Sunil Kumar Deshmukh ·
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Fungi Bioactive Metabolites

Integration of Pharmaceutical Applications

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Preface

Natural products have been the mainstay for bioactive compounds, which have propelled the development of pharmaceuticals, cosmeceuticals, and agrochemicals. All biological matrices present on the Earth comprise naturally occurring substances that may prove to be beneficial as a medicine and personal care products or could provide solutions to crop protection and production. It may not be out of place to mention that fungi were recognized as one of the tactical organisms which could propel the process of drug discovery and developed subsequent to identification of the first beta-lactam antibiotic, penicillin, from *Penicillium notatum* (now *Penicillium rubens*). Prior to this, plants were the mainstay for discovering medicinal compounds based on the earlier studies done by shamans and healers. Microbes are ubiquitous in their existence and so are fungi.

Fungi are eukaryotic microorganisms, which exhibit a heterotrophic mode of nutrition, evolutionarily closer to animals than plants. The study of fungi, their forms, life cycle, and physiological and biochemical patterns that influence their growth and reproduction is known as mycology. These are considered as one of the most diverse forms of life-forms with respect to their morphological, ecological, and nutritional modes. They constitute the largest organismic group after insects, which have been estimated to be existing between 2.8 and 3.3 million species within different ecosystems on the Earth, of which only 150,000 species have been described.

Many secondary metabolites (bioactive compounds) produced by fungi have been successfully developed into medicines. The major fungal secondary metabolites which have been developed into medicinal agents (drugs) beyond penicillin comprise cephalosporins (antibiotic, *Cephalosporium acremonium*), griseofulvin (antifungal, *Penicillium griseofulvum*), echinocandins (antifungal, *Aspergillus nidulans* var. *roseus* ATCC 58397), mycophenolic acid (immunosuppressant, *Penicillium* sp.), cyclosporine (immunomodulatory agent, *Tolypocladium inflatum*), and lovastatin (cholesterol reducing, *Aspergillus terreus*). Thus, fungi hold immense possibilities as bioresources of new chemical entities, which could be developed into new medicines. The recent development in the field of genomics and analytical

instrumentation has paved way to shift the exploration of these new chemical entities more rationally and target specifically, as compared to the phenotypic screening methods. Hence, in this book, we have tried to bring in the latest concepts in screening fungi from varied ecological niches for fueling the drug discovery and development in industry as well as in academia.

This book has been aptly divided into eight sections. The first part of the book addresses the role of bioactive compounds isolated from the endophytic fungi, wherein the emphasis has been laid on the molecules which are into pharmaceutical development, possessing anti-infective properties and possibilities to be developed into therapeutic interventions for neurodegenerative disorders. Another interesting facet of fungi is to associate itself with other microorganisms such as algae in lichens, and during this interaction, they produce some novel chemistries. So, the second part of the book is basically oriented on the discovery of bioactive compounds from endolichenic fungi with pharmaceutical potential by Sri Lankan and Indian researchers. The third part of the book emphasizes the role of marine fungi in discovery of new pharmacophores as well as in antibacterial drug discovery and development. The fourth part addresses the role of fungal secondary metabolites in the development of medicinals belonging to different classes of drugs. The emphasis is on antibacterial drug, immunosuppressants, anticancer agents, antifungals, and anti-inflammatory and antithrombotic compounds. As fungi also exist in unique environments and extreme conditions, they become a lucrative source of unique secondary metabolites with potential to be exploited for drug discovery and development. This is the theme of the fifth part of the book. Several techniques at molecular level have been developed to enhance the yield of secondary metabolites for commercial production. These comprise one strain many compounds (OSMAC), co-cultivation, and epigenetic modulation. These have been addressed in the sixth part. The seventh part addresses other products of fungal metabolism, the pigments and peptidyl/non-peptidyl compounds, which possess medicinal properties that could be exploited in the process of drug discovery and development. The last part of this book emphasizes on the role of bioinformatics and biotransformation carried out by fungi in the production of novel medicinal agents.

To summarize, this book is a treatise on all aspects of fungi and their potential for pharmaceutical drug development and would generate much interest among the readers to undertake mycological research using advanced tools and techniques in propelling drug discovery research.

Mumbai, India — Sunil K. Deshmukh
Belo Horizonte, Brazil — Jacqueline A. Takahashi
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About the Editors

Sunil Kumar Deshmukh is Scientific Advisor to Greenvention Biotech, Uruli Kanchan, Pune, India, and Agpharm Bioinnovations LLP, Patiala, Punjab, India. The veteran industrial mycologist spent a substantial part of his career in drug discovery at Hoechst Marion Roussel Limited [now Sanofi India Ltd.], Mumbai, and Piramal Enterprises Limited, Mumbai. He has also served TERI-Deakin Nanobiotechnology Centre, TERI, New Delhi, and as an Adjunct Associate Professor at Deakin University, Australia. He has to his credit 8 patents, 150 publications, and 20 books on various aspects of fungi and natural products of microbial origin. He is President of the Association of Fungal Biologists (AFB) and a past president of the Mycological Society of India (MSI). He is also a fellow of the Mycological Society of India, the Association of Biotechnology and Pharmacy, the Society for Applied Biotechnology, and Maharashtra Academy of Science. Dr. Deshmukh serves as a referee for more than 20 national and international journals. He has approximately four decades of research experience in getting bioactive compounds from fungi and keratinophilic fungi.

Jacqueline Aparecida Takahashi is a Full Professor at the Universidade Federal de Minas Gerais (Brazil). For more than two decades, she has been studying routes to modulate fungal secondary metabolism, and she has been dedicated in recent years to issues related to access to Brazilian biodiversity, in reference to international treaties. She is a member of the Disciplinary Nucleus of Bioactive Natural Products of the Montevideo Group Universities Association (AUGM). She has to her credit 8 patents, 183 publications, and 12 book chapters on natural products from plants and fungi. She is also a member of the Brazilian Society of Chemistry (SBQ) and of the Brazilian Society for the Progress of Science (SBPS). Dr. Takahashi serves as a referee for more than 20 national and international journals.

Sanjai Saxena is currently an academic and researcher with over two decades of experience in biotech research and development. He is currently a full professor in the Department of Biotechnology, Thapar Institute of Engineering and Technology (Patiala), India. He has embarked upon his journey as an innovator turned entrepreneur and founded a startup, Agpharm Bioinnovations LLP, with a pursuit to address the ever-arising unanswered pharmaceutical and agricultural sector needs. He continually focuses on exploring and exploiting novel biomolecules drawn from biological systems and their interactions in nature, particularly plant-microbe interactions. Prof. Saxena focuses his research primarily on microbial secondary metabolites, microbial diversity and bioprospecting, biochemistry, and drug discovery. His research work has resulted in significant extramural funding, 4 patents, over 78 peer-reviewed publications in referred research journals, 12 book chapters, and 1 book on applied microbiology to his credit. Dr. Saxena is a Fellow of Association of Advancement of Biodiversity Science, Karnataka. Prof. Saxena serves as a referee for approximately 25 peer-reviewed journals apart from being on the editorial board of a couple of them.

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Part I
Bioactive Compounds from Endophytic Fungi

Chapter 1
Recent Advances in Pharmaceutically Important Compounds from Endophytic Fungi

Jacqueline A. Takahashi, João Gabriel S. Marques, Mariana R. Ferreira, Thiago P. Santos, and Gustavo O. C. Rosário

Abstract The study of endophytic fungi as a source of new bioactive molecules is an area that has grown very much within the chemistry of natural products, probably due to advances in the techniques of isolation, identification, and dereplication of fungal species and the exploration of new biomes and niches for the collection of host plants. Secondary metabolites produced by endophytes have been shown to be active against the most diverse types of biological targets, such as numerous tumor cell lines, parasites, and etiological agents causing neglected diseases (HIV, Chagas disease, Leishmaniasis, etc.). More recently, a significant number of metabolites of endophytic fungi have also been successfully studied against several important targets of the SARS-CoV-2 virus. This chapter presents recent data on fungal metabolites that have distinguished biological activity and potential for further drug development.

Keywords Endophyte fungi · Biomes · Cancer · Neglected diseases · SARS-CoV-2 · Drug development

1 Introduction

Endophytic fungi are species that live inside plant tissues, throughout the entire or partial life cycle without affecting morphological characteristics or causing damage to the host plant. Instead, several studies have shown that these species offer physiological and ecological support to the host plants (Manganyi and Ateba 2020). Other studies have sought to understand the relationship between these fungi and the regulation of plant growth, plant protection, and resilience to stress

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(Fadiji and Babalola 2020; Tiwari and Bae 2022; Toppo et al. 2022). Endophytic fungi are also the target of studies related to their influence on the biosynthesis of secondary metabolites by host plants (Caruso et al. 2020) and the effect of fungal diversity on the ecosystem (Adnan et al. 2022). This kind of fungi has a prolific and creative biosynthesis, capable of producing secondary metabolites of various classes such as terpenoids (Amirzakariya and Shakeri 2022; Galindo-Solís and Fernández 2022), polyketides (Liu et al. 2016), alkaloids (Daley and Cordell 2021), xanthones (Khattab and Farag 2022), and sulfur-containing compounds (Fan et al. 2022). The production of secondary metabolites with complex and unusual chemical structures such as 2,3-dihydro-1H-indene analogs (Cui et al. 2018) and molecules containing 6/6/6/5/5 tetracyclic skeleton has also been described (Zhang et al. 2022). Wen et al. recently reviewed 220 new compounds with rare or novel structures or skeleton structures possessing diverse biological activities from endophytic fungi from 82 journal articles between 2011 and 2021 (Wen et al. 2022).

In addition to secondary metabolites, it is worth to briefly mention the importance of fungi in the production of extracellular enzymes, many of which have therapeutic applications (Raghav et al. 2022; Bhadra et al. 2022). Several industrially important enzymes are produced extracellularly by fungi: among them amylases, produced by endophytic species isolated *from Euterpe precatory* (Batista et al. 2022) and *Albizia lebbeck* (Mathur et al. 2022), and asparaginases, produced by endophytes of the plant *Mandevilla catimbauensis* (Araújo-Magalhães et al. 2021). L-asparaginase is used as a drug in acute lymphocytic leukemia chemotherapy, and it is produced by a number of fungi using submerged and solid-state fermentation. The use of L-asparaginase is preferred over chemical drugs due to the lack of toxicity (Bhadra et al. 2022). This enzyme can be produced by bacteria, but the final product has some toxicity issues, raising the interest of the pharmaceutical industry to find safe sources of this enzyme. Promising research in this area concerns in the isolation of L-asparaginase from *T. pinophilus*, an endophyte isolated from the rhizomes of *Curcuma amada* (Araújo-Magalhães et al. 2021). Several other enzymes, usually with hydrolytic action, are usually excreted by microorganisms during the fermentation process and have several industrial and pharmacological applications such as cellulases (Khalil et al. 2021), laccases, gelatinases (Marsola et al. 2022), lipases (Alves et al. 2018), and xylanases (Amobonye et al. 2021). Lipases have therapeutic potential as antifungal and leishmanicidal agents (Alves et al. 2018), while amylases are useful in the diagnosis of cystic fibrosis (Bhadra et al. 2022).

Studies with endophyte fungi are progressing all over the world, and much information is available. Li et al. (2018) reviewed the antitumor activity of alkaloids, isocoumarins, polyketides, quinones, steroids, terpenes, and other classes of compounds from 109 strains of endophytic fungi (3 phyla, 7 classes, and 50 genera), reported between 2014 and 2017. A total of 118 anti-inflammatory compounds from diverse chemical classes (alkaloids, anthraquinones, azaphilones, butenolides, coumarins, cytochalasans, glycosides, lactones, sesquiterpenoids, quinones, xanthones) reported in the last two decades (until February 2019) as endophytic fungal metabolites were reviewed by Pal et al. (2020). The antibacterial profiling, chemical structures, and mode of action of 451 metabolites reported from January 2015 to

April 2021 of anthraquinones, sesquiterpenoids, chromones, xanthones, phenols, quinones, quinolones, piperazines, coumarins, and cyclic peptides classes were recently reported (Deshmukh et al. 2022a). The present chapter focuses on aspects less explored, such as fungal distribution, methodological approaches to study endophyte species, recent developments, current pharmacological targets, and metabolites recently described from endophyte fungal species, to add complimentary discussion in the area.

2 Biomes, Regions, and Plant Families

Research with endophytic fungi has been carried out worldwide, thanks to the ubiquity of these microorganisms. Therefore, there are no major restrictions for the isolation of fungi in relation to the biome where the host plant is located. On the contrary, endophytic species that produce bioactive metabolites have been related from the most different biomes and these microorganisms are always associated to an ecological and sustainable production of compounds to feed the pharmaceutical industry in a nearby future (Zhao et al. 2020; Ancheeva et al. 2020). The marine environment is one of the main sources of new endophytic fungi nowadays. The marine endophytes have been isolated mainly from plants like seagrass, mangrove plants, algae, sponge, and corals. Marine sediments have also been frequently described as sources of fungi, although they cannot be considered endophytes. The success of marine plants-derived endophytic fungi can be justified by the different metabolism of these organisms, as a result of coevolution in an environment constantly subjected to osmotic stress.

Sharma et al. (2020) reviewed the therapeutic applications of endophytes of several genera (*Alternaria*, *Aspergillus*, *Botryosphaeria*, *Colletotrichum*, *Fusarium*, *Paecilomyces*, *Penicillium*, and *Pestalotiopsis*, among others) harbored in Asian medicinal plants from Bangladesh, China, India, Indonesia, Japan, Malaysia, South Korea, Sri Lanka, and Thailand. The authors also discussed the interaction of fungal endophytes with the host plants. The arid ecosystem of the Thar Desert of Rajasthan is a rich repository of ethnomedicinal plants which, in turn, contain endophyte species able to produce pharmaceutically important metabolites (Yadav and Meena 2021). Indonesian fungal endophytes from medicinal plants with antibacterial, antifungal, and anti-enzymatic activity have been reported by Mamangkey et al. (2022). Endophytic fungi from the Himalayan region have been pointed out as important targets in the search for new antitumor and anti-HIV agents. The Himalayan region is also an important hotspot, with unique microorganisms, plants, and other underexplored genetic resources (Banyal et al. 2021). Santos et al. (2022) reviewed the metabolites produced by 36 Brazilian endophyte fungi (2015–2021), with a focus on terpenoids. Sixty-seven metabolites of this class were identified and they were associated with several biological activities such as antidepressant, anti-cancer, antimalarial, among others (Santos et al. 2022). On another study, Savi et al. (2019) described endophyte species isolated in Brazil

between 2012 and 2017 covering rainforest, *caatinga*, *cerrado*, Atlantic Forest, *pampa*, and *pantanal*, the major Brazilian biomes. The authors highlighted the importance of studying species from global biodiversity hotspots since some endophytes are host-specific. *Empetrum rubum*, a medicinal plant from Austral South America, furnished 83 endophytic fungal isolates, including some fungal taxa uncommonly reported as endophytes (*Allantophomopsis cytisporea*, *Humicolopsis cephalosporioides*, *Phacidium grevilleae*, *Pseudocercospora pseudophacidioides*, *Pseudocercospora brackenicola*, and *Pseudopithomyces* sp.) (Ferreira et al. 2021).

Several endophytic fungi of previously unknown taxa were identified from the Eastern Mountain Avens (*Geum peckii*), a globally rare and endangered perennial plant found only at two spots in Canada and endemic to the alpine sites in the White Mountains (New Hampshire, USA) (Adams et al. 2021). The fungi isolated are of future interest for restoration and conservation purposes from a mycological perspective, and they offer novel options for the recovery of novel secondary metabolites.

Multiple species of fungal endophytes have been isolated from the most diverse plant families such as Rubiaceae (Cruz et al. 2020), Brassicaceae (Poveda et al. 2022), Amaryllidaceae (Caruso et al. 2020), as well as from *Citrus* plants (Nicoletti 2019), and ornamental species (Gioia et al. 2020). The specific interest for some botanical families as source of endophyte species has been increasing in the recent years, many times related to the identification of fungi producing specific metabolites or rare compounds. Other times, the interest is associated with the numerous biological activities associated with secondary metabolites biosynthesized by the host plant or by the associated endophyte, as occurs with the Rubiaceae family. The chemical diversity of metabolites isolated from Rubiaceae endophyte species is high, and these metabolites have demonstrated potential in the treatment of neurodegenerative diseases, and in the control of inflammation and hyperglycemia (Cruz et al. 2020; López et al. 2021).

Some peculiarities make some botanical families the target of studies with specific objectives. A good example is the Brassicaceae family which has species with "remarkable peculiarities," related to the production of defense compounds of the glucosinolate class (Poveda et al. 2022). These compounds are involved in plant defense making difficult the co-existence of fungi inside the plant. For the establishment of endophytic fungi in plants of the Brassicaceae family, it is necessary to depress plant defense mechanisms, increase the plant tolerance to the fungus, or suppress the defense mechanisms associated with the establishment of fungi. This dynamic makes the study of the interaction of endophytic fungi–Brassicaceae plants of great interest for the understanding of the mechanism of fungus–plant symbiosis formation. Research in this area has excellent future perspectives for agricultural applications such as plant disease prevention and biological control (Macías-Rubalcava and Garrido-Santos 2022; Poveda et al. 2022).

Amaryllidaceae species have important applications in culinary, especially as component of exotic foods, and this botanical family comprises medicinal plant species, although the family is neglected as sources of endophyte fungi. Caruso et al. reported that although the dominant fungal endophytes isolated from

Amaryllidaceae family are endemic from several taxonomically different hosts, there are some species and tissue-dependent endophytic species in this botanical family (Caruso et al. 2020). These authors highlighted the prominence of Amarylloidaceae subfamily as source of alkaloids that can be produced under stimuli and via in vitro synthesis, aiming at several applications. In Amaryllidaceae plants, fungal endophytes are better adapted to the morphological and physiological conditions found in the plants' roots.

Endophytes from plants of Citrus genus are mostly studied to verify pathogenicity issues and ecological features (Nicoletti 2019), while some botanical families have peculiar relationship with endophyte species. Plants from Orchidaceae family form a mutualistic association with some specific fungi during the germination phase, depending on the nutrition provided by the fungal species to initiate the seedling cycle (Bhatti and Thakur 2022). The diversity of fungal endophytes from Orchidaceae family is also of great interest since these fungi have several industrial applications (Chua and Ting 2021).

Although endophyte fungi are not harmful for the host plants, these fungi may interfere in the environment once outside the host. In this way, some preoccupation is arising about the endophyte present in plants imported for ornamental purposes, and regulatory agencies have been studying possible threats to ecosystems and biosecurity that may arise during intercontinental trade (Gioia et al. 2020). Therefore, issues about the negative impacts of foreign fungal endophytes that may be accidentally introduced in foreign ecosystems are an interesting point for discussion among authorities and scientists.

3 Handling Endophytic Fungi

Several authors strongly suggest that the success of endophytic fungi as a prolific source of new bioactive compounds, benefited from modern technologies for purification, characterization, and biological screening of active metabolites, as well as useful new approaches such as genetic engineering, amplification of gene clusters, mutagenesis, and refinement of fungal cultures (Rai et al. 2022). Starting from the beginning, the classical methods for the isolation of endophytic fungi consist in the addition of sterile fragments of the host plant in agar plates, following with the transference of macromorphologically different species until obtaining pure colonies. Enrichment of the culture media can help in increasing the variety of fungal endophytes isolated. An interesting work recently published by Reis, Lorenzi, and Vale in 2022 presents and discusses some useful methods used to collect and sample plant material, disinfection, culture media choices, morphotypes grouping, and molecular identification of endophyte fungi. The authors also discuss issues related to ITS rRNA region and molecular phylogeny, methods for studying chemical diversity, and cultivation-independent methods, being a good guide for starting working in this area (Reis et al. 2022).

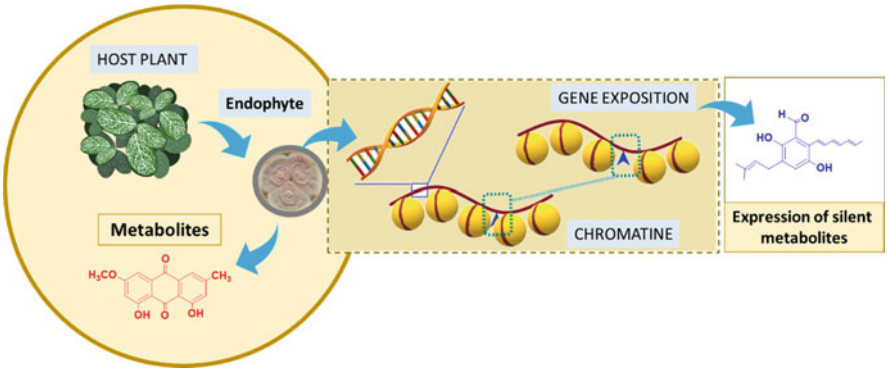

Fig. 1.1 Production of silent secondary metabolites after gene exposition by epigenetic modifiers

After isolating the endophyte species, the fermentation process for obtaining the secondary metabolites of endophytic fungi usually follows the general methodology used for fungi cultivation. The fungus is transferred from the solid storage medium (usually potato dextrose agar, PDA) and inoculated into a basic culture medium such as PDB (potato dextrose broth) to prepare a seed culture. The use of a seed culture prevents direct inoculation of the fungus, usually stored at low temperature, into the culture medium that will be used for fermentation. This procedure allows reactivation of fungal metabolism and increases the amount of inoculum, making the cultivation more productive. The use of rice as a substrate for the production of secondary metabolites has been used by a significant number of research groups (Chen et al. 2021; Ramos et al. 2022).

Co-culture (Oliveira et al. 2022) modifications in the growing conditions (Lima et al. 2018) and mutation are important approaches for optimizing metabolites production by fungi (Deshmukh et al. 2022a; Takahashi et al. 2020). These tools can induce silent gene clusters related to the production of cryptic metabolites. The genetic tools available in recent decades have allowed a great advance in the knowledge of the vast metabolome of fungi (Nagarajan et al. 2021). The production of fungal secondary metabolites can be induced by small molecules, such as epigenetic modifiers (Toghueo et al. 2020) that act on DNA methyltransferases (e.g., 5-azacytidine, hydralazine, and procainamide) or histone deacetylases (e.g., suberoylanilide hydroxamic acid). These molecules provide changes in the chromatin structure allowing the expression of secondary metabolite-related genes hitherto silent in the chromatin (Mapook et al. 2022) (Fig. 1.1).

The biosynthesis of cryptic metabolites and yield improvement have been achieved using the combination of OSMAC (one strain, many compounds) approach with other methodologies, such as genome mining. Simple modifications such as the introduction of halogens to the culture medium can lead to the incorporation of the halogen in the metabolite's chemical structure. Using this approach, 12 new cytochalasins, including phomopchalasin E (bromine) (**1**) and phomopchalasins F (iodine), were isolated from *Phomopsis* sp. QYM-13 (Chen et al. 2022a). The

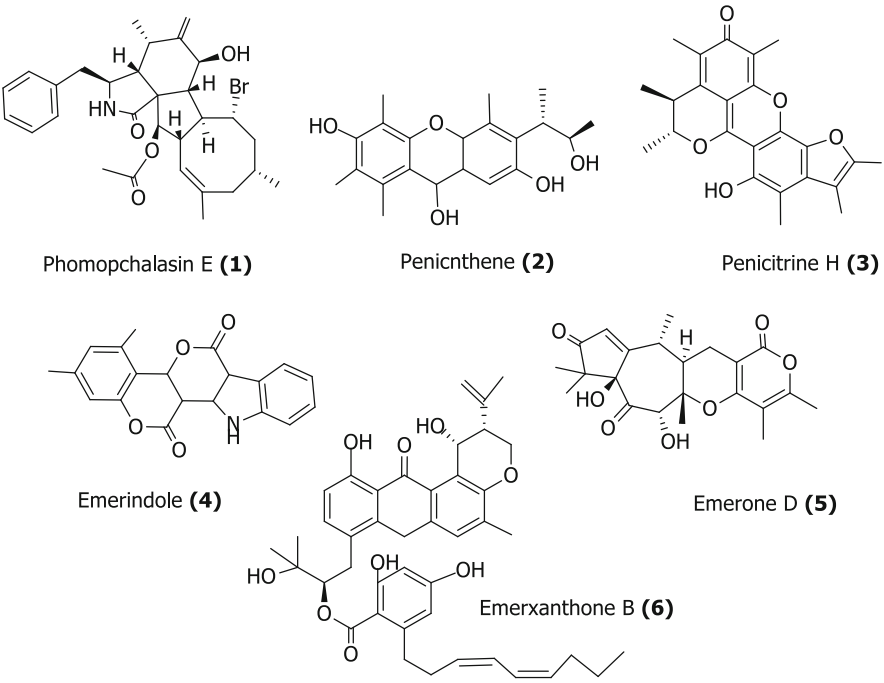

Fig. 1.2 Chemical structures of the compounds produced vunder OSMAC conditions (**1–6**)

metabolic alteration caused by OSMAC can also lead to the synthesis of new metabolites. *Penicillium* sp. T2–11 afforded penicnthene (**2**) and penicitrione H (**3**), new metabolites of xanthene and citrinin types. Penicnthene is the first example of a xanthene derivative with a 3-hydroxy-2-butyl chain. These metabolites showed acetylcholinesterase inhibition (28.03 ± 0.15 and 23.62 ± 0.37 μM, respectively), and the activity of penicitrione H (**3**) was supposed to be related to the methyl group located at C-6 (Li et al. 2022a). *Asordaria conoidea* was submitted to OSMAC modulation. Three extracts were obtained, one of them with allelochemical activity, showing that the different cultivation conditions led to different pools of metabolites (Arruda et al. 2022). *Emericella* sp. XL029, under OSMAC conditions, produced seven new compounds, including emerindole (**4**), emerone D (**5**), and emerxanthone B (**6**) (Xian et al. 2022). The chemical structures of the compounds are presented in Fig. 1.2. *Macrophomina phaseolina*, endophyte isolated from the host plant *Brugmansia aurea*, was studied with and without the effect of the epigenetic modifier sodium valproate, a histone deacetylase inhibitor, combined with OSMAC modifications. The results showed that the metabolite profile was modified. Among the changes, *M. phaseolina* produced four new and several volatile compounds (Singh et al. 2022).

4 Innovative Pharmacological Targets for Bioactive Fungal Metabolites

The demand for biologically active compounds for the production of new drugs is a very dynamic area. The development of new drugs can be oriented to increased absorption, prolongation of effect, higher specificity with decreased side effects, and lower negative drug interactions. Innovative drugs may be necessary for the treatment of new diseases, as occurred recently with the COVID-19 pandemic, a situation that involved the research of substances capable of acting on biological targets present in the SARS-CoV-2 virus, as well as antiviral drugs with new mechanisms of action. The development and application of vaccines are essential for coping with endemic viruses, such as HIV, dengue fever, Chikungunya, and Zica in various regions of the world (Costa and de Oliveira 2020; Banyal et al. 2021), but new drugs are also necessary since these diseases are spreading out (Takahashi et al. 2021; Lacerda et al. 2022).

To exemplify, it is estimated that almost half of the individuals contaminated with HIV have died and around 37 million people are living with HIV worldwide (Bertagnolio et al. 2022). In addition, patients with pathologies such as AIDS are more propense to acquire other diseases, as recently reported during the COVID-19 pandemic. Figures also show that there is still a great demand for the development and availability of new medicines with lower toxicity and enhanced tolerability to virus mutations (Banyal et al. 2021).

Equally important in this context is the development of antibiotics for the control of resistant bacteria, which causes the death of millions of people annually. In this area, research for new drugs should be constant since it is virtually impossible for bacterial strains to become resistant to available drugs. Several works have shown that metabolites biosynthesized by endophytic fungi are active against resistant bacterial strains (Manganyi and Ateba 2020). Isotetrahydroauroglaucin (**7**) and cristatumin B (**8**), metabolites produced by an *Aspergillus niger* species isolated from the host plant *Opuntia ficus-indica*, are examples of antibacterial metabolites as promising leads for the development of antibiotics effective against resistant strains (Fig. 1.3). Isotetrahydroauroglaucin (**7**) and cristatumin B (**8**) were active against Gram-positive and Gram-negative resistant bacteria, respectively (Elkady et al. 2022). Antibiotic activity was the most impactful event in the history of fungal metabolites as a source of new drugs. Between 2015 and 2021 were reported over 450 new fungal metabolites, potentially useful in the treatment of infections by resistant bacteria, although it is stressed the need for further evaluation by standardized studies using reference strains for a comparative analysis of the inhibiting potential of these metabolites (Deshmukh et al. 2022a).

New drugs are also needed for the treatment of neglected diseases such as malaria, chagas disease, leishmaniasis, parasitic diseases, among others. These pathologies remain a serious public health problem and generally affect the most deprived populations living in tropical regions (Costa and de Oliveira 2020). A

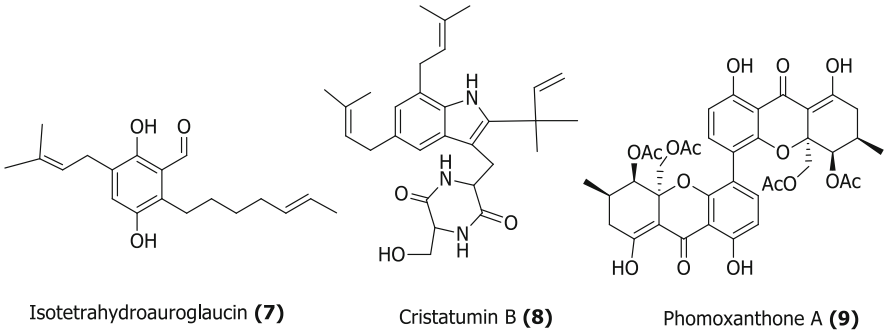

Fig. 1.3 Chemical structures of the some of the antibacterial compounds (**7, 8**) and antileishmanial compound (**9**)

Paecilomyces species isolated as endophytic from *Schnella splendens*, a typical plant of the Amazon, produces phomoxanthone A (**9**), a metabolite with pronounced inhibitory effect against promastigote forms of *Leishmania amazonensis* and against epimastigote forms of *Trypanosoma cruzi* (Ramos et al. 2022). Trypanocidal natural products have also been described by Nascimento et al. (2020), while natural products active against *Toxoplasma gondii* were reported from Mazzone et al. (2022).

Fungi can also cause human diseases of difficult treatment and new antifungal drugs for human use are extremely necessary. Several fungal infections are silent or seen only as esthetic problems related to skin, nails, and hair colonization. However, fungal diseases can acquire severity, reaching sepsis and death if untreated, with a mortality rate similar to tuberculosis (Bongomin et al. 2017). Some prominent fungal diseases are chronic pulmonary aspergillosis, cryptococcal meningitis complicating HIV/AIDs, *Pneumocystis jirovecii* pneumonia, invasive aspergillosis, histoplasmosis, fungal asthma, and fungal keratitis. The prevalence of invasive fungal infections has been increasing in recent decades among immunocompromised population, mainly HIV-positive individuals, organ transplant recipients, and cancer patients (Pfaller et al. 2019). Another fungal disease of great importance is candidemia, an invasive candidiasis caused by different species of *Candida* sp., which has approximately 700,000 individuals infected annually in the world (Bongomin et al. 2017). The literature has shown the emergence of resistance to antifungals of echinocandin-R and fluconazole classes in *C. glabrata* and *C. tropicalis* isolates (Pfaller et al. 2019).

Endophyte fungi are prolific sources of antifungal metabolites, which are produced to ensure the survival of a fungal species in the presence of another fungus (Oki et al. 2021). Antifungals naturally produced by endophytic fungi have been used for the treatment of mucormycosis, a very destructive invasive human disease caused by several mucorales, typically present in immunocompromised patients (Hashem et al. 2022). Deshmukh et al. (2018) listed 127 antifungal metabolites produced by Coelomycetes, 40 produced by *Hyphomyces*, and 3 produced by

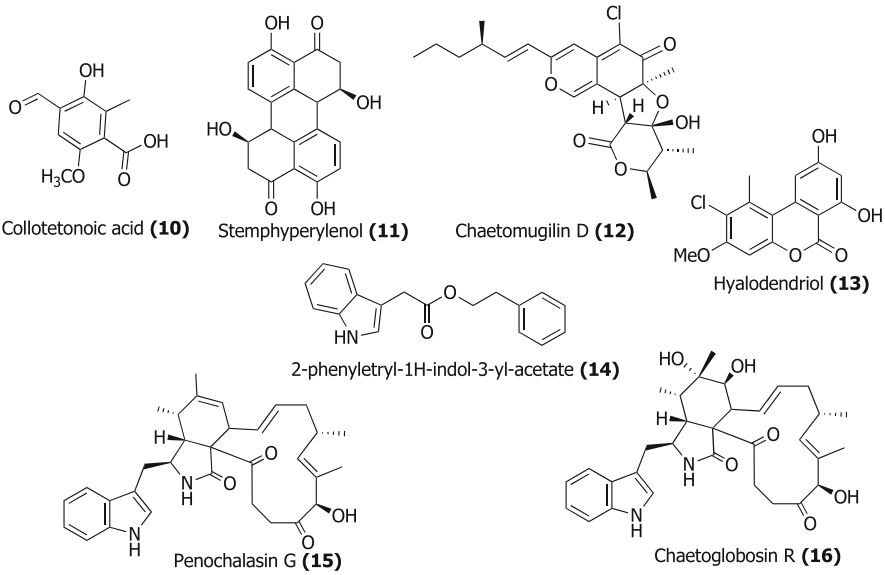

Fig. 1.4 Chemical structures of antifungal compounds (**10–16**)

Basidiomycetes of endophytic origin. Among the antifungal metabolites described in Deshmukh's review, there are polyfunctionalized phenolic derivatives (colletonoic acid, polycyclic metabolites, macrocycles, phenylindol derivative, and chlorinated metabolites) (**10**–**15**). Endophyte species are also known to produce some metabolites with complex chemical structures, as can be exemplified by chaetoglobosin R (**16**) (Fig. 1.4).

Antifungal metabolites containing the pyrone nucleus are commonly produced by endophyte species, as exemplified by ficipyrone A (**17**), rhizopycnin D (**18**), and phomopsolide A (**19**), which are α-pyrone, dibenzo-α-pyrone, and dihydropyrone derivatives, respectively. Anthraquinones like emodin (**20**) are also common fungal metabolites and molecules with steroidal and terpenoid biosynthetic origin can be exemplified by calvasterol A (**21**) and the oxygenated guaiane-type sesquiterpene (**22**), respectively (Deshmukh et al. 2022a) (Fig. 1.5).

More restricted demands, equally important, comprise the search for innovative drugs aimed at the treatment of chronic and rare diseases, individuals allergic to conventional drugs, and immunosuppressed patients. Innovation in this area can lead to a decrease in the cost of production, distribution, and sale, increasing people's access to drug treatments.

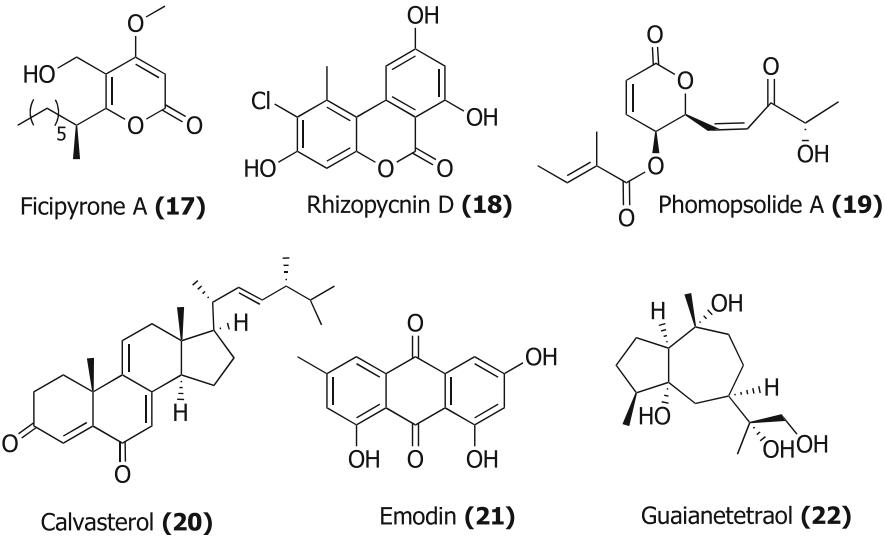

Fig. 1.5 Chemical structures of Antifungal metabolites containing the pyrone nucleus (**17–22**)

5 Promising Endophytic Fungi Metabolites Recently Described

Secondary metabolites produced by endophytic fungi have been widely studied in the search for applications in agriculture (Fadiji et al. 2022; Fadiji and Babalola 2020) especially the production of volatile compounds (Santos et al. 2022), including those that can bring sustainability to agricultural activities (Kaddes et al. 2019). Development of anti-phytopatogenic agents is also suggested (Zhao et al. 2020). Other applications concern food (Manganyi and Ateba 2020) and cosmetics (Mahlangu and Tai 2022) industries. This is an area that is showing huge progress over the last two decades (Pal et al. 2020; Zheng et al. 2021). Fungal metabolites have been studied as leads for the development of new drugs to treat diseases, such as cancer (Kousar et al. 2022; Bai et al. 2022), inflammatory (Cui et al. 2018; Liao et al. 2018; Pal et al. 2020), infectious (Deshmukh et al. 2022a), and neurological problems (Mapook et al. 2022), as antioxidants (Bedi et al. 2021) and, recently, antiviral fungal metabolites against the SARS-CoV-2 virus due to the COVID-19 pandemic (Takahashi et al. 2021; Deshmukh et al. 2022b). In vitro screenings were also related (ElNaggar et al. 2022). Among them, cancer is undoubtedly one of the most important long-lasting targets for the development of new drugs. This disease is the second cause of death in the world and its incidence is increasing every year.

Although hereditary factors, food habits, and occupational exposure are related to specific types of cancer, this disease often affects people regardless of age or social group. Treatment is currently based on chemotherapy, but the low specificity of the available treatments severely weakens the patient due to several major side effects,

making treatment complex and costly. In addition, tumor cells may develop resistance to the drugs in use, and it is therefore necessary to find new selective drugs with fewer side effects for cancer treatment (Rai et al. 2022). An interesting review paper about endophyte fungi published in 2022 covered different chemical and biological aspects, such as the mechanisms of action, current challenges, and future perspectives of fungal metabolites active against Kaposi's sarcoma, human papillary thyroid carcinoma, human pancreatic, ovarian, hepatic, lung, human lymphoma, human skin carcinoma, and breast cancer cell lines (Kousar et al. 2022). Many times, the antitumor activity is associated with uncommon natural products with unrevealed mechanisms of action. Some examples of metabolites with antitumor and other activities are given in Table 1.1, and the chemical structures of the representative metabolites are presented in Figs. 1.6 and 1.7.

It is noteworthy that the fungal species present different cytotoxic profiles, represented by activity against different targets, as is the case of the action against a large number of human cancer cell lines. The metabolites cited in Table 1.1 also represent the action in other pathologies that can be associated with cancer, such as inflammatory processes and bacterial infection. The latter can be caused by opportunistic bacterial contamination of immune-depressed individuals during cancer chemotherapy.

Some of the compounds presented in Table 1.1 were isolated for the first time from endophytes and represent novel compounds, as is the case of fusaroxazin (**29**), a novel 1,4- oxazine-xanthone derivative, produced by *Fusarium oxysporum* (endophyte isolated from *Vicia faba*) with cytotoxic effect toward A549, HCT-116, and MCF-7 tumor cell lines (Mohamed et al. 2022).

In terms of biotechnological applications and product development, the potential of secondary metabolites in medicine is of major importance, but more investments are still needed in this area to accelerate biotechnological advance toward the development of new drugs and drug candidates (Mapook et al. 2022).

6 Developments Related to Bioactive Metabolites from Endophyte Fungi

Chemical diversity is an important ally in the search for new pharmacologically active drugs. The biosynthesis of specific classes of metabolites can be related to the fungal species and also to the environment from where the host species is collected. Molecules with xanthone moiety, for example, are commonly isolated from marine-derived endophytic fungi (Veríssimo et al. 2022; Khattab and Farag 2022), but novel chemical scaffolds have been isolated from most species of endophyte fungi (Liu et al. 2020).

One of the most representative classes of secondary metabolites produced by endophytic fungi are alkaloids (Pal et al. 2020; Deshmukh et al. 2022a), as well exemplified by the well-known alkaloid's paclitaxel, camptothecin, chincona, and

Table 1.1 Bioactive metabolites reported from endophytic fungal species and respective activity

Endophyte fungal species [host]	Bioactive metabolite [IC_{50}]	Activity	References
Aspergillus terreus[a]	Asperimide C (**23**) [0.78 ± 0.06 μM]	Inhibition of LPS-activated NO production in RAW264.7 cells	Liao et al. (2018)
	Asperimide D (**24**) [1.26 ± 0.11 μM]		
Cladosporium sp.[b]	Aspulvinone D (**25**) [10.3 ± 0.6 μM]	Inhibition of NLRP3 inflammasome activation	Liang et al. (2022)
	Aspulvinone M (**26**) [9.4 ± 0.6 μM]		
	Aspulvinone R (**27**) [7.7 ± 0.6 μM]		
Diaporthe sp.[c]	Diaporindene A (**28**) [8.5 μM]	Inhibition of LPS-activated NO production in RAW 264.7 cells	Cui et al. (2018)
	Diaporindene B [5.9 μM]		
	Diaporindene C [4.2 μM]		
	Diaporindene D [4.2 μM]		
Fusarium oxysporum[d]	Fusaroxazin (**29**) [HCT-116 2.1 μM]	Cytotoxic effect toward tumor cell lines	Mohamed et al. (2022)
	[MCF-7 1.8 μM] [A549 3.2 μM]		
Montagnulaceae sp.[e]	Rubiginosin B (**30**) [9.2 ± 0.9 μM]	Inhibition of LPS-activated NO production in RAW 264.7 cells	Luo et al. (2017)
	Montagnuphilone B (**31**) [25.5 ± 1.1 μM]		
	Montagnuphilone E (**32**) [39.6 ± 1.8 μM]		
Paraboeremia selaginellae[f]	NK-A 17e233 (**33**) [5.75 μM]	Anti-toxoplasma activity with low or no cytotoxicity in human cell lines	Mazzone et al. (2022)
	Cyperin (**34**) [27.22 μM]		
	ES-242-1 (**35**) [7.38 μM]		
	ES-242-3 (**36**) [17.99 μM]		
	5S,6S-phomalactone [5.13 μM]		
Paraphaeosphaeria sporulosa[g]	Paraphaeone E [25 μM]	Antibiotic activity against *pseudomonas syringae pv. Actinidiae*	Chen et al. (2022b)
	Paraphaeone F (**37**) [50 μM]		
	Acremonone E [50 μM]		
	Epicoccone [100 μM]		
Penicillium brefeldianum[h]	Penipentenone A (**38**) [10.88 μM]	Inhibition of SW620 and ASPC-1 cells	Bai et al. (2022)
	Brefeldin C [2.71 ± 0.21 μM]		
Penicillium sclerotiorum ZJHJJ-18[i]	Sclerazaphilone C (**39**) [7.87 ± 2.04 μM]	Inhibition of LPS-activated NO production in RAW 264.7 cells	Jiang et al. (2022)
	Sclerazaphilone D (**40**) [6.30 ± 0.35 μM]		
	Sclerazaphilone E (**41**) [9.45 ± 1.39 μM]		
Penicillium sp.[j]	Pinselin [5.9 to 7.5 μg/mL]	Suppress con A-induced T-cell and LPS-induced B-cell proliferation	Liu et al. (2016)
	Sydowinin A (**42**) [6.5 to 7.1 μg/mL]		

(continued)

Table 1.1 (continued)

Endophyte fungal species [host]	Bioactive metabolite [IC_{50}]	Activity	References
Phoma bellidis[k]	Phomaone A (**43**) [14.99 μM]	Cytotoxicity against MCF-7, PC-3, and DU145	Zhang et al. (2022)
	Oblongolide J [22.26 μM]		
Phomopsis asparagi DHS-48[l]	Phomoparagin B [21.6 ± 1.7 μM]	Inhibition of interleukin-2 (IL-2) transcription	Feng et al. (2022)
	Phomopchalasin A (**44**) [32.8 ± 2.4 μM]		
Phomopsis asparagi LSLYZ-87[m]	Phomasparapyrone B [50 μM]	Inhibition of NO accumulation induced by LPS on BV-2 cells	Liu et al. (2022)
Phomopsis sp.[n]	Phomopchalasin F [7.4 μM]	Cytotoxicity against MDA-MB-435	Chen et al. (2022a)
	Cytochalasin U (**45**) [8.2 ± 0.9 μM]		
	Cytochalasin J [4.9 ± 0.6 μM]		
	Cytochalasin H [0.2 ± 0.1 μM]		
Talaromyces amestolkiae YX1[o]	Amestolkolide B (**46**) [1.6 ± 0.1 μM]	Inhibition of NO production in LPS-activated AW264.7 cells	Chen et al. (2018)

A-549 (lung cancer), DU145 (prostate cancer), HCT-116 (colon cancer), MCF-7 (breast cancer), PC-3 (prostate cancer), and RAW 264.7 (macrophage cell line established from a tumor induced by the Abelson leukemia virus)
LPS lipopolysaccharide, *NO* nitric oxide
[a]*Suriana maritima* [South China]
[b]*Paris polyphylla var. yunnanensis* [China]
[c]*Excoecaria agallocha* [China]
[d]*Vicia faba* [China]
[e]*Persicaria amphibia* [North America]
[f]*Philodendron monstera* [Central America]
[g]*Actinidia chinensis* [China]
[h]*Houttuynia cordata* [SW Asia]
[i]*Hibiscus tiliaceus* [Pacific coastal zone]
[j]*Sonneratia apetala* [China]
[k]*Tricyrtis maculate* [Eastern Asia]
[l]*Rhizophora mangle* [China]
[m]*Acanthus ilicifolius* [China]
[n]*Kandelia candel* [Southeast Asia]
[o]*Kandelia obovate* [Eastern Asia]

huperzine A. Several classes of alkaloids isolated from fungi possess complex chemical structures, as shown in Fig. 1.8. Therefore, the total synthesis of many bioactive alkaloid compounds can be unfeasible or economically unviable. Therefore, natural sources of bioactive alkaloids are sought out worldwide for pre-clinical and clinical studies.

It is widely reported that alkaloids and other secondary metabolites are biosynthesized by both endophytic fungi and the host plant, although this point is

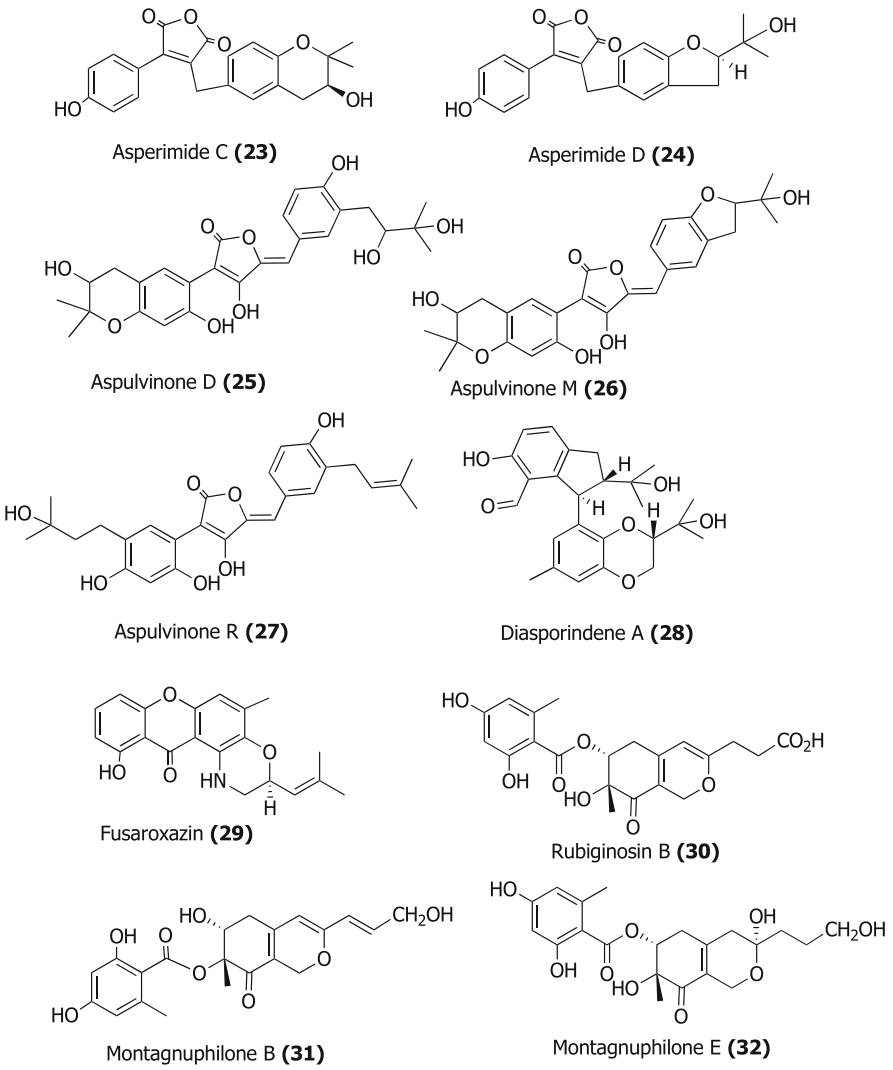

Fig. 1.6 Chemical structures of representative metabolites described in Table 1.1 (**23–32**)

still a topic for which in-depth studies are suggested. Nevertheless, many examples of co-production are reported in the literature, such as vinblastine and vincristine, minor antitumor bis-monoterpene indole alkaloids produced in very low yield by the plant species *Catharanthus roseus*. As research with these alkaloids progressed, it was discovered that endophytic fungi isolated from *C. roseus* produce these indole alkaloids and structurally related bioactive derivatives at encouraging yields (Daley and Cordell 2021).

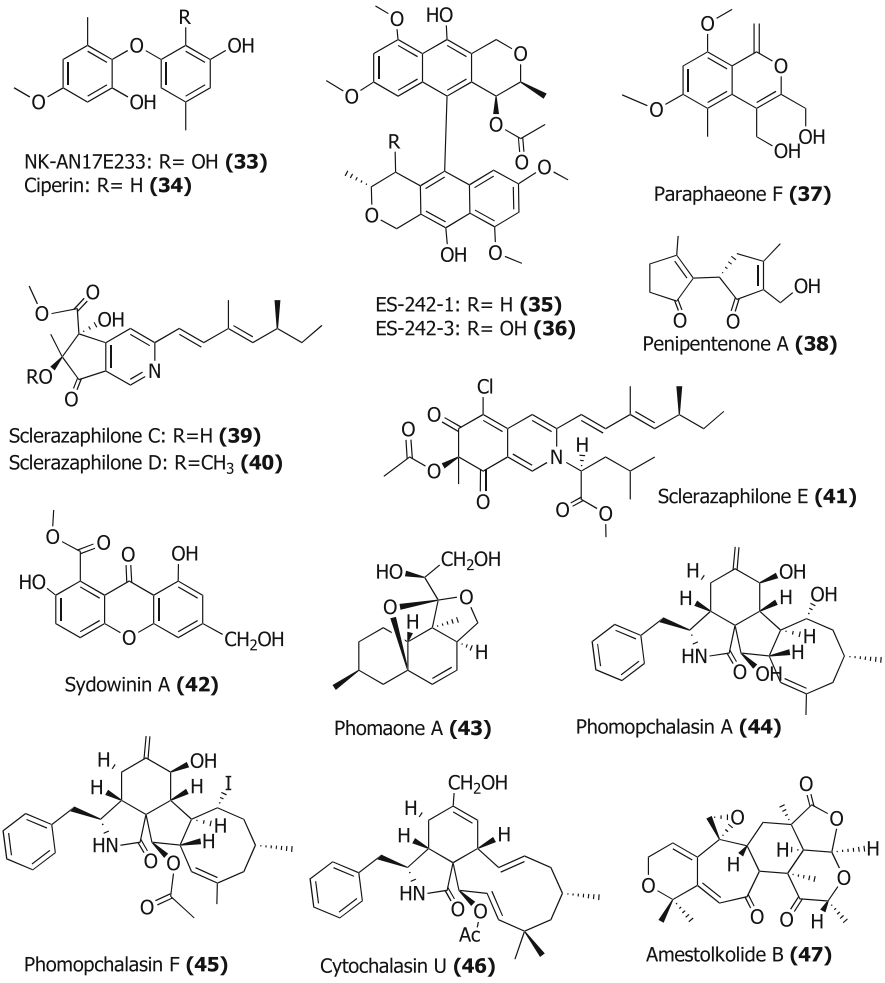

Fig. 1.7 Chemical structures of representative metabolites described in Table 1.1 (**33**–**47**)

Indole alkaloids are a group of representative fungal metabolites comprising ergot alkaloids, produced by the species *Claviceps purpurea* and other species of the phylum Ascomycota. The ergot alkaloids interact strongly with serotonin, dopamine, and adrenergic receptors, causing several useful biological effects in the treatment of Parkinson's disease, dementia, uterine hemorrhage, and migraines. *Claviceps*-infected rye contributes with half of the global production of ergot alkaloids (Yao et al. 2022a). Taking into consideration the high structural complexity of the natural bioactive ergot alkaloids, as represented by compound (**48**), total synthesis of this metabolite is challenging, making the research on endophyte fungi with improved production of ergot alkaloids an interesting research area. Suitable endophyte strains must have minimum tendency to degeneration, and few

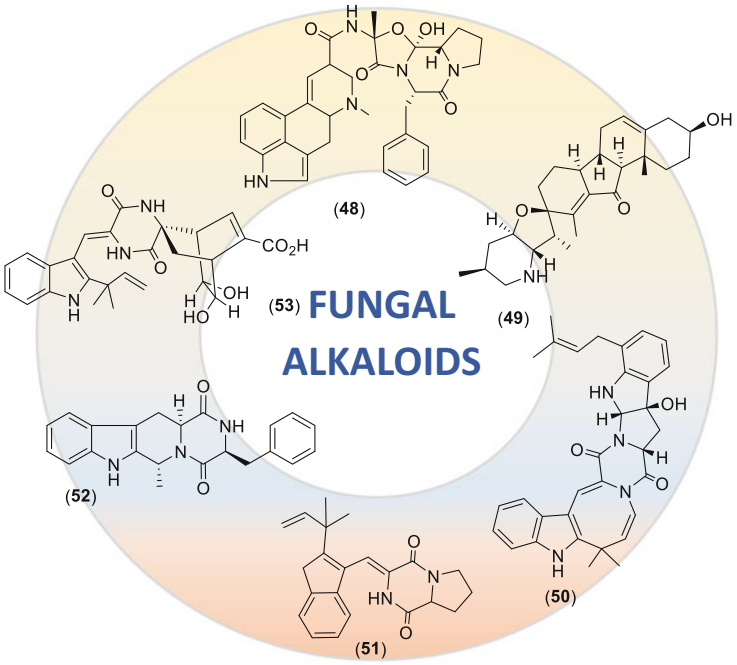

Fig. 1.8 Chemical diversity of fungal alkaloids produced by endophytic fungi

biosynthetic routes to ensure long utilization of the strain and ease separation of the product. Since a screening step must precede the production development, the use of high-speed automatized tools, to select promising strains, would allow the improvement of natural ergot alkaloids production (Wong et al. 2022).

Jerveratum-type steroidal alkaloids were described as fungal metabolites with antifungal and anti-cancer activity. This alkaloid (**49**) acts synergistically with fluconazole and inhibits β-1,6-glucan biosynthesis in *S. cerevisiae*. Structure activity studies have shown that the NH group is essential for activity (Kubo et al. 2022). Diketopiperazine alkaloids have been described from several fungal species and with different biological activities. An alkaloid isolated from *Penicillium* sp. TW58–16 (**50**) was active against standard strains and drug-resistant clinical isolates of *Helicobacter pylori* in vitro (Tian et al. 2022), while a brevianamide analogue (**51**) exhibited potent cytotoxic activity against the HGC-27 cell line with IC_{50} stronger than that of cisplatin (Li et al. 2022b). A diketopiperazine-type alkaloid (**52**) isolated from the endophytic fungus *Penicillium expansum* showed anti-inflammatory activity against LPS-induced nitric oxide (NO) production in RAW264.7 mouse macrophages (IC_{50} 25.65 μM) (Yao et al. 2022b). Chevalinulin A (**53**), an indole diketopiperazine alkaloid containing an unprecedented spiro[bicyclo[2.2.2]octane-diketopiperazine skeleton with significant in vivo proangiogenic activity in transgenic zebrafish, was isolated from *Aspergillus chevalieri* CS-122 (Yan et al. 2022).

Aspergillus species are proliferous sources of bioactive secondary metabolites (Hagag et al. 2022). Figure 1.8 presents the chemical structures of some alkaloids isolated by endophytic fungi, to show the huge chemical diversity of these fungal metabolites.

Terpenoids are the largest group of secondary metabolites (Galindo-Solís and Fernández 2022) and have industrial great potential for cosmetic, food, and pharmaceutical industries. In the period of 2017–2019, only in China, 200 terpenoids biologically active were reported, mainly sesquiterpenoids, all of them produced by endophytic fungi (Zhao et al. 2020). In their review, Zhao et al. pointed that most of the endophyte fungal species were from *Trichoderma, Aspergillus, Bipolaris, Penicillium, Phomopsis,* and *Alternaria* genera, isolated from leaves, roots, branches, inner tissues, thallus, stems of plants, as well as from fruiting bodies. Paclitaxel, camptothecin, and fusidic acid are examples of bioactive terpenoids. In the period 2011–2020, 516 terpenoids were described from endophyte fungi, most of them of sesquiterpenoid type (Amirzakariya and Shakeri 2022), although meroterpenoids have also been highlighted.

A pentacyclic triterpene, lupeol acetate, commonly found in plants was isolated from *Colletotrichum gigasporum*, an endophyte from *Withania somnifera*. Interestingly, this triterpene was described as an anti-obesity agent due to its capacity of inhibiting pancreatic lipase (IC_{50} 16.62 ± 1.43 μg/mL) and its mechanism of action was studied (Patil et al. 2021). *Zingiber griffithii* was described as the host of the endophytic fungus *Hypomontagnella monticulosa*. The endophyte produced a sesterterpenoid active against Panc-1, NBT-T2, and HCT116 tumor cell lines (IC_{50} 0.71, 0.30, and 0.67 ppm, respectively) (Lutfia et al. 2021). Meroterpenoids, ophiobolin-type sesterterpenoids, drimane-type sesquiterpenoids, and other terpenes were reported from *Aspergillus* sp. RR-YLM-12, an endophyte from the red algae *Rhodomela confervoides*. Some of these metabolites were reported as antimicroalgal (Fang et al. 2021).

Phomopsis prunorum, an endophytic fungal species isolated from leaves of *Hypericum ascyron,* produces (+)- Phomoterpene A (**54**), a new phenolic bisabolane-type sesquiterpenoid with activity against the plant pathogenic bacteria *Pseudomonas syringae pv. Lachrymans* (MIC 15.6 μg/mL) (Qu et al. 2020). *Penicillium chrysogenum* MT-12, an endophytic fungus isolated from the medicinal plant *Huperzia serrata,* produced a new diterpenoid named Penicichrysogene A (**55**). This diterpenoid is capable of inhibit ATP release of thrombin-activated platelets (IC_{50} 42.7 ± 3.5 μM) (Qi et al. 2022). Another diterpenoid (**56**) with a new type of indole scaffold with a rare 6/5/6/6/6/6/5 heterocyclic system bearing an aromatic ring C was reported from *Penicillium* sp. (strain ZO-R1–1) and named Shearilicine. This Indonesian strain was isolated from roots of *Zingiber officinale*, a medicinal plant used locally to treat cold, cough, and rheumatism among other illnesses. This cytotoxic diterpenoid was active toward L5178Y (IC_{50} 3.6 μM) and A2780 (IC_{50} 8.7 μM) cells (Ariantari et al. 2019). *Talaromyces pinophilus* produced a new fusicoccane diterpene, pinophicin A (**57**). The endophyte was obtained from the aerial parts of *Salvia miltiorrhiza* (Zhao et al. 2021). This novel meroterpenoid was isolated from the plant endophytic fungus of *Penicillium* sp. sh18. Isopenicin A

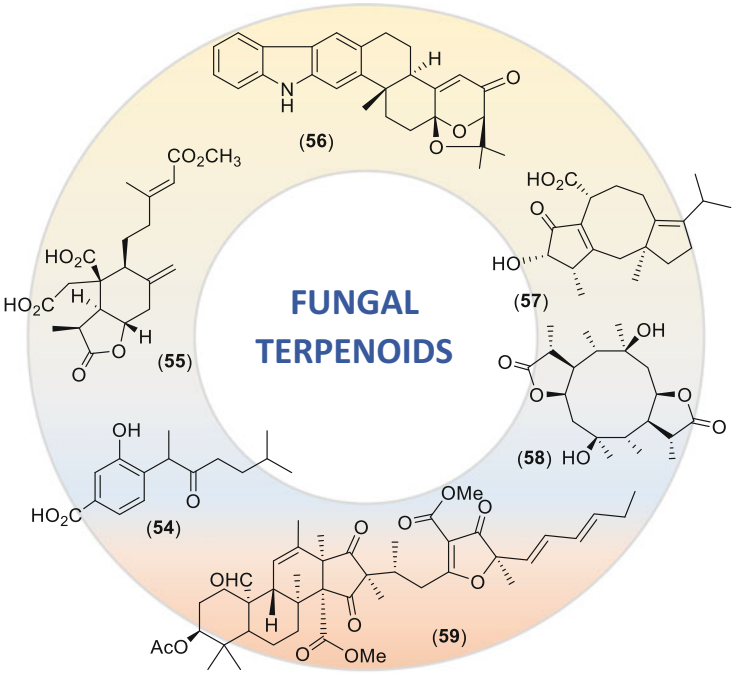

Fig. 1.9 Chemical diversity of fungal terpenoids produced by endophytic fungi

(**58**) inhibits tumor by inducing G2/M cell cycle arrest and cell apoptosis. Moreover, this metabolite is a new chemotype for discovery and the development of microtubule inhibitors (Chen et al. 2020). Diaporpenoid A (**59**) is a new diterpenoid, with anti-inflammatory activity. This metabolite, produced by *Diaporthe* sp. QYM12, an endophyte isolated from the host plant *Kandelia candel*, inhibits the production of nitric oxide (NO) in lipopolysaccharide (LPS)-induced RAW264.7 cells (IC_{50} 21.5 μM) (Chen et al. 2021). Figure 1.9 contains some examples of the chemical diversity of fungal terpenoids produced by endophytic fungi.

7 Perspectives

Although fungi are an endless source of novel bioactive metabolites, the research of endophyte species is still in its childhood. The re-isolation of secondary metabolites already known is very common, but this is not a bad outcome per se. Re-isolation can provide higher yields of useful metabolites and, also, can bring about new biological activities. With the evolution of analytical and spectroscopical tools, minor novel metabolites are expected to be isolated, revealing novel chemical structures and new pharmacophoric groups, consequently, new mechanisms of action for several industrial applications (Singh et al. 2021).

Plants are capable of adapting their metabolism to survive in challenging environments, causing a cascade of internal physiological modifications that interfere in the fungi that inhabit their interior. The metabolic changes that occur in the host plant also affect the variety of species and the metabolism of the endophytes. Comprehensive studies on the variability of endophytic species of the same plant collected from different biomes are still scarce and advanced research tools have been useful in this area (Adnan et al. 2022). Meta-genomics has been pointed out as a robust tool to quickly identify endophytes, with the objective of selecting promising species for the selection of a pool of beneficial fungi that can be explored focusing on biotechnological development (Toppo et al. 2022).

Another relevant issue for the study in this area is the relationship between the biosynthesis of endophytic fungi in loco (within the host plant) and the in vitro biosynthesis (laboratory conditions) since the internal environment of the host plant is unique and cannot be imitated in vitro. Consequently, the biosynthesis of fungal secondary metabolites that depends on enzymes, metals, cofactors, and other conditions provided by the host plant is still to be better explored, although culture medium supplementation may provide some help in this regard.

Development of novel approaches to produce metabolites from uncultivable endophytes and the search for new species are among the perspectives toward the progress of new drugs from endophyte fungi (Rai et al. 2022). Interdisciplinary approaches, application of omics, chromatin control, next-generation of sequencing, and genetic tools are also mentioned as a way to improve the release of toxin-free fungal products in the next two decades (Bielecka et al. 2022; Mapook et al. 2022). Additionally, it has been demonstrated that the ecological relationship of fungi in an ecosystem is dynamic, requiring metaproteomic and meta-transcriptomic assessments, and studies to define evolutionary and ecosystem functionality of the endophyte species (Adnan et al. 2022). Therefore, fungal endophytes are still a major reservoir of novel metabolites with the expectation of new discoveries in the near future.

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Chapter 2
Recent Advances in Anti-Infective Compounds Produced by Endophytic Fungi

Sanjay Kumar, Indu Pathania, Takaaki Kamishima, Yoshitaka Koseki, Hitoshi Kasai, and Inder Pal Singh

Abstract Endophyte is the combined form of two Greek words, which are endon (i.e. within) and phyto (i.e. plants). Endophytic microorganisms (mainly bacteria and fungi) live inside the plant for habitat, food and protection without any harmful effect on the host plant. Endophytic fungi are a highly diverse group of fungi colonized in the host plant tissue and produce diverse chemical classes of secondary metabolites such as alkaloids, steroids, terpenoids, flavonoids, glycosides, xanthones, isocoumarins, quinones, phenyl propanoids, lignans, lactones, benzopyranones, tetralones, cytochalasins and enniatins. These secondary metabolites have been reported to have various biological activities such as anticancer, immunomodulatory, insecticidal, antibacterial, antifungal, antitubercular, antiplasmodial, antiviral, anti-inflammatory and anti-oxidant. In this chapter, we mainly focused on recently reported and related antibacterial, antimycobacterial, antifungal, antiviral and antiplasmodial secondary metabolites isolated from endophytic fungi.

Keywords Antibacterial · Antifungal · Antiviral · Fungi · Secondary metabolites · Antiparasitic

1 Introduction

Endophyte biology is a relatively new field, with origins dating back to the nineteenth century. De Bary coined the word ‘endophyte’ of botanical origin in 1866 (De Bary 1866). Endophyte is derived from the two Greek roots: ‘endon’ which

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means ‘within’ and ‘phyto’ which means ‘plants’ (Jalgaonwala et al. 2011). The word was originally used for any organism living within a plant, but with the development of science, broader definitions have been given nowadays. In general, endophytic microorganisms such as bacteria and fungi live in the tissues, leaves and roots of plants for reasons like habitat, food and protection. They may or not affect the host plant, and also have a symbiotic relationship. In addition, they inhabit or colonize plant tissues at specific or arbitrary stages of their life cycle, and do not immediately harm the host plant (Petrini 1991).

Initially, researchers prospected that the relationship between host plants and endophytes was symbiotic. However, the initial hypothesis was disproved by the proposed one by Schulz and Boyle (2005). According to their hypothesis, endophytes acquire high virulence as pathogens when they colonize host tissues and secrete several exo-enzymes. The defence mechanism arising on the host plants as a response to colonization shows little difference between pathogens and endophytes. Pathogens often overcome the defensive chemicals produced by hosts causing a disease, tissue damage or even death. On the other hand, endophytes can colonize host tissues without damage to the hosts in the process of overcoming the defence reaction. Therefore, the difference between pathogens and endophytes depends on whether they injure the hosts during colonization. Furthermore, the collapse of the exquisite balance due to any stress caused by external or internal factors can lead to the death of hosts and/or endophytes. The individual species of more than 300,000 plants identified to date are hosts for one or hundreds of endophytes and are not host-specific (Strobel and Daisy 2003). Therefore, a single endophytic species can exist in multiple different hosts or different tissues within the same host (Jalgaonwala et al. 2011). Endophytes belonging to various taxonomic groups have been identified, including bacteria such as mycoplasma and actinomycetes, and fungi, among which fungi have been the most extensively studied endophytic group (Bandara et al. 2006; Rana et al. 2019).

Fungal endophytes are versatile and common, symbiotic with plants growing in almost all geo-climatic conditions on the earth (Patil et al. 2016). Some of the endophytic fungi can produce compounds with the same or equivalent properties as that of their host plant (Strobel and Daisy 2003; Zhao et al. 2010). Those ones are well known as a source of secondary metabolites with bioactivity, and some of them are certainly useful for the therapy of diseases affecting mankind.

Various metabolites that have attractive functional properties such as antibiotics, anticancer agents, biological control agents and other bioactive compounds have been discovered and isolated from endophytic fungi up to the present. However, it has not yet been identified which diseases most of them are effective against. Hence, accelerating the study on endophyte biology may lead to the discovery of new products of unprecedented therapeutic value (Kusari and Spiteller 2011). Additionally, endophytic fungi give the researchers like chemists, microbiologists, ecologists and agronomists, challenging themes for discovering novel compounds that could help face problems like cancer, AIDS and infectious diseases.

In this chapter, we describe some beneficial pharmaceutical and medicinal compounds with anti-infective potential derived from endophytic fungi. Since there are

abundant compounds to introduce, we have mainly focused on recently reported or representative examples.

2 Role of Endophytic Research with Respect to Drug Discovery

The notable features of endophytes are the production of their secondary metabolites (Schulz et al. 2002). Enormous attractive secondary metabolites have been isolated to date, and many studies have been conducted in a wide range of fields, including pharmaceutical and medicinal chemistry. For instance, some of the secondary metabolites are known to have excellent bioactive effects like anticancer, antibacterial and anti-inflammatory, and some of them are actually used as pharmaceuticals in clinical conditions. The endophytes also produce several useful and novel enzymes that could be used in the biotechnology field for various purposes like degradation and biotransformation.

One of the most famous secondary metabolites discovered from endophytes is penicillin isolated from *Penicillium notatum* in 1928 (Fleming 1929). Starting with this discovery, various antibiotics have been developed successively, and continue to save human lives to date. However, human beings have not yet overcome fungal and bacterial infections, and the threat of antimicrobial resistance (AMR) has recently arisen and must be addressed urgently (Fischbach and Walsh 2009). To confront AMR, there is an urgent need for novel antibiotics, which will be an alternative to the currently available broad-spectrum antibiotics. Even though the problem of morbidity and mortality associated with AMR is not new, AMR has recently been increasing at a significant rate due to bacteria that have acquired resistance to multiple groups of antimicrobial agents. Moreover, the decline in the development and marketing of new antimicrobial agents worsens the situation. O'Neill's report predicts that with the current increasing trend of AMR (Fischbach and Walsh 2009), an estimated ten million lives per year may be lost to AMR-related diseases by 2050 (O'Neill 2016). To avoid this worst-case scenario, it is necessary to develop novel antimicrobial agents with chemical skeletons and/or antimicrobial mechanisms different from those of conventional drugs. Endophytic fungi can assemble mechanisms to resist pathogenic invasion by producing secondary metabolites. Some of antibiotics isolated from endophytic fungi would act against broad or specific pathogens at extremely low concentrations (Bo et al. 1998); Umino et al. 1973). Thus, many scientists pay attention to discovering novel compounds among the secondary metabolites of endophytes that have high potential for the treatment of infectious diseases.

Another field that has been drawing attention in recent years is anticancer agents derived from endophytes. Today, cancer is raging as a worldwide disease. According to a statistical survey in 2018, more than 18.1 million people were affected by this incurable disease, of whom 9.6 million passed away (Umino et al. 1973). Current

cancer treatment systems are classified as surgery in the early stages and chemotherapy or radiotherapy in the later stages, which have significant side effects for the patient (Bray et al. 2018; Burish and Tope 1992; Vijayalakshmi et al. 2010). Due to the frequent disease recurrence and chemoresistance, treatment strategies to target cancer are required strongly. According to a report on the anticancer drugs, it was found that approximately 47% of the total 155 anticancer drugs approved by 2006 were derived from natural products or semi-synthesized from lead compounds isolated from natural compoundse (Newman and Cragg 2007). Additionally, it is estimated that more than 85% of plants have not yet been explored for their bioactive properties (Pieters and Vlietinck 2005). Thus, there is a great potential for undiscovered novel compounds with anticancer activities.

The discovery of attractive bioactive metabolites from endophytic fungi has a potential to increase the options of lead compounds that may be developed into novel pharmaceuticals. On the other hand, it is extremely inefficient to develop effective drugs without any clues. Therefore, it will accelerate the development of novel drugs that can solve important problems such as high efficacy, low toxicity, low cost and low environmental impact.

Compounds derived from discovered or undiscovered endophytes have great potential for the development of novel drugs that can save human lives. The following section introduces some representative compounds and their beneficial bioactive effects on human health. Endophytic fungi protect their host plant from a range of diseases (bacteria, fungi, viruses and protozoa) by creating bioactive compounds. Endophytic chemicals exhibit antibacterial, antifungal, antiviral and antiprotozoan properties. Due to the increase in the ability of bacteria for drug resistance after repeated use of a known antibiotic, novel antibiotic sources are constantly sought after. In an axenic culture, several endophytic fungi produce antibiotic substances that are active against bacteria harmful to people and plants. Compared to synthetic products, natural compounds made from endophytic fungi are more effective across a wider spectrum and are less hazardous. It has been observed that secondary metabolites from endophytic fungi have promising antibiotic actions against a variety of bacteria (Fig. 2.1, Table 2.1).

3 Antibacterial Metabolites

The severity of antibiotic resistance was acknowledged by Aharwal and colleagues who also stressed the significance of creative approaches to research and development (Aharwal et al. 2016). Endophytes have been shown in several studies to produce secondary metabolites as part of their defence mechanisms against pathogenic invasion. Low molecular weight, organic, naturally occurring chemicals known as bioactive metabolites are created by microbes and have antimicrobial effects at low concentrations on other microorganisms (Manganyi and Ateba 2020).

Recent studies have shown the investigation of Cupressaceae related natural sources for endophytic fungi. Highly bioactive endophytic *Alternaria* fungi were

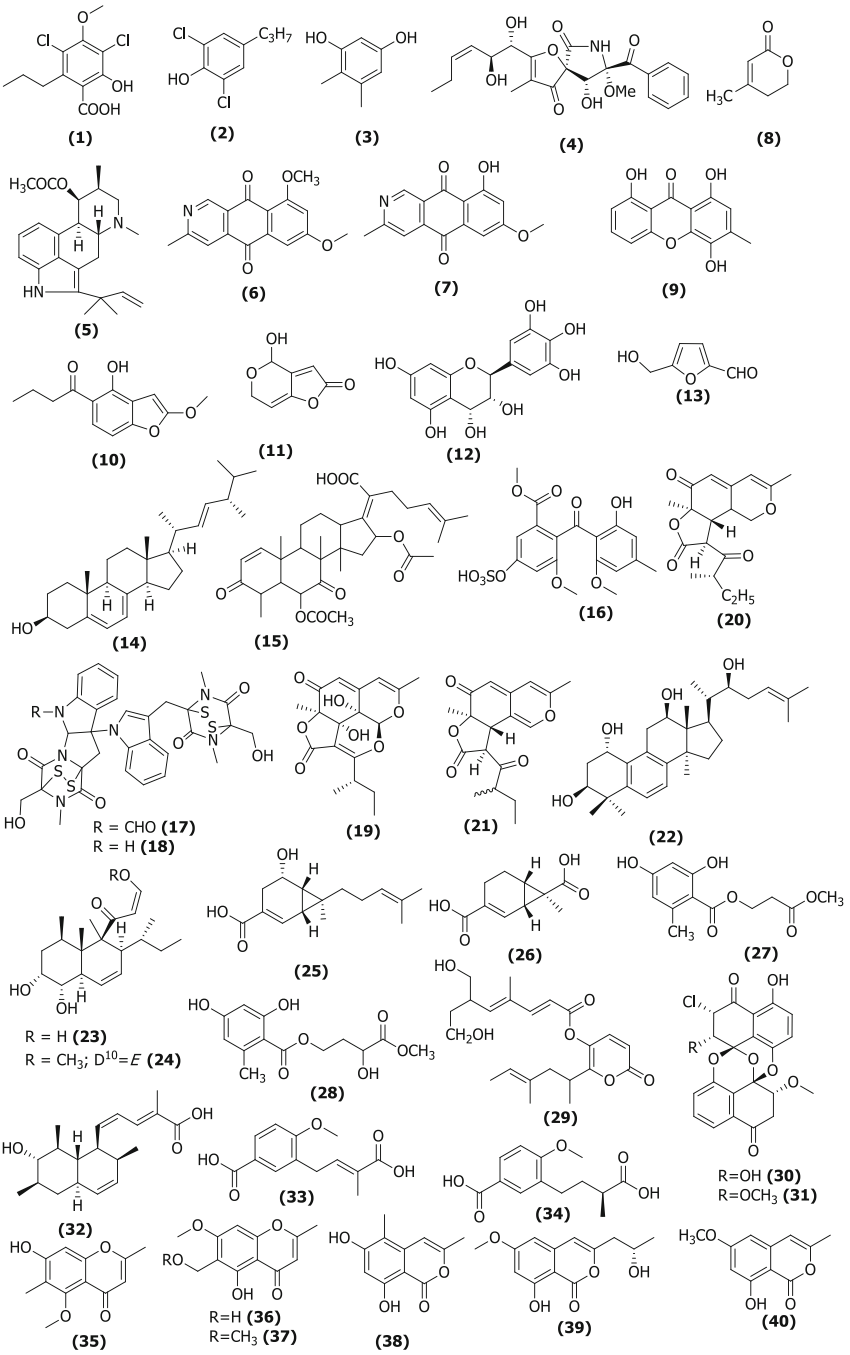

Fig. 2.1 Structures of Antibacterial metabolites from endophytic fungi

Table 2.1 List of anti-bacterial metabolites from endophytic fungi

S. no.	Endophytic fungus strain	Compound isolated	Target bacteria	Plant source	Anti-bacterial activity	References
1.	*Alternaria alternata*, *Alternaria pellucida*, and *Alternaria tangelonis*	*A. pellucida* POE_{182}, *A. alternata* CAE_{92} CSE_{177}, CSE_{165}, POE_{43}, POE_{104}, POE_{72}.	*Bacillus* sp., *Erwinia amylovora*, and *Pseudomonas syringae*	Cypress trees (*Cupressus arizonica*, *Cupressus sempervirens* var. *cereiformis*, and *Thuja orientalis*)	MIC (minimum inhibitory concentration)—7.8–31.2 μg mL^{-1}, 31.2–125 μg mL^{-1} and 15.6–62.5 μg mL^{-1}	Soltani and Hosseyni Moghaddam (2014)
2.	*Chaetomium* sp. HQ-1, *Fusarium* sp. HQ-7 and *Fusarium* sp. HQ-9	Differanisole A (**1**), 2,6-dichloro-4-propylphenol (**2**), 4,5-dimethylresorcinol (**3**)	*Listeria monocytogenes*, *Staphylococcus aureus*	*Astragalus chinensis*	MIC—16 to 128 μg/mL	Li et al. (2019)
3.	*Aspergillus* sp. EJC08	Pseurotin A (**4**), Fumigaclavine C (**5**)	*B. subtilis*	*Bauhinia guianensis*	MIC—7.81 μg/mL and 15.6 μg/mL	Pinheiro et al. (2013)
4.	*Colletotrichum* and *Fusarium* species	EtOAc extracts of *Fusarium* sp.	Gram positive bacteria	*Cinnamomum mercadoi*	MIC - 2.1–4.2 mg/mL	Marcellano et al. (2017)
5.	*Microsporum gypseum* and *Penicillium marneffei*	Crude extract of fungal strain	*Staphylococcus aureus*, *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*	*Cymodocea serrulata*, *Halophila ovalis*, and *Thalassia hemprichii*	<10 μg/mL	Supaphon et al. (2013)
6.	*Curvularia protuberata* and *Penicillium citrinum*	Ciprofloxacin cyclo (phenylalanylprolyl)	*S. aureus*, *S. enteric*, and *S. Typhi*	*Digitaria bicornis* and *Paspalidium flavidum*	ZOI—10.0– 30.0 mm for different bacterial strains	Nischitha and Shivanna (2021)
7.	*Lophiostoma*	Scorpinone (**6**) 5-Deoxybostrycoidin (**7**) and 4-methyl-5,6-dihydro-2 H-pyran-2-one (**8**)	*R. Solanacearum*	*Eucalyptus exserta*	ZOI—6.43 ± 0.69 to 9.86 ± 0.75	Mao et al. (2021)
8.	*Exserohilumrostratum*	Ravenelin (**9**)	*Bacillus subtilis* and *Staphylococcus aureus*	*Stemona* sp.	MIC—125 μg/mL and 1.95 μg/mL	Pina et al. (2021)

9.	*Epicoccum nigrum*	Radicinol derivative and 1-(4-Hydroxy-2-methoxybenzofuran-5-yl) butan-1-one (**10**)	*B. subtilis*, *E. coli*, and *S. aureus*	*Acanthus ilicifolius*	MIC value—25 and 50 μg/mL	Yan et al. (2021)
10.	*Nigrospora*	5*E*,9*E*-Farnesyl acetone, Columellarin, Totarene, Laurenan-2-one and 8*S*,13-Cedranediol, sclareolide, and epi-a-bisabolol	*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *P. aeruginosa*	*Ocimum basilicum*	MIC—81–250 μg/mL	Atiphasaworn et al. (2017)
11.	*P. canescens*, *P. commune* and *A. alternata*	3-Methyl-1-butanol and phenylethyl alcohol	*B. subtilis*, *S. aureus*, *B. cereus*, *E. coli*	*Olea europaea*	MIC ≤ 0.095 mg/mL	Malhadas et al. (2017)
12.	*Penicillium setosum*	Patulin (**11**) Leucodelphinidin (**12**) dihydroquercetin, Kaempferol, and Quercetin	*S. aureus* and *E. coli*	*Withania somnifera*	MIC—8 μg/mL	George et al. (2019)
13.	*Fusarium oxysporum*	5-Hydroxymethylfurfural (HMF) (**13**) and octadecanoic acid	*Enterococcus Faecalis*, *Enterococcus gallinarum*, *Bacillus cereus*	*Sceletium tortuosum*	ZOI—(>10 mm)	Manganyi et al. (2019)
14.	*C. globosum* and *Fusarium*	Securinega-type alkaloids	*E. coli*, *P. aeruginosa*, *S. aureus*	*Securinega suffruticosa*	MIC—0.5 mg/mL	Du et al. (2020)
15.	*O. senegalensis*	3-Methyl-1,2-cyclopentanedione, terrain, 9,12-octadecadienoic acid (*Z*, *Z*)-methyl ester, (*E*)-9-octadecenoic acid, methyl ester and rosenonolactone	*E. coli* and *S. aureus*	*Vernonia anthelmintica*	ZOI—26 and 19 mm	Niu et al. (2022)

(continued)

Table 2.1 (continued)

S. no.	Endophytic fungus strain	Compound isolated	Target bacteria	Plant source	Anti-bacterial activity	References
16.	*Cochliobolus* sp.	1-(2-aminoethyl)-aziridine	MRSA, VRSA, *B. cereus*, *B. subtilis*, and *S. aureus*	*Andrographis paniculata*	MIC—15.62–125 μg/mL	Santra et al. (2022)
17.	*Aspergillus fumigatus*	Ergosterol (**14**), Helvolic acid (**15**), and Monomethyl sulochrin-4-sulphate (**16**)	*S. aureus*	*Albizia lucidior*	MIC—15.63, 1.95, and 3.90 μg/mL, respectively	Hussein et al. (2022)
18.	*A. alternata*	Isotalaroflavone (2), (+/)-50 Dehydroxytalaroflavone (3a/3b), (+)-talaroflavone (4b)	Xanthomonas oryzae	*Cercis chinensis*	MIC—0.5–64 μg/mL	Zhao et al. (2021)
19.	*Chaetomium* sp	6-Formamide-chetomin (**17**) and chetomin (**18**)	*Escherichia coli*, *S. aureus*, *enterococcus faecalis*	*Huperzia serrata*	MIC—0.78 μg/mL	Yu et al. (2018)
20.	*Colletotrichum* sp.	Colletotrichones A–C (**19–20**), Chermesinone B (**21**)	*E. coli*, *Bacillus subtilis*	*Buxus sinica*	MIC—0.1 μg/mL	Wang et al. (2016)
21.	*Diaporthe* sp.	19-nor-lanosta-5 (10),6,8,24-tetraene-1,3,12,22S-tetraol (**22**)	*Pseudomonas aeruginosa*, *Staphylococcus aureus*	*Mahonia fortunei*	MIC—1 to>10 μg/ml	Li et al. (2015)
22.	*Eupenicillium* sp. LG41	Eupenicinicols A (**23**) and B (**24**), Eupenicisirenins A (**25**) and B (**26**)	*Staphylococcus aureus*, *Escherichia coli*	*Xanthium sibiricum*	MIC—1 to >10 μg/mL	Li et al. (2014)
23.	*Fusarium solani* JK10	7-Desmethyl fusarin C	*E. coli*, *Acinetobacter* sp	*Chlorophora regia*	MIC-10 or more than 10 μg/mL	Kyekyeku et al. (2017)
24.	*Penicillium cataractum* SYPF 7131	Penicimenolidying, A (**27**), B (**28**) Rasfonin (**29**)	*S. aureus*	*Ginkgo biloba*	MIC—65–10 μg/mL	Wu et al. (2018a, b)

25.	*Lasiodiplodia theobromae* ZJ-HQ1	Preussomerins, chloropreussomerins A (**30**) and B (**31**)	*Staphylococcus aureus*	*Acanthus ilicifolius*	MIC—1.6 and 13 μg/mL	Chen et al. (2016)
26.	*Penicillium ochrochloronthe*	Trichodermic acid (**32**)	*Bacillus substilis*, *Staphyloccocus aureus*, *Proteusbacillm vulgaris*, *Escherichia coli*	Taxus roots	MIC—12.5 to 100 μg/mL	Zhao et al. (2019)
27.	*Rhizopycnis vagumn*	Rhizopycnis acids A (**33**) and B (**34**)	*B. subtilis*, *P. lachrymans*, *R. Solanacearum*	*Nicotiana tabacum*	IC_{50} = 16.1–81.3 μg/mL	Wang et al. (2018)
28.	*Xylomelasma* sp.	(2,6-Dimethyl-5-methoxyl-7-hydroxylchromone (**35**) 6-hydroxymethyleugenin (**36**), 6-Methoxymethyleugenin (**37**), Isoeugenitol (**38**), Diaporthin (**39**), 8-Hydroxy- 6-methoxy-3-methylisocoumarin (**40**)	*B. subtilis*, *S. haemolyticus*, *A. tumefaciens*, *Erwinia carotovora*, *Ralstonia solanacearum*, *Xanthomonas vesicatoria*	*Salvia miltiorrhiza*	MIC—25–100 μg/mL	Lai et al. (2021)

found on cypress trees such *Cupressus arizonica*, *Cupressus sempervirens* var. *cereiformis* and *Thuja orientalis*. Additionally, it was discovered that the extracts of *Alternaria* exhibit strong antibacterial properties. The target phytopathogenic bacteria *Bacillus* sp., *Erwinia amylovora* and *Pseudomonas syringae* and the model target fungus *Pyricularia oryzae* HS-1390 were used to test the activity of the *Alternaria* extracted compounds. The extracellular extracts had bacteriostatic and bactericidal effects in the range of 7.8–31.2 μg/mL. The intracellular metabolites were bactericidal at concentrations between 31.2–125 μg/mL and 15.6–62.5 μg/mL, respectively. *A. pellucida* POE182 was more efficient against Gram-positive bacteria, whereas *A. alternata* CAE92 and *A. tangelonis* POE103 were more effective against Gram-negative bacteria (Soltani and Hosseyni Moghaddam 2014). Three fungal endophytes with antibacterial activity were isolated from *Astragalus chinensis* and identified as *Chaetomium* sp. HQ-1, *Fusarium* sp. HQ-7 and *Fusarium* sp. HQ-9 showed potential antibiotic activity and was thus selected for large-scale fermentation. Further fermentation of strain HQ-1 led to the discovery of three compounds, i.e. differanisole A (**1**), 2,6-dichloro-4-propylphenol (**2**) and 4,5-dimethylresorcinol (**3**). All three compounds possess potent antibacterial activity against *Listeria monocytogenes, Staphylococcus aureus* and methicillin-resistant *S. aureus*, with Minimum Inhibitory Concentration (MIC) values ranging from 16 to 128 μg/mL (50 μg/mL ampicillin as a positive control). These findings suggest that the endophytic fungi from *A. chinensis* have the potential to be used as a bactericide (Liu et al. 2019a, b).

Aspergillus sp. from *Bauhinia guianensis* was evaluated for antimicrobial activity. Two known alkaloids pseurotin A (**4**) and fumigaclavine C (**5**) were isolated. The purified alkaloids were about ten times more active than the ethyl acetate extract from which they were isolated. Fumigaclavine C was found to be the most active showing a MIC of 7.81 μg/mL against *B. subtilis.* The MIC of pseurotin A was 15.6 μg/mL (penicillin, vancomycin and tetracycline 25 μg/mL each were used as positive controls) (Pinheiro et al. 2013). *Cinnamomum* species have been widely studied for their endophytic fungi of pharmacological importance. Twelve endophytes were isolated from the bark of *C. mercadoi*. All endophytes exhibited antibacterial activity against Gram-positive bacteria. However, only two of the four endophytes were subjected to secondary screening and showed wide-spectrum activity and inhibited the growth of all test bacteria. EtOAc extracts displayed the highest antibacterial activity. Fungal isolates from the inner bark of *C. mercadoi* belong to nine different genera of filamentous fungi. The composition of fungi included the Ascomycetes *Colletotrichum* and *Fusarium*; the Zygomycetes *Cunninghamella* and *Mucor* and the Deuteromycetes *Aspergillus, Penicillium, Pestalotiopsis*, *Phomopsis* and *Rhizoctonia*. The ethyl acetate extract of *Fusarium* sp. isolated from the bark of *Cinnamomum mercadoi* showed MIC of 2.1, 4.2, 4.2, and 3.8 mg/mL against *E. coli, E. aerogenes, S. aureus* and *B. cereus,* respectively (Marcellano et al. 2017).

Endophytic fungi are found in three common seagrasses in southern Thailand which were explored to produce antimicrobial metabolites. One hundred and sixty endophytic fungi derived from *Cymodocea serrulata* (family Cymodoceaceae),

Halophila ovalis and *Thalassia hemprichii* (family Hydrocharitaceae) were screened for the production of antimicrobial compounds against ten human pathogenic microorganisms including *Staphylococcus aureus*, a clinical isolate of methicillin-resistant *S. aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cryptococcus neoformans* and clinical isolates of *Microsporum gypseum* and *Penicillium marneffei*. Among the active fungi, seven isolates including *Hypocreales* sp. from *C. serrulata, Trichoderma* sp. *H. ovalis*, *Penicillium* sp., *Fusarium* sp., *Stephanonectria* sp. and an unidentified endophyte PSU-ES190 from *T. hemprichii* exhibited strong antimicrobial activity against human pathogens with MIC of less than 10 μg/mL. The inhibitory extracts at concentrations of four times their MIC destroyed the targeted cells as observed by scanning electron microscopy (Supaphon et al. 2013).

The diverse endophytic fungal associations in the aerial regions of perennial grasses *D. bicornis* and *P. flavidum* have 70 species of 29 genera and 71 species of 30 genera, respectively, which were documented. Methanolic and ethyl acetate extracts of fungal species showed effective antibacterial and antagonistic properties. *Curvularia protuberata* and *Penicillium citrinum*, two fungal endophytes, showed strong antagonistic and antibacterial activity. The experiment was arranged in a randomized complete block design and carried out in triplicate using amoxicillin, chloramphenicol or ciprofloxacin as positive controls. Among the endophytic fungi showing antagonism in vitro, three isolates from *P. flavidum* showed high activity and another three isolates from *D. bicornis* showed moderate antibacterial activity against *S. aureus, S. enteric* and *S. typhi* (Nischitha and Shivanna 2021). *Lophiostoma* sp. Eef-7 produced 2-azaanthraquinone derivatives with little antibacterial action when exposed to *Ralstonia solanacearum*. The endophytic fungus *Lophiostoma* sp. linked to *E. exserta* produced three known compounds, scorpinone (**6**), 5-deoxybostrycoidin (**7**) and 4-methyl-5,6-dihydro-2 H-pyran-2-one (**8**). Antibacterial activities of isolated compounds were tested against *R. solanacearum* and the inhibition zones observed at 64 μg were 9.86 and 9.58 mm for the first two compounds. The inhibition zone diameter of streptomycin sulphate was 13.03 mm at 6.25 μg (Mao et al. 2021).

In the biomass extracts of the fungus *Exserohilum rostratum*, the natural compound ravenelin (**9**) was identified and is a promising candidate for hit and lead development. Along with antiplasmodial and trypanocidal activities, it showed strong antibacterial activity. According to the findings, ravenelin inhibited both *Bacillus subtilis* and *Staphylococcus aureus* (MIC values of 7.5 μM and 1.95 μg/mL, respectively; amoxicillin and terramycin were used as positive controls). Additionally, ravenelin has antiparasitic efficacy against *P. falciparum* (IC_{50} value of 3.4 ± 0.4 μM), *Trypanosoma cruzi* (IC_{50} value of 5.0 μM), and both the epimastigote and intracellular amastigote forms of *Trypanosoma cruzi* (IC_{50} value of 9.0 μM) (Pina et al. 2021).

Marine natural products play a significant role in the drug discovery and development process, one such example is mangrove-derived endophytic fungus *Epicoccum nigrum* obtained from *Acanthus ilicifolius*. Six new polyketides, including one coumarin, two isocoumarins, dihydroradicinin and two benzofuranone

derivatives, together with seven known analogues were isolated from the culture of the mangrove endophytic fungus *Epicoccum nigrum* SCNU-F0002. The results showed that isolated compound 1-(4-hydroxy-2-methoxybenzofuran-5-yl) butan-1-one (**10**) showed antibacterial activity with MIC values between 25 and 50 μg/mL against *B. subtilis* (ATCC 6538), *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538). Triadimefon and ciprofloxacin were used as positive controls for fungi and bacteria, respectively (Yan et al. 2019). About 14 fungal endophytes were isolated from the *Ocimum basilicum* var. thyrsiflora leaves. The antibacterial activity of crude extracts from all endophytic fungi was tested against nine human bacterial pathogens including *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Vibrio cholerae* and *Vibrio parahaemolyticus*. The crude extract from *Nigrospora* MFLUCC16-0605 exhibited broad-spectrum antibiotic activity with MIC values ranging from 7.81 to 250 μg/mL (chloramphenicol was used as a positive control) and high antioxidant activity (IC_{50} value of 15.36 μg/mL) comparable to the trolox and gallic acid, respectively (Atiphasaworn et al. 2017). Another study reveals the antimicrobial potential of three fungal endophytes from leaves of *Olea europaea* L. The ethyl acetate extracts inhibited a wide spectrum of microorganisms at a MIC of ≤0.095 mg/mL. *P. canescens* and *A. alternata* isolated from *O. europaea* possess antimicrobial activity against tested Gram-positive and Gram-negative bacteria and yeasts (Malhadas et al. 2017).

Fractionation of the crude extract of *P. setosum* was done using semi-preparative HPLC and the separated active fraction showed antibacterial efficacy with a MIC of 8 μg/mL against both *S. aureus* and *E. coli*. The yield of the active fraction was 7 mg and antibacterial activity against *S. aureus* and *E. coli* was obtained as a zone of inhibition of 19.5 ± 0.6 and 20 ± 0.4 mm, respectively (20 μL from 2 mg/mL). The compounds present in the active fraction were confirmed as patulin (**11**), leucodelphinidin (**12**) and other minor compounds like quercetin, taxifolin and kaempferol (George et al. 2019). A recent study determined the antimicrobial properties of secondary metabolites produced by endophytic fungi isolated from *Sceletium tortuosum* L. The secondary metabolites isolated from the *Fusarium oxysporum* were ethanol, octadecanoic acid, phenylmethyl ester, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 5 hydroxymethylfurfural. 5-Hydroxymethylfurfural (**13**) was the most abundant secondary metabolite produced by *F. oxysporum*. The diameter of the inhibition zone of bacterial growth was found to be between 2 and 10 mm (Manganyi et al. 2019).

Neurasthenia and newborn malnutrition are two conditions that are treated with the roots of *S. suffruticosa*. Therefore, *S. suffruticosa* endophytic fungi were investigated and tested for antimicrobial activity. The Minimum Bactericidal Concentration (MBC) and the MIC ranged from 0.5 to 2 mg/mL, respectively. The major active ingredients in *S. suffruticosa* are alkaloids of the securinega class. Strong antimicrobial activity was observed in the endophytic fungus *C. globosum* and *Fusarium* sp., which had alkaloid concentrations of 0.231% and 0.170%, respectively. The best bacteriostatic activity was demonstrated by *C. globosum* ethyl acetate extracts. *S. aureus* was effectively inhibited by the crude extracts of

C. globosum and *Fusarium* sp. 3 (1 mg/mL). The inhibition of *E. coli*, *P. aeruginosa* and *E. faecalis* required a crude extract concentration of at least 0.50 mg/mL (Du et al. 2020). *Vernonia anthelmintica* is another very effective source of antibacterial and cytotoxic activity. The isolates included species of *Aspergillus calidoustus* NR-10, *Ovatospora senegalensis* NR-03, *Chaetomium globosum* NR-04, *Thielavia subthermophila* NR-06, *Aspergillus keveii* XJF-23 and *Aspergillus terreus* XJF-3. *O. senegalensis* NR-03's crude extract demonstrated potent antibacterial activity against the *E. coli* and *S. aureus* (zone of inhibition 26 and 19 mm) as well as mild antifungal inhibition (7.5 mm ZOI) against *Candida albicans*. *O. senegalensis* NR-03's dichloromethane extract yielded 52 distinct components, the primary constituents were 9,12-octadecadienoic acid (*Z*, *Z*)-methyl ester (6.39%), (E)-9-octadecenoic acid, methyl ester (6.15%), rosenonolactone (2.11%), 3-dimethyl-1,2-cyclopentanedione (1.41%) and terrein (33.71%) (Niu et al. 2022).

Cochliobolus sp. APS1, an endophyte of *Andrographis paniculata*, produces a plethora of natural bioactive compounds among which multipotent alkaloid 1-(2-aminoethyl) aziridine exhibited antibacterial, antibiofilm and antilarval potency. The MIC and MBC values of the ethyl-acetate culture extract ranged from 15.62 to 250 μg/mL against ten pathogenic microorganisms (including MRSA and VRSA) (Santra et al. 2022). *Aspergillus fumigatus*, isolated from *Albizia lucidior* leaves, was investigated for antimicrobial activity of isolated metabolites and their probable mode of action. Out of 42, three metabolites ergosterol (**14**), helvolic acid (**15**) and monomethyl sulochrin-4-sulphate (**16**) showed good activity with MIC values of 15.63, 1.95, and 3.90 μg/mL, respectively, compared to ciprofloxacin (Hussein et al. 2022). *Cercis chinensis* derived fungus *A. alternata* ZHJG5 yielded five polyketide derivatives, all of the studied microorganisms were effectively inhibited by these compounds at minimum inhibitory doses ranging from 0.5 to 64 μg/mL (Zhao et al. 2021).

The endophytic fungus *Chaetomium* sp. M336 produced solid fermentation products that contained two novel compounds, 6-formamide-chetomin (**17**) and chaetomin (**18**). These compounds were identified after intensive spectroscopic analysis, including 1D and 2D NMR and HR-ESI-MS. Compound **17** had strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* ATCC 6539 and *Enterococcus faecalis* with a MIC value of 0.78 μg/mL (Yu et al. 2018).

An endophytic fungus, *Colletotrichum* sp. BS4 from a well-known boxwood plant *Buxus sinica* used in traditional Chinese medicine yielded new compounds, colletotrichones A (**19**), C (**20**) and one known compound chermesinone B (**21**). Compound **19** demonstrated substantial antibacterial activity against environmental microorganisms with a MIC of 0.1 μg/mL. *E. coli* was also susceptible to compound **21**. Moreover, compound **20** was equally effective as streptomycin against the clinically significant pathogen *S. aureus* (Wang et al. 2016).

A novel lanostanoid, 19-nor-lanosta-5(10),6,8,24-tetraene-1,3,12,22S-tetraol (**22**), containing an aromatic ring was discovered from an endophytic fungus called *Diaporthe* sp. LG23 that was isolated from the leaves of the Chinese medicinal plant *Mahonia fortunei*. Both Gram-positive and Gram-negative bacteria were

significantly inhibited by compound **22**, particularly clinical isolates of *Streptococcus* pyogenes, *Pseudomonas aeruginosa* and a human pathogenic strain of *Staphylococcus aureus* with a MIC value of 1 to >10 μg/mL (Li et al. 2015).

Eupenicillium sp. LG41, an endophytic fungus found in the roots of the Chinese medicinal plant *Xanthium sibiricum*, was the source of two new compounds with the decalin moiety, eupenicinicols A (**23**) and B (**24**), two new sirenin derivatives, eupenicisirenins A (**25**) and B (**26**), and four other known compounds. The antibacterial efficacies of these compounds were examined against the clinically significant *Staphylococcus aureus* and *Escherichia coli*, as well as *Bacillus subtilis* and *Acinetobacter* sp. BD4, which is generally found in soil with a MIC value of 1 to >10 μg/mL (Li et al. 2014). Seven 7-desmethyl fusarin C compounds were isolated from the endophytic fungus *Fusarium solani* JK10 found in the root of the Ghanaian medicinal plant *Chlorophora regia*. These compounds showed antibacterial activity against *E. coli* and *Acinetobacter* sp. with a MIC value of 10 or more than 10 μg/mL (Kyekyeku et al. 2017) The leaves, stems and roots of *Ginkgo biloba* L. yielded a total of 58 fungal isolates from 24 different genera. Among these, *Penicillium cataractum* SYPF 7131, an endophytic fungal strain, had the most potent antibacterial activity. Compounds penicimenolidyu A (**27**), penicimenolidyu B (**28**) and rasfonin (**29**) were isolated from the strain fermentation broth and had a MIC value of 65–10 μg/mL and had considerable inhibitory action against *S. aureus* (Wu et al. 2018a, b).

Lasiodiplodia theobromae ZJ-HQ1, an endophytic fungus, yielded two novels chlorinated preussomerins, chloropreussomerins A (**30**) and B (**31**) as well as nine previously identified preussomerin analogues, from the healthy leaves of the marine mangrove *Acanthus ilicifolius.* The isolated compounds exhibited significant activities against *Staphylococcus aureus* with MIC values between 1.6 and 13 μg/mL (Chen et al. 2016).

A solid-substrate fermentation culture of *Penicillium ochrochloronthe* linked taxus roots yielded three 3,4,6-trisubstituted pyrone derivatives and trichodermic acid (**32**). The MIC values for these substances ranged from 12.5 to 100 μg/mL, selectively exhibiting antibacterial action against *Bacillus substilis, Micrococcus luteus, Staphyloccocus aureus, Bacillus megaterium, Salmonella enterica, Proteusbacillm vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli* and *Enterobacter aerogenes* (Zhao et al. 2019).

Two novel anisic acid derivatives, rhizopycnis acids A (**33**) and B (**34**), were isolated from the fermentation cultures of the endophytic fungus *Rhizopycnis vagumn* from the healthy tissues of *Nicotiana tabacum*. Both substances were the first methylbutanoic/methylbutenoic acid group-containing anisic acid derivatives discovered in fungi. IC_{50} values for **33** and **34** ranged from 16.1 to 81.3 μg/mL against six tested bacteria (Wang et al. 2018).

From the culture of an endophytic fungus, *Xylomelasma* sp. obtained from the curative herb *Salvia miltiorrhiza* Bunge, five chromone derivatives (2,6-dimethyl-5-methoxyl-7-hydroxylchromone) (**35**), 6-hydroxymethyleugenin (**36**), 6-methoxymethyleugenin (**37**) and isoeugenitol (**38**); and isocoumarin congeners, namely diaporthin (**39**) and 8-hydroxy- 6-methoxy-3-methylisocoumarin (**40**) were

discovered. The isolated compounds demonstrated inhibitory activities against several bacteria with MIC ranges of 25–100 μg/mL (Lai et al. 2021).

3.1 Antimycobacterial Compounds from Endophytes

The microorganisms (*Mycobacterium tuberculosis*) that cause tuberculosis (TB) often damage the lungs. The most potent first-line TB medications, isoniazid and rifampicin are ineffective against multidrug-resistant tuberculosis (MDR-TB). Using second-line medications, MDR-TB can be treatable and curable. The alternatives for second-line therapy, however, are few and demand prolonged chemotherapy (lasting at least 9 months and may be 20 months). In 2021, the WHO South-East Asian Region saw the largest number of new cases of TB (46%), followed by the WHO African Region (23%) and the WHO Western Pacific (18%) (www.who.int). It has been observed that secondary metabolites from endophytic fungi have promising antimycobacterial action against a variety of bacteria (Table 2.2, Fig. 2.2).

In the Huizhou Mangrove Nature Reserve in Guangdong Province, China, healthy leaves of *K. obovate* were host to the endophytic *Talaromyces* sp. (HZ-YX1), which produced the alkaloid talaramide A (**41**). Talaramide A demonstrated strong inhibitory efficacy against mycobacterial PknG with an IC_{50} value of 18.2 μg/mL (Chen et al. 2017). Together with a precursor, tenellone C (**42**), the new isoprenylisoindole alkaloid diaporisoindole A (**43**), was isolated from the endophytic *Diaporthe* sp. SYSU-HQ3 derived from the mangrove plant *Acanthus ilicifolius* collected from Shankou in Guangxi province, China. These compounds exhibited inhibitory efficacy against *M. tuberculosis* protein tyrosine phosphatase B (MptpB) with IC_{50} values of 1.77 and 2.2 μg/mL (Cui et al. 2017). A fungus BCC 25093 was isolated from an unidentified mangrove wood procured from Surat Thani province, Thailand. Palmarumycin P1 (**44**) and decaspirons A (**45**), members of the spirodioxynaph-thalenes, group exhibited antitubercular action against *M. tuberculosis* H37Ra, with MIC values of 1.56 and 3.13 μg/mL, respectively (Bunyapaiboonsri et al. 2015).

A natural anthraquinone nigrosporin (**46**) isolated from a mangrove endophytic fungus *Nigrospor* showed an inhibitory effect against *Mycobacterium tuberculosis*. Another anthraquinone, 4-deoxybostrycin (**47**) was isolated from the fungus *Alternaria eichhorniae* obtained from *Nigrospora oryzae*. Their MIC values against a variety of mycobacterial strains were established and compared to those of commercially available control medications such as streptomycin (SM), isoniazid (INH), rifampicin (RFP) and ethambutol (EMB). Additionally, 4-deoxybostrycin showed good inhibition against the two MDR *M. tuberculosis* clinical isolates K2903531 and 0907961, which was even better than that of the first line of anti-TB medications (Wang et al. 2013).

Another research demonstrated the antimycobacterial action of penialidin C (**48**), which was isolated from the endophyte *Penicillium* sp. that was hosted by *Garcinia nobilis*. Citromycetin showed a MIC of 31.2 μg/mL, while penialidin C, which was

Table 2.2 List of anti-mycobacterial metabolites from endophytic fungi

S. no.	Endophytic fungus strain	Compounds isolated	Target bacteria	Plant source	Anti-mycobacterial activity	Reference
1.	*Talaromyces* sp	Talaramide A (**41**)	Mycobacterial PknG	*K. Obovate*	IC_{50}–18.2 μg/mL	Chen et al. (2017)
2.	*Diaporthe* sp	Tenellone C (**42**), Diaporisoindole (**43**)	*M. Tuberculosis* protein tyrosine phosphatase B (MptpB)	*Acanthus ilicifolius*	IC_{50}–1.77 and 2.2 μg/mL	Cui et al. (2017)
3.	BCC 25093	Palmarumycin P1 (**44**), Decaspirone A (**45**)	*M. Tuberculosis* H37Ra	Mangrove wood	MIC—1.56 μg/mL and 3.13 μg/mL	Bunyapaiboonsri et al. (2015)
4.	*Alternaria eichhorniae*	Nigrosporin (**46**), 4-Deoxybostrycin (**47**)	*M. Tuberculosis*	*Nigrospora oryzae*	MIC—172.25–12.50 μg/ml	Wang et al. (2013)
5.	*Penicillium* sp	Penialidin C (**48**)	*Mycobacterium smegmatis*	*Garcinia nobilis*	MIC—15.6 μg/mL	Jouda et al. (2016)
6.	*Aspergillus* N830	Kaurene diterpene, β-Sitosterol, brassicadiol, aspergillitine and β-sitosterol ferulate	*Mycobacterium tuberculosis*	*Gleditsia caspia*	MIC—15.63–125 μg/mL	Wang et al. (2013)
7.	*Phoma* sp.	Cyclopenta[b]fluorene ring systems, phomapyrrolidones A (**49**)–C	*M. Tuberculosis*	*Saurauia scaberrinae*	MIC—5–50 μg/mL	Wijeratne et al. (2013)
8.	*Colletotrichum* sp., *Fusarium* sp., *Guignardia* sp., *Phomopsis* sp., *Phoma* sp., and *Microdochium* sp.	Javanicin (**50**)	*Mycobacterium phlei*, *M. tuberculosis*	*Rhoeo spathacea*	MIC—25–50 μg mL	Alvin et al. (2016)

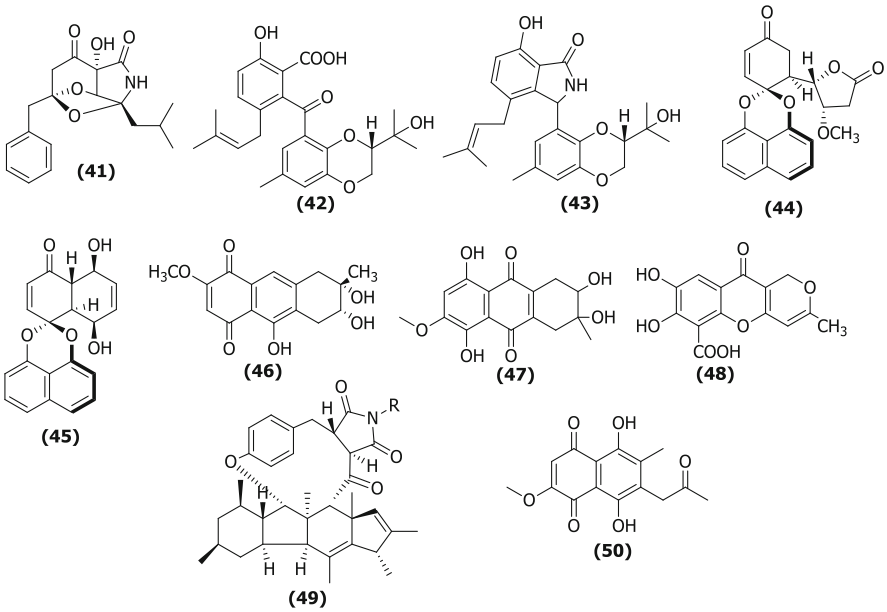

Fig. 2.2 Structures of anti-mycobacterial metabolites from endophytic fungi

the active molecule, had a MIC of 15.6 µg/mL (Jouda et al. 2016). The stems of *Gleditsia caspia* Desf. were used to create a fungal culture of *Aspergillus* N830, which yielded a novel kaurene derivative with a new 6/6/6/5/6 ring system structure. Using the Alamar Blue Assay (MABA), the compound demonstrated a promising antitubercular action with a MIC value of 124.5 µM. Six previously identified compounds were also isolated and showed great MIC values against *Mycobacterium tuberculosis*, ranging from 15.63 µg/mL (26.5 µM) to 125 µg/mL (500 µM), in comparison to the positive control isoniazid, whose MIC value was 0.24 µg/mL (1.75 µM), suggesting them as potential natural antitubercular agents (Moussa et al. 2021).

Three novel alkaloids with cyclopenta[b]fluorene ring systems, phomapyrrolidones A (**49**)–C, were discovered from an endophytic fungal strain *Phoma* sp. mycelium extract inhabiting *Saurauia scaberrinae*. At subcytotoxic concentrations, these compounds showed minimal antitubercular action (Wijeratne et al. 2013).

Rhoeo spathacea's flowers, leaves and roots were found to have fungal endophytes, which revealed a community that included *Colletotrichum* sp., *Fusarium* sp., *Guignardia* sp., *Phomopsis* sp., *Phoma* sp. and *Microdochium* sp. Ethyl acetate extracts of the fungal isolates exhibited antiproliferative activity against at least one of the seven bacterial and mycobacterial test strains. The crude extract from a *Fusarium* sp. strain which exhibited strong antiproliferative activity against *Mycobacterium tuberculosis* resulted in the isolation of the polyketide javanicin (**50**)

effective against *Mycobacterium phlei* (MIC = 50 μg mL) and *M. tuberculosis* (MIC = 25 μg mL) (Alvin et al. 2016).

4 Antifungal Metabolites

Fungi are a diverse group of creatures that are frequently found on surfaces, in water, soil and the air, as well as on our skin and within our bodies. Moulds can exacerbate asthma or allergy sufferers' breathing issues, and different varieties of fungi can infect nails and result in skin rashes. There are now just four kinds of antifungal medications accessible, and there are not many candidates in the clinical development stage, making fungal diseases a serious danger to public health. The majority of fungal diseases lack quick and accurate diagnostics, and those that do exist are neither generally accessible nor inexpensively available worldwide.

While resistance to the newly released medicine appears to have grown, particularly in patients receiving long-term therapy, the trends of new antifungals being launched onto the market have remained minimal. Given their enormous potential for the extraction and screening of new antibiotics for various pharmaceutical uses, natural microbial products have largely gone untapped as a source alternative. Endophytic fungi are a common source of new chemical compounds possessing antifungal properties. This section presented the endophytic fungi from medicinal plants that have been shown to produce antifungal metabolites and their potential as antifungal medicines in the last ten years (www.who.int). It has been observed that secondary metabolites from endophytic fungi have promising antifungal action against a variety of fungi (Table 2.3, Fig. 2.3).

Aplosporella javeedii, an endophytic fungus associated with the host plant *Orychophragmus violaceus*, produced six novel polyketides called aplojaveediins A–F. Both the microbroth dilution experiment and the agar plate diffusion assay revealed that compound aplojaveediin A (**51**) has antifungal action against the hyphal form of the *Candida albicans* strain ATCC 24433. The microbroth dilution experiment revealed that the MIC of compound **51** against the hyphae form of the *C. albicans* strain ATCC 24433 was 100 μM. A time-kill assay was carried out as an extension of the antifungal assay. *Candida albicans* strain ATCC 24,433 hyphae cells were incubated with compound **51** at 400 μM (=fourfold MIC) using hygromycin B (474 μM = fourfold MIC) as a positive control drug. This discovery highlights the fungicidal ability of compound **51** (Gao et al. 2020a, b).

Sixteen bioactive endophytic fungi including *Alternaria alternata*, *Alternaria pellucida*, *Ascorhizoctonia* sp., *Aspergillus fumigatus*, *Aspergillus niger*, *Aurobasidium* sp., *Cladosporium porophorum*, *Fusarium oxysporum* and *Penicillium viridicatum* were isolated from the plants of Cupressaceae family. The secondary metabolites of four endophytic fungi showed antifungal efficacy against *A. fumigatus* and *A. niger*. High antifungal activity against *A. niger* was demonstrated by secondary metabolites from *Trichoderma koningii* CSE32 and *Pyrenochaeta* CSE134. Secondary metabolites from *Trichoderma koningii*, namely

Table 2.3 List of antifungal metabolites from endophytic fungi

S. no	Endophytic fungi strain	Compound isolated	Target fungi	Plant source	Anti- fungal activity	Reference
1.	*Aplosporella javeedii*	Aplojaveediins A (**51**)-F	*Candida albicans*	*Orychophragmus violaceus*	MIC—100 μM	Gao et al. (2020a, b)
2.	*Aspergillus fumigatus* and *Aspergillus niger*	6-Pentyl-α-pyranone (**52**) and 6-(4-oxopentyl)-2*H*-pyran-2-one (**53**)	*A. fumigatus*, *A. niger*	*Juniperus virginiana*, *Platycladus orientalis*, and *Cupressus arizonica*	ZOI—0.9 ± 1.00 at 7.8 mg mL^{-1}	Li et al. (2019) and Erfandoust et al. (2020)
3.	*Botryosphaeria ramosa*	Isocoumarin derivatives, botryospyrones A (**54**), natural tryptamines, (3aS, 8aS)-1-acetyl-1, 2, 3, 3a, 8, 8a-hexahydropyrrolo [2,3b] indol-3a-ol (**55**)	*Penicillium italicum*	*Myoporum bontioides*	MIC—450.4–57.3 μM	Wu et al. (2019)
4.	*Botryosphaeria dothidea*	Stemphyperylenol (**56**) Chaetoglobosins, ceramide derivative, 3-hydroxy-*N*-(1-hydroxy-3-methylpentan-2-yl)-5-oxohexanamide	Alternaria solani	*Melia azedarach*	MIC of 1.57 μM	Xiao et al. (2014)
5.	*Mycosphaerella* sp.	2-Amino-3,4-dihydroxy-2-25 (hydroxymethyl)-14-oxo-6,12-eicosenoic acid (**57**) and myriocin (**58**)	*Cryptococcus neoformans* and *C. gattii*	*Eugenia bimarginata*	MIC-1.95 to 7.82 μM and from 0.49 to 7.82 μM	Pereira et al. (2016)
6.	*Emericella* sp.	5-(Undeca-3′,5′,7′-trien-1′-yl) furan-2-ol (**59**) and 5-(undeca-3′,5′,7′-trien-1′-yl) furan-2-carbonate (**60**)	*Rhizoctorzia solani*, *Verticillium dahlia*, *Helminthosporium maydis*, *Fusarium oxysporum*, *F. tricinctum*,	*Panax notoginseng*	MIC-50 to 6.3 μg/mL	Wu et al. (2018a, b)

(continued)

Table 2.3 (continued)

S. no	Endophytic fungi strain	Compound isolated	Target fungi	Plant source	Anti- fungal activity	Reference
			Botryosphaeria dothidea, and *Alternaria fragriae*			
7.	*Hypoxylon anthochroum*	7-Hydroxy-4,6-dimethyl-3*H*-isobenzofuran-1-one (**61**), 7-methoxy-4,6-dimethyl-3*H*-isobenzofuran-1-one (**62**), 6-formyl-4-methyl-7-methoxy-3*H*-isobenzofuran-1-one (**63**), 7-methoxy-4-methyl-3*H*-isobenzofuran-1-one (**64**)	*Fusarium oxysporum*, *Alternaria alternata*, *Pythium aphanidermatum*, *and Phytophthora capsica*	*Gliricidia sepium*	IC_{50}—50 to 600 µg/mL	Fernández-Pastor et al. (2021)
8.	*Trichoderma longibrachiatum* and *Syncephalastrum racemosum*	Ethyl acetate extracts and methanol fractions of *T. longibrachiatum* isolates (MF1 and MF5)	*F.oxysporum*, *Sclerotinia sclerotiorium*, *Rhizoctonia solani*, and *Botrytis cinerea*	*Markhamia tomentosa*	MIC—1000 µg/mL	Ibrahim et al. (2017)
9.	*Diaporthe eucalyptorum*	Eucalyptacid A (**65**) and eucacalactam B (**66**), eugenitol (**67**), cytosporone C (**68**), 4-hydroxyphenethyl alcohol (5), 1-(4-hydroxyphenyl) ethane-1,2-diol (**69**), N-(2-hydroxy-2-phenylethyl) acetamide (7), and phomopene (**70**)	*Alternaria solani*	*Melia azedarach*	MIC—6.25 to 50 µM	Gao et al. (2020a, b)

10.	*Paraconionthyrium* sp	Globosuxanthone A (**71**), vertixanthone (**72**), hydroxyvertixanthone (**73**), 3,8-dihydroxy-1-methy1-9*H*- xanthen-9-one (4), and danthron (**74**), danthron (**75**)	*Fusarium graminearum*, *Fusarium solani*, and *Botrytis cinerea*	*Azadirachta indica*	MIC—4-130 μg/mL	Miao et al. (2020)
11.	*Phyllosticta* sp.	Guignardones (C) (**76**), meroterpenes, dioxolanone derivatives	*Rhizoctonia solani*, *Fusarium graminearum*, and *Botrytis cinerea*	*Osmanthus fragrans*	Inhibition ratio—48.43%, 40.98%, and 49.53% at 50 μg/mL, 200 μg/mL	Yan et al. (2021)
12.	*Xylaria* sp. SNB-GTC2501	Piliformic acid (**77**) and cytochalasin D (**78**)	*C. Gloeosporioides*	*P. cupana*	MIC—128 μg/mL	Elias et al. (2018)
13.	*Arthrinium arundinis*	Diorcinol M,N,O	*Mucor hiemalis*	*Nicotiana tabacum*	MIC—8 and 4 μg/mL	Zhang et al. (2018)
14.	*Aspergillus clavatus*	24-hydroxylergosta-4,6,8 (14),22-tetraen-3-one, 4,40-dimethoxy-5,50-dimethyl-7,70-oxydicoumarin, orlandin	*F. Oxysporum*, *C. musae*, and *P. italicm*	*Myoporum bontioides*	MIC—244.73, 195.79, and 61.18 μM	Li et al. (2017)
15.	*Saccharicola bicolor*	Bicolorins B (**79**) and D (**80**)	*Pythium dissimile*	*Bergenia purpurascens*	MIC—6.2 and 8.5 μg/mL	Zhao et al. (2020)
16.	*Biscogniauxia mediterranea*	5-methylmellein (**81**)	*Phomopsis obscurans*, *F. Oxysporum*, *C. fragariae*	*Opuntia humifusa*	63.5% growth suppression at 150 μM at 120 h	Silva-Hughes et al. (2015)
17.	*Chaetomium globosum*	Chaetoglobosin A (**82**) and D (**83**)	*Sclerotinia sclerotiorum*	*Ginkgo biloba*	IC_{50} values of 0.35 and 0.62 μg/mL	Zhang et al. (2013a, b)
18.	*Alternaria alternata*	Altenusin (**84**)	*C. albicans*	*Terminalia chebula*	FIC—0.078 to 0.188	Phaopongthai et al. (2013)
19.	*Berkleasmium*	Palmarumycin C_8 (**85**)	*M. oryzae*	*Dioscorea zingiberensis*	IC_{50}—9.1 μg/mL	Shan et al. (2014)

(continued)

Table 2.3 (continued)

S. no	Endophytic fungi strain	Compound isolated	Target fungi	Plant source	Anti- fungal activity	Reference
20.	*Trichoderma* sp.	Dichlorodiaportinolide (**86**) and dichlorodiaportin (**87**)	*Colletotrichum musae* and *Rhizoctonia solani*	*Myoporum bontioides*	MIC—6.25 to 150 μg/mL	Li et al. (2016)
21.	*Trichoderma koningiopsis*	Koninginin O (**88**), koninginin Q, and 7-O-methylkoninginin D	*F. Oxysporum* and *P. cucumerina*	*Panax notoginseng*	MIC—128 μg/mL	Liu et al. (2016a, b)
22.	*Trichoderma brevicompactum* 0248	Trichodermin (**89**)	*Rhizoctonia solani* and *B. cinerea*	*Allium sativum*	EC_{50}—0.25 μg/mL and 2.02 μg/mL	Shentu et al. (2014)

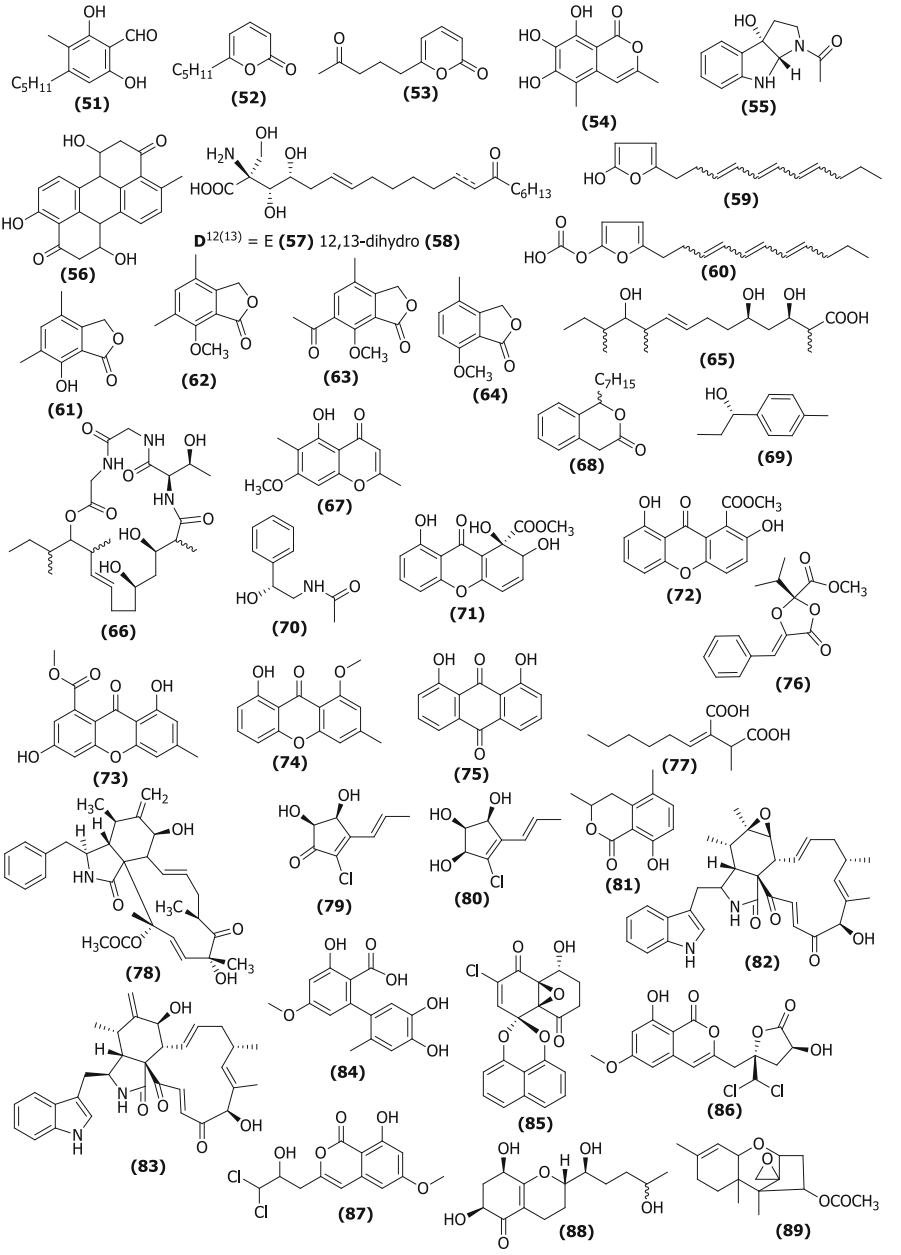

Fig. 2.3 Structures of Antifungal metabolites from endophytic fungi

6-pentyl-α-pyranone (**52**) and 6-(4-oxopentyl)-2H-pyran-2-one (**53**) showed high antifungal activity against *A. fumigates* (Li et al. 2019) (Erfandoust et al. 2020).

Botryospyrones A (**54**) and a new natural tryptamine (3aS, 8aS)-1-acetyl-1, 2, 3, 3a, 8, 8a-hexahydropyrrolo [2,3b] indol-3a-ol (**55**) were isolated from a marine mangrove endophytic fungus called *Botryosphaeria* from the leaves of *Myoporum bontioides*. Inhibitory activities of isolated compounds on *Fusarium oxysporum* and *Fusarium graminearum* were all greater than those of triadimefon, a common agricultural fungicide. Botryospyrones A (**54**) had a MIC value of 112.6 μM against *F. oxysporum*. Compound **55** had roughly 12, 3- and 18-times greater activity against *F. oxysporum*, *P. italicum* and *F. oxysporum,* respectively (Wu et al. 2019).

In another study by Zhibo et al. the solid culture of the endophytic fungus *Botryosphaeria dothidea* from the stems of white cedar (*Melia azedarach*) gave 15 known compounds, including chaetoglobosins C and F, as well as two new metabolites, α-pyridone derivative, a ceramide derivative. The antibacterial, antioxidant and cytotoxic properties of these metabolites were assessed in vitro (Xiao et al. 2014). Stemphyperylenol (**56**) demonstrated significant antifungal activity against the plant disease caused by *Alternaria solani*, comparable to the widely used fungicide carbendazim (Hu et al. 2020).

Two eicosanoic acids from *Mycosphaerella* sp., 2-amino-3,4-dihydroxy-25-(hydroxymethyl)-14-oxo-6,12-eicosenoic acid (**57**) and myriocin (**58**), were tested for their antifungal properties against *Cryptococcus neoformans* and *C. gattii*. The compounds demonstrated antifungal activity against several isolates of *Candida neoformans* and *Candida gattii*, with MIC values for compound **57** and compound **58** ranging from 0.48 to 1.95 μM and 0.49 to 7.82 μM, respectively (Pereira et al. 2016). 5-(undeca-3′,5′,7′-trien-1′-yl) furan-2-ol (**59**) and 5-(undeca-3′,5′,7′-trien-1′-yl) furan-2-carbonate (**60**), two novel alkylated furan derivatives, were discovered from the crude extract of an endophytic fungus *Emericella* sp. XL029 associated with the leaves of *Panax notoginseng*. Compound **59** showed considerable action against all investigated fungi with MIC ranging from 25 to 3.1 μg/mL, while compound **60** demonstrated activity across the board with MIC ranges of 50–12.5 μg/mL. The MIC values of compounds **59** and **60** ranged from 50 to 6.3 μg/mL (Wu et al. 2018a, b).

A chemical study of the culture medium and mycelium organic extracts of the endophytic fungus *Hypoxylon anthochroum* Gseg1 led to the isolation of three known isobenzofuranones, 7-hydroxy-4,6-dimethyl-3*H*-isobenzofuran-1-one (**61**), 7-methoxy-4,6-dimethyl-3H isobenzofuran-1-one (**62**), 6-formyl-4-methyl-7-methoxy-3*H*-isobenzofuran-1-one (**63**) together with 7-methoxy-4-methyl-3*H*-isobenzofuran-1-one (**64**), which was reported for the first time as a natural product. All of the compounds significantly and concentration-dependently suppress the development of pathogens. IC_{50} for the isolated compounds ranged from 0.64 to 2.61 mM when compared with carbendazim as control (Sánchez-Fernández et al. 2020).

Endophytic fungi namely *Trichoderma longibrachiatum* and *Syncephalastrum racemosum* were isolated from the leaves of *M. tomentosa*. In the poisoned food assay, the ethyl acetate extracts and methanol fractions of *T. longibrachiatum*

isolates (MF1 and MF5) and the methanol fraction of *S. racemosum* isolates (MF3) showed a MIC value of 1000 μg/mL (Ibrahim et al. 2017). The endophytic fungus *Diaporthe eucalyptorum* KY-9 that had been isolated from *Melia azedarach* produced two biosynthetically related new metabolites, eucalyptacid A (**65**) and eucacalactam B (**66**), as well as six known substances, eugenitol (**67**), cytosporone C (**68**), 1-(4-hydroxyphenyl) ethane-1,2-diol (**69**) and N- (2-hydroxy-2-phenylethyl) acetamide (**70**). The MICs of compounds **65**, **68**, **69** and **70** against *Alternaria solani* ranged from 6.25 to 50 μM. Strain KY-9 is an unexplored resource for the creation of biological control agents to stop the spread of the pathogenic fungus *A. solani* (Gao et al. 2020a, b).

The strain YM 311593 (*Paraconiothyrium* sp.) was isolated from the fruits of *Azadirachta indica*. The fermentation broth extract of the strain yielded four xanthones and one anthraquinone. They were identified as globosuxanthone A (**71**), vertixanthone (**72**), hydroxyvertixanthone (**73**), 3,8-dihydroxy-1-methy1-9H-xanthen-9-one (**74**) and danthron (**75**). The broth microdilution technique was used to test the antifungal activity of compounds **71–75** against four phytopathogens. One of them, globosuxanthone A (**71**), had MIC values of 4, 8 and 16 μg/mL against *Fusarium graminearum*, *Fusarium solani* and *Botrytis cinerea*, respectively, demonstrating significant antifungal activity. Compound **72** had MIC of 16, 32 and 64 μg/mL, respectively, and moderately inhibited the growth of *F. graminearum, F. solani and P. oryzae*. With MIC of 8 and 64 μg/mL, respectively, compound **73** also demonstrated significant inhibitory action against *F. graminearum and B. cinerea*. Compounds **74** and **75** showed moderate antifungal activity (MICs of 128 μg/mL) against *P. oryzae* (Miao et al. 2020).

The endophytic fungus *Phyllosticta* sp. WGHL2 provided four novel meroterpenes, guignardones U-X, 11 well-known meroterpenes and three well-known dioxolanone derivatives. With an inhibition of 48.43%, 40.98% and 49.53% at 50 μg/mL, respectively, against *Rhizoctonia solani*, *Fusarium graminearum* and *Botrytis cinerea* in antifungal assays, dioxolanone guignardianone C (**76**) showed broad-spectrum antifungal activity. Additionally, compound **76** effectively inhibited *B. cinerea* spore germination at 200 μg/mL and showed a modest protective effect against *B. cinerea* in vivo (Yan et al. 2021).

Piliformic acid (**77**) and cytochalasin D (**78**) were extracted using chromatographic methods from two species of *Xylaria*, with the help of tests using *Colletotrichum gloeosporioides*. This demonstrates the antifungal activity of piliformic acid and cytochalasin D against *C. gloeosporioides*, with MIC of 2.92 and 2.46 mol/mL, respectively (Elias et al. 2018).

Fresh tobacco leaves were used to isolate and purify the endophytic fungus *Arthrinium arundinis* TE-3 from *Nicotiana tabacum* L. Three prenylated diphenyl ethers from this fungal strain showed MICs of 8 and 4 μg/mL and showed specific antifungal activity against *Mucor hiemalis* (Zhang et al. 2018).

Two novel coumarin derivatives and two well-known bicoumarins were isolated from the root of *Myoporum bontioides* by the endophytic fungus *Aspergillus clavatus*. Isolated compounds showed strong broad-spectrum inhibitory activities with MIC values 244.73, 195.79 and 61.18 μM against all three pathogenic fungi,

F. oxysporum, *C. musae* and *P. italicm*, and triadimefon, which was used as the positive control, had MIC values 340.43, 272.39 and 170.24 μM, respectively (Li et al. 2017). Bicolorins A–I, a group of nine novel halogenated cyclopentenones, were isolated from endophytic fungus *Saccharicola bicolour* of *Bergenia purpurascens* together with three other known cyclopentenones. Isolated compounds showed antifungal activity against five phytopathogenic fungi (*Uromyces viciae-fabae*, *Pythium dissimile*, *Gibberella zeae*, *Aspergillus niger* and *Sclerotinia sclerotiorum*). Bicolorin B (**79**) and D (**80**), in particular, showed potent antifungal activity against *P. dissimile* with MIC values of 6.2 and 8.5 μg/mL, respectively, in comparison to the positive control cycloheximide (MIC of 8.6 μg/mL). Additionally, both in vitro and in vivo tests showed **80** to be a powerful antifungal against *S. sclerotiorum* (Zhao et al. 2020).

5-Methylmellein (**81**) was isolated from the endophytic fungus *Biscogniauxia mediterranea* Ohu 19B found on *Opuntia humifusa* (Cactaceae) and evaluated in vitro microdilution broth experiment against seven plant pathogens, including *Colletotrichum acutatum*, *C. fragariae*, *C. gloeosporioides*, *Fusarium oxysporum*, *B. cinerea*, *Phomopsis obscurans* and *P. viticola*. *Phomopsis obscurans* was most vulnerable (63.5% growth suppression) at 150 μM at 120 h. At 300 μM after 48 h, the best growth inhibition of *F. oxysporum* (20.1%) was achieved. Compound **81** stimulated *B. cinera* and *C. fragariae* at lower doses (75 and 150 μM), but *C. acutatum* and *C. gloeosporioides* were stimulated at all levels (Silva-Hughes et al. 2015).

Another ascomycete genus, *Chaetomium*, is a prolific producer of active compounds. *Chaetomium globosum* CDW7, an endophyte from *Ginkgo biloba*, was used to isolate chaetoglobosin A (**82**) and chaetoglobosin D (**83**). Both the compounds demonstrated antifungal efficacy against *Sclerotinia sclerotiorum* with IC_{50} values of 0.35 and 0.62 μg/mL, respectively, in comparison to carbendazim (0.17 μg/mL) (Zhang et al. 2013a, b).

The endophytic fungus *Alternaria alternata* Tche-153 from *Terminalia chebula* yielded altenusin (**84**), a biphenyl derivative, which showed strong synergistic activity against *C. albicans* when combined with each of the three azole medications, ketoconazole, fluconazole or itraconazole, using the disc diffusion method and the microdilution checkerboard technique. The fractional inhibitory concentration (FIC) index ranged from 0.078 to 0.188 (Phaopongthai et al. 2013).

Diepoxin, palmarumycin C11, palmarumycin C12, cladospirone B 1,4,7-trihydroxy-8-(spirodioxy-1′,8′-naphthyl)-7,8-dihydronaphthalene and palmarumycin were all extracted from *Berkleasmium* sp., an endophyte associated with *Dioscorea zingiberensis* from Hubei Province, China. The antifungal activity of several compounds was tested against *M. oryzae* spore germination. They prevented *M. oryzae* spore germination with IC_{50} values ranging from 9.1 to 124.5 μg/mL. Among the compounds examined, palmarumycin C8 (**85**) had the most inhibitory action (IC_{50} 9.1 μg/mL), however it was less potent than the positive control carbendazim (IC_{50} 6.3 μg/mL) (Shan et al. 2014).

The endophytic fungus *Trichoderma* sp. isolated from the roots of *Myoporum bontioides* contained dichlorodiaportinolide (**86**) and dichlorodiaportin (**87**), which

showed MIC values ranging from 6.25 to 150 μg/mL against *Colletotrichum musae* and *Rhizoctonia solani*, however they were inactive against *Penicillium italic* and *Fusarium graminearum* (MIC values >200 μg/mL) (Li et al. 2016).

Koninginin O (**88**) produced by the endophytic fungus *Trichoderma koningiopsis* YIM PH30002 growing on *Panax notoginseng* showed antifungal properties against *Fusarium oxysporum, Fusarium solani, Fusarium flocciferum, Plectosphaerella cucumerina* and *Alternaria panax,* which are root rot diseases causing pathogens in *P. notoginseng*. The MIC for koninginin O against *F. oxysporum* and *P. cucumerina* was 128 μg/mL (Liu et al. 2016a, b). *Trichoderma brevicompactum* 0248, an endophytic fungus strain identified from *Allium sativum*, was the source of the trichodermin (**89**). Trichodermin showed strong inhibitory efficacy against *Rhizoctonia solani* and *B. cinerea*, with an EC_{50} of 0.25 and 2.02 μg/mL, respectively, but was only moderately effective against *Colletotrichum lindemuthianum* (EC_{50} = 25.60 μg/mL) (Shentu et al. 2014).

5 Antiviral Metabolites

Virus is nucleic acid (DNA or RNA) containing protein coated submicroscopic infectious agent that lives inside living cells and uses the machinery of the host cell for replication. It can infect all living organisms including plants, animals and microorganisms. Several diseases in humans caused by viral infections are respiratory viral diseases (e.g. flu, parainfluenza virus infection, severe acute respiratory syndrome (SARS), COVID, etc.), gastrointestinal viral diseases (e.g. norovirus infection, rotavirus infection, astrovirus infection, etc.), exanthematous viral disease (e.g. measles, rubella, chickenpox/shingles, roseola, smallpox, fifth disease, chikungunya virus infection, etc.), hepatic viral diseases (e.g. hepatitis A, hepatitis B, hepatitis C, etc.), cutaneous viral diseases (e.g. warts, oral herpes, genital herpes, etc.), haemorrhagic viral diseases (e.g. Ebola, Lassa fever, dengue fever, yellow fever, etc.), neurologic viral diseases (e.g. polio, viral meningitis, viral encephalitis, rabies, etc.) and human immunodeficiency virus (HIV). Tobacco mosaic virus is the first discovered virus. In 1892, Dimitri Ivanovski reported the extract of infectious tobacco leaves remain infectious after passing through a Chamberland filter candle. He concluded the infectious agents are smaller than bacteria. In 1892, Martinus Beijerinck named the infectious agent as tobacco mosaic virus (Lecoq 2001).

Viruses complete their life cycle in three steps starting from entry, then genome replication and finally exit from host cell. In the entry step, the viral capsid or glycoprotein interacts with the receptor of the host cell, which then penetrates the virus particle into the cell followed by uncoating, in which the virus sheds its capsid. The naked viral genome is utilized for gene expression and viral genome replication. Finally, the viral proteins and viral genomes are accumulated and assembled to form new virions. The assembled virions are released from the host cell through cell lysis, budding and exocytosis (Ryu 2017). Secondary metabolites isolated from

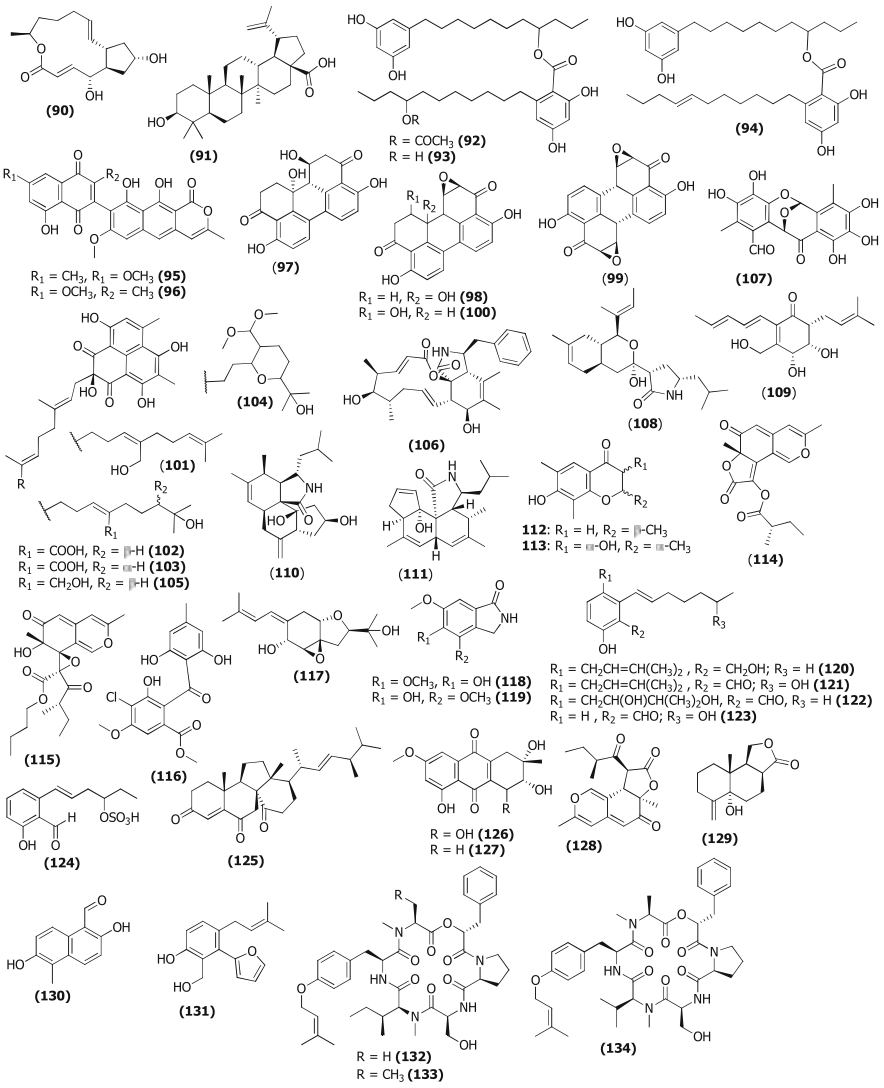

Fig. 2.4 Structures of Antiviral metabolites from endophytic fungi

endophytic fungi have been reported for promising antiviral activity against diverse range of viruses (Fig. 2.4, Table 2.4).

Brefeldin-A (BFA, 90) is a 13-carbon macrolide lactone also known as ascotoxin or cyanein or decumbin or lunatin or sinergisidin. It was reported for diverse biological activities such as antifungal (Crabbe and Betina 1994; Talukdar et al. 2021), antiproliferative (Ishii et al. 1989; Sausville et al. 1996), antimitotic, cytostatic (Wang et al. 2002) and antiviral (Betina 1992; Tamura et al. 1968). It was recently reported that BFA can inhibit TNFα-induced necroptosis (Shi et al. 2022).

Table 2.4 List of antiviral metabolites from endophytic fungi

S. no.	Endophytic fungal species/strain	Metabolites	Host plant	Target virus	IC_{50} or EC_{50}#	Reference
1.	*Penicillium* sp.	Brefeldin-A (**90**)	Soil around *Angelica keiskei* root	DENV-1	61.32 ± 13.5 nM	Raekiansyah et al. (2017)
				DENV-2	54.6 ± 0.9 nM	
				DENV-3	57.9 ± 0.1 nM	
				DENV-4	65.7 ± 6.3 nM	
				Japanese encephalitis virus	58.4 ± 0.3 nM	
				Zika virus	54.8 ± 0.4 nM	
2.	*Phomopsis sp*	Betulinic acid (**91**)	*Diospyros carbonaria*	DENV-NS5	4.1 ± 3.1 μM	Peyrat et al. (2017)
3.	*Cytonaema* sp.	Integracin A (**92**) Integracin B (**93**) Integracin C (**94**)	Twigs of *Quercus ilex*	HIV-1 integrase	3.2 μM	Singh et al. (2002)
					6.1 μM	
					3.5 μM	
4.	*Penicillium chrysogenum*	Xanthoviridicatin E (**95**)	Leaves of unidentified plant	HIV-1 integrase	6 μM	Singh et al. (2003)
		Xanthoviridicatin F (**96**)			5 μM	
5.	*Alternaria tenuissima*	Altertoxin I (**97**) Altertoxin II (**98**) Altertoxin III (**99**) Altertoxin V (**100**)	Stem tissues of *Quercus emoryi*	HIV-1	1.42 μM	Bashyal et al. (2014)
					0.21 μM	
					0.29 μM	
					0.09 μM	

(continued)

Table 2.4 (continued)

S. no.	Endophytic fungal species/strain	Metabolites	Host plant	Target virus	IC_{50} or EC_{50}#	Reference
6.	*Aspergillus* sp.	Asperphenalenone A (**101**) Asperphenalenone B (**102**) Asperphenalenone C (**103**) Asperphenalenone D (**104**) Asperphenalenone E (**105**)	*Kadsuralongi pedunculata*	HIV-1	4.5 μM	Pang et al. (2017)
					2.4 μM	
				H1N1	0.28 ± 0.01 μM	Zhao et al. (2021)
7.	*Aspergillus* sp.	Cytochalasin Z8 (**106**)	*Kadsuralongi pedunculata*	HIV-1	9.2 μM	Pang et al. (2017)
8.	*Aspergillus* sp.	Epicocconigrone A (**107**)	*Kadsuralongi pedunculata*	HIV-1	6.6 μM	Pang et al. (2017)
9.	*Periconia* sp.	Pericoannosin A (**108**) Periconiasin F (**110**) Periconiasin J (**111**) Periconone B (**109**)	*Annona muricata*	HIV-1	69.6 μM	Zhang et al. (2016)
					29.2 μM	
					25 μM	Liu et al. (2016a, b)
					18 μM	
10.	*Phomopsis sp*	Phomochromanone A (**112**), Phomochromanone B (**113**), Phomopsones B (**114**), Phomopsones C (**115**)	Fresh stems of *Achyranthes bidentata*	HIV-1	99 μM	Yang et al. (2020a, b)
					147 μM	
					6.1 μM	
					0.5 μM	
11.	*Pestalotiopsis theae*	Chloroisosulochrin (**116**)	Leaves of *Fagara zanthoxyloides*	RSV	4.22 ± 1.03 μM	Uzor et al. (2016)
12.	*Pestalotiopsis theae*	Pestalotheol C (**117**)	Branches of unknown tree	HIV-1_{1A1}	16.1 μM#	Liu et al. (2019a, b)
13.	*Emericella* sp.	Emerimidine A (**118**) Emerimidine B (**119**)	Inner bark *Aegiceras corniculatum*	H1N1	42.07 μg/mL	Zhang et al. (2011)
					62.05 μg/mL	

14.	*Pestalotiopsis* sp.	Pestalol A (**120**) Pestalol B (**121**) Pestalol C (**122**) Pestalol D (**123**) Pestalol E (**124**)	Stem of *Aegiceras corniculatum*	H1N1/H3N2	18.9/ 22.6 μM	Sun et al. (2014)
					28.7/ 27.7 μM	
					40.0/ 42.6 μM	
					48.0/ 48.0 μM	
					49.9/ >50 μM	
15.	*Phomopsis* sp.	Dankasterone A (**125**)	Stem of *Aconitum carmichaeli*	H5N1	3.56 μM	Ma et al. (2014)
16.	*Nigrospora* sp.	4-dehydroxyaltersolanol A (**126**) 6-*O*-demethyl-4-dehydroxyaltersolanol A Altersolanol B (**127**)	Root of *Aconitum carmichaeli*	H5N1	8.35 ± 1.41 μg/ mL	Zhang et al. (2016)
					2.59 ± 1.22 μg/ mL	
					7.82 ± 1.86 μg/ mL	
17.	*Nigrospora* sp.	Chermesinone B (**128**)	Root of *Aconitum carmichaeli*	H1N1	0.80 ± 0.29 μg/ mL	Zhang et al. (2016)
18.	*Phoma* sp.	Phomanolide (**129**)	Root of *aconitum vilmorinianum*	H1N1	2.96 ± 0.64 μg/ mL	Liu et al. (2019a, b)
19.	*Pestalotiopsisvaccinii*	Vaccinal A (**130**) Vaccinol J (**131**)	*Kandelia candel*	EV71	19.2 μM	Wang et al. (2014)

[#]Respective value is EC_{50}, all others are IC_{50}

BFA is produced by various endophytic fungi such as *Paecilomyces* sp./*Aspergillus clavatus* (isolated from the bark of the host plant *Taxus mairei* and *Torreya grandis* (Wang et al. 2002)). *Penicillium melinii/Penicillium janthinellum* (isolated from the roots of the host plant *Panax ginseng*) (Zheng et al. 2013), *Penicillium brefeldianum* (Bai et al. 2022), *Eupenicellium brefeldianum* (isolated from the host plant *Arisaema erubescens*) (Hai-yan et al. 2008), *Scedosporium apiospermum* (isolated from the host plant *Bauhinia guianensis*) (Cordeiro et al. 2019), *Penicillium cyaneum* and *Cladosporium* sp. (isolated from the host plant *Quercus variabilis*) (Wang et al. 2007) and *Acremonium* sp. (isolated from healthy twigs of the host plant *Knema laurina*). In 1958, Brefeldin-A was first isolated by Singleton et al. from the fungus *Penicillium decumbens* (Singleton et al. 1958) followed by isolation from other *Penicillium* sp. (Abraham and Arfmann 1992). In 1968, Tamura et al. reported that brefeldin-A (BFA) showed antiviral activity against herpes simplex viral strain HF (HSV) and Nawcastle disease viral strain Miyadare (NDV) by the agar-diffusion plaque-inhibition method (Tamura et al. 1968). Finter observed that the growth of myxovirus was inhibited by BFA at concentrations between 0.03 and 25 μg/mL (Finter 1970). Cheung et al. observed that BFA arrests the maturation and egress of HSV during early stages of infection by inhibiting the transportation of enveloped particles from the endoplasmic reticulum (ER) to Golgi complexes (Cheung et al. 1991).

Raekiansyah et al. isolated the endophytic fungal strain *Penicillium* sp. FKI-7127 from soil around the root of the plant *Angelica keiskei* (family Umbelliferae). Brefeldin-A (BFA) was isolated from the ethyl alcohol extract of the cultured endophytic fungal broth of *Penicillium* sp. FKI-7127. It showed antiviral activity against dengue viruses (DENV-1, 2, 3, and 4), Zika virus (ZIKV) and Japanese encephalitis virus (JEV) with IC_{50} values at 48 h post infection 61.32 ± 13.5, 54.6 ± 0.9, 57.9 ± 0.1, 65.7 ± 6.3, 54.8 ± 0.4 and 58.4 ± 0.3 nM, respectively. The time-of-addition study showed that BFA inhibited the maturation of the DENV, ZIKV and JEV (Raekiansyah et al. 2017). It blocked the transportation of glycoprotein from the ER to the Golgi apparatus preventing the formation and release of the viruses from infected cells. An additional study by Raekiansyah et al. showed BFA could not block intracellular protein transport in the mosquito cell line (Raekiansyah et al. 2017).

Peyrat et al. isolated betulinic acid (91) from the H_2O/MeOH extract of an endophytic fungus *Phomopsis* sp. and its host plant *Diospyros carbonaria* (EtOAc extract of the bark). Betulinic acid showed antiviral activity, evaluated by the dengue polymerase assay on the RNA-dependant RNA polymerase (RdRp) domain of the non-structural protein 5 (NS5) with an IC_{50} value 4.3 ± 3.1 μM (Peyrat et al. 2017). Betulinic acid was reported to have a diverse range of biological activities such as anti-HIV, antibacterial, antimalarial, anti-inflammatory, antihelmintic, antinociceptive, anti-HSV-1 and anticancer activities (Moghaddam et al. 2012).

Singh et al. isolated an endophytic fungus *Cytonaema* sp. from living twigs of *Quercus ilex* (family Fagaceae) in El Pardo, province of Madrid, Spain. Integracins A (**92**) B (**93**) and C (**94**) were isolated from the methyl ethyl ketone extract of the cultured fungal broth and evaluated for HIV-1 integrase inhibitory activities.

Integracins A, B and C inhibited the coupled reaction of HIV-1 integrase with IC_{50} values of 3.2, 6.1 and 3.5 μM, respectively, whereas they were found to be 10–30 times less active in the strand transfer assay with IC_{50} values of 32, 17 and 88 μM, respectively (Singh et al. 2002). Xanthoviridicatin E (**95**) and F (**96**) were isolated from the methyl ethyl ketone extract of the cultured broth of the endophytic fungus *Penicillium chrysogenum*. The fungal strain was isolated from the leaves of an unidentified plant, in Camino Cuzco Amazonico, Peru. Xanthoviridicatin E and F inhibited the HIV-1 integrase coupled reaction with IC_{50} values 6 and 5 μM but they did not inhibit the stand transfer reaction ($IC_{50} > 100$ μM) (Singh et al. 2003).

Altertoxins I (**97**), II (**98**), III (**99**) and V (**100**) were isolated from the EtOAc extract of the endophytic fungal fermented broth *Alternaria tenuissima*. The fungal strain was isolated from the stem tissues of *Quercus emoryi* (family Fagaceae). Isolated compounds inhibited HIV-1_{LAV} replication with IC_{50} values 1.42, 0.21, 0.29 and 0.09 μM, respectively (Bashyal et al. 2014).

Pang et al. isolated asperphenalenone A–E (**101–105**), cytochalasin Z8 (**106**) and epicocconigrone A (**107**) from the methanolic extract of an endophytic fungus *Aspergillus* sp. The fungal strain was isolated from the plant *Kadsura longipedunculata* (family Schisandraceae). Asperphenalenone A, D, cytochalasin Z8 and epicocconigrone A showed anti-HIV activity against VSV-G pseudo-typed viral supernatant (HIV-1) with IC_{50} values 4.5, 2.4, 9.2 and 6.6 μM, respectively (IC_{50} values of positive control lamivudine and efavirenz were 0.1 and 1.4×10^{-3} μM, respectively). Asperphenalenone B (**102**) did not show good anti-HIV activity, whereas asperphenalenone C (**103**) was not tested (Pang et al. 2017). Asperphenalenone E was reported for antiviral activity against influenza A H1N1 with an EC_{50} value of 0.28 ± 0.01 μM and protected the mice from lethal influenza A H1N1 infection (Venkatesan et al. 2022). Pericoannosin A (**108**), periconone B (**109**) and periconiasin F (**110**) and J (**111**) were isolated from the EtOAc extract of an endophytic fungus *Periconia* sp. (host plant *Annona muricata*). They showed anti-HIV activity against VSV-G pseudo-typed viral supernatant (HIV-1) with IC_{50} values 69.6, 18, 29.2 and 25 μM, respectively (Liu et al. 2017, 2016a, b; Zhang et al. 2013a, b). Phomochromanone A (**112**), B (**113**), phomopsone B (**114**) and C (**115**) were isolated from the EtOAc extract of an endophytic fungus *Phomopsis* sp. The fungal strain was isolated from the fresh stems of *Achyranthes bidentata* (family *Amaranthaceae*). These compounds inhibited the VSV-G pseudotyped viral supernatant (HIV-1) with IC_{50} values 99, 147, 7.6 and 0.55 μM, respectively (Yang et al. 2020a, b).

Li et al. isolated pestalotheols A–D from the endophytic fungus *Pestalotiopsis theae,* which was isolated from the branches of an unidentified tree near the Jianfeng Mountain, Hainan Province, China (Li et al. 2008). Later, *P. theae* was also isolated from the fresh leaves of the host Nigerian plant *Fagara zanthoxyloides.* Chloroisosulochrin (**116**) isolated from the cultured broth of the endophytic fungus showed antiviral activity against respiratory syncytial virus (RSV) with an IC_{50} of 4.22 ± 1.03 μM (IC_{50} value of positive control ribavirin was 4.91 ± 1.85 μM) (Uzor et al. 2016). In 2020, it was isolated from tea tree *Camellia sinensis* (Theaceae) in Hangzhou, China (Guo et al. 2020). Pestalotheols A–D were tested for anti-HIV

activity against HIV-1_{LAI} in C8166 cells by the MTT assay. Pestalotheol C (**117**) showed anti-HIV replication with EC_{50} and CC_{50} values of 16.1 and 163 μM, respectively (EC_{50} value of positive control indinavir sulphate was 8.18 nM) (Li et al. 2008).

Zhang et al. isolated an endophytic fungus *Emericella* sp. from the inner bark of the mangrove plant *Aegiceras corniculatum* (family Myrsinaceae). Isoindolones derivatives (emerimidine A and B), isoindolone-sesquiterpenoids and meroterpenoids were isolated from the EtOAc extract of both cultured broth mycelium of an endophytic fungus *Emericella* sp. and evaluated for antiviral activity against influenza A virus (H1N1) in MDCK cell monolayers by the CPE inhibition assay. Emerimidine A (**118**) and B (**119**) showed moderate inhibitory activity with IC_{50} values of 42.07 and 62.05 μg/mL (IC_{50} value of positive control ribavirin was 24.60 μg/mL) (Zhang et al. 2011). An endophytic fungal strain *Pestalotiopsis* sp. was also isolated from the mangrove plant *A. corniculatum* (inner stem). Pestalols A–E (**120–124**) were isolated from the EtOAc extract of the cultured mycelium containing broth of the endophytic fungus. Isolated compounds showed antiviral activity against virus H1N1 with IC_{50} values 18.9, 28.7, 40.0, 48.0 and 49.2 μM, respectively. Pestalols A–D also showed activity against virus H3N2 with IC_{50} values 22.6, 27.7, 42.6 and 48.0 μM, respectively (Sun et al. 2014).

Dankasterone A (**125**) was isolated from the EtOAc extract of the cultured broth of an endophytic fungus *Phomopsis* sp. The fungal strain was isolated from the stem of *Aconitum carmichaeli* (family Ranunculaceae). Dankasterone A showed inhibitory activity against influenza A (H5N1) pseudovirus with an IC_{50} value of 3.56 μM (Ma et al. 2014). A fungal strain of *Nigrospora* sp. was also isolated from *A. carmichaeli* (root) in Lijiang County, Yunnan Province, China. 6-*O*-demethyl-4-dehydroxyaltersolanol A (hydroanthraquinone derivative), 4-dehydroxyaltersolanol A (**126**), altersolanol B (**127**) and chermesinone B (**128**) were isolated from the EtOAc extract of the mycelia free cultured broth of an endophytic fungus *Nigrospora* sp. Isolated compounds were tested by the CPE inhibition assay against influenza virus A/Puerto Rico/8/34 (H1N1) in MDCK cell monolayers. Chermesinone B showed most potent antiviral activity with an IC_{50} value of 0.80 ± 0.29 μg/mL (CC_{50} = 184.75 ± 1.34 μg/mL). The hydroanthraquinone derivative was found to be three times less potent than **128** with an IC_{50} value of 2.59 ± 1.22 μg/mL, whereas 4-dehydroxyaltersolanol A (**126**) and altersolanol B (**127**) were found about ten time less potent than **128** with IC_{50} values 8.35 ± 1.41 and 7.82 ± 1.86 μg/mL, respectively (Zhang et al. 2016). The selective index of chermesinone B was >200, therefore it can be a key compound for further research and the development of anti-influenza drug. It was first time isolated from the EtOAc extract of the mycelia free cultured broth of the endophyte fungus *Penicillium chermesinumm* (fungal strain was isolated from the stem of the mangrove plant *Kandelia candel*) (Huang et al. 2011). Phomanolide (**129**) (14-nordrimane-type sesquiterpenoid) was isolated from the EtOAc extract of the cultured broth of an endophytic fungus *Phoma* sp. (isolated from roots of *Aconitum vilmorinianum*). It was also reported for antiviral activity against influenza virus

A/Puerto Rico/8/34 (H1N1) with IC_{50} values 2.96 ± 0.64 μg/mL (Liu et al. 2019a, b).

Vaccinal A (**130**) and vaccinol J (**131**) were isolated from the EtOAc extract of the cultured broth of the endophytic fungus *Pestalotiopsis vaccinii*. The fungal strain was isolated from the branch of the mangrove plant *K. candel*. Isolated compounds (vaccinal A and vaccinol J) showed better antiviral activity than positive control ribavirin (IC_{50} 177.0 μM) against enterovirus 71 (EV71) with IC_{50} values 19.2 and 30.7 μM, respectively (Wang et al. 2017, 2014).

Selim et al. isolated 132 endophytic fungal strains from 18 different Egyptian medicinal plants. The isolated fungi were cultivated, extracted with EtOAc and evaluated for antiviral activity against herpes simplex virus type-2 (HSV-2, DNA virus) and vesicular stomatitis virus (VSV, RNA virus). The extract of the endophytic fungus *Pleospora tarda* (host pant *Ephedra aphylla*) showed promising antiviral activity against both HSV-2 and VSV with inhibitory activity 40.7% and 15.2%, respectively. Pullularins A–C (**132–134**) were isolated from the EtOAc extract of an endophytic fungus (*Pullularia* sp.) fermented broth. The fungal strain was isolated from a leaf of *Culophyllum* sp. (family Guttiferae). Pullularins A (**132**) and B (**133**) showed anti-HSV-2 activity with IC_{50} values 3.3 and 49 μg/mL, respectively, whereas pullularin C (**134**) was inactive against HSV-2. Pullularins A–C also showed antimalarial activity against *Plasmodium falciparum* K1 with IC_{50} values 3.6, 3.3 and 9.8 μg/mL, respectively (Selim et al. 2018).

6 Antiprotozoal Metabolites

Protozoans (eukaryotic, unicellular, heterotrophic organisms) are either parasites or free-living organisms. Parasitic protozoans can infect humans, animals and plants and cause different diseases such as malaria, leishmaniasis, African sleeping sickness and American sleeping sickness (in human), coccidiosis, cvine theileriosis, and babeciosis (in animals) and phloem necrosis (in plants).

6.1 Antimalarial Metabolites

Malaria is a life-threatening disease caused by parasite *Plasmodium* species which is transmitted to people through the bites of infected female *Anopheles* mosquitoes. There are five *Plasmodium* species responsible for malaria such as *P. falciparum*, *P. vivax, P. ovale, P. malariae* and *P. knowlesi*. *P. falciparum* is the deadliest malaria parasite in the African continent, whereas *P. vivax* is the dominant malaria parasite in most countries outside of the African continent. In 2021, WHO estimated 247 million malarial cases and 6,19,000 malarial deaths worldwide. The African region contributes 95% of all malaria cases and 96% of deaths (www.who.int/news-room/fact-sheets/detail/malaria). Malarial parasites are continuously developing resistance

against currently used antimalarial drugs. It is very important to discover new antimalarial drugs to kill or prevent the antimalarial drug resistant *Plasmodium* species.

Elfita et al. isolated two endophytic fungal strains (BB3 and BB4) from *Tinaspora crispa* L. Two alkaloids (2,5-dihydroxy-1-(hydroxymethyl)pyridin-4-on (**135**) and 7- hydroxy-3,4,5-trimethyl-6-on-2,3,4,6-tetrahydroisoquinoline-8-carboxylic acid (**136**)) were isolated from the EtOAc extract of cultured broth of fungal strains BB3 and BB4, respectively. These alkaloids **136** and **135** showed antimalarial activity against *P. falciparum* 3D7 with IC_{50} values 0.127 and 0.129 μM, respectively (Elfita et al. 2011). Hemtasin et al. isolated the endophytic fungal strain *Phomopsis archeri* from the cortex stem of *Vanilla albidia*. The EtOAc extract of the cultured fungal strain showed antimalarial activity against *P. falciparum* with an IC_{50} value 5.0 μg/mL. Three sesquiterpenes, phomoarcherins A–C, were isolated from the EtOAc extract of the cultured fungal strain. Phomoarcherin B (**137**) showed activity against *P. falciparum* (K1, multidrug-resistant strain) with an IC_{50} value 0.79 μM (Hemtasin et al. 2011). Moreno et al. isolated cercosporin (**138**) and cercosporin analogue (**139**) from the EtOAc extract of the mycelium of an endophytic fungus *Mycosphaerella* sp. The fungal strain was isolated from the plant *Psychotria horizontalis* (family Rubiaceae) in Panama. Isolated compounds showed antimalarial activity against *P. falciparum* (Indochina W2, chloroquine-resistant strain) with IC_{50} values 1.03 ± 0.03 and 2.99 ± 0.40 μM, respectively. Compounds **138** and **139** also showed activity against *Leishmania donovani* (IC_{50} values 0.46 ± 0.05 and 0.64 ± 0.05 μM, respectively) and *Trypanosoma cruzi* (IC_{50} values 1.08 ± 0.03 and 0.78 ± 0.07 μM, respectively) (Moreno et al. 2011).

Ferreira et al. obtained the endophytic fungal strain *Diaporthe miriciae* from the leaves and adventitious roots of the endemic neotropical plant *Vellozia gigantea* (family Velloziaceae). Epoxycytochalasin H (**140**) was isolated from the dichloromethane extract of cultured endophytic fungus *D. miriciae* which showed antimalarial activity against two strains of *P. falciparum* (D6 and W2) with IC_{50} values of 52 and 39 ng/mL, respectively (Ferreira et al. 2017). Kumarihamy et al. isolated three known cytochalasins, 19,20-epoxycytochalasins C (**141**) and D (**142**) and 18-deoxy-19,20-epoxycytochalasin C (**143**), from the EtOAc extract of the cultured broth of an endophytic fungus *Nemania* sp. The fungal strain was isolated from the leaves of *Torreya taxifolia* (family Taxaceae). Compounds **141–143** showed antimalarial activity against *P. falciparum* D6 and W2 with IC_{50} values 0.07, 0.04, 0.56 and 0.05, 0.04, 0.29 μM, respectively (IC_{50} values of positive control chloroquine and artemisinin *P. falciparum* D6 and W2 were 0.03, 0.02 and 0.31, 0.01 μM, respectively) (Kumarihamy et al. 2019).

Fusaripeptide A (**144**) (cyclodepsipeptide) was isolated from the EtOAC extract of the cultured endophytic fungal strain *Fusarium* sp. which was isolated from the root of *Mentha longifolia* (family Lamiaceae). It showed antimalarial activity against *P. falciparum* D6 with an IC_{50} value of 0.34 μM (IC_{50} value of positive control artemisinin was 0.57 μM) (Ibrahim et al. 2018). Another cyclopeptide, pipecolisporin (**145**), was isolated from the methyl ethyl ketone extract of the cultured endophytic fungus *Nigrospora oryzae*. The fungal strain was obtained

from the roots of *Triticum* sp. of family Poaceae. Pipecolisporin showed antimalarial activity against *P. falciparum* 3D7 (chloroquine sensitive strain) with an IC_{50} value of 3.21 μM. It also showed activity against *T. cruzi* with an IC_{50} value of 8.46 μM (Fernández-Pastor et al. 2021).

Ravenelin (**146**), a xanthone, was isolated from the EtOAC extract of the endophytic fungus (isolated from *Phanera splendens* belonging to family Leguminosae). It showed antimalarial activity against *P. falciparum* 3D7 with an IC_{50} value of 3.4 ± 0.4 μM. It also showed activity against epimastigote and intracellular amastigote forms of *T. cruzi* with IC_{50} values 5 ± 1, 9 ± 2 μM, respectively (Pina et al. 2021). Aurasperone A (**147**), a dimeric naphthopyran, was isolated from the EtOAc extract of the cultured endophytic fungus *Aspergillus niger* (obtained from *Terminalia catappa* of family Combretaceae). It showed antimalarial activity against *P. falciparum* 3D7 and *P. falciparum* INDO (chloroquine-resistant strain) with IC_{50} values 4.17 and 3.08 μM, respectively (Toghueo et al. 2021). Recently, it was also reported for antiviral activity against SARS CoV-2 with an IC_{50} value of 12.25 μM (IC_{50} value of positive control remdesivir was 10.11 μM) (ElNaggar et al. 2022).

6.2 Anti-Leishmanial Metabolites

Leishmaniasis is parasitic disease caused by more than 20 *Leishmania* species. These parasites are transmitted through the bites of infected female phlebotomine sandflies. More than 90 species are known to transmit these parasites. There are three main forms of the disease such as visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis. The cases of VL, also known as kala azar, mainly occur in Brazil, East Africa and in India. About 90,000 new cases occur worldwide in every 1 year but only 25–40% are reported in WHO (www.who.int). CL is the most common form of leishmaniasis. It causes skin lesions, ulcers on exposed parts of the body, leaving life-long scars and serious disability or stigma. About one million new cases of CL occur worldwide annually. Mucocutaneous leishmaniasis may cause the destruction of mucous membranes of the nose, mouth and throat. Endophytic fungi produce different chemical classes of compounds that showed promising anti-leishmanial activity (Fig. 2.5, Table 2.5).

Martinez-Luis et al. isolated pseurotin A (**148**), 14-norpseurotin A (**149**), FD-838 (**150**), pseurotin D (**151**) and fumoquinone B (**152**) from the EtOAc extract of an endophytic fungus (*Aspergillus* sp.). The fungal strain was isolated from the mature leaves of *Guapira standleyana* (family Nyctaginaceae) in Panama. Isolated compounds (**148–152**) showed anti-leishmanial activity against *L. donovani* (LD-1S/MHOM/SD/00-strain1S) with IC_{50} values 5.8, 4.4, 0.2, 0.5 and 0.5 μM, respectively. Compounds **149–152** also showed weak antimalarial activity against *P. falciparum* IC_{50} values 16.9, 14.9, 12.5 and 5.4 μM, respectively (Martínez-Luis et al. 2012).

Ibrahim et al. isolated stigmasterol derivatives (22*E*,24*R*)-stigmasta-5,7,22-trien-3-β-ol (**153**), stigmast-4-ene-3-one (**154**) and stigmasta-4,6,8(14), 22-tetraen-3-one

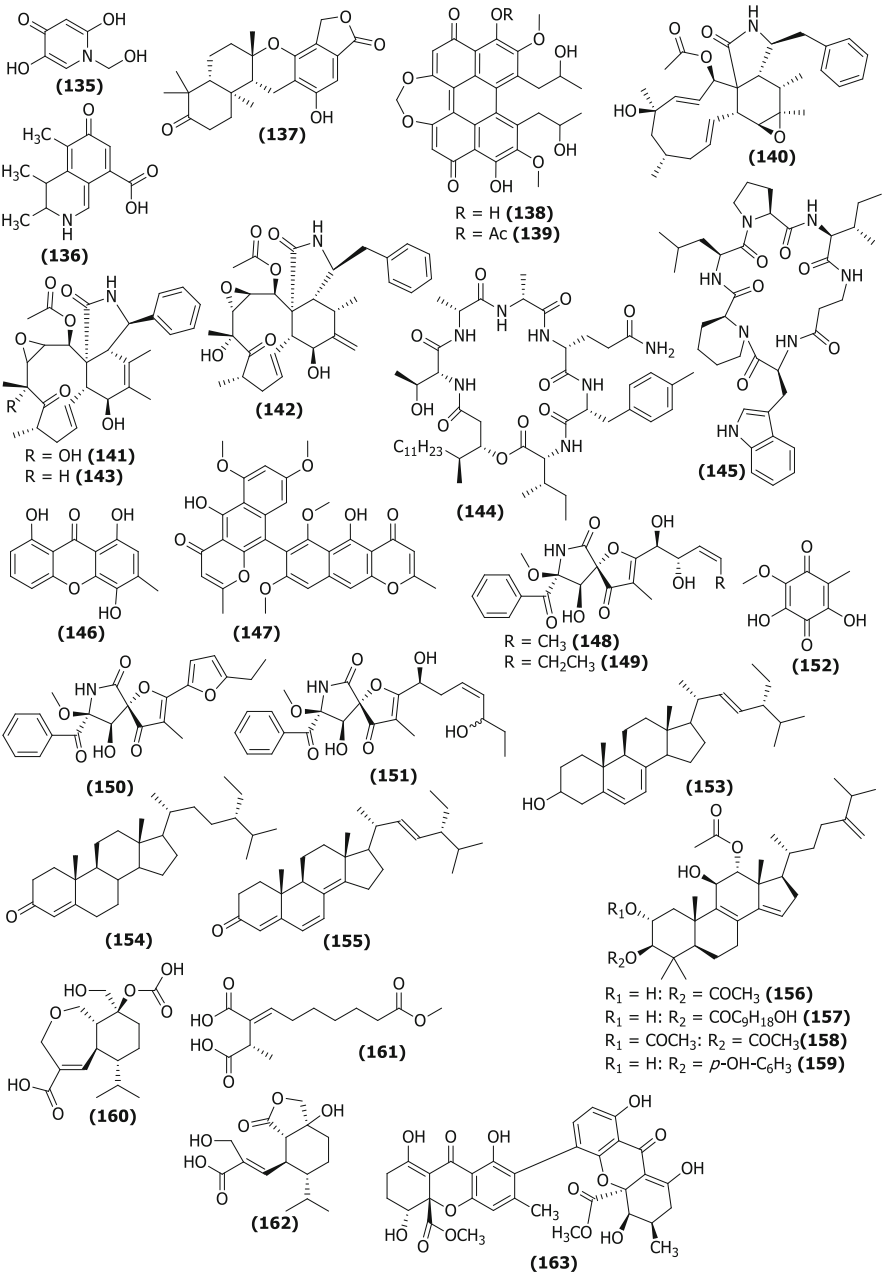

Fig. 2.5 Structures of Anti-protozoal metabolites from endophytic fungi

Table 2.5 Anti-protozoal metabolites from endophytic fungi

S. no.	Endophytic fungal species/strain	Metabolites	Host plant	Target virus	IC_{50}	Reference
1.	BB3	2,5-dihydroxy-1-(hydroxymethyl)pyridin-4-on (**135**)	*Tinospora crispa*	*P. falciparum3D7*	0.127 μM	Elfita et al. (2011)
2.	BB4	7- hydroxy-3,4,5-trimethyl-6-on-2,3,4,6-tetrahydroisoquinoline-8-carboxylic acid (**136**)			0.129 μM	
3.	*Phomopsis archeri*	Phomoarcherin B (**137**)	Stem of *vanilla albidia*	*P. Falciparum* W2	0.79 μM	Hemtasin et al. (2011)
4.	*Mycosphaerella* sp.	Cercosporin (**138**)	*Psychotriahorizontalis*	*P. Falciparum* W2	1.03 ± 0.03 μM	Moreno et al. (2011)
				L. donovani	0.46 ± 0.05 μM	
				T. cruzi	1.08 ± 0.03 μM	
		Acetate of cercosporin (**139**)		*P. Falciparum* W2	2.99 ± 0.40 μM	
				L. donovani	0.64 ± 0.05 μM	
				T. cruzi	0.78 ± 0.07 μM	
5.	*Diaporthemiriciae*	Epoxycytochalasin H (**140**)	Leaves and root *Vellozia gigantea*	*P. Falciparum* D6	52 ng/mL	Ferreira et al. (2017)
				P. Falciparum W2	39 ng/mL	
6.	*Nemania* sp.	19,20-epoxycytochalasins C (**141**) and D (**142**)	Leaves of *Torreya taxifolia*	*P. Falciparum* D6	0.04 μM	Kumarihamy et al. (2019)
				P. Falciparum W2	0.04 μM	
7.	*Nemania* sp.	18-deoxy-19,20-epoxy-cytochalasin C (**143**)	Leaves of *Torreya taxifolia*	*P. Falciparum* D6	0.56 μM	Kumarihamy et al. (2019)
				P. Falciparum W2	0.29 μM	

(continued)

Table 2.5 (continued)

S. no.	Endophytic fungal species/strain	Metabolites	Host plant	Target virus	IC_{50}	Reference
8.	*Fusarium* sp.	Fusaripeptide A (**144**)	Root of *Mentha longifolia*	*P. Falciparum* D6	0.34 μM	Ibrahim et al. (2017)
9.	*Nigrospora oryzae*	Pipecolisporin (**145**)	Root of *Triticum* sp.	*P. Falciparum* 3D7	3.21 μM	Fernández-Pastor et al. (2021)
				T. cruzi	8.46 μM	
10.	*Exserohilum rostratum*	Ravenelin (**146**)	*Phanera splendens*	*P. Falciparum* 3D7	3.4 ± 0.4 μM	Pina et al. (2021)
				T. cruzi (epimastigote)	5 ± 1 μM	
					9 ± 2 μM	
11.	*Aspergillus Niger*	Aurasperone A (**147**)	*Terminalia catappa*	*P. Falciparum* 3D7	4.17 μM	Toghueo et al. (2021)
				P. Falciparum INDO	3.08 μM	
12.	*Aspergillus* sp.	Pseurotin A (**148**)	Leaves of *Guapira standleyana*	*L. donovani*	5.8 μM	Martínez-Luis et al. (2012)
		14-Norpseurotin A (**149**)			4.4 μM	
13.		FD-838 (**150**)			0.2 μM	
		Pseurotin D (**151**)			0.5 μM	
		Fumoquinone B (**152**)			0.5 μM	
14.	*Aspergillus terreus*	(22*E*,24*R*)-Stigmasta-5,7,22-trien-3-β-ol (**153**)	Roots of *Carthamus lanatus*	*L. donovani* promastigote	4.61 μg/mL	Ibrahim et al. (2015)
		Stigmast-4-ene-3-one (**154**)			6.31 μg/mL	
		Stigmasta-4,6,8(14), 22-tetraen-3-one (**155**)			22.03 μg/mL	
15.	*Fusarium* sp.	Integracide F (**156**) Integracide G (**157**) Integracide H (**158**) Integracide J (**159**)	Roots of *Mentha longifolia*	*L. donovani* promastigote	3.74 μg/mL	Ibrahim et al. (2016a, b)
					2.53 μg/mL	

					4.75 μg/mL	
					3.29 μg/mL	
16.	*Nectriapseudotrichia*	10-acetyl trichoderonic acid A (**160**)	*Caesalpinia echinata*	*Leishmania (Viannia) braziliensis* amastigote	21.4 μM	Cota et al. (2018)
17.	*Nectriapseudotrichia*	6′-acetoxy-piliformic acid (**161**)	*Caesalpinia echinata*	*Leishmania (Viannia) braziliensis*	28.3 μM	Cota et al. (2018)
		Hydroheptelidic acid (**162**)		Amastigote	24.8 μM	
18.	*Purpureocillium lilacinum*	Purpureone (**163**)	Roots of *Rauvolfia macrophylla*	*L. donovani*	0.87 μM	Lenta et al. (2016)
				P. Falciparum	12.34 μM	

(**155**) from the EtOAc extract of the cultured endophytic fungus *Aspergillus terreus*. The fungal strain was isolated from the roots of *Carthamus lanatus* (family Asteraceae). Compounds **153–155** inhibited *L. donovani* promastigotes with IC_{50} values 4.61, 6.31 and 22.03 μg/mL, respectively (Ibrahim et al. 2015). Other stigmasterol derivatives, integracides F (**156**), G (**157**), H (**158**) and J (**159**), were isolated from the EtOAc extract of an cultured endophytic fungal strain *Fusarium* sp. which was obtained from the roots of *Mentha longifolia* L. (family Labiatae). Integracides F, G, H and J showed anti-leishmanial activity against *L. donovani* promastigotes with IC_{50} values 3.74, 2.53, 4.75 and 3.29 μg/mL, respectively (IC_{50} value of positive control, pentamidine, was 2.1 μg/mL) (Ibrahim et al. 2016a, b).

10-Acetyl trichoderonic acid A (**160**), 6′-acetoxy-piliformic acid (**161**) and hydroheptelidic acid (**162**) were isolated from the EtOAc extract of *Nectria pseudotrichia*, obtained from the tree *Caesalpinia echinata* (family Caesalpinieae). Compounds inhibited intracellular amastigote forms of *Leishmania (Viannia) braziliensis* with IC_{50} values 21.4, 28.3 and 24.8 μM, respectively (Cota et al. 2018).

Lenta et al. isolated endophytic fungus *Purpureocillium lilacinum* from the roots of *Rauvolfia macrophylla* (family Apocynaceae). The EtOAc extract of fungal mycelia showed anti-leishmanial activity against *L. donovani* (IC_{50} = 0.174 μg/mL). Purpureone (**163**) (ergochromone derivative) was isolated from the same extract which showed activity against *L. donovani* with an IC_{50} value 0.87 μM. It also showed antimalarial activity against *P. falciparum* NF54 with an IC_{50} value 12.64 μM (Lenta et al. 2016).

6.3 Anti-Trypanosomal Metabolites

American trypanosomiasis (Chagas disease) and African trypanosomiasis (sleeping sickness) are parasitic disease, which are caused by parasitic protozoa *T. cruzi* and *T. bruci,* respectively. These parasites are transmitted to humans from either infected humans or animals by the bite of an infected vector such as triatomine bugs and tsetse fly (*Glossina* genus), respectively. These parasites can also be transmitted from infected mother to newborn baby because they can cross the placenta. *T. bruci* can also be transmitted orally (food-borne), or through blood/blood products, organ transplantation and laboratory accidents. It is estimated that about six to seven million people are infected with *T. cruzi* worldwide, mostly in Latin America (www.who.int). Secondary metabolites, isolated from endophytic fungi, were reported for their promising anti-trypanosomal activity (Fig. 2.6, Table 2.6). Aly et al. isolated 11α-methoxycurvularin (**164**), 11β-methoxycurvularin (**165**), 5-chloro-6,8,10-trihydroxy-1-methoxy-3-methyl-9(10H)-anthracenone (**166**) and trichodimerol (**167**) from the EtOAc extract of a cultured endophytic fungus *Penicillium* sp. The fungal strain was obtained from the fresh healthy stem tissues of *Limonium tubiflorum* (family Rutaceae) in Egypt. The isolated compound showed anti-trypanosomal activity against *T. brucei* with MIC values of 9.68, 4.96, 9.75 and 6.29 μM, respectively (Aly et al. 2011).

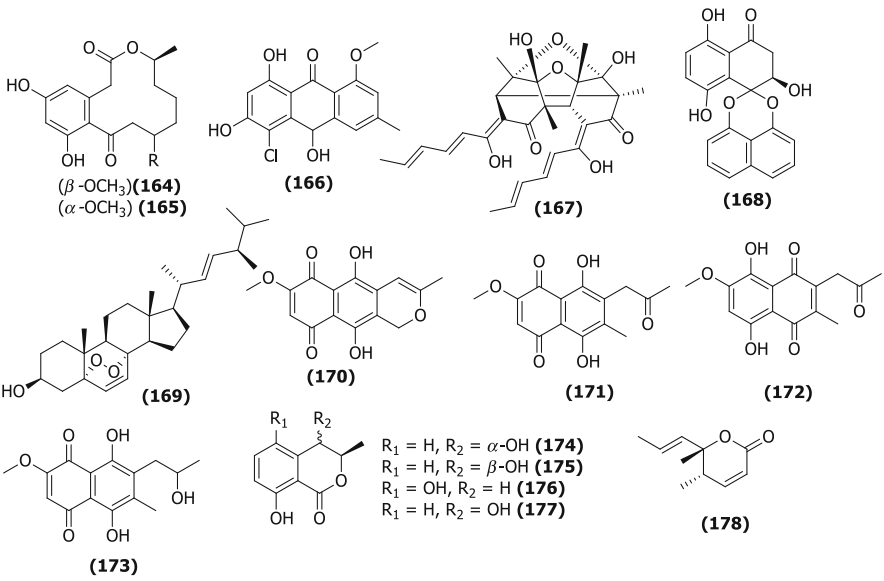

Fig. 2.6 Structures of anti-trypanosomal metabolites from endophytic fungi

Cladospirone B (168) and desmethyl-lasiodiplodin were isolated from the EtOAc extract of the cultured endophytic fungus *Lasiodiplodia theobromae* (obtained from the leaves of *Vitex pinnata*). Isolated compounds showed anti-trypanosomal activity against *T. brucei* with MIC values of 17.8 and 22.5 μM, respectively (Kamal et al. 2017). Ergosterol peroxide (**169**) isolated from the EtOAc extract of the cultured endophytic fungus *Aspergillus* flocculus showed anti-trypanosomal activity against *T. brucei* with a MIC value of 3.12 μM. The fungal strain was isolated from the fresh stems of *Markhamia platycalyx* (family Bignoniaceae) (Tawfike et al. 2019).

Mazlan et al. isolated endophytic fungal strains Fusarium sp. and *L. theobromae* from root and stem of the mangrove plant *Avicennia lanata*, respectively. 1,4-Naphthoquinones, anhydrofusarubin (**170**), javanicin (**171**), dihydrojavanicin (**172**) and solaniol (**173**) were isolated from *Fusarium* sp., whereas dihydroisocoumarins (trans (**174**) and cis (**175**), 4,8-dihydroxy-3-methylisochroman-1-one, 5-hydroxymellein (**176**) and mellein (**177**)) were isolated from the EtOAc extract of a cultured endophytic fungi *L. theobromae*. Solaniol was found most potent against *T. brucei* among isolated compounds from either *Fusarium* sp. or *L. theobromae* with a MIC value of 0.32 μM (MIC value of positive control, suramin, was 0.11 μM). Compound **170** (MIC = 1.20 μM), 2 (MIC = 0.60 μM) and 3 (MIC = 1.90 μM) was three, two and six times less potent, respectively, than compound **173** against *T. brucei*. Compounds **174–177** isolated from *L. theobromae* were found less potent than compounds isolated from *Fusarium* sp. with MIC values 3.2, 8.2, 2.4 and 5.2 μM, respectively, against *T. brucei brucei* (Mazlan et al. 2020).

Table 2.6 Anti-trypanosomal metabolites from endophytic fungi

S. no.	Endophytic fungal species/strain	Metabolites	Host plant	Target virus	MIC/ IC_{50} #	Reference
1.	*Penicillium* sp.	11α-Methoxycurvularin (**164**)	Stem tissues of *Limonium tubiflorum*	*T. brucei brucei*	9.68 μM	Aly et al. (2011)
		11β-Methoxycurvularin (**165**)			4.96 μM	
		5-Chloro-6,8,10-trihydroxy-1-methoxy-3-methyl-9(10*H*)-anthracenone (**166**)			9.75 μM	
		Trichodimerol (**167**)			6.29 μM	
2.	*Aspergillus flocculus*	Ergosterol peroxide (**169**)	Stems of *Markhamia platycalyx*	*T. brucei brucei*	3.12 μM	Tawfike et al. (2019)
3.	*Fusarium* sp.	Anhydrofusarubin (**170**)	Root of *Avicennia lanata*	*T. brucei brucei*	1.2 μM	Mazlan et al. (2020)
		Javanicin (**171**)			0.6 μM	
		Solaniol (**173**)			0.3 μM	
4.	*Lasiodiplodia theobromae*	*Trans* 4,8-Dihydroxy-3-methylisochroman-1-one (**174**)	Stem of *Avicennia lanata*	*T. brucei brucei*	3.2 μM	Mazlan et al. (2020)
		Cis 4,8-Dihydroxy-3-methylisochroman-1-one (**175**)			8.2 μM	
		5-Hydroxymellein (**176**)			2.4 μM	
		Mellein (**177**)			5.2 μM	
		Cladospirone B			17.8 μM	
		Desmethyl-lasiodiplodin	Leaves of *Vitex pinnata*		22.5 μM	Kamal et al. (2017)
5.	*Aspergillus pseudonomiae*	(+)-Phomolactone (**178**)	Arial part of *euphorbia umbellate*	*T. cruzi* epimastigote	0.86 μM*	Gusmão et al. (2021)
		(+)-Phomolactone (**178**)		*T. cruzi* trypomastigote	0.41 μM#	
6.	*Fusarium* sp.	Dihydrojavanicin	Root of *Avicennia lanata*	*T. brucei brucei*	1.9 μM	Mazlan et al. (2020)

* Respective value is IC_{50}, all others are MIC values

Gusmao et al. isolated (+)-phomolactone (**178**) from the EtOAc extract of the cultured endophytic fungus *Aspergillus pseudonomiae* and evaluated for trypanocidal activity. The fungal strain was obtained from the arial part of *Euphorbia umbellate* (family Euphorbiaceae). (+)-**178** inhibited both epimastigote and trypomastigote form of *T. cruzi* with IC_{50} values 0.86 and 0.41 μM, respectively (IC_{50} values of positive control benznidazole were reported to be 30.78 and 4.88 μM against *T. cruzi* epimastigote and trypomastigote, respectively) (Gusmão et al. 2021).

7 Future Prospects and Challenges

The derived compounds from endophytic fungi possess an immense potential in curing various diseases such as cancer, infection, tuberculosis, heart, inflammatory diseases and so on. In fact, these are used as antimicrobial, antiviral, immunosuppressive agents, etc., saving a large number of human lives. In developing endophyte biology, one should pay attention to their biological properties. Host plants usually establish symbiotic relationships by having endophytes protect them from a variety of biotic and/or abiotic stresses. Moreover, their signalling processes still have unexplained fields. Therefore, it is possible to obtain desired secondary metabolites from endophytes by understanding this symbiosis more deeply.

In addition, the work on endophytic fungi can help to prevent neurodegenerative diseases (NDs) and modulation of heat shock response. Misfolding of proteins in the brain and aggregation of various protein bodies occur irreversibly, resulting in the development of NDs represented by Huntington's, Parkinson's and Alzheimer's diseases. Unfortunately, there are no breakthrough treatments for these diseases to date, and many people worldwide are still suffering, and waiting for the discovery of curative treatment and/or novel medicines. Normally, proper protein homeostasis in cells is appropriately regulated by the molecular chaperone system that functions to remove misfolded protein aggregates from the body. The chaperone system generates heat shock proteins (HSPs) (Schlesinger 1990). They play an important role in protein–protein interactions such as folding and assisting in the establishment of proper protein formation and prevention or the improvement of protein aggregation by misfolding (Borges and Ramos 2005). The regulation of these HSPs is governed by heat shock factor 1 (HSF1) (Salamanca et al. 2011). HSF1 is a transcription factor involved in protein maintenance and expression and, under non-stress conditions, exists as an inactive monomer, predominantly throughout the nucleus and cytoplasm. According to recent reports, the levels of monomeric HSF1 are identified to be reduced markedly in NDs or similar conditions (Huang et al. 2018; Ingenwerth et al. 2016). Therefore, increasing the amount of HSF1 to an appropriate value may ameliorate NDs caused by protein aggregation (Calderwood and Murshid 2017; Singh et al. 2018).

More interestingly, HSPs and their associated factors also play a noteworthy role in cancer epidemiology and related fields. Recent reports have shown that high expression of HSPs may determine the presence of tumours (Albakova et al. 2021).

Cancer cells inadvertently cause protein misfolding because they divide more frequently than normal cells. To avoid the immune system and the apoptotic machinery of cells, they force to take over the role of normal HSF1 and maintain proper cellular homeostasis. Furthermore, HSF1 disrupts the HER2/NEU-mediated cellular senescence process and promotes malignant transformation (Meng et al. 2010). From these facts, HSPs are correlated with cancer information such as production, lifespan, up-regulation and/or down-regulation of tumour cells (Yildiz et al. 2022). In addition, the inhibition of HSF1 has been attracting attention as one of the targets for cancer therapy because it inhibits signal transduction pathways and apoptosis associated with carcinogenesis.

Many pharmaceuticals have been discovered and developed from endophytes for the treatment of various diseases due to the great efforts of scientists. Antibiotics including beta-lactams, aminoglycosides and tetracyclines derived from natural products or led by semi-synthesis have been developed, however the antimicrobial activity of conventional drugs, especially those developed based on their chemical structures, is attenuated by AMR. Therefore, it is necessary to develop new kinds of antimicrobial agents with chemical skeletons and/or antimicrobial mechanisms different from those of conventional drugs. In recent years, novel skeletal compounds based on carbon five-membered ring with antimicrobial activity like pentenomycins and hygrophorones were discovered and synthetic studies and evaluation of their antimicrobial activity have been vigorously carried out (Arjona et al. 2007; Lübken et al. 2006; Umino et al. 1973).

Anticancer agents like taxol, camptothecin and podophyllotoxin have been isolated from endophytic fungi (Amna et al. 2006; Hartwell and Schrecker 1951; Stierle et al. 1993). Moreover, studies on the formulation thereof such as DDS and prodrugs are being actively conducted for coping with drug resistance and reducing side effects (Kasai et al. 2012; Koseki et al. 2016; Rautio et al. 2008; Shibata et al. 2022; Taemaitree et al. 2021). Ergoflavin isolated from endophytic fungus has shown anti-inflammatory and anticancer activities (Deshmukh et al. 2009; Franck et al. 1965). Subglutinol A isolated from *Fusarum subglutinans* from *Tripterygium wilfordii* (Lee et al. 2012; Park et al. 2019), guaiazulene and its derivatives obtained from *Lactarius indigo* (Harmon et al. 1980; Maruoka et al. 2022) have been the subject of numerous biological and synthetic studies.

As is evident from the above cases, proceeding with the discovery of novel secondary metabolites from endophyte fungi may give opportunities to discover more pharmaceutically important compounds to cure diseases. At the same time, new treatments may be developed by devising compounds that have already been discovered. Endophyte biology has infinite possibilities as a challenging field for humanity to overcome various diseases.

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Chapter 3
Fungal Endophytes: An Accessible Natural Repository for Discovery of Bioactive Compounds

Malik Muzafar Manzoor, Zahoor Ahmed Wani, and Syed Riyaz-Ul-Hassan

Abstract Microorganisms are proficient synthesizers of natural product molecules that are often bioactive and a source of various antibiotics and other pharmaceutical agents. The demand for new bioactive molecules is ever-increasing due to the emergence of new diseases, novel pathogens, and drug-resistant microbes. It is imperative to extend our search for molecules with novel bioactivities from unique and unexplored niches. Since the discovery of microbial Taxol (**1**), from a fungus inhabiting the tissues of *Taxus brevifolia*, endophytes, the microbes living within the plant tissues in mutualistic associations, have become an important source for the discovery of bioactive molecules. Since the endophytic associations are plant-specific, each plant forms a unique niche for the accumulation of a specific set of microbial species. The endophytes also pass through various stages of evolution within the plant system to evolve their natural product arsenal. Consequently, numerous plants are being analysed for the isolation and characterization of their endophytes for bioactive molecules and enzymes. Here, we report the progress of research and the current status of endophyte biology and biochemistry for the production of bioactive natural products.

Keywords Fungi · Endophytes · Bioactive metabolites · Bioprospecting · Natural products · Secondary metabolites

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1 Endophytic Natural Products (ENPs): An Introduction

In any ecosystem, plants are subjected to innumerable environmental challenges and these environmental challenges can be from biotic stressors, like pathogens and parasites or abiotic stressors, like temperature, drought, salinity, and floods. Plants are known to produce a diverse array of secondary metabolites to overcome these environmental challenges. Howitz and Sinclair proposed the hypothesis of xenohormesis, which states that plants and associated microbes share homologous gene clusters capable of producing secondary metabolites under stress conditions (Howitz and Sinclair 2008). These homologous gene clusters are horizontally transferred between the host plant and microbes which are cross activated by plant hosts or endophytes under some emergencies. Plants in nature are engaged with a diverse array of microorganisms and this multispecies crosstalk results in complex and transient metabolic flux across the interacting partners that have a direct bearing on their phenotype and functional traits (Wani et al. 2015). Every natural product produced by the plant or microbe is potentially a functional trait developed by the interacting organisms sharing the same habitat and plays a central role in their interaction and survival (Kusari et al. 2012).

