

Current Status and Future of Antifungal Therapy for Systemic Mycoses

Joshua D. Nosanchuk*

Department of Medicine, Division of Infectious Diseases, Albert Einstein College of Medicine, Bronx, NY, USA

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Abstract: Since the 1950s there has been an increase in the incidence of invasive fungal disease. The first successful systemically administered antifungal drug, amphotericin B, was introduced in the 1950s and, until very recently, was considered the best therapeutic drug for severe mycoses. The development of new antifungals to treat systemic disease has been slow compared to that of antibacterial compounds, with the introduction of only a single new class of drugs over the past 20 years. This review discusses the antifungal drugs that are clinically in use and summarizes interesting new applications and patents from the US Patent and Trademark Office.

Keywords: Fungi, antifungal, combination therapy, antibody.

INTRODUCTION

The incidence of invasive fungal disease has dramatically increased over the past few decades paralleling the rising number of immunocompromised patients. The major risk factors for severe fungal disease include administration of broad-spectrum antibiotics, corticosteroids and cytotoxic agents, intravenous catheters, invasive medical procedures, and Human Immunodeficiency Virus (HIV) infection. Unfortunately, there has not been a correspondingly rapid development of therapeutics to combat invasive fungal disease. Antifungal therapy has been limited by toxicities and by resistance. Mechanisms of resistance to the available antifungals that have been described include the expression of efflux pumps to reduce drug accumulation, alteration of target proteins, and modification of membrane sterol composition [1]. The clinical consequences of antifungal resistance is evident in treatment failures as well as in changes in the changing prevalences of fungi, such as for *Candida* spp. and emerging moulds, causing disease [2, 3]. *Candida* spp. are the fourth most common cause of bloodstream infection in the US [4] with an attributable mortality rate of approximately 40% [5]. Currently, the incidence of aspergillosis in the US ranges from 0.5% after autologous hematopoietic stem cell transplantation to 3.9% after transplantation from an unrelated donor [6]. In these patients, mortality 3 months after diagnosis of aspergillosis was 53.8% in autologous transplant recipients and 84.6% in those with unrelated donor transplants [6]. These data clearly show that our current antifungal armamentarium is not adequate.

Antifungal drug development has lagged far behind that of antibacterial agents. Fungi are eukaryotes and, despite the presence of a cell wall, fungi are more similar to mammalian cells on a cellular level than to bacteria. Additionally, fungi replicate more slowly than bacteria and are often difficult to quantify, particularly for moulds, which complicates efficacy assessments. The term mould defines growth characterized

by the presence of hyphal structures that are tubular, branching filaments typically 2-10 μm in diameter with cells separated by septae. Moulds frequently grow as mycelium, which refers to a complex, tangled mass of hyphae. Reproduction occurs via germination of conidia that are produced from the transformation of a hyphal cell or from a specialized conidiogenous cell. Yeast are unicellular spherical to ellipsoid fungal cells of 3-15 μm in diameter that typically reproduce by budding. This review will provide an overview of medications currently in clinical use and describe therapeutics recently disclosed by the US Patent and Trademark Office for treatment of invasive fungal infections.

There are three main targets of drugs currently used for systemic therapy of invasive mycoses: the polyenes and azoles target the cell membrane, the antimetabolite 5-fluorocytosine (flucytosine, Ancobon, Valeant Pharmaceuticals International) interferes with DNA and RNA synthesis, and echinocandins affect the cell wall (Fig. 1). In January, 2001, the echinocandins became the first of a new class of antifungal approved since the first azole was approved twenty years earlier.

POLYENES

Amphotericin B (Amphocin, Pharmacia and Upjohn) is the polyene primarily in use therapeutically. Amphotericin B is a product of *Streptomyces nodosus* and is a 38-membered lactone ring with a covalently linked amino sugar moiety (Fig. 2A). The polyene compounds are so named because of the alternating conjugated double bonds that constitute a part of their macrolide ring structure. Amphotericin B was noted to have antifungal activity in 1953, and was approved for use in the USA in 1957. Amphotericin B selectively and irreversibly binds fungal cell membrane sterols [7]. The interaction of the antifungal with membrane sterols results in the formation of transmembrane pores, allowing for the leakage of ions and small molecules resulting in cellular damage or death [8]. The avidity of amphotericin B for ergosterol, the main fungal sterol, is greater than that for cholesterol [9, 10]. Also, the pore formation with ergosterol has a 100-fold longer half-life than pores formed after binding cholesterol [11]. Until the introduction of the

*Address correspondence to this author at the Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461; E-mail: nosanchuk@aecom.yu.edu

