Rev Esp Quimioterap, June 2006; Vol. 19 (Nº 2): 130-139

Review

Antifungal agents: Mode of action in yeast cells

A.J. Carrillo-Muñoz[1], G. Giusiano[2], P.A. Ezkurra[3] and G. Quindós[3]

[1]Department of Microbiology, ACIA, Barcelona, Spain; [2]Department of Mycology, Instituto de Medicina Regional, Universidad Nacional del Nordeste, Resistencia, Argentina; and [3]Department of Immunology, Microbiology and Parasitology, Faculty of Medicine, Universidad del País Vasco, Bilbao, Spain

SUMMARY

Different kinds of mycoses, especially invasive, have become an important public health problem as their incidence has increased dramatically in the last decades in relation to AIDS, hematological malignancies, transplant recipients and other immunosuppressed individuals. Management of fungal infections is markedly limited by problems of drug safety, resistance and effectiveness profile. Current therapy for invasive mycoses uses a relatively reduced number of antifungal drugs, such as amphotericin B, fluconazole and itraconazole. Other new antifungal agents from old and new chemical families, like voriconazole, posaconazole, ravuconazole, caspofungin and micafungin, have been introduced into the armamentarium for fungal infections management. This review is focused on the mode of action of those antifungal drugs used against pathogenic yeasts. The interaction of amphotericin B with ergosterol and other membrane sterols results in the production of aqueous pores of drug and the ergosterol biosynthetic pathway is the target of the allylamines, phenylmorpholines and azole antifungal agents. The main molecular target of azole antifungals is the cytochrome P-450 protein Erg11p/Cyp51p. Echinocandins, a new class of antifungal drugs, are fungal secondary metabolites that act against beta-1-3-D-glucan synthesis. The phenylmorpholines, of which amorolfine is the sole representative in human therapy, affect two targets in the ergosterol pathway: Erg24p (delta 14 reductase) and Erg2p (delta 8–delta 7 isomerase). The sordarins group are protein synthesis inhibitors that work by blocking the function of fungal translation elongation factor 2. Other protein inhibitors are zofimarin, BE31045, SCH57504, xylarin, hypoxysordarin and GR135402. In order to overcome the problems derived from the exploitation of azole drugs, macrolides and echinocandins, novel targets were explored. Proposed antifungal drugs have been developed against potential targets like the N-myristylation of fungal proteins, with inhibitors like myristate and histidine analogues or myristoylpeptide derivatives, aminobenzothiazoles, quinolines and benzofurans. Polymerization of cell wall carbohydrates from uridine di-phospho sugars is another potential target.

Key words: Antifungal drugs - Mode of action - Amphotericin B - Azole antifungals - Allylamines - Echinocandins

Antifúngicos: mecanismo de acción en células de levaduras

RESUMEN

Las micosis, especialmente las invasoras, se han convertido en un importante problema de salud al aumentar espectacularmente su incidencia durante las últimas décadas en pacientes con sida, neoplasias hematológicas, trasplantes y otros tipos de inmunosupresión. Su tratamiento está muy limitado por problemas de eficacia, resistencia y seguridad farmacológicas, y actualmente se utiliza un número relativamente reducido de antifúngicos, como amfotericina B, fluconazol e itraconazol. Otros nuevos antifúngicos, procedentes tanto de recientes familias químicas como de las clásicas, se han introducido en los protocolos de las infecciones fúngicas. Esta revisión se centra en el mecanismo de acción de los antifúngicos utilizados frente a levaduras patógenas. La interacción de amfotericina B con ergosterol y otros esteroles de membrana da como resultado la producción de poros acuosos y la vía biosintética del ergosterol es la diana sobre la que actúan las alilaminas, las fenilmorfolinas y los azoles. La principal diana molecular de los azoles es la proteína Erg11p/Cyp51p del citocromo P-450. Las equinocandinas son metabolitos secundarios fúngicos que inhiben la síntesis de beta-1-3-D-glucano. Las fenilmorfolinas, de las que amorolfina es la única utilizada en humanos, afecta a dos dianas en la vía del ergosterol: Erg24p (delta 14 reductasa) y Erg2p (delta 8-delta 7 isomerasa). Las sordarinas son inhibidores de la síntesis proteica que bloquean la función del factor de elongación 2. Otros inhibidores proteicos son zofimarina, BE31045, SCH57504, xilarina, hipoxisordarina y GR135402. Con objeto de superar los problemas derivados del abuso de azoles, macrólidos y equinocandinas, se han explorado nuevas dianas y posibles antifúngicos frente a ellas,como los inhibidores de la N-miristilación de las proteínas fúngicas, por ejemplo miristato y análogos de la histidina o derivados miristoil peptidicos, aminobenzotiazoles, quinolinas y benzofuranos. La polimerización de los hidratos de carbono de la pared celular procedentes de azúcares uridina difosfato es otra posible diana.

Palabras clave: Antifúngicos - Mecanismo de acción - Amfotericina B - Azoles - Alilaminas - Equinocandinas

INTRODUCTION

Different kinds of mycoses, especially invasive, have become an important public health problem as their incidence has increased dramatically in the last decades in relation to AIDS, hematological malignancies, transplant recipients and other immunosuppressed individuals (1–8). Fungal infections remain a major direct cause of death in patients who are treated for a malignant disease, and emerging resistance is also an important problem (1–9). These immunocompromised patients are mainly infected by *Candida, Aspergillus, Cryptococcus* and other opportunistic fungi. *Candida albicans* is most often associated with serious invasive fungal infections, but other *Candida* species and yeast-like organisms (*Trichosporon*, *Blastoschizomyces* and *Malassezia*) have emerged as etiological agents of severe mycoses (1–9).

Management of fungal infections is markedly limited by problems of drug safety, resistance and effectiveness profiles. Current therapy for invasive mycoses uses a relative reduced number of antifungal drugs, such as amphotericin B, fluconazole and itraconazole. Other new antifungal agents, from old and new chemical families, like voriconazole, posaconazole, ravuconazole, caspofungin and micafungin, have been introduced into the armamentarium for fungal infections management. Some other molecules such as albaconazole and anidulafungin among others probably will be introduced soon for clinical use (10–14).

While amphotericin B is still considered the gold standard for severe mycoses treatment (4, 15, 16), its severe acute and chronic toxicities, such as renal function impairment, limit its clinical use (15–17). The azoles and other drug families are being used even more frequently for treatment of candidemia and cryptococcosis. However, the low susceptibility of emerging fungal pathogens (mainly filamentous fungi) to classical azole derivatives, such as fluconazole or ketoconazole, has widened research interests to many other chemical compounds with better microbiological profiles.

