Appl Microbiol Biotechnol (2002) 58:46–57
DOI 10.1007/s002530100822

MINI-REVIEW

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Antifungal antibiotics

Received: 25 May 2001 / Received revision: 21 August 2001 / Accepted: 24 August 2001 / Published online: 10 November 2001

Abstract The search for new drugs against fungal infections is a major challenge to current research in mycotic diseases. The present article reviews the current types of antifungal infections, the current scenario of antifungal antibiotics, and the need and approaches to search for newer antifungal antibiotics and antifungal drug targets.

Introduction

Among the different types of drug prevailing in the market, antifungal antibiotics are a very small but significant group of drugs and have an important role in the control of mycotic diseases. The need for new, safe and more effective antifungals is a major challenge to the pharmaceutical industry today, especially with the increase in opportunistic infections in the immunocompromised host. The history of new drug discovery processes shows that novel skeletons have, in the majority of cases, come from natural sources (Bevan et al. 1995). This involves the screening of microorganisms and plant extracts, using a variety of models (Shadomy 1987). Today, the emphasis is on the exploration of unusual and previously ignored ecosystems, using a variety of selected novel targets (Cragg et al. 1997; Von Dohren and Grafe 1997; Hegde et al. 2001; Phoebe et al. 2001). However, exceptions do exist, such as the fluoroquinolones, the azoles and the allylamines, which have all come from synthetic chemistry programs.

Historical background

The discovery of antifungal antibiotics dates back to 1939 when Oxford et al. (1939) reported the isolation of the first antifungal antibiotic, griseofulvin or "curling factor". Brian et al. (1949) described the curling effect on the fungus *Botrytis allii*. It was found to be produced by *Penicillium griseofulvum* and *P. janczewskii* Zal; and its structure was elucidated by Grove and McGowan (1947) and Brian et al. (1949). Griseofulvin inhibits the growth of various species of *Epidermophyton*, *Microsporum* and *Trichophyton*. It is fungistatic and is bound to cell lipids. In addition, it inhibits nuclear division, depending on the concentration used (Gull and Trinci 1973). Cycloheximide (also known as actidione and naramycin A) was discovered by Whiffen et al. (1946) in a streptomycin-yielding culture of *Streptomyces griseus*. It was first isolated in crystalline form by Leach et al. (1947) and its chemical structure was determined by Kornfeld et al. (1949). The first member of the group of polyene macrolide antifungal antibiotics, tentatively named fungicidin, was discovered by Hazen and Brown (1950) and was later renamed nystatin. Since 1950, more than 90 different members of this group have been described; and more are being discovered each year (Gil et al. 1984). Polyene macrolides, although light-sensitive and highly toxic (due to the nonspecific binding to all sterols, causing membrane permeability and inhibition of cytochrome P-450 and the electron transport chain), are used in synergism with other agents. Amphotericin B, a polyene macrolide which is still a "gold standard" for the treatment of the most severe invasive fungal infections, was discovered in the 1950s and marketed in 1957. This was followed by the lipopeptides in the 1970s. Since then, the search for new, safer, broad-spectrum antifungal antibiotics with greater potency has been progressing slowly Kobayashi and Medoff 1977; Martin 1977). One reason for the slow progress compared to antibacterials is that, like mammalian cells, fungi are eukaryotes; and therefore agents that inhibit protein, RNA or DNA biosynthesis in fungi have greater potential for toxicity to the host as well (Georgopapadakou and Walsh 1994). The second reason is that, until recently, the incidence of life-threatening fungal infections was perceived as being too low to warrant aggressive research by the

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pharmaceutical companies (Georgopapadakou and Walsh 1996).

The need for antifungals

Fungi are a large group with about 250,000 species, of which more than 300 species have been reported to be potentially pathogenic to humans (Guarro et al. 1997). Fungal infections have been gaining prime importance because of the morbidity of hospitalized patients (Beck-Sague and Jarvis 1993). Approximately 90% of human fungal infections are caused by *Aspergillus*, *Candida*, *Cladosporium*, *Epidermophyton*, *Microsporum* and *Trichophyton* spp (Dasgupta 1998; Pathak 1998). Of major concern are the cases of systemic mycoses caused by *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Fusarium* sp., *Histoplasma capsulatum* and *Pneumocystis carinii*, which have been increasing over the years. Dasgupta (1998) reports a 400% increase over the past two decades. The frequency of candidiasis has increased ten-fold, so that *Candida albicans* has become the fourth most common culture isolate. Invasive pulmonary aspergillosis is a leading cause of attributable mortality in bone-marrow transplant recipients (Beck-Sague and Jarvis 1993). The cause of concern is further enhanced by the fact that these pathogens and several others (e.g. *H. capsulatum*, *Coccidioides immitis*) which cause respiratory ailments in otherwise healthy patients may result in severe systemic infections in immunocompromised individuals (Perfect and Schell 1996; Dasgupta 1998).

Market evolution

Antifungal agents now constitute 15–16% of the total activity in the infective area (Dasgupta 1998); and the world market for the antifungals is reported to be expanding at a rate of 20% per annum (Khan and Gyanchandani 1998). Although synthetic drugs contribute to a major proportion of the antifungals used, natural antifungals have their own place in the antimycotic market (Cragg et al. 1997). The interest in searching for new molecules has been enhanced with the development of new approaches and ideas, such as the use of genetically engineered microbes and cells as screening organisms, and newer sources of bioactive materials, like marine organisms, coral reefs and sponges from ecological niches (Monghan and Tkacz 1990; Jacob and Zasloff 1994; Persidis 1998; Fudou et al. 2001a; Phoebe et al. 2001).

Approaches to the discovery of antifungals

The earliest classical method used to detect antifungals is the whole-cell bioassay technique, e.g. using susceptible and resistant species of *Aspergillus* and *Candida*. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration are determined and the best compound is selected. Subsequently, the antifungal profile of the compound is determined, using a wide range of pathogens. Finally, the compound has to be proven to be active on in vivo models. This classical method is still used today, because it targets the growth of whole organism (Barry 1980; Shadomy et al. 1985; McGinnis and Rinaldi 1991). However, this method cannot identify the target specificity or the mechanism of action of the compounds.

Today, with the greater knowledge of fungal metabolism, efforts are being made to inhibit specific enzymes in the metabolic pathway of the fungus. For example, inhibition of glucan and/or chitin synthase, which leads to a lack of cell-wall formation. Inhibition of squalene epoxidase and/or squalene synthetase is another target that has been chosen (Tanimoto et al. 1996; Wills et al. 2000; Table 3). There are several new targets that are being explored for new antimycotics:

