A General Overview on Past, Present and Future Antimycotics

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Abstract: Since the discovery of amphotericin B in 1955 the armamentarium of antimycotic drugs now embraces many new chemical classes: azoles, allylamines and candins. However, despite the wide variety in chemical structure, there is a lack of diversity in terms of mechanism of action. The mechanism of action of the main classes of antimycotics as well as the therapeutic value of some representatives is discussed. Some challenges to innovation will be highlighted that when overcome will herald more effective therapeutic interventions. Finally, we will list antimycotics that are at a late stage of development.

Keywords: *Candida* spp., dermathophytes, antimycotics, fungicidal, drug development.

INTRODUCTION

During the past two decades the incidence of fungal infections has increased dramatically. This is especially the case in cancer patients, transplant recipients and patients with AIDS. In addition, patients receiving broad-spectrum antibiotics, corticosteroids, cytotoxic agents or parental nutrition are prone to fungal infection [1]. So far, notable advances in antifungal therapeutics were achieved with the development of less toxic formulations of amphotericin B, the introduction of improved triazoles, the advent of the echinocandin lipopeptides and the recent finding of broad-spectrum benzoxaboroles.

In this review, we provide the reader with a general overview of the current classes of antimycotics and their principal applications, focusing on both systemic and topical fungal infections. For more in-depth literature regarding novel azole agents under development such as isavuconazole and the more recently introduced posaconazole, we refer to other excellent reviews in this regard [2-4]. In addition, we comment on some of the hurdles that need to be overcome in order to improve safety and pharmacokinetic profiles of today's antimycotics. Additionally, we will discuss the *in vitro – in vivo* enigma. Finally, some examples of tomorrow's antimycotics are listed.

SURVEY OF THE MAIN CLASSES OF ANTIMYCOTICS AND THEIR PRINCIPAL APPLICATIONS

Classes of Currently Used Antimycotics

Systemic antimycotics are mostly reserved for the treatment of onychomycosis, tinea capitis, superficial and systemic candidiasis, and prophylaxis and treatment of invasive fungal infections [5]. Topical antimycotics are generally used for the treatment of superficial mycoses unless the infection is widespread or involves an extensive area or is resistant to initial therapy. The routinely used antimycotics for topical and systemic mycoses are grouped into eight different classes based on their mode of action [6,7]. Their respective mechanisms are depicted in Fig. (**1**). Azoles inhibit two cytochrome P450 (CYP) enzymes, lanosterol 14 α-demethylase and 22 Δ-desaturase, involved in the biosynthesis of the major lipid component of the fungal plasma membrane, ergosterol (Fig. **1A**). The resulting ergosterol depletion and accumulation of precursor sterols alters the normal permeability and fluidity of the plasma membrane which impact the action of membrane-bound enzymes such as those involved in cell wall synthesis. Allylamines and thiocarbamates inhibit the early steps of ergosterol biosynthesis, more specifically squalene epoxidase, resulting in the accumulation of the sterol precursor squalene (Fig. **1A**). High squalene levels may increase membrane permeability, leading to disruption of cellular organization. Polyene macrolides interact with ergosterol by forming a complex with its two hydrophobic side chains, resulting in the formation of pores leading to enhanced proton permeability, leakage of vital components and, ultimately, death of the organism (Fig. **1B**). Fluorinated pyrimidines, such as 5-fluorocytosine (5-FC), interfere with DNA, RNA and protein synthesis in the fungal cells (Fig. **1C**). Candins target the fungal cell wall enzyme complex β-1,3-D-glucan synthase and as such inhibit the biosynthesis of the structural glucan component of the cell wall, which makes the cell vulnerable to osmotic lysis (Fig. **1D**). Griseofulvin, only active against dermatophytes, acts via a different mechanism. It includes inhibition of the hyphal cell wall synthesis, binding to RNA, interference with nucleic acid synthesis and inhibition of microtubules essential for mitosis and cytoplasmic transport processes (Fig. **1E**). Oxaboroles have the novel action of inhibiting fungal