Molecular targets have included 14-alpha-demethylase for azole derivatives, ergosterol biosynthesis for polyenes, and beta-1,3-glucan synthetase for echinocandins (14). Nevertheless, others like DNA or mitotic inhibitors, sordarins, antimicrobial peptides and aromatic di-cations have been investigated (11, 14). Different disadvantages have been reported, such as adverse reactions, fungistatic behavior instead of fungicidal activity, and resistance emergence for some azole derivatives and other drugs (3). The absence of host selectivity and a higher toxicity profile characterize polyenes in clinical practice (18). A narrow spectrum of activity against some fungal pathogens is related to echinocandins (19, 20). The ideal antifungal agent of the future should have a broad spectrum of fungicidal activity without mechanism-based host toxicity. In order to find a "golden" antifungal drug, a golden target is required. It must be founded in a broad spectrum for fungal pathogens being essential for fungal cell viability but not in human cells to avoid toxicity problems. Nevertheless, progress in that area is slow in comparison with antibacterial agents. Among the explored fungal cell targets, the cell wall is preferred for many of these agents; others may abound, but the difficulty in translating those targets into meaningful new drugs is well illustrated by the paucity of new classes of compounds over the past 20 years.

GENERAL PATHWAYS FOR ANTIFUNGAL MODE OF ACTION

Targets for the classical antifungal agents

Fungal sterols

The fungal sterols are most prolific group of antifungal agents widely used in clinical practice. They target fungal membrane sterols such as polyenes, azole derivatives, allylamines and thiocarbamates.

INTERACTION WITH MEMBRANE STEROLS

Polyene antifungals

The structure of polyene antifungals constitutes a macrolide ring of 26–28 carbons with polyunsaturations, closed by an ester or lactone, where hydroxyl groups confer the amphipatic character of the molecule. More than 100 different compounds classified as heptaenes or tetraens have been described, but amphotericin B and nystatin are the most widely used. Amphotericin B (Fig. 1) is based on the complex formation between antifungal molecule and ergosterol

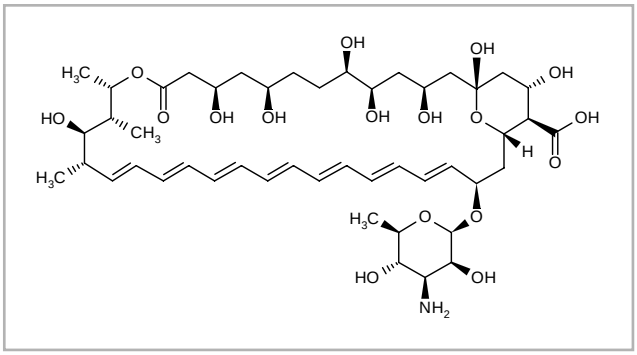

Figure 1. Amphotericin B.

membrane. This is an interactive model with pores across the membrane formed by eight amphotericin B molecules linked hydrophobically to the ergosterol membrane. This action produces an altered permeability and leakage of vital cytoplasmic components, and finally the cell dies (21, 22). Other damage related to an oxidative damage have been seen in *Candida albicans* cell (21, 22).

Amphotericin B toxicity results from the poor ability of the drug to differentiate ergosterol from fungal cell and cholesterol in mammalian cell. The clinical interest in amphotericin B is due to the fact that resistant isolates are rarely recovered from patients, and that resistant mutants are difficult to obtain in the laboratory (22), where minimal inhibitory concentrations are generally lower than 2 µg/ml aginst yeasts (23). Nevertheless, mutations are possible in *ERG3* and *ERG11* genes in *C. albicans* and *Candida glabrata* producing ergosterol depletion (22). Toxicity problems have lessened with the introduction of lipid-based drug delivery systems such as amphotericin B and nystatin formulations (13, 14, 16, 18, 24–33). Lipid carriers or liposome encapsulation, vesicles with cationic lipids, complexes or emulsions, surfactants, or cochleates are being developed and commercial available in most countries. Molar ratio and chemical composition of different formulations of liposome have different percentages of recovery, diameter of particles and acute toxicity levels. Overall, these forms of amphotericin B have a lower affinity for mammalian cells and improve the pharmacological properties of the active antifungal drug (13, 14, 18). The mode of action is similar, but a complementary action of phospholipases is needed to deliver active drug inside cells (34). Nevertheless, in phospholipase-deficient mutants of *C. albicans* and *Cryptococcus neoformans* no effect of extracellular fungal phospholipase activity has been demonstrated (35).

Amphotericin B colloidal dispersion is a formulation with this antifungal compound entrapped into a stable complex with sodium cholesteryl sulphate (molar ratio of 1:1). Transport of amphotericin B is made by forming a colloidal suspension in aqueous solutions as disk-like colloidal particles. Amphotericin B lipid complex is an amphotericin B formulation associated with a biodegradable phospholipid matrix (5% molar) composed of L-alpha-dimyristoyl-phosphatidyl choline and L-alpha-dimyristoyl-phosphatidyl glycerol from which the drug is released by cell phospholipases (34). Liposomal amphotericin B includes the antifungal drug inside unilamellar liposomes composed of hydrogenated soy phosphatidylcholine, distearoyl phosphatidylglycerol and cholesterol and containing 10 mol% of amphotericin B (13, 14, 18). A selective transfer of amphotericin B is made between the liposome and the target, the reticulo-endothelium or the foci of infection avoiding the uptake by mammalian cells (18).

Nystatin is obtained from *Streptomyces noursei*. Nystatin binds to ergosterol in the fungal membrane, producing membrane permeability changes which allow the release of K^+, sugars and metabolites (13, 14, 18). Disruption of the cell membrane is believed to be responsible for fungal death, but modes of action of amphotericin B and nystatin have differences (13, 14, 18, 30–33). Liposomal nystatin is a multilamellar liposomal formulation of nystatin, which contains nystatin, dimyristoylphosphatidyl choline and dimyristoylphosphatidyl glycerol in a ratio of 1:7:3 (by weight) (13, 14, 18, 30–33).

Other new formulations of amphotericin B and new polyenes with related modes of action are also under development. Oral cochleate–amphotericin B is a lipid-based drug delivery system (24). Composition of the carrier is based on stable phospholipid-calcium precipitates of phosphatidylserine (24). These molecules form a multilayer structure of continuous, spiralized solid lipid bilayer without internal aqueous space, requiring the fusion of the carrier with the target cell (24). Dioctadecyldimethylammonium bromide is a cationic lipid that in water solutions can form bilayer vesicles or bilayer fragments electrostatically stabilized and able to entrap amphotericin B and miconazole (25). NS-718 (Nippon Shinyaku Co., Japan) was prepared by encapsulating amphotericin B with lipid nanospheres composed of equal amounts of ovolecithin and soybean oil or lecithin-based oil and water microemulsion (26, 36), core-forming blocks of amphiphilic diblock copolymers based on methoxypoly (ethylene oxide)-block-poly (L-aspartate), PEO-b-p(L-aspartate) (37). The association with N-methyl-N-D-fructose with amphotericin B methyl ester (38) improves water solubility of amphotericin B; this could be the reason for the important reduction of toxicity by means of the formation of the monomeric form of the active drug. Another formulation is formed with nanoparticles of amphotericin B by complex coacervation using polyethylenimine and dextran sulphate (ratio 1:2) and zinc sulphate (stabilizer) (29). Conjugation of amphotericin B with oxidized arabinogalactan has generated a highly water-soluble amphotericin B–oxidized arabinogalactan conjugate (>100 mg/ml) (26). Other polyenes obtained from fungi (fungal metabolites), such as calbistrins or deformylcalbistrin, have an antifungal activity against *C. albicans*, but further studies are needed to compare the efficacy against different fungal infections (38).

