

# Antifungal agents: their diversity and increasing sophistication

FRANK C. ODDS

Aberdeen Fungal Group, Molecular & Cell Biology, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK

Since the 1950s, antifungal drug discovery has identified three classes of natural products (griseofulvin, polyenes and echinocandins) and four classes of synthetic chemicals (allylamines, azoles, flucytosine and phenylmorpholines) with clinical value against fungal infections. For life threatening fungal disease, the polyene amphotericin B is still a common choice despite toxic side-effects. The azoles remain the most widely used group of anti fungals active against a wide range of mycoses, benefiting from creative chemistry to boost their effectiveness. More recently, the echinocandins show great promise, with caspofungin licensed for clinical use in 2002 and two other molecules close to registration. New advances in molecular genetics afford the promise of revealing new antifungal targets, together with new agents to inhibit those targets specifically.

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Anyone who has ever suffered the irritation of athlete's foot or thrush (and that includes most adults!) knows something of the availability of medicines to treat fungal diseases. For common, superficial infections like the two mentioned above, it is now possible to purchase appropriate antifungal agents over the counter and without prescription. Gone are the days of non-specific remedies such as Whitfield's ointment (which stings unmercifully) and Gentian Violet (which leaves unsightly stains); modern antifungal drugs are distinguished by the selectivity of their antifungal action and their low tendency to evoke serious side-effects. Their existence is testimony to a long and persistent history of research that has steadily yielded new classes of antifungal molecules so we now have a sizeable armoury of medicines for treating all types of fungal diseases. Not all branches of antifungal development have been equally productive, but the many types of compounds available have given us great insights into useful antifungal targets and molecular motifs with antifungal potential.

Figure 1 shows a historical perspective of antifungal agents that are used clinically. While some classes of antifungal molecules that were first brought into use in the 1950s have not been fruitful areas for further development, others – particularly the azole antifungals – have been a fruitful source of ever more potent and specific pharmaceuticals.

## Griseofulvin

First tested as an antifungal agent in humans in the 1950s, griseofulvin was the earliest chemical that could be claimed to have a selective inhibitory activity against fungi. It is a secondary metabolite of the fungus *Penicillium griseofulvum* and thus the first example of a product from one fungus being used to attack another. It blocks the assembly of microtubules within susceptible fungal cells, without exerting similar effects on mammalian cells. Its activity spectrum, among all types of pathogenic fungi, is limited to the dermatophytes, which cause 'ringworm' infections of skin, nails and hair. The agent is taken by mouth and, in countries where poverty and distance to hospitals reduce patients' compliance with a complex regime, single doses of 2-3 g are given by doctors, although this approach is less efficacious than a longer period of repeated treatment. Griseofulvin has proved its worth over many years, particularly in the successful treatment of scalp ringworms. Somewhat strangely, no other clinically useful agents acting on the microtubule target have been discovered since griseofulvin.

## The polyenes

Polyenes are one of nature's most frequently produced chemical structures. Natural products with conjugated double bonds (poly '-enes') include terpenes, vitamin A and many essential oils in addition to molecules such as

	polyketides	polyenes	azoles	pyrimidines	echinocandins	allyamines
1950s	griseofulvin	amphotericin B nystatin	chlormidazole			
1960s			topical imidazoles	flucytosine		
1970s			systemic imidazoles		cilofungin	
1980s			systemic triazoles			naftifine terbinafine
1990s					broad-spectrum echinocandins	

**Fig 1** Discovery timelines of major classes and subclasses of antifungal agents for treatment of mycoses in humans. Dates shown correspond to introduction of the agents into clinical trials, rather than discovery of antifungal activity in vitro. Several types of agent are not shown in the figure, including the one example of a clinically usable phenylmorpholine agent (amorolfine), introduced in the 1980s.

amphotericin B and nystatin which are used as antifungal agents for human diseases. These antifungal molecules, produced by streptomycete bacteria, bind to sterols in cell membranes and cause them to leak cellular constituents. Because they bind with up to 10 times more avidity to ergosterol, the sterol in fungal membranes, than to cholesterol – the human membrane sterol – they show selective antifungal activity. Nystatin can only be used as a topical agent, in the form of creams, pessaries, etc. However, amphotericin B is formulated for intravenous use, and the molecule still represents the mainstay for treatment of life-threatening fungal diseases. Its broad antifungal spectrum makes it an ideal choice for immediate use when the exact cause of a probable fungal infection has not been established. Indeed, it is probable that most fungal diseases are treated ‘empirically’ before an infecting species has been determined. Tests for fungal identification and for diagnosis of deep-seated fungal diseases remain slow and, often, uncertain.

