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PII: S0734-9750(19)30027-8
DOI: https://doi.org/10.1016/j.biotechadv.2019.02.008
Reference: JBA 7352

To appear in: *Biotechnology Advances*

Received date: 22 November 2018
Revised date: 23 January 2019
Accepted date: 16 February 2019

Please cite this article as: J.C.G. Cortés, M.-Á. Curto, V.S.D. Carvalho, et al., The fungal cell wall as a target for the development of new antifungal therapies, Biotechnology Advances, https://doi.org/10.1016/j.biotechadv.2019.02.008

The fungal cell wall as a target for the development of new antifungal therapies

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ABSTRACT

In the past three decades invasive mycoses have globally emerged as a persistent source of healthcare-associated infections. The cell wall surrounding the fungal cell opposes the turgor pressure that otherwise could produce cell lysis. Thus, the cell wall is essential for maintaining fungal cell shape and integrity. Given that this structure is absent in host mammalian cells, it stands as an important target when developing selective compounds for the treatment of fungal infections. Consequently, treatment with echinocandins, a family of antifungal agents that specifically inhibits the biosynthesis of cell wall (1-3)β-D-glucan, has been established as an alternative and effective antifungal therapy. However, the existence of many pathogenic fungi resistant to single or multiple antifungal families, together with the limited arsenal of available antifungal compounds, critically affects the effectiveness of treatments against these life-threatening infections. Thus, new antifungal therapies are required. Here we review the fungal cell wall and its relevance in biotechnology as a target for the development of new antifungal compounds, disclosing the most promising cell wall inhibitors that are currently in experimental or clinical development for the treatment of some invasive mycoses.

ARTICLE INFO

Keywords: Mycoses, Invasive fungal infection, Fungi, Fungal cell wall, Glucan synthase, Antifungal drugs, Echinocandins, Fungal drug resistance, Multidrug-resistant fungal species, Combination therapy

1. Introduction

Invasive mycoses have been growing during the past several decades, becoming a major threat for public health. The cause of this is mainly due to the vulnerable immunocompromised population created by the advances in modern medicine (organ transplantation and chemotherapy) and the HIV/AIDS syndrome (Hopke et al., 2018; Perfect, 2017). In addition, outbursts of mycetomas, a disease of subcutaneous fungal infections endemic of tropical and subtropical regions, present multiple medical, health and socioeconomic adversities for affected patients (Bakhiet et al., 2018). Over 300 million people suffer from severe fungal diseases, which can cause chronic illness and death in the most severe cases. As a result, over 1.6 million people die annually because of mycoses (Denning, 2017; Editorial, 2017; LIFE, 2017). The problem of invasive mycoses is also aggravated by the emergence of fungi resistant to the available antifungal treatments. Isavuconazole, from the azol class, was the last compound approved for the treatment of some mycoses in 2015; however, no new antifungal classes have been authorized for medical practice since 2006 (reviewed by Denning and Bromley, 2015; McCarthy et al., 2017; Perfect, 2017).

The discovery of new antifungals is challenging because as eukaryotes, fungi contain relatively few differential targets with respect to human host cells that can be employed for developing new antifungal compounds (McCarthy et al., 2017). Currently, four major classes of antifungal agents are utilized as a remedy for invasive mycoses: triazoles that block the synthesis of the plasma membrane ergosterol; the polyene amphotericin B that disrupts the fungal plasma membrane by binding ergosterol; the pyrimidine analogue flucytosine, which blocks the synthesis of both DNA and RNA; and echinocandins that bind to and inhibit the enzyme responsible for the synthesis of the (1-3)β-D-glucan present in the fungal cell wall (reviewed by Denning and Bromley, 2015; Perfect, 2017).

The most common human pathogenic microorganisms responsible for fungal diseases are species from the genera *Aspergillus* and *Candida*, *Cryptococcus neoformans*, *Pneumocystis jirovecii* and several dimorphic fungal species such as *Histoplasma capsulatum* (**Table 1**). Recent global analyses have estimated the annual incidence of fungal diseases in >300,000 cases of invasive aspergillosis, >3,000,000 of chronic pulmonary aspergillosis, >750,000 of invasive candidiasis, >1,000,000 of cryptococcosis and ~500,000 of pneumonia due to *P. jirovecii* (**Table 1**) (Bongomin et

al., 2017; Brown et al., 2012a; GAFFI). Despite the effectiveness of some antifungal treatments, the mortality rates from invasive mycoses remain significantly high, reaching approximately 20-40% for invasive candidiasis and 20-30% for invasive aspergillosis, and being even higher (≥50%) for other invasive fungal infections caused by other molds (Andes et al., 2012; Marr et al., 2015; Perfect, 2017). Studies analyzing *Cryptococcus* isolates collected from different countries have led to estimate the rate of mortality for disseminated cryptococcosis in a range of 15-70% (Bratton et al., 2012; Brown et al., 2012a; Nyazika et al., 2016; Park et al., 2009). Therefore, there is a critical necessity for discovering and improving new more effective and cheaper antifungals with a more rapid fungicidal action and a broader-spectrum of activity, including against highly resistant fungi (Brown et al., 2012b; Perfect, 2017).

For hospitalized patients, the main life-threatening nosocomial infections are invasive candidiasis (candidemia) and to a lower extent, invasive aspergillosis and *Pneumocystis* pneumonia. Globally, nosocomial infections suppose an estimated annual burden of 1,200,000 cases with an estimated annual mortality of >35% (GAFFI, 2015). *Candida* bloodstream infection (candidemia) is an indicator of invasive candidiasis that is found in about 40% of the cases (GAFFI, 2015). A global study has shown that from 256,882 *Candida* isolates collected in 142 hospitals from 41 countries and over a 11-year period from 1997 to 2007, more than 90% of infections were caused by five *Candida* species, *C albicans* (65%), *C. glabrata* (11%), *C. tropicalis* (7%), *C. parapsilosis* (6%) and *C. krusei* (2%) (Alangaden, 2011; Pfaller et al., 2010). Similarly, from 24,179 cases of nosocomial bloodstream infections analyzed in 49 hospitals from the United States between 1995 and 2002, 1,980 cases were candidemia with the most frequent isolated species being *C. albicans* (54%), *C. glabrata* (19%), *C. tropicalis* (11%), *C. parapsilosis* (11%) and *C. krusei* (2%) (Alangaden, 2011; Wisplinghoff et al., 2004). The percentage of crude mortality related to candidemia was 47%, with the lowest one caused by *C. parapsilosis* (28%) and the highest one by *C. krusei* (59%) (Alangaden, 2011; Wisplinghoff et al., 2004). More recent studies have shown that the annual incidence rates for candidemia oscillate in a range from 2 per 100,000 to 26 per 100,000. Considering a low average rate of 5 per 100,000 (Rodríguez-Tudela et al., 2015) and a global population of 7 billion, it has been estimated 350,000 candidemia and 875,000 invasive candidiasis cases per year (GAFFI, 2015). In intensive care units, the rates of candidemia are much higher being reported 6.5 cases per 1,000 admissions in hospitals from India (Chakrabarti et al., 2015; GAFFI, 2015). These data show that

the number of cases each year in India could reach approximately 676,000, with an estimated mortality of about 50%. In France, the rates of candidemia are rising every year, with the mortality associated to candidemia remaining above 50% (Bitar et al., 2014; GAFFI, 2015; Lortholary et al., 2014).

The biotechnological and bioeconomic appeal of the fungal cell wall lies in its importance in the development and improvement of new and existing antifungal strategies against a wide-spectrum of human-pathogenic fungi (Meyer et al., 2016). The cell wall is not only essential for the survival of the fungus, but is absent from human host cells (**Fig. 1**). Consequently, the enzymes that synthetize the essential cell wall components have been traditionally considered as an outstanding target for developing specific antifungal agents (**Fig. 2**) (reviewed by Debono and Gordee, 1994; Georgopapadakou and Tkacz, 1995). In addition to the newest echinocandins clinically in use (caspofungin, micafungin, and anidulafungin; **Fig. 2** and **Table 2**), which inhibit the synthesis of cell wall (1-3)β-D-glucan, in this review we discuss the new classes of cell wall antifungals and their mode of action, with a special focus on the most encouraging candidates that are undergoing clinical trials for combating infectious diseases caused by invasive fungal species (**Fig. 2** and **Table 3**). Moreover, we will also describe the new and existing naturally derived, synthetic and semi-synthetic antifungals that target cell wall synthesis and can be active against the most infective fungi responsible for life-threatening mycoses (**Fig. 2** and **Table 4**).

2. The fungal cell wall

The cell wall is a structure that encases the fungal cell, protecting it against the osmotic pressure inside the cell, thus conferring mechanical protection and maintaining the cellular shape. Disruption of the cell wall structure has a large impact on fungal cell survival, causing the rupture of the plasma membrane and cell lysis. However, the cell wall structure is more than a simple rigid shell; instead, it is a highly changing structure that performs a major task allowing diverse morphogenesic processes such as cell growth, cytokinesis and the development of specialized types of fungal cells (reviewed by Free, 2013; Gow et al., 2017; Hopke et al., 2018; Levin, 2011).

In fungal infections the cell wall is the structure where the pathogen and host establish contact, being essential for fungal pathogenicity and virulence (reviewed by Geoghegan et al., 2017). The cell wall provides adherent properties vital for the

invasion of the host tissues and protects against the defensive machinery of the host (reviewed by Lipke, 2018). Since the main elements of the cell wall of all fungal pathogens are absent in animal cells including humans and other mammals, the cell wall promotes adaptive and innate immune responses of the host (reviewed by Erwig and Gow, 2016; Hopke et al., 2018). As a result, the cell wall is the source of most antigens used in the diagnosis of human mycoses (reviewed by Farhour et al., 2018; Gow et al., 2017; Ostrosky-Zeichner, 2012), and given its essential role in fungal cell integrity, it is considered an outstanding target for the discovery and improvement of new antifungal drugs (reviewed by Debono and Gordee, 1994; Georgopapadakou and Tkacz, 1995; Ribas et al., 2014).

2.1. Composition and structure of the fungal cell wall

The fungal cell wall is built of matrix components embedded and linked to microfibers comprising long chains of polysaccharides (**Fig. 1** and **Fig. 2**) (Chaffin, 2008; Gow et al., 2017; Klis et al., 2014; Latge and Beauvais, 2014; Orlean, 2012; Wang et al., 2018). The cell wall contains an inner layer that in most fungi is composed of branched (1-3)β-D-glucan, with both (1-6)β-D-glucan and some of the chitin connected to the non-reducing ends of the (1-3)β-D-glucan, and an outer layer of glycoproteins (**Fig. 1** and **Fig. 2**) (Cabib and Arroyo, 2013; James et al., 1990; Klis et al., 2001; Klis et al., 2002; Latge, 2007; Lesage and Bussey, 2006). All of these polymers are connected to one another building a strong network that prevents the release of glycoproteins to the extracellular environment (reviewed by Chaffin, 2008; Gow et al., 2017; Klis et al., 2014; Latge and Beauvais, 2014; Orlean, 2012; Wang et al., 2018). When examined through transmission electron microscopy, the fungal cell wall structure exhibits an external electron-dense layer and an internal electron-transparent layer (Kitajima, 2001; Osumi, 1998; Sipiczki, 2016; Walker et al., 2018). Depending on the fungus, the edge of the outer layer of glycoproteins can exhibit a brush-like appearance (Osumi, 2012; Reese et al., 2007; Walker et al., 2018; Walther et al., 1984). Examination of the ultrastructure of regenerating cell walls originated from protoplasts of *Saccharomyces cerevisiae*, *C. albicans* and *Schizosaccharomyces pombe* showed fibrous structures constituted of microfibrils of (1-3)β-D-glucan, surrounded by a large number of granular particles (Kopecka and Kreger, 1986; Osumi, 2012). In the

case of the fission yeast *S. pombe*, the interfibrillar space of the emerging cell wall is filled up with amorphous particles of galactomannan (Osumi, 2012).

The main cell wall glucan is the (1-3)β-D-glucan (65-90% of the total cell wall β-glucans), whose content in the cell wall varies from 50-55% in *S. pombe* and *S. cerevisiae* to 25-30% in the filamentous fungus *Aspergillus fumigatus* (Gastebois et al., 2009; Lipke and Ovalle, 1998; Orlean, 2012; Pérez et al., 2016). This glucan is made up of glucose monomers linked by (1-3)β bonds, although there are also (1-6)β-, (1-3)α- and (1-4)α-D-glucans. Typically the (1-3)β-D-glucan is composed by a main backbone made of (1-3)β-D-glucan and branched with (1-6)β-linked (1-3)β-D-glucan side chains. The proportion of (1-6)β branches varies depending on the organism. The other β-glucan of the fungal cell wall is the (1-6)β-D-glucan that accounts for 5-12% in *S. cerevisiae* cell walls and 21% in *C. albicans* cell walls (Klis et al., 1997; Lesage and Bussey, 2006; Lipke and Ovalle, 1998; Manners et al., 1973b; Orlean, 2012). In an acapsular *C. neoformans* mutant strain the cell wall seems to be enriched in (1-6)β-D-linked glucose residues (Gilbert et al., 2010; Wang et al., 2018). The structure of this polysaccharide varies among fungi, with the most branched (1-6)β-D-glucan being detected in *S. pombe*. The (1-6)β-D-glucan chains are shorter than those of (1-3)β-D-glucan and do not exhibit a fibrillar conformation (Magnelli et al., 2005; Manners et al., 1973a; Manners et al., 1973b). Chitin is a polymer of N-acetylglucosamine monomers linked by (1-4)β bonds that makes microfibrils stabilized through hydrogen-bonds, which exhibit a huge tensile strength (Cabib and Kang, 1987). This polysaccharide appears in the cell wall of all fungal species examined so far, except in *S. pombe*, and probably the rest of *Schizosaccharomyces* species. Chitin only constitutes approximately 1-3% of the *S. cerevisiae* cell wall polysaccharides and 10-20% of the *A. fumigatus* and *Neurospora crassa* cell walls (Bowman and Free, 2006; Kapteyn et al., 1997; Lesage and Bussey, 2006). The chitin present in the basidiomycetous yeast *C. neoformans* is mostly deacetylated to chitosan (Gow et al., 2017). In addition, this fungus has a layer of melanin that may assemble near the chitin/chitosan layer, although its precise location is still unknown (Gow et al., 2017). Branched (1-3)β-D-glucan is connected to other α- and β-glucans, to chitin and to glycoproteins (**Fig. 2**) conferring mechanical strength to the cell wall, which is vital for maintaining the integrity of the fungal cell. (1-6)β-D-glucan, on the other hand, act as cement within the network by covalently binding to (1-3)β-D-glucan, chitin, and glycoproteins (Fontaine et al., 2000; Iorio et al., 2008; Kapteyn et al., 1996; Kollar et al., 1995; Kollar et al., 1997).

The internal layer of the cell wall of the yeasts *S. pombe* and *C. neoformans* and several filamentous and dimorphic fungi also contains linear (1-3)(1-4)α-D-glucan attached to the (1-3)β-D-glucan (**Fig. 1**) (Bacon et al., 1968; Edwards et al., 2011; Grun et al., 2005; Henry et al., 2011; Yoshimi et al., 2017). Budding yeasts like *S. cerevisiae* or *C. albicans* do not contain this polysaccharide. This linear (1-3)(1-4)α-D-glucan is constituted of approximately 260 glucose units, comprising two linear chains, each constituted of about 120-130 monomers of glucose linked by (1-3)α bonds and connected at the reducing end by a bridge of approximately 10 units of (1-4)α-D-linked glucose (Grun et al., 2005). The total content of this polymer in the cell wall accounts for 35-40% in *A. fumigatus*, 35-46% in the virulent yeast *C. neoformans* and dimorphic fungi *Paracoccidioides brasiliensis, H. capsulatum, Blastomyces dermatitidis*, and 28-32% in *S. pombe* (Grun et al., 2005; Henry et al., 2011; Pérez et al., 2016). Solid-state NMR spectroscopy combined with dynamic nuclear polarization and analysis of the glycosyl linkages of the cell walls of *A. fumigatus* and *Aspergillus niger* revealed the distribution of (1-3)α-D-glucan in all cell wall layers (Kang et al., 2018). The inner wall core comprises a hydrophobic and rigid scaffold of chitin and (1-3)α-D-glucan that is covered by a hydrated and soft matrix of interconnected (1-3)α-D-glucan, (1-3)β-D-glucan and (1-6)β-D-glucan. The outer wall region is formed by a dynamic layer of glycoproteins and a minor fraction of (1-3)α-D-glucan (Kang et al., 2018). The (1-3)α-D-glucan is crucial for the virulence of several fungal pathogens. Thus, the absence of (1-3)α-D-glucan causes the loss of the surface capsule in *C. neoformans* (Reese and Doering, 2003) and also prevents fungal pathogenesis (Reese et al., 2007). There is also a correlation between the content of (1-3)α-D-glucan and the virulence of several dimorphic fungal species (Hogan and Klein, 1994; Klimpel and Goldman, 1988; Rappleye and Goldman, 2006; San-Blas et al., 1977). The (1-3)α-D-glucan of *H. capsulatum* hyphae prevents the efficient immune recognition by the host receptor Dectin-1 of the underlying (1-3)β-D-glucan (Gow et al., 2017; Rappleye et al., 2007). Furthermore, the (1-3)α-D-glucan is important as a factor of aggregation for *Aspergillus* (Fontaine et al., 2010; Yoshimi et al., 2013). In *S. pombe* the (1-3)α-D-glucan possess a vital structural function providing rigidity to the cell wall (Alfa et al., 1993). Similar to the (1-3)β-D-glucan, the (1-3)α-D-glucan of *S. pombe* is vital for the integrity of the cell (Cortés et al., 2012; Hochstenbach et al., 1998; Katayama et al., 1999). During cell wall biogenesis, this polymer could be implicated in the general development of glucan bundles (Osumi et al., 1989). In addition, the (1-3)α-D-glucan is essential for conferring

to the primary septum the adhesion strength required to counteract the internal turgor pressure during septum degradation and cell separation (Cortés et al., 2012). The (1-3)α-D-glucan presents some similarities with the pectin of plants, as both are vital for cell wall adhesion and cell separation, preserving the association between polymers in equivalent structures, the primary septum of fungal cells and the middle lamella of plant cells (Cortés et al., 2012; Iwai et al., 2002; Roberts and Gonzalez-Carranza, 2007).

