Novel antifungal drugs

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There have been many new developments in antifungal therapy in the past few years. Some antifungal drugs have been reformulated to reduce toxicity (e.g. new lipid formulations of polyenes), and new derivatives of drugs have been developed to enhance potencies. The search for unique drug targets will be enhanced by the availability of sequencing data from whole genome sequencing projects.

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Abbreviations

AMB	amphotericin B
EF	elongation factor
FLU	fluconazole
GGT	geranylgeranyltransferase
ITZ	itraconazole
VOR	voriconazole

Introduction

The need for novel, broad-spectrum antifungal agents is increasingly important in today's medical arena. With the increase in immuno-compromised patients, the physician is constantly being presented with a growing number of patients with a variety of fungal pathogens. AIDs, poor nutrition, the use of broad-spectrum antibiotics, indwelling surgical devices, and a variety of treatment regimens such as cancer chemotherapy or immunosuppressive therapy prior to transplantation all contribute to the growth of this patient population.

Candida albicans remains the most frequently encountered human fungal pathogen, although physicians have reported a disturbing trend in the number of 'non-albicans' species encountered, some of which exhibit naturally occurring resistance to antifungal drugs already in use [1–4]. There has been a concomitant increase in morbidity due, in part, to the number of serious invasive *Aspergillus* infections, prominence of pneumocystis infections, as well as the number of patients presenting with previously rare pathogens such as *Fusarium*, *Scedosporium* and *Exophiala*. Although it is desirable to develop compounds having a broad spectrum of activity, it is also important to keep in mind that fungi represent one of the most diverse collections of organisms in the biosphere. Thus, there may be a commercial niche for developing certain narrow spectrum agents as well.

The fungi, like their hosts, are eukaryotic organisms, making it difficult to choose intracellular targets whose inhibition would not also be deleterious to the host cell. Of the four classes of antifungal compounds currently in use, three affect ergosterol: the polyenes, azoles, and allylamines. The fluoropyrimidine 5-fluorocytosine (5-FC) achieves its specificity through a converting enzyme not present in mammalian cells. Excellent reviews have recently described the mechanism of action and characteristics of each of the major compounds within these classes [5,6•,7••,8]. This review will limit comments to investigational compounds recently approved or still under development. See Table 1 for a general overview of antifungal agents.

Inhibitors of fungal cell membranes

Polyenes

The only polyene currently approved for systemic use is amphotericinB (AMB). Its primary advantages include its fungicidal activity against most clinically relevant pathogens, and the low occurrence of resistance, despite decades of use. The primary disadvantage of AMB has been its nephrotoxicity; however, new lipid formulations have recently been approved that address this issue. Ambisome, Abelcet and Amphocil/Amphotech all exert relatively similar efficacies with considerably fewer side effects than AMB [9]. Composition of the lipid bilayer containing the polyenes (i.e. choice of lipid, molar ratio of lipids, and size of the vesicle) appears to contribute to slight differences in efficacy as a result of both redistribution of the antifungal drug to tissues and the selective release of active AMB from the complex [10•,11].

Nystatin, like its structurally related cousin AMB, binds ergosterol and destabilizes fungal membranes. Its toxicity has limited its use to topical infections; however, with the success of the liposomal versions of AMB, Aronex (TX) has devised a novel formulation of this classic drug in order to increase the drug's therapeutic index [12,13]. Liposomal nystatin (Nyotran) is currently undergoing phase III trials for presumed candidemia in neutropenic patients. A phase II study demonstrated that Nyotran was 67% effective for candidiasis. Most impressive was its activity for 9/15 patients whose infections were previously resistant to other antifungals (T Wallace, Abstracts of the Third International Antifungal Drug Discovery Summit, Princeton NJ, 1997). Although improvements in formulation have led to an enhanced therapeutic index for this compound, cost, as well as competition from an established liposomal AMB market, may limit its usage.

Azoles

There are a wide variety of azoles that have *in vitro* efficacy against fungi, but only a few have had significant clinical utility. Azoles inhibit cytochrome P450-dependent lanosterol 14-alpha-demethylase, causing accumulation of methylated sterols, depletion of ergosterol, and

Table 1

An overview of antifungal agents.