The problem with intravenous amphotericin B treatment is that the low selectivity of the antifungal action of this molecule often results in serious toxic effects, particularly kidney damage. To solve this problem, various advances in drug formulation technology have been applied to amphotericin B. Today, patients with serious mycoses are commonly treated with lipid-based formulations of the compound, which reduce kidney toxicity to very low levels without impairing efficacy. Even newer formulations, known as cochleates and arabinogalactan conjugates, are being developed to reduce even further the problems of safely administering amphotericin B to very sick patients.

### Antifungal imidazoles and triazoles

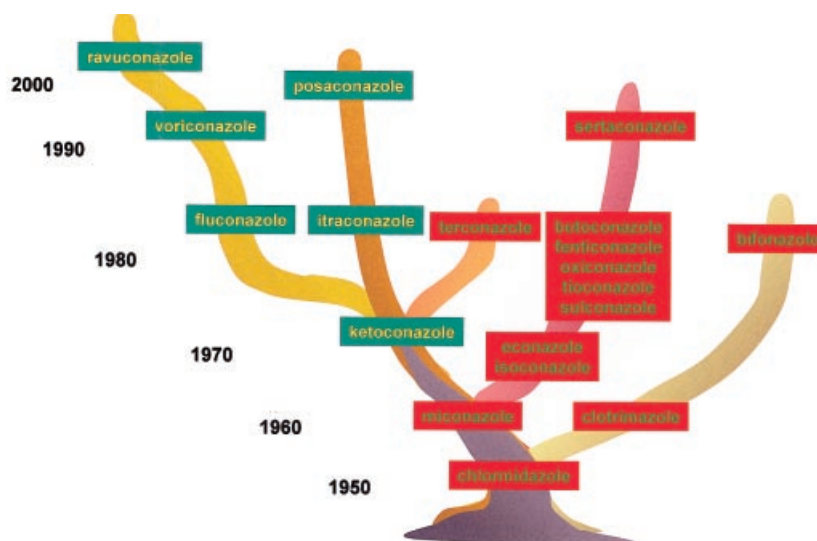
The third class of antifungal agent shown in Fig 1 is the group of compounds known collectively as ‘azoles’. Chemically they all have either an imidazole or a triazole group joined to an asymmetric carbon atom as their functional pharmacophore; they all work by blocking the active site of an enzyme variously known as lanosterol 14 $\alpha$ -demethylase or cytochrome P450<sub>DM</sub>. This action means that the azoles inhibit the synthesis of normal membrane sterols in fungi. Lack of ergosterol in a fungal membrane seriously cripples the fungus and leaves it unable to grow and develop in the normal way.

More than any other antifungal class, the azoles have been steadily refined and improved upon over the course of almost 50 years. The earliest azole for clinical use, chlormidazole, was really not a very good pharmaceutical, but the ease with which variants on the chlormidazole chemical structure could be synthesized and tested led to steady progress with azole antifungal agents. Figure 2 shows most of the agents that are now in clinical use in the form of a timed tree. Each branch represents a new fundamental chemical modification to a previous structure, so the tree illustrates the way in which successive strokes of human ingenuity among pharmaceutical chemists has given us the wide diversity of azoles we can now draw on to treat mycoses of almost all types.

Figure 3a shows the main features of molecules that have branched from the part of the tree started with miconazole. Almost all the azole antifungals have an active structure of this type. The atoms shown as ‘X’ in Fig 3a are halogens; the earlier molecules had chlorine

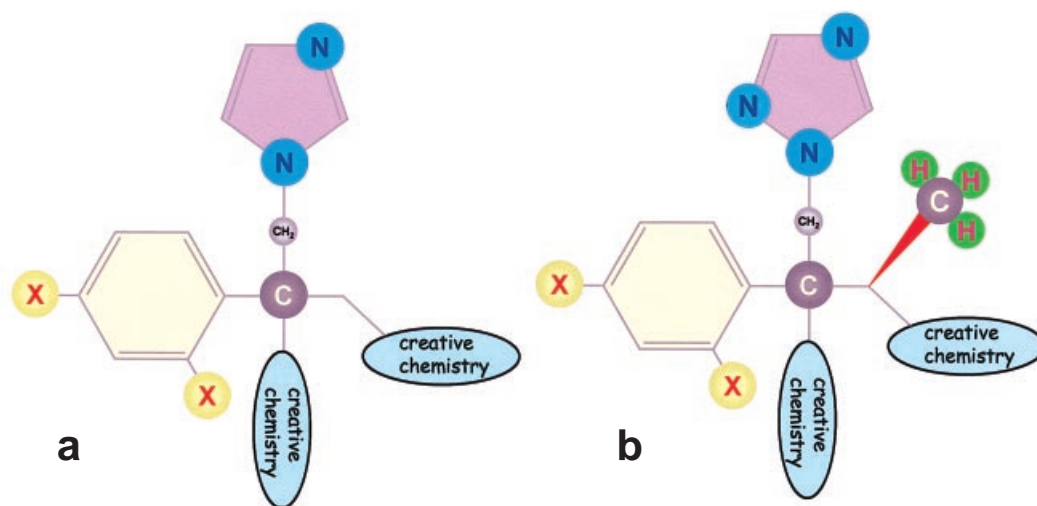
atoms in these positions, but fluorines have taken their place in more recent molecules. The portions labelled as 'creative chemistry' indicate the parts of azole molecules that form the main differences between each individual azole illustrated in the tree of Fig 2. Two changes in the 'fixed' part of the azole pharmacophore that have been made in the light of research are indicated in Fig 3b. Addition of an extra nitrogen atom to put a triazole ring in place of the original imidazole at the head of the structure seems to confer both increased breadth of antifungal spectrum and reduced potential toxicity on the molecule. For some azoles the addition of a methyl group adjacent to the focal asymmetric carbon (Fig 3b) ensures they can inhibit many filamentous fungi as well as yeasts.