Microorganisms are considered a treasure hunt for the isolation of biomolecules with potential therapeutic importance. From the discovery of antibiotics, like penicillin (**2**) from *Penicillium notatum* by Alexander Fleming in 1928, to other life-saving drug molecules like Paclitaxel from *Taxomyces andreanae* (Stierle et al. 1993), microorganisms have contributed numerous molecules to natural product repositories having potential pharmaceutical importance. It is reported that approximately 15–20 natural products with different chemical types are produced by an individual microorganism, and another report suggests more than 70,000 ENPs of microbial origin out of which approximately 33,500 are bioactive (Berdy 2012; Wani et al. 2022). Studies based on the estimation of microbial populations have conservatively revealed that only about 1% of bacteria and 5% of fungal species have been identified and explored for human welfare while as the rest remain unexplored (Staley et al. 1997; Wani et al. 2022). The endophytic microbes are subjected to various selective pressures from biotic and abiotic factors which lead to the activation of cryptic/orphan biosynthetic pathways resulting in the production of an increased repertoire of ENPs which usually are not produced under standard culture conditions (Kusari et al. 2014). Challenging the microorganisms through various methods and modulating their secondary metabolism have resulted in several new molecules in recent times. Microorganisms are also challenged in nature by extreme environmental conditions that prompt them to evolve for new ecological settings, thus activating gene clusters that do not express under the optimum conditions, therefore they are metabolically more active than their free-living counterparts (Strobel 2006; Riyaz-Ul-Hassan et al. 2012).

1.1 Endophytes as a Treasure Hunt for ENPs

The term "secondary metabolite" refers to the low molecular mass compounds/natural products derived from primary molecules or long enzymatic pathways of the organism. Secondary metabolites are not required for the growth, development, and reproduction of the organism under normal conditions. The production of secondary metabolites/natural products by endophytic microorganisms is often related to the stage of morphological differentiation of the microorganism (Keller et al. 2005; Singh et al. 2017; Narayanan and Glick 2022) and is induced by certain selection pressures in a given ecological niche. These secondary metabolites mostly induce specific phenotypic functions such as crosstalk with associated organisms, chemical warfare/defence, and stress adaptation in the endophyte (Kusari et al. 2014). The microorganisms are known to produce natural products in axenic culture conditions in the stationary phase when the nutrient status becomes a limiting factor. These natural products are of different types or belong to different classes of compounds and show a broad spectrum of bioactivity as an antibiotic, antiparasitic, immunosuppressant, anticancer, antimycotic, and anti-cholesterol (Mou et al. 2021; Deshmukh et al. 2022). In addition to their pharmaceutical value, many ENPs also play an important role within their host organisms as signalling molecules, pigments, defence molecules, or virulence factors. Endophytic microorganisms produce important classes of secondary metabolites including polyketides (e.g. rapamycin (**3**), lovastatin (**4**)), non-ribosomal peptides (e.g. sirodesmin G (**5**)), terpenes (T-2 toxin (**6**)), steroids (penicisteroid A (**7**)), alkaloids (cryptocin (**8**)), phenolic compounds (colletotric acid (**9**)), aliphatic compounds (brefeldin A (**10**)), and peptides (cryptocandin (**11**)) (Table 3.1).

The production of ENPs under axenic cultures has many constraints depending upon the culture conditions, fermentation parameters, and microbial strains. The production of bioactive natural products from endophytes has so far been made by fermentation of axenic monocultures under standard culture conditions (Scherlach and Hertweck 2009). The sustained production of bioactive ENPs by an endophyte under standard culture conditions requires constant expression of the biosynthetic genes responsible for producing the natural product. However, it has been observed that there is a substantial reduction in the production of ENPs upon repeated sub-culturing under axenic monoculture conditions, which is one of the key challenges in achieving their commercial production. It is suggested that the failure in a constant expression of the biosynthetic genes required for the desired metabolite and non-expression of the cryptic biosynthetic gene clusters present in the endophytes under standard culture conditions is responsible for the reduction in secondary metabolite production in axenic cultures (Kusari et al. 2014). Thus, even if a microorganism has the potential to biosynthesize a particular secondary metabolite, it may not be produced under standard laboratory conditions. This deactivation of cryptic biosynthetic gene clusters under culture conditions leads to the production of a far lesser diversity of secondary metabolites than the actual repertoire of endophyte secondary metabolomes. This great challenge is obscuring the true potential of

Table 3.1 Bioactive compounds produced by endophytic microorganisms isolated from different host plants

Host plant	Endophyte	Bioactive compound/ activity	References
Taxus brevifolia	*Taxomyces andreanae*	Taxol (**1**)	Stierle et al. (1993)
Nothapodytes foetida	*Entrophospora infrequens* SW116	Taxol (**1**) and taxane	Stierle et al. (1993)
Tripterygium wilfordii	*Cryptosporiopsis* cf. *quercina*	Cryptocandin (**11**)	Strobel et al. (1999)
Torreya taxifolia	*Pestallotiopsis microspora*	Pestaloside, caryophyllene, sesquiterpenes, 2-hydroxydimeninol, pestalotiopsins A and B	Lee et al. (1995) and Pulici et al. (1996a, b, 1997)
Catharanthus roseus	*Alternaria* sp.	Vinblastine	Guo et al. (1998)
Garcini adulcis	*Phomopsis* sp.	Phomoxanthones A and B	Isaka et al. (2001)
Kennedia nigriscans	*Streptomyces coelicolor*	Munumbicins	Castillo et al. (2002)
Grevillea pteridifolia	*Streptomyces NRRl 30,566*	Kakadumycins	Castillo et al. (2003)
Monstera sp.,	*Streptomyces* sp. (MSU-2110)	Coronamycin	Ezra et al. (2004)
Erythrina crista-galli	*Phoma* sp.	Phomol	Weber et al. (2004)
Monstera sp.	*Streptomyces* sp.	Coronamycin	Ezra et al. (2004)
Nothapodytes foetida	*Entrophospora infrequens*	Camptothecin (**36**)	Puri et al. (2005)
Podophyllum hexandrum	*Trametes hirsuta*	Podophyllotoxin (**33**)	Puri et al. (2006)
Hypericum perforatum	*Chaetomium globosum*	Hypericin	Kusari et al. (2008)
Juniperus recurva	*Fusarium oxysporum*	Podophyllotoxin (**33**)	Kour et al. (2008)
Azadirachta indica	*Chloridium* sp.	Javinicin (**38**)	Kharwar et al. (2009)
Camptotheca acuminata	*Fusarium solani*	Camptothecin (**36**)	Kusari et al. (2009)
Phlegmariurus cryptomerianus	*Blastomyces* sp., *Botrytis* sp.	Hupzine A	Ju et al. (2009)
Lycopodium serratum	*Penicillium* sp.	Huperzine A	Zhou et al. (2009)
Mimusops elengi	Ascomycetes species	Ergoflavin	Deshmukh et al. (2009)

(continued)

Table 3.1 (continued)

Host plant	Endophyte	Bioactive compound/ activity	References
Manihot esculenta (cassava)	*Paenibacillus* sp. *IIRAC-30*	Lipopeptides	Canova et al. (2010)
Taxus	*Metarhizium anisopliae*	Taxol (**1**)	Sonaimuthu and Johnpaul (2010)
Aegiceras corniculatum	*Emericella* sp.	Emerimidine A and B, emeriphenolicins A and D	Zhang et al. (2011)
Ginkgo biloba	*Chaetomium globosum*	Chaetoglobosin G (**60**), fumitremorgin C, Gliotoxin	Li et al. (2011)
Marine red alga (*Laurencia*)	*Penicillium chrysogenum* QEN-24S	Penicisteroids A and B	Gao et al. (2011)
Salvia miltiorrhiza	*Trichoderma atroviride* D16	Tanshinone IIA and tanshinone I	Ming et al. (2012)
Ginkgo biloba	*Fusarium oxysporum*	Ginkgolide B	Cui et al. (2012)
Forsythia suspensa	*Colletotrichum gloeosporioides*	Phillyrin	Zhang et al. (2012)
Dysoxylum binectariferum	*Fusarium proliferatum*	Rohitukine (**37**)	Kumara et al. (2012)
Clidemia hirta	*Cryptosporiopsis* sp	(R) 5-hydroxy-2-methylchroman-4-one (**40**)	Zilla et al. (2013)
Cedrus deodara	*Talaromyces* sp.	Ramulosin, epoformin	Kumar et al. (2013)
Capsicum annuum	*Alternaria alternata*	Capsaicin	Devari et al. (2014)
Fritillaria unibracteata	*Fusarium redolens*	Imperialine-3β-d-glucoside and peimisine	Pan et al. (2015)
Dracaena draco L.	*Botryodiplodia theobromae* Pat.	Norharman, capsiamide, coumarin and isocoumarins	Zaher et al. (2015)
Bostrychia tenella	*Penicillium decaturense; P. waksmanii*	Cytochalasin D (**53**)	de Felício et al. (2015)
Crocus sativus	*Phialophora mustea*	Phialomustins A-D (**41–44**)	Nalli et al. (2015)
Rosa damascena	*Cercospora* sp.	Fulvic acid and Anhydro fulvic acid	Bashir et al. (2022)
Glycyrrhiza glabra	*Phoma* sp.	Thiodiketopiperazine derivatives	Arora et al. (2016)
Sonneratia apetala	*Penicillium* sp.	Lipopolysaccharides, concanavalin A	Liu et al. (2016)
Glycyrrhiza glabra		Diapolic acid A-B	

(continued)

Table 3.1 (continued)

Host plant	Endophyte	Bioactive compound/ activity	References
	Diaporthe terebinthifolii		Nalli et al. (2017)
Nymphaea nouchali	*Chaetomium globosum*	Chaetoglobosin A (**56**) and C (**62**)	Dissanayake et al. (2016)
Hibiscus rosa-sinensis	*Pseudomonas oryzihabitans*	Asparaginase	Bhagat et al. (2016)
Elleanthus sp.	*Muscodor yucatanensis*	Brefeldin A (**10**)	Qadri et al. (2017)
Arbutus unedo	*Talaromyces pinophilus*	Ferrirubin, herquline B, 3-O-methylfunicone	Vinale et al. (2017)
Pyrenacanthavolubilis Hook.	*Bacillus subtilis* (KY741853) and *Bacillus amyloliquefaciens* (KY741854)	Camptothecin (**36**)	Soujanya et al. (2017)
Glycyrrhiza glabra	*Fusarium solani*	Fusarubin	Shah et al. (2017)
Zingiber cassumunar	*Arthrinium* sp. MFLUCC16–1053	β-Cyclocitral, 3*E*-cembrene A, laurenan-2-one, sclareol, 2*Z*,6*E*-farnesol, cembrene, β-isocomene and γ-curcumene	Pansanit and Pripdeevech (2018)
Pelargonium sidoides	*Alternaria* sp.	Linoleic acid and cyclodecasiloxane	Manganyi et al. (2019)
Melaleuca leucadendra	*Nigrospora aurantiaca*	Nigaurdiol, ergasterol, bostrycin	Safwan et al. (2021)

endophytes as microbial factories for the industrial production of desired bioactive metabolites.

1.2 Polyketides, an Important Class of Bioactive ENPs

Polyketides represent a class of compounds that are named after biosynthetic intermediates that carry multiple keto groups. Fungi are a prolific source of polyketides (PKs), which display remarkable structural diversity and biological activity (Fig. 3.1). The polyketide pathway is one of the main metabolic routes that result in the formation of fungal bioactive molecules. PKs utilize chemistry common to fatty acid biosynthesis. They are polymers synthesized from simple carboxylic acid derivatives of methyl malonyl-CoA, acetyl-CoA, and malonyl-CoA into linear chains by effective decarboxylative condensation. In some cases, the

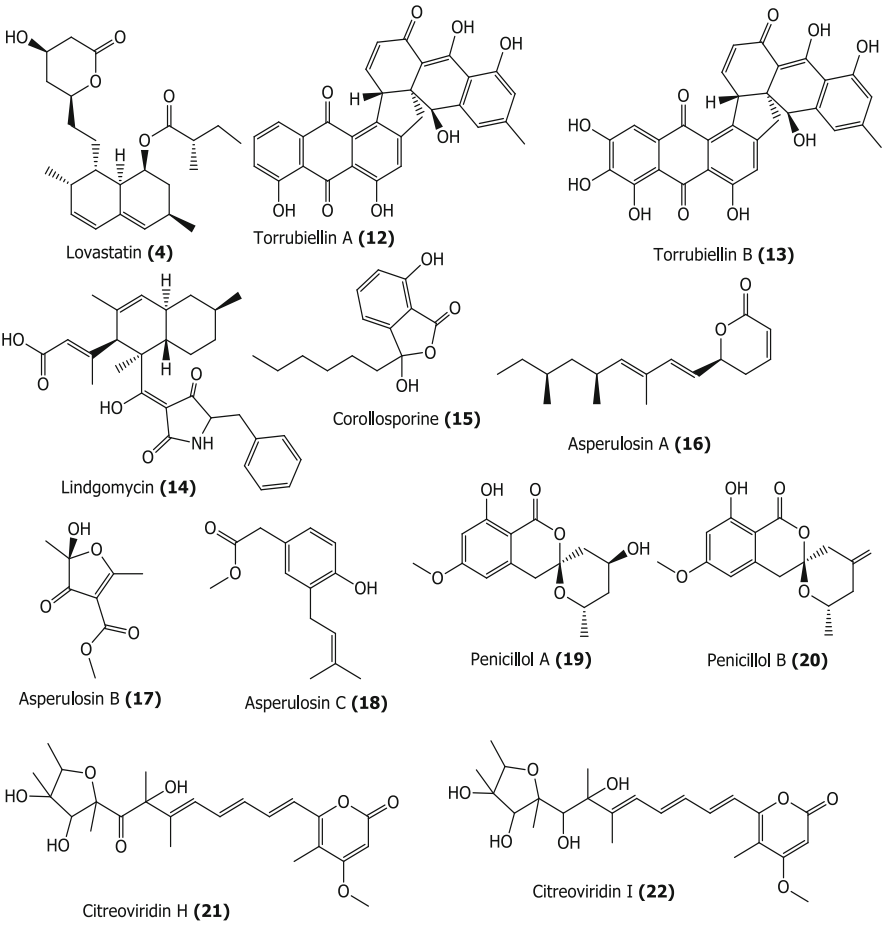

Fig. 3.1 Chemical structure of various polyketides derived from endophytes

resulting β-keto groups are then subjected to reductive modification. The fungal polyketide statins, such as lovastatin (**4**) for example, are among the most successful cholesterol-lowering agents in the market (Crawford and Townsend 2010). Chemical investigation of filamentous fungus *Penicillium citrinum* isolated from the flowers of *Ocimum tenuiflorum* L. led to the discovery of 14 polyketides, some of which showed antibacterial activity against *Staphylococcus aureus* ATCC 29213 (Lai et al. 2013). Recently in 2018 a group of researchers from CAS Key Laboratory of Tropical Marine Bio-Resources and Ecology, Chinese Academy of Sciences, China, isolated six new polyketides including two octaketides, one each chromone and α-pyrone derivative, and two highly substituted phthalides from a mangrove-associated fungus *Diaporthe* sp. (SCSIO 41011). Antiviral evaluation results

revealed that substituted phthalides displayed significant anti-viral activities against three influenza A virus subtypes (Luo et al. 2018).

In some cases, pathogenic fungi produce certain polyketides with dimeric structures. One such example is that of dimeric anthraquinones, torrubiellins A and B (**12**, **13**) produced by *Torrubiella* species, an orthopod pathogenic fungus known to parasitize spiders, scale insects, and hoppers. These compounds showed antibacterial activity against *Bacillus cereus,* antifungal activity against *Candida albicans*, and cytotoxic activity against three human cancer lines (KB, NCI-H187, and MCF-7) (Isaka et al. 2012). Lindgomycin (**14**) is an unusual polyketide antibiotic isolated from a variety of marine and terrestrial fungi belonging to the family Lindgomycetaceae, which is reported to exhibit antifungal activity against *Candida albicans* and plant pathogenic fungi *Septoria tritici.* Lindgomycin (**14**) also shows antibacterial activity with IC_{50} of 2–6 μM against a number of Gram-positive bacteria (Marfori et al. 2002). Corollosporine (**15**) is another polyketide derived from the marine fungi *Corollospora maritime*, showing concentration-dependent antibacterial activity against *Staphylococcus aureus* (Liberra et al. 1998). Xu et al. (2021) reported two new polyketide compounds asperulosins A and B (**16**, **17**), as well as a new prenylated small molecule asperulosin C (**18**) from the fungus *Aspergillus rugulosa*. Asperulosins A (**16**) was found to exhibit potential anti-inflammatory activities by suppressing immune response in lipopolysaccharide (LPS)-induced RAW264.7 cells by blocking the NF-κB pathway and decreasing the production of COX-2 and iNOS. Recently four new polyketide compounds isocoumarins penicillol A (**19**) and penicillol B (**20**) containing spiroketal rings and two novel citreoviridin derivatives citreoviridin H (**21**) and citreoviridin I (**22**) were reported from *Penicillium* sp. (BJR-P2) an endophytic fungus found in mangroves. All the isolated compounds were evaluated for their anti-inflammatory activity. Penicillol B (**20**) was found to be more effective than indomethacin used as a positive control, at inhibiting lipopolysaccharide (LPS)-induced NO (nitric oxide) generation in RAW 264.7 cells, with half-maximal inhibitory concentration (IC_{50}) values of 12 M (Chen et al. 2022).

1.3 Small Peptides as Bioactive ENPs

The synthesis of peptidic natural products in fungi is carried out via two different mechanisms. The bulk of peptide metabolites is produced by enzymes present in the non-ribosomal peptide (NRP) pathway (Arnison et al. 2013). These multimodular, highly specialized enzymes known as non-ribosomal peptide synthetases (NRPSs), synthesize the peptidic backbones from both proteinogenic and non-proteinogenic amino acids. The second method involves ribosomally synthesized and post-translationally modified peptides (RiPPs), which are used to create very large peptidic NPs with molecular weights generally about 1000 Da (Arnison et al. 2013). Two novel cyclic peptides, talaromins A (**23**) and B (**24**) (Fig. 3.2), were discovered through chemical analysis of the endophytic fungus *Talaromyces wortmannii* that was isolated from *Aloe vera*. Based on NMR spectroscopic and

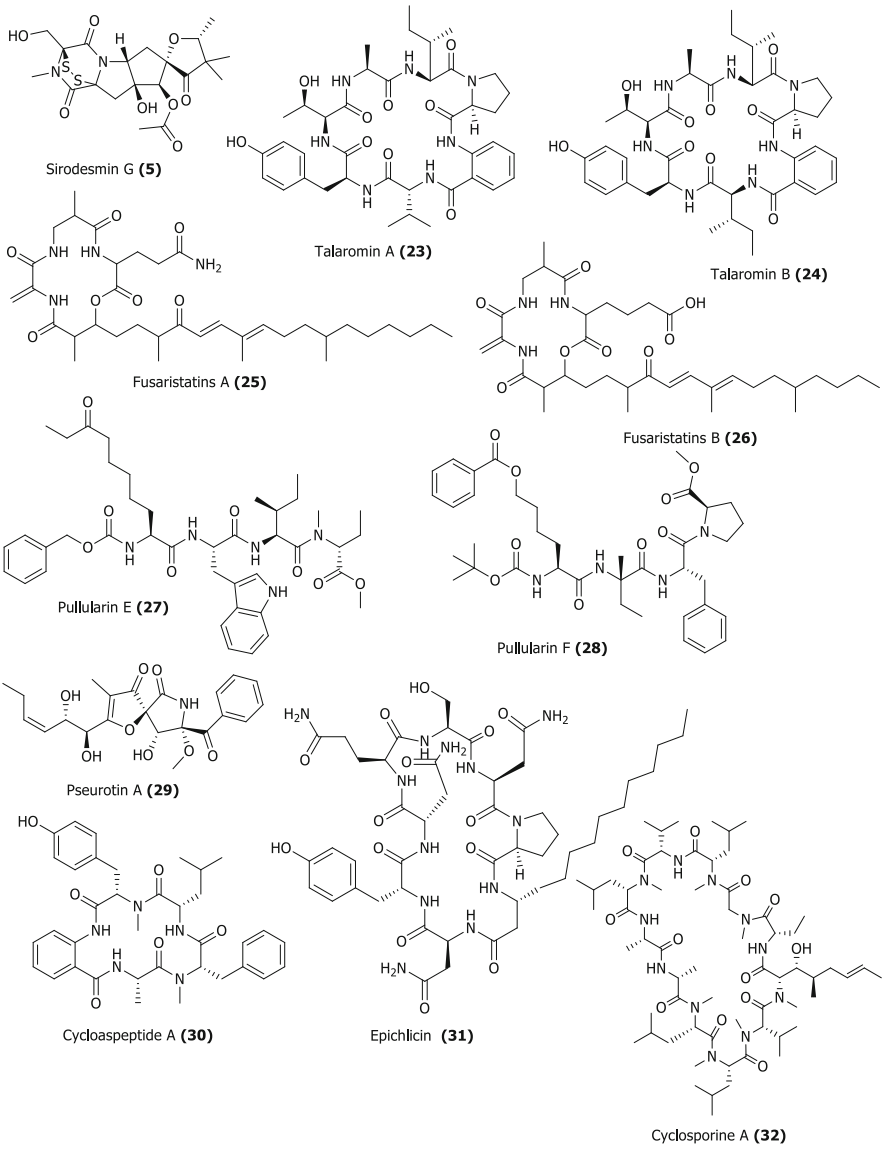

Fig. 3.2 Chemical structures of bioactive peptides, isolated from the endophytes

mass spectrometric analysis, their structures were established. The absolute configurations of the amino acids were determined using Marfey's Method. Further, it was found that both the cyclopeptides contain ring systems comprised of α-amino acid residues connected to β-amino acid (Bara et al. 2013).

Similarly, several genes encoding non-ribosomal peptide synthetases were identified by Lane and coworkers in *Epichloë festucae* an endophyte of *Lolium perenne* (Bara et al. 2013). *A* group of researchers from China isolated two novel cyclic

pentapeptides (L-Phe-L-Leu[1]-L-Leu[2]-L-Leu[3]-L-Ile and (3*S*,4*R*)-dihydroxy-(6*S*)-undecyl-α-pyranone) from a fungus isolated from mangrove *Avicennia marina.* While assessing the isolated peptides for anti-cancer activity, it is reported that cyclo-(L-Phe-L-Leu[1]-L-Leu[2]-L-Leu[3]-L-Ile) exhibited inhibitory activity against the human cancer cell line Bel-7402 with 67% cell viability at a dose of 15 μg mL^{-1} (Li et al. 2004). Another study on endophytic fungus isolated from the leaf of *Kandelia candel* reported two novel cyclic depsipeptides along with the three known cyclodipeptides cyclo-(Leu-Leu), cyclo-(Leu-Tyr) and cyclo-(Phe-Gly) being active against MCF-7 human breast cancer cell lines (Huang et al. 2007). In 2004, Strobel and colleagues identified a combination of novel peptide antibiotics known as coronamycin. They found that it is delivered by verticillate endophytic *Streptomyces* sp. from an epiphytic vine, *Monstera* sp. found in the Amazon forests of Peru. Coronamycin was found active against *pythiaceous* fungi and *Cryptococcus neoformans* with an IC_{50} of 9.0 ng/mL (Ezra et al. 2004). Two novel cyclic lipopeptides, fusaristatins A (**25**) and B (**26**), were isolated from *Fusarium* sp. Fusaristatin A (**25**) and fusaristatin B (**26**) are reported to moderately inhibit Topoisomerases I and II at IC_{50} of 73 and 98 μM, respectively. Moreover, both the lipopeptides demonstrated inhibitory activity against lung cancer cells LU 65 with IC_{50} values of 23 and 7 μM, respectively (Shiono et al. 2007). Two new peptides pullularins E (**27**) and F (**28**) were isolated from the endophytic fungus (*Bionectria ochroleuca*) of the mangrove plant (*Sonneratia caseolaris*). These peptides demonstrated strong to moderate cytotoxic action against mouse lymphoma cells (L5178Y) (Ebrahim et al. 2012). Two cyclopeptides (pseurotin A (**29**) and cycloaspeptide A (**30**)) were isolated from *Penicillium janczewskii,* an endophytic fungus of Chilean gymnosperm *Prumnopitys andina.* Both the isolated compounds demonstrated low cytotoxicity towards human lung cells with an IC_{50} of greater than or equal to 1000 mM. Pseurotin A (**29**) also showed antibacterial activity against phytopathogenic bacteria *Erwinia carotovora* and *Pseudomonas syringae* (Schmeda-Hirschmann et al. 2008). Another cyclic peptide (epichlicin (**31**)) isolated from *Epichloe typhina,* an endophytic fungus of *Phleum pretense* L, was found to inhibit spore germination of *Cladosporium phlei,* a pathogenic fungus of the timothy plant (Seto et al. 2007). Hybrid peptide-polyketides described as curvularides A–E isolated from *Curvularia geniculate,* an endophyte of *Catunaregam tomentosa,* showed antifungal activity against *Candida albicans* and also showed synergistic activity with fluconazole drug (Chomcheon et al. 2010). Several peptides of non-ribosomal origin are important because they act as antibiotics or immunosuppressive drugs. It is reported that a cyclic peptide cyclosporine A (**32**) obtained from an endophytic fungus, *Penicillium fellutanum* , exhibits anticancer activity (Wu et al. 2022).

2 ENPs with Anticancer Potential

Since the discovery of Taxol from an endophytic fungus *Taxomyces andreanae* inhabiting *Taxus brevifolia*, there has been a paradigm shift in endophytic research, and more focus was laid on obtaining bioactive metabolites with significant pharmaceutical values (Stierle et al. 1993). As taxol (**1**) is a natural-source cancer drug derived from the bark of the Yew tree (*T. brevifolia*), hence its production from an endophytic microbe is of great significance. Similarly, podophyllotoxin (**33**) was isolated from an endophytic fungus *Trametes hirsuta*, present in the host plant *Podophyllum hexandrum* (Puri et al. 2006), podophyllotoxin (**33**) was extracted from another endophytic fungus *Fusarium oxysporum* found in *Juniperus recurva* (Kour et al. 2008). Podophyllotoxin is used on the skin as a topical treatment of external genital warts caused by some types of the human pappilomavirus and other warts. Podophyllotoxin (**33**) is also used as starting compound for the synthesis of anticancer drug etopside (**34**) and teniposide (**35**). These drugs are used for lung cancer, testicular cancer, neuroblastoma, hepatoma, and other tumours.

Another ENP with antitumour potential is camptothecin (**36**), isolated from an endophytic fungus *Entrophospora infrequens*, harbouring the host plant *Nothapodytes foetida* (Puri et al. 2005). Camptothecin is used as a chemotherapeutic agent in the treatment of leukaemia (Jones et al. 1997). Other plant metabolites isolated from endophytes include rohitukine (**37**) (Kumara et al. 2012), javinicin (**38**) (Kharwar et al. 2009), paclitaxel (taxol)(**1**) (Sreekanth et al. 2011), piperine (**39**) (Verma et al. 2011), and so on and these metabolites are reported to possess antitumour, anti-inflammatory, and immunomodulatory properties (Fig. 3.3). In most of the above-mentioned studies, the endophytes have been mostly acquired from individual plants sporadically. There is enough literature revealing the synthesis of plant-derived compounds by endophytes rather than hosts. However, the phylogenetic origin of genes related to the biosynthesis pathway of such plant-derived compounds in host plants and their microbial endophytes is still unclear.

The novel stereo bioactive secondary metabolite, (R)-5-hydroxy-2-methylchroman-4-one (HMC) (**40**), isolated from the endophytic fungus *Cryptosporiopsis* sp. H2-1, associated with *Clidemia hirta* showed anti-proliferative activity against different cell lines with cytotoxicity window of 6–12 times lower in normal cells as compared to susceptible leukaemic HL-60, MOLT-4, and K-562 cells (Pathania et al. 2015). In the aforementioned leukaemic cell lines, it induces apoptosis via intrinsic and extrinsic pathways. Further in myeloid and lymphoid leukaemia cells, HMC was found to cause caspase-dependent apoptosis and significantly attenuate the p-STAT-3 transcription factor. The study showed how endophytes can aid in the identification and development of new anticancer therapies.

Similarly, four novel bioactive compounds, phialomustin A–D (**41–44**), were isolated from an endophytic fungus *Phialophora mustae* associated with *Crocus sativus* (Nalli et al. 2015). Phialomustin A–D were screened for cytotoxic activity against four distinct human cancer cell lines, including pancreatic (MIAPaCa2), lung (A549), colon (HCT 116), and human breast cancer (T47D). Interestingly,

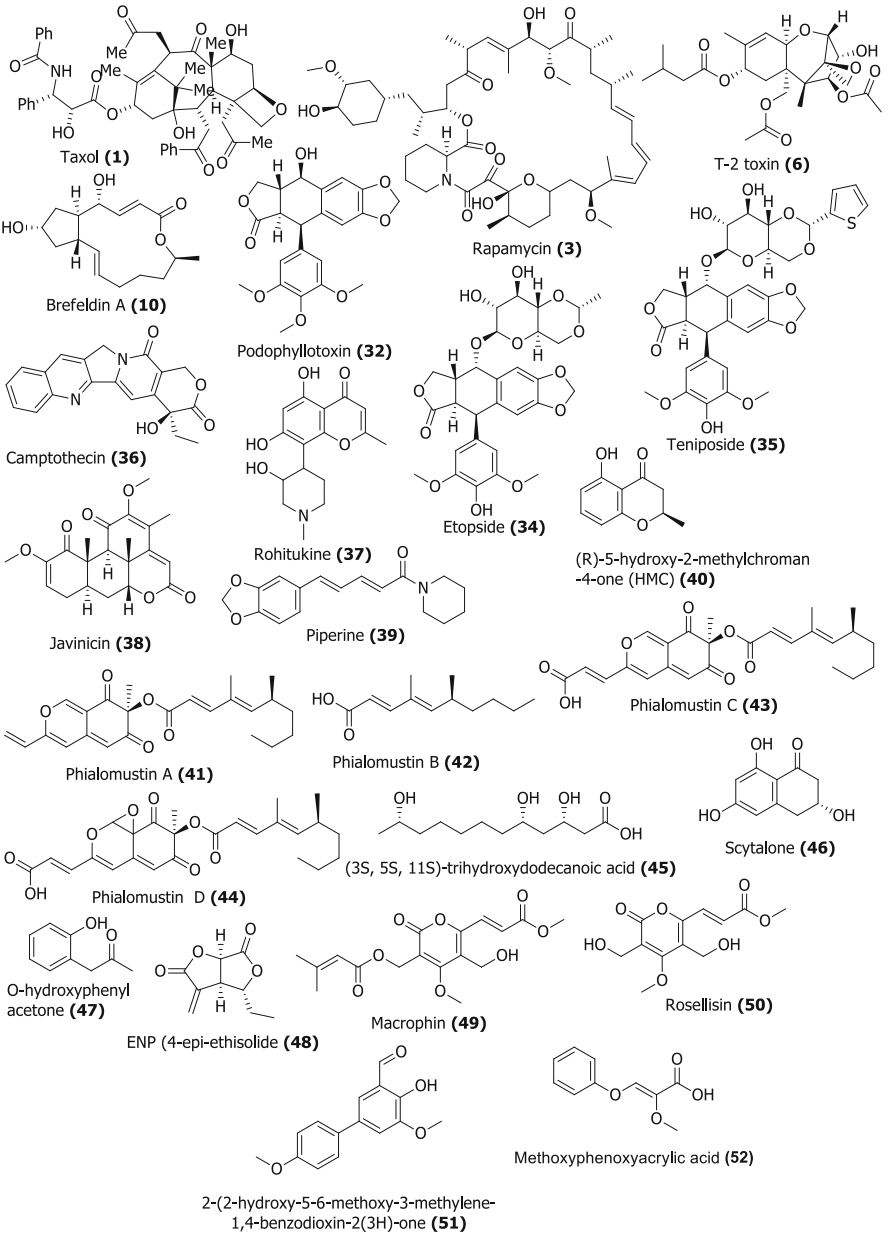

Fig. 3.3 Chemical structure of ENPs synthesized by various endophytes with anticancer potential

phialomustin B (**42**), which is an unusual unsaturated fatty acid having two double bonds at C-3, C-5 and two methyls at C-4, C-6, exhibited potent cytotoxic potential in T47D cell lines (IC_{50} 1 mM), while phialomustin A (**41**), C (**43**), and D (**44**)

showed promising cytotoxicity in T47D (IC_{50} 7–10 mM). Three bioactive molecules (3S, 5S, 11S)-trihydroxydodecanoic acid (**45**), scytalone (**46**), and o-hydroxyphenyl acetone (**47**) were isolated from *Cladosporium tenuissimum*, an endophyte of *Pinus wallichiana* (Naseer et al. 2017). (3S, 5S, 11S)-trihydroxydodecanoic acid (**45**) showed significant cytotoxic activity against the human breast cancer cell line (MCF-7) with an IC_{50} of 15 μg/mL. A similar study was conducted in which three new natural products were derived from an endophytic fungus *Cryptosporiopsis* sp. (Kumar et al. 2017). These ENPs were evaluated for cytotoxic activity against six cancer cell lines, in which one ENP (4-epi-ethisolide (**48**) showed moderate activity with IC_{50} values 11 μM against human myeloid leukaemia cell line HL-60. In another study four known metabolites, macrophin (**49**), rosellisin (**50**), 2-(2-hydroxy-5-6-methoxy-3-methylene-1,4-benzodioxin-2(3H)-one (**51**), and methoxyphenoxyacrylic acid (**52**), were isolated from *Phoma macrostoma*, a fungal endophyte associated with *Glycyrrhiza glabra* (Nalli et al. 2019). Of these compounds, macrophin (**49**) showed prominent cytotoxic activity against the MDA-MB-231, T47D, MCF-7, and MIAPaCa-2 cancer-cell lines with IC_{50} values of 14.8, 8.12, 13.0, and 0.9 μM, respectively. Further, the developed acylated analogs of macrophin (**49**) were found more potent than the parent molecule against MDMB-231 and MIAPaCa-2 cancer cell lines. Figure 3.3 depicts the structures of various compounds, isolated from endophytes, with anticancer potential.

3 ENPs with Antimicrobial Potential

Endophytic fungi are considered favourable natural bioactive compound reservoirs. The discovery of new bioactive secondary metabolites from endophytic microorganisms is vital to overcome the growing levels of drug resistance in many pathogenic fungi. Aggressive fungal infections are considered the main cause of illness and mortality in immunocompromised people (Gandji et al. 2018). The structure of some endophyte-based compounds with potential antimicrobial activity has been presented in Fig. 3.4. The cytochalasin alkaloids are a class of structurally related fungal metabolic products characterized by a highly substituted perhydroisoindolone moiety which is fused with a 9- to 15-membered macrocyclic ring (Wei et al. 2017). Till date, more than 300 cytochalasin analogs have been isolated from many genera of basidiomycetes and ascomycetes, including *Chaetomium, Aspergillus, Penicillium, Spicaria, Xylaria, Phomopsis,* and *Phoma*. Many cytochalasins display a variety of biological functions, including cytotoxicity, antibacterial activity, and phytotoxicity (Xue et al. 2012). There are reports of 14 cytochalasins with moderate to strong antifungal activity that were isolated from fungal endophytes. Endophytic fungi of *Xylaria* sp. isolated from the leaves of the Guarana plant are reported to produce cytochalasin D (**53**).

Cytochalasin D (**53**) showed fungistatic efficacy with a MIC of 2.46 mM against the phytopathogen *Colletotrichum gloeosporioides* that causes anthracnose disease (Elias et al. 2018). Active cytochalasin alkaloids isolated from *Xylaria* sp., an

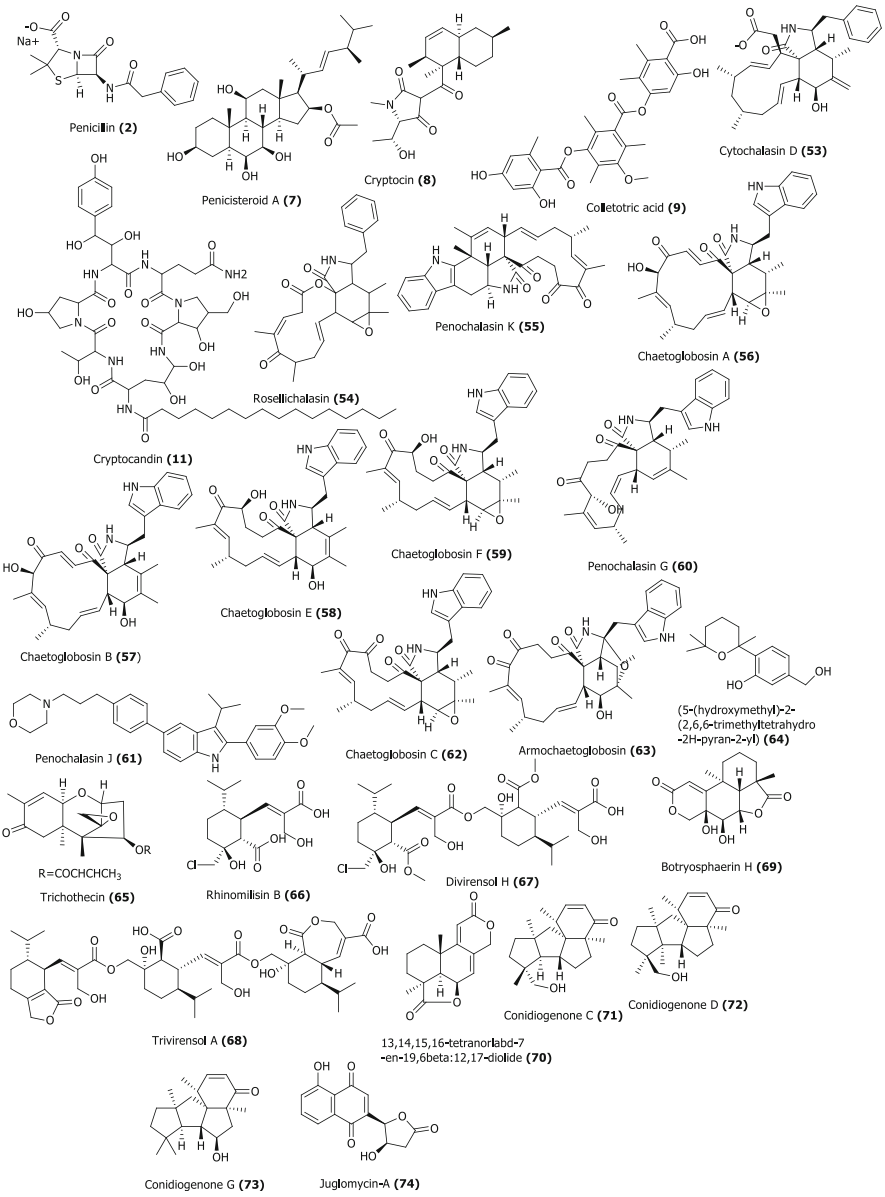

Fig. 3.4 Chemical structure of ENPs synthesized by various endophytes with antimicrobial potential

endophytic fungus of *Toona sinensis*, showed potent fungicidal activity at MIC 12.5 μM against the phytopathogen *Gibberella saubinetti* (Zhang et al. 2014). Similarly, the antifungal metabolite rosellichalasin (**54**) was isolated from *Aspergillus capensis*, and was found to have good biocontrol potential in *Brassica napus*

(Qin et al. 2019). Penochalasin K (**55**) an unusual chaetoglobosin having a rare six-cyclic 6/5/6/5/6/13 fused ring system isolated from the solid culture of the mangrove endophytic fungus *Penicillium chrysogenum* demonstrated robust selective activity against *C. gloeosporioides* and *Rhizoctonia solani* (Zhu et al. 2017).

Chaetoglobosins A (**56**), B (**57**), E (**58**), F (**59**), and penochalasin G (**60**) were obtained from *Chaetomium globosum*, an endophytic fungus isolated from the seeds of *Panax notoginseng*. Some of the isolated chaetoglobosins showed promising inhibitory activity against root rot causing pathogenic fungi. For instance, chaetoglobosin E (**58**) and penochalasin G (**60**) were found to possess potent activity against *Epicoccum nigrum* with the MICs <2 μM (Li et al. 2016). Similarly, penochalasin J (**61**), a new chaetoglobosin along with two known chaetoglobosins, chaetoglobosin C (**62**) and armochaetoglobosin I (**63**), were isolated from the mangrove endophytic fungus *P. chrysogenum*. All three chaetoglobosin were found to have potent antifungal activity (Huang et al. 2016).

Terpenoids produced by fungi are the most prevalent and structurally diverse secondary metabolites with a wide range of pharmacological activities. *Lophodermium* sp., an endophyte isolated from the needles of *Pinus strobes,* was found to produce sesquiterpene (5-(hydroxymethyl)-2-(2′,6′,6′-trimethyltetrahydro-2H-pyran-2-yl)) (**64**), a natural antifungal agent against the rust fungus *Microbotryum violaceum* (Sumarah et al. 2011). Similarly, *Trichothecium roseum* LZ93, an endophyte of *Maytenus hookeri*, is reported to have antimycotic potential against various phytopathogens. Trichothecin (**65**), an ENP isolated from this fungus, exhibits weak to moderate inhibitory activity against the phytopathogenic fungi *Gaeumannomyces graminis*, *Phytophthora infestans*, *Typhula incarnate*, *A. solani*, etc. (Zhang et al. 2010). Rhinomilisin B (monosesquiterpene) (**66**) and divirensol H (**67**) (sesquiterpene), and trivirensol A (**68**) an unprecedented trimeric sesquiterpene, were purified from *T. virens* FY06 and were found to demonstrate potent antifungal activities (Hu et al. 2019). Two tetranor labdane diterpenoids, botryosphaerin H (**69**) and 13,14,15,16- tetranorlabd-7-en-19,6β:12,17-diolide (**70**), isolated from *Botryosphaeria* sp. P483, an endophyte of *Huperzia serrate, showed* strong antifungal activity against *G. graminis*, *F. moniliforme*, *F. solani*, *F. oxysporum*, and *Pyricularia oryzae* (Chen et al. 2015). Similarly, three diterpenes conidiogenones C (**71**), D (**72**), and G (**73**) isolated from *Leptosphaeria* sp., an endophyte of *Panax notoginseng*, exhibited antifungal activity against *Rhizoctonia cerealis* and *Verticillium dahliae* (Chen et al. 2019). *Aspergillus fumigatus* isolated from *Melia azedarach* was found to produce 39 metabolites, out of them 16 compounds showed broad-spectrum antifungal activities against various phytopathogenic fungi (Li et al. 2012).

Two thiodiketopiperazine derivatives obtained from *Phoma* sp., a fungal endophyte associated with *Glycyrrhiza glabra*, showed significant antimicrobial activity (Arora et al. 2016). Both compounds are reported to inhibit the growth of *Staphylococcus aureus* and *Streptococcus pyogenes*, with IC_{50} values of less than 10 μM.

The compounds were found to act synergistically with streptomycin while producing varying effects in combination with ciprofloxacin and ampicillin. The compounds inhibited bacterial transcription/translation in vitro, and also inhibited

staphyloxanthin production in *S. aureus*. Another study reported the antimicrobial potential of juglomycin A (**74**) isolated from a bacterial endophyte *Streptomyces achromogenes* (Ahmad et al. 2020). They also reported juglomycin A (**74**) reduces biofilm formation in *E. coli* through the downregulation of *fimH* gene and alpha-haemolysin-related gene (hlyA), thereby resulting in the reduction of virulence in *E. coli*. Additionally, juglomycin A (**74**) caused post-antibiotic effect in *E. coli* while inhibiting bacterial transcription and translation under in vitro settings (Ahmad et al. 2020).

4 Molecular Crosstalk Underlying ENPs Production

As the focus on exploring the plant metabolites from the endophytic microbial partner increases, the molecular mechanisms underlying multispecies crosstalk gained much impetus. These multispecies crosstalks may be at the chemical level or physiological level or cellular level or gene level. The most studied mechanism is the theory of horizontal gene transfer (HGT), which suggests the transfer of the gene clusters, responsible for the biosynthesis of secondary metabolites in the host plant, between the plant and its endophytes (Strobel and Daisy 2003). The hypothesis of xenohormesis also states that plants and associated microbes share homologous gene clusters capable of producing secondary metabolites under stress conditions. These homologous gene clusters are horizontally transferred between the host plant and microbes which are cross activated by plant hosts or endophytes under some emergencies. Interestingly, recent reports also suggest the existence of independent biosynthetic pathways in endophytic microorganisms (Staniek et al. 2008). However, it also seems logical that a microorganism producing similar metabolites to those of the host plant may have more chances of thriving in the host tissues because of its resistance to the key metabolites. Since taxol (**1**) is a fungicide in nature, therefore it was interesting to observe that yew trees producing taxol harbour fungi that also produce taxol. This mystery was solved by Soliman and co-workers (2015), they elucidated the role of a taxol producing endophytic fungus in the defence mechanism of the host plant. The endophytic fungus, *Paraconiothyrium* sp. sequesters taxol in intracellular hydrophobic bodies (HBs) and this fungicide containing HBs are released by the fungus upon sensing pathogens at the pathogen entry points. The fungicide laced HBs coalesce to form remarkable extracellular barriers at the pathogen entry points (Soliman et al. 2015). Thus, it makes a novel plant defence mechanism against the phytopathogens. Also, the taxol producing endophytic fungus, *T. andreanae* has a *taxadiene synthase* (txs) gene different than the txs gene of the host plant. This indicates that microorganisms possess independent pathways for the production of such high-value natural products. Since then, Taxol (**1**) has been detected in many endophytic strains isolated from different plant species (Mousa and Raizada 2013; Chen et al. 2015).

Undoubtedly, endophytic microorganisms are metabolically more proficient than their free-living counterparts and they have the potential to produce an exceedingly

high number of secondary metabolites, related or unrelated to the host plant. The endophytic microorganisms residing inside the plant tissues must have undergone rigorous screening before establishment and successful colonization, during the process their genetic as well as metabolic machinery evolved to survive in the host tissue, thus activating the production of molecules that help the microorganism to evade host plant defence mechanisms. Secondly, to establish a balanced antagonistic relationship the endophyte produces several molecules/phytotoxins in the host plant (Strobel and Daisy 2003; Strobel 2006). Recently, it is proposed that the epigenetic modifications in the endophytes due to host plant metabolites may result in turning on some of its otherwise "silent biosynthetic pathways" (Riyaz-Ul-Hassan et al. 2012; Keller 2019).

The whole genome sequencing of microorganisms opened a new area in the field of natural product research and drug discovery programmes (Sagita et al. 2021). Large-scale microbial genome sequencing started from the whole-genome shotgun sequencing of *Haemophilus influenza*, which demonstrated that microbial genome sequences can be obtained with unimagined ease and rapidity (Fleischmann et al. 1995). Our understanding of the genetics and enzymology of microbial natural product biosynthesis has also led to the identification and analysis of genes/clusters involved in the biosynthetic pathways in sequenced microbial genomes (Fischbach and Walsh 2006). Bentley and colleagues reported that the gene clusters coding for natural products in *Streptomyces coelicolor* are more than the natural products of the microorganism known so far (Bentley et al. 2002). Similar observations have been reported in several diverse sequenced micro-organisms like *S. avermitilis* (Ikeda et al. 2003), *Pseudomonas fluorescens* (Paulsen et al. 2005), *Aspergillus* (Bok et al. 2006), *Saccharopolyspora erythraea* (Oliynyk et al. 2007), and *Salinispora tropica* (Udwary et al. 2007). Over the past few years, genome mining of microorganisms for the discovery of new natural products and/or biosynthetic pathways has rapidly advanced as a new field in endophyte biology (Corre and Challis 2009; Sagita et al. 2021; Wani et al. 2022).

The above findings strongly support the one-strain-many-compounds (OSMAC) approach, according to which the metabolite profile of microorganisms can be altered by varying the growth conditions under standard culture conditions (Bode et al. 2002; Pan et al. 2019). Therefore, it can be suggested that many potentially useful natural products of microbial origin await discovery. The endophytes thus represent a hidden bio-resource, mostly unexplored, keeping in view the plant biodiversity of the world and the fact that each plant investigated is found to harbour endophytic microorganisms.

5 Conclusion

Endophytic microbes hold the promise of obtaining a huge repertoire of bioactive natural products which can be used as an effective and feasible alternative source of phytochemicals and pharmaceuticals. The ENPs have a great diversity in chemical

structures and biological activities, opening an exciting area of research to understand the production, utilization, and commercialization of the bioactive metabolites of endophytes. It is important to unravel the molecular crosstalk and the information processing networks involved in the biosynthetic machinery of these important bio-molecules and based on the information examine the possible manipulation of this machinery to produce unnatural bioactive molecules from the endophytic microbes. With the advent of gene sequencing technologies and biosynthetic engineering or activation of the cryptic gene clusters through epigenetic manipulation, gene inactivation, regulation of global regulators, and pathway-specific transcription factors (TFs), new bioactive molecules can be developed with improved activities. Elucidation of the molecular mechanisms behind the complex regulatory network will not only provide a deeper insight into how microbes translate environmental signals into secondary metabolite biosynthesis but will also warrant the identification of novel secondary metabolites and a deeper understanding of their ecological role.

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Part II
Endolichenic Fungi: a Source of New Chemical Entities

Chapter 4
Endolichenic Fungi as a Source of Pharmaceutically Active Compounds

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Abstract Around the world, fungi are found in a wide range of biological and environmental niches, where they serve as saprobes, symbionts, and pathogens. Among them, there are certain types of fungi that are capable of residing within the internal tissues of other organisms asymptomatically, without showing any outward signs of infection. One such example of them is the endolichenic fungi, which are a large and diverse group of fungi that live within the thalli of lichens. Similar to endophytic fungi, which live quiescently inside plant tissues, endolichenic fungi are also known to produce a diverse array of secondary metabolites with various bioactivities. A considerable number of these compounds are novel, with unique structures, or those that have been isolated from natural sources for the first time. Furthermore, there has been a renewed interest among the scientific community in the search for novel, natural product-based therapeutics, due to the threat of various emerging and re-emerging human diseases. It is against this background that the present chapter provides an overview of 108 different compounds that have been isolated from endolichenic fungi that possess one or more properties out of cytotoxic, antifungal, antioxidant, antibacterial, antiviral and anti-inflammatory activities, with an emphasis on novel compounds.

Keywords Endolichenic fungi · Bioactive compounds · Secondary metabolites · Antimicrobial activity · Cytotoxic activity

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1 Introduction

Natural products, such as secondary metabolites from plants, fungi and animals have been used for the treatment of human diseases since ancient times (Atanasov et al. 2021). Their distinctive advantages over synthetic compounds such as large chemical and structural diversity, higher molecular weights, steric complexity, and advanced binding capabilities allow them to remain important sources of novel therapeutics to this day. However, many pharmaceutical companies scaled down their work on natural products due to mainly, the time-consuming nature of the isolation of natural products and their structural elucidation. With the advent of new technologies such as high throughput screening and automated separation techniques, new methods to improve and accelerate the drug discovery process are being rapidly developed. Also, alternative drug discovery processes have not been quite successful in generating as many drug leads as was desired. Within this context, there has been a renewed interest in natural product research, especially in the search for novel compounds with antimicrobial, anticancer, immunosuppressive, and anti-Alzheimer properties. This was reflected, at first, by the enthusiasm within the scientific community to investigate novel sources of natural products, such as endophytic fungi for their bioactive metabolites, but more recently, attention has been diverted to endolichenic fungi as well.

As is commonly known, lichens are a symbiotic association between fungi (mycobiont) and one or more photobiont partners, such as algae or cyanobacteria (Henskens et al. 2012). These organisms are often slow-growers, but are very drought-tolerant and can flourish in a variety of substrates and harsh ecological conditions. These distinct features of lichens have been found to provide other microorganisms with a unique and long-lasting biological niche (Purvis and Hector 2000). In addition to lichenized fungi, another class of non-obligate micro-fungi known as endolichenic fungi also live inside the internal tissues of lichen thalli without harming them. Within the lichen thalli, it has been estimated that around 13,500 different groups of filamentous fungi species exist asymptomatically (Kellogg and Raja 2017). Most of these endolichenic fungal species are found in the subphylum Pezizomycotina which is the most abundant, as well as the most ecologically and morphologically diverse subphylum among the ascomycetes (Suryanarayanan and Thirunavukkarasu 2017; Schoch et al. 2009).

2 Pharmaceutically Active Compounds Isolated from Endolichenic Fungi

Endolichenic fungi are a rich source of novel bioactive compounds with the potential to be developed as therapeutic candidates. The secondary metabolites produced by these organisms include various classes of substances like steroids, quinones, terpenoids, peptides, xanthones, and sulfur-containing chromenones, which exhibit a

range of biological activities including anticancer, antiviral, antibacterial, antifungal, and anti-Alzheimer's activities (Suryanarayanan and Thirunavukkarasu 2017). Therefore, this chapter presents an overview of the cytotoxic, antimicrobial, antioxidant, and anti-inflammatory compounds that have been derived from endolichenic fungi up until 2022.

2.1 Cytotoxic Compounds Isolated from Endolichenic Fungi

Cancer is the largest cause of death worldwide, accounting for almost 10 million fatalities in 2020. Among the various types of cancers breast, lung, colon, and rectal cancers are the most prevalent. Cancer arises as a result of the transformation of normal cells into tumor cells in a multi-stage process that normally goes from a pre-cancerous lesion to a malignant tumor (WHO 2022). Hence, scientists across the globe are in pursuit of developing novel therapeutic agents to combat cancer. Natural products play a key role in this pursuit as a large number of drugs that were approved as anticancer drugs were of natural origin (Kharwar et al. 2011; Cragg et al. 1997). Widely used anticancer drugs such as paclitaxel, camptothecin, and vincristine are a few examples of such drugs that have been isolated and developed from endophytic fungi (Stierle et al. 1993; Puri et al. 2005; Lingqi et al. 2000). However, endolichenic fungi have not yet been widely explored for bioactive compounds. Therefore, this section provides an in-detail summary of novel cytotoxic compounds isolated from endolichenic fungi.

A cytotoxic compound is a chemical species that is capable of destroying cancer cells by inhibiting their growth and development (Veeresham 2012). Table 4.1 provides details of 47 novel compounds with cytotoxic activity, their corresponding IC_{50} values, the endolichenic fungi from which they were isolated, host lichen species of the endolichenic fungi, cell lines, and positive controls used.

A number of cytotoxic compounds isolated from endolichenic fungi were reported to comprise of novel and unique chemical structures. Phaeosphaerins A–F (**1–6**) isolated from the endolichenic fungus *Phaeosphaeria* sp. collected from the lichen *Heterodermia obscurata* belong to a group of sparsely found naturally occurring perylenequinonoid pigments (Zhou and Liu 2010; Li et al. 2012). These perylenequinonoid pigments are capable of absorbing light and then transforming themselves into an electronically excited triplet state that can react with oxygen to generate highly cytotoxic reactive oxygen species (Daub et al. 1992; Daub and Ehrenshaft 2000; Daub et al. 2005). The study by Li et al. reports that these species can kill cancer cells in vitro with accumulation in lysosomes. Furthermore, it was reported that the cytotoxic effects are potentially enhanced when exposed to light (Li et al. 2012). These compounds exhibited highly potent cytotoxic activity against human prostate cancer PC3, DU145, and LNCaP cell lines in comparison with the positive control Adriamycin (Li et al. 2012). The chemical structures of novel compounds with cytotoxic activity isolated from endolichenic fungi are depicted below (Fig. 4.1).

Table 4.1 Novel compounds derived from endolichenic fungi with cytotoxic activity

S. no.	Fungal species	Host	Compound	Cell line (IC_{50} in μM)	Reference
1	*Phaeosphaeria* sp.	*Heterodermia obscurata*	Phaeosphaerin A (**1**)	PC (5.84); DU145(10.77); LNCaP (10.76)	Li et al. (2012)
2			Phaeosphaerin B (**2**)	PC (2.42); DU145(9.54); LNCaP (2.67)	
3			Phaeosphaerin C (**3**)	PC (6.11); DU145(15.21); LNCaP (14.35)	
4			Phaeosphaerin D (**4**)	PC (9.74); DU145(20.19); LNCaP (10.11)	
5			Phaeosphaerin E (**5**)	PC (5.25); DU145(16.25); LNCaP (10.11)	
6			Phaeosphaerin F (**6**)	PC (5.45); DU145(16.66); LNCaP (11.84)	
	Positive control		Adriamycin	PC (5.84); DU145(10.77); LNCaP (2.67)	
7	*Ulocladium* sp.	*Everniastrum* sp.	Ophiobolin P (**7**)	KB (6.18); HepG2(1.48)	Wang et al. (2013)
8			Ophiobolin Q (**8**)	KB (2.38); HepG2(1.32)	
9			Ophiobolin R (**9**)	KB (2.91); HePG2(1.18)	
10			Ophiobolin S (**10**)	KB (4.91); HepG2(1.40)	
11			Ophiobolin T (**11**)	KB (3.01); HepG2 (0.24)	
	Positive control		Etoposide	KB (4.7); HepG2 (1.93)	
12	*Ulospora bilgramii*	*Umbilicaria* sp.	Ulosporin G (**12 a and b**)	A549(1.3); MCF-7 (1.3); KB (2.2)	Xie et al. (2020)
13			Ulosporin A (**13 a and b**)	A549(28.2); MCF-7 (11.6); KB (16.6)	
	Positive control		Adriamycin	A549 (5.0); MCF-7(2.5); KB (2.2)	
14	*Floricola striata*	*Pseudosyphellaria* sp.	Floricolin K (**14**)	A2780 (25.1); MCF-7(13.4)	Xu et al. (2018)
15			Floricolin L (**15**)	A2780 (21.4); MCF-7(17.9)	
16			Floricolin M (**16**)	A2780 (40.1); MCF-7(17.5)	
17			Floricolin N (**17**)	A2780 (14.9); MCF-7 (11.7); A549 (27.8)	

18			Floricolin O (**18**)	A2780 (3.4); MCF-7(19.9); A549 (40.1)	
19			Floricolin P (**19**)	A2780 (8.6); MCF-7(16.7)	
20			Floricolin T (**20**)	MCF-7(38.5); A549 (12.5)	
	Positive control		Adriamycin	A2780 (0.5); MCF-7(1.3); A549 (1.3)	
21	*Aspergillus unguis*	*Xanthoria* sp.	Asperunguisin C (**21**)	HT-29 (24.2); A549 (6.2); U251(12.6); U87(16.4); SMMC-7721(15.2); HepG2 (20.1)	Li et al. (2019)
22			Asperunguisin A (**22**)	A549 (16.3); HepG2(8.2)	
23			Asperunguisin B (**23**)	SMMC-7721(25.4)	
24			Asperunguisin E (**24**)	HT-29 (24.2)	
	Positive control		Adriamycin	HT-29 (3.8); A549 (2.9); U251(2.9); U87 (1.1); SMMC-7721(0.8); HepG2 (1.9)	
25	*Myrothecium innundatum*	*Ramalina* sp.	Myrotheol A (**25**)	K562 (28.9); RKO (51)	Basnet et al. (2019)
26			Myrotheol B (**26**)	K562 (63.9); RKO (62.3)	
27			Myrotheside C (**27**)	K562 (28.6)	
28			Myrotheside D (**28**)	K562 (32.5); RKO (68.8)	
	Positive control		Taxol	K562 (0.5); RKO (0.1)	
29	*Xylaria psidii*	*Amandiena medusulina*	(Z)-3-{(3-acetyl-2-hydroxyphenyl) diazenyl}-2,4-dihydroxybenzaldehyde (**29**)	NCI-H292 (27.2)	Santhirasegaram et al. (2020)
	Positive control		Paclitaxel	NCI-H292 (12.6)	
30	*Coniochaeta* sp.	*Xanthuria mandschurica*	Conioxepinol A (**30**)	HeLa (103.8)	Wang et al. (2010b)
31			Conioxepinol B (**31**)	HeLa (36.2)	
32			Conioxepinol C (**32**)	A549 (83.6); MDA-MB-231 (112.4)	
33			Conioxepinol D (**33**)	A549 (40.9); MDA-MB-231 (41.4)	
	Positive control		5- Fluorouracil	HeLa (10.0); A549 (4.17); MDA-MB-231 (4.45)	
34	*Preussia africana*	*Ramalina calicaris*	Preussochromone A (**34**)	A549(8.34); HeLa (25.52); HCT116 (25.87)	Zhang et al. (2012)
35			Preussochromone C (**35**)		

(continued)

Table 4.1 (continued)

S. no.	Fungal species	Host	Compound	Cell line (IC_{50} in μM)	Reference
				A549(5.75); MCF-7 (41.17); HeLa (29.96); HCT116 (15.1)	
	Positive control		Cisplatin	A549(5.51); MCF-7 (11.62); HeLa (9.3); HCT116 (11.3)	
36	*Aspergillus versicolor*	*Lobaria quercizans*	Diorcinol G (**36**)	PC3(24.0); A549(23.9); A2780(31.0); MDA-MB-231(19.0); HePG2(24.3)	Li et al. (2015)
37			Diorcinol H (**37**)	MDA-MB-231(43.2)	
	Positive control		Cisplatin	PC3 (18.0); A549(19.9); A2780(14.9); MDA-MB-231 (18.0)	
38	*Aspergillus* sp.	*Cetrelia* sp.	Isocoumarindole A (**38**)	PaCa-2(1.63); AsPC-1 (5.53)	Chen et al. (2019)
	Positive control		Gemcitabine	PaCa-2(1.02); AsPC-1 (20.1)	
39	*Neurospora terricola*	*Everniastrum cirrhatum*	Terricollene A (**39**)	HeLa (53.3); MCF-7 (59.2)	Zhang et al. (2009)
	Positive control		5-Fluorouracil	HeLa (10.0); MCF-7 (15.0)	
40	*Chaetomium elatum*	*Everniastrum cirrhatum*	Xanthoquinodin A4 (**40**)	SMMC-7721 (19.18); A549 (25.47); MCF-7 (31.17); SW480 (18.85)	Chen et al. (2013)
41			Xanthoquinodin A5(**41**)	HL-60 (26.62); SMMC-7721 (16.81); A549 (18.6); MCF-7 (23.96); SW480 (16.19)	
42			Xanthoquinodin A6 (**42**)	HL-60 (3.75); SMMC-7721 (2.87); A549 (2.04); MCF-7 (5.64); SW480 (6.44)	
43			Xanthoquinodin B4 (**43**)	HL-60 (3.01); SMMC-7721 (27.7); A549 (25.13); MCF-7 (30.03)	
44			Xanthoquinodin B5 (**44**)	HL-60 (4.74); SMMC-7721 (14.99); A549 (11.38); MCF-7 (30.03)	
	Positive control		Cisplatin	HL-60 (1.86); SMMC-7721 (6.13); A549 (7.27); MCF-7 (15.27); SW480 (16.23)	

45	*Phanerochaete sordida*	*Bactrospora myraidea*	12,14-dihydroxy-3-methyl-3,4,5,6,7,8, 9, 10-octohydro-1H-benzo[−c] [1] oxacyclododecin-1-one (**45**)	CAL-27 (13.65)	Weerasinghe et al. (2022)
	Positive control		Doxorubicin	CAL-27(01.20)	
46	Sp. ELF000039	*Parmotrema* *Austrosinense*	(3R)-5-hydroxymellein (**46**)	B16F1(92.2); B16F10(99.6); HaCaT (96.5)	Zhao et al. (2016)
	Positive control		–	–	
47	*Talaromyces* sp.	*Xanthoparmelia angustiphylla*	Talaromycin A (**47**)	THLE (46.6); MDA-MB-231(24.6)	Yuan et al. (2018)
	Positive control		–	–	

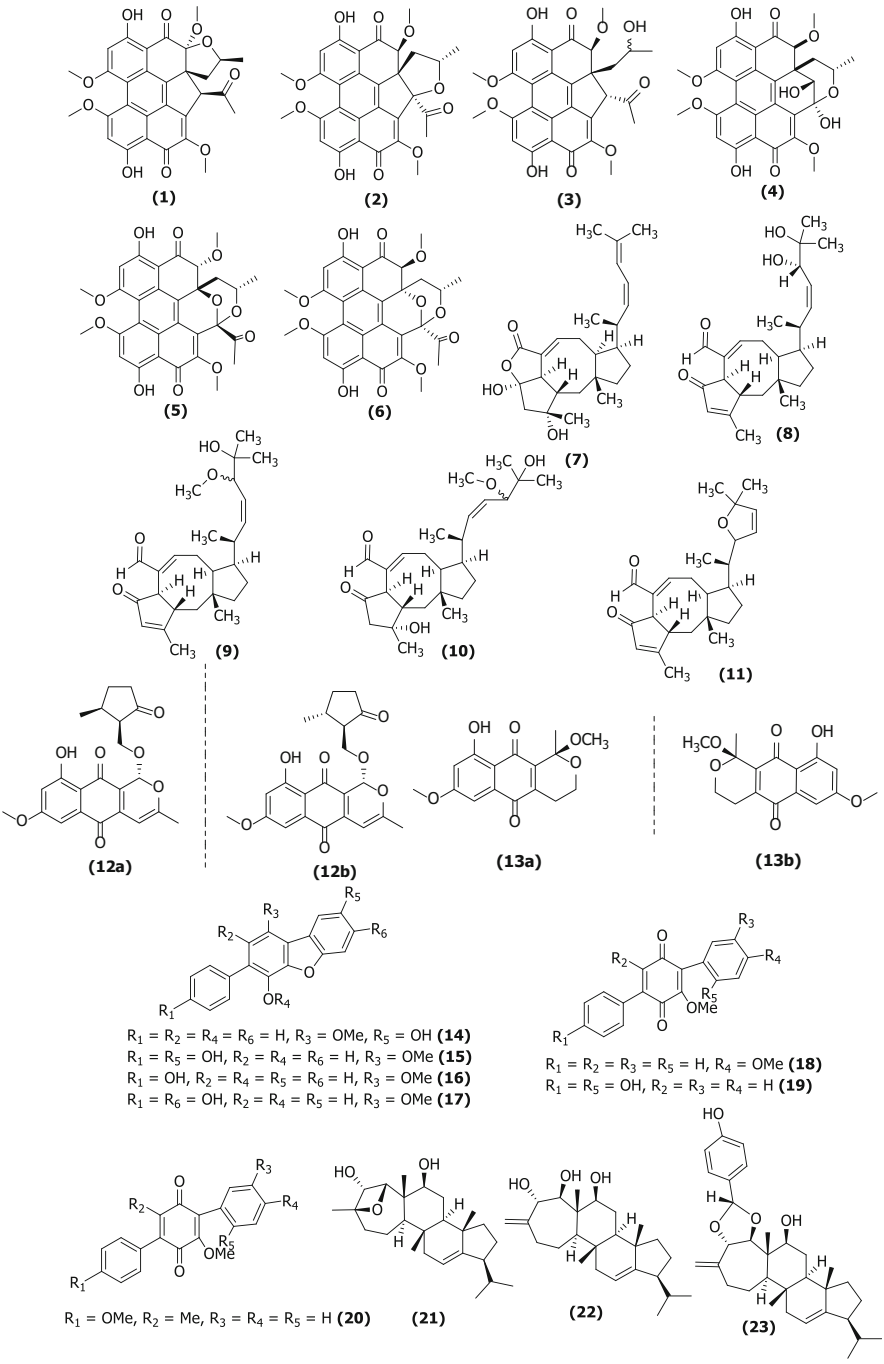

Fig. 4.1 Structures of novel compounds derived from endolichenic fungi with cytotoxic activity

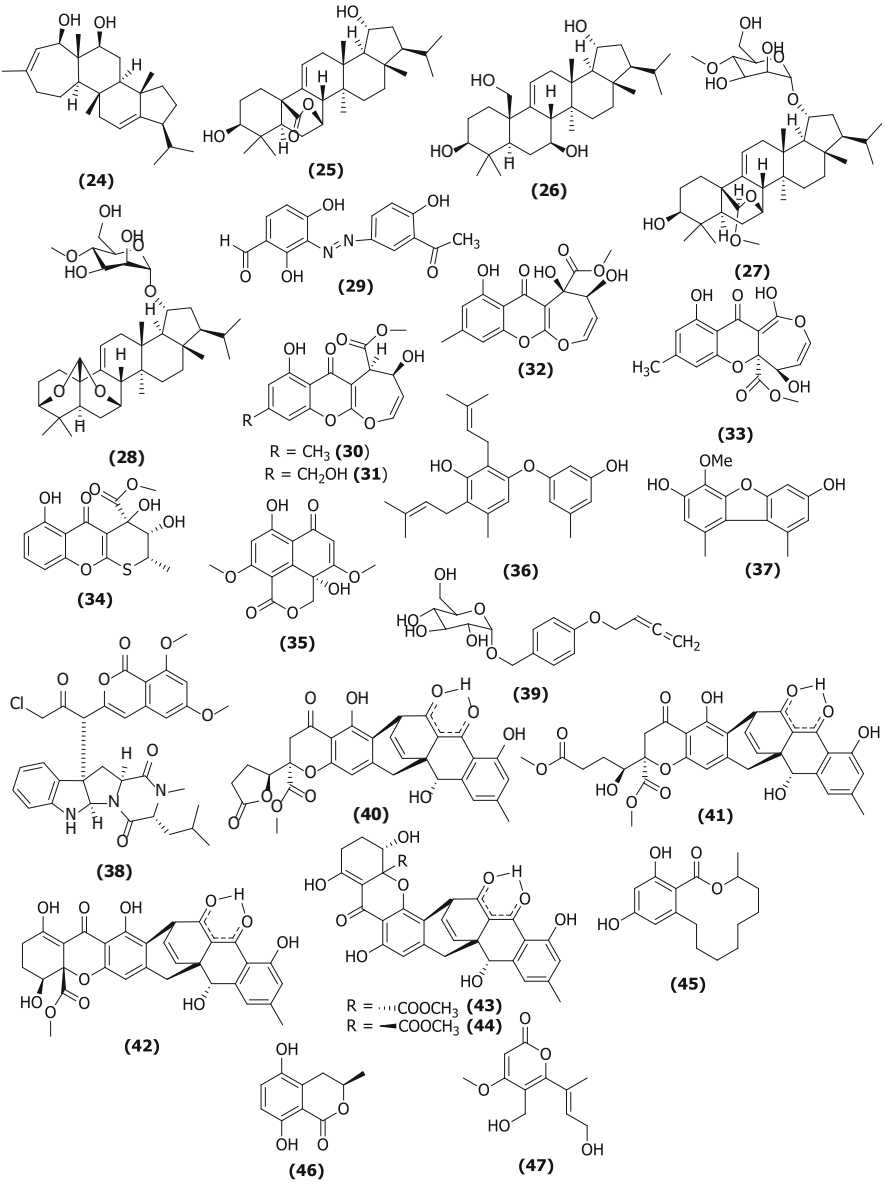

Fig. 4.1 (continued)

Ophiobolins P–T (**7–11**) belong to a group of sesterterpenes that are characterized by an unusual tri-cyclic (5-8-5) ring system. They are a group of bioactive compounds that occur naturally and possess significantly strong cytotoxic activity against HepG2 human hepatoma cells with activity greater than the positive control, etoposide (Wang et al. 2013). A study that evaluated the cytotoxic compounds

produced by *Ulospora bilgramii* obtained from the lichen species *Umbilicaria* sp. isolated the new heptaketides ulosporin G (**12 a and b**) and ulosporin A (**13 a and b**) that possessed cytotoxic activity against A549 human lung carcinoma cells, MCF-7 breast carcinoma cells, and KB human oral epidermoid carcinoma cells. The study noted that the central quinone moiety and the substituents at C-1 may play a significant role in improving the cytotoxicity of the two compounds (Xie et al. 2020).

In another study carried out to identify the cytotoxic compounds present in the endolichenic fungi *Floricola striata* isolated from the lichen *Pseudosyphellaria* spp. led to the discovery of *p*-terphenyls, floricolins K–P (**14–19**), and T (**20**). The isolated compounds showed cytotoxic activity against the human ovarian cancer cell line A2780, human breast carcinoma cell line MCF-7, and human non-small-cell lung cancer A549 cell lines. A notable finding presented in this study was the multidrug resistance (MDR) reversal activity of floricolin O (**18**). It was discovered that floricolin O (**18**) enhanced the sensitivity of MCF-7/ADR cells 39-fold at 10 μM toward adriamycin through modulating P-glycoprotein-mediated drug exclusion (Xu et al. 2018). This is an excellent example of the diverse variety of bioactive chemicals found in endolichenic fungi.

The novel asperane-type sesterterpenoid, asperunguisin C (**21**), isolated from the endolichenic fungus *Aspergillus unguis* showed strong cytotoxic activity against human cancer cell line A549 recording an IC_{50} value of 6.2 μM in comparison to the positive control adriamycin with an IC_{50} of 2.9 μM. Furthermore, the study discovered that the cytotoxic activity was a result of the G0/G1 cell cycle arrest caused by DNA damage followed by cellular apoptosis (Li et al. 2019). Another example of novel naturally occurring scaffolds is the isolation of myrotheside C and D (**27** and **28**) from the endolichenic fungus *Myrothecium inundatum* which is the first example of naturally occurring 4-O-methyl-α-D-mannosides. Myrotheol A and B (**25** and **26**), two new arborinane-type triterpenes, isolated from the same endolichenic fungal species also showed cytotoxic activity (Basnet et al. 2019).

It has been found that conioxepinol A–C (**30–32**), isolated from the *Coniochaeta* fungal species, are closely related in structure to the relatively rare xanthepinone and the microsphaeropsones, an oxepinochromenone class of fungal metabolites consisting of the 4H-oxepino[2,3-b]chromen-6(5H)-one skeleton (Liermann et al. 2009; Wang et al. 2010b). In addition, conioxepinol D (**33**) isolated from the same endolichenic fungus has been found to possess a structure that is closely related to that of brocaenols (Bugni et al. 2003; Wang et al. 2010b). Preussochromone A (**34**), the first example of a thiopyranchromenone that occurs naturally was isolated from the endolichenic fungus, *Preussia africana.* Thiopyranchromenone is a class of compounds that possess the 3,4-dihydrothiopyrano[2,3-b]chromen-5(2H)-one skeleton that was previously only found in synthetic compounds displaying considerable cytotoxicity against the tested cell lines.

Isocoumarindole A (**38**), isolated from the endolichenic fungus *Aspergillus* sp. CPCC 400810, has an unprecedented skeleton containing indole diketopiperazine alkaloid moieties connected by a carbon−carbon bond and a chlorinated isocoumarin. In addition, it has been described as a novel polyketide synthetase−non-ribosomal peptide synthetase (PKS−NRPS) hybrid metabolite. It

displayed strong cytotoxic activity with an IC_{50} value of 5.53 μM against the human pancreatic adenocarcinoma cell line AsPC-1 in comparison to the positive control gemcitabine that recorded an IC_{50} value of 20.10 μM (Chen et al. 2019).

2.2 *Antifungal Compounds Isolated from Endolichenic Fungi*

It has been necessary to find antifungal drugs with improved potency and better compatibility due to the new and re-emerging forms of fungal infections, particularly those that occur during allogeneic bone marrow transplants, organ transplants, cancer therapy, and in immunocompromised individuals (Lockhart and Guarner 2019). The many types of topical and systemic fungal infections are combated by a fairly small number of antifungal substances (Agrawal et al. 2020). While resistance to the currently available antifungal medicine appears to be increasing, particularly in patients receiving long-term treatment, the introduction of new antifungals to the market remains limited (Agrawal et al. 2020; Fuentefria et al. 2018). Despite natural microbial products having an immense potential for the extraction and screening of novel metabolites for pharmaceutical purposes, they have generally been underutilized as an alternate source of antifungal compounds (Fuentefria et al. 2018). Due to this reason, there have been several studies which focused on the possibility of isolating compounds with antifungal properties from endolichenic fungi (Table 4.2; Fig. 4.2).

2.3 *Antioxidant Compounds Isolated from Endolichenic Fungi*

Biological systems generate superoxide radicals, hydrogen peroxide, and hydroxyl radicals as metabolic byproducts, which are commonly known as reactive oxygen species (ROS) (Pizzino et al. 2017; Navarro-Yepes et al. 2014; Sato et al. 2013). These ROS must be maintained at an optimum concentration in cells to prevent them from causing harmful effects. The imbalance between the amount of ROS that are produced and accumulate in cells and tissues and the body's capacity to detoxify them results in oxidative stress (Pizzino et al. 2017). A vast amount of evidence suggests that oxidative stress plays a role in the onset and/or progression of multiple diseases, including cancer, cardiovascular disease, diabetes, atherosclerosis, and a number of other metabolic disorders (Taniyama and Griendling 2003). An antioxidant is defined as a chemical that considerably slows down or stops the oxidation of a substrate when present in low quantities compared to oxidizable substrate (Halliwell 1995). It has been discovered that natural antioxidants are particularly effective at halting the damage brought on by oxidative stress.

Table 4.2 Novel compounds derived from endolichenic fungi with antifungal activity

S. no.	Endolichenic fungus	Host	Compound	Test fungus and MIC (μg/mL)	Positive control and MIC (μg/mL)	Ref. no.
01	*Pestalotiopsis* sp.	*Cetraria islandica*	Ambuic acid derivative 1 (**48**)	*V. dahlia* [64] and *F. oxysporum* [8]	Ketoconazole | *V. dahlia* [1] and F. oxysporum [1]	Yuan et al. (2017)
			Ambuic acid derivative 2 (**49**)	*V. dahlia* [64] and *F. oxysporum* [32]	Ketoconazole | *V. dahlia* [1] and *F. oxysporum* [8]	
			Ambuic acid derivative 3 (**50**)	*V. dahlia* [64], *F. oxysporum* [64], *R. solani* [64]	Ketoconazole | *V. dahlia* [1], *F. oxysporum* [8], *R. solani* [8]	
			Ambuic acid derivative 4 (**51**)	*V. dahlia* [16], *F. oxysporum* [8],	Ketoconazole | *V. dahlia* [1] and F. oxysporum [8]	
			Ambuic acid derivative 5 (**52**)	*V. dahlia* [16], *F. oxysporum* [8], *R. solani* [64], *F. graminearum* [8]	Ketoconazole | *V. dahlia* [1], *F. oxysporum* [8], *R. solani* [8], *F. graminearum* [8]	
02	*Talaromyces funiculosus*	*Diorygma hieroglyphicum*	Funiculosone (**53**)	*C. albicans* [35]	–	Padhi et al. (2019)
03	*Periconia* sp.	*Parmelia* sp.	(E)-3-(8-hydroxy-7-methyl-1-oxoisochroman-3-yl) acrylic acid (**54**)	*A. niger* [31], *C. alblicans* [500]	Cycloheximide | *A. niger* [< 16], *C. alblicans* [[< 16]	Wu et al. (2015b)
			5,6-dihydroxy-7-methoxy-2,3-dimethyl-4*H*-chromen-4-one (**55**)	*A. niger* [31], *C. alblicans* [500]	Cycloheximide | *A. niger* [< 16], *C. alblicans* [[< 16]	
			6-((3S,4S,E)-3,4-dihydroxypent-1-en-1-yl)-2*H*-pyran-2-one (**56**)	*A. niger* [31], *C. alblicans* [500]		
			(S)-3-hydroxy-3-(2-oxo-2H-pyran-6-yl) propanoic acid (**57**)	*A. niger* [31], *C. alblicans* [500]		

04	*Periconia* sp.	*Parmelia* sp.	Pericoterpenoid A (**58**)	*A. niger* [31], *C. alblicans* [500]	Cycloheximide | *A. niger* [< 16], *C. alblicans* [[< 16]	Wu et al. (2015a)
05	*Aspergillus niger*	*Parmotrema ravum*	Aspergyllone (**59**)	*C. parapsilosis* [52]	Pyrophen | *C. parapsilosis* [35]	Padhi et al. (2020)
06	*Biatriospora* sp.	*Pseudosyphellaria* sp.	Biatriosporin D (**60**)	*C. albicans* IC_{80} [16]	Terbinafine | *C. albicans* IC_{80} [16]	Zhou et al. (2016)
			Biatriosporin K (**61**)	*C. albicans* IC_{80} [64]	Terbinafine | *C. albicans* IC_{80} [16]	
07	CR1546C	*Sticta fuliginosa*	(R)-4,6,8-trihydroxy-3,4-dihydro-1(2H)-naphthalenone (**62**)	*C. albicans* IC_{50} [78.2]	Amphotericin B | *C. albicans* IC_{50} [1.3]	Kim et al. (2014)
			6,8-dihydroxy-(3R)-(2-oxopropyl)-3,4-dihydroisocoumarin (**63**)	*C. albicans* IC_{50} [71.2]	Amphotericin B | *C. albicans* IC_{50} [1.3]	
08	*Floricola striata*	*Umbilicaria* sp.	Floricolin A (**64**)	*C. albicans* IC_{80} [16]	–	Li et al. (2016)
			Floricolin B (**65**)	*C. albicans* IC_{80} [8]	–	
			Floricolin C (**66**)	*C. albicans* IC_{80} [8]	–	
			Floricolin D (**67**)	*C. albicans* IC_{80} [64]	–	
09	*Coniochaeta* sp.	*Xanthoria mandschurica*	Coniothiepinol A (**68**)	*F. oxysporum* IC_{50} [13.12]	Carbendazim |*F. oxysporum* IC_{50} [0.44]	Wang et al. (2010a)

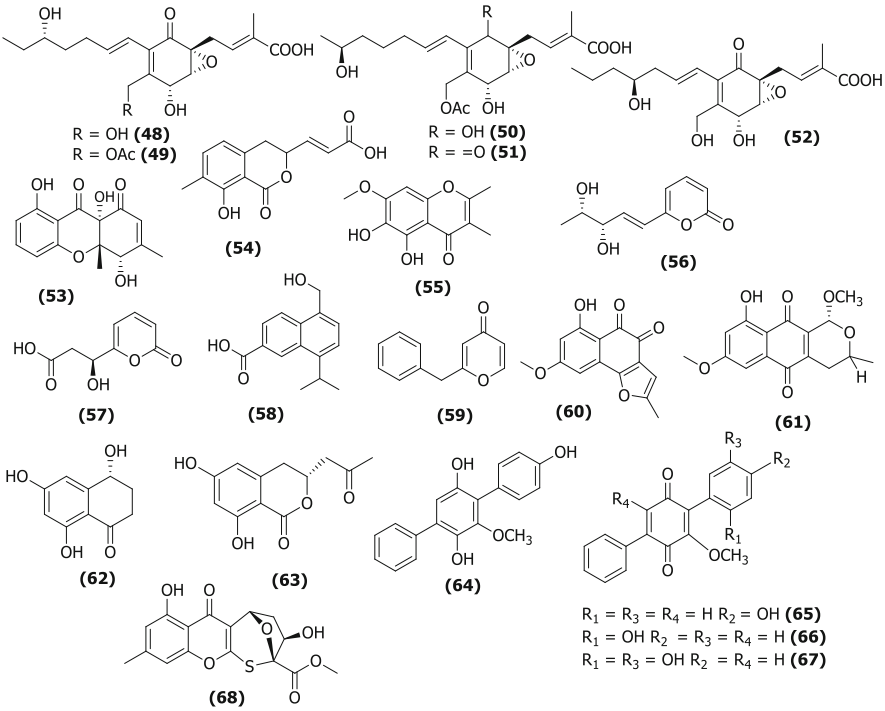

Fig. 4.2 Structure of novel compounds derived from endolichenic fungi with antifungal activity

Herein, we have systematically evaluated compounds isolated from endolichenic fungi and provide a detailed summary of the fungal species, host plant species, the antioxidant activities, the positive control used, and their corresponding IC_{50} values (Table 4.3; Fig. 4.3).

2.4 Antibacterial Compounds Isolated from Endolichenic Fungi

Antibiotic resistance has been dubbed by the World Health Organization as one of the most serious threats to public health and food security of the modern world (WHO 2020). Antibiotic resistance refers to the ability of bacteria to evade the effects of antibacterial drugs (also termed antibiotics), owing to them developing resistance by various mechanisms (Munita and Arias 2016). While this is a natural phenomenon, the overuse and misuse of antibiotics have resulted in the rapid acceleration of the development of antibiotic resistance over the last two decades (Agrawal et al. 2020). This has inevitably led to more frequent and persistent infections, increased risk of disease spread, severe illness, and death (Chinemerem

Table 4.3 Compounds derived from endolichenic fungi with antioxidant activity

Sr. No.	Fungal species	Host	Compound	Activity	Positive control	Activity	Ref. No.
1	*Penicillium citrinum*	*Parmotrema* sp.	5′-acetyl-3,5,7′-trimethoxy-3'H-spiro [cyclohexa [2,4] diene-1,1′-isobenzofuran]-3′,6-dione (**69**)[a]	IC_{50} = 159.7 μg/mL	BHT	–	Samanthi et al. (2015a)
2			4-acetyl-2′-Hydroxy-3′,5′,6-trimethoxy biphenyl-2-carboxylic acid (**70**)[a]	IC_{50} = 68.6 μg/mL			
3	*Penicillium citrinum*	*Parmotrema* sp.	10-Ethylidene-2,4,9-trimethoxy-10,10a-dihydro-7,11-dioxa-benzo[b] heptalene-6,12-dione (**71**)[a]	IC_{50} = 120.1 μg/mL	BHT	–	Wickramarachchi et al. (2019)
4	*Daldinia eschscholzii*	*Parmotrema* sp.	8-methoxynaphthalen-1-ol (**72**)	IC_{50} = 10.2 μM	BHT	–	Manthrirathna et al. (2020)
5	*Dothideomycetes* sp.	*Pertusaria laeviganda*	Norlichexanthone (**73**)[a]	ORAC value =0.0202 mol TE/g	AA	ORAC value = 0.0290 mol TE/g	Kawakami et al. (2019)
6	*Aspergillus chevalieri* SQ-8	*Lepraria incana*	2-(E-3-heptenyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)-benzaldehyde (**74**)	IC_{50} = 10.6 μM	AA	IC_{50} = 10.0 μM	Lin et al. (2021)
7			Tetrahydroauroglaucin (**75**)	IC_{50} = 11.0 μM			
8			Flavoglaucin (**76**)	IC_{50} = 11.5 μM			
9			2-(1′,5′-heptadienyl)-3,6-dihydroxy-5-(3″-methyl-2″--butenyl)benzaldehyde (**77**)	IC_{50} = 12.3 μM			
10	Sp. ELF000039	*Parmotrema austrosinense*	(3R)-5-hydroxymellein (**46**)	IC_{50} = 30.8 μg/mL	AA	IC_{50} = 40.8 μg/mL	Zhao et al. (2016)
11	*Curvularia trifolii*	*Usnea* sp.	5-methoxy-4,8,15-trimethyl-3,7-dioxo-1,3,7,8,9,10,11,12,13,14,15,15α-dodecahydrocyclododeca [de]isochrome ne-15-carboxylic acid (**78**)[a]	IC_{50} = 68.6 mg/ml	BHT	–	Samanthi et al. (2015b)

(continued)

Table 4.3 (continued)

Sr. No.	Fungal species	Host	Compound	Activity	Positive control	Activity	Ref. No.
12	*Ulocladium* sp.	*Everniastrum* sp.	Altenusin (**79**)	IC_{50} = 52.8 μM	AA	IC_{50} = 51.7 μM	Wang et al. (2012)
13			Alterlactone (**80**)	IC_{50} = 99.0 μM.			
14	*Neurospora ugadawe*	*Graphis tsunodae*	Neurosporalol 1 (**81**)[a]	IC_{50} = 3.48 μg/mL	BHT	IC_{50} = 4.50 μg/mL	Maduranga et al. (2021)
15			Neurosporalol 2 (**82**)[a]	IC_{50} = 5.03 μg/mL			
16	*Phanerochaete sordida*	*Bactrospora myriadea*	12,14-dihydroxy-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-benzo[−c][1] oxacyclododecin-1-one (**83**)[a]	IC_{50} = 58: 91 μM	BHT	IC_{50} = 55.20 μM	Weerasinghe et al. (2022)

BHT butylated hydroxytoluene, *AA* ascorbic acid

[a]Indicates novel compounds isolated from endolichenic fungi.

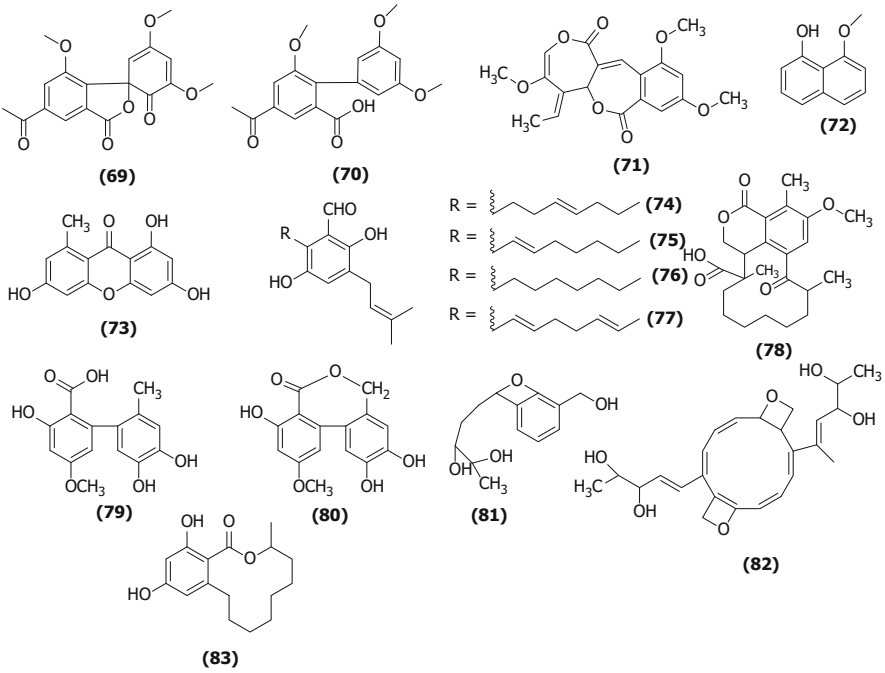

Fig. 4.3 Structures of compounds derived from endolichenic fungi with antioxidant activity

Nwobodo et al. 2022). Therefore, antibiotic resistance has generated an increased interest among scientists to discover novel, potent antibiotic drug leads from previously overlooked natural sources, such as endolichenic fungi. Table 4.4 summarizes the novel compounds that have been isolated from endolichenic fungi, which display promising antibacterial properties (Fig. 4.4).

Endolichenic fungi have the potential to serve as a valuable source of new antibacterial drug leads. This is evident by the fact that almost all of the novel compounds included in Table 4.2 are active against more than one type of test bacteria. Five new ophiobolane sesterterpenes, which were named as ophiobolins P–T (**7–11**) by the authors, were isolated from the endolichenic fungus *Ulocladium* sp. using the One Strain Many Compounds (OSMAC) method. Out of these compounds, ophiobolin P (**7**) displayed moderate antibacterial activity against *B. subtilis* (MIC = 31.3 μM) and methicillin-resistant *S. aureus* (MRSA) (MIC = 62.5 μM), while ophiobolin T (**11**) displayed moderate antibacterial activity against *B. subtilis* (MIC = 15.6 μM), MRSA (MIC = 31.3 μM), and the Bacille Calmette–Guerin (BCG) strain (MIC = 31.3 μM) (Wang et al. 2013).

A previously undescribed substituted dihydroxanthene-1,9-dione named funiculosone (**53**) was isolated together with its two analogs, mangrovamide J (**86**) and ravenelin (**87**), from the endolichenic fungus *Talaromyces funiculosus.* All these compounds showed antibacterial activity with IC_{50} values of 23–104 μg/mL when

Table 4.4 Novel compounds derived from endolichenic fungi with antibacterial activity

Sr. no.	Endolichenic fungus	Host	Compound	Test bacterium	Activity	Positive control	Activity	Ref. no.
1	*Pestalotiopsis* sp.	*Clavaroids* sp.	18-O-acetylambuic acid (**84**)	*S. aureus*	$IC_{50} = 27.8$ μM	AMP	$IC_{50} = 1.4$ μM	Ding et al. (2009)
2	*Talaromyces funiculosus*	*Diorygma hieroglyphicum*	Funiculosone (**53**)	*E. Coli*	$IC_{50} = 25$ μg/mL	–	–	Padhi et al. (2019)
				S. aureus	$IC_{50} = 58$ μg/mL			
3			Mangrovamide J (**86**)	*E. coli*	$IC_{50} = 64$ μg/mL			
				S. aureus	$IC_{50} = 104$ μg/mL			
4			Ravenelin (**87**)	*E. coli*	$IC_{50} = 23$ μg/mL			
				S. aureus	$IC_{50} = 96$ μg/mL			
5	*Ulocladium* sp.	*Everniastrum* sp.	6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3H-furo[3,2-c] isochromene-2,5-dione (**88**)	*B. subtilis*	$IC_{50} = 94.4$ μM	Gentamicin	$IC_{50} < 0.1$ μM	Wang et al. (2012)
6	*Ulocladium* sp.	*Everniastrum* sp.	Ophiobolin P (**7**)	*B. subtilis*	MIC = 31.3 μM	Gentamicin	MIC = 0.10 μM	Wang et al. (2013)
				MRSA	MIC = 62.5 μM	Vancomycin	MIC = 0.7 μM	
7			Ophiobolin T (**11**)	*B. subtilis*	MIC = 15.6 μM	Gentamicin	MIC = 0.10 μM	
				BCG	MIC = 31.3 μM	Hygromycin	MIC = 0.68 μM	
				MRSA	MIC = 31.3 μM	Vancomycin	MIC = 0.7 μM	
8	*Aspergillus chevalieri*	*Lepraria incana*	Asperglaucin A (**89**)	*P. Syringae*	MIC = 6.25 μM	Ampicillin sodium	MIC = 3.12 μM	Lin et al. (2021)
						Fosfomycin sodium	MIC = 50.0 μM	
				MRSA	MIC = 25.0 μM	Ampicillin sodium	MIC = 12.5 μM	
						Fosfomycin sodium	MIC = 25.0 μM	
				B. cereus	MIC = 6.25 μM	Ampicillin sodium	MIC = 3.12 μM	
						Fosfomycin sodium	MIC = 12.5 μM	
				R. Solanaearum	MIC = 100.0 μM	Ampicillin sodium	MIC = 12.5 μM	
						Fosfomycin sodium	MIC = 50.0 μM	

9			Asperglaucin B (**90**)	*P. Syringae*	MIC = 6.25 μM	Ampicillin sodium	MIC = 3.12 μM	
						Fosfomycin sodium	MIC = 50.0 μM	
				MRSA	MIC = 50.0 μM	Ampicillin sodium	MIC = 12.5 μM	
						Fosfomycin sodium	MIC = 25.0 μM	
				B. cereus	MIC = 6.25 μM	Ampicillin sodium	MIC = 3.12 μM	
						Fosfomycin sodium	MIC = 12.5 μM	
				R. Solanaearum	MIC = 50.0 μM	Ampicillin sodium	MIC = 12.5 μM	
						Fosfomycin sodium	MIC = 50.0 μM	
10	CR1546C[a]	*Sticta fuliginosa*	(R)-4,6,8-trihydroxy-3,4-dihydro-1(2H)-naphthalenone (**62**)	*B. subtilis*	IC_{50} = 104.2 μg/mL	Streptomycin sulfate	IC_{50} = 5.2 μg/mL	Kim et al. (2014)
11			6,8-dihydroxy-(3R)-(2-oxopropyl)-3,4-dihydroisocoumarin (**63**)	*B. subtilis*	IC_{50} = 106.4 μg/mL	Streptomycin sulfate	IC_{50} = 5.2 μg/mL	
12	*Coniochaeta* sp.	*Xanthoria mandschurica*	Coniothiepinol A (**68**)	*E. Faecium*	IC_{50} = 3.93 μg/mL	Ampicillin	IC_{50} = 0.51 μg/mL	Wang et al. (2010a)
				E. faecalis	IC_{50} = 11.51 μg/mL	Ampicillin	IC_{50} = 2.61 μg/mL	
13			Coniothienol A (**91**)	*E. Faecium*	IC_{50} = 2.00 μg/mL	Ampicillin	IC_{50} = 0.51 μg/mL	
				E. faecalis	IC_{50} = 4.89 μg/mL	Ampicillin	IC_{50} = 2.61 μg/mL	
14	*Daldinia* sp.		Daldispone A (**92**)	*S. aureus*, *E. faecalis*, *B. cereus*	Inactive			Gu et al. (2021)
		–	Daldispone B (**93**)	*S. aureus*	MIC = 32 μg/mL	–	–	
15				*E. faecalis*	MIC = 16 μg/mL			
				B. cereus	MIC = 32 μg/mL			

[a]Indicates the strain of the endolichenic fungi

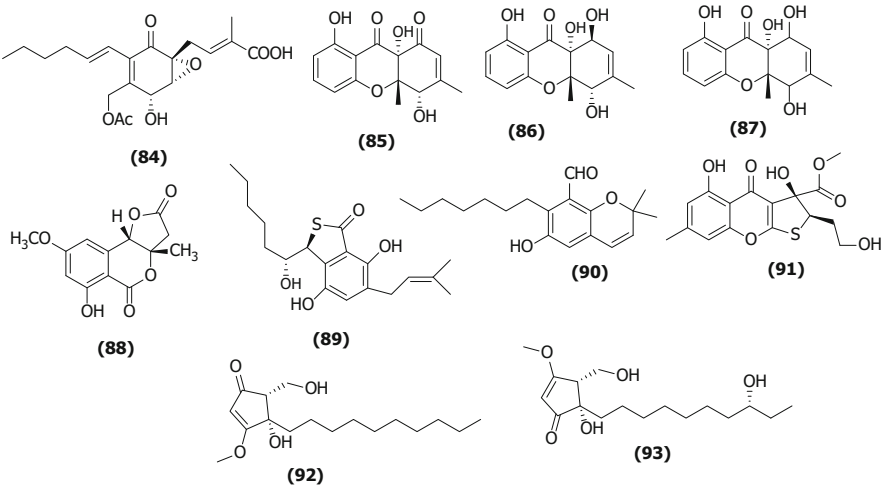

Fig. 4.4 Structures of novel compounds derived from endolichenic fungi with antibacterial activity

assayed against *E. coli* and *S. aureus* (Padhi et al. 2019). In another study, a new naphthalenone, (R)-4,6,8-trihydroxy-3,4-dihydro-1(2H)-naphthalenone (**62**) and a new isocoumarin, 6,8-dihydroxy-(3R)-(2-oxopropyl)-3,4-dihydroisocoumarin (**63**) were isolated from a *Xylariaceae* sp. which is an endolichenic fungal species from the Costa Rican lichen *Sticta fuliginosa*. Both these compounds were active against *B. subtilis,* with (R)-4,6,8-trihydroxy-3,4-dihydro-1(2H)-naphthalenone (**62**) having an IC_{50} value of 104.2 μg/mL, and 6,8-dihydroxy-(3R)-(2-oxopropyl)-3,4-dihydroisocoumarin (**63**) with an IC_{50} value of 106.4 μg/mL against this bacterium (Kim et al. 2014).

The first naturally occurring thiepinol (coniothiepinol A) (**68**) and thienol (coniothienol A) (**91**) were isolated from the endolichenic fungus *Coniochaeta* sp. Coniothiepinol A (**68**) was found to possess a unique 8-oxa-2-thia-bicyclo [3.2.1]octane skeleton, and also displayed antibacterial properties against *E. faecium* (IC_{50} = 3.93 μg/mL) and *E. faecalis* (IC_{50} = 11.51 μg/mL). On the other hand, coniothienol A (**91**) also displayed antibacterial activity against these two test bacteria, where its IC_{50} value against E. *faecium* was 2.00 μg/mL and against *E. faecalis* its was 4.89 μg/mL (Wang et al. 2010a).

Finally, a study reported the isolation of two new cyclopentenone derivatives named daldispone A (**92**) and daldispone B (**93**) from an endolichenic fungus, *Daldinia* sp. Out of the two compounds, the second one, daldispone B (**93**) showed moderate antibacterial activities against *S. aureus* (MIC = 32 μg/mL), *E. faecalis* (MIC = 16 μg/mL), and *B. cereus* (MIC = 32 μg/mL) (Gu et al. 2021).

2.5 Antiviral Compounds Isolated from Endolichenic Fungi

Only 10 out of more than 220 viruses known to infect humans are now treated with antiviral medications that have received clinical approval (Adamson et al. 2021). Indeed, the SARS-CoV-2 outbreak has highlighted the urgent need for therapeutic agents that can be quickly mobilized for the treatment of emerging or re-emerging viral illnesses. In the early phases of an emergent, highly pathogenic viral outbreak, the accessibility of safe and effective therapies is crucial in the absence of a viable vaccine. Hence, a few studies have investigated the antiviral properties of some compounds isolated from endolichenic fungi, in the search for such novel therapies. Both novel and previously isolated compounds from endolichenic fungi which display antiviral properties are summarized in Table 4.5 (Fig. 4.5).

Alternariol (**94**) and several of its analogs have been previously isolated from the common fungal genus *Alternaria* (Aly et al. 2008). However, there were no reports on the isolation of this compound from genus *Nigrospora* until the study done by He et al. (2012). Specifically, alternariol (**94**) was isolated from endolichenic *Nigrospora sphaerica*, and it was found to be active against the Herpes Simplex Virus, with an IC_{50} value of 13.5 μM (He et al. 2012).

Daldispols A (**96**) and C (**97**) are novel phenolic metabolites that were isolated from the endolichenic fungus *Daldinia* species, which displayed comparable antiviral activity against the Influenza A virus ($IC_{50} = 12.7$ μM and $IC_{50} = 6.4$ μM respectively). Importantly, both their activities were stronger than the positive control, ribavirin ($IC_{50} = 21.7$ μM). In another study, two new cyclopentenone derivatives named daldispones A and B (**92** and **93**) were isolated from the same endolichenic fungus. These two compounds also exhibited potent anti-influenza A virus activities, with IC_{50} values of 16.0 and 7.4 μM, respectively. Therefore, both were more potent against Influenza A than the positive control ribavirin, which had an IC_{50} value of 21.72 μM (Gu et al. 2021). Even though a significant number of compounds isolated from endolichenic fungi were found to possess antibacterial and/or antifungal activities, only a very limited number of studies had evaluated such compounds for their antiviral activity. Hence, this paucity of research focused on isolating antiviral compounds from endolichenic fungi highlights the need for more studies in this area.

2.6 Anti-Inflammatory Compounds Isolated from Endolichenic Fungi

Inflammation is generally defined as the innate immune response to potentially harmful stimuli such as pathogens, injury, and metabolic stress (Antonelli and Kushner 2017). Even though localized inflammation results in benefits such as the destruction of pathogens and enhances wound healing, chronic, excessive inflammation has negative health repercussions (Kiecolt-Glaser et al. 2010). Hence, anti-

Table 4.5 Novel and previosly isolated compounds derived from endolichenic fungi with antiviral activity

Sr. no.	Endolichenic fungus	Host	Compound	Test virus	Activity (IC_{50})	Positive control	Activity (IC_{50})	Ref. no.
1	*Nigrospora sphaerica*	*Parmelinella wallichiana*	Alternariol (**94**)	Herpes simplex virus	13.5 μM	–	–	He et al. (2012)
2			Alternariol-9-methyl ether (**95**)	Herpes simplex virus	21.3 μM	–	–	
3	*Daldinia* sp.	–	Daldispol A (**96**)	Influenza A virus	12.7 μM	Ribavirin	21.7 μM	Zhang et al. (2021)
4			Daldispol C (**97**)	Influenza A virus	6.4 μM	Ribavirin	21.7 μM	
5			2-phenylethyl-β-D-glucopyranoside (**98**)	Influenza A virus	12.5 μM	Ribavirin	21.7 μM	
6			(5R,7R)-5,7-dihydroxy-2- methyl-5,6,7,8-tetrahydro-4*H*-chromen-4-one (**99**)	Influenza A virus	16.1 μM	Ribavirin	21.7 μM	
7			(5R,7S)-5,7-dihydroxy-2-methyl-5,6,7,8-tetrahydro-4*H*-chromen-4-one (**100**)	Influenza A virus	9.0 μM	Ribavirin	21.7 μM	
8			Daldispone A (**92**)	Influenza A virus	16.0 μM	Ribavirin	21.72 μM	
9			Daldispone B (**93**)	Influenza A virus	7.4 μM	Ribavirin	21.72 μM	

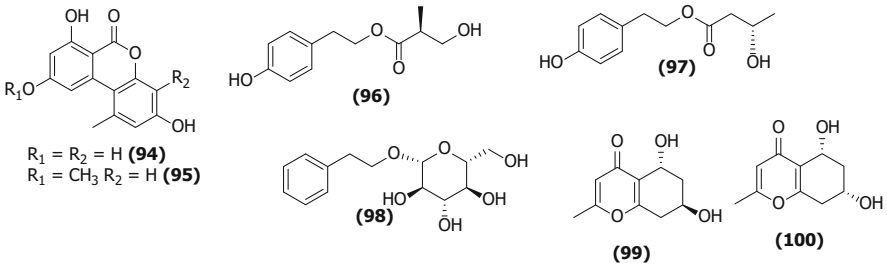

Fig. 4.5 Structure of novel compounds derived from endolichenic fungi with antiviral activity

inflammatory drugs have been developed and used over decades to maintain tissue homeostasis and attenuate the damage that may have resulted from an exaggerated inflammatory response (Azab et al. 2016). Natural products have been a major contributor to the discovery and development of anti-inflammatory drugs. One such notable example is aspirin, which was derived from the bark of the Willow tree. Even though a large number of anti-inflammatory drugs have been developed during the past century, many have been associated with adverse effects (McCarberg and Gibofsky 2012). Hence, there is a need for the development of novel anti-inflammatory therapies with increased effectiveness and decreased side effects. Consequently, we have summarized the compounds with anti-inflammatory activity isolated from endolichenic fungi (Table 4.6; Fig. 4.6).

A total of eight compounds showing anti-inflammatory activity is summarized in the present section. Amongst the compounds reported here, there are four novel compounds isolated from endolichenic fungi, and four previously isolated compounds with promising anti-inflammatory activity. A previously undescribed polyketide javanicol E (**101**) and (+)-terrein (**102**) were evaluated for their anti-inflammatory activity against lipopolysaccharide-activated RAW 264.7 murine macrophages, where both these compounds were found to show moderate anti-inflammatory activity. This study has further reported that the presence of an intact terrein unit played a major role in the anti-inflammatory activity of these compounds (Xu et al. 2020).

In a study that investigated anti-inflammatory compounds isolated from the endolichenic fungus *Phoma* sp. EL002650, the compound phomalichenone A (**103**) was isolated. It was identified as a bioactive compound capable of diminishing the protein expression levels of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) enzymes. It is also capable of decreasing the mRNA expression levels of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α) Kim et al. (2018). This study also used the inhibition of nitric oxide in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages in order to evaluate the anti-inflammatory activity. The human red blood cells (HRBC) membrane stability assay was another commonly used bioassay for evaluating the anti-inflammatory action of compounds derived from endolichenic fungi (Weerasinghe et al. 2022; Maduranga et al. 2021). Furthermore, the limited

Table 4.6 Compounds derived from endolichenic fungi with anti-inflammatory activity

Sr. no.	Fungal Species	Host	Compound	Activity	Positive control	Activity	Ref no.
1	*Eupenicillium javanicum*	*Parmelia* sp.	Javanicol E (**101**)[a]	IC_{50} = 17.00 μM	Luteolin	80%[a]	Xu et al. (2020)
2			(+)-Terrein (**102**)	IC_{50} = 13.46 μM			
3	*Phoma* sp. EL002650		Phomalichenone A (**103**)[a]	IC_{50} = 9.4 μM	–	–	Kim et al. (2018)
4			(E)-1-(2,4-dihydroxy-3-(2-hydroxyethyl)-6-methoxyphenyl)but-2-en-1-one (**104**)	IC_{50} = 7.4 μM			
5	*Curvularia trifolii*	*Usnea* sp.	5-methoxy-4,8,15-trimethyl-3,7-dioxo-1,3,7,8,9,10,11,12,13,14,15,15α-dodecahydrocyclododeca[de]isochrome Ne-15-carboxylic acid (**105**)[a]	IC_{50} = 310 μM	–	–	(Samanthi et al. (2015a, 2015b)
6	*Aspergillus chevalieri* SQ-8	*Lepraria incana*	Isodihydroauroglaucin (**106**)	IC_{50} = 12.2 μM	Quercetin	IC_{50} = 10.0 μM	Lin et al. (2021)
7			Neoechinulin C (**107**)	IC_{50} = 12.8 μM			
8	*Phanerochaete sordida*	*Bactrospora myriadea*	12,14-dihydroxy-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-benzo[−c][1]oxacyclododecin-1-one (**108**)[a]	IC_{50} = 254.79 μM	Aspirin	IC_{50} = 134.16 μM	Weerasinghe et al. (2022)

[a]Percentage inhibition at a concentration of 50 μM

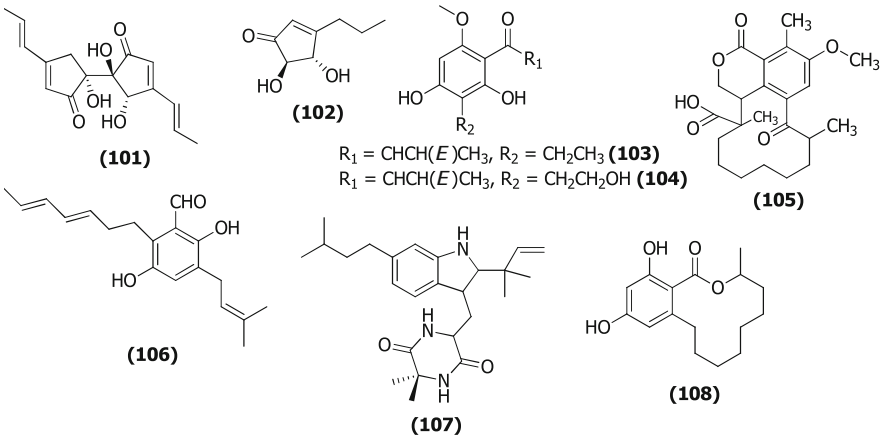

Fig. 4.6 Structure of compounds derived from endolichenic fungi with anti-inflammatory activity

number of studies that have been carried out to identify anti-inflammatory compounds from endolichenic fungi indicates that a plethora of bioactive compounds is awaiting to be explored.

3 Classification of Bioactive Compounds According to Selected Taxa of Their Endolichenic Fungi

A majority of the endolichenic fungi that displayed pharmaceutically important bioactivities belonged to the order Pleosporales. A total of 43 out of the 114 compounds were derived from endolichenic fungi that belong to the above order. Out of these 43 compounds, 22 were cytotoxic, 11 displayed antifungal activity, 4 displayed antioxidant activity, 3 displayed antibacterial activity, while one showed anti-inflammatory activity. Within the order Pleosporales, the reported bioactive compounds were isolated from endolichenic fungi belonging to seven families, namely, Phaeosphaeriaceae, Pleosporaceae, Sporomiaceae, Teichosporaceae, Periconiaceae, Biatriosporaceae, and Testudinaceae.

The second and third most abundant orders in terms of the endolichenic fungi that display pharmaceutically important bioactivities were Eurotiales and Xylariales, respectively. A total of 23 compounds isolated from endolichenic fungi of order Eurotiales were found to display almost all of the bioactivities described in this chapter. Out of these compounds, a majority (eight) of them were cytotoxic, while another six were antioxidant compounds. There were 22 compounds that had been isolated from endolichenic fungi belonging to the order Xylariales, and most of them exhibited antimicrobial properties. This was the only order from which antiviral compounds had been isolated from endolichenic fungi.

Therefore, endolichenic fungi belonging to the orders Pleosporales, Eurotiales, and Xylariales display a particular propensity toward producing pharmaceutically useful bioactive compounds.

4 Summary Statement

Endolichenic fungi are a special group of fungi that are capable of living within the thalli of lichens via quiescent infections. Owing to their unique ecology, these organisms are known to produce a wide range of bioactive secondary metabolites, many of which are novel, and possess unprecedented structures. Understanding this potential of endolichenic fungi as a promising source of pharmaceutically important drug leads, the present chapter has reviewed over 100 compounds that have been isolated from them. Among them are 47 compounds with cytotoxic activity, 22 compounds with antifungal activity, 16 compounds exhibiting antioxidant activity, 12 compounds with antibacterial activity, 9 antiviral compounds, and eight anti-inflammatory compounds. Furthermore, a significant number of these compounds had comparable activity in comparison with their respective positive controls. As such, it is clear that endolichenic fungi can serve as a promising source of bioactive secondary metabolites that can be developed into efficient and effective therapeutic agents.

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Chapter 5
Endolichenic Fungi, an Emerging Source of Bioactive Compounds: A Pharmaceutical Perspective

Kaveri Pawar, Shamana Gondalia, Chaitrali Shevkar, EDE Venkata Gopal, and Abhijeet S. Kate

Abstract Endolichenic fungi (ELF), residing within the thallus of lichen, which is a symbiotic association of algae and fungi, are emerging as an alternative source for bioactive secondary metabolites. The complex miniature ecosystem compels endolichenic fungi to produce diverse chemical scaffolds, thus proving a potential resource for novel bioactive molecules. The chemical diversity and pharmacological benefits of secondary metabolites from endolichenic fungi have been investigated in numerous studies. This chapter highlights 40 potent molecules selected after a critical analysis of 672 documented molecules (2007–2022) from ELF. The inclusion criteria for selection are as follows: cytotoxicity less than 2μM, antibacterial less than 5μM, antifungal less than 10μg/mL, antioxidant less than 5 mg/mL, anti-inflammatory less than 10μM, anti-Alzheimer's less than 10μM, and AChE inhibition less than 50μg/mL. This chapter concisely states the bioactive secondary metabolites from ELF, along with the challenges, and future directives to convert them into pharmaceutical leads.

Keywords Endolichenic fungi · Anticancer · Antibacterial · Anti-inflammatory · Antioxidant · Antifungal · Anti-Alzheimer's

1 Introduction

Natural products have been crucial in the establishment of pharmaceutical discoveries and the efficient management of healthcare. Doxorubicin (Adriamycin®), Captopril (Capoten®), Metformin (Fortamet®), Empagliflozin (Jardiance®), Tirzepatide (Mounjaro™), and many other bioactive compounds have been developed from natural scaffolds (Newman and Phil 2022). Nature's enormous untapped biodiversity offers a guaranteed source of a wide range of compounds with the

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potential to address unmet medical needs (Atanasov et al. 2021; Sytar and Smetanska 2022). Lichen is one such biologically diverse ecosystem comprising symbiotic associations between a dominant fungal mycobiont, one or more algal or cyanobacterial photobionts, and a multifarious platoon of other microorganisms (Galun and Bubrick 1984). Traditional medicine makes an extensive use of lichens, with 60 different lichen genera listed in various ancient texts including *Xanthoparmelia* (North America and Africa), *Lethariella* (China), *Cetraria* (Europe), *Parmotrema*, and *Hypotrachyna* (India). Further, throughout the world (except Australia), the *Usnea* genus has been used to treat cough, discomfort, wound healing, and obesity (Ranković 2015). In addition, lichens are being used for the treatment of blood and heart disorders, spermatorrhoea, bleeding piles, diabetes, bronchitis, tuberculosis, and dyspepsia (Calcott et al. 2018; Mitrović et al. 2011). The intricacies of lichen-dwelling species and their symbiotic relationship lead to the formation of a diverse range of secondary metabolites. However, small colonies of slow-growing lichens cannot provide enough compounds to support the requirements of pharmaceutical industries (Zambare and Christopher 2012).

Endolichenic fungi (ELF) are primarily filamentous fungi present asymptomatically inside lichen thalli (Fig. 5.1). These endolichenic fungi were discovered while culturing lichen-forming fungus (mycobiont), and they are akin to endophytic fungi that reside within plant tissues (Suryanarayanan and Thirunavukkarasu 2017). ELF are distinct from both lichenicolous fungi (surface fungus) and mycobiont fungi in terms of taxonomy and ecology (Arnold et al. 2009). The majority of isolated species of ELF are members of the subdivision Pezizomycotina including genera like *Aspergillus, Sporomiella, Curvularia, Chaetomium, Xylariaceae, Phaesophaeria, Phialophora, Uiocladium, Xylaria, Xylariaceae, Xylariales*, *and Colchibolus* (U'Ren et al. 2010). The number of species reported in the abundant genus of ELF along with their secondary metabolites were studied and illustrated in Fig. 5.2. Although the number of species reported in the genus *Aspergillus* and *Xylaria* were the same, the number of secondary metabolites from *Aspergillus* were almost fourfold higher than the later genus indicating *Aspergillus* has been more explored in terms of isolated molecules which was followed by the genus *Uiocladium*. The competitive environment provided by the lichen-associated micro ecosystem compelled ELF to produce bioactive scaffolds (Kellogg and Raja 2017). A number of reviews have been recently published describing ELF metabolites, including alkaloids, terpenoids, quinones, xanthones, macrocyclic lactones, and polyketides (Wethalawe et al. 2021; Agrawal et al. 2020; Kellogg and Raja 2017).

A critical analysis was carried out using the reported data listed in multiple platforms like Sci-Finder, PubMed, PubChem, and Science Direct. It has been observed that a total of 192 reports are published describing secondary metabolites of endolichenic fungi in the period of 2007–2022. A total of 672 documented molecules with ELF origins were found in this survey, of which 145 molecules were unique to ELF. These molecules were investigated by various pharmacological assays to explore their biological properties such as antimicrobial, anticancer, antioxidant, anti-inflammatory, anti-Alzheimer's, and AChE inhibitory activity. These compounds were categorized according to their biological activities as well

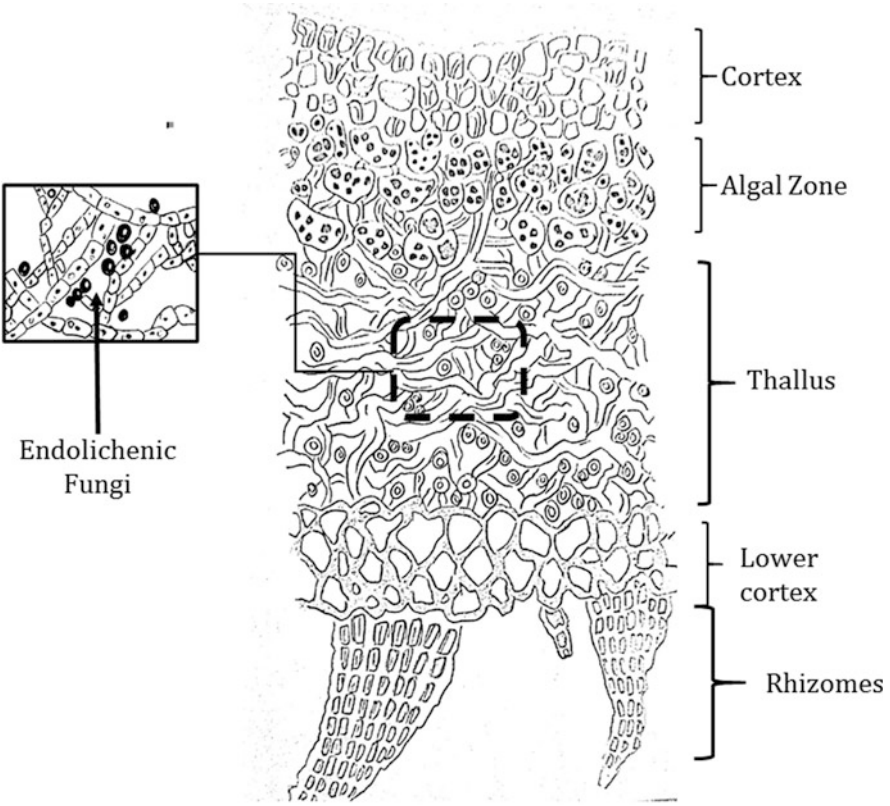


Fig. 5.1 Cross section of lichen showing embedded endolichenic fungi (ELF) in thallus

as their potency. It is necessary to mention that few compounds were having more than one activity. The prominent observations indicated that 83 anticancer molecules, 44 antibacterial, and 64 antifungals are reported from ELF among others.

In this chapter, the potent metabolites of ELF are included categorizing them into antibacterial compounds, anti-inflammatory, anti-Alzheimer's, and anticancer compounds. The inclusion criteria for the molecule were as follows: cytotoxicity less than 2μM, antibacterial less than 5μM, antifungal less than 10μg/mL, antioxidant less than 5 mg/mL, anti-inflammatory less than 10μM, anti-Alzheimer's less than 10μM, and AChE inhibition less than 50μg/mL. In all, 40 potent bioactive compounds isolated from ELF are discussed along with their in vitro and in vivo pharmacological results. With an estimated 20,000 lichen species in the world, there is still a sizable pool of potential ELF that could produce bioactive natural compounds and hence, a huge scope for further research in this field (Feuerer and Hawksworth 2007).

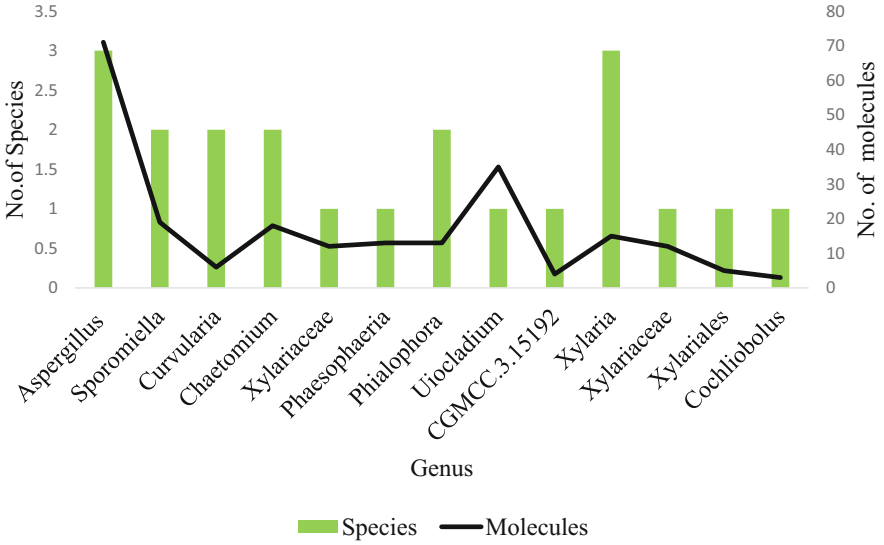


Fig. 5.2 Selected ELF genus with a number of species and molecules isolated from them

2 Anticancer Compounds

Ophiobolins, hypocrellins A, apiosporamide, ulosporin G, and isocoumarin indole A are the reported anticancer compounds from ELF possessing cytotoxicity less than 2μM. Ophiobolin is a subclass of sesterterpenes having a tricyclic 5-8-5 carbotricyclic scaffold possessing anticancer activity. Since its discovery in 1999, numerous ophiobolins and their derivatives have been isolated from various sources like plant fungi, endolichenic fungi, and marine fungi (Xie et al. 2020; Zhu et al. 2018). *Ulocladium* sp., an endolichenic fungi showed the presence of different ophiobolins, namely ophiobolin P (**1**), opiobolin Q (**2**), opiobolin R (**3**), ophiobolin S (**4**), ophiobolin T (**5**), 6-epi-ophiobolin G (**6**), and 6-epi-ophiobolin K (**7**) (Fig. 5.3). These compounds were evaluated for anticancer activity (in-vitro) against human epithelial carcinoma (KB cell lines) and human hepatocellular carcinoma (HepG2 cell lines) using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. It is a colourimetric test for determining the number of viable cells based on the cleavage of the tetrazolium ring in MTT by dehydrogenase enzymes in active mitochondria of living cells. The observed anticancer activities of ophiobolins against KB cell line were 6.18μM (**1**), 2.38μM (**2**), 2.91μM (**3**), 4.91μM (**4**), 3.01μM (**5**), 1.37μM (**6**), and 4.7μM (**7**). In the case of HepG2 cell line, the cytotoxicity values were 1.48μM (**1**), 1.32μM (**2**), 1.18μM (**3**), 1.40μM (**4**), 0.24μM (**5**), 0.37μM (**6**), and 1.93μM (**7**) (Wang et al. 2013). The structure–activity relationship study revealed three major observations such as the attachment of hydroxyl groups at positions C-3, C-6, and C-14, the configuration at position C-6, and the location of the tetrahydrofuran ring between positions C-14 and C-17

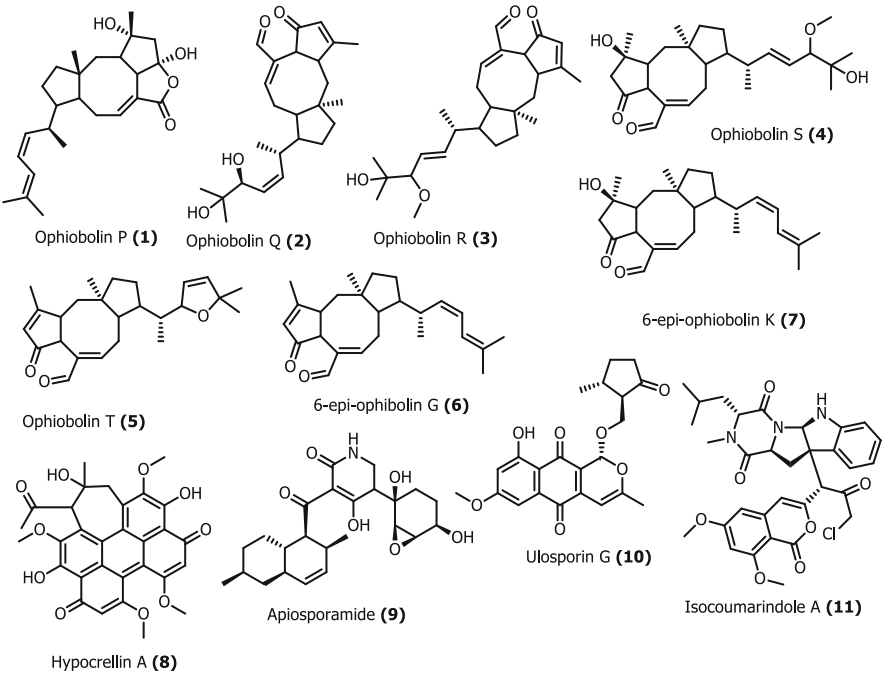


Fig. 5.3 Selected anticancer molecules from endolichenic fungi

and C-5 and C-21 are critical for biological activities (Masi et al. 2019). Further in-vivo and toxicity studies are needed to prove the candidature of ophiobolins in drug development.

Hypocrellins A (HA) (**8**) (Fig. 5.3) is an effective photosensitizer known to exert outstanding light-induced antiviral, antibacterial, and anticancer action via modulating a number of signalling pathways. It is a perylenequinones-type pigment isolated from the endolichenic fungus *Phaeosphaeria* sp. With an IC_{50} value of 0.14μM, HA demonstrated strong anticancer activity against A549 cell lines. The compound inhibits the proliferation of A431 cell lines through the activation of the ROS-mediated JNK and NF-κB pathways causing apoptosis and autophagy (Niu et al. 2020). The fungal–bacterial co-culture of *Shiraia* sp. S9 and *Pseudomonas fulva* SB1 significantly enhanced HA yield via the exogenous ATP production. A study on nanoformulation revealed promising results as biocompatible HA-Fe(III) nanoparticles (HA-Fe(III) NPs) showed in vitro activity 1.3μg mL^{-1}, 0.85μg mL^{-1}, and 1.6μg mL^{-1} towards A549, SKOV-3, and cis-A549 cells (cisplatin resistant), respectively, when irradiated with red light (600 nm) (Li et al. 2021). In vivo experiments were also conducted using HA-Fe(III) NPs on the 4T1 tumour bearing mouse model. In this study, mice having a tumour size of 50 mm^3 were divided into three groups ($n = 5$) randomly in which the control group received phosphate buffered saline (PBS) with 15 min of 628 nm laser radiation after 6 h of administration. In the second group, a dosage of 5 mg kg^{-1} of HA-Fe (III) NPs was

administered through the tail vein in the dark and not given any laser irradiation. The third group had received the same dose of HA-Fe(III) NPs (5 mg kg^{-1}) and then, after 6 h, the animals were given 628 nm laser radiation for 15 min. The therapy was given on the first, third, and fifth day and a fourfold decrease in the tumour weight was observed (Wang et al. 2022).

Apiosporamide (**9**) (Fig. 5.3) is a pyridine alkaloid that was isolated from *Apiospora montagnei*, an endolichenic fungus inhabiting the lichen *Cladonia* sp. The compound showed potent in vitro anticancer activity ($IC_{50} = 2.1\mu M$) against the mouse lymphoma cell line (L5178Y). Apiosporamide was isolated from marine-derived fungus *Arthrinium* sp. UJNMF0008 as well and it has shown inhibition against human osteosarcoma cell lines U2OS ($IC_{50} = 19.3\mu M$) and MG63 ($IC_{50} = 11.7\mu M$). The structure–activity relationship study revealed the cytotoxicity was completely lost upon acetylation of 23-OH and aromatization from C-20 to C-25, but the activity was boosted 10-fold upon the removal of the hydroxy group at position N-16 (Wang et al. 2017; Zhang et al. 2019). Ulosporin G (**10**) (Fig. 5.3), a heptaketide, was derived from *Ulospora bilgramii* with host lichen *Umbilicaria* sp. There are a total of ten analogues of ulosporins that have been isolated from this fungus and among all, ulosporin G showed better activity against human epithelial carcinoma (KB), human breast cancer cell line (MCF-7), and human lung cancer cell line (A549) with IC_{50} values of 3.0, 1.3, and 1.3μM, respectively. The presence of ether linkage at position 1 might be responsible for the improved activity (Xie et al. 2020). Isocoumarin indole A (**11**) (Fig. 5.3) is a hybrid molecule obtained from non-ribosomal peptide synthase and polyketide synthase pathways. It is isolated from an endolichenic fungus *Aspergillus* sp. residing in lichen *Cetrelia* sp. from China. It demonstrated noteworthy cytotoxicity against human pancreatic adenocarcinoma cell lines AsPC-1 and MIA-PaCa-2 with IC_{50} values of 5.53 and 1.63μM, respectively (Chen et al. 2019).

3 Anti-Microbial Compounds

The need for new antimicrobial drugs has risen as a result of the evolution of microbe resistance to almost all of the presently used antibiotics and the unexpected rise of serious viral infections (Strobel et al. 2004). In 2016, Ban Ki-Moon, the outgoing UN secretary general, said that the problem of antibiotic-resistant microorganisms may be resolved by creating novel antimicrobial drugs (Sarasan et al. 2017). After the discovery of Penicillin G in 1928, the substantial contribution of fungal species to the production of antibiotics became apparent (Agrawal et al. 2020). Endophytic fungi, a subtype of symbiotic fungus, are known to create a variety of antibacterial compounds that are effective in both healthcare and agriculture. ELF, like many other symbiotic fungi, is involved in the production of many secondary metabolites, which function as a defensive mechanism for the lichen against abiotic and biotic stresses (Nguyen et al. 2013). The identification of several antimicrobial secondary metabolites in ELF offers a possible solution to the never-ending demand for new

antimicrobial drugs. However, in order to change their pharmacological and toxicological properties and make them more effective, these compounds may need to undergo additional modifications. Antimicrobials from ELF, including antibacterial and antifungal agents, are described here (Wethalawe et al. 2021).

3.1 Antibacterial Compounds

In the last 20 years, vancomycin-resistant *Enterococcus faecium* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and other antibiotic-resistant bacteria have posed significant challenges (Nannini et al. 2010). Moreover, these strains have gained resistance to two modern medicines, daptomycin and linezolid. Further, issues like MDR and XDR-TB are difficult to detect and have unexpected results. The quest for novel and innovative bioactive scaffolds that may be present in ELF is the greatest priority step for tackling all of these current difficulties.

Key antibacterial compounds from ELF are Griseoxanthone C (**12**), 6-O-methylnorlichexanthone (**13**), and Norlichexanthone (**14**). They belong to the xanthone group which is known to produce antibacterial effects due to their γ-pyrone rings. These compounds have been isolated from *Ulocladium* sp. from the lichen *Everniastrum* sp. and tested against *B. subtilis* using the microtitre plate assay. The inhibition is measured by determining the optical density at 578 nm after 24 h incubation with bacterial strain. Compounds **12**, **13**, and **14** showed their sensitivity towards *B. subtilis* with IC_{50} values of 1.29, 1.43, and 2.25μM, respectively. The presence of the methoxy group in **12** and **13** as compared to the hydroxy group in **14** resulted in better antibacterial effects against *B. subtilis* (Wang et al. 2012).

ELF *Coniochaeta* sp., residing in the *Xanthoria mandschurica* host lichen, has served as a source of the very first naturally present thiepinol, coniothiepinol A (**15**) along with thienol and coniothienol (**16**) (Fig. 5.4). Coniothiepinol A has the distinct

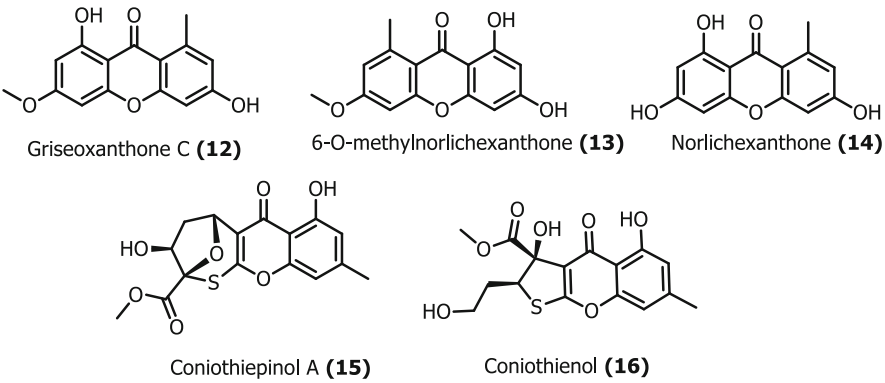


Fig. 5.4 Selected antibacterial molecules from endolichenic fungi

4,5-dihydro-2H-thiepino[2,3-b]chromen-6(3H)-one scaffold. Both the compounds were evaluated for antibacterial activity against *Enterococcus faecium* and *Enterococcus faecalis* by the inhibition assay in a 96-well plate and measuring the absorbance at 590 nm in a microplate reader. In this method 10^6 cells/mL were added to the 96-well plate and then treated with the dilutions of the compound and the inhibition was measured after 24 h. Coniothiepinol A inhibited *Enterococcus faecium* and *Enterococcus faecalis* with IC_{50} of 3.93 and 11.51μg/mL respectively. While the IC_{50} of coniothienol A against *Enterococcus faecium* and *Enterococcus faecalis* were 2.00 and 4.89μg/mL, respectively (Wang et al. 2010).

3.2 Antifungal Compounds

The re-emergence of fungal infections during allogeneic bone marrow transplants, cancer therapy, and organ transplants has necessitated the discovery of antifungal compounds having improved efficacy and compatibility. Only a few antifungal drugs are currently prescribed to treat the many types of topical and systemic fungal infections (Lockhart and Guarner 2019). As a result, endolichenic fungus secondary metabolites are an important source of antifungal drugs. A few potent antifungal molecules isolated from endolichenic fungus are described here. Cordyol C (**17**) and 3,7-dihydroxy-1,9-dimethyldibenzofuran (**18**) isolated from *Aspergillus versicolor* an ELF from the lichen *Lobaria quercizans* exhibited significant antifungal activity at the tested concentration of 8μg/mL against *Candida albicans* with the broth microdilution approach (Li et al. 2015).

Floricolins A–J were discovered from the ELF *Floricola striata* with lichen host *Umbilicaria* sp. These compounds were tested against *C. albicans* using a broth microdilution checkerboard assay. Floricolin B (**19**) and floricolin C (**20**) were found to have MICs of 8μg/mL with 80% reduction in the growth of *C. albicans.* Floricolin C acts as a fungicide by making the plasma membrane more permeable, as seen by the influx of propidium iodide. Recent research suggests that the α, β-unsaturated carbonyl group of floricolins may react with GSH or thiol-group proteins, resulting in an imbalance of the intracellular redox potential and the formation of reactive oxygen species (ROS). Alternately, floricolins may disrupt mitochondria, promoting the accumulation of ROS. These accumulated ROS eventually cause mitochondrial dysfunction, nuclear fragmentation, and apoptosis. Sydowiol A (**21**) and sydowic acid (**22**) (Fig. 5.5) are bisabolane sesquiterpenoids identified from endolichenic fungus *Aspergillus versicolor* residing in *Lobaria quercizans*, a lichen. These compounds at 8μg/mL have shown growth inhibition of *C. albicans* when tested by the microdilution technique (Li et al. 2015).

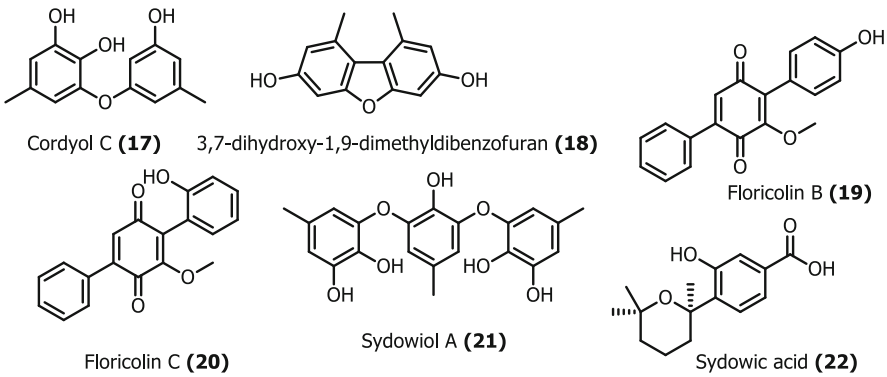


Fig. 5.5 Selected antifungal molecules from endolichenic fungi

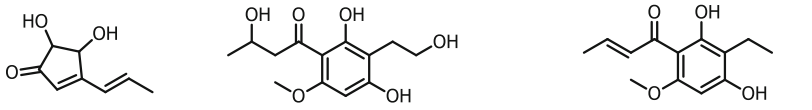


Fig. 5.6 Selected anti-inflammatory molecules from endolichenic fungi

4 Anti-Inflammatory Compounds

Immune cells, blood vessels, and chemical messengers are engaged in inflammatory conditions which can be alleviated by blocking specific pathways and preventing the release of inflammatory mediators (Ferrero-Miliani et al. 2007). Multiple compounds identified from the endolichenic fungus, such as (+)-terrein (**23**), (*E*)-1-(2, 4-dihydroxy-3-(2-hydroxyethyl)-6-methoxyphenyl) but-2-en-1-one (**24**), and phomalichenone A (**25**) (Fig. 5.6), are reported to have strong anti-inflammatory activity. (+)-Terrein, isolated from *Aspergillus terreus*, is reported to have a wide range of bioactivities including anticancer and anti-inflammation. The compound has been studied for its anti-inflammatory activity against pulpal inflammation in pulp cells, which revealed that its anti-inflammatory properties prevented LPS-induced activation of AKT but not MAPKs, and its aftereffects reduced LPS-induced expression of adhesion molecules such as ICAM-1 and VCAM-1, and terrein protected HDF cells from oxidative stress and suppressed SASP inflammatory indicators such as COX-2, ICAM-1, TNF-α, and IL-1 in a study where oxidative stress-induced inflammation in old HDF cells brought on by the Nrf2/ERK1/2/HO signalling pathway. The compound was found to suppress age-related signalling, including p-EKR1/2 (Lee et al. 2008; Lee et al. 2015; Raistrick and Smith 1935).

Phomalichenone A and (*E*)-1-(2, 4-dihydroxy-3-(2-hydroxyethyl)-6-methoxyphenyl) but-2-en-1-one are phenolic compounds isolated from the

endolichenic source *Phoma* sp. (EL002650). Both these structures are having the same scaffold but differences in hydroxylation at various positions of phomalichenone A. These compounds produce anti-inflammatory effect through nitric oxide production in LPS-induced RAW 264.7 macrophages and the observed IC_{50} values were 9.4 and 7.4μM, respectively. Phomalichenone A in LPS-stimulated RAW264.7 cells suppressed mRNA transcription of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6. The study suggested the unsaturation on the side chain of phomalichenone A might be contributing to its bioactivity (Kim et al. 2018; Ayer and Jimenez 1994).

5 Anti-Alzheimer Compounds

Alzheimer's disease (AD) is a neurodegenerative condition characterized by amyloid β peptide aggregation in fibrillary plaques (Rochet and Lansbury 2000). The approach for AD prevention involves reducing the neurotoxic activity of Aβ, suppressing the β- and γ-secretases, eliminating Aβ from the brain, and preventing Aβ aggregation (Baglioni et al. 2006). Nodulisporiviridin E (**26**), nodulisporiviridin G (**27**), inoterpene B (**28**), violaceol I (**29**), and violaceol II (**30**) (Fig. 5.7) are compounds isolated from several endolichenic fungi that have been shown to have potent activity in various investigations involving Alzheimer's disease.

Nodulisporiviridin A–H are viridins class of molecules isolated from *Nodulisporium* sp. (No. 65-17-2-1). The anti-Alzheimer potentials of these compounds were evaluated by the ThT assay to evaluate the $A\beta_{42}$ aggregation inhibition at 100μM concentration and observed 99.0% and 98.8% inhibition for

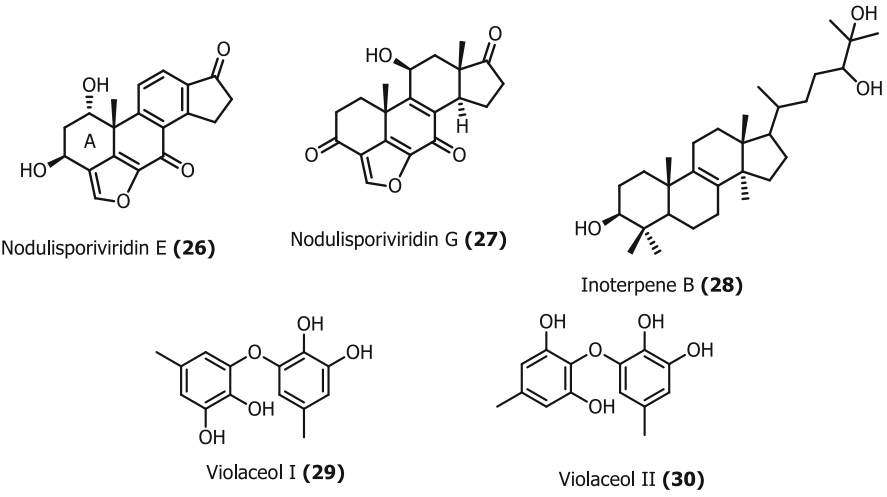


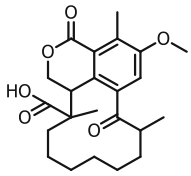
Fig. 5.7 Selected Anti-Alzheimer's molecules from endolichenic fungi

nodulisporiviridin E and G, respectively. Further, it was noted that nodulisporiviridin G showed an IC_{50} value of 1.2μM showing the best potency among the tested molecules. Further, these molecules were studied to check improvement in the AD flies' models by checking the short-term memory, with memantine as a positive control. The flies were subjected to a Pavlovian Olfactory Learning, during this test, and about 100 flies were exposed to methylcyclohexanol or octanol for 60 s at a time, with a 45-s break between each odour. When flies smell the first scent, at the same time they also get a foot shock, a 1.5 s pulse of 60 V with 3.5 s intervals. The immediate memory of the flies is quantified and referred as learning, by giving a choice to the flies in the T-maze between two odours. After that, they are captured in their respective arms, anaesthetized and counted. From how the flies were spread out in the T-maze, a PI was made. If PI = 0, it means that the flies cannot remember an odour that was paired with a foot shock. If PI = 100, it means that all of the flies ran into the other T-maze arm to avoid the odour that was paired with a foot shock. The short-term memory assay resulted in the PI of 49.9 and 42.5 for nodulisporiviridin E and G, respectively. Therefore, the 3-carbonyl group is likely to play a crucial role in the inhibition of $A\beta_{42}$ aggregation, whereas the modifications to rings C and D might have a minor impact on the activity (Zhao et al. 2015).

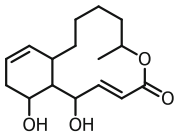
Violaceol I and violaceol II belong to the class of diphenyl ethers isolated from a strain of endolichenic fungus *Aspergillus* sp. (No. 16-20-8-1). Violaceol II differs from violaceol I in which the methyl group is present at the fourth position and hydroxy group at the second position instead of 3′ and 5′ positions. The diphenyl ethers are said to be having anti- $A\beta_{42}$ aggregation inhibitory activity as violaceol I and violaceol II showed 74.7% and 73.1% inhibition at 100μM with IC_{50} values of 5.1 and 2.3μM, respectively (H. Zhao et al. 2014). Inoterpene B is a lanostane-type triterpenoid, first isolated from the white-rot fungus *Inonotus obliquus* (Hymenochaetaceae) and also isolated from the endolichenic source from the fungal strain *Nodulisporium* sp. (No. 65-12-7-1). The Aβ42 aggregation inhibitory activity of inoterpene B was found to be 48.6% at 100μM when evaluated using the ThT test with epigallocatechin gallate (EGCG) as a positive control (Nakamura et al. 2009; Zheng et al. 2013).

6 Antioxidant Compounds

Polyketides from the endolichenic fungi are known to have potent free radical scavenging activity. A chemical characterization study of endolichenic fungus *Curvularia trifolii* collected from Sri Lanka revealed polyketides, namely 5-methoxy 4,8,15-trimethyl-3,7-dioxo-1,3,7,8,9,10,11,12,13,14,15,15 adodecahydrocyclododeca [de]isochromene-15-carboxylic acid (**31**) and 1,14-dihydroxy6-methyl-6,7,8,9,10,10a,14,14aoctahydro-1H benzo[f] [1] oxacyclo-dodecin-4 (13H)-one (**32**) (Fig. 5.8). These molecules contain a macrocyclic ring structure specifically macrocyclic ketone and macrocyclic lactone, respectively.



5-methoxy- 4,8,15-trimethyl-3,7-dioxo-1,3,7,8,9,10,11,12,13,14,1 5,15a-dodecahydrocyclododeca[de]isochromene-15-carboxylic acid **(31)**



1,14-dihydroxy-6-methyl-6,7,8,9,10,10a,14,14a-octahydro -1H-benzo[f][1]oxacyclododecin-4(13H)-one **(32)**

Fig. 5.8 Selected anti-oxidant molecules from endolichenic fungi

Both the molecules display potent antioxidant activity in in vitro conditions with IC_{50} and 1.3 and 4.0 mg/mL, respectively, in the DPPH antioxidant assay (Samanthi et al. 2015).

7 AChE Inhibitors

AChE inhibitors or anti-cholinesterases prevent the cholinesterase enzyme from degrading acetylcholine, hence, prolonging and enhancing the effects of the neurotransmitter. Acetylcholinesterase is involved in inhibiting impulse transmission by the breakdown of the neurotransmitter acetylcholine in several cholinergic pathways in the central and peripheral nervous systems (Colovic et al. 2013). Promising AChE (Acetylcholinesterase) and BChE (butyrylcholinesterase) inhibitors are produced by various natural sources including endolichenic fungi. Ophiosphaerellins A−I (bicyclo[4.1.0] heptenones) and their biosynthetic analogues ophiosphaerekorrins A−B (oxaspiro[4.4]nonenone) showed AChE inhibitory activity, isolated from *Ophiosphaerella korrae* (Li et al. 2018).

The compounds were initially assessed for their capability to prevent AChE utilizing a thin-layer-bioautographic technique (Marston et al. 2002). It is a simple and efficient bioautographic enzyme assay method in which TLC plates was prepared using acetylcholinesterase and butyrylcholinesterase enzymes to evaluate the plant extracts and pure compounds. The transformation of naphthyl acetate into naphthol and the production of the corresponding purple diazonium dye with Fast Blue B salt allowed enzyme activity identification. White spots will appear on the TLC plate's dye-coloured background when cholinesterase inhibitors are present in the extract/compound. The assay's sensitivity was evaluated using the alkaloids galantamine and physostigmine, which are acetylcholinesterase inhibitors. For ophiosphaerellin C (**33**), ophiosphaerellin I (**34**), and ophiosphaerekorrin A (**35**) (Fig. 5.9) to inhibit AChE a minimum quantity is required 1.25, 30, and 10μg, respectively, on the TLC plate method. Ophiosphaerellin C was found to be a potent AChE inhibitor with the observed activity at 1.25μg (Li et al. 2018).

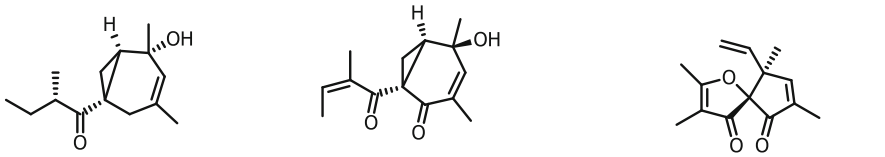
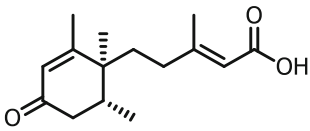


Fig. 5.9 Selected AChE inhibitor molecules from endolichenic fungi

Fig. 5.10 Selected phytotoxic molecules from endolichenic fungi



Structure–activity relationship study based on the natural analogues of ophiosphaerellins indicated that the retention of stereo centres (C-1 and C-5) is necessary for the AChE inhibitory activity. The configuration of 1S and 5R may be necessary for the activity since ophiosphaerellin C has shown better potency than the other tested ones. Ophiosphaerekorrin A have oxaspiro[4.4]nonenone scaffold in their structures and have double bond in the furan ring. This compound was found to be biologically active, while its analogue with the absence of the double bond was inactive indicating the bioactivity of ophiosphaerekorrin scaffold might be due to the unsaturated furan ring.

8 Phytotoxic Compounds

Phytotoxicity is defined as a delay in seed germination, an obstruction to plant development, or any other unfavourable influence on plants caused by specific substances (phytotoxins) or cultivating conditions (Blok et al. 2019). Sesquiterpenoids, perylenequinones, and aromatic polyketides derived from the endolichenic fungus *Ohiospharella korrae* of the lichen *Physeiceae physica* showed significant cytotoxicity against the A549 cell line. *Arabidopsis thaliana* was used as a test model to screen these metabolites to check inhibitory effect on root elongation activity. In this study, ophiokorrin (**36**) (Fig. 5.10) demonstratedgrowth inhibition on root development during the germination of *A. thaliana* with an IC_{50} value of 18.06μg mL^{-1} which was equivalent to a well-established phytohormone abscisic acid (Li et al. 2019).

9 Antithrombotic Compounds

Platelet aggregation and coagulation are the two fundamental processes that lead to thrombogenesis (clot creation). While active platelets bind to fibrinogen strands during platelet aggregation, coagulation is a complicated series of enzymatic processes that result in the creation of fibrin strands. Antithrombotic medications prevent the development of fibrin strands (anticoagulants), prevent platelet aggregation (antiplatelet medications), and break existing clots (fibrinolytic) (Becker 2013). An endolichenic fungus *Xylaria* sp. is a rich source of unusual molecules with various biological functions. A *Xylaria* sp. from lichen *Parmelia* sp. showed the presence of xylarins A–D (**37–40**) (Fig. 5.11) containing 5/6/55/6 polycyclic scaffold with a dihydrobenzofurone unit and an isoindoline unit. In case of xylarin C–D, an additional N, N-dimethylaniline unit is present (Becker 2013). Iterative polyketide synthases (IPKSs) are responsible for the biosynthesis of xylarins (Cox 2007).

Xylarins have shown antithrombic action in the Zebrafish larvae model using 24-well cell culture plates filled with AB zebrafish larvae at 3 dpf (days post-fertilization) with 10 larvae per well divided into three groups' vehicle (0.4% DMSO,) control (aspirin), and the model group. The plates were incubated at 28 ° C for 1 h, and the zebrafish larvaes' morphology was examined by staining them with 1.0 mg/mL O-dianisidine dye for 10 min. The thrombus of the zebrafish larvaes' caudal vein was then observed under a fluorescence microscope and measured in accordance with the staining intensity (SI) of erythrocytes. It was observed that xylarin B exhibits very marginal antithrombic activity, while xylarin A having 2S and 3'R configuration exhibited considerable antithrombic activity at a concentration of 5μM. Xylarin A and xylarin B were found to be non-toxic when tested in cytotoxic assay (Xu et al. 2021).

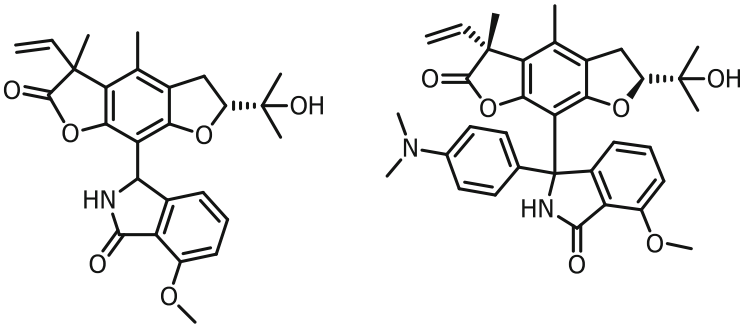


Fig. 5.11 Selected antithrombotic molecules from endolichenic fungi

10 Conclusion

Endolichenic fungi are emerging as an alternative source of novel bioactive scaffolds. Since its first report from 2007, more than 672 molecules have been isolated from endolichenic fungi out of which 145 are unique to ELF. In this chapter, we discussed the potent scaffolds derived from ELF for various pharmacological activities, such as ophobolins for anticancer, phomalichenone for anti-inflammatory, xylarins for antithrombotic, and viridin analogues for anti-Alzheimer's, among others. The literature on these compounds was thoroughly examined and the critical outcomes, including in-vitro and in-vivo animal studies, were discussed. This chapter clearly shows that ELF secondary metabolites have enormous potential for development as therapeutic candidates; nevertheless, additional investigations such as toxicity, pharmacokinetics, and clinical trials are required. One of the most evident challenges is low isolation yield, which necessitates the use of one strain many compounds (OSMAC), response surface method (RSM), genetic tools, and other strategies such as cocultivation and epigenetic modification. Thus, more efforts in this direction will be required to witness better pharmaceutical leads.

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Part III
Marine Fungi as a Source of Medicinal Compounds

Chapter 6
Antibiofilm Metabolites from Sponge-Derived *Aspergillus*, *Penicillium*, and *Fusarium* for the Antibiotic Pipeline

RuAngelie Edrada-Ebel, Amenay Michael, Fatemah Alsaleh, and Hannah Binti Zaharuddin

Abstract Antibacterial resistance has recently been a huge burden on the pharmaceutical industry and a global threat to human's health. Biofilm formation is a complex group of one or more microorganisms grouped together forming an extracellular polymeric substance (EPS) matrix that restricts the effectivity of antimicrobial compounds causing bacterial resistance infections. The discovery of new antibiofilm agents to serve us in the antibiotic pipeline is in huge demand as an increasing number of pathogenic bacteria are no longer responding to current antibiotics, resulting in less efficient treatment of infections. The marine environment is a novel source of thousands of natural products that possess various biological activities. Marine sponges are optimal sources for unique chemical scaffolding associated with symbiotic interactions. Marine sponges and their symbionts account for about 30% of the compounds discovered in the past few decades. This literature review will delve into fungal metabolites extracted from sponge-derived *Aspergillus, Penicillium,* and *Fusarium* from 2007 to 2022 covering their antibiotics and antibiofilm potentials. Metabolites collected from various locations could be unique to the collection location due to external factors, such as temperature, pH, salinity, and predators. This review chapter explores the impact of both geographical region and environmental conditions on secondary metabolite production and briefly outlines the current methods used for bioprospecting new antimicrobial agents. Current literature has shown the vast potential of sponge-derived fungal metabolites as potential novel antibiotics to combat the imminent threat of antibiotic resistance globally. Marine sponges are still relatively unexplored and have not been fully utilised as a latent source of new scaffolding and approaches to create novel antibiotics.

Keywords Sponge-derived fungus · Antibiofilm · Antibacterial · Aspergillus · Penicillium · Fusarium · Marine sponges

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1 Introduction

1.1 Antibiofilm Mechanism of Action Versus Antibiotic Resistance

Controlling antibacterial resistance is a primary global concern where current available antibiotics are not as beneficial as before as those treatments can only prevent infection by either directly killing or inhibiting the microorganism causing the disease. Current antibiotics cause a potential interruption in either primary processes or structures in the bacterial cell. This either kills the bacterium or slows down bacterial growth (Tagliabue and Rappuoli 2018). Therefore, it is important to discover new targets that could aid in solving this problem.

Biofilms have a very complex antibacterial resistance that is causing an urgent threat to human health, which urges the pharmaceutical industry to develop new effective antibiofilm agents. Biofilms are sessile, complex microbial communities that encompass microbial pathogens in a self-generated extracellular polymeric substance (EPS) matrix that embeds itself to biotic and abiotic surfaces or aggregates without attaching itself to any surface (Roy et al. 2018). EPS is composed of mucus and DNA originating from the host and its self-produced polysaccharides, lipids, extracellular DNA (eDNA), and proteins. The formation of biofilm has a pivotal role in antimicrobial resistance and promotes the survival of microbial pathogens in uncongenial environments (Parrino et al. 2019). Microbes inhabiting biofilms are up to around a 1000-fold significantly more resistant to antibiotics compared to their planktonic, free-living counterparts (Li and Lee 2017). Forming biofilm protects microorganisms from harsh conditions, such as pH changes, mechanical forces, drugs, and host immune cells, enabling them to develop bacterial resistance (Li and Lee 2017). Therefore, an antibiofilm agent is crucial to tackling the growing antibiotic resistance and understanding how biofilm forms could help design and develop drugs that prevent it (Roy et al. 2018).

Microbial cells preserve the intrinsic and adaptive mechanisms of antimicrobial resistance from their typical planktonic counterparts. The transition from a free-living to a complex matrix environment could act as a physical and chemical barrier for the antimicrobials. Microorganisms embedded in biofilm could produce inhibitory enzymes, which would inactivate antimicrobial activity, further reducing its efficacy, and conjointly generating efflux pumps to reduce the cell permeability of antibiotics (Lebeaux et al. 2014). Furthermore, oxygen availability varies in different regions within the biofilm, which tends to significantly increase the efficacy of antimicrobials on surfaces with more exposure to oxygen, whereas oxygen and nutrient-deprived regions of the biofilm would drive the microorganisms dormant (Roy et al. 2018). Dormancy promotes a reduction in sensitivity to some antimicrobials when microbial cells divide infrequently as antimicrobials will only target actively dividing cells. Additionally, microbial cells in the biofilm have a higher tendency to mutate compared to its planktonic strain, promoting a horizontal transfer of antibiotic-resistant genes in the biofilm environment when it is exposed to a lethal

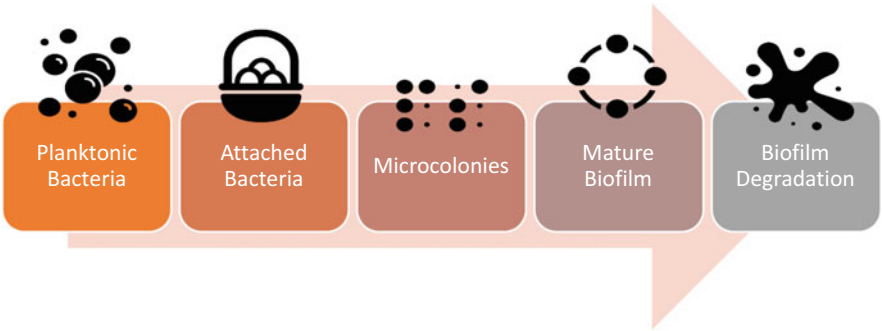


Fig. 6.1 Schematic diagram of biofilm formation

concentration of the antimicrobial (Ma and Bryers 2013). This complex mechanism of resistance leads to a significant increase in the minimum inhibitory concentration (MIC) for antimicrobials to be effective, which could bring the risk of in vivo toxicity (Roy et al. 2018).

Biofilm is the ability of microorganisms to produce a complex matrix by sticking into any surface, living or non-living, including essential medical devices such as vents and catheters, causing severe infections (Kostakioti et al. 2013). The biofilm formation process has several stages (Fig. 6.1) attachment to surfaces, production of a complex extracellular polymeric substance (EPS), maturation, and dispersal to invade another location (Hancock et al. 2021). Biofilms can grow on a variety of surfaces and are known to cause infections that are naturally chronic as preventing bacterial infection is reliant on both one's immune system and antibiotics administration. Biofilms act as chemical or physical barriers to limit the entry of antimicrobials causing antibiotic resistance (Singh et al. 2017).

Antibiofilm agents are molecules that prevent biofilm genesis. The development of new antibiofilm agents for the antibiotic pipeline is of utmost importance. Antibiofilm mechanisms are a new approach to help tackle antibacterial resistance and are demonstrated to either affect biofilm formation or degradation. These naturally derived compounds target different microorganisms via different mechanisms of action. The bioprospecting for new antibiofilm agents should find an effective agent that does not solely inhibit planktonic cell growth as this would impact on exerting selection pressure for antibiotics that target planktonic cell growth thus, increasing microbial evolution. Although planktonic bacteria are known to be more sensitive to traditional antibiotics, they are commonly found in biofilms where traditional antibiotics have no effect on them (Deng et al. 2022).

Some of the modes of action of the compounds are discussed below. Killing bacteria under biofilms is not easy (Stowe et al. 2011), so a combination therapy of an antibiotic and an antibiofilm agent is needed to get better results. Certain enzymes can disperse the EPS in a biofilm, which protects the microbes and exposes them to antimicrobial agents. DNase-I can digest the extracellular DNA, and another

enzyme, glycoside hydrolase, can break extracellular polysaccharide substances, which facilitates the aggregation of bacteria (Kaplan 2009).

The increase in antibiotic resistance in biofilms could be caused by several reasons including the limitation of antimicrobials to diffuse through the biofilm matrix, enzyme-mediated resistance, genetic adaption, heterogeneity in metabolism and growth rate, quorum sensing (QS), and many others (Singh et al. 2017). The development of new antibiofilm agents for the antibiotic pipeline is of utmost importance. Antibiofilm mechanisms are a new approach to help tackle antibacterial resistance and are demonstrated to either affect biofilm formation or degradation. Antibiofilm mechanisms inhibit the formation process of biofilm or by disrupting existing biofilm (Parrino et al. 2019). Several strategies to target biofilms include inhibiting the adherence of the microbial cells on surfaces, and interfering with the fundamental intercellular signalling and regulatory mechanisms within the biofilm (Parrino et al. 2019). Disrupting biofilm architectures target the components of EPS and the three-dimensional structure, thus converting the microbial cells' biofilm environment into its planktonic state (Fleming and Rumbaugh 2017). QS is the system of communication between bacterial cells. QS activates specific signals to facilitate the adaptation of bacteria to their respective environmental condition. This involves the process of synthesising, sensing, and reacting to chemical signalling molecules as extracellular autoinducers (AIs). Autoinducers of the QS system are chemical signalling molecules that synchronise the communication within the system (Pompilio et al. 2022). QS inhibitors (QSIs) achieve antibacterial activity by interrupting virulence factor expression, biofilm formation, bacterial migration, and secretion regulation, which are all regulated by QS. QS inhibitors are less selective on bacteria that could barely cause drug resistance to those types of antibiotics. QSIs' ability to inhibit QS signalling prevents biofilm formation (Zhou et al. 2014). The QS system is a bacterial cell-to-cell signalling mechanism that instigates the synchronisation of factors contributing to the virulence of a microbial colony, including biofilm production and survival of microbial cells in their harsh environments. Thus, reducing bacterial effectiveness without causing drug-resistance highlighting the importance of QSIs as an alternative for novel antibiotics (Yu et al. 2021). At this point, the major aim of antibiotics research is to focus on developing new chemical approaches to cause disruption in these signalling molecules, leading to an antibiofilm effect.

Another antibiofilm mechanism of action is the cleavage of peptidoglycan in the bacterial cell wall, which prevents biofilm formation by changing the composition of the proteins and teichoic acids found in the cell wall, thus affecting the binding to extracellular molecules (Roy et al. 2018). Moreover, targeting bacterial amyloids is vital in preventing biofilm development as these proteins are essential in cell-to-cell or cell-to-surface interaction (Evans et al. 2018). Making the cell membrane more permeable using an antibiofilm agent could be effective based on laboratory results performed in animal models to heal wounds (Weigelt et al. 2021). Antimicrobial peptides can also be used as antibiofilm agents by binding to the lipopolysaccharide (LPS) and destabilising the lipid layer, thus affecting the cell membrane integrity and breaking down the EPS matrix (Luo and Song 2021).

Table 6.1 List of bioassays used to determine the mechanism of action of antibiofilm compounds

Mechanism of action of antibiofilm	Bioassay followed
Membrane distribution: Destruction of membrane potential or membrane permeabilisation	– Minimum biofilm inhibitory concentration (MBIC)
	– Minimum biofilm eradication concentration (MBEC)
	– Membrane demoralisation (DiBAC4(3) assay)
	– Membrane damage (Sytox green, PI, Syto-9, ATP release)
Cell signalling quorum sensing or twitching motility	– MBIC
EPS degradation	– MBEC
Stringent response inhibition	– MBIC
	– MBEC
	– Co-precipitation; ^{31}P NMR
Other targets	– MBIC
	– MBEC e.g., gene down-regulation/targeting

Understanding the mechanism of action of antibiofilm compounds is an important step in developing them into clinically used drugs. Some biological assays have been carried out to determine the mechanism of action of various secondary metabolites and are briefly summarised in Table 6.1 (Raheem and Straus 2019). The limitation of most studies on new potential antibacterial was that not all of them were tested for antibiofilm activity, thus their capability to inhibit biofilm formation remains unclear.

Antibiotic resistance develops because of improper or overuse of antibiotics due to empirical prescribing. Patients infected by resistant microorganisms are left with fewer treatment options, resulting in increased morbidity, mortality, and healthcare expenses (Reygaert 2018). The UK strategy for antimicrobial resistance advises that prescribing should be evidence-based, increasing professional and public awareness of antimicrobial resistance, encouraging researchers for new diagnostics and alternative antibiotics, and collaborating internationally for learning and sharing data from best practices available (Courtenay et al. 2019).

There are several ways where microorganisms develop resistance to antibiotics, including efflux pump, drug inactivation, limited drug uptake, biofilm formation, and modifying their drug target, to name some (Hawkey 1998). However, microorganisms in biofilms develop antibiotic resistance due to spore formation, protection of the biofilm by the EPS matrix, and the slow or incomplete penetration of the antibiotics to the biofilm, thus resulting in low doses reaching the microbes (Sharma et al. 2019), and the slow growth and metabolic rates within the biofilm communities work against antibiotics that target fast dividing cells (Davies 2003).

1.2 Marine Sponge Symbionts as a Potential Source of Antimicrobials

Symbiosis can be defined as "*different organisms living together*" (Oulhen et al. 2016). Two organisms coexist in long-term proximity for nutritional advantages and can be mutualistic, parasitical, or beneficial to only one symbiont in nature (Aslam et al. 2022). Furthermore, the microorganism may exploit its host to generate secondary metabolites, anaerobic metabolism, photosynthesis carbon fixation, and nitrification (Abdelaleem et al. 2022). Symbioses' categorisation depends on the symbionts' ability to survive independently, and they can be categorised by either "obligatory" or "facultative" symbionts. "Obligatory" symbioses are classed as two symbionts dependent on one another for survival, and "facultative" symbioses, also called "optional", are when both symbionts, respectively, can survive independently (Aslam et al. 2022). A symbiosis relationship is a long-term biological interaction between two different biological organisms where both organisms cause no harm to each other but live together in a way that benefits them all. Symbiotic relationships basically include a parasite and a host where, in this case, marine sponges are the hosts, and micro-organisms are the parasitic symbionts. There are three main types of symbiotic relationships, which include mutualism, parasitism, and commensalism. Mutualism is when both organisms benefit, whereas parasitism is when the parasite takes advantage of the host, and commensalism is when one organism benefits, but the other is neither helped nor harmed. Mutualism is the most common relationship in marine sponges (Li 2009).

The marine environment has been known to be one of the natural places that offer a diverse variety of bioactive compounds providing potentially effective antibiofilm agents. Marine sponges live in the ocean, where most of the species are associated with microbes. Although marine sponges are soft-bodied species and do not have the ability to physically defend themselves, they are still known to have protection against microbes and pathogens. In consequence, sponges are in need to rely on a chemical defence to help them survive. Studies and investigations led to the answer that marine sponges tend to have a symbiotic relationship with microorganisms to sustain themselves (Lopanik 2014). Marine sponges are a habitat of many diverse microorganisms, where some microbes reach about 40% of the total sponge biomass. So far, more than 9000 species of sponges from marine and freshwater have been identified and grouped into four broad classes, namely Demospongiae, Calcarea, Hexactinellida, and Homoscleromorpha (Carrier et al. 2022). Most sponges are found in tropical, polar, and temperate regions (Taylor et al. 2007). The global sponge microbiome project that aimed to study the sponge microbial diversity revealed more than 39 microbial phyla from some sponges collected from various geographical regions (Moitinho-Silva et al. 2017). In addition to bacteria and viruses, some eukaryotes, such as microalgae and fungi, are harboured within sponges and have symbiotic relationships (Carrier et al. 2022).

Sponges are filter-feeders, which mean that when water enters through the tiny pores on the sponge surface to enter the canal, microorganisms and organic particles

are filtered out and then absorbed within the sponge. As multicellular filter-feeding organisms, sponges are highly efficient at circulating water containing nutrients and waste removal through their many pores (Kiran et al. 2018). Their feeding mechanism exposes them to different microbes in the surrounding environment. Many microbes can evade the sponge digestive system and tolerate the sponge defence mechanisms, thus allowing them to proliferate inside the sponge and contribute to symbiosis. The symbionts are generated from vertical and horizontal transmissions, where the former is through reproduction, and the latter is via the feeding process (Taylor et al. 2007). Any microorganisms that can tolerate the digestive process and immune response are able to inhabit sponges. Filter-feeding increases the chance of establishing symbiotic relationships between sponges and microbes (Schönberg 2016). Micro-organisms usually harbour on marine sponge surfaces, their canal systems, and intercellular spaces. Marine sponges offer rich nourishment and safe habitats for those micro-organisms as both the surfaces and the internal spaces of marine sponges are more nutrient-richer than the surrounding seawater (Carrier et al. 2022). Marine sponges (phylum Porifera) are optimal sources for unique chemistry associated with symbiotic interactions. Marine sponges have well-defined pore structures that allow them to host diverse varieties of marine microorganisms (Al-Saleem et al. 2022). Marine organisms and microbes are considered the main source of secondary metabolites with antimicrobial activity.

The role of microorganisms in symbiosis with sponges is not fully understood. However, it is believed that they contribute to the synthesis of secondary metabolites, which are vital for the defence mechanisms of sponges against pathogens, predators, and other competitors for space and nutrients (Flórez et al. 2015). Additionally, microbes play a crucial role in the carbon dioxide fixation, uptake and conversion of dissolved organic substances, photosynthesis, vitamin synthesis, nitrification, and nitrogen fixation. In turn, sponges provide a habitat for these microbes and a source of nutrients by filtering large volumes of seawater containing essential nutrients. Sponges can differentiate their symbionts from other organisms via their innate immunity (Pita et al. 2018; Taylor et al. 2007). Microorganisms stabilise the sponge skeleton to help build chemical defence against other pathogens, thus gaining them protection. Additionally, sponge symbionts are also responsible for sponge metabolism, growth, and adaptation. Microbial symbionts play a critical role in the maintenance of sponge health, including being a nutrient source, shielding sponges against oxidative stress, providing UV protection, and eliminating toxic waste products (Zhang et al. 2022).

As symbionts could be responsible for the production of bioactive secondary metabolites, studies have experimentally indicated that those compounds defend their respective host marine sponges. Moreover, these metabolites play an ecological role for the sponge as a barrier against competitors, microbes, and marine fouling organisms (Abdelaleem et al. 2022). Symbioses of microbial symbionts within marine sponges result in the generation of secondary metabolites with significantly varying structures and therapeutic bioactivities that hold vast antimicrobial potential (Aslam et al. 2022). Marine symbionts have recently been a potential target of

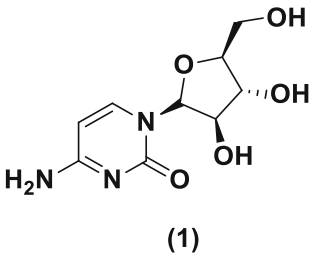


Fig. 6.2 The drug cytarabine to treat leukaemia developed from a marine sponge

pharmacological investigations as those symbionts could be a potential source for the development of new antimicrobials and antibiofilm drugs (Amelia et al. 2022).

The diversity of secondary metabolites by marine-derived microorganisms is the direct result of the marine environment's hyper-salinity and high pressure. These secondary metabolites' scaffolds are more diverse compared to those produced by terrestrial species. The secondary metabolites produced by sponge-derived microorganisms exhibit various properties, such as cytotoxic, antimicrobial, anti-inflammatory, antioxidant, antiplasmodial, antihypercholesterolaemic, and neuroprotective activities. In addition to this, the secondary metabolites could also act as growth regulators, virulence factors, and communication signals with organisms (Al-Saleem et al. 2022). The biological activities of these secondary metabolites could be more potent than synthetic compounds, and in 40% of their chemical scaffolds, these natural products occupy a chemical space that synthetic molecules do not (Aslam et al. 2022).

The marine environment is a novel source of thousands of biologically active secondary metabolites. Approximately, 30% of all the products were derived from marine sponges (Brinkmann et al. 2017), and they also account for about 75% of all anticancer drugs (Koopmans et al. 2009). These naturally active products are a source of many molecules with promising pharmacological potential in drug discovery. Some have already been used to produce antimicrobials, antimalarials, antifungal, antiviral, antifouling, anti-inflammatory, and anticancer agents. However, only a few molecules have antibiofilm effects. Marine sponge metabolites have garnered increasing interest among researchers since the development of the drug cytarabine **(1)** (arabinofuranosylcytidine or ara-C as shown in Fig. 6.2) to treat leukaemia (Stowe et al. 2011). Currently, few marine-derived products are approved as drugs by the responsible authorities, namely the Food and Drug Administration (FDA) and the Medicines and Healthcare Products Regulatory Agency (MHRA), while there is an increasing number of marine natural products that are now in pre-clinical and clinical trials to treat various human diseases, such as viruses, cancer, pain, Alzheimer, and infectious diseases (Lindequist 2016).

The aim of this review is to analyse whether marine invertebrates are chemically protected against pathogenic microbes in the ocean by antibiofilm metabolites from endosymbiotic fungi, with a special focus on *Aspergillus, Penicillium,* and *Fusarium* species derived from marine sponges. Additionally, this chapter demonstrates the significance of geographical regions of the species and its metabolites production,

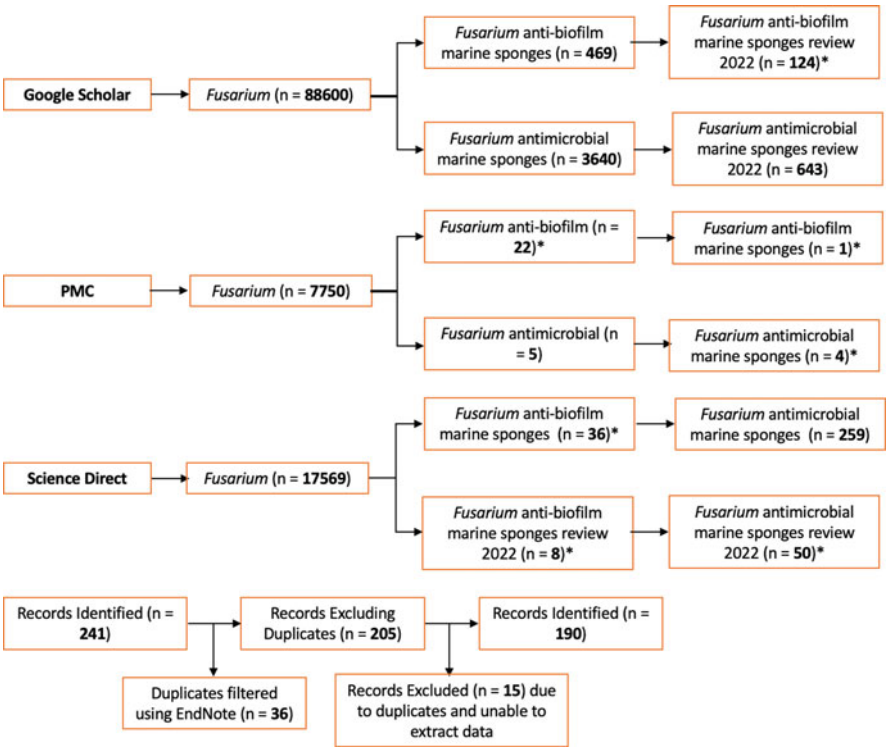


Fig. 6.3 An example of a flowchart illustrating keywords and the number of records obtained used to source the relevant papers to review the literature on *Fusarium* species

and the various methods used in the extraction of its antibiofilm metabolites. In the last 15 years, there has been a significant increase in global antibiotic consumption, hence the increasing rise of the emergence of antimicrobial resistance (AMR) in the twenty-first century (Prestinaci et al. 2015; Browne et al. 2021). The objectives of this chapter are to analyse and examine various literature within the previous 15 years (2007–2022) on the antibiofilm metabolites of the sponge-derived fungal species, particularly those of the most widely investigated genera: *Aspergillus, Penicillium,* and *Fusarium.* Secondly, to be able to correlate metabolite production and the productivity of the extracted metabolites with the geographical distribution of the host species.

It has been hypothesised that marine-derived fungal strains, such as *Aspergillus, Penicillium,* and *Fusarium* species, found on marine sponges provide marine invertebrates chemical protection against pathogens in the ocean through exhibiting biological properties via their antimicrobial and antibiofilm metabolites. Online databases, such as PubMed, Google Scholar, and Science Direct, were used to search literature papers and review papers from 2007 to 2022 as illustrated in Fig. 6.3. Some of the keywords used were antibiofilm, antibiotics, marine sponges, and review 2022, which are listed below in Table 6.2.

Table 6.2 Keywords used, and the number of hits obtained from different databases

Keywords	Number of hits		
	PubMed	Google Scholar	Science Direct
Antimicrobials	886,695	167,000	293,073
Aspergillus	32,007	524,000	81,035
Fusarium	7750	88,600	17,569
Antibiofilm	6361	19,200	4880
Marine sponges	5549	42,100	21,064
Antimicrobial marine sponges	943	17,700	3486
Aspergillus marine sponges	108	16,300	1641
Antibiofilm marine	30	3740	210
Antimicrobial marine sponges review 2022 sponges	19	11,800	140
Antibiofilm marine sponges review	3	934	9
Aspergillus marine sponges review 2022	1	3330	57
Antibiofilm, *Penicillium*, review 2022	0	1690	23

2 Secondary Metabolites and New Potential Antibiotics

Metabolites are small molecules that are the end products of a metabolic reaction and are characterised by either primary or secondary metabolites. Primary metabolites are products that are used by living organism's development, reproduction, and growth and are also essential for maintaining physiological processes. On the other hand, secondary metabolites, which are known as natural products, are organic compounds produced after the rapid growth phase from living organisms, such as bacteria, fungi, plants, and animals, as well as marine organisms, such as sponges. Secondary metabolites are usually small molecules with a molecular weight of $<$1500 Dalton (Da), which are not involved in physiologic processes as they are not required for either the growth or the reproduction of the living organism. However, their production plays an essential role in ecological interactions and other activities (Jiang and Pawliszyn 2014). Secondary metabolites serve their natural resources a selective advantage and are classified according to their function, biosynthesis, and structural diversity, depending on their chemical structure. Organising secondary metabolites depending on their chemical structure would consist of five main classes that include alkaloids; fatty acid-derived substances; polyketides, terpenoids, and steroids; enzyme cofactors; and non-ribosomal polypeptides. The major role of secondary metabolites is to act as (1) weapons against other pathogens attacking their host, (2) metal transportation agents, (3) symbionts, (4) sexual hormones, and lastly (5) differentiation effectors (Oladipo et al. 2022).

Marine secondary metabolites turned out to be an attractive field of chemistry that is being used as a source of medicine where over 200 new compounds are being isolated each year with 23% of them being approved as drugs. The spectrum of

biological activities performed by those secondary metabolites is broad, where they can act as antitumour, cancer-preventive, anti-inflammatory, and antimicrobial including antiviral, antifungal, antimalarial, antibiofilm, and other activities. Nearly half of the metabolites described were for their anticancer potential due to long-term intense research in this field for decades. The class of antibacterial compounds is ranked the second largest number of metabolites, which would conceivably lead to a new era of developing a pipeline of novel antibiotics (Hong et al. 2022). As the ocean is known to be the largest habitat on earth containing vast biological and chemical diversity of marine organisms, secondary metabolites produced by these organisms have a remarkable role in protecting their host with unique chemical defence. This gives us the opportunity to identify new chemical mechanisms and use them for therapeutic applications to serve us pharmaceutically in developing new drugs derived from marine natural products. As these secondary metabolites putatively protect their host organisms in a unique mechanism of action, then, hypothetically, these natural products could also as potential drugs serve human beings in curing several diseases (Lee et al. 2021).

Numerous secondary metabolites have been isolated from marine natural products (MNPs) through investigations of the symbiotic relationship between marine organisms and microbes. Marine sponges are rich in microbial communities, leading to the discovery of a plethora of novel metabolites annually. Several of these metabolites have been found to exhibit antitumour, antiviral, antioxidant, and antimicrobial properties (Balakrishnan et al. 2014). Similarly, new therapeutic agents have been discovered through secondary metabolites of sponge-derived fungi. These secondary metabolites have also been shown to exhibit antimicrobial, cytotoxic, antioxidant, and antibiofilm activities (Al-Saleem et al. 2022). Moreover, some of these secondary metabolites' scaffolds are not produced by terrestrial species and are unique only to the marine ecosystem (Al-Saleem et al. 2022). This part of the chapter will highlight metabolites derived from *Aspergillus, Penicillium,* and *Fusarium* sp., the most commonly derived fungus from various marine sponge families that showed promising antibiotic activity (El-Bondkly et al. 2021). It is important to note that while many metabolites have been identified, not all possess antibiotic properties and may have alternative pharmaceutical applications. Therefore, those that do not possess antibiotic activity have been excluded from this review.

2.1 Antimicrobials

Multidrug resistance is a growing global health crisis that is a direct consequence of abuse and prolonged use of antibiotics. The development of novel antibiotics is a pressing need to combat the growing resistance against conventional antibiotics occurring globally. The rate of discovery and distribution of novel antibiotics currently fall behind the increasing rate of bacterial resistance (Li et al. 2022b).

2.1.1 Aspergillus Antimicrobial Metabolites

Chevalone E (**2**), a compound from *Aspergillus similanensis* derived from a sponge collected from the Thailand coastline, showed a synergistic effect against *S. aureus* when combined with oxacillin, with a *minimum inhibitory concentration (MIC)* value of >256 μg/mL, against many gram-positive and gram-negative microbes, including *Candida albicans,* but exhibited no other effect on its own. The loss of activity of chevalone E was accounted for by the absence of specific substituents on the meroditerpene structure (Prompanya et al. 2014). The presence of specific functional groups drove the antibiotic or antibiofilm activity of the extracts. For instance, the absence of the β-acetoxyl group on C-3 and a free 4-hydroxy-6-methyl-2H-pyran-2-one ring on C-15 on the meroditerpenoid structure of chevalone E (**2**) indicated the loss of its antimicrobial activity against the test microbes (Prompanya et al. 2014).

A literature review from 1975 to 2016 listed 77 butyrolactone derivatives isolated from the genus *Aspergillus*, where 11 compounds were derived from marine sponges (Ibrahim et al. 2017). Butyrolactone I (**3**) was a weak inhibitor of *Bacillus cereus* with an MIC value of 64 mg/mL but displayed an outstanding efficacy against *Clavibacter michiganensis* (Ibrahim et al. 2017). The compound butyrolactone VI (**4**) isolated from *Aspergillus* sp. extracted from *Cliona chilensis* collected from Chile (Pacific Ocean) was effective only against gram-positive *Clavibacter michiganensis* with a minimum inhibitory quantity (MIQ) value of 50 μg when evaluated using the disc diffusion method (San-Martin et al. 2011).

An *Aspergillus* species derived from the sponge, *Chondrilla nucula*, collected from the Aegean Sea in Turkey afforded 13 compounds, where four compounds, asperchondols A (**5**) and B (**6**), expansol D (**7**), and diorcinol (**8**), showed antibacterial activities against *S. aureus, S. epidermidis*, and *Enterococcus faecium* (Liu et al. 2016). Expansol D (**7**) exhibited more potent activity against *E. faecium* than the positive control moxifloxacin, a quinolone antibiotic as positive control with MIC values of 6.25 and 7.78 μM, respectively (Liu et al. 2016). The presence of specific functional groups in certain positions of the phenyl ring played a vital role in the antibiotic activity.

Tubingenoic anhydride A (**9**), from A. *tubingensis* isolated from *Ircinia variabilis* collected from the Mediterranean Sea, was effective against *Neurospora crassa.* The MIC was 300 μM (Koch et al. 2014). The mode of action was reported as affecting the integrity of cell walls. From an Indonesian sponge, *Xestospongia exigua, A. versicolor* was isolated and given seven tricyclic chromones. One of which is aspergillitine ***(10)***, which showed a moderate antibacterial effect on *Bacillus subtilis* (Lin et al. 2003).

Another three active 1,3,5-bisabolatriene-7,15-diol congeners are aspergiterpenoid A (**11**), sydonic acid (**12**), and sydonol (**13**), which were isolated from an *Aspergillus* strain derived from *Xestospongia testudinaria* collected from the South China Sea. Sydonic acid had the same MIC value of 2.5 μM as the positive control ciprofloxacin, a broad-spectrum quinolone antibiotic, when assessed on

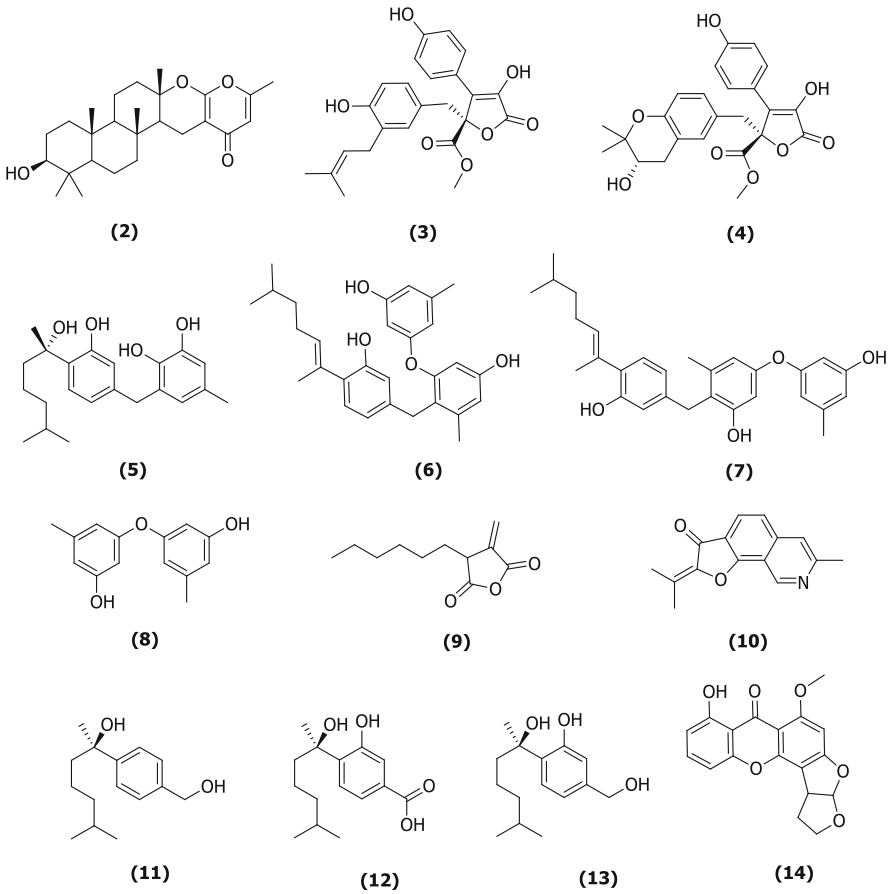


Fig. 6.4 Structure of antimicrobial metabolites isolated from *Aspergillus* sp.

Sarcina lutea (Li et al. 2012). Moreover, another compound, 5-methoxydihydrosterigmatocystin (**14**), afforded by *A. versicolor* isolated from *Hymeniacidon perleve from* Bohai Sea, China, was effective against *B. subtills* and *S. aureus* (Cheng et al. 2020).

The relatively comparable potencies of expansol D (**7**) and sydonic acid (**12**) to their counterpart antibiotic drug standards illustrate the potential of some of the marine-derived *Aspergillus* metabolites (Fig. 6.4) for the antibiotic pipeline.

2.1.2 *Penicillium* Antimicrobial Metabolites

Penicillium is one of the most abundant and ubiquitous fungi that can be found in various ranges of different habitats, especially marine sponges, and has a huge worldwide distribution. The genus *Penicillium* is known to have a large economic

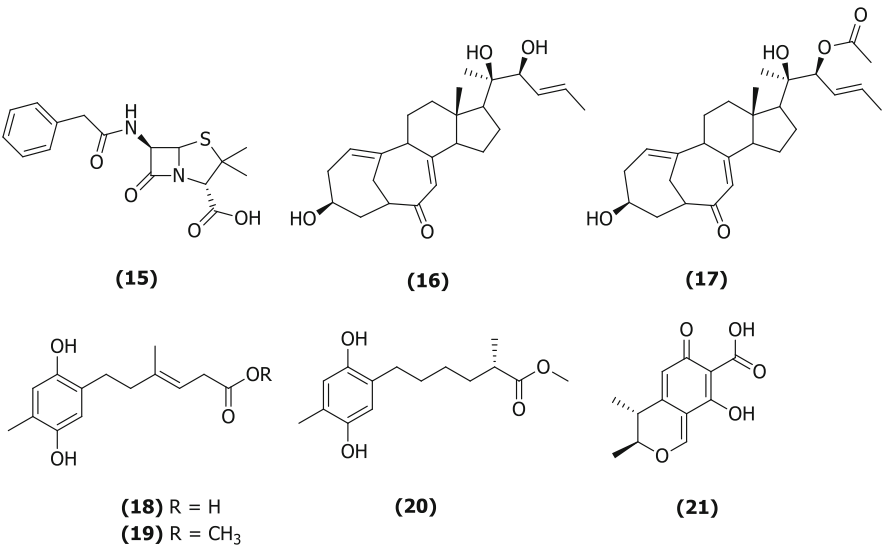


Fig. 6.5 Structure of antimicrobial metabolites isolated from *Penicillium* species

impact on human health, where it can produce a plethora of secondary metabolites that could serve us in both harmful and beneficial ways. Those secondary metabolites have been a valuable promising lead for the pharmaceutical industry. *Penicillium* secondary metabolites (Fig. 6.5) have different biological mechanisms, such as mycotoxins, antibacterial, immunosuppressants: ciclosporins, and cholesterol-lowering agents: statins (Toghueo and Boyom 2020). *Penicillium* was known to produce the β-lactam antibiotic, penicillin (**15**), as the first broad-spectrum antibiotic substance discovered in history. Penicillin is a drug of fungal origin derived from *Penicillium* mould and discovered by a Scottish physician and microbiologist Sir Alexander Fleming. This drug was discovered back in 1928 when Sir Alexander noticed a growing mould on a Petri dish of *Staphylococcus* bacteria where he indicated that the mould was preventing the growth of bacteria by producing antibacterial self-defence chemicals (Tagliabue and Rappuoli 2018). Within the last couple of years, the same story repeats itself with marine sponges highlighting the importance of the role of *Penicillium* in expanding the antibiotic pipeline. The *Penicillium* genus was found to be amongst those that live on marine sponges and has antibacterial chemical-defence, which would be able to protect its hosts from pathogens.

Penicillium comprises more than 200 species that have varied chemical structures. Alkaloids and polyketides are the most common chemical structures in antibacterial *Penicillium* species. *Penicillium* is a remarkable source of synthesising bioactive alkaloids (Zhang et al. 2020). Alkaloids have unique structures that enhance them with unique pharmacological activities and the same applies to polyketides. In regard to both alkaloids and polyketides produced by *Penicillium*,

those compounds are recently been used in the production of new novel therapeutic antibacterial compounds that could tackle the barrier of antibacterial resistance's threat to human's health (Pang et al. 2019).

Two different steroids isocyclocitrinol A (**16**) and 22-acetylisocyclocitrinol A (**17**), isolated from *Penicillium citrinum* from an *Axinella* sponge collected from Papua, New Guinea, exhibited antibacterial activity against *Staphylococcus epidermidis* and *Enterococcus durans* (Cheng et al. 2020). Both steroids **16** and **17** were first described as anti-inflammatory agents.

Penicillium sp. HDN151272 fungal strain was isolated from an Antarctica sponge found in Pyrdz Bay. The fungal extract gave a unique high-performance liquid chromatography (HPLC) profile. Through investigations of the three hydroquinone polyketides, ketidocillinones A–C (**18**, **19**, and **20**) showed antibacterial activity. Both compounds **19** and **20** were tested to have broad spectrum antibacterial activity against *Pseudomonas aeurigenosa, Mycobacterium phlei,* and Methicillin-resistant coagulase-negative staphylococci (MRCNS) (Shah et al. 2020).

Citrinin (**21**) was isolated from various *Penicillium* strains and exhibited its antibacterial activity on several pathogenic bacterial species. Citrinin was isolated from two different regions: the Fiji Islands and the Indonesian coastline, which share the same waters of the Pacific Ocean. Citrinin obtained from the Fiji islands was extracted from *Penicillium* sp. FF001 and tested against *S. aureus*, rifampicin-resistant *S. aureus*, wild type *S. aureus,* and vancomycin-resistant *Enterococcus faecium* (Indraningrat et al. 2016). Citrinin from Indonesia was extracted from *P. citrinum* WK-P9 and tested against *B. subtilis JH642* (Sabdaningsih et al. 2020). The same compound had unique antibacterial activity exhibiting different minimum inhibitory concentration (MIC) values against different microorganisms. Citrinin assayed on various *S. aureus* strains had an average MIC value of 1.95 μg/mL (Indraningrat et al. 2016) while against *B. subtilis,* citrinin was less active with an MIC value of 16 μg/mL (Sabdaningsih et al. 2020).

2.1.3 *Fusarium* Antimicrobial Metabolites

Within the recent 15 years, new therapeutic agents have been reported through secondary metabolites of sponge-derived fungi *Fusarium*. These secondary metabolites have been shown to exhibit antimicrobial, cytotoxic, antioxidant, and antibiofilm activities (Al-Saleem et al. 2022). Moreover, some of these secondary metabolites' scaffolds are not produced by terrestrial species and are unique only to the marine ecosystem (Al-Saleem et al. 2022).

Secondary metabolites from marine sponge-derived *Fusarium* possess antibacterial properties against an array of pathogenic bacteria. Sponge-derived fungi Fusarium produces variable potent extracts that are currently used in antimicrobial drugs. Zearalenone (**22**), for example, is a 14-membered β-resorcyclic macrolide that was obtained from *Fusarium* sp. 05ABR26 living in symbiosis with marine sponges in Japan's Miura Peninsula. Zearalenone exhibited significant

inhibitory activity against *Pyricularia oryzae* with an MIC of 6.25 μg/mL (Karpinski 2019).

Fusarium sp. LY019, associated with the sponge *Suberea mollis* found in the Red Sea, afforded two dimeric alkaloids fusarypyridines A and B. Both fusarypyridines A (**23**) and B (**24**) displayed moderate antibacterial activity against *S. aureus* and *E. coli*. The compounds are the first natural products to possess a novel 1,4-bis (2-hydroxyl-1,2-dihydropyridin-2-yl) butane-2,3-dione backbone. Additionally, both symmetrical dihydropyridine moieties exhibited a highly oxygenated substitution pattern. Fusarypyridine B differed from fusarypyridine A by the occurrence of two additional hydroxy groups (Shaala et al. 2021). Metabolites fusaripyridines A and B showed moderate activity against *E. coli*. Both compounds **23** and **24** inhibited *E. coli* with an MIC of ≥32 μM, respectively. However, the inhibition zones shown by both metabolites were slightly different with fusaripyridine A (**23**), with an inhibition zone of 9 mm, and fusaripyridine B (**24**), with an inhibition zone of 7 mm (Shaala et al. 2021).

The sponge species from Pulau Tinggi, Malaysia, that was associated with *F. proliferatum* was not identified in the published literature (Mosadeghzad et al. 2012). *F. proliferatum* afforded tyrosol (4-hydroxy phenethyl alcohol) (**25**), 2,5-furandimethanol (**26**), adenosine (**27**), and linoleic acid (**28**). Metabolites **25** and **26** showed antibacterial activity against MRSA. The study did not provide the MIC values for compounds **25** and **26** against MRSA. However, inhibition zones were used to describe the potency and sensitivity of the metabolites against MRSA. Tyrosol (**25**) showed an inhibition zone of 7 mm against MRSA, whereas 2,5-furandimethanol (**26**) exhibited an inhibition zone of 8 mm (Mosadeghzad et al. 2012). *S. aureus* developed resistance to methicillin by augmenting the number of resistant genes of each strain via horizontal gene transfer. The horizontal gene transfer in MRSA promotes resistance against conventional antibiotics, thus posing a huge risk if the pathogen invades patients systemically.

