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Fungal endophytes: A potential source of antifungal compounds

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1. ABSTRACT

The prevalence of invasive fungal infections has increased significantly during organ transplantation, cancer chemotherapy and allogeneic bone marrow transplantation. However, only a limited number of antifungal agents are currently available for the treatment of life-threatening fungal infections. Although new antifungal agents have been introduced in the market, the development of resistance to antifungal drugs has become increasingly apparent, especially in patients with long term treatment. Microbial natural products have always been an alternative natural source for the isolation of novel molecules for various therapeutic applications. Endophytes are the microorganisms that colonize internal tissues of all plant species and represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural and industrial arenas. In the present review several metabolites obtained from endophytic fungi with a potential as antifungal agents are mentioned with bioactivity including volatile organic compounds. The compounds reported here with a diverse scaffold can be a potential starting point for new antifungal agents either as such or after chemical modification.

2. INTRODUCTION

The role of fungi as a source of antibiotics was first established by the pioneering work of Sir Alexander Fleming in 1928 leading to the discovery of Penicillin (1), followed by the work of Brotzu who found Cephalosporin C (2, 3). These discoveries are milestones in the discovery of antibacterials from fungi. The discovery of Griseofulvin (4) gave the first antifungal agent of fungal origin. Well known antifungals are Amphotericin B, Nystatin and Cycloheximide but they possess inherent toxicity problems. Antifungal compounds can also be used as agrochemicals to control plant diseases. But such chemicals used as agrochemicals have various health hazard problems. Hence there is a need for naturally occurring compounds which are less toxic, target specific, easily degradable and can compete with synthetic pesticides. The whole area of entomopathogenic fungi as biopesticides has been developed with crop destroying pests in mind, specially Helicoverpa armigera. Compounds like Echinocandin (5), Mulundocandin (6), Papulacandin (7) and Pneumocandin (8) isolated from fungi have been developed as antifungal agents for human fungal infections eg. Caspofungin, Micafungin, Anidulafungin (9-11). These molecules are beta- (1,3)-glucan synthesis inhibitors and have anti-

Pneumocystis carinii activity. Pneumocystis carinii infection is now very common in immunocompromised hosts. It is the most common lethal infection in patients with AIDS. Pseudomycins, represent a family of lipopeptides that are active against plant and human pathogenic fungi and are isolated from Pseudomonas syringae, a plant-associated bacterium (12). It can be a candidate molecule for use in human medicine especially after structural modification which can remove mammalian toxicity (13-14). The problem of resistance against these drugs and also against the other existing drugs e.g. azoles (Ketoconazole, Miconazole, Itraconazole, Fluconazole, Voriconazole, Posaconazole etc) still exists (15-16).

Natural products from medicinal plants and microorganisms are the most consistent and productive source for the "first-in-class" drugs (17). Recently, a great deal of interest has been generated by discovery of remarkable pharmacological agents from endophytic fungi (18). There is a whole area of endophytic fungi that has not really been explored or exploited in any great detail.

Endophytes living asymptomatically within plant tissues have been found in almost all plant studied to date (19). They play a major role in physiological activities of host plants influencing enhancement of stress, insect, nematode and disease resistance (20-23). Endophytes can also accelerate plant growth and nitrogen fixing capabilities of host plants (24-25). Endophytes constitute a valuable source of bioactive secondary metabolites (26-29) and would be a source of new drugs of biotechnological importance and plant disease management programs (22, 30-31).

Each of the nearly 300,000 species of land plant on earth is likely to host one or more endophyte species (32). Strobel and Daisy (18) commented that endophytes could be a goldmine of secondary metabolites. Pestalotiopsis sp. can be considerd as "the E. coli of the rain forests" and P. microspora, a "microbial factory" of bioactive secondary metabolites. According to them a variety of chemical structures are seen such as Taxol, Torreyanic acid, Ambuic acid, Cryptocandin, Subglutinol A and B and others.

New organisms and many novel natural products from endophytic fungi inhibit or kill a wide variety of harmful microorganisms like bacteria, fungi, viruses and protozoans that affect humans and animals (33). Endophytes do produce secondary metabolites in culture, but the temperature, composition of the medium and the degree of aeration will affect the amount and kind of compounds produced (34), including steroids, xanthones, phenols, isocoumarins, perylene derivatives, quinines, furandiones, terpenoids, depsipeptides and cytochalasins (33-37).

Here we are covering the metabolites obtained from endophytic fungi, belonging to various classes of fungi along with unidentified fungi and their potential as antifungal agent. Many of these compounds are shown in Table 1.

3. ANTIFUNGAL COMPOUNDS PRODUCED BY ENDOPHYTIC FUNGI

3.1. Compounds produced by coelomycetes

The genus Pestalotiopsis exist as an endophyte in most of the world's rainforests and are extremely biochemically diverse. Some examples of products coming from this group of endophtyes are described herein. Ambuic acid (1) (Figure 1) is a highly functionalized cyclohexenone and was isolated from endophytic fungi Pestalotiopsis microspora, P. guepinii and Monochaetia sp. from rainforest plants. It was active against Pythium ultimum with an MIC of 7.5 mcg/ml and also active against several Fusarium sp., Diplodia natelensis and Cephalosporium gramineum but was found inactive against phytopathogenic fungi namely Pyricularia oryzae, Rhizoctonia solani, Botrytis cinerea and human pathogenic fungi suggesting that the role played by this compound in the fungus plant relationship is that of providing protection to the plant by virtue of its antimycotic activity (38-39). Ambuic acid also targets the quorum sensing mediated virulence expression of gram-positive bacteria (40). Pestacin (2) and Isopestacin (3) (Figure 1) were obtained from culture fluids of P. microspora, an endophyte isolated from the combretaceaous tree, Terminalia morobensis. Pestacin and isopestacin display antimycotic as well as antioxidant activities (41- 42).

Another species of Pestalotiopsis namely P. jesteri, an endophytic fungal species isolated from the inner bark of small limbs of a Fragraea bodenii located at the singsing grounds of the Aluakambe village from the Septik river area of Papua New Guinea, produces the highly functionalized cyclohexenone epoxides, Jesterone (4) and Hydroxyjesterone (5) (Figure 1). Jesterone was found active againt Pythium ultimum, Aphanomyces sp., Phytophthora citrophthora, Phytophthora cinnamomi, Sclerotinia sclerotiorum, Rhizoctonia solani, Geotrichum candidum and Pyricularia oryzae with MIC of 25, 6.5, 25, 6.5, 100, 25, >100 and 25 mcg/ml. Hydroxyjesterone was also found active against Aphanomyces sp. and Phytophthora cinnamomi with MIC of 125 and 62.5 mcg/ml (43).

Pestalachloride A-C (6-8) (Figure 1), three new chlorinated benzophenone derivatives were isolated from an endophytic fungus Pestalotiopsis adusta. The fungus was isolated from unidentified tree in Xinglong near Dongzai Hainan Province, Republic of China. These compounds were evaluated for antifungal activity against the plant pathogenic fungi namely Fusarium culmorum, Gibberella zeae and Verticillium aibo-atrum. Pestalachloride A displayed potent antifungal activity against F. culmorum, with an IC_{50} value of 0.89 micromolar, while Pestalachloride B exhibited remarkable activity against G. zeae, with an IC_{50} value of 1.1 micromolar whereas Pestalachloride C did not show noticeable *in-vitro* antifungal activities against F.

Table 1. Antifungal compounds reported from endophytic fungi

Sr. No	Fungus	Plant Source	Compounds isolated
1	*Pestalotiopsis microspora, P. guepinii and Monochaetia* sp.	*Taxus baccata, Torreya taxifolia, Taxus wallichiana, Wollemia nobelis, Dendrobium speciosum*	Ambuic acid (1)
2	*Pestalotiopsis microspora*	*Terminalia morobensis*	Pestacin (2), Isopestacin (3)
3	*Pestalotiopsis jesteri*	*Fragraea bodenii*	Jesterone (4), Hydroxyjesterone (5)
4	*Pestalotiopsis adusta*	Unidentified tree	Pestalachlorides A (6), B (7), C (8)
5	*Pestalotiopsis foedan*	Unidentified tree	Pestafolide A (9), Pestaphthalide A (10) , Pestaphthalide B (11)
6	*Pestalotiopsis fici*	Unidentified tree	Pestalofone A (12), B (13), C (14), D (15), E (16)
7	*Phomopsis* sp. ZSU-H76	*Excoecaria agallocha*	Cytosporone B (17), Cytosporone C (18)
8	*Dothiorella* sp., strain HTF3	*Avicennia marina*	Cytosporone B (17)
9	*Phomopsis* sp.	*Costus* sp.	Phomoxanthone A (19)
10	*Phomopsis* sp.	*Adenocarpus foliolosus*	Phomosine A - G (20-26), 6-hydroxy- 6-isopropylcyclohex-1-enecarboxylic acid (27) , 1aS,3R,4R,4aR,6S,7R,8aS)-7-chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octahydro-1aH-naphtho (1-b)oxirene- 4-carboxylic acid (28) , 5-methylmellein (29) , 4-hydroxy-5-methylmellein (30) , 2-quinazolin- 4 (3H)-one (31) , Alternariol (32)
11	*Phomopsis* sp. YM 311483	*Azadirachta indica*	Five lactones (33-37)
12	*Phomopsis* sp	*Erythrina crista-galli*	Phomol (38)
13	*Phomopsis* sp. strain E99401	Unknown plant	Cerulenin (39)
14	*Phomopsis cassiae*	*Cassia spectabilis*	Ethyl 2,4-dihydroxy-5,6- dimethylbenzoate (40), Phomopsilactone (41)
15	*Phomopsis* sp.	*Laurus azorica*	Cycloepoxylactone (42)
16	*Phomopis cassiae*	*Cassia spectabilis*	Two distereoisomer of 3,9,12-trihydroxycalamenene (43-44), 3,12-dihydroxycalamenene (45), 3,12-dihydroxycadalene (46), 3,11,12-trihydroxycadalene (47)
17	*Phoma* sp.	*Saurauia scaberrinae*	Phomodione (48)
18	*Phoma* sp.	*Lycium intricatum*	Pyrenophorol (49), (–)-dihydropyrenophorin (50), 4-acetylpyrenophorol (51), 4-acetyldihydropyrenophorin (52), *Cis*-dihydropyrenophorin (53), Tetrahydropyrenophorin (54), *Seco*-dihydropyrenophorin (55), 7-acetyl *seco*-dihydropyrenophorin (56), *Seco*-dihydropyrenophorin-1,4-lactone (57)
19	*Morinia longiappendiculata*	*Santolina rosmarinifolia, Helichrysum stoechas, Calluna vulgaris, Thymus mastichina*	Moriniafungin (58)
20	*Colletotrichum gloeosporioides*	*Artemisia mongolica*	Colletotric acid (59)
21	*Colletotrichum* sp.	*Artemisia annua*	3beta,5alpha-dihydroxy-6beta-acetoxy-ergosta-7,22-diene (60), 3beta,5alpha-dihydroxy-6beta-phenylacetyloxy-ergosta-7,22-diene (61), 3beta -hydroxy-ergosta-5-ene (62), 3beta -hydroxy-5alpha,8alpha -epidioxy-ergosta-6,22-diene (63)
22	*Colletotrichum gloeosporioides*	*Cryptocarya mandioccana*	Cis-4-hydroxy-6-deoxyscytalone (64), (4R)-4,8-dihydroxy-alpha-tetralone (65)
23	*Exophiala* sp.	*Adenocarpus foliolosus*	Exochromone (66)
24	*Cryptosporiopsis quercina*	*Tripterigeum wilfordii*	Cryptocandin A (67)
25	*Cryptosporiopsis* sp.	*Pinus sylvestris*	Echinocandins A (68), B (69), D (70), H (71)
26	*Pezicula* sp.	*Fagus sylvatica*	Echinocandins A (68), B (69), D (70), H (71)
27	*Cryptosporiopsis quercina*	*Phleum pratense*	Cryptocin (72)
28	*Hormonema* sp. (ATCC 74360)	*Juniperus communis*	Enfumafungin (73)
29	*Epichloe typhina*	*Phleum pratense*	Epichlicin (74)
30	*Edenia gomezpompae*	*Callicarpa acuminata*	Preussomerin EG1 (75), Preussomerin EG2 (76), Preussomerin EG3 (77)
31	Endophytic strain E99297	*Cistus salvifolius*	5- (1,3-Butadien-1-yl)-3- (propen-1-yl)-2 (5H)-furanone (78)
32	*Botryosphaeria rhodina*	*Bidens pilosa*	Botryorhodine A (79), B (80), C (81), D (82)
33	*Dinemasporium strigosum*	*Calystegia sepium*	Dinemasone A (83), B (84), C (85)
34	*Chaetomium globosum*	*Ginkgo biloba*	Chaetoglobosin A (86), C (87)
35	*Xylaria* sp. PSU-D14	*Garcinia dulcis*	Sordaricin (88)
36	*Xylaria* sp. F0010	*Abies holophylla*	Griseofulvin (89), 7-dechlorogriseofulvin (90)
37	PSU-N24.	*Garcinia nigrolineata*	Griseofulvin (89)
38	*Xylaria* sp.	*Palicourea marcgravii*	2-hexyl-3-methylbutanodioic acid (91), Cytochalasin D (92)
39	*Xylaria* sp.	*Ginkgo biloba*	7-amino-4-methylcoumarin (93)
40	*Penicillium paxilli* PSU-A71	*Garcinia atroviridis*	Penicillone (94), Pyrenocine A (95), Pyrenocine B (96)
41	*Verticillium* sp.	*Rehmannia glutinosa*	2,6-dihydroxy-2-methyl-7- (prop-1*E*-enyl)-1-benzofuran-3 (2*H*)-one (97), Massariphenone (98), Ergosterol peroxide (99)
42	*Arthrinium phaeospermum*	Unidentified grass	Arthrichitin (100)
43	*Nigrospora* sp. YB-141	*Azadirachta indica*	Solanapyrone C (101), Solanapyrone N (102), Solanapyrone O (103), Nigrosporalactone (104), Phomalactone (105)
44	*Trichoderma harzianum*	*Llexcornuta Lindl*	Trichodermin (106)
Sr. No	**Fungus**	**Plant Source**	**Compounds isolated**
45	*Nodulisporium* sp.	*Erica arborea*	Nodulisporin D (107), E (108), F (109), (3S,4S,5R)- 2,4,6-trimethyloct-6-ene-3,5-diol (110), 5-hydroxy-2-hydroxymethyl- 4H-chromen-4-one (111), 3-(2,3-dihydroxyphenoxy)- butanoic acid (112), Benzene 1,2,3 triol (113)
46	*Aspergillus clavatus* and *Paecilomyces* sp.	*Taxus mairei* and *Torreya grandis*	Brefeldin A (114)
47	*Eupenicillium brefeldianum*	*Arisaema erubescens*	Brefeldin A (114)