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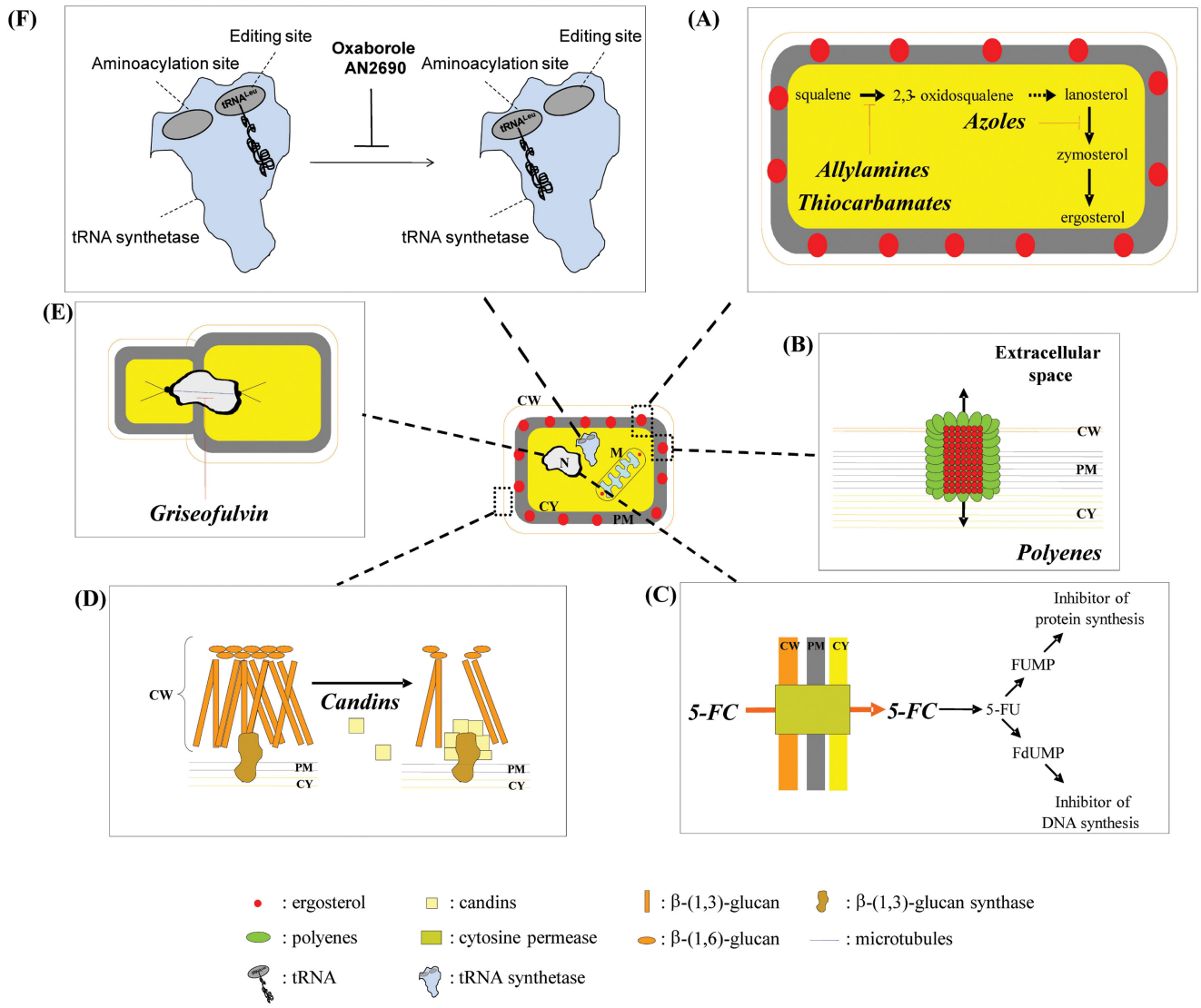

Fig. (1). Schematic representation of the mechanism of action of the different classes of antifungal agents. (**A**) Azoles, allylamines and thiocarbamates, (**B**) polyenes, (**C**) 5-fluorocytosine, (**D**) candins, (**E**) griseofulvin and (**F**) oxaboroles. (CW = cell wall, M = mitochondrion, PM = plasma membrane, CY = cytoplasm, 5-FU = 5-fluorouracil, FUMP = 5-fluorouridine monophosphate and FdUMP = 5-fluorodeoxyuridine monophosphate).

cytoplasmic leucyl-tRNA synthetase by trapping $tRNA^{Leu}$ in the editing site. This trapping of enzyme-bound $tRNA^{Leu}$ prevents catalytic turnover, thus inhibiting synthesis of leucyl-$tRNA^{Leu}$ and consequently blocking fungal protein synthesis [4] (Fig. **1F**).

In the following section, current treatment options for management of both types of mycoses (i.e. superficial and systemic) are discussed in more detail.

Superficial Mycoses

Superficial fungal infections are usually confined to the outer layers of skin, hair and nails. Generally, they are caused by dermatophytes (especially the genera *Trichophyton, Microsporum* and *Epidermophyton*), but also yeasts (e.g. *Candida*) and non-dermatophyte molds (e.g. *Scopulariopsis brevicaulis)* can be the infectious agents. In immune competent individuals, they usually have debilitating effects on a person's quality of life, whereas in the immune compromised population they can cause more complicated symptoms.

Physicians use topical antifungal agents as their first-choice medication to treat dermatologic diseases caused by superficial infections. However, oral treatment is recommended in difficult to access infected areas such as toe-nails, large infected surface, immune compromised host or recurrent infection showing poor response to topical agents [2].

Topical formulations such as creams, aerosols, shampoos, lotions, gels and lacquers of azoles (e.g. miconazole, ketoconazole, econazole, tioconazole, oxiconazole, clotrimazole, bifonazole and sertaconazole), allylamines (e.g. terbinafine and naftifine), ciclopiroxolamine and amorolfine are available. They are indicated for treatment of superficial infections such as tinea corporis, tinea cruris, tinea pedis (also known as athlete's foot), pityriasis versicolor,

onychomycosis (when nail involvement is <50% and if no matrix involvement exists), cutaneous candidiasis, seborrheic dermatitis and *Candida*-associated diaper dermatitis [8-17]. For topical applications the choice of the vehicle to deliver the antifungal compound is of great importance and should be strongly linked to the condition of the involved and immediately surrounding non-involved skin areas. It is known for example that a more hydrophobic and protecting vehicle is better adapted to the diaper environment and that the right choice of formulation can shorten the treatment for example in seborrheic dermatitis.