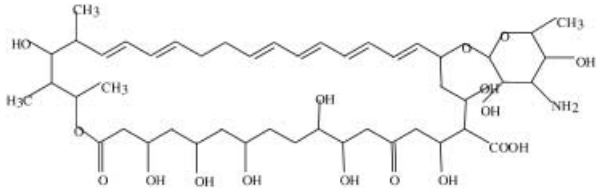
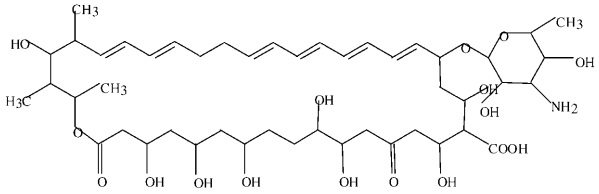
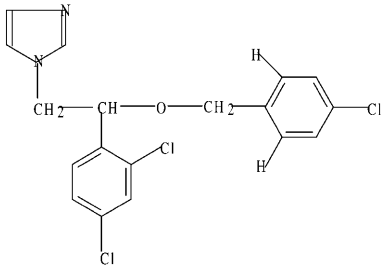
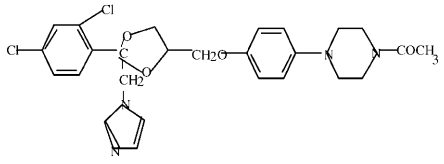
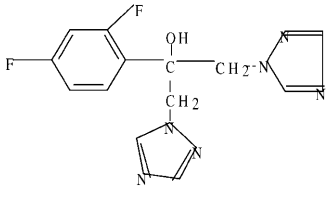
1. Specific inhibition of cell wall biosynthesis, particularly the glucan synthesis complex of enzymes (Mio et al. 1997).
2. Cell wall associated “adhesions”. These are mannoproteins that exist in the cell wall periphery (Wills et al. 2000).
3. Inhibition of synthesis of specific lipids of plasma membrane (Monk and Perlin 1994).
4. Serine/threonine phosphatases in cell wall biogenesis, particularly in *Saccharomyces cerevisiae*. The target may be the proteins they phosphorylate. But are they present in *Candida*? This is not clear (Hemenway and Heitman 1999).
5. Secreted aspartyl proteinases (Ross et al. 1990; Sato et al. 1994).
6. Translation elongation factors, EF3 is the focused target, but EF2 is also being investigated (Qin et al. 1990; Colthurst et al. 1991; Selva et al. 1997).

The success of such screening models is dependent on the access to adequate supplies of the enzymes and/or the proteins required. The latest advances in molecular biology techniques provide the solution and this in turn leads to the implementation of high-throughput screening and better chances of success (Bevan et al. 1995; Fostel and Lartey 2000).

Antifungal antibiotics in current use

Antifungal agents have a wide application in human medicine, agriculture and veterinary medicine (Misato and Yamaguchi 1977; Vandamme 1984). Five major classes of systemic antifungal compounds are currently in clinical use: the polyene antibiotics, the azole derivatives, the allylamines and thiocarbamates, the morpholines and the nucleoside analogs (Table 1; Georgopapadakou and Walsh 1996). The first three are targeted against ergosterol, the major fungal sterol in the plasma membrane. They are ineffective against *Pneumocystis*

Table 1 Major classes of antifungal agents

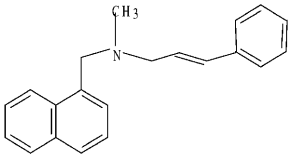
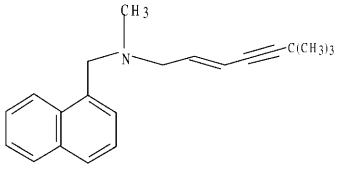
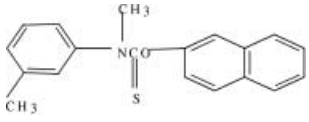
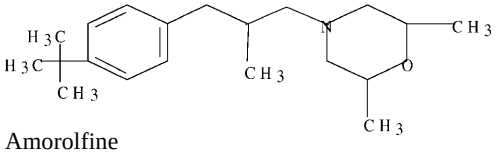
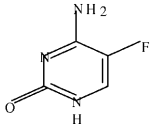
Class and compound	Route of administration	Mechanism of action	Structure
Polyene			
Amphotericin B, Nystatin	Systemic, topical	Interact with ergosterol, thereby disrupting the cytoplasmic membrane	 Amphotericin
			 B Nystatin
Azoles			
Miconazole, Ketoconazole, Fluconazole	Topical, systemic, systemic	Interact with cytochrome P-450; inhibit C-14 demethylation of lanosterol, causing ergosterol depletion and accumulation of aberrant sterols in the membrane	 Miconazole
			 Ketoconazole
			 Fluconazole

carinii, which has cholesterol instead of ergosterol, which it possibly acquires from its mammalian host (Kaneshiro et al. 1989). The fourth inhibits sterol synthesis and the fifth class targets the DNA synthesis. Griseofulvin, a nuclear division and membrane tubule inhibitor, and lipopeptides that are known to act on *Pneumocystis carinii* belong to a miscellaneous class of compounds (Gull and Trinci 1973; Morris et al. 1994).

Toxicity and drug resistance problems

Amphotericin B is used in the treatment of serious disseminated dimorphic fungal and yeast infections, caused by *Blastomyces*, *Candida*, *Cryptococcus* and *Histoplasma* spp. However, it causes nephrotoxicity, reduction of renal blood flow, nausea, vomiting and anorexia. Nystatin, although too toxic for systemic use, is mainly ap-

Table 1 (continued)

Class and compound	Route of administration	Mechanism of action	Structure
Allylamine and thiocarbamate			
Naftifine, Terbinafine, Tolnaftate	Topical, systemic, topical	Inhibit oxidosqualene cyclase, thereby causing ergosterol depletion and accumulation of squalene oxides in the membrane	 Naftifine  Terbinafine  Tolnaftate
Morpholine			
Amorolfine	Topical	Inhibit sterol reductase and isomerase; only the former is essential	 Amorolfine
Nucleoside analogue			
5-Fluorocytosine (5FC)	Systemic	Is deaminated to 5-FU causes miscoding and inhibits DNA synthesis.	 5-Fluorocytosine

plied topically in cases of mucous membrane candidiasis. Griseofulvin causes hepatotoxicity and gastrointestinal distress but is used for the treatment of certain dermatophyte infections, caused by *Epidermophyton*, *Microsporum* and *Trichophyton* spp. 5-Fluorocytosine interferes with DNA synthesis and causes bone-marrow toxicity, leukopenia and liver enzyme elevations. It is used in adjuvant therapy with amphotericin B. Most of the azoles are toxic and cause hepatic necrosis and abdominal cramping. They are mainly used topically in the treatment of candidiasis, coccidiodal meningitis, cutaneous dermatophytes and histoplasmosis (Iyer 1998). The echinocandins (glucan synthesis inhibitors) and nikkomycins (chitin synthesis inhibitors) are used in combination with amphotericin B and have relatively lower toxicity, compared with the polyenes (Decker et al. 1990, 1991; Bartizal et al. 1997). Thus, a number of factors affect and limit the use of some of the existing antifungal antibiotics, which necessitates a search for newer antifungal antibiotics to control existing problems. These include low potency, poor solubility, limited or inconvenient dosage forms, narrow clinical spectrum, rapid emergence of resistant strains and drug toxicity (Shadomy 1987; Bennett 1990; McGinnis and Rinaldi 1991; Odds 1993). The mechanisms of drug resistance involve: (1) changes in membrane permeases (Kurtz 1998), (2) changes in cellular efflux mechanism (Clark et al. 1996), (3) changes to a particular fungal "activase" whose action is required before that agent becomes metabolically active (Kurtz 1998) and (4) mutations that render the target enzyme less sensitive or insensitive to the antimycotic agent. For some targets, cryptic bypass pathways can substitute for the otherwise essential cell product. In addition, regulatory mutations that increase

