

Chapter 1

MARINE-DERIVED FUNGI: A SOURCE OF BIOLOGICALLY POTENT AND NOVEL CHEMICALS-A REVIEW

I. Introduction

i) General

Terrestrial microbes are well recognized as a source of novel metabolites, many of which possess valuable biological properties. Indeed, many of the pharmaceutical, agrochemical and crop protection industry are based on the exploitation of microbial natural products. Over the last five decade the search for new drugs has expanded, to those produced by marine macro-organisms and over a last two decade or so it has extended towards microbes obtained from marine sources (Bugni and Ireland, 2010). Fungi isolated from substrates such as marine plants (algae, sea grasses), invertebrates (ascidians, sponges, tunicates, etc), vertebrates (fish), sands, sediments and seawater itself, have been found to yield novel bioactive natural products. This review covers the literature on novel bioactive metabolites of marine-derived fungi published in the first half of 2010. The review also presents the trend so far observed in isolation of novel molecules from different types of species of fungi, source of fungi, their biosynthetic classification and the biological activities. It also gives brief overview of progress of the marine fungal chemistry over the years.

ii) Marine habitats and associated fungi as bioactive resource

The world's oceans represent a resource of huge dimension for natural product chemists; however, the chemistry of marine fungi until last two decades was relatively neglected area of marine natural products chemistry. Published reviews shows the importance of these organisms as potential sources of pharmaceutical leads (Ebel, 2006; Ebel, 2010; Rateb and Ebel, 2011). More specifically, fungi from the marine environment are fast growing as source of potential bioactive resource as suggested by the diversity of secondary metabolites isolated so far.

Though sourced from marine substrates, the marine character of these microbes may not be absolute. Therefore most fungi are referred as "marine-derived" since the fungi obtained from marine ecosystem can be obligate and facultative fungal species. Kohlmeyer (1979) defined obligate marine fungi as those "that grow and sporulate exclusively in a marine or estuarine (brackish water) habitat; facultative

marine are fungi from freshwater or terrestrial areas also able to grow in the natural marine environment.” Apparently, the majority of fungi inhabiting the world have not yet been described, which implicates fungi to represent an enormous source for natural products with diverse chemical structures and biological activities. Of special interest are creative fungal strains. Creativity in this sense is defined as the ability to produce compounds of interest for human activities (Dreyfuss & Chapela, 1994). Even if the natural function of secondary metabolites often is unknown, it is assumed that they play an important role in chemical defence and communication (Gloer 1995). This suggests that the biosynthesis of secondary metabolites does not occur randomly but it is correlated with ecological factors. Many of them have been suggested to act as pheromones, antifeedants or repellents, and as regulators in the development of organism (Sterner, 1995). Whether a marine derived microbe is obligate or facultative, or its status remains unknown, these organisms are quite capable of contributing to bio-prospecting and the discovery of new generation drugs.

iii) Bioactive metabolite symbioses: fungi as an alternative for the supply of natural products.

Marine invertebrates have been and continue to be a prolific source of novel and structurally diverse natural products. Often these compounds display potent and selective bioactivities that trigger biomedical interest. Unfortunately, the supply of the bioactive natural product is usually insufficient to meet the demands of pre-clinical and clinical development. A large-scale collection of the source marine invertebrate can be difficult due to scarcity of the organism, and can also have negative environmental consequences. In addition, natural supplies can fluctuate, either seasonally or due to environmental changes. Ideally, an efficient chemical synthesis of the desired natural product could be achieved; however the structural complexity of many natural products such as bryostatin 1 and swinholide A requires inefficient multistep syntheses that cannot meet the demands of pre-clinical and clinical development. Identification of a simpler, more easily synthesized structure which retains the biological activity is another option, but

the best scenario would be to have a supply of the natural product that can be generated inexpensively and reproducibly in the lab under controlled conditions.

In many cases, marine chemical ecological studies have revealed that the molecule is biosynthesized by associated microbial symbionts. Symbiotic fungi that can be cultivated in the laboratory and still produce the bioactive metabolite could be subjected to fermentation technology to produce larger amounts of the compound. Such a process eliminates large scale collection of the source marine macro organisms.

iv) Biology and ecology of marine fungi

Based on the distinction between obligate and facultative marine fungi, it can clearly be stated that among the former there are taxa which are exclusively found in the marine environment, while by definition, this is not the case for the latter. A recent monographic treatment of filamentous marine fungi listed a total of 530 species in 321 genera, among them 424 species within Ascomycota (in 251 genera), 94 species of anamorphic fungi (in 61 genera) and only 12 species within Basidiomycota (in 9 genera) (Jones et al., 2008). Marine fungal strains have been obtained from virtually every possible marine habitat, including inorganic matter (soil, sediments, sandy habitats, artificial substrates, and the water column), marine microbial communities, marine plants (algae, sea grasses, driftwood and other higher plants, especially mangrove plants), marine invertebrates (most notably sponges, but also corals, ascidians, holothurians, bivalves, crustaceans) and vertebrates (mainly fishes). However, it is worth mentioning that the fraction of culturable isolates is very low, i.e. in the range of 4% or less, with regard to the overall estimated biodiversity, similar to the situation with bacteria. As described above, marine plants, especially from mangrove habitats, harbour an enormous diversity of fungal associates. Several individual marine habitats are tremendous sources of fungal diversity and they have been so far underestimated. There is a pronounced difference regarding the biological diversity accessible by cultivation-based methods, and the diversity evident from molecular biological studies which rely on gene sequences present in environmental samples. In terms of global fungal diversity, a commonly used estimate is that while 74,000 fungal species

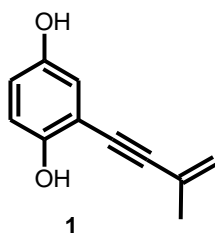
have been described, the overall expected diversity amounts to 1.5 million species.

v) *Historical perspective*

Historically, fungi have influenced many aspects of human life and development. It was the antibiotic production by fungi that have provided the stimulus for an entire era of drug discovery research. The earliest report indicating the antibacterial potential of fungi occurred in 1876 when Tyndall described the antagonistic effect of a *Penicillium* sp. on bacteria (Florey et al., 1949). In 1896, Gosio reported that he had isolated small amounts of a crystalline substance that had antibacterial properties. The compound was later confirmed to be mycophenolic acid. Although, there were numerous reports describing antibiotic properties of fungi in the late 1800s and early 1900s, the landmark discovery came in 1929 when Sir Alexander Fleming described the effects of *Penicillium notatum* and its metabolite, penicillin on bacteria. Unfortunately, the importance of his work was not fully realized until the early 1940s when a group at Oxford began investigating penicillin for use as an antibiotic in humans. After the potential of penicillin was elaborated, Giuseppe Brotzu 1945, began investigating seawater samples near a sewage outlet in Sardinia for antibiotic producing microbes (Abraham and Loder, 1972). Brotzu hypothesized that self-purification of water might be due in part to bacterial antagonism. Later, he obtained a fungus from a seawater sample that exhibited antibacterial activity and noted that it was similar to *Cephalosporium acremonium* (now named *Acremonium chrysogenum*). This observation led to the discovery of cephalosporin C.

In the intervening years, the fungi were not in the focus of drug discovery work, but interest in fungi resurged when cyclosporin A was isolated from *Tolypocladium inflatum* in 1976 and was approved for clinical use as an immunosuppressant in 1983. However, the rediscovery of high numbers of previously described metabolites has to some extent precluded the study of traditional terrestrial sources of fungi and led researchers to explore unique habitats, such as the marine environment, for fungi with potentially new biosynthetic diversity. Over the past many years fungi have proven to be a

valuable resource for the discovery of novel natural products. Although cephalosporin C was isolated from a marine derived fungus, studies reporting chemistry from marine derived fungi were rare until the 1990s. A few studies were performed between 1970 and 1990 followed by a nearly exponential growth through the 1990s. Although, siccayne (**1**) was most likely the first antibiotic isolated from an obligate marine fungus, the compound had been previously isolated from a terrestrial species (Kupka et al., 1981).



vi) Current status of marine mycochemistry

Overall, research on marine-derived fungi has led to the discovery of more than 1000 new natural products including many that have novel carbon skeletons, indicating that marine-derived fungi have the potential to be a rich source of pharmaceutical leads.