Due to poor skin penetration profiles, topical antifungals are not ideal for (i) superficial infections of hair and nails, such as tinea capitis, tinea barbae, tinea unguium and onychomycosis (when nail involvement is >50%), and (ii) in skin infections in which these topical antimycotics give no to little cure after one or two weeks therapy [2,13,18-20]. In these cases, oral rather than topical, administrated azoles and allylamines are recommended [13,15]. Additionally, oral griseofulvin can be used as an alternative therapy if the infection is caused by dermatophytes [21].

The importance of onychomycosis is often underestimated as it affects the quality of life of the patient. Far beyond being a simple "cosmetic problem", infected nails can serve as chronic reservoirs for infection or colonization which can give rise to recurrent skin mycoses [22]. Since onychomycosis represents such an important superficial mycosis, we will go into more detail in its treatment options. For about 40 years, griseofulvin was the only antifungal agent available for treatment of onychomycosis. However, its rather limited efficacy, its poor pharmacokinetic profile and its high recurrence rate prompted researchers to seek more effective drugs. Currently, three agents, terbinafine, itraconazole and fluconazole, are used as oral therapy for the treatment of onychomycosis [15,23]. Although oral monotherapy is effective in onychomycosis, oral regimens in combination with topical adjuvant therapy is considered to improve the therapeutic outcome [24,25]. Currently used combination therapies comprise combinations of oral terbinafine or itraconazole with 5% amorolfine nail lacquers [26]. Another type of film forming presentation containing ciclopirox (Penlac™) is used as topical treatment in immune competent patients with mild to moderate onychomycosis of fingernails and toenails without involvement of lunula due to *Trychophyton rubrum* [27]. AN2690 (5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole), a novel boron-containing small molecule designed to penetrate nails, is believed to be a potential successor of ciclopirox lacquers. As discussed later, this drug, with much better nail penetration kinetics than ciclopirox, has been shown to have broad spectrum antifungal activity *in vitro* and is currently in clinical trials to treat onychomycosis topically [28-30].

Systemic Mycoses

During the past 20 years, the incidence of systemic mycoses in humans has increased dramatically, especially due to the growth of the immune compromised patient population and the increased use of invasive devices (such as central venous catheters) and implants (such as prosthetic cardiac valves). A number of surveys have revealed that in the beginning of 2000 more than 50% of *Candida* infections were caused by *C. albicans* [31]. However, non-*Candida albicans* spp. such as *C. glabrata* are being increasingly isolated as causal agent for systemic mycoses [32]. Apart from *Candida* spp., infections by yeasts such as *Cryptococcus neoformans* as well as by filamentous fungi (moulds) such as *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., *Penicillium* spp. and *Zygomycetes* spp. are also increasingly reported since 2000 [33-35]. Besides, a myriad of pathogens, such as e.g. *Pneumocystis jiroveci, Histoplasma capsulatum, Paracoccidioides brasiliensis* and *Coccidioides immitis*, are in rise [36-39]. Strikingly, mortality rate associated with systemic *Candida* and *Aspergillus* infections still sticks high, 35-64% for *Candida* up to 100% for *Aspergillus* in organ transplant recipients [40-42].

The basis of systemic mycoses management goes back to the 1940's with the discovery of the antifungal activity of sulfonamides. During the two decades that followed, amphotericin B (which is still considered to be the gold standard for the treatment of most life-threatening mycoses) became available. Since then, the following drugs were approved: 5-fluorocytosine (early 1960s), intravenous miconazole and oral ketoconazole (late 1970s), fluconazole and itraconazole (1980s), caspofungin (2001), voriconazole (2002) and micafungin (2005). Today these drugs, grouped by chemical class as, fluorinated pyrimidines, polyenes, azoles and candins, are still used in practice for several types of systemic mycoses. Some specific examples are listed below:

(i) Fluorinated Pyrimidines

5-fluorocytosine (Ancotil™) is used to treat systemic mycoses caused by *Candida* spp., *Cryptococcus* spp. and *Aspergillus* spp. [43]. Intrinsic resistance in *Candida* spp. to this molecule is uncommon [44], though as with other antimetabolites the emergence of drug resistance can be a problem. Therefore, 5-fluorocytosine is preferentially used in combination with another antifungal agent. Along with Amphotericin B, 5-fluorocytosine is known as a premium combination therapy for the treatment of *Cryptococcus meningitis* [45].

(ii) Polyenes

Amphotericin B deoxycholate (AmB; Fungizone™) is a polyene with a very broad spectrum of activity including most yeasts and filamentous fungi [34]. It is useful in treating candidiasis, cryptococcosis, histoplasmosis, blastomycosis, aspergillosis as well as zygomycosis [46,47]. Side effects are common, occurring in 50-90% of cases and are principally nephrotoxicity or infusion-related. However, there is rationale that the renal toxicity usually associated with longterm use of AmB deoxycholate is reduced or even absent when liposomal formulations of AmB are used as treatment regimen: liposomal AmB (L-AmB), AmB lipid complex (ABLC) and AmB colloidal dispersion (ABCD). L-AmB, rather then AmB, has become the standard therapy [46].

