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Recent Approaches to Antifungal Therapy for Invasive Mycoses

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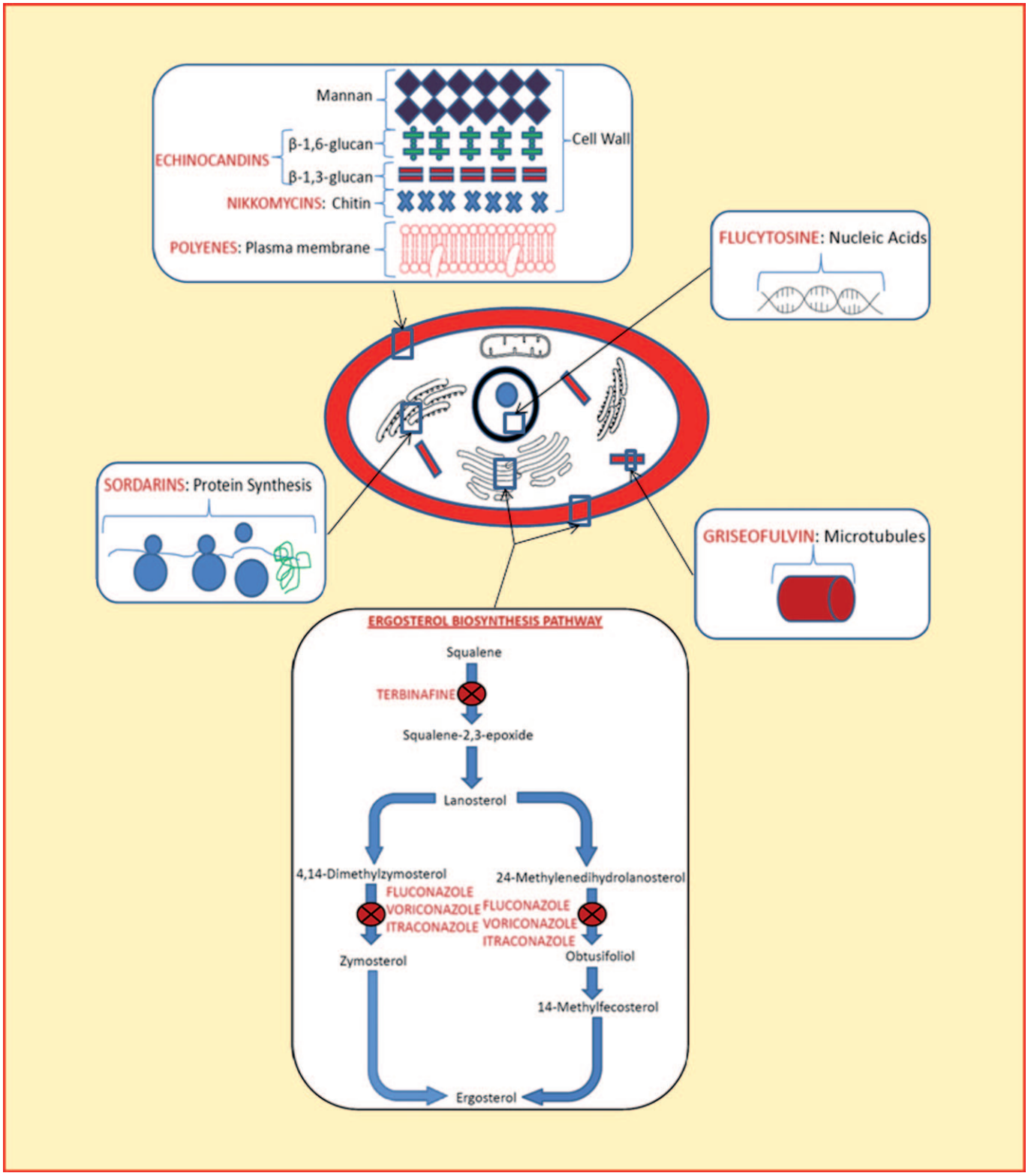

Invasive fungal infections with primary and opportunistic mycoses have become increasingly common in recent years and pose a major diagnostic and therapeutic challenge. They represent a major area of concern in today's medical fraternity. The occurrence of invasive fungal diseases, particularly in AIDS and other immunocompromised patients, is life-threatening and increases the economic burden. Apart from the previously known polyenes and imidazole-based azoles, newly discovered triazoles and echinocandins are more effective in terms of specificity, yet some immunosuppressed hosts are difficult to treat. The main reasons for this include antifungal resistance, toxicity, lack of rapid and microbe-specific diagnoses, poor penetration of drugs into sanctuary sites, and lack of oral or intravenous preparations. In addition to combination antifungal therapy, other novel antimycotic treatments such as calcineurin signaling pathway blockers and vaccines have recently emerged. This review briefly summarizes recent developments in the pharmacotherapeutic treatment of invasive fungal infections.

1. Introduction

The incidence of invasive fungal infections (mycoses) has continued to increase over the past two decades through widespread soil or airborne transmission of the systemic fungal pathogens. These mycoses are a major cause of morbidity and mortality and are difficult to diagnose and treat. The large number of immunocompromised patients, especially transplant recipients, are the most vulnerable sufferers, as mycoses grow rapidly and infection spreads quickly, hence treatment becomes cumbersome. A study conducted in 49 hospitals in the United States over a span of three years revealed that *Candida* spp. are the fourth most common cause of nosocomial bloodstream infections.[1] In addition to *Aspergillus* spp., other newly discovered moulds such as *Fusarium* spp., *Scedosporium* spp., and *Zygomycetes* have emerged as the leading causes of various mycotic infections, with high mortality rates in hematopoietic stem cell transplant recipients. Treatment of these infections is limited with currently available antifungals.[2]

At present, several antimycotic agents are known, but some critically ill and susceptible patients remain difficult to treat. The main reasons for this include delayed diagnosis, drug toxicity and development of antifungal drug resistance, drug bioavailability, and a lack of oral or intravenous preparations. Although the problem of toxicity has been significantly addressed by the development of several broad-spectrum imidazole-, triazole-, and echinocandin-based derivatives for specific use, drug resistance remains a problem with newly discovered antifungal drugs.[3] Despite recent advances in chemotherapy, the treatment of most fungal diseases is far from satisfactory. This review briefly summarizes recently available fungicides as well as novel targets, such as the calcineurin signaling pathway, for some compounds currently under evaluation, and the possibility of vaccination for the treatment of invasive mycoses.

2. Antifungal Agents

Herein, both systemic and superficial fungicides, which have been or are currently under evaluation for use in combating invasive fungal pathogens, are broadly classified into polyenes (e.g. amphotericin B), azoles (e.g. voriconazole), newly introduced echinocandins (e.g. caspofungin), and various other antifungal agents (e.g. allylamines such as terbinafine).

2.1. Polyenes

This family constitutes the major class of fungal antibiotics. Although many polyenes have been isolated from *Streptomyces* species, only amphotericin B (AmB), nystatin, pimaricin, and candicidin have shown therapeutic application (Table 1). Only AmB and nystatin are in current widespread use; the others have become a commodity of the past. Structurally, these drugs contain conjugated *trans*-configured double bonds in a large lactone macrolide ring system, and thus exhibit characteristic UV/Vis absorption spectra (Figure 1). Most importantly, the polar hydroxy groups are attached opposite to the double bonds; this structural characteristic gives an amphipathic nature to these molecules, which in turn is responsible for their biological activity.[4,5] In addition, studies of the interaction between polyene antibiotics and cholesterol[6–8] have shown that polyenes interact with the ergosterol component of the fungal membrane and lead to the formation of a complex with the hydrophobic outer core. The inner core of the polyene–sterol complex is hydrophilic due to the presence of hydroxy groups, and provides a necessary aqueous pore, which causes cell leakage and ultimately cell death. Nystatin forms pores similar to those of amphotericin B, but are smaller due to the shorter hydrophobic region. Interestingly, the pimaricin–cholesterol complex does not lead to the formation of aqueous pores, but does affect cell permeability.

Amphotericin B, a 'gold standard' of antifungal therapy, was initially isolated by Gold and co-workers in 1955 from *Streptomyces nodosus*, recovered from soil samples obtained at Tembladora on the banks of the Orinoco river in Venezuela.[9] Its broad-spectrum in vitro antifungal activity was investigated in the 1960s against most fungal pathogens, yet some AmB-resistant variants such as *Trichosporan beigelii*, *Aspergillus terreus*, *Pseudallesheria boydii*, *Malassezia furfur*, and *Fusarium* species were also found.[10–14] AmB was determined to be active against *Candida albicans* and most other *Candida* species.[15] Owing to its poor water solubility, AmB is not absorbed from the gastrointestinal tract when administered orally; however, an oral preparation is available for the treatment of oral mucosal candidiasis. Although intravenous AmB treatment is the backbone of effective therapy against invasive fungal infec-

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Table 1. The polyene class of antifungal agents.

Entry	Drug	Trade Name	Route[a]	Current Status	Approval Year
1	amphotericin B (AmB)	Fungizone (Apothecon Products, Princeton, NJ, USA)	i.v., p.o.	licensed	1958
2	AmB lipid complex	Abelcet (Liposome, Princeton, NJ, USA)	i.v.	licensed	1995
3	AmB cholesteryl sulfate	Amphotec (SEQUUS Pharmaceuticals, Menlo Park, CA, USA)	i.v.	licensed	1996
4	liposomal AmB	AmBisome (Fujisawa Healthcare, Deerfield, IL, USA)	i.v.	licensed	1997
5	liposomal nystatin	Nyotran (Aronex-Roche, USA)	i.v.	under development	–
6	topical nystatin	Mycostatin (Bristol-Myers Squibb, USA)	topical	licensed	1982
7	pimaricin	Natacyn (Alcon, Switzerland)	ophthalmic	licensed	1982
8	candicidin	Vanobid (Sanofi-Aventis, USA)	topical	discontinued	–

[a] i.v. = intravenous; p.o. = peroral.

Mahendra Nath was born in Unnao, Uttar Pradesh (India) on May 4, 1971. He is currently reader (organic chemistry) in the department of chemistry at the University of Delhi. He earned his PhD (1996) in chemistry from the Central Drug Research Institute, Lucknow (India) under the supervision of Dr. Vishnu Ji Ram. After one year (1996–97) at the medicinal chemistry division of the CDRI as a research associate (CSIR), he worked as a postdoctoral fellow (1997–1998) in the department of medical microbiology and immunology at the University of Alberta, Edmonton (Canada). Following one-year postdoctoral stays, he joined Lupin Laboratories Ltd. in Mandideep (India) as a scientist (1999–2001) in the department of organic synthesis. In December 2001, he was appointed as an NIH postdoctoral researcher in the department of chemistry at Indiana University, Bloomington, IN (USA), where he worked with Professor Jeffrey M. Zaleski for three years prior to joining the University of Delhi. In addition to various aspects of synthetic, heterocyclic, and medicinal chemistry, his current research is focused on novel antimicrobial agents and peripheral functionalization of porphyrins and biomimetic metalloporphyrins to develop new phototherapeutic agents for applications in photodynamic therapy.

Bijoy P. Mathew was born in 1983 in India. He received his BSc (honors, 2004) and MSc (2006) degrees in chemistry from the University of Delhi. He recently completed his MPhil degree in chemistry for which he prepared certain heterocyclic molecules that show significant antifungal activities. He is currently working on his PhD in medicinal chemistry in the department of chemistry at the University of Delhi, under the supervision of Dr. Mahendra Nath. His research interests are concentrated mainly on the development of a library of new compounds as antifungal agents and the study of their structure–activity relationships.

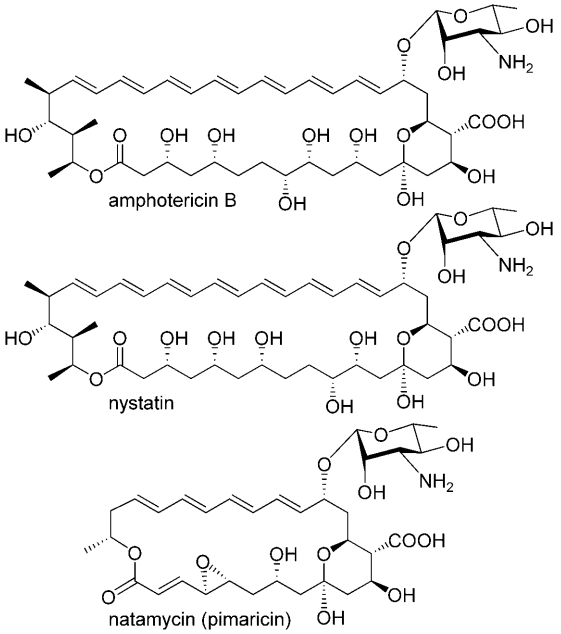

Figure 1. Structures of amphotericin B, nystatin, and natamycin.

tions, topical preparations are also available for the treatment of some superficial fungal infections. The most serious side effect of AmB is nephrotoxicity. The manifestations of nephrotoxicity are azotemia, decreased glomerular filtration, and loss of urinary concentrating capacity, renal loss of sodium and potassium ions, and renal tubular acidosis.[16] The renal injury decreases the production of erythropoietin and leads to normocytic-normochromic anemia.[17] A study has shown that about 30% of individuals under AmB treatment succumb to acute renal failures (ARF). Furthermore, increased mortality, prolonged hospital stays, and increased economic costs due to ARF exacerbate the problem.[18,19]

To avoid the side effects associated with AmB, various lipid formulations are available that are less toxic and can therefore be administered in larger doses. Lipid formulations that are currently on the market include an AmB colloidal dispersion (Amphocil/Amphotec, Intermune Pharmaceuticals, USA), an AmB lipid complex (Abelcet, Enzon Pharmaceuticals, USA), and liposomal AmB (AmBisome, Gilead Sciences, USA).

The AmB colloidal dispersion (ABCD) is an AmB–cholesteryl sulfate complex that forms a colloidal suspension in aqueous solutions, thus allowing the transport of AmB in disk-shaped colloidal particles.[20,21] The efficacy of this formulation is similar to that of parent AmB. ABCD is generally administered intravenously at doses of 3–6 mg kg^{-1}.[22] One of the major disadvantages of ABCD relative to other lipid formulations is an increased incidence of acute infusion-related toxic reaction (hypoxia and chills).[23] ABCD is currently licensed for use in the treatment of invasive fungal infections when the patient is refractory toward AmB or when the drug is unacceptably toxic. Its potency in different clinical settings is under investigation. However, the higher incidence of acute toxic reactions to ABCD appears to be a significant drawback and limits its clinical use relative to the other lipid formulations.

Another lipid formulation, AmB–lipid complex (ABLC), consists of amphotericin B complexed with a biodegradable phospholipid matrix composed of L-α-dimyristoylphosphatidylcholine and L-α-dimyristoylphosphatidylglycerol, which releases amphotericin B through the action of cellular phospholipases.[24] Its in vitro activity is similar to the parent amphotericin B deoxycholate. After ABLC administration, the level of AmB in the blood is decreased, but it appears in higher concentrations in the liver, spleen, and lungs. The mechanism by which ABLC decreases toxicity relative to conventional preparations has been a topic of interest. It has been speculated that the ribbon-like structure of the AmB–lipid complex limits the quantity of free drug which thus lowers the toxicity of ABLC.[25] Similar to other lipid formulations, nephrotoxicity is greatly diminished. ABLC has been approved for the treatment of invasive fungal infections as a second-line therapy. Moreover, it has been recommended for the long-term treatment of serious mycoses refractory to conventional antifungal therapy.

Liposomal AmB (L-AMB) is composed of amphotericin B complexed with hydrogenated soy phosphatidylcholine (HSPC), distearoylphosphatidylglycerol (DSPG), and cholesterol.[26] Unlike other lipid formulations of AmB, it is a true liposome composed of unilamellar lipid vesicles. Inclusion of cholesterol in the liposomal structure contributes to the decreased toxicity of the formulation, increased bilayer stability, and a lower affinity for mammalian cholesterol.[27] Thus AmB is selectively transferred to the fungal target from the liposome, avoiding uptake by mammalian cells. As usual, nephrotoxicity is decreased, but mild infusion-related side effects,[28] anaphylactic reactions,[29] reversible hepatic dysfunction, and hypernatremia have been observed. The approved dosages for intravenous use are 3 mg kg^{-1} for empirical fungal infection, 3–5 mg kg^{-1} for systemic mycoses, and 6 mg kg^{-1} for *Cryptococcus* meningitis in adults. Interestingly, liposomal amphotericin B is also licensed for the treatment of the protozoan infection of kala-azar or visceral leishmaniasis.[30,31]

As illustrated by various in vivo and in vitro clinical studies, the lipid-associated AmB formulations are less toxic than the conventional amphotericin B deoxycholate. In spite of this, the high cost and lack of information about their efficacy in the treatment of proven invasive candidiasis limits their use as a first-line therapy. A new formulation of AmB, NS-718 (Nippon Shinyaku Co., Japan), was developed through the use of lipid nanospheres. Each vial of NS-718 contains amphotericin B encapsulated in nanoparticles composed of egg lecithin, soybean oil, and maltose.[32,33] To curtail toxicity arising from agglomeration of AmB particles in aqueous solution, various water-soluble formulations are under development, including amphotericin B methyl ester associated with *N*-methyl-*N*-D-fructose,[34] AmB encapsulated in micelles formed by poly(ethylene oxide)-*block*-poly(L-aspartate),[35] as well as AmB conjugated with oxidized arabinogalactan.[36] These water-soluble formulations prevent agglomeration of AmB molecules and thus show a significant decrease in nephrotoxicity.[37]

Nystatin was discovered by Brown and Hazen in 1949 from *Streptomyces noursei*, obtained from soil samples in Virginia, USA.[38] The similarity between fungal and mammalian cell membranes, namely the presence of sterols in both cell types (cholesterol in mammals and ergosterol in fungi), means that polyenes can form pores that affect the permeability of both pathogen and host cells. However, ergosterol-containing membranes are more sensitive to nystatin than those possessing cholesterol.[39,40] Therefore, nystatin can be therapeutically applied in the treatment of fungal infections. However, nystatin's low absorption in the gut and high toxicity are limiting factors in its application as an oral or topical therapy. Its spectrum of antifungal activity is broader than that of amphotericin B, and therefore the intravenous administration of nystatin could be more effective. Furthermore, like L-AMB, nystatin can be incorporated into liposomes (Nyotran, Aronex Pharmaceuticals, USA); in this form it does not lose its therapeutic properties, while its toxicity is profoundly decreased. The liposomal formulation of nystatin is as effective as liposomal amphotericin B, and even more active than AmB deoxycholate or AmB–lipid complex.[41] Importantly, nystatin in this form is also effective against amphotericin B-resistant infections.[42–46] It is active toward a wide variety of fungal pathogens including yeasts and moulds such as *Candida*, *Aspergillus*, *Histoplasma*, and *Coccidioides*. It is currently in late phase III clinical trials.

Three linear polyene antibiotics, mediomycins A and B, and clethramycin (Figure 2), were recently isolated from *Streptomy-*

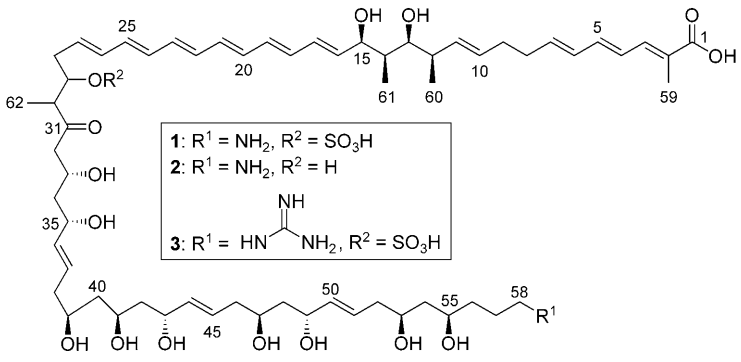

Figure 2. Structures of mediomycin A (**1**), mediomycin B (**2**), and clethramycin (**3**).

ces mediocidicous (ATCC 23936). All three compounds contain a conjugated oxotriene group and a hexaene moiety. Among these, clethramycin had been isolated earlier from *Streptomyces hygroscopicous*. All three compounds were shown to exhibit a broad spectrum of activities against yeasts and various filamentous fungi.[47]

2.2. Azoles

This is the second largest class of compounds active against fungal infections. Imidazole-based drugs such as clotrimazole, miconazole, and ketoconazole were among the first azole compounds explored. Of these, only ketoconazole was available for systemic treatment. Later, triazole-based antifungal drugs such as fluconazole, itraconazole, voriconazole, posaconazole, and ravuconazole appeared on the market. These drugs, particularly voriconazole, posaconazole, and ravuconazole, are more specific in their mode of action and are applicable as both superficial and systemic fungicidal agents.

