New triazoles and echinocandins: mode of action, in vitro activity and mechanisms of resistance

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Different types of mycoses, especially invasive mycoses caused by yeasts and molds, are a growing problem in healthcare. The most notable explanation for this increase is a rise in the number of immunocompromised patients owing to advances in transplantation, the emergence of AIDS and a rise in the number of invasive surgical procedures. Despite advances in medical practice, some therapeutic problems remain. In addition, intrinsic or acquired antifungal resistance may pose a serious problem to antifungal therapy. A new generation of triazole agents (voriconazole, posaconazole, isavuconazole, ravuconazole and albaconazole) and the recent class of the echinocandins (caspofungin, micafungin and anidulafungin) have become available, and represent an alternative to conventional antifungals for serious fungal infection management. Currently, only two of the recent triazole generation (voriconazole and posaconazole) and all three echinocandins are available for clinical use. More precisely, voriconazole and posaconazole are indicated for the treatment of invasive fungal infections and the echinocandins for the treatment of specific candidiasis. Voriconazole and posaconazole have a very broad spectrum of antifungal activity that includes Candida species, and filamentous and dimorphic fungi. Their activity extends to both fluconazole- and itraconazole-resistant strains of Candida. A major difference between posaconazole and voriconazole is that posaconazole has activity against Zygomycetes including Mucor spp., Rhizopus spp. and Cunninghamella spp., and voriconazole has no activity against this class of fungi. Ravuconazole, isavuconazole and albaconazole have shown very potent in vitro activity against species of Candida, Cryptococcus and Aspergillus, and they are currently in various stages of development. All three echinocandin agents, caspofungin, micafungin and anidulafungin, are similar in their spectrum of activity. Echinocandins do not possess in vitro activity against important basidiomycetes, including Cryptococcus, Rhodotorula and Trichosporon. This review attempts to deliver the most up-to-date knowledge on the mode of action and mechanisms of resistance to triazoles and echinocandins in fungal pathogens. In addition, the in vitro activity data available on triazoles and echinocandins are reported.

Keywords: albaconazole • anidulafungin • caspofungin • isavuconazole • micafungin • posaconazole • ravuconazole • voriconazole

Different kinds of mycoses, especially invasive, have become an important public health problem as their incidence has increased dramatically in the last decades [1]. Individuals who are severely immunocompromised are particularly vulnerable to infection from unusual molds and yeasts that are often found naturally in the environment [2,3]. Besides the most commonly isolated Candida and Aspergillus spp., new emerging opportunistic fungi such as Mucor, Fusarium, Zygomycetes or Scedosporium spp. have also emerged as significant pathogens in recent years. In addition, the recent surge of antifungal agents is selecting resistant strains of susceptible species and is shifting the population of fungal pathogens towards yeast genera that are intrinsically resistant, such as Cryptococcus, Rhodotorula and Trichosporon spp., molds of the genus Fusarium and members of the Zygomycetes family [3–5]. This changing pattern in fungal infections has driven the need for new agents with additional activities, even if amphotericin B is still considered the gold standard for the treatment of severe mycoses [6,7]. Advances made during the past...
few years have led to the introduction of several new options for treating serious fungal infections. New antifungal agents from old and new chemical families such as the triazoles (voriconazole and posaconazole) and echinocandins (caspofungin, micafungin and anidulafungin) have been approved for use. This article focuses on the mode of action, mechanisms of resistance and in vitro activity of recent triazole and echinocandin agents.

**Triazole antifungal agents**

The triazoles are synthetic compounds that have three nitrogen atoms in the five-member azole ring. Fluconazole and itraconazole were the first triazoles in clinical practice. Newer triazoles such as voriconazole and posaconazole have been developed to overcome the limited efficacy of fluconazole against *Aspergillus* and other molds, and to improve the pharmacokinetic profile of itraconazole [8].

Voriconazole was approved by the US FDA in May 2002 for the treatment of invasive aspergillosis and infections caused by *Scedosporium apiospermum* and *Fusarium* spp. in cases of intolerance of or refractoriness to other antifungal agents. In November 2003, a license was granted for its use in the treatment of esophageal candidiasis, and in December 2004 for the treatment of disseminated candidiasis. In January 2005, an indication for primary treatment of invasive candidiasis (including candidemia) in non-neutropenic patients was approved. In Europe, voriconazole has been approved by the European Medicines Agency (EMEA) for the treatment of invasive aspergillosis, serious infections caused by *Fusarium* spp. and *S. apiospermum*, and fluconazole-resistant serious invasive Candida infections (including candidemia) in non-neutropenic patients [23].

Posaconazole has been approved by the EMEA (2005) for the prophylaxis of invasive Aspergillus and Candida infections in patients 13 years of age and older who are at a high risk of developing these infections due to being severely immunocompromised, such as hematopoietic stem cell transplantation recipients with graft-versus-host disease or those with hematologic malignancies with prolonged neutropenia from chemotherapy. Subsequently, posaconazole has also been approved for the treatment of oropharyngeal candidiasis, including oropharyngeal candidiasis refractory to itraconazole and/or fluconazole.

Posaconazole has been authorized by the EMEA (2005) for the treatment of the following fungal infections in adults:

- Invasive aspergillosis in patients with disease that is refractory to amphotericin B or itraconazole, or in patients who are intolerant of these medicinal products;
- Fusariosis in patients with disease that is refractory to amphotericin B or in patients who are intolerant of amphotericin B;
- Chromoblastomycosis and mycetoma in patients with disease that is refractory to itraconazole or in patients who are intolerant to itraconazole;
- Coccidioidomycosis in patients with disease that is refractory to amphotericin B, itraconazole or fluconazole or in patients who are intolerant of these medicinal products;
- Oropharyngeal candidiasis: as a first-line therapy in patients who have severe disease or are immunocompromised, in whom response to topical therapy is expected to be poor.

Currently, the new agents isavuconazole, ravuconazole and albaconazole are emerging from preclinical research and moving to clinical development [6,9].

**Mode of action of triazoles**

Triazole antifungal agents inhibit the ergosterol biosynthesis pathway via the inhibition of 14-α-demethylase, the enzyme that removes the methyl group at position C-14 of precursor sterols. *ERG11* is the name of the gene that encodes the yeast 14-α-lanosterol demethylase (Erg11p). Inhibition of this enzyme leads to accumulation of aberrant sterol intermediates (14-α-methylerstols) on the fungal surface, which results in the arrest of fungal growth [10–14]. In filamentous fungi, two homologous genes, *CYP51A* and *CYP51B*, encoding two different 14-α-sterol demethylases, Cyp51A and Cyp51B, have been described [15]. In yeasts, the Erg11p uses lanosterol as an exclusive substrate, and in filamentous fungi, both enzymes are used as lanosterol and/or eburicol substrates.

A second target for azole antifungals, sterol δ14 desaturase (Cyp61), has been described [16]. This is also a cytochrome P450 enzyme involved in the last step of ergosterol biosynthesis [16].

The influx into fungal cells and the affinity of various azole derivatives to the various cytochrome P450 isoenzymes are different [3,11,12,14,17,18]. Consequently, the various azole antifungals differ in their in vitro potency and spectrum of fungi affected [19].

**Antifungal spectrum & activity of triazoles**

**Voriconazole**

Voriconazole has been shown to have potent activity against a wide range of clinically relevant yeasts and molds [20]. Recently, the Clinical and Laboratory Standards Institute (CLSI) established some provisional breakpoints for voriconazole, classifying isolates with a MIC of 1 µg/ml or less as susceptible and those with a MIC of 4 µg/ml or more as resistant [21,22].

The drug is fungicidal in vitro for the majority of *Aspergillus* spp. [23] and *C. krusei* [24]. By contrast, it appears to exhibit fungistatic activity against *Candida* spp. [25]. Zygomycetes are known to be resistant to voriconazole in vitro and in vivo [26].

