Agricultural and Biological Sciences Journal
Vol. 1, No. 5, 2015, pp. 206-216
http://www.aiscience.org/journal/absj

Antifungal Agents That Target Fungal Cell Wall Components: A Review

Babatunde I. Aderiye*, Olusola A. Oluwole

Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria

Abstract

The fungal cell wall represents an exclusive structure that confers mechanical strength and osmotic resistance to fungal cells. The fungal cell wall's structure contains mainly mannan, chitins and glucans in different proportions depending on the species. These structural components are unique to fungi and are currently being investigated as targets for novel antifungals. Currently, glucan component of the cell wall is the predominant target for therapeutic applications and developments of antifungals. Three classes of glucan inhibitors in application includes the lipopeptides e.g. echinocandins, the glycolipid papulacandins and the acid terpenoids including enfumafungin and ascosterosides. These agents are known 1,3-β-d glucan synthesis inhibitors and have been shown to possess antifungal activity *in vitro* as well as *in vivo* in many different animal models against yeasts e.g. *Candida* and filamentous fungi e.g. *Aspergillus.* The other targets, chitin and mannan are however being investigated given the limited applications of some of the glucan synthesis inhibitors. Chitin inhibitors available includes the related polyoxins and nikkomycins produced by *Streptomyces* while those that target the mannan component of the cell wall are Pradimicins and benanomicins by *Actinomadura* spp. Both groups have been found to possess broad spectrum activity against most medically important fungi e.g. *Aspergillus fumigatus*, *Candida* and *Coccidioides immitis*. The mechanism of action of these agents as well as their activity of against fungal pathogens is discussed.

Keywords

Fungi, Glucan, Chitin, Mannan, Cell Wall, Echinocandins

Received: June 9, 2015 / Accepted: June 25, 2015 / Published online: July 17, 2015

1. Introduction

The cell wall is an extracellular feature that is present in fungi, plant cells and bacteria (Cooper and Sunderland, 2000). The fungal cell wall is a dynamic structure that protects the cell from changes in osmotic pressure and other environmental stresses, while allowing the fungal cell to interact with its environment (Bowman and Free, 2006). The structure and biosynthesis of a fungal cell wall is exclusive to the fungi, differing from the mammalian cells and is therefore an excellent target for the development of anti-fungal drugs. The fungal cell wall is a vital and complex structure containing mannoproteins, chitins and glucans. Chitin and glucan components of the cell wall are unique and essential to fungi (Georgopapadakou and Tkacz, 1995) and unlike sterols; they have no mammalian counterpart and are the targets of antifungals. The composition of the cell wall varies between species of fungi but a major components of many fungal cell walls is β1,3-glucan (Hector, 1993). Glucan is an essential carbohydrate component of all fungal cell walls, comprising 30%–60% of the fungal cell wall and forms a layer of network which acts as a scaffold for other macromolecules (Klis *et al*., 2001; 2002).

The cell wall composition of filamentous fungi differs in some respects from that of yeasts (Bernand and Latge, 2001). These differences include a higher concentration of chitin, and, in *Aspergillus fumigatus*, the presence of a poly N-acetylgalactosamine polymer and a novel linear b-1,3/1,4

* Corresponding author
E-mail address: jadesolaaderiye@yahoo.com (B. I. Aderiye)

glucan (Fontaine *et al*., 2000; Beauvais *et al*., 2001). In *Aspergillus fumigatus* and *Cryptococcus neoformans*, many of the same polysaccharides and mannoproteins are found in the cell wall, but the organization appears to be quite different (Reese and Doering, 2003; Douglas, 2009).

Giving the unique nature of the fungal cell wall, any disruption in cell wall integrity should affect growth. Also, the pathways for the synthesis of these cell wall precursors represent unexplored targets for new antifungals that have modes of action that are different from currently used therapeutics (Selitrennikoff and Nakata, 2003).

The discovery of antifungal agents that possess selective toxicity against the eukaryotic fungal cell remains an important scientific challenge (Debono and Gordee, 1994). This review looks at the antifungal agents that target fungal cell wall components and their mechanism of activity.

2. Antibiotics That Target Glucan Components

Inhibitors of glucan synthesis have been shown to possess antifungal activity *in vitro* as well as *in vivo* in many different animal models. There are three general structural classes of 1,3-β- d glucan synthesis inhibitors (Douglas, 2001). The first class, the lipopeptides, includes the echinocandins, the aerothricin lipopeptidolactones and the arborcandins. The second class of inhibitors is the glycolipid papulacandins, which consist of a modified disaccharide linked to two fatty acyl chains. The third and recently discovered class, are represented by enfumafungin, ascosterosides, arundifungin and ergokonin A (Douglas, 2009). Of the antifungal agents that are glucan inhibitors, the echinocandins are the most prominent in therapeutic use.

2.1. Echinocandins

The echinocandins are first-line agents for treating severe invasive fungal infections (IFIs), being fungicidal against yeast and fungistatic against molds (Jiménez-Ortigosa *et al*., 2014). The echinocandins are one of the newest classes of antifungal agents that act by inhibiting cell wall synthesis. The echinocandins are semi-synthetic lipopeptides that are derived from fermentation products from several different fungi (Hector, 1993). Echinocandins function by inhibiting the synthesis of β -1,3-D-glucan, an essential component of the fungal cell wall (Eshwika *et al*., 2013). They specifically target the FKS1 genes in fungal organisms that encode for the components of the enzyme glucan synthase, an enzyme necessary for synthesis of 1,3-β -D glucan, an essential component of the cell wall of susceptible fungi (Gubbins and Anaissie, 2009). These agents bind rapidly and irreversibly to β 1, 3-d-glucan synthase and cause rapid death in certain pathogens.

The echinocandins exhibit potent *in vitro* and *in vivo* fungicidal activity against *Candida* species, including azole-resistant pathogens. Caspofungin was derived from *Glarea lozoyensis,* while micafungin and anidulafungin are fermentation bye- products of the fungi *Coleophoma empetri* F-11899 and *Aspergillus nidulans,* respectively. Echinocandins have antifungal activity against the most common yeasts and molds, although these agents generally lack activity as single agents against *Cryptococcus neoformans* and *Zygomycetes*. The proportion of the fungal cell wall composed of glucan varies widely between different species of fungi. 1, 3- β-D-glucan is more predominant in the cell walls of *Candida* and *Aspergillus* species (especially *C. albicans* and *A. fumigatus*) than in yeast forms of dimorphic fungi. Likewise, the cell walls of mycelial forms of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Paracoccidioides braziliensis* contain significant amounts of 1,3-β-D-glucan, while zygomycetes lack this target component. However, these characteristics do not always predict echinocandin activity. For instance, the cell wall of *Cryptococcus neoformans* contains 1, 3-β-D-glucan but the echinocandins demonstrate little activity against this pathogen. This suggests that there are likely additional (or alternate) components of the mechanism of action of the echinocandins (Feldmesser *et al*., 2000; Maligie and Selitrennikoff, 2005). Growth of *A. fumigatus* and several other filamentous fungi is significantly inhibited by the echinocandins.