The mode of action of azoles is depicted in Figure 3. They mainly inhibit the cytochrome P450-dependent enzyme lanosterol demethylase (14α-sterol demethylase or $P450_{DM}$), a

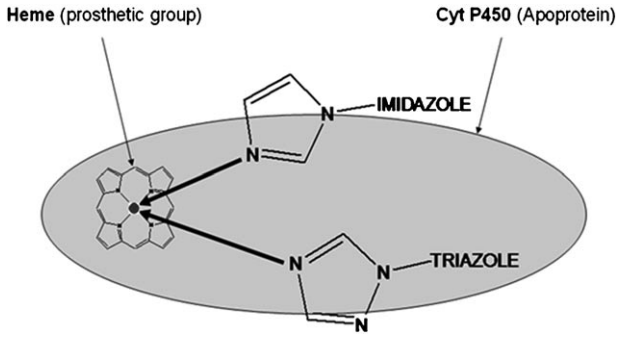

Figure 3. A schematic diagram showing the binding of cytochrome P450 with azole-based fungicides.

major component of the fungal plasma membrane.[48,49] Although this enzyme is also present in mammals, at required therapeutic concentrations, azoles have greater affinity for fungal $P450_{DM}$. Therefore, exposure of fungi to azoles causes a depletion of ergosterol and an accumulation of 14α-methylated sterol. This disrupts both the structure of the membrane and its functions in nutrient transport and chitin synthesis. The net effect is inhibition of fungal growth. Ergosterol also has a hormone-like ("sparking") function in fungal cells, which stimulates growth and proliferation. This function may be disrupted when ergosterol is depleted by $>90\%$.

Resistance to azoles can occur by mutations that modify the target molecule or by the expression of efflux pumps that hinder the accumulation of the drug in fungal cells. Combinations of both mechanisms have been detected in some *C. albicans* isolates; in clinical terms, however, the consequences of azole resistance have been minimal so far, except in cases of AIDS-associated oropharyngeal *Candida* infections.[50]

At present the second generation[51] of triazole molecules includes voriconazole, ravuconazole, and posaconazole (Table 2). The structure of voriconazole and ravuconazole are related to fluconazole, whereas posaconazole bears a close resemblance to itraconazole (Figure 4). Furthermore, slight differences in the structure of these azoles are responsible for their variation in antifungal potency, bioavailability, drug interaction, and toxicity.

Voriconazole was approved in May of 2002 by the US Food and Drug Administration (FDA) for the primary treatment of acute invasive aspergillosis and salvage therapy for infrequent but serious fungal infections caused by the pathogens *Scedosporium apiospermum* and *Fusarium* species. Later, it was also approved for the treatment of invasive candidiasis. Voriconazole interacts with various drugs (benzodiazepines, prednisolone, digoxin, terfenadine, cisapride, astemizole, etc.) that are also substrates of cytochrome P450 3A4. This causes a problem

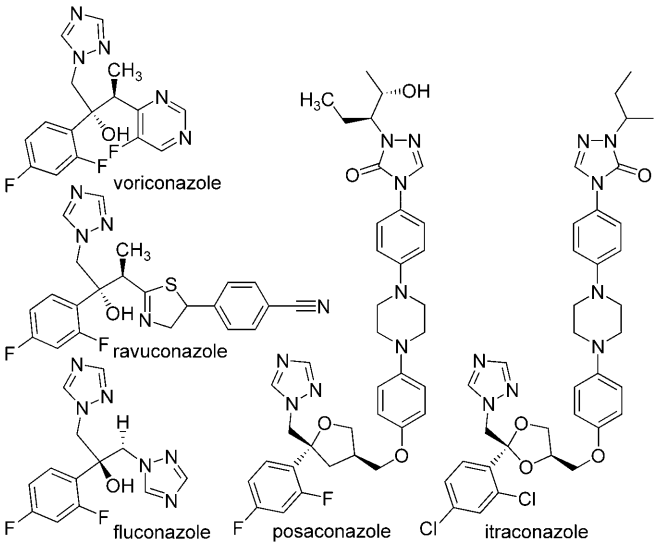

Figure 4. The azole class of antifungal agents.

Table 2. The azole class of antifungal agents.

Entry	Drug	Trade Name	Route[a]	Current Status	Approval Year
1	fluconazole	Diflucan (Pfizer)	i.v., p.o.	licensed	1993
2	itraconazole	Sporanox (Janssen)	i.v., p.o.	licensed	1992
3	voriconazole	Vfend (Pfizer)	i.v., p.o.	licensed	2002
4	posaconazole	Noxafil (Schering)	p.o.	licensed	2006
5	ravuconazole	Ravuconazole (Eisai/Bristol-Myers Squibb)	p.o.	discontinued	–

[a] i.v. = intravenous; p.o. = peroral.

for physicians when prescribing multiple medicines for seriously immunocompromised patients.[52,53]

Voriconazole is available in both oral and intravenous forms with excellent bioavailability (90%). Furthermore, it is metabolized by the liver, and its excretion is not affected by renal failure. An evaluation of the clinical records of all patients treated with voriconazole at the Fred Hutchinson Cancer Institute indicates the rise of resistant species, such as *Zygomyces* and *Candida glabrata*, responsible for bloodstream infection.[54] The use of voriconazole as a first-line therapy for the treatment of invasive aspergillosis resulted in significantly fewer deaths and increased hospital-free survival of patients.[55]

Posaconazole is a synthetic analogue of SCH-51048, which is highly soluble in water.[56] It was approved by the FDA in 2006 for prophylaxis against invasive *Aspergillus* and *Candida* infections. As with voriconazole, it also shows interaction with other drugs, but to a lesser extent relative to other triazoles.[57] Posaconazole is orally bioavailable and well tolerated by healthy human adults. Its toxicity profile is quite favorable, as concluded from a variety of clinical studies.[58–60] Recently, it was found that posaconazole is effective against mucormycosis, a deadly fungal infection caused by *Rhizopus oryzae*.[61]

Ravuconazole (BMS-207147) is another broad-spectrum antifungal triazole[62] originally discovered by Eisai Co. Ltd. (Japan) for the treatment of systemic fungal infections. Bristol-Myers Squibb signed a licensing agreement with Eisai and carried out the clinical development of ravuconazole. It has shown a high degree of in vitro efficacy against a wide range of fungi, including *Candida* spp., *C. neoformans*, other yeast species, and fluconazole-resistant isolates.[63,64] However, it does not appear to be active in vitro against *Fusarium*, *Scedosporium prolificans* and several species of *Mucorales*. An in vitro evaluation of ravuconazole against 923 clinical isolates of non-dermatophyte filamentous fungi indicated that the drug is active against *Aspergillus* spp., other species of hyaline filamentous fungi, black moulds, and zygomycetes.[65] In comparison with voriconazole, ravuconazole was found active and has shown 56% inhibition of *Mucorales*. Moreover, a comparative evaluation of the efficacy of ravuconazole and fluconazole against esophageal candidiasis in HIV patients revealed that a better success rate was achieved with ravuconazole (86%) than with fluconazole (78%).[66] By 1999, ravuconazole was undergoing phase II clinical trials in the US. However, in 2004 Eisai terminated the collaboration with Bristol-Myers Squibb, and further clinical studies of this drug were discontinued. In a quest to develop new and effective triazole derivatives, researchers have recently synthesized a series of 1-([1*H*]-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-[(4-substituted-phenyl)-piperazin-1-yl]-propan-2-ol derivatives as inhibitors of cytochrome P450 14α-demethylase (CYP51). Preliminary in vitro results show that all the synthesized compounds have significant antimycotic effects.[67]

2.3. Echinocandins

The echinocandins are secondary metabolites produced from cyclopentamine, which is formed by fermentation in fungi such as *Zalerion arboricola* and *Aspergillus nidulans*. They are water-soluble cyclic lipopeptides that are amphiphilic in nature.[68,69] Furthermore, echinocandins act as inhibitors of the synthesis of β-1,3-glucan, an important component of the fungal cell wall, by blocking the action of a pathway enzyme, most likely β-1,3-glucan synthase. However, structural studies of the enzyme complex in *S. cerevisiae* revealed that the complex consists of two proteins, Fks1p and Fks2p, which are regulated by a GTP binding peptide, Rho1p, and by elements of the calcineurin pathway (Figure 5).[70] Although echinocandins

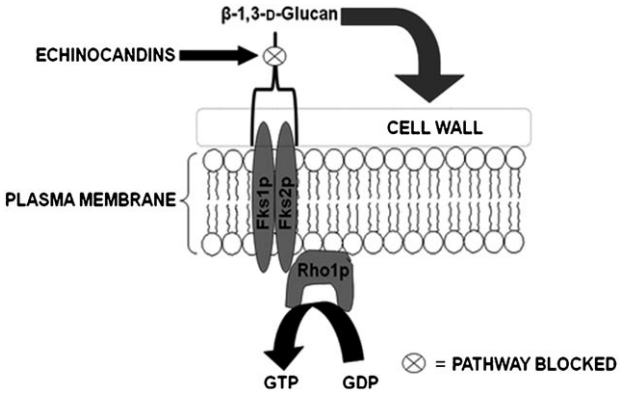

Figure 5. A diagram showing the inhibition of β-1,3-D-glucan synthesis.

bind to Fks1p, their noncompetitive inhibitory effects on glucan synthesis do not necessarily imply that Fks1p itself is the catalytic subunit. Moreover, it is still not clear whether the echinocandin binding site on Fks1p is internal or external to the cell membrane.[52]

The most important drugs in the echinocandin class include caspofungin, micafungin, and anidulafungin (Figure 6 and Table 3).[71] Caspofungin (CFN) was approved by the FDA in 2001 for salvage therapy in cases of invasive aspergillosis for which resistance has appeared toward other antifungal agents such as AmB and lipid-associated formulations of AmB. It is also effective against esophageal and oropharyngeal candidiasis. Furthermore, CFN is fungicidal against *Candida* spp., but fungistatic against *Aspergillus* spp.[72,73] It is not active against *Zygomycetes*, *Cryptococcus neoformans*, or *Fusarium* species at clinically relevant concentrations.[74] The oral bioavailability of CFN is negligible,[75] and therefore the route of administration is intravenous. The recommended dosage is 70 mg day^{-1} for the treatment of invasive aspergillosis, and the duration of therapy depends on the severity of the infection. Caspofungin is cleared by non-oxidative hepatic metabolism, and therefore, the dosages must be decreased for patients with significant hepatic impairment; however, dosage adjustment is not necessary for patients with renal failure. The side effects of CFN are minor, and include fever, headache, nausea, phlebitis, rash, and elevated hepatic enzyme levels.[76]

Micafungin (MFN) is a macrocyclic semisynthetic derivative of the echinocandin-like lipopeptide FR901379, which was isolated from the culture broth of *Coleophoma empetri*, a plant pathogen associated with post-harvest fruit rot in cranberries.[77] It consists of a hexapeptide nucleus with a complex aro-

