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Past, Present, and Future of Antifungal Drug Development

P.K. Shukla, Pratiksha Singh, Ravindra Kumar Yadav, Smriti Pandey, and Shome S. Bhunia

Abstract Fungi are eukaryotic, single cell or multicellular organisms which cause a wide range of human diseases ranging from superficial skin to invasive life-threatening infections. Over the last couple of decades the incidence of life-threatening fungal infections has increased seriously as the patients of AIDS, cancer, organ transplant and immune-compromised population have increased. Though a significant progress has been made in the discovery of antifungal agents in the form of polyenes, azoles and allylamines yet the antifungal therapy poses severe challenge because of the side effects, narrow spectrum of activity and recently development resistance among patients against the present antifungals. This chapter deals with the current antifungal agents, their spectrum of activity, mode of action, limitations, current challenges in antifungal therapy, and new avenues for future developments.

Keywords Allylamines, Antifungal therapy, Azole, Cell membrane, Ergosterol, Immunocompromise, Monoclonal antibodies, Pathogenic fungi, Polyenes

Contents

P.K. Shukla (✉), P. Singh, R.K. Yadav, and S. Pandey
Division of Microbiology, CSIR-Central Drug Research Institute, Lucknow 226031, India
e-mail: pk_shukla@cdr.res.in

S.S. Bhunia
Division of Medicinal & Process Chemistry, CSIR-Central Drug Research Institute, Lucknow 226031, India

1 Introduction

Fungi are one of the extensively spread organisms on earth and have great environmental and medical importance. The kingdom fungi contains about 1.5 million [1] different species which are either unicellular or multicellular eukaryotic, heterotrophic organisms that can be divided into biotrophs: which obtain their nutrients from a living host (plant or animal), saprotrophs: which obtained their nutrients from animals or dead plants, and necrotrophs: which infect a living host and kill host cells to obtain their nutrients [2].

Besides being beneficial organisms for humans in bio-production of alcohol and bakery, fungal species like *Aspergillus* sp., *Penicillium* sp., and *Acremonium* sp. are associated with the production of enzymes and antibiotics. Along with the above positive

impacts certain species adversely affect the crops and humans by producing diseases. A number of fungi have been reported as causal agents of human and animal infections and the first published record of infection in human is a case of oral manifestation of *Candida albicans* infection that was recorded in 1665 as a fatal disease [3].

In the atmosphere fungi are present from temperate to subtropical and tropical areas, and these organisms are mostly non-pathogenic and can cause infection under certain compromised conditions like immune suppression which may be due to various factors [4]. The fungi can cause infection of any part of the body starting from the hair of the scalp to nails of the toe web. However these infections are opportunistic in nature and the fungi causing these infections are categorized as opportunistic pathogens. The true pathogenic fungi are only four in number and these are *Coccidioides*, *Paracoccidiodes*, *Blastomyces*, and *Histoplasma* [5]. Fortunately the geographic distribution of these fungi is known to restricted area [6]. In case of superficial fungal infections the value is more of cosmetic in nature and man hour loss in terms and public nuisance. However the systemic infections pose a serious challenge in the form of early and accurate diagnosis as well as treatment [7].

A number of antifungal agents as described in this chapter are available in the market. Barring amphotericin B almost all the known antifungal agents are fungistatic in nature. Amphotericin B considered to be the gold standard of the antifungal agents is fungicidal; however its use is very much limited due to its side effects, particularly nephrotoxicity [5]. The fungal infections have emerged into prominence after the onset of AIDS and HIV infections where these infections may prove to be fatal to the host [6]. The number of antifungal agents is limited as compared to antibacterial drugs because of the fact that the fungus is an eukaryotic organism that parasitizes an eukaryotic host where the narrow range of physiologic difference between them cause difficulties in developing safe and broad spectrum antifungal agents. There are limited number of classes of antifungal agents to combat fungal infections with limitations of toxicity and development of drug resistance [8, 9].

2 Challenges in Antifungal Therapy

The major challenge in the treatment of mycoses is the timely and correct diagnosis of the disease. This is the first very important step which is mainly dependent on the clinical symptoms, which are very peculiar in case of superficial infections like raised erythematous margins of the lesions with prominent scaling and many times present with itching. However, in case of systemic infections the symptoms are very often common to those caused by other bacterial infections particularly in case of the infections of the lung. Then comes the step of obtaining the sample from the site of infection which may be achieved through scraping from the active sites of the infection in case of the involvement of the skin (margins), hair, nail, and sputum in case of lung infection, blood in case of systemic infection, etc. The samples thus collected are subjected to direct microscopic examination using wet mount, KOH preparations or fungal specific stains such as lactophenol cotton blue. In case of

deep seated infections biopsy is often required for establishing the correct diagnosis. From the obtained clinical specimen cultures are made generally in Sabouraud's dextrose agar at 30–53°C. Very often the fungi take longer periods to grow and thus result in the delay in diagnosis of mucoses.

Advances in biological techniques particularly the molecular one have opened avenues for diagnostic methods that are not dependent on culture of the organisms. Specific metabolites and molecular probes are often used for the detection and identification of fungal infections [10–13]. PCR (polymerase chain reaction) has exhibited its utility in the diagnosis of microbial infections inclusive of mycoses [14–18]. In the genome the most conserved region is the ribosomal DNA having capability of phylogenetic divergence [19]. The rRNA gene has a large subunit (LSU) 28S rRNA and small subunit (SSU) 18S rRNA and 5.8S rRNA. The internal transcribed spacer (ITS) region I (ITSI) and ITSII are found between SSU rRNA and 5.8S rRNA and between 5.8S rRNA and LSU rRNA respectively and are more variable than the rest of the ribosomal gene subunits. In addition the intergenicspacer (IGS) region I (IGSI) and IGSII occur between the LSU and SSU sequence [20]. Further the single-stranded conformation polymorphism (SSCP) technique to identify sequence variations in a single strand of DNA due to its adoption to a unique conformation under non-denaturing conditions [18] has been used by various researchers [21–24]. Such molecular approaches have the advantage of detecting fungi directly in the clinical specimen and provide much faster and more sensitive fungal detection than the conventional culture-based methods.

The next important step in the direction of therapy is in vitro sensitivity tests for the isolated fungal strain against the available antifungal agents. This is achieved by exposing the test fungus against the known concentrations of various antifungal agents and determining the minimal inhibitory concentration values. This may be achieved by either disc diffusion method or more precisely by the twofold serial dilution method as per guidelines of the CLSI (Earlier NCCLS). There are a number of antifungal agents available for the treatment of mycoses. However their usefulness has been limited either by their selective activity or more recently this situation is further complicated because of the development of resistance in the fungal pathogens against the existing antifungal agents.

3 Available Antifungal Drugs, Spectrum of Activity, and Development of Resistance

The availability of antifungal agents is limited for therapy and the use of these drugs is further restricted by the issue of safety, resistance, and their efficacy profiles. Understanding the mode of action of different antifungal agents is an important prerequisite to explore drug resistance mechanisms. The emergence of resistance against drugs is an evolutionary process based on natural selection of organisms

that enhances their ability to survive and multiply in presence of drug. Investment of a considerable amount of energy is required by competitive microbial communities for the production and elaboration of antimicrobial agents [25]. The evolution of resistance against antimicrobial agent is ubiquitous in nature and microbes evolve various strategies to combat the action of drugs. The development of new antibiotics is outpaced by the evolution of drug resistance due to which progressing our knowledge towards understanding evolutionary mechanisms gains utmost importance. The present antifungal arsenal has been discussed below.

4 Polyenes

The Polyene antibiotics discovered in late 1950s have a broad spectrum fungicidal activity and were isolated from different species of *Streptomyces* which are soil born [26] (Fig. 1). Chemically the polyenes are the molecules that contain polyhydroxylic lactone ring of 20–40 carbon atoms with 4–7 conjugated double bonds, that's why they are hydrophobic in nature. These are known to bind to the main component of fungal cell membrane, the ergosterol and result in the formation of transmembrane channels that allow the leakage of cell contents along with K+ and Na+ ions leading to the damage and death of the fungal cells [27]. The affinity of polyenes for ergosterol in fungal cell wall is higher than the affinity for cholesterol in mammalian cell; therefore they are less toxic to the latter. Yet this non-negligible toxicity cannot be ignored and explains the high toxicity associated with several side effects. Of the several polyene antibiotics only three, nystatin, natamycin, and amphotericin B, are in clinical use despite their side effects.

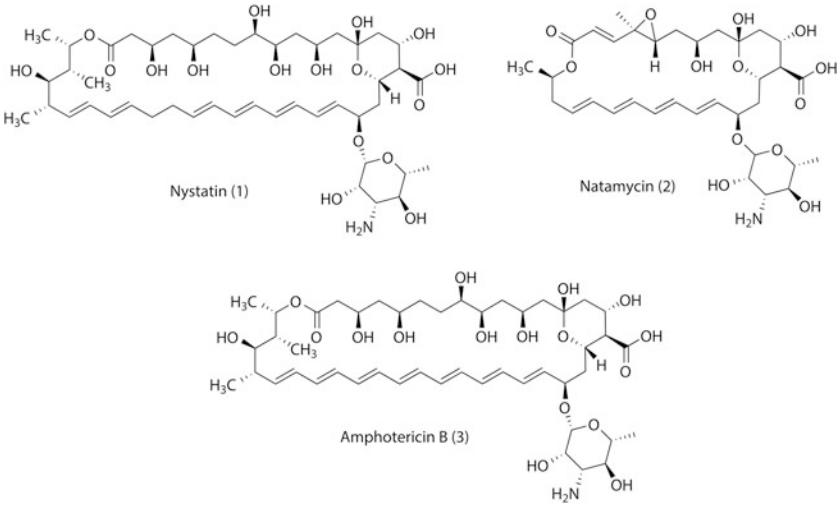

Fig. 1 Structure of polyene antibiotics

4.1 Nystatin

Nystatin (**1**) is the first antifungal agent introduced for the clinical use which was discovered by E.L. Hazen and R.F. Brown in 1944 while doing their research in the division of Laboratories and Research, New York State Department of Health which was published in 1950 [28, 29]. It was isolated from an actinomycete *Streptomyces noursei* which is commercially described as mycostatin and is active against many moulds and yeast infections [29, 30]. Nystatin is insoluble in water and sparingly soluble in organic solvents. It is unstable under moist conditions, heat, and light sensitive and therefore stored in cold and dark places [31]. Nystatin structure has been resolved by chemical degradation and X-ray crystallography [32]. It consists of a 38-membered macrolide lactone ring containing single tetraene and diene moieties separated by two methylene groups [33].

This drug is not absorbed through oral route but is effective topically for oropharyngealcandidosis. Nystatin was licensed for use in 1951 and due to its greater potential activity that caused toxicity in the system its use has been restricted to topical administration for superficial (mucosal) *Candida* infections of the oropharynx, esophagus, and intestinal tract.

Later on a liposomal preparation of nystatin was prepared that enhanced survival and reduced the tissue burden of *Aspergillus* in experimental neutropenic rabbits with invasive pulmonary aspergillosis and mice with disseminated aspergillosis [34, 35].

