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Targeting the fungal cell wall: current therapies and implications for development of alternative antifungal agents

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Fungal infections are a worldwide problem associated with high morbidity and mortality. There are relatively few antifungal agents, and resistance has emerged within these pathogens for the newest antifungal drugs. As the fungal cell wall is critical for growth and development, it is one of the most important targets for drug development. In this review, the currently available cell wall inhibitors and suitable drug candidates for the treatment of fungal infections are explored. Future studies of the fungal cell wall and compounds that have detrimental effects on this important outer structural layer could aid in antifungal drug discovery and lead to the development of alternative cell wall inhibitors to fill gaps in clinical therapies for difficult-to-treat fungal infections.

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Fungal infections are a global health problem, where about 20% (325 species where 87 have been described since 1980) of all known human pathogens are fungi [1,2]. The severity of fungal infections can range from superficial, affecting the skin or nails, to severe, causing life-threatening invasive or disseminated infections. The most severe infections occur primarily in patients with an underlying disease or the immunocompromised patient population including cancer patients, hematopoietic stem cell or organ transplant recipients, those on a long-term corticosteroid regime, and HIV patients [3–6]. Some of the most common opportunistic fungal pathogens responsible for infections are *Candida*, *Aspergillus* and *Cryptococcus*, but many other fungi can cause infections including the dimorphic endemic fungi *Blastomyces*, *Histoplasma*, *Coccidioides*, *Paracoccidioides*, and fungi frequently found in the environment such as *Fusarium*, *Scedosporium*, *Penicillium*, and members of the Mucorales [7–12]. Currently, clinically relevant antifungal agents are divided into five classes based on the structure of the therapeutic compound and the mode of action, and they consist of the polyenes, azoles, allylamines, pyrimidines, and echinocandins [13,14]. Most of these antifungal agents either directly target the membrane (polyenes) or a component of the membrane (allylamines and azoles), while the most recently approved class, the echinocandins, target the fungal cell-wall complex β (1-3)-glucan synthase [15,16]. Although the current antifungal drugs have enabled management of fungal infections, rising drug resistance (azoles, echinocandins), high toxicity (polyenes), and poor oral availability (polyenes, echinocandins), have left an opportunity for improvement in therapeutic antifungal compounds [15,17,18]. In this review, we will cover the available antifungal drugs that target the cell wall and their challenge to eliminate the fungal infection and consider advancements in the identification of new lead compounds and strategies for targeting the fungal cell wall.

The structure of the cell wall is unique to fungi, and because it is absent in humans, the fungal cell wall is a promising target for antifungal drugs. One of the main roles of the fungal cell wall is to provide protection from environmental stresses including osmotic stress, which can result in cell lysis and provide passive protection against the entrance of potentially harmful macromolecules [19]. The cell wall of most fungi consists of polysaccharides (mainly glucans and chitins) and glycoproteins (Figure 1A). The inner layers of the cell wall include chitin and glucan, where 50–60% of the dry weight of the cell wall is made up of β (1-3)-glucan; therefore, its synthesis is essential for proper cell wall formation and normal development of the cell [20]. Chitin, which is mostly present in

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Figure 1. Structure of the fungal cell wall and overview of compounds that target components. The fungal cell wall is composed of layers of chitin and glucan with an outer layer of mannoproteins and other proteins linked by a glycosylphosphatidylinositol anchor to the cell wall (A). An overview of antifungal agents that either directly target an element of the cell wall or impede their biosynthesis (B).

filamentous fungi, consists of β -1,4-linked *N*-acetylglucosamine and is covalently linked to β (1-3)-glucan [21,22]. Chitin synthesis is mediated by chitin synthases (CHS) [23–25]. Chitin provides the structural integrity and physical strength of the cell wall. When the structure of chitin and/or the biosynthesis of the polymer becomes disrupted, the wall becomes disordered and can lead to lysis and cell death [19]. The outer layer of the cell wall consists of homopolymers of mannose, 3–5% protein and 1–2% phosphate [26]. Mannan does not exist as a polysaccharide in the cell wall, but instead is found covalently bound to cell wall-associated proteins forming mannosylated glycoproteins [27–29]. Cell wall glycoproteins modified with *N*- and *O*-linked oligosaccharides are attached to the cell membrane. Proteins are firmly attached to the cell wall via covalent bonds between the *N*- and *O*-linked sugar moiety or a glycosylphosphatidylinositol (GPI) anchor to the chitin and/or glucan polymers [26,30,31]. Proteins located on the cell wall carry out a wide range of functions and are involved in adhesion to a surface, trafficking of macromolecules, cell-to-cell interactions, and providing protection against xenobiotic toxic substances. Each one of these cell wall components is vital for the normal development of the fungal cell and can be considered a potential target for antifungal agents (Figure 1B).