48	*Cladosporium* sp.	*Quercus variabilis*	Brefeldin A (114)
49	CR377 (*Fusarium* sp.)	*Selaginella pallescens*	CR377 (115)
50	*Aspergillus niger*	*Cynodon dactylon*	Rubrofusarin B (116), Fonsecinone A (117), Asperpyrone B (118),, Aurasperone A (119)
51	*Coniothyrium* sp.	*Sideritis chamaedryfolia*	1-hydroxy-5-methoxy-2-nitro-naphthalene (120), 1,5-dimethoxy-4-nitronaphthalene (121), 1-hydroxy-5-methoxy-2,4-dinitronaphthalene (122), 1,5-di-methoxy-4,8-dinitronaphthalene (123), 1-hydroxy-5-methoxynaphthalene (124)
52	*Periconia* sp.	*Taxus cuspidata*	Periconicin A (125), Periconicin B (126)
53	*Nodulisporium* sp.	*Juniperus cedre*	3-hydroxy-1- (2,6-dihydroxyphenyl)butan-1-one (127), 1- (2,6-dihydroxyphenyl)butan-1- one (128), 1- (2-hydroxy-6-methoxyphenyl)butan-1-one (129), 5-hydroxy-2-methyl-4*H*-chromen-4-one (130), 2,3-dihydro-5-hydroxy-2-methylchromen-4-one (131), 2,3-dihydro-5-methoxy-2-methylchromen-4-one (132), 8-methoxynaphthalen-1-ol (133), 1,8-dimethoxynaphthalene (134), Nodulisporin A (135), Nodulisporin B (136), Daldinol (137), Nodulisporin C (138), (4E,6E)-2,4,6-trimethylocta-4,6-dien-3-one (139)
54	*Penicillium* sp.	*Hopea hainanensis*	Monomethylsulochrin (140), Rhizoctonic acid (141), Asperfumoid (142), Physcion (143), 7,8- dimethyl-iso-alloxazine (144), 3,5-dichloro-panisic acid (145)
55	*Curvularia* sp.	*Ocotea corymbosa*	2-methyl-5-methoxy-benzopyran-4-one (146) , (2'S) 2- (propan-2'-ol)-5-hydroxy-benzopyran-4-one (147)
56	*Aspergillus fumigatus* CY018	*Cynodon dactylon*	Asperfumoid (142), Physcion (143), Fumigaclavine C (148), Fumitremorgin C (149), Helvolic acid (150)
57	*Aspergillus clavatonanicus*	*Torreya mairei*	Clavatol (151), Patulin (152)
58	*Aspergillus niger* EN-13	*Colpomenia sinuosa*	Asperamide A (153), Asperamide B (154)
59	*Aspergillus niger* EN-13	*Colpomenia sinuosa*	5,7-dihydroxy-2- (1- (4-methoxy-6-oxo-6H-pyran-2-yl)-2-phenylethylamino)-(1,4) naphthoquinone (155)
60	*Microdochium bolleyi*	*Fagonia cretica*	(12R)-12-hydroxymonocerin (156), (12S)-12-hydroxymonocerin (157), (3R,4R,10R)-4 (2-4) (4) (158), Monocerin (159)
61	*Neoplaconema napellum* IFB-E016	*Hopea hainanensis*	Neoplaether (160)
Sr. No	Fungus	Plant Source	Compounds isolated
62	*Chalara* sp. (strain 6661)	*Artemisia vulgaris*	Isofusidienol A- D (161- 164)
63	*Blennoria* sp.	*Carpobrotus edulis*	Blennolide A -G (165-171), Secalonic acid B (172)
64	*Periconia atropurpurea*	*Xylopia aromatica*	Periconicin B (126), 6,8-Dimethoxy-3- (2'-oxo-propyl)-coumarin (173) and 2,4-dihydroxy-6- ((1'E,3'E)-penta-1', 3'-dienyl)-benzaldehyde (174)
65	F-042,833, Undetermined Coelomycetes	*Olea europea* var *europea*	Arundifungin (175)
66	F054,289, sterile mycelium	*Quercu silex*	Arundifungin (175)
67	Fungus culture MF6020	Not reported	Khafrefungin (176)
68	E99291	*Cistus salvifolius*	Ascosteroside A -B (177- 178)
69	E99204	*Quercus ilex*	Sphaeropsidin A (179)
70	Mycelia sterila	*Cirsium arvense*	6,8-diacetoxy-3,5-dimethylisocoumarin (180), 3-Acetyl-6-hydroxy-4-methyl-2,3-dihydrobenzofuran (181)
71	Unidentified endophytic fungus (6760)	*Trifolium dubium*	Pyrenocine A (95), F –H (182-184)

culmorum, G. zeae and V. aibo-atrum (IC_{50} > 100 micromolar) (44).

Pestafolide A (9) (Figure 1), a new reduced spiroazaphilone derivative and Pestaphthalide A and B (10-11) (Figure 1), two new isobenzofuranones were isolated from an endophytic fungus Pestalotiopsis foedan. The fungus was isolated from the branch of an unidentified tree near Dongzai, Hainan Province, Republic of China. The isolated compounds were evaluated for antifungal activity against Candida albicans (ATCC 10231), Geotrichum candidum (AS2.498) and Aspergillus fumigatus (ATCC 10894) in agar diffusion assays. Pestafolide A (9) displayed antifungal activity against Aspergillus fumigatus (ATCC 10894), with a zone of inhibition of 10 mm at 100 mcg/disk. Pestaphthalide A (10) showed activity against Candida albicans (ATCC 10231), with 13 mm zone of inhibition and Pestaphthalide B (11) showed activity against Geotrichum candidum (AS2.498) with 11 mm zone of inhibition when tested at the same level (Fluconazole: 18–28mm zones of inhibition for C. albicans, A. fumigatus, and G. candidum at 100 mcg/disk) (45).

Pestalofone A–E (12-16) (Figure 1), five new cyclohexanone derivatives were isolated from Pestalotiopsis fici, isolated from the branches of an unidentified tree in suburb of Hangzhou, Zhejiang Province, Republic of China. Pestalofones A–E were evaluated for activities against Candida albicans (ATCC 10231), Geotrichum candidum (AS2.498) and Aspergillus fumigatus (ATCC 10894). Pestalofone C and E showed significant antifungal activity against A. fumigatus, with IC_{50} / MIC values of 1.10/35.3 and 0.90/31.2 micromolar, respectively (The positive control Fluconazole showed IC_{50}/MIC values of 7.35/163.4 micromolar) (46).

Phomopsis is another genus which exist as an endophyte associated with most of the plants and are extremely biochemically diverse. Here are some examples

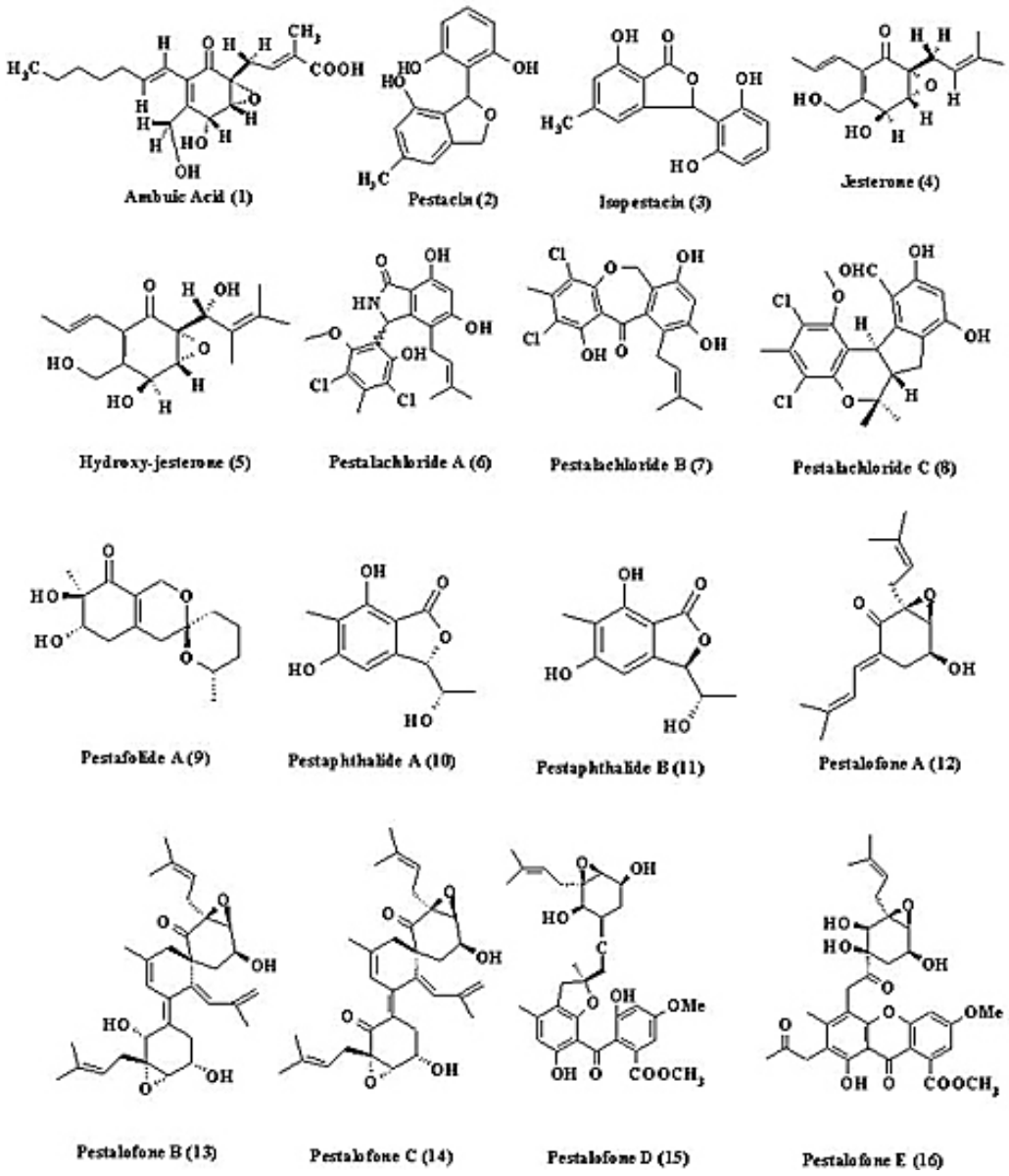

Figure 1. Structures of antifungal metabolites isolated from coelomycetes

of bioactive metabolites products by this endophytic genus. Cytosporone B and C (17-18) (Figure 2) were isolated from an endophytic fungus Phomopsis sp. ZSU-H76 obtained from the stem of mangrove tree Excoecaria agallocha Linn from Dongzai, Hainan, China. Both the compounds were found active against Candida albicans and Fusarium oxysporum with an MIC ranging from 32 to 64 mcg/ml (47). Cytosporone B (17) was also reported from an endophytic fungus, Dothiorella sp., strain HTF3 isolated from mangrove plant Avicennia marina at the estuary of Jiulong River, Fujian Province. It showed activities against fungi namely Aspergillus niger, Trichoderma sp. and Fusarium sp. with MIC of 0.125, 62.5 and 62.5 mcg/ml, respectively (48).