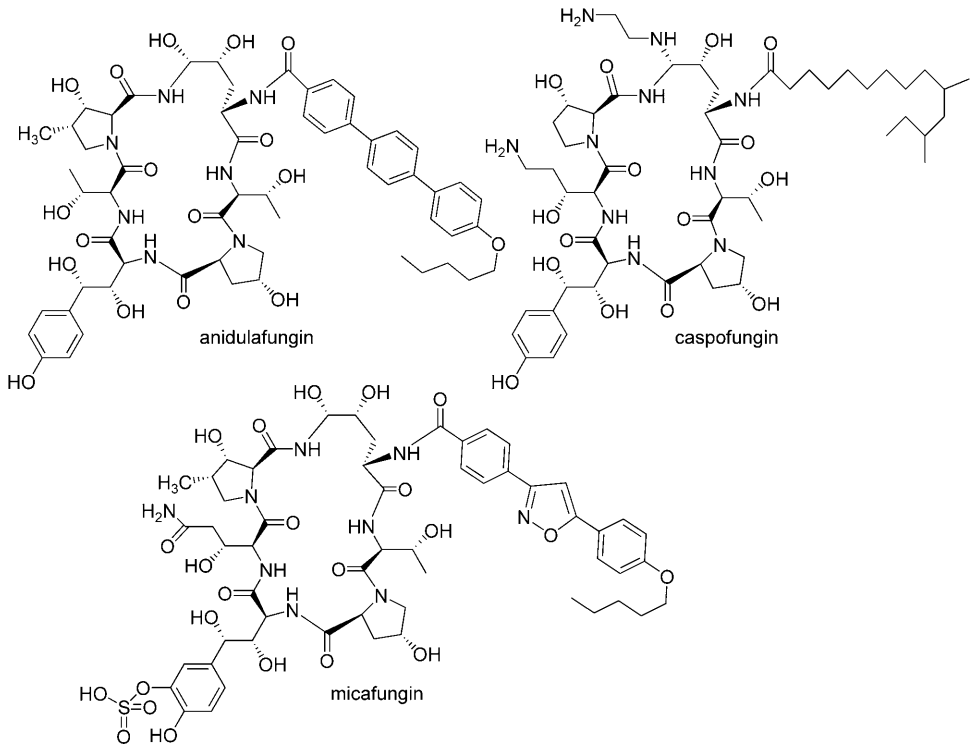

Figure 6. Structures of semisynthetic echinocandins.

matic side chain (3,5-diphenyl-substituted isoxazole) that acylates the N-terminus of the cyclic peptide.[78] MFN was approved in 2005 for the prevention of *Candida* infections in patients undergoing hematopoetic stem cell transplantation or for the treatment of esophageal candidiasis. It has a broad spectrum of activity against *Candida* and *Aspergillus* species. This echinocandin is also active against the mycelial forms of dimorphic fungi, including azole- and polyene-resistant isolates. MFN seems to be well tolerated, with a favorable safety profile. The most common side effects include rash, chills, nausea, vomiting, diarrhea, headache, and leukopenia. Possible histamine-mediated symptoms such as pruritus, facile swelling, and vasodilations have also been reported. Drug interactions are minimal in this antifungal class. However, on the basis of some clinical studies,[79] it is recommended that patients receiving sirolimus or nifedipine in combination with MFN be monitored for symptoms of toxicity. Furthermore, a recent study has suggested that MFN is a mild inhibitor of cyclosporine metabolism and that cyclosporine levels should be monitored.[80]

Anidulafungin is the most recently FDA-approved (2006) echinocandin for the treatment of esophageal candidiasis, peritonitis, and intra-abdominal abscess. Its in vitro activity is similar to other echinocandins. A clinical study compared anidulafungin (100 mg day^{-1} for one day, then 50 mg day^{-1}) with fluconozole (200 mg day^{-1}) in a randomized double-blind trial involving 601 patients with esophageal candidiasis. Endoscopic success rates were similar between the two treatments (97.4 and 98.7%, respectively). However, relapse rates were higher (53%) in patients treated with anidulafungin than in those treated with fluconazole (19%). This higher relapse rate may have resulted from the relatively lower doses of anidulafungin used in this trial.[81] Similar to other echinocandins, anidulafungin is well tolerated. The most common side effects reported from clinical trials were hypotension, vomiting, constipation, nausea, fever, hypokalemia, and elevated hepatic enzyme levels. Because it is not a substrate of the cytochrome P450 system, its interaction with cyclosporine, voriconazole, tacrolimus, AmB, or rifampicin is insignificant.

Recently, echinocandin-like lipopeptides (FR209602, FR209603, and FR209604; Fujisawa Pharmaceuticals Co. Ltd.; Figure 7) were evaluated for their fungicidal activity. These compounds showed efficacy against *C. albicans* and *A. fumigatus* by inhibiting the synthesis of 1,3-β-glucan; MIC values ranged from 0.02 to 0.04 μg mL^{-1} by microbroth dilution assay. FR209602 and FR209603 showed good efficacy by subcutaneous injection against *C. albicans* in a murine model of systemic infection, with ED_{50} values of 2.0 and 1.9 mg kg^{-1}, respectively.[82]

2.4. Other antifungal agents

In addition to the three major classes of fungicides discussed above, other classes of compounds such as allylamines, flucytosine, griseofulvin, sordarins, nikkomycins, ciclopiroxolamine, 1,3-dithian-2-ylidenes, and many other pyrrole derivatives are

Table 3. The echinocandin class of antifungal agents.

Entry	Drug	Trade Name	Route[a]	Current Status	Approval Year
1	caspofungin	Cancidas (Merck)	i.v.	licensed	2001
2	micafungin	Mycamine (Astellas Pharmaceuticals)	i.v.	licensed	2005
3	anidulafungin	Eraxis (Vicuron and Pfizer)	i.v.	licensed	2006

[a] i.v. = intravenous.

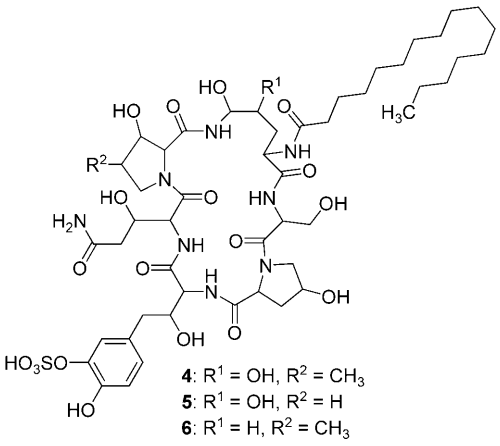

Figure 7. Structures of FR209602 (**4**), FR209603 (**5**), and FR209604 (**6**).

being added to the arsenal of antifungal agents (Figures 8 and 9).

Allylamines such as terbinafine, butenafine, (Figure 8) and naftifine are synthetic fungicidal agents that are reversible, noncompetitive inhibitors of squalene epoxidase, an enzyme that, together with squalene cyclase, converts squalene to lanosterol, which ultimately forms ergosterol in the fungal cell.[48] Butenafine and naftifine are topical preparations, whereas terbinafine (Lamisil, SF86-327; Sandoz Pharmaceuticals) is an oral systemic agent. Terbinafine has shown good in vitro activity against *Aspergillus* spp., *Fusarium* spp., and other filamentous fungi, but variable efficacy against yeasts. In in vivo models, it has not been very effective against invasive aspergillosis, systemic sporotrichosis, systemic candidiasis, or pulmonary cryptococcosis. However, terbinafine was found to be very effective against triazole-resistant strains of *Aspergillus* spp., *Candida* spp., and *P. boydii*, when administered with azoles and amphotericin B.[83]

Flucytosine (5-fluorocytosine; 4-amino-5-fluoro-2-pyrimidone; Figure 8) is the only available antimetabolite type of antifungal drug, marketed as Ancobon by Roche Laboratories. Flucytosine works as an antifungal agent through its conversion into 5-fluorouracil within target cells. Fluorouracil is incorporated into RNA, where it causes premature chain termination, and it inhibits DNA synthesis through effects on thymidylate synthase. For this mechanism of action, target cells must possess cytosine permease to internalize the flucytosine molecule, cytosine deaminase to convert flucytosine to 5-fluorouracil, and uracil phosphoribosyltransferase to convert 5-fluorouracil into a substrate for nucleic acid synthesis. Most filamentous fungi lack these enzymes, and hence the useful spectrum of flucytosine is limited to pathogenic yeasts (*Candida* spp. and *C. neoformans*).[84] This drug is selectively toxic to fungi because mammalian cells lack cytosine permease and do not convert flucytosine into 5-fluorouracil. Side effects in the form of gastrointestinal intolerance and bone marrow depression have been observed. Rash, hepatotoxicity, headache, confusion, hallucinations, sedation, and euphoria have also been reported.

Griseofulvin (Figure 8), which is produced by a number of species of *Penicillium*, was named after the first organism (*P. griseofulvum*), from which it was isolated. The earliest known inhibitory agent specific to fungal species was griseofulvin. The precise mechanism of action of this compound is still unknown, but the favored explanation is that it interferes with polymerized microtubule assembly and thus inhibits fungal mitosis by disrupting the mitotic spindle.[85] The selective toxicity of griseofulvin for fungi is only moderate and its spectrum of action is restricted mainly to the dermatophyte fungi (the cause of ringworm and athlete's foot). The typical dose for microcrystalline form is 500–1000 mg day^{-1}. Adverse reactions of griseofulvin are uncommon. Nausea, diarrhea, headache, skin eruptions, and photosensitivity are occasionally observed. Hepatotoxicity and neurological side effects may rarely occur.[86] For many years griseofulvin had been the first-line treatment for dermatophytosis. However, with the emergence of alternatives such as itraconazole and terbinafine, its use has been limited.

The nikkomycins are competitive inhibitors of fungal chitin synthases, which are necessary for fungal cell wall synthesis. Chitin is a linear polymer of β-(1,4)-linked *N*-acetylglucosamine

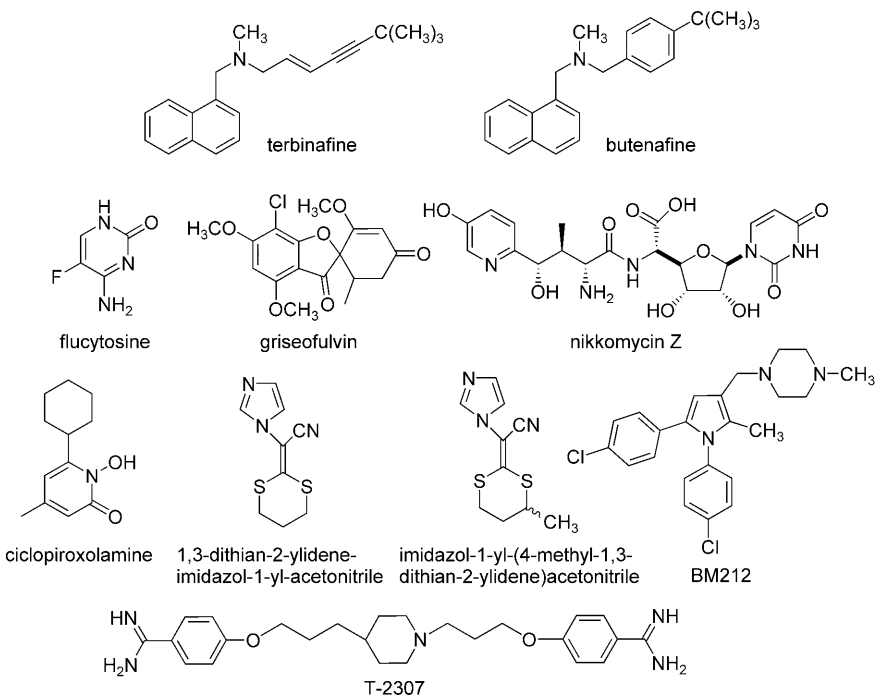

Figure 8. Various antifungal agents.

residues and is synthesized on the cytoplasmic surface of the plasma membrane. The polymerization of *N*-acetylglucosamine for the formation of chitin is catalyzed by chitin synthase. The nikkomycin compound class shows good in vitro activity but in vivo activity is poor. Nikkomycin Z (Figure 8) is the only analogue that was shown to be effective both in vitro and in vivo against the dimorphic fungi, *C. immitis* and *B. dermatitidis*, but only moderately effective in vitro against *C. albicans*, *C. neoformans*, and *H. capsulatum*. In addition, nikkomycin Z has shown in vitro synergic activity when used in combination with either fluconazole or itraconazole.[83]

The antifungal agent ciclopiroxolamine (6-cyclohexyl-1-hydroxy-4-methyl-2[1*H*]-pyridone) has an alternative mode of action based on the intracellular depletion of essential constituents of the fungal cell, resulting in growth inhibition followed by fungal death (Figure 8). Members of the hydroxypyridone class of antifungal agents are considered to be blockers of the G_1/S phase initiation of the cell cycle. The broad target spectrum of hydroxypyridones includes yeasts, dermatophytes, and other filamentous fungi.[87] Ciclopiroxolamine has been tested for efficacy against head and neck dermatitis and the results were promising.[88] Furthermore, in a comparative study, it was demonstrated that ciclopiroxolamine as a 1% cream was noninferior to ketoconazole as a 2% foaming gel, in mild to moderate facial seborrheic dermatitis.[89]

Some 1,3-dithian-2-ylidene derivatives (Figure 8) were synthesized, characterized, and evaluated for their antifungal activity. The compounds, 1,3-dithian-2-ylideneimidazol-1-yl-acetonitrile and imidazol-1-yl-(4-methyl-1,3-dithian-2-ylidene)acetonitrile, displayed better antifungal activity against *Aspergillus fumigates* than ketoconazole.[90]

Recently, several new compounds with 1,4-diaryl- and 1,5-diarylpyrrole skeletons have also been synthesized and evaluated for their antimicrobial activities. Many analogues of the antimycobacterial compound BM212 (Figure 8) have exhibited promising antifungal, antibacterial, and selective COX-2 inhibitory activities.[91] Additionally, a novel arylamidine (T-2307, Toyama Chemical Co. Ltd.; Figure 8) has demonstrated potent antifungal and growth inhibitory activities against pathogenic yeasts and filamentous fungi.[92]

Sordarins (Glaxo Wellcome S.A.; Figure 9) are a class of potential antifungal agents that inhibit protein synthesis in pathogenic fungi. The primary target for sordarin activity has been identified as elongation factor 2 (EF2). The mechanism involves stabilization of the EF2–ribosome complex, causing a halt to fungal protein synthesis.[93,94] Out of the many derivatives of sordarins synthesized, GM211676, GM193663, GM222712, and GM237354 showed activity in some animal models of candidiasis, coccidioidomycosis, histoplasmosis, aspergillosis, and pneumocystosis.[95–97]

Azasordarins (GlaxoSmithKline; Figure 9) are a new family of fungicides structurally characterized by the presence of a 6-methylmorpholin-2-yl group in place of the sugar moiety in sordarins. Six azasordarins demonstrated significant activity against emerging fungal pathogens.[98]

In a search for new lead compounds toward potent antimicrobial agents, novel *N*-morpholinoacetyl-2,6-diarylpiperidin-4-

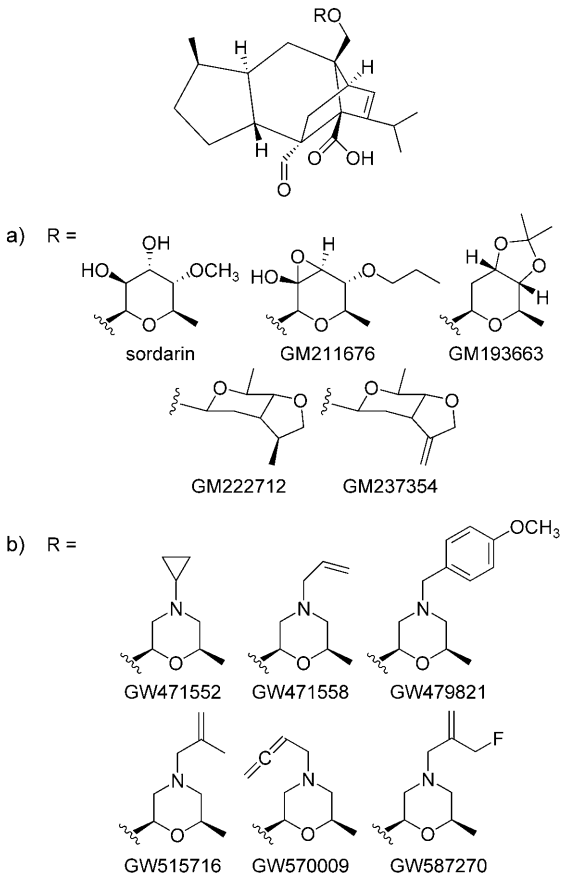

Figure 9. Inhibitors of fungal amino acids and protein synthesis: analogues of a) sordarin and b) azasordarin.

ones were synthesized, and their in vitro antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and antifungal activity against *C. albicans*, *Rhizopus* spp., *Aspergillus niger*, and *Aspergillus flavus* were evaluated.[99] Thus the quests for developing more specific antifungal agents are still underway.

3. Recently Emerging Treatments

Two novel strategies are under development for the treatment of invasive fungal infections. These developments are described below.

3.1. Calcineurin as an antifungal drug target

Calcineurin is a highly conserved serine- and threonine-specific protein phosphatase necessary for signal transduction, and is regulated by the Ca^{2+}–calmodulin complex.[100] It is an essential enzyme involved in various important biological processes such as T-cell activation,[101] muscle hypertrophy,[102,103] memory development,[104,105] activation of nitric oxide synthase,[106] suppression of cytokine production,[107] regulation of vascular inflammation,[108] and regulation of Na^+/K^+ ATPase activity in mammalian cells.[109] Only recently, however, attention has

been focused on calcineurin as a possible target for antifungal drugs.

Calcineurin is a heterodimer composed of a catalytic subunit (calcineurin A) and a regulatory subunit (calcineurin B). Upon calcineurin-dependent signal transduction, intracellular Ca^{2+} levels increase, thereby activating the binding of calmodulin and calcineurin B to Ca^{2+}. This causes calcineurin to undergo a conformational change that displaces an autoinhibitory domain from the active site of the catalytic calcineurin A subunit. This activated complex then catalyses the dephosphorylation of transcription factor (TF), thereby facilitating the translocation of dephosphorylated transcription factor (dTF) to the nucleus. Translocated dTF, in turn, transcribes the gene necessary for signal transduction (Figure 10).[110,111] Steinbach et al. recently published an excellent review article that covers this area in greater detail.[112]

The calcineurin signaling pathway is essential for fungal growth and pathogenesis. It is responsible for glucan synthesis,[113] ion homeostasis,[114,115] cell cycle control,[116] and various other processes. Studies based on the effect of cyclosporine A (CsA) and FK506 (tacrolimus) on fungal calcineurin highlight the susceptibility of the fungal pathogens to these immunosuppressants.[117–121] It has been reported that both CsA and FK506 are toxic to *Cryptococcus neoformans* at 37 °C in in vitro; therefore, these compounds are thought to be good candidates for anticryptococcal activity in in vivo. To prove this, experiments were performed with CsA and FK506 separately on *Cryptococcus*-infected mice and rabbits. It was found that both drugs decrease cryptococcal pulmonary infections, but cause cryptococcal meningitis, suggesting that the immunosuppressive effect dominates over fungal inhibition under the conditions in vivo.[122] Therefore, there is a need for the development of non-immunosuppressive analogues. To this end, certain non-immunosuppressive analogues of CsA such as (γ-OH)MeLeu^4-Cs (211-810), D-Sar(α-SMe)^{3}Val^2-DH-Cs (209-825), and FK506 derivative L-685 818 have been prepared and evaluated for their fungicidal activity.[122] These molecules have displayed promising efficacy against various pathogens. Furthermore, Audrey et al. demonstrated that the fungicidal activity of caspofungin can be enhanced if it is administered in combination with calcineurin inhibitors (FK506, CsA, FK520, and L-685 818) against *Aspergillus* species.[123]

3.2. Vaccination against mycoses

In recent years, the concept of developing vaccines against mycoses has attracted interest because of the increased incidence of various invasive fungal infections worldwide.[124] Moreover, it has been found that fungal carbohydrates can induce the production of antibodies that enhance host resistance, and numerous fungal proteins can trigger T-cell-mediated immunity. Therefore, various carbohydrate- and protein-based antigens have been identified as good candidates for the development of vaccines that can provide protective immunity against several fungal pathogens.

The immune system includes basic cellular effectors such as neutrophils, macrophages, and dendritic cells as well as non-cellular effectors such as collectins, complement and natural antibodies against fungal pathogens; these play a crucial role in determining the antimycotic response. Fungi initially come into contact with host cell-surface receptors, which can bind or interact with various receptor sites, thus eliciting a host response. The host response to fungal infection varies, as mycoses differ in their genetic, morphological, and biochemical nature.

Generally, the selection of a carbohydrate antigen for developing a vaccine is based on its accessibility to host immune response rather than the chemical composition of the antigen. Recent immunological studies suggest that monoclonal antibodies are potential therapeutic reagents for the treatment of *Cryptococcus neoformans* infections. In fact, *C. neoformans* infections induce the release of large amounts of soluble capsular polysaccharide in the tissues, and this mediates a variety of harmful effects on the host immune system. Furthermore, the level of serum antibodies against the capsular polysaccharide decreases in most patients.