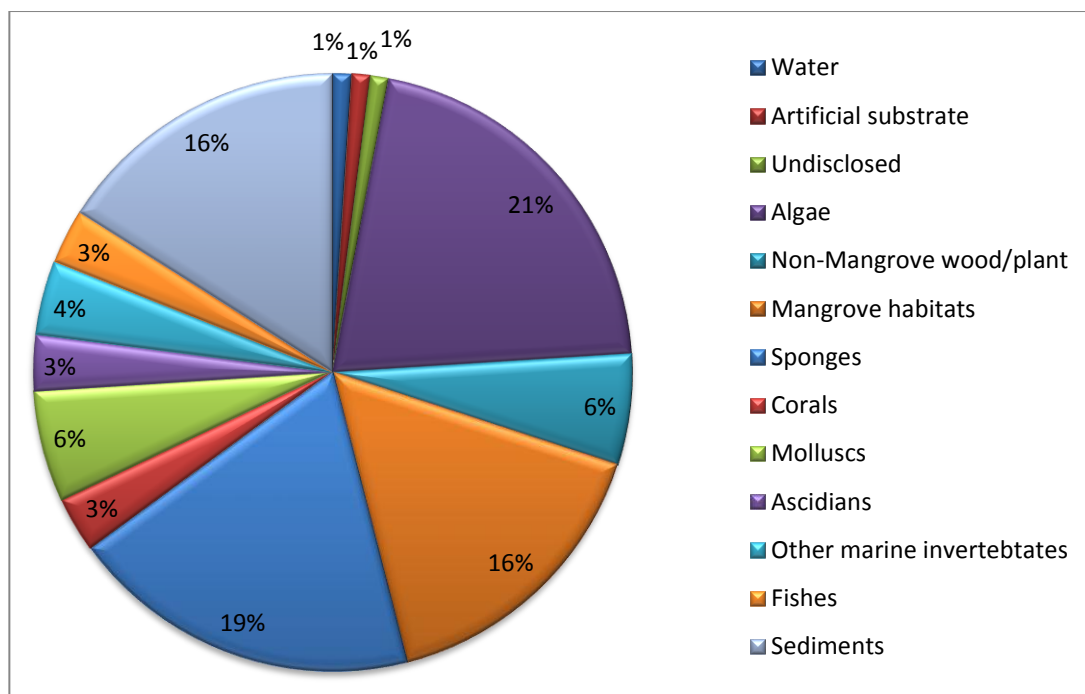


Fig 1.1: Distribution of fungal metabolites according to source of fungi

Fig. 1.1 gives an overview of new chemical structures from marine-derived fungi reported in the literature until mid-2010 classified according to the source of the fungi. Based on these considerations, it can be estimated that roughly two-thirds of all new compounds reported from marine fungi are derived from isolates from living matter, while the remaining compounds are due to fungi from non-living sources, most notably sediments. Within the individual groups, algae are the predominant sources for fungal diversity, closely followed by sponges and mangrove habitats. An interesting newly emerging source is the deep sea, and only a handful of reports exist describing new secondary metabolites from fungi derived from this habitat, all of them actually obtained from deep sea sediments.

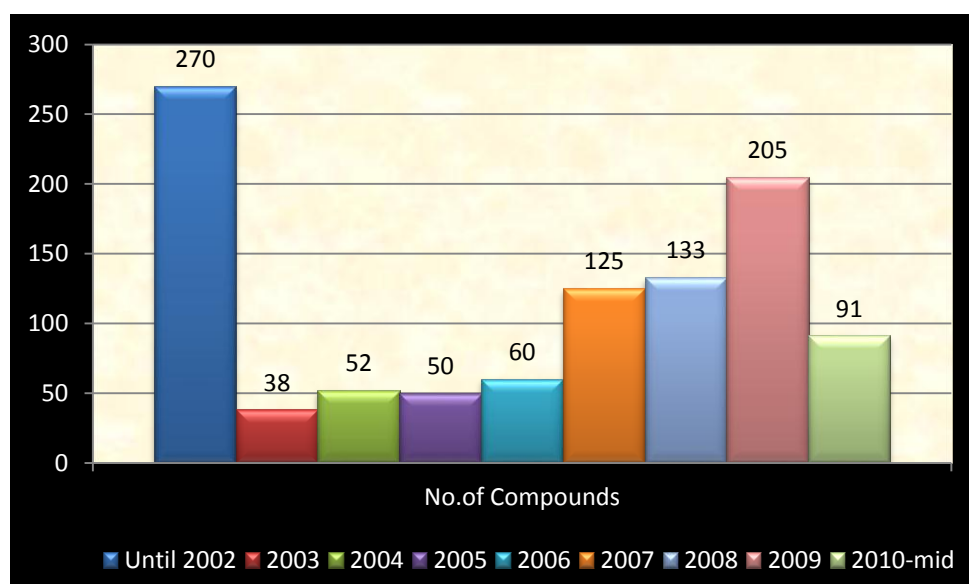


Fig 1.2: No. of fungal metabolites isolated over each year

From the number of new compounds published each year (**Fig.1.2**), it is evident that natural products chemistry of marine-derived fungi is rapidly developing, and still has not reached its climax. While, from the beginning until 2002, 272 new structures had been reported (Bugni and Ireland, 2004); in 2009 alone more than two-thirds of this figure was reached and it appears that similar figure will also be achieved in 2010, which is evident from the number of metabolites isolated in first half the year (95 new metabolites). The total number of new natural products from marine-derived fungi currently exceeds 1000.

Fig 1.3 gives an overview of new chemical structures from marine-derived fungi reported in the literature until mid-2010, based on their putative biogenetic origin. As evident from **Fig 1.3**, polyketides play a dominant role, and if prenylated polyketides and nitrogen-containing polyketides (grouped as alkaloids in this overview) are taken into account, their total share will exceed 50% of all new natural products from marine-derived fungi, which is similar to the situation of terrestrial fungi.

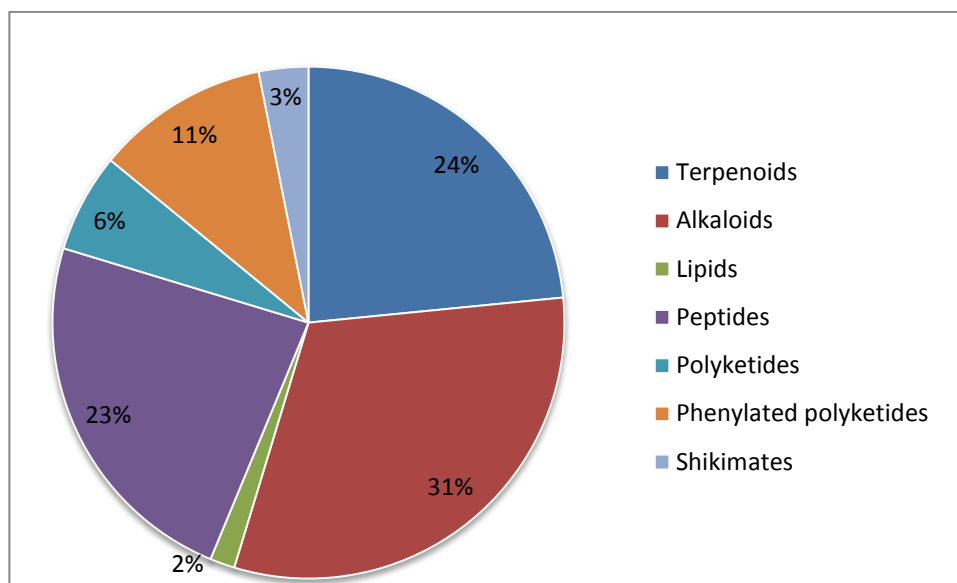


Fig 1.3: Fungal metabolites distribution according to their biosynthetic classification