Phomoxanthone A (19) (Figure 2), a dimeric xanthone was isolated from an endophytic fungus Phomopsis sp., isolated from the stem of Costus sp. (Costaceae) growing in the rain forest of Costa Rica. It showed moderate inhibition of Ustilago violacea at a concentration of 10mg/ml. Phomoxanthone A (19) showed strong activity against phytopathogenic fungi namely Phytophthora infestans, Botrytis cinerea, Pyricularia oryzae and Ustilago violacea (49).

Thirteen metabolites viz Phomosine A–G (20-26), 6-hydroxy- 6-isopropylcyclohex-1-enecarboxylic acid (27), (1aS,3R,4R,4aR,6S,7R,8aS)-7-chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octahydro-1aH-naphtho (1-b)oxirene-4-carboxylic acid (28), 5-methylmellein (29), 4-hydroxy-5-methylmellein (30), 2-quinazolin-4 (3H)-one (31) and Alternariol (32) (Figure 2) were isolated from the endophytic fungus Phomopsis sp. isolated from

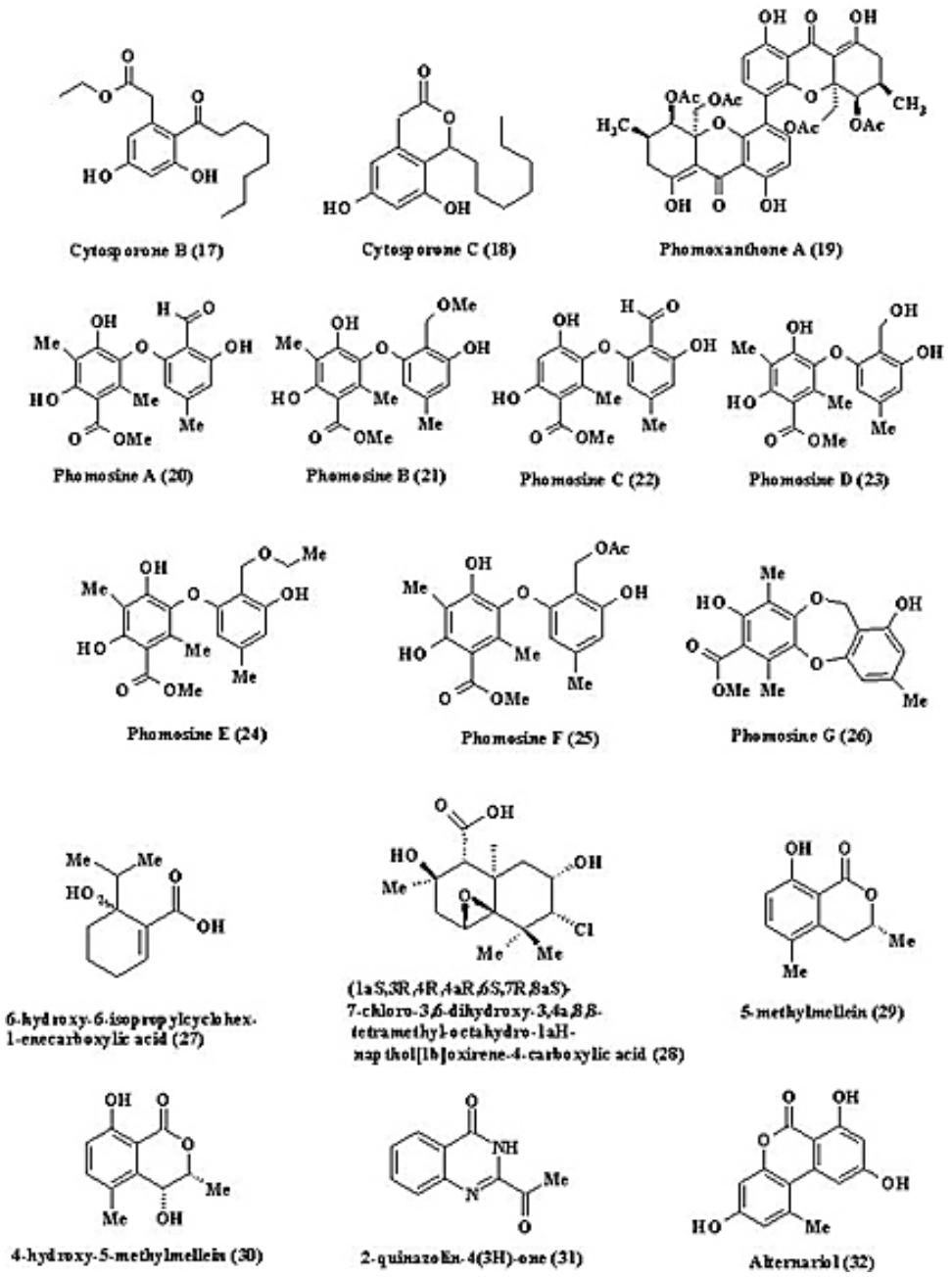

Figure 2. Structures of antifungal metabolites isolated from coelomycetes

Adenocarpus foliolosus from Gomera. The fungicidal properties of these compounds were evaluated against Microbotryum violaceum. Metabolites 20, 22, 26 and 29 exhibited moderate inhibitory activity (50).

Five 10-membered lactones (33-37) (Figure 3) were isolated from Phomopsis sp. YM 311483, obtained from the stem of Azadirachta indica growing in Yuanjiang Country, a tropical region in Yunnan province, People's Republic of China. These lactones were evaluated for their antifungal activity against seven plant pathogens namely Aspergillus niger, Botrytis cinerea, Fusarium avenaceum, F. moniliforme, Helminthosporium maydis, Penicillium islandicum and Ophiostoma minus, using the dose-dependent paper-disk diffusion method. All five compounds showed weak antifungal activities. Compound (36) was the most potent, with MIC values in the range of 31.25-500 mcg/ml, interestingly compound (36) was more active than compound (35), even though their structures differed only in the position of the acetoxy substituent (51).

Phomol (38) (Figure 3), a polyketide lactone was isolated from Phomopsis sp., isolated from medicinal plant Erythrina crista-galli. It exhibited antiinflammatory, weak

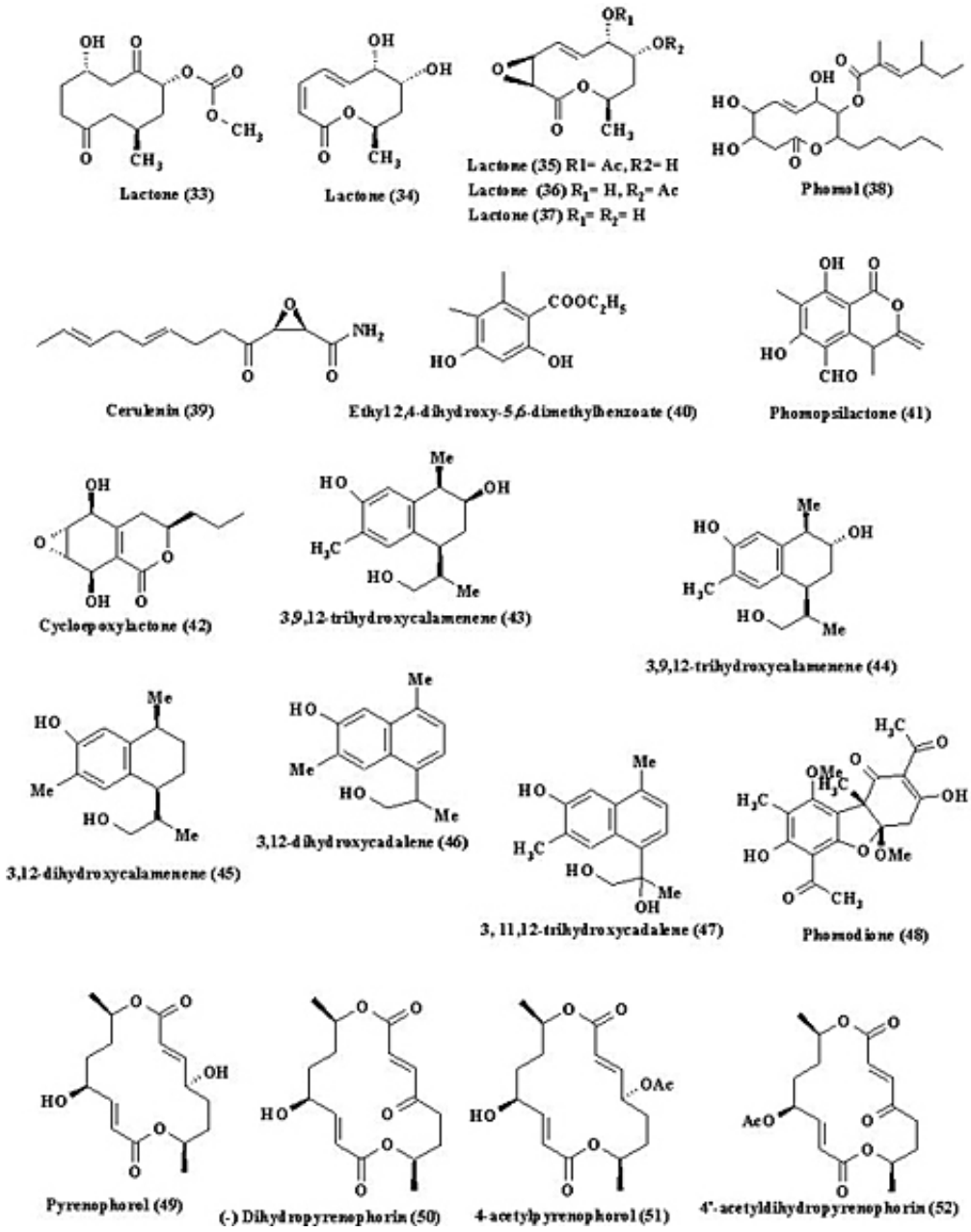

Figure 3. Structures of antifungal metabolites isolated from coelomycetes

cytotoxic activity, antibacterial and antifungal activity against both yeast and filamentous fungi (52).

Cerulenin (39) (Figure 3) was isolated from an endophytic fungus Phomopsis sp., isolated from an asymptomatic leaf of a tropical rainforest tree from French Guyana (53). It is an inhibitor of fatty acid and polyketide synthases with known anti-Candida activity (54).

Two new metabolites, Ethyl 2, 4-dihydroxy-5,6-dimethylbenzoate (40) and Phomopsilactone (41) (Figure 3) were isolated from Phomopsis cassiae, an endophytic fungus in Cassia spectabilis. Both the compounds displayed strong antifungal activity against the phytopathogenic fungi Cladosporium cladosporioides and C. sphaerospermum and the detection limit for both the compounds was 1 mcg, the same as for the positive control Nystatin (55).

Cycloepoxylactone (42) (Figure 3), was isolated from an endophytic fungus Phomopsis sp. isolated from the leaves of Laurus azorica growing in Gomera, Spain. It exhibited good antifungal activity against Microbotryum violaceum with radius of 10mm at the concentration of 50 mcg/disk (56).

Cadinane sesquiterpenes derivatives namely two diastereoisomers of 3,9,12-trihydroxycalamenenes (43-44), 3,12-dihydroxycalamenene (45), 3,12-dihydroxycadalene (46) and 3,11,12-trihydroxycadalene (47) (Figure 3) were isolated from Phomopis cassiae, isolated from Cassia spectabilis collected from Brazil. The antifungal activity of these compounds was evaluated against the phytopathogenic fungi Cladosporium cladosporioides and C. sphaerospermum. The compound 3,11,12-trihydroxycadalene (47) was the most active compound and the detection limit for the compound was found to be 1.0 mcg , comparable with the same amount of the standard Nystatin. The compound 3, 12-dihydroxycadalene (46) also exhibited potent activity against two fungi tested (57).

Phomodione (48) (Figure 3), was isolated from a Phoma sp., an endophyte on a Guinea plant (Saurauia scaberrinae). Phomodione exhibited antifungal activity against Pythium ultimum, Sclerotinia sclerotiorum and Rhizoctonium solani, with MIC between 3 and 8 mcg/ml (58).

Pyrenophorol (49), a known macrolide and (–) dihydropyrenophorin (50), together with four new analogues; 4-acetylpyrenophorol (51), 4-acetyldihydropyrenophorin (52), (Figure 3) cis-dihydropyrenophorin (53) and tetrahydropyrenophorin (54) and three novel ring-opened derivatives named seco-dihydropyrenophorin (55), 7-acetyl seco-dihydropyrenophorin (56) and seco-dihydropyrenophorin-1,4-lactone (57) (Figure 4) were isolated from Phoma sp., an endophytic fungus isolated from Lycium intricatum from Gomera, Spain. All the nine compounds exhibited antifungal activity against Microbotryum violaceum (59).

Moriniafungin (58) (Figure 4), a novel sordarin analog with potent antifungal activity was isolated from Morinia longiappendiculata isolated from stems of four plant species namely Santolina rosmarinifolia, Helichrysum stoechas, Thymus mastichina and Calluna vulgarisin collected from central Spain. Moriniafungin exhibited an MIC of 6 mcg/ml against Candida albicans and IC_{50} ranging from 0.9 to 70 mcg/ml against a panel of clinically relevant strains namely Candida albicans (MY1055), Cryptococcus neoformans (MY 2062), Cr. neoformans (ATCC 66031), Candida glabrata (MY1381), C. parapsilosis (ATCC 22019), C. krusei (ATCC 6258) and C. lusitaniae (MY1396) (60- 61).