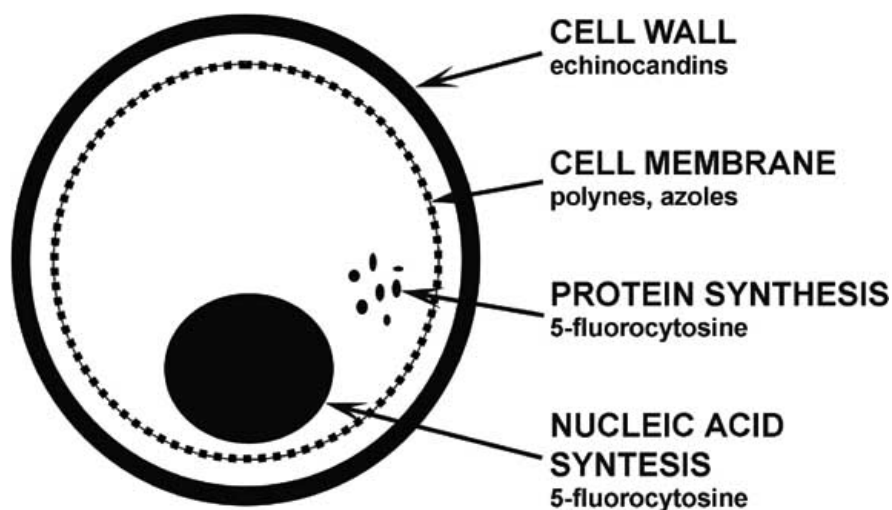


Fig. (1). Sites of action of antifungal drugs currently in use.

echinocandins and the newer azoles, amphotericin has long been the “gold standard” antifungal. A primary reason for this is that resistance is extremely rare, with notable exceptions being *Trichosporon beigelii*, *Aspergillus terreus*, *Pseudallescheria boydii*, *Malassezia furfur*, *Fusarium* spp., and *Candida lusitanae* [12].

The major drawbacks of amphotericin B use are the significant side effects associated with its use and that intravenous administration is required for treatment of invasive mycoses. Significant toxicities include fever, chills, arrhythmia, hypotension, respiratory distress, type IV renal tubular acidosis, renal failure, and anemia. The significant toxicity of the medication led to the introduction of lipid-based products in the 1990s. The three available drugs are ABLC (Abelcet, Enzon Pharmaceuticals), ABCD (Amphotec, InterMune), and Ambisome (Astellas), with Ambisome the only true liposomal form of amphotericin B. These products are significantly more expensive (30-60 times more costly), but have reduced side effects. The two major indications for these lipid-based antifungals are intolerance to standard amphotericin B or refractory disease. Similar efforts are underway to bring a second polyene, nystatin, to market in a liposomal form (Nyotran, Aronex) [13].

AZOLES

The azoles are the second class to target the fungal cell membrane. The major groups of azoles are the imidizoles and the triazoles, which have five-membered organic rings containing either two or three nitrogen molecules, respectively (Fig. 2B). The azoles inhibit cytochrome P450-dependent 14 α -lanosterol demethylation [14], which is a critical step in fungal cell membrane ergosterol synthesis [15]. Azoles affect both cell and mitochondrial membranes. The imidizoles are generally used topically. In 1981, the imidazole ketoconazole (Nizoral, Janssen), became the first systemically used azole, but it is presently rarely used in the US since the newer azoles have less toxicity. The triazoles have largely replaced ketoconazole since they have better potency and improved toxicity profiles. Since the azoles affect P450 enzyme activity, their main toxicities are due to interactions with other compounds that induce or inhibit this

system. The triazoles in use, in both an oral and intravenous form, are itraconazole (Sporanox, Janssen), fluconazole (Difucan, Pfizer Inc.) and voriconazole (Vfend, Pfizer Inc.), with posaconazole (SCH56592, Schering) and ravuconazole (BMS-207147, Bristol-Myers Squibb) remaining in preclinical development. Voriconazole and the investigational azoles have a broader spectrum of activity and fewer side effects compared to the older azoles. In fact, voriconazole can be superior to amphotericin B in the treatment of difficult mould infections such as aspergillosis [16].

ANTIMETABOLITE

Flucytosine has been available since 1972 and it is the only antimetabolite available for the treatment of systemic fungal infections. Flucytosine is a fluorine analogue of cytosine (Fig. 2C) that functions as an inhibitor of thymidylate synthetase. The drug is only available in oral form and the bioavailability is 78-89% [17]. The major toxicities include bone marrow suppression, myocardial toxicity, and renal failure. The spectrum of activity is rather limited, but includes *Candida* spp., *C. neoformans*, and some black moulds. Cryptococcosis is the only mycoses for which the use of flucytosine is routinely recommended, and only when administered in combination with amphotericin B [18]. Rapid resistance occurs if flucytosine is used as monotherapy [19, 20].