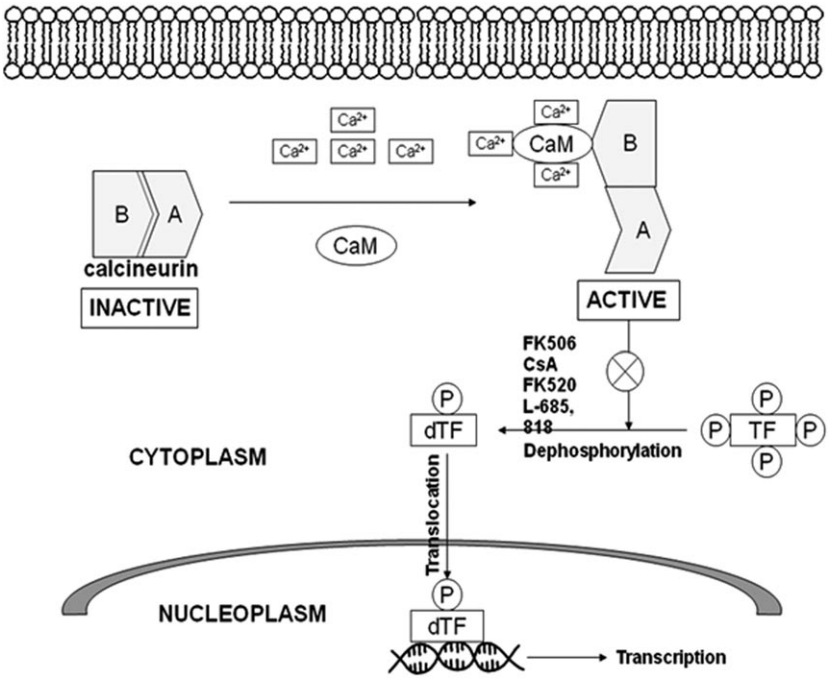

Figure 10. The calcineurin signaling pathway: CaM, calmodulin; TF, transcription factor; dTF, dephosphorylated transcription factor.

Several research groups have shown that the administration of antibodies specific for the glucuronoxylomannan (GXM) component of the polysaccharide capsule of *C. neoformans* are protective against murine cryptococcal infections and can enhance the therapeutic efficacy of amphotericin B, fluconazole, and flucytosine.[125] These results, together with the rapid clearance of capsular polysaccharide after antibody administration in humans and mice,[126] suggest that antibody therapy could play an important role in the treatment of human cryptococcocis.

It was recently found that the conjugation of *C. neoformans* capsular heptasaccharide with a protein carrier affords a highly immunogenic compound that induces an antibody response.[121] Arturo et al. observed an antibody response to GXM in mice immunized with 10 μg of heptasaccharide–protein complex (Figure 11).[127]

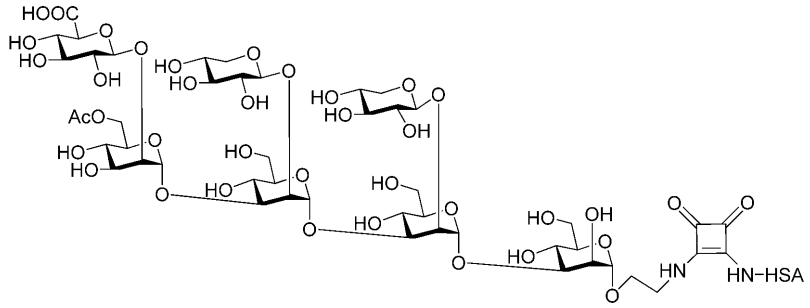

Figure 11. Structure of a heptasaccharide protein conjugate (HSA = human serum albumin).

Antibodies against β-1,2-linked mannotriose or mannobiose (another antigen) have been shown to protect mice against hematogenously disseminated candidiasis involving both *C. albicans* and *C. tropicalis*.[128] Furthermore, such protection is expected to be elicited against *Candida* spp. that produce β-1,2-linked oligomannosides. Recently, the fungal cell wall polysaccharide β-1,3-glucan was identified as a possible antigen for the production of protective antibodies.[129,130] On immunization, mice were successfully protected by β-glucan-induced antibodies against experimental candidiasis and aspergillosis. Therefore, β-glucan may be a good lead for the development of vaccines against fungal infections.

Many proteins that are produced by pathogenic fungal strains are glycosylated, yet a majority of the studies with proteins in medical mycology have used recombinant proteins generated in *E. coli*. To the best of our knowledge, there are no published reports of a comparison between the protective efficacy of glycosylated and non-glycosylated proteins. It has been found that the mannosylated form of ovalbumin enhances its capacity as an antigen, as assessed by T-cell proliferation.[131] Hence, the addition of carbohydrate moieties to immunogenic protein antigens could enhance their efficacy or decrease the quantity required to achieve protection. Another approach to enhancing the potency of fungal vaccines is to create vaccines that contain more than one antigen. The use of such an approach would increase the potency of a single antigen and engage a broader repertoire of T-cell families.

In vaccinology there are many examples of highly efficacious vaccines against various infectious diseases. These vaccines are derived from live attenuated infectious agents such as viruses, including polio, mumps, rubella, measles, influenza, rotavirus, and varicella.[132] The advantage of using a live agent is that the fungus replicates at the site of infection and induces a strong immune response, which recapitulates natural immunity to disease. By contrast, protein or killed agents might not reach the endogenous antigen-processing pathway and induce $CD8^+$T-cell responses. These immune responses provide protective immunity against several mycoses including *H. capsulatum*, *B. dermatidis*, *P. brasiliensis*, *C. neoformans*, and *P. carinii*.[133–135] To this end researchers have reported that a targeted mutant of *C. immitis* elicited an immune response upon administration to mice as a vaccine after deleting two of its chitinase genes. It seemed to be a safer alternative, as in this case the fungus is unable to convert to a virulent state. Therefore, these genetically altered pathogenic species offer a good candidate for the development of live attenuated vaccines.

4. Summary and Outlook

Over the past decade, several new antimycotic agents have been licensed, and some of these are also available on the market. However, there is still an urgent need for new, safer and more economical alternatives for antifungal therapy. Although the drugs discussed above are useful and are the current backbone of antifungal treatments, they often either suffer from a certain degree of toxicity or quickly succumb to fungal resistance through their large-scale use. As a result, physicians are now shifting toward combination therapies, in which the synergistic action of multiple antifungal drugs is used to combat infection and to decrease the emergence of resistant strains. Unlike the clear progress in the synthesis of many model compounds, there are fewer reports of progress in the development of non-immunosuppressive agents that block the activity of fungal calcineurin, which is a potential target for future antifungal drugs. With the increase in knowledge about fungus–host interactions, vaccines against both primary fungal pathogens and prevalent opportunistic fungi are becoming a reality. The preliminary results obtained from vaccine studies on the prevention of coccidioidomycoses have encouraged the pursuit of vaccine development against ever-growing spread of fungal disease posed by various mycoses. Although mouse models of human fungal pathogens have served well in defining candidate antigens and vaccine formulations, there is the need to conduct the clinical trials in humans. With ongoing advances in modern medicine, it is expected that a wealth of new drugs that are nontoxic and that have higher selectivity will emerge in the near future.

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Keywords: antimycotic agents · azoles · calcineurins · echinocandins · medicinal chemistry · polyenes · vaccines