cellular levels of an essential enzyme can also provide a means of resistance to a particular agent. For instance, increased production of lanosterol demethylase enables *Candida albicans* to withstand antimycotic agents such as amphotericin B (Kurtz 1998; Wills et al. 2000). In theory, fungal cells sometimes induce enzymes that can degrade or inactivate the antimycotic agent to which they are exposed. So far, this mechanism has not been demonstrated for any clinical antifungal agent, although there are numerous examples of antibacterials, such as β-lactams and β-lactamases, where this occurs. Each of the mechanisms which have been observed among fungal pathogens involves vertically transmitted mutations in the fungal genome. Investigators have not recorded any examples of plasmid-mediated resistance in fungi (White 1997; Kurtz 1998). Pathogenic fungi present a threat not only to immunocompromised patients with immune systems weakened by AIDS, aggressive cancer chemotherapy, or drugs aimed at foiling rejection of transplanted organs but also to others, particularly when microbes are resistant to antifungal agents (McGinnis and Rinaldi 1991; Odds 1993). Otherwise healthy patients hospitalized for elective surgery, for example, are potential prey for fungi transmitted through catheters, prostheses and other invasive devices. Studies confirm a surge in the rates of nosocomial fungal infections. Recent studies have found examples of fungal resistance to agents such as 5-fluorocytosine, amphotericin B and the azoles. An estimated 5–10% of patients with advanced AIDS have oropharyngeal infections with *Candida* that are difficult to treat with currently available and readily used drugs, notably fluconazole. In view of all these limitations of the existing agents, the need for more effective and eco-friendly therapeutic agents is increasing. (Ben-Yaacov et al. 1995; Phoebe et al. 2001).

New targets for novel antimycotics

Promising new compounds have recently been identified in an effort to supplement the relatively sparse portfolio of antifungal drugs. Many of these compounds have defined mechanisms of action against fungal cells and have, in some cases, aided the identification of new selective targets in fungi.

The fungal cell membrane and cell wall are structures that are essential for the fungus to survive. They differ from those in the mammalian host and consequently present attractive targets for new antifungals (Cabib et al. 1988; Calderone and Brown 1991). Chitin and glucan have been targeted mainly. Chitin, the structural component of the cell wall, is synthesized by a family of enzymes known as chitin synthases I, II and III (CS I, II and III) in *Candida albicans* and *Saccharomyces cerevisiae*. The loss of function of any one of them can only be partially fulfilled by the remaining enzymes (Shaw et al. 1991; Sudoh et al. 1993). Nikkomycins and polyoxins belong to a family of peptide-nucleoside antimycotic agents that are substrate analogs of UDP-*N*-acetylglucosamine, the essential building block for chitin biosynthesis (Georgopapadakou and Tkacz 1995). Individual chitin synthases vary in their susceptibility to these substrate analog inhibitors. In general, the nikkomycins inhibit all CS enzymes from *C. albicans* and kill *C. albicans* and other fungi with high chitin content, but they do not kill *S. cerevisiae*. However, their relative activity in vitro does not correspond to the MIC of these agents against whole cells. Other factors, such as the efficiency of peptide transport in cells, the relative activity of peptidases and the biological role played by the nikkomycin-sensitive enzyme in cells, contribute to the overall activity of these agents in intact cells (Decker et al. 1990; Kurtz 1998).

The steps in the biosynthesis of the fungal cell wall polymer, glucan, have been well studied (Cabib et al. 1988; Tkacz 1992; Georgopadakou and Tkacz 1995). Glucan synthase inhibitors are now known to be effective against *Pneumocystis carinii*, which is being seen in AIDS patients with increasing frequency. Several natural lipopeptides or lipopolysaccharides, including a number of echinocandins, pneumocandins, papulacandins, mulundocandins and benanomycins have been reported to have effect on glucan synthase and inhibit the synthesis of glucan in whole fungal cells (Roy et al. 1987; Takeuchi et al. 1988; Schwartz et al. 1989; Schmatz et al. 1992; Kurtz and Douglas 1997). They also block enzyme reactions that are specific to this biosynthetic pathway. Hence, several members of this group of inhibitors are of particular interest as antifungal agents for potential clinical use (Kurtz 1998). Mannoproteins, which comprise as much as 50% of the carbohydrate present in fungal membranes, represent a potential target. The greatest concentration of cell-wall mannoproteins is found at the periphery of the cell wall, where it constitutes the major antigenic component of such cells. The proposed mechanism of compounds such as benanomycins and pradimicins is an initial calcium-dependent complexing of their free carboxyl group with the saccharide portion of cell-surface mannoproteins, followed by action on the membrane, causing leakage of intracellular potassium (Sawada et al. 1990; Ueki et al. 1993). So far, no single cell wall-associated mannoproteins has been identified as essential for viability, by gene disruption experiments. This probably suggests that individual mannoproteins probably may not make an attractive target for researchers seeking to develop antimycotic agents.

Like its mammalian counterpart, the fungal plasma membrane contains sterols and phospholipids as its major lipid components and functions as a permeability barrier, a conduit for the transport of small molecules and signals, and a matrix for proteins. A key factor for its functions is its fluidity, which is determined by its lipid composition (Georgopapadakou and Walsh 1996; Wills et al. 2000). Fungal sterols, which are structurally distinct from their mammalian counterparts, have been studied extensively in attempts to obtain novel antifungals (Weete 1989; Mercer 1993). New targets actively pursued in the ergos-

Table 2 Important antifungal compounds acting on new target sites

New target site	Name/class of compound
Fungal cell wall inhibitors	
Chitin synthase inhibitors	Polyxins, nikkomycins
Glucan synthase inhibitors	Echinocandins, papulacandins
Mannoprotein inhibitors	Benanomicins, pradimicins
Ergosterol synthesis inhibitors	
Sphingolipid inhibitors	Sphinofungins
Proton ATPase inhibitors	Folimycins, bafilomycins
DNA and topoisomerase inhibitors	Pentamidine, Bisbenzimidazoles
Intermediary metabolism	
Amino acid synthesis inhibitors	Cispentacin
Other cellular functions	
Microtubule inhibitors	Benomyl

terol biosynthesis pathway are oxidosqualene cyclase and ▲^{24}methyl transferase. The latter has no mammalian counterpart (cholesterol is not methylated at C-24) and is thus a particularly attractive target (Oehlschlager and Czyzewska 1992; Georgopapadakou and Walsh 1996).

Fungal phospholipids synthesized by pathways that are basically similar to their mammalian counterpart are also one of the new targets (Carman and Henry 1989). Sphingolipids are essential membrane components of both mammalian cells and fungi and are localized primarily on the outer leaflet of the fungal cytoplasmic membrane (Patton and Lester 1991; Mandala et al. 1997; Mandala and Harris 2000). They have also been pursued as potential targets, as in the case of sphinofungins and folimycins (Georgopapadakou and Walsh 1996). The plasma membrane H^+ ATPase is an integral membrane protein belonging to the P-type class of ion-translocating ATPases and has been a target site for bafilomycins (Muroi et al. 1993). It is an abundant, essential enzyme involved in the maintenance of electrochemical proton gradients and the regulation of intracellular pH (Monk and Perlin 1994; Georgopapadakou and Walsh 1996).