The external layer of the cell wall is more heterogeneous than the internal layer when compared between organisms (**Fig. 1**). In budding yeasts *S. cerevisiae* and in *C. albicans* the external layer contains a large amount of highly mannosylated proteins that are mainly connected to the (1-6)β-D-glucan and to the (1-3)β-D-glucan-chitin core through a glycosylphosphatidylinositol (GPI) remnant. Other proteins containing internal repeats (PIR) are covalently associated to (1-3)β-D-glucan via an alkali-soluble linkage (**Fig. 2**) (Ecker et al., 2006; Klis et al., 2010; Klis et al., 2001; Mrsa et al., 1997; Mrsa and Tanner, 1999; Ruiz-Herrera et al., 2006). The cell wall glycoproteins are extensively modified with carbohydrates both N- and O-linked, which are principally or entirely composed by mannose units to form a polymer known as mannan (Gow et al., 2017). Glycoproteins constitute 30-50% of the cell wall dry weight of *S. cerevisiae* or *C. albicans*. In some fungi the backbone of mannan contains single units or branches of diverse surgars such as galactomannan, rhamnomannan, rhamnogalactomannan, glucogalactomannan, etc. (Bowman and Free, 2006; Leal et al., 2010). *S. pombe* and *A. fumigatus* glycoproteins represent around 15-20% of the cell wall dry weight and contain galactomannans instead of mannans (Ballou et al., 1994; Beauvais et al., 2014; Fontaine et al., 2000; Horisberger et al., 1978). In addition, the hyphae of *A. fumigatus* also contain galactosaminoglycan (Gow et al., 2017). Since a reduction in the content of *S. pombe* (1-3)α- or (1-3)β-D-glucan causes the loss or reduction of the outer layer of galactomannan (Cortés et al., 2012; Ribas et al., 1991b), the glycoprotein layer might be also connected to the underlying glucan fibrils in this yeast. *C. neoformans* exhibits an external capsule required for virulence that is composed of glucuronoxylomannan and glucuronoxylomannogalactan and is connected to the internal (1-3)α-D-glucan (Heiss et al., 2009; Reese et al., 2007). In this sense, several *C. neoformans* capsule-deficient strains exhibiting reduced virulence have been also reported (Bulmer et al., 1967; Fromtling et al., 1982; Jacobson et al., 1982).

2.2. Biosynthesis of the cell wall polysaccharides

When the cell wall is weakened, the internal turgor pressure of the fungal cells can cause cell lysis and release of the cytoplasmic content; thus, cell wall integrity must always be preserved to ensure the survival of the fungal cell (Cabib and Arroyo, 2013). During the distinct morphogenesis phases of the life cycle, the fungal wall must be continuously reconstructed, which involves the rupture of the old cross-linkages, the insertion into the cell wall of new synthetized chains of polysaccharides and proteins and the formation of new cross-linkages (Cabib and Arroyo, 2013). Polysaccharides such as chitin, (1-3)β-D-glucan and (1-3)α-D-glucan are synthesized in the plasma membrane by transmembrane enzymes or enzymatic complexes. The synthesis of (1-6)β-D-glucan appears to be more intricate and is not well known. Glycoproteins initiate their synthesis in the endoplasmic reticulum, are extended in the Golgi and then exported to the plasma membrane and cell wall by the secretory pathway (Cabib and Arroyo, 2013; Gow et al., 2017; Orlean, 2012). All synthases use nucleoside diphosphate sugars as substrate and catalyze the donation of a monosaccharide unit to the growing chains of new polysaccharides (reviewed by Cabib and Arroyo, 2013; Gow et al., 2017; Orlean, 2012).

2.2.1. (1-3)β-D-glucan

Fungal (1-3)β-D-glucan chains are synthesized by the enzyme complex (1-3)β-D-glucan synthase (GS) (EC 2.4.1.34, UDP-glucose:1,3-β-D-glucan 3-β-glucosyltransferase). Then, the synthesized (1-3)β-D-glucan chains are extruded to the periplasmic space located between plasma membrane and cell wall, where they are incorporated into the cell wall (Cabib and Kang, 1987; Maligie and Selitrennikoff, 2005; Orlean, 1982; Shematek et al., 1980; Taft and Selitrennikoff, 1988). The GS activity can be extracted from the plasma membrane and fractionated into soluble and membrane-bound insoluble fractions, containing the guanosine triphosphate (GTP)-binding regulatory and the catalytic subunits, respectively. Each fraction alone is inactive, but when both fractions are mixed the activity is reconstituted (Kang and Cabib, 1986). The GTPase Rho1 acts as the regulatory subunit that when linked to GTP triggers the activation of the catalytic subunit, and Rho1 is regulated by switching between an inactive GDP-linked status and an active GTP-linked status (Arellano et al., 1996; Drgonova et al., 1996). To activate the GS catalytic subunit, Rho1 must be

attached to the plasma membrane via a C-terminal prenylated modification tail of the protein (Arellano et al., 1998; Díaz et al., 1993; Inoue et al., 1999).

The antifungal family of echinocandins bind the GS catalytic subunit directly inhibiting the biosynthesis of (1-3)β-D-glucan (**Fig. 2**) (Bartizal et al., 1992; Cassone et al., 1981; Debono and Gordee, 1994; Douglas et al., 1994b; Sawistowska-Schroder et al., 1984). The *FKS/GSC* genes encoding the putative catalytic subunit of the GS were first isolated in *S. cerevisiae* through different cloning strategies that identified two *FKS* genes (Castro et al., 1995; Douglas et al., 1994a; Douglas et al., 1994b; El-Sherbeini and Clemas, 1995; Garrett-Engele et al., 1995; Inoue et al., 1995; Mazur et al., 1995; Parent et al., 1993; Ram et al., 1995). Later on, a third *FKS* gene was found (Dijkgraaf et al., 2002; Ishihara et al., 2007; Lesage et al., 2004). Although none of the *FKS* genes are essential in the budding yeast, the combined absence of *FKS1* and *FKS2* causes cell lethality (Inoue et al., 1995; Mazur et al., 1995). The fungal GS Fks and the plant callose synthase CalS proteins form a well-conserved GS family present in both fungi and plants. Fungal and plant GS enzymes share a high sequence homology (54% similarity and 25% identity) and both are responsible for the synthesis of a (1-3)β-D-glucan polymer, which in plants is named callose and presents a very specialized localization and function (Cortés et al., 2002; Chen and Kim, 2009; Hong et al., 2001). Thus, all Fks/CalS GS are large integral membrane proteins with an estimated molecular weight of more than 200 kDa. These proteins contain 15-16 predicted transmembrane domains divided into two hydrophobic regions separated through a highly conserved central hydrophilic region, which exhibits an identity of >80% among all studied Fks proteins (Johnson and Edlind, 2012). Orthologues of the *FKS* genes have been identified in all the analyzed fungi, encoding proteins whose degree of identity varies from 56% (*C. neoformans*) to 83% (*C. glabrata*) (reviewed by Hori and Shibuya, 2018; Latge, 2007; Lesage and Bussey, 2006; Pérez and Ribas, 2004). *Aspergillus* species and *C. neoformans* contain a single and essential *FKS1* gene (Beauvais et al., 2001; Douglas et al., 1994a; Kelly et al., 1996; Staab et al., 2010; Thompson et al., 1999). In *C. albicans* and *C. glabrata* three *FKS/GSC/GSL* orthologues have been described (Katiyar et al., 2006; Mio et al., 1997). *GSC1/FKS1* is the only essential gene in *C. albicans*, suggesting that this gene might be coding the major GS, with *GSL1/FKS2* carrying out a dispensable or different function and with the expression of *GSL2* mRNA not being detected (Douglas et al., 1997; Thompson et al., 1999), while *FKS1* and *FKS2* have overlapping roles in *C. glabrata* (Katiyar et al., 2006).

S. pombe genome contains four *bgs/fks* genes. In contrast to budding yeast and other fungi, where the GS catalytic subunits have partially overlapping roles, the four *bgs* genes code for essential proteins. Bgs1, Bgs3 and Bgs4 act throughout vegetative growth, while Bgs2 acts only throughout the sexual phase of the life cycle. The localization of the three vegetative Bgs proteins is restricted to the growing ends during polarized growth and to the cell middle during cell division (reviewed by Cortés et al., 2016; Pérez et al., 2016). Phenotypic analyses of several *bgs1*$^{+}$ mutants, combined with the ultrastructural observation of the septa built in cells coming from the germination of spores lacking Bgs1, proved that Bgs1 is responsible for the synthesis of the linear (1-3)β-D-glucan and the formation of the primary septum structure (Cortés et al., 2002; Cortés et al., 2007; Ishiguro et al., 1997; Le Goff et al., 1999; Liu et al., 2002; Liu et al., 1999). The Bgs2-dependent GS activity results vital for maturation of the spore wall (Liu et al., 2000; Martín et al., 2000). The function of Bgs3 is not yet known, although it may have a role during polarized cell growth (Martín et al., 2003). Most of the GS activity detected *in vitro* is due to Bgs4, which synthetizes the branched (1-3)β-D-glucan of the cell wall. This Bgs4-dependent β-glucan is vital for the cell integrity and the construction of both primary and secondary septum (Cortés et al., 2005; Martins et al., 2011; Muñoz et al., 2013). Currently, Bgs4 is the only GS of *S. pombe* whose mutants exhibit reduced levels of both *in vitro* GS activity and the corresponding *in vivo* cell wall β-glucan (Ribas et al., 1991a). Because their non-overlapping roles, the Bgs proteins are excellent molecules for developing new inhibitors of the (1-3)β-D-glucan synthesis.

2.2.2. (1-6)β-D-glucan

Both the site and mechanism of biosynthesis of (1-6)β-D-glucan are largely unidentified. Immunoelectron microscopy in *S. pombe* led to suggest that the synthesis of the backbone of (1-6)β-D-glucan starts in the endoplasmic reticulum, follows in the Golgi and is concluded at the plasma membrane (Humbel et al., 2001). In contrast, in *S. cerevisiae* (1-6)β-D-glucan is mostly detected at the plasma membrane, suggesting that the synthesis of this polymer occurs mainly at the cell surface (Aimanianda et al., 2009; Montijn et al., 1999; Vink et al., 2004). The genes involved in the (1-6)β-D-glucan synthesis were first studied in *S. cerevisiae* through the identification of the *KRE* mutants, able to survive and grow in the presence of the K1 killer toxin. The killer toxin

kills the cells by binding to the cell wall (1-6)β-D-glucan as the primary target, subsequently altering the plasma membrane (Kasahara et al., 1994a; Kasahara et al., 1994b; Page et al., 2003). To date many *KRE* genes and their paralogues, *SKN1* and *KNH1*, have been related to the (1-6)β-D-glucan synthesis in *S. cerevisiae* and some fungal pathogens (Aimanianda et al., 2009; Gilbert et al., 2010; Herrero et al., 2004; Lussier et al., 1998; Nagahashi et al., 1998; Shahinian and Bussey, 2000). In *S. cerevisiae*, *KRE5* and *KRE9* seem to play a major role in the synthesis of (1-6)β-D-glucan (Brown and Bussey, 1993; Meaden et al., 1990; Shahinian and Bussey, 2000). *C. neoformans KRE5*, *KRE6*, and *SKN1* encode proteins that are also implicated in the synthesis of (1-6)β-D-glucan (Gilbert et al., 2010). In *C. albicans* the absence of CaBig1 protein, which is similar to *S. cerevisiae* Big1, induces a decrease in the amount of (1-6)β-D-glucan in the cell wall (Umeyama et al., 2006). *S. cerevisiae KRE* genes have been also implicated in the synthesis of (1-3)β-D-glucan and *FKS* genes in the synthesis of (1-6)β-D-glucan (Dijkgraaf et al., 2002). Similarly, *S. pombe* Sbg1, which is paralogous to *S. cerevisiae* Kre6 and Skn1, has been also implicated in the synthesis of the linear (1-3)β-D-glucan of the primary septum during cytokinesis (Sethi et al., 2016).

2.2.3. Chitin

The enzyme chitin synthase uses UDP-N-acetylglucosamine as substrate to synthetize microfibrils of chitin that are extruded to the periplasmic space (**Fig. 2**). In *S. cerevisiae* three chitin synthase activities (chitin synthase I, chitin synthase II, and chitin synthase III) have been described, with the subsequent catalytic subunits being the integral membrane proteins Chs1, Chs2 and Chs3, respectively (reviewed by Cabib and Arroyo, 2013; Cabib et al., 2001; Roncero and Sánchez, 2010; Schmidt et al., 2002). The chitin provided by Chs1 is required as a replacement for preserving cell integrity during cell separation (Cabib et al., 1989; Cabib et al., 1992). Chs2 synthetizes the chitin found in the primary septum during septation (Shaw et al., 1991) and Chs3 synthesizes the chitin of both a ring at the site where bud emergence occurs and the cell wall, and is also required for the synthesis of the remedial septum formed in the absence of Chs2 (Cabib and Schmidt, 2003; Kollar et al., 1995).

Chs3 is also required for the accumulation of chitin in response to cell wall stress (reviewed by Popolo et al., 2001). Several *S. cerevisiae* cell wall mutants have been reported to exhibit increased levels of chitin as a compensatory mechanism to

reestablish the cell wall integrity (Bulik et al., 2003; Kapteyn et al., 1999a; Kapteyn et al., 1999b; Popolo et al., 1997; Ram et al., 1998). Because the absence of Chs3 causes a reduction in the content of chitin in some of these cell wall mutants (Bulik et al., 2003; Carotti et al., 2002; García-Rodríguez et al., 2000; Valdivieso et al., 2000), it has been proposed that Chs3 is responsible for the compensatory increase of chitin levels in response to cell wall damage (Popolo et al., 2001). Similarly, *C. albicans* cells treated with echinocandin display high levels of cell wall chitin (Walker et al., 2008). This increase in the content of cell wall chitin confers some resistance to echinocandin both in cell cultures and infected mice (Lee et al., 2012; Plaine et al., 2008). In addition, it has been hypothesized that the high levels of chitin induced by the presence of echinocandin may favor the acquisition or selection of mutations in the *FKS* genes (Munro, 2012). In agreement, *C. albicans* cells with high levels of chitin and recovered from infected mice had acquired at low frequency a point mutation that confers echinocandin resistance to the GS catalytic subunit Fks1, suggesting that the reduced echinocandin susceptibility of *C. albicans* cells in mice models could be due to either the high content of cell wall chitin or the acquisition of *FKS1* point mutations (Lee et al., 2012).

Based on the evolution of their sequences of amino acids, two families of fungal chitin synthases, which are divided into seven chitin synthase classes, have been identified (Gow et al., 2017; Morozov and Likhoshway, 2016; Roncero, 2002), although only four classes have been found in filamentous fungi (Bowen et al., 1992; Fernandes et al., 2016). Depending on the fungus, the number of *CHS* genes fluctuates from 1 to 20. *A. fumigatus*, *Aspergillus nidulans* and *C. neoformans* have eight non-essential *CHS* genes encoding putative chitin synthases (Lenardon et al., 2010). In contrast, *C. albicans* contains four *CHS* genes, but only *CHS1* is vital for cell survival (Munro et al., 2001). Although chitin has not been detected in the cell wall of *S. pombe* (Bush et al., 1974), its genome contains two homologous *chs* genes. Chs2 homolog is required for the actomyosin ring integrity during cytokinesis (Martín-García and Valdivieso, 2006), while Chs1 participates in the synthesis of the chitosan required for proper maturation of the ascospores produced through sexual differentiation (Arellano et al., 2000).

2.2.4. (1-3)α-D-glucan

The (1-3)α-D-glucan synthase catalytic subunit is a multidomain protein containing a putative synthase domain located in the cytoplasm, numerous transmembrane domains and a transglycosylase domain located in the periplasmic space (Grun et al., 2005; Vos et al., 2007). The first gene coding for the (1-3)α-D-glucan synthase was detected in *S. pombe* and named *ags1*$^{+}$*/mok1*$^{+}$ (Hochstenbach et al., 1998; Katayama et al., 1999). The protein Ags1/Mok1 is essential for the integrity of the cell during both polar growth and mainly cell separation, and for maintaining the primary septum adhesion during cell separation (Cortés et al., 2012). *S. pombe* contains four more genes encoding (1-3)α-D-glucan synthases, of which at least three, together with Ags1, play different roles throughout sexual differentiation (Cortés et al., 2012; García et al., 2006). *N. crassa* has exclusively one (1-3)α-D-glucan synthase, Ags1, whose absence causes defects in the conidial cell wall (Fu et al., 2014). Deletion of *C. neoformans AGS1* removes the capsule material and causes the loss of fungal virulence (Reese and Doering, 2003; Reese et al., 2007). In *H. capsulatum* deletion of the single *AGS1* gene depletes the cell wall (1-3)α-D-glucan and reduces virulence (Edwards et al., 2011; Rappleye et al., 2004). In this fungus, the synthesis of (1-3)α-D-glucan also requires the functions of the predicted α-amylase Amy1 and the UTP-glucose-1-phosphate uridylyltransferase Ugp1 (Marion et al., 2006). *A. fumigatus* presents three Ags proteins (Beauvais et al., 2005; Maubon et al., 2006), and although their simultaneous absence is dispensable, it affects the conidial cell wall and fungal virulence (Beauvais et al., 2013; Henry et al., 2011).

