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REVIEW

# Endophytes and associated marine derived fungi—ecological and chemical perspectives

**Abdessamad Debbab · Amal H. Aly · Peter Proksch**

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**Abstract** The potential of endophytes and algal or invertebrate associated marine derived fungi as promising sources of structurally unprecedented bioactive natural products is undeniable and continues to attract broad attention. This review highlights new bioactive fungal metabolites reported in 2011 until April 2012, as well as known compounds for which novel biological activities have been disclosed. All compounds are grouped according to their reported biological activities which include cytotoxic, anti-infective, as well as radical scavenging, enzyme inhibition, anti-fouling and anti-parasitic activities. Overall, 178 fungal metabolites, including 138 new natural products are presented. Furthermore, new insights into fungal-host interaction, chemical communication, and chemo-ecological roles of fungal metabolites, as well as new strategies for bioprospecting are presented.

**Keywords** Fungi · Endophytes · Associated marine fungi · Bioactive metabolites · Natural products · Biosynthetic pathways

## Introduction

Studies on fungal-host interactions in plant and animal systems aiming at improving our understanding of these associations and their impact on the environment are on the rise. Such host organisms have been long considered as autonomous regulated by their genetic code and cellular physiology, while in reality their internal tissues represent unique ecological niches for diverse communities of symbiotic microbes which often contribute in multiple ways to host fitness (Barrow et al. 2008). The potential of fungal-host interactions for advancing discovery in therapeutical and agricultural applications is continuing to gain recognition. Over the last decades, fungal endosymbionts emerged as a vast untapped reservoir of metabolic diversity yielding a significant number of interesting bioactive natural products that are of great pharmacological potential (Aly et al. 2010, 2011a,b; Debbab et al. 2010, 2011; Rateb and Ebel 2011; Blunt et al. 2012; Newman and Cragg 2012). On the other hand, the mutualistic interaction between host plants and endophytic fungi offers a tool for biological control of plant diseases which may improve crop yields and result in the production of novel defence compounds with potential as new agrochemicals of natural origin (Sikora et al. 2008).

Our basic understanding of fungal morphology, taxonomy and molecular profiles was for a long time derived from fungal strains which were successfully isolated and cultured on artificial media. Yet, advanced techniques including extraction and amplification of fungal DNA from colonized host tissues followed by DGGE, light and electron microscopy combined with the use of specific stains to selectively highlight fungal wall components (chitin) with minimal background staining of host tissue, chemical analysis, and molecular markers, allowed detection and quantification of complex microbial communities in host tissues, showing that 90–99 % of endosymbiotic fungi cannot survive under laboratory conditions (Amann et al. 1995; Gange et al. 1999; Maheshwari 2006; Selosse et al. 2004; Duong et al. 2006; Tao et al. 2008). This enormous diversity indicates that fungal endosymbionts still hold great promises as natural sources of drugs and drug leads.

In this review, we present new bioactive secondary metabolites or known compounds from endophytic and

A. Debbab (✉) · A. H. Aly (✉) · P. Proksch (✉)
Heinrich-Heine-Universität Düsseldorf,
Institut für Pharmazeutische Biologie und Biotechnologie,
Universitätsstr. 1, Geb. 26.23,
40225 Düsseldorf, Germany
e-mail: abdessamad.debbab@uni-duesseldorf.de
e-mail: amal.hassan@uni-duesseldorf.de
e-mail: proksch@uni-duesseldorf.de

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associated marine derived fungi for which hitherto undescribed bioactivities were reported from 2011 till early 2012. Emphasis is on endophytes isolated from higher plants including mangroves, as well as on fungi associated with marine algae or invertebrates. The review is a continuation of our earlier reports dealing with bioactive metabolites recovered from endophytes and marine derived fungi (Aly et al. 2010a,b; Debbab et al. 2011). All compounds are grouped according to their biological activities including cytotoxic, anti-infective, radical scavenging, enzyme inhibition, anti-fouling and anti-parasitic activities. In total 178 compounds, comprising 138 new natural products, are presented. In addition, new insights on fungal-host interaction, communication, and potential ecological roles recently published for endophytic and marine-derived fungi, as well as new strategies for manipulating biosynthetic genes and triggering the production of novel secondary metabolites by fungi are presented.

## Endophytic fungal-host interaction

Fungal associations with land plants date back from early evolutionary times. Examination of thin petrographic sections of a 400 million year old Rhynie chert plant, *Nothia aphylla*, showed the presence of three endophytic fungal species in root tissues (Krings et al. 2007). Like any form of symbiosis, fungal-host interactions are extremely variable with respect to their impact on both partners. In most cases the fungal partner exploits resources from the associated host through a parasitic or commensal interaction, whereas in mutualistic interactions the host is able to take advantage of the inhabiting fungus in return. It is believed that co-evolution of endophytes and their host plants influence natural products patterns of both partners, probably affecting endophyte-host communication and host adaptation to environmental challenges (Gunatilaka 2006).

Endophytic fungi have been found in every plant species examined to date, where they spend all or part of their life cycle residing asymptomatically within plant tissues (Saikkonen et al. 1998). These fungi may contribute to the overall performance of host plants by improving their fitness, photosynthetic efficiency, nutrient and water use, growth rate, reproductive success, or by acting as chemical defenses against herbivores, pathogens, or competitors (Schulz and Boyle 2005; Strobel 2006; Herre et al. 2007; Singh et al. 2011), by sharing genes and secondary metabolites that allow plants to tolerate abiotic or biotic stress and thus adapt to changing environmental conditions (Barrow et al. 2008; Singh et al. 2011). They may accordingly have a significant influence on plant biogeography, evolution, and community structure in terrestrial ecosystems (Rodríguez et al. 2009). Furthermore, transferring such endophytes from cell cultures of host plants to non-host plants would offer great promises as a revolutionary biotechnological approach to develop new and improved cultivars for potential agricultural applications (Barrow et al. 2008).

Several studies correlated improved plant tolerance to abiotic stresses upon pathogenic or mutualistic microbial infections with an observed increase in antioxidant or osmolyte concentrations and/or in antioxidant enzymes activities (Rouhier and Jacquot 2008). This may explain the development of systemic acquired resistance in plants following pathogenic infections where healthy plant parts gain more resistance to a subsequent infection by either the same or another microbe (Singh et al. 2011).

The root colonizing endophytic fungus *Piriformospora indica* was discovered in association with woody shrubs in the Indian Thar desert and was found to improve plant fitness of a variety of host plants by growth enhancement under normal and stress conditions (Verma et al. 1998; Schäfer et al. 2007). The fungus was reported to activate nitrate reductase and glucan-water dikinase enzymes resulting in increased nitrate acquisition and/or starch degradation in *Arabidopsis* and tobacco roots (Sherameti et al. 2005). Further studies indicated involvement of cytokinins in *P. indica* induced growth promotion of *Arabidopsis* plants, while auxins had little or no effect (Vadassery et al. 2008).

In addition to growth promotion, *P. indica*, originally isolated from desert plants, was found to induce drought stress tolerance of *Arabidopsis* and Chinese cabbage (*Brassica rapa*) by stimulation the expression of stress-related genes in leaves (Oelmüller et al. 2009; Sun et al. 2010). In Chinese cabbage colonized by *P. indica* the activities of peroxidases, catalases and superoxide dismutases in the leaves were increased within 24 h in response to drought stress. The fungus also increased the amount of chloroplast-localized $Ca^{2+}$ sensing receptor protein, which regulates stomatal function in response to elevations of external $Ca^{2+}$ by modulating cytoplasmic $Ca^{2+}$ concentration (Weinl et al. 2008; Sun et al. 2010). Furthermore, the drought induced decrease in photosynthetic efficiency and the degradation of chlorophylls and thylakoid proteins were delayed (Sun et al. 2010).

*P. indica* also induced salt tolerance to a salt-sensitive barley cultivar (*Hordeum vulgare*) by increasing the rate of metabolic activity to compensate salt-induced inhibition of leaf metabolism (Criddle et al. 1989; Baltruschat et al. 2008), by induction of antioxidant enzymes (Baltruschat et al. 2008), and by enhancing the ratio of reduced to oxidized ascorbate (Waller et al. 2005). The latter neutralizes oxygen

free radicals and acts as a primary substrate in the ascorbate-glutathione cycle to detoxify hydrogen peroxide (Foyer and Noctor 2000). It may also act by accelerating root elongation and increasing root biomass (Córdoba-Pedregosa et al. 2005). Furthermore, *P. indica* enhanced the biosynthesis of polyamines and lowered that of ethylene by increasing methionine synthase levels (Peškan-Berghöfer et al. 2004).

In vitro co-culture experiments demonstrated that endophytic fungi may inhibit the growth of phytopathogens (Yue et al. 2000; Arnold et al. 2003), as well as other coexisting endophytic fungi (Espinosa-García et al. 1993). Metabolites of the endophytic fungus *Muscodor yucatanensis*, isolated from the leaves of *Bursera simaruba* (Burseraceae) collected from a tropical forest in the Ecological Reserve El Eden, Quintana Roo, Mexico, were found to play a possible allelopathic role in its interaction with its host plant and other organisms. The compounds were found to inhibit the growth of other endophytic fungi as well as of important phytopathogens, and to reduce germination and root growth of dicotyledonous and monocotyledonous plants. These results suggested that mutualistic interactions of *M. yucatanensis* with its host plants may increase host defensive responses against pathogens and/or competitors to the host or to the fungus itself by the production of bioactive secondary metabolites (Macías-Rubalcava et al. 2010).

Endophytes were also reported to inhibit or prevent pathogen growth thus justifying their possible employment as biological control agents. Inoculation of endophytic *Chaetomium globosum* in wheat, and even solely applying its culture filtrate, reduced the severity of *Pyrenophora tritici-repentis* infections, which cause tan spot in wheat leaves. Infected host tissues accumulated extracellular proteins, yet the intercellular washing fluid of inoculated leaves showed no in vitro inhibition of the pathogen. These observations suggested an antagonistic effect of the endophyte or its secondary metabolites by activation of host defences rather than direct antagonism (Istifadah and McGee 2006).

In many cases enhanced pest resistance was correlated to the production of bioactive secondary metabolites by the endophytes or the host-endophyte association thus altering plant chemistry (Mei and Flinn 2010; Gange et al. 2012). Vertically transmitted endophytic fungi of the genus *Neotyphodium* are considered as useful insect biocontrol agents. In a recent study they were found to increase resistance of infected host grasses including perennial ryegrass, *Lolium perenne*, tall fescue, *Festuca arundinacea*, and meadow fescue, *Festuca pratensis*, against the corn flea beetle, *Chaetocnema pulicaria*. In addition to being an economically important pest of maize in the United States, this insect also feeds on many other cereal and grass species. The endophytes reduced feeding and survival of *C. pulicaria* by antixenosis rather than antibiosis, as indicated by preference and nonpreference feeding tests using a variety of grass-endophyte associations with variable alkaloid spectra showing varying effects according to host and endophyte species. Infected plants showed less feeding damage and lower fecal pellet numbers (Ball et al. 2011).

Consequently, colonisation by endophytic fungi may be considered as a promising environmentally-friendly tool to achieve improved plant germplasm for increasing sustainable and renewable crop production without the use of harmful chemicals. It is important to note that insects consume plant leaf material containing a variety of endophytic species in addition to secondary metabolites produced or induced by these fungi. Accordingly, interactions between these fungi within leaf tissues may affect secondary metabolite profiles and thus insect feeding and fitness (Gange et al. 2012).

## Marine-derived fungal-host interaction

Marine invertebrates such as sponges, ascidians and soft corals are well known to house numerous microorganisms within their tissues including fungi which may be detected directly by microscopy or indirectly by metagenomic surveys (Olson and Kellogg 2010). They were found to have physiological and ecological roles for the fungal-host consortium which comprise nutritional enhancement, stabilization of host skeleton, and secondary metabolite production. However, compared to terrestrial fungi, which were intensively investigated over the past decades, marine fungi still remain an underexplored group in the marine habitat and only very few reports can be found in the literature, which is in sharp contrast to their bacterial counterparts (Zhou et al. 2011b).

Sponge driven currents produced during filter feeding result in inhaling microorganisms from ambient seawater which mostly reside permanently in the sponge mesohyl if not phagocytised by the sponge (Thakur et al. 2004). In some cases such inhaled microbes may develop sponge-specific associations which can be maintained by vertical transmission (Taylor et al. 2007). It was reported that microorganisms may account for up to 40 % of sponge volume and greatly influence sponge biology, chemistry and evolution (Webster and Taylor 2012).

Being soft-bodied sessile organisms not able to move and lacking a hard outer protective shell, sponges are highly susceptible to marine predators. Hence it was concluded that sponges rely on chemical rather than on physical defence (Burns et al. 2003). Endosymbionts may contribute to sponge defence by ecological competition

with pathogens for space and nutrients, parasitizing or eradicating invading pathogens, altering host physiology to prevent invasion, and stimulating host innate immune system to rapidly respond to pathogens (Selvin et al. 2010). Sponge-associated fungi may have a potential role in the chemical defence of their hosts against pathogens, predators and foulers by the production of bioactive secondary metabolites, or by supplying precursors for the biosynthesis of defence metabolites by sponges, as well as defence enzymes such as extracellular phospholipases (Taylor et al. 2007; Selvin 2009; Ding et al. 2011). Furthermore, in a study investigating fungi associated with ten species of marine sponges in the South China Sea, polyketide synthase (PKS) or non-ribosomal peptide synthase (NRPS) genes were mainly detected in fungal isolates with antimicrobial activity against *Pseudomonas fluorescens*, *Staphylococcus aureus*, and *Bacillus subtilis*, suggesting their contribution to sponge host chemical defence processes by biosynthesizing antimicrobial compounds (Zhou et al. 2011a,b). Furthermore, endosymbionts may play a nutritional role for sponges by producing hydrolytic enzymes able to convert complex organic matter swirled into the host by filter feeding into easily accessible nutritional sources (Selvin et al. 2010). On the other hand, microbial symbionts presumably benefit from their sponge hosts which offer generous nutrient supply, as well as protection from predators or high levels of light within sponge tissues (Taylor et al. 2007).

It was suggested that disturbances in symbiosis due to environmental stress may affect sponge health, growth rates or resistance to predation, fouling and disease (Webster and Taylor 2012). Similarly, observed shifts in the composition of diverse and metabolically active endosymbionts inhabiting corals in response to environmental changes indicated their possible contribution to the ability of their hosts to adapt or acclimatize to climate changes or environmental stress (Reshef et al. 2006; van Oppen et al. 2009). This fact gains enormous interest considering currently observed rapid environmental changes and degradation of marine ecosystems (Webster and Taylor 2012).

## Fungal-host communication

Symbiotic microorganisms must have evolved to overcome or manipulate host defence systems in order to be able to establish a stable association with their hosts (Pieterse and Dicke 2007; Robert-Seilaniantz et al. 2007). The latter is assumed to be mediated by biochemical and/or genetic communication between symbionts and hosts, where a specific form of communication probably results in the expression of a symbiotic interaction under particular environmental factors (Singh et al. 2011). Examples include disturbing the defense signaling network of host plants, or reprogramming host metabolism by modifying hormonal homoeostasis and antioxidant contents (Robert-Seilaniantz et al. 2007; Göhre and Robatzek 2008).

Interestingly, most pathogens and mutualists share the same initial phases of infection and colonization (Rodriguez et al. 2004). Hence, plants probably differentiate between beneficial and harmful microbes by specific recognition and early signalling processes and consequently determine the kind of interaction expressed (Singh et al. 2011). The increase of intracellular calcium levels in plant cells, a second messenger in numerous plant signaling pathways, was found to be one of the early signalling events following infection. Potential pathogens activate plant defense responses through receptor-mediated cytoplasmic calcium elevation, which through a signal chain of events results in defense-related gene induction and phytoalexin accumulation by activation of ion fluxes at the plasma membrane ($H^+/Ca^{2+}$ influxes, $K^+/Cl^-$ effluxes), an oxidative burst and MAPK activation (Blume et al. 2000; Vadassery and Oelmüller 2009). This may only control or slow down pathogen spread, as pathogen-derived virulence molecules may suppress plant defense responses, thus allowing the pathogen to successfully invade the plant. Endophytic plant-fungus interactions lead to sequencial cytoplasmic and nuclear calcium elevations resulting in a better plant performance. Factors influencing the specificity of calcium response include calcium signature, amplitude, duration, frequency and location, selective activation of calcium channels in cellular membranes, and stimulation of calcium-dependent signalling components (Vadassery and Oelmüller 2009). Furthermore, individual fungal species are able to extend the symbiotic continuum by expressing either mutualistic or pathogenic interactions depending on host genotype (Redman et al. 2001). For example, *Colletotrichum gloeosporioides* was identified as a pathogen of strawberry, but as a mutualist in tomato plants (Redman et al. 2001; Rodriguez and Redman 2008).

On the other hand, molecular mechanisms involved in marine invertebrate-microbial associations were found to include selective receptor-ligand interactions through highly specific immunological cross-reactions by which the host permits the symbiotic microorganism to recognize its specific point of colonization and retains it there (Selvin et al. 2010). This recognition and maintenance of specific symbiotic microorganisms by the marine host are achieved by production of sponge lectins (Müller et al. 1981), surface glycans (McFall-Ngai 1994), or antibiosis where symbionts are able to adapt to antibiotics produced by the host (Foster et al. 2000).

