Antifungals: what's in the pipeline

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The therapeutic landscape for mycotic infections is shifting. New generation azoles that are active against clinically relevant, drug-resistant fungal pathogens have improved bioavailability, half-lives and safety profiles. Acylated cyclic peptide inhibitors of β(1,3)glucan synthesis with origins as fungal metabolites provide an alternative and highly-selective mode of action, targeting cell-wall biogenesis in important pathogens such as *Candida* and *Aspergillus* species. The development, in each structural class, of compounds that have advanced to late-stage clinical trials is summarized in this review.

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Abbreviation
MIC minimum inhibitory concentration

Introduction

Azoles that inhibit sterol formation and polyenes that bind to mature membrane sterols have been the mainstays of antifungal therapy for two decades or more. The utility of fluconazole is being undermined by the development of resistance among pathogenic *Candida* spp. and by the inherent resistance of molds (for example, *Aspergillus* spp.), and there is a great need for an alternative to amphotericin B in light of its toxicity and cost as a parenteral liposomal formulation [1]. These limitations, together with the rising incidence of systemic life-threatening fungal infections associated with immunosuppression and neutropenia, have prompted the development of promising new antifungal compounds in two structural classes that have progressed to late-stage clinical trials. Voriconazole, ravuconazole and posaconazole are the newest members of the azole group. Inhibitors of glucan synthesis, namely caspofungin, anidulafungin and micafungin, offer the long-desired possibility of moving away from membrane sterols to the cell wall as the target of selective action. Our intent is to review the salient qualities of compounds from both of these structural classes that allowed their advancement from drug leads to clinical candidates.

New and improved azoles

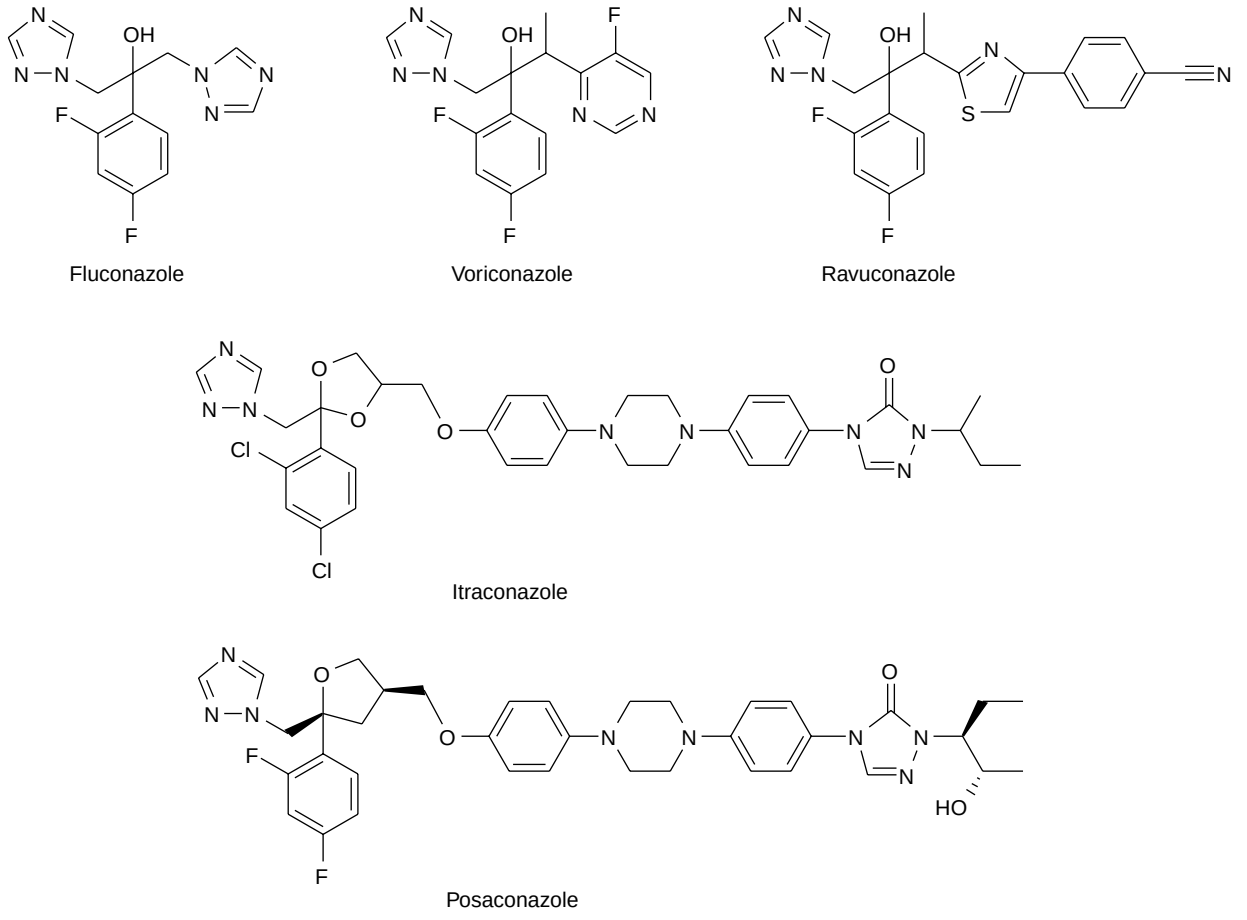
The focus in the azole arena has been on triazoles (compounds with azole rings containing three nitrogen atoms; see Figure 1). Voriconazole (a.k.a UK-109496) is related to fluconazole but has a fluorinated pyrimidine heterocycle replacing one triazolyl moiety. Ravuconazole (a.k.a BMS-207147, ER30346) also resembles fluconazole and voriconazole but contains a 4-thiazolyl benzonitrile instead of the fluorinated pyrimidine. Posaconazole (a.k.a. SCH 56592) more closely resembles itraconazole, with a furan ring instead of a ketal ring, an absolute stereochemistry of the five-carbon hydroxylated side chain (S,S), and fluoro substitutions. These newer compounds bring improved potency and a much broader spectrum than previous azoles, a spectrum that includes excellent activity against fluconazole-resistant yeasts, as well as enhanced activity against most filamentous species. All three compounds are active orally; voriconazole can also be administered intravenously.

