

# Efficacy of some natural compounds as antifungal agents

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## ABSTRACT

Natural sources have been important for the development of new active molecules for many years. Various small molecules with unique chemical skeleton and potent bioactivities were discovered through various sources like plants, marine products, and microorganisms, etc., which are considered as very important part of the nature. A number of potent antifungals have been originated from various natural sources. This account describes structure and activities of selected agents isolated from various natural sources.

**Key words:** Antifungals, biological activities, chemical structure, natural sources

## INTRODUCTION

In the immunological comprised patients single mold spore can initiate the deadly process. Patients on chemotherapy or immunosuppression, imposed for organ transplantation and those with chronic diseases such as AIDs, recurrent infection, cystic fibrosis, and diabetes are also at risk.<sup>[1,2]</sup> One of the factors aiding the spread of fungal disease has been the widespread use of broad-spectrum antibiotics, which eliminate or decrease the nonpathogenic bacterial populations that normally compete with fungi. Another has been the increased number of individuals with reduced immune responses caused by the acquired immunodeficiency syndrome or by the action of immunosuppressant drugs, or cancer chemotherapeutic agents. Besides their increasing frequency, fungal infections are still associated with an unacceptably high mortality, up to 40% in bloodstream infections caused by *Candida albicans*<sup>[3]</sup> and more than 50% in invasive aspergillosis.<sup>[4]</sup> The most commonly recognized causes of opportunistic invasive fungal infections (IFIs) traditionally are *C. albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*.<sup>[5]</sup> Along with the widespread use of antifungal prophylaxis, the epidemiology of IFIs has shifted toward non-*albicans* *Candida*,

non-*fumigatus* *Aspergillus*, opportunistic yeast-like fungi (e.g. *Trichosporon* and *Rhodotorula* spp.), zygomycetes, and hyaline moulds (e.g. *Fusarium* and *Scedosporium* spp.).<sup>[6]</sup> The limitations of current antifungal drugs, increased incidence of systemic fungal infections, and rapid development of drug resistance have highlighted the need for the discovery of new antifungal agents, preferably with novel mechanism of action.<sup>[7,8]</sup> While synthetic efforts remain the mainstream in antifungal drug discovery, as evidenced by the fact that 18 out of 23 antifungal drugs approved from 1980 to 2002 are synthetic (83% belongs to single azole class), unique antifungal natural products have demonstrated remarkable success in this regard.<sup>[9]</sup> For example, two major drug classes currently in use (amphotericin B, the gold standard and armamentarium, and the lipopeptide caspofungin) are the important antifungal drugs approved in recent years, are derived from natural products.<sup>[10]</sup> Natural products provide opportunities for new drug leads. This account describes structure and activities of selected agents isolated from various natural sources with the aim to examine the recent developments toward discovering novel antifungal drugs of natural origin. However, we have excluded certain compounds that are often termed “nuisance” compounds, because they show a variety of biological activities.

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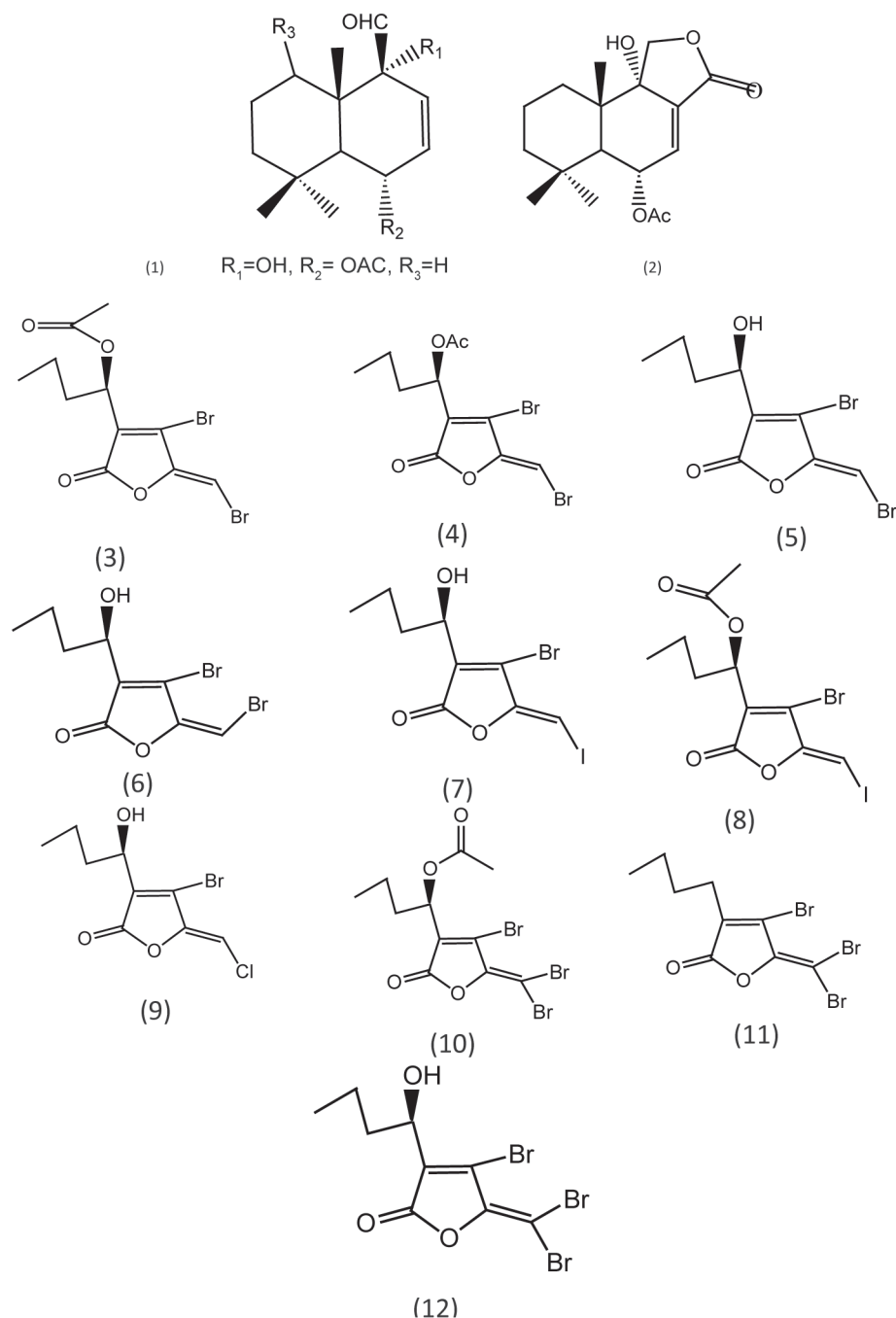
## ANTIFUNGALS FROM NATURAL SOURCES