Interestingly, pathogenic coral microbes were detected in apparently healthy sponge tissues of *Agelas tubulata* and *Amphimedon compressa* from Florida reefs (Negandhi et al. 2010). Similarly, *Aspergillus sydowii*, a pathogen of gorgonian sea fans, was isolated from healthy *Spongia obscura* collected in the Bahamas. This may indicate that, in analogy to endphytic fungi, marine-derived fungi are able to express mutualistic or pathogenic interactions depending on the colonized host. Alternatively, these disease-associated microbes may be opportunists, which infect only stressed coral tissues (Ein-Gil et al. 2009).

## Unravelling silent biosynthetic pathways

The production of numerous potentially valuable compounds by microorganisms occurs only under specific conditions and hence researchers often fail to detect them upon culturing the producing organism on standardized laboratory media. The reason may be a large metabolic background or unfavourable culture conditions. It may also be that corresponding biosynthesis genes for such "cryptic" or "orphan" pathways are not expressed in the laboratory, due to lack of signal molecules, or that encoded secondary metabolites have very low production rates and thus escape detection. It is evident that the biosynthetic potential of microorganisms is underinvestigated, as the number of genes encoding for natural products in many bacteria and fungi by far exceeds the known secondary metabolites produced from these organisms (Scherlach and Hertweck 2009; Cichewicz 2010; Brakhage and Schroeckh 2011).

Efforts to optimize secondary metabolite production by manipulating nutritional or environmental factors in many cases enhanced secondary metabolite biosynthesis leading to the discovery of new natural products. In this context, production of novel natural products was achieved by applying the "OSMAC" (One Strain MAny Compounds) approach, which is based on the modification of easily accessible cultivation parameters including media composition, aeration, temperature or shape of culturing flask (Grond et al. 2002; Bode et al. 2002). Similarly, endophytic *Paraphaeosphaeria quadriseptata* was triggered to produce six new metabolites by using distilled instead of tap water for preparing the medium (Paranagama et al. 2007). Application of stress conditions may also influence secondary metabolite biosynthesis in microorganisms. UV mutagenesis as well as addition of tricyclazole, an inhibitor of dihydroxynaphthalene biosynthesis, to spirobisnaphthalene-producing *Sphaeropsidales* sp. resulted in the discovery of the 14-membered macrolide mutolide, thus indicating a possible impact of enzyme inhibitors on natural product profiles (Bode et al. 2002).

It is assumed that interaction between organisms inhabiting the same or different species underlies the observed vast diversity of natural products. Thus, the same approach may be applied to the laboratory by performing mixed fermentation experiments (Scherlach and Hertweck 2009). Challenging marine-derived *Emericella* sp. with the marine actinomycete *Salinispora arenicola*, in co-culture, induced production of two new cyclic depsipeptides, emericellamides A and B (Oh et al. 2007). Similarly, the soil-dwelling bacterium *Streptomyces rapamycinicusthese* was found to specifically activate a previously unrecognized PKS cluster in *Aspergillus nidulans*, which encoded for the production of orsellinic acid, its derivative lecanoric acid, and the cathepsin K inhibitors F-9775A and F-9775B, by modification of fungal histones (Nützmann et al. 2011).

Chemical screening of extract libraries combined with genome sequencing studies represent a new powerful tool to predict chemical structures encoded by orphan genetic loci and hence may direct the search for new, relevant metabolites (Nguyen et al. 2008). While scanning *Aspergillus nidulans* genome sequence for putative biosynthesis genes three copies of genes encoding for proteins related to anthranilate synthase were detected. These enzymes catalyse the transformation of chorismate to anthranilic acid in tryptophane biosynthesis. Presence of multiple copies, however, indicated involvement in secondary metabolic pathways. As anthranilic acid is known as a precursor of quinazoline, quinoline and acridine alkaloids, *A. nidulans* was investigated for alkaloidal compounds leading to the discovery of four novel prenylated quinoline alkaloids, aspoquinolones A–D (Scherlach and Hertweck 2006).

A new strategy to trigger the biosynthesis of fungal natural products is based on the discovery that transcription of fungal genes is often controlled by epigenetic regulation such as histone deacetylation and DNA methylation. Histone modifications and DNA methylation communally operate to modify chromatin thereby regulating gene expression or silencing in fungi and other organisms. Thus, it is assumed that epigenetic modifiers may be applied for modulating secondary metabolite production (Scherlach and Hertweck 2009; Cichewicz 2010). Accordingly, twelve fungi were treated with DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors in a dose dilution series. Eleven strains were found to produce new or enhanced levels of secondary metabolites (Williams et al. 2008; Henrikson et al. 2009). Examples of commonly used DNMT inhibitors include 5-azacytidine and 5-aza-20-deoxycytidine, and the HDAC inhibitors hydroxamic-acid-containing compounds or cyclic peptides such as trichostatin A and trapoxin B, respectively (Cichewicz 2010). An increase in carotenoid production by *Neurospora crassa* cultures was achieved by addition of low doses of 5-azacytidine ($\leq 30\,\mu$M), whereas higher doses (100 and $300\,\mu$M) decreased carotenoid levels and altered reproductive structures (Kritsky et al. 2001). The same compound

triggered the biosynthesis of two new galactose-conjugated polyunsaturated polyketides in *Diatrype* sp. (Cichewicz 2010). Similarly, addition of 1 $\mu$M trichostatin A to *Alternaria alternata* and *Penicillium expansum* significantly increased the concentrations of numerous hitherto unidentified natural products (Shwab et al. 2007). Furthermore, addition of epigenetic modifiers to *A. niger* cultures resulted in increased transcriptional rates among most of its PKS, NRPS and hybrid PKS-NRPS (HPN) biosynthetic gene clusters, whereas less than 30 % of these gene clusters were transcribed when the organism was grown in absence of the modifiers (Fisch et al. 2009). In a further study implying molecular-based gene manipulation, deletion of *cclA* gene in *A. nidulans* resulted in a significant decrease in methylation of histone H3. Thus, this gene presumably encodes for a protein component of the Set1-containing COMPASS complex catalyzing methylation of histone H3. The *cclA* deletant was found to produce several silent secondary metabolites, including monodictyophenone, emodin and its derivatives, and to inhibit the growth of wild-type *A. nidulans*. 2-Hydroxyemodin, which exhibited significant anti-fungal and anti-bacterial activities, was assumed to mediate the inhibitory activity of the *cclA* deletant. Hence, it can be concluded that changes in chromatin levels are involved in the suppression or activation of biosynthetic gene clusters (Cichewicz 2010; Giles et al. 2011).

A more specific approach involves activation of pathway-specific regulatory genes encoding for presumed activator proteins. The detection of a putative hybrid PKS-NRPS gene in the genome of *A. nidulans* with no corresponding natural product indicated that this gene locus is silent under standard fermentation conditions. A presumed activator gene was also identified within the cluster, and its homologous overexpression under the control of an inducible promoter resulted in the activation of the biosynthetic pathway and the production of two novel pyridone alkaloids, aspyridones A and B, isolated after scale-up fermentation (Bergmann et al. 2007).

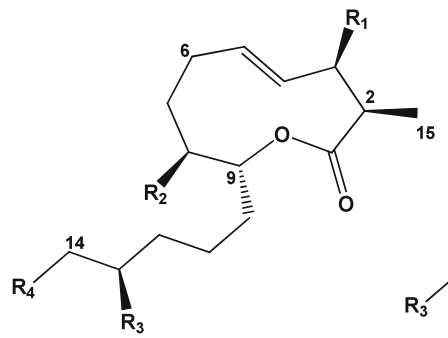
It is worth mentioning that since the discovery of the paclitaxel (taxol®) producing endophytic fungus *Taxomyces andreanae* from the yew plant *Taxus brevifolia* (Stierle et al. 1993), comprehensive examination of endophytic fungi isolated from a significant number of different terrestrial plants for the production of paclitaxel was conducted (Stierle et al. 1995; Soca-Chafre et al. 2011). Furthermore, genes encoding for taxadiene synthetase, which is responsible for the formation of the unique taxane-skeleton, as well as for phenylpropanoyl transferase, catalyzing the final acylation of the core structure for ultimate efficacy of the drug, were identified in *T. andreanae* (Staniek et al. 2009). Similarly, taxane biosynthetic genes were detected in endophytic *Phomopsis* sp. and *Cladosporium langeronii*, isolated from *Wollemia nobilis*, thus indicating the biosynthesis of paclitaxel to be an inherent genetic trait of endophytes (Staniek et al. 2010). Thus, it could be assumed that finding the "genetic on-switch" to enhance the expression of such clusters (Newman and Cragg 2012) may increase paclitaxel yields from its fungal producers and accordingly facilitate its industrial production.

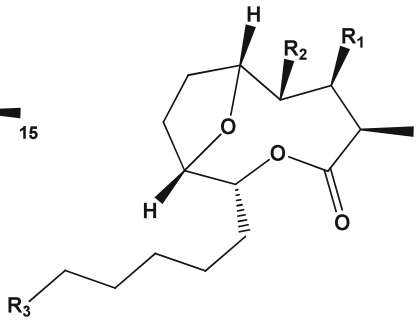
## Newly reported examples of bioactive compounds from endophytic and associated marine derived fungi

In this part we present an overview on natural products reported from endophytic and associated marine derived fungi during the period from 2011 until April 2012 that were selected based on their bioactivities. This overview is intended to be a continuation of our previous reviews covering this field (Aly et al. 2010, 2011a,b; Debbab et al. 2010, 2011).

### Cytotoxic secondary metabolites

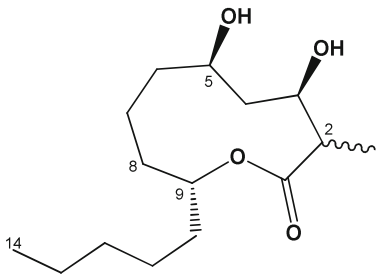
Twelve new cytospolides F–Q (**1–12**) and two decytospolides A and B (**13** and **14**), together with seven known metabolites, were isolated from *Cytospora* sp., an endophytic fungus of *Ilex canariensis* (Aquifoliaceae) an evergreen shrub from Gomera, Spain. The structures were elucidated by means of detailed spectroscopic analysis, chemical interconversion and X-ray single crystal diffraction. Furthermore, the absolute configuration of the isolated new products was assigned by modified Mosher's method as well as solution- and solid-state TDDFT ECD calculations. Cytospolides F−L (**1–7**) may biogenetically derive from precursor A (**CPA**) via dehydration involving the 5-OH group, followed by oxidation at C-8, C-13, and/or C-14. Further oxidation of OH-3 would result in the reactive $\alpha,\beta$-unsaturated ketone precursor B, the C-3 carbonyl and the $\Delta^{4,5}$ bond. The precursor B (**DPB**) leads to decytospolides A and B (**13** and **14**) by an initiation of a ring cleavage on the lactone function, followed by intramolecular oxa-Michael addition of 9-OH to the $\alpha,\beta$-unsaturated ketone and decarboxylation of the $\beta$-ketocarboxylic acid derivative. All compounds were tested for their cytotoxic activity against human tumor cell lines, including lung adenocarcinoma (A549), colon (HCT116), hepatocarcinoma (QGY), malignant melanoma (A375), and leukemic (U937) cells by the MTT method, using adriamycin as a positive control. Among the tested compounds, **11** showed the strongest activity against cell lines A-549, QGY, and U973 with $IC_{50}$ values of 6.25, 48.23 and 86.16 $\mu$M, respectively, whereas, **12** was selective and inhibited the growth of the cell line A-549 cell line with an $IC_{50}$ value of 36.89 $\mu$M (Lu et al. 2011).



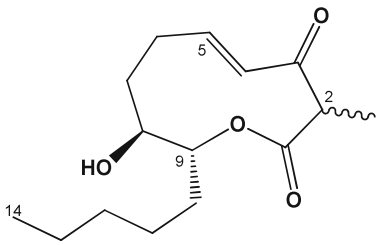




**1** $R_1 = R_2 = R_3 = OH, R_4 = H$
**2** $R_1 = OAc, R_2 = R_3 = OH, R_4 = H$
**3** $R_1 = R_3 = OAc, R_2 = OH, R_4 = H$
**4** $R_1 = R_2 = OH, R_3 = OAc, R_4 = H$
**5** $R_1 = R_2 = OH, R_3 = H, R_4 = OAc$
**6** $R_1 = R_3 = OH, R_2 = R_4 = H$
**7** $R_1 = OH, R_2 = R_4 = H, R_3 = OAc$

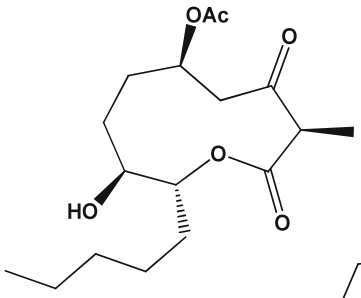
**8** $R_1 = R_2 = OH, R_3 = H$
**9** $R_1 = R_2 = OH, R_3 = OAc$
**10** $R_1 = =O, R_2 = R_3 = H$



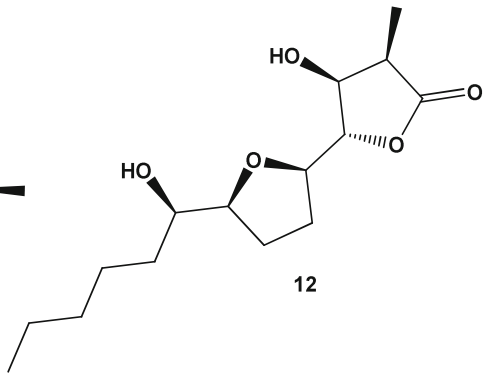

CPA



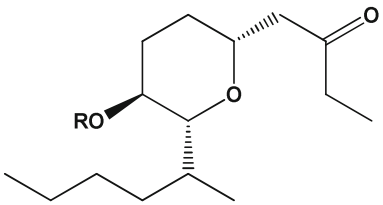

DPB




**11**



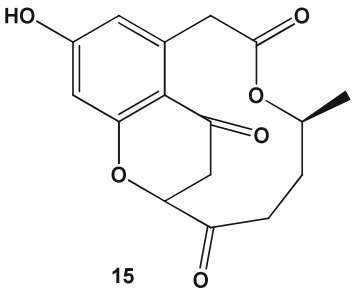

**12**




**13** R = H
**14** R = Ac

Chemical investigation of the endophytic fungus *Penicillium* sp. isolated from *Limonium tubiflorum* (Rutaceae) growing in Egypt afforded four new compounds of polyketide origin, including two macrolides named penilactone (**15**) and 10,11-epoxycurvularin (**16**), a dianthrone, neobulgarone G (**17**), and a sulfinylcoumarin, sulfimarin (**18**),

along with 12 known metabolites. The structures of all compounds were assigned by comprehensive spectral analysis (1D and 2D NMR) and mass spectrometry. Compounds **17**–**18** as well as the known **19**–**20** showed pronounced activity against *Trypanosoma brucei brucei* S427 with mean MIC values ranging from 4.96 to 9.75 $\mu$M. Moreover, when tested against three human tumor cell lines, including human erythromyeloblastoid leukemia (K562), human T cell leukemia (Jurkat) and human histiocytic lymphoma (U937) cells, **17**–**18** as well as the known **21**–**22** showed selective growth inhibition against Jurkat and U937 cell lines with $IC_{50}$ values ranging from 1.8 to 13.3 $\mu$M. Moreover, the compounds were examined for their effect on TNF$\alpha$-induced NF-$_K$B activity in K562 cells, using a luciferase reporter gene assay, to identify the mechanism of action. The obtained results indicated that **17**–**18**, **21** and **22** significantly reduced TNF$\alpha$-triggered NF-*k*B activation as expressed by their $IC_{50}$ values of 4.7, 10.1, 5.6, and 1.6 $\mu$M, respectively (Aly et al. 2011a,b).