The fungal cell wall

The fungal cell wall can compose approximately 40% of the total cell volume and range in thickness from 0.1 to 1.0 μm [32,33], which can be further extended by the presence of an outer capsule layer in *Cryptococcus neoformans* and *Histoplasma capsulatum*. The main components of the cell wall of most fungi, chitin and β (1-3)-glucan, are surrounded by a gel-like substrate comprised primarily of α (1-3)-glucans and galactomannoproteins.

Chitin

An integral component and defining feature of the fungal cell wall, chitin is a polymer of β 1,4-linked *N*-acetylglucosamine; composing only one to two percent of the total dry weight in *Saccharomyces cerevisiae* but in considerable quantities in filamentous fungi (10–20%) with important roles in maintaining the mechanical strength and structural integrity of the fungal cell wall [19,21,22,34,35]. Since chitin does not exist in human cells, this component is an attractive target for antifungal therapies with low toxicity for humans. As an indication of the importance of chitin to the fungal cell, inhibition of chitin biosynthesis can result in several detrimental effects ranging from a delay in cell wall maturation and septum and bud ring formation, to a delay or defect in cell division resulting in suboptimal growth of the fungus [36,37].

CHSs are a family of enzymes that are localized to the plasma membrane and are responsible for the biosynthesis of chitin [23]. Filamentous fungi harbor multiple different classes of CHSs within their genomes, while typically three classes of CHSs are present in yeast [38]. Of the three CHS in *S. cerevisiae*, Chs3p is the primary CHS where it is responsible for generating a majority of the chitin found in the cell wall (approximately 80–90%) [36,38]. Mutants of *CHS3* have reduced chitin levels, poor growth and defects in cell wall integrity [36,37]. *Aspergillus fumigatus* has seven CHSs (*CHSA-G*), and each of these genes has been systematically disrupted demonstrating none of the *CHS* genes are essential in this fungus [39]. Single mutants of the *CHSA-C*, *-F* and *-G* genes appear to have no discernible difference from the wild-type fungus [37,39,40]; however, disruption of *CHSD* and *CHSE* results in a 20% and a 30% reduction in the production of chitin, respectively [41,42]. Seven CHSs (encoded by *CHS1-7*) are present in the genome of *Neurospora crassa* [43–46]. Disruption of *CHS6*, *CHS7*, and a double mutant of *CHS1;CHS3* results in lower *N*-acetylglucosamine (0.34, 0.30, 0.35 $\mu\text{g}/\text{ml}$, respectively) [43–47]. Chitin is synthesized in *Candida albicans* by four *CHS* genes, where *CHS1* is essential for viability. Chs2p is responsible for the production of about 40% of the chitin present in hyphal cells, whereas Chs3p and Chs8p synthesize short- and long-chitin microfibrils, respectively [48–52]. A proportion of chitin is deacetylated to chitosan by chitin deacetylases, and the quantity of chitosan can vary greatly between fungal species. For instance, only a small proportion of chitin is deacetylated to chitosan in *C. albicans* (<5%); however, a majority of chitin is deacetylated to chitosan in *C. neoformans* (~65%) [53]. It is postulated that chitin deacetylation may provide the polymer with more flexibility, while decreasing the availability of chitin to be degraded by chitinases [53].