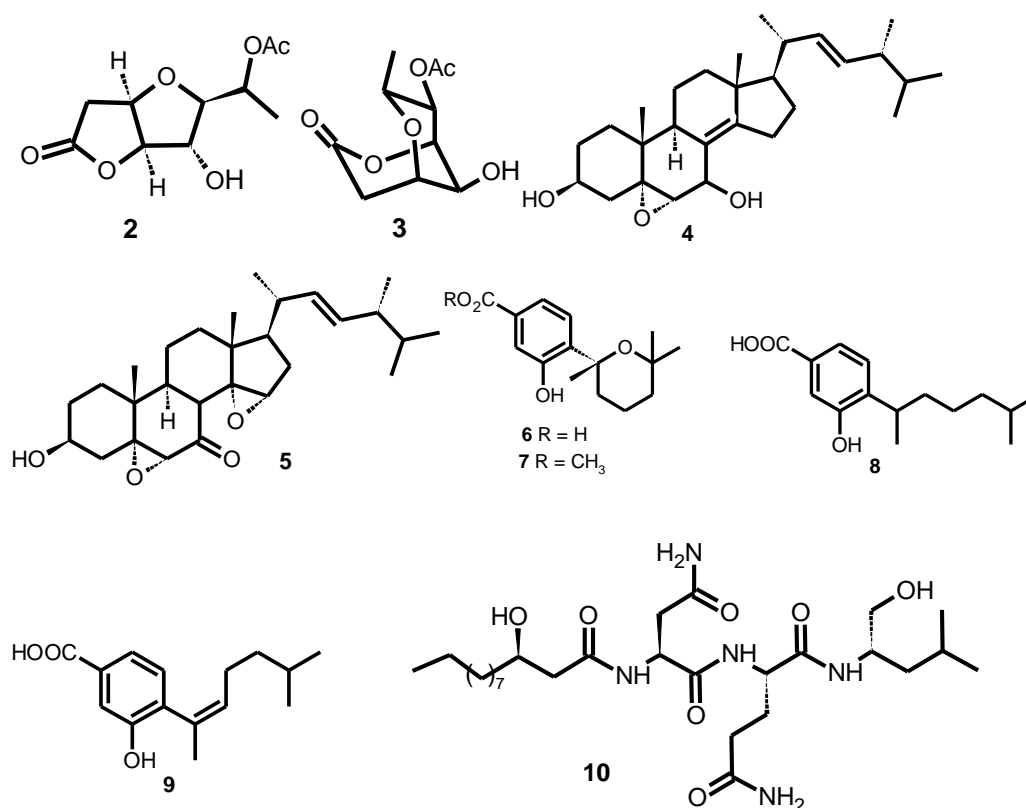
II. Metabolites from marine derived fungi reported in first half of 2010

i) Metabolites from *Aspergillus* sp.

Protulactones A (**2**) and B (**3**) were reported from an *Aspergillus* sp., isolated from an inter-tidal sediment sample collected in Korea (Sohn and Oh, 2010). Both the unusual furo[3,2-b] furan ring system present in **2** and the dioxabicyclo[3.3.1]nonane ring system as found in **3** appear to be unprecedented in fungi, at least in the genus *Aspergillus*, but are present in plant-derived metabolites such as goniofufurone from *Goniothalamus giganteus* (Fang et al., 1990), or a tetraketide from *Euscaphis japonica* (Takeda et al., 2000).

A. awamori isolated from soil around the mangrove plant *Acrostichum speciosum* in Hainan, China, yielded the two new oxidized sterols, (22*E*)-7 α -methoxy - 5 α ,6 α -epoxyergosta-8(14),22-dien-3 β -ol (**4**) and (22*E*)-3 β -hydroxy-5 α ,6 α ,8 α ,14 α -

diepoxyergosta-22-en-7-one (**5**), both of which displayed weak cytotoxic activity towards the lung cancer cell line A549 (Gao et al., 2010). Conformational analysis on the basis of the observed NOEs in the ROESY spectrum indicated that the cyclohexene oxide system in ring B of **5** adopted an *endo*-boat rather than a half-chair conformation.

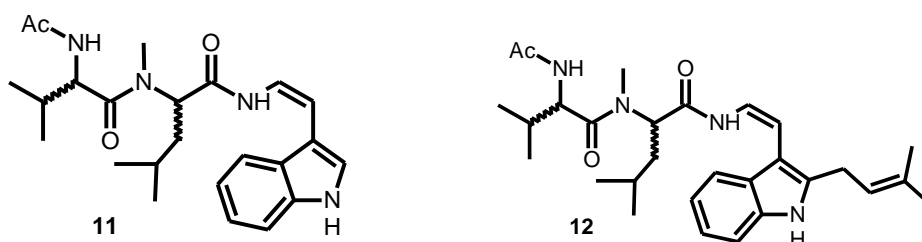


Four new phenolic bisabolane-type sesquiterpenoids, (+)-sydowic acid (**6**), (+)-methyl sydowate (**7**), 7-deoxy-7,14-didehydrozydonic acid (**8**) and 7-deoxy-7,8-didehydrozydonic acid (**9**), together with the known (+)-sydonic acid were isolated from an *Aspergillus* sp., obtained from the Chinese gorgonian *Dichotella gemmacea* (Wei et al., 2010a). The enantiomer of **6** [(-)-sydowic acid], as well as the corresponding racemate and sydonic acid, had previously been obtained from terrestrial strains of *Aspergillus sydowi* (Hamasaki et al., 1978; 1975a, 1975b). Compounds **6**, **7** and sydonic acid exhibited weak antibacterial activity against *Staphylococcus aureus*, but were inactive against methicillin resistant *S. aureus*.

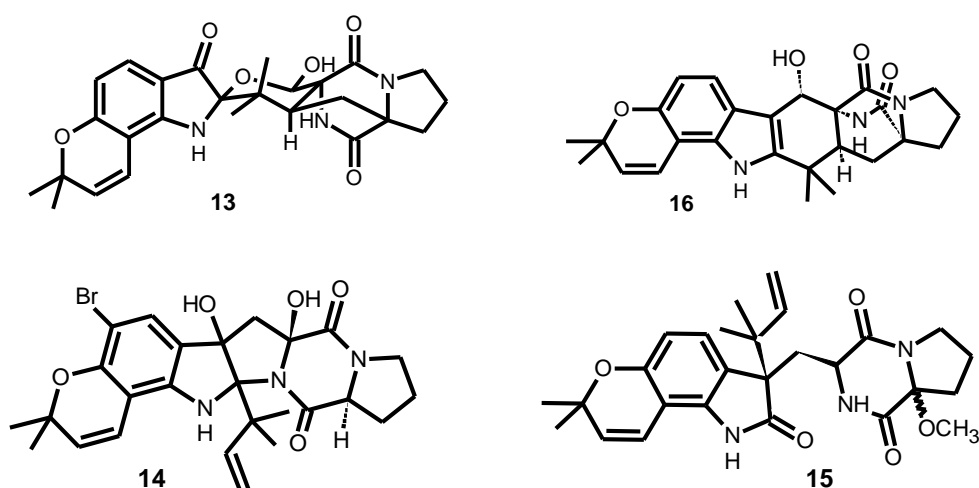
A new lipopeptide, fellutamide C (**10**), was isolated from a strain of *A. versicolor*, which was obtained from the Korean sponge *Petrosia* sp (Lee et al., 2010). Compound **10** displayed mid-range cytotoxicity against several cancer cell lines,

and is a derivative of the previously described fellutamides A and B, metabolites of a marine fish-derived *Penicillium fellutanum*, (Shigemori et al 1991), which however were not detected in the extract of *A. versicolor*.

Japanese brown alga *Sargassum* sp. derived fungus *Aspergillus* sp. produced terpeptin derivatives, JBIR-81 (**11**) and JBIR-82 (**12**), along with terpeptin (Izumikawa et al., 2010). The latter is a mammalian cell cycle inhibitor initially described from *A. terreus* (Kagamizono et al, 1997). All three terpeptins displayed free radical scavenging activities as expressed by their ability to protect N18- RE-105 cells against L-glutamate toxicity, and were considerably more potent than the positive control, α -tocopherol.