ECHINOCANDINS

The echinocandins are the newest agents clinically available for use. They are water-soluble large amphipathic polypeptides that are only available in intravenous forms (Fig. 2D). The drugs inhibit 1,3- β -glucan synthetase [21, 22] resulting in the reduction of cell wall 1,3- β -glucans, the major glucan in many fungi. Organisms such as *C. neoformans*, which have mainly 1,6- β -glucan and β -glucans, are resistant [23]. The major toxicities include drug interactions, phlebitis, and fever. Caspofungin (Cancidas, Merck and Co., Inc.) is FDA approved for use empirically in febrile neutropenic patients, for candidal infections, and for aspergillosis in the setting of refractory disease or

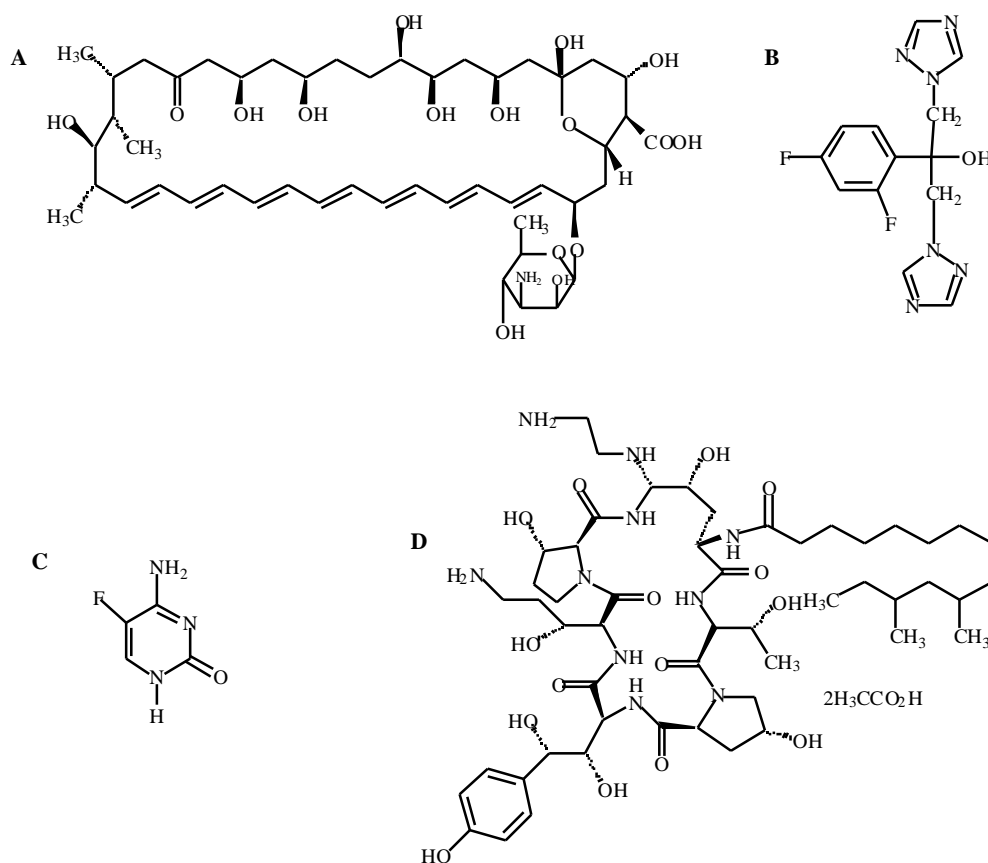


Fig. (2). Chemical structures of representative [A] polyenes (amphotericin B), [B] azoles (fluconazole), [C] antimetabolite (flucytosine), and [D] echinocandins (caspofungin).

intolerance to amphotericin B. In 2005, micafungin (Mycamine, Astellas) became the second FDA approved echinocandin, with indications for prophylaxis of *Candida* infection in stem cell transplant patients and for treatment of *Candida* esophagitis. A third echinocandin, anidulafungin (VER-002, V-echinocandin, LY303366, Vicuron Pharmaceuticals), has completed phase III clinical trials and should be available in the near future.

COMBINATION THERAPY

Recently, increased interest in combination therapy has developed. The goal of combination therapy is to increase efficacy, reduce toxicity, minimize resistance development, and extend the spectrum of activity. As mentioned, the only currently standard recommended combination therapy for invasive disease is with amphotericin B and flucytosine for cryptococcosis, which results in more rapid clearance of the fungus and improved outcomes [24]. Since experimental studies using combinations of polyenes and azoles have shown varied results from antagonism to synergy [reviewed in [25], concerns regarding potential antagonism in human mycoses have led clinicians to avoid their combination. Since polyenes and azoles both target ergosterol, the fear is that the depletion of ergosterol by an azole will result in a reduction in target sites for amphotericin B thereby diminishing the potency of the polyene [26, 27]. The addition of the echinocandins to the antifungal

armamentarium has greatly increased the capacity for combination therapy. In fact, caspofungin used in combination with amphotericin [28, 29] or voriconazole [30] for aspergillosis in immunosuppressed patients can improve clinical outcomes.

RECENT PATENTS ON THERAPEUTICS FOR INVASIVE MYCOSES

Recently published patent applications and issued patents have largely focused on the utilization of drugs used in combination with existing antifungals. Methods for improving delivery or providing new methods of delivery have also been reported. Additionally, there are several novel therapeutics described. The following descriptions highlight particularly interesting reports.