2.1.1. Caspofungin

Caspofungin was the first member of the echinocandins to be licensed for use (Deresinski and Stevens, 2003; Grover, 2010) and shows excellent *in vitro* and in *vivo* activity against *Candida* and *Aspergillus* species (Maertens *et al*., 2004; Zaoutis *et al*., 2009). Caspofungin is a water-soluble amphipathic lipopeptide with a molecular mass of 1213 kDa that is a semisynthetic derivative of pneumocandin B0, a fermentation product of *Glarea lozoyensis* (Deresinski and Stevens, 2003). The caspofungin-mediated disruption of glucan synthesis results in the formation of an osmotically delicate atypical cell wall and subsequent osmolysis of the cell at high concentrations (Deresinski and Stevens, 2003). Caspofungin has a long fatty acid side chain that may allow intercalation in the bi-layer of the fungal cell membrane where it may interact with, and disrupt the function of the enzyme β -1,3-D-glucan synthase (Denning, 2003).

Caspofungin is a noncompetitive inhibitor of the enzyme -(1,3)-glucan synthase, which catalyzes the polymerization of uridine diphosphateglucose (UDP-glucose) into -(1,3)-glucan,

a structural component of the fungal cell wall responsible for maintaining integrity and rigidity. When -(1,3)-glucan synthesis is inhibited, ballooning out of the weakened cell wall occurs as a result of the high osmotic pressure of the protoplast and causes cell lysis (Stone *et al*., 2002). Caspofungin has showed fungicidal against *Candida* species (Bartizal *et al*., 1997). Caspofungin is active in vitro against both azole-susceptible and resistant Candida species isolates, including *Candida krusei*, regardless of the mechanism of azole resistance (Pfaller *et al.,* 2001). *Candida lusitaniae*, which is commonly resistant to amphotericin B, is susceptible to caspofungin (Bartizal *et al*., 1997; Espinel-Ingroff, 1998).

Powles *et al*. (1998) found out that Caspofungin was active *in vitro* and in the treatment of experimental *Pneumocystis carinii* infection of mice. Krishnan *et al*. (2005) reported that caspofungin was fungistatic against *A. fumigatus*. The drug causes significant injury, and perhaps lysis, to the hyphal tips of actively growing cells, this may prevent the organism from spreading beyond the initial site of infection. Also, caspofungin was found to inhibit the growth (via 1,3-β-D-glucan synthase inhibition) of several rare molds, including *Alternaria* sp., *Curvularia* sp., *Scedosporium apiospermum* and *prolificans*, *Acremonium* sp., *Bipolaris* sp., and *Trichoderma* sp. (Kahn *et al*., 2006). Caspofungin was also effective in patients with invasive aspergillosis who were refractory to or intolerant of standard antifungal agents (Keating and Figgitt, 2003). Caspofungin provides an alternative to triazoles or amphotericin B in oesophageal candidiasis and an alternative to amphotericin B in invasive candidiasis, as well as being an effective salvage therapy in invasive aspergillosis (Keating and Figgitt, 2003).

Other fungi besides members of the *Candida* and *Aspergillus* genera have been evaluated for caspofungin susceptibility in animal models. *Pneumocystis carinii* is a major cause of morbidity and mortality in HIV-infected patients, and caspofungin was found to be potent in a rat model of *P. carinii* pneumonia (Powles *et al*., 1998). However, there are no in vitro susceptibility correlates for evaluating the activity of echinocandins against *P. carinii* because this organism has not been grown in culture (Douglas, 2009). Another human respiratory pathogenic fungus (*C. immitis*) produces disseminated disease when injected into mice, and the infection can be effectively treated with caspofungin, which prolongs survival and reduces CFU in several different target organs (Gonzalez *et al*., 2001).

2.1.2. Anidulafungin

Anidulafungin is a semi-synthetic product of echinocandin B, itself a fermentation product of the mold *Aspergillus nidulans*. Anidulafungin inhibits enzyme complex 1,3-β-D-glucan synthase and thereby inhibits fungal 1,3-β-D-glucan synthesis. Glucan is a major structural component of the cell wall of many pathogenic fungi that is not present in mammalian cells (Denning, 2003). A difference in glucan content determines the exceptional activity of anidulafungin in fungi and the rareness of side effects in humans. The inhibition leads to lysis of the fungal cell wall, and cell death. Anidulafungin exhibits low MICs against mycelial forms of *H. capsulatum*, *B. dermatitidis*, and *C. immitis*, but hhig MICs against the yeast forms (Espinel-Ingroff, 1998).

Anidulafungin has potent in vitro fungicidal activity against a broad range of *Candida* species, including *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, Candida famata, Candida rugosa*, and *Candida stellatoidea* (Odabasi *et al*., 2004). Anidulafungin is also effective against species of *Candida* that are inherently resistant to azoles *(Candida krusei)*, amphotericin B *(Candida lusitaniae)*, or other echinocandins *(C. parapsilosis)* (Vasquez and Sobel, 2006). Anidulafungin has also demonstrated excellent in vitro activity against several species of *Aspergillus* (Zhanel *et al*., 1997; Serrano *et al*., 2003). Anidulafungin also shows synergestic effects in vitro in combination with amphotericin B against *Aspergillus* species and *Fusarium* species isolates as well as when combined with itraconazole or voriconazole against *Aspergillus* species (Philip *et al*., 2003).

The safety and effectiveness of anidulafungin, as well as its novel pharmacokinetic characteristics, make it a suitable alternative antifungal compound for therapy of muco-cutaneous candidiasis, candidemia, IC, and, particularly, for antifungal-refractory mucosal candidiasis (Vasquez and Sobel, 2006). Anidulafungin appears to have several advantages over other antifungal drugs. It provides a broad spectrum of activity with proven potency against a wide array of *Candida* species, including azole- or polyene resistant species. Anidulafungin also has in vitro and in vivo activity against *Aspergillus* species, comparable to caspofungin and micafungin (Vasquez and Sobel, 2006).

2.1.3. Micafungin

Micafungin is an echinocandin; semisynthetic water-soluble lipopeptide. It is synthesized by a chemical modification of a fermentation product of *Coleophoma empetri* F-11899. Like other echinocandins, micafungin inhibits production of 1,3-b-d-glucan synthase that is needed for the synthesis of cell wall glucans which provide the structural integrity and osmotic stability to the fungus. The action of the echinocandin in inhibiting cell wall synthesis results in lysis. Micafungin exhibits fungicidal activity against *Candida* spp. and fungistatic activity against *Aspergillus* spp. (Sucher *et al*., 2009). Micafungin has good activity against *C. albicans, C. glabrata, C. krusei, C. tropicalis*, *and C. parapsilosis* and has

been shown, including those strains that are azole resistant. There is activity against dimorphic fungi, especially the mycelial forms of these organisms. Micafungin has been shown to possess activity against *Aspergillus* spp. including *A. fumigatus* and *A. terreus*. Micafugin demonstrates activity against the mycelialforms of the dimorphic fungi *Histoplasma capsulatum, Blastomyces dermatitidis, and Coccidioides immitis,* but only limited activity vs. yeast forms of these fungi; pooractivity is demonstrated against Zygomycetes and hyaline moulds including *Fusarium*and *Pseudallescheria boydii*; no activity against *C. neoformans* (Kaufman and Carver, 2008; Sucher *et al*., 2009).

2.1.4. Aerothricin

They have been identified as novel members of liposaccharide glucan synthesis inhibitors. Aerothricins, like natural product molecules, act as antifungal drugs that inhibit the formation of the -1,3-D-glucan component of the cell wall, but they are less water soluble than the related semi-synthetic molecules. The semi-synthetic molecules contain various basic amino acids and a large series of amino-alkyl groups (Schwartz, 2001).