Glucans

β (1-3)-glucan is a long, linear polymer of glucose that is important for cell wall structural integrity and is enriched in the inner layer of the two-layered cell wall. β (1-3)-glucan is the major component of cell wall glucan (40–55%); however β (1,6)-, mixed β (1-3)- and β (1-4)-, α (1-3)-, and α (1-4)-linked glucans have also been found in various fungal cell walls [54–56]. β (1-3)-glucan is covalently linked to polymers of β (1-6)-glucan and chitin; this network of covalently bound polymers are able to interact with adjacent polymers in other chains through hydrogen bonds, ultimately forming a complex three-dimensional network of microfibrils [57,58]. Based on the composition of the components in this network, there is a large degree of variation among fungal pathogens; for instance, in *A. fumigatus* the polysaccharides are primarily galactomannan and β (1-3 and 1-4)-glucans, whereas in *C. albicans* they are mainly β (1-6)-glucans [59,60]. β (1-3)-glucans are synthesized by the highly conserved β (1-3)-glucan synthase complex, which is composed of a catalytic domain (Fks), responsible for the biosynthesis of the polymer, and a regulatory (Rho1) subunit [55,56,61]. Disruption of the *FKS* gene yields mutants with cell wall defects and decreased viability [56]. Filamentous fungi encode a single essential *FKS* gene, while two *FKS* genes have been identified in *S. cerevisiae* (*FKS1/FKS2*) [61]. The predicted membrane topology of Fks1p shows two large hydrophobic domains separated by a large hydrophilic domain, several transmembrane helices and a cytoplasmic N-terminus [62].

Mannans & mannoprotein

The outer layer of the cell wall consists of heavily mannosylated glycoproteins that are modified with *N*- and *O*-linked oligosaccharides [26,30,31], where the structures of the oligosaccharide side chains are diverse between fungi. Some

well-characterized examples include the cell walls of the model yeasts *S. cerevisiae* and *C. albicans*, which contain proteins that are glycosylated with mannose-rich oligosaccharides (mannans), while the oligosaccharide side chains of *N. crassa* and *A. fumigatus* glycoproteins are composed of both mannose and galactose residues, referred to as galactomannan [63,64]. Mannose polymers are linked to the protein through asparagine and threonine (*N*-glycosidic) or serine (*O*-glycosidic) residues. Outer chains of branched mannan that contains α -(1-2), α -(1-3), β -(1-2), β -(1-6) and phosphodiester linkages are attached to the *N*-mannan core through an α -(1-6)-backbone [26,30,31]. The *O*-mannan consists of a simple, unbranched linear carbohydrate, which is comprised of a series of α -(1-2)-linkages [31]. Mannoproteins are covalently attached to β (1-3)-glucan or β (1-6)-glucan via either their oligosaccharide sidechains or GPI anchors [31,65]. Several mannosyltransferases genes (*MNN1-2, 4-6, 9-12, 14*) are required for normal cell wall mannan content in *C. albicans*, and deletion of each gene results in a defect in cell wall function [65,66].

Cell wall proteins

The outer layer of the cell wall contains proteins involved in surface adhesion and plays an important role in colonization and biofilm formation, including the Als (agglutinin-like sequence) and Hwp (hyphal wall protein) proteins [67,68]. One major difference between *Candida* and *Saccharomyces*, which are closely related, is that *Candida* species produce an extensive amount of adhesion proteins that are an important determinant of pathogenicity [69]. In *C. albicans*, the *ALS* family consists of eight genes that are engaged in cell adhesion [68,69]. Among them, Als1 and Als3 are considered the most important for biofilm formation [70,71]. The Als proteins have hydrophobic N and C termini, where the N terminus is involved in ligand binding, and the C terminus is modified by the addition of a GPI anchor [70], which is essential for linking the Als protein to the fungal cell wall [69].

As Hwp1 is involved in adhesion, it localizes to the cell surface and undergoes extensive modification followed by covalent attachment via a GPI anchor located at the C-terminus to β -glucan in the cell wall. This protein is involved in controlling hyphal surface gene expression with several other potential functional properties [72]. Hwp1 is rich in the amino acids proline (27%) and glutamine (16%), which may function to maintain the conformation of the polypeptide chains and binding [72,73]. The C terminus of Hwp1 is rich in serine and threonine, which provide *O*-glycosylation sites predicted to function by extending ligand-binding domains into the extracellular space. Strains without Hwp1 show less adhesion to human epithelial cells and are attenuated in virulence in a murine model of infection [74].