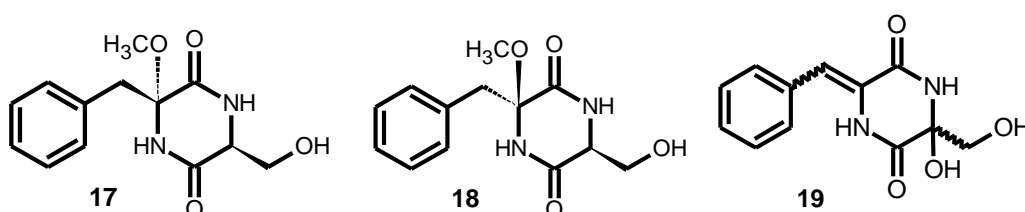


Notoamides O–Q (**13–15**) and notoamide R (**16**) were reported from *Aspergillus* sp. (Tsukamoto et al., 2010), but the latter compound appears to be identical to 21-hydroxystephacidin A, a metabolite of Marine fungi *A. ostianus*, which also produced notoamide F (Kito et al., 2009). Compound **13** possesses a hemiacetal/hemiaminal ether function, which is unprecedented in the notoamide class of compounds.

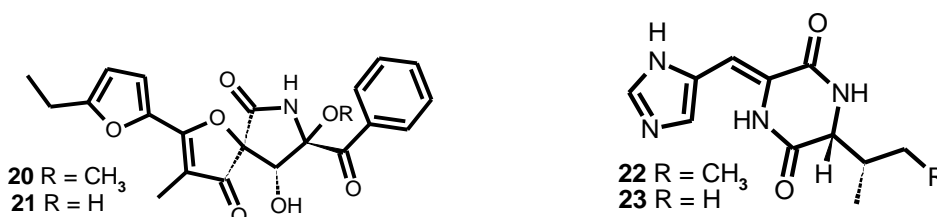


Three new dioxopiperazine metabolites **17–19** were isolated from a strain of *A. fumigatus* which was obtained from marine sediments collected in China.

Compounds **17-19** were devoid of cytotoxic activity (Zhao et al., 2010). The fungus *A. sydowi*, isolated from marine sediments collected in China, was identified as the producer of two additional pseurotin derivatives, azaspirofurans A (**20**) and B (**21**), characterized by the presence of an additional furan ring in the side chain (Ren et al., 2010). Compound **20** exhibited moderate cytotoxicity against the A549 cell line.



The fungus *Aspergillus*, isolated from an unidentified Japanese calcareous sponge, produced JBIR-74 (**22**) and JBIR-75 (**23**), which were devoid of cytotoxic or antimicrobial activity (Takagi et al., 2010).

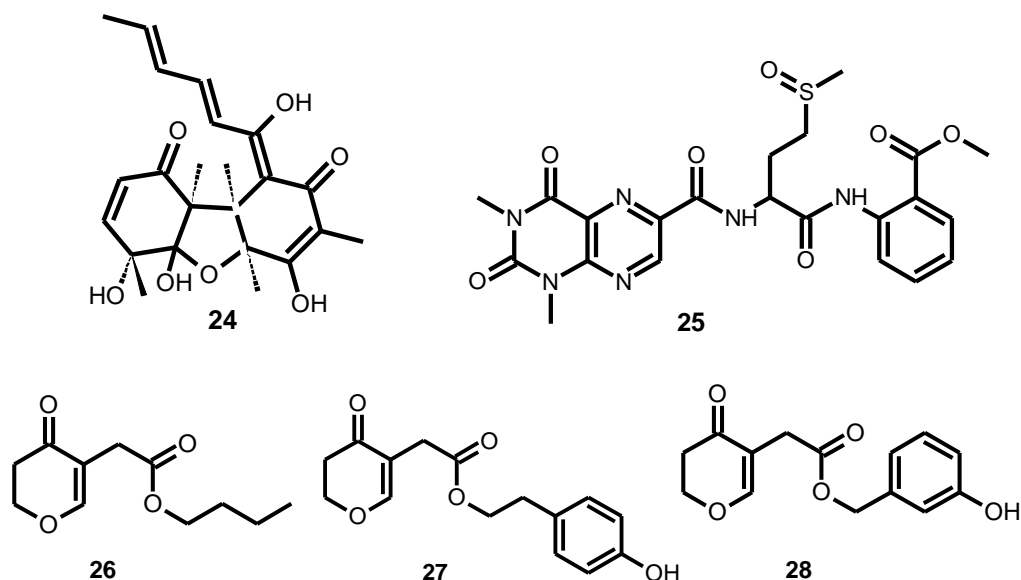


ii) Metabolites from *Penicillium* sp.

An isolate of *P. citrinum* obtained from an unidentified Japanese demosponge, yielded JBIR-59 (**24**), along with a series of known sorbicillinoids (Ueda et al., 2010). Based on structural considerations, **24** is assumed to be formed by reaction of sorbicillin and the known trihydroxyquinol USF 406A, a metabolite of the soilborne fungus *Mortierella* sp.

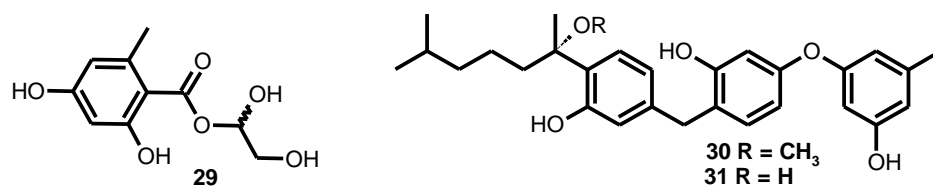
A *Penicillium* sp., isolated from the red alga *Laurencia* sp. collected in the Bahamas Islands, yielded penilumamide (**26**), an unusual alkaloid combining a lumazine system with L-methionine sulfoxide and an anthranilic acid ester (Meyer et al., 2010). The 1,3-dimethyllumazine-6-carboxamide moiety within a natural product had previously only been reported from the freshwater leech *Limnatis nilotica* (Voerman et al., 2005). Compound **25** was inactive when tested for

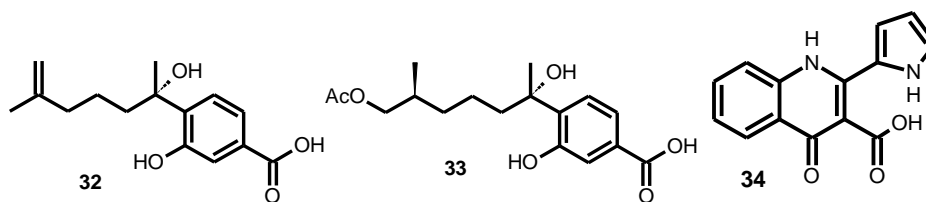
cytotoxic and antimicrobial properties, and also did not affect cellular Ca^{2+} signalling in neuroendocrine cells. The fungus *Penicillium* sp., isolated from the tree *Clerodendrum inerme* growing in the inter-tidal zone of the South China Sea, yielded three new esters of desoxyapatulinic acid **26-28** (Wu et al., 2010). Desoxyapatulinic acid, which was also detected in the culture broth, is a common mycotoxin initially reported from *Penicillium patulum* (Scott et al., 1972).



The endophytic fungus *P. commune*, which was isolated from the Chinese semi-mangrove plant *Hibiscus tiliaceus*, afforded a new ester of orsellinic acid with glycerol (**29**), besides a suite of mostly structurally simple known metabolites (Yan et al., 2010).

Another Chinese mangrove (*Excoecaria agallocha*) derived fungus *P. expansum* was identified as the producer of expansols A (**30**) and B (**31**), bisabolane sesquiterpenoids substituted with a diphenyl ether unit, and two new phenolic bisabolane sesquiterpenoids, (S)- (+)-11-dehydrosydonic acid (**32**) and (7S,11S)- (+)-12-acetoxysydonic acid (**33**) (Lu et al., 2010). Compound **30** exhibited moderate cytotoxicity against the HL-60 cell line, while **31** inhibited the proliferation of A549 and HL-60 cells, while **32** and **33** were inactive.