4.2 Natamycin

Natamycin (**2**) also known as pimaricin has been reported to be produced during fermentation process by a soil inhabiting microorganism *Streptomyces natalensis* [36]. It is sparingly soluble in water and has been found to exhibit antifungal activity at low concentrations. Natamycin is being used in the treatment of mycotic keratitis an infection of the cornea especially the cases caused by the species of *Aspergillus* and *Fusarium* [37]. It is normally used as topical antifungal agent in the form of cream or drops where it exhibits absorption in very low quantities in the body. This antibiotic is very little absorbed from the GI tract and therefore not recommended for use against systemic fungal infections [38].

4.3 Amphotericin B

Amphotericin B (**3**) is a polyene antifungal agent which is produced by *Streptomycin nodosus* [39]. According to the modern pharmacological standards, it is notified that amphotericin B, an antifungal agent, is a very old drug and since long times it was

the only therapeutic option for the treatment of invasive mycoses. This compound is amphoteric in nature with a primary amino group attached to the mycosamine ring and a carboxyl group on the macrocycle [40]. Amphotericin B forms deep yellow crystals that are sparingly soluble in organic solvents but insoluble in water [41].

Though it is not well absorbed after oral administration, it exhibits a wide spectrum of activity that is fungicidal in nature [42]. This drug can be used as an oral/topical formulation for the treatment of mucosal candidosis and intravenous amphotericin B for invasive fungal infections as a successful therapy [43]. It is proposed by most clinical medical mycologists as the drug of choice for all forms of invasive aspergillosis and cutaneous mucormycosis, blastomycosis, paracoccidioidomycosis, histoplasmosis, fusariosis, severe and moderate cryptococcal meningitis, coccidioidomycosis, candidosis, and *Candida* infections of the central nervous system [9]. The side effect of amphotericin B therapy causes serious nephrotoxicity where almost each patient contracts some defect in renal function [44].

The amphotericin B molecule is largely lipophilic and forms pore in the fungal membranes but does not cause pore formation in the mammalian cell membrane because its partition coefficient is lower for cholesterol which form the main constituent of mammalian cell membrane instead of ergosterol, which is found in fungal membrane. The drug gets saturated in fungal cell and leads to its lysis due to its higher partition coefficient for ergosterol. The fungicidal activity of amphotericin B is mediated by its binding with ergosterol that is supplemented by the secondary mechanism of membrane permeabilization through channel formation. In a recent study the cytocidal activity of amphotericin B has been attributed due to its ability to extract ergosterol from lipid bilayers by forming large, extramembranous aggregates [45–49]. Use of amphotericin B has certain limitations as its intravenous administration is associated with side effects such as fever, chills, headache, nausea, vomiting and nephrotoxicity. To overcome this problem different commercial lipid-based formulations of amphotericin B are available that cause less toxicity.

The clinically useful and established novel formulations are lipid combinations with amphotericin B, encapsulated in liposomes or in ribbon-like and disc-like lipid complexes while the others studied are amphotericin B–cochleate preparation and an arabinogalactan complex. To overcome the nephrotoxiciy of standard amphotericin B lipid formulation of amphotericin B can be used. The lipid formulation is very expensive as compared to the native formulation [25, 50]. Occurrence of resistance to polyenes in *C. albicans* is a rare event but recently increasing cases of resistance have been reported [51]. Filamentous fungi exhibit greater resistance to polyenes than yeasts. *Aspergillus terreus* is generally amphotericin B resistant whereas *A. fumigates* and *A. flavus* are becoming gradually more resistant [52]. Polyene resistance could be developed by reducing the substrate to which it binds, i.e., ergosterol content in plasma membrane. Mutation in *ERG3* gene lowers the ergosterol content of plasma membrane leading to accumulation of alternative sterols, causing amphotericin B resistance. The polyene resistance is also associated with increased catalase activity, which increases its oxidative tolerance [53].

A biochemical hypothesis for amphotericin B resistance has been given by Hamilton Miller that the altered sterol content of the resistant cells should bind to smaller amounts of polyene than do susceptible cells, hence may become resistant.

5 Azoles

Azoles were first introduced in 1960s as derivatives of N-substituted imidazole such as econazole, ketoconazole, miconazole, and clotrimazole, and is the most widely used class of antifungal agents [54] (Fig. 2). The azoles form a group of fungistatic agents with broad spectrum activity and are classified into two groups: the imidazoles and the triazoles. These antifungals inhibit the cytochrome P450 dependent enzyme lanosterol 14-alpha-demethylase that converts the lanosterol (the main sterol found in fungal cell wall) to ergosterol and thus results in the depleted ergosterol in the cell membrane causing cell death [55]. Azole antifungals are widely used in the treatment of systemic and topical (athletes foot, ringworm etc.) fungal infections. However azoles being fungistatic have a disadvantage due to recurrence of fungal infections.

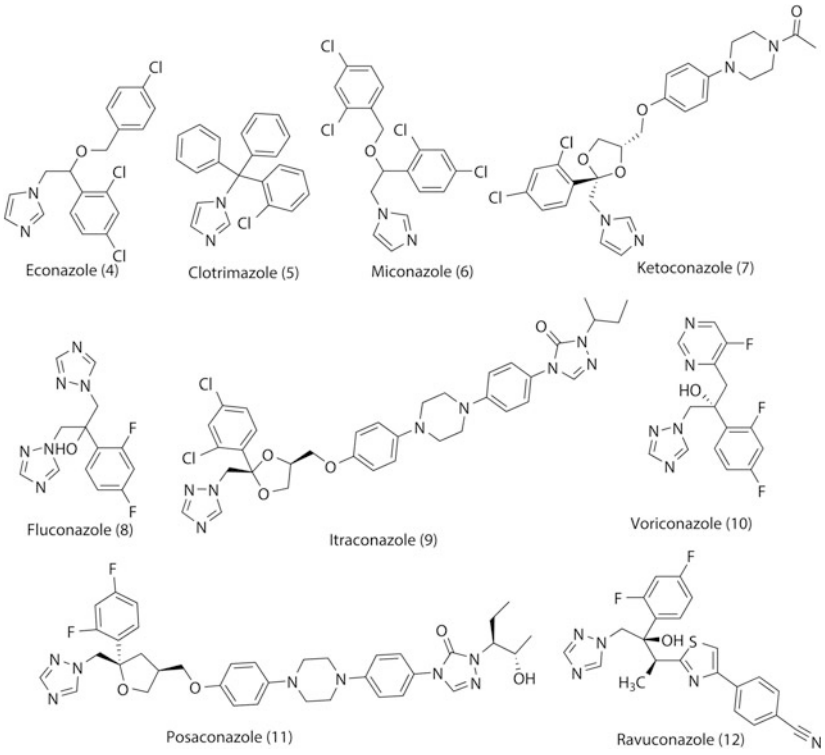

Fig. 2 Structure of azole class of antifungals

5.1 Econazole

Econazole nitrate chemically 1-[2-[(4-Chlorophenyl) methoxy]-2-(2,4-dichlorophenyl)-ethyl]-1*H*-imidazole (**4**) is a white crystalline nitric acid salt of econazole. It is slightly soluble in water, ether, and alcohol, sparingly soluble in chloroform, and soluble in methanol [56]. This antifungal is commonly used as the nitrate salt for antifungal therapy [57] mainly in the form of a cream to treat tinea corporis, tinea pedis, athlete's foot, tinea cruris, tinea versicolor and cutaneous candidiasis. However about 3% of treated patients have been reported to exhibit side effects like burning, itching, erthema, and pruritic rash [58].

5.2 Clotrimazole

Clotrimazole (**5**) was first described in 1969 from At Bayer Research Laboratories. 1-(*o*-Chloro-alpha,alpha-diphenyl benzyl)imidazole (clotrimazole) is a white crystalline solid that is sparingly soluble in water but soluble in alcohol and most organic solvents [59]. This antifungal is also known as Canesten or Lotrimin and is the first imidazole derivative which was developed for the treatment of human mycoses. It played an important role in the treatment of fungal infections such as vaginal yeast infections, oral thrush, ringworm, athlete foot, and jock itch. It is a vital medicine in the list of WHO [60].

Clotrimazole kills fungal cell by altering the permeability of fungal cell wall and binds to phospholipids in the cell membrane that inhibit the biosynthesis of ergosterol and sterols for the cell membrane production which results in loss of intracellular elements and cellular death [61]. Clotrimazole is a broad spectrum antifungal agent used in the treatment of infections caused by dermatophytes, yeasts, and *Malassezia furfur*.

5.3 Miconazole

Miconazole (**6**) is a synthetic imidazole antifungal agent that is poorly soluble in water and most of the organic solvents [62]. It also has some antibacterial action and antiparasitic properties. The mode of action of miconazole is inhibiting the synthesis of ergosterol [63]. It is used for the treatment of topical fungal infections including vaginal candidiasis [64], onychomycosis [65], tineapedis [66], and pityriasis versicolor [67]. It has also been moderately successful in the treatment of systemic mycoses [68].

5.4 *Ketoconazole*

Ketoconazole (**7**), discovered in 1976, is a member of imidazole synthetic compounds series which has a broad spectrum antifungal profile. 1-Acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2(1*H*-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy] phenyl]piperazine is a weak basic compound that occurs as a white crystalline solid [69]. Ketoconazole is a racemic compound, consisting of the *cis*-2*S*,4*R* and *cis*-2*R*,4*S* isomers and it has seen that the 2*S*,4*R* isomer was more active than its 2*R*,4*S* enantiomer [70].

Ketoconazole was the first available compound for the oral treatment of systemic fungal infections in the early 1980s [71]. Ketoconazole is less soluble in water and administered orally [72] and in a range of formulations for topical administration such as creams (in treatment of cutaneous candidasis, pityriasis versicolor, candidal paronychia) and shampoos [73]. It shows toxic effects against yeast and interferes with other membrane lipids or enzymes. Ketoconazole inhibits the enzyme cytochrome P450 14-alpha-demethylase (P45014DM) which plays an important role in sterol biosynthesis pathway that leads from lanosterol to ergosterol [74].

High oral dose of ketoconazole may cause hepatotoxicity. Higher therapeutic doses may also produce endocrine abnormalities by reduction in circulating testosterone levels and blocks both testicular and adrenal androgen biosynthesis [75]. Ketoconazole is highly protein bound, has poor CNS penetration, and is not suitable for treating CNS infections. There is no intravenous preparation [76]. Oral ketoconazole is effective in patients with candidosis, coccidioidomycosis, blastomycosis, histoplasmosis, paracoccidioidomycosis, and cutaneous dermatophyte infections [77].

Later on the first generation triazoles such as fluconazole and itraconazole were introduced which are the imidazoles having five membrane ring atoms with one, two, and three nitrogen molecules. Fluconazole and itraconazole exhibited a broader antifungal activity spectrum as compared to the imidazoles and had a significant improved safety profile in comparison of amphotericin B and ketoconazole. Despite their prevalent use they face certain clinical limitations such as suboptimal level of activity spectrum, development of resistance and toxicity. In order to rectify these problems, several analogues have been derived. The second generation triazoles such as voriconazole, ravuconazole, and posaconazole possess higher potency and have increased efficacy against the emerging pathogens. Azoles perform their activity on cell membrane by inhibiting the ergosterol biosynthesis [78]. The major targets of most azoles are gene *ERG11* encoded cytochrome P450 lanosterol 14α-demethylase, it leads to generation of faulty intermediate namely 14-methylergosta-8, 24(28)-dien-3,6-diol, which is toxic and is responsible for inhibition of fungus [79]. Increase in azole resistance is mainly due to its fungistatic nature instead of fungicidal. Resistance against fluconazole among HIV patients with OPC is the direct consequence of excess use of itraconazole and fluconazole [80]. About one-third population of patients with AIDS has azole resistant

C. albicans in their oral tract [81]. *Candida* species employs various mechanisms to develop resistance against azoles as follows:

Over expression of efflux pumps: *C. albicans* overexpresses the efflux pumps in response to drug which results in efflux of drugs from cells thus reducing the drug concentration at action site. Two gene families namely *MDR* (Multi-Drug Resistance) genes of the major facilitator class and *CDR* genes belonging to the ATP-binding super cassette family. Up-regulation of *CDR* genes is responsible for resistance against most azoles while *MDR* encoded pumps exhibit narrow fluconazole specific spectrum [82].