Antifungal agents targeting the cell wall in clinical use

The structure of the cell wall is necessary for the survival and growth of fungal cells; it also provides a barrier from the environmental stress. Damage to the cell wall structure leads to efflux of cytoplasmic molecules, cell membrane disruption, osmotic fragility, and suppression of fungal growth [19]. Efforts have been made to develop a drug to inhibit cell wall activity, but currently available antifungal drugs do not inhibit fungal infection. In this section, we will discuss the currently available antifungal agents that act on the cell wall (Figure 1B).

Inhibitors of β (1-3)-glucan synthase, echinocandins

The precursor to the group known as the echinocandins were discovered in the 1970s from *Aspergillus nidulans* and *Aspergillus rugulosus* [75]. The production and assembly of glucan requires a series of enzymes and regulatory networks that are mostly specific for fungi, and therefore a prime target for antifungal compounds. The echinocandins are the most recent class of antifungal agents approved by the US FDA for clinical use and consist of caspofungin, anidulafungin, and micafungin (Figure 2 & Table 1). The antifungal activity of echinocandins is derived by their ability to act as a noncompetitive inhibitor of β (1-3)-glucan synthase [76]. The inhibition of β (1-3)-glucan synthesis causes a decrease in the β (1-3)- and β (1-6)-glucan network, leading to a disordered and osmotically unstable cell wall, which results in fungal cell death and/or decreased host tissue damage [77,78]. Furthermore, inhibition of β (1-3)-glucan synthase influences several other key components of both the fungal cell membrane, where there is a reduction in the ergosterol content, and the cell wall, where there is an increase in the chitin content and ultrastructural changes [79]. While the primary *in vitro* mode of action of echinocandins is understood to be cell wall inhibition, an *in vivo* mode of action of these compounds is tied to the host immune response [80]. Clearance of a *C. albicans* systemic infection in a murine model by caspofungin is dependent on the immune receptor, dectin-1, which binds to fungal cell wall β (1-3)-glucan. *Candida albicans* decreases the efficiency of dectin-1 detection of β (1-3)-glucan by covering it with a layer of mannosylated glycoproteins (mannan), which interfere with the glucan–dectin-1 interaction [80]. This is referred to as masking, and greater exposure of β (1-3)-glucan due to

Figure 2. Echinocandins, β (1,3)-glucan synthase inhibitors. Caspofungin, micafungin, and anidulafungin are inhibitors of fungal β (1,3)-glucan synthesis and are approved by the US FDA.

Table 1. Summary of antifungal drugs in the two major classes targeting cell wall components.

Target site	Drug	Source	Indication
Inhibitors of β (1,3)-glucan synthase	Caspofungin	Lipopeptide product of <i>Aspergillus nidulans</i> or <i>Aspergillus rugulosus</i>	Candidemia, refractory aspergillosis
	Anidulafungin	Lipopeptide product of <i>A. nidulans</i> or <i>A. rugulosus</i> and acetylation by <i>Actinoplanes utahensis</i>	Candidiasis, salvage therapy for aspergillosis
	Micafungin	<i>Coleophoma empetri</i>	Candidiasis
Inhibitors of chitin synthesis	Polyoxin	<i>Streptomyces cacaoi</i>	
	Nikkomycin Z	<i>Streptomyces tendae</i>	Coccidioidomycosis

Data taken from [77,83].

Figure 3. Chitin synthase inhibitors. Nikkomycins, polyoxin, and plagiochin are chitin synthase inhibitors and are structurally similar to UDP-*N*-acetylglucosamine. Their inhibitory activity derive from their ability to bind with a high affinity to the catalytic site of chitin synthases.

mutations or drug treatment is referred to as unmasking. Studies suggest that the *in vivo* activity of caspofungin affects the structure of the cell wall and increases β (1-3)-glucan exposure to dectin-1, which increases the response of the immune cells to the fungus [80–82].