Compound/Class	MOA	Comments
Amphotericin B/polyene	Selective binding to ergosterol, major sterol of fungal membranes	Fungicidal Broad spectrum Intravenous Little resistance observed Significant nephrotoxicity
Abelcet/polyene Ambisome Amphotec	Selective binding to ergosterol, major sterol of fungal membranes	Liposomal formulations of AMB Similar efficacy as AMB Reduced toxicity observed
Nyotran/nystatin	Selective binding to ergosterol, major sterol of fungal membranes	Liposomal formulation of nystatin Lowered toxicity compared to nystatin
5-Fluorocytosine (5FC)/ nucleoside analog	Selective conversion to toxic intermediate (5-fluorouridine)	Most often given in combination with AMB for Cryptococcal meningitis Poor activity against filamentous fungi Significant resistance observed
Miconazole/azoles Ketoconazole	Selective inhibition of fungal cytochrome P450-dependent lanosterol-14-α-demethylase	Static activity against yeasts, dimorphic fungi, dermatophytes Generally fungistatic activity
Fluconazole/triazoles Itraconazaole Voriconazole Posaconazole UR-9825 SYN-2869 BMS-207147	Selective inhibition of fungal cytochrome P450-dependent lanosterol-14-α-demethylase	Broad spectrum including *Aspergillus* spp FLU-resistant *C. albicans* strains and non-albicans strains increasing (VOR and POS effective against FLU-R isolates) Efficacious in immune compromised models
LY303366/candins Caspofungin FK-463	Fungal β-1,3-glucan synthase inhibitors	Partly fungicidal Broad spectrum except for *Cryptococcus*, *Fusarium*, *Sporothrix*, *Trichosporon* CAS has intravenous formulation only Efficacious in immune compromised models
BMS181184/pradimicins	Calcium-dependent binding to mannoproteins in cell wall	Broad spectrum except for *Fusarium* Oral Hepatotoxicity led to discontinuation
Nikkomycin/nikkomycins	Chitin synthase inhibitor	Liposomal formulation of nikkomycin Limited spectrum for fungi Effective against cells with high chitin content
Terbinafine/allylamines	Squalene epoxidase inhibitor	Fungicidal Active against dermatophytes Topical and oral formulations
Basifungin/aureobasidins	Inositol-P ceramide synthase inhibitor	Fungicidal Broad spectrum (*Aspergillus* intrinsically less susceptible)
Sordarin/Sordarins	Selectively binds to fungal EF2/ribosomal stalk proteins	Fungicidal Broad spectrum

MOA, mode of action.

inhibition of cell growth [5]. Sensitivity of other P450-dependent enzymes accounts for their primary mode of toxicity. Although azoles demonstrate a broad spectrum of activity with less toxicity than AMB, they are not generally fungicidal.

The triazoles, itraconazole (ITZ; Jansen) and fluconazole (FLU; Pfizer, New York, NY), are approved for clinical use. Fluconazole's major impact in the clinic is for oral candidiasis in HIV-infected patients. Because of its low toxicity profile, it is preferred as first-line therapy. Unfortunately, resistance is becoming a major problem, both in the form of newly emerging pathogens, and selection for a subpopulation of formerly rare, but intrinsically FLU-resistant non-albicans species [14].

A number of new triazole antifungals are currently under preclinical or clinical evaluation for antifungal therapy. They include voriconazole (VOR; Pfizer, New York, NY), SCH 56592 (POS; Schering-Plough, Madison NJ; now called posaconazole), BMS-207147 (BMS; Bristol Meyers Squibb, Princeton, NJ), UR-9825 (UR; Uriach, Spain), and Syn2869 (SYN; Syn-Phar, Japan). All are active *in vitro* against a wide variety of clinical yeasts, dermatophytes and

filamentous fungi [15–21], and in immunocompromised animal models [22–28]. In general, VOR, SCH, BMS and UR are all more active than ITZ and FLU against *C. albicans* spp. including FLU-resistant isolates, *C. tropicalis* and *C. krusei* strains [29–35], whereas SYN is similar to ITZ [21]. The advantages of these newest members are, first, their *in vivo* efficacy, especially in models of compromised infections, second, their broader spectrum of activity, including *Aspergillus* spp., non-albicans *Candida* spp., and FLU-resistant *C. albicans* strains, third, better bioavailability, and fourth, their apparent fungicidal activity in some infections [24]. The major disadvantage is in their target, which is non-essential. Of the growing list of azoles, VOR and posaconazole are furthest along in clinical development (phase II/III). Both are apparently well tolerated with minimal adverse effects reported. It is anticipated that these compounds will be very useful in the clinical setting.