Modification of target: Mutations in the *ERG11* gene, which encodes lanosterol 14α-demethylase, decrease azole affinity to the target site. Fluconazole has been used against a variety of mycotic infections and resistance to this antifungal has been documented. The two yeasts *Candida glabrata* and *C. krusei* with inherent low susceptibilities to fluconazole have been reported at a greater frequency from patients [83].

Up-regulation of target enzyme: *Candida* isolates overexpress the *ERG11* gene which results in reduced azole susceptibility [84]. The overexpression of gene results in accumulation of target molecules.

Development of alternative pathways: Organisms express alternative genes in order to bypass the pathway. Azole exposure results in ergosterol depletion from the membrane and leads to accumulation of toxic metabolite namely 14α-methyl 3, 6-diol. Additive mutation in *ERG3* gene prevents the formation of this toxic product from 14α-methyl fecosterol and leads to accumulation of nontoxic sterols [85].

5.5 *Fluconazole*

Fluconazole (**8**) was formulated in 1981 and marketed in 1990. It is a novel bi-striazole which is metabolically stable, water soluble, low lipophilicity, and plasma protein binding antifungal agent. Fluconazole acts by inhibiting ergosterol enzyme biosynthesis in fungal cells through inhibition of a cytochrome P450 enzyme dependent 14 alpha-sterol demethylase [86]. This leads to the accumulation of methylated sterols which break fungal membrane structure resulting in growth arrest. Fluconazole antifungal is administered orally, intravenously, or both and is used to treat broad spectrum of fungal infections and has a very low incidence of side effects. It is used to treat *Candida* infections of the vagina ("yeast infections"), mouth, throat, and bloodstream [87]. It is also used to prevent infections in people with weak immune systems, including those due to cancer chemotherapy, bone marrow transplantation patients, premature babies, and oropharyngeal candidosis, neutropenia, sporotrichosis infections [87, 88].

5.6 Itraconazole

Itraconazole (**9**) discovered in 1984 is another triazole antifungal agent with broad spectrum antifungal activity [89]. It contains a weakly basic 1,2,4-triazole and a non-basic 1,2,4-triazol-3-one moieties in its structure and requires an acidic environment for optimum solubilization and oral absorption [90].

It is insoluble in water and available in oral form. It is active against *Aspergillus*, *Candida*, and *Cryptococcus* species [91]. Itraconazole has been useful in the treatment of chronic cavitary pulmonary disease, extrapulmonary blastomycosis, disseminated non-meningeal histoplasmosis, osseous/articular and lymphocutaneoussporotrichosis in non-immunosuppressed patients [92]. Itraconazole has recently been repositioned as anticancer agent [93]. Traconazole is the only inhibitor in this class that has been exposed to reduce both hedgehog signaling pathway and angiogenesis. These different actions are unrelated to inhibition of the cytochrome P450 lenosterol 14 alpha demethylase. The anti-angiogenic action of itraconazole is associated with inhibition of glycosylation VEGFR2, phosphorylation, trafficking, and cholesterol biosynthesis pathways.

5.7 Voriconazole

Voriconazole (**10**) is a low molecular weight, water soluble broad spectrum triazole effective against the treatment of invasive aspergillosis and esophageal candidiasis [94, 95]. It shows activity against *Aspergillus* spp., *Fusarium* spp., *Candida* spp., *Cryptococcus neoformans*, Fusarium, and Scedosporium infections including the fluconazole resistant or less susceptible spp. of *C. glabrata* and *C. krusei* [96, 97]. It showed serious drug–drug interactions and side effects like skin rash and transaminase elevation and hallucinations [98–102].

5.8 Posaconazole

Posaconazole (**11**), a triazole antifungal drug, was approved by the US FDA in September 2006 for the prophylaxis of invasive *Aspergillus* and *Candida* infections in severely immune-compromised patients [103]. It shows in vitro activity against *Aspergillus*, *Candida* spp., *Cryptococcus* spp., and *Histoplasma* spp. and also effective against infections caused by the zygomycetes than voriconazole [8, 104]. The most common side effects of posaconazole are gastrointestinal complaints, nausea, vomiting, abdominal pain, headache, elevation of liver enzymes, and skin rash [105–107].

5.9 Ravuconazole

Ravuconazole (**12**), a triazole, is a broad spectrum antifungal agent. It shows activity against *Candida* spp. even isolates that are resistant to fluconazole, *Aspergillus*, *Cryptococcus*, and many dermatophytic fungi [107–109]. Ravuconazole shows long elimination half-life and high protein binding [110, 111].

5.10 Other Azoles

5.10.1 Imidazoles

Azoles being popular as antifungal agents have been considered for various modifications (Fig. 3). Among the imidazoles, a series of *N*-[(1,1′-biphenyl)-4-ylmethyl]-1*H*-imidazol-1-amine derivatives (**13**) reported by Setzu et al. [112] showed better antifungal activity with substitutions at 2-position (R_1) of the phenyl ring compared to substitution at the 4- position (R_2) when tested in *Candida neoformans*. However the most potent compound in the series with chloro substitutions at both 2 and 4 positions (R_1 and R_2) of the phenyl ring had a MIC value of 0.8 μg/mL against *Trichophyton rubrum* compared to miconazole (0.4 μg/mL). Imidazole modifications were also made by introducing nitro group at 5-position resulting in potent antifungal compounds. The analogs of **14** having R_1 substituted by morpholineor piperidine, R_2 and X substituted by H showed good activity against *Sclerophoma pityophila* [113]. Effective antifungal activity was also observed in another series of 5-nitro imidazoles having phenyl piperidine

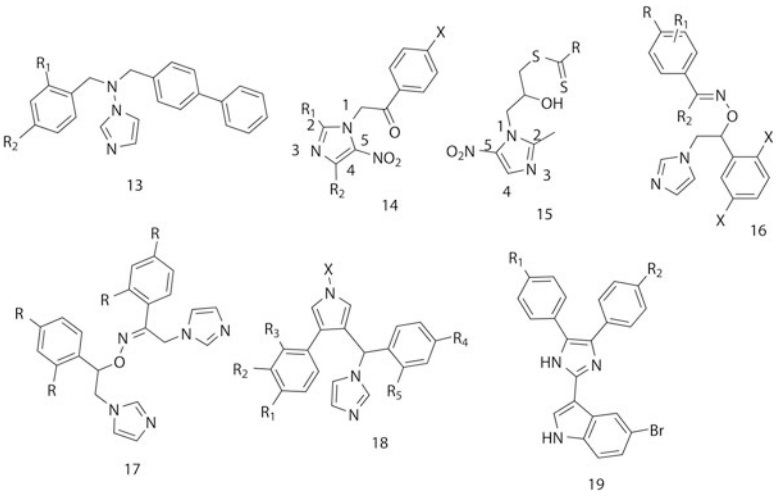

Fig. 3 Structure of imidazole containing molecules as antifungal agents

substitution at R separated by 2-hydroxypropyl methanedithioatespacer (**15**) [114]. The compound had MIC = 3 μg/mL against *Trichophyton tonsurans*. However the compound was less effective than miconazole (MIC = 0.2 μg/mL) or ornidazole (MIC = 0.8 μg/mL). In another imidazole containing series 2-(1*H*-imidazol-1-yl)-1-phenylethanone-*O*-2-(1*H*-imidazol-1-yl)-1-phenyl-ethyl oxime derivatives (**16**) were synthesized by inverting the oxime group present in oxiconazole [115]. The most active compound in the series having substitutions at R=Ethoxy morpholine, R_1=H, R_2=Me, and X=Cl is an effective antifungal compound against *C. glabrata* (MIC = 0.06 μg/mL), *C. parapsilosis* (MIC = 0.004 μg/mL), and *C. albicans* (MIC = 1 μg/mL). Another compound in the series where X=F also showed good antifungal activity in the above fungal species [*C. glabrata* (MIC = 0.25 μg/mL), *C. parapsilosis* (MIC = 0.03 μg/mL), and *C. albicans* (MIC = 8 μg/mL)]. In the same series modification of the R_2 to imidazole and substitution at R=F resulted in less active compound (**17**) with MIC values of 4, 8, 2 μg/mL in *C. glabrata*, *C. parapsilosis*, and *C. albicans*, respectively. In another series of imidazole derivatives having a pyrrole ring (**18**) it was observed that compounds having R_1=Cl, R_2=R_3=R_4=R_5=H, and R_3=CH_3 (MIC = 0.062 μg/mL), C_3H_7 (MIC = 0.016 μg/mL), CH_2–c–C_3H_5 (MIC = 0.016 μg/mL), CH_2=CH_2 (MIC = 0.032 μg/mL), CH_2–CH=CH_2 (MIC = 0.016 μg/mL), CH_2–CH=$C(CH_3)_2$ (MIC = 0.065 μg/mL) had comparable activity with miconazole (MIC = 0.062 μg/mL) and itraconazole (MIC = 0.062 μg/mL) and better than fluconazole (MIC = 0.25 μg/mL) in *C. albicans* [116]. In a series of 2,4,5- trisubstituted imidazoles (**19**), the best compounds had an indole moiety at the 2-position of the imidazole ring while the 4 and 5 positions were having substituted phenyl moiety . Three compounds [1: (R_1=R_2=F), 2: (R_1=Cl, R_2=H), 3:(R_1=Br, R_2=H)] in the series showed MIC = 8 μg/mL in *C. albicans* [117, 118].