2.2.5. Glycoproteins

The proteins, covalently and non-covalently linked or associated to the cell wall, can be modified with N-glycans, O-manno-oligosaccharides and a GPI anchor as they traverse the endoplasmic reticulum-to-Golgi pathway of secretion (reviewed by Orlean, 2012). These structures are initially attached to the proteins in the lumen of the endoplasmic reticulum and are later extended and processed in the Golgi to form the glycoproteins that will be either placed in the plasma membrane or secreted to the periplasmic space to be incorporated together with the rest of polysaccharides of the cell wall (reviewed by Orlean, 2012). The proteins of the outer layer of the cell walls of *S. cerevisiae* and *C. albicans* are highly glycosylated via the incorporation of α- and β-linked oligomannosyl monomers by the action of Golgi mannosyltransferases using GDP-mannose as substrate (reviewed by Gow et al., 2017; Orlean, 2012). Many

glycoproteins carry N-linked glycans, with the endoplasmic reticulum-made core structure Man(10-14)-GlcNAc(2)-Asn being similar in all eukaryotes. The outer chains comprise 50-200 additional α-linked mannose monomers, with a long (1-6)α-D-linked backbone branched with short side chains with (1-2)α- and (1-3)α-D linkages (Gow et al., 2017; Lipke and Ovalle, 1998). The O-linked sugars are short linear chains of α-linked mannose residues. The structure of mannan can be significantly different depending on the fungi. However, a comparative genomic analysis has shown that orthologues of the *S. cerevisiae* (1-6)α-D-mannosyltransferase *OCH1* gene are found in the genomes of *A. fumigatus* and other filamentous fungi (Lambou et al., 2010). Filamentous fungi and fission yeast cell wall glycoproteins are distinctive because they contain galactose in addition to the mannose found in all fungal glycoproteins (Fontaine et al., 2000; Simpson et al., 1972; Walther et al., 1988). Galactose units are found in the terminal positions of the N-glycan structural core and of the outer chains of α-linked mannose. *S. pombe* O-linked sugars consist of small tri- and tetrasaccharides of α(l,2)-linked mannosylmannose and α(l,2)-linked galactosylmannose (Ballou et al., 1994). *S. pombe* O-mannosyltransferases are crucial for cell wall integrity, septation and cell viability (Willer et al., 2005). Furthermore, the absence of *S. pombe* Gmd3/Alg11, which is homologous to the *S. cerevisiae* mannosyltransferase Alg11, causes a general reduction in both mannose and galactose residues of the outer layer of glycoproteins (Umeda et al., 2000). The capsule of *C. neoformans* is mainly composed of glucuronoxylomannan forming a backbone of (1-3)α-D-linked mannose units with branches of glucuronic acid in (1-2)β linkage and xylose in (1-2)β and (1-4)β linkages (Wang et al., 2018). The capsule is also composed of a smaller polymer of glucuronoxylomannogalactan, which is formed by a backbone of (1-6)α-D-linked galactose units with branches of galactose, mannose and a variable number of β-linked glucuronic acid and xylose residues (Heiss et al., 2009; Wang et al., 2018).

2.3. Biosynthesis of the GPI precursor and remodeling of the protein-linked GPIs

The glycosylphosphatidylinositol (GPI) is a posttranslational modification to attach cell surface proteins to the plasma membrane and is well conserved in all eukaryotes (Umemura et al., 2003). In fungi, the GPI facilitates the cell wall cross-linkages between glycoproteins and (1-6)β-D-glucan (Kapteyn et al., 1995; Kapteyn et al., 1996; Kollar et al., 1995; Lu et al., 1995; Van Der Vaart et al., 1996). GPI cross-linkage to

glycoproteins is essential for fungi survival (Leidich et al., 1995; Lipke and Ovalle, 1998; Vossen et al., 1997), providing a valuable target for developing new antifungal agents (**Fig. 2**). Furthermore, restricted GPI synthesis leads to aberrant wall biogenesis and limited growth (Vossen et al., 1997).

Briefly, GPI synthesis begins in the cytoplasmic side of the membrane of the endoplasmic reticulum. Once synthetized, GlcNAc-PI is de-N-acetylated, and probably translocated to the luminal side of the endoplasmic reticulum membrane (reviewed by Orlean, 2012). Next, the inositol ring in GlcN-PI is acylated by the essential acyltransferase Gwt1, making it resistant to cleavage by PI-specific phospholipase C. The *GWT1* gene was identified in *C. albicans* as a dosage-dependent suppressor of the phenotypes produced by 1-[4-butylbenzyl] isoquinoline (BIQ), a compound that blocks the incorporation of GPI proteins into the cell wall (Tsukahara et al., 2003; Umemura et al., 2003). Next, the GlcN-(acyl)PI is extended by addition of four α(l,2)- and α(l,6)-linked mannoses, and at the same time Etn-P moieties are added to three of the four mannoses by Mcd4 and other ethanolamine phosphotransferases (Orlean, 2012; Wiedman et al., 2007). Then, the resulting structure is transferred by transamidation to proteins containing a GPI-signal anchor domain at the C-terminus (reviewed by Orlean, 2012). The final remodeling of protein-bound GPIs continues in the endoplasmic reticulum lumen and includes the removal of the inositol palmitoyl group and the *sn*-2 acyl chain of diacylglycerol, the addition of a $C_{26:0}$ acyl chain to the *sn*-2 position and the replacement of diphosphatidic acid with ceramide phosphate or of diacylglycerol with ceramide. Following on, new α(l,2)- or α(l,3)-linked mannoses are added to the previous four in the Golgi, before the GPI protein reaches the cell surface to be integrated into the cell wall (reviewed by Orlean, 2012).

The fungal Gwt1 enzyme is an excellent target for the design of new antifungals because it displays low homology (<30% amino acid sequence identity) with its closest mammalian orthologue, and its function is critical to maintain the cell wall integrity and to enable the fungal cell adhesion (Hata et al., 2011; Mutz and Roemer, 2016). As a consequence, one of the new cell wall-specific antifungal classes that is currently undergoing clinical development for the treatment of invasive mycoses (see below), specifically Gwt1 targets for blocking the incorporation of GPI-linked proteins to the cell wall (**Fig. 2**) (Hager et al., 2018; Shaw et al., 2018). Similarly, because inhibition of Mcd4 blocks the cell growth of a wide-range of pathogenic fungi, inhibitors of this

ethanolamine phosphotransferase have the potential to be developed as new antifungal drugs (Mann et al., 2015).

3. Echinocandin derivatives approved for therapeutic use

The echinocandins are the novel class of approved antifungal compounds to treat invasive mycoses. Echinocandins bind in a non-competitively fashion to the catalytic subunit of GS to block the biosynthesis of the essential (1-3)β-D-glucan of fungal cell walls (**Fig. 2**) (Douglas et al., 1994b; Huttel, 2017; Pérez et al., 1981; Yamaguchi et al., 1985). Natural echinocandins are produced by several fungi that cannot be used in clinical practice because of their toxicity and low solubility. Thus, marketable caspofungin, micafungin and anidulafungin are semisynthetic chemical derivatives of natural echinocandins (**Table 2**) (Denning, 2003; Huttel, 2017).

Echinocandins are non-ribosomal cyclic hexapeptides differentiated by the acyl lipid side chain N-linked to the dihydroxyornithine residue (Huttel, 2017). Caspofungin contains a fatty acid side chain and is originated from pneumocandin B0 through a chemical modification of the hexapeptide scaffold. Micafungin is generated from the echinocandin B FR901379 by replacement of the fatty acid side chain with an aromatic complex side chain. Anidulafungin is echinocandin B with the fatty acid side chain replaced with an alkoxytriphenyl chain (**Table 2**) (Denning, 2003; Vicente et al., 2003). The position and conformation of these fatty acid side chains is critical for their antifungal activity, presumably because this side chain is intercalated with the bilayer of phospholipids of the fungal plasma membrane (Denning, 2003; Odds et al., 2003).

Although there is no doubt that echinocandins bind to the GS catalytic subunit, their effects inhibiting the synthesis of (1-3)β-D-glucan do not certainly imply a direct inhibition of the catalytic subunit itself. Likewise, it is not known whether they bind to the GS enzyme in the extracellular or cytoplasmic side of the cell membrane (Odds et al., 2003). Echinocandins binding to the plasma membrane domains that concentrate GS could influence (1-3)β-D-glucan production. This is supported by the fact that disruption of the fungal plasma membrane by the action of theonellamides, which specifically bind to the sterol-rich domains of the cell membrane, highly increases the cell wall (1-3)β-D-glucan in a Bgs1- and Rho1-dependent manner (Nishimura et al., 2010). Echinocandins exhibit different fungicidal activity that depends on the fungal species, displaying a broad fungicidal spectrum against most *Candida* species and a

fungistatic activity with *A. fumigatus*. Due to their impressive safety profile and potent killing activity, echinocandins have emerged as the primary treatment for systemic candidiasis (Emri et al., 2013; Geddes-McAlister and Shapiro, 2018; Kathiravan et al., 2012; Pappas et al., 2016a, b).

4. Mechanisms of fungal resistance to echinocandins

Therapies for invasive mycoses are extremely limited (Geddes-McAlister and Shapiro, 2018; Healey and Perlin, 2018). Traditionally, the azole antifungals have been used as the first-line therapy to treat *C. albicans* infections. However, the emergence of non-albicans *Candida* species that quickly develop resistance to azoles (*C. glabrata*) or are naturally azole-resistant (*C. krusei*) has conducted to the extensive utilization of echinocandins (Geddes-McAlister and Shapiro, 2018; Healey and Perlin, 2018). The emergence of echinocandin resistances threatens the effective treatment of mycoses. Although the incidence of echinocandin resistance among *C. albicans* and other *Candida* species is still moderately rare, it is progressively encountered (Ben-Ami and Kontoyiannis, 2012; Castanheira et al., 2010; Dannaoui et al., 2012; Pfaller et al., 2011a). *C. glabrata* and *Candida auris* infections have increased considerably, supposing a serious public health problem because many isolates exhibit resistance to both azoles and echinocandins (Geddes-McAlister and Shapiro, 2018; Healey and Perlin, 2018; Perlin et al., 2017; Sharma et al., 2016). The rise of azole resistance among *A. fumigatus* isolates is also a significant concern (Chowdhary et al., 2017; Guinea et al., 2018; Verweij et al., 2009). Similarly, *Aspergillus terreus* is intrinsically resistant to amphotericin B and exhibits high rates of azoles and echinocandins resistance both *in vitro* and *in vivo* (Geddes-McAlister and Shapiro, 2018). To decrease the emergence of these highly drug-resistant fungal species, an improved combination of therapeutic compounds is required. However, given the restricted arsenal of available antifungals, the discovery and development of new antifungals is an urgent need (Geddes-McAlister and Shapiro, 2018).

Echinocandin resistance is related to recurrent or continued drug treatments, though the resistance can be also developed after short periods (Lewis et al., 2013). One of the most common mechanisms by which microorganisms acquire resistance to antimicrobials is through gene mutations that alter the encoded drug target conformation thus reduce drug binding and efficacy (reviewed by Cowen et al., 2014; Revie et al.,

2018; Robbins et al., 2017). Clinical fungal resistance to echinocandins involves modifications of the GS Fks subunits through mutations in two "hot spot" regions highly conserved of the Fks proteins (**Fig. 3**) (Park et al., 2005; Perlin, 2007; Rocha et al., 2007; Walker et al., 2010).

The adaptation of the fungus to sub-lethal doses of antimicrobial drug is another mechanism that can also lead to the emergence of drug resistance. In the case of echinocandins, a specific type of adaptation termed the paradoxical effect, whereby cell growth is reestablished at echinocandin concentrations considerably higher than the reported minimal inhibitory concentration (Stevens et al., 2005; Wiederhold et al., 2005). The result is a cell growth inhibition at lower drug concentrations, followed by drug adaptation and fungal cell survival at higher concentrations of drug, and again a new inhibitory effect when the echinocandin concentration continues increasing. Cell adaptation to echinocandins does not depend on *FKS* mutations (Fleischhacker et al., 2008; Wiederhold, 2007), but eventually spontaneous resistance mutations can be generated in those fungal cells that live long enough (Healey and Perlin, 2018). In *A. fumigatus* and *C. albicans* this mechanism seems to be due, at least partially, to the transcription increase of chitin synthases upon echinocandin exposure (Robbins et al., 2017). Activation of multiple pathways within the fungal cell in response to a cell stress, including the activation of the cell wall integrity signaling mediated via protein kinase C (PKC), the phosphatase calcineurin and the molecular chaperone Hsp90, is vital in order to enable echinocandin adaptation and compensatory mechanisms such as the upregulation of chitin synthases (**Fig. 3**) (reviewed by Cowen et al., 2014; Revie et al., 2018; Robbins et al., 2017). Thus, combined treatment of inhibitors of the chitin synthase and echinocandins is clearly more efficient than treatment of each compound individually (Lenardon et al., 2010; Munro, 2013; Walker et al., 2008).

Although the echinocandins and other semisynthetic GS inhibitors possess a broad-spectrum of activity, acting against an extensive range of species of *Aspergillus, Candida* and other pathogenic fungi (Debono and Gordee, 1994; Emri et al., 2013; Thompson et al., 1999), all (1-3)β-D-glucan synthesis inhibitors developed so far are inactive against *C. neoformans* assayed *in vivo* in animal models (Thompson et al., 1999). The fact that the Fks1 protein of this fungus is essential, has led to propose that its GS enzyme could be intrinsically resistant to the inhibition by echinocandins. However, the *in vitro* analysis of the *C. neoformans* GS has shown that its GS activity is very susceptible to the echinocandins caspofungin and cilofungin, indicating that the

natural echinocandin resistance of this fungus is due to other mechanisms distinct from Fks resistance (Coelho and Casadevall, 2016; Feldmesser et al., 2000; Maligie and Selitrennikoff, 2005).

S. pombe vegetative cells require three vital Bgs/Fks GS (see above) and are susceptible to the main classes of GS antifungals, acidic terpenoids (enfumafungin), echinocandins (caspofungin, pneumocandin and aculeacin) and glycolipids (papulacandin B) (Castro et al., 1995; Font de Mora et al., 1990; Martins et al., 2011; Miyata et al., 1980; Varona et al., 1983). All Bgs present in their conserved hot spots sequences the amino acids associated with natural susceptibility to antifungals. However, in this yeast the resistance to antifungals is specifically caused by mutations in the hot spots of *bgs4*$^{+}$ while maintaining the wild-type *bgs1*$^{+}$ and *bgs3*$^{+}$ sequences. Also, the absence of mutants of *bgs1*$^{+}$ and *bgs3*$^{+}$ among all of the isolated mutants exhibiting resistance to GS antifungals, suggests that Bgs1 and Bgs3 might be naturally occurring intrinsic resistant subunits (Castro et al., 1995; Martins et al., 2011). The study of *bgs4*$^{+}$ mutations conferring cellular resistance to papulacandins, acidic terpenoids and echinocandins extended the resistance to a larger hot spot region and to a third new hot spot region (Martins et al., 2011). Further analysis of these novel mutations will aid to better understand the mechanism of action of GS inhibitors and the fungal resistance to GS inhibitors.

5. Combination therapy

Overcoming the current emergence of multidrug-resistant fungi is challenging because of the lack of novel antifungal drugs being developed. Simultaneous treatment with two antifungal drugs is a promising therapeutic strategy of either additive or synergistic effect, expanding both therapeutic range and longevity of available antifungals by increasing the drug efficacy and reducing the acquisition of drug resistance (reviewed by Revie et al., 2018; Robbins et al., 2017). Fungal biofilms exhibit increased resistance to most antifungal drugs. Combination of multiple antifungal agents displays some efficacy at disrupting biofilms in cell cultures, therefore, enhancing the antifungal activity to impair the emergence of resistance (Tobudic et al., 2010; Zeng et al., 2014).

Drug combinations can also target the mechanisms for paradoxical effect involved in drug adaptation and that could lead to the emergence of drug resistance. For example,

inhibition of the cellular response to stress by targeting the phosphatase complex calcineurin or the chaperone protein Hsp90 with non-antifungal drugs has a promising therapeutic potential, because it induces a synergic effect that increases the activity of both azoles and echinocandins while weakening the acquisition of mutations that confer fungal drug resistance (Cordeiro Rde et al., 2014; Cowen and Lindquist, 2005; Cruz et al., 2002). The calcineurin pathway is necessary for cell wall integrity in *C. neoformans* and for both germination of conidia and growth of hyphae in *A. fumigatus*. Accordingly, the calcineurin inhibitor FK506 (tacrolimus) displays an efficient antifungal activity against a broad-spectrum of pathogenic fungal species. FK506 cannot be used as an antifungal compound because of its strong immunosuppressive activity, but several FK506 analogs (9D-, 9DP-, 31OD-, and 9D31OD-FK506) with lower cytotoxicity and immunosuppressive activity have been developed (Lee, Y. et al., 2018). These analogs, except 9DP-FK506, display an efficient antifungal activity against *A. fumigatus*, *C. albicans* and *C. neoformans*, and 31OD- and 9D31OD-FK506 are also synergistic with fluconazole. Thus, in a mice model of systemic cryptococcosis, combination of 9D31OD-FK506 with fluconazole significantly extends the survival of infected mice (Lee, Y. et al., 2018). It would be interesting to know whether these FK506 analogs can also show some synergism with the echinocandins.

The novel class of GPI biosynthesis inhibitors (see below) exhibit *in vivo* synergies with multiple echinocandins. This synergistic effect could be caused because the GS-containing plasma membrane would be more accessible to echinocandin molecules in the absence of the cell wall outer layer of GPI-linked glycoproteins (Mutz and Roemer, 2016). The discovery of new compounds that show inhibitory addition or synergy with existing antifungals could help to identify new therapeutic combinations. As a result, it was found that, through a large-scale screening for pharmacologically active compounds, the diethylenetriaminepentaacetic acid (DTPA) chelator increases the activity of echinocandins against resistant isolates of *C. albicans* (Polvi et al., 2016). Similarly, a large-scale screening of chemicals identified a synergistic inhibitory effect between the antibiotic colistin, which is a drug that increases the yeast membrane permeability, and aminocandin (see below) against several *Candida* species (Zeidler et al., 2013).