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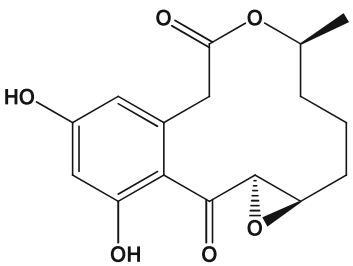


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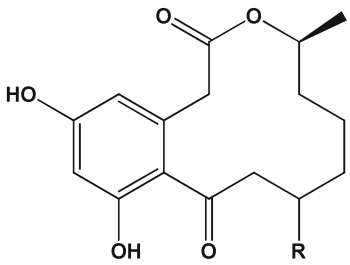
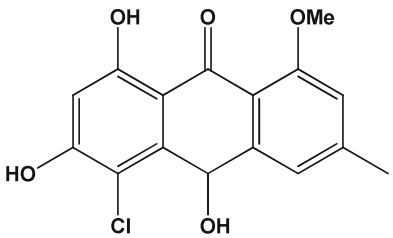



**17** R = $\beta$-OMe
**18** R = $\alpha$-OMe

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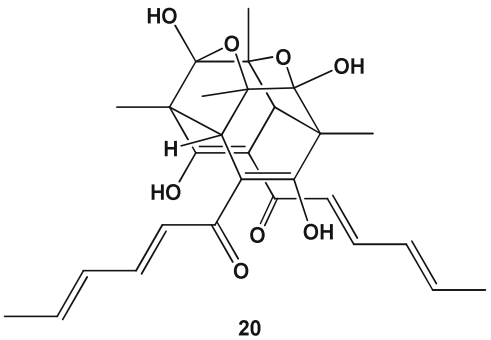


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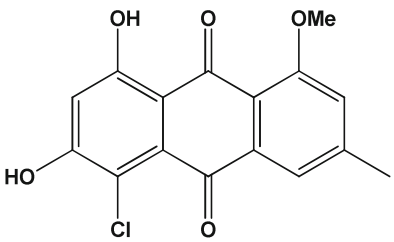


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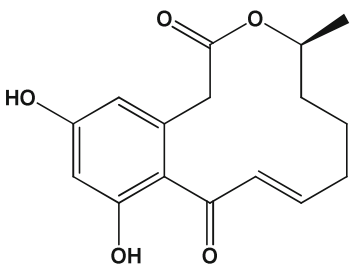
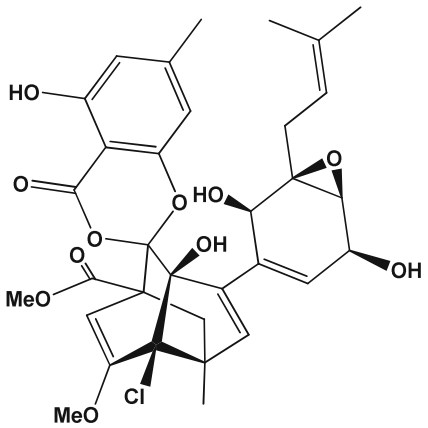



21

Liu et al. described three novel spiroketal derivatives, named chloropupukeanolides C–E (**23**–**25**), which are derived from chlorinated tricyclo-[4.3.1.03,7]-decane (pupukeanane) and 2,6-dihydroxy-4-methylbenzoic acid moieties, in addition of seven known products. All metabolites were isolated from the scale-up fermentation extract of *Pestalotiopsis fici*, an endophytic fungus of the branches of *Camellia sinensis* (Theaceae) collected in a suburb of Hangzhou, China. The structures of **23**–**25** were elucidated primarily by NMR measurements as well as mass spectrometry. Their relative configurations were deduced by comparison with those of the known metabolites chloropestolide A (Liu et al. 2009), chloropupukeanolides A and B (Liu et al. 2010), likewise isolated from the same fungus. The absolute configuration of **23** was assigned by X-ray crystallography and those of **24** and **25** by quantumchemical CD calculations. Biogenetically, chloropupukeanolides C-E (**23**–**25**) are presumably derived from the oxidation-induced Diels-Alder reaction pathway as the known chloropupukeananin (Liu et al. 2008), chloropestolide A, chloropupukeanolides A and B, and chloropupukeanone A (Liu et al. 2010), via the putative biosynthetic precursors iso-A82775C and pestheic acid (Liu et al. 2008). The new metabolites **23**–**25** were tested for their cytotoxicity against two human tumor cell lines including epithelial carcinoma (HeLa) and colon adenocarcinoma (HT29) cells. Compounds **23** and **24** showed significant cytotoxicity against both cell lines, with $IC_{50}$ values ranging from 1.2 to 7.9 $\mu$M, with a higher activity than the known positive control 5-fluorouracil, which gave $IC_{50}$ values of 10.0 and 15.0 $\mu$M (Liu et al. 2011).

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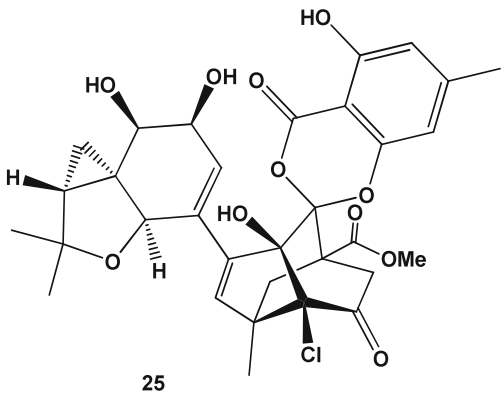
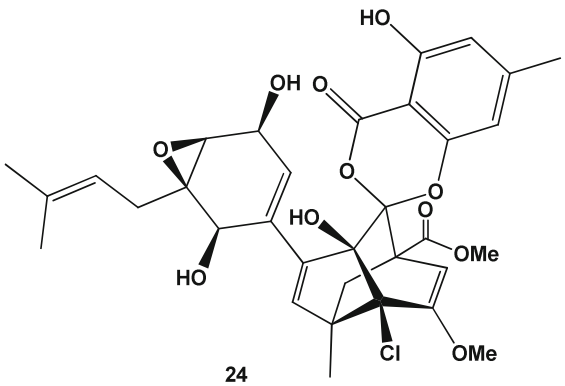


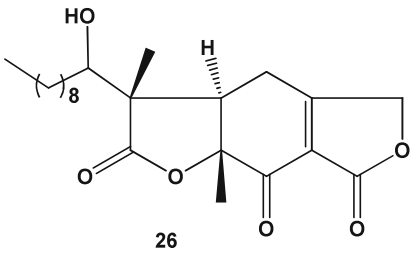

25




24

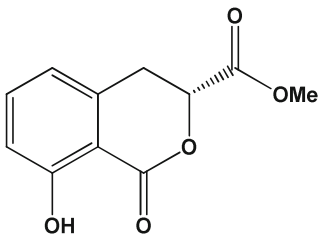
Annulosquamulin (**26**), a new dihydrobenzofuran-2,4-dione derivative, in addition to 10 known secondary metabolites, were isolated from the *n*-BuOH-soluble fraction of the endophytic fungus *Annulohypoxylon squamulosum* BCRC 34022, derived from the stem bark of the medicinal plant *Cinnamomum* sp. (Lauraceae) collected from Fu-Shan Botanical Garden, I-lan County, Taiwan. The structures of the isolated compounds were elucidated by means of 1D and 2D NMR spectroscopy and by HRESIMS. Annulosquamulin (**26**) comprises a dihydrobenzofuran-2,4-dione skeleton, a 1-hydroxydecyl side chain, and a $\gamma$-lactone ring. The relative configuration of **26** was deduced from inspection of NOESY spectra, comparison with similar compounds, as well as by the help of the molecular modeling program CS CHEM 3D Ultra 10.0, with MM2 force-field calculations for energy minimization. Furthermore, **26** was evaluated for its in vitro cytotoxicity against MCF-7 (human breast adenocarcinoma), NCIH460 (non-small-cell lung cancer) and SF-268 (glioblastoma) cells by the MTT method with actinomycin D as positive control. **26** possessed moderate

cytotoxicity against MCF-7, NCI-H460, and SF-268 cancer cell lines with $IC_{50}$ values of 8.4, 8.9 and 6.5 $\mu$M, respectively (Cheng et al. 2012).




26

Cultures of endophytic *Alternaria tenuissima* yielded a new isocoumarin, tenuissimasatin (**27**), together with 11 known compounds. The endophyte had been isolated from the bark of *Erythrophleum fordii* Oliver (Leguminosae), collected at Nanning, Guangxi Province, China. The new compounds as well as the known metabolites were identified by NMR spectroscopy and mass spectrometry. Furthermore, the absolute configuration of tenuissimasatin was obtained by CD calculation. All compounds were tested for their cytotoxic activities toward five human tumor cell lines, including intestinal epithelial (HCT-8), hepatoma (Bel-7402), gastric cancer (BGC-823), lung adenocarcinoma (A549) and ovarian cancer (A2780) cells. Among them, only the known product 7,8,9-hexahydroperylene (**28**) (Okuno et al. 1983) showed selective cytotoxic activity against HCT-8 cells ($IC_{50}$ 1.78 $\mu$M), while the other compounds were only weakly active ($IC_{50}$>10 $\mu$M) (Fang et al. 2012).

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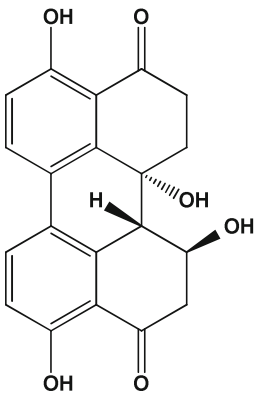
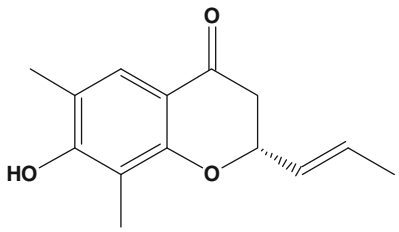



28

The fungus P-1 was isolated from healthy stem tissues of the plant *Huperzia serrata* (Lycopodiaceae), which was collected in Xishuangbanna Tropical Plant Garden, China. Chemical investigation of the chloroform extract yielded a new chromone derivative, (2S)-2,3-dihydro-7-hydroxy-6,8-dimethyl-2-[(E)-prop-1-enyl]-chroman-4-one (**29**) along with seven known metabolites. The structures of the isolated compounds were elucidated by spectroscopic methods, including extensive 2D NMR as well as mass spectrometry. Furthermore, the absolute configuration of **29** was obtained by CD spectroscopy. When tested in vitro against epithelial carcinoma (HeLa) and hepatocellular liver carcinoma (HepG2) human cancer cell lines, only the known metabolite sorbicillin (**30**) exhibited potent cytotoxic activity against HeLa cells ($IC_{50}$ 1.6 $\mu$M) and weak activity against HepG2 cells (27.2 $\mu$M). 2′,3′-Dihydrosorbicillin (**31**) showed moderate activity against HeLa cells ($IC_{50}$ 7.4 $\mu$M) and weak activity against HepG2 cells ($IC_{50}$ 44.4 $\mu$M) (Ying et al. 2011).

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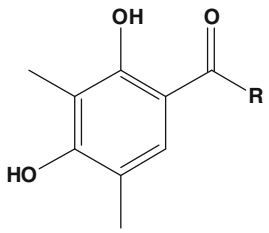
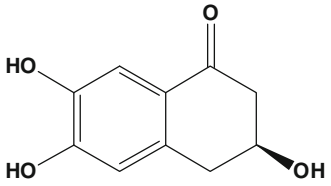



**30** R = -CH=CH-CH=CH-$CH_3$
**31** R = -$CH_2$-$CH_2$-CH=CH-$CH_3$

*Phoma* sp. ZJWCF006, isolated from healthy tubers of the medicinal plant *Arisaema erubescens* (Araceae), collected from Wencheng County of Zhejiang Province, China, was identified as a source of the new $\alpha$-tetralone derivative, (3*S*)-3,6,7-trihydroxy-$\alpha$-tetralone (**32**), together with three known congeners. **32** is a new member of the $\alpha$-tetralone class of metabolites and its absolute configuration was established by circular dichroism (CD) spectroscopy. When tested for cytotoxic activity, only the known cercosporamide (**33**) exhibited cytotoxic activity against six human tumor cell lines, including colon adenocarcinoma grade II (HT-29), hepatic carcinoma (SMMC-772), breast adenocarcinoma (MCF-7), promyelocytic leukemia (HL-60),

gastric carcinoma (MGC80-3), as well as murine leukemia (P388) cells, with $IC_{50}$ values of 9.3±2.8, 27.87±1.78, 48.79±2.56, 37.57±1.65, 27.83±0.48, and 30.37±0.28 $\mu$M, respectively (Wang et al. 2012a).

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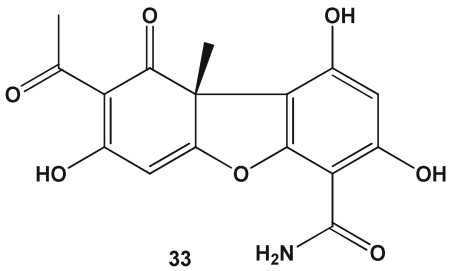
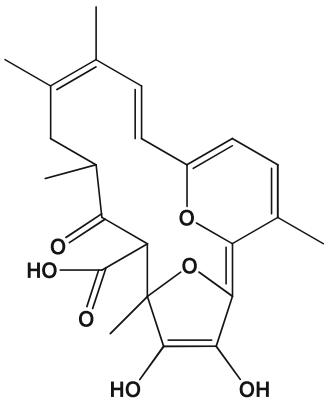


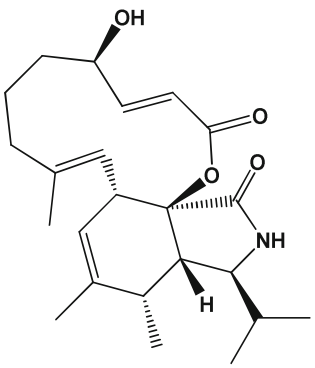

33

Cultures of endophytic *Chaetomium globosum* L18, isolated from fresh healthy leaves of *Curcuma wenyujin* (Zingiberaceae), collected in Zhejiang Province, Wenzhou, China, yielded a new metabolite named chaetoglobosin X (**34**). **34** showed similarities to chaetoglobosin A regarding its spectroscopic data (Ni et al. 2008). All compounds were evaluated for their anticancer activity against gastric cancer (MFC) and hepatic cancer (H22) murine cell lines. Chaetoglobosin X displayed the strongest cytotoxicity against H22 cells ($IC_{50}$ 7.5 $\mu$M) and moderate cytotoxicity against MFC cells ($IC_{50}$ 15.0 $\mu$M), whereas the other compounds were inactive against both cell lines (Wang et al. 2012a,b).




34

The extract of *Trichoderma gamsii*, isolated from the traditional Chinese medicinal plant *Panax notoginseng* (Araliaceae), displayed cytotoxic activity against human epithelial carcinoma (HeLa) cells. Chemical study of the ethyl acetate extract of this fungal strain, when fermented on slants of potato dextrose agar, afforded two new cytochalasans, including trichalasin A (**35**) and trichalasin B (**36**), in addition to several known derivatives. The structures of **35**–**36** were unambiguously elucidated based on extensive NMR spectroscopy and HRMS analysis. Their absolute configurations were tentatively assigned to be the same as those of the known derivatives aspochalasins I and J based on biogenetic considerations. Aspochalasin J (**37**) displayed weak inhibitory activity with an $IC_{50}$ value of 27.8 $\mu$M, when tested against HeLa cells, whereas the other compounds showed only moderate activity ($IC_{50}$>40 $\mu$M) (Ding et al. 2012).

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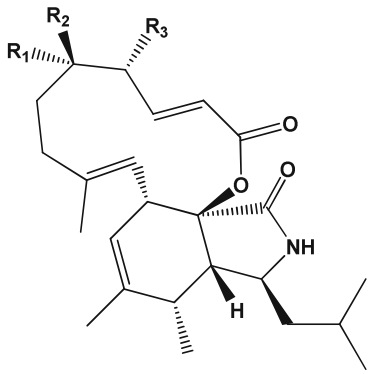
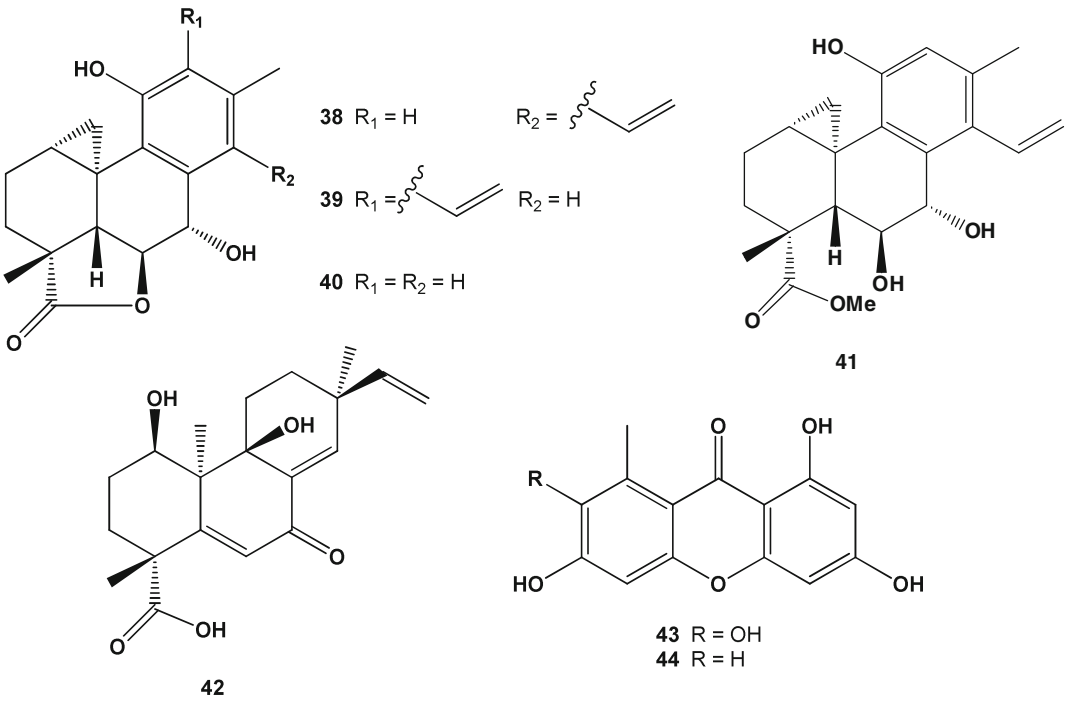