Aureobasidins

Basifungin is a cyclic depsipeptide with good *in vitro* and *in vivo* activity against a number of pathogenic fungi including most *Candida* species, *Crytococcus neoformans*, *Histoplasma capsulatum* and *Blastomyces dermatididis*, with poor activity against *Aspergillus* spp. and dermatophytes [36]. This compound inhibits phosphatidyl-inositol:ceramide phosphoinositol transferase (IPC synthase), which is encoded by an essential gene, *AUR1*, in *Saccharomyces cerevisiae* [37,38]. Other natural products, kafrefungin and rustmicin also inhibit IPC synthase [39,40]. More recently, *AUR1* homologs have been identified in *Candida*, *Schizosaccharomyces pombe* and several *Aspergillus* spp. [41,42], suggesting that the relatively poor potency against *Aspergillus* [36] may be due to poor penetration rather than absence of the target.

Although Basifungin shows fungicidal activity and has a unique mode of action, its limited potency against *Aspergillus* is a major drawback. Whether this compound is successful as a sole entity depends in large part on its human pharmacokinetics. Alternatively, it could potentially be used in combination with efflux pump inhibitors to extend its spectrum, or further evaluated in order to identify new leads with a broader spectrum.

Inhibitors of fungal cell wall

The fungal cell wall is an ideal target for the search for novel, fungicidal compounds. Several of the enzymes involved in the biosynthesis of the cell wall are unique to fungi and, thus, suitable as targets, including chitin and glucans synthases [43,44]. Early inhibitors of these enzymes failed in discovery; however, newer derivatives have enhanced potency, better solubility and improved efficacy in animal models.

Echinocandins and pneumocandins

β-1,3-glucan synthase is the target of both the echinocandins and pneumocandins [45,46,47••]. LY-303366 (LY; Eli Lilly, Indianapolis, IN) is a derivative of cilofungin, an early echinocandin B analog that has a limited spectrum. LY is both orally and parenterally active and more potent than its predecessor. It has *in vitro* and *in vivo* activity against numerous clinical isolates of *C. albicans* including non-albicans species, *B. dermatididis*, *H. capsulatum*, *Aspergillus fumigatus* and the cystic form of *Pneumocystis carinii* [48–51]. MK0991 (MK; Merck, Rahway, NJ), now called caspofungin, has partly fungicidal activity *in vitro* against some *Candida* spp., especially FLU-resistant strains, non-albicans spp., *A. fumigatus* and some dimorphic fungi [52–55]. In animal models it prolongs survival and reduces tissue counts against disseminated candidiasis and aspergillosis infections [50,51,56], as well as demonstrating efficacy in compromised murine pulmonary pneumocystosis and histoplasmosis models [57,58]. FK-463 (FK; Fujisawa) shows similar efficacy *in vitro* and *in vivo* to the other members of this class [59–61]. However, it should be noted that due to their mechanism of action, none of these 'candins' are effective against *Cryptococcus* or *Trichosporon*, which utilize relatively little β-1,3 glucan [49]. Nevertheless, they all have excellent activity against other fungi.

Comparisons with compounds already in use demonstrate that MK and LY are generally more active *in vitro* against a variety of yeast and filamentous fungi [52,55]. Direct head-to-head comparisons against the azole posaconazole demonstrate differences in spectrum: in general, *in vitro* potency of posaconazole is either equivalent or better than the echinocandins for 13/15 species of molds and for 6/7 yeasts [49]. Further, posaconazole is active against *C. neoformans* and *Trichosporon beigelii*, two fungi for which the candins are ineffective [49]. All three echinocandins are currently undergoing human clinical trials. A clear advantage of LY over MK is its oral activity. Initial human pharmacokinetic studies were reported as favorable for both compounds with only minor, reversible adverse events. Lilly has recently licensed the further development of LY-303366 to Versicor (Freemont, CA). Because of their novel mechanism of action, antifungal potency and relatively broad-spectrum activity, these echinocandins may someday become valuable tools against fungal disease.

Nikkomycins

Members of this class of compounds have also been known for many years. They appear to act competitively as substrate analogs of UDP-*N*-acetyl-glucosamine in preventing the synthesis of chitin. Although chitin synthesis is an essential function, multiple isozymes present in fungi add a level of complexity. The potency of an inhibitor may thus depend on the isoform's relative effectiveness in building a cell wall as well as its affinity to a given enzyme. For example, in strains where Chs2 activity is significant, nikkomycin may be relatively ineffective as it is very active *in vitro* against *S. cerevisiae* Chs3 but not Chs2 [62]. Nikkomycin has a relatively narrow spectrum as a solo agent but has recently been shown to have either additive or synergistic effects in combination with azoles against a number of human pathogens [63]. Furthermore, it is

efficacious in murine models of blastomycosis [64] and histoplasmosis [65]. Permeability, in addition to the redundancy of genes, will be a major hurdle for this compound since peptides in the host bloodstream may alter the dipeptide permease-dependent entry of compound into cells.