Colletotric acid (59) (Figure 4) was isolated from Colletotrichum gloeosporioides, an endophytic fungus colonized inside the stem of Artemisia mongolica and inhibited the growth of the pathogenic fungus Helminthosporium sativum with MIC value of 50 mcg/ml (62).

3beta,5alpha-dihydroxy-6beta-acetoxy-ergosta-7,22-diene (60), 3beta,5alpha-dihydroxy-6beta-phenylacetyloxy-ergosta-7,22-diene (61), 3beta-hydroxy-ergosta-5-ene (62) and 3beta-hydroxy-5alpha,8alpha-epidioxy-ergosta-6,22-diene (63) (Figure 4) were characterized from the culture of Colletotrichum sp., an endophyte isolated from inside the stem of Artemisia annua. These compounds exhibited antifungal activities against Candida albicans and Aspergillus niger (MICs: 50–100 mcg/ml) (63).

Cis-4-hydroxy-6-deoxyscytalone (64) and (4R)-4, 8-dihydroxy-alpha-tetralone (65) (Figure 4) were isolated from endophytic fungus Colletotrichum gloeosporioides residing in healthy leaves of Cryptocarya mandioccana. The antifungal activity of these two compounds was evaluated and the detection limit of these compounds required to inhibit growth of the phytopathogenic fungi Cladosporium cladosporioides and C. sphaerospermum was 5.0 mg, comparable with the positive control Nystatin (64).

Exochromone (66) (Figure 4), a highly substituted chromone dimer was isolated from Exophiala sp. isolated from Adenocarpus foliolosus, growing on the hills of Baranco de Los Jargus, Gomera. Exochromone showed a radial zone of inhibition of 9 mm at the concentration of 1mg/ml against Microbotryum violaceum (65).

3.2. Compounds produced by ascomycetes

Cryptocandin A (67) (Figure 5), an antifungal lipopeptide was isolated and characterized from the endophytic fungus, Cryptosporiopsis quercina isolated from stem of Tripterigeum wilfordii (66). This compound contains a number of unusual hydroxylated amino acids and a novel amino acid, 3- hydroxy-4-hydroxymethylproline and a beta- (1,3)-glucan synthesis inhibitor. Cryptocandin A is active against some important human fungal pathogens including Candida albicans and Trichophyton sp. and also against a number of plant pathogenic fungi, including Sclerotinia sclerotiorum and Botrytis cinerea. Similarly a group of peptides, Echinocandin A, B, D and H (68-71) (Figure 5), also a beta- (1,3)-glucan synthesis inhibitors were isolated from endophytic Cryptosporiopsis sp. and Pezicula sp. in Pinus sylvestris and Fagus sylvatica and showed to be antifungal (67). Cryptocin (72) (Figure 5), a tetramic acid is an antifungal compound also obtained from Cryptosporiopsis cf quercina isolated from the inner bark of the stem of Tripterigeum wilfordii. This unusual compound possesses potent activity against Pyricularia oryzae, with MICs of 0.39 mcg/ml, a causal agent of rice blast disease, as well as a number of other plant pathogenic fungi (68).

Enfumafungin (73) (Figure 5), a triterpenoid glucoside was produced by Hormonema sp. (ATCC 74360) an endophytic fungus isolated from Juniperus communis. Enfumafungin shows interesting antifungal spectrum and its effect on the morphology of Aspergillus fumigatus was shown to be comparable to that of the glucan synthase inhibitor, Pneumocandin B (69).

Epichlicin (74) (Figure 5), a novel cyclic peptide was isolated from Epichloe typhina, an endophytic fungus from Phleum pratense. Epichlicin showed inhibitory activity toward the spore germination of Cladosporium phlei, a pathogenic fungus of Epichloe typhina plant at an IC_{50} value of 22 nanomolars (70).

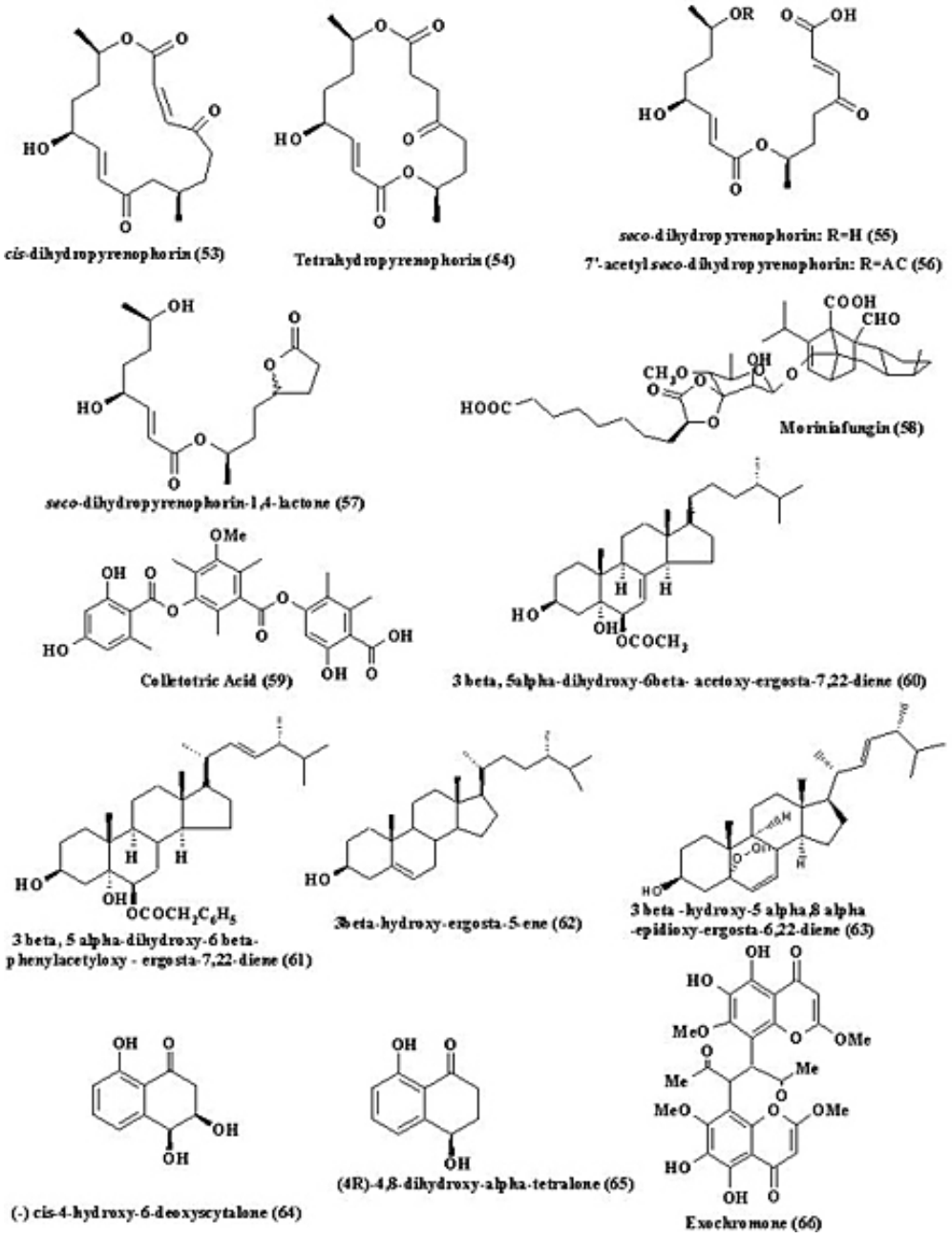

Figure 4. Structures of antifungal metabolites isolated from coelomycetes

New naphthoquinone spiroketals, namely Preussomerin EG1–EG3 (75-77) (Figure 5), were isolated from the mycelium of Edenia gomezpompae, isolated from the leaves of Callicarpa acuminata (Verbenaceae) collected from the ecological reserve El Eden, Quintana Roo, Mexico. The new spiroketals displayed significant growth inhibition against all the phytopathogens namely Phytophthora capsici, P. parasitica, Fusarium oxysporum and F. solani with IC_{50} values in the range of 20 to 170 mcg/ml (71).

Weber *et al* (53) reported 5- (1,3-Butadien-1-yl)-3- (propen-1-yl)-2 (5H)-furanone (78) (Figure 5) from an endophytic strain E99297, isolated from the twig of Cistus salvifolius, which was previously shown by Kopcke *et al* (72) to belong to the Sarcosomataceae (order Pezizales). The compound was isolated on the basis of its anti-Candida activity and was identical to 5- (E)-buta-1,3-dienyl-3 (E)-propenyl-5Hfuran-2-one isolated by Kopcke *et al* (72).

Depsidones, Botryorhodine A–D (79-82) (Figure 6) were produced by Botryosphaeria rhodina isolated from the stems of the medicinal plant Bidens pilosa (Asteraceae). Botryorhodine A exihibited an MIC of 26.03 and 191.60

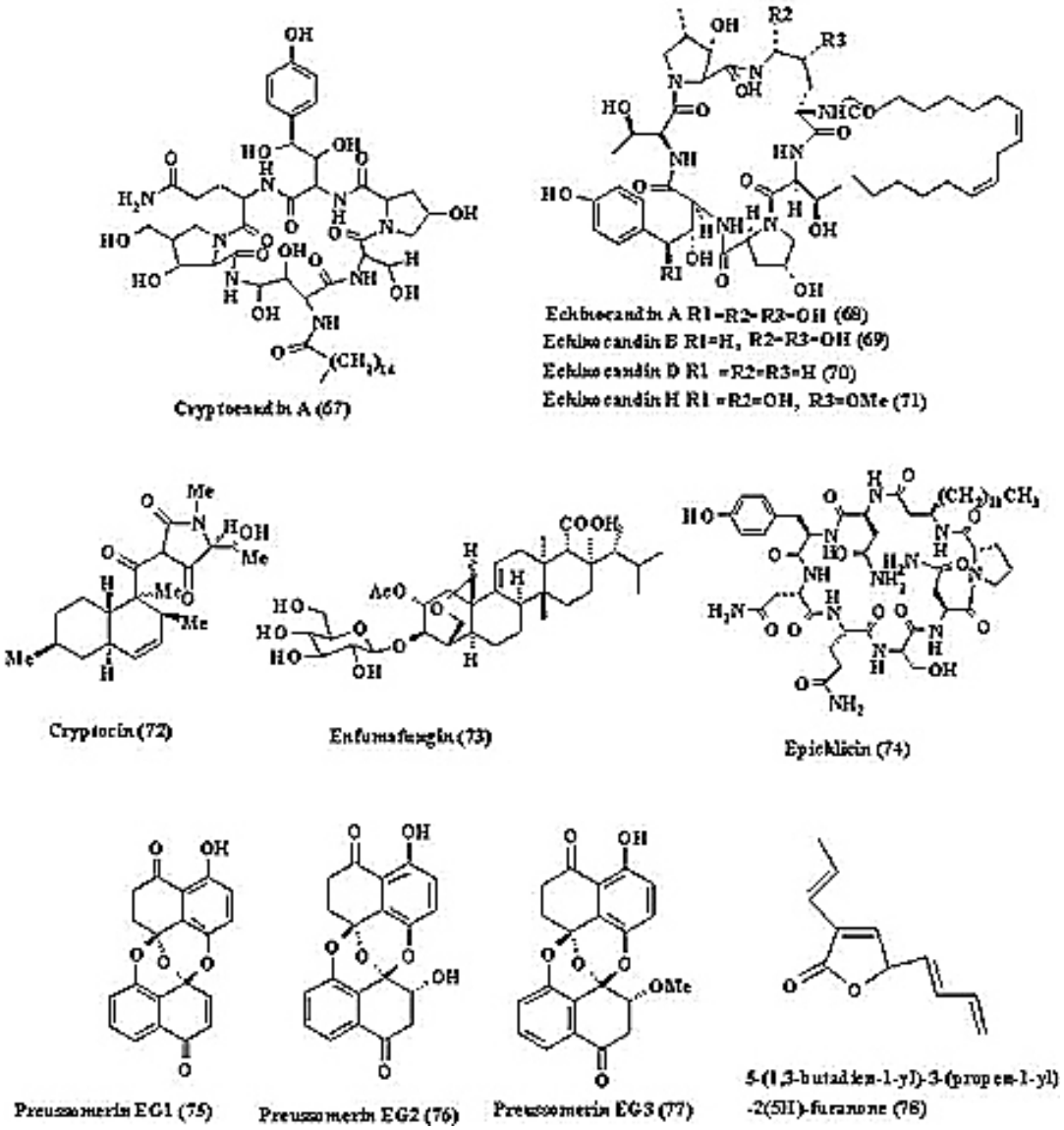

Figure 5. Structures of antifungal metabolites isolated from ascomycetes

micromolar against Aspergillus terreus and Fusarium oxysporum respectively, whereas the compound botryorhodine B exihibited an MIC of 49.70 and 238.80 micromolar against Aspergillus terreus and Fusarium oxysporum, respectively (73).