Combination Therapy

The combination of cyclic hexapeptides, echinocandin derivatives, with other classes of antifungal drugs for treatment of fungal pathogens is reported in [31]. As discussed above, precedence for the use of lipopeptides exists, since echinocandins have been used in clinical investigations (reviewed in [32]). The authors provide evidence for efficacy using microdilution minimal inhibitory concentration [MIC] assays as evidence for efficacy. For example, the MIC for an *A. fumigatus* isolate was 2 $\mu\text{g/ml}$ for amphotericin B, 0.03 $\mu\text{g/ml}$ for lipopeptide [I], and

0.0078 $\mu\text{g/ml}$ for the combination of the drugs. Synergy was reported for the lipopeptide in combination with amphotericin B, itraconazole, nikkomycin X (an experimental cell wall agent), or flucytosine against *Aspergillus* spp., *C. albicans*, *C. neoformans*, and other fungi.

A novel combination therapy is disclosed in [33]. The invention describes the administration of an echinocandin in combination with a glycopeptide for invasive mycoses. Glycopeptides are antibacterial drugs, with vancomycin and teicoplanin being the main clinically used agents [34]. The author details the surprising finding that the efficacy of an echinocandin can be significantly improved when co-administered with a glycopeptide having a substituent comprising at least about 8 carbon atoms. With the addition of such a glycopeptide, the dose of the echinocandins could be lowered to reduce toxicity and drug cost while maintaining potency. The invention states that the antifungal and antibacterial drugs can be administered in the same or separate formulations simultaneously or sequentially. The description includes data showing synergy with test compounds, resulting in fractional inhibitory concentrations (FIC) for *Aspergillus* spp. ranging from 0.16 to 0.31 $\mu\text{g/ml}$.

Tetracyclines are widely used antibiotics as they have bacteriostatic activity against a broad variety of Gram positive, Gram negative, and atypical pathogens [35]. A method for inhibiting fungal growth with substituted tetracycline compounds is described in [36]. Substituted tetracycline compounds include tetracycline compounds that have at least one constituent replaced (Fig. 3). The new tetracyclines have lower MICs for the targeted fungal organisms than the unsubstituted form. An additional consideration is that the substituted tetracycline may have less toxicity than the parent compound. However, the utility of monotherapy with these drugs is unclear in the setting of invasive disease, since the potency of the compounds is not well described. Importantly, the knowledge that certain tetracyclines possess limited antifungal activity both alone and in synergy with amphotericin B [37-39] certainly influenced the development of the invention. In a continuation application by the authors [40], they extend their claims to include the use of these novel tetracycline compounds in combination therapy with standard antifungals. They propose that the substituted tetracyclines will be synergistic when used with these drugs. The agents can be given as a mixture or separately at the same or different times. Experimental data is provided indicating the FIC for combinations of a substituted tetracycline with amphotericin B ranging from 0.063-0.125 $\mu\text{g/ml}$ for diverse fungi. Importantly, the efficacy of amphotericin B was achieved with the combination when 8 to 10 fold less amphotericin B was used, which would substantially reduce the toxicity of the polyene.

The use of a substance referred to as FKI-0076 (Fig. 4) or derivatives thereof as an antifungal agent is described in [41]. The compound is produced by *Talaromyces flavus* FKI-0076, which has been deposited in the International Patent Organism Depository, Japan under the identifier FERM BP-7037. The patent describes that FKI-0076 enhances the activity of miconazole, an azole that is used

