ELSEVIER

International Journal of Antimicrobial Agents 17 (2001) 81–91

INTERNATIONAL JOURNAL OF
Antimicrobial
Agents

www.ischemo.org

Review

Current and future approaches to antimycotic treatment in the era of resistant fungi and immunocompromised hosts

J. Bastert [a], M. Schaller [a], H.C. Korting [a,*], E.G.V. Evans [b]

[a] *Klinik und Poliklinik für Dermatologie und Allergologie der Ludwig-Maximilians-Universitat München, Frauenlobstr. 9–11, 80337 Munchen, Germany*

[b] *Department of Microbiology, University of Leeds, Leeds, UK*

Received 11 May 2000; accepted 28 September 2000

Abstract

Due to the ever-increasing number of immunocompromised patients, both localised and life-threatening systemic fungal infections are on the increase. Conventional treatment is of limited help, not in the least due to a less optimum benefit-to-risk ratio. Moreover, emerging pathogens with reduced antimicrobial susceptibility and the development of resistance in *Candida albicans* form a new challenge. Fortunately, conventional antimycotics have been improved and entirely new ones are on the horizon as well as alternative approaches such as immunoreconstitution. © 2001 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

Keywords: Antimycotics; Antimicrobial resistance; Immunoreconstitution; Targets

1. Introduction

Due to the progress made in modern medicine and in the fields of intensive care, haematooncology and transplantation in particular, the number of immunocompromised patients is on the increase. Modern antibacterial chemotherapeutic agents contribute a great deal to a better prognosis in immunocompromised states but they also contribute to an increased number of systemic fungal infections, which can affect survival. Candida and, increasingly, Aspergilli are the major pathogens in this situation [1,2]. The rapidly increasing number of HIV-infections presents another group of immunocompromised hosts susceptible to fungal infections [3,4]. So far, fungal infections, particularly those in immunocompromised hosts, have been a major therapeutic challenge. The situation has been made worse over recent years with the emergence of resistance among fungal pathogens. Resistance has been seen mainly following treatment of *Candida albicans* infections in HIV-infected patients with the triazole fluconazole, which is currently the most frequently used antifungal in this situation [5]. In some cases, resistance to fluconazole confers cross-resistance to other azoles. Fluconazole has been reported to cause a pathogen shift from *C. albicans* to inherently less sensitive species such as *C. glabrata* and *C. krusei* [6–10]. Antifungal resistance of *C. albicans* can be traced back to various changes that occur at a molecular level resulting in increased availability of lanosterol-delta-14-demethylase and efflux-pumps [3,8,9,11]. It is obvious that new antimycotics developed as a result of our increased understanding of potential molecular targets will enlarge our therapeutic armamentarium and enable us to resist the threat of severe fungal infections. In this review, both conventional and new targets for antifungal agents and the corresponding active compounds are discussed in parallel (Fig. 1).

* Corresponding author. Tel.: +49-89-51604615; fax: +49-89-51604602.

PII: S0924-8579(00)00323-X

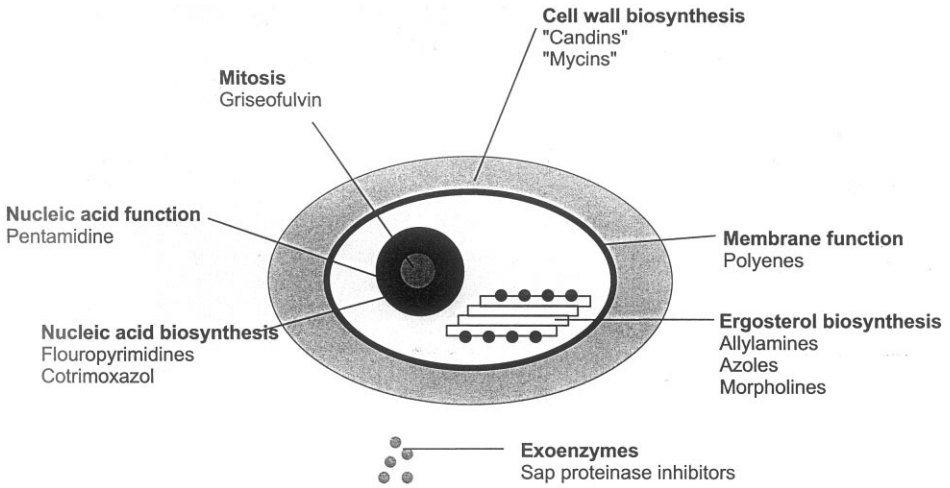

Fig. 1. Mechanism of action of different antimycotics.

2. Conventional antimycotics

In 1952, amphotericin B was introduced for the systemic treatment of fungal infections. The natural compound, an amphoteril heptaene, can be given both systemically and topically (Table 1). It interacts with ergosterol, the major component of the cytoplasmic membrane of fungal cells, by forming a complex with its two hydrophobic chains. This results in the formation of minute pores, linked to leakage and cell death [12–14]. Although cholesterol and not ergosterol is the major component of the cytoplasmic membrane of mammalian cells, there is enough similarity between these two molecules to explain the major toxicity problems that occur with systemic application of amphotericin B, particularly those linked to the kidneys [15]. While liposomal encapsulation of amphotericin B has been shown to be efficacious in increasing the benefit-to-risk-ratio by lowering renal toxicity, the drug is still not well tolerated in the majority of patients, especially those treated with higher doses [16].