The tree in Fig 2 illustrates how, with time, the trend with azoles has shifted away from topically useful azoles for the common, superficial infections to compounds with systemic bioavailability that can treat life-threatening mycoses. This trend accompanies the clinical problems of growing numbers of patients who receive immunosuppressive treatments for malignancies and for transplantation surgery and who



**Fig 2** Growth of the azole 'tree', indicating approximate dates of commencement of clinical trials with each agent or group of agents. Each branch of the tree represents a series of chemically related compounds.

are thus rendered highly vulnerable to fungal infection. Three new triazole antifungals are now emerging into clinical use: voriconazole (licensed in 2002), posaconazole and ravuconazole. All of these azoles have very broad antifungal spectra and each shows individual benefits in terms of their effects in clinical trials. In the future the azole tree may still grow further as more novel modifications of the basic structures shown in Fig 3 prove to add potential clinical benefit.



**Fig 3** Active pharmacophore of an azole antifungal. (a) The structure common to the majority of the earliest azoles, with an imidazole ring (pink) N-linked through a  $\text{CH}_2$  group to an asymmetric carbon atom. The 2,4 di-halogen-substituted benzene ring is common to all azoles ( $\text{X} = \text{Cl}$  or  $\text{F}$ ). The ellipses labelled "creative chemistry" indicate the portions of the molecule that can be (and have been) varied significantly to create different azole antifungal molecules. (b) the most recent advances in azole chemistry involve substituting a triazole ring (pink) in place of an imidazole and, in some molecules, a methyl group (centre right) adjacent to the asymmetric carbon atom.

## Flucytosine

Flucytosine (see Fig 1) is a unique antifungal agent. It has no siblings or progeny in its antifungal class. The compound was first made as a potential anti-cancer drug. Its antifungal activity was picked up in separate screening research and developed to give us a compound that still has some value as adjunctive treatment with amphotericin B in clinically difficult infections such as meningitis caused by *Cryptococcus neoformans*. Its antifungal specificity comes from the fact that fungi, but not human cells, possess the enzymes needed to take up flucytosine and convert it internally to 5-fluorouracil, a compound that is highly toxic to all eukaryotic systems. Fluorouracil becomes incorporated in fungal DNA and RNA and blocks synthesis of both these vital molecules, preventing cell proliferation.

## Allylamines

The allylamine class of antifungal agents is very well known and widely represented among plant fungicides, but has given rise to surprisingly few compounds of clinical value. Naftifine was developed as an allylamine agent for topical use. Its success and antifungal potency led to much research for variants with systemic bioavailability, but only the single compound terbinafine has ever emerged with the combination of efficacy and safety needed for a pharmaceutical agent. Terbinafine has been a very valuable antifungal for dermatophyte infections; it has become the widest used treatment for nail infections caused by fungi. Like the azoles, the allylamines act to block fungal ergosterol synthesis, but they act much earlier in the biosynthetic pathway. Very many laboratories have tried to design novel allylamines for the clinic, but with no success in the course of more than 20 years.

## Echinocandins

The final class of antifungal molecules listed in Fig 1 is the echinocandins. These agents are natural products: secondary metabolites of fungi. The prototype agent echinocandin B was isolated in the 1970s from a culture of *Aspergillus nidulans* var. *echinulatus*. Ironically, its present-day semi-synthetic offspring are being developed for the treatment of *Aspergillus* infections! Echinocandin B was one of a crop of novel molecules, all natural products found in the 1970s, that achieved the 'holy grail' of antifungal biochemists by inhibiting or impairing the function of

the fungal cell wall. Fungal cell wall polysaccharides have long been regarded as ideal targets for antifungal activity, because mammalian cells are bounded by membranes without a cell wall. The echinocandins and papulacandins both inhibit synthesis of  $\beta$ -1,3 glucan polymers, and are therefore fungicidal for species where the wall is dependent on such glucans for their structural integrity. Nikkomycins inhibit synthesis of chitin in fungal walls, while pradimicins and benanomicins bind to cell wall mannoproteins and induce fatal permeability changes in the fungal cell envelope. Of all these agents, only the echinocandins have been successfully developed for clinical use.