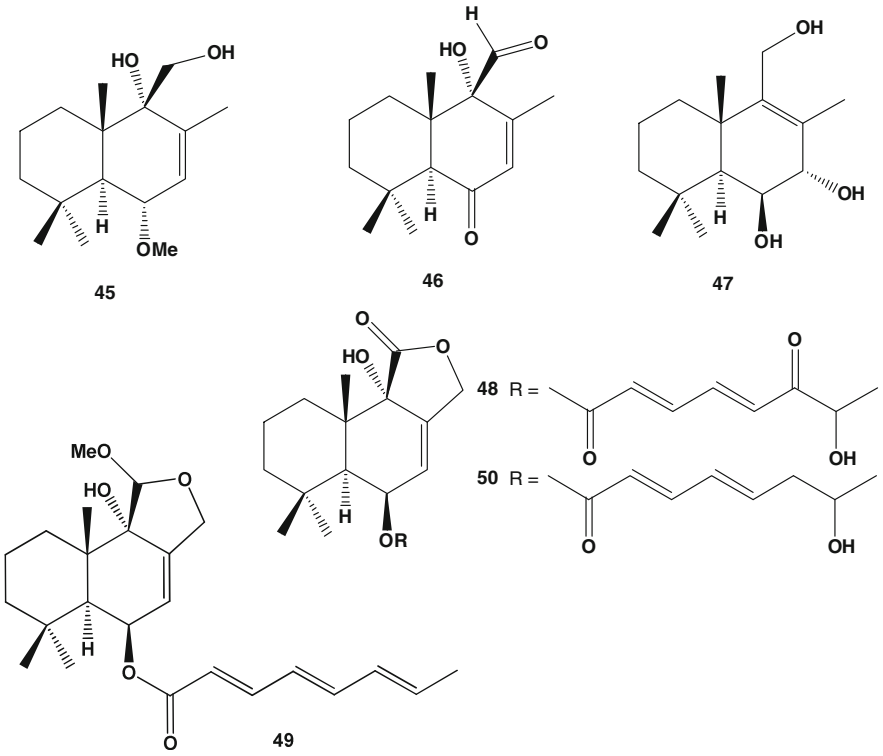

**36** $R_1 = R_3 = H$, $R_2 = OH$
**37** $R_1 = R_3 = OH$, $R_2 = H$

Bioassay-guided fractionation of a methanolic extract of the sponge derived fungus *Arthrinium* sp. afforded ten natural products including five new diterpenoids, arthrinins A-D (**38**–**41**) and myrocin D (**42**). The sponge

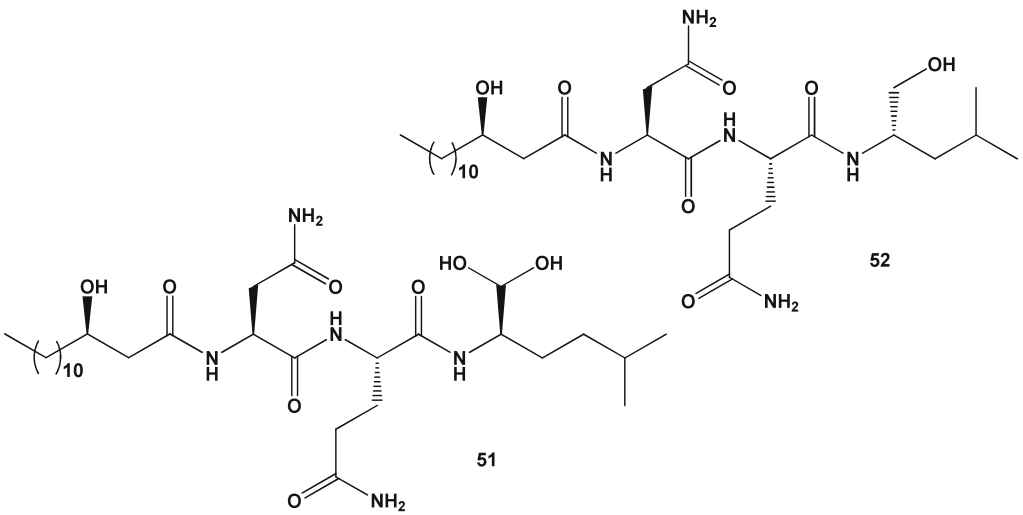
was collected from the Adriatic Sea near Italy and was identified as *Geodia cydonium* (Geodiidae). The structures of isolated metabolites were unambiguously elucidated based on extensive NMR and HR-MS analyses. Furthermore, the absolute configuration of arthrinins A–D (**38**–**41**) was established by interpretation of their ROESY spectra as well as by the convenient Mosher method performed in NMR tubes. Using the MTT assay, all isolated compounds were tested for their in vitro antiproliferative activity against four different tumor cell lines, including mouse lymphoma (L5178Y), human erythromyeloblastoid leukemia (K562), human ovarian cancer (A2780) and cisplatin-resistant ovarian cancer cells (A2780CisR). Among the tested compounds, only the known metabolite anomalin A (**43**) exhibited strong and selective activities with $IC_{50}$ values of 0.40, 4.34, and 26.0 $\mu$M against L5178Y, A2780, and A2780CisR tumor cell lines, respectively. However, it was not active against the K562 cell line. The isolated compounds were also tested against 16 protein kinases to identify possible mechanisms of action of the active metabolites. Both known compounds **43** and norlichexanthone (**44**) inhibited one or more of the tested kinases by at least 40 %, suggesting that inhibition of protein kinases may be one of the major mechanisms contributing to their antiproliferative activity (Ebada et al. 2011).



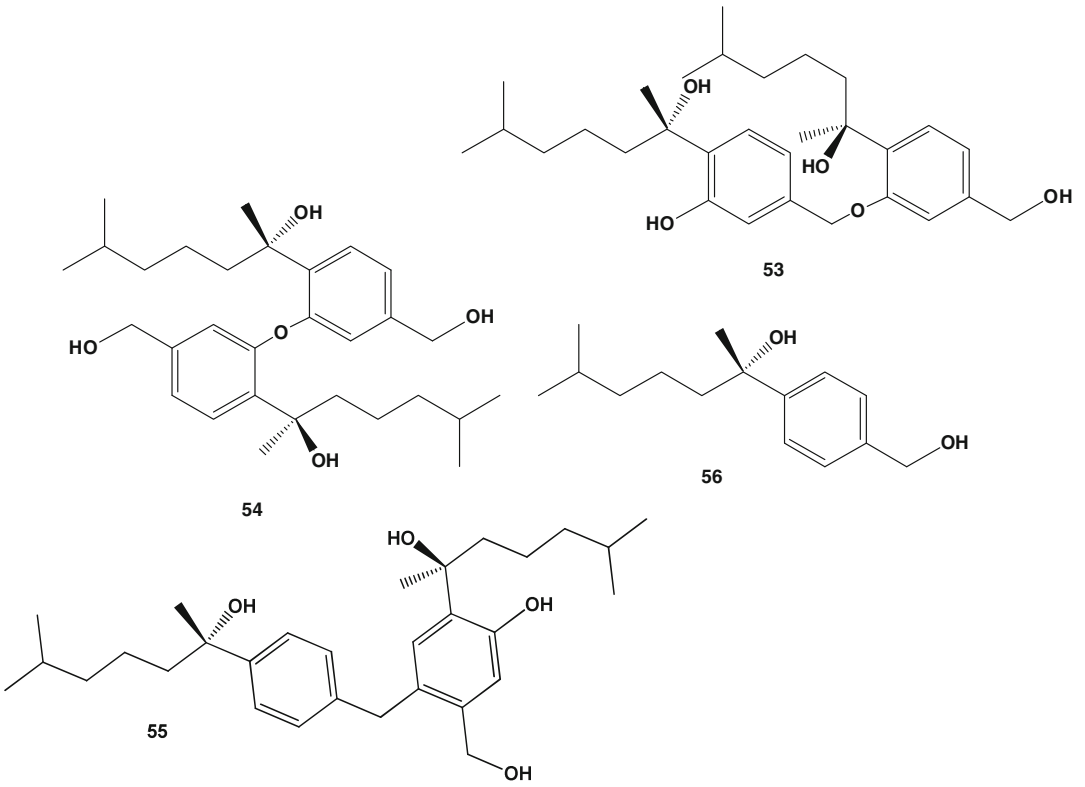

Cultures of *Aspergillus ustus*, isolated from the mangrove plant *Acrostichum aureum* (Pteridaceae) growing in Guangxi Province, China, yielded five new drimane sesquiterpenes (**45**–**49**) together with 14 known analogues. When tested for their cytotoxicities against murine leukemic (P388), human promyelocytic leukemia (HL-60), human erythromyeloblastoid leukemia (K562) and human hepatocellular carcinoma (BEL-7402) cells, only **48** exhibited moderate cytotoxicity against the P388 cell line with an $IC_{50}$ value of 8.7 $\mu$M, whereas the other compounds were inactive. Interestingly, the differences of cytotoxicities between **48** and the known congener **50** against the P388 cell line indicated that the carbonyl group at C-6′ is necessary for activity (Zhou et al. 2011a,b).



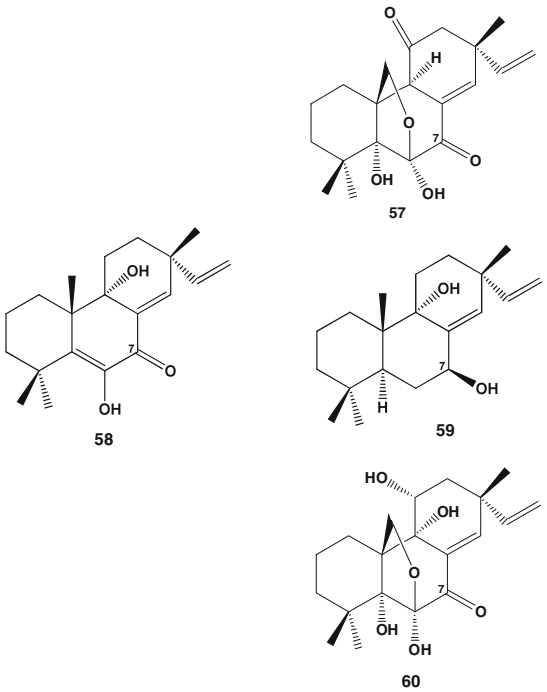

Lee et al. reported a new cytotoxic lipopeptide, fellutamide F (**51**), isolated from *Aspergillus versicolor*, together with three known derivatives. This fungal strain was isolated from the sponge *Petrosia* sp. (Petrosiidae) collected by hand at the coast of Jeju Island, Korea. Even though **51** differs from the known congener **52** only by replacement of the carbinol group by an acetal group, **51** showed strong cytotoxicity toward five human solid tumor cell lines, including lung cancer (A549), ovarian cancer (SK-OV-3), skin cancer (SK-MEL-2), CNS cancer (XF498) and colon cancer (HCT15) cells, with $IC_{50}$ values of 3.4, 2.3, 1.3, 0.3 and 0.2 $\mu$M, respectively. Interestingly, cytotoxic potencies of **51** against XF498 and HCT15 cells were comparable to those of the anticancer agent doxorubicin. In contrast, **52** showed lower potency with $IC_{50}$ values of 35.9, 25.9, 5.5, 4.2 and 3.4 $\mu$M, respectively (Lee et al. 2011).



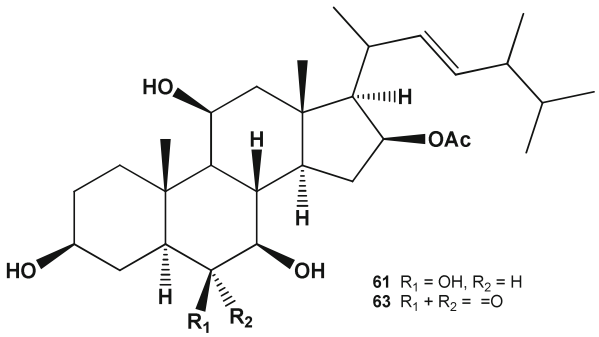
Cytotoxicity-guided fractionation of the EtOAc extract of the marine-derived fungus *Aspergillus* sp. afforded three new phenolic bisabolane sesquiterpenoid dimers, disydonols A-C (**53**–**55**). The fungal strain was isolated from the sponge *Xestospongia testudinaria* (Petrosiidae) collected from the South China Sea. When tested for their cytotoxic activity in vitro against human hepatoma (HepG-2) and human cervical (Caski) cells, compound **53** exhibited moderate in vitro cytotoxicity toward these two cell lines with $IC_{50}$ values of 19.2 and 25.5 $\mu$M. Compound **55** showed selective activity against these two cell lines with $IC_{50}$ values of 6.2 and 21.7 $\mu$M, respectively, whereas **54** was found to be inactive ($IC_{50}$>200 $\mu$M). From a biosynthetic perspective (Cichewicz et al. 2005), the absolute configuration of **53** was tentatively assigned based on co-occurrence with **55** and the known (*S*)-(+)-sydonol (**56**). This could explain the cytotoxicity results which showed that 7*S*, 7′*S* configuration resulted in increased activity (Sun et al. 2012).

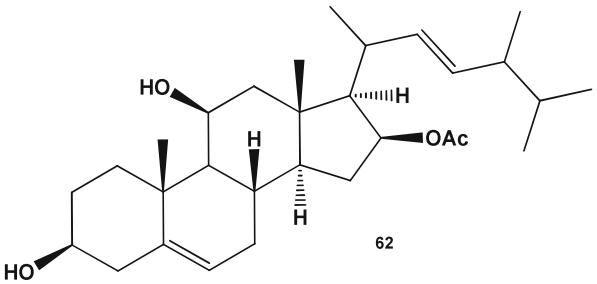



Three new pimarane diterpenes (**57**–**59**) as well as the known diaporthin B (**60**) were isolated from *Epicoccum* sp. HS-1, a marine-derived fungus of the sea cucumber *Apostichopus japonicas* (Stichopodidae). The structures of **57**–**59** were identified by NMR and MS, and their absolute configuration was obtained by comparison of their CD spectra with that of the known **60**. Compounds **57** and **60** exhibited relatively strong cytotoxic activities against human KB cell line with $IC_{50}$ values of 10.1 and 10.6 $\mu$M, and against KBv200 cells with $IC_{50}$ values of 6.8 and 17.9 $\mu$M, respectively. In contrast, **58** showed weaker activities against KB and KBv200 cells with $IC_{50}$ values of 65.6 and 45.8 $\mu$M, respectively, while the activity of **59** toward both cell lines was above 320 $\mu$M. This could be related to the presence of different substituents at C-7 (ketones in **57**, **58** and **60**; hydroxyl group in **59**) (Xia et al. 2012).



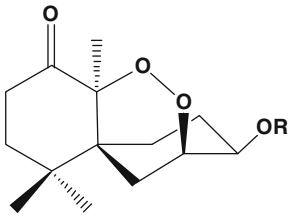

In addition to seven known steroid derivatives, Gao et al. reported two new polyoxygenated steroids, namely, penicisteroids A and B (**61**–**62**) from the culture of *Penicillium chrysogenum* QEN-24S, an endophytic fungus of an unidentified marine red algal species of the genus *Laurencia* (Rhodomelaceae). Compound **61** was the first steroid having tetrahydroxy and C-16-acetoxy groups. Its absolute configuration was assigned by application of the modified Mosher's method. All isolated compounds were tested for their cytotoxicity against seven tumor cell lines. Penicisteroide A (**61**) displayed selective activity against epithelial carcinoma (HeLa), pancreatic carcinoma (SW1990), and lung cancer (NCI-H460) cells with $IC_{50}$ values of 29.6, 61.2 and 79.1 $\mu$M, respectively, while the other compounds displayed only weak activity. Compound **61** was the strongest cytotoxic compound and it was the only steroid possessing a hydroxyl group at C-6 compared to **62** and **63**, a structural feature most likely responsible for its cytotoxic activity (Gao et al. 2011a).



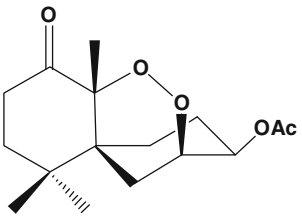




The fungus *Talaromyces flavus*, which was isolated from leaves of a mangrove plant *Sonneratia apetala* (Lythraceae), collected on the coastal saltmarsh of the South China Sea, afforded four new norsesquiterpene peroxides, talaperoxides

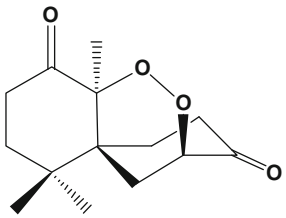
A-D (**64**–**67**), as well as one known analogue, steperoxide B (**68**). Their structures were elucidated mainly by 1D and 2D NMR as well as mass spectrometry. Furthermore, the absolute configurations of **64**, **65**, and **68** were obtained by single-crystal X-ray diffraction. All compounds were further evaluated for their cytotoxic activity against human cancer cell lines, including breast (MCF-7, MDA-MB-435), hepatoma (HepG2), cervical (HeLa), and prostatic (PC-3) cancer cells, using the MTT method. Compounds **65** and **67** showed cytotoxicity toward all tested cancer cell lines with $IC_{50}$ values between 2.8 and 9.4 $\mu$M. In particular, compound **67** showed promising growth inhibitory effects towards MDA-MB-435, HepG2, and PC-3 cells with $IC_{50}$ values of 3.6, 3.6 and 2.8 $\mu$M, respectively. Interestingly, when tested at a concentration of 50 $\mu$g/mL against several pathogenic microorganisms, such as *Staphylococcus aureus* (ATCC 27154), *Escherichia coli* (ATCC25922), *Sarcina ventriculi* (ATCC 29068), *Pseudomonas aeruginosa* (ATCC 25668), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 13496), none of the compounds showed inhibitory effects (Li et al. 2011a).