(iii) Azoles

Fluconazole (Diflucan™) is available in oral or intravenous formulations and is well tolerated, with fewer drug interactions than other azoles. It is inactive against non-dermatophyte filamentous fungi. Itraconazole (Sporanox™) has a broader activity spectrum than fluconazole. It is active

against yeasts and moulds with the exception of *Fusarium* spp., *Scedosporium* spp. and the Zygomycetes. Although some precaution with prescription of this drug is required in patients with evidence of ventricular dysfunction such as congestive heart failure, both oral and intravenous formulations are widely used for non-immuno compromised patients [48]. The spectrum of voriconazole (Vfend^{TM}) is similar to that of itraconazole, but includes several emerging moulds such as *Fusarium* spp. and *Scedosporium* spp. [49]. Voriconazole may require some precaution since it is prone to metabolization by the cytochrome (CYP) P450 enzyme system and therefore has potential drug interactions [50]. In Europe, both oral and intravenous formulations of voriconazole are licensed for the treatment of fluconazole-resistant invasive *Candida* infections such as *C. krusei*. Furthermore, voriconazole is presently the drug of choice against invasive aspergillosis [51]. For an overview on the activity spectra of the comparator azole drugs fluconazole, itraconazole and voriconazole we refer to a study of Sabatelli and colleagues that included 19000 clinically important strains of yeasts and molds collected from 200 medical centers worldwide over a 10-year time span [52].

(iv) Candins

Caspofungin (Cancidas^{TM}) is used as an intravenous formulation for the treatment of invasive *Candida* spp. and *Aspergillus* spp. infections [53]. It has good activity against *C. glabrata* but *C. parapsilosis* may respond less to treatment [54]. Lack of susceptibility is also reported for important basidomycetes such as *Cryptococcus* spp., *Rhodotolura* spp. and *Trichosporon* spp. [2].

Fungicidal *versus* Fungistatic Action

It is essential to treat patients with life-threatening mycoses with broad-spectrum fungicidal antimycotics, as early initiation of effective systemic antifungal treatment is essential for a successful clinical outcome in these patients. However, clinical clues for diagnosis are sparse and early microbiological proof of e.g. invasive aspergillosis is rare. For such patients, it is essential to treat them with broad-spectrum fungicidal antimycotics. In practice, the new generation of azoles like voriconazole and posaconazole, as well as amphotericin B or caspofungin are used to treat such patients [55]. However, while amphotericin B is fungicidal against filamentous fungi, including *Aspergillus* spp., it is also characterized by a high cytotoxicity in general [56]. Echinocandins like caspofungin are rather fungistatic against *Aspergillus* spp. and their complex structure results in high production costs [57]. In contrast to the fungistatic activity of triazoles against *Candida* spp. (including itraconazole and voriconazole), these azoles are fungicidal against *Aspergillus* spp. [58].

CURRENTLY USED ANTIMYCOTICS WITH MULTIPLE MECHANISMS OF ACTION

Antimycotics with Additional Mode of Antifungal Action

There exists some heterogeneity in the mode of action amongst azole antifungals. Besides ergosterol biosynthesis inhibition, the earlier azoles (such as miconazole and ketoconazole) are reported to have a more complex mode of action, inhibiting several membrane-bound enzymes as well as membrane lipid biosynthesis [59]. Interestingly, miconazole exerted a prominent effect on enzymes involved in the production and breakdown of reactive oxygen species (ROS) [60-62]. The production of ROS together with the simultaneous inhibition of peroxidative defence enzymes has been held responsible for miconazole's fungicidal action against *C. albicans* [44]. Moreover, miconazole was recently demonstrated to induce stabilization of the actin cytoskeleton in yeast prior to induction of intracellular ROS accumulation [63]. Additionally, the complexity of the mode of action of azoles is further demonstrated by the induction of farnesol production in susceptible *Candida* spp. upon incubation with fluconazole, ketoconazole, clotrimazole and miconazole [64]. Not only azoles, but also polyenes were demonstrated to have an additional mode of antifungal action. More specifically, amphotericin B was shown to induce increased endogenous ROS levels in *C. albicans* and concommitantly programmed cell death or apoptosis [65].

Ancillary Properties of Azoles

Cutaneous fungal infections are often superinfected by bacteria. Therefore, antifungals should preferentially possess antibacterial activities, especially when destined for topical application. This is the case for the azoles miconazole and ketoconazole which exert antibacterial activities. Miconazole is bactericidal against gram positive bacteria such as *Propionibacterium acnes* and *Staphylococcus aureus* at low concentrations, while ketoconazole has only a bacteriostatic activity at high concentrations [66].

Fungal skin lesions are mostly accompanied by inflammatory reactions, hence concomitant anti-inflammatory activity as an adjunct property in one molecule may be important. Although there are indications that ketoconazole has some inflammatory activity, glucocorticosteroids are often incorporated in antifungal ointments to achieve anti-inflammatory effect.

Fungal infections are often favored by excessive sebum production. Indeed, growth of *Malassezia* spp. and maintenance of seborrhoeic dermatitis and pityriasis versicolor are highly dependent on lipid production [67]. Consequently, drugs that, ancillary inhibit the production and flow of sebum are highly desirable for the treatment of these cutaneous diseases.

Adjuvant Therapy

It is known that the simple modification of the calcium concentration in culture media highly influences the antifungal activity of azoles. In fact, calcium addition decreases their activity whereas chelating this cation increases its activity [68]. This observation led to the combined use of azoles with calcineurin inhibitors such as cyclosporine A and tacrolimus. The effect was synergistic and resulted in potent fungicidal activity against *C. albicans* [69].