Evidence for the involvement of *Candida* aspartyl proteinase (CAP) in virulence is convincing but not definitive. However, the role of proteinase in pathogenicity is that aspartyl proteinase deficient mutants of *C. albicans* are found to be significantly less lethal than the parent wild-type strain or partial revertants from the mutants. Thus, CAP is another attractive target for antifungal drugs against *C. albicans, C. parapsilosis*, *C. tropicalis*, etc. (Ross et al. 1990; Homma et al. 1993). Some other target sites have also proved to be successful in the case of synthetic antifungal compounds. These include ergosterol synthesis inhibitors (like zaragozic acid; Chen et al. 1994) inhibitors of DNA function and topoisomerases (like pentamidine), inhibitors of amino acid synthesis (like cispentacin) and microtubule inhibitors (like benomyl; Jones et al. 1990; Iwasaki 1993). Attempts have also begun to exploit all these targets to obtain novel antifungal compounds. Table 2 summarizes important antifungal compounds acting on new target sites. However, factors such as narrow spectrum of activity, susceptibility to efflux pumps, protein binding, serum inactivation and poor pharmaceutical properties restrict their use in the clinic. Even so, these compounds are novel leads for synthetic modifications that could lead to the discovery of future antifungal drugs (Fostel and Lartey 2000). Table 3 mentions the important new antifungal antibiotics with a variety of mechanisms of action discovered during the past decade or so.

Inhibitors of cell wall biosynthesis

Corynecandin is a novel glycolipid analogous to the structural variant of papulacandin, named chaetiacandin and isolated from *Coryneum modonium* (Gunawardana et al. 1997). Mer-WF3010 is another new member of the papulacandin class, isolated from the culture broth of *Phialophora cyclaminis* and similar to papulacandin B in that it inhibits the growth of *Candida albicans* and *C. kefyr* but not the growth of *A. fumigatus* or *C. neoformans*. Fusacandin, isolated from *Fusarium sambucinum*, is a structural variant of chaetiacandin (Lartey 1997) and is active against *C. albicans*. An iron-binding compound, identified as pyochelin and isolated from a novel species of *Pseudomonas*, is found to inhibit glucan synthase activity (Phoebe et al. 2001). Arthrichitin, a cyclic depsipeptide identified through screening for chitin synthesis inhibitors, is produced by *Arthrinium phaeospermum*, whereas LL15G256γ is produced by the marine fungus *Hypoxylon oceanicum* and has a broad-spectrum activity against *Candida, Trychophyton* and several phytopathogens (Fostel and Lartey 2000).

Selective inhibitors of fungal protein and amino acid synthesis

Sordarin, an antifungal agent bearing the tetracyclic diterpene aglycone sordaricin, is isolated from *Sordaria araneosa* in 1971, but its mode of action was only elucidated after the discovery of a closely related analog,

Table 3 Novel antifungal antibiotics of recent origin

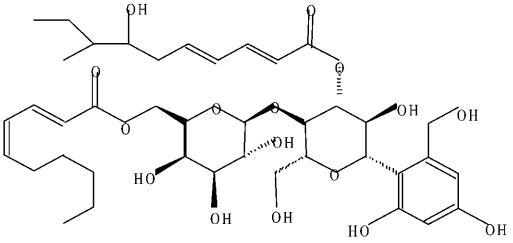
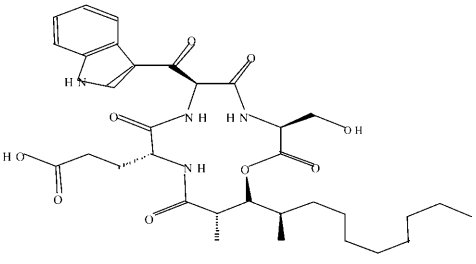
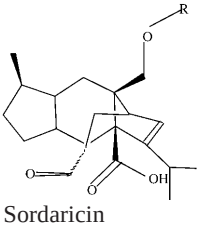
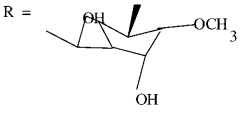
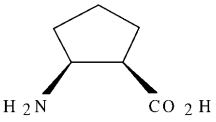
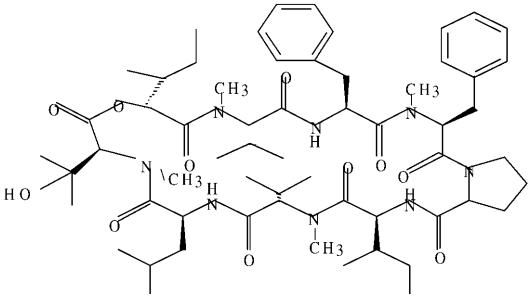
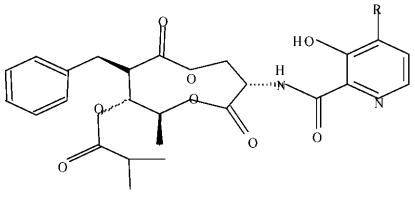
Name of antifungal antibiotic/source	Activity against	Mechanism of action	Structures
Corynecandin; *Coryneum modonium*	*Candida albicans*	Inhibits cell wall biosynthesis	 Corynecandin
Mer-WF3010; . *Phialophora cyclaminis*; Fusacandins; *Fusarium sambucinum*; Pyochelin; *Pseudomonas* sp	*C. albicans*, *C. kefyr*, *C. albicans*, *Candida* sp., *Aspergillus fumigatus*		
Arthrichitin; *Arthrinium phaeospermum*	*Candida* sp.		 Arthrichitin
LL15G256α; *Hypoxylon oceanicum*	*Trychophyton* sp., phytopathogens		
Sordarin; *Sordaria araneosa*	*C. albicans*	Inhibitors of fungal protein synthesis	 Sordaricin
GR135402; *Graphium putredinis* BE31405; *Penicillium minioluteum*	*C. kefyr*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*		 Sordarin (a glycone of sordarins)
Cispentacin: *Bacillus cereus*	*C. albicans*	Inhibits amino acid synthesis	 Cispentacin
	C. albicans		
FR109615; *Streptomyces setonii*	*Candida* sp.		
	Aspergillus sp.		

Table 3 (continued)

Name of antifungal antibiotic/source	Activity against	Mechanism of action	Structures
Azoxybacilin; *Bacillus cereus*			
Aureobasidin A; *Aureobasidium pullulans*	*Candida* sp.	Inhibits sphingolipid synthesis	 Aureobasidin A
	C. neoformans		
Rustmicin (galbonolide A), galbonolide B; *Micromonospora* sp.	*C. albicans*, *C. neoformans*		
Khafrefungin; sterile fungus from a Costa Rican plant	*C. albicans*, *C. neoformans*		
UK2A, UK3A; *Streptomyces* spp 517-02	*C. albicans*, . *Aspergillus* sp		
Haliangicin; *Haliangium luteum*	*C. albicans*	Inhibits electron transport	 UK2A R=OCH_3, UK3A, R=H

GR135402 from *Graphium putredinis*. A further analog, BE31405, is isolated from *Penicillium minioluteum* (Kinsman 1998). Cispentacin from *Bacillus cereus* and FR109615 from *Streptomyces setonii* are unnatural cyclic β-amino acids. These compounds and their analogs, such as BAY108888, appear to have a dual mode of action (Konishi et al. 1989). Cispentacin acts by interfering with amino acid transport and cellular regulation of amino acid metabolism (Capobianco et al. 1993). Azoxybacilin, an aliphatic amino acid with an azoxy side-chain, was isolated from *B. cereus* and has a broad spectrum of antifungal activity (Fujiu et al. 1994).