Mechanism of action and spectrum of potency

The mechanism of action of azoles has been well established. They inhibit the cytochrome P450 that demethylates lanosterol at C14, depleting intracellular ergosterol and causing methylated sterols to accumulate [2–4]. Although *ERG11* (the gene encoding lanosterol demethylase) is essential in *Saccharomyces cerevisiae* and *Candida albicans*, inhibition of the step it mediates in these yeasts yields a static response that has been the biggest drawback to the use of fluconazole and itraconazole. Azoles bind to cytochrome P450s with different affinities through the N4 position of the triazole ring as a sixth ligand for the heme iron. The selectivity of the azoles for fungal cytochrome P450s is presumed to reside in the binding of the N1 aromatic group to specific amino acids that are not well conserved in mammalian proteins [5••]. Genes with homology to *S. cerevisiae ERG11* have been identified in all fungi examined, and the proteins they encode have a great deal of homology (65%–83% similarity to proteins of *S. cerevisiae*), supporting the broad spectrum of azole activity.

The three new triazoles have an added spectrum and potency against clinically relevant fungal species. Generally, ravuconazole, posacoanzole and voriconazole are more active than itraconazole and fluconazole against a wide variety of *Candida*: *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and azole-resistant isolates of *C. albicans*. The newer agents demonstrate a broad, although not identical, spectrum against filamentous and dimorphic fungi and less common yeasts [6–9]. In animal models of disseminated fungal disease (candidiasis and aspergillosis), all of these azoles demonstrate *in vivo* efficacy in normal and immunocompromised hosts that is either equivalent to or superior to approved azoles [10]. Published reports on the efficacy of posaconazole include a wide array of fungal diseases including cryptococcosis [11], blastomycosis [12], histoplasmosis [13], coccidioidomycosis [14], fusariosis [15] and phaeohyphomycosis [16]. Although efficacy of voriconazole and ravuconazole against all of these diseases has not

Figure 1



Structures of approved and investigational azoles. Voriconazole and ravuconzole most closely resemble fluconazole, but with the following modifications: voriconazole has a fluorinated pyrimidine heterocycle replacing one triazoyl moiety of fluconazole, and ravuconazole contains a 4-thiazolyl benzonitrile instead of voriconazole's fluorinated pyrimidine. Posconazole is prepared as a single isomer, and is structurally related to itraconazole, but with the following modifications: poscanozole has a furan ring instead of a ketal ring, and absolute sterochemistry of the five-carbon hydroxylated side chain, and fluoro substitutions.

yet been reported in animal models, the expectation is that they will be potent against a wide spectrum of fungi *in vivo*.