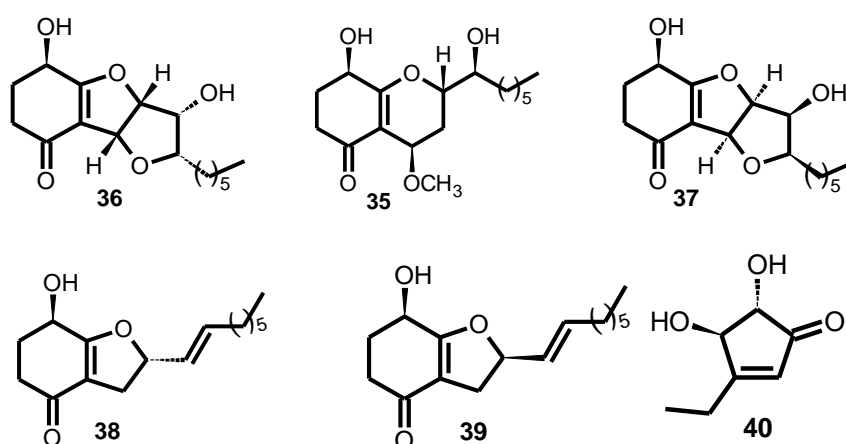




Yet another *Penicillium* sp., isolated from the Chinese mangrove plant *Acanthus ilicifolius* was the producer of isomeric pyrrolyl 4-quinolinone alkaloid penicinoline (**34**) (Shao et al., 2010a). Upon methylation of this compound, an N-methylated lactam was unexpectedly formed. Compound **34** exhibited potent cytotoxicity towards the 95-D and HepG2 cell lines, and also displayed strong insecticidal activity against *Aphis gossypii*.

iii) Metabolites from *Trichoderma* sp.

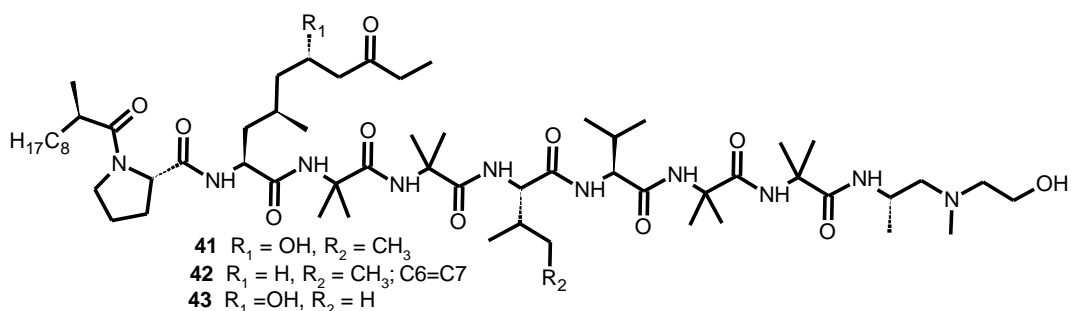
The fungus *T. koningii*, isolated from marine mud of the South China Sea, produced 7-O-methylkoninginin D (**35**) and trichodermaketones A–D (**36-39**), together with four koningin derivatives (Song et al., 2010). Koninginin D (**39**) had previously been reported from a terrestrial strain of *T. Koningii* (Dunlop et al., 1989). Compounds **35-39** did not display antimicrobial activity against methicillin-resistant *Staphylococcus aureus* or *Candida albicans*, but **36** showed synergistic antifungal activity against *C. Albicans* with ketoconazole, but at rather high concentrations.



The fungus *Trichoderma* sp. was isolated from marine sediment in the South China Sea and found to produce a new cyclopentenone, trichoderone (**40**), besides a known steroid (You et al., 2010). Compound **40** displayed selective cytotoxicity

towards six cancer cell lines and were moderately active, probably by inducing apoptosis.

The fungal strain *Trichoderma* sp., isolated from an unidentified marine sponge of undisclosed geographic origin, produced three new aminolipopeptides, trichoderins A (**41**), A1 (**42**) and B (**43**) (Pruksakorn et al., 2010). Compounds **41-43** are characterized by the presence of a 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD) moiety, which they share with previously described aminolipopeptides such as the leucinostatins or trichopolyns, metabolites of *P. lilacinum* (Arai et al., 1973) and *T. polysporum*, (Fuji et al, 1978) respectively. Compounds **41-43** showed potent anti-mycobacterial activity against *Mycobacterium smegmatis*, *M. bovis* and *M. tuberculosis*, with MIC values in the range 0.02–2.0 mg/ml, and were effective against both actively growing and dormant states

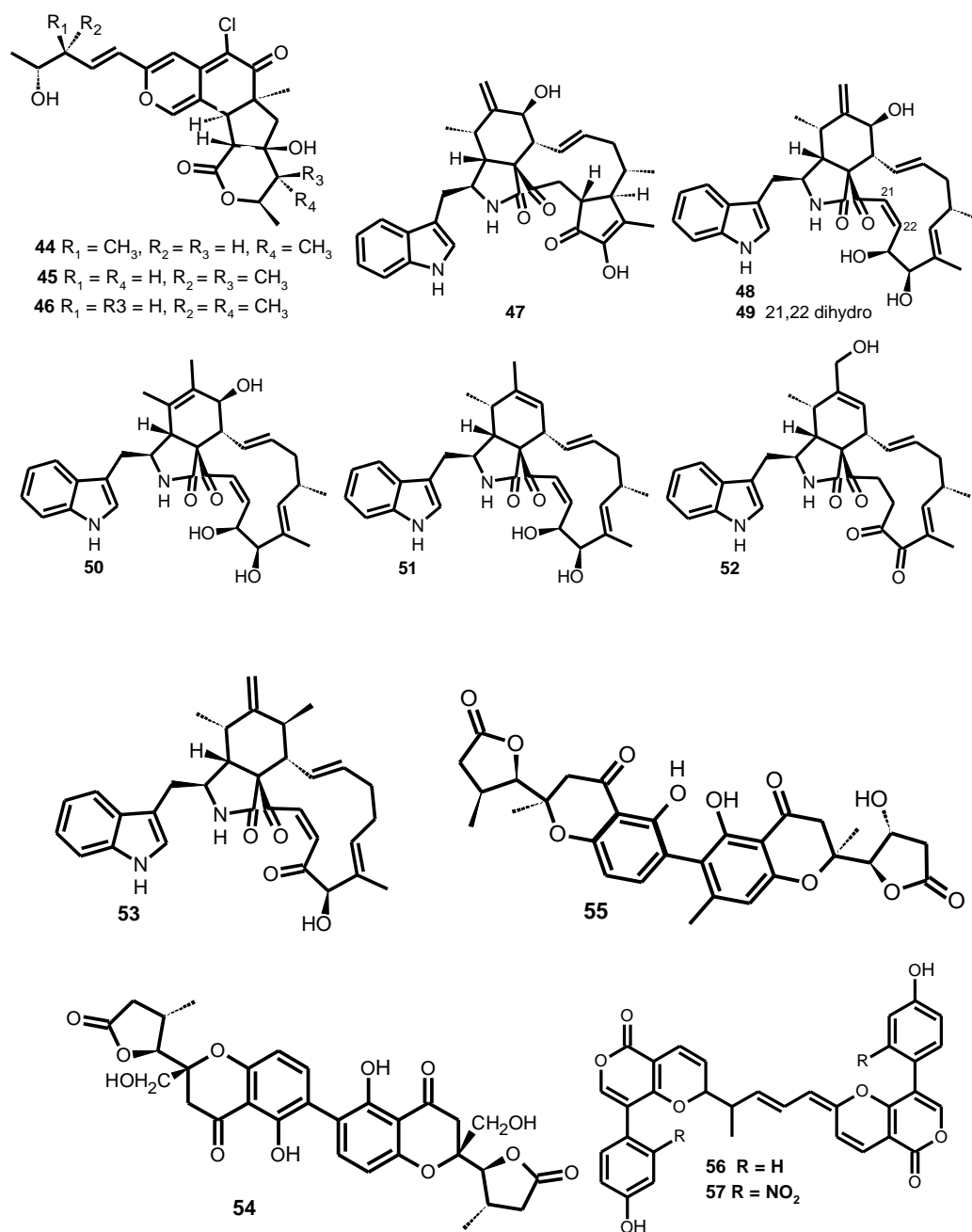


iv) Metabolites from *Chaetomium globosum*

11-epi-chaetomugilin A (**44**) and 40-epichaetomugilin A (**45**), were discovered from fungus *Chaetomium globosum*, originally isolated from the Japanese fish *Mugil cephalus* (Muroga et al., 2010). Both the compounds are stereoisomers of chaetomugilins A (**46**) previously isolated from the same culture (Yamada et al., 2008). Compound **44** exhibited moderate activity against P388 and HL-60 cell lines, but marginal activity against L1210 and KB cell lines, whereas **45** was only marginally active against all cell lines.