5.10.2 Triazoles

Bile conjugates of fluconazole (**20**) (Fig. 4) have shown good antifungal activity when the R position of the steroid moiety is substituted by H or OH, the activity was in between 2.18 and 25 μg/mL when evaluated in different fungal species (*S. schenckii*, *C. albicans*, *C. parapsilosis*) [119]. The triazole derivatives of 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-cyclopropyl-*N*-substituted-amino)-2-propanol (**21**) were effective antifungal agents, most of them had broad antifungal activity with MIC_{80} less than 0.125 μg/mL [120]. The compounds having R=CH_3, CH_2CH_3, CH_2CHCH_2, $(CH_2)_3CH_3$, $(CH_2)_4CH_3$, $(CH_2)_6CH_3$, $(CH_2)_7CH_3$ were the most potent ones having MIC_{80} in the range of 0.125–8 μg/mL against *C. albicans*, *C. parapsilosis*, *C. neoformans*, *C. tropicalis*, *T. rubrum*, *A. fumigatus*, *M. canis*, and *F. compacta*. Fluconazole at similar bioassay condition showed MIC_{80} range of 0.5–32 μg/mL in the fungal species mentioned above. In this series retention of antifungal activity was observed when the R group is substituted by benzyl group (**22**) having different substituents at the phenyl ring [X=H, 3F, 3Cl,

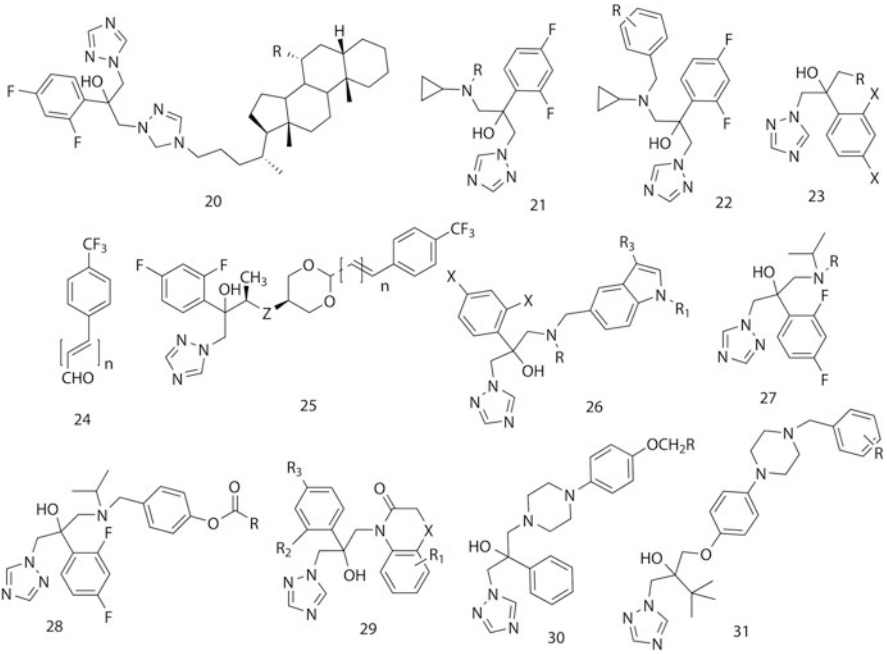

Fig. 4 Structure of molecules having triazole structure and fluconazole modifications

$3CH_3$, 4-NO_2, $2NO_2$, 2CN, 4CN, (2,4-Cl), $2CH_3$, $4CH_3$, 4F]. All these compounds had MIC_{80} value less than 0.125 μg/mL in *C. albicans*. Heterocyclic derivatives of fluconazole having N1-Indazole, indole, indoline, benzimidazole, azaindole, and benztriazole (**23**) were also synthesized where the R=N1-indazole and X=Cl, Cl substitution was the most potent candidate ($MIC_{80} = 0.0007$ μg/mL) than fluconazole ($MIC_{80} = 0.020$ μg/mL) against *C. albicans* [121]. In this series (**23**) better antifungal activity was observed by the replacement of N1-indazole by azaindole moiety having X=Cl, Cl ($MIC_{80} = 0.0031$ μg/mL) and X=F, F ($MIC_{80} = 0.007$ μg/mL). Another compound where R=3-ethoxycarbonylmethyl-1*H*-indole and X=Cl, Cl also showed good antifungal activity ($MIC_{80} = 0.006$ μg/mL). The syntheses of triazole derivatives with varying olefinic chain length for two series have been reported where in first case the optimum chain length of $n = 2$ having the structure **24** and varying olefinic chain length ($n = 0$–3) in structure **25** has shown excellent in vitro activity against *Candida*, *Cryptococcus*, and *Aspergillus* spp. with antifungal activity MIC ranging 0.016–0.125 μg/mL [122]. This is better than fluconazole that is having the MIC range 0.5–4 μg/mL in the above-mentioned species. A series of 1-[((hetero)aryl- or piperidinylmethyl) amino]-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols evaluated against *C. albicans* and *A. fumigatus* showed compound **26** having X=F, F, R=R_3=H, and R=N-Boc to be the most potent one ($MIC_{80} = 3$ ng/mL) and better than fluconazole ($MIC_{80} = 190$ ng/mL). In this series methyl substitution of the nitrogen atom in the linker reduces the activity 20 times

($MIC_{80} = 60$ ng/mL) when compared to **26** [123]. A series of triazole derivatives targeting lanosterol 14α-demethylase (CYP51) with a general structure 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-isoproyl-*N*-substituted-amino)-2-propanol depicted good antifungal activity when R=4-$H_3COC_6H_4$, 4-$H_5C_2OC_6H_4$ with MIC_{80} ranging 0.0156–1 μg/mL in **27**. In the same series different esters at 4-position of the phenyl ring having R=CH_3, CH_2CH_3, $CH_2CH_2CH_3$ in **28** had MIC_{80} in the range of 0.0156–64 μg/mL [124]. A series of fluconazole derivatives (**29**) with benzothiazinone substituent depicted slightly better antifungal activity. The compound **29** (X=S, R_1=H, R_2=R_3=F) (0.25 μg/mL) showed improved activity than the benzoxazinone replacement [X=O, R_1=H, R_2=R_3=F (0.5 μg/mL)] but both were found to be better than fluconazole (1 μg/mL) [125]. Another synthesized triazole containing compound (**30**) based on QSAR study with R=4-FC_6H_4, 4-$CONH_2C_6H_4$, 4-C_5H_4N was having comparable activity (0.0625–0.5 μg/mL) with itraconazole (1–2 μg/mL) when tested in *A. fumigatus*, *C. parapsilosis*, *C. tropicalis*, *C. neoformans*, *M. lauosum*, and *T. rubrum* with best activity in *M. lauosum* [126]. A series of triazole compounds having hydrophobic substitution or CN group with the general structure **31** (R=3,4- $(CH_3)_2$, 4-tBu, CN) was having comparable potency (0.125–64 μg/mL) with fluconazole (1–64 μg/mL) and itraconazole (0.125–1 μg/mL) [127]. A series of carboxylic acid esters of fluconazole showed higher activity than fluconazole against *C. albicans* (ATCC 14053) in SDB medium. The carboxylic acid esters of fluconazole having R=*O*-2-bromooctanoyl and *O*-11-bromoundecanoyl (**32**) (Fig. 5) have MIC values of 111 μg/mL and 198 μg/mL as compared to fluconazole that is having an MIC

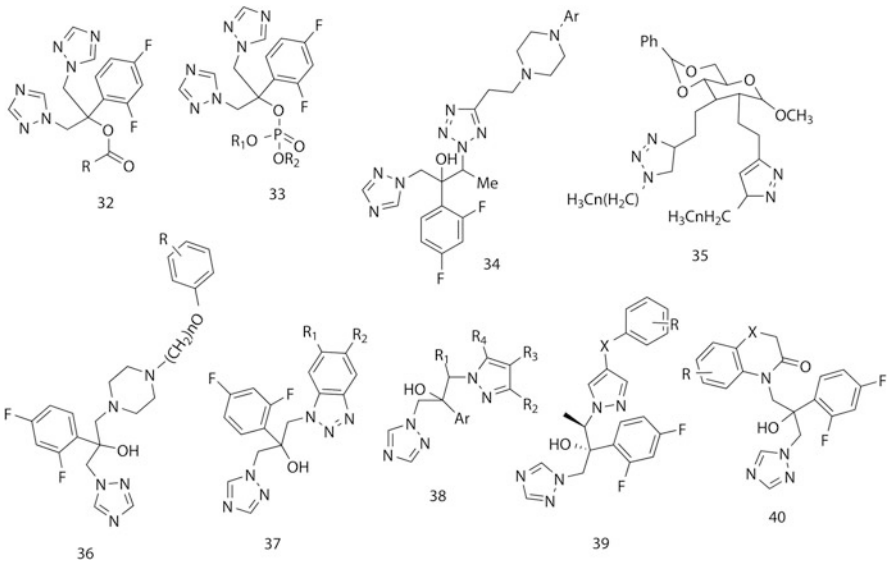

Fig. 5 Molecular structures having triazole moiety and fluconazole modifications

value greater than 4,444 μg/mL under similar bioassay conditions. Another series of fatty alcohol phosphate triester derivatives **33** has also been synthesized where compounds having R_1=$CNCH_2CH_2$: R_2=*n*-$C_{11}H_{23}$, R_1=$CNCH_2CH_2$: R_2=*n*-CH_2=CH–C_9H_{18}, R1=CH_3: R_2=*n*-$C_{11}H_{23}$, R_1=CH_3: R_2=*n*-CH_2=CH–C_9H_{18}, R_1=CH_3: R_2=*n*-C_8H_{17} have MIC values ranging from 12 to 1,658 μg/mL [128]. A series of triazole derivatives having 5-substituted tetrazole ring and having Ar =2-*n*BuOC_6H_6 attached to piperazine (**34**) is the most active with MIC values of 1.0–8.0 μg/mL, against *Candida* sp. [129]. In another series involving D-glucose derivatives of 1,2,3-Triazoles (**35**), chain length is important for antifungal activity with $n = 8$ having 14 times better activity than fluconazole with no activity when the chain length was increased to $n = 12$ [130]. Substituted 1,2,4-triazole and benzotriazole derivatives having phenoxypropyl piperazine side chains showed the linker length of three carbon atoms ($n = 3$) between piperazine and the phenyl ring to be crucial for antifungal activity (**36**). Compounds with R=H was having an MIC of 0.0156 μg/mL; however substitution at R by CH_3 (2,3,4 positions), 4-C$(CH_3)_3$, 4-Cl, 3-NO_2, 4-Br has good antifungal activity against *C. albicans* with MIC values ranging from 0.0156 to 0.25 μg/mL [131]. Benztriazole having no substitution (R_1=R_2=H) at 5, 6 positions was found to have an MIC value of 0.8 μg/mL while substitution at R_1=R_2=CH_3 and R_1=R_2=NO_2 was found to have same MIC value of 1.6 μg/mL in *C. albicans* (**37**) [129]. In the triazole series following the structural requirements in fluconazole a halogenated phenyl ring and tertiary alcoholic oxygen is preserved (**38**). In this series compounds having a phenyl ring with one halogen or trifluoro substituent were found to be active in *Candida* spp., *Aspergillus* spp., and *C. neoformans* with MIC ranging from 0.015 to 8 μg/mL. The most active compound in the series had an MIC $\leq$ 0.015 μg/mL in *C. parapsilopsis* while having good activity for *C. krusei* (MIC = 0.25 μg/mL) and *C. glabrata* (MIC = 1 μg/mL). A series of triazole molecules were synthesized where imidazole ring (A) was connected with variable spacer (X) to a substituted phenyl ring (**39**). The active compounds in the series were found to have X=C–C, C=C, C≡C, imidazolidine-2-one, 1*H*-imidazol-2(3*H*)-one, and R=4-Cl, 4-F with MIC_{80} ranging from 0.015 to 4 μg/mL in the *Candida* sp. (*C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. neoformans*). All the compounds have better activity in *C. albicans* with an MIC_{80} of $\leq$0.015 μg/mL as compared to fluconazole (MIC_{80} = 4 μg/mL) [132, 133]. A series of triazoles were synthesized where one triazole ring of fluconazole was modified into benzoxazinone (X=O, $n = 1$), benzothiazinone (X=S, $n = 1$), and benzoxazolinone (X=O; $n = 0$) moiety, with the most active compounds **40** having R=H, Cl and MIC = 0.06 μg/mL in *C. glabrata* [134].

6 Pyrimidine Analogue

5-Fluorocytosine or flucytosine (5-FC) (**41**) (Fig. 6), an antimetabolite, was first synthesized in 1957 and its antifungal property discovered in 1964 [135]. It is used for the treatment of invasive mycoses where it is effective against yeasts [136]. 5-FC is a fluorine analogue which inhibits nucleotide biosynthesis as it enters inside the fungal cells via cytosine permease and get deaminated to 5-fluorouracil (5-FU) by cytosine deaminase. 5-FU is a specific inhibitor of an enzyme essential for DNA synthesis namely thymidylate synthetase. This antifungal is selectively toxic to fungi as there is little or no cytosinedeaminase in mammalian cells [137]. The drug application is limited by the high prevalence of resistance in fungal species. Surveys conducted by Defever et al. and Stiller et al. [85, 137] on *C. albicans* estimated that 50–60% of the *Candida* isolates were susceptible, 30–40% were partially resistant along with 4–6% were highly resistant. 5-FC is administered in combination with other drugs such as fluconazole and amphotericin Bat present and rarely used as a sole agent. Resistance against 5-FC is developed due to mutational loss of permease activity. The resistance caused due to decreased uptake of 5-FC is prevalent in *C. glabrata* and *S. cerevisiae*, but this phenomenon is of least importance in case of *C. albicans* or *C. neoformans*. The mutational loss of the pyrimidine salvage enzymes forms the basis of resistance in laboratory or clinical strains of *C. neoformans* and *C. albicans* [138–140].

7 Allylamines

Allylamines form the newly developed class of ergosterol synthesis inhibitors. They are functionally and chemically very distinct from other classes of ergosterol binding antifungal agents [141] (Fig. 6). Allylamines inhibits the early steps of ergosterol biosynthesis leading to accumulation of squalene and absence of other

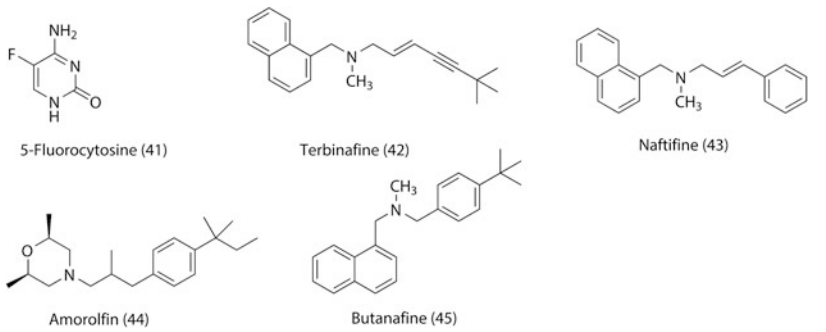

Fig. 6 Structure of pyrimidine and allylamine antifungal agents

sterol derivatives [142, 143]. Although clinical failures have been reported in treatment cases of allylamines yet human pathogenic fungi do not exhibit any associated resistance. Its resistance mechanism is poorly understood and further researches are required in this area. Important members of this group include naftifine and terbinafine.

7.1 Terbinafine

In Europe terbinafine (**42**) became available in 1991 whereas it got approval in the USA in 1996 [144]. Its hydrochloride salt is crystalline hydrophobic in nature but soluble in methanol, dichloromethane, and ethanol. This antifungal is mainly effective for dermatophytic fungi and used for superficial infections [145]. Terbinafineis recognized as inhibitor of fungal ergosterol biosynthesis by inhibiting squaleneepoxidase, an essential component of fungal cell. Fungal cell death is due to accumulation of squalene, which may increase permeability leading to disruption of cellular organization. Terbinafine hydrochloride may induce sub-acute cutaneous erythematous and people with this have been advised to know the possible risks with their physicians before the start of therapy [146].

A number of adverse drug reactions and side effects have been reported with oral terbinafine hydrochloride which may possibly due to longer duration of treatment and due to its extensive distribution in the body [144, 147].

7.2 Naftifine

Naftifine (**43**) is a synthetic, broad spectrum, allylamine antifungal agent which is used as a topical medication for the treatment of fungal infections. Naftifine hydrochloride is a white crystalline powder that is soluble in polar solvents such as ethanol and methylene chloride [148]. Naftifine hydrochloride, with potent in vitro antifungal activity against dermatophytes, was found to be effective against *tinea cruris*, *tinea corporis*, and *tinea pedis* as a topical agent [149]. It has shown very good activity against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Epidermophyton floccosum*, and *Microsporum canis*, *Microsporum audouini*, and *Microsporum gypseum*; and fungistatic activity against *Candida* species including *Candida albicans* [150, 151]. The mode of action of naftifine is not so clear but it seems to block the sterol biosynthesis via inhibition of the squalene 2,3-epoxidase enzyme [152]. This inhibition results in the accumulation of squalene, which is known to be toxic to fungi.

7.3 *Amorolfin*

Amorolfine (**44**) is a new topical water soluble antifungal drug of the morpholine derivatives. It inhibits D14 reductase and D 7-D8 isomerase which reduce ergosterol and accumulates in the fungal cytoplasmic membrane. This antifungal is used for the treatment of infections caused by dermatophyitic fungi and has been very effective in the treatment of onychomycosis [153, 154].

7.4 *Butenafine*

Butenafine (**45**) is a new synthetic benzylamine which has a broad spectrum of antifungal activity and used for the topical treatment of dermatophytoses caused fungi such as *Trichophyton mentagrophytes*, *Microsporum canis*, and *Trichophyton rubrum*. Its structure and mode of action are similar to allylamines as it inhibits sterol synthesis by blocking squalene epoxidation resulting in depletion of egosterol which is an essential lipid component of fungal cell membrane [147, 155]. The dermatophytes isolated from *Tinea cruris* have been found to be susceptible to both terbinafine and butenafine. The butenafine 1% cream has been found to exhibit supremacy over 1% terbinafine cream with statistically significant difference [156].

8 Indoles

Several compounds incorporating the indole moiety have also been reported as antifungal agents (Fig. 7). A series of 1*H*-Indole-4,7-diones derivatives have been synthesized by masking the indole nitrogen atom with CH_3 or with substituted phenyl groups (**46**, **47**). The compounds having substituted phenyl ring were active for *C. krusei*, *C. neoformans*, and *A. niger* with the most active compound having R_2=Cl (MIC = 0.8 μg/mL; *Candida krusei*) and a methyl ester attached to 3-position of the indole ring in **47**. A series of 5,6-bis(arylthio)-1*H*-indole-4,7-diones (**48**) showed moderate activity with an MIC range of 1.6–100 μg/mL with the most active compound (MIC = 1.6 μg/mL) having R_1=Cl, R_2=H for *Candida tropicalis*. The other substitutions such as R_1=CH_3, H and R_2=H, Cl, Br, I, OCH_3, CH_3; R_1=H, CH_3, F, Cl and R_2=H, Cl, Br, F, OH in all the 1*H*-Indole-4,7-dione series had potent antifungal activity with MICs ranging from 0.8 to 100 μg/mL [157]. The aminoguanidine derivatives of *N*-arylsulfonyl-3-acylindoles indicated that incorporation of electron donating groups at R_1 and R_2 improve antifungal activity. Variations were also made regarding the length of alkyl chain at R_3 (methyl, ethyl, propyl) (**49**). The compounds with R_1=4-Me, R_2=H, R_3=Me (*P. oryzae* = 79.64%, *A. alternata* = 79.15%, *B. sorokinianum* = 82.28%) and

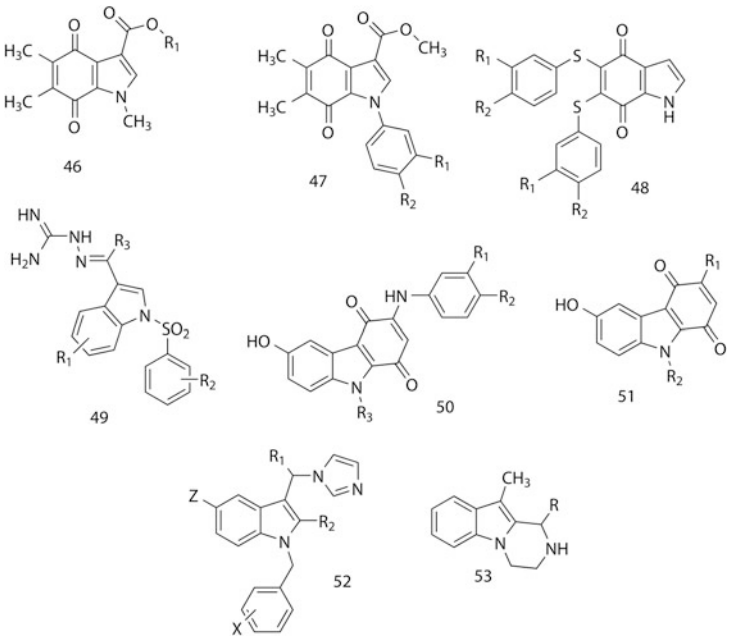

Fig. 7 Structure of indole antifungals

R_1=R_2=4-Me, R_3=Me (*P. oryzae* = 84.84%, *A. alternata* = 82.98%, *B. sorokinianum* = 80.58%) had good antifungal activity [158].

A series of compounds having indole fused with benzoquinone moiety having substitutions R_1=H, OH, F; R_2=CH_3O, H, CH_3, Br, Cl, I, F, OH, R_3=C_2H_5, CH_3, n-Pr (**50**) had potent antifungal activity with MIC 6.3–100 μg/mL in the *Candida* and *Aspergillus* sp. [159]. In another series of indole (**51**) substitution at R_1=CH_3CH_2S, H; R_2=C_2H_5, CH_3, *n*-Pr resulted in compounds with MIC of 1.6–100 μg/mL [159]. A series of 1-benzyl-3-(imidazol-1-ylmethyl)indole derivatives (**52**) showed that compound having Z=H, R_1=H, R_2=CH, and X=4-Cl to be the most potent in the series with an MIC of 1 μg/mL against *C. albicans* (CA980001). Compounds having Z=H/H/H/H/H (substitution for five compounds C1/C2/C3/C4/C5 at position Z), R_1=CH_3/H/i-propyl/H/*n*-butyl, R_2=H/H/H/H/H and X=4-Cl/4-F/4-Cl/2,4-diCl/4-Cl have MIC values of 3, 4,5,5,3.5 μg/mL respectively for the *C. albicans*. However none of these compounds are better than fluconazole (MIC = 0.02 μg/mL). Most of these compounds were less potent for *A. fumigatus* (AF980003) with the best compound (MIC = 8 μg/mL) having Z=Br, R_1=H, R_2=H, and X=2-Cl and 16 times less active than itraconazole [160]. Compounds having substituted-10-methyl-1,2,3,4-tetrahydro-pyrazino[1,2-a]indoles structure (**53**) with R=4-ClC_6H_4 was the most potent in the series having MIC values of 31.25, 15.62, and 31.25 μg/mL against *A. niger*, *A. fumigatus*, and *A. flavus*, respectively [161].