Dinemasone A–C (83-85), (Figure 6) were isolated from Dinemasporium strigosum, isolated from the roots of Calystegia sepium from the shores of Baltic Sea, Wustrow, Germany. The dinemasone A and B exhibited considerable activity against Microbotryum violaceum (74).

Chaetoglobosin A and C (86- 87) (Figure 6), were characterized from an endophytic Chaetomium globosum isolated from the leaves of Ginkgo biloba. Both the compounds displayed marked inhibitory activity against Mucor miehei by agar diffusion method with the zone of inhibition of 25 and 15 mm in diameter at 10 mcg/disk respectively (75).

Sordaricin (88) (Figure 6), a known antifungal metabolite, was isolated from an endophytic fungus Xylaria sp. PSU-D14, isolated from the leaves of Garcinia dulcis, collected in Songkhla Province, Thailand. It exhibited moderate antifungal activity against Candida albicans ATCC 90028 with a MIC value of 32 mcg/ml (76).

Griseofulvin (89) and 7-dechlorogriseofulvin (90) (Figure 6) were isolated from Xylaria sp. F0010 isolated from Abies holophylla. Compared to 7-dechlorogriseofulvin, griseofulvin showed high in-vivo and in-vitro antifungal activity and effectively controlled the development of plant pathogenic fungi namely

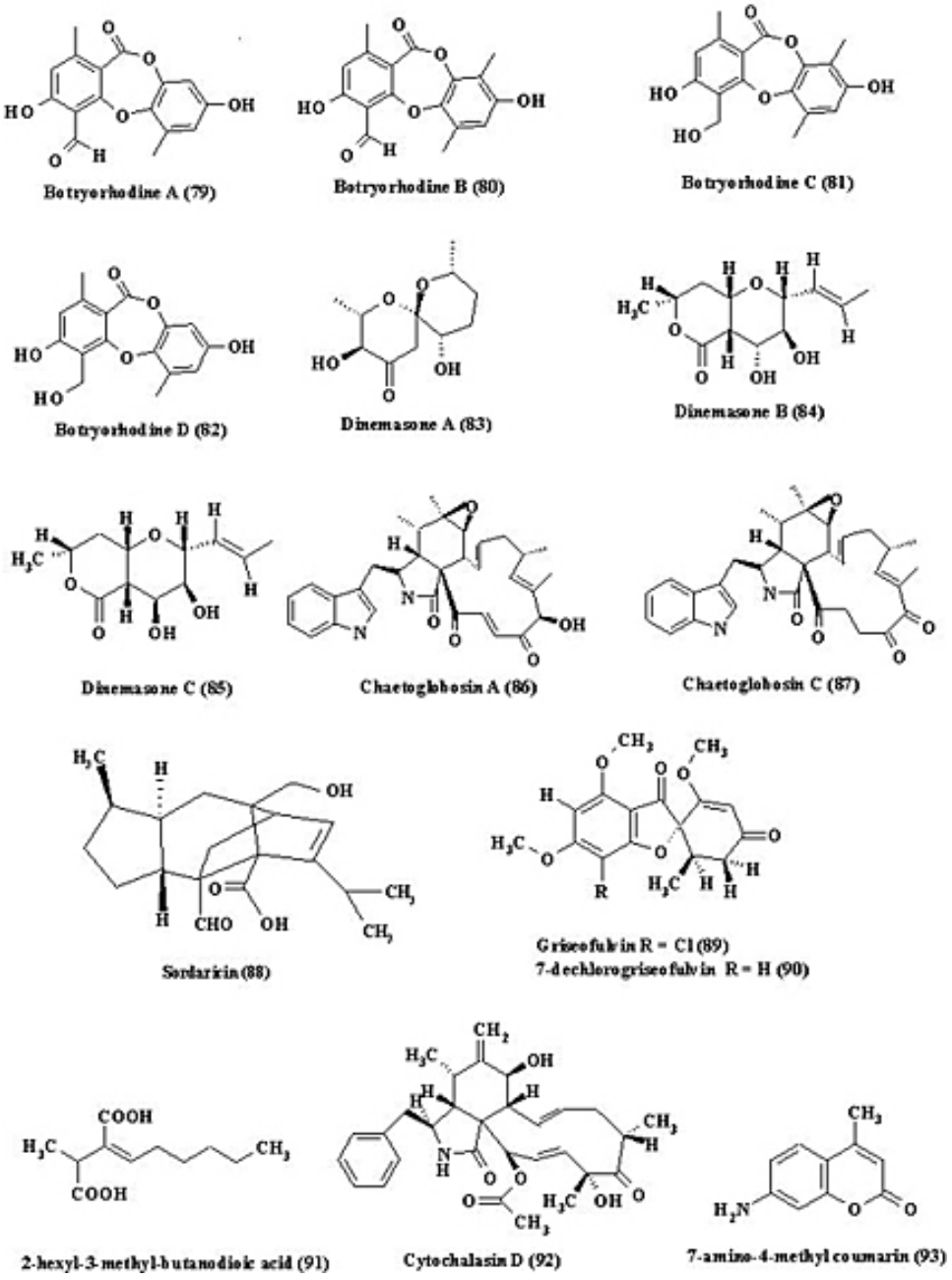

Figure 6. Structures of antifungal metabolites isolated from ascomycetes

Magnaporthe grisea, Corticium sasakii, Puccinia recondita and Blumeria graminis f. sp. hordei at doses of 50 to 150 mcg/ml, depending on the disease (77). Griseofulvin (89) (Figure 6) was also isolated from an endophytic fungus PSU-N24 isolated from Garcinia nigrolineata. It displayed strong antifungal activity against Microsporum gypseum SH-MU-4 with a MIC value of 2 mcg/ml (78).

2-hexyl-3-methyl-butanodioic acid (91) and Cytochalasin D (92) (Figure 6) were isolated from an endophytic fungus Xylaria sp. isolated from Palicourea marcgravii. These compounds were found active against phytopathogenic fungi Cladosporium cladosporioides and C. sphaerospermum (79). 7-amino-4-methylcoumarin (93) (Figure 6) was isolated from an endophytic Xylaria sp., obtained from Ginkgo biloba L. The compound showed strong *in-vitro* antifungal activities with MIC of 15, 40, and 25 mcg/ml against Candida albicans, Penicillium expansum and Aspergillus niger respectively (80).

3.3. Compounds produced by hyphomycetes

A new pyrone derivative, Penicillone (94), together with Pyrenocine A and B (95–96) (Figure 7), were

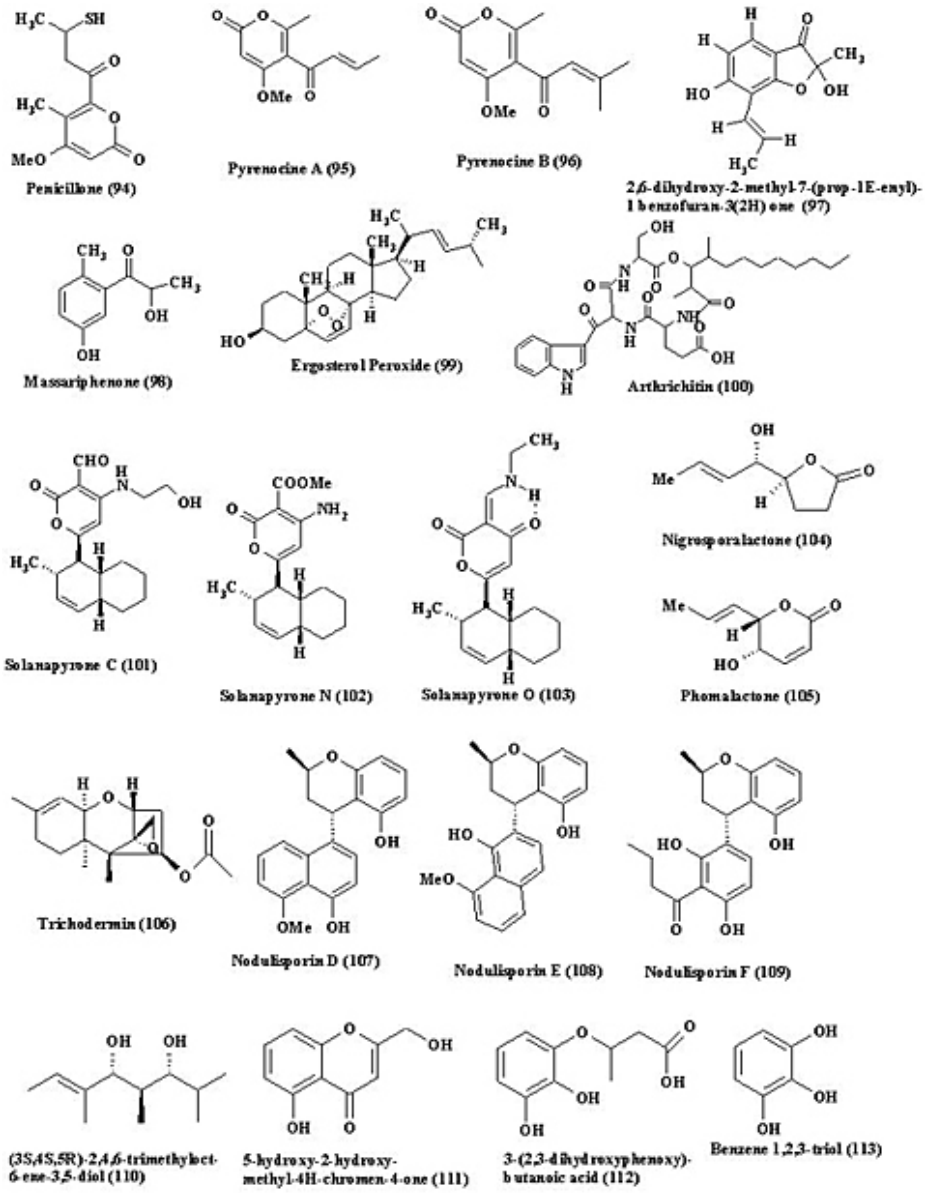

Figure 7. Structures of antifungal metabolites isolated from hyphomycetes

isolated from the endophytic fungus Penicillium paxilli PSU-A71, isolated from the leaves of Garcinia atroviridis, collected in Songkhla Province, Thailand. All the compounds were tested for antifungal activity against Microsporum gypseum SH-MU-4. Pyrenocine B (96) showed mild activity with a MIC value of 32 mcg/ml while penicillone (94) and pyrenocine A (95) were much less active than pyrenocine B (96) with MIC values of 64 and 128 mcg/ml, respectively (81).

2,6-dihydroxy-2-methyl-7-(prop-1E-enyl)-1-benzofuran-3 (2H)-one (97), Massariphenone (98) and Ergosterol peroxide (99) (Figure 7) were reported from Verticillium sp. isolated from roots of wild Rehmannia glutinosa from Wushe County, Henan Province, People's Republic of China. Minimum morphological deformation concentration (MMDC) of compounds (97-99) towards Pyricularia oryzae P-2b was 1.95, 125.0 and 7.8 mcg/ml respectively. Compound (97) exhibited the strongest antibiotic activity but compound (98) only slightly inhibited growth of Septoria sp. and Fusarium sp. Compounds (97) and (99) clearly inhibited biomass accumulations at a low concentration (0.97 mcg/ml) in liquid culture. Growth of the Verticillium sp. itself was also inhibited to some degree by compounds (97) and (99). Compound (97) showed selective inhibition and inhibited pathogens more strongly; in contrast compound (99) displayed no selectivity (82).

Arthrichitin (100) (Figure 7), a cyclic depsipeptide was isolated from Arthrinium phaeospermum obtained from unidentified grass (83). It was also isolated

as LL15G256γ from the marine fungus Hypoxylon oceanicum (84-85). Arthrichitin has a broad-spectrum of activity against Candida sp., Trichophyton sp. and several phytopathogens. *In-vitro* testing has shown that arthrichitin inhibits membrane preparations of fungal chitin and glucan synthases, with a greater potency against chitin synthase. Fungal cells exposed to arthrichitin undergo morphological changes similar to the effects of chitin synthase inhibitor, Polyoxin B (83). The morphological effects of arthrichitin occur at concentrations that are tenfold below those required to inhibit chitin synthase. This suggests the existence of another target for arthrichitin, possibly an isozyme of chitin synthase not present in the membrane preparation (86). It is also possible that the compound alters regulatory processes in the cell cycle that affect the cell wall. The in-vitro potency of arthrichitin is too low for its use in the clinic. However, it has been suggested that the development of analogs, such as those based on the much larger cyclic peptides, the echinocandins, might yield congeners with improved activity (86).

The solanapyrone analogues, Solanapyrone C, N and O (101–103), Nigrosporalactone (104) and Phomalactone (105) (Figure 7) were isolated from Nigrospora sp. YB-141, an endophytic fungus isolated from Azadirachta indica. All the compounds were tested for their antifungal properties against seven phytopathogenic fungal strains namely Aspergillus niger, Botrytis cinerea, Fusarium avenaceum, Fusarium moniliforme, Helminthosporium maydis, Ophiostoma minus and Penicillium islandicum. All five compounds showed antifungal activities against B. cinerea with MIC values in the range of 31.25–250 mcg /ml (87).