Inhibitors of sphingolipid biosynthesis

These include aureobasidin A produced by *Aureobasidium pullulans*, which belongs to the family of potent cyclic depsipeptide antifungals. Rustmicin (galbonolide A), galbonolide B and analogous antifungal macrolides isolated from *Micromonospora* sp. are inhibitors of sphingolipid biosynthesis. Khafrefungin isolated from an unidentified sterile fungus from a Costa Rican plant is active against *C. albicans* and *C. neoformans* (Fostel and Lartey 2000). Spinofungins, myriocin, lipoxamycin and viridiofungins are other compounds that have been reported (Zweerink et al. 1992; Horn et al. 1994; Mandala et al. 1997).

Inhibitors of electron transport

These include a series of related antibiotics with a nine-membered dilactone ring, such as UK2A and UK3A, which have been isolated from *Streptomyces* sp. 517-02. This class of compounds has broad-spectrum antifungal activity against *Aspergillus* sp., *C. albicans* and others.

Also, UK2A and UK3A do not have antibacterial activity or significant toxicity (Ueki et al. 1997). A series of respiratory inhibitors (nearly 20 compounds) have been found from myxobacteria (Sasse et al. 1999). Haliangicin isolated from a culture broth of the marine myxobacterium, *Haliangium luteum*, is a member of this class of compounds with a β-methoxyacrylate moiety which specifically inhibits the electron transport within the complex III of respiratory chain, like other β-methoxyacrylate inhibitors, such as myxothiazols, melithiazols and cystothiazoles (Fudou et al. 2001a, b).

Pneumocandin Do, a new member of the echinocandin class of antifungal agents isolated from the filamentous fungus *Zalerion arboricola*, has been found to be a potent inhibitor of *Pneumocystis carinii* (Morris et al. 1994). Tricholin, a ribosome inactivating protein isolated from the culture broth of *Trichoderma viride*, has been shown to exert fungicidal effects on *Rhizoctonia solani* (Lin et al. 1994). Azoxybacilin, a novel antifungal agent produced by *Bacillus cereus* NR2991 shows potent antifungal activity against mycelial fungi such as *Aspergillus fumigatus* and *Trichophyton mentagrophytes* (Fujiu et al. 1994). Soraphen, known to have inhibitory activity against numerous fungi, was isolated from *Sorangium cellulosum* (Myxococcales; Gerth et al. 1994). WF11899A, B, C, the novel antifungal lipopeptide antibiotics belonging to the echinocandin class, possess potent in vitro antifungal activities against *Candida* sp. (Iwamoto et al. 1994a, b). Another new antifungal antibiotic from *Fusarium* sp. K432, Fusarielin A, shows antifungal activity against *Aspergillus fumigatus*, *Pyricularia oryzae*, *Rhizoctonia solani* and *Rhizopus chinensis* (Kobayashi et al. 1995), whereas an antibiotic from a *Bacillus* sp. YL-03709B shows antifungal activity against *Pichia angusta* and *Rhodotorula acuta* (Shibazaki et al. 1996; Sugawara et al. 1996). Dorrigocins A and B isolated from *Streptomyces platensis* subsp. *rosaceus* strain have moderate antifungal activity against some *Aspergillus* and *Fusarium* spp., but not against yeasts (Karwowski et al. 1994). Recently, four compounds (SCH 378161, SCH 217048, SCH 378199, SCH 378167) isolated from a taxonomically unidentified fungus were found to selectively inhibit the human NK_2 receptor, tachykinin (Hegde et al. 2001).

Perspectives for new antifungals

Although a number of compounds with a variety of skeletons and acting on various fungal targets have been reported, there is no real break-through in antifungal chemotherapy. The invasive and long-term nature of fungal infections, whether superficial or systemic, causes major hurdles in their treatment. Greater emphasis may be required on studying the invasive process in order to arrest it. The dimorphic switch from the budding to the pathogenic mycelial form in *Candida* is another target, for example.

A much greater investment into antifungal research is required and must be paralleled by a systematic study of the metabolic pathways of various pathogenic fungi. With the recent, vast strides in molecular biological techniques, it is now possible to isolate genes and promoters of specific enzymes and to clone and express them in heterologous hosts. This gives a handle on studying enzyme inhibition, transcription inhibition and the specific function of the enzyme itself. In the area of the search for new antifungals, this must be enhanced.

Among the main hurdles in antimycotic therapy are the lack of water-soluble compounds and the inherent toxicity of the different molecules. Nephrotoxicity and hepatotoxicity are major problems. Formulations with site-directed conjugates might be the solution, e.g. liposomes and amphotericin B.

In order to address the problem of antifungal drug resistance, three complementary strategies are mainly used. The first is the direct study of the molecular mechanisms of the resistance and the methods to circumvent them; the second is the discovery and/or synthesis of compounds with greater potency, better pharmacokinetic properties and acting on specific targets; and the third is to search for new targets in the pathogens. This approach includes cloning of appropriate genes, purification of target enzymes, rigorous characterization of their catalytic functions and probing structure–function relationships by molecular biological methods (such as site-specific mutagenesis) and chemical means (inhibitor and affinity label design). Fungal topoisomerase I is a good target for the discovery of novel antifungal agents that stabilize the cleavage complex formed by this enzyme and DNA (Fostel et al. 1992; Fostel and Montgomery 1995). Elongation factor 3 (EF-3), an essential requirement of fungal translational machinery, is another attractive drug target. However, no inhibitors of EF-3 have been identified so far. The success or failure of antifungal drug therapy is dependent not only on the drug, but also on several other factors. The best way to improve antifungal drug therapy is to improve the immune status of the host. Cytokines, such as granulocyte colony-stimulating factor, may be useful in the treatment of fungal disease (Alexander and Perfect 1997; Natarajan et al. 1997). Another important strategy in treating fungal disease is the elimination of the disease focus. The removal of fungus-contaminated foreign objects or the surgical removal of abscesses can also reduce the fungal burden and allow the host and antifungal drugs to clear the infection, even with strains that are resistant to standard antifungal therapy (White et al. 1998).

In addition to the above, there are virulence factors that may be tackled, such as melanin production, phospholipases in pathogen penetration and glucuronoxylomannan as a racemic conjugate with the tetanus toxoid (GXH-TT) (Wills et al. 2000). It is clear that, with the advent of combinatorial chemistry, fungal genomics and molecular biology techniques, high-throughput screening will gain greater emphasis and possibly greater success. The agents discussed in this Mini-Review represent important antifungal compounds that have been isolated, either from natural sources or synthesized. The review has tried to emphasize the naturally occurring skeletons.

In the coming years, in-depth studies of biological processes associated with fungal infections should result in newer, more specific target sites. Current trends in research in this area suggest that the present focus on screening molecules from natural sources will continue. However, more emphasis towards molecular modeling for the rational design of lead molecules, based on structure–activity relationships, drug–receptor interaction and derived steric and physico-chemical properties is expected in the search for the next generation antifungals.

References

Alexander BD, Perfect JR (1997) Antifungal resistance trends towards the year 2000: implications for therapy and new approaches. Drugs 54:657–678

Barry AL (1980) Procedure for testing antibiotics in agar media: theoretical considerations. In: Lorain V (ed) Antibiotics in laboratory medicine. Waverly Press, Baltimore, pp 1–23

Bartizal K, Gill CJ, Abruzzo GK, Flattery AM, Kong L, Scott PM, Smith JG, Leighton CE, Bouffard A, Dropinski JF, Balkovec J (1997) In vitro preclinical evaluation studies with the echinocandin antifungal MK-0991 (L-743,872). Antimicrob Agents Chemother 41:2326–2332

Beck-Sague CM, Jarvis WR (1993) Secular trends in the epidemiology of nosocomial fungal infections in the United States 1980–1990. J Infect Dis 167:1247–1251

Bennett JE (1990) Antimicrobial agents: antifungal agents. In: Gillman AG, Rall TW, Nies AS, Taylor P (eds) Goodman and Gilman's pharmacological basis of therapeutics, 8th edn. Pergamon Press, Elmsford, New York, pp 1165–1181

Ben-Yaacov R, Becker JM, Oppenheim JM, Oppenheim A, Goldway M, Schmidt R, Jiang W, Clifford J, Koltin Y (1995) Multi drug resistance in *Candida albicans*. J Cell Biochem 19B:146

Bevan P, Ryder H, Shaw I (1995) Identifying small-molecule lead compounds: The screening approach to drug discovery. Trends Biotechnol 13:115–121

Brian PW, Curtis PJ, Hemming HG (1949) A substance causing abnormal development of fungal hyphae produced by *Penicillium janczewskii* Zal. Trans Br Mycol Soc 29:173

Cabib E, Bowers B, Sburlati A, Silverman SJ (1988) Fungal cell-wall synthesis: the construction of a biological structure. Microbiol Sci 5:370–375

Calderone RA, Brown PC (1991) Adherence and receptor relationships of *Candida albicans*. Microbiol Rev 55:1–20

Capobianco JO et al (1993) Anti-*Candida* activity of cispentacin: the active transport by amino acid permeases and possible mechanisms of action. Biochem Biophys Res Commun 190: 1037–1044

Carman GM, Henry SA (1989) Phospholipid biosynthesis in yeast. Annu Rev Biochem 58:636–669

Chen TS, Petuch B, MacConnell J, White R, Dezeny G, Arison B, Bergstrom JD, Colwell L, Huang L, Monaghan RL (1994) The preparation of zaragozic acid analogues by directed biosynthesis. J Antibiot 47:1290–1294

Clark FS, Parkinson T, Hitchcock CA, Gow NA (1996) Correlation between rhodamine 123 accumulation and azole sensitivity in *Candida* species: possible role for drug efflux in drug resistance. Antimicrob Agents Chemother 40:419–425

Colthurst DR, Santos M, Grant CM, Tuite MF (1991) *Candida albicans* and three other *Candida* species contain an elongation factor structurally and functionally analogous to elongation factor 3. FEMS Microbiol Lett 80:45–50

Cragg GM, Newman DJ, Snader KM (1997) Natural products in drug discovery and development. J Nat Prod 60:52–60

Dasgupta F (1998) Antifungal agents – past, present and future possibilities. In: Varma RS, Khan IK, Singh AP (eds) Antifungal agents past, present, future prospect. National Academy of Chemistry and Biology, Lucknow, pp 29–54

Decker H, Walz F, Bormann C, Fiedler HP, Zahner H, Heitsch H, Konig WA (1990) Metabolic products of microorganisms 255. Nikkomycins Wz and Wx, new chitin synthetase inhibitors from *Streptomyces tendae*. J Antibiot 43:43–48

Decker H, Heitsch H, Konig WA, Fiedler HP (1991) Structure–activity relationships of the nikkomycins. J Gen Microbiol 137:1805–1813

Fostel JM, Lartey PA (2000) Emerging novel antifungal agents. Curr Trends Drug Discov Today. 5:25–32

Fostel JM, Montgomery DA (1995) Identification of the aminocatechol A-3253 as an in-vitro poison of DNA topoisomerase I from *Candida albicans*. Antimicrob Agents Chemother 39:586–592

Fostel JM, Montgomery DA, Shen LL (1992) Characterization of DNA Topoisomerase I from *Candida albicans* as a target for drug discovery. Antimicrob Agents Chemother 36:2131–2138

Fudou R, Iizuka T, Yamanaka S (2001a) Haliangicin, a novel antifungal metabolite produced by a marine myxobacterium 1. Fermentation and biological characteristics. J Antibiot 54:149–152

Fudou R, Iizuka T, Sato S, Ando T, Shimba N, Yamanaka S (2001b) Haliangicin, a novel antifungal metabolite produced by a marine myxobacterium 2. Isolation and structural elucidation. J Antibiot 54:153–156

Fujiu M, Sawairi S, Shimada H, Takaya H, Aoki Y, Okuda T, Yokose K (1994) Azoxybacilin, a novel antifungal agent produced by *Bacillus cereus* NR2991, production, isolation and structure elucidation. J Antibiot 47:833–835

Georgopapadakou NH, Tkacz JS (1995) The fungal cell wall as a drug target. Trends Microbiol 3:98–104

Georgopapadakou NH, Walsh TJ (1994) Human mycoses: drugs and targets for emerging pathogens. Science 264:371–373

Georgopapadakou NH, Walsh TJ (1996) Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother 40:279–291

Gerth K, Bedorf N, Irschik H, Hofle G, Reichenbach H (1994) The soraphens: a family of novel antifungal compounds from *Sorangium cellulosum* (Myxobacteria) I. Soraphen $A_{1\alpha}$: fermentation, isolation, biological properties. J Antibiot 47:23–31

Gil JA, Paloma L, Martin JF (1984) The polyenes: properties, biosynthesis and fermentation. In: Vandamme EJ (ed) Biotechnology of industrial antibiotics, vol 22. Dekker, New York, pp 513–529

Grove JF, McGowan JC (1947) Identity of griseofulvin and "curling factor". Nature 160:574

Guarro J, Llop C, Aguilar C, Pujol I (1997) Comparison of in vitro antifungal susceptibilities of conidia and hyphae of filamentous fungi. Antimicrob Agents Chemother 41:2760–2762

Gull K, Trinci APJ (1973) Griseofulvin inhibits fungal mitosis. Nature 244:292

Gunawardana G, Rasmussen RR, Scherr M, Frost D, Brandt KD, Choi W, Jackson M, Karwowski JP, Sunga G, Malmberg LH, West P, Chen RH, Kadam S, Clement, JJ, McAlpine JB (1997) Corynecandin: a novel antifungal glycolipid from *Coryneum modonium*. J Antibiot 50:884–886

Hazen EL, Brown R (1950) Two antifungal agents produced by soil actinomycetes. Science 112:423

Hegde VR, Puar MS, Dai P, Pu H, Patel M, Anthes JC, Richard C, Terracciano J, Das PR, Gullo V (2001) A family of depsi-peptide fungal metabolite, as selective and competitive human tachykinin receptor (NK_2) antagonists: fermentation, isolation, physico-chemical properties, and biological activity. J Antibiot 54:125–135

Hemenway CS, Heitman J (1999) Calcineurin: structure, function and inhibition. Cell Biochem Biophys 30:115–151

Homma M, Chibana H, Tanaka K (1993) Induction of extracellular proteinase in *Candida albicans*. J Gen Microbiol 139: 1187–1193

Horn WS, Smith JL, Bills GF et al (1994) Sphingofungins E and F: novel serine palmitoyltransferase inhibitors from *Paecilomyces variotii*. J Antibiot 47:376–379

Iwamoto T, Fujie A, Sakamoto K, Tsurumi Y, Shigematsu N, Yamashita M, Hashimoto S, Okuhara M, Kohsaka M (1994a) WF11899A, B and C, novel antifungal lipopeptides I. Taxonomy, fermentation, isolation and physico-chemical properties. J Antibiot 47:1084–1091

Iwamoto T, Fujie A, Nitta K, Hashimoto S, Okuhara M, Kohsaka M (1994b) WF11899A, B and C, novel antifungal lipopeptides II. Biological properties. J Antibiot 47:1092–1097

Iwasaki S (1993) Antimitotic agents: chemistry and recognition of tubulin molecule. Med Res Rev 13:183–198

Iyer SV (1998) Antifungal agents – past, present and future possibilities. In: Varma RS, Khan IK, Singh AP (eds) Antifungal agents past, present, future prospect. National Academy of Chemistry and Biology, Lucknow, pp 3–26

Jacob L, Zasloff M (1994) Potential therapeutic applications of magainins and other antimicrobial agents of animal origin. In: Boman HG, Marsh J, Goode JA (eds) Antimicrobial peptides. (Ciba Foundation Symp 186) Wiley, Chichester

Jones SK, Hall JE, Allen MA, Morrison SD, Ohemeng KA, Reddy VV, Geratz JD Tidewell RR (1990) Novel pentamidine analogues in the treatment of experimental *Pneumocystis carinii* pneumonia. Antimicrob Agents Chemother 34:1026–1030

Kaneshiro ES, Cushion MT, Walzer PD, Jayasimhulu K (1989) Analysis of pneumocystis fatty acids. J Protocol 36:695–705

Karwowski JP, Jackson M, Sunga G, Sheldon P, Poddig JB, Kohl WL, Kadam S (1994) Doorigocins: novel antifungal antibiotics that change the morphology of ras-transformed NIH/3T3 cells to that of normal cells. I. Taxonomy of the producing organism, fermentation and biological activity. J Antibiot 47: 862–869

Khan ZK, Gyanchandani A (1998) Trends in antifungals: past, present and future. In: Varma RS, Khan ZK, Singh AP (eds) Antifungal agents past, present, future prospect, National Academy of Chemistry and Biology, Lucknow, pp 55–128

Kinsman OS (1998) Isolation and characterization of an antifungal antibiotic (GR 135402) with protein synthesis inhibition. J Antibiot 51:41–49

Kobayashi GS, Medoff G (1977) Antifungal agents: recent developments. Annu Rev Microbiol 31:291–308

Kobayashi H, Sunaga R, Furihata K, Morisaki N, Iwasaki S (1995) Isolation and structures of an antifungal antibiotic, fusarielin A and related compounds produced by *Fusarium* sp. J Antibiot 48:42–52

Konishi M, Nishio M, Saitoh K, Miyaki T, Oki T, Kawaguchi H (1989) Cispentacin, a new antifungal antibiotic I. Production, isolation, physicochemical properties and structure. J Antibiot 42:1749–1755

Kornfeld EC, Jones RJ, Parke TV (1949) The structure and chemistry of actidione, an antibiotic from *Streptomyces griseus*. J Am Chem Soc 71:150–159

Kurtz MB (1998) New antifungal drug targets: a vision for the future. ASM News 64:31–35

Kurtz MB, Douglas CM (1997) Lipopeptide inhibitors of fungal glucan synthase. J Med Vet Mycol 35:79–86

Lartey PA (1997) Challenges in antibacterial and antifungal drug discovery. In: Awouters F (ed) XIV International symposium on medicinal chemistry. Elsevier, Rotterdam, pp 205–218

Leach B, Ford J, Whiffen A (1947) Actidione, an antibiotic from *Streptomyces griseus*. J Am Chem Soc 69:474

Lin A, Lee TM, Rern JC (1994) Tricholin, a new antifungal agent from *Trichoderma viride*, and its action in biological control of *Rhizoctonia solani*. J Antibiot 47:799–805

Mandala SM, Harris GH (2000) Isolation of novel inhibitors of sphingolipids synthesis: australifungin, viridiofungins, rustimicin and khafrefungin. Methods Enzymol 311:335–348

Mandala SM, Thornton RA, Frommer BR, Dreikorn S, Kurtz MB (1997) Viridiofungins, novel inhibitors of sphingolipid synthesis. J Antibiot 50:339–343

Martin JF (1977) Biosynthesis of polyene macrolide antibiotics. Annu Rev Microbiol 31:13–38

McGinnis MR, Rinaldi MG (1991) Antifungal drugs: mechanisms of action, drug resistance, susceptibility testing and assays of activity in biological fluids. In: Lorain V (ed) Antibiotics in laboratory medicine. Williams and Wilkins, Baltimore, pp 198–257

Mercer EI (1993) Inhibitors of sterols biosynthesis and their applications. Prog Lipid Res 32:357–416

Mio T, Adachi SM, Tachivana Y (1997) Cloning of the *Candida albicans* homolog of *Saccharomyces cerevisiae* GSC1/FKS1 and its involvement in 1,3-β-glucan synthesis. J Bacteriol 179:4096–4105

Misato TK, Yamaguchi I (1977) Use of antibiotics in agriculture. Adv Appl Microbiol 21:53–88

Monghan RL, Tkacz JS (1990) Bioactive microbial products: focus upon mechanism of action. Annu Rev Microbiol 44:271–301

Monk BC, Perlin DS (1994) Fungal plasma membrane protein pumps as promising new antifungal targets. Crit Rev Microbiol 20:209–223

Morris SA, Schwartz RE, Sesin DF, Masurekar P, Hallada TC, Schmatz DM, Bartizal K, Hensens OD, Deborah ZL (1994) Pneumocandin D_o, a new antifungal agent and potent inhibitor of *Pneumocystis carinii*. J Antibiot 47:755–764

Muroi M, Takasu A, Yamasaki M, Takatsuki A (1993) Folimycin (concanamycin A), an inhibitor of V-type H+-ATPase, blocks cell surface expression of virus-envelope glycoproteins. Biochem Biophys Res Commun 193:999–1005

Natarajan U, Brummer E, Stevens DA (1997) Effect of granulocyte colony-stimulating factor on the candidacidal activity of polymorphonuclear neutrophils and their collaboration with fluconazole. Antimicrob Agents Chemother 41:1575–1578

Odds FC (1993) Resistance of yeasts to azole-derivative antifungals. J Antimicrob Chemother 22:473–481

Oehlschlager AC, Czyzewska E (1992) Rationally designed inhibitors for sterol biosynthesis. In: Sutcliffe J, Georgopapadakou NH (eds) Emerging targets in antibacterial and antifungal chemotherapy. Chapman and Hall, New York, pp 437–475

Oxford AE, Raistrick H, Simonart P (1939) Studies on the biochemistry of microorganisms 60. Griseofulvin, C17H17O6Cl, a metabolic product of *Penicillium griseofulvum* Dierckx. Biochem J 33:240

Pathak AN (1998) Antifungal agents to manage mycotic infections. In: Varma R S, Khan IK, Singh AP (eds) Antifungal agents past, present, future prospect. National Academy of Chemistry and Biology, Lucknow, pp 149–186

Patton JL, Lester RL (1991) The phosphoinositol sphingolipids of *Saccharomyces cerevisiae* are highly localised in the plasma membrane. J Bacteriol 173:3101–3108

Perfect JR, Schell WA (1996) The new fungal opportunists are coming. Clin Infect Dis 22:5112–5118

Persidis A (1998) Extremophiles. Nat Biotechnol 16:593–594

Phoebe CH, Combie J, Albert FG, Tran KV, Cabrera J, Correira HJ, Guo Y, Lindermuth J, Rauert N, Galbraith W, Selitrennikoff CP (2001) Extremophilic organisms as an unexplored source of antifungal compounds. J Antibiot 54:56–65

Qin S, Xie A, Bonato MC, McLaughlin CS (1990) Sequence analysis of the translational elongation factor 3 from *Saccharomyces cerevisiae*. J Biol Chem 265:1903–1912

Ross IK, De Bernardis F, Emerson GW, Cassone A, Sullivan P (1990) The secreted aspartate proteinase of *Candida albicans*: physiology of secretion and virulence of a proteinase-deficient mutant. J Gen Microbiol 136:687–694

Roy K, Mukhopadhyay T, Reddy GCS, Desikan KR, Ganguli BN (1987) Mulundocandin, a new lipopeptide antibiotic I. Taxonomy, fermentation, isolation and characterization. J Antibiot 40:275–280

Sasse F, Boehlendorf B, Hermann M, Kunze B, Forche E, Steinmetz H, Hoefle G, Reichenbach H (1999) Melithiazols, new β-methoxyacrylate inhibitors of the respiratory chain isolated from myxobacteria. J Antibiot 52:721–729

Sato T, Nagai K, Shibazaki M, Abe K (1994) Novel aspartyl proteinase inhibitors YF-0200R A and B. J Antibiot 47:566–570

Sawada Y, Mumata KI, Murakami T, Tanimichi H, Yamamoto S, Oki T (1990) Calcium dependent anticandidal action of pradimicin A. J Antibiot 43:715–721

Schmatz DM, Abruzzo G, Powles MA, McFadden DC, Balkovec JM, Black RM, Nollstadt K, Bartizal K (1992) Pneumocandins from *Zalerion arboricola* IV. Biological evaluation of natural and semisynthetic pneumocandins for activity against *Pneumocystis carinii* and *Candida* species. J Antibiot 45:1886–1891

Schwartz RR, Giacobbe RA, Bland JA, Monaghan RL (1989) L-671,329, a new antifungal agent. I. Fermentation and isolation. J Antibiot 42:163–167

Selva E, Montanini N, Stella S, Soffientini A, Gastaldo L, Denaro M (1997) Targeted screening for elongation factor Tu binding antibiotics. J Antibiot 50:22–26

Shadomy S (1987) Preclinical evaluation of antifungal agents. In: Fromtling RA (ed) Recent trends in the discovery, development and evaluation of antifungal agents. Prous Science, New Jersey, pp 8–14

Shadomy S, Espinel-Ingroff A, Cartwright RY (1985) Laboratory studies with antifungal agents: susceptibility tests and bioassays. In: Lenette EH, Balows AS, Hausler WJ Jr (eds) Manual of clinical microbiology, 4th edn. American Society for Microbiology, Washington, D.C. pp 991–999

Shaw JA, Bowers B, Silverman SJ, Valdivieso MH, Duran A, Cabib E (1991) The function of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. J Cell Biol 114:111–123

Shibazaki M, Sugawara T, Nagai K, Shimizu Y, Yamaguchi H, Suzuki K (1996) YM-47522, a novel antifungal antibiotic produced by *Bacillus* sp. I. Taxonomy, fermentation, isolation and biological properties. J Antibiot 49:340–344

Sudoh M. Nagahashi S, Doi M, Ohta A, Takagi M, Arisawa M (1993) Cloning of the chitin synthase 3 gene from *Candida albicans* and its expression during yeast-hyphal transition. Mol Gen Genet 241:351–358

Sugawara T, Shibazaki M, Nakahara H, Suzuki K (1996) YM-47522, a novel antifungal antibiotic produced by *Bacillus* sp. II. Structure and relative stereochemistry. J Antibiot 49: 345–348

Takeuchi T, Hara T, Naganawa H, Okada M, Hamada M, Umezawa H, Gomi S, Sezaki M, Kondo S (1988) New antibiotics, benanomicins A and B from an actinomycete. J Antibiot 41:807–811

Tanimoto T, Onodera K, Hosoya T, Takamatsu Y, Kinoshita T, Tago K, Kogen H, Fujioka T, Hamano K, Tsujita Y (1996) Schizostatin, a novel squalene synthase inhibitor produced by the mushroom, *Schizophyllum commune* I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J Antibiot 49:617–623

Tkacz JS (1992) Glucan biosynthesis in fungi and its inhibition. In: Sutcliffe J, Georgopapadakou NH (eds) Emerging targets in antibacterial and antifungal chemotherapy. Chapman and Hall, New York, pp 495–523

Ueki M, Kusumoto A, Hanafi M, Shibata K, Tanaka T, Taniguchi M (1997) UK-3A, a novel antifungal antibiotic from *Streptomyces* sp. 517–02: fermentation, isolation, structural elucidation and biological properties. J Antibiot 50:551–555

Ueki T, Numata KI, Sawada Y, Nishio M, Ohkuma H, Toda S, Kamachi H, Fukagawa Y, Oki T (1993) Studies on the mode of antifungal action of pradimicin antibiotics. II. D-mannopyranoside binding site and calcium binding site. J Antibiot 46:455–469

Vandamme EJ (1984) Antibiotic search and production: an overview. In: Vandamme EJ (ed) Biotechnology of industrial antibiotics, vol 22. Dekker, New York, pp 3–31

Von Dohren H, Grafe U (1997) General aspects of secondary metabolism In: Rehm HJ, Reed G (eds) Biotechnology: products of secondary metabolism, vol 7, 2nd edn VCH/Wiley, Weinheim, pp 2–55

Weete JD (1989) Structure and function of sterols in fungi. Adv Lipid Res 23:115–167

Whiffen AJ, Bohonos N, Emerson RL (1946) The production of an antifungal antibiotic by *Streptomyces griseus*. J Bacteriol 56:283–291

White TC (1997) Antifungal drug resistance in *Candida albicans*. ASM News 63:427–433

White TC, Marr KA, Bowden RA (1998) Clinical, cellular and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 11:382–402

Wills EA, Redinbo MR, Perfect JR (2000) New potential targets for antifungal development. Emerg Ther Targets 4:265–296

Zweerink MM, Edison AM, Wells GB, Pinto W, Lester RL (1992) Characterization of a novel, potent and specific inhibitor of serine palmitoyltransferase. J Biol Chem 267:25032–25038