[1] M. B. Edmond, S. E. Wallace, D. K. McClish, M. A. Pfaller, R. N. Jones, R. P. Wenzel, *Clin. Infect. Dis.* **1999**, *29*, 239–244.
[2] a) K. A. Marr, R. A. Carter, F. Crippa, A. Wald, L. Corey, *Clin. Infect. Dis.* **2002**, *34*, 909–917; b) A. Wald, W. Leisenring, J. A. V. Burik, R. A. Bowden, *J. Infect. Dis.* **1997**, *175*, 1459–1466.
[3] J. Bastert, M. Schaller, H. C. Korting, E. G. V. Evans, *Int. J. Antimicrob. Agents* **2001**, *17*, 81–91.
[4] W. Oroshnik, L. C. Vining, A. D. Mebane, W. A. Taber, *Science* **1955**, *121*, 147–149.
[5] P. Ganis, G. Avitabile, W. Mechlinski, C. P. Schaffner, *J. Am. Chem. Soc.* **1971**, *93*, 4560–4564.
[6] E. Borowski, J. Zielinski, T. Ziminski, L. Falkowski, P. Kolodziejczyk, J. Golik, E. Jereczek, H. Adlercreutz, *Tetrahedron Lett.* **1970**, *11*, 3909–3914.
[7] R. A. Demel, F. J. L. Crombag, L. L. M. V. Deenean, S. C. Kinsky, *Biochim. Biophys. Acta Biomembr.* **1968**, *150*, 1–14.
[8] B. D. Kruijff, R. A. Demel, *Biochim. Biophys. Acta Biomembr.* **1974**, *339*, 57–70.
[9] W. Gold, *Antibiotic Annals* **1995**, 579–586.
[10] T. J. Walsh, G. P. Melcher, M. G. Rinaldi, J. Lecciones, D. A. McGough, P. Kelly, J. Lee, D. Callender, M. Rubin, P. A. Pizzo, *J. Clin. Microbiol.* **1990**, *28*, 1616–1622.
[11] D. A. Sutton, S. E. Sanche, S. G. Revankar, A. W. Fothergill, M. G. Rinaldi, *J. Clin. Microbiol.* **1999**, *37*, 2343–2345.
[12] M. Walsh, L. White, K. Atkinson, A. Enno, *Aust. N. Z. J. Med.* **1992**, *22*, 265–268.
[13] P. Francis, T. J. Walsh, *Oncology* **1992**, *6*, 133–144.
[14] S. Arikan, M. Lozano-Chiu, V. Paetznick, S. Nangia, J. H. Rex, *J. Clin. Microbiol.* **1999**, *37*, 3946–3951.
[15] a) G. S. Kobayashi, G. Medoff, *Annu. Rev. Microbiol.* **1977**, *31*, 291–308; b) M. A. Fischer, S. H. Shen, J. Haddad, W. F. Tarry, *Antimicrob. Agents Chemother.* **1989**, *33*, 1443–1446.
[16] R. D. Meyer, *Clin. Infect. Dis.* **1992**, *14*, S154–S160.
[17] A. C. Lin, E. Goldwasser, E. M. Bernard, S. W. Chapman, *J. Infect. Dis.* **1990**, *161*, 348–351.
[18] D. W. Bates, L. Su, D. T. Yu, G. M. Chertow, D. L. Seger, D. R. J. Gomes, E. J. Dasbach, R. Platt, *Clin. Infect. Dis.* **2001**, *32*, 686–693.
[19] A. J. Ullmann, M. A. Sanz, A. Tramarin, R. A. Barnes, W. Wu, B. A. Gerlach, K. J. Krobot, W. C. Gerth, *Clin. Infect. Dis.* **2006**, *43*, e29–e38.
[20] L. S. S. Guo, R. M. Fielding, D. D. Lasic, R. L. Hamilton, D. Mufson, *Int. J. Pharm.* **1991**, *75*, 45–54.
[21] R. M. Fielding, P. C. Smith, L. H. Wang, J. Porter, L. S. S. Guo, *Antimicrob. Agents Chemother.* **1991**, *35*, 1208–1213.
[22] a) E. J. Anaissie, G. N. Mattiuzzi, C. B. Miller, G. A. Noskin, M. J. Gurwith, R. D. Mamelok, L. A. Pietrelli, *Antimicrob. Agents Chemother.* **1998**, *42*, 606–611; b) B. A. Oppenheim, R. Herbrecht, S. Kusne, *Clin. Infect. Dis.* **1995**, *21*, 1145–1153.
[23] M. H. White, R. A. Bowden, E. S. Sandler, M. L. Graham, G. A. Noskin, J. R. Wingard, M. Goldman, J. A. V. Burik, A. McCabe, J. S. Lin, M. Gurwith, C. B. Miller, *Clin. Infect. Dis.* **1998**, *27*, 296–302.
[24] a) J. W. Hiemenz, T. J. Walsh, *Clin. Infect. Dis.* **1996**, *22*, S133–S144; b) C. E. Swenson, W. R. Perkins, P. Roberts, I. Ahmad, R. Stevens, D. A. Stevens, A. S. Janoff, *Antimicrob. Agents Chemother.* **1998**, *42*, 767–771.
[25] A. S. Janoff, W. R. Perkins, S. L. Saletan, C. E. Swenson, *J. Liposome Res.* **1993**, *3*, 451–471.
[26] a) J. P. Adler-Moore, R. T. Proffitt, *J. Liposome Res.* **1993**, *3*, 429–450; b) R. T. Proffitt, J. A. Moore, S. M. Chiang, US6770290, **2004**.
[27] J. Tollemar, L. Klingspor, O. Ringden, *Clin. Microbiol. Infect.* **2001**, *7*, 68–79.
[28] T. J. Walsh, V. Yeldandi, M. McEvoy, C. Gonzalez, S. Chanock, A. Freifeld, N. I. Seibel, P. O. Whitcomb, P. Jarosinski, G. Boswell, I. Bekersky, A. Alak, D. Buell, J. Barret, W. Wilson, *Antimicrob. Agents Chemother.* **1998**, *42*, 2391–2398.
[29] R. B. S. Laing, L. J. R. Milne, C. L. S. Leen, G. P. Malcolm, A. J. W. Steers, *Lancet* **1994**, *344*, 682.
[30] I. Molina, V. Falco, M. Crespo, C. Riera, E. Ribera, A. Curran, J. Carrio, M. Diaz, S. V. del Saz, R. Fisa, P. Lopez-Chejade, I. Ocana, A. Pahissa, *J. Antimicrob. Chemother.* **2007**, *60*, 837–842.
[31] V. J. Ram, M. Nath, *Curr. Med. Chem.* **1996**, *3*, 303–316.
[32] M. A. Hossain, S. Maesaki, M. S. Razzaque, K. Tomono, T. Taguchi, S. Kohno, *J. Antimicrob. Chemother.* **2000**, *46*, 263–268.
[33] Y. Tomii, *Curr. Pharm. Des.* **2002**, *8*, 467–474.
[34] A. J. Carrillo-Munoz, G. Giusiano, P. A. Ezkurra, G. Quindos, *Rev. Esp. Quimioterap.* **2006**, *19*, 130–139.
[35] M. L. Adams, D. R. Andes, G. S. Kwon, *Biomacromolecules*, **2003**, *4*, 750–757.
[36] T. Ehrenfreund-Kleinman, T. Azzam, R. Falk, I. Polacheck, J. Golenser, A. J. Domb, *Biomaterials* **2002**, *23*, 1327–1335.
[37] R. Falk, A. J. Domb, I. Polacheck, *Antimicrob. Agents Chemother.* **1999**, *43*, 1975–1981.
[38] E. L. Hazen, R. Brown, *Science* **1950**, *112*, 423.
[39] S. C. Kinsky, *J. Bacteriol.* **1962**, *83*, 351–358.
[40] J. Kotler-Brajtburg, G. Medoff, G. S. Kobayashi, S. Boggs, D. Schlessinger, R. C. Pandey, K. L. Rinehart, *Antimicrob. Agents Chemother.* **1979**, *15*, 716–722.
[41] A. J. Carrillo-Munoz, G. Quindos, C. Tur, M. T. Ruesga, Y. Miranda, O. del Valle, P. A. Cossum, T. L. Wallace, *J. Antimicrob. Chemother.* **1999**, *44*, 397–401.
[42] E. M. Johnson, J. O. Ojwang, A. Szekely, T. L. Wallace, D. W. Warnock, *Antimicrob. Agents Chemother.* **1998**, *42*, 1412–1416.
[43] M. A. Ghannoum, L. B. Rice, *Clin. Microbiol. Rev.* **1999**, *12*, 501–517.
[44] D. W. Denning, P. Warn, *Antimicrob. Agents Chemother.* **1999**, *43*, 2592–2599.
[45] F. Offner, V. Krcmery, M. Boogaerts, C. Doyen, D. Engelhard, P. Ribaud, C. Cordonnier, B. D. Pauw, S. Durrant, J. P. Marie, P. Moreau, H. Guiot, G. Samonis, R. Sylvester, R. Herbrecht, *Antimicrob. Agents Chemother.* **2004**, *48*, 4808–4812.
[46] K. L. Oakley, C. B. Moore, D. W. Denning, *Antimicrob. Agents Chemother.* **1999**, *43*, 1264–1266.
[47] P. Cai, F. Kong, P. Fink, M. E. Ruppen, R. T. Williamson, T. Keiko, *J. Nat. Prod.* **2007**, *70*, 215–219.
[48] N. H. Georgopapadakou, T. J. Walsh, *Antimicrob. Agents Chemother.* **1996**, *40*,279–291.
[49] T. C. White, K. A. Marr, R. A. Bowden, *Clin. Microbiol. Rev.* **1998**, *11*, 382–402.
[50] D. Sanglard, F. C. Odds, *Lancet Infect. Dis.* **2002**, *2*, 73–85.
[51] G. Petrikkos, A. Skiada, *Int. J. Antimicrob. Agents* **2007**, *30*, 108–117.
[52] F. C. Odds, A. J. P. Brown, N. A. R. Gow, *Trends Microbiol.* **2003**, *11*, 272–279.
[53] a) A. J. Ullmann, *Curr. Med. Res. Opin.* **2003**, *19*, 263–271; b) M. A. Ghannoum, D. M. Kunhe, *Eur. J. Med. Res.* **2002**, *7*, 242–256.
[54] Y. Li, M. H. Nguyen, H. Derendorf, S. Cheng, C. J. Clancy, *Antimicrob. Agents Chemother.* **2007**, *51*, 2985–2987.
[55] J. R. Wingard, R. Herbrecht, J. Mauskopf, H. T. Schlamm, A. Marciniak, C. S. Roberts, *Transplant Infect. Dis.* **2007**, *9*, 182–188.
[56] A. H. Groll, S. C. Piscitelli, T. J. Walsh, *Adv. Pharmacol.* **1998**, *44*, 343–500.
[57] D. Wexler, R. Courtney, W. Richards, C. Banfield, J. Lim, M. Laughlin, *Eur. J. Pharm. Sci.* **2004**, *21*, 645–653.
[58] N. D. Greer, *Proc. (Bayl. Univ. Med. Cent.)* **2007**, *20*, 188–196.
[59] R. Courtney, S. Pai, M. Laughlin, J. Lim, V. Batra, *Antimicrob. Agents Chemother.* **2003**, *47*, 2788–2795.
[60] I. I. Raad, J. R. Graybill, A. B. Bustamante, O. A. Cornely, V. Gaona-Flores, C. Afif, D. R. Graham, R. N. Greenberg, S. Hadley, A. Langston, R. Negroni, J. R. Perfect, P. Pitisuttithum, A. Restrepo, G. Schiller, L. Pedicone, A. J. Ullmann, *Clin. Infect. Dis.* **2006**, *42*, 1726–1734.
[61] J. Kok, N. Gilroy, C. Halliday, O. C. Lee, D. Novakovic, P. Kevin, S. Chen, *J. Infect.* **2007**, *55*, e33–e36.