Trichodermin (106) (Figure 7) was characterized from Trichoderma harzianum, an endophytic fungus from Llex cornuta. Plant experimental results showed that trichodermin exhibited significant protective effect to early blight on tomato and damping-off on cucumber. The EC_{50} values of trichodermin inhibiting the mycelia growth of Alternaria solani and Rhizoctonia solani were 3.35 and 3.59mg/L, respectively. In addition, protective and therapeutic effects of trichodermin at 100 mg/L against A. solani and R. solani were 97.8%, 98.1% and 96.7%, 97.3%, respectively (88).

Nodulisporins D–F (107-109), (3S,4S,5R)- 2,4,6-trimethyloct-6-ene-3,5-diol (110), 5-hydroxy-2-hydroxymethyl-4H-chromen-4-one (111), 3- (2,3-dihydroxyphenoxy)-butanoic acid (112) and Benzene-1,2,3-triol (113) (Figure 7) were isolated from Nodulisporium sp. an endophytic fungus, isolated from the plant Erica arborea from Gomera. These compounds were found active against Microbotryum violaceum (89).

Wang *et al* (90) isolated Brefeldin A (114) (Figure 8), with a wide range of biological activities, namely antifungal, antiviral, antimitotic, antiinflammatory and antitumor (91-95) from Aspergillus clavatus and Paecilomyces sp. endophytic in Chinese Taxus mairei and Torreya grandis. Brefeldin A was also isolated from an endophytic fungal strain of Eupenicillium brefeldianum, isolated from a Chinese traditional medicinal plant Arisaema erubescens (96) and from an endophytic fungal strain of Cladosporium sp, isolated from a Chinese traditional medicinal plant Quercus variabilis (97).

CR377 (115) (Figure 8), a new pentaketide was isolated from fungus CR377 (Fusarium sp.) isolated from the interior of a surface-sterilized pieces of Selaginella pallescens stem tissue, collected in the Guanacaste Conservation Area of Costa Rica. CR377 was tested against Wisconsin and 109 strain of Candida albicans by agar diffusion method. The compound exhibited inhibition zones of 20 and 24 mm against both Candida albicans strains: Wisconsin and 109 respectively. Nystatin, a positive control produced inhibition zones of 19 and 22 mm against the Wisconsin and 109 strains respectively at the concentration of 100 units (approximately 30mcg) (98).

Four known naphtho-gamma-pyrones, Rubrofusarin B (116), Fonsecinone A (117), Asperpyrone B (118) and Aurasperone A (119) (Figure 8) were isolated from Aspergillus niger IFB-E003, an endophyte isolated from leaves of Cynodon dactylon collected from Yancheng Biosphere reserve, People's Republic of China. These compounds exhibited growth inhibition against Trichophyton rubrum and Candida albicans with MICs ranging between 1.9 to 15.6 mcg/ml (99).

Four natural nitro metabolites, 1-hydroxy-5-methoxy-2-nitro-naphthalene (120), 1,5-dimethoxy-4-nitronaphthalene (121), 1-hydroxy-5-methoxy-2,4-dinitronaphthalene (122) and 1,5-di-methoxy-4,8-dinitronaphthalene (123) known from chemical synthesis but new as natural products, were isolated together with 1-hydroxy-5-methoxynaphthalene (124) (Figure 8) from an endophytic fungus, Coniothyrium sp. isolated from the shrub Sideritis chamaedryfolia, from an arid habitat near Alicante, Spain. Compounds (120-122) and (124) showed excellent activity against Microbotryum violaceum (100).

Fusicoccane diterpenes, named Periconicin A and B (125-126) (Figure 8) with antibacterial activities were isolated from an endophytic fungus Periconia sp., OBW-15, collected from small branches of Taxus cuspidate (101). Periconicin A (125) showed potent inhibitory activity against the agents of human mycoses, including Candida albicans, Trichophyton mentagrophytes and T. rubrum, with MIC in the range of 3.12-6.25 mcg/ml (102).

3-hydroxy-1- (2,6-dihydroxyphenyl)butan-1-one (127), 1- (2,6-dihydroxyphenyl)butan-1-one (128) (Figure 8), 1- (2-hydroxy-6-methoxyphenyl)butan-1-one (129), 5-hydroxy-2-methyl-4H-chromen-4-one (130), 2,3-dihydro-5-hydroxy-2-methylchromen-4-one (131), 2,3-dihydro-5-methoxy-2-methylchromen-4-one (132), 8-methoxynaphthalen-1-ol (133), 1,8-dimethoxynaphthalene (134), Nodulisporin A (135), Nodulisporin B (136), Daldinol (137), Nodulisporin C (138) and (4E,6E)-2,4,6-trimethylocta-4,6-dien-3-one (139) (Figure 9) were isolated from Nodulisporium sp. from Juniperus cedre from Gomera Island. Compounds (127-139) were tested at the concentration of 0.25 mg/filter disc for antifungal

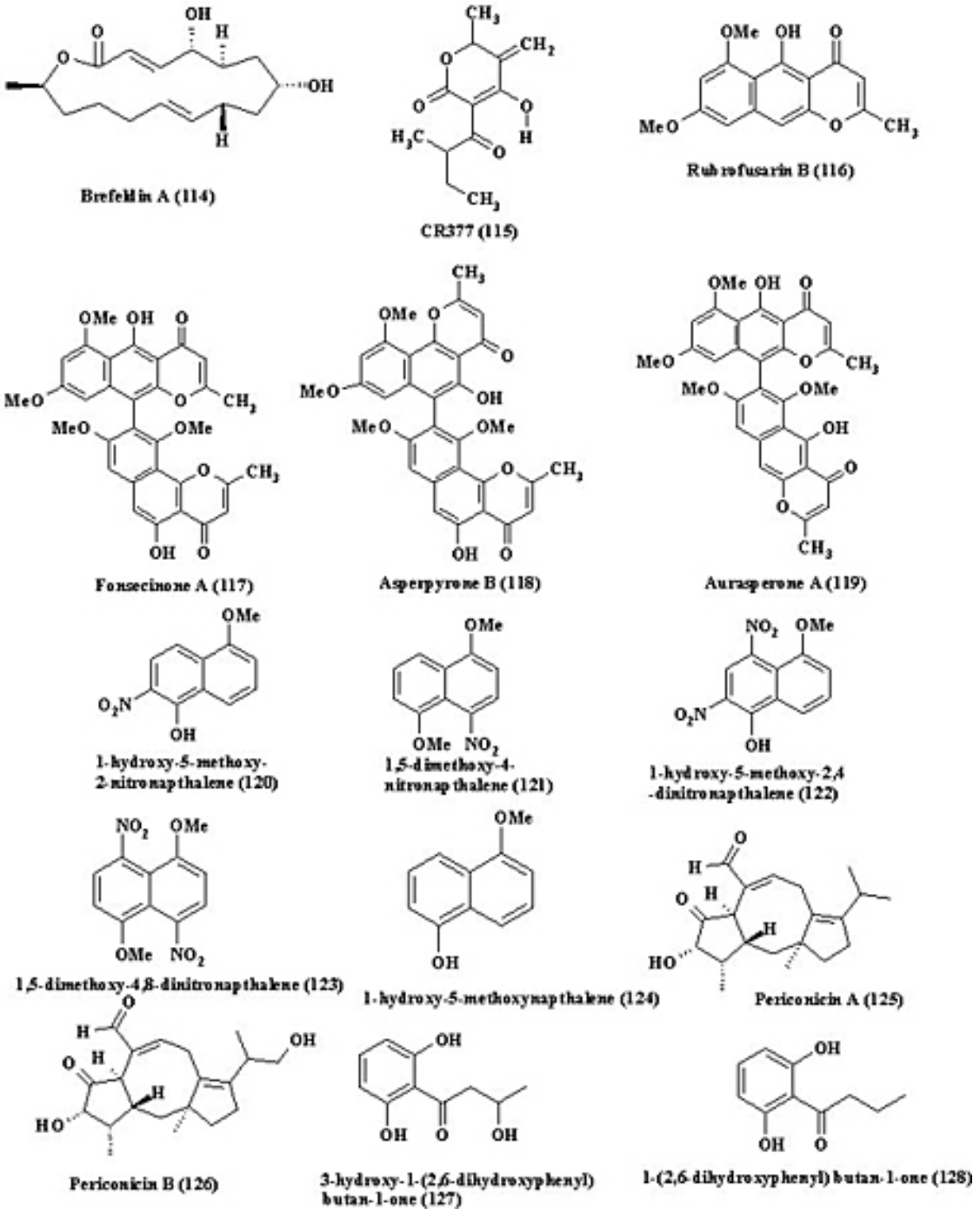

Figure 8. Structures of antifungal metabolites isolated from hyphomycetes

activity. Compounds (128-139) showed activity against Microbotryum violaceum while compounds (128), (130), (132), (134) and (139) showed activity against Septoria tritici (103).

Monomethylsulochrin (140), Rhizoctonic acid (141), Asperfumoid (142), Physcion (143), 7,8-dimethyl-iso-alloxazine (144) (Figure 9) and 3,5-dichloro-p-anisic acid (145) (Figure 10) were identified from Penicillium sp. isolated from the leaf of Hopea hainanensis. All of the six isolated compounds were subjected to antifungal activity against three human pathogenic fungi namely Candida albicans, Trichophyton rubrum and Aspergillus niger. Compounds (141-143) and (145) inhibited the growth of C. albicans with MICs of 40.0, 20.0, 50.0 and 15.0 mcg/ml, respectively and compound (145) showed growth inhibition against A. niger with MICs of 40.0 mcg/ml (104).

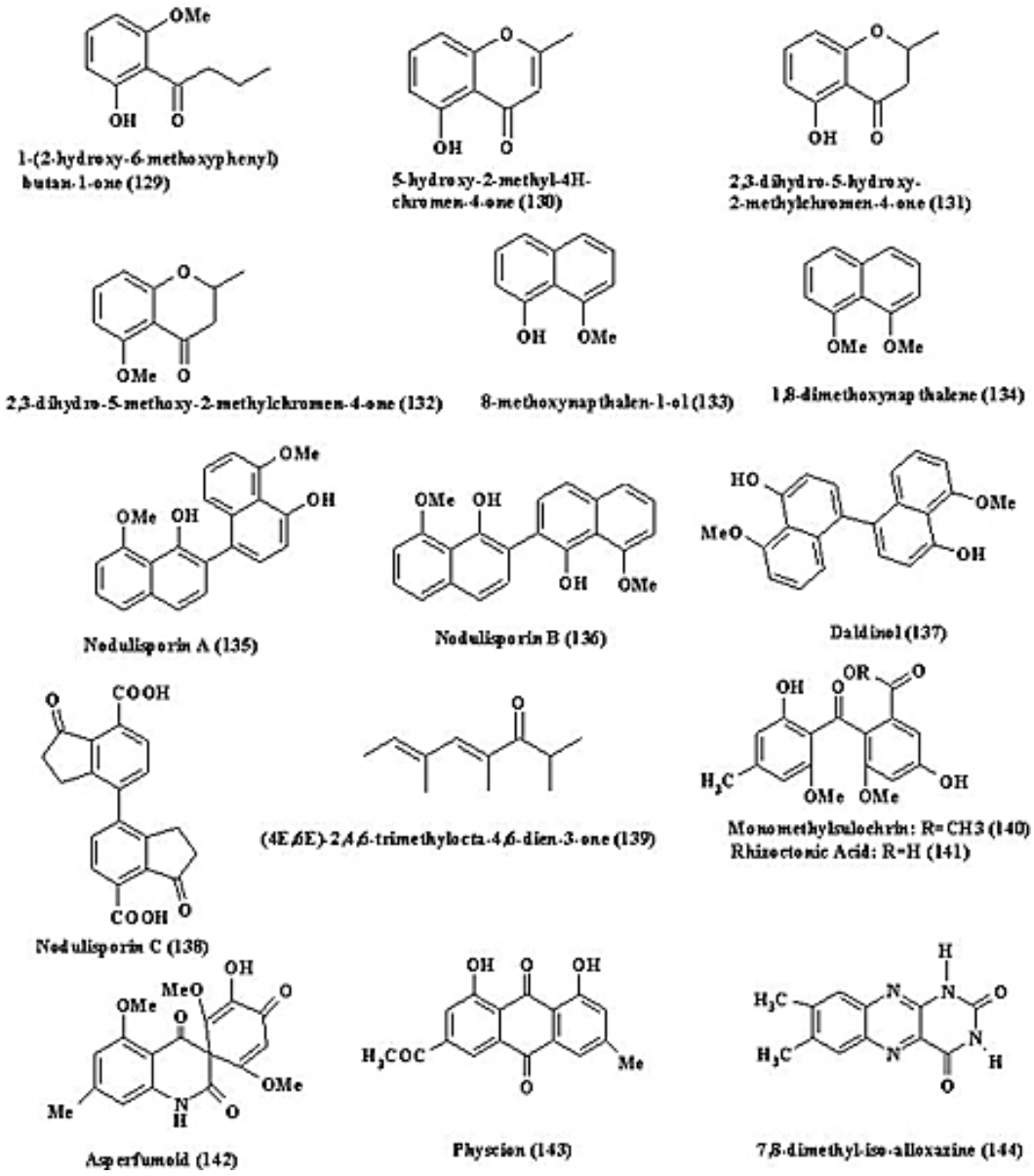

Figure 9. Structures of antifungal metabolites isolated from hyphomycetes

Benzopyran derivatives, 2-methyl-5-methoxy-benzopyran-4-one (146) and (2'S) 2- (propan-2'-ol)-5-hydroxy-benzopyran-4-one (147) (Figure 10) were isolated from Curvularia sp. obtained from the leaves of Ocotea corymbosa, a native plant of the Brazilian Cerrado. Compound (146) and (147) showed weak in-vitro antifungal activity against Cladosporium sphaerospermum and C. cladosporioides, showing detection limit of 10 mcg for both substances. Nystatin was used as a positive control showing a detection limit of 1mcg (105).