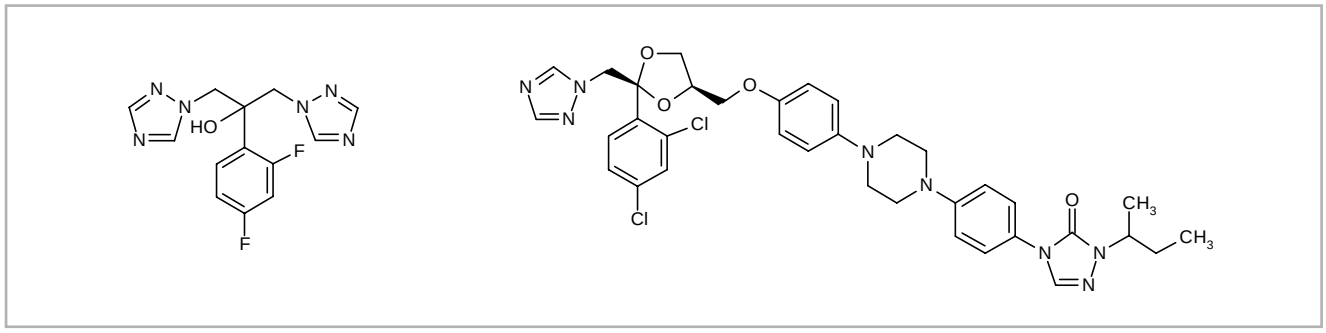

Figure 2. Fluconazole and itraconazole.

Ergosterol biosynthesis

The first generation of these antifungal drugs were the N-substituted imidazoles (late 1960s) and later the triazoles (fluconazole, itraconazole) (Fig. 2) and new triazole derivatives (voriconazole, ravuconazole, posaconazole and albaconazole) (Fig. 3). The mode of action of azole derivatives is based on the ergosterol biosynthesis pathway inhibition at different steps (21, 22, 39). Ergosterol is the major component of the fungal cell membrane. Its essential function is as a bioregulator of membrane fluidity, asymmetry and integrity (21, 22, 39). This is the target of azole derivatives and allylamine antifungal agents (2, 14, 21, 39). Earlier imidazole derivatives had a complex mode of action inhibiting several membrane-bound enzymes as well as membrane lipid biosynthesis (21, 22, 39). The activity of some enzymes in reactions can be affected in fungal cell between acetic acid and ergosterol as a result of the action of azole derivatives (14, 21, 22, 39). Due to the fact that the same P-450 enzymes produce cholesterol in mammalian liver cells, azoles can also block this biosynthesis being the basis of their adverse effects (14-alphademethylation) (14, 21, 22, 39–41). Nevertheless, new triazole derivatives have a higher target specificity (42–35).

Cytochrome P-450 lanosterol 14-alpha-demethylase, encoded by the *ERG11* gene for Erg11p, is the point of action of fluconazole, voriconazole, itraconazole and posaconazole (14, 21, 22, 42–45). Some mutations in this gene can confer fluconazole resistance in yeasts. The pathway, at lanosterol step, is not blocked when the enzyme is inhibited by azole derivatives, although lanosterol concentration can be increased (22). The 14-methyl group of lanosterol is important to generate 14-methylated intermediates; one of these is toxic (14-methylergosta-8,24(28)-dien-3,6-diol) and responsible for producing fungal inhibition (22). Some

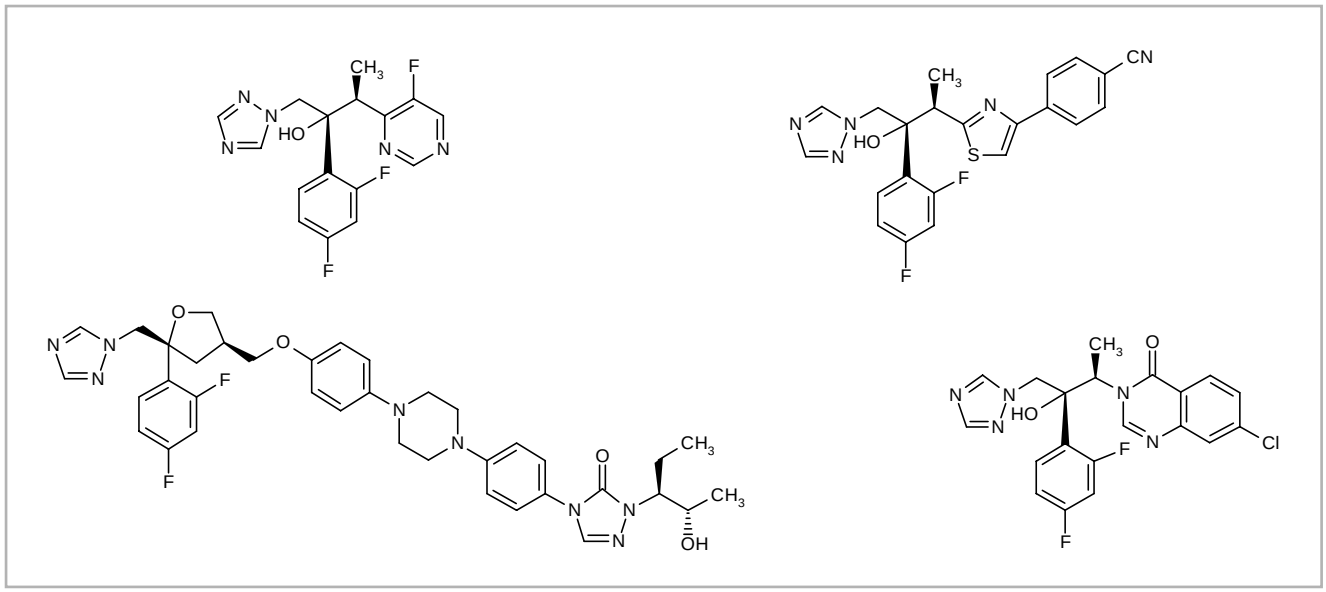

Figure 3. Voriconazole, ravuconazole, posaconazole and albaconazole.

authors found that the fungal inhibition was based in this toxic intermediates accumulation, more than in lack of ergosterol permeabilizing the plasma membrane (union with phospholipid) (22). This toxic diol model is not generally accepted for *C. albicans* (46).

C14-sterol reductase ends the C-14 modification reactions initiated by cytochrome P-450 lanosterol 14-alpha-demethylase. Antifungal attack at this target by fenpropimorph in *Saccharomyces cerevisiae* produces an accumulation of ignosterol (ergosta-8,14-dienol) (22). This is a toxic compound which perturbs membrane and inhibits uptake of glucose and pyridines (22).

C4-sterol methyl oxidase, encoded by the *ERG25* gene, removes the two C4-methyl groups, and the accumulated 4,4-dimethylzymosterol is a non-tolerated membrane sterol. In *S. cerevisiae* it produces a sterol auxotroph (22).