Linoleic acid (**28**) is an essential fatty acid; however, this compound was not found to exert any bacterial activity. Adenosine (**27**) is a free nucleotide that is produced abundantly by marine sponges but is not common in other animals. Adenosine showed antibacterial activity against the bacteria *Aeromonas hydrophila*. Additionally, tyrosol (**25**) did not exert any antibiofilm activity despite being a well-known quorum sensing molecule in the yeast *Candida albicans* (Chen et al. 2004). Moreover, metabolites **25** and **26** isolated from this sponge-derived *Fusarium* species inhibited MRSA.

From the Java Sea, aromatic polyketides karimunones A (**29**) and B (**30**) were afforded by *Fusarium* sp. KJMT.FP.4.3 derived from inner tissues of the Indonesian sponge, *Xestospongia* sp. Karimunone A (**29**) was inactive, whereas karimunone B (**30**) possessed significant activity against multi-drug resistant *Salmonella enterica* ser. *Typhi* with an MIC value of 125 μg/mL (Sibero et al. 2019). *Salmonella enterica serotype typhi* is a gram-negative bacterium that is associated with typhoid fevers. The pathogen's plasma-mediated antimicrobial resistance genes and ability to evade defence systems and trigger virulence in the body allow the pathogen to cause

disease and grow resistant to various broad-spectrum antibiotics used in Salmonella-related infections (Khan and Shamim 2022).

Additionally, *Fusarium* sp. ALAA-20 associated with sponge *Aplysina fistularis* found in the Caribbean Sea yielded the statin lovastatin (**31**). Lovastatin exhibited antibacterial activity against multiple drug-resistant *Staphylococcus*, *Pseudomonas*, and *Klebsiella* species. Lovastatin showed antibacterial activity against six *Staphylococcus* strains, three *Pseudomonas* strains, and two *Klebsiella* strains. *Staphylococcus* strains that were sensitive to lovastatin are *S. capitis*, *S. sciuri*, *S. aureus*, *S. saprophyticus*, *S. carnosus,* and *S. schleiferi*. Lovastatin showed activity against *S. aureus* at an MIC of 8 μM (El-Bondkly et al. 2020). *Pseudomonas aeruginosa*, *P. syringae,* and *P. savastanoi* were found to be sensitive to lovastatin. In addition to that, *K. pneumoniae* and *K. oxytoca* were two *Klebsiella* strains that were also sensitive to lovastatin (El-Bondkly et al. 2020). Lovastatin indeed exhibited activity against multiple drug-resistant *Pseudomonas* and *Klebsiella* species (El-Bondkly et al. 2020). Klebsiella species particularly are pathogens that cause severe diseases and mortality in inpatient populations. *Klebsiella* species pathogens are associated with pneumonia, UTIs, and septicaemia, which often results in septic shock (Singh et al. 2016).

Although the number of metabolites isolated from sponge-associated *Fusarium* is limited, fusarypyridines A and B (**22** and **23**), tyrosol (**25**), 2,5-furandimethanol (**26**), adenosine (**27**), karimunone B (**30**), and lovastatin (**31**), all exhibited antibacterial activity against a plethora of pathogenic bacteria. The sensitivity of well-known drug-resistant bacteria species against these *Fusarium* metabolites (Fig. 6.6) suggests that there is a potential to further exploit sponge-derived *Fusarium* as a source for further development of novel antibiotics in the future to combat the growing antibacterial resistance globally.

Lovastatin (**31**) has been traditionally indicated for primary cardiovascular disease prevention, hypercholesterolaemia, and hyperlipidaemia (Taylor et al. 2011). Similar to lovastatin, adenosine is not typically indicated for bacterial infections but is usually indicated for arrhythmias and diagnosing supraventricular tachycardias (Wilbur and Marchlinski 1997). Antimicrobial activities found in statins and nucleotides produced uniquely by marine sponge-derived *Fusarium* could propose an expansion of functionality for these compounds. This could suggest an opportunity for further research on the possible use of statins and nucleosides for a quicker and more efficient solution to address the looming growth of antibacterial resistance. Novel drug discovery and development is cost-consuming and incredibly slow-paced due to regularity requirements to bring them to the market. Drug repositioning for diseases offers the advantage of reduced development costs and a shorter development period because of using de-risked compounds. The approved or experimental drug used would have already been screened in a safety assessment in humans and preclinical models during most of the early stages of the clinical trials, and this would not be required at the drug repurposing stage (Pushpakom et al. 2019). Drug repositioning, also known as drug repurposing, is defined as the process of identifying new indications for approved or experimental drugs outside of their traditional medical indication (Ashburn and Thor 2004).

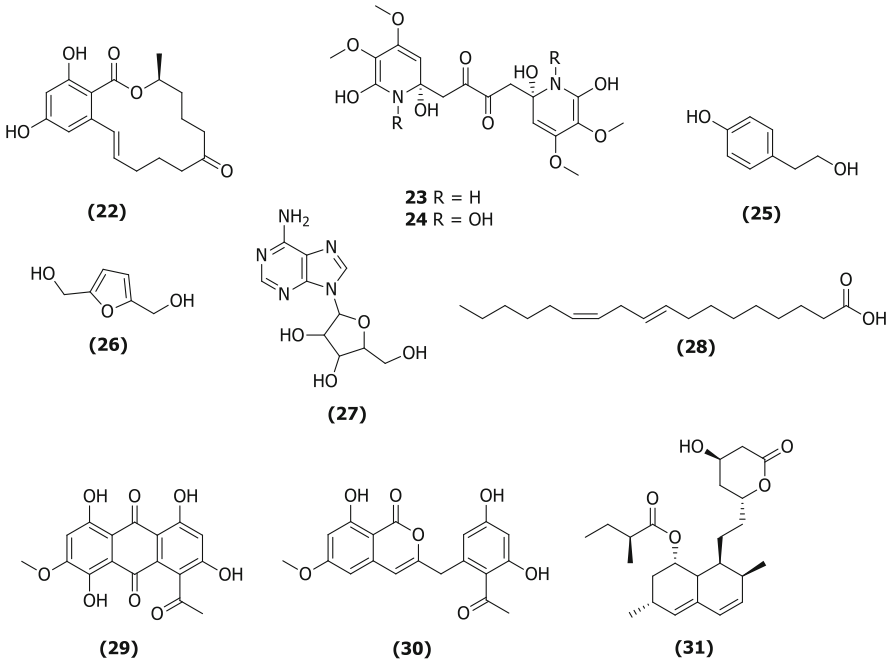


Fig. 6.6 Structure of antimicrobial metabolites isolated from *Fusarium* sp.

2.2 *Antibiofilms*

Biofilms are the cause of about 80% of chronic infections and antibiotic resistance, so tackling their formation should be a priority (Jamal et al. 2018). Some common biofilm-forming microbes are *S. aureus, P. aeruginosa, E. coli, K. pneumoniae,* and so on (Nadar et al. 2022). Marine sponge symbionts are an excellent source of metabolites that could be a novel source for antibiofilm drug discovery. About 12% of all the antibiofilm compounds discovered in the marine environment were isolated from fungi, mainly from *Aspergillus* and *Penicillium*. Recently, 129 compounds from MNPs have been found to have antibiofilm activity. These compounds contain alkaloids, terpenoids, polyketides, peptides, fatty acids, and fatty acyls. Some compounds have both antibacterial and antibiofilm effects, whereas some metabolites have antibiofilm activity without any antibiotic result, meaning bacterial growth is unaffected (Deng et al. 2022).

2.2.1 *Aspergillus* Antibiofilm Metabolites

Aspergillus is a widely available symbiont of mangroves, algae, coral reefs, and marine sponges in different geographical locations. It produces many metabolites

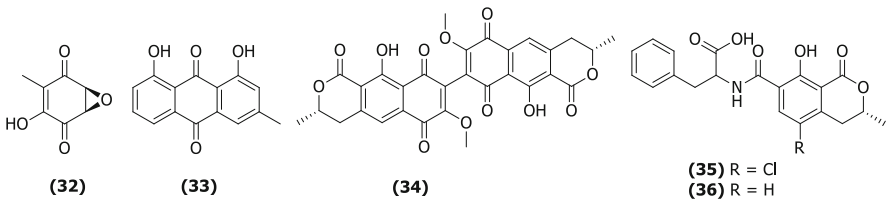


Fig. 6.7 Structure of antibiofilm metabolites isolated from *Aspergillus* sp.

(Fig. 6.7) that could be used for various purposes. The same metabolites can be isolated from various symbionts of marine sponges, such as bacteria or fungi.

Terreic acid (**32**) was isolated from *Aspergillus terreus* DMTMGK004 (Pompilio et al. 2022; Kong et al. 2018). *A. terreus* is a symbiont commonly found in coral reefs, sponges, algae, and mangroves. Compound **32** was assessed on *Streptococcus pneumoniae, Haemophilus influenzae*, and *K. pneumoniae* and showed a promising result. Compound **32** was also evaluated on *E. coli* and inhibited the biofilm formation of the bacteria. It was reported that the antibiofilm mechanism of action of the compound involved the inhibition of EPS production (Pompilio et al. 2022).

Additionally, chrysophanol (**33**), isolated from *Aspergillus candidus* derived from *Epipolasis* sp., was collected off the coast of Similan Island in Thailand. Compound **33** was described to inhibit 70% biofilm formation in *E. coli and P. aeruginosa* on catheters coated with the compound (Hafez Ghoran et al. 2022). The mechanism of action of chrysophanol (**33**) entails inhibiting quorum sensing, and the communication between cells that helps bacteria adjust to any fluctuations by regulating their gene expression (Teixeira-Santos et al. 2022).

Xanthomegnin (**34**), ochratoxin A (**35**), and B (**36**) were isolated from *Aspergillus elegans* associated with *Monanchora unguiculata* and collected from the Indian Ocean (Gupta et al. 2018; Durães et al. 2021). Antibiofilm activity of compounds **34** and **35** was evaluated on *S. aureus*. It showed a higher percentage (>80%) of inhibition of the biofilm formation, although the mechanism of action was not mentioned (Durães et al. 2021).

From the 28 antibacterial compounds from sponge-derived *Aspergillus* metabolites listed in Table 6.2, only xanthomegnin (**34**), from *A. elegans*, was described to exhibit both antibacterial and antibiofilm activity against *S. aureus*. Alternatively, ochratoxin A (**35**), also isolated from *A. elegans*, only possessed antibiofilm activity but no antibacterial effect. Additionally, the presence of a chlorine functional group in ochratoxin A (**35**) but not ochratoxin B (**36**) resulted in a difference in their biological activity, where the former had an antibiofilm effect, but the latter had antibacterial activity. Here, chlorine indicated a vital role in the antibiofilm activity.

From the described antibacterial metabolites of sponge-derived *Aspergillus* in the literature, only 17% were assessed against inhibition for biofilm formation (Fig. 6.8). Therefore, further evaluation of the metabolites would later benefit in determining their effectiveness against biofilm formation. Those described metabolites that showed antibiofilm activity do not interfere with bacterial growth. Thus, combining those antibiofilm metabolites with those designated as bactericidal or bacteriostatic

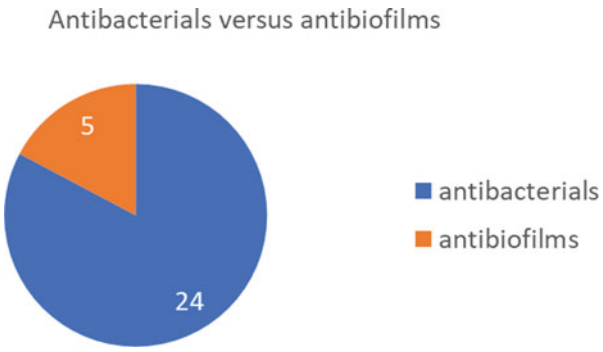


Fig. 6.8 Number of described antibacterial and antibiofilm metabolites isolated from sponge-derived *Aspergillus* reported in the literature between 2007 and 2022

could help tackle global microbial resistance, as about 80% of microbial resistance is due to biofilm formation (Jamal et al. 2018).

2.2.2 *Penicillium* Antibiofilm Metabolites

Penicillium is one of the most promising genera that could provide novel structures for the development of new effective antibiofilm agents. *Penicillium* extracts have been identified to have components (Fig. 6.9) that can act as antibiofilm agents by quorum sensing (QS) inhibition. Patulin (**37**) and penicillanic acid (**38**) extracted from *Penicillium* are known to cause QS inhibition (Asma et al. 2022).

The dipeptide *cis*-cyclo(leucyl-tyrosyl) (**39**) isolated from *Penicillium* sp. derived from the sponge *Axinella corrugata* collected from Arvoredo Biological Marine Reserve in Brazil (Nicoletti and Trincone 2016) was tested against *S. epidermidis* and showed antibacterial activity with up to 85% reduction in biofilm formation at 1000 μg/mL (Indraningrat et al. 2016). Percentages of biofilm formation inhibition caused by different concentrations of compound **39** were at 60%, 65%, and 85% for 0.25, 0.50, and 1.0 mg mL^{-1}, respectively. However, a 2.0 mg mL^{-1} concentration caused no antibiofilm activity whereas afforded bacterial growth inhibition. The concentration 0.125 mg mL^{-1} had no significant activity in inhibiting biofilm formation (Indraningrat et al. 2016). Unfortunately, neither mechanism of action of both antibacterial and antibiofilm activity was explained for this compound.

Taken together, our findings indicate that some compounds have both antibacterial and antibiofilm activities at the same time. However, not all antibacterial compounds have antibiofilm activity, and some can act as antibiofilm but not antibacterial. As examples, both polyketide erubescensoic acid (**40**) and xanthonopyrone SPF-3059-26 (**41**) extracted from *Penicillium erubescens* KUFA0220 from the sponge *Neopetrosia* located at the Samaesan Island, Thailand, was tested for their capability to prevent biofilm formations on four strains. Results indicated that it had no antibiofilm activity against *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, or *Enterococcus faecalis* ATCC 2921, whereas it showed significant antibiofilm activity against *E. coli* ATCC 25922 (Kumla et al. 2019).

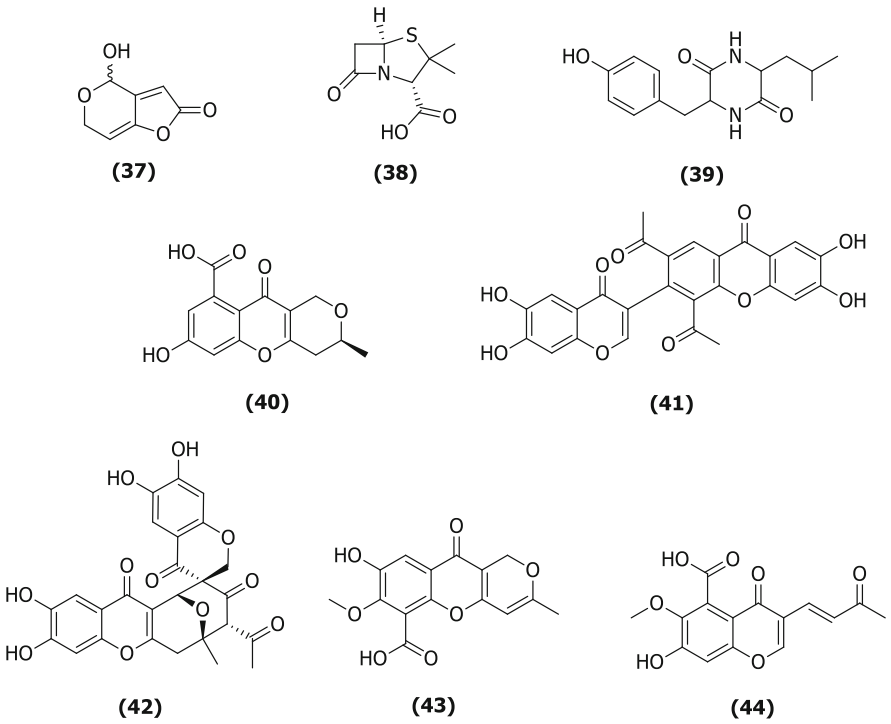


Fig. 6.9 Structure of antibiofilm metabolites isolated from *Penicillium* sp.

On the other hand, compounds **40** and **41** showed no antibacterial activity when tested against three gram-positive bacteria: *S. aureus* ATCC 29213, *E. faecium* ATCC 19434, and *E. faecalis* ATCC 29212 and two gram-negative bacteria: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Antibacterial tests were not carried out for the following compounds that were isolated from the same strain and location: erubescenschromone B (**42**), penialidin D (**43**), and 7-hydroxy-6-methoxy-4-oxo-3-[(1*E*)-3-oxobut-1-en-1-yl]-4*H*-chromen-5-carboxylic acid (**44**), whilst when tested for antibiofilm activity, it showed similar activity to metabolites **40** and **41**. This highlights the fact that the mechanism of action of inhibiting biofilm formation can sometimes be different from the antibacterial mechanism of action (Kumla et al. 2019) where antibiofilm agents focus on indirect strategies of causing inhibition of biofilm formation (Raheem and Straus 2019), while antibacterial agents focus on direct strategies of either killing or slowing down the growth of bacteria (Tagliabue and Rappuoli 2018; Etebu and Arikekpar 2016).

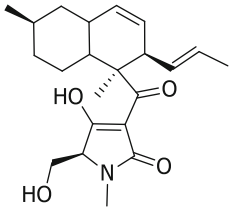
2.2.3 *Fusarium* Antibiofilm Metabolites

From the Atlantic Ocean, the English Channel, the North Sea, and the Mediterranean Sea, the tetramic acid equisetin (**55**) was isolated from three different *Fusarium*

strains associated with the sponge *Tethya aurantium* (Wiese et al. 2011). Additionally, equisetin isolated from *Fusarium equiseti* and *F. heterosporum* showed potent antibacterial activities against methicillin-resistant *S. aureus* (MRSA)*, Enteroccus faecalis, E. casseliflavus, B. subtilis, B. amyloliquefaciens,* and vancomycin-resistant Enterococci (VRE) (Gomes et al. 2021; Wiese et al. 2011; Chen et al. 2021). Equisetin is the most effective agent, significantly exhibiting the lowest MIC value against *S. aureus* compared to three other *Fusarium* metabolites **32**, **33**, and **34**. Equisetin isolated from *F. equiseti* and *F. heterosporum* inhibited the growth of MRSA with an MIC of 1 μM (Chen et al. 2021).

The marine-derived fungi species *Fusarium* suppresses biofilm production and alters its structure by inhibiting quorum-sensing (QS) systems via the secondary metabolite **45** (Fig. 6.10). Equisetin isolated from *Fusarium* sp. Z10 possessed antibiofilm properties against *P. aeruginosa* (Pompilio et al. 2022). Equisetin downregulates the gene expression for prompting the QS system. Inhibiting the QS system results in the pathogenicity reduction of the microbial cells without influencing their growth (Zhang et al. 2018). Additionally, equisetin was also found to be more efficacious against vancomycin-resistant *Enterococci* than most conventional antibiotics (Chen et al. 2021). *Enterococci* is an anaerobic gram-positive coccus that lives symbiotically with humans in the gastrointestinal tract. *Enterococci* is often associated with urinary tract infections (UTI) and intraabdominal infections. VRE is still a problem in many hospitals despite the various treatments and efforts available currently (Levitus et al. 2022). Equisetin's significant efficacy against VRE may pose a potential progress forward in options to reduce VRE's cost and health burden in hospitals.

Despite all sponge-derived *Fusarium,* metabolites mentioned in this review possessed antibacterial activity; only one metabolite that is equisetin exhibited antibiofilm activity. Equisetin, tested against *P. aeruginosa,* showed quorum sensing (QS) inhibitory effects (Zhang et al. 2018; Wiese et al. 2011; Pompilio et al. 2022). Quorum sensing is an intracellular communication mechanism that regulates virulence gene expression, swarming motility, and biofilm formation. QS systems enable bacteria to regulate their cell density by releasing autoinducers to synchronise cell-to-cell communication and adapt to environmental changes. Treating bacterial infections by targeting QS systems has been found to reduce the pathogenicity of said pathogens without altering their growth (Zhang et al. 2018). This is particularly significant with *P. aeruginosa*, a common gram-negative bacterium that is abundant in the environment. *P. aeruginosa* infections still pose a large risk in immune-



(45)

Fig. 6.10 Structure of antibiofilm metabolite isolated from *Fusarium* sp.

suppressed patients as in cancer patients, HIV, and burn victims where their flesh is exposed and more vulnerable (Li et al. 2022b). Even in patient demographics with competent immune systems, *P. aeruginosa* infections are difficult to treat due to their biofilm-based properties (Stewart and William Costerton 2001). *P. aeruginosa* easily develops resistance to conventional antibiotics due to their ability to form biofilms. Their ability to form biofilms is the underlying cause why *P. aeruginosa* is difficult to eradicate and an imminent threat in healthcare (Doshi et al. 2011). However, *P. aeruginosa*'s virulence factors and motility are predominantly controlled by QS systems. Equisetin targeting the QS systems provides a new method to eradicate *P. aeruginosa* infections. This study pushes a new direction of treating bacterial infections prone to resistance by discovering more QS inhibitors or utilising well-known ones.

Some of the antibacterial modes of action reported for the fungal metabolites involved inhibiting the efflux pump, disruption of the cell wall, or synergistic activity with other metabolites. On the other hand, the antibiofilm mode of action included inhibiting EPS formation or obstructing quorum sensing. Most of the published research would still require further studies to gain knowledge on the mechanism of antibacterial activity for the reported compounds (Pompilio et al. 2022). Table 6.3 summarises key information on metabolites, particularly focusing on antibiofilm and antibacterial properties. The table highlights strains from *Aspergillus, Penicillium,* and *Fusarium* sp derived from various sponge species collected from different geographical regions.

3 Global Distribution of Antibacterial Sponge-Derived Fungal Metabolites

The process of biosynthesising secondary metabolites is dependent on a variety of factors. Environmental conditions are the most important factor that could impact secondary metabolite production. Metabolite production depends on several environmental factors including temperature, water activity, interrelationship with other microorganisms, pH, and lastly the way those factors interact in nature. Secondary metabolite production in fungi is often associated with their growth and development. Factors that affect marine fungal growth include salinity, temperature, hydrostatic pressure in the deep-sea, and the anoxic conditions of the sediment. Adding on that, light might have an impact on the reproductive behavior of marine fungi, whereas hydrocarbon and plastic pollution in the sea could have an impact on fungal growth behavior. The availability of nutrients is also expected to impact the metabolism process in sponges, thus affecting fungal secondary metabolite production (Jones et al. 2022). The salinity of the water impacts metabolite production but whilst marine fungi are known to be widely tolerant to changes in the salinity of the ocean with regards to mycelial growth, spore germination, and sporulation, thus being more capable of adapting to changes in their marine environment. Lastly, the

Table 6.3 Summary of identified metabolites with antibiotic activities reported from 2007 to 2022. Highlighted rows represent metabolites reported for their antibiofilm activity

Sponge host	Species/metabolite	Geographical source	Microbial test panel/ microorganisms tested	Activity and/or mechanism of action	Reference
A. *Aspergillus* strains					
Rhabderemia sp.	*Aspergillus similanensis*	Thailand	*MRSA*	Synergistic activity with oxacillin	Prompanya et al. (2014)
	Chevalone E (**2**)				
Chondrilla nucula	*Aspergillus* sp.	Aegean Sea (Turkey) (Mediterranean Sea)	*S. aureus; S. epidermidis; E. faecium*	Antibacterial	Liu et al. (2016)
	Asperchondols A (**5**) and B (**6**), Expansol D (**7**), Diorcinol (**8**)				
Cliona chilensis; Unidentified sp.	*Aspergillus* sp.	Chile (Pacific Ocean); La Cruz (Costa Rica)	*B. cereus; C. michiganensis*	Antibacterial; antifungal	Ibrahim et al. (2017); San-Martin et al. (2011)
	Butyrolactone I (**3**), Butyrolactone VI (**4**)				
Ircinia variabilis	*Aspergillus tubingensis*	Mediterranean Sea	*Neurospora crassa*	Cell wall disruption	Koch et al. (2014)
	Tubingenoic anhydride A (**9**)				
Xestospongia exigua	*Aspergillus versicolor*	Bali Sea (Indonesia)	*B. subtilis*	Antibacterial	Lin et al. (2003)
	Aspergillitine (**10**)				
Monanchora unguiculta	*Aspergillus elegans*	Indian Ocean	*S. aureus*	Efflux pump inhibitors	Durães et al. (2021)
	Ochratoxin B (**36**), *Vioxanthin* (**49**), *Viomellein* (**50**), *Xanthomegnin* (**34**)				

Xestospongia testudinaria	*Aspergillus* sp. *Aspergiterpenoid* (**11**), *Sydonol* (**13**), *Sydonic acid* (**12**)	South China Sea	*E. coli; Micrococcus tetragenus; S. albus*	Antibacterial	Li et al. (2012)
Hymeniacidon perleve	*Aspergillus versicolor* 5-methoxydihydro-Sterigmatocystin (**14**)	Bohai Sea (China)	*B. subtills*; *S. aureus*	Antibacterial	Cheng et al. (2020)
Petrosia sp.	*Aspergillus versicolor* Averantin; methyl averantin; Versiconol; Nidurufin; Averufin	Jeju Island (Korea)	*S. aureus; S. pyogenes; MRSA*	Antibacterial	Lee et al. (2010)
Tethya aurantium	*Aspergillus* sp. Austalide R (**46**)	Mediterranean Sea	*Vibrio* sp.	Antibacterial	Zhou et al. (2014)
Callyspongia sp.	*Aspergillus* sp. Versiconol B	Xuwen County (China)	*S. aureus; MRSA*	Antibacterial	Hafez Ghoran et al. (2022)
Unidentified sponge	*Aspergillus terreus* *DMTMGK004; terreic acid* (**32**)	*Location not reported*	*S. pneumoniae; H. influenzae; K. pneumoniae; E. coli*	*Inhibit EPS production*	Pompilio et al. (2022)
Epipolasis sp.	*Aspergillus candidus* *Chrysophanol* (***33***)	*Similan Island (Thailand)*	*E. coli; P. aeruginosa*	*Inhibiting quorum sensing*	Teixeira-Santos et al. (2022)
B. *Penicillium* strains					
Melophus sp.	*Penicillium* sp. *FF001* Citrinin (**21**)	Lau group, Fiji Islands (10 m)	*S. aureus,* rifampicin-resistant *S. aureus,* wild type *S. aureus* and VRE	Antibacterial	Indraningrat et al. (2016)
Pseudoceratina purpurea	*Penicillium citreonigrum* Unidentified metabolites	Indonesia	Unidentified		El-Bondkly et al. (2021)

(continued)

Table 6.3 (continued)

Sponge host	Species/metabolite	Geographical source	Microbial test panel/ microorganisms tested	Activity and/or mechanism of action	Reference
Axinella corrugata	*Penicillium* sp.	*Arvoredo biological marine reserve, Brazil (ND)*	*S. epidermidis*	*Anti-biofilm*	Indraningrat et al. (2016)
	Dipeptide cis-cyclo (Leucyl-Tyrosyl) (**39**)				
Antarctica sponge	*Penicillium sp HDN151272,* Ketidocillinones A-C (**18–20**)	Prydz Bay	*Pseudomonas aeruginosa, Mycobacterium phlei,* and MRCoNS		Shah et al. (2020)
Unidentified	*Penicillium* sp. *YPGA 11*	Deep-sea	*S. aureus* ATCC 25913		Cheng et al. (2018)
	Unidentified metabolites				
Tedania anhelans	Penicillium chrysogenum	Indian Ocean	*Myobacterium tuberculosis* H37Ra, *M. avium, M. fortitum, M. smegmatis, M. vaccae, S. aureus, Aeromonas hydrophila, P. aeruginosa, Vibrio cholerea*		Visamsetti et al. (2016)
	Unidentified metabolites				
Unidentified	*Penicillium* sp. *LS54*	Data not given	*V. harveyi*		Liu et al. (2019)
	Penicillilactone A; Rugulosin A				
Chondrus ocellatus (seaweed)	*Penicillium echinulatum pt-4*	Pingtan Island, China	*E. coli*		Kuml et al. (2014)
	Arisugacin K				
Rhizophora stylosa (mangrove plant)	*Penicillium hrysogenum* PXP55	Hainan, China	*E. aerogenes*		Ma et al. (2016)
	Chrysogeside B				

Antarctic sponge	*Penicillium* sp. *HDN151272* Phenolic polyketide	Antarctic	*P. aeruginosa*		Li et al. (2022a)
Chinese marine sponge	*Penicillium brasilianum WZXY-m1 22–9* Meroterpenoid brasilianoid A	China	Not given		Mayer et al. (2017)
Axinella sp.	*Penicillium citrinum* Isocyclocitrinol A (**16**) 22-acetyl-isocyclocitrinol A (**17**)	Papua, New Guinea	*Staphylococcus epidermidis*; *Enterococcus durans*		Cheng et al. (2020)
Tethya aurantium	*Penicillium chrysogenum* strain LF066 Cillifuranone	Mediterranean	*Staphylococcus epidermidis Enterococcus durans*		Cheng et al. (2020)
Suberea sp.	*Penicillium citrinum strain* WK-P9; Citrinin (**21**)	Indonesia	*Bacillus subtilis* JH642		Sabdaningsih et al. (2020)
C. *Fusarium* strains					
Suberea mollis	*Fusarium* sp. LY019 Fusarypyridine A (**23**), Fusarypyridine B (**24**)	Red Sea	*S. aureus; E. coli*	Antibacterial	Shaala et al. (2021) and Alves et al. (2022)
Tethya aurantium	*Fusarium* sp. Z10, *F. equiseti, F. heterosporum* Equisetin (**55**)	Atlantic Ocean, the English Channel, north sea & Mediterranean Sea	*P. aeruginosa,* MRSA, VRE *E. faecalis*, *B. subtilis*, *B. amyloliquefaeciens*, *S. aureus*	Anti-biofilm	Zhang et al. (2018), Wiese et al. (2011), Pompilio et al. (2022), Gomes et al. (2021), Wiese et al. (2011), Chen et al. (2021)
Unidentified	*Fusarium proliferatum* Tyrosol (**25**), 2,5-Furandimethanol (**26**) Adenosine (**27**)	Pulau Tinggi, Malaysia	MRSA; *Aeromonas hydrophila*	Antibacterial	Mosadeghzad et al. (2012)

(continued)

Table 6.3 (continued)

Sponge host	Species/metabolite	Geographical source	Microbial test panel/ microorganisms tested	Activity and/or mechanism of action	Reference
Xestospongia sp.	*Fusarium* sp. KJMT. FP.4.3 Karimunone B (**30**)	Karimunjawa National Park, Indonesia	MDR *Salmonella enterica* ser. *Typhi*	Antibacterial	Sibero et al. (2019)
Aplysina fistularis	*Fusarium* sp. ALAA-20	Caribbean Sea	Drug resistant *Staphylococcus*, *Pseudomonas* & *Klebsiella* sp.	Antibacterial	El-Bondkly et al. (2020)
	Lovastatin (**31**)				

type of extraction process of marine-derived secondary metabolites depends on some factors that influence the type of metabolites isolated. Those factors include parameters, such as the polarity of the solvent used for extraction, temperature, and pressure (Varijakzhan et al. 2021).

Marine sponges possess a wide distributional range, thriving in both tropical and polar regions and occupying a range of depths, from shallow waters to depths of up to 600 m (Jones et al. 2022). A recent study has highlighted the vast ecological niche occupied by marine sponges (Xu et al. 2022). Furthermore, more than 2700 secondary metabolites were discovered in marine sponges between 2009 and 2018 (Xu et al. 2022; Hong et al. 2022). This led to the isolation of approximately 18,000 microbial metabolites, many of which possess potential biological applications. Several factors affect the geographical distribution of marine sponges. These factors include nutrients, pH, temperature, sedimentation, salinity, hydrodynamics (mixing and flow), and predators, thus, in turn, affecting secondary metabolite production. Sponges affected by the same external factors are expected to produce the same metabolites (Bayona et al. 2020).

3.1 Antibacterial

More than 120 naturally active compounds were isolated from *Aspergillus* alone from various marine sources (Wong Chin et al. 2021). This review chapter mentions only 28 secondary metabolites (Figs. 6.4, 6.7, and 6.11) afforded by *Aspergillus* strains associated with marine sponges from various geographical regions.

Sponge-derived *Aspergillus* species that were collected from the Mediterranean included *A. tubingensis*, a symbiont with *Ircinia variabilis* as well as other *Aspergillus* species from *Chondrilla nucula* and *Tethya aurantium*. Isolated metabolites were identified as expansol D (**7**), diorcinol (**8**), tubingenoic anhydride A (**9**), austalide R (**46**), asperchondols A (**47**), and B (**48**). Moreover, the metabolites from fungal samples obtained from Indian Ocean sponges were xanthomegnin

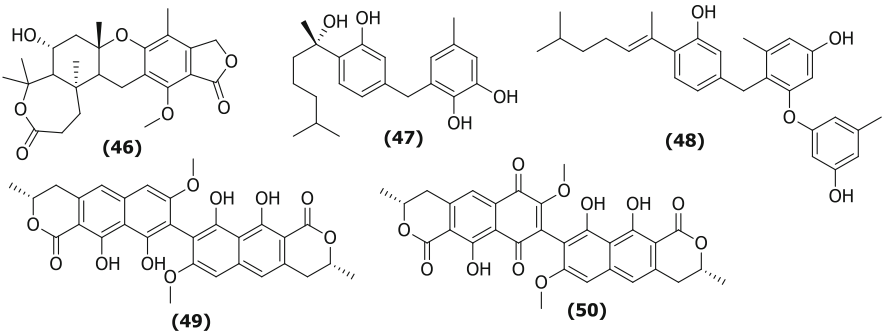


Fig. 6.11 Structure of other antibacterial metabolites from sponge-derived Aspergillus

(**34**), ochratoxin A (**35**) and B (**36**), vioxanthin (**49**), and viomellein (**50**). These metabolites were extracted from *Aspergillus elegans,* which was derived from *Monanchora unguiculta* (Fig. 6.11).

Sponge-derived *Aspergillus* samples were collected from Thailand, Chile, La Cruz (Costa Rica), Bali Sea (Indonesia), the South China Sea, Bohai Sea (China), Jeju Island (Korea), Xuwen County (China), and Similan Island (Thailand). From Thailand, *A. similanensis* was derived from the sponge *Rhabderemia* sp., and *A. candidus* was isolated from the sponge *Epipolasis* sp. *A. versicolor* was found in three sponge genera *Xestospongia exigua* from the Bali Sea (Indonesia), *Hymeniacidon perleve* from Bohai Sea (China), and *Petrosia* sp. from Jeju Island (Korea). Geographical and physical parameters of the three locations from where *A. versicolor* was isolated are presented in Table 6.4. The metabolites extracted from the sponge-derived *Aspergillus* were not similar. Overall, the same species of *Aspergillus* could be found in different geographical locations affording unique metabolites.

Aspergillus has been isolated from sponges collected from both tropical and temperate ocean temperatures (Wang et al. 2016). The incubation temperature of the *A. versicolor* isolated from the three genera of sponges ranges from 25°C to 28 °C. From the data represented in Table 6.3, the average water temperatures on Bali Sea, Bohai Sea, and Jeju Island are 28, 24.7, and 20.6 °C, respectively. There is a significant difference of about 8 °C in temperature between the Bali Sea and Jeju Island waters. The pH of the three seas is almost similar, ranging from 7.8 to 8.2, and the average salinity is about 32‰. The combination of all these factors affects the metabolite synthesis of the symbionts. For this literature review, no literature was found for sponge-derived *Aspergillus* from the Antarctic and Arctic waters to make the comparison in extreme conditions. This shows that there is still a lot to be explored from the polar areas for bioprospecting essential metabolites for antimicrobial drug discovery. The geographical distribution of antimicrobial sponge-derived fungal metabolites from the genus *Aspergillus* from 2007 to 2022 is briefly summarised in Fig. 6.12.

The three sponge hosts (Table 6.4) for *A. versicolor* were quite diverse, taxonomically from three sponge genera and two different families. The sponge species are morphologically not similar, differing in their sizes and spicule structures. The stony sponge is a name given to *Petrosia* sp., bread sponge to *Hymeniacidon perleve,* and giant barrel sponge to *Xestospongia exigua.* The structural difference among the sponges could contribute to variations in metabolite production. As its name indicates, the giant barrel is enormous, so it can host more microorganisms than the smaller stony or bread sponge. The size can affect which microorganisms to host since the more competitive microbes will likely settle in and use more available nutrients than the other less competitive microbes.

Table 6.4 Geographical and physical parameters of the three geographical locations from where the sponge-derived *Aspergillus versicolor* originated

Geographic location	GPS location	Distance from the equator (miles)	Sponge host	Average sea water temp (°C)	*Aspergillus* incubation temp (°C)	Sea water pH	Avg Sea water % Salinity
Bali Sea (Indonesia)	S 0° 47.3565′	345.47	*Xestospongia exigua*	28	27	8.2	32
	E 113° 55.2796’						
Bohai Sea (China)	N 38° 50.9722′	*2418.27*	*Hymeniacidon perleve*	24.7	28	8.2	31
	E 119° 47.3355’						
Jeju Island (Korea)	N 33° 29.3407′	2314.60	*Petrosia* sp.	20.6	25	7.8	34
	E 126° 29.8981’						

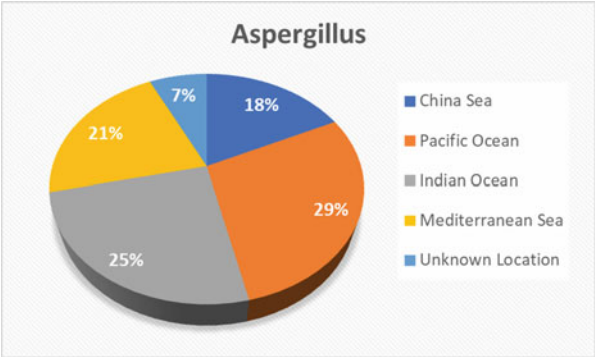


Fig. 6.12 Percentages of antibacterial sponge-derived *Aspergillus* metabolites from various geographical regions as reported from 2007 to 2022

Table 6.5 Geographical distribution of antibacterial sponge-derived *Penicillium* metabolites

Compound name	Ocean	Cold or tropical water
Citrinin (21)	South Pacific Ocean. Pacific Ocean	Tropical
Cis-cyclo (Leucyl-Tyrosyl) (39)	Atlantic Ocean	Cold
Cyclo(L-Tyrosyl-L-Prolyl)	Pacific Ocean	Tropical
Ketidocillinones A–C (18–20)	Antarctica	Cold
Arisugacin K	Pacific Ocean	Tropical
Chrysogeside B	Pacific Ocean	Tropical
Meroterpenoid brasilianoid A	Pacific Ocean	Tropical
Cillifuranone	Atlantic Ocean	Cold
Isocyclocitrinol A (16), 22-acetylisocyclositrinol A (17)	Pacific Ocean	Tropical

3.2 Antibacterial Sponge-Derived Penicillium *Metabolites*

Secondary metabolites from sponge-derived *Penicillium* are also widely distributed in the marine environment. There was a diverse set of compounds isolated from various *Penicillium* species collected from different regions as presented in Table 6.3. Metabolite production of unique active antibacterial compounds in *Penicillium* varies in South Asia, Mediterranean, Antarctica, and even the Middle East. Table 6.5 lists the compounds isolated from *Penicillium* and from which oceans the host organisms were collected, highlighting the variation of compounds isolated from both cold and tropical waters, whereas Fig. 6.12 shows the percentage distribution of sponge-derived *Penicillium* metabolite production in both cold and tropical waters.

As earlier mentioned, the literature has strongly implied that the type of ocean water, whether it is tropical or cold, influences the type and number of metabolites produced by marine sponges (Steffen et al. 2022). Marine fungi are widely distributed worldwide while some types of metabolites are restricted to be found in specific

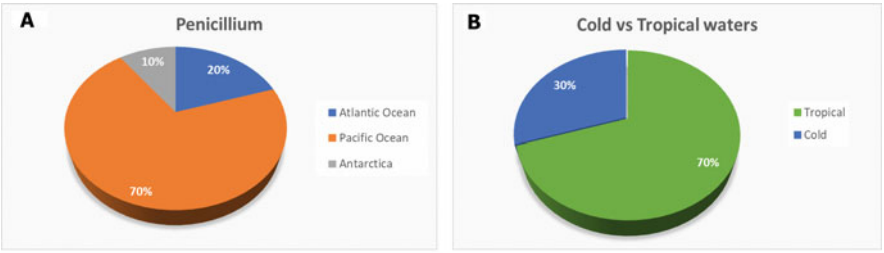


Fig. 6.13 Percentages of antibacterial sponge-derived *Penicillium* metabolites from (**a**) various geographical regions and (**b**) in cold versus tropical waters as reported from 2007 to 2022

tropical geographical regions, subtropics, temperate, or polar waters. However, the literature has also shown that several metabolites isolated from the tropics overlapped other regions as sponge-derived fungal metabolites have been widely distributed between temperate and tropical sea regions (Petrini et al. 1993). Many marine-derived fungi such as *Penicillium* have a huge capability to adapt to sea life due to their wide tolerance to environmental conditions such as sea water temperature (Jones et al. 2022). As presented in Table 6.5 and Fig. 6.13, most metabolites were isolated from the Pacific Ocean, which is known to be tropical in the Asian Pacific Region but turns cold in the west.

3.3 Antibacterial Sponge-Derived Fusarium *Metabolites*

Certain *Fusarium* strains could be found living in symbiosis with sponges in different geographical regions and may produce distinctive compounds depending on their region. Sponge-derived *Fusarium* metabolites from various geographical regions showed antibacterial activity against test microorganisms, such as *E. coli*, *S. aureus*, and MRSA. *Fusarium* species have been described to be in symbiosis with varying species of sponges, producing compounds unique from their free-living and terrestrial counterparts. These unprecedented compounds produced by marine sponge-derived *Fusarium* highlight their competency in novel antibiotic development (Shaala et al. 2021). Table 6.6 outlines the secondary metabolites found in different geographical areas categorised by the sea from where the host organisms were collected. Geographically categorised as listed under Table 6.3, Pulau Tinggi of Malaysia is encompassed by the South China Sea, and the region Karimunjawa of Indonesia falls under the Java Sea region. Figure 6.14 illustrates the percentages of antibacterial sponge-derived Fusarium metabolites in various geographical regions as well as between cold and tropical waters as reported in the last 15 years.

Fusarium equiseti and *Fusarium heterosporum* were isolated from the sponge species *Tethya aurantium* often found in the Atlantic Ocean, the English Channel, North Sea and Mediterranean Sea (Wiese et al. 2011; Gomes et al. 2021).

Table 6.6 Geographical distribution of antibacterial sponge-derived *Fusarium* metabolites

Compound name	Geographical area
Fusarypyridines A (**23**)	Red Sea
Fusarypyridines B (**24**)	Red Sea
Equisetin (**55**)	Atlantic Ocean
Equisetin (**55**)	English Channel
Equisetin (**55**)	Mediterranean Sea
Equisetin (**55**)	North Sea
Tyrosol (**25**)	South China Sea
2,5-furandimethanol (**26**)	South China Sea
Adenosine (**27**)	South China Sea
Karimunone B (**30**)	Java Sea
Lovastatin (**31**)	Caribbean Sea

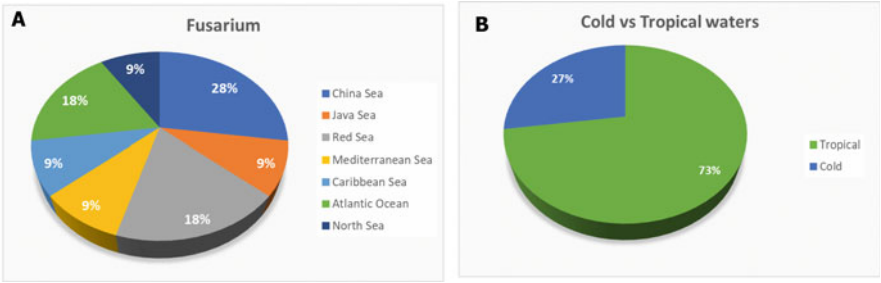


Fig. 6.14 Percentages of antibacterial sponge-derived Fusarium metabolites in (**a**) various geographical regions and (**b**) between cold and tropical waters as reported from 2007 to 2022

Furthermore, *Fusarium* sp. *ALAA-20,* which afforded lovastatin, was obtained from a Caribbean sponge (El-Bondkly et al. 2020).

From three studies, sponge-derived *Fusarium* isolated from European waters that include the Atlantic Ocean, the English Channel, the Mediterranean Sea, and the North Sea produced the metabolite equisetin (Chen et al. 2021; Wiese et al. 2011; Gomes et al. 2021). Taking this into account, the European waters are on the cold-water spectrum except for the Mediterranean Sea having moderately warmer temperatures up to a maximum of 28 °C. From the warm and tropical waters, sponge-derived *Fusarium* obtained from the Red Sea afforded two unique metabolites fusarypyridines A and B (Alves et al. 2022). Consequentially, three metabolites that include tyrosol, 2,5-furandimethanol, and adenosine were isolated from the South China Sea (Mosadeghzad et al. 2012), karimunone B from the Java Sea (Sibero et al. 2019), and lovastatin from the Caribbean Sea (El-Bondkly et al. 2020). In this case scenario, the warm-water regions that were quite distant from each other did produce unique metabolites compared to the colder regions of European waters. However, despite differences in the chemistry of the metabolites gained from sponge-associated *Fusarium* species collected from various water temperatures, various metabolites can possess antibacterial activity against the

same pathogenic species. Equisetin exhibited antibacterial activity against *S. aureus* as well as lovastatin, fusarypyridines A and B. Albeit, equisetin has the lowest MIC value at 1 μM against *S. aureus* compared to lovastatin, fusarypyridines A and B (Chen et al. 2021; Gomes et al. 2021; Wiese et al. 2011).

In this review chapter covering the period 2007–2022, equisetin was isolated from three different *Fusarium* species: *Fusarium* sp. *Z10*, *Fusarium equiseti*, and *Fusarium heterosporum*. All three *Fusarium* species were symbionts of the same sponge, *Tethya aurantium* was obtained from four different geographical regions. This could suggest that equisetin is potentially a chemotaxonomic marker for the *Fusarium* genus. Chemotaxonomy is defined as "the classification and identification of organisms by their natural variability and similarities in their biochemical composition". Expanding the knowledge of chemotaxonomic markers is still essential to detect overlaps of similarities and differences between varying strains of the same species as well as intraspecies comparisons (Mattarelli and Sgorbati 2018). The overlap of metabolite production and sponge host species could improve bioprospecting processes of identifying the organism, creating a more streamlined classification, and improving the efficiency of manufacturing novel products.

4 Bioprospecting Antibiofilm Metabolites

4.1 What Is Bioprospecting?

Biodiversity prospecting or bioprospecting is the search for natural sources, such as plants, animals, and all living organisms that would enable pharmaceutical industries to produce novel medicinal products that can be developed to serve as beneficial for the treatment of certain diseases. Bioprospecting is a potent way that supports the discovery of novel drugs. The process of bioprospecting usually involves sample collection of materials, which would be further investigated for their pharmacological effectiveness. The bioprospecting process must be accomplished in an ethical and sustainable means without causing any damage to the environment. Our oceans, that is the marine environment, are one of the most abundant habitats for bioprospecting due to the diversity of its organisms and microbial population. The capability of marine organisms to cope with their habitat is augmented by marine-derived metabolites that act as chemical weapons against pathogens to protect sessile marine invertebrates in an ecologically diverse and competitive habitat. Bioprospecting illuminates the role of these biologically active metabolites and their importance to be developed into new medicines. Recently, bioprospecting the role of marine-derived metabolites in treating antibiotic-resistant infection has never been more essential to be considered with the global rise of new infectious diseases (Karthikeyan et al. 2022).

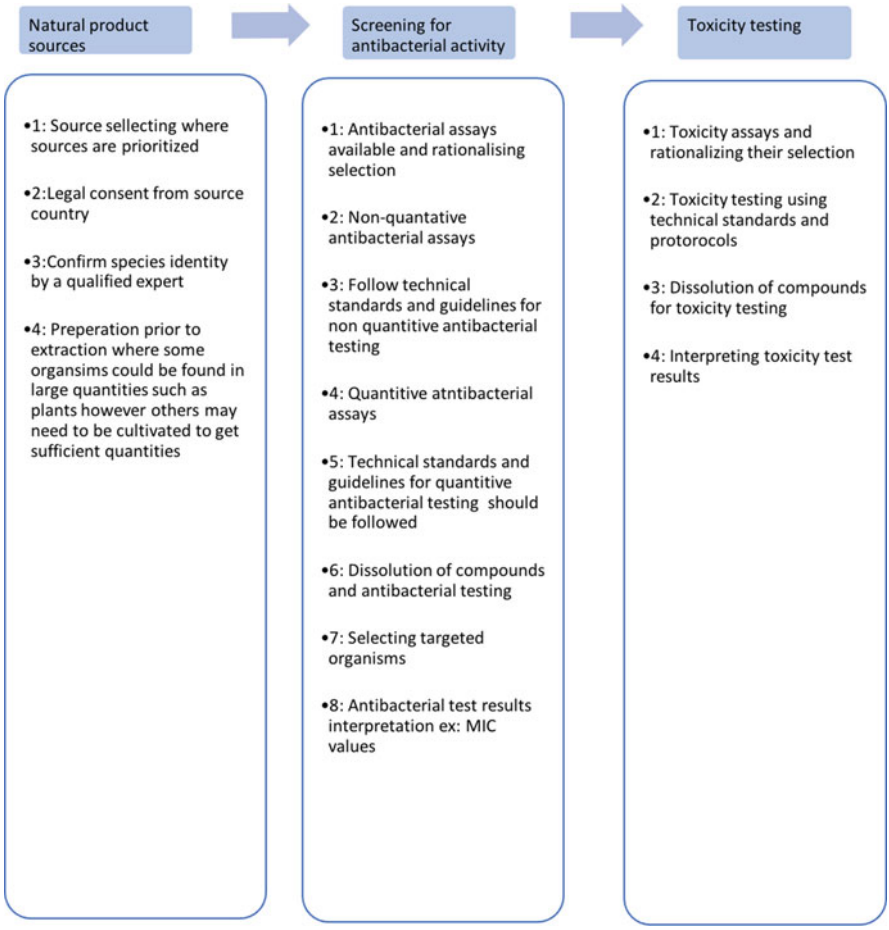


Fig. 6.15 The process of bioprospecting antibacterial agents

4.2 Methods Used for Bioprospecting

Bioprospecting is the primary research base used by pharmaceutical industries that facilitated the discovery of currently used antibiotics. A bioprospecting method involves the process of extraction, screening, and isolation of bioactive compounds from microorganisms, tests for potency and efficacy, as well as tests for toxicity of a new drug compound. Figure 6.15 shows the complete detailed process of bioprospecting for new antibacterial agents (Cushnie et al. 2020).

5 Summary and Conclusion

In the past two decades, many metabolites have been discovered from fungi, associated with different families of marine sponges. These metabolites can be used for various applications, such as antiparasitic, antibacterial, antibiofilm, anticancer, anti-inflammatories, and antienzymatic activities, with some others as a good resource of materials for research purposes (Rodrigues 2016). This chapter highlighted the importance of marine sponge-derived fungal metabolites in solving the global threat of bacterial resistance infections by producing new effective antibacterial and antibiofilm agents as new novel drugs serving the pharmaceutical industry and humans' health.

The marine ecosystem serves as a prolific source of a wide array of secondary metabolites, many of which possess biologically relevant properties. Marine sponges harbour many diverse microorganisms in which they take part in symbiosis. The symbionts, such as bacteria, viruses, and fungi, are vital in producing metabolites that sponges use to defend against pathogens and predators. So far, about 39 microbial phyla and about 30% of all marine natural products have been discovered from sponges.

Antibiotics are first-line treatments to treat pathogenic bacterial infections. Bacterial resistance against existing antimicrobial drugs has exponentially risen globally due to the incorrect use and abuse of antibiotics. Consequently, it is crucial to address this rise in resistance at the highest priority because the rate of discovery and distribution of novel antibiotics currently fall behind the increasing rate of bacterial resistance (Li et al. 2022b). The World Health Organisation (WHO) defined the absence of cross-resistance as a measure of how innovative a novel antibacterial is. Traditionally, the three criteria for an innovative novel antibacterial would include (1) a new chemical scaffolding, (2) must bind to a new target and (3) applies a new mode of action. These three criteria are established predictors for the absence or lack of cross-resistance (Gomes et al. 2021).

Biofilm formation has recently been a serious threat to humans' health because it restricts the effectiveness of antimicrobial compounds causing antibiotic resistance. Controlling antibiotic resistance is a global concern as current clinically used antibacterial, which follow direct strategies of either killing or inhibiting bacterial growth are no longer effective. Thus, developing new antibacterial strategies targeting antibiofilm activities would achieve the desired outcome of treating bacterial resistance infections (Yu et al. 2021).

Biofilm is the capability of microbes to produce unique environments that help them overcome harsh conditions. Understanding the stages of biofilm formation is the key to developing a solution to multidrug resistance. Antibacterial strategies differ from antibiofilm schemes as each could follow different paths of mechanisms of action where antibacterial compounds follow direct strategies of killing the pathogen and antibiofilm agents ensure an indirect scheme. Some compounds could have both antibacterial and antibiofilm activity (Indraningrat et al. 2016). However, some compounds have antibacterial activity without being able to have

antibiofilm activity and vice versa (Kumla et al. 2019). Compounds can target a specific stage and prevent microbes from forming a biofilm. These compounds cover a broad chemical diversity, such as alkaloids, terpenoids, polyketides, peptides, fatty acids, and fatty acyls. The number of new compounds from sponge-derived fungi has been currently increasing.

Most fungal species in the marine ecosystem live in symbiosis with marine sponges. Metabolites isolated from sponge-derived fungi make up 28% of novel compounds isolated from marine fungi (Bugni and Ireland 2004). Marine-derived fungi are a prolific source of secondary metabolites that are valuable for developing novel antibacterial and antibiofilm agents. Recently, there has been an increasing interest in the field of fungal secondary metabolites isolated from marine sponges to be developed as new antibiofilm compounds. A marine sponge is a rich source of novel metabolites, which is worth screening for microbial samples. Marine symbionts protect marine sponges by producing antimicrobial metabolites that could be developed into antibacterial novel drugs (Lopanik 2014). Secondary metabolites isolated from marine-derived microbes were not just known for antibacterial and antibiofilm activities but also other activities, such as antitumour, cancer-preventive, anti-inflammatory, and other activities (Hong et al. 2022).

This review covered literature between 2007 and 2022. From sponge-derived *Aspergillus* that originated from various geographical locations except from the polar regions, 28 compounds were found. Only five compounds were found to have an antibiofilm effect with their respective mechanisms of action. The remaining 23 compounds were only reported for their antibacterial activity. Some of the promising metabolites described were expansol D (**7**) and sydonic acid (**12**).

Penicillium is one of the endosymbiotic fungi that is richly found in marine sponges and has been known for decades for its antibacterial activity. *Penicillium* secondary metabolites were found mostly in tropical waters than in other regions. In addition, there were very limited studies for cold water regions (Jones et al. 2022). Both steroids isocyclocitrinol A (**16**) and 22-acetyl-isocyclocitrinol A (**17**) isolated from *P. citrinum* were first described as anti-inflammatory agents but later showed beneficial for their antibacterial activity against certain microorganisms (Cheng et al. 2020). Indirect strategies such as QS inhibitions is the mechanism of action usually followed by *Penicillium* secondary metabolites to instigate antibacterial activity, which showed an effective outcome in some studies (Yu et al. 2021).

Despite the discovery of natural products produced by fungi associated with sponges, there is still a significant lack of evidence and research available on the bioactivity of metabolites produced by sponge-derived *Fusarium*. Only eight distinct secondary metabolites have been described from various sponge-derived *Fusarium* species that were found to have significant antimicrobial properties, while only one metabolite, equisetin (**45**), exhibited antibiofilm activity. Secondary metabolites from sponge-derived *Fusarium* target a variety of pathogenic bacteria species including resistant strains such as MRSA, VRE, and multi-drug resistant *Salmonella enterica* ser. *Typhi*. Furthermore, antibacterial activity exhibited by metabolites lovastatin (El-Bondkly et al. 2020) and adenosine (Mosadeghzad et al. 2012) has

the potential to create opportunities to repurpose these compounds outside of their traditional indications.

Further studies should be conducted to reinforce the design and development of the new metabolites into drugs for use by humans. The mechanism of action of most of the metabolites reviewed has not been described. Therefore, the mode of action of these potential antimicrobial metabolites is recommended in future scientific research as this would save some time and funds for the experts doing further drug development activities by not repeating the steps of assessing the mechanism of action. More effort and funds should be provided for scientists to perform complete pharmacodynamic and pharmacokinetic relationships and conduct clinical trials. This will save many lives and healthcare costs if effective drugs are designed and developed in the future.

Biologically active metabolites like those mentioned in this review have also been described from other marine organisms, such as corals, algae, clams, and mangroves. This indicates how rich the marine environment is in natural products, with a potential for different pharmaceutical purposes. The new compounds may have various biological activities, such as anticancer, antiparasitic, anti-inflammatory, antiviral, antioxidants, antimicrobials, antienzymatic, and antimalarials. Although many are under clinical trials, only a few compounds have been approved by the FDA and MHRA.

Overall, this review concluded that sponge-derived fungi are a novel source of secondary metabolites that could be used in a future antibiotic pipeline to treat infections and reduce antibiotic resistance. Marine sponges are excellent sources of unique chemical scaffolding associated with symbiotic interactions. Well-defined pore structures of marine sponges enable them to host a plethora of marine micro-organisms (Al-Saleem et al. 2022). Symbioses of microbial symbionts within marine sponges result in the generation of secondary metabolites with significantly varying structures and therapeutic bioactivities that hold vast antimicrobial potential while 40% of their chemical scaffolds occupy a chemical space that traditional synthetic molecules do not (Aslam et al. 2022). Additionally, sponge-derived fungi could produce unique metabolites specific to their geographical regions that augment to the chemical diversity of these compounds.

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Chapter 7
Marine Fungi as a Bioresource of Medicinal Entities

Siya Kamat, Disha Sureesh, Suraj Modi, Madhuree Kumari, and C. Jayabaskaran

Abstract The search for new drugs is the aim of drug discovery research. Even today, biodiversity is the inspirational source of novel metabolites, and marine environments have proven to possess an exceptional chemical space. Marine fungi have received immense attention as sustainable producers of unique secondary metabolites with promising bioactive properties, such as immunomodulatory, anti-cancer, anti-inflammatory, antimicrobial, and antivirals. Marine fungi are often found in symbiotic association with higher organisms, such as tunicates, corals, sponges, mollusks, cnidarians, and fish, as well as seaweeds, mangroves, and kelps. They are also found in seawater, deep-sea sediments, and the intertidal region. As an adaptive evolution, the marine fungal metabolites possess unique carbon skeletons, halogenated decorations, functional groups, and heterocyclic structures that contribute to more significant bioactivity than terrestrial sources. The roadmap for a marine natural product to the market involves several stages, including sampling, isolation and taxonomic identification of fungi, extraction of secondary metabolites, primary screening for specific bioactivities, isolation of a pure compound, secondary screening for target identification using genetic and biochemical screens, yield enhancement, in vivo studies, preclinical and clinical analysis, costing, and market approval. The advances in genomics, metabolomics, data science, automation, and robotics have immensely eased the roadmap and accelerated novel compound discovery. The generation of compound libraries has allowed the exploration of new bioactivities for known structures. However, to limit the dereplication of known compounds, efforts must be undertaken to explore marine-specific fungi and innovative marine-specific culture conditions. This chapter aims to highlight the incredible potential of

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marine fungi as a bioresource of medicinal compounds and the several advancements in natural product research that can accelerate the approval of a fungal drug.

Keywords Marine fungi · Endophytes · Natural products · Anticancer · Secondary metabolites

1 Introduction

Marine fungi are considered the most recent source of medicinal entities in comparison with higher organisms of the same ecosystem. Oceans host a rich species diversity and cover ~70% of the Earth's surface. Owing to the harsh marine conditions, competition, salinity, pollution, varying temperatures, light, and predation, adaptive evolution favored the production of diverse chemical entities called secondary metabolites. The sheer structural diversity of these compounds has contributed to their immense bioactivities, even at minuscule amounts compared to terrestrial bioactive compounds. The large water cover of our planet presents immense scope for discovering new marine natural products (MNPs). The 8000 km long Indian coastline, the coastlines of Northern and Southern American continents, Australia, the Gulf, European coasts, Southeast Asian seas, and the Poles have been explored to varying extents for marine natural products (Kamat et al. 2022a; Faulkner 2001; Blunt et al. 2007; Khotimchenko et al. 2022; Fotedar et al. 2009; San-Martín et al. 2008).

Marine organisms have demonstrated outstanding potential to produce structurally novel secondary metabolites. Their biological properties have attracted immense attention from the pharmaceutical industry; therefore, there are drug discovery programs specifically focused on marine-derived microbial natural products (Sebak et al. 2022). Lurbinectidin (**1**), vedotin (**2**), trabectidin (**3**), ziconotide (**4**), and cytarabine (**5**) are some of the marine anticancer drugs approved by the US FDA until 2022. These structures were inspired by marine natural products sourced from invertebrates, such as sponges, cone snails, mollusks, and tunicates. Plinabulin (**6**), oligo-fucoidan (**7**), marizomib (**8**), and some monomethyl auristatin E (**9**) conjugates derived from marine sources are currently undergoing clinical trials as anticancer drugs (Kamat et al. 2022a).

With the advent of biotechnology, there has been an upward trend in discovering novel marine natural products from marine microorganisms. Microbial sources of secondary metabolites, particularly marine fungi, have garnered more attention as numerous chemical compounds have been isolated from them with a wide range of bioactive properties, such as anticancer, anti-inflammatory, antiviral, and antibacterial (Ahmad et al. 2019). The immunomodulatory cyclosporine A (**10**) were initially derived from marine fungi, including *Verticillium* sp., *Neocosmospora vasinfecta, Microdochium nivale, and Tolypocladium inflatum* (Kebede et al. 2017). The immunosuppressive macrocyclic lactone sirolimus (rapamycin) (**11**) was also derived from the marine fungus *Streptomyces hygroscopicus* (Sehgal 2003). Recent developments in marine mycology and natural product research have opened a

pandora's box of medicinal entities. It is observed that a single compound has a myriad of bioactive properties (Jiménez 2018). Rapamycin was initially observed to have antibiotic properties. However, subsequent studies have revealed its impressive antiproliferative and immunosuppressive properties that have revolutionized organ transplantation procedures. It is also a potent anticandidal compound on par with amphotericin B (**12**). However, its antifungal property was not pursued because of its strong immunosuppressive activity (Sehgal 2003).

Most fungi yielded bioactive extracts or new MNPs were isolated as endophytes from macroalgae or seaweeds, sponges, sediments, cnidarians, or other invertebrates. The most commonly studied genus is *Aspergillus,* which has produced many bioactive compounds with heterocyclic cores, which are otherwise hard to synthesize. They are also known to synthesize a wide range of flavones (**13**), cyclic peptides (**14**), diketopiperazines (**15**), glycosides (**16**), and so on. Other common prolific genera include *Penicillium, Cladosporium, Alternaria, Chaetomium, Epicoccum, Fusarium, Talaromyces,* and so on (Carroll et al. 2019). There are several fungal genera that require very specific culturing conditions for cultivation in laboratory settings. Extremophilic fungi, such as halophiles, thermophiles, and psychrophiles, also require specialized equipment for cultivation. Marine fungi can solve the material supply drawback of the marine drug development process. Although chemical synthesis of bioactive natural products has proved enormously beneficial, certain chemical structures are tough to synthesize or are simply non-sustainable. Marine fungal sources coupled with industrial biotechnology can be employed to perform fermentation of the fungal source for the large-scale production of the desired compound. Salinosporamide A or marizomib (**8**) is the first MNP obtained in large quantities from saline fermentation of a marine actinobacteria that has entered clinical trials. Thus, marine fungi are rightfully a sustainable source of drug discovery (Kamat et al. 2022a). In this chapter, we aim to highlight the immense medicinal potential of marine fungi-derived natural products.

2 Sources of Marine Fungi

Marine fungi can be found in all marine habitats, including planktonic communities, marine plants (i.e., algae, seagrasses, seaweeds, mangrove plants, and driftwood), marine invertebrates (sponges, coral, ascidians, bivalves, crustaceans, etc.), vertebrates (mainly fishes), and inorganic matter (Jones 2011).

2.1 Plant Sources

2.1.1 Marine Algae and Seaweeds

Marine algae, which include phytoplankton and macroalgae, are a diverse group of organisms ranging in size from unicellular to highly complex giant kelps. Macroalgae (seaweeds) occupy the littoral zone, which includes green algae, brown algae, and red algae, and the micro algae are found in both benthic and littoral habitats and throughout the ocean waters as phytoplankton (Garson 1989). Some of the earliest records of marine fungi were of species growing on marine algae (Jones et al. 2012). Bugni and Ireland (2004) commented that fungi isolated or growing on algae were the second largest source of marine fungi.

Filamentous fungi inhabiting or associated with marine algae are referred to as marine algicolous fungi (MAFs) (Ji and Wang 2016). These fungi reside in the inner tissues as endophytes or on the surfaces as epiphytes of marine algae. A wide array of partnerships exists between algae and fungi. These range from loose commensal association between algae and fungi as in primitive lichens, obligate symbiotic association termed mycophycobioses between the systemic marine fungi and the macroalgae, parasitism where the fungi are pathogens causing disease in the host, and saprobic association where fungi grow on senescent to moribund algae (Damare et al. 2006). The genera with the most marine species are the lichens *Verrucaria* and *Collemopsidium* and the genera growing parasitically on various seaweeds: *Pontogeneia*, *Chadefaudia*, *Haloguignardia,* and *Spathulospora*. Common hosts are the larger Phaeophyta (e.g., *Fucus*, *Laminaria,* and *Sargassum* species) (Jones et al. 2012). Table 7.1 and Fig. 7.1 list some examples of marine algae derived fungi.

Marine endophytic fungi that live inside the living tissues of seagrasses and macroalgae constitute an interesting ecological group of fungi (Raghukumar et al. 1992, 1995). The observation that the spores of *Acremonium fuci* associated with *F. serratus* can germinate only in the presence of tissues of the alga and not in seawater alone points to host specificity among seaweed endosymbionts and adds credence to the view that marine-derived fungi are not mere migrants from land habitats (Zuccaro et al. 2004). With reference to the species diversity of the endomycobiota harbored by seaweeds, the red and brown algae support a higher species diversity than the green algae (Suryanarayanan et al. 2010). The short life cycle of some of the green algal species and the characteristically slow growth of the endosymbionts could together be responsible for the green algae harboring a low diversity of these fungi (Dighton and White 2017). Although the common endophyte genera, such as *Phyllosticta, Colletotrichum, Phoma, and Pestalotiopsis,* observed among terrestrial plants are absent from the seaweeds, the pattern of distribution of endosymbionts is like that of the endophyte assemblages in terrestrial plants with a few dominant fungal species of low host specificity able to colonize taxonomically disparate seaweeds (Suryanarayanan et al. 2010).

Table 7.1 Bioactive metabolite isolated from marine fungi associated with marine algae and seaweed

Host	Fungus	Bioactive metabolite	Activity/ application	Reference
Enteromorpha intestinalis	*Penicillium* sp.	Communesin (**25**)	Cytotoxic	Numata et al. (1993)
Saroassum tortile	*Leptosphaeria* sp.	Leptosins **26**)	Cytotoxic	Takahashi et al. (1995a)
Actinotrichia fragilis	*Penicillium citrinum*	Citrinadin A (**27**)	Anti-cancer	Tsuda et al. (2004)
Polysiphonia violacea	*Apiospora montagnei*	Diterpene (**28**)	Anti-cancer	Klemke et al. (2004)
Sargassum thunbergii	*Aspergillus versicolor*	Asperversin A (**29**)	Antibacterial	Miao et al. (2012)
Avrainvillea longicaulis	*Penicillium* sp.	11, 1′-Dideoxyverticillin A (**30**)	Cytotoxic	Son et al. (1999)
Valomia utricularis	*Chaetomium* sp.	Chaetominedione (**31**)	Enzyme-inhibitory	Abdel-Lateff (2008)
Ceramium sp.	*Phaeosphaeria spartinae*	Spartinol C (**32**)	Enzyme-inhibitory	Elsebai et al. (2009)
Enteromorpha sp.	*Coniothyrium cereale*	Coniolactone (**33**)	Antibacterial	Elsebai et al. (2010)
Halodule wrightii	*Scytalidium* sp.	Halovirs A (**34**)	Antiviral	Rowley et al. (2003)
Carpopeltis cornea	*Aspergillus parasiticus*	Parasitenone (**35**)	Antioxidative	Son et al. (2002)
Ulva pertusa	*Chaetomium globosum*	Cytoglobosin C (**36**)	Cytotoxic	Cui et al. (2010)
Halymenia acuminata	*Aspergillus terreus*	Terreusinone (**37**)	UV-A absorbing	Lee et al. (2003)
Ceramium sp.	*Phaeosphaeria spartinae*	Spartinoxide (**38**)	Enzyme-inhibitory	Elsebai et al. (2010)
Chondrus ocellatus	*Penicillium echinulatum*	Arisugacin K (**39**)	Antibacterial	Li et al. (2014)
Chaetomorpha sp.	*Chaetomium globosum*	Chrysin (**40**)	Anti-cancer	Kamat et al. (2020b)

Environmental factors such as salinity gradient may select fungal species that colonize seaweeds (Mohamed and Martiny 2011). Another factor that can determine the composition of the endosymbiont assemblage is the antifungal metabolites produced by seaweeds to deter fungal colonization (Kubanek et al. 2003; König et al. 2006; Lam et al. 2008). Adaptations by a few fungal species to tolerate or detoxify these compounds along with the constant availability of host tissue for colonization in seaweed beds can lead to the evolution of generalist endosymbionts capable of colonizing taxonomically unrelated seaweeds. Such adaptations can possibly explain the constant presence of certain marine-derived fungal genera,

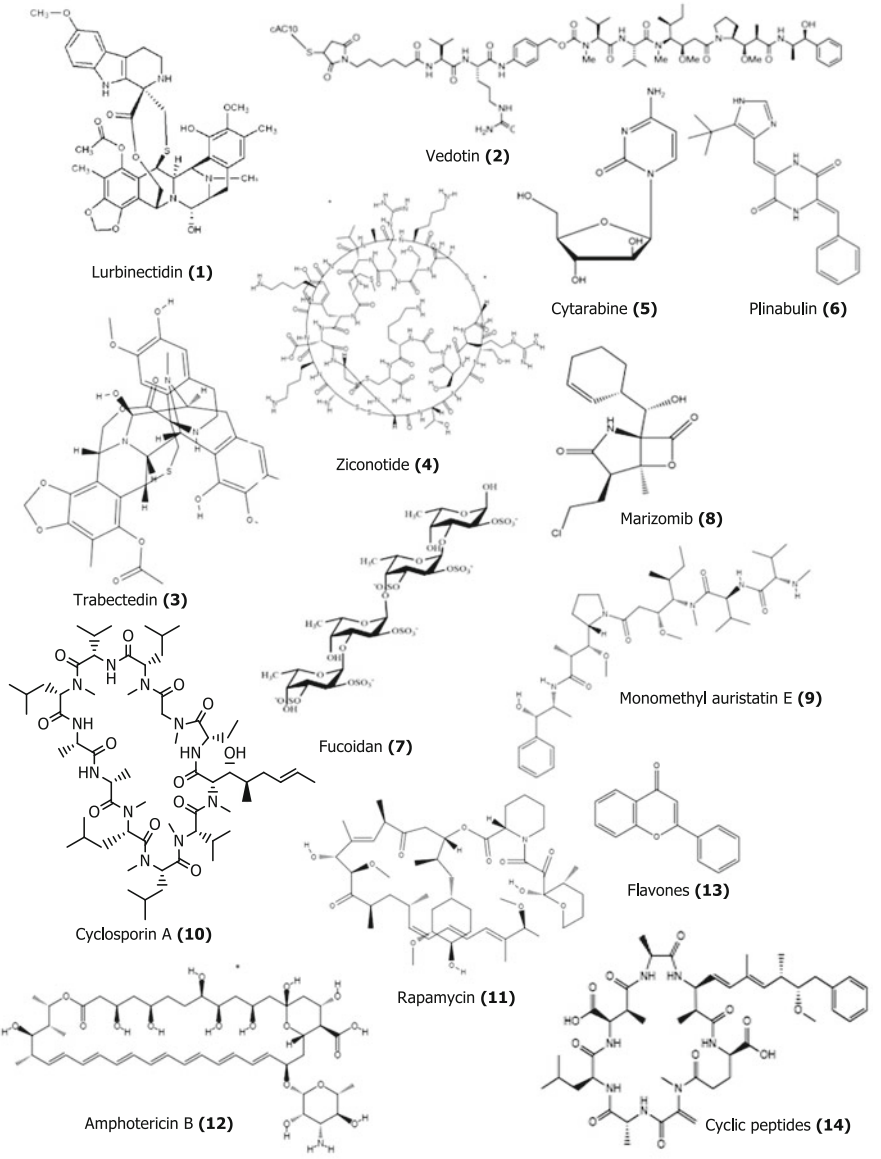

Fig. 7.1 Representative chemical structures of bioactive metabolites isolated from marine fungi associated with marine algae and seaweeds

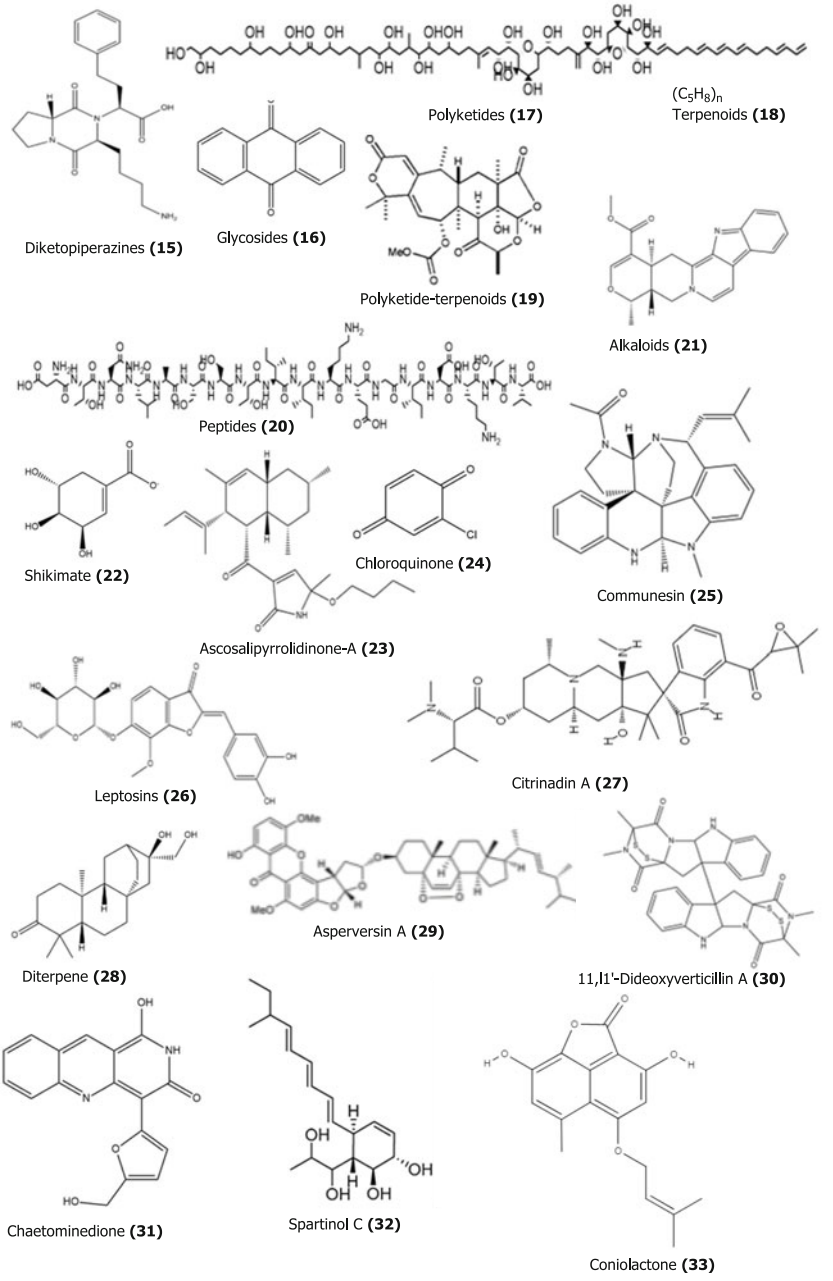

Fig. 7.1 (continued)

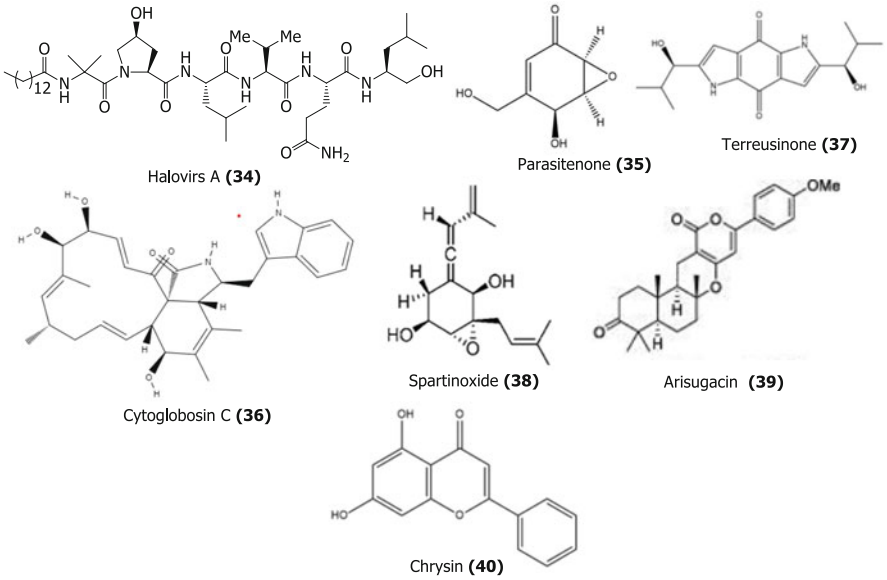

Fig. 7.1 (continued)

such as *Aspergillus, Cladosporium*, and *Penicillium*, as endosymbionts in different seaweeds (König et al. 2006; Zuccaro et al. 2008).

In the last few decades, interesting bioactive secondary metabolites from marine endophytic fungi have been reported with relevant clinical targets, some of which are novel. This has led to the discovery of several antibacterial, antifungal, antimalarial, antitumor, antiviral, and several such pharmacologically useful compounds from them (Mayer and Hamann 2004). The secondary metabolites can be categorized into polyketides (**17**), terpenoids (**18**), polyketide-terpenoids (**19**), peptides (**20**), alkaloids (**21**), and shikimate (**22**) derivatives according to their main scaffolds and putative biogenetic origin (Rateb and Ebel 2011). To cite one example, a novel polyketide ascosalipyrrolidinone A (**23**) was isolated from the marine fungus *Aschochyta salicorniae* associated with the marine green alga *Ulva* species (Osterhage et al. 2000). This compound showed antiplasmodial activity toward *Plasmodium falciparum* strain K1, a strain resistant to chloroquinone (**24**), and strain NF 54, susceptible to standard anti-malarials. The biosynthesis of these metabolites is dependent on ecological, physical, and biological factors; therefore, small changes in these conditions can generate an entirely new set of metabolites (Pejin and Karaman 2017).

The Antarctic macroalgal community is unique compared to the tropical and temperate ecosystems and is characterized by a high degree of endemism and the presence of cold-adapted species (Oliveira et al. 2009). Furbino et al. 2018 isolated saprobic fungi from Antarctic macroalgae, which are able to produce enzymes with the potential to degrade the algal biomass and may be involved in a complex network

of biogeochemical cycles and cycling of organic matter, which may be available to other marine organisms in the food web. The agarolytic and/or carrageenolytic Antarctic fungi obtained in the study may be useful in further processes involving the biological extraction of agar, carrageenan, or their byproducts, such as bioactive oligosaccharides and galactose present in the algal biomass, for use as substrates of third-generation bioethanol.

2.2 Animal Sources

Marine fungi have also been identified as pathogens and symbionts in marine animals. Bioactive compounds have already been isolated from marine invertebrates, such as sponges, corals, and tunicates. Therefore, it is only obvious that the fungi associated with marine invertebrates have also been explored for bioactive compounds. Fungi associated with marine vertebrates, which includes fishes, have also been investigated for bioactive metabolites that can serve as drugs (Amend et al. 2019).

2.2.1 Sponges

Isolating a fungus from a marine organism does not show that the fungus grows there since sponges filter feed from the surrounding seas, which contain spores of terrestrial fungi coincidentally found in these waters (Kohlmeyer 1974) but Rot et al. (2006) point out that some fungal associations with sponges may not be accidental as some of the sponge mitochondrial introns are of fungal origin, perhaps as a result of horizontal gene transfer. The finding that sponges have (1 → 3)-β-d-glucan-binding proteins on their cell surfaces for fungal recognition also lends credence to this view (Perović-Ottstadt et al. 2004). Another pointer in this direction is the observation that sponges can be symptomless carriers of the fungal pathogen *Aspergillus sydowii* (Bain. and Sart.) Thom and Church, which causes the death of sea fans (Ein-Gil et al. 2009).

It is only in recent years that the fungal symbionts of marine sponges have received any attention, mainly related to their capacity to produce novel bioactive metabolites (Blunt et al. 2007). Filamentous ascomycete fungi of the orders eurotiales, capnodiales, pleosporales, and hypocreales (Höller et al. 2000; Wang et al. 2008; Paz et al. 2010; Thirunavukkarasu et al. 2012) are commonly associated with sponges. Among the marine-derived fungi from various marine sources, those associated with sponges have higher species diversity and are prolific producers of novel secondary metabolites including antitumor, antibiotic, antifungal, antialgal, anti-insect, antioxidant, and acetylcholine esterase inhibitors (e.g., Kjer et al. 2010; Lee et al. 2010; Rateb et al. 2010; Almeida et al. 2011; Chu et al. 2011; Thirunavukkarasu et al. 2012). To cite a few examples, *Dreschlera hawaiiensis* elaborates novel spiciferone derivatives (**41**) (Edrada et al. 2000), *Cladosporium*

herbarum synthesizes two novel macrolides: pandangolide 3 and 4 (**42**, **43**) (Jadulco et al. 2001), *Cladosporium* sp. synthesizes a novel hexaketide (**44**) (Gesner et al. 2005), *Paecilomyces lilacinus* produces two novel α-pyrones (**45**) (Elbandy et al. 2009), *Arthrinium* sp. produces myrocin D, a novel diterpenoid (**46**) (Ebada et al. 2011), and *Aspergillus versicolor* synthesizes a novel lipopeptide (**47**) (Lee et al. 2010).

Therefore, fungal symbionts of sponges are a promising source of novel drugs and drug leads. It is important to note that the same species of fungus isolated from different sponge species have different secondary metabolite profiles (e.g., Jadulco et al. 2002). Secondary metabolism in fungi is influenced by chemical and physical factors, such as carbon and nitrogen sources, pH, light, and temperature (Yu and Keller 2005).

2.2.2 Corals

Coral reefs provide an excellent habitat for fungi. Fungi grow both on the surface and interior of scleractinian corals. They are found both within the polyps and calcium carbonate skeleton of such corals. In the latter case, they exist as endoliths by penetrating the calcium carbonate structures. Coral reef fungi are also found as endobionts, being associated with living organisms, such as coral polyps, sponges, and holothurians, or as saprotrophs in coral mucus, plant detritus, sediments, and water column. They are not only responsible for occasional coral diseases but may also have a mutualistic role in healthy corals (Le Campion-Alsumard et al. 1995).

Coral reefs are a rich source of fungi that produce secondary metabolites. Namikoshi et al. (2000) have reported two new compounds, paecilospirone (**48**) and phomopsidin (**49**), and seven known compounds, griseofulvin (**50**), fusarielin A (**51**), chaetoglobosin A (**52**), deoxyfusapyrone (**53**), verrucarins J and L acetate (**54**, **55**), and fusapyrone (**56**), from marine-derived fungi collected in tropical and sub-tropical coral reef environments.

The report of aspergillosis disease in the sea fan *Gorgonia* (Smith et al. 1996) has kindled a worldwide interest in the mycoflora of soft corals. Fungi have been isolated from the gorgonian soft coral, *Gorgonia ventalina* collected from Puerto Rico (Toledo-Hernández et al. 2007). Koh et al. (2000) isolated 16 fungal genera and 51 species, including two yeasts from ten species of Gorgonian corals in Singapore by culture-dependent technique. The microbial metagenome of *Porites astreoides* collected from Bocal del Toro, Panama, showed fungi to be the dominant community, contributing 38% to the genome of the coral (Wegley et al. 2007). The soft coral genus *Sarcophyton* hosts a wide diversity of marine fungi that interact with the soft corals in multiple ways and are reported to be an important source of sesquiterpenes (**57**). The earliest reports on sesquiterpenes from soft-coral associated fungi are the isolation and characterization of the hirsutane sesquiterpenes (**58**) and hirsutanol derivatives (**59**) from the marine fungus *Chondrostereum* sp. isolated from the soft coral *Sarcophyton tortuosum* (Li et al. 2011).

2.2.3 Sea Urchins

Sea urchins are most commonly found in the shallow sub tidal zone on rocky bottoms and are harvested for various bioactive compounds (Chandra 2020). New antimicrobial anthraquinones—monodictyquinone A (LIX) (**60**) and anthcolorins A, E, and F (**61–63**)—were isolated from marine-derived fungi of genus *Monodictys* and *Aspergillus*, respectively, which were originally isolated from the sea urchin *Anthocidaris crassispina* (El-Beih et al. 2007; Nakanishi et al. 2013). Table 7.2 and Fig. 7.2 summarize some examples of bioactive compounds isolated from marine invertebrate-derived endophytic fungi.

2.2.4 Marine Vertebrates: Fish

Although marine organisms are well known for regulating the ecological balance of the ecosystem, they have also enriched the diversity. Furthermore, the vast difference in ecomorphological marine life essentially establishes symbiont relationships for survival (Grassle 2013). Marine vertebrates and microorganisms including fungi mutually establish symbiotic relationships. Isolating and exploring such microsymbiont like fungi intrigued researchers to explore more as they produce various potential bioactive compounds (Fig. 7.3). For example, fumiquinazolines A, B, and C (**77–79**) were isolated from fungus *Aspergillus fumigatus*, which is usually present in the gastrointestinal tract of the marine fish *Pseudolabrus japonicus*. Further functional study suggested that it can cause moderate cytotoxicity, which is confirmed by studying its cytotoxicity against the P-388 lymphocytic leukemia cells (Numata et al. 1992; Takahashi et al. 1995b). Furthermore, fumiquinazolines A, C, and F (**77**, **79**, **80**) showed anti-proliferation activity against mouse CDC2-mutant (tsDT210) cells (Takahashi et al. 1995a; Resende et al. 2019). Similarly, two groups led by Tanaka reported chaetomugilins A, B, C, D, E, and F (**81–86**) from a fungal strain *Chaetomium globosum* OUPS-T106B-6 isolated from the marine fish *Mugil cephalus* collected in the Katsuura Bay, Japan. Functional studies concluded that chaetomugilins A-F (**81–86**) showed different degrees of hydroxylation and methylation and exhibited cytotoxic effect against promyelocytic leukemia (HL-60) cell lines with IC_{50} values ranging between 1.3 and 18.7 μM (Yamada et al. 2008; Yasuhide et al. 2008). There are many more marine vertebrate-associated fungi that need to be explored in order to find such bioactive molecules.

2.3 Mangrove Soil

Mangroves are salt tolerating plant species often found in tropical and subtropical intertidal coastal regions. Although mangrove forests consist of only 1% of total terrestrial forest land, it has wide complex biodiversity and play vital roles in coastal

Table 7.2 Bioactive metabolites isolated from marine fungi associated with marine invertebrates

Host type	Host name	Fungus	Bioactive metabolites	Activity	Reference
Sponges	*Stelletta* sp.	*Acremonium* sp.	Sesquiterpenoids (**64**)	Anti-inflammatory	Zhang et al. (2009)
	Geodia cydonium	*Arthrinium* sp.	Norlichexanthone (**65**), anomalin A (**66**)	Antitumor	Ebada et al. (2011)
	Myxilla incrustans	*Microsphaeropsis* sp.	Microsphaeropsisin (**67**)	Antimicrobial	Höller et al. (1999)
	Xestospongia testudinaria	*Aspergillus aculeatus* Lizuka	Aspergillusol A ((**68**)	Enzyme-inhibitory	Ingavat et al. (2009)
	Tethya aurantium	*Alternaria* sp.	Phomenin A and B (**69**, **70**)	Phytotoxin	Wiese et al. (2011)
	Suberites domuncula	*Petriella* sp.	Cyclic tetrapeptide (**71**)	Cytotoxic	Proksch et al. (2008)
Corals	*Sarcophyton* sp.	*Pseudallescheria boydii*	3,30-cyclohexylidenebis(1H-indole) (**72**)	Cytotoxic	Yuan et al. (2019)
	Sarcophyton sp.	*Alternaria* sp.	Ampelanol (**73**)	Cytotoxic	Aly et al. (2008)
Sea urchins	*Anthocidaris crassispina*	*Aspergillus* sp.	Spiculisporic acid B and D (**74**,**75**)	Antibacterial	Wang et al. (2012)
	Anthocidaris crassispina	*Alternaria* sp.	Altercrasin A (**76**)	Cytotoxic	Yamada et al. (2015)

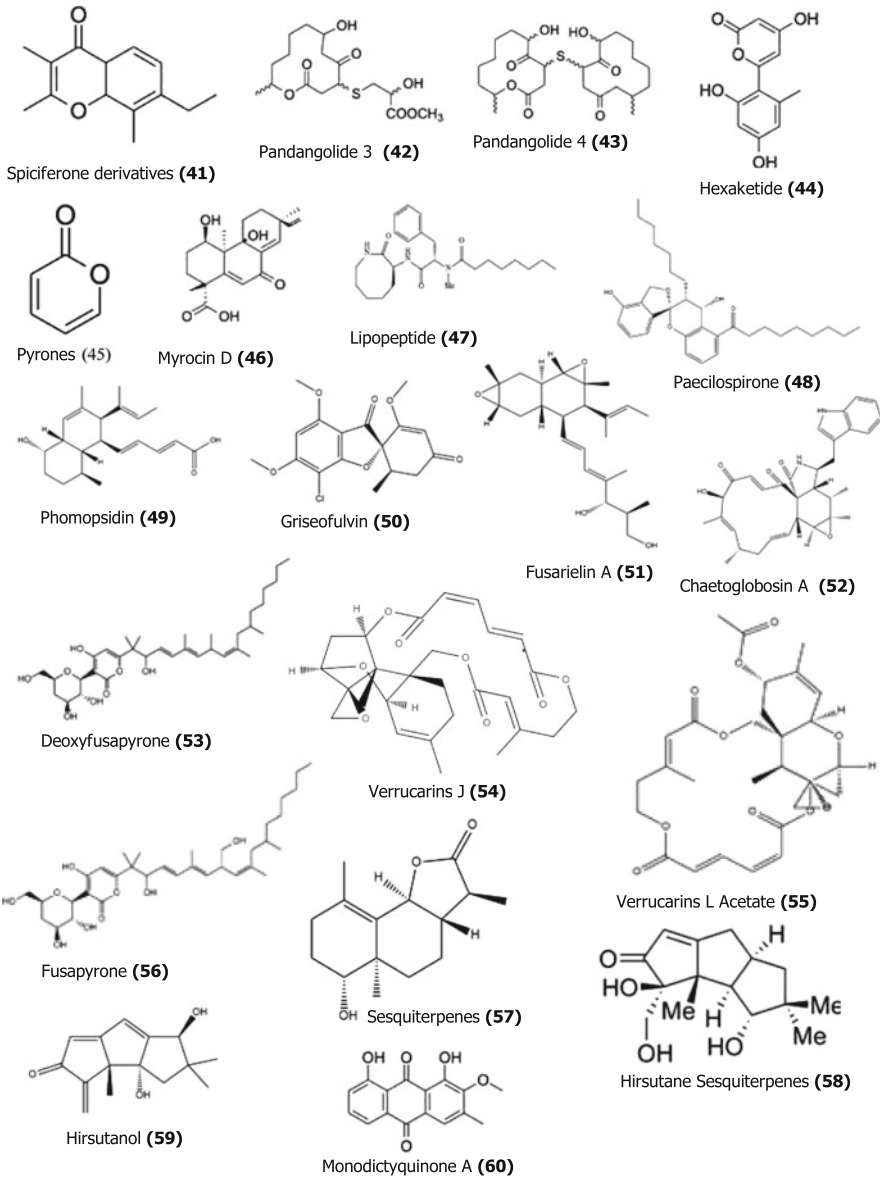

Fig. 7.2 Representative chemical structures of bioactive metabolites isolated from marine fungi associated with marine invertebrates

ecology, in sustaining and securing the coastal community (Nyanga 2020). Mangroves thriving in sea water inhabiting the edge of sea and land make it a hugely complex and diverse ecosystem in terms of soil composition and marine organisms. Mangrove ecosystems are well known for having fungal endophytes, which

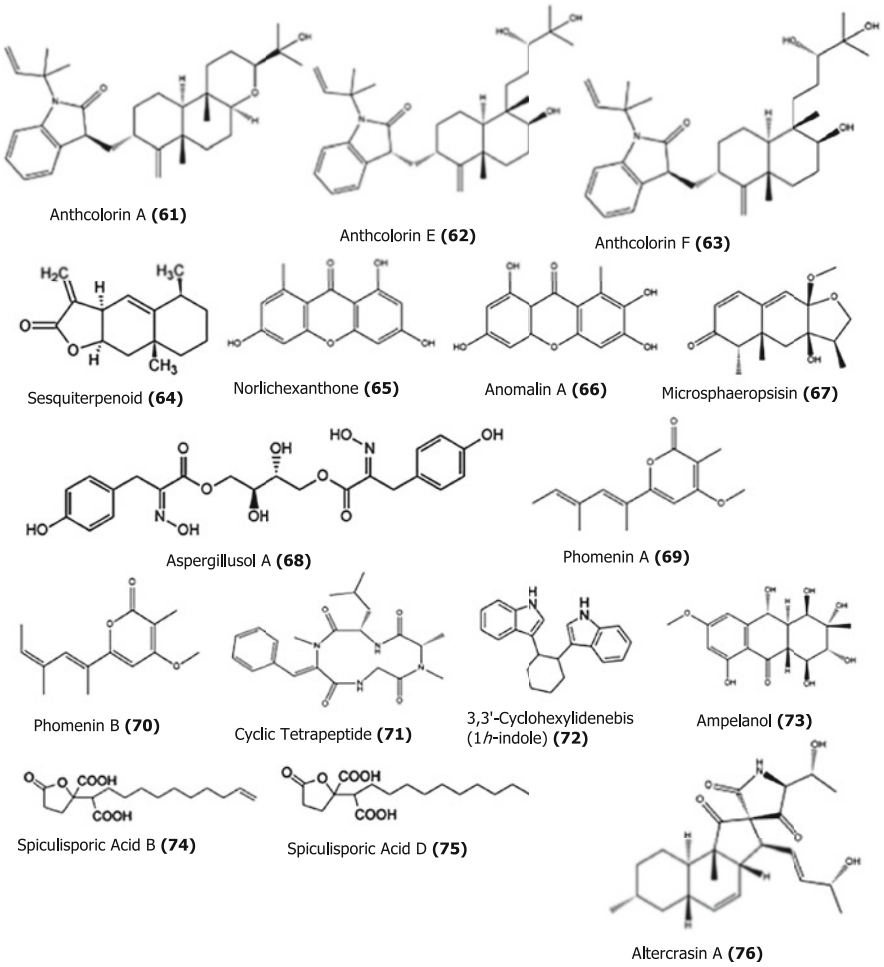

Fig. 7.2 (continued)

produces potential bioactive compounds (Fig. 7.4). Various fungal species have been reported in mangrove ecosystem including mangrove soils and plants with their biomedical applications. For example, Liu et al. 2018 reported mangrove derived from a fungus of the genus *Ascomycota* producing several polyketides, such as +/− ascomindone D (**87**) having a novel 2,3-diaryl indole moiety and two novel prenylated polyketides ascomfurans C (**88**) and ascomarugosin A (**89**). Functionally, both forms of enantiomers (+) and (−) ascomindone D (**87**) showed significant anti-inflammatory activities by inhibiting nitrous oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 246.7 mouse macrophages with IC_{50} values of 17.0 and 17.1 μM, respectively. (Liu et al. 2018). Similarly, Liu et al. (2016) reported mangrove-derived fungal endophyte *Penicillium* sp. ZJ-SY2 produces a new benophenone—methyl peniphenone (**90**). Functional studies showed that the

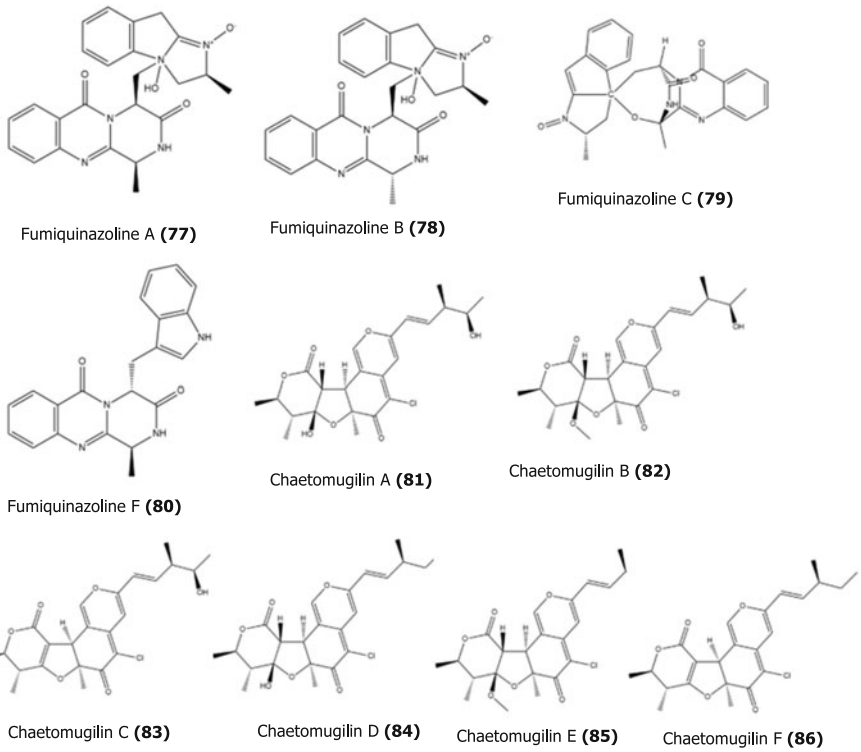

Fig. 7.3 Representative chemical structures of bioactive metabolites isolated from marine vertebrates

compounds act as immunosuppressants. Interestingly, it was observed that the two novel compounds had the highest immunosuppressant activities with IC_{50} values from 5.9 to 9.3 μg/mL, while the rest had moderate immunosuppressant activity. This was confirmed by studying the immunosuppressant activity of all compounds in Con A-induced (T cell) and LPS-induced (B cell) proliferation spleen lymphocytes of BALB/c mice (7–10 weeks old) (Liu et al. 2016). In another study, three new polyketides, namely asperochrins A–C (**91–93**), along with 12 known related derivatives, were isolated from *Aspergillus ochraceus* MA-15 present in rhizosphere soil of marine mangrove plant *Bruguiera gymnorrhiza*. Furthermore, functional analysis revealed that out of 15 compounds, chlorohydroaspyrones A and B (**94**, **95**) chlorohydroasperlactones A (**96**), penicillic acid (**97**), and (R)-7-hydroxymellein (**98**) showed potent anti-proliferative activity against aquatic pathogenic bacteria including *Vibrio harveyi*, *Aeromonas hydrophila*, and *Vibrio anguillarum* with IC_{50} values ranging from 0.5 to 64.0 mg/mL (Liu et al. 2016).

Two new depsipeptides named sarooclide A and saroclide B (**99**) were isolated from the mangrove-derived fungus *Sarocladium kiliense* HDN11–112. Structurally, saroclide A and B (XCIX) (**99**, **100**) were found with epimer of L- and D form of

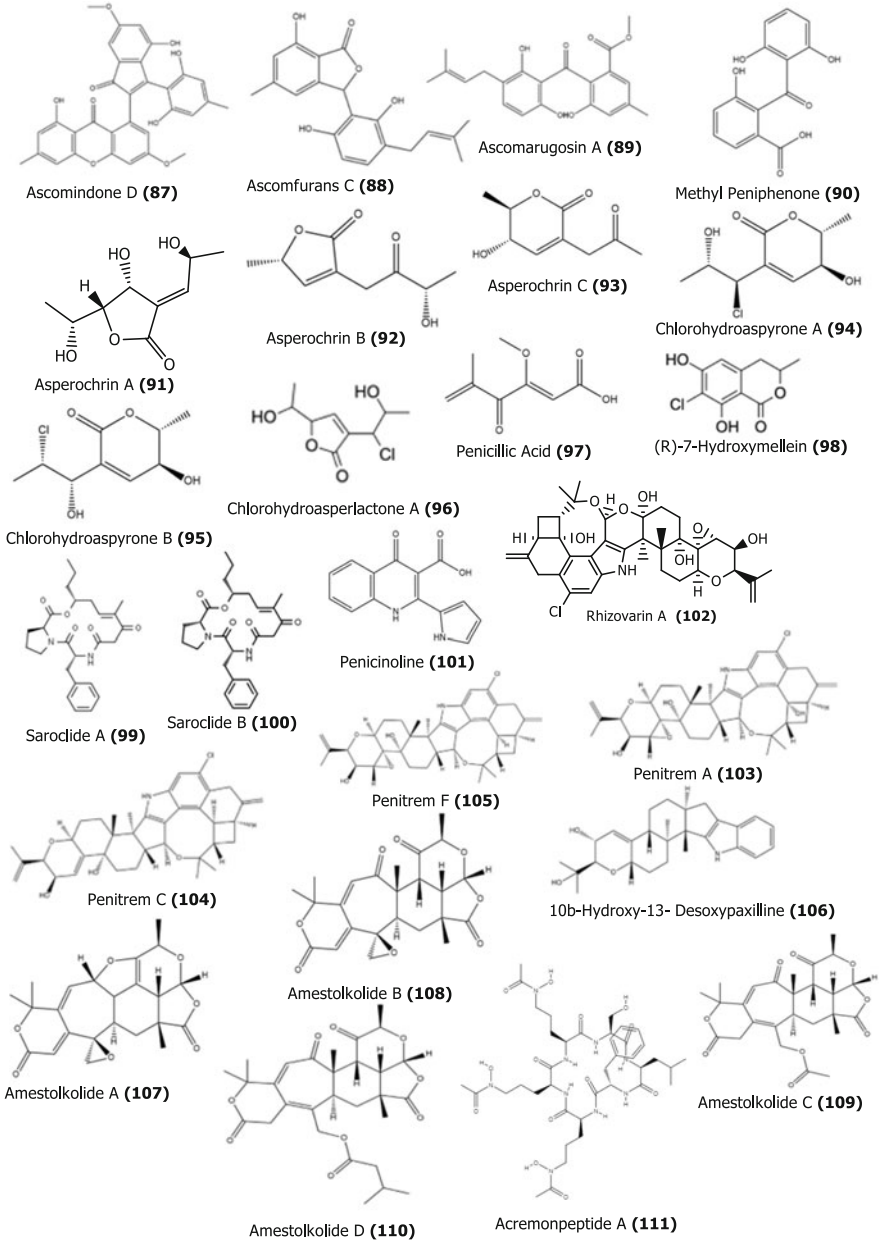

Fig. 7.4 Representative chemical structures of bioactive metabolites isolated from mangrove and marine sediment derived fungi

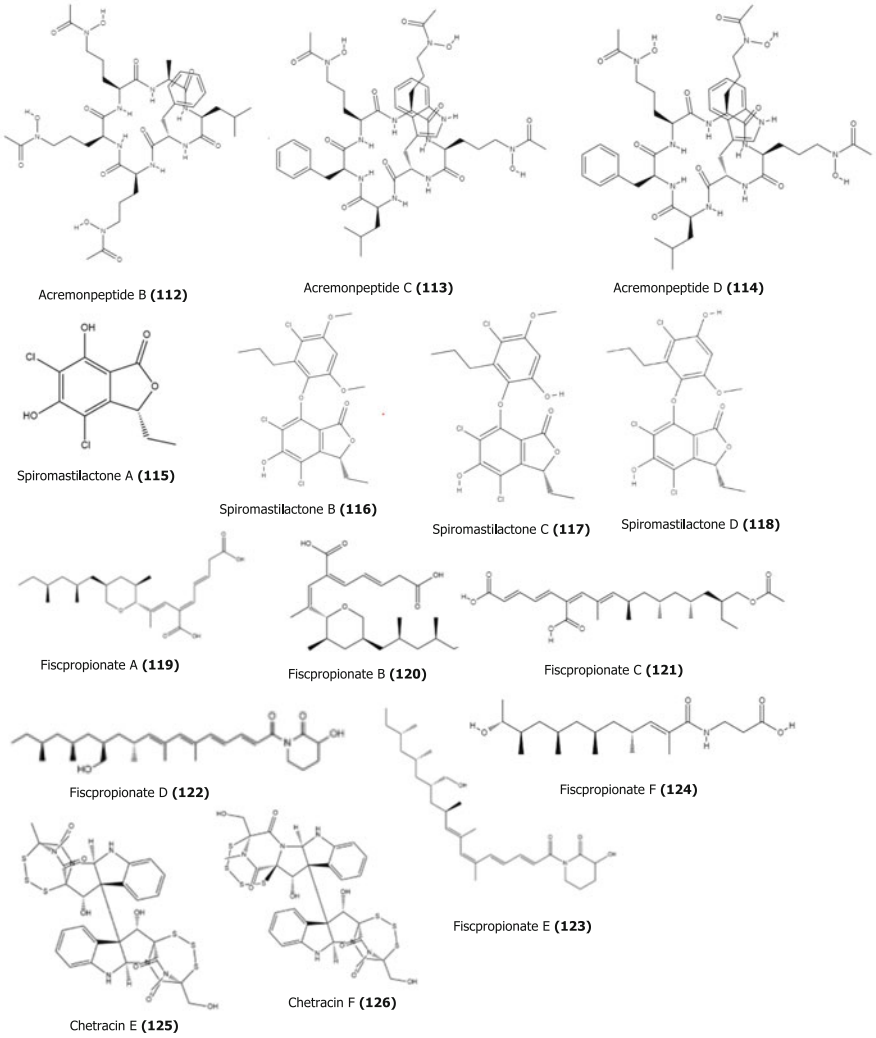

Fig. 7.4 (continued)

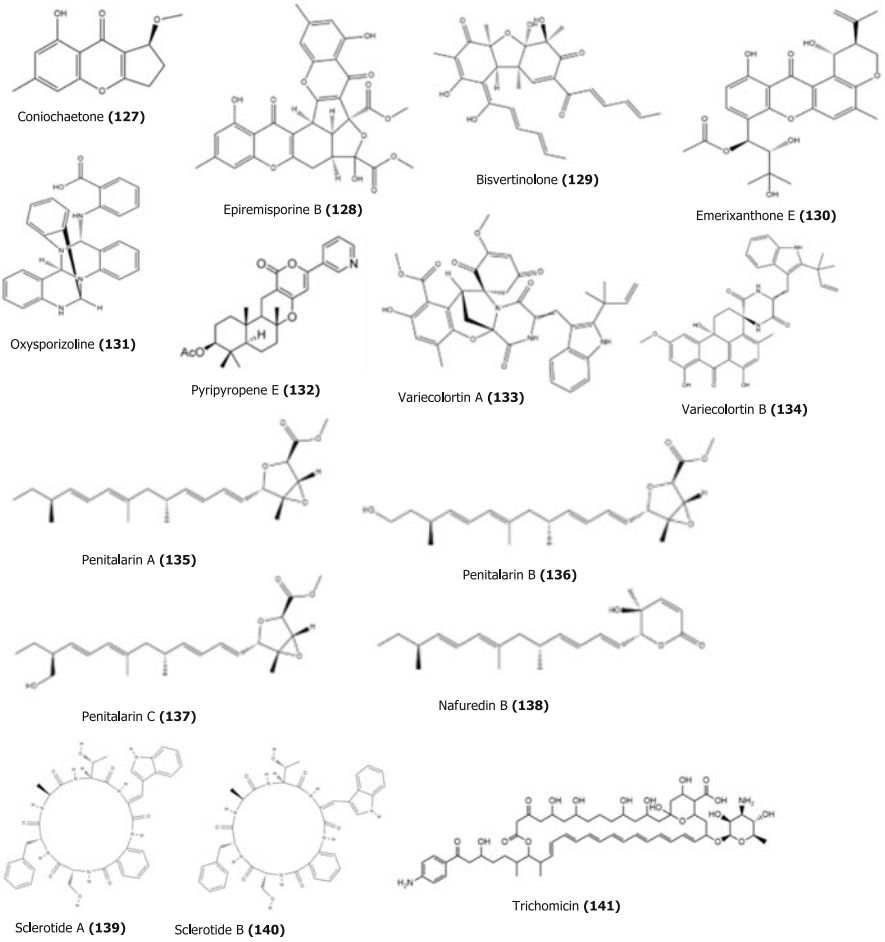

Fig. 7.4 (continued)

proline. Functionally, both compounds did not show the obvious cytotoxic effect against the cell lines including HeLa, HEK-293, and bacterial cells *E. coli* and *B. subtilis* but it showed significant lipid lowering effect as observed in the levels of oleic acids (Liu et al. 2016; Guo et al. 2018). Another study found the bioactive compound alkaloid pyrrolyl 4-quinolinone isolated from mangrove-derived fungus *Penicillium* sp. and thereby named penicinoline (**101**). In the functional study, penicinoline (**101**) showed a potent cytotoxic effect in HepG2 with IC_{50} values of 0.57 and 6.5 μg/mL, respectively. There are many more studies reported about bioactive secondary metabolites derived from fungal endophytes residing in the mangrove ecosystem. Below, Table 7.3 lists some of the bioactive compounds along with their bioactive effects.

Table 7.3 Bioactive compound isolated from mangrove derived fungi

Compounds	Mangrove	Isolation place	Activity	References
Rhizovarin A (**102**)	*Mucor irregularis* QEN-189	Hainan Island, China	Cytotoxic (cell line A549 and/or HL-60 (promyelocytic leukemia) cancer cell lines	Gao et al. (2016)
Penitrem A, C and F (**103–105**)				
10β-Hydroxy-13-desoxypaxilline (**106**)				
Ascomindone D (**87**)	*Ascomycota* sp. SK2YWS-L	Shankou Mangrove Nature Reserve in Guangxi Province, China	Inflammatory activity against LPS-activated NO production in RAW264.7 cells in vitro	Liu et al. (2018)
Asperochrins A, B, and C (**91–93**)	*Aspergillus ochraceus* MA-15	Hainan Island, China	Antibacterial activity against aquatic pathogenic bacteria *Aeromonas hydrophila*, *Vibrio anguillarum*, and *Vibrio harveyi*	Liu et al. (2015)
Amestolkolides A, B, C and D (**107–110**)	*Talaromyces amestolkiae YX1*	Zhanjiang Mangrove Nature Reserve in Guangdong Province, China	Amestolkolide B showed strong anti-inflammatory activity in vitro by inhibiting nitric oxide (NO) production in lipopolysaccharide activated in RAW264.7 cells	Chen et al. (2018)

2.4 Marine Sediment

Marine sediment is the mixture of insoluble materials deposited on the sea floor mainly because of nature's activities like volcanos, erosion of continents, and cosmic debris (Dunlea et al. 2018). Marine sediments have extreme environmental conditions like low pressure and so on and enforce the inhabitants to evolve tolerance in extreme conditions. Biodiversity in the marine sediment makes survival very competitive, which affects the organism's metabolism. Hence, to adapt to these conditions, such organisms produce various secondary metabolites allowing them to survive. Fungi being comparatively lesser in population than bacteria and archaea in marine sediment produce various metabolites, antimicrobial moieties, or other essential biomolecules in order to adapt to harsh conditions (Yurchenko et al. 2021). Interestingly, exploring such microorganisms led to the discovery of rare bioactive compounds having potential biomedical applications, especially in therapeutics (Fig. 7.4). For example, four new hydroxymate natural product cyclopeptides named acremonpeptide A-D (**111–114**) were obtained from marine fungus *Acremonium persicinum* SCSIO 115. Interestingly, these compounds showed significant antiviral activities against herpes simplex virus with EC_{50} values of 16, 8.7,

and 14 μM, respectively (Luo et al. 2019). In another study, Niu et al. (2016) reported deep sea derived *Spiromasic* sp. that produces a novel class of bioactive phenolic lactones—spiromastilactone A-D (**115–118**), and further functional analysis revealed that most of the tested compounds have antiviral activities against WSN influenza virus with considerable cytotoxicity. Moreover, spiromastilactone D (**118**) exhibited the most potent antiviral activities by inhibiting drug resistant clinical isolates of influenza A and B viruses. Mechanistically, it suggested that spiromastilactone D (**118**) disrupts the hemagglutinin protein (HA)-sialic acid receptor interaction, which essentially helps influenza viruses attach and enter the host (Niu et al. 2016).

Besides antiviral, various bioactive compounds isolated from sea sediment derived fungus have antibacterial activities. For example, Liu et al. (2019) recently reported that deep sea derived fungus *Aspergillus fischeri* FS452 produces six novel polypropionate derivatives fiscpropionates A–F (**119–124**), of which fiscpropionates A-D (**119–122**) showed significant antibacterial activities against *Mycobacterium tuberculosis* specifically to its protein, tyrosine phosphatase B (MptpB). The reported IC_{50} values for the same were 5.1, 12, 4.0, and 11 μM, respectively (Liu et al. 2019). A novel compound named anthraquinone (**60**) isolated from the fungus *Aspergillus versicolor* in the West Pacific Ocean was reported as a potent agent against MRSA. Mechanistically, it inhibits AmpC β-lactamase and topoisomerase activities, revealed through molecular docking studies (Wang et al. 2017). A study reported two new compounds epipolythiodiketopiperazines, named chetracins E and F (**125**, **126**), isolated from the fungus *Acrostalagmus luteoalbus* HDN13–530 in Liaodong Bay, the Bohai Sea, which showed significant cytotoxicity against the cancer cell lines including A549, HCT116, K562, H1975, and HL-60. Mechanistically, chetracin E and F (**125**, **126**) bind to the C-terminal of heat shock protein 90α (Hsp90α) by forming an H-bond and hydrophobic interaction (Yu et al. 2017). Another fungus *Aspergillus niger* isolated from sea sediment of the Northeast Brazilian coast, the Atlantic Ocean, produced a new cytotoxic furan, which potentially showed a cytotoxic effect against the HCT-116 cell line, with IC_{50} values 2.9 μg/mL (Uchoa et al. 2017). There are many such studies that have been reported. Table 7.4 lists some recent examples of bioactive compounds from marine sediment derived fungi.

3 How Can Marine Fungal Compounds Serve as Novel Drugs?

The currently approved MNPs approved or enrolled in clinical trials encompass peptides, antibody-drug conjugates, alkaloids, lactams, macrolides, polysaccharides, and terpenes. The diversity and complexity of these structures have resulted in the development of different production processes and costs for each drug. Scalable total synthesis and solid phase peptide synthesis are routinely employed to mass produce

Table 7.4 Bioactive compound isolated from marine sediment derived fungi

Compounds	Fungus	Source	Bioactive application	References
Coniochaetone (**127**)	*Penicillium* sp. SCSIO Ind16F01	The Indian Ocean	Antiviral against enterovirus EV71	Liu et al. and (2017)
			Cytotoxic effect against cell lines K562, MCF-7, SGC7901	
Epiremisporine B (**128**)			Antiviral against enterovirus EV71, influenza A virus subtype H3N2	
			Cytotoxic against cell lines K562, MCF-7, and SGC7901	
Fiscpropionates A–D (**119–122**)	*Aspergillus fischeri* FS452	Indian Ocean	Antibacterial (*M. tuberculosis* protein tyrosine phosphatase B inhibition)	Liu et al. (2019)
Bisvertinolone (**129**)	*Aspergillus protuberus* MUT3638	Hammerfest fiord, the Barents Sea	Antibacterial against *S. aureus*	Corral et al. (2018)
Emerixanthone E (**130**)	*Emericella* sp.	The South China Sea	Antibacterial (*E. coli*, *K. pneumoniae*, *Enterococcus faecalis*, *A. baumannii*, *Aeromonas hydrophila, and S. aureus*)	Fredimoses et al. (2018)
Oxysporizoline (**131**)	*Fusarium oxysporum*	Suncheon Bay, South Korea, Korea Strait	Antibacterial (MRSA, multidrug resistant *S. aureus*)	Nenkep et al. (2016)
Pyripyropene E (**132**)	*Aspergillus fumigatus* YK-7	Yingkou, the Bohai Sea	Cytotoxicity against U937 cell line	Wang et al. (2015)
Variecolortin A and B (**133**, **134**)	*Eurotium* sp. SCSIO F452	The South China Sea	Cytotoxicity (SF-268, HepG2 cell lines)	Zhong et al. (2018)

the desired known compound. However, the investigation of new MNPs or extracts starts from sampling to structure elucidation. The detailed protocols for the isolation of marine-derived endophytic fungi by Kjer et al. (2010) have led to an increase in fungal-derived bioactive natural products. The protocols have been used to isolate endophytic fungi from marine algae (Kamat et al. 2020a, b; Kumari et al. 2018; Sajna et al. 2020; Taritla et al. 2021), invertebrates (Chen et al. 2019), mangrove plants (Ju et al. 2015), and sponges (Küppers et al. 2017). The PharmaSea project by the European Union is a classic example of an academic-driven project started in 2012 for the discovery of novel bioactive compounds (Chemistry World n.d.). The project aimed to bring marine biodiscovery closer to the pharmaceutical industry. Under this project, several marine samples were collected from different zones of the ocean. Furthermore, a microbial library was developed with 1000s of strains followed by screening of fungal extracts for specific bioactivity and searching for active molecular leads or chemical structures against inflammation,

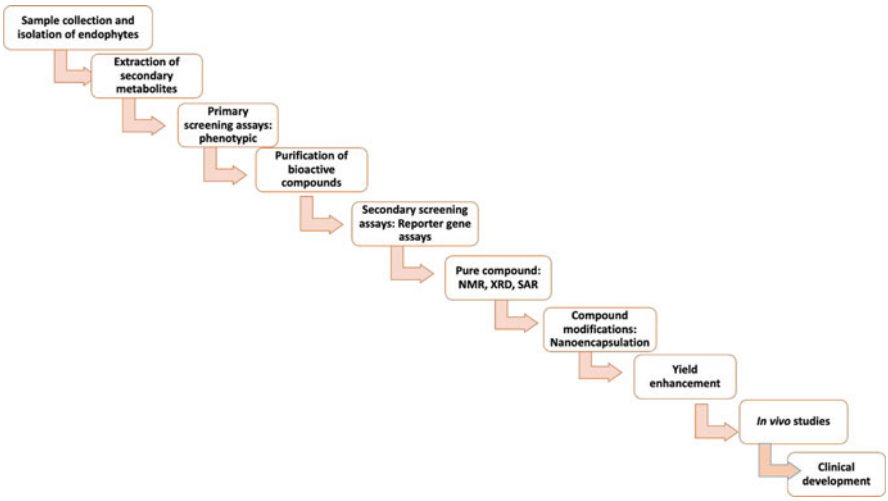

Fig. 7.5 A schematic of developing a drug from a marine fungal source

neurodegenerative conditions, and bacterial infections. Fungi also presented sustainable opportunities for scale-up. The basic pipeline of isolating a bioactive secondary metabolite has been explained in detail elsewhere (Kamat et al. 2022a). In this section, we will try to describe the forthcoming efforts and new additions to the pipeline that lead marine fungal natural products to clinical trials and beyond. The latest advancements in the field of marine natural products have been in the following areas (Zhang et al. 2016; Papon et al. 2022):

- investigation of relatively unculturable microorganisms or extremophiles
- investigation of the uncharacterized natural productome
- methods to increase the efficiency of finding novel chemical structures and eliminating those that are already identified (dereplication)
- better screening platforms for understanding the bioactivity of compounds, extracts, and fractions
- tackling compound bioavailability issues

Figure 7.5 illustrates the different stages of developing a medicinal natural product from a marine fungus. After extensive rRNA sequencing and taxonomic studies, it was revealed that $<0.1\%$ of marine microbes have been discovered, of which only a small fraction is culturable. Also, among the already known marine fungi, it is now known that many have silent biosynthetic gene clusters that can be activated by epigenetic manipulation. *Ascomycota* sp. from deep-sea sediments of the Indian Ocean was cultured on rice solid media. A co-culture of *Talaromyces aculeatus* from the Indian Ocean and *Penicillium variabile* from the mangroves of Fujian, China, led to the production of four new polyketides, penitalarins A–C (**135–137**), and nafuredin B (**138**) with anticancer properties. Halophilic fungi growing in nutrient-limited conditions led to the identification of several cyclic

hexapeptides called sclerotides A–B (**139**, **140**). Recent advances in marine sampling equipment and modern genomic technologies are significantly contributing to making full use of this precious bioresource (Zhang et al. 2018).

A critical part of natural product research is structure elucidation, and, therefore, analytical and statistical approaches have emerged. These methods and algorithms prioritize the efforts toward the discovery of new chemical scaffolds and derivates of known bioactive compounds and, therefore, enhance the repository of natural products. Mass spectrometry-based networking, NMR based association networking, compound activity mapping, in silico fragmentation prediction, AI-based structure prediction, and bio chemometrics are some of the strategies that allow the investigation of new leads in natural product extracts (Caesar et al. 2021). An integration of metabolomics and genomics has generated a lot of data, and it is now a high throughput pipeline of natural product discovery.

Cytotoxicity evaluation assays, such as the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay, resazurin reduction assay, antibacterial assay, have been routinely deployed in primary screening of fungal extracts and compounds. Efforts in this direction across the world have generated large libraries of natural product crude extracts, purified natural product fractions, and natural product-inspired bioactive compounds (Wilson et al. 2020).

The National Cancer Institute (NCI) of the United States produced a library of ~1,000,000 partially pure natural product fractions. Such efforts have led to the development of innovative cell-based and cell-free high throughput screening bioassays. This has eased the investigation of the synergistic effect of two or more compounds in a multi-well plate. Robotic drug dispensing and plate handling equipment have further enhanced the quick screening of large drug libraries (Tegally et al. 2020).

For the successful translation of a natural product into a pharmaceutical product, its target needs to be identified with high precision readouts. Cytotoxic compounds are phenotypically screened for cellular mechanisms, such as angiogenesis, senescence, reactive oxygen levels (ROS) apoptosis, autophagy, and other hallmarks of cancer cells. Recent studies have included other mechanisms of cell death, including ferroptosis, pyroptosis, and necroptosis (Kamat et al. 2022a). The second level of screening deals with identifying molecular targets of the drug. These targets can be specific proteins involved in immunomodulation, proliferation and growth, nuclear transport, and cellular metabolism. Therefore, reporter gene assays using a luciferase or fluorescence protein are used for efficiently measuring the read out of a specific target (Wilson et al. 2020). The molecular mechanism of trichomicin (**141**), a novel anticancer compound identified from *Trichoderma harzianum,* was investigated in several human cancer cell lines. Using the reporter gene assays, it was noted that trichomicin (**141**) reduces IL-6 levels and phosphorylation of STAT3. The compound could also reverse drug resistance in cancer cells (Zhu et al. 2020). Furthermore, the compound induced biophysical changes in the cell architecture, cell membrane, nucleic acids, proteins, and lipids are also studied using force microscopy, Raman spectroscopy, Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy, Dynamic Light Scattering (DLS) Spectroscopy, Flow

Cytometry, and so on (Aparanji and Kamat 2022; Kamat et al. 2022a; Kumari et al. 2022). Chrysin (XL) is a flavone reported from the marine fungus *Chaetomium globosum* (Kamat et al. 2020b) from the Konkan coast of India. Since the compound had bioavailability issues, it was nanoencapsulated using Polylactic-co-glycolic acid (PLGA) polymer (Kamat et al. 2022b). The anticancer effects of nano-chrysin were mapped using ATR-FTIR and flow cytometry. In HeLa cells, the nano-chrysin treatment induced membrane disruption, increased externalization of phosphatidylserine, DNA denaturation, degradation of α helical and β sheet protein structures, and lipid peroxidation. These bio-macromolecular changes led to the apoptosis of HeLa cells (Kamat et al. 2022c).

4 Challenges and Future Aspects

Exploration of the pharmaceutical potential of marine fungi is relatively new, and it still must face multiple challenges to reach the end user. Here, we have summarized the main challenges and the path ahead:

4.1 Untapping the Potential of Marine Fungi

Oceans are a vast and diverse world, with completely different flora and fauna. Many of the marine fungi are either not discovered or not cultivable in lab conditions (Agrawal et al. 2018). Endophytic microbes may carry an additional requirement of their host or host-derived metabolites for their successful growth and cultivation (Kamat et al. 2022a). Advancements in bioinformatics, metagenomics and metaproteomic based approaches, genetic engineering, and culture-independent strategies can open new avenues for isolating unique metabolites from marine fungi (Srinivasan et al. 2021). Improved mass spectroscopy techniques integrated with high-throughput screening and metagenetics can be used for screening and bioprospecting multiple marine fungi rapidly with increased accuracy (Colonia et al. 2021).

Oceans and seas have uneven topography, which are often difficult to explore. Advanced oceanography techniques with the latest instrumentation can help the explorers to dive deep and discover new fungal strains.

4.2 Quantity of Secondary Metabolites Produced.

Marine fungi-derived secondary metabolites are produced in very minute quantities. For preclinical studies and commercialization of the active principle, it is important that they should be produced in considerable quantity. Although fungi are the

sustainable source of synthesis of secondary metabolites, they need optimization of culture and growth conditions, use of elicitors and precursors, co-culturing, optimization of culture media, and extraction conditions for enhanced production and extraction of desired secondary metabolite (Kumari et al. 2018; Kamat et al. 2020b). Further research is required to scale-up the production of marine-derived secondary metabolites from fungal biofactories.

4.3 Understanding of Complete Secondary Metabolites Synthesis Pathway in Marine Fungi

Marine fungi are a recent addition in bioprospecting for their pharmaceutical potential. Many of the natural products isolated from them are either unique or modification of existing natural products (Wang et al. 2021; Shabana et al. 2021). For scaling-up the synthesis of secondary metabolites, their chemical synthesis or semisynthesis, it is necessary to understand their molecular pathways. Almeida et al. (2021) elucidated the structure and pathways of tryptophan-derived natural marine alkaloids and synthesized their derivatives, which were potential antimicrobials. They also imply different novel mechanisms to exhibit anticancer, antimicrobial, or other bioactivities, which should be researched thoroughly. Continuous research in this field will yield novel pathways and mechanisms with the potential for industrial translation.

4.4 Sustainable Drug Delivery

One of the most important challenges faced by any of the natural products is their sustainable and targeted drug delivery (Kamat et al. 2022a). The toxicity of the potential secondary metabolites on non-targeted cells is an important concern that needs to be addressed in all natural products. Recent developments in nanotechnology-based drug delivery systems, such as nanoencapsulation, liposome, and micelles-based drug delivery, have promised the efficient and sustainable delivery of secondary metabolites. Furthermore, coating secondary metabolites with biomolecules or conjugated nanoparticles ensures the avoidance of non-targeted toxicity inside the cells (Sahu et al. 2021; Saka and Chella 2021).

4.5 Industrial Transition and Commercialization

There is a huge gap in the discovery of marine fungi derived secondary metabolites and their industrial transition. The lack of understanding of biosynthetic pathways

and their production in minute quantity hampers their clinical trials and further commercialization. Nevertheless, the advancement in the latest isolation, screening, and molecular technologies will bridge the gap between the lab and the industry. The number of marine-derived drug getting regulatory approval for their bioactivities are continuously increasing (Kamat et al. 2022a). After embarking on the latest technologies and research in marine fungi derived secondary metabolites, many more novel and potential drugs can be expected, paving their path to large-scale production and commercialization.

5 Conclusions

Marine natural products have increasingly proven to have structural diversity and impressive bioactive properties against many human ailments. Marine microbial natural products, particularly those that are derived from fungi, are observed to be very potent. While there is some overlap between natural products isolated from marine fungi and marine macro-organisms, there is a unique chemical space solely produced by marine fungi. It includes unique carbon skeletons with some halogenated decorations and heterocyclic structures that reflect their medicinal properties. Fungi are a sustainable source of natural products and present opportunities for yield enhancement. The successful inclusion of marine fungal natural products into the pharmaceutical market is dependent upon the synergistic efforts of technical advances in marine sampling, growing, and culturing unique fungi, instrumentation for extraction and structure elucidation, compound library generation, high throughput screening platforms, dereplication methods, yield enhancement, and so on. The genomic and metabolomic technologies are further bringing the marine microbial natural product research on the precipice of a new age. Even the careful curation of chemical databases and software tools has contributed to a data-rich environment using which one can accelerate the discovery of a new compound. However, most of the fungal sources used belonged to the conventional genera. To make novel natural product discoveries, we need to venture into studying the understudied fungi and optimize marine specific culture conditions. We hope that this will spark the diversity of the marine natural productome and lead to novel drug discoveries.

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Chapter 8
Natural Bioactive Products from Marine Fungi Against Bacterial Infection

Minakshi, Shaurya Prakash, Hemlata Kumari, and Antresh Kumar

Abstract In spite of the different antibiotics available in the market, the need for new antibacterial agents is still in high demand. This is because of continuously increasing antimicrobial resistance which makes treatment ineffective to control superficial and invasive bacterial infections and has become a primary cause of global issues. These global issues appealed to us to find out a novel drug discovery against these infections. For the last decades, marine microorganisms especially marine-derived fungi have been considered to be a rich source of therapeutic agents and helpful for humankind to produce a multitude of bioactive secondary metabolites, important for preventing different ailments. These marine fungal natural products possess cytotoxic, anticancer, antiviral, antibacterial, or antifungal activities. Marine fungal-derived bioactive compounds can broadly belong to alkaloids, polyketides, coumarins, polyphenols, acetophenones, and xanthones groups. A number of anti-infective agents derived from different microbial sources are currently used for the treatment of different ailments. As antifungal- Helicascolide C, isolated from *Daldinia eschscholzii* KT32, anticancer-meroterpenes from *Penicillium* sp., and antibacterial as Cephalosporin C, from a *Cephalosporium* sp., diketopiperazine produced by *Aspergillus* sp., ascochital from marine ascomycete *Kirschsteiniothelia maritima.,* amino lipopeptides trichoderins A, A1, and B from a marine *Trichoderma* sp., pestalon from marine *Pestalotia* sp. The present chapter is focused on describing different antimicrobial secondary metabolites originating from marine-derived fungal sources and synthesis and regulatory conditions and action mechanisms in response to drug-sensitive and resistant bacteria pathogens.

Keywords Secondary metabolites · Antimicrobial resistance (AMR) · Gram-positive bacteria · Gram-negative bacteria · Multidrug resistant (MDR) bacteria

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1 Introduction

Modern antimicrobial research is facing an enormous barrier with the rise of multidrug resistance. According to the World Health Organization, over 700,000 people worldwide died every year from drug-resistant bacterial infections, caused by priority pathogens that are linked to high mortality and high drug resistance (Mancuso et al. 2021). The spread of antibiotic-resistant bacteria on a global scale necessitates for the identification of new and effective antimicrobial agents. The primary method for developing a novel antimicrobial agent for last more than 50 years has used a semi-synthetic modification of natural compounds (Aminov 2010). However, pathogenic microbes build antibiotic resistance, and available medications are temporarily effective. The issue of microbial resistance can be solved by the identification of unique natural compounds isolated from biological sources that are able to restrict the cell growth of resistant pathogens because of their distinct chemical composition and targets (Kumar et al. 2019; Kumar et al. 2018a). To overcome the problem of antimicrobial resistance (AMR), marine fungi have gained much attention and proved a significant source of new antibacterial agents (Youssef et al. 2019). The marine ecosystem covers approximately half of the world's total biodiversity and has its natural habitat (Huan Nan et al. 2021). Oceans have provided a smug base for biological activities on the earth. The marine environment is one of the least unexplored sources of marine microorganisms (bacteria, fungi, actinomycetes, cyanobacteria, and diatoms) that are potential producers of bioactive secondary metabolites (Hasan et al. 2015). Characteristically, the marine ecosystem is relatively competitive for survival from different predators and opponents. To protect themselves from other competitors and in self-defense, a multitude of bioactive compounds tend to synthesize by the organism that can easily obstruct other's growth. Among the diverse range of marine microorganisms, fungi and bacteria particularly have gained an important role as a rich source of biologically active secondary metabolites against different ailments such as antibacterial, anticancer, and antifungal. A range of compounds isolated from marine-derived fungi are in use such as pestalon (**1**), cladospolide E (**2**), xestodecalactone B (**3**), helicascolide C (**4**), circumdatin G (**5**), and torreyanic acid (**6**) (Fig. 8.1) (Bajpai 2016; Deshmukh et al. 2018). Sponges, soft corals, invertebrates, and other organisms are the common host of different marine fungi that habitat symbiotic, parasitic, and commensal relationships with each other (Gonçalves et al. 2022). The nature of marine fungi can be obligate and facultative which is considered to be more pharmaceutically important as compared to terrestrial fungi.

Even after the discovery of penicillin and cephalosporin from marine fungi, the marine microflora especially fungi remained unexplored. An estimation of around 1.5–5.1 million different kinds of fungi exists worldwide. Among them, marine fungi contribute less than 1% which is also poorly understood. It has been documented that approximately 12,500 marine fungi species have been identified and over a million marine fungal species are yet to be discovered. As of now, there were only 1901 marine fungal species described which were distributed into

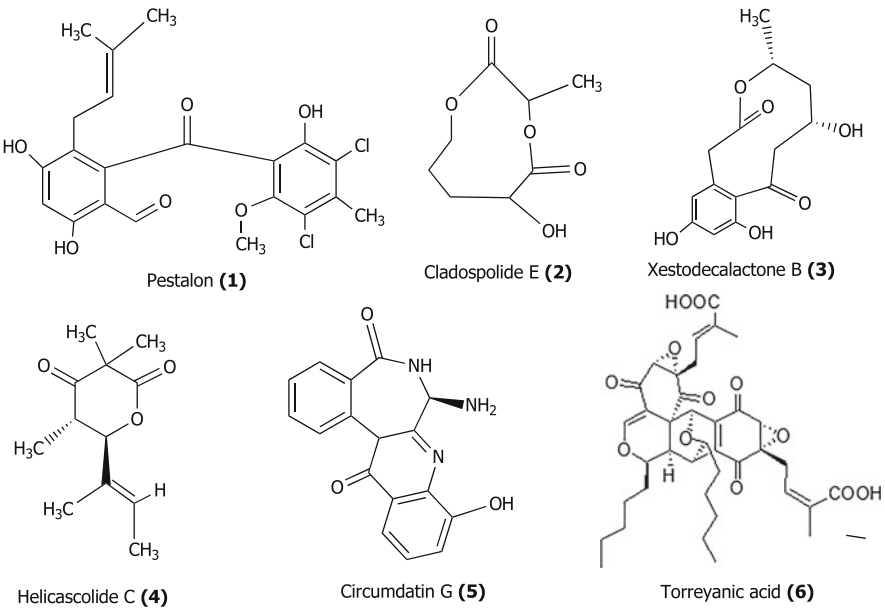

Fig. 8.1 Structure of compounds isolated from marine-derived fungi having antimicrobial activity

769 genera, 226 families, 88 orders, and 22 classes. The existing known fungi were classified into seven phyla termed *Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Aphelidiomycota, Mucoromycota,* and *Mortierellomycota.* Secondary metabolites isolated from marine fungi were found to be more effective, because of their adverse habitat conditions that grow under high salinity, high pressure, temperature variation, nutrients scarcity, and competition with bacteria, viruses, and other fungi (Bajpai 2016; Gonçalves et al. 2022). The first antibacterial agent isolated from marine fungi *Acremonium chrysogenum* was cephalosporin in 1945. The discovery of cephalosporin fueled the exploration of marine microorganisms as a source of antibacterial agents which initiated the golden era for exploring different natural products from marine fungi (Muñiz et al. 2007). More than 3500 marine fungi-derived secondary metabolites have already been reported which have different therapeutic potentials and many more remain undiscovered (Carroll et al. 2019). Alkaloids, terpenes, macrolides, tetracyclines, and aminoglycosides are chemical classes of secondary metabolites produced by marine fungi which show tremendous antifungal, antitumor, anticancer, antiproliferative, antibacterial, and cytotoxic activity (Hasan et al. 2015). This chapter highlights different secondary metabolites, their structure, and therapeutic activities that were isolated from different marine fungi. These bioactive compounds with antibacterial activity against both Gram-positive and Gram-negative bacteria contribute about 76% of new natural products from marine-derived fungi.

2 Fungal Secondary Metabolites Biosynthetic Pathway

Secondary metabolites are structurally heterogenic low-molecular weight, molecules synthesize naturally in different organisms from bacteria to plants. Unlike primary metabolites, these compounds are not directly required for the growth of the organisms. It has long been understood that factors such as carbon and nitrogen, temperature, salinity stress, light, and pH influence secondary metabolite production. As a result, modifications to growth conditions can significantly alter the range and quantity of secondary metabolites synthesized in fungi. A variety of stimuli were used to generate secondary metabolites producing conditions for marine fungi. The growth and nutrition conditions are considered to be key factors for the synthesis and induction of secondary metabolite production in microbes (Kebede et al. 2017). These metabolites are the end result of enzymatic cascades that begin when backbone enzymes such as polyketide synthases (PKSs), nonribosomal peptide synthetases (NRPSs), terpene cyclases (TCs), and dimethylallyl tryptophan synthases (DMATSs) respectively, catalyze the condensation or rearrangement of simple primary metabolites, like acetyl-CoA, amino acids, resulting into more complex secondary metabolites. Marine fungi produced five different kinds of secondary metabolites such as polyketides, cyclic terpenes, nonribosomal peptides, indoles, alkaloids, and hybrid. Marine alkaloids are nitrogenated small molecules, containing a primary amine group with amphiphilic, rigid, and low globular nature that tend to aggregate more efficiently in Gram-negative pathogens, resulting to facilitate a potential antibacterial activity (Willems et al. 2020). Marine macrolides are a premium natural product with a polyene backbone that has highly oxygenated macrocyclic lactone. Significant efforts have been made over the past few decades to identify and define the chemical and biological characteristics of macrolide. Different macrolides have been isolated from marine fungi that were found to be highly effective against different ailments (Das et al. 2022). Marine fungi also synthesize therapeutically relevant cyclic peptides which display a wide range of antibacterial properties against bacteria, protozoa, fungi, and viruses (Ribeiro et al. 2022). In addition, different polyketide-mediated natural substances have also been isolated from marine fungi with different biological activities (Gomes et al. 2013).

These secondary metabolites are synthesized through different biosynthetic pathways but they also showed some common interlinks (Keller et al. 2005). In general, the biosynthesis genes coding for fungal secondary metabolites are located in a gene cluster that spans a few tens of kilobases (Kumar et al. 2018b; Kumar et al. 2019). The biosynthetic gene cluster of the fungal secondary metabolite is primarily clustered either in a single locus or a different locus of the same chromosome. It is rare and an exception to have such gene clusters located at different chromosomes in fungi (Keller 2019).

3 Bioactive Compounds Derived from Marine Fungi

An essential aspect of marine-originated microorganisms is their ability to produce secondary metabolites. A large number of biologically active compounds were isolated from marine ecosystems, especially from marine fungi, which have shown great potential to use as antimicrobial and cosmeceutical agents. These bioactive compounds from marine-derived belong to the diverse nature of secondary metabolites.

The different biosynthetic pathways involved in the secondary metabolites synthesis are summarized in Fig. 8.2. Polyketides are one of the largest categories of natural secondary metabolites, with various biological activities valuable to the pharmaceutical industry. The polyketide synthases (PKSs) are enzyme complexes that catalyze carbon skeleton assembly of polyketides from coenzyme-A fatty esters, which are made from short-chain fatty acids like acetate, propionate, and butyrate (Gomes et al. 2013). PKSs are multidomain proteins that are related to eukaryotic fatty-acid synthases and contain similar domain structures. Like nonribosomal peptide synthetase, PKSs are also known as multidomain enzymes comprised of an acyltransferase (AT), ketoacyl CoA synthase (KS), dehydratase (DH), ketone-reducing enoyl reductase (ER), keto reductase (KR), and acyl carrier (ACP) domains. PKSs are classified into three classes, PKS I, II, and III, which have identical enzymatic functions, but variations in chain initiation and termination steps. Among them, PKS I is the most complex and versatile in nature, further sub-categorized into iterative and noniterative types (Kumar and Kumar 2019). After

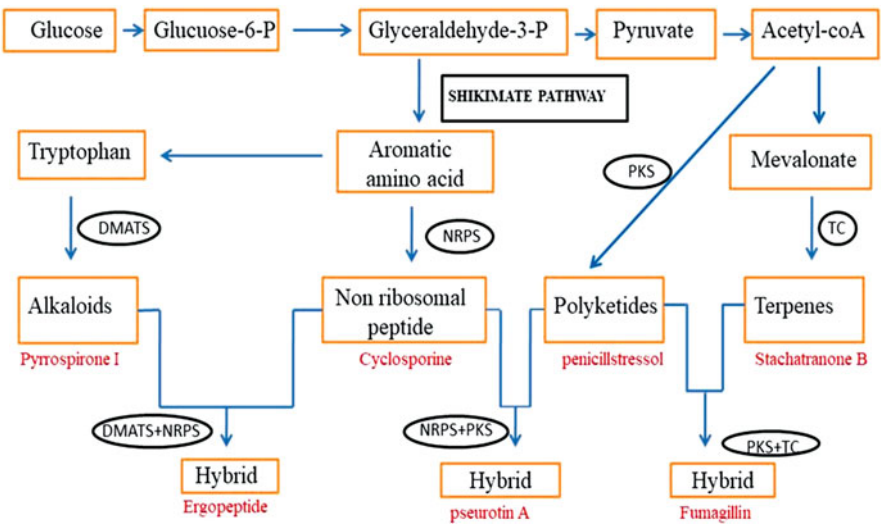

Fig. 8.2 Biosynthetic pathways of secondary metabolites. *NRPS* nonribosomal peptide synthetase, *PKS* polyketide synthase, *TC* terpene cyclase, *DMATS* dimethylallyl tryptophan synthase (Assaf et al. 2020)

polyketides, terpenes are a dominating group of marine-derived secondary metabolites. All terpenes are composed of several isoprene units, that can be linear or cyclic, saturated or unsaturated. Common classes of terpenes include monoterpenes, which are generated from geranyl pyrophosphate; sesquiterpenes, which are generated from farnesyl pyrophosphate; and diterpenes and carotenoids, which are generated from geranyl-geranyl pyrophosphate (Ebel 2010). The key enzyme in terpene synthesis is terpene cyclase, which is responsible for producing a variety of terpenes from different diphosphates. Although terpene cyclases have structural homology, they have little primary sequence similarity and seem to have diverged relatively from a common ancestor (Saleem et al. 2007). Nonribosomal peptide or mixed polyketide-nonribosomal peptide secondary metabolites derived from marine bacteria, cyanobacteria, and fungi make up more than 50% of drugs currently in clinical use (Agrawal et al. 2017). Nonribosomal peptides are derived from both proteinogenic amino acids and nonproteinogenic amino acids by multidomain, multimodular enzymes named, nonribosomal peptide synthetases (NRPSs) (Keller et al. 2005). All the secondary metabolites present in micro to higher organisms belong to these classes or their hybrid.

4 Marine-Derived Fungi Effective Against Different Bacterial Pathogens

4.1 Marine-Derived Compounds Effective to Gram-Positive Bacterial Pathogens

SCOPE (Surveillance and Control of Pathogens of Epidemiologic Importance) reported that the infection caused by Gram-positive bacteria has dramatically increased and it reached up to 78% in bloodstream infections in the last two decades (Gerberding et al. 2000). Gram-positive bacteria like *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus pneumonia,* and *Enterococci* are the leading infectious pathogens, causing different acute and chronic infections. The formation of biofilm and a variety of virulence factors secreted by bacteria are some of the key determinants that contributed to therapeutic failure, predominantly after emerging drug resistance against topical antibiotics (Kumar et al. 2018a, b). At present, penicillin, cloxacillin, daptomycin, linozolide, cephalosporins, and quinupristin-dalfopristin are commonly used against leading drug-resistant pathogens as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), vancomycin-intermediate-sensitive/resistant *S. aureus* (VISA and VRSA), coagulase-negative *Staphylococcus* (CoNS), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) (Woodford and Livermore 2009; Shariati et al. 2020). Continuously emerging drug resistance in Gram-positive pathogens demands, new antimicrobial agents from natural microbial sources. Marine-derived fungi-associated marine substrates including marine sponges, fishes, mangroves, and algae

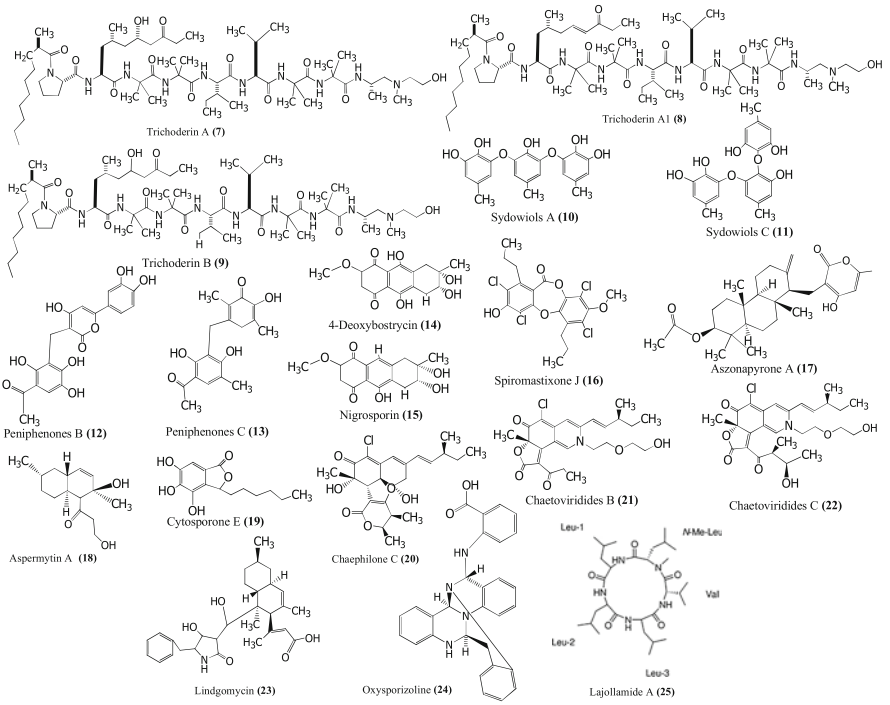

Fig. 8.3 Structures of marine fungi-derived bioactive compounds effective against gram-positive bacteria

produced a variety of secondary metabolites which possess a tremendous antibacterial nature. We summarize most of the known antimicrobial compounds isolated from marine-derived fungi (Wisplinghoff et al. 2003). Marine sponge-derived *Trichoderma* sp. isolated three compounds trichoderins A (**7**), A1 (**8**), and B (**9**) (Fig. 8.3) which were found effective against *Mycobacterium smegmatis*, *Mycobacterium bovis, Bacillus Calmette-Guerin* (BCG), and *Mycobacterium tuberculosis* H37Rv having a minimum inhibitory concentration (MIC) ranging from 0.02 to 2.0 μg/mL (Pruksakorn et al. 2010, 2011).

Sydowiols A (**10**) and C (**11**) (Fig. 8.3) isolated from *Aspergillus sydowii* from an East China sea sediment potentially blocked tyrosine phosphatases A (mPTPA) activity of *M. tuberculosis* with IC_{50} 14 and 24 μg/mL, respectively (Liu et al. 2013). Similarly, tyrosine phosphatases B (mPTPB) activity of *M. tuberculosis* was observed to inhibit by peniphenones B (**12**) and C (**13**) compounds, isolated from the mangrove fungal strain of *Penicillium dipodomyicola* synthesized (Fig. 8.3) (Li et al. 2014). Natural anthraquinone, 4-Deoxybostrycin (**14**) and its deoxyderivative, nigrosporin (**15**) (Fig. 8.3) isolated from mangrove endophytic fungus *Nigrospora* sp. showed an inhibitory effect against multidrug-resistant (MDR) *Mycobacterium tuberculosis* H37Rv (Wang et al. 2013). The chlorinated and prenylated

benzophenone, pestalon (**1**) extracted from marine fungal strain CNL-365, *Pestalotia* sp. (deuteromycete) showed a commendable antimicrobial effect against vancomycin-resistant *Enterococcus faecium* at 78 ng/mL concentration. Spiromastixone J (**16**) (Fig. 8.3) (chlorodepsidones) isolated from *Spiromastix* sp. MCCC 3A00308 also ceased the growth of *S. aureus* ATCC 29213 and vancomycin-resistant bacteria *E. faecalis* and *E. faecium* (VRE) at a range of 0.125–8 μg/mL concentration of MIC (Niu et al. 2014). A similar effect of pestalon was also observed against methicillin-resistant *S. aureus* (MRSA) with a MIC value of 37 ng/mL (Cueto et al. 2001). Similarly, a meroterpenoid aszonapyrone A (**17**) (Fig. 8.3) obtained from coral-derived fungus *Neosartorya laciniosa* KUFC 7896 was found to be strongly active against vancomycin-resistant *E. faecium* (Gomes et al. 2014). Recently, aspermytin A (**18**) (Fig. 8.3), a polyketide from a Marine-derived *Aspergillus* sp. strongly diminishes sortase (SrtA) activity, in *S. aureus* ATCC6538p, resulting in disruption of bacterial adherence to fibronectin-coated surfaces (Park et al. 2020). An endophytic fungal strain *Leucostoma persoonii,* isolated from red mangrove, *Rhizophora mangle* synthesized cytosporone compounds in the presence of epigenetic modifiers, among which cytosporone E (**19**) (Fig. 8.3) showed strong antibacterial and antibiofilm activities against MRSA at 72 μM concentration (Beau et al. 2012). Another study state that isolated azaphilone such as chaephilone C (**20**) chaetoviridides B (**21**) and C (**22**) (Fig. 8.3) from deep-sea *Chaetomium globosum* shows strong staphylocidal activity against MRSA (Wang et al. 2018). Lindgomycin (**23**) (Fig. 8.3) synthesized in *Lindgomyces* LF327 and KF970 fungi, isolated from Baltic Sea sponge showed inhibitory efficacy against *S. epidermidis,* and Methicillin-resistant *S. epidermidis* (MRSE) and *S. aureus* (Willems et al. 2020; Wu et al. 2015). In Suncheon Bay, Korea, the marine fungus *F. oxysporum* produced oxysporizoline (**24**) (Fig. 8.3) showed antibacterial properties against MRSA, and multidrug-resistant *S. aureus* in an antimicrobial assay (MDRSA) (Ibrahim et al. 2021). The marine-derived filamentous fungus *Asteromyces cruciatus* 763, produces a pentapeptide compound named lajollamide A (**25**) (Fig. 8.3). This pentapeptide showed an antibacterial effect against *S. epidermidis and B. subtilis* at a concentration of 100 μM (Gulder et al. 2012).

4.2 Marine-Derived Fungal Compounds Effective Against Gram-Negative Bacteria

Infections caused by Gram-negative bacteria have increased globally in recent years due to multitude reasons. These infections are frequently more common than Gram-positive infections. According to CDC's report, it is estimated that up to two million people were infected with Gram-positive or Gram-negative bacterial infections with 0.2 million fatalities caused every year in the US (Dadgostar 2019). Earlier studies revealed that the prevalence of Gram-negative infections in hospital-acquired settings is relatively two- to threefold higher than the Gram-positive pathogens (Alhumaid et al. 2021).

Table 8.1 List of marine fungi-derived bioactive compounds effective against gram-positive bacteria

Compound	Source	Activity against	MIC	Reference
Trichoderins A (**7**), A1 (**8**), and B (**9**)	*Trichoderma* sp.	*Mycobacterium smegmatis*	0.02–2.0 μg/mL	Pruksakorn et al. (2010, 2011)
Sydowiols A (**10**) and C (**11**)	*Aspergillus sydowii*	*M. tuberculosis*	14 and 24 μg/mL	Liu et al. (2013)
Peniphenones B (**12**), Peniphenones C (**13**)	*Penicillium dipodomyicola* HN4-3A	*M. tuberculosis*	0.16 and 1.37 μM	Li et al. (2014)
4-Deoxybostrycin (**14**), Nigrosporin (**15**)	*Nigrospora* sp.	*M. tuberculosis* H37Rv	15 and 20 μg/mL	Wang et al. (2013)
Pestalon (**1**)	Strain CNL-365, *Pestalotia* sp.	VRE and *S. aureus*	78 and 37 ng/mL	Cueto et al. (2001)
Spiromastixone (**16**)	*Spiromastix* sp. MCCC 3A00308	*S. aureus* ATCC 29213, vancomycin-resistant *E. faecalis,* and *E. faecium* (VRE)	0.125–8 μg/mL	Niu et al. (2014)
Aszonapyrone A (**17**)	*Neosartorya laciniosa* KUFC 7896	*Enterococcus faecium*	16 μg/mL	Gomes et al. (2014)
Aspermytin A (**18**)	*Aspergillus* sp.	*S. aureus* ATCC6538	146 μM	Park et al. (2020)
Cytosporone E (**19**)	*Leucostoma persoonii*	MRSA	72 μM	Beau et al. (2012)
Chaephilone C (**20**) and Chaetoviridides B (**21**) & C (**22**)	*Chaetomium globosum*	MRSA	7.3–7.8 μg/mL	Wang et al. (2018)
Lindgomycin (**23**)	*Lindgomyces* strains LF327 and KF970	*S. epidermidis*, *S. aureus*, and MRSE	2.2–5.1 μM	Willems et al. (2020); Wu et al. (2015)
Oxysporizoline (**24**)	*F. oxysporum*	MRSA, multidrug-resistant *S. aureus* (MDRSA)	6.25 μg/mL	Ibrahim et al. (2021)
Lajollamide A (**25**)	*Asteromyces cruciatus* 763	*S. epidermidis, B. subtilis*	100 μM	Gulder et al. (2012)

Gram-negative pathogens frequently cause bacteremia, ventilator-associated pneumonia (VAP), urinary tract infections (UTIs), and intra-abdominal infections (IAI) and are also responsible for the majority of fatalities (Denkinger et al. 2013) (Table 8.1).

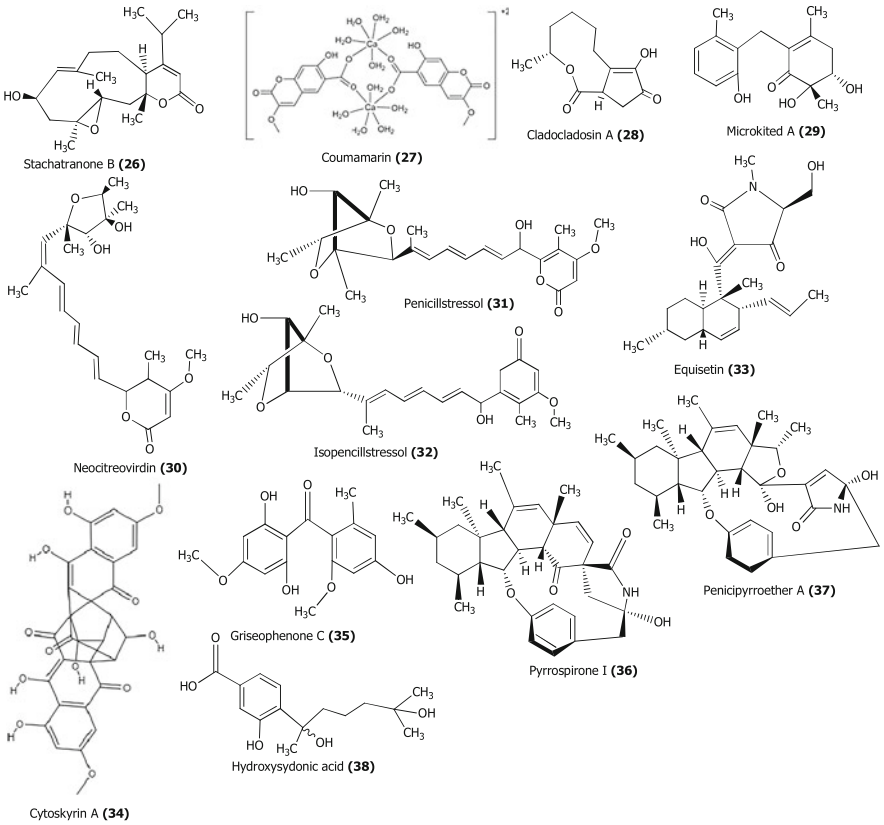

Fig. 8.4 Structure of marine fungi-derived bioactive compounds effective against gram-negative bacteria

The leading Gram-negative pathogens include *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa* which contribute to almost 27% of all nosocomial infections. In long-term medication, treatment and widespread use of antibiotics are responsible for increment in multidrug resistance (MDR) in Gram-negative infections. The risk of MDR has been significantly increasing and emerging as a global issue, particularly in extensively drug-resistant (XDR) bacteria. Although, the pipeline for new agents against resistant Gram-negative bacterial pathogens is limited but bioactive secondary metabolites isolated from different sources including marine-derived fungi found to be successful to restrict associated infections. The carbapenem-resistant *Acinetobacter baumannii* is a leading Gram-negative pathogen. For instance, stachatranone B (**26**) (Fig. 8.4) isolated from cultures of a marine-derived fungi *Stachybotrys chartarum* showed a strong inhibitory effect against *A. baumannii* (Yang et al. 2019). Likewise, coumamarin (**27**) (Fig. 8.4) synthesized from *Aspergillus sydowii* and cladocladosin A (**28**) (Fig. 8.4) produced and isolated from mangrove-derived endophytic fungus *Cladosporium cladosporiodes* both

exhibited strong antibacterial activity against *P. aeruginosa* (Zhang et al. 2020; Hamed et al. 2019). Similarly, microkited A (**29**) (Fig. 8.4), a diphenylketone derivative was obtained from *Anthogorgia ochracea* also showed potential antibacterial activity against *P. aeruginosa* (Liu et al. 2020). Neocitreovirdin (**30**), and two polyene-pyrone polyketide penicillstressol (**31**) and isopencillstressol (**32**) (Fig. 8.4) were isolated from a metal stressed strain of marine fungi of *Penicillium* sp. BB1122. These cryptic metabolites tend to kill clinical isolates of *P. aeruginosa* (Auckloo et al. 2017). Equisetin (**33**) (Fig. 8.4), first member of 3-decalinoyltetramic acid family which synthesized in *Fusarium* sp. Z10 showed antibiofilm and antivirulent activity to *P. aeruginosa* (Zhang et al. 2018). An endophytic mangrove fungus, *Conocarpus erectus* is capable to synthesize a bisanthraquinone compound known as cytoskyrin A (**34**) (Fig. 8.4). This compound has a potent bacteriostatic and bactericidal activity against *E. coli* imp BAS849 by targeting DNA damage (Singh et al. 2007). Mangrove-derived fungi *Nigrospora* sp. MA75-produced griseophenone C (**35**) (Fig. 8.4), a cochlioquinone showed a significant antibacterial against *E. coli,* with MIC values of 2 μg/mL (Shang et al. 2012).

Likewise, marine fungi *Penicillium* sp. ZZ380 isolated from a wild sea crab *Pachygrapsus crassipes* produced pyrrospirone I (**36**), and penicipyrroether A (**37**) (Fig. 8.4), alkaloids. Both compounds show antibacterial activity against *E. coli* (Song et al. 2018, 2019). Deep-sea sedimented-derived fungus *Aspergillus versicolor* SD-330 produced a group of several bisabolene-type sesquiterpenoids such as hydroxysydonic acid (**38**) (Fig. 8.4), and this bioactive molecule was capable to show growth inhibitory effect against *E. coli* with MIC value ranging from 1.0 to 8.0 μg/mL (Li et al. 2019).

4.3 Broad-Spectrum Antimicrobial Effect of Marine-Derived Fungal Secondary Metabolites

Bioactive compounds derived from marine fungi have been shown potentially lead against both Gram-positive and Gram-negative bacteria. Pyranonigrin F (**39**) (Fig. 8.5), a bioactive compound isolated from *Penicillium brocae* MA-231, an endophytic fungus that recovered from the marine mangrove plant *Avicennia marina*. This pyranonigrin F (**39**) showed inhibitory action against both Gram-positive and Gram-negative bacteria *S. aureus, Vibrio harveyi,* and *Vibrio parahemolyticus* (Meng et al. 2015). Likewise, xanthone derivatives, emerixanthones A (**40**), C (**41**), and E (**42**) (Fig. 8.5) extracted from marine-derived *Emericella strain* SCSIO05240 showed antibacterial effect against *A. baumannii, E. coli, S. aureus,* and *Klebsiella pneumoniae* (Fredimoses et al. 2014, 2019). The *Penicillium* sp. ZZ380 isolated from a wild crab (*Pachygrapsus crassipes*), produced secondary metabolites pyrrospirone C (**43**), and F (**44**) (Fig. 8.5). Both these potentially show antibacterial activity against *E. coli* and MRSA (Song et al. 2018). The indoles represent a significantly larger class of antibacterial marine

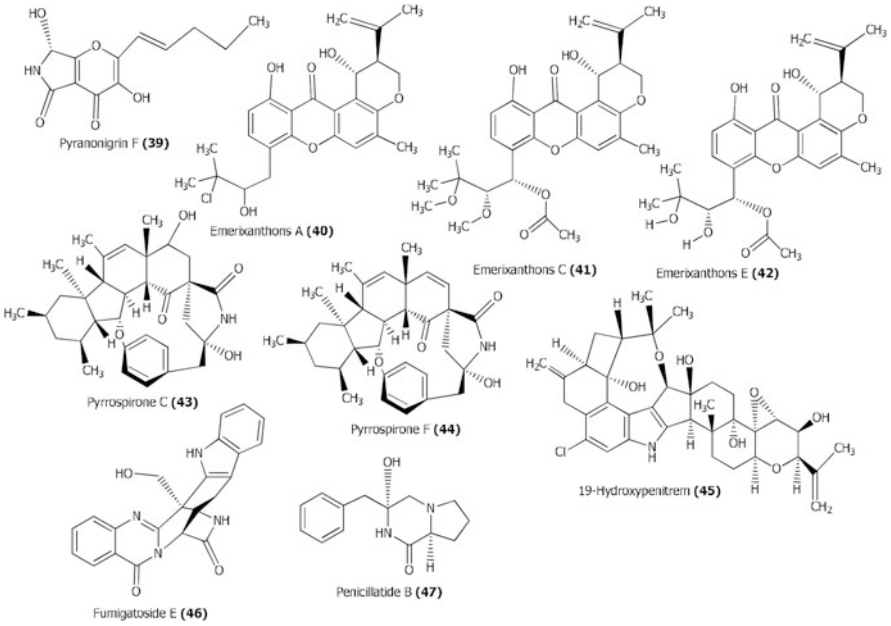

Fig. 8.5 Structures of marine-derived fungal secondary metabolites having broad spectrum antibacterial activity

alkaloids. Over the last 5 years, different indole compounds have been investigated. For instance, 19-hydroxypenitrem A (**45**) (Fig. 8.5) (indole-diterpenoid), a product of the algal-associated fungus *Aspergillus nidulans* EN-330 made susceptible to *S. aureus, E. coli, Edward siellatarda*, and *Vibrio anguillarum* (Zhang et al. 2015).

Another quinazoline indole, named fumigatoside E (**46**) (Fig. 8.5) was isolated from *Aspergillus fumigatus* SCSIO41012 that was capable to show broad-spectrum antibacterial activity against *S. aureus*, *K. pneumonia*, and *Acinetobacter baumannii* (Zhang et al. 2022; Limbadri et al. 2018). A Penicillium strain recovered from Red Sea tunicate *Didemnum* sp. synthesized penicillatide B (**47**) (Fig. 8.5), diketopiperazines that also showed a broad-spectrum antibacterial effect to Gram-positive and negative strains. However, it showed higher cell susceptibility to *V. anguillarum* than to *S. aureus* (Youssef and Alahdal 2018).

4.4 Marine-Derived Fungal Secondary Metabolites Effective Against Multidrug Resistant Bacterial Strains

Despite significant advancement in infection control and antimicrobial therapy, the spread of antibiotic resistance poses a serious threat primarily to individuals with

impaired immune systems. Particularly, multidrug-resistant bacteria leave few viable antibacterial options and are responsible for high mortality. Despite this, current clinical practice recommendations recommend using marine-derived fungi as a reliable therapeutic option for diseases that are resistant to a variety of drugs (Table 8.2).

The most out breaking threats related to the majority of hospital-acquired infections are caused by the ESCAPE pathogens (*Enterococcus faecium*, *S. aureus*, *Clostridium difficile*, *Acinetobacter* species, *P. aeruginosa*, and *Enterobacteriaceae*), these pathogens are able to easily "escape" the effects of antimicrobial drugs (Peterson 2009). Marine-derived fungi have also proved effective against MDR bacteria (Table 8.3).

The best-known example of a marine-derived antibacterial agent is cefiderocol (**48**) which is also known as "trojan horse" and targets the serine and metallo-lactamase carbapenemase producer bacterial pathogens and other Gram-negative pathogens. Strikingly, Food and Drug Administration (FDA) and European Medicines Agency (EMA) both approved FDA the cefiderocol as a broad-spectrum antibacterial agent (Simner and Patel 2020). By exploiting active iron transporters to penetrate bacterial cells, the novel siderophore antibiotic is able to bypass drug resistance caused by porin channel mutations and increased efflux pumps (Bonomo 2019). Likewise, 4-deoxybostrycin (**14**) also prescribed against multidrug-resistant (MDR) *Mycobacterium tuberculosis* H37Rv. Pestalon (**1**), a benzophenone and spiromastixone J (**17**) efficiently work against vancomycin-resistant *E. faecium* and *E. faecalis*. More in list lindgomycin (**24**) and oxysporizoline (**25**) secondary metabolites show moderate to high antibacterial efficacy against *Staphylococcus epidermidis,* and *Methicillin-resistant S. epidermidis* (MRSE), *S. aureus.* The chemical structure of molecules **1, 14, 17, 24–25,** and **48** is mentioned in Fig. 8.6.

5 Conclusion

Infection caused by multidrug-resistant bacterial strains is emerging global issue and it is something difficult to treat because these bacteria show resistance against most of antibiotic and sometimes to all available drugs. The number of therapies currently available for bacterial infections treatment and to combat the multidrug resistance issue are limited. Although marine ecosystems, especially marine-derived fungi are few agents with promising antimicrobial activity against sensitive and multidrug-resistant Gram-positive or Gram-negative bacteria. In this chapter, marine fungi isolated bioactive compounds were described that belong to various classes of secondary metabolites. Their unique chemical structure and low minimum inhibitory concentration values make them the most prominent antibacterial agents. All the isolated secondary metabolites showed moderate to high antibacterial activity either by killing the bacteria or inhibiting their growth, reducing their virulence nature by gene expression alteration, or either by inhibiting the formation of Biofilm of Gram-positive and Gram-negative sensitive and multidrug-resistant bacteria. Despite the

Table 8.2 List of marine fungi-derived bioactive compounds effective against gram-negative bacteria

Compound	Source	Compound type	Active against	MIC	Reference
Stachatranone B (**26**)	*Stachybotrys chartarum*	Dolabellane terpenoids	*A. baumannii*	47.9 μM	Yang et al. (2019)
Coumamarin (**27**)	*Aspergillus sydowii*	NA	*P. aeruginosa*	NA	Hamed et al. (2019)
Cladocladosin A (**28**)	*Cladosporium cladosporiodes*	Macrolide	*P. aeruginosa*	17.8 μM	Zhang et al. (2020)
Microkited A (**29**)	*Microsphaeropsis* sp. RA10–14	Diphenylketone	*P. aeruginosa*	0.19 μg/mL	Liu et al. (2020)
Neocitreovirdin (**30**)	*Penicillium* sp. BB1122	Polyketide	*P. aeruginosa*	4 μg/mL	Auckloo et al. (2017)
Penicillstressol (**31**)	*Penicillium* sp. BB1122	Polyketide	*P. aeruginosa*	4 μg/mL	Auckloo et al. (2017)
Isopencillstressol (**32**)	*Penicillium* sp. BB1122	Polyketide	*P. aeruginosa*	4 μg/mL	Auckloo et al. (2017)
Equisetin (**33**)	*Fusarium* sp. Z10	3-decalinoyltetramic acid	*P. aeruginosa*	NA	Zhang et al. (2018)
Cytoskyrin A (**34**)	*Cytospora* sp. CR200	Bisanthraquinone	*E. coli imp BAS849*	0.03–0.25 μg/mL	Singh et al. (2007)
Griseophenone C (**35**)	*Nigrospora* sp. MA75	NA	*E. coli*	2 μg/mL	Shang et al. (2012)
Pyrrospirone I (**36**)	*Penicillium* sp. *ZZ380*	Alkaloid	*E. coli*	2 μg/mL	Song et al. (2018, 2019)
Penicipyrroether A (**37**)	*Penicillium* sp. *ZZ380*	Alkaloid	*E. coli*	3 μg/mL	Song et al. (2018, 2019)
Hydroxysydonic acid (**38**)	*Aspergillus versicolor* SD-330	Sesquiterpenoids	*E. coli*	1.0 μg/mL	Li et al. (2019)

Table 8.3 Broad spectrum antibacterial activity of marine-derived fungal secondary metabolites

Compound	Source	Active against	MIC	Reference
Pyranonigrin F (**39**)	*Penicillium brocae* MA-231	*S. aureus, Vibrio harveyi,* and *Vibrio parahemolyticus*	0.5 mg/mL	Meng et al. (2015)
Emerixanthons A (**40**), C (**41**), and E (**42**)	*Emericella strain* SCSIO05240	*Acinetobacter baumanni E. coli, S. aureus Klebsiella pneumoniae*	50 μg/mL	Fredimoses et al. (2014, 2019)
Pyrrospirone C (**43**), F (**44**)	*Penicillium* sp. ZZ380	*E. coli* and MRSA	2.0–5.0 μg/mL	Song et al. (2018)
19-Hydroxypenitrem (**45**)	*Aspergillus nidulans* EN-330	*S. aureus, E. coli, Edward siellatarda*, *Vibrio anguillarum*	16–64 mg/mL	Zhang et al. (2015)
Fumigatoside E (**46**)	*Aspergillus fumigates* SCSIO 41012	*S. aureus, K. pneumonia Acinetobacter baumannii*	6.25–12.5 μg/mL	Zhu et al. (2018)
Penicillatide B (**47**)	*Penicillium* strain	*S. aureus*, *V. anguillarum*	100 μg/disc	Youssef and Alahdal (2018)

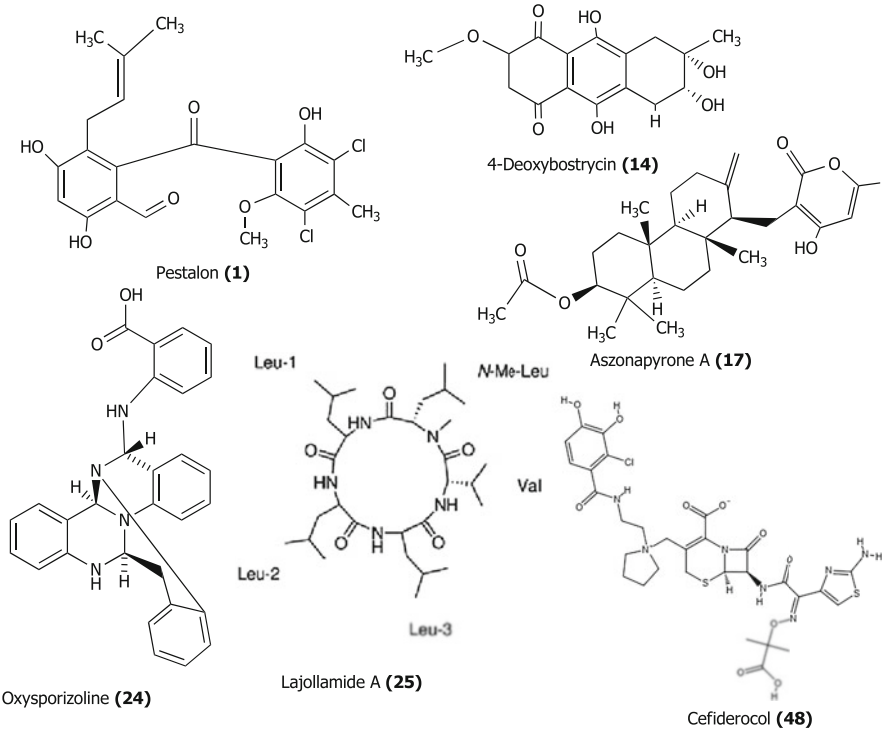

Fig. 8.6 Structure of marine-derived fungal secondary metabolite effective against multidrug resistant bacterial strains

high antibacterial potential of marine fungi, exploration of this group is not up to the mark. If researchers go deep down into marine fungi to discover bioactive compounds, it became easy to fight current and forthcoming infections in the world.

6 Future Prospects

The incidences of bacterial infections and emerging drug resistance bacterial elicit a need for the development of sustainable and potential alternatives for prevention of bacterial infections. Fungal secondary metabolites synthesized in marine fungi have gained an attention for prevention of various bacterial infections caused by drug-resistant pathogens. Marine fungal flora has proven their potential against different ailments. It is only a fraction of the total marine fungal flora that has been explored so far, which indicates toward the possibilities of immense therapeutic significance harbored by them which remains to be explored against other infectious or noninfectious health conditions.

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Part IV
Fungi as a Bioresource of Pharmacologically Active Agents

Chapter 9
Penicillium: A Treasure Trove for Antimycobacterial and Antioxidant Metabolites

Mehak Kaur, Hishita Peshwani, and Mayurika Goel

Abstract *Penicillium*, belonging to the phylum Ascomycota, is one of the most studied genera for its therapeutic properties. Based on ascocarps, it can be divided into two groups: *Eupenicillium* and *Talaromyces*. Bioactive secondary metabolites with antimicrobial, anticancer, antimycobacterial, antioxidant, anti-inflammatory, insecticidal, and biocontrol properties have been isolated from various species of this genus. The agricultural, biotechnological, and pharmaceutical applications of these metabolites are currently being investigated. This chapter highlights the potential of antimycobacterial, and antioxidant compounds isolated from *Penicillium* spp., including alkaloids, chromones, penicillanic acids, polyketides, steroids, terpenoids, and their derivatives. Biotechnological interventions, such as cocultivation, epigenetics, and genetic engineering, applied to enhance bioactivities, are elaborated. Furthermore, biologically synthesized metallic nanoparticles to augment the production of such metabolites in the genus would also be discussed.

Keywords Fungi · *Penicillium* · Antimycobacterial · Antioxidant · Bioactive compounds

1 Introduction

With the increasing technological advancements for bioprospecting natural sources to obtain bioactive metabolites, the focus on microorganisms for novel products has incremented exponentially. They are being widely exploited as continuous sources to meet the demands of ever-growing pharmaceutical industry. Around one-third of the new molecular entities (NMEs) approved by the food and drug administration (FDA) are from natural sources, out of which microbes share a good portion. Since 2000, 77% of licensed antibiotics have been made from natural ingredients sourced from microorganisms. The first FDA-approved fungal NME was an antibiotic named

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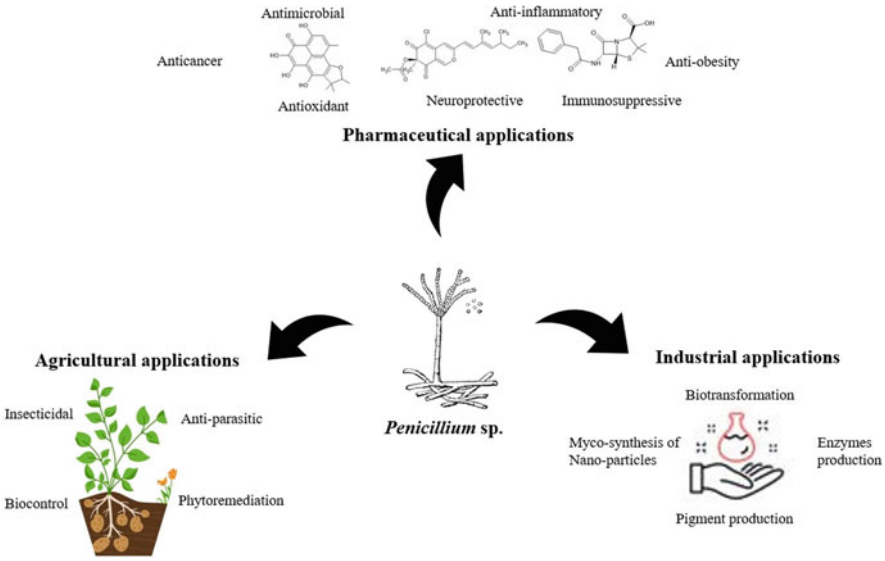

Fig. 9.1 Applications of secondary metabolites obtained from *Penicillium* sp.

phenoxy-methyl penicillin from *Penicillium* in 1953 and since then the genus has been one of the heavily studied fungal genera with more than 300 recognized species (Samson and Pitt 2000; Patridge et al. 2016). Sir Alexander Fleming's unintentional discovery of penicillin opened a new gate of fungal secondary metabolites with medicinal potential for humans, plants, and other biotechnological applications (Fig. 9.1) (Fleming 1980; Demain and Elander 1999). More than 500 compounds have been identified from different species of *Penicillium* with antimicrobial, anticancer, antioxidant, antiparasitic, antidiabetic, anti-inflammatory, neuroprotective, and immunosuppressive properties. Most compounds have been identified for their antitumor properties followed by antimicrobial properties (Nicoletti and Trincone 2016; Toghueo and Boyom 2020). Among the number of bioactivities, antimycobacterial and antioxidant potential are reviewed for the sake of this chapter. The biotechnological interventions that have been used recently to bioprospect and develop bioactive metabolites as active pharmaceutical agents have also been elaborated along with the mycosynthesis of pharmaceutically active nanoparticles.

2 Antimycobacterial Activity of *Penicillium*

Tuberculosis (TB) is a chronic respiratory disorder caused by *Mycobacterium*. *Mycobacterium* pathogen is the etiology of not only tuberculosis but also leprosy and atypical infections known as nontuberculous mycobacteria (NTM) infections. NTM are opportunistic pathogens affecting those with weakened immune systems.

TB was the foremost cause of death from a single communicable disease, until SARS-CoV-2. The World Health Organization (WHO) had established plans to eradicate TB by 2050. However, owing to the recent outbreak of the coronavirus, there was an increment of 3.6% in the incidence rate of TB between 2020 and 2021, halting the reductions that averaged 2% each year for the past two decades. The COVID-19 (coronavirus disease-2019) pandemic continues to impair TB diagnosis and treatment, driving the global TB targets off course. In 2021, 10.6 million people were diagnosed with TB worldwide, with more cases in men (56.5%) than in women (32.5%) and children being the least affected (11%). Approximately 1.6 million deaths occurred in the past year from TB and related infections (WHO 2022).

The only licensed vaccine for TB is BCG-Bacille Calmette Guerin (CDC 2019). However, it is not used extensively as it is not 100% effective. MVA85A was a failed booster vaccine for BCG (Macleod 2018). Rifampicin (RMP) is the most promising first-line anti-TB drug available in the market. RMP has been used for the past 50 years in combination with ethambutol, isoniazid, and pyrazinamide. TB treatment regimens last for 1–6 months. Inconsistent treatment and misuse of these drugs have accelerated the development of resistant strains. In addition, the emergence of MDR-TB (multidrug resistant TB) and XDR-TB (extensive-drug resistant TB), led to the current TB therapeutic crisis. Moxifloxacin and levofloxacin have been used to treat MDR-TB (Zheng et al. 2021). Bedaquiline (Sirturo®, FDA) is also available in the market but is less effective. The emergence of antibiotic resistance is substantially faster as compared to the slow pace of novel antibiotic discovery. The pharmaceutical industry has started to bioprospect natural sources for antimycobacterial compounds, with filamentous fungi showing substantial potential as cures. They serve as excellent alternative pharmaceuticals due to their low cost of production, vast diversity, and abundance of bioactive metabolites. For its ability to combat mycobacterial growth, *Penicillium* is one of the most exploited genera with *P. chrysogenum* being the most researched species.

Various compounds extracted from *Penicillium* spp. inhabiting *Garcinia nobilis* have displayed promising antimycobacterial activity (refer to Table 9.1) against *M. smegmatis* (Jouda et al. 2016). Furthermore, Andrioli et al. (2022), isolated a compound named emodin (**4**) from *P. citrinum*, endophytic fungus isolated from the plant *Ocotea notata*. They tested its antimycobacterial activity (via MTT assay) against various strains of *M. bovis* and *M. tuberculosis*. They also went a step further and performed a macrophage viability assay and determined a selectivity index (SI). Emodin exhibited the lowest MIC_{50} (minimum inhibitory concentration to inhibit the growth of the organism by 50%) value, less than 30% cytotoxicity, and an SI of 20, making it the most active compound among the six compounds studied. This compound also presented high activity against the NTM strain *M. kansasii*. Emodin was selective toward mycobacteria, protecting the microbiota, making it a promising candidate for a twofold treatment of TB and NMT infections (Fig. 9.2).

Penicillium chrysogenum and *Talaromyces* spp. isolated from the fruits and leaves of *Solanum mauritianum* exhibited broad-spectrum antimycobacterial activity against *M. smegmatis* and *M. bovis.* (Pelo et al. 2020). Another method that can be used to determine the antimycobacterial potential is the luciferase reporter phage

Table 9.1 Antimycobacterial activity of various species from the genus *Penicillium* (Fig. 9.2)

S. no.	Crude extracts of fungal endophytes		MIC/IC50 values	Against	Reference
	Species	Compound isolated			
1	*Talaromyces* spp. (strain HZ-YX1)	Talaramide A (**1**)	55[c]	*Mycobacterium PknG*	Prasher et al. (2020)
2	*P. chrysogenum* (F)	Extract	0.02[a]	*M. bovis, M. smegmatis*	Pelo et al. (2020)
3	*P. chrysogenum* (L)		0.01[a]	*M. bovis, M. smegmatis*	
4	*Talaromyces* sp.		0.03[a]	*M. bovis, M. smegmatis*	
5	*P. chrysogenum* DSOA	Extract	1[a]	*M. fortuitum*	Visamsetti et al. (2016)
6	*Penicillium* sp.	Brefeldin A (**2**)	0.25[a]	*M.smegmatis*	Jouda et al. (2016); Ferreira et al. (2019)
7		Citromycetin (**3**)	0.0312[a]	*M.smegmatis*	
8		Penialidin A (**5**)	0.0625[a]	*M.smegmatis*	
9		Penialidin B (**6**)	0.0625[a]	*M.smegmatis*	
10		Penialidin C (**7**)	0.0156[a]	*M.smegmatis*	
11		p-Hydroxy-phenyl-glyoxalaldoxime (**10**)	0.0625[a]	*M.smegmatis*	
12	*P. glabrum*		3.13[a]	*M. madagascariense, M. indicus pranii*	Kowanga et al. (2018)
13	*P. limosum*		0.78[a]	*M. madagascariense, M. indicus pranii*	
14	*P. janthinellum*		6.25[a]	*M. madagascariense, M. indicus pranii*	
15			6.25[a]	*M. madagascariense, M. indicus pranii*	
16	*P. charlesii*		3.13[a]	*M. madagascariense, M. indicus pranii*	
17	*P. sacculum*		12.5[a]	*M. madagascariense, M. indicus pranii*	

18	*Eupenicillium* spp.		12.5[a]	*M. madagascariense, M. indicus pranii*	
19	*P. dipodomyicola* HN4-3A	Peniphenone B (**8**)	0.063[b]	*MptpB*	Deshmukh et al. (2020)
		Peniphenone C (**9**)	0.452[b]	*MptpB*	
20	*Talaromyces* spp.	Talaramide A (**1**)	18.2[b]	*Mycobacterial PknG*	
21	*P. citrinum*	Emodin (**4**)	0.4 ± 0.1[c]	*M. bovis (BCG)*	Andrioli et al. (2022)
22			5.0, 5.3, 8[c]	*M.tuberculosis (H37Rv, M442, M299)*	
23			1.9, 9.1, 23.9[c]	*M. kansasii (12,478, 4404, 8835)*	

[a]MIC values in mg/mL
[b]IC_{50} values in mg/mL
[c]MIC_{50} values in μM

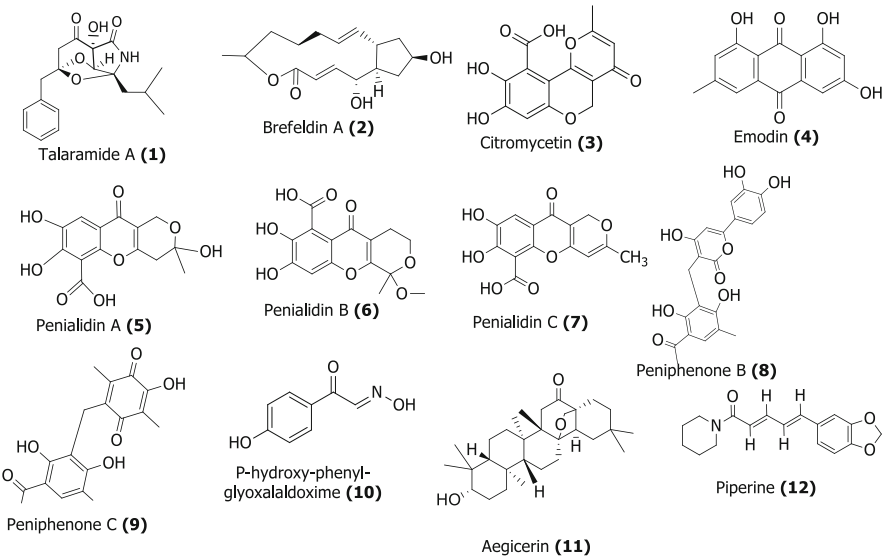

Fig. 9.2 Secondary metabolites from the genus *Penicillium* possessing antimycobacterial potential

(LRP) assay. The reduction in the relative light unit (RLU) by more than 50% in accordance with the control indicates antimycobacterial activity. Radhakrishnan et al. (2011) used this method for fungal bioprospecting Sundarbans mangrove forests and found that *Penicillium* spp. exhibited a 60% RLU reduction against *M. tuberculosis* H37Rv.

An endophyte's defense response to stress affects the amount and type of biosynthetic metabolites produced. Deshmukh et al. (2020), researched mangrove-associated fungi and deciphered that their stress tolerance makes them an ideal source of diverse chemical metabolites. Of the 183 compounds isolated (anti-infectives), 17 possessed antimycobacterial properties. An alkaloid, talaramide A (**1**), was extracted from *Talaromyces* spp. from *K. obovata* leaves. This compound exhibited potent inhibitory activity, with an IC_{50} value of 18.2 mg/mL against mycobacterial PknG. Antimycobacterial peniphenone B (**8**) and C (**9**) were also isolated from *P. dipodomyicola* HN4-3A, an endophytic fungus from the plant *Acanthus ilicifolius.* Likewise, Kowanga et al. (2018) investigated the antimycobacterial efficacy of endophytes harboring the saline waters of Lake Magadi. *P. glabrum, P. decumbens, and P. charlesi* had MIC value of 3.13 mg/mL against *M. madagascariense*, while *P. limosum* and *P. sacculum* had MIC values of 0.78 mg/mL. *Eupenicillium* spp. showed no antimycobacterial activity. Marine sponge-associated endophytes have also been recently explored for novel drugs. Visamsetti et al. (2016) reported an antimycobacterial compound produced by *P. chrysogenum* DSOA extracted from the marine sponge, *Tedania anhelans.* The MIC values ranged between 0.03 and 1.00 mg/mL. Showing moderate activity, the extract completely inhibited *M. tuberculosis* and *M. vaccae* at an MIC of 0.03 mg/

mL. This report also indicated that DSOA might be shielding mutations in antimycobacterial compound-producing pathways (Fig. 9.2).

3 Antioxidant Activity of *Penicillium*

When a covalent link between two atoms is broken and one electron stays with each newly produced atom, a free radical is created (an atom or molecule with one unpaired electron). In human physiology, they boost the immune system, promote cell signaling, and play a critical role in apoptosis, all of which serve as protection from disease. However, they can also harm essential macromolecules in cells, contributing to the aging process, the development of cancer, and the onset of cardiovascular disorders. Reactive oxygen species (ROS), also known as free radicals, play a significant role in the pathogenesis of many different conditions, most notably carcinogenesis, cardiovascular disease, ischemia, Alzheimer's disease, early aging, arteriosclerosis, liver injury, inflammation, diabetes mellitus, skin damage, and arthritis (Song and Yen 2002; Valentão et al. 2002). By neutralizing or stabilizing free radicals, antioxidants halt these chain events, and by self-oxidizing, they block subsequent oxidation reactions (Bhattacharya 2014; Valentão et al. 2002). The prevention of oxidative cell damage is aided by the presence of natural antioxidants such as vitamin C, glutathione, and antioxidant enzymes including superoxide dismutases (SOD), catalases, and peroxidases in the cells (Pisoschi and Pop 2015). Numerous phytochemicals, including vitamins C, E, α-tocopherol (Baines and Seal 2016), β-carotene (Wang et al. 2015), flavonoids, tannins, coumarins, xanthones, terpenoids, and other phenolic compounds, are among the many dietary antioxidants available which are isolated from fungi, algae, and plants (Faustino et al. 2019).

As one of the most effective producers of bioactive secondary metabolites, microorganisms are of tremendous biotechnological interest in the fermentative processes to develop antioxidant compounds. *Penicillium* has been studied for producing various phenolic antioxidants such as curvulic acid (**13**), 2,3-hydroxybenzoic acid (**14**), atrovenetin (**15**), and a chlorine-containing pigment sclerotiorin (**16**) (Lu et al. 2008; Mapari et al. 2005). Various sources of *Penicillium* such as marine water, hypersaline water, soil, plants, and lichen have been explored to isolate known and novel antioxidant compounds (Mishra et al. 2022a). Sun et al. (2009) identified antioxidant polysaccharides from marine *Penicillium* spp. F23-2 while Canturk et al. (2017) explored *P. flavigenum* from hypersaline waters of Turkey and isolated phenolic acids with antioxidant properties. Halotolerant species *P. citrinum* B-57 yielded two novel antioxidant compounds penicitrinone C (**17**) and penicitrinol B (**18**) (Lu et al. 2008). Jakovljevic and team (Jakovljevic et al. 2014, 2018) surveyed various *Penicillium* species obtained from wastewaters and found secondary metabolites with antioxidant properties. Team of Arora (Arora and Chandra 2011; Arora et al. 2012) surveyed soil-borne *P. citrinum* and *P. granulatum* for antioxidant compounds. Rhizospheric fungi *P. citrinum* isolated

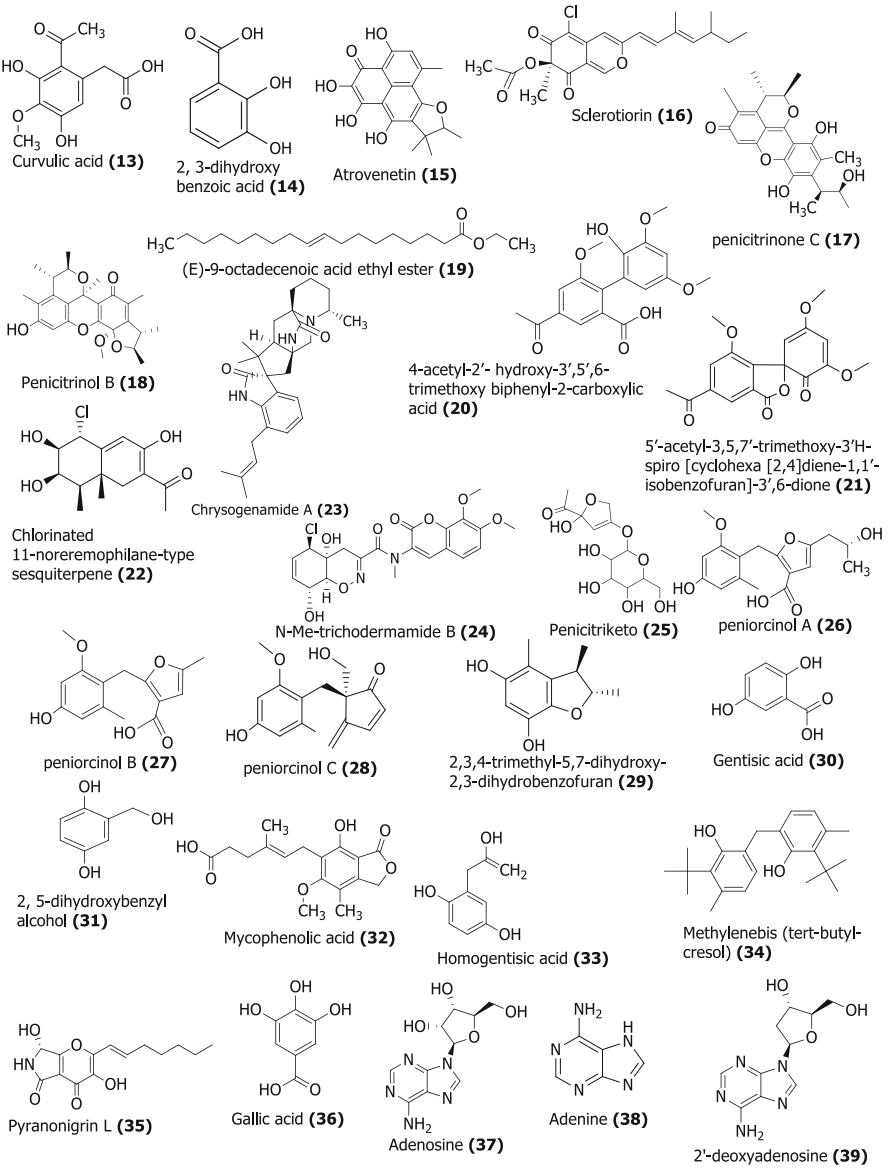

Fig. 9.3 Antioxidant secondary metabolites isolated from the genus *Penicillium*

by Sahu et al. (2022) also exhibited antioxidant activity owing to its secondary metabolite (E)-9-octadecenoic acid ethyl ester (**19**) (Fig. 9.3).

In the search of novel antioxidant compounds, many endophytic and endolichenic sources of *Penicillium* have also been exploited (Mishra et al. 2020; Agrawal et al. 2020). Endolichenic fungus *P. citrinum* isolated from *Parmotrema* species yielded

two novel antioxidants 4-acetyl-2′- hydroxy-3′,5′,6-trimethoxy biphenyl-2-carboxylic acid (**20**) and 5′-acetyl-3,5,7′-trimethoxy-3'H-spiro (cyclohexa [2,4] diene-1,1′-isobenzofuran)—3′,6-dione (**21**) with high antioxidant activity (Samanthi et al. 2015). Isolation of chlorinated 11-noreremophilane-type sesquiterpene (**22**) (Yuan et al. 2017), chrysogenamide A (**23**) (Lin et al. 2008), N-Me-trichodermamide B (**24**) (Yang et al. 2017), penicitriketo (**25**) (Wang et al. 2014), and peniorcinols A-C (**26–28**) (Bai et al. 2021) have been done from various *Penicillium* spp. isolated as endophytic fungi (Table 9.2). Pigmented extracts such as red pigment from *P. purpurogenum* and sclerotiorin (**16**) from *P. frequentans* and endophyte *P. mallochii* also exhibited antioxidant activity (Chidananda and Sattur 2007; dos Santos et al. 2019). *P. citrinum* is one of the most explored species for novel antioxidant metabolites (Fig. 9.3).

4 Biotechnological Interventions in *Penicillium* for Enhanced Metabolite Production

4.1 Antimycobacterial Potential

Penicillium is one of the most exploited genera of ascomycetous fungi for its antimicrobial activity against *Mycobacterium*. The mycobacteriaceae family comprises 190 species, amid which *M. tuberculosis, M. avium,* and *M. leprae* are so far the most significant pathogens affecting global health (Ferreira et al. 2019). The first antibiotic in existence, penicillin was derived from *P. chrysogenum*, which has also been documented to have remarkable antimycobacterial potential. Various physiological states of mycobacteria and its microenvironment should be accounted while developing new antimycobacterial drugs. There have been studies exploring the impact of environmental factors such as salinity, waterlogging, type of media used, and more on the metabolite production in microorganisms. Upscaling from the bench scale to the pilot scale requires optimization of several growth factors. Techniques and experimental designs such as response surface methodology (RSM), one factor at a time (OFAT), and Plackett Burman (PB) design, can be used to drastically improve product yield, cut costs, time, and process variability. Amarendra et al. (2015) used a combination of classical OFAT for media optimization and statistical PB to inspect the importance of selected factors on marine *P. chrysogenum* DSOA. Glucose and calcium nitrate were identified as appropriate carbon and nitrogen sources as they increased activity by 3.75- and 1.625-fold, respectively. Amino acids have been found to inhibit the production of antimycobacterial metabolites. There was a substantial difference between the secondary metabolites produced, depending on the basal medium used. In defined media (NB—nutrient broth), high levels of initial pH, sodium chloride concentration, incubation period, and low levels of calcium nitrate and glucose were

Table 9.2 Antioxidant activity of metabolites isolated from the genus *Penicillium* (Fig. 9.3)

S. No.	Species	Source	Antioxidant metabolite	Reference
1	*P. janthinellum* Biourge	Lab cultured	Curvulic acid (**13**), homogentisic acid (**33**), methylenebis (tert-butyl-cresol) (**34**)	Nakakita et al. (1984)
2	*P. roquefortii* IFO 5956	Lab cultured	2,3-dihydroxy benzoic acid (**14**)	Hayashi et al. (1995)
3	*P. paraherquei*	Soil	Atrovenetin (**15**)	Ishikawa et al. (1991)
4	*P. frequentans*	Lab cultured	Sclerotiorin (**16**)	Chidananda and Sattur (2007)
5	*P. mallochii*	Plant—*Himatanthus* sp	Sclerotiorin (**16**)	dos Santos et al. (2019)
6	*P. citrinum* B-57	Salt field	Pennicitrinone C (**17**) and penicitrinol B (**18**)	Lu et al. (2008)
7	*P. citrinum*	Rhizospheric region	(E)-9-octadecenoic acid ethyl ester (**19**)	Sahu et al. (2022)
8	*P. citrinum*	Lichen—*Parmotrema* species	4-acetyl-2′-hydroxy-3′,5′,6-trimethoxy biphenyl-2-carboxylic acid (**20**), and 5′-acetyl-3,5,7′-trimethoxy-3'H-spiro [cyclohexa [2,4] diene-1,1′--isobenzofuran]-3′,6-dione (**21**)	Samanthi et al. (2015)
9	*P. citreonigrum*	Plant—*Dryopteris setosa* (Thunb.)	Chlorinated 11-noreremophilane-type sesquiterpene (**22**), and mycophenolic acid (**32**)	Yuan et al. (2017)
10	*P.chrysogenum* no. 005	Plant—*Cistanche deserticola* Y. C. Ma	Chrysogenamide A (**23**)	Lin et al. (2008)
11	*P. janthinellum*	Marine waters	N-Me-trichodermamide B (**24**)	Yang et al. (2017)
12	*Penicillium* spp	Plant—*Salicornia herbacea* Torr	Penicitriketo (**25**)	Wang et al. (2014)
13	*P. brefeldianum* F4a	Plant—*H. cordata*	Peniorcinols A–C (**26–28**)	Bai et al. (2021)
14	*P. citrinum* F5	Soil	2,3,4-trimethyl-5,7-dihydroxy-2,3-dihydrobenzofuran (**29**) and gentisic acid (**30**)	Chen et al. (2002)
15	*P. commune*	Lab cultured	Gentisyl alcohol (2, 5-dihydroxybenzyl alcohol, GA) (**31**)	Ishikawa et al. (1991)
16	*P. adametzii* BF-0003	Hot-spring	Pyranonigrin L (**35**)	Uchida et al. (2016)

(continued)

Table 9.2 (continued)

S. No.	Species	Source	Antioxidant metabolite	Reference
17	*Pencillium* spp.	Plant—*Acer ginnal*	Gallic acid (**36**)	Qi et al. (2009)
18	*Penicillium* spp. YY-20	Plant—*Ginkgo biloba*	Adenosine (**37**), adenine (**38**) and 2′-deoxyadenosine (**39**)	Yuan et al. (2013)
19	*P. brevicompactum*	Wastewaters		Jakovljevic et al. (2018)
20	*P. chrysogenum*	Wastewaters		Jakovljevic et al. (2014)
21	*P.chrysogenum* Snef1216	Lab cultured		Sikandar et al. (2020)
22	*P. citrinum*	Soil		Arora and Chandra (2011)
23	*P. citrinum*	Plant—*Digitaria bicornis*		Nischitha and Shivanna (2022)
24	*P. cyclopium*	Wastewaters		Jakovljevic et al. (2018)
25	*P. duclauxii*	Lab cultured	Lectins	Singh and Walia (2019)
26	*P. flavigenum*	Hypersaline lake	Phenolic acids	Canturk et al. (2017)
27	*P. fumiculosum*	Wastewaters		Jakovljevic et al. (2014)
28	*P. granulatum*	Soil		Arora et al. (2012)
29	*P. griseoroseum*	Lab cultured	Lectins	Singh and Walia (2019)
30	*P. oxalicum*	Plant—*Citrus Limon* (L.)		Kaur et al. (2020)
31	*P. pinophilum* Hedgc.	Plant—*Alloteropsis cimicina*		Nischitha and Shivanna (2021)
32	*P. proteolyticum*	Lab cultured	Lectins	Singh and Walia (2019)
33	*P. purpurogenum*	Soil		Padmapriya and Murugesan (2016)
34	*Penicillium* spp. F23–2	Marine waters	Polysaccharides	Sun et al. (2009)

profitable. On the contrary, complex media (GA—glycerol asparagine) required high levels of all five factors (Amarendra et al. 2015).

Multidrug resistant (MDR) mycobacteria may use various strategies to resist antimycobacterial agents. To list a few, modifications of the outer membrane, making it impermeable to drugs, capability to employ efflux pumps to pump out drugs, and inhibiting-enzyme production (β-lactamases). Additionally, MDR forms may modify or bypass normal drug-binding proteins. Plant secondary metabolites play an inhibitory role in ATP-binding cassette transporters (ABC transporters) systems, and consequently have a hand in reversing antimycobacterial resistance. Aegicerin (**11**), a secondary metabolite isolated from *Clavija procera,* has been proved to reverse MRD in resistant *M. tuberculosis*. Likewise, piperine (**12**) inhibits the overexpression of Rv1258c, a mycobacterial efflux protein in *M. tuberculosis* (Shahid et al. 2013). With the increasing prevalence of resistant strains, there is a need not only for novel drugs but also for drugs working through novel mechanisms. Ferreira et al. (2019) outlined the potential use of metal complex-associated natural products focused on enhancing their antimycobacterial potential. Pan et al. (2011) screened the metal complexes of fusaric acid and reported an increase in antimycobacterial activity in Cadmium (II) and Copper (II) complexes. This research can be further taken down to nanoscale (Fig. 9.2).

Having antimycobacterial properties is not sufficient, the compound is also required to show low or no cytotoxicity and selectivity to mycobacteria with no effect on the rest of the microbiota, as the treatments available for tuberculosis are long-term. Andrioli et al. (2022) tested various antimycobacterial compounds isolated from *P. citrinum* for cytotoxicity by using a macrophage viability assay. While bioprospecting for drugs, effect should be studied synergistically with the commercially available drugs, which might provide new alternatives for multidrug regimens. Among the vast array of natural sources explored so far, metabolites such as flavonoids, peptides, alkaloids, sterols, and terpenoids have shown antimycobacterial potential. They can easily be included in regular balanced diets and can act as a multidrug regimen (Fig. 9.2).

4.2 Antioxidant Potential

Biotechnological interventions to activate silent biosynthetic gene clusters (BGCs) in this genus have led to the isolation of various novel antioxidants (Mishra et al. 2021). One of the most explored strategies is one strain many compounds approach/OSMAC (Bode et al. 2002) in which alterations of physical parameters can lead to the activation or augmentation of several BGCs that would have remained silent otherwise. Liu et al. (2018) have isolated five novel antioxidants compounds raistrickiones A–E (**40**–**44**) from *P. raistrickii* by changing the thermal conditions. Modifications in the salt concentrations led to isolation of a new compound tanzawaic acid Z1 (**45**) with antioxidant activity from *Penicillium* spp. S1a1 (Ebada and Ebrahim 2019) while change in the carbon source led to the isolation

of antioxidants penicnthene (**46**) and penicitrinone H (**47**) from *Penicillium* spp. T2–11 (Li et al. 2022). Even after being exposed to various media, the endophytic fungus *P. dangeardii,* isolated from *Lysidice rhodostegia,* did not produce any new bioactive compounds, but genome mining enabled the discovery of several novel compounds including antioxidants didangelone B (**48**)**,** tridangelone C (**49**), dangelone G (**50**), polyketide Scheme 1385568 (**51**), and (±)-asperlone A (**52**) (Wei et al. 2021). Epigenetic modifications via histone deacetylases (HDAC) inhibitors led to the discovery of three novel antioxidants syringic acid (**53**), sinapic acid (**54**), and acetosyringone (**55**) from *P. brevicompactum* (El-Hawary et al. 2018). Metabolic profiling of *P. claviforme* also led to the identification of known antioxidants kurilensoside F, cetrimonium, phalluside-1, obtusin, maculosin, and onchidal (**56–61**) (Ali Shah et al. 2022) (Fig. 9.4).

The process of creating nanoparticles (NPs) from endophytic fungi with augmented antioxidant properties has also gained a lot of attention (Mishra et al. 2022b). *Penicillium* by the process of mycosynthesis has produced a variety of bioactive NPs, a good section of which have antioxidant properties. Mostly silver NPs have been created with high antioxidant activity by *Penicillium* spp., *P. citrinum* CGJ-C1, *P. italicum,* and *P. oxalicum* (Govindappa et al. 2016; Taha et al. 2019; Danagoudar et al. 2020; Gupta et al. 2022). Fungal consortium of marine isolated *P. oxalicum* and *Fusarium hainanense* also resulted in the production of silver NPs with high antioxidant activity as compared to the ones formed by the individual fungi (Thakor et al. 2022). Gold NPs from endophytic fungus *P. citrinum* and silver NPs from endophytic fungus *Penicillium* spp. were one of the first reported endophytic fungi NPs from this genus with antioxidant properties (Manjunath et al. 2016; Govindappa et al. 2016). Selenium NP from *P. expansum* ATTC 36200 and iron oxide NP from *Penicillium* spp. were also found to be exhibiting significant antioxidant activity (Hashem et al. 2021; Zakariya et al. 2022).

5 Conclusion

For more than a century, the pathogen *Mycobacterium* has been causing consequential morbidity and deaths in humans. With the COVID-19 pandemic reversing years of WHO's efforts, it is imperative to invest and advance from R&D to commercial scales. As listed in this chapter, several compounds have been reported to have antimycobacterial properties, but further research and work has not been conducted. In our literature screening, we found emodin to be the most researched compound so far, yet it has not even made it to the market. Therefore, novel, safe, efficient, accessible, and inexpensive antitubercular drugs are urgently required. In terms of antimycobacterial activity, a few areas have yet to be fully investigated, such as the potential of mycosynthesized nanoformulations, plant-based/edible vaccines, and more. With the advent of nanobiotechnology, natural sources can be exploited even further, taking antimycobacterial research a step ahead. The full antimycobacterial

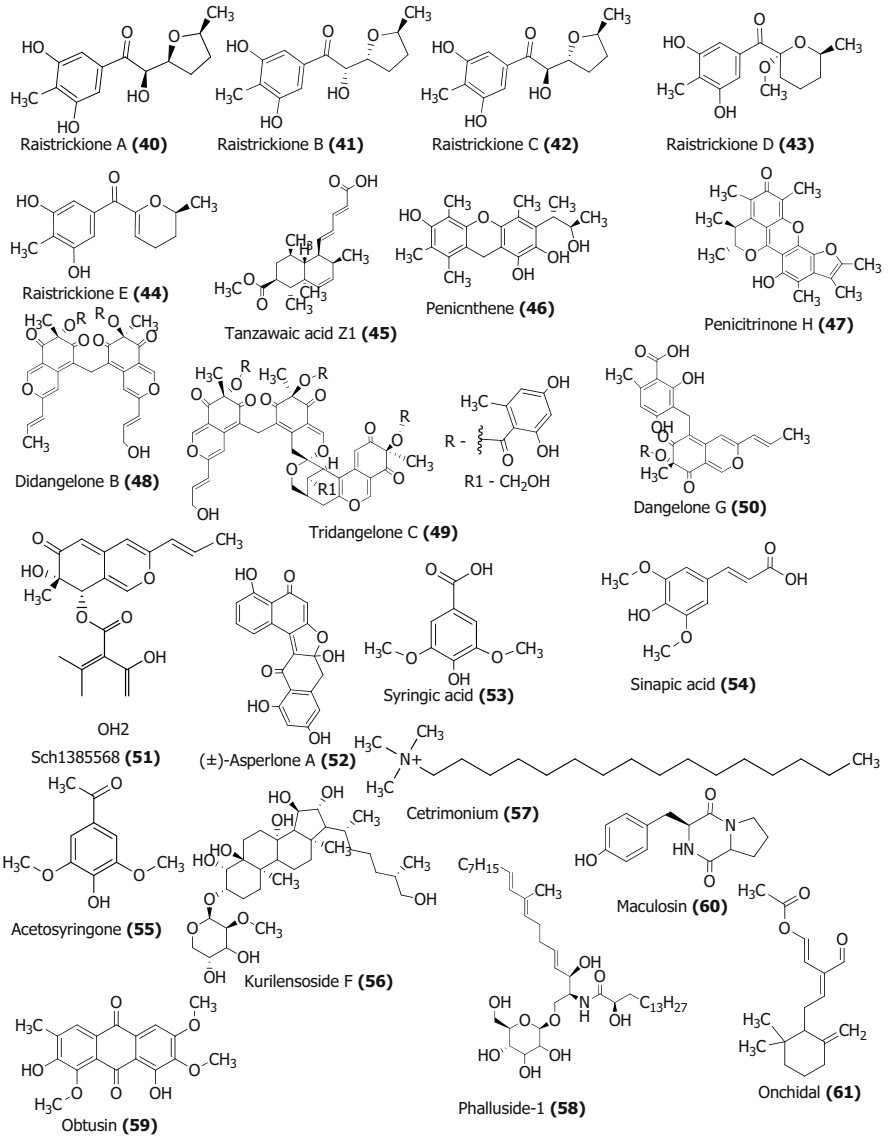

Fig. 9.4 Secondary metabolites isolated from the genus *Penicillium* through biotechnological interventions

potential of *Penicillium* is yet to be explored, and by extent the potential of endophytes as cures for tuberculosis.

Consumers' rising health consciousness and preference for natural additives have prompted researchers to hunt for natural substitutes for synthetic antioxidants. In comparison to higher plants, the microorganisms may enable simpler fermentation

setup and quicker generation of natural antioxidants. Antioxidants are now being researched for their potential use as chemical warfare countermeasures. Using antioxidants to neutralize or reduce the negative effects brought on by exposure to "chemical warfare agents" and "toxic industrial chemicals" appears to be a potential strategy for creating medical countermeasures. Natural antioxidants were recommended as a possible replacement for hazardous synthetic antioxidants such as ethoxyquin used in the animal feed industry. Utilizing the enormous variety of antioxidant substances and pigments from the *Penicillium* genus can be a good place to start for this long-lasting change.

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Chapter 10
Metabolites from Fungi: A Promising Source of Lead Compounds Against Cancer

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Abstract The diversity of fungi represents a fabulous and remarkable asset of sources of bioactive compounds with vast potential for pharmaceutical applications. Especially in Oncology, there is a relentless search for new drugs that allow for more effective and long-lasting treatments with fewer side effects. Innovations in fungi isolation, cultivation, genetic engineering, and metabolic extraction techniques have raised the probability of obtaining fungal metabolites and their derivatives from the most diverse ecosystems worldwide. We review the recent studies on antineoplastic compounds originating from fungal secondary metabolites, which can provide essential alternatives to reinforce the arsenal of chemotherapeutic agents to fight cancer.

Keywords Fungal metabolites · Drug discovery · Chemotherapy · Cancer · Biodiversity

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1 Historical Overview: Discovery of Anticancer Drugs from Fungi

Cancer is a generic name that encompasses a large group of diseases, fundamentally characterized by uncontrolled cell growth that results in the loss of cell functions and is often associated with metastasis to other organs. The origin of cancer is related to genetic phenomena and epigenetic modifications that disrupt the normal signaling pathways responsible for coordinating cell death, proliferation, and differentiation (Koeffler et al. 1991; Baylin and Jones 2016; Tomasetti et al. 2017). Genetic modifications in the DNA are regarded as the most relevant factor for the development of cancer since changes in the nucleotide sequence within the promoter or coding regions of a gene, as well as epigenetic events, can alter the expression of driver genes, promoting tumorigenesis and leading to the formation of cancer (Baylin and Jones 2016; Tomasetti et al. 2017; Hanahan 2022). Mutations can occur by intrinsic errors in the cellular machinery of DNA replication or by exposure to exogenous and endogenous environmental carcinogens (Youn and Simon 2011). Although some of the genetic and epigenetic alterations in DNA that predispose to cancer are inherited (5–10% of cases), the vast majority (90–95%) of cancer cases are induced by exposure to external factors, such as ultraviolet or ionizing radiations, xenobiotics (e.g., tobacco, alcohol, pesticides), infectious organisms (e.g., hepatitis B and C viruses, Epstein-Barr virus, human papillomavirus, human T-cell lymphotropic virus types 1 and 2, and *Helicobacter pylori*), and inadequate nutrition (Bouvard et al. 2009; Ali and Bhattacharya 2014).

The loss of genome stability is a relevant feature related to tumor progression. High mutation rates added to numerical and structural chromosomal alterations and activation of specific pathways combine to lead to cell proliferation without any control and resistance to cell death. As genomic instability is associated with the response to treatment with immunotherapy, studies on these factors have been increasing exponentially in recent times (Chen et al. 2022). Additionally, other factors are also essential to build the microenvironment that enables the development of cancer. These factors are currently known as "hallmarks of cancer." They include the reprogramming of cellular metabolism and epigenome, acquisition of phenotypic plasticity by cancer cells and cancer-associated cells, stimulation of angiogenesis and inflammation, evasion from growth suppressors and immune system agents, and polymorphic microbiomes, among others (Hanahan and Weinberg 2011; Wang et al. 2018; Fares et al. 2020; Hanahan 2022). The phenotypic plasticity of cancer cells allows the occurrence of epithelial-to-mesenchymal transition (EMT), a reversible process which seems to be at the basis of the establishment of cancer stem cells (CSC), both strictly related to chemotherapeutic resistance, evasion from the immune system, invasiveness, and metastasis of certain types of cancer (Sato et al. 2016).

Cancer ranks as a leading cause of death and a significant barrier to increasing life expectancy in every world country. According to GLOBOCAN Database 2020, published online by the International Agency for Research on Cancer (IARC) from

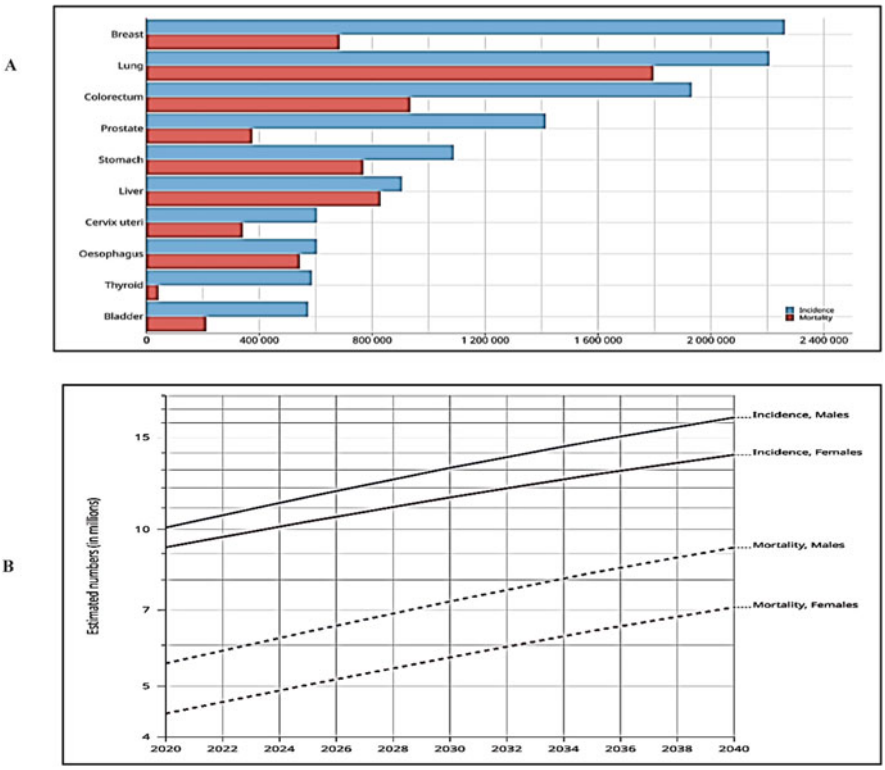

Fig. 10.1 Worldwide cancer incidence versus mortality, for both sexes and all ages, (**a**) for the year 2020, and (**b**) from 2020 to 2040, according to GLOBOCAN Database 2020 (adapted from Ferlay et al. 2020a, b, under permission of IARC/WHO)

the World Health Organization (WHO 2020), breast, lung, colorectal, prostate, stomach, and liver cancer accounted for 50.7% of the new cases worldwide, while lung, colorectal, liver, stomach, and breast cancer were responsible for 50.3% of the deaths related to this disease, only in 2020 (Ferlay et al. 2020a). Moreover, a total of approximately 19.3 million new cases and almost 10 million deaths of cancer were estimated worldwide in 2020 (Ferlay et al. 2020a), considering a population of almost 7.8 million, with an expected increase to 30.2 million (56.7%) new cases and 16.3 million (63.7%) deaths by 2040 (Ferlay et al. 2020b) (Fig. 10.1).

Cancer treatment is based on a multidisciplinary approach. The first approach is surgery, with the tumor lesion preferably resected with free surgical margins. Then the patient undergoes chemotherapy and/or radiotherapy. The treatment includes hormone therapy in some specific cases, such as breast and prostate cancer (Devita et al. 2018). Chemotherapy uses drugs as single or combined agents, which have a cytotoxic effect that leads tumor cells to death or a cytostatic effect that at least prevents tumors from growing. However, in recent years, new therapies have been included in the anticancer arsenal, such as targeted drugs, angiogenesis inhibitors,

and immunotherapy (e.g., Devita et al. 2018; Nasirmoghadas et al. 2021; Conforti et al. 2022; Zampetidis et al. 2022). Despite the evolution in cancer treatment, the mortality is still high, and new drugs are needed in the context of metastatic disease to increase the overall survival of patients, progression-free survival, and cure.

Natural products have been extensively exploited in the search for anticancer agents. In their review, Newman and Cragg (2020) brought up the anticancer drugs on the market from 1981 to 2019. Among 247 drugs approved, 185 were small natural entities, such as secondary metabolites, their derivatives, and even one botanical natural product. The latest reviews comprising fungal metabolites, published by Hridoy et al. (2022), Takahashi et al. (2022), and Contigli et al. (2023), also highlighted the potential of fungi to produce compounds with anticancer properties. These findings reinforce the need to continue investigating natural products from all sources in searching for lead compounds to reinforce the arsenal of chemotherapeutic treatments, which could contribute to overcoming the main obstacles of current treatments, such as adverse side effects, chemoresistance, and disease recurrence.

The kingdom of fungi is a highly complex group of eukaryotic organisms encompassing yeasts, mods, and mushrooms. Besides being known for their toxic compounds causing damage to crop, or human and animal diseases, they are also recognized for their nutritional and therapeutic properties, sources of essential medicines, such as penicillin and statins, among many others. To date, around 148,000 fungal species have been identified, although the diversity of these organisms is estimated to be around 3.8 million (Hawksworth and Lücking 2017; Zhou and May 2022). The diversity of species, allied to their distinct habitats, allows the premise that fungi represent a rich unexploited source of new lead metabolites in the pharmaceutical field. These compounds display scaffolds originated from diverse metabolic pathways, especially fungal secondary metabolites, which are classified into various classes such as polyketides, alkaloids, shikimate derivatives, and terpenoids (Goyal et al. 2017; Barbero et al. 2018; Keller 2019).

Fungi and their secondary metabolites occupy a prominent position in drug discovery since several compounds originating from this taxon, as well as their natural and synthetic derivatives, have been intensively studied and used in the development of new therapeutic drugs (Newman and Cragg 2020; Hridoy et al. 2022; Huang and Zhang 2022; Tiwari and Bae 2022). Since Stierle et al. (1993) revealed that the endophytic fungi *Taxomyces andreanae* could synthesize the diterpenoid taxol (**1**) (paclitaxel, BMS), a well-known chemotherapeutic drug firstly isolated from *Taxus brevifolia* tree, many other fungal sources of this compound were revealed. And although the controversy disclaimed by Heinig et al. (2013) about the unlikely natural biosynthesis of taxol (**1**) by fungi, many new fungal secondary metabolites have been discovered and applied in cancer therapy, such as camptothecin (**2**) (Amna et al. 2006; Shweta et al. 2010), vinblastine (**3**) (Guo and Kunming 1998; Okouneva et al. 2003), vincristine (**4**) (Zhang et al. 2000; Tung et al. 2002), cercosporamide (**5**) (Wang et al. 2012), and alternariol (**6**) (Singh et al. 2014) (Fig. 10.2).

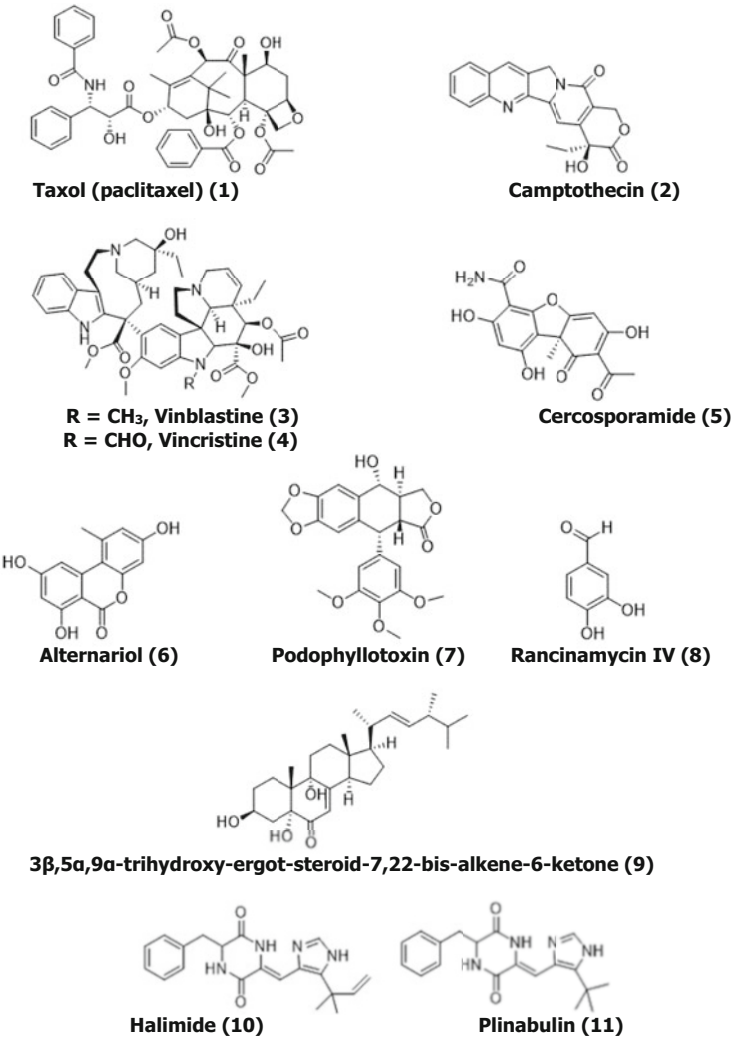

Fig. 10.2 Some fungal secondary metabolites and derivatives in clinical use as chemotherapeutic drugs and patented compounds

Other compounds obtained from medicinal mushroom species are being used as adjuvants for cancer treatment, like the polysaccharides schizophyllan from *Schizophyllum commune*, lentinan from *Lentinus edodes*, and krestin from *Trametes versicolor* (Patel and Goyal 2012). Some fungal metabolites and derivatives with patented cancer treatment applications include: (i) podophyllotoxin (**7**) (Patent US20040248265A1, 2004), the precursor of teniposide and etoposide, produced by a variety of endophytic fungi, such as *Phialocephala fortinii* isolated from the ethnomedicinal plant *Podophyllum peltatum* (Eyberger et al. 2006);

(ii) rancinamycin IV (**8**), derived from the endogenetic fungus *Aspergillus fumigatus* HWT5, obtained from the medicinal *Schisandra chinensis* (Patent CN103966109B, 2016); (iii) 3β,5α,9α-trihydroxy-ergot-steroid-7,22-bis-alkene-6-ketone (**9**), isolated from the edible mushroom *Volvariella volvacea* (Patent CN107474092A, 2017) (Takahashi et al. 2022). Another example of fungal metabolite with promising anticancer activity is halimide (**10**), isolated from marine-derived fungi and used as a lead for the development of plinabulin (**11**), which is in clinical phase III for the treatment of non-small cell lung cancer (Copmans et al. 2022) (Fig. 10.2).

Nowadays, these chemotherapeutic drugs continue to be isolated from other fungal species, and efforts are being directed to innovate in bioprocesses to shift from plant-based to fungal-based production and scale up an economically sustainable production that supports a still-growing pharmaceutical market. For instance, the endophytic fungi *Epicoccum nigrum* (TXB502), isolated from *Taxus baccata*, has been explored to produce taxol (**1**) (El-Sayed et al. 2020). The endophytic fungi *Phyllosticta elongata* (MH458897) from the medicinal plant *Cipadessa baccifera* (Dhakshinamoorthy et al. 2021), as well as *Aspergillus flavus* from *Astragalus fruticosus*, have been used in new techniques of camptothecin (**2**) production (El-Sayed et al. 2022). Moreover, novel potential sources of podophyllotoxin (**7**) were found among the strains of endophytic fungi from *Penicillium* sp., *Trametes* sp., *Aspergillus* sp., *Purpureocillium* sp., and *Ganoderma* sp., isolated from the ethnomedicinal plant *Dysosma difformis* (Thi Tran et al. 2022).

The search for fungal secondary metabolites with anticancer properties remains an important avenue for bioprospecting since this source of compounds brings many advantages in terms of productivity and environmental preservation.

2 Promising Fungal Metabolites and Derivatives with Anticancer Effects

The great diversity of fungal species (Schueffler and Anke 2014), associated with their location in different microhabitats, shows the enormous potential of these microorganisms to produce new metabolites. The biological diversity of fungi, and the biological functions and ecological significance of fungal secondary metabolites, particularly of phylum Ascomycota and Basidiomycota, is due to the diversification of biosynthetic genes and gene clusters and their differential expression in diverse ecosystems. While some fungal metabolites are associated with negative impacts on human and animal health or agriculture, there is an unmatchable diversity of compounds with useful biotechnological applications. Many of these present a large potential for the generation of new medicines.

More than 300 compounds of diverse classes, such as alkaloids, terpenes, polyketides, quinones, lactones, spirobisnaphthalenes, and others with remarkable medicinal properties, including cytotoxic and antitumor activities, were listed by Paluszczak et al. (2018) and Mohammadi et al. (2020). The anticancer potential of

fungal compounds can be ranked according to many factors; thus, for this review, we split them according to their main fungal source: (i) compounds isolated from mushrooms (Basidiomycetes), which are already explored for their nutritional, nutraceutical or traditional curative properties (regardless from the identification of the bioactive principle); (ii) bioactive compounds produced by endophytic fungi, which maintain symbiotic relationships with plants, including the production of mutually beneficial secondary metabolites; and (iii) metabolites discovered in other classes of fungi, that maintain symbiotic relationships to other organisms (e.g., sponges, algae) and/or colonize diverse biomes (e.g., thermophilic, marine). From each category of fungi, a search in the literature was done comprising the last 5 years (from 2018 to 2022), and results are presented in Tables 10.1, 10.2, and 10.3. Those fungal compounds that displayed a wide range of anticancer targets, lower IC_{50}, advanced studies of their biological mechanism of action, and/or indication to clinical trials, were further described in the next topics and presented in the accompanying figures.

2.1 Edible and Medicinal Mushrooms

Edible and medicinal mushrooms have received considerable attention as potential weapons against cancer due to their polysaccharides, polysaccharides-protein complexes, and low molecular weight secondary metabolite contents. These fungi have been used for centuries, and continue to be used today as functional foods, sources of traditional medicines with chemopreventive properties, alleged to reduce the risk of developing cancer, and even as natural chemotherapeutics based on their absent or reduced side effects. According to our review in the major scientific search databases (Web of Science and Scopus), there are more than 3000 publications, 29% of them in the last 5 years, addressing the efficacy of mushrooms in cancer treatment.

Scientific investigations on mushroom bioactive metabolites reveal that many of these natural entities exhibit in vitro cytotoxic effects in cancer cell lines derived from human tissues, and a few selected compounds were evaluated in experimental in vivo models. However, only a fraction of these molecules has passed through the initial phases of clinical trials (Hridoy et al. 2022; Takahashi et al. 2022; Contigli et al. 2023). Low-molecular weight compounds isolated from edible and non edible mushrooms, as well as their antitumor potential and anticarcinogenic effects, were reviewed by Nandi et al. (2019a). Some of these metabolites efficiently interfere with tumor initiation and progression by activating tumor-suppressive mechanisms, while others possess properties of reducing the risk of cancer development.

Zhao and Zheng (2021) reviewed the antitumor potential of bioactive metabolites from the mushroom *Inonotus obliquus* described from 1990 to 2020. The diverse bioactive metabolites conferred to *I. obliquus* a great potential as a therapeutic agent for tumor prevention and treatment, as a supplemental natural drug to reduce side toxicity of chemo- or radiotherapy, or even be used as a therapeutic agent for

Table 10.1 Anticancer potential of secondary metabolites and derivatives from edible and medicinal mushrooms (2018–2022)

Compound	Chemical class	Source (fungi species)	Cancer target/biological effect	Reference
2-(3,4-Dihydroxyphenyl)-6-[(E)-2-(3,4-dihydroxyphenyl) ethenyl]-5'-methylspiro[2H-furo[3,2-c] pyran-3,2'-furan]-3',4-dione (**12**)	Flavonoid	Mushroom *Fulvifomes fastuosus*	Selective cytotoxicity to tumor Hep-G2 cells (IC_{50} 20.8 μg/mL) versus nontumoral CC-1 cells (IC_{50} 167.00 μM), but microscopical evidence of apoptosis for both cells	Fernando et al. (2019)
			In silico analysis indicate high absorption and clearance; targets to metalloproteinases, glyoxalase I, and receptor protein-tyrosine kinase erbB1; compatibility with lapatinib	
Grifolin (**13**)	Terpenoids	Mushroom *Albatrellus flettii*	Cytotoxicity to tumor HeLa, SW480 and HT29 cells (IC_{50} 27.4, 35.4 and 30.7 μM)	Yaqoob et al. (2020)
			Inhibition of KRAS expression on tumor SW480 and HT29 cells	
		Mushroom *Albatrellus confluens*	Cytotoxicity to tumor PC-3 and HT-29 cells (IC_{50} 32.2 and 60.8 μM)	Dube et al. (2022)
Neogrifolin (**14**)		Mushroom *Albatrellus flettii*	Cytotoxicity to tumor HeLa, SW480 and HT29 cells (IC_{50} 24.3, 34.6 and 30.1 μM)	Yaqoob et al. (2020)
			Inhibition of KRAS expression on SW480 and HT29 cells	
		Mushroom *Albatrellus confluens*	Cytotoxicity to tumor PC-3 and HT-29 cells (IC_{50} 28.6 and 45.6 μM)	Dube et al. (2022)
Confluentin (**15**)		Mushroom *Albatrellus flettii*	Cytotoxicity to tumor HeLa, SW480 and HT29 cells (IC_{50} 25.9, 33.5 and 25.8 μM)	Yaqoob et al. (2020)
			Cell cycle arrest (G2/M phase) and apoptosis, with increased expression of cleaved caspase-3, on SW480 cells at 50 μM	
			Inhibition of KRAS expression on SW480 and HT29 cells	

Geranyl-2-orcinol		Synthetic	Cytotoxicity to tumor PC-3 and HT-29 cells (IC_{50} 59.9 and 88.2 μM)	Dube et al. (2022)
Geranylgeranyl-2-orcinol			Cytotoxicity to PC-3 and HT-29 cells (IC_{50} 16.1 and 33.7 μM)	
3β,7β,15β-Trihydroxy-11,23-dioxolanost-8-en-26-oate	Lanostane triterpenoids	Medicinal mushroom *Ganoderma luteomarginatum*	Cytotoxicity to tumor A549 and SMMC-7721 cells (IC_{50} 37.93 and 46.21 μM)	Li et al. (2021)
(17Z)-3β,7β,15β-Trihydroxy-11,23-dioxolanost-8,17(20)-dien-26-oate			Cytotoxicity to tumor HGC-27 cells (IC_{50} 6.82 μM) with dose-dependent apoptosis	
(20E)-15β-Hydroxy-3,7,11,23-tetraoxolanost-20(22)-en-26-oate			Cytotoxicity to tumor A549 cells (IC_{50} 13.67 μM)	
Lanosta-7,9(11)-dien-3β-acetyloxy-24,25-diol			Cytotoxicity to tumor K562 cells (IC_{50} 8.59 μg/mL)	Ma et al. (2022)
Lanosta-7,9(11)-dien-3-oxo-24,26-diol-25-methoxy			Cytotoxicity to tumor K562, BEL-7402 and SGC-7901 cells (IC_{50} 16.05, 24.27 and 33.38 μg/mL)	
Lanosta-7,9(11),24-triene-3β-ol-26-al			Cytotoxicity to K562 cells (IC_{50} 17.38 μg/mL)	
(24*R*,25*S*)-lanosta-7,9(11)-dien- 3β,24,26-triol-25-methoxy			Cytotoxicity to K562 and BEL-7402 cells (IC_{50} 11.69 and 20.05 μg/mL)	
Lucidumol A			Cytotoxicity to K562 cells (IC_{50} 16.95 μg/mL)	
Ganoderiol A			Cytotoxicity to K562, BEL-7402 and SGC-7901 cells (IC_{50} 6.64, 13.49 and 15.62 μg/mL)	
Ganoderiol F			Cytotoxicity to K562 cells (IC_{50} 8.82 μg/mL)	
Ganoderic acid A (**16**)		Medicinal mushroom *Ganoderma lucidum*	Cytotoxicity to human tumor MDA-MB-231 cells (EC_{50}>50 μM), with no effect on nontumoral BJ cells	Martínez-Montemayor et al. (2019)
			Dose- and time-dependent cytotoxicity to tumor U251 cells	Cheng and Xie (2019)

(continued)

Table 10.1 (continued)

Compound	Chemical class	Source (fungi species)	Cancer target/biological effect	Reference
			Apoptosis of U251 cells, with downregulation of Bcl-2 and upregulation of Bax and activated caspase-3	
			Autophagy of U251 cells, with increased beclin1and LC3 II and decreased P-62	
			Inhibition of PI3K/Akt pathway, with decreased expression of p-Akt, p-mTOR, p-P70S6K, and cyclin D1 on U251 cells	
			Reduced migration and invasion of U251 cells	
			Cytotoxicity and apoptosis of tumor IOMM-Lee cells ($NDRG2^{low}$), with no effect on human nontumoral neurons and arachnoid cells	Das et al. (2020)
			Upregulation of NDRG2 protein expression and Bax; downregulation of MMP-9, p-P13K, p-Akt, p-mTOR and Wnt-2 on IOMM-Lee cells	
			Reduction of 60% on IOMM-Lee-induced meningioma tumors on xenograft SCID mouse model, after 14-day treatment with 10 mg/kg	
Ganoderic acid DM (**19**)			Cytotoxicity and apoptosis of tumor IOMM-Lee cells ($NDRG2^{low}$), with no effect on human nontumoral neurons and arachnoid cells	Das et al. (2020)
			Upregulation of NDRG2 protein expression and Bax; downregulation of MMP-9,	

			p-P13K, p-Akt, p-mTOR and Wnt-2 on IOMM-Lee cells	
			Reduction of 60% on IOMM-Lee-induced meningioma tumors on xenograft SCID mouse model, after 14-day treatment with 10 mg/kg	
			Time- and dose-dependent inhibition of proliferation of tumor A549 and NCI-H460 cells, with no effect on nontumoral BEAS2B cells	Xia et al. (2020)
			Apoptosis of A549 and NCI-H460 cells, with downregulation of Bcl-2 expression and upregulation of Bax expression, activated caspase-3 and PARP	
			Autophagy on tumor cells evidenced by morphological alterations, increased LC3B-II, reduced p-PI3K, p-Akt and p-mTOR (Akt/mTOR pathway)	
Calvasterol B	Triterpene sterols	Medicinal mushroom *Ganoderma lucidum*	Cytotoxicity to tumor K562, BEL-7402 and SGC-7901 cells (IC_{50} 22.81, 47.63 and 26.06 μg/mL)	Ma et al. (2022)
Ergosterol (**17**)			Cytotoxicity to human tumor SUM-149 cells at 64 μM, with no effect on nontumoral BJ cells	Martínez-Montemayor et al. (2019)
		Pholiota populnea	Cytotoxicity to tumor Colo 205 and Colo320 cells (IC_{50} 4.9 and 6.5 μM), but also to nontumoral MRC-5 cells (IC_{50} 0.5 μM)	Yazdani et al. (2022)
			Additive effect with doxorubicin against drug-resistant Colo320 cells	

(continued)

Table 10.1 (continued)

Compound	Chemical class	Source (fungi species)	Cancer target/biological effect	Reference
5,6-dehydroergosterol		Medicinal mushroom *Ganoderma lucidum*	Cytotoxicity to tumor SUM-149 cells (EC_{50}>50 μM), with no effect on nontumoral BJ cells	Martínez-Montemayor et al. (2019)
Ergosterol peroxide (**18**)			Dose-dependent cytotoxicity to human tumor SUM-149, MDA-MB-231 and SUM-190 cells (EC_{50} 34, 19 and 43 μM), and to murine tumor UoCB-1 and SUP-B15 cells (EC_{50} 9.86 and 13.55 μM), with no effect on nontumoral BJ and MCF10A cells	
			Cell cycle arrest (G1 phase) and decreased proliferation (clone formation) of SUM-149 cells	
			Apoptosis of SUM-149 cells, with increased caspase-3/7 activity, decreased expression of phosphorylated/total Akt1 and total Akt2, PARP cleavage, and decreased expression of BCL-XL, cyclin D1 and c-Myc; ROS formation in MDA-MB-231, SUM-149 and MCF-7 cells	
			Migration and invasion inhibition of SUM-149 cells at 10 μM	
Ergosterol peroxide sulfonamide		Ergosterol peroxide derivative	Enhanced and selective cytotoxicity to SUM-149 cells (EC_{50} 12 μM), with no effect on nontumoral BJ cells	

Astrakurkurol (**20**)	Triterpenoid	Edible mushroom *Astraeus hygrometricus*	Dose-dependent cytotoxicity to tumor Hep3B cells (IC_{50} 22.6 μM), with effects on the reproductive viability and cell-cycle arrest at the G0/G1 phase	Nandi et al. (2019b)
			Apoptosis of Hep3B cells, with upregulation of pro-apoptotic factors Fas, cleaved caspase-8 (extrinsic pathway), cleaved caspase-9 and -3, and Bax (intrinsic pathway); cleavage of Bid and PARP; ROS generation; downregulation of antiapoptotic factors Bcl-2, Akt; blocking of NF-κB	
			Inhibition of migration/invasion of Hep3B cells	
			Dose-dependent cytotoxicity to tumor Hep3B cells (IC_{50} 65.92 μM), with effects on the reproductive viability, and cell-cycle arrest at the G0/G1 phase, with no effect on nontumoral Thle2 cells at the same dose	Nandi et al. (2022)
			Apoptosis of Hep3B cells, with upregulation of proapoptotic factors FADD, Fas, cleaved caspase-8 (extrinsic pathway), phosphorylated p53 and Bax, cleaved caspase-9 and -3 (intrinsic pathway); cleavage of Bid and PARP; downregulation of intrinsic antiapoptotic factors Bcl-2 and Bcl-xL, ROS production, release of mitochondrial cytochrome c	
			Inhibition of migration/invasion of HepG2 cells, through downregulation of MMP-2 and MMP-9	

(continued)

Table 10.1 (continued)

Compound	Chemical class	Source (fungi species)	Cancer target/biological effect	Reference
Hispidin (**21**)	Polyketide	Medicinal mushroom *Phellinus linteus*	Dose-dependent cytotoxicity to tumor BxPC-3 and AsPC-1 cells	Chandimali et al. (2018)
			Dose-dependent upregulation of phosphorylated p53, cleaved caspase-3 and cleaved PARP, and downregulation of NF-κB and Bcl-2 on BxPC-3 cells	
			Downregulation of NANOG, SOX2 (stemness markers) and CD44 (CSC marker) on BxPC-3 cells and $CD44^+$ CSC subpopulation	
			On $CD44^+$ BxPC-3 CSC, decreased viability, proliferation (2D and 3D cultures), migration and invasion; cell cycle arrest at the G1 phase and increased apoptosis	
			Synergistic effects with gemcitabine on $CD44^+$ BxPC-3 CSC, eliciting cell cycle arrest (G1 phase), apoptosis, inhibition of proliferation, migration and invasion, downregulation of NF-κB and Bcl-2, caspase-3 activation, and low expression of stem cell markers	

Table 10.2 Anticancer potential of secondary metabolites and derivatives of endophytic fungi (2018–2022)

Compound	Chemical class	Source (fungi species)	Plant host	Cancer target/biological effect	Reference
Trichothecin (**22**)	Trichothecenes (sesquiterpenoids)	*Alternaria* sp. sb23	*Schisandra sphenanthera*	Cytotoxicity to tumor HT-29 (IC_{50} 9.38, 5.29, and 9.02 μM) and MCF-7 cells (IC_{50} 4.59, 0.89, and 0.92 μM), synergically enhanced in HT-29 cells by TRAIL (150 ng/mL) treatment (IC_{50} 0.94, 0.05, and 0.09 μM)	Gao et al. (2020)
Trichothecinol A (**23**)					
8-Dihydrotrichothecinol A (**24**)					
8-n-Butyrylneosolaniol (**25**)		*Fusarium sporotrichioides*	*Rauvolfia yunnanensis*	Cytotoxicity to tumor Huh-7 and MRMT-1 cells (IC_{50} 265.9 and 279.4 nM)	Wang et al. (2022)
				Dose-dependent cell cycle arrest at G2/M phase in Huh-7 cells	
				Apoptosis of Huh-7 cells, through decreased mitochondrial transmembrane potential, production of ROS, mitochondrial cytochrome c release, cleaved caspase-9 and -3, cleaved PARP	
8-Isobutyrylneosolaniol				Cytotoxicity to tumor Huh-7 and MRMT-1 cells (IC_{50} 479.7 and 462.4 nM)	
12′-Hydroxyroridin	Trichothecene macrolides (sesquiterpenoids)	*Paramyrothecium roridum*	*Morinda officinalis*	Cytotoxicity to tumor SF-268 (IC_{50} 0.26–16.2 μM), NCI-H460 (IC_{50} 0.05–12.0 μM), and HepG2 cells (IC_{50} 0.19–12.5 μM), but also to nontumoral LX-2 cells (IC_{50}	Liu et al. (2020)
12,13-Deoxyroridin E					
14′-Hydroxymytoxin B					
Miophytocen A					
Myrothecine A					

(continued)

Table 10.2 (continued)

Compound	Chemical class	Source (fungi species)	Plant host	Cancer target/biological effect	Reference
Mytoxin B				0.18–8.7 μM), versus chemotherapeutic cisplatin (IC_{50} 6.63, 2.35, 9.71, and 7.37 μM, respectively)	
Roridin A					
Trichoverrol B					
Verrucarin J					
Verrucin A					
Myrothecine H (**26**)				Cytotoxicity to tumor SF-268 and HepG2 cells (IC_{50} 6.75 and 6.72 μM), but also to nontumoral LX-2 cells (IC_{50} 7.95 μM), versus cisplatin (IC_{50} 6.63, 2.35, and 7.37 μM, respectively)	
				Dose- and time-dependent cell cycle arrest (G1 phase) in HepG2 cells	
				Dose- and time-dependent apoptosis (28.4%) of HepG2 cells, through decreased mitochondrial membrane potential, release of mitochondrial cytochrome c, cleavage of PARP, and phosphorylation of JNK	
Myrothecine I (**27**)				Cytotoxicity to tumor SF-268 and HepG2 cells (IC_{50} 0.20 μM), but also to nontumoral LX-2 cells (IC_{50} 0.21 μM), versus cisplatin (IC_{50} 6.63, 2.35, and 7.37 μM, respectively)	

				Dose- and time-dependent cell cycle arrest (G1 phase) in HepG2 cells	
				Dose- and time-dependent apoptosis of HepG2 cells (33.4%), through decreased mitochondrial membrane potential, release of mitochondrial cytochrome c, cleavage of PARP, and phosphorylation of JNK	
Pchaeglobosal B (**28**)	Cytochalasans	Endophytic *Chaetomium globosum* (P2-2-2)	Host not indicated	Cytotoxicity to tumor A549, HepG2, HT29, MCF-7, and CT26 cells (IC_{50} 1.04–9.90 μM)	Peng et al. (2020)
				Apoptosis and cell cycle arrest induced on CT26 cells (>10 fold; S phase) at 3–4 μM, and on HT29 cells (>13 fold; G0/G1 phase) at 12–15 μM	
19,20-Epoxy cytochalasin C		*Rosellinia sanctae-cruciana*	Medicinal *Albizia lebbeck*	Cytotoxicity to tumor MOLT-4 cells (IC_{50} 6.0–25.0 μmol/L)	Sharma et al. (2018)
19,20-Epoxy cytochalasin D					
Cytochalasin C					
Cytochalasin D					
Jammosporin A					
Cytochalasin H (**29**)		*Phomopsis liquidambari*	Mangrove (plant host not identified)	Cytotoxicity to tumor A549 cells (IC_{50} 159.50 μM), with cell cycle arrest at the G2/M phase and sub-G1 peaks	Ma et al. (2018)
				Apoptosis of A549 cells evidenced by DNA fragmentation, decreased mitochondrial transmembrane potential,	

(continued)

Table 10.2 (continued)

Compound	Chemical class	Source (fungi species)	Plant host	Cancer target/biological effect	Reference
				increased expression of proapoptotic proteins (Bax, P53, and cleaved caspase-3), and decreased expression of antiapoptotic proteins (Bcl-xL, Bcl-2, and full-length caspase-3)	
				Inhibited migration of A549 cells	
5,6-Dihydro-7-oxo-19,20-epoxycytochalasin C		*Xylaria* cf. *curta*	*Solanum tuberosum*	Cytotoxicity to tumor HL-60 cells (IC_{50} 10.04, 1.11 and 13.31 μM)	Wang et al. (2019b)
19-Epi-cytochalasin P1					
19,20-Epoxycytochalasin C					
7-O-Acetyl-6-epi-19,20-epoxycytochalasin P				Cytotoxicity to tumor HL-60 and MCF-7 cells (IC_{50} 37.16 and 26.64 μM)	
7-O-Acetyl-19,20-epoxycytochalasin D				Cytotoxicity to tumor HL-60 and MCF-7 cells (IC_{50} 25.83 and 34.03 μM)	
Cytochalasin D		Endophytic *Xylaria longipes*	Host not indicated	Cytotoxicity to tumor HL-60 and SMMC-7721 cells (IC_{50} 4.17–22.69 μM)	Wang et al. (2019c)
Cytochalasin P				Cytotoxicity to tumor HL-60, SMMC-7721, MCF-7, and SW480 cells (IC_{50} 26.74–37.18 μM)	
6,7-Dihydro-7-oxo-cytochalasin C				Cytotoxicity to tumor HL-60 and MCF-7 cells (IC_{50} 26.02 and 36.44 μM)	

6,7-Dihydro-7-oxo-deacetylcytochalasin C				Cytotoxicity to HL-60 and MCF-7 cells (IC_{50} 12.05 and 27.24 μM)	
Zygosporin D				Cytotoxicity to HL-60 cells (IC_{50} 6.43 μM)	
Perennisubacin A		Plant-pathogenic *Perenniporia subacida*	Host not indicated	Cytotoxicity to tumor SMMC-7721 cells (IC_{50} 4.17 μM)	Guo et al. (2021)
Perennisubacin C				Cytotoxicity to SMMC-7721 cells (IC_{50} 37.6 μM)	
				Nitric oxide inhibition in SMMC-7721 cells (IC_{50} 18.4 μM)	
Aspochalasin P		*Aspergillus* sp. FT1307	*Heliotropium* sp.	Selective cytotoxicity to tumor A2780 cells (IC_{50} 17.29 μM) and no effect against nontumoral EA. hy926 cells	Qader et al. (2021)
Diaporthol A	Polyketides	*Diaporthe* sp. ECN-137	*Phellodendron amurense*	Inhibition of TGF-β1-elicited migration of tumor MDA-MB-231 cells at 20 μM	Nakashima et al. (2018)
Diaporthol B					
Bellidisin A		*Phoma bellidis*	Medicinal *Tricyrtis maculata*	Cytotoxicity against tumor HL-60 cells (IC_{50} 20.62 μM)	Wang et al. (2019a)
Bellidisin D				Cytotoxicity to tumor HL-60, A549, MCF-7, SW480, SMMC-7721 cells (IC_{50} 3.40–15.25 μM), more potent than cisplatin (IC_{50} 4.86–27.70 μM)	
4,5-Dihydroxy-6'(6'--methylsalicyloxy)-2-hydroxymethyl-2-cyclohexenil-one		*Epicoccum sorghinum*	*Arundo donax*	Cytotoxicity to tumor A549, HepG2, and MDA-MB-231 cells (IC_{50} 1.86–18.31 μM), versus doxorubicin (IC_{50} 0.2–0.47 μM)	Chang et al. (2021)
Gentisyl alcohol					
6-(Hydroxymethyl)benzene-1,2,4-triol					

(continued)

Table 10.2 (continued)

Compound	Chemical class	Source (fungi species)	Plant host	Cancer target/biological effect	Reference
Alterperylenol		*Alternaria* sp. (MG1)	*Vitis quinquangularis*	Cytotoxic to NCI-H1299 (98%) and HT-1080 (96.2%) at 20 μM	Tian et al. (2021)
Fusaketide A (**30**)		Entomogenous *Fusarium equiseti* (LGWB-9)	*Harmonia axyridis*	Cytotoxic to tumor HeLa, Huh-7, and MCF-7 cells (IC_{50} 46.0–69.7 μg/mL), versus cisplatin (IC_{50} 2.1–8.3 μg/mL)	Liu et al. (2021)
Fusarisetin B (**31**)				Cytotoxic to tumor HeLa, Huh-7, MCF-7, and MGC-803 cells (IC_{50} 2.4–46.5 μg/mL), versus cisplatin (IC_{50} 2.1–8.3 μg/mL)	
				Apoptosis of MGC-803 cells, with increased expression of Bax and p53, and lower expression of Bcl-2	
				Inhibition of migration and invasion of MGC-803 cells	
N-(3'-Chloro-5'-oxobutyl)-1-methyl-5-phenyl-1*H*-pyrrole3-carboxamide	Pyrrole alkaloid	*Aspergillus* sp. (RL1)	*Phyllanthus niruri*	Selective cytotoxicity to tumor T-47D cells (IC_{50} 8.3 μg/mL) compared to nontumoral Vero cells (IC_{50} 124 μg/mL), Selectivity Index 12.2	Yuniati et al. (2019)
				Cell cycle impairment of T-47D cells (S phase) at 0.9 μg/mL	
(±)-Asperteretone E	Butenolide derivative	*Aspergillus terreus*	*Hypericum perforatum*	Cytotoxicity to tumor AsPC-1, SW1990 and PANC-1 cells (IC_{50} 9.5–15.6 μM)	Deng et al. (2020)

3β-5α-Dihydroxy-6-β-phenylacetyloxy-ergosta-7,22-diene	Benzoquinones	*Chaetomium cupreum*	*Mussaenda luteola*	Cytotoxicity to tumor MCF-7 cells (52%) at 100 μg/mL	Shylaja and Sathiavelu (2019)
				Antioxidant effect indicated by the DPPH scavenging potential (72.07%) at 100 μg/mL	
6-(Heptacosa-18′ Z enyl)-2-(-18′′ hydroxyl-1′′ enyl-19′′ oxy)-3 hydroxy benzoquinone				Cytotoxicity to tumor MCF-7 cells (49%) at 100 μg/mL	
				Antioxidant effect indicated by the DPPH scavenging potential (71.63%) at 100 μg/mL	
Acrocalysterol B	Steroid	*Acrocalymma* sp.	*Sinomenium acutum*	Cytotoxicity to tumor HeLa, HCC-1806 and RKO cells (IC_{50} 19.14, 18.37 and 19.64 μM)	Yang et al. (2022)
Giluterrin (**32**)	Prenylated indole alkaloid	*Aspergillus terreus* P63	*Axonopus leptostachyus*	Cytotoxicity to tumor 786-0 and PC-3 cells (IC_{50} 22.93 and 48.55 μM), but also to nontumoral HaCat cells (IC_{50} 49.79 μM)	Gubiani et al. (2019)
				Inhibition of proliferation of 786-0 (58%) and PC-3 cells (77%) at the respective IC_{50}	
(4S,8aR,12S,12aR)-12-Hydroxy-4-methyl-4,5,6,7,8,8a,12,12a-octahydro-1H-3-benzoxecine-2,9-dione (Sch-642305) (**33**)	Macrocyclic lactone	Endophytic strain MF31b11 (not identified)	Medicinal *Achyrocline satureioides*	Selective cytotoxicity to tumor C6 and U138MG cells (IC_{50} 1.1 and 7.6 μg/mL), with no effects on rat primary astrocyte cells (nontumoral)	Pedra et al. (2018)
				Decreased colony formation (70%) of C6 cells at 4.36 μM	
				Cell cycle arrest (G2/M phase) and enhanced apoptosis (10%) of C6 cells at 1 μg/mL	

(continued)

Table 10.2 (continued)

Compound	Chemical class	Source (fungi species)	Plant host	Cancer target/biological effect	Reference
				Inhibition of migration of C6 (20%) and U138MG cells (36%) at 1.98 μM	
				Increased superoxide dismutase and catalase activities, enhanced sulfhydryl content, decreased ROS on C6 cells	
Altersolanol B (**34**)	Tetrahydroanthraquinone	*Stemphylium solani*	*Morinda citrifolia* (Noni)	Cytotoxicity to tumor MCF-7 and MDA-MB-231 cells (IC_{50} 5.5 and 21.3 μM), without effect on nontumoral MCF-10A cells	Siraj et al. (2022)
				Cell cycle arrest of MCF-7 cells at the G0/G1 phase, with downregulation of cyclin D1, CDK4 and CDK2, and increased p21Waf1/Cip1 and p53	
				Apoptosis of MCF-7 cells, with activation of caspase-9 and PARP, increased Bax and decreased Bcl-2; inhibition of the oncogenic PI3K/AKT pathway, with decreased phospho-AKT, phospho-FOXO1, phospho-4E-BP1, GSK-3β, and NF-κB; inhibition of the oncogenic ERK1/2 pathway, with decreased phospho-MEK1/2 and phospho-ERK1/2, and increased phospho-MKK4 and phospho-p38	

Chaetopseudeurin B	[11]-Chaetoglobosins (Cytochalasan alkaloids)	*Pseudeurotium bakeri* P1-1-1	Chinese macrocoma plant (Orthotrichaceae)	Selective cytotoxicity to tumor A549, A427, HCT116, HT-29, MCF7, HeLa, and HepG2 cells (IC_{50} 4.7 to 9.1 μM), compared to nontumoral LO2 cells (IC_{50} 44.1 μM)	Duan et al. (2021)
				Dose-dependent cell cycle arrest (G2/M) in MCF-7 and A427 cells	
				Dose-dependent apoptosis of MCF-7 and A427 cells, with increased expression of Bax, mitochondrial cytochrome c release, cleaved caspase-3, cleaved PARP, and decreased expression of Bcl-2 on MCF-7	
Chaetopseudeurin E				Selective cytotoxicity to tumor A549, A427, HCT116, HT-29, MCF7, HeLa, and HepG2 cells (IC_{50} 6.6–10.6 μM), compared to nontumoral LO2 cells (IC_{50} 35.5 μM)	
				Dose-dependent cell cycle arrest (G2/M) and apoptosis of MCF-7 and A427 cells	
Chaetopseudeurin F				Selective cytotoxicity to A549, A427, HCT116, HT-29, MCF7, HeLa, and HepG2 cells (IC_{50} 4.8–12.2 μM), compared to nontumoral LO2 cells (IC_{50} 36.3 μM)	
				Dose-dependent cell cycle arrest (G2/M) and apoptosis of MCF-7 and A427 cells	

(continued)

Table 10.2 (continued)

Compound	Chemical class	Source (fungi species)	Plant host	Cancer target/biological effect	Reference
Chaetopseudeurin G				Selective cytotoxicity to tumor A549, A427, HCT116, HT-29, MCF7, HeLa, and HepG2 cells (IC_{50} 6.8–11.2 μM), compared to nontumoral LO2 cells (IC_{50} 41.8 μM)	
				Dose-dependent cell cycle arrest (G2/M) and apoptosis of MCF-7 and A427 cells	
Chaetopseudeurin M (**35**)				Cytotoxic effects against seven human cancer cell lines (IC_{50} 5.1–10.8 μM)	Duan et al. (2022)
				Dose-dependent cell cycle arrest (G2/M) in tumor MCF-7 cells	
				Dose-dependent apoptosis of MCF-7 cells, with increased expression of pro-apoptotic proteins (Bax, cleaved caspase-3), decreased expression of anti-apoptosis proteins (cyclin B1 and Cdk1, Bcl-2), and release of mitochondrial cytochrome c	
Greensporone A (**36**)	Resorcylic acid lactone polyketide	*Rhinocladiella similis*	*Agriophyllum squarrosum*	Cytotoxicity to tumor HCT116 and HeLa cells (IC_{50} 43.51 and 40.61 μM)	Li et al. (2019b) (see also Table 10.3)
Phomaones B-E	Oblongolides	*Phoma bellidis*	*Tricyrtis maculata*	Cytotoxicity to tumor MCF-7, DU145 and SW480 cells (IC_{50} 12.45–49.84 μM)	Zhang et al. (2022)

Swainsonine	Indolizidine alkaloid (mycotoxin)	*Alternaria oxytropis*	*Oxytropis glabra*	Cytotoxicity to tumor A549 and HeLa cells (IC_{50} 10.93 and 66.69 μM), in comparison to cisplatin (IC_{50} 8.73 and 14.68 μM)	Tan et al. (2019)
β-11-Methoxy curvularine	Macrolides	*Aspergillus* sp. FT1307	*Heliotropium* sp.	Selective cytotoxicity to tumor A2780 cells (IC_{50} 11.76 μM) and no effect against nontumoral EA. hy926 cells	Qader et al. (2021)
Brefeldin A derivatives		*Penicillium brefeldianum* f4a	not indicated	Cytotoxicity to tumor H358, SW620 and ASPC-1 (KRAS mutants), PC-3, and HepG2 cells	Bai et al. (2022)
Penipentenone A	Dicyclopentenone			Cytotoxicity to tumor SW620 and ASPC-1 cells (KRAS mutants)	
Resveratrol	Resorcinol	*Quambalaria cyanescens*	*Vitis vinifera*	Cytotoxicity to tumor A549 cells (82%)	Meshram et al. (2022)
				Antioxidant activity (83%)	
Bipolacochlioquinone C	Meroterpenoids	*Bipolaris victoriae* S27	*Rubia podantha*	Cytotoxicity and migration inhibition of tumor HCT116 and MDA-MB-231 cells	Feng et al. (2022)
				Suppression of the NF-κB-associated protein expression (phospho-p65) on MDA-MB-231 cells	
Cochlioquinone A				Cytotoxicity and migration inhibition of tumor HCT116 and MDA-MB-231 cells	
				Suppression of NF-κB-associated protein expression (phospho-IκBα and phospho-p65) on MDA-MB-231 cells at 20 μM	

Table 10.3 Anticancer potential of key secondary metabolites and derivatives of fungi from diverse biomes (2018–2022)

Compound	Chemical class	Source (fungi species)	Ecosystem/ host organism	Cancer target/biological effect	Reference
(-)-(*3R, 6R*) Hyalodendrin (**36**)	Epidithiodioxopiperazines	*Paradendryphiella salina* PC 362H	Brown alga *Pelvetia caniculata*	Selective cytotoxicity to tumor MCF-7, stem-like invasive MCF7-Sh-WISP2, and drug-resistant MDA-MB-231 cells (IC_{50} 216.3, 142.0 and 132.5 nM), HeLa cells (IC_{50} 69.0 nM) and a panel of 15 colorectal cancer cell lines (IC_{50} 40.0–163.7 nM), compared to nontumoral 3T3-F442A and C19 cells (IC_{50} 305.0 and 252.5 nM)	Dezaire et al. (2020)
				Increased phosphorylation of p53 (Ser15) and γ-H2AX, decreased phosphorylation of PRAS40, indicating double-strand DNA damage on MCF7-Sh-WISP2 cells	
				Decreased expression of HSP60, HSP70 and SOD2 proteins, involved in oxidative stress, on MCF7-Sh-WISP2 cells	
(-)-(3*R*, 6*R*) Bisdethiodi (methylthio) hyalodendrin				Cytotoxicity to tumor MCF7 and MCF7-Sh-WISP2 cells (IC_{50} 42 and 68 μM), but also to nontumoral 3T3-F442A cells (IC_{50} 26 μM)	
Asperpyrone B	Naphto-c-pyrones	*Aspergillus foetidus* KMM 4694	Marine sediment	Cytotoxicity to tumor 22Rv1 cells (IC_{50} 95.54 μM)	Antonov et al. (2021)
Rubrofusarine B				Cytotoxicity to 22Rv1 cells (IC_{50} 88.7 μM)	
				Induce reproductive death by inhibiting colony formation of 22Rv1 cells (68.55%)	

Fansecinone A				Cytotoxicity to 22Rv1 cells (IC_{50} 13.13 μM)	
				Induce reproductive death by inhibiting colony formation of 22Rv1 cells (88.71%)	
Kojic acid	Pyrone derivative	*Penicillium chrysogenum*	Sponge *Cliona* sp.	Cytotoxicity to tumor HCT116 and HEP-2 cells (IC_{50} 23.4 and 30.8 μg/mL).	Al-Saleem et al. (2022)
Penicitrinone A	Polyketides	*Penicillium citrinum* (SCSIO 41017)	Sponge *Callyspongia* sp.	Cytotoxicity to tumor MCF7 cells (IC_{50} 1.3 μM), comparable to taxol (IC_{50} 1.4 μM)	Salendra et al. (2021)
16α-Methylpregna-17α,19-dihydroxy-(9*R*,11*R*)-epoxy-4-ene-3,18-dione-20-acetoxy				Cytotoxicity to tumor SF-268, MCF-7, HepG-2, and A549 cells (IC_{50} 13.5–18.0 μM).	
Globosuxanthone F		*Pleosporales* sp. (NBUF144)	Sponges from mesophotic coral	Cytotoxic to tumor CCRF-CEM cells (IC_{50} 0.46 μM)	Zhou et al. (2021)
1,3-Dihydroxy-6-methyl-7-methoxyanthraquinone (**37**)		Thermophilic *Thermomyces lanuginosus* (KMM 4681)	Marine sediment	Selective cytotoxicity to drug-resistant tumor 22Rv1 cells (65%), compared to nontumoral PNT-2 cells (35%) at 100 μM	Sobolevskaya et al. (2021)
				Induce reproductive death of 22Rv1 cells (70%), on colony formation assay, at 50 μM	
Sulochrin (**38**)	Benzophenone polyketide	*Aspergillus falconensis*	Marine	Molecular docking analysis indicate affinity to CDK-2 (ΔG = -25.03 Kcal/mol), TOP-2 (ΔG = -12.11 Kcal/mol)	El-Kashef et al. (2021)

(continued)

Table 10.3 (continued)

Compound	Chemical class	Source (fungi species)	Ecosystem/ host organism	Cancer target/biological effect	Reference
				and MMP-13 proteins (ΔG =- -33.83 Kcal/mol)	
				Cytotoxicity to murine tumor L5178Y cells (IC_{50} 5.1 μM), but not to human tumor MDA-MB-231 cells, up to 100 μM	
				Cell migration inhibition of MDA-MB-231 cells at 70 μM	
Cytochalasin E (**39**)	Cytochalasin	*Aspergillus clavatus*	Soil and animal manure	Cytotoxicity to tumor A549 cells (50%) at 0.5 μM	Takanezawa et al. (2018)
				Effects on cell morphology, including complete disruption and depolymerization of cytoskeleton actin filaments, and pronounced increase of binucleated cells, indicating cytokinesis failure	
				Evidence of autophagy inhibition by increasing LC3-II and p62 levels	
Myceliothermophins A, E and F	Polyketide-amino acids	*Thermothelomyces thermophilus* ATCC 42464	Thermophilic	Cytotoxicity to tumor DLD-1, Hep3B, HepG2, and HGC-27 cells (IC_{50} 0.08 to 0.93 μg/mL)	Gao et al. (2019)
Greensporone A (**40**)	Resorcylic acid lactone polyketides	*Halenospora* sp.		Cytotoxicity to breast cancer MDA-MB-435 cells (IC_{50} 14.1 μM)	

			Freshwater (facultative marine)	and leukemic K562, U937 and AR230 cells (IC_{50} 17–42 μM)	El-Elimat et al. (2014); Prabhu et al. (2019)
				Dose-dependent cell cycle arrest at subG0/G1 for K562 and U937 cells	
				Dose-dependent apoptosis of K562 and U937 cells, through activation of caspase-3 and -9, cleavage of PARP, double-strand DNA damage (increased γ-H2AX), suppression of AKT and FOXO-1 phosphorylation, suppression of activated GSK, suppression of IAPs (XIAP, cIAP-1), increased Bax/Bcl-2 ratio, increased cytosolic cytochrome c, increased intracellular ROS, and GSH depletion	
Greensporone C (**41**)				Cytotoxicity to breast cancer MDA-MB-435 cells (IC_{50} 2.9 μM), intestinal cancer HT-29 cells (IC_{50} 7.5 μM), and to leukemic K562 (90%), U937 (96%), and AR230 cells (83%) at 50 μM, with no effect on normal PBMC	El-Elimat et al. (2014); Prabhu et al. (2018)
				Dose-dependent cell cycle arrest at subG0/G1 for K562 and U937 cells	
				Dose-dependent apoptosis of K562 and U937 cells, through activation of caspase-3 and -9, cleavage of PARP, double-strand DNA damage	

(continued)

Table 10.3 (continued)

Compound	Chemical class	Source (fungi species)	Ecosystem/ host organism	Cancer target/biological effect	Reference
				(increased γ-H2AX), suppression of AKT phosphorylation, suppression of IAPs (XIAP, cIAP-1, and cIAP-2), increased Bax/Bcl-2 ratio, increased cytosolic cytochrome c, increased intracellular ROS, and GSH depletion	
Aspersclerolide A	γ-hydroxyl butenolides	*Aspergillus sclerotiorum*	Saline soil	Cytotoxicity to tumor HL-60 and A549 cells (IC_{50} 6.5 and 8.9 μM)	Ma et al. (2019)
Aspersclerolide C				Cytotoxicity to tumor HL-60 and A549 cells (IC_{50} 12.1 and 16.7 μM)	

preventing tumor metastasis post therapy. Besides, evidence suggests a potential to reduce the incidence of tumorigenesis in healthy people.

The structure of pharmaceutically active secondary metabolites, isolated from different genera of edible and medicinal mushrooms, with promising activities against tumors, was also described by Dai et al. (2021). The biological mechanisms of action of these compounds against various types of tumors, regarding apoptosis, autophagy, angiogenesis, oxidative stress, metastasis, multidrug resistance, and gut microenvironment, were discussed in that review.

Neergheen et al. (2022) reviewed the cellular signaling pathways involved in the inhibition of tumor cell growth as a target for mushrooms and their bioactive compounds and the associated challenges for the molecules therein to be successfully considered as preventive/therapeutic agents against cancer. These authors concluded that the potential health benefits of mushrooms against cancer are due to the multitargeted effects of the diverse and multifunctional groups of active compounds.

The flavonoid extracted from *Fulviformes fastuosus* mushroom, 2-(3,4-dihydroxyphenyl)-6-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]-5′-methylspiro [2H-furo[3,2-c] pyran-3,2′-furan]-3′,4-dione (**12**) (Table 10.1, Fig. 10.3), showed selective cytotoxicity to hepatoma Hep-G2 cells, compared to nontumoral CC-1 epithelial cells (Selective index = 8.03) (Fernando et al. 2019). The in silico predictive pharmacological analysis revealed a great potential to act safely in the human body without producing any side effects. Probable targets are matrix metalloproteinases, glyoxalase I and receptor protein-tyrosine kinase erbB1, and the compound is also compatible with the known cancer drug lapatinib. The authors suggested that this flavonoid from *F. fastuosus* can be administered through beverages to be used as an adjuvant drug in the treatment of hepatoma.

Mushrooms of the genus *Albatrellus* are well known for producing terpenoids such as grifolin (**13**), neogrifolin (**14**), and confluentin (**15**), under investigation by their in vitro anticancer effects (Table 10.1, Fig. 10.3). Grifolin (**13**) and neogrifolin (**14**), extracted from *Albatrellus flettii* and *A. confluens*, were recently tested for their ability to inhibit cancer cells from endocervical adenocarcinoma (HeLa), colorectal carcinoma (SW480) and adenocarcinoma (HT-29), and prostate adenocarcinoma (PC-3) (Yaqoob et al. 2020; Dube et al. 2022). Confluentin (**15**) from *A. flettii* was also cytotoxic to HeLa, SW480 and HT29, and induced cell cycle arrest at the G2/M phase and cleavage of caspase-3, eliciting apoptosis of SW480 cells (Yaqoob et al. 2020). Moreover, Yaqoob et al. (2020) showed that these terpenoids act through the suppression of KRAS expression, which is a potential target due to its role in cancer cell proliferation.

Some mushrooms from the *Ganoderma* genus have been used for centuries as traditional remedies against cancer by Asian populations, highlighting the potential of their bioactive metabolites as promising drug leads (Paterson 2006; Suárez-Arroyo et al. 2017; Oke et al. 2022). The rare *G. luteomarginatum* is one of these species under scientific investigation. Among three cytotoxic lanostane triterpenoids derived from ganoderic acid, extracted from *G. luteomarginatum* fruiting bodies, (17Z)-3β,7β,15β-trihydroxy-11,23-dioxolanost-8,17(20)-dien-26-oate was the most

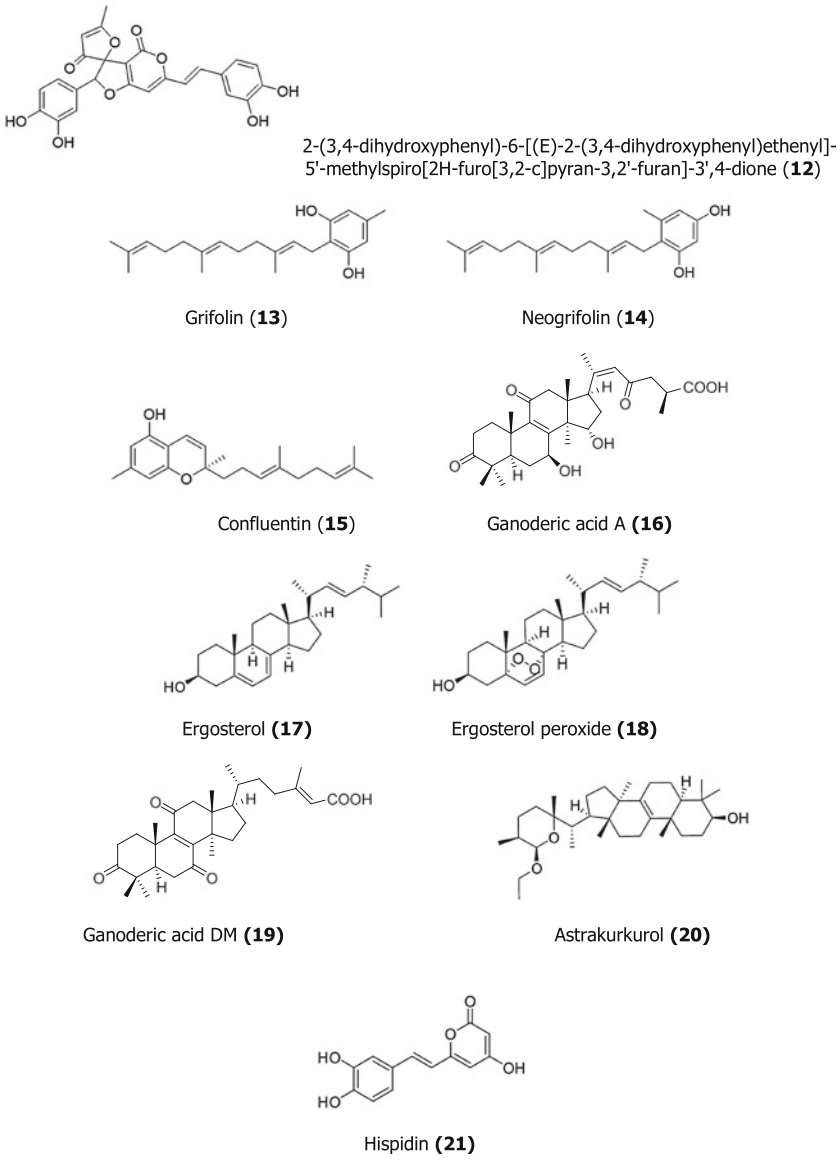

Fig. 10.3 Secondary metabolites from edible and medicinal fungi with promising mechanisms of action against cancer

potent to inhibit proliferation and inducing apoptosis of the human gastric adenocarcinoma HGC-27 cell line (Table 10.1). Structure-activity relationships indicated that ganoderic alcohols, like this one, have more potent cytotoxicity than the corresponding ganoderic acid derivatives (Li et al. 2021). According to another study conducted by Ma et al. (2022), from eight ergostane steroids and 26 lanostane

triterpenoids isolated from *G. luteomarginatum*, one ergostane steroid (calvasterol B) and seven triterpenoids presented cytotoxic effects against the human cell line K562 (chronic myelogenous leukemia). Lanosta-7,9(11)-dien-3β-acetyloxy-24,25-diol, ganoderiol A and ganoderiol F were the most potent, and their structure-activity relationships were also discussed (Table 10.1).

Ganoderma lucidum (known as reishi) is another species of biotechnological interest, used as ancient medicine against cancer (Sharif et al. 2018). The review of Suárez-Arroyo et al. (2017) demonstrated the significant anticancer activity of this mushroom against triple-negative inflammatory breast cancer models. Martínez-Montemayor et al. (2019) elucidated the most abundant chemical constituents of *G. lucidum* as ganoderic acid A (**16**), ergosterol (**17**), 5,6-dehydroergosterol, ergosterol peroxide (**18**), and palmitic acid (Table 10.1, Fig. 10.3). The authors studied the efficacy of these metabolites against a panel of cell lines derived from aggressive human breast carcinoma (MDA-MB-23, SUM-149, and SUM-190) and murine leukemia (UoCB-1 and SUP-B15). Ganoderic acid A (**16**) and 5,6-dehydroergosterol shown modest cytotoxicity ($EC_{50} > 50$ μM) to triple-negative breast carcinoma cell lines, respectively, mesenchymal-like MDA-MB-231 and epithelial-like inflammatory SUM-149, with no effect on nontumoral skin fibroblast BJ line, demonstrating the selectivity of these compounds to those cancer cells. Ergosterol (**17**) was also more effective against SUM-149 cells at 64 μM and presented no toxicity to BJ cells. The most promising secondary metabolite was ergosterol peroxide (**18**), which displayed significant cytotoxic effects (EC_{50} 9.86 to 43.0 μM) against MDA-MB-231, SUM-149, and SUM-190 ($HER2^+$ inflammatory breast carcinoma) and against two murine leukemia cell lines (UoCB-1 and SUP-B15), with no relevant effects on two nontumoral human cell lines (BJ, and MCF10A mammary epithelial cells). Ergosterol peroxide (**18**) has been shown to disrupt cell cycle kinetics on inflammatory breast carcinoma SUM-149, decreasing cell proliferation and clonal expansion. Apoptosis was another observed effect upon treatment of SUM-149 cells with the compound, as a result of increased activity of caspase-3 and -7, decreased expression of phosphorylated Akt1 plus total Akt1 and total Akt2 (protein kinase B family members), activation of poly (ADP-ribose) polymerase (PARP), decreased expression of Bcl-xL (antiapoptotic factor), cyclin D1 (related to cell cycle regulation and normally overexpressed on breast cancer) and c-Myc (involved in cell proliferation and constitutively expressed on cancer cells), and oxidative stress through the generation of intracellular reactive oxygen species (ROS). Moreover, even in low doses, ergosterol peroxide (**18**) presented antimigratory and antiinvasion effects on SUM-149, an additional mechanism of action that can be explored against aggressive cancer cells (Martínez-Montemayor et al. 2019).

The anticancer effects of ganoderic acid A (**16**) and ganoderic acid DM (**19**), originally isolated from *G. lucidum*, have been studied on diverse in vitro and in vivo models (Liu et al. 2009; Wu et al. 2012; Cheng and Xie 2019; Das et al. 2020; Xia et al. 2020) (Table 10.1). On human glioblastoma U251 cell line treated with ganoderic acid A (**16**), Cheng and Xie (2019) observed a dose- and time-dependent reduction on cell viability, provoked by an upregulation of autophagy, which led to

cell death by apoptosis. Molecular evidence of these effects was given by the increase in beclin 1 and LC3 II, p62 decrease (markers of autophagy), concomitant with Bcl-2 increase, Bax decrease, and caspase-3 activation (proapoptotic factors). The inhibition of cell migration and invasion was also induced by ganoderic acid A (**16**). The inactivation of the PI3K/Akt/mTOR signaling pathway seems to be underlying the responses of glioblastoma cells to ganoderic acid A (**16**), since the expression of p-Akt, p-mTOR, p-P70S6K, and cyclin D1 was downregulated. Similar results were observed using human lung adenocarcinoma A549 and lung non small cell carcinoma NCI-H460 cell lines treated with ganoderic acid DM (**19**) (Xia et al. 2020). No effect was observed on the nontumoral BEAS2B lung cell line. Cancer cells showed morphological and molecular evidence of autophagy and apoptosis, with upregulated expression of LC3B-II, reduced expression of p-PI3K, p-Akt, and p-mTOR (indicating the inhibition of the PI3K/Akt/mTOR pathway), plus reduced Bcl-2 expression, upregulated Bax expression, and activation of caspase-3 and PARP. Das et al. (2020) studied the effects of ganoderic acids A (**16**) and DM (**19**) on human anaplastic meningioma, an aggressive brain cancer characterized by low expression of NDRG2 (N-myc downstream-regulated gene 2). IOMM-Lee meningioma cells were susceptible to apoptosis upon ganoderic acids A (**16**) or DM (**19**) exposure, but human nontumoral neurons and arachnoid cells, used as controls, presented no effect. Interestingly, NDRG2 protein and Bax expression were upregulated, while levels of MMP-9, p-P13K, p-Akt, p-mTOR, and Wnt-2, related to survival pathways, were diminished. Moreover, using an in vivo model of IOMM-Lee xenographic meningioma tumors on SCID mice, treatment with 10 mg/kg of ganoderic A (**16**) or DM (**19**) for 14 days elicited a 60% reduction in tumors' size.

Another lanostane-type triterpenoid of interest is astrakurkurol (**20**) (Table 10.1, Fig. 10.3), derived from the edible mushroom *Astraeus hygrometricus*, and tested in an in vitro model using human cell lines derived from hepatocarcinoma (Nandi et al. 2019b, 2022). Authors revealed that this compound was able, in a dose-dependent manner, to reduce cell viability (IC_{50} 22.6 μM for Hep3B, and 65.92 μM for HepG2) and proliferative capacity, to induce cell-cycle arrest at the G0/G1 phase, and to elicit cell death through the intrinsic and extrinsic apoptotic pathways. The mechanism of apoptosis elicited by astrakurkurol (**20**) was related to the overexpression of proapoptotic factors (Fas, p53, Bax), leading to caspase-8 cleavage, Bid truncation (tBid), cleavage of PARP, and oxidative stress through generation of ROS, but it was also related to the inhibition of survival pathways, through downregulation of antiapoptotic factors (Bcl-2, Bcl-xL, Akt), increasing caspase-9 and -3 cleavage, and blocking the activation of nuclear factor κB (NF-κB). Astrakurkurol (**20**) was also involved in migration and invasion inhibition on both cell lines, and at least for HepG2, the effect is related to the downregulation of matrix metalloproteinase MMP-2 and MMP-9 (Nandi et al. 2019b, 2022).

Hispidin (**21**) (Table 10.1, Fig. 10.3), a polyphenolic metabolite extracted from the medicinal mushroom *Phellinus linteu*s (and also from *Inonotus* sp.), has been revealed by successive studies as a potent cytotoxic agent against different cancer cell lines, derived from human lung carcinoma (A549), endocervical

adenocarcinoma (SGC-7901), hepatocarcinoma (HepG2), colorectal carcinoma (HCT116), and pancreatic adenocarcinoma (Capan-1, BxPC-3, AsPC-1) (Nguyen et al. 2016; Lv et al. 2017; Chandimali et al. 2018). This secondary metabolite presented a dose-dependent effect on the viability of BxPC-3 cells (among other cell lines), inducing the activation of the proapoptotic factors p53, caspase-3, and PARP, and suppressing the antiapoptotic NF-κB and Bcl-2 (Chandimali et al. 2018). Interestingly, $CD44^+$ CSC subpopulation constitutes 90% of the BxPC-3 cell line, and hispidin (**21**) exerted a specific effect on this subpopulation, suppressing CD44 markers and stemness markers, such as NANOG and SOX2. Additionally, this polyketide affected the viability and proliferation of the $CD44^+$ BxPC-3 CSC subpopulation, both on 2D (monolayer) and 3D (spheroid) cultures, by eliciting cell cycle arrest at the G1 phase and increasing apoptosis. $CD44^+$ CSC's migration and invasion were also affected. Moreover, hispidin (**21**) behaved as a chemosensitizer of $CD44^+$ BxPC-3 CSC, synergically enhancing the chemotherapeutic effect of gemcitabine, as shown by caspase-3 activation and subexpression of NANOG, SOX2, CD44, Bcl-2, and NF-κB (Chandimali et al. 2018). This in vitro evidence justifies the choice of hispidin (**21**) as a potential candidate for further studies.

2.2 *Endophytic Fungi*

Endophytic fungi are major inconspicuous components of fungal diversity (Arnold and Lutzoni 2007; Pant et al. 2021). This group of fungi, known to live asymptomatically inside the plant, have representatives mainly from the phylum Ascomycota and Basidiomycota (Kousar et al. 2022). The diversity of these endophytic fungi can vary with the host plant, although some genera, such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Piriformospora*, are most frequently found (Nisa et al. 2015). Endophytic fungi are specific to particular host plant families or genera (Zhang and Yao 2015; Oki et al. 2016, 2021). Some studies indicate that a greater symbiotic relationship between endophytic fungi and host plants may favor a higher association of secondary metabolites produced between both groups.

Traditional medicine worldwide uses plants for plenty of diseases (Oyenihi et al. 2021; Qadir and Raja 2021), and scientific studies revealed that, very often, endophytic fungi are actually producing the bioactive compounds previously related to their host plants. These studies show the use of endophytic fungi as a promising alternative for producing bioactives on a larger scale. Generally, the compounds produced per plant present low concentrations. Taxol (**1**) (Fig. 10.2) concentrations found in *Taxus baccata* bark, for example, range from 0.064 to 8 g per tree (Nadeem et al. 2002). This promising alternative for producing bioactive compounds has attracted the attention of numerous researchers. The latest review about putative anticancer compounds from plant-derived endophytic fungi covered the end of 1990 to November 2021 (Hridoy et al. 2022).

Reviewing the scientific search databases of Web of Science and Scopus, we identified over 800 articles indicating the use of secondary metabolites synthesized by endophytic fungi for cancer treatment, with half published in the last 5 years (2018 to 2022). More than 200 of these fungal compounds are already known, including paclitaxel/taxol (**1**), camptothecin (**2**), podophyllotoxin (**7**) (Fig. 10.2), and alkaloids, among others (Uzma et al. 2018; Prajapati et al. 2021; Hridoy et al. 2022). These molecules have been tested on more than 60 cancer strains, including those with the highest incidence and mortality in the population, such as breast, lung, and prostate cancer (Hridoy et al. 2022).

Taxol (**1**) (Fig. 10.2) is the best-known and the most studied secondary compound produced by endophytic fungi and has already been applied in the chemotherapeutic treatment of breast, lung, ovarian, and prostate cancer (Naik 2019; Rai et al. 2022; Tiwari and Bae 2022). Over 200 endophytic fungi from 40 genera (such as *Pestalotiopsis*, *Sporormia*, *Trichothecium*, *Tubercularia, Seimatoantlerium*, *Alternaria*, *Penicillium, Fusarium,* among others) have been described as producers of taxol (**1**) (Naik 2019).

Camptothecin (**2**) (Fig. 10.2) is also a chemotherapeutic drug with a broad spectrum of anticancer activities, already in use against colon, breast, ovarian, and small cell lung cancers. It is responsible for inhibiting cell viability and inducing apoptosis in different types of cancer (Kousar et al. 2022). New biosynthetic sources were recently evaluated among endophytic fungi. Camptothecin (**2**) was successfully obtained from the optimized in vitro culture of *Phyllosticta elongata*, isolated from the medicinal plant *Cipadessa bacifera*, demonstrating cytotoxic activity against A549 adenocarcinoma cells, comparable to the standard commercial compound (Dhakshinamoorthy et al. 2021). This bioactive metabolite was also purified from the endophytic *Aspergillus flavus*, isolated from *Astragalus fruticosus*, showing cytotoxic activity against the cancer cell lines HepG2 (hepatocarcinoma), MCF7 (invasive breast carcinoma), and HCT29 (colorectal carcinoma) (El-Sayed et al. 2022).

Podophyllotoxin (**7**) (Fig. 10.2) is another promising compound, explored as a biosynthetic precursor of chemotherapeutic drugs such as teniposide and etoposide (Clark and Slevin 1987; Bohlin and Rosen 1996; Kusari et al. 2009; Kumar et al. 2021). This compound has shown strong anticancer activity at the metastasis stage, and it is used to treat leukemia and testicular, lung, prostate, and ovarian cancer (Uzma et al. 2018; Li et al. 2019a). Podophyllotoxin (**7**) has often been found as a secondary metabolite of various fungal species, including those from the endophytic strains *Chaetomium globosum*, *Chaetomium* sp., and *Pseudallescheria* sp., recently isolated from the ethnomedicinal plant *Ginkgo biloba* by He et al. (2020). The authors observed that those fungal extracts containing podophyllotoxin (**7**) could inhibit cell proliferation and migration and induce apoptosis of HeLa cervical carcinoma, under in vitro conditions, and reduce the size of HeLa xenographic tumors transfected to nude mice. Thi Tran et al. (2022) also identified the presence of podophyllotoxin (**7**) among the metabolites obtained from different genera of endophytic fungi isolated from *Dysosma difformis*, such as *Penicillium* sp., *Trametes* sp., *Purpureocillium* sp., *Aspergillus* sp., and *Ganoderma* sp.. In vitro

assays using these extracts demonstrated cytotoxic activity against human acute leukemia (HL-60), lung carcinoma (SK-LU-1), and hepatocellular carcinoma (Thi Tran et al. 2022).

Trichothecenes are a class of secondary metabolites produced by different genera of fungi, shown to be effective in immunomodulation and prevention of cancer, also exhibiting strong activity against various cancer strains (Amagata et al. 2003; Wu et al. 2017; Gao et al. 2020; Liu et al. 2020; Wang et al. 2022). Trichothecin (**22**), trichothecinol A (**23**), and 8-dihydrotrichothecinol A (**24**) (Table 10.2, Fig. 10.4), obtained from an endophytic strain of *Alternaria* sp., isolated from *Schisandra sphenanthera*, demonstrated significant cytotoxicity to colorectal carcinoma cell line HT-29 and triple-negative breast carcinoma MCF-7. Moreover, treatment with tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-sensitized HT-29 cells, known to be TRAIL-resistant, enhancing the cytotoxic effect of these trichothecenes and indicating their possible role in inducing apoptosis (Gao et al. 2020).

The secondary metabolite 8-n-butyrylneosolaniol (**25**) (Table 10.2, Fig. 10.4) was the most active among five trichothecenes produced by the endophytic fungus *Fusarium sporotrichioides*, isolated from *Rauvolfia yunnanensis*, presenting cytotoxic effects against human hepatoma Hub-7 and mouse mammary tumor MRMT-1 cells (Wang et al. 2022). That trichothecene caused cell cycle arrest at the G2/M phase and apoptosis of Hub-7 cancer cells in a dose-dependent manner. The mechanism of cell death elicited by 8-n-butyrylneosolaniol (**25**) involved the apoptotic intrinsic pathway, evidenced by the disruption of mitochondrial membrane potential, production of ROS, the release of mitochondrial cytochrome c, activation of caspase-9 and -3, and activation of PARP.

Among the 12 trichothecene macrolides produced by the endophyte *Paramyrothecium roridum*, found in *Morinda officinalis*, myrothecine H (**26**) and I (**27**) (Table 10.2, Fig. 10.4) were the most effective against cancer cell lines derived from human glioma (SF-268) and hepatocarcinoma (HepG2), results comparable to cisplatin, used as a positive control (Liu et al. 2020). Unfortunately, all these compounds were also cytotoxic to nontumoral human hepatic stellate cells. Nevertheless, myrothecines (**26–27**) induced cell cycle arrest (at the G1 phase) and apoptosis of HepG2 in a dose- and time-dependent mode. As for other trichothecenes, the intrinsic pathway of apoptosis was confirmed by a decreased mitochondrial membrane potential, release of mitochondrial cytochrome c, cleavage of PARP, and phosphorylation of JNK (c-Jun N-terminal protein kinase) protein. Taken together with the previous results obtained with other trichothecenes, these findings suggest that this class of fungal metabolites deserves to be studied more in searching for potential drug leads toward cancer treatment.

Among the most promising fungal secondary metabolites, the class is represented of cytochalasans is represented by several compounds that have aroused interest for their biological effects on tumor cell lines (Ma et al. 2018; Sharma et al. 2018; Wang et al. 2019b, c; Peng et al. 2020; Guo et al. 2021; Qader et al. 2021) (Table 10.2). One of them, pchaeglobosal B (**28**), isolated from a strain of the endophytic fungus *Chaetomium globosum*, revealed to be cytotoxic to different cancer cell lines,

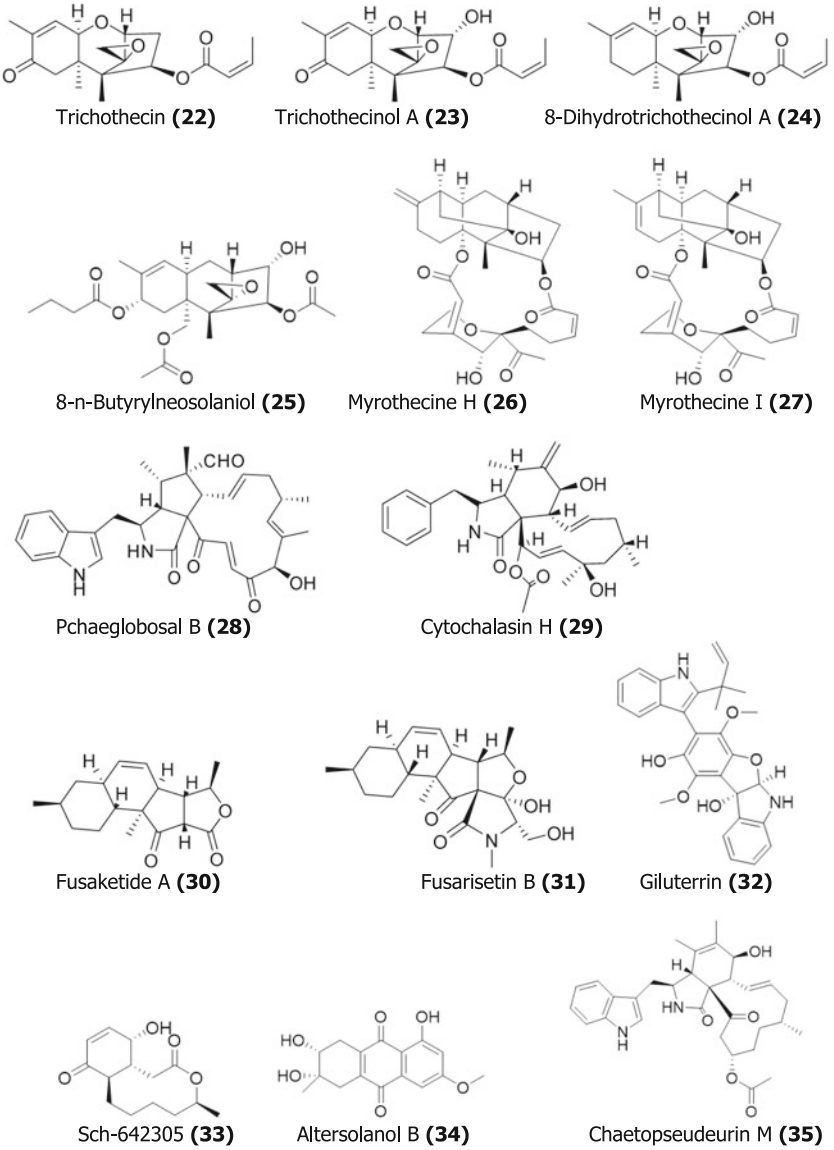

Fig. 10.4 Secondary metabolites from endophytic fungi with promising mechanisms of action against cancer

originated from human lung adenocarcinoma (A549), hepatocarcinoma (HepG2), triple-negative breast carcinoma (MCF-7), and human (HT29) and murine (CT26) colorectal carcinomas (Peng et al. 2020). The in vitro effect of pchaeglobosal B (**28**)

on the colorectal carcinoma cell lines was related to cell cycle arrest at different phases and to a significant increase in death by apoptosis (Table 10.2, Fig. 10.4).

Cytochalasin H (**29**), a cytochalasan isolated from the mangrove endophytic fungus *Phomopsis liquidambari*, has its mechanism of action studied by Ma et al. (2018). Using the human adenocarcinoma A549 line, authors observed the effect of cytochalasin H (**29**) on cancer cell migration, cell cycle arrest at G2/M, and death by apoptosis. Apoptosis was evidenced by an increased expression of proapoptotic proteins (Bax, p53, and active caspase-3) versus a decrease in antiapoptotic proteins (Bcl-xL, Bcl-2, and inactive caspase-3) (Table 10.2, Fig. 10.4). Previous studies of Takanezawa et al. (2017) on the antitumoral effects of cytochalasins on the same cancer cell line, indicated a partial disruption of cytoskeleton actin filaments, as well as an increase in binucleated cells induced by cytochalasin H (**29**), which can explain the effect on the cell cycle kinetics observed by Ma et al. (2018). Moreover, upon treatment of A549 cells with cytochalasin H (**29**), there was evidence of autophagy inhibition, with increasing levels of LC3-II and p62 markers, justifying the impairment in the self-renewal potential of these tumor cells.

Other polyketides with anticancer properties have been highlighted in recent studies (Nakashima et al. 2018; Li et al. 2019b; Wang et al. 2019a; Chang et al. 2021; Liu et al. 2021; Tian et al. 2021). Especially interesting examples are fusaketide A (**30**) and fusarisetin B (**31**), obtained from *Fusarium equiseti* (LGWB-9), an entomogenous fungus isolated from the ladybug *Harmonia axyridis* (Table 10.2, Fig. 10.4). Both metabolites were able to inhibit the proliferation of a panel of cancer cell lines, such as HeLa (endocervical adenocarcinoma), Huh-7 (hepatocarcinoma), MCF-7 (breast carcinoma), and MGC-803 (gastric carcinoma). Fusarisetin B (**31**), further evaluated on MGC-803 line, also demonstrated to inhibit cell migration and invasion, and to elicit apoptosis through the upregulation of Bax and p53 and the downregulation of Bcl-2 (Liu et al. 2021).

Giluterrin (**32**) is a novel carbon skeleton derived from prenylated indole alkaloid, produced by the endophytic fungus *Aspergillus terreus* from *Axonopus leptostachyus* (Table 10.2, Fig. 10.4). Although this compound affected the viability of nontumoral human keratinocytes (HaCat), used as control, it demonstrated strong antiproliferative activity against human cell lines from renal carcinoma (786-0) and metastatic prostate carcinoma (PC-3), significantly reducing clone formation (Gubiani et al. 2019), which justifies further studies to understand its mechanism of action and propose possible derivatives to overcome the toxicity to healthy cells.

The macrocyclic lactone Sch-642305 (**33**) (Table 10.2, Fig. 10.4), obtained from an endophytic strain of the medicinal plant *Achyrocline satureioides*, presented selective cytotoxic effects against glioma cells lines from rat (C6) and human (U138MG) origin, with no visible effects on rat primary astrocyte cells (control). Besides inhibiting migration on both glioma cells, the compound affected clonal proliferation and cell cycle kinetics (arrest at the G2/M phase) of C6 glioma cells. An increase in apoptosis was also observed in these cells through the modulation of mitochondrial redox status, evidenced by the superoxide dismutase and catalase upregulation and downregulation of ROS generation (Pedra et al. 2018).

Altersolanol B (**34**) (Table 10.2, Fig. 10.4) is a tetrahydroanthraquinone isolated from the endophytic *Stemphylium solani* colonizing the plant *Morinda citrifolia*. Studied in a breast cancer model by Siraj et al. (2022), this bioactive compound selectively reduced the viability of human invasive breast carcinoma (MCF-7) and triple-negative breast carcinoma (MDA-MB-231) cell lines without affecting human mammary epithelial cells (MCF-10A) used as control. Altersolanol B (**34**) downregulated the expression of cyclin D1, CDK4, and CDK2, and upregulated the expression of p21Waf1/Cip1 and p53, eliciting cell cycle arrest at the G0/G1 phase. Carcinoma cells treated with the compound undergo apoptosis through the intrinsic mitochondrial pathway, as evidenced by the activation of caspase-9 and PARP, expression of Bax expression, and downregulation of Bcl-2 (Siraj et al. 2022). Aggressive tumors, such as those represented in this study, generally present an overactivation of the PI3K/Akt/mTOR signaling pathway, which is involved in multiple steps of carcinogenesis, such as proliferation, invasion, and metastasis (Rascio et al. 2021). Interestingly, altersolanol B (**34**) showed an inhibitory effect on this pathway through the downregulation of phosphorylated factors, glycogen synthase kinase 3β (GSK-3β), and NF-κB (Siraj et al. 2022). The extracellular signal-regulated kinase 1/2 (ERK1/2) cascade also plays a role in carcinogenesis, involving cellular events such as proliferation, differentiation, survival, and apoptosis (Wortzel and Seger 2011). Again, altersolanol B (**34**) could affect this signaling pathway, inhibiting the phosphorylation of MEK1/2 (mitogen-activated protein kinase/ERK) and ERK1/2, and upregulating phosphorylated MKK4 and p38 (Siraj et al. 2022). These remarkable results encourage further studies about the potential role of altersolanol B (**34**) as a chemotherapeutic drug lead.

Another interesting class of endophytic metabolites is the [11]-chaetoglobosin, represented by chaetopseudeurins B, E, F, G, M (**35**) (Table 10.2, Fig. 10.4), which were obtained by Duan et al. (2021, 2022) from a fungal strain of *Pseudeurotium bakeri*. All compounds presented cytotoxic effects against a panel of human cancer cell lines derived from lung adenocarcinoma (A427, A549), invasive breast carcinoma (MCF-7), endocervical adenocarcinoma (HeLa), colorectal carcinoma (HCT116), adenocarcinoma (HT-29), and hepatocarcinoma (HepG2). These compounds also elicited cell cycle arrest (at G2/M) and apoptosis on the tested cell lines. Regarding chaetopseudeurin B and chaetopseudeurin M (**35**), it was shown that the intrinsic apoptotic pathway was involved in cell death since the compounds were able to upregulate Bax expression and activate caspase-3 (proapoptotic factors), while downregulating the expression of antiapoptosis cyclin B1 and Cdk1, and Bcl-2, releasing mitochondrial cytochrome c (Duan et al. 2021, 2022).

Other classes of secondary compounds produced by endophytic fungi, such as alkaloids (Tan et al. 2019; Yuniati et al. 2019), sterols (Yang et al. 2022), diphenyl ether derivatives (Nakashima et al. 2018), butenolide derivatives (Deng et al. 2020), benzoquinones (Shylaja and Sathiavelu 2019), oblongolides (Zhang et al. 2022), and macrolides (Liu et al. 2020; Qader et al. 2021; Bai et al. 2022), also have been noted for their antiproliferative activity against several human cancer cell lines, being presented on Table 10.2.

2.3 *Fungi from Other Biomes*

In addition to traditional edible and medicinal mushrooms and endophytic fungi, which have provided a substantial variety of chemotherapeutic drug leads, many other species originating from diverse environments, including other host-living fungi, have been highlighted in the production of anticancer compounds in vitro and some synthetic analogs have progressed into clinical trials.

Penicillium species from the most diverse ecosystems have been extensively investigated as an important source of cytotoxic compounds. In the literature survey of De Carvalho et al. (2021), between 1984 and 2020, a total of 76 compounds and one enzyme originated from *Penicillium* spp. were classified as antileukemia agents, based on the in vitro effects against a variety of human cell lines derived from myeloid or lymphocytic leukemia. The source of these compounds was mostly from marine *Penicillium* species (62%), followed by endophytic (15%), soil originated (14%), and extremophiles (9%). Regarding their biosynthetic classes, 57% were polyketides, 25% alkaloids, and a small number of terpenoids (9%) and peptides and proteins (9%) was also found. The biological mechanisms of action of these metabolites were so diverse as their chemical structures, resulting in cytotoxic effects such as cell cycle arrest or cell death by apoptosis. Interestingly, the enzyme L-asparaginase, found in some *Penicillium* strains, has an important cytotoxic effect on acute lymphocytic leukemia, due to its role in the hydrolysis of L-asparagine, an essential amino acid for the growth of neoplastic lymphoblasts, and whose absence leads to cell death (De Carvalho et al. 2021).

In recent years, most attention has been given to marine fungi and their secondary metabolites (Sugumaran et al. 2022). Endozoic fungi have been recovered from marine sponges, and some species have been gaining increasing importance as promising sources of numerous unique bioactive compounds, some with anticancer activities. In particular, *Penicillium chrysogenum* isolated from *Cliona* sp. of Red Sea is a promising natural source of many ß-lactam antibiotics and biologically active compounds with anticancer activities (Al-Saleem et al. 2022). One of the secondary metabolites of *P. chrysogenum,* kojic acid, showed cytotoxic activity against human cell lines from colorectal carcinoma (HCT116) and larynx carcinoma (Hep2) (Table 10.3). Compounds such as rubrofusarine B, fansecinone A, and asperpyrone A (Table 10.3), produced by a marine strain of *Aspergillus foetidus* isolated from the Sea of Okhotsk, exhibited significant inhibitory activity on prostate cancer 22Rv1 cells. Rubrofusarine B and fansecinone A were able to inhibit cell colony formation, inducing the reproductive death of this cell line (Antonov et al. 2021).

Dezaire et al. (2020) explored the diversity of fungi from a specific niche, marine algae, as a new source of natural products capable of inhibiting phenotypes involved in cancer aggressiveness, like the MCF7 epithelial cancer cell line. Among 99 isolated fungal strains, the authors indicated *Paradendryphiella salina* as one of the most promising fungi. This fungus produces active compounds, such as (−)-(*3R, 6R*) hyalodendrin (**36**) (Table 10.3, Fig. 10.5), an interesting fungal toxin previously

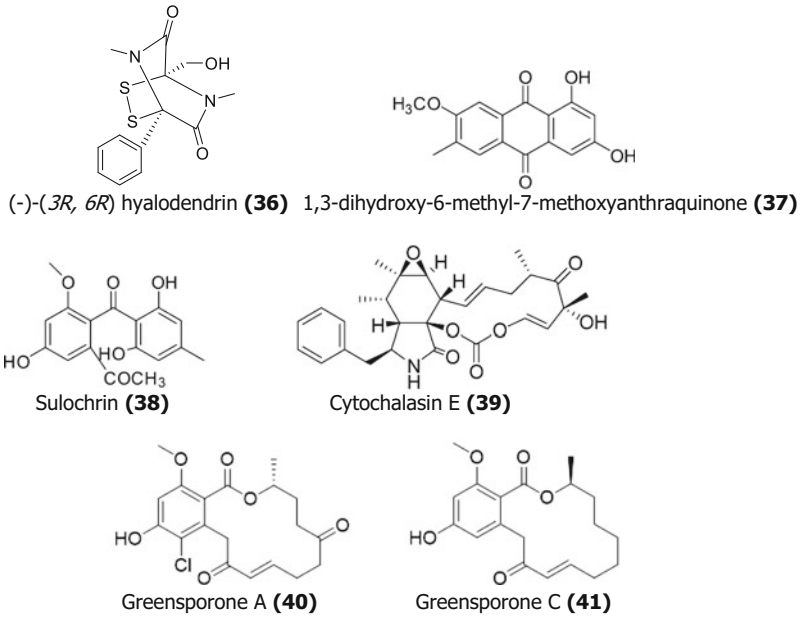

Fig. 10.5 Secondary metabolites originated from fungi from diverse biomes with promising mechanisms of action against cancer

associated with antifungal and antibacterial properties, with selective cytotoxicity to human invasive breast carcinoma (MCF7 and stem-like MCF7-Sh-WISP2), drug-resistant triple-negative breast carcinoma (MDA-MB-231), endocervical adenocarcinoma (HeLa), among other colorectal cancer cell lines with invasive and drug-resistant phenotypes, regardless some effects on nontumoral murine preadipocytes (3T3-F442A) used as control. This compound seems to act by causing DNA damage to MCF7-Sh-WISP2 cells through the phosphorylation of p53 and γ-H2AX and dephosphorylation of PRAS40, and also inducing cell oxidative stress, as indicated by the downregulation of HSP60, HSP70, and SOD2 proteins (Dezaire et al. 2020).

The polyketide 1,3-dihydroxy-6-methyl-7-methoxyanthraquinone (**37**) (Table 10.3, Fig. 10.5), isolated from the marine fungus *Thermomyces lanuginosus*, also presented selective cytotoxicity to a drug-resistant cell line derived from human prostate carcinoma (22Rv1), compared to a nontumoral human prostate cell line (PNT-2). The metabolite strongly affected the ability to self-renewal (clonal expansion) of 22Rv1 (Sobolevskaya et al. 2021).

Using the OSMAC (One Strain Many Compounds) strategy and modifying culture conditions to change the fungal metabolic profile, El-Kashef et al. (2021) isolated several compounds from the Red Sea (Egypt) fungus *Aspergillus falconensis*, including the polyketide sulochrin (**38**) (Table 10.3, Fig. 10.5). Further molecular docking analysis revealed that this compound has an affinity to human enzymes, such as CDK-2 (cyclin-dependent kinase 2) and TOP-2 (DNA

topoisomerase II), as well as to MMP-13 (matrix metalloproteinase 13). In vitro assays demonstrate its selective cytotoxicity to murine lymphoma cell line L5178Y and the ability to inhibit migration of MDA-MB-231 cells, although not affecting their viability.

Cytochalasin E (**39**) (Table 10.3, Fig. 10.5), another member of the cytochalasan class, was originally produced by *Aspergillus clavatus*, a fungus encountered in soil and animal manure. Takanezawa et al. (2018) observed a significant reduction in the viability of A549 cells treated with low concentrations of the compound (IC_{50} 0.5 μM), with visible effects on cell morphology, including a complete disruption and depolymerization of cytoskeleton actin filaments and a pronounced increase in binucleated cells, which could indicate cytokinesis failure. Moreover, cytochalasin E (**39**) inhibited cell autophagy by increasing LC3-II and p62 levels, like previous results observed for other cytochalasins (Takanezawa et al. 2017, 2018).

Freshwater fungi are also an underexplored source of important secondary metabolites with anticancer properties, such as greensporones (Table 10.3, Fig. 10.5). The mechanism of action of greensporone A (**40**) and C (**41**), isolated from the fungi *Halenospora* sp., was unraveled in a series of elegant studies conducted by El-Elimat et al. (2014) and Prabhu et al. (2018, 2019), using a panel of cancer cell lines. Both compounds were found to be cytotoxic to the triple-negative breast carcinoma cell line MDA-MB-435 and to leukemic cell lines (K562, U937, and AR230). Greensporone C (**41**) was also effective against colorectal adenocarcinoma HT-29 cells (El-Elimat et al. 2014; Prabhu et al. 2018, 2019). On K562 and U937 cells, these metabolites induced cell cycle arrest at the subG0/G1 phase and elicited apoptosis via the extrinsic and intrinsic pathways. On the PI3K/AKT pathway, effects were related to the downregulation of activated AKT and its downstream targets (forkhead box-O (FOXO), glycogen synthase kinase (GSK3) is the acronym of glycogen synthase kinase-3, and inhibitor of apoptosis proteins —- IAPs is the acronym of inhibitor of apoptosis proteins). The ability of greensporone A (**40**) and C (**41**) eliciting the mitochondrial apoptotic pathway involves altering Bax/Bcl-2 ratio and enhancing cytosolic cytochrome c, which activates caspases-9 and -3 and consequent cleavage of PARP, as well as an increase of ROS level and a decrease in glutathione (GSH). Additional experiments were done to confirm the role of these greensporones (**40**–**41**) in apoptosis. Pretreatment of K562 cells with caspase-inhibitor z-VAD-FMK abrogated greensporone A (**40**) effects on caspase-induced apoptosis. Pretreatment with a ROS scavenger abrogated greensporone C (**41**) effects on apoptosis. One possible application of these greensporones as chemotherapeutic adjuvants was explored using K562 cells treated with 10 μM of greensporone A (**40**) or C (**41**) plus 10 μM of chemotherapeutic imatinib, revealing that the conjugated treatment potentiated the apoptotic effect on those leukemic cells.

3 Future Perspectives: What's Next in the Pathway of Anticancer Drug Discovery from Fungal Compounds

Novel lead compounds from the fungal origin are being highlighted as alternative or adjuvant treatments to conventional chemical drugs due to their high biodiversity, innovative modes of action, multiple and combined effects on cellular targets, apparent safe action on different tissues, biocompatibility, and bioavailability. Although more than 1500 compounds were isolated from fungi between 1992 and 2020, and no fungal secondary metabolites or their derivatives have been approved as anticancer drugs until 2020 (Keller 2019; Yuan et al. 2020). Considering this scenario, several research groups pointed out the importance of bringing together natural product chemistry and synthetic strategies of active compounds with genetic engineering and/or other biotechnological approaches to introduce in the market a fungal-originated anticancer drug (Schueffler and Anke 2014; Keller 2019; Yuan et al. 2020; Hridoy et al. 2022; Takahashi et al. 2022; Contigli et al. 2023).

In 2014, Wasser (2014) described the perspectives, advances, evidence, challenges, and future development of medicinal mushroom science in the twenty-first century. His work brought critical unsolved problems in the study of medicinal mushrooms that are still now to be addressed by the scientific community and that could be fundamentally applied to the study of any other group of fungi with chemotherapeutic applications. The reviews of Takahashi et al. (2022) and Contigli et al. (2023) returned to these topics, updating the state of art of technological tools applicable to drug discovery.

As we present in this review, and other authors recently pointed out (Hridoy et al. 2022; Kousar et al. 2022; Takahashi et al. 2022; Contigli et al. 2023), the biological mechanisms of action of most fungal secondary metabolites, selected by their in vitro anticancer properties, are not entirely unraveled at the cellular and molecular levels. Therefore, efforts should be made to identify molecular targets and affected signaling and metabolic pathways in cancer cells. Additionally, selectivity for oncologic tissues associated with safety for healthy tissues must be ensured thus, representative panels of drug-resistant tumor cells, as well as nontumor cells, should be used at this phase of discovery.

There are many biological mechanisms of action through which chemotherapeutic drugs affect cancer cells and avoid the progression of tumorigenesis. The choice of new lead drugs among the potential candidates of fungal origin depends on deeply investigating these mechanisms, looking for molecules capable of eliciting long-lasting cytotoxic effects on cancer cell populations and minor side effects on healthy cells. Multiple and combined effects on cellular targets are also advantages to overlap possible mechanisms of chemoresistance.

Further preclinical studies should also be conducted to simulate, as much as possible, the pharmacokinetics of potential new drugs that takes place in vivo, considering both the tumor microenvironment and the systemic level. For that achievement, many technological platforms can be used, such as in vitro cultures of 3D spheroids and organoids and ex vivo organotypic cultures (Lancaster and

Knoblich 2014; Supadmanaba et al. 2021), as well as alternative in vivo models, minimizing and replacing experiments on sentient animals (Kendall et al. 2018; Lopes et al. 2021), and multiomics analysis (Chakraborty et al. 2018; Chaudhary et al. 2018; Nam et al. 2021).

In silico studies are also a potent tool to elucidate chemical interactions between bioactive compounds and their molecular targets and to estimate their chemotherapeutic and potentially toxic in vivo effects (Carracedo-Reboredo et al. 2021). As Aguirre et al. (2018) pointed out in their work, in silico approaches can be used in parallel to conventional in vitro and in vivo studies to build integrated platforms for developing innovative chemotherapeutic drugs. These authors evaluate the antitumor and epigenetic modulatory potential of phenolic compounds from edible and medicinal mushrooms, based on their structural similarities to reference compounds previously characterized by other researchers as anticancer agents. For example, hesperetin, obtained from the medicinal mushroom *G. lucidum*, was found to be an analog to curcumin, related to epigenetic activities on DNA methyltransferase (DNMT), histone acetyltransferase (HAT), and histone deacetylase (HDAC). The flavonoid myricetin, present in *Pleurotus ostreatus* (oyster mushroom), was structurally related to compounds with known inhibitory effects on DNA methyltransferase (DNMT) and topoisomerase I, as well as alkylating agents. While naringin, a second flavonoid found in *P. ostreatus*, was structurally similar to compounds with antimitotic action, such as vinblastine sulfate and vincristine sulfate, among others (Aguirre et al. 2018).

In silico studies, such as molecular docking analysis, offers an exciting tool for screening new molecules since structure-related biological effects can be inferred before undergoing time and money-consuming in vitro assays. El-Kashef et al. (2021) used this approach to select some promising compounds isolated from marine *Aspergillus falconensis*, studying their potential affinity to some human enzymes related to cancer formation. Sulochrin (**38**) was one of the selected compounds that displayed stability within the active sites of cyclin-dependent kinase 2 (CDK-2), human DNA topoisomerase II (TOP-2), and matrix metalloproteinase 13 (MMP-13), justifying its choice for further in vitro studies.

Yuan et al. (2020) also discussed the importance of better understanding the biosynthetic gene clusters of promising anticancer fungal metabolites. There is an urge to intensify the studies in synthetic biology techniques such as genomic sequencing, gene synthesis, and CRISPR-Cas 9 genome editing. These techniques allied to epigenetic activation, and transcriptional regulators activation, among others, might broaden the anticancer fungal natural products library (Takahashi et al. 2018). Furthermore, biosynthetic knowledge and metabolic engineering could be applied to increase the production of new potential compounds for preclinical and clinical trials.

The synthesis of analogs has been one strategy not only to improve the antitumoral action of fungal metabolites but also to develop new approaches to transport these active metabolites to their target. For instance, nanoparticles are good examples of drug-delivery vectors and have been explored in the treatment and diagnosis of human cancer (Ali et al. 2020). Akther et al. (2019) explored this

approach by synthesizing silver nanoparticles which contained the endophytic fungal cell filtrate of *Botryosphaeria rhodina*, isolated from *Catharanthus roseus*. The mycosilver nanoparticles showed high cytotoxicity against the lung carcinoma A549 cell line. Moreover, this study suggested that silver nanoparticles may either undergo endocytosis or penetrate the cancer cell membrane and cause DNA damage, evidenced by the DNA fragmentation assay.

In conclusion, it is urgent to add scientific evidence on the physiological and pharmacological effects of fungal bioactive compounds and derivatives as chemotherapeutic lead drugs, applying complementary strategies of in vitro, in silico, and in vivo preclinical studies, and moving forward clinical trials.

4 Final Considerations

The biotechnological use of fungi is intrinsically related to human culture, from the historical evolution of traditional foods and ancient remedies to the modern development of functional food, nutraceutical products, and pharmaceutical drugs. For centuries, higher basidiomycetes and other taxonomic groups of fungi, including the symbiotic species hidden on their hosts, have been used in many countries as natural prophylactic and curative medicines against cancer. Traditional and alternative health practices are recognized worldwide as essential resources to complement conventional health systems, especially in the prevention of chronic and age-related diseases (WHO 2019). They are also potential sources of insights for new pharmaceuticals. Bioactive metabolites from a large number of fungi species are being identified, isolated, and studied to determine their molecular, cellular, and systemic mechanisms of action to prevent or inhibit carcinogenesis (Nandi et al. 2019a; Dai et al. 2021; De Carvalho et al. 2021; Hridoy et al. 2022; Kousar et al. 2022; Neergheen et al. 2022; Takahashi et al. 2022; Contigli et al. 2023). Their properties include: (i) prevention of oncogenesis; (ii) selective cytotoxicity to cancer cells; (iii) inhibition of proliferation, migration, and invasion of cancer cells; (iv) adjuvant effect for conventional chemotherapeutic drugs with less adverse effects; and (v) indirect mechanisms of action on the immune system. Further efforts are needed to fill gaps in the scientific evidence of the physiological and pharmacological effects of fungal metabolites in order to support the development of drug leads that will presumably expand the therapeutic arsenal toward cancer.

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Chapter 11
Edible Mushrooms Substances as Natural Prevention in Autoimmunological Diseases

Katarzyna Kała, Jan Lazur, Katarzyna Sułkowska-Ziaja, and Bożena Muszyńska

Abstract Edible mushrooms have been among the foods present in the human diet for centuries. They were valued for their taste and nutritional potential, but also for their health-promoting properties. Several edible mushroom activities have been proven which predominantly comprise of antioxidant, immunostimulant, antimicrobial, antiatherosclerotic or anti-inflammatory effects. Despite numerous reports on the therapeutic importance of mushrooms, it has long been recognized that their low dietary and therapeutic potential is lacking, so a comprehensive analysis of the content of substances with health-promoting activity, with a particular focus on their potential bioavailability and actual importance to the human body, is essential. Mushroom fruiting bodies are a source of primary metabolites, secondary metabolites, and biologically active bioelements that are important for the proper functioning of the human body. Reports in recent years indicate that edible mushroom extracts show activity, particularly against immunological diseases. Knowledge of the physiology of the immune system is constantly evolving—understanding the detailed mechanisms of the immune response enables the development of increasingly effective and safer drugs. One of the directions of new drug development is the pharmacotherapy of chronic inflammation, which is often associated with autoimmune diseases. A proper diet and supplementation based on edible mushroom-based products can be the crucial factors in preventing and treating this type of disorder.

Keywords Medicinal mushrooms · Immune system · Disease prevention · Polysaccharides · Lectins · Phenolic compounds · Bioelements · Functional foods

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1 Introduction

Autoimmune diseases can be caused by chronic inflammation, so preventing their development is crucial in the prophylaxis of them (Fugger et al. 2020). An essential factor in preventing the development of the autoimmune disease process is modulating the activity of the human immune system (Navegantes et al. 2017). The inflammatory response is caused by the natural response of the human immune system to damaging factors, i.e., physical, chemical agents or pathogens. Acute inflammation in the body is a short-term process that usually undergoes spontaneous extinction, but can sometimes progress to a chronic, debilitating state (Chen et al. 2017; Varela et al. 2018). Chronic inflammation is a distinctive process in the pathogenesis of many diseases, such as autoimmune, cardiovascular, metabolic, neurodegenerative or cancerous ones. Deficiencies of antioxidants, vitamins, and bioelements with anti-inflammatory properties (e.g., zinc, magnesium, selenium) in the body, as well as age or the condition of the body, can affect the ineffective extinction of inflammation (Okin and Medzhitov 2012; Chen et al. 2017).

Knowledge of the physiology of the immune system is constantly evolving, and understanding the detailed mechanisms of the immune response makes it possible to create more effective and safer drugs and encourages research on the prevention of this condition. One direction of new drug development is the pharmacotherapy of chronic inflammation, often associated with autoimmune diseases (Wang and du Bois 2015; Fugger et al. 2020).

For both dietary and medicinal purposes, mushrooms have been used for thousands of years and are one of the oldest documented natural medicinal resources, especially for supporting the immune system (Fugger et al. 2020; Venturella et al. 2021).

The long history of the use of medicinal mushrooms of the genus *Polyporus* is evidenced by the fruiting bodies found next to Ötzi. In 1991, tourists found the body of a man in the Alps at an altitude of more than 3000 meters above sea level, which was initially thought to belong to a tragically deceased tourist, but over time it became clear that the remains were more than 5300 years old and surprisingly well-preserved thanks to being frozen in a glacier. The discovery caused a worldwide sensation, and the person who found it named Ötzi (Iceman). A species of wood decay mushroom, *Fomitopsis officinalis*, was found next to his corpse. Ötzi, according to the scientific examination of samples from his body, suffered from many diseases, such as atherosclerosis (which refutes the claim that it is a modern disease of civilization), arthritis, ulcer disease, parasitic diseases, and many wounds on the body. The researchers found that Ötzi was also infected with *Trichuris trichiura*, a parasitic nematode species that causes abdominal pain. *Fomitopsis officinalis* exhibits all the actions that were effective in the ailments of the "Iceman"; for this reason, he had it with him, as he used it as a remedy that offsets his illnesses, especially the associated inflammation. This history proves that the fruiting bodies of this mushroom, now recognized as a medicinal species, were one of the oldest

natural resources effective in extinguishing inflammation and treating many conditions (Stamets and Zwickey 2014; Grienke et al. 2014).

Mushrooms have been essential medicinal raw materials in the traditional folk medicine of many cultures, and so, for example, in Japanese medicine, the species *Lentinula edodes* (Shiitake mushroom), in Chinese traditional medicine, *Ganoderma lucidum* (commonly Lingzhi or Reishi) were considered a panacea. Recent scientific research confirms the therapeutic effects of traditionally used mushrooms, now classified as medicinal species (Grienke et al. 2014; Drori et al. 2016).

According to current knowledge, the fruiting bodies of edible mushrooms are known to be excellent dietary ingredients and are also all on the list of medicinal mushrooms. They are an excellent source of carbohydrates, including the dietary fiber chitin (**1**), which also has antiatherosclerotic and detoxifying effects on the human body (Bhambri et al. 2022; El-Ramady et al. 2022). Mushroom fruiting bodies are a valuable source of wholesome protein, which contains proteinogenic amino acids (including exogenous ones); hence they can be a dietary alternative to animal products (which explains why they were called forest meat in traditional medicine) (Bell et al. 2022). Edible mushrooms are low in calories due to their low-fat content. However, they are rich in polyunsaturated fatty acids that are valuable for health, including those with anti-inflammatory activity and that activate the human body's immune system (e.g., α-linolenic acid). The dietary and medicinal value of edible mushrooms has been proven and confirmed by determining biologically active compounds of health-promoting importance in their fruiting bodies (Kała et al. 2021; Bell et al. 2022).

An analysis of the incidence of civilization diseases accompanied by inflammation may indicate that free radicals cause them. It has been scientifically proven that oxygen-free radicals play an important role in biological processes, such as the detoxifying reactions of the cytochrome P450 system, the synthesis of prostaglandins or the reactions taking place in the mitochondrial respiratory chain (Guerrero-Castillo et al. 2017; Bell et al. 2022). Oxygen-free radicals are, unfortunately, also activators of lipid peroxidation, which in turn promotes damage to genetic material as a result of cross-reactions of malondialdehyde (one of the main products of peroxidation) with nitrogenous bases, which are components of nucleic acids (Sánchez 2016; Bell et al. 2022). Worldwide, cancer, along with cardiovascular and autoimmune diseases, are the leading causes of death and the available treatments, besides not always being effective, often have a number of side effects and put a strain on the weakened human body. For this reason, natural and safer treatments are increasingly being considered in research, and the next challenge is to develop preventive methods, primarily those that will use a properly balanced diet (Friedman 2016; Bell et al. 2022).

Based on the results of scientific research, edible mushrooms can be included among functional foods and recommended as a valuable component of the daily diet, since their fruiting bodies are rich in components such as polysaccharides, phenolic and indolic compounds, mycosterols, fatty acids, carotenoids, vitamins (primarily water-soluble: C, B_1, B_2, B_6 and in fats: D and E) and bioelements (e.g., zinc, magnesium, copper, selenium) (Das et al. 2021; Kumar et al. 2021; Kała et al. 2021).

The content of the mentioned compounds and bioelements in the fruiting bodies results directly in numerous biological activities: antioxidant, anticancer, immunostimulant, antiatherosclerotic, neuroprotective, antidepressant, anti-allergic, antibacterial, antiviral or hypoglycemic, but most importantly they have anti-inflammatory properties and can be effective against diseases caused by the human immune system (Rathore et al. 2019; Bach et al. 2019).

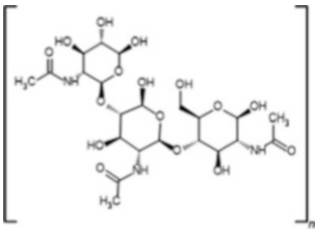
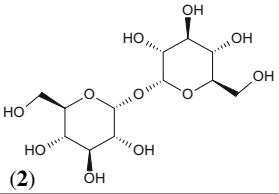
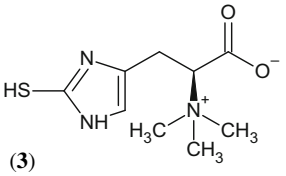
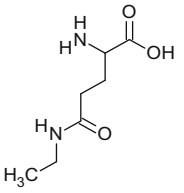
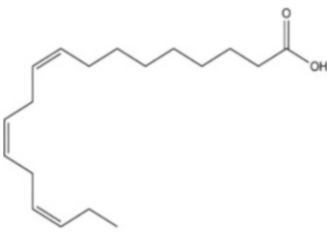
2 Primary Metabolites: Significant Developments

2.1 Carbohydrates

Carbohydrates are a common group of compounds present in mushrooms (Table 11.1). L-Fucose (6-deoxy-L-galactose) is a simple carbohydrate commonly found in the fruiting bodies of mushrooms showing moisturizing, antiaging and anti-inflammatory effects. In inflammatory diseases, L-fucose can suppress allergic reactions, such as contact dermatitis. The distribution of L-fucose in the skin keratinocytes of psoriasis patients was found to be quite different from that in the skin of healthy people (Ma et al. 2021b; Wang et al. 2021). Mannitol, a polyol with antioxidant properties, is also a carbohydrate commonly found in the fruiting bodies of edible mushrooms (Meena et al. 2015). Trehalose (**2**), a disaccharide in mushroom fruiting bodies, acts as a spare material and protects proteins found in the filaments from denaturation. A study using a subarachnoid hemorrhage model using RAW 264.7 macrophages and vascular endothelial cells (HUVECs) found that trehalose (**2**) can inhibit the expression of proinflammatory proteins (cyclooxygenase-2, inducible nitric oxide synthase iNOS), and also inhibits the degradation of the IκB-α subunit, an inhibitor of the nuclear transcription factor NF-κB. The carbohydrate has also been shown to reduce lipid peroxidation and the release of arachidonic acid from cell membrane phospholipids due to the action of reactive oxygen species (Echigo et al. 2012). An example of the trehalose (**2**), content of dried fruiting bodies of one of the world's most popular mushrooms, *Agaricus bisporus*, ranges from 1 to 3% of dry weight (Muszyńska et al. 2017).

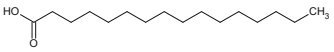
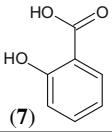
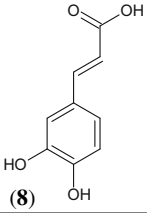
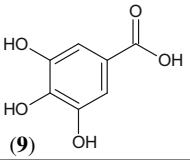
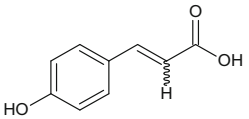
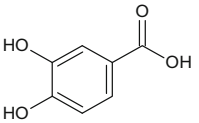
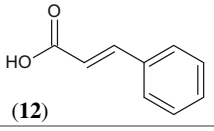
Polysaccharides are particularly valuable for improving immunity and modulating the defense response of the human body. Various types of polysaccharides have been shown to be produced in mushroom fruiting bodies, which can be both water-soluble and water-insoluble. Some of them are built from residues of one type of monosaccharide—homopolysaccharides, while others from heteropolysaccharides: connections to peptides (proteoglycans) or steroids that build the walls of fungal filaments, built from residues of different monosaccharides. Heteropolysaccharides are also polymers of N-acetylaminoglucose—chitin and chitosans (deacetylated forms of chitin) (Yu et al. 2023). These compounds, acting as dietary fiber, contribute, among other things, to the protection of the intestinal mucosa (Chou et al. 2013; Belkaid and Hand 2014). The role of probiotic bacteria in the proper functioning of the immune system is increasingly pointed out (Belkaid and Hand 2014). Mushroom

Table 11.1 Biologically active compounds present in selected mushroom species

Name of compounds and number of figure	Chemical structure	Biological activity determined in mushroom material	References
Carbohydrates			
Chitin	 (**1**)	Blood glucose level regulation Obesity prevention Reducing cholesterol levels	Satitsri and Muanprasat (2020)
Trehalose	 (**2**)	Anti-inflammatory Antioxidant	Echigo et al. (2012), Abd-Elsalam et al. (2021), and Korolenko et al. (2022)
Amino acids			
Ergothioneine	 (**3**)	Anti-inflammatory Antimutagenic Antioxidant Chemoprevention Radioprotection	Cheah and Halliwell (2012), Asahi et al. (2016), Halliwell et al. (2018), and Borodina et al. (2020)
Theanine	 (**4**)	Lowering blood pressure Neurotransmitter	Türközü and Şanlier (2017)
Lipids			
α-Linolenic acids	 (**5**)	Protective factor in breast cancer	Wang et al. (2020)

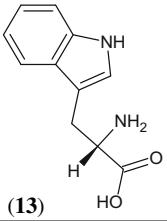
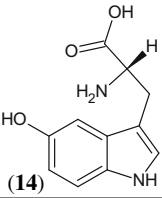
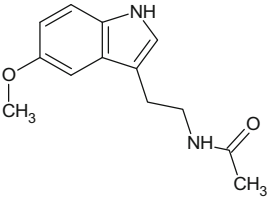
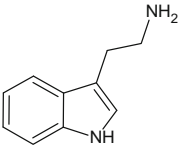
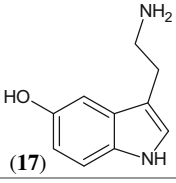
(continued)

Table 11.1 (continued)

Name of compounds and number of figure	Chemical structure	Biological activity determined in mushroom material	References
Palmitic acid	 (**6**)	Protective factor in breast cancer	Liu et al. (2022)
Secondary metabolites			
Phenolic compound			
Benzoic acid	 (**7**)	Antioxidant	Velika and Kron (2012)
Caffeic acid	 (**8**)	Antioxidant Anti-inflammatory	Kassa et al. (2021) and Zielińska et al. (2021)
Gallic acid	 (**9**)	Antioxidant	Bai et al. (2021)
Coumaric acid	 (**10**)	Antioxidant	Boz (2015)
Protocatechuic acid	 (**11**)	Antioxidant Anti-inflammatory	Khan et al. (2015) and Safaeian et al. (2018)
Cinnamic acid	 (**12**)	Antioxidant	Ruwizhi and Aderibigbe (2020)

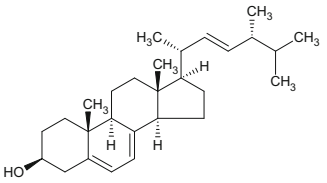
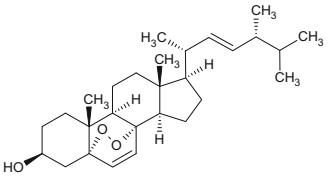
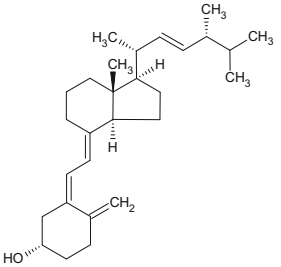
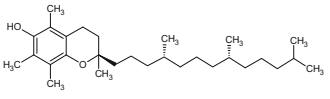
(continued)

Table 11.1 (continued)

Name of compounds and number of figure	Chemical structure	Biological activity determined in mushroom material	References
Nonhallucinogenic indole compounds			
L-tryptophan	 (**13**)	Neurotransmitter Precursor to indole compounds	Fukuwatari and Shibata (2013) and Muszyńska et al. (2014)
5-Hydroxy-L-tryptophan	 (**14**)	Neurotransmitter	Connelly et al. (2020)
Melatonin	 (**15**)	Antioxidant Anti-inflammatory Neurotransmitter Prevention of neurodegradative diseases	Nabavi et al. (2019) and Gunata et al. (2020)
Tryptamine	 (**16**)	Neurotransmitter	Zohairi et al. (2023)
Serotonin	 (**17**)	Antialergic Anti-inflammatory Depression therapy Neurotransmitter	Artigas (2013), Wu et al. (2019a)

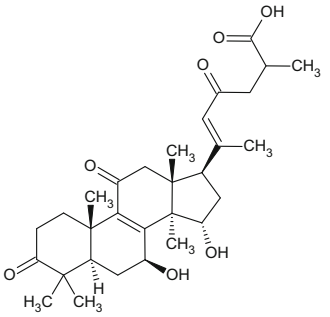
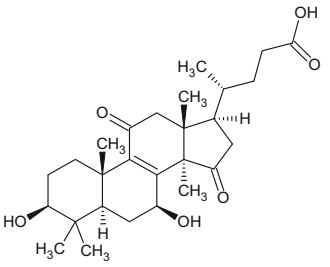
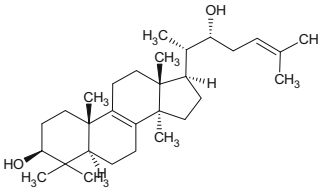
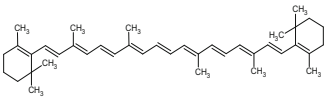
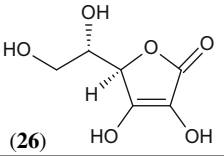
(continued)

Table 11.1 (continued)

Name of compounds and number of figure	Chemical structure	Biological activity determined in mushroom material	References
Sterols			
Ergosterol	 (**18**)	Anti-inflammatory Antitumor Hepatoprotective	Zhabinskii et al. (2022)
Ergosterol peroxide	 (**19**)	Anti-inflammatory	Merdivan and Lindequist (2017)
Tocopherols			
Ergocalciferol	 (**20**)	Anti-inflammatory Antitumor	Cavalier et al. (2013) and Chien et al. (2017)
Tocopherol	 (**21**)	Anti-inflammatory Anticancer Prevention of peroxidation of cellular phospholipids	Abraham et al. (2019) and Wallert et al. (2019)

(continued)

Table 11.1 (continued)

Name of compounds and number of figure	Chemical structure	Biological activity determined in mushroom material	References
Isoprenoids			
Ganoderic acid	 (**22**)	Anti-inflammatory Antibacterial Antiviral	Ma et al. (2021a), Ryu et al. (2021), and Wang and Lu (2021)
Lucidenic acid	 (**23**)	Anti-inflammatory Antibacterial Antiviral	Zheng et al. (2023)
Inotodiol	 (**24**)	Anti-inflammatory	Nguyen et al. (2022)
Other compounds			
β-Carotene	 (**25**)	Antioxidant	Young and Lowe (2018)
Ascorbic acid	 (**26**)	Antioxidant	Njus et al. (2020)

polysaccharides are among the prebiotics that stimulate the growth of proper intestinal bacterial flora (Chou et al. 2013; Belkaid and Hand 2014). Chitin (**1**), chitosans, and glucans have also been shown to lower total and LDL cholesterol, reduce fat absorption in the gastrointestinal tract, and regulate glycemia, so they may protect against the development of cardiovascular disease, obesity, and diabetes (Friedman 2016; Bell et al. 2022; Yu et al. 2023). The total fiber content varies on average from 27 to 38 g per 100 g of edible fruiting bodies (Nile and Park 2014).

β-glucans found in the fruiting bodies of mushrooms in the Basidiomycota taxon, due to their broad spectrum of action in the immune system, play the role of biological response modifiers (Yu et al. 2023). These compounds are also attributed with antioxidant properties, preventing DNA damage and destroying carcinogenic metabolites (Friedman 2016). The effect of β-glucans on the production of both pro- and anti-inflammatory cytokines has also been demonstrated, and another of the mechanisms of action of β-glucans is the binding to Pattern Recognition Receptors of immune cells, as pathogen-associated molecular patterns (Patin et al. 2019; Yu et al. 2023). Such receptors include dictyin-1, complement receptor 3 (CR3, CD11b/CD18) or Toll-Like receptors. Thus, β-glucans activate the proliferation and maturation of immune cells, and stimulate the activation of macrophages and NK cells (Yu et al. 2023).

In ongoing clinical trials using *Trametes versicolor* fruiting body extracts, containing β-glucan (Krestin), the immune system has been stimulated and survival rates of colorectal cancer oncology patients have been shown (He et al. 2022) (Fig. 11.1).

Immunomodulatory and anti-inflammatory effects have been demonstrated for glucans obtained from the fruiting bodies of *Inonotus obliquus*. In vitro studies of these compounds demonstrated inhibitory properties of NF-κB, COX-2, and iNOS signaling pathway activity in RAW 264.7 cells (Ma et al. 2013). One of the better-studied (1→6)-β-D-glucan with a linear structure, obtained from extracts of *Agaricus bisporus* fruiting bodies, has proven repressive effects on proinflammatory genes in in vitro studies using the human monocytic leukemia macrophages cell line, and causes inhibition of the lipopolysaccharide (LPS)-induced inflammatory response through repression of COX-2 and interleukin 1 (IL-1) protein expression (Smiderle et al. 2013).

2.2 Amino Acids and Proteins

The medicinal properties of edible mushroom fruiting body extracts are related to the amino acids (both endogenous and exogenous) they contain, which modulate prostaglandin metabolism (Table 11.1). The anti-inflammatory and immunomodulatory properties of the arboreal edible and medicinal species *Pleurotus ostreatus* (Fig. 11.2) are due to the content of essential amino acids such as leucine, isoleucine, tyrosine, and phenylalanine in its fruiting bodies (Tagkouli et al. 2020).

Fig. 11.1 *Trametes versicolor* (L.) Lloyd (photo by Katarzyna Sułkowska-Ziaja)

Arginine—an amino acid found in this species (but also in other edible mushrooms), which inhibits the growth of cancer cells and can reduce the risk of metastasis. Supplementation of cancer patients with arginine has been shown to have beneficial effects on the immune system, weight gain, and improved body condition and survival time for patients. This action is due to the delaying of tumor growth and the appearance of metastases, as well as the production of nitric oxide, which is needed for blood vessels (Tagkouli et al. 2020; Chen et al. 2021).

Ergothioneine (**3**) is another important nonprotein amino acid, exogenous to humans (i.e., it must be supplied with the food we intake). Deficiency of this amino acid can be a risk factor for neurodegenerative diseases, among others. A good source of this compound is the fruiting bodies of edible mushrooms. The antioxidant and anti-inflammatory effects of *Agaricus bisporus* as well as many other edible mushrooms are due to the presence of this histidine derivative in their fruiting bodies (Chen et al. 2012). Ergotioneine also exhibits antimutagenic activity. Studies in the HR-1 mouse model (hairless mice) in vivo showed that ergothioneine (**3**) isolated from *Coprinus comatus* significantly reduced the amount of DNA damage and inhibited UV-B-induced inflammation. Furthermore, ergothioneine (**3**) shows chemoprotective and radioprotective activity (Asahi et al. 2016).

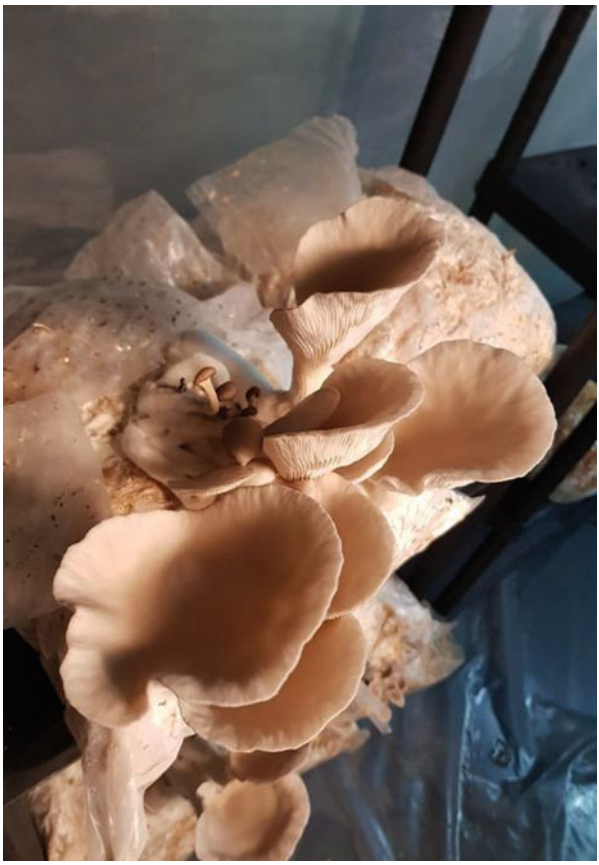


Fig. 11.2 *Pleurotus ostreatus* (Jacq. Fr.) Kummer (photo by Piotr Zięba)

On the other hand, *Imleria badia* fruiting bodies are a source of theanine (-γ-glutamylethylamide) (**4**), a neurotransmitter that stabilizes mood, reduces stress, and extends effective working time, to which it is due its popularity. It also has blood pressure-lowering effects, and helps fight obesity. Theanine (**4**), shows similar effects to synthetic anticancer substances such as irinotecan, doxorubicin or cisplatin, hence its effectiveness during cancer therapy (Roupas et al. 2012; Muszyńska et al. 2020). All of these characteristics mean that new, efficient sources for obtaining this compound are still being searched for. Chinese scientists in 2007 developed a way to obtain theanine (**4**), from *Imleria badia* mycelium by submerged fermentation (Roupas et al. 2012).

2.3 Glycoproteins: Lectins

The fruiting bodies of edible mushrooms have also been shown to be rich in biologically active proteins linked to polysaccharides (glycoproteins). Lectins are

glycoprotein-like compounds that are essential for the normal growth of mushroom fruiting bodies (Ismaya et al. 2020). They are also a protective factor for these organisms against toxins from the environment, such as pesticides, as well as bacteria and viruses. Mushroom lectins have the ability to selectively bind to membrane carbohydrates of various cell types, thus playing an important role in regulating the immune system. Lectins can affect cell adhesion, contribute to lymphocyte activation, and have potent antiproliferative properties. Mushrooms represent a huge and still poorly understood reservoir of lectins with potentially therapeutic properties (Hassan et al. 2015).

In the in vitro studies, they have been shown to inhibit the proliferation of cancer cells, such as breast cancer, without the accompanying cytotoxicity of lectins, which have been isolated from extracts from the fruiting bodies of *Agaricus bisporus* and the Chinese species *Tricholoma mongolicum*, among others (Singh et al. 2016; Singh et al. 2015). Derived from *Tricholoma mongolicum* fruiting body extracts, the lectins showed properties that activated mouse macrophages by affecting the production of nitric oxides, MAF and TNF, and effectively inhibited the growth of tumor cells (sarcoma) (Singh et al. 2015).

The effect of regulating cell proliferation by lectins from *Agaricus bisporus* has been studied as a therapeutic agent in the treatment of psoriasis (action on skin keratinocytes) and glaucoma and other eye diseases (against retinal epithelial cells) (Muszyńska et al. 2017; Ismaya et al. 2020).

Mushroom lectins have also been studied as compounds with therapeutic potential in the prevention and treatment of diabetes, due in part to their induction of pancreatic islet β-cell divisions. The study aimed to determine the extent of β-cell regeneration in mice and to understand the mechanisms of their proliferation. The analysis was based on measurements of glucose levels and the degree of insulin secretion after administration of mushroom lectins. By comparing the results, the effect of *Agaricus bisporus* lectins on reducing blood glucose levels, increasing glucose tolerance, and increasing pancreatic islet β-cell mass was proven (Wang et al. 2012). Immunoregulatory properties were found for lectins extracted from *Volvariella volvacea* fruiting body extracts, which stimulate Th-1 lymphocyte population in mice by inducing IL-2 and IFN-γ expression through a calcium ion-dependent pathway (Li et al. 2021).

2.4 Lipids

The lipids found in the fruiting bodies of edible mushrooms have been shown to support immunomodulatory processes in the human body, as they are composed of unsaturated fatty acids (Table 11.1) (D'Angelo et al. 2020; Bell et al. 2022). Polyunsaturated fatty acids (PUFAs) are precursors of eicosanoids, signaling molecules essential for the proper regulation of cellular processes in muscles, blood vessels, nerve cells, and the immune system. Eicosanoids have been found to provide homeostasis between inflammatory and anti-inflammatory processes

Fig. 11.3 *Imleria badia* (Fr.) Vizzini. (photo by Paweł Stasiowski)

(Dennis and Norris 2015). PUFAs include ω-3, ω-6 i ω-9 acids. It is now known that maintaining an adequate ratio of ω-3 to ω-6 fatty acids in the diet is key to preventing the development of cardiovascular disease or cancer (D'Angelo et al. 2020). The ω-6 acids (especially arachidonic acid) facilitate the production of prostaglandins, or proinflammatory hormones. In turn, ω-3 acids have an inhibitory effect on inflammation, as they compete with ω-6 acids in the body by reducing their concentration in tissues and limiting their reactions with enzymes. The α-linolenic acid (ALA) (**5**) is an essential ingredient for proper nutrition, a precursor of long-chain PUFA ω-3. Immunomodulatory effects have been demonstrated for this compound (Gdula-Argasińska et al. 2015; D'Angelo et al. 2020). In a study of the composition of extracts from several species of mushrooms, the following fatty acids were found to be the most common: linoleic (**5**) (C18: 2 ω-6), oleic (C18: 1 ω-9), and palmitic (**6**) (C16: 0) (Sande et al. 2019; D'Angelo et al. 2020).

In experiments using LPS-activated mouse RAW 264.7 macrophages, the anti-inflammatory effect of biomass extracts from in vitro cultures of *Imleria badia* was proven. The mechanism of this action, among others related to the high content of unsaturated fatty acids, involved inhibition of the expression of COX-2, prostaglandin E2 synthase (cPGES), p50 and p65 subunits of NF-κB. The biomass extract of *Imleria badia* (Fig. 11.3) had a particularly high content of ALA acid, up to 5%, which was not found in the fruiting bodies (Grzywacz et al. 2016).

Fatty acids were determined in extracts obtained from *Cantharellus cibarius* fruiting bodies, for which agonist activity against Peroxisome Proliferator-Activated Receptor γ (PPARγ) was tested. PPARγ receptors play a special role in lipid and carbohydrate metabolism, adipocyte differentiation, and regulation of inflammatory processes. PPARγ agonists have been shown to inhibit the development of insulin resistance and thus are effective antidiabetic agents, as well as exhibiting therapeutic effects for immune-mediated diseases, including inflammatory diseases and certain cancers (Hong et al. 2012). Palmitic acid (**6**), linoleic acid, and α-linolenic acid (**5**), found in the fruiting bodies of edible mushrooms such as *Agaricus bisporus*, may

have a protective effect against hormone-dependent breast cancer. The protective mechanism involves inhibiting the activity of the aromatase enzyme involved in the synthesis of estrogen (Muszyńska et al. 2017).

3 Secondary Metabolites: Significant Developments

3.1 Phenolic Compounds

Secondary metabolites found in the fruiting bodies of edible mushrooms with proven immunomodulatory, including antioxidant and anti-inflammatory properties are phenolic compounds (Table 11.1) (Friedman 2016; Kała et al. 2021; Bell et al. 2022). The mechanism of antioxidant action of these compounds is that they can be electron donors, so they neutralize reactive oxygen species protecting cells from damage. These compounds have also been shown to have the ability to chelate bioelements (e.g., Fe, Cu) that can generate reactive oxygen species. These compounds further inhibit the formation of free radicals by inhibiting enzymes, mainly from the oxidase group, such as cyclooxygenase, lipoxygenase, microsomal monooxygenase, NADH oxidase, protein kinase C, and S-glutathione transferase (Kumar et al. 2021). Many of the mentioned enzymes are involved in the inflammatory process. The content of phenolic compounds determined in various mushroom species and their antioxidant activity have been determined and described in the scientific literature (Reis et al. 2012; Muszyńska et al. 2013a, b; Sánchez 2016). These reports indicate a significant content of phenolic compounds in mushroom fruiting bodies and extracts derived from them. The most common phenolic compounds in mushrooms for which antioxidant activity has been demonstrated are gallic, protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, cinnamic, and coffee acids (Reis et al. 2012; Muszyńska et al. 2013a, b; Kała et al. 2021).

One of the first species studied in whose fruiting bodies phenolic compounds were demonstrated was *Imleria badia*, and the highest amounts of phenolic compounds such as *p*-coumaric acid (**10**), protocatechuic acid (**11**), and *p*-hydroxybenzoic acid, cinnamic acid (**12**) were found in them. The total content of phenolic compounds in this species was as high as 48.25 mg/kg d.w. In a study of the content of phenolic compounds in *Imleria badia* fruiting bodies and their antioxidant activity, it was found to be 99.2% in the linoleic acid oxidation test (Podkowa et al. 2021). On the contrary, the cultivated species *Agaricus bisporus* contains phenolic acids such as gallic, caffeic, ferulic, *p*-coumaric, and protocatechuic acids (**11**) in its fruiting bodies (Liu et al. 2013; Kała et al. 2017). A high content (about 15 mg/g d.w.) of one of the most active antioxidants in mushrooms, caffeic acid, is contained in species such as *Cantharellus cibarius*, *Agaricus bisporus*, *Boletus edulis* (Fig. 11.4), *Calocybe gambosa*, *Hygrophorus marzuolus*, *Lactarius deliciosus* (Reis et al. 2012; Muszyńska et al. 2013a, b).

Anti-inflammatory and antioxidant activity has been proven for caffeic acid. In vitro studies using cocultures of human vascular HUVEC cells and TNF-α-induced

Fig. 11.4 *Boletus edulis* Bull. (photo by Bożena Muszyńska)

U937 monocytes demonstrated the potential role of caffeic acid (**8**) in the treatment of inflammatory changes in cardiovascular disease. A significant reduction in the adhesion of monocytes to HUVEC cells was observed in cultures supplemented with caffeic acid. Furthermore, coffee acid inhibited the expression of IL-8, MPC-1 (chemoattractant protein 1), and NF-κB translocation to the cell nucleus in monocytes (Mei and Chen 2023).

Immunomodulatory and especially anti-inflammatory properties associated with the activity of phenolic compounds found in extracts derived from fruiting bodies of edible mushrooms: *Cantharellus cibarius*, *Hygrophorus marzuolus*, *Agaricus bisporus*, *Boletus edulis*, *Lactarius deliciosus* i *Pleurotus ostreatus* were tested in vitro using LPS-activated RAW 264.7 macrophages. The experiments showed an inhibitory effect of mushroom fruiting body extracts on the expression of inflammatory markers such as IL-6 and IL-1β and NO production. The greatest anti-inflammatory efficacy in the case study was demonstrated for *Cantharellus cibarius*, *Agaricus bisporus,* and *Lactarius deliciosus* species (Bhambri et al. 2022; El-Ramady et al. 2022). In another experiment, the anti-inflammatory properties of *Elaphomyces granulatus* species were proven. Inhibition of COX-2 activity in RAW 264.7 macrophages and antioxidant activity of the obtained extracts were found. The obtained effect was probably related to the content of phenolic compounds, most notably syringic acid (Elsayed et al. 2014).

3.2 Indole Compounds

Scientific studies have shown that edible mushrooms are also a rich source of precursors and neurotransmitters that are indole derivatives and exhibit antioxidant

and immunomodulatory properties, such as, for example, L-tryptophan (**13**), 5-hydroxy-L-tryptophan (**14**), serotonin (**17**), tryptamine (**16**), and melatonin (**15**) (Table 11.1) (Kała et al. 2017). These compounds have particularly strong effects on the immune and nervous systems of animals. Among the indole derivatives present in mushrooms, those with hallucinogenic effects (such as psilocybin) and those with nonhallucinogenic effects—L-tryptophan (**13**), 5-hydroxy-L-tryptophan (**14**), serotonin (**17**), or tryptamine (**16**) have been found (Muszyńska et al. 2014; Kała et al. 2017).

Mushrooms of the *Cantharellus cibarius* species contain a significant amount of serotonin, which is 17.61 mg/100 g d.w., as well as smaller amounts of L-tryptophan (**13**), 5-hydroxy-L-tryptophan (**14**), melatonin (**15**), 5-methyltryptophan, or indole and indole-3-acetonitrile. Studies have shown that the content of indole compounds in biomass from in vitro cultures can be much higher than in fruiting bodies (Muszyńska et al. 2016a). The content of nonhallucinogenic indole compounds (and their release into artificial digestive juices so as to confirm their potential bioavailability) was studied in the fruiting bodies of *Agaricus bisporus, Boletus edulis*, *Imleria badia*, *Lactarius deliciosus*, *Leccinum scabrum* (Fig. 11.5), *Suillus luteus*, *Suillus bovinus*, *Pleurotus ostreatus*, *Armillaria mellea,* and *Tricholoma equestre* (Kała et al. 2017).

In the fruiting bodies subjected to these experiments (release into artificial digestive juices), the following indole compounds were identified and determined: L-tryptophan (**13**), 5-hydroxy-L-tryptophan (**14**), melatonin (**15**), serotonin (**17**), tryptamine (**16**), and 5-methyltryptamine, indicating that these popular edible species are a good dietary source of them (Muszyńska et al. 2016b; Kała et al. 2017). It should be noted that in the human body, L-tryptophan (**13**) present in mushrooms undergoes numerous transformations and can be converted into other indole

Fig. 11.5 *Leccinum scabrum* (Bull.) Gray (photo by Bożena Muszyńska)

derivatives with high biological activity, such as serotonin (**17**), melatonin (**15**), and niacin (vitamin B_3) (Fukuwatari and Shibata 2013).

Melatonin (**15**), a broad-spectrum compound in the human body, is also an effective anti-immune disease agent by scavenging free radicals and protecting cells from damage. The compound also protects against the development of an inflammatory response. It has been shown that melatonin (**15**) can regulate cytokine production by preventing the translocation of NF-κB into the cell nucleus. Moreover, it can affect the reduction of damage that causes acute inflammation. This is due to the inhibition of the generation of adhesion molecules by leukocytes, affecting their migration process (Muszyńska et al. 2014). Melatonin (**15**) has also been shown to have a positive effect on the course of immune-mediated neurodegenerative diseases, such as dementia, Alzheimer's and Parkinson's diseases, and multiple sclerosis (Cardinali 2019). In an in vitro model, indole derivatives such as melatonin, acetylserotonin, and 6-methylxytryptamine have been shown to have additional properties that reduce lipid peroxidation (Muszyńska et al. 2014).

The idea that emotional state can affect immune system function can be explained, among other things, by the effects of serotonin. Serotonin (5-hydroxytryptamine) (**17**) is a neurotransmitter synthesized in the central nervous system and also in mast cells, platelets, and enterochromatophilic cells of the intestines. Associated mainly with the treatment of depression, serotonin also plays a role in regulating immune system cell activation and migration through its effects on the receptor pathway, as well as through the process of protein serotonylation (Shajib and Khan 2015). In LPS-activated monocytes, serotonin (**17**) has been shown to affect the activity of these cells, prevent apoptosis, and regulate the production of cytokines and chemokines (Herr et al. 2017). Changes in serotonin (**17**) levels have been observed in inflammatory bowel disease, rheumatoid arthritis, asthma, and allergies. Scientific research indicates that serotonin derivatives reduced allergic lung inflammation in a C57BL/6 mouse model by 70–90% (Abdala-Valencia et al. 2012). In addition, a decrease in allergen-induced transglutaminase 2 expression was observed, as well as a decrease in protein serotyping in the pulmonary endothelium and a decrease in leukocyte and eosinophil migration. Serotonin (**17**) addition to cell cultures was also shown to result in a decrease in TNF-α-induced protein serotonylation. This indicates an important role for serotonin and its derivatives in the process of leukocyte migration, which may be important in the treatment of allergic diseases (Abdala-Valencia et al. 2012).

3.3 Vitamins

Metabolic processes in the human body result in the production of free radicals, mainly reactive oxygen and nitrogen species, which in excess can lead to damage to cellular structures and the formation of immune diseases (Sánchez 2016; Kumar et al. 2021). Among the particularly active antioxidants contained in mushrooms are

also vitamins (especially B vitamins), ascorbic acid, carotenoids, tocopherols (**21**) (Table 11.1) (Bell et al. 2022; Gopal et al. 2022).

In scientific studies, vitamin composition was determined in five species of mushrooms *Termitomyces microcarpus*, *Termitomyces tyleranus*, *Termitomyces clypeatus*, *Volvariella speciosa,* and *Polyporus tenucuilus*, among others. The dried mushrooms were found to contain primarily folic acid, niacin, vitamin C, and thiamine, as well as small amounts of riboflavin, β-carotene, and α-tocopherol (**21**). However, pantothenic acid, biotin, and cobalamin were not detected in these species (Nakalembe et al. 2015). The significant content of B vitamins, such as thiamin, riboflavin, biotin, pyridoxine, was determined in fruiting bodies of *Agaricus bisporus* and *Cantharellus cibarius*. These species are also a good source of tocopherols, carotenoids, and vitamin C (Muszyńska et al. 2016a; Muszyńska et al. 2017).

Tocopherols, of which α-tocopherol (**21**) is the most active, exhibit anticancer, immunomodulatory, anti-inflammatory effects, and prevent the peroxidation of cell membrane phospholipids (Jiang 2014).

Essential for proper vision, carotenoids are also powerful antioxidants. Particularly rich sources of the carotenoids, β-carotene (**25**) and lycopene are the fruiting bodies of *Suillus bovinus* (fruiting body β-carotene content of about 15.26μg/g d.w.), as well as *Cantharellus cibarius* (Muszyńska et al. 2016a; Szwajkowska-Michałek et al. 2022).

Ergosterol (a precursor to vitamin D) (**18**), ergocalciferol (a precursor to vitamin D_2) (**20**), and other sterols (including cerevisterol, ergosta-7,22-dienol, ergosta-5,7-dienol, ergosta-7-enol, β-sitosterol, ergosterol peroxide (**19**), 7-dehydrostigmasterol) have been detected in many mushroom species. Among other things, the fruiting bodies of *Agaricus bisporus* are a rich source of ergosterol (**18**) (about 61.5 mg/100 g d.w.) and ergocalciferol (**20**) (Muszyńska et al. 2017).

Cantharellus cibarius is also a good source of vitamin D, with ergocalciferol (**20**) content even after prolonged storage of dried specimens in the range of 0.12–6.3μg/g d.w. (Jiang 2014; Muszyńska et al. 2016a). Furthermore, exposure of mushrooms to UV-B and UV-C irradiation has been found to allow them to increase their vitamin D_2 content, which has proven immunomodulatory and immunosupporting effects (Strange 2015; Drori et al. 2016). The latest research on vitamin D points to its widespread effects in the human body. Its deficiency has been associated with the development of hypertension, metabolic syndrome and diabetes, inflammatory bowel syndrome, and some types of cancer (Strange 2015; Álvarez-Mercado et al. 2023). It is indicated that even more than half of the world's population suffers from a deficiency of this vitamin (Stepien et al. 2013).

One of the mechanisms of the immunomodulatory action of ergosterol (**18**) and its derivatives is the inhibition of NF-κB translocation to the cell nucleus and therefore the prevention of the expression of proinflammatory genes (Drori et al. 2016). Ergosterol (**18**), present in the fruiting bodies of edible mushrooms (e.g., *Imleria badia* and *Agaricus bisporus*), exhibits anti-inflammatory and anticancer effects (Szwajkowska-Michałek et al. 2022). An ergosterol-rich extract derived from *Lentinula edodes* also showed anti-inflammatory properties in a mouse in vitro

model. In C57B1/6 mice with concanavalin A-induced liver inflammation, supplementation with *Lentinula edodes* extract enriched with vitamin D via UV-B radiation resulted in a significant reduction in liver damage. An improvement in the histopathological appearance of the liver was demonstrated, as well as a decrease in the plasma levels of INF-γ and aminotransferases. In addition, the anti-inflammatory effect of significant amounts of vitamin D and mushroom extract was found to be synergistic (Drori et al. 2016). In another experiment, supplementation with powdered *Agaricus bisporus* fruiting bodies enriched with vitamin D_2 after 4 weeks was shown to contribute to a significant reduction in levels of hsCRP protein, a specific information and marker of inflammation in humans (Stepien et al. 2013). Ergosterol peroxide (**19**) mushroom origin suppressed LPS-induced TNF-α secretion and IL-1α/β expression in RAW 264.7 cells. Ergosterol peroxide (**19**) and ergosterol (**18**) suppressed LPS-induced DNA-binding activity of NF-κB and C/EBPβ, and inhibited the phosphorylation of p38, JNK, and ERK MAPKs. Ergosterol peroxide downregulated the expression of low-density lipoprotein receptor (LDLR) regulated by C/EBP, and HMG-CoA reductase (HMGCR) in RAW 264.7 cells (Yuan et al. 2019).

3.4 Isoprenoids

A group of mushroom compounds with significant immunomodulatory activity are isoprenoids (Table 11.1), for this reason there is a great amount of scientific work on the acquisition of new active terpenoid compounds from medicinal mushroom species (Yaoita et al. 2014). Among the triterpenoids obtained from extracts of *Ganoderma lucidum* fruiting bodies, more than 100 different compounds have been identified so far, of which about 50 are characteristic only of this species. These are mainly ganodermic and lucidenic acids, as well as ganoderols and ganoderiols. The anti-inflammatory effect of triterpene extracts from this species has been demonstrated in LPS-induced macrophages. The extract contributed significantly to the downregulation of COX-2 expression and inhibition of prostaglandin E2 production in RAW 264.7 cells. As immunomodulatory activity, the study observed reduced iNOS activity and decreased NO secretion, as well as reduced TNF-α and IL-6 secretion. The mechanism of anti-inflammatory action of the tested extracts was based on inhibition of NF-κB and AP-1 signaling pathways. It is noteworthy that terpenoids from *Ganoderma lucidum* show antibacterial and antiviral activity against Epstein-Barr or HIV-1 viruses (Wu et al. 2019b).

Compounds from the terpenoid group with immunomodulatory, including anti-inflammatory, antiproliferative, and anticancer potential were also isolated from other arboreal mushrooms (Sułkowska-Ziaja et al. 2018). One of them is *Poria cocos*. The anti-inflammatory activity of extracts from this species of mushroom was demonstrated in an in vitro model using RAW 264.7 macrophages. A decrease in the generation of proinflammatory mediators was observed through inactivation of the NF-κB signaling pathway (Jeong et al. 2014). In contrast, the anticancer activity of

mushroom terpenoids was tested on the U937 line. Concentration-dependent and time-dependent significant antiproliferative and proapoptotic effects of the tested extracts were shown to be associated with release of cytochrome C into the cytosol, degradation of PARP, activation of caspases -3, -8, and -9, and loss of mitochondrial membrane potential. The results show the potential of *Poria cocos* in the treatment of leukemia, which belongs to hematopoietic malignancies (Choi 2015). A number of triterpenes with similar inhibitory effects on the NF-κB signaling pathway have been isolated from another species, *Inonotus obliquus* (Ma et al. 2013). An example is inotodiol (**24**) that induces a characteristic type of maturation in DCs through phosphatidylinositol-3-kinase activation independent of NF-κB, and inotodiol (**24**)-treated DCs enhance T cell proliferation and IL-2 secretion (Maza et al. 2021). *Antrodia camphorata* extracts, on the other hand, inhibit the proliferation of cancer cells, and have been shown to reduce NO, TNF-α, and IL-12 (Long et al. 2019).

3.5 Bioelements

Mushrooms show a high ability to accumulate bioelements from the substrate, so they can be a valuable source of them in the diet. Among the bioelements with immunomodulatory, antioxidant, and anti-inflammatory properties accumulated by mushrooms are zinc, copper, iron, and selenium, among others (Reczyński et al. 2013; Brzezicha-Cirocka et al. 2019). The fruiting bodies of such popular mushrooms as *Boletus edulis*, *Cantharellus cibarius*, *Agaricus bisporus,* and *Imleria badia* are rich sources of these elements (Reczyński et al. 2013; Muszyńska et al. 2015). A micronutrient found in mushrooms that has important immune system-supporting effects is zinc (Reczyński et al. 2013; Muszyńska et al. 2015). The study also showed good accumulation of zinc(II) ions in biomass from in vitro cultures (Fig. 11.6) from media enriched with this element (Reczyński et al. 2013; Muszyńska et al. 2015).

Zinc is a cofactor for about 300 enzymes in the human body, and also exhibits immunoregulatory and antioxidant activity. Zinc's role in antioxidant processes is related to the regulation of superoxide dismutase (SOD) activity and the regulation of gene expression (including metallothioneins), which effectively bind free radicals (Grzywacz et al. 2015). It is known that this element can affect both the specific and nonspecific immune response of the body. Zinc is a micronutrient essential for the proper functioning of all types of immune cells, influencing the proliferation, differentiation, and proper function of neutrophils, macrophages, NK cells, and T and B lymphocytes. It also ensures a proper balance between Th1 and Th2 lymphocyte function by regulating the secretion of cytokines, the primary signaling elements in the immune system (Prasad 2014; Grzywacz et al. 2015). Disruption of zinc homeostasis or chronic zinc deficiency contributes to weakened immunity, increases the production of proinflammatory cytokines, and may influence the transition of inflammation into a chronic state affecting the organism negatively (Prasad 2014).

Fig. 11.6 Submerged in vitro mycelial cultures (photo by Katarzyna Kała)

The results show anti-inflammatory effects of *Cantharellus cibarius* biomass extracts from zinc-enriched in vitro cultures. A decrease in proinflammatory proteins COX-2 and cPGES was observed in A549 lung epithelial cells activated with LPS and supplemented with the extracts (Gdula-Argasińska et al. 2018). Other scientific studies have shown anti-inflammatory and antioxidant effects of *Imleria badia* biomass extracts extracted from zinc(II) enriched media in RAW 264.7 macrophages. Cells incubated with mushroom extracts and activated with LPS showed a significant decrease in the expression of cPGES, NF-κB p50, COX-2, and NF-κB p65 proteins and an increase in the expression of GSTM1 (Grzywacz et al. 2016).

Edible mushrooms are one of the best dietary sources of selenium (Dogan et al. 2016). The selenium content of fruiting bodies ranges from about 0.5 to 20 mg/kg d. w., although some species contain much more, for example, selenium content of 400 mg/kg d.w. of fruiting bodies was found in the fruiting bodies of *Albatrellus prescaprae* (Dogan et al. 2016; Kora 2020). The selenium content in the fruiting bodies of *Agaricus bisporus*, on the other hand, is lower, at up to 0.150 mg/kg d.w. (Maseko et al. 2014; Dogan et al. 2016). This bioelement is essential for the proper functioning of the immune system. Selenium in association with proteins forms selenoproteins involved in the normal differentiation and proliferation and activation of immune cells, thereby influencing the innate and adaptive immune response (Avery and Hoffmann 2018). Selenium's immunoregulatory function is also manifested through its effects on key leukocyte functions, i.e., such as phagocytosis, adhesion, and migration, as well as cytokine secretion, which may be important in autoimmune diseases. Selenoproteins also play a role in antioxidant processes in cells. Selenium is an important factor in the struggle against free

radicals, due in part to its presence in the structure of superoxide dismutase (SOD) or glutathione peroxidase (Huang et al. 2012; Maseko et al. 2014; Avery and Hoffmann 2018).

Copper, like zinc, is a cofactor for many enzymes, including those with protective effects against oxidative stress. These enzymes include superoxide dismutase (SOD) and ceruloplasmin, a major serum oxidase that plays an important role in copper transport and iron homeostasis. Copper has been shown to play an important role in regulating inflammatory processes (Isaev et al. 2020). Mushroom species such as *Lycoperdon perlatum* and *Macrolepiota procera* stand out for their high content of this bioelement (Brzezicha-Cirocka et al. 2019). Certain amounts of this bioelement were also detected not only in fruiting bodies, but in the mycelium of species *Agaricus bisporus*, *Lentinula edodes*, or *Cantharellus cibarius* (Kała et al. 2021).

4 Conclusions

The composition of substances in the fruiting bodies of edible mushrooms obtained from the natural state depends on factors such as climatic conditions, environmental conditions, pollution, and weather occurring during the growth period, the growth stage of the mushrooms and also the way they are stored and processed (Muszyńska and Sułkowska-Ziaja 2012; Muszyńska et al. 2013a, b; Nakalembe et al. 2015). Mushrooms of the same species, from different populations and sites can differ significantly in the content of individual bioactive compounds of health-promoting importance. For example, *Cantharellus cibarius* harvested in Poland contained *p*-hydroxybenzoic, protocatechuic (**11**), sinapic, cinnamic acids in amounts ranging from 1.29 to 3.32 mg/kg d.w. In contrast, *Cantharellus cibarius* from Spain showed contents of caffeic (**8**), gallic (**9**), *p*-hydroxybenzoic, ferulic, homogentistic, protocatechuic (**11**), pyrogallol, myricetin, and catechin acids in amounts ranging from 5.82 to as much as 316.76 mg/kg d.w. for homogentistic acid (Muszyńska et al. 2016a).

It is worth noting that the described properties are often determined for alcoholic extracts, while under the conditions of including mushrooms in the diet we still have limited data. As a result of processing and storage of mushrooms, the amount of phenolic compounds, vitamins or free amino acids can significantly decrease. For example, in the case of fruiting bodies of *Agaricus bisporus*, a reduction of up to 90% in free amino acids was observed during 6 months of storage (Muszyńska et al. 2017). Nevertheless, the medicinal properties of mushrooms even after processing are very important. Mushroom lectins, on the other hand, appear to be stable compounds during processing. The effects of heating, freezing, acidification, alkalinization, and drying simulating culinary processing on the activity of lectins from *Agaricus bisporus* and *Auricularia polytricha*, were studied, among others. These treated lectins were shown to retain the ability to induce TNF-α and NO synthesis and exhibit stable immunostimulatory effects in RAW264.7 cells. This demonstrates the strong therapeutic potential of mushrooms (Ditamo et al. 2016). The effect of

heat treatment on the content of indole compounds in edible mushroom species such as *Armillaria mellea*, *Imleria badia*, *Boletus edulis*, *Cantharellus cibarius*, *Lactarius deliciosus,* and *Pleurotus ostreatus* was also studied. A decrease of about 48% in 5-hydroxy-L-tryptophan (**14**) content was observed for *Auricularia polytricha* species compared to nonheat-treated samples. Indole compounds still detectable after thermal processing included L-tryptophan (**13**) or serotonin (Muszyńska and Sułkowska-Ziaja 2012; Muszyńska et al. 2013a, b). Blanching (exposure to boiling water for a short time) of *Pleurotus ostreatus* fruiting bodies did not significantly affect the content of β-glucans, while the content of phenolic compounds and thus their activity decreased significantly. However, the experiment showed that some of the active phenolic compounds remained in the water used, which may be a guide for consumers to use it in further processing (Lam and Okello 2015).

Summarizing the data discussed, it is important to be aware that the medicinal properties of mushrooms, including their immunomodulatory effects, are influenced by a wide variety of factors. These include environmental factors, but also include how the mushrooms are prepared for consumption and how they are stored. The basic nutrients contained in mushrooms and their secondary metabolites exhibit multifaceted beneficial effects on immune function. This is primarily antioxidant activity (Kumar et al. 2021). Some chemical compounds in mushrooms affect the proliferation and differentiation of lymphocyte populations, as well as the migration and adhesion of these cells. Other compounds inhibit the synthesis of inflammatory mediators, i.e., cytokines, interleukins, prostaglandins or nitric oxide, by inhibiting proinflammatory signaling pathways associated with the nuclear receptor NF-κB (Jakopovic et al. 2021).

In conclusion, mushroom fruiting bodies are a source of primary and secondary metabolites and biologically active bioelements, the properties of which we are only beginning to discover. Recent reports indicate that edible mushroom extracts have beneficial health-promoting and therapeutic effects, including, in particular, immune-mediated diseases (Rathore et al. 2019; Kumar et al. 2021).

Declaration of Competing Interest The authors declare that they have no competing financial interests.

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Chapter 12
New Antifungal Drugs: Discovery and Therapeutic Potential

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Abstract There has been an increase in invasive fungal infections, which are a universal problem due to the longer average lifespan, the number of immunocompromised patients associated with high morbidity/mortality rates, and the appearance of resistance to antifungals. In addition, certain fungal infections that emerged during the COVID-19 pandemic reinforced the action of searching for new antifungal alternatives. One example is the fungus *Aspergillus fumigatus* for which mortality reached 80% among severe COVID-19 patients infected with it. Despite the urgent need for more antifungal drug options, no new classes of antifungal drugs have become available over the last two decades. Current antifungal therapies have many limitations: poor oral bioavailability, narrow therapeutic indexes, and emerging drug resistance, and it is essential to discover and develop novel drugs, which can overcome these limitations and be added to the antifungal armamentarium.

This review is an extension of another one published in 2016, and it covers the discovery and potential of the most promising antifungal agents developed since then: **fosmanogepix** (**1**) (a novel Gwt1 enzyme inhibitor), **ibrexafungerp** (an enfumafungin derivative, the first representative of a novel class of structurally distinct glucan synthase inhibitors, and triterpenoids), **rezafungin** (an echinocandin designed to be given as a weekly dose), **encochleated amphotericin B** (CAMB), **oteseconazole** (**VT-1161**), **VT-1598**, **VT-1129** and **opelconazole PC945**, (novel tetrazole-specific Cyp51 inhibitors), and **olorofim** (a novel dihydroorotate dehydrogenase enzyme inhibitor). These new drugs, as well as new agents developed as new formulations of existing antifungal classes, offer advantages over current therapies and new mechanisms of action for overcoming resistance, improving safety profiles, reducing interactions, and improving the potential role of these drugs in relation to unmet needs in the future.

Keywords Fungi · Infections · Armamentarium · Immunocompromised · Mortality

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1 Introduction

Invasive fungal infections, including those caused by yeasts and molds, can be significant causes of morbidity and mortality in immunocompromised hosts, in those with multiple comorbidities, and occasionally in immunocompetent hosts. Invasive fungal infection carries a high morbidity, mortality, and economic cost. In recent times, there has been a rising incidence of fungal infection and antifungal resistance, and the current antifungal agents are often limited by drug toxicities and drug interactions, both of which have prompted the development of novel antifungal agents (Stewart and Paterson 2021).

Due to the warmer temperature caused by climate change worldwide, fungi may become more of a health risk. This is because fungi classified as pathogenic could undergo mutations, which might presumably make them more resistant to heat or give them greater disease-causing potential, leading to more serious infections as the temperatures rise. This association between increased temperature and pathogenicity raises concerns about new infectivity. Heat stress favors and increases the number of genetic mutations (Gusa et al. 2023).

In view of all these effects, the antifungal drug development pipeline will need to respond to a growing need for new agents to effectively treat fungal disease without concomitant toxicity or drug tolerance issues. In this review, we have selected several antifungal agents in development for which there are human data from phase 1, phase 2, and/or phase 3 clinical trials. They show great promise in offering clinicians better agents to treat these difficult infections. The agents, mechanism of action, susceptibility data, and stage of development of all these new antifungals are summarized below (Kim et al. 2022).

2 Target Fungal Cell Wall and Membrane Synthesis

2.1 Fosmanogepix/Manogepix (A Novel Gwt1 Enzyme Inhibitor, AMPLYX)

Manogepix (MGX, APX001A, Amplyx Pharmaceuticals Inc., this company now belongs to Pfizer, Inc.), formerly discovered by Eisai Co., Ltd., as E1210 is the active moiety of the N-phosphonooxymethyl prodrug fosmanogepix (**1**) (Fig. 12.1) (APX001) with broad clinical activity against yeasts and molds (Nakamoto et al. 2010; Miyazaki et al. 2011). Following oral or intravenous administration, systemic phosphatases rapidly convert fosmanogepix into manogepix (Shaw and Ibrahim 2020). This compound is a first-in-class antifungal that blocks glycosylphosphatidylinositol (GPI) production by targeting the highly conserved fungal enzyme **GPI-anchored wall transfer protein 1** (Gwt1). The GPI compounds are important for the construction of the cell wall and the maintenance of homeostasis via a highly conserved pathway. (McCarty and Pappas 2021). GPI-anchored

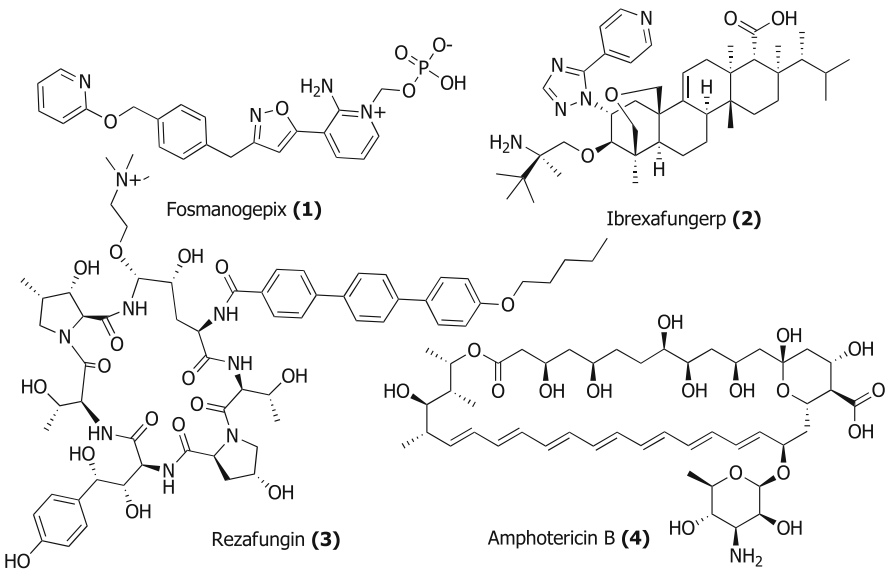

Fig. 12.1 Target fungal cell wall and membrane synthesis inhibitors in development

proteins are found in eukaryotic organisms, playing a crucial role in fungal adhesion to the host cells (Hoenigl et al. 2021b).

2.1.1 Mechanism of Action

The mechanism of action of MGX is novel as it inhibits Gwt1 (an inositol acyltransferase), which prevents proper localization of mannoproteins, which are essential for cell wall integrity and fungal growth (Mota Fernandes et al. 2021; Watanabe et al. 2012). In addition, manogepix appears to be fungal Gwt1-specific, but does not prevent the inositol acylation of GPI in human cells, suggesting that the compound is selective toward fungal cells (Watanabe et al. 2012).

2.1.2 Spectrum of Activity

Fosmanogepix/manogepix has broad-spectrum activity against a large array of yeast and filamenting fungi. Potent in vitro activity has been reported against *Candida* spp., including isolates of *C. albicans, C. auris,* and *C. glabrata* that are resistant to the azoles and echinocandins. However, a lack of in vitro activity against *C. krusei* has been described for this compound (Pfaller et al. 2011; Arendrup and Jorgensen 2020; Zhu et al. 2020).

In addition, activity has also been reported against *Cryptococcus neoformans* and *C. gattii, Coccidioides* spp., *Aspergillus* spp., including azole-resistant *A. fumigatus, Fusarium* solani, *Scedosporium prolificans*, *Lomentospora prolificans*, *Pseudallescheria boydii,* and other rare molds (Jorgensen et al. 2020; Rivero-Menendez et al. 2019a; Badali et al. 2021). This effect of activity on microorganisms other than *Candida* yeast is very significant in comparison with the echinocandins, which lack activity against non-*Candida* yeasts (Miyazaki et al. 2011; Arendrup et al. 2018b). In contrast, the activity observed against members of the Mucorales family is limited (Rivero-Menendez et al. 2019a).

2.1.3 Mouse Model Studies

The data obtained from mouse model experiments of both oropharyngeal and disseminated candidiasis performed with APX001 and APX001A showed high rates of survival and reduced CFU levels of fungi in lung, kidney, and brain, even against azole-resistant strains (Zhao et al. 2018; Shaw et al. 2018). These data were an excellent basis to support their clinical development and for performing clinical trials.

In a comparison of fosmanogepix with anidulafungin in an immunocompromised mouse model of a disseminated disease study, better activity was observed with fosmanogepix (Hager et al. 2018a). The activity of this new compound against *Cryptococcus* spp. in a cryptococcal meningitis mouse model is equivalent to that shown by fluconazole (Shaw et al. 2018).

In addition, a combination of manogepix with liposomal amphotericin B in a mouse model showed a strong synergistic effect reducing lung fungal burden and improving survival in invasive pulmonary aspergillosis and reducing both lung and brain fungal burden and improving survival in mucormycosis (Ibrahim et al. 2021).

Moreover, recent work has demonstrated activity of this compound in mouse models against *Rhizopus delemar* (Gebremariam et al. 2017a) and both in vitro and in vivo activity against *Rhizopus arrhizus/oryzae*, a frequent cause of mucormycosis in humans (Gebremariam et al. 2020).

2.1.4 Clinical Studies

Clinical studies of fosmanogepix show that it was well-tolerated when administered orally or intravenously in clinical phase 1 studies, and it was fast-tracked by the US FDA in September 2019 for seven invasive fungal infections, including candidiasis, aspergillosis, scedosporiosis, fusariosis, mucormycosis, cryptococcosis, and coccidioidomycosis. The phase 1 studies examined single- and multi-dose ascending regimens. Doses were well-tolerated, and no significant adverse effects were observed (Hodges et al. 2017).

Fosmanogepix is currently in phase 2 clinical trial for invasive candidiasis in humans (Amplyx Pharmaceuticals 2020). The phase 2 study was a single-arm proof-of-concept study in candidemia (Pappas et al. 2020). There were no severe adverse treatment-related events and no discontinuations due to adverse effects. There is a similar ongoing similar phase 2 study for *Aspergillus* and rare molds, and a phase 3 candidemia study is in development.

2.2 *Ibrexafungerp (An Enfumafungin Derivative, Glucan Synthase Inhibitor, Scynexis)*

Ibrexafungerp (**2**) (Fig. 12.1) (MK-3118 and SCY-078; developed by Scynexis, Jersey City, NJ, USA) is the first representative of a novel class of structurally distinct oral glucan synthase inhibitors called triterpenoids, which are semisynthetic derivatives of enfumafungin natural product lead (Peláez et al. 2000).

The development of this natural product presented different challenges, but thanks to the scientist's perseverance that was able to resolve fermentation, potency, oral bioavailability, and other pharmacology issues and the synthesis of the new derivative ibrexafungerp (Apgar et al. 2021). The compound is structurally similar to the echinocandins and is an inhibitor of β-1,3-glucan synthase but with a different binding site from that of the echinocandins, but with only some overlap in the binding region (Apgar et al. 2021).

In June 2021, SCYNEXIS announced FDA approval of BREXAFEMME® (ibrexafungerp tablets) as the first and only oral non-azole treatment for vaginal yeast infections.

2.2.1 Mechanism of Action

Similarly to the echinocandins, ibrexafungerp disrupts fungal cell wall synthesis inhibiting the biosynthesis of (1 → 3)-β-D-glucan with fungicidal activity against *Candida* spp. and fungistatic activity on *Aspergillus* spp. (Bowman et al. 2002; Ghannoum et al. 2018). Ibrexafungerp is a semisynthetic derivative of the naturally occurring hemiacetal triterpene glycoside enfumafungin, which incorporates a pyridine triazole at position 15 of the core phenanthropyran carboxylic acid ring system and a 2-amino-2,3,3-trimethyl-butyl ether at position 14 to enhance its antifungal potency and pharmacokinetic properties. Therefore, this new derivative compound is the first to be developed in the novel class of triterpenoid antifungals (Wring et al. 2017). Ibrexafungerp has some advantages compared to echinocandins, such as oral bioavailability, broad activity against pan-resistant *C. auris,* and maintaining activity against most echinocandin-resistant *Candida* spp. (Jacobs et al. 2022).

Although the mechanism of action for ibrexafungerp and echinocandins is similar, the binding sites for both antifungal drugs are different with only a partial

overlap. This results in very limited cross-resistance between ibrexafungerp and echinocandins (Pfaller et al. 2013; Jimenez-Ortigosa et al. 2017). Even, ibrexafungerp has potent activity against echinocandin-resistant *C. glabrata* isolates with FKS mutations (Jimenez-Ortigosa et al. 2017; Nunnally et al. 2022).

2.2.2 Spectrum of Activity/in Vivo Susceptibility Data

In vitro studies with ibrexafungerp have demonstrated activity against *Aspergillus* and *Candida* spp., with slightly weaker activity against *C. lusitaniae and C. krusei* compared to another *Candida* spp. Ibrexafungerp shows high activity against *Candida* isolates that are resistant to azoles (e.g., *C. albicans*, *C. krusei*, and *C. glabrata*), but activity against echinocandin-resistant *Candida* spp. is variable (Schell et al. 2017b). In addition, this compound has shown activity against several resistant strains of *Candida auris* (Schell et al. 2017a; Ghannoum et al. 2020), and even *C. auris* biofilms treated with ibrexafungerp show reduced metabolic activity and thickness as compared to untreated control biofilms (Larkin et al. 2017).

Ibrexafungerp has also shown in vitro activity against *C. parapsilosis*, which is known to have elevated echinocandin minimal inhibitory concentrations (MICs). This is comparable to or better than that of the echinocandins (Nunnally et al. 2019). The modal MIC of ibrexafungerp against *C. parapsilosis* is also similar to the modal MIC observed against other common *Candida* species causing clinical disease, *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* (Pfaller et al. 2017a).

Ibrexafungerp has fungistatic activity against *Aspergillus* spp. growth (MEC range $<$ 0.06 to 4 μg/mL) (Ghannoum et al. 2018) and so it is a potent inhibitor of *A. fumigatus*, *A. niger*, *A terreus*, cryptic species (Rivero-Menendez et al. 2021), and azole-resistant strains (Ghannoum et al. 2018; Pfaller et al. 2013; Jimenez-Ortigosa et al. 2014). The compound does not show significant activity against other important fungal pathogens such as *Fusarium* for the Mucorales, but it is very active against *Alternaria* and *Cladosporium* spp. (Lamoth and Alexander 2015; Ghannoum et al. 2020). It is also active against *Pneumocystis jirovecii,* but this has only been demonstrated in animal models, not in human trials. It does retain activity against *Candida* FKS-1 and FKS-2 mutations including those demonstrated in certain strains of *Candida glabrata* (Nunnally et al. 2019).

Regarding mouse studies, in an invasive candidiasis model with WT and echinocandin-resistant (ER) *C. glabrata*, ibrexafungerp significantly reduced kidney fungal burden in both groups as compared to the placebo. Reduced tissue fungal burden and improved survival with ibrexafungerp versus control were also observed in immunocompromised mice with disseminated *C. auris* (Ghannoum et al. 2019). In a murine model of disseminated aspergillosis, treatment with ibrexafungerp led to a significant reduction in *Aspergillus* kidney burden and serum galactomannan (GM) levels and improved survival as compared to the control (Borroto-Esoda et al. 2017).

The combination of ibrexafungerp and isavuconazole also demonstrates synergy in a neutropenic rabbit model of experimental invasive pulmonary aspergillosis. As compared to isavuconazole alone, mice treated with ibrexafungerp and isavuconazole had significantly improved survival, decreased pulmonary infarct scores, and diminished serum GM levels (Petraitis et al. 2020). Ibrexafungerp is also efficacious in a murine model of *Pneumocystis murina* pneumonia, in which reductions in asci burden and improvements in survival were similar to those of trimethoprim–sulfamethoxazole and significantly better than in untreated controls (Barat et al. 2019).

2.2.3 Clinical Development

There are several completed and ongoing phase 2 and phase 3 clinical trials with ibrexafungerp.

In a first published study (phase 2) of a multicenter clinical trial, 27 patients with invasive candidiasis were randomized to receive step-down therapy in one of three treatment arms: two dosing regimens of ibrexafungerp (1000 mg loading dose followed by 500 mg daily or 1250 mg loading dose followed by 750 mg daily) or standard of care (SOC) following initial echinocandin therapy for 3 to 10 days. The study achieved its main objective of providing target exposure to ibrexafungerp in over 80% of subjects; the compound also demonstrated excellent efficacy with no dose-limiting adverse events (Spec et al. 2019). Similar rates of adverse events were observed across study arms (vomiting and diarrhea) and did not require drug discontinuation.

In the DOVE Trial, a second multicenter trial, women with acute vulvovaginal candidiasis (VVC) were randomized to receive ibrexafungerp 300 mg bid or a single-dose fluconazole 150 mg. Overall, 51 subjects received ibrexafungerp and 24 received fluconazole. At day 10, the clinical cure rates for ibrexafungerp and fluconazole were 51.9% and 58.3%, respectively; at day 25, patients with no signs or symptoms were 70.4% and 50.0% among ibrexafungerp and fluconazole recipients, respectively (Angulo et al. 2019). Ibrexafungerp was well-tolerated, with the most common treatment-related adverse events being mild gastrointestinal discomfort.

Ibrexafungerp has also been studied for the treatment of VVC. Day 10 and day 25 clinical cure and mycological eradication rates were similar or improved with ibrexafungerp 300 mg twice daily × 2 doses compared to fluconazole 150 mg × 1 dose. Diarrhea was the most common adverse event in the ibrexafungerp arm, observed in 10% of subjects (Cadet et al. 2019).

In a phase 3 open-label, single-arm study of ibrexafungerp in patients with refractory or intolerant fungal diseases (FURI; NCT03059992). An interim analysis was performed on 20 patients with proven or probable invasive candidiasis ($N = 11$) or severe mucocutaneous candidiasis ($N = 9$). Eleven (55%) patients achieved a complete or partial response, and six (30%) had stable disease. The most common treatment-related adverse events were gastrointestinal (Cornely et al. 2019).

In an ongoing open-label clinical trial, (CARES; NCT0336381), with ibrexafungerp in India and the United States for treatment of *Candida auris* infection, two patients enrolled have been reported so far so successfully treated with ibrexafungerp (Juneja et al. 2019). Other clinical trials (NCT03672292) including a multicenter, randomized, double-blind study in patients with invasive pulmonary aspergillosis to evaluate the efficacy and safety of ibrexafungerp and voriconazole are being performed (Juneja et al. 2019).

In summary, the FURI outcomes for ibrexafungerp by fungal disease type demonstrated a favorable therapeutic response for the majority of patients with a variety of serious fungal infections for whom current treatments have failed or who are intolerant to them, or whom currently available oral antifungals are not adequate due to resistance and IV therapy is no longer feasible or desirable. The data presented include 113 patients enrolled in the FURI study who had completed treatment through October 2021. Of these patients, the most common fungal disease was invasive candidiasis/candidemia (IC) in 56 (49.6%), followed by severe mucocutaneous candidiasis in 32 (28.3%), refractory vulvovaginal candidiasis (VVC) in 14 (12.3%), and invasive aspergillosis (IA) in 11 (9.7%). Complete or partial response was reported in 62.5% of patients with IC, with an additional 23.2% reporting stable disease. For patients with severe mucocutaneous candidiasis, 53.1% reported complete or partial response, and 34.4% reported stable disease. Most subjects with refractory VVC also responded favorably, with 71.4% achieving clinical improvement. Six of 11 patients with invasive aspergillosis achieved complete/partial response or stable disease.

Finally, there is an ongoing phase 2 combination study (SCYNERGIA Study) of ibrexafungerp plus voriconazole versus voriconazole monotherapy for treatment of invasive pulmonary aspergillosis (McCarty and Pappas 2021). During a current European study, it was observed that hospital stay was prolonged because of parenteral therapy in 21% of cases with invasive *Candid*a infections (Hoenigl et al. 2021b) owing to a lack of reliable oral alternatives.

However, ibrexafungerp will fill the gap and will likely be used for primary and oral step-down therapy of invasive *Candida* infections. In addition, it is an oral antifungal drug with limited contraindications, resistance, or potential drug–drug interactions as well as high tissue penetration and broad and fungicidal activity particularly against yeasts, including *C. auris*. Moreover, in the case of invasive pulmonary aspergillosis, it may have a role as therapy for resistant cases, particularly in combination therapy with an azole or amphotericin B lipid formulations (Hoenigl et al. 2021a).