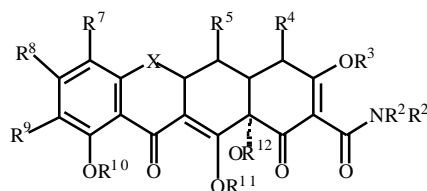


Fig. (3). Basic chemical structure of substituted tetracycline. X is $\text{CHC}(\text{R}^{13} \text{Y}'\text{Y})$, CR^6 , R^6 , S, NR^6 , or O; R^2 , R^2 , R^4 , and R^{4n} are hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, alkylamino, arylalkyl, aryl, heterocyclic, heteroaromatic or a prodrug moiety; R^4 is NR^4 , R^{4n} , alkyl, alkenyl, alkynyl, hydroxyl, halogen, or hydrogen; R^3 , R^{11} and R^{12} are each hydrogen, or a pro-drug moiety; R^{10} hydrogen, a prodrug moiety, or linked to R^9 to form a ring; R^5 is hydroxyl, hydrogen, thiol, alkanoyl, aroyl, alkaroyl, aryl, heteroaromatic, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, alkylamino, arylalkyl, alkyl carbonyloxy, or aryl carbonyloxy; R^6 and R^{6n} are hydrogen, methylene, absent, hydroxyl, halogen, thiol, alkyl, alkenyl, alkynyl, aryl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, alkylamino, or an arylalkyl; R^7 is hydrogen, halogen, nitro, alkyl, alkenyl, alkynyl, aryl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, arylalkyl, amino, arylalkenyl, arylalkynyl, or $-(\text{CH}_2)_0-3\text{NR}^{7c}\text{C}(=\text{W})\text{WR}^{7a}$; R^9 is hydrogen, halogen, nitro, alkyl, alkenyl, alkynyl, aryl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, arylalkenyl, amino, arylalkenyl, arylalkynyl, thionitroso, or $-(\text{CH}_2)_0-3\text{NR}^{9c}\text{C}(=\text{Z})\text{ZR}^{9a}$; Z is $\text{CR}^{9d}\text{R}^{9e}$, S, NR^{9b} or O; Z' is O, S, or NR^{9f} ; W is $\text{CR}^{7d}\text{R}^{7e}$, S, NR^{7b} or O; W' is O, NR^{7f} S; R^{7a} , R^{7b} , R^{7c} , R^{7d} , R^{7e} , R^{9a} , R^{9b} , R^{9c} , R^{9d} , and R^{9e} are hydrogen, acyl, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, alkylamino, arylalkyl, aryl, heterocyclic, heteroaromatic or a prodrug moiety; R^8 is hydrogen, hydroxyl, halogen, thiol, alkyl, alkenyl, alkynyl, aryl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, alkylamino, or an arylalkyl; R^{13} is hydrogen, hydroxy, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, alkylamino, or an arylalkyl; and Y' and Y are each independently hydrogen, halogen, hydroxyl, cyano, sulfhydryl, amino, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylsulfanyl, alkylsulfonyl, alkylamino, or an arylalkyl, or salts thereof.

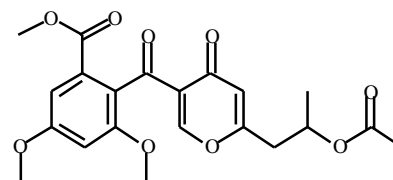


Fig. (4). Structure of the substance FKI-0076 produced by *T. flavus* FKI-0076. FKI-0076 is a yellow oily substance with a molecular weight of 418 by fast atom bombardment mass spectrometry and a molecular formula of $\text{C}_{21}\text{H}_{22}\text{O}_9$.

primarily in topical formulations, against *C. albicans* and *S. cerevisiae*. Although the report claims that the compound alone has activity against diverse fungi, the MICs reported for *A. niger*, *M. racemosus*, *C. albicans*, and *S. cerevisiae* were $>125 \mu\text{g/ml}$. Finally, the patent claims that the compound can reduce the development of resistance, but no data is provided to support this claim. No description

Table 1. Antifungal Drugs Approved by the US FDA

Class	Drug	Year Approved
Polyene	Amphotericin B	1957
	Amphotericin B lipid complex [Abelcet]	1995
	Amphotericin B cholesteryl sulfate [Amphotec]	1996
	Liposomal amphotericin B [Ambisome]	1997
Antimetabolite	Flucytosine	1972
Azole	Ketaconazole	1981
	Fluconazole	1990
	Itraconazole	1992
	Voriconazole	2002
Echinocandin	Caspofungin	2001
	Micafungin	2005

regarding a mechanism of action for FKI-0076 is given. The evidence supports further consideration for the use of this compound in combination therapy, particularly with azoles.

Cell Wall

The identification of imidazo[1,2-a]pyridine derivative compounds that inhibit 1,6- β -glucan synthetase is reported in [42]. As with the echinocandins that interfere with the synthesis of 1,3- β -glucan, these substances should be relatively safe since mammalian cells lack cell walls and do not produce 1,6- β -glucans. Heretofore, there are no known substances that 1,6- β -glucan synthetase and drugs with the capacity to target fungi that have higher concentrations of 1,6- β -glucans than 1,3- β -glucans, such as *C. neoformans* [23], would indeed be valuable. The report presents limited data regarding the activity of the compounds as antifungals. The MICs of several of the compounds is shown to be very high against yeast, but one substance, #8, had MICs of <0.063 to *S. cerevisiae* and *C. glabrata*. These compounds have great potential as antifungal therapeutics, and they may also be used to potentiate the effects of the echinocandins.

Cell Membrane

As described, lipid formulations of amphotericin B are useful therapeutics given the reduced toxicity associated with their use. A novel process for solubilizing amphiphilic drugs by a commercially feasible process, specifically to improve the preparation of liposomal amphotericin B, is detailed in [43]. Phospholipid molecules are polar, with a characteristic hydrophilic ionizable head and a hydrophobic tail composed of long fatty acid chains. In the presence of water, phospholipids spontaneously form a sphere consisting of a bilayer where the fatty acid tails point into the newly formed membrane's interior and the polar heads point toward the aqueous solution. The process allows for the deposition of additional substances, such as a polyene, into the aqueous

interior during the liposome formation. The process allows deposition of amphotericin B in various quantities of volatile solvent followed by spray drying to facilitate commercial production. The dried form can be stored in a lyophilized state for subsequent hydration and clinical use without significant alteration in size, toxicity, or function.