C-24 sterol methyl transferase, encoded by the *ERG6* gene, catalyzes a reaction not present in the cholesterol biosynthesis pathway (22), being an interesting target for selective antifungal drugs design. The effects produced in *S. cerevisiae* and *C. albicans* as a result of enzyme interaction are slow growth, poor mating, poor uptake of tryptophan, increased permeability, cation and resistance to amphotericin B (22). In addition, antifungal inhibition at this point should have a potent synergism with some existing clinical antifungal drugs increasing the antifungal susceptibility by membrane permeability to terbinafine, cycloheximide, fenpropimorph and tridemorph but not to azole derivatives (22).

Delta 5,6-desaturase, encoded by the *ERG3* gene, transforms tolerated 14-methyl intermediates into the toxic compound 14-methylergosta-8,24[28]-dien-3,6-diol (20). *ERG3* gene inactivation confers azole resistance in *C. albicans* and *S. cerevisiae* but not in *C. glabrata* and other species (22).

Previous reactions in the ergosterol biosynthesis pathway can be exploited by other antifungal families. Beta-hydroxymethylglutarate reductase, encoded by the *HMG1* gene in *C. albicans*, is the target of some statins (lovastatin, zocor) that can synergically act with fluconazole (22). A reduction of minimal inhibitory drug concentrations requires high concentrations of lovastatin (*C. albicans* or with other azole derivatives in *S. cerevisiae*) (22). This mode of synergic action could be related to a low reduction of lovastatin, which could potentiate azole effects (22). Allylamine antifungals (terbinafine and naftifine) are also ergosterol biosynthesis pathway inhibitors at squalene epoxidase; this reaction is encoded by the *ERG1 gene* (22, 47). Their mode of action is achieved by inhibiting earlier but different steps of ergosterol biosynthesis pathway than azole derivatives (22, 47). The inhibition site is located at squalene-epoxidation, producing an accumulation of the sterol precursor squalene and the absence of any other sterol intermediate (22, 47). This effect produces fungal death rather than ergosterol deficiency through an ergosterol depletion and accumulation of squalene (22, 47). An overexpression of *ERG1* has been demonstrated in cells exposed to terbinafine (*C. albicans, S. cerevisiae, Aspergillus fumigatus*) (22).

Inhibition of nucleic acids

Flucytosine is a fluorinated prymidine active against many yeasts (21, 48, 49) with a mode of action based on interference with pyrimidine metabolism, RNA/DNA and protein synthesis (21, 22, 49). The activity is mediated by a permease that drives the flucytosine inside the fungal cell (21, 22, 49). Thus, it is converted to 5-fluorouracil by a cytosine deaminase, and later, by UMP pyrophosphorylase into 5-fluorouridylic acid, which is further phosphorylated and incorporated into RNA, resulting in disruption of protein synthesis. 5-Fluorouracil is converted to 5-fluorodeoxyuridine monophosphate, a potent inhibitor of thymidylate synthase (21, 22, 49). This enzyme is involved in DNA synthesis and the nuclear division process (21, 22, 49).

Other antifungal drugs under investigation have modes of action at DNA/RNA molecules (13, 14, 22). Yatakemycin belongs to a new family of drugs isolated from *Streptomyces* spp. and chemically related to the anticancer molecules duocarmycins whose mode of action is based on the alkylation of DNA molecule (50, 51). Icofungipen (PLD-118) (Pliva Pharmaceutical Company, Croatia) was previously known as BAY 10-8888 (Bayer, Germany) (52). Icofungipen is a derivative of cispentacin, a cyclic beta–amino acid originally isolated from *Bacillus cereus* (53). Its mode of action is based on inhibiting intracellular isoleucyl-tRNA (a vital enzyme in protein synthesis and cell growth), a new mechanism of action with the advantage of a higher active accumulation in susceptible fungi (53).

Cell wall

The cell wall has historically been considered the third target for antifungal agents development. The cell wall contains mannoproteins, chitins, and alpha- and beta-glucans and plays an important role in protection, cell morphology, cell rigidity, metabolism, ion exchange and filtration, antigenic expression, primary interaction with the host and resistance to host cell–mediated immune functions (20–22, 54). This composition is not found elsewhere in other organisms, providing some selective and toxic advantages

over the modes of action of other antifungal agents (13, 14, 20–22, 54, 55). The yeast cell wall is a multilayered structure of these compounds, especially mannoproteins, that can modulate the molecular architecture of the cell wall (21, 54). Cell wall mass in *C. albicans* is composed of chitin and beta-glucan plus mannoprotein (up to 80%). In this yeast, the outer layers have beta-1,6-glucan, while the inner layers are composed of beta-1,3-glucan (21, 54).

The main targets in yeast cell wall are chitin synthesis for nikkomycins (21, 55) and also inhibitors of glucan synthesis for aculeacins, echinocandins, papulacandins, acidic terpenoids (arundifungin, enfumafungin, ascosteroside or ergokonin A) and others that have been previously and widely reviewed (13, 14, 21, 22, 56, 57).

Beta-glucan synthetase inhibitors

Echinocandins are chemically modified molecules from fungi (20). They include: caspofungin, derived from pneumocandin Bo (56) and produced by *Glarea lozoyensis* (56); micafungin derived from echinocandin B and produced by *Coleophoma empetri* (58); and anidulafungin, derived from echinocandin B and produced by *Aspergillus nidulans* (59) (Fig. 4).

The mode of action of echinocandins is based on the inhibition of cell wall beta-glucan synthesis, a specific non-competitive inhibition (beta-glucan synthase) (13, 14, 20–22, 39, 56–59). Promising data from research on echinocandins is the absence of cross-resistance with other antifungal drugs (20, 22). Nevertheless, gene mutations can induce resistance, and the development of a secondary multidrug (echinocandin-azole)-resistant yeast strain as been described (60). The main effect of echinocandins is glucan inhibition but a secondary effect is obtained by means of a reduction of the ergosterol and lanosterol content concomitant with a increased chitin content of cell wall (13, 14, 20–22, 39, 56–59). This produces cytological and ultrastructural changes, such as growth of pseudohyphae, thickened cell wall, buds failing to separate from mother cells, cells becoming osmotically sensitive and lysis being restricted to the growing tips of budding cells (13, 14, 20–22, 39, 56–59).

New trends, other targets and other antifungals agents

Other investigations in antifungal drugs are related to the discovery and use of protein synthesis pathways such as sordarins (39, 56), which are not present in the mammalian cells or mitochondrial electron transport inhibition (56, 61). Thus, novel targets have been explored in an attempt to overcome the problems derived from the exploitation of the three traditional targets for azoles, polyenes and echinocandins. These problems are focused on non-discrimination of ergosterol-cholesterol targets (problems of selectivity and toxicity) of polyenes as well as the development of resistance associated with the use of 14-alpha-demethylase and 1,3-beta-glucan synthase (problems of fungistatic effect *versus* fungicidal activity and also toxicity) (62).

Proposed antifungal drugs have been developed against potential targets like the N-myristylation of fungal proteins. Inhibitors like myristate and histidine analogues or myristoylpeptide derivatives, aminobenzothiazoles, quinolines, benzofurans and polymers of cell wall carbohydrates from uridine di-phospho sugars has been described (62-67). Protein N-myristoyl transferase is essential for *in vitro* viability of *C. albicans* and *Cryptococcus neoformans* A series of potent non-peptidic inhibitors of *C. albicans* protein N-myristoyl transferase are benzothiazoles, exemplified by UK-356,417, UK-362,091 and UK-370,753.