6. Inhibitors of the fungal cell wall in clinical development for therapeutic use

The clinical use of echinocandins (caspofungin, micafungin and anidulafungin) is highly valuable because they do not exhibit the toxicity and drug-drug interactions of polyenes and azoles (Wiederhold, 2018). However, lipopeptide echinocandins cannot be administered orally as they exhibit both a high molecular weight and high instability at low pH (Kofla and Ruhnke, 2011). Echinocandins have not been approved for topical formulation due to reduced chemical stability, preventing the treatment of some *Candida* infections such as vulvovaginal candidiasis (Locke et al., 2018). Therefore, successful echinocandin treatment requires daily intravenous administration, which can be problematic for those patients suitable for the treatment with oral drugs but affected by an azole-resistant fungus, and that in addition reside outside of the healthcare facilities. In an attempt to solve these problems, compounds with longer half-lives that could be administered more sporadically or suitable for oral formulation are currently under development (Wiederhold, 2018).

6.1. CD101 (rezafungin)

CD101 (rezafungin) is an echinocandin derivative currently being developed by Cidara Therapeutics (San Diego, CA, USA), which has a modification in the choline moiety at the cyclic echinocandin core (**Fig. 2** and **Table 3**). This modification confers increased solubility and remarkable chemical and metabolic stability, opening up the possibility of a more sporadic administration and also enables topical formulations for skin and vaginal infections (González-Lara et al., 2017; Krishnan et al., 2017; Locke et al., 2018). The spectrum of antifungal activity of CD101 is similar to that of currently approved echinocandins, exhibiting potent activity against both *Aspergillus* and *Candida* species (Hall et al., 2017; Pfaller et al., 2017; Pfaller et al., 2016). Like caspofungin and anidulafungin, CD101 exhibits a low potential for the acquisition of *FKS* mutations that confer echinocandin resistance in several *Candida* species (Locke et al., 2016). Also, CD101 and micafungin exhibit comparable inhibition of the *in vitro* GS activity on wild-type and several isolates resistant to echinocandin of both *C. albicans* and *C. glabatra* (Zhao et al., 2016).

6.2. SCY-078 (ibrexafungerp)

The acidic terpenoid enfumafungin is a hemiacetal triterpene glycoside derivative that was first identified by Merck Sharp & Dohme (MSD, Whitehouse Station, NJ, USA) as a specific inhibitor of GS (Peláez et al., 2000). A program developed to identify and evaluate derivatives of enfumafungin, conducted by MSD in collaboration with Scynexis (Jersey City, NJ, USA), led to the production of several semi-synthetic derivatives and the selection of SCY-078 (formerly MK-3118), which is presently in clinical trials at its phase 3 (**Table 3**) (Apgar et al., 2015; Heasley et al., 2012). SCY-078 and enfumafungin-derived inhibitors have the benefit of both oral and intravenous formulations. SCY-078 is a potent inhibitor of fungal GS modified from enfumafungin by tethering its alkyl groups proximal to the basic amino group C3 side chain to prevent oxidative N-demethylation (González-Lara et al., 2017; Heasley et al., 2012; Hector and Bierer, 2011). Given that SCY-078 and other enfumafungin derivatives are structurally different from echinocandins, the *FKS* mutations conferring drug resistance to echinocandins cause, in some cases, a different result with enfumafungin derivatives. SCY-078 exhibits an excellent *in vitro* GS inhibition efficacy in wild-type *Candida* species and in both wild-type and azole-resistant *Aspergillus* species, but is more variable against non-*Aspergillus* molds (Jiménez-Ortigosa et al., 2014; Lamoth and Alexander, 2015; Pfaller et al., 2013a, b; Walker et al., 2011). Preclinical results show an equivalent level of efficacy of SCY-078 against *Candida* species with respect to caspofungin, while the *in vivo* inhibitory activity against aspergillosis presents a slightly inferior response compared to that of caspofungin (Hector and Bierer, 2011; Lepak et al., 2015).

6.3. Nikkomycin Z

Given that chitin is vital for maintaining the integrity of the fungal cell wall, its inhibition is an appealing objective for developing novel antifungal treatments. Nikkomycin Z was originally isolated by Bayer Pharmaceutical (Berlin, Germany) in the decade of 1970, and later developed by the now closed Shaman Pharmaceuticals (San Francisco, CA, USA) (Nix et al., 2009). Its development was renewed again in 2007 by the University of Arizona, but the conducted clinical trials were prematurely terminated because of recruitment challenges and the lack of financial funding (**Table 3**). Nikkomycins and polyoxins are structural analogs of the UDP-N-acetylglucosamine that competitively inhibit chitin synthases by binding to their catalytic sites (Cabib,

1991). Orally administered to mice, nikkomycin Z exhibits a potent fungicidal activity against *B. dermatitidis*, *Coccidioides immitis* and *H. capsulatum* (Hector et al., 1990). Nikkomycin Z exhibits low antifungal activity against *A. fumigatus* (Fortwendel et al., 2009), while it is able to inhibit the cell growth of *C. albicans* (Becker et al., 1983; Chapman et al., 1992). Interestingly, the combined treatment of nikkomycin Z and caspofungin shows a synergistic inhibitory effect against *A. fumigatus* and *C. albicans*, highlighting the potential of combination therapies to enhance fungicidal activity against yeasts and molds (Fortwendel et al., 2009; Walker et al., 2008).

6.4. APX001

APX001/E1211 (Amplyx Pharmaceuticals, San Diego, CA, USA) is a broad-spectrum antifungal agent with both intravenous and oral bioavailability. This compound is an N-phosphonooxymethyl pro-drug which is quickly and entirely metabolized to the active moiety APX001A/E1210 (initially discovered by Eisai Co., Tokyo, Japan) by the action of systemic alkaline phosphatases. The antifungal action of APX001 is fungistatic, exhibiting low toxicity levels like those of fluconazole. APX001 displays highly selective antifungal activity by targeting the fungal Gwt1 protein to inhibit the synthesis of GPI and linkage of essential mannoproteins to the surface of the cell wall (**Fig. 2** and **Table 3**) (Hata et al., 2011; Watanabe et al., 2012). This antifungal agent displays an excellent efficacy in *in vivo* cell culture assays against species of *Candida*, including some fluconazole-resistant isolates, *Aspergillus*, *Cryptococcus* and some mold species that are problematic to handle (Castanheira et al., 2012; Miyazaki et al., 2011; Pfaller et al., 2011b; Pfaller et al., 2011c; Shaw et al., 2018; Zhao et al., 2018). In animal models, APX001 administered orally has proven to be highly effective against disseminated candidiasis produced by azole-resistant *C. albicans* and *C. tropicalis*, aspergillosis caused by *Aspergillus flavus* (combined with voriconazole or caspofungin) and *A. fumigatus* and disseminated fusariosis caused by *Fusarium solani* (Hata et al., 2011). In a cryptococcal meningitis model, APX001 and other analogs have also shown some effectiveness when combined with fluconazole (Shaw et al., 2018).

7. Current inhibitors of (1-3)β-D-glucan synthesis in development

In the past years several (1-3)β-D-glucan synthesis inhibitors, presenting a wide-range of activity against the major infectious fungal species and oral bioavailability, have emerged as potential candidates for clinical development (Hector and Bierer, 2011). In addition, the existing GS inhibitors may also be used as initial scaffolds for synthetically creating more efficient and useful antifungal compounds.

7.1. Echinocandin B derivatives

New semi-synthetic echinocandin derivatives are the focus of some patents issued to Aventis Pharma (Mumbai, India). These patents state that aculeacin, cilofungin, echinocandin B, pneumocandin A_0, pneumocandin B_0 and pneumocandin C_0 might be exploited as synthetic starting points for synthetizing new echinocandin derivative inhibitors (Hector and Bierer, 2011). The efficiency of semi-synthetic compounds against several *Aspergillus*, *Candida* and *Cryptococcus* strains has been cited, but biological data has not been provided. It has been also claimed that several of the echinocandin B derivatives show good efficiency against the *in vitro* GS of *A. fumigatus* and *C. albicans* (Hector and Bierer, 2011).

7.2. Pyridazinone derivatives

The identification of bioactive molecules in a high-throughput screen combined with analyses of their mechanism of action allowed the identification of piperazinyl-pyridazinones derivatives as a new class of compounds that inhibit the target GS enzyme (Schering-Plough Corporation, Kenilworth, NJ, USA, now fused with MSD, and Albany Molecular Research, Albany, NY, USA, **Table 4**). These small molecules display both a comparable *in vitro* GS inhibition to that of echinocandins and a strong correlation between of GS enzyme inhibition and *in vivo* antifungal effectiveness. Furthermore, they induce a similar cell lysis to that of other traditional GS inhibitors. In contrast to echinocandins, these compounds exhibit efficacy when orally administered to mice infected with *C. glabrata* (Butts and Krysan, 2012; Hector and Bierer, 2011; Walker et al., 2011).

7.3. Papulacandins

Papulacandins are naturally occurring glycolipid antifungals characterized by containing a benzannulated spiroketal unit and were originally isolated by Ciba-Geigy (Basel, Switzerland), now known as Novartis after its fusion with Sandoz (**Table 4**) (Traxler et al., 1977). The unsaturated side chains of papulacandins are required for their activity. Papulacandins inhibit the synthesis of (1-3)β-D-glucan by targeting the GS enzyme, and are very active and specific against several yeasts, but less active against molds and largely inactive against bacteria and protozoa (Baguley et al., 1979; Font de Mora et al., 1990; Pérez et al., 1981; Traxler et al., 1977; Varona et al., 1983). Comparison between echinocandins, enfumafungin and papulacandins has shown that papulacandins are the most potent inhibitors of the *in vitro* GS activity, several orders of magnitude more effective than other inhibitors (Martins et al., 2011). However, owing to the limited activity of papulacandins observed in studies in animal models, neither papulacandin B nor any of their analogs have been further improved (Vicente et al., 2003). Some new papulacandin derivatives have been developed during the past years. For example, several papulacandin D derivatives with moderate biological activity have been synthetized by the modification of two partly unsaturated acyl chains linked to sugars (van der Kaaden et al., 2012). New and already known papulacandins have been identified by MSD through high-throughput separation approaches used to isolate natural products effective as antifungals (Roemer et al., 2011). Also, a new papulacandin class named pestiocandin has recently been described as being more effective against multidrug-sensitive yeasts than against wild-type yeasts (Sakai et al., 2018).

7.4. Poacic acid

Screening for diferulates isolated from lignocellulosic hydrolysates, exhibiting antifungal activity, allowed the identification of poacic acid (diferulate, 8-5-DC) as a new cell wall antifungal that targets the (1-3)β-D-glucan polysaccharide and inhibits both *in vivo* synthesis of (1-3)β-D-glucan and *in vitro* GS activity (**Table 4**) (Piotrowski et al., 2015). This natural product causes lysis of cells and is synergistic with fluconazole and caspofungin. Poacic acid inhibits the cell growth of fungi *S. cerevisiae*, *Sclerotinia sclerotiorum*, *Alternaria solani* and the oomycete *Phytophthora sojae*. A sole application of poacic acid to leaves of soybean infected with *S. sclerotiorum* can reduce the infection progression (Piotrowski et al., 2015). However, *Candida* species

display different sensitivity to poacic acid and therefore, the spectrum of activity against disseminated candidiasis may differ (Lee, K.K. et al., 2018).

7.5. Other inhibitors of the GS enzyme

Other cyclic peptides have been proposed to inhibit the GS activity (**Table 4**) (Hector and Bierer, 2011; Vicente et al., 2003). Aminocandin (Aventis Pharma) is a new echinocandin with excellent *in vitro* and *in vivo* inhibitory activity against both *Aspergillus* and *Candida* (Andes et al., 2003; Warn et al., 2005, 2010). This drug is efficient at inhibiting the first step of biofilm formation by *C. albicans* (Cateau et al., 2007). The echinocandin-related cryptocandin (HMV Corporation, Alpine, UT, USA) exhibits an inhibitory activity against *C. albicans* and *Trychophyton* species (Strobel et al., 1999). Arborcandins (Daiichi Sankyo, Tokyo, Japan) are other GS inhibitors which are structurally different from echinocandins. These compounds exhibit potent fungicidal activity against *Candida* species and fungistatic activity against *A. fumigatus* (Ohyama et al., 2000). Similarly, aerothricin3/FR901469 (Chugai Pharmaceutical, Tokyo, Japan and Basilea Pharmaceutica, Basel, Switzerland) is a macrocyclic lipopeptidolactone comprised of ring of 12 amino acids and a 3-hydroxypalmitoyl moiety that inhibits the GS activity. This compound is efficient against most *Candida* species and all tested *Aspergillus* species. Fks1 and Fks2 of *S. cerevisiae* exhibit differential sensitivity to this inhibitor (Fujie et al., 2001; Kondoh et al., 2002). The synthetic antifungal drug GSI578 is a piperazine propanol derived ([2,6-Difluoro-phenyl)-carbamic acid 3-[4-benzothiazol-2-yl-piperazine-1-yl]-propyl ester; Chugai Pharmaceutical) that also inhibits the GS activity and displays antifungal effect against *A. fumigatus* and *C. albicans* (Kondoh et al., 2005). New modifications of several acids (chlorogenic, quinic and caffeic acid) associated with an H2N-orn-4-(octyloxy) aniline group inhibit partially the GS activity and display antifungal effect (Ma et al., 2010). Finally, novel synthetic homoallylamines, tetrahydroquinolines, quinolines and chalcones are inactive *in vivo* against *Aspergillus* and *Candida* species, but are extremely active with dermatophytes, in particular *Epidermophyton floccosum*, and show a remarkable *in vitro* inhibitory effect of both chitin synthase and GS activities (López et al., 2001; Urbina et al., 2000; Vargas et al., 2003). Macrocyclic lactone glycolipids isolated from plant Convolvulaceous species have a structure resembling

that of papulacandins and show an *in vitro* GS inhibitory activity similar to that of papulacandin (Castelli et al., 2002).

8. Novel inhibitors of (1-6)β-D-glucan and GPI synthesis

The main weakness of all GS inhibitors is their apparent inefficiency against disseminated cryptococcosis. One theoretical explanation for the resistance observed in *C. neoformans* is that the capsule of the fungus could block the entrance of inhibitors and consequently the inhibition of the GS located in the plasma membrane. However, this hypothesis seems improbable because the growth of both acapsular and encapsulated strains is inhibited with a similar concentration of echinocandin (Feldmesser et al., 2000). Another plausible hypothesis is that cell wall melanin could obstruct echinocandins from reaching the GS in the plasma membrane of this fungus, since a reduction in melanin increases susceptibility to GS antifungals (Coelho and Casadevall, 2016). However, it is unknown whether the reduction of melanin is accompanied by a reduction of other cell wall components, which could be equally responsible for the obstruction of the access of antifungals to the GS. It is considered that the cell wall of acapsular *C. neoformans* is enriched in glucose residues with (1-6)β-D linkages. The (1-6)β-D-glucan is vital for the cell integrity of *S. cerevisiae*, which contains a lower amount of this polysaccharide. Another hypothesis that may explain *C. neoformans* resistance to specific GS antifungals is that since echinocandins do not inhibit the synthesis of this (1-6)β-D-linked polymer, the supposed higher amount of (1-6)β-D-glucan would protect cell integrity from the (1-3)β-D-glucan reduction caused by GS-specific antifungals (Feldmesser et al., 2000; Gilbert et al., 2010; James et al., 1990).

Thus, the discovery and improvement of new compounds that specifically inhibit the (1-6)β-D-glucan synthesis could be critical for circumventing the resistance of *C. neoformans* and other fungal species which are naturally resistant to GS inhibitors. In addition, the (1-6)β-D-glucan is vital for the *C. albicans* virulence (Herrero et al., 2004; Umeyama et al., 2006). The novel class of bicyclic heteroaryl ring derivatives (D75-4590, D11-2040 and D21-6076; Daiichi Sankyo, Tokyo, Japan) target Kre6 to inhibit the biosynthesis of (1-6)β-D-glucan (**Table 4**). These small molecules display *in vivo* inhibitory efficacy both in cell cultures and in animal models against a broad-range of *Candida* species (Hector and Bierer, 2011; Kitamura et al., 2009a; Kitamura et al.,

2009b; Kitamura et al., 2010; Takeshita et al., 2010). Unfortunately, like GS inhibitors, bicyclic heteroaryl ring derivatives are ineffective against *C. neoformans* and molds. D11-2040 presents a synergistic effect with caspofungin and fluconazole for *C. albicans* growth inhibition and, interestingly, this inhibitor also induces a slight reduction in the minimal inhibitory concentrations of itraconazole, amphotericin B and caspofungin in *A. fumigatus* and *C. neoformans* (Hector and Bierer, 2011; Kitamura et al., 2009a; Kitamura et al., 2009b; Kitamura et al., 2010; Takeshita et al., 2010).

GPI-linked proteins are mostly connected to the cell wall through the (1-6)β-D-glucan chains. Recently, the novel inhibitor of GPI biosynthesis APX001A/E1210 (see above and **Table 3**) has shown good inhibitory effectivity with *C. neoformans*, as well as with a broad-spectrum of clinically important fungal pathogens (Miyazaki et al., 2011; Pfaller et al., 2011b; Pfaller et al., 2011c; Shaw et al., 2018). Several synthetic APX001 derivatives also present activity against *C. neoformans* (Miyazaki et al., 2011; Pfaller et al., 2011b; Pfaller et al., 2011c; Shaw et al., 2018). Screening for chemically induced haploinsufficiency by using a library of *C. albicans* heterozygous deletion mutants has allowed the identification of four molecules that inhibit GPI biosynthesis. G365 and G884 target the acyltransferase Gwt1, whereas M743 and M720 target the ethanolamine phosphotransferase Mcd4. These compounds display antifungal efficiency against a wide-spectrum of pathogenic yeasts and molds. In a murine infection model, M720 is active against systemic candidiasis and the combination of Gwt1 and Mcd4 inhibitors induces a potent synergistic effect (reviewed by Mutz and Roemer, 2016). In addition, the small molecule Gepinacin (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) is another inhibitor of Gwt1 that exhibits broad-spectrum antifungal activity (**Table 4**). Interestingly, GPI inhibitors cause cell wall β-glucans to be exposed to the medium, stimulating the recognition of fungi by the immune system of the host (Mann et al., 2015; McLellan et al., 2012).