Therefore, the FDA granted Qualified Infectious Disease Product (QIDP) and Fast Track designations for the oral and IV formulations of ibrexafungerp for the indications of invasive candidiasis (IC), including candidemia, and invasive aspergillosis (IA) and has granted Orphan Drug Designation for the IC and IA indications. The European Medicines Agency (EMA) has granted ibrexafungerp Orphan Medicinal Product designation for the indication of IC. Ibrexafungerp was formerly known as SCY-078.

2.3 *Rezafungin (Second-*Generation *Echinocandins, CIDARA)*

Rezafungin (**3**) (Fig. 12.1) (formerly, SP3025 and CD101; Cidara Therapeutics, San Diego, CA, USA) is considered the first member of second-generation echinocandins with enhanced PK/PD pharmacometrics (Thompson III et al. 2021), which arose from an iterative search to identify novel echinocandin that offered alternative dosing regimens to already approved agents (James et al. 2017). While rezafungin does not add new utility when choosing empiric therapy, it does have the potential to expand treatment options in the context of echinocandin resistance, especially with respect to *C. glabrata* (Beyda et al. 2012; Alexander et al. 2013; Pfaller et al. 2019).

The unique pharmacokinetic properties allowing for weekly, rather than daily, dosing also afford a treatment option that does not require an indwelling catheter, in situations where fluconazole is either contra-indicated or ineffective. This novel drug is currently being investigated in two phase III trials (ReSTORE, NCT03667690, and ReSPECT, NCT04368559) to assess its therapeutic use in candidemia and other invasive candidiasis and its potential to prevent invasive fungal disease (IFD) (McCarty and Pappas 2021).

2.3.1 Mechanism of Action

Rezafungin is a novel agent in the echinocandin antifungal drug class that inhibits the cell wall enzyme complex (1 → 3)-β-D-glucan synthesis. Rezafungin is a structural analog of anidulafungin, but it is differentiated by a choline moiety at the C5 ornithine position, conferring increased stability and solubility (Sofjan et al. 2018). This modification results in a considerably longer half-life because non-enzymatic chemical degradation occurs on the hemiaminal of anidulafungin (James et al. 2017). Due to its long half-life, rezafungin has the advantage of once-weekly dosing as compared to other drugs within the echinocandin class that require daily dosing.

Rezafungin was designed to optimize PK properties and avoid hepatotoxicity, by reducing degradation while maintaining the potent antifungal activity and safety profile of the echinocandin class. Rezafungin is stable to biotransformation in liver microsomes or hepatocytes, reducing the risk of hepatotoxicity, similar to other echinocandins. As with other echinocandins, in vitro cytochrome inhibition studies suggest minimal interaction with CYP450 enzymes (Ong et al. 2016).

2.3.2 Spectrum of Activity/In Vivo Susceptibility Data

Rezafungin has potent in vitro activity that is comparable to that of other members of the echinocandin class against WT and azole-resistant *Candida* species, as well as

WT and azole-resistant *Aspergillus* species (similar to other echinocandins, rezafungin has higher MICs (MIC_{50} 1 μg/mL/MIC_{90} 2 μg/mL) against *C. parapsilosis* mirrored with other common *Candida species*.

For *C. lusitaniae* and *C. auris* isolates, MICs were 0.25 μg/mL by CLSI methodology (Pfaller et al. 2020; Tóth et al. 2019).

MIC of 0.5 μg/mL were found for *C. metapsilosis* and 1 μg/mL for *C. orthopsilosis* and *C. guilliermondii* (Tóth et al. 2019). *Candida parapsilosis* was the least susceptible organism, with MICs up to 4 μg/mL (Arendrup et al. 2018a). For isolates harboring *FKS* mutations, elevated rezafungin MICs were described with a similar or slightly better activity against mutant *Candida* spp. compared to other echinocandins (Pfaller et al. 2017b; Helleberg et al. 2020). In summary, rezafungin demonstrates potent in vitro activity against most wild-type and azole-resistant *Candida* spp., including *C. auris*. Rezafungin also has potent activity versus common dermatophytes (e.g., *Trichophyton mentagrophytes, T. rubrum, Microsporum gypseum, and Epidermophyton floccosum*).

Furthermore, the SENTRY surveillance program evaluated the minimum inhibitory concentration of rezafungin from 2014 to 2018 for *Candida*, *Aspergillus*, and *Cryptococcus* (Pfaller et al. 2017a, b, c, 2020). Results from the 2020 Pfaller study demonstrated that 95–100% of *Candida* isolates were susceptible, with variability based on species. Against *Aspergillus fumigatus* and *A. flavi*, rezafungin had MIC_{50} and MIC_{90} values of ≤0.008–0.015 and 0.015–0.03, respectively, equal to or within 1 dilution of the currently available echinocandins. Against *A. flavus*, *A. niger*, and *A. terreus*, rezafungin was shown to be active with minimum effective concentrations (MECs) of ≤0.008–0.03 μg/mL (Pfaller et al. 2016). Rezafungin also has activity against cryptic species, including *A. calidoustus*, *A. lentulus*, *A. thermomutatus*, and *A. udagawae* (Wiederhold et al. 2018a). Rezafungin activity against *Aspergillus* spp. is therefore comparable to that of other echinocandins. When tested against *Cryptococcus*, the MIC_{50} and MIC_{90} were again in line with the echinocandins at >4 for both values. As with other echinocandins, rezafungin is inactive against non-*Aspergillus* molds, *Trichosporon*, and *Rhodotorula* isolates with MICs >8 μg/mL (Garcia-Effron 2020); Pfaller et al. 2017b).

In immunocompromised mouse models of *C. albicans,* including azole-resistant *C. albicans*, *C. glabrata*, and *C. parapsilosis* and *A. fumigatus* infection, decreased fungal tissue burden and improved 10-day survival, respectively, were observed with rezafungin as compared to controls (Ong et al. 2016). Rezafungin also had activity in a mouse model of disseminated *C. auris*, leading to decreased fungal tissue burden as compared to amphotericin B and control (Hager et al. 2018b). Echinocandins are not commonly used to treat or prevent *Pneumocystis jirovecii* infections. However, rezafungin was efficacious as a prophylaxis against *Pneumocystis murina* in an immunosuppressed mouse model, supporting its potential for development for the prevention of *Pneumocystis* pneumonia in immunocompromised hosts (Cushion and Ashbaugh 2019). A significant decrease in the number of trophic nuclei and a reduced count of cystic forms have been demonstrated in the rezafungin-treated groups, with a comparable efficacy to the active control trimethoprim–sulfamethoxazole (Miesel et al. 2021).

In a disseminated infection mouse model of aspergillosis caused by *A. fumigatus*, similar survival rates were shown with rezafungin compared to amphotericin B treatment (Miesel et al. 2019). In vivo efficacy has also been demonstrated in a murine model of disseminated aspergillosis caused by an azole-resistant *A. fumigatus* isolate harboring a TR34/L98H *CYP51A* mutation (Wiederhold et al. 2019a).

2.3.3 Clinical Development

Phase 1 rezafungin safety studies, in which participants receiving either a single dose or up to 3 weekly doses, did not reveal any deaths or severe adverse events, and no participants withdrew from the study (Sandison et al. 2017). The weekly dosing regimen was confirmed with high plasma exposures. In addition, there was minimal renal excretion of this compound. An additional phase 1 study has shown no effect on QTc or other aspects of the cardiac electrical rhythm (Flanagan et al. 2020).

A phase 2 multicenter, randomized, double-blind trial (STRIVE; NCT02734862) was published in 2020, evaluating two different dosing strategies compared to caspofungin (Thompson III et al. 2021). This study included 207 adult patients with candidemia and/or invasive candidiasis and compared the efficacy and safety of treatment with rezafungin versus caspofungin with fluconazole step-down once clinically stable (Thompson III et al. 2021). Two different regimens of rezafungin (400 mg once weekly and 400 mg 1 week first, followed by 200 mg weekly) were tested against standard dose caspofungin (70 mg loading dose followed by 50 mg daily for ≤4 weeks). Outcomes for primary and secondary endpoints were similar across the three groups and comparable to the published literature of the currently approved echinocandins. Rezafungin was also well-tolerated. The most common adverse events—hypokalemia, diarrhea, and vomiting—were observed in similar proportions of patients in the rezafungin and caspofungin groups.

Based on the promising results of STRIVE, two phase 3 clinical trials are ongoing. One of them (ReSTORE; NCT03667690) compares rezafungin versus caspofungin for the treatment of candidemia and invasive candidiasis are ongoing. The ReSTORE trial is the progression of STRIVE for the treatment of candidemia and invasive candidiasis. The 400 mg followed by 200 mg weekly dosing regimen was chosen for final development.

The other ongoing phase 3 trial (ReSPECT; NCT04368559) compares rezafungin to standard of care for prophylaxis of IFD due to *Candida* spp., *Aspergillus* spp., and *Pneumocystis* in patients undergoing allogeneic hematopoietic cell transplantation. The comparator arm in this study is a combination of oral azole and trimethoprim sulfamethoxazole. The primary outcome is fungal-free survival at day 90. In both phase 3 trials, rezafungin is dosed 400 mg for the first week followed by 200 mg once weekly.

Rezafungin has received U.S. FDA QIDP and Fast Track designations for the prevention of invasive fungal infections and QIDP, Fast Track, and orphan drug designations for the treatment of invasive candidiasis. Therefore, its use as a

prophylactic agent for preventing invasive fungal disease caused by *Candida* spp., *Aspergillus* spp., and *P. jirovecii* could replace current multidrug regimens, and the once-weekly dosing with limited drug–drug interactions may also be attractive for prophylaxis during the early phase after solid organ transplantation (e.g., liver) (McCarty and Pappas 2021; Hoenigl et al. 2021b).

2.4 Encochleated Amphotericin B (MAT2203, CAmB-MATINAS, Cell Membrane Inhibitor)

Lipid formulations of amphotericin B (AmB) (**4**) (Fig. 12.1) are a significant improvement over AmB-D (CAmB; Matinas BioPharma, Bedminster, NJ, USA) in terms of drug-associated renal toxicity, but there are still significant electrolyte disturbances with their use, such as hypokalemia and hypomagnesemia. Also, infusion-related toxicity effects such as fever, chills, and hypotension are seen with lipid formulations of AmB. Therefore, in order to avoid these significant toxicities, an orally administered encochleated AmB-d (CAmB) has been developed as a novel lipid nanocrystal, which is designed for oral therapy for serious fungal infections. CAmB is comprised of a solid lipid bilayer along with calcium, which is rolled into a spiral form, where amphotericin B molecules (or other biologically active molecules) are entrapped (Segarra et al. 2002). The drug is made biologically available when the cochlea is taken up by a phagocytic cell, and the gradient between the high calcium concentration in the cochlea and the lower cytoplasmic calcium concentration triggers the cochlea to unfold and open thereby releasing amphotericin B molecules. The cochlea structure provides protection against degradation in unfavorable environments such as low pH in the stomach, while allowing for targeted intracellular delivery into macrophages and reticuloendothelial cells (McCarty and Pappas 2021).

2.4.1 Mechanism of Action

Amphotericin B (AmB), a polyene antifungal agent, disrupts fungal cell wall synthesis by binding to ergosterol to form pores that allow leakage of intracellular contents, resulting in potent fungicidal activity against a wide range of yeasts and molds. However, AmB and its lipid formulations are only available via intravenous injection due to low solubility, a tendency to self-aggregate in aqueous media, and low permeability (Torrado et al. 2013). Encochleated AmB (CAmB) is a novel formulation that allows for oral administration with reduced toxicity. Cochleates form a multilayered structure composed of a negatively charged lipid (phosphatidylserine) and a divalent cation (calcium), which protects AmB from degradation within the gastrointestinal tract (Aigner and Lass-Flörl 2020). AmB is released to the fungus only when the cochleates interact with the target cells.

2.4.2 Spectrum of Activity/In Vivo Susceptibility Data

Comparable in vitro activity against *Candida* spp. and *Aspergillus* spp. is observed with CAmB and deoxycholate AmB. The encochleated formulation of AmB-d demonstrates retention of the broad-spectrum activity of the parent compound in murine models of candidiasis and aspergillosis (Zarif et al. 2000; Delmas et al. 2002).

In both studies, oral CAmB and intraperitoneal deoxycholate amphotericin B demonstrated a similar improvement in survival and reduction in tissue fungal burden as compared to untreated control animals (Delmas et al. 2002; Santangelo et al. 2000). Furthermore, CAmB was evaluated in a mouse model of cryptococcal meningoencephalitis where CAmB plus flucytosine had similar efficacy to parenteral AmB plus flucytosine and demonstrated potent activity (Lu et al. 2019).

2.4.3 Clinical Development

So far, human studies have been limited to several phase1 and 2 trials of safety, tolerability, and efficacy. A recently published phase 1 study among 35 volunteers with a history of recovered HIV-associated CNS cryptococcosis (EnACT 1) demonstrated that the drug is generally tolerated well up to a dose of 1.5 g daily, above which significant bloating, abdominal pain, and diarrhea were common adverse effects (Skipper et al. 2020). In this trial study with C–AmB, the traditional side effects of parenteral amphotericin B have not been seen.

Three phase 2 studies have been performed or are underway among adults with a variety of conditions. A phase 2a single-arm study of CAmB (NCT02629419) for refractory mucocutaneous candidiasis is ongoing. Preliminary results indicate that all enrolled patients met the primary endpoint of ≥50% improvement in clinical signs and symptoms. CAmB was well-tolerated at 400 and 800 mg, with no observed renal or hepatic toxicity.

In a second phase 2 study of CAmB (NCT02971007) 200 and 400 mg and fluconazole 150 mg for VVC in 137 patients, lower rates of clinical cure and more adverse events were observed with CAmB 200 and 400 mg as compared to fluconazole. There were no serious adverse events (Clinicaltrials.gov 2020; Jacobs et al. 2022). The third trial, phase 1 and 2 studies of CAmB (EnACT 2; NCT04031833) for the treatment of cryptococcal meningitis in HIV-infected patients in Uganda, is ongoing. In this trial, CAmB is being administered as primary/induction therapy (orally) and compared to AmB-d intravenously. Measures of outcome include clinical and mycological (cerebrospinal early fungicidal activity).

In summary, the promise of CAmB is the potential for broad-spectrum antifungal activity delivered orally to the target organ with very little, if any, of the typical toxicity associated with AmB administered intravenously, regardless of formulation (lipid versus deoxycholate). Larger comparative clinical trials will be necessary to determine if this agent has a potential role in serious systemic fungal infections, but

data at this point show significant promise in selected populations. Due to the results of all of these trials, CAmB has FDA-granted Fast Track, QIDP, and orphan drug designations for the treatment of invasive candidiasis and aspergillosis, prevention of IFDs in patients undergoing immunosuppressive therapy, and treatment of cryptococcosis (Jacobs et al. 2022; McCarty and Pappas 2021).

3 Tetrazole Inhibitors

3.1 Oteseconazole (VT-1161), VT-1598, VT-1129 (Mycovia, Novel Tetrazole-Specific Cyp51 Inhibitors)

Oteseconazole (VT-1161) (**5**), VT-1598 (**6**), and VT-1129 (**8**) (Fig. 12.2) (Mycovia Pharmaceuticals, Inc., Durham, NC, USA) are next-generation azoles. Oteseconazole (VT-1161) is the first of several tetrazole agents, which have in common a much greater affinity for fungal CYP51 compared to human cytochrome enzymes. The compound was designed to achieve greater selectivity, fewer side effects, and improved efficacy compared to currently available azoles (Hoekstra et al. 2014; Warrilow et al. 2014). Based on this observation, one would expect fewer drug–drug interactions with this class of compounds and less direct toxicity.

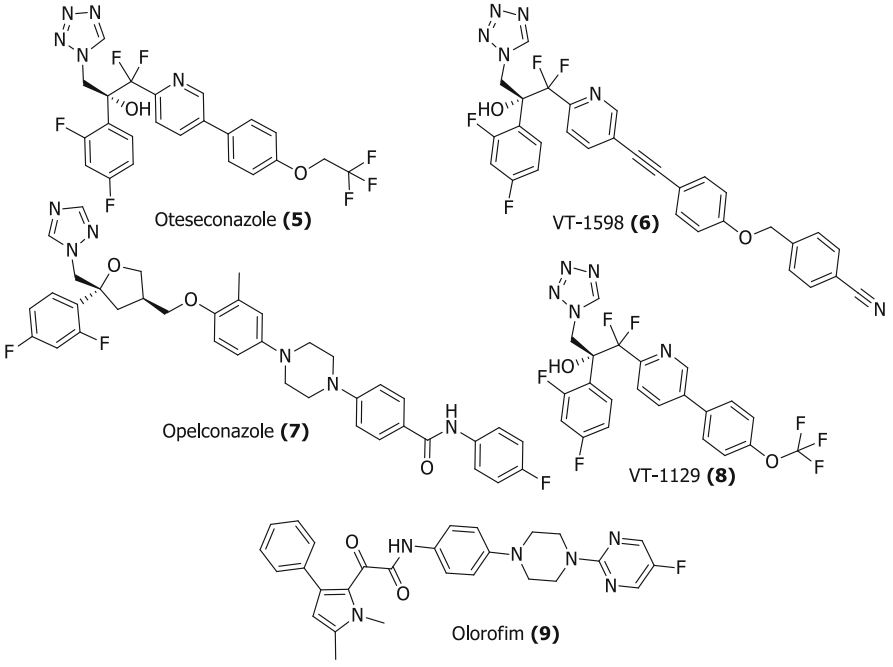

Fig. 12.2 Tetrazoles and olorofim antifungal agents in development

The target enzyme of tetrazoles (lanosterol 14α-demethylase), including oteseconazole, is the same as for other azoles; however, the affinity for the target enzyme is greater than for triazoles such as fluconazole (McCarty and Pappas 2021).

3.1.1 Mechanism of Action and Spectrum of Activity/In Vivo Susceptibility Data

Oteseconazole (VT-1161), VT-1598, and VT-1129 (Mycovia Pharmaceuticals, Inc.) are next-generation azoles in which selective inhibition of the fungal enzyme CYP51 is more readily achieved by replacing the 1-(1,2,4-triazole) metal-binding group with a tetrazole (Hoekstra et al. 2014). Oteseconazole, VT-1598, and VT-1129 have potent and broad in vitro activity against *Cryptococcus* spp., *Candida* spp. including *C. krusei* and fluconazole and echinocandin-resistant *C. glabrata*, dermatophytes, certain endemic fungi such as *Coccidioides* species, and selected Mucorales species (Schell et al. 2017b; Nishimoto et al. 2019a, b). VT-1598 has the broadest spectrum, which includes *C. auris*, molds (*Aspergillus* spp. and *Rhizopus* spp.), and endemic dimorphic fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides posadasii,* and *C. immitis*) (Wiederhold et al. 2018b, 2019b; Garvey et al. 2020).

In murine models of CNS coccidioidomycosis, VT-1598 treatment leads to improved survival and reduced fungal burden in brain tissue as compared to fluconazole. Oteseconazole has also demonstrated efficacy in murine models of pulmonary and CNS coccidioidomycosis and disseminated mucormycosis due to *Rhizopus arrhizus* var. *arrhizus* (Shubitz et al. 2015; Gebremariam et al. 2017b).

3.1.2 Clinical Development

The FDA has granted QIDP, fast track, and orphan drug designation to VT-1598 for the treatment of coccidioidomycosis (Valley fever) (Wiederhold et al. 2018c). VT-1598 is in phase 1 studies (NCT04208321). Oteseconazole is in phase 3 clinical trials for the treatment of both acute and recurrent vulvovaginal candidiasis (VVC) (NCT02267382, NCT03562156, NCT03561701) after demonstrating safety and efficacy in a phase 2 study, and it has FDA QIDP and Fast Track designations for this indication. In a randomized placebo-controlled, double-blind, dose-ranging study of women with recurrent VVC, women receiving any of four regimens of VT 1161 experienced a significant reduction in recurrent VVC compared to placebo (Brand et al. 2018). The compound was very well-tolerated, so there were few early discontinuations during the study when administered on a weekly basis at doses ranging from 150 to 300 mg weekly.

A phase 2 trial for toenail onychomycosis demonstrated higher week 48 cure rates with oteseconazole (32% to 42%) versus placebo (0%) (NCT02267356) (Elewski et al. 2021). This study compared 1 of 4 dosing regimens of oral VT 1161 to placebo, each administered for 10 to 22 weeks. The active regimens included 300 or 600 mg VT-1161 administered daily for 2 weeks, followed by weekly dosing, with the

randomized dose for either 10 or 22 weeks. Study drug was well-tolerated with no evidence of hepatotoxicity or QT prolongation in this trial involving 259 patients.

As an agent that is only available in an oral formulation, it remains unclear whether oteseconazole will have a role in the treatment of more serious invasive fungal infections such as candidemia and invasive candidiasis. Nonetheless, the potential for a systemically administered tetrazole compound with significantly less potential for drug–drug interactions and direct toxicity is very appealing, and it could represent a significant step forward for the azole class of drugs. Oteseconazole is currently under FDA consideration for approval as an agent for the treatment of recurrent VVC (McCarty and Pappas 2021; Jacobs et al. 2022).

3.2 Opelconazole (PC945, Pulmocide, Novel Tetrazole-Specific Cyp51 Inhibitors)

Opelconazole (**7**) (Fig. 12.2) (PC945, developed by Pulmocide Ltd., London, UK) is a first-in-class inhaled antifungal drug from the class of broad-spectrum triazoles.

3.2.1 Mechanism of Action

PC945 is a novel triazole antifungal agent that was designed and optimized for inhalation via commonly available nebulizers, for the treatment and prevention of invasive fungal infections of the sinopulmonary tract (Cass et al. 2021). The structure of PC945 is similar to but different from that of posaconazole. The structures are similar in that they have 2,4-difluorophenyl and 1H-1,2,4-triazole substitutions on the asymmetric carbon atom. However, PC945 differs structurally as it has a central oxolane ring (rather than a dioxolane ring) and a long hydrophobic 3-ylmethoxy-3-methylphenyl[piperazin-1-yl]-N-(4-fluorophenyl) benzamide substitution. This hydrophobic moiety likely contributes to the sustained intrapulmonary concentrations of PC945 (Hoenigl et al. 2021b).

After inhalation, opelconazole shows efficacy primarily in the lungs (systemic concentrations are minimal), making it a promising agent for treating pulmonary aspergillosis in non-neutropenic patients without disseminated infection. Its primary mechanism of action is familiar, being comparable to established azoles, given that opelconazole also contains the typical heterocyclic triazole scaffold. By inhibiting lanosterol 14α-demethylase (CYP51A1), lanosterol conversion to ergosterol is inhibited, leading to a reduction in ergosterol synthesis and, hence, the dysfunction of the fungal membrane structure, preventing further growth (Colley et al. 2017).

The chemical and physical attributes of opelconazole, namely they have a high amount of lipophilic compounds and micronized drug particles, result in high local concentrations of the drug, prolonged lung retention, slow absorption by the lung, and, as a consequence, low plasma concentrations (Cass et al. 2021). These findings

are promising given the low potential for systemic adverse drug effects and drug–drug interactions. Additionally, some data suggest cellular persistence of opelconazole in local immune and epithelial cells, which is potentially valuable in terms of use in prophylaxis or enhancement of antifungal activity (Baistrocchi et al. 2017; Colley et al. 2019).

3.2.2 Spectrum of Activity/In Vivo Susceptibility Data

Opelconazole or PC945 shows broad-spectrum antifungal activity, including against *Candida* spp. and *Aspergillus* spp. PC945 has shown in vitro activity against azole-susceptible *A. fumigatus* [median MIC 0.031 μg/mL (IQR 0.02–0.031 μg/mL)] and most azole-resistant *A. fumigatus*. In general, opelconazole has shown in vitro superiority over posaconazole, itraconazole, and voriconazole in azole-susceptible and azole-resistant strains of *A. fumigatus* (Colley et al. 2017).

Opelconazole activity against *A. terreus* is comparable to that of posaconazole and more potent than that of voriconazole; however, PC945 has poor in vitro activity against *A. flavus* and *A. niger*.

PC945 lacks activity against most Mucorales; although a MIC 2 μg/mL was observed for *Rhizopus arrhizus/oryzae*. PC945 is generally more active against *Candida albicans* (both azole-susceptible and azole-resistant strains), *C. glabrata*, *C. gattii*, *C, neoformans,* and *C. krusei*, than voriconazole, and it shares equal potency with posaconazole (Colley et al. 2017). Using a global collection of 50 emerging clinical *Candida auris* isolates, PC945 had more potent in vitro activity than posaconazole, voriconazole, and fluconazole [PC945 GM MIC (MIC_{50}, MIC_{90}): 0.14 μg/mL (0.13, 1 μg/mL)] (Rudramurthy et al. 2019). It is remarkable that potent inhibition was noted considering that *C. auris* could potentially be a pathogen with limited treatment options due to its multi-drug-resistant nature (Gebremariam et al. 2020).

Remarkable synergistic effects were reported in an in vitro human alveolus bilayer model when opelconazole was administered apically combined with basolateral posaconazole or voriconazole, proving to be an in vitro model of concomitant topical lung and systemic therapy. Interestingly, these effects were not present when combining posaconazole or voriconazole locally and systemically, highlighting the superiority of opelconazole pharmacokinetics regarding local drug activity as, compared to established azoles (Colley et al. 2019). Regarding mouse models, combination therapy with intranasal PC945 and oral posaconazole was also evaluated in immunocompromised neutropenic mice with azole-susceptible *A. fumigatus* infection. Suboptimal dosages of PC945 and posaconazole were administered simultaneously and day 7 survival improved to 83% (Colley et al. 2019).

As a potential prophylactic agent, PC945 was administered in the same *A. fumigatus*-infected mouse model and yielded greater inhibition of fungal load in lung tissue and GM concentrations in BALF and serum as compared to a shorter

duration, suggesting that the antifungal effects of PC945 accumulated in the lung upon repeat dosing (Kimura et al. 2017).

3.2.3 Clinical Development

A phase 3 study of PC945 was performed for adults with, limited or no alternative treatment options, for the treatment of invasive pulmonary aspergillosis as part of a combined antifungal regimen. In a report of two lung transplant recipients with bronchial anastomotic masses due to *A. fumigatus*, PC945, administered in combination with systemic antifungal agents, was well-tolerated, and clinical resolution of infection was observed (Pagani et al. 2020).

In an ongoing study, opelconazole has shown promising results when used as part of its compassionate use program, as a last resort in patients who have not responded to standard antifungal therapies. The results were also promising in an open-label randomized phase IIb study carried out in 2021, focusing on the safety and tolerability of opelconazole prophylaxis for treating invasive aspergillosis in lung transplant recipients. Lately, the emergence of primary airway invasive aspergillosis in non-traditional risk groups has increased the interest in inhaled antifungal agents. In order to achieve local efficacy, high systemic concentrations are required, which are frequently associated with complications that limit treatment. Topical or inhaled administration may maximize local efficacy while avoiding systemic toxicity, thereby decreasing the need for high drug concentrations and potential toxicity.

Particularly for combination therapy approaches in invasive aspergillosis, opelconazole would be a new therapeutic option of potentially great value in high local concentration without systemic drug concentrations and toxicity. This is, specifically attractive for primarily airway invasive aspergillosis in non-neutropenic patients, including those with COVID-19-associated pulmonary aspergillosis (Castanheira et al. 2012; Hoenigl 2020). Other roles may include antifungal prophylaxis after lung transplantation, in the intensive care unit setting or in other settings where mold-active prophylaxis is not yet firmly established and in combination therapy with a systemic antifungal agent for patients with angioinvasive pulmonary aspergillosis.

4 Target Nucleic Acid Metabolism

4.1 Olorofim (F2G, LTD, a Novel Dihydroorotate Dehydrogenase Enzyme Inhibitor)

Olorofim (**9**) (Fig. 12.2) (F901318, developed by F2G, Inc., Manchester, UK, Fig. 12.2) is the first member of a novel class of antifungals known as the orotomides, which target nucleic acid metabolism. The compound was found by

screening a library of >300,000 small molecules (Oliver et al. 2016). It has progressed through pre-clinical studies and phase 1 human trials to an ongoing phase 2 clinical trial.

4.1.1 Mechanism of Action

After discovery, researchers determined that the mechanism of action is through inhibition of fungal growth by the dihydroorotate dehydrogenase enzyme. The enzyme functions in the production of pyrimidine biosynthesis. Olorofim has no activity against human dihydroorotate dehydrogenase, which limits the compounds for targeting drug toxicity (Rauseo et al. 2020; Negri et al. 2018). Interruption of pyrimidine synthesis impairs nucleic acid production and leads to the arrest of hyphal extension (Oliver et al. 2016). The compound itself has poor water solubility and is highly protein-bound, but it has excellent tissue distribution including the kidney, liver, lung, and brain (at lower levels) (Rauseo et al. 2020).

4.1.2 Spectrum of Activity/In Vivo Susceptibility Data

Susceptibility testing of various molds shows very broad, but not universal anti-mold activity (Jorgensen et al. 2018). However, olorofim does entail significant novelty and a spectrum of activity that will be relevant in the future.

Aspergillus spp. nearly uniformly have low MICs to olorofim (Du Pré et al. 2018). This includes azole-resistant (including *A. terreus* and cryptic species of *Aspergillus*), with generally lower MICs than currently approved anti-mold agents (Buil et al. 2017; Lackner et al. 2018; Rivero-Menendez et al. 2019b). Of the common hyaline molds, olorofim exhibits overall good activity against *Fusarium solani species complex* and *Fusarium oxysporum*, with some isolates demonstrating MICs of up to 4 mg/L, while others demonstrate low MICs (Georgacopoulos et al. 2021; Badali et al. 2021).

The in vitro activity of olorofim is extended to both mold and yeast phases of *Talaromyces, Trichophyton*, and *Penicillium* (Singh et al. 2021; Zhang et al. 2021). In addition, olorofim exhibits activity against several clinically important groups of fungi, including dimorphic molds (e.g., *Histoplasma* and *Coccidioides* spp.), hyaline hyphomycetes (e.g., *Aspergillus* spp.), and dematiaceous molds (e.g., *Scedosporium* spp.) (Rauseo et al. 2020; Oliver et al. 2016; Wiederhold 2020; Fothergill et al. 2015). *Scedosporium* and *Lomentospora* are frequently resistant to all available treatment options; however, olorofim shows potent activity with much lower MICs compared to anti-mold triazoles and amphotericin (Kirchhoff et al. 2020a; Rivero-Menendez et al. 2020).

Regarding murine models involving profound neutropenia and chronic granulomatous disease with disseminated and pulmonary aspergillosis, respectively, the olorofim was administrated by intraperitoneal leading to significantly reduced serum GM levels and organ fungal DNA burden, resulting in and improved survival as

compared to controls (Seyedmousavi et al. 2019). In a murine model of acute sinopulmonary aspergillosis due to *A. flavus*, olorofim had antifungal activity comparable to posaconazole resulting in a decrease in GM, histologic clearance of lung tissue, and survival (Negri et al. 2018).

4.1.3 Clinical Data

Multiple ascending single and multiple-dose phase 1 studies (NCT04171739) were carried out (Kennedy et al. 2015, 2017a, b, c). One study was a drug–drug interaction study with itraconazole and rifampicin. Olorofim was well-tolerated in healthy volunteers, and short infusion durations were safe while maintaining desired exposures.

An open-label, single-arm, phase 2b clinical trial (FORMULA-OLS; NCT03583164) of olorofim for the treatment of IFDs due to resistant fungi including azole-resistant aspergillosis, scedosporiosis, and lomentosporiosis is being evaluated. Olorofim has successfully treated two reported cases of Lomentospora infections, one localized and the other disseminated, which is notable given the essentially pan-resistant nature of *L. prolificans* (Chen et al. 2020; Tio et al. 2020). It has also been used in combination with posaconazole for the treatment of disseminated coccidioidomycosis, after multiple different antifungal treatment regimens had failed previously (Harvey et al. 2020).

Therefore, the European Medicines Agency Committee for Orphan Medicinal Products granted orphan drug status to olorofim for the treatment of invasive aspergillosis and scedosporiosis in March 2019. Olorofim also received designation from the U.S. FDA as a breakthrough therapy in 2019 and as an orphan drug in 2020 (ClinicalTrials.gov 2021).

Olorofim is not only associated with very low MICs but also has antibiofilm activity, so it could be considered as a new treatment option (Kirchhoff et al. 2020a, b). Moreover, olorofim will play a very important role in the treatment of endemic mycoses, particularly coccidioidomycosis, as well as infections caused by *T. marneffei* (Zhang et al. 2021), and *Madurella mycetomatis.*

5 Final Evaluation

Multiple evolutions in the fungal domain, such as prolonged antifungal therapy, emerging fungal resistance, and disadvantages of existing antifungals, demand that new antifungal drugs be developed in clinical practice. Available treatment options for mold infections are scarce and have limitations, especially due to the increasing reports of drug resistance. The increased incidence of *Aspergillus* spp. and Mucorales infections among COVID-19 patients reminds us of what is lacking in our antifungal treatment armamentarium. Novel antifungals and treatment strategies are needed to improve the outcomes of these difficult-to-treat infections.

There are several drugs in the late stage of development. Some of them are described throughout this review/chapter. For example, some antifungals like SCY-078 and olorofim are better in resistance profile. Others, such as CD101, SCY-078, and Coch-AmB, have a major advantage in formulation and administration. Some triazoles have advantages over other azoles because of their improved safety profile while preserving the efficacy of azoles. Lastly, a new antifungal class, the orotomides, is being developed to reduce cross-resistance and allow oral administration. In the case of ibrexafungerp, additional clinical data are needed to better define its future role in mold infections; however, the pre-clinical data generated thus far are promising, justifying further development and its approval by the FDA as the first and only oral non-azole treatment for vaginal yeast infections: SCYNEXIS and BREXAFEMME® (ibrexafungerp tablets). Novel therapeutic approaches including antifungal combinations may also improve outcomes and warrant further investigation in general.

Moreover, additional molecules are still in development, so the future will tell which of them will finally enter the drug market. Of course, more research is needed after commercialization in order to assess effectivity, safety, and resistance in clinical practice and in special patient populations. In addition, efforts should be made to discover other strategies in the approach to fungal infections, such as repurposing drugs, host immune cell-targeted approaches, and antifungal biological agents (Perfect 2017). Lastly, effort and coordination in health policies at global and national levels are required to ensure equitable access to antifungals, and an antifungal stewardship program is needed to optimize the use of antifungal agents.

In summary, the reviews presented in this chapter provide useful information and insight into the development of new, safe molecules with antifungal mechanisms of action aimed at different cellular targets, providing data that will help in the search for effective methods to control fungal pathogens, especially those resistant to current therapeutic agents.

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Chapter 13
Fungal Enzyme Inhibitors: Potent Repository of Lead Compounds to Curb Cancer

Lokesh Gambhir and Neha Kapoor

Abstract Fungi are considered to be storehouse of novel biologically and structurally diverse compounds with immense therapeutic potential. Novel therapeutic moieties have been discovered from fungi and subsequently deployed in pharmaceutical industry as antimicrobial, anticancer, neuroprotective, and antioxidant moieties. Tremendous research is being carried out in the exploration of fungal secondary metabolites for anticancer therapy of which enzyme inhibitors are the novel targets. Various enzymes, viz. histone deacetylase (HDAC), DNA topoisomerases, telomerase, cyclooxygenase (COX-2), angiotensin-converting enzyme, phosphatidylinositol-3-kinase enzymes, are considered to be drug targets for the treatment of various tumors. Many therapeutic molecules belonging to the different classes of organic compounds are reported to be potent anticancerous molecules, but none of the molecule is commercialized due to various adverse effects. The present chapter will outline various classes of enzymes and their inhibitors which can be exploited as effective anticancer agents.

Keywords Enzyme inhibitors · Cancer · HDAC · Fungal metabolites · COX-2 · Topoisomerases

1 Introduction

1.1 Cancer

Cancer is reported to be the second principal cause of mortality across the globe. As per WHO (2022), 19.3 million new cancer cases and 10 million cancer deaths incurred worldwide in 2020. Among the newly reported cancer cases, breast cancer

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is leading with 2.3 million cases (11.7%) followed by lung (2.21 million, 11.4%), colon and rectum (1.93 million, 10%), and prostate cancer (7.3%). The Global Cancer Statistics 2020 reported that the incidence and mortality rate of cancer are rising at exponential pace and global cancer burden is projected to escalate by 47% by the year 2040 (Sung et al. 2021). The conventional treatments (surgery, radiation, chemotherapy) are generally implemented either alone or in combination to achieve greater efficacy. Regardless of the serious adverse effects of conventional interventions, chemotherapy is the main characteristic treatment modality for most of the cancers irrespective of the clinical extent of disease at presentation (Jemal et al. 2008; Saini and Twelves 2021). Due to the ever-increasing prevalence of cancer, a more comprehensive and target-specific molecular therapy as an alternative to conventional treatment is a dire requirement (Bedard et al. 2020; Min and Lee 2022). Various small molecules are being exploited as potent drug candidates for curbing different types of cancer (Hoelder et al. 2012). Multiple molecular targets have been embroiled in past decade for developing putative anticancer modality (Zhong et al. 2021). Out of the pool, targets which are specifically overexpressed in cancer cells serves a promising tool for developing selective therapeutic drug (Zhu and Leung 2020; Robinson and Schiemann 2022). Since antiquity, natural products harbored from plants, microorganisms, and other sources have contributed a lot to the therapeutic industry (Newman and Cragg 2020).

Till date, approximately 35% of the naturally occurring products/secondary metabolites with 15% belonging to microbial origin, 25% to plants, and nearly 5% extracted from animal source are approved by the US Food and Drug Administration (FDA) as potential therapeutics (Calixto 2019). Fungi are ubiquitous group of diverse microorganisms with remarkable ability to emanate plethora of chemically diverse and biologically active compounds to be exploited in pharmaceutical and biotechnological industries (Aly et al. 2011). Since the marvelous discovery of penicillin (**1**) (Fleming 1929), a β-lactam antibiotic from *Penicillium rubens* (Houbraken et al. 2011), an era of exploration of fungal secondary metabolites as antibiotics and antimicrobial agents was ushered. The benchmark discovery of these antibiotics served as stepping stone for the development of next-generation therapeutic moieties of fungal origin. Many fungi-derived secondary metabolites such as cyclosporine (**2**) as an immunosuppressive drug and lovastatin (**3**) as cholesterol lowering drug (Manzoni and Rollini 2002) are frequently being deployed in the market. Emphasizing on the infinite dimension of biodiversity in the fungal kingdom is based upon the concept that only a limited fraction of fungal species has been explored for bioactive compounds. In past two decades, many studies have documented the therapeutic potential of novel fungal secondary metabolites belonging to class of polyketides, diterpenes, phenols, peptides, terpenoids as anticancer, antioxidant, anti-inflammatory, antidiabetic, and immunosuppressive agents indicating that the wealth of compounds needs to be discovered from fungi (Zhan et al. 2007; Rönsberg et al. 2013; Uesugi et al. 2016; Zhang et al. 2018; Khan et al. 2020; Conrado et al. 2022; Deshmukh et al. 2023).

1.2 Rationale of Selecting Enzyme Inhibitors for Anticancer Therapy

The metabolic enzymes work in a concerted and sequential manner to express their biological function. Targeting metabolic enzymes has revolutionized the field of drug discovery, as drugs targeting specific enzymes are of immense utility for assessment of their three-dimensional structures and mechanisms (Robertson 2007). Specific enzyme inhibitors are of great importance to deduce the mechanism of disease emergence and diagnosis (Rufer 2021). Hence, the research is more focused on exploration of natural products as potent drugs against specific enzymes for the probable discovery of new therapeutic molecules (Kumar et al. 2015). There exist a number of enzymes with the potential to be exploited as potent targets. Many enzyme inhibitors of endopeptidases, exopeptidases, glycosidases, lipases, alkaline phosphates, esterases, and tyrosinase have been recovered from microbial sources (Umezawa 1972; Fernandes and Kerkar 2017) for the treatment of various medical conditions including hypertension, obesity, inflammation, diabetes, Alzheimer's disease (AD), carcinogenesis, metastasis, viral infection, and autoimmune diseases (Ebrahimi et al. 2017; Zaki et al. 2020).

2 Selective Enzyme Targets for Discovering Endophytic Fungal Inhibitors

2.1 DNA Topoisomerases

In last two decades, it is well documented that existing anticancer therapeutic molecules have a narrow activity spectrum and impose toxic effects also. Keeping this view in mind, researchers are continuously in search of novel specific targets, against which anticancer moieties can be explored. DNA is the one of the prime targets for exploration of novel cancer therapeutics drugs (Hurley 2002). DNA topoisomerases are one of the important enzymes involved in many cellular processes such as DNA replication, transcription, recombination, chromosome assembly, and segregation by regulating the topological state of DNA (Wang 1996; Nitiss 2009). DNA topoisomerases alter the topological structure of DNA by creating and resealing DNA strand breaks to facilitate the passage of other DNA strands. DNA topoisomerase (topo) I and II trigger the DNA relaxation by creating a transient single-strand and double-strand breaks in the duplex DNA, respectively. The incision mediated by DNA topo I involves the nucleophilic attack of hydroxyl group of tyrosine residue of active site onto the phosphodiester bond of DNA at the site of cleavage. This attack results in the formation of phosphotyrosine bond between DNA and enzyme generating a cleavable covalent binary intermediate complex of DNA topo I. On the other hand, topo II catalyzes DNA double-strand break and rejoining in an ATP-dependent fashion to eliminate superhelical twists and

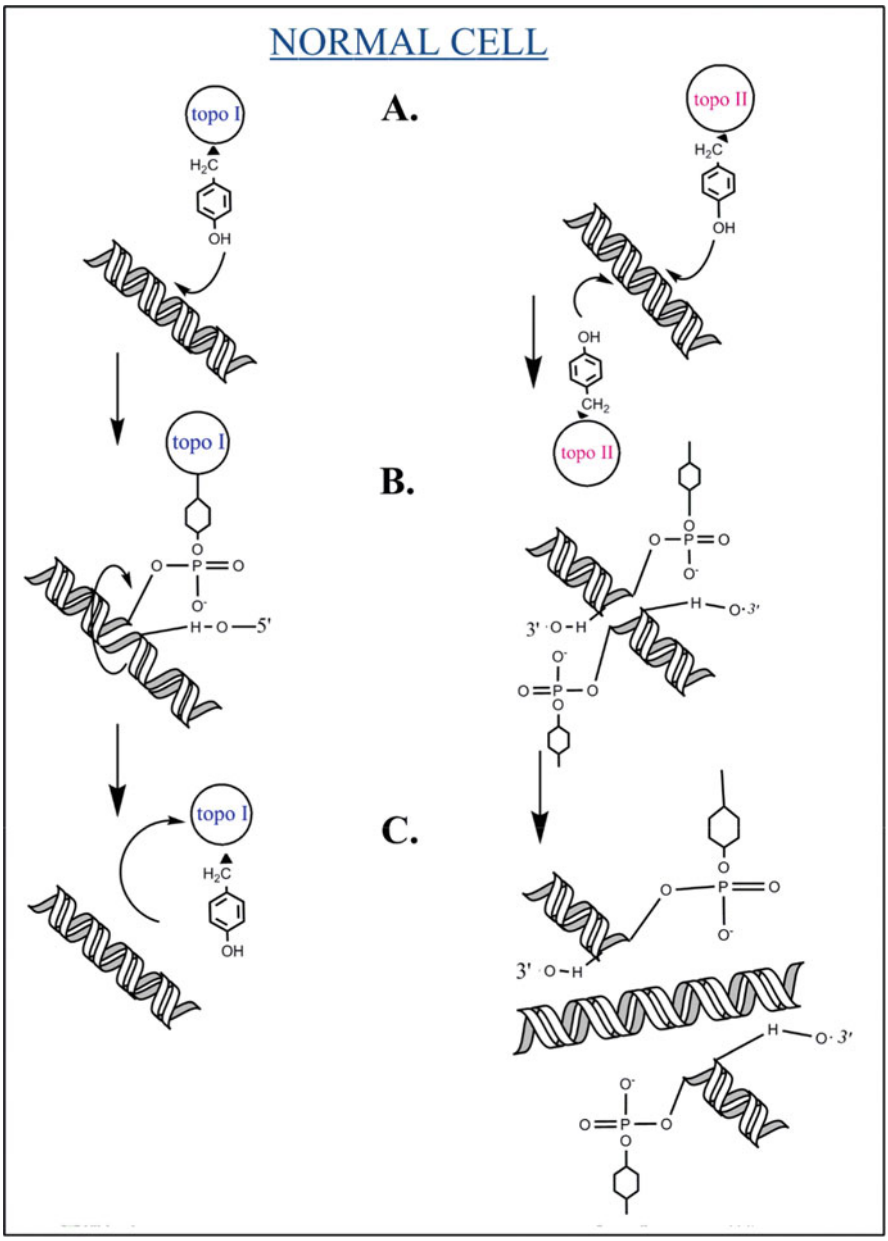

Fig. 13.1 Mechanism of DNA topoisomerase I and II inducing the DNA relaxation in normal cells

intramolecular DNA knots. Topo II also results in formation of cleavable covalent DNA enzyme complex similar to that of topo I by forming two phosphotyrosyl bonds (Fig. 13.1). The relaxation of DNA is achieved by hydrolysis of an ATP

molecule, due to which the enzyme undergoes a conformational change from open to closed clamp form thereby allowing the unbroken double-stranded DNA passage through the protein–DNA gate (Schoeffler and Berger 2008). Eukaryotic DNA topoisomerase I and II are potential target for the development of therapeutic moieties against various types of cancer such as lung, prostate, colon and ovarian, and hematological malignancies such as leukemia (AML, ALL, etc.) and lymphosarcoma (Giles and Sharma 2005).

Drugs targeting DNA topoisomerase act by blocking the process of DNA replication in tumor cells by binding to the 3′-phosphotyrosyl intermediate, thereby stabilizing the DNA topo (I and II) cleavable complex due to the stable single-strand and double-strand break (Hsiang and Liu 1988; Chen and Liu 1994). Nonetheless, these stable breaks are converted to irreversible double-strand breaks on stumbling with DNA replication fork and ultimately trigger cell death of tumor cells (Fig. 13.2) (Li and Liu 2001). Many drugs such as camptothecin (**4**) and its derivative topotecan and irinotecan inhibited the eukaryotic DNA topoisomerase I. mAMSA (**5**), etoposide (VP-16), and adriamycin (**6**) exhibit potent antineoplastic activity mediated by inhibition of mammalian DNA topoisomerase II (Tewey et al. 1984; Liu 1989; Spiridonidis et al. 1989; Kollmannsberger et al. 1999; Mathijssen et al. 2002; Pommier 1998). There exist many reports for fungal species serving as a repository of novel compounds exhibiting significantly potent topoisomerase inhibitory activity. (+)-3,3′,7,7′,8,8'hexahydroxy-5,5′-dimethylbianthraquinone (2240A) isolated from mangrove endophytic fungus No. 2240 (Tan et al. 2008) inhibited hTopo I enzyme. Further, ethyl acetate extracts of 19 fungal endophytes, of which 83.3% isolates recovered from *Brine fern*, 62.5% isolates from *Bruguiera gymnorrhiza*, and 83.3% isolates of *Heritiera littoralis* inhabiting mangrove reserve in Qiao Island of Zhuhai Guangdong province in the south of China were reported to exhibit significantly potent topoisomerase I inhibitory activity (Xiaoling et al. 2010).

Camptothecin (**4**), a natural compound from the Chinese herb *Camptotheca acuminata,* was found to exert promising anticancer activity owing to its linkage with DNA topoisomerase II (Hsiang et al. 1985). Further, camptothecin (**4**) was recovered from an endophytic fungus *Entrophospora infrequens* obtained from *Nothapodytes foetida*, two endophytic *Fusarium solani* strains MTCC 9667 and MTCC 9668 (Amna et al. 2006), and an endophytic fungus, *Fusarium solani* strain ATLOY-8, recovered from *Chonemorpha fragrans* (Moon) Alston with promising topoisomerase I and II inhibitory activity (Clarance et al. 2019). Two novel cyclic lipopeptides, fusaristatins A (**7**) and B (**8**), purified from rice cultures of a *Fusarium* sp. YG-45 isolated from the stem of *Maackia chinensis* exhibited moderate inhibitory effect on topoisomerases I (IC_{50}: 73 mM) and II (IC_{50}: 98 mM) without cleavable complexes (Shiono et al. 2007).

Alternariol (**9**), a mycotoxin produced by *Alternaria alternata*, possessing genotoxic properties was found to exert this property by inhibiting the DNA topoisomerase I and II enzyme (Fehr et al. 2009). Secalonic acid D (SAD) (**10**) recovered from the marine lichen-derived fungus *Gliocladium* sp. T31, collected from marine sediments in South Pole, exhibited potent inhibitory activity on topo I via in vitro plasmid supercoil relaxation assay and EMSA. The SAD (**10**) exhibited

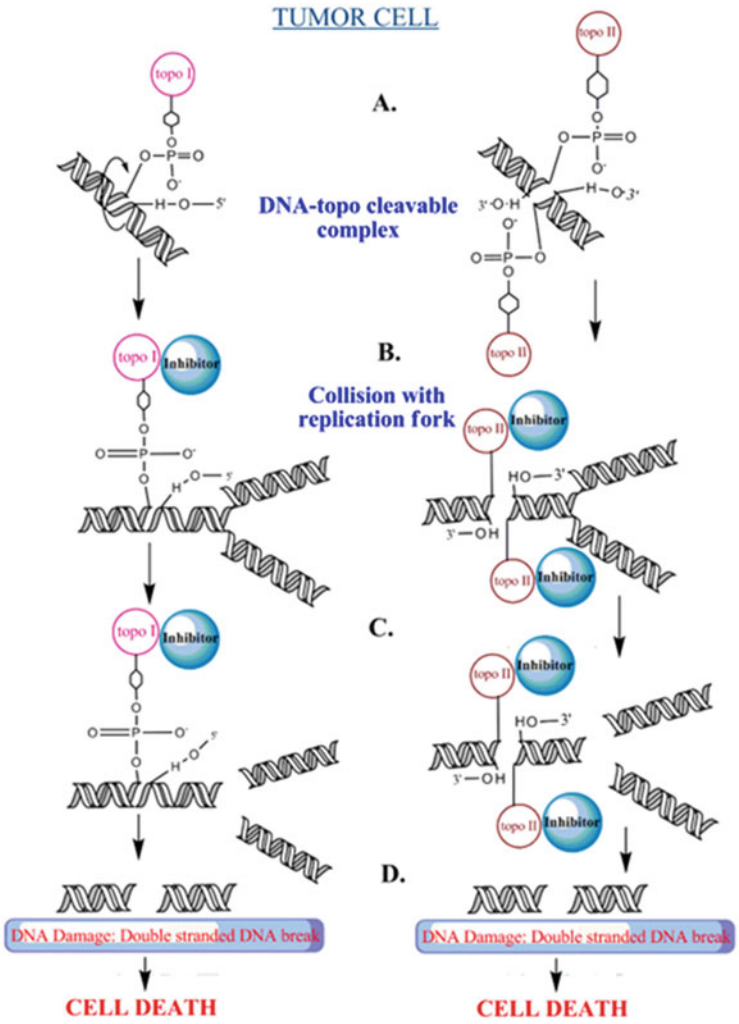

Fig. 13.2 Schematic representation of the drugs/inhibitors of DNA topoisomerase blocking the process of DNA replication to induce single- and double-strand breaks leading to tumor cell death

minimum inhibitory concentration (MIC) of 0.4 μM against topo I (Hong 2011). Fomitellic acids A (**11**) and B (**12**), the triterpenoid compounds, recovered from basidiomycete *Fomitella fraxinea* inhibit selectively the activities of mammalian DNA polymerases α and β (Mizushina 2000). These triterpenoids were potent inhibitors of calf DNA polymerase, rat DNA polymerase, and human DNA topo I and II. Recent advancements and discovery of novel and potent DNA topoisomerase inhibitors have led to a major shift from DNA enzymology to the therapeutic development. The progressive discovery of novel therapeutic moieties targeted against topoisomerase enzymes led to understand the mechanism of cell killing

and DNA repair pathways in rational drug designing for cancer therapeutics (Schneider et al. 1990).

2.2 Telomerase

Telomeric DNA sequence, a short tandem hexameric sequence, and telomerase are potent targets for cancer therapy (Trybek et al. 2020; Fragkiadaki et al. 2022). Telomere, a terminal ribonucleoprotein complex, consists of tandem repeat units of short hexameric sequence $(TTAGG)_n$ with an average length of approximately 10–15 Kb in humans (Blackburn 1991). During repeated cycles of chromosomal division, the terminal telomeric ends are shortened in length to maintain the integrity and stability of genetic information present in the linear chromosomes. Multiple chromosomal end-fusion events and shortening of telomeric sequences due to end-replication and end-protection problems lead to growth arrest and loss of cell viability. In normal somatic cells, the end-replication and end-protection problems are catered by employing a very unique telomere maintenance mechanism tightly regulated by the enzyme telomerase involved in catalyzing the addition of hexameric repeat unit at the terminal ends of chromosomes. In cancerous cells, the shortening of telomere is a major barrier to progression of malignancy which hinge upon the activation of telomerase to regain the proliferative immortalization (Jafri et al. 2016). Hence, the indefinite cell proliferation, a hallmark of malignancy, is governed by high expression of telomerase enzyme in cancer cells. Therefore, this distinctive feature has evolved the telomerase enzyme as an attractive and putative target for drug development in cancer therapeutics (Ahmed and Tollefsbol 2003).

In contrast to conventional therapies, telomerase inhibition enables much specific and highly promising future cancer therapy by virtue of differential expression of telomerase between cancer and normal cells (Betori et al. 2020; Mutlu 2017). Many studies documented the reduction in progression of different cancer cell types, viz. bone marrow, prostate, breast, and pancreas cancer cells, by employing certain synthetic telomerase inhibitors (Nakajima et al. 2003; Zisuh et al. 2012; Holysz et al. 2013; Graham and Meeker 2017; Li et al. 2020). Apart from synthetic chemical compounds, natural sources have also been reported to be putative source of bioactive moieties capable of inhibiting the expression of telomerase (Ganesan and Xu 2018). Many fungi also have been reported to be the sources of telomerase inhibitors. Thelavin B (**13**), a polyphenolics compound purified from a fungus *Thielavia terricola* (Kitahara et al. 1981), exhibited telomerase inhibitory activity with an IC_{50} value of 32 μM in TRAP assay (Togashi et al. 2001). Telomerase inhibitory studies of an antibiotic, UCS 1025A (**14**), recovered from *Acremonium* sp. KY 4917 (Nakai et al. 2000; Agatsuma et al. 2002) were carried out in yeast cell by utilizing TRAP assay. The compound was found to directly inhibit telomerase activity with IC_{50} value of 1.3 μM (Agatsuma et al. 2002). Furthermore, many reports including purification of diazaphilonic acid (**15**), belonging to azaphilone class of compound, from a fungus *Talaromyces flavus* (Tabata et al. 1999)

completely inhibited the telomerase with IC_{50} value of 50 μM. Many fungal species, viz. *Alternaria* sp., *Penicillium funiculosum,* are reported to produce alterperylenol (Okuno et al. 1983) (**16**) and wortmannin (**17**), a steroid metabolite respectively which were found to be potent inhibitor of Akt kinase enzyme which has directly influence the increased activity of human telomerase reverse transcriptase (hTERT) (Akiyama et al. 2002). It phosphorylates hTERT and upregulates its activity without altering the expression level of hTERT (Kang et al. 1999).

2.3 Angiotensin-Converting Enzyme (ACE)

Angiotensin-converting enzymes (ACE) are membrane bound glycoprotein involved in central metabolic pathway rennin–aldosterone pathway. A dipeptide, WF-10129 (**18**), composed of L-tyrosine and one novel amino acid purified from *Doratomyces putredinis* exhibited angiotensin-converting enzyme (ACE) inhibitory activity with IC_{50} value of 1.4 × 10 μM (Ando et al. 1987). Further, ethyl acetate extracts of five *Pestalotiopsis* species recovered from the *Terminalia arjuna* and *Terminalia chebula* from the riverbanks of Nanjangud and Mysore region of South Karnataka, India, were found to exhibit ACE-I inhibitory activity. The IC_{50} value of these isolates ranged from 21 to 37 μg/mL (Tejesvi et al. 2008).

DEAD/DEAH box RNA helicase-like protein recovered from the mycelial water extract of *Pleurotus pulmonarius and Ganoderma lucidum* inhibited ACE-I enzyme with an IC_{50} value of 12 μg/mL (Ibadallah et al. 2015; Ansor et al. 2013). Many other active peptides are reported from medicinal mushrooms with potential to inhibit ACE-I enzyme. Other reported ACE inhibitors were from an active tripeptide of *Tricholoma giganteum* (IC_{50} = 0.04 mg/mL) (Lee et al. 2004), an active pentapeptide of *Pholiota adiposa* (IC_{50} = 0.044 mg/mL) (Koo et al. 2006), and two oligopeptides from *Pleurotus cornucopiae* (IC_{50} = 0.46 and 1.14 mg/mL, respectively) (Jang et al. 2011). Three new thiodiketopiperazines, geospallins A–C (**19–21**) isolated from the culture of the deep-sea sediment-derived fungus *Geosmithia pallida* FS140, were found to exhibit ACE inhibitory activity with IC_{50} values of 29–35 μM (Sun et al. 2018). Two dipeptides (Cys-Cys and Cys-Arg) purified from *Xerocomus badius* fermented shrimp processing waste were found to exhibit ACE inhibition with IC_{50} values of 4.37 ± 0.07 μM and 475.95 ± 0.11 μM, respectively (Gao et al. 2018).

2.4 Histone Deacetylase (HDAC)

Histone deacetylase (HDAC), a metalloenzyme, acts as a transcriptional co-repressor and triggers the hydrolysis/deacetylation of acetylated L-lysine side chains of histone proteins altering the chromatin remodeling and affecting the gene regulation. The HDAC-dependent catalysis proceeds by the polarizing the carbonyl

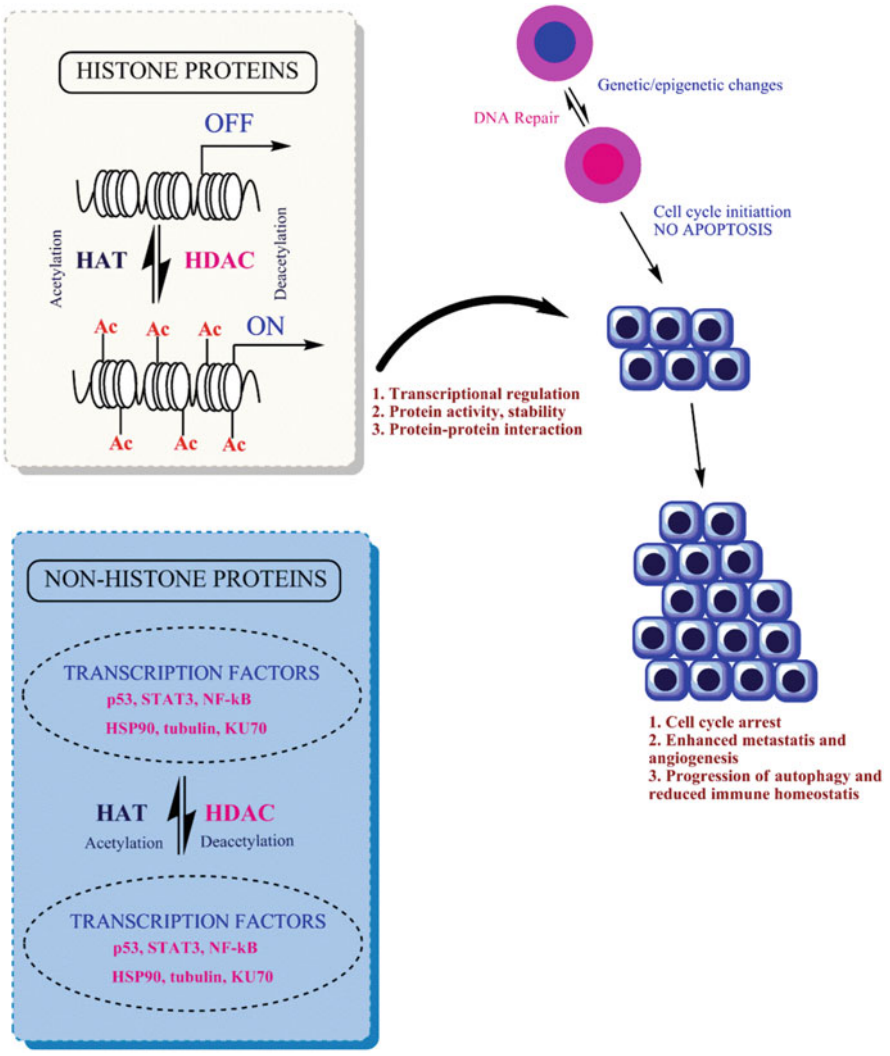

Fig. 13.3 Mode of Histone deacetylase (HDAC) in cancer progression

group of substrates by formation coordinating complexes with Zn^{2+} ion (Shein and Shohami 2011). HDAC inhibitors are emerging class of drugs for carcinogenesis. The active compounds act by blocking the substrate interaction with the catalytic site of HDACs and allow hyperacetylation of histone tails (Xu et al. 2007). HDAC inhibitors often exploit chelate interactions with the catalytic Zn^{2+} ion forming five- and six-membered coordination ring complexes.

HDAC inhibitors exhibit anticarcinogenic properties by affecting various unsynchronized cellular processes, viz. inhibition of cell cycle checkpoints, differentiation processes, apoptosis induction in tumor cells (Shanmugam et al. 2022)

(Fig. 13.3). Henceforth, HDAC are the prime drug target for treatment of different types of cancer such as cardiorenal, neurodegeneration, and psychiatric disorders. Suberoylanilide hydroxamic acid (SAHA) was the first commercially approved HDAC inhibitor cancer chemotherapy, and the crystal structure of the HDLP-SAHA complex was the first to reveal a hydroxamate-Zn^{2+} chelate complex in an HDAC-related deacetylase (Lombardi et al. 2011).

Two polyketides namely epicocconigrones A (**22**) and epicoccolide B (**23**) recovered from the endophytic fungus *Epicoccum nigrum* isolated from leaves of *Mentha suaveolens* collected in Morocco inhibited histone deacetylase (HDAC) activities with IC_{50} values of 9.8 and 14.2 μM, respectively (El Amrani et al. 2014). Depudecin (**24**) (Fig. 13.4), structurally like epoxyketone side chain, isolated from the fungus *Alternaria brassicicola* exhibited HDAC inhibitory activity with an IC_{50} of 4.7 μM (Kwon et al. 1998). Two new cyclotetrapeptides, trapoxins A (**25**) and B (**26**), were isolated from the culture broth of *Helicoma ambiens* RF-1023 (Itazaki et al. 1990)-inhibited HDAC enzyme.

2.5 DNA Polymerase

A number of compounds used for cancer chemotherapy exert their effects by inhibiting DNA replication. New inhibitors of DNA polymerases, therefore, could be potential candidates for new anticancer drugs. Two novel tetrols, namely nodulisporol (**27**) and nodulisporone (**28**), and 3,5-dimethyl-8-methoxy-3,4-dihydroisocoumarin (**29**) purified from a fungus *Nodulisporium* sp. selectively inhibited the activity of human DNA polymerase λ (pol λ) with an IC_{50} value of 168, 82 and 49 μM, respectively (Kamisuki et al. 2007). Kasanosins A (**30**) and B (**31**), novel azaphilones, recovered from the *Talaromyces* sp. derived from seaweed, selectively inhibited the activities of eukaryotic DNA polymerases β and λ (pols β and λ) in family X of pols. The IC_{50} values of kasanosins A (**30**) on rat pol β and human pol λ were 27.3 and 35.0 μM, respectively (Kimura et al. 2008). Penicilliosis A (**32**) and B (**33**) are novel 5-methoxy-3(2H)-furanones isolated from cultures of a fungus (*Penicillium daleae* K.M. Zalessky) derived from a sea moss. These compounds selectively inhibited the activities of eukaryotic Y-family DNA polymerases (pols) (i.e., pols η, ι, and κ). Mouse pol ι, belonging to mammalian Y-family pols, activity was most strongly inhibited by Penicilliosis A and B, with IC_{50} values of 19.8 and 32.5 μM respectively (Kimura et al. 2009).

Formosusin A (6Z,8E,10E)-variotin, a new cis-olefin analog of variotin (**34**) isolated from the cultures of the fungus *Paecilomyces formosus,* selectively inhibited the activity of mammalian DNA polymerase β (pol β) in vitro, with an IC_{50} of 35.6 μM (Mizushina et al. 2014). 1-deoxyrubralactone, a novel rubralactone derivative 6hydroxy-8-methoxy-1-methyl-1,2,3a,9b-tetrahydrocyclopenta[c] isochromene-3,5-dione (**35**) isolated from cultures of a fungal strain derived from sea algae, selectively inhibited the activities of families X and Y of eukaryotic DNA polymerases (pols). The IC_{50} values of isolated compound on rat pol β and human

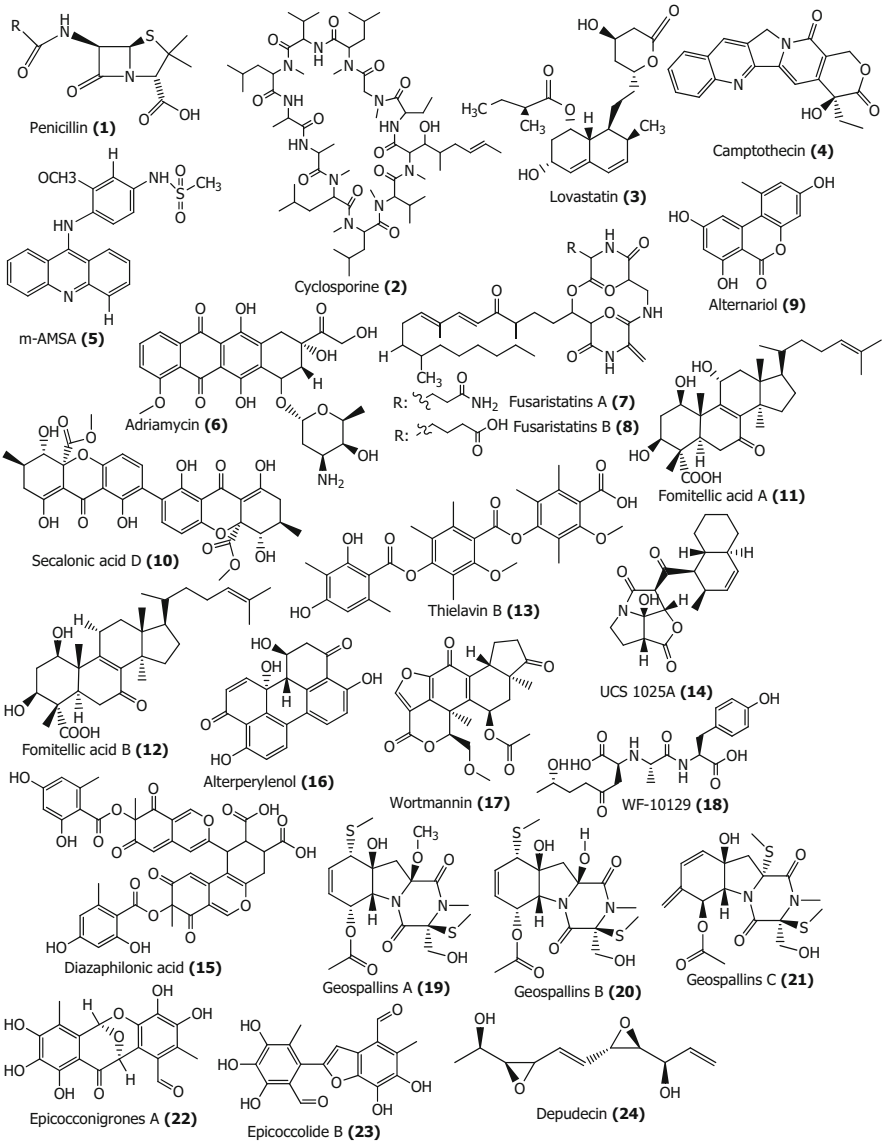

Fig. 13.4 Representative structure of bioactive fungal metabolites (**1–3**) and various fungal enzyme inhibitors of DNA topoisomerase (**4–12**), telomerase (**13–17**), angiotensin-converting enzyme (**18–21**), and histone deacetylase (**22–24**) exhibiting anticancer activity

pol κ were 11.9 and 59.8 μM, respectively (Naganuma et al. 2008). Hymenoic acid (**36**) is a natural compound isolated from *Hymenochaetaceae* sp.; the recovered compound is a novel sesquiterpene, trans-4-[(1′E,5'S)-5-carboxy-1′-hexenyl] cyclohexanecarboxylic acid. This compound selectively inhibited the activity of

human DNA polymerase λ (pol λ) in vitro, and 50% inhibition was observed at a concentration of 91.7 μM (Nishida et al. 2008). Two phenalenone-skeleton-based compounds were isolated from a marine-derived fungus *Penicillium* sp., sculezonone-B (SCUL-B) (**37**) and sculezonone-A (SCUL-A) (**38**), upon DNA polymerase activity. Both compound-inhibited bovine DNA polymerases α and γ moderately affected the activity of DNA polymerase ε. SCUL-A (**37**) inhibited pol β (IC50 = 17 μM); SCUL-B (**38**) has only a weak influence upon this polymerase ($IC_{50} = 90$ μM). The *Ki* values of SCUL-B (**38**) were calculated to be 1.8 and 6.8 μM for DNA polymerases α and γ, respectively. The *Ki* of DNA polymerase β for SCUL-A was 12 μM and that for DNA polymerase α, 16 μM (Perpelescu et al. 2002).

2.6 *Methionine Aminopeptidase 2 (Met AP-2)*

Methionine aminopeptidase belongs to the class of metalloprotease involved in eliminating the N-terminal methionine from newly synthesized proteins. The enzyme has two classes: Type 1 and Type 2 having a crucial role in co-translational regulation of protein synthesis. Type 2 (Met AP-2) class plays an important role in growth and development of new blood vessels, tumors, and metastasis and thus is a potent drug target in the anticancer therapy (Bradshaw 2004; Mauriz et al. 2010).

A natural compound, fumagillin (**39**), recovered from fungus *Aspergillus fumigates* (Hanson and Eble 1949) was found to strongly inhibit the angiogenesis process by blocking the enzymatic activity of Met AP-2 in an irreversible manner (Sin et al. 1997; Hou et al. 2009). Many of the derivatives of Fumagillin, viz. Ovalicin (**40**), cis-fumagillin (**41**), RK-805 (**42**) (Fig. 13.5), RK-95113 (**43**), TNP-470 (**44**), were found to be potent and specific inhibitors of Met AP-2 in different types of tumor cell types (Kwon et al. 2000). Ovalicin (**40**), a sesquiterpene purified from the fungus *Pseudorotium ovalis* (Sigg and Weber 1968), exhibited promising antitumor and immunosuppressive and anti-angiogenic activity (Corey et al. 1994). A novel compound cis-fumagillin methyl ester purified from the ethyl acetate extracted fermentation broth of *Penicillium janczewskii* strongly inhibited the Met AP-2 enzyme with an IC_{50} value of 6.3 nM (Kwon et al. 2000). Afterward, many of the fungal species were reported for the secretion of bioactive compounds capable of inhibiting the Met AP-2 enzyme in a specific manner. 5-Demethylovalicin (**45**) recovered from the fermentation broth of fungus *Chrysosporium lucknowense* inhibited the recombinant human Met AP-2 with an IC_{50} value of 17.7 nM) (Son et al. 2002). A study documented by Asami et al. 2006 reports the isolation of fungal species *Aspergillus fumigatus var. fumigatus* RK95–113 from soil collected in Wako, Saitama, Japan. The isolated strain RK95–113 was found to secrete a compound, RK-95113(**43**) with potent anti-angiogenic activity like that of fumagillin (**39**) and its derivatives.

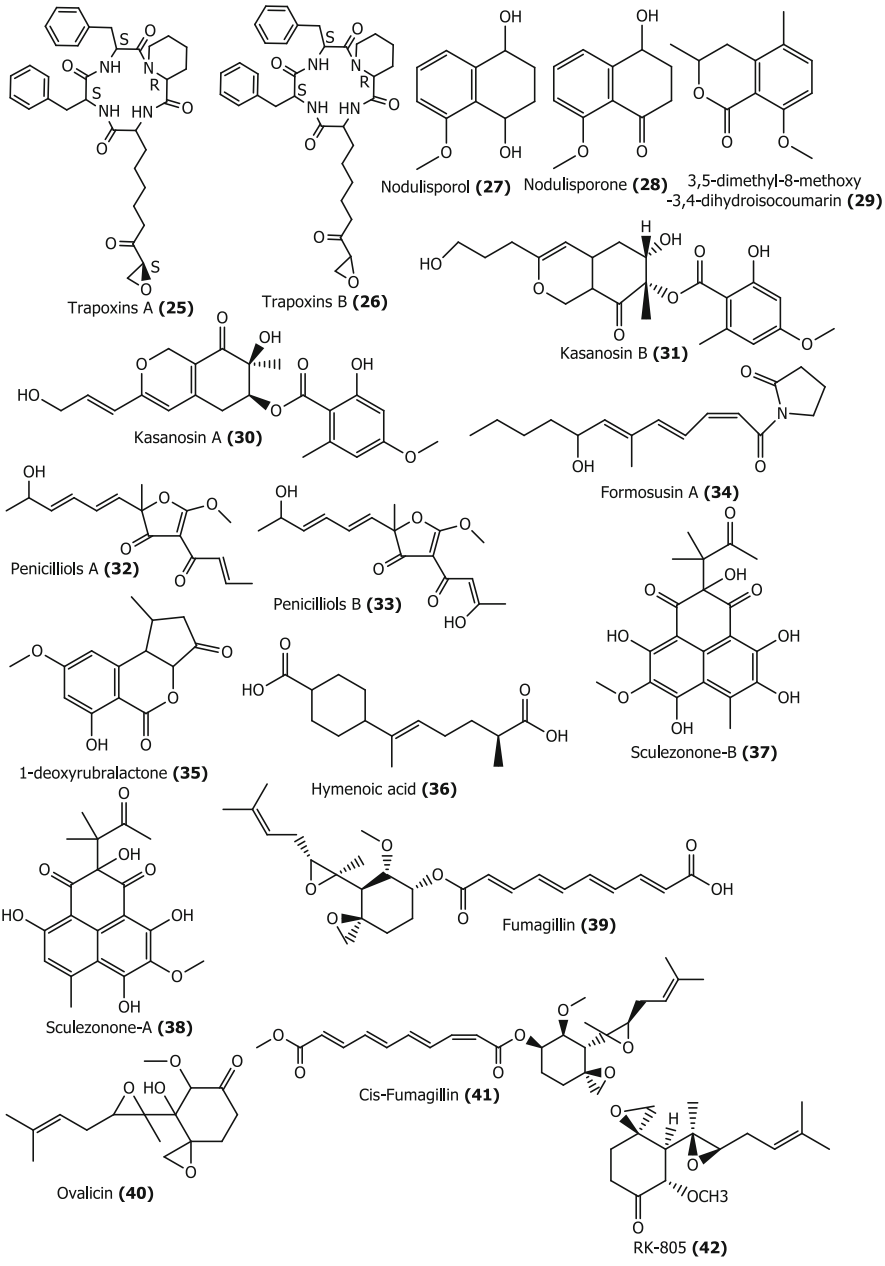

Fig. 13.5 Representative structure of fungal enzyme inhibitors with potent anticancer activity. Histone deacetylase (**25–26**), DNA polymerase (**27–38**), methionine aminopeptide-2 (**39–42**)

2.7 Caspase-1

Caspase-1, also known as interleukin-1-converting enzyme (ICE), belongs to class of cysteine protease responsible for converting interleukin-1ß to its mature form in monocytes. It acts as a bridging moiety between inherited and acquired inflammatory diseases and dysregulated inflammasome activity (Thornberry et al. 1992; Schroder and Tschopp 2010; Thul et al. 2017). The increased activity of caspase-1 enzyme along with chronic inflammatory episodes (Watson et al. 1998) has been reported to be associated with many devastating medical complications including obesity-dependent diabetes, multiple sclerosis, different types of cancers such as breast cancer, pancreatic cancer, acute leukemia, and melanoma (Dinarello 2011; Tu et al. 2008; Molla et al. 2020). Hence, caspase-1 inhibitors are considered to be potential drug target for cancer therapy, Alzheimer, osteoarthritis, rheumatoid arthritis, etc. (Rohn 2010; Zitvogel et al. 2012; Celardo et al. 2013; Shalini et al. 2015). The development of novel caspase-1 inhibitors not only will open up biomes for novel chemotherapeutics but will also pave the way for investigation of signal transduction pathways.

A total of 15 polyketide molecules were purified from *Penicillium rubrum* recovered from 270 m Pit Lake in Montana. Out of 15 compounds, berkazaphilone B (**46**) and C (**47**) inhibited caspase-1 enzyme with IC_{50} value of 25 μM and berkazaphilone A (**48**) and penisimplicissin (**49**) exhibited complete inhibitory action at a concentration of 250 μM (Stierle et al. 2012a). Two novel drimane sesquiterpene lactones, namely berkedrimanes A (**50**) and B (**51**), purified from the Berkeley Pit extremophilic fungus *Penicillium solitum* inhibited the signal transduction enzymes caspase-1 and caspase-3 by blocking the production of interleukin 1-β in the induced promonocytic leukemia cell line assay. Berkedrimanes A (**50**) exhibited IC_{50} value of 100 μM against caspase-1 and 200 μM against caspase 3 followed by berkedrimanes B (**51**) with IC_{50} value of 90 μM and 50 μM against caspase 1 and 3, respectively (Stierle et al. 2012b).

2.8 Cyclooxygenase (COX)

Cyclooxygenase (COX) is the vital enzyme involved in the biogenesis of prostaglandins, thereby playing a key role in the pathophysiological process of many debilitating health conditions such as inflammation and carcinogenesis. Out of the three isoforms of COX (COX-1, COX-2, and COX-3), the isoform COX-1 is constitutively expressed in normal tissues and is essential for regular physiological process by regulating the production of prostaglandins. The inhibition of COX-1 results in serious health complications such as ulcers of gastrointestinal tract (Brune and Patrignani 2015). However, the COX-2 enzyme is a major pro-inflammatory mediator which is overexpressed in response to any inflammatory event (Khan et al. 2007) and carcinogenesis (Wang and DuBois 2010; Vosooghi and Amini 2014;

Mohsin et al. 2022). COX-2 enzyme contributes to the progression of carcinogenesis by inhibiting the process of apoptosis, increasing the rate of angiogenesis, invasion, and metastatic effects of cancer cells (Tsujii et al. 1998; Jiang et al. 2001; Sánchez-Alcázar et al. 2003; Hashemi Goradel et al. 2018). Many studies have documented the chronic overexpression of COX-2 in various premalignant, malignant, and metastatic forms of different cancers, viz. lung, colorectal (Sheng et al. 2020), prostate (Gupta et al. 2000), breast (Bhutani et al. 2021), pancreatic (Hu et al. 2017), esophageal, and skin malignancy (Moon et al. 2020). Many nonsteroidal anti-inflammatory drugs (NSAIDs) and natural products are reported to inhibit the COX-1 and COX-2 enzyme to reduce the prostaglandin synthesis, thereby blocking the progression of cancer and induce the metastasis (Steinmeyer 2000). Hence, the vigorous research is underway from various natural resources in search of novel inhibitors of COX-2 enzyme, being a prime drug target for the cancer treatment.

There are few reports providing the evidence of discovery of novel COX-2 inhibitors from various fungal species. Botryoisocoumarin A (3S-5, 8-dihydroxy-3-hydroxymethyldihydroisocoumarin) (**52**), a novel mellein derivative, is purified from the ethyl acetate extract of endophytic fungus *Botryosphaeria* sp. KcF6 inhabiting the inner fruit part of the mangrove plant *Kandelia candel exerts promising* COX-2 inhibitory activity with an IC_{50} value of 6.51 μM (Ju et al. 2015). An enantiomeric meroterpenoid, (±)-Lucidumone (**53**), is purified from the *Ganoderma lucidum* exhibited significantly potent COX-2 inhibitory profiles. The (−)-Lucidumone (**54**) exerted the selective and potent inhibition of COX-2 by directly binding with an amino acid residue of Tyr385 with an IC_{50} value of 5.62 μM as compared to (+)-Lucidumone (**53**) with IC_{50} value of 25.95 μM (Yan et al. 2019). The research happening in this domain led to the discovery of alternaritin B (**55**) and alternaritin C (**56**) (Fig. 13.6), two novel polyketides and isoxanalteric acid I (**57**), from the ethyl acetate extracts of endophytic fungi *Alternaria* sp. MG1 inhabiting *Vitis quinquangularis* (Wu et al. 2019) with inhibitory activities against COX-2 enzyme. Alternaritin B (**55**) exhibits significantly potent COX-2 inhibition with IC_{50} value of 1.0 μM in conjunction to previous studies. However, alternaritin C (**56**) and isoxanalteric acid I (**57**) inhibited COX-2 with IC_{50} value of 7.0 μM and 7.0 μM, respectively, which was comparable to the previous reports (Tian et al. 2021). Different lanostane triterpenoids from various fungal species, viz. *Ganoderma lucidum, Poria cocos, Laetiporus sulphureus, Inonotus obliquus, Antrodia camphorata*, and *Daedalea dickinsii*, have also been reported to exhibit the COX-2 inhibition (Ríos and Andújar 2015). Ganoderic acids (**58**) from *Ganoderma lucidum* exerts the inhibition of cyclooxygenase (COX)-2 by inducing the expression of heme oxygenase (HO)-1 through the PI3K/AKT-nuclear factor 2 (Nrf2) pathways (Choi et al. 2014). Fomitopinic acids A (**59**), fomitopinic acid B (**60**), and their glycosides named fomitosides A-J (**61–70**) from *Fomitopsis pinicola* also demonstrated the inhibition of COX-1 and COX-2 in a prostaglandin biosynthesis assay in vitro (Yoshikawa et al. 2005).

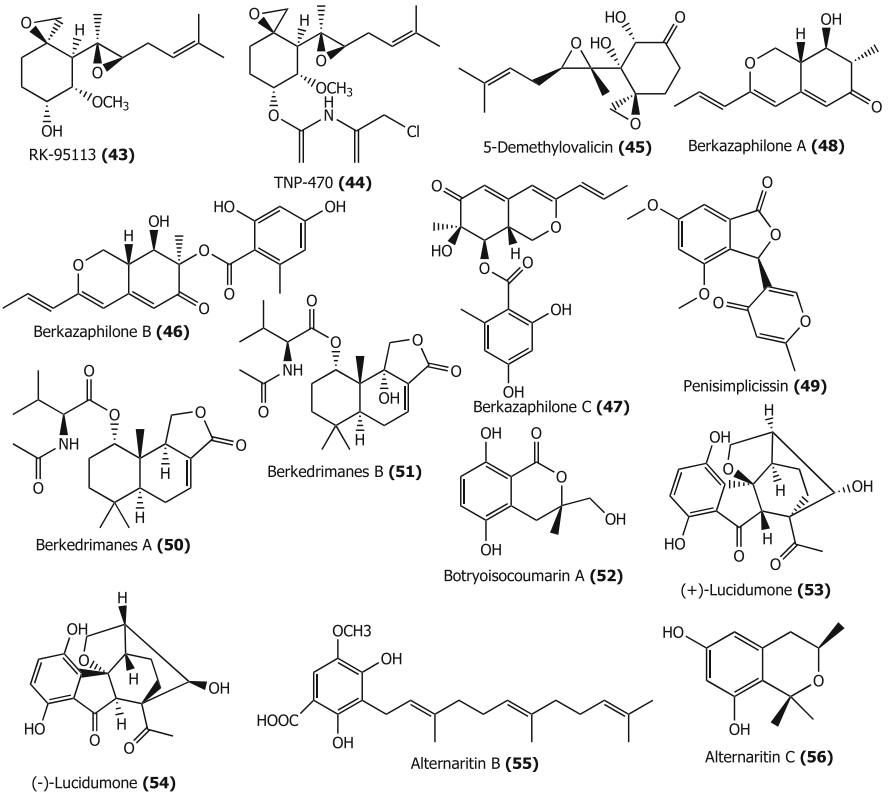

Fig. 13.6 Representative structure of fungal enzyme inhibitors with potent anticancer activity. Methionine aminopeptide-2 (**43–45**), caspase-1 (**46–51**), COX-2 (**52–56**)

2.9 Phosphatidylinositol 3′ Kinases (PI3Ks)

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that play a key role in regulating diverse cellular processes such as cell cycle progression, growth, angiogenesis, migration, cellular metabolism adhesion, survival, and motility (Akinleye et al. 2013). PI3Ks are considered to be a significant secondary messenger and plays imperative role in PI3K/AKT/mTOR signaling pathway (Osaki et al. 2004). The PI3Ks utilize phosphorylated phosphatidylinositols as an intermediate molecule to transmit signals from the cell surface to the cytoplasm leading to stimulation of several effector kinase pathways, including BTK, AKT, PKC, NF-kappa-B, and JNK/SAPK pathways and results in stable persistence and growth of normal cells. Being a crucial player in averting apoptosis and prompting normal cell proliferation, the imbalance in PI3K signaling pathway is utilized by cancer cells for enlarged proliferative ability, resistance to apoptosis, tissue invasion, and metastasis (Shaw and Cantley 2006). PI3K has been recognized as an attractive molecular target for novel anticancer molecules (Arcaro and Guerreiro 2007). In the last few years,

several classes of potent and selective small-molecule PI3K inhibitors have been developed, and at least 15 compounds have progressed into clinical trials as new anticancer drugs (Yang et al. 2019a, b).

Wortmannin (**71**), a furanosteroid metabolite purified from *Penicillium wortmannin* (Brian et al. 1957), is an inhibitor of phosphatidylinositol 3′ kinases (PI3Ks) and phosphatidylinositol 3′ kinase-related kinases (PIKKs), such as DNA-dependent protein kinase (Powis et al. 1994). Cyclo (L-leucyl-L-leucyl) and brevianamide F (**72**) isolated from *Colletotrichum gloeosporioides* (an endophytic fungus from *Uncaria rhynchophylla*) exhibited potent PI3K α inhibitory activity with IC_{50} values of 38.1 and 4.8 μM, respectively (Yang et al. 2019a, b). *Trichoderma Viride*, producing viridin, was shown to exhibit irreversible PI3K inhibition with IC_{50} of 5 nM. Interestingly, LY294002 (Vlahos et al. 1994) used extensively as a tool to inhibit PI3K was developed on the basis of quercetin, a natural flavanoid. Further, kaempferol (**73**) produced by *Fusarium chlamydosporum* inhabiting *Tylophora indica* induced G2/M cell cycle arrest in cancer cells by inhibiting PI3K pathway (Chaturvedi et al. 2014). Similarly, alkaloid dehydronotoamide C (**74**) (Fig. 13.7) produced by *Aspergillus versicolor* inhabiting *Casearia balansae* inhibited proliferation in cancer cells by abrogating PI3K (Dietschmann et al. 2022). Thus, PI3K has been at the center of prime targets for discovering novel cancer therapeutics (Sabbah et al. 2021).

3 Concluding Remarks

Developing novel cancer therapeutics paradigm has shifted from chemically synthesized compounds to drug discovery from natural origin. Pleiotropic side effects of conventional therapies have been a roadblock in the efficient management of cancer. Thus, a strategy focusing on the selective inhibition of the key enzymes pivotal for cancer growth has been at the focal point of drug discovery. Plant and microbial sources have been a repository of medicinal chemistries. Among the pool, the past two decades have seen the endophytic fungi as a putative repository to harness novel chemistries with higher therapeutic gain. The present chapter provides a comprehensive overview of the key enzymes that are being targeted for discovering novel selective inhibitors produced by endophytic fungi. Recent advances in genome mining and systems biology may further pave the way for the efficient transition of the investigated fungal enzyme inhibitors from bench to clinic. The denouement of harnessing putative endophytic fungi-derived secondary metabolite as an ideal anticancer drug is a promising feat in the green drug revolution.

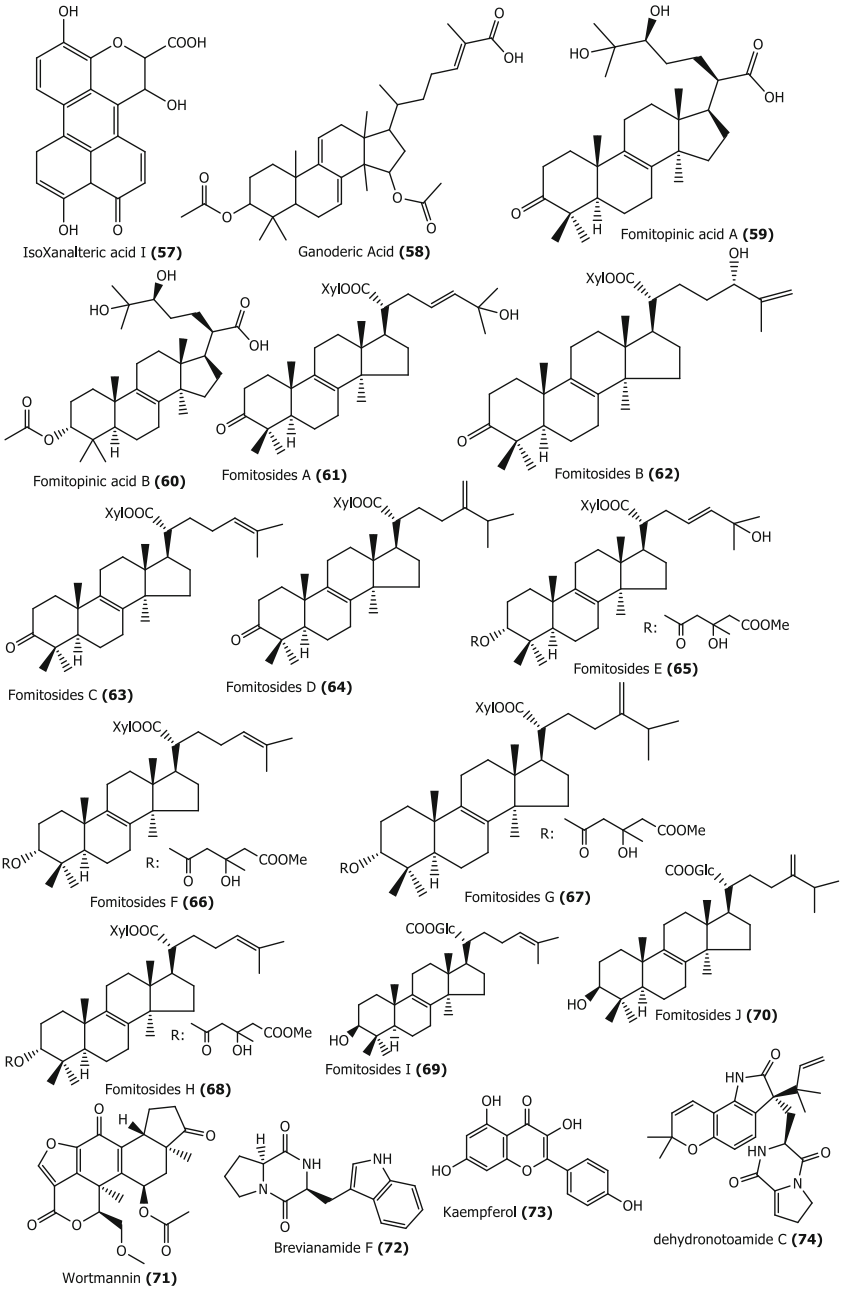

Fig. 13.7 Representative structure of fungal enzyme inhibitors with potent anticancer activity. COX-2 (**57–70**), phosphatidylinositol 3′ kinases (**71–74**)

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Chapter 14
Bioactive Metabolites from Fungi with Anti-Inflammatory and Antithrombotic Properties: Current Status and Future Perspectives for Drug Development

Alexandros Tsoupras and Kyeesha Glenn Davi

Abstract Bioactives of natural origin have recently gained a lot of ground in drug discovery due to their enormous structural diversity, diverse pharmacological activities, safety, and inherent binding capacity with other biomolecules. However, the high demand for new agents for the prevention and therapy of inflammation-related chronic disorders has not yet been sufficiently addressed by the drug discovery process. This gap highlights the relevance of intensifying studies to reach sustainable employment of the huge world biodiversity, such as microorganisms, including fungi of biotechnological and agro-food medical interest that can be easily cultured/engineered as sustainable sources of natural bioactives for drug development. Thus, fungi are important sources of such bioactive compounds studied and applied for different purposes, specifically, in the pharmaceutical area, such as the development of antibiotics, immunomodulators, immunosuppressants, enzyme inhibitors, and antiviral, hypercholesteremic, antineoplastic/antitumor, and anti-inflammatory agents. Within this chapter, several examples of bioactive metabolites produced by different fungi species with proven pharmacological effects and beneficial anti-inflammatory and antithrombotic potential are thoroughly reviewed. After recent updates in the field of "omics" and "one strain many compounds" (OSMAC) approaches, within this chapter the emerging use of fungal endophytes as strong unconventional sources of biologically active natural compounds with many producing pharmacologically valuable specific plant-derived anti-inflammatory products is also presented. Overall, this chapter explores the current and future perspectives of the use of fungal metabolites with potent anti-inflammatory and antithrombotic properties and subsequent health benefits with potential pharmaceutical applications as supplements and/or drugs.

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1 Introduction

Inflammation is a complex biological reaction by which the human body responds to tissue damage and to various harmful stimuli, including toxic compounds, damaged cells, and infectious agents and pathogens. In addition, inflammation plays a vital role in tissue repair and regeneration toward homeostasis (Tsoupras et al. 2018). Generally, acute and controlled inflammation is beneficial, while unresolved and chronic inflammatory response can occur due to the continuous and unresolved presence of the triggering agent(s) and risk factors, and due to a subsequent inappropriate immune response, that usually results in further tissue injury, destruction, and several other harmful inflammatory manifestations (Tsoupras et al. 2018).

Thus, it has now been well established that unresolved and chronic inflammation and its thrombo-inflammatory manifestations are major causes of several chronic disorders (Tsoupras et al. 2018; Furman et al. 2019), such as atherosclerosis and cardiovascular diseases (CVD) (Tsoupras et al. 2018, 2019; Furman et al. 2019), renal (Tsoupras et al. 2007, 2018), and neurodegenerative disorders (Tsoupras et al. 2018), tumor and metastatic procedures (Tsoupras et al. 2018; Tsoupras et al. 2009; Lordan et al. 2019), asthma, allergy and several autoimmune diseases (Tsoupras et al. 2018), and persistent infections, including HIV infection (Tsoupras et al. 2012a), SARS-COV-19 infection (Tsoupras et al. 2020a; Zabetakis et al. 2020), periodontitis (Tsoupras et al. 2006; Antonopoulou et al. 2003), sepsis (Tsoupras et al. 2011a), and their associated inflammatory conditions and comorbidities (Tsoupras et al. 2018). Interestingly, the presence of one or more of such pathological conditions further triggers and propagates the co-development of other ones through further inflammatory activation and a subsequent vicious cycle of inflammatory, thrombotic, and oxidative manifestations (Fig. 14.1) (Tsoupras et al. 2018, 2019, 2007, 2009, 2012a, 2020a, 2006, 2011a; Furman et al. 2019; Lordan et al. 2019; Zabetakis et al. 2020; Antonopoulou et al. 2003).

Several compounds of natural and/or synthetic origin with anti-inflammatory actions have been extensively studied for potential benefits and amelioration of the inflammatory burden in these disorders, with natural bioactives being on the hotspot lately, especially for the food/feed supplements and drug industries (Tsoupras et al. 2018, 2007, 2009, 2020a, b, 2022a, b, c, 2011b; Lordan et al. 2019; Zabetakis et al. 2020, 2022; Conde et al. 2021; Glenn-Davi et al. 2022; Verouti et al. 2013; Karantonis et al. 2022). Natural products have been relevant sources for drug discovery and the development of medicines since ancient times. Several such bioactive compounds of natural origin have been found in plants, animals, or marine organisms, and microorganisms of different environments (aquatic, terrestrial, or air), with emphasis being given to natural bioactives derived from sustainable sources on a circular economy approach.

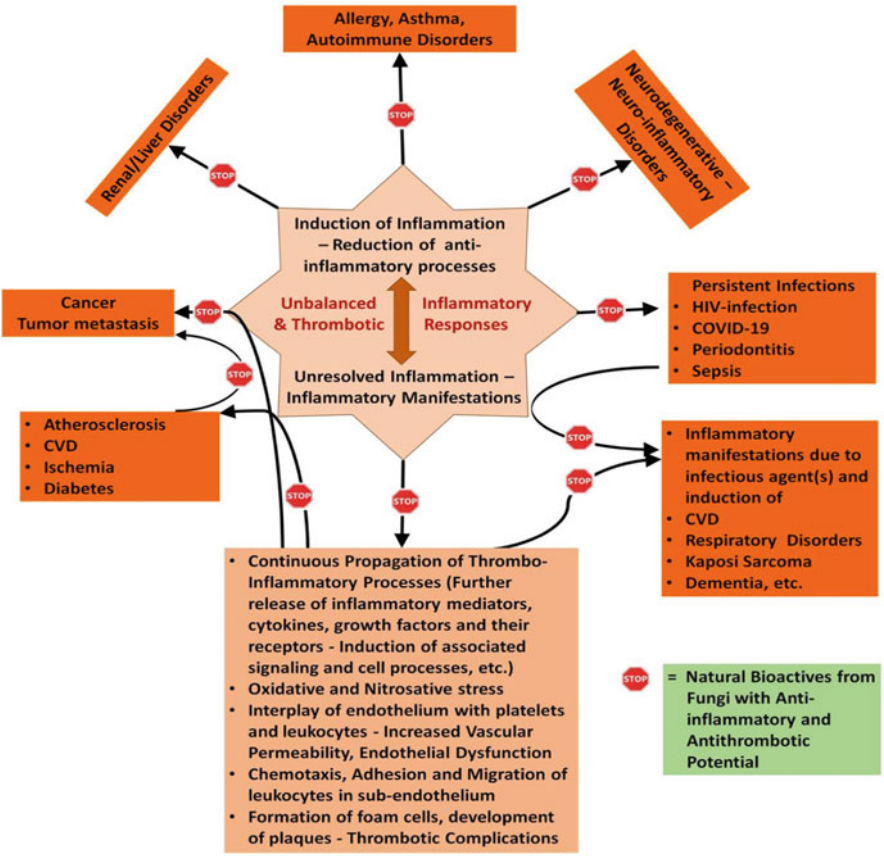

Fig. 14.1 Unbalanced inflammatory and thrombotic responses due to the presence/co-presence of several risk factors can lead to continuous and unresolved inflammation with subsequent thrombo-inflammatory manifestations that are implicated in the initiation, propagation, and development of several inflammation-related chronic disorders. Such thrombo-inflammatory processes are also implicated in the induction of a chronic disorder(s) due to the unresolved presence of another one of these disorders. Natural bioactives, and especially those from microorganisms of biotechnological and agro-food-medical interest, including fungi bioactives with anti-inflammatory and antithrombotic properties are potential candidates for drug development against inflammation, thrombosis, and the prevention of inflammation-related chronic disorders

Among these natural sources, the cultivation of microorganisms as sustainable sources for isolating their bioactive metabolites has gained ground in the drug and supplements industries. Attention has been given to microorganisms with low toxicity and well-established tolerance in human nutrition and other non-toxic applications, not only well-established ones used in fermentation processes, including yeasts for the production of fermented foods (Moran et al. 2021; Fragopoulou et al. 2004) and bacteria for bioethanol production (Tsoupras et al. 2012b), but also microorganisms used in other fields, such as fungi of bio-agro/food technological

applications like those used as natural entomopathogenic agents for organic cultures (Tsoupras et al. 2022b).

Fungi are a group of heterotrophic and essentially aerobic, with limited anaerobic capabilities, and eukaryotic microorganisms (McGinnis and Tyring 1996). Fungi can occur as yeasts, molds, or as a combination of both forms. Yeasts are microscopic fungi consisting of solitary cells that reproduce by budding. Molds, in contrast, occur in long filaments known as hyphae, which grow by apical extension. Hyphae can be sparsely septate to regularly septate and possess a variable number of nuclei. Regardless of their shape or size, fungi are all heterotrophic and digest their food externally by releasing hydrolytic enzymes into their immediate surroundings (absorptive nutrition). Other characteristics of fungi are the ability to synthesize lysine by the L-α-adipic acid biosynthetic pathway and possession of a chitinous cell wall, plasma membranes containing the sterol ergosterol, 80S rRNA, and microtubules composed of tubulin.

Apart from these general properties of fungi, lately they have been proposed as a promising sustainable source of an enormous number of natural bioactives, which have made significant contribution to almost each sphere of human, plant, and veterinary life. Natural compounds obtained from fungi have proved their value in nutrition, agriculture, and healthcare. Primary metabolites, such as amino acids, enzymes, vitamins, organic acids, and alcohol, are used as nutritional supplements and in the production of industrial commodities through biotransformation (Singh et al. 2017). In addition, highly bioactive fungi compounds have also been found to be some secondary metabolites (SMs) of fungi, meaning organic biomolecules with a low molecular weight that form at the end or near the stationary phase of growth, and are not directly associated with growth, development, and reproduction of fungi. SMs are not only essential for the growth and development of their microbial producer, but they can also increase the tolerance of microorganisms to different types of environmental stresses and hostile conditions and, consequently, their survival rate. On this accord, and since the unanticipated discovery of antibiotic penicillin by Fleming in 1929, this has drawn the interest of scientists to investigate the therapeutic role of microbial products not only for combating life-threatening infections as antimicrobial and antiparasitic agents, but also for several other healthcare applications, such as their use as antitumor, anti-inflammatory, anticoagulant and antithrombotic agents, enzyme inhibitors, anabolics, hemolytics, hypocholesterolemics and vasodilators, anesthetics, anthelmintics, and immunosuppressants (Singh et al. 2017).

Bioactive SMs are produced mainly by fungi of soil microbiota, marine environments, or extreme environments, but also of underexplored niches, including plant-associated fungi called endophytes, which live in symbiotic relationships with host plants (Conrado et al. 2022). They usually are obtained by extraction and other separation procedures, and they are primarily used in the biopharmaceutical industry and as ingredients in functional foods due to their capability to reduce inflammatory and infectious diseases in human beings and animals and thus increase life expectancy (Singh et al. 2017; Conrado et al. 2022).

Health care is the largest end-user market for microbes like fungi and such beneficial microbial products, which reflects the importance of microbe-based biopharmaceutical industry (Singh et al. 2017). To date, among the approved SMs by the US Food and Drug Administration (FDA) for drug discovery, ~ 15% belongs to microbial origin. In microbial groups of fungi and their products, for example, enzymes, fermented foods, animal feed, antibiotics, pharmacy products, and pigments have contributed significantly to humans and many biotechnological industries, as well as for several other applications such as the use of SMs from a basidiomycetous fungus, for example, *Ganoderma lucidum* and *Trametes versicolor*, as flavoring compounds, in flavored coffee, and in cosmeceutical products (Shankar and Sharma 2022). In addition, recent research has demonstrated that among fungi species, the endophytes are a great source of bioactive SMs. These microorganisms have functional diversity and, in association with plants, can produce a plethora of SMs (Conrado et al. 2022).

Fungi-derived nutrients and bioactive compounds have great potential as ingredients of functional foods, supplements, and drugs, since they can exhibit various beneficial effects along with medicinal properties and additional health benefits, including anti-inflammatory effects. From the several applications of fungi metabolites within this chapter, the current status of the anti-inflammatory and antithrombotic benefits of several fungi bioactives will be extensively reviewed, with emphasis on compounds with potential use in the development of supplements and drugs for the prevention and treatment of a range of inflammatory disorders and associated chronic diseases.

2 Fungi as Sustainable Sources for Bioactive Compounds of Natural Origin

Fungi are a miscellaneous group of organisms comprising several beneficial and damaging biological activities, which have a major impact on flora and fauna of the biome. For example, fungi are known to produce some potent antibiotics such as penicillin and cephalosporin. On the other hand, some fungal species are able to synthesize and excrete extremely toxic metabolites, such as aflatoxins, curvularin, and helminthosporin. These filamentous fungi show huge diversity in their metabolites including lipids, polyketides, alkaloids, quinones, and carotenoids. The metabolic diversity of fungal genera is extraordinary, making it possible for them to produce metabolites with various biological activities such as antioxidant, anticancer, antiviral, antimicrobial, and anti-inflammatory (Mehta et al. 2022).

The optimum extraction of such natural bioactives from fungi can be performed by controlling and optimizing their growth conditions and using low-cost and fast approaches, thus representing an economically valid and biosustainable alternative production of natural bioactives for the development of supplements and drugs. Usually, the isolation of a fungi strain for the development of a pharmaceutical

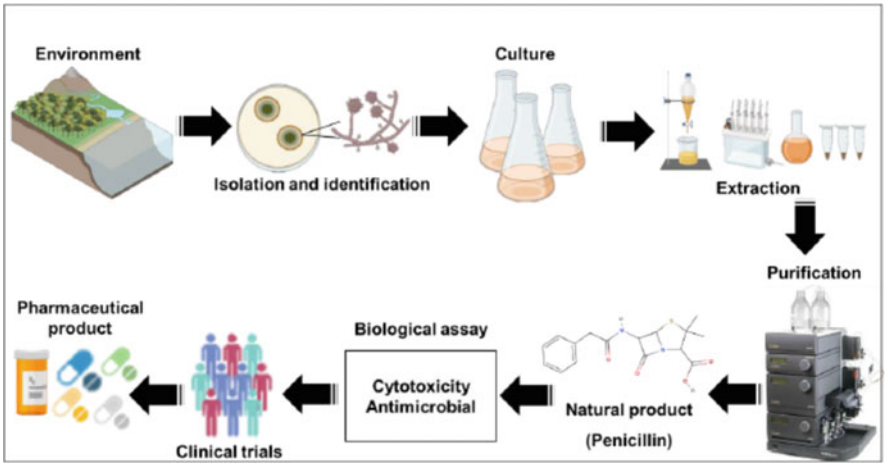

Fig. 14.2 Representation of fungal strain isolation and development of medicines containing natural products as the bioactive component. Penicillin is being used just as an example. Reproduced from Conrado et al. (2022)

product begins with the isolation of the fungus strain from diverse environmental sources (e.g., water, plants, and soil) and the culture of the microorganisms. The product of the culture is processed for extraction and purification of fungi metabolites. Subsequently, biological assays and clinical trials are performed to define the possibility of future pharmaceutical applications of the SM (Fig. 14.2).

However, there are several limitations and drawbacks in this field, such as the handling and isolation of new strains. There are several well-described isolation processes of fungi, including that for endophytes; however, there is some challenge in isolating new fungi species, mainly due to the current techniques applied for the cultivation and strain purification that usually have remained the same in the last years. For obtaining new species, it is mandatory to design new approaches that are able to simulate the condition of the natural habitat from which the microorganisms were isolated. In this aspect, it is essential to overcome challenges related to growing conditions such as pH, temperature, nutrients, and preservation in the laboratory. Fungal culture for obtaining its bioactive metabolites can be performed in either solid-state fermentation (SSF) or submerged fermentation (SmF). The first one, on a solid substrate, can produce a high concentration of metabolites. The SmF occurs in aqueous liquid nutrient media, and it is the most widely used due to the ease of control of fermentation parameters such as pH, temperature, dissolved oxygen, and types of culture media (Conrado et al. 2022).

Also, there is an ongoing search for highly productive fungi for desired compounds followed by their strain improvement through epigenetic modulations, mutations, and genetic engineering to make them suitable for industrial applications. Furthermore, the elucidation of their complete biosynthesis route is more than needed, including all the enzymes and related genes involved through "omics"—

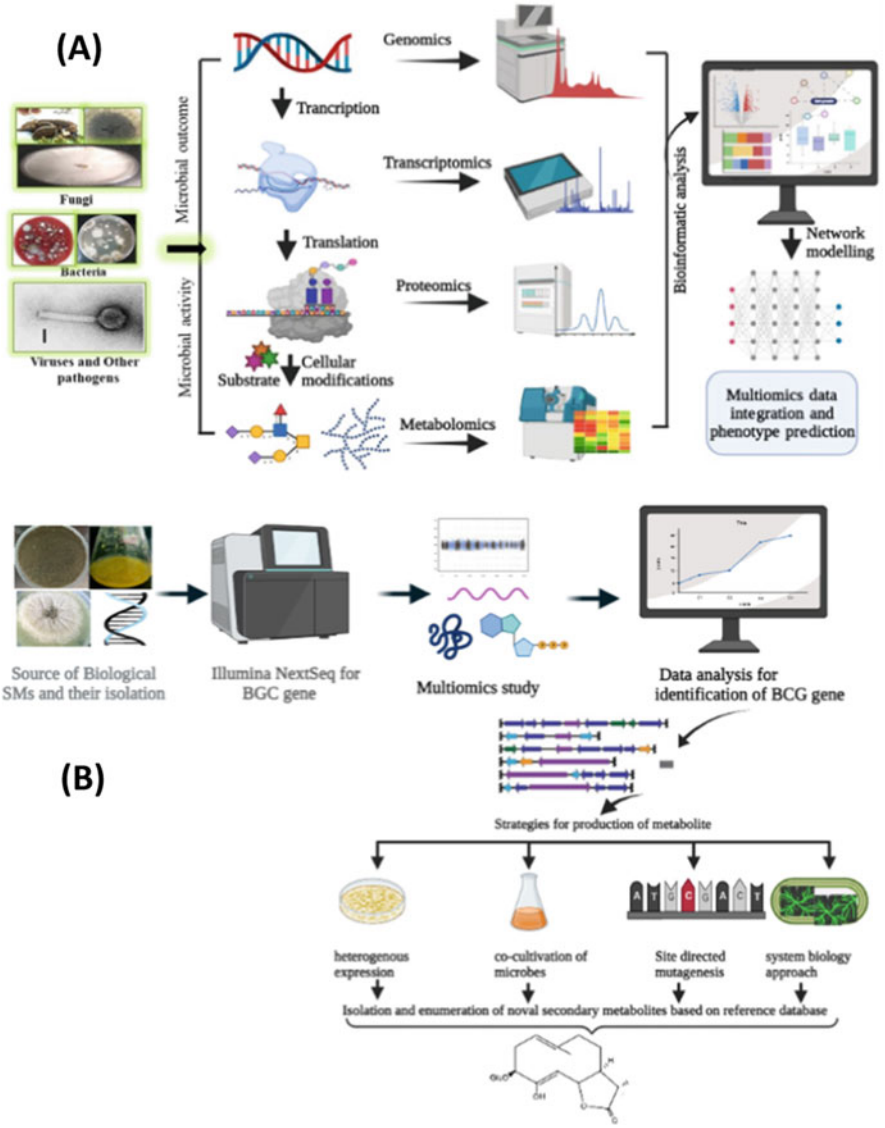

Fig. 14.3 (**a**) Flow chart depicts multi-omic tool with data integration and artificial intelligence modeling used for fungal metabolite studies. (**b**) The use of multi-omics for screening and isolating novel fungal secondary metabolites. Reproduced from Shankar and Sharma (2022)

genomics, transcriptomics, proteomics, and metabolomics—to regulate and manipulate the biosynthesis process for improved productivity (Fig. 14.3). Alternatively, the identified biosynthetic pathway of the bioactive compounds can be assembled and mimicked in convenient systems, offering an approach to produce target compounds with ease (Shankar and Sharma 2022; Singh et al. 2021).

Once the limitations for microorganism isolation, culture, genetic manipulation, and characterization through omics of the biosynthetic route have been overcome, the next step is to proceed with extraction, purification, physicochemical characterization of the SMs, structural elucidation, and metabolomics with multi-omics, along with bioassay techniques for evaluating their bioactivities and structure–activity relationships. In addition, new and advanced omic approaches are needed to screen newer fungi, increase the pool of novel metabolites with their molecular interaction, and exploit the fungal cultures with their metabolic capacity (Fig. 14.3) (Shankar and Sharma 2022). All these processes are very well-described and widely applied in natural product research and have enabled the rapid characterization of valuable novel fungi-derived natural products, which along with cytotoxicity tests and targeted clinical trials have allowed the appropriate design, development, and release in the market of drugs and supplements containing fungi metabolites (Figs. 14.2 and 14.3).

3 Fungi Bioactive Metabolites with Anti-Inflammatory and Antithrombotic Properties as Candidates for the Development of Supplements and Drugs

Within this section, the most important and promising fungi metabolites with anti-inflammatory and antithrombotic potential that are/can be used for the development of supplements and drugs against inflammation-related disorders are presented. These are fungi-derived vitamins, carotenoid-colored metabolites, phenolic compounds/pigments, lipid bioactives, alkaloids, terpenes, polyketides, and several other bioactive fungi metabolites, which possess various biological activities including anticancer, anti-inflammatory, antithrombotic, antimicrobial, and antioxidant potential against chronic disorders.

3.1 Fungi-Derived Vitamins and Bioactive Carotenoid-Based Colored Pigments

Micronutrients known as vitamins are crucial for sustaining the body's normal physiological functions and are required in small quantities. As mammals are unable to produce these essential nutrients, it is imperative to obtain them through dietary supplementation from external sources to ensure balanced metabolism in all living organisms (Shimizu 2001; Gupta and Gupta 2015). Certain vitamins act as coenzymes that assist in the biochemical reactions catalyzed by enzymes. For example, vitamin K is essential for normal blood clotting and plays a vital role in activating receptors that facilitate transcription mechanisms in bone tissues, making it a valuable treatment option for osteoporosis (Berg et al. 2002; Bolander 1997). The

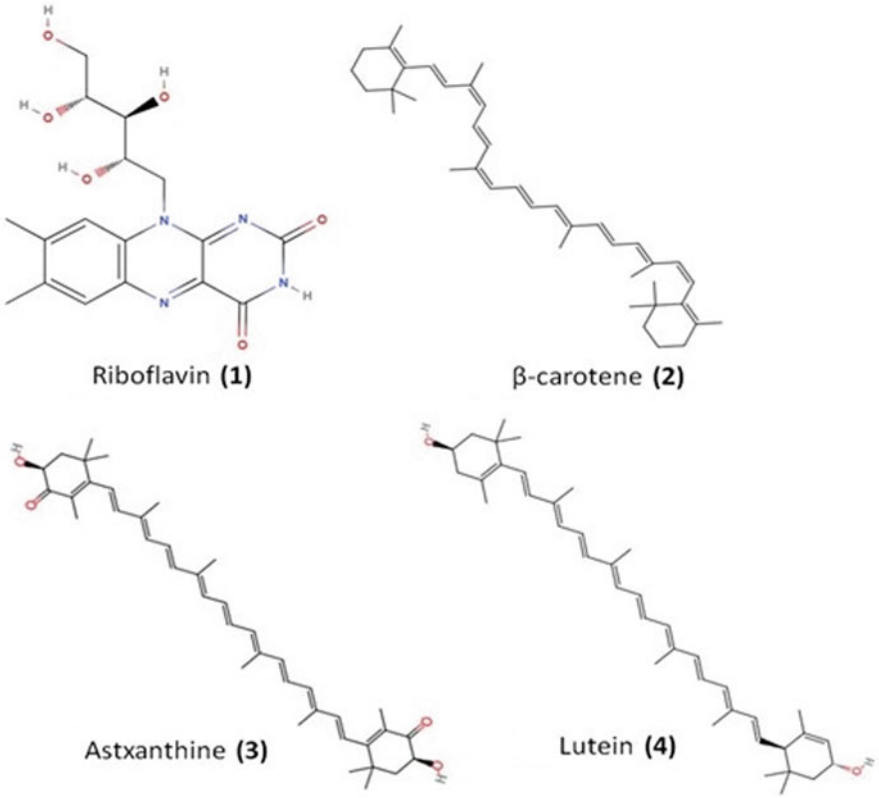

Fig. 14.4 Chemical structures of natural fungi vitamins and bioactive carotenoid pigments with anti-inflammatory potential. Structures were obtained from https://molview.org/ (accessed on April 2, 2023)

presence of vitamin A is necessary as a precursor to rhodopsin and other visual pigments, and it also plays a role in activating specific gene transcription processes that aid in growth and development (Berg et al. 2002). Microorganisms, such as fungi, naturally produce vitamins during regular metabolism, which are commonly used as food additives, health supplements, and therapeutic agents (Singh et al. 2017). To commercially produce fungi-derived vitamins, suitable fungi can be utilized in direct fermentation or through a combination of chemical and microbiological processes (Shimizu 2001; Bhalla et al. 2007). Characteristic examples of industrial production of vitamins from several fungi species are riboflavin (**1**) and β-carotene (**2**) (Fig. 14.4) (Bhalla et al. 2007; Demain 1999; Survase et al. 2006; FAO/WHO. 2001; Wang et al. 2012a; Mata-Gómez et al. 2014).

Vitamin B2, also known as riboflavin, is a water-soluble vitamin that is necessary for growth and reproduction. A lack of this essential vitamin can lead to cheilosis and dermatitis in humans. *Eremothecium ashbyii* and *Ashbya gossypii*, which are closely related ascomycetes, are commonly utilized microorganisms for the fermentative

production of riboflavin (Demain 1999; Survase et al. 2006). Of the two, *A. gossypii* is the preferred choice due to its remarkable efficiency in producing riboflavin. It can generate up to 40,000 times the amount of vitamin necessary for its own growth (Survase et al. 2006).

Provitamin A, called β-carotene, is necessary for healthy growth, reproduction, and vision. Night blindness, changes in the skin, and mucosal membranes are all brought on by a vitamin A deficiency or malabsorption (FAO/WHO. 2001). The formation of β-carotene involves the fungi *Blakeslea trispora*, *Phycomyces blakesleeanus*, *Mucor circinelloides*, *Rhodotorula* spp., and *Choanephora cucurbitarum*. Due to their high yield of β-carotene, *Blakeslea trispora* and *Phycomyces blakesleeanus* are chosen and employed for submerged fermentation in industrial production (Wang et al. 2012a; Mata-Gómez et al. 2014).

Carotenoid-based bioactive pigments have been found in a variety of fungi, primarily those found in marine habitats, while engineered fungi such as yeasts can also be used for their production in industrial scale (Elbandy 2022; Galasso et al. 2017; Elbandy et al. 2009; Takahashi et al. 2020). Even though microalgae are the primary source of carotenoids used in industry, however, alternative organisms like fungi may be of use in other fields such as the cosmetics or pharmaceutical industries (Ambati et al. 2014; Maoka 2011; Corinaldesi et al. 2017). Thus, while the focus of carotenoid production has been on algal sources, marine fungi and pigmented yeasts can also be used for de novo synthesis of such biomolecules (Corinaldesi et al. 2017).

More specifically, several carotenoids are synthesized by various yeast species that have been isolated from the marine environment. Notably, the genera *Xanthophyllomyces*, *Rhodotorula*, and *Phaffia* have been utilized to produce one of the most bioactive carotenoids used in supplements, astaxanthin (**3**) (Fig. 14.4) (Ambati et al. 2014). Despite yeasts and bacteria yielding smaller quantities of astaxanthin compared to algae, they have the advantage of quicker rates of growth and more straightforward cultivation techniques (Mata-Gómez et al. 2014; Bumbak et al. 2011). Marine organisms, notably fungi and prokaryotes, exhibit tremendous potential for the creation and commercialization of novel antioxidant compounds and carotenoids, which can be used in a variety of industries (Corinaldesi et al. 2017). Marine carotenoids offer numerous advantages due to their potent antioxidant, repairing, antiproliferative, and anti-inflammatory properties. They can be employed to provide skin photoprotection against the detrimental impact of solar UV radiation or as nutraceutical/cosmeceutical components to safeguard against oxidative stress-induced diseases (Nichols and Katiyar 2010; Berthon et al. 2017; Gonzalez et al. 2011).

Fungi-derived α- and β-carotene (**2**), lutein (**4**), and astaxanthin (**3**) are examples of exogenous antioxidants that are crucial in preventing oxidative damage caused by free radicals via their scavenging activity (Birben et al. 2012; D'Orazio et al. 2012). These compounds have lipophilic properties, which allow them to act as a natural defense for both marine and earth-bound plants by crossing the cellular membrane. Their ability to cross the blood–brain barrier allows them to be biologically active in organs such as the brain (Elbandy 2022).

Di Tomo et al. (2012) found that the addition of carotene and lycopene was able to reduce TNF-α induced inflammatory response in human umbilical vein endothelial cells (HUVECs). These antioxidant molecules inhibited monocyte (U937)–endothelial adhesion while also limiting gene expression of the vascular adhesion proteins, VCAM-1, ICAM-1, and E-selectin. Lycopene and β-carotene helped sustain nitric oxide (NO) bioavailability resulting in control of the cellular redox environment (Di Pietro et al. 2016; Bian et al. 2023).

Lutein (**4**) is another carotenoid with strong antioxidant capacity and an important component of macular pigment in the retina, and thus, it possesses wide applications in pharmaceutical, food, feed, and cosmetics industries. Besides extraction from plant and algae, microbial fermentation using engineered cell factories of fungi (mostly engineered yeasts, such as *Saccharomyces cerevisiae*) to produce lutein has emerged as a promising route (Bian et al. 2023). Dwyer et al. (2001) research, comprising in vitro, in vivo, and epidemiological studies, demonstrates lutein's potential as a protective agent against the early stages of atherosclerosis (Dwyer et al. 2001). Their co-culture model of lipoprotein oxidation with the artery wall revealed a significant dose-dependent reduction in the chemotactic signal for monocytes in the presence of lutein (0.1, 1, 10, and 100 nM), resulting in the inhibition of monocyte inflammation associated with LDL in the artery wall. This effect was observed in both apoE-null and LDL receptor-null mice, wherein an enriched lutein diet led to a significant reduction in atherosclerotic lesion size in the aortic arch. Furthermore, high plasma lutein levels were correlated with the progression of intima-media thickness (Dwyer et al. 2001).

The red pigment astaxanthin (**3**) can inhibit peroxidation by the protection of lipid bilayers from free radicals. The scavenging properties of this marine fungi carotenoid are associated with the molecular structure (Fig. 14.6), which is thought to be responsible for its 10 times higher antioxidant activity compared to previously mentioned carotenoids like β-carotene and lutein (Galasso et al. 2017; Gammone et al. 2015).

Astaxanthin was also found to reduce COX-2 expression-related neuroinflammation (Si and Zhu 2022). Endothelial function was improved in resistance arteries with astaxanthin enriched diet in hypertensive rats (Monroy-Ruiz et al. 2011). In addition to this effect, a decrease in oxidative stress was also observed and an improvement in NO bioavailability. 10 μM of astaxanthin was able to reduce pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, previously induced by the treatment with 100 μM of H_2O_2 (Galasso et al. 2017; Si and Zhu 2022).

It has also been proposed that astaxanthin's anti-inflammatory and anti-apoptotic effects in the neural system are also related to elevated myelin basic protein (MBP) expression and decreased levels of caspase-3, iNOS, granulocyte colony-stimulating factor, and growth-related oncogene (Elbandy 2022; Aslankoc et al. 2022). Furthermore, astaxanthin decreased microglial activation and the expression of various pro-inflammatory cytokines, which further resulted in the suppression of neuroinflammation. The anti-inflammatory activity of astaxanthin was demonstrated by the nuclear translocation of NFκB p65, as well as by the suppression of p38 and Erk1/2 phosphorylation (Elbandy 2022; Pietrasik et al. 2022; Zhao et al. 2021).

3.2 *Fungi-Derived Classic and Complex Bioactive Phenolic Compounds/Pigments with Anti-Inflammatory and Antithrombotic Properties*

Several fungi produce a plethora of phenolic compounds, phenylpropanoids and aromatic amino acids. Such secondary metabolites consist of phenolic compounds and are secreted from the fungal metabolic pathways and/or the plant metabolic pathway (of plants that symbiote with endophyte fungi), which is called the shikimate pathway. Metabolites of this group include compounds like folic acid and salicylic acid, which help in reducing inflammation and pain, while they also produce the antioxidant drug resveratrol (**5**) (Fig. 14.5) (Singh et al. 2017, 2021). Phenolic compounds have many functions, while due to the high antioxidant effect and free radical scavenging property, phenylpropanoid compounds have major attention in the area of human health, and nowadays, their functions are updated by the use of multi-omic tools (Singh et al. 2017, 2021; Shankar and Sharma 2022).

Resveratrol (*trans*-3,5,4′-trihydroxystilbene) is the most well-established polyphenol used as drug and is found mostly in grapes, berries, and fermented alcoholic beverages like wine, where *S. cerevisiae* is used as the main yeast for the fermentation, but can also be made by some fungi. It is a phytoalexin, i.e., a low molecular weight secondary metabolite. *Alternaria* sp. 61, isolated from Merlot cobs, can also produce resveratrol, as well as recombinant *S. cerevisiae* upon feeding of coumaric acid or L-tyrosine (Sanchez and Demain 2017; Shi et al. 2012; Shin et al. 2012). Resveratrol has exhibited potent antioxidant and anti-inflammatory activities, as well as anti-platelet aggregation and anti-atherogenic properties, and thus, it possesses beneficial effects against inflammation, carcinogenesis, oxidation, aging, diabetes, and renal and neurodegenerative disease (Tsoupras et al. 2007, 2009; Fragopoulou et al. 2004; Sanchez and Demain 2017; Sánchez-Fidalgo et al. 2010). Moreover, resveratrol was also found to reduce the levels of the potent inflammatory mediator and platelet-activating factor (PAF) by inhibiting its synthesis and thus reducing the inflammatory status (Tsoupras et al. 2007, 2009). Such bioactive phenolic compounds from fungi like resveratrol, which can reduce the levels and/or inhibit the activities of thrombo-inflammatory mediators like PAF and thrombin, may potentially reduce the risk of developing chronic disorders (Tsoupras et al. 2018, 2009, 2022a).

Moreover, a phenolic fraction derived from the HPLC separation of a bioactive extract from *B. bassiana* exhibited potent anti-inflammatory and antithrombotic activities against PAF in rabbit platelets (Tsoupras et al. 2022b). However, more studies are needed in order to elucidate the bioactive phenolic molecules that can be derived from such entomopathogenic fungus.

A mushroom species with high functional and nutraceutical potential of high market value is *Agaricus subrufescens* (synonymy *Agaricus blazei* and *Agaricus brasiliensis*). It is commercialized in several countries such as Brazil (brand name "Sun mushroom"), China (Ji Song Rong), and Japan (Himematsutake). Sun mushroom contains polyphenols and polysaccharides and is known to decrease oxidative

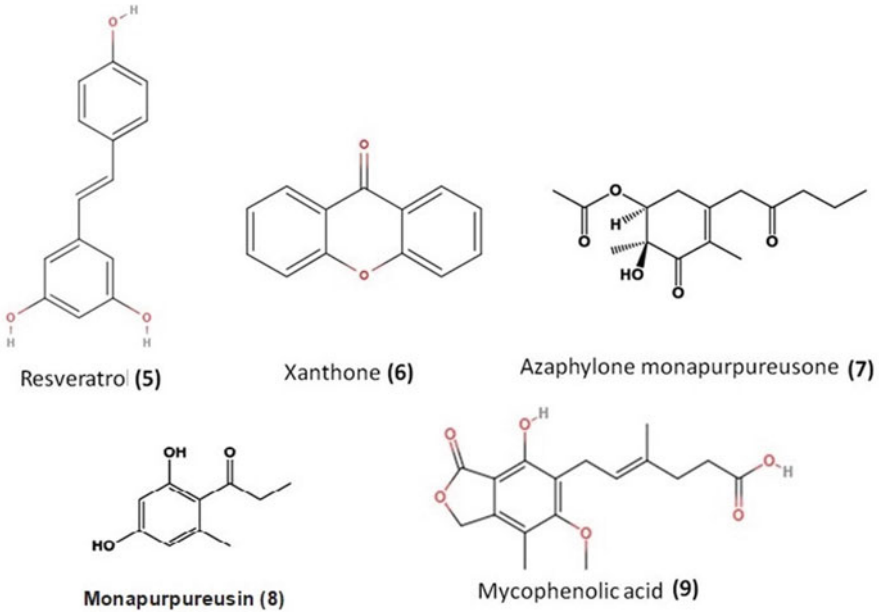

Fig. 14.5 Chemical structures of fungi bioactive phenolic compounds/pigments with anti-inflammatory potential. Most of the structures were obtained from https://molview.org/ (accessed on April 2, 2023), while (**7**) and (**8**) were reproduced from Takahashi et al. (2020)

stress and prevent non-transmitted chronic disorders (NTCD); it is indicated to have antioxidant, antitumor, anti-inflammatory, and immunomodulatory properties (Shankar and Sharma 2022; Takahashi et al. 2020; Navegantes-Lima et al. 2020).

For example, *Agaricus brasiliensis* extract rich in polyphenols showed anti-inflammatory, immunomodulatory, and antioxidant effects in the cecal ligation and puncture (CLP) sepsis model (Navegantes-Lima et al. 2020). More specifically, the aqueous extract of *A. brasiliensis* reduced systemic inflammatory response and improved bacteria clearance and mice survival. In addition, *A. brasiliensis* decreased the oxidative stress markers in serum, peritoneal cavity, heart, and liver of septic animals, as well as ROS production (in vitro and in vivo) and *tert*-butyl hydroperoxide-induced DNA damage in peripheral blood mononuclear cells from healthy donors in vitro. In conclusion, the aqueous extract of *A. brasiliensis* was able to increase the survival of septic animals by a mechanism involving anti-inflammatory, immunomodulatory, and antioxidant protective effects (Navegantes-Lima et al. 2020).

Xanthone (**6**) (Fig. 14.5) is the basis of several xanthones, including fungi-derived xanthones, which are another class of bioactive phenolic compounds showing antioxidant and anti-inflammatory properties and potential medicinal benefits (Feng et al. 2020). For example, the anti-neuroinflammatory assay of Hypoxylon xanthone A from soil fungus *Hypoxylon* sp., as manifested by the inhibitory effect on LPS-induced NO production in BV-2 microglial cells, indicated almost the same

inhibitory effect as the antibiotic minocycline in a dose-dependent manner within the concentration of 1–50 μM, suggesting that hypoxylon xanthone A could be a new potential neuroinflammation inhibitor (Takahashi et al. 2020; Xiao et al. 2020).

Another example to enhance the quantity of easily available phenolic pigments from fungi is engineered *Monascus* species by subjecting them to mutations. One of these mutant strains of *M. purpureus* was grown in fermented rice extract, leading to the production of two new compounds—the azaphylone monapurpureusone (**7**) and the brownish natural product monapurpureusin (**8**) (Fig. 14.5). These compounds and their derivatives produced by reactions with other biomolecules (i.e., the water-soluble red pigments monascorubramine and rubropunctatin) have exhibited remarkable superoxide radical scavenging activity, either comparable to or surpassing the control gallic acid, and superior anti-inflammatory activity in comparison with the control quercetin (Wu et al. 2019). In general, *Monascus* pigments come in 54 different varieties. They have a remarkable range of functions, including antibacterial, anticancer, antimutagenesis, antidiabetes, anti-obesity, anti-inflammatory, cholesterol-lowering, immunosuppressive, and hypotensive (Sanchez and Demain 2017; Feng et al. 2012; Lee and Pan 2012).

Some classic and/or more complex fungi phenolic molecules can reduce the inflammatory response indirectly as immunosuppressants. For example, mycophenolic acid (**9**) is a mesoterpenoid with a phenolic functional group, synthesized by the fungi *Penicillium brevicompactum*, *Penicillium stoloniferum*, and *P. echinulatum*, with a primary use as an immunosuppressive drug and especially to prevent organ transplant rejection. It reversibly inhibits inosine monophosphate dehydrogenase, the enzyme that controls the rate of synthesis of guanine monophosphate in the de novo pathway of purine synthesis used in the proliferation of B and T lymphocytes, which concludes to immunosuppressant and reduced inflammatory response (Patel et al. 2017; Anderson et al. 1988; Bills and Gloer 2016).

3.3 Fungi Lipid Bioactives with Anti-Inflammatory and Antithrombotic Properties

3.3.1 Fungi-Derived Anti-Inflammatory and Antithrombotic Fatty Acids

Fungi produce several unsaturated fatty acids, in a combination that usually has anti-inflammatory potential (Fig. 14.6). For example, almost all fungi contain the monounsaturated fatty acid (MUFA) oleic acid (OA; 18:1 omega 9) (**10**), which has shown strong anti-inflammatory and antithrombotic properties against several inflammatory mediators, including PAF (Nunez et al. 1990). In addition, various fungi produce also several of the long-chain polyunsaturated fatty acids (PUFA), such as gamma linoleic acid (GLA; 18:3 omega-6) and alpha-linolenic acid (ALA; 18:3 omega-3) (**11**) and (**12**) (i.e., from *Mucor circinelloides)*, arachidonic acid (ARA; 20:4 omega-

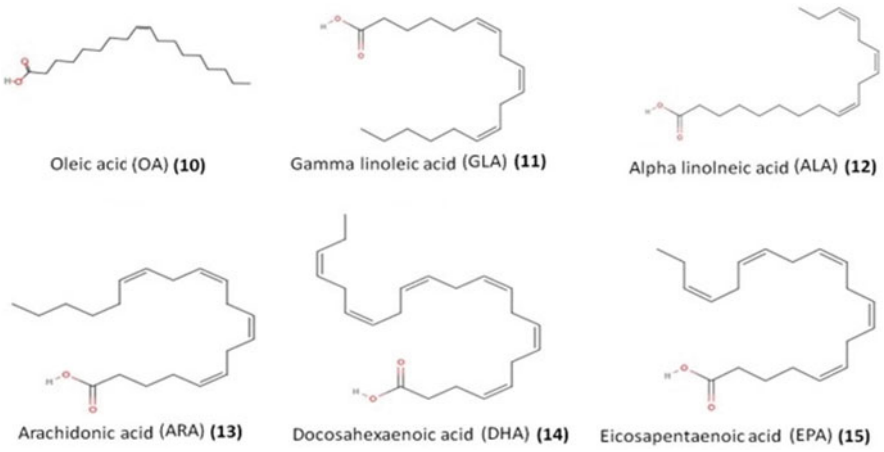

Fig. 14.6 Chemical structures of fungi bioactive unsaturated fatty acids with anti-inflammatory potential. Structures were obtained from https://molview.org/ (accessed on April 2, 2023)

6) (**13**) (i.e., from *Mortierella alpine)*, docosahexaenoic acid (DHA; 22:6 omega-3) (**14**) (i.e., from *Crypthecodinium cohnii spp.)*, and eicosapentaenoic acid (EPA; 20:5 omega-3) (**15**) (i.e., from genetically modified *Y. lipolytica*. The oil produced from fungi has much higher levels of EPA than natural oils. EPA is important for the anti-inflammatory activity of fish oils, thus contributing to cardiovascular and joint health (Tsoupras et al. 2022a; Sanchez and Demain 2017). Consumption of such PUFA-containing oily products with a low ratio of omega-6/omega-3 PUFA promotes an anti-inflammatory potential since diets and foods containing such a ratio have been found to possess strong anti-inflammatory health benefits against several inflammation-related disorders (Simopoulos 2008). PUFAS are the principal component of cell membrane phospholipids and thus are used heavily in the infant formula industries (especially ARA and DHA). EPA and DHA can be utilized to avoid heart issues. They control cell fluidity, the attachment of certain enzymes to cell membranes, and the transmission of signals and other metabolic activities. They are involved in the manufacture of eicosanoids, leukotrienes, prostaglandins, and resolvins, which have anti-inflammatory, anti-arrhythmic, and anti-aggregatory properties. Several of them promote cardiovascular health, and others increase visual function and cognition in newborns and adults (Sanchez and Demain 2017).

3.3.2 Polar Lipids

Polar lipids are important biomolecules and structural elements for all cells in nature, with a plethora of diverse bioactivities. Polar lipids are amphiphilic molecules that generally have two fatty acids esterified to a glycerol- or a sphingosine-based backbone and a phosphorus functional group for phospholipids or a sugar for glycolipids that is linked to a head group (Fig. 14.7a). Lately, it has been found

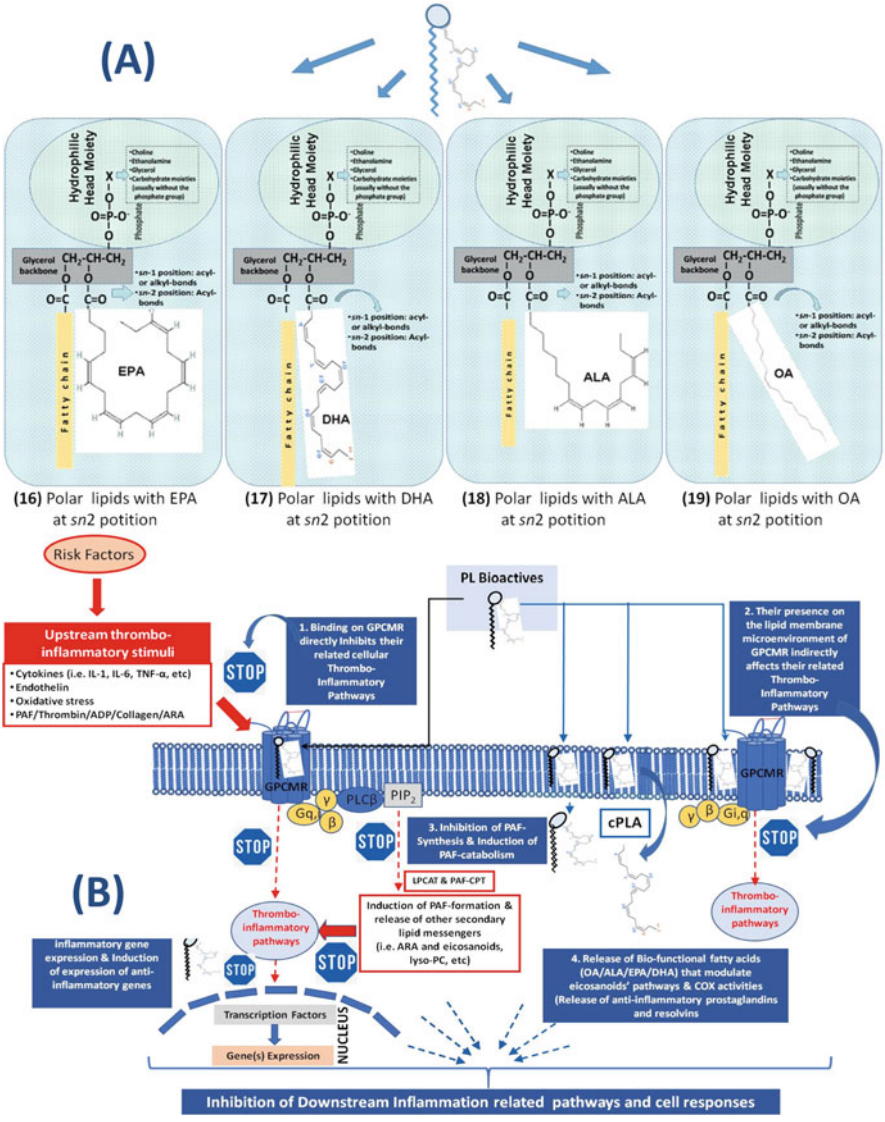

Fig. 14.7 Structures (**a**) and mechanisms of action (**b**) of classic bioactive polar lipids with anti-inflammatory and antithrombotic properties; (**a**) bioactive polar lipids with unsaturated fatty acids at their *sn*2 position of their glycerol backbone. *EPA* eicosapentaenoic acid (C20:5 omega-3), *DHA* docosahexaenoic acid (C22:6 omega-3), *ALA* alpha-linolenic acid (C18:3 omega-3), *OA* oleic acid (C18:1 omega-9). (**b**) Modes of beneficial actions of bioactive polar lipids (in blue color) against the thrombo-inflammatory related pathways and cell responses (red color). *PL* polar lipids, *PAF* platelet-activating factor, *ADP* adenosine 5′ diphosphate, *GPCMR* G-protein-coupled membrane receptors, *cPLA2* cytoplasmic phospholipase A2, *LPCAT and PAF-CPT* basic regulatory biosynthetic enzymes of PAF, *ARA* arachidonic acid, *COX* cyclooxygenase. Reproduced with modifications from Tsoupras et al. (2022a)

that bioactive polar lipids with unsaturated fatty acids in their structures (usually at the *sn*2 position), such as polar lipids with EPA (**16**), polar lipids with DHA (**17**), polar lipids with ALA (**18**), and polar lipids with OA (**19**) (Fig. 14.7a), possess higher bioavailability of their unsaturated fatty acids throughout the body, due to their amphiphilic properties, while most importantly they possess strong anti-inflammatory and antithrombotic properties against several mediators and inflammatory pathways, by a variety of mechanisms of actions (Fig. 14.7b), with promising health benefits against atherosclerosis and CVD, cancer and metastatic procedures, renal and neurodegenerative disorders, persistent infections and associated inflammatory manifestations, allergy and asthma, sepsis, etc. (Tsoupras et al. 2018, 2022a).

For example, such bioactive polar lipids have modulated or even reduced the formation of arteriosclerotic plaques, by reducing the levels of the inflammatory and thrombotic mediator, PAF, and its atherogenic effects. Apart from modulating PAF metabolism toward reduced PAF levels, such fungi-derived bioactive polar lipids have also inhibited the inflammatory and thrombotic pathways of both PAF and thrombin, while they have reduced the platelet activation and aggregation induced by well-established platelet agonists, collagen, and ADP (Moran et al. 2021; Fragopoulou et al. 2004; Tsoupras et al. 2022b). More specifically, it has recently been found that several yeasts used for the production of fermented foods and beverages (Moran et al. 2021; Fragopoulou et al. 2004) and fungi of bio-agro/food technological applications like those used as natural entomopathogenic agents for organic cultures, such as *Beauveria bassiana* (Tsoupras et al. 2022b), contain such bioactive polar lipids with strong anti-inflammatory and antithrombotic properties against the thrombo-inflammatory mediators, PAF and thrombin, and against the well-established platelet agonists, collagen, and ADP (Moran et al. 2021; Fragopoulou et al. 2004; Tsoupras et al. 2022b).

It seems that the anti-inflammatory and antithrombotic anti-PAF properties of these fungi-derived polar lipid bioactives occur either through affecting beneficially PAF metabolism toward reduction in its levels of homeostatic ones and/or through inhibiting the binding of PAF on its receptor and thus inhibiting PAF-related inflammatory and thrombotic pathways and activities (Fig. 14.7b) and subsequently reducing the risk for PAF-associated inflammatory chronic disorders such as atherosclerosis, CVD, and cancer (Tsoupras et al. 2018, 2009, 2022a). Moreover, apart from the strong anti-inflammatory and antithrombotic properties of the whole structures of such bioactive polar lipids, it has also been proposed that once the rich in unsaturated polar lipids have surpassed the intestine barrier and are bound to and transferred from plasma lipoproteins to the cell membranes of all tissues, there a cytoplasmic phospholipase A2 (PLA2) releases their unsaturated fatty acids from their structure of these membrane-bound polar lipids, while the released unsaturated fatty acids interacts with the eicosanoids pathways (COX-enzymes) for reducing and resolving inflammation and the inflammatory cell-response (Fig. 14.7b).

3.3.3 Other Fungi-Derived Lipid Bioactives with Anti-Inflammatory and Antithrombotic Properties

Several other fungi-derived lipid bioactives have also been found to possess anti-inflammatory and antithrombotic potential. For example, ergosterol (**20**) (Fig. 14.8), the main sterol present in various fungal membrane cells, has a range of activities including cell cycle control, regulation, and permeability, as well as several health-promoting effects such as, anti-inflammatory, antioxidant, anticancer, and anti-hypercholesteremic (Rousta et al. 2023). Ergosterol is also implicated in the synthesis of vitamin D2 (ergocalciferol) by conversion of ergosterol by UV radiation. This form of vitamin D2 is converted to calcitriol, the biologically active form of vitamin D by the body. Vitamin D is an important vitamin for the prevention of various metabolic diseases like hypertension, diabetes, and cancer (Feng et al. 2020). Deficiency of vitamin D has been associated with COVID-19 mortality (Tsoupras et al. 2020a, b; Zabetakis et al. 2020).

Vitamin D and its analogs like paricalcitol have also exhibited strong anti-inflammatory and antithrombotic properties by inhibiting the inflammatory actions of PAF in vitro in platelets, they reduced PAF synthesis in vitro in cell cultures of mesangial cells, while in an in vivo trial daily administration of paricalcitol in hemodialysis patients for a month resulted also in modulation of PAF metabolism toward reduced PAF synthesis and increased PAF catabolism and thus toward reduced PAF levels in their blood, which subsequently resulted in reduced levels

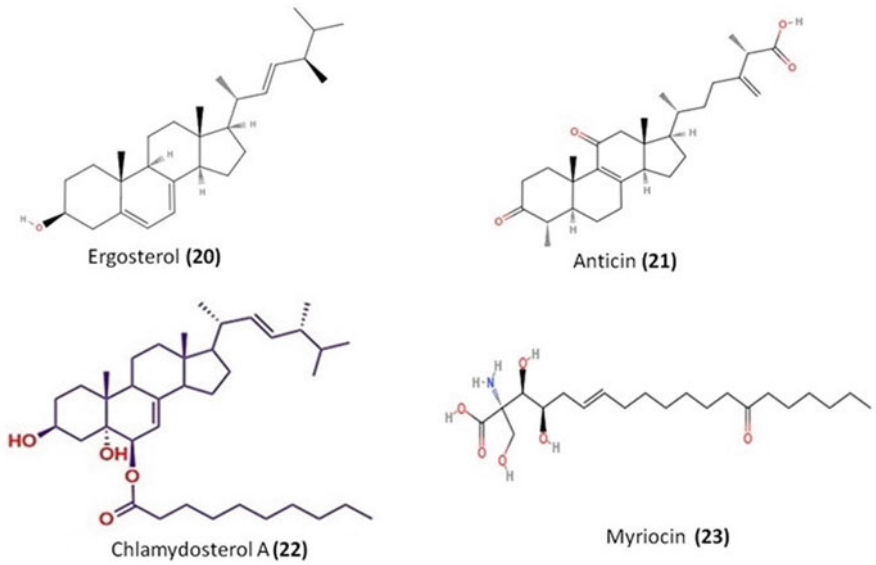

Fig. 14.8 Chemical structures of other fungi lipid bioactives with anti-inflammatory potential. Structures of (**20**), (**21**), and (**23**) molecules were obtained from https://molview.org/ (accessed on April 2, 2023), while (**22**) was reproduced from Al-Rabia et al. (2021)

of other inflammatory cytokines and a beneficial decrease in the inflammatory status in the blood of these patients versus control subjects (Verouti et al. 2013). Since the rich diet source of vitamin D is animal products, the fungi as the only vegetarian source of vitamin D are highly considered.

Another class of fungi-derived bioactive lipid compounds of increasing prominence found in edible mushrooms includes antcins (**21**) (Fig. 14.8) and steroids that contain an ergostane-type skeleton and are produced by *Antrodia* species such as *Antrodia cinnamomea* and *Antrodia salmonea* (Takahashi et al. 2020). Studies suggest that these compounds are promising agents in the treatment of cancer, inflammation, diabetes, and diseases resulting from oxidative stress (Takahashi et al. 2020). A study tracking 36,499 middle-aged and elderly Japanese men over an average of 13.2 years found a positive relationship between regular mushroom consumption and decreased incidence of prostate cancer (Zhang et al. 2020).

Two other ergosterol derivatives namely chlamydosterols A (**22**) (Fig. 14.8) [(22*E*,24*R*)-ergosta-7,22-diene-3β,5$^{\alpha}$,6β-triol 6-decanoate] and ergosta-5,7,22-triene-3β-ol, from the EtOAc extract of the endophytic fungus *Fusarium chlamydosporum* isolated from *Anvillea garcinia* (Asteraceae) leaves growing in Saudi Arabia, showed a 5-lipoxygenase (5-LOX) inhibitory potential compared to a classic 5-LOX inhibitor, indomethacin (Al-Rabia et al. 2021).

Some classic and/or more complex fungi lipid molecules can reduce the inflammatory response indirectly as immunosuppressants. For example, myriocin (ISP-I) (**23**) (Fig. 14.8) is an amino acid lipid produced by the fungus *Isaria sinclairii*, whose structural model is used as a template for the synthesis of fingolimod, commercially available as Gilenya. Used in the treatment of multiple sclerosis through the activation of its structure, this drug connects with extracellular G protein-coupled receptors, preventing the release of lymphocytes that enter the central nervous system and thus indirectly prevent the neuroinflammatory response of sclerosis (Fujita et al. 2000).

3.4 Alkaloids

Alkaloids are an important group of nitrogen-containing secondary metabolites, such as caffeine, cocaine, nicotine, morphine, and strychnine, which consist of one or more nitrogen atoms within their heterocyclic ring. Alkaloids have several biological activities including anti-inflammatory properties (Mehta et al. 2022; Debnath et al. 2018). For example, the addition of pyrrole alkaloid into the cell of fungus *Curvularia* sp. IFB-Z10, associated with white croaker, induces the generation of an anti-inflammatory metabolite named curindolizine (**24**) (Fig. 14.9) (Mehta et al. 2022; Han et al. 2016). In general, several species of *Curvularia* are known to synthesize various bioactive alkaloid compounds including curvulamine, curindolizine, and curvupallides, which show antimicrobial and anti-inflammatory activities (Mehta et al. 2022). Moreover, marine fungi-derived indole alkaloids are also very promising and an active group of molecules. They possess various

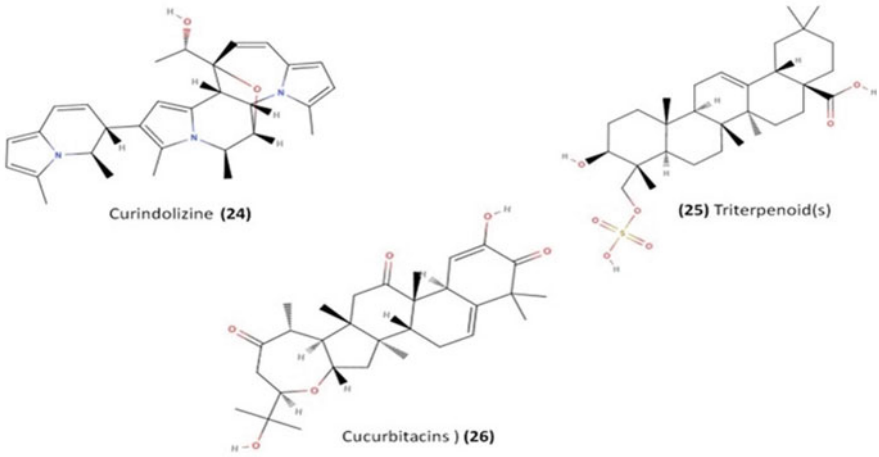

Fig. 14.9 Chemical structures of characteristic fungi-derived alkaloids and terpenoids with anti-inflammatory potential. Structures were obtained from https://molview.org/ (accessed on April 2, 2023)

biological activities like antiparasitic, cytotoxic, serotonin and antagonistic realms, anti-inflammatory, and antiviral (Shankar and Sharma 2022; Gul and Hamann 2005).

3.5 Terpenoids

Terpenes are a major class of bioactive metabolites that are produced from a higher class of Polyporales group of basidiomycetes. These compounds are structural constituents of another group of metabolites that are formed through the linkage of isoprene units. Different NMR and other omic tools confirm the member of the terpenoid class of compounds such as steroids, menthol, and taxol, which have key components for anticancer drugs and β-carotene properties. These bioactive compounds possess anti-inflammatory properties and anti-infection, anticancer, antiproliferative, and antiangiogenic medicinal benefits (Shankar and Sharma 2022). Mushroom secondary metabolites triterpenes (**25**) (Fig. 14.9), abundant in reishi mushroom (*Ganoderma lucidum*), inhibit various pro-inflammatory mediators, such as TNF-α, interleukin-6, nitrogen monoxide free radical, prostaglandin E, nuclear factor-kappa B (NF-κB), and cyclooxygenase-2 (COX-2), while *Poria cocos* mushroom incorporates lanostane triterpenoids (**25**) (Fig. 14.9) that improve inflammation and treat tumors (Bell et al. 2022). Cucurbitacins (**26**) (Fig. 14.9) belong to cucurbitane-type tetracyclic triterpenoid saponins that are abundant in several natural sources, while they are also present in some fungi. Several studies have shown that cucurbitacins have significant biological activities, such as anti-inflammatory,

antioxidant, antimalarial, antimicrobial, hepatoprotective, and antitumor potential (Varela et al. 2022).

3.6 Other Fungi Metabolites with Well-Established Pharmaceutical Health Benefits That Also Exhibit Anti-Inflammatory Effects

A plethora of well-established pharmaceutical compounds have been derived from several fungi species. These compounds have been used against specific pathological conditions with high specificity and pharmacological actions. Nevertheless, there are a lot of studies lately indicating the potential pleiotropic effects of several of these drugs. Thus, apart from their classic pharmaceutical actions, the newly observed pleiotropic effects and multifunctional applications of these compounds, and especially their potential anti-inflammatory and antithrombotic properties, may provide additional perspectives for the appropriate choice of a drug/drug combination against a specific inflammation-related condition where anti-inflammatory actions will provide further health benefits by reducing the inflammatory burden. Characteristic examples of such fungi-derived metabolites and their derivatives that are used as several drugs and also possess pleiotropic effects, including anti-inflammatory potency, are further analyzed below.

3.6.1 Drugs Containing Fungal Polyketides and Their Derivatives with Anti-Inflammatory Potential

Fungal polyketides are common metabolites present in large groups of ascomycete and basidiomycete fungi. All groups of microbial polyketides are synthesized by multi-domain polyketide synthases (PKSs), which are structurally diverse. These secondary metabolites contain also a fatty group and are formed through the acetate pathway within the cell by coupling of acetate groups. In the fatty acid group, crude fat is considered under all categories of lipid compounds, including fatty acids, monoglycerides, diglycerides, triglycerides, sterols, and phospholipids. These groups of metabolites include antibiotics, statins (lowers cholesterol levels), and antidepressant drugs. Characteristic pharmaceutically modified polyketides that are used as drugs are the cholesterol-lowering statin, lovastatin (**27**) (Fig. 14.10), and the anti-mycotic drug griseofulvin (**28**) (Shankar and Sharma (2022).

Statins are one of the most well-established cholesterol-lowering drugs used for the prevention and treatment of cardiovascular disorders. In 1976, Akira Endo at the Sankyo Company, Tokyo, identified the first inhibitor of the HMG-CoA reductase, which drives down the levels of intracellular cholesterol, thereby embarking on the long journey of evolution of statin drugs (Sultan et al. 2019). Several statins are fungi metabolites, such as lovastatin, a bioactive polyketide commercially sold as

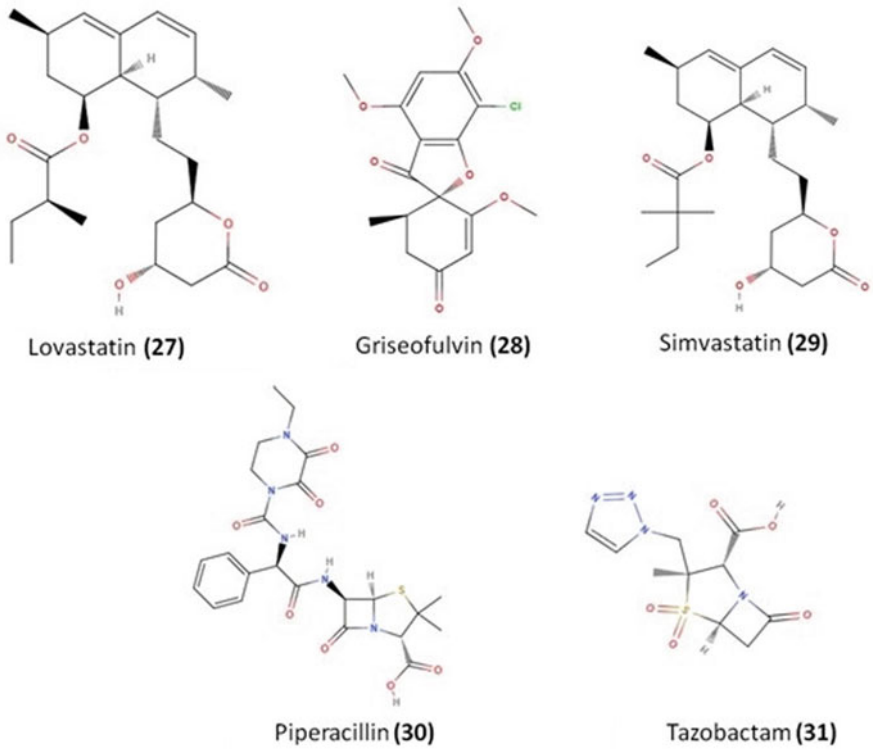

Fig. 14.10 Chemical structures of characteristic fungi-derived drugs that also possess anti-inflammatory properties. Structures were obtained from https://molview.org/ (accessed on April 2, 2023)

mevacor, and its semisynthetic product, simvastatin (**29**) (Fig. 14.10), which is on the market as zocor and simvador. Lovastatin biosynthesis has been reported in several fungal species, including *Aspergillus terreus*, *Monascus purpureus*, *Penicillium citrinum*, *Paecilomyces viridis*, *Penicillium purpurogenum*, *Pleurotus* sp., and *Trichoderma viride*, with the latent being the species used for industrial production.

These statins block 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and prevent the conversion of mevalonate into cholesterol, being the most frequently used drugs in the treatment of hypercholesterolemia to reduce the risk of cardiovascular diseases and to manage abnormal lipid levels by inhibiting the endogenous production of cholesterol in the liver. Another fungi-derived statin is compactin, which is produced by the fungi *P. citrinum* and *Penicillium solitum* and is commercially available as pravachol, selektine, or lipostat, while it also reduces the risk of cardiovascular diseases through the same pathway and is also used as a substrate for the biotransformation or partial chemical synthesis of mevastatin.

Additional studies have indicated several important pleiotropic applications of statins, with lovastatin for example to be used as an antimicrobial and agent for

treatments of cancers and bone diseases (Conrado et al. 2022). Nevertheless, apart from their proposed beneficial cardioprotective properties through lowering cholesterol levels, recent studies have also demonstrated several side effects of statin use, such as diabetes mellitus, cancer, early premature cataracts, hearing loss, suicidal ideation, peripheral neuropathy, depression, benign intention tremors and Parkinson's disease, pulmonary interstitial pneumonitis and dysfunctional breathing, interstitial cystitis and bladder wall instability, herpes zoster, impotency, and cognitive impairments, while several researchers have also suggested that statin treatment ultimately offer little protection against cardiovascular risk by their effects on cholesterol levels (Sultan et al. 2019).

Interestingly, some but not all statins, including simvastatin with an intact lactone ring, have also exhibited in vitro a strong anti-inflammatory inhibitory effect against the pro-inflammatory and thrombotic mediator, platelet-activating factor (PAF), and its inflammatory signaling in platelets. Moreover, incubation of human mesangial cells in the form of simvastatin with an intact lactone ring resulted in decreased PAF biosynthesis toward reduced PAF levels. Furthermore, in six simvastatin-treated volunteers a reduction in PAF biosynthesis was observed in their leukocytes explaining the lower levels of PAF in the blood of these subjects, while total cholesterol and low-density lipoprotein cholesterol were also significantly decreased. Within the same period of this administration, high-density lipoprotein cholesterol, triacylglycerol, the effective concentration (EC_{50}) for inducing 50% of PAF-induced plasma-rich platelet aggregation, and lag time of plasma oxidation were unaffected in these participants. Since PAF is implicated in several inflammation-related chronic disorders, the observed anti-inflammatory and cardioprotective anti-PAF effects of statins should be further evaluated as part of their pleiotropic activities, which can provide novel perspectives on the use of statins against chronic disorders (Tsantila et al. 2011).

Griseofulvin is a natural product that was first discovered and isolated from *Penicillium griseofulvum*. In addition to *Penicillium*, griseofulvin may be isolated from other genera of ascomycetes. Griseofulvin has a wide range of applications, from agriculture to medicine. In medicine, griseofulvin has been widely used as an antifungal drug and in treating ringworm and dermatophyte infections in humans and animals due to its low toxicity. This fungistatic has gained increasing interest for multifunctional applications in the last decades due to its anticancer and antiviral potential (Aris et al. 2022). Moreover, it has recently been found that griseofulvin has exhibited indirect anti-inflammatory protection against the inflammatory burden of SARS-CoV-2 infection, through its vasodilation and increased capillary blood flow effects, by beneficially affecting the ACE2 signaling of the renin–angiotensin–aldosterone system (RAAS) toward reduction in inflammation. It seems that griseofulvin binds to ACE2 and competes against SARS-CoV-2 binding to the extracellular domain of ACE2, therefore improving ACE2 vasodilation and tissue protection anti-inflammatory functions. These findings suggest that griseofulvin and its derivatives might be considered when developing future SARS-CoV-2 treatment options (Aris et al. 2022).

Further studies reported the effectiveness of griseofulvin in the treatment of non-fungal skin inflammation diseases, suggesting that it may also have anti-inflammatory and immunomodulatory potential. This is while, earlier studies have shown that griseofulvin is effective in treating shoulder-hand syndrome and a few other inflammatory, rheumatic conditions, such as posttraumatic reflex dystrophies and scapulohumeral periarthritis. Additionally, griseofulvin has been shown effective for inflammatory skin diseases through inducing inhibitory effects on vascular cell adhesion molecule 1 (VCAM-1) in both TNF-alpha and IL-1 stimulated human dermal microvascular endothelial cells (HDMEC) (Aris et al. 2022).

3.6.2 Fungi-Derived Antibiotics with Anti-Inflammatory Properties

Apart from their general and well-established antimicrobial actions, several antibiotics have also exhibited specific anti-inflammatory and antithrombotic potency. For example, the semisynthetic derivatives of penicillins, the firstly reported fungi-derived β-lactam antibiotics originally obtained from *Penicillium* molds, principally *P. chrysogenum* and *P. rubens*, namely the β-lactam antibiotic piperacillin (**30**) and the beta-lactamase antibiotic tazobactam (**31**) (Fig. 14.10), which are widely used in combination for the management and treatment of serious, hospital-acquired infections, including sepsis, were also found to possess strong anti-inflammatory and antithrombotic properties against the activities and the synthesis of the inflammatory and thrombotic mediator, PAF, and in lesser potency against thrombin (Tsoupras et al. 2011a).

More specifically, the combination of piperacillin–tazobactam inhibited strongly PAF-induced aggregation of both washed rabbit platelets and rabbit plasma-rich platelets in a concentration-dependent manner, while when combined with other antibiotics such as netilmycin and amikacin, they synergistically inhibited more potently PAF actions in these cells. Higher concentrations of these antibiotics were needed in order to inhibit platelet aggregation induced by the classic thrombotic mediator, thrombin, suggesting that they have higher specificity against the PAF-related signaling pathway of inflammation and thrombosis. In addition, these antibiotics were also able to inhibit PAF synthesis of rabbit leukocytes, suggesting that they can also reduce PAF levels during inflammatory activation of leukocytes and platelets. These newly found properties of fungi-derived antibiotics used in sepsis suggest that apart from their general actions, these drugs may present additional beneficial anti-inflammatory and antithrombotic effects against the onset and establishment of sepsis and of other microbial-induced inflammatory manifestations and associated disorders, by inhibiting the PAF/PAF receptor inflammatory and thrombotic signaling and/or the thrombin/protease-activated receptor-1 thrombotic signaling, as well as by reducing PAF levels through both PAF biosynthesis inhibition and PAF catabolism induction (Tsoupras et al. 2011a). These promising in vitro results need to be further studied and confirmed by in vivo tests, in order to optimize the efficacy of antibiotic treatment in sepsis and other microbial-induced inflammatory conditions.

3.7 Other Fungi Metabolites with Anti-Inflammatory Properties and Health Benefits

Apart from all these bioactive metabolites, a variety of several other molecules of different structures and activities have also been found in fungi, with a direct or indirect anti-inflammatory and antithrombotic potential (Fig. 14.11). Classic example of such molecules is cinnabarinic acid (**32**). *Pycnoporus*, a white-rot basidiomycete mushroom, secretes enzymes and pigments that break down cellulose, hemicelluloses, and lignin. Specifically, the species *P. sanguineus* produces cinnabarinic acid, which is highly effective at inhibiting the growth of bacteria and common foodborne pathogens. Cinnabarinic acid has been proposed as a potential therapeutic agent in the treatment of neuroinflammatory diseases. Given these powerful properties, *Pycnoporus* should be further studied for applications in food and medicine (Shankar and Sharma 2022; Fazio et al. 2017).

Fungi are classified based on the presence of the essential long-chain polymer, chitin, and its derivative chitosan (**33**). Like other fungal-derived compounds, they possess antimicrobial effects and research has suggested they could be used for the production of new therapeutics with antitumor, anti-inflammatory, and antihypertensive activities (Rousta et al. 2023).

Another structural component of many microbial organisms like fungi, yeasts, and mushrooms is β-glucan (**34**). This glucose polymer is a common ingredient in skin care products due to its positive effects on wound healing, skin moisture, and oxidative stress. Additionally, β-glucan has been proposed as a treatment for several health conditions including, cancer, inflammatory bowel disease, and arthritis (Rousta et al. 2023).

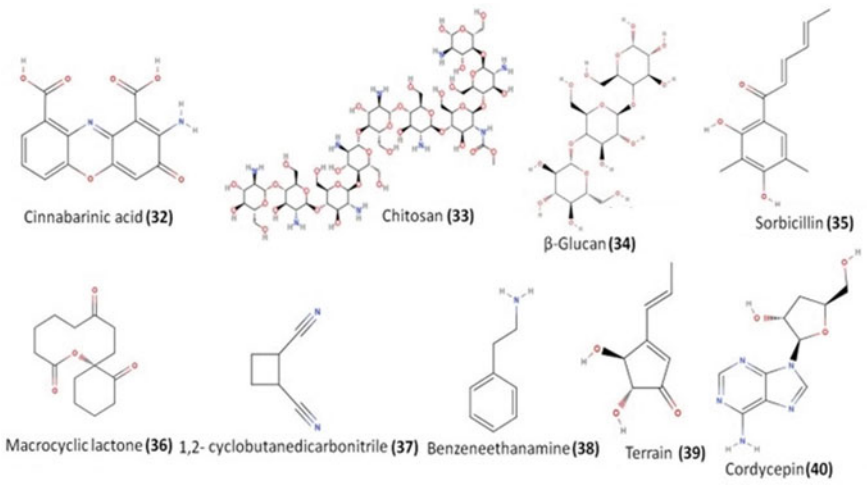

Fig. 14.11 Chemical structures of other fungi metabolites with anti-inflammatory properties. Structures were obtained from https://molview.org/ (accessed on April 2, 2023)

Hexaketide metabolites known as sorbicillinoids were initially discovered in 1948 as a penicillin contaminant. It is composed of the base sorbicillin (**35**) structure with cyclization at the carboxylate terminus. Great advances have been made in the structural elucidation of sorbicillinoids derived from fungi with 62 additional structures discovered between 2016 and 2021. These compounds have many bioactivities, including anti-inflammatory, antiviral, cytotoxic, and antibacterial characteristics. Fungi from the *Pezizomycotina subphylum* of the *Ascomycota phylum*, including species like *Aspergillus* sp., *Penicillium notatum*, and *Trichoderma* sp., generate sorbicillinoids. From maritime habitats, certain sorbicillinoid-producing fungus have been isolated. Due to their health-promoting properties, fungal sorbicillinoids have shown great potential in the pharmaceutical industry (Conrado et al. 2022).

Both macrocyclic lactone and macrocyclic ketone (**36**) were recently isolated from filamentous fungi from various species of *Curvularia*. They have been posed as potential bioactive compounds for application in agricultural and pharmaceutical industries. They seem to possess radical scavenging properties contributing to antioxidant effects. Additionally, they have been found to possess anti-inflammatory and anticancer activities (Mehta et al. 2022).

A. alternata has been found to produce bioactive endophytes, which have potential to inhibit the HIV and reduce the viral load. Specifically, 1,2-cyclobutanedicarbonitrile (**37**) and benzeneethanamine (**38**) have been found to possess anti-inflammatory, antimicrobial, and antioxidant properties. Thus, these bioactive compounds have potential to serve as treatments for HIV infection (Nzimande et al. 2022).

In vitro studies have demonstrated the anti-inflammatory and antioxidant properties of terrain (**39**), a secondary metabolite produced by *A. terreus.* These significant medicinal properties, combined with the high initial yield of this metabolite, facilitate large-scale production and technological advancements in the crude extract of *A. terreus* for the prevention of certain non-transmissible chronic disorders (NTCDs) associated with aging (Takahashi et al. 2020; Asfour et al. 2019; Lee et al. 2015).

Cordyceps militaris is a well-known mushroom that possesses beneficial nutraceutical properties and produces several metabolites. The global market offers numerous nutraceuticals containing cordyceps that support the immune system, vascular system, cognition, anticancer and antioxidant activities, and mental health. The primary metabolite produced by *C. militaris*, cordycepin (**40**), is effective in reducing hyperlipidemia and the accumulation of LDL, total cholesterol, triglycerides, and other lipids caused by high-fat diets. Cordycepin has various pharmacological properties, including anti-inflammatory, immunomodulatory, antioxidant, anti-aging, anticancer, antiviral, cardio, and hepatoprotective effects. *Cordyceps sinensis*, which contains cordycepin, stimulates the production of interleukin-10, an anti-inflammatory cytokine. Cordycepin products, which contain adenosine receptor agonists, are being studied as potential treatments for COVID-19 pneumonia and brain protection (Takahashi et al. 2020; Ashraf et al. 2020).

4 Plant-Derived Compounds from Fungal Endophytes with Potential Anti-Inflammatory and Antithrombotic Benefits

Many plant-derived compounds are actually synthesized by symbiotic/parasitic fungi living inside plants, called endophytes, rather than by the plants themselves. In recent years, increasing researchers have demonstrated the ability of endophytic fungi derived from important medicinal plants to produce the same bioactive metabolites as their host plants. It is now established that endophytes can produce, induce, and modify a variety of plant-derived metabolites inside and outside of host plants, which opens up new possibilities for producing medicinal compounds from endophytes, since only a small fraction of plant species have been studied for their endophytes and their produced bioactives. Thus, as the demand for natural products increases endophytes are becoming more attractive targets for isolating typical host-derived compounds. Given that medicinal plants are a rich source of therapeutic compounds, exploring their endophytes is crucial for identifying and isolating such compounds (Singh et al. 2021; White 2018; Xingyuan et al. 2022). Characteristic examples are nonribosomal peptides, polyketides, terpenes, alkaloids, coumarins, flavonoids, lignans, saponins, quinones, xanthones, and miscellaneous compounds, for which their health benefits and mode(s) of action have been extensively presented elsewhere by Singh et al. (2021), Toghueo (2019), and Nicoletti and Fiorentino (2015). Moreover, many anti-inflammatory compounds currently isolated from medicinal plants have been identified in the metabolome of endophytic fungi. We are presenting in this section a few numbers of well-established and promising bioactive metabolites with anti-inflammatory and antithrombotic potential, which are produced by the interaction(s) of endophytes with their host plants, as summarized in Table 14.1, along with subsequent associated health benefits and/or putative side effects.

5 Conclusions

Within this chapter, it was presented that bioactive fungi metabolites, such as phenolics, bioactive polar lipids, alkaloids, terpenoids, and other miscellaneous compounds, are a new type of promising products for drug and supplements' development with multi-purpose and multi-target aspects, as these active substances do not regulate the inflammatory response through a single reaction path, rather than through their pleiotropic protective effects against several thrombo-inflammatory signaling pathways. The research on the action mechanism of modern pharmacological research shows that bioactive secondary metabolites produced from natural fungi or by engineered species can effectively interact with relevant immune cells, inflammatory factors, and intestinal flora, play an anti-inflammatory role, possess antithrombotic benefits and repair the inflammatory reaction disorder of the body.

Table 14.1 Bioactive metabolites derived from the interaction(s) between plants and fungal endophytes, with anti-inflammatory and antithrombotic potential and several other health benefits

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
Piperine	• Inhibition of LPS—induced expression of IRFs and its associated pro-inflammatory responses, such as inhibition of PGE2 and NO.	• Anticancer.	• In high doses it might slow blood clotting and thus might increase the risk of bleeding in people with bleeding disorders.	*Piper longum, Piper nigrum*	*Periconia sp.*	*Piper longum*	Singh et al. (2021), Verma et al. (2011), Chithra et al. (2014a, b), Chithra et al. (2017), Stojanović-Radić et al. (2019), Park et al. (2007), Ziegenhagen et al. (2021)
	• Inhibition of platelet aggregation induced by collagen, AA, and PAF.	• Antimicrobial.	• Bioavailability-enhancing ability for certain drugs that can increase the risk of unintended deleteriously increased or adverse drug effects.		*Colletotrichum gloeosporioides*	*Piper nigrum*	
	• Decrease of TNF-α, inducible iNOS, and COX-2.	• Antidepressant.	• In high doses it can cause disturbance of spermatogenesis and of maternal reproductive and embryotoxic effects.		*Mycosphaerella* sp.	*Piper nigrum*	
	• Inhibition of IL-1β, TNF-α, IL-6, expression of iNOS, activities of MMP-3 and MMP-13, of mRNA expression for ADAMTS-4, and ADAMTS-5.	• Hepatoprotective.			*Phomopsis* sp.	*Oryza sativa*	
	• Reduction in activation of the STAT1 and its associated inflammatory signal transduction pathways involved in	• Bioavailability-enhancing ability for certain drugs and nutrients.					

	different pathologies correlated to the inflammatory process. • Inhibition of the release of Th-2-mediated cytokines involved in inflammatory cascades.						
Aconite-like alkaloids (i.e., aconitine) • Subsequent inhibition of inflammation-induced increased vascular permeability and/or edema formation. • Can protect the heart, platelets, and vascular smooth muscle in DIC.	• Inhibition of inflammatory pathways induced by histamine, serotonin, acetic acid, carrageenan.	• Anticancer. • Analgesic. • Anti-neuralgic.	• Cardiotoxic. • Bradycardia. • Hypotension. • Ventricular dysrhythmia. • Neurotoxic. • Inhibition of release of neurotransmitters.	*Aconitum* spp.	*Cladosporium cladosporioides*	*Aconitum leucostomum*	Singh et al. (2021), Yang et al. (2013), Singhuber et al. (2009), Hikino et al. (1980), Hikino et al. (1982), and Zhao et al. (2012)
Sanguinarine	• Inhibition of monocyte attachment to cytokine-activated HUVEC by inhibiting PAF synthesis induced by TNF-α and IL-1β. • Inhibition of tumor invasiveness-induced inflammatory manifestations by inhibiting the expression of MMP-9 and COX-2.	• Anticancer. • Antimicrobial.	• Toxicant. • Hepatotoxic.	*Macleaya cordata, Sanguinaria canadensis*	*Fusarium proliferatum*	*Macleaya cordata*	Wang et al. (2014), Singh and Sharma (2018), Park et al. (2014), Shu et al. (2021), Jeng et al. (2007), Lou et al. (2021), and Sneddon et al. (2006)

(continued)

Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	• Inhibitory effect on the collagen pathway of platelet activation and aggregation, and reduction in thrombus formation.	• Antioxidant.	• Pro-apoptotic properties in normal cells (through several mechanisms, including its action on the Na + -K + -ATPase transmembrane protein).				
	– Inhibition of the phosphorylation of multiple components in the collagen-specific receptor GPVI signaling pathway.	• Anthelmintic.	• Induce apoptosis and adversely influence embryonic development.				
	– Inhibition of collagen-induced increase in the intracellular Ca2+ concentration ([Ca2+]i).	• Neuroprotective.	• Carcinogen (might be associated with pre-carcinoma within oral or skin potentially).				
	– Inhibition of integrin αIIbβ3 outside-in signaling via reducing β3 and Src (Tyr-416) phosphorylation.	• Cardioprotective, when an excess of activated platelets is involved.					
	– Inhibition of platelet activation and aggregation induced by arachidonic acid, collagen, U4619,						

	and thrombin, by activating adenylate cyclase, by inhibiting platelet Ca2+ mobilization, TXB2 production, as well as by suppressing COX-1 enzyme activity.						
Capsaicin	• Inhibition of adipose tissue inflammatory responses via decreasing adipose tissue macrophages and levels of inflammatory adipocytokines (TNF-α, MCP-1, IL-6, and leptin].	• Antilithogenic.	• Potent irritant—can cause irritation, pain, and burning of mucous membranes.	*Capsicum annuum* (red pepper)	*Alternaria alternata*	*C. annuum*	Devari et al. (2014), Choi et al. (2000), Hogaboam and Wallace (1991), Munjuluri et al. (2021), and Chang et al. (2023)
	• Inhibition of PAF-induced superoxide production by inhibiting PAF-activated elicited SOCE via PLC activation, which results in inhibiting the subsequent superoxide production.	• Thermogenic.	• Topical patch administration can cause local erythema, pain, edema, and other relevant adverse effects.				
	• Inhibition of platelet activation and aggregation induced by PAF, thrombin, and calcium ionophore (A23187).	• Gastro-stimulatory. • Antidiabetic. • Cardioprotective. • Anticancer.	• Vasoconstrictive effects in people with underlying inflammatory conditions.				
Plant-derived phenolics—coumarins	*Anti-inflammatory and antithrombotic bioactivities, molecular targets, and mode(s) of action*	*Other health benefits*	*Putative side effects—toxicity*	*Plant source*	*Endophytic source*	*Host plant*	*Reference*

(continued)

Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
Isofraxidin	• Depletes infiltrating inflammatory cells (Kupffer cells and macrophages) and inflammatory cytokines (TNF-α and IL-6) in liver cells.	• Antioxidant.	Minimal cytotoxic side effects	*Acanthopanax senticosus or Eleutherococcus senticosus,*	*Biscogniauxia cylindrospora*		Yamazaki and Tokiwa (2010), Yim et al. (2017), Majnooni et al. (2020), Lin et al. (2020), and Chen et al. (2020)
	• Reduces inflammation mediators (COX-2, NO, PGE2, and iNOS) and cytokines (TNF-α and IL-6), as well as the expression of MMP-3 and MMP-13 and NF-κB activation in human cells that were induced with IL-1β.	• Anticancer and cytotoxic activities.		*Apium graveolens* (Siberian ginseng)			
	• Downregulation of several signaling pathways including NO, PGE2, TNF-α, IL-6, ADAMTS, COX-2, iNOS, MMPs, NF-kβ, and TLR4 for the treatment of inflammatory diseases such as osteoarthritis and pain.	• Cardioprotective effects.					

	• Inhibits NLRP3 inflammasome and thus exhibits cardioprotective effects and alleviates MI.	• Anti-hypertension effects.					
	• Inhibits platelet aggregation and activation induced by ADP, AA, and collagen.	• Hypolipidemic (regulates lipid metabolism, by reducing triglyceride accumulation and release of associated signaling molecules and thus protects from related disorders, and reduces hepatic lipogenesis).					
	• Ameliorates influenza virus A-induced lung inflammation, as well as it reduces the expressions of platelet activation biomarkers (P-selectin and CD61), decreases the serum levels of TNF-α, IL-1β, IL-6, and MIP-2, suppresses peripheral platelet aggregation, and prolongs the survival time of infected animals.	• Potent hyperpigmentation agent.					
Umbelliferone	• Reduction in inflammation-induced elevated levels of hepatic and cerebral NO,	• Antioxidant.	Minimal cytotoxic side effects	*Artemisia scoparia,*	*Penicillium* sp.	*Avicennia*	Yu et al. (2015), Germoush et al. (2018), Wang et al. (2019), Wang et al.

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	TNF-α, IL-1β, and glutamine.						(2015), Muthu et al. (2016), Chen et al. (1995), Cruz et al. (2020), Mazimba (2017), and Sim et al. (2015)
	• Suppresses hepatic LPS binding protein, TLR4, NF-kB, and TNF-α levels and gene expression increases in response to chronic alcohol intake.	• Anti-hyperglycemic.		*Scopolia carniolica*			
	• Reduces tumor-induced inflammatory markers (TNF-α and IL-1β) in colon carcinogenesis.	• Antimicrobial.		*(syn. Scopolia japonica),*			
	• Alleviates LPS-induced inflammatory responses by downregulating TLR4/MyD88/NF-κB signaling.	• Anticancer.		*Viburnum prunifolium*			
	• Ameliorates cerebral ischemia–reperfusion injury via upregulating the PPAR-γ expression and suppressing NLRP3 inflammasome.	• Hepatoprotective.					
	• Inhibition of LOX and COX.	• Antidiarrheal and antiulcerogenic.					

	• Inhibits platelet activation and aggregation induced by AA, collagen, and PAF.	• Protector against the side effects of anti-inflammatory agents.					
Plant-derived Phenolics—flavonoids	*Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action*	*Other health benefits*	*Putative side effects—toxicity*	*Plant source*	Endophytic source	*Host plant*	*Reference*
Quercetin	• Inhibits PAF activities and PAF synthesis, while it induces PAF catabolism, and subsequently ameliorates PAF-related inflammatory signaling and associated inflammatory manifestations.	• Antioxidant.	• Rarely reported.	*Fruits, vegetable*	*Aspergillus nidulans, Aspergillus oryzae*	*Ginkgo biloba*	Cheng et al. (2012), Qiu et al. (2010), Ebada et al. (2016), Srivastava et al. (2016), Colunga Biancatelli et al. (2020), Marunaka et al. (2017), Costa et al. (2016), Gormaz et al. (2015), Morikawa et al. (2003), Pace-Asciak et al. (1995), Balestrieri et al. (2003), and Vallance et al. (2019)
	• Inhibitory effects on AA and on the activity and production of the leukotriene/prostaglandin pathways' associated pro-inflammatory mediators, such as LOX, COX, TNF-α, and PLA2.	• Anticancer.	• May cause mild headache and upset stomach.		*Annulohypoxylon squamulosum*	*Cinnamomum*	
	• Suppression of PGE2, TNF-α, RANTES, MIP-2, and the mRNA for COX2 in carrageenan induced inflammation.	• Antiviral.	• Some interactions with certain drugs leading to altered drug bioavailability that might interfere with the effects of medication.		*Nigrospora oryzae*	*Loranthus micranthus* sp.	
	• Reduces the production of IL-1α, IL-6, IL-8, TNF-α, and PARP-1, and downregulating VCAM-1.	• Antidiabetic.					

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	• Reduces LPS-induced release of NO and inflammatory cytokines via induction of PON2 which also possesses anti-inflammatory activity.	• Cardioprotective.					
	• Decrease in thrombin formation in platelets.	• Neuroprotective.					
	• Inhibition of platelet aggregation induced by thrombin, ADP, TXA2, collagen, PAF, AA, and U46619 (a synthetic TxA2 mimetic).	• Antiallergic.					
	• Inhibitor of multiple kinases involved in platelet activatory signaling pathways.	• Anti-hypertensive (via anti-inflammatory and anti-oxidant actions, as well as via regulation of ion transporters and channels).					
	• Inhibits calcium mobilization induced by a low dose of thrombin, ADP, collagen, and U46619, and subsequently it reduces PS enrichment of platelets' surface, and the						

	associated thrombin generation.						
	• Antagonistically blocks TxA2 receptors and thus TxA2 signaling for platelet activation.						
	• Inhibits TxB2 production in platelets in response to collagen, and it reversibly inhibits COX-1 and the conversion of AA to prostaglandin H2 (PGH2) by preventing its entry into the active site of COX-1, while it also inhibits thromboxane synthase required to convert PGH2 into TxA2.						
Curcumin	• Influences/modulates inflammatory chemokines and chemokine receptors toward anti-inflammatory protection of several inflammation-related disorders.	• Anti-inflammatory protection and preventive properties in healthy subjects, aged subjects, and menopausal women.	• In general, safe, well-tolerated, and without any adverse side effects.	*Curcuma* spp.	*Chaetomium globosum*	*Curcuma wenyujin*	Rakha et al. (2022), Wang et al. (2012b), Yan et al. (2014), Fadus et al. (2017), Shah et al. (1999), Hussain et al. (2022), and Panknin et al. (2023)
	• Prevent atherosclerosis and platelet aggregation.	• Antioxidant.	• The intended use of curcumin as a food additive is generally recognized as safe by the U.S. Food and Drug Administration.				

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	• Through regulating many processes involved in platelet aggregation, curcuminoids collectively demonstrated detectable antiplatelet activity.	• Antitumor and anti-angiogenetic.	• As a component of turmeric, curcumin may interact with prescription drugs and dietary supplements.				
	• Lowers platelet and leukocyte adhesion.	• Anti-atherosclerotic and cardioprotective.	• In high amounts, it may be unsafe for women during pregnancy.				
	• Modulates the endothelium to reduce platelet adhesion.	• Neuroprotective.	• It may cause side effects, such as nausea, diarrhea, hives, or dizziness.				
	• Inhibits the expression of platelet/endothelial cell adhesion molecule 1, netrin G1, delta-like 1, and plasma cell endoplasmic reticulum protein 1, which causes cell adhesion and migration.	• Anti-arthritic.	• Between 2004 and 2022, there were 10 cases of liver injury caused by cucumin herbal and dietary supplement.				
	• Inhibit activation of platelets to form thrombosis/embolism, reduces P-selectin, E-selectin, and GP	• Anti-inflammatory protection against chronic liver diseases.	• Curcumin is a contact allergen.				

	IIb/IIIa and inhibits PDGF.						
	• Inhibits platelet aggregation induced by PAF, AA, collagen epinephrine, and ADP.	• Antidiabetic.	• Preliminary clinical studies in cancer patients consuming high doses of curcumin (up to 8 grams per day for 3–4 months) showed no toxicity, though some subjects reported mild nausea or diarrhea.				
	• Suppresses TXB2 production and inhibits TXA2 and mobilization of intracellular Ca2+ and associated signaling in platelets, and elevates prostacyclin and 12-LOX enzyme activities.	• Protects against gastrointestinal disorders.					
	• Reduces acute inflammation brought on by carrageenan.	• Protects against inflammatory manifestations of persistent infections (periodontitis, gingivitis, stomatitis, COVID-19, HIV infections, etc.)					
	• Downregulates of COX-2, LOX, TNF-α, IL-1, IL-2, IL-6, IL-8, and Janus kinases.	• Protects against inflammatory manifestations of autoimmune disorders (rheumatoid arthritis, multiple sclerosis, psoriasis, etc.)					
	• Downregulates the expression of pro-inflammatory cytokines and chemokines via NF-kB inactivation.	• Anti-lipidemic and anti-hypercholesterolemic.					
	• Reduced mortality and coagulopathy	• Beneficial effects on clinical outcomes					

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	induced by infections and LPS-induced intravascular coagulation by decreasing TNF-α level.	and/or biomarkers of obesity-associated metabolic disorders or musculoskeletal disorders, where inflammation is a key driver.					
Kaempferol	• It blocks the expression of inflammatory cytokines (IL-1B and TNF-α), COX-2 protein, and inducible NO synthase (iNOS).	• Antitumor.	• Rarely reported.	*Fruits, vegetables, medicinal herbs*	*Annulohypoxylon boveri var. microspora*	*Cinnamomum* sp.	Vallance et al. (2019), Sadeghi et al. (2023), Hegde et al. (2023), Chaturvedi et al. (2014), Huang et al. (2014), Chen and Chen (2013), and Imran et al. (2019)
	• Suppress the inflammatory responses, in LPS-stimulated inflammation via NF-κB and MAPK pathway, and inhibition of inflammatory factors such as iNOS, COX-2, TNF-α, and IL-6.	• Chemopreventive agent in cancer treatment.	• Poor oral bioavailability, which is increased by the use of nanocarriers.		*Annulohypoxylon squamulosum*	*Tylophora indica*	
	• Reduces the Th2 inflammatory cytokines.	• Antibacterial.	• High intake may cause adverse side effects. For example, in tumor patients who are deficient in iron and/or folic acid may exhibit abnormal side effects, since it reduces the		*Fusarium chlamydosporum*	*Podophyllum hexandrum*	
	• Inhibitory effects against platelet activation stimulated by thrombin, collagen, AA, ADP, and PAF.	• Antidiabetic.			*Mucor fragilis*		
		• Antioxidant.					

	• Inhibits pro-coagulant protein-ase activity, fibrin clot formation, blood clot and thrombin (or collagen/epineph-rine)-stimulated platelet activation, thrombosis, and coagulation.	• Cardioprotective. • Neuroprotective. • Hepatoprotective. • Antiallergic.	bioavailability of iron and/or reduces the level of folic acid in the cells.				
Rutin	• Inhibition of expression of COX-2 and iNOS via inhibition of p38 MAPK and c-Jun N-terminal kinase (JNK), and decreases the secretion of TNF-α.	• Antioxidant.	• Generally consid-ered safe when con-sumed in the amounts found in foods.	*Aegle marmelos*	*Anonymous*	*Pteris multifida, Nerium olean-der, Ginkgo biloba, Aegle marmelos*	Balestrieri et al. (2003), Choi et al. (2015a), Ren et al. (2019), Fan et al. (2007), Ganeshpurkar and Saluja (2017), Enogieru et al. (2018), Rakotondrabe et al. (2023), Zaragozá et al. (2021), Sheu et al. (2004), Choi et al. (2021), and Choi et al. (2015b)
	• Upregulates SIRT-1, Nrf-2, and HO-1 and downregulates JAK-2/STAT3 pathways, as well as inflammatory markers.	• Anticancer.	• Higher doses of rutin in supplements may cause specific side effects, including headache, rashes, muscle tension changes in heartbeat, a high white blood cell count (leukocytosis), blurred vision, fluid accumulation in knees, upset stomach.	*Ginkgo biloba,*	*Chaetomium* sp.		
	• Reduces the levels of pro-inflammatory bio-markers and cytokines (NO, PGE2, TNF-α, and IL-6).	• Neuroprotective.	• May interact with medications for diabetes; people with diabetes should monitor blood sugar levels closely, especially if adding rutin to a	*Nerium oleander,*	*Xylaria* sp.		

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
			regimen that already includes blood sugar-lowering medications.				
	• Inhibits dense granule secretion in platelets.	• Mitigates hyperglycemia and hyperlipidemia, while increasing insulin amount.	• Should be avoided if you have been prescribed warfarin, as it may reduce the medication's anticoagulant (blood thinning) effect.	*Pteris multifida*	*Aspergillus flavus*		
	• Antiplatelet activity through the blocking of fibrinogen GPIIb/IIIa receptors, the suppression of the platelet activation, as well as the pro-aggregate effect of calcium ionophore.	• Reduces the induced damage of the kidney, liver, and pancreas.					
	• Inhibition of platelet aggregation induced by ADP, collagen, and PAF.	• Cardioprotective.					
	• Antiplatelet activity through inhibition of activation of phospholipase C, followed by inhibition of protein kinase C activity and TXA2 formation, thereby leading to inhibition of the phosphorylation of P47	• Protects against metabolic disease.					

	and intracellular Ca(2+) mobilization, finally resulting in inhibition of platelet aggregation in platelets stimulated by collagen or PAF.						
	• Reduces fibrin clot and prolongs aPTT, PT, and CT, and decreases the activity of pro-coagulant protein, thrombin.	• Anti-arthritic.					
	• Protective effect against collagen and epinephrine (or thrombin) induced acute thromboembolism.						
Silymarin	• Anti-inflammatory actions via ameliorating the release and levels of inflammatory and immunological biomarkers and cytokines, such as CRP, TNFα, INF-γ, IL-6, IL-1β, and IL-12β, as well as by impairing NF-κB-dependent transcription and by impeding NLRP3 inflammasome activation.	• Anticancer—antimetastatic.	• Can lead to disruptions of the thyroid system by blocking the uptake of thyroid hormones into the cells by their receptors. Subsequently, the administration of silymarin during pregnancy is especially thought to be dangerous, potentially leading to the Allan–Herndon–Dudley syndrome, a brain development disorder that causes both moderate-to-severe intellectual disability and problems with speech and movement.	*Silybum marianum*	*Aspergillus iizukae*	*Silybum marianum*	Chen et al. (2002), Ganeshpurkar and Saluja (2017), Ramasamy and Agarwal (2008), Vargas-Mendoza et al. (2014), Dixit et al. (2007), Karimi et al. (2011), El-Elimat et al. (2014), Breschi et al. (2002), Stanca et al. (2013), De La Lastra et al. (1992), Rui (1991), Pourová et al. (2019), and Zhang et al. (2023)
		• Antioxidant.					

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	• Anti-inflammatory properties in cirrhotic liver by reducing the expression and levels of PAF-biosynthetic enzymes.		• Asymptomatic liver toxicity (hyperbilirubinemia and elevation of alanine aminotransferase) in prostate cancer patients.				
	• Inhibits platelet aggregation induced by collagen, AA, and ADP, the transformation of prostaglandin H2 to TXA2 by thromboxane synthase and antagonistically inhibits thromboxane receptors, while it also decreases the expression of platelet activation markers, such as P-selectin and an active form of αIIbβ3.	• Cardioprotective.	• Is also devoid of embryotoxic potential.				
	• Influence human blood coagulation by decreasing the platelet aggregation via inhibition of the COX activity.	• Hepatoprotective—antihepatitic.					
	• Membrane-stabilizing action preventing or inhibiting membrane peroxidation of fatty	• Antiallergic.					

	acids and subsequent inflammation by inhibiting LOX and the formation of associated prostaglandins.						
	• Anti-inflammatory and antioxidant properties against ALD, NAFLD, and hepatocellular carcinoma, by regulating the activation of including AMPK, Farnesoid X receptor, nuclear factor erythroid 2-related factor-2, PPARs, phosphatidylinositol-3-kinase, and lysyl oxidase-like proteins, and their associated signaling pathways.	• Neuroprotective.					
	• Anti-ulcerogenic effect of silymarin related to its inhibitory mechanism of enzymatic peroxidation by the LOX pathway, avoiding leukotriene synthesis.	• Antidiabetic.					
		• Antiviral.					
		• Anti-hypertensive.					
		• Immunomodulatory.					
		• Photoprotective.					
		• Detoxification.					
Apigenin	• Inhibits PGE2, COX-2, IL-1β, and IL-6 and downregulates the COX-2 expression and activity via	• Antioxidant.	• In general, it is safe and tolerable in a variety of in vivo and in vitro studies and clinical trials.	*Matricaria* spp. *and vegetables*	*Chaetomium globosum*	*Cajanus cajan*	Balestrieri et al. (2003), Wadhwa et al. (2022), Johannes et al. (2016), Shukla and Gupta (2010), Panda and Kar (2007), Gao

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	suppressing the LPS-induced protein tyrosine kinase signal transduction.						et al. (2012), Sato et al. (1994), Viola et al. (1995), and Salehi et al. (2019)
	• Inhibits PAF activities and PAF synthesis and subsequently ameliorates PAF-related inflammatory signaling and associated inflammatory manifestations.	• Anticancer.	• It can cause hepatorenal toxic only at high doses of 500 mg/kg and 1000 mg/kg.				
	• Inhibit the release of IL-1β, IL-6, and TNF-α through suppressing the NF-κB signaling pathway.	• Neuroprotective.					
	• Prevents the IκB degradation and nuclear translocation of the NF-κB.	• Regulates hyperglycemia, thyroid dysfunction, and lipid peroxidation.					
	• Activates different anti-inflammatory pathways, including p38/MAPK and phosphatidylinositol 3-kinase (PI3K)/AKT.	• Exerts anxiolytic and sedative effects.					
	• Can inhibit neuroinflammation by suppressing CD38 overexpression.						

	• Inhibits platelet aggregation induced by AA and U46619 (a synthetic TxA2 mimetic).						
	• Inhibits calcium mobilization induced by a low dose of thrombin, ADP, collagen, and U46619, and subsequently, it reduces PS enrichment of platelets' surface and the associated thrombin generation.						
	• Antagonistically blocks TxA2 receptors and thus TxA2 signaling for platelet activation.						
	• Inhibits TxB2 production in platelets in response to collagen, and it reversibly inhibits COX-1 and the conversion of AA to prostaglandin H2 (PGH2) by preventing its entry into the active site of COX-1, while it also inhibits thromboxane synthase required to convert PGH2 into TxA2.						