[62] J. Bartroli, E. Turmo, M. Alguero, E. Boncompte, M. L. Vericat, L. Conte, J. Ramis, M. Merlos, J. Garcia-Rafanell, J. Forn, *J. Med. Chem.* **1998**, *41*, 1855–1868.
[63] T. Yamazumi, M. A. Pfaller, S. A. Messer, A. Houston, R. J. Hollis, R. N. Jones, *Antimicrob. Agents Chemother.* **2000**, *44*, 2883–2886.
[64] M. A. Pfaller, S. A. Messer, R. J. Hollis, R. N. Jones, D. J. Diekema, *Antimicrob. Agents Chemother.* **2002**, *46*, 1723–1727.
[65] M. Cuenca-Estrella, A. Gomez-Lopez, E. Mellado, G. Garcia-Effron, A. Monzon, J. L. Rodriguez-Tudela, *Antimicrob. Agents Chemother.* **2005**, *49*, 5136–5138.
[66] M. Beale, F. Q. Telles, D. Banhegyi, N. Li, P. F. Pierce in: *41st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)*, December 16–19, **2001**, Chicago, IL (USA); Washington DC, ASM, **2001**.
[67] Q. Y. Sun, J. M. Xu, Y. B. Cao, W. N. Zhang, Q. Y. Wu, D. Z. Zhang, J. Zhang, H. Q. Zhao, Y. Y. Jiang, *Eur. J. Med. Chem*. **2007**, *42*, 1226–1233.
[68] F. Benz, F. Knusel, J. Nuesch, H. Treichler, W. Voser, R. Nyfeler, W. Keller-Schierlein, *Helv. Chim. Acta* **1974**, *57*, 2459–2477.
[69] A. A. Adefarati, O. D. Hensens, E. T. T. Jones, J. S. Tkacz, *J. Antibiot.* **1992**, *45*, 1953–1957.
[70] C. M. Douglas, *Med. Mycol.* **2001**, *39*, 55–66.
[71] a) H. V. Bossche, *Expert Opin. Ther. Pat.* **2002**, *12*, 151–167; b) Y. Hori, Y. Tsurumi, S. Takase, H. Hatanaka, K. Sakamoto, S. Hashimoto, H. Ohki, T. Tojo, K. Matsuda, K. Kawabata, US6331521, **2001**.
[72] K. L. Oakley, C. B. Moore, D. W. Denning, *Antimicrob. Agents Chemother.* **1998**, *42*, 2726–2730.
[73] F. C. Odds, M. Motyl, R. Andrade, J. Bille, E. Canton, M. Cuenca-Estrella, A. Davidson, C. Durussel, D. Ellis, E. Foraker, A. W. Fothergill, M. A. Ghannoum, R. A. Giacobbe, M. Gobernado, R. Handke, M. Laverdiere, W. Lee-Yang, W. G. Merz, L. Ostrosky-Zeichner, J. Peman, S. Perea, J. R. Perfect, M. A. Pfaller, L. Proia, J. H. Rex, M. G. Rinaldi, J. L. Rodriguez-Tudela, W. A. Schell, C. Shields, D. A. Sutton, P. E. Verweij, D. W. Warnock, *J. Clin. Microbiol.* **2004**, *42*, 3475–3482.
[74] a) G. K. Abruzzo, A. M. Flattery, C. J. Gill, L. Kong, J. G. Smith, V. B. Pikounis, J. M. Balkovec, A. F. Bouffard, J. F. Dropinski, H. Rosen, H. Kropp, K. Bartizal, *Antimicrob. Agents Chemother.* **1997**, *41*, 2333–2338; b) M. F. Pfaller, F. Marco, S. A. Messer, R. N. Jones, *Diagn. Microbiol. Infect. Dis.* **1998**, *30*, 251–255.
[75] S. C. Deresinski, D. A. Stevens, *Clin. Infect. Dis.* **2003**, *36*, 1445–1457.
[76] E. G. Arathoon, E. Gotuzzo, L. M. Noriega, R. S. Berman, M. J. DiNubile, C. A. Sable, *Antimicrob. Agents Chemother.* **2002**, *46*, 451–457.
[77] N. P. Wiederhold, J. S. Lewis II, *Expert Opin. Pharmacother.* **2007**, *8*, 1155–1166.
[78] D. W. Denning, *Lancet* **2003**, *362*, 1142–1151.
[79] T. Sakaeda, K. Iwaki, M. Kakumoto, M. Nishikawa, T. Niwa, J. S. Jin, T. Nakamura, K. Nishiguchi, N. Okamura, K. Okumura, *J. Pharm. Pharmacol.* **2005**, *57*, 759–764.
[80] M. F. Hebert, R. W. Townsend, S. Austin, G. Balan, D. K. Blough, D. Buell, J. Keirns, I. Bekersky, *J. Clin. Pharmacol.* **2005**, *45*, 954–960.
[81] D. S. Krause, A. E. Simjee, C. van Rensburg, J. Viljoen, T. J. Walsh, B. P. Goldstein, M. Wible, T. Henkel, *Clin. Infect. Dis.* **2004**, *39*, 770–775.
[82] R. Kanasaki, F. Abe, S. Furukawa, K. Yoshikawa, A. Fujie, M. Hino, S. Hashimoto, Y. Hori, *J. Antibiot.* **2006**, *59*, 145–148.
[83] V. T. Andriole, *J. Antimicrob. Chemother.* **1999**, *44*, 151–162.
[84] M. A. Pfaller, S. A. Messer, L. Boyken, H. Huynh, R. J. Hollis, D. J. Diekema, *Antimicrob. Agents Chemother.* **2002**, *46*, 3518–3521.
[85] K. Gull, A. P. J. Trinci, *Nature* **1973**, *244*, 292–294.
[86] H. C. Korting, M. Schafer-Korting, H. Zienicke, A. Georgii, M. W. Ollert, *Antimicrob. Agents Chemother.* **1993**, *37*, 2064–2068.
[87] A. J. Carrillo-Munoz, S. Brio, R. Alonso, O. del Valle, P. Santos, G. Quindos, *Int. J. Antimicrob. Agents* **2002**, *20*, 375–379.
[88] P. Mayser, J. Kupfer, D. Nemetz, U. Schafer, M. Nilles, W. Hort, U. Gieler, *Skin Pharmacol. Physiol.* **2006**, *19*, 153–158.
[89] O. Chosidow, C. Maurette, P. Dupuy, *Dermatology* **2003**, *206*, 233–240.
[90] a) V. J. Ram, M. Nath, P. K. Shukla, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2137–2140; b) V. J. Ram, M. Nath, P. K. Shukla, IN185788, **2001**.
[91] M. Biava, G. C. Porretta, G. Poce, S. Supino, G. Sleiter, *Curr. Org. Chem.* **2007**, *11*, 1092–1112.
[92] J. Mitsuyama, N. Nomura, K. Hashimoto, E. Yamada, H. Nishikawa, M. Kaeriyama, A. Kimura, Y. Todo, H. Narita, *Antimicrob. Agents Chemother.* **2008**, *52*, 1318–1324.
[93] J. M. Dominguez, J. J. Martin, *Antimicrob. Agents Chemother.* **1998**, *42*, 2279–2283.
[94] L. Capa, A. Mendoza, J. L. Lavandera, F. G. D. L. Heras, J. F. Garcia-Bustos, *Antimicrob. Agents Chemother.* **1998**, *42*, 2694–2699.
[95] K. V. Clemons, D. A. Stevens, *Antimicrob. Agents Chemother.* **2000**, *44*, 1874–1877.
[96] A. Martinez, P. Aviles, E. Jimenez, J. Caballero, D. Gargallo-Viola, *Antimicrob. Agents Chemother.* **2000**, *44*, 3389–3394.
[97] A. Martinez, J. Regadera, E. Jimenez, I. Santos, D. Gargallo-Viola, *Antimicrob. Agents Chemother.* **2001**, *45*, 1008–1013.
[98] E. Herreros, M. J. Almela, S. Lozano, F. G. D. L. Heras, D. Gargallo-Viola, *Antimicrob. Agents Chemother.* **2001**, *45*, 3132–3139.
[99] G. Aridoss, S. Balasubramanian, P. Parthiban, S. Kabilan, *Eur. J. Med. Chem.* **2007**, *42*, 851–860.
[100] F. Rusnak, P. Mertz, *Physiol. Rev.* **2000**, *80*, 1483–1521.
[101] N. A. Clipstone, G. R. Crabtree, *Nature* **1992**, *357*, 695–697.
[102] M. A. Sussman, H. W. Lim, N. Gude, T. Taigen, E. N. Olson, J. Robbins, M. C. Colbert, A. Gualberto, D. F. Wieczorek, J. D. Molkentin, *Science* **1998**, *281*, 1690–1693.
[103] E. N. Olson, R. S. Williams, *Cell* **2000**, *101*, 689–692.
[104] F. Shibasaki, U. Hallin, H. Uchino, *J. Biochem.* **2002**, *131*, 1–15.
[105] I. M. Mansuy, M. Mayford, B. Jacob, E. R. Kandel, M. E. Bach, *Cell* **1998**, *92*, 39–49.
[106] T. M. Dawson, J. P. Steiner, V. L. Dawson, J. L. Dinerman, G. R. Uhl, S. H. Snyder, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9808–9812.
[107] I. M. Conboy, D. Manoli, V. Mhaiskar, P. P. Jones, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6324–6329.
[108] H. Satonaka, E. Suzuki, H. Nishimatsu, S. Oba, R. Takeda, A. Goto, M. Omata, T. Fujita, R. Nagai, Y. Hirata, *Circ. Res.* **2004**, *94*, 693–700.
[109] A. Aperia, F. Ibarra, L. B. Svensson, C. Klee, P. Greengard, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7394–7397.
[110] J. Aramburu, J. Heitman, G. R. Crabtree, *EMBO Rep.* **2004**, *5*, 343–348.
[111] R. Sugiura, S. O. Sio, H. Shuntoh, T. Kuno, *Cell. Mol. Life Sci.* **2001**, *58*, 278–288.
[112] W. J. Steinbach, J. L. Reedy, R. A. Cramer, Jr., J. R. Perfect, J. Heitman, *Nat. Rev. Microbiol.* **2007**, *5*, 418–430.
[113] S. A. Parent, J. B. Nielson, N. Morin, G. Chrebet, N. Ramadan, A. M. Dahl, M. J. Hsu, K. A. Bostian, F. Foor, *J. Gen. Microbiol.* **1993**, *139*, 2973–2984.
[114] I. Mendoza, F. Rubio, A. Rodriguez-Navarro, J. M. Pardo, *J. Biol. Chem.* **1994**, *269*, 8792–8796.
[115] D. Guerini, E. Gracia-Martin, A. Gerber, C. Volbracht, M. Leist, C. G. Merino, E. Carafoli, *J. Biol. Chem.* **1999**, *274*, 1667–1676.
[116] M. Mizunuma, D. Hirata, K. Miyahara, E. Tsuchiya, T. Miyakawa, *Nature* **1998**, *392*, 303–306.
[117] H. Hatanaka, T. Kino, S. Miyata, N. Inamura, A. Kuroda, T. Goto, H. Tanaka, M. Okuhara, *J. Antibiot.* **1988**, *41*, 1592–1601.
[118] I. B. Barthelmess, M. Tropschug, *Curr. Genet.* **1993**, *23*, 54–58.
[119] T. Breuder, C. S. Hemenway, N. R. Movva, M. E. Cardenas, J. Heitman, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5372–5376.
[120] P. Garrett-Engele, B. Moilanen, M. S. Cyert, *Mol. Cell. Biol.* **1995**, *15*, 4103–4114.
[121] K. P. High, *Transplantation* **1994**, *57*, 1689–1700.
[122] M. C. Cruz, M. D. Poeta, P. Wang, R. Wenger, G. Zenke, V. F. J. Quesniaux, N. R. Movva, J. R. Perfect, M. E. Cardenas, J. Heitman, *Antimicrob. Agents Chemother.* **2000**, *44*, 143–149.
[123] A. Odom, M. D. Poeta, J. Perfect, J. Heitman, *Antimicrob. Agents Chemother.* **1997**, *41*, 156–161.
[124] J. E. Cutler, G. S. Deepe, Jr., B. S. Klein, *Nat. Rev. Microbiol.* **2007**, *5*, 13–28.
[125] L. R. Martinez, D. Moussai, A. Casadevall, *Infect. Immun.* **2004**, *72*, 3674–3679.
[126] L. E. Yauch, M. K. Mansour, S. M. Levitz, *Infect. Immun.* **2005**, *73*, 8429–8432.
[127] S. Oscarson, M. Alpe, P. Svahnberg, A. Nakouzi, A. Casadevall, *Vaccine* **2005**, *23*, 3961–3972.
[128] Y. Han, J. E. Cutler, *Infect. Immun.* **1995**, *63*, 2714–2719.
[129] A. Torosantucci, C. Bromuro, P. Chiani, F. D. Bernardis, F. Berti, C. Galli, F. Norelli, C. Bellucci, L. Polonelli, P. Costantino, R. Rappuoli, A. Cassone, *J. Exp. Med.* **2005**, *202*, 597–606.
[130] A. Casadevall, L. A. Pirofski, *Trends Mol. Med.* **2006**, *12*, 6–9.

[131] J. S. Lam, M. K. Mansour, C. A. Specht, S. M. Levitz, *J. Immunol.* **2005**, *175*, 7496–7503.
[132] *Red Book: Report of the Committee on Infectious Disease* (Ed.: L. Pickering), American Academy of Pediatrics, Elk Grove Village, **2006**.
[133] M. Wüthrich, H. I. Filutowicz, T. Warner, G. S. Deepe, Jr., B. S. Klein, *J. Exp. Med.* **2003**, *197*, 1405–1416.
[134] G. S. Deepe, Jr., *J. Immunol.* **1994**, *152*, 3491–3500.
[135] F. McAllister, C. Steele, M. Zheng, E. Young, J. E. Shellito, L. Marrero, J. K. Kolls, *J. Immunol.* **2004**, *172*, 1132–1138.

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