Although their modes of action are not well understood, some of these molecules can be active against a broad range of pathogenic fungi, including those resistant to current therapies (62–67). They may also act synergistically with other antimicrobial compounds of saliva and must be classified as prototypic molecules that could be new antifungal agents of natural or synthetic sources (68, 69). Among the chitin synthesis inhibitors and mannoprotein synthesis inhibitors, nikkomycins, polyoxins, phellinsin A and arthrichthin as well as pradimicin/benanomycin have been described but discontinued in the second class of antifungal drugs (70).

Chemically modified tetracyclines, known as antibacterial agents (CMT-3) (71), have shown reduced antifungal activity *in vitro* against *C. albicans*. Their mode of action, different from their antibacterial activity, is based on a non-conventionally exploited target for antifungal agents in intracellular organelles (depolarization of mitochondrial membranes, nucleus or endoplasmatic reticulum) (71). The interaction of CMT-3 with these organelles may result in inhibition of some metabolic pathways in the oxidative phosphorylation or protein synthesis. In the same way, other antibacterial substances, bacterial histidine kinase inhibitors (RWJ-49815, RWJ-49968, RWJ-61907), inhibit *C. albicans*, producing a general membrane damage (72). Other antifungal drugs can be found within a variety of molecules such as rapamycin (TOR pathway) (73, 74), aureobasidin (inhibition of sphingolipid biosynthesis pathway at

inositol phosphorylceramide synthase) (75); derivatives of aminoacyl tRNA synthetase (75); natural peptides (histatins, lactoferrin and cyclic amino-acid analogues) (PDL-118, BAY 10-8888, cispentacin) (76). Cationic peptides (DHVAR 4), with similar structure to salivary histatins (76), can disrupt the fungal cell membrane of *C. albicans* in the same way as lactoferrin B and LBF 17–30 (76). Ciclopiroxolamine [6-cyclohexyl-1-hydroxy-4-methyl-2[1H]-pyridone]) hydroxypyridone class antifungals, is still clinically used for topical management of fungal infections (77).

An alternative mode of action is based on the intracellular depletion of some essential molecules for the fungal cell,

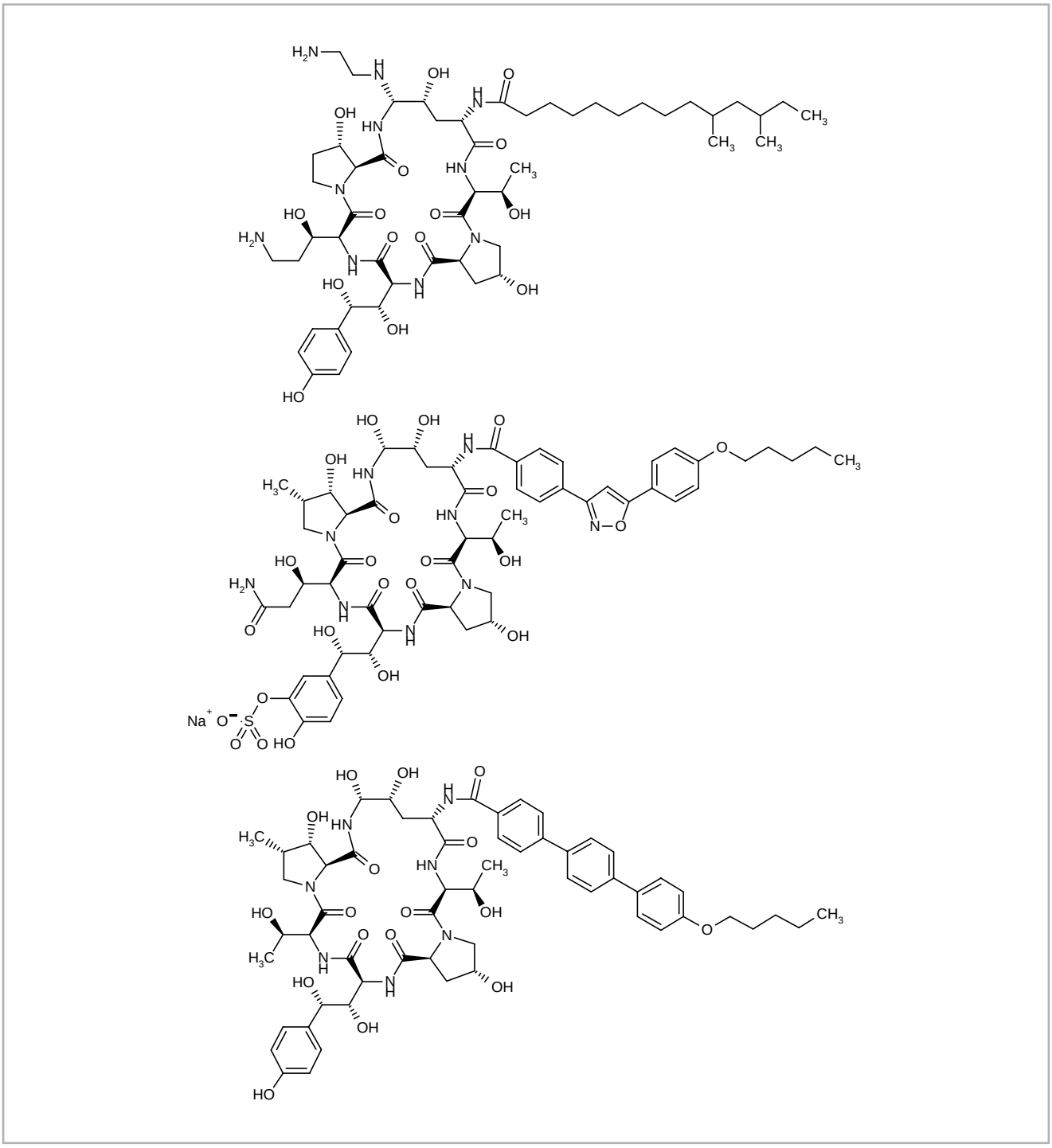

Figure 4. Caspofungin, micafungin and anidulafungin.

resulting in growth inhibition or in fungal death. This class of antifungal agents is considered blockers of the G1/S phase initiation (78). Sphingolipid synthesis inhibitors are antifungal compounds with another mode of action, specially inhibition of serine palmitoyltransferase (sphingofungins A–F, lipoxamycin, viridofungins A–C), ceramide synthase (fumonisins, australifungin), inositol phosphoceramide synthase (aureobasidins A–R, khafrefungin, galbonolide B or the mencionated macrolide rustimicin and minimoidin) with varied attractive properties and differential characters (14, 56, 57). Other peptides are produced by bacteria (iturin, bacillomycins, syringomycins, syringostatins, syringotoxins, cepacidines, nikkomycins) or by fungi (A-192411.29, L-693.989, L-731,373, L-733,560) (57). Zeamatin, frangufoline, nummularine and rugosamine A are peptides from plants with *in vitro* antifungal activity against pathogenic yeast and *Aspergillus* spp. (57). Additionally, other peptides, such as the histatins (Hsn5) and the cationic peptide MUC7 20-Mer (an amino-acid residue peptide derived from human and primate salivary mucin) developed a markedly different antifungal activity potenciated from those of miconazole or histatin. These drugs are suitable for antifungal combined therapy (78) against pathogens based in their mitochondrial mode of action. In the mitochondria, histatins disrupt the non-lytic loss of ATP and cause the cell death after the cell cycle disruption (68, 79, 80). The presence of these peptides in the mouth could be useful in the control of fungal infections of the oral cavity, while their action has been demonstrated against biofilm and adherence formation by *C. albicans* (80). In addition, severe side effects could be reduced in humans by a more selective mode of action in comparison with other antifungal agents. Nevertheless, more clinical trials should be undertaken in order establish their safety profile in light of the encouraging clinical efficacy results.