All three echinocandins in clinical use are semisynthetic lipopeptide derivatives of natural products and have efficacy against *Candida* spp. and *Aspergillus* spp. (Table 1) [81]. The main difference between caspofungin, micafungin, and anidulafungin resides in the side chain, where they possess a fatty acid side chain, a complex aromatic substituent and an alkoxytriphenyl group, respectively (Figure 2) [81]. This side chain modification increases glucan synthase inhibitory activity and decreases their toxicity to the host cells [81]. The long tail fatty acid chain of caspofungin allows interaction of this compound with the fungal cell membrane, leading to disruption of the function of the enzyme Fks1. Mutations in two hot spot regions of the *FKS1* gene lead to conformational changes in the enzyme resulting in lower susceptibility to echinocandins [82,84]. These mutations happen in highly conserved regions of the *FKS1* gene termed HS1 (positions 641–649) and HS2 (positions 1345–1365) [84,85].

The echinocandins are safer than other classes of antifungal agents; however, a significant drawback is their poor oral bioavailability and high protein binding affinity [86,87]. They are metabolized by the liver and distributed into the lung, liver, and spleen, but not into the CNS and eye [88], and are therefore not recommended for meningial or eye infections.

Inhibitors of chitin synthase

Several compounds that affect chitin synthesis have been identified, primarily the nikkomycins, polyoxins, and plagiochin (Figure 1B). Nikkomycin Z and polyoxins are antifungal drugs that are pyrimidine nucleosides linked to a di- or tripeptide moiety and therefore have structural similarities to UDP-*N*-acetylglucosamine, the substrate of CHS (Figure 3 & Table 1) [77]. Nikkomycins were first isolated from *Streptomyces tendae*, and their antifungal activity derives from being competitive inhibitors for the catalytic site of CHS where they bind with higher affinity than *N*-acetylglucosamine [77,89]. The nucleoside moiety varies in different nikkomycins (nikkomycins X and Z). Studies with *S. cerevisiae* have demonstrated that this drug can inhibit chitin synthase I and III, but not II, and has various degrees of inhibitory activity to other CHSs [90]. Nikkomycin Z has synergism with other antifungal agents including the Fks inhibiting echinocandins and the ergosterol biosynthesis inhibiting triazoles [91–93]. Nikkomycins have been shown to have clinically relevant antifungal activity *in vitro* against *Candida parapsilosis*, *Coccidioides immitis*, and *Blastomyces dermatitidis* [94], while *in vivo* models of fungal infections have indicated that nikkomycin Z may have some efficacy for the treatment of coccidioidomycosis, blastomycosis, and perhaps histoplasmosis [94]. However, other fungi including most of the *Candida* spp., *C. neoformans*, *Aspergillus* spp., *Fusarium* spp., and other opportunistic fungi have been reported to be resistant to nikkomycins perhaps due to their degradation and difficulty to penetrate the cell membrane [91,94,95]. Combination therapy of the chitin synthase inhibitor nikkomycin Z and an echinocandin enhanced the treatment of *A. fumigatus*, *C. albicans*, *Rhizopus* spp., and *C. immitis* with resistance to echinocandins [18].

Polyoxins are similar to nikkomycins having selective inhibition against CHS in fungi (Figure 3 & Table 1) [94]. First isolated from *Streptomyces cacaoi* var. *asoensis* in the 1960s, the polyoxins are a group of 12 structurally related compounds, which have been named alphabetically from A to L. The competition between the nucleoside moieties of the polyoxins and UDP-*N*-acetylglucosamine for the active site of the enzyme causes inhibition of the chitin synthase. Polyoxins contain two unusual amino acids, carbamoylpolyoxamic and polyoximic acids. In this competition, the carbamoylpolyoxamic acid moiety plays a critical role in the binding of polyoxins to the enzyme [96]. The carbamoylpolyoxamic acid moiety associates with the chitin synthetase due to hydrophobic interactions while the terminal carbamoyloxy group hydrogen bonds with the enzyme through an amide group [97]. Polyoxins are divided into two groups, one having polyoximic acid (III) and a negative rotation, while the other group does not contain polyoximic acid and has a positive rotation [95]. Polyoxins had been used with some success against *C. neoformans*, *C. albicans*, *Aspergillus flavus* and *S. cerevisiae*, although resistance to polyoxins has been reported in *C. albicans* mainly due to their poor transport through the cell membrane [98].