HURDLES TO OVERCOME IN THE SEARCH FOR NEW ANTIMYCOTICS

Although today's approved antimycotics such as the widely prescribed azoles possess a broad activity spectrum,

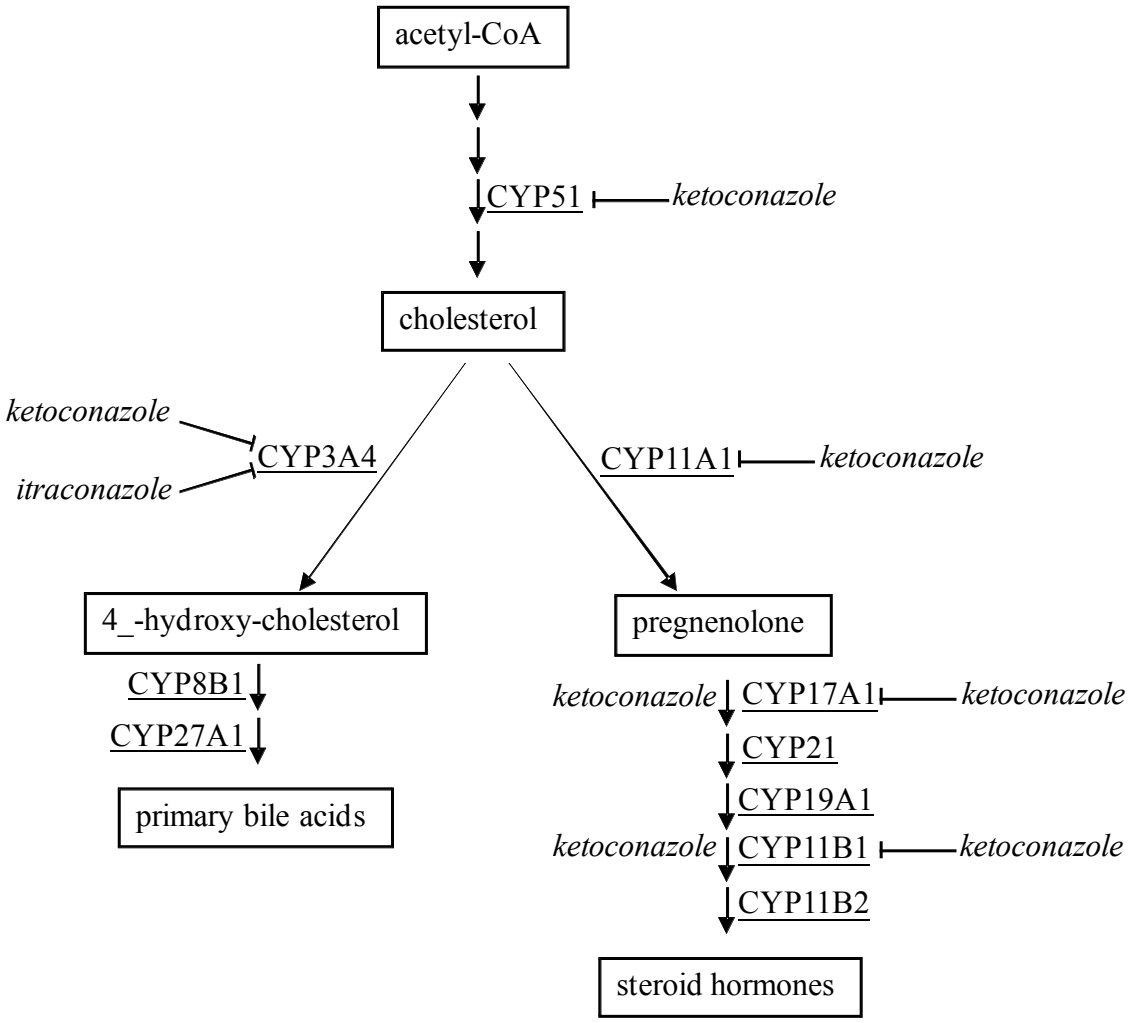

Fig. (2). Schematic overview of cholesterol metabolism, focusing on the pathways that can be inhibited by ketoconazole and itraconazole. In boxes key molecules of cholesterol metabolism are given. The different CYP enzymes responsible for the different enzymatic steps are shown underlined. Inhibition of CYP enzymes by azoles is indicated.

relatively favorable kinetics and low toxicity, their properties are subject to improvement. To achieve in the near future key improvements three main areas of research are actively pursued:

i. Enhanced fungicidal action

ii. Reduced incidence of resistance

iii. Greater selectivity for inhibiting fungal CYP isoforms.

Safety Profile

Current azole antifungals (e.g ketoconazole, itraconazole, miconazole and fluconazole) can inhibit both fungal and mammalian CYPs. At concentrations >100nM, ketoconazole inhibits the fungal CYP51, but also the mammalian CYP51 that plays an important role in cholesterol biosynthesis (Fig. **2**). Additionally, at these higher concentrations, ketoconazole also affects the activity of enzymes involved in catabolism of cholesterol. More specifically, ketoconazole inhibits 17-hydroxylase-17,20-lyase (CYP17), the cholesterol side chain cleavage enzyme (CYP11A1), and the 11ß-hydroxylase (CYP11B1) (Fig. **2**) [70]. Itraconazole is almost devoid of effects on steroid metabolism [71]. However like ketoconazole, itraconazole inhibits CYP3A4, a major drug-metabolizing P450 isoform that also plays a role in cholesterol metabolism in the human liver [72]. The 50% inhibitory concentrations (IC50s) of ketoconazole and itraconazole for CYP3A4 inhibition were 11.7 and 32.6 nM, respectively [73]. Fluconazole and miconazole are potent inhibitors of CYP2C9, which plays a major role in the metabolism of drugs such as phenytoin, S-warfarin and a range of nonsteroidal anti-inflammatory drugs [74,75]. The search for future azole antifungals that show pronounced selectivity for fungal CYP's will be advantaged by the recent research related to the production of strains of *S. cerevisiae*, humanized with respect to the amino acids encoded at the CYP 51 (ERG11) yeast locus [76].