2.1.5. Pneumocandins

The pneumocandins in particular have been successfully used to develop an antifungal drug that has been recently approved by the FDA. This semi-synthetic pneumocandin, caspofungin acetate, is an aza-substituted derivative of pneumocandin B0 (Bouffard *et al*., 1996). Pneumocandins are natural products derived from the fermentation of the fungus Glarea lozoyensis (Schwartz *et al*., 1992, Bills *et al*., 1999). The introduction of additional amino groups in the peptide ring of pneumocandin B0 increased the solubility of the molecule and the potency against fungal pathogens by two orders of magnitude (Bouffard *et al*., 1994). The compound has been shown to be effective in vivo in animal models of disseminated candidiasis, aspergillosis, coccidiomycosis and pneumonia caused by *Pneumocystis carinii* (Abruzzo *et al*., 1997; Gonzalez *et al*., 2001) resulting in morphological alterations of hyphae in filamentous fungi with hyphae abnormally grown, shortened, stunted and highly branched with bipolar or vesicular tips, swollen germ tubes and frequent balloon-like cells (Vicente *et al*., 2001; Kobayashi *et al*. 2005).

2.1.6. Mulunocandin

Mulundocandin is an echinocandin-like lipopeptide obtained from a variant of *Aspergillus sydowii* (Roy *et al*., 1987). It has antifungal activity against *Candida* strains, including fluconazole-resistant isolates, and is being developed by Aventis (Romainville, France). However, this compound is poorly active against other non-*Candida albicans* isolates and is inactive against *Cryptococcus neoformans* (Hawser *et al*., 1999). The biological activity of mulundocandin and structural elucidation studies with the compound have been described previously (Mukhopadhyay *et al*., 1989; Hawser *et al*., 1999).

2.1.7. Plant Based Antifungals That Target Fungal Cell Wall

Pitrowski *et al*. (2015) reported the discovery of a new antifungal compound that targeted the cell wall of fungi. The compound, poacic acid was found in lignocellulosic hydrolysates of grasses. Morphological analysis carried out in the study revealed that cells treated with poacic acid behaved similarly to cells treated with other cell wall-targeting drugs. Using morphological analysis and chemical genomics, they observed that poacic acid inhibited β-1,3-glucan synthesis in vivo and in vitro, possessed rapid lysis ability by actually by directly binding β-1,3-glucan. They concluded that, poacic acid is a natural antifungal agent against yeasts (*Saccharomyces cerevisiae*), economically significant fungi and oomycete plant pathogens (*Sclerotinia sclerotiorum, Alternaria solani,* and *Phytophthora sojae*).

2.2. Papulacandin

(We removed the subheading Glycolipids as Papulacandins are the only available representative of the group)

The papulacandins are a series of naturally occurring antifungal agents whose isolation and characterization were initially reported by Traxler *et al*. (1977). Five structurally related papulacandins A, B, C, D and E were identified based on the fermentation, isolation, physico-chemical properties and their biological activities with papulacandin B the main component (Traxler *et al*., 1977). They contain a benzannulated spiroketal unit, which has been the signature of a wide series of bioactive natural products (Kaaden *et al*., 2012). It is a highly amphophilic substance with glucose and galactose residues and two long-chain unsaturated fatty acids. It does not cause the release of potassium ions from yeast cells hence differing in its activity from the polyene antibiotics. The papulacandins are a family of modified disaccharides with fatty acyl side chains that possess reasonable antifungal activity against most species of *Candida*, but little activity against *C. neoformans* or filamentous fungi. Both papulacandin A and B exhibited in vivo efficacy against an induced disseminated *C. albicans* infection in mice, albeit at high doses (180 and 80 mg/kg, respectively) and when dosed subcutaneously rather than orally (Traxler *et al*., 1977; Douglas, 2009).

Observations of growing *C. albicans* cells exposed to papulacandin were consistent with an effect on cell wall synthesis, since buds viewed under the microscope appeared

to burst, and quiescent cells were much less susceptible to lysis. Metabolic labeling of cells incubated with papulacandins revealed preferential inhibition of incorporation of radiolabeled glucose into the cell wall polysaccharides. When the wall components were fractionated, the alkali-insoluble fraction, which is enriched in glucan rather than mannan, was specifically affected (Baguley *et al*., 1979). Papulacandin B, at low concentrations, partially but selectively inhibited the incorporation of glucose into cells of *Saccharomyces cerevisiae* and *Candida albicans* (Baguley *et al*., 1979). More recent work with papulacandin B and some new members of the papulacandin family has demonstrated direct, noncompetitive inhibition of microsomal GS activity (Perez *et* al., 1983; Ohyama *et al*., 2002).

2.3. Terpene Glycosides

The terpene glycosides are a distinct class of GS inhibitors (Onishi *et al.,* 2000, Yang *et al*., 2003). Like the papulacandins, these compounds preferentially inhibit incorporation of radiolabeled glucose into glucan in the whole-cell labeling experiments, and directly interfere with microsomal GS activity. The spectrum of antifungal activity includes many *Candida* species, with little to no activity against *C. neoformans* or bacteria. The effect on *Aspergillus* species was distinct – hyphae exposed to these compounds were highly branched, shortened and stunted, with bipolar or vesicular tips, swollen germ tubes, and frequent balloon-like cells (Cabello *et al.,* 2001). Activity of the terpene glycosides against *C. albicans* in liquid micro-broth dilution assays was significantly reduced in the presence of osmotic support.

2.3.1. Enfumafungin

Enfumafungin is one among several new fungal triterpenoid glycosides isolated from the fermentation of *Hormonema* sp. (Onishi *et al*., 2000) that present potent *in vitro* antifungal activity by inhibiting the β-(1,3)-glucan synthase (Peel *et al*., 2010). It is one of the structurally distinct natural product class of GS inhibitors identified others including (ascosterocide, arundifungin, and ergokonin A) (Onishi *et al*., 2000). Pelaez *et al*. (2000) screened the drug for its antifungal activity and found it to possess both *in vitro* and *in vivo* activity against *Candida* and *Aspergillus* species although the *in-vivo* activity was moderate. A synthetic derivative of Enfumafungin MK-3118 is in clinical trials as an oral antifungal agent (Motyl *et al*., 2010). An investigation on the activity of the new derivative by Jimenez-Ortigosa *et al*. (2014) showed that MK-3118 was highly active on most *fks*-mediated echinocandin-resistant strains, especially those from *C. albicans* and *C. glabrata*. It was also active on *Aspergillus* spp. at high concentrations of the drug and was active against a highly resistant strain.

MK-3118 has also been shown to retain *in vitro* activity against both azole- and echinocandin-resistant strains of *Candida* (Walker *et al*., 2011; Heasley *et al*. 2012) with potent in vivo activity against *Candida* and *Aspergillus* spp. (Pfaller *et al*. 2013a,b). Importantly, although echinocandins and enfumafungin both target the GS enzyme (encoded by the Fks1-encoding catalytic subunit and GTPase regulatory subunit Rho1), drug-resistant mutations to each GS inhibitor class map to fks1 but do not display cross-resistance, emphasizing that the two molecules have distinct mechanisms of GS inhibition (Walker *et al*. 2011).