Plagiochin E is a phenolic antifungal macrocyclic bisbibenzyl compound that was first identified from liverwort, *Marchantia polymorpha* L. (Figure 3 & Table 1) [99]. The MIC of plagiochin E was 16 mg/ml against strains of *C. albicans*. Transmission electron microscopy of *C. albicans* cells treated with plagiochin E showed the structure of the cell was extremely damaged suggesting the antifungal activity of plagiochin E was associated with its effect on the cell wall [96]. Plagiochin E alters the expression of several chitin synthase genes in *C. albicans*, where the transcripts of *CHS1* were significantly decreased while the expression of *CHS2* and *CHS3* was upregulated [96]. *Candida albicans* exposed to plagiochin E displayed the presence of phosphatidylserine at the cytoplasmic membrane, a marker for the early stage of apoptotic events [100]. Plagiochin E acts synergistically with fluconazole on fluconazole-resistant *C. albicans* strains suggesting it could be used in combinational therapy [99].

Antifungal agents in development

In addition to the antifungal drugs that are currently FDA approved for clinical use, there are several compounds in clinical development. In this section, we summarize and discuss these new compounds in clinical trials, in particular those compounds with a different mode of action.

Rezafungin

Rezafungin (formerly CD101) is a highly stable echinocandin similar in structure to anidulafungin with efficacy against *Aspergillus* spp. and *Candida* spp. (Figure 4) [101,102]. Importantly, CD101 has *in vitro* antifungal activity against *Candida auris* and other *Candida* strains that are resistant to other echinocandins [101,103]. A key characteristic of CD101 is the therapeutically favorable pharmacokinetic properties where the compound displays prolonged plasma stability when compared with anidulafungin and minimal degradation during storage [104]. This compound has a long half-life and very low clearance in animal models when compared with anidulafungin [105]. These advantages allow rezafungin to remain longer inside the patient at a therapeutic level, thereby potentially decreasing the frequency of doses to patients. Rezafungin is currently in Phase III clinical trials for the treatment of candidemia and invasive candidiasis.

Ibrexafungerp

Ibrexafungerp (formerly SCY-078) is a glucan synthase inhibitor, which is a semisynthetic derivative of enfumafungin, a triterpene glycoside natural product. While both the echinocandins and ibrexafungerp inhibit glucan synthase, the structure of SCY-078 is significantly different from the echinocandins and has been designated the first member of the triterpene class of antifungal agents (Figure 4). *In vitro*, SCY-078 has antifungal efficacy (MIC levels below 0.5 µg/ml) against *Aspergillus* and, importantly, *Candida* strains that are resistant to the echinocandin glucan synthase inhibitors [106–109]. SCY-078 has good activity against the multidrug-resistant *C. auris* [110], and is the only β (1-3)-glucan inhibitor that showed some antifungal activity against the filamentous fungus *Scedosporium prolificans* [111]. This drug is currently in Phase I clinical development for intravenous formulation and initiating Phase III clinical trials as an oral formulation for the treatment of invasive candidiasis.

E1210

The glycosylphosphatidylinositol anchor is involved in attaching proteins to the fungal cell wall and cell membrane. The compound E1210 is capable of inhibiting inositol acylation via the Gwt1 protein in biosynthesis of the GPI anchor (Figure 4) [112]. The critical role of Gwt1 is to maintain the integrity of the fungal cell wall and enable the

Figure 4. Structure of antifungal agents in various stages of clinical development. Illustration of chemical structures of rezafungin (CD101), ibrexafungerp (SCY-078), E1210, pradimicin A, and poacic acid.

fungal cell to adhere to the host mucosal surface. E1210 has broad spectrum *in vitro* fungicidal activity at 1–2 µg/ml against many clinically important fungi including most *Candida* spp. (*Candida krusei* is an exception), *Aspergillus* spp., *Fusarium* spp. and *Scedosporium* spp. [112–114]. In *C. albicans*, E1210 is able to inhibit germ tube and biofilm formation due to a decrease in the adherence to the surface [112,113]. While E1210 displayed antifungal efficacy in murine models of candidiasis, aspergillosis, and fusariosis, there was no inhibitory activity observed against Pig-W (phosphatidylinositol-glycan biosynthesis, the human ortholog) even at concentrations as high as 100 µM.