Protein inhibitors

The sordarin are protein synthesis inhibitors with a mode of action that blocks the function of fungal but not human translation elongation factor 2 (39, 56). Different sordarin derivatives have different spectra of susceptible species for reasons that are not yet clear but may be related to the problems of penetration of these agents into target fungi (56, 81). Nevertheless, their high specificity for the fungal target and the relative way to obtain new sordarin variants hold promise for positive future developments with this series of antifungal drugs.

Other compounds

Although development of some antifungal drugs has been discontinued, some studies are available searching alternative modes of action, antifungal spectrum and activity (13, 14): zofimarin, BE31045, SCH57504, xylarin, hypoxysordarin and GR135402. Some psychotropic drugs of the phenothiazine and thioxanthene, sertraline and 5-hydroxytryptamine (serotonin), a typical selective serotonin reuptake inhibitor, are also under investigation in the same way that magainins, dermaseptins, cecropins A and B, skin-PYY, HSn-5, CAY-1, MUC7 20-Mer, thananin, drosomycin and defensins produced by insects, amphibians or mammalians (mainly by human and rabbit neutrophils), some of the damaging cell membranes of *C. albicans* (55, 66, 77, 82-85).

Other substances produced by *Aspergillus* spp., like alpha-sarcin from *A. giganteus*, the antifungal protein that acts by permeabilization of the fungal membranes, have similar structures to defensins (86). An inactivation of the ribosome function has been observed in ribotoxins (87), but the mode of action is unclear although it is related to the interaction with anionic membrane phospholipids in some filamentous fungi that are sensitive to this protein in opposition with others.

Although the need for new drugs is clear, progress in that area is slow and unpredictable, requiring a long time for therapeutical tools to enter into the large list of investigational molecules listed clinical use.

ACKNOWLEDGMENTS

Authors were in part supported by grants from the Fondo de Investigación Sanitaria del Ministerio de Sanidad de España (project PI030662/2003) and from the Departamento de Industria, Comercio y Turismo del Gobierno Vasco-Eusko Jaurlaritza (project IE019, subproject Diamolfun).

Correspondence: Dr. Alfonso J. Carrillo-Muñoz, Department of Microbiology, ACIA, P.O. Box 10178, E-08080 Barcelona, Spain. Tel./Fax: +34 93 429 71 20. E-mail: acarrillo@ya.com.

REFERENCES

1. Quindós, G. *Mycoses at the dawn of the XXI century.* Rev Iberoam Micol 2002; 19: 1-4.
2. García-Ruiz, J.C., Amutio, E., Pontón, J. *Invasive fungal infection in immunocompromized patients.* Rev Iberoam Micol 2004; 21: 55-62.
3. Pontón, J., Rüchel, R., Clemonds, K.V. et al. *Emerging pathogens.* J Mycology 2000; 38 (Suppl. 1): 225-236.
4. Sandven, P. *Epidemiology of candidemia.* Rev Iberoam Micol 2000; 17: 73-81.
5. Hazen, K.C. *New and emerging yeast pathogens.* Clin Microbiol Res 1995; 8: 462-478.