Pradimicins

The pradimicin family of antifungals exerts its selectivity by calcium-dependent binding of cell surface mannoproteins leading to cell membrane leakage and loss of viability [66]. These compounds exhibit broad *in vitro* and *in vivo* activity [67,68]. In a direct comparison with AMB, the compound is 40–50 fold less active, but also 130-fold less toxic [67]. Azole and 5FC-resistant strains remain susceptible. Interestingly, the pradimicins have demonstrated antiviral activities *in vitro*, perhaps via a critical interaction with mannose-containing polysaccharides on the viral coat surfaces.

BMS 181184 is a water-soluble hydroxy-analog derivative of pradimicin with a good *in vitro* and *in vivo* spectrum [69]. Only *Fusarium*, with its relatively low mannoprotein content, is relatively unaffected by doses of BMS 181184 as high as 64 μg/ml *in vitro* [70]. It remains to be seen whether other filamentous fungi may also be relatively resistant to compounds with this mechanism of action. Unfortunately, reports of elevated liver transaminases in human volunteers led to the discontinuation of this particular compound despite its unique mode of action. Additional screens for less toxic compounds have not been reported, although further investigations into compounds inhibiting this target could lead to new drug structures.

Geranylgeranyltransferase inhibitors

Cell wall integrity requires a functional geranylgeranyltransferase (GGT) and while a human ortholog has been identified, there is only about 20% homology between the fungal and mammalian GGT. At a recent meeting, Mitotix (Cambridge, MA) revealed that it has identified a number of selective active-site inhibitors targeted specifically against GGT in the micromolar to nanomolar range: some appear fungicidal, as expected (V Berlin, 4th International Antifungal Drug Discovery & Development Summit, Princeton, NJ, 10–11 March 1999). It will be interesting to see the spectrum of activity of these inhibitors and whether they maintain their good activity in animal models.

Inhibitors of protein synthesis

Sordarins

The search for suitable unique targets within the fungal ribosome has been very challenging. With the exception of elongation factor 3 (EF3), the structural and sequence similarity between fungal and mammalian ribosomal RNAs, subunits and soluble factors is remarkable. This 120 kDa soluble factor was originally discovered in *S. cerevisiae* and has subsequently been identified in other fungal pathogens [71–73]. It has been shown by gene knockout experiments to be essential for cell viability in *S. cerevisiae* [71]. Unfortunately, although several EF3 inhibitors have been identified, none are selective.

Sordarins are highly specific inhibitors of fungal translation. Several derivatives are active against *C. albicans*, including FLU-resistant strains, C. *tropicalis*, *C. kefyr*, but their activity against *C. glabrata* and *C. parapsilosis* is poor [74]. Their spectrum does not include *A. flavus*, *A. fumigatus* or *Fusarium* strains, but they are active in rat model against both forms of *P. carinii* [75]. The relatively poor activity against some fungi and the high degree of specificity for fungal ribosomes is somewhat surprising, given the recent findings that a highly conserved protein, EF2, is the target of the sordarins [76,77,78•]. Analysis of other resistant mutants has recently uncovered a number of additional interactions within the ribosome itself. This provides a possible explanation for the selectivity of these compounds especially if specificity is governed by multiple points of interaction between the compound and the ribosome [79••,80]. The ability of the sordarins to selectively inhibit fungal translation underscores the possibility that other essential proteins, as well as EF2, may be important targets in the search for novel antifungals.

N-myristoyltransferase inhibitors

A number of inhibitors targeted towards N-myristoyltransferase (NMT) have appeared recently. The transfer of myristate, a 14-carbon fatty acid, from CoA to the terminal glycine of certain proteins has been shown to be essential in *C. albicans*, *C. neoformans* and other fungi [81–84]. A knockout of the *nmt1* gene in *S. cerevisiae* is inviable [85]. Not only have peptidometic [86] and nonpeptidometic [87•] inhibitors with selectivity towards the human enzyme been described, but also a crystal structure of NMT is available which should aid in rational drug design [88]. It will be interesting to see whether compounds can be derived that will be sufficiently selective for this target since a putative protein with very high homology (around 64%) has been identified in both *C. elegans* and *D. melanogaster*.

Conclusions

There have been many new developments in recent years in antifungal therapy. Some 'old friends' have been reformulated and demonstrated to be less toxic, while newer derivatives of other classes have enhanced potencies and improved pharmokinetics. In addition, exponential increase in the sequence information provided by a number of whole genome projects [85,89–92] has provided a wealth of new targets to be validated and screened for new leads. Innovative research, the application of genomics technologies to the field, and continued improvements in delivery systems will ultimately yield many novel antifungal drugs in the near future.

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