Pradimicins

Pradimicins were first reported in 1988 from *Actinomadura* spp. with broad-spectrum antifungal activities (Figure 4) [115,116]. The mechanism of action of pradimicin is dependent on binding to mannan in the presence of Ca^{2+} ions, which causes a disruption in the integrity of the cell membrane [115]. These compounds show low toxicity in mammalian cells [115]. A new pradimicin derivative, BMS-181184, has *in vitro* antifungal activity against many clinically relevant fungi including *Candida* spp., *C. neoformans*, *Aspergillus* spp., dematiaceous molds, and members of the Mucorales [117,118].

Poacic acid

Poacic acid is primarily found in members of the grass family (Poaceae) of plants. This compound has antifungal activity against a broad range of fungi, in particular, against *S. cerevisiae* (IC_{50} of 111 µg/ml, 324 µM) (Figure 4) [119]. Poacic acid localizes to the cell wall and causes rapid cell lysis by directly binding to β (1-3)-glucan [119]. In addition to *S. cerevisiae*, poacic acid showed antifungal activity against *Sclerotinia sclerotiorum*, *Alternaria solani*, and *Phytophthora sojae* [119]. A significant synergistic effect exists between poacic acid and caspofungin, and to a lesser extent between poacic acid and nikkomycin Z [119]. The synergism with caspofungin suggests that poacic acid targets the cell wall via a mechanism that is independent of the mode of action of caspofungin [119]. Caspofungin and poacic acid both cause rapid cell leakage, but in nonactively growing cells, the cell leakage due to treatment

with poacic acid is significantly greater than caspofungin [119]. This suggests an alternative mode of action for poacic acid such as a disruption in cell wall integrity rather than enzymatic inhibition by binding directly to β (1-3)-glucan [119]. Poacic acid is localized to the cell surface and targets the β (1-3)-glucan layer leading to turgor pressure and a weakened cell wall [119].

Future discovery & development of potential antifungal drugs

Antifungal resistance is a significant concern, and new treatment strategies are necessary to address this issue. A better understanding of the synthesis of the cell wall precursors, the cell wall assembly, and mechanisms or cellular alterations that perturb this cellular structure, could provide insight into possible targets for the development of new antifungal agents. Due to increased incidence of resistance to currently used antifungal drugs, it is imperative to identify alternative modes of action for new generations of therapeutic agents to be developed.

Facilitate the recognition of fungal pathogens by immune cells

As indicated above, the fungal cell wall is an intricate, ordered cellular structure composed of primarily of manno-proteins, β -glucans, and chitin that protects the fungus from recognition by the innate immune system. Fungal pathogens possess a pathogen-associated molecular pattern (PAMP), which can be recognized by host immune cells such as macrophages, neutrophils, and dendritic cells via a pattern recognition receptor [120].

The dectin-1 receptor, a C-type lectin, recognizes β -glucans with β (1-3)- and β (1-6)-linkages and elicits a proinflammatory response via TNF α to destroy the pathogen [121,122]. The interaction between dectin-1 and β (1-3)-glucans requires a minimum of 10 or 11 glucosyl residues [123–125]. Dectin-1 recognition is very specific and does not recognize monosaccharides or carbohydrates with different linkages. β (1-3)-glucan is enriched in the inner layer of the cell wall, which is covered, or masked, by a layer of heavily glycosylated mannoproteins (mannan), which inhibit binding and recognition by the pattern recognition receptor dectin-1 [126]. Most opportunistic fungal pathogens such as *C. albicans* mask their PAMPs making it difficult for the immune system to detect [126,127]. The mechanism of PAMP masking and the recognition by the immune system is still unclear, and further understanding of fungal masking and the ability of the cell to escape recognition by the host immune system may aid in the development of an effective antifungal drug. Antifungal agents like caspofungin disrupt the cell wall architecture and cause unmasking of the β (1-3)-glucan layer allowing greater binding by dectin-1. This greater binding is associated with increased recognition of *Candida* spp. by macrophages, and increased elicitation of cytokines like TNF α . A screen for mutants that exhibit a phenotype similar to the unmasking of the β (1-3)-glucan layer caused by caspofungin could be used to identify potential drug targets in the future.