The first semi-synthetic echinocandin developed for human use was cilofungin. This agent was limited by a relatively narrow antifungal spectrum; it was used to treat a small number of patients but toxic effects – now almost certainly known to result from the intravenous vehicle rather than cilofungin itself – led to its abandonment. Meanwhile, careful research on structure-activity relationships in echinocandin molecules led to their modification to enhance both spectrum and potency. Three echinocandin molecules are now in advanced development. Caspofungin was licensed for clinical use in 2002; anidulafungin and micafungin are close to registration. All three molecules have an antifungal spectrum that includes most *Candida* spp., many *Aspergillus* spp. and *Pneumocystis carinii*, among others. All three are administrable only by intravenous injection, but data for caspofungin indicate their safety and tolerability are among the best of all types of injectable antifungal agents.

## Other antifungal agents

Not shown in Fig 1 are a small number of antifungal agents that have either not achieved world-wide acceptance comparable to the agents shown, or they have failed so far to turn pre-clinical potential into clinical use. One such agent is amorolfine, the sole representative of the phenyl-morpholine chemical class, well known in agriculture but used as a topical broad-spectrum agent in just a few countries. The sordarins are a class of molecules produced by fungi that inhibit protein synthesis in susceptible fungal species by binding to elongation factor 3. In the late 1990s the sordarins looked the most promising novel agents in development, but the two companies that were doing research on the class have never identified a candidate molecule for clinical use; sordarin development at present seems to have halted.

## Lessons from antifungal history

From the earliest antifungal agents to the present day there has been a steady stream of newly discovered antifungal drugs that have greatly advanced our ability to manage fungal disease successfully. The drugs now marketed clinically for antifungal use act against a very wide range of molecular targets: cell wall glucan synthesis, membrane ergosterol, ergosterol synthesis, DNA and RNA synthesis and microtubule assembly. Inhibitors of fungal chitin synthesis and protein synthesis are known, but have not been developed to clinical use. This list of known antifungal targets is almost as large as the list of known targets for antibacterial agents. The current pharmaceutical armoury of antifungals is a clear cause for satisfaction, not for gloom. However, we still do not have agents that fulfil every one of the criteria that a physician would set as desiderata for antifungal drugs. They need to be active against those fungi causing infections which we cannot yet depend on eradicating (e.g. *Fusarium* spp., *Scedosporium* spp., Zygomycota). They need to be formulated for both oral and parenteral administration; they need to be extremely safe; and they need to be as cheap as possible. The search for new antifungal agents therefore must go on.

The problem for antifungal chemotherapy can be regarded as a problem of comparative biochemistry: the eradication of one eukaryotic organism residing within another. All the agents we now know of as antifungals were discovered first as inhibitors of fungal growth; their targets were determined later. Thanks to technological advances in molecular genetics we can now call on novel disciplines with names such as 'genomics', 'transcriptomics' and 'proteomics' to reveal antifungal targets we had never previously thought of. We then hope to discover novel agents that specifically inhibit those targets. This reversed approach to antifungal discovery one day will bear new fruit to hang on the branches of the antifungal tree; however, we should not expect progress to be rapid. Drug discovery is never a fast business. Fourteen years elapsed between the discovery of chlormidazole, the first clinically useful azole, and its next successors, clotrimazole and miconazole. A similar period of time elapsed between cilofungin, the first useful

echinocandin, and its current successors. Finding the lead compound that affects a novel target is due cause for celebration but, as the sordarins and the nikkomycins bear witness, the road between discovery and a product that fills the clinical and economic desiderata for an antifungal agent is much longer – and a lot more difficult – than discovery researchers ever imagine.

The current set of clinically available antifungal agents includes three classes of natural products (griseofulvin, polyenes, echinocandins) and four classes of synthetic chemicals (allylamines, azoles, flucytosine and phenylmorpholines). We therefore cannot abandon interest in biodiversity as a source of natural antifungal products – indeed, if we add the sordarins and nikkomycins as 'also-rans' to the list of possibles, natural products exceed synthetic chemicals. Nor can we underestimate or undervalue the skill of medicinal chemists at synthesizing novel compounds to underwrite our antifungal future. Novel chemistry is always the keystone of drug discovery. Our future research needs to ensure we continue to achieve as much chemical diversity as we determine new genome sequences and identify novel targets.

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