Another endophytic fungus *Chaetomium globosum* derived from the Chinese green alga *Ulva pertusa* produced seven new cytochalasan derivatives of the chaetoglobosin class, cytoglobosins A–G (**47-53**), together with the structurally related known compounds, isochaetoglobosin D and chaetoglobosin F.

Compounds **49** and **50** exhibited moderate cytotoxic activity against the A-549 cell line.

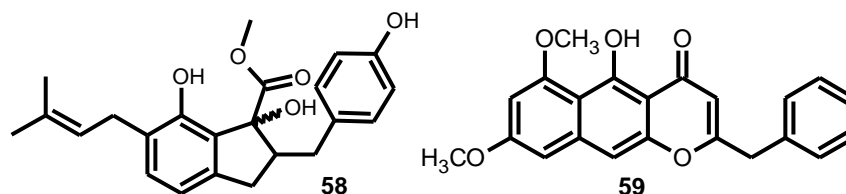


v) Metabolites from *Phomopsis* sp

The endophytic fungus *Phomopsis* sp., isolated from the Chinese mangrove tree *Excoecaria agallocha*, produced phomopsis-H76 A (**54**), a dimeric chromanone closely related to monodictyochrome B (**55**), as well as phomopsis-H76 B (**56**),

and C (**57**), structurally unique dimeric pyrano[4,3-b]pyran-5(2H)-ones (Yang et al., 2010).

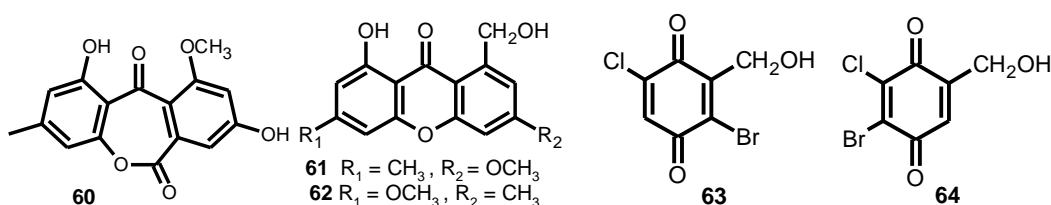
Phomoidene A (**58**), a rare example of an indene derivative from nature, was described from the fungus *Phomopsis* sp., obtained from a sediment sample in a Chinese mangrove habitat, but was inactive when tested for cytotoxic properties (Chen et al., 2010).



A new naphtho- γ -pyrone, 2-benzyl-5-hydroxy-6,8-dimethoxy-4H-benzo[g]chromen-4-one (**59**), was reported from the endophytic fungus *Phomopsis* sp., isolated from the Chinese mangrove tree *Excoecaria agallocha* (Huang et al., 2010a).

vi) Metabolites from *Phoma* sp.

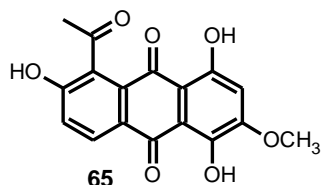
A new lactone, 1,8-dihydroxy-10-methoxy-3-methyldibenzo[b,e]oxepine-6,11-dione (**60**), and two new xanthenes, 1-hydroxy-8-(hydroxymethyl)-6-methoxy-3-methyl-9H-xanthen-9-one (**61**) and 1-hydroxy-8-(hydroxymethyl)-3-methoxy-6-methyl-9H-xanthen-9-one (**62**) were reported from the endophytic fungus *Phoma* sp., isolated from the roots of the Chinese mangrove plant *Avicennia marina* (Pan et al., 2010). Compounds **60-62** were inactive when tested for cytotoxic properties.



Bromochlorogentisylquinones A (**63**) and B (**64**) are metabolites of the fungus *Phoma herbarum*, isolated from the Korean red alga *Gloiopeltis tenax*, which were formed upon addition of calcium bromide to the culture medium, together with the known chlorogentisyl alcohol and gentisyl alcohol (Nenkep et al., 2010).

All compounds displayed significant radical scavenging properties in the DPPH assay.

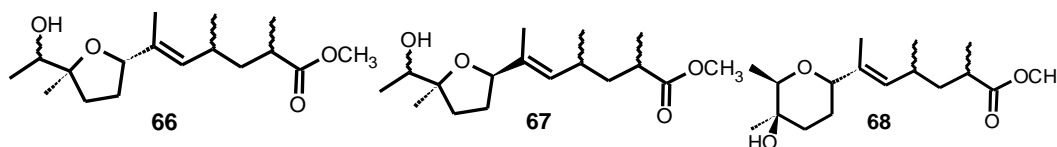
vii) Metabolites from Fusarium sp.



A new anthraquinone, 5-acetyl-2-methoxy-1,4,6-trihydroxy-anthraquinone (**65**), besides a series of known naphthoquinones from the endophytic fungus *Fusarium* sp. from an undisclosed Chinese mangrove plant is the only report on this genus in first half of 2010 (Shao et al., 2010b).

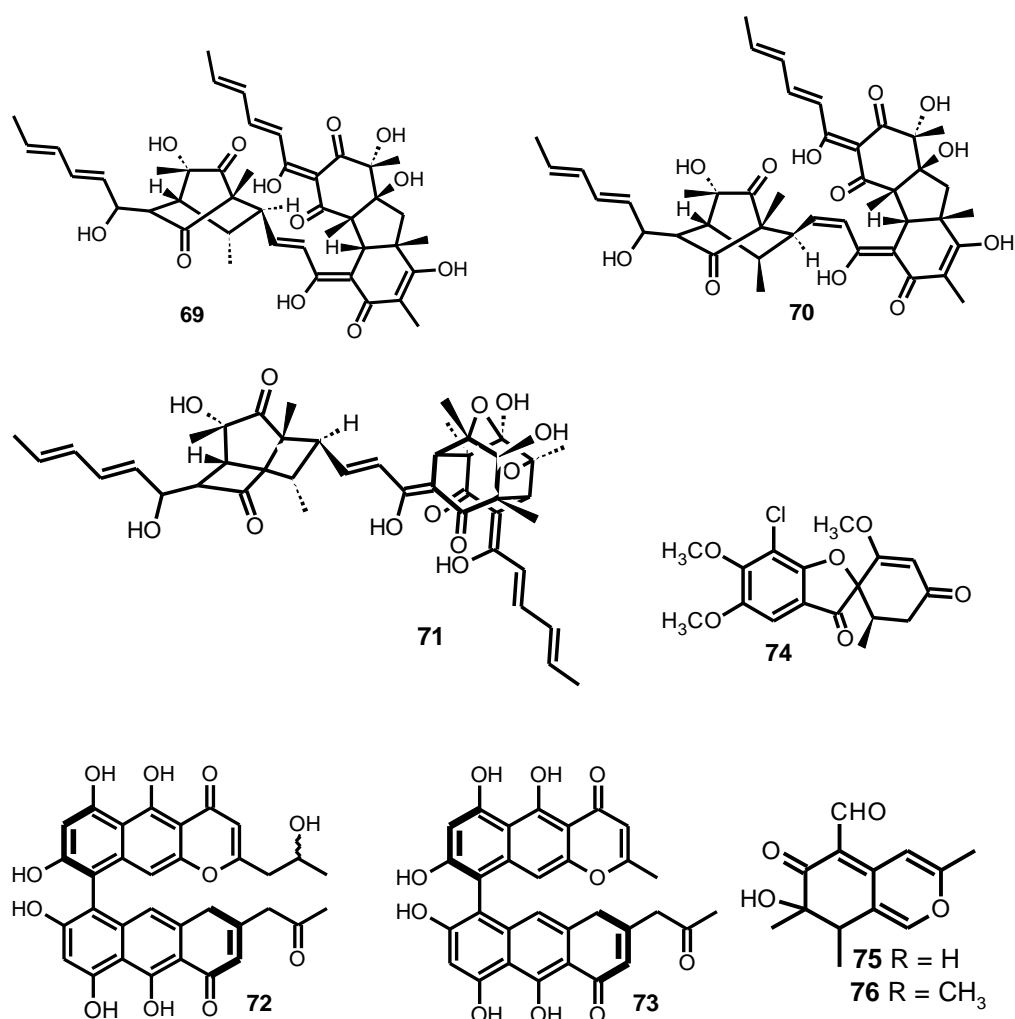
viii) Metabolites from other species

The fungus *Rhizopus* sp. was isolated from the Chinese bryozoan *Bugula* sp., and was found to produce aspericins A–C (**66-68**), along with the related asperic acid (Wang et al., 2010). Asperic acid, so far the only compound reported from nature with the same carbon skeleton as present in **66-68**, had previously been obtained from a saltwater culture of sponge-derived *Aspergillus niger* (Varoglu and Crews, 2000). When tested for cytotoxic properties against four different cell lines, only **68** displayed moderate activity.