**64** R = Ac
**68** R = H




**65**

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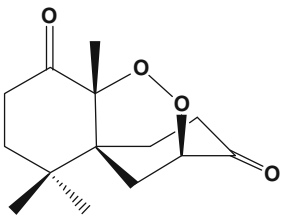
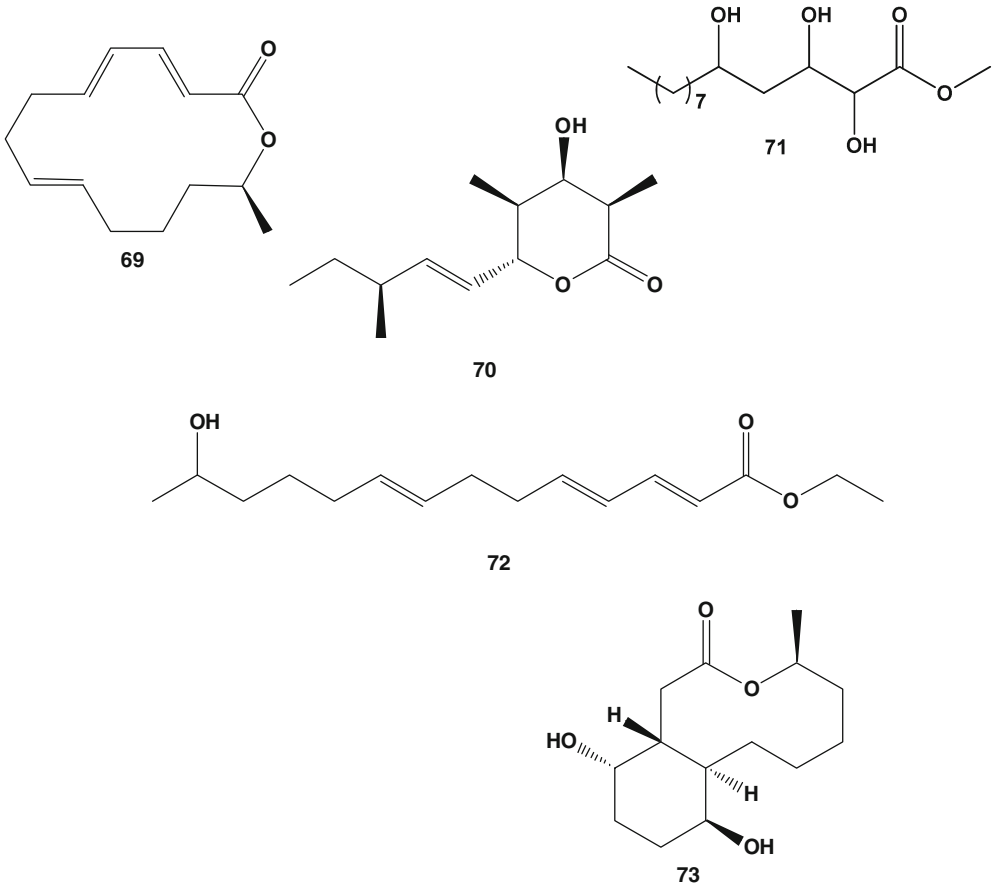
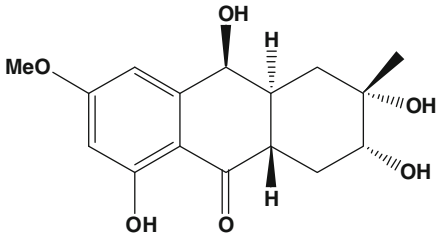



**67**

Chemical investigation of the endophytic fungus *Phomopsis* sp., isolated from *Alpinia malaccensis* (Zingiberaceae), afforded four new cytotoxic metabolites, including benquione (**69**), LMA-P2 (**70**), LMA-P3 (**71**) and benquinol (**72**), together with four known products. **69**–**72** were identified based on their NMR spectra as well as by HRMS, and the absolute configuration of **70** was confirmed by X-ray crystallography. Benquoine (**69**) is a new 14-membered lactone generated from cyclization of benquinol (**72**). All isolated compounds were tested for their cytotoxic activity against colonic epithelial (HCT-116) cancer cells. The known LMA-P1 (**73**) displayed the strongest cytotoxicity with an $IC_{50}$ value of 0.041 $\mu$M, whereas benquoine had a lower activity ($IC_{50}$ 0.21 $\mu$M) (Adelin et al. 2011).




Eleven new polyketides, including five new hydroanthraquinone derivatives, tetrahydroaltersolanols C–F (**74–77**), dihydroaltersolanol A (**78**), and five new alterporriol-type anthranoid dimers, alterporriols N–R (**79**–**83**), along with seven known analogues were produced by *Alternaria* sp. ZJ-2008003. This strain was isolated from inner tissues of the soft coral *Sarcophyton* sp. (GX-WZ-20080011) (alcyoniidae) collected from the Weizhou coral reef in the South China Sea. The structures and the relative configurations of the isolated compounds were elucidated using comprehensive spectroscopic methods (NMR and MS) as well as single-crystal X-ray crystallography. Furthermore, the absolute configuration of **80** was assigned by using the modified Mosher's method. Compounds **74**–**81** were evaluated for their cytotoxic activity against human colon carcinoma (HCT-116), human breast cancer (MCF-7/ADR), human prostatic cancer (PC-3), and human hepatoma (HepG2 and Hep3B) cells. The known altersolanol C (**84**) was the most active metabolite among the monomeric anthranoids, exhibiting $IC_{50}$ values between 2.2 and 8.9 $\mu$M, while the other monomers which lack the paraquinone moiety were inactive ($IC_{50}$>100 $\mu$M). These results indicated that the paraquinone moiety was important for cytotoxic activity, as described previously (Debbab et al. 2009). In addition, **81** was found to inhibit the growth of PC-3 and HCT-116 cells with $IC_{50}$ values of 6.4 and 8.6 $\mu$M, respectively (Zheng et al. 2012).

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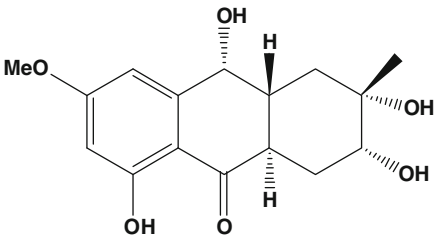


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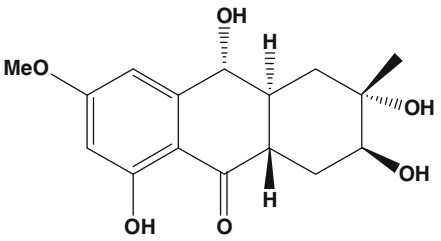


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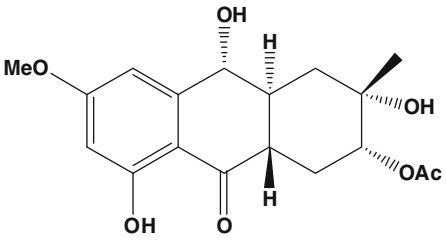


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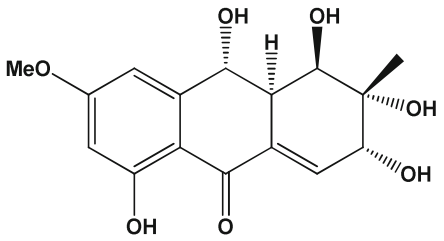


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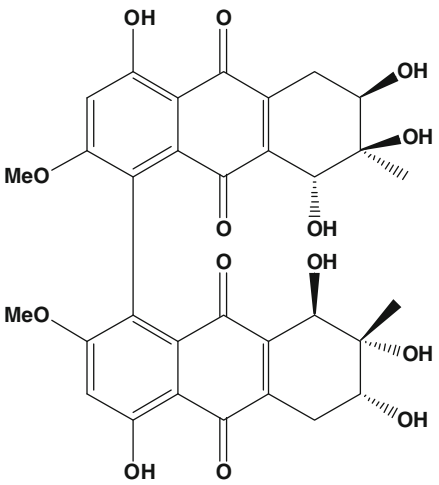


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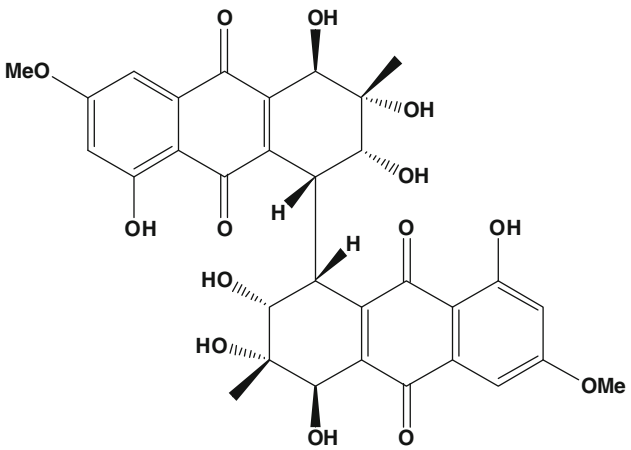


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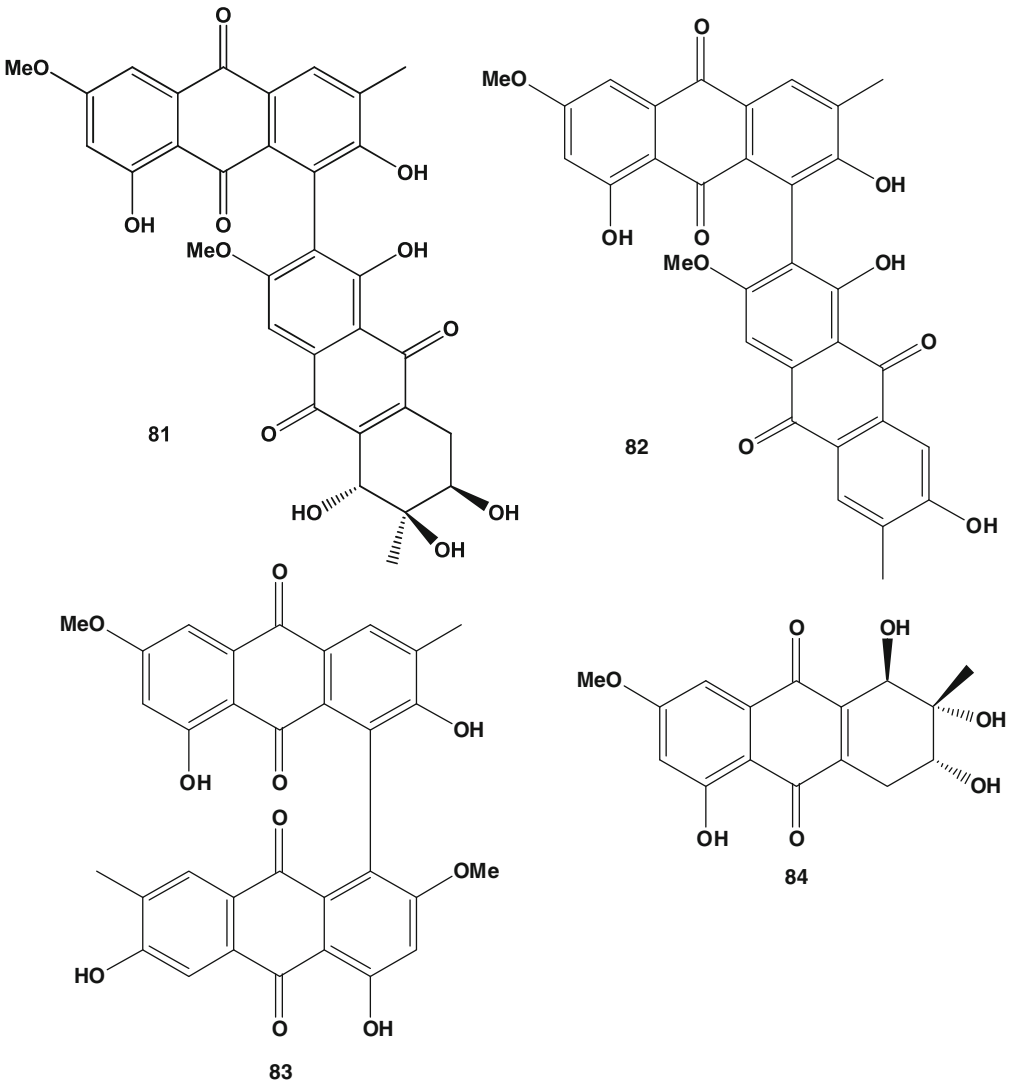
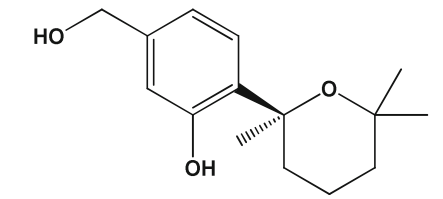


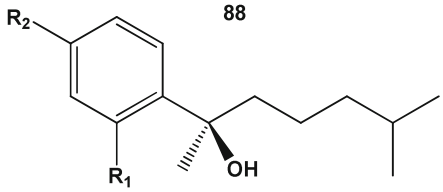

**Anti-infective secondary metabolites**

Fermentation broth of the marine-derived fungus *Aspergillus* sp., isolated from the sponge *Xestospongia testudinaria* (Petrosiidae) collected from the South China Sea, yielded four new bisabolane-type sesquiterpenoids, including aspergiterpenoid A (**85**), (−)-sydonol (**86**), (−)-sydonic acid (**87**), and (−)-5-(hydroxymethyl)-2-(2′,6′,6′-trimethyltetrahydro-2Hpyran-2-yl)phenol (**88**) together with the known (*Z*)-5-(hydroxymethyl)-2-(6′-methylhept-2′-en-2′-yl)phenol. The structures were established by NMR spectroscopic techniques and mass spectrometric analysis, and the absolute configurations were assigned by measuring optical rotation and comparison with related known analogues. The antibacterial activity of **85**–**88** was studied, using microplate assay, against eight bacterial strains, e.g. six pathogenic bacteria *Staphylococcus albus*, *Bacillus subtilis*, *Bacillus*

*cereus*, *Sarcina lutea*, *Escherichia coli*, *Micrococcus tetragenus*, and two marine bacterial strains *Vibrio Parahaemolyticus* and *Vibrio anguillarum*. Compound **85** exhibited weak antibacterial activity against *E. coli* and *M. tetragenus*. Compound **86** exhibited strong inhibitory activity against *S. albus* and *M. tetragenus* with MIC (minimum inhibiting concentrations) values of 5.0 and 1.25 $\mu$M, respectively, whereas **88** featured MIC values of 5.0 and 2.50 $\mu$M against *S. albus* and *B. subtilis*, respectively. Compound **87** and the known (*Z*)-5-(hydroxymethyl)-2-(6′-methylhept-2′-en-2′-yl)phenol showed a broad spectrum of antibacterial activity with MIC values ranging from 2.5 to >20.0 $\mu$M (Li et al. 2012a).

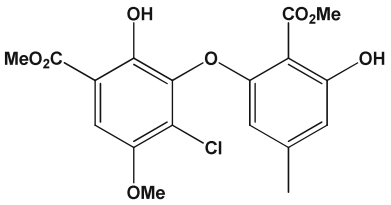



**88**




**85** $R_1$ = H, $R_2$ = $CH_2OH$
**86** $R_1$ = OH, $R_2$ = $CH_2OH$
**87** $R_1$ = OH, $R_2$ = COOH

The mangrove-derived fungus *Pestalotiopsis* sp. PSU-MA69 was isolated from a branch of *Rhizophora apiculata* (Rhizophoraceae), which was collected in Sutun province, Thailand. The ethyl acetate extract of this fungus exhibited antifungal activity against *Candida albicans* NCPF3153, and *Cryptococcus neoformans* ATCC90112. Chemical investigation afforded nine new secondary metabolites, including four diphenyl ethers, pestalotethers A-D (**89–92**), three chromones, pestalochromones A-C (**93–95**), one xanthone, pestaloxanthone (**96**) and one new butenolide, pestalolide (**97**), in addition to eleven known products. Compounds obtained in sufficient amounts were evaluated for antifungal activity against *C. albicans* NCPF3153 and *C. neoformans* ATCC90112. Compound **97** showed weak antifungal activity against both fungal strains with equal MIC values of 653.1 $\mu$M. Compounds **89**, **90** as well as the known metabolites pestheic acid (**98**), chloroisosulochrin dehydrate (**99**) and chloroisosulochrin (**100**) were mildly active against *C. neoformans* with MIC values of 505.1, 591.7, 523.6, 574.7 and 546.4 $\mu$M, respectively, but were inactive against *C. albicans*. The remaining compounds were inactive against both *C. albicans* and *C. neoformans*. Interestingly, compounds **89**, **90**, pestheic acid and chloroisosulochrin dehydrate that feature a chlorine substituent displayed better antifungal activity against *C. neoformans* than **92**, **96** and isosulochrin dehydrate (**101**) which lack a chlorine substituent (Klaiklay et al. 2012).