**Susceptibility studies**

*Candida*

Recently, the results of an investigation demonstrated the broad-spectrum in vitro activity of voriconazole, relative to that of fluconazole, against 1024 yeasts tested, in particular fluconazole-resistant isolates, such as *C. krusei* [27]. Voriconazole and selected comparators were tested against 6970 invasive isolates of *Candida* spp. from sites worldwide [28]. Voriconazole was comparable in spectrum against the recently isolated *Candida* spp.
to fluconazole, but it showed a spectrum of activity greater than that of itraconazole. The MIC\textsubscript{90} values for voriconazole against all Candida spp. were 0.25 µg/ml or less [28]. Candida albicans was the most susceptible species (MIC\textsubscript{90}: 0.03 µg/ml) and Candida glabrata was the least susceptible species (MIC\textsubscript{90}: 1–2 µg/ml) [28].

A global evaluation of voriconazole susceptibility was performed on 7191 Candida spp. from 78 centers between 2004 and 2007 [29]. Voriconazole was very active (MIC\textsubscript{90}/MIC\textsubscript{90}: 0.008/0.25 µg/ml) and among Candida species, C. glabrata demonstrated the highest MIC values (MIC\textsubscript{90}/MIC\textsubscript{90}: 0.25/2.0 µg/ml) [29].

Recently, Enache-Angoulvant et al. evaluated the activity of voriconazole and caspofungin against 143 clinical isolates belonging to 18 uncommon Candida spp. [30]. At a concentration of 1 µg/ml or less both drugs inhibited 99% of the strains tested [30]. Two international surveys tested a total of 5329 clinical isolates of Candida spp. against voriconazole. All strains were susceptible to voriconazole and presented a MIC\textsubscript{90} value of 1 µg/ml or less [31,32].

Non-Candida yeast isolates
The in vitro susceptibilities of 237 isolates of Cryptococcus neoformans to voriconazole and two comparator agents were also examined in the aforementioned international survey [31]. A total of 100% of the C. neoformans isolates were susceptible, with MICs of 1 µg/ml or less for voriconazole [31]. Voriconazole showed in vitro activity against 1811 clinical isolates of C. neoformans obtained from 100 laboratories in five geographic regions worldwide [33]. Overall, 99% of all isolates tested were susceptible to voriconazole at a MIC of 1 µg/ml [33].

In a recent susceptibility study, 122 isolates of non-C. neoformans/non-Cryptococcus gattii spp. were analyzed against voriconazole and comparator agents. Voriconazole was active against most of the isolates [34]. Voriconazole appears to be broadly active against 8717 yeast isolates tested from 2001 to 2007 in a recent survey [35]. A total of 22 different species were isolated, of which C. neoformans was the most common (31.2% of all isolates). Overall, Cryptococcus (32.9%), Saccharomyces (11.7%), Trichosporon (10.6%) and Rhodotorula (4.1%) were the most commonly identified genera. The overall percentages of isolates in each category (susceptible, and resistant) were 78.0 and 12.5%, and 92.7 and 5.0% for fluconazole and voriconazole, respectively. Voriconazole was considerably more active than fluconazole against all of the non-candidal yeasts, although it was not particularly active against Cryptococcus albidus (62.5%) or any of the species of Rhodotorula (23.0–54.1%) [35].

Molds
Voriconazole showed excellent in vitro activity against Aspergillus spp. with a MIC\textsubscript{90} value of 1 µg/ml or less [32]. In other susceptibility studies, voriconazole has exhibited pronounced activity against most Aspergillus spp. (MIC\textsubscript{90}: 0.01–2 µg/ml) [36,37]. Voriconazole is also effective against Fusarium spp., Scedosporium spp., Histoplasma capsulatum, Blastomyces dermatitidis and Coccidioides spp. [26,38–40]. Voriconazole exhibit the lowest MICs (MIC range: ≤0.03–0.5 µg/ml) for Scytalidium spp. in a comparative study against 32 clinical isolates of Scytalidium dimidiatum and Scytalidium hyalinum [41].

In addition, voriconazole exhibits broad-spectrum activity at a concentration of 0.1 µg/ml against dermatophytes [42]. MIC\textsubscript{90} values for Epidermophyton spp. are 1 µg/ml [43].

The in vitro activity of voriconazole against dermatophytes, Scopulariopsis brevicaulis and other opportunistic fungi as agents of onychomycosis has been evaluated [44]. Results showed a high antifungal activity of voriconazole against dermatophytes (MIC\textsubscript{90}: 0.25 µg/ml). For S. brevicaulis, the in vitro activity of voriconazole was considerably lower (MIC\textsubscript{90}: 16 µg/ml) [44].

Posaconazole
Posaconazole showed an expanded spectrum of activity against most species of Candida, Cryptococcus, Aspergillus, Zygomycetes, and other opportunistic and endemic fungal pathogens collected from medical centers worldwide [10,31,33,45–47]. From the group of triazole antifungal agents, posaconazole is the only compound for which interpretive breakpoints have not been established for Candida spp. For purposes of comparison, Pfaller et al. applied the MIC breakpoints established for voriconazole to posaconazole (susceptible: ≤1 µg/ml; resistant: ≥4 µg/ml) [48]. Some reports highlight the fungicidal action of posaconazole against a number of clinically relevant Candida species [24,49].

Susceptibility studies
Candida & Cryptococcus
The in vitro activities of posaconazole, voriconazole and fluconazole against 3932 isolates of Candida species and 237 isolates of C. neoformans obtained from over 100 medical centres worldwide during 2001 and 2002 were examined [31]. Posaconazole and voriconazole were very active against Candida species (97–98% susceptible at MICs of 1 µg/ml) and C. neoformans (98–100% susceptible at MICs of 1 µg/ml). C. albicans (MIC\textsubscript{90}: 0.015–0.03 µg/ml) was the most susceptible species of Candida to both agents and C. glabrata (MIC\textsubscript{90}: 1–2 µg/ml) was the least susceptible. These two triazoles were relatively comparable in both spectrum and potency, and exhibited an improved spectrum of activity relative to fluconazole against all Candida species [31]. In an international survey, the in vitro activities of posaconazole were compared with those of itraconazole and fluconazole against 3685 clinical isolates of Candida spp. and C. neoformans. Posaconazole was very active against all Candida spp. and C. neoformans (MIC\textsubscript{90}: 0.5 µg/ml). C. albicans was the most susceptible species of Candida (MIC\textsubscript{90}: 0.06 µg/ml) and C. glabrata was the least susceptible (MIC\textsubscript{90}: 4 µg/ml). Posaconazole was more active than itraconazole and fluconazole against all Candida spp. and C. neoformans [50].

The posaconazole MIC\textsubscript{90} for 1903 yeast isolates from France was 1 µg/ml (range: ≤0.015–8 µg/ml). A total of 90% of isolates with fluconazole MICs of 8 µg/ml or more (n = 509) and 90% of those with voriconazole MICs of 2 µg/ml or more (n = 80) were inhibited by 2 and 8 µg/ml of posaconazole, respectively.
C. neoformans isolates were highly susceptible to posaconazole, and slightly higher posaconazole MIC₉₀ values than those of voriconazole were found [51].

The MIC₉₀ values for posaconazole against 1811 clinical isolates of C. neoformans were 1 µg/ml or less [33].

In a recent study, MIC₉₀ and MIC₉₀ for posaconazole against all yeasts (n = 18,351) were 0.063 and 1.0 µg/ml or less, respectively [45].

Molds

Posaconazole also showed potent in vitro activity against a globally diverse collection of molds (n = 4499) [45]. The MIC₉₀ and MIC₉₀ values for posaconazole against all molds were 0.125 µg/ml and 1.0 µg/ml or less, respectively. Among the triazoles, posaconazole was the only agent that exhibited consistent activity against the Zygomycetes [45].

Recently, the in vitro susceptibilities of 217 Zygomycetes to posaconazole in comparison with other antifungal agents were evaluated [52]. For the Mucorales as a whole, amphotericin B was the most active antifungal agent, with the majority of strains displaying MICs near the suggested breakpoint of 1 µg/ml or less. Posaconazole appeared to be the second most active agent against various genera and species, and is an exception among the azoles. Approximately 75% of the isolates appeared to be susceptible to posaconazole [52].

Recently, the antifungal susceptibility profiles of 77 clinical strains of Mucorales species were analyzed [53]. Amphotericin B was the most active agent against all isolates, except for Cunninghamamella spp. and Apophysomyces spp. isolates. Posaconazole was the azole drug which showed the best in vitro activity. The geometric mean of the MICs was 2 µg/ml or less for all species but C. bortolatae [53]. This corroborates other reports [45, 46, 54, 55]. Posaconazole has MIC₉₀ values ranging from 1 to over 16 mg/ml against Fusarium spp. [56, 57]. Posaconazole has shown potent in vitro activity against Aspergillus spp. [58–60]. Diekema et al. examined the in vitro activity of posaconazole, caspofungin, voriconazole, ravinouconazole, itraconazole and amphotericin B against 448 recent clinical mold isolates [59]. Posaconazole, voriconazole and caspofungin were found to be more potent than amphotericin B against Aspergillus fumigatus [59].

The new triazoles and caspofungin were more potent than amphotericin B against Aspergillus terreus. Excellent activity against clinical (n = 48) and environmental (n = 31) isolates of A. terreus was observed (MIC₉₀: 0.12 µg/ml) [60].

Posaconazole showed a potent in vitro inhibitory activity against Rhizopus spp., B. dermatitidis, Coccidioides immitis, Coccidioides posadasi and H. capsulatum [58, 61–63].

Isavuconazole

Isavuconazole (BAL4815) is a promising novel broad-spectrum triazole in late-stage clinical development that has proven active in vitro against Aspergillus and Candida spp.

Susceptibility studies

Candida & Cryptococcus

Seifert et al. compared the in vitro activities of isavuconazole and five other antifungal agents against 296 Candida isolates that were recovered from blood cultures [64]. For isavuconazole, MIC₅₀/MIC₉₀ ranged from 0.002/0.004 µg/ml for C. albicans to 0.25/0.5 µg/ml for C. glabrata. Overall, on the basis of MIC₉₀ values, isavuconazole was as active as amphotericin B, itraconazole and voriconazole (each at 0.5 µg/ml) and more active than fluconazole (2 µg/ml) and fluconazole (8 µg/ml). In terms of MIC₉₀ values, isavuconazole was more active (0.004 µg/ml) than amphotericin B (0.5 µg/ml), itraconazole (0.008 µg/ml), voriconazole (0.03 µg/ml), fluconazole (0.125 µg/ml) and fluconazole (8 µg/ml) [64]. The MIC₉₀ and MIC₉₀ values for isavuconazole against Candida spp. (n = 218) were 0.25 and 1.0 µg/ml, respectively [56].

Isavuconazole was compared with six other antifungal agents against 162 C. neoformans isolates from Cuba [65]. Isavuconazole and posaconazole seem to be potentially active drugs for treating cryptococcal infections with MIC₉₀ values of 0.016 µg/ml [65].

The antifungal activity against a total of 128 Cryptococcus isolates including 86 isolates of C. neoformans (28 of serotype A, 25 of serotype D and 33 of the hybrid AD serotype) and 42 isolates of C. gattii (30 of serotype B and 12 of serotype C) was evaluated [66]. Isavuconazole, posaconazole and voriconazole demonstrated excellent potency against each isolate and serotype, including isolates with reduced fluconazole susceptibilities. The MIC₉₀ and MIC₉₀ values for isavuconazole (<0.015 and 0.06 µg/ml for C. neoformans, and 0.03 and 0.06 µg/ml for C. gattii, respectively) were lower or equivalent to the corresponding values of all other antifungals [66].

Molds

An in vitro investigation evaluated the antifungal activities of isavuconazole, voriconazole and fluconazole against relevant molds [26]. Isavuconazole and voriconazole had MIC₉₀ and MIC₉₀ values of 1 and 1 µg/ml, and 0.5 and 1 µg/ml against Aspergillus spp., respectively [26].

The in vitro activity of isavuconazole was compared with that of itraconazole, voriconazole, caspofungin and amphotericin B against 118 isolates of Aspergillus comprising four different species (A. fumigatus, A. terreus, Aspergillus flavus and Aspergillus niger) [67]. For all isolates, geometric mean MIC values and ranges (in µg/ml) were:

- Isavuconazole: 0.620 and 0.125–2.0
- Itraconazole: 0.399 and 0.063 ≥ 8.0
- Voriconazole: 0.347 and 0.125–8.0
- Caspofungin: 0.341 and 0.125–4.0
- Amphotericin B: 0.452 and 0.06–4.0

No significant differences in susceptibility to isavuconazole were seen between species and in contrast to itraconazole no isolates demonstrated MICs over 2.0 µg/ml. For all isolates, geometric mean minimum fungicidal concentration (MFC) values and ranges (in µg/ml) were:

- Isavuconazole: 1.68 and 0.25 ≥ 8.0
- Itraconazole: 1.78 and 0.06 ≥ 8.0
- Voriconazole: 1.09 and 0.25 ≥ 8.0
- Amphotericin B: 0.98 and 0.25 ≥ 4.0 [67]
Isavuconazole demonstrated potent in vitro activity against Aspergillus spp. in a study testing 118 clinical isolates of *A. fumigatus, A. flavus, A. terreus* and *A. niger* according to the CLSI method for broth dilution antifungal susceptibility testing of filamentous fungi [7].

Recently, a study showed isavuconazole to be active against a wide range of *Aspergillus* conidia and hyphae, and demonstrated activity against *A. terreus*, an amphotericin B-resistant species [68]. The average geometric means of MICs for isavuconazole against *A. fumigatus, A. flavus, A. terreus* and *A. niger* were 0.63, 0.76, 0.68 and 2.36, respectively [68]. In the latter study, isavuconazole presented limited antifungal effects against 36 Zygomycetes fungi [68].

The in vitro activity of isavuconazole was compared with those of amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole against 300 clinical isolates of *Pseudallescheria boydii, Paecilomyces lilacinus, Fusarium* spp., * Bipolaris spicifera, Curvularia lunata, Alternaria alternata, Exophiala* spp., *Rhizopus arrhizus, Mucor circinelloides, Absidia corymbifera, B. dermatitidis, H. capsulatum* and *C. poudasi* [69]. The triazoles were relatively uniform in that they showed strong in vitro inhibitory activity against most of the tested fungi. The results suggest that isavuconazole is a broad-spectrum antifungal agent, effective against a wide range of molds in vitro [69].

Isavuconazole has shown limited activity against isolates of *Sporothrix schenckii* (MIC: 2–8 µg/ml) and *Fusarium* spp. (MIC: 1–16 µg/ml) [6].

Potent activity of isavuconazole has been demonstrated against the dermatophytes *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans, Epidermophyton floccosum* and *Microsporum canis* [6]. The mean MIC of isavuconazole was 0.1 µg/ml for all strains, including six *T. rubrum* strains [6].

**Ravuconazole**

Ravuconazole is an extended-spectrum investigational triazole agent that is highly active in vitro against *Candida* spp., *C. neoformans* and other yeast species, even against the majority of fluconazole-resistant isolates of yeasts [28,70–74].

**Susceptibility studies**

Candida & Cryptococcus

The in vitro activities of ravuconazole and voriconazole were compared with those of amphotericin B, flucytosine, itraconazole and fluconazole against 6970 isolates of *Candida* spp. obtained from over 200 medical centers worldwide [28]. Both ravuconazole and voriconazole were very active against all *Candida* spp. (MIC$_{90}$: ≤0.25 µg/ml); however, a decrease in the activities of both these agents was noted among isolates that were susceptible or dose dependent (MIC: 16–32 µg/ml) and resistant (MIC: ≥64 µg/ml) to fluconazole. Moreover, over 94% of *C. krusei* isolates that were resistant to fluconazole and itraconazole were sensitive to ravuconazole (MIC$_{90}$: ≤1 µg/ml) [28].

Cuenca-Estrella *et al.* investigated the in vitro activities of ravuconazole and four other antifungal agents against 1796 clinical yeast isolates, including fluconazole-susceptible and -resistant strains [75]. Ravuconazole was active against the majority of fluconazole-resistant isolates; however, for 102 out of 562 (18%) resistant isolates, mainly *Candida tropicalis, C. glabrata* and *C. neoformans*, ravuconazole MICs were 1 µg/ml or more [78].

A total of 464 yeast isolates, including 453 bloodstream *Candida* isolates, were tested for susceptibility to ravuconazole and comparator agents [76]. Ravuconazole, posaconazole and caspofungin displayed a broad spectrum of activity against these isolates, with MICs of 1 µg/ml or lower in 92, 56 and 100% of bloodstream *Candida* isolates, respectively [76].

The antifungal susceptibilities of a large, globally diverse collection of 1811 clinical isolates of *C. neoformans* were determined [34]. Ravuconazole was very active against *C. neoformans* (99% susceptible at a MIC of 1 µg/ml) [34].

In a study with 57 strains of *C. gattii*, ravuconazole has shown activity superior to that of amphotericin B, itraconazole, ketoconazole and voriconazole against 57 strains of *C. gattii* (MIC of 0.05 µg/ml for all isolates) [77].

The in vitro activity of ravuconazole and currently marketed antifungal agents against a total of 1397 *Candida* spp. isolates was compared [32]. Ravuconazole and voriconazole demonstrated enhanced potency against *C. albicans* (MIC$_{90}$: ≤0.008 µg/ml [both]), *Candida parapsilosis* (MIC$_{90}$: 0.12 µg/ml [both]), *C. glabrata* (MIC$_{90}$: 1 µg/ml [both]), *C. tropicalis* (MIC$_{90}$: 0.12 µg/ml [both]), *C. krusei* (MIC$_{90}$: 0.5 µg/ml [both]) and other *Candida* spp. (MIC$_{90}$: 0.5 and 0.25 µg/ml, respectively) [32].

Molds

Against the Zygomycetes, ravuconazole and itraconazole were the most active azoles, with modal MICs of 0.5–2 µg/ml [78]. An International Surveillance Program of *Aspergillus* spp. reported MIC$_{90}$ values for ravuconazole of 1 µg/ml against 73 clinical isolates [32]. Cuenca-Estrella *et al.* analyzed the in vitro activities of ravuconazole against 575 clinical strains of *Aspergillus* spp. and 348 nondermatophyte non-*Aspergillus* spp. Ravuconazole was active against *Aspergillus* spp., other hyaline filamentous fungi, black molds and some *Mucorales* [74]. Species such as *Scedosporium prolificans,* *Fusarium* spp., and *Scopulariopsis* were resistant to ravuconazole and *Candida* spp. [79,80].

Ravuconazole showed very poor activity against *Fusarium* spp. and against the species of the *P. boydii* complex [79,80]. Ravuconazole showed good in vitro activity against *P. lilacinus* and poor activity against *Paecilomyces variotii* [81].

**Albaconazole**

Albaconazole (UR-9825) is a new, broad-spectrum triazole antifungal agent presently under clinical investigation. It has shown potent activity against a broad range of organisms, including pathogens resistant to other antifungals [82,83].

**Susceptibility studies**

Candida & Cryptococcus

Ramos *et al.* evaluated the in vitro activity of albaconazole, and compared it with that of fluconazole and itraconazole against 283 clinical isolates of *Candida* spp [83]. Albaconazole was more potent against *Candida* spp. than both fluconazole
Resistance to triazoles

The growing prominence of antifungal azole resistance has emerged as a serious problem in patients receiving antifungal therapy [13,90]. Extensive biochemical studies highlighted a significant diversity in the molecular mechanisms by which yeasts can become resistant toazole antifungals [13,91–93]. Since drug resistance can develop as a stepwise process over time, these mechanisms may combine with each other [10,94–96]. Resistance to azoles can result from:

• Altered cellular accumulation of azole antifungals

• Increased levels of the azole cellular target Erg11p and/or decreased affinity of azoles to Erg11p

• Ergosterol biosynthesis pathway modification [3,17]

Altered cellular accumulation of azole antifungals

The development of active efflux pumps results in decreased drug concentrations at the site of action. Two main classes of efflux pumps have been shown to be involved in antifungal resistance: the ATP-binding cassette (ABC) transport family and the major facilitator superfamily (MFS) [94,97]. Azole-resistant clinical isolates overexpress the CDR1 and CDR2 genes, which encode two homologous transporters of the ABC family, and/or the MDRI gene, which encodes a transporter of MFS [11]. Among the studies examining multiple matched azole-susceptible and -resistant sets of isolates, some isolates only overexpress MDRI, whereas others only overexpress CDR1 and CDR2 [94,98]. Upregulation of the CDR genes appears to confer resistance to multiple azoles, whereas upregulation of the MDRI gene alone leads to fluconazole resistance exclusively [98,99–101]. Evidence that these mechanisms (MDR and CDR efflux pumps) may act individually, sequentially and in concert has been derived by studying serial azole-resistant C. albicans isolates from AIDS patients with oropharyngeal candidiasis, as well as from patients with invasive diseases [93,94,96,102,103]. In vitro studies have demonstrated that the cross-resistance may occur with fluconazole and other azole compounds [104,105]. Multiple mechanisms of resistance is combined in isolates displaying high-level fluconazole resistance [95]. Cross-resistance between fluconazole and ravuconazole (and likely extended-spectrum triazoles) is such that the fluconazole MIC result may be used as a surrogate marker to predict susceptibility and resistance to ravuconazole among clinical isolates of Candida spp. [106]. The mechanisms most often involve the upregulation of genes encoding the ABC efflux transporters [94,105,107,108]. In C. glabrata, the primary mechanism of resistance to fluconazole involves upregulation of the CDR1 and CDR2 genes, resulting in resistance to multiple azoles [109]. Borst et al. observed the rapid development ofazole drug resistance (fluconazole, itraconazole and voriconazole) following in vitro exposure to fluconazole among C. glabrata isolates that had never previously been exposed toazole antifungal agents [107].

Failure to accumulate azole antifungals can also be the consequence of impaired drug influx. It is conceivable that intracellular azole penetration could be affected by the biophysical properties of the cell membrane; for example, low ergosterol levels, which may change the membrane fluidity [11,110].

and itraconazole, even against some C. albicans and C. krusei isolates with decreased susceptibility to fluconazole (MIC: 16 μg/ml) [83].

The activity of albaconazole was compared with that of fluconazole in vitro, and albaconazole was found to be very active against all 12 C. neoformans isolates tested, including an isolate for which the fluconazole MIC was 64 μg/ml [84]. The albaconazole MICs for the majority of cryptococcal isolates were between 0.0024 and 0.156 μg/ml [84].

Albaconazole and ravuconazole showed the best activities (MIC: 0.04 and 0.05 μg/ml, respectively) in a study evaluating the susceptibilities of 57 strains of C. gattii to nine antifungal agents [77]. In another study, the MICs and MFCs of albaconazole, voriconazole, and fluconazole against 55 strains of C. gattii, were assessed. Geometric mean values of 0.02 and 0.03 μg/ml for albaconazole and voriconazole, respectively, were found. MFCs were also very low for albaconazole and voriconazole (geometric mean values of 0.023 and 0.07 μg/ml, respectively) [88].

Molds

Capilla et al. compared the in vitro activities of albaconazole and those of amphotericin B against 77 strains of opportunistic filamentous fungi [40]. They included ten isolates of A. fumigatus, 11 isolates of A. flavus, 11 isolates of A. niger, ten isolates of Fusarium solani, ten isolates of P. variotii, ten isolates of P. lilacinus, ten isolates of Chaetomium globosum, two isolates of Scytalidium lignicola and three isolates of S. dimidiatum. The MIC ranges and MIC90 values were: 0.06–0.125 and 0.125 μg/ml for A. fumigatus, respectively; 0.06–0.25 and 0.25 μg/ml for A. flavus, respectively; and 0.06–0.5 and 0.5 μg/ml for A. niger, respectively. The new triazoles demonstrated excellent activity against the strains of P. variotii and P. lilacinus tested. The MIC90 was 0.125 μg/ml for both species [40]. The MIC values of both antifungals were relatively high for C. globosum; however, those of albaconazole (MIC90: 2 μg/ml) were clearly lower than those of amphotericin B (MIC90: 16 μg/ml). Albaconazole and amphotericin B were also poorly active against the two species of Scytalidium tested (MIC90 of albaconazole: >16 μg/ml for both species; MIC90 of amphotericin B for S. lignicola and S. dimidiatum: 2 and 4 μg/ml, respectively) [40].

Ortoneda et al. confirmed the generalized resistance of Fusarium spp. (MIC90: 16–32 μg/ml) against albaconazole [86]. However, an additive effect was registered for most of the Fusarium species tested when albaconazole was combined with amphotericin B [86]. Like ravuconazole, albaconazole demonstrated poor in vitro activity against the species of the P. boydii complex [79]. Albaconazole also showed activity against S. apiospermum and S. prolificans [87].

Malassezia, Trypanosoma cruzi

Albaconazole showed an in vitro profile similar to those of the different antifungals tested (MIC: 50.06 μg/ml for all the strains) against 70 strains of Malassezia spp. [88]. Albaconazole is the most potent azole derivative tested against the protozoan parasite Trypanosoma cruzi [89].
**Increased levels of the azole cellular target Erg11p and/or decreased affinity of azoles to Erg11p**

Some studies have looked at the potential of higher levels of the target enzymes or its altered regulation to confer resistance to triazoles or at least lower the susceptibility [111]. In a resistant cell, several genetic alterations have been identified that are associated with the ERG11 gene of *C. albicans*, including point mutations in the coding region, overexpression of the gene, gene amplification, and gene conversion or mitotic recombination. Mutations in other genes of the sterol biosynthesis pathway (such as ERG3) can contribute to resistance [115].

Alterations in the affinity ofazole agents for Erg11p is another important mechanism of resistance that has been described in fluconazole-resistant strains of *C. albicans* [116]. The innate resistance of *C. krusei* to fluconazole appears to be mainly due to the diminished sensitivity of the target Erg11p to inhibition by this drug [117,118]. In addition, other investigations reported that efflux pumps can contribute to *C. krusei* fluconazole resistance [119]. Evidence exists that the improved activity of the new triazoles such as voriconazole and posaconazole versus *C. krusei* is due to the increased affinity of these agents for the target compared with fluconazole [118,120].

**Ergosterol biosynthesis pathway modification**

Alteration of the ergosterol biosynthetic pathway, particularly a defect in *δ^1,5*-desaturase (encoded by the ERG3 gene), an enzyme responsible for the conversion of ergosta-7,22-dienol into ergosterol, has been described. The enzyme *Δ^5*,-desaturase is also thought to be responsible for the accumulation of the toxic metabolite 14-α-methylergosta-8,24(28)-dien-3b,6a-diol in the fungal membrane when yeast cells are exposed to triazoles, and therefore the inactivation of this gene suppresses toxicity and causesazole resistance [9,121].

In *A. fumigatus*, azole drug resistance has been described for both laboratory mutants and clinical strains, and has mainly been attributed to alterations in the target enzyme (Cyp51a) [122,123]. Targeted disruption of the *CYP51A* gene in different icalonazole-resistant *A. fumigatus* strains resulted in strains with similar patterns of decreased susceptibility toazole drugs, confirming that Cyp51a is the target of azole antifungals [123]. A study of five clinical *A. fumigatus* isolates exhibiting reduced susceptibility to itraconazole and other triazole drugs revealed that all of the five strains harbored mutations in CYP51A, resulting in the replacement of methionine at residue 2 by valine, lysine or threonine [124]. A new *A. fumigatus* resistance mechanism conferring in vitro cross-resistance toazole antifungals has been described, where mutations in the promoter region of CYP51A lead to overexpression of the protein product [125]. Likewise, Verweij et al. observed the emergence of multiple triazole resistance in *A. fumigatus* isolates that were highly resistant to itraconazole, and showed elevated MICs of voriconazole, posaconazole and the experimental azole rauconazole [125,126].

Recently, Nascimento et al. found that in addition to a mutation at G54 in the CYP51A target, itraconazole-resistant isolates of *A. fumigatus* also exhibited high-level expression of two genes, MDR3 and MDR4, which encode for drug efflux pumps [122].

**Echinocandins**

The echinocandins are synthetically modified lipopeptides, originally derived from the fermentation broths of various fungi [127]. They include: caspofungin, derived from *fungicandin B* and produced by *Gliarrowzezys* [128]; micafungin derived from *echinocandin B* and produced by *Coleosophora empeyi* [129]; and anidulafungin derived from *echinocandin B* and produced by *Aspergillus nidulans* [130].

Caspofungin was the first agent of the echinocandin class. It was approved by both the FDA and the EMEA in 2001. Its therapeutic indications include the empirical therapy of presumed fungal infections in febrile, neutropenic adult patients, the treatment of invasive aspergillosis in adult patients whose disease is refractory to, or who are intolerant of, other antifungal agents, and the treatment of candidemia and some specific Candida infections (intra-abdominal abscesses, peritonitis, pleural cavity infections and oesophagitis).

Micafungin and anidulafungin share with caspofungin an identical spectrum of *in vitro* activity against *C. albicans*, non-*albicans* species of *Candida* and *Aspergillus* species, as well as several but not all pathogenic molds. Micafungin was approved by the FDA in 2005 and the EMEA in 2008, while anidulafungin was approved by the FDA in February 2006. Both echinocandins are indicated for the treatment of candidemia and some specific Candida infections (intra-abdominal abscesses, peritonitis, pleural cavity infections and oesophagitis). Since 2008, micafungin has also been approved for the prophylaxis of *Candida* infections in patients undergoing hematopoietic stem cell transplantation.

Micafungin has been evaluated as a salvage therapy for invasive aspergillosis, but remains investigational for this indication [134].

**Mode of action of echinocandins**

Echinocandin drugs inhibit the 1,3- and 1,6-d-glucan synthase [122]. Glucan synthase is an enzyme complex that is involved in the synthesis of 1,3-β-d-glucan, a glucose polymer crucial to the structure and integrity of the cell wall of several common fungal pathogens [132]. Glucan synthase is thought to contain a catalytic subunit, encoded by the three homologous genes FKS1, FKS2 and FKS3, and a regulatory subunit, the small GTPase Rholp. FKS1 and FKS2 encode a pair of integral membrane proteins with 16 predicted transmembrane domains that share 88% identity. The product of the third gene, FKS3, is 72% identical to Fks1p and Fks2p [133].

Fks1p and Fks2p are related proteins thought to be the target of the echinocandin drugs. Specific mutations of *C. albicans* Fks1 and of *C. glabrata* Fks2 as a potential basis for reduced echinocandin susceptibility have been demonstrated [134,135]. The two catalytic subunits have different roles but can partially substitute for each other. Disruption of both FKS1 and FKS2 is lethal [133]. Deletion of FKS1 leads to a decrease in β-glucan and an increase in chitin and mannoprotein levels in the cell wall [136]. Deletion of FKS2 causes no obvious cell wall defect, although the fks1 fks2 double mutant is nonviable, which suggests that in vegetative growth, Fks1p and Fks2p are alternative subunits with essentially...
overlapping functions [133]. The role of FKS3 remains unknown, but a fks3-null mutant has no apparent cell wall defects or genetic interactions with FKS1 or FKS2 [137].

Rho1p acts as a molecular switch that monitors and receives upstream signals of cell morphogenesis [138]. Changes in 1,3-β-D-glucan synthesis result in changes leading to osmotic instability and lysis of the fungal cell [10,127,139]. The proportion of the fungal cell wall of glucan varies widely between different species of fungi, while Zygomycetes lack this target component [140]. However, these characteristics do not always predict echinocandin activity, thus additional mechanisms of action should be considered [4,141].

**Antifungal spectrum & activity of echinocandins**

*In vitro* studies have shown echinocandins to be effective against a wide range of fungal pathogens, including yeasts and mold. [106,139,142–145]. The echinocandins appear to have cidal activity against *Candida* spp. and static activity against *Aspergillus* spp. [132].

Recently, the CSLI Antifungal Subcommittee has recommended a susceptibility breakpoint of 2 µg/ml or less for anidulafungin, caspofungin and micafungin, while designating all isolates with a MIC over 2 µg/ml as nonsusceptible [144].

**Susceptibility studies**

*Candida*

A summary of *in vitro* activity by the echinocandins against *Candida* spp. is provided in Table 1.

Anidulafungin, caspofungin and micafungin appear to have good antifungal activity for most isolates of *Candida* spp., including those that are either amphotericin B-resistant or fluconazole- and itraconazole-resistant, such as *C. glabrata* [146]. All agents have higher MIC values against *C. parapsilosis* and *Candida guilliermondii* than the MICs against other species of *Candida*. Echinocandins have no activity against *C. neoformans*, *Trichosporon* species and Zygomycetes organisms [146].

The *in vitro* activity of micafungin against 2656 invasive isolates of *Candida* spp. collected from 60 medical centers worldwide in 2004 and 2005 have been studied [147]. Overall, micafungin was very active against *Candida* (MIC50/MIC90: 0.015/1 µg/ml; 96% inhibited at a MIC of ≤1 µg/ml, 100% inhibited at a MIC of ≤2 µg/ml) and comparable to caspofungin (MIC50/MIC90: 0.03/0.25 µg/ml; 99% inhibited at a MIC of ≤2 µg/ml) [147].

An international collection of 8197 isolates of *Candida* spp. obtained from 91 medical centers between 2001 and 2004 was tested for susceptibility to caspofungin [148]. The MIC distributions generated for 8197 clinical isolates of *Candida* spp. provide a robust data set for both common and uncommon species of *Candida* and reveal two important findings. First, isolates for which caspofungin MICs exceeded 1 µg/ml rarely occurred in clinical situations. Only 25 (12 *C. parapsilosis*, six *C. guilliermondii*, two *C. rugosa*, and one each of *C. albicans*, *C. glabrata*, *C. krusei*, *Candida lusitaniae* and *C. tropicalis*) out of 8197 (0.3%) clinical isolates exhibited decreased susceptibilities to caspofungin with MICs of 2 µg/ml or more. Second, the MIC distributions identified two broad groups among the nine different species tested. The most susceptible species (MIC90: 0.03–0.06 µg/ml) were *C. albicans*, *C. glabrata*, *C. tropicalis*, *Candida kefyr* and *Candida pelliculosa*, whereas *C. parapsilosis* (MIC90: 0.5 µg/ml), *C. guilliermondii* (MIC90: 1 µg/ml), *C. krusei* (MIC90: 0.5 µg/ml) and *C. lusitaniae* (MIC90: 0.5 µg/ml) were all significantly less susceptible to caspofungin [148].

The results of the *in vitro* activities of anidulafungin, caspofungin and micafungin against 5346 invasive isolates of *Candida* spp. collected from over 90 medical centers worldwide demonstrate the excellent spectrum and potency of all three echinocandins. More than 99% of 5346 invasive isolates of *Candida* spp. were inhibited by 2 µg/ml or less of all three echinocandins [145]. These results confirm data from another antifungal susceptibility survey of 2000 bloodstream *Candida* isolates [104]. The slight differences in potency *in vitro* observed in both studies among the three echinocandins for given species of *Candida* have been shown to be normalized by the addition of serum to the *in vitro* test system [149].

A total of 1038 yeast isolates, mostly (84%) recovered from blood or sterile sites and consecutively received at the French National Reference Center for Mycoses and Antifungals in 2005–2006, were prospectively analyzed for their *in vitro* susceptibilities to caspofungin and micafungin [150]. The most susceptible species (MIC90: ≤1 and ≤0.125 µg/ml for caspofungin and micafungin, respectively) were *C. albicans*, *C. glabrata* and *C. tropicalis*, whereas *C. parapsilosis*, *C. guilliermondii* and *C. krusei* exhibited higher MICs (MIC90: ≥2 and ≥0.25 µg/ml for caspofungin and micafungin, respectively) [150].

MIC90 values of 0.06 µg/ml were found for micafungin as well as anidulafungin against *Candida* species recovered from Canadian intensive care unit patients [151].

Caspofungin, micafungin and anidulafungin demonstrated potent *in vitro* activity against all invasive *Candida* isolates except *C. parapsilosis* and *C. guilliermondii*, which were less susceptible to the echinocandins (MIC90: 1–2 µg/ml) [152]. Anidulafungin was quite active against 2500 *Candida* species (MIC90: ≤2 µg/ml). *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. kefyr* were the most susceptible species of *Candida* (MIC90: 0.06–0.12 µg/ml), and *C. parapsilosis*, *C. lusitaniae* and *C. guilliermondii* were the least susceptible (MIC90: 0.5–2 µg/ml). Notably, 100% of *C. glabrata* and *C. krusei* isolates were inhibited by 0.25 µg/ml of anidulafungin [143].

In another study, anidulafungin was very active against *Candida* spp. isolates (MIC90: ≤0.5 µg/ml), except *C. parapsilosis* (MIC90: 4 µg/ml) and two *C. guilliermondii* isolates (MIC: ≥32 µg/ml) [153].

The *C. parapsilosis* isolates recovered from a burn unit were susceptible to anidulafungin, but were less so to caspofungin and micafungin [154].

An *in vitro* investigation evaluated the antifungal activities of anidulafungin, itraconazole and amphotericin B against 156 fluconazole-resistant clinical isolates of *Candida* spp. Anidulafungin was more potent than either itraconazole or amphotericin B against *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis*, even against isolates with itraconazole MICs of 1 µg/ml or less. Anidulafungin was less potent *in vitro* against *C. parapsilosis* and *C. guilliermondii* isolates [155].
Out of 880 clinical yeast isolates, anidulafungin was more active when compared with fluconazole and itraconazole for *C. albicans* (MIC$_{90}$: 0.06 µg/ml), *C. tropicalis* (MIC$_{90}$: 0.06 µg/ml), *C. glabrata* (MIC$_{90}$: 0.12 µg/ml), *C. krusei* (MIC$_{90}$: 0.06 µg/ml) and *C. lusitaniae* (MIC$_{90}$: 1 µg/ml), as well as the less-often encountered yeast species. Anidulafungin was less active against *C. parapsilosis*, *C. guilliermondii* and *Candida famata* (MIC$_{50}$: 1–2 µg/ml) [156].

Even though the MIC values for *C. parapsilosis* tend to be higher than the MIC values for other species of *Candida*, this has not led to clinically significant differences in outcomes [157].

Aspergillus & Fusarium

*In vitro* and at clinically relevant concentrations, the echinocandins do not usually cause complete inhibition of *Aspergillus* growth but induce morphological hyphal changes. The minimum effective concentration (MEC), defined as the lowest drug concentration at which short, stubby and highly branched hyphae are observed, has been introduced for the determination of echinocandin activity against *Aspergillus* spp. *in vitro* [156,158,159]. This method requires considerable practice since there is a risk that drug dilutions in the microdilution trays could dry out when testing slow-growing molds. Anidulafungin also exhibited excellent activity against 68 *Aspergillus* spp. (MEC$_{90}$: ≤0.03 µg/ml) [156].

### Table 1. *In vitro* activity of echinocandins against *Candida* spp.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Antifungal agent</th>
<th>Isolated (n)</th>
<th>MIC$_{90}$ (µg/ml)</th>
<th>MIC range (µg/ml)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>Anidulafungin</td>
<td>9648</td>
<td>0.01–0.12</td>
<td>≤0.0002–4</td>
<td>[104,143,145–147,151–153,155,156]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>14,251</td>
<td>0.06–1</td>
<td>0.007 ≥ 8</td>
<td>[104,145–148,150,152]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>5313</td>
<td>0.03–0.5</td>
<td>0.007–1</td>
<td>[104,145,146,150,151]</td>
</tr>
<tr>
<td><em>Candida dubliniensis</em></td>
<td>Anidulafungin</td>
<td>110</td>
<td>0.03–4</td>
<td>0.03–8</td>
<td>[104,146]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>195</td>
<td>0.05</td>
<td>0.01–1</td>
<td>[104,146]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>58</td>
<td>0.03–0.5</td>
<td>0.03–1</td>
<td>[104,146]</td>
</tr>
<tr>
<td><em>Candida famata</em></td>
<td>Anidulafungin</td>
<td>35</td>
<td>2–8</td>
<td>0.01 ≥ 16</td>
<td>[145,146]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>37</td>
<td>1–4</td>
<td>0.06 ≥ 8</td>
<td>[145,146]</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>Anidulafungin</td>
<td>2607</td>
<td>0.03–8</td>
<td>≤0.008–8</td>
<td>[104,143,145,146,151–153]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida guillermondii</em></td>
<td>Anidulafungin</td>
<td>177</td>
<td>2–4</td>
<td>0.06–4</td>
<td>[143,145,146,152]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>430</td>
<td>1–2</td>
<td>0.03 ≥ 8</td>
<td>[145–148,150,152]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>189</td>
<td>1–2</td>
<td>0.015–2</td>
<td>[145–147,152]</td>
</tr>
<tr>
<td><em>Candida kefyr</em></td>
<td>Anidulafungin</td>
<td>73</td>
<td>0.12–0.5</td>
<td>0.007–0.5</td>
<td>[143,145,146]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>140</td>
<td>0.015–0.5</td>
<td>0.007–1</td>
<td>[145–148]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>79</td>
<td>0.06–0.25</td>
<td>0.015–0.5</td>
<td>[145–147,150]</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>Anidulafungin</td>
<td>414</td>
<td>0.06–0.5</td>
<td>≤0.01–8</td>
<td>[104,145,146,151–153]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>729</td>
<td>0.25–2</td>
<td>0.015–4</td>
<td>[104,145–148,150]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>310</td>
<td>0.12–0.25</td>
<td>0.015–4</td>
<td>[104,145–147,150]</td>
</tr>
<tr>
<td><em>Candida lusitaniae</em></td>
<td>Anidulafungin</td>
<td>204</td>
<td>0.12–8</td>
<td>0.03 ≥ 8</td>
<td>[104,143,145,146,152]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>344</td>
<td>0.25–2</td>
<td>0.015–4</td>
<td>[104,145–148,150,152]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>148</td>
<td>0.06–2</td>
<td>0.03–2</td>
<td>[104,145–147,150,152]</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>Anidulafungin</td>
<td>1821</td>
<td>0.13–8</td>
<td>0.01 ≥ 8</td>
<td>[104,143,145,146,151–153]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>3884</td>
<td>0.5–4</td>
<td>0.007 ≥ 8</td>
<td>[104,145–148,150,152]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>2145</td>
<td>0.06–8</td>
<td>0.015 ≥ 8</td>
<td>[104,145–147,150,152]</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>Anidulafungin</td>
<td>1867</td>
<td>0.06–2</td>
<td>≤0.003–32</td>
<td>[104,143,145,151–153]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>2505</td>
<td>0.06–1</td>
<td>0.007 ≥ 8</td>
<td>[104,145,148,150,152]</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>1902</td>
<td>0.06–2</td>
<td>0.007 ≥ 8</td>
<td>[104,145,146,150–152]</td>
</tr>
</tbody>
</table>

MIC: Minimum inhibitory concentration.
In a recent study, the susceptibilities of 11 A. fumigatus, eight A. terreus and eight A. flavus isolates to caspofungin, micafungin and anidulafungin were studied by a CLSI M38-A broth microdilution-based method [160,161]. All echinocandins therefore exerted comparable levels of maximal metabolic inhibition against Aspergillus spp. at concentrations that were differentially increased for germinated versus nongerminated conidia [160].

Caspofungin generated low MICs and MECs against Aspergillus, but not for Fusarium. While MICs increased inconsistently when the incubation time was prolonged, MEC appeared as a stable and potentially relevant end point in testing in vitro caspofungin activity [162].

In a comparative study, anidulafungin and several fungal-focused agents were tested against a total of 68 strains of Aspergillus spp. [163]. Anidulafungin was the most active antifungal agent tested, with a MIC of 0.03 µg/ml or less [163].

No differences in susceptibility patterns were observed between clinical (n = 48) and environmental (n = 31) isolates of A. terreus against echinocandins [60]. Caspofungin showed higher MECs (MEC90: 2 µg/ml) than anidulafungin (MEC90: 0.03 µg/ml) and micafungin (MEC90: 0.02 µg/ml) [60].

Resistance to echinocandins
Clinical exposure to echinocandins is growing, and the development of full resistance or reduced susceptibility to echinocandins appears to be a rare event [1,132].

It has been shown that the acquired echinocandin resistance in susceptible fungi is due to mutations within highly conserved regions of FKS1 and FKS2, genes encoding subunits of the glucan synthase enzyme complex [164–170]. Specific mutations in FKS1 genes from Saccharomyces cerevisiae and C. albicans mutants are described [166]. Amino acid substitutions Fe639I, V641K and S645F, S645P and S645Y (C. albicans) helped define a region (CaFKS1 Phe641–Pro649), the so-called ‘hot-spot 1’, which confers reduced susceptibility to caspofungin [166]. Most of the mutations were between 641 and 648 of the Fks1p, and most common among these was the mutation Ser645 [166]. A reduced susceptibility to caspofungin in strains of C. albicans is associated with mutations in FKS1 at codon 645 in which serine is replaced by proline, tyrosine or phenylalanine [135]. The analysis of a C. albicans strain (MIC ≥8) in a patient with recurrent esophagitis revealed that it contained a serine-to-proline substitution at position 645 in the FKS1 gene [168]. In addition, new mutations in FKS1 were discovered. Among clinical isolates of C. albicans harboring mutations, six patterns were observed involving amino acid changes at positions 641, 645, 649 and 1358. [171]. Notably, Hakki et al. reported a strain of C. krusei from a leukemic patient that displayed reduced susceptibilities to echinocandin drugs [71,172]. The strain emerged during therapy with caspofungin and was subsequently shown to contain a heterozygous mutation, T80K, in the FKS1 gene, resulting in altered sensitivity of the glucan synthase enzyme complex to inhibition by echinocandin drugs [167].

Mutations in FKS1 and FKS2 have also been linked with echinocandin resistance in C. glabrata [169].

Despite a report suggesting that overexpression of CDR2 efflux can result in increased MICs to echinocandins [173], other authors refuted the efflux pumps as a possible echinocandin resistance mechanism. In a comprehensive study, overexpression of C. albicans Cdr1p, Cdr2p or Mdr1p does not produce significant changes in echinocandin susceptibility [174]. A recent survey of 351 fluconazole-resistant Candida isolates revealed that the organisms are inhibited by caspofungin at standard MIC90 doses [175]. A dual-uptake model is proposed to account for caspofungin transport. At low drug levels at or below approximately 1 µg/ml, a high-affinity facilitated-diffusion carrier is suggested to mediate drug uptake into the cell. At higher drug levels, nonspecific drug uptake could occur through normal diffusion pathways across the bilayer of the plasma membrane. Disruption of this system represents a potential mechanism of resistance [176]. Different resistance mechanisms have been suggested for other fungal organisms. Overexpression of Sbe2p, a Golgi protein that is involved in the transport of cell wall components, may confer resistance to caspofungin in S. cerevisiae [177].

Two reports provide evidence that the modification of Fks1p in A. fumigatus is necessary and sufficient to confer resistance to echinocandin drugs [178,179]. Moreover, there is another report of reduced susceptibility to caspofungin in a clinical A. fumigatus isolate with increased expression of the FKS1 gene [180]. In addition, C. neoformans and the emerging fungal pathogens Fusarium spp., Scedosporium spp. and members of the Zygomycetes family are intrinsically resistant to echinocandins [181]. The mechanism of this inherent resistance in not related to echinocandin target alteration. Echinocandin resistance in C. neoformans is intriguing, given that the gene encoding the 1,3-β-D-glucan synthase FKS subunit is essential for C. neoformans growth and that 1,3-β-D-glucans are found in the cell wall. Malige et al. demonstrated that C. neoformans is resistant to caspofungin by a mechanism unrelated to 1,3-β-D-glucan synthase resistance [4].

Conclusion
Both the newer triazoles and echinocandins represent a significant advance in the treatment of serious fungal infections. The new triazoles enjoy a very broad spectrum of target fungal species.

Voriconazole is active against most Candida and Aspergillus spp. It has significant activity against S. apiopermum and Fusarium spp. Voriconazole is also active in vitro against the endemic fungi (Coccidioides spp., B. dermatitidis and H. capsulatum), as well as against C. neoformans and Trichosporon spp. Posaconazole has a wide range of antifungal activity, which includes Candida spp. resistant to older azoles, C. neoformans, Aspergillus spp., Rhizopus spp., B. dermatitidis, C. immitis, H. capsulatum, and other opportunistic filamentous and dimorphic fungi.

A major difference between posaconazole and voriconazole is that posaconazole has activity against Zygomycetes including Mucor spp., Rhizopus spp. and Cunninghamella spp., and voriconazole has no activity against this class of fungi.

Ravuconazole shows a broad spectrum of in vitro activity against a wide range of fungi, including Candida spp., C. neoformans, Aspergillus spp., H. capsulatum and C. immitis.
Isavuconazole has potent *in vitro* activity against *Candida* spp. and *Aspergillus* spp., including *A. fumigatus*, *A. flavus*, *A. terreus* and *A. niger*.

Albaconazole has also been reported to have excellent activity against species of *Candida*, *Cryptococcus* and *Aspergillus*. However, the role of the newer triazoles (isavuconazole, ravuconazole and albaconazole) that are currently under development will be clarified over the next years.

Given that theazole antifungals share a common mechanism of action and, in most cases, of resistance, a concern for the development of cross-resistance among the azoles has been indicated by many authors. Of note, some studies indicate that multiple mechanisms of resistance are combined in isolates displaying high-level fluconazole resistance. The cross-resistance between fluconazole and ravuconazole has been observed with *C. glabrata* and other *Candida* spp.

The echinocandins have a broad spectrum of activity and are similar to each other with respect to *in vitro* activity against *Candida* spp., with micafungin and anidulafungin having similar MICs that are generally lower than those of caspofungin. All agents have higher MIC values against *C. parapsilosis*, *C. krusei* and *C. guilliermondii* than the MICs against other species of *Candida*. The intrinsic reduced *in vitro* activity reported here for *C. parapsilosis* and *C. guilliermondii* is unclear, since patients infected with these two species have responded to echinocandin therapy. The echinocandins demonstrate similar *in vitro* activity against *Aspergillus* spp., but only caspofungin is approved for treatment in patients who are intolerant of or refractory to other therapies. However, testing the *in vitro* activity of echinocandins against *Aspergillus* spp. is complicated, and it is unclear how MEC or MIC correlates with echinocandin treatment outcome. There is ongoing research to standardize susceptibility testing for the echinocandins, and caution must be used when drawing clinical conclusions from the available methods.

Other difficult-to-treat fungi such as *C. neoformans*, *Fusarium* spp., *Trichosporon* spp. and Zygomycetes organisms are intrinsically resistant.

At present, resistance to the echinocandins is rare among clinical isolates and it is associated with point mutations in the FKS1 gene of the glucan synthase enzyme complex.

**Expert commentary**

This review focuses on the mode of action of the new antifungal triazole generation and echinocandins. Additionally, the representative literature on the *in vitro* activity, and mechanisms of resistance to triazoles and echinocandins in fungal pathogens is reported.

Voriconazole and posaconazole are relatively comparable in both spectrum and potency, and exhibited an improved spectrum of activity relative to fluconazole against all *Candida* species. Voriconazole is generally the most active of the triazoles against *S. apiospermum* followed by posaconazole. Comparing posaconazole and voriconazole against *Aspergillus* spp. and other filamentous fungi (including *Fusarium, Rhizopus* and *Mucor* spp.), posaconazole was the most active agent. A major difference between posaconazole and voriconazole is that posaconazole has activity against Zygomycetes, while voriconazole has no activity against this class of fungi.

The role of the newer triazoles that are currently under development (isavuconazole, ravuconazole and albaconazole) will also be clarified over the next years. Currently, these agents appear to be effective against a broad range of organisms, including pathogens resistant to other antifungals.

Given the shared mechanisms of action and resistance within theazole class, concerns regarding the development of cross-resistance are understandable. Based on *in vitro* studies, it appears that cross-resistance may be important in opportunistic yeast and molds. *In vitro* studies have demonstrated that cross-resistance may occur with fluconazole and otherazole compounds. Multiple mechanisms of resistance are combined in isolates displaying high-level fluconazole resistance. Cross-resistance between fluconazole and ravuconazole (and likely extended-spectrum triazoles) is such that the fluconazole MIC result may be used as a surrogate marker to predict susceptibility and resistance to ravuconazole among clinical isolates of *Candida* spp. The *in vitro* cross-resistance between posaconazole, voriconazole and ravuconazole is also well described for *A. fumigatus*, and the primary mechanisms of resistance involves point mutations in the target enzyme. Data from *in vitro* susceptibility studies support the use of voriconazole and posaconazole in the treatment of a number of invasive fungal infections.

The three echinocandin drugs discussed herein share target, spectrum, *in vitro* potency and mechanism of resistance. Unlike the azoles, the echinocandins have cidal activity against *Candida* species and static activity against *Aspergillus* species. On the basis of the *in vitro* studies, the spectrum of echinocandins activity is limited to the treatment of invasive *Candida* and *Aspergillus* infections. Fortunately, the echinocandins have proven to be worthy options in the treatment of azole-resistant *Candida* infections. Acquired echinocandin resistance is rare. On the other hand, the intrinsic echinocandin resistance of yeast genera such as *C. parapsilosis*, *Rhodotorula* and *Trichosporon* spp., molds of the genus *Fusarium*, and members of the Zygomyces family, represents a major limitation to echinocandins in clinical use.

**Five-year view**

The availability of new antifungal drugs with improved efficacy and safety profiles represents an attractive strategy for the treatment and prophylaxis of infections against current and emerging fungal pathogens, such as *Aspergillus* species and *Zygomycetes*. Both echinocandins and new triazole derivatives have characteristics that render them potentially suitable agents against some resistant fungi. Antifungal susceptibility testing *in vitro* is assuming an increasing role in antifungal drug selection, as a means of tracking the emergence of antifungal resistance. The introduction of echinocandins is an important advance in the treatment of systemic fungal infections by reducing the problems of cross-resistance to azoles. Intrinsic reduced echinocandin *in vitro* activity has been reported for *C. parapsilosis*, *C. lusitaniae* and *C. guilliermondii*; however, whether this will affect clinical outcomes need to be clarified in the future.
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Key issues

- As recent epidemiologic trends have confirmed the increasing importance of infections caused by resistant fungal species, there is a need to deal with the most recent antifungal agents, triazoles and echinocandins, which have been added to antifungal armamentarium.
- In vitro susceptibility results may improve and promote strategies to prevent the emergence and spread of antifungal-resistant organisms.
- Voriconazole and posaconazole, belonging to the second-generation triazole agents, have a very broad spectrum of antifungal activity that includes Candida species, and filamentous and dimorphic fungi.
- The use of fluconazole as a surrogate marker for resistance to recent triazoles is an important issue that must be understood in order to provide optimal therapy for infected patients.
- Posaconazole is the broadest spectrum azole to date. Cross-resistance of posaconazole with fluconazole is uncommon.
- Posaconazole is the only licensed azole with activity against Zygomycetes.
- All echinocandins have an excellent activity against a wide range of fungal pathogens, including yeasts and molds, among them several Aspergillus spp.
- The intrinsic echinocandin resistance of yeast genera such as Cryptococcus, Rhodotorula and Trichosporon spp., molds of the genus Fusarium, and members of the Zygomycetes family represents a major limitation to the clinical use of echinocandins.

References

Papers of special note have been highlighted as:

- of considerable interest

A global evaluation of voriconazole activity

Diekema DJ, Messer SA, Hollis RJ


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**Evaluated fungal efflux-mediated drug resistance. A possible strategy to overcome effects due to efflux upregulation is envisaged.**


Described the analysis of a new molecular mechanism responsible for a phenotype of *Aspergillus fumigatus* cross-resistance to azole drugs.

**The prevalence of azole resistance in *A. fumigatus* isolates that were resistant to itraconazole is investigated.**


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The Clinical and Laboratory Standards Institute Antifungal Subcommittee utilized this study to propose interpretative breakpoints for the MIC testing of anidulafungin, caspofungin and micafungin against Candida species.


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