The antifungal metabolites, named Asperfumoid (142), Physcion (143), Fumigaclavine C (148), Fumitremorgin C (149) and Helvolic acid (150) (Figure 10) were obtained from an endophytic fungus, Aspergillus fumigatus CY018 isolated from the leaf of Cynodon dactylon. Compounds (142, 143, 148-150) inhibited the growth of Candida albicans with MICs of 75.0, 125.0, 31.5, 62.5, and 31.5 mcg/ml, respectively. The MIC of ketonazole used as a positive reference against C. albicans was 31.5 mcg /ml respectively (106).

Aspergillus clavatonanicus, an endophytic fungal strain from Taxus mairei yielded Clavatol (151) and Patulin (152) (Figure 10). Both compounds exhibited *in-vitro* inhibitory activity against several plant pathogenic fungi, namely Botrytis cinerea, Didymella bryoniae, Fusarium oxysporum f. sp. cucumerinum, Rhizoctonia solani and Pythium ultimum (107).

Asperamide A and B (153-154) (Figure 10), a sphingolipid and their corresponding glycosphingolipid

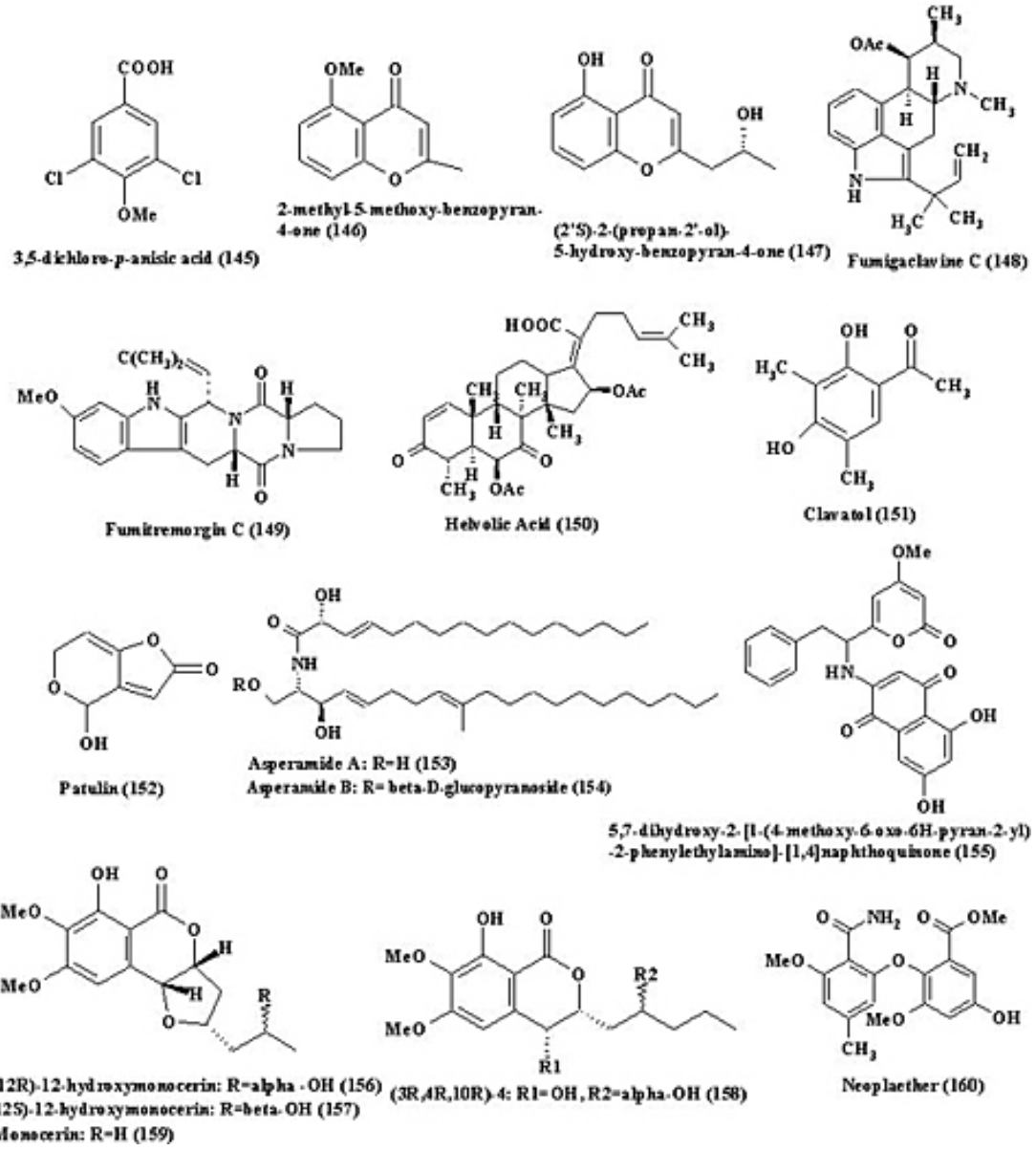

Figure 10. Structures of antifungal metabolites isolated from hyphomycetes

possessing a hitherto unreported 9-methyl-C20-sphingosine moiety, were characterized from Aspergillus niger EN-13, an endophytic fungus isolated from marine brown alga Colpomenia sinuosa along the Qingdao coastline of Shandong Province, People's Republic of China. Asperamide A (153) displayed moderate activity against Candida albicans with a zone of inhibition of 20mm (108). A new naphthoquinoneimine derivative namely, 5, 7-dihydroxy-2-(1-(4-methoxy-6-oxo-6H-pyran-2-yl)-2-phenylethylamino)- (1,4) naphthoquinone (155) (Figure 10) was also reported from Aspergillus niger EN-13 isolated from the inner tissue of the marine brown alga Colpomenia sinuosa. It displayed moderate antifungal activity against Candida albicans with an inhibitory zone (10 mm) at 20 mg/well (6 mm) (109).

Isocoumarin derivatives, (12R)-12-hydroxymonocerin (156), (12S)-12-hydroxymonocerin (157), (3R,4R,10R)-4 (2-4) (158) and Monocerin (159) (Figure 10) were isolated from Microdochium bolleyi, an endophytic fungus from Fagonia cretica, from the semiarid coastal regions of Gomera. Compounds (157), (158) and (159) exhibited good antifungal activity while compound (156) exhibited moderate antifungal activities against Microbotryum violaceum (110).

A new diphenyl ether, Neoplaether (160) (Figure 10) was isolated from the culture of Neoplaconema napellum IFB-E016, an endophytic fungus residing in the healthy leaves of Hopea hainanensis from Hainan Island, People's Republic of China. In-vitro antifungal activity of Neoplaether was examined, using Aspergillus niger,

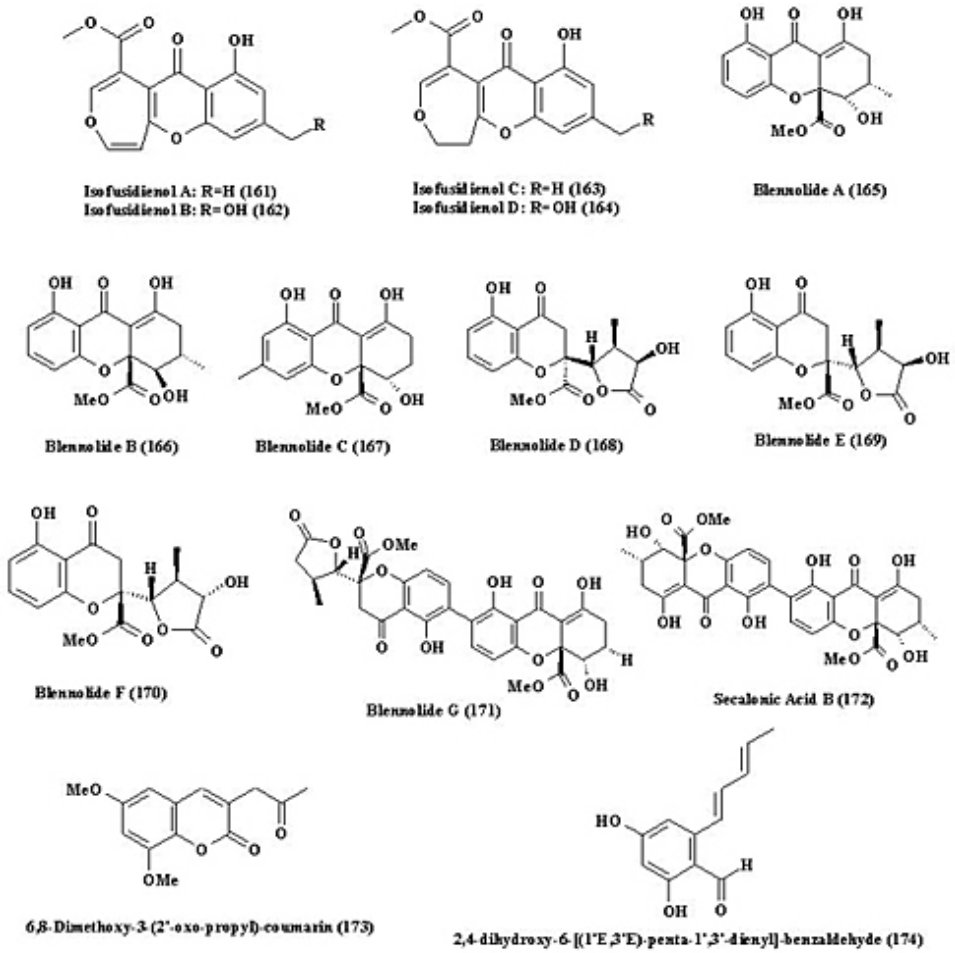

Figure 11. Structures of antifungal metabolites isolated from hyphomycetes

Candida albicans and Trichophyton rubrum as test organisms, and it showed obvious activity against C. albicans with an MIC value of 6.2 mcg/ml (that of Amphotericin co-assayed as positive control was 1.5 mcg/ml), whereas no growth inhibition to A. niger and T. rubrum could be discerned when it was tested at 100 mcg/ml (111).

Isofusidienol A–D (161-164) (Figure 11), were produced by Chalara sp. (strain 6661), an endophytic fungus isolated from Artemisia vulgaris. All the isofusidienol compounds exhibited antifungal activity against Candida albicans (112).

Blennolide A–G (165 - 171), seven unusual chromanones, were isolated together with Secalonic acid B (172) (Figure 11) from Blennoria sp., an endophytic fungus from a succulent Carpobrotus edulis growing on Gomera, in the Canary Islands. All the compounds showed strong antifungal activity against Microbotryum violaceum (113).

6,8-Dimethoxy-3- (2'-oxo-propyl)-coumarin (173) and 2,4-dihydroxy-6- ((1'E,3'E)-penta-1', 3'-dienyl)-benzaldehyde (174) (Figure 11), in addition to a known compound Periconicin B (126), were isolated from Periconia atropurpurea, obtained from the leaves of Xylopia aromatica, a native plant of the Brazilian Cerrado. These compounds were evaluated against Cladosporium sphaerospermum and C. cladosporioides. Only compound (174) exhibited strong antifungal activity against both fungi, showing detection limit of 1.0 mcg, comparable to nystatin (used as a positive control). Compound (173) did not show any antifungal activity and compound (126) showed a relatively weak detection limit of 25.0 mcg (114).