AmB (still the mainstay of therapy for some serious infections), is besides its extreme potency also quite famous for its renal toxicity and infusion-related side-effects [77]. Dose-limiting nephrotoxicity directly relates to the mode of action of AmB when it selectively binds to fungal ergosterol but also, to a lesser extent, to human membrane-associated low density lipoproteins such as cholesterol. On the other hand, toll-like receptor 1 and 2 play a major role in the infusion-related, immunomodulatory side-effects [76].

Resistance Development

To date, several mechanisms have been proposed to explain azole resistance of *C. albicans*, including (i) reduced accumulation of the azole due to reduced uptake or due to an active transport out of the cell, (ii) overproduction or mutation of the target enzyme [78] and (iii) the increased occurrence of biofilms.

(i) Interactions between sterols and phospholipids in the cytoplasmic membrane affect membrane fluidity and asymmetry and consequently influence the transport of substances across the membranes. A decrease in azole uptake by the fungal cell may result from changes in the sterol and/or the phospholipid composition of the fungal cell membrane. Hence, intracellular accumulation of azoles can be reduced by the lack of drug penetration because of low ergosterol levels. However, an important cause of reduced intracellular accumulation of the drug is the active transport of the drug out of the cell. In *C. albicans*, two families of drug efflux pumps are described: ATP-binding cassette transporters and major facilitator proteins.

(ii) Upregulation of the *ERG11* gene, which encodes the major target enzyme of the azoles lanosterol 14-α-demethylase, has been observed in azole-resistant *C. albicans* and *C. glabrata* isolates [79-81]. However, other studies have reported no significant change in expression levels of the *ERG11* gene in azole-resistant clinical isolates of *C. glabrata* [82,83]. This indicates that overexpression of the *ERG11* gene probably is not critical for the development of azole resistance. Direct evidence for certain mutations of Erg11p resulting in decreased affinity to the drug was provided by biochemical analysis of heterologously expressed enzymes. The affinity of fluconazole for lanosterol 14-α-demethylase containing the mutations Y132H, G464S or R467K was reduced as compared with the wild-type enzyme, confirming that these naturally occurring mutations indeed caused drug resistance in clinical *C. albicans* isolates [56,84,85].

(iii) Another emerging source of antifungal resistance is the occurrence of a biofilm. Biofilms are extracellular matrices produced by microbes themselves. They serve to help organisms attach to living or non-viable surfaces. Presence of biofilms may markedly reduce the susceptibility of a microbe to antimicrobial agents by either reducing the accessibility or more fundamentally to the phenotypic change undergone by the microbe [86,87]. Recent surveys estimate that to date about 65% of all human microbial infections involve biofilms and that since 2005 the majority of invasive diseases produced by *C. albicans* are associated with biofilm growth [88-96]. The most important phenotype is a reduced susceptibility to conventional antimycotics and to the host immune system [97-102]. In 2004, Mateus and co-workers demonstrated that drug efflux pumps play a role in the drug resistance of early biofilms [82,103]. In contrast, resistance of mature biofilms does not rely on the known antifungal efflux pumps [82,84]. It has been hypothesized that a change in membrane sterol composition during biofilm formation might explain resistance to amphotericin B and the azoles. In addition, the MAPK Mkc1p seems to be a regulator of azole resistance in mature biofilms [104].

Like *Candida* spp., *Aspergillus* spp. can be prone to azole resistance. Multiple mechanisms are known to be responsible, with differing degrees of azole-cross resistance, including mutations in the CYP51A gene at positions 54, 98, 138, 220 and 448 [105]. Although efflux pump-mediated resistance also occurs in *Aspergillus* spp. as in *Candida* spp., most often CYP mutations underly azole-resistance in *Aspergillus* spp. [2].

Besides azole resistance, it is worth to mention that candin resistance is frequently reported in clinical isolates of *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis*. Here, the resistance phenotype is associated with amino acid substitions in two 'hot-spot' regions of Fks1, the major subunit of glucan synthase [106]. The Fks1 mutations are known to be genetically dominant and confer cross-resistance to all echinocandin drugs. First evidence exists that Fsk1 mutations can cause resistance in yeast as well as in moulds such as *Aspergillus fumigates,* and that this mechanism may be pervasive in fungal kingdom [105].

Pharmacokinetics

Optimal active drug concentration at the site of infection is another important target for improved therapeutics. Subtle structural modifications may result in striking differences in plasma pharmacokinetics, as in case of AmB and its lipid formulations L-AmB, ABLC and ABCD. The latter three derivatives display pharmacokinetic profiles that are influenced by diverse disposition of respective lipid moieties, whereas liberated AmB its pharmacokinetic behavior is independent of lipids [107]. Triazoles such as e.g. fluconazole, itraconazole, voriconazole, pramiconazole, posaconazole, albaconazole, ravuconazole and isavuconazole are to a certain extent so-called 'look-alikes', though they differ from each other in species-specificity, potency as well as in their pharmacokinetics. Compared to the oldies fluconazole and intraconazole, the newer triazoles have in general an improved specify for fungal CYP, a different metabolic profile and a longer half-life in blood [106-109]. Metabolisation in the liver where the cytochrome P450 (CYP) system is involved is frequent for all triazoles and requires some precaution during patient management. This alertness is not required for candins although they are also rapidly taken up by pheripheral tissues such as the liver. In the first 24h, this liver uptake appears to be the main route of elimination from plasma for all three candin prototypes, i.e. caspofungin, micafungin and anidulafungin [106].