6. Ellis, M., Richardson, M., de Pauw, B. *Epidemiology.* Hosp Med 2000; 61: 605-609.
7. Walsh, T.J., Groll, A., Hiemenz. J., Fleming, R., Roilides, E., Anaissie, E. *Infections due to emerging and uncommon medically important fungal pathogens.* Clin Microbiol Infect 2004; 10 (Suppl. 1): 48-66.
8. Giusiano, G., Mangiaterra, M., Rojas, F., Gámez, V. *Azole resistance in neonatal intensive care units*. J Chemother 2005; 17: 347-350.
9. Giusiano, G., Mangiaterra, M., Rojas, F., Gámez, V. *Yeast species distribution in neonatal intensive care units in northeast Argentina.* Mycoses 2004; 47: 300-303.
10. Antachopoulos, C., Walsh, T.J. *New agents for invasive mycoses in children*. Curr Opin Pediatr 2005; 17: 78-87.
11. De Pauw, B.E. *New antifungal agents and preparations.* Int J Antimicrob Agents 2000; 16: 147-150.
12. Steinbach, W.J., Benjamin, D.K., Steinbach, W.J., Benjamin, D.K. *New antifungal agents under development in children and neonates.* Curr Opin Infect Dis 2005; 18: 484-489.
13. Carrillo-Muñoz, A.J., Brió, S., Quindós, G. *A new generation of antifungal drugs*. Rev Iberoam Micol 2001; 18: 2-5.
14. Carrillo-Muñoz, A.J., Quindós, G., López-Ribot, J.L. *Current developments in antifungal agents.* Curr Med Chem Anti Infective Agents 2004; 3: 297-323
15. Ostrosky-Zeichner, L., Marr, K.A., Rex, J.H., Cohen, S.H. *Amphotericin B: Time for a new "gold standard"*. Clin Infect Dis 2003; 37: 415-425.
16. Lemke, A., Kiderlen, A.F., Kayser, O. *Amphotericin B.* Appl Microbiol Biotechnol 2005; 68: 151-162.
17. Ellis, M.E., al-Hokail, A.A., Clink, H.M. et al. *Double-blind randomized study of the effect of infusion rates on toxicity of amphotericin B.* Antimicrob Agents Chemother 1992; 36: 172-179.
18. Zotchev, S.B. *Polyene macrolide antibiotics and their applications in human therapy.* Curr Med Chem 2003; 10: 211-223.
19. Johnson, M.D., Perfect, J.R. *Caspofungin: First approved agent in a new class of antifungals*. Expert Opin Pharmacother 2003; 4: 807-823.
20. Wiederhold, N.P., Lewis, R.E., Wiederhold, N.P., Lewis, R.E. *The echinocandin antifungals: An overview of the pharmacology, spectrum and clinical efficacy.* Expert Opin Investig Drugs 2003; 12: 1313-1333.
21. Ghannoum, M.A., Rice, L. *Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance.* Clin Microbiol Review 1999; 12: 501-517.
22. Akins, R.A. *An update on antifungal targets and mechanisms of resistance in Candida albicans.* Med Mycol 2005; 42: 285-318.
23. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard–Second Edition. NCCLS document M27-A2. NCCLS, Wayne, Pennsylvania, 2002.
24. Santangelo, R., Paderu, P., Delmas, G. et al. *Efficacy of oral cochleate-amphotericin B in a mouse model of systemic candidiasis.* Antimicrob Agents Chemother 2000; 44: 2356-2360.
25. Lincopan, N., Mamizuka, E.M., Carmona-Ribeiro, A.M. *In vitro activity of a novel amphotericin B formulation with synthetic cationic bilayer fragments*. J Antimicrob Chemother 2003; 52: 412-418.
26. Espuelas, M.S., Legrand, P., Campanero, M.A. et al. *Polymeric carriers for amphotericin B: In vitro activity, toxicity and therapeutic efficacy against systemic candidiasis in neutropenic mice.* J Antimicrob Chemother 2003; 52: 419-427.
27. Fukui, H., Koike, T., Nakawa, T. et al. *Comparison of LNS-AmB, a novel low-dose formulation of amphotericin B with lipid nanosphere (LNS), with commercial lipid-based formulations.* Int J Pharm 2003; 28: 101-112.
28. Brime, B., Molero, G., Frutos, P., Frutos, G. *Comparative therapeutic efficacy of a novel lyophilised amphotericin B lecithin-based oil-water microemulsion and deoxycholate-amphotericin B in immunocompetent and neutropenic mice infected with Candida albicans.* Eur J Pharm Sci 2004; 22: 451-458.
29. Tiyaboonchai, W., Woiszwillo, J., Middaugh, C.R. *Formulation and characterizaction of amphotericin B-polyethylenimine-dextran sulfate nanoparticles.* J Pharm Sci 2001; 90: 902-914
30. Arikan, S., Rex, J.H. *Nystatin LF (Aronex/Abbott).* Curr Opin Investig Drugs 2001; 2: 488-495.
31. Alonso-Vargas, R., González-Alvarez, L., Ruesga, M.T. et al. *In vitro activity of a liposomal nystatin formulation (Nyotran) against Cryptococcus neoformans.* Rev Iberoam Micol 2000; 17: 90-92.
32. Ng, A.W., Wasan, K.M., López-Berestein, G. *Liposomal polyene antibiotics*. Methods Enzymol 2005; 391: 304-313.
33. Carrillo-Muñoz, A.J., Quindós, G., Tur, C. et al. *In-vitro antifungal activity of liposomal nystatin in comparison with nystatin, amphotericin B cholesteryl sulphate, liposomal amphotericin B, amphotericin B lipid complex, amphotericin B desoxycholate, fluconazole and itraconazole.* J Antimicrob Chemother 1999; 44: 397-401.
34. Swenson, C.E., Perkins, W.R., Roberts, P. et al. *In vitro and in vivo antifungal activity of amphotericin B lipid complex: Are phospholipases important?* Antimicrob Agents Chemother 1998; 42: 767-771.
35. Gottfredsson, M., Jessup, C.J., Cox, G.M., Perfect, J.R., Ghannoum, M.A. *Fungal phospholipase activity and susceptibility to lipid preparations of amphotericin B.* Antimicrob Agents Chemother 2001; 45: 3231-3233.
36. Tomii, Y. *Lipid formulation as a drug carrier for drug delivery.* Curr Pharm Des 2002; 8: 467-474.
37. Adams, M.L., Andes, D.R., Kwon, G.S. *Amphotericin B encapsulated in micelles based on poly(ethylene oxide)-block-poly(L-amino acid) derivatives exerts reduced in vitro hemolysis but maintains potent in vivo antifungal activity.* Biomacromolecules 2003; 4: 750-757.
38. Stewart, M., Capon, R.J., Lacey, E., Tennant, S., Gill, J.H. *Calbistrin E and two other new metabolites from an Australian isolate of Penicillium striatisporum.* J Nat Prod 2005; 68: 581-584.
39. Odds, F.C., Brown, A.J., Gow, N.A. *Antifungal agents: Mechanisms of action.* Trends Microbiol 2003; 11: 272-279.
40. Hossain, M.A., Ghannoum, M.A. *New investigational antifungal agents for treating invasive fungal infections.* Expert Opin Investig Drugs 2000; 9: 1797-1813.
41. Hossain, M.A., Ghannoum, M.A. *New developments in chemotherapy for non-invasive fungal infections.* Expert Opin Investig Drugs 2001; 10: 1501-1511.
42. Courtney, R., Pai, S., Laughlin, M., Lim, J., Batra, V. *Pharmacokinetics, safety, and tolerability of oral posaconazole administered in single and multiple doses in healthy adults.* Antimicrob Agents Chemother 2003; 47: 2788-2795.
43. Arikan, S, Rex, J.H. *New agents for the treatment of systemic fungal infections–Current status.* Expert Opin Emerg Drugs 2002; 7: 3-32.
44. Arikan, S., Rex, J.H. *Ravuconazole (Eisai/Bristol-Myers Squibb).* Curr Opin Investig Drugs 2002; 3: 555-561.
45. Bartrolí, J., Turmo, E., Algueró, M. et al. *New azole antifungals. 3. Synthesis and antifungal activity of 3-substituted-4(3H)-quinazolinones.* J Med Chem 1998; 41: 1869-1882.
46. Bard, M., Lees, N.D., Turi, T. et al. *Sterol synthesis and viability of erg11 (cytochrome P450 lanosterol demethylase) mutations in Saccharomyces cerevisiae and Candida albicans.* Lipids 1993; 28: 963-967.
47. Leber, R., Fuchsbichler, S., Klobucnikova, V. et al. *Molecular mechanism of terbinafine resistance in Saccharomyces cerevisiae.* Antimicrob Agents Chemother 2003; 47: 3890-3900.