9. Concluding remarks and future perspectives

In the 1990s the magnitude of fungal diversity was estimated at approximately 1.5 million species (Hawksworth, 2001), of which over 300 are human pathogens. *Aspergillus*, *Candida*, *Cryptococcus* and *Pneumocystis* species are the main cause of severe fungal diseases in humans. Over 1.6 million people die yearly due to severe fungi-related diseases. Most mycoses developed in hospitals, particularly by

immunocompromised patients infected with HIV or those undergoing chemotherapy, are still closely associated with high mortality rates. The situation is also aggravated because of the emergence of mycoses due to resistant species of *Candida*, *Aspergillus* and some *Cryptococcus*, which has dramatically reduced the number of available therapeutic options (Denning, 2017; Editorial, 2017; Geddes-McAlister and Shapiro, 2018; LIFE, 2017). In order to alleviate this unfortunate situation, mycologists have proposed three lines of attention that should be solved during the next few years (Brown et al., 2012a; Brown et al., 2012b; Denning and Bromley, 2015; Editorial, 2017), of which the current understanding of the interactions between the fungus-specific cell wall and host cells will play a pivotal role.

First of all, the early diagnosis and therapeutic monitoring of mycoses through improved and affordable methods are required for the effective early implementation of antifungal treatments (Brown et al., 2012a; Brown et al., 2012b; Denning and Bromley, 2015; Editorial, 2017). The cell wall delivers most antigens used to diagnose human mycoses. Antibodies raised against mannans of the outer cell wall layer are appealing for the diagnosis of systemic candidiasis, aspergillosis and cryptococcosis. The (1-3)β-D-glucan has also emerged as a tool for diagnosis of mycoses in patients infected with HIV (reviewed by Farhour et al., 2018; Gow et al., 2017; Ostrosky-Zeichner, 2012). Since cell wall polysaccharides present in the serum or plasma of infected immunocompromised patients correlate with fungal growth, several diagnostic methods for testing the content of (1-3)β-D-glucan and/or mannan are currently undergoing clinical trials for *Pneumocystis* (ClinicalTrials.gov identifier: NCT03613025), *Candida* (ClinicalTrials.gov identifier: NCT03674359, NCT03468803, NCT03090334) and *Aspergillus* (ClinicalTrials.gov identifier: NCT02104479, NCT01695499).

Secondly, safer, cheaper and more effective new antifungal drugs are needed to treat infections of multidrug-resistant fungi (Brown et al., 2012a; Brown et al., 2012b; Denning and Bromley, 2015; Editorial, 2017). Currently, three antifungals that target the synthesis of essential polymers of the cell wall are undergoing clinical development. The novel echinocandin CD101 and the enfumafungin derivative SCY-078 inhibit the production of (1-3)β-D-glucan, while the pro-drug AXP001 impedes the cell wall surface incorporation of GPI-linked proteins. These compounds exhibit a broad-spectrum activity to treat the main invasive fungal infections and, importantly, they can be orally administered and some of them even display improved chemical stability (see above).

And finally, advances in our knowledge of the interactions involving fungal and host cells have allowed the design and exploration of new panfungal vaccines and immunotherapeutic strategies, particularly useful for the treatment of immunocompromised individuals affected by mycoses (reviewed by Armstrong-James et al., 2017; Armstrong-James and Harrison, 2012; Lionakis and Levitz, 2018). Since innate recognition of (1-3)β-D-glucan, mannan, and chitin/chitosan on the fungal cell wall can induce strong adaptive immune responses, these polysaccharides can be used as adjuvants in the development of vaccines, which might protect against a broad-spectrum of mycoses. In fact, the cell wall of heat-killed *Saccharomyces* is an effective vaccine against *Aspergillus*, *Coccidioides* or *Candida*. Mannan or glucans alone also delivered substantial protection (Stevens et al., 2011). A vaccine consisting of conjugated-(1-3)β-D-glucan (laminarin) has revealed to be immunogenic and protective in mice infected with *Candida*, *Aspergillus* or *Cryptococcus* species (Torosantucci et al., 2005). Also, recombinant vaccines against *C. albicans*, based on epitopes of mannose trisaccharides conjugated to cell wall-associated proteins, are able to induce protection (Gow et al., 2017; Ibrahim et al., 2013).

Immunocompromised individuals are usually unable to mount protective responses after vaccination. Therefore, the efficiency of antibodies or T-cells to prevent and/or treat mycoses has been examined. In models of systemic aspergillosis, candidiasis, cryptococcosis and histoplasmosis, mice vaccinated with monoclonal antibodies against surface-exposed epitopes become moderately protected from a subsequent lethal infection (Armstrong-James et al., 2017). Radioimmunotherapy using a monoclonal antibody directed against the glucuronoxylomannan of the *C. neoformans* capsule is more efficient than amphotericin B in murine cryptococcosis (Bryan et al., 2010). T-cells expressing chimeric antigens of the Dectin-1 receptor that recognize the cell wall β-glucans lead to *in vivo* hyphal growth inhibition and cell damage of *Aspergillus*, both in cell cultures and in animal models (Kumaresan et al., 2014).

In sum, because the cell wall is specifically indispensable for fungal cells to infect mammalian hosts, it will continue to be a pivotal target for the design and discovery of safer and more effective treatments for the prevention and remedy of invasive mycoses in future years. The discovery of new drugs or immunotherapies directed against conserved cell wall enzymes that are still unexplored as targets for antifungal therapy, such as the essential (1-3)α-D-glucan synthases and (1-3)β-D-glucan remodeling glucanosyltransferases, will help to expand the variety of available antifungals and to

provide new, more effective and wider-range drug combinations for treating new and emerging multidrug-resistant fungal species.

Acknowledgements

We thank Emma Keck for language revision. This publication was supported by the grants BIO2015-69958-P from the Ministry of Science, Innovation and Universities (MICIU) (http://www.ciencia.gob.es/) and CSI068P17 from the Regional Government of Castile and Leon (JCyL/FEDER) and the European Regional Development Fund (ERDF) (https://www.jcyl.es/).

References

Aimanianda, V., Clavaud, C., Simenel, C., Fontaine, T., Delepierre, M., Latge, J.P., 2009. Cell wall β-(1,6)-glucan of *Saccharomyces cerevisiae*: structural characterization and *in situ* synthesis. J. Biol. Chem. 284(20), 13401-13412.

Alangaden, G.J., 2011. Nosocomial fungal infections: epidemiology, infection control, and prevention. Infect. Dis. Clin. North. Am. 25(1), 201-225.

Andes, D., Marchillo, K., Lowther, J., Bryskier, A., Stamstad, T., Conklin, R., 2003. *In vivo* pharmacodynamics of HMR 3270, a glucan synthase inhibitor, in a murine candidiasis model. Antimicrob. Agents Chemother. 47(4), 1187-1192.

Andes, D.R., Safdar, N., Baddley, J.W., Playford, G., Reboli, A.C., Rex, J.H., Sobel, J.D., Pappas, P.G., Kullberg, B.J., Mycoses Study, G., 2012. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. Clin. Infect. Dis. 54(8), 1110-1122.

Apgar, J.M., Wilkening, R.R., Greenlee, M.L., Balkovec, J.M., Flattery, A.M., Abruzzo, G.K., Galgoci, A.M., Giacobbe, R.A., Gill, C.J., Hsu, M.J., Liberator, P., Misura, A.S., Motyl, M., Nielsen Kahn, J., Powles, M., Racine, F., Dragovic, J., Habulihaz, B., Fan, W., Kirwan, R., Lee, S., Liu, H., Mamai, A., Nelson, K., Peel, M., 2015. Novel orally active inhibitors of β-1,3-glucan synthesis derived from enfumafungin. Bioorg. Med. Chem. Lett. 25(24), 5813-5818.

Arellano, M., Cartagena-Lirola, H., Nasser Hajibagheri, M.A., Durán, A., Valdivieso, M.H., 2000. Proper ascospore maturation requires the *chs1*$^{+}$ chitin synthase gene in *Schizosaccharomyces pombe*. Mol. Microbiol. 35(1), 79-89.

Arellano, M., Coll, P.M., Yang, W., Durán, A., Tamanoi, F., Pérez, P., 1998. Characterization of the geranylgeranyl transferase type I from *Schizosaccharomyces pombe*. Mol. Microbiol. 29(6), 1357-1367.

Arellano, M., Durán, A., Pérez, P., 1996. Rho1 GTPase activates the (1-3)β-D-glucan synthase and is involved in *Schizosaccharomyces pombe* morphogenesis. EMBO J. 15(17), 4584-4591.

Armstrong-James, D., Brown, G.D., Netea, M.G., Zelante, T., Gresnigt, M.S., van de Veerdonk, F.L., Levitz, S.M., 2017. Immunotherapeutic approaches to treatment of fungal diseases. Lancet Infect. Dis. 17(12), e393-e402.

Armstrong-James, D., Harrison, T.S., 2012. Immunotherapy for fungal infections. Curr. Opin. Microbiol. 15(4), 434-439.

Bacon, J.S., Jones, D., Farmer, V.C., Webley, D.M., 1968. The occurrence of α(1-3)glucan in *Cryptococcus*, *Schizosaccharomyces* and *Polyporus* species, and its hydrolysis by a *Streptomyces* culture filtrate lysing cell walls of *Cryptococcus*. Biochim. Biophys. Acta 158(2), 313-315.

Baguley, B.C., Rommele, G., Gruner, J., Wehrli, W., 1979. Papulacandin B: an inhibitor of glucan synthesis in yeast spheroplasts. Eur. J. Biochem. 97(2), 345-351.

Bakhiet, S.M., Fahal, A.H., Musa, A.M., Mohamed, E.S.W., Omer, R.F., Ahmed, E.S., El Nour, M., Mustafa, E.R.M., Sheikh, A.R.M.E., Suliman, S.H., El Mamoun, M.A.G., El Amin, H.M., 2018. A holistic approach to the mycetoma management. PLoS Negl. Trop. Dis. 12(5).

Ballou, C.E., Ballou, L., Ball, G., 1994. *Schizosaccharomyces pombe* glycosylation mutant with altered cell surface properties. Proc. Natl. Acad. Sci. U. S. A. 91(20), 9327-9331.

Bartizal, K., Abruzzo, G., Trainor, C., Krupa, D., Nollstadt, K., Schmatz, D., Schwartz, R., Hammond, M., Balkovec, J., Vanmiddlesworth, F., 1992. *In vitro* antifungal activities and *in vivo* efficacies of 1,3-β-D-glucan synthesis inhibitors L-671,329, L-646,991, tetrahydroechinocandin B, and L-687,781, a papulacandin. Antimicrob. Agents Chemother. 36(8), 1648-1657.

Beauvais, A., Bozza, S., Kniemeyer, O., Formosa, C., Balloy, V., Henry, C., Roberson, R.W., Dague, E., Chignard, M., Brakhage, A.A., Romani, L., Latge, J.P., 2013. Deletion of the α-(1,3)-glucan synthase genes induces a restructuring of the conidial cell wall responsible for the avirulence of *Aspergillus fumigatus*. PLoS Pathog. 9(11), e1003716.

Beauvais, A., Bruneau, J.M., Mol, P.C., Buitrago, M.J., Legrand, R., Latge, J.P., 2001. Glucan synthase complex of *Aspergillus fumigatus*. J. Bacteriol. 183(7), 2273-2279.

Beauvais, A., Fontaine, T., Aimanianda, V., Latge, J.P., 2014. *Aspergillus* cell wall and biofilm. Mycopathologia 178(5-6), 371-377.

Beauvais, A., Maubon, D., Park, S., Morelle, W., Tanguy, M., Huerre, M., Perlin, D.S., Latge, J.P., 2005. Two α(1-3) glucan synthases with different functions in *Aspergillus fumigatus*. Appl. Environ. Microbiol. 71(3), 1531-1538.

Becker, J.M., Covert, N.L., Shenbagamurthi, P., Steinfeld, A.S., Naider, F., 1983. Polyoxin D inhibits growth of zoopathogenic fungi. Antimicrob. Agents Chemother. 23(6), 926-929.

Ben-Ami, R., Kontoyiannis, D.P., 2012. Resistance to echinocandins comes at a cost: the impact of *FKS1* hotspot mutations on *Candida albicans* fitness and virulence. Virulence 3(1), 95-97.

Bitar, D., Lortholary, O., Le Strat, Y., Nicolau, J., Coignard, B., Tattevin, P., Che, D., Dromer, F., 2014. Population-based analysis of invasive fungal infections, France, 2001-2010. Emerg. Infect. Dis. 20(7), 1149-1155.

Bongomin, F., Gago, S., Oladele, R.O., Denning, D.W., 2017. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. J. Fungi (Basel) 3(4).

Bowen, A.R., Chen-Wu, J.L., Momany, M., Young, R., Szaniszlo, P.J., Robbins, P.W., 1992. Classification of fungal chitin synthases. Proc. Natl. Acad. Sci. U. S. A. 89(2), 519-523.

Bowman, S.M., Free, S.J., 2006. The structure and synthesis of the fungal cell wall. Bioessays 28(8), 799-808.

Bratton, E.W., El Husseini, N., Chastain, C.A., Lee, M.S., Poole, C., Sturmer, T., Juliano, J.J., Weber, D.J., Perfect, J.R., 2012. Comparison and temporal trends of three groups with cryptococcosis: HIV-infected, solid organ transplant, and HIV-negative/non-transplant. PLoS One 7(8), e43582.

Brown, G.D., Denning, D.W., Gow, N.A., Levitz, S.M., Netea, M.G., White, T.C., 2012a. Hidden killers: human fungal infections. Sci .Transl. Med. 4(165), 165rv113.

Brown, G.D., Denning, D.W., Levitz, S.M., 2012b. Tackling human fungal infections. Science 336(6082), 647.

Brown, J.L., Bussey, H., 1993. The yeast *KRE9* gene encodes an O glycoprotein involved in cell surface β-glucan assembly. Mol. Cell. Biol. 13(10), 6346-6356.

Bryan, R.A., Jiang, Z., Howell, R.C., Morgenstern, A., Bruchertseifer, F., Casadevall, A., Dadachova, E., 2010. Radioimmunotherapy is more effective than antifungal treatment in experimental cryptococcal infection. J. Infect. Dis. 202(4), 633-637.

Bulik, D.A., Olczak, M., Lucero, H.A., Osmond, B.C., Robbins, P.W., Specht, C.A., 2003. Chitin synthesis in *Saccharomyces cerevisiae* in response to supplementation of growth medium with glucosamine and cell wall stress. Eukaryot. Cell 2(5), 886-900.

Bulmer, G.S., Sans, M.D., Gunn, C.M., 1967. *Cryptococcus neoformans.* I. Nonencapsulated mutants. J. Bacteriol. 94(5), 1475-1479.

Bush, D.A., Horisberger, M., Horman, I., Wursch, P., 1974. The wall structure of *Schizosaccharomyces pombe*. J. Gen. Microbiol. 81(1), 199-206.

Butts, A., Krysan, D.J., 2012. Antifungal drug discovery: something old and something new. PLoS Pathog. 8(9), e1002870.

Cabib, E., 1991. Differential inhibition of chitin synthetases 1 and 2 from *Saccharomyces cerevisiae* by polyoxin D and nikkomycins. Antimicrob. Agents Chemother. 35(1), 170-173.

Cabib, E., Arroyo, J., 2013. How carbohydrates sculpt cells: chemical control of morphogenesis in the yeast cell wall. Nat. Rev. Microbiol. 11(9), 648-655.

Cabib, E., Kang, M.S., 1987. Fungal 1,3-β-glucan synthase. Methods Enzymol. 138, 637-642.

Cabib, E., Roh, D.H., Schmidt, M., Crotti, L.B., Varma, A., 2001. The yeast cell wall and septum as paradigms of cell growth and morphogenesis. J. Biol. Chem. 276(23), 19679-19682.

Cabib, E., Sburlati, A., Bowers, B., Silverman, S.J., 1989. Chitin synthase 1, an auxiliary enzyme for chitin synthesis in *Saccharomyces cerevisiae*. J. Cell Biol. 108(5), 1665-1672.

Cabib, E., Schmidt, M., 2003. Chitin synthase III activity, but not the chitin ring, is required for remedial septa formation in budding yeast. FEMS Microbiol. Lett. 224(2), 299-305.

Cabib, E., Silverman, S.J., Shaw, J.A., 1992. Chitinase and chitin synthase 1: counterbalancing activities in cell separation of *Saccharomyces cerevisiae*. J. Gen. Microbiol. 138(1), 97-102.

Carotti, C., Ferrario, L., Roncero, C., Valdivieso, M.H., Duran, A., Popolo, L., 2002. Maintenance of cell integrity in the *gas1* mutant of *Saccharomyces cerevisiae* requires the Chs3p-targeting and activation pathway and involves an unusual Chs3p localization. Yeast 19(13), 1113-1124.

Cassone, A., Mason, R.E., Kerridge, D., 1981. Lysis of growing yeast-form cells of *Candida albicans* by echinocandin: a cytological study. Sabouraudia 19(2), 97-110.

Castanheira, M., Duncanson, F.P., Diekema, D.J., Guarro, J., Jones, R.N., Pfaller, M.A., 2012. Activities of E1210 and comparator agents tested by CLSI and EUCAST broth microdilution methods against *Fusarium* and *Scedosporium* species identified using molecular methods. Antimicrob. Agents Chemother. 56(1), 352-357.

Castanheira, M., Woosley, L.N., Diekema, D.J., Messer, S.A., Jones, R.N., Pfaller, M.A., 2010. Low prevalence of *fks1* hot spot 1 mutations in a worldwide collection of *Candida* strains. Antimicrob. Agents Chemother. 54(6), 2655-2659.