THE *IN VITRO – IN VIVO* ENIGMA

The minimum inhibitory concentration (MIC) of an antifungal drug as determined *in vitro* is expected to predict and correlate with the behavior of that drug in infected tissues. Indeed, manufacturers of antimycotics often quote low MIC values in their communication as implied evidence of therapeutic potency. In reality, however, the correlation is not always so straightforward. When multiple reports of the

correlation of therapeutic outcome with *in vitro* susceptibility are examined (reviewed in [110]), a pattern commonly referred to as the '90-60 rule', emerges. The 90-60 rule observes that infections due to susceptible isolates respond to appropriate therapy approximately 90% of the time, whereas infections due to resistant isolates (or infections treated with inappropriate antibiotics) respond approximately 60% of the time. Why does this response follow the 90-60 rule rather than a 100-0 rule? Some possibilities exist to explain this. First, the *in vitro* susceptibility might vary if a different testing method is used. For example the standard Clinical and Laboratory Standards Institute (CLSI) broth dilution techniques for antifungal susceptibility testing use planktonic populations, and, therefore, do not enable true evaluation of antifungal efficacy against *Candida* biofilms. Additionally, the CLSI method may not be the method of choice for the evaluation of slow growing organisms. In the latter case, the method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), is highly recommended. Both, CLSI and EUCAST (two microdilution methods) have made it possible to identify break points (so called MIC values) for specific antifungals against specific test-organisms [111,112]. Amongst others, determination of fungicidal activity, flow cytometry, ergosterol quantification, colorimetric microdilution, disk diffusion and agar dilution methods are also in use for susceptibility testing. No surprise that also between those methods subtle differences may exist. A popular commercial method in use today to overcome the problem of the different conditions between different labs when measuring MIC values are the so-called E-test strips [111]. In principle, those strips are based on a combination of the concepts of the dilution and diffusion tests. Like dilution methods, E-test directly quantifies antifungal susceptibility in terms of discrete MIC values. As the E-test consists of a predefined and continuous concentration gradient, the MIC values obtained can be more precise than values from conventional procedures based on discontinuous two-fold serial dilutions [113].

Second, the problem might be linked to inter-patient variability in terms of pharmacokinetics, lack of host response or production of toxins. Hence, individual susceptibility testing should be considered as a part of the process of predicting whether a given patient will respond to therapy. In essence research into the development of diagnostics is advocated.

Third, some caution should be exercised in the case of the assessment of the *in vitro* efficacy of imidazole antifungal agents using MIC determination. In practice these data are subject to large inter-laboratory variability. Notable variables include the nature of culture medium, temperature, pH, duration of incubation, inoculum size, phase of fungal growth, the presence of serum, leukocytes, cornified cells and keratins [114-116]. Keratins for example were found to affect the terbinafine efficacy in a clinically relevant onychomycosis *in* vitro test model. Indeed, the terbinafine concentration needed to block invasive mycelia that were formed after inoculation of human nail powder with *Trichophyton rubrum* was found to be 1 µg/ml after 4 weeks exposure, which is much higher than MICs $\leq$ 0.03 µg/ml in standard NCCLS MIC assays techniques [92]. Accordingly, *in vitro* results should be interpreted with caution and some *in vitro* susceptibility test results may poorly reflect *in vivo* efficacy of the imidazole drugs [117]. Ketoconazole, the first imidazole used for oral treatment, represents a notable example. Its observed *in vivo* efficacy in experimental models of dermatomycoses and candidiasis is distinctly superior than might be expected from its *in vitro* activity against the same species [93].

EMERGING ANTIMYCOTICS

Using the following selected examples we illustrate that the hunt for novel, improved antimycotics with better efficacy and safety profiles and less drug-drug interactions is very active.

In addition to caspofungin (Cancidas™, Merck&Co), two new echinocandins, micafungin (Mycamine™, Fujisawa Healthcare) and anidulafungin (Eraxis™, Pfizer), appeared in 2005 and 2006, respectively [118-121]. Primarily, these echinocandins are designed for the treatment of deep mycoses. At present, it is not yet clear whether they will be used to treat dermatophytes infections.

Voriconazole (Vfend™, Pfizer) and posaconazole (Noxafil™, Schering-Plough) are two broad spectrum triazole antifungal agents that were recently approved. In common with other azoles, they act principally by inhibition of fungal CYP51 [122,123]. Currently, voriconazole is approved for a multitude of indications (i.e. invasive aspergillosis, candidemia in non-neutropenic patients, disseminated candidal infections, esophageal candidiasis and infections caused by *Scedosporium apiaspermum* and *Fusarium* species), whereas, to our knowledge, posaconazole is only approved for two indications (i.e. prophylaxis of invasive *Aspergillus* and *Candida* infections in high-risk patients and first in-line treatment for candidiasis). Clinical studies in dermatophyte infections are ongoing.