9 Quinolines

In a quinoline series (Fig. 8) compounds **54** having nitro substitutions at 5 and 7 positions of the quinoline ring and hydroxyl group at the 8 position had less antifungal activity ($MIC_{80} = 1.95$ μmol/L) compared to fluconazole ($MIC_{80} = 0.06$ μmol/L) against *C. albicans*. Two other compounds **55** and **56** had similar activity ($MIC_{80} = 1.95$ μmol/L) in *C. albicans*, the former had the quinoline ring substituted at position 8 by OH group and at position 2 by *N*-phenylethanimine moiety having 4′-OH substituent at the phenyl ring and the latter had same substitution at the quinoline ring (8-OH group) but a saturated linker with a methoxy group attached to the carbon next to the amine group with a phenyl ring having 2,5 diCl and 4-NO_2 substitution [162]. In another series of quinoline derivatives compounds having substitution at the 2-position by γ-pyridyl ring and at the C4 (R_1) and/or C8 (R_2) by methyl or isopropyl groups were found to be active. Substitutions at the same position by α-Furyl or α-thienyl group yielded inactive compounds (**57**). However some compounds having the γ-pyridyl ring were devoid of antifungal activity that indicated the importance of substituents at the C4/C8 position to be important for antifungal activity. The most active compounds in the series had C4=methyl and C8=methyl, isopropyl with an MIC value of 12.5 μg/mL [163]. The derivatives of norfloxacin (1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazinyl) quinoline-3-carboxylic acid) having R=3-(2,4-dichlorophenyl)propyl-2-en-1-one and 2-(2-methoxyphenoxy)ethyl-1-one (**58**) were found to inhibit the growth of *R. solani* by 83% and 94% at a concentration of 200 mg/L that is comparable to carbendazim (**59**) (100% inhibition under similar bioassay conditions) [164]. A series of 5-methyl benzothieno[3,2-b] quinolinium compounds were synthesized where two compounds having R=3-

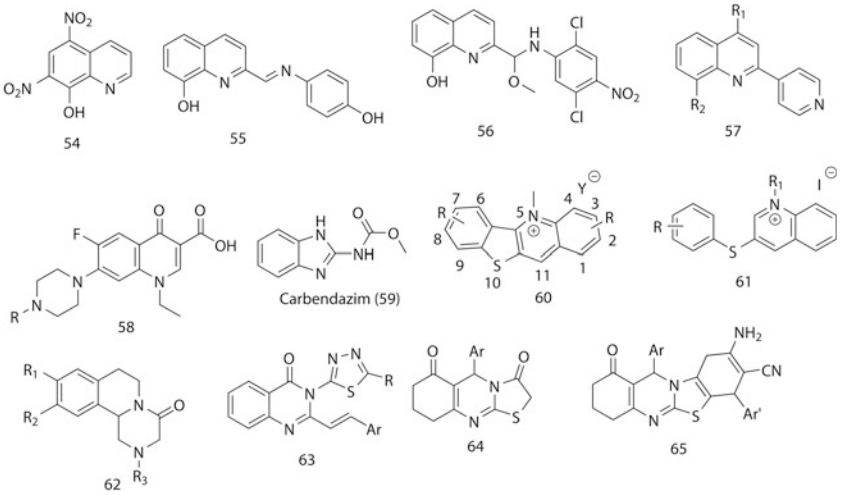

Fig. 8 Structure of quinoline, quinazoline, quinazolinone, and isoquinoline antifungals

OMe, 4-Cl and for both Y=OTf (**60**) [*C. neoformans* (IC_{50} = 6 μg/mL), *C. albicans* (IC_{50} = 1.5 μg/mL), *A. fumigatus* (IC_{50} = 0.4 μg/mL)] and R=4-Cl, Y=OTf [*C. neoformans* (IC_{50} = 4 μg/mL), *C. albicans* (not determined), *A. fumigatus* (IC_{50} = 6 μg/mL)] were observed to be active [165]. The seco analog (**61**) of the benzothienoquinoline (**60**) resulted in *N*-methyl-3-phenylthio-quinolinium salt. In this series the most active compound having R=H and R_1=5-cyclohexylpentyl group was found to be active in *C. neoformans* (IC_{50} = 0.5 μg/mL), *C. albicans* (IC_{50} = 2.7 μg/mL), *A. fumigatus* (IC_{50} = 8.6 μg/mL), *C. krusei* (IC_{50} = 0.7 μg/mL) [165]. In the isoquinoline analog hexahydro-4*H*-pyrazino[2,1-a]isoquinolin-4-one series the most potent compound had better activity than fluconazole (2–64 μg/mL) with R_1=H, R_2=F, R_3=$(CH_2)_8CH_3$ in **62** and an MIC range of 4–16 μg/mL against different fungal species such as *T. rubrum*, *C. neoformans*, *M. gypseum*, and *A. fumigatus* [166].

10 Quinazolines

In the quinazoline class (Fig. 8) the most potent compound (**63**) had R=*m*-ClC_6H_4 and Ar=*p*-$CH_3C_6H_4$ group having MIC values of 13.70, 17.07, 16.62 μg/mL against *A. nigers*, *C. albicans*, and *F. oxysporum*, respectively. At the same bioassay condition clotrimazole had slightly better activity (*A. nigers* = 12.98 μg/mL, *C. albicans* = 6.21 μg/mL, and *F. oxysporum* = 10.78 μg/mL) than the most potent compound in the series [167]. In the quinazoline class of compounds, the compound **64** (Ar=*p*-FC_6H_4) and **65** (Ar=*p*-FC_6H_4, Ar′=*p*-ClC_6H_4) showed less antifungal activity than Ticonazole (trosyd) [168].

11 Napthalenes

In a series of naphthalene derivatives (Fig. 9) compounds having R=7 or 8-NO_2 group at the naphthalene ring of **66** with X=S, Se showed better or comparable activity (MIC = 0.53–25 μg/mL) than fluconazole (MIC = 25 μg/mL) on *S. cerevisiae*. Better antifungal activity was also observed in *S. cerevisiae* (MIC = 3.12 μg/mL) when the NO_2 group was replaced by R=7-SO_2NH_2 and X=S, Se. One of the analogs of **66** having X=S and 7-SO_2NH_2 substitution was also active (MIC = 0.53 μg/mL) towards *C. neoformans* like fluconazole (MIC = 0.53 μg/mL) [169]. The butenafine derivative (**67**) with R=CH_3 (MIC = 0.125 μg/mL) had comparable activity to butenafine (MIC = 0.125 μg/mL) in *C. neoformans*. The terbinafine derivative (**68**) with R=CH_3 retained antifungal activity (MIC = 0.5 μg/mL) towards *C. neoformans* comparable to Butenafine (MIC = 0.25 μg/mL); however R=CH_2F, CHF_2, CF_3, and CN resulted in less active compounds [170].

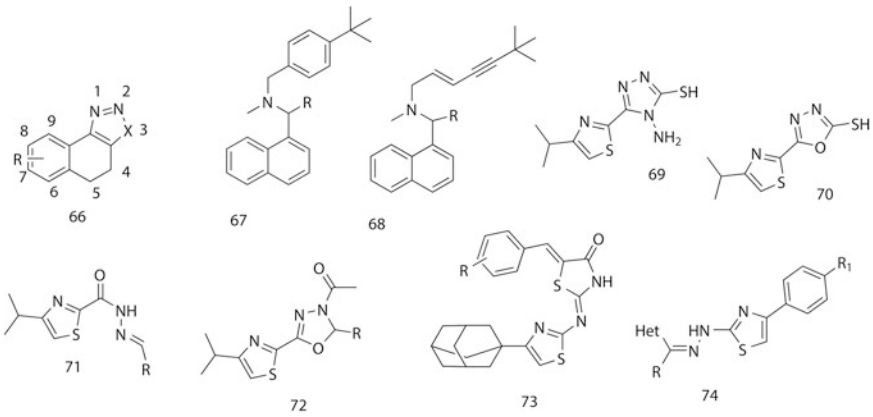

Fig. 9 Structure of naphthalene and thiazole antifungals

12 Thiazoles

In a series of thiazole derivatives (Fig. 9) compound having the structure **69** had an MIC of 8 μg/mL in *C. tropicalis* and in *A. niger*. The compound was also active in *S. cerevisiae* with MIC of 16 μg/mL. Another compound with structure (**70**) was also active in *C. tropicalis*. Compounds having the general structure of **71** with R=4-OH–C_6H_4 was active (MIC = 16 μg/mL) in *C. tropicalis* while R=2,3-di-ClC_6H_5 was active in *S. cerevisiae* with MIC of 16 μg/mL. Compounds with general structure **72** having R=C_6H_5, 3,4,5 -$(OCH_3)_3$–C_6H_2, 4-OH–C_6H_4, 2,3-diCl–C_6H_3 showed good activity with MIC ranging from 16 to 31.25 μg/mL in *S. cerevisiae*, *C. tropicalis*, and *A. niger* [171]. A series incorporating thiazole, thiazolidinone, and adamantine structures were synthesized where all the compounds were more potent than ketoconazole and bifonazole (**73**) under same biological assay condition. The various substituents at R=2-Cl, 3-Cl, 4-Cl, 2-NO_2, 3-NO_2, 4-NO_2, 4-OH, (4-OH and 3-OCH_3), (4-OH and 3,5-OCH_3) and 4-OCH_3 of **73** were having MIC in the range of 0.52–2.38 μg/mL in different fungal species (*P. funiculosum*, *P. ochrochloron*, *T. viride*, *A. fumigatus*, *A. niger*, *A. flavus*, *A. versicolor*, *F. fulvum*) [172, 173]. A series of [4-(4′-substituted-phenyl)thiazol-2-yl]hydrazine derivatives (**74**) showed better activity in *C. glabrata* and *C. albicans* with MIC values within 0.125–16 μg/mL. Under same assay conditions clotrimazole was found to have MIC values in the range of 2–8 μg/mL in both *C. glabrata* and *C. albicans* while fluconazole antifungal activity (MIC) varied from 4 to 16 μg/mL in *C. glabrata* and 4–64 μg/mL in *C. albicans*. The most active compounds for *C. albicans* (MIC = 0.125 μg/mL) had Het=Thiophen-2-yl, Pyridin-3-yl, Pyridin-4-yl, Benzodioxol-5-yl, Indol-3-yl, Coumarin-3-yl, R=H, CH_3 and R1=CH_3, OCH_3 [174].

13 Echinocandins

Echinocandins (**75**) (Fig. 10) are the most recent antifungals available for use. Echinocandins are water soluble, large hetrodimeric amphipathic polypeptides. This antifungal drug inhibit 1,3- β-D-glucansynthetase, resulting in damage of the cell wall of fungi, cell lysis, and cell death and are also called as "penicillin of antifungals" [175, 176]. Echinocandins are poorly absorbed through oral route; therefore they are administered intravenously to cure the localized and systemic fungal infections. It has a broad range of activity against all *Candida* species, also used in empirically in febrile neutropenia and stem cell transplant. At present medically used echinocandins like caspofungin, micafungin, and anidulafungin are semisynthetic derivatives with clinical use due to their solubility, antifungal spectrum, and pharmacokinetic properties [177].