Most noteworthy are reports of fungicidal activity for a number of clinically relevant human pathogens *in vitro* [7,11,17–19,20•,21], and *in vivo* (sterilization of tissues in some infection models against *Coccidioides immitis*, *Aspergillus fumigatus* and *Blastomyces dermatididis*) [12,14,22]. Fungicidal activity was defined for *in vitro* experiments as the inability for outgrowth of the organism following removal of drug, and for *in vivo* experiments as the sterilization of tissues based on culture results.

Direct comparisons of *in vitro* activities between the three azoles have been reported for *Candida* bloodstream isolates [23] and for *Aspergillus* spp. [21]. All had potencies against *Candida* spp. that were comparable to one another, substantially greater than fluconazole, and slightly more active than itraconazole. Comparisons for *Aspergillus* spp. also demonstrated nearly equipotent activity except for *A. niger* (ravuconazole, MIC_{90} = 4.0 μg/ml; voriconazole and posaconazole, MIC_{90} = 0.5 μg/ml each) and *A. fumigatus*, which was more susceptible to posaconazole compared to voriconazole or ravuconazole (MIC_{90} values of 0.12, 0.5 and 0.5 μg/ml, respectively).

Fluconazole-resistant organisms

One factor behind the development of newer azoles was the rapid appearance of fluconazole-resistant organisms during long-term treatment. Each of the newer compounds has increased potency against recent fluconazole-resistant isolates. The azole resistance mechanisms that have been documented in fungi [24] include: first, upregulation of efflux pumps; second, alterations in amino acid sequence of the target enzyme with concomitant reduction in affinity for the inhibitor; and third, bypass mutations that enable

fungi to cope with altered membrane structure. Some resistant strains have been seen that use combinations of these mechanisms. Cross-resistance occurs when a genetic change leads to parallel increases in minimum inhibitory concentration (MIC) for two or more compounds. In complex situations in which resistance is often multifactorial, parallel increases in MIC are not necessarily indicative of true cross-resistance within a single isolate. Although such correlations have been documented between voriconazole and fluconazole, voriconazole and itraconazole [25,26], and traconazole and fluconazole [27] for *Candida* infections, it is important to note that posaconazole, voriconazole and ravuconazole have substantial potency against recent fluconazole-resistant clinical isolates. Furthermore, posaconazole is not a substrate for *MDR*1 efflux, one of the more common resistant mechanisms [28].

Clinical trials involving azoles

Voriconazole, posaconazole and ravuconazole also have favorable pharmacokinetics and bioavailability that have prompted further evaluation in human clinical trials. Voriconazole and posaconazole are currently in phase III trials and may become available before ravuconazole. Clinical experience with voriconazole and posaconazole have been postive; both are generally better-tolerated than amphotericin B. Furthermore, on the basis of efficacy in animal models and *in vitro* tests, they should demonstrate better efficacy against a wider spectrum of organisms than currently marketed triazoles, especially in the immuocompromised host.

Inhibitors of glucan synthesis: candins

A distinctly different mode of action is offered by three other compounds that are now in advanced stages of development. They are semisynthetic derivatives of acylated cyclic hexapeptide antibiotics discovered during the mid-1970s as agents that cause osmotic fragility and lysis of fungal cells. Representatives from this group of natural products (collectively, the candins) are shown in Figure 2, along with drug candidates that have been synthesized from them. Although the natural candins were potently fungicidal for a variety of *Candida* species, they had drawbacks as drug candidates. For example, FR901379 (a.k.a. WF11899A) was the only candin with appreciable water solubility, a consequence of an ionizable sulfate group (R1) on the homotyrosine [29]. Aqueous solubility, improved potency and a wider antifungal spectrum could be provided to pneumocandin B_0 by selectively reducing the 3-hydroxyglutamine (R2) to 3-hydroxyornithine and by replacing the C-5 hydroxyl of 4,5-dihydroxyornithine (R4) with a 1,2-diaminoethyl moiety [30]. These changes provide ionizable amines, and the effect of the latter was twofold. It stabilized the hemiaminal linkage that links the 3-hydroxyproline and ornithine residues (required for bioactivity), and it provided more satisfactory pharmacokinetics [31]. A water-soluble derivative, now known as caspofungin (a.k.a. Cancidas®, MK991, L-743,872), incorporates both modifications and retains the acyl side chain present in pneumocandin B_0. In contrast, the drug candidates anidulafungin (a.k.a. LY303366, VER-002, V-echinocandin) and micafungin (a.k.a. FK463) are made, respectively, from echinocandin B and FR901379, in each case by enzymatic deacylation of the natural product and addition of a synthetic side chain (R5). The derivatives have increased antifungal activity and lower hemolytic potential compared with their natural precursors [29,32]. Unlike the other semisynthetics, anidulafungin has an aqueous solubility of <0.1 mg/mL [33].

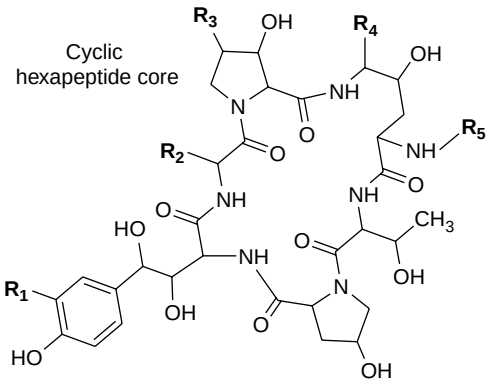
Mode of action

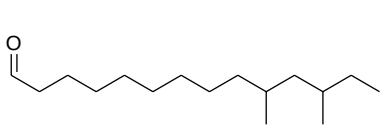
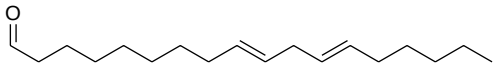
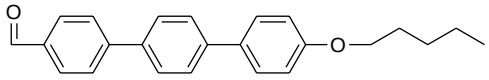
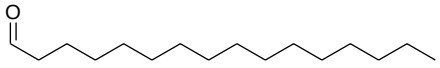
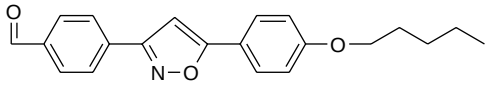
Candins and their semisynthetic derivatives cause the lysis of susceptible fungi at the points of cell wall synthesis in the tips of emerging hyphae or buds. Lysis results from inhibition of the synthesis of β-1,3-glucan, which is a major structural element in the walls of ascomycetes and certain other fungi. This polymer is synthesized by a complex composed of a large (>200 kDa) integral membrane protein and a small prenylated GTPase that works as a regulator in concert with a protein kinase [34,35•]. In *A. fumigatus*, the complex may also include a 100-kDa protein homologous to an H^+-ATPase, and a 160-kDa homolog of bacterial ATP-binding-cassette (ABC) β-1,2-glucan transporter [36••]. With membrane preparations from *C. albicans* that catalyze the transfer of glucose from UDP-glucose to β-1,3-glucan, polymerization is inhibited with IC_{50} values of 70–600 nM for the natural compounds and 1 nM for a semisynthetic derivative [30]. The IC_{50} represents the concentration at which 50% inhibition is achieved. The transmembrane protein in *S. cerevisiae* is encoded by either of two homologous genes designated *FKS1* and *FKS2*, and *FKS* homologs have been found in *C. albicans*, *A. fumigatus* and *Cryptococcus neoformans*. Analysis of the *FKS1* protein sequence predicts 16 transmembrane domains. Regions of the protein show homology with the cellulose synthase of *Acetobacter xylinum*, and a carboxy-terminal sequence contains the putative motif for UDP-glucose binding, suggesting that this protein comprises the catalytic center for glucan polymerization. Mutations in *S. cerevisiae* that confer a high degree of resistance to glucan-synthesis inhibitors map to the *FKS*1 locus, as may be expected if the inhibitors interact with the *FKS*1 protein. However, the predominant proteins in *C. albicans* membrane preparations specifically labeled by a photoactivatable crosslinking analog of anidulafungin are 40 kDa and 18 kDa in size, and neither one is the *Candida FKS* homolog [37]. These findings appear to add another layer of complexity to the understanding of glucan synthase and inhibitors like anidulafungin.

Efficacy of semisynthetic candins

A wide variety of clinically important *Candida* species and *A. fumigatus* (both amphotericin-sensitive and amphotericin-resistant strains) are sensitive to the semisynthetic candins *in vitro* [38] and in animal models of disseminated infection [39]. The *Candida* species that are relatively insensitive to marketed azoles include *C. glabrata*, *C. krusei*,

Figure 2



	R_1	R_2	R_3	R_4	R_5
Pneumocandin B_0	H	$NH_2(CO)CH_2CHOH$	H	OH	
Caspofungin	H	$NH_2(CH_2)_2CHOH$	H	$NH(CH_2)_2NH_2$	
Echinocandin B	H	CH_3CHOH	CH_3	OH	
Anidulafungin	H	CH_3CHOH	CH_3	OH	
FR901379	$HOSO_3$	$NH_2(CO)CH_2CHOH$	CH_3	OH	
Micafungin	$HOSO_3$	$NH_2(CO)CH_2CHOH$	CH_3	OH	

Current Opinion in Microbiology

Structures of natural and semisynthetic glucan-synthesis inhibitors. Despite the high degree of structural similarity among the natural antibiotic compounds, organisms that produce them are found throughout the fungal kingdom: *Aspergillus nidulans* (order Eurotiales) produces echinocandin B, the archetype of this class [50]; and *Glarea lozoyensis* (order Leotiales) and *Coleophoma empedri* produce pneumocandin B_0 and FR901379, respectively [38,51]. The fungi modify the peptide cores with different acyl chains (R_5), which are likely to be of polyketide origin [52]. Natural variation in the peptide core structure occurs in the residues marked by R_1, R_2 and R_3. Dihydroxyhomotyrosine (R_1 = H) is sulfated in FR901379. The threonine residue (with R_2) of echinocandin B is replaced by 3-hydroxyglutamine in pneumocandin B_0 and FR901379. The occurrence of 3-hydroxy-4-methylproline (R_3) appears restricted in nature to antibiotics of this class; it arises by cyclization of leucine [53]. In pneumocandin B_0, this position is occupied by 3-hydroxyproline. The chemical modifications indicated at R_2, R_4 and R_5 are discussed in the text.

C. lusitaniae, *C. parapsilosis* and *C. tropicalis*. *C. albicans* strains expressing azole resistance via a multidrug efflux mechanism are also susceptible [40]. The glucan synthesis inhibitors are efficacious in rat and mouse models of pneumonia caused by *Pneumocystis carinii*, the fungal nature of which has only recently been appreciated [41], but for *Histoplasma* infections, the data are conflicting [42]. Although susceptibility *in vitro* extends to a number of other filamentous pathogens [43,44], the clinical utility of semisynthetic candins for infections caused by these agents remains to be established.

Clinical trials involving semisynthetic candins

What initially animated the search for candins was the premise that β-1,3-glucan and the enzymatic machinery for its synthesis are not part of mammalian metabolism, making the potential of the inhibitors for selective antifungal action high and the likelihood for mechanism-based mammalian toxicity low. At doses that were efficacious in animals, the semisynthetic candins were well tolerated [31,45]. All three candidates have passed the safety portion of human clinical trials (Phase I). Biliary excretion is an important route of elimination of these compounds [46].

After a one-hour infusion of radiolabeled caspofungin in healthy human volunteers, excretion was slow, with 35% and 41% of the administered label recovered in the feces and urine, respectively, over 27 days [47]. A day after drug administration, the plasma contained either intact caspofungin or a rearrangement product arising by disruption of the hemiaminal linkage. Thereafter, the latter became the major circulating form. Being extensively hydroxylated, caspofungin was refractory to oxidative bioconversion and did not interfere with oxidation of other drugs. The primary labeled products in the urine were small hydrolytic products. Both caspofungin and anidulafungin display serum half-lives of several hours, allowing maintenance of active drug above the MIC levels of various pathogens for extended periods [31,45,46]. Anidulafungin is being evaluated in Phase II trials for the parenteral treatment of candidiasis and aspergillosis; micafungin has also reached Phase II trials [48,49]. Caspofungin is being developed as a once-a-day parenteral drug for systemic mycoses and has received approval from the United States Food and Drug Administration as therapy for aspergillosis patients who are refractory to or intolerant of standard therapies.

Conclusions

A new generation of azoles and the semisynthetic candins are emerging from the research pipeline. Clinical findings and experience will now define the role that is appropriate for each compound in combating fungal infections. The relative potency, selectivity and efficacy of the compounds, and the potential for synergy and drug interactions will be clarified as the populations of patients treated with the new agents grow. Entering into the pipeline are compounds from other structural classes that target different functions within the fungal cell. As an example, sordarin analogs are under investigation to determine whether their ability to inhibit the function of fungal-specific elongation factor 2 during protein synthesis offers a realistic hope for the development of a novel class of antifungal agents [49].

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- •• of outstanding interest

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