(continued)

Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	• Inhibitor of multiple kinases involved in platelet activatory signaling pathways.						
Dimer of epicatechin (DoE)	• Anti-inflammatory effects on the inflammatory response of inflammation-induced paw edema by inhibiting carrageenan-induced inflammation.	• Antioxidant.	• In an animal study the effective dosage for survival was found 1.25 g/kg (Gp V) and the lethal dosage was 1.5 g/kg (Gp IV). Above 1.5 g/kg of the DoE produced hypersensitivity, righting reflex, tremors, and convulsions leading to the death of the animal.		*Curvularia australiensis FC2AP*	*Leaves of Aegle marmelos*	Mehta et al. (2022), Attiq et al. (2021), Guo et al. (2023), Mani et al. (2021), and Matsui (2015)
	• Minimizes the accumulation of leukocytes, proteins, and fluids and decreases vascular permeability and cellular infiltration.	• Anticancer.					
	• Inhibits STAT3 activation induced by PDGF.	• Cardioprotective.					
	• Inhibits platelet aggregation.	• Anti-hypertensive.					
		• Anti-obesity.					
		• Antidiabetic.					
Chrysin	• Inhibits PGE2 production in whole blood, due to direct	• Antibacterial.		*Passiflora incarnata*	*Alternaria alternata, Colletotrichum capsici,*	*Passiflora incarnata*	Singh et al. (2021), Cooper et al. (2004), and Santos-Buelga and Scalbert (2000)

	inhibition of COX-2 enzymatic activity.				*Colletotrichum taiwanense*		
	• Inhibits TXB2 production in whole blood, suggesting that it strongly inhibits COX-1 activity.	• Antidiabetic.					
	• Inhibits PAF receptor binding, and thus PAF-associated inflammatory signaling.	• Anxiolytic.					
		• Hepatoprotective.					
		• Anti-aging.					
		• Anticancer.					
Luteolin	• Inhibits PAF activities and PAF synthesis and subsequently ameliorates PAF-related inflammatory signaling and associated inflammatory manifestations and disorders.	• Antioxidant.	• Safe and tolerable in a variety of in vivo and in vitro studies and clinical trials.	*Fruits, vegetables*	*Annulohypoxylon boveri var.*	*Cinnamomum* sp.	Cheng et al. (2012), Pace-Asciak et al. (1995), Shukla and Gupta (2010), Viola et al. (1995), Seetharaman et al. (2017), Saadawi et al. (2012), Jang et al. (2017), Lopez-Lazaro (2009), Zhao et al. (2014), and Lee et al. (2023)
	• Prevents inflammation and allergic response in the lungs and nose by inhibiting inflammatory cytokines: IL-4, IL-5, and IL-13.	• Neuroprotective.	• May have adverse effects for autistic children, people with colitis, and pregnant people	*Medicinal herbs*	*Microspora*	*Cajanus cajan*	
	• Inhibits PGE2, COX-2, IL-1β, and	• Antimicrobial.			*Aspergillus fumigatus*		
	IL-6 and downregulates the COX-2 expression and activity via suppressing the LPS-induced protein tyrosine kinase signal transduction.						

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Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	• Inhibit the release of IL-1β, IL-6, and TNF-α through suppressing the NF-κB signaling pathway.	• Cardioprotective.					
	• Inhibitory effect on collagen, ADP, thrombin, and U46619-induced platelet aggregation, as well as the adhesion of thrombin-activated platelets to collagen or fibrinogen.	• Antidiabetic.					
	• Suppresses platelet aggregation by inhibiting the TxA2 receptor, and strongly inhibits TxA2 production in agonist-induced platelets.	• Immunomodulatory.					
	• Targets both EGFR and KDR genes that induce the phosphorylation of AKT and the AKT-mediated activation of PI3K, which has been implicated in playing an important role	• Anticancer. • Antiallergic. • Skin protection. • Due to its anti-inflammatory properties, luteolin is being researched as a potential complementary					

	in platelet activation, suggesting that its effect on the PI3K-Akt signaling pathway is highly relevant to its antithrombotic therapeutic effect.	treatment for multiple sclerosis.					
Plant-derived Saponins—Terpenes	*Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action*	*Other health benefits*	*Putative side effects—toxicity*	*Plant source*	*Endophytic source*	*Host plant*	*Reference*
Diosgenin (phytosteroid sapogenin)	• Reduction in the levels of several inflammatory mediators, including NO and IL-1 and IL-6 in LPS/IFN-γ-induced inflammation.	• Antitumor and antiproliferative activities.	• Safe and tolerable in several studies.	*Dioscorea* spp.	*Paecilomyces* sp. *Cephalosporium* sp.	*Paris polyphylla var. Yunnanensis*	Singh et al. (2021), Lu et al. (2021), Yanoshita et al. (1996), Cao et al. (2007), Jesus et al. (2016), Patel et al. (2012), and Gong et al. (2011)
	• Inhibited the extracellular and intracellular superoxide anion generation by blocking the cAMP, PKA, cPLA 2, PAK, MAPK, and AKT signaling pathways.	• Anti-atherosclerotic and cardiovascular protection.	• In high amounts has exhibited toxic effects in animal models as an emerging contaminant, induced transgenerational reproductive toxic effects due to its endocrine disrupting nature.		*Fusarium* sp.	*Dioscorea nipponica*	
	• Reduces the adhesive capacity of vascular smooth muscle cells and the TNF-α mediated induction of ICAM-1 and VCAM-1 in these cells by inhibiting the MAPK/Akt/NF-κB	• Neuroprotective.					

(continued)

Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	signaling pathway and ROS production.						
	• Regulates adipokine expression and ameliorates endothelial dysfunction via regulation of AMPK and thus protects endothelial functions against inflammatory insults.	• Antiallergic.					
	• Inhibits IL-1-β-induced expression of inflammatory mediators, including MMP 3 and 13, iNOS, and COX-2 against skin inflammation, while it also increases the expression of VEGF, angiopoietin, and endothelial tyrosine kinase receptor against rheumatoid arthritis.	• Hypoglycemic.					
	• Antithrombotic activity through improving the anticoagulation function, inhibiting ADP-induced platelet	• Protective effects against metabolic diseases (diabetes, obesity, and dyslipidemia, including					

	aggregation and thrombosis.	hypercholesterolemia) and several infections.					
Ginsenosides (steroid glycosides and triterpene saponins)	• Anti-inflammatory agents by targeting different steps of the NF-κB signaling - pathway, ameliorating the symptoms of severe inflammation-related manifestations and associated illnesses.	• Antioxidation.	• Likely safe when taken for up to 6 months.	*Panax*	*Camarosporium* sp., *Dictyochaeta* sp.	*Aralia elata* *Panax ginseng*	Nicoletti and Fiorentino (2015), Salehi et al. (2019), Zhang et al. (2013), Maurya et al. (2022), Wu et al. (2012), Wu et al. (2013), Luo et al. (2013), Jin et al. (2017), Jang et al. (2023), Huo et al. (2023), and Luo et al. (2020)
	• Participate in anti-inflammatory response by inhibiting the action of inflammatory mediators (iNOS and COX-2) and by downregulating the expression and secretion of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α.	• Antitumor and anti-proliferative effects.	• Hormone-like effects that could be harmful when used long-term (trouble sleeping, severe rash, liver damage, and severe allergic reactions).		*Penicillium* sp.	*Panax notoginseng*	
	• Can inhibit inflammatory factors related to liver fibrosis, including TNF-α, IL-1β, caspase-1, and IL-6, in a JNK- and p38/ERK-dependent manner.	• Cardioprotective.	• Caution is needed in patients with auto-immune disorders that need immunosuppres-sion, since ginsenosides can enhance and increase the activity of the immune system.		*Aspergillus* sp., *Fusarium* sp., *Verticillium* sp. *Aspergillus* sp., *Fusarium* sp.		
	• Anti-inflammatory regulation and activities of immune cells (T cells and macrophages).	• Neuroprotective.	• Caution is also needed in the case of bleeding conditions due to their strong antithrombotic effects.				

(continued)

Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	• Influence the NAMPT/NAD+/SIRT, AMPK/SIRT1/PGC-1α, Nrf2/HO-1, PKCs/PARPs/NF-κB, and AMPK/Nrf2/mTOR signaling pathways, thereby regulating NAD+ metabolism to prevent and treat various diseases.	• Antimicrobial.	• Likely unsafe in infants and children (fatal poisoning in newborns).				
	• Antiplatelet effects due to their interference on multiple signal pathways of platelet aggregation induced by thrombin, collagen, ADP, U46619, and PAF, which lead to attenuation on several significant events, including TXA2 production, [Ca2+]i generation, αIIb/β3 activation, ATP release, and MAPK phosphorylation.		• Unsafe when taken by mouth during pregnancy.				
	• Suppression on proliferation and migration of VSMCs, which also help to delay thrombosis.						

Ginkgolides and bilobalide (terpenes)	• Inhibit PAF activities and PAF synthesis and subsequently ameliorates PAF-related inflammatory signaling and associated inflammatory manifestations and disorders.	• Neuroprotective.	• Side effects occur very rarely if preparations from controlled cultivation and standardized processing are used.	*Ginkgo biloba*	*Pestalotiopsis uvicola*	*Ginkgo biloba*	Tsoupras et al. (2020b), Tsoupras et al. (2011b), Luo et al. (2020), Irfan et al. (2020), Valli and Giardina (2002), and Sarkar et al. (2020)
	• Inhibition of STAT-3 pathway in high-glucose-stimulated endothelium.	• Antiallergic.	• Gastrointestinal complaints (bloating, cramps, and heartburn) headache, dizziness, and a general feeling of discomfort.		*Fusarium oxysporum*		
	• Suppression of COX-2, NO, TNF-α, IL-6, and IL-1β during LPS-induced inflammation.	• Analgesic.	• Increased bleeding tendency, platelet dysfunction.				
	• Inhibition of NF-κB activity by NF-κB-specific suppressor IκBα in CCL4-induced inflammatory hepatotoxicity.	• Anti-viral.	• CNS hemorrhage in patient previously stable of warfarin.				
	• Inhibition of IL-6, IL-8, MCP-1, and TNF-α. activation of NF-κB signaling during LPS-induced inflammation.	• Antitumor.	• In pregnancy, they are contraindicated for safe use due to insufficient data.				
	• Antithrombotic-antiplatelet effects against the PAF,	• Anti-atherosclerotic and cardioprotective.					

(continued)

Table 14.1 (continued)

Plant-derived alkaloids	Anti-inflammatory and antithrombotic bioactivities; molecular targets and mode(s) of action	Other health benefits	Putative side effects—toxicity	Plant source	Endophytic source	Host plant	Reference
	thrombin, collagen and ADP-associated pathways of platelet activation-aggregation.	• Protective effects in cardio-cerebral ischemia–reperfusion injuries.					
		• Beneficial against cerebrovascular disease by improving blood flow, reducing ischemia–reperfusion injury or inhibiting platelets.					
		• Hepatoprotective.					

LPS: lipopolysaccharide, *IRFs:* interferon regulatory transcription factors, *PGE1*: prostaglandine-E1, *PGE2*: prostaglandine-E2, *NO*: nitric oxide, *AA*: arachidonic acid, *PAF*: platelet-activating factor, *TNF-α*: tumor necrosis factor alpha, *iNOS*: nitric oxide synthase, *COX-2*: cyclooxigenase-2, *IL-1β:* interleukin 1-beta, *IL-6*: interleukin 6, *MMP*: matrix metalloproteinase, *ADAMTS:* a disintegrin and metalloproteinase with thrombospondin motifs, *STAT1*: signal transducer and activator of transcription 1, *DIC*: disseminated intravascular coagulation, *HUVEC*: human umbilical vein endothelial cells, *MCP-1*: monocyte chemoattractant protein-1, *SOCE*: store-operated Ca2+ entry, *PLC*: phospholipase C, *TLR4*: toll-like receptor 4, *NF-kβ*: nuclear factor-kappa beta, *ROS*: *reactive oxygen species*, *ACC*: acetyl coenzyme A carboxylase, *FAS*: fatty acid synthase, *HMGC*: 3-hydroxyl-3-methylglutaryl-CoA synthase 2, *ADP*: adenosine 5′ diphosphate, *MI*: myocardial infraction, *NLRPs*: family of leucine-rich repeat containing proteins, *PPAR-α/γ*: peroxisome proliferator-activated receptor alpha/gamma, *LOX:* lipoxygenase, *PLA2*: phospholipase A2, *RANTES*: regulated upon activation, normal T-cell-expressed and presumably secreted, *MIP-2*: macrophage inflammatory protein-2, *VCAM-1*: vascular cell adhesion molecule-1, *PON2*: serum paraoxonase/arylesterase 2, *TXA*: thromboxane A, *TXB*: thromboxane B, *PDGF*: platelet-derived growth factor, *aPTT*: activated partial thromboplastin time, *PT*: prothrombin time, *CT*: closure time, *ALD*: alcoholic liver disease, *NAFLD:* non-alcoholic fatty liver disease, *CRP*: C-reactive protein, *PS*: phosphatidylserine

Advances have been made in the understanding of the mechanism of action of various fungi metabolites, but many questions remain unanswered. For the past few years, there has been an exponential growth in the field of fungi-derived medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects.

At present, most of the studies are based on in vitro research and/or animal models, while the anti-inflammatory and antithrombotic mechanism of these fungi-derived metabolites in human body by targeted trials has not yet been fully described for all fungi compounds, as well as for combinations of fungi metabolites. Therefore, the action mechanism of fungi-derived bioactives in the process of anti-inflammatory response needs to be further studied. The research on the separation process and the study of structure–activity relationship of monomer components of fungi-derived products should be built upon, as well as the putative synergistic effects of multi-targeting anti-inflammatory fungi metabolites. In the future, active components in fungi and mixtures of fungi compounds with synergistic effects can be analyzed with the help of modern research means, such as network pharmacology, modern multi-omics and metabolomics, artificial intelligence-based docking modeling molecular biology, genetics, and epigenetics, to determine the relevant anti-inflammatory response mechanism and provide a basis for the clinical application of fungi-derived metabolites.

Moreover, the library of fungi-derived anti-inflammatory and antithrombotic bioactives has dramatically been increased by the inclusion of specific plant-derived medicinal compounds efficiently biosynthesized by hundreds of endophytic fungi. Nonetheless, the exciting progress that has been made in the field of functional genomics, genome mining and genome scanning, fermentation technology, green combinatorial chemistry, and systems biology might remove the roadblocks in the way of commercial success of this innovative approach. The design of endophyte-dependent enhanced in vivo and in vitro production of plant-derived valuable metabolites is of prime importance for the pharmaceutical industries and provides a plethora of valuable compounds with future perspectives for the healthcare systems and for a "green drug revolution."

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Chapter 15
Progress in the Development of Fungal Metabolites as New Drugs for Human Mycoses

Jacqueline Aparecida Takahashi

Abstract Fungi have a fascinating track record related to producing bioactive substances that have become medicines or inspired the synthesis of drug leads. On the other hand, there are several fungal species that cause damage to human health, agriculture, materials, and animals, some of which lead to conditions that can acquire great severity when reaching vital organs. Mycoses deserve special attention when they are installed in immunocompromised individuals, neonates, and patients debilitated by other diseases, as occurred during the COVID-19 pandemic. Thus, the development of antifungal drugs for several applications is very relevant worldwide. These drugs can be obtained through multiple strategies, highlighting the biosynthesis of antifungal metabolites by the fungi themselves. These metabolites are substances usually involved in the defense of a species that cohabitates in an environment where there are other fungi. Since these metabolites can be produced in vitro, their isolation, identification, and development as new antiviral drugs are an important area of research and drug development. This chapter presents an overview of some important fungal infections in humans, the drugs developed for the control of these microorganisms, mechanisms of action, chemical structures, and properties of some fungal metabolites with antifungal activity recently described in the literature, with potential application in pharma industry.

Keywords Filamentous fungi · Secondary metabolites · Antifungals · Candidiasis · COVID-19-associated fungal infections · SDG3

1 Introduction

Infections of fungal origin affect over 25% of the world's population. The superficial infections reach over a billion people in the world (Huang et al. 2019). These infections can be caused by opportunist yeast or by filamentous fungal species and

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are classified as superficial, cutaneous, subcutaneous, and systemic (Ma et al. 2021). In superficial mycosis, the fungus is installed in the corneal extract of epithelial tissue, in the hair, and in the skin in general. Since most of the superficial mycoses are not dangerous, fungal infections are frequently underestimated (Gnat et al. 2021). Invasive mycoses that affect the dermis, muscles, and connective tissues, reaching larger regions of the human body, are severe and deserve attention. Fungi can reach the lung, where an infection can be developed, affecting several organs of the human body. Systemic mycoses are therefore serious threats associated with high mortality rates, mostly in immunocompromised, and organ-transplanted patients, premature newborns, elderly people, and other patients severely affected by debilitating diseases such as cancer (Butts and Krysan 2012; Shafiei et al. 2020). At this point, it is important to emphasize that new antifungals are necessary to achieve the objective of sustainable development SDG3, that is, to decrease the mortality of live births. Among the fungal species capable of causing systemic infections, there are *Candida* sp., *Aspergillus* sp., *Cryptococcus* sp., *Pneumocystis* sp., and zygomycetes species such as *Rhizopus* sp. Superficial mycoses reach a billion people in the world, while invasive infections affect millions of patients (Heard et al. 2021). Clinical evidence shows that even fungi considered harmless to human beings can cause invasive infections under suitable conditions, especially in immunocompromised patients, because the virulence is frequently associated with the health of the host (Dworecka-Kaszak et al. 2020; Rokas 2022). The complexity of fungal infections becomes more worrisome due to the growing development of drug resistance during the evolution of fungal pathogenicity, and the number and scope of action of antifungal drugs are very limited. In addition, the resistance to antifungals follows the pattern of antibiotics resistance; i.e., there is a clear acceleration trend (Witzany et al. 2020). The suitability of antifungal drugs to pharma applications must attend parameters such as dosage, side effects, and effectiveness, and pharmacodynamic and pharmacokinetic patterns need to be determined for each application and individual conditions. In this way, the development of novel antifungal drugs for the most diverse applications is highly desirable (Alegbeleye et al. 2022; De Lucca 2007; Alghuthaymi et al. 2021).

Nature is known as a source of bioactive molecules that contributed to the development of new drugs and molecules used for the most diverse purposes throughout the human history (Takahashi et al. 2020; Pimenta et al. 2021; Brtko 2022). Fungal metabolites occupy a place of special interest to the pharmaceutical industry, as source of antibiotics, immunosuppressants, antitumor and cholesterol-lowering agents, as well as source of drug that leads to treat several major diseases (Deshmukh et al. 2022; Agrawal et al. 2023). Antimicrobial agents, active against bacteria and fungi, can be considered the most important fungal metabolites used by the humankind. The biosynthesis of antimicrobial agents by fungi is closely related to their survival, as an in situ mechanism of defense, since, in nature, these agents are essential to fight cohabitant microbes (Takahashi and Lucas 2008; Oliveira et al. 2019). This ability to produce antifungal agents has been observed by scientists, and, with the development of new niches, new fungal species have been described and identified more easily, due to the advance of techniques for the identification and dereplication of fungal species. In addition, fermentation processes have also been

improved in recent decades, and new methodologies for bio-monitored isolation and structural elucidation have ensured prosperity in this area (Wijeratne et al. 2004; Lucas et al. 2007; Takahashi et al. 2013).

2 Development and Mechanisms of Action of Antifungal Drugs

Several chemicals have been used throughout history for the treatment of fungal infections, such as compounds containing sulfur and iodine compounds, but most of the current antifungal agents are from polyene, pyrimidine, allylamine, azole, and echinocandin classes (Langfeldt et al. 2022). Antifungals have also been obtained from plants, repurposing approaches, or they can be chemically synthesized (Butts and Krysan 2012), and some promising antifungal agents are being evaluated in clinical trials (Heard et al. 2021). In relation to antifungals produced by fungi, it is important to highlight the historical role of griseofulvin (**1**), isolated from *Penicillium griseofulvum*. Griseofulvin (**1**) action occurs at the microtubules level, preventing the separation of chromosomes, and inhibiting the cell division (Pawar et al. 2019). Concomitantly, the discovery of nystatin (**2**) was reported from *Streptomyces noursei* (Rai et al. 2022). However, nystatin is not indicated for systemic use due to its toxicity, although it can be used topically for the treatment of specific skin infections.

Amphotericin B was isolated from *Streptomyces nodosus* in mid-1950s and started to be used for the treatment of systemic mycoses (Cavassin et al. 2021). However, despite having a broad spectrum of action (*Candida* sp., *Aspergillus* sp., *Cryptococcus* sp., *Fusarium* sp., among others, it is still a toxic drug and reported to cause nephrotoxicity (Laniado-Laborín and Cabrales-Vargas 2009). Amphotericin B (**3**) and nystatin (**2**) are polyene compounds; i.e., they contain highly conjugated systems consisting of many carbons in the form of a macrocycle. Their chemical structures are also characterized by the presence of free hydroxyl groups (Fig. 15.1). The mechanism of action of polyene antifungals occurs directly in the cell membrane, destroying their integrity, and forming transmembrane channels after binding directly to ergosterol (Carolus et al. 2020).

Antifungals of the azole class were reported in middle 1940s and entered the market in the following decades. Figure 15.2 presents some of the antifungal compounds discovered or licensed from 1950 to 2020 (Heard et al. 2021).

Azole molecules act on ergosterol biosynthesis when interacting with cytochrome P-450. Antifungals of azole group can be divided into first, second, and third generations. First-generation triazoles were developed in the 1990s with the advantage of being safer than some other antifungal drugs, expanding the options for the treatment of opportunistic and endemic fungal infections (Sheehan et al. 1999; Lockhart et al. 2021). Second-generation triazoles appeared in the early 2000s. They have improved the activity spectrum and lower risk of drug–

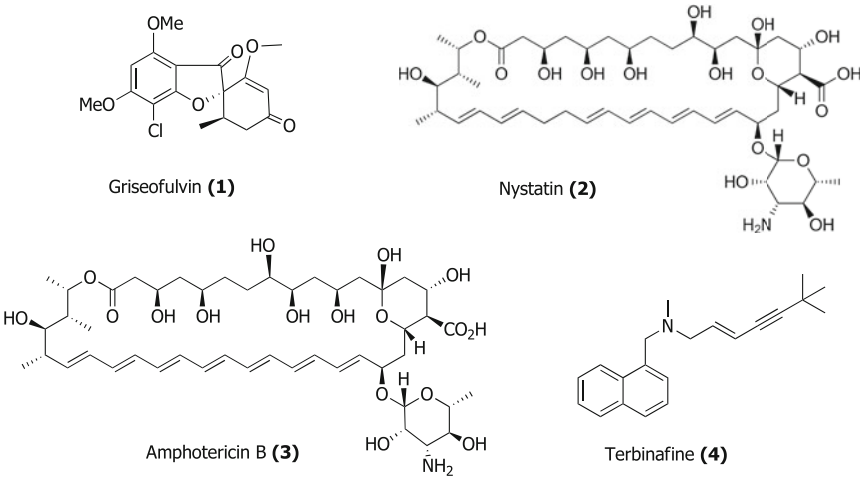

Fig. 15.1 Chemical structures of Griseofulvin, Nystatin, Amphotericin B, and Terbinafine

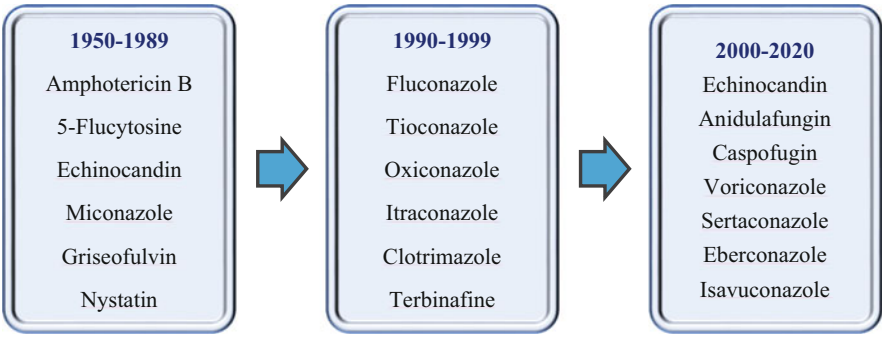

Fig. 15.2 Some antifungals developed from 1950 to 2020 (Heard et al. 2021)

drug interactions (Shafiei et al. 2020). Second- and third-generation imidazoles are used to treat systemic infections, although they present side effects such as hepatotoxicity. Imidazoles are characterized by an aromatic heterocyclic ring containing C-N bond and two nitrogen atoms. Triazoles have three nitrogen atoms in the ring structure (Shafiei et al. 2020). The chemical structures of some representative azole molecules can be seen in Fig. 15.3.

Drugs that act on the fungal cytoplasm membrane should be able to selectively destroy the fungal membrane without degrading the human cell membrane (Huang et al. 2019). The fungal cell membrane has, as peculiarity, the presence of ergosterol, and antifungal agents can act on the synthesis of ergosterol or by altering the physical integrity of the membrane. Inhibition of ergosterol synthesis occurs by inactivation of enzymes that participate in the biosynthesis of this steroid (Cavassin et al. 2021). Consequently, there is accumulation of toxic intermediates and,

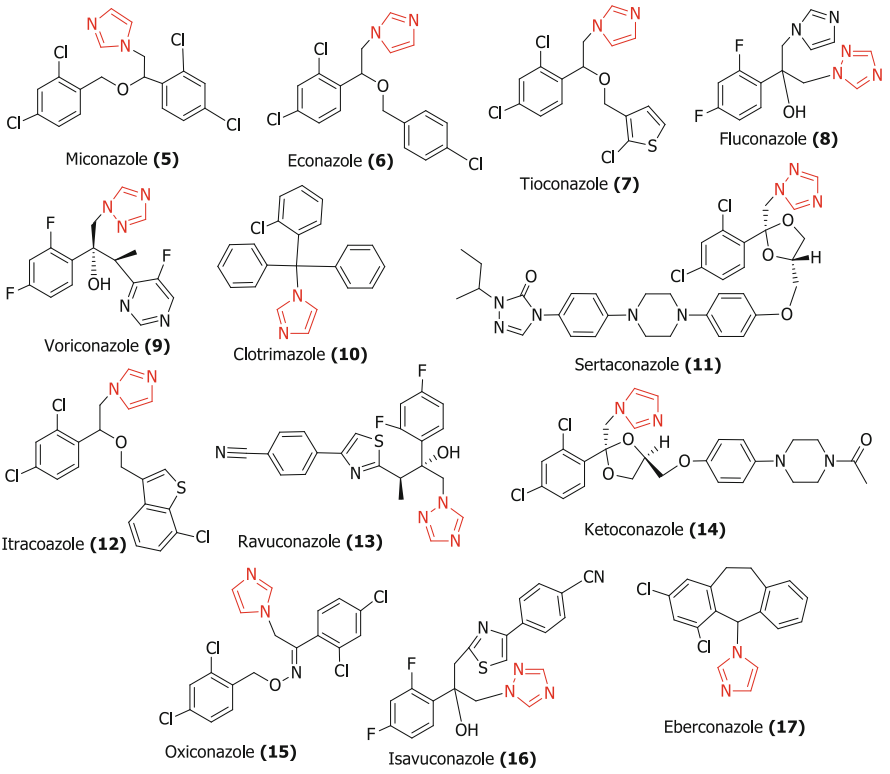

Fig. 15.3 Chemical structures of some representative molecules of azole class

therefore, installation of fungal growth. Terbinafine (**4**) is an allylamine with systemic activity and can be used topically or orally for the treatment of dermatophyte infections, cutaneous candidiasis, among other fungi, capable of interfering in the synthesis of ergosterol. Some antifungals modify the permeability of the fungal cytoplasmic membrane, forming pores that allow the output of important intracellular materials such as proteins, carbohydrates, and ions, which culminates in the death of the fungus. Another mechanism of antifungal action consists of inhibiting the synthesis of chitin and glycans, causing changes in the cell wall that protects the fungus (Brauer et al. 2023).

Some intrinsic characteristics of the fungi themselves also challenge the development of efficient antifungal drugs. For example, within the same strain of fungi, there are species with different degrees of virulence. If the treatment is sized in general, there will be selection of more virulent species. In addition, it is rare to perform sensitivity tests and determination of efficient doses for the treatment of mycoses, whether cutaneous or systemic (Denning 2022). Thus, the use of subdoses may occur, with selection of more virulent species, as described above, while unnecessary prolonged use of antifungal can lead to the development of resistance mechanisms. Finally, the formation of biofilms is quite common and the entry of

medicines into this type of ecosystem is difficult, demanding research (Cavassin et al. 2021).

The recent increase in resistance to antifungals causes much concern (Brauer et al. 2023). Several events that have occurred in recent years have led to this increase, and the use of antifungals after transplants and the increase in immunosuppression cases of patients infected with the HIV virus can be mentioned. The limited number of antifungals available for human use requires the development of new antifungal agents, preferably having new mechanisms of action, selective toxicity, structural stability, low cost, and targets unique to fungi (Butts and Krysan 2012; Heard et al. 2021). Antifungal drugs for oral administration are also very welcome. Although it is desired that antifungal drugs have a broad spectrum of action, it is important that the mechanism of action does not corroborate the development of fungal resistance. In addition, new antifungals need to meet pharmacokinetic criteria, i.e., present good absorption, distribution, and be excreted efficiently. Despite the ongoing studies with some antifungal molecules in clinical trials (Fig. 15.4), the development of new antifungals needs to be dynamic, as there are still no drug candidates capable of fulfilling all the necessary pharmacokinetic and pharmacodynamic requirements, besides that the phenomenon of microbial resistance makes the demand for new drugs constant.

3 Production of Antifungal Metabolites by Fungi

Fungi are prolific sources of biologically active chemicals that can be produced using simple fermentation methodology using liquid or solid substrates. The bioactive compounds are usually released to the culture medium and can be recovered by extraction followed by purification techniques (Rustamova et al. 2020). In addition to the natural production of bioactive metabolites, biosynthesis of new compounds can be activated through alterations incorporated during the fermentation process (Gomes and Takahashi 2016). However, a fungus can slow down its natural metabolism when it is isolated, since the lack of other microbes prevents the production of defense metabolites, such as antifungal agents. On the other hand, the modern genomic tools proved that fungi biosynthetic potential is underestimated and underexplored, since they have a huge number of silent biosynthetic routes capable of producing complex molecules that are not usually expressed. Genome mining and omics tools are promising in this field (Heard et al. 2021). Alterations in the fermentation process, with insertion of microbial DNA, pH modification, decrease in the nutrients supply, and introduction of biosynthesis inductors can result in metabolic diversification, i.e., expression of new compounds, which occurs through activation of silenced metabolic routes (Takahashi et al. 2022).

This scenario shows that the production of antimicrobial agents by fungi is a promising alternative for the production of new drugs (Talukdar et al. 2020). The success in this area is owed to the great advance in taxonomic identification techniques, isolation, and structural characterization of new molecules, as well as

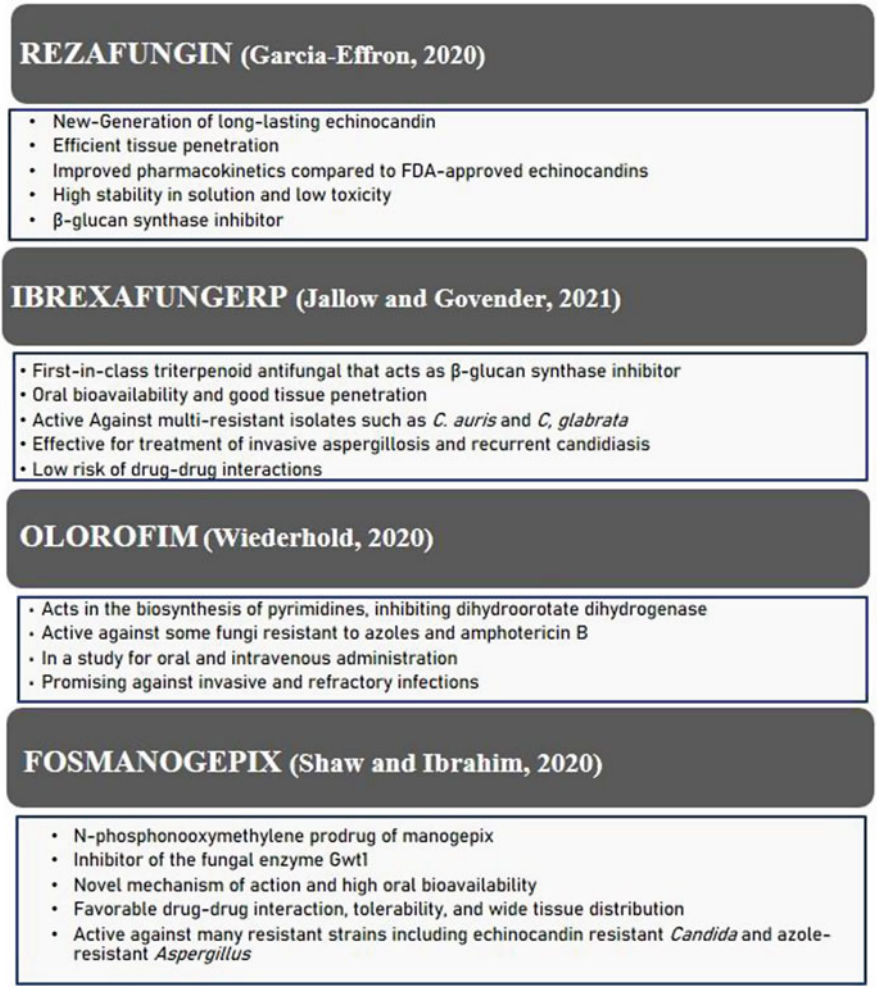

Fig. 15.4 Comparison of characteristics and antifungal profile of rezafungin, ibrexafungerp, olorofim, and fosmanogepix

the emergence of new fermentation techniques (Lucas et al. 2007; Petit et al. 2009; Takahashi et al. 2022). Many reports on the antifungal activity of fungal extracts are reported in the literature (dos Reis et al. 2019). Although the identification of the metabolite responsible for the activity is desirable, in many cases, synergistic effects between the compounds present in the extracts were proposed to explain the higher antimicrobial activity of the extracts, as exemplified by the extract containing phenolic metabolites produced from an engineered yeast species (*Saccharomyces cerevisiae* strain N2) compared to the activity of pure compounds (Ng et al. 2019).

A tryprostatin derivative, spirotryprostatin A (**18**), isolated from *A. fumigatus* was active against *C. albicans* (Zhang et al. 2018). Methoxyl spirotryprostatin B (**19**), a

compound with related structure, is active against *C. albicans* (Zhang et al. 2016). Other metabolites related to spirotryprostatin were isolated from another *Aspergillus fumigatus* species, derived from deep-sea sediment. Some of the new metabolites were secofumitremorgins A (**20**) and B (**21**) (inseparable mixture), 10R-15-methylpseurotin A (**22**), and 1,4,23-trihydroxy-hopane-22,30-diol (**23**), as well as the known metabolite cyclotryprostatin B (**24**) (Yan et al. 2022). Some of the metabolites isolated by Yan et al. were active against *F. graminearum* and *F. oxysporum* and maybe able to inhibit human fungal pathogens. Cyclotryprostatin B (**24**), 3-hydroxyfumiquinazoline A (**25**), and fumitremorgin (**26**) are active against *C. albicans* (Zhang et al. 2016).

Two indole diketopiperazines (luteoalbusin A (**27**) and T988 C (**28**)) were isolated from a bioguided screening from the Antarctic fungus *A. luteoalbus* CH-6 showed promising activity against *C. albicans* (Table 15.1, Fig. 15.5) (Shi et al. 2022). Fusarithioamide B (**29**), a new benzamide derivative isolated from an endophyte *Fusarium* species, also showed antifungal activity against *C. albicans*, as well as inhibitory effect on the phytopathogenic species *G. candidum* and *T. rubrum* (Ibrahim et al. 2018). The endophyte was isolated from *Anvillea garcinia* leaves inner tissues, and the fermentation was carried out in solid rice medium.

A series of sorbicillinoids produced by *T. longibrachiatum* were screened for their action over several phytopathogenic fungi: *Alternaria brassicicola*, *B. cinerea*, *Colletotrichum coccodes*, *Cladosporium cucumerinum*, *Cylindrocarpon destructans*, *Magnaporthe oryzae*, and *Phytophthora infestans* (Ngo et al. 2021). In the work, bisvertinolone (**30**), bisorbicillinol (**31**), bisvertinoquinol (**32**), epoxysorbicillinol (**33**), oxosorbicillinol (**34**), and cyclonerodiol (**35**) showed activity against the phytopathogenic species addressed in the assay. Bisvertinolone (**30**) was the most promising metabolite, with good activity against *C. cucumerinum*, *C. destructans*, and *M. oryzae* (MIC 50, 25, 50 μg/mL, respectively), and outstanding activity against *P. infestans* (MIC 6.3 μg/mL) (Ngo et al. 2021). In another work, the anti-phytopathogenic activities of bipolins I (**36**) and J (**37**) (sesquiterpenoid-xanthone adducts) were evaluated against four plant pathogens *Phytophthora infestans*, *Alternaria solani*, *Rhizoctonia solani*, and *Fusarium oxysporum*. Bipolins I (**36**) and J (**37**) were not active against *P. infestans* and *R. solani*, but showed promising inhibition of *A. solani* (8 and 16 μg mL^{-1}, respectively). Bipolin I (**36**) was as active as the positive control hygromycin B against *F. oxysporum* (64 μg mL^{-1}) (He et al. 2019) (Table 15.1). Chemical structures can be observed in Fig. 15.6.

The activity of seiricardine D (**38**), a new cyclic sesquiterpene, isolated from an endophytic species of *Cytospora* sp., against the *Magnaporthe oryzae,* was described by Deng et al. (2020). The metabolite was not active, but an ergostane derivative (22*E*, 24*R*)5, 8-epidioxy-5α, 8α-ergosta-6,9(11), 22-trien-3 ß-ol) isolated in the same study was active against *C. albicans*. Two new sclerotioramine derivatives (isochromophilone XIV (**39**) and isochromophilone XV (**40**)) and two known compounds, sclerotioramine (**41**) and (+)-sclerotiorin (**42**) (Fig. 15.7), were isolated from a fungus (*P. mallochii*) isolated from the gut of *Ectropis oblique* (Zhang et al. 2019). Sclerotiorin (**42**) was previously reported as an antifungal metabolite (Lucas

Table 15.1 Antifungal metabolites produced by different fungal species and their activity against filamentous fungi and yeasts

Fungal species	Material tested	Antifungal activity profile	Reference
Alternaria tenuissima OE7	Ethyl acetate fraction OE7	*C. albicans* (IZ 12 ± 1.0–16 ± 1.0 mm); *C. tropicalis* (7 ± 1.0–14 ± 1.0 mm)	Chatterjee et al. (2020)
Aspergillus fumigatus	Spirotryprostatin G (**18**)	*C. albicans* (MIC 0.39 μg/mL)	Zhang et al. (2018)
	Spirotryprostatin B (**19**)		Zhang et al. (2016)
Aspergillus fumigatus SD-406	Secofumitremorgins A (**20**) and B (**21**) (inseparable mixture), 10R-15-methylpseurotin A (**22**), 1,4,23-trihydroxy-hopane-22,30-diol (**23**), cyclotryprostatin B (**24**)	*F. Graminearum* (MIC 4 μg/mL)	Yan et al. (2022)
		F. Graminearum (MIC 16 μg/mL)	
		F. Graminearum (MIC 32 μg/mL)	
		Fusarium graminearum (MIC 64 μg/mL)	
Aspergillus fumigatus	3-hydroxyfumiquinazoline A (**25**), and fumitremorgin (**26**)	*C. albicans*	Zhang et al. (2016)
		Active	
A. luteoalbus CH-6	Luteoalbusin A (**27**)	*C. albicans* (MIC 12.5 μM)	Shi et al. (2022)
	T988 C (**28**)	*C. albicans* (MIC 6.25 μM)	
F. chlamydosporium	Fusarithioamide B (**29**)	*C. albicans* (MIC 1.9 ± 0.31 μg/mL); *G. candidum* (MIC 6.9 ± 0.64 μg/mL); *T. rubrum* (MIC 5.2 ± 0.79 μg/mL)	Ibrahim et al. (2018)
Trichoderma longibrachiatum SFC100166	Bisvertinolone (30)	*P. infestans* (MIC 6.3 μg/mL)	Ngo et al. (2021)
	Bisorbicillinol (31)	*P. infestans* (MIC 100 μg/mL)	
	Bisvertinoquinol (32)	*M. oryzae* (MIC 200 μg/mL)	
	Epoxysorbicillinol (33)	*P. infestans* (MIC 50 μg/mL)	
	Oxosorbicillinol (34)	*P. infestans* (MIC 25 μg/mL)	
	Cyclonerodiol (35)	*P. infestans* (MIC 400 μg/mL)	
Bipolaris eleusines	Bipolin I (**36**)	*A. solani* (MIC 8 μg mL^{-1})	He et al. (2019)
	Bipolin J (**37**)	*A. solani* (MIC 16 μg mL^{-1})	
Cytospora sp.	Seiricardine D (**38**)	*Magnaporthe Oryzae* (839.6 μg mL^{-1})	
Penicillium mallochii CCH01	Isochromophilone XIV (**39**)	*C. graminicola* (IC_{50} > 50.0 μg/mL)	Zhang et al. (2019)
	Isochromophilone XV (**40**)	*C. graminicola* (IC_{50} 29.9 μg/mL)	
	Sclerotioramine (**41**)	*C. graminicola* (IC_{50} 9.7 μg/mL)	
	(+)-Sclerotiorin (**42**)	*C. albicans* (IZ 10.5 mm)	

(continued)

Table 15.1 (continued)

Fungal species	Material tested	Antifungal activity profile	Reference
Aspergillus niger	Aspergyllone (**43**)	*C. parapsilosis* (IC_{50} 52 μg/mL)	Padhi et al. (2020)
	Carbonarone A (**44**)	*C. krusei* (IC_{50} 373 μg/mL)	
	Aurasperone A (**45**)	*C. albicans* (IC_{50} 103 μg/mL)	
		C. krusei (IC_{50} 31 μg/mL)	
Colletotrichum coccodes	Ethyl acetate extract	*C. albicans* (IZ 21 ± 1.0)	
Phyllosticta capitalensis	Ethyl acetate extract	*C. albicans* (IZ 22 ± 0.57)	
Penicillium decumbens	3,11-dihydroxy-6,8-dimethyldodecanoic acid (**46**)	Inactive	Lin et al. (2018)
	Trichopyrone B (**47**)	Inactive	
	Sorbicillin (**48**)	*C. albicans* (MIC 50 μM)	
	Penicillone A (**49**)	Inactive	
Xylaria cf. *curta*	Curtachalasin C (**50**)	Resistance reversal activity against fluconazole-resistant *C. albicans*	Wang et al. (2019)

et al. 2007; Bao et al. 2010). Lin and colleagues successfully prepared several sclerotiorin analogs with simplified structures and improved activity (Lin et al. 2012). The authors proposed four major structure activity relationships based on their results. Shortly, (i) the key role of the hydroxyl group present in the quaternary center of azaphilone skeleton, (ii) the possibility of improving the activity according to the halogen introduction at C-5, (iii) the role of electron-withdrawing and (iv) electron-donating substituents on the phenyl ring (Lin et al. 2012). Sclerotioramine (**41**) is an antifungal metabolite of azaphilone class active against *C. albicans*, *C. parapsilosis*, *C. tropicalis,* and *C. krusei* (Lucas et al. 2007; Wang et al. 2010). The compounds isolated from *P. mallochii* were obtained on a fermentation process developed under the action of the epigenetic modifier suberoylanilide hydroxamic acid (SAHA) and were capable of inhibiting several fungal species, including *Curvularia lunata* and *Curvularia clavata*. The activity of this metabolite against *Curvularia species* is of great interest, since species of this genus are considered one of the most relevant fungi involved in invasive phaeohyphomycoses, a human disease highly prevalent in immunosuppressed patients (Tóth et al. 2020). *C. lunata*-associated phaeohyphomycoses, the most identified *Curvularia* species in this pathology, may cause severe complications, reaching the central nervous system with fatal outcomes (Carter and Boudreaux 2004). Zhang et al. (2019) also reported the activity of (+)-sclerotiorin active against *C. albicans* (Table 15.1).

Echinocandins are fungal natural products of non-ribosomal lipopeptides class, capable of obstructing cell wall biosynthesis through the inhibition of 1,3-β-D-glucan synthase and a variety of glycoproteins (Carolus et al. 2020). The mechanism of action does not affect human cells, reducing the toxicity of this drug in relation to

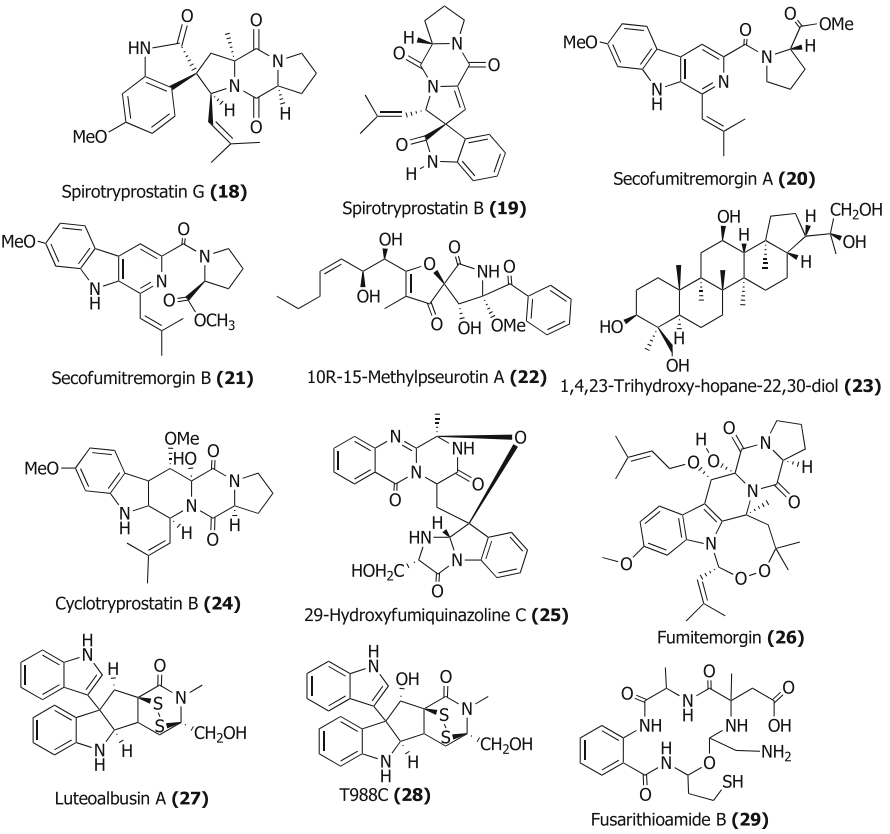

Fig. 15.5 Chemical structures of compounds (18–29)

others without specificity. However, they have demonstrated several advantages over azoles, polyenes, and flucytosine drugs (Szymański et al. 2022). They were discovered in the 1970s as metabolites of fungi such as *Aspergillus nidulans* and *Aspergillus rugulosus* with effective antifungal activity (Szymański et al. 2022). Their chemical structures are very complex to be synthesized on a route viable for industrial production. Therefore, the most successful approach is the production of semisynthetic derivatives, well represented by the cyclic lipopeptides anidulafungin, caspofungin, and micafungin which have potent antimycotic activity (Hüttel 2021) (Fig. 15.8). Other semisynthetic analogs were also produced, but water solubility, oral availability, and toxicity issues, along with low antifungal effect led to the discontinuation of the studies, as was the case of cilofungin (Balkovec et al. 2014). Although the success of echinocandins in the treatment of infections caused by several fungal species, the treatment is restricted to hospitalized patients and cannot be administered to pregnant women due embryotoxicity (Szymański et al. 2022).

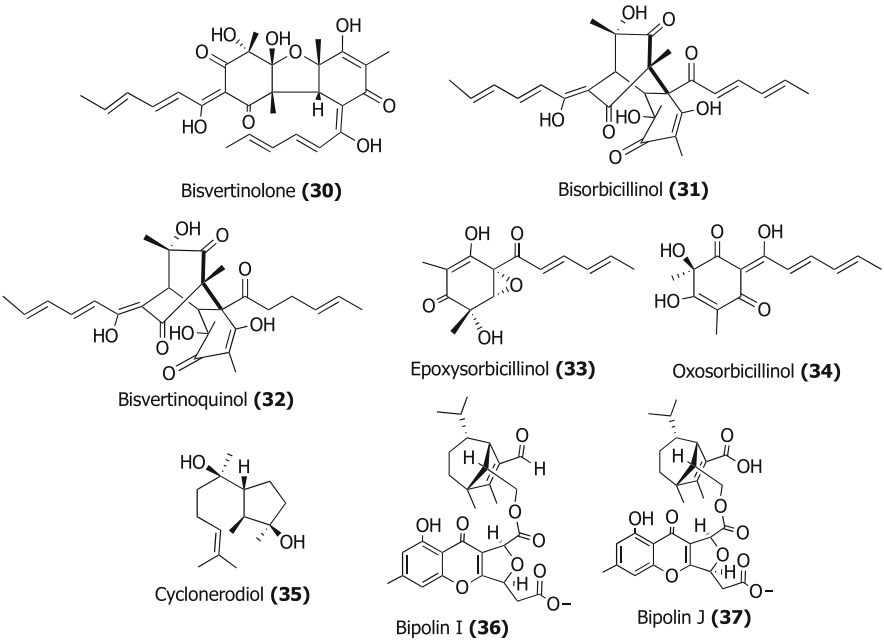

Fig. 15.6 Chemical structures of compounds (30–37)

4 Some Current Major Applications for Antifungal Fungal Metabolites

4.1 Treatment of COVID-19-Associated Fungal Infections

The severity of the SARS-CoV-2 pandemic, which caused a severe respiratory syndrome that overwhelmed the world in 2020 and 2021, also evoked the need for the development of new antimicrobial agents because COVID-19 was frequently characterized by secondary bacterial and fungal co-infections, which increased the severity and mortality rate of the disease (Duarte and Takahashi 2022). Since the onset of the pandemic, COVID-19-associated pulmonary aspergillosis, the first invasive fungal disease associated with COVID-19 infection, has been widely reported, sometimes related to azole-resistant species of *Aspergillus* sp. (Basile et al. 2022; Hoenigl et al. 2022). Aspergillosis can be caused by species such as *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *A. terreus,* and *A. parasiticus* (Shafiei et al. 2020), and laboratory diagnosis, although effective to characterize the infection, is not efficiently applied to prevent nosocomial transmission of COVID-19 (Basile et al. 2022).

Other fungal infections associated with COVID-19 patients deserve attention, such as mucormycosis, pneumocystosis, cryptococcosis, endemic mycoses, and candidiasis (Basile et al. 2022). Although mucormycosis is rare in

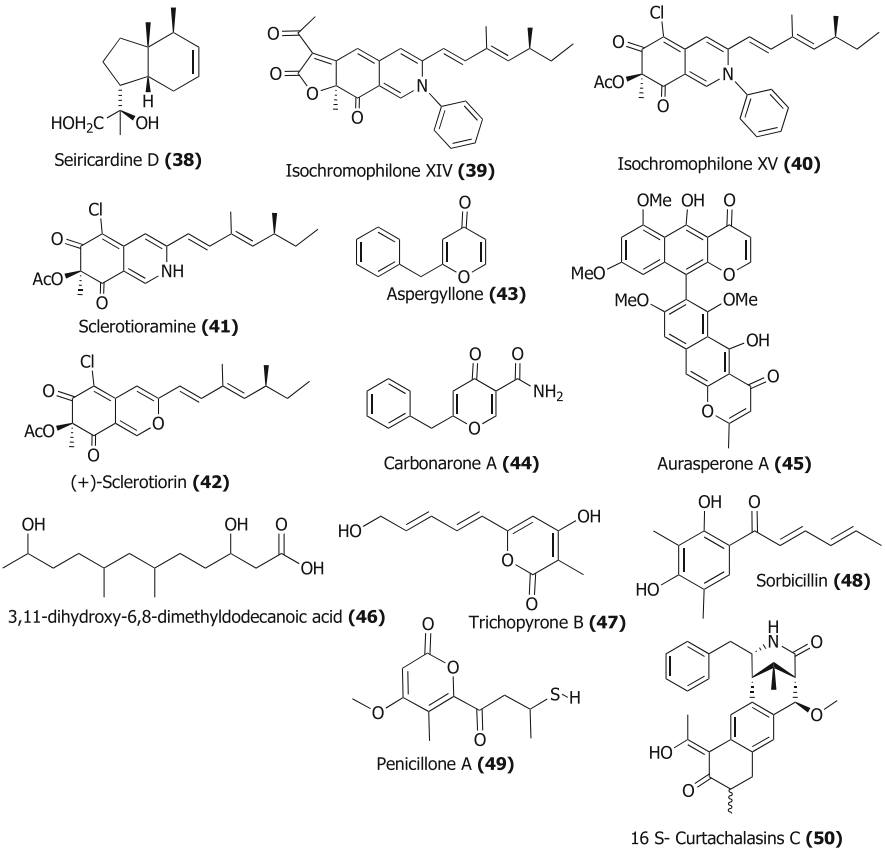

Fig. 15.7 Chemical structures of compounds (38–50)

immunocompetent individuals, this infection was related to thousands of COVID-19 patients with uncontrolled diabetes, especially in India, establishing a picture of great severity, since the diagnosis and, consequently, the treatment of mucormycosis may not occur, since the symptoms of this infection can be confused with the symptoms of COVID-19 (Arbune et al. 2023). Intravenous liposomal amphotericin is the preferable pharmacological treatment of mucormycosis that can be caused by fungi from *Rhizopus*, *Mucor,* and *Rhizomucor* genera, after spores' inhalation, ingestion of contaminated food, or direct contamination of damaged skin or mucosa (Jafarzadeh et al. 2022; Skiada et al. 2022; Arbune et al. 2023).

Candidiasis is a disease of global concern, considered a public health and economic problem. Invasive candidiasis is the second most reported fungal infection associated with COVID-19; therefore, it deserves attention (Basile et al. 2022). Most of the invasive yeast infections are caused by *Candida* species, mainly *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis,* and *C. glabrata* (Shafiei et al. 2020). The latter is acquiring resistance to fluconazole by overexpression of multidrug efflux

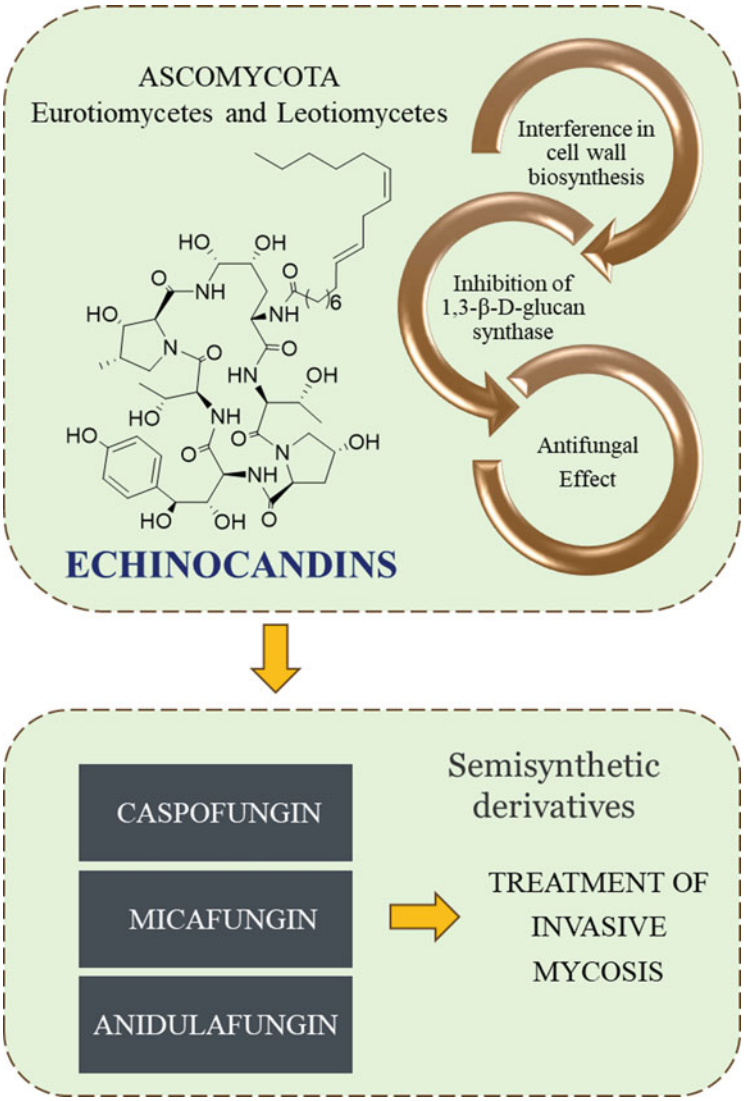

Fig. 15.8 Natural and semisynthetic echinocandins (Denning 2002; Hüttel 2021)

pumps, which leads to the possibility of cross-resistance. Together, *C. glabrata* and *C. auris* showed the highest mortality rates in patients with COVID-19 (Basile et al. 2022). *Candida dubliniensis* and *C. auris* are also reported as opportunistic species that can lead to severe infections (Rokas 2022). More recently, it has been found that yeasts of the genus *Candida*, present in the fungal mycobiome, may contribute to inflammatory bowel disease and are worrisome in patients undergoing abdominal surgery (Underhill and Braun 2022). Oral candidiasis is another opportunistic

infection caused by *C. albicans* and other *Candida* species that requires long treatment, especially in infants and HIV patients (Rai et al. 2022). Invasive *Candida* infections have also been reported in patients with COVID-19. The magnitude of this infection is considered underestimated, since the differential diagnosis of the main species involved in the process, *Candida auris*, has a high cost and is not routinely performed in several countries (Hoenigl et al. 2022).

4.2 Development of New Drugs to Rare and Neglected Fungal Diseases

Mycoses that have restricted geographical distribution affect specific population groups or are rare tend to be even more underestimated than superficial mycoses. However, several of these infections have a severe phase and potential to spread, becoming endemic. In other situations, life-threatening mycoses may manifest very early, in preterm infants, and treatment with antifungals of first choice, such as voriconazole (**10**) brings severe side effects, especially in the liver of babies (Mehler et al. 2022). Thus, new drugs to combat the etiological agents of these diseases are also of great interest.

Lobomycosis is an infection caused by the fungus *Lacazia loboi*, whose distribution is concentrated in the Amazon rainforest region, although it has already been reported in other countries. The fungus targets both humans and dolphins, but due to the regional distribution, epidemiological data are still insufficient. A serious aggravating factor is the late manifestation of this disease, making it difficult to locate the origin of the fungus and the time of contamination. Several cases of lobomycosis have been related to ecotourists, a class of individuals that has been growing a lot in recent decades (Gonçalves et al. 2022).

5 Final Remarks and Perspectives

There are still many other challenges in the fungal infections field, such as the need for a significant decrease in fungal human infections. Research should be able to conquer deeper knowledge about infections caused by nonculturable fungal species, as well as fungal infections restricted to some regions of the world (Gonçalves et al. 2022). Recovery of epidemiological data can help avoid the spread of fungi species that are currently restricted to specific territories. For mycoses already installed, the development of new drugs must take into consideration the need to avoid triggering resistance, maintaining the desirable characteristics for absorption, effectiveness, and low toxicity. Chemical stability should be observed so that new antifungals can be administered orally. In this scenario, new echinocandin derivatives have been developed to fulfill the current needs and some are already in clinical trials, and they

are promising new antifungal antibiotics, with the advantage of being related to reduced rates of fungal resistance. Some new targets of fungal inhibition with significant differences with human metabolism are arising, bringing hope to the development of novel drugs with reduced toxicity (Amich 2022). Immunotherapeutic approaches have also been emerging in this field (Armstrong-James et al. 2017). Hopefully, the new generation of antifungal drugs will be effective and fully accessible by all infected people, regardless of their economic and health condition.

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Part V
Fungi from Extremophilic Environment/Unique Ecosystems

Chapter 16
Uncovering the Desert Fungal Enigma: An Attractive Resource for Biopharmaceuticals

Pruthviraj Chavan and Shivankar Agrawal

Abstract Fungi reside everywhere on the earth, from the Arctic to the tropics. Fungi are described as an exceptionally capable organism living in extremely inhospitable environments. Fungi also represent a promising biological reservoir for novel chemical entities and open new avenues in the frontiers of drug discovery. This chapter will highlight various bioactive molecules obtained from desert fungi and emphasis recent advanced technological developments that are enabling natural product-based drug discovery from fungi, highlight selected applications, and discuss key opportunities.

Keywords Bioactive metabolites · Desert fungi · Diversity · Endophytes · Extreme environments

1 Introduction

Life evolved on earth under very extreme conditions. Continued exploration of the biosphere has led to further discoveries of life in environments that were previously thought to be uninhabitable pushing the boundaries of where life can still exist and greatly expanding the notion of terrestrial habitability. It is expanding. Over 75% of our earth is covered by hostile environments, which create a favorable condition for the emergence of a unique type of biodiversity and specialized adaptations (Fitz et al. 2007). A striking number of microorganisms have been discovered during the past few decades in habitats with temperatures as high as +121 °C and as low as −25 °C, or capable of surviving at pH levels of 0 or 13, or exposed to harmful radiation or toxic substances, or in saturating salt concentrations (Merino et al. 2019; Rampelotto 2013). These discoveries have greatly increased our understanding of how extremophiles and extreme-tolerant microbes adapt, live, and function in the extremes, helping to shed light on the origins of life on earth by demonstrating unusually high levels of diversity and complexity (Rothschild and Mancinelli 2001).

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Initially, more effort has been mainly on bacteria or archaea to understand their survival in such environment. But it is now becoming evident that fungi are exceptionally capable of living in extremely inhospitable environments, showing evidence of resistance that is even higher than prokaryotes. Fungi are the most adaptable phylogenetic lineage in the tree of life (TOL) due to their high level of ecological versatility and morphological plasticity (McLaughlin et al. 2009), as they can live in almost any habitat (Blackwell 2011), thrive in a variety of conditions, and adopt a variety of lifestyles, such as saprotrophs, symbionts, or parasites. Some very extremophilic or extreme-tolerant lineages, which have been shown to originate from lichenized and rock-inhabiting lifestyles, have evolved as a result of interactions with other living forms. Fungi come under the furthermost stress-resistant eukaryotes (Sterflinger et al. 2012). The extreme hot and frigid desert environments have been shown to be rich in unexpected amount of fungal species diversity. They have exhibited a wide range of adaptive mechanisms (Santiago et al. 2018; Onofri et al. 2007). Although novel fungal species have been discovered from desert ecosystem, their utility in bioprospecting campaigns is limited. Considering this rationale, this chapter encompasses the recent progress being done in the exploration of biopharmaceuticals potential of desert fungi.

2 Biopharmaceuticals from Desert Fungi

Desert plant-derived fungus *Alternaria* sp. HM 134 was the source of five new polyketides alternafurones A (**1**) and B (**2**), alternapyrones M-O (**3–5**), together with 14 known compounds (**6–19**). Out of these compounds, **1**, **2,** and **4** showed definite inhibitory activities against isocitrate dehydrogenase 1 gene (IDH1 R132h) with IC_{50} values of 29.38, 19.41, and 14.14 μg/mL, respectively, and remaining seven compounds (**6, 7, 9–12, 14**) showed potent protein tyrosine phosphatase 1B (PTP1B) inhibitory activity with IC_{50} values ranging from 0.97 to 89.80 μg/mL. Polyketides Alternafurones A and B represent an unprecedented 6/5/6 skeleton core (Li et al. 2022).

A novel skin-whitening agent Comoclathrin (**20**) was produced by endophytic *Comoclathris* strains which was isolated from Andalusia desert plants stems of *Sedum sediforme* (S. Alhamilla, Almeria, Spain) and *Nerium oleander* (Tabernas, Almeria, Spain). Two new compounds (**21–22**), along with the known compounds graphostrin B (**23**) and brevianamide M (**24**), were also obtained. Comoclathrin showed the strongest anti-tyrosinase activity at IC_{50} value of 0.16 μM, which was 90 times higher than positive control kojic acid (IC_{50} 14.07 μM). Moreover, Compound 20 also showed no significant cytotoxicity against a panel of cancer cell lines HepG2, A2058, A549, MCF-7 and MIA PaCa-2 and normal BJ fibroblasts (Georgousaki et al. 2022).

Phaeosphspirone (**25**), an undescribed polyketide with a unique 6/5/5/6-fused tetracyclic system, along with two known analogs, herbarin (**26**) and O-methylherbarin (**27**), were isolated from the culture broth of a desert plant *Bassia*

dasyphylla endophytic fungus *Phaeosphaeriaceae* sp. Both herbarin and O-methylherbarin displayed moderate cytotoxicity against three cancer cell lines HepG2, HCT116, and B16 with IC_{50} = 4.62/3.64, 2.56/1.22, and 2.25/1.97 μM, respectively (Tan et al. 2022).

Desert endophytic fungi *Fusarium* sp. HM166 was the source of seven undescribed carotane sesquiterpenoids, fusanoids A–G (**28–34**), along with one known analog (**35**) and two known sesterterpenes (**36–37**). Cytotoxic evaluation showed that compound 34 exhibited cytotoxic activity against human hepatoma carcinoma cell line (Huh-7) and human breast cell lines (MCF-7 and MDA-MB-231), while compound (**29**) showed activity against MCF-7 with IC_{50} value of 43.38 μM, whereas compounds **31–36** were inactive against all the tested cell lines. Compounds (**31**) and (**37**) showed potent inhibitory activities against the IDH1R132h mutant IC_{50} value of 22.27 and 13.99 μM. (Zhang et al. 2022) (Fig. 16.1).

Three rare new cytochalasan derivative chaetoglobosins B1-B3 (**38–40**) along with four known metabolite chaetoglobosins (**41–44**) were isolated from chemically engineered crude broth of desert soil-derived fungus *Chaetomium madrasense* 375. Potent anticancer activity against four human cancer cells (A549, HCC827, SW620, and MDA-MB-231) and two drug-resistant HCC827 cells (gefitinib-resistant, osimertinib-resistant) was observed in compound 43 (Guo et al. 2021).

The desert plant-associated endophytic fungus *Chaetomium globosum* (Chaetomiaceae) gave an undescribed chromene-4,7(4aH)-dione-tetramic acid PKS-PKS-NRPS hybrid, globosumin (**45**), and globosumone (**46**), an undescribed azaphilone, together with 10 known metabolites spiciferone A (**47**), chaetoviridin E (**48**), chaetoviridin A (**49**), chaetoglobosin A (**50**), chaetoglobosin C (**51**), isochaetoglobosin D (**52**), chaetoglobosin E (**53**), chaetoglobosin F (**54**), chaetoglobosin G (**55**), and chaetoglobosin F_{ex} (**56**). Chaetoglobosin A displayed biological effects against the seedling growth of *Arabidopsis thaliana* (Brassicaceae) in a dose-dependent manner, and this compound also exhibited biological activity against two cancer cell lines, A549 and HepG2, with IC_{50} values of 6.82 and 38.62 μM, respectively (Zhang et al. 2021). Fusapyrone A (**57**), a new c-pyrone derivative, along with six previously described compounds, 3-ethyl-2,5-pyrazinedipropanoic acid (**58**), neovasinin (**59**), neovasifuranone A (**60**), isolumichrome (**61**), protocatechuic acid (**62**), and adenosine (**63**) was isolated from the rice fermentation of desert fungus *Fusarium* sp. CPCC 401218. Fusapyrone A was found to have weak antiproliferative activity for Hela cells, with an IC_{50} of 50.6 μM (Zhen et al. 2021).

Desert plant endophytic fungus *Chaetosphaeronema hispidulum* was the source of four resorcylic acid lactones, hispidulactone F (**64**), hispidulactone B (**65**), 2 R, 4R-sonnerlactone (**66**), and 2 R, 4S-sonnerlactone (**67**), which were originated from the polyketide pathway. Compounds hispidulactone F and 2 R, 4S-sonnerlactone inhibit HepG2 cell proliferation at the IC_{50} values of 61.05 and 107.69 $\mu M/L^{-1}$, respectively, with a dose-dependent manner (Zheng et al. 2020). Hispidulones A and B (**68–69**) are two new phenalenone analogs isolated from desert endophytic fungus *Chaetosphaeronema hispidulum*. NMR analysis reveals that compound (**68**)

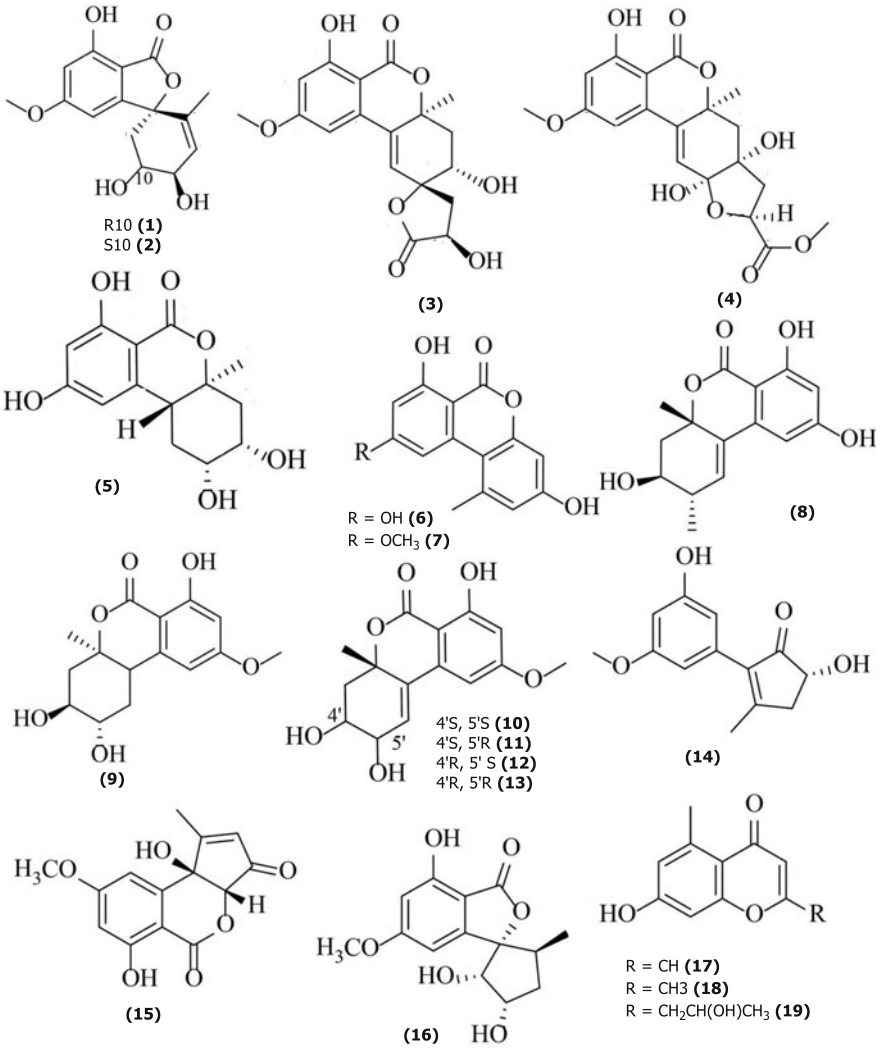

Fig. 16.1 Biologically active compounds isolated from desert fungi (**1–19**)

possesses a cyclohexa-2,5-dien-1-one moiety, whereas compound (**69**) contains a hemiacetal OCH_3 group, which is very rare in the structures of phenalenone analogs. Hispidulone B demonstrated cytotoxic activities against three cancer cell lines A549, Huh7, and HeLa with IC_{50} values of 2.71, 22.93, and 23.94 μM (Zhang et al. 2020). Fungus, *Monosporascus eutypoides*, which was isolated from the root of the desert plant *Agriophyllum squarrosum* gave two new compounds, monosporasols A (**70**) and B (**71**), and two known compounds, pestaloficin C (**72**) and arthrinone (**73**). Unfortunately, none of these compounds showed cytotoxicity against HeLa, HCT-8, A549, and MCF-7 cells (Guo and Zou 2020).

Two new cytotoxic cytochalasans, Chaetomadrasins A (**74**) and B (**75**) along with six known analogs (**76–81**), were purified from the desert soil-derived Fungus *Chaetomium madrasense* 375. Both new compounds displayed moderate cytotoxicity against HepG2 human hepatocellular carcinoma cells with an IC_{50} value of 8.7 and 19.4 μM, respectively; however, the positive control (cis-platin) showed IC_{50} value 3.14 μM (Guo et al. 2019). The locoweed endophytic fungus *Alternaria oxytropis* was the source of 10 undescribed guaiane-type sesquiterpenoids oxytropiols A-J (**82–91**), and the mycotoxin swainsonine (**92**). Fungal toxin swainsonine showed cytotoxicity against two cancer cell lines, A549, and HeLa at IC_{50} values of 10.93 and 66.69 μM, respectively, whereas compound **82** displayed biological effects on the root growth of *Arabidopsis thaliana* (Tan et al. 2019). Trematosphones A (**93**) and B (**94**) were obtained from the desert plant endophytic fungus *Trematosphaeria terricola*. Further in vitro antidepressant activity using corticosterone-induced damage in PC12 cells model showed that Trematosphone A exhibited a protective effect against induced injury in PC 12 cells at 6.25 μM concentration (Song et al. 2019). A desert grass associated novel rhizospheric fungus PAAN135 was the source of a new antibacterial cyclo-L-prolylglycine diketopiperazine (**95**) (Nasim et al. 2018) (Figs. 16.2, 16.3, 16.4, and 16.5).

Barceloneic acid A (**96**), barceloneic lactone (**97**), and 2′-O-methyl-barceloneate (**98**) were obtained from the desert plant endophytic fungus *Embellisia chlamydospore*. Compounds **97** and **98** significantly retarded the root growth of *Arabidopsis thaliana* (Li et al. 2018a). Hispidulactones A–E (**99–103**) along with a new natural product (**104**) and four known ones (**105–108**) with different ring systems were obtained from the fungus *Chaetosphaeronema hispidulur*. However, only natural product (**104**) showed cytotoxic activity against three HCT116, Hela, and MCF7 cancer cell lines with IC_{50} values at 54.86, 4.90, and 20.04 μM, respectively (Zhang et al. 2018).

Endophytic fungus *Phoma betae* inhabiting in the desert plant *Kalidium foliatum* was the source of new spiciferone F (**109**) including two new analogs spiciferones G (**110**) and H (**111**) together with four known ones spiciferone A (**112**), spiciferol A (**113**), (**114**), and (**115**). Spiciferone F displayed inhibition against MCF7 cells with a half MIC value at 7.73 μM (Tan et al. 2018). Three new polyketides 4,6,8-trihydroxy-5-methyl-3,4-zihydronaphthalen-1 (2H)—one (**116**), 5,7-dihydrox y-3—(1-hydroxyethyl)—3,4- dimethylisobenzofuran-1(3H)-one (**117**), and 1-(4-hydroxy-6-methoxy- 1,7-dimethyl-3-oxo-1,3-dihydroisobenzofuran-1-yl) ethyl acetate (**118**) together with seven known analogs (**119–125**) were obtained from desert fungus *Paraphoma* sp. Unfortunately, none of these compounds displayed cytotoxic activities (Li et al. 2018b). A thermophilic fungi *Aspergillus japonicus* isolated form the Egyptian desert soil was the source of nigragillin (**126**) and nigerazine B (**127**). Nigragillin showed antibacterial activities against *B. subtilis*, *E. coli*, *N. lactamica*, and *S. aureus* (Magdy et al. 2017). An endophytic fungus *Alternaria* sp. AST0039, obtained from the leaf of desert plant *Astragalus lentiginosus*, was the source of (−)-(10E,15S)-4,6-dichloro-10(11)-dehydrocurvularin (**128**), (−)-(10E,15S)-6-chloro-10(11)-dehydrocurvularin (**129**), (−)-(10E,15S)-10(11)-dehydrocurvularin (**130**), and alterperylenepoxide A (**131**).

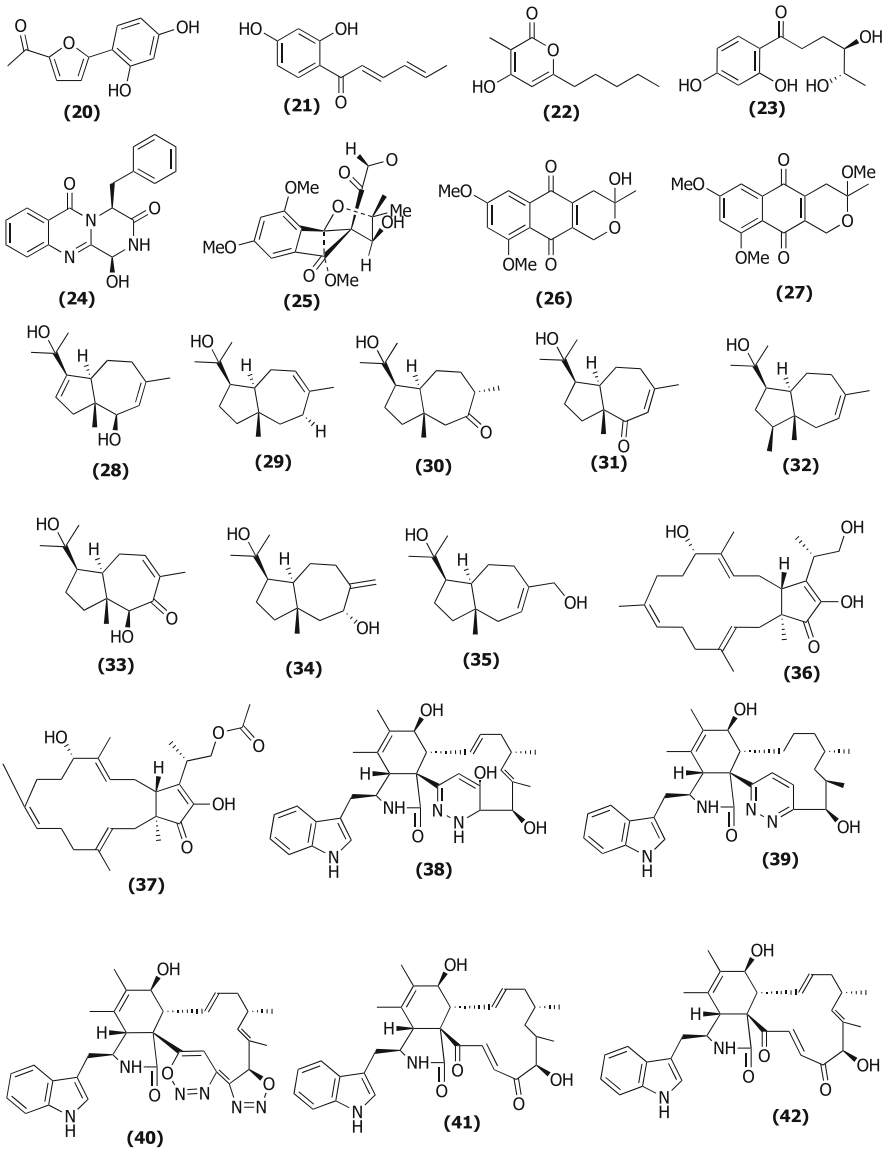

Fig. 16.2 Biologically active compounds isolated from desert fungi (**20–42**)

Only **129** and **130** were found to be cytotoxic below a concentration of 5.0 μM with no apparent selectivity for any of the cell lines tested (Bashyal et al. 2017). A desert soil fungus, *Aspergillus terreus,* gave a novel compound Amhezole (**132**), which showed growth inhibition of *Candida albicans* at the concentration of 7.81 μg/mL (Awaad et al. 2017). Hormonemates A–D (**133–136**), hormonemate (**137**), and hormonemate E (**138**) and F (**139**) were isolated from fungus *Dothiora*

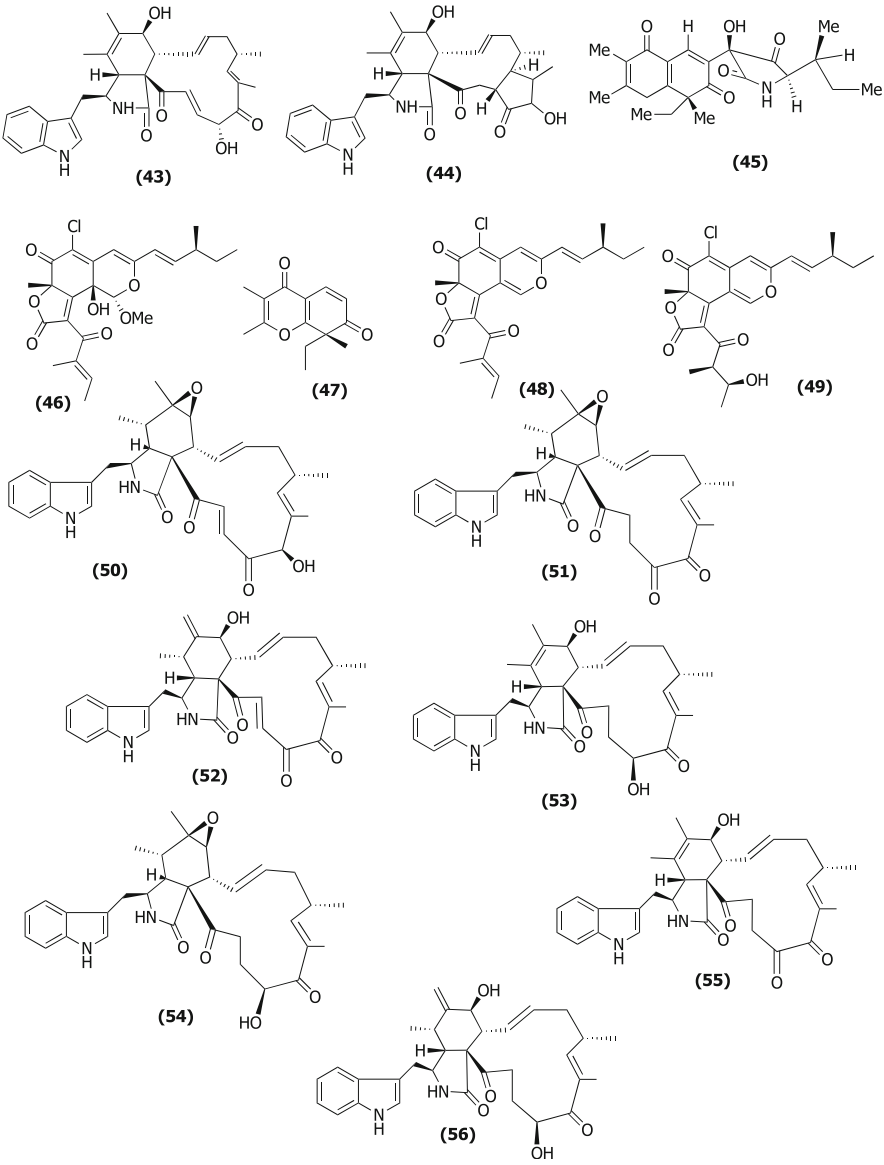

Fig. 16.3 Biologically active compounds isolated from desert fungi (**43–56**)

sp. Hormonemate B with IC_{50} 11.1 μM and hormonemate A with IC_{50} 15.6 μM showed cytotoxic activity against human breast cancer cells (MCF-7). Furthermore, all hormonemates were active against human hepatic cancer cells (HepG2) with an IC_{50} around 30 μM. Except hormonemate E ($IC_{50} = 36.4$ μM), none of them were

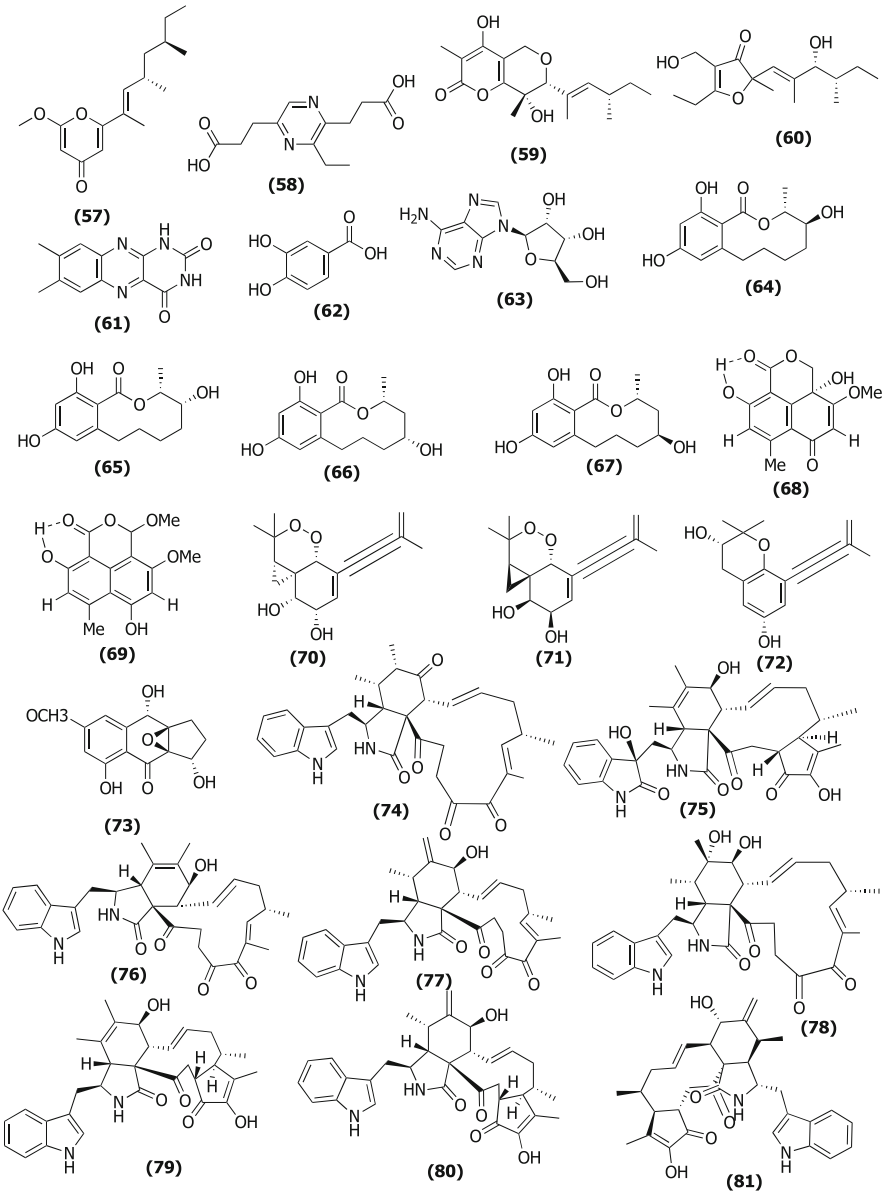

Fig. 16.4 Biologically active compounds isolated from desert fungi (**57–81**)

active against the pancreatic cell line (MiaPaca_2) at the highest concentration tested (Perez-Bonilla et al. 2017).

The mycelia and the culture broth of fungal endophyte *Montagnulaceae* sp. DM0194, gave seven azaphilones, montagnuphilones A–G (**140–146**), together

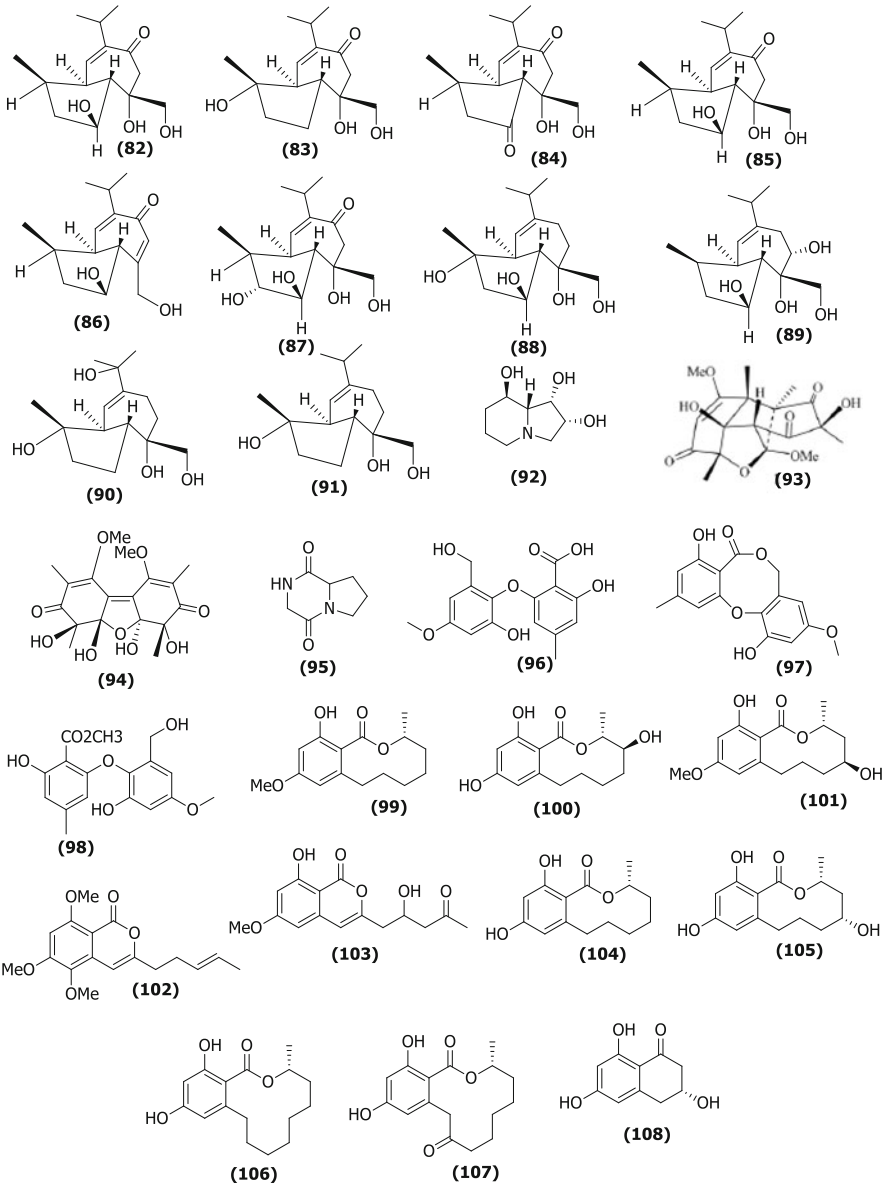

Fig. 16.5 Biologically active compounds isolated from desert fungi (**82–108**)

with previously known azaphilones (**147–150**). Out of then only compounds **147**, **144**, and **141** displayed NO inhibitory activity with IC_{50} values of 9.2, 25.5, and 39.6 μM, respectively (Luo et al. 2017). Five new isopimarane diterpenes, smardaesidins A–E (**151–155**) and two new 20-nor-isopimarane diterpenes, smardaesidins F (**156**) and G (**157**), together with sphaeropsidins A (**158**) and C–F

(**160–163**), were obtained from fungal strain, *Smardaea* sp. AZ0432, which living inside the photosynthetic tissue of the moss *Ceratodon purpureus*. Chemical reduction of sphaeropsidin A afforded sphaeropsidin B (**159**), further catalytic hydrogenation of same compound yielded 7-O-15,16-tetrahydrosphaeropsidin A (**164**) and its new derivative, 7-hydroxy-6-oxoisopimara-7-en-20-oic acid (**165**). The acetylation and diazomethane reaction of sphaeropsidin A afforded two of its known derivatives, 6-O-acetylsphaeropsidin A (**166**) and 8,14-methylenesphaeropsidin A methyl ester (**167**), respectively. Methylation of compound **160** yielded sphaeropsidin C methyl ester (**168**). Biologically, only the compounds **156**, **160**, and **164** displayed significant cytotoxic activity. More importantly, sphaeropsidin A showed cell-type selectivity in the cytotoxicity assay and inhibited migration of metastatic breast adenocarcinoma (MDA-MB-231) cells at subcytotoxic concentrations (Wang et al. 2011) (Fig. 16.6).

A desert endophytic strain of *Fusarium tricinctum* was the source of two new rare irregular sesquiterpenes, tricinonoic acid (**169**) and tricindiol (**170**), and the known furanopyrrolidones, NG-391 (**171**), and NG-393 (**172**) (Bashyal and Leslie Gunatilaka 2010). A desert endophytic fungal strain *Phyllosticta spinarum* gave five new metabolites, (+)-(5S,10S)-4′-hydroxymethylcyclozonarone (**173**), 3-ketotauranin (**174**), 3R-hydroxytauranin (**175**), 12-hydroxytauranin (**176**), and phyllospinarone (**177**)**,** together with tauranin (**178**). All compounds were found inactive against a panel of five cancer cell lines, and only tauranin showed apoptosis in PC-3 M cell line with IC_{50} of 3.5 μM (Wijeratne et al. 2008). Sonoran desert plant-associated *Aspergillus tubingensis* gave a dimeric naphtho-c-pyrone asperpyrone D (**179**), nine known naphtho-c-pyrones (**180–188**), funalenone (**189**), and a cytotoxic cyclic penta-peptide, malformin A1 (**190**) (Zhan et al. 2007). Desert plant-associated fungal strains *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii* were the source of five new isocoumarins, paraphaeosphaerins A–C (**191–193**) and chaetochiversins A and B (**194–195**) biogenetically related to monocillin I (**196**) and radicicol (**197**), a new chroman-4-one, aposphaerin C (**198**), and three known chromones, eugenetin (**199**), 6-methoxymethyleugenin (**200**), and 6-hydroxymethyleugenin (**201**) (Wijeratne et al. 2006a). Ethyl acetate extract of Sonoran Desert fungal strain, *Chaetomium globosum*, afforded a new dihydroxanthenone, globosuxanthone A (**202**), a new tetrahydroxanthenone, globosuxanthone B (**203**), two new xanthones, globosuxanthone C (**204**) and D (**205**), 2 hydroxyvertixanthone (**206**), and two known anthraquinones (**207–208**). Only globosuxanthone A exhibits strong cytotoxicity by disrupting the cell cycle in either G2/M or S phase against seven human solid tumor cell lines (Wijeratne et al. 2006b). Three new orsellinic acid esters globosumones A-C (**209–211**) and three known compounds, orsellinic acid (**212**), orcinol (**213**), and trichodion (**214**), were obtained from desert endophytic *Chaetomium globosum*. Only globosumones A and B showed moderate inhibition of cell proliferation of four cancer cell lines, NCI-H460 (nonsmall cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS glioma), and MIA Pa Ca-2 (pancreatic carcinoma), and normal human fibroblast cells (WI-38) (Bashyal et al. 2005). A *Penicillium* sp. obtained from the from the rhizosphere of *Fallugia paradoxa* of the Sonoran Desert was the source of a new anthraquinone (**215**)

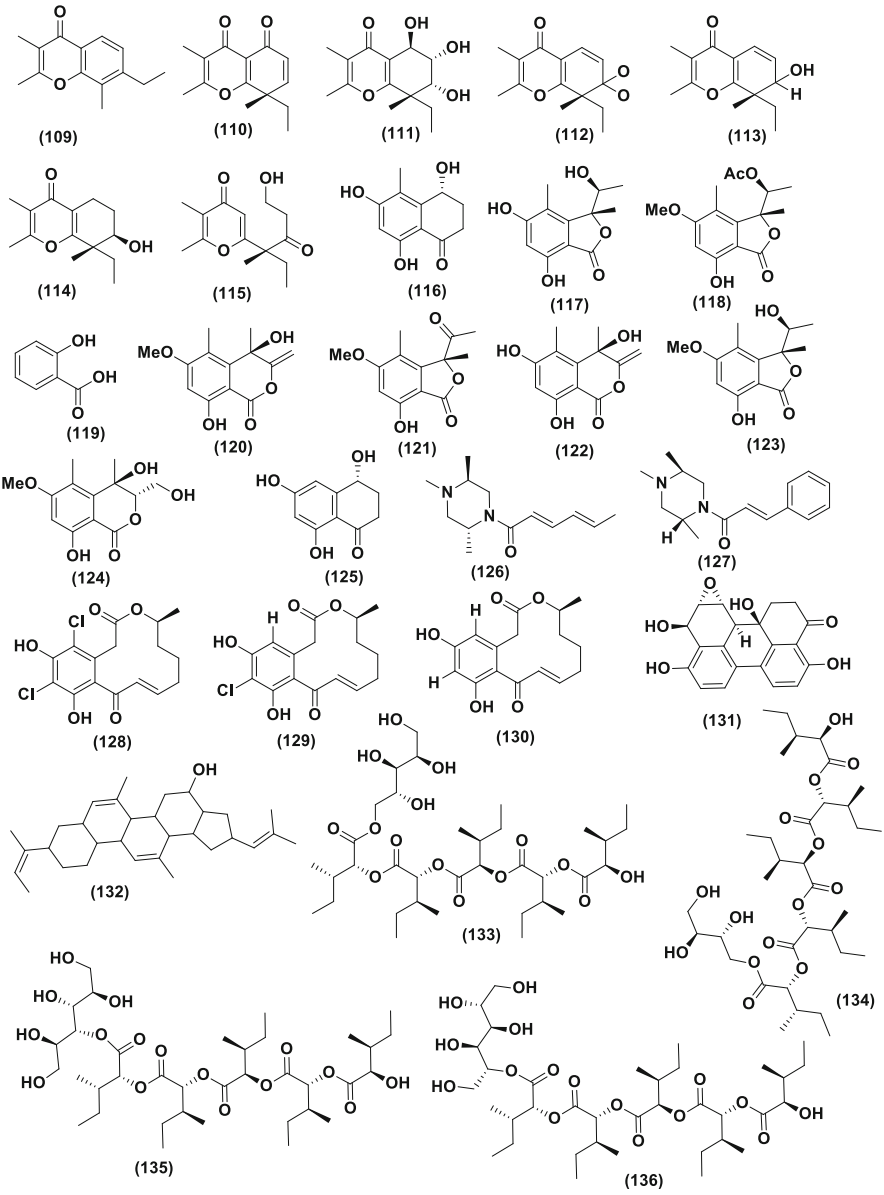

Fig. 16.6 Biologically active compounds isolated from desert fungi (**109–136**)

along with known anthraquinone (**216**) and cytotoxic curvularins (**217–219**) (Zhan et al. 2004). Three new cytotoxic cytochalasans, Aspochalasins I, J, and K (**220–222**) along with four known cytochalasans, aspochalasins C (**223**), D (**224**), and E (**225**) and TMC-169 (**226**) were obtained from *Aspergillus flavipes* fungus of

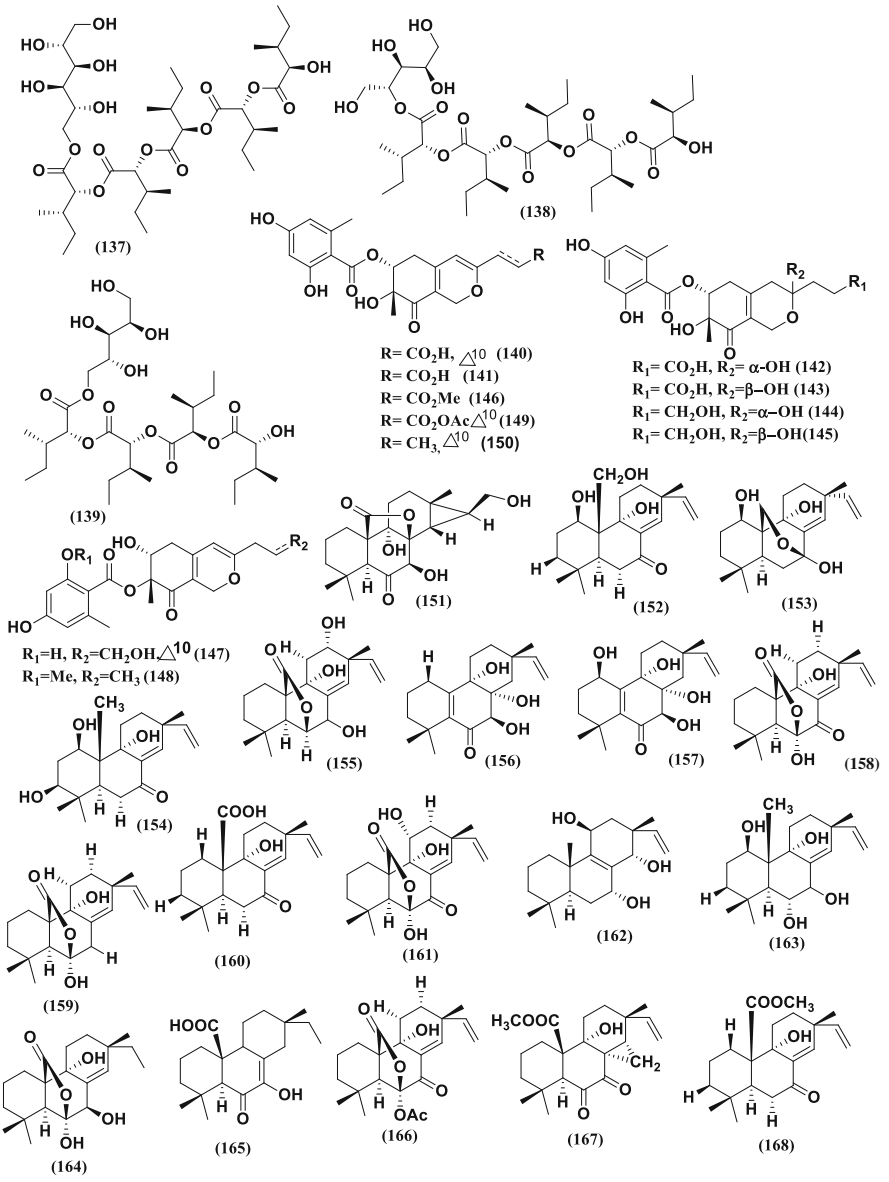

Fig. 16.7 Biologically active compounds isolated from desert fungi (**137–168**)

the Sonoran Desert. A weak to moderate cytotoxicity was observed in all compounds against NCIH460, MCF-7, and SF-268 cancer cell lines; however, none of them showed significant selectivity (Zhou et al. 2004) (Figs. 16.7, 16.8, and 16.9).

Sonoran Desert-associated *Aspergillus terreus* gave a novel cyclopentanedione, asterredione (**227**), two new terrecyclic acid A derivatives, (+)-5(6)-dihydro-6-

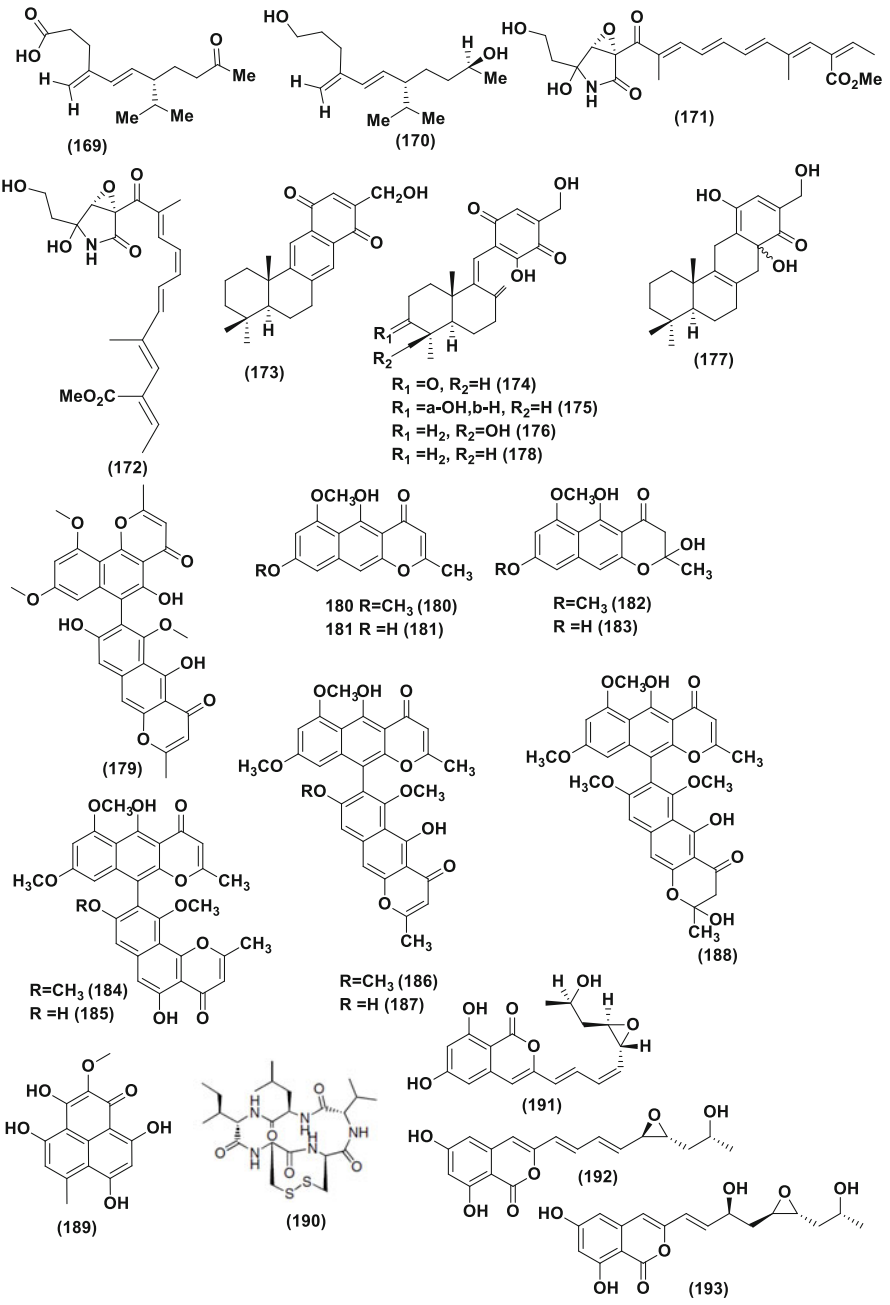

Fig. 16.8 Biologically active compounds isolated from desert fungi (**169–193**)

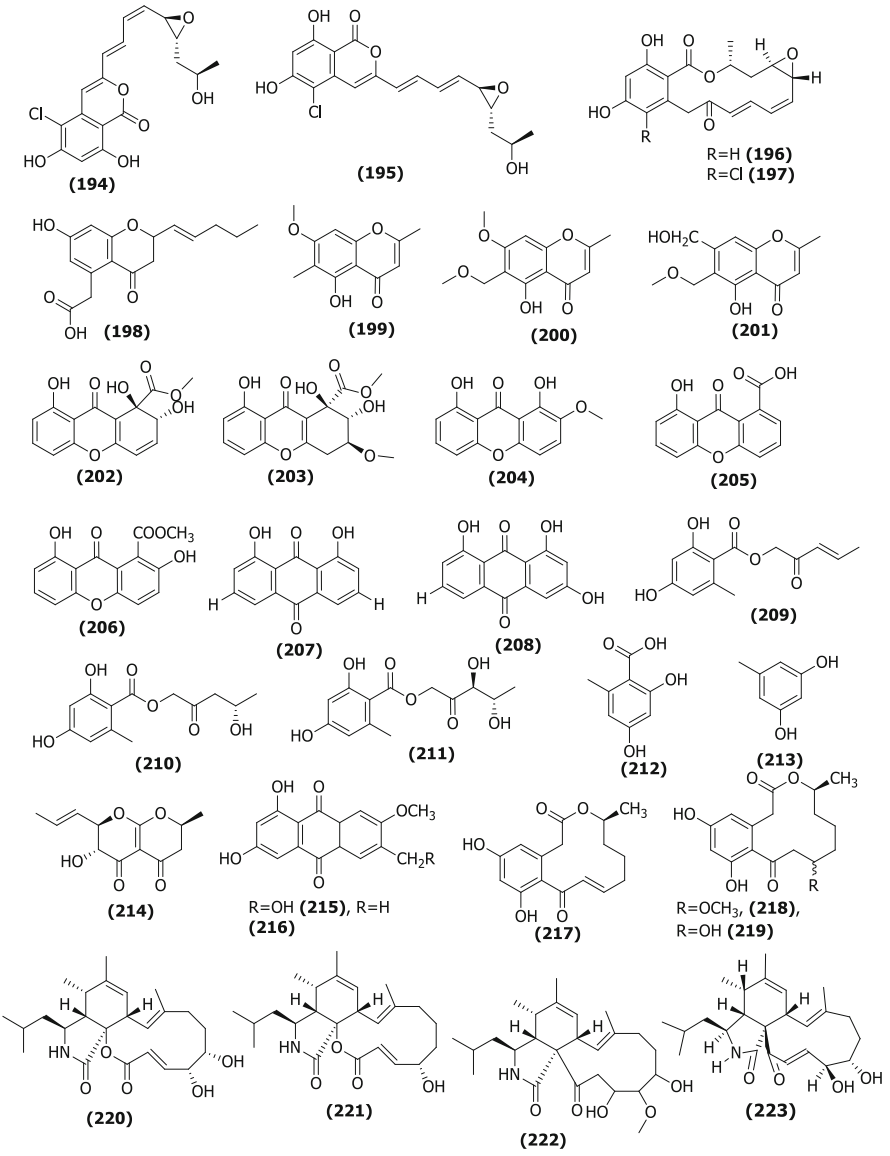

Fig. 16.9 Biologically active compounds isolated from desert fungi (**194–223**)

methoxyterrecyclic acid A (**228**) and (+)-5(6)-dihydro-6-hydroxyterrecyclic acid A (**229**). Except these five known molecules, (+)-terrecyclic acid A (**230**), (−)-quadrone (**231**), betulinan A (**232**), asterriquinone D (**233**), and asterriquinone C-1 (**234**), (−)-isoquadrone (**235**), 5(6)-dihydro-terrecyclic acid A (**236**), and (+)-terrecyclic acid A methyl ester (**237**) were also obtained. All compounds showed a

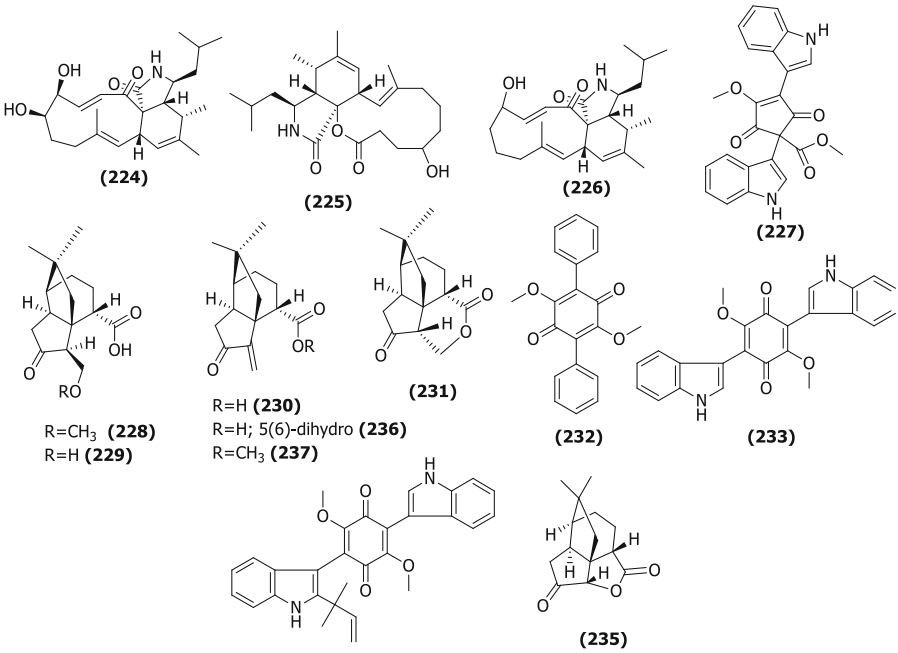

Fig. 16.10 Biologically active compounds isolated from desert fungi (**224–235**)

moderate cytotoxicity against panel of three sentinel cancer cell lines, NCI-H460 (nonsmall cell lung cancer), MCF-7 (breast cancer), and SF-268 (CNS glioma) (Wijeratne et al. 2003) (Fig. 16.10).

3 Future Prospect

With increased focus on investigating the natural products of microorganisms from unusual or specialized niches may increase the chances of finding novel compounds. Fungi are increasingly being explored as production platforms for bioactive metabolites with diverse chemical structures (Agrawal et al. 2018, 2020, 2022; Deshmukh et al. 2022). Approximately 1% of total fungi have been studied so far, and still millions of fungal species need to be studied and characterized. Hot deserts are characterized by exceptionally limited availability of water and nutrients, extreme temperatures with wide day/night excursion, strong winds, and high ultraviolet (UV) radiation (Makhalanyane et al. 2015). Desert microbial communities have evolved to flourish in harsh environments and play a crucial part in ecosystem bioprocesses. Desert microbial communities differ from biomes in moderate or tropical climates in terms of composition, function, and phylogenetic diversity (Fierer et al. 2012). Fungi are the most stress-resistant eukaryotes, capable of

growing under water shortage and tolerating desiccation by producing spores (Sterflinger et al. 2012). Fungi include a diverse range of saprobes, parasites, or symbionts, and they can also be pathogens or endophytes. Different groups of fungi are adapted to the desert environment, comprising terricolous fungi, fungi associated with plants, hyphomycetes, yeasts, and microcolonial fungi (Sterflinger et al. 2012). Although molecular studies of fungal diversity have started to emerge in recent years (Buee et al. 2009; Santiago et al. 2018; Moreno et al. 2014), the fungal communities of deserts are still poorly understood, particularly with regard to human pathogenic fungi (Kellogg and Griffin 2006). Despite the recent beginnings of molecular investigations of fungal diversity, in fact, desert mycological studies are mainly based on culture-dependent methods, which detect only a fraction of the fungal community (Sterflinger et al. 2012). Future research on the regulation of fungal secondary metabolism and the variety of endophytes may benefit from more sophisticated molecular biology techniques. Another fascinating area of research is examining how the endophyte–host connection is shaped by the viral or bacterial endosymbionts of endophytes. Optimizing the production of secondary metabolites by endophytic fungi under laboratory conditions will require a deeper comprehension of host–endophyte relationships at the molecular and genetic levels, of biogenetic gene cluster regulation, and of the effects of environmental changes and culture conditions on gene expression. This will eventually result in bioactive medicines and therapeutic candidates with great medical value. Research has revealed that metabolic engineering and random mutagenesis TALEN (transcription activator-like effector nucleases), CRISPR (clustered regulatory interspaced short palindromic repeats)-Cas9, and ZFN (zinc finger nucleases), MALDI-IMS (matrix-assisted laser desorption ionization-imaging mass spectrometry), and UPLC–TOF–MS (ultra-high-performance liquid chromatography–quadrupole time of flight mass spectroscopy) can be employed as effective techniques to improve the production of secondary metabolites and their identification (Tiwari and Bae 2022; Peng et al. 2021). Metabolic engineering involves a protoplast fusion of UL50-6 and UV40-19 fungal strains which was found to increase taxol production by 20–25% compared to parental strains (Zhao et al. 2011). Transgenic endophyte strain of *Phomopsis* sp. produced using genome shuffling yields deacetylmycoepoxydiene (DAM) (a potent antitumor drug) by 200-fold more than nontransgenic strains (Wang et al. 2016). Many fungal biosynthetic gene clusters are inactive in typical laboratories. The coculturing technique activates numerous dormant pathways for the synthesis of secondary metabolites and helps to replicate the biological environment. One practical way to increase the yield of secondary metabolites is coculturing (Peng et al. 2021). Coculturing *Corynespora cassiicola* SUK2 and *Colletotrichum fructicola* SUK1 showed 1.4-fold increase in yield of camptothecin (CPT) than that of monocultures (Bhalkar et al. 2016). Ahamed and Ahring (2011) demonstrated that *Gliocladium roseum* an endophytic fungus when cocultivated with *E. coli*, the fungus produce of hydrocarbons, was increased by 100-fold; thus, coculturing can act as an alternative for increasing metabolite yield. A study showed that bacteria when placed in physical contact with fungus they release chitinolytic enzymes which facilitate the entry of bacteria into the mycelium, endophytic fungi undergo histone

modification stimulated by acetyltransferase (HAT) Saga/Ada complex ultimately resulting in PKS (AN7909) and NRPS (AN7884) gene clusters of *Aspergillus nidulans* when cocultured with *Streptomyces hygroscopicus* (Nützmann et al. 2011). Fungi are not only employed to potentiate the production of secondary metabolites by other fungi and bacteria, but also to manufacture secondary metabolites themselves when cocultured with other microbes. Due to the complexity of the interactions between the bacteria, further research into the metabolic pathways that the coculture approach activates in the microbes is necessary. However, researchers need to focus on using the coculture approach to produce the desired bioactive molecule. To sum it all up, dessert fungi are a rich source of secondary metabolites with a variety of pharmacological value; thus, researchers must focus immediately on creating sustainable methods for the isolation and production of bioactive compounds at commercial levels.

4 Conclusion

The difficulty in attaining the ideal growing conditions and high-cost participation are the main obstacles to the development, manufacturing, and extraction of extremophilic fungi on a laboratory scale. The generation of sufficient biomass for extensive industrial output continues to be quite challenging. The amount of information about extremophiles is growing, yet it is still insufficient given the enormous range of substances and species that exist. Additional study in this field may reveal additional bioactive compounds with clinically significant properties. Extremophilic fungus can be thought of as a kind of "chemical factory" in general. Therefore, it may be assumed that the desert fungus might be the new science's armory as a source of bioactive chemicals.

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Chapter 17
Bioactive Metabolites Produced by Fungi Present in Antarctic, Arctic, and Alpine Ecosystems

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Abstract Hostile and harsh environments to most organisms represent more than 80% of the earth's surface. These extreme environments shelter interesting microbial communities that can live in habitats that combine polyextremophile conditions, including cold, hot, dry, oligotrophic conditions, and high solar radiation over the year. Microorganisms inhabiting extreme ecosystems can have specialized metabolism to adapt to physicochemical conditions different from those that most organisms can withstand. These microbes are called extremophiles. For this reason, extreme regions represent a natural laboratory to study different aspects of its resident extremophile microorganisms, including fungi able to produce bioactive compounds. In this context, extremophile fungi present in environments such as Antarctica, the Artic, and alpine regions such as the Alps may have unusual biochemical metabolic pathways that produce secondary metabolites that can be used as possible prototypes for pharmaceuticals in medicine and pesticides for use in agriculture, as well as for industrial uses. Recent studies with these fungal

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extremophiles have searched for new compounds with biological functions such as cytotoxicity, antimicrobial, lipid-lowering ability, antioxidant, nematocidal, anti-inflammatory, antimalarial, antitumor, herbicidal, and antifouling activities. This review summarizes recent literature on extremophile fungi that are sources of metabolites for use in medicine, agriculture, and industry.

Keywords Bioprospecting · Extremophile · Fungi · Polar regions, secondary metabolites

1 Introduction

Considered the second most diverse group of organisms, with about five million estimated species (Blackwell 2011), only a small fraction (~100,000 species described) of fungal species has been described until now (Hibbett et al. 2011), and only a minor proportion of these fungi have been explored for their pharmacological potential (Ibrar et al. 2020). Microorganisms live in complex ecosystems where they compete and interact with other organisms and where they must evolve traits that guarantee their survival. One of these traits is the production of metabolites that provide an advantage in the physical and biological environment in which the fungus is found (Brakhage and Schroeckh 2011).

Major secondary metabolites include antibiotics, pigments, toxins, ecological effectors of competition and symbiosis, pheromones, pesticides, antitumor agents, and growth promoters (Demain 1998). According to Rothschild and Mancinelli (2001), in addition to extremophile organisms providing fundamental data for the knowledge of molecular and evolutionary biology, these organisms have great economic potential, as they can be a source of metabolites of interest to the agrochemical and pharmaceutical industries (Cantrell et al. 2012; Newman and Cragg 2020; Sparks et al. 2023).

To survive in cold ecosystems, such as polar and alpine regions, it is necessary to overcome abiotic conditions and ecological pressure such as food and space competition, predation, high UV radiation, and low temperatures, different adaptation strategies are employed by resident organisms. One of these is the production of secondary metabolites (Rosa et al. 2019; Ibrar et al. 2020; dos Santos et al. 2022). Fungi known as psychrophiles and psychrotolerants (extremophile microbes) can be considered valuable sources for the study of secondary metabolites with antimicrobial, lipid-lowering ability, antioxidant, nematocidal, anti-inflammatory, antimalarial, antitumor, herbicidal, and antifouling activities, which can be used as prototypes for future pharmaceuticals and pest management chemicals.

2 Polar and Alpine Ecosystems: A Natural Habitat of Psychrophilic and Psychrotolerant Fungi

Approximately 80% of the biosphere experiences seasonally or permanently cold temperatures (Margesin et al. 2007). These environments, although often considered extreme for most organisms, are the habitat of different species of microorganisms, which grow and reproduce in conditions that restrict human life (Ibrar et al. 2020). Although the first extremophile microorganism was isolated in the 1860s, studies in the field and the acceptance that there were other organisms capable of surviving in extreme conditions only gained momentum after more than a century (Wilson and Brimble 2009). Recently, extremophile microbes have gained more attention due to the search for bioactive compounds, based on the hypothesis that competition for survival in extreme environments favors the production of unusual metabolites (Rosa et al. 2019; Ibrar et al. 2020). Psychrophilic fungi present a great unexplored potential regarding the production of bioactive compounds, reportedly producing cytotoxic compounds, antimicrobials, antioxidants, anti-inflammatory, antifouling, biosurfactants, herbicides, among others (Godinho et al. 2015; Gomes et al. 2018; Ibrar et al. 2020).

The psychrophilic and psychrotolerant fungi are considered extremophile microorganisms, characterized by the ability to grow at low temperatures. According to Morita (1975), psychrophiles are microorganisms that have optimum growth temperature of around 15 °C or below, maximum growth temperature of around 20 °C and minimum growth temperature of 0 °C or below. In addition, psychrotolerant microorganisms are those that are able to grow at low temperatures, but have optimum and maximum growth temperatures above 15 and 20 °C, respectively (Morita 1975). Despite the predominantly cold nature of the biosphere and the first reports of psychrotolerant microorganisms occurring in the 1880s, research on the subject remained scarce for decades (Moyer and Morita 2007).

Ecosystems characterized by the presence of more than one parameter considered extreme are common, and microorganisms capable of surviving in this kind of environment are called polyextremophiles (Rothschild and Mancinelli 2001). According to Robinson (2001), several physiological mechanisms of cold tolerance by fungi have been proposed, and it is possible that a combination of these strategies is employed and that there is not a single adaptation, as all the cell components of a psychrophile or psychrotolerant fungus must be functional. Thus, the actual role (s) of each physiological and ecological mechanism is/are extremely complex.

Since approximately 15% of the earth's surface lies in the polar regions (Fig. 17.1), the Arctic and Antarctic represent important sites for research on psychrophilic and psychrotolerant microorganisms (Jansen and Painter 1974). The Arctic is generally defined by biologists as the lands beyond the climatic limit of trees (Robinson 2001). In contrast, Antarctica is the coldest continent in the world and has several characteristics that make it an extreme environment (Rosa et al. 2019). On the Antarctic continent, oligotrophic conditions, high incidence of ultraviolet radiation, and drought and low temperatures are stressful conditions for

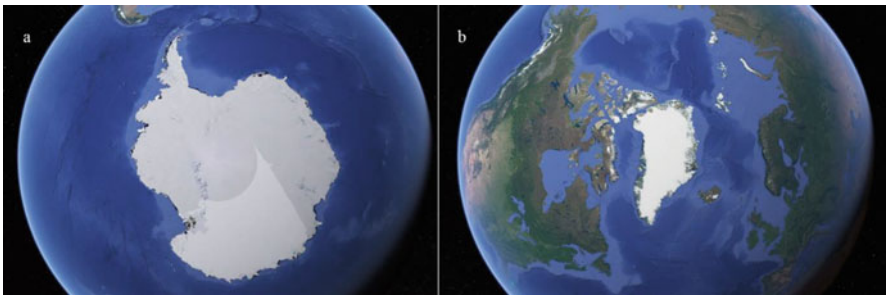

Fig. 17.1 (**a**) Antarctic and (**b**) Arctic regions: hotspot of extremophile microorganisms. Images obtained from https://earth.google.com/web

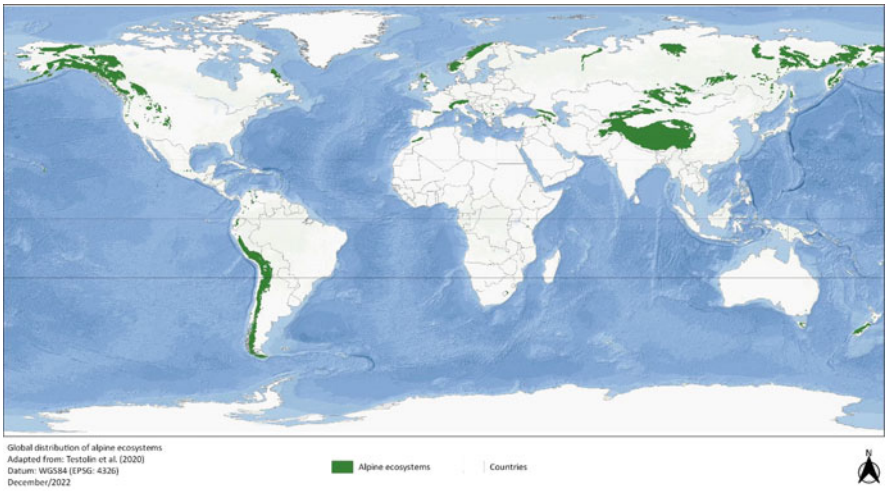

Fig. 17.2 Major alpine regions across the earth

microorganisms (Ruisi et al. 2007). Temperatures average below 0 °C and in certain regions can range from −20 to −50 °C, such as the McMurdo Dry Valleys (Onofri et al. 2007). Temperatures at Antarctic latitudes are generally lower than temperatures at equivalent latitudes in the Arctic, where there are no terrestrial ecosystems similar to those found in continental Antarctica (Robinson 2001). In addition, although the air temperatures at these locations are extremely low, the temperature of the substrates where the fungi grow can be higher, especially at some times of the year (Robinson 2001).

Alpine ecosystems (Fig. 17.2) are usually defined as areas occurring above the limit of trees, though they can be found below this line in some regions, due to geology, geomorphology, and microclimate (Rundel and Millar 2016). Even though this definition might be similar to the definition of the Arctic, there are differences in the stress conditions that microbes are exposed to in these regions. The European

Alpine region, for example, has higher maximum temperatures, lower minimum temperatures, recurring temperature fluctuations, higher precipitation, lower atmospheric pressure, and higher intensity of solar radiation (Margesin 2012). According to Testolin et al. (2020), about 73% of the total area of alpine ecosystems are found in the Asian continent, followed by 15% located in South America, 9% in North America, 2% in Europe, and 1% in Oceania and Africa. Similar to those of polar ecosystems, alpine psychrophilic microorganisms play important ecological roles in their natural habitats, such as involvement in litter decomposition and CO_2 release (Margesin 2012).

Fungi known as psychrophiles or psychrotolerant are subject to different types of stress caused mainly by low temperatures, such as decreased membrane fluidity (induced by fatty acid tails of phospholipids becoming rigid due to less movement), decreasing permeability to molecules such as oxygen and glucose, and formation of ice crystals (that can damage membranes), resulting in death of the cell (Rafiq et al. 2019). Additionally, cooling to low temperatures reduces the rate at which chemical reactions occur, increases the viscosity of water, and denatures protein (Robinson 2001). This decrease in chemical reaction rates is explained by Arrhenius' law, which postulates that the frequency of molecular collisions is directly related to temperature. Thus, adjustments in the control of molecule assembly and the secondary structure of nucleic acids are necessary to ensure proper rates of transcription and translation (Marx et al. 2007). Increased water viscosity is also a stressful factor for these microorganisms by affecting diffusion processes (Robinson 2001; Duarte et al. 2018).

3 Bioactive Compounds from Extremophile Fungi from the Antarctic

Fungi are microorganisms with a great ability to synthesize secondary metabolites with different biological activities. According to Rosa et al. (2019), one of the interesting strategies for finding new metabolites of pharmaceutical importance is searching for fungi isolated from underexplored, geographically isolated, extreme environments, such as the Antarctic. Antarctica is a remote, inhospitable, and most unexplored environment on the earth that harbors the coldest and driest climate known (Rogers et al. 2007; Vaca and Chávez 2019). Temperature is the key factor, which exerts selective pressure on the growth of fungi and leads to structural and functional changes in the biomolecules along with the integrity of cellular components. These unique biomolecules are of strong interest to natural product chemists and biotechnologists (Ibrar et al. 2020). Therefore, the ability of Antarctic fungi to survive in extreme conditions suggests that they may possess unusual biochemical pathways that allow them to generate new compounds (Santiago et al. 2012; Rosa et al. 2019).

Fungi are considered prolific producers of antimicrobial compounds throughout history (Ibrar et al. 2020), and extreme environments could be a unique and

unexplored habitat to detect species and strains able to produce new antibiotics (Zucconi et al. 2020). Some secondary metabolites and their respective activities, produced by different species of extremophile fungi and their habitats and collection sites, are listed in Table 17.1. Li et al. (2008) reported the antibacterial and antifungal activities of geomycins B (**1**) and C (**2**) (Fig. 17.3) obtained from the fungus *Geomyces* sp. (now *Pseudogymnoascus*) isolated from a soil sample collected at the Fildes Peninsula of King George Island. Geomycin B showed antifungal activity against *Aspergillus fumigatus* with IC_{50}/MIC values of 0.86/29.5 μM (the positive control fluconazole had IC_{50}/MIC values of 7.35/163.4 μM). Geomycin C had antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae*, with IC_{50}/MIC values of 17.3/75.8 and 36.2/151.5 μM, respectively (the positive control antimicrobial peptide AMP had IC_{50}/MIC values of 1.40/4.50 and 7.20/18.0 μM, respectively), and against *Escherichia coli*, with IC_{50}/MIC values of 12.9/30.3 μM (the positive control streptomycin showed IC_{50}/MIC values of 1.05/11.2 μM) (Li et al. 2008).

Brunati et al. (2009) collected 160 filamentous fungi and 171 yeasts from 11 lakes, and 47 (29%) of the filamentous fungi showed antimicrobial activity. Two bis-anthraquinone metabolites identified as rugulosin (**3**) and skyrin (**4**) (Fig. 17.3) were obtained from the most active isolate *Penicillium chrysogenum* isolated from a saline lake in the Vestfold Hills. The compounds rugulosin and skyrin exhibited antimicrobial activity against *S. aureus* with MIC values of 1.84 and 1.85 μM, respectively, and against *Moraxella catarrhalis* with MIC values of 460.82 nM and 1.85 μM, respectively (the positive control penicillin G had an MIC of 80.53 nM for both microorganisms).

The extract of the endophytic fungus *Mortierella alpina* isolated from the moss *Schistidium antarctici* found in Admiralty Bay, King George Island, revealed the presence of potential antimicrobials: pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) (**5**) and pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl) (**6**) (Fig. 17.3). The pure compounds were not tested, but the crude extract of *M. alpina* exhibited antibacterial activity with a MIC value of 26.9 μg mL^{-1} against *E. coli* and 107 μg mL^{-1} against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Besides these two compounds, some fatty acids were identified as mystic, palmitic, stearic, heptadecanoic, oleic, eicosenoic, gamma-linoleic, and arachidonic (Melo et al. 2014).

Amphotericin B (**7**) (Fig. 17.3) was isolated from an organism other than the bacterium *Streptomyces nodosus* for the first time by Svahn et al. (2015). The metabolite was secreted from the isolate *Penicillium nalgiovense* recovered from a soil sample from a recently abandoned Adélie Penguin (*Pygoscelis adeliae*) nest located on Paulet Island. The compound showed an MIC value of 135.26 nM against *Candida albicans* (Svahn et al. 2015).

The compounds meleagrin, neoxaline, and questiomycin A were isolated from the fungus *Penicillium* sp., obtained from a soil sample collected in King George Island, Antarctic Peninsula. Questiomycin A (**8**) (Fig. 17.3) had potent antituberculosis activity against *Mycobacterium tuberculosis* with an MIC value of 3.91 μM, an MIC value close to the control drug isoniazid (2.04 μM). Meleagrin,

Table 17.1 Summary of secondary metabolites and their respective activities, produced by different species of extremophile fungi and their habitats and collection sites

Region/specie	Habitat	Activity	Compounds	Formula	Collection site	Reference
Antarctica						
Geomyces sp.	Soil	Antibacterial and antifungal	Geomycins B and C	$C_{34}H_{30}O_{15}Na$ (**1**) and $C_{34}H_{28}O_{14}Na$ (**2**)	Fildes Peninsula, King George Island	Li et al. (2008)
Penicillium chrysogenum	Lake	Antibacterial	Rugulosin	$C_{30}H_{22}O_{10}$ (**3**)	Vestfold Hills	Brunati et al. (2009)
			Skyrin	$C_{30}H_{18}O_{10}$ (**4**)		
Mortierella alpina	Endophytic of moss	Not analyzed	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	$C_{11}H_{18}N_2O_2$ (**5**)	Admiralty Bay, King George Island	Melo et al. (2014)
			Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)	$C_{14}H_{16}N_2O_2$ (**6**)		
Penicillium nalgiovense	Penguin's nest	Antifungal	Amphotericin B	$C_{47}H_{73}NO_{17}$ (**7**)	Paulet Island	Svahn et al. (2015)
Penicillium sp.	Soil	Antituberculosis	Questiomycin A	$C_{12}H_8N_2O_2$ (**8**)	Great Wall Station	Wang et al. (2015)
Aspergillus ochraceopetaliformis	Soil	Antiviral	Ochraceopone A	$C_{23}H_{30}NaO_9$ (**9**)	Great Wall Station	Wang et al. (2016)
			Isoasteltoxin	$C_{23}H_{30}NaO_7$ (**10**)		
			Asteltoxin	$C_{23}H_{30}O_7$ (**11**)		
Aspergillus sydowii	Marine sediment	Antibacterial	Cyclo-(L-Trp-L-Phe)	$C_{20}H_{19}N_3O_2$ (**12**)	Great Wall Station	Li et al. (2018)
			(7*S*, 11*S*)-(+)-12-hydroxysydonic acid	$C_{15}H_{22}O_5Na$ (**13**)		
Acrostalagmus luteoalbus	Soil	Antibacterial and antifungal	Luteoalbusin A	$C_{23}H_{20}N_4O_3S_2$ (**14**)	Fildes Peninsula	Shi et al. (2022)
			T988 C	$C_{23}H_{20}N_4O_4S_2$ (**15**)		

(continued)

Table 17.1 (continued)

Region/specie	Habitat	Activity	Compounds	Formula	Collection site	Reference
Pseudogymnoascus sp.	Soil	Cytotoxic and antibacterial	Ganodermasides A and B	$C_{28}H_{40}O_2$ (**16**, **17** and **18**)	Fields Peninsula	Shi et al. (2021)
			Ganodermasides B and D			
Oidiodendron truncatum	Soil	Cytotoxic	Chetracin B	$C_{30}H_{28}N_6O_8S_5$ (**19**)	Great Wall Station	Li et al. (2012)
			Chetracin C	$C_{30}H_{28}N_6O_8S_6$ (**20**)		
			Chetracin D	$C_{34}H_{40}N_6O_8S_4$ (**21**)		
			Melinacidin IV	$C_{30}H_{28}N_6O_8S4$ (**22**)		
			T988 C	$C_{23}H_{20}N_4O_4S_2$ (**15**)		
			T988 A	$C_{23}H_{20}N_4O_4S_3$ (**23**)		
Penicillium sp.	Marine sludge	Cytotoxic	Chloro-trinoreremophilane sesquiterpene	$C_{14}H_{15}ClO_4$ (**24**)	Prydz Bay	Wu et al. (2013)
Penicillium sp.	Marine sludge	Cytotoxic	Eremophilane-type sesquiterpene compounds	$C_{15}H_{24}O_2$ (**25**)	Prydz Bay	Lin et al. (2014)
				$C_{18}H_{24}O_6$ (**26**)		
Microbispora aerate	Penguin excrements	Cytotoxic	Microbiaeratin	$C_{18}H_{20}N_4O_2S$ (**27**)	Livingston Island	Ivanova et al. (2007)
Penicillium crustosum	Sediment	Cytotoxic	Fusaperazine F	$C_{19}H_{24}N_2O_3S$ (**28**)	Prydz Bay	Liu et al. (2018)
Penicillium granulatum	Sediment	Antiallergic	Spirograterpene A	$C_{20}H_{30}O_3$ (**29**)	Prydz Bay	Niu et al. (2017)
			Conidiogenone I	$C_{20}H_{32}O_3$ (**30**)		
			Conidiogenone C	$C_{20}H_{30}O_2$ (**31**)		
Penicillium sp.	Sponge	Anti-inflammatory	Curvulone C	$C_{16}H_{20}O_6$ (**32**)	Ross Sea	Ha et al. (2017)
			Curvulone B	$C_{17}H_{22}O_6$ (**33**)		
			Curvularin	$C_{16}H_{20}O_5$ (**34**)		
			(11*R*,15*S*)-11-hydroxycurvularin	$C_{16}H_{20}O_6$ (**35**)		

			(11*S*,15*S*)-11-hydroxycurvularin	$C_{16}H_{20}O_6$ (**36**)		
			(11*R*,15*S*)-11-methoxycurvularin	$C_{17}H_{22}O_6$ (**37**)		
			(11*S*,15*S*)-11-methoxycurvularin	$C_{17}H_{22}O_6$ (**38**)		
			(10*E*,15*S*)-10,11-dehydrocurvularin	$C_{16}H_{18}O_5$ (**39**)		
			(10*Z*,15*S*)-10,11-dehydrocurvularin	$C_{16}H_{18}O_5$ (**40**)		
Penicillium glabrum	Sediment	Anti-inflammatory	Neuchromenin	$C_{13}H_{12}O_5$ (**41**)	Ross Sea	Ha et al. (2020)
			Myxotrichin C	$C_{14}H_{10}O_6$ (**42**)		
			Deoxyfunicone	$C_{19}H_{18}O_7$ (**43**)		
Pleosporales sp.	Moss	Anti-inflammatory	Alternate C	$C_{16}H_{16}O_6$ (**44**)	King George Island	Dong et al. (2021)
			Alternariol	$C_{14}H_{10}O_5$ (**45**)		
Aspergillus sp.	Lichen	Anti-neuroinflammatory	5-methoxysterigmatocystin Sterigmatocystin	$C_{19}H_{15}O_7$ (**46**) $C_{18}H_{13}O_6$ (**47**)	King George Island	Cao et al. (2022)
Arctic						
Penicillium algidum	Soil	Cytotoxic and antimalarial	Psychrophilin D	$C_{24}H_{24}N_4O_5$ (**48**)	Zackenberg, Greenland	Dalsgaard et al. (2005)
			Cycloaspeptide A and D	$C_{36}H_{43}N_5O_6$ (**49**) and $C_{35}H_{41}N_5O_6$ (**50**)		
Penicillium griseofulvum		Antimicrobial	Griseofulvin	$C_{17}H_{17}ClO_6$ (**51**)	Greenland	Frisvaad et al. (2004)
			Fulvic acid	$C_{14}H_{12}O_8$ (**52**)		
			Mycelianamide	$C_{22}H_{28}N_2O_5$ (**53**)		
			Roquefortine C and D	$C_{22}H_{23}N_5O_2$ (**54**) and $C_{22}H_{25}N_5O_2$ (**55**)		
			Chanoclavine I	$C_{16}H_{20}N_2O$ (**56**)		
			Elymoclavine	$C_{16}H_{18}N_2O$ (**57**)		

(continued)

Table 17.1 (continued)

Region/specie	Habitat	Activity	Compounds	Formula	Collection site	Reference
Penicillium jamesonlandense	Soil	Antibacterial, antifungal, and antimalarial	Griseofulvin	$C_{17}H_{17}ClO_6$ (**51**)	Greenland	Frisvad et al. (2006)
			Penicillic acid	$C_8H_{10}O_4$ (**58**)		
			Cycloaspeptide A	$C_{36}H_{43}N_5O_6$ (**49**)		
			Tryptoquivalin	$C_{27}H_{26}N_4O_7$ (**59**)		
			Chrysogine	$C_{10}H_{10}N_2O_2$ (**60**)		
			Pseurotin A	$C_{22}H_{25}NO_8$ (**61**)		
Eutypella sp. D-1	Soil	Antibacterial and cytotoxic	Libertellenone M	$C_{20}H_{24}O_4$ (**62**)	Ny-Ålesund District	Wang et al. (2018)
			Libertellenone N	$C_{21}H_{28}O_4$ (**63**)		
			Libertellenones A–C	$C_{20}H_{28}O_4$ (**64**), $C_{20}H_{26}O_4$ (**65**) and $C_{20}H_{28}O_5$ (**66**)		
			Eutypellone A	$C_{20}H_{30}O_4$ (**67**)		
			Kaempulchraol W	$C_{20}H_{32}O_3$ (**68**)		
Eutypella sp. D-1	Soil	Cytitoxic and anti-inflammatory	Libertellenones O–S	$C_{24}H_{30}O_7$ (**69**), $C_{25}H_{32}O_7$ (**70**), $C_{24}H_{32}O_6$ (**71**), $C_{26}H_{36}O_7$ (**72** and **73**)	Ny-Ålesund District	Yu et al. (2018a)
			Eutypellenones A and B	$C_{26}H_{34}O_7$ (**74**), and $C_{27}H_{36}O_7$ (**75**), $C_{26}H_{34}O_7$ (**76**), and $C_{21}H_{28}O_6$ (**77**)		
			Libertellenones H and J			
Eutypella sp. D-1	Soil	Antibacterial, antifungal, and cytotoxic	Eutypellenoids A–C	$C_{26}H_{34}O_8$ (**78**, **79**) and$C_{26}H_{34}O_7$ (**80**)	Ny-Ålesund District	Yu et al. (2018b)
			Eutypenoid C	$C_{26}H_{34}O_8$ (**81**)		
Nectria sp.	Soil	Antibacterial and cytotoxic	Nectriatones A–B	$C_{20}H_{26}O$ (**82**) and $C_{13}H_{18}O_2$ (**83**)	Ny-Ålesund District	Yu et al. (2018c)
			Nectriatone C	$C_{13}H_{20}O_2$ (**84**)		
			Ilicicolin E and D	$C_{23}H_{27}ClO_4$ (**85**) and $C_{23}H_{29}ClO_4$ (**86**)		
			Deacetylchloronectrin	$C_{23}H_{31}ClO_5$ (**87**)		

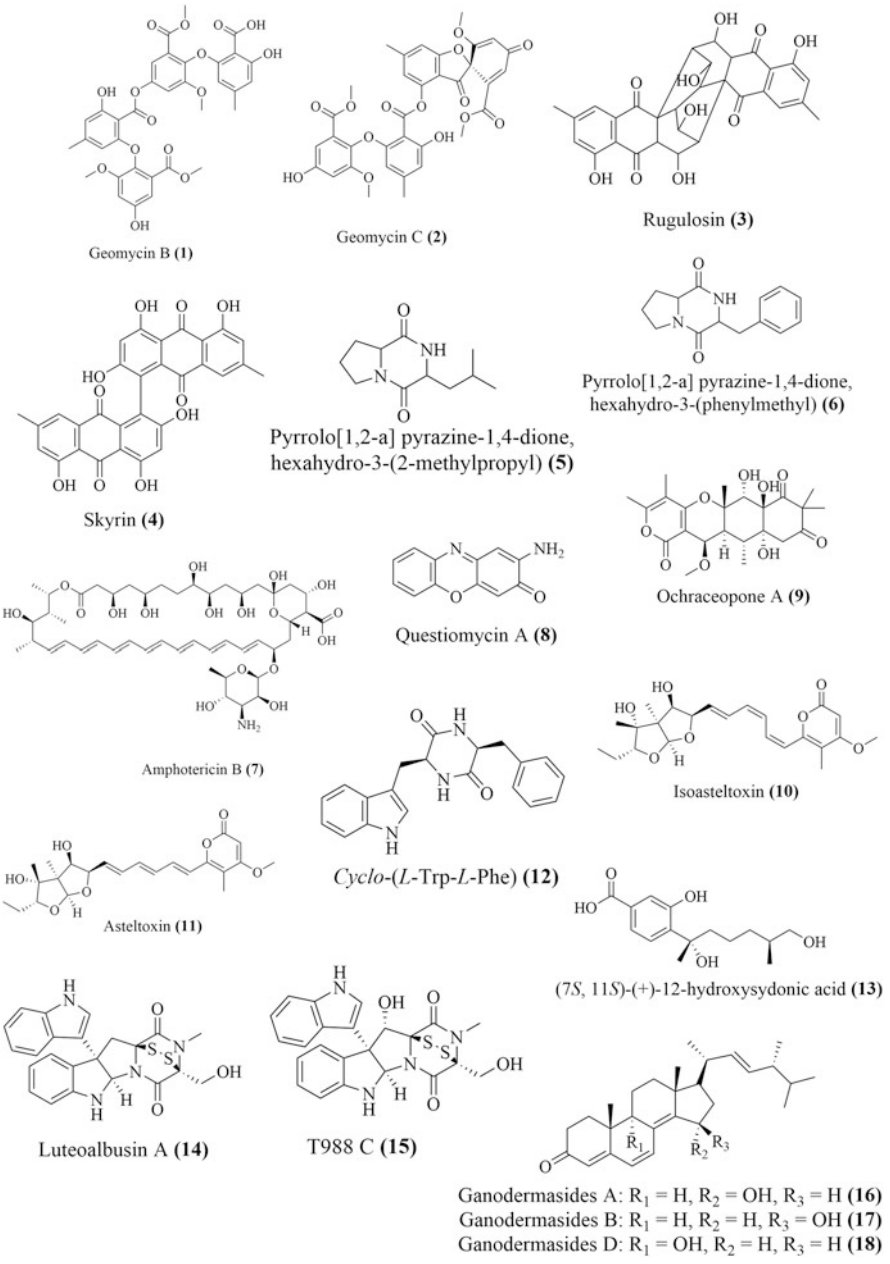

Fig. 17.3 Secondary metabolites obtained from extremophile fungi from the Antarctic (**1–18**)

neoxaline, and questiomycin A had significant in vitro cytotoxicities against the K562, MCF-7, A549, U937, Hela, DU145, HL60, and HT29 cell lines, with IC_{50} values ranging from 2.73 to 17.7 μM (Wang et al. 2015). At King George Island, Wang et al. (2016) recovered *Aspergillus ochraceopetaliformis* from soil samples that produced the compounds such as ochraceopone A (**9**), isoasteltoxin (**10**), and asteltoxin (**11**) (Fig. 17.3). These compounds exhibited antiviral activities against the H1N1 and H3N2 influenza viruses with IC_{50} values of >20.0/12.2 (ochraceopone A), 0.23/0.66 (isoasteltoxin), and 0.54/0.84 μM (asteltoxin).

One new alkaloid, acremolin C, and four known compounds, cyclo-(L-Trp-L-Phe) (**12**), 4-hydroxyphenylacetic acid, (7S)-(+)-hydroxysydonic acid, and (7S, 11S)-(+)-12-hydroxysydonic acid (**13**) (Fig. 17.3), were isolated from *Aspergillus sydowii*, fungi recovered from a marine sediment sample at King George Island. All the compounds were evaluated for their antibacterial activities; among them, compounds **12** and **13** showed moderate inhibition against methicillin-resistant *Staphylococcus epidermidis* (MRSE) with MIC values of 1.49 and 1.63 μM and methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC values of 2.99 and 3.27 μM, respectively (the positive control tigecycline had MIC values of 204.9 and 426.9 nM, respectively) (Li et al. 2018).

Shi et al. (2022) reported the isolation of two new α-pyrones, acrostalapyrones A and B, along with one known analog, multiforisin G, and three known indole diketopiperazines: luteoalbusin A (**14**), gliocladine C, and T988 C (**15**) (Fig. 17.3) by bioactivity tracking, combined with molecular networking. The secondary metabolites were obtained from the fungus *Acrostalagmus luteoalbus* (CH-6) isolated from soil samples collected from the Fildes Peninsula, King George Island. All the isolated compounds were evaluated for their antifungal and antibacterial activities, and luteoalbusin A and T988 C showed strong antimicrobial activities. Luteoalbusin A showed significant antimicrobial activities against *Candida albicans* and *Aeromonas salmonicida* with the same MIC value of 12.5 μM for both microbes. Compound T988 C exhibited broad-spectrum antimicrobial activities against *C. albicans*, *A. salmonicida*, *Photobacterium halotolerans*, *Pseudomonas fulva,* and *S. aureus* with the MIC values of 6.25, 3.125, 25, 25, and 25 μM, respectively. This compound displayed antibacterial activity two times higher than that of the antibiotic drug ciprofloxacin (6.25 μM) against *A. salmonicida* (Shi et al. 2022).

An isolate of *Pseudogymnoascus* sp. genus, known to be a kind of psychrophilic pathogenic fungus that is ubiquitously distributed in Antarctica, was isolated by Shi et al. (2021) from a soil sample of the Fields Peninsula, King George Island. It yielded compounds such as ganodermasides A (**16**), B (**17**), and D (**18**) (Fig. 17.3). Ganodermasides B and D showed antibacterial activities against the marine-fouling bacteria *A. salmonicida* with the MIC values of 30 and 36 μM, respectively (positive control ciprofloxacin with MIC value of 7.8 μM). Concomitant compounds ganodermasides A and B exhibited cytotoxic activities against human breast cancer (MDAMB-231), colorectal cancer (HCT116), and hepatoma (HepG2) cell lines with the IC_{50} values ranging from 21 to 30 μM. Li et al. (2012) reported the isolation of two new epipolythiodioxopiperazines, named chetracins B and C (**19** and **20**), and five new diketopiperazines, named chetracin D (**21**) (Fig. 17.4) and oidioperazines

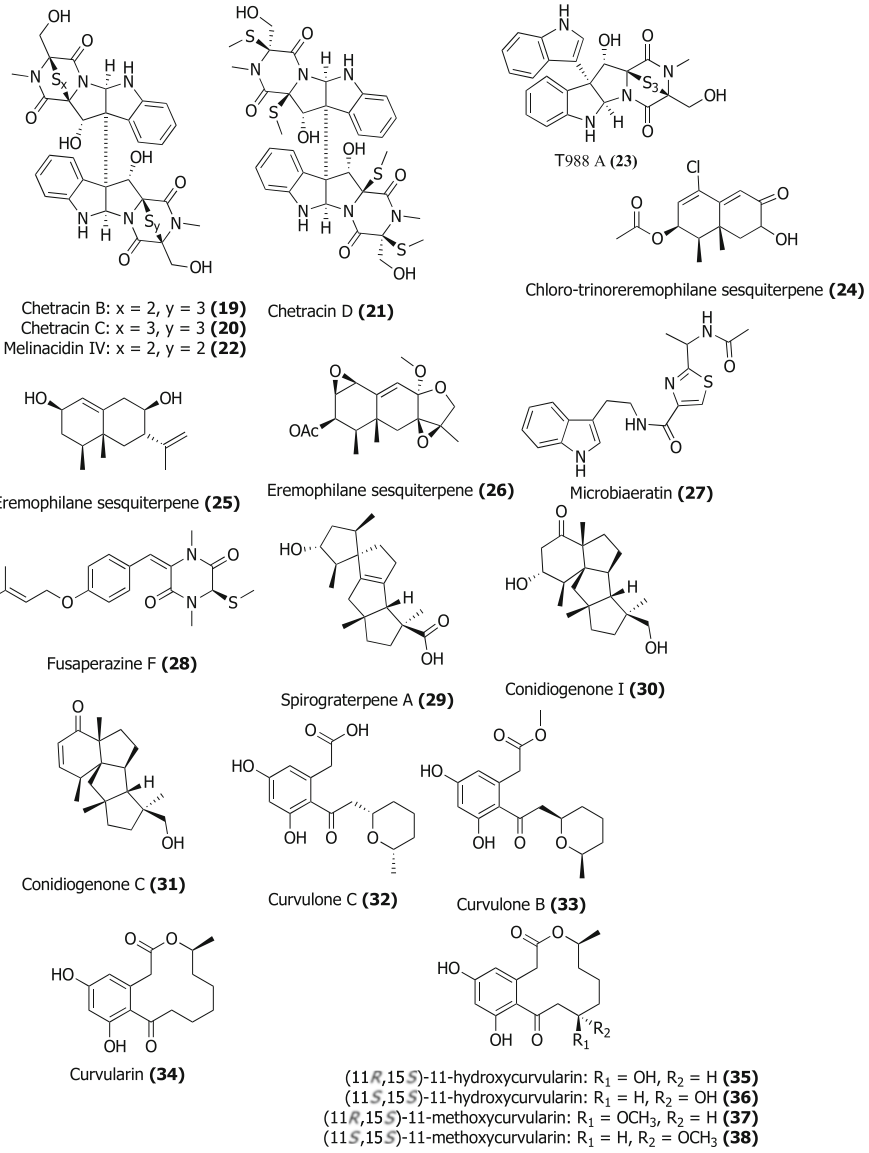

Fig. 17.4 Secondary metabolites obtained from extremophile fungi from the Antarctic (**19–38**)

A–D, along with the known natural products melinacidin IV (**22**), T988 B, T988 C (**15**), T988 A (**23**) (Fig. 17.4), chetoseminudin C, and cyclo-$_{L}$-Trp-$_{L}$Ser from the fungus *Oidiodendron truncatum* isolated from soil under lichens at King George Island. All the compounds were evaluated against a panel of five human tumor cell lines: HCT-8 (colorectal), BEL-7402 (hepatocellular adenocarcinoma), BGC-823 (gastric), A-549 (lung adenocarcinoma), and A-2780 (ovarian). Compounds **19** and **22** exhibited potent activity in the nanomolar range with the IC_{50} 3–28 nM against the human cell lines HCT-8, BEL-7402, BGC-823, A-549, and A-2780. In contrast, the compounds **20**–**21** and **15**–**23** showed moderate activity in the nano- to micromolar range, with IC_{50} values of 5 nM–1.8 μM.

Wu et al. (2013) reported the isolation of a new chloro-eremophilane sesquiterpene named chloro-trinoreremophilane sesquiterpene (**24**) (Fig. 17.4) from *Penicillium* sp. isolated from marine sludge in Prydz Bay, Antarctica. The compound was evaluated for cytotoxic activity against HL-60 and A549 cell lines and showed IC_{50} values of 11.8 and 12.2 μM, respectively (Wu et al. 2013). From the same fungus, Lin et al. (2014) reported the isolation of two new eremophilane-type sesquiterpene compounds [$C_{15}H_{24}O_2$ (**25**) and $C_{18}H_{24}O_6$ (**26**) (Fig. 17.4)]. Cytotoxicity studies revealed moderate activity, with IC_{50} values of 45.8 and 28.3 μM against HL-60 cells and 82.8 and 5.2 μM against A-549 cell lines, respectively (Lin et al. 2014). Ivanova et al. (2007) described the isolation of *Microbispora aerata* from penguin excrement collected on Livingston Island, Antarctica. Two compounds, microbiaeratin (**27**) (Fig. 17.4) and microbiaeratinin, were obtained from the culture. Low antiproliferative and cytotoxic effects of microbiaeratin were determined with L-929 mouse fibroblast cells, K-562 human leukemia cells ($GI_{50} > 50$ μg/mL), and HeLa human cervix carcinoma ($CC_{50} > 50$ μg/mL). Liu et al. (2018) reported the cytotoxic activity of the compound fusaperazine F (**28**) (Fig. 17.4) isolated from *Penicillium crustosum* obtained from the Antarctic sediment from Prydz Bay. This compound exhibited cytotoxicity against K562 cell, with an IC_{50} value of 12.7 μM.

A novel spiro-tetracyclic diterpene, spirograterpene A (**29**), and two biosynthetically related cyclopianes, conidiogenone I (**30**) and conidiogenone C (**31**) (Fig. 17.4), were isolated from the fungus *Penicillium granulatum*, recovered from a sediment sample at a depth of 2284 m from the Prydz Bay. The antiallergic activity of the compounds was measured, and spirograterpene A had an antiallergic effect on immunoglobulin E (IgE)-mediated rat mast RBL-2H3 cells with 18% inhibition compared to 35% inhibition for the positive control (loratadine, at the same concentration of 20 μg mL^{-1}). Conidiogenone I and conidiogenone C exhibited weak effects with inhibition of 4% and 10%, respectively, at 20 μg mL^{-1} (Niu et al. 2017).

The anti-inflammatory effects of Antarctic fungal compounds were studied by Ha et al. (2017). The authors described the isolation of a new curvularin derivative, named curvulone C (**32**), along with eight known curvularin-type polyketides: curvulone B (**33**), curvularin (**34**), (11R,15S)-11-hydroxycurvularin (**35**), (11S,15S)-11-hydroxycurvularin (**36**), (11R,15S)-11-methoxycurvularin (**37**), (11S,15S)-11-methoxycurvularin (**38**) (Fig. 17.4), (10E,15S)-10,11-dehydrocurvularin (**39**) (Fig. 17.5)**,** and (10Z,15S)-10,11-dehydrocurvularin (**40**) (Fig. 17.5) from the marine-derived fungal strain Penicillium sp. obtained from an

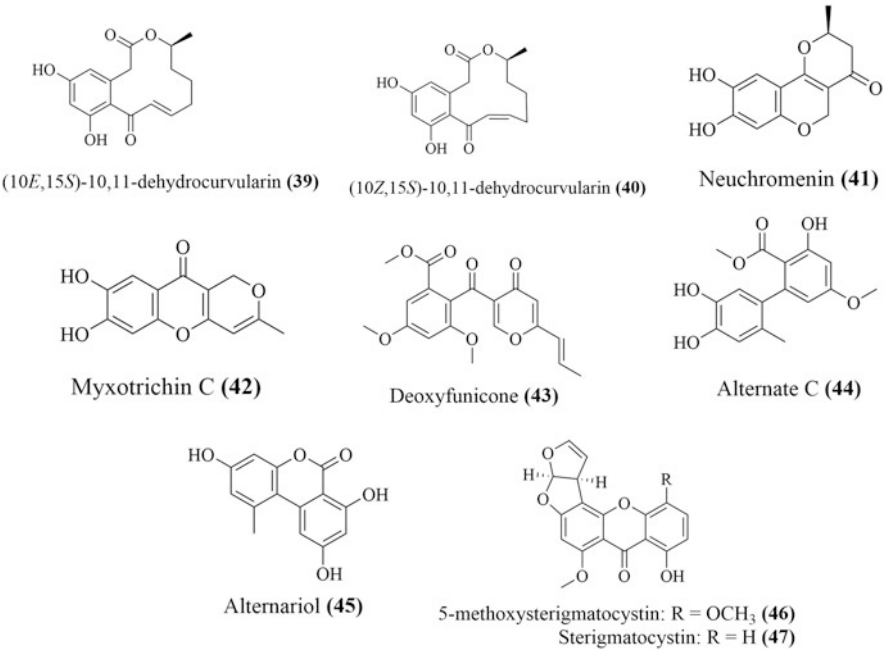

Fig. 17.5 Secondary metabolites obtained from extremophile fungi from the Antarctic (**39–47**)

unidentified sponge collected in the Ross Sea, Antarctica. All the compounds were evaluated in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Compounds **34–40** (Figs. 17.4 and 17.5) strongly inhibited LPS-induced overproduction of nitric oxide (NO) and prostaglandin E2 (PGE2) with IC_{50} values ranging from 1.9 to 18.1 μM and from 2.7 to 18.7 μM, respectively. In the evaluation of signal pathways involved in these effects, the most active compound, (10E,15S)-10,11-dehydrocurvularin, attenuated the expression of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) in LPS-stimulated RAW264.7 macrophages.

The compound (10E,15S)-10,11-dehydrocurvularin suppressed the upregulation of pro-inflammatory mediators and cytokines via the inhibition of the nuclear factor-κB (NF-κB) signaling pathway, but not through the mitogen-activated protein kinase (MAPK) pathway (Ha et al. 2017). *Penicillium glabrum*, also obtained from sediments collected at the Ross Sea, yielded a new citromycetin (polyketide) derivative and four known secondary fungal metabolites: neuchromenin (**41**), asterric acid, myxotrichin C (**42**), and deoxyfunicone (**43**) (Fig. 17.5). The compounds were evaluated for their anti-inflammatory activity, and the compounds such as neuchromenin, myxotrichin, and deoxyfunicone showed inhibitory activity against the excessive production of nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated BV2 microglial cells, with IC_{50} values of 2.7, 28.1, and 10.6 μM, respectively. These compounds also inhibited the excessive production of NO, with IC_{50} values of 4.7, 41.5, and 40.1 μM, respectively, in LPS-stimulated

RAW264.7 macrophage cells, and inhibited LPS-induced overproduction of prostaglandin E_2 in both cellular models (Ha et al. 2020). The anti-inflammatory effects of the compounds alternate C (**44**), altenusin, alternariol (**45**) (Fig. 17.5), and altenuene obtained from the fungal taxa *Pleosporales* sp., isolated from moss collected from the Marian Cove on King George Island, were evaluated in HaCaT human keratinocytes by Dong et al. (2021). The compounds alternate C and alternariol inhibited the secretion of interleukin-8 and interleukin-6 in tumor necrosis factor-α/interferon-γ-treated HaCaT cells, and these compounds also showed inhibitory effects on CCL5 and CCL22 (Dong et al. 2021).

Cao et al. (2022) evaluated the anti-neuroinflammatory potential of compounds from *Aspergillus* sp. isolated from a lichen collected on King George Island in lipopolysaccharide (LPS)-stimulated BV2 cells. Anti-neuroinflammatory effects of the 5-methoxysterigmatocystin (**46**), sterigmatocystin (**47**) (Fig. 17.5), aversin, and 6, 8-O-dimethylversicolorin A were evaluated by measuring the production of NO, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 in LPS-activated microglia at non-cytotoxic concentrations. The compounds 5-methoxysterigmatocystin and sterigmatocystin had significant effects on NO production and mild effects on TNF-α and IL-6 expression inhibition. The authors concluded that these sterigmatocystins present in the *Aspergillus* sp. are promising candidates for the treatment of neuroinflammatory diseases.

Because of the rapid evolution and spread of herbicide resistance in weeds (Gaines et al. 2020; Heap 2022), the search for herbicides with new chemical structures and new molecular targets is badly needed for resistance management (Duke and Dayan 2022). Phytotoxins from fungi are a good source of compounds with both new structures and new molecular targets (Dayan and Duke 2014). Although some studies have looked for compounds with such structures and activities from extremophile fungi, many of them only describe the activity of crude extracts. For example, Godinho et al. (2015) described the herbicidal activity from the extracts of *Penicillium brevicompactum*, isolated from soil collected from ice-free areas of the Union Glacier region in the southern Heritage Range, Continental Antarctica against *Lactuca sativa* (lettuce) and *Agrostis stolonifera* (bentgrass). Gomes et al. (2018) reported the herbicidal activity of crude extracts of some isolates of *Penicillium*, *Mortierella,* and *Pseudogymnoascus* genus obtained soil samples in the Robert, Nelson, King George, and Penguin islands of the South Shetland Archipelago. Among them, *Pseudogymnoascus destructans* displayed strong herbicidal activity against *Allium schoenoprasum* and *L. sativa*.

From glacial ice samples of Antarctica, de Menezes et al. (2020) determined that the fungi *P. palitans*, *Penicillium* sp. 2, *Penicillium* sp. 6, and *Penicillium* sp. 7 showed selective herbicidal activity. The extracts of these fungi were examined using ^{1}H NMR analysis to search for the presence of aromatic compounds, which revealed the presence of highly functionalized secondary metabolites that were confirmed by the presence of proton signals consistent with aromatic/heteroaromatic ($\delta H = 6–9.5$) compounds, suggesting that these fungi can potentially produce secondary bioactive compounds.

From marine deep-sea sediments of Southern Ocean, Ogaki et al. (2020) detected herbicidal activity of compounds produced by the fungi *Acremonium fusidioides*, *Penicillium allii-sativi*, *Penicillium chrysogenum*, *Penicillium palitans,* and *Penicillium solitum* that could bring about total inhibition of germination of the seeds of *A. schoenoprasum* and *L. sativa*. Additionally, *A. fusidioides* and *Pseudogymnoascus verrucosus* produced compounds with selective herbicidal activities.

4 Bioactive Compounds Produced by Extremophiles Fungi from Arctic Ecosystems

According to Bratchkova and Ivanova (2011), the Arctic region is located at the northernmost part of the Earth and includes the Arctic Ocean and all or parts of Canada, Russia, Greenland, the United States, Norway, Sweden, Finland, and Iceland. The Arctic's landscapes consist of a vast, ice-covered ocean, surrounded by treeless permafrost (permanently frozen ground), and the climate is characterized by cold winters and cool summers (Bratchkova and Ivanova 2011). The large amounts of sea ice and the remoteness of the Arctic make access to study sites difficult and expensive, and this is probably the reason for the low number of studies on marine fungi from the Arctic (Hagestad et al. 2020).

Food-borne penicillia are commonly found in refrigerated foods (Frisvad and Samson 2004) that are similar to those in Arctic glacier ice, and some studies have found no or very few differences between the secondary metabolite profiles of isolates from this microbial group found in refrigerated foods and cold ecosystems, such as Artic regions (Frisvad 2008). Sonjak et al. (2005) compared the secondary metabolite production of several isolates of *Penicillium crustosum* obtained from glacial ice from the Arctic Archipelago of Svalbard and further isolates from many different sources, and the authors detected that all isolates produced terrestric acid, roquefortine C, penitrem A, and viridicatins. In contrast, andrastin A was detected for the first time as a secondary metabolite produced by *P. crustosum* from melted snow, sea, and glacial ice collected in Arctic Archipelago of Svalbard. This is an example that it could be possible to find new secondary metabolites in already well-characterized microbial strains from other species from cold habitats (Frisvad 2008). However, it is important to emphasize that *P. crustosum* is a species with highly conserved secondary metabolite profile properties (Sonjak et al. 2005). Thus, new species may be more interesting candidates for finding new compounds (Frisvad 2008).

The psychrotolerant fungus *Penicillium algidum*, isolated from soil of Zackenberg, Greenland, was cultivated, and a new cyclic nitropeptide, psychrophilin D (**48**), and two known cyclic peptides, cycloaspeptide A (**49**) and cycloaspeptide D (**50**) (Fig. 17.6), were isolated and tested in antimicrobial, antiviral, anticancer, and antiplasmodial assays (Dalsgaard et al. 2005). Psychrophilin D (**48**) exhibited moderate activity in a murine leukemia cell assay, with an ID_{50} of 22.52 μM, and cycloaspeptide A (**49**) and D (**50**) exhibited moderate activity against *Plasmodium*

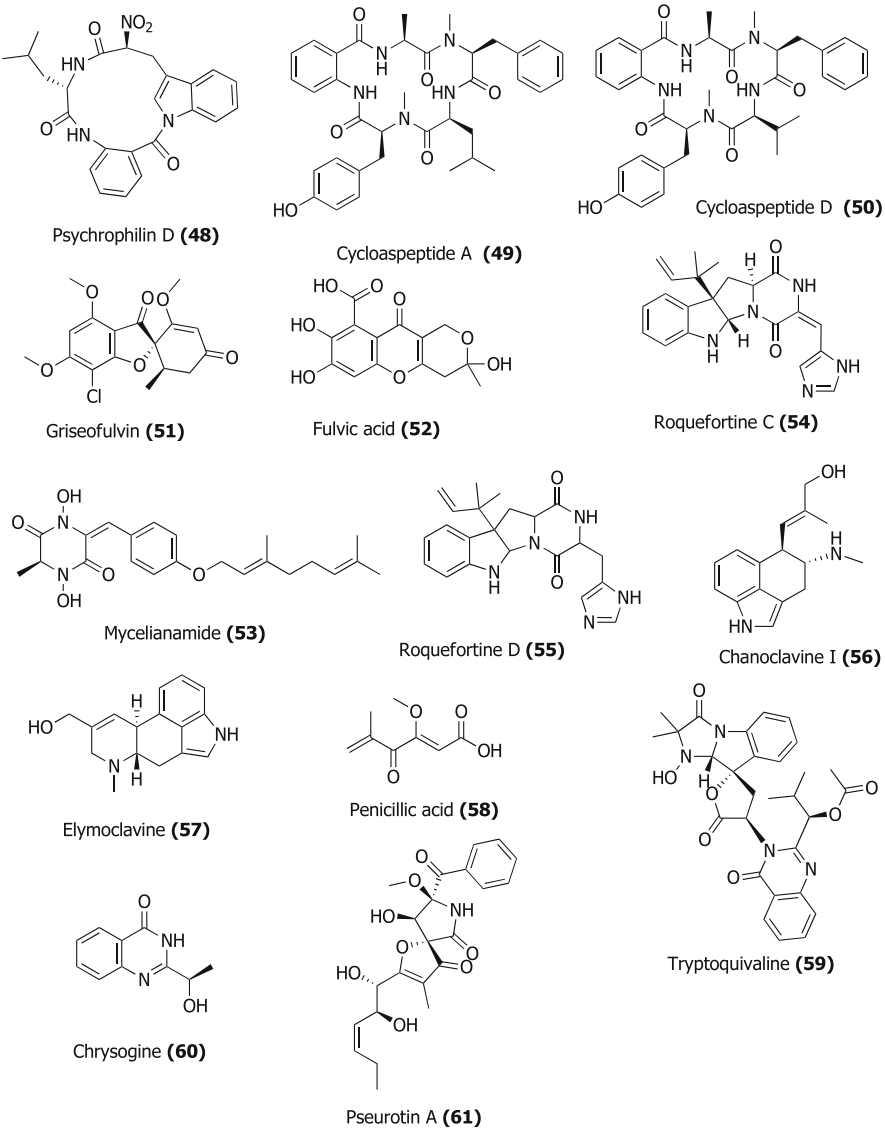

Fig. 17.6 Secondary metabolites obtained from extremophile fungi from the Arctic (48–61)

falciparum, with IC_{50} values of 5.45 and 7.48 μM, respectively (Dalsgaard et al. 2005). Frisvaad et al. (2004) described a serie of secondary metabolites with antimicrobial activity produced by *Penicillium griseofulvum* isolates, collected in Greenland, and identified as griseofulvin (**51**), fulvic acid (**52**), mycelianamide (**53**), roquefortine C (**54**) and D (**55**), chanoclavine I (**56**), and elymoclavine (**57**) (Fig. 17.6). Frisvad et al. (2006) described that *Penicillium jamesonlandense* is a novel species from soil samples taken from Greenland and detected that all isolates of this fungus produced the glucose-derived kojic acid, the polyketides griseofulvin (**51**) and penicillic acid (**58**) (Fig. 17.6) (known for antifungal and antibacterial activity, respectively), the amino acid-derived compounds cycloaspeptide A (**49**) (previously studied with antimalarial activity), tryptoquivalins (**59**), and chrysogine (**60**), and the phenylalanine- and hexaketide-derived pseurotin (**61**) (Fig. 17.6).

The secondary metabolite profile of a strain of *Eutypella* sp., obtained from high-latitude soil of the Arctic, has been extensively studied (Wang et al. 2018; Yu et al. 2018a, b). Wang et al. (2018) isolated from the culture extract of *Eutypella* sp. two new pimarane diterpenes identified as libertellenone M (**62**) and libertellenone N (**63**) and five known compounds [libertellenones A–C (**64**–**66**), eutypellone A (**67**), and kaempulchraol W (**68**) (Fig. 17.7)]. Compound **64** had weak antibacterial activity against *E. coli*, *Bacillus subtilis,* and *Vibrio vulnificus*, each with MIC values of 48.12 μM, while compounds **63** and **64** showed moderate cytotoxic activity against K562 and MCF-7 cell lines with IC_{50} values of 7.67 and 9.57 μM, respectively.

Yu et al. (2018a) isolated seven new pimarane-type diterpene derivatives, libertellenones O–S (**69–73**) and eutypellenones A and B (**74** and **75**) (Fig. 17.7), together with two known compounds, libertellenones H and J (**76** and **77**) (Fig. 17.7). All compounds exhibited cytotoxicity against the cell lines (HeLa, MCF-7, HCT-116, PANC-1, and SW1990) cells, with IC_{50} values in the range of 0.3 to 29.4 μM, and libertellenone Q (**71**) presented similar IC_{50} values or even better than the positive control used in the assay. Additionally, eutypellenones A and B (**74** and **75**) showed anti-inflammatory activity, as they could dose-dependently inhibit the activity of NF-κB and exhibited significant inhibitory effects on NO production induced by lipopolysaccharide. Yu et al. (2018b) investigating the secondary metabolite profile of the same strain of *Eutypella* sp. isolated three new pimarane diterpenes, eutypellenoids A–C (**78–80**) (Fig. 17.8), together with a known compound, eutypenoid C (**81**) (Fig. 17.8). Antibacterial, antifungal, and cytotoxic activity were evaluated and eutypellenoid B (**79**) had antibacterial activity against *S. aureus* and *E. coli* with MIC values of 16.85 and 16.85 μM, respectively. Additionally, the same compound showed antifungal activity against *Candida parapsilosis*, *C. albicans*, *C. glabrata,* and *C. tropicalis* with MIC values of 16.85, 16.85, 33.71, and 67.43 μM, respectively, and moderate cytotoxic activity against HCT-116 cell line with an IC_{50} value of 3.7 μM.

Yu et al. (2018c) studied the secondary metabolites produced by the fungus *Nectria* sp., isolated from a soil sample obtained from Ny-Ålesund on the Arctic Island of Spitzbergen, Svalbard. The authors identified two new cyclohexanone derivatives, nectriatones A–B (**82**, **83**), and one new natural product, nectriatone C

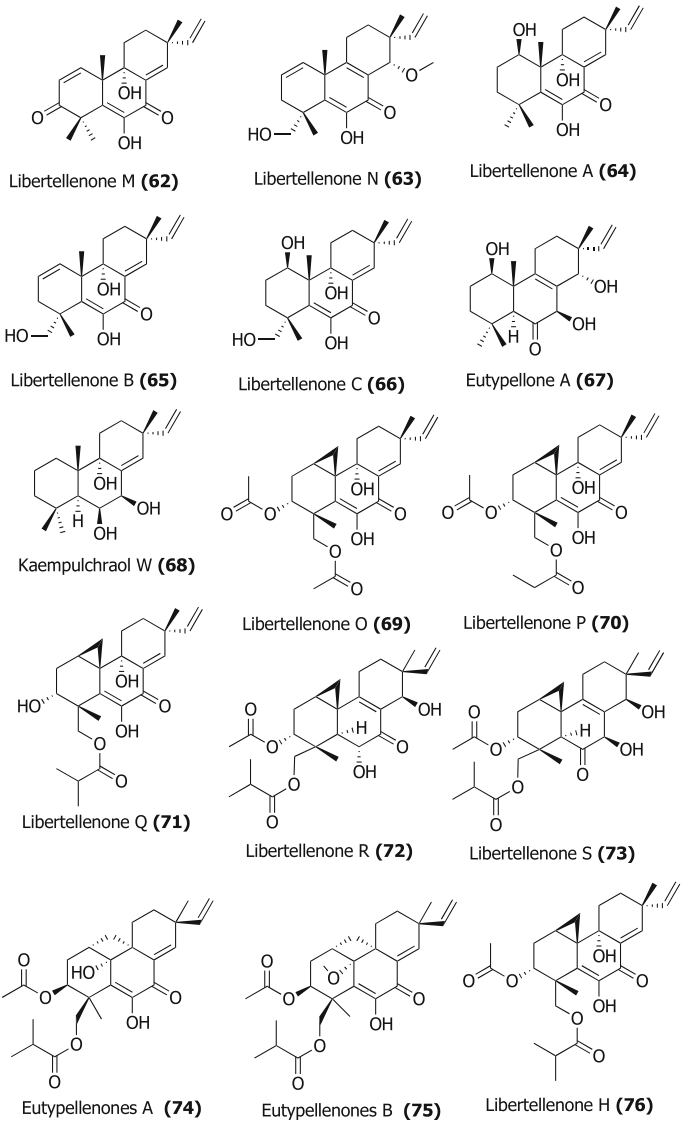

Fig. 17.7 Secondary metabolites obtained from extremophile fungi from the Arctic (**62–76**)

(**84**), together with three known phenolic sesquiterpene derivatives, identified as ilicicolin E (**85**) and D (**86**) and deacetylchloronectrin (**87**). Compounds nectriatone A (**82**), ilicicolin E (**85**) and D (**86**), and deacetylchloronectrin (**87**) (Fig. 17.8) were cytotoxic to all the human cancer cell lines tested (SW1990, HCT-116, MCF-7 and K562), with IC_{50} values ranging 0.43 to 42.64 μM. Only ilicicolin E exhibited

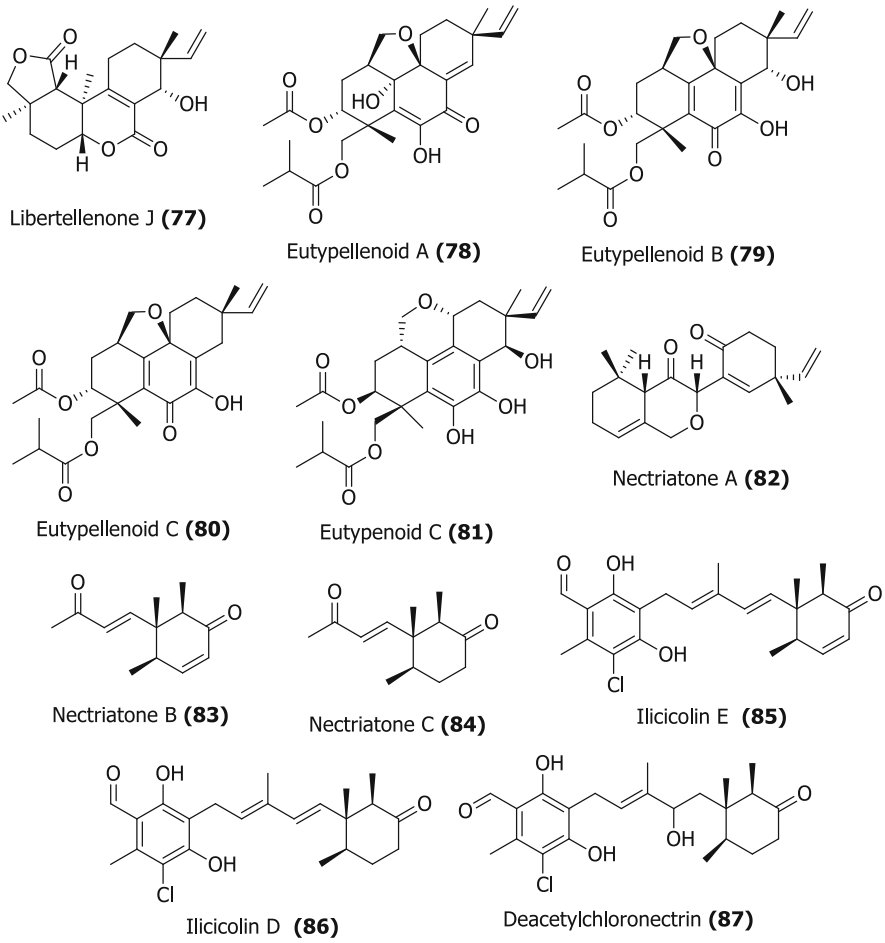

Fig. 17.8 Secondary metabolites obtained from extremophile fungi from the Arctic (77–87)

antibacterial activity against *E. coli*, *B. subtilis*, and *S. aureus* (MIC 9.92, 4.96, and 9.92 μM, respectively).

The endolichenic fungus *Cochliobolus kusanoi*, obtained from a lichen collected in Ny-Ålesund, Arctic, showed significant antimicrobial activity, against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *C. albicans* (ATCC 10213), using the Oxford cup method (Yang et al. 2019). From the EtOAc extract of liquid cultures of *Cochliobolus kusanoi*, three substances were identified as phomalone (1-(2,4-dihydroxy-3-[2-hydroxyethyl]-6-methoxyphenyl) butan-1-one and two derivatives (1-(2,4-dihydroxy-3-[2-hydroxyethyl]-6-methoxyphenyl)-3-hydroxybutan-1-one, and LL-D253α (7-hydroxy-8-[2-hydroxyethyl]-5-methoxy2-methylchro-man-4-one. While the compound (1-(2,4-dihydroxy-3-[2-hydroxyethyl]-6-methoxyphenyl)-3-hydroxybutan-1-one was a new product,

phomalone (1-(2,4-dihydroxy-3-[2-hydroxyethyl]-6-methoxyphenyl) butan-1-one and LL-D253α (7-hydroxy-8-[2-hydroxyethyl]-5-methoxy2-methylchro-man-4-one produced by *C. kusanoi* were isolated for the first time.

5 Bioactive Compounds from Extremophiles Fungi from Alpine Ecosystems

Alpine ecosystems (high-altitude habitats above the tree line) are the only cold biogeographic unit found on all continents and latitudes. They are characterized by a history of climate change and a wide range of microhabitats and are considered global biodiversity hotspots, harboring many endemic species (Körner 2003). The fungi found in these cold-dominated regions can exist as mutualists, increasing nutrient uptake in alpine plants; as decomposers, replenishing nutrients in poor alpine soils; and as pathogens, affecting populations of alpine plants (Körner 1999; Haselwandter 2007).

Despite the fact that fungi have been considered a factory of bioactive secondary metabolites with great biotechnological application (Patil et al. 2016), no article was found reporting the isolation and characterization of bioactive compounds derived from fungi found in the Alps. However, some papers were found demonstrating activities derived from the crude extracts produced by these fungi. Clericuzio et al. (2021) reported the antimicrobial activity of 16 different species of the *Basidiomycota*, with the majority belonging to the genera *Cortinarius*, *Mycena,* and *Ramaria*, all of them collected in the Italian Alps. Although 12 extracts displayed good activity against *P. aeruginosa*, the highest activity was from *Cortinarius nanceiensis*.

Rhodiola spp. are rare and endangered alpine plants widely used as medicines and food additives. Their main effective ingredients (salidrosides and *p*-tyrosol) have pharmacological effects and antioxidant properties (Cui et al. 2015). Cui et al. (2015) isolated 347 endophytic fungi of *Rhodiola crenulata*, *R. angusta,* and *R. sachalinensis* inhabiting the alpine ecosystem in China, and the antioxidant activity of these fungi was investigated for the first time. These fungi were identified and grouped into four phyla: *Ascomycota*, *Basidiomycota*, *Mucoromycota,* and *Glomeromycota*. The antioxidant assay showed that the DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical-scavenging rates of 114 isolates (63.33%) were >50%, and those of five isolates (Rct45, Rct63, Rct64, Rac76, and Rsc57) were >90%. The EC_{50} (effective concentration at which reactive oxygen species and other reactive radicals or ions were scavenged by 50%) values from five antioxidant assays suggested a significant potential of these fungi in scavenging DPPH radical, $^1O_2$ and OH radicals, as well as scavenging nitrite and acting as a Fe^{2+} chelator. Other research provided the first evidence that Rac12 (*Lachnum* sp.) could produce salidrosides and *p*-tyrosol. The results suggested that versatile endophytic fungi associated with *Rhodiola* sp. could be explored as potential sources of new antioxidant products.

Lv et al. (2010) investigated the antimicrobial potential of endophytic fungi obtained from *Saussurea involucrata* collected in the alpine ecosystem in China. A total of 49 fungi were isolated, and extracts from the fermentation broth of these fungi were tested for antimicrobial activity against pathogenic microorganisms using the agar diffusion method. Twelve isolates belonging to *Fusarium* sp., *Phaeosphaeria* sp., *Cylindrocarpon* sp., *Leptosphaeria* sp., *Cladosporium* sp., and *Phoma* sp. taxa. exhibited antimicrobial activity against at least one test microorganism, whereas the remainder yielded no activity. There were six strains active against *Bacillus subtilis*, one against *S. aureus*, nine against *C. albicans*, six against *Cryptococcus neoformans,* and eight against *Aspergillus fumigatus*. Moreover, five strains showed a broader spectrum of antimicrobial activity against test bacteria and fungi, respectively, and four displayed strong inhibition of pathogenic fungi. None of the fungal broths inhibited the test bacteria *E. coli*. The results indicate that endophytic fungi isolated from *S. involucrata* are diverse in species and a potential source of antimicrobial agents.

6 Summary

Although there are several studies carried out from different fungi collected at the poles and alpine regions in recent years, most of these works aim to evaluate the biodiversity of the fungal community and to explore and understand the physiological adaptations present in these microorganisms that allow their survival in hostile environments. There is a relative lack of studies aimed to identify species and strains from these regions able to produce bioactive compounds that could be templates for new pharmaceuticals and/or pesticides. Such bioprospecting studies with fungi from other parts of the world are much more common. Unfortunately, a significant proportion of the studies on the biological activities of fungi from alpine and polar regions have only been conducted with crude extracts. Further studies are needed to isolate and identify the compounds responsible for the activities found, mainly those with potential to produce molecules to combat neglected tropical diseases or with herbicidal activities to use in agriculture. Another interesting point relating to natural product research is the lack of in vivo tests necessary to better understand the structural moieties of the active compounds responsible for bioactivities. Therefore, most of the bioprospecting and isolation studies of active compounds are carried out using in vitro tests. Among the regions discussed here, the Antarctic continent is the one with the most studies about isolation of active compounds from fungi, followed by the Arctic and lastly alpine regions. Regarding the fungal genera studied, different species of *Penicillium* are frequently isolated from samples of cold ecosystems and have been extensively studied for the isolation of secondary metabolites with biological activity. The discovery of new fungi species from Antarctic, Arctic, and alpine soils and vegetation and the isolation of new compounds from these fungal strains indicate that some secondary metabolites may be entirely unique to cold ecosystems. Thus, we can conclude from these studies that the fungal

communities present in the Antarctic, Arctic, and alpine regions are extremely interesting because they are sources of novel, bioactive compounds that are needed as prototypes to be used in the pharmaceutical and agrochemical industries.

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Part VI
Novel Strategies to Screen or Enhance Secondary Metabolite Production

Chapter 18
Enhancing Chemical Diversity of Fungal Secondary Metabolite by OSMAC Strategy

Wangjie Zhu and Huawei Zhang

Abstract Fungal secondary metabolites (SMs) with therapeutic potential have played and continue to play an important role in new drug discovery. These substances are biosynthetically formed through a series of sequential reactions catalyzed by SM biosynthetic gene cluster (BGC)-encoded enzymes. However, most of these BGCs are silent or expressed in low level under conventional cultivation and fermentation conditions. It frequently results in the rediscovery of the same metabolites from various strains, and the speed of discovery of novel fungal SM is slowing down. In order to alleviate this challenge, one strain many compounds (OSMAC) strategy has become the most effective and simple approach to awaken these cryptic BGCs. A great number of successful examples using this strategy to enhance chemical diversity of fungal SM are comprehensively summarized in this chapter.

Keywords OSMAC strategy · Fungal secondary metabolite · Medium composition · Culture condition · Coculture · Epigenetic modification

1 Introduction

Fungal microbes have extremely abundant biodiversity since they are ubiquitous in various natural environments including the aquatic, soils, marine sediments, polar regions, vascular plants, insects, and animals. Numerous chemical studies demonstrate that fungi are a prolific source of secondary metabolites (SMs) with diverse chemical structures and a wide variety of biological properties. In the past few years, compounds that have been isolated from fungi more than half displayed antibacterial, antifungal, or antitumor activity (Keller 2019). These leading SMs possessing therapeutic effects are critically important for new drug development (Deng et al. 2013; Gunatilaka 2006; Rateb et al. 2011). Traditional chemical investigation of fungal SMs mainly focuses on extraction and isolation of

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structurally and highly bioactive compounds from fermentation broth and mycelium. However, these processes are becoming inefficient due to high rate of the rediscovery (Wasil et al. 2013). It is commonly believed that a large portion of SM biosynthetic gene clusters (BGCs) are silenced under standard fermentation conditions (Scherlach and Hertweck 2009; Schneider et al. 2008).

Great advances in genome sequencing, bioinformatic algorithms, phylogenetic sleuthing, and increasing ease in genome manipulations have been made in the post-genomic era and definitely promoted the discovery of therapeutic agents from fungi (Kueck et al. 2014). Among these ways to stimulate silent BGCs in fungi, variation of culture conditions has been considered as the simplest and most effective strategy, which had been originally termed as "one strain many compounds (OSMAC)" by professor Zeeck and coworkers (Bode et al. 2002). During the past two decades, OSMAC strategy had been constantly improved and widely applied in the field of microbe natural product chemistry (Pan et al. 2019). Currently, this approach consists of several culture methods, including variation of medium, changing condition, and coculture as well as adding epigenetic modifier or biosynthetic precursor. By extensive literature search of Dictionary of Natural Products (DNP) and SciFinder databases, a total of 476 SMs (**1**–**476**) had been isolated and characterized from fungal strains using OSMAC strategy. Based on cultivation method, these natural products are respectively introduced herein.

2 Variation of Culture Medium

Variations of medium include medium composition (C/N ratio), salinity, and metal ion, which regulate the degree and pattern of gene expression and result in production of various SMs in fungi. One hundred and thirty five new SMs (**1–135**) were discovered by changing the composition of culture medium.

2.1 Medium Composition

Generally, carbon and nitrogen sources are major components in the culture medium with C/N ratios and play an important role in the production of SMs (Basiacik Karakoc and Aksoz 2004; Brzonkalik et al. 2012; Dinarvand et al. 2013). The carbon source not only provides the basis for building biomass and represents the source of energy for all heterotrophs, but also delivers carbon units for SMs. The nitrogen source is required for the synthesis of essential proteins and nucleic acids, and likewise N-containing units for SMs. Studies have shown that the types of used carbon and nitrogen sources have a significant effect on fungal SMs (Ruiz et al. 2010; Singh et al. 2017). Notably, the consumption of carbon and nitrogen-based medium components can greatly affect the pH of the cultivation. Thus, fungi cultured in medium containing different components may exhibit differently adapted

metabolism and express specific sets of biosynthetic genes, which produced specialized metabolites.

One marine-derived strain *Asteromyces cruciatus* 763 was shown to produce a new pentapeptide lajollamide A (**1**) when cultivated in the Czapek-Dox broth containing arginine solely as nitrogen source rather than $NaNO_3$, which was missed in the normal Czapek-Dox medium (Gulder et al. 2012). A novel furan ester derivative (**2**), a compound that has toxicity activity against HCT-116 cancer cell line, was isolated from a sediment-derived strain *Aspergillus niger* BRF-074 which was cultivated in MPDB (malt peptone dextrose broth) medium (Uchoa et al. 2017). A fungus *Aspergillus* sp. from Waikiki Beach (Honolulu, HI) generated six isotopically labeled metabolites (**3–8**) when grown on the deuterium-enriched Czapek broth (Wang et al. 2015a), whereas this strain was found to produce a novel prenylated indole alkaloid, waikialoid A (**9**), when cultivated in PDB medium, which played potent inhibitory effect on biofilm formation of *Candida albicans* (Wang et al. 2012a).

Five new polyketides (**10–14**) were produced by a marine-derived *Cladosporium sphaerospermum* 2005-01-E3 in rice-based medium (Wu et al. 2014). While this strain was fermented on the soybean flour, two new hybrid polyketides (**15–16**) were detected and characterized (Yu et al. 2015). Strain *Dothideomycete* sp. CRI7 growing in PDB medium led to the isolation of two azaphilone derivatives (**17–18**) and a novel tricyclic polyketide (**19**). However, three new polyketides (**20–22**) were produced when this strain was grown in PDB broth prepared from a commercial potato powder. Moreover, this fungus synthesized other SMs (**20–21** and **23–25**) in Czapek malt medium. Bioassay results indicated that compounds **19** and **24** displayed a broad spectrum of cytotoxic activity (Hewage et al. 2014; Senadeera et al. 2012). Fermentation of strain *Fusarium tricinctum* colonizing the rhizomes of *Aristolochia paucinervis* afforded three new fusarielins (**26–28**), in which compound **26** possessed cytotoxic effect on human ovarian cancer cell line A2780 with an IC_{50} value of 12.5 μM, while these metabolites were not detected when cultivated in normal rice medium supplemented with fruit and vegetable juice (Hemphill et al. 2017). A new diketopiperazine (**29**) isolated from *Eurotium rubrum* MPUC136 cultured in wheat medium displays more powerful bioactivity than that from the Czapek-Dox agar medium and has shown to have cytotoxicity against B16 melanoma cell line with an IC_{50} value of 60 μM (Kamauchi et al. 2016) (Fig. 18.1).

One rhizosphere fungus *Paraphaeosphaeria quadriseptata* produced a known C18 polyketide monocillin I together with several analogs when incubated in PDA medium constituted with tap water (Wijeratne et al. 2004) and produced six new trihydroxybenzene lactones, cytosporones F-I (**30–35**), when the tap water was changed as distilled water (Paranagama et al. 2007). Similarly, one new naphthalopyran compound (**36**), which possesses an unusual oxygenated aromatic structure with a lactone bridge, could be metabolized by the fungus *Penicillium hordei* grown on plant tissue agar (macerated tulip, oatmeal, yellow onion, and red onion), while it was not detected in CYA (caffeic acid agar), MEA (malt extract agar), and YES (yeast extract with supplements) media (Overy et al. 2005). When cultivated in rice medium, a hard coral-derived fungus *Scopulariopsis* sp. was shown

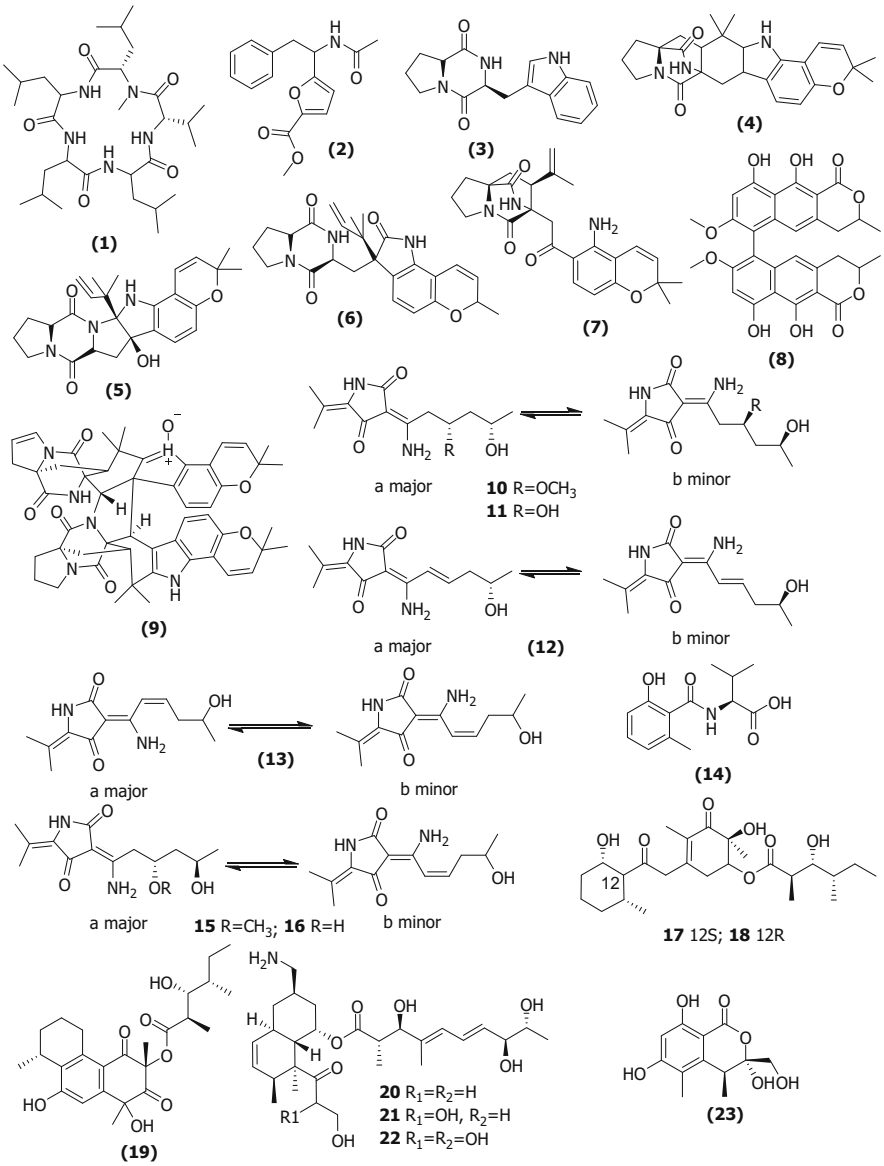

Fig. 18.1 Second metabolites obtained from fungi using various medium composition (**1**–**23**)

to afford six SMs, including xanthone derivatives (**37–38**), phenolic bisabolane-type sesquiterpenes (**39–40**), one new alkaloid (**41**), and one new α-pyrone derivative (**42**) (Elnaggar et al. 2016). Interestingly, when cultivated in the protein-rich white bean medium, this strain could biosynthesize a new naphthoquinone derivative (**43**)

and two new triterpenoids (**44–45**). All isolated compounds were evaluated for their cytotoxic, antibacterial, and antitubercular activities (Elnaggar et al. 2017).

Phytochemical study of a filamentous soil fungus *Talaromyces wortmannii* cultivated in maize culture medium led to the separation of three new polyketones (**46–48**), which were absent in rice or dextrose agar media and these metabolites displayed inhibitory activities against NFRD (fumarate reductase) with IC_{50} values of 8.8, 11, and 13 μM, respectively (Liu et al. 2016). Interestingly, four novel 22-membered macrolides (**49–52**), exhibiting in vitro moderate cytotoxic activities against human cancer cell lines (Dong et al. 2006), and four novel tetraene lactones (**53–56**), showing potent inhibitory effects on cathepsin B (Dong et al. 2009), were detected when this strain has grown in the still-cultured medium (2.5% soybean meal and 97.5% rice). A new sesquiterpene derivative (**57**) and two known compounds were obtained from the endophytic fungus *Trichocladium* sp. isolated from roots of *Houttuynia cordata* which was cultured on peas medium instead of rice medium (Tran-Cong et al. 2019). A sponge-derived fungus *Aspergillus* sp. LS34, using solid rice medium, resulted in the isolation and identification of two new compounds, asperspin A (**58**) and asperther A (**59**), while isolated seven known compounds in PDB medium (Li et al. 2019) (Fig. 18.2).

2.2 Salinity

Salinity is an important factor in determining many aspects of the chemistry of natural water and biochemical process within cultivation system. It is a thermodynamic state variable that regulates physical characteristics such as osmotic pressure involved in microbial growth and metabolism (Blunt et al. 2017). Suitable salinity is needed for normal fungal growth. Fungi exposed to different types of media supplemented with various halogens may trigger their synthesis pathway to restore osmotic imbalance, thus activating different hidden SMs biosynthetic gene clusters.