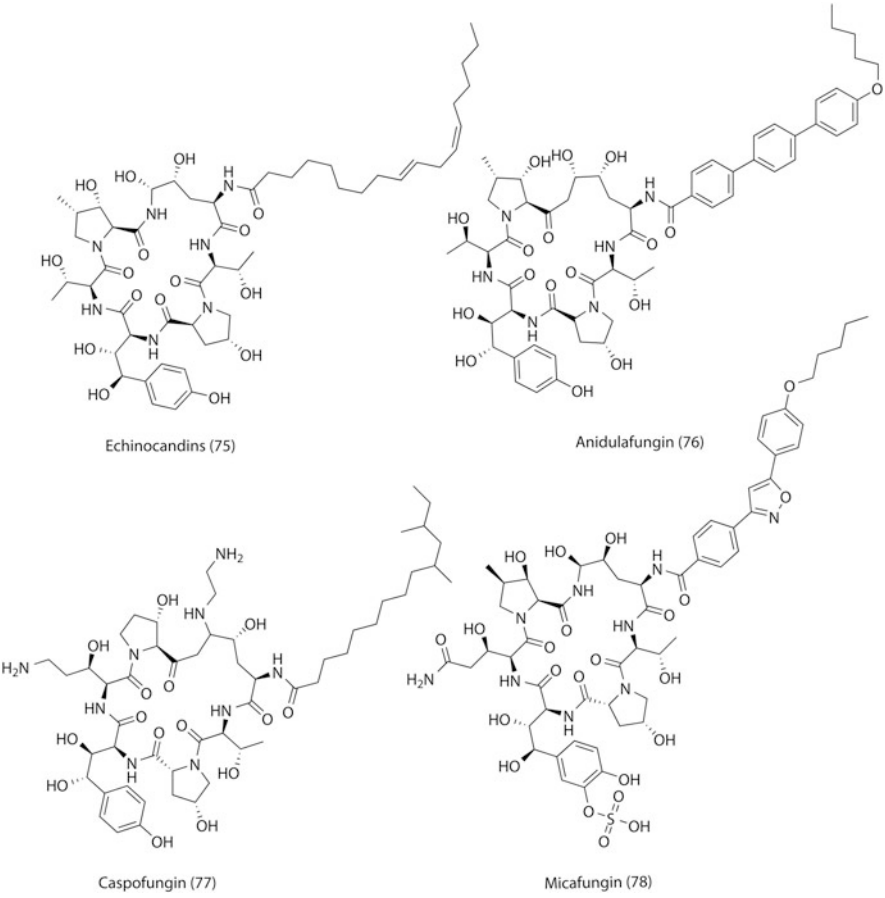

Fig. 10 Structure of echinocandin antifungals

13.1 Anidulafungin

Anidulafungin (**76**) is a semisynthetic lipopeptide antifungal approved by Food and Drug Administration. It was buildup by Eli Lily under clinical development at Vicuron Pharmaceuticals. It is the fermented product of the mold *Aspergillus nidulans*. Anidulafungin is used for the treatment of the persons who have high risk for serious fungal infections include patients with organ transplantation or hematopoietic stem cell transplantation, HIV infection/AIDS, malignancies, high-dose steroid therapy, and invasive A*spergillus* infections [178]. It inhibits β-1,3-D-glucan synthase as glucan is a major structural component of the cell wall of pathogenic fungi, resulting in cell death.

13.2 Caspofungin

Caspofungin (**77**) is a semi-synthetic water soluble lipopeptide antifungal drug which belongs to member of echinocandins. Caspofunginis is a fermented product of the fungus *Glareal-ozoyensis*. Caspofungin is administered intravenously and it inhibits the synthesis of component beta-(1,3)-D-glucan of fungal cell wall [179]. It is used for the treatment of fungal infections such as *Candida* infection (intra-abdominal abscesses, pleural cavity, perotonotis infections and esophagitis) and invasive aspergillosis [180].

13.3 Micafungin

Micafungin (**78**) is an echinocandin antifungal agent which was approved by FDA in March 2008. Micafungin is administered through intravenous route. Beta-(1,3)-D-glucanan is an essential component of fungal cell wall and the production of which is inhibited by micafungin. This drug is used in the treatment of infections caused by *Candida* sp. [181].

14 Miscellaneous

Diverse structural classes of compounds have been evaluated for antifungal activity. A series of benzoxazole derivatives (**79**) (Fig. 11) with fluorine substitution at different position of the phenyl ring were synthesized. All these compounds were synthesized as isosteric analogues of benzoheterocyclic-*N*-myristoyltransferase inhibitors. The most potent compound against *C. tropicalis* ($MIC_{80} = 0.0625$ μg/mL) had R=2-F substitution on the phenyl ring with better antifungal activity than

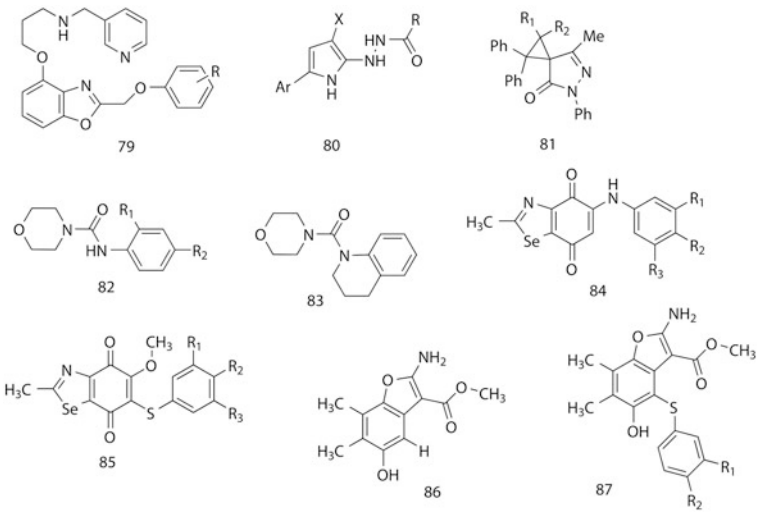

Fig. 11 Structure of different miscellaneous antifungals

fluconazole (*C. tropicalis*: $MIC_{80} = 4$ μg/mL) while another compound having R=2,3,4-trifluoro substitution in the phenyl ring had equipotent activity ($MIC_{80} = 0.25$ μg/mL) in *C. albicans*, *C. parasilosis*, and *C. tropicalis*. The compound (R=2,3,4-trifluoro substituted phenyl ring) had equivalent activity like fluconazole ($MIC_{80} = 0.25$ μg/mL) against *C. albicans* and better activity than fluconazole in *C. parasilosis* ($MIC_{80} = 4$ μg/mL) and *C. tropicalis* ($MIC_{80} = 4$ μg/mL) [182]. In a series of 2-Acylhydrazino-5-arylpyrroles (**80**) the most active compound with X=CN, Ar=4-OMePh and R=Et as substituent had an MIC of 0.39 μg/mL in *C. albicans* that is equipotent to amphotericin B (MIC = 0.39 μg/mL) and better than fluconazole (MIC = 0.78 μg/mL) under similar bioassay condition. The compound also showed good activity in other fungal species [*C. glabrata* (MIC = 0.78 μg/mL), *C. parapsilosis* (MIC = 0.78 μg/mL), *C. krusei* (MIC = 0.78 μg/mL)]. Substitution with R=iPr, 4-OMeBz when X is $-COOC_2H_5$ decreases activity drastically (MIC >100 μg/mL); however with X=CN fungal activity for R=iPr improved to great extent (MIC = 3.12 μg/mL) as observed against *C. albicans*. Hence the CN group is vital for antifungal activity [183]. A series of antifungal compounds having spiro[cyclopropane-1,4′-pyrazol-3-one] as the basic structural moiety (**81**) with R_1=H, CH_3 and R_2=CO_2Me, CO_2Et, CO_2iPr, CO_2tBt, CN, $CONEt_2$ had weak antifungal activity (MIC = 25 μg/mL) in *C. albicans* as compared to miconazole and itraconazole (MIC = 2 μg/mL) [184]. In a series of *N*-alkyl substituted urea derivatives two compounds having R1=F, R_2=H and R_1=H, R_2=F had MIC values of 3.1 and 3.5 μg/mL against *T. rubrum*as compared to ketoconazole (MIC = 3.9 μg/mL) on the same species (**82**). None of the compounds in the series are better than ketoconazole for *A. niger* (MIC = 7.8 μg/mL) except an analogue having the structure **83** had an MIC = 12.5 μg/mL [185]. A series of 5-Arylamino- and 6-arylthio-4,7-

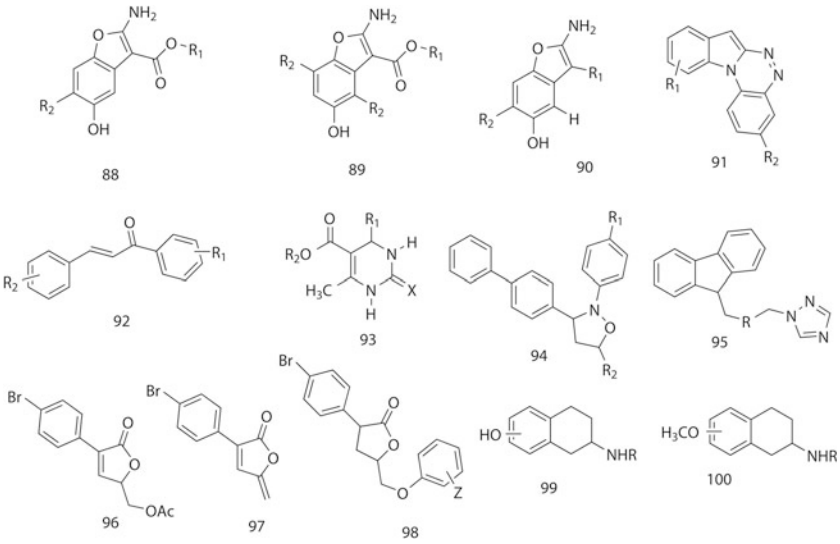

Fig. 12 Structure of different chemical class of antifungals

dioxobenzoselenazoles were synthesized where the best compound with R_1=R_3=Cl, R_2=H (**84**) had an MIC = 1.6 μg/mL better than 5-Fluorocytosine (MIC = 12.5 μg/mL) in *C. albicans*. The compound had MIC value of 3.2 μg/mL in *C. tropicalis* (5-Fluorocytosine : MIC = 12.5 μg/mL). The other active compounds in the series (**85**) had MIC values of 3.2 μg/mL (R_1=R_3=H, R_2=NO_2), 6.3 μg/mL (R_1=R_2=R_3=H), 6.3 μg/mL (R_1=R_3=H, R_2=F), 6.3 μg/mL (R_1=R_3=H, R_2=CH_3), 6.3 μg/mL (R_1=R_2=R_3=H) against *C. albicans*. The activity of other compounds in the series varied from 6.3 to 50 μg/mL in *C. tropicalis*, *C. krusei*, *A. niger*, and *A. flavus* [186]. A series of benzofuran compounds (**86–90**) (Figs. 11 and 12) with different substitutions R_1=H, CH_3, C_2H_5, CN and R_2=H, CH_3, Cl on the phenyl ring have good antifungal activity in *C. albicans*, *C. tropicalis*, *C. Krusei*, *A. niger*, *A. flavus*, and *C. neoformans* (MIC = 1.6–50-μg/mL). The two best compounds against *C. albicans* with R_1=CH_3, R_2=H (**89**) (Fig. 12) and R_1=C_2H_5, R_2=H (**89**) were equipotent (MIC = 1.6 μg/mL) and was better than 5-Fluorocytosine (MIC = 6.3 μg/mL) and fluconazole (MIC = 50 μg/mL). These two compounds were also active in *C. tropicalis* and *A. niger* with both having MIC of 3.2 μg/mL in the two fungal species [187]. In the benzotriazine series the most active compound having R_1=H, R_2=H (**91**) was more potent than hymexazol [188]. In the chalcones (**92**) compounds with R_1=H, 4-Br and R_2=H, 4-NO_2, 2-NO_2 had good antifungal activity with the potent compounds having electron withdrawing substituents at the para position of the phenyl ring [189].