Castelli, M.V., Cortés, J.C.G., Escalante, A.M., Bah, M., Pereda-Miranda, R., Ribas, J.C., Zacchino, S.A., 2002. In vitro inhibition of 1,3-β-glucan synthase by glycolipids from convolvulaceous species. Planta Med. 68(8), 739-742.

Castro, C., Ribas, J.C., Valdivieso, M.H., Varona, R., del Rey, F., Durán, A., 1995. Papulacandin B resistance in budding and fission yeasts: isolation and characterization of a gene involved in (1,3)β-D-glucan synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. 177(20), 5732-5739.

Cateau, E., Levasseur, P., Borgonovi, M., Imbert, C., 2007. The effect of aminocandin (HMR 3270) on the in-vitro adherence of *Candida albicans* to polystyrene surfaces coated with extracellular matrix proteins or fibronectin. Clin. Microbiol. Infect. 13(3), 311-315.

Coelho, C., Casadevall, A., 2016. Cryptococcal therapies and drug targets: the old, the new and the promising. Cell. Microbiol. 18(6), 792-799.

Cordeiro Rde, A., Macedo Rde, B., Teixeira, C.E., Marques, F.J., Bandeira Tde, J., Moreira, J.L., Brilhante, R.S., Rocha, M.F., Sidrim, J.J., 2014. The calcineurin inhibitor cyclosporin A exhibits synergism with antifungals against *Candida parapsilosis* species complex. J. Med. Microbiol. 63(Pt 7), 936-944.

Cortés, J.C.G., Carnero, E., Ishiguro, J., Sánchez, Y., Durán, A., Ribas, J.C., 2005. The novel fission yeast (1,3)β-D-glucan synthase catalytic subunit Bgs4p is essential during both cytokinesis and polarized growth. J. Cell Sci. 118(Pt 1), 157-174.

Cortés, J.C.G., Ishiguro, J., Durán, A., Ribas, J.C., 2002. Localization of the (1,3)β-D-glucan synthase catalytic subunit homologue Bgs1p/Cps1p from fission yeast suggests that it is involved in septation, polarized growth, mating, spore wall formation and spore germination. J. Cell Sci. 115(Pt 21), 4081-4096.

Cortés, J.C.G., Konomi, M., Martins, I.M., Muñoz, J., Moreno, M.B., Osumi, M., Durán, A., Ribas, J.C., 2007. The (1,3)β-D-glucan synthase subunit Bgs1p is responsible for the fission yeast primary septum formation. Mol. Microbiol. 65(1), 201-217.

Cortés, J.C.G., Ramos, M., Osumi, M., Pérez, P., Ribas, J.C., 2016. The Cell Biology of Fission Yeast Septation. Microbiol. Mol. Biol. Rev. 80(3), 779-791.

Cortés, J.C.G., Sato, M., Muñoz, J., Moreno, M.B., Clemente-Ramos, J.A., Ramos, M., Okada, H., Osumi, M., Durán, A., Ribas, J.C., 2012. Fission yeast Ags1 confers the essential septum strength needed for safe gradual cell abscission. J. Cell Biol. 198(4), 637-656.

Cowen, L.E., Lindquist, S., 2005. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. Science 309(5744), 2185-2189.

Cowen, L.E., Sanglard, D., Howard, S.J., Rogers, P.D., Perlin, D.S., 2014. Mechanisms of Antifungal Drug Resistance. Cold Spring Harb. Perspect. Med. 5(7), a019752.

Cruz, M.C., Goldstein, A.L., Blankenship, J.R., Del Poeta, M., Davis, D., Cardenas, M.E., Perfect, J.R., McCusker, J.H., Heitman, J., 2002. Calcineurin is essential for survival during membrane stress in *Candida albicans*. EMBO J. 21(4), 546-559.

Chaffin, W.L., 2008. *Candida albicans* cell wall proteins. Microbiol. Mol. Biol. Rev. 72(3), 495-544.

Chakrabarti, A., Sood, P., Rudramurthy, S.M., Chen, S., Kaur, H., Capoor, M., Chhina, D., Rao, R., Eshwara, V.K., Xess, I., Kindo, A.J., Umabala, P., Savio, J., Patel, A., Ray, U., Mohan, S., Iyer, R., Chander, J., Arora, A., Sardana, R., Roy, I., Appalaraju, B., Sharma, A., Shetty, A., Khanna, N., Marak, R., Biswas, S., Das, S., Harish, B.N., Joshi, S., Mendiratta, D., 2015. Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Med. 41(2), 285-295.

Chapman, T., Kinsman, O., Houston, J., 1992. Chitin biosynthesis in *Candida albicans* grown in vitro and in vivo and its inhibition by nikkomycin Z. Antimicrob. Agents Chemother. 36(9), 1909-1914.

Chen, X.Y., Kim, J.Y., 2009. Callose synthesis in higher plants. Plant Signal Behav. 4(6), 489-492.

Chowdhary, A., Sharma, C., Meis, J.F., 2017. Azole-Resistant Aspergillosis: Epidemiology, Molecular Mechanisms, and Treatment. J. Infect. Dis. 216(suppl_3), S436-S444.

Dannaoui, E., Desnos-Ollivier, M., García-Hermoso, D., Grenouillet, F., Cassaing, S., Baixench, M.T., Bretagne, S., Dromer, F., Lortholary, O., French Mycoses Study, G., 2012. *Candida* spp. with acquired echinocandin resistance, France, 2004-2010. Emerg. Infect. Dis. 18(1), 86-90.

Debono, M., Gordee, R.S., 1994. Antibiotics that inhibit fungal cell wall development. Annu. Rev. Microbiol. 48, 471-497.

Denning, D.W., 2003. Echinocandin antifungal drugs. Lancet 362(9390), 1142-1151.

Denning, D.W., 2017. Calling upon all public health mycologists. Eur. J. Clin. Microbiol. Infect. Dis. 36(6), 923-924.

Denning, D.W., Bromley, M.J., 2015. Infectious disease. How to bolster the antifungal pipeline. Science 347, 1414–1416.

Díaz, M., Sánchez, Y., Bennett, T., Sun, C.R., Godoy, C., Tamanoi, F., Durán, A., Pérez, P., 1993. The *Schizosaccharomyces pombe cwg2*$^{+}$ gene codes for the β subunit of a geranylgeranyltransferase type I required for β-glucan synthesis. EMBO J. 12(13), 5245-5254.

Dijkgraaf, G.J., Abe, M., Ohya, Y., Bussey, H., 2002. Mutations in Fks1p affect the cell wall content of β-1,3- and β-1,6-glucan in *Saccharomyces cerevisiae*. Yeast 19(8), 671-690.

Douglas, C.M., D'Ippolito, J.A., Shei, G.J., Meinz, M., Onishi, J., Marrinan, J.A., Li, W., Abruzzo, G.K., Flattery, A., Bartizal, K., Mitchell, A., Kurtz, M.B., 1997. Identification of the *FKS1* gene of *Candida albicans* as the essential target of 1,3-β-D-glucan synthase inhibitors. Antimicrob. Agents Chemother. 41(11), 2471-2479.

Douglas, C.M., Foor, F., Marrinan, J.A., Morin, N., Nielsen, J.B., Dahl, A.M., Mazur, P., Baginsky, W., Li, W., El-Sherbeini, M., Clemas, J.A., Mandala, S.M., Frommer, B.R., Kurtz, M.B., 1994a. The *Saccharomyces cerevisiae FKS1 (ETG1)* gene encodes an integral membrane protein which is a subunit of 1,3-β-D-glucan synthase. Proc. Natl. Acad. Sci. U. S. A. 91(26), 12907-12911.

Douglas, C.M., Marrinan, J.A., Li, W., Kurtz, M.B., 1994b. A *Saccharomyces cerevisiae* mutant with echinocandin-resistant 1,3-β–D-glucan synthase. J. Bacteriol. 176(18), 5686-5696.

Drgonova, J., Drgon, T., Tanaka, K., Kollar, R., Chen, G.C., Ford, R.A., Chan, C.S., Takai, Y., Cabib, E., 1996. Rho1p, a yeast protein at the interface between cell polarization and morphogenesis. Science 272, 277-279.

Ecker, M., Deutzmann, R., Lehle, L., Mrsa, V., Tanner, W., 2006. Pir proteins of *Saccharomyces cerevisiae* are attached to β-1,3-glucan by a new protein-carbohydrate linkage. J. Biol. Chem. 281(17), 11523-11529.

Editorial, 2017. Stop neglecting fungi. Nat. Microbiol. 2, 17120.

Edwards, J.A., Alore, E.A., Rappleye, C.A., 2011. The yeast-phase virulence requirement for α-glucan synthase differs among *Histoplasma capsulatum* chemotypes. Eukaryot. Cell 10(1), 87-97.

El-Sherbeini, M., Clemas, J.A., 1995. Nikkomycin Z supersensitivity of an echinocandin-resistant mutant of *Saccharomyces cerevisiae*. Antimicrob. Agents Chemother. 39(1), 200-207.

Emri, T., Majoros, L., Toth, V., Pocsi, I., 2013. Echinocandins: production and applications. Appl. Microbiol. Biotechnol. 97(8), 3267-3284.

Erwig, L.P., Gow, N.A., 2016. Interactions of fungal pathogens with phagocytes. Nat. Rev. Microbiol. 14(3), 163-176.

Farhour, Z., Mehraj, V., Chen, J., Ramendra, R., Lu, H., Routy, J.P., 2018. Use of (1-->3)-β-d-glucan for diagnosis and management of invasive mycoses in HIV-infected patients. Mycoses.

Feldmesser, M., Kress, Y., Mednick, A., Casadevall, A., 2000. The effect of the echinocandin analogue caspofungin on cell wall glucan synthesis by *Cryptococcus neoformans*. J. Infect. Dis. 182(6), 1791-1795.

Fernandes, C., Gow, N.A.R., Gonçalves, T., 2016. The importance of subclasses of chitin synthase enzymes with myosin-like domains for the fitness of fungi. Fungal Biol. Rev. 30(1), 1-14.

Fleischhacker, M., Radecke, C., Schulz, B., Ruhnke, M., 2008. Paradoxical growth effects of the echinocandins caspofungin and micafungin, but not of anidulafungin, on clinical isolates of *Candida albicans* and *C. dubliniensis*. Eur. J. Clin. Microbiol. Infect. Dis. 27(2), 127-131.

Font de Mora, J., Valentín, E., Herrero, E., Sentandreu, R., 1990. Glycoprotein molecules in the walls of *Schizosaccharomyces pombe* wild-type cells and a morphologically altered mutant resistant to papulacandin B. J. Gen. Microbiol. 136(11), 2251-2259.

Fontaine, T., Beauvais, A., Loussert, C., Thevenard, B., Fulgsang, C.C., Ohno, N., Clavaud, C., Prevost, M.C., Latge, J.P., 2010. Cell wall α1-3glucans induce the aggregation of germinating conidia of *Aspergillus fumigatus*. Fungal Genet. Biol. 47(8), 707-712.

Fontaine, T., Simenel, C., Dubreucq, G., Adam, O., Delepierre, M., Lemoine, J., Vorgias, C.E., Diaquin, M., Latge, J.P., 2000. Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. J. Biol. Chem. 275(36), 27594-27607.

Fortwendel, J.R., Juvvadi, P.R., Pinchai, N., Perfect, B.Z., Alspaugh, J.A., Perfect, J.R., Steinbach, W.J., 2009. Differential effects of inhibiting chitin and 1,3-β-D-glucan synthesis in ras and calcineurin mutants of *Aspergillus fumigatus*. Antimicrob. Agents Chemother. 53(2), 476-482.

Free, S.J., 2013. Fungal cell wall organization and biosynthesis. Adv. Genet. 81, 33-82.

Fromtling, R.A., Shadomy, H.J., Jacobson, E.S., 1982. Decreased virulence in stable, acapsular mutants of *Cryptococcus neoformans*. Mycopathologia 79(1), 23-29.

Fu, C., Tanaka, A., Free, S.J., 2014. *Neurospora crassa* 1,3-α-glucan synthase, AGS-1, is required for cell wall biosynthesis during macroconidia development. Microbiology 160(Pt 8), 1618-1627.

Fujie, A., Iwamoto, T., Sato, B., Muramatsu, H., Kasahara, C., Furuta, T., Hori, Y., Hino, M., Hashimoto, S., 2001. FR131535, a novel water-soluble echinocandin-like lipopeptide: synthesis and biological properties. Bioorg. Med. Chem. Lett. 11(3), 399-402.

GAFFI, Fungal Disease Frequency. Global Action Fund For Fungal Infections https://www.gaffi.org/why/fungal-disease-frequency/ (Last accessed on January 15, 2019).

GAFFI, 2015. Appendix 3: Burden of Fungal Diseases. Global Action Fund For Fungal Infections https://www.gaffi.org/wp-content/uploads/Appendices-3-V3.pdf (Last accessed on January 15, 2019).

García-Rodríguez, L.J., Trilla, J.A., Castro, C., Valdivieso, M.H., Durán, A., Roncero, C., 2000. Characterization of the chitin biosynthesis process as a compensatory mechanism in the *fks1* mutant of *Saccharomyces cerevisiae*. FEBS Lett. 478(1-2), 84-88.

García, I., Tajadura, V., Martín, V., Toda, T., Sánchez, Y., 2006. Synthesis of α-glucans in fission yeast spores is carried out by three α-glucan synthase paralogues, Mok12p, Mok13p and Mok14p. Mol. Microbiol. 59(3), 836-853.

Garrett-Engele, P., Moilanen, B., Cyert, M.S., 1995. Calcineurin, the Ca^{2+}/calmodulin-dependent protein phosphatase, is essential in yeast mutants with cell integrity defects and in mutants that lack a functional vacuolar H^{+}-ATPase. Mol. Cell. Biol. 15(8), 4103-4114.

Gastebois, A., Clavaud, C., Aimanianda, V., Latge, J.P., 2009. *Aspergillus fumigatus*: cell wall polysaccharides, their biosynthesis and organization. Future Microbiol. 4(5), 583-595.

Geddes-McAlister, J., Shapiro, R.S., 2018. New pathogens, new tricks: emerging, drug-resistant fungal pathogens and future prospects for antifungal therapeutics. Ann. N. Y. Acad. Sci.

Geoghegan, I., Steinberg, G., Gurr, S., 2017. The Role of the Fungal Cell Wall in the Infection of Plants. Trends Microbiol. 25(12), 957-967.

Georgopapadakou, N.H., Tkacz, J.S., 1995. The fungal cell wall as a drug target. Trends Microbiol. 3(3), 98-104.

Gilbert, N.M., Donlin, M.J., Gerik, K.J., Specht, C.A., Djordjevic, J.T., Wilson, C.F., Sorrell, T.C., Lodge, J.K., 2010. *KRE* genes are required for β-1,6-glucan synthesis, maintenance of capsule architecture and cell wall protein anchoring in *Cryptococcus neoformans*. Mol. Microbiol. 76(2), 517-534.

González-Lara, M.F., Sifuentes-Osornio, J., Ostrosky-Zeichner, L., 2017. Drugs in Clinical Development for Fungal Infections. Drugs 77(14), 1505-1518.

Gow, N.A.R., Latge, J.P., Munro, C.A., 2017. The Fungal Cell Wall: Structure, Biosynthesis, and Function. Microbiol. Spectr. 5(3).

Grun, C.H., Hochstenbach, F., Humbel, B.M., Verkleij, A.J., Sietsma, J.H., Klis, F.M., Kamerling, J.P., Vliegenthart, J.F., 2005. The structure of cell wall α-glucan from fission yeast. Glycobiology 15(3), 245-257.

Guinea, J., Verweij, P.E., Meletiadis, J., Mouton, J.W., Barchiesi, F., Arendrup, M.C., Subcommittee on Antifungal Susceptibility Testing of the Escmid European Committee for Antimicrobial Susceptibility Testing, E.-A., Arendrup, M.C., Arikan-Akdagli, S., Barchiesi, F., Castanheira, M., Chryssanthou, E., Friberg, N., Guinea, J., Jarv, H., Klimko, N., Kurzai, O., Lagrou, K., Lass-Florl, C., Mares, M., Matos, T., Meletiadis, J., Moore, C.B., Mouton, J.W., Muehlethaler, K., Rogers, T.R., Andersen, C.T., Velegraki, A., 2018. How to: EUCAST recommendations on the screening procedure E.Def 10.1 for the detection of azole resistance in *Aspergillus fumigatus* isolates using four well azole-containing agar plates. Clin. Microbiol. Infect.

Hager, C.L., Larkin, E.L., Long, L., Zohra Abidi, F., Shaw, K.J., Ghannoum, M.A., 2018. In Vitro and In Vivo Evaluation of the Antifungal Activity of APX001A/APX001 against *Candida auris*. Antimicrob. Agents Chemother. 62(3).

Hall, D., Bonifas, R., Stapert, L., Thwaites, M., Shinabarger, D.L., Pillar, C.M., 2017. *In vitro* potency and fungicidal activity of CD101, a novel echinocandin, against recent clinical isolates of *Candida* spp. Diagn. Microbiol. Infect. Dis. 89(3), 205-211.

Hata, K., Horii, T., Miyazaki, M., Watanabe, N.A., Okubo, M., Sonoda, J., Nakamoto, K., Tanaka, K., Shirotori, S., Murai, N., Inoue, S., Matsukura, M., Abe, S., Yoshimatsu, K., Asada, M., 2011. Efficacy of oral E1210, a new broad-spectrum antifungal with a novel mechanism of action, in murine models of candidiasis, aspergillosis, and fusariosis. Antimicrob. Agents Chemother. 55(10), 4543-5145.

Hawksworth, D.L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited*. Mycol. Res. 105(12), 1422-1432.

Healey, K.R., Perlin, D.S., 2018. Fungal Resistance to Echinocandins and the MDR Phenomenon in *Candida glabrata*. J. Fungi (Basel) 4(3).