Compared to that grown in seawater, one marine-derived fungus *Aspergillus unguis* CRI282-03 was shown to yield three new brominated depsidones (**60–62**), a new depsidone (**63**) in KI broth and two new orcinol derivatives (**64–65**) in KBr medium, while compounds **62** and **64** possess aromatase inhibitory effects (Sureram et al. 2012, 2013). Nine new polyketides (**66–74**), which were absent in the broth containing KI or deionized water, were obtained by the fungus *Dothideomycete* sp. CRI7 isolated from *Tiliacora triandra* when cultivated in the medium supplemented with KBr and seawater (Wijesekera et al. 2017).

Chemical investigation of one symbiotic stain *Aspergillus* sp. D from *Edgeworthia chrysantha* yielded five known heterocyclic alkaloids from normal Czapek medium, while a new meroterpenoid (**75**) and four known analogs were obtained from Czapek medium with 3% salt (Zhang et al. 2018a, b). One mangrove-derived endophyte *Wallemia sebi* PXP-89 cultivated in 10% NaCl broth led to the isolation of a new cyclopentanol pyridine alkaloid (**76**), which was not detected in normal medium (Peng et al. 2011). When cultivated in the medium containing 10%

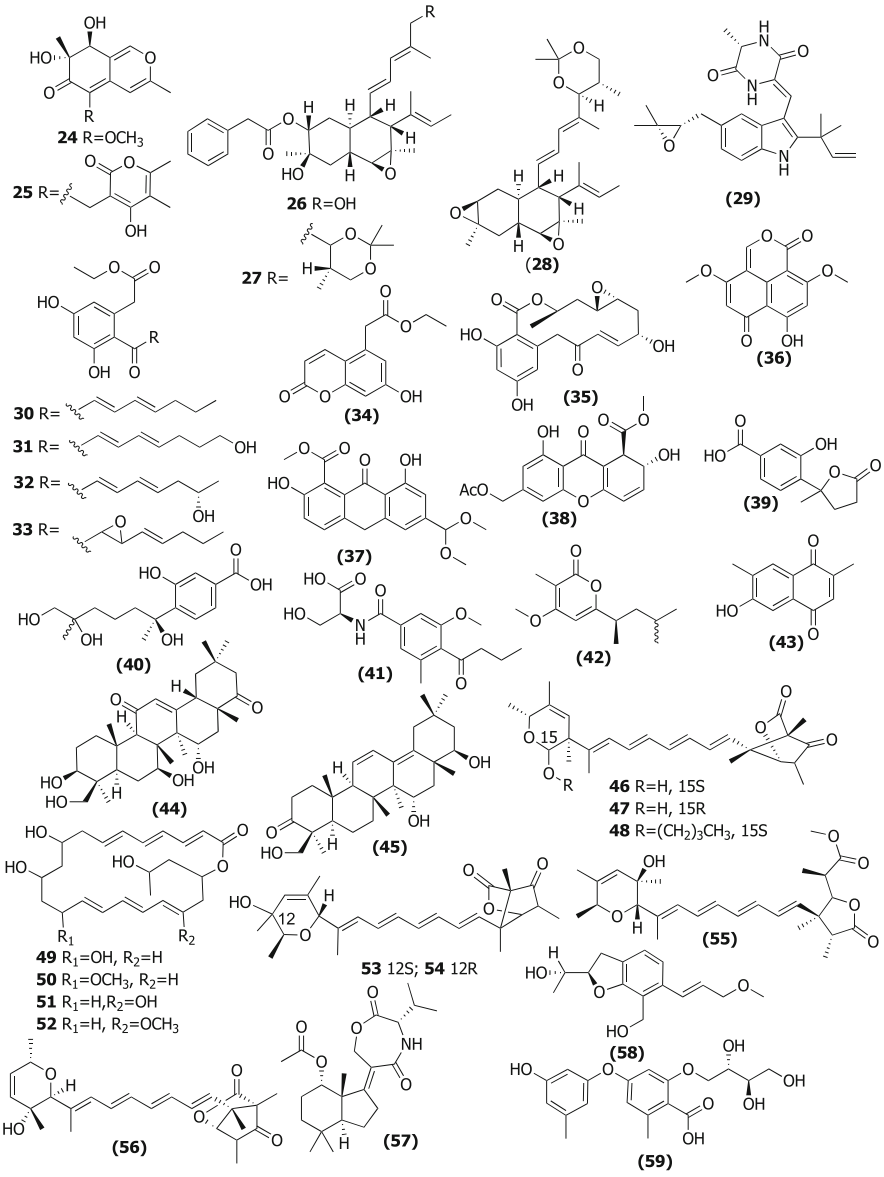

Fig. 18.2 Second metabolites obtained from fungi using medium composition (**24**–**59**)

sea salt, strain *Spicaria elegans* KLA03 was shown to biosynthesize a new antimicrobial diacrylic acid (**77**) (Wang et al. 2011). Two rare epidithiodiketopiperazines, gliovirin and pretrichodermamide A, were detected in 1.5% NaCl broth of a marine-derived strain *Trichoderma* sp. TPU199, while this strain produced a new iodine

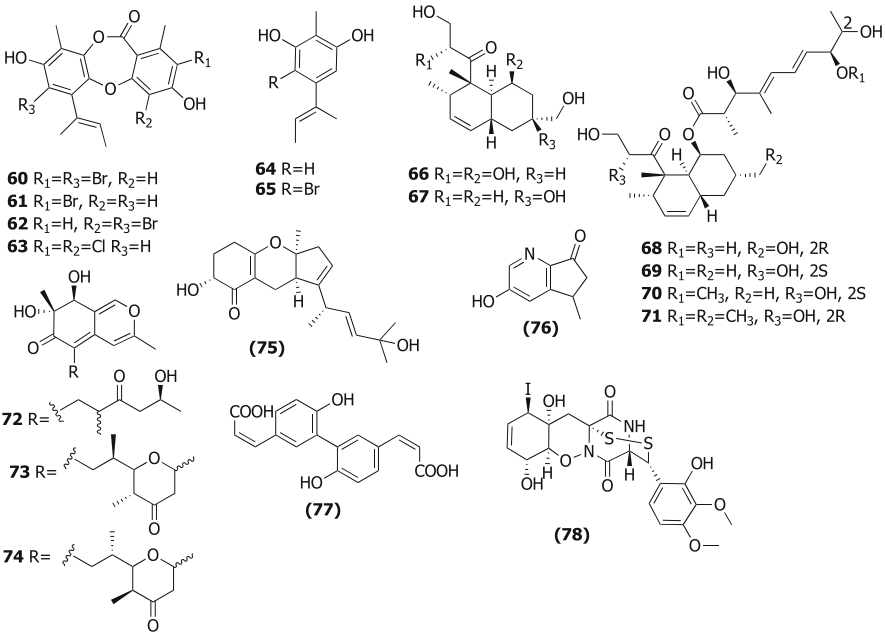

Fig. 18.3 Second metabolites obtained from fungi exposed to different types of media supplemented with various halogens (**60**–**78**)

derivative (**78**) from freshwater medium with 3.0% NaI and 3.0% NaBr as well as 5-bromo-5-deoxy derivative (Yamazaki et al. 2015a) (Fig. 18.3).

Twelve new cytochalasins, phomopchalasins D-O (**79**–**90**), including one brominated (**80**) and two iodinated cytochalasins (**81** and **83**), were isolated from the mangrove-derived fungus *Phomopsis* sp. QYM-13 treated with 3% NaBr or 3% KI in potato liquid medium and compound **80** exhibited significant cytotoxicity against human cancer cell line MDA-MB-435 with IC_{50} was 7.4 μM (Chen et al. 2022). A sponge-derived fungus *Pestalotiopsis heterocornis* XWS03F09 cultivated on 3% artificial sea salt in the rice media led to biosynthesize 12 compounds, including eight new polyketide derivatives, heterocornols Q-X (**91**–**98**), one new ceramide (**99**), and three known analogues (Lei et al. 2021). Fermentation of the endophytic fungus *Aplosporella javeedii* on solid rice medium with either 3.5% $NaNO_3$ or 3.5% monosodium glutamate led to yield 11 new lactam derivatives, aplosporellins A-K (**100**–**110**), compared to fungal controls grown only on rice (Gao et al. 2020). The addition of a mixture of salts ($MgSO_4$, $NaNO_3$, and NaCl) to solid Czapek medium induced the endophytic fungus *Bulgaria inquinans* to produce nine additional new secondary metabolites (**111**–**119**), most of them not detectable in cultures lacking the salt mixture (Ariantari et al. 2019). A marine-derived fungus *Aspergillus falconensis* cultivated on solid rice medium containing 3.5% NaCl yielded two new chlorinated azaphilones, falconensins O and P (**120**–**121**), while replacing NaCl with 3.5% NaBr induced the accumulation of three additional new azaphilones, falconensins Q-S (**122**–**124**) (El-Kashef et al. 2020) (Fig. 18.4).

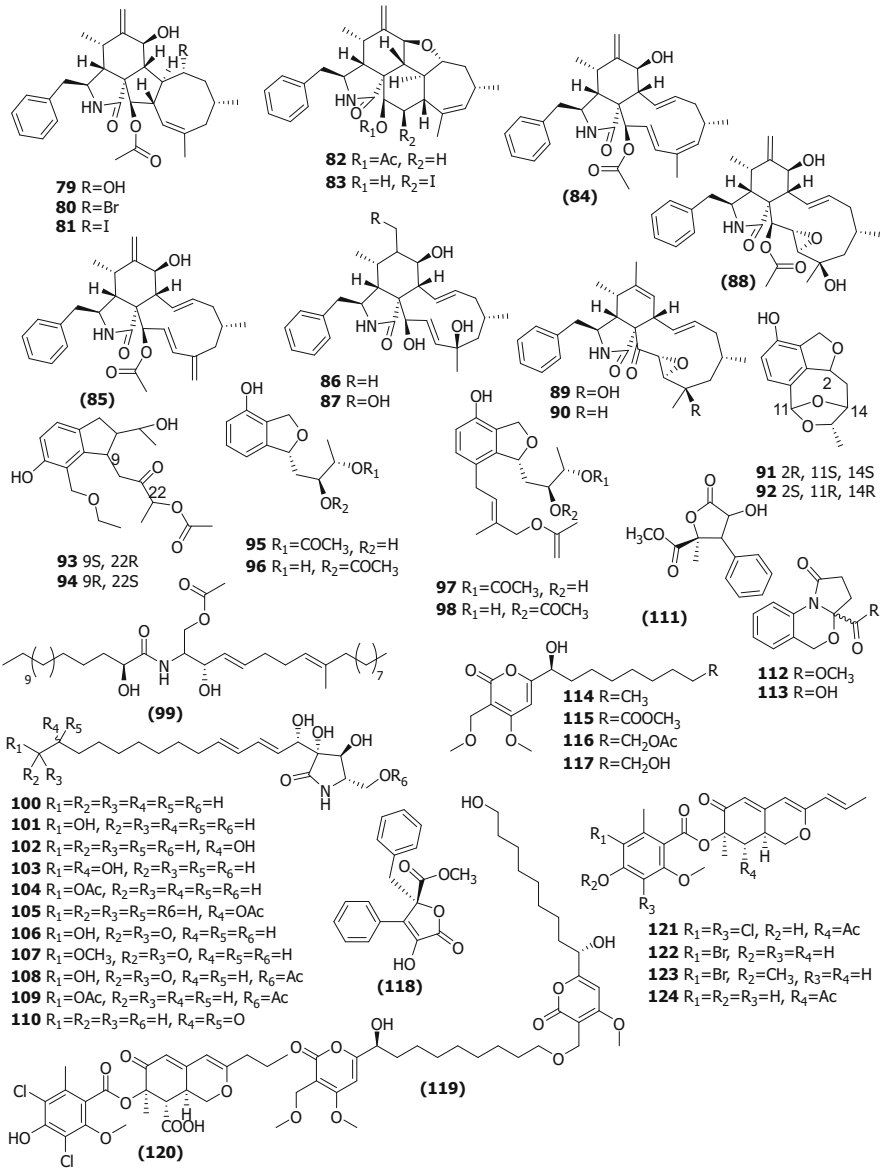

Fig. 18.4 Second metabolites obtained from fungi exposed to different types of media supplemented with various halogens (**79**–**124**)

2.3 Metal Ion

Metal ion affects physiological structure and function of fungi, which led to the production of various SMs. The interaction between metal ion and microbe is usually assumed in three pathways, including causing reactions in cells, conserving energy in the process of dissimilation, and assimilating reactions.

One marine-derived strain *Ascotricha* sp. ZJ-M-5 was shown to produce a new 3,4-split ring lanolin alkyl triterpene (**125**) and a new cyclonerols derivative (**126**), when cultivated in eutrophic medium made up with sea salt (Xie et al. 2013), and three new caryophyllene derivatives (**127**–**129**) were detected in modified Czapek Dox medium, while compound **128** was absent in the fermentation broth without Mg^{2+} (Wang et al. 2014b). Strain Aspergillus sclerotiorum C10WU derived from hydrothermal vent sediment could biosynthesis three new alkaloids (**130**–**132**) under normal medium, but this strain metabolized one unelucidated compound when grown in the stressed culture medium with Cu^{2+} as a supplement. Likewise, two compounds, namely deoxytryptoquivaline and tryptoquivaline A (**133**–**134**), were purified from the normal extract of *Aspergillus clavatus* C2WU, while a new metabolite **135** was found in the normal medium adding Cu^{2+} and Cd^{3+} (Jiang et al. 2014) (Fig. 18.5).

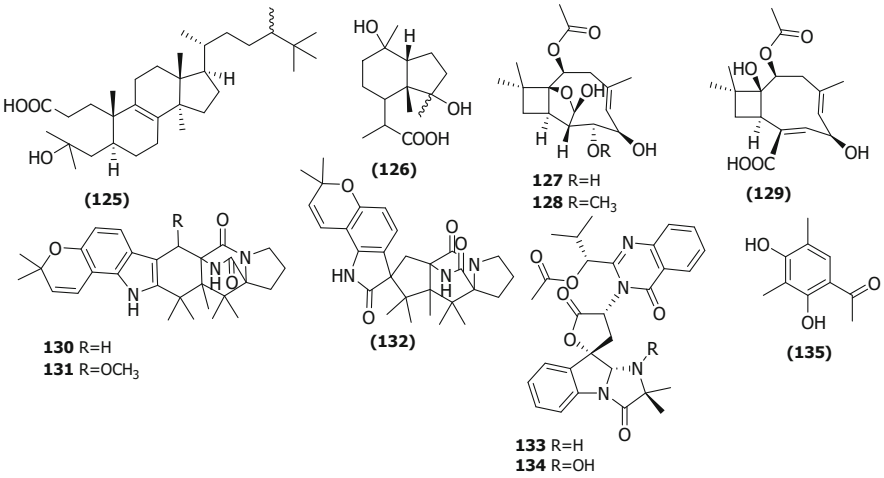

Fig. 18.5 Second metabolites obtained from different types of media supplemented with various metal ion (**125**–**135**)

3 Change of Cultivation Condition

Suitable cultivation conditions, such as appropriate temperature, pH, oxygen concentration, and cultivation status, are essential for the growth and biochemical reactions of fungi and to activate silent gene clusters. In this part, 182 SMs (**136–317**) of fungi resulting from changes in culture conditions were summarized.

3.1 Temperature

Chemical diversity of fungal SMs is directly influenced by enzyme activity, which is susceptible to cultivation temperature. Generally, the higher the cultivation temperature is, the faster the enzyme deactivation rate will be (Feller et al. 1994). Five new polyketide, named raistrickiones A-E (**136–140**), were isolated from *Penicillium raistrickii* in response to the fermentation temperature setting at 15 °C instead of 28 °C, with other conditions unchanged. These compounds exhibited moderate radical scavenging activities against 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) (Liu et al. 2018).

3.2 pH

During fungus fermentation process, the decomposition and utilization of nutrients as well as the accumulation of SMs usually causes the variation of medium pH, which not only affects the activity of each enzyme, but also affects the surface charge of the membrane, thus affecting the growth of fungi and biosynthesis of SMs (Gibson et al. 1988; Tan et al. 1998).

Acidic medium (pH = 5) dramatically increased the production of bioactive compounds of a mangrove-derived fungus *Rhytidhysteron rufulum* AS21B, including two new antitumor spirobisnaphthalenes (**141–142**) which were not detected in neutral medium (Siridechakorn et al. 2017).

3.3 Oxygen Concentration

Changes in oxygen supply can affect the biochemical reactions and activate different sets of functional gene clusters for different SMs production (Sato 1990). [^{13}C]-labeled acetates and a small amount of [$^{18}O_2$] were used to investigate the biosynthetic pathway of aspinonene (**143**) in the culture broth of *Aspergillus ochraceus* DSM-7428. It is interesting that a new compound aspyrone (**144**) was produced by increasing dissolved oxygen concentration during fermentation, accompanied by

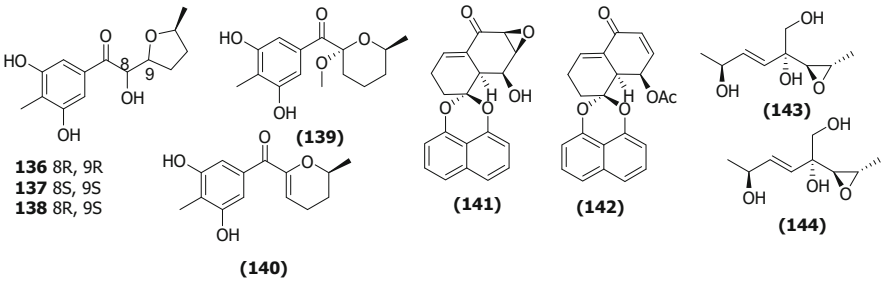

Fig. 18.6 Second metabolites obtained from fungi by change of cultivation conditions (**136**–**144**)

reduced amounts of compound **143** under an oxygen-enriched atmosphere (Fuchser et al. 1995) (Fig. 18.6).

3.4 Cultivation Status

Cultivation status can directly affect fungus metabolic process, including solid or liquid, static or dynamic. Compared with solid and static cultivation, liquid and dynamic modes not only ensure the full contact of fungi and nutrients, but also affect their biochemical reactions by changing oxygen supply and activating functional gene clusters.

One marine sponge-derived fungus *Arthrinium arundinis* ZSDS1-F was shown to metabolize a novel naphthalene glycoside (**145**) (Wang et al. 2014a), five cytochalasins (**146–150**) (Wang et al. 2015b), and three alkaloids (**151–153**) when cultivated in a rotary liquid medium (Wang et al. 2015c), when in static rice medium only phenethyl 5-hydroxy-4-oxohexanoate (**154**) was traced (Li et al. 2017c), and among this metabolites, compounds **151–154** possessed in vitro cytotoxicity against cancer cell lines A549, BGC823, Huh-7, K562, H1975, MCF-7, HL60, U937, Hela, and MOLT-4 with IC_{50} values in range of 0.24–45 μM. In addition, compounds **151** and **153** displayed acetylcholine esterase (AchE) inhibitory activity with IC_{50} values of 47 and 0.81 μM, respectively. Strain *Aspergillus versicolor* ZLN-60 could generate four new prenylated diphenyl (**155–158**) in static liquid condition (Gao et al. 2013), with four other novel cyclic peptides (**159–163**) detected in solid medium (Peng et al. 2014). Biological tests indicated that compound **157** displayed moderate cytotoxicity against Hela and K562 cancer cell lines with IC_{50} values of 31.5 and 48.9 μM, respectively. Chemical study of one marine-derived fungus *Aspergillus terreus* cultivated in 11 different culture conditions indicated that static agar was ideal for the accumulation of antifungal lovastatins (**164–165**) and 7-desmethylcitreoviridin (**166**), which were absent in the shaking fermentation (Adpressa and Loesgen 2016) (Fig. 18.7).

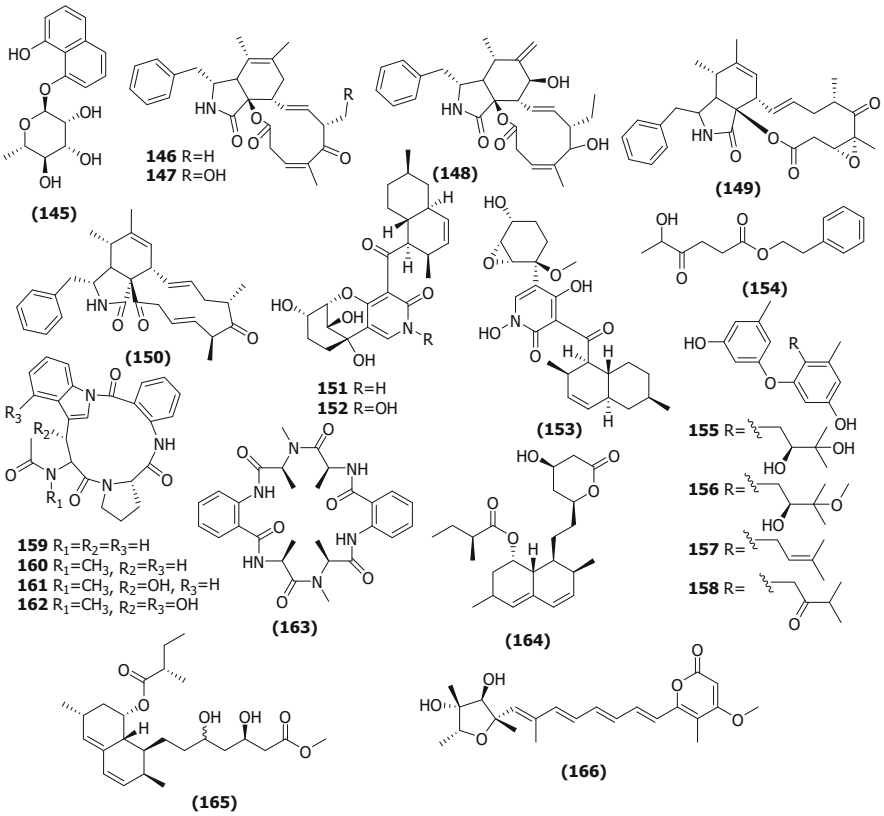

Fig. 18.7 Second metabolites obtained from fungi by change of cultivation conditions (**145**–**166**)

One fungal strain *Myxotrichum* sp. isolated from lichen *Cetraria islandica* was shown to make one novel austdiol analog (**167**), three new fulvic acid derivatives (**168–170**), and a new citromycetin analog (**171**) in rotary PDB medium (Yuan et al. 2013), while four new polyketides (**172–175**) were acquired in rice medium under static fermentation status. And compound **174** was shown to restrain *Arabidopsis* seeds root markedly with the inhibition rate of 75.9% at 8 μg/mL (Yuan et al. 2016). Chemical investigation of one symbiotic strain *Nodulisporium* sp. (No. 65-12-7-1) from the lichen *Everniastrum* sp. resulted in the isolation of two rarely 4-methyl-progesteroids (**176–177**) when grown in rice medium (Zheng et al. 2013), and this strain biosynthesized 10 novel nodulisporisteroids (**178–187**) in shaking PDB medium (Zhao et al. 2015). Two new prenyl phenols (**188–189**), one indole alkaloid echinuline (**190**) and one anthraquinone fiscione (**191**), were biosynthesized by *Lentinus strigellus* under static condition, while this strain could produce benzopyrans (**192–195**) together with panepoxydone (**196**) and isopanepoxydone (**197**) in shaking fermentation broth (Barros-Filho et al. 2012; Zheng et al. 2009).

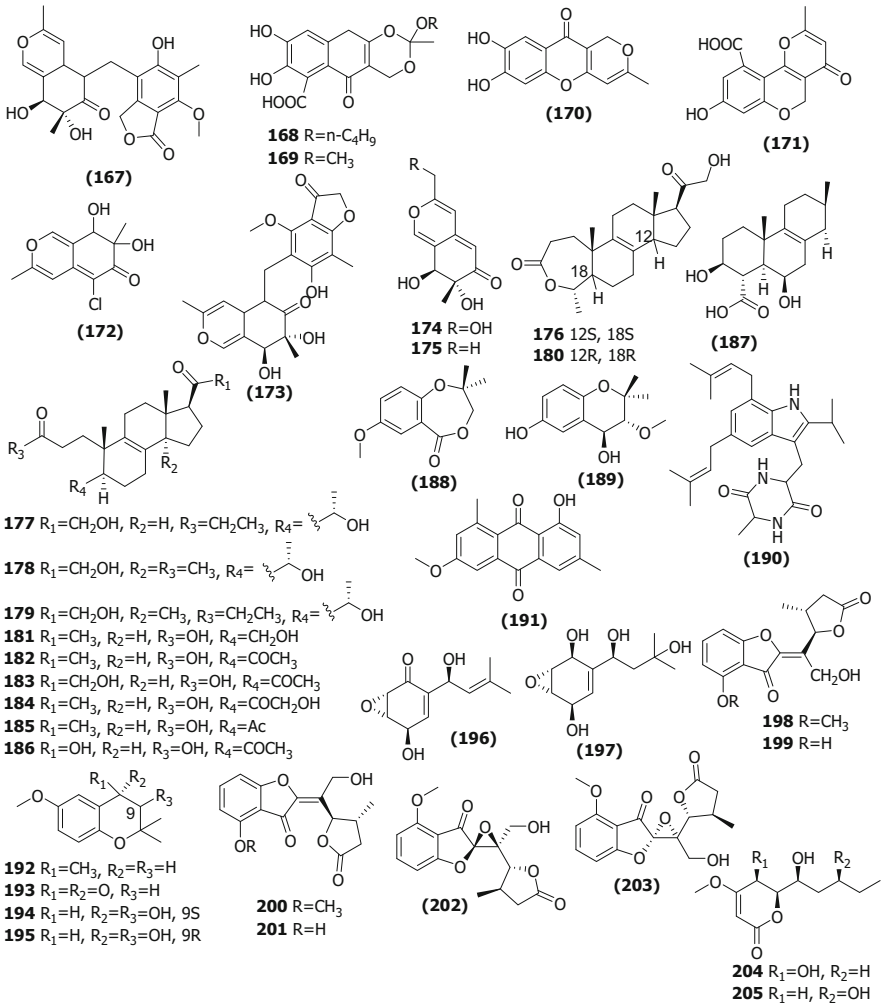

Fig. 18.8 Second metabolites obtained from fungi by change of cultivation conditions (**167**–**205**)

A fungal strain *Paraphaeosphaeria photiniae*, inhabiting *Roystonea regia* collected from Jianfeng Mountain (China), was shown to yield six new unique benzofuranone-derived γ-lactones (**198–203**) when cultivated in shaking liquid medium (Ding et al. 2009), while in rice medium, two different δ-lactone derivatives (**204–205**) were detected (Ding et al. 2012) (Fig. 18.8).

When grown on solid PDA medium, one mangrove-derived fungus *Penicillium brocae* MA-231 could yield six new disulfide-bridged diketopiperazine derivatives (**206**–**211**), and bioassay results showed that compounds **206**, **207**, **210,** and **211** had cytotoxic activities against Du145, Hela, HepG2, MCF-7, NCI-H460, SGC-7901,

SW1990, SW480, and U251 tumor cell lines with IC_{50} values ranging from 0.89 to 9.0 μM (Meng et al. 2014). Five new penicibrocazines (**212–216**), four new thiodiketopiperazine alkaloids (**217–220**), and two new *N*-containing *p*-hydroxyphenopyrrozin derivatives (**221–222**) were detected when this strain was cultivated in liquid media (PDB or Czapek medium), in which compounds **212–214** displayed antimicrobial activities against *Staphylococcus aureus* with MIC values of 32.0, 0.25, and 8.0 μg/mL and compounds **214–216** exhibited potent antimicrobial effect on *Gaeumannomyces graminis* with MIC values of 0.25, 8.0, and 0.25 μg/mL, respectively. Bioactivity test showed that compound **221** had powerful inhibitory effect on *Fusarium oxysporum* and *Staphylococcus aureus* (Meng et al. 2015b, 2017).

Chemical investigation of one marine sponge-derived strain *Penicillium adametzioides* AS-53 yielded two new bisthiodiketopiperazine derivatives (**223–224**) from shaking PDB broth, whereas two new acorane sesquiterpenes (**225–226**) were isolated in its static rice medium, in which compound **223** showed strong lethality against brine shrimp with an LD_{50} value of 4.8 μM and a broad spectrum of antimicrobial effect (Liu et al. 2015). Six novel azaphilone derivatives (**227–232**) as major SMs were obtained from rotary PDB medium of one marine-derived strain *Penicillium commune* QSD-17, whereas other new compounds isophomenone (**233**) and 3-deacetylcitreohybridonol (**234**) were detected in its static rice medium. Compounds **230–232** showed cytotoxic activity against human pancreatic tumor cell line SW1990 (Gao et al. 2011, 2012) (Fig. 18.9).

Three novel penipanoids (**235–237**) were characterized from one marine-derived strain *Penicillium paneum* SD-44 grown in static rice medium (Li et al. 2011). The exploration of changing fermentation conditions of strain SD-44 to a seawater-based culture broth under dynamic fermentation condition gave five new anthranilic acid derivatives (**238–242**), in which compounds **238** and **242** exhibited inhibitory activity toward human colon cancer RKO cell lines with IC_{50} values of 8.4 and 9.7 μM, respectively (Li et al. 2013). One deep sea-derived fungus *Penicillium* sp. F23-2 biosynthesized terpenoids, diketopiperazines, and meleagrin alkaloids when incubated in sea-water-based culture medium under static condition (Du et al. 2009, 2010), whereas five new nitrogen-containing sorbicillinoids (**243–247**) were metabolized when incubated in PYG (peptone yeast glucose) medium under shaking status (Guo et al. 2013). Strain *Penicillium alliisativi* conducted on the oat static fermentation could biosynthesize 9 new andrastones (**248–256**) (Xie et al. 2021).

When grown in rice medium, one endophytic strain of *Pestalotiopsis fici* from *Camellia sinensis* was found to be a prolific producer of bioactive SMs, including pupukeanane chloride (**257**) (Liu et al. 2008a), chloropestolide A (**258**) (Liu et al. 2009a), seven isoprenylated chromones (**259–265**) (Liu et al. 2010), three highly functionalized compounds (**266–268**) (Liu et al. 2009b), and three cytotoxic pupukeanane chlorides (**269–271**) (Liu et al. 2011). Among these metabolites, compound **258** possessed potent inhibitory effects on HeLa and HT29 cells with GI_{50} values of 0.7 and 4.2 μM, respectively, by in vitro cytotoxic assays. Besides, five new cyclopropane derivatives (**272–276**) were obtained when this strain

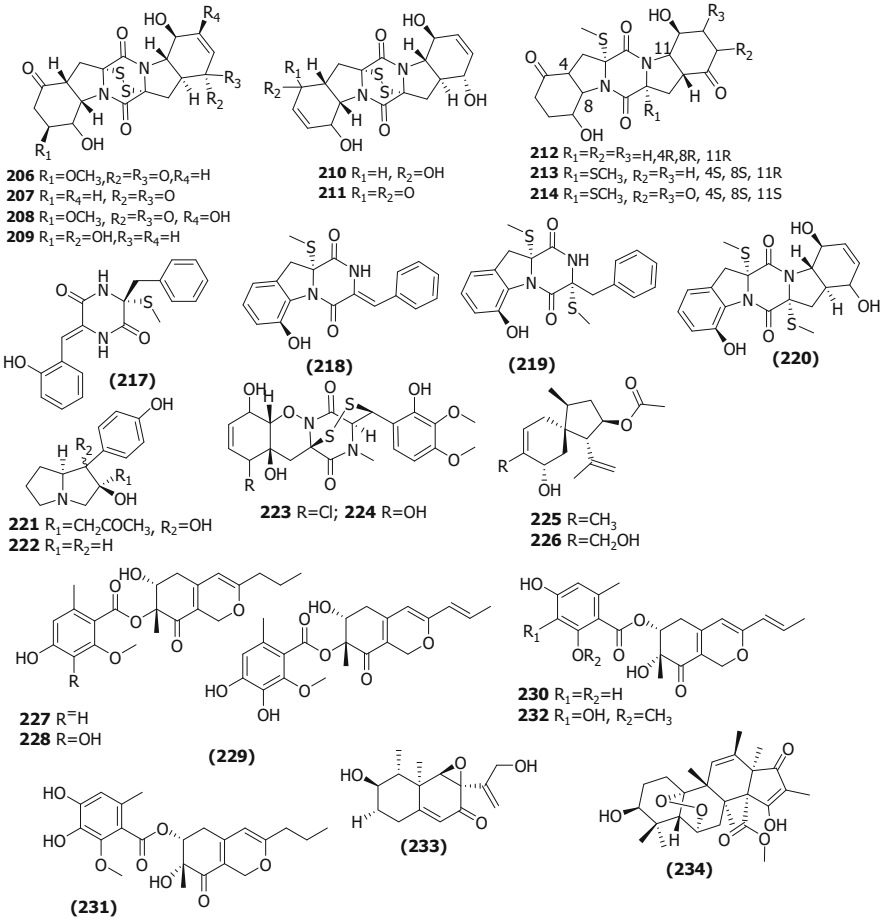

Fig. 18.9 Second metabolites obtained from fungi by change of cultivation conditions (**206**–**234**)

cultivated in shaking liquid medium (Liu et al. 2008b). An endophytic fungus *Pestalotiopsis foedan*, residing in *Bruguiera sexangul*, synthesized a new reduced spiro azaphilone derivative (**277**) together with two new isobenzofuranones (**278**–**279**) in solid GYM medium (Ding et al. 2008), and a pair of novel spiro-γ-lactone enantiomers (**280**–**281**) were identified in liquid-modified PDB medium (Yang and Li 2013) (Fig. 18.10).

An endophytic fungus *Phomopsis* sp. sh917 isolated from fresh stems of *Isodon eriocalyx var. laxiflora* collected in Kunming Botanical Garden of China yielded six new polyketides (**282**–**287**) on solid rice medium, but metabolized a new polyketide (**288**) in shaking liquid FM4 medium (Tang et al. 2017). Nine new cytochalasins Z7-Z15 (**289**–**297**), one novel spicochalasin (**298**), five new aspochalasins (**299**–**303**), and three new aspochalasin derivatives (**304**–**306**) were synthesized by

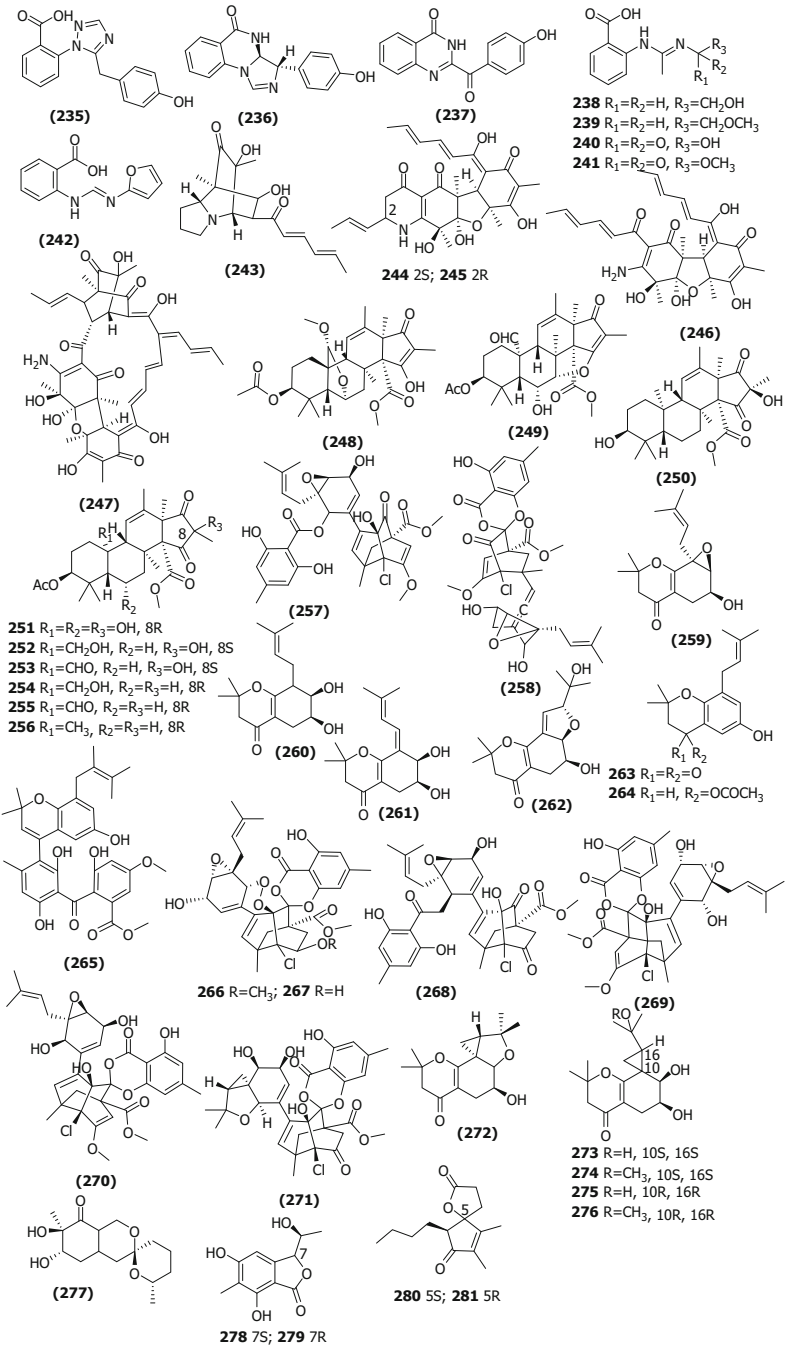

Fig. 18.10 Second metabolites obtained from fungi by change of cultivation conditions (**235**–**281**)

a marine-derived fungus *Spicaria elegans* KLA03 in the seawater-based medium under static fermentation status, while a new aromatic polyketide (**307**) was obtained by this strain under shaking seawater medium, and compounds **289** and **290** displayed strong cytotoxicity against P388 and A-549 cancer cell lines with IC_{50} values in range of 8.4–99 μM (Lin et al. 2009b, 2010; Liu et al. 2005, 2006, 2008c; Luan et al. 2014). Two antifungal polyketides (**308–309**) were characterized from rice medium of *Ulocladium* sp. that was isolated from the lichen *Everniastrum* sp. (Wang et al. 2012b), whereas three new tricycloalternarenes F-H (**310–312**) and five ophiobolane sesterterpenes (**313–317**) were detected in liquid Czapek or PDB medium. Biological assays indicated that compounds **313** and **317** exhibited moderate antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis* and displayed strong in vitro cytotoxicity against cancer cell lines KB and HepG2 (Wang et al. 2013a, b) (Fig. 18.11).

4 Cocultivation with Other Strain(s)

In one culture medium, the relationship between one strain and other(s) may be competitive, antagonistic, or friendly. Cocultivation of two or more strains usually has positive effect of an enhanced production of known compounds or an accumulation of cryptic compounds that are not detected in common condition (Bohni et al. 2013; Marmann et al. 2014). This effect may result from the production of enzymes that activate metabolite precursors or that other strain(s) may induce epigenetic modifications of the producer strain. In fungi, there are two methods: cocultivation of fungus and other fungal strain and fungus and other microorganism. Forty nine new SMs (**318–366**) detected by fungus cocultivation with other strains were summarized in this part.

4.1 Fungus and Other Fungal Strain

When cocultivated with *Mycogone rosea* DSM 12973, an endophytic strain *Acremonium* sp. Tbp-5 from the European yew (*Taxus baccata* L.) could yield three new lipoaminopeptides (**318–320**) (Degenkolb et al. 2002). Chemical investigation of the mixed fermentation broth of two epiphytic strains *Aspergillus* sp. FSY-01 and FSW-02 from marine mangrove *Avicennia marina* led to the isolation of a novel alkaloid (**321**), which had antibacterial activity against *Bacillus dysenteriae*, *Bacillus proteus*, and *E. coli* (Zhu et al. 2011). The production of three 2-alkenyl-tetrahydropyran analogs (**322–324**) was provoked by *Chaunopycnis* sp. CMB-MF028 in the mixed culture with a partner strain *Trichoderma hamatum* CMB-MF030 (Shang et al. 2017). One marine-derived *Penicillium citrinum* could biosynthesize two new compounds (**325–326**) featuring in a unique tetracyclic framework when cocultivated with *Beauveria felina*, whereas neither strain could

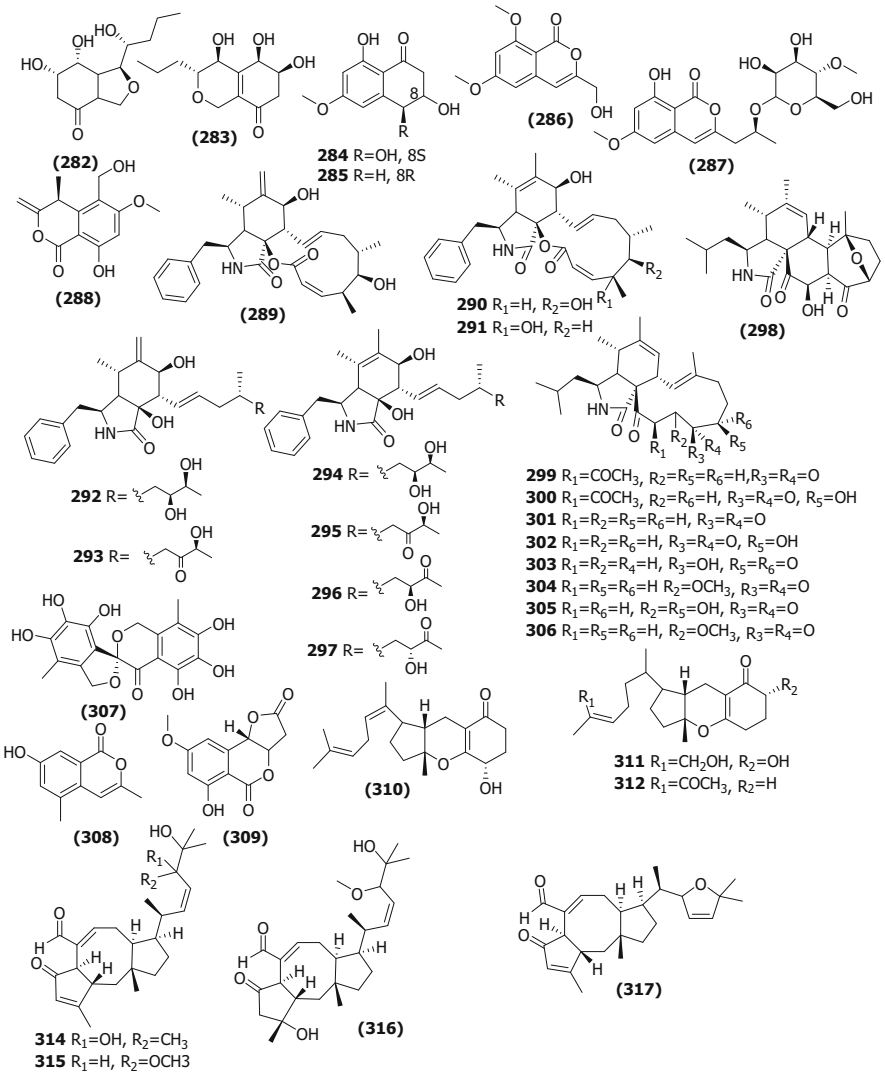

Fig. 18.11 Second metabolites obtained from fungi by change of cultivation conditions (**282**–**317**)

produce these compounds in axenic medium and antimicrobial assay showed that compounds **325** and **326** had strong inhibitory effects on *S. aureus* and *E. coli* (Meng et al. 2015a). Four new polyketides (**327–330**) were detected in a dual culture of a deep-sea-derived fungus *Talaromyces aculeatus* and a mangrove-derived fungus *Penicillium variabile*, while these compounds were not identified in single culture and compounds **330** displayed strong cytotoxicity against human cancer cell lines such as A549, K562, HCT-116, HeLa, MCF-7, and HL-60 with IC_{50} values ranging from 1.2 to 9.8 μM (Zhang et al. 2017b) (Fig. 18.12).

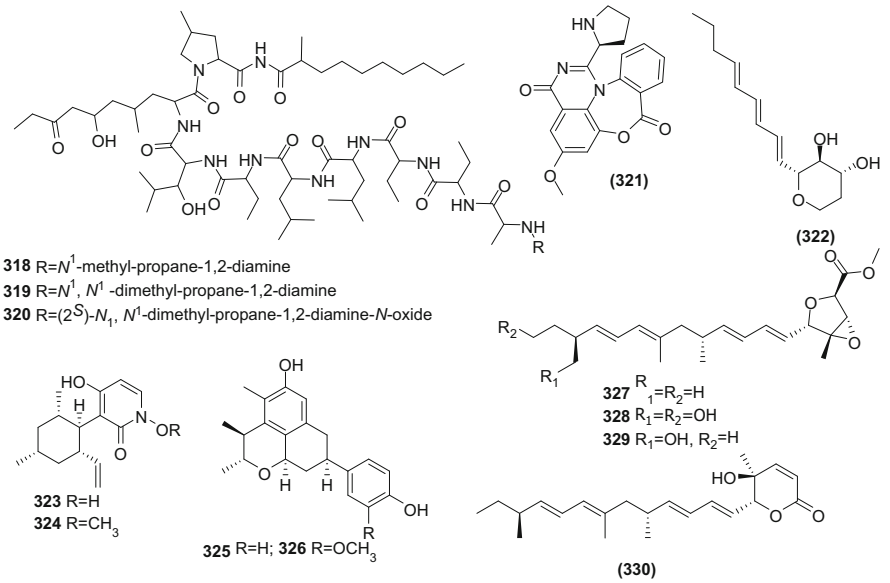

Fig. 18.12 Second metabolites obtained from co-cultivation of fungi with other fungi (**318**–**330**)

4.2 Fungus and Other Microorganism

Cocultivation of one fungal strain *Aspergillus terreus* with bacteria *Bacillus cereus* and *B. subtilis* resulted in the yield of two novel butyrolactones (**331–332**), which were absent in single culture medium (Chen et al. 2015). An endophyte *Chaetomium* sp. from *Sapium Ellipticum* (Euphorbiaceae) metabolized two novel shikimic acid analogs (**333–334**) and four new butenolide derivatives (**335–338**) when cocultivated with *Pseudomonas aeruginosa*, while none of these chemicals was traced in axenic medium (Ancheeva et al. 2017). Strain *Bacillus subtilis* 168 trpC2 was shown to greatly activate the biosynthesis of three novel chemicals (**339–341**) of fungal endophyte *Fusarium tricinctum* during coculture process and these compounds were not duplicated in axenic fungal culture (Ola et al. 2013).

A new pyridone alkaloid (**342**) was isolated from the mixed culture extract of *Paecilomyces lilacinus* and *Salmonella typhimurium*, which had 57.5 ± 5.50% of AChE inhibition (Teles and Takahashi 2013). Coculture of an endophyte *Pestalotiopsis* sp. from *Drepanocarpus lunatus* with *B. subtilis* was found to biosynthesize two novel sesquiterpenoids (**343–344**), while two new compounds (**344–346**) emerged in axenic culture (Liu et al. 2017). The mixed cultivation of *Trichoderma* sp. 307 colonizing in *Clerodendrum inerme* and one bacterium *Acinetobacter johnsonii* B2 detected two new sesquiterpenes (**347–348**) and three novel de-*O*-methyl lasiodiplodins (**349–351**) in which compounds **349** and **350** displayed potent α-glucosidase inhibitory effect with IC_{50} values of 25.8 and

54.6 μM, respectively (Zhang et al. 2017a). Mixed fermentation of *Aspergillus austroafricanus* with *B. subtilis* or *Streptomyces lividans* afforded several diphenyl ethers, including one new austramide (**352**) (Ebrahim et al. 2016). Two novel *N*-formyl alkaloids (**353–354**) were characterized from a mixed fermentation of *Aspergillus fumigatus* and *Streptomyces peucetius*, and compound **354** exhibited in vitro cytotoxic effect on cancer cell line NCI-60 with an IC_{50} value of 1.12 μM (Zuck et al. 2011). Cocultivation of *Aspergillus fumigatus* MR2012 with *Streptomyces leeuwenhoekii* C34 in ISP2 medium resulted in the yield of a new luteoride derivative (**355**) and a new pseurotin derivative (**356**) with none of these compounds could be detected in axenic culture (Elsard et al. 2015; Wakefield et al. 2017) (Fig. 18.13).

Physical interaction of *Aspergillus nidulans* RMS011 with *Streptomyces hygroscopicus* was found to trigger biosynthesis of four new aromatic polyketides (**357–360**), which were absent in the axenic medium (Schroeckh et al. 2009). Biosynthesis of a new hexadienedioxic acid (**361**) and a new phenol derivative (**362**) was achieved by coculture of *Streptomyces coelicolor* A3(2) M145 and strain *Aspergillus niger* N402 (Wu et al. 2015). Cocultivation of one marine-derived fungus *Emericella* sp. CNL-878 with *Salinispora arenicola* CNH-665 resulted in the higher yields of two novel antimicrobial cyclic depsipeptides (**363–364**) than axenic culture (Oh et al. 2007). Two new compounds, ochraspergillic acids (**365–366**), which are both adducts of dihydropenicillic acid and *o*- or *p*-aminobenzoic acid, were characterized from the coculture of the fungus *Aspergillus ochraceus* with *Bacillus subtilis* (Frank et al. 2019).

5 Epigenetic Modifier(s)

Epigenetic modifiers are those chemicals that are able to change microbial characteristics in correspondence to alteration of their epigenetic status, such as DNA methyltransferase (DNMT) inhibitor and histone deacetylase (HDAC) inhibitor. The addition of these modifiers usually suppresses the activity of related enzymes in the biosynthetic pathway and promotes the progress of other metabolic pathways (Seyedsayamdost 2014). 88 SMs (**367**–**454**) from fungi obtained by adding epigenetic modifier(s) were mentioned in this part.

5.1 DNMT Inhibitor

When located in a gene promoter, DNA methylation typically acts to repress gene transcription and causes chromatin structure changes in the corresponding regions, preventing the binding of specific transcription factors and suppressing gene expression (Araujo et al. 2001). 5-Azacytidine (5-Aza) is the most common DNMT inhibitor used to modify the function of microbe DNA followed by repressing

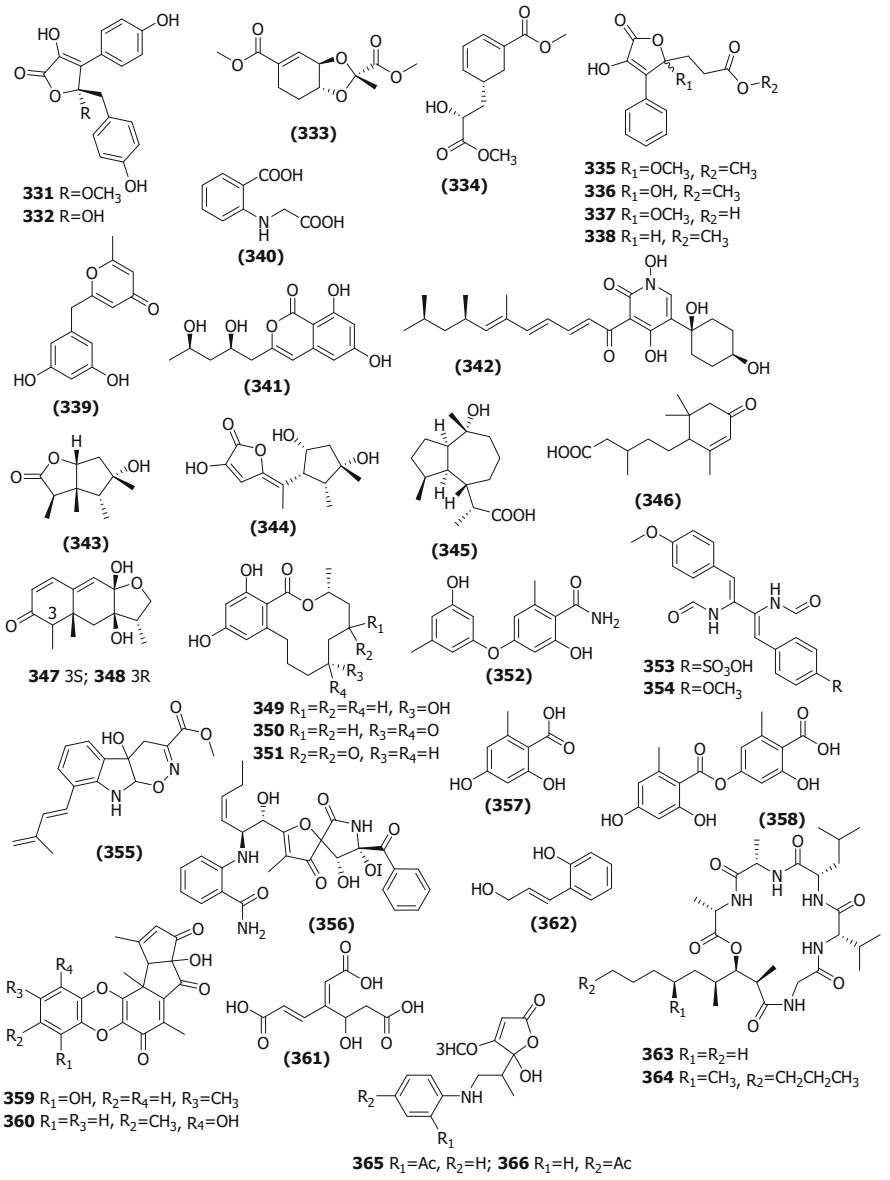

Fig. 18.13 Second metabolites obtained from co-cultivation of fungi with other microorganisms (**331**–**366**)

gene transcription. Chemical investigation of a marine-derived fungus *Aspergillus sydowii* afforded three novel bisabolane-type sesquiterpenoids (**367–369**) when its culture medium was supplied with 5-Aza (Chung et al. 2013b). An

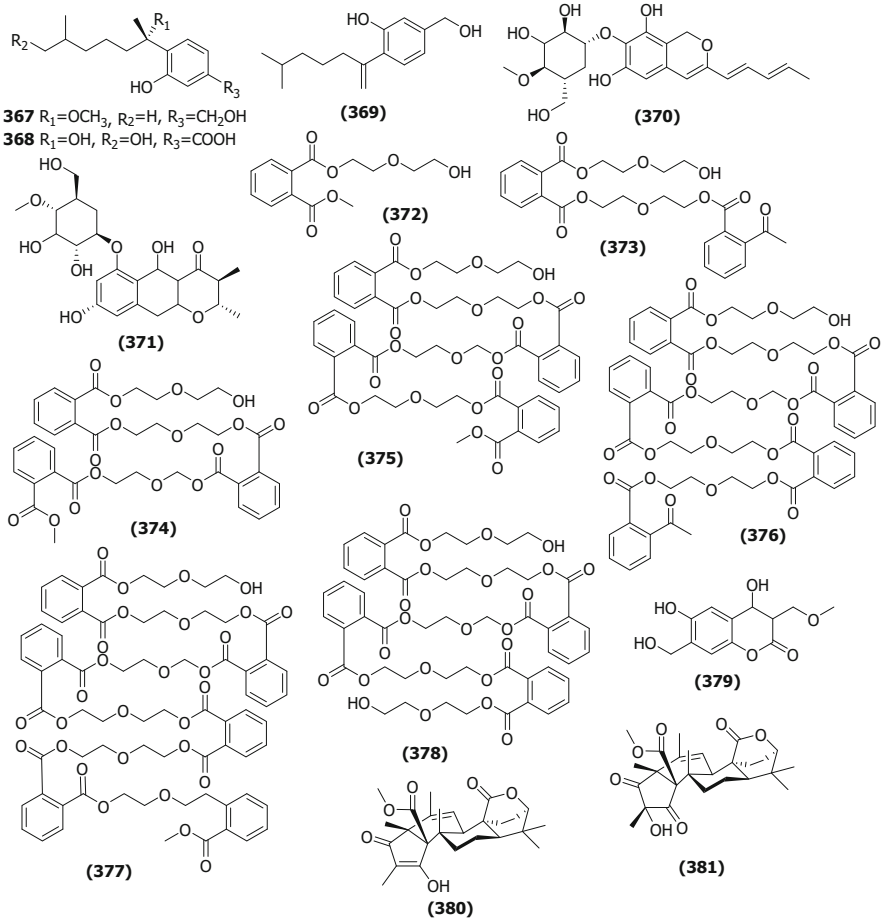

Fig. 18.14 Second metabolites obtained from fungi using DNMT inhibitors as Epigenetic Modifier (s) (**367**–**381**)

entomopathogenic fungus *Cordyceps indigotica* yielded a novel aromatic polyketide glycoside (**370**) when cultivated in PDB media, while the strain produced another unusual glycoside (**371**) when 5-Aza was added in the medium (Asai et al. 2012e). Several other examples that supplement 5-Aza as epigenetic modifier in culture medium could lead to the discovery of new metabolites as were also reported, such as seven novel diethylene glycol phthalate esters (**372–378**) from a marine-derived strain *Cochliobolus lunatus* TA26–46 (Chen et al. 2016), one new coumarin (**379**) from *Pestalotiopsis crassiuscula* NBRC 31055 associated with *Fragaria chiloensis* (Yang et al. 2014), and two novel meroterpenes (**380–381**) from *Penicillium citreonigrum* (Wang et al. 2010) (Fig. 18.14).

5.2 HDAC Inhibitor

The acetylation or deacetylation of histone affects its binding to DNA in microbe, with many chemical modifications in the tail of histone that regulate the gene expression. The introduction of hydrophobic acetyl group into the *N*-terminal lysine residues of histone could increase the electrostatic attraction and steric hindrance between histone and DNA, which is conducive to facilitate the depolymerization of DNA and the binding of transcription factors (Cole 2008; Fukuda et al. 2006). Suberoyl bishydroxamic acid (SBHA), suberoylanilide hydroxamic acid (SAHA), and nicotinamide are the most common HDAC inhibitors, with several other chemicals such as valproic acid and phenylbutyrate (Moore et al. 2012).

Many reports suggested that the presence of SAHA in culture medium could result in the biosynthesis of new SMs, such as a novel metabolite nygerone A (**382**) from a soildwelling fungus *Aspergillus niger* ATCC 1015 (Henrikson et al. 2009), three novel cyclodepsipeptides (**383–385**) from *Beauveria feline* (Chung et al. 2013a), one novel chlorinated polyketide (**386**) from *Daldinia* sp. (Du et al. 2014), and a new cyclodepsipeptide of hybrid EGM-556 (**387**) from a marine sediment-derived fungus *Microascus* sp. (Vervoort et al. 2011).

In SBHA-treated culture medium, *Chaetomium indicum* could characterized two novel spironolactone polyketides (**388–389**) and six novel prenylated aromatic polyketides (**390–395**) (Asai et al. 2013b, c). Similarly, when exposed to SBHA, four new 2,3-dihydrobenzofurans (**396–399**) and a new aromatic polyketide (**400**) were detected from an entomopathogenic fungus *Cordyceps annullata* (Asai et al. 2012c), six new aromatic polyketides (**401–406**) were synthesized by *Cordyceps indigotica* (Asai et al. 2012f), two new fusaric acid derivatives (**407–408**) were produced by *Fusarium oxysporum* associated with medicinal plant *Datura stramonium* L. (Chen et al. 2013), and three novel prenylated tryptophan analogs (**409–411**) were metabolized by an entomopathogenic fungus *Torrubiella luteorostrata* (Asai et al. 2011) (Fig. 18.15).

Supplement of nicotinamide in culture medium of *Chaetomium cancroideum* could generate three novel polyketides (**412–414**) (Asai et al. 2016). Strains *Eupenicillium* sp. LG41 and *Graphiopsis chlorocephala* exposed in this inhibitor had the similar effect, the former supplied two new decalin-containing compounds (**415–416**) (Li et al. 2017a) and the later afforded a serious of new benzophenones (**417–418**) and diverse new C^{13}-polyketides (**419–427**) (Asai et al. 2012d, 2013a). Adding sodium valproate into potato dextrose medium causing *Macrophomina phaseolina* metabolized 3-acetyl-3-methyldihydro-furan-2(3H)-one (**428**) (Singh et al. 2022) (Fig. 18.16).

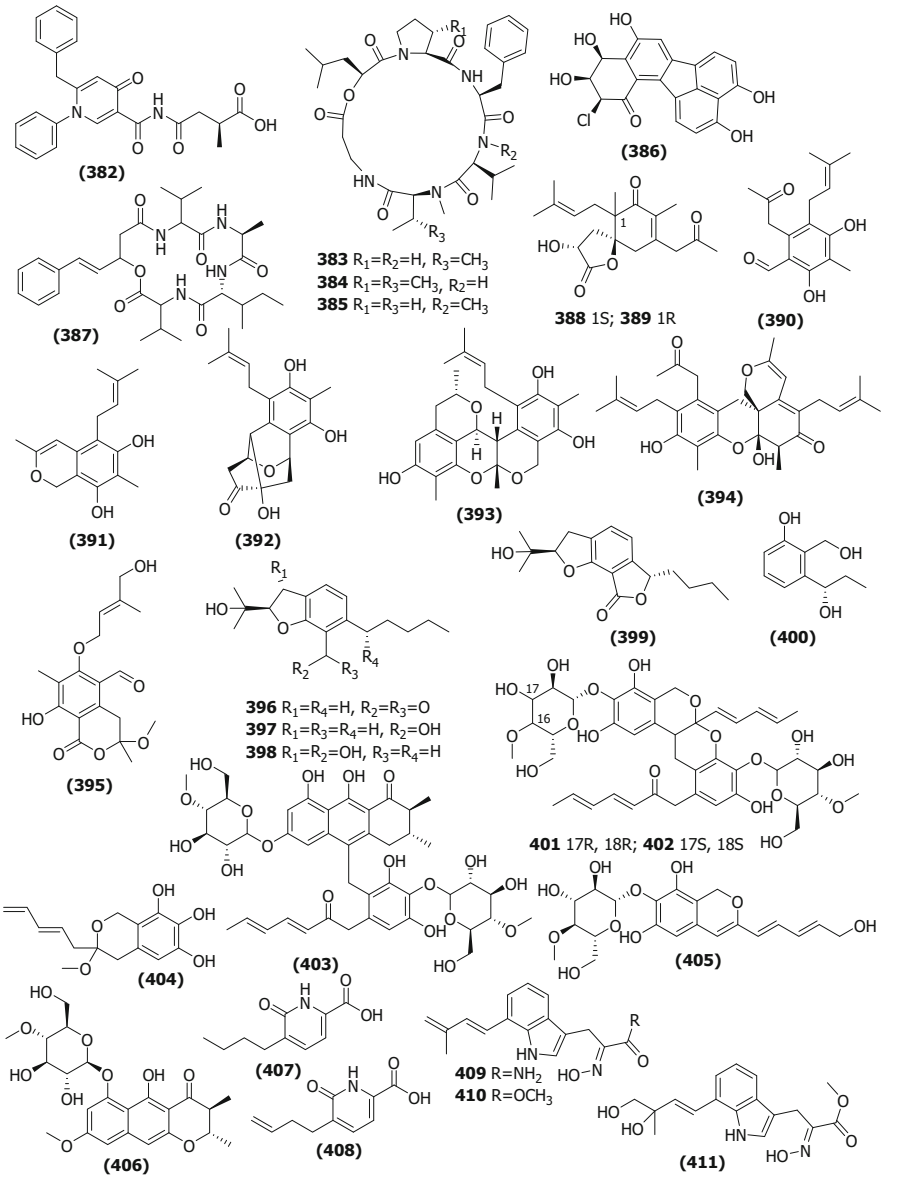

Fig. 18.15 Second metabolites obtained from fungi using HDAC inhibitors as Epigenetic Modifier (s) (**382**–**411**)

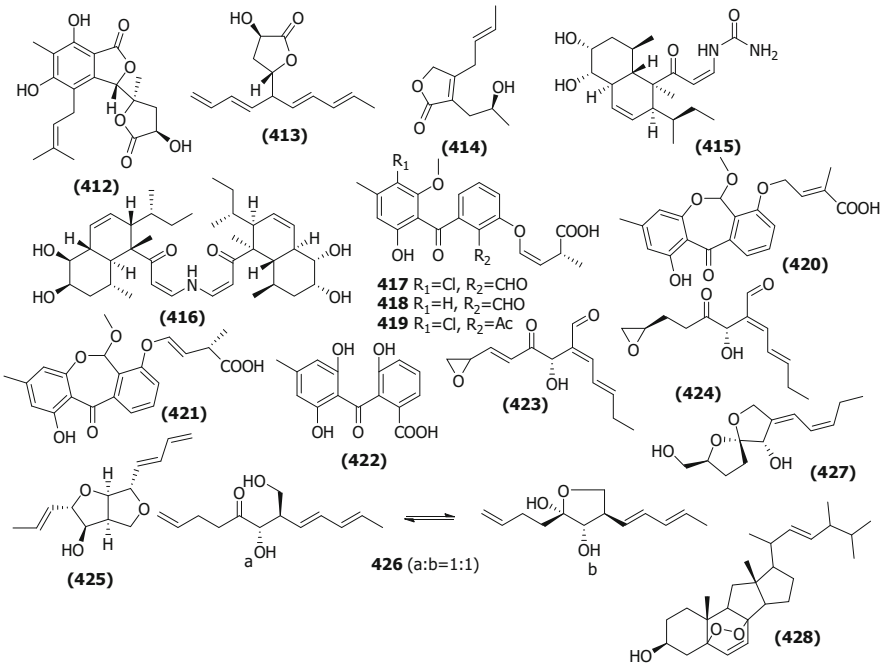

Fig. 18.16 Second metabolites obtained from fungi using HDAC inhibitors as Epigenetic Modifier (s) (**412**–**428**)

5.3 Multiple Chemical Epigenetic Modifiers

Interactions between epigenetic features play an important role in regulation of gene expressing or silencing in microorganisms, and these chemicals could regulate the activity of genomic regions of varying sizes, from single genes to entire domains and chromosomes. Epigenetic markers could also interact with other nuclear proteins to work together to form chromatin structures and to create genomic functional discrete regions that induce the production of new SMs (Tammen et al. 2013).

One symbiotic strain *Alternaria* sp. from medicinal plant *Datura stramonium Linn.* generated four new aromatic polyketides (**429**–**432**) and a new tenuazonic acid (**433**) when incubated in medium containing 5-Aza and/or SBHA, which were absent in normal culture medium, with the yield of these SMs being higher in the medium of adding HDAC and DNMT inhibitors than that of addition of any other inhibitors (Sun et al. 2012). Chemical investigation of one marine-derived fungus *Aspergillus* sp. SCSIOW2 exposed with an integration of SBHA and 5-Aza led to the isolation of three new eremophilane-type sesquiterpenes (**434**–**436**) together with a new diphenylether-*O*-glycoside (**437**) (Li et al. 2017b; Wang et al. 2016a). Exposure of one strain *Cladosporium cladosporioides* to 5-Aza resulted in

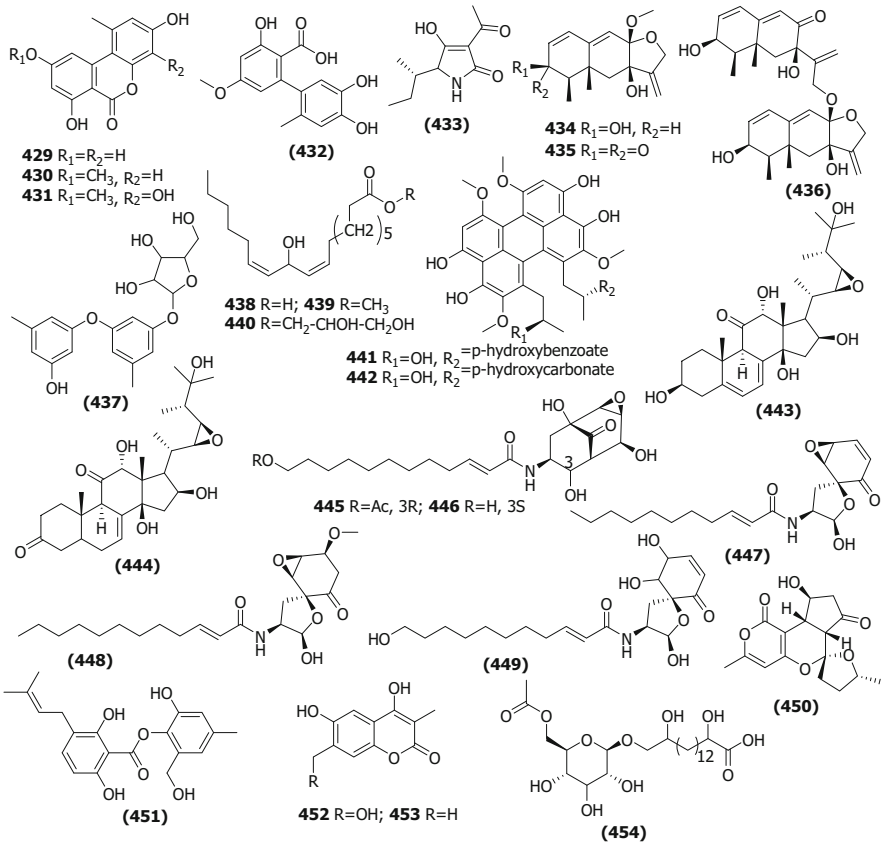

Fig. 18.17 Second metabolites obtained from fungi using multiple chemical Epigenetic Modifier (s) (**429**–**454**)

substantially increased biosynthesis of three oxylipins (**438**–**440**), whereas SBHA induced the yield of two new perylenequinones (**441**–**442**) (Williams et al. 2008).

Concomitant supplement of SBHA and *N*-phthalyl-*L*-tryptophan to the fermentation medium of an entomopathogenic fungus *Gibellula formosana* induced the formation of two new highly oxidized ergosterols (**443**–**444**) and five new isariotin analogs (**445**–**449**) (Asai et al. 2012a). The same method was applied to *Isaria tenuipes*, which resulted in the yield of one new polyketide (**450**) (Asai et al. 2012b). Three novel aromatics (**451**–**453**) were detected in the culture medium of *Pestalotiopsis acacia* from *Taxus brevifolia* which was supplied with SBHA and 5-Aza (Yang and Li 2013). Application of this approach also stimulated strain *Ustilago maydis* to produce a new glycolipid ustilagic acid C (**454**) (Yang et al. 2013) (Fig. 18.17).

6 Other Factors

6.1 Enzyme Inhibitor

Beside DNMT and HDAC, other microbial enzymes also played important roles in regulating the biosynthesis of SMs, such as monooxygenase and hydroxylase. Some chemicals can selectively inhibit the activities of these enzymes in the biosynthetic pathway and promote the progress of other metabolic pathways, such as metyrapone, tricyclazole, jasplakinolide, and DMSO.

Chemical study of *Chaetomium subaffine* in the presence of metyrapone (an inhibitor of cytochrome P450) led to purification of five new polyketides (**455–459**) and two new less oxidized analogs (**460–461**) (Oikawa et al. 1992). A soil-derived strain *Phoma* sp. SNF-1778 was shown to yield a new cytochalasin (**462**) when inoculated with metyrapone (Kakeya et al. 1997). When added with the F-actin inhibitor jasplakinolide in culture medium, one marine sponge-derived fungus *Phomopsis asparagi* could afford three unusual cytotoxic compounds, chaetoglobosin-510 (**463**), chaetoglobosin-540 (**464**), and chaetoglobosin-542 (**465**) (Christian et al. 2005). Two novel bisnaphthalene compounds (**466–467**) were characterized from *Sphaeropsidales* sp. F-24′707 cultured with tricyclazole, which was shown to inhibit the regular biosynthesis of 1,8-dihydroxynaphthalene (Bode and Zeeck 2000). Continuous study showed that metyrapone supplementation in the culture of *Spicaria elegans* led to the isolation of two novel 7-deoxy-cytochalasins (**468–469**) (Lin et al. 2009a). One marine-derived strain *Trichoderma* cf. *brevicompactum* elicited an unprecedented epidiketopiperazine (**470**), which has a trisulfide bond between the α-β positions of two amino acid residues, by adding DMSO to its natural seawater medium (Yamazaki et al. 2015b).

6.2 Biosynthetic Precursor

Biosynthetic precursor refers to one chemical that is apt to be directly incorporated into the final product. Adding various biosynthetic precursors in the fermentation medium may change biosynthesis pathways of SMs (Ramm et al. 2017).

An endophytic fungus *Penicillium crustosum* treated with ferulic acid and quinic acid or cinnamic acid and 3,4-(methylenedioxy) cinnamic acid could generate a mycophenolic acid (**471**) and 5-hydroxy-7-methoxy-4-methylphthalide (**472**) (Valente et al. 2013). Three novel cytochalasins Z_{21}–Z_{23} (**473–475**) were isolated from one marine-derived fungus *Chaetomium indicum* KLA03 when cultivated in medium supplied by *L*- and *D*-tryptophan (Wang et al. 2011). *Trichocladium* sp. cultivated on the rice medium adding 2% tryptophan yielded a new bismacrolactone (**476**) (Tran-Cong et al. 2019) (Fig. 18.18).

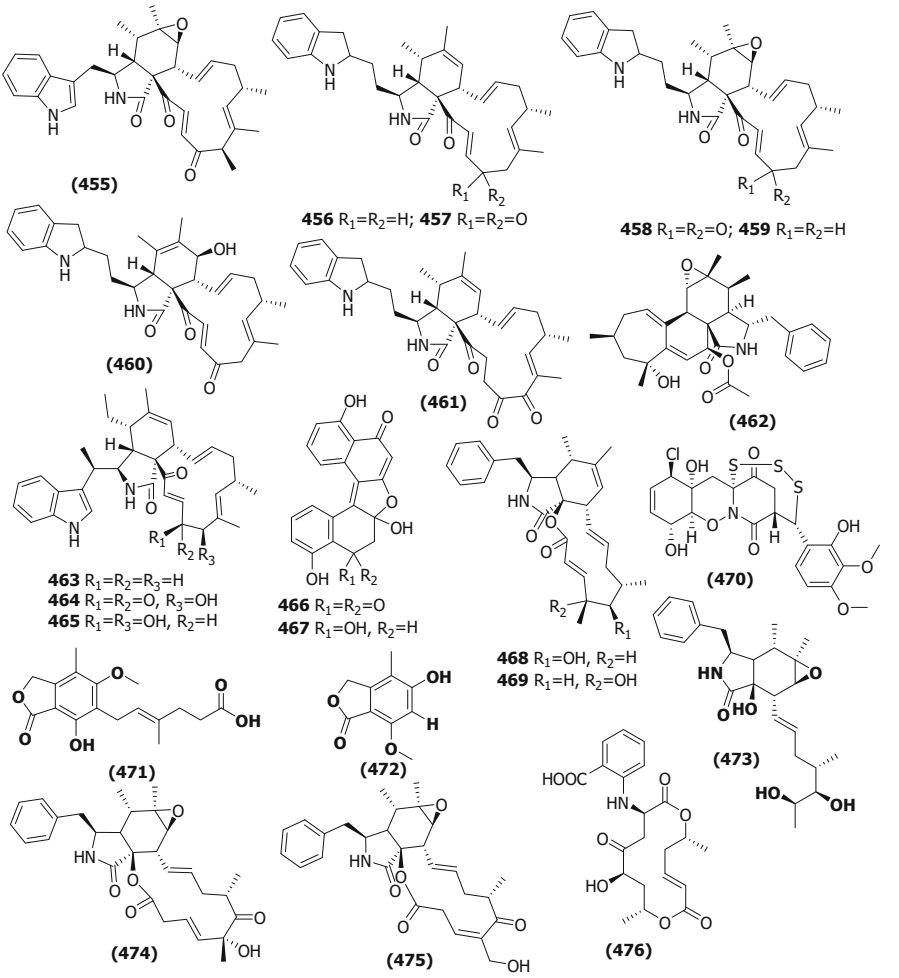

Fig. 18.18 Second metabolites obtained from fungi using Enzyme Inhibitor (**455**–**476**)

7 Conclusions

In summary, chemical diversity of fungal SMs is susceptible to the variation of culture conditions, such as medium composition, temperature, pH, salinity, culture status, axenic or mixed culture, epigenetic modifier, and biosynthetic precursor. Conventional culture method of fungi is limited to the expression of a large number of metabolic pathways that many SMs could not be biologically synthesized. OSMAC strategy can provide a simple, quick, and effective approach for enhancing chemical diversity of SMs to obtain new leading compounds through modification of

culture condition to awaken SM BGC(s). Therefore, OSMAC strategy is an important alternative and extensively used way to enhance chemical diversity of fungal SM. Combination of new approaches with OSMAC strategy to assist in isolating and identifying bioactive SMs with tiny amount is of vital importance for future research, including precision high-throughput screening, microbe genomes mining (Hug et al. 2018), and LC-MS/MS-based molecular networking analysis (Wang et al. 2016b).

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Chapter 19
Epigenetic Regulation of Fungal Secondary Metabolites for the Enhancement of Therapeutically Active Compounds

Shaurya Prakash, Hemlata Kumari, Minakshi, and Antresh Kumar

Abstract The microbial flora has always been a center of interest for the identification of novel bioactive secondary metabolites (SMs) used against a diversity of infections and ailments. These bioactive compounds are produced by the hosts in response to different stress conditions. Novobiocin naturally synthesized in *Streptomyces spheroids*, Echinocandin B from *Emericella rugulosa*, Penicillin from *Penicillium chrysogenum*, and Gentamycin from *Micromonospora purpurea* are such examples. In nature, the secondary metabolites genes, either cryptic or microbial bioactive compounds, synthesize in trace amounts under specific environmental conditions, physiological conditions which make their bioavailability scarcer. Different strategies such as induced stress stimuli, cloning the genes under the control of strong promoters, downregulating the expression of negative regulators, epigenetic manipulations, etc. have been tried to increase SM bioavailability. The posttranslational modifications of histones proteins render to regulate the secondary metabolites and have also been implied. Here, in this chapter, we emphasized the epigenetic regulation for enhancement of the production of therapeutically significant bioactive compounds in fungi. DNA wrapped around the histones proteins undergo different posttranslational modifications that facilitate chromatin DNA packaging around the histones proteins either loose (euchromatin) or tight (heterochromatin). Such alterations in the histones proteins epigenetically modulate the expression of genes that are responsible for regulating different metabolic events. Relevance of different epigenetic approaches such as the use of Histone deacetylase inhibitors (HDACs) or DNA methyl transferase inhibitors (DNMTs) concern different fungal bioactive compounds. These modifications could be intrinsically programmed or affected externally. Different studies have provided supportive arguments about the use of epigenetic modifiers for increasing the bioavailability of different fungal bioactive compounds. These epigenetic modifiers either act locally or globally for inducing or

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repressing the secondary metabolites synthesis genes. Different epigenetic modifiers have been earlier studied for the modulation of secondary metabolite synthesis in fungi. For instance, 5-azacytidine, hydralazine hydrochloride, trichostatin A, trapoxin B, etc. The chapter elaborates on a more developed understanding of histone modifiers for activation/induction of secondary metabolites yield of pharmacologically significant fungal-derived bioactive compounds.

Keywords Epigenetics · Fungi · Therapeutically active secondary metabolites · Histones · Biosynthetic gene clusters

1 Introduction

In recent times of rapid global development, drastic changes in the lifestyles of people are being observed. Other than the perks of developments, a major setback that the entire community is exposed to is a horizon of new diseases and health conditions that are more lethal and advanced than preexisting ones. For instance, mucormycosis, a recent secondary fungal epidemic infection, when traced back for its epidemiology, was found to be linked to diabetes and other predisposing conditions (Prakash and Kumar 2022). Similarly, the occurrence of lifestyle disease, diabetes, is more prominent among the population of developing and developed nations which is turning up into a life-threatening form. However, the existing medications and therapies for such ailments are expensive and not affordable to the common man. Antimicrobial resistance to existing medications is another alarming problem that needs to be addressed by means of sustainable medications/therapeutics. *Staphylococcus aureus* is among the most prominent bacterial pathogens that are very readily acquiring antimicrobial resistance which makes it the need of the hour for the identification and characterization of novel antimicrobials against the same (Kumari et al. 2019). It is an urgent need to develop sustainable, affordable, and cost-effective medicines and therapies against these unseen enemies. Enhancement of yield of existing natural therapeutics is one of the lucrative approaches that could be a better option for providing a stable and sustainable solution. Nature has provided us with a never-ending treasure of resources and one such resource for yielding therapeutically active secondary metabolites compounds is the fungal flora. The trend of harnessing the fungal flora for the isolation of therapeutically relevant biomolecules began with the discovery of penicillin from *Penicillium* molds in 1928 which has proven a remarkable milestone in the discovery of the isolation of therapeutically significant bioactive compounds. Fungi are well known for their ability to produce a multitude of products ranging from antibiotics to toxins and a variety of enzymes useful to mankind and remain to be one of the most important resources for the discovery of unique bioactive molecules. Fungal secondary metabolites have proved to serve as important drugs such as antibacterial; penicillin (**1**) and alamethicin (**2**) isolated from *P. chrysogenum*, and *Trichoderma viridie*,

respectively, anti-hypercholesterolemic lovastatin (**3**), isolated from *Aspergillus terreus*, anti-cancerous taxol (**4**) from *A. fumigatus*, antifungal echinocandin B (**5**) from *A. nidulans*, antimalarial codinaeopsin (**6**) from fungal isolate CR127A, immunosuppressant bredinin (**7**) from *Eupenicillium brefeldianum*, and antifungal such as griseofulvin (**8**) (Fig. 19.1) isolated from *Penicillium griseofulvum* are some such examples. The complex genomic organization of the fungal plethora poses a major challenge to the research community. The attempts to resolve these complexities led to the exploration of the enormous reserves of bioactive compounds which may potentially be of therapeutic significance. The secondary metabolite synthesis genes were identified, characterized, and designated as biosynthetic gene clusters (BGCs).

Most of the fungal-derived bioactive secondary metabolites have been well-identified for the survival and growth of the organism. They also protect their territory from pathogenic invasions or help the hosts to display counter-reaction against adverse growth conditions or environmental stimulus (Kumar and Kumar 2019). The biosynthesis of fungal secondary metabolites is stage-specific and synthesized under certain environmental conditions. It has been documented that most of the secondary metabolite's gene clusters remain silent under normal conditions. The activation of these genes remains an issue under laboratory conditions. Moreover, the biosynthesis of secondary metabolites in trace amounts is also another challenge. Different groups of studies have been employed to overcome these issues and make the bioavailability of secondary metabolites synthesis easier and more abundant. There has been a recent shift in research from synthetic medication to the characterization of BGCs for the identification of bioactive compounds of therapeutic significance. The research regarding BGCs is, however, not easy because these genes are mostly cryptic or silent. Another challenge includes the synthesis of secondary metabolites (SMs) in trace levels, inside the host. Hence, the isolation of SMs becomes an expensive and time-consuming process. To make available SMs, more economical, genomics, proteomics, and metabolomics approaches have been used to activate and upregulate the expression of these BGCs of induction of SMs synthesis (Begani et al. 2018; Cichewicz 2010; Ochi and Hosaka 2013; Scherlach and Hertweck 2009, 2021). OSMAC (one strain many compounds), coculturing, genetic manipulation, induction of global regulators, pathway-specific regulation, reporter mutant selection, promoter engineering, and construction of heterologous expression systems are such examples that have been attempted, but they come up with an array of limitations such as time-consuming, laborious, altered metabolic profiling, lack of selection markers, etc. (Baral et al. 2018; Fan et al. 2017; González-Menéndez et al. 2019; Harvey et al. 2018; Jiang et al. 2021; Pfannenstiel and Keller 2019; Romano et al. 2018; Rutledge and Challis 2015; Toghueo et al. 2020).

The epigenetic regulation approach is considered to be one of the most promising ways to activate and upregulate the biosynthesis of SMs. This

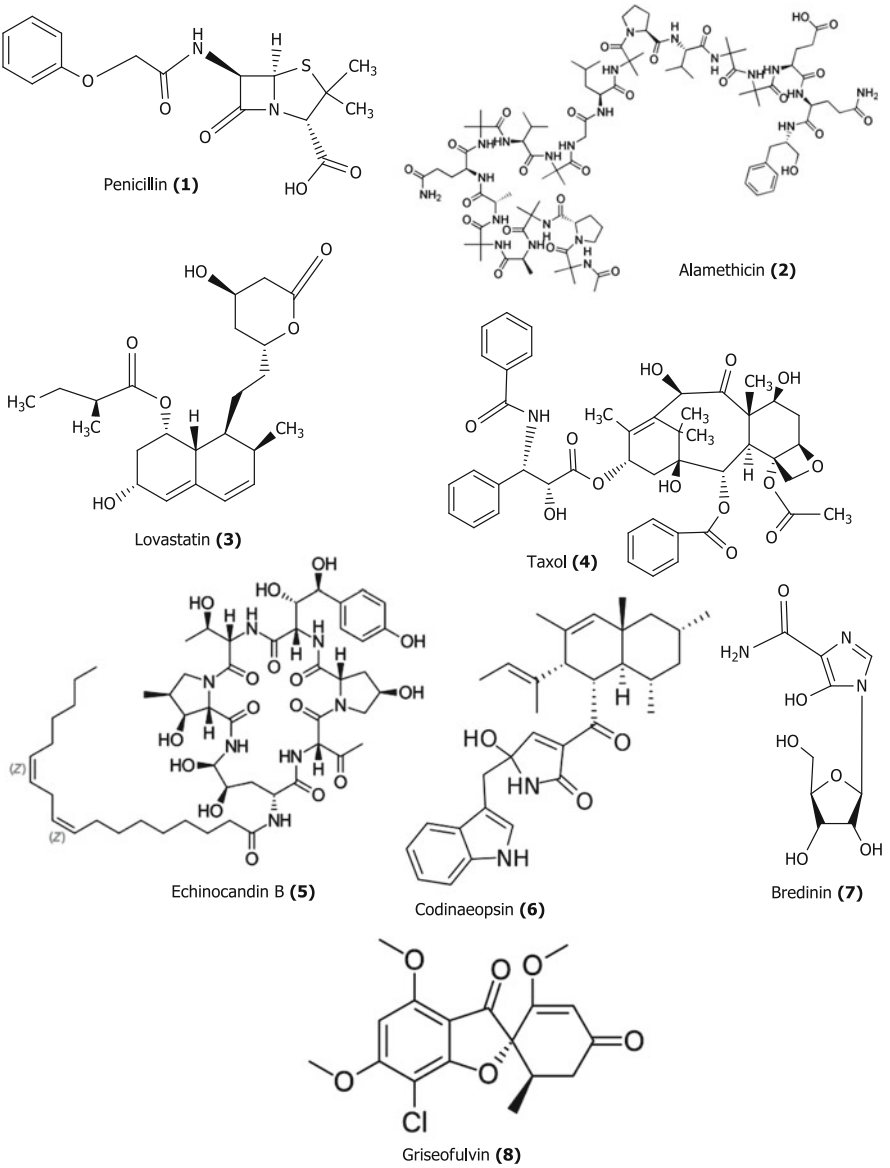

Fig. 19.1 Structure of fungal secondary metabolites used as a drug

approach has been studied extensively and displayed immense opportunities for activating the silent genes as well as increasing the expression level of pre-activated genes of interest.

2 Epigenetic Modifications in Fungi

Epigenetics comes from the word epigenesist, a term coined by Waddington in 1942 for explaining the phenomena of phenotypic changes without change in the genotype of an organism (Aghcheh and Kubicek 2015). Epigenetics is the study of how a stimulus (chemical or environmental) affects the expression pattern of a gene without leading to any turnaround in the genome of the organism. It acts upon histone proteins, wrapped around chromatin DNA, and posttranslationally modifies them to cause chromatic alterations which facilitate regulation of gene expression of different genes including secondary metabolite biosynthetic genes. This close relationship between histone proteins and gene expression makes histones serve as a prime candidate for epigenetic regulation. However, epigenetic alterations caused at different levels of chromatin DNA led to cause several diseases, such as breast cancer, where DNA methylation of the BRCA1 gene reduces its expression levels and increases the risk of morbidity (Tang et al. 2016). Upon histone modification, *Mycobacterium tuberculosis*-engulfed macrophages switched off the expression of the IL-12B gene, resulting to cause weak immunity that potentiates *M. tuberculosis* growth (Chandran et al. 2015). Epigenetics regulation has also been extensively studied in the modulation of therapeutically bioactive compounds synthesized in different biological systems such as plants, bacteria, and fungi. The epigenetic modification for the activation of BGCs has shown significant success. The chromatin structure among fungi serves as a regulator of cellular metabolism where the chromatin-modifying enzymes play a crucial role in the regulation of secondary metabolome. Changes in the chromatin structure are found to be a prerequisite for the functioning of the cell and may lead to alterations in the transcriptional response of the cells. These alterations are regulated by intrinsic cellular or extrinsic environmental factors. Different histone modifiers tend to epigenetically modulate the level of gene expression involved in the secondary metabolites' biosynthetic gene clusters. DNA methylation, Histone modification, RNA interference, etc. are some examples of epigenetic modifications that occur in fungi. The alterations in chromatin can be affected by local, global, or interspecies-based regulation. The in-cluster regulatory gene(s) present in BGCs are the main local regulator of the SM synthesis pathway.

The epigenetic modifications on BGCs help to activate silent genes and also upregulate the expression of pre-activated genes. Histone deacetyltransferase (HDAC) and Histone acetyltransferase (HAT) are the intrinsic or local modifiers that modify gene expression levels to different extents (Albright et al. 2015). In contrast, environmental stimuli responsive global regulators allow for modulation of the transcriptional activity of different BGCs resulting in modulation of the production of SMs. Loss of function study of CclA, a Bre2 ortholog responsible for methylation of H3 and H4, has led to identifying novel SMs in fungi. Monodictyphenone (**9**), emodin (**10**), and their analogs are bioactive molecules

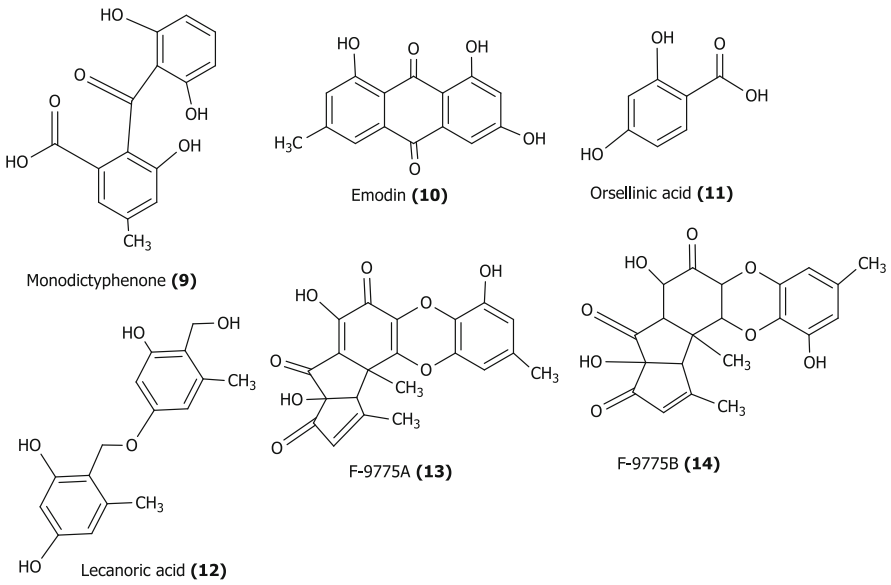

Fig. 19.2 Structures of secondary metabolites activate under interspecies interactions

synthesized in *A. nidulans* (Bok et al. 2009). Moreover, interspecies interaction also epigenetically affects SM production (Schroeckh et al. 2009). An earlier study on cell-cell interactions between *A. nidulans* and *Streptomyces rapamycinicus* displayed some remarkable shifts in the SM profile of the fungal counterpart. Some SMs that were previously silent were found to activate under interspecies interactions. For instance, orsellinic acid (**11**), lecanoric acid (**12**), F-9775A (**13**), and F-9775B (**14**) synthesized in *A. nidulans* were induced after cell-cell interaction with *Streptomyces rapamycinicus*. The activation of orsellinic acid (**11**), lecanoric acid (**12**), F-9775A (**13**), and F-9775B (**14**) (Fig. 19.2) was further corroborated by supplementing the anacardic acid and suberoylanilide hydroxamic acid (SAHA) which repressed and induced the orsA, orsellinic acid synthesis gene, respectively (Nützmann et al. 2011).

Histones are lysine and arginine-rich proteins of basic nature which specifically present in the eukaryotic nucleus that has a significant role in DNA packaging and regulation of different metabolic processes such as DNA replication, recombination, repair, and transcription (Hauer and Gasser 2017). They are structural components of nucleosome assembly to act as a spool around which the DNA winds up. The winding up of DNA around nucleosome assembly protects the DNA from entangling and getting damaged. The histone superfamily comprises five subfamilies, of which

Fig. 19.3 Structural arrangement of chromatin DNA on histone proteins: Condensed heterochromatin and relaxed euchromatin

H1 and H5 act as linker histones and H2A, H2B, H3, and H4 are core histones. The octamer structure of the nucleosome comprised two units of H2A, H2B H3, and H4 of histones proteins. In contrast, the linker histone binds with the nucleosome at the start and end of DNA to lock it in place (Kumar et al. 2020). The abundance of lysine and arginine contributes to the overall positive charge of histones which facilitates the binding of negatively charged DNA tightly around it. The histone DNA interaction can be of several types, such as Salt bridge and H-bond between amino acid side chain and phosphates on DNA, H-bond between DNA and amide group on the main chain of histone proteins, nonpolar bonding between DNA sugar and histone, etc. The final structure of histone features a long tail at the N-terminal end which serves as a site for posttranslational modification. Histones undergo posttranslational modification which affects their ability to interact with the DNA and thus affects the nucleosome integrity.

Some modifications tighten the binding of histone and DNA (heterochromatin), thus making it less accessible for interaction with transcription factor and vice versa (euchromatin) (Fig. 19.3).

2.1 Histone Modifications

The understanding of posttranslational histone modifications has developed since the early 1960s. There occur different types of histone modifications, such as acetylation, phosphorylation, methylation, deimination, ADP (Adenosine diphosphate) ribosylation, ubiquitylatiion, sumoylation, tail clipping, etc. (Bannister and Kouzarides 2011). Histone acetylation and deacetylation are known to contribute to gene expression levels by regulating the DNA-histone interaction (Hyndman and Knepper 2017; Javaid and Choi 2017). The cascades of methylation and demethylation have also been attention seekers for their involvement in processes of gene regulation (Poças-Fonseca et al. 2020). The process of methylation could occur at sites such as lysine (mono-, di- or trimethylation) and arginine (mono- or dimethylation). The transcriptional repressive heterochromatic and active

Fig. 19.4 Nucleosome structure with protruding tails of histones

euchromatic modifications take place at H3 and H4. The lysine in the H3 and H4 of the euchromatin occur to be hyperacetylated while H3K4 is trimethylated, whereas in the heterochromatin, the H3K9 is trimethylated and others are hypoacetylated. The acetylation and phosphorylation of histones tend to increase the overall negativity of the histones which in turn hinder the histone DNA interactions, leading to a less compact chromatin structure. The role of histone SUMOylation is studied to be associated with transcriptional repression through the modification of H4, H2A, and H2B by SUMO (small ubiquitin modifier) family proteins. Much developed understanding has been established about the interactive network of SUMO with the members of the Velvet complex (involved in reproduction and secondary metabolites synthesis in yeast) and SAGA (Spt-Ada-Gcn5 acetyltransferase) complex (transcriptional facilitator in yeast) (Aghcheh and Kubicek 2015). Likewise, other histone modifications also play their respective roles in the regulation and activation of a different set of genes among different hosts. As discussed previously, it is the tail of histones that gets subjected to modifications (Fig. 19.4).

2.1.1 Histone Acetylation and Deacetylation

This mechanism of histone modifications involves the incorporation of the acetyl group to the lysine residue in the N-terminal tail that projects out from the histone core of the nucleosome assembly. Acetylation of histones hampers their positive charge resulting to decrease interactions between the DNA and histones. Acetylation causes relaxation in the condensed conformation of the chromatin which allows the expression of the gene to be transcribed. The process is catalyzed by agents falling under the category of histone acetyltransferases (HATs). Various types of HATs are involved in the metabolic process of cells. Some major HATs belong to the GNAT family (SAGA, SILK, STAGA, ADA and A2 complexes, Gcn5, Elp3, HPA2), MYST family (Tip60, MOF, MOZ, MORF), SRC1, ATF-2, etc. (Marmorstein and

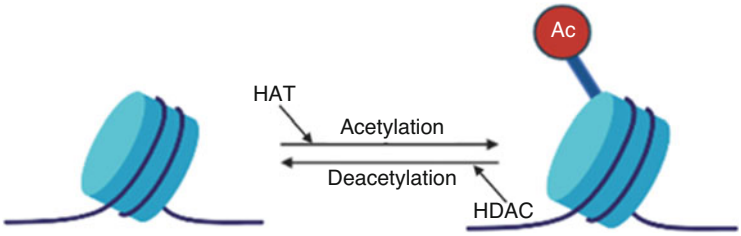

Fig. 19.5 Histone acetylation and deacetylation

Roth 2001). HATs drive the transfer of the acetyl group from acetyl-coenzyme A to the lysine. Due to acetylation, the overall positive charge of the histone tail neutralizes which affects the nucleosome assembly by hindering the interaction between H4 and H2A domains. This configurational shift makes the DNA more exposed to a higher number of transcription factors, activating some genes which would be silent otherwise (Broekhuis et al. 2022) (Fig. 19.5). It is well-studied that histone acetylation modulates the expression of different genes, resulting to alter various metabolic processes including secondary metabolites synthesis.

In contrast, the process of histone deacetylation carried out by histone deacetylases (HDAC) mediated catalysis which is exactly opposite to what occurs during histone acetylation. The deacetylation of histones proteins tends to facilitate the binding of histone and DNA tighter, resulting to cause silencing or downregulation of genes. Due to deacetylation, the coiling of DNA around histone is more condensed, and hence less part remains available for interaction with the transcription factors. Similar to acetylation, deacetylation also occurs at the lysine residues (Seto and Yoshida 2014). The application of HDACi for secondary metabolite regulation has proven to be fruitful. The use of SAHA, SBHA (Suberoyl bis-hydroamicspiepr acid), Trichostatin A, Valproic acid, nicotinamide, and sodium butyrate is involved in the regulation of several fungal SMs. desmethylisaridin E (**15**), desmethylisaridin C2 (**16**), and isaridin F (**17**) synthesized in *Beauveria felina*, meroterpenoids (**18, 19**) in *Aspergillus terreus*, fumiquinazoline C (**20**) in *A. fumigatus*, piperine (**21**) in *Diaporthe* sp., fellutamides A-D (**22–25**) in *A. nidulans*, ergometrine (**26**) in *Claviceps purpurea*, Terrein (**27**) in *Aspergillus terreus*, resveratrol (**28**) in *Xylaria psidii*, nygerone A (**29**) in *Aspergillus niger*, Chrysogine (**30**) in *Penicillium chrysogenum*, tryptophan analogs (**31–33**) in *Torrubiella luteorostrata*, 13-angeloyloxy-diplosporin (**34**) in *Phomopsis* sp., tenuipyrone (**35**) in *Isaria tenuipes*, mollipilins (**36, 37**) in *Chaetomium moliipilium*, syringic acid (**38**), sinapic acid (**39**), acetosyringone (**40**) in *Penicillium brevicompactum*, eupenicinicols C (**41**) and D (**42**) in *Eupenicillium* sp., and 5-bromozeaenol in *Cochliobous lunatus*, cytosporone B (**43**), and E (**44**) in *Leucostoma persoonii* are such examples.

2.1.2 Histone Methylation and Demethylation

The process of histone methylation takes place on the lysine and arginine residues of the histone side chain. The histone methylation and demethylation modification doesn't affect the overall charge of histones and hence doesn't alter the conformation of nucleosome assembly (Fig. 19.6). There may be one, two, or three substitutions of methyl group on the lysine, while arginine may have mono- or di-methylations. The methyl group borrowed from S-adenosyl methionine (SAM). The process of lysine methylation is catalyzed by histone lysine methyl transferase (HKMT). The SUV39H, DotI, DIM5, PRC2 (Polycomb repression complexesspiepr 2), and SET7/9 are some classes of histone methyl transferases (Bannister and Kouzarides 2011; Tamaru et al. 2003; Xiao et al. 2003). The event of arginine methylation is carried out by type I and type II categories of arginine methyl transferase (Bedford and Clarke 2009; Wolf 2009). The transfer of the methyl group takes place from SAM to the ω-guanidino group of the arginine residue. It was until 2004 that histone methylation was thought to be a stable modification, but the identification of lysine-specific demethylase I (LSD1) triggered the arguments about the demethylation of histones. LSD1 was found as a FAD utilizing enzyme which requires protonated nitrogen for the demethylation of mono- and di- methylated lysine residues (Shi et al. 2004).

2.1.3 Histone Phosphorylation

This type of histone modification is dependent upon the catalytic activity of kinases and phosphatases to transfer phosphate groups at the N-terminal histone tail (Oki et al. 2007). Phosphorylation is more vivid and takes place mostly on serine, threonine, and tyrosine residues of histone proteins. The kinases facilitate phosphate transfer from ATP to the hydroxyl group of target amino acid in the side chain. The addition of the phosphate group alters the overall charge configuration, leading to modify chromatin structure. Not much is clear about the histone phosphatase activity, but the occurrence of specific histone phosphorylation indicates the presence of high phosphatase activity within the nucleus (Goto et al. 2002; Sugiyama et al. 2002).

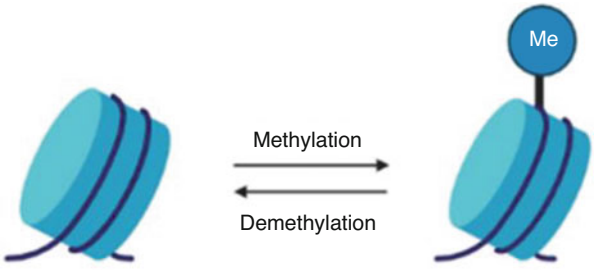

Fig. 19.6 Histone methylation and demethylation

2.1.4 Histone Succinylation

This type of posttranslational modification is specific to the lysine (Ksuc) residue of histone and nonhistone proteins among both prokaryotes and eukaryotes, either enzymatically or nonenzymatically. It is one of the most recently identified and studied modifications. It has been found that sites for succinylation are localized at the promoter region. Such modifications also enable the host to adapt for changes in environmental conditions such as carbon concentration and also enables host such as *Mycobacterium tuberculosis* to gain antibiotic resistance (Xie et al. 2015). The addition of succinyl group (-CO-CH_2-CH_2-CO_2H) from succinyl-CoA to ε-amine of lysine leads to a charge shift from +1 to −1 state at physiological pH which invites other modifications such as acetylation and methylation to occur. Unlike other histone modifications, Ksuc occurs at both, the linker as well as core histones region. The Ksuc destabilizes the nucleosome stability and increases the DNA unwrapping at a significant rate to affect the transcription (Jing et al. 2018). Experiments have revealed that the succinylation at the promoter region is an indication of transcriptional initiation. In yeast, the global succinylation levels are regulated by the levels of galactose, since it serves as a modulator of succinyl-CoA concentration. A study conducted on *Aspergillus flavus* showed that lysine succinylation contributed to the aflatoxin production and its pathogenicity and also facilitated the metabolism of the cell (Ren et al. 2018).

2.1.5 Histone Crotonylation

Crotonylation is yet another posttranslational modification that is specific to the lysine residue of histone and nonhistone proteins. The donor for lysine crotonylation is a thioester-linked sulfhydryl group, named crotonyl-CoA, which covalently crotonylates the ε-amino group of lysine. No selective writers or drivers of crotonylation are reported yet, instead HATs and HDACs are reported to carry out such modifications. For example, GNAT and MYST families. Gcn5 and EsaI corotonylate at the K9, K14, K18, K23, K27 of H3 and K5, K8, K12 and, K16 of H4 respectively. Interestingly, these are the same K residues where HATs catalyze to acetylate as well (Kollenstart et al. 2019). Class I and III HDACs serve as crotonylation erasers and show histone decrotronylation (HDCR) activity. Earlier studies showed that crotonylation is mediated by the beta-oxidation of fatty acids (Gowans et al. 2019). Proteomics analysis of *Trichophyton rubrum* was performed to assess gross crotonylation. Data revealed that a total of 1574 proteins got crotonylated both in the conidial and mycelial stages. Out of which, 53 crotonylation sites were observed in histone proteins. All the identified crotonylated proteins were found to be involved in metabolic, virulence and pathogenicity, and cellular processes. For instance, a total of 30 efflux transporters were found to be crotonylated which contributes to drug resistance.

2.1.6 Histone Biotinylation

This is yet another type of lysine-specific histone modification which is catalyzed by biotinidase and holocarboxylase synthetase enzymes. The biotinidase catalyzes to remove the biotin group from biocytin which later reacts with holocarboxylase synthetase in ATP-dependent manner to histones. Earlier studies identified K9, K13, K125, K127, and K129 of H2A, K4, K9, and K128 of H3, K8, and K12 in H4, the sites for this such kind of modification from human and other eukaryotes (Hassan and Zempleni 2008). The biotinylated histones are reported to involve in gene regulation, cell proliferation, and cellular response to DNA damage. A study conducted on *Candida albicans* revealed that 16 out of 32 total lysine residues present in H2B and H4 were found to be biotinylated. However, biotinylation in other histones proteins suggested the functional disparity in humans and *C. albicans*. Chromatin modification via biotinylation served as a reservoir of biotin in the pathogenic *C. albicans* (Hasim et al. 2013).

2.1.7 Histone Malonylation

It is a reversible PTM where the negatively charged malonyl group is prominently coupled with positively charged lysine and fewer with arginine and glutamate. This modification amends the electrostatic force around the modified lysine residue. The malonyl group gets transferred from malonyl-CoA, an intermediate metabolite in the de-novo fatty acid synthesis pathway (Zhang et al. 2023). A study performed on *Sanhuangporus sanghuang*, a known medicinal mushroom for understanding the role of lysine malonylation, revealed a total of 714 sites for malonylation in 255 different proteins with a total of 26 different enzymes participating in malonylation which involved triterpene or polysaccharide biosynthesis pathways (Wang et al. 2021).

2.1.8 Histone SUMOylation

SUMOylation is a multistep PTM that plays a significant regulatory role in several physiological pathways, such as DNA repair, transcription, cell cycle progression, immune response, intracellular transport, and viral defense. SUMO stands for small ubiquitin-like modifiers whose C-terminal glycine is coupled with isopeptide linkage with the lysine residue of the acceptor protein. Studies conducted on *Candida*, *Aspergillus*, and *Cryptococcus* species revealed that conjugation of SUMO protein is an important driving factor in cell differentiation, stress tolerance, capsule and biofilm formation, colony forming abilities, conidiation, etc. (Gupta et al. 2020). A

study conducted on *A. nidulans* revealed that deletion of SumO (identified and characterized SUMOylation machinery) led to self-sterility and increased sensitivity towards DNA damaging agents and DNA synthesis inhibitors (Wong et al. 2008).

2.1.9 Histone Ubiquitination

Ubiquitin-mediated protein degradation is a regulatory mechanism of cellular functions. Ubiquitin is a 76 amino acid protein, highly conserved whose activity is triggered by ubiquitin-activating enzyme E1 by ATP hydrolysis, which conjugates with ubiquitin-conjugating enzyme E2. The activated ubiquitin-E2 complex binds to E3 ubiquitin ligase, resulting in the C-terminal glycine of ubiquitin covalently binding with the lysine residue of the substrate. This process repeatedly takes place forming a poly-ubiquitin chain which is recognized by proteasome for degradation. Ubiquitin is synthesized by cleavage of precursor proteins such as UBI1, UBI2, UBI3, or to itself UBI4. UBI4 is a poly-ubiquitin protein (five ubiquitin repeats) that is upregulated under stress conditions. The role of ubiquitination has been well-studied in fungi. Any defect in ubiquitination resulted to cause sporulation, pathogenicity, growth, and abnormal conidia morphology (Oh et al. 2012). Studies among different fungal species such as *Candida albicans*, *Cryptococcus neoforms*, *Magnaporthe oryzae*, *Saccharomyces cerevisiae,* etc. led to the similar findings (Cao and Xue 2021).

2.1.10 Other Histone Modifications

Several other less prominent histone modifications take place in different fungal species which were not well characterized for their specific functions. These modifications include propionylation, butyrylation, deamidation, ethylation, piperidation, and proline isomerization which are distributed at H3, H4, and H2B histones. The consequence of these modifications is needed to be studied and characterized as their effects on the fungal genome.

2.2 DNA Methylation

DNA methylation is a metabolic process of methyl group addition from SAM to cytosine (fifth carbon), yielding 5-methylcytosine. DNA methylation results to extend a major groove of the DNA, thereby causing repression of gene expression (Fig. 19.7). It is involved in gene imprinting, chromosome inactivation, and silencing (Jin et al. 2011). However, the reason for the natural occurrence of DNA

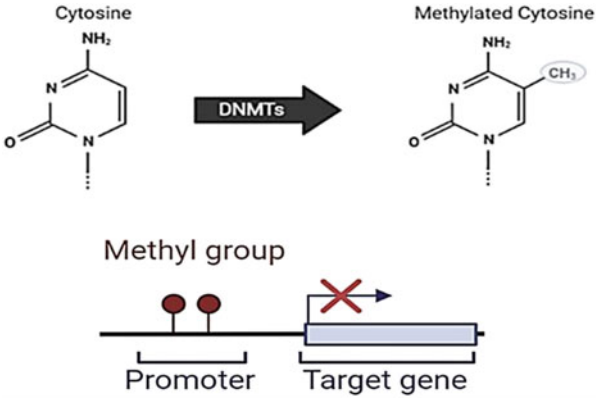

Fig. 19.7 DNA methylation and its effect on gene expression

methylation in fungi remains a subject of study. DNA methyltransferase (DNMTs) class of DNA-modifying enzymes catalyze DNA methylation and are mainly involved in epigenetic regulation. DNMTs are comprised of five different members termed DNMT1, -DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT1 performs methylation of hemi-methylated DNA and is found near the replication foci during the S-phase (Jin et al. 2011). DNMT2 catalyzes methylation of cytosine-38 located at the anticodon loop of aspartic acid-coded tRNA (Goll et al. 2006). In addition, DNMT3A and 3B preferably methylate the unmethylated CpG dinucleotides and encourage de novo methylation. Inhibition of DNA methylation activity also led to induction and upregulation of several fungal secondary metabolites of therapeutic importance. 5′ azacytidine is a widely explored and applied epigenetic modifier which acts as a DNA methyl transferase inhibitor (DNMTi). 5′ azacytidine led to modulating various fungal secondary metabolites such as bisabolane-type sesquiterpenoids (**45–50**) in *Aspergillus* sp., sclerotiorin (**51**), sclerotioramine (**52**), dechloroisochromophilne III (**53**) in *Penicillium citeronigrum*, camptothecin (**54**) in *Botryosphaeria rhodina*, Penicillin (**1**) in *A. nidulans*, oxylipins (**55–57**) in *Cladosporium cladosporioides*, lunalides A and B (**58**) in *Diatrype* sp., carotenoids in *Neurospora crassa*, and isosulochrin (**59**) in *Chaetomium* sp.

DNMT3L is structurally homolog to DNMT3A and 3B, but has no catalytic activity; it facilitates binding other DNMTs for methylation(Kareta et al. 2006). In contrast, DNMTs also help to trigger the expression of cryptic genes (Ramesha et al. 2021).

2.3 RNA-Mediated Regulation

The role of noncoding RNAs in chromatin remodeling emerged as an intense area of investigation in the recent past. Non-coding RNAs are a subgroup of RNAs recently known to play a significant role in epigenetic regulation (Fig. 19.8). Such regulatory RNAs are mainly divided into two groups termed short-chain noncoding RNA

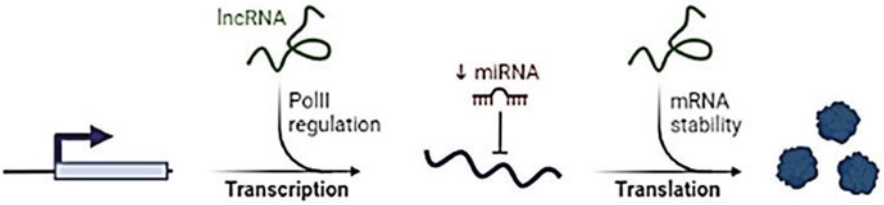

Fig. 19.8 Scheme of RNA-mediated regulation. lncRNA regulating the activity of enzymes and miRNA regulating the stability of mRNA

(sncRNA) and long-chain noncoding RNAs (lncRNA). The size of short-chain noncoding RNA (sncRNA) and long-chain noncoding RNAs (lncRNA) is shorter than 30 nucleotides and longer than 200 nucleotides, respectively. These short or long noncoding RNAs modulate transcription (antisense) activity, thereby involved in chromatin remodeling. The micro RNAs (miRNAs), short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), extracellular RNAs (exRNAs), and small cajal body-specific (scaRNAs) are some examples of short noncoding RNAs. In contrast, long noncoding RNAs include X-linked X-inactive-specific transcript (Xist), HOX antisense intergenic RNA (HOTAIR), intronic noncoding RNAs (incRNAs), and sense/antisense long noncoding RNAs (lncRNAs) (Fernandes et al. 2019; Hombach and Kretz 2016). The short-chain noncoding RNA interacts with the Piwi clade of Argonaute (AGO) proteins and guides the AGO proteins for the regulation of gene expression. Recent studies revealed that siRNA is predicted to facilitate DNA and histone methylation for gene silencing (Bayne and Allshire 2005; Kawasaki and Taira 2004; Morris et al. 2004). However, an earlier report showed that miRNAs did not directly silence the gene expression by disrupting the mRNA, but this was due to alteration of chromatin structure or chromatin remodeling enzymes (Denis et al. 2011; Yuan et al. 2011). Natural antisense transcripts (NATs) are a subset of lncRNAs involved in chromatin remodeling and transcriptional interference. The exact mechanism behind the activity of lncRNAs has not been well deciphered yet. However, it is proposed that lncRNAs either participate in chromosome inactivation or facilitate genome imprinting (Wu and Bernstein 2008; Yang and Kuroda 2007). Several studies have revealed that RNAi-mediated gene regulation affects the levels of gene (s) expression, suggesting to be involved in a variety of cellular functions. The level of patulin, a potential mycotoxin and an antimicrobial agent, was regulated by RNAi-mediated epigenetic modification (Sanzani et al. 2012).

3 Relevance of Epigenetic Modifications in Fungal SM Synthesis

There are various epigenetic modifications taking place among the fungal species which affect several metabolic processes in the fungi. To survive under any adverse conditions, fungi tend to cope the stress by modulating their genomic and proteomic profiles to produce counter biomolecules that help them to survive. The biomolecules that help to cope with the surrounding stimulus are referred to as secondary metabolites (SMs). The genomic complexity of the fungal flora has immense potential for synthesizing an array of bioactive secondary metabolites (SMs) which especially synthesize under different environmental parameters such as nutrient availability, surrounding pH, competition, and oxidative or environmental stress. The bioactive secondary metabolites also have shown a significant therapeutic relevance for humans, plants, and animals. SMs synthesizing genes are mostly silent or minimally expressed under normal physiological conditions, making their bioavailability very scarce. Researchers tried to activate these genes for increasing the production of secondary metabolites (SMs) by employing various approaches. Epigenetic modification is the one that displayed promising outcomes. These modifications either lead to an increase in the transcription of some genes by loosening the DNA wrap around histones which increases the interaction between transcription factors (TFs) and genes or downregulate the expression of genes by masking the promoters or several other factors to convert it into heterochromatin. Here, we discuss the role of epigenetic modifications on secondary metabolite production in fungi. For the very first time, the role of chromatin remodeling in SM production in fungi was reported in *A. nidulans*. Lae A, a methyltransferase, has been identified as a global regulator in *Aspergillus species* that remodeled the chromatin by histone methylation. Other than Lae A, some examples of histone methylation regulators are COMPASS (complex proteins associated with Set1) and CclA (Wu and Yu 2015). The knockout mutant of LaeA disrupted the sterigmatocystin (ST) synthesis. In contrast, its overexpression was catalyzed to induce penicillin (**1**) and lovastatin synthesis genes in *Aspergillus* species (Wu and Yu 2015). The deletion of a deacetylase coding gene hdaA resulted to increase SM production in *Clacarisporium arbuscula* (Okada and Seyedsayamdost 2017). The epigenetic modifications led to activating cryptic genes to identify the novel SMs in fungi. Monodictyphenone, emodin, and its analogs were reported to activate cryptic genes in *A. nidulans* by epigenetic modifications (Bok et al. 2009). Studies on *Fusarium* species revealed several findings such as methylation of fourth lysine residue of H3 acts as a transcriptional activator for BGCs. Moreover, the knockout mutant of histone methyl transferase encoded gene, Set1, led to repress fumonisins and deoxynivalenol (DON) synthesis in *Fusarium species* (Gu et al. 2017; Liu et al. 2015; Studt et al. 2017). Interestingly, the loss of Set1 increased the bioavailability of fusarins and

Table 19.1 List of novel fungal compounds activated in response to epigenetic regulators

Species	Modifier	Compound	Activity	Reference
Aspergillus sp.	5'azacytidine	Bisabolane-type sesquiterpenoids (**45–50**)	Antidiabetic, anti-inflammatory, antibacterial	Bai et al. (2018); Garnaud et al. (2016); Jasim et al. (2019); Jeon et al. (2015)
Eupenicillium sp.	Nicotinamide	Eupenicinicols C (**41**) and D (**42**)	Antibacterial and cytotoxic	Cichewicz (2010)
Aspergillus terreus	Trichostatin A	Meroterpenoids (**18–19**)	Alpha glucosidase inhibitor	Costa et al. (2001)
Chaetomium moliipilium	Nicotinamide	Mollipilins (**36–37**)	Anticancer	Anjum and Xuewei (2022)
Beauveria feline	SAHA	Desmethylisaridin E (**15**), Desmethylisaridin C2 (**16**), and isaridin F (**17**)	Anti-inflammatory	Chung et al. (2013)

bikaervin in *F. fujikuroi* (Studt et al. 2017). On contrary, deletion of CclA, methyltransferase gene in Aspergillus species induces expression of different gene clusters. An example of SM gene downregulation by histone methylation is the effect of H3K9me3, which represses the ST gene cluster (Reyes-Dominguez et al. 2010). In contrast, loss of methylation from H3K9me3 in *F. graminearum* increased aurofusarin levels and decreased DON levels. Some examples of histone methyltransferases in fungi are SUV39 (methylate H3K9), SET1 (methylate H3K4), EZH1/2 (methylate H3K27), SET2 (methylate H3K36), DOT1 (methylate H3K79), RmtA (methylate H4R3), and RmtC (methylate H4 and H2A). The list of histone demethylase in fungi includes LSD1 (specificity not established but may demethylate H3K4), JHDM3A/JMJD2A (may demethylate H3K4 and H4K20), and RPH1 (demethylate H3K36me3 and K36me2) (Etier et al. 2022). Histone acetylation and deacetylation, other highly characterized epigenetic modifications, have been reported to modulate SM synthesis in fungi. GcnE (a homolog of GCN5) found in *A. nidulans* noticeably impaired the synthesis of sterigmatocystin, terrequinone, and penicillin up to some level. It was shown that H3K9ac and H3K14ac in A. nidulans by GcnE were localized in promoter region of ST biosynthesis gene aflR, regulating the level of aflatoxin. Loss of HdaA (histone decaetylase) also increased the ST and penicillin biosynthesis. Deacetylation of H3K4, H3K9, H3K14, H3K18, and H3K27 by FgSa3 downregulation in *F. graminearum* completely aborted the DON biosynthesis. However, similar modifications led to upregulate zearalenone and orsellinic acid (**11**) synthesis (Hueza et al. 2014; Kong et al. 2018). Various different therapeutically significant fungal SMs have been newly identified by these modifications listed in Table 19.1. The structure of such SMs is mentioned in Fig. 19.9. On the other hand, the synthesis of fungal bioactive was induced by epigenetic modifications as shown in Table 19.2 and Fig. 19.10. Some compounds that are obtained upon epigenetic modification but have not been characterized (Table 19.3) for their bioactivity are mentioned in Fig. 19.11.

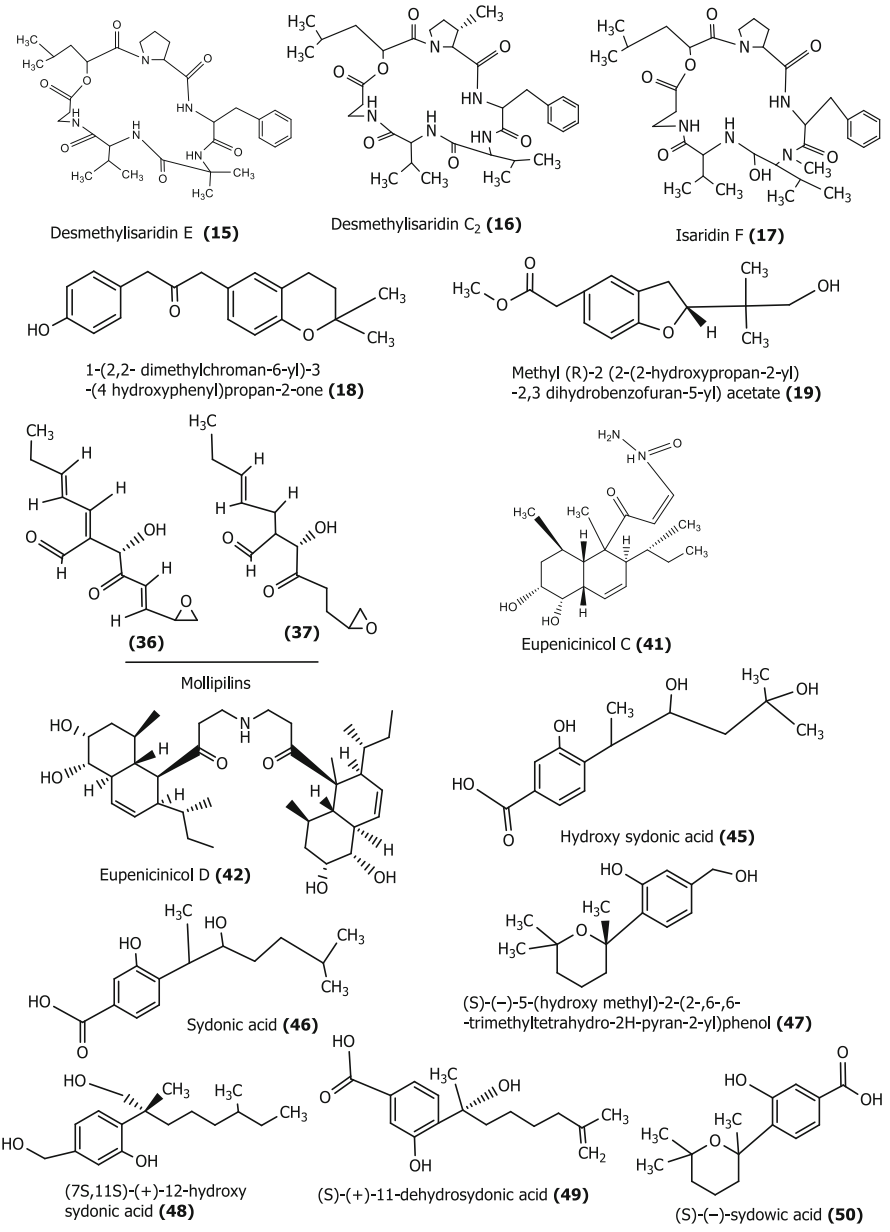

Fig. 19.9 Structure of novel fungal bioactive compounds identified by epigenetic regulation: [18–19]: Meroterpenoids; [45–50] Bisabolane-type sesquiterpeniods

Table 19.2 Induction of therapeutic active secondary metabolites enhanced by epigenetic regulators

Species	Modifiers	Bioactive compound	Activity	Reference
Aspergillus fumigatus	Valproic acid	Fumiquinazoline C (**20**)	Antibacterial, antifungal, antitumor	Magotra et al. (2017)
Diaporthe sp.	SAHA	Piperine (**21**)	Dietary supplement	Jeon et al. (2015)
Botryosapaeriar hodina	5'azacytidine	Camptothecin (**54**)	Insecticidal, apoptosis inducer, and anticancer	Vasanthakumari et al. (2015); Asai et al. (2012a)
Claviceps purpurea	SAHA	Ergometrine (**26**)	Obstetrics	Asai et al. (2012b)
Aspergillus nidulans	SAHA	Fellutamide A-D (**22–25**)	Proteasome inhibitor	Asai et al. (2012c)
Leucostoma persooniii	Sodium butyrate	Cytosporone B (**43**), and E (**44**)	Antimalarial and antibacterial	Belofsky et al. (2000)
Aspergillus terreus	SAHA	Terrein (**27**)	Anti-inflammatory, antibiosis, antitumor	Akone et al. (2016); Chen et al. (2019)
Penicillium citeronigrum	5'azacytidine	Sclerotiorin (**51**), Sclerotioramine (**52**), Dechloroisochromophilne III (**53**)	Antibacterial and antifungal	Wang et al. (2010)
Xylaria psidii	SAHA	Resveratrol (**28**)	Antitumor, antioxidant, antiviral, phytoestrogenic	Dwibedi et al. (2019); Risuleo and La Mesa (2019)
Penicillium brevicompactum	Nicotinamide	Syringic acid (**38**), sinapic acid (**39**), acetosyringone (**40**)	Antioxidant and anti-proliferative	El-Hawary et al. (2018)
Aspergillus nidulans	5'azacytidine	Penicillin (**1**)	Antibacterial	Bok et al. (2009)

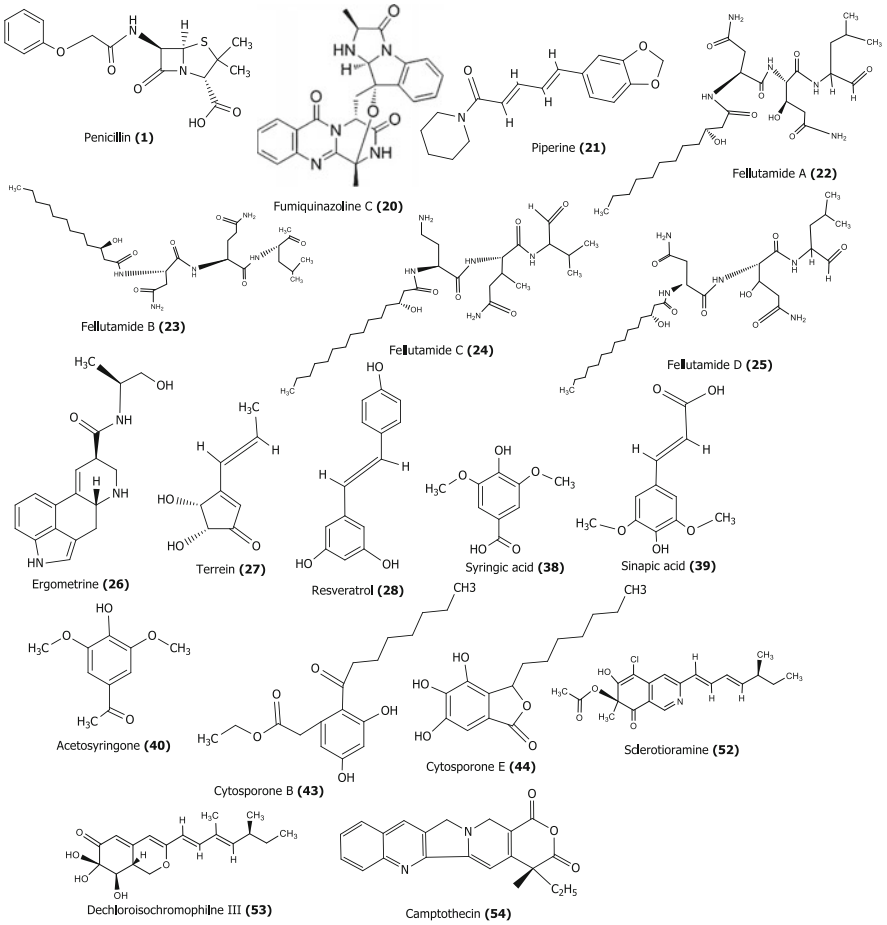

Fig. 19.10 Structure of bioactive compounds induced by epigenetic modification

4 Conclusion

The high-paced and changing lifestyle is leading to cause numerous health conditions among individuals. With the increasing health anomalies, there is a need for the development and identification of sustainable medications. The diversity among fungal flora makes them a suitable candidate for the identification of the same. The recent biological advancements have allowed for attempts to upscale the bioavailability of therapeutically relevant SMs from the fungal hosts. Epigenetic modifications have been tried as a tool for the regulation of the synthesis level of these SMs. These modifications are mainly rendered at the histones proteins wrapped around the chromatin which leads to altered interactions with chromatin DNA, resulting in gene

Table 19.3 Epigenetically synthesized SMs with unknown therapeutic functions

Species	Modifiers	Compound	Reference
Cladosporium cladosporioides	5'azacytidine	Oxylipins (**55–57**)	Cichewicz (2012); Jeon et al. (2015)
Diatrype sp.	5'azacytidine	Lunalides A and B (**58**)	Ying et al. (2021)
Neurospora crassa	5'azacytidine	Carotenoids	Zwergel et al. (2016)
Aspergillus Niger	SAHA	Nygerone A (**29**)	Baker et al. (2008)
Penicillium chrysogenum	SAHA	Chrysogine (**30**)	Zhang et al. (2019)
Torrubiella luteorostrata	SBHA	Tryptophan analogs (**31–33**)	Costa et al. (2001)
Phomopsis sp.	SBHA	13-angeloyloxy-diplosporin (**34**)	Brosch et al. (2001)
Cochliobous lunatus	Sodium butyrate	5-bromozeaenol	Kritsky et al. (2001)
Isaria tenuipes	SBHA	Tenuipyrone (**55**)	Montanini et al. (2014)
Chaetomium sp	SAHA/ 5'azacytidine	Isosulochrin (**59**)	Silva et al. (2004)

induction or gene repression due to the formation of either euchromatin or heterochromatin structure, respectively. These modifications worked by modulating synthetic genes to induce secondary metabolites synthesis or also led to induce cryptic genes for the synthesis of novel compounds. The reason behind inducing secondary metabolite synthesis by epigenetic regulation is to make chromatin DNA accessible for gene expression which leads to a shift in the overall transcript profile of the host without changing the genetic makeup of the host. This transcriptomic shift sometimes induces genes for SMs that were silent at normal conditions. Induction of chromatin modifications can be assessed both at the local, especially at the gene cluster of the SMs via modulating transcription activity of in clustered regulatory protein, or global level by counter-response exhibited by hosts in response to the external stress stimuli. These factors are the epigenetic inducers modulating several SMs without changing the genetic makeup of the host. The epigenetic approach can prove to be highly advantageous for the identification of novel bioactive compounds of therapeutic significance as well as for increasing the bioavailability of the known but scarce therapeutically significant fungal bioactive compounds or secondary metabolites.

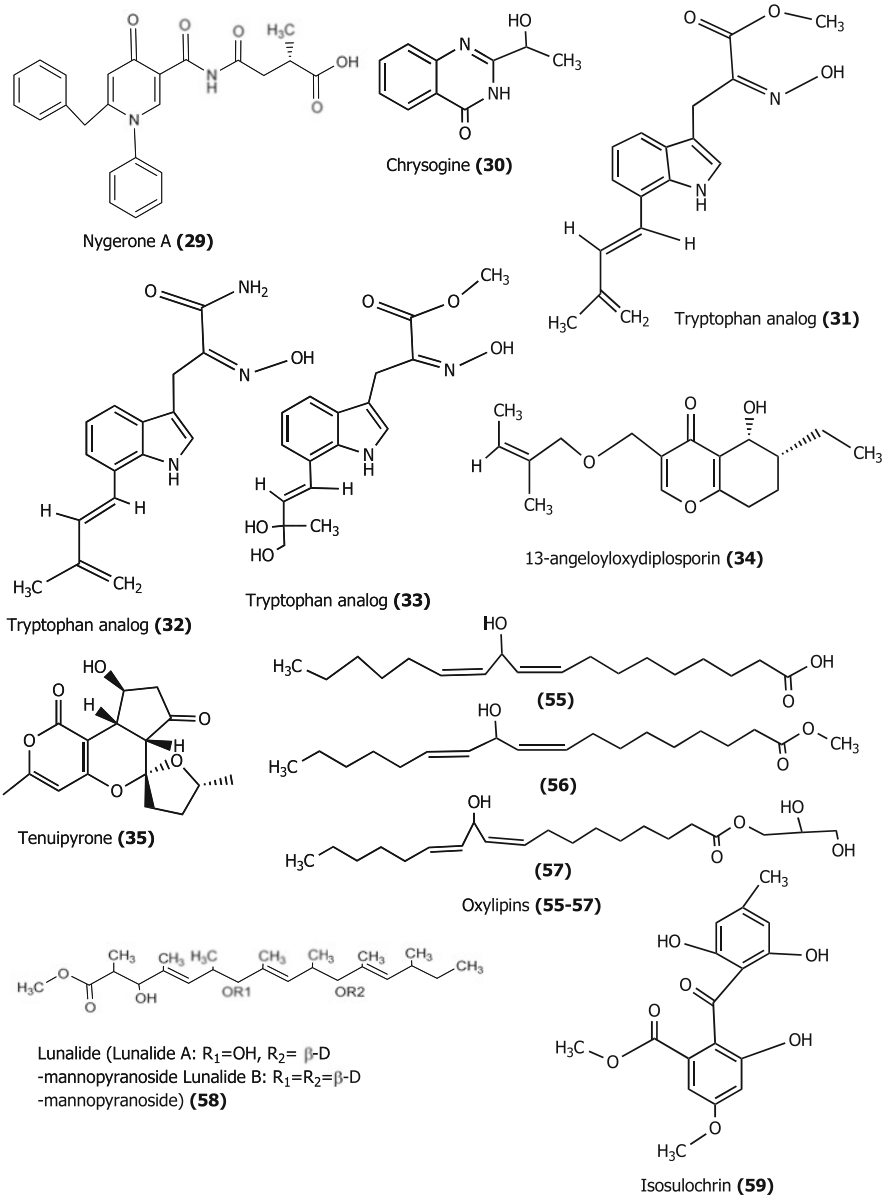

Fig. 19.11 Structure of compounds synthesized with unknown function by epigenetic modification

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Chapter 20
Strategies for Enhancing the Production of Echinocandin

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Abstract Fungi are well known for their ability to naturally produce a multitude of secondary metabolites (SMs) which act as a weapon of defense to protect themselves against parasites and predators. The synthesis of SMs is a multistep process, stage-specific, and more amiable in the late-log or stationary phase. Echinocandins are potent first-line antifungals naturally synthesized from different fungal strains like *Aspergillus rugulovalvus, Zalerion arboricola,* and *Papularia sphaerosperma*. These important antifungals require a lower dosage, are less toxic to the host, and have quick fungicidal and fungistatic potential against a variety of fungal pathogens by targeting to inhibit 1,3-β-D-glucan synthase activity. The treatment of echinocandins is somewhat costlier and impracticable than other available antifungals. A group of investigators has made efforts to develop cost-effect echinocandin treatment. Different forms of echinocandins are now known that can be synthesized either naturally or semisynthetically from different strains. However, in apparent time, various semisynthetic derivatives of echinocandins such as anidulafungin, caspofungin, and micafungin have been developed. In this chapter, we will focus on different strategies employed for the induction of echinocandin production, in particular for reducing the cost of echinocandin treatment against fungal infections.

Keywords Echinocandins · Antifungal · Media optimization · Surfactants · Microparticles · Genetic regulation

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1 Introduction

Echinocandins are cyclic lipo-hexapeptide comprised of non-proteinogenic amino acids and are majorly synthesized by filamentous fungi in response to different stress conditions. The cyclic lipo-hexapeptide structure of echinocandins is primarily assembled by non-ribosomal peptide synthase (NRPS) assembly (Kumar et al. 2018). These antifungals are potent fungicidal and fungistatic in nature which show antifungal activity against *Candida, Aspergillus* sp., and other fungal pathogens (Mroczyńska and Brillowska-Dąbrowska 2020; Prakash and Kumar 2023). Mechanistically, echinocandin blocks glucan synthesis by restricting β-1,3-glucan synthase activity, which is one of the major components of the fungal cell wall. As compared to polyenes and azoles, echinocandins show superior benefits as they are relatively less toxic which gains interest in their efficient biosynthesis (Szymanski et al. 2022). The most crucial step in the echinocandin research field was the discovery of Echinocandin B (**1**), pneumocandin A_0 (**2**), and pneumocandin B_0 (**3**). However, caspofungin (**4**), a semisynthetic echinocandin, was synthesized from pneumocandin B_0, a fermented product of *Glarea lozoyensis*. Though filamentous fungi naturally produce echinocandins like pneumocandin A_0 (**2**) and B_o (**3**), echinocandin B (**1**), and FR901379 (**5**), echinocandin B (**1**) was the first echinocandin class antifungal that was extracted in 1974 from the fermented product of the fungus *Aspergillus delacroxii* (also known as *Aspergillus nidulans*) with good antifungal activity. Later on, another echinocandin B producing filamentous fungi *Aspergillus rugulosus* NRRL 8039 and 8113 and other echinocandin-producing strains have been isolated. To date, more than 20 different natural fungal sources of echinocandin production have been discovered (Hüttel 2017). The naturally synthesized echinocandins have been further used as a main precursor for semisynthetically echinocandins synthesis, thereby three semisynthetically echinocandins, caspofungin (caspofungin acetate) (**4**), anidulafungin (**6**), and micafungin (**7**), have been developed as shown in (Fig. 20.1) (Hüttel 2021). Caspofungin is a semisynthetic water-soluble lipopeptide derived from pneumocandin B_o, and it was produced from the fermented product of *Glarea lozoyensis* (Letscher Bru and Herbrecht 2003). *Similarly,* anidulafungin was obtained by substitution of the linoleic acid side chain by a terphenylacyl chain. It is well elucidated that biosynthetic genes for fungal secondary metabolites are located in the cluster form which is more often located on the same chromosome. In contrast, two separate gene clusters of secondary metabolites in *Aspergillus nidulans* are present on two different chromosomes. These include the biosynthesis gene clusters of the echinocandin B, meroterpenoids, austinol, and dehydroaustinol. The purpose of such evolution divergent of gene clusters distribution on two separate chromosomes remained a question for study. The biosynthetic gene clusters of the fungal secondary metabolites encode for the large multidomain(s) non-ribosomal peptide synthetases (NRPSs) or polyketide synthases (PKSs) along with various oxygenases and hydrolyses, required for synthesis and maturation of secondary metabolites. Both NRPS and PKS systems are comprised of at least three basic

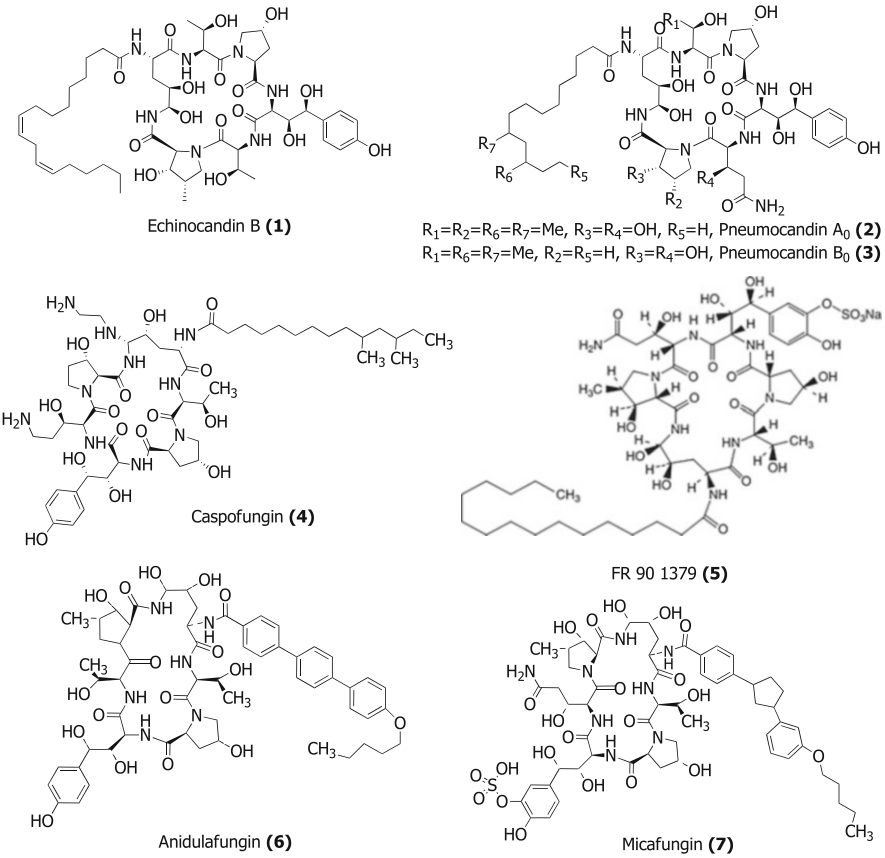

Fig. 20.1 The structure of different antifungals belongs to echinocandin class

domains called as an adenylating domain (AD), required for amino acid activation; a thiolation domain (a peptidyl carrier protein), which helps to covalently bind the activated amino acid; a third one called condensation domain, responsible for peptide bond formation. Echinocandin B (**1**) is comprised of a linoleic acid sidechain and six amino acid residues namely, 3,4-dihydroxyhomo-tyrosine, 3-hydroxy-4-methylproline, 4,5-dihydroxy-ornithine, 4-hydroxyproline, and two threonine residues. It has been established that modified homotyrosine and proline are essentially important for the antifungal efficacy of echinocandins. A hydrophobic linoleic acid chain attached to the echinocandin B (**1**) acts as a hook that helps to anchor it into the fungal cell wall, thereby necessary for antifungal activity. The biosynthetic pathway for echinocandin B (**1**) production has been recently studied. Echinocandin biosynthesis in fungi belongs to NRPS systems and biosynthetic genes of echinocandin B arranged into two gene clusters termed *ecd* (echinocandin) and *hty* (L-homotyrosine) located on two separate chromosomes which involved in assembly and maturation of echinocandin B, respectably (Kumar et al. 2019; Cacho et al. 2012). Disruption of

hty gene cluster, located on a separate chromosome, led to the loss of the production echinocandin B, indicating to require homotyrosine for echinocandin B production in *Emericella rugulosa*. The regulation of echinocandins production is not well explored. However, biosynthetic gene clusters of fungal secondary metabolites are reported to be controlled both at global and local levels under various environmental conditions. The regulatory gene present within the gene cluster entirely controls a specific secondary metabolite pathway or various stimulus present in entire genomes globally control the synthesis of secondary metabolites. These stimuli can be various different nutrients (carbon and nitrogen sources), temperature, light, pH, reactive oxygen species, hypoxic conditions, biofilm formation and limited iron availability, or stimulus derived from interspecies interactions. The impact of these stimuli can vary in different fungal species. Different sets of literature have also revealed that echinocandins biosynthesis primarily takes place between the late exponential and initial decline phase and is usually silent during the initial stage of growth phase (Kumar and Kumar 2019). Moreover, the yield of secondary metabolites is usually very low, hence their purification of secondary metabolite in sufficient amount is challenging and expensive. Hence, there is a need for optimization of conditions for increasing the natural yield of the compounds (Emri et al. 2013). A group of earlier studies focused to develop different parameters such as optimization of various environmental conditions, growth conditions, chemical modifications for the improvement of production yield, and better stability and solubility of echinocandins. The various factors or parameters which affect echinocandin production include pH, temperature, oxygen supply, carbon source, nitrogen source, and adding microparticles, lipids and surfactant in fermentation medium which have been successfully employed that help to augment the production yield of echinocandins (Niu et al. 2021; Kumar and Kumar 2019). Some of the important inducing parameters for echinocandins production have been summarized.

2 Temperature

Temperature is a major parameter for the growth and biosynthesis of different metabolites in microorganisms. Generally, alteration in temperature can affect the activity of enzyme and cell growth which leads to alter metabolite activity of cell (Zou et al. 2019). Temperature for cell growth and secondary metabolites synthesis are sometimes independent to each other. The temperature for secondary metabolites production is often lower than the culture growth temperature. Hence, events of cell growth and secondary metabolites biosynthesis are sometimes independent of each other (Tóth et al. 2011). There are many examples of temperature effect on secondary metabolite production in different microorganisms which have been studied. Similarly, *A. flavus* was able to tolerate a broad range of temperature (15–45 °C) for growth, but the synthesis of aflatoxin B1 was optimally observed at the temperature range of 25–30 °C (Abdel-Hadi et al. 2012). The cell mass of *Mortierella alpinia* was optimally grown at 25 °C; however, this fungal strain thrives in the arachidonic synthesis at 20 °C (Peng et al. 2010). In case of echinocandin B production by

A. nidulans, the optimum temperature for cell growth and echinocandin B biosynthesis is reported between 30 °C and 24–28 °C, respectively. To resolve the problem of requirement of two separate temperature ranges, a two-stage temperature control system was introduced (Zou et al. 2019; Feng et al. 2016). This two-stage temperature control strategy has been applied in many fermentation processes to obtain desired metabolite products. The maximum growth of *Aspergillus nidulans* was observed at 30 °C from first-sixth day of incubation, leading to accelerated growth. The higher echinocandin production yield was measured when fermented culture was shifted from 30 to 25 °C after sixth days of incubation for next 6 days. In initial period of fermentation process, echinocandin B production was not detectable which was started to be observed from fourth day of incubation and attained maximum echinocandin production on 12th day of incubation. This time-dependent two-stage temperature shifting resulted to increase the echinocandin B in *A. nidulans* up to1.3-fold, as compared to single temperature. Similar two-stage temperature conditions were also met for the production of echinocandin-FR901379 and it was optimally produced between 24 and 28 °C (Zou et al. 2019). The temperature effect on pneumocandin B_0 production has also been studied in *G. lozoyensi* which optimally grows between 23.5 and 25 °C. No viable growth was observed at 28 °C and above (Li et al. 2018). The temperature shift of the fermented culture also enhanced the validamycin A production in *Streptomyces hygroscopicus* (Wei et al. 2012).

3 pH

pH is another important parameter for the fermentation process that helps to optimize secondary metabolite production. It is a general notion that alteration in pH affects the overall growth of the fermented culture and metabolic profiling of the host. The pH of the growth medium should be maintained constant at its optimal value to attain optimal synthesis of echinocandins. Alteration of pH in fermented culture also shows global regulatory effect in secondary metabolites production. The PacC and CCAAT-binding complex (CBC) are the pH responsive elements present in fungi which are able to control the gene clusters of different secondary metabolites (Brakhage 2013). Earlier reports referred that pH displayed to modulate the secondary metabolite production at different extent. Among members of *Aspergillus* species, the role of pH has been studied and it has been revealed that optimal pH can lead to increase in bioavailability levels of certain metabolites by several folds. For example, the levels of aflatoxin and sterigmatocystin were found to be upregulated by fivefold when grown in pH range of 4–5 in comparison to their levels at pH8 (Keller et al. 1997).

The pH of the growth medium also affects the levels of certain enzymes among the hosts (such as acid phosphatase, alkaline phosphatase, and phosphodiesterase) and of certain permeases (such as those for 7-amino-n-butyrate) (Caddick et al. 1986). Considering echinocandin synthesis, echinocandin-producing *A. nidulans* survive in a growth medium having a pH range between 3.5 and 9.0 pH (Caddick

et al. 1986). However, it was also reported that the acidic medium pH is considered to be the most preferable for the production of echinocandins (Kumar and Kumar 2019). In contrast, the optimal pneumocandin level was reported at pH5.2 and is considered to be an ideal pH and no significant change in pneumocandin production was observed between 4.5 and 6.5 pH. However, cells grown over 4.0 pH or higher affect the stability of pneumocandin (Li et al. 2018). Similarly, at acidic pH, different fungal strains induce secondary metabolites production. PacC, a pH responsive transcription factor, has been shown to modulate the SM profile of host as well as supporting their growth at adverse pH conditions (Li et al. 2022).

4 Effects of Medium

Media composition is one of the major factors which directly influence the cell and metabolites' growth. The media required for microbial culture growth should have all nutritional components such as carbon source, nitrogen source, vital amino acids, vitamins, growth factors, and minerals. Despite cell mass, medium composition is also crucial for achieving maximal yield of host synthesized biomolecules. Evaluation of different components of growth medium also helps to achieve maximum yield of bioactive secondary metabolites in fungi. Optimization of carbon and nitrogen sources of the growth medium can promote the production of eucalyptene A and xyloketal A in *Xylaria* sp. by 1.32–1.53-fold (Xiaobo et al. 2006).

The media used for host growth and echinocandin production are seed medium and fermentation medium. Some echinocandin producing growth media have been optimized containing glucose (4%), Soybean powder (2%), KH_2PO_4(0.1%), and trace amount of $CaCl_2$, HBO_3, $CuCl_2$, and $(NH_4)_5MO_7O_2$ with pH5.0. To scale up the echinocandin production, fermentation media contain higher concentration mannitol (8%), glucose (2%) as a carbon source, and peptone (2%) used as a nitrogenous source along with K_2HPO_4 (0.25%) of pH-6.8 (Song et al. 2018). In another study, the synthesis of echinocandin was carried out using seed medium (pH6.6–6.8) having glucose (1%), glycerin (1%), and soybean meal (2.5%). However, the seed medium was optimized that contains peanut oil (2%), glycerin (1%), peptone (0.86%), soybean meal (4%), K_2HPO_4 (0.5%), and trace amount of different salts ($MgSO_4$, $CaCl_2$, $FeSO_4$. $7H_2O$, $MnSO_4$ with pH6.6–6.8) (Niu et al. 2021). The effect of fermented media optimization has also been reported to increase the production of FR901379, a type of echinocandin which potentiated the overall yield of almost 30-fold. Optimized media was achieved by changing the concentrations of different media constituents and addition of other supplements in the basal media (CMA media). Highest production of FR901379 is in the media containing soluble starch (6%), rice oil 3%, soybean flour 2%, cotton seed flour (1%), wheat germ (1%), KH_2O_4 (0.5%), and $MgSO_4.7H_2O$ (0.2%) by mutant M-7 (Kanda et al. 2009).

An additional reporting about enhanced echinocandin production reveals 1.4-fold increase in the production of echinocandin B (ECB) by optimizing the basal media.

Media constituents at the stated concentrations and standard growth parameters were used for optimized growth, which differed from the basal requirements. Peptone (0.86%), K_2HPO_4 (0.84%), mannitol (9.7%), and L-ornithine (0.53%) at pH6.6 and temperature 25 °C were the optimizations employed for increased echinocandin production (Zou et al. 2015). The cell grown in defined medium is also another approach to increase production of specific secondary metabolites because of defined medium that contains known components, which essentially required for cell growth. A defined medium commonly contains carbon, nitrogen, phosphorous, and different metals. For the echinocandin production, varying concentrations of carbon, nitrogen, and other components have been utilized for its optimal production. Some of these important factors are summarized below.

4.1 Effects of Carbon and Nitrogen Source

Carbon source is an essential component of fermentation media which can influence the morphological characterization of mycelium, metabolite production, and cell growth. Filamentous fungus like *Aspergillus nidulans* basically grows in three morphological forms of short hyphal fragments, different size pallets, and dispersed mycelium, which show important relationship with fermentation titer and quality of product (Antecka et al. 2016). It is well versed that mannitol, glucose, glycerol, and methyl-oleate are considered the basic carbon sources used in fermentation process of echinocandin synthesis. Earlier research revealed that supplementation of methyl-oleate in growth medium tends to increase the uptake of the precursor valine during the synthesis of polyether antibiotics by *Streptomyces hygroscopicus* (Mouslim et al. 1997). Similarly, these carbon sources supplemented with equal molar concentration carbon have also been investigated for their effect on echinocandin production which induces the echinocandin yield.

Among the mannitol, glucose, glycerol, and methyl-oleate carbon sources, methyl oleate was found to be the optimal carbon source which produces maximum amount of echinocandin as compared to mannitol, glucose, and glycerol (Niu et al. 2020). In another research, a nitrogen source is used as L-threonine which was identified as a crucial precursor amino acid responsible for echinocandin production (Cacho et al. 2012; Chen et al. 2013). The time-dependent effect of L-threonine concentration inside the cell was tested and found that concentration of L-threonine remains same on fourth day and started to decrease after seventh day. Growth medium supplemented with methyl oleate induced L-threonine production with a 275 mg/L concentration on seventh day, which was observed approximately 2.5-fold greater than mannitol (106 mg/L) (Niu et al. 2020). These findings indicated that L-threonine might be an important echinocandin B precursor.

In case of pneumocandin, B_0 10% mannitol was the preferred carbon source which not only increased production, but also accelerated optimal synthesis faster than other carbon sources (Li et al. 2018). In the scaled-up fermentation, high-cost mannitol was swapped out by less expensive fructose. The group of investigators has demonstrated that mannitol is one of the best carbon sources. However, other carbon

sources also show the complementary effect similar to mannitol. Different studies have also used mixed carbon source for production of echinocandin production and reported that mannitol and glucose used in combination increase production of pneumocandin B_0. It was speculated that mannitol potentiates to generate more amount NADPH as compared to other carbon source. However, glucose used as mixed carbon source tends to induce cell growth as it is radially absorbed by the cells (Qin et al. 2016).

For growth and development of microorganism, nitrogen is also an essential component of nutrient media. A wide range of nitrogen sources have been utilized for production of echinocandin. Ammonium and glutamine are the most preferred nitrogen sources for fungus, and apart from these, some non-preferred nitrogen sources like amides, uric acid, purines, nitrate, pyrimidines, and amines are used in absence of preferred nitrogen sources (Kumar and Kumar 2019; Wong et al. 2007). The pneumocandin producing *G. lozoyensis* is grown with nitrogen sources such as peptone, soyabean powder, corn steep liquor, corn gluten meal, and Cotton-seed powder-Corn gluten meal. Out of them, highest pneumocandin production was measured in Cotton-seed powder-Corn gluten meal followed by peptone. However, the solubility of cotton-seed meal and corn gluten meal in growth medium was sparingly soluble and some undissolved parts of cottonseed meal and corn gluten meal remain available in the medium which tend to increase the viscosity of medium, leading to poor oxygen transport during the fermentation. This limitation of cottonseed meal and corn gluten meal containing medium defers it and prefers peptone in place of it (Qin et al. 2016).

4.2 Effects of Microparticles

Filamentous fungi like *Aspergillus nidulans* show a complex morphology in suspension culture, which often has a significant impact on their production characteristics, like production of various antibiotics, enzymes, etc. (Driouch et al. 2011). Mycelium morphology is also an essential factor for metabolism of filamentous fungi. Microparticles is one of the critical factors that affect the cell growth and secondary metabolites production in fungi. Microparticle elicited variable effects on secondary metabolites synthesis depending on the size of microparticles. Hence, the size of microparticle altered the echinocandins production. Microparticles mainly affect the mycelial morphology of microorganisms and change it from clumped cells to single hyphae. Adequate supply of oxygen and nutrients in unclamped cells is also a reason of enhanced productivity of biomass and enzymatic activities (Walisko et al. 2012). There are many applications which are studied on the basis of microparticles' effects on filamentous microorganisms, like effect of talcum powder and alumina particles is checked on the production of fructo-furanosidase in *A. niger*. (Driouch et al. 2011). To check the effects of microparticles on echinocandin production, different types of microparticles have been administered in the fermentation medium for the purpose of enhancement of echinocandins production and

improvement of the morphology of mycelium (Niu et al. 2020). In an earlier report, two types of microparticles, named as talcum powder (45 μm dia) and glass beads (2 mm dia), have been supplemented with nutrient medium for induction of echinocandin B production in *Aspergillus nidulans*. The findings of this study revealed that talcum powder enhanced the production of echinocandin B up to 47.5% as compared to control (medium without microparticles). It has been observed that talcum powder facilitates interspaces between cell mass which leads to loosely grow the mycelial growth over talcum powder. These interspaces allowed nutrients and oxygen to flow more thoroughly and easily at inner core of the cell mass, leading to improve the echinocandin B production. In contrast, glass bead-supplemented fermented medium relatively suppressed the production of echinocandin B as it did not support cell growth over beads, thereby shearing force of beads tend to cause a compact mycelial growth. The shear-force of beads also limits nutrients and oxygen transport, resulting to decrease echinocandin B production under glass beads conditions (Niu et al. 2020).

4.3 Effects of Surfactants

As earlier studies revealed that echinocandin B is an intracellularly synthesized secondary metabolite which efflux outside through specialized transport machinery (Chen et al. 2017; Niu et al. 2021; Yang et al. 2020). Surfactants tend to help in secretion of intracellularly synthesized metabolites by increasing the cell membrane permeability which slows down the feedback inhibition. Different kinds of surfactants have been used in support of synthesis and induction of echinocandins production. Tween 80, Tween 20, and SDS are some common examples of surfactants which have been studied previously. The supplementation of tween 80 surfactant in the fermented medium increased the synthesis of echinocandin level up to 19.1% with significantly decreased dry cell weight up to 9.8%. (Liu et al. 2021). This is due to the amphiphilic nature of tween 80 which mimics phospholipids' structure of cell membrane and easily fits into lipid bilayer, resulting in increased cell permeability, and induces echinocandin production. Moreover, addition of SDS in the fermented medium remarkably suppressed the cell dry weight and echinocandin production sharply on concentration-dependent manner and showed toxic effect for *A. nidulans* growth. In contrast, *Glarea lozoyensis* elicited an opposite effect that is capable to increase pneumocandin B_0 production on 13th day of incubation of SDS (0.1%) administration into fermentation medium (Yuan et al. 2019).

5 Genetic Regulation in Echinocandin Production

Different earlier reports investigated the effect of the genetic manipulations in the gene cluster of echinocandin which stimulates the production by different extent. The induction of echinocandin production was observed due to transcriptional

activation of echinocandin biosynthesis genes through regulatory cascades (Soukup et al. 2012). Genetic regulation is also a parameter for enhancement of echinocandins' production. Among all the living entities, the regulation of their metabolic profiles is widely controlled by different transcription factors (TFs). Transcription factors are regulatory proteins which modulate expression of genes involved in several biological processes. The transcription factors can be categorized based on their ability to modulate different cellular processes, such as those involved in supporting growth at different pH, temperature, reactive oxygen species, hypoxic conditions, biofilm formation, and iron availability conditions.

Different transactional global regulators have been reported in fungi that showed regulatory role under different stimulatory conditions. PacC, CreA, AreA/B, Yap1-HapC/E, LaeA-VeA/B, and HapX and SAGA-ADA are some examples of regulatory proteins that involved in pH, Carbon, Nitrogen, Redox, Light, Iron, and interspecies responsive conditions, respectively. The pH-responsive PacC factor activates after cleavage by Pal signal transduction component that leads to yield PacC27. The activated PacC modulates gene expression of secondary metabolites under acidic or alkaline pH conditions. It suppresses gene regulation under acidic pH conditions and supports growth under alkaline conditions (Tilburn et al. 1995). Hence, PacC expression in *A. nidulans* was found to be minimal under acidic conditions, but increased greatly either at neutral to alkaline growth conditions (Li et al. 2022). Similarly, CreA, a carbon responsive transcription factor of fungi, is involved in the regulation of secondary metabolites production in carbon metabolism. CreA reported to negatively regulate the penicillin synthesis in *P. chrysogenum* when it was grown in presence of glucose (Cepeda García et al. 2014). AreA and AreB are nitrogen-responsive regulators that modulate secondary metabolism production (Pfannmüller et al. 2017). However, they primarily control the nitrate assimilation under nitrogen starvation conditions (Michielse et al. 2014). Iron responsive transcription factor, HapX, binds with a CBC complex (HapB, HapC, and HapE) under iron depleted conditions to activate genes responsible for iron acquisition (Hortschansky et al. 2007). The synthesis of penicillin and aflatoxin is reported to be regulated by HapX and Yap1, respectively, that act to activate CBC complex in *P. chyrsogenum* and *A. parasiticus*, respectively (Brakhage 2013).

A very few pathways-specific transactional regulatory proteins have also been studied previously in echinocandin production. Deletion study of GLOXY4 in *G lozoyensis* resulted to inhibit pneumocandin A_0 production and enhance pneumocandin B_0 production by 9.5-fold. The knockout of GLOXY4 tends to replace 3S-hydroxyl-4S-methyl-L-proline with 3S-hydroxyl-L-proline at sixth position of pneumocandin B_0, which leads to induce excessive production of pneumocandin B_0 (Chen et al. 2015). This report indicates that the GLOXY4 has a role in 3S-hydroxyl-4S-methyl-L-proline formation. Another report stated that the level of echinocandins can also be enhanced by duplicating the different genes present in gene cluster or by switching the native promoter to a strong constitutive promoter (Li et al. 2018). Overexpression of transcription factor encoding gene, *frbF,* increased the production of FR901469 (echinocandin like molecule) by up to 3.4 times (Matsui et al. 2016). The recent study has also revealed to decipher the role

Table 20.1 List of mutants developed for the enhancement of echinocandin production in fungi

Parent strain	Mutant strain	Mutagenesis method	Increased Yield	Reference
A. nidulans ZJB-0817	*A. nidulans* ULN-59	UV irradiation, lithium chloride, and sodium nitrite treatment	1583.1 ± 40.9 mg/L (2.1-fold)	Zou et al. (2015)
A. nidulans ULN-59	*A. nidulans* ZJB12073	UV and microwave irradiation	1656.3 ± 40.3 mg/L (1.9-fold)	Hu et al. (2016)
A. nidulans ZJB12073	*A. nidulans* ZJB19033	Atmospheric and room temperature plasma (ARTP) treatment, medium optimization	2425.9 ± 43.8 mg/L (1.3-fold compared to unoptimized media)	Hu et al. (2020)
Zalerion arboricola ATCC 20868	*Z. arboricola* ATCC 74030	N-nitroso-N-methylurethane (NMU) and N-methyl-N-nitro-N-nitrosoguanidine (NTG)	2.5-fold	Masurekar et al. (1992)
G. Lozoyensis CCTCC M2014416	N. A	Low temperature adaptive laboratory evolution (ALE)	32% higher than parent	Song et al. (2018)
G. lozoyensis ATCC 74030	*G. Lozoyensis* Q1	Atmospheric and room temperature plasma (ARTP) treatment	1.39-fold increase	Qin et al. (2016)

of in-clustered transcription factor present in the biosynthetic genes of Echinocandin B in *Emericella rugulosa* NRRL 11440. The knockout mutant of ecdB (a transcription factor of echinocandin B) led not to hamper the bioavailability or production of echinocandin B (Kumar and Kumar 2019).

Site-specific mutations have also been attempted for upscaling the secondary metabolite production in different organisms, *Beauveria bassiana, Monascus purpureus,* etc. (Xiong et al. 2021; Zhang et al. 2022). For increasing echinocandin B production, site-specific mutations have not been studied very widely. The random mutations have been employed in echinocandin-producing strains such as *A. nidulans, Z. arboricola,* and *G. lozoyensis* as shown in Table 20.1. Random mutations by the means of UV irradiation, atmospheric and room temperature plasma (ARTP) treatment, low temperature adaptive laboratory evolution (ALE), microwave irradiation, and chemical mutagens have shown significant increase in ECB biosynthesis levels (Zou et al. 2015; Hu et al. 2016, 2020; Masurekar et al. 1992; Song et al. 2018; Qin et al. 2016).

6 Conclusion

This chapter discussed various growth parameters and strategies for enhancement of echinocandin production such as temperature, pH, media composition, etc. The discussed contributing factors have shown significant effects in the echinocandin

synthesis levels. Optimized concentrations of media constituents such as carbon and nitrogen sources and supplementation with microparticles and surfactants have laid major impact on the echinocandin biosynthesis. The combination of methyl oleate, mannitol, and glucose has been designated the best carbon source for echinocandins production; however, ammonium and glutamine have proven to be the best nitrogen source. Supplementation of microparticles such as talcum powder and glass beads has also significantly upregulated the echinocandin biosynthesis by regulating the mycelial morphology. Echinocandins are often synthesized intracellularly which turned to release extracellularly after administration of surfactants in the fermented culture and release outside the cell. The highest production of echinocandin B and pneumocandin B_0 was optimized with Tween 80 and SDS administration in the growth medium. The temperature required for cellular growth and echinocandin production has also been discussed and temperature shift strategy was found the most promising for maximum echinocandins yield. Different set of studies determined that echinocandins production are the highest acquired below than the cell growth temperature. Additionally, acidic pH was found the most preferable for echinocandin production. However, some strategies such as site-specific mutations, chromatin remodelling, and effect of global and local transcription factors need to be studied extensively for maximizing the yield of echinocandins.

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Part VII
Other By Products of Fungal Metabolism with Medicinal Applications

Chapter 21
Fungal Pigments: Applications and Their Medicinal Potential

Dhionne Correia Gomes

Abstract Color plays an important role in many segments of food, cosmetic, and textile industry; however, in the last century, the deliberate use of synthetic pigments demonstrated the urge for natural alternatives. Pigments produced by fungi have many advantages over synthetic ones, such as low toxicity, the ease of controlled production, the possibility of optimization, and the independence of external seasonal factors. Some metabolites produced by fungi are already commercialized as pigments, notably β-carotene, produced by *Blakeslea trispora*, and *Monascus* pigments, found in more than 20 types of products in oriental markets. Pigments produced by fungi are divided into melanins, carotenoids, and polyketides, the latter containing the classes anthraquinones, hydroxyanthraquinones, naphthoquinones, and azaphilones, representing a wide range of colors, including the commonly obtained hues yellow, orange, and red, in addition to brown and dark pigments, such as melanins polymers, and also atypical colors, such as the blue-green pigment xylindein, produced by *Chlorociboria aeruginascens*. Those pigments are mainly produced by fungi as a defense mechanism against UV light, but also have other important biological functions related to the transport of metals, differentiation, interaction with other organisms, either by symbiosis or competition, or even defense against insects and other animal predators. For this reason, these substances are biologically active, and their functions can be extrapolated to the pharmaceutical industry, with many reports in the literature of their potential as antimicrobial, antifungal, antiviral, antioxidant, cytotoxic, nematicidal, anti-inflammatory, and antitumor agents. However, industrial use of fungal pigments still faces some challenges such as the search for better production yields, the targeted synthesis of only one specific compound or color, and more essentially, a mycotoxin-free production process. Many studies have shown the importance of factors related to the fungus cultivation such as temperature, medium agitation/oxygenation, carbon source, nitrogen source, and pH, the last two being considered the most important factors related to the production of colored metabolites. However, the chemical reaction of pigments with components of the culture medium must also be

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considered, as an example the oxidation of azaphilones, leading to the conversion of yellow pigments into orange, and the reaction of the same class of substances with amines and nitrogenated compounds, which leads to the production of red pigments in some species. Fungal pigments are gaining ground in different industrial branches, and new uses for pigments are constantly emerging, such as their application as biosensors and as electron acceptors in biofuel cells.

Keywords Color · Fungal pigments · Secondary metabolites · Food industry · Chromophore · Bioactivity

1 Introduction

1.1 Synthetic Dyes

Colors are essential for living beings, as many important biologicals processes, like metal transportation and photosynthesis, rely deeply on pigments. It's safe to say that colored molecules helped living beings not only to adapt, but to thrive in this planet (Sajjad et al. 2020). The perception of color by animals and humans is key for many biological roles, like camouflaging defense mechanisms, aposematic warning signs, in promoting sexual attraction, and in our food choice inclinations (Meruvu and dos Santos 2021). Ultimately, all life on earth depends on light energy from the sun, which is converted into other forms of energy or biomass by photosynthetic organisms, via pigments such as chlorophylls in plants and bacteriochlorophylls and phycobilins in microbial photosynthesis. Visible light, which ranges from 380 to 750 nm, is absorbed in a molecular region usually composed of sequential conjugated double bonds named chromophore. The energy then can be transferred to auxochromes, which are functional groups bonded to the chromophore, and the resulting emitted light gives us the perception of color. Many organisms produce a wide variety of pigments, but surely microorganisms stand out (Khan et al. 2021).

For humans, pigments have always been an important part of the culture, since prehistoric era, when they were used for decoration and for preservative and architectural purposes (Meruvu and dos Santos 2021). Insects and plants were historically used as the main source of pigments, until 1856, when the first synthetic dye, mauvine, was produced by Sir William Henry Perkin (Sajjad et al. 2020). After that, boosted by the industrial revolution, synthetic dyes' research and production grew exponentially in order to meet the market's demand (Heo et al. 2018). Dyes are usually small molecules with aryl ring, easily soluble in water or other solvents, that tend to adhere to surfaces either via physical adsorption or mechanical retention, and therefore, highly used in textile processes. On the other hand, pigments are those colorants usually insoluble in water that tend to form bigger particles, with 1–2 μm (Shindy 2016). Despite solubility, natural compounds are usually called pigments.

Colorants are important to maintain products' quality and acceptance, for example, in food industry, where pigments are used to make food look more attractive (Sajid and Akbar 2018). However, many of the first synthetic dyes were produced

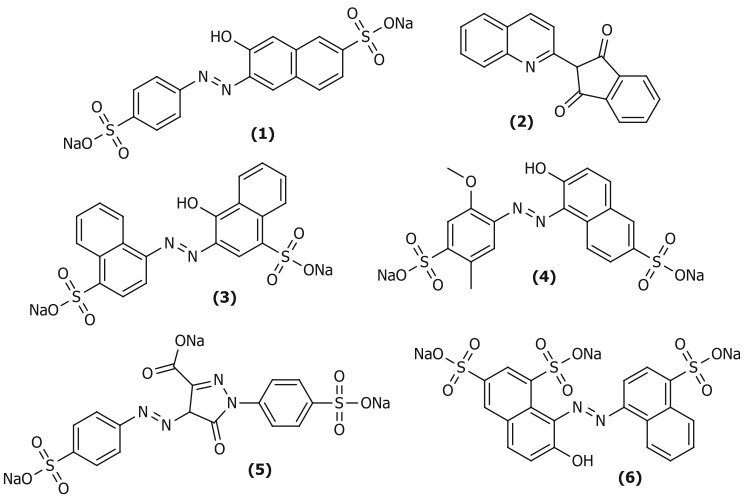

Fig. 21.1 Synthetic dyes used in food industry

using toxic compounds like lead, mercury, copper, chromium, sodium chloride, benzene, and toluene. Toxicological studies in the last century proved many largely used pigments to be harmful to humans and toxic to the environment (Sajjad et al. 2020). Some dyes used in the textile industry are carcinogenic and may trigger allergic reactions to humans, besides requiring strong acids, alkalis, solvents, and heavy metal catalysts in their production (Venil et al. 2020). The consumption of six pigments used in the food industry (sunset yellow FCF (**1**), quinoline yellow (**2**), carmoisine (**3**), allura red (**4**), tartrazine (**5**), and ponceau 4R (**6**)), known as "the Southampton six", showed in Fig. 21.1, was linked in 2007 to hyperactivity in children (McCann et al. 2007). Some of these pigments were also related to allergic reactions like urticaria, dermatitis, angioedema, and the exacerbation of asthmatic symptoms (Poorniammal et al. 2021). In 2009, the European Food and Safety Authority (EFSA) started a reevaluation and lowered the acceptable daily intake of the aforementioned sunset yellow (**1**), quinoline yellow (**2**), and ponceau 4R (**6**) (Palacio-Barrera et al. 2019). Along with that, many other synthetically derived colorants were banned by the FDA, including ethyl acrylate, benzophenone, eugenyl methyl ether (methyl eugenol), pulegone, myrcene, and pyridine (Sajjad et al. 2020). However, there is still an important share of the market dedicated to FDA- and EU-approved synthetic pigments, such as mono and di-azo dyes, triarylmethane derivatives, xanthenes dyes, quinophthalones, and indigoid compounds, along with some mineral dyes such as titanium dioxide, calcium carbonate, and iron oxides (Rana et al. 2021). Azo dyes, such as the Southampton six, are the most used synthetic food colorants; however, they are reported as toxic, since they can be metabolized by the intestinal microbiota into carcinogenic products (Poorniammal et al. 2021). This deliberate use of synthetic dyes in the last century led to a demand

of natural pigments that tend to be less toxic to humans and to the environment (Tirumale and Wani 2018).

1.2 Natural Fungal Pigments

Natural pigments are obtained from many sources, mainly plants and microalgae, and proved to be better alternatives to synthetic ones because of their fast production, easy processing, and bioactivity (Venkatachalam et al. 2018b). Natural pigments are considered safe if they are nontoxic, nonallergic, biodegradable, noncarcinogenic, and if they render no risk to the environment (Khan et al. 2021). Fungi represent another valuable source of natural pigments, since they can be obtained in large quantity, using cheap culture medium (Sajid and Akbar 2018). Also, the industrial production of fungal metabolites is independent of geographic and seasonal constraints, can be better controlled and optimized via bioreactor fermentation, and can be genetically manipulated. Many fungal pigments discovered in the last decades are highly stable towards biotic (microbes and enzymes) and abiotic (light, heat, and pH) agents (Venkatachalam et al. 2020). This makes it possible for fungal pigments replace even natural pigments such as curcumin and betacyanin, widely used, but with low stability (Palacio-Barrera et al. 2019). It has been shown that *Talaromyces* pigments are more stable to ozone food processing than traditional food colorants, and they are good alternatives in juice industry (Aguilar et al. 2018). Actually, the high stability of fungal pigments is proved in ancient samples of burnt soil and coal dating back to the medieval period (738–1411 CE), where *Hypoxylon fragiforme* azaphilones were found. Mitorubin type azaphylones, orsellinic acid, along with seven novel molecules, namely rutilin C-D and fragirubin A-E, could be identified in the sample, as it remained stable for all these years, mainly due to a waxy layer protecting the stromata that kept the pigments unexposed to the environment (Surup et al. 2018).

Large-scale use of natural fungal pigments has only started recently, as it depends first on consumers' acceptance and also on the development of biotechnological techniques and subsequential regulatory approval. It is relevant that many other fungal-derived ingredients are being approved and used in the food industry, like thickening or gelling agents, flavor enhancers, polyunsaturated fatty acids, flavor compounds, vitamins, essential amino acids, and acidulants (Venkatachalam et al. 2018b). Nowadays, some fungal pigments are already being marketed, like polyketide pigments from *Monascus* species, Arpink red (**7**) from *Penicillium oxalicum*, riboflavin (**8**) from *Ashbya gossypii*, astaxanthin (**9**) from *Xanthophyllomyces dendrorhous*, β-carotene (**10**) from *Blakeslea trispora,* and lycopene (**11**) from *Erwinia uredovora* and *Fusarium sporotrichioides*, showed in Fig. 21.2 (Sajid and Akbar 2018; Venkatachalam et al. 2018a). A yearly growth of 5–10% is projected for the natural colorants market, reaching revenues of $1620 million by 2023, while synthetic dyes market is projected to grow 3–5%. Carotenoids global market is expected to reach $2000 million by 2026, and riboflavin ranging from $7790 to

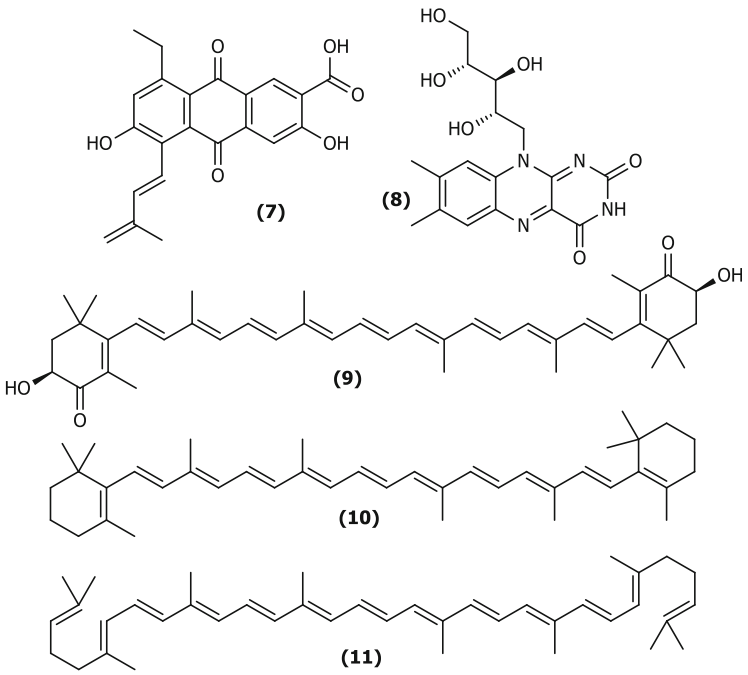

Fig. 21.2 Fungal pigments marketed

$10,300 until 2024 (Meruvu and dos Santos 2021). However, the market price of natural pigments is still high due to low production levels (Heo et al. 2018). Some authors report that, even with its high costs, natural pigments can still be competitive due to its natural and safe labels (Poorniammal et al. 2021).

Most fungal pigments discovered nowadays are derived from *Penicillium* (and its correlated genus *Talaromyces*) and *Aspergillus*, followed by *Fusarium* and *Cladosporium* genera. Pigments are not correlated to fungi grown, but to their protection and competition in natural habitat, and therefore, many of these molecules are bioactive (Venkatachalam et al. 2018a). For example, polyketides and melanins protect the fungus against environmental stress, carotenoids are important against photo-oxidations, and flavins act as enzymatic cofactors (Venkatachalam et al. 2018b). Aurofusarin, a bis-naphthoquinone produced by *Fusarium* species, is important to their protection against animal predators such as springtails, woodlice, and mealworms (Xu et al. 2019). Fungal pigments in higher fungi can start local and global processes, for example, defense and carbon cycling, respectively (Tauber et al. 2018). Pigment production is species-specific, and the wide variety and ubiquity of fungi in nature also indicate a wide variety of molecules and, consequentially, hues (Venkatachalam et al. 2020). This potential is extended by the fact that approximately 90% of biosynthetic gene clusters for fungal secondary metabolites remain silent or cryptic under laboratory conditions (Paillìè-Jiménez et al. 2020). Even though genes in fungi are clustered, the production of secondary

metabolites is many times branched, affected by different enzymes and by the media constituents. For this fact, many times a pigment's initial structure can be modified and lead to the formation of many other molecules. In *Penicillium chrysogenum*, for example, the production of the yellow pigment chrysogine starts with the gene-mediated condensation of anthranilic acid and alanine into the intermediate 2-(2-aminopropanamido)benzoic acid. From this molecule, at least 13 different chrysogine-related compounds can be formed (Viggiano et al. 2018). All those facts show that, even though many new molecules are being discovered in recent years, there is an even greater potential for more findings, due also to new developments regarding media manipulation and postproduction (Venkatachalam et al. 2020). The number of genetically identified natural product pathways in fungi usually exceeds the number of known compounds from the same species, the reason why most natural products remain as "dark matter" (Tauber et al. 2018).

Fungi usually produce a mix of pigments, and the fungal colorimetric range is genus and/or species-specific, being an important descriptive characteristic (Valenzuela-Gloria et al. 2021). Most pigment-producing fungi tend to form molecules that absorb low wavelengths, since it requires a shorter chromophore that is easier to synthetize. For this reason, yellow and orange pigments are the most common in fungi extracts, as the yellow emodin (**12**) and felinone A (**13**), and the orange questin (**14**) and sclerotiorin (**15**). Many recent studies focus on finding red colorants, since there is a high demand for this hue in the textile, food, cosmetics, and pharmaceutical industries, and there are few natural alternatives to synthetic red dyes. Some red pigment produced by fungi are rubrocristin (**16**) and atrorosin S (**17**) (Lebeau et al. 2020a). Purple pigments are rarer, and most natural purple pigments used today are anthocyanins from red cabbage and betalain from red beet (Lebeau et al. 2019). Viopurpurin (**18**) is a metabolite produced by *Aspergillus melleus* with dark-purple color (Melo et al. 2019). However, pigments with unusual colors that absorb higher wavelengths have been described, as an example, the blue albatrellin (**19**), produced by *Alabtrellus flettii* (dos Santos and Bicas 2021), green cycloleucomelone (**20**), produced by *Aspergillus niger* (Melo et al. 2019), and also some magenta unidentified pigment produced by *Phoma herbarum* (Chiba et al. 2006). The structures of the pigments are described in Fig. 21.3, along with the proper chromophore highlighted.

2 Fungal Pigments Sources

Pigment producing fungi can be found in many habitats. One of the most unusual records is the deterioration of paper cultural heritage. Fungi, most of them from the genera *Aspergillus* and *Penicillium*, are recorded to grow in books, prints, drawings, watercolors, engravings, and other works of art based on paper, producing mostly brown stains. From a total of 80 different colorants detected, polyketide quinones, especially hydroxyanthraquinones, are the most predominant (Melo et al. 2019).

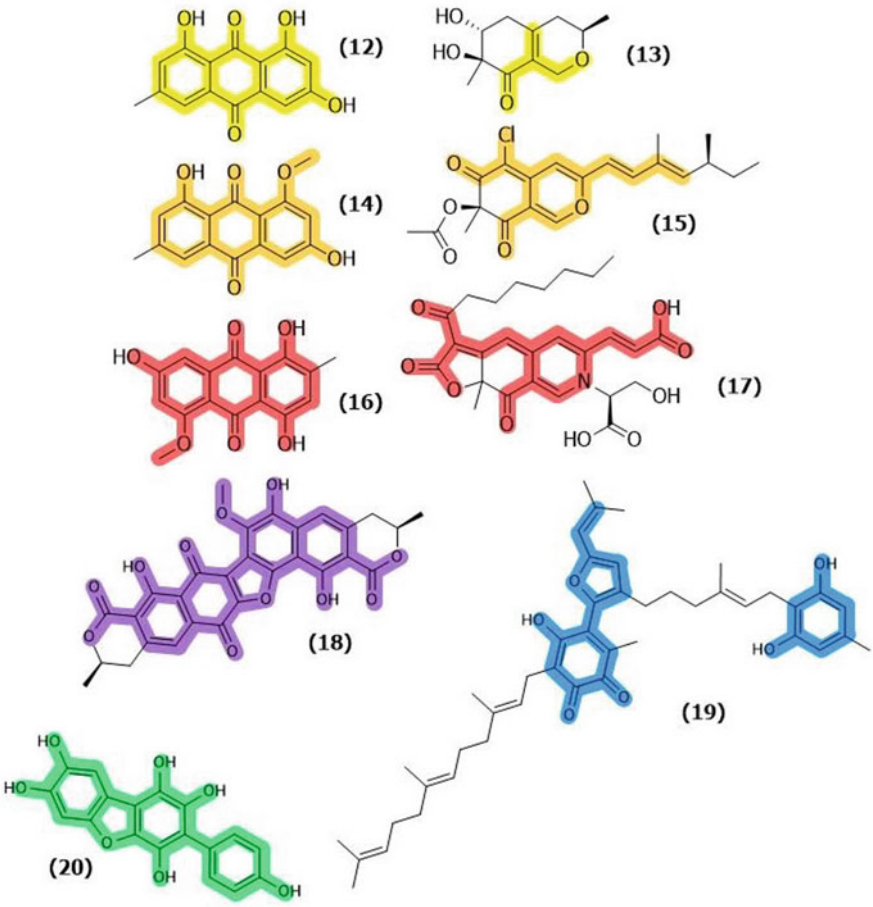

Fig. 21.3 Fungal pigments and their chromophores

In extreme conditions, such as the deep sea and the polar desert, it is expected to find fungi with a high expression of secondary metabolites, since their production, and more specifically pigments production, is fundamental for fungal survival. In the cryosphere, for example, the production of pigments and morphological alterations are some of the mechanisms that fungi possess in order to endure the many stress conditions in this habitat, such as extremely low temperatures, oligotrophic conditions, freeze–thaw cycles, ultraviolet radiations, and higher salinity. For this reason, Antarctic fungi have great physiological plasticity and capacity of producing many metabolites. Most common Antarctic fungi metabolites are melanins and carotenoid derivatives such as phoenicoxhantin (**21**) and γ-carotene (**22**) that are useful to not only absorb UV radiation, but also act as cryoprotectants, antioxidants, and antimicrobials (Sajjad et al. 2020). On the same way, marine fungi, which are adapted to survive not only the halophilic conditions, but also low temperature, high pressure,

and dark, tend to produce a variety of pigments. Marine fungi can be found in sponges, algae, but are more successful in niches like mangrove, because of their rich organic matter (Deshmukh et al. 2018). Pigment-producing marine fungi can tolerate radiation and hydration/dehydration cycles better than nonpigmented (Venil et al. 2020). The so-called "halophilic pigments" represent a new class of substances with promising uses in pharmaceuticals and cosmetology (Sajid and Akbar 2018). Halophilic pigments, with a wide range of colors, have been reported in the genera *Aspergillus*, *Cladosporium*, *Eurotium*, *Monascus*, *Penicillium*, *Talaromyces*, and *Trichoderma* (Venkatachalam et al. 2020). Anthraquinones, naphthoquinones, and hydroxyanthraquinones are found in marine environments, but fungal azaphilones stand out. They proved to not only provide resistance to environmental hazards, but also to enhance ecological interactions with sponges, corals, and associated microorganisms. Some marine pigments are halimide (**23**) from *Aspergillus ustus*, and the marron chaetoglobin (**24**), chaetoglobosin (**25**) and chaetomugilin (**26**), and purple cochliodinol (**27**), isolated from *Chaetomium globosum* (Meruvu and dos Santos 2021). Marine pigments are similar to those produced by terrestrial fungi, but some are only found in marine environments, such as the yellow anthracene glycoside asperflavin-ribofuranoside (**28**), extracted from *Microsporum* sp. (Sajid and Akbar 2018).

Another source of pigments are the spalting fungi that grow inside wood producing stains that vary greatly in color. The spalted wood is traditionally used by woodworkers in art and craft pieces, but in recent years, many studies focus on extracting and identifying these pigments (Robinson et al. 2018). Fungi can change the color of wood by direct pigment production or by other factors like dehydration or discoloration caused by degradation of lignin. The most important spalting fungi are *Chlorociboria* spp., producing blue-green stains caused mainly by the metabolite xylindein (**29**), and *Scytalidium cuboideum*, that produces draconin-red (**30**), causing red stains in wood (Pittis et al. 2018). Besides that, *S. cuboideum* and *Scytalidium ganodermophthorum* can produce, respectively, other red and yellow unidentified metabolites. These spalting pigments are highly stable, and some vibrant blue-green pigments produced by *Chlorociboria aeruginosa* can still be seen in wood art pieces from the 1400s to 1700s. These pigments also have high UV-light resistance and are potential substitutes for anilines in textile dyeing (Gutierrez et al. 2021). The main issue regarding spalting fungi is pigment extraction, since it requires great amounts of organic solvents, mainly dichloromethane. Natural oils like linseed oil are being tested as an alternative to organic solvents, but many pigments suffer complete discoloration in this media (Robinson et al. 2018).

Regarding fungi growing in caves, there's also a great potential for discovering new pigments. First, this is because this habitat contains extreme environmental conditions that can select microorganisms able to produce bioactive metabolites. Also, many of these ecosystems are yet to be discovered. In Brazil solely, it is estimated that there are more than 100,000 caves, where most of their underground biota is still unknown. The pigments oosporein (**31**), dihydrotrichodimerol (**32**), and oravactaene (**33**) were isolated from the cave fungi *Lecanicillium aphanocladii*, *Epicoccum nigrum,* and *Penicillium flavigenum* (Tavares et al. 2018). Endophytic

fungi produce pigments with great antimicrobial activity, as reported against *Klebsiella pneumoniae*, *Staphylococcus aureus, Salmonella typhi,* and *Vibrio cholera*. This activity is superior to commercially available antibiotics such as streptomycin (Visalakchi and Muthumary 2014). Also, many fungal metabolites are active against multiresistant species such as *S. aureus* and *Mycobacterium tuberculosis* (Deshmukh et al. 2015). The rust fungi, or "rusts", are a group of plant pathogens belonging to the order *Puccinales*, with the ability to cause colored rusty-like infections. Spores provide color to those fungi, with *Uredinopsis pteridis* producing white spores, *Puccinia striiformis* yellow-orange, and *Puccinia graminis* reddish brown. The color is due to carotenoid accumulation in lipid droplets in the cytoplasm and other pigments associated to the cell wall (Wang et al. 2018a). Figure 21.4 shows the most common pigments found in extreme habitats.

3 Fungal Pigments Applications

Fungal pigments are very versatile and are useful in several branches of the industry, one of the most important being in fabric dyeing. These compounds have great potential in textile industries because of their excellent colorfastness and staining properties (Venil et al. 2020). Basidiomycetous fungi were traditionally used by ancient cultures to dye silk and wool, but due to difficulties in bulk production, filamentous fungi are now used (Kalra et al. 2020). Most common fungi in this area are: *Monascus purpureus*, *Monascus ruber*, *Emericella nidulans*, *Fusarium verticillioides*, *Isaria farinosa*, *Thermomyces* sp., and *Penicillium marneffei*, besides the spalting fungi *C. aeruginosa* and *S. cuboideum* (Meruvu and dos Santos 2021). The applications of *Monascus* like pigments, produced by *Penicillium* sp., to dye textile, cotton, wool, leather, paper, and paint were patented (Mapari et al. 2011). Due to its high stability, spalting pigments xylindein (**29**) and draconin-red (**30**) are successful in dyeing many types of fabric without needing additional chemicals (Venil et al. 2020). The pigment draconin-red (**30**) from *S. cuboideum* not only is effective to dye fabrics, but also showed high UV-light resistance, even after machine washing and crocking. This pigment also can be used in coatings, since it persisted after 500 h of weathering, showing its potential as an additive in decking finishes, to help delay greying (Gutierrez et al. 2021). Draconin-red (**30**), when applied to fabric using linseed oil as carrier, and going through a heating process, is able to form a stable, shiny fabric surface, with potential use in waterproof garments (Agurto et al. 2020).

Since fungal pigments can absorb UV-light when applied to fabrics, they can protect human skin from harmful UV radiation. Also, a yellow pigment from *Thermomyces* sp., with antimicrobial properties, is reported as potential coating in medical equipments like bandages, masks, and wound dressings (Poorniammal et al. 2021). Other examples are the red pigment bostrycin, from *Nigrospora aurantiaca*, pH and temperature stable, useful to dye cotton fibers (Suwannarach et al. 2019a), ankaflavin from *Penicillium* sp., with strong affinity with wool, due to its ionic

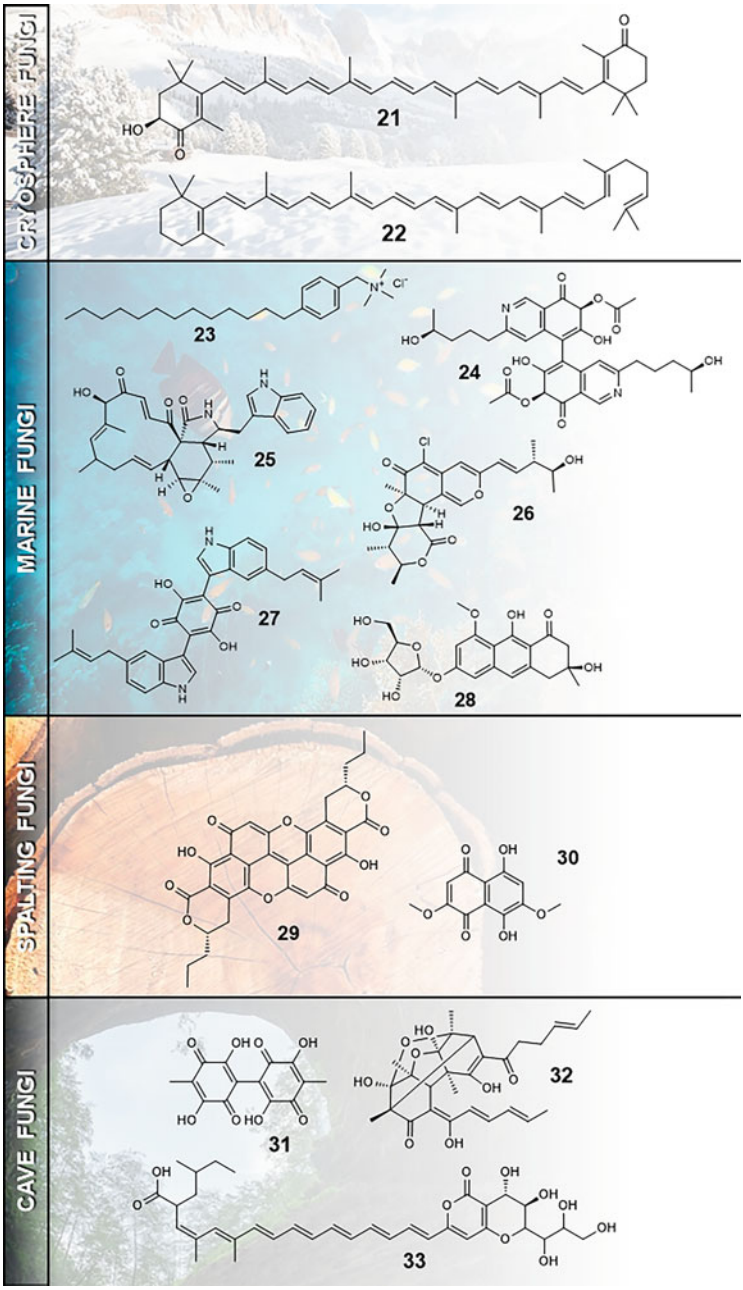

Fig. 21.4 Fungal pigments found on extreme environments

Fig. 21.5 Red yeast rice (*Monascus ruber*) and oncom (*Neurospora intermedia*)

nature (Afshari et al. 2015), and cynodontin from *Curvularia lunata*, reported as good substitute to the synthetic Disperse blue 7 and Acid green 28 (Venil et al. 2020).

In food preparations, pigmented fungi improve digestibility and nutritional characteristics, and the excreted pigments can also provide bioactivity. However, these pigments must be compatible with the food flavor, safety, and must have a minimal impact on the price (Sen et al. 2019). *Neurospora intermedia* is an orange edible fungus traditionally used for preparation of the Indonesian food oncom (Gmoser et al. 2019). *Monascus* pigments have been used in Oriental/Asian countries for many years, especially in the food and beverage industry, where more than 20 kinds of products are known (Venkatachalam et al. 2018a). In Fig. 21.5, *Monascus* and *Neurospora intermedia* food derivatives are shown. Some *Monascus* species produce edible pigments like monacolins, dimerumic acid, ergosterol, and γ-aminobutyric acid, presenting bioactivity (Chen et al. 2015). In short, the pigments' ability to neutralize free radicals indicates their potential in preventing various diseases such as cardiovascular, autoimmune disorders, cancer, and diabetes. Antioxidant activity is reported for a great number of fungal pigments like carotenoids, violacein, and naphthaquinones (Sajid and Akbar 2018). The bioactivity of each pigment group will be further discussed on topics 4–6.

4 Pigment Production

The industrial production of fungal pigments depends on basically three main factors: the pigment yield, the absence of mycotoxin, and the pigment stability and purity. These parameters can be improved via media manipulation, genetic manipulation, and other techniques (Kalra et al. 2020). Also, most fungi produce a mixture of intra and extracellular pigments, and another challenge is to obtain only one specific color tone (Venil et al. 2020).

4.1 Pigment Yield

Many fungi are naturally great colorant producers, for example the genus *Penicillium*, which is recognized as more efficient and profitable than any other microorganism. These fungi can produce high amounts of enzymes and pigments, and also secrete them out of the cell and, as most of these metabolites are stable and water-soluble, they are easy to purify (Poorniammal et al. 2021). Water-soluble pigments are most suitable for industrial production, since they are easier to mass produce, and to extract, without needing hazardous organic solvents (Heo et al. 2018).

The easiest way to enhance pigment yield is by inducing biomass growth, and therefore, the accumulation of intracellular pigments (Meruvu and dos Santos 2021). However, it is known that, in fungi, biomass growth and pigment production are negatively correlated (Venil et al. 2020). Actually, pigment production often occurs after fungal growth has ceased or due to an environmental signal, such as nutrient limitation or external stress. This shift from primary to secondary metabolism occurs in order to preserve fungal energy sources and the production of essential metabolites for better growth conditions (Gmoser et al. 2019). Secondary metabolism, and consequentially, the production of pigments, is influenced by global regulators. These mediators are responsible for directly or indirectly activating specific genes, and they respond to many abiotic factors, like nutrient availability, light, temperature, and pH (Lebeau et al. 2019).

Light influences not only pigment production in fungi, but also the whole metabolism. One of the most important secondary metabolites master regulators in fungi, LaeA-VeA, is affected by light. Studies with an endophytic unidentified fungus show that, in presence of light, the mycelium acquires a dark green color, and when cultivated in the dark, the fungus does not produce any pigment, and its secondary metabolism is directed to taxol production. In this specific case, the photoreceptor opsin is responsible for light perception and pigment production, and when the fungus is growing inside the plant, the photoreceptor is not active, and taxol is produced to complement the host defense mechanism (Soliman and Raizada 2018). It is supposed that fungi, at least Basidiomycetes, possesses a complex photosensitizer system used as defense mechanism that resembles the one from plants. This defense mechanism is based on the pigment activation via light absorption and the production of a lethal sign, which can be a radical form of the molecule itself, a singlet oxygen species, a configurational isomer (cis/trans), or a new molecule photochemically formed. For example, the fruiting bodies of the mushroom *Cortinarius austrovenetus*, whenever is nibbled by insects, generates red-violet stains on the bite site. This is due to the formation of hypericin, a pigment produced by oxidation of austrovenetin, once the site is exposed to light (Siewert 2021).

In *Neurospora intermedia*, light is necessary to produce pigments, and the highest amount of pigment is achieved with continuous light exposure. However, fungal cells exposed for both 1 min and 1 h also produced high quantities of pigments, showing that even a short amount of light exposure is enough to trigger pigment

production mechanisms (Gmoser et al. 2019). For *Dacryopinax spathularia* and the yeast *Rhodotorula glutinis*, light is necessary for carotenoids production (Ramesh et al. 2019). The light wavelength should also be considered, as exemplified by *Neurospora* species, in which red light has no effect on pigment production, but blue and also white light stimulate it (Gmoser et al. 2019). Blue, along with green light, is reported to stimulate bikaverin production in *Fusarium oxysporum* (Palacio-Barrera et al. 2019).

In media optimization, submerged fermentation (SF) is favored against solid state fermentation (SSF) due to the facility of adjusting media parameters, such as temperature, pH, incubation time, nutritional sources, aeration, agitation rate, and so on (Venkatachalam et al. 2020). However, the lower water content in solid media can be considered a stress factor to stimulate metabolite production in many fungi (Molelekoa et al. 2021). Some techniques are used to evaluate the effect of sole parameters, and also the interactions between them in pigment production and, regarding that, Response surface methodology (RSM) is one of the most successful. In recent years, Artificial neural network (ANN) showed the power of computational science in predicting fungal behavior and metabolite production. In *Monascus purpureus*, an ANN model was able to predict the effects of fermentation parameters on red pigment production with a high correlation (Singh et al. 2015).

The carbon and nitrogen source are the major factors regarding secondary metabolites production. Some carbon sources like glucose and nitrogen sources ammonium sulphate, peptone, and amino acids are reported to induce pigment formation (Ramesh et al. 2019). A carbon:nitrogen ratio equal or higher than seven promotes high pigmentation in *Fusarium oxysporum*, while for *Aspergillus chevalieri*, the ideal C:N is among 10–20 (Palacio-Barrera et al. 2019). However, even small shifts in media composition can significantly alter the products extracted. *Fusarium graminearum*, for example, is a well-known producer of aurofusarins, but changes in media composition, especially in the pH and nitrogen source, can redirect fungal metabolism to prioritize citreoisocumarins production (Westphal et al. 2018). Growing the fungi in a "minimal" nutrient medium, such as the Defined minimal dextrose (DMD) broth, can be considered a stress factor that enhances pigment production, since the fungus has to grow fast and develop protective strategies (Lebeau et al. 2019). Genomic studies concerning *M. purpureus* show a high expression of genes correlated to pigment production on carbon starvation (Pailliè-Jiménez et al. 2020). Nitrogen content affects pigment profile and also its solubility, especially for azaphilone polyketides. These pigments can react with organic nitrogen sources to form red, water-soluble pigments (Lebeau et al. 2020b). In *Talaromyces albobiverticillius*, a change of media color can be noticed during fermentation, from yellow to red, due to this process (Venkatachalam et al. 2020). The effects of nitrogen sources on azaphilone formation will be discussed on the item 5.

The pH affects pigment production, in a species-specific way, and is considered an environmental stress factor, since it stimulates isolation and protection of the mycelium through pigment production (Hernández et al. 2019). For most filamentous fungi, including *Monascus* species, the optimal pH is slightly acidic, between

5.5 and 6.5, while for *Rhodotorula* is between 4.0 and 4.5, and for *Penicillium* and *Talaromyces* species, a neutral or alkaline media (7.0–9.0) is ideal (Venkatachalam et al. 2020). However, different pH values can induce different responses on the same species, and consequentially result in different metabolites. The carotenoids β-carotene and lycopene can be produced by the same fungi, but the first one is favored in alkaline pH, and the latter is better produced at acidic pH (Hernández et al. 2019; Rana et al. 2021). It is important to reassure that fungal metabolism focuses either on its growth or on metabolites production, as reported for many fungi of the genera *Penicillium*, *Fusarium*, *Talaromyces*, *Wojnowiciella*, *Epicoccum,* and *Phoma*, in which a pH between 7 and 9 is ideal for pigment production, while a pH 4 stimulates biomass production (Molelekoa et al. 2021). An acidic pH can also help the hydrolysis of substrates, as well as react with some products, what enhances their extraction to the media, as noticed for *Monascus* species, and also *Talaromyces albobiverticillius* (Venkatachalam et al. 2020). For these genera, the oxidation of pigments, especially hydrophobic molecules, can also occur, inserting an oxygen atom in the molecule and helping their excretion (Venkatachalam et al. 2018a).

The parameter "time" must be considered, since pigments can deteriorate in media. In the aforementioned *T. albobiverticillius*, maximum pigment production is achieved with 149 h, and after that, deterioration causes discoloration of the media (Venkatachalam et al. 2020). Regarding temperature, all fungi possess an ideal value that stimulates maximum growth and biomass production, but this parameter can be controlled in order to direct metabolism to pigment production. In *Monascus purpureus*, there is a high red pigment production at 30 °C, while at 40 °C, most yellow pigments are excreted (Venkatachalam et al. 2019). Agitation of the media also affects, usually stimulating pigment production, since it increases the amount of dissolved oxygen, promoting fungal development (Venkatachalam et al. 2020).

In general, NaCl concentration usually stimulates pigment formation, since high osmolarity is a well-described fungal stress condition, but osmotic stress has distinct effects in each species. *Penicillium murcianum* reaches its highest production in saline conditions (6–7.5 g/L NaCl) (Hernández et al. 2019), while *T. albobiverticillius*, is stimulated by a higher concentration (9%). However, low salinity can be a stress factor for marine species (Venkatachalam et al. 2019), as for *Talaromyces australis*, a marine fungus with its highest pigment content in the absence of salt (Hernández et al. 2019).

Other factors seem to influence pigment production, such as high phosphate content and high acidity that inhibit fluorescent pigment formation, and sulphate, capable of restraining color formation, even in trace amounts. The accumulation of organic acids in the media, by *Monascus ruber*, also appears to inhibit pigmentation (Ramesh et al. 2019). Penicillin addition in *Neurospora intermedia* media can stimulate carotenoid biosynthesis (Gmoser et al. 2019), and glutamic acid significantly enhances *Monascus* pigment production yield, from 48.4 to 215.4 mg/L (Zhang et al. 2017). Elicitors are exogenous compounds with the ability to selectively and rapidly stimulate gene expression and metabolite production. Fungal elicitors include the mycelium (untreated, concentrated, or its extract), cell wall degradation components, peptides, and proteins. The *Rhodotorula glutinis* biomass,

after drying, resuspension, and sterilization, was able to increase carotenoid production by *Cordyceps militaris* (Tang et al. 2019).

The cost of production should be considered in industrial processes, and, for this reason, many alternative media are being pointed out as substitutes for traditional ones. The use of agricultural waste can fulfill this goal and also contribute to diminish the amount of toxic waste generated in the process (Pailliè-Jiménez et al. 2020). Using orange processing waste, *Penicillium purpurogenum* was able to produce *Monascus*-like pigments, without excreting any mycotoxin (Kantifedaki et al. 2018). Corncob and other lignocellulosic matrices, if previously hydrolyzed, can be used by fungi able to metabolize xylose as carbon source, such as *Talaromyces atroroseus* (Morales-Oyervides et al. 2020). The red pigment bikaverin was produced by *Fusarium oxysporum* using rice flour medium (Meruvu and dos Santos 2021), while *Neurospora crassa* was able to produce β-carotene in a mixture of 60% tapioca by-product and 40% tofu waste (Gmoser et al. 2019).

Genetic manipulation techniques are successful to increase pigments' yield in fungi. Actually, mammalian and plant pigments, like hemoglobin and nonmicrobial carotenoids, have already been expressed in microorganisms (Khan et al. 2021). In *Fusarium graminearum*, the overexpression of transcription factor AurR1 increased three times aurofusarin production. In the same species, a knockout mutant for this gene was created, presenting no pigmentation after growth (Westphal et al. 2018). Recombinant DNA technology is used to alter the activity of enzymes related to carotenoids synthesis (Gmoser et al. 2017). Genome mining allows a thorough study of fungal genome, including those gene clusters suppressed under laboratory conditions. With the identification of a potential cluster, it can be engineered into a heterologous host for large-scale production (Kalra et al. 2020). With this aim, CRISPR-Cas9 technique is reported as an optimal method to create fungal cell factories (Meruvu and dos Santos 2021).

4.2 Mycotoxin Production

Since many applications of fungal pigments are on food, pharmaceutical, and cosmetic products, it is important to discuss mycotoxin production. Three species of *Aspergillus* sp., namely *Aspergillus glaucus*, *Aspergillus cristatus,* and *Aspergillus repens*, produce a wide array of pigments of different colors; however, some of these colored metabolites are recognized toxins, such as secalonic acid, oxaline, tanzawaic acid A, cyclochlorotine, islanditoxin, luteoskyrin, erythroskyrin, rugulosin, and aspergilolide A. This indicates that not all pigmented compounds are safe and must undergo toxicological evaluations. Even if the fungus does not produce toxic pigments, its extract should be searched for any other harmful substance, and the best-known example concerns the genera *Monascus*. The *Monascus* azaphilones, known as "Monascus-like pigments", were historically and extensively evaluated regarding its toxicity and recognized as safe; however, many *Monascus* species produce citrinin, a nephrotoxic and hepatotoxic mycotoxin

(Venkatachalam et al. 2018a), along with the cholesterol-lowering drug mevinolin (Venkatachalam et al. 2018b). For this reason, *Monascus* pigments were banned from US and EU markets. The only *Monascus* product approved by the FDA as a dietary ingredient is Monascus 8000 F (monacolin J from *Monascus pilosus*) (Kim and Ku 2018).

Several *Penicillium* and *Talaromyces* species can produce Monascus-like pigments, without producing citrinin, being considered safer options for industrial production. *Talaromyces atroroseus* is a successful example of a high yield red colorant producer, with no toxic metabolites on its extract. However, in the same genus, *Talaromyces purpurogenus* also produces red pigments, but the mycotoxins rubratoxin A and B and luteoskyrin are found in the media (Venkatachalam et al. 2020). A rhubard-wine contaminated with this fungus, and with a high concentration of rubratoxin, induced immediate liver transplant in a teenager after consumption (Venkatachalam et al. 2018b). Genetic manipulation is being used to redirect fungi metabolites pathway and assure a production of pigments free of toxins. A random mutant, namely *M. purpureus* YY-1, was reported as low citrinin producer and the repression of this metabolic route led to an increase on pigment content. Also, the transcriptional activator CtnA was identified as related to citrinin synthesis, and its deletion significantly decreased citrinin production (Liang et al. 2018). Another genetically modified strain, named *M. purpureus* SM001, is also reported as free of citrinin and a potential strain to produce pigments for the food industry (Blanc et al. 1995).

Nevertheless, many studies evaluated the toxicity of fungal pigments, and generally, they can be considered safe. Spalding pigment xylindein showed no toxicity when tested against zebra fish (Almurshidi et al. 2021). The pigment 2-(1,5, dimethylhexyl)-3,5-dimethyl 1-6-hydro-l,4-benzoquinone, from *Penicillium europium,* also was confirmed as nontoxic by the LD_{50} test (Khan et al. 2021). The commercially available pigment β-carotene, along with its producer's (*Blackslea trispora*) extract, is considered safe to humans, as studies did not detect any toxic effect (Poorniammal et al. 2021).

4.3 Pigment Stability

The last steps in pigment industrial production are the extraction and purification, which are fundamental for the process because many pigments can deteriorate due to high temperatures or strong solvents. To avoid this, extracellular and water-soluble pigments are preferred, since they require no organic solvent on its extraction and are considered safe (Kalra et al. 2020). Even some greener extraction alternatives are being studied such as Pressurized liquid extraction (PLE) that uses eco-friendly solvents such as water, methanol, and ethanol, with high pressure, in order to better remove pigments from the media. *Monascus*-like pigments were successfully extracted from a *Talaromyces* sp. media through this methodology (Lebeau et al. 2020b). Aqueous two-phase system (ATPS) extraction is also based on the use of

nontoxic solvents, and its success on extracting pigments is reported for *Talaromyces albobiverticillius*, *Emericella purpurea*, *Paecilomyces marquandii,* and *Trichoderma harzianum* (Lebeau et al. 2020a). This method in based on liquid-liquid fractionation between ionic solutions and requires mild conditions of pH, temperature, and pressure (Kalra et al. 2020). Pigment stability can also be improved in postproduction, via microencapsulation, nanoemulsion, and nanoformulations, that can enhance a pigment shelf life and improve its solubility, increasing its range of applications (Rana et al. 2021).

Microbial pigments can be categorized as carotenoids, polyketides, and melanin, as described below.

5 Carotenoids

Carotenoids represent the main natural pigment group produced by plants and microorganisms, with more than 1000 compounds described and more than 700 source organisms (Sajjad et al. 2020). They are hydrophilic molecules, mainly terpenoids of 40 carbon atoms (Rapoport et al. 2021). This group is characterized by a polyene hydrocarbon chain that may be terminated by rings and possesses extra oxygen atoms. They can be classified as carotenes, which are simple hydrocarbons, such as torulene (**34**), lycopene (**11**), and α- to ε-carotenes, or xanthophylls, also known as "oxygen-containing carotenoids", if these molecules are functionalized with keto, hydroxyl, and/or carboxyl groups, such as lutein (**35**) and zeaxanthin (**36**), shown in Fig. 21.6. Carotenoids chromophore consists of a long-conjugated chain, with the ability to absorb blue light (440–520 nm), what gives them a range of color from yellow to deep red, depending on the structure (Sajjad et al. 2020). The conjugated double bonds are responsible for their color and physicochemical properties, and it is known that at least seven of these bonds are necessary for obtaining a colored carotenoid (Rapoport et al. 2021). This conjugated polyene chain also offers

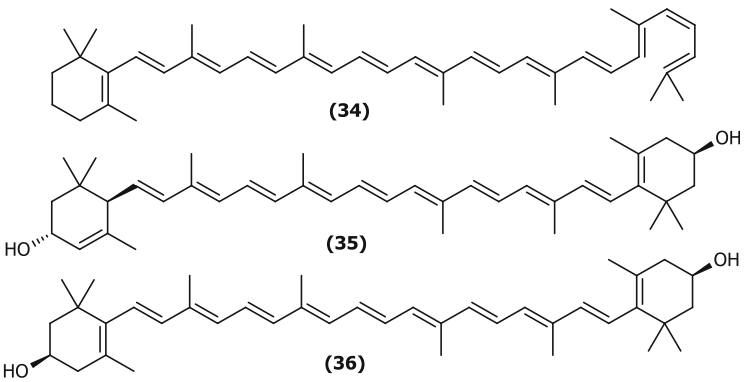

Fig. 21.6 Carotenoids produced by fungi

a chemical reaction site against oxidizing agents, what gives its characteristic activity against photo-oxidation (Venil et al. 2020).

Carotenoids are found in all plants, as it plays a crucial role in photosynthesis, helping the absorption of light and removing excess solar energy, in order to protect chlorophyll (Rapoport et al. 2021). The main role of carotenoid in fungal development is protection against reactive forms of oxygen and radiation, and more than 200 species are reported as carotenoid producer, belonging to the genera *Aschersonia*, *Aspergillus*, *Blakeslea*, *Fusarium*, *Mucor*, *Neurospora*, *Phycomyces*, *Rhodotorula*, *Rhodosporium*, *Sporidobolus,* and *Xanthophyllomyces*. In fungi, the most notable carotenoid producer is *Blakeslea trispora*, with β-carotene (**10**) production reports as high as 17 g/L of culture medium, besides also producing lycopene (**11**) with high yields (Dufossé 2016). β-carotene (**10**) is the typical carotenoid in Zygomycetes, but also found in the Ascomycetes *Penicillium* sp., *Aspergillus giganteus*, *Sclerotinia sclerotiorum,* and in the Basidiomycetes *Rhodosporidium* sp., *Sclerotium rolfsii*, and *Sporidiobolus pararoseus* media (Meruvu and dos Santos 2021). In Ascomycetes and Basidiomycetes, other carotenoids such as torularhodin, hydroxytorulene, canthaxanthin, and astaxanthin are found (Tang et al. 2019).

Carotenoids are not synthesized by humans and animals, and therefore need to be obtained from the diet. β-carotene, α-carotene, and β-cryptoxanthin are called provitamin A carotenes, since they are converted into vitamin A by enzymes inside the human body. For this reason, carotenoids' consumption can prevent vitamin A deficiency, which is essential for the promotion of growth, embryonal development, and visual function. Carotenoids are fundamental in protecting the retina against cataract and other age-related macular degenerations that can lead to blindness, even in children. Non-provitamin A carotenoids, like lutein (**35**) and zeaxanthin (**36**), are important for eye health too, since they reduce the risk of age-related eye diseases, but also promote better visual performance, improving contrast sensitivity, glare tolerance, and photo-stress recovery (Rapoport et al. 2021).

Since carotenoids are lipophilic, they are found in fungal membranes and other lipophilic compartments, like lipid droplets, where they act as antioxidants. Polar carotenoids can also regulate membrane fluidity (Rapoport et al. 2021). Due to its bioactivity as antioxidants and free radical scavenger, carotenoids can reduce the incidence of coronary heart disease and carcinomas, including lung, breast, prostate, and colorectal cancers. The antioxidant properties are tightly correlated with their chemical structure, and it is proved that the opening of the β-ionone ring and the increase of the conjugated double bond chain, consequently the chromophore's increase, are the two most important factors regarding the capacity of peroxyl radical scavenging (Lin and Xu 2020).

Carotenoids are used to provide color in animal feed industry, however, its uses on human food are limited, especially due to its poor water solubility and low thermal stability (Tang et al. 2019; Venil et al. 2020). Canthaxanthin is already used in candy, fish, meat, cheese, snacks, beverages, beer, and wine (Sajid and Akbar 2018). Some studies with marine fungi show anti-obesity activity of carotenoids with an allenic bond and two hydroxyl groups at the end of the polyene

chromophore (Lin and Xu 2020). Along with that, torularhodin, produced by yeasts of the genera *Rhodotorula* and *Sporobolomyces,* has strong antimicrobial activity and can be used in the production of films for coating medical implants, or even as a new antibiotic (Rapoport et al. 2021). Also, carotenoids show potential as ingredients in sunscreen/sunblock face creams and antiaging masks. Red and yellow carotenoids increase sun protection factor by, respectively, 36.5% and 13%, besides providing antioxidant properties (Meruvu and dos Santos 2021). Lycopene is already used in face cream and other products (Sajid and Akbar 2018).

6 Polyketides

Fungal polyketides are tetraketides and octoketides, possessing eight C2 units, synthetized by polyketide synthases (PKS), from acetyl CoA and malonyl CoA. They possess a wide array of colors, ranging from red, yellow, orange, brown, and black, and can be classified into anthraquinones, hydroxyanthraquinones, naphthoquinones, and azaphilones (Venil et al. 2020). Many genera are reported as polyketide producers, the most important being *Monascus*, *Talaromyces*, *Emericella*, *Paecilomyces,* and *Trichoderma* (Lebeau et al. 2020a). In marine fungi, they are the predominant pigments (Venkatachalam et al. 2019). Polyketides in fungi are related to protection to many environmental stressors, like desiccation, extreme temperatures, and irradiation. Besides that, polyketides play a fundamental role on fungal development, as they act as antimicrobial, antioxidant, in protecting against photo oxidation, inhibition of mutagenesis, enhancement of immune response, and inhibition of tumor development (Venkatachalam et al. 2019). Despite the production during log phase, poliketydes show no direct implications on fungal growth (Lebeau et al. 2020a).

6.1 Anthraquinones and Hydroxyanthraquinones

Anthraquinones are molecules with a 9,10-dioxoanthracene structure, with three benzene rings and two ketone groups in the center. They can be produced by fungi as dimeric structures, known as bisanthraquinones. Anthraquinones are formed as yellow compounds, with absorption spectra at 405 nm, whereas the substituents in the molecule can produce various hues, ranging from yellow, orange, red, brown, and violet (Venkatachalam et al. 2018a). When hydrogen atoms are replaced by hydroxyl groups, the molecule is called hydroxyanthraquinone. Most pigments belonging to this category are 1,8-dihydroxy and 1, 5,8-trihydroxy anthraquinone derivatives, and they can be found either in free form or glycosylated, what makes them water-soluble (Venil et al. 2020). Among them, *Fusarium* pigments chrysophanol (**37**), catenarin (**38**), erythroglaucin (**39**), and tritisporin (**40**) stand out (Lebeau et al. 2019) and are shown in Fig. 21.7. Chrysophanols are also reported

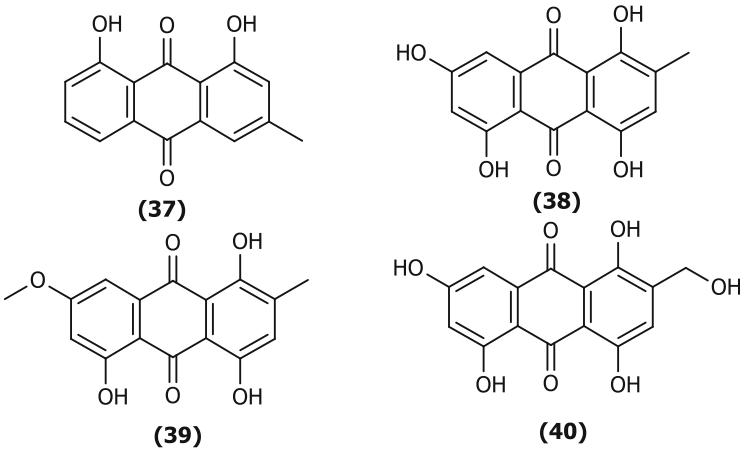

Fig. 21.7 Hydroxyanthraquinones produced by fungi

for *Trichoderma harzianum*, *Trichoderma polysporum*, *Trichoderma viridae*, *Trichoderma aureoviride*, *Aspergillus* sp., *Penicillium citrinum*, and *Fomes annosus* (Meruvu and dos Santos 2021). In *Aspergillus glaucus*, *Aspergillus cristatus,* and *Aspergillus repens*, the following hydroxyanthraquinoid pigments are found: yellow emodin (**12**) and physcion, orange questin (**14**), and red rubrocristin (**16**), besides the aforementioned catenarin (**38**) and erythroglaucin (**39**) (Venkatachalam et al. 2018b).

Arpink Red (**7**) is an anthraquinone produced by *Penicillium oxalicum* and commercially marketed as food colorant (Venil et al. 2020). *Aspergillus*, *Eurotium*, *Emericella*, *Fusarium*, *Penicillium*, *Mycosphaerella,* and *Microsporum* species produce anthraquinones pigments that can exhibit antimicrobial, herbicidal, and insecticidal properties (Venkatachalam et al. 2018a). Aspergilols H and I, hydroxyanthraquinones isolated from *Aspergillus versicolor*, are reported as potent antivirals towards HSV-1 (Huang et al. 2017). Two novel pigments, namely harzianumnones A and B, from *Trichoderma harzanium* showed cytotoxic activity against HeLa cell line (Shi et al. 2018). Many pigments isolated from *Drechslera rostrate* and *Eurotium tonpholium* possess anti-leishmanial activity, such as 1,3,8-trihydroxy-6-methyl-anthraquinone, aloe-emodin 8-O-glucopyranoside, 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone, and 1,4,5-trihdroxy-7-ethoxy-2-methyl-anthraquinone (Awaad et al. 2014). Due to hydroxyanthraquinones' good light fastness, excellent brightness, and ability to form complexes with metals like aluminum, barium, calcium, copper, and iron, they have great potential as textile dyes for cotton, wool, and nylon. Besides the bioactivity reported, the anthraquinone three rings structure suggests that they can interact with DNA, and for this reason, they are used in small doses (Venil et al. 2020).

6.2 Naphthoquinones

Naphthoquinones are polyketides possessing a naphthalene backbone, with two ketone groups in one ring, being able to form the structures 1,2-naphtoquinone or 1,4-naphtoquinone (Meruvu and dos Santos 2021). More than 100 naphtoquinones are found in filamentous fungi, with the red pigments bikaverin (**41**), aurofusarin (**42**), fusarubin (**43**), bostrycoidin (**44**), javanicin (**45**), produced mainly by *Fusarium* species. The pigments are shown in Fig. 21.8. Naphtoquinones are reported as the main response to environmental stress in *Fusarium* genus (Lebeau et al. 2019). *Monascus* species can form 1,4-naphtoquinones structures similar to their traditional azaphilone pigments, but in small quantities (Venil et al. 2020).

Due to their ability to protect DNA, naphthoquinones are reported as antimicrobial, anti-inflammatory, and anticancer agents (Venil et al. 2020). Dimeric naphthoquinones possess antimicrobial activity towards Gram + and − bacteria, besides all yeasts from the genus *Candida* (Westphal et al. 2018), as an example fusarubin, produced by *Fusarium solani* (Tirumale and Wani 2018). Bostrycin is an antibacterial compound, but also a phytotoxic agent, besides promoting the inhibition of lung, prostate, and gastric cancer cells. This pigment is reported as potential color fixative agent in food processing (Suwannarach et al. 2019a). Trypethelonamide A and 5-hydroxytrypethelone, novel naphthaquinones isolated from *Trypethelium eluteriae*, possess antioxidant and cytotoxic activities against many cancer cell lines (Meruvu and dos Santos 2021). The wood spalding fungi pigments xylindein (**29**) and draconin-red (**30**), from *Chlorociboria* sp. and *S. cuboideum,* have naphtoquinone-like structures, with many already discussed applications (Venil et al. 2020).

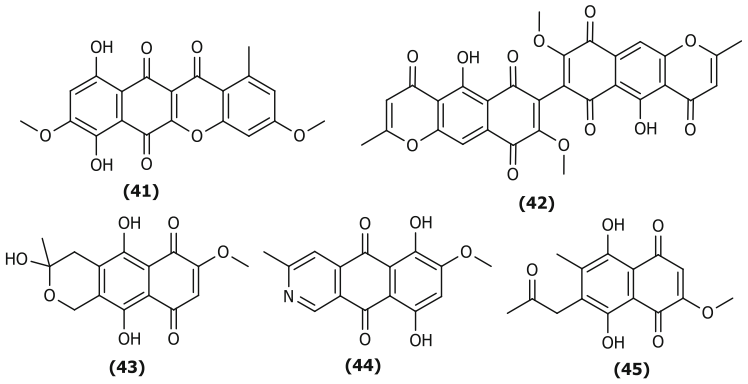

Fig. 21.8 Naphthoquinones produced by fungi

6.3 *Azaphilones*

Azaphilones have pyrone-quinone structures, with a high-oxygenated bicyclic core and a chiral quaternary center, characterized by its thermostability and easy derivatization, and produced by numerous ascomycetous and basidiomycetous fungi including the genera *Aspergillus*, *Penicillium*, *Chaetomium*, *Talaromyces*, *Pestalotiopsis*, *Phomopsis*, *Emericella,* and *Epicoccum*, as well as *Monascus* and *Hypoxylon* (Tirumale and Wani 2018; Venil et al. 2020). They are formed via reaction between acetic acid and five malonic acid, and further transesterification with medium chain fatty acids (Venil et al. 2020). The vinylogous γ-pyridones backbone is responsible for many activities reported for azaphilones such as antimicrobial, antifungal, antiviral, antioxidant, cytotoxic, nematocidal, and anti-inflammatory (Tirumale and Wani 2018). Azaphilones are divided into dimeric azaphilones, dimeric spiroazaphilones, bicyclic azaphilones, and azaphilones with lactone rings (Meruvu and dos Santos 2021). *Monascus* pigments, the yellow monascin (**46**) and ankaflavin (**47**), the orange rubropunctatin (**48**) and monascorubrin (**49**), and the red rubropunctamine (**50**) and monascorubramine (**51**), are the most well-described polyketides (Isbrandt et al. 2020) and are shown in Fig. 21.9.

Azaphilones have the distinctive feature of reacting with ammonia and other nitrogenated groups in the media such as proteins, amino acids, and nucleic acids, resulting in water-soluble pigments (Venkatachalam et al. 2018b). These compounds are red or purple vinylogous γ-pyridones that exchange the pyrane oxygen atom for nitrogen, as described for rubropunctamine (**50**) and monascorubramine (**51**), formed via reaction with ammonia, and chlorofusin, that reacts and incorporates a macrocyclic polypeptide (Tirumale and Wani 2018). The red azaphilones N-threonine-monascorubramine (**52**) and N-glutaryl-rubropunctamine (**53**), produced by *Talaromyces albobiverticillius*, are examples of pigments synthetized via the aforementioned mechanism (Venkatachalam et al. 2018b). Actually, many *Talaromyces* species, such as *T. aculeatus*, *T. pinophilus*, *T. purpurogenus*, *T. funiculosus*, *T. amestolkiae*, *T. ruber,* and *T. stolii,* can produce *Monascus*-like pigments and also their amino acid derivatives (Venkatachalam et al. 2018a). Atrorosins are a class of red pigments produced by *T. atroroseus*, also with

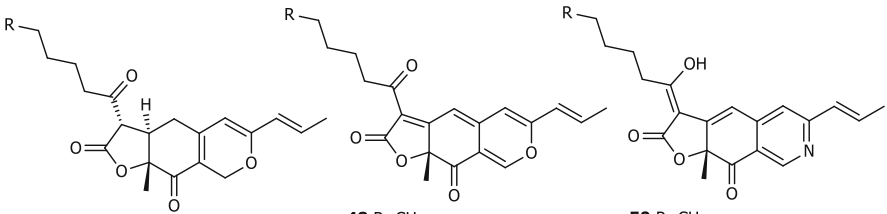

Fig. 21.9 Azaphilones produced by Fungi

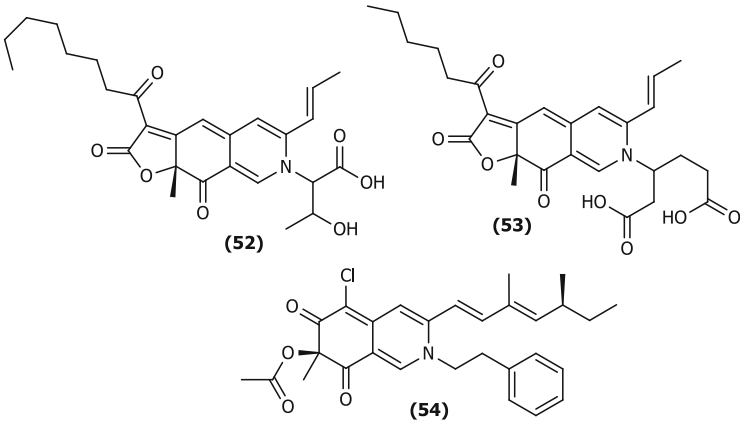

Fig. 21.10 Nitrogenated Azaphilones produced by fungi

incorporation of amino acids but, unlike other azaphilones, this fungus produces exclusively cis-isomers and can react with all L-amino acids, besides proline, as it is a secondary amine (Isbrandt et al. 2020). This red-azaphilone formation can also happen via biotransformation, as described for orange sclerotiorin, produced by *Penicillium sclerotiorum* and modified by *Beauveria bassiana,* to form red N-ethylbenzene-sclerotioramine (**54**) (Gomes and Takahashi 2016). The nitrogenated azaphilones are depicted in Fig. 21.10.

Monascus fermented food are produced on an industrial scale, and their pigments are traditionally used in meats, sausages, flavored milk, tomato ketchup, and fish pastes. Studies regarding flavoring yoghurt with these pigments found good market reception (Meruvu and dos Santos 2021). Also, *Monascus* pigments have high antioxidant, anti-inflammatory, and anti-cholesterol activity (Sajid and Akbar 2018), and monascorubramine (**51**) is reported as an antimicrobial agent. Ankaflavin (**47**), rubropunctatin (**48**), and monascorubramine (**51**) possess good anticancer activity (Tirumale and Wani 2018), and the latter two are also found in *Talaromyces* species, with no production of mycotoxin, the main problem in *Monascus* species (Lebeau et al. 2020b).

Orevactaene is an azaphilone produced by *Epicoccum nigrum*, with high antioxidant properties (Tirumale and Wani 2018). Penicilones A-D, from the marine fungi *Penicillium janthillum*, have excellent activity against multiresistant *Staphylococcus aureus* (MRSA) (Chen et al. 2017). The dimers citrofurans A-D, from *Aspergillus* sp., have inhibitory activity against LPS-induced NO production (Yin et al. 2017). Penicitol A, a citrinin-like azaphilone dimer, produced by *Penicillium chrysogenum*, presents cytotoxicity against HeLa, HEK-293, HCT-116, and A549 cancer cell lines (Guo et al. 2015). Many other azaphilones show activity against cancer cell lines, as monascin, monaphilone A and B, from *Monascus* sp., felinone A from *Beauveria feline*, xylariphilone from *Xylariales* sp., and aspergillusone C from *Aspergillus clavatus* (Sajid and Akbar 2018; Venil et al. 2020). Monacolin K, commercially known as lovastatin, produced by *Monascus* sp., is a serum cholesterol lowering

drug due to its inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which controls the biosynthesis of cholesterol (Venil et al. 2020). Along with pharmaceutical applications, *Monascus* azaphilones possess bright colors and excellent capability to absorb UV rays, what indicates their potential as additives in cosmetics (Chen et al. 2015).

7 Melanins

Melanins are negatively charged hydrophobic indolic or phenolic polymers with high molecular weight and brownish-black color, widely distributed in animals, plants, and microorganisms (Suwannarach et al. 2019b). In fungi, melanin protects against stress factors such as ionizing radiation, desiccation, extreme temperatures, enzymatic lysis, oxidizing and antifungal agents, and UV radiations (Sajjad et al. 2020). In *Sclerotium rolfsii*, even a small amount of melanin present in cell wall is used as antioxidant to promote its growth (Song 2018). In *Cryptococcus neoformans*, it is shown that melanized cells are more likely to survive heat and cold shock, when compared to non-melanized species. Also, in endophytic fungi, melanin helps plants to dissipate heat and neutralize reactive oxygen species (Mattoon et al. 2021). It can absorb a broad range of the electromagnetic spectrum and protect the fungi from photo-induced damage, besides providing a physical barrier to defend the cell and protect it against osmotic stress (Sajid and Akbar 2018; Smith and Casadevall 2019). For these reasons, melanins are recognized as a distinctive trait for fungi living in extreme conditions such as the Antarctic desert (Pacelli et al. 2020). For example, in the damaged reactor at Chernobyl, as well as the surrounding soils, it were found a large number of melanized fungi (Zhdanova et al. 2000).

Melanins form a highly pigmented structure named microsclerotia that protects spores and guarantees its survival in soil for years (Wang et al. 2018b). Microsclerotia have many outer layers of pigment parenchyma cells and an inner layer containing colorless cells (Song 2018). Many filamentous fungi are reported as melanin productors, such as those belonging to the genera *Alternaria*, *Armillaria*, *Aspergillus*, *Auricularia*, *Cladosporium*, *Epicoccum*, *Eurotium*, *Magnapothe*, *Ochroconis*, *Penicillium*, *Phomopsis*, *Sporothri*, *Stachybotrys* and *Wangiella*, along with the yeasts *Aureobasidium pullulans*, *Candida albicans*, *Cryptococcus neoformans*, *Hormoconis resinae,* and *Kluyveromyces marxianus* (Suwannarach et al. 2019b).

Melanins are a very heterogenous group regarding the structure, composition, and functional use (Smith and Casadevall 2019). In fungi, they can be formed through polymerization of two different precursors, 3,4-dihydroxyphenylalanine (DOPA) and 1,8-dihydroxynaphthalene (DHN) (Suwannarach et al. 2019b). DOPA melanins (**55**) are mainly found in Basidiomycetes, while DHN are found mostly in Ascomycetes (Pacelli et al. 2020). DOPA melanins (**55**) are divided into black eumelanin and yellow-red pheomelanin, which differ in nitrogen (5.1–9% and 8–11%, respectively)

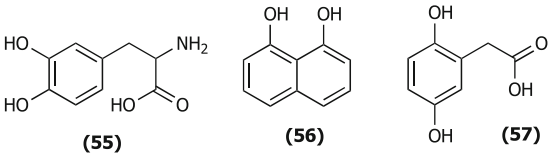

Fig. 21.11 Precursors of eumelanin and pheomelanin (**55**), allomelanin (**56**), and pyomelanin (**57**)

and sulfur content (0–1% and 9–12%, respectively). On the other hand, DNH melanins (**56**), classified as allomelanins, have only trace amounts of nitrogen (Suwannarach et al. 2019b). There is also evidence of pyomelanins (**57**) in fungi, with a biosynthetic route similar to eumelanin, but formed via the breakdown of aromatic amino acids, especially tyrosine (Smith and Casadevall 2019). The precursors used in the formation of each type of melanin are shown in Fig. 21.11.

Fungi can accumulate melanin in cell walls, but also excrete to the extracellular medium. In the mushroom *Auricularia auricula*, melanin can represent up to 10% of fungal dry mass, and therefore, is a good industrial source of this pigment. In *Armillaria cepistipes* and *Amorphotheca resinae*, melanin is excreted in high yields, respectively 27.98 and 4.6 g/L of media (Oh et al. 2021). Usually, allomelanins are attached to the inner side of the cell wall and eumellanins can be secreted to neutralize toxic environmental compounds and, for this reason, the latter is preferred in industrial processes, since it is easier to purify (Mattoon et al. 2021).

The most well-known property of this pigment is the ability to absorb radiation, and this is important in many industrial products nowadays. They are used in gelatin coatings for pork lard packaging to reduce the oxidative rancidity and promote stability. Melanin from *Aspergillus nidulans* and *Aspergillus alternata* has potential applications in skin care products due to antioxidant properties and UVB protection (Meruvu and dos Santos 2021). Actually, melanin has already been used in cosmetics, eyeglasses, sunscreens, sun blocks, and immobilization of radioactive waste like uranium (Sajid and Akbar 2018). It has been proved in studies with mice that even when melanin is consumed, it can protect gastrointestinal tract from radiation and also reduce oxidative stress in the liver, besides improving spleen parameters and reducing the production of inflammatory cytokines. Some studies even consider using melanins to make spacesuits and protect astronauts from dangerous space radiation (Mattoon et al. 2021).

Melanin and its intermediates can induce oxidative stress in cells through production of free radicals, and this is an important virulence factor. For this reason, melanized fungi tend to be more pathogenic to humans, since it can neutralize the oxidative bursts of the innate immune system (Smith and Casadevall 2019). Melanin from *Inonotus obliquus* showed in vitro effects against tumor growth and also diabetes mellitus (Mattoon et al. 2021). Actually, melanin is being used in the treatment of metastatic melanoma (Sajid and Akbar 2018). This compound shows antimicrobial activity and is used in the manufacture of anticancer drugs, suture threads, face masks, sunglass lenses, and skin care creams (Meruvu and dos Santos 2021). Melanins can also be used in water waste treatment due to their ability in capturing contaminants, especially heavy metals, via adsorption. Melanin from

Amorphotheca resinae is capable of remove cooper II and lead II from solutions, reaching maximum binding capacity in less than 2.5 h (Oh et al. 2021). Uranium absorption in water waste from mines is also reported for melanin. Volatile organic compounds (VOC), that can be toxic to humans in long time exposures, can be absorbed and neutralized by melanins, with VOC reduction rate of more than 96% after 48 h (Mattoon et al. 2021).

8 Final Considerations

The potential of fungal pigments in various industrial segments has been demonstrated in several studies in recent years. New molecules are increasingly being discovered, and fungi are being recognized as important producers of compounds with valuable bioactivity. Riboflavin (vitamin B2) (**8**), for example, is essential to human metabolism as it is necessary to synthetize the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), involved in redox reactions to metabolize carbohydrates, fats, and proteins (Meruvu and dos Santos 2021). Besides that, they are widely used as yellow food colorants in ice cream, beverages, and desserts (Sajid and Akbar 2018). Many fungi are reported as riboflavin producers, such as the yeasts *Candida guilliermondii*, *C. biodinii*, *C. acetobutylicum*, *C. famata*, *C. flaveri*, *C. ghoshii*, *C. parapsilosis*, *Pichia guilliermodii*, *Sarcoscypha occidentalis*, and *Torulopsis candida*, besides the filamentous fungi *Ashbya gossypii*, *Aspergillus niger*, and *Aspergillus fumigatus*. This helps increasing fungi recognition as safe and profitable producers of pigments, and they can be an important alternative to natural products, replacing plants, microalgae, and bacteria (Meruvu and dos Santos 2021).

Not only that, but fungal pigments are finding new and exciting uses in segments not explored until now. One of those is its usage in fuel biocells that requires functionalized molecules, called electronic shuttles, to help electron transportation to enzymes and energy production. Fungal pigments have been tested, showing an increase in reaction rates in fuel biocells, and the fact that these molecules are usually nontoxic and biodegradable makes them good candidates as electronic shuttles (da Silva et al. 2022). In photovoltaic devices, molecules with high electron acceptance are also required for the conversion of light into electricity. Photons excite these molecule's electrons from its valence band to the conduction band, and then the energy is transferred to an external circuit. Novel solar cells, called dye-sensitized solar cells, are using synthetic and natural pigments, showing good efficiency even in low irradiance conditions (Orona-Navar et al. 2021). Xylindein (**29**) was tested in optoelectronic devices, showing good photoresponse and high photostability and electron mobility (Giesbers et al. 2019).

Regarding their uses in the textile industry, fungal pigments' potential has been proven. However, now fungal pigments are being evaluated to be used in digital textile inkjet printing. This method provides a more technological and precise way of dyeing, allowing a fast printing of complex patterns directly onto fabrics. Spalding

fungi pigments are the most well-suited for this application, and draconin-red (**30**), from *S. cuboideum*, was successful to dye cotton and polyester fabrics using this technique (Gutierrez et al. 2019).

It is important to keep searching for greener alternatives to synthetic compounds used in pharmaceutical, food, and textile industry. Some tools are available to make this prospection more efficient, the most important being the "omics" strategies. Melabolomics and proteomics, in association with genetic engineering approaches, can detect metabolites in fungal media, even in trace amounts and can help the study of fungal metabolism, in order to activate silent genes that may produce novel pigments (Paillié-Jiménez et al. 2020). Some tools can also help producers to easily identify pigments in samples, such as RAMAN spectroscopy, and able to detect pigments via surface direct analysis, even in untreated samples, in a fast and nondestructive way (Tauber et al. 2018). Natural fungal pigments benefit producers, since fungi represent a cheap and highly productive source, but also the consumers, due to the health benefits that many of these molecules can provide.

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Chapter 22
A Brief Insight into Peptide and Non-Peptide Drugs of Fungal Origin

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Abstract Microorganisms are valuable resources for obtaining bioactive compounds, which have proven to be very useful in various domains, e.g., agriculture, food and pharmaceutical industries. The kingdom of fungi constitutes unicellular and multicellular eukaryotic microorganisms. Various types of molecules identified from fungi have shown very fruitful medicinal and biological functions. Consequently, fungi have been recognized to be a vital resource for isolating compounds that have achieved success for treating infectious as well as non-infectious diseases. In this chapter, we are highlighting certain important characteristics, mainly molecular structural properties and key pharmacological activities of secondary metabolites discovered from different species and genera of fungi. The secondary metabolites that we have chosen are also commercially known drug compounds with very important therapeutic activities. We have classified these metabolites into two categories: peptide drugs and non-peptide drugs. Five non-ribosomal cyclic peptides: caspofungin, micafungin, anidulafungin, rezafungin and cyclosporine constitute the peptide drugs category (1000–1500 Daltons). While all these five peptides are anti-fungal agents, cyclosporine is also a popular immunosuppressant drug. Caspofungin, micafungin and anidulafungin are approved drugs that are available in market, whereas rezafungin is currently under clinical trials. In the non-peptide drugs category, we have selected small molecule secondary metabolites (intact molecular mass: 200–600 Daltons), which are griseofulvin, fusidic acid, mycophenolic acid, fingolimod and irofulven. Griseofulvin and fusidic acid are anti-fungal and anti-bacterial drugs, respectively. Mycophenolic acid and fingolimod

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are immunosuppressant drugs. Irofulven is an anticancer agent, which is currently under clinical trials.

Keywords Drugs · Fungi · Secondary metabolites · Peptides · Small molecules

1 Introduction

Fungi are not only known to cause infections and diseases, but also have proven to be useful in numerous applications, such as in food industries, for production of recombinant proteins, etc. (Rantasalo et al. 2019; Ntana et al. 2020; Chugh et al. 2022). Fungi have been extensively utilized in pharmaceutical industries as well, mainly to produce therapeutic molecules by processing the fermentation products (Fernández de Ullivarri et al. 2020). In the pharma laboratories, the molecules of fungal origin are developed not only for producing antibiotics, but also for various other pharmacological and medicinal applications such as anticancer, anti-fungal, anti-viral, anti-inflammatory, immunosuppressant, etc. (Kück et al. 2014; Chugh et al. 2022). Although several bioactive molecules have been isolated from diverse fungal genera and species, only a few molecules have been approved to be sold as safe drugs in the market. Various fungal molecules that have been found to be of immense potency in different kinds of in vitro experiments, have not cleared the clinical trials at different stages, which may be attributed to toxicity or adverse side-effects of those molecules. Consequently, the naturally derived molecules are subjected to chemical modifications (viz., semi-synthetic drugs) or the fungus would be subjected to certain genetic modifications, with the hope to produce compounds having better safety and more efficacy.

For this chapter, we have chosen to describe about 10 compounds that have been derived from different genera and species of fungi, among which eight are approved and commercially available drugs, while two are currently under clinical trials. Based on the molecular structure, we have classified these 10 compounds into two different categories: peptide and non-peptide drugs, wherein each category consists of five representative compounds. Caspofungin, micafungin, anidulafungin, rezafungin and cyclosporine are five representative examples of peptide drugs. The biosynthesis of these peptides involves multi-modular enzymes called non-ribosomal peptide synthetases (NRPSs) and hence are called as 'non-ribosomal peptides' (Bills et al. 2014; Hüttel 2021). Caspofungin, micafungin, anidulafungin and rezafungin are well-known for their anti-fungal action, whereas cyclosporine is a popular immunosuppressant drug. In the non-peptide drugs category, we have chosen to delineate some important aspects of griseofulvin, fusidic acid, mycophenolic acid, fingolimod and irofulven. Griseofulvin is an anti-fungal drug, while fusidic acid is an anti-bacterial drug. Mycophenolic acid and fingolimod are immunosuppressant agents, whereas irofulven has been shown to possess anticancer activity. It can be realized that all these 10 compounds are secondary metabolites (Conrado et al. 2022). In the ensuing sections, salient features covering the

molecular structural properties and some key pharmacological actions of each compound are illustrated.

2 Peptide Drugs

2.1 Echinocandin Class of Drugs

In the 'WHO Model List of Essential Medicines - 22nd List (2021)', some peptidic drugs of fungal origin such as micafungin, anidulafungin and caspofungin have been included that are meant for anti-fungal treatment. These are in fact semisynthetic compounds, whereby the naturally occurring molecules isolated from the fungi were subjected to a variety of chemical modifications, to obtain more potent and less toxic drugs, with better stability (Balkovec et al. 2013; Szymański et al. 2022). Table 22.1 summarizes a few details of these drugs.

Glucans account for approximately 50–60% of the cell wall components in fungi such as *Candida* spp. and *Saccharomyces* spp. (Szymański et al. 2022). However, β-(1,3)-D-glucan is not found in animal cells, and hence, its biosynthetic pathway becomes a better target for anti-fungal drug discovery (Szymański et al. 2022). Inability of the fungus to synthesize β-1,3-D-glucan can perturb its cell wall integrity causing osmotic instability and cell death (Kofla and Ruhnke 2011). Echinocandin class of compounds elicits their antifungal activity by inhibiting the biosynthesis of

Table 22.1 Summary of the details of peptide drugs of fungal origin

S. no.	Name of the semisynthetic peptide	Trade name or commercial name	Details of approval[a]	Name of the natural precursor peptide	Name of the fungal source of the precursor
1.	Caspofungin acetate	CANCIDAS	FDA approval in 2001 & approval in Europe in 2002	Pneumocandin B_0	*Glaria lozoyensis*
2.	Micafungin	MYCAMINE	FDA approval in 2005 & approved in Europe in 2008	FR901379	*Coleophoma empetrii*
3.	Anidulafungin	ERAXIS (US); ECALTA (EU)	FDA approval in 2006	Echinocandin B	*Aspergillus nidulans; Aspergillus rugulosus*
4.	Rezafungin	–	Currently under phase III clinical trials	Anidulafungin	–

FDA United States Food and Drug Administration

Note: The hydroxyproline and homotyrosine residues have been found to be essential for the anti-fungal function of echinocandins (Szymański et al. 2022)

[a] Guinea (2023); Szymański et al. (2022); Pfaller et al. (2020); Zhao and Perlin (2020); Thompson et al. (2021)

β-(1,3)-D-glucan (Szymański et al. 2022; Zhao and Perlin 2020; Hector 1993). These compounds exhibit fungicidal activity against several fungi including *Candida* species and are fungistatic to the *Aspergillus* genus (Szymański et al. 2022; Zhao and Perlin 2020; Hector 1993). Because of their higher efficacy and less toxicity, echinocandins became more preferrable over the azoles and amphotericin B (Balkovec et al. 2013; Chen et al. 2015). So, these are regarded as first-line drugs for treating candidemia and invasive candidiasis (Zhao and Perlin 2020). Furthermore, the echinocandins have been observed to be useful even against the azole-resistant strains, since the mechanism of anti-fungal action of echinocandins is entirely different from that of azoles (Emri et al. 2013).

Echinocandins are a class of cyclic lipopeptides first reported from *Aspergillus* species, and echinocandin B was the major product of the complex produced by *A. nidulans* and *A. rugulosus* (Hector 1993). Echinocandin B is the precursor of anidulafungin, whereby the naturally occurring aliphatic sidechain is replaced by aromatic sidechain in the semi-synthetic anidulafungin (Szymański et al. 2022). Anidulafungin was approved by FDA in 2006 and sold under the trade name ERAXIS in the USA and ECALTA is its commercial name in Europe (Szymański et al. 2022).

2.1.1 Caspofungin (Brand Name: Cancidas)

Caspofungin acetate—CANCIDAS® (Merck & Co. Inc.) was the first in the echinocandin class to be approved as an anti-fungal drug by FDA in the year 2001 and approved in Europe in 2002 (Balkovec et al. 2013; Szymański et al. 2022) (Fig. 22.1). Caspofungin acetate is actually a semi-synthetic product made from the starting molecule, pneumocandin B_0 (Balkovec et al. 2013; Szymański et al. 2022). Pneumocandins are a class of peptidic molecules that were found from the fermentation of fungus *Glarea lozoyensis* (Balkovec et al. 2013; Chen et al. 2015). Pneumocandin A_0 was the predominant fermentation product, whereas pneumocandin B_0 was a minor product obtained from the wild-type *G. lozoyensis* (Balkovec et al. 2013; Chen et al. 2015). After several efforts, a mutant of *G. lozoyensis* was isolated that produced pneumocandin B_0 as the major fermentation product (Balkovec et al. 2013; Chen et al. 2015). Thus, pneumocandin B_0 became the precursor for CANCIDAS®.

Caspofungin is completely soluble in methanol and water but only partially soluble in ethanol. It is available as lyophilized powder and some of the excipients are sodium hydroxide, mannitol, acetic acid and sucrose. After reconstitution, caspofungin can be stored for 24 h in refrigerator (Kofla and Ruhnke 2011).

Candidemia is the fourth dominant disease with 20% and 47% mortality rates in paediatric and intensive care unit patients, respectively, by causing bloodstream infections in various hospitalized patients. Candida infections have caused serious mortality in patients with immunosuppression and there is no effective treatment for invasive candidiasis. Caspofungin is used for the treatment of not only candidemia/candidiasis, but also for other diseased conditions such as Febrile neutropenia and

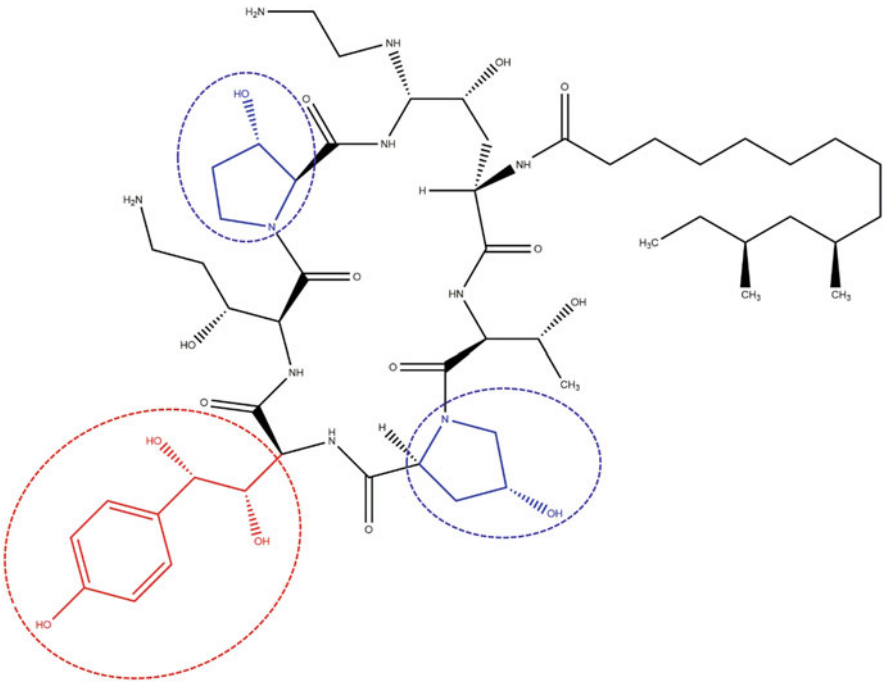


Fig. 22.1 Caspofungin; DrugBank Accession Number DB00520, MW: 1093.331; Mol. Formula: $C_{52}H_{88}N_{10}O_{15}$

invasive aspergillosis. As per IDSA (Infectious Diseases Society of America), the anti-fungal caspofungin is recommended to the patients with high risk of persistent febrile neutropenia regardless of broad-spectrum antibiotic therapy (Dongmo Fotsing and Bajaj 2022). In a double-blind trial study, the efficacy of caspofungin was observed in 73.4% patients having invasive candidiasis, whereas amphotericin B efficacy was observed in only 61.7% patients. Caspofungin was superior with 80.7% response of patients when compared with amphotericin B which showed 64.9% response of patients only, as per analysis of patients having prespecified criteria. Caspofungin has efficacy for the treatment of invasive candidiasis, but it is more specific for Candidemia. It has less toxicity than amphotericin B when administered via intravenous. The postsurgical infected patients including candida peritonitis and intraabdominal abscess has shown high response for caspofungin (Mora-Duarte et al. 2002). Andes et al. (2012) reported that echinocandins are more preferred than azoles for the initial treatment of candidemia. The rate of mortality is low in association with echinocandin class of drugs, when compared to other drugs like triazole or polyene class. The efficacy of echinocandin was also reported in patients with wide range of severe illness for both *C. albicans* and non-albicans groups. The loading dosage is 70 mg following 50 mg for once in a day. The higher dosage up to 150 mg/day has been studied via clinical trials resulting in high efficacy and some dose-dependent responses in patient subgroups (Cornely et al. 2007; Betts

et al. 2009; Andes et al. 2012). Caspofungin has been approved for the usage in children between the age of 12 months and 17 years. The area under concentration-time curve for 24 h (AUC_{O-24}) for children receiving 1 mg/kg/day is importantly smaller than the adult dose consuming 50 mg/day. The exposure observed in children for a dose of 50 mg/m^2/day is comparable to that of adult patients being treated with 50 mg/day (Walsh et al. 2005). In the liver, spontaneous disintegration occurs by hydrolysis resulting in opening of the ring structure of the peptide followed by N-acetylation, thereby giving rise to two inactive metabolites (Stone et al. 2002; Walsh et al. 2005). Caspofungin half-life is 9–11 h along with the additional gamma-phase half-life of 45 h. The elimination rate of caspofungin is slow with the clearance rate of 10–12 mL/min from plasma. Caspofungin reduces AUC by 20% when it interacts with tacrolimus. In vitro studies show that caspofungin has positive interaction with both CsA and tacrolimus against *Aspergillus fumigatus* due to calcineurin inhibiting delayed filamentation observed morphologically under microscopic analysis (Steinbach et al. 2004). Caspofungin has higher frequency for certain liver-related laboratory abnormalities (1–15%) (Cappelletty and Eiselstein-McKitrick 2007).

Echinocandins (caspofungin and micafungin) combined with triazoles (voriconazole, itraconazole and posaconazole) showed no effect in the case of patients with CAPA (Coronavirus Disease 2019—associated pulmonary aspergillosis) in spite of effective results in laboratory studies, but it showed positive result with LAmB (Liposomal amphotericin B) (Nakaya et al. 2023). Echinocandins combined with mucorales (amphotericin B) has no efficacy as they focus mainly on beta-(1,3)-D-glucan synthase, whereas *Mucor* spp. has beta-(1,6)-D-glucan synthase in their cell walls. Caspofungin has been reported to inhibit the expression of fungal gene FKS, but no proper evidence is known for its use to treat Mucorales (Meena et al. 2023).

Through a computational study, Liu and Wang found that caspofungin is a potential inhibitor of SARS-CoV 2 main protease M^{Pro}, from which Itoh et al. hypothesized that caspofungin may be applicable for treating COVID-19 patients with fungal co-infection (Liu and Wang 2020; Itoh et al. 2021). Caspofungin is not only a popular antifungal drug, but also known to influence the immune system. This was demonstrated in the case of fungal sepsis, whereby the hypercytokinemia condition was prevented through the involvement of spleen tyrosine kinase pathways, upon treating with caspofungin (Itoh et al. 2021).

2.1.2 Micafungin (Brand Name: Mycamine)

Micafungin is another candidate in the echinocandin class, whose precursor FR901379 was originally identified from *Coleophoma empetri* (Hashimoto 2009) (Fig. 22.2). After subjecting the FR901379 to different types of modifications, the semisynthetic micafungin was approved by FDA in 2005 and in Europe in 2008 (Szymański et al. 2022). Micafungin is a sulfated echinocandin, whereby the presence of sulfate group renders it water soluble (Hashimoto 2009). It is sold under the name MYCAMINE by Astellas Pharma Inc. (Szymański et al. 2022).

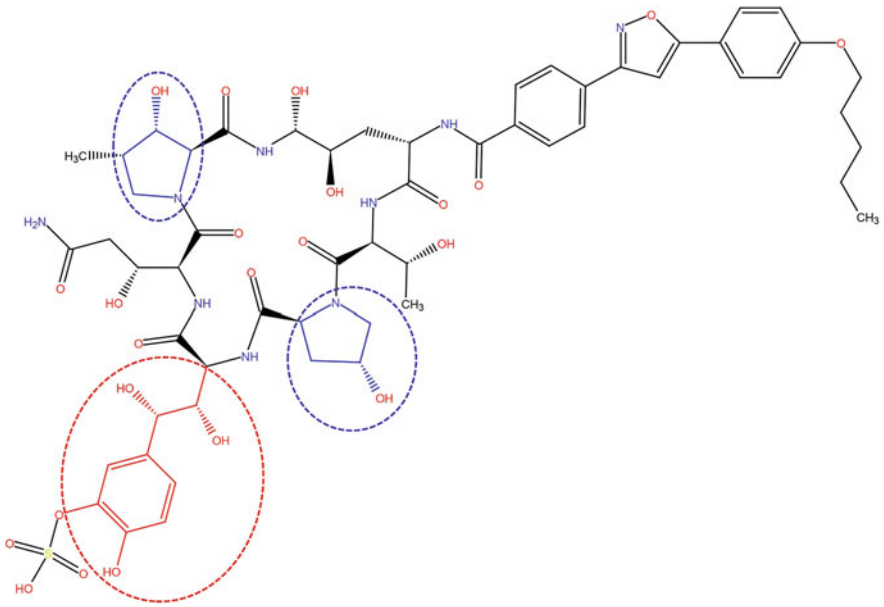


Fig. 22.2 Micafungin; DrugBank Accession Number DB01141, MW: 1270.274; Mol. Formula: $C_{56}H_{71}N_9O_{23}S$

Apart from fluconazole, micafungin is another drug that is approved for the treatment of *Candida* spp. infection (Gastine et al. 2019; de la Torre and Reboli 2014; Farowski et al. 2010). The European Medicines Agency approved it for the treatment of all types of invasive candidiasis, oesophageal candidiasis and also prophylaxis. It is an attractive drug for the treatment of invasive- and azole-resistant oesophageal candidiasis. It is recommended for the treatment of invasive candidiasis, and *Candida* prophylaxis infection, in the patients with allogeneic haematopoietic stem cell transplantation or suffering from granulocytopenia for almost more than 10 days. It is indicated for adolescents, children and adults. It is administered via intravenous and has anti-fungal potential with no toxicity (Kofla and Ruhnke 2011).

Some investigations have shown that even the lowest concentration of micafungin can affect cell wall of fungi such as changes in morphology and sometimes cell death of *Candida albicans*. In vitro studies of micafungin against *C. parapsilosis* showed less effectiveness than other *Candida* spp. But, in vivo studies reported fungicidal activity against *C. parapsilosis* similar to other *Candida* spp. It also showed high anti-fungal effect against clinical isolates of various *Aspergillus* spp. Micafungin is more active compound for the treatment of the central nervous system (CNS) aspergillosis in experimental murine than caspofungin, voriconazole and liposomal amphotericin B. No additional dosage is necessary for patients after hemodialysis, as no pharmacokinetic difference was observed with

micafungin, as it has higher propensity to bind to proteins mainly to albumin. Micafungin shows linear pharmacokinetic profile in premature neonates, young infants with short terminal half-life and rapid clearance of micafungin doses, when compared to children and adults (Farowski et al. 2010).

Micafungin is considered as a pregnancy category C agent. In order to avoid reactions due to histamine, it is infused for over 1 h. In vitro studies reported that 2 mg/L and 5 mg/L of micafungin can be used as lock solutions against *C. albicans* biofilm by reducing its intermediate metabolic activity and thereby not allowing the mature biofilms to grow on silicone catheters (de la Torre and Reboli 2014). Some significant precaution or warning related to the toxicity of micafungin was issued based on the observation of foci development of hepatocytes and hepatocellular tumors in the rat model, after 3 months of the treatment. However, its effect is not similar in humans, yet careful monitoring of liver function was advised during the administration of micafungin drug. If the elimination of aspartate aminotransferase/alanine aminotransferase is observed in considerable quantity, then the termination of the micafungin treatment is recommended. Micafungin therapy is considered as "careful risk – benefit analysis" for the patients who has been suffering from severe liver dysfunction or disease including malignancy (Schonfeld et al. 2008; Sidhu et al. 2009) [Northbrook, IL: Astellas Pharma US, Inc.; 2012, www.ema.europa.eu/docs/en. Accessed November 13, 2013].

In comparison with LAmB, micafungin has more effectiveness as well as less cost for the treatment of candidiasis and candidemia. Australian Hospital data showed that the use of micafungin is a lower cost treatment and the length-of-stay is also shorter for patients. The success rate of micafungin therapy was found to be better than caspofungin, concluding that micafungin is more cost-effective though there are no significant differences between these two drugs (Sidhu et al. 2009; Schonfeld et al. 2008). It is considered as salvage therapy showing high efficacy for the patients with high risk of aspergillosis although only a few groups of patients have received this monotherapy (de la Torre and Reboli 2014).

2.1.3 Anidulafungin (Brand Name: Ecalta, Eraxis)

Anidulafungin is another drug belonging to the group of echinocandins that is used to control and cure the infection such as candidiasis and aspergillosis. As already mentioned (*vide supra*), it is obtained by modifying the natural echinocandin B, whose aliphatic sidechain is replaced with an aromatic group (Fig. 22.3).

It leads to hemolysis and eryptosis causing cell damages, leakage of phospholipids due to translocation of phosphatidylserine on the cell surface of erythrocytes. It happens when p38 kinase is activated and Ca^{2+} ions enter inside the cell causing high Ca^{2+} cytosolic activity, energy reduction and activation of caspases along with oxidative stress. However, 1.5 μg/mL concentration of anidulafungin significantly reduces the percentage of swollen erythrocytes, whereas 3 μg/mL enhances the erythrocytes cell shrinkage significantly. The statistical data show that at the concentration of 6 μg/mL when applied for 24 h, anidulafungin causes hemolysis of erythrocytic cells (Peter et al. 2016; Houšť et al. 2020).

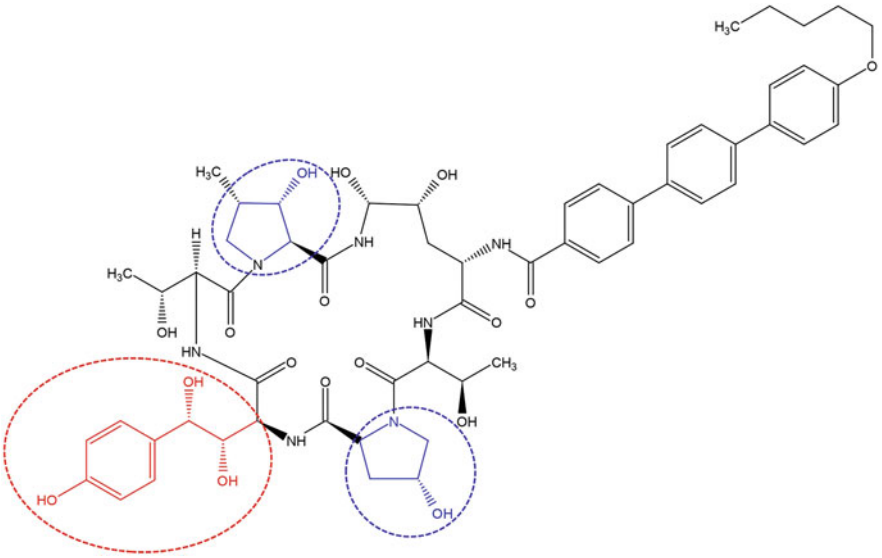


Fig. 22.3 Anidulafungin; DrugBank Accession Number DB00362, MW: 1140.2369, Mol. Formula: $C_{58}H_{73}N_7O_{17}$

Anidulafungin is used for the treatment of invasive candidiasis only in adults above the age of 18 and not effected by granulocytopenia. For the reconstitution, anidulafungin is provided in the form of lyophilized powder. Water is used for vial reconstitution in the case of injection, whereas for infusion, it is diluted using 0.9% sodium chloride or 5% glucose. A linear pharmacokinetic profile has been observed on the dosage of 15–130 mg, once a day and after loading dose, a steady state could also be observed along with low systemic inter-subject variability. Micafungin spontaneously undergoes biotransformation into an open-ring structured peptide, which is excreted in the bile. Liver metabolism is not considered for anidulafungin yet. It does not act as an inducer, substrate or an inhibitor for isoenzymes cytochrome P450. The volume (30–50 L) and half-life (0.5–1 h) of rapid distribution of anidulafungin is similar to the volume of total body fluid, and it significantly binds to human plasma proteins (>99%), but the distribution of this drug in specific tissues in humans is not yet reported. Thus, the penetration of anidulafungin via blood-brain barrier or into cerebrospinal fluid (CSF) is not yet observed or reported. The animal model studies have shown that its concentration in tissues is 10 times greater than in plasma. The concentration-time profile of plasma is featured through half-life span of elimination (24 h), whereas the clearance of anidulafungin is 11 h and the terminal elimination profile of anidulafungin is 40–50 h. As per population pharmacokinetic analysis, body weight impacts the variability of clearance of drugs, whereas there is as yet no clinically reliable data which shows the impact of body weight for the clearance of anidulafungin drug. The recommended standard loading dosage is 200 mg with 100 mg once a day. The usage of this drug is not yet approved for

children, yet it has been tested in the case of patients of the age 2–17 years suffering from neutropenia, in the concentration of 0.75 or 1.5 mg/kg/day, which is similar to 50 or 100 mg/day concentration administrated to the adult patients (Kofla and Ruhnke 2011).

Anidulafungin inhibits the virus entry process in the SFTSV (severe fever with thrombocytopenia syndrome virus) infection by 10 times reducing the copies of intracellular viral RNA than the control in cell line studies. Dose dependent behavior was observed, when the virus was injected with different doses of anidulafungin and preincubated in cell culture medium. At the dose of 20 μM of anidulafungin, the diameter of SFTSV virion is ~80 nm similar to normal size, whereas the virion size was 90–100 and 110–120 nm at the higher doses of 50 and 100 μM, respectively. Increase in virion size makes them unstable, eventually causing their disruption and leading to suppression of SFTSV infection along with decrease in protein expression. It has been shown that anidulafungin interferes with the fusion of virus with plasma membrane and inhibits HSV-1 (herpes simplex virus 1) and SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). In an exhaustive in-silico screening involving 450 FDA-approved drugs, anidulafungin was found to bind with angiotensin converting enzyme 2 (ACE 2) receptor with high affinity, and hence, it was taken up for in vitro experiments, which also indicated very favorable interaction with ACE 2 S-glycoprotein (Ahamad et al. 2022).

2.1.4 Rezafungin (Drug Bank Accession Number DB16310)

Rezafungin (earlier CD101) is a new echinocandin, whose molecular structure is very similar to anidulafungin, wherein the only difference is that rezafungin contains choline amino ether linkage at the C5 position of the ornithine residue (Zhao and Perlin 2020). In the pharmacokinetic studies conducted in beagle dogs, the half-life of CD101 was found to be about five times longer than the half-life of anidulafungin (James et al. 2017). Because of its longer pharmacokinetic properties, rezafungin could exhibit efficacy very similar to other echinocandin drugs with less frequency dosing regimen (Thompson et al. 2021; Zhao and Perlin 2020; Sofjan et al. 2018). The targets and mechanism of action of rezafungin is almost the same as that of other echinocandin drugs; however, it has better safety profile, and hence, higher doses of rezafungin may be administered (Sofjan et al. 2018; Garcia-Effron 2020). Rezafungin has now entered the Phase 3 clinical trials, and therefore, it is regarded as a next-generation echinocandin drug (Szymański et al. 2022; Zhao and Perlin 2020; Sofjan et al. 2018).

2.2 Cyclosporine (Brand Names: Sandimmune, Cequa, Gengraf)

Cyclosporins are a class of cyclic undecapeptides and are rich in N-methylated amino acid residues (Wang et al. 2017). Different variants or analogs of cyclosporin

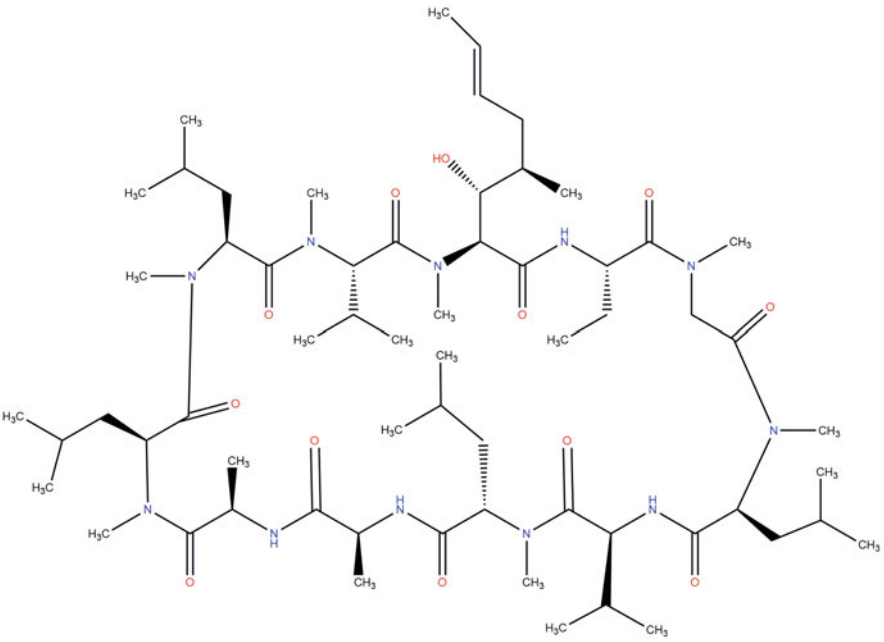


Fig. 22.4 Cyclosporine; DrugBank Accession No. DB00091, MW: 1202.635; Mol. Formula: $C_{62}H_{111}N_{11}O_{12}$

have been identified in various fungal genera and species, such as *Tolypocladium* sp., *Aspergillus* sp., *Trichoderma* sp. etc.; however, cyclosporin A has been thoroughly investigated (Wang et al. 2017). Cyclosporin A is one of the popular immunosuppressive drugs used in the patients undergoing organ transplantation, so as to prevent allogeneic post-transplant organ rejection such as kidney, liver or heart. It inhibits T-cell activation by inhibiting calcineurin. More specifically, cyclosporine binds with cyclophilin A in the cytoplasm of T cells and forms cyclosporine—cyclophilin complex, interfering with the phosphatase activity, via the enzyme calcineurin (Fig. 22.4).

Cyclophilin A acts as a mediator for several cardiovascular diseases, Alzheimer's disease, certain inflammatory diseases and amyotrophic lateral sclerosis. Cyclophilin expression increases in certain inflammatory condition during psoriasis and rheumatoid arthritis as well (Ma-Lauer et al. 2020; Glowacka et al. 2020). Therefore, cyclosporine finds very useful applications in various fields such as dermatology, transplantology, ophthalmology, rheumatology and nephrology. Additionally, the anti-fungal and anti-inflammatory activities of cyclosporin A are also known (Wang et al. 2017). Other variants of cyclosporine have also been found to possess immunosuppressant and anti-fungal activities (Wang et al. 2017).

Cyclosporine is used in certain autoimmune diseases and rheumatoid arthritis which shows no response towards methotrexate. For amyotrophic lateral sclerosis

(ALS) and graft Vs host disease (GVHD), it acts as a second-line agent. Some of the FDA approved indications for which cyclosporine is used are as follows:

- When methotrexate cannot treat rheumatoid arthritis.
- Solid organ such as lung, kidney or liver transplantation.
- For psoriasis patients, who do not respond for even a single systemic therapy, including adults and non-immune compromised patients.
- Patients with severe plaque psoriasis and recalcitrant, ALS or also called as Lou Gehrig disease and its several variants.
- Treatment of focal segmental glomerulosclerosis, which is a part of nephrotic syndrome and can't be treated with corticosteroids.

There are certain indications, which are not approved by FDA such as allergic conjunctivitis, ulcerative colitis, Henoch—Schonlein purpura nephritis, autoimmune hepatitis, histiocytosis, keratoconjunctivitis, pure red cell aplasia, etc. wherein cyclosporine is not used (Tapia et al. 2022). Cyclosporine has no effects on suppression of bone marrow-related immune reaction in animal and human models as per the research studies. Animal studies showed that cyclosporine has no adverse effects on function of phagocytic process and suppress only cell-mediated immune response (Masi et al. 2019; Liddicoat and Lavelle 2019). Based on several studies, it was concluded that the patients with lethal injury of reperfusion along with myocardial infarct size can be reduced, if cyclosporine is administrated during the time lap of reperfusion in the patients with STEMI (ST elevation MI). Some investigations have shown that cyclosporine is better for patients with Acute Myocardial Infarction (AMI), whereas a few other studies have also reported that placebo has adverse effect, when compared to cyclosporine. Cyclosporine is much tolerable by the patients with AMI, when compared with other drugs such as placebo (Rahman et al. 2018).

It has been reported that either cyclosporine as monotherapy or combined with other immunosuppressant drug can be considered for the treatment of patients with hepatitis C-virus (HCV) positive infection (Glowacka et al. 2020). In vitro studies have shown that cyclosporine inhibits hepatitis B-virus (HBV) replication in dose-dependent manner by interfering with the signalling pathway of calcium in the cytoplasm of cell. The concomitant therapy of cyclosporine led to decrease in the expression of Hepatitis B surface antigen and Hepatitis B core e antigen with the increment of Hepatitis B core antigen in the nucleus of hepatic cells (Motaparthi et al. 2014). Some studies even reported that the analogs of cyclosporine have more inhibitory potential than cyclosporin A, as antiviral agents. There is also a study showing lack of correlation between the antiviral activity and binding properties of cyclophilin and calcineurin (Watashi et al. 2014). The use of cyclosporine for the treatment of patients with HBV infected cells has moderate risk of reactivation of those cells during psoriasis treatment, which includes the concomitant need of Anti-HBV prophylactic therapy along with the monitoring of DNA of HBV for every 3 months (Piaserico et al. 2019). In vitro studies on HCV infection have unveiled that the cyclosporine has the potential for the inhibition of protein and RNA expression in HCV cells. The pre-treatment of cyclosporine on the hepatic cell cultures revealed

significant inhibition of HCV (Glowacka et al. 2020). In contrast, some case studies reported the possibility of the development of herpes zoster virus infection (herpes zoster meningitis) in the patients administered with cyclosporine drug (Tsurukawa et al. 2016).

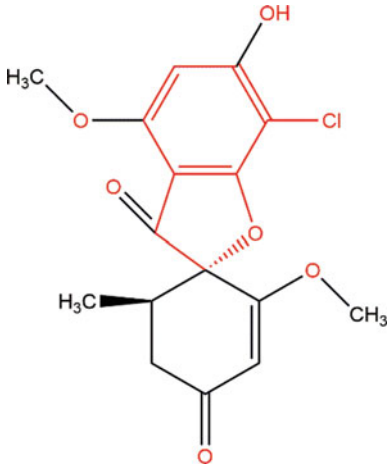
Further, replication of SARS was inhibited by non-toxic amount of cyclosporine with almost undetected levels of protein and viral RNA in cell culture models, as well as in the human coronavirus 229E model. High-throughput screening experiments showed that cyclosporine inhibit functional interaction of cyclophilin and viral proteins and their replication. However, certain part within the cell culture was not resistant to MERS infection completely despite using higher concentrations of cyclosporine. Both in vitro and ex vivo studies involving cyclosporin proved inhibition or reduction of MERS-CoV replication (Li et al. 2018; Glowacka et al. 2020). One of the most common problems due to the administration of cyclosporine as immunosuppressor agent is, urinary tract infection, which occurs after renal transplantation. Not only urinary tract infection, but it also leads to reduction in the expression of TLR4 along with cytokine production and reduction in the neutrophil migration with an increased level of bacterial infection (Sadio et al. 2018; Glowacka et al. 2020).

3 Non-peptide Drugs

3.1 Griseofulvin (Brand Name: Gris-Peg*)*

Griseofulvin is an organoheterocyclic compound, which can be categorized under the class benzofurans [Drug Bank Accession Number DB00400]. It is also a spiro compound (Fig. 22.5). Griseofulvin is an antifungal metabolite produced by *Penicillium* spp. It was first isolated from *P. griseofulvum,* and it was also found to be

Fig. 22.5 Griseofulvin



produced by other Ascomycetes such as *Aspergillus* sp. and *Xylaria* sp. (Xu et al. 2021). Griseofulvin has also been identified in an endophytic *Nigrospora* sp., which was isolated from the root of the medicinal plant *Moringa oleifera* lam (Zhao et al. 2012). This drug is administered orally for infections caused by *Microsporum* or *Trichophyton* fungi. In humans, it targets keratin—type 1 cytoskeletal 12 and forms keratin-griseofulvin complex. When griseofulvin reaches the site of infection, its mechanism of action begins by binding to fungal tubulin and altering the mitosis process. However, the mechanism of action is still not completely understood. Since it is poorly absorbed from gastrointestinal tract, it is prescribed to take after a calorie dense food and avoid alcohol. It has a half-life of 9–21 h, and it has only fewer side effects.

Because griseofulvin binds to tubulin, which is a major component in the microtubule assembly, it also has anticancer activity (Das and Paul 2018; Schmeel et al. 2017; Singh et al. 2008). Moreover, in a recent computational study, griseofulvin and its derivatives have been shown to possess therapeutic potential to treat COVID-19 (Aris et al. 2022). Griseofulvin analogues have also been shown to inhibit phytopathogenic fungi both in vitro and in vivo (Bai et al. 2019). Further, several investigations provide encouraging hints that griseofulvin derivatives can be used to develop new potent agricultural fungicides (Xu et al. 2021). Furthermore, analysis of genome sequences has shown that seven genes responsible for the biosynthesis of griseofulvin are very much conserved across various fungal species (Aris et al. 2022).

3.2 Fusidic Acid (Brand Name: Fucibet, Fucidin, Fucithalmic*)*

Fusidic acid (FA) is an organic compound belonging to the class of 'steroids' and sub class: 'steroid ester' [DrugBank Accession Number DB02703] (Fig. 22.6a). It is produced by the fungus *Fusidium coccineum* and extracted from the mycelium (Godtfredsen et al. 1962; Fernandes 2016). Interestingly, FA and its analogue, 16-desacetyl FA has been discovered in an endophytic fungus, *Acremonium pilosum* F47 also (Tian et al. 2021). It is a bacteriostatic metabolite, which aids in preventing dermal infections particularly caused by Gram-positive bacteria such as *Staphylococcus* and *Streptococcus* species (Fernandes 2016; Garcia Chavez et al. 2021). It elicits antibacterial activity by specifically inhibiting elongation factor-G (EF-G) during the translocation step, thereby interfering with the protein synthesis (Fernandes 2016). It has been used to treat skin infections for many years in Europe, Canada and Australia (Fernandes 2016; Garcia Chavez et al. 2021). The resistance to this drug may be due to the mutations in FusA gene that codes for the EF-G (Fernandes 2016). Fusidic acid also inhibits chloramphenicol acetyltransferase in *Salmonella typhi* and *Escherichia coli*. FA is a competitive inhibitor of chloramphenicol acetyltransferase-I (CAT-I), but does not competitively inhibit other CAT

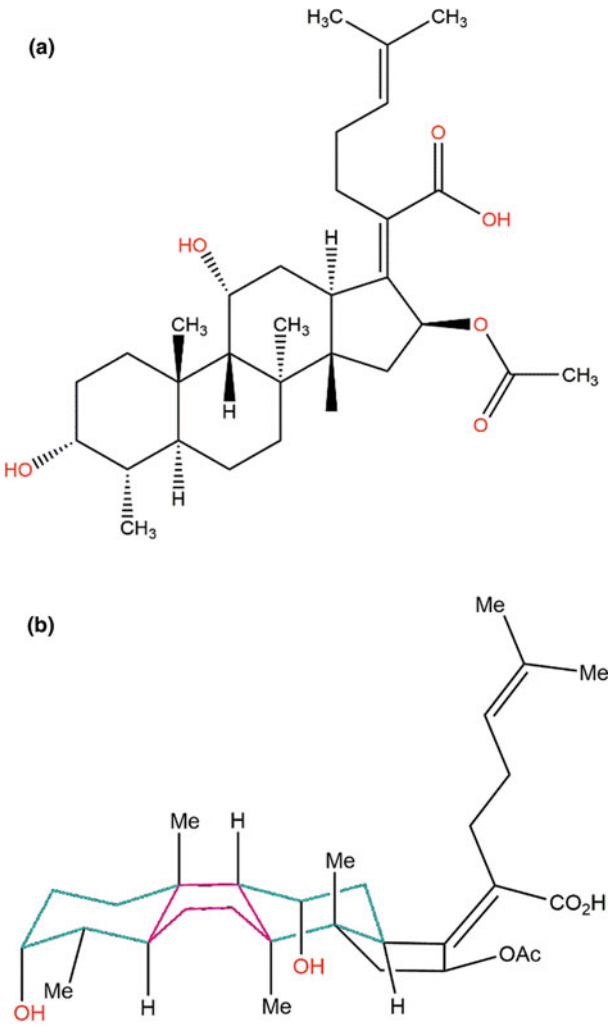


Fig. 22.6 (**a**) Fusidic acid (FA). (**b**) Chair-boat-chair conformation of FA

variants (Biswas et al. 2012). The hydroxyl group of FA forms a strong hydrogen bond with the sidechain of His193 of CAT-I, while the hydrophobic steroidal ring group of FA favourably interacts with the sidechains of amino acid residues such as phenylalanine, leucine and valine of CAT-I (Biswas et al. 2012).

In order to avoid irritation, it should be taken along with food. Its half-life in adult is around 5 to 6 h. FA is administered via oral, intra-venous and topical routes (Garcia Chavez et al. 2021). It is commercially available in the form of eyedrops and creams but it is also available in systemic dosage forms as pills and injections. It is

important to note that total synthesis of this drug is very difficult due to its unique chair-boat-chair conformation (Fig. 22.6b) (Garcia Chavez et al. 2021). Nevertheless, synthesizing derivatives of FA has been attempted in search of compounds that can show better efficacy on the resistant strains of bacteria. In this regard, Garcia Chavez et al. have demonstrated good efficacy of a particular synthetic analogue of FA against the FA-resistant strain of *S. aureus* in a soft-tissue murine infection model (Garcia Chavez et al. 2021). One of the synthetic derivatives of FA was reported to exhibit anti-sepsis activity, by potently inhibiting stimulator of interferon genes, STING (Long et al. 2022).

3.3 Mycophenolic Acid (Brand Name: Myfortic*)*

Mycophenolic acid (MPA) (Fig. 22.7) is an organoheterocyclic compound belonging to the class 'isocoumarins' and subclass 'isobenzofuranones' [DrugBank Accession Number DB01024]. MPA was discovered for the first time from the fungus *Penicillium stoloniferum* (Bentley 2000). *P. brevicompactum* is another main producer of MPA; however, MPA has been found in other species of *Penicillium* as well (Vinokurova et al. 2005; Lu et al. 2009; Mahmoudian et al. 2021). It has higher propensity to bind to proteins, especially to albumin. It is an FDA approved immunosuppressive drug, which inhibits de novo purine biosynthesis by reversibly binding to inosine monophosphate dehydrogenase (IMPDH). The de novo purine biosynthetic pathway is critical for the proliferation of T and B lymphocytes (Sintchak et al. 1996; Jonsson and Carlsten 2001). So, MPA inhibits the proliferation of T and B lymphocytes, and hence, it could be applied as an immunosuppressive agent (Hood and Zarembski 1997).

The enteric coated tablet of MPA dissolves well in neutral pH of the intestine. It is suggested to be taken after 2 h of eating or in the empty stomach and the route of elimination is through urine in the form of mycophenolic acid glucuronide (MPAG). It has a half-life between 8 and 16 h. Adverse effects of this drug are seen only at higher doses. The prodrug of MPA, mycophenolate mofetil, CellCept® is also prescribed for preventing organ rejections during the transplants of kidney, heart and liver [https://www.cellcept.com/].

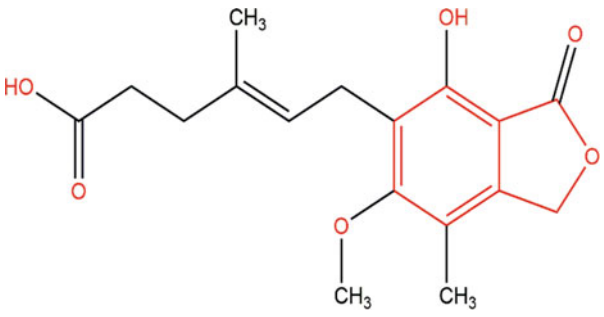


Fig. 22.7 Mycophenolic acid (MPA)

MPA has also been shown to exert potent in vitro anticancer activity through various molecular pathways, whereby it was found to inhibit cell proliferation in nine different cell lines (Dun et al. 2013). Additionally, in the wake of the COVID-19 pandemic, when various types of drugs were analyzed for repurposing, a computational investigation showed that MPA could bind to papain-like protease (PLP) of SARS CoV (Mohamed et al. 2021). With regard to the molecular basis of biosynthesis, the polyketide synthase gene mpaC was found to be responsible for the biosynthesis of MPA backbone, as observed from the gene deletion study conducted in *P. brevicompactum* (Regueira et al. 2011). Furthermore, seven genes involved in the biosynthesis of MPA in *P. roqueforti* were identified through gene silencing (Del-Cid et al. 2016). The gene corresponding to O-methyltransferase, which catalyzes the methylation of demethyl MPA to MPA has also been characterized in *P. brevicompactum* NRRL 864 (Zhang et al. 2015).

3.4 Fingolimod (Brand Name: Gilenya*)*

Fingolimod is a biologically active sphingolipid metabolite (Fig. 22.8). It is chemically synthesized from the compound myriocin, which is derived from the culture broths of the entomopathogenic fungus *Isaria sinclairii* (Chiba 2020). Though myriocin itself showed good immunosuppressive activity in vitro, it was observed to be toxic in vivo (Chiba 2020). However, the chemically modified form of myriocin, FTY720 (fingolimod) which did not have three consecutive chiral centres, was found to be an immunosuppressant with same potency and less toxic than myriocin (Adachi et al. 1995). The earlier investigations on FTY720 were done to understand its function in the cases of organ transplantation, such as skin and cardiac allografts (Adachi and Chiba 2007). Even in recent studies, the beneficial role of FTY720 has been reported in the cases of heart transplantation models (Ahmed et al. 2020; Chen et al. 2021). In the year 2010, it was commercially approved for treating multiple sclerosis (MS), despite that the synthesis of this compound began in 1995 (Thomas et al. 2017; Volpi et al. 2019). In fact, it is the first drug to be approved by FDA to treat relapsing MS by oral therapy (Chun et al. 2019). Interestingly, it was approved by both FDA and the European Medicines Agency to treat even the

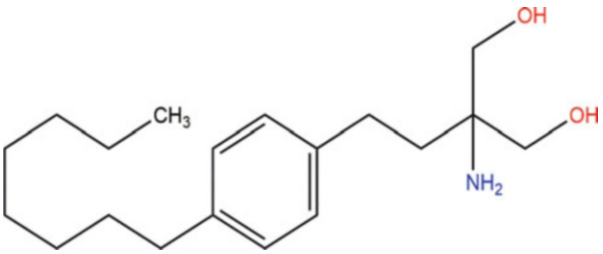


Fig. 22.8 Fingolimod (FTY720)

pediatric patients with relapsing-remitting form of MS (Volpi et al. 2019; Zaffaroni 2021). Fingolimod is actually regarded as a pro-drug of the phosphorylated form. Fingolimod phosphate is a functional antagonist of Sphingosine 1-phosphate receptor 1 ($S1P_1$) on lymphocytes, thereby modifying the lymphocyte migration to the inflammatory site (Massberg and von Andrian 2006; Chun et al. 2019). Its half-life is about 6 to 9 days. In addition to its immunosuppressant activity, fingolimod was also demonstrated to restrict the growth of *Candida albicans* both in vitro and in a mouse candidiasis model (Najarzadegan et al. 2022). The motivation to test fingolimod for anti-fungal activity by Najarzadegan et al. actually arises from the reports on the consistent anti-fungal activity of myriocin against *Candida* and *Aspergillus* species. Furthermore, clinical trials are underway to examine the effect of fingolimod for treating COVID-19 patients as well [DrugBank Accession Number DB08868].

3.5 Irofulven

Irofulven is an organic spiro compound with a cyclohexenone ring and a cyclopropyl ring (Fig. 22.9). It is actually a semisynthetic compound derived from Illudin S, which was originally identified from a toxic jack-o-lantern mushroom, *Omphalotus illudens* (Staake et al. 2016). It elicits an antitumor activity through DNA damage and it selectively initiates apoptosis of tumor cells (Kelner et al. 2008). Adduct formation with macromolecule and arresting S-phase during cell cycle are a few other mechanisms involved in antitumor activity (van Laar et al. 2004a). A study by Kelner et al. showed that DNA damage caused by irofulven is repaired by transcription-coupled nucleotide excision repair (TC-NER) (Kelner et al. 2008; Börcsök et al. 2021).

van Laar et al. (2004a) conducted investigations on human pancreatic carcinoma cell lines to test the efficacy of irofulven and the outcome of this study is the following. When irofulven was tested against MiaPaCa pancreatic xenografts, a significant curative activity was observed at both daily and intermittent dosages.

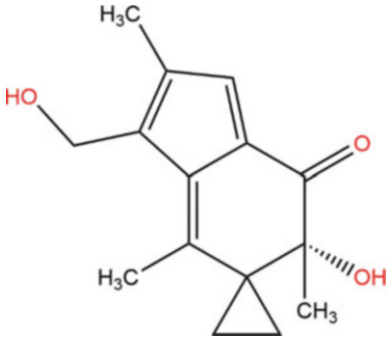


Fig. 22.9 Irofulven

Further, combination of irofulven with gemcitabine showed higher efficacy than the single drug. In another study, van Laar et al. (2004b) demonstrated in vitro antiproliferative activity against some ovarian cancer cell lines (such as IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8 and SK-OV-3) and on tumor specimens. The cancer cell lines were characterized by sulforhodamine B, while tumor specimens were analyzed by Human tumor colony-forming assays. Due to the potential activity of irofulven against ovarian cells in both in vitro and in vivo studies, it has been approved for further clinical trials (van Laar et al. 2004b). Irofulven was also demonstrated to possess cytotoxic as well as genotoxic activity on certain standard *Salmonella typhimurium* strains TA98, TA100 and TA104 (Glatt et al. 2014).

Additionally, two different derivatives of irofulven was observed to exhibit better antitumor efficacy than the irofulven itself under both in vitro and in vivo conditions (Staake et al. 2016). Further, clinical trials are currently underway on irofulven [https://clinicaltrials.gov/ct2/show/NCT00062257; DrugBank Accession Number DB05786].

The aforementioned details of these five non-peptide drugs are summarized in Table 22.2.

4 Perspective for Future

Fungi have indeed proven to be a treasure trove for multipurpose applications encompassing a wide range of areas such as agriculture, pharmaceutics and environmental biotechnology. The fungal secondary metabolites have been quite attractive for pharmaceutical industries leading to the production of various successful drugs (Conrado et al. 2022). Particularly, in the scenario of increasing number of cases of drug resistance towards various types of diseases, the search for novel drug compounds with improved potency will not cease, for which fungi will continue to be sought after. It appears that the future of endophytic fungi is bright for the discovery, development and production of drugs and agrochemicals, especially when combined with application of mass spectrometry-based-metabolomics (George et al. 2019; Xu et al. 2021; Zheng et al. 2021; Nagarajan et al. 2021).

Table 22.2 Summary of small molecule secondary metabolites of fungal origin

Secondary metabolites Drug Bank Accession No.	Molecular formula and mol. mass	Fungal source	Chemical class	Commercial name	Therapeutic activity
Griseofulvin	$C_{17}H_{17}ClO_6$	*Penicillium* sp.	Benzofuran, Spiro compound	Gris-peg, Grisovin, Grisofit, Fulcin etc.	Anti-fungal, to treat infections caused on hair, skin and nails
DB00400	352.766				
Fusidic acid (FA)	$C_{31}H_{48}O_6$	*Fusidium coccineum*	Steroid	Fucibet, Fucidin, Fucithalmic	Anti-bacterial to treat dermal infection
DB02703	516.7092				
Mycophenolic acid (MPA)	$C_{17}H_{20}O_6$	*Penicillium stoloniferum*	Benzofuran (Isobenzofuran)	Myfortic	Immunosuppressant during organ transplantation
DB01024	320.337				
Fingolimod	$C_{19}H_{33}NO_2$	*Isaria sinclairii*	Sphingolipid	Gilenya	Immunomodulatory to treat multiple sclerosis
DB08868	307.4708				
Irofulven	$C_{15}H_{18}O_3$	*Omphalotus illudins*	Spiro compound, cyclohexenone	Clinical trials phase-3	Anticancer
DB05786	246.3016				

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Chapter 23
Kojic Acid from *Aspergillus wentii:* A Journey from Isolation to Application

Prabha Devi, Rajesh Parvatkar, Rani Rajamanikam, Solimabi Wahidullah, and Narsinh Thakur

Abstract Marine-derived fungi are widely known to produce a plethora of priceless biologically active secondary metabolites. The main objective of this study was to produce kojic acid as an exclusive secondary metabolite in the fermentation medium of *Aspergillus wentii* (MTCC 5374). Firstly, we describe the isolation of endophytic fungi from a marine alga followed by its growth- optimization in the laboratory to give maximum yield of kojic acid and finally its mass culturing for obtaining crude product. This paper also describes a simple, single-step extraction and column chromatographic purification of the crude extract to produce 98% pure kojic acid. Commonly used thin-layer chromatography (TLC) and advanced instrumentation techniques using NMR, IR and MS is reported for its easy identification. Kojic acid (5-hydroxy-2-hydroxymethyl-γ-pyrone) having a molecular formula $C_6H_6O_4$ is a white, crystalline, water-soluble organic acid enzymatically produced from *Aspergillus* sp. It possesses potential application in the field of medicine, food, cosmetics and agriculture. Its outstanding application as a skin whitening agent by inhibiting formation of tyrosine is schematically represented assigning tyrosinase enzyme to act as a rate determining step in the production of melanin during hyperpigmentation.

Keywords Kojic acid · *Aspergillus wentii* · Commercial production · Application

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1 Introduction

Marine natural sources play a significant role in producing biologically active compounds useful in the treatment and prevention of human diseases. Since ancient times, it is seen that fungi belonged to one of the richest sources of secondary metabolites for human use (Cimmino et al. 2015; Kornienko et al. 2015). Recent studies revealed that fungi established a symbiotic association with marine macroalgae (Zuccaro et al. 2008; Harvey and Goff 2010). The lack of the immune system in seaweed and its sedentary lifestyle have led to the evolution of newer compounds from its associated micro-biota, causing both seaweed and its associated fungi to enhance tolerance to various biotic and abiotic stresses (Egan et al. 2013). Some of these metabolites demonstrated cytotoxic, antioxidant or antimicrobial activities that led to the discovery of several new compounds or same compounds with increased activity against pathogens (Sarasan et al. 2017; Flewelling et al. 2013a, b). One of the first most potent known antibiotics was penicillin (Penicillin F) discovered by Alexander Fleming (1929) which was later (1940s) developed into a medicine by Chain et al. (1940). Extensive research, following the discovery of penicillin, resulted in thousands of newer compounds possessing broad-spectrum biological activities. Few examples of marine-derived fungi producing useful compounds like anthraquinone derivative named Aspergiolide A produced by *Aspergillus glaucus* exhibited cytotoxicity against cell line K562 and P388 (Du et al. 2007). Marine-derived *Penicillium* sp. exhibited antitumor activities by meleagrin D and E and roquefortine H and I (Du et al. 2010). Marine fungi *Ampelomyces* sp. produced potent antimicrobial and antifouling compounds (Kwong et al. 2006). Marine-derived fungus *Cladosporium* sp. exhibited antibiotic and antifouling activity (Xiong et al. 2009). Marine-derived fungus *Fusarium* sp. possessed novel antimitotic and antifungal activities due to the formation of fusarielin E (Gai et al. 2007). Yet, another marine-derived fungus *Pseudoallescheria* produced novel antibacterial compound dioxopiperazine, dehydroxybis-dethio-bismethylthio-gliotoxin exhibiting potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Li et al. 2006). Marine-derived fungus *Exophiala* sp. produced chlorohydroaspyrones A and B, as well as aspyrone, asperlactone and penicillic acid showing antibacterial activity (Zhang et al. 2008). Kojic acid is an organic acid produced biologically by microbes (fungi/bacteria), such as *Aspergillus oryzae*, *Penicillium* or *Acetobacter* species (Nohynek et al. 2004) during fermentation of various carbon containing substrates (Chen et al. 1991a). Initially, kojic acid was extracted from a mould (*Aspergillus oryzae*) grown on rice or soyabean (*A. sojae*). Glucose was found to be the best carbon source precursor for the synthesis of kojic acid. During fermentation, glucose directly produces kojic acid through a multistep reaction without causing cleavage of carbon chain (Kitada and Kukimbara 1971; Bajpai et al. 1981).

This chapter deals with the exploitation of marine-derived fungi as a potential source of kojic acid, a compound useful in medicine (against several diseases), food, agriculture and cosmetics (Kayahara et al. 1990; Le Blanch and Akers 1989; Gomes

et al. 2001; Smith and Lindsay 2001). In medicine, kojic acid is used mainly as an antibiotic, anti-inflammatory and painkiller (Chen et al. 1991a). Being a free radical scavenger, it acts as an antioxidant, a property that makes it useful as nutraceuticals in the food industry. Kojic acid is used against blackening of fruits and vegetables (Chen et al. 1991a), precursor for flavor enhancers (Le Blanch and Akers 1989) and anti-browning agent (Chen et al. 1991b). Cosmeceutical compounds are substances which add therapeutic value to cosmetics. In cosmetics, it finds wide application as a skin whitening agent (Ohyama and Mishima 2010).

Due to its significant potentials, kojic acid continues to attract attention and in view of this, we experimented on the production of kojic acid using *Aspergillus* wentii, under laboratory conditions and optimized fermentation medium (FM) for significant production of kojic acid. Considering the high price and poor availability coupled with the benefits of kojic acid, this study may be extremely beneficial for kojic acid-utilizing industry for its mass production using cheap and readily available raw material enzymatically from *Aspergillus* sp. coupled with simple process of production, detection and purification. An attempt is also made to understand how tyrosinase can act as a rate determining step in melanin production and how it could play a major role in preventing discoloration of fruits and vegetables.

2 The Approach

2.1 Collection of Green Alga from Goa Coast, India, and Isolation of Endophytic Fungi

Green algal sample *Caulerpa sertularioides* was collected from Anjuna (15°54′N & 73°51′E to 73°52′E), Goa coast, India, in sterile polythene bags and transported to the laboratory. Isolation of endophytic fungi was carried out as described earlier (Prabha Devi et al. 2009). Briefly, immediately on reaching the laboratory, the marine sample was rinsed in sterile seawater and cut into small pieces aseptically using a sterile scalpel. Small portions of the alga were vortexed in sterile filtered seawater for 10 s. Washings were discarded and algal pieces were placed on potato dextrose agar (PDA) plates spiked with tetracycline (100 mg/L) and streptomycin (20 mg/L) to prevent bacterial contamination. The plates were incubated for 2 weeks to allow the fungus to grow and sporulate. Fungal hyphae were picked and separately subcultured, repeatedly to obtain pure isolate of the cultures which were later identified at Agharkar Research Institute, Pune. Among the various cultures, *Aspergillus wentii* was used for further study. It was deposited at the Institute of Microbial Technology bearing Accession No. MTCC 5374.

2.2 Kojic Acid Production

Portions of agar were cut from a fully grown culture plate and added to flasks containing 2 L of the fermentation medium (FM). The fermentation medium (FM) comprised of glucose 100 g L^{-1}, yeast extract 5 g L^{-1}, KH_2PO_4, 1 g L^{-1}, $MgSO_4$. 7H2O, 0.5 g L^{-1}, and peptone 6 g L^{-1} prepared in seawater: distilled water (1:1, 17 ppt).

Mass culturing was carried out in 5 L flasks (5 nos.), each containing 2 L of fermentation medium. The media were sterilized in an autoclave (121 °C) for 15 min, cooled to room temperature and inoculated with *Aspergillus wentii*. The culture was incubated at 27 °C for 15–20 days with occasional swirling. The formation of kojic acid in the medium was detected by spotting a drop of the aseptically removed broth on a ferric chloride-sprayed TLC plate. The reddish-brown color on the plate indicated the presence of kojic acid formation. More intense color indicated higher concentration of kojic acid.

2.2.1 Crude Extract Preparation and Purification of Kojic Acid

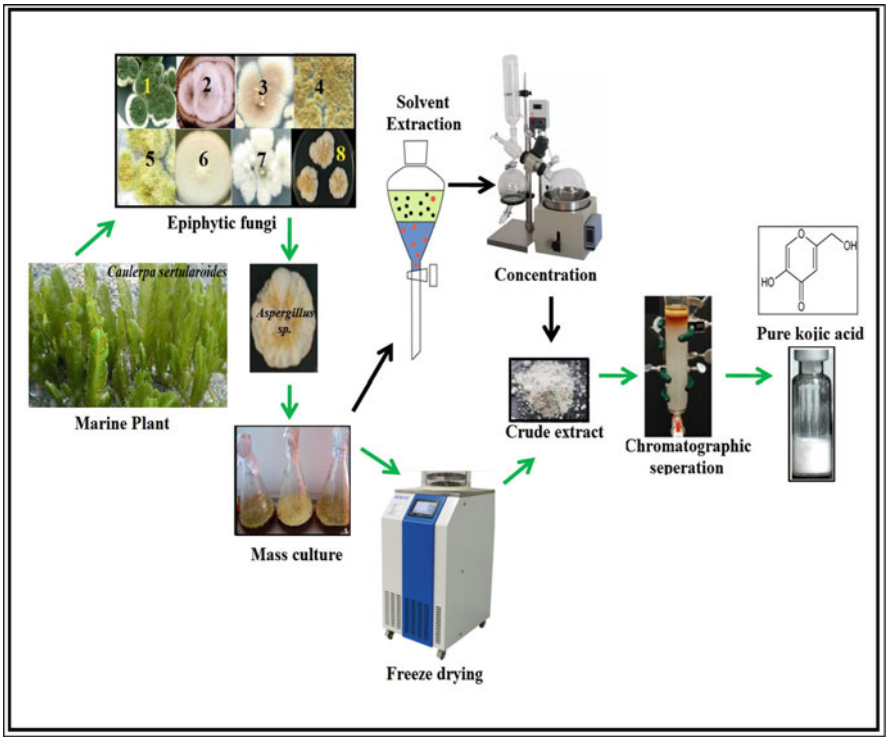
At the end of the fermentation period, fungal mycelia were filtered out from the broth. Extraction was carried out in two ways: (a) butanol extraction of the fermentation medium followed by pooling the solvent and vacuum concentration of the same to obtain crude extract. (b) The mycelia-free medium was frozen followed by lyophilization till the crude extract was obtained in the powdered form. Finally, slurry of the crude extract (obtained by both the methods a & b) was prepared and loaded onto a glass silica gel column. Initially, the column was eluted with 100% chloroform to remove the less polar chemical constituents and colored impurities if any. This was followed by elution of the column with chloroform:methanol (95:5 v/v) to obtain pure (98%) kojic acid which was further purified by repeated column chromatography.

2.2.2 Spectroscopic Identification

TLC was conducted on Kieselgel $60F_{254.}$ Mass spectra were obtained both in the positive and negative ion mode, on a QTOF-XL MS/MS Applied Biosystem instrument equipped with MDS Sciex Analyst Software (Concord Ontario, Canada). NMR spectra were recorded on a Bruker Avance Spectrometer (300 MHz) in deuterated dimethyl sulfoxide (DMSO) containing tetramethylsilane as an internal reference. Comparison of ^{1}H and ^{13}CNMR spectra with those available in the literature identified the pure compound, and its 2D NMR analysis (Table 23.1) helped to confirm its structure.

Table 23.1 HMQC and HMBC data of kojic acid

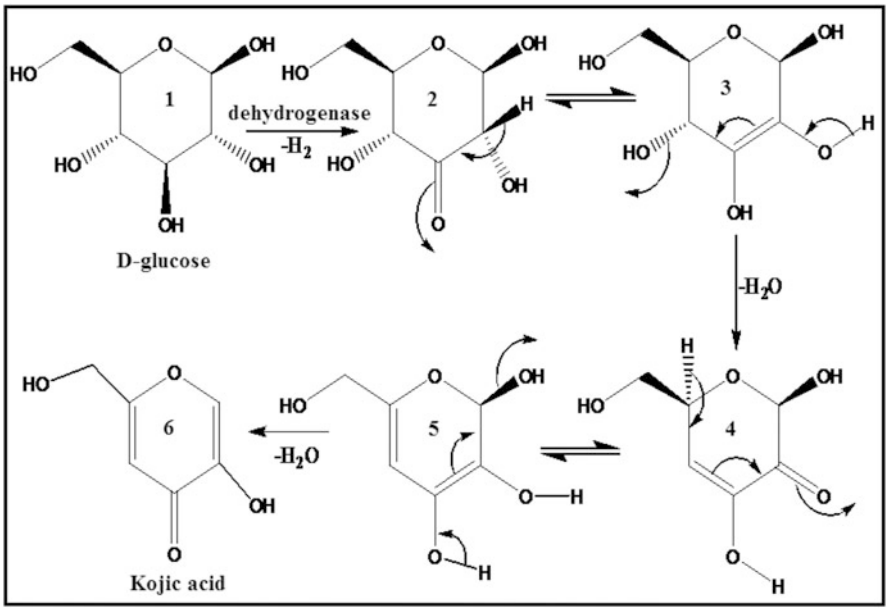
HMQC		HMBC	
δH	δC	δH	δC
4.28	58.9 (CH_2)	8.02	145,170,176
6.33	109.4 (CH)	6.33	145,170
8.00	138.7 (CH)	4.28	110,170



Scheme 23.1 Process involving isolation of endophytic fungi, mass culturing, extraction of crude mixture, purification and identification of pure compound

3 The Outcome

Following the approval of kojic acid in 1988, several other cosmetics possessing skin lightening ingredients were launched in the early 1990s leading to skin lightening cosmetics boom in Asia. In the present study, we describe the isolation of several endophytic fungi from marine sources (Scheme 23.1). This was followed by screening of the fungi to produce kojic acid as the main secondary metabolite in the fermentation medium. After initial screening, we found *Aspergillus wentii* (Plate 8) to be an excellent source to produce kojic acid and hence the same was used for further study. Optimization of culture growth condition (28 ± 1 °C, stationary condition, 20 days incubation) and medium (glucose (100 g/L), yeast extract (5 g/



Scheme 23.2 Mechanism of enzymatic conversion of D-glucose to kojic acid in the fermentation medium

L), KH_2PO_4 (1 g/L), $MgSO_4$ (0.3 g/L) and peptone (6 g/L) dissolved in seawater: distilled water (1:1, salinity 17 ppt) was realized. The yield of kojic acid was 20 g/L using this medium. The cheapest way to produce kojic acid enzymatically from fungi was earlier reported by Rosfarizan and Ariff (2020), and we describe its mechanism schematically (Scheme 23.2). Briefly, D-glucose (**1**) on oxidation at C-3 produces keto D-glucose (**2**) which enolises by the removal of C-2 proton to form enol (**3**). This enol further dehydrates losing –OH at C-4 producing unsaturated keto compound (**4**), which in turn enolises losing C-5 proton followed by the elimination of water forming kojic acid (**6**).

High productivity of kojic acid (40 g/L) was earlier reported from *Aspergillus* sp. by Bajpai et al. (1981) as well as by Futamura et al. (2001). However, the estimation of kojic acid by them was measured calorimetrically which could be an overestimation, wherein the aflatoxin/pigments in the medium could interfere with the kojic measurements. In our study, we determined kojic acid by dry weight method and the pure crystalline kojic acid was collected in vials as a dry product. The presence of aflatoxin as well as pigments adds to the complexity of the purification process and hence the production cost.

3.1 Preferred Method of Extraction of Kojic Acid

In general, under natural product research, solvent extraction was the most convenient method of extracting the fermentation medium using solvents immiscible in the aqueous medium to dissolve the compounds present in the medium. Kojic acid was extracted by the routine solvent-solvent extraction technique wherein butanol was the preferred solvent considering the polarity of the extracting solvent and the solubility of kojic acid. The organic solvent was pooled together, evaporated to dryness and the resulting crude extract contained the compound of interest. The resulting aqueous phase is generally discarded after the solvent extraction process is complete. However, since our compound is water soluble discarding the aqueous phase would result in total loss of major amount of our compound. Secondly, this method using butanol extraction was more tedious as it had a high boiling point (117 °C) and hence was more cumbersome to evaporate off butanol to obtain the crude extract. Under the existing conditions, the production of kojic acid is expensive and hence not economically viable for scale up. To make the production of kojic acid economical for commercial applications, we tried exploring a second method described below for the extraction of the same.

In the second preferred method wherein the fermentation medium was directly concentrated using a lyophilizer, the resulting crude powder was richer in the kojic fraction (Scheme 23.1, green arrow route). This method greatly decreased the extraction time and cost in obtaining the crude powder. The crude powder thus obtained by both the above methods was used for further purification. Purification of kojic acid from the crude fraction was also a simplified process involving simple glass column chromatography separation eluted with suitable solvents to obtain highly pure kojic acid [Scheme 23.1].

3.2 Unique Technique for the Detection of Kojic Acid

Pure kojic acid formed both in the fermentation medium as well as during chromatographic elution was detected by the presence of intense reddish-brown color on $FeCl_3$-sprayed TLC plates. The intense brown color was due to the oxidation reaction between $FeCl_3$ and the OH group from kojic acid to form a colored ferric phenoxide complex.

3.3 Characterization of Kojic Acid

Kojic acid has a m.p. of 154 °C and a molecular weight of 142 amu as evident from ESI-MS analysis. In the negative ion mode, ESI-MS (Fig. 23.1a) showed an intense $[M\text{-}H]^-$ ion at m/z 141 and in positive ion mode (Fig. 23.1b), it showed peaks at m/z

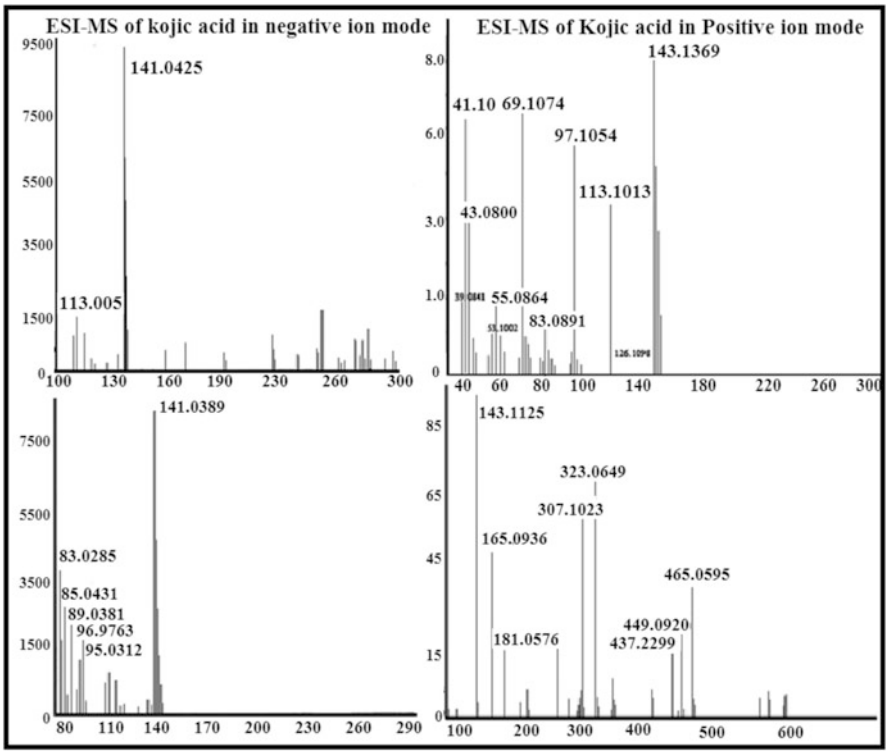


Fig. 23.1 (**a**) ESI-MS of kojic acid in the negative ion mode. (**b**) ESI-MS of kojic acid in the positive ion mode

143 $[M + H]^+$, m/z 165 $[M + Na]^+$ and m/z 181 $[M + K]^+$. Thus, presence of protonated, sodiated or potassiated ion can be used to detect the presence of kojic acid in the fermentation medium. ESI-MS in positive ion mode also displayed additional peaks at m/z 307 $[2M + Na]^+$, m/z 323 $[2M + K]^+$, m/z 449 $[3M + Na]^+$ and m/z465 $[3M + K]^+$.

IR spectrum (KBr pellet) of kojic acid exhibited absorption at 3176 and 1660 cm^{-1} due to hydroxyl (–OH) and α, β-unsaturated carbonyl group, respectively (Fig. 23.2). The proton (^1H) NMR spectrum at 300 MHz in DMSO (Fig. 23.3, Table 23.2) indicated two singlets, each for one olefinic proton at δ 6.33 and 8.02. A doublet integrated for two protons was evident at δ 4.28 ($J = 4.5$ Hz) which indicated the presence of a methylene group adjacent to an oxygen function. Two D_2O exchangeable protons at δ5.68 (triplet) and broad singlet at δ9.05 were assigned to –OH groups. One –OH is next to methylene (methylene doublet at δ4.28 is reduced to a singlet on D_2O exchange) and the other –OH on a quaternary carbon. The signal at δ9.05 was assigned to –OH and not –NH on the basis of even molecular weight. A less intense signal at δ 2.5 and an intense broad singlet at δ 3.3 were assigned to the methyl groups of DMSO-d_6 and the HOD (in DMSO). The carbon

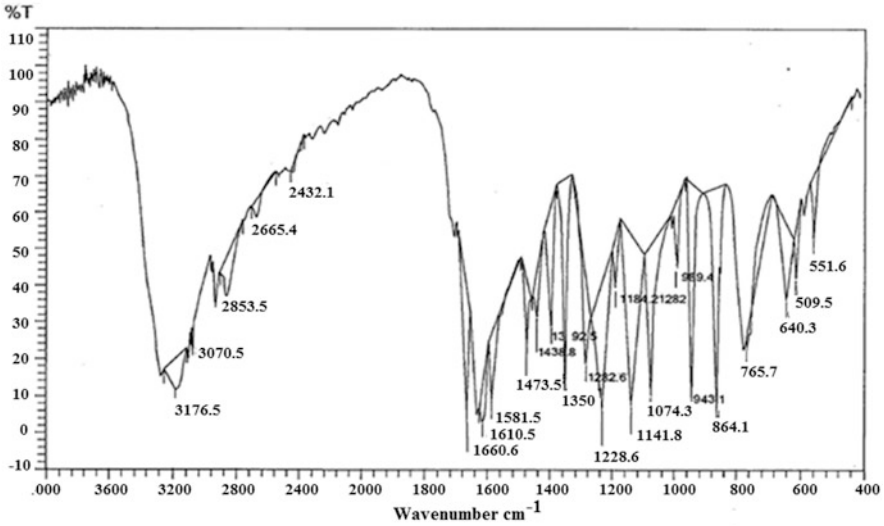


Fig. 23.2 IR spectrum of kojic acid

(^{13}C) NMR spectrum at 300 MHz in DMSO (Fig. 23.4, Table 23.2) of compound 1 showed signals which on the basis of DEPT experiments were inferred as three quaternary carbons at δ 173, 167 and 145, two methines at δ 109 and δ 138 and one methylene carbon at δ 58.

Proton-carbon chemical shift correlation for all the carbons directly bonded to protons was established by a HMBC experiment (Table 23.1). The HMBC experiment establishes the long range proton-carbon chemical shift correlation. The spectrum showed correlation of H-6 proton with C-5, C-2 and C-4; the olefinic H-3 proton showed correlation with C-5 and C-2. Similarly methylene protons H-7 correlated with C-2 and C-3. Thus, compound 1, on the basis of the above spectral data, was identified as 2-hydroxymethyl-5-hydroxy-ɣ-pyrone (kojic acid). The data is well in agreement with the values reported in the literature (Wilson 1917).

It was a breakthrough in medicine and cosmetic technology when kojic acid was approved as the first skin-lightening ingredient. Kojic acid efficiently treated melasma, solar lentigines, hyper pigmentation etc. The epidermal basal layer of skin contains melanocyte which produce the dark pigment called melanin imparting skin and hair their dark color. Inside the melanocyte, the amino acid, tyrosine, works as the substrate to activate enzyme tyrosinase to produce the melanin pigment. Tyrosinase is a copper-containing enzyme which catalyzes the production of melanin from tyrosine by oxidation. Although melanin protects skin cells from UV damage, its over accumulation causes dark spots to appear on the skin. Kojic acid is especially effective in inhibiting production of transmitters, reactive oxygen species and inflammation, thus preventing melanin production and hence preventing skin darkening. Tyrosinase is thus regarded as a rate determining step in the biosynthesis of melanin involving tyrosinase-catalyzed conversion of tyrosine to

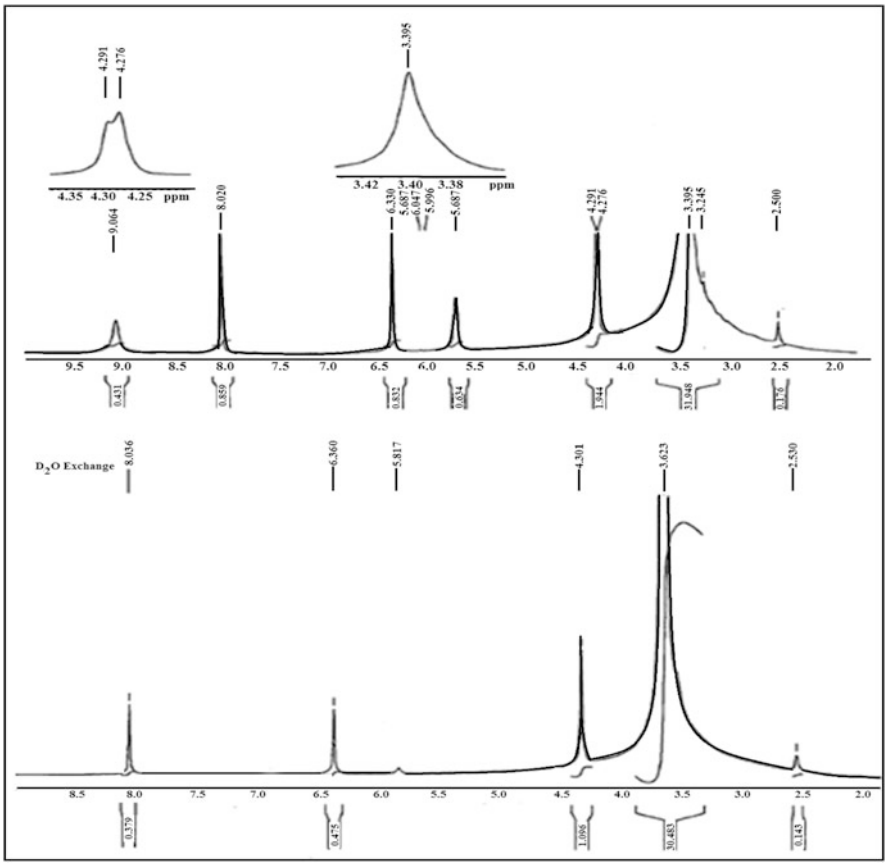


Fig. 23.3 [1]HNMR spectrum of kojic acid

DOPAquinone and rearrangement of DOPAchrome to DHICA and DHI (Scheme 23.3). Diminished tyrosinase activity causes lower pigment production (Castro-Sowinski et al. 2007). Wang et al. (2019) reported that copper has the tendency to increase tyrosinase activity while Fe and Ni enhances tyrosinase expression and hence melanin production.

In medicine, kojic acid is used as an antibiotic since it inhibits the growth of bacteria, fungus and viruses. In food industry, it is used as a flavor enhancer (Le Blanch and Akers 1989), in the preservation of fruits and vegetables as well as to prevent food browning by inhibiting polyphenol oxidase, an enzyme responsible for the blackening of agricultural products (Chen et al. 1991a). Kojic acid is widely used in Japanese diet as it is believed to be beneficial to health. Kojic acid and its derivatives are added to cut flowers to keep flower color fresh and meat color unchanged. Of late, kojic acid may also have other uses in future for the production

Table 23.2 ^{1}H and ^{13}C NMR data of kojic acid

Carbon no.	δH (DMSO)	δH (D_2O exchange)	δC (DMSO)
2			167.5 (s)
3	6.33(s,1H)	6.33 (s)	109.3 (d)
4			173.4 (s)
5			145.1 (s)
6	8.02 (s,1H)	8.02	138.7 (d)
7	4.28 (d, 2H, $J = 4.5$ Hz)	4.28 (s)	58.9 (t)
5-OH	9.05 (s, 1H)		
7-OH	5.68 (t,1H)		

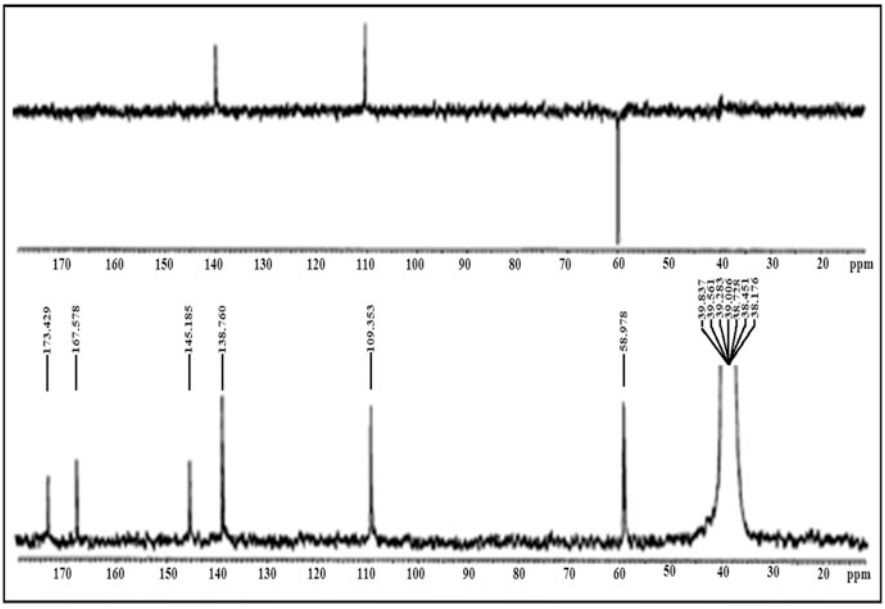
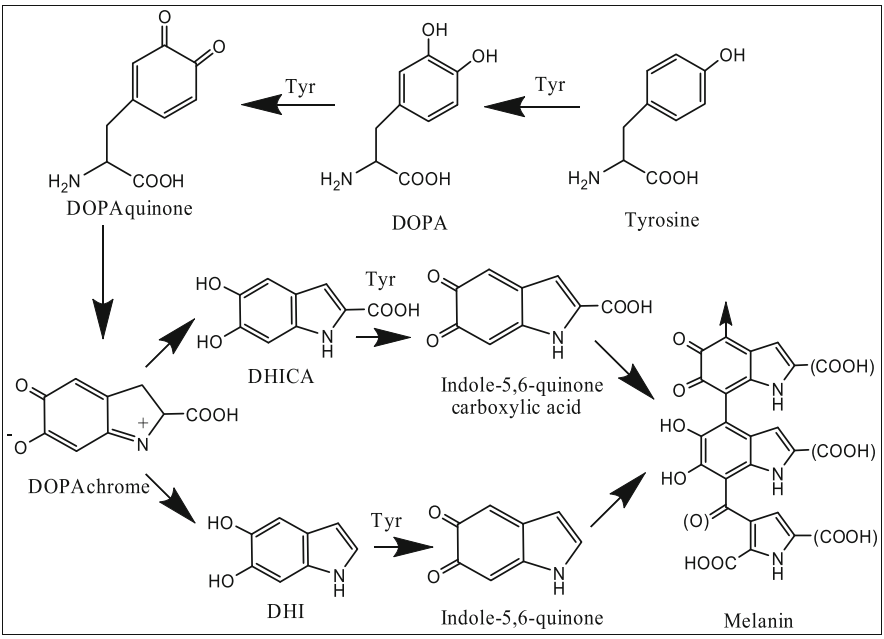


Fig. 23.4 ^{13}CNMR and DEPT spectra of kojic acid

of novel biodegradable plastics, if it can be produced industrially at a reasonable cost (Futamura et al. 2001). Kojic acid in conjugation to Chitosan is stated to improve antibacterial activity against both Gram-positive and Gram-negative bacteria (Liu et al. 2014). For all the above reasons, and in the light of the importance and proven utility of kojic acid, extensive screening of *Aspergillus* species has been established to detect the organism that produces exclusively kojic acid as a secondary metabolite. The best fermentation medium and large commercial scale production calls for economical methods to meet the future demands of kojic acid.



Scheme 23.3 Mechanism showing how tyrosinase is the rate determining step in the biosynthesis of melanin

4 Conclusion

Justification as to why such an intense study on kojic acid and its easy detection is required is because of its vast application in the field of medicine, food industry, cosmetics and agriculture. Also, till date, there is no concern raised on safety aspects of kojic acid due to the long history of its industrial applications and human exposure. A strategy for increasing the production of kojic acid economically, rapid detection of the compound in the fermentation medium, followed by its extraction, easy purification and identification of the economically important metabolite formed the main objective of the study. A probable mechanism for the enzymatic conversion of D-glucose to kojic acid is described (Scheme 23.2) and mechanism to show that tyrosinase is a rate determining step in melanin biosynthesis (Scheme 23.3) is also discussed in this study.

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Part VIII
Bioinformatics and Fungal Biotransformations in Pharmaceutical Drug Development

Chapter 24
How Does Bioinformatics Play a Role in Fungal Drug Discovery?

Akanksha Jaiswar and Nivedita Rai

Abstract Despite all the medical advancements and discoveries over the past several decades, approximately 30% of all known diseases can be currently treated with available pharmacological agents. However, the discovery of new pharmaceutical drugs is one of the most important tasks in biomedical research, both scientifically and economically. Moreover, fungi are prolific producers of a wide variety of natural products. Recent developments in synthetic biology, genetics, bioinformatics, and natural product chemistry have significantly improved our ability to mine fungal genomes for the discovery of novel medications. Thus, to combat the emergence of antibiotic resistance and drug interactions, particularly in immunocompromised patients, there is an urgent need for novel antifungal drugs with novel mechanisms of action. Similarly, drug discovery is a complex process in which drug design is based on mainly two approaches, such as receptor-based drug design and ligand-based drug design, respectively. In this book chapter, we mainly emphasize combinatorial approaches from genomics, proteomics, and bioinformatics research in the development and identification of novel drugs, with a special focus on human mycoses. In addition to this, the framework of bioinformatics findings, advantages, and limitations of fungal drug discovery was obtained by data integration from pharmacology and other associated fields.

Keywords Bioinformatics · Drug candidates · Genomics · Transcriptomics · Pharmaceutical drugs · Antifungal agents · Natural products · Proteomics

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1 Introduction

Over the past few decades, computational techniques have become the most valuable tools for drug investigations. According to the International Human Genome Sequencing Consortium, bioinformatics is an interdisciplinary science of managing and analyzing biological data utilizing cutting-edge computational techniques (Miozza et al. 2020; Luscombe et al. 2001; International Human Genome Sequencing Consortium 2022). The exploitation of genomics, transcriptomics, proteomics, population genetics, and target-based screening platforms are all included in this interdisciplinary science of bioinformatics (Gangotia et al. 2021; Misra et al. 2019). Bioinformaticians can facilitate drug discovery programs by using high-throughput molecular data to compare diseases and their carrier genes with healthy controls (Xia 2017). The first stage of drug discovery begins with the identification of an illness with well-defined symptoms that lower the quality of life. A "desirable medicine" is typically defined as a chemical (which could be a simple molecule or a sophisticated protein) or chemical combination that lessens symptoms without having a significant negative impact on the patient. Other qualities of a desirable drug molecule include a low likelihood of drug resistance, low negative environmental effects, affordability, and financial success (David et al. 2009; Drews and Ryser 1997; Davies and Davies 2010; Boxall et al. 2012). The major attributes, like higher potency, low chemical reactivity-related toxicity, increased efficacy, ease of administration, and affordability, are the preconditions for the new drug to be considered for marketing authorization (Naci et al. 2015; Kiriiri et al. 2020).

As a natural component of the ecosystem, fungi can be found in every conceivable niche, including soil, air, water, and even hospitals (Summerbell et al. 1989). However, fungi are newly developing pathogens (Hajek and St. Leger 1994). For instance, *Candida auris* is multi-drug-resistant by nature, was initially identified in Japan in 2009, and has since spread to 17 nations across five continents, posing a risk to public health (Spivak and Hanson 2018; Jeffery-Smith et al. 2018). Across the country, fungal infections are a severe health issue (Ravikant et al. 2015). While invasive fungal infections are less frequent, superficial infections brought on by dermatophytes affect about 25–30% of the general population (Havlickova et al. 2008). The frequency will result in a high mortality rate. Majority of the fungal infections are mainly caused by the four genera like *Cryptococcus, Candida, Pneumocystis,* and *Aspergillus* (Schmiedel and Zimmerli 2016; Wurster et al. 2017). During the COVID-19 pandemic, the gravity of fungal infections was mainly highlighted in the form of secondary infections in the intensive care unit. Two-thirds of hospitalized coronavirus patients are also infected with *C. auris,* which may lead to adverse outcomes and additional strain on healthcare resources (Chowdhary and Sharma 2020; Chowdhary et al. 2020). Among the available *Aspergillus* species, only a very few, i.e., *A. fumigatus* and *A. flavus,* are infecting humans. The type of infections in aspergillosis causes invasiveness, chronic pulmonary, and ubiquitous aeroallergen (mainly causing allergies and asthma) (Bencurova et al. 2018; Bouz and Doležal 2021). *Pneumocystis jirovecii* is another common respiratory pathogen that

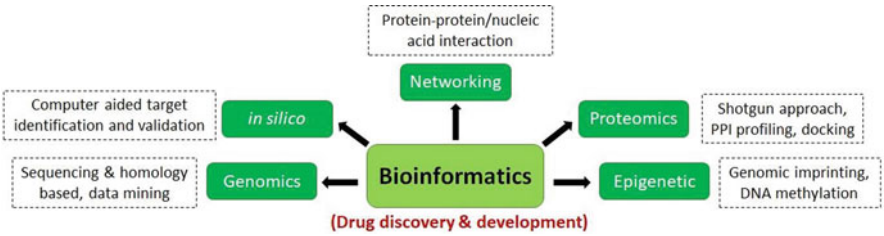

Fig. 24.1 Role of bioinformatics in drug discovery and development

infects individuals with impaired immunity, especially those suffering from HIV. Other groups of fungal infections are associated with organ transplant patients and individuals on prolonged immunosuppressive therapy. Fungal infections have become an overlook emerging disease, and early diagnostics and treatment are life-saving solutions before it is too late (Brown et al. 2012).

This book chapter emphasizes how the bioinformatics approach can speed up the search for antifungal drugs with therapeutic efficacy in order to provide a comprehensive source for desirable medications. Also, an overview of the potency of different central metabolic pathways and their key enzymes as potential antifungal drug targets (Fig. 24.1) is provided.

While each of these methods has its own advantages and disadvantages, together they provide a potent tool for identifying metabolic drug targets for future drug development.

2 Genomic Analysis for Understanding Fungal Pathogenesis

Genomic analysis can provide valuable insights into the molecular mechanisms underlying fungal pathogenesis and host-pathogen interactions and can help identify potential targets for the development of new drugs or control strategies (Aguileta et al. 2009a, b; Amselem et al. 2011). There are several methods and approaches that can be used for genomic analysis of fungal pathogens, including classical genetics, cell biology, and biochemistry, as well as modern, comprehensive, and high-throughput omics techniques supported by appropriate bioinformatics tools (Aguileta et al. 2009a, b; Alves and Pozza 2009; Hadwiger 2009). Current approaches for genomics analysis include whole genome resequencing using reference-based genome assembly and de novo assembly for a novel species the genomes were constructed from a large number of short/long DNA fragments (i.e., contigs) (Bentley 2006; Sohn and Nam 2018).

Various methods are available for genome assembly including Illumina for short read sequencing, Pacific Biosciences and Oxford Nanopore for long read sequencing, i.e., beneficial for a repetitive genome (Cuomo 2017). High throughput de novo

assembly used for a wide variety of newly emerged and rarely observed fungal species, e.g., *Candida albicans* commonly causing candidemia. The clinical epidemiology of *Candida* spp. grows now as new emergent and rare species, e.g., *C. auris*, *C. glabrata*, and *C. inconspicua*. Within European countries, the type strain of *C. inconspicua* was de novo assembled along with 10 additional isolates (Mixão et al. 2019). The recent pathogenic evolution of *C. inconspicua* from highly heterozygous genome to event loss of heterozygosity will clearly improve our understanding about variant analysis. The single nucleotide polymorphism (SNP) reconstruction reveals the bifurcation into two distinct clades, i.e., not correlated with geographical distribution. It also represents a hybrid nature of *C. inconspicua* species with high propensity of antifungal resistance (Lockhart et al. 2017). Similarly in the distinct geographic regions, the simultaneous emergence of four clades of *C. auris* isolates has been deciphered by comparative genomic analysis. The gene expansion contribution in antifungal resistance and virulence across *C. auris* clades revealed by phylogenomics and gene family changes. Additionally, *C. auris* isolates (at positions Y132, K143, and F126) contain increased copy number and mutations of the *ERG11* gene is azole multi-drug resistance (MDR) (Lockhart et al. 2017; Marichal et al. 1990). It can also improve our understanding of the evolution and spread of fungal pathogens, and facilitate the discovery of new fungal species and the characterization of their pathogenic potential (Amselem et al. 2011).

Another intriguing technique for examining the molecular underpinnings of fungal-plant interactions and diseases is transcriptomics or the global investigation of gene expression at the mRNA level (Bhadauria et al. 2007; Oh et al. 2008; Wise et al. 2007; Takahara et al. 2009). In the transcriptomics study, gene expression at the mRNA level has been used in conjunction with newer techniques such as differential display, cDNA-amplified fragment-length polymorphism, suppression subtractive hybridization, serial analysis of gene expression, expressed sequence tags (ESTs), and DNA microarrays for a better understanding of transcription in these organisms (Bhadauria et al. 2007; Oh et al. 2008; Wise et al. 2007; Takahara et al. 2009; Venkatesh et al. 2005; Wang et al. 2009; Fekete et al. 2009).

To investigate the antifungal resistance genes availability, an RNA-seq experiment is carried out in *C. auris* strains B8441 and B11210. The gene expression profiling after exposure to two antifungal drugs (e.g., voriconazole (VCZ) and amphotericin B) on *C. auris* strains reflect that the B11210 strain is resistant and B8441 strain is susceptible to amphotericin B. Whereas, these strains (i.e., B11210 and B8441) display an elevated MIC to VCZ and a low MIC to VCZ, respectively. The differentially expressed genes (DEGs) after drug exposure on the B8441 strain show increased expression of small sets of genes. Overall, 39 genes were induced in response to amphotericin B, 21 genes responded towards the VCZ drug, and 14 genes were induced by both drugs (Muñoz et al. 2018). These techniques, along with targeted mutagenesis or transgenic studies, are revealing molecular host-pathogen crosstalk and the intricate mechanisms underlying pathogenesis and host avoidance.

3 Proteomic Analysis for Understanding Fungal Pathogenesis

In the early 90's, in vitro studies of individual components like the genes and proteins were investigated one at a time. Later on, the strategy shifted starting with structural genomics, transcriptomics, and proteomics to recently gaining multi-Omics. The complexity of living organisms is difficult to fully elucidate by a single approach. For instance, the identification of potential pathogenic/virulence factors is made possible by the merger of genomics and proteomic analysis. Both sequence-based and structure-based analyses were used in functional annotations (Amera et al. 2020). Different pathogenic/virulence factors are deployed by plant pathogenic fungi to infect their host (Gonzalez-Fernandez and Jorrin-Novo 2012). It is a natural secondary metabolite named "fungal phytotoxins" that work against the host's defenses, transporters, and components of the signal transduction system. Fungal phytotoxins protect the plant pathogenic fungi from the host's defense system, penetration effectors, and enzymes that break down the host's defenses (Vurro et al. 2018; Pandey et al. 2019; Garrido et al. 2010; Rush et al. 2005). Comparative proteomics, pathogen-induced protein expression, and immunoproteomics are three strategies that, in combination with functional genomics, microarray analysis, immunochemical models, or infection model systems, provide conclusive knowledge on protein function in fungi.

With recent advancements in mass spectrometry (MS), 2-D mass spectrometry, and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry-based techniques, fungal research on host–fungal interactions, and antifungal development has profoundly progressed over the years (Ball et al. 2019; Han et al. 2008). The gene expression patterns like post-translational modifications (PTMs), alternative splicing isoforms, protein product quantification, and protein-protein interaction networks are collectively observed in proteomics profiling (Cox and Mann 2007).

There are mainly three MS-based proteomics strategies, i.e., top-down, bottom-up, and targeted proteomics are employed for protein identification and characterization (Fig. 24.2). In top-down proteomics, intact protein/ions or large protein

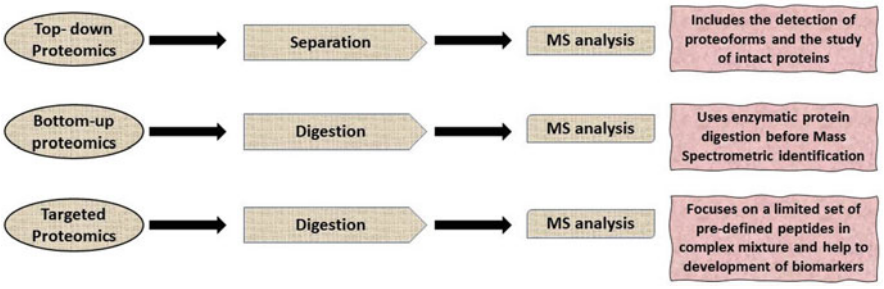

Fig. 24.2 Schematic representation of MS-based workflow of proteomics approaches

fragments were analyzed by liquid chromatography (LC)-MS/MS for unique proteoform separation based on their size and peak isolation (Ball et al. 2019; Melby et al. 2021; Gonzalez-Fernandez and Jorrin-Novo 2012). Bottom-up proteomics is the most mature approach where proteolytic digestion of intact proteins is analyzed by LC-MS/MS for unbiased identification and characterization of proteins. Due to high sensitivity, quantitative accuracy and reproducibility of targeted proteomics gain significant popularity. It can monitor hundreds of peptides in a single analysis by LC-selected reaction monitoring (SRM) for enzyme and pathway identification, characterization, and quantification of specific proteins and is mainly associated with biomarker designing (Gao et al. 2020). The MS workflow begins with sample preparation, solubilization, and protein denaturation by using chaotropic agent (e.g., guanidine hydrochloride, and urea) and surfactants (e.g., sodium dodecyl sulfate and sodium deoxycholate) (Ball et al. 2019; Han et al. 2008). An adequate amount of high-quality sample protein/peptide is required for MS-based proteomics analysis (Han et al. 2008). Further MS-based proteomics data analysis, visualization, and interpretation require sophisticated bioinformatics platforms like OpenSWATH, DIA-Umpire, and MaxQuant (Röst et al. 2014; Tsou et al. 2015; Cox and Mann 2008). All these proteomics approaches are currently being used in fungal pathogenesis and host–fungal interactions and antifungal development.

4 Benefits and Limitations of Proteomic Versus TRANSCRIPTOMIC Analysis in Antifungal Drug Discovery

Proteomic and transcriptomic analysis are two approaches used for studying the effects of antifungal drugs and discovering new ones, but each technique has its own limitations and strengths. Both approaches involve the study of proteins and genes, respectively, but there are some key differences between the two approaches that may make one more suitable than the other in certain situations. One advantage of proteomic analysis is that it can provide more direct information about the functional state of a cell or organism. This is because proteins are the primary functional molecules in cells, and changes in protein expression or activity can directly affect cellular processes (Doyle 2011). Proteomic analysis can also provide information about post-translational modifications, which are chemical changes that occur to proteins after they are translated from mRNA (Ramazi and Zahiri 2021). These modifications can play a significant role in the function and regulation of proteins, and may not be detected by transcriptomic analysis (Ramazi and Zahiri 2021).

However, proteomic analysis has several limitations in antifungal drug discovery. One limitation is that proteins are often difficult to purify and isolate, especially in large quantities. This can make it challenging to obtain sufficient amounts of protein for analysis. In addition, proteins are often modified post-translationally, which can

complicate the interpretation of proteomic data (Chandramouli and Qian 2009). Finally, the proteomic analysis may not accurately reflect the abundance of proteins, as the levels of protein synthesis and degradation can vary independently of transcriptional regulation (Ramazi and Zahiri 2021; Chandramouli and Qian 2009).

On the other hand, transcriptomic analysis has some advantages over proteomic analysis. One advantage is that mRNA is a more stable molecule than proteins, so it is easier to detect and quantify changes in gene expression over time. In addition, transcriptomic analysis can provide information about the regulation of gene expression at the transcriptional level, which can help to identify potential regulatory targets for the development of new drugs (Tseng et al. 2021; Cools and Hammond-Kosack 2013).

However, the transcriptomic analysis also has several limitations. One limitation is that RNA is often unstable, which can make it difficult to preserve the integrity of RNA samples for analysis. In addition, the transcriptomic analysis may not accurately reflect the levels of protein synthesis, as the stability and translation efficiency of mRNA can vary independently of transcriptional regulation. Finally, a transcriptomic analysis may not provide information about post-transcriptional regulation or the function of non-coding RNA.

Overall, the choice between proteomic and transcriptomic analysis will depend on the specific goals of the study and the questions being asked. Both approaches can provide valuable insights into the molecular mechanisms underlying fungal pathogenesis and the effects of antifungal drugs, and may be used in combination to provide a more comprehensive understanding of these processes.

5 Fungal Secondary Metabolites and Human Health

In the current world scenario, antibiotics are mainly resistant to known drugs, causing major hurdles for the medicinal and agriculture sectors. Most antibiotics, anticancer, and immunosuppressive drugs are largely derived from natural products (Skellam 2019). These natural products are potent bioactive in nature and often have a low molecular weight; they are also known as secondary metabolites. Fungi are a rich source of such metabolites, and various other important drugs are isolated from the fungal organisms (Bills and Gloer 2017; Newman and Cragg 2016). All approved therapeutic agents from 1981 to 2019 were derivatives of natural products or their mimics, which are now recognized as drug entities by the FDA (Newman and Cragg 2020). Fungi are abundant producers of various important drugs, like the β-lactam (a combination of Penicillins and Cephalosporins), Penicillin G and V, Pleuromutilin, Retapamulin antibiotics, anti-fungal (e.g., Echinocandin B and Griseofulvin), immunosuppressants (e.g., Mycophenolic acid and Cyclosporine A), and hypercholesterolemia treating drugs (e.g., Lovastatin and Compactin). Apart from this, fungal metabolites have other biological activities, including broad-range fungicides (e.g., strobilurins A–D), carcinogens (e.g., aflatoxin), growth hormones (e.g., gibberellic acid), and antioxidants in cosmetics (e.g., kojic acid) (Skellam

2019; Bills and Gloer 2017; Karwehl and Stadler 2017). Overall, 197 unique natural products obtained from fungi mainly belong to non-ribosomal peptides, polyketides, terpenoids, and alkaloids or their derivatives (Li et al. 2016).

Various types of mycotoxins (aflatoxin, patulin, fumonisin, ochratoxin, trichothecene, cytochalasin, deoxynivalenol, etc.) have been secreted from fungi and have a negative impact on mammalian health. Mycotoxins are not only contaminated food for humans and livestock but also potent disease-prone agents in humans (Bills and Gloer 2017). The consumption of these mycotoxin-contaminated foods causes the death of humans and animals alike. All these secondary metabolites are mainly secreted to protect the fungi from predators, and UV radiation and to compete with other microorganisms, which have a profound effect on human health (Reddy and Raghavender 2007). With emerging interest in natural products, the current pharmaceutical industry is taking as a lead structure in drug discovery. Little bio-engineering will improve these product analogs and activate the silent biosynthetic pathways. Improving the production of natural products in native/heterogenous hosts or in in-vitro conditions will start at the DNA or protein level. Advanced analytical procedures like transcriptomics and metabolomics may require further characterization (Barkal et al. 2016; Rodrigues et al. 2016).

6 Human: Antifungal Drug Targets

Antifungal drugs are medications that are used to treat fungal infections in humans. Fungal infections can affect various body parts, including the skin, nails, mouth, throat, lungs, and urinary tract, and can range from mild to severe. Depending on the type of fungal infections, they can be exogenous (e.g., dermatomycoses), endogenous (e.g., candidiasis), and exo-endogenous (e.g., cryptococcosis). Fungal infections are broadly of two types: superficial infection and invasive fungal infection (IFI) (Mota Fernandes et al. 2021). Superficial infections mainly affect the skin, mucous membrane, and keratin region whereas, invasive infections are more life-threatening and affect the bloodstream, and various organs like the lungs, liver, heart, kidneys, and brain (Mota Fernandes et al. 2021). Superficial infections although not life-threatening and have a greater incidence than IFI (Roemer and Krysan 2014; Hasim and Coleman 2019). The IFIs predominantly cause serious illnesses in immunocompetent and immunocompromised individuals having an organ transplant or chemotherapy or individuals having immunosuppressive infections like HIV/AIDS (Roemer and Krysan 2014; Hasim and Coleman 2019). The most prevalent opportunistic pathogenic fungus is the *Candida* species, *Cryptococcus neoformans* (also known as *Filobasidiella neoformans*), *Aspergillus species*, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Fusarium species* (Sandhu et al. 1995; Taylor et al. 1999; Rinaldi 1989). Antifungal drugs are used to kill or inhibit fungal growth and can be administered orally, intravenously, or topically depending on the location and severity of the infection (https://www.cdc.gov/fungal/diseases/index.html).

Table 24.1 Different classes of antifungal drugs and their modes of action

Drug class	Example drugs	Target	Mode of action
Azoles	Fluconazole, itraconazole	Ergosterol synthesis	Inhibit the synthesis of ergosterol, weaken fungal cell membrane
Polyenes	Amphotericin B	Cell wall synthesis	Bind to ergosterol, disrupt cell wall structure
Allylamines	Terbinafine	N-linked glycans synthesis	Inhibit the synthesis of N-linked glycans, inhibit fungal growth
Echinocandins	Caspofungin	1,3-beta-D-glucan synthesis	Inhibit the synthesis of 1,3-beta-D-glucan, inhibit fungal growth

Approximately, 1.7 million individuals annually died due to severe fungal infection, all these fungal infections are mainly associated with the species that belong to one of the four genera: *Candida, Cryptococcus, Aspergillus*, *Histoplasma*, and *Pneumocystis* (Kainz et al. 2020; Brown et al. 2012; Richardson and Lass-Flörl 2008; Victoria Castelli et al. 2017). Other emerging fungal pathogens include molds such as *Fusarium*, *Scedosporium*, *Penicillium* species, and *Mucorales* that are significantly more prevalent due to the excessive use of antifungals in agriculture (Seyedmousavi et al. 2017). There are several types of antifungal drugs that are used to treat human fungal infections, including azoles, polyenes, allylamines, and echinocandins. Each of these drug classes targets specific enzymes or pathways in fungi and has different modes of action and spectra of activity.

The following are the examples of antifungal drug targets:

(a) Ergosterol synthesis: Azoles (such as fluconazole and itraconazole) inhibits the synthesis of ergosterol, sterol is an essential component of the fungal cell membrane. By disrupting ergosterol synthesis, azoles weaken the fungal cell membrane and inhibit the growth of fungi (Mota Fernandes et al. 2021; Rauseo et al. 2020).
(b) Cell wall synthesis: Polyenes (such as amphotericin B) bind to ergosterol in the fungal cell membrane and disrupt the structure of the cell wall, leading to leakage and fungal death (Victoria Castelli et al. 2017).
(c) N-linked glycans: Allylamines (such as terbinafine) inhibit the synthesis of N-linked glycans, which are sugars that are required for the formation of the fungal cell wall. By disrupting N-linked glycans synthesis, allylamines inhibit the growth of fungi (Mota Fernandes et al. 2021; Rauseo et al. 2020; Smith et al. 2015).
(d) 1,3-beta-D-glucan synthesis: Echinocandins (such as caspofungin) inhibit the synthesis of 1,3-beta-D-glucan, a polysaccharide (Mota Fernandes et al. 2021; Chamilos et al. 2016) (Table 24.1).

All these drugs, azoles, polyenes, and echinocandins are used to treat *Candida albicans* infection; however, resistance has been developed against all the three (Wijnants et al. 2021). One more specific feature of *C. albicans* pathogenicity is their biofilm formation ability, biofilm is a highly heterogeneous architecture made of cellular and non-cellular elements. The biofilm colonized coating in oral and vaginal

epithelia, and implanted medical devices such as heart valves, venous, and urinary catheter (Pappas et al. 2016; Jiménez-Expósito et al. 2004; Uppuluri et al. 2011). Only two drug classes, amphotericin B and echinocandins as a group, appear to have in vitro efficacy and a limited antifungal spectrum against this biofilm (Uppuluri et al. 2011; Mazu et al. 2016). As a result, developing novel anti-fungal therapies and regulation is the current need to save human life.

7 Structural Biology and Interactome-Based Approaches in Antifungal Drug Discovery

Structural biology and interactome-based approaches are two methods that can be used in the discovery and development of new antifungal drugs (Segura-Cabrera et al. 2013). Structural biology involves studying the three-dimensional structures of biological molecules, such as proteins and enzymes, and determining how these structures relate to their functions (Rotonda et al. 1996). This information can be used to design and develop drugs that specifically target and inhibit the activity of proteins or enzymes involved in fungal pathogenicity (Jadhav et al. 2017).

Interactome-based approaches, on the other hand, involve identifying and characterizing the protein-protein interactions (PPIs) within a pathogen, and using this information to identify potential targets for drug development (Rotonda et al. 1996). These approaches can be used to identify proteins involved in key pathways such as ergosterol synthesis, cell wall synthesis, or N-linked glycans synthesis, and to design drugs that specifically target these pathways. Both structural biology and interactome-based approaches can be combined with other methods, such as bioinformatics analysis or chemical synthesis, to provide a more comprehensive understanding of the mechanisms of action of antifungal drugs and to optimize their effectiveness.

8 Bioinformatics Software and Databases for Antifungal Drug Discovery

Bioinformatics analytics has potentially increased drug target identification, drug candidate screening, and refinement, along with characterizing the drug side effects and predicting drug resistance. Bioinformatics software and databases are tools that can be used to analyze, visualize, and interpret biological data, and can facilitate the discovery and development of novel antifungal drugs.

Bioinformatics software and databases that are commonly used in antifungal drug discovery includes the following:

1. Sequence analysis software: Tools such as **BLAST** (Basic Local Alignment Search Tool) and **ClustalW** can be used to compare and align genomic

sequences, and to identify conserved regions or motifs that may be important for protein function (Camacho et al. 2009; Ye et al. 2006; Thompson et al. 1994). The most commonly used program to compare different genome is blastp (Camacho et al. 2009).

2. Molecular visualizing software: Programs such as **PyMOL** (Yuan et al. 2017; Rosignoli and Paiardini 2022) and **Swiss-PdbViewer** (Kanehisa and Goto 2000) can be used to visualize and analyze the three-dimensional structures of proteins and other biomolecules, and to design and optimize antifungal drug as well.
3. Pathway databases: Resources such as **KEGG** (Kyoto Encyclopedia of Genes and Genomes) and **BioCyc** can be used to map and analyze metabolic pathways and enzymes that are involved in fungal metabolism, and to identify potential targets for antifungal drugs (Kanehisa and Goto 2000; Karp et al. 2019).
4. Gene expression databases: Repositories such as Gene Expression Omnibus (**GEO**) and ArrayExpress can be used to analyze and compare gene expression patterns in different fungal species under different conditions, and to identify potential targets for antifungal drugs (Barrett et al. 2012).
5. Drug discovery databases: Resources such as **DrugBank** and **PubChem** are most commonly used databases to search and evaluate existing antifungal drugs, and also identify potential leads for new antifungal drug development (Wishart et al. 2006; van Dijk and Bonvin 2009).

The genome databases such as Ensembl Fungi (https://fungi.ensembl.org/index.html), FungiDB (http://FungiDB.org), *Saccharomyces* Genome Database (SGD, http://www.yeastgenome.org), and *Candida* Genome Database (CGD, http://www.candidagenome.org/) etc. have been developed to provide comprehensive integrated biological information about various fungal genome (Binkley et al. 2014; Stajich et al. 2012; Wong et al. 2013). The collaboration of the Joint Genome Institute of the US Department of Energy has developed an interactive web-based tools for fungal genome (https://jgi.doe.gov/our-science/science-programs/fungal-genomics/1000-fungal-genomes/) to support the integration, for comparative genomics of fungi and other 'omics' data information. The 1000 fungal genomes from across the fungal tree of life are mainly focusing on phylogenetic diversity, genome-centric analyses, and community annotation of kingdom fungi (Grigoriev et al. 2014).

Various other in silico approaches were employed for the candidate identification of virulence pathogenicity of fungi/plants (da Silva et al. 2014). However, Flex Analysis Software (Pernstål et al. 2019) was used to create peak list files and spectral analysis files. Similar searches were conducted using MASCOT software (http://www.matrixscience.com) (Brosch et al. 2009) using Tandem mass spectra against the NCBIprot database. Additionally, the discovered proteins were functionally annotated using the Uniprot database (Uniprot.org) and classified into various groups using the Clusters of Orthologous Genes (COG) database for microbial genome annotation and comparative genomics (Galperin et al. 2019). TargetP was additionally employed to forecast the localization of the discovered proteins within the cell (Emanuelsson et al. 2000). By using blastp, homologs of potential pathogenic or virulence factors were found in the genomic sequences (Gish and States

1993). Three-dimensional protein models of identified pathogenicity or virulence factors were predicted by RaptorX software (Källberg et al. 2014) (http://raptorx.uchicago.edu), and validated using bioinformatics tools like RAMPAGE (Lovell et al. 2003), ProFunc (Laskowski et al. 2005), DALI server (Holm and Rosenström 2010), and ProQ server (Wallner and Elofsson 2003). Identified pathogenicity and virulence factors were annotated using the NCBI conserved domain database (CDD) (Marchler-Bauer et al. 2010), InterProScan (Quevillon et al. 2005), SMART (Letunic et al. 2012), and ScanProsite (De Castro et al. 2006).

9 Conclusion

In conclusion, bioinformatics plays a crucial role in fungal drug discovery by providing tools and resources for the analysis, visualization, and interpretation of biological data, and by enabling the identification and characterization of potential targets for the development of new drugs. Bioinformatics approaches can be used to analyze genomic sequences, protein structures and functions, metabolic pathways, and gene expression patterns, and to identify conserved regions, motifs, or pathways that are essential for the growth and survival of fungal pathogens. Various tools and databases, such as sequence analysis software, molecular modeling software, pathway databases, gene expression databases, and drug discovery databases, can facilitate the identification and evaluation of potential targets for antifungal drugs, and can help to optimize and refine the design and development of new drugs.

By integrating bioinformatics with other methods, such as structural biology, interactome-based approaches, or high-throughput screening, researchers can gain a more comprehensive and integrated understanding of the biology and pathogenicity of fungal pathogens, and can accelerate the discovery and development of new and more effective antifungal therapies.

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Chapter 25
Biotransformation of Steroids: History, Current Status, and Future Prospects

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Abstract Spreading of new diseases has elevated the need to synthesis novel derivatives from currently used drugs. Corticosteroidal drugs are potent drugs that are involved in treating different diseases, and their market is expected to witness continuous growth due to the emergence of new diseases as COVID-19 and spread of chronic diseases. Therefore, continuous efforts are made to synthesis and construct new steroidal analogues showing potency and reduced side effects. Biotransformation can achieve this in an ecofriendly and efficient way. Different microorganisms can be used in a biotransformation process depending on their produced enzymes, which attack the structure of the chemical substrate in a highly specific way resulting in the production of new analogues. Out of different microorganisms used for this purpose, fungi are attracting extra attention. Hence, in this review, we described chemical structure of steroids to show how biotransformation can affect their structure. Moreover, we highlighted history of microbial transformation of steroids, types of biotransformations, and different techniques used using whole viable cells, spores, immobilized cells, or enzymes focusing on fungal enzymes involved in steroids biotransformation. We also highlighted the role of the genus *Cunninghamella* as a biotechnological tool in steroid biotransformation. Finally, future prospects concerning steroid fungal biotransformation were discussed.

Keywords Biotransformation · Bioconversion · Steroids · Analogues · Fungi · *Cunninghamella*

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1 Introduction

There is a raising need to find new bioactive compounds capable of showing activity against recently emerging and already existing diseases. One of the ways to find such compounds is to synthesis new derivatives or in other words, use already known compounds as a base to produce new ones. To achieve this, biotransformation represents the perfect process in an economic and efficient way. Biotransformation is microbial transformation of compounds using endogenous and exogenous enzymes to yield new compounds or derivatives of the parent compound. Employing microorganisms to get desired compounds have many advantages over using chemical techniques as shown in Table 25.1.

Microbial transformation of steroids is one of the most successful examples of employing biotransformation for the synthesis of different bioactive compounds. Steroids are compounds that are naturally made by the body, or can be obtained through chemical or microbial synthesis for medical uses. Steroids are potent, and known for having long half-lives in the blood stream (Larsson et al. 1976). A breakthrough was made in the field of steroids when the anti-inflammatory property of cortisone and its therapeutic action in treatment of rheumatoid arthritis was discovered (Hench et al. 1949). It was then noticed that many steroids besides being anti-inflammatory also showed other physiological properties. Synthesized steroids have therapeutic uses especially as anti-inflammatory, diuretic, immunosuppressive, progestational, anabolic, and contraceptive agents (Barnes et al. 1993; Patel and Savjani 2015). Steroids have also been previously used to treat osteoporosis, and some types of breast and prostate cancer. Moreover, as replacement agents in the treatment of adrenal insufficiencies (Walsh et al. 1996; Johnston 1997; Johnston and Dowsett 2003), in coronary heart disease prevention (Frye and Leonard 1999), as anti-fungal agents (Chen et al. 1998; Kumar et al. 2010), as anti-obesity agents (Suzuki 1993; Suzuki et al. 1998). On the other hand, due to the importance of

Table 25.1 Comparison between chemical synthesis and biotransformation

	Chemical synthesis	Biotransformation
Specificity	Chemicals are usually not that specific	Substrate and regiospecific site specificity, stereoselectivity
Effect on steroid structural integrity	May compromise the steroid structural integrity	Will not affect steroid structural integrity
	Many reagents may degrade one or more of the rings	No need to use reagents
Need for protection and deprotection	Protection and deprotection are often needed to achieve the required regiochemical and stereochemical outcomes	No need for protection or deprotection
Impact on environment	May include using environmental hazardous chemicals	Safer on environment
Reaction conditions	Require special reaction conditions	Require mild reaction conditions

progesterone in repairing of myelin sheath of damaged nerves, some steroids are capable of limiting the severity of spinal cord damages (Short et al. 2000; Bydon et al. 2014). The contributions of steroidal drugs extend to the antiviral field, mainly for inhibiting human immunodeficiency virus (HIV) integrase (Dombrowski et al. 2000; Arthan et al. 2002). Recently, some studies have described the role of steroids in reducing, reversing the severity of the COVID-19 induced cytokine storm (Kolilekas et al. 2020; Chatterjee et al. 2020; Shanshal 2020; Sarkar et al. 2021). The therapeutic potential of some steroidal hormones depends on their reaction with specific receptors that act as transcription factors in the gene expression regulation process (Rupprecht and Holsboer 1999). Some steroids, such as dehydroepiandrosterone (DHEA), progesterone, 17β-estradiol, allopregnanolone, afoxolaner, ganaxolone/pregnenolone, and its sulfated derivatives are considered neurosteroids, due to their action at the level of the central nervous system (Baulieu and Schumacher 2000; Mellon and Griffin 2002; Cano-Flores et al. 2019). On the other hand, some steroids are used in veterinary medicine (Bruner 2006; Dembek et al. 2017). In spite of the previously mentioned uses of steroids, their associated side effects are relatively limiting their use. Hypercortisolism may cause cutaneous impacts that includes hyperpigmentation, truncal obesity, acanthosis nigricans, acne, ecchymoses after minor trauma, hirsutism, petechia, and striae (Buchman 2001; Meena et al. 2017), nevertheless majority of symptoms are completely reversible after stopping medication. Hypokalemia (low level of potassium in the blood serum) is sometimes associated with corticosteroid therapy (Powell 1969). Similarly, myopathy was also reported to be associated with hypercortisolism (Cushing 1964). Moreover, hyperglycemia, mood alterations, hyperactivity, hypertension, insomnia, among other side effects have been reported in some patients during treatment with corticosteroids (Buchman 2001). Regardless of the reported side effects of some steroids, more than 300 steroidal drugs are currently available in markets, and steroids production is ranked second after antibiotics in the pharmaceutical market (Cano-Flores et al. 2019). In the last few years, steroids world markets reached 10 billion US$ and their production is exceeding 1,000,000 tons per year (Sultana 2018; Cano-Flores et al. 2019). Moreover, corticosteroids market is expected to witness an annual growth of 4.3%, during the forecast period (2020–2025), which is related to the spread of many chronic diseases, and the increasing number of geriatric populations. Hence, continuous efforts are made to discover and identify novel steroids and/or construction of new analogues that show potency and reduced side effects. In this review, the chemical structure of steroids was described to contribute in understanding their origin and how biotransformation can affect their structure. Moreover, we highlighted the history of microbial transformation of steroids, and the use of different microorganisms for this purpose. Additionally, types of biotransformations and some of the known and recent steroid biotransformation techniques using whole viable cells, spores, immobilized cells, or enzymes were presented focusing on different fungal enzymes involved in steroids biotransformation which are causing their high specificity. We also highlighted the role of the genus *Cunninghamella* as a biotechnological tool in steroid biotransformation. Finally, future prospects concerning steroid fungal biotransformation were discussed.

2 Chemical Structure of Steroids

Steroids are natural compounds that are widely distributed in bile salts, adrenocortical and sex-hormones, alkaloids, insect molting hormones, sapogenins, and some antibiotics (Sultan and Raza 2015). Generally, all steroids originate from one basic structure, a cyclopentanoperhydrophenanthrene nucleus (sterane) which consists of four fused rings (Fig. 25.1) (Fernandes et al. 2003). Generally, the activity of steroids depends on their type, structure, spatial orientation, and reactivity of the different functional groups that exist in the tetracyclic core as well as the oxidation state of the rings. For example, the presence of an oxygenated function in C-11β is critical for the anti-inflammatory activity (Cano-Flores et al. 2019); the hydroxyl function in C-17β determines androgenic characteristics; the aromatization of ring A confers estrogenic activity; and corticosteroids have the 3-keto-4-ene group and the pregnane side chain at C-17 (Flickinger 2010; Donova and Egorova 2012). Cortisone is very potent anti-inflammatory agent against rheumatoid arthritis and skin diseases. By changing its structure through making a 1,2 double bond in ring A of the cortisone molecule, prednisone which have more potent anti-inflammatory agent is produced. Molecules of steroids have several asymmetric centers which makes their total synthesis very difficult. Hence, the need for microbial transformation of steroids has been risen as easier, potent, and reliable alternative to chemical synthesis. Microbial transformations are superior over chemical transformations due to their reaction substrate specificity, regiospecificity, stereospecificity, relatively low cost, and mild reaction conditions (Borges et al. 2009). Briefly, microbial transformations can be considered as a good biotechnological tool to conduct reaction steps which can hardly be accomplished by chemical reactions.

3 Microbial Transformation of Steroids

Although processes involving oxidation of the Δ^5–3β-hydroxyl structure to Δ^4–3-oxo (Koester 1937) and reduction of 17-keto group (Schoeller 1937) were patented in 1937, the importance of biotransformation was highlighted in 1952 after Murray and Peterson (1952) have patented the process of 11α-hydroxylation of progesterone using *Rhizopus arrhizus*. Microbial conversion of progesterone to corticosteroids is employed in pharmaceutical industry in a single step through introducing an oxygen function at C-11 position for the preparation of 11β-hydroxy progesterone. Microbial hydroxylations and dehydrogenations have become industrially important for the production of steroid hormones and their analogues. Other examples of industrially important biotransformations are the microbial production of androstanes by degradation of side chains of some sterols such as cholesterol or sitosterol (Capek et al. 1966; Sih and Rosassa 1976; Donova et al. 2005; Panek et al. 2020; Rohman and Dijkstra 2021).

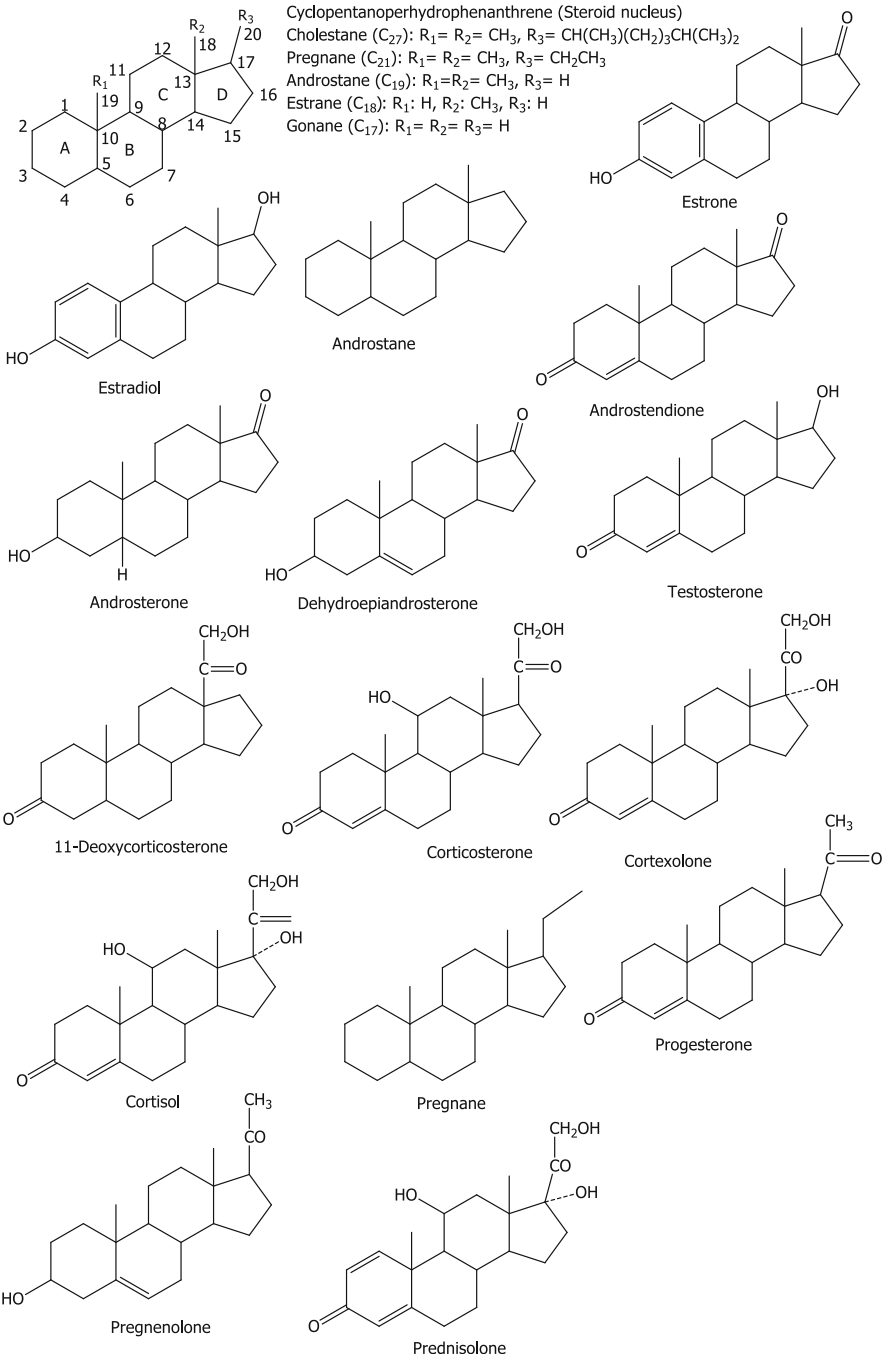

Fig. 25.1 Basic steroid structures. Steroid nucleus Cyclopentanoperhydrophenanthrene, some esterane, androstane

3.1 Microorganisms Employed in Biotransformation

Microorganisms are currently employed as potent biotechnological tools for the manufacture of various products in different pharmaceutical and industrial fields. Concerning steroids biotransformation, the ability to attack the steroid skeleton is not limited to a definite group of microorganisms, it has been previously reported in bacteria, yeasts, actinomycetes, algae, and even protozoa (Akhrem and Titov 1970; Manosroi et al. 1999; Hakki et al. 2008; Xu and Li 2020). As mentioned previously, the biotransformation of first steroids was performed to produce testosterone from dehydroepiandrosterone using *Corynebacterium* sp. Later cholesterol was produced from 4-dehydroeticholanic and 7-hydroxycholestrol using *Nocardia* spp. Currently, studies and efforts are made in order to screen for and isolate novel promising microbes capable of biotransforming different compounds to be used in the field of pharmaceutical industry. However, filamentous fungi are considered as the most potent and versatile producers of many biotransforming enzymes (Ohlson et al. 1980; Sonomoto et al. 1983; Schlosser et al. 1993; Manosroi et al. 1999; Asha and Vidyavathi 2009). Progesterone biotransformation was performed using the whole cells of *Circinella muscae* to yield 9α-hydroxyprogesterone, 14-α-hydroxyprogesterone, and 6β, 14α-dihydroxyprogesterone after 6 days of incubation at 26 °C. Similarly, the same strain was used to conduct biotransformation of testosterone enanthate yielding 8β,14α-dihydroxytestosterone, 6-β-hydroxytestosterone, and 9α-hydroxytestosterone (Zoghi et al. 2019). Structural conversion of androsta-1,4-diene-3,17-dione (ADD) and 3-oxo-androstane steroids,—androst-4-ene-3,17-dione (AD) were achieved with a remarkable yield of 7α-, 7β-, 11α-, and 14α-hydroxylated derivatives, as well as 17β-reduced and 1 (2)-using strains belonging to the fungal genera *Absidia*, *Acremonium*, *Beauveria*, *Cunninghamella*, *Doratomyces*, *Drechslera*, *Fusarium,* and *Gibberella* (Kollerov et al. 2020).

3.2 Types of Biotransformation Reactions

Microbial transformation of steroids takes place by the enzymes formed by the active microorganisms. These enzymes cause many changes in steroid nucleus. Types of biotransformation reactions include oxidations, reductions, hydrolysis, condensation, isomerization, and formation of C-C bonds or hetero-atom bonds. Oxidation reactions include hydroxylation, epoxidation, dehydrogenation of C-C bonds, oxidation of alcohols and aldehydes, oxidative degradation of alkyl, carboxyalkyl or ketoalkyl chains, oxidative removal of substituents, oxidative deamination, oxidation of heterofunctions, and oxidative ring fission. On the other hand, reduction reactons include reduction of organic acids, aldehydes, ketones and hydrogenation of C-C bonds, reduction of heterofunctions, dehydroxylation, and reductive elimination of substituents. Hydrolysis reactions include hydrolysis of esters, amines,

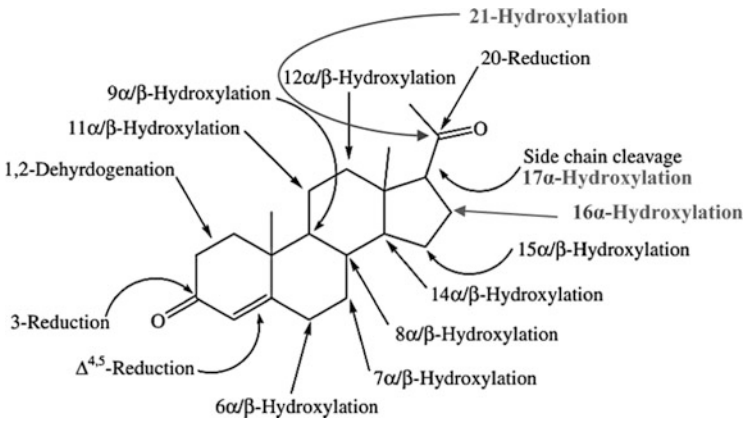

Fig. 25.2 Schematic presentation showing major sites of biotransformation reactions

amides, lactones, ethers, and lactams. Finally, condensation reactions include dehydration, O- and N-acylation, glycosidation, esterification, lactonization, and amination, while isomerization reactions are as in the case of migration of double bonds or oxygen functions racemization, and rearrangements. Microbial modifications in the steroid skeleton have been previously reported in numerous literatures (Mamoli and Vercelone 1937; Nobile et al. 1955; Constantinides 1980; Fraga et al. 2005; Nassiri-Koopaei and Faramarzi 2015; Yildirim and Kuru 2017; Yildirim et al. 2020). Major sites of biotransformations reaction are illustrated in Fig. 25.2.

4 Steroid Biotransformation Techniques

4.1 Whole Viable Cells

Dispensability, powder aggregation, and low solubility in water are considered as the main problems reported during fungal steroid biotransformation (Goetschel and Bar 1992; Nassiri-Koopaei and Faramarzi 2015). Hence, overcoming these limitations is critically important to enhance biotransformation process. Improving the permeability of viable cells has been considered as a solution to some of these problems through employing fed-batch systems, substrate micronization, cell wall permeabilization using surfactants (as Tweens, Triton X-110, and X-114), and antibiotics (as polymyxin B, vancomycin, bacitracin, and ethambutol) (Ni and Chen 2004; Malaviya and Gomes 2008; Vijayan and Raghu 2020). Other compounds including organic solvents, or cyclodextrins, glycine, lecithin, protamine, nonapeptide, and polyethyleneimine dimethyl formamide were also reported for being capable of transferring steroids across microbial cell wall (Wang et al. 2004a, b, 2005; Nassiri-Koopaei and Faramarzi 2015).

4.2 Using Whole-Cell/Spores Immobilized Biocatalysts

Whole-cell immobilization has been widely applied in fungal biotransformation of steroids in order to maintain longer half-life, and minimize chances of losing enzyme activity (Carballeira et al. 2009; Nassiri-Koopaei and Faramarzi 2015). It was previously reported that viable immobilized cells offer advantages over conventional fermentation methods, including reusability of immobilized biocatalysts, high volumetric reaction rates, convenient downstream processing, high biomass retention in the reactor in continuous processes, improved operational and storage stabilities, and increase in product yield (Junter and Jouenne 2004; Nassiri-Koopaei and Faramarzi 2015). Various immobilization methods have been used for whole-cell steroid microbial biotransformation including entrapment, adhesion to solid carrier surfaces, microencapsulation (Giorgi et al. 2019; dos Santos et al. 2020). Synthetic polymers including silicon-based polymers, photo-crosslinked resins, polyurethane foams, and calcium alginate beads coated with a polyurea layer have been also investigated (Nassiri-Koopaei and Faramarzi 2015). Many literatures have described the use of immobilized spores for steroid biotransformations. Schlosser et al. (1993) investigated 15 α-hydroxylation of 13-ethyl-gon-4-en-3,17-dione using free cells, calcium alginate-immobilized, and β-cyclodextrin-immobilized *Penicillium raistrickii i* 477 cells. For biotransformation of progesterone to 11α-hydroxyprogesterone by *Aspergillus ochraceus*, a novel cell immobilization technique was developed for increasing substrate partition to the gel matrix, through coating a thin layer of polyurea on the surface of calcium alginate beads (Houng et al. 1994; Chen et al. 1994). On the other hand, for 11α-hydroxylation of progesterone, Kulkarni et al. (1998) have immobilized *Aspergillus niger* NCIM 589 spores on high-density polyethylene (HDPE), using polyethyleneimine. Hydrocortisone was produced from cortexolone-21-acetate using Absidia orchidis entrapped in calcium alginate gel in a cosolvent-containing media (Wang et al. 1998). The enhanced production of 17α-hydroxyprogesterone from progesterone through biotransformation using *Curvularia lunata* ATCC 12017 was conducted employing a hydroxypropyl-β-cyclodextrin complexation technique (Manosroi et al. 2008). However, 15α-hydroxylation of D-ethylgonendione was performed using *Penicillium raistrickii* in deep eutectic solvents containing system (Mao et al. 2020).

4.3 Enzymes in Steroids Biotransformation

Generally, biotransformation enzymes can be considered as suitable alternatives for active pharmaceutical components production, as they are highly selective, have a lower cost, more environmentally friendly, and are less time consuming (Manosroi et al. 2007). However, modifying the fungal steroid-transforming enzymes specificity is currently conducted by site-directed mutagenesis. Some of the main enzymatic classes underlying fungal biotransformation reactions are illustrated in Fig. 25.3.

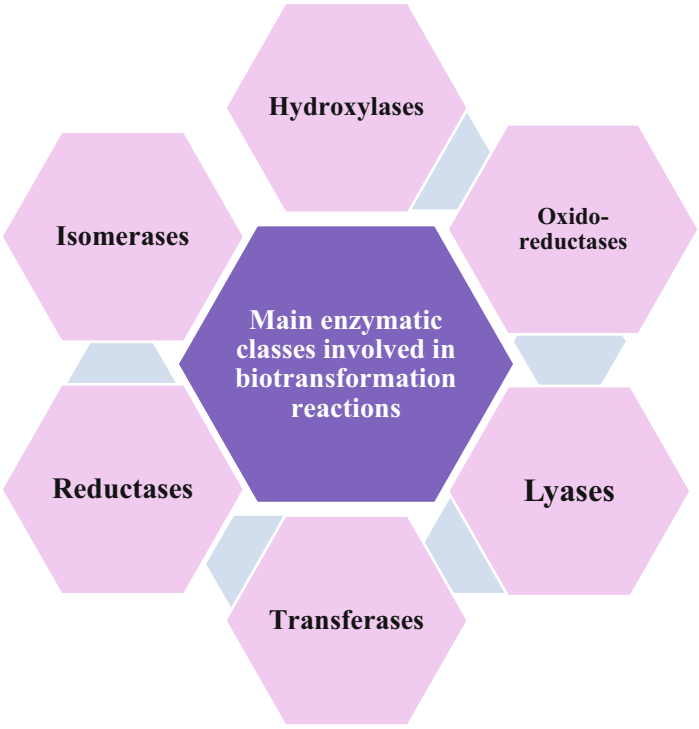

Fig. 25.3 Some of the main fungal enzymatic classes involved in biotransformation reactions

4.3.1 Fungal Enzymes Involved in Steroids Biotransformation

Many fungal enzymes in general, and hydroxylases and oxidoreductases in particular have been used in pharmaceuticals production, mainly for the production of anti-inflammatories. For example, 7α-hydroxylase is used in bile acids production; 5α-reductase is used in neurosteroids production; 14α-hydroxylase contributes in cardio-active steroids production; 3β-HSD, 17β-HSD, 5α-reductase, and Δ1-dehydrogenase are used in androgens production; and Δ1-dehydrogenase, 11-α-hydroxylase, and 11β-hydroxylase are used for progestins production (Yazdi and Hosseini 2002; Fernandes et al. 2003; Faramarzi et al. 2009; Nassiri-Koopaei and Faramarzi 2015). Major fungal enzymes involved in biotransformation process are hydroxylases, oxidoreductases, dehydrogenases, and lyases.

Hydroxylases

These enzymes insert a hydroxyl group in a highly specific, cost effective way in carbon atom of a steroid molecule. They represent the predominant fungal enzymes

in steroid biotransformation reactions (Bhosale et al. 2006; Borges et al. 2009; dos Santos et al. 2020). Many fungi are able to hydroxylate nearly every carbon atom in the steroidal structure in a unique way. *Fusarium culmorum* is used to obtain 15α-Hydroxy-17α-ethyl-19-nortestosterone from 17α-Ethyl-19-nortestosterone. *Rhizopus stolonifers* is used for the biotransformation of oxandrolone to 9-α-hydroxyoxandrolone. *Acremonium strictum* can convert 17α-methyltestosterone to 6β, 17β-dihydroxy-17β-methylandrosta-1,4-dien-3-one, and 3,17β-dihydroxy-17-β-methylestra-1,3,5(10)-triene. Hydroxylases include 1β, 2β, 4 (on aromatic ring), 5α, 6β, 7α, 7β, 9α, 10β, 11α, 11β, 12α, 12β, 14, 15α, 15β, 16α, 17α, 19, 20, 21, 22, and 26. It should be noted that 11α, 11β, and 16α-hydroxylases are regarded as the most commercially important hydroxylases (Fernandes et al. 2003; El-Kadi and Mostafa 2004; Faramarzi et al. 2007; Yang et al. 2007; Peart et al. 2012; Nassiri-Koopaei and Faramarzi 2015).

5α-Reductases

It is also known as 3-oxo-5α steroid 4-dehydrogenases, and its function is to reduce the double bond in steroids such as androgens, estrogens, and bile acids. Some of the reported producers of this enzymes include *Penicillium decumbens*, *Penicillium chrysogenum*, and *Penicillium crustosum* (Cabeza et al. 1999; Murray 2000).

Oxidoreductases

One of the most famous oxidoreductases is Laccase which is an oxidase that catalyzes the reduction of ketones to alcohols. Laccase has been employed to remove estrogenic compounds from environmental samples (Tamagawa et al. 2006; Lloret et al. 2010; Ueda et al. 2012). Other examples on oxidation reactions include conversion of testosterone to 7β-hydroxytestosterone by *Chaetomium* sp.; the action of *Penicillium lilacinum* on pregnenolone to produce progesterone; the biotransformation of 19-nortotestosterone to 19-norandrostenedione by *Absidia glauca*. On the other hand, examples on reduction reactions are adrenosterone conversion to 11-ketotestosterone by *Cunninghamella elegans*; production of 3β-hydroxy-17α-oxa-D-homo-5α-androstan-17-one from dehydroepiandrosterone using *Penicillium glabrum*; and biotransformation of androst-4-en-3,17-dione to 17-β-hydroxyandrost-1,4-dien-3-one by *Acremonium strictum* (Nassiri-Koopaei and Faramarzi 2015).

β-Hydroxysteroid Dehydrogenase/Δ5-Δ4-Isomerases

These enzymes are responsible for the oxidation and isomerization of Δ5–3 β-hydroxysteroid precursors into Δ4-ketosteroids, catalyzing an essential step in

the formation of all classes of active steroid hormones (Simard et al. 2005; Hunter et al. 2009).

17 β-Hydroxysteroid Dehydrogenases

These enzymes are capable of catalyzing reduction or oxidation at C_{17}; hence, they are essential in controlling the biological potency of steroid hormones, also these enzymes can metabolize other substrates like alcohols, fatty acids, bile acids, and retinols (Adamski and Jakob 2001). Producers of these includes *Pleurotus ostreatus, Cochliobolus lunatus, Cylindrocarpon radicicola,* and *Trimatostroma salinum* (Itagaki and Iwaya 1988; Rižner et al. 1996; Mindnich et al. 2004; Donova et al. 2005).

Steroid C-1/C-2 Dehydrogenases

These enzymes are not common in fungi except for *Fusarium solani* (Ahmed et al. 1996).

C-17–C-20 Lyase

This is one of the main enzymes responsible for androgens biosynthesis in *Nectria haematococca* (*Fusarium solani*) (Ahmed et al. 1996).

5 Recent Techniques for Selective Transformations

One of the most efficient techniques used for steroid transformation is conducted using fungal spores, which survive harsh surrounding conditions and tolerate toxic components (Wolken et al. 2003). Many examples were successfully employed for fungal spores used in biotransformation of steroids such as 11α-hydroxylation performed using *Aspergillus ochraceus*, and 11β-hydroxylation conducted by the help of *Stachylidium theobromae* (Wolken et al. 2003).

On the other hand, performing biotransformation in two-phase systems has been used to improve product yield. For example, aqueous organic two-phase systems are frequently performed to enhance fermentations yield where lipophilic substrates and products are existing (Leon et al. 1998; Cruz et al. 2002; Arabi et al. 2009). Applying this technique keep concentration of substrate constant in the aqueous phase, and facilitate extracting of products. It should be noted that used organic solvents to the fungus provides adequate partition and mass transfer characteristics (Leon et al. 1998; Wu et al. 2011). However, due to environmental impacts of using solvents along with their risks on human health, supercritical fluids were introduced

biotransformations as safer alternatives (Carvalho et al. 2009; Brandenbusch and Sadowski 2010).

Another strategy used to improve biotransformation productivity is using two-stage substrate addition strategy (Lu et al. 2006). Also, biotransformation is performed in a cloud point system that is prepared by the help of using nonionic surfactants which offer a micro-aqueous environment to make sure that cells are viable and their enzymes are active. Biotransformation is conducted using water vesicles containing the biocatalyst that is dispersed in the surfactant-rich continuous phase in a homogenous way. Hence, the product is protected from degradation as it is extracted back into the continuous phase. Also, many factors such as substrate inhibition, organic phase, and toxicity are considerably reduced (Wang et al. 2005, 2008). An example for using the cloud point system was transformation of cholesterol to androst4-ene-3,17-dione and androst-1,4-diene-3,17-dione using *Mycobacterium* spp. NRRL B 3683 (Wang et al. 2004b).

Using organic media in biotransformations can negatively affect this process due to low interfacial mass transfer area and cell viability loss. Therefore, microemulsions and liposomes are used as alternative biotransformation systems. In comparison with biphasic systems, microemulsions can successfully increase the interfacial area mass transfer. Nevertheless, lower cells bioactivity and overall productivity are noticed as a result of the long exposure to the organic solvent (Nassiri-Koopaei and Faramarzi 2015). This problem can be solved by using liposomes (Stefan et al. 2002).

6 *Cunninghamella* as Potent Biotechnological Tool in Steroid Biotransformation

The enzymatic machinery owned by microorganisms in general and fungi in particular represents the core for all their contributions in biotransformation processes and preparative reactions. Diverse fungal genera have been reported for their ability to metabolize a wide variety of compounds. However, *Cunninghamella* is considered as one of the most extensively studied fungi. *Cunninghamella* is capable of metabolizing various compounds in regio- and stereo-selective ways via phase I (oxidative) and phase II (conjugative) biotransformation mechanisms (Zhang et al. 1996), which made it one of the promising and strong biotechnological tools in the field of biotransformation (Fig. 25.4). Till now, only 14 species were identified under the genus *Cunninghamella* (Nguyen et al. 2017). Potency of the cytochrome P450 enzyme systems of one of the *Cunninghamella* species (*C. elegans*) has been used in neutralizing different polycyclic aromatic hydrocarbon, pollutants, and dyes (Lisowska and Dlugonski 2003; Nguyen et al. 2017). Moreover, the cytochrome P450 of *C. elegans* is responsible for the hydroxylation, N-oxidation, and N-demethylation of different tricyclic antidepressants (Zhang et al. 1996; Nguyen et al. 2017). *Cunninghamella* species are employed to synthesis biologically active

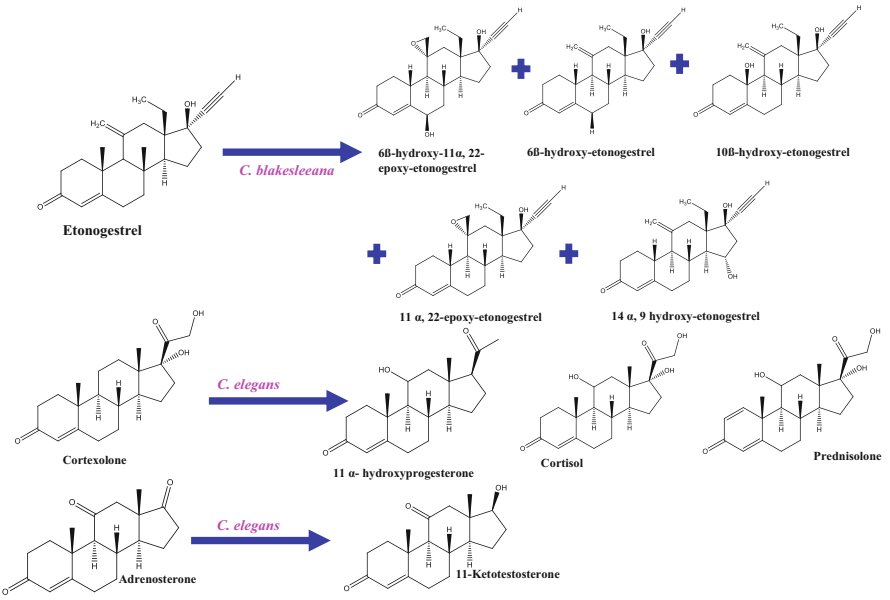

Fig. 25.4 Examples of some steroids biotransformation reactions conducted using *Cunninghamella* species

derivatives from known steroidal drugs. For example, cortexolone is fermented by *C. blakesleeana* or *C. elegans* yielding cortisol; 17α-hydroxycorticosterone; Prednisolone; 20β-hydroxy cortisol; and cortisone) (O'Connell et al. 1955; Allam et al. 2011). Adrenosterone which is used as muscle gaining and fat loss supplement is transformed by *C. elegans* giving 9α-hydroxyadrenosterone; 11-ketotestosterone; 6β-hydroxyadrenosterone; 9α-hydroxy-11-ketotestosterone; and 6β-hydroxy-11-ketotestosterone (Choudhary et al. 2007).

Biotransformation of the steroid mesterolone which is used for treatment of low testosterone levels was conducted using *C. blakesleeana* resulted in seven compounds which are 1α-methyl-1β,11β,17β-trihydroxy-5α-androstan-3-one; 1α-methyl-1β,6α,17β-trihydroxy-5α-androstan-3-one; 1α-methyl-7α,11β,17β-trihydroxy-5α-androstan-3-one; 1α-methyl-1β,11α,17β-trihydroxy-5α-androstan-3-one; 1α-methyl-11α,17β-dihydroxy-5α-androstan-3-one; 1α-methyl-6α,17β-dihydroxy-5α-androstan-3-one; and 1α-methyl-7α,17β-dihydroxy-5α-androstan-3-one (Ahmad et al. 2014).

Both *Cunninghamella blakesleeana* and *C. echinulata* together with *Macrophomina phaseolina* have contributed in catalyzing the biotransformation of the veterinary drug steroid, mibolerone, yielding 10β,17β-dihydroxy-7α,17α-dimethylestr-4-en-3-one; 6β,17β-dihydroxy-7α,17α-dimethylestr-4-en-3-one; 6β,10β,17β-trihydroxy-7α,17α-dimethylestr-4-en-3-one; 11β,17β-dihydroxy-(20-hydroxymethyl)-7α,17α-dimethylestr-4-en-3-one; 1α,17β-dihydroxy-7α,17α-dimethylestr-4-en-3-one; 1α,11β,17β-trihydroxy-7α,17α-dimethylestr-4-en-3-one;

and 11β,17β-dihydroxy-7α,17α-dimethylestr-4-en-3-one (Siddiqui et al. 2017). *Cunninghamella echinulata* and *C. blakesleeana were used to synthesize* derivatives of the antileishmanial steroid, nandrolone. Biotransformation of nandrolone resulted in production of seven metabolites, 10β,12β,17β-trihydroxy-19-nor-4-androsten-3-one; 10β,16α,17β-trihydroxy-19-nor-4-androsten-3-one; 6β,10β,17β-trihydroxy-19-nor-4-androsten-3-one; 10β,17β-dihydroxy-19-nor-4-androsten-3-one; 6β,17-β-dihydroxy-19-nor-4-androsten-3-one; 10β-hydroxy-19-nor-4-androsten-3,17-dione; and 16β,17β-dihydroxy-19-nor-4-androsten-3-one (Baydoun et al. 2014).

Norandrostenedione (19-nor-4-androsten-3,17-dione) was microbially converted by *C. blakesleeana* ATCC 8688A into 6α,10 β -dihydroxy-19-nor-4-androsten-3-one; 10 β -hydroxy-19-nor-4-androsten-3,17-dione; 6 β,10 β,17 β -trihydroxy-19-nor-4-androsten-3-one; and 10 β,17 β -dihydroxy-19-nor-4-androsten-3-one (Chegaing et al. 2020). Biotransformation of the oral contraceptive drug, desogestrel, using *C. elegans* produced 13β-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17β-ol-3,6-dione; 13β-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3β,6β,17β-triol; 13β-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β,17β-diol-3-one; 13β-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17β-ol-3-one; 13β-ethyl-11-epoxy-18,19-dinor-17α-pregn-4-en-20-yn-17β-ol-3-one; and 13β-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-10β,17β-diol-3-one (Ibrahim et al. 2020).

7 Conclusion and Future Prospects

Emergence of new serious diseases such as COVID-19 as well as the extended list of lethal diseases that threaten human life have forced researchers to accelerate their forces to screen for novel powerful drugs or synthesis bioactive new compounds. Microbial transformation of drugs in general, and steroids in particular, has facilitated obtaining desired products when compared with chemical ways. It is also ecofriendly without the need to use harmful chemicals. Fungal enzymatic machinery is the most potent and promising biotechnological tool that can be employed to conduct various types of reactions. Therefore, fungal biotransformation can be used to synthesis different derivatives from a biologically active steroidal drug, which can contribute in obtaining more potent compounds, or compounds showing different pharmaceutical applications other than those known for their parent compound. Further studies should be conducted to screen for and identify potent microorganisms in general, and fungi in particular which are capable of performing highly specific biotransformation processes. Moreover, in vitro and in vivo studies should be designed and performed in order to evaluate obtained novel derivatives compounds along with the evaluation of their cytotoxicity and compatibility to be used for human or animals. Dedicated websites should be constructed, developed, and kept updated with the recent available information about reported transformation reactions performed by the help of specific species to facilitate getting information.

Introducing techniques including nanotechnology may facilitate the uptake of the substrate by the biotransforming microorganism and increase overall productivity. Also, applying genetic manipulation on potent biotransforming microorganisms can contribute in the development of new clones with promising biotransformation potentials. On the other hand, each biotransformation reaction should be well-studied to eliminate the risk of producing undesired or harmful products. Hence, pharmaceutical companies can use it to produce required safe drugs in more efficient way.

Conflict of Interest

The authors declare that there is no conflict of interest.

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