2.3.2. Arundifungin

Arundifungin is a novel acidic terpenoid antifungal compound produced by *Arthrinium arundinis*. Arundifungin was found to cause the same morphological alterations pattern in *Aspergillus fumigatus* hyphae as observed in echinocandins, further supporting the idea that arundifungin belongs to a new class of glucan synthesis inhibitors (Cabello *et al*., 2001). Moreover, its antifungal spectrum was comparable to those of echinocandins and papulacandins, preferentially inhibiting the growth of Candida and *Aspergillus* strains, with very poor activity against *Cryptococcus* (Onishi *et al*., 2000). Arundifungin inhibited normal polarized hyphal growth and shortened, stunted, highly branched hyphae were observed, with polar tips, swollen germ tubes and frequently ballooned cells (Cabello *et al*., 2001).

2.3.3. Ergokonin A

Ergokonin A, a sulfated carboxysteroid, was first isolated from *Trichoderma koningii* in 1991 (Angustiniak *et al*. 1991), and from *T. viride* (Kumeda *et al*., 1994) but only a limited characterization of the compound was provided and its mode of action was not clearly identified. It was however rediscovered by Merck scientists from *T. longibrachiatum* (Vicente *et al*., 2001). The compound shows similar activity as a (1,3)-b-D-glucan synthase inhibitor observed in lipopeptides pneumocandin Bo and aculeacin. The active substance exhibited an antifungal activity against *Candida* and *Aspergillus* (Vicente *et al*., 2001). Vicente *et al*. (2001) investigation revealed that the antifungal spectrum of ergokonin A was very wide, inhibiting the growth of many *Candida spp., S. cerevisiae* and most of the filamentous fungi. Examination of *A. fumigatus* cells after treatment with ergokonin A revealed some relevant changes in hyphal morphology, similar to that produced by semisynthetic pneumocandin L-733560 and other (1,3)-b-D-glucan synthase inhibitors e.g. FR207944, an antifungal triterpene glucoside from *Chaetomium* sp. (Kobayashi *et al*., 2005).

2.3.4. Ascosteroside

Ascosteroside, closely related to PF-1032 in structure, was first discovered by the Bristol-Meyers Squibb research team (Gorman *et al*. 1996). The producing organism, an ascomycetous fungus *Ascotricha amphitricha*, was isolated from soil in Kenya, Africa. Ascosteroside was discovered independently from the genus *Ellisiodothis* by a joint research team of Meiji Seika and Mitsubishi Kasei, Japan under the name of MK6059 (Konno *et al*. 1997). The compound is active against several *Candida* species, *Saccharomyces cerevisiae* and against filamentous fungi (*Trichophyton mentagrophytes* and *Aspergillus nidulans*) and showed activity *in vivo* in mice infected with *Candida*, resulting in increased survival time comparable to what obtained in ketoconazole (Gorman *et al*., 1996). Onishi *et al*. (2000) found out that ascosteroside showed high fungicidal activity against *Candida glabrata*, a *Candida* species that showed resistance to other glucan synthase inhibitors. Its activity is exhibited by inhibiting normal hyphae growth in filamentous fungi and alteration of the ovoid shape of yeasts (Onishi *et al*., 2000).

2.4. Aculeacin A

Aculeacin A is the main component of a new family of antibiotic complex produced by *Aspergillus aculeatus* (Mizuno *et al*., 1977). Aculeacin A is a cyclopeptide-containing long-chain fatty acid, representing a new class of antibiotics. It has a relatively narrow antifungal spectrum in vitro and is highly active against some groups of yeasts e.g. *Candida* and *Torulopsis* (Yamaguchi *et al*., 1977).

The chemical structure of aculeacin A containing a cyclopeptide moiety and a long-chain fatty acid, palmitic acid, is closely related to that of echinocandin B which possesses linoleic acid instead (Satoi *et al*., 1977; Iwata *et al*., 1982). Aculeacin A is fungicidal for growing cells of *C. albicans*. Mizoguchi *et al*., 1977 in their study found out cytological and biochemical evidence that Aculeacin A selectively inhibits the cell wall synthesis of growing S*accharomyces cerevisiae*. This was also confirmed by Baguley *et al*. (1970) who tested the antibiotics against *S. cerevisiae* and *Candida albicans*. In 1982, Yamaguchi *et al*. found out that even at low concentrations, Aculeacin A possesses fungicidal effect against actively growing *Candida albicans* and other yeasts resulting in cytolysis but not non growing cultures.

3. Chitin Synthase Inhibitors

Chitin and chitosan are hallmark polysaccharides that are present in all known fungal pathogens and not in humans. Inhibition of chitin synthesis has therefore been proposed as an attractive target for antifungal therapies (Lenardon *et al*., 2010). Chitin is synthesized by plasma membrane associated proteins which accept substrate from the cytosol and extrude chitin polymers into the wall. In *Saccharomyces cerevisiae,* chitin synthesis encompasses at least three synthases with different characteristics (Choi *et al*. 1994) although they all catalyse the formation of glycosidic bonds using uridine diphospho-N-acetylglucosamine. Chitin synthases are specifically inhibited by two related families of antibiotics, referred to as polyoxins and nikkomycins produced by *Streptomyces cacaoi* var. *asoensis* and *S. tendae*, respectively (Gooday 1990c). They are nucleoside peptide antibiotics that act as competitive inhibitors of chitin synthases by imitating UDP-GlcpNAc. Fungi susceptible to these inhibitors show characteristic morphological features, particularly bulging hyphae and swelling tips. However, susceptibility to polyoxins and nikkomycins is highly variable between fungal species owing to differences in their transport rate through the cell membrane (Debono and Gordee 1994, Tariq and Devlin 1996). However, no Chitin synthase inhibitor has ever progressed into clinical practice (Munro and Gow, 1995) with Nikkomycin Z still in development (Chaudhary *et al*., 2013). Nikkomycins and polyoxins are potent and specific against class I enzymes but are less effective inhibitors of other classes of CHS enzymes, and of fungal growth *in vivo* (Guaghran *et al.,* 1994).

3.1. Polyoxins

Polyoxins are potent inhibitors of chitin synthetases in fungi (Li *et al*., 2012). Polyoxins (A-M), a group of peptidyl nucleoside antibiotics were isolated from the culture broth of *Streptomyces cacaoi* (Isono *et al*., 1969). Due to their structural similarity to UDP-*N*-acetylglucosamine, polyoxins act as competitive inhibitors of chitin synthetases and display potent inhibitory activity against phytopathogenic fungi (Hori *et al*., 1971, 1974).

3.2. Nikkomycins

Nikkomycins are nucleoside-peptide antibiotics produced by *Streptomyces* species with antifungal activities through the inhibition of chitin synthesis. Like polyoxins, they act as competitive analogs of the substrate UDP-*N*-acetylglucosamine for chitin synthase (Cabib, 1991). Lack of chitin in the cell wall eventually leads to osmotic lysis (Becker *et al*., 1981). The antifungal activity of nikkomycin Z, one of the naturally derived nikkomycins, has been well described (Guaghran *et al*., 1994; Bormann *et al*., 1996; Georgopapapdakou *et al*., 1996). Among the medically important fungi, *Coccidioidis immitis* and *B. dermatitidis* are susceptible to nikkomycin Z both *in vitro* and *in vivo* (Hector *et al*., 1990; Perfect *et al*., 1991). Hector *et al*. (1990)

reported prolonged survival of mice infected with *Coccidioides immitis* and *Blastomyces dermatitidis* when treated with nikkomycin Z.

4. Mannan-Binding Antibiotics

Pradimicins and benanomicins are antifungal antibiotics produced by *Actinomadura* spp. (Takeuchi *et al*. 1988, Tomita *et al*. 1990). Pradimicins-benanomicins are generally fungicidal (Fung-Tomc and Bonner, 1997). These antibiotics exhibit broad spectrum antifungal activities and are fungicidal (Oki *et al*. 1990). The mechanism of action depends on the binding to mannan in the presence of calcium ions. This complex disrupts the integrity of the cell membrane (Walsh and Giri 1997). Both groups of compounds show low toxicity in mammalian cell models (Lyman and Walsh 1992) and semisynthetic derivatives have been prepared and studied (Fung-Tomc *et al*. 1995).

4.1. Pradimicins

Pradimicins are a recent class of nonpeptidic benzonaphtacenequinone antifungal compounds currently being introduced for therapeutic purposes (Walsh and Giri, 1997). An actinomycete strain, *Actinomadura hibisca,* was found to produce pradimicin A (PRM-A) and showed which shows activity against systemic fungal infections (Oki *et al*., 1988). The pradimicin structure is characterized by an aglycone of dihydrobenzo (alpha) naphthacenequinone with substitutions by a D-amino acid and hexose sugar. Pradimicins prossess a novel mechanism of action consisting of a specific binding recognition to terminal D-mannosides of the cell wall of *Candida albicans*, resulting in the formation of a ternary complex consisting of D-mannoside, pradimicin, and calcium that leads to disruption of the integrity of the fungal cell membrane. Pradimicin in the form of BMS-181184 has broad-spectrum in vitro antifungal activity against *Candida* spp. *Cryptococcus neoformans*, *Aspergillus* spp., dematiaceous molds, and the *Zygomycetes*. *Fusarium* spp. are comparatively resistant to high concentrations of pradimicin. Initial in vivo studies indicate that pradimicins have antifungal activity against experimental murine disseminated candidiasis and disseminated aspergillosis.

4.2. Benanomicins

Benanomicin A is an antifungal antibiotic produced by *Actinomadura spadix* MH193-16F (Takeuchi *et al.,* 1988), and one of a new family of benzo-[a]-naphthacenequinone antibiotics (Gomi *et al.,* 1988; Tomita *et al.,* 1990). Preliminary reports showed that benanomicin A has in-vitro activity against several pathogenic *Candida* spp., *Cryptococcus neoformans, Aspergillus* spp. and *Trichophyton* spp. (Kondo *et al.,* 1991). Watanabe *et al*. (1996) in their study found out that benanomicin A binds selectively to mannan or mannan-derived polysaccharide moieties localized on the cell envelope of fungi. They also observed that a carboxylic acid in the D-alanine moiety and a sugar moiety in the benanomicin molecule are essential for both binding and antifungal activities against growing *S. cerevisiae*. Benanomicin A binds to various yeast mannans which differ in glycosidic linkages. They therefore concluded that binding of benanomicin A to the mannan portion of fungal cells is essential for exertion of the antifungal activity.

5. Conclusion

The compounds discussed in this review represents the antifungal drugs that target the cell wall components of yeasts and filamentous fungi in clinical development. As the development in antifungal therapy continues, a better understanding of the mechanism of action of the antifungals available should help to improve their activity and to identify new antifungal targets. Also, the pathways for the synthesis of these cell wall precursors represent possible targets for new antifungals that have modes of action that are different from currently used therapeutics. With the advent of fungal genomics, an increase in the number of molecular targets useful for antifungal drug discovery is expected which will bypass the challenge of structural similarities of eukaryotic cells that have limited the progress of antifungals currently employed in therapeutics. This will set the stage for the development of an antifungal portfolio to rival the diversity of antibacterial drugs resulting in the increase the armamentarium of drugs active against systemic fungal infections.

References

[1] Abruzzo GK, Flattery AM, Gill CJ *et al*. 1997 Evaluation of the echinocandin antifungal MK-0991 (L- 743,872): efficacies in mouse models of disseminated aspergillosis, candidiasis and cryptococcosis. Antimicrob Agents Chemother. 41: 2333–8.

[2] Angustiniak H, Forsche E, Reichenbach H, Wray V, Grafe U, and Hofle G. Isolerung und Struktur Aufklarung von Ergokonin A und B, zwei neue antifungish Sterol-antibiotika aus *Trichoderma konningii. Liebigs Ann Chem* 1991; 361–366.

[3] Baguley, B.C., Römmele, G., Gruner, J. and Wehrli, W. Papulacandin B: an Inhibitor of Glucan Synthesis in Yeast Spheroplasts. *European Journal of Biochemistry*, 1979; 97: 345–351. doi: 10.1111/j.1432-1033.1979.tb13120.x

[4] Bartizal K, Gill CJ, Abruzzo GK, Flattery AM, Kong L, Scott PM, Smith JG, Leighton CE, Bouffard A, Dropinski JF, et al. *In vitro* preclinical evaluation studies with the echinocandin antifungal MK-0991 (L-743,872). *Antimicrob Agents Chemother* 1997; 41: 2326–2332.

[5] Beauvais A, Bruneau JM, Mol PC, et al. Glucan synthase complex of *Aspergillus fumigatus. J. Bacteriol.* 2001; 183:2273–9.

[6] Bernard M, Latge, JP. *Aspergillus fumigatus* cell wall: composition and biosynthesis. *Med Mycol* 2001; 39 (Suppl 1):9–17.

[7] Bills GF, Platas G, Pela ´ez F, Masurekar P. Reclassification of a pneumocandin-producing anamorph*, Glarea lozoyensis* gen. et sp. nov.,previously identified as *Zalerion arboricola. Mycol. Res.* 1999; 103: 179–92.

[8] Bouffard FA, Dropinski JF, Balkovec JM et al. L-743,872, a novel antifungal lipopeptide: synthesis and structure-activity relationships of new azasubstituted pneumocandins. [abstract F27]. In: *Proceedings of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans. American Society for Microbiology*: Washington, DC, 1996; 104.

[9] Bouffard FA, Zambias RA, Dropinski JF et al. Synthesis and antifungal activity of novel cationic pneumocandin Bo derivatives. *J Med Chem* 1994; 37: 222–5

[10] Bowman, S. M. and Free, S.J. "The structure and synthesis of the fungal cell wall". *BioEssays* 2006; 28 (8): 799–808.

[11] Cabello MA, Platas G, Collado J, Diez MT, Martin I, Vicente F, Meinz M, Onishi JC, Douglas C, Thompson J, Kurtz MB, Schwartz RE, Bills GF, Giacobbe RA, Abruzzo GK, Flattery AM, Kong L, Pelaez F. Arundifungin, a novel antifungal compound produced by fungi: biological activity and taxonomy of the producing organisms. *Int Microbiol* 2001; 4(2):93-102.

[12] Chaudhary PM, Tupe SG, Deshpande MV. Chitin synthase inhibitors as antifungal agents. *Mini Rev Med Chem.* 2013; 13(2):222-36.

[13] Choi, W.J., Santos, B., Duran, A. and Cabib, E.. Are yeast chitin synthases regulated at the transcriptional or the posttranslational level? *Mol. Cell. Biol*. 1994; 14: 7685-7694.