Heasley, B.H., Pacofsky, G.J., Mamai, A., Liu, H., Nelson, K., Coti, G., Peel, M.R., Balkovec, J.M., Greenlee, M.L., Liberator, P., Meng, D., Parker, D.L., Wilkening, R.R., Apgar, J.M., Racine, F., Hsu, M.J., Giacobbe, R.A., Kahn, J.N., 2012. Synthesis and biological evaluation of antifungal derivatives of enfumafungin as orally bioavailable inhibitors of β-1,3-glucan synthase. Bioorg. Med. Chem. Lett. 22(22), 6811-6816.

Hector, R.F., Bierer, D.E., 2011. New β-glucan inhibitors as antifungal drugs. Expert. Opin. Ther. Pat. 21(10), 1597-1610.

Hector, R.F., Zimmer, B.L., Pappagianis, D., 1990. Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis. Antimicrob. Agents Chemother. 34(4), 587-593.

Heiss, C., Klutts, J.S., Wang, Z., Doering, T.L., Azadi, P., 2009. The structure of *Cryptococcus neoformans* galactoxylomannan contains β-D-glucuronic acid. Carbohydr. Res. 344(7), 915-920.

Henry, C., Latge, J.P., Beauvais, A., 2011. α1,3 glucans are dispensable in *Aspergillus fumigatus*. Eukaryot. Cell 11(1), 26-29.

Herrero, A.B., Magnelli, P., Mansour, M.K., Levitz, S.M., Bussey, H., Abeijon, C., 2004. *KRE5* gene null mutant strains of *Candida albicans* are avirulent and have altered cell wall composition and hypha formation properties. Eukaryot. Cell 3(6), 1423-1432.

Hochstenbach, F., Klis, F.M., van den Ende, H., van Donselaar, E., Peters, P.J., Klausner, R.D., 1998. Identification of a putative α-glucan synthase essential for cell

wall construction and morphogenesis in fission yeast. Proc. Natl. Acad. Sci. U. S. A. 95(16), 9161-9166.

Hogan, L.H., Klein, B.S., 1994. Altered expression of surface α-1,3-glucan in genetically related strains of *Blastomyces dermatitidis* that differ in virulence. Infect. Immun. 62(8), 3543-3546.

Hong, Z., Delauney, A.J., Verma, D.P., 2001. A cell plate-specific callose synthase and its interaction with phragmoplastin. Plant Cell 13(4), 755-768.

Hopke, A., Brown, A.J.P., Hall, R.A., Wheeler, R.T., 2018. Dynamic Fungal Cell Wall Architecture in Stress Adaptation and Immune Evasion. Trends Microbiol. 26(4), 284-295.

Hori, Y., Shibuya, K., 2018. Role of *FKS* Gene in the Susceptibility of Pathogenic Fungi to Echinocandins. Med. Mycol. J. 59(2), E31-E40.

Horisberger, M., Vonlanthen, M., Rosset, J., 1978. Localization of α-galactomannan and of wheat germ agglutinin receptors in *Schizosaccharomyces pombe*. Arch. Microbiol. 119(2), 107-111.

Humbel, B.M., Konomi, M., Takagi, T., Kamasawa, N., Ishijima, S.A., Osumi, M., 2001. In situ localization of β-glucans in the cell wall of *Schizosaccharomyces pombe*. Yeast 18(5), 433-444.

Huttel, W., 2017. Structural diversity in echinocandin biosynthesis: the impact of oxidation steps and approaches toward an evolutionary explanation. Z. Naturforsch C. 72(1-2), 1-20.

Ibrahim, A.S., Luo, G., Gebremariam, T., Lee, H., Schmidt, C.S., Hennessey, J.P., Jr., French, S.W., Yeaman, M.R., Filler, S.G., Edwards, J.E., Jr., 2013. NDV-3 protects mice from vulvovaginal candidiasis through T- and B-cell immune response. Vaccine 31(47), 5549-5556.

Inoue, S.B., Qadota, H., Arisawa, M., Watanabe, T., Ohya, Y., 1999. Prenylation of Rho1p is required for activation of yeast 1, 3-β-glucan synthase. J. Biol. Chem. 274(53), 38119-38124.

Inoue, S.B., Takewaki, N., Takasuka, T., Mio, T., Adachi, M., Fujii, Y., Miyamoto, C., Arisawa, M., Furuichi, Y., Watanabe, T., 1995. Characterization and gene cloning of 1,3-β-D-glucan synthase from *Saccharomyces cerevisiae*. Eur. J. Biochem. 231(3), 845-854.

Iorio, E., Torosantucci, A., Bromuro, C., Chiani, P., Ferretti, A., Giannini, M., Cassone, A., Podo, F., 2008. *Candida albicans* cell wall comprises a branched β-D-(1-->6)-glucan with β-D-(1-->3)-side chains. Carbohydr Res 343(6), 1050-1061.

Ishiguro, J., Saitou, A., Durán, A., Ribas, J.C., 1997. *cps1*$^{+}$, a *Schizosaccharomyces pombe* gene homolog of *Saccharomyces cerevisiae FKS* genes whose mutation confers hypersensitivity to cyclosporin A and papulacandin B. J. Bacteriol. 179(24), 7653-7662.

Ishihara, S., Hirata, A., Nogami, S., Beauvais, A., Latge, J.P., Ohya, Y., 2007. Homologous subunits of 1,3-β-glucan synthase are important for spore wall assembly in *Saccharomyces cerevisiae*. Eukaryot. Cell 6(2), 143-156.

Iwai, H., Masaoka, N., Ishii, T., Satoh, S., 2002. A pectin glucuronyltransferase gene is essential for intercellular attachment in the plant meristem. Proc. Natl. Acad. Sci. U. S. A. 99(25), 16319-16324.

Jacobson, E.S., Ayers, D.J., Harrell, A.C., Nicholas, C.C., 1982. Genetic and phenotypic characterization of capsule mutants of *Cryptococcus neoformans*. J. Bacteriol. 150(3), 1292-1296.

James, P.G., Cherniak, R., Jones, R.G., Stortz, C.A., Reiss, E., 1990. Cell-wall glucans of *Cryptococcus neoformans* Cap 67. Carbohydr. Res. 198(1), 23-38.

Jiménez-Ortigosa, C., Paderu, P., Motyl, M.R., Perlin, D.S., 2014. Enfumafungin derivative MK-3118 shows increased in vitro potency against clinical echinocandin-resistant *Candida* Species and *Aspergillus* species isolates. Antimicrob. Agents Chemother. 58(2), 1248-1251.

Johnson, M.E., Edlind, T.D., 2012. Topological and mutational analysis of *Saccharomyces cerevisiae* Fks1. Eukaryot. Cell 11(7), 952-960.

Kang, M.S., Cabib, E., 1986. Regulation of fungal cell wall growth: a guanine nucleotide-binding proteinaceous component required for activity of (1,3)-β-D-glucan synthase. Proc. Natl. Acad. Sci. U. S. A. 83(16), 5808-5812.

Kang, X., Kirui, A., Muszynski, A., Widanage, M.C.D., Chen, A., Azadi, P., Wang, P., Mentink-Vigier, F., Wang, T., 2018. Molecular architecture of fungal cell walls revealed by solid-state NMR. Nat. Commun. 9(1), 2747.

Kapteyn, J.C., Montijn, R.C., Dijkgraaf, G.J., Van den Ende, H., Klis, F.M., 1995. Covalent association of β-1,3-glucan with β-1,6-glucosylated mannoproteins in cell walls of *Candida albicans*. J. Bacteriol. 177(13), 3788-3792.

Kapteyn, J.C., Montijn, R.C., Vink, E., de la Cruz, J., Llobell, A., Douwes, J.E., Shimoi, H., Lipke, P.N., Klis, F.M., 1996. Retention of *Saccharomyces cerevisiae* cell wall proteins through a phosphodiester-linked β-1,3-/β-1,6-glucan heteropolymer. Glycobiology 6(3), 337-345.

Kapteyn, J.C., Ram, A.F., Groos, E.M., Kollar, R., Montijn, R.C., Van Den Ende, H., Llobell, A., Cabib, E., Klis, F.M., 1997. Altered extent of cross-linking of β1,6-glucosylated mannoproteins to chitin in *Saccharomyces cerevisiae* mutants with reduced cell wall β1,3-glucan content. J. Bacteriol. 179(20), 6279-6284.

Kapteyn, J.C., Van Den Ende, H., Klis, F.M., 1999a. The contribution of cell wall proteins to the organization of the yeast cell wall. Biochim. Biophys. Acta 1426(2), 373-383.

Kapteyn, J.C., Van Egmond, P., Sievi, E., Van Den Ende, H., Makarow, M., Klis, F.M., 1999b. The contribution of the O-glycosylated protein Pir2p/Hsp150 to the construction of the yeast cell wall in wild-type cells and β1,6-glucan-deficient mutants. Mol. Microbiol. 31(6), 1835-1844.

Kasahara, S., Ben Inoue, S., Mio, T., Yamada, T., Nakajima, T., Ichishima, E., Furuichi, Y., Yamada, H., 1994a. Involvement of cell wall β-glucan in the action of HM-1 killer toxin. FEBS Lett. 348(1), 27-32.

Kasahara, S., Yamada, H., Mio, T., Shiratori, Y., Miyamoto, C., Yabe, T., Nakajima, T., Ichishima, E., Furuichi, Y., 1994b. Cloning of the *Saccharomyces cerevisiae* gene whose overexpression overcomes the effects of HM-1 killer toxin, which inhibits β-glucan synthesis. J. Bacteriol. 176(5), 1488-1499.

Katayama, S., Hirata, D., Arellano, M., Pérez, P., Toda, T., 1999. Fission yeast α-glucan synthase Mok1 requires the actin cytoskeleton to localize the sites of growth and plays an essential role in cell morphogenesis downstream of protein kinase C function. J. Cell Biol. 144(6), 1173-1186.

Kathiravan, M.K., Salake, A.B., Chothe, A.S., Dudhe, P.B., Watode, R.P., Mukta, M.S., Gadhwe, S., 2012. The biology and chemistry of antifungal agents: a review. Bioorg. Med. Chem. 20(19), 5678-5698.

Katiyar, S., Pfaller, M., Edlind, T., 2006. *Candida albicans* and *Candida glabrata* clinical isolates exhibiting reduced echinocandin susceptibility. Antimicrob. Agents Chemother. 50(8), 2892-2894.

Kelly, R., Register, E., Hsu, M.J., Kurtz, M., Nielsen, J., 1996. Isolation of a gene involved in 1,3-β-glucan synthesis in *Aspergillus nidulans* and purification of the corresponding protein. J. Bacteriol. 178(15), 4381-4391.

Kitajima, Y., 2001. [Ultrastructure and molecular biochemistry on pathogenic fungal cells: the architecture of septal cell walls of dermatophytes]. Nihon Ishinkin Gakkai Zasshi 42(2), 55-59.

Kitamura, A., Higuchi, S., Hata, M., Kawakami, K., Yoshida, K., Namba, K., Nakajima, R., 2009a. Effect of β-1,6-glucan inhibitors on the invasion process of *Candida albicans*: potential mechanism of their in vivo efficacy. Antimicrob. Agents Chemother. 53(9), 3963-3971.

Kitamura, A., Someya, K., Hata, M., Nakajima, R., Takemura, M., 2009b. Discovery of a small-molecule inhibitor of β-1,6-glucan synthesis. Antimicrob. Agents Chemother. 53(2), 670-677.

Kitamura, A., Someya, K., Okumura, R., Hata, M., Takeshita, H., Nakajima, R., 2010. In vitro antifungal activities of D11-2040, a β-1,6-glucan inhibitor, with or without currently available antifungal drugs. Biol. Pharm. Bull. 33(2), 192-197.

Klimpel, K.R., Goldman, W.E., 1988. Cell walls from avirulent variants of *Histoplasma capsulatum* lack α-(1,3)-glucan. Infect. Immun. 56(11), 2997-3000.

Klis, F.M., Brul, S., De Groot, P.W., 2010. Covalently linked wall proteins in ascomycetous fungi. Yeast 27(8), 489-493.

Klis, F.M., Caro, L.H., Vossen, J.H., Kapteyn, J.C., Ram, A.F., Montijn, R.C., Van Berkel, M.A., Van den Ende, H., 1997. Identification and characterization of a major building block in the cell wall of *Saccharomyces cerevisiae*. Biochem. Soc. Trans. 25(3), 856-860.

Klis, F.M., de Groot, P., Hellingwerf, K., 2001. Molecular organization of the cell wall of *Candida albicans*. Med. Mycol. 39 Suppl 1, 1-8.

Klis, F.M., de Koster, C.G., Brul, S., 2014. Cell wall-related bionumbers and bioestimates of *Saccharomyces cerevisiae* and *Candida albicans*. Eukaryot. Cell 13(1), 2-9.

Klis, F.M., Mol, P., Hellingwerf, K., Brul, S., 2002. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 26(3), 239-256.

Kofla, G., Ruhnke, M., 2011. Pharmacology and metabolism of anidulafungin, caspofungin and micafungin in the treatment of invasive candidosis: review of the literature. Eur. J. Med. Res. 16(4), 159-166.

Kollar, R., Petrakova, E., Ashwell, G., Robbins, P.W., Cabib, E., 1995. Architecture of the yeast cell wall. The linkage between chitin and β(1-3)-glucan. J. Biol. Chem. 270(3), 1170-1178.

Kollar, R., Reinhold, B.B., Petráková, E., Yeh, H.J., Ashwell, G., Drgonová, J., Kapteyn, J.C., Klis, F.M., Cabib, E., 1997. Architecture of the yeast cell wall. β(1-6)-glucan interconnects mannoprotein, β(1-3)-glucan, and chitin. J. Biol. Chem. 272(28), 17762-17775.

Kondoh, O., Inagaki, Y., Fukuda, H., Mizuguchi, E., Ohya, Y., Arisawa, M., Shimma, N., Aoki, Y., Sakaitani, M., Watanabe, T., 2005. Piperazine propanol derivative as a novel antifungal targeting 1,3-β-D-glucan synthase. Biol. Pharm. Bull. 28(11), 2138-2141.

Kondoh, O., Takasuka, T., Arisawa, M., Aoki, Y., Watanabe, T., 2002. Differential sensitivity between Fks1p and Fks2p against a novel β -1,3-glucan synthase inhibitor, aerothricin3. J. Biol. Chem. 277(44), 41744-41749.

Kopecka, M., Kreger, D.R., 1986. Assembly of microfibrils *in vivo* and *in vitro* from (1-3)-β-D-glucan synthesized by protoplasts of *Saccharomyces cerevisiae*. Arch. Microbiol. 143(4), 387-395.

Krishnan, B.R., James, K.D., Polowy, K., Bryant, B.J., Vaidya, A., Smith, S., Laudeman, C.P., 2017. CD101, a novel echinocandin with exceptional stability properties and enhanced aqueous solubility. J. Antibiot. (Tokyo) 70(2), 130-135.

Kumaresan, P.R., Manuri, P.R., Albert, N.D., Maiti, S., Singh, H., Mi, T., Roszik, J., Rabinovich, B., Olivares, S., Krishnamurthy, J., Zhang, L., Najjar, A.M., Huls, M.H., Lee, D.A., Champlin, R.E., Kontoyiannis, D.P., Cooper, L.J., 2014. Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection. Proc. Natl. Acad. Sci. U. S. A. 111(29), 10660-10665.

Lambou, K., Perkhofer, S., Fontaine, T., Latge, J.P., 2010. Comparative functional analysis of the OCH1 mannosyltransferase families in *Aspergillus fumigatus* and *Saccharomyces cerevisiae*. Yeast 27(8), 625-636.

Lamoth, F., Alexander, B.D., 2015. Antifungal activities of SCY-078 (MK-3118) and standard antifungal agents against clinical non-*Aspergillus* mold isolates. Antimicrob. Agents Chemother. 59(7), 4308-4311.

Latge, J.P., 2007. The cell wall: a carbohydrate armour for the fungal cell. Mol. Microbiol. 66(2), 279-290.

Latge, J.P., Beauvais, A., 2014. Functional duality of the cell wall. Curr. Opin. Microbiol. 20, 111-117.

Le Goff, X., Woollard, A., Simanis, V., 1999. Analysis of the *cps1* gene provides evidence for a septation checkpoint in *Schizosaccharomyces pombe*. Mol. Gen. Genet. 262, 163-172.

Leal, J.A., Prieto, A., Bernabe, M., Hawksworth, D.L., 2010. An assessment of fungal wall heteromannans as a phylogenetically informative character in ascomycetes. FEMS Microbiol. Rev. 34(6), 986-1014.

Lee, K.K., Kubo, K., Abdelaziz, J.A., Cunningham, I., de Silva Dantas, A., Chen, X., Okada, H., Ohya, Y., Gow, N.A.R., 2018. Yeast species-specific, differential inhibition of β-1,3-glucan synthesis by poacic acid and caspofungin. The Cell Surface 3, 12-25.

Lee, K.K., Maccallum, D.M., Jacobsen, M.D., Walker, L.A., Odds, F.C., Gow, N.A., Munro, C.A., 2012. Elevated cell wall chitin in *Candida albicans* confers echinocandin resistance *in vivo*. Antimicrob. Agents Chemother. 56(1), 208-217.

Lee, Y., Lee, K.T., Lee, S.J., Beom, J.Y., Hwangbo, A., Jung, J.A., Song, M.C., Yoo, Y.J., Kang, S.H., Averette, A.F., Heitman, J., Yoon, Y.J., Cheong, E., Bahn, Y.S., 2018. *In Vitro* and *In Vivo* Assessment of FK506 Analogs as Novel Antifungal Drug Candidates. Antimicrob. Agents Chemother. 62(11).

Leidich, S.D., Kostova, Z., Latek, R.R., Costello, L.C., Drapp, D.A., Gray, W., Fassler, J.S., Orlean, P., 1995. Temperature-sensitive yeast GPI anchoring mutants *gpi2* and *gpi3* are defective in the synthesis of N-acetylglucosaminyl phosphatidylinositol. Cloning of the *GPI2* gene. J. Biol. Chem. 270(22), 13029-13035.