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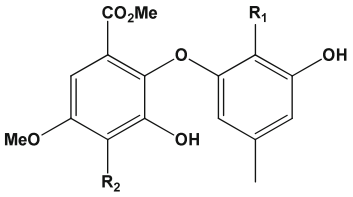
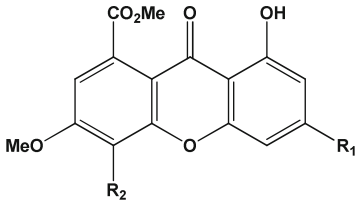
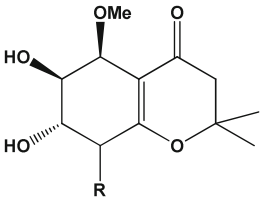


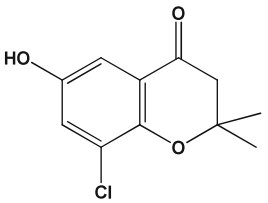

**89** $R_1$ = COOMe, $R_2$ = Cl
**90** $R_1$ = H, $R_2$ = Cl
**92** $R_1$ = COOMe, $R_2$ = H
**98** $R_1$ = COOH, $R_2$ = Cl



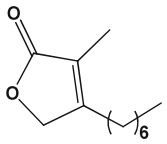

**96** $R_1$ = $CH_2OH$, $R_2$ = H
**99** $R_1$ = Me, $R_2$ = H
**101** $R_1$ = Me, $R_2$ = Cl




**93** R = $\beta$–Cl
**94** R = $\alpha$–Cl




**95**

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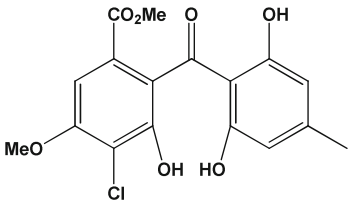
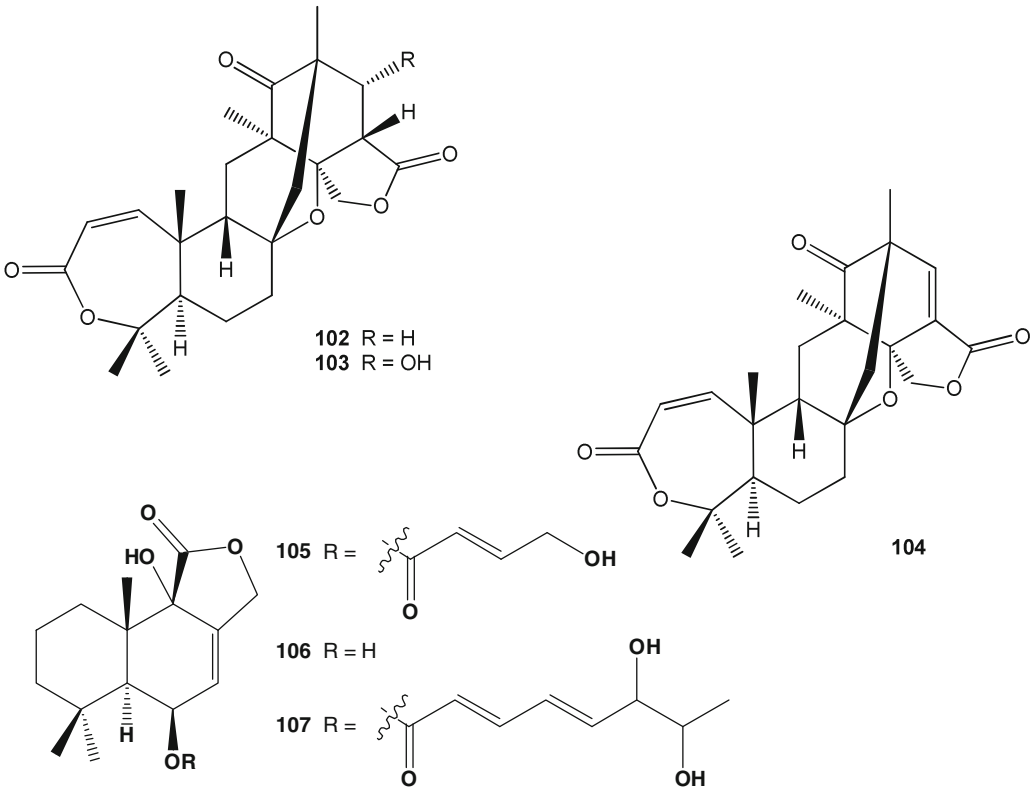


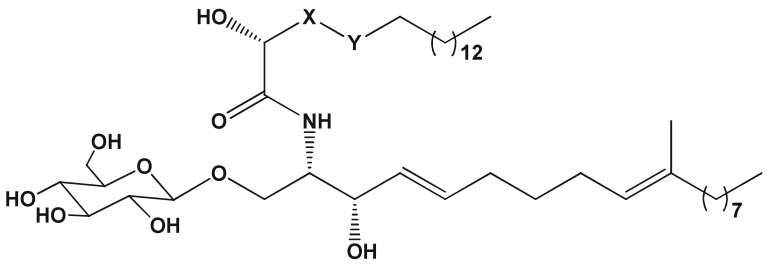

**100**

Cohen et al. reported three novel meroterpenoids, insuetolides A–C (**102–104**) as well as the new (*E*)-6-(40-hydroxy-20-butenoyl)-strobilactone A (**105**), from the EtOAc extract of the marine-derived fungus *Aspergillus insuetus* (OY-207), which was isolated from the Mediterranean sponge *Psammocinia* sp. (Irciniidae). Insuetolides **102–104** revealed a new carbon skeleton derived from the cyclization of farnesyl and 3,5-dimethylorsellinic acid. When tested towards *Neurospora crassa*, **102** and the known metabolites strobilactone A (**106**) and (E,E)-6-(60,70-dihydroxy-20,40-octadienoyl)-strobilactone A (**107**) exhibited anti-fungal activity with MIC values of 140, 242, and 162 $\mu$M, respectively (Cohen et al. 2011).

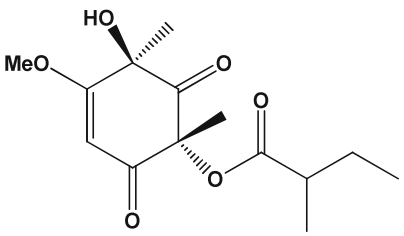



Two new antibacterial cerebroside derivatives, named flavusides A and B (**108** and **109**), in addition to four known secondary metabolites were isolated from the $CH_2Cl_2$-MeOH fraction of marine-derived *Aspergillus flavus*. The fungus was isolated from the surface of the edible green alga, *Codium fragile* (Codiaceae), collected in GeoMun Island, Yeosu, Korea. The structure and absolute stereochemistry of the two cerebrosides were assigned on the basis of carbon chemical shifts, optical rotation values, comparison with related known metabolites, and Tandem FAB-MS/MS experiments. Compounds **108**, **109**, and the known phomaligol A (**110**) exhibited mild antibacterial activity

against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. **108** and **109** showed MIC values of 20.7 $\mu$M toward *S. aureus* and 41.4 $\mu$M against methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus* (MRSA), whereas **110** showed a MIC of 109.9 $\mu$M against *S. aureus* and methicillin-resistant *S. aureus* and of 220.1 $\mu$M toward multidrug-resistant *S. aureus*. Cerebrosides are glycosphingolipids, containing ceramide and a single sugar residue (glucose or galactose) at C-1. The hydrophobic ceramide substructure (sphingoid base and an amide-linked fatty acyl chain) is reported to exhibit antitumor/cytotoxic, anti-HIV-1, neuritogenic, anti-hepatotoxic, immunosuppressive, immunomodulatory, cyclooxigenase-2 inhibitory, antifungal, antimicrobial, and antifouling activities (Mansoor et al. 2007; Yang et al. 2011).

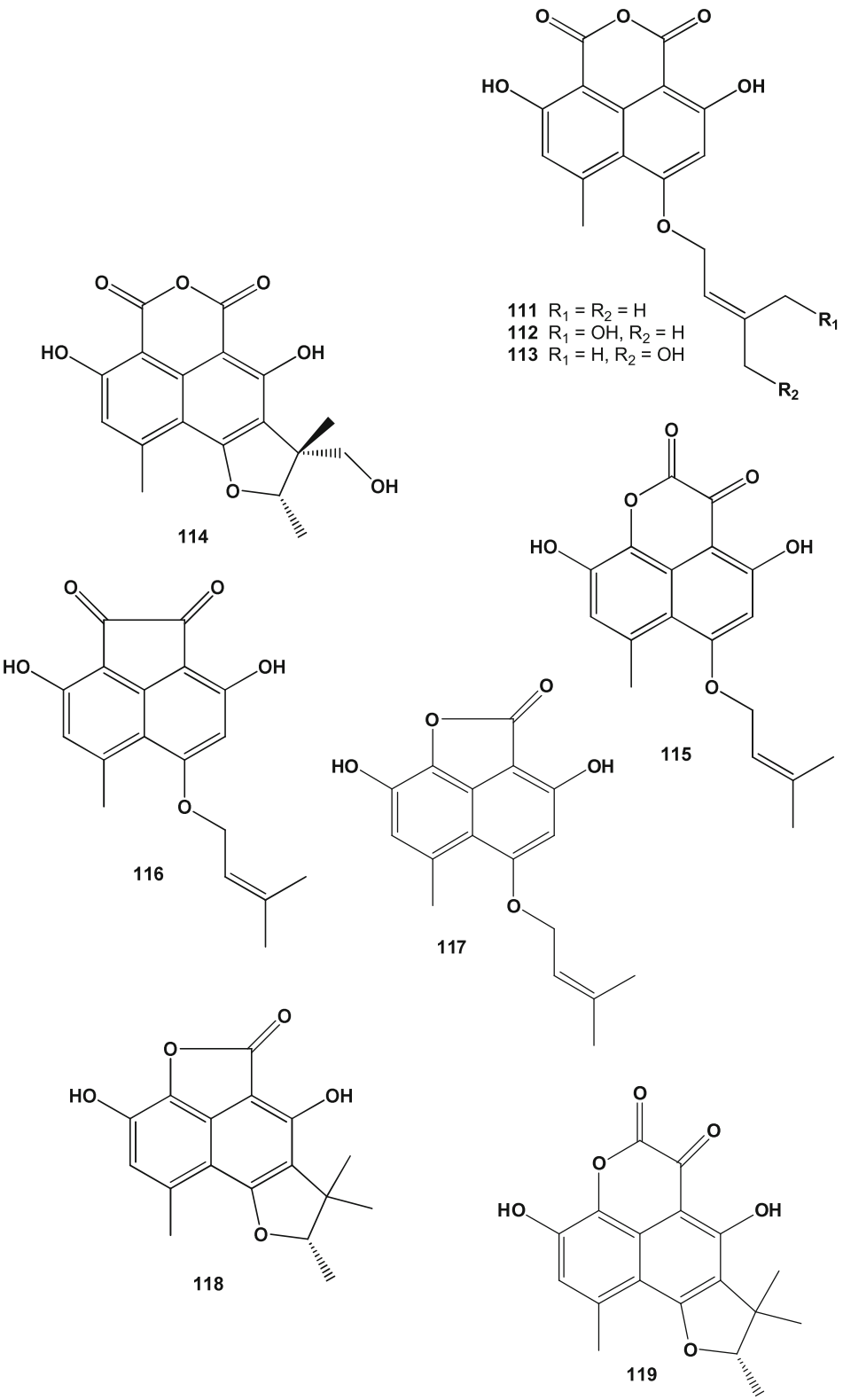



**108** X-Y = $CH_2CH_2$
**109** X-Y = CH=CH



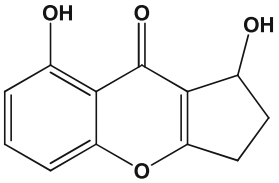

**110**

Seven new phenalenone derivatives **111**–**117**, along with five known natural products, were isolated and identified from the marine-derived fungus *Coniothyrium cereal* which was obtained from the green alga *Enteromorpha* sp. (Ulvaceae). Their structures were established from extensive spectroscopic analysis on the basis of NMR spectroscopic studies, mass spectrometry, UV as well as IR spectroscopy. When tested for their antibacterial activity toward *Staphylococcus aureus* SG 511, compounds **115**, **116** as well as the known metabolites (−)-7,8-dihydro-3,6-dihydroxy-1,7,7,8-tetramethyl-5H-furo-[2′,3′:5,6]naphtho[1,8-bc]furan-5-one (**118**), and (−) sclero-derolide (**119**) inhibited the growth of *S. aureus* SG 511 with MIC values of 24, 66, 52, and 24 $\mu$M, respectively. This result suggested that the antibacterial activity correlated with the presence of a diketo-lactone ring as found in **115** and **119**, whereas cyclisation of the hemiterpene unit does not influence the activity. Furthermore, compounds **112**, **114** and **117** exhibited considerable inhibition zones (>15 mm) in agar diffusion assays against *Mycobacterium phlei* (Elsebai et al. 2011).

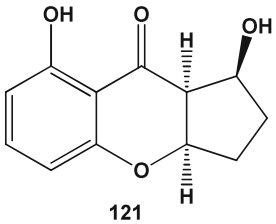



Bioassay-guided isolation of antimicrobial secondary metabolites from the endophytic fungus *Diaporthe* sp. P133, isolated from *Pandanus amaryllifolius* (Pandanaceae), yielded two new benzopyranones, diaportheone A and B (**120** and **121**). Biological evaluation of the antitubercular activity of **120** and **121** against a virulent strain of

*Mycobacterium tuberculosis* H37Rv showed MIC values of 100.9 $\mu$M for **120** and 3.5 $\mu$M for **121** (Bungihan et al. 2011).

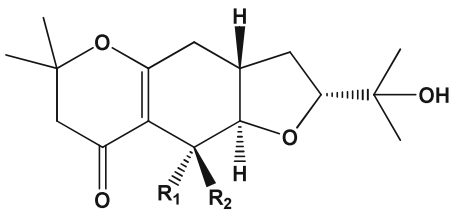



**120**

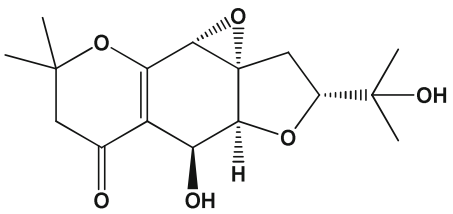



**121**

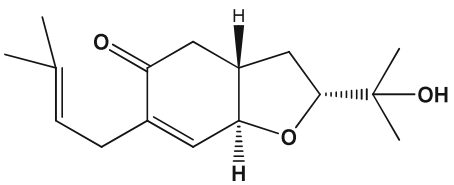
Qin et al. investigated an unidentified ascomycete which was isolated from *Arbutus unedo* (Ericaceae). When cultured on biomalt solid agar medium, this fungal strain produced four new compounds, pestalotheols E-H (**122–125**), along with the known metabolite anofinic acid (**126**). Pestalotheols **122–125** are new compounds exhibiting a chromenone-type core structure. Biogenetically, these compounds could be derived from two isoprene units and a polyketide (Li et al. 2008). All isolated compounds were tested for their antifungal, antibacterial, and algicidal properties toward *Microbotryum violaceum*, *Escherichia coli*, *Bacillus megaterium*, and *Chlorella fusca*. Interestingly, all compounds showed antifungal, antibacterial, and algicidal properties. Compounds **124–126** showed strong antibacterial activity. In particular, the antibacterial activity of **124** against the Gram-negative bacterium *E. coli* (12 mm) and of **126** against *E. coli* (12 mm) and *B. megaterium* (12 mm) was comparable to that of the positive controls penicillin (14 mm) and tetracycline (18 mm) (Qin et al. 2011).



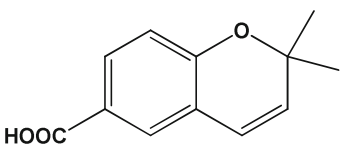

**122** $R_1$ = H, $R_2$ = OH
**123** $R_1$ = OH, $R_2$ = H




**124**



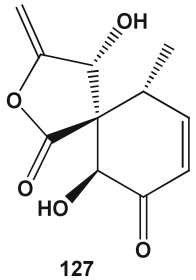

**125**



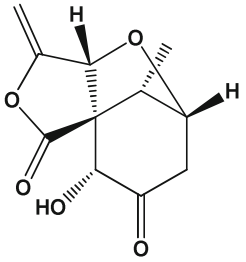

**126**

Three novel compounds with spiro-5, 6-lactone ring skeleton, including massarigenin D (**127**), spiromassaritone (**128**) and paecilospirone (**129**), were found in the fermentation broth of *Massrison* sp. The fungus was isolated from roots of *Rehmannia glutinosa* (Phrymaceae) collected from Wushe County, Henan Province, China. The structures were established by a variety of one- and two-dimensional NMR experiments as well as by mass spectrometry. Compounds **127–129** were tested in vitro for their antifungal activity toward *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum* and *Aspergillus fumigatus*. **127–129** showed antifungal activity against all pathogens tested with MIC values ranging from 1.1 to 142.8 $\mu$M. Antifungal activities of spiromassaritone (**128**) and paecilospirone (**129**) were comparable with those of griseofulvin and ketoconazole, whereas spiromassaritone (**128**) exhibited stronger activity against *Candida albicans* and *Cryptococcus neoformans* than griseofulvin (Sun et al. 2011).