Ravuconazole (Bristol-Myers Squibb) is another new member of the azole family that acts selectively on the fungal CYP51 [124]. Since this triazole has not yet advanced to phase 3 clinical trials, it is too early to predict whether or not this drug will reach the market and, if so, for which specific indications. Considering its antifungal spectrum, ravuconazole would be a good candidate for treatment of non-*Candida albicans* infections, including dermatophyte infections [125-128].

Hyphanox™, a late phase drug candidate of Stiefel Laboratories, is an oral formulation of itraconazole (a melt extrusion form) being developed for the treatment of various fungal infections, including vaginal candidiasis, tinea pedis and onychomycosis. It is anticipated that this 200 mg tablet formulation will improve patient compliance and reduce pharmacokinetic variability that is frequently encountered with current therapies. Apart from fluconazole, most azoles can show high inter-patient variability in terms of serum AUC and C_{max} levels. A notable food effect is also observed.

Pramiconazole (Stiefel Laboratories) is a new member of triazole class which is in phase 2 development for the treatment of superficial infections caused by dermatophytes *(e.g. Trichophyton* spp., *Microsporum canis* and *Epidermaphyton*), yeasts (*Candida* spp. and *Malassezia* spp.) and many other fungi [18,88]. At present, seven Phase 2a and

one Phase 2b studies are completed. These studies comprise a pilot and a dose-finding trial in pityriasis versicolor and pilot trials in seborrheic dermatitis, tinea pedis, tinea cruris and tinea corporis [12,18,129-132]. In all studies, both primary and secondary endpoints were met. A drug efficacy study for use in onychomycosis is in progress.

Albaconazole (Laboratorios Uriach & Cía. S.A.) is a new triazole with a potent, broad spectrum of antifungal activity (inclusive *Candida* spp., *Cryptococcus* spp. and *Aspergillus* spp.), good pharmacokinetics, low toxicity and excellent oral bioavailability (nearby 80% in rats and 100% in dogs) [133].

Posaconazole is a member of the azole class of antifungals recently approved for the prophylaxis and treatment of invasive fungal infection. It has proven fungistatic activity against most *Candida* spp., *Cryptococcus* spp. and *Trichosporon* spp, and, on top, has superior activity to the other azoles against *Zygomycetes* isolates [134].

Basilea Pharmaceutica Ltd selected a new broad spectrum azole prodrug (BAL8857) that has the potential to treat mucocutaneous and invasive fungal infections as well as onychomycosis [135,136]. After oral or intravenous administration, BAL-8557 is rapidly cleaved into isavuconazole, in a reaction catalysed by human plasma esterases. Being a broad spectrum water-soluble drug, BAL8857 was in 2008 the subject of two phase III studies that investigated the safety, tolerability and efficacy in the prevention of fungal diseases and the pharmacokinetics of the antifungal drug, namely NCT00413439 and NCT00444366 [134].

Abafungin (Abasol^{TM}, York Pharma) is a promising drug candidate of the arylguanidine family that possess fungicidal activity against dermatophytes, yeasts and moulds, regardless of whether the etiologic agents are in the growth or dormant phase [137]. A 1% cream formulation has reached an advanced stage of development and may be indicated not only for Candida intertrigo and tinea infections, but also for skin diseases caused by yeasts such as pytiriasis versicolor.

The new imidazole derivative luliconazole (Nihon Nohyaku Co. Ltd.) showed promising *in vitro* and *in vivo* activity when compared to bifonazole, terbinafine and lanoconazole. Besides many yeast species, luliconazole is active against several dermatophyte- and mold species [138,139]. In line with luliconazole, some 2,4,5-tri-substituted imidazole derivatives from Lorus Therapeutics have antifungal activity against *C. albicans*.

Anti-invasins (Microbia) represent a class of broad spectrum antifungals that have been selected using a morphogenetic transformation assay in *C. albicans* [140].

Sordarins (Diversa Corporation) were recently selected as naturally derived antifungal compounds with a novel mode of action. Interestingly, they show activity against azole-resistant fungi [116].

AN 2690 is the novel benzoxaborole (Anacor) in clinical development that shows promising preliminary results. In a reported trial, 60 subjects with mild to moderate, KOH positive, distal subungual onychomycosis were treated with either AN2690 5.0% or 7.5% once daily for a 6 month treatment period followed by a 6 month treatment-free follow-up period [27].

Efungumab (formerly know as Mycograb) is a monoclonal antibody targeting heat shock protein 90 (HSP90). *In vitro* and first clinical data show that Efungumab shows activity against *Candida* spp. when used alone and synergism when combined with fluconazole, caspofungin, and amphotericin B. Although further safety data are needed, it looks like Efungumab may become a new antifungal agent with unique mechanism of action for treatment of invasive candidiasis [141].

CONCLUSIONS

Over the last fifty years, several important discoveries have lead to the current approved armamentarium of antimycotics, including polyenes, imidazoles, triazoles, allylamines and candins. Our review of representative examples of these classes of drugs, illustrates their therapeutic value in the fight against many pathogenic fungi. Failure rates in management of fungal infections are still significant, consequently it is not surprising that there is still an unmet medical need to further improve antimycotic therapy. We believe that based on our ever expanding knowledge base, interdisciplinary research bringing together chemical, molecular and clinical expertise can help make the antimycotic drugs of tomorrow fulfill the promise of today.

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