[14] Cooper, G.M. and Sunderland, M.A. *The Cell: A Molecular Approach.* 2nd edition**.** Sinauer Associates Inc., U.S. 2000; Pp 1.

[15] Debono, M. and Gordee, R.S. Antibiotics that inhibit cell wall development. *Annu. Rev. Microbiol*. 1994; 48: 471-497.

[16] Denning DW. Echinocandin antifungal drugs. *Lancet* 2003; 362:1142-51.

[17] Deresinski S, Stevens DA. Caspofungin. *Clin Infect Dis* 2003; 36: 1445 – 1457.

[18] Douglas, C.M., *Antimicrobial Drug Resistance* Volume 1 Lerner, S.A. Ouellette, M. and Sobel, J.D. (Eds.). Humana press, London 2009.

[19] Espinel-Ingroff A. Comparison of In vitro activities of the new triazole SCH56592 and the echinocandins MK-0991 (L-743, 872) and LY303366 against opportunistic filamentous and dimorphic fungi and yeasts. *J Clin Microbiol*. 1998; 36:2950–6

[20] Feldmesser M, Kress Y, Mednick A, et al. The effect of the echinocandin analogue caspofungin on cell wall glucan synthesis by *Cryptococcus neoformans*. *J Infect Dis*. 2000; 182: 1791–5.

[21] Fontaine T, Simenel C, Dubreucq G, et al. Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. *J Biol Chem* 2000; 275:27594–607.

[22] Fung-Tomc, J.C. and Bonner, D.P. Recent developments in pradimicin-benanomicin and triazole antibiotics. *Expert Opin Investig Drugs*. 1997; 6(2):129-45.

[23] Fung-Tomc, J.C., Minassian, B., Huczko, E., Kolek, B., Bonner, D.P. and Kessler, R.E. *In vitro* antifungal and fungicidal spectra of a new pradimicin derivative, BMS-181184. *Antimicrob. Agents Chemother*. 1995; 39: 295-300.

[24] Gaughran, J.P., Lai, M.H., Kirsch, D.R., and Silverman, S.J. Nikkomycin Z is a specific inhibitor of *Saccharomyces cerevisiae* chitin synthase isozyme Chs3 in vitro and in vivo. *J. Bacteriol* 1994; 176: 5857-5860.

[25] Georgopapadakou NH, Tkacz JS. The fungal cell wall as a drug target. *Trends Microbiol*. 1995; 3(3):98-104.

[26] Gomi, S. M. Sezaki, S. Kondo, T. Hara, H. Naganawa and T. Takeuchi: The structures of new antifungal antibiotics benanomicins A and B. *J. Antibiotics* 1988; 41: 1019-1028.

[27] Gonza´lez GM, Tijerina R, Najvar LK et al. Correlation between antifungal susceptibilities of *Coccidioides immitis* in vitro and antifungal treatment with caspofungin in a mouse model. *Antimicrob. Agents Chemother*. 2001; 45: 1854–9.

[28] Gooday, G.W. Inhibition of chitin metabolism. In: *Biochemistry of cell walls and membranes in fungi*. P.J. Kuhn, A.P.J. Trinci, M.J. Jung, M.W. Goosey and L.G. Copping (eds.). Springer-Verlag, Berlin. 1990c; 61-79.

[29] Gorman JA, Chang L-P, Clark J, Gustavson DR, Lam KS, Mamber SW, Pirnik D, Ricca C, Fernandes PB, and O'Sullivan J. Ascosteroside, a new antifungal agent from Ascotricha amphitricha. I. Taxonomy, fermentation and biological activities. *J. Antibiot*. 1996; 49:547–552.

[30] Gubbins, P.O. and Anaissie, E.J. Antifungal therapy In: *Clinical Mycology*. Elsevier Health Sciences 2009; Chapter 7.

[31] Hawser, H. Borgonovi, M. Markus, A. and Isert, D. Mulundocandin, an Echinocandin-like Lipopeptide Antifungal Agent: Biological Activities *In Vitro*. *J. Antibiotics*. 1999; 52(3): 305-310.

[32] Heasley BH, Pacofsky GJ, Mamai A, Liu H, Nelson K, Coti G, Peel MR, Balkovec JM, Greenlee ML, Liberator P, et al. Synthesis and biological evaluation of antifungal derivatives of enfumafungin as orally bioavailable inhibitors of β-1,3-glucan synthase. *Bioorg. Med. Chem. Lett*. 2012; 22: 6811–6816.

[33] Hector R.F., Zimmer B.L., Pappagianis D. Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis. *Antimicrob. Agents Chemother*. 1990; 34:587–593.

[34] Hector RF. Compounds active against cell walls of medically important fungi. *Clin. Microbiol. Rev* 1993; 6:1,

[35] Hori M, Eguchi J, Kakiki K, Misato T: Studies on the mode of action of polyoxins. VI. Effect of polyoxin B on chitin synthesis in polyoxin-sensitive and resistant strains of *Alternaria kikuchiana. J Antibiot* 1974; 27(4):260-266.

[36] Hori M, Kakiki K, Suzuki S, Misato T: Studies on the mode of action of polyoxins. Part III. Relation of polyoxin structure to chitin synthetase inhibition. *Agr Biol Chem* 1971; 35(8):1280-1291.

[37] Isono K, Asahi K, Suzuki S: Studies on polyoxins, antifungal antibiotics. 13. The structure of polyoxins. *J Am Chem Soc* 1969; 91(26):7490-7505.

[38] Iwata, K., Yamamoto, Y., Yamaguchi, H. and Hiratani, T. *In-vitro* studies of aculeacin A, a new antifungal antibiotic. *J. Antibiotics.* 1982; 35: 203-209.

[39] Jiménez-Ortigosa, C. Paderu, P. Motyl, M.R. Perlina, D.S. Enfumafungin Derivative MK-3118 Shows Increased In Vitro Potency against Clinical Echinocandin-Resistant *Candida* Species and *Aspergillus* Species Isolates. *Antimicrobial Agents and Chemotherapy* 2014; 58(2): 1248 –1251.

[40] Kahn JN, Hsu M, Racine F, et al. Caspofungin susceptibility in *Aspergillus* and non-*Aspergillus*molds: inhibition of glucan synthase and reduction of -D-1,3 glucan levels in culture. Antimicrob Agents Chemother. 2006; 50: 2214–16.

[41] Kaufman CA, Carver PL Update on echinocandin antifungals. *Semin. Respir. Crit. Care Med.* 2008; 28: 211–219.

[42] Keating G. and Figgitt D. Caspofungin: a review of its use in oesophageal candidiasis, invasive candidiasis and invasive aspergillosis. *Drugs* 2003; 63(20): 2235-63.

[43] Klis, F.M. De-Groot P. and Hellingwerf, K. Molecular organization of the cell wall of *Candida albicans Medical Mycology* 2001; 39(1): 1-8.

[44] Klis, F.M., Mol, P., Hellingwerf, K. and Brul, S. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* 2002; 26: 239–256.

[45] Kobayashi, M. Yoshimura, S. Kinoshita, T.; Hashimoto, M.; Hashimoto, S. Takase, S. Fujie, A. Hino, M. and Hori, Y. FR207944, an Antifungal Antibiotic from *Chaetomium* sp. No. 217 I. Taxonomy, Fermentation, and Biological Properties. *Biosci. Biotechnol. Biochem.* 2005; 69: 1029-1032.

[46] Kondo, S.; S. Gomi, D. Ikeda, M. Hamada, T. Takeuchi, H. Hoshino and J. Seki: Antifungal and antiviral activities of benanomicins and their analogues. *J. Antibiotics* 1991; 44: 1228-1236.

[47] Konno F, Sasaki T, Chiba N, Harimaya K, Kanda M, Mikawa T, and Sato Y. *Novel antibiotic MK6059, process for the preparation of the same, and drug composition containing the same*. WO9738005, 1997; 16 Oct.

[48] Krishnan S, Manavathu EK, Chandrasekar PH. A comparative study of fungicidal activities of voriconazole and amphotericin B against hyphae of *Aspergillus fumigatus. J Antimicrob Chemother*. 2005; 55: 914–20.

[49] Kurtz MB, Heath IB, Marrinan J, Dreikorn S, Onishi J, Douglas C. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-beta-D-glucan synthase. *Antimicrob Agents Chemother* 1994; 38:1480.

[50] Lenardon, M.D., Munro, C.A and. Gow, N.A.R. Chitin synthesis and fungal pathogenesis *Curr Opin Microbiol*. 2010; 13(4): 416–423 doi: 10.1016/j.mib.2010.05.002.

[51] Li, J. Li, L. Feng, C. Chen, Y and Tan, H. Novel polyoxins generated by heterologously expressing polyoxin biosynthetic gene cluster in the sanN inactivated mutant of *Streptomyces ansochromogenes*. *Microbial Cell Factories* 201; 11:135 doi:10.1186/1475-2859-11-135.

[52] Lyman, C.A. and Walsh, T.J. Systemically administered antifungal agents. *Drugs*. 1992; 44: 9-35.

[53] Maertens J, Raad I, Petrikkos G, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin. Infect. Dis.* 2004; 39: 1563 – 1571.

[54] Maligie MA and Selitrennikoff CP. *Cryptococcus neoformans* resistance to echinocandins: (1,3) beta-glucan synthase activity is sensitive to echinocandins. *Antimicrob Agents Chemother*.2005; 49:2851–6.

[55] Mizoguchi, J.; Saito, T. Mizuno K. and Hayano, K. On the mode of action of anew antifungal antibiotic, aculeacin A: inhibition of cell wall synthesis in *Saccharomyces cerevisiae*. *J. Antibiotics* 1977; 30: 308-313.

[56] Mizuno, K., Yagi, A., Satoi, S., Takada, M., Hayashi, M., Asano, K. and Matsuda, T. Studies on aculeacin. I. Isolation and characterization of aculeacin A. *J. Antibiotics*. 1977; 30: 297- 302.

[57] Motyl MR, Tan C, Liberator P, Giacobbe R, Racine F, Hsu MJ, NielsenKahn, Bowman JJ, Douglas C, Hammond M, Balkovec JM, Greenlee ML, Meng D, Parker D, Peel M, Fan W, Mamai A, Hong J, Orr M, Ouvry G, Perrey D, Liu H, Jones M, Nelson K, Ogbu C, Lee S, Li K, Kirwan R, Noe A, Sligar J, Martensen P. *Abstr. Intersci. Conf. Antimicrob. Agents Chemother. Boston, MA*, abstr. 2010; F1-847.

[58] Mukhopadhyay T., Ganguli B. N., Fehlhaber H. W., Kogler H., Vertesy L. Mulundocandin, a new lipopeptide antibiotic. II. Structure elucidation. *J. Antibiot*. (Tokyo) 1987; 40:281–289.

[59] Munro, C.A., and Gow, N.A.R. Chitin biosynthesis as a target for antifungals. In *Antifungal Agents: Discovery and Mode of Action*. Dixon, G.K., Copping, L.G., and Hollomon, D.W. (eds). Oxford: Bios Scientific, 1995; 161- 171.

[60] Odabasi Z, Paetznick VL, Rodriguez JR, Chen E, Ostrosky-Zeichner L. In vitro activity of anidulafungin against selected clinically important mold isolates. *Antimicrob Agents Chemother* 2004; 48:1912-15.

[61] Ohyama T, Iwadate-Kurihara Y, Hosoya T, Ishikawa T, Miyakoshi S, Hamano K, Inukai M. *F-10748 A1, A2, B1, B2, C1, C2, D1 and D2,* novel papulacandins. *J. Antibiot.* (Tokyo), 2002; 55(8): 758–763.

[62] Oki, T., M. Konishi, K. Tomatsu, K. Tomita, K. I. Saitoh, M. Tsunakawa, M. Nishio, T. Miyaki, and H. Kawaguchi. Pradimicin, a novel class of potent antifungal antibiotics. *J. Antibiotics* 1988; 41:1701-1704.

[63] Oki, T., Tenmyo, O., Hirano, M., Tomatsu, K. and Kamei, H. Pradimicins A, B and C: new antifungal antibiotics. *J. Antibiot*. 1990; 43: 763-770.

[64] Onishi J, Meinz M, Thompson J, Curotto J, Dreikorn S, Rosenbach M, Douglas C, Abruzzo G, Flattery A, Kong L, et al. Discovery of novel antifungal (1,3)-β-d-glucan synthase inhibitors. *Antimicrob Agents Chemother* 2000; 44: 368–377.

[65] Ostrosky-Zeichner L, Rex JH, Pappas PG, et al. (Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. *Antimicrob Agents Chemother* 2003; 47:3149.

[66] Peel M, Fan W, Mamai A, Hong J, Orr M, Ouvry G, Perrey D, Liu H, Jones M, Nelson K, Ogbu C, Lee S, Li K, Kirwan R, Noe A, Sligar J, Martensen P, Balkove J, Greenlee CM, Meng D, Parker D, Wildonger K, Liberator P, Abruzzo G, Flattery A, Galgoci A, Giacobbe R, Gill C, Hsu MJ, Misura A, Nielsen J, Powles M, Racine F, Dragovic J, Habulihaz B, Balkovec J. 2010. Enfumafungin derivatives: orally active glucan synthase inhibitors, abstr F1-845. *Abstr. Intersci. Conf. Antimicrob. Agents Chemother.* Boston, MA.

[67] Peláez F, Cabello A, Platas G, Díez MT, González A, Basilio A, Martín I, Vincente F, Bills GF, Giacobbe RA, Schwartz RE, Onishi JC, Meinz MS, Arbruzzo GK, Flattery AM, Kong L, Kurtz MB. The discovery of enfumafungin, a novel antifungal compound produced by endophytic *Hormonema* species, biological activity, and taxonomy of the producing organisms. *Systematic and Applied Microbiology* 2000; 23: 333–343.

[68] Perez, P., I. Garcia-Acha, and A. Duran, Effect of papulacandin B on the cell wall and growth of *Geotrichum lactis*. *J. Gen. Microbiol.*, 1983; 129 (Pt 2): 245–250

[69] Perfect J.R., Wright K.A., Hector R.F. Synergistic interaction of nikkomycin and cilofungin against diverse fungi. In: *Recent progress in antifungal therapy*. Yamaguchi H. (eds) (Marcel Dekker, New York, N.Y), 1991; 369–379.

[70] Pfaller MA, Messer SA, Motyl MR, Jones RN, Castanheira M. Activity of MK-3118, a new oral glucan synthase inhibitor, tested against *Candida* spp. by two international methods (CLSI and EUCAST). *J. Antimicrob. Chemother.* 2013a; 68: 858–863.