Phytochemical analysis of *P. costaricense* parts leads to the isolation and identification of  $\delta$ -toco trienol,  $\beta$ -sitosterol, and other active constituents. Cinnamodial (1) and cinnamosmolide (2) were found to have high activity against *Alternaria alternate* (MIC=3.9 $\mu$ g/ml), *C. albicans*, and *Wangiella dermatides* (MIC=15.6 $\mu$ g/ml). Compound (2) showed less potent antifungal activities than (1) but was more effective against *C. albicans* CNIA (MIC = 23.4 $\mu$ g/ml).<sup>[11]</sup>

Twentyfive natural products mainly halogenated furanones were isolated from the temperate red algae *Delisea pulchra*. The activity was found to be very strong against fungus *P. oxalis* for 18 compounds, 10 compounds (3-12) maintained strong activity at 0.1 µg/ml while two compounds 10 and 12 have shown activities comparable to miconazole [Figure 1].<sup>[12]</sup>

Carneic acids (13, 14) isolated as major constituents of the stromata of *hyposylon carneum* have shown moderate antifungal activity against various fungal infections.<sup>[13]</sup> Hopeanolin (15), an unusual resveratral trimer with an *ortho*-quinone nucleus,

from stem bark of *Hopea exalata* demonstrated very good antifungal activity [Figure 2]. This plant has six known stibenoids, shorempinol (16), vaticanol (17),  $\alpha$ -viniferin (18), pauciflorol A (19), vaticanol A (20), and *trans*-3,5,4'-trihydroxystilbene 2-C-glucoside (21) have shown antifungal activities in the MIC value range 0.1–22.5 µg/ml.<sup>[14]</sup> Two new macrolides, swinholdes I (22) and hurghadolide A (23, 24) of which hurghadolide A possesses an unprecedented asymmetric 42-membered dilactone moiety were reported. Compounds 23 and 24 have been demonstrated to be active against *C. albicans*.<sup>[15]</sup>



**Figure 1:** Halogenated furanones obtained from algae as antifungal agents

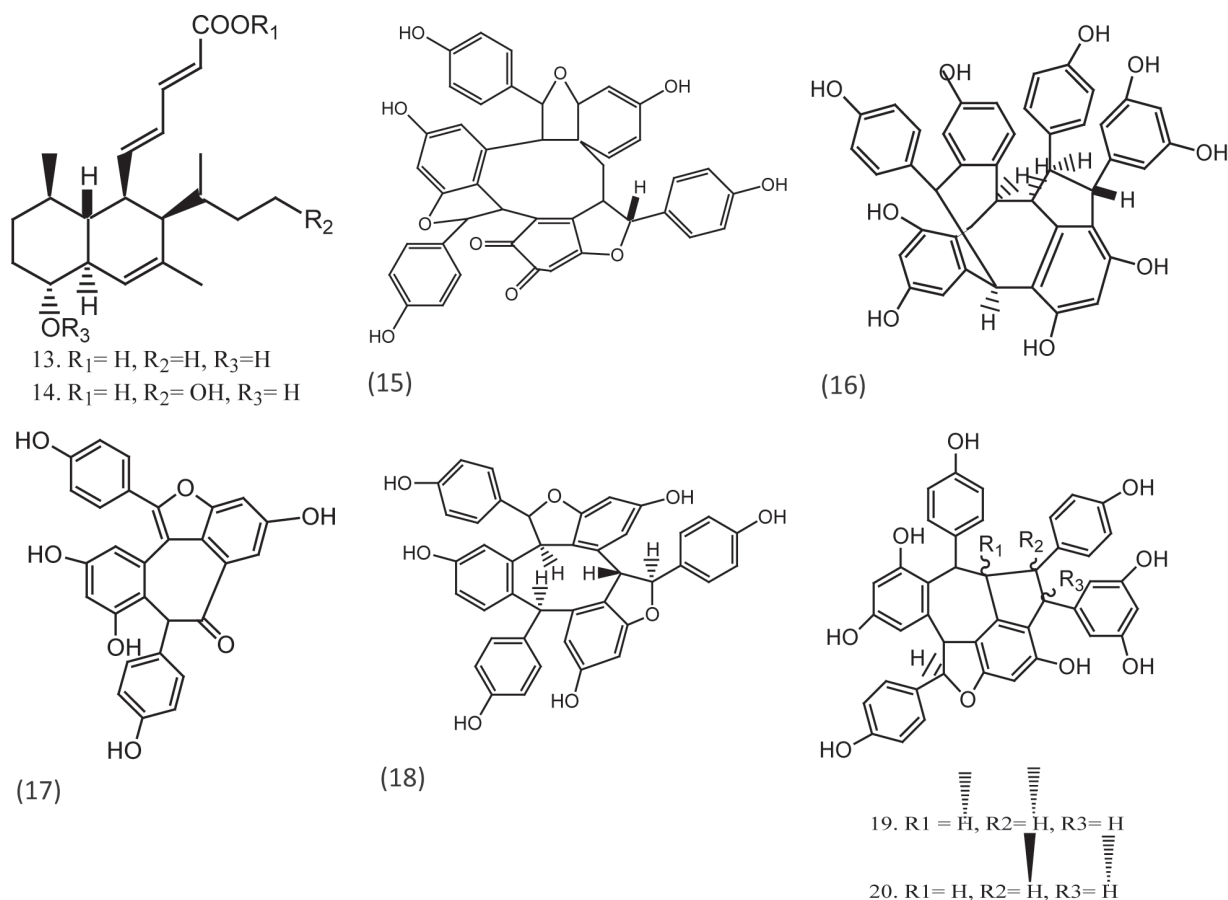
Marine-derived macrolides latrunculins A and B of the red sea sponge *Negombata magnifica* are the marine products. Latrunculin B (25) together with latrunculin T (26) was isolated and certain semisynthetic analogs (27, 28, 29, and 30) were reported to have potent activity against *C. albicans*, the presence of  $\alpha\beta$  lactones in the ring is responsible for the activities.<sup>[16]</sup>

Five new *ent*-labdane diterpenoids, 3-*o*- $\beta$ -D-glucopyranosyl-14,19-dideoxyandrographolide (31), 14-deoxy-17-hydroxyandrographolide (32), 19-*o*-[ $\beta$ -D-glucopyranosylandrographolide (33), 12-s-hydroxy andrographolide (34), and andrographatoside (35) were isolated from the aerial part of *andrographis paniculata* and all compounds were active against various fungal strains [Figure 3].<sup>[17]</sup>

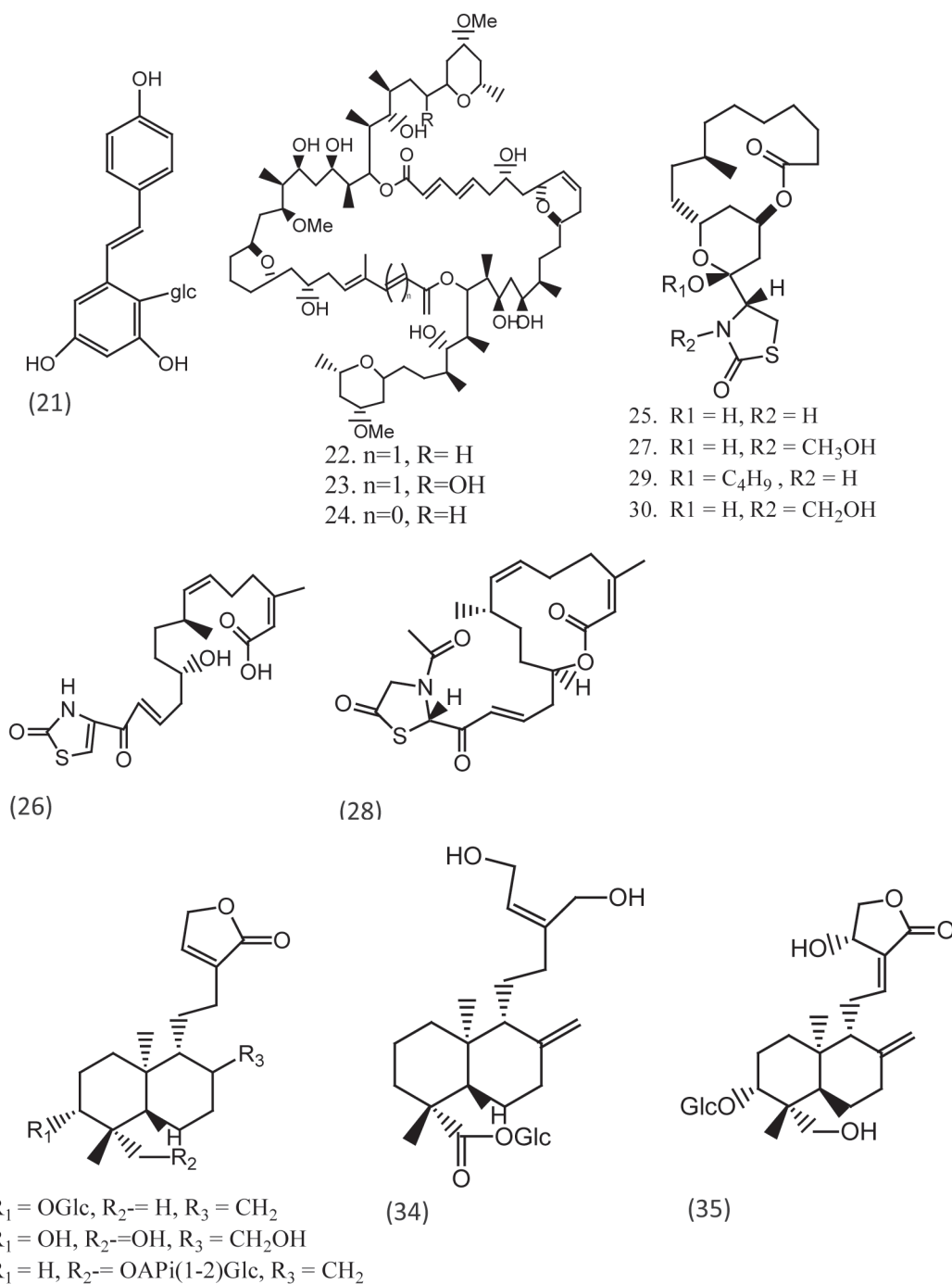
Three new *N*-methyl-4-hydroxy-2-pyridinone analogs, 6-epioxysporidinone (36), were isolated from the fungus *Fusarium oxysporum* (N17B). The compound showed selective fungistatic activity against *Aspergillus fumigatus*.<sup>[18]</sup> Four dialkylresorcinols (37-40) were isolated from the liquid culture of *Pseudomonas* sp. K19. These compounds were reported to inhibit *A. fumigatus* and *Fusarium culmorum* at 50  $\mu$ g/ml.<sup>[19]</sup> Three linear sesquiterpene lactones, anthecotulide (41), hydroxyl anthecotulide (42), and acetoxanthecotulide (43) were isolated from aerial parts of *Anthemis auriculata*.<sup>[20]</sup>

Two new azapilones named rotiorinols (44, 45) and stereoisomer (-)-rotiorin (46), and a known compound rubrotiorin (47) were isolated from the fungus *Chaetorium cupreum* CC3003. Compounds exhibited activity against *C. albicans* with  $IC_{50}$  values of 10.5, 16.7, 24.3, and 0.6, respectively [Figure 4].<sup>[21]</sup> Crysostriones A (48) and B (49), two new 2-acylcyclopentene-1,3-dione derivatives, were isolated from the fruiting body of the Basidiomycete *Hygrophorus chrysodon* found in mushrooms have shown activity against *Fusarium verticillioides*.<sup>[22]</sup> Two new peptaibols, septocylindrin A and septocylindrin B, were obtained from the fungus *Septocylindrium* sp.<sup>[23]</sup> A new marine -derived macrolides as neopeltolide (50) were reported to have activity against fungal pathogen *C. albicans* with MIC of 0.62  $\mu$ g/ml.<sup>[24]</sup> The natural product dihydroferulic acid (DFA, 51) and the six synthesized derivatives of it were examined for antifungal activities. Test fungi included *Saccharomyces cerevisiae*, *A. fumigatus*, and *A. flavus*.<sup>[25]</sup> Six new minor dammarane triterpenoids (52, 53) were isolated from the roots of *Gentiana rigescens*. These are the glycosides and have shown antifungal activity against the pathogen *Glomerella cingulata*.<sup>[26]</sup>

Four solanapyrone analogs (solanapyrones, 54-57) were reported from an unidentified fungicolous fungus and showed activity against *A. flavus* and *F. verticillioides*, and *C. albicans*.<sup>[27]</sup> Two volatile benzaldehyde derivatives (58, 59) were isolated from a



**Figure 2:** Compounds with quinone, furan nucleus



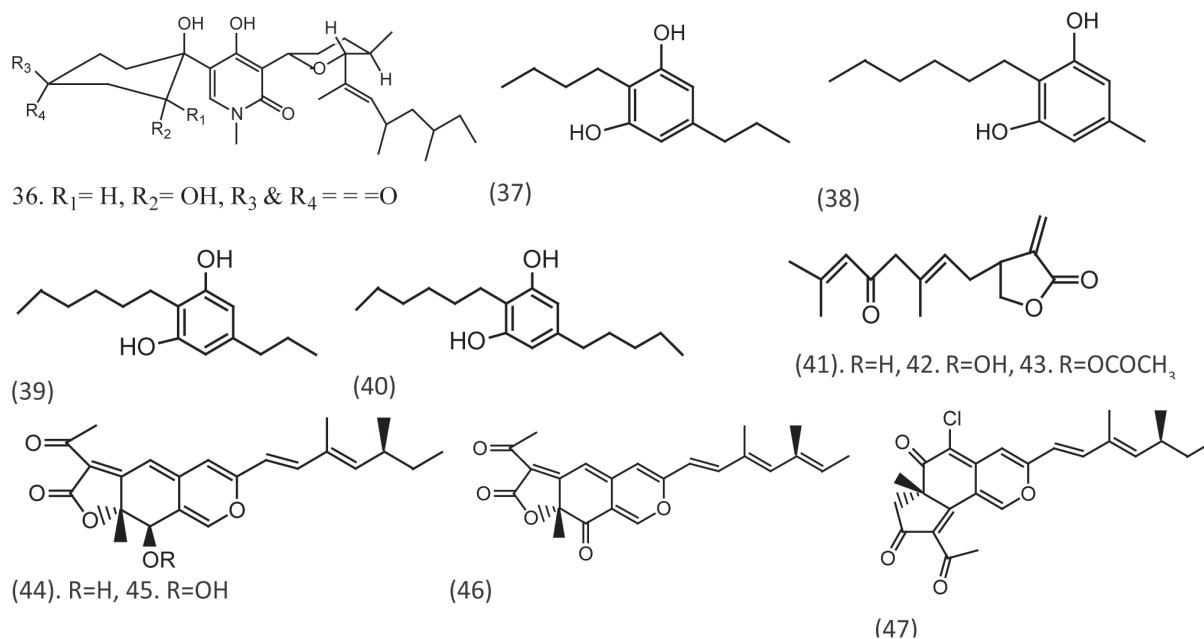
**Figure 3:** Compounds with thiazoles and macrolide structures

*Basidiomycete Sacodontia crosea* found to be active against various pathogenic fungi. The aerial part of *Centaurea pullata* resulted into compounds (60, 61) which is reported to have potent activity [Figure 5].<sup>[28]</sup>

Leaves of *Piper scutifolium* yielded two new isobutyl amides, scutifolium A and scutifoliamide B, which were reported as fungistatics.<sup>[29]</sup> Compound phaeosphenone (62, 63) was isolated from *Staphylococcus aureus* and inhibited the growth of *C. albicans* with an MIC of 8  $\mu\text{g}/\text{ml}$ .<sup>[30]</sup> 4-Terpenyl cannabinolate (64) which is isolated from *Cannabis sativa* have shown activity against

*C. albicans* ATCC 90028 with an  $\text{IC}_{50}$  value of 8.5  $\mu\text{g}/\text{ml}$ .<sup>[31]</sup> 1 $\alpha$ ,5 $\alpha$ -Dioxy-11  $\alpha$ -hydroxyurs-12-en-3-one (65) and ursane triterpenoid (66) exhibited modest activity against *E. coli*, *C. albicans* with the MIC 31.9  $\mu\text{M}/\text{L}$  and 36.3  $\mu\text{M}/\text{L}$ , respectively.<sup>[32]</sup>

Pestafolide A (67), a new reduced spiro azaphilone derivative, and pestaphthalides A (68) and B (69) were isolated from *Pestalotiopsis foedan*. These compounds have shown activity against *C. albicans* (ATCC 10231), *Geotrichum candidum*, and *A. fumigates* (ATCC 10894).<sup>[33]</sup>



**Figure 4:** Compounds reported with antifungal activity from different chemical categories

Lipophilic extracts of the stem bark of *Buddleja globosa* were found to have antifungal activity at 125  $\mu\text{g/ml}$  against three dermatophytic fungal species *Trichophyton rubrum*, *Trichophyton interdigitale*, and *Epidermophyton floccosum*. The known compounds were the diterpene buddlejone (70), the bisditerpene maytenone, and the two sesquiterpenes buddledin A and buddledin B, while the novel compound was characterized as the diterpene deoxybuddlejone (71).<sup>[34]</sup>

From witches' broom diseased bamboo, *Phyllostachys bambusoides*, *N-p*-coumaroylserotonin, and *N-feruloylserotonin* were isolated. *N-p*-Coumaroylserotonin possesses antifungal activity against *Aciculosporium takei*, the causal agent of witches' broom of bamboo. A new antifungal substance against rice blast fungus (*Pyricularia oryzae*), 5-(8'-*Z*-heptadecenyl) resorcinol, was isolated from etiolated rice seedlings together with a mixture of its homologs with  $C_{13}$ ,  $C_{15}$ , and  $C_{17}$  saturated alkyl chains. Its structure was determined by  $^1\text{NMR}$ ,  $^{13}\text{C-NMR}$ , and MS spectra. Its  $\text{ED}_{50}$  was 40  $\mu\text{g/ml}$ .<sup>[35]</sup>

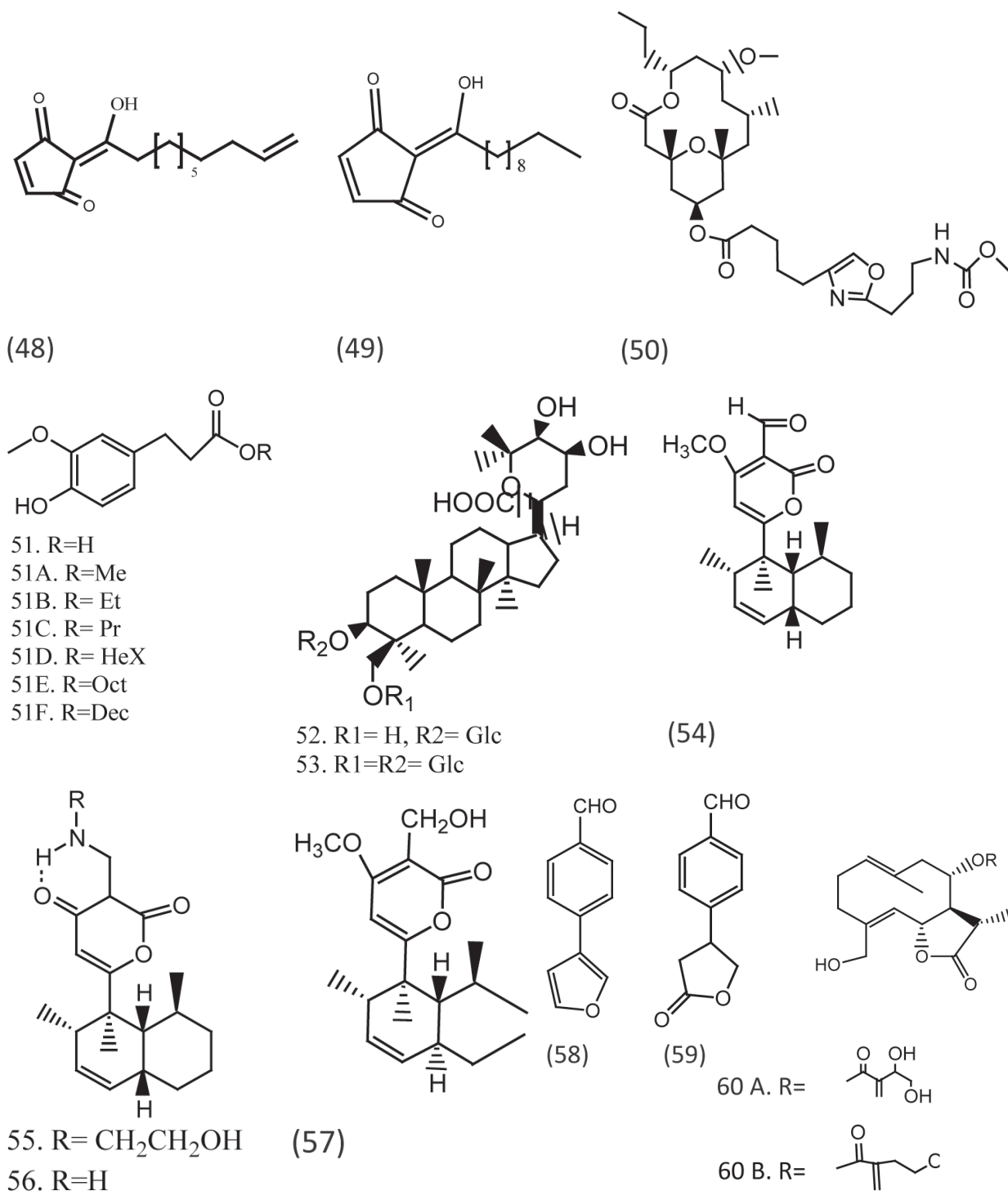
Two antifungal terpenoids, 6-isopropyltropolone  $\beta$ -glucoside and 5-(3-hydroxy-3-methyl-*trans*-1-butenyl)-6-isopropyltropolone  $\beta$ -glucoside, named by us cupressotropolone A and B, respectively, were isolated from the bark of Italian cypress, in response to infection by the fungus *Diplodia pinea* f. sp. *cupressi*. These tropolone glucosides inhibited *in vitro* germination of spores of *Diplodia pinea* f. sp. *cupressi*, *Seiridium cardinale*, *Alternaria alternata*, and *Verticillium dahliae*.<sup>[36]</sup> The antifungal activity of *Monilinia fructicola* from some naturally occurring pterocarpanes were reported with  $\text{ED}_{50}$  values around  $2 \times 10^{-5}$   $\mu\text{g/ml}$ .<sup>[37]</sup>

Secondary metabolites were extracted from culture filtrates of three isolates of the mycoparasitic biocontrol agent *Verticillium biguttatum*. The two antifungal metabolites were 1-[2',3'-dihydroxy-5'-(hydroxymethyl)phenyl]-3-methyl-but-

2-ene (72) and 1-[2'-hydroxy-3'-methoxy-5'-(hydroxymethyl)phenyl]-3-methyl-but-2-ene (72, 73). These new metabolites were named bigutol and methylbigutol, respectively. Low concentrations of each metabolite inhibited mycelial extension of *Rhizoctonia solani* (minimum inhibitory concentration 138  $\mu\text{g/ml}$ ). Production of these antifungals by *V. biguttatum* suggests that antibiosis may play a role during biocontrol by this mycoparasite, particularly of plant diseases caused by *R. solani*.<sup>[38]</sup> Four new phenalenone-type phytoalexins, named musanolones (74–77), have been isolated from infected rhizomes of banana plants (*Musa acuminata*; AAA cultivar Grand Nain). All the compounds have reported to have a strong inhibitory activity on the growth of the germination tube of *F. oxysporum* f. sp. *cubeense* race 4.<sup>[39]</sup>

Bioassay-guided fractionation resulted in the isolation of four antifungal agents from the roots of *Cudrania cochinchinensis*. Two of these were new compounds, cudraxanthone S [1,3,5,6-tetrahydroxy-2-(1,1-dimethyl-2-propenyl)xanthone] (78) and cudraflavanone B (2',4',5,7-tetrahydroxy-6-prenylflavanone) (79). These compounds exhibited antifungal activities against *C. neoformans*, *A. fumigatus*, and *A. nidulans* (MICs, 2–8  $\mu\text{g/ml}$ ) [Figure 6].<sup>[40]</sup>

Vitis vinifera antimicrobial peptide (Vv-AMP1), isolated from *Vitis vinifera*, shown to encode a plant defensin. Recombinant Vv-AMP1 showed nonmorphogenic antifungal activity against a broad spectrum of fungi, probably altering the membrane permeability of the fungal pathogens. Recombinant Vv-AMP1 was extremely heat-stable and showed strong antifungal activity against a broad spectrum of plant pathogenic fungi, with very high levels of activity against the wilting disease causing pathogens *F. oxysporum* and *Verticillium dahliae*. The Vv-AMP1 peptide did not induce morphological changes on the treated



**Figure 5:** Compounds with remarkable antifungal activities

fungal hyphae, but instead strongly inhibited hyphal elongation. A propidium iodide uptake assay suggested that the inhibitory activity of Vv-AMP1 might be associated with altering the membrane permeability of the fungal membranes.<sup>[41, 42]</sup>

Dalbergia is a genus of trees, shrubs, and woody climbers widely distributed in tropical and sub-tropical regions. It possesses good antimicrobial activity.<sup>[43]</sup> Terminalia chebula

plant was also reported to have antifungal activity.<sup>[44]</sup> Entada abyssinnica (Fabaceae) leaves used by herbalists from the Lake Victoria region, Kenya. *E. abyssinnica* could be a rich source of antimicrobial agents, especially antifungals (zones of inhibition were between 9.00 and 14.10 mm) against *C. albicans*, *Sa. typhi*, and *St. aureus*.<sup>[45]</sup> *Butea monosperma* has been reported for their antimicrobial activities.<sup>[46]</sup> *Nyctanthes arbortristis* have also shown great potential for antifungal activity.<sup>[47]</sup>

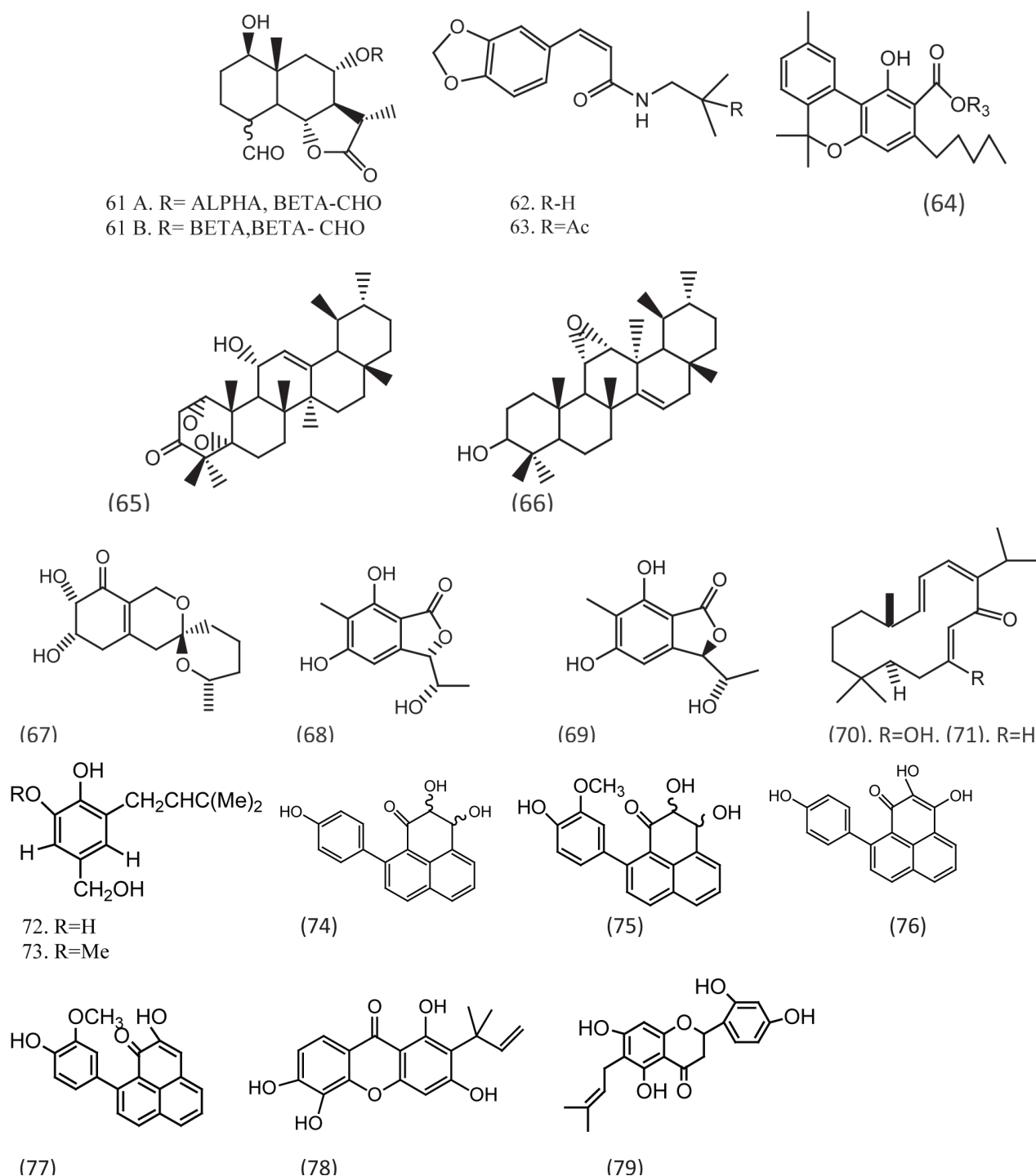


Figure 6: Compounds with good antifungal activities

## CONCLUSION

A large number of metabolites from marine invertebrates, from plants, and from various other natural sources have been reported to inhibit the pathogenic fungi. These compounds represent a wide variety of structural classes ranging from cinnamodial sterols, furanones, quinines, and resorcinol to terpenoids in natural origin category. The spread of multidrug-resistant

strains of fungus and the reduced number of drugs available make it necessary to discover new classes of antifungals and compounds that inhibit these resistant mechanisms. This has led to a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties. In these natural sources, a series of molecules with antifungal activity against different strains of fungus have been found, which are of great importance

to humans and plants. It is hoped that other chemists will embark on the search for potent antifungal agents.

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Articles can be submitted online from <http://www.journalonweb.com>. For online submission, the articles should be prepared in two files (first page file and article file). Images should be submitted separately.

1) **First Page File:**

Prepare the title page, covering letter, acknowledgement etc. using a word processor program. All information related to your identity should be included here. Use text/rtf/doc/pdf files. Do not zip the files.

2) **Article File:**

The main text of the article, beginning with the Abstract to References (including tables) should be in this file. Do not include any information (such as acknowledgement, your names in page headers etc.) in this file. Use text/rtf/doc/pdf files. Do not zip the files. Limit the file size to 1024 kb. Do not incorporate images in the file. If file size is large, graphs can be submitted separately as images, without their being incorporated in the article file. This will reduce the size of the file.

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Submit good quality color images. Each image should be less than **4096 kb (4 MB)** in size. The size of the image can be reduced by decreasing the actual height and width of the images (keep up to about 6 inches and up to about 1800 x 1200 pixels). JPEG is the most suitable file format. The image quality should be good enough to judge the scientific value of the image. For the purpose of printing, always retain a good quality, high resolution image. This high resolution image should be sent to the editorial office at the time of sending a revised article.

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