48. Quindós, G., Ruesga, M.T., Martín-Mazuelos, E. et al. *In-vitro activity of 5-fluorocytosine against 1,021 Spanish clinical isolates of Candida and other medically important yeasts.* Rev Iberoam Micol 2004; 21: 63-69.
49. Kerridge, D. *Mode of action of clinically important antifungal drugs.* Adv Microb Physiol 1986; 27: 1-72.
50. Parrish, J.P., Kastrinsky, D.B., Wolkenberg, S.E., Igarashi, Y., Boger, D.L. *DNA alkylation properties of yatakemycin.* J Am Chem Soc 2003; 25: 10971-10976.
51. Igarashi, Y., Futamata, K., Fujita, T. et al. *Yatakemycin, a novel antifungal antibiotic produced by Streptomyces spp. TP-A0356.* J Antibiot (Tokyo) 2003; 56: 107-113.
52. Yeates, C. *Icofungipen (PLIVA).* Curr Opin Investig Drugs 2005; 6: 838-844.
53. Petraitiene, R., Petraitis, V., Kelaher, A.M. et al. *Efficacy, plasma pharmacokinetics, and safety of icofungipen, an inhibitor of Candida isoleucyl-tRNA synthetase, in treatment of experimental disseminated candidiasis in persistently neutropenic rabbits.* Antimicrob Agents Chemother 2005; 49: 2084-2092.
54. Marcilla, A., Valentin, E., Santandreu, R. *The cell wall structure: Developments in diagnosis and treatment of candidiasis.* Int Microbiol 1998; 1: 107-116.
55. Ruiz-Herrera, J., San-Blas, G. *Chitin synthesis as target for antifungal drugs.* Curr Drug Targets Infect Disord 2003; 3: 77-91.
56. Vicente, M.F., Basilio, A., Cabello, A., Peláez, F. *Microbial natural products as a source of antifungals.* Clin Microbiol Infect 2003; 9: 15-32.
57. De Lucca, A.J., Walsh, T.J. *Péptidos antifúngicos: Origen, actividad y potencial terapéutico.* Rev Iberoam Micol 2000; 17: 116-120.
58. Jarvis, B., Figgitt, D.P., Scott, L.J. *Micafungin*. Drugs 2004; 64: 969-982.
59. Vázquez, J.A. *Anidulafungin: A new echinocandin with a novel profile.* Clin Ther 2005; 27: 657-673.
60. Moudgal, V., Little, T., Boikov, D., Vázquez, J.A. *Multiechinocandin- and multiazole-resistant Candida parapsilosis isolates serially obtained during therapy for prosthetic valve endocarditis*. Antimicrob Agents Chemother 2005; 49: 767-769.
61. Fujita, K., Tani, K., Usuki, Y., Tanaka, T., Taniguchi, M. *Growth inhibition dependent on reactive oxygen species generated by C9-UK-2A, a derivative of the antifungal antibiotic UK-2A in Saccharomyces cerevisiae.* J Antibiot (Tokyo) 2004; 57: 511-517.
62. Georgopapadakou, N.H. *New cell wall targets for antifungal drugs.* Expert Opin Investig Drugs 2001; 10: 269.
63. Bujdakova, H., Kuchta, T., Sidoova, E., Gvozdjakova, A. *Anti-Candida activity of four antifungal benzothiazoles.* FEMS Microbiol Lett 1993; 112: 329-333.
64. Lodge, J.K., Jackson-Machelski, E., Devadas, B. et al. *N-myristoylation of Arf proteins in Candida albicans: An in vivo assay for evaluating antifungal inhibitors of myristoyl-CoA:Protein N-myristoyltransferase*. Microbiology 1997; 143: 357-366.
65. Kawasaki, K., Masubuchi, M., Morikami, K. *Design and synthesis of novel benzofurans as a new class of antifungal agents targeting fungal N-myristoyltransferase. Part 3.* Bioorg Med Chem Lett 2003; 13: 87-91.
66. Ebiike, H., Masubuchi, M., Liu, P. *Design and synthesis of novel benzofurans as a new class of antifungal agents targeting fungal N-myristoyltransferase. Part 2.* Bioorg Med Chem Lett 2002; 12: 607-610.
67. Selitrennikoff, C.P., Nakata, M. *New cell wall targets for antifungal drugs.* Curr Opin Investig Drugs 2003; 4: 200.
68. Bobek, L.A., Situ, H. *MUC7 20-Mer: Investigation of antimicrobial activity, secondary structure, and possible mechanism of antifungal action.* Antimicrob Agents Chemother 2003; 47: 643.
69. Jacob, M.R., Walker, L.A. *Natural products and antifungal drug discovery.* Methods Mol Med 2005; 118: 83-109.
70. López, S.N., Castelli, M.V., de Campos, F. *In vitro antifungal properties structure-activity relationships and studies on the mode of action of N-phenyl, N-aryl, N-phenylalkyl maleimides and related compounds*. Arzneimittelforschung 2005; 55: 123-132.
71. Liu, C.H., Meng, J.C., Zou, W.X., Huang, L.L., Tang, H.Q., Tan, R.X. *Antifungal metabolite with a new carbon skeleton from Keissleriella spp. YS4108, a marine filamentous fungus*. Planta Med 2002; 68: 363-365.
72. Deschenes, R.J., Lin, H., Ault, A.D., Fassler, J.S. *Antifungal properties and target evaluation of three putative bacterial histidine kinase inhibitors.* Antimicrob Agents Chemother 1999; 43: 1700-1703.
73. Singh, N., Heitman, J. *Antifungal attributes of immunosuppressive agents: New paradigms in management and elucidating the pathophysiologic basis of opportunistic mycoses in organ transplant recipients*. Transplantation 2004; 77: 795-800.
74. Wong, G.K., Griffith, S., Kojima, I., Demain, A.L. *Antifungal activities of rapamycin and its derivatives, prolylrapamycin, 32-desmethylrapamycin, and 32-desmethoxyrapamycin.* J Antibiot (Tokyo) 1998; 51: 487-491.
75. Sugimoto, Y., Sakoh, H., Yamada, K. *IPC synthase as a useful target for antifungal drugs.* Curr Drug Targets Infect Disord 2004; 4: 311-322.
76. Nikawa, H., Fukushima, H., Makihira, S., Hamada, T., Samaranayake, L.P. *Fungicidal effect of three new synthetic cationic peptides against Candida albicans.* Oral Dis 2004; 10: 221-228.
77. Niewerth, M., Schaller, M., Korting, H.C., Hube, B. *Mode of action of ciclopiroxolamine on Candida albicans.* Mycoses 2002; 45 (Suppl. 1): 63-68.
78. Baev, D., Li, X.S., Dong, J., Keng, P., Edgerton, M. *Human salivary histatin 5 causes disordered volume regulation and cell cycle arrest in Candida albicans.* Infect Immun 2002; 70: 4777-4784.
79. Wei, G.X., Bobek, L.A. *In vitro synergic antifungal effect of MUC7 12-mer with histatin-5 12-mer or miconazole*. J Antimicrob Chemother 2004; 53: 750-758.
80. Kavanagh, K., Dowd, S. *Histatins: Antimicrobial peptides with therapeutic potential.* J Pharm Pharmacol 2004; 56: 285-289.
81. Domínguez, J.M., Martín, J.J. *Identification of elongation factor 2 as the essential protein targeted by sordarins in Candida albicans.* Antimicrob Agents Chemother 1998; 42: 2279-2283.
82. Lass-Flörl, C., Fuchs, D., Ledochowski, M., Speth, C., Dierich, M.P., Würzner, R. *Antifungal properties of 5-hydroxytryptamine (serotonin) against Candida species in vitro*. J Med Microbiol 2003; 52: 169-171.
83. Lass-Flörl, C., Dierich, M.P., Fuchs, D., Semenitz, E., Ledochowski, M. *Antifungal activity against Candida species of the selective serotonin-reuptake inhibitor, sertraline.* Clin Infect Dis 2001; 33: 135-136.
84. Park, Y., Lee, D.G., Hahm, K.S. *HP(2-9)-magainin 2(1-12), a synthetic hybrid peptide, exerts its antifungal effect on Candida albicans by damaging the plasma membrane.* J Pept Sci 2004; 10: 204-209.
85. Renault, S., De Lucca, A.J., Boue, S., Bland, J.M., Vigo, C.B., Selitrennikoff, C.P. *CAY-1, a novel antifungal compound from cayenne pepper.* Med Mycol 2003; 41: 75.
86. Theis, T., Wedde, M., Meyer, V., Stahl, U. *The antifungal protein from Aspergillus giganteus causes membrane permeabilization*. Antimicrob Agents Chemother 2003; 47: 588.
87. Holden, D.W., Tang, C.M., Smith, J.M. *Molecular genetics of Aspergillus pathogenicity*. Antonie Van Leeuwenhoek 1994; 65: 251-255.