[71] Pfaller MA, Messer SA, Motyl MR, Jones RN, Castanheira M. *In vitro* activity of a new oral glucan synthase inhibitor (MK-3118) tested against *Aspergillus* spp. by CLSI and EUCAST broth microdilution methods. *Antimicrob. Agents Chemother.* 2013b; 57: 1065–1068.

[72] Pfaller MA, Messer SA, Mills K. *et al.* Evaluation of Etest method for determining caspofungin (MK-0991) susceptibilities of 726 clinical isolates of *Candida* sp. *J. Clin. Microbiol.* 2001; 39:4387.

[73] Philip A, Odabasi Z, Rodrigues JR, et al. Program and abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology; *In vitro* synergy testing of anidulafungin (ANID) with itraconazole (ITR) and voriconazole (VOR) against *Aspergillus* spp. and *Fusarium* spp [abstract M-988]; 2003; 452.

[74] Powles MA, Liberator P, Anderson J, et al. Efficacy of MK-991 (L- 743,872), a semisynthetic pneumocandin, in murine models of *Pneumocystis carinii. Antimicrob Agents Chemother* 1998; 42:1985–9.

[75] Reese, A.J. and T.L. Doering, Cell wall alpha-1,3-glucan is required to anchor the *Cryptococcus neoformans* capsule. Mol Microbiol, 2003; 50(4): 1401–1409.

[76] Roy K., Mukhopadhyay T., Reddy C.G.S., Desikan K.R., Ganguli B.N. Mulundocandin, a new lipopeptide antibiotic. I. Taxonomy, fermentation, isolation and characterization. J. Antibiot. (Tokyo) 1987; 40:275–280.

[77] Satoi, S., Yagi, A., Asano, K., Mizuno, K. and Watanabe, T. Studies on aculeacin II. Isolation and characterization of aculeacins B, C, D, E, F and G. *J. Antibiotics*. 1977; 30: 303-307.

[78] Schwartz, R. Cell wall active antifungal agents. *Expert Opin. Therapeutic Patents* 2001; 11**:**1761-1772.

[79] Schwartz RE, Sesin DF, Joshua H et al. Pneumocandins from *Zalerion arboricola*. I Discovery and isolation. *J. Antibiotics* 1992; 45: 1853–66.

[80] Selitrennikoff, C.P. and Nakata, M. New cell wall targets for antifungal drugs. *Curr Opin Investig Drugs.* 2003; 4(2):200-205.

[81] Serrano MC, Valverde-Conde A, Chávez M, et al. In vitro activity of voriconazole, itraconazole, caspofungin, anidulafungin (VER002, LY303366) and amphotericin B against Aspergillus spp. *Diagn. Microbiol. Infect. Dis.* 2003; 45:131-5.

[82] Stone JA, Holland SD, Wickersham PJ, Sterrett A, Schwartz M, Bonfiglio C, Hesney M, Winchell GA, Deutsch PJ, Greenberg H, et al. Single- and multiple-dose pharmacokinetics of caspofungin in healthy men. *Antimicrob Agents Chemother* 2002; 46**:** 739–745.

[83] Sucher AJ, Chahine EB, Balcer HE. Echinocandins: the newest class of antifungals. *Ann Pharmacother.* 2009; 43:1647–1657.

[84] Takeuchi, T., Hara, T., Naganawa, H., Okada, M., Hamada, M., Umezawa, H., Gomi, S., Sezaki, M. and Kondo, S. New antifungal antibiotics, benanomicins A and B from an *Actinomycete*. J. Antibiot. 1988; 41: 807-811.

[85] Tariq, V.N. and Devlin, P.L. Sensitivity of fungi to nikkomycin Z. *Fungal Gen. Biol.* 1996; 20: 4-11.

[86] Tomita, K., Nishio, M., Saitoh, K., Yamamoto, H., Hoshino, Y., Ohkuma, H., Konishi, M., Miyaki, T. and Oki, T. Pradimicins A, B and C: new antifungal antibiotics. I. Taxonomy, production, isolation and physico-chemical properties. J. *Antibiot*. 1990; 43: 755-762

[87] Traxler, P., Gruner, J., Auden, J.A. Papulacandins, a new family of antibiotics with antifungal activity, I. Fermentation, isolation, chemical and biological characterization of papulacandins A, B, C, D and E. *J. Antibiot*. 1977; 30(4):289-96.

[88] Vazquez JA. and Sobel, JD. Anidulafungin: A Novel Echinocandin. *Clin Infect Dis*. 2006; 43 (2): 215-222. doi: 10.1086/505204.

[89] Vicente, M.F., Cabello, A., Platas, G., Basilio, A., Diez, M.T., Dreikorn, S., Giacobbe, R. A., Onishi, J. C., Meinz, M., Kurtz, M. B., Rosenbach, M., Thompson, J., Abruzzo, G., Flattery, A., Kong, L., Tsipouras, A., Wilson, K. E., and Pelaez, F., Antimicrobial activity of ergokonin A from *Trichoderma longibrachiatum*. *J. Appl. Microbiol*., 2001; 91, 806–813.

[90] Wagner, C., Graninger W, Prester I.E, et al. The echinocandins: Comparison of their Pharmacokinetics, pharmacodynamics and clinical applications. *Pharmacology* 2006; 78: 161–177.

[91] Walker SS, Xu Y, Triantafyllou I, Waldman MF, Mendrick C, Brown N, Mann P, Chau A, Patel R, Bauman N, et al. Discovery of a novel class of orally active antifungal β-1,3-d-glucan synthase inhibitors. *Antimicrob. Agents Chemother.* 2011; 55: 5099–5106.

[92] Walsh, T.J. and Giri, N. Pradimicins: a novel class of broadspectrum antifungal compounds. *Eur. J. Clin. Microbiol. Infect. Dis.* 1997; 16: 93-97.

[93] Watanabe M, Gomi S, Tohyama H, Ohtsuka K, Shibahara S, Inouye S, Kobayashi H, Suzuki S, Kondo S, Takeuchi T, Yamaguchi H. Binding of benanomicin A to fungal cells in reference to its fungicidal action. *J. Antibiot*. 1996; 49(4):366-73.

[94] Yamaguchi, H. Hiratani, T. Iwata K. and Yamamoto Y. Studies on the mechanism of antifungal action of Aculeacin a. *The Journal of Antibiotics* 1982; 35(2): 210-219.

[95] Yang SW, Buevich A, Chan TM, Terracciano J, Chen G, Loebenberg D, Patel M, Boehm E, Gullo V, Pramanik B, Chu M. A new antifungal sterol sulfate, Sch 601324, from *Chrysosporium* sp. J Antibiot. 2003; 56(4): 419-22.

[96] Zaoutis TE, Jafri HS, Huang LM, et al. A prospective, multicenter study of caspofungin for the treatment of documented *Candida* or *Aspergillus* infections in pediatric patients. *Pediatrics* 2009; 123: 877 – 884.

[97] Zhanel GG, Karlowsky JA, Harding GAJ, et al. In vitro activity of a new semisynthetic echinocandin, LY-303366, against systemic isolates of *Candida* species, *Cryptococcus neoformans, Blastomyces dermatitidis,* and *Aspergillus* species. *Antimicrob. Agents Chemother* 1997; 41:863-5.