A method for delivery by aerosolization of amphotericin B, or other antifungal compounds, for prophylaxis or treatment of pulmonary mycoses is described in [44]. The approach discussed by the authors has a significant drawback that is largely overlooked. Although they indicate that the MIC of the antifungal needs to be determined for the microbe prior to the administration of the drug, there is no discussion regarding the facts that the determination of the MIC for most fungi takes 48 to 72 h and MIC methods have not been standardized for all fungal organisms. A better clinical approach would be to administer an empiric dose of the antifungal pending further microbiological data. The aerosolized antifungal would maintain appropriate drug levels for approximately one week. An important benefit of pulmonary administration would be a reduction in systemic toxicities. However, the medication would not be effective against organisms outside of the pulmonary tissues and treatment of a disseminated infection would require the use of the aerosol in combination with a second systemic antifungal.

The cost-efficient development of an immediate release itraconazole capsule is illustrated in [45]. Itraconazole is a triazole that is commercially available in capsules, injections, and oral solutions. The authors describe active pellets consisting of an inert starting seed, itraconazole, a binder, and, optionally, an alkaline agent that are subsequently pressed into a tablet or loaded into a capsule. The invention principally improves on prior oral forms since there is no need for a seal coat, which substantially reduces manufacturing time and costs. The starting seed could consist of a synthetic or biological compound, but the preferred seed is a sugar sphere. Since the typical dose of itraconazole is 200-400 mg/D with each 100 mg capsule costing approximately US\$9.30, the proposed advantage of the new formulation of itraconazole would be the ability to achieve high oral drug bioavailability at a reduced price.

The production of azole derivatives that potentially have improved potency and a broad spectrum of activity is reported in [46]. The authors generally propose the manufacture of compounds comprised of a five to seven membered heterocyclic ring containing one to four heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur; phenyl or a substituted phenyl (Fig. 5). Although the experimental compounds did not always have improved MICs compared to available antifungals, the novel compounds were significantly more potent when tested against certain *Candida* species. The disclosure states that *in vivo* testing has been performed, but the results are not provided. Given their improved activity against *Candida*, these compounds may be a useful addition to our antifungal armamentarium.

Two novel products from a recently identified *Streptomyces* sp. with potential antifungal activity are reported in [47]. The strain has been deposited as

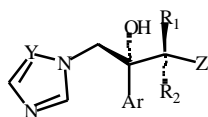


Fig. (5). General structure for the derivation of novel azoles. Ar is a five to seven membered heterocyclic ring and the preferred Ar is halogen substituted phenyl and the more preferred halogen substituted phenyl is 2,4-difluorophenyl; R₁ and R₂ are either hydrogen, straight chain or branched alkyl groups and the more preferred combination is when R₁ is methyl and R₂ is hydrogen, Y is CH or N; Z includes heterocyclic rings such as an imidazole or indole. An example is 1-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]-1-(2-hydroxyethyl)-3-[4-(1H-1-tetrazolyl)phenyl]thio-urea.

Streptomyces sp. K99-5041 in the International Patent Organism Depository, Japan under the permanent depository number FERM BP-8272. When grown in diverse conditions, the microbe produces K99-5041-C1x and K99-5041-C2x (Fig. 6), which are both readily obtained from the medium. The authors describe that the compounds have a similar activity to that of statins (which inhibit hydroxymethylglutaryl-coenzyme A reductase, a rate-limiting step in cholesterol synthesis) and azoles in that the compounds inhibit lanosterol synthase. Lanosterol synthase is an enzyme essential for the production of lanosterol by cyclization of 2,3-oxidosqualene. There are currently no drugs clinically available that inhibit fungal lanosterol synthase. The 50% inhibition concentration for lanosterol synthase activity for K99-5041-C1x and K99-5041-C2x is 15 and 18 μ M, respectively. However, the application does not provide antifungal efficacy data or toxicity information. Hence, the compounds are theoretically useful as antifungal agents as well as for the prevention and treatment of diseases caused by hyperlipidemia, such as atherosclerosis.

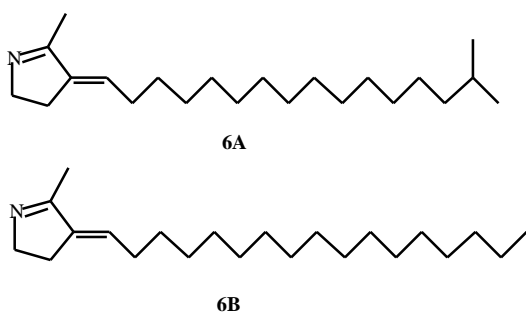


Fig. (6). Chemical structures K99-5041-C1x [A] and K99-5041-C2x [B] that are produced by *Streptomyces* sp. K99-504. K99-5041-C1x is a pale yellow oily substance and K99-5041-C2x is a white oily substance. Both compounds have a molecular formula of C₂₂H₄₁N and a molecular weight of 319.