Lenardon, M.D., Munro, C.A., Gow, N.A., 2010. Chitin synthesis and fungal pathogenesis. Curr. Opin. Microbiol. 13(4), 416-423.

Lepak, A.J., Marchillo, K., Andes, D.R., 2015. Pharmacodynamic target evaluation of a novel oral glucan synthase inhibitor, SCY-078 (MK-3118), using an in vivo murine invasive candidiasis model. Antimicrob. Agents Chemother. 59(2), 1265-1272.

Lesage, G., Bussey, H., 2006. Cell wall assembly in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. 70(2), 317-343.

Lesage, G., Sdicu, A.M., Menard, P., Shapiro, J., Hussein, S., Bussey, H., 2004. Analysis of β-1,3-glucan assembly in *Saccharomyces cerevisiae* using a synthetic interaction network and altered sensitivity to caspofungin. Genetics 167(1), 35-49.

Levin, D.E., 2011. Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. Genetics 189(4), 1145-1175.

Lewis, J.S., 2nd, Wiederhold, N.P., Wickes, B.L., Patterson, T.F., Jorgensen, J.H., 2013. Rapid emergence of echinocandin resistance in *Candida glabrata* resulting in clinical and microbiologic failure. Antimicrob. Agents Chemother. 57(9), 4559-4561.

LIFE, 2017. Leading International Fungal Education: The burden of fungal disease http://www.life-worldwide.org/media-centre/article/the-burden-of-fungal-disease-new-evidence-to-show-the-scale-of-the-problem (Last accessed on January 15, 2019).

Lionakis, M.S., Levitz, S.M., 2018. Host Control of Fungal Infections: Lessons from Basic Studies and Human Cohorts. Annu. Rev. Immunol. 36, 157-191.

Lipke, P.N., 2018. What We Do Not Know about Fungal Cell Adhesion Molecules. J. Fungi (Basel) 4(2).

Lipke, P.N., Ovalle, R., 1998. Cell wall architecture in yeast: new structure and new challenges. J. Bacteriol. 180(15), 3735-3740.

Liu, J., Tang, X., Wang, H., Balasubramanian, M., 2000. Bgs2p, a 1,3-β-glucan synthase subunit, is essential for maturation of ascospore wall in *Schizosaccharomyces pombe*. FEBS Lett. 478(1-2), 105-108.

Liu, J., Tang, X., Wang, H., Oliferenko, S., Balasubramanian, M.K., 2002. The localization of the integral membrane protein Cps1p to the cell division site is dependent on the actomyosin ring and the septation-inducing network in *Schizosaccharomyces pombe*. Mol. Biol. Cell 13(3), 989-1000.

Liu, J., Wang, H., McCollum, D., Balasubramanian, M.K., 1999. Drc1p/Cps1p, a 1,3-β-glucan synthase subunit, is essential for division septum assembly in *Schizosaccharomyces pombe*. Genetics 153(3), 1193-1203.

Locke, J.B., Almaguer, A.L., Donatelli, J.L., Bartizal, K.F., 2018. Time-Kill Kinetics of Rezafungin (CD101) in Vagina-Simulative Medium for Fluconazole-Susceptible and Fluconazole-Resistant *Candida albicans* and Non-*albicans Candida* Species. Infect. Dis. Obstet. Gynecol. 2018, 7040498.

Locke, J.B., Almaguer, A.L., Zuill, D.E., Bartizal, K., 2016. Characterization of In Vitro Resistance Development to the Novel Echinocandin CD101 in *Candida* Species. Antimicrob. Agents Chemother. 60(10), 6100-6107.

López, S.N., Castelli, M.V., Zacchino, S.A., Domínguez, J.N., Lobo, G., Charris-Charris, J., Cortés, J.C.G., Ribas, J.C., Devia, C., Rodríguez, A.M., Enriz, R.D., 2001. In vitro antifungal evaluation and structure-activity relationships of a new series of chalcone derivatives and synthetic analogues, with inhibitory properties against polymers of the fungal cell wall. Bioorg. Med. Chem. 9(8), 1999-2013.

Lortholary, O., Renaudat, C., Sitbon, K., Madec, Y., Denoeud-Ndam, L., Wolff, M., Fontanet, A., Bretagne, S., Dromer, F., French Mycosis Study, G., 2014. Worrisome trends in incidence and mortality of candidemia in intensive care units (Paris area, 2002-2010). Intensive Care Med. 40(9), 1303-1312.

Lu, C.F., Montijn, R.C., Brown, J.L., Klis, F., Kurjan, J., Bussey, H., Lipke, P.N., 1995. Glycosyl phosphatidylinositol-dependent cross-linking of α-agglutinin and β1,6-glucan in the *Saccharomyces cerevisiae* cell wall. J. Cell Biol. 128(3), 333-340.

Lussier, M., Sdicu, A.M., Shahinian, S., Bussey, H., 1998. The *Candida albicans KRE9* gene is required for cell wall β-1,6-glucan synthesis and is essential for growth on glucose. Proc. Natl. Acad. Sci. U. S. A. 95(17), 9825-9830.

Ma, C.M., Abe, T., Komiyama, T., Wang, W., Hattori, M., Daneshtalab, M., 2010. Synthesis, anti-fungal and 1,3-β-D-glucan synthase inhibitory activities of caffeic and quinic acid derivatives. Bioorg. Med. Chem. 18(19), 7009-7014.

Magnelli, P.E., Cipollo, J.F., Robbins, P.W., 2005. A glucanase-driven fractionation allows redefinition of *Schizosaccharomyces pombe* cell wall composition and structure: assignment of diglucan. Anal. Biochem. 336(2), 202-212.

Maligie, M.A., Selitrennikoff, C.P., 2005. *Cryptococcus neoformans* resistance to echinocandins: (1,3)β-glucan synthase activity is sensitive to echinocandins. Antimicrob. Agents Chemother. 49(7), 2851-2856.

Mann, P.A., McLellan, C.A., Koseoglu, S., Si, Q., Kuzmin, E., Flattery, A., Harris, G., Sher, X., Murgolo, N., Wang, H., Devito, K., de Pedro, N., Genilloud, O., Kahn, J.N., Jiang, B., Costanzo, M., Boone, C., Garlisi, C.G., Lindquist, S., Roemer, T., 2015. Chemical Genomics-Based Antifungal Drug Discovery: Targeting Glycosylphosphatidylinositol (GPI) Precursor Biosynthesis. ACS Infect. Dis. 1(1), 59-72.

Manners, D.J., Masson, A.J., Patterson, J.C., 1973a. The structure of a β-(1-3)-D-glucan from yeast cell walls. Biochem. J. 135(1), 19-30.

Manners, D.J., Masson, A.J., Patterson, J.C., Bjorndal, H., Lindberg, B., 1973b. The structure of a β-(1-6)-D-glucan from yeast cell walls. Biochem. J. 135(1), 31-36.

Marion, C.L., Rappleye, C.A., Engle, J.T., Goldman, W.E., 2006. An α-(1,4)-amylase is essential for α-(1,3)-glucan production and virulence in *Histoplasma capsulatum*. Mol. Microbiol. 62(4), 970-983.

Marr, K.A., Schlamm, H.T., Herbrecht, R., Rottinghaus, S.T., Bow, E.J., Cornely, O.A., Heinz, W.J., Jagannatha, S., Koh, L.P., Kontoyiannis, D.P., Lee, D.G., Nucci, M., Pappas, P.G., Slavin, M.A., Queiroz-Telles, F., Selleslag, D., Walsh, T.J., Wingard, J.R., Maertens, J.A., 2015. Combination antifungal therapy for invasive aspergillosis: a randomized trial. Ann. Intern. Med. 162(2), 81-89.

Martín-García, R., Valdivieso, M.H., 2006. The fission yeast Chs2 protein interacts with the type-II myosin Myo3p and is required for the integrity of the actomyosin ring. J. Cell Sci. 119(Pt 13), 2768-2779.

Martín, V., García, B., Carnero, E., Durán, A., Sánchez, Y., 2003. Bgs3p, a putative 1,3-β-glucan synthase subunit, is required for cell wall assembly in *Schizosaccharomyces pombe*. Eukaryot. Cell 2(1), 159-169.

Martín, V., Ribas, J.C., Carnero, E., Durán, A., Sánchez, Y., 2000. *bgs2*$^{+}$, a sporulation-specific glucan synthase homologue is required for proper ascospore wall maturation in fission yeast. Mol. Microbiol. 38(2), 308-321.

Martins, I.M., Cortés, J.C.G., Muñoz, J., Moreno, M.B., Ramos, M., Clemente-Ramos, J.A., Durán, A., Ribas, J.C., 2011. Differential activities of three families of specific β(1,3)glucan synthase inhibitors in wild-type and resistant strains of fission yeast. J. Biol. Chem. 286(5), 3484-3496.

Maubon, D., Park, S., Tanguy, M., Huerre, M., Schmitt, C., Prevost, M.C., Perlin, D.S., Latge, J.P., Beauvais, A., 2006. *AGS3*, an α(1-3)glucan synthase gene family member of *Aspergillus fumigatus*, modulates mycelium growth in the lung of experimentally infected mice. Fungal Genet. Biol. 43(5), 366-375.

Mazur, P., Morin, N., Baginsky, W., El-Sherbeini, M., Clemas, J.A., Nielsen, J.B., Foor, F., 1995. Differential expression and function of two homologous subunits of yeast 1,3-β-D-glucan synthase. Mol. Cell. Biol. 15(10), 5671-5681.

McCarthy, M.W., Kontoyiannis, D.P., Cornely, O.A., Perfect, J.R., Walsh, T.J., 2017. Novel Agents and Drug Targets to Meet the Challenges of Resistant Fungi. J. Infect. Dis. 216(suppl_3), S474-S483.

McLellan, C.A., Whitesell, L., King, O.D., Lancaster, A.K., Mazitschek, R., Lindquist, S., 2012. Inhibiting GPI anchor biosynthesis in fungi stresses the endoplasmic reticulum and enhances immunogenicity. ACS Chem. Biol. 7(9), 1520-1528.

Meaden, P., Hill, K., Wagner, J., Slipetz, D., Sommer, S.S., Bussey, H., 1990. The yeast *KRE5* gene encodes a probable endoplasmic reticulum protein required for (1----6)-β-D-glucan synthesis and normal cell growth. Mol. Cell. Biol. 10(6), 3013-3019.

Meyer, V., Andersen, M.R., Brakhage, A.A., Braus, G.H., Caddick, M.X., Cairns, T.C., de Vries, R.P., Haarmann, T., Hansen, K., Hertz-Fowler, C., Krappmann, S., Mortensen, U.H., Penalva, M.A., Ram, A.F.J., Head, R.M., 2016. Current challenges of research on filamentous fungi in relation to human welfare and a sustainable bio-economy: a white paper. Fungal Biol. Biotechnol. 3, 6.

Mio, T., Adachi-Shimizu, M., Tachibana, Y., Tabuchi, H., Inoue, S.B., Yabe, T., Yamada-Okabe, T., Arisawa, M., Watanabe, T., Yamada-Okabe, H., 1997. Cloning of the *Candida albicans* homolog of Saccharomyces cerevisiae *GSC1*/*FKS1* and its involvement in β-1,3-glucan synthesis. J. Bacteriol. 179(13), 4096-4105.

Miyata, M., Kitamura, J., Miyata, H., 1980. Lysis of growing fission-yeast cells induced by aculeacin A, a new antifungal antibiotic. Arch. Microbiol. 127(1), 11-16.

Miyazaki, M., Horii, T., Hata, K., Watanabe, N.A., Nakamoto, K., Tanaka, K., Shirotori, S., Murai, N., Inoue, S., Matsukura, M., Abe, S., Yoshimatsu, K., Asada, M., 2011. In vitro activity of E1210, a novel antifungal, against clinically important yeasts and molds. Antimicrob. Agents Chemother. 55(10), 4652-4658.

Montijn, R.C., Vink, E., Muller, W.H., Verkleij, A.J., Van Den Ende, H., Henrissat, B., Klis, F.M., 1999. Localization of synthesis of β1,6-glucan in *Saccharomyces cerevisiae*. J. Bacteriol. 181(24), 7414-7420.

Morozov, A.A., Likhoshway, Y.V., 2016. Evolutionary history of the chitin synthases of eukaryotes. Glycobiology 26(6), 635-639.

Mrsa, V., Seidl, T., Gentzsch, M., Tanner, W., 1997. Specific labelling of cell wall proteins by biotinylation. Identification of four covalently linked O-mannosylated proteins of *Saccharomyces cerevisiae*. Yeast 13(12), 1145-1154.

Mrsa, V., Tanner, W., 1999. Role of NaOH-extractable cell wall proteins Ccw5p, Ccw6p, Ccw7p and Ccw8p (members of the Pir protein family) in stability of the *Saccharomyces cerevisiae* cell wall. Yeast 15(10A), 813-820.

Munro, C.A., 2012. Echinocandin resistance in human pathogenic fungi. Expert. Rev. Anti. Infect. Ther. 10(2), 115-116.

Munro, C.A., 2013. Chitin and glucan, the yin and yang of the fungal cell wall, implications for antifungal drug discovery and therapy. Adv. Appl. Microbiol. 83, 145-172.

Munro, C.A., Winter, K., Buchan, A., Henry, K., Becker, J.M., Brown, A.J., Bulawa, C.E., Gow, N.A., 2001. Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis of the septum and for cell integrity. Mol. Microbiol. 39(5), 1414-1426.

Muñoz, J., Cortés, J.C.G., Sipiczki, M., Ramos, M., Clemente-Ramos, J.A., Moreno, M.B., Martins, I.M., Pérez, P., Ribas, J.C., 2013. Extracellular cell wall β(1,3)glucan is required to couple septation to actomyosin ring contraction. J. Cell Biol. 203(2), 265-282.

Mutz, M., Roemer, T., 2016. The GPI anchor pathway: a promising antifungal target? Future Med. Chem. 8(12), 1387-1391.

Nagahashi, S., Lussier, M., Bussey, H., 1998. Isolation of *Candida glabrata* homologs of the *Saccharomyces cerevisiae KRE9* and *KNH1* genes and their involvement in cell wall β-1,6-glucan synthesis. J. Bacteriol. 180(19), 5020-5029.

Nishimura, S., Arita, Y., Honda, M., Iwamoto, K., Matsuyama, A., Shirai, A., Kawasaki, H., Kakeya, H., Kobayashi, T., Matsunaga, S., Yoshida, M., 2010. Marine antifungal theonellamides target 3β-hydroxysterol to activate Rho1 signaling. Nat. Chem. Biol. 6(7), 519-526.

Nix, D.E., Swezey, R.R., Hector, R., Galgiani, J.N., 2009. Pharmacokinetics of nikkomycin Z after single rising oral doses. Antimicrob. Agents Chemother. 53(6), 2517-2521.

Nyazika, T.K., Hagen, F., Machiridza, T., Kutepa, M., Masanganise, F., Hendrickx, M., Boekhout, T., Magombei-Majinjiwa, T., Siziba, N., Chin'ombe, N., Mateveke, K., Meis, J.F., Robertson, V.J., 2016. *Cryptococcus neoformans* population diversity and clinical outcomes of HIV-associated cryptococcal meningitis patients in Zimbabwe. J Med Microbiol 65(11), 1281-1288.

Odds, F.C., Brown, A.J., Gow, N.A., 2003. Antifungal agents: mechanisms of action. Trends Microbiol. 11(6), 272-279.

Ohyama, T., Kurihara, Y., Ono, Y., Ishikawa, T., Miyakoshi, S., Hamano, K., Arai, M., Suzuki, T., Igari, H., Suzuki, Y., Inukai, M., 2000. Arborcandins A, B, C, D, E and F, novel 1,3-β-glucan synthase inhibitors: production and biological activity. J. Antibiot. (Tokyo) 53(10), 1108-1116.

Orlean, P., 2012. Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. Genetics 192(3), 775-818.

Orlean, P.A., 1982. (1,3)-β-D-Glucan synthase from budding and filamentous cultures of the dimorphic fungus *Candida albicans*. Eur. J. Biochem. 127(2), 397-403.

Ostrosky-Zeichner, L., 2012. Invasive Mycoses: Diagnostic Challenges. Am. J. Med. 125(1 Suppl), S14-24.

Osumi, M., 1998. The ultrastructure of yeast: cell wall structure and formation. Micron 29(2-3), 207-233.

Osumi, M., 2012. Visualization of yeast cells by electron microscopy. J Electron Microsc (Tokyo) 61(6), 343-365.

Osumi, M., Yamada, N., Kobori, H., Taki, A., Naito, N., Baba, M., Nagatani, T., 1989. Cell wall formation in regenerating protoplasts of *Schizosaccharomyces pombe*: study by high resolution, low voltage scanning electron microscopy. J. Electron Microsc. (Tokyo) 38(6), 457-468.

Page, N., Gerard-Vincent, M., Menard, P., Beaulieu, M., Azuma, M., Dijkgraaf, G.J., Li, H., Marcoux, J., Nguyen, T., Dowse, T., Sdicu, A.M., Bussey, H., 2003. A *Saccharomyces cerevisiae* genome-wide mutant screen for altered sensitivity to K1 killer toxin. Genetics 163(3), 875-894.

Pappas, P.G., Kauffman, C.A., Andes, D.R., Clancy, C.J., Marr, K.A., Ostrosky-Zeichner, L., Reboli, A.C., Schuster, M.G., Vázquez, J.A., Walsh, T.J., Zaoutis, T.E., Sobel, J.D., 2016a. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin. Infect. Dis. 62(4), e1-50.

Pappas, P.G., Kauffman, C.A., Andes, D.R., Clancy, C.J., Marr, K.A., Ostrosky-Zeichner, L., Reboli, A.C., Schuster, M.G., Vázquez, J.A., Walsh, T.J., Zaoutis, T.E., Sobel, J.D., 2016b. Executive Summary: Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin. Infect. Dis. 62(4), 409-417.

Parent, S.A., Nielsen, J.B., Morin, N., Chrