
History of the development of azole derivatives

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ABSTRACT

Until the 1940s, relatively few agents were available for the treatment of systemic fungal infections. The development of the polyene antifungals represented a major advance in medical mycology. Although amphotericin B quickly became the mainstay of therapy for serious infections, its use was associated with infusion-related side-effects and dose-limiting nephrotoxicity. The continued search for new and less toxic antifungals led to the discovery of the azoles several decades later. Ketoconazole, the first available compound for the oral treatment of systemic fungal infections, was released in the early 1980s. For almost a decade, ketoconazole was regarded as the drug of choice in nonlife-threatening endemic mycoses. The introduction of the first-generation triazoles represented a second major advance in the treatment of fungal infections. Both fluconazole and itraconazole displayed a broader spectrum of antifungal activity than the imidazoles and had a markedly improved safety profile compared with amphotericin B and ketoconazole. Despite widespread use, however, these agents became subject to a number of clinically important limitations related to their suboptimal spectrum of activity, the development of resistance, the induction of hazardous drug–drug interactions, their less than optimal pharmacokinetic profile (itraconazole capsules), and toxicity. In order to overcome these limitations, several analogues have been developed. These so-called ‘second-generation’ triazoles, including voriconazole, posaconazole and ravuconazole, have greater potency and possess increased activity against resistant and emerging pathogens, in particular against *Aspergillus* spp. If the toxicity profile of these agents is comparable to or better than that of the first-generation triazoles and drug interactions remain manageable, then these compounds represent a true expansion of our antifungal arsenal.

Keywords Antifungal, azole, overview

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INTRODUCTION

Despite the implementation of several preventive measures and the use of antifungal chemoprophylaxis, physicians have witnessed an increased incidence of both mucosal and invasive fungal infections during the past two decades [1–4]. This increase is linked with progress in medical technology and novel therapeutic options and appears to be multifactorial. The widespread use of quinolone prophylaxis in neutropenic cancer patients and the availability of broad-spectrum antibacterial agents has virtually eliminated early

death due to bacterial sepsis, thereby setting the stage for fungal colonisation and putting patients at risk for subsequent mycotic infections. Medical procedures have become more invasive and aggressive; the accompanying disruption of protective anatomical barriers as a result of indwelling catheters, therapy-induced mucositis, viral infections, and graft-versus-host disease, or following major abdominal surgery or associated with extensive burns, allows fungi to reach normally sterile body sites [5]. In addition, the community of vulnerable patients is continuously expanding as a result of the spread of human immunodeficiency virus (HIV) infections, the increased use of (novel) immunosuppressive drugs in autoimmune disorders and to prevent or treat rejection in the expanding area of transplant medicine, the popularity of dose-escalated, often myelo-ablative cytotoxic therapy, the

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improved survival rate in premature infants, and the availability of sophisticated life-saving medical techniques [5–7]. Unfortunately, the attributable mortality rate of (systemic) fungal infections remains high [8,9]. This may partly be explained by the difficulty of diagnosing these infections at an early stage of their development, because definite proof often requires time-consuming and labour-intensive approaches that cannot always be achieved in these severely ill patients. However, an additional explanation may be found in shortcomings of the current antifungal armamentarium.

Clearly, progress in the development of new antifungals has lagged behind antibacterial research, a fact that can be explained by at least two factors. First, before the HIV-era, the occurrence of fungal infections was believed to be too low to warrant aggressive research by the pharmaceutical industry. Second, the 'apparent' lack of a highly selective fungal target, not present in other eukaryotic (including mammalian) cells, precluded the development of new agents. Until recently, the arsenal that was available for the treatment of systemic fungal infections was limited in number and consisted mainly of the polyene antibiotic amphotericin B, some azole derivatives, the allylamines–thiocarbamates and 5-flucytosine. With the exception of 5-flucytosine, all other agents acted by interfering with the structural or functional integrity of the fungal plasma membrane, either by physical disruption or by blocking the biosynthesis of membrane sterols. The past decade, however, has witnessed an expansion of basic and clinical research in antifungal pharmacology and many companies have launched new compounds, including several new azole compounds and the candins [5].

MECHANISM OF ACTION

For a detailed discussion of the mechanism of action, the reader is referred to original work by Vanden Bossche *et al.* [10–12] and a recent review article by White *et al.* [13]. Azole antifungals are divided into the imidazoles (e.g. miconazole and ketoconazole) and the triazoles (e.g. itraconazole, fluconazole, voriconazole). The latter group has three instead of two nitrogen atoms in the azole ring. All of the azoles operate via a common mode of action: they prevent the synthesis of ergosterol, the major sterol component of fungal plasma

membranes, through inhibition of the fungal cytochrome P450-dependent enzyme lanosterol 14- α -demethylase. The resulting depletion of ergosterol and the concomitant accumulation of 14- α -methylated precursors interferes with the bulk function of ergosterol in fungal membranes and alters both the fluidity of the membrane and the activity of several membrane-bound enzymes (e.g. chitin synthase). The net effect is an inhibition of fungal growth and replication. In addition, a number of secondary effects, such as inhibition of the morphogenetic transformation of yeasts to the mycelial form, decreased fungal adherence, and direct toxic effects on membrane phospholipids, have been reported [14].

Unfortunately, as a result of the nonselective nature of the therapeutic target, cross-inhibition of P450-dependent enzymes involved in mammalian biosynthesis has been responsible for some toxicity, although significantly lower and less severe with fluconazole, itraconazole and voriconazole than with the older compounds. The improved toxicity profile of the triazoles compared to the imidazoles (especially endocrine-related side-effects) can be explained by their greater affinity for fungal rather than mammalian P450-enzymes at therapeutic concentrations [15].

HISTORY OF AZOLES

Although the first report of antifungal activity of an azole compound, benzimidazole, was already described in 1944 by Woolley, it was not until after the introduction of topical chlormidazole in 1958 that researchers became interested in the antifungal activity of azole compounds [16]. In the late 1960s, three new topical compounds were introduced: clotrimazole, developed by Bayer Ag (Germany), and miconazole and econazole, both developed by Janssen Pharmaceutica (Belgium) [17].

The in-vitro activity of clotrimazole against dermatophytes, yeasts, and dimorphic as well as filamentous fungi, is well-established and comparable to that of amphotericin B for many pathogens [18]. However, unacceptable side-effects following oral administration [19] and unpredictable pharmacokinetics as a result of the induction of hepatic microsomal enzymes [20] have limited the use of clotrimazole to the topical treatment of dermatophytic infections and superficial candida infections, including oral thrush and vaginal candidiasis.

Miconazole, a phenethyl imidazole synthesised in 1969, was the first azole available for parenteral administration (although not before 1978). Like other azoles, it interferes with the biosynthesis of fungal ergosterol, but at high concentrations, miconazole may also cause direct membrane damage that results in leakage of cell constituents. The drug has a limited spectrum of activity including dermatophytes, *Candida* species, dimorphic fungi, and *Pseudallescheria boydii*. The agent has proven to be an effective topical antifungal agent, but toxicity associated with the vehicle used for intravenous administration has limited its parenteral use [21], although it has been used successfully in the treatment of systemic candida infections, pseudallescheriasis and some refractory cases of cryptococcal meningitis [22,23]. Miconazole has recently been withdrawn from the market.

In 1981, the Food and Drug Administration (FDA) approved the systemic use of ketoconazole, an imidazole derivative synthesised and developed by Janssen Pharmaceutica [24]. For almost a decade it would be regarded as the standard and was the only available oral agent for the treatment of systemic fungal infections. Until the introduction of the triazoles, ketoconazole was indicated as the drug of choice in chronic mucocutaneous candidiasis [25] and as an effective alternative to amphotericin B in less severe (nonimmunocompromised) cases of blastomycosis [26], histoplasmosis [26], and paracoccidioidomycosis [27]; in coccidioidomycosis, the relapse rate after discontinuation of the drug was high [28]. Ketoconazole has not been adequately evaluated in deep-seated candida infections or cryptococcosis and was ineffective in aspergillosis and mucormycosis.

Over the years, a number of clinically relevant shortcomings of this compound became evident:

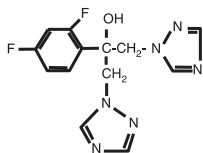
- The absorption of orally administered ketoconazole showed considerable interindividual variation and was markedly influenced by gastric pH [29].
- An intravenous formulation was not available.
- The drug penetrated the blood–brain barrier poorly and could therefore not be recommended for the treatment of fungal meningitis [30,31].
- Ketoconazole was largely fungistatic and proved to be less effective in immunocompromised patients [17].

- The use of ketoconazole was associated with several dose-related (gastrointestinal) side-effects [26]; in addition, ketoconazole could cause symptomatic, even fatal, drug-induced hepatitis [32].
- When given in doses exceeding 400 mg daily, ketoconazole might reversibly inhibit the synthesis of testosterone and cortisol, resulting in a variety of endocrine disturbances, including rare cases of adrenal insufficiency [33].
- A number of clinically important, often unpredictable, drug interactions (e.g. to cyclosporine) have been reported [34].

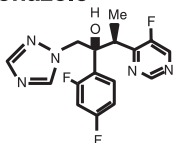
Thus, the poor response rates and frequent recurrences of major fungal infections, as well as the toxicity associated with ketoconazole therapy, led to the search for a second chemical group of azole derivatives, namely the triazoles. In general, the triazoles demonstrate a broader spectrum of antifungal activity and reduced toxicity when compared with the imidazole antifungals. Tercanazole, the first triazole marketed for human use, was active in the topical treatment of vaginal candidiasis and dermatomycoses.

Fluconazole (Figure 1a), a broad-spectrum triazole antifungal developed by Pfizer and approved for use in early 1990, covers many of the shortcomings of the imidazoles. In contrast to ketoconazole, fluconazole is highly water soluble and can be given intravenously to seriously ill patients. After oral administration, absorption is essentially complete (~90% bioavailability) and not influenced by gastric pH [35]. In contrast to ketoconazole, fluconazole enters the cerebrospinal fluid (CSF) extremely well, with CSF levels of almost 80% of the corresponding serum levels [36]. The serum half-life allows once-daily dosing, and, also in contrast to ketoconazole, renal clearance is the major route of elimination of fluconazole, with 70–80% of unchanged drug excreted in the urine [37]. Given this favourable pharmacokinetic profile (Table 1), fluconazole has been studied extensively in various clinical settings, both in prophylaxis and in therapy. The drug is approved for the treatment of oropharyngeal, oesophageal, vaginal, peritoneal and genito-urinary candida infections, disseminated candidiasis (including chronic disseminated candidiasis) and cryptococcal meningitis. Fluconazole also has good activity against coccidioidomycosis and is a good alternative to ketoconazole in chronic mucocutaneous candidiasis.

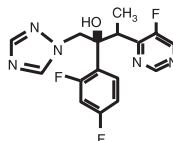
(a) Fluconazole



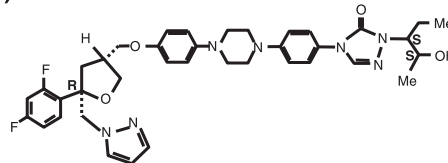
(b) Itraconazole



(c) Voriconazole



(d) Posaconazole



(e) Ravuconazole

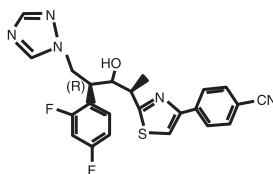


Fig. 1. Chemical structures of old and new triazoles.

Fluconazole has no clinically meaningful activity in infections caused by filamentous fungi. In addition, the drug is relatively safe (even at daily doses up to 1600 mg) and does not interfere with the synthesis of testosterone or cortisol. Also, fluconazole has fewer drug interactions than does ketoconazole. The clinical experience with fluconazole is reviewed by Dr Pfaller in this issue.

The initial enthusiasm for fluconazole, however, has been challenged by two recent developments: the evolving spectrum of fungal pathogens and the development of azole-resistance.

The evolving spectrum of fungal pathogens

In virtually every North American and European medical centre, hospital-acquired infections caused by *Candida* species—both superficial and deep-seated forms—have increased substantially over the past two decades. According to the National Nosocomial Infection Surveillance Program from the Centers for Disease Control and Prevention (CDC) and the Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) study, *Candida* species now account for 7–8% of all nosocomial bloodstream infections in the USA [38,39]. Although *Candida albicans* remains the most commonly encountered pathogenic yeast, a number of recent studies have demonstrated a shift towards infection with

'non-*albicans*' *Candida* species. In the SCOPE study, 50% of isolates from bloodstream infections were non-*albicans* species, including *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. lusitaniae* [39]. Some species are considered less virulent than *C. albicans* but are, on the other hand, inherently less susceptible to fluconazole. Recent data, however, have revealed important differences between patient groups at risk (e.g., oncology versus nononcology), between institutions and between countries [9]. Although the cause of this changing spectrum is multifactorial and has not been evaluated systematically, the selective pressure resulting from the widespread use of fluconazole has most probably contributed to the higher proportion of non-*albicans* species [40]. Nevertheless, links between spectral changes and prior use of fluconazole have not yet been firmly proved; additional factors such as institution-related differences in anti-infective protocols and treatment-specific factors may be equally important for fungal colonisation and subsequent infection [41].

In addition, major observational and autopsy surveys, both in Europe and the USA, have shown that the incidence of invasive aspergillus infections—a pathogen not covered by fluconazole—has increased dramatically over the past two decades [2,3].

Fungal infections have been important complications among patients with HIV from the begin-

Table 1. Comparative data of available triazole antifungal agents

Parameter	Fluconazole	Itraconazole	Voriconazole
Formulation	oral suspension tablets intravenous	oral solution capsules intravenous	tablets intravenous
Oral bioavailability (%)	> 90	55	> 85
Influence of food ↓ (solution)	none	↑ (capsules)	↓
Influence of ↑ pH	none	↓ (capsules only)	none
T_{\max} (h)	1–2	1.5–4.0	< 2
Steady-state plasma concentration (mg/L)	10	1	2 (200 mg bid)
Protein binding (%)	< 10	> 95	60
V_d (L/kg)	0.7–0.8	10.7	2
Principal route of elimination	renal	hepatic	hepatic
Elimination half-life	27–37 h	21–64 h	6–9 h
Cl (mL/min.kg)	0.23	3.80	~ 3
Unchanged drug in urine (%)	80	< 1	< 5
Relative CSF levels (%)	> 60–80	< 1	> 50
Important drug interactions	++	+++	+++
Toxicity			
Nephrotoxicity	–	–	–
Hepatotoxicity	+	+	+
Relevant other toxicity	–	–	visual
Dose reduction in renal failure	yes	no	?
Dialysable	yes	no	?

CSF = cerebrospinal fluid; Cl = clearance.

ning of the pandemic. Population-based surveillance studies, conducted by the CDC and others, have clearly reflected the impact of highly active antiretroviral therapy (HAART) on the incidence and spectrum of these infections in HIV-positive patients. The incidence of cryptococcal meningitis (from 10% to <3%), mucosal candidiasis, aspergillosis, and many other fungal infections (histoplasmosis, penicilliosis marneffeii) has decreased spectacularly with the use of protease inhibitors [42,43].

Although *Candida* and *Aspergillus* species still represent the vast majority of fungal isolates encountered in human pathology, a battery of new species—both yeasts and filamentous fungi—is increasingly recognized as opportunistic pathogens. Of particular concern is the fact that many of these so-called ‘emerging’ pathogens are not covered by fluconazole, including *Trichosporon* spp., *Fusarium* spp., *Scedosporium prolificans*, members of the *Mucoraceae*, and dematiaceous or darkly pigmented fungi [44,45].

The development of azole-resistance

Until the 1990s, acquired resistance to azole antifungals was uncommon. In recent years,

however, particularly as a result of the liberal use of fluconazole in immunosuppressed and critically ill patients, clear patterns of resistance have emerged. However, the lack of an established definition of resistance remains problematic when analysing the scope of this problem. Clearly, the continuous or intermittent administration of any antifungal agent may select for overgrowth of intrinsically resistant or less susceptible strains or species, as has been documented in oncology patients receiving fluconazole prophylaxis. This phenomenon, however, was not reported in a very large study of female patients with, or at risk of, HIV infection. Unfortunately, resistance has too often been defined as ‘clinically resistant’, referring to a patient whose infection has progressed or persisted despite antifungal therapy. Classical resistance refers to treatment failure in association with high or rising minimum inhibitory concentrations (MICs) for the same fungal strain while receiving therapy: a definition not used by many publications. Besides, key features such as pharmacodynamic parameters, fungal virulence factors, host factors and differences in susceptibility testing methods further impair the analysis of a possible link between MIC and outcome [46]. Therefore, the

National Committee for Clinical Laboratory Standards has recently developed a standardized and reproducible method for the *in vitro* susceptibility testing of yeasts to azoles (measuring true microbial resistance) [47].

Apart from this, it is obvious from the literature that in-vitro resistance for *C. albicans* has emerged rapidly during the pre-HAART era in HIV/acquired immune deficiency syndrome (AIDS) patients with oropharyngeal or oesophageal candidiasis [48]. In this setting, up to 20% of *C. albicans* strains have become resistant to fluconazole, depending on the duration and total cumulative dose of fluconazole given, the frequency of exposure and the patient's CD4⁺ cell count. Fortunately, at least in developed countries, the introduction of HAART and the subsequent host immune reconstitution has led to a marked reduction in the number of HIV-associated opportunistic fungal infections; as such, azole-resistant isolates from AIDS patients are now seldom encountered, although it remains to be seen whether this improvement will be maintained. In addition, it is critical to recognize the concept of dose dependency in azole-susceptibility when approaching the therapy of these infections [49]. For example, AIDS patients with oropharyngeal candidiasis caused by strains with low MICs will typically respond to low doses of fluconazole. In contrast, patients with 'susceptible, dose-dependent' strains may still respond to fluconazole therapy provided that higher doses of 400–800 mg/day are given. In general, yeast in-vitro resistance remains manageable in HIV/AIDS patients.

It is evident that the same phenomenon of azole resistance could also arise in deep-seated candida infections and candidaemia. However, evidence of emerging resistance in this setting remains largely equivocal. Based on a number of large surveillance studies in developed countries, there is no evident trend towards an increased acquired resistance to fluconazole for bloodstream isolates of *C. albicans* [46]. Although an increased prevalence of *C. krusei* and *C. glabrata* bloodstream infections has been seen in oncology patients, bone marrow transplant recipients and patients in intensive care units, it remains debatable whether this is (solely) the result of the introduction of fluconazole or not.

In 1992, itraconazole (Figure 1b), a broad-spectrum triazole from Janssen Pharmaceutica,

was approved by the Food and Drug Administration. Compared to ketoconazole, itraconazole was less toxic and showed a broad spectrum of activity against *Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii* and some phaeohyphomycetes [50]. Since its introduction, itraconazole has gradually replaced ketoconazole as the treatment of choice for non-meningeal, nonlife-threatening cases of histoplasmosis, blastomycosis and paracoccidioidomycosis (amphotericin B for severe or meningeal cases). Itraconazole also has considerable spectral advantages over fluconazole with greater activity in aspergillosis and sporotrichosis [51]. However, fluconazole demonstrates a more favourable pharmacological and toxicity profile.

Unlike fluconazole, itraconazole is highly lipid soluble and when first introduced, itraconazole was formulated in capsular form only. This formulation became widely used for the treatment of onychomycosis, superficial fungal infections, endemic systemic infections, systemic aspergillus infections, and to a much lesser extent, systemic candida infections. The prophylactic efficacy has been demonstrated in a number of trials in patients with neutropenia or with HIV infection [52]. However, because absorption of itraconazole capsules can be erratic and low blood concentrations (< 500 ng/mL) have been associated with treatment failure, many clinicians disapprove of its use in patients who were suffering from therapy-induced mucositis or who were receiving antacids [53]. A novel oral formulation of itraconazole, containing the solubilizing excipient hydroxypropyl- β -cyclodextrin, has therefore been developed (FDA approved in 1997). When the capsules and oral solution are compared, the bioavailability of the solution is approximately 60% greater than that of the capsules. This value was obtained in healthy volunteers [54], as well as in various patient groups, including neutropenic patients [55], HIV patients [56], and patients following autologous and allogeneic transplantation [57].

Recently, an intravenous formulation has been developed. In patients with haematological malignancy, patients with advanced HIV infection, or those in intensive care units, high and steady-state plasma concentrations can be achieved within 2–3 days (as opposed to 1–2 weeks when

using capsules) using intravenous itraconazole, 400 mg/day for 2 days followed by 200 mg/day for 5 days. Subsequent administration of itraconazole oral solution or capsules, 400 mg daily, will maintain these high plasma levels [58]. Thus, the availability of new formulations has introduced more flexibility in the use of itraconazole. However, when compared with fluconazole, clinical experience remains limited, especially with the intravenous formulation.

Although the availability over the past decade of both fluconazole and itraconazole represents a major advance in the management of systemic fungal infections, these triazole antifungal drugs have some important limitations.

Several azole–drug interactions may result in hazardous and unpredictable toxicity, especially in patients receiving chemotherapy (e.g. vincristine), transplant recipients (e.g. cyclosporine A, tacrolimus) and AIDS patients (e.g. indinavir, ritonavir). The potential for drug interactions was greater with ketoconazole, but similar interactions have been described with the triazoles. One type of azole–drug interaction may result in decreased plasma concentrations of the azole, either as a result of decreased absorption or increased metabolism of the azole. A second type of interaction may result in increased toxicity of the coadministered drug via interference with the cytochrome P450 systems that are involved in the metabolism of many drugs [59].

Their antifungal spectrum remains suboptimal, especially when considering the growing diversity of offending species. The activity of fluconazole is limited to dermatophytes, *C. neoformans*, *C. albicans* and the dimorphic fungi. Although itraconazole displays a broader spectrum of activity, including *Aspergillus* spp. and some yeast strains that are intrinsically resistant to fluconazole, neither compound has documented activity against some of the emerging pathogens, such as *Fusarium* spp., *Scedosporium* spp. and the Zygomycetes.

Clinical resistance associated with microbiological resistance has been reported, both for fluconazole (mainly *C. albicans*) and itraconazole (including *Aspergillus* spp.) [60]. Meanwhile, several mechanisms of azole-resistance have been identified, including enhanced efflux of the azole by up-regulation of multidrug efflux pumps, alterations in the cellular target of azoles (Erg11p), and modification of the ERG11 gene at the molecular level [61]. Of note is that these mech-

anisms mediate cross-resistance amongst the azoles [62].

Given these shortcomings, the characteristics of the ideal azole can easily be deduced: the agent should be available in oral and intravenous dosage forms, demonstrate a broad spectrum of activity, covering both yeast as well as classic and emerging filamentous fungi, be fungicidal, display a good pharmacokinetic profile with minimal drug interactions, be stable to resistance and be cost-effective. Whether the 'second-generation' triazole derivatives that are currently under clinical development or that have recently been launched on the market will eliminate or reduce these shortcomings remains to be seen.

Voriconazole (Figure 1c), structurally related to fluconazole, was developed by Pfizer Pharmaceuticals as part of a programme designed to enhance the potency and spectrum of activity of fluconazole [63]. Voriconazole displays wide-spectrum in-vitro activity against fungi from all clinically important pathogenic groups such as *Candida* spp., *Aspergillus* spp., *C. neoformans*, dimorphic fungi, dermatophytes, and some of the emerging mould pathogens including *Fusarium* spp., *Penicillium*, *Scedosporium*, *Acremonium* and *Trichosporon*. Members of the zygomycetes still appear to be resistant. Compared to reference triazoles, voriconazole is several-fold more active than fluconazole and itraconazole against *Candida* spp. However, *C. albicans* isolates with decreased susceptibility to fluconazole and itraconazole also demonstrate significantly higher MICs for voriconazole, and isolates (*Candida* as well as *Aspergillus*) that are highly resistant to both fluconazole and itraconazole show apparent cross-resistance to voriconazole. The drug is orally and parenterally active but exhibits complex pharmacokinetics. Interestingly, animal studies have revealed good penetration into the CSF and central nervous system. The promising in-vitro activity has been confirmed in a range of infections in immunosuppressed animal models where voriconazole proved to be more effective than amphotericin B, fluconazole and itraconazole. Data from phase II and III clinical trials indicate that voriconazole is a promising agent for the treatment of oropharyngeal candidiasis in AIDS patients, oesophageal candidiasis, and acute and chronic invasive aspergillosis, including cerebral aspergillosis. A number of cases have reported activity in unusual mould infections, such as scedosporiosis and

fusariosis. However, voriconazole is not yet the ideal azole, because the agent is not devoid of the classical azole–drug interactions and class-related side-effects (including severe cases of hepatic dysfunction [64]). Furthermore, dose-related transient visual disturbances (without morphological correlates) have been reported in up to 10% of patients receiving this agent. Hence, additional work regarding (visual) safety, drug–drug interactions, metabolism and exposure (given the genetic polymorphism of CYP2C19), and emergence of (cross)-resistance is needed. The current status of voriconazole is reviewed by Dr Donnelly and Dr De Pauw in this issue.

Posaconazole (Figure 1d), a hydroxylated analogue of itraconazole developed by Schering-Plough Research Institute, possesses potent broad-spectrum activity against opportunistic and endemic fungal pathogens, including zygomycetes and some of the dematiaceous moulds. *In vitro*, the drug is highly active against *Aspergillus* spp. and at least eightfold more potent than fluconazole against *Candida* spp. Similar to voriconazole, posaconazole was more effective than amphotericin B, fluconazole and itraconazole in animal studies [65]. Ravuconazole (Figure 1e), another derivative of fluconazole developed by Bristol-Myers Squibb, represents the third second-generation triazole. Ravuconazole has a broader antifungal spectrum than fluconazole and itraconazole, particularly against strains of *C. krusei* and *C. neoformans*. *In vitro*, ravuconazole is not active against *Fusarium* spp. and *Pseudallescheria boydii* [65]. Both posaconazole and ravuconazole are currently undergoing phase II and III clinical trials.

CONCLUSION

Until the 1940s, relatively few agents were available for the treatment of systemic fungal infections. The development of the polyene antifungals, first nystatin and later amphotericin B, represented a major advance in medical mycology. Although amphotericin B quickly became the mainstay of therapy for serious infections, its use was associated with a number of infusion-related side-effects and dose-limiting nephrotoxicity. The continued search for new and less toxic antifungals led to the discovery of the azoles several decades later. Ketoconazole, the first available compound for the oral

treatment of systemic fungal infections, was released in the early 1980s. For almost a decade, ketoconazole was regarded as the drug of choice in nonlife-threatening endemic mycoses. The introduction of intravenous and oral fluconazole in 1990 and oral itraconazole in 1992 represented a second major advance in the treatment of fungal infections. Both first-generation triazoles displayed a broader spectrum of antifungal activity than the imidazoles and had a markedly improved safety profile compared with amphotericin B and ketoconazole. Fluconazole was used frequently for prophylaxis and treatment of candidal and cryptococcal infections, especially in the pre-HAART era, whereas itraconazole, despite its erratic absorption, became the drug of choice for the treatment of less severe forms of histoplasmosis and blastomycosis, and an attractive alternative to amphotericin B for the treatment of select cases of invasive aspergillosis. Although expanded uses have been suggested for both agents—in prophylaxis as well as in therapy—they also became subject to a number of clinically important limitations, including suboptimal spectrum of activity, development of resistance, induction of hazardous drug–drug interactions, less than optimal pharmacokinetic profile (itraconazole capsules), and toxicity. To overcome these limitations, several structural analogues have been developed and tested in various stages of clinical development. Three of these so-called ‘second-generation’ triazoles, including voriconazole, posaconazole and ravuconazole, appear to have greater potency and possess increased activity against resistant and emerging pathogens. All three agents are active following oral administration and have demonstrated promising antifungal activity *in vitro* and in animal models. These second-generation triazoles seem particularly promising for the treatment of *Aspergillus* infections and for unusual (but emerging) opportunistic infections that are otherwise covered only by amphotericin B or not covered at all. However, further clinical investigation is warranted to identify the role of these agents in the future treatment of systemic fungal infections. If the toxicity profile of these agents is comparable to or better than that of the first-generation triazoles and drug interactions remain manageable, then these compounds represent a true expansion of our antifungal arsenal.

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