A deep-sea isolate of *Phialocephala* sp., collected from sediments at a depth of 5059 m yielded three new metabolites, trisorbicillinones B–D (**69-71**), all of which exhibited only very weak cytotoxicity (Li et al., 2010a). The former member of the series trisorbicillinone A was obtained from the same strain in previous studies, which was moderately cytotoxic against P388 and HL60 cells (Li et al., 2007).

In a screening effort directed at discovering tyrosine kinase inhibitors using human umbilical vein endothelial cell lysate, out of a total of 200 fungal strains tested, only the fungus *Hypocrea vinosa* isolated from beach sand collected in Japan displayed activity. Chemical analysis revealed the presence of two new bisnaphtho-g-pyrones, hypochromins A (**72**) and B (**73**), besides the closely related known SC2051, the diketocongener of **72**, which so far has only been reported in the patent literature (Miki et al., 2010). All three compounds inhibited tyrosine kinase activity in HUVEC lysate, and in addition also inhibited HUVEC proliferation, migration, and tubule formation, thus displaying antiangiogenic potential.

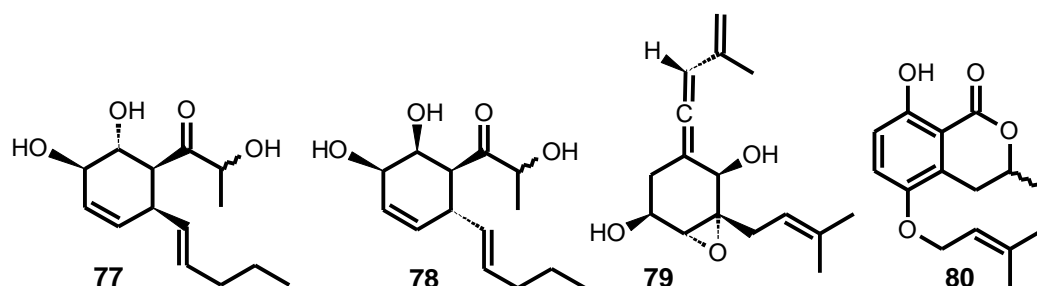


The fungus *Sporothrix* sp., isolated from an undisclosed mangrove at the South China Sea coast, yielded the new griseofulvin derivative **74** (Wen et al., 2010).

The endophytic fungus *Talaromyces* sp., isolated from the Chinese mangrove plant *Kandelia candel*, yielded two new isochromenones 7-epiaustdiol (**75**) and 8-O-methylepiaustdiol (**76**), besides a diverse series of known polyketides Liu et al., 2010).

Nigrosporanenes A (**77**) and B (**78**) were isolated from fungus *Nigrospora* sp, which was obtained from the sea fan *Annella* sp. collected Thailand (Rukachaisirikul et al. 2010). Compound **77** displayed moderate cytotoxicity towards MCF-7 and Vero cells, while **77** and **78** also showed weak radical scavenging activity.

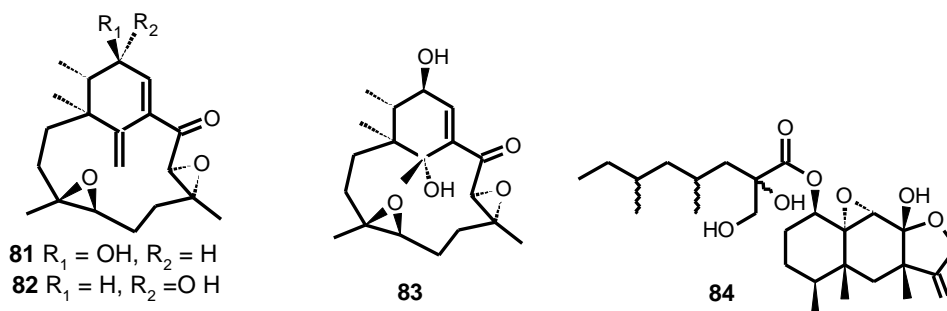
The endophytic fungus *Phaeosphaeria spartinae*, isolated from a German red alga of the genus *Ceramium*, was the source of spartinoxide (**79**), which is the enantiomer of the known compound A82775C (Elsebai, et al., 2010). The latter was previously reported from an unknown fungus of the order *Sphaeropsidales* (Sanson et al., 1991). Compound **79** showed potent inhibition of human leukocyte elastase (HLE), but was inactive against bovine trypsin acetyl cholinesterase from *electrophorus electricus*, and porcine cholesterol esterase.



8-Hydroxy-3-methyl-5-(3-methylbut-2-enyloxy)isochroman-1-one (**80**) was detected in the culture broth of the fungus *Cephalosporium* sp., isolated from an undisclosed Chinese mangrove plant (Wei et al., 2010b).

An unidentified fungus that was isolated from the surface of the Japanese brown alga *Ishige okamurae*, and based on DNA sequence analysis was grouped into the order *Dothideales* was identified as the producer of phomactin I (**81**), 13-epi-phomactin I (**82**) and phomactin J (**83**) (Ishino et al., 2010). Phomactins are an intriguing class of fungal diterpenes which have raised considerable attention due

to their activity as platelet-activating factor (PAF) antagonists (Goldring and Pattenden, 2006), and were initially discovered in cultures of the fungus *Phoma* sp. obtained from the shell of the crab *Chionoecetes opilio* (Sugano et al., 1994), and it is therefore remarkable that the unidentified fungus in this study was taxonomically not closely related to the genus *Phoma*.

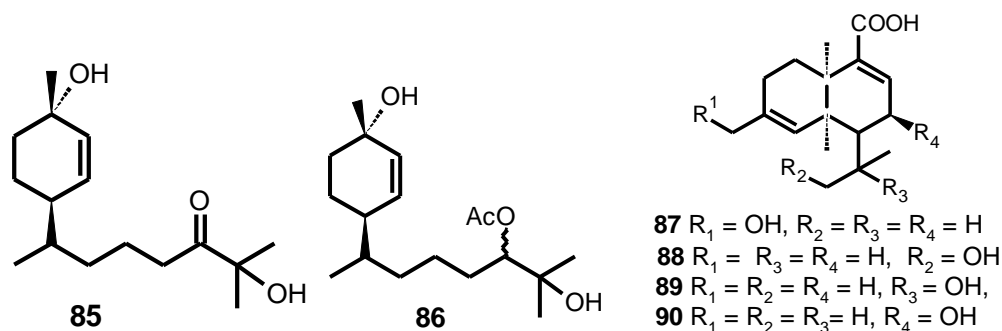


A marine-derived ascomycete related to the genus *Cryptosphaeria*, isolated from an unidentified ascidian in the Bahamas, produced cryptosphaerolide (**84**), an eremophilane type sesquiterpenoid bearing both an exo-methylene and an ester function (Oh et al., 2010). **84** is related to berkleasmin A, a metabolite of the terrestrial saprobic fungus, *Berkleasium nigroapicale* (Isaka et al., 2009). In the Mcl-1/Bak fluorescence resonance energy transfer (FRET) assay, **84** displayed inhibitory activity towards the Mcl-1 protein, a cancer drug target involved in apoptosis. In addition, **84** also showed significant cytotoxicity against the HCT-116 human colon carcinoma cell line, with IC50 values in the lower mM range.