In the pyrimidinone series three compounds having substitutions as R_1=C_6H_5, 4-$Me_2NC_6H_5$, 4-$Me_2NC_6H_5$ and R_2=C_2H_5 and X=S, S, O (**93**) had MIC = 0.35 μg/mL against *A. niger*. Another compound having R_1=2-HOC_6H_4, R_2=C_2H_5, X=O prevents the radical growth of *T. koningii* after 24 and 48 h completely (100%)

[190]. The isoxazolidine derivatives (**94**) having R_1=OCH_3, F and R_2=C_6H_5, $COOC_2H_5$, CH_2OH had MIC values ranging from 2.5 to 3 mM in *A. flavus* that is comparable to nystatin (3 mM) [191]. In the carbazole series introduction of azole (imidazole or 1,2,4-triazole) ring increased activity with better antifungal activity (2–4 μg/mL) for R=C_4H_8, C_2H_4 in (**95**) [192]. In the 3,5-disubstituted furanones series compounds with structure (**96**) and (**97**) had equal MIC values of 0.49 μmol/L in *C. albicans*. Other compounds in the series having Z=4-OCH_3, 4-I, 3-Br and 4-$COOCH_3$ had MIC values of 0.97, 0.48, 0.97, and 0.48 μmol/L in *C. albicans*. Modification in the phenyl ring with Z=3-COOH, 4-COOH, 4-OH (**98**) also resulted in active compounds against *C. albicans* with MIC values of 0.48, 0.97, and 0.97 μmol/L, respectively. Amphotericin B and fluconazole had MIC values of 0.03 and 1 μmol/L in the same assay system for *C. albicans* [193]. In the 2-amino tetraline series compounds with R=$(CH_2)_9CH_3$ had better antifungal activity with the two potent compounds having 5-OH and 5-OCH_3 substitutions in the phenyl ring having equal MIC values of 0.3125 μmol/L against *C. albicans* (**99**). Another compound having R=$(CH_2)_8CH_3$ and 6-OCH_3 was active (MIC = 0.0625 μmol/L) in *C. albicans* strain resistant to fluconazole (MIC >64 μmol/L) (**100**) [194].

15 Cationic Peptides

The cationic peptides are small cationic and amphipathic molecules isolated from plants, mammals, and microorganisms with antifungal activity with great potential for development as new therapeutic agents [195]. Cecropins isolated from the hemolymph of the giant silk moth (*Hyalophora cecropia*) is constituted by 35–37 residues with a strongly basic N-terminal linked to a neutral C-terminal by a flexible glycine–proline link. Both *Hyalophora* and *Drosophila* Cecropin (Cecropin A and B) inhibited growth of *S. cerevisiae*, *D. uninucleata*, *G. candidum*, and *M. anisopliae* in MICs ranging from 0.4 to 4 mM [196]. The LD_{50} value of Cecropin was also evaluated on germinating and non-germinating *A. flavus*, *A. fumigatus*, *A. niger*, *F. moniliforme*, and *F. oxysporum*. Cecropin B had LD_{50} values of 3.0, 0.5, 2, 0.2, and 1 μM in *A. flavus*, *A. fumigatus*, *A. niger*, *F. moniliforme*, and *F. oxysporum* respectively while for non-germinating *F. Moniliforme* and *F. oxysporum* the LD_{50} value was 0.2 μM for both species. Dermaseptin peptides found in skin secretions of Phyllomedusinae frogs reported in the same study had LD_{50} values of 4, 0.05, 2, 0.3, and 0.8 μM in *A. flavus*, *A. fumigatus*, *A. niger*, *F. moniliforme*, and *F. oxysporum* [197]. Indolicin, the shortest linearly occurring peptide consisting of 39% tryptophan and 23% proline (ILPWKWPWWPWRR), is found in the cytoplasmic granules of bovine neutrophil. Indolicin disrupt the structure of cell membranes as examined on interaction with *T. beigelii* [198]. Histatins are histidine rich peptides isolated from human saliva and had strong antifungal activity in different *Candida* spp. (C. *albicans*, *C. glabrata*, *C. guillermondii*, *C. krusei*, *C. lambica*, *C. parapsilosis*, *C. pseudo -tropicalis*, *C. stellatoidea*, and *C. tropicalis*) with histatin 5 showing the strongest

fungicidal activity against *C. albicans* (MIC = 100 μM) [199]. Magainins from *Xenopuslaevis* (the African frog) had antifungal activity against *Candida* spp., *C. neoformans*, and *Saccharomyces cerevisiae*. Magainin 2 acts as an antifungal against *C. neoformans* (MIC = 6.25 μg/mL), *C. glabrata* (MIC = 25.0 μg/ml), *C. tropicalis* (MIC, 12.5 μg/mL), and *C. krusei* (MIC = 12.5–25.0 μg/mL) with relatively low activity against *C. albicans* (MIC > 80 μg/mL) [200, 201]. Bombinin-H isolated from skin of *Bombina* genus are glycine rich peptides active against fungi, especially bombinin-like peptides-1 in *C. albicans* (MIC = 3–0.4-μM). Bombinins H2 and H4 also have antifungal activity against *C. albicans*, *C. guillermondii*, and *C. tropicalis*. Bombinin H2 had MIC values of 3.1, 1.3, 1.1 μM in C. *albicans*, *C. guillermondii*, and *C. tropicalis* respectively while Bombinin H4 had MIC values of 1.6, 0.7, and 0.6 μM for the above species [202, 203]. The antifungal activities of the amphibian cationic peptides have been reported elsewhere [204]. The cationic peptides bind to cholesterol and ergosterolin fungal cell membranes leading to fungal lysis [205]. Dolastatin 10, a synthetic cationic peptide, targeted at intracellular tubulin and inhibits microtubule assembly and tubulin-dependent GTP binding and have effective fungicidal activity against *C. neoformans* [206].

16 Monoclonal Antibodies

Since the fungi are eukaryotic organisms, a character shared with the host, it is difficult to develop a safe drug like antibacterials which are directed against prokaryotic organisms. In view of this an approach directed towards monoclonal antibodies against at least most common fungal pathogens like *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* is desirable. Identification and characterization of the proteins that are immunologically dominant and exhibit strong immune responses during mycoses could have vital repercussions for evolving new diagnostic, prophylactic, and therapeutic techniques for mycoses. Therefore efforts focused on the discovery of useful inhibitors of fungal specific, chitin, cell wall glucan and mannoprotein biosynthesis may play a very important role. In the absence of a safe and wide spectrum antimycotic agent, efforts may be directed for the development of monoclonal antibodies (MAbs). The MAbs have improved the specificity of immune procedures and have served as useful research methods and tools such as isolation, purification, and characterization of microbial antigens and development of assays methods for antibody and antigen detection [207–209]. In market there are many monoclonal antibodies available against a number of challenging diseases like cancer and many more diseases (Table 1). The antibodies are either developed in mouse which may be humanized, chimeric or in humans. The MAbs often exhibit adverse reactions like HAMA which is common for MAbs developed in mouse.

To overcome these types of side effects, an approach leading to the identification of active peptide sequences from the hypervariable regions of the hybridoma clone

Table 1 List of monoclonal antibodies approved for therapy

Antibody	Brand name	Approval date	Type	Indication
MuromonabCD3 [210]	Orthoclone OKT3	1986	Murine	Transplant rejection
Abciximab [211]	Reopro	1994	Chimeric	Cardiovascular disease
Daclizumab [212]	Zenapax	1997	Humanized	Transplant rejection
Rituximab [213]	Rituxan, Mabthera	1997	Chimeric	Non-Hodgkin lymphoma
Trastuzumab [214]	Herceptin	1998	Humanized	Brest cancer
Palivizumab [215]	Synagis	1998	Humanized	Respiratory syncytical virus
Infliximab [216]	Remicade	1998	Chimeric	Several autoimmune disorders
Basiliximab [217]	Simulect	1998	Chimeric	Transplant rejection
Gemtuzumab [218]	Mylotarg	2000	Humanized	Acute myelogenous leukemia
Alemtuzumab [219]	Campath	2001	Humanized	Chronic lymphocytic leukemia
Efalizumab [220]	Raptiva	2002	Humanized	Psoriasis
Adalimumab [221]	Humira	2002	Human	Several autoimmune disorders
Ibritumomab tiuxetan [222]	Zevalin	2002	Murine	Non-Hodgkin lymphoma (with yttrium-90 or indium-111)
Bevacizumab [223]	Avastin	2004	Humanized	Colorectal cancer, age-related macular degeneration
Cetuximab [224]	Erbitux	2004	Chimeric	Colorectal cancer, head and neck cancer
Omalizumab [225]	Xolair	2004	Humanized	Mainly allergy-related asthma
Natalizumab [226]	Tysabri	2006	Humanized	Multiple sclerosis and Crohn's disease
Panitumumab [227]	Vectibex	2006	Human	Colorectal cancer
Ranibizumab [228]	Lucentis	2006	Humanized	Macular degeneration
Eculizumab [229]	Soliris	2007	Humanized	Paroxysmal nocturnal hemoglobinuria
Certolizumab [230]	Cimzia	2008	Humanized	Crohn's disease
Ustekinumab [231]	Stelara	2009	Human	Psoriasis
Golimumab [232]	Simponi	2009	Human	Rheumatoid and psoriatic arthritis, ankylosing spondylitis
Canakinumab [233]	Ilaris	2009	Human	Muckle–Wells syndrome

(continued)

Table 1 (continued)

Antibody	Brand name	Approval date	Type	Indication
Ofatumumab [234]	Arzerra	2009	Human	Chronic lymphocytic leukemia
Tocilizumab [235]	RoActemra, Actemra	2010	Humanized	Rheumatoid arthritis
Denosumab [236]	Prolia	2010	Human	Bone loss
Ipilimumab [237]	Yervoy	2011	Human	Metastatic melanoma
Belimumab [238]	Benlysta	2011	Human	Systemic lupus erythematosus
Brentuximab				
Vedotin [239]	Adcetris	2011	Chimeric	Hodgkin lymphoma, systemic anaplastic large cell lymphoma
Pertuzumab [240]	Perjeta	2012	Humanized	Breast cancer
Adotrastuzumab Emtansine [241]	Kadcyla	2013	Humanized	Breast cancer
Obinutuzumab [242]	Gazyva	2013	Humanized	Chronic lymphocytic leukemia
Siltuximab [243]	Sylvant	2014	Chimeric	Castleman disease
Vedolizumab [244]	Entyvio	2014	Humanized	Ulcerative colitis, Crohn's disease
Ramucirumab [245]	Cyramza	2014	Human	Gastric cancer
Secukinumab [246]	Cosentyx	2015	Human	Psoriasis

may be helpful. This way a library of peptide sequences may be synthesized and evaluated for antifungal activity which may have specific activity against fungi. The peptide sequences thus generated may not only have specific antifungal activity but may also result in specific diagnostic tolls.

17 Conclusions

Fungal diseases are global health problem with rising prevalence of infections in immunocompromised hosts related to cases of cancer, AIDS, diabetes, cystic fibrosis and in invasive surgical procedures. The three major fungal diseases in immunocompromised subjects are candidosis, aspergillosis, and cryptococcosis. Azoles, the most common clinically antifungals among the other candidates (polyenes, pyrimidines, allylamines, and echinocandins), suffer from developing

resistance with drug–drug interactions and drug toxicity. This chapter presented the most common antifungals used for human health and also a brief update about the latest developments in antifungal agents.

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