Protein Synthesis Inhibitors and Antimetabolites

Sordarins are a class of drugs that inhibit protein synthesis by reversibly binding to elongation factor 2 (EF2) [48, 49]. EF2 is an essential protein catalyzing ribosomal translocation during protein synthesis and is highly

conserved in all eukaryotes. The first sordarin was isolated from the fungus *Sordaria areneosa* [50]. Experimental sordarin compounds have been reported to be efficacious in the treatment of candidiasis, aspergillosis, coccidioidomycosis, histoplasmosis, and pneumocystosis [51-55]. The use of C-11-hydroxysordarins (Fig. 7A) as antifungal agents is reported in [56]. Despite the high degree of sequence homology of EF2s from diverse eukaryotes, the described compounds specifically interact with fungal EF2s resulting in selective inhibition of protein synthesis in these organisms [57]. The sordarins described have a broad spectrum of antifungal activity, including important pathogenic fungi such as *Candida* spp., dimorphic fungi, *C. neoformans*, and filamentous moulds. The patent also details the use of a sordarin compound with a second antifungal. A second application also from Merck describes a sordarin derived from the basic formulae depicted in figure 7A by fermentation (Fig. 7B) with improved efficacy to diverse fungal species [58]. A drawback not mentioned in these disclosures is that the $t_{1/2}$ of sordarins in several animal species has been shown to be very short, indicating that high and repeated dosing may be required. Nevertheless, the high specificity of these compounds for fungal EF2, the apparent lack of significant toxicity, and the relative ease with which new sordarin variants can be synthesized suggests that these drugs could be extremely useful therapeutics.

Novel 6,7-disubstituted-5,8-quinolinedione compounds (Fig. 8) are proposed as antifungal drugs in [59]. These compounds function as antimetabolites of coenzyme Q to inhibit mitochondrial coenzyme-Q dependent succinoxidase and electron transport in *Saccharomyces cerevisiae* [60]. MIC data for 20 synthesized 6,7-disubstituted-5,8-quinolinedione derivatives showed that amount of drug required to inhibit growth of *Candida* spp. and *C. neoformans* was less than or similar to fluconazole and ketoconazole *in vitro*. Some of this data has been published elsewhere [61, 62]. In a rat model of candidemia, a synthesized quinolinedione significantly prolonged survival compared to an equivalent dose of ketoconazole. No toxicity data is provided.

Compounds that inhibit microbial NAD synthetase (Fig. 9) are described in [63]. These compounds have diverse uses, including the capacity to inhibit or kill fungi by reducing or eliminating microbial production of NAD. The compounds are comprised of two aryl moieties joined by a suitable linker. Though details are not provided, the report claims that the compounds are highly effective against fungal pathogens, while having only moderate toxicity in mammals.

Antifungal Proteins

Antifungal proteins that are analogues of the radish seed antifungal protein 2 [Rs-AFP2] are detailed in [64]. The amino acid sequence of Rs-AFP2 is QKLCQRPSGT WSGVCGNNA CKNQCIRLEK ARHGSCNYVF PAHK CICYFP C, and the mutants identified are 80% homologous. The patent details the generation of the mutants and provides some microbiological data using plant fungal pathogens. The authors state that these peptides can also be effective as food preservatives and as therapeutics for humans. The vector

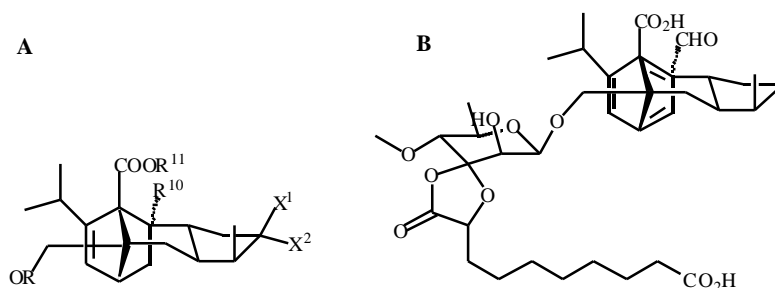


Fig. (7). [A] Basic structure for C-11-hydroxysordarins and [B] a derivative compound.

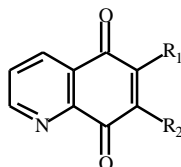


Fig. (8). General formula for derivatives of a 6,7-disubstituted-5,8-quinolinedione. R₁ is a C₁₋₂₀ alkylmercapto or phenylamino group substituted by up to 3 groups selected from a halogen atom and an aceto group; R₂ represents a thiocyno or a C₁₋₂₀ alkylmercapto group.

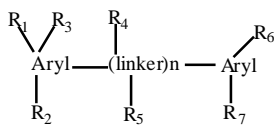


Fig. (9). General formula for NAD synthetase inhibitors where n is an integer from 1 to 12; R₁₋₇ are independently H, an unsubstituted or a substituted cyclic or aliphatic group, a branched or unbranched group; the linker is a cyclic or aliphatic, branched or an unbranched alkyl, alkenyl, or an alkynyl group and the linker may also contain heteroatoms.

selected for production and secretion of the peptides is *S. cerevisiae*. This yeast is not affected by the presence of Rs-AFP2 at concentrations as high as 500 µg/ml, which raises a potential concern about the ability of the protein to affect human pathogenic yeast. Nevertheless, there is increasing interest in the application of peptide and protein drugs in the treatment of infectious diseases [65]. However, their clinical utility has proven difficult since proteolysis occurs in the circulatory system, the agents are rapidly cleared by the kidneys, and there are concerns regarding immunogenicity. A number of approaches have been introduced to circumvent these difficulties, including targeted alterations in the primary peptide structure, conjugation to polymers, and incorporation into liposomes. A second patent [US6875744] discloses information about short bioactive peptides containing phenylalanine, leucine, alanine, and lysine residues that can be used in antibacterial, antifungal, anticancer, and other biological applications. The peptides are 5 to 22 amino acids in length and comprised of at least 80% phenylalanine, leucine, alanine, and lysine residues and no more than 20% phenylalanine and tryptophan residues.

Although the examples provided are shown to be effective against bacterial species, the MICs are generally 50 µg/ml against *S. cerevisiae* and *C. albicans*. These patents again support the potential activity of peptides as anti-infectives and designed antifungal proteins are highly likely to be clinically useful in the future [66, 67].

Radioimmunotherapy

The utilization of radiolabeled monoclonal antibodies for the treatment of infectious diseases is reported in [68]. Radioimmunotherapy [RIT] is well established as a treatment modality for cancer, but there is limited information about its use against infectious diseases. RIT is a therapeutic modality that depends on antibody-antigen interactions and utilizes antibodies radiolabeled with therapeutic radioisotopes to deliver lethal doses of radiation to target cells. The inventors describe mouse models, where radiolabeled monoclonal antibodies were effective in the treatment of lethal infections of *C. neoformans* [69] and of the bacterium *Streptococcus pneumoniae* [70]. In addition, RIT with a novel monoclonal antibody to *H. capsulatum* killed the fungus *in vitro* [71]. This novel approach may have particular utility in the setting of drug resistance or when an immunosuppressed host is incapable of complete eradication of the pathogen [72]. Side effects of radiation need to be considered, though the investigators report low toxicity due to this treatment modality [73]. The major drawbacks of this treatment are that it is not empiric (i.e. the organism must be identified) and an appropriate monoclonal antibody or peptide must be available for chelating to the radioisotope.

CURRENT AND FUTURE DEVELOPMENTS

This review summarizes the antifungal drugs in use clinically for invasive mycoses and described recent published disclosures to the US Patent and Trademark Office that have potential to impact our ability to combat fungal infections in the future. Clinicians are currently faced with a broad variety of disseminated mycoses in diverse immunologically normal and compromised individuals. As the number of highly immunosuppressed individuals continues to grow and the complexity of the medications administered to these patients similarly increases, the management of disseminated mycoses has grown more difficult. The addition of the echinocandins and the newer azoles has significantly improved our ability to combat disease, but there continues to be an urgent need for more potent drugs. Despite aggressive management, the prognosis

Table 2. Recent Antifungal Applications and Patents

COMBINATION THERAPY	
Cyclic hexapeptides with other antifungals	[31]
Echinocandin and glycopeptide	[33]
Substituted tetracycline with other antifungals	[36, 40]
FKI-0076 and azoles	[41]
CELL WALL	
1,6- β -glucan synthetase inhibitor	[42]
CELL MEMBRANE	
Improved liposomal amphotericin B formulations	[43]
Aerosolization of amphotericin B	[44]
Immediate release itraconazole capsule	[45]
Novel azoles	[46]
Lanosterol synthase inhibitors	[47]
PROTEIN SYNTHESIS INHIBITORS AND ANTIMETABOLITES	
C-11-hydroxysordarins that bind elongation factor 2	[56]
Substituted quinolinediones function as antimetabolites of coenzyme Q	[59]
Compounds that inhibit NAD synthetase	[63]
ANTIFUNGAL PROTEINS	
Radish seed antifungal protein 2 analogues	[64]
RADIOIMMUNOTHERAPY	
Radiolabeled specific antibodies	[68]

of invasive fungal disease, in particular those caused by filamentous fungi, continues to be dismal, with mortality rates exceeding 80% in selected categories of patients. There are abundant un-exploited targets in fungi that have been identified. For example the cell wall is rich in targets [74], yet little development has focused on inhibiting melanin formation which is significantly associated with virulence in fungi [75]. Ongoing genome analysis projects may also reveal novel targets. Adjunctive therapies, such as granulocyte infusions and the administration of immunomodulators (i.e. gamma interferon or colony stimulating factors) may also dramatically improve outcomes. Combinational regimens of antifungal drugs with monoclonal antibodies [76] or therapies utilizing 'killer' antibodies or peptides [77] also hold great promise. Radiolabeled antibodies may also be important therapeutics in the setting of drug resistant fungi. Finally, it is essential that proper clinical trials be performed with these novel compounds or combination of drugs to fully elucidate their efficacy and toxicity in patients.

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