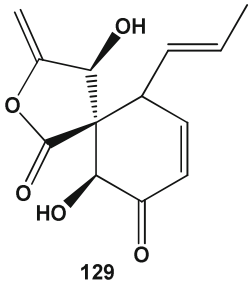
Compounds with a rare spiro-5,6-lactone ring skeleton have previously been reported to be antibiotically active against murine leukemia and to extend the life time of infected mice (Nakayama et al. 1992).




127

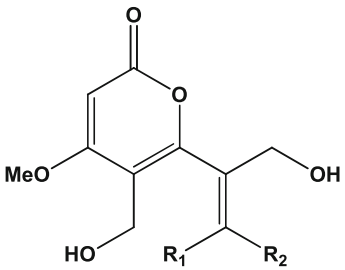



128

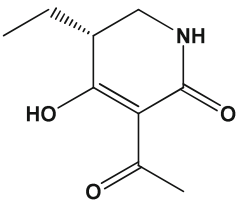



129

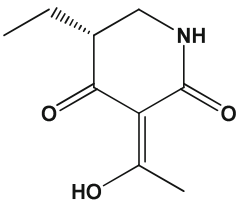
Antimicrobially guided isolation of an extract of *Chaetomium globosum*, isolated from *Cynodon dactylon* (Poaceae), yielded four new secondary metabolites, chaetoglocins A–D (**130–133**). When tested against the Gram-positive bacteria *Bacillus subtilis* CICC10285, *Streptococcus pyogenes* ATCC19615, *Mirococcus luteus* CMCC(B) 28001, *Mycobacterium smegmatis* CGMCC1.562, and against the Gram-negative bacteria *Escherichia coli* ATCC35218 and *Pseudomonas aeruginosa* CICC10351, only compounds **130** and **131** exhibited moderate antibacterial activity with MIC values ranging from 35.4 to 141.6 and from 70.8 to 141.6 $\mu$M, respectively (Ge et al. 2011).




**130** $R_1$ = H, $R_2$ = Me
**131** $R_1$ = Me, $R_2$ = H

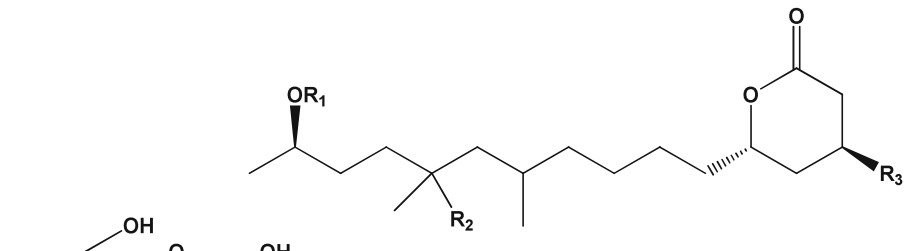



132

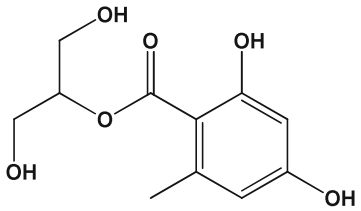



133

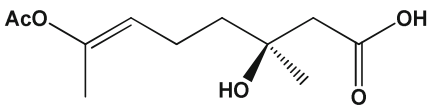
Chemical investigation of the endophytic fungus *Penicillium chrysogenum* QEN-24S, isolated from an unidentified marine red algal species of the genus *Laurencia* (Rhodomelaceae), which was collected from the Weizhou Island of southern China Sea, afforded four new secondary metabolites identified as penicitides A and B (**134** and **135**), one glycerol derivative 2-(2,4-dihydroxy-6-methylbenzoyl)-glycerol (**136**) and one monoterpene derivative penicimonoterpene (**137**), together with one known glycerol analogue. Interestingly, **134** and **135** feature a unique 10-hydroxy- or 7,10-dihydroxy-5,7-dimethylundecyl moiety present as substituent at C-5 of the $\alpha$-tetrahydropyrone ring, a structural feature not reported previously for natural products. The isolated metabolites were evaluated for antifungal activity against *Aspergillus niger* and *A. brassicae*. Only **137** displayed selective and potent activity against the pathogen *A. brassicae* with an inhibition zone of 17 mm in diameter at a concentration of 20 $\mu$g/disk, while the positive control amphotericin B exhibited an inhibition zone of 18 mm. The remaining compounds were inactive (Gao et al. 2011b).




**134** $R_1 = R_2 = H, R_3 = OH$
**135** $R_1 = H, R_2 = R_3 = OH$

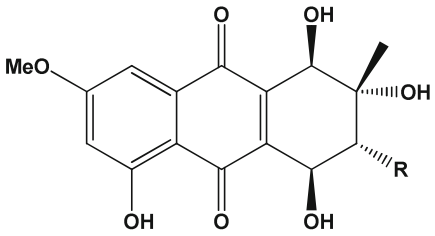



**136**

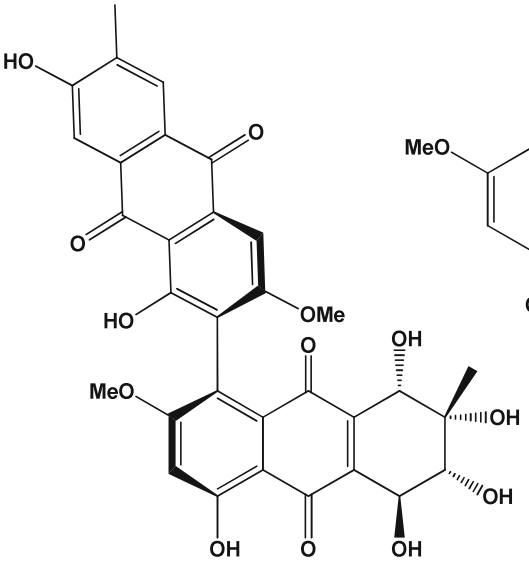



**137**

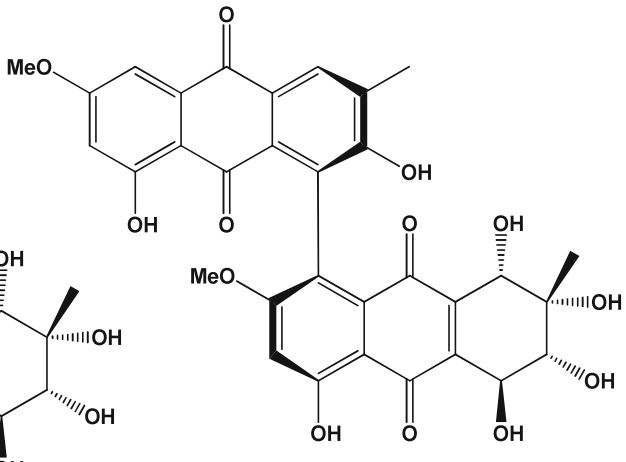
Three new anthracene derivatives, including tetrahydroanthraquinone **138** and the tetrahydroanthraquinone heterodimers **139** and **140**, together with four known metabolites, were obtained from *Stemphylium globuliferum*. *S. globuliferum* was isolated from the Moroccan medicinal plant *Mentha pulegium* (Lamiaceae). Detailed analysis of the spectroscopic data allowed the unambiguous determination of the new structures and revision of the structure of alterporriol C and its atropisomer (Suemitsu et al. 1988; Okamura et al. 1993), as well as that of alterporriol G. The absolute configurations of **138**–**140** were assigned by calculation of their CD spectra, which also allowed the configurational assignment of altersolanol A (**141**) and the determination of the axial chirality of the known alterporriols D and E (**142** and **143**), likewise isolated from *S. globuliferum*. All isolated compounds were analysed for their antimicrobial activity against several pathogenic microorganisms, including *Streptococcus pneumonia*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Aspergillus fumigatus* and *Candida albicans*. The known altersolanol A (**141**) inhibited the growth of most pathogenic microorganisms tested (MIC between 23.2 and 186.0 $\mu$M), whereas **139**, alterporriol D (**142**) and alterporriol E (**143**) showed likewise inhibition of bacteria but were inactive against fungi (Debbab et al. 2012).



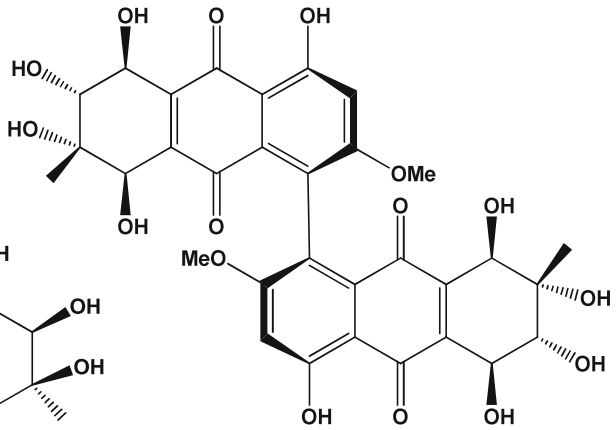

**138** R = OAc
**141** R = OH



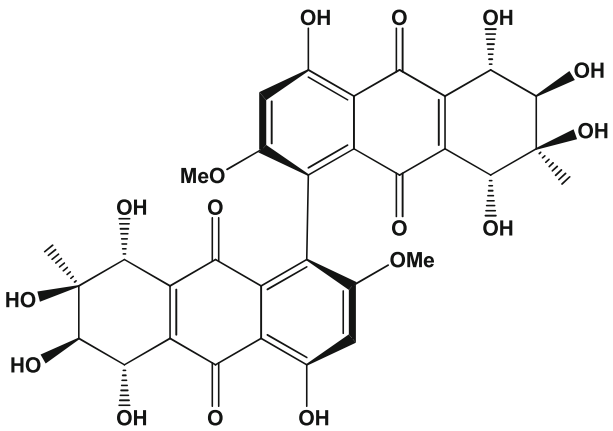

**140**




**139**

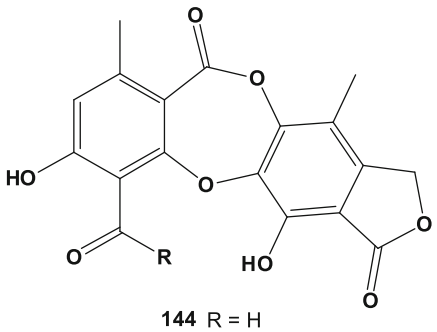



**142**



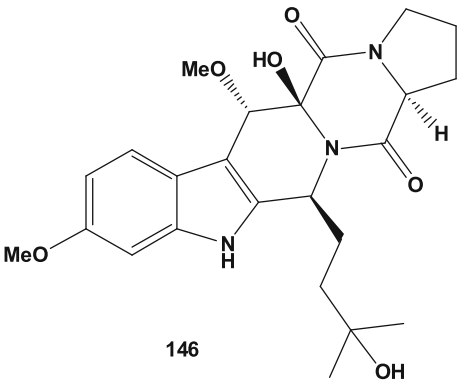

**143**

*Cordyceps dipterigena*, an endophyte from *Desmotes incomparabilis* (Rutaceae) collected in Coiba National Park, Veraguas, Panama, was found to strongly inhibit mycelial growth of the plant pathogenic fungus *Gibberella fujikuroi*, the causative agent of bakanae disease in rice crops which results from overproduction of the plant growth hormone gibberellic acid. Chemical investigation of the endophytic fungal strain yielded two new depsidone metabolites, cordycepsidones A and B (**144** and **145**), which were identified as being responsible for the antifungal activity. Compound **144** exhibited strong and dose-dependent antifungal activity against the phytopathogens *G. fujikuroi* and *Pythium ultimum* with MIC values of 23.3 and 3.4 $\mu$M, respectively, but was less potent against the *G. fujikuroi* anamorph *Fusarium subglutinans*. However, **145** showed a general reduction in activity in the antifungal assays, denoting the importance of the aldehyde function for the biological activity of **144**. Both compounds were inactive in bioassays for malaria (*Plasmodium falciparum*), leishmaniasis (*Leishmania donovani*), Chagas's disease (*Trypanosoma cruzi*), and cytotoxicity at 10 $\mu$g/mL, indicating selective antifungal activity. The compounds were also inactive against several bacterial strains even at a concentration of 50 $\mu$g/mL (Varughese et al. 2012).

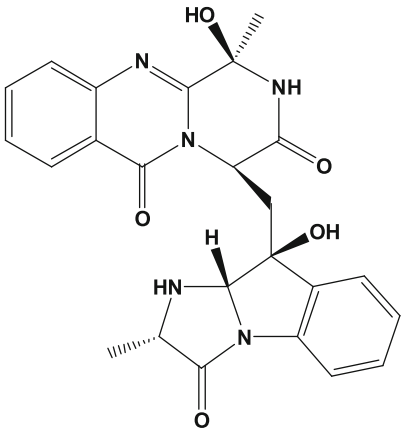



**144** R = H
**145** R = OH

Two new alkaloids, 12$\beta$-hydroxy-13$\alpha$-methoxyverruculogen TR-2 (**146**) and 3-hydroxyfumiquinazoline A (**147**), were isolated from the fermentation broth of *Aspergillus fumigatus*, isolated from the stem bark of *Melia azedarach* (Meliaceae) collected at Yangling, Shaanxi province, China. Evaluation of the in vitro antifungal activities of the compounds against a panel of phytopathogenic fungi including *Botrytis cinerea*, *Alternaria solani*, *A. alternata*, *Colletotrichum gloeosporioides*, *Fusarium solani*, *F. oxysporum*, and *G. saubinettii*, showed MIC values of 13.7–54.7 and 27.1–216.9 $\mu$M for **146** and **147**, respectively. Upon testing their toxicity against brine shrimps **146** and **147** showed only weak toxicity with $LC_{50}$ values of 132.8 and 175.3 $\mu$M, respectively (Li et al. 2012a,b).



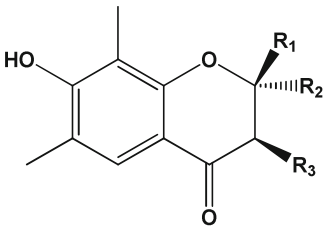

**146**

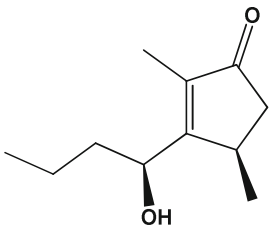



**147**

Two new chromones, phomochromone A and B (**148** and **149**), and one new cyclopentenone derivative, phomotenone (**150**), together with six known compounds were obtained from *Phomopsis* sp., isolated from *Cistus monspeliensis* (Cistaceae), through a bioassay-guided procedure. The structure of **150** shows similarity to

the phytohormone jasmonic acid indicating a possible role of **150** in modulating fungal interaction with its host plant. Compounds **148–150** showed moderate antifungal (*Microbotryum violaceum*), antibacterial (*Escherichia coli*, *Bacillus megaterium*), and antialgal (*Chlorella fusca*) activities with inhibition zone radii ranging from 5 to 10 mm (Ahmed et al. 2011).



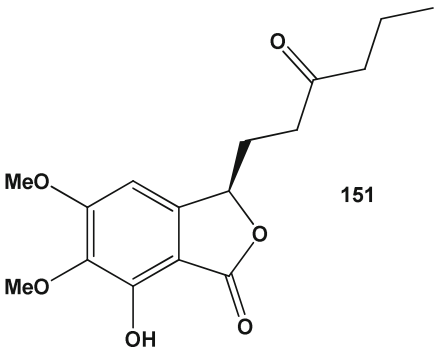




150

**148** $R_1 = R_3 = H, R_2 = Me$
**149** $R_1 = Me, R_2 = H, R_3 = OH$

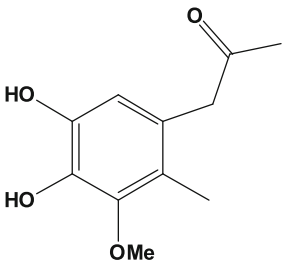
## Antioxidant secondary metabolites

Colletotrialide (**151**), a new phthalide isolated from the endophytic fungus *Colletotrichum* sp., showed potent antioxidant activity when tested in a modified oxygen radical absorbance capacity (ORAC) assay with 2.4 ORAC units. The fungus was isolated from from *Piper ornatum* (Piperaceae), which was collected from the Tai Rom Yen National Park, Surat Thani Province, Thailand. The antioxidant potential of **151** (1 $\mu$M) was compared with that of Trolox, a water-soluble vitamin E analogue, and expressed as ORAC units, where 1 ORAC unit equals the net protection of $\beta$-phycoerythrin produced by 1 $\mu$M Trolox (Tianpanich et al. 2011).




151

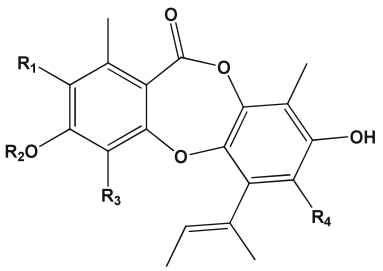
Chemical investigation of marine-derived *Aspergillus versicolor* resulted in the isolation of a new aromatic polyketide, aspergillin A (**152**). The fungus was obtained from the sponge *Petrosia* sp. (Petrosiidae) collected off the coast of Jeju Island, Korea. In comparison with standard antioxidants, **152** showed antioxidant activity comparable to that of butylated hydroxyanisole, and siginificantly higher than that of butylated hydroxytoluene (Li et al. 2011b).



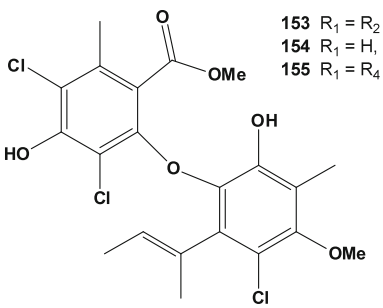

152

Three new depsidones, aspergillusidones A–C (**153–155**), and a new diaryl ether, aspergillusether A (**156**), together with three known depsidones and a synthetically known pyrone, were isolated from the marine-

derived fungus *Aspergillus unguis*. The fungal strain was isolated from an unidentified marine sponge collected from the Royal Navy Base at Tub-La-Mu bay, Pang-nga Province, Thailand. The isolated compounds were evaluated for aromatase inhibitory and radical scavenging activities. Aspergillusidone C (**155**) showed the most potent aromatase inhibitory activity with an $IC_{50}$ value of 0.7 $\mu$M, while $IC_{50}$ values detected for depsidones **153** and **154** were 2.2 and 4.1 $\mu$M, respectively. Inactivity of **156** indicated that the structural features of depsidones are important for aromatase inhibitory activity. Furthermore, **153** and **154** inhibited superoxide anion radical formation in the xanthine-xanthine oxidase assay with $IC_{50}$ values of 16.0 and <15.6 $\mu$M, respectively. Due to the limited amount of **154** determination of the exact $IC_{50}$ value was not possible. All compounds were found to be inactive or only weakly active when testing them for cytotoxicity against several human cancer cell lines (Sureram et al. 2012).

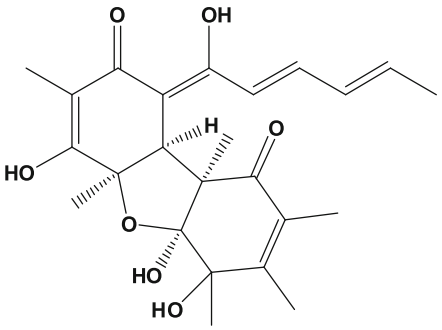



**153** $R_1 = R_2 = R_3 = H$, $R_4 = COOH$
**154** $R_1 = H$, $R_2 = Me$, $R_3 = R_4 = Cl$
**155** $R_1 = R_4 = Cl$, $R_2 = R_3 = H$



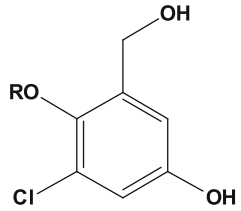

**156**

From *Penicillium citrinum*, isolated from a marine sponge belonging to the Demospongiae class, collected offshore of Ishigaki island, Okinawa Prefecture, Japan, a new metabolite JBIR-124 (**157**) was isolated and characterized. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of **157** was evaluated using $\alpha$-tocopherol as a positive control ($IC_{50}$ 9.0 $\mu$M). JBIR-124 (**157**) showed DPPH radical scavenging activity with an $IC_{50}$ value of 30 $\mu$M (Kawahara et al. 2012).



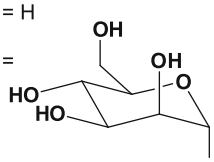

**157**

Chlorogentisyl alcohol (**158**), originally isolated from the marine-derived fungus *Aspergillus* sp., obtained from the marine red alga *Hypnea saidana* (Hypneaceae) collected in Tongnyeong, Gyeongnam Province, Korea (Li et al. 2005), was biotransformed by marine-derived *Chrysosporium synchronum* resulting in a new glycosidic metabolite, 1-*O*-($\alpha$-D-mannopyranosyl)chlorogentisyl alcohol (**159**). The transforming fungus *C. synchronum* was isolated from the surface of edible brown alga *Sargassum ringgoldium* (Sargassaceae) collected at Yokji Island of GyeongNam, Korea. Both **158** and **159** showed significant radical-scavenging activity in the DPPH assay with $IC_{50}$ values of 1.0 and 4.7 $\mu$M, respectively, being thus more active than the positive control L-ascorbic acid ($IC_{50}$ 20.0 $\mu$M) (Yun et al. 2011).



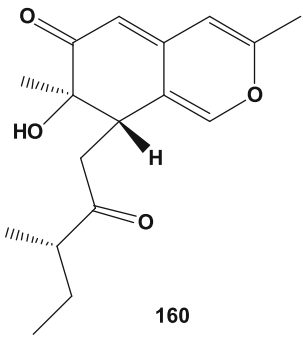

158 R = H

159 R =

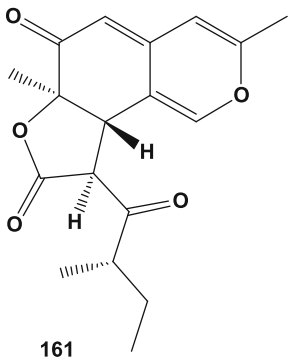



## Other biological activities

Three new azaphilones, chermesinones A–C (**160–162**), three new *p*-terphenyls (6′-*O*-desmethylterphenyllin, 3-hydroxy-6′-*O*-desmethylterphenyllin, 3″-deoxy-6′-*O*-desmethylcandidusin B) (**163–165**), and two known *p*-terphenyls, were obtained from the endophytic fungus *Penicillium chermesinum*, isolated from the mangrove plant *Kandelia candel* (Rhizophoraceae) collected at the South China Sea in Guangdong Province, China. All compounds were tested for their in vitro inhibitory activities in $\alpha$-glucosidase and acetylcholinesterase enzyme assays. Compounds **163**, **164**, and the known 3,3″-dihydroxy-6′-*O*-desmethylterphenyllin (**166**) exhibited strong inhibition of $\alpha$-glucosidase with $IC_{50}$ values of 0.9, 4.9, and 2.5 $\mu$M, respectively, showing significantly higher effects than the positive control genistein ($IC_{50}$ 9.8 $\mu$M). Compound **160** showed a weaker inhibitory effect with an $IC_{50}$ value of only 24.5 $\mu$M. In contrast, **165** and the known 6′-*O*-desmethylcandidusin B (**167**), featuring a furan ring in their structures, showed inhibitory activity in an acetylcholinesterase assay with $IC_{50}$ values of 7.8 and 5.2 $\mu$M, respectively. The remaining compounds (**161 and 162**) showed no inhibition of both enzymes ($IC_{50}$>100 $\mu$M) (Huang et al. 2011).




160

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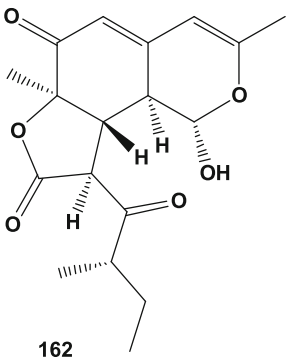


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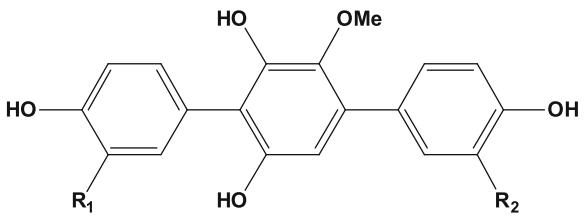
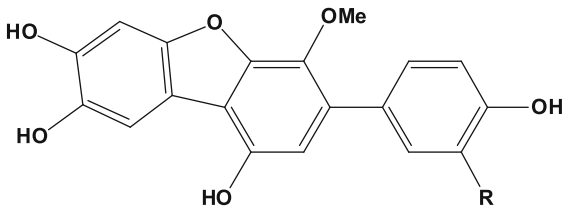
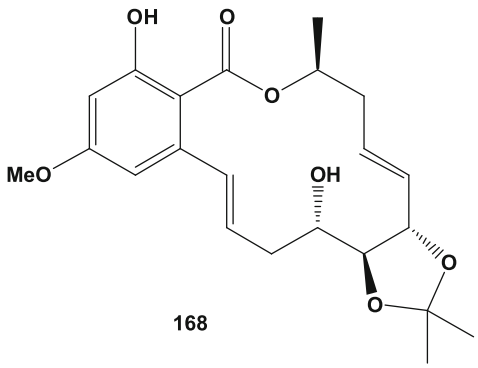



163 $R_1 = R_2 = H$
164 $R_1$ = OH, $R_2$ = H
166 $R_1 = R_2$ = OH

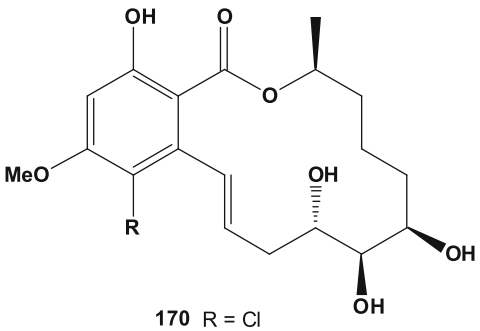



165 R = H
167 R = OH

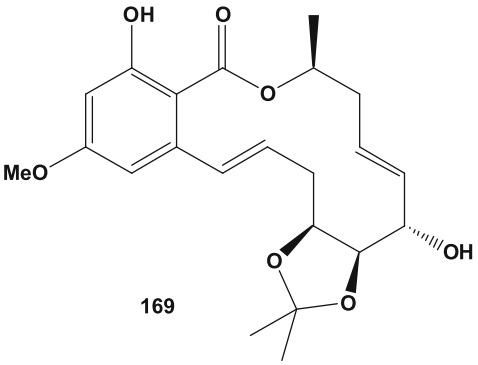
Three new 14-membered resorcylic acid lactones, two bearing a rare natural acetonide group, cochliomycins A and B (**168** and **169**), and one compound with a 5-chloro-substituted lactone, cochliomycin C (**170**), together with four known analogues, were isolated from cultures of *Cochliobolus lunatus*, a fungus obtained from the gorgonian *Dichotella gemmacea* (Ellisellidae) collected from the Weizhou coral reef in the South China Sea. The isolated resorcylic acid lactones were evaluated for their antifouling activity against the barnacle *Balanus amphitrite*. Cochliomycin A (**168**) and the known zeaenol (**171**), LL-Z1640-1 (**172**), and paecilomycin F (**173**) completely inhibited larval settlement of *B. amphitrite* at a concentration of 20.0 $\mu$g/mL. Cochliomycin A (**168**) showed a significant inhibitory activity even at a concentration of 5.0 $\mu$g/mL (12.4 $\mu$M), but it was also toxic to the larvae at this concentration. Furthermore, **168** and **171–173** showed potent antifouling activities at nontoxic concentrations with $IC_{50}$ values of 3.0, 13.7, 14.6 and 48.9 $\mu$M, respectively. These values were lower than the standard requirement of an $IC_{50}$ of 25 $\mu$g/mL established by the U.S. Navy program as an efficacy level for natural antifouling agents and indicated for the first time antifouling activities for this class of metabolites (Shao et al. 2011b).



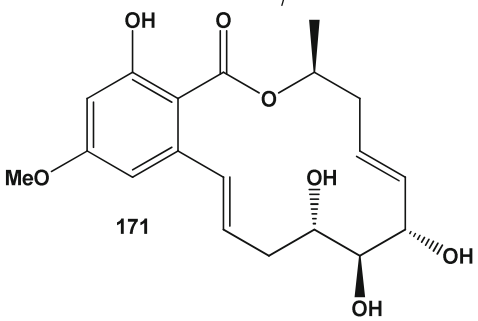

**168**



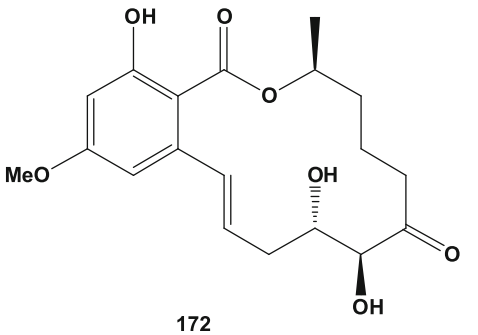

**170** R = Cl
**173** R = H




**169**



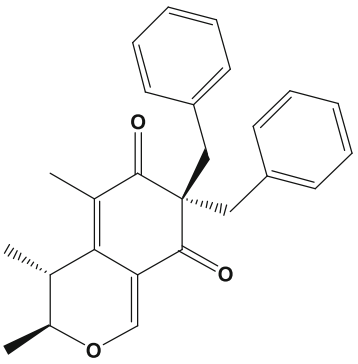

**171**



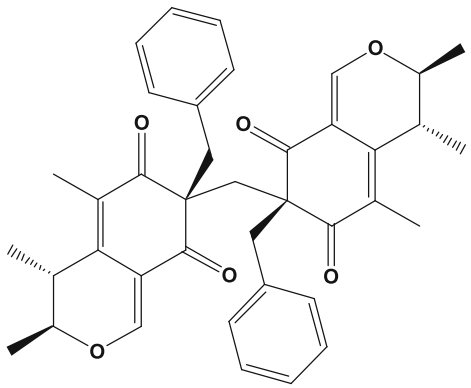

**172**

A culture of a marine-derived *Aspergillus* sp. yielded two novel benzylazaphilone derivatives having an unprecedented carbon skeleton, aspergilone A (**174**) and its symmetrical dimer with a unique methylene bridge, aspergilone B (**175**). The fungus was isolated from the gorgonian *D. gemmacea* collected from the South China Sea. Aspergilone A (**174**) exhibited strong inhibition of larval settlement of *B. amphitrite* at nontoxic concentration with an $IC_{50}$ value of 19.9 $\mu$M. The compound also showed selective in vitro cytotoxicity toward the human cancer cell lines HL-60 (promyelocytic leukemia), MCF-7 (breast adenocarcinoma) and A-549 (lung carcinoma) with $IC_{50}$ values of 8.3, 64.8

and 95.9 $\mu$M, respectively. Aspergilone B (**175**), however, was inactive in the cytotoxicity assays, indicating the importance of the monomeric form for the observed activity (Shao et al. 2011a,b).

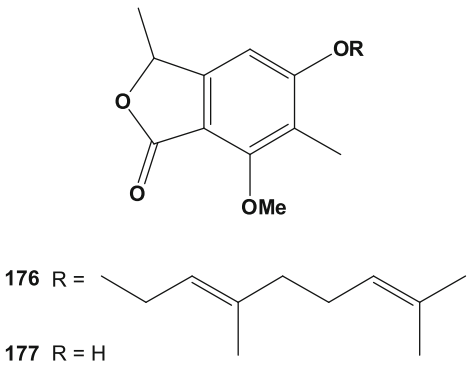


174



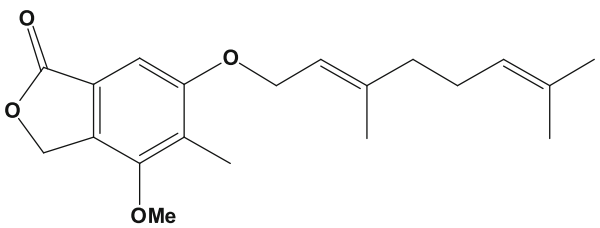
175

The marine-derived fungus *Stachylidium* sp. when grown on a biomalt medium supplemented with sea salt afforded three new phthalide derivatives, marilones A–C (**176–178**). The source fungus was isolated from the sponge *Callyspongia flammea* (Callyspongiidae), which was collected at Bear Island, Sydney, Australia. Marilone A (**176**) showed antiplasmodial activity against *Plasmodium berghei* liver stages with an $IC_{50}$ value of 12.1 $\mu$M. In contrast, marilone C (**178**) showed no activity even at a concentration of 25 $\mu$M, indicating that the methyl substituent of the furanone ring and/or the position of the ketone functionality are essential for the observed activity of **176**. On the other hand, marilone B (**177**) was tested on a panel of 44 psychoactive receptors, including 11 serotonin receptors, where it exhibited a selective antagonistic effect against the serotonin receptor 5-$HT_{2B}$ with a $K_i$ value of 7.7 $\mu$M. Interestingly, the marilones were produced only on solid biomalt medium supplemented with sea salt, and were not detected in other media such as Czapek or YPM (Almeida et al. 2011).



176 R =

177 R = H



178

## Conclusion

Advanced technologies allowing a better detection, identification, and monitoring of microbial inhabitants are improving our understanding of the complex microbial dynamics in various ecosystems. Microbial endosymbionts can modify their host organisms at genetic, physiological, chemical and ecological levels, thus inducing extreme changes in their response and adaptation to their environments. In this context, it is important to identify key endophytes that can improve the competitive ability of a certain plant under specific environmental conditions, in part by the production of bioactive

secondary metabolites. Such endophytes may have potential agricultural applications including the development of modified plant germplasm for native and crop plants which shows improved capabilities for tolerating specific environmental stresses caused by global changes.

The great diversity of fungal populations inhabiting plants and marine invertebrates suggests the presence of a plethora of novel unexplored fungal strains estimated to exceed a million new species (Maheshwari 2006; Johri 2006). Thus, terrestrial and marine endosymbiotic microorganisms still represent a vast untapped reserve of secondary metabolites which can be exploited for therapeutical and agricultural applications. Taking into account the growing needs of modern medicine for new drugs or drug leads, a continuous supply of new chemical entities is of great necessity. Thus, it is essential to find alternative strategies to promote the discovery of novel secondary metabolites and compensate for the inadequacy of traditional methods, thereby unravelling the hidden wealth of fungal natural products. Great potential is expected by further investigating and targeting the epigenome for finding new secondary metabolites from fungi and other organisms, which will be facilitated by advances in modern molecular techniques, sequencing technologies, combined with genomic and transcriptomic approaches.

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