Targeting the fungal cell wall pathways

Several enzymatic steps are involved in the biosynthetic process of chitin. Chitin synthesis is mediated by PKC, the Ca²⁺ calcineurin, and the high-osmolarity glycerol (HOG) signaling pathways [128]. Activation of these pathways in the presence of antifungal drugs reduces susceptibility and enables the cells to adapt to the treatment. Additionally, the mannosyltransferases and glycosyltransferases located in the Golgi apparatus, which are responsible for the synthesis of the N-linked and O-linked oligosaccharides that reside on the exterior mannoproteins, are another potential molecular target for the development of antifungal compounds.

Disruption of pathways for expression of fungal adhesion proteins

A variety of adhesion proteins reside on the fungal cell wall and are vital to the infection process where they aid in colonization and adhesion to host tissue. The expression of Als3 and Hwp1 are governed by a network of signal transduction pathways including the cAMP-PKA pathway (Ras1, Tpk1, Tpk2, Efg1) [129]. Each of the components in these pathways is involved in the correct expression of cell surface adhesion factors, and impeding any of the genes in this pathway could reduce fungal cell adhesion and biofilm formation. Hence, a better understanding of the fungal cell wall adhesion proteins and how they are regulated could lead to potential alternative drug targets for the treatment of fungi.

Drug delivery system for direct targeting to the fungal cells

Although a large number of synthetic and macromolecular antifungal drugs are available, most of them fail to have a sufficient effect on the site of infection. The site-specific release of a drug which directly targets the pathogen, will improve the impact of the drug, even for a drug-resistant pathogen which requires a concentrated dose of

the drug. Nanoparticles as drug delivery vehicles were first introduced by Paul Ehrlich in 1954, wherein the drug was directly attached to targeted cells without affecting the healthy ones [130]. Nanoparticles such as liposomes, ethosomes, and niosomes are used as drug delivery systems to precisely target the fungal cell wall. Nanoparticles can be functionalized with materials such as mannan binding protein or Flo1p, which binds to the fungal surface, and could then release the antifungal compound(s) and disrupt the fungal cell wall. This approach could potentially reduce host toxicity while enhancing aqueous drug solubility, bioavailability, and antifungal efficacy [131].

Conclusion

The essential nature of the fungal cell wall makes it a prime target for antifungal agents. While many current therapies in clinical use already target the cell wall, as a better understanding of this structure is revealed, additional therapeutic drugs can potentially be developed for treatment options.

Future perspective

Fungal infections have increased in recent years [132–134], and cause a high degree of morbidity and mortality in patients with weakened immunity. Current available antifungal agents do not meet the expectations for eradicating fungal infections due to the difficulties in selective killing of eukaryotic fungal cells without adverse toxicity to humans. In order to change this situation, a new antifungal drug should possess an extended spectrum of activity against fungi, reduce dosing frequency, oral availability, and have no toxicity to human cells. One of the difficult challenges in antifungal drug development is identifying a suitable target that fulfills all of these criteria. The fungal cell wall is an ideal candidate to develop future antifungal drugs as it is a vital component of the fungus, it is exposed to the external environment, and it is unique to fungi and not present in other eukaryotes [135].

Executive summary

- Currently, five class of antifungal agents are used clinically, where the newest class, the echinocandins, targets the cell wall.
- The fungal cell wall is a complex interwoven network composed of primarily of chitin, glucans and various glycoproteins.
- Chitin is synthesized from monomers of *N*-acetylglucosamine by membrane-bound chitin synthases.
- β (1-3)-glucan is the major component of cell wall glucans, although multiple other linkages occur in the cell wall. The glucans are synthesized by the glucan synthase complex where the Fks subunit is responsible for the biosynthesis.
- The echinocandins are noncompetitive inhibitors of Fks, and there are three currently approved for clinical use that differ in their side chain.
- The chitin synthase inhibitor nikkomycin Z is currently in development for coccidioidomycosis.
- Multiple other compounds with antifungal activity that target components of the cell wall are in various stages of clinical development, including another echinocandin (rezafungin), a triterpene glucan synthase inhibitor (ibrexafungerp), and a compound that targets the glycosylphosphatidylinositol anchor of cell wall-localized proteins (E1210).
- Future approaches to antifungal drug development concerning the cell wall include alteration of the cell wall to facilitate recognition by immune cells, inhibition of pathways regulating cell wall development and targeting critical proteins localized to the cell wall.

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