3.4. Compounds produced by unidentified fungus

Cabello *et al* (115) reported a novel acidic steroid Arundifungin (175) (Figure 12), a beta- (1,3)-glucan synthesis inhibitor from F-042,833, isolated from twig of Olea europea var europea an undetermined coelomycetes and F054,289 a sterile mycelium from leaves of Quercu silex isolated from Ontigola, Madrid, Spain. Arundifungin cause the same pattern of hallmark morphological alteration in Aspergillus fumigatus hyphae as echinocandins, which supports the idea that arundifungin, belongs to the class of glucan synthesis inhibitors. It exhibits antifungal activity against a range

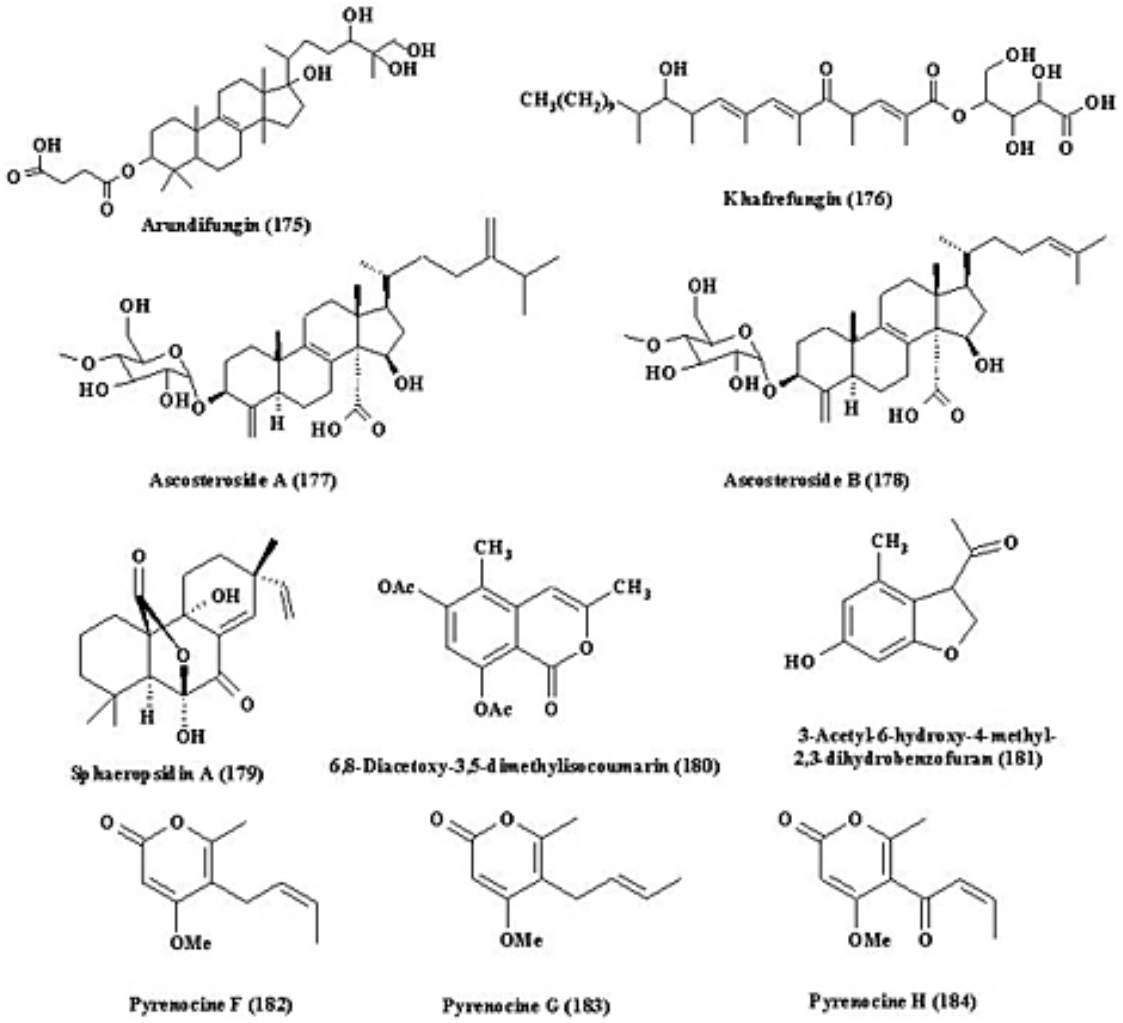

Figure 12. Structures of antifungal metabolites isolated from unidentified fungus

Candida sp. with MIC over 2-8 mcg/ml, whereas it is only 1mg/ml for Aspergillus fumigatus.

Khafrefungin (176) (Figure 12) was isolated from an unidentified sterile fungus (MF 6020) cultured from a Costa Rican plant sample. It inhibits fungal sphingolipid synthesis, at the step in which phosphoinositol are transferred to ceramide, resulting in accumulation of ceramide and loss of all of the complex sphingolipids. *In-vitro*, khafrefungin inhibits the inositol phosphoceramide synthase of Candida albicans with an IC_{50} of 0.6 nanomolar. Khafrefungin inhibited the growth of Candida albicans, Cryptococcus neoformans and Saccharomyces cerevisiae in liquid culture with MIC of 2, 2 and 15.6 mcg/ml respectively. Khafrefungin does not inhibit the synthesis of mammalian sphingolipids thus making this the first reported compound that is specific for the fungal pathway (116).

Ascosteroside A and B (177–178) (Figure 12) were isolated from E99291, an endophyte from shoots of Cistus salvifolius. Both the compounds showed antifungal activity against a wide range of saprophytic, plant and human pathogenic fungi (53). Ascosteroside A was originally isolated from Ascotricha amphitricha as a metabolite with anti-Candida activity (117), which is now known to be due to inhibition of beta- (1,3)-glucan synthesis (118).

Sphaeropsidin A (179) (Figure 12) was isolated from E99204, an endophyte from an asymptomatic leaf of Quercus ilex and exhibited antifungal activity against yeast and filamentous fungi (53). Sphaeropsidins are a group of pimarane diterpenes known from the anamorphic fungi Sphaeropsis sapinea f. sp. cupressi and Diplodia mutila (119 –120).

6,8-diacetoxy-3,5-dimethylisocoumarin (180), 3-Acetyl-6-hydroxy-4-methyl-2,3 dihydrobenzofuran (181) (Figure 12), were isolated from mycelia sterila, an endophytic fungus from the Canadian thistle Cirsium arvense growing in Lower Saxony, Germany. The

compounds were tested against three fungal test organisms namely Mycotypha microspora, Eurotium repens and Ustilago violacae. Compound (180) showed moderate antifungal activity against all tested fungi where as compound (181) was moderately antifungal against Eurotium repens (121).

Pyrenocine A (95), Pyrenocine F–H (182–184) (Figure 12), were isolated from an unidentified endophytic fungus (6760) isolated from Trifolium dubium from Wustrow near the Baltic Sea. Pyrenocine A (95) and G (183) exhibited considerable activity against Microbotryum violaceum (122).

4. VOLATILE ORGANIC COMPOUNDS FROM FUNGUS

Strobel *et al* (123) reported at least 28 volatile organic compounds (VOC) from xylariaceaous endophytic fungus Muscodor albus (isolate 620), isolated from small limbs of Cinnamomum zeylanicum located in the Lancetilla Botanical Garden near La Ceiba, Honduras which posses antimicrobial properties. These VOC's are a mixture of gases of five classes consisting primarily of various alcohols, acids, esters, ketones and lipids. The most effective class was the esters, of which 1-butanol, 3-methyl-acetate was the most active biologically. The VOC effectively inhibited and killed certain fungi and bacteria, within a period of 1 to 3days. Most test organisms were completely inhibited, and in fact killed namely Pythium ultimum, Phytophthora cinnamomi, Rhizoctonia solani, Ustilago hordei, Stagnospora nodorum, Sclerotinia sclerotiorum, Aspergillus fumigatus, Verticillum dahliae, Tapesia yallundae, Candida albicans, Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis. However the growth of Fusarium solani and Cercospora beticola, were only partially inhibited. The antibiotic effect of the VOC's of M. albus is strictly related to the synergistic activity of the compounds in the gas phase. The volatiles of M. albus did not kill M. albus itself or its close relative Xylaria sp., although they did inhibit the growth of Xylaria sp. Other species of Muscodor is Muscodor roseus, an endophyte from small limbs of a fern-leafed (Grevillea pteridifolia) in the Northern Territory of Australia and is also known to produce similar type of VOC's (124).

Recently another species of Muscodor namely Muscodor crispans, isolated from Ananas ananassoides (wild pineapple) growing in the Bolivian Amazon Basin produces VOC's; namely propanoic acid, 2-methyl-, methyl ester; propanoic acid, 2-methyl-; 1-butanol, 3-methyl-;1-butanol, 3-methyl-, acetate; propanoic acid, 2-methyl-, 2-methylbutyl ester; and ethanol. The VOC's of this fungus was effective against a wide range of plant pathogens, namely Pythium ultimum, Phytophthora cinnamomi, Sclerotinia sclerotiorum and Mycosphaerella fijiensis (the black sigatoka pathogen of bananas), and the serious bacterial pathogen of citrus, Xanthomonas axonopodis pv. citri. The VOC's of M. crispans killed several human pathogens, including Yersinia pestis, Mycobacterium tuberculosis and Staphylococcus aureus. Muscodor crispans is only effective against the vegetative cells of Bacillus anthracis, but not against the spores. Artificial mixtures of the fungal VOC's were both inhibitory and lethal to a number of human and plant pathogens, including three drug-resistant strains of Mycobacterium tuberculosis (125).

The mechanism of action of the VOC's of Muscodor spp. on target fungi and bacteria is unknown. A microarray study of the transcriptional response analysis of Bacillus subtilis cells exposed to M. albus VOC's showed that the expression of genes involved in DNA repair and replication increased, suggesting that VOC's are inducing some type of DNA damage in cells, possibly through the effects of one of the naphthalene derivatives (125).

The VOC's of M. albus kill many of the pathogens that affect plants, people and even buildings (123, 126). The term "mycofumigation" has been applied to the practical aspects of this fungus. The first practical demonstration of its effects against a pathogen was the mycofumigation of covered smut infected barley seeds for a few days resulting in 100% disease control (123). This technology is currently being developed for the treatment of fruits in storage and transit (127). Soil treatments have also been effectively used in both field and green house situations (128-130). In these cases, soils are pretreated with a M. albus formulation in order to preclude the development of infected seedlings.

5. OUTLOOK

From the extensive data quoted in this paper by the authors on antifungals and earlier numerous publications on a wide variety of bioactive compounds such as anticancer to name just one, it becomes obvious that endophytes are a feasible source for the discovery of novel molecules yet there are so much more to be discovered. Initially endophyte was an area of interest for the botanists, but after the discovery of interesting bio-active molecules it opened the field to structural chemists and biochemists. The interdisciplinary approach, wherein the knowledge of the botanist is combined with the technology and structural tools of the chemist can lead to real success in this area.

The tropical and sub-tropical rain forests have been used for the isolation of endophytes with an assumption that, the conditions prevailing in these ecosystems would require plants to be armed with antifungal and antibacterial allies. Other possible fruitful areas might include endophytes from plants growing in high alpine regions, desert areas, mangroves, marine weeds etc. as they are struggling to survive in harsh conditions.

Historically it has been assumed that the active ingredients are synthesized by the plant itself, but the discovery that microbial endophytes are capable of synthesizing bio-active compounds has opened up a whole new area of research. Plants used by indigenous people for medicinal purposes and that are endemic to a particular area may be a good source for endophyte isolation (18). A medicinal plant may be growing in the different part of the

world and is used in different treatments by different indigenous groups. The endophytes from the same medicinal plant should be investigated from different part of the world for bioactive metabolites and correlated with the activity (131).

The distribution of endophytes within a plant host needs to be studied as some are ubiquitous to all parts of the host, whereas others are found in the roots or roots and stem only, in the stem and leaves only, or only in the leaves. The distribution study will put a light on whether the medicinal property of a particular plant part is plant character or is it because of a specific endophyte residing in that plant part (131).

While studying the endophytic fungi from a particular plant or a group of plants the taxonomic identification is must, which actually should be mandatory for publication or documentation purpose. This will help in correlating the metabolites of host plant and the metabolites of endophytes harboring the same plant.

Not all endophytes are culturable (132), therefore, apart from isolating culturable endophytes from different taxonomic groups of plants and plants growing in different habitats, shotgun metagenomics for endophyte community analysis and function-based screening of their metagenomic libraries could be used to harness the unculturable and truly cryptic endophytes from environmental samples for bioactive metabolites (133).

Endophytic fungal species should be screened for their secondary metabolite spectrum under different growth conditions; culture parameters such as composition of growth medium, aeration, pH and the presence of certain enzyme inhibitors that can change dramatically the secondary metabolite profile and even induce the synthesis of several new metabolites (134).

The review of the literature indicates that novel chemotypes directed against the pathogenic fungi have come from the endophytic microbial source. It is well known that resistance development in pathogens has become a major hazard, for example azole resistant Candida albicans and effective molecules against such multidrug resistant microbes have not been easy to find. The mode of action of majority of compounds reported in this review is not known. The mode of action based screening offers a great scope for the isolation of novel antifungal lead from endophytic fungi. There are compounds like Cryptocandin, Echinocandin A, B, D, H, Sordaricin, Arundifungin, Khafrefungin which are isolated from endophytes and working through specific mode of action and can be a drugable candidate after chemical modification. For example, Echinocandin B was initially isolated from soil fungi; later from an endophytic fungi, and developed as antifungal drug, Anidulafungin by Vicuron Pharmaceuticals (VER002, LY-303366) (135).

There is a great need of culture collection for this group of fungi with trained mycologists having classical and molecular background. The collection will help not only in getting novel secondary metabolites for various pharmaceutical and agricultural applications but also for applications like biotransformation, enzyme production etc to name few.

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Abbreviations: mcg: microgram, ml: milliliter, AIDS: Acquired Immuno Deficiency Syndrome, MIC: Minimum Inhibitory Concentration, sp: species, IC_{50}: half maximal inhibitory concentration, EC_{50}: half maximal effective concentration, mg: milligram, mm: millimeter, ATCC: American Type Culture Collection, VOC: Volatile Organic Compound

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