Over the past 10–20 years, the ergosterol biosynthesis pathway has been elucidated in sufficient detail to allow interference at various stages. This applies to the azoles in particular, which were introduced in the second half of the 1970s. While most early congeners can only be applied topically for pharmacokinetic reasons a few benzimidazoles such as miconazole and ketoconazole can also be given systemically. The same applies to newer congeners belonging to the class of triazoles, such as itraconazole and fluconazole (Fig. 2). These drugs interfere with the activity of the cytochrome P450-dependent enzyme lanosterol-delta-14-demethylase [17]. As fungal P450 is related to human P450, toxicity with these drugs is an issue, particularly with ketoconazole. Although systemic application of ketoconazole has had to be restricted to a subgroup of original indications, there is no such problem with itraconazole and fluconazole, although these agents can interfere with the metabolism of some commonly used medical drugs. While a parenteral form of itraconazole has not been licensed, fluconazole can be given both by the peroral and parenteral route. Due to teratogenicity in animals, use of these drugs is a major concern in females of child-bearing age. While polyenes such as amphotericin B act as fungistatic agents due to their mode of action, the azoles can be fungicidal in some contexts. Although the polyenes have a broad spectrum, which includes activity against *Candida* spp. and *Aspergillus* spp., unlike the azoles, they do not inhibit dermatophytes. Fluconazole, on the one hand, is not very active against *Aspergillus* spp., whereas itraconazole will inhibit the fungus. While clinically relevant antifungal resistance has not been detected with amphotericin B, resistance of *C. albicans* to fluconazole has become a major problem in orogastrointestinal candidosis in HIV-infected patients [18,19]. Today, up to 10% of strains isolated from a given patient population are likely to exhibit minimum inhibitory concentrations of 78 mg/l or more, which corresponds to clinical resistance [7]. This may be due to increased expression of Ben- and Cdr1-genes, leading to the increased presence of energy-dependent efflux-pumps [3,6]. Within the next few years, voriconazole is likely to become the

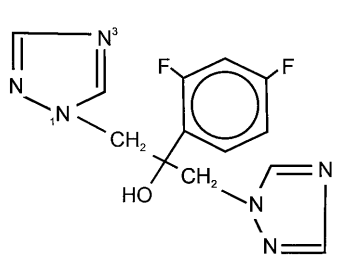

Fig. 2. Structure of fluconazole.

Table 1
Current antimycotics[a]

Major chemical classes	Antifungals	Target/mechanism of action	Spectrum	Resistance	Side-effects
Polyenes	Amphotericin B (s,l), nystatin (l)	Complex with ergosterol causes membrane disruption	*Candida* spp., *Aspergillus* spp.	*P. carinii*, *Trichosporon* spp., *Fusarium* spp.	Nephrotoxicity
Azoles	(a) Imidazole (l, s) miconazole, ketoconazole, (b) Triazoles (s) — itraconazole, fluconazole	Inhibit cytochrome P450 51A1-mediated sterol 14-α-demethylation	Broad	*P. carinii*	Rare hepatoxicity, gynaecomastia, adrenal insufficiency, teratogenicity
Allylamines	Naftifine (l), terbinafine (s,l)	Inhibit squalene epoxidase	Dermatophytes, broad spectrum in vitro	*P. carinii*	Rarely adverse effects
Fluoropyrimidines	Flucytosine (5-FC) (s)	Interference with RNA	Limited spectrum, including *Candida* spp., in combination with amphotericin B	Primary and secondary resistance, precluding monotherapy	Reduces renal toxicity of amphotericin B
Morpholines	Amorolfine (l)	Inhibits δ-14-reductase and δ-7-δ-8-isomerase	Dermatophytes, *Candida* spp.	Inactive when given orally for life-threatening mycoses	
Benzofuranes	Griseofulvin (s)	Inhibits guanine	Dermatophytes	*Candida* spp.	Induction of LE, teratogenicity

[a] l, Local therapy; s, systemic therapy.

most important new azole compound due primarily to its clear-cut activity against *Aspergillus* spp. [19,20].

Allylamines such as terbinafin, which bind to squalene epoxidase, inhibit ergosterol biosynthesis at an even earlier level than the azoles [21–23]. While the clinical activity against *Candida* spp. and many other pathogenic fungi is limited, the allylamines are approximately 10–20-times more active in vitro against dermatophytes than the azoles, with minimum inhibitory concentrations ranging from 0.001–0.05 mg/l for *Trichophyton rubrum* [24,25]. The mode of action of these antifungal agents is fungicidal rather than fungistatic [26,27]. Terbinafine is well-tolerated by most patients although some experience a temporary loss of taste and, rarely, toxic epidermal necrolysis. The morpholines, currently represented by amorolfine, inhibit ergosterol biosynthesis at a very late stage by interfering with delta-14-reductase and delta-7-delta-8-isomerase. Being fungistatic by nature, amorolfine is only moderately active, mainly against dermatophytes and yeasts. So far, only the topical route of application has been exploited [28].

After the introduction of griseofulvin in 1960, this drug was used widely to treat dermatophyte infections [13]. It interferes with intracellular microtubule production leading to fungistatic activity. While its efficacy in major indications such as onychomycosis is limited, concern with the side-effects of this drug has increased over the past few years [29]. While griseofulvin is not generally lethal, it has been shown to cause serious problems in patients with hepatic porphyria.

Although it is not recommended for monotherapy, due to well-defined problems with both primary and secondary resistance, the antimetabolite-related drug flucytosine is still used in combination with amphotericin B for the therapy of invasive Candida or Cryptococcus infections [30–33].

3. Immunoreconstitution

Recently, a deeper insight into the nature of the pathogen *Pneumocystis carinii*, which causes pneumonitis in many HIV-infected patients, has shown that this pathogen belongs to the Kingdom of fungi. Conventional drugs for the treatment of pertinent infections include the antibacterial drug cotrimoxazole combined with the anti-protozoal agent pentamidine [33,34]. Faced with the increasing problems with antifungal therapy, it is considered wise not only to attack the pathogen directly but also to reconstitute the compromised immune status of the patient as far as possible [30,35]. This approach, termed immunoreconstitution, is currently based on the application of cytokines, such as granulocyte-monocyte colony stimulating factor, gamma interferon (-IF), interleukin 1 and tumour necrosis factor [5]. The combination of an antifungal agent such as amphotericin B, and a cytokine such as -IF, looks particularly promising in vitro [36]. Instead of immunomodulatory agents, antibacterial agents have also been considered for combination with antifungal treatment. In experimental animals, inhibition of fungal DNA-gyrase by the quinolones ciprofloxacin and trovafloxacin is considered helpful when combined with amphotericin B or fluconazole [37].

4. Targets

During the past few years, the search for new antimycotic agents has focussed largely on the development of new azoles [14,15,38–42]. In this context, more elaborate approaches to drug development have been implemented, including multiple automatic structure evaluation, which has contributed to the screening of a series of 71 different triazoles. It has been possible to relate the molecular structure to the desired (antimycotic) activity and the unwanted teratogenic activity [43]. Using this method, certain congeners with increased desired activity and decreased unwanted activity could be identified, i.e. drugs with an increased therapeutic index [29,44]. Using recently developed molecular biology tools, the genes encoding particular enzymes belonging to the ergosterol biosynthesis pathway could be classified, allowing the discrimination of those which are essential and, thereby, qualify as targets for antimycotic intervention [45]. Modification of the polyene-structure is also an issue. Changing the amphotericin B molecule at the C13-position led to increased selectivity and reduce unwanted side-effects on the host [15]. Certainly, the components of the cytoplasmic membrane are not the only targets currently under investigation. A critical reduction of the availability of certain ions has already been the focus in the development of various antimycotics of the hydroxypyridone class encompassing ciclopiroxolamine and rilopirox [46]. Recently, calprotectin, a member of the S100 protein family, has been under consideration as a chelating agent for zinc, which is an essential element for fungal growth. This concept has been shown to be valid, at least in the context of *C. albicans* [47]. While the cytoplasmic membrane, as the innermost part of the fungal cell wall, was considered a potential target, the outer parts of the wall now look more interesting [21,48]. The major components consist of mannans and glucans. These molecules are, by and large, unrelated to components of the host cell, which should mean low toxicity. The complete lack of a functional cell wall should result in immediate cell death and represent fungicidal activity. In addition to the development of agents interfering with various aspects of fungal structure and function, it is considered useful to clarify the

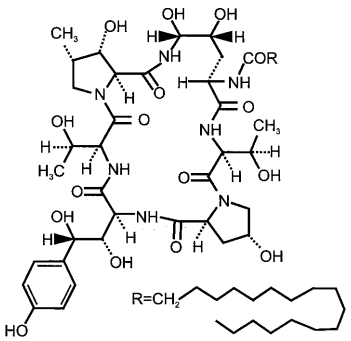

Fig. 3. Structure of tetrahydroechinocandin B.

role of various fungal virulence factors in more detail [49,50]. This applies to adherence and penetration in particular, which are the first two stages of the pathogenic chain [21,48].

5. New antimycotics

Among the new agents interfering with the production and/or integrity of the fungal cell wall, the so-called candins have, at least in part, been developed up to the more advanced stages of clinical drug evaluation [3,33,51–53]. The pneumo-, papulo- and echinocandins (Fig. 3) are active against most pathogens causing systemic mycoses [15,53,54]. By interfering with the FKS-gene, pertinent substances disrupt -1,3-glucan-synthase, which is not compatible with the formation of an intact cell wall [55–62] (Table 2). Although the target does not appear to be closely related to any in the mammalian host, moderate reversible haemolysis is a concern, as is the existence of primary resistant strains. In particular, the echinocandin LY295337 proved to be highly active against Candida in vitro. In human disease, it was found to be as effective as fluconazole and amphotericin B in several trials [63–66]. Activity was also observed against fluconazole-resistant strains of *Candida* [67]. LY303366 is another echinocandin, which has already been used, successfully in animal models [64,68,69]. Although it is active against several *Candida* spp., including *C. albicans*, as well as *Aspergillus* spp., it is less active against *C. parapsilosis* and is not active at all against *Cryptococcus neoformans* [70]. L-733560 is a water-soluble pneumocandin, which is active, in vitro against both azole-susceptible and resistant *Candida* spp. [52,71,72].

The so-called mycins are another group of antimycotic agents, which act, on the fungal cell wall. They comprise the nikkomycins, pramidicins and benanomycins [20,73,74]. Nikkomycin inhibits the enzyme chitin-synthase [3,75,76]. Pramidicins and benanomycins form an insoluble complex with mannoproteins in the fungal cell wall in the presence of calcium and, thus, compromise the integrity of the cell wall [73,77,78]. Pramidicin FS is formed by the addition of D-serine to pramidicin S, although this does not change its activity in vitro or in vivo [79]. Pramidicin Q is an aglycon, which inhibits fungal α-glucosidase. It is formed by splitting dihydrobenzo-(α)-naphthacenequinone. By substitution of the d-amino- and hexose-sugar moiety, it binds to terminal dimannosides of *C. albicans* compromising cell wall integrity [35].

During the past few years, secreted aspartic proteinase (Sap) has been characterised as a major virulence factor of *C. albicans* (Fig. 4) [80–82]. Sap play a major role in both adhesion and penetration, the first and most relevant steps in pathogenesis [83–85]. In the adherence assay based on human keratinocytes grown in vitro, the specific aspartic proteinase inhibitor pepstatin A was able to reduce Candida adhesion to the host cells in a dose-dependent manner [86]. The application of pepstatin A demonstrated a protective role in several experimental infections with *C. albicans* (Fig. 5). However, this inhibitor is unlikely to be useful as an antifungal agent as it also acts on host proteinases and is taken up by the liver. Other inhibitors of Sap might also be relevant in animal and human infections, and pertinent drugs have recently been introduced in the treatment of HIV-infection, compounds such as saquinavir (Fig. 6) and indinavir inhibit HIV-proteinase, which also belongs to the class of aspartic proteinases. According to X-ray crystallographic analyses, there is a close structural relationship between HIV-proteinase and Sap. More recently, it has been possible to demonstrate a dose-dependent inhibitory effect of both compounds in an in vitro assay [87]. The concentrations resulting in marked inhibition were within the range of concentrations reached in man in the treatment of HIV-infection. These findings confirm those obtained by molecular modelling approaches. It has been speculated that the tremendous reduction in frequency of oral candidosis in HIV-infected patients

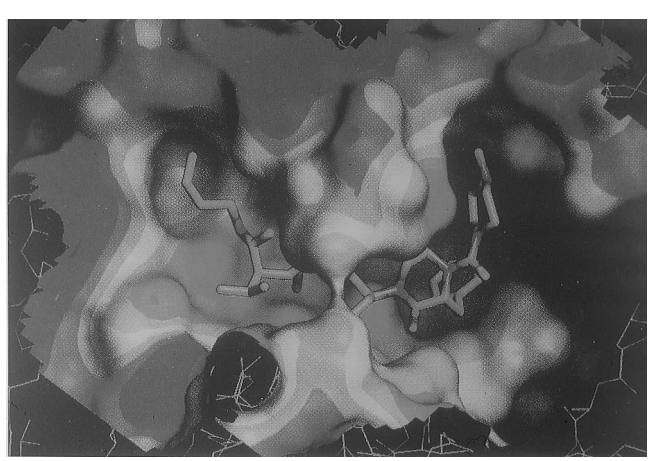

Fig. 4. Stereo-diagram of Sap2 and inhibitor A70450 [101].

Table 2
Future antimycotics[a]

Major chemical classes	Antifungals	Target/mechanism of action	Spectrum	Resistance	Side-effects
Echinocandins (s)	Echinocandin B, LY-303366, LY-295337, LY-307853	Inhibit β-1,3-glucan-synthase non-competetively	*Candida* spp., *Aspergillus* spp. *H. capsulatum*, *P. carinii*	*C. neoformans*, *Mucor* spp., *Fusarium* spp.	Low toxicity, Haemolysis
Pneumocandins (s)	L-743872, L-733560, L-705589, L-731373	Inhibit β-1,3-glucan-synthase non-competetively	*Candida* spp., *Aspergillus* spp. *P. carinii*	*C. neoformans*, *C. albicans* with single mutant echinocandin target gene 1-1	Low toxicity, haemolysis
Papulacandins (s)	Papulacandin B, Chaetiacandin	Inhibit β-1,3-glucan-synthase non-competetively	*Candida* spp.		
Nikkomycins (s)	Nikkomycin Z	Inhibit chitin synthase 3	*Coccidioides* spp.	*Saccharomyces cerevisiae*	
Pramidicins (s)	Pramidicin A, B, C, D, E, M, N, O, P and Q, FS, FB BMS-181184	Bind to mannoproteins of the cell wall	*Candida* spp., *C. neoformans*, *Aspergillus* spp. Zygomycetes	*Trichophyton mentagrophytes*, *Fusarium* spp.	No major end-organ toxicity, discoloration of urine, elevation of hepatic transaminases
Benanomicin (s)	Benanomicin A (ME 1451)	Binds to mannoproteins of the cell wall	C*andida* spp., *P. carinii*, *C. neoformans*, *A. fumigatus*		

[a] l, Local therapy, s, systemic therapy.

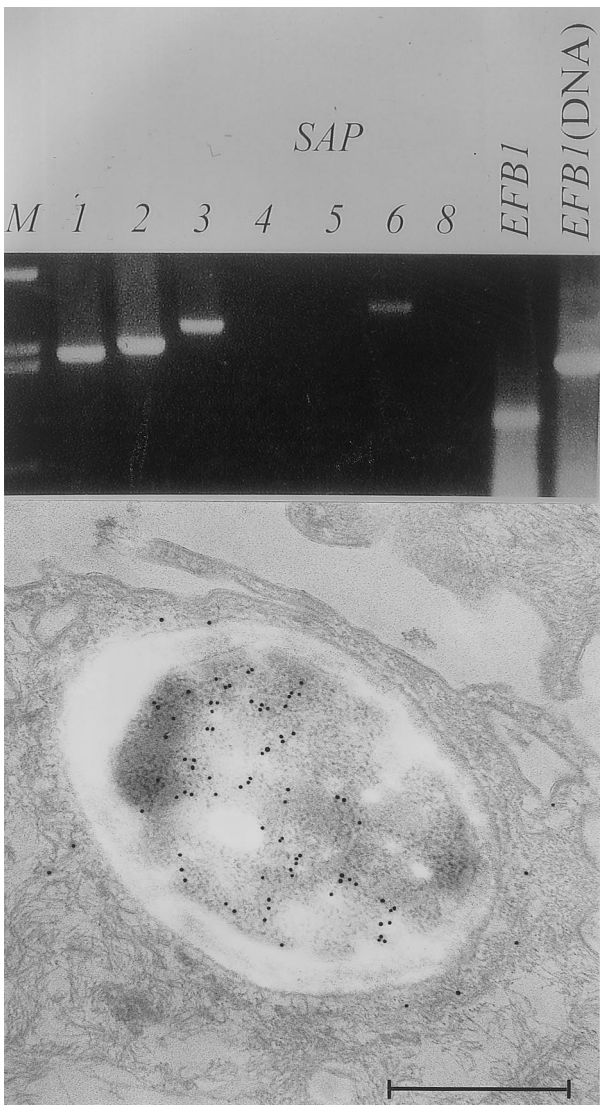

Fig. 5. Detection of secreted aspartic proteinase in oral candidosis in man. Demonstration of expression of *SAP* 1–3,6 by reverse transcription-polymerase chain reaction (RT-PCR; top). Elongation Factor B1 (EFB1) was used to demonstrate the cDNA origin of the template (lane 9). Amplification of genomic DNA with the same set of primers is shown on the extreme right. Molecular mass marker — pBR322 DNA/MvaI (M). Immunoelectron microscopy demonstration of Sap 1–3 using pre-embedding immunogold labelling. Bar represents 0.5 m ([80–82]).

observed after the introduction of HIV-proteinase inhibitors into clinical practice might not only be due to an improved immune status, determined by CD4 cell counts, but also to a direct effect on Sap [88]. This target looks more relevant in the context of future antimycotic drug development as the molecular Koch's/Henle's postulates have been fulfilled, and expression of the protein in man has been demonstrated unambiguously [80,82]. In parallel, new compounds have been developed which address Sap as the direct target — A70450 has been shown to be active against a variety of different fungi including *C. albicans*. Moreover, this compound proved to be efficacious in animal experiments. Most interestingly, Sap is not only found in *Candida* spp. but can also be detected in *Aspergillus* spp. [89–92].

6. Drugs indicated for other diseases

Facing the obvious need for completely new antimycotic agents, the chemical entities developed to date for indications other than mycoses should also be considered (Table 3). This approach looks particularly rewarding as the toxicity profile of the drug under consideration is known from the start. The anti-ulcer drug omeprazole, which acts as an inhibitor of ATPase in the proton-pump in the cytoplasmic membrane of oxyntic cells of the gastric mucosa, might be of interest for inhibiting the corresponding enzyme in fungi [93–95]. Beta-blocking agents used for cardiovascular diseases such as arterial hypertension, might also inhibit tissue penetration by fungi [96]. Zaragozic acids lower serum-cholesterol by inhibiting squalene-synthase and this may be of interest in the context of ergosterol biosynthesis in the fungal cell [97]. The natural product tacrolimus, currently used as an immunosuppressive agent in various indications ranging from the prevention of renal transplant rejection to psoriasis vulgaris, binds to an intracellular protein present in mammalian and fungal cells. The complex inhibits calcineurin which leads to the decay of *C. neoformans* cells grown in vitro at 36°C. Unfortunately, in animal experiments, an immunosuppressive effect was observed with this compound due to inhibition of T-cell activation, resulting in death of all experimental animals due to systemic mycosis. However, the principle might still be valid. This is currently under investigation using the analogue L-685818 that does not exhibit any immunosuppressive activity [98].

7. Unconventional approaches

Antimycotic agents, which are of potential use in human diseases, are not only found in fungi but also in protozoa and higher organisms. The cytotoxic hydroxypyridone, fusaricide, secreted by *Fusarium* spp. is one

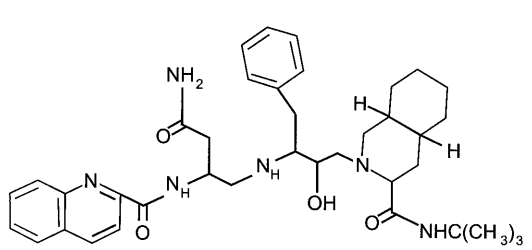

Fig. 6. Structure of saquinavir.

Table 3
Drugs indicated for other diseases but with known antimycotic potency[a]

Major chemical classes	Antifungals	Target/mechanism of action	Spectrum	Resistance	Side-effects
Antacids (s)	Acid-activated omeprazol	Targets the P-type ATPase (plasma membrane proton pump)	*S. cerevisiae* (according to in vitro tests)		
β-Hydroxy-ethylamines	H0733, H0734, H1891, S1978, S6363, S8802, S0384, S1195	Inhibitory effect on tissue penetration	*C. albicans*, *T. mentagrophytes*		
Immuno-suppressant (s)	Tacrolimus (FK 506)	FK 506 together with FKBP 12 (intracellular protein) inhibit calcineurin, blocks T-cell activation	*C. neoformans*		Immunosuppression overweighs antifungal action in vivo (murine model)
Quinolones (s)	Ciprofloxacin, trovafloxacin	Inhibit DNA-gyrase	*C. albicans* (only in combination with amphotericin B or fluconazole in a murine model)		
Azasqualenes, II (s)	Squalene maleimide	Inhibits 2,3-oxidosqualene cyclases	*C. albicans*, *S. cerevisiae* (murine)		
Tetracyclic diterpene glycosides	Sordarin	Block ribosomal trans-location by stabilising fungal elongation factor 2-ribosome complex	*S. cerevisiae* (only in vitro)		
Zaragozic acids (ZA) as antimycotica	ZA-A, B, C, D and F group	Inhibit squalene synthase and fungal ergosterol synthesis	Various *Candida* spp., Filamentous fungi, *S. cerevisiae*	All fungi that produce ZA	Lowers Cholesterol

[a] l, Local therapy; s, systemic therapy.

example [99]. Another is a peptide belonging to the family of tachyplesines and a protein known as anti-LPS-factor which are to be found in the horseshoe crab. These agents not only inhibit the growth of Gram-negative and Gram-positive bacteria but also fungi [100].

8. Conclusion

In conclusion, there is hope that the increased need for antifungal agents will be met by new antimycotic agents in the foreseeable future. These agents might vary from modification of existing conventional agents, such as the azoles, to entirely new compounds. They are likely to be developed either by the new approach of rational drug design based on exact characterisation of molecular targets, or by systematic screening of natural agents. It looks probable that immunoreconstitution will play a major role in the improvement of the therapeutic armamentarium.

References

[1] Becker WK, Cioffi WG, McManus AT, et al. Fungal burn wound infection, a 10-year experience. Arch Surg 1991;126:44–8.

[2] Gelfand MS. Candidiasis: implications for intensivists and pulmonologists. Semin Resp Crit Care Med 1997;18:225–34.

[3] Vanden Bossche HV, Warnock D, Dupont B, et al. Mechanisms and clinical impact of antifungal drug resistance. J Med Vet Mycol 1994;32(Suppl. 1):189–202.

[4] White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 1998;11:382–402.

[5] Roilides E, Dignani MC, Anaissie EJ, Rex JH. The role of immunoreconstitution in the management of refractory opportunistic fungal infections. Med Mycol 1998;36:12–25.

[6] Albertson GD, Niimi M, Cannon RD. Jenkinson HF. Multiple efflux mechanisms are involved in *Candida albicans* fluconazole resistance. Antimicrob Agents Chemother 1996;40:2835–41.

[7] Kelly SL, Lamb DC, Kelly DE, et al. Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS-patients caused by defective sterol 5,6-desaturation. FEBS Lett 1997;400:80–2.

[8] Sanglard D, Ischer F, Monod M, Bille J. Susceptibilities of *Candida albicans* multidrug transporter mutants to various antifungal agents and other metabolic inhibitors. Antimicrob Agents Chemother 1996;40:2300–5.

[9] Sanglard D, Kuchler K, Ischer F, Pagani JL, Monod M, Bille J. Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve multidrug transporters. Antimicrob Agents Chemother 1995;39:2378–86.

[10] Tuite MF. Discovery and development of new systemic antifungals. Trends Biotechnol 1996;14:219–20.

[11] Sanglard D, Ischer F, Monod M, Bille J. Cloning of *Candida albicans* genes conferring resistance to azole antifungal agents: characterization of CDR2, a new multidrug ABC transporter gene. Microbiology 1997;143:405–16.

[12] Hartsel S, Bolard J. Amphotericin B: new life for an old drug. Trends Pharmacol Sci 1996;17:445–9.

[13] Mechlinski W, Schaffner CP, Ganis P, Avitabile G. Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B, Tetrahedron Lett 1970, 3873–3876.

[14] Viviani MA, de Marie S, Graybill JR, Yamaguchi H, Anaissie E, Caillot D. New approaches to antifungal chemotherapy. Med Mycol 1998;36:194–206.

[15] Taylor AW, Costello BJ, Hunter PA, MacLachlan WS, Shanks CT. Synthesis and antifungal selectivity of new derivatives of amphotericin B modified at the C-13 position. J Antibiot Tokyo 1993;46:486–93.

[16] Prentice HG, Hann IM, Herbrecht R, et al. A randomized comparison of liposomal versus conventional amphotericin B for the treatment of pyrexia of unknown origin in neutropenic patients. Br J Haematol 1997;98:711–8.

[17] Joseph-Horne T, Hollomon D, Loeffler RST, Kelly SL. Altered P450 activity associated with direct selection for fungal azole resistance. FEBS Lett 1995;374:174–8.

[18] Alexander BD, Perfect JR. Antifungal resistance trends towards the year 2000. Drugs 1997;54:657–78.

[19] Barry AL, Brown SD. In vitro studies of two triazole antifungal agents (voriconazole (UK-109, 496) and fluconazole) against *Candida* species. Antimicrob Agents Chemother 1996;40:1948–9.

[20] Stevens DA. New directions in antifungal therapy. Jpn J Med Mycol 1997;38:141–4.

[21] Georgopapadakou NH. Antifungals targeted to the cell wall. Exp Opin Invest Drugs 1997;6:147–50.

[22] Georgopapadakou NH, Bertasso A. Effects of squalene epoxidase inhibitors on *Candida albicans*. Antimicrob Agents Chemother 1992;36:1779–81.

[23] Georgopapadakou NH, Walsh TJ. Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob Agents Chemother 1996;40:279–91.

[24] Balfour JA, Faulds D. Terbinafine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. Drugs 1992;43:259–84.

[25] German Collaborative Dermatophyte Drug Susceptibility Study Group, Korting HC, Ollert M, Abeck D. Results of German multicenter study of antimicrobial susceptibilities of Trichophyton rubrum and Trichophyton mentagrophytes strains causing tinea unguium. Antimicrob Agents Chemother 1995;39:1206–8.

[26] Graybill JR, Burgess DS, Hardin TC. Key issues concerning fungistatic versus fungicidal drugs. Eur J Microbiol Infect Dis 1997;16:42–50.

[27] Rashid A. New mechanisms of action with fungicidal antifungals. Br J Dermatol 1996;46:1–6.

[28] Haria M, Bryson HM. Amorolfine. A review of its pharmacological properties and therapeutic potential in the treatment of onychomycosis and other superficial fungal infections. Drugs 1995;49:103–20.

[29] Korting HC, Schäfer-Korting M, Zienicke H, Georgii A, Ollert MW. Treatment of tinea unguium with medium and high doses of ultramicrosize griseofulvin compared with that with itraconazole. Antimicrob Agents Chemother 1993;37:2064–8.

[30] Graybill JR. The future of antifungal therapy. Clin Infect Dis 1996;22:S166–78.

[31] Kauffman CA, Carver PL. Antifungal agents in the 1990s. Current status and future developments. Drugs 1997;53:539–49.

[32] Vanden Bossche HV, Dromer F, Improvisi I, Lozano-Chiu M, Rex JH, Sanglard D. Antifungal drug resistance in pathogenic fungi. Med Mycol 1998;36:119–28.

[33] Bartlett MS, Current WL, Goheen MP, et al. Semisynthetic echinocandins affect cell wall deposition of *Pneumocystis carinii* in vitro and in vivo. Antimicrob Agents Chemother 1996;40:1811–6.

[34] Fishman JA. Treatment of infection due to *Pneumocystis carinii*. J Antimicrob Chemother 1998;42:1309–14.
[35] Sawada Y, Tsuno T, Ueki T, Yamamoto H, Fukagawa Y, Oki T. Pradimicin Q, a new pradimicin aglycone, with α-glucosidase inhibitory activity. J Antibiot Tokyo 1993;46:507–10.
[36] Herrmann JL, Dubois N, Fourgeaud M, Basset D, Lagrange PH. Synergic inhibitory activity of amphotericin-B and interferon against intracellular *Cryptoccocus neoformans* in murine macrophages. Antimicrob Agents Chemother 1994;34:1051–8.
[37] Sugar AM, Liu XP, Chen RJ. Effectiveness of quinolone antibiotics in modulating the effects of antifungal drugs. Antimicrob Agents Chemother 1997;41:2518–21.
[38] Erol DD, Calis U, Demirdamar R, Yulug N, Ertan M. Synthesis and biological activities of some 3,6-disubstituted thiazolo (3,2-b) (1,2,4) triazoles. J Pharm Sci 1995;84:462–5.
[39] Karyotakis NC, Dignani MC, Hachem R, Anaissie EJ. Activities of D0870, a novel triazole, against *Candida lusitaniae* and *Trichosporon beigelii* in experimental murine infections. Antimicrob Agents Chemother 1995;39:571–3.
[40] Venkateswarlu K, Denning DW, Manning NJ, Kelly SL. Comparison of D0870, a new triazole antifungal agent, to fluconazole for inhibition of *Candida albicans* cytochrome P-450 by using in vitro assays. Antimicrob Agents Chemother 1996;40:1382–6.
[41] Wardle HM, Law D, Moore CB, Mason C, Denning DW. In vitro activity of D0870 compared with those of other azoles against fluconazole-resistant *Candida* spp. Antimicrob Agents Chemother 1995;39:868–71.
[42] Yotsuji A, Shimizu K, Araki H, et al. T-8581, a new orally and parenterally active triazole antifungal agent: in vitro and in vivo evaluations. Antimicrob Agents Chemother 1997;41:30–4.
[43] Klopman G, Ptchelintsev D. Antifungal triazole alcohols: a comparative analysis of structure-activity, structure-therapeutic index relationships using the Multiple Computer-Automated Structure Evaluation (Multi-CASE) methodology. J Comput Aided Mol Des 1993;7:349–62.
[44] Schaefer-Korting M, Blechschmidt J, Korting HC. Clinical use of oral nystatin in the prevention of systemic candidosis in patients at particular risk. Mycoses 1996;39:329–39.
[45] Lees ND, Skaggs B, Kirsch DR, Bard M. Cloning of the late genes in the ergosterol biosynthetic pathway of *Saccharomyces cerevisiae* — a review. Lipids 1995;30:221–6.
[46] Korting HC, Grundmann-Kollmann M. The hydroxypyridones: a class of antimycotics of its own. Mycoses 1997;40:243–7.
[47] Clohessy PA, Golden BE. The mechanism of calprotectin's candidastatic activity appears to involve zinc chelation. Biochem Soc Trans 1996;24:309S.
[48] Braga PC, Dal Sasso M, Maci S, Piatti G, Dannhorn DR, Bohn M. Inhibition of *Candida albicans* adhesiveness to human buccal and vaginal cells by sub-inhibitory concentrations of rilopirox. Arzneimittelforschung 1995;45:84–7.
[49] Del Poeta M, Toffaletti DL, Rude TH, Dykstra CC, Heitman J, Perfect JR. Topoisomerase I is essential in *Cryptococcus neoformans*: role in pathobiology and as an antifungal target. Genetics 1999;152:167–78.
[50] Deschenes RJ, Lin H, Ault AD, Fassler JS. Antifungal properties and target evaluation of three putative bacterial histidine kinase inhibitors. Antimicrob Agents Chemother 1999;43:1700–3.
[51] Bartizal K, Abruzzo GK, Trainor C, et al. In vitro antifungal activities and in vivo efficacies of 1,3-β-D-glucan synthesis inhibitors L-671,329, L-646,991, tetrahydroechinocandin B and L-687,781, a papulacandin. Antimicrob Agents Chemother 1992;36:1648–57.
[52] Bartizal K, Scott T, Abruzzo GK, et al. In vitro evaluation of the pneumocandin antifungal agent L-733560, a new water-soluble hybrid of L-705589 and L-731373. Antimicrob Agents Chemother 1995;39:1070–6.
[53] Denning DW. Echinocandins and pneumocandins — a new antifungal class with a novel mode of action. J Antimicrob Chemother 1997;40:611–4.
[54] Brummer E, Chauhan SD, Stevens DA. Collaboration of human phagozytes with LY 303366 for antifungal activity against *Aspergillus fumigatus*. J Antimicrob Chemother 1999;43:491–6.
[55] Debono M. Echinocandin lipopeptide antifungal agents: new agents and recent chemical modification studies. Exp Opin Ther Patents 1995;5:771–86.
[56] Debono M. The echinocandins: antifungals targeted to the fungal cell wall. Exp Opin Invest Drugs 1994;3:821–9.
[57] Debono M, Gordee R. Antibiotics that inhibit fungal cell wall development. Annu Rev Microbiol 1994;48:471–97.
[58] Debono M, Turner WW, LaGrandeur L, et al. Semisynthetic chemical modification of the antifungal lipopeptide echinocandin B (ECB): structure–activity studies of the lipophilic and geometric parameters of polyarylated acyl analogs of ECB. J Med Chem 1995;38:3271–81.
[59] Douglas CM, D'Ippolito JA, Shei G, et al. Identification of the FKS1 gene of *Candida albicans* as the essential target of 1,3-β-D-glucan synthase inhibitors. J Antimicrob Chemother 1997;41:2471–9.
[60] Douglas CM, Marrinan JA, Li W, Kurtz MB. A *Saccharomyces cerevisiae* mutant with echinocandin-resistant 1,3-β-D-glucan synthase. J Bacteriol 1994;176:5686–96.
[61] Kurtz MB, Douglas CM. Lipopeptide inhibitors of fungal glucan synthase. J Med Vet Mycol 1997;35:79–86.
[62] Kurtz MB, Douglas C, Marrinan J, et al. Increased antifungal activity of L-733,560, a water-soluble, semisynthetic pneumocandin, is due to enhanced inhibition of cell wall synthesis. Antimicrob Agents Chemother 1994;38:2750–7.
[63] Aller AI, Martin-Mazuelos E, Morilla MD, Montero O. Antifungal activity of LY295337 in vitro against clinical isolates of *Candida* spp. Chemotherapy 1995;41:276–80.
[64] Krishnarao TV, Galgiani JN. Comparison of the in vitro activities of the echinocandin LY 303366, the pneumocandin MK-0991, and fluconazole against *Candida* species and *Cryptococcus neoformans*. Antimicrob Agents Chemother 1997;41:1957–60.
[65] Pfaller MA, Messer SA, Coffman S. In vitro susceptibilities of clinical yeast isolates to a new echinocandin derivative, LY 303366, and other antifungal agents. Antimicrob Agents Chemother 1997;41:763–6.
[66] Uzun O, Kocagoz S, Cetinkaya Y, Arikan S, Unal S. In vitro activity of a new echinocandin, LY303366, compared with those of amphotericin B and fluconazole against clinical yeast isolates. Antimicrob Agents Chemother 1997;41:1156–7.
[67] Karlowsky JA, Harding GAJ, Zelenitsky SA, et al. In vitro kill curves of a new semisynthetic echinocandin, LY-303366, against fluconazole-sensitive and -resistant *Candida* species. Antimicrob Agents Chemother 1997;41:2576–8.
[68] Ernst ME, Klepser ME, Wolfe EJ, Pfaller MA. Antifungal dynamics of LY 303366, an investigational echinocandin B analog, against *Candida* spp. Diagn Microbiol Infect Dis 1996;26:125–31.
[69] Zornes LL, Stratford RE. Development of a plasma high-performance liquid chromatographic assay for LY 303366, a lipopeptide antifungal agent, and its application in a dog pharmacokinetic study. J Chromatogr B 1997;695:381–7.
[70] Zhanel GG, Karlowsky JA, Harding GA, et al. In vitro activity of a new semisynthetic echinocandin, LY-303366, against systemic isolates of *Candida* species, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and *Aspergillus* species. Antimicrob Agents Chemother 1997;41:863–5.

[71] Abruzzo GK, Flattery AM, Gill CJ, et al. Evaluation of water-soluble pneumocandin analogs L-733560, L-705589, L-731373 with mouse models of disseminated aspergillosis, candidiasis and cryptococcosis. Antimicrob Agents Chemother 1995;39:1077–81.
[72] Vazquez JA, Lynch M, Sobel JD. In vitro activity of a new pneumocandin antifungal agent, L-733560 against azole-susceptible and -resistant *Candida* and *Torulopsis* species. Antimicrob Agents Chemother 1995;39:2689–91.
[73] Walsh TJ, Giri N. Pradimicins: a novel class of broad-spectrum antifungal compounds. Eur J Clin Microbiol Infect Dis 1997;16:93–7.
[74] Wardle HM, Law D, Denning DW. In vitro activity of BMS-181184 compared with those of fluconazole and amphotericin B against various *Candida* spp. Antimicrob Agents Chemother 1996;40:2229–31.
[75] El-Sherbeini M, Clemas JA. Nikkomycin Z supersensitivity of an echinocandin-resistant mutant of *Saccharomyces cerevisiae*. Antimicrob Agents Chemother 1995;39:200–7.
[76] Gaughran JP, Lai MH, Kirsch DR, Silverman SJ. Nikkomycin Z is a specific inhibitor of *Saccharomyces cerevisiae* chitin synthase isozyme Chs3 in vitro and in vivo. J Bacteriol 1994;176:5857–60.
[77] Fung-Tomc JC, Minassian B, Huczko E, Kolek B, Bonner DP, Kessler RE. In vitro antifungal and fungicidal spectra of a new pradimicin derivative, BMS-181184. Antimicrob Agents Chemother 1995;39:295–300.
[78] Furumai T, Saitoh K, Kakushima M, et al. BMS-181184, a new pradimicin derivative. Screening, taxonomy, directed biosynthesis, isolation and characterization. J Antibiot Tokyo 1993;46:265–74.
[79] Saitoh K, Suzuki K, Hirano M, Furumai T, Oki T. Pradimicins FS and FB, new pradimicin analogs: directed production, structures and biological activities. J Antibiot Tokyo 1993;46:398–405.
[80] Schaller M, Hube B, Ollert MW, et al. In vivo expression and localization of *Candida albicans* secreted aspartyl proteinases during oral candidiasis in HIV-infected patients. J Invest Dermatol 1999;112:383–6.
[81] Schaller M, Korting HC, Schaefer W, Bastert J, Chen WC, Hube B. Secreted aspartic proteinase (Sap) activity contributes to tissue damage in a model of human oral candidosis. Mol Microbiol 1999;34:169–80.
[82] Schaller M, Schaefer W, Korting HC, Hube B. Differential expression of secreted aspartyl proteinases in a model of human oral candidosis and in a patient samples from the oral cavity. Mol Microbiol 1998;29:605–15.
[83] Apad-Zapatero C, Goldman R, Muchmore SW, et al. Structure of a secreted aspartic protease from *C. albicans* complexed with a potent inhibitor: implications for the design of antifungal agents. Protein Sci 1996;5:640–52.
[84] Hube B. *Candida albicans* secreted aspartyl proteinases. Curr Top Med Mycol 1996;7:55–69.
[85] Odds FC. *Candida* species and virulence. ASM News 1994;60:313–8.
[86] Ollert MW, Wende C, Goerlich M, et al. Increased expression of *Candida albicans* secretory proteinase, a virulence factor, in isolates from human immunodeficiency virus-positive patients. J Clin Microbiol 1995;33:2543–9.
[87] Korting HC, Schaller M, Eder G, Hamm G, Böhmer U, Hube B. Effects of the human immunodeficiency virus (HIV) proteinase inhibitors saquinavir and indinavir on in vitro activities of secreted aspartyl proteinases of *Candida albicans* isolates from HIV-infected patients. Antimicrob Agents Chemother 1999;43:2038–42.
[88] Hoegl L, Thoma-Greber E, Roecken M, Korting HC. HIV protease inhibitors influence the prevalence of oral candidosis in HIV-infected patients: a 2-year study. Mycoses 1998;41:321–5.
[89] Reichard U. The significance of secretory and structure-associated proteases of *Aspergillus fumigatus* for the pathogenesis of invasive aspergillosis. Mycoses 1998;41(Suppl. 1):78–82.
[90] Reichard U, Eiffert H, Rüchel R. Purification and characterization of an extracellular aspartic proteinase from *Aspergillus fumigatus*. J Med Vet Mycol 1995;33:279.
[91] Reichard U, Monod M, Rüchel R. Molecular cloning and sequencing of the gene encoding an extracellular aspartic proteinase from *Aspergillus fumigatus*. FEMS Microbiol Lett 1995;130:69–74.
[92] Rüchel R. Proteinase. In: Bennett JE, Hay RJ, Peterson PK, editors. New Stratagies in Fungal Diseases. Edinburgh: Churchill Livingstone, 1992:17–31.
[93] Monk BC, Mason AB, Abramochkin G, Haber JE, Seto-Young D, Perlin DS. The yeast plasma membrane proton pumping ATPase is a viable antifungal target. I. Effects of the cysteine-modifying reagent omeprazole. Biochim Biophys Acta 1995;1239:81–90.
[94] Monk BC, Mason AB, Kardos TB, Perlin DS. Targeting the fungal plasma membrane proton pump. Acta Biochim Pol 1995;42:481–96.
[95] Monk BC, Perlin DS. Fungal plasma membrane proton pumps as promising new antifungal targets. Crit Rev Microbiol 1994;20:209–23.
[96] Hanel H, Kirsch R, Schmidts HL, Kottmann H. New systematically active antimycotics from the beta-blocker category. Mycoses 1995;38:251–64.
[97] Bergstrom JD, Dufresne C, Bills GF, Nallin-Omstaed M, Byrne K. Discovery, biosynthesis and mechanism of action of the zaragozic acids: potent inhibitors of squalene synthase. Annu Rev Microbiol 1995;49:607–39.
[98] Odom A, del Poeta M, Perfect J, Heitman J. The immunosuppressant FK506 and its nonimmunosuppressive analog L-685,818 are toxic to *Cryptococcus neoformans* by inhibition of a common target protein. Antimicrob Agents Chemother 1997;41:156–61.
[99] McBrien KD, Gao Q, Huang S, et al. Fusaricide, a new cytotoxic *N*-hydroxypyridone from *Fusarium* spp. J Nat Prod 1996;59:1151–3.
[100] Saito T, Kawabata S, Shigenaga T, et al. A novel big defensin identified in horseshoe crab hemocytes: isolation, amino acid sequence and antibacterial activity. J Biochem Tokyo 1995;117:1131–7.
[101] Cutfield SM, Dodson EJ, Anderson BF, et al. The crystal structure of a major secreted aspartic proteinase from *Candida albicans* incomplexes with two inhibitors. Structure 1995;3:1261–71.