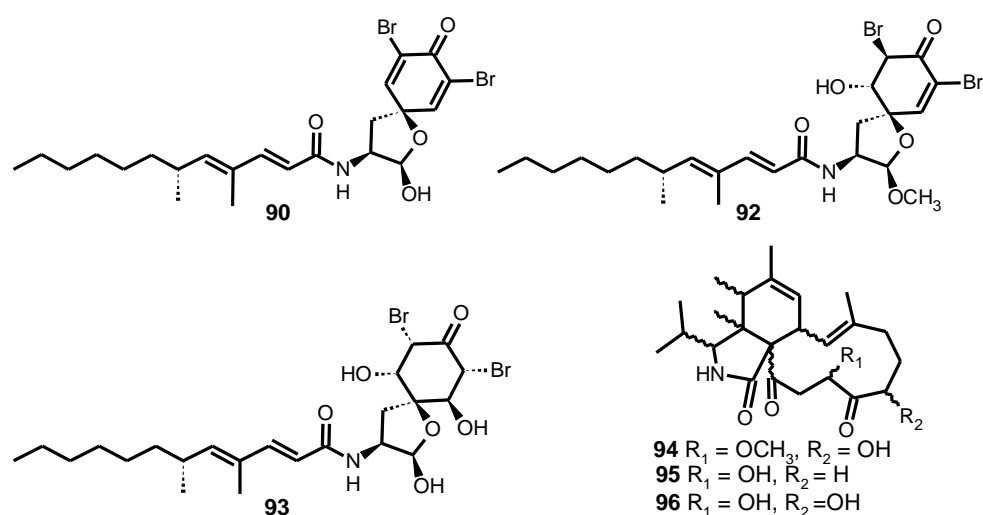
Verticinols A (**85**) and B (**86**) are two new hydroxylated bisabolane-type sesquiterpenes which were reported from the fungus *Verticillium tenerum*, isolated from an unidentified marine alga (Almeida et al., 2010a). Although **85** and **86** were tested for a variety of effects, i.e. antibacterial, antifungal, antialgal, antiplasmodial, antiviral, and cytotoxic activity as well as protein kinase inhibition or fat-accumulation inhibitory activity against 3T3-L1 murine adipocytes, they did not display significant activity in any of these test systems.

Four new hydroxylated sclerosporin derivatives 15-hydroxysclerosporin (**87**), 12-hydroxysclerosporin (**88**), 11-hydroxysclerosporin (**89**) and 8-hydroxysclerosporin (**90**), besides the known (+)-sclerosporin were produced by the fungus *Cadophora malorum*, isolated from the green alga *Enteromorpha* sp. Upon long-term fermentation in a medium supplemented with artificial sea salt

(Almeida et al., 2010a). Sclerosporin was initially characterised as a sporogenic metabolite of a terrestrial isolate of *Sclerotinia fruticula*, and is a rare example of a fungal-derived cadinane-type sesquiterpenes (Katayama et al., 1979). Compounds **87-90** were subjected to a variety of assays, but were found devoid of significant biological activity, apart from **90**, which showed a weak fat-accumulation inhibitory activity against 3T3-L1 murine adipocytes.



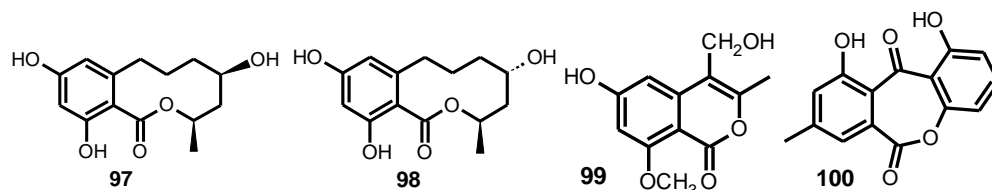
Fungus *Gymnascella dankaliensis*, isolated from *Halichondria japonica* collected off Osaka in Japan was known to produce gymnastatins F–H and the fatty acid- or polyketide-derived gymnamide (Amagata et al., 2006). The same fungus yielded the brominated analogues gymnastatins I–K (**91-93**), when cultured in a bromine-containing medium. All of them exhibited potent cytotoxicity towards the murine P388 lymphocytic leukemia cell line. Moreover, **91** and **92** displayed pronounced activity against a panel of 39 human cancer cell lines (Amagata et al., 2010).



An investigation of fungus *Spicaria elegans* yielded aspochalasin congeners, aspochalasins R–T (**94-96**), all of which proved inactive in terms of cytotoxic effects (Lin et al., 2010).

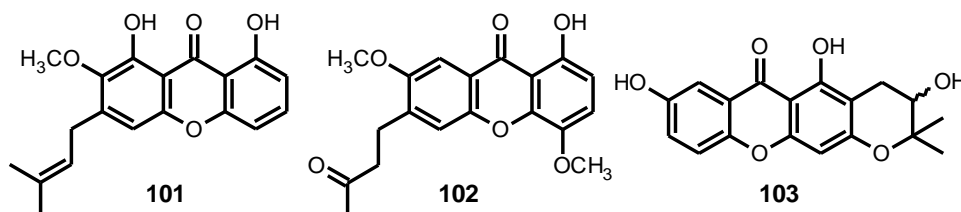
Metabolites from Unidentified fungi

An unidentified endophytic fungus, isolated from the Chinese mangrove plant *Sonneratia apetala*, produced two new 10-membered lactones, (3*R*,5*R*)-sonnerlactone (**97**) and its (3*R*,5*S*) diastereoisomer (**98**) (Li et al., 2010b). Both **97** and **98** displayed weak inhibitory effects towards the multi-drug resistant cancer cell line KV/MDR.



An unidentified endophytic fungal strain isolated from the Chinese mangrove *Bruguiera gymnorrhiza* produced two new aromatic lactones, 1,10-dihydroxy-8-methyl dibenzo[b,e]oxepine-6,11-dione (**99**) and 3-(hydroxymethyl)-6,8-dimethoxy-2H-chromen-2-one (**100**), along with a known coumarin derivative and the 8-demethyl congener of **100**, which so far had only been obtained synthetically, but not as a natural product (Huang et al., 2010b).

1,7-Dihydroxy-2-methoxy-3-(3-methylbut-2-enyl)-9H-xanthen-9-one (**101**) and 1-hydroxy-4,7-dimethoxy-6-(3-oxobutyl)-9H-xanthen-9-one (**102**) were reported from an unidentified endophytic fungus, isolated from the Chinese mangrove tree *Avicennia marina* (Huang et al., 2010c). Compounds **101** and **102** displayed mild cytotoxicity towards the KB and KBV200 cell lines.



3,5,8-Trihydroxy-2,2-dimethyl-3,4-dihydropyrano[3,2-b]xanthen-6(2H)-one (**103**) was reported from an unidentified endophytic fungus, obtained from a Chinese mangrove tree of the genus *Avicennia* (Huang et al., 2010d).

x) Concluding remarks

It may be concluded that the ubiquitous fungi *Aspergillus* and *Penicillium* continues to be the prolific producers of new metabolites (**Fig 1.4**)

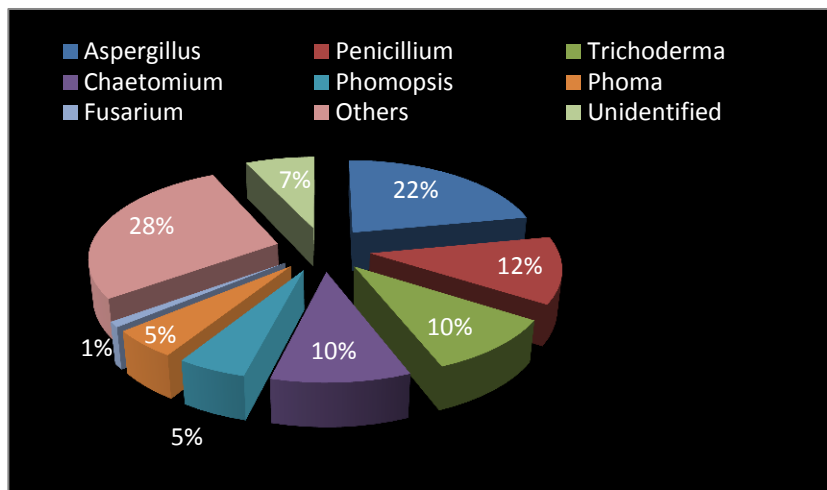


Fig 1.4 Distribution of metabolites reported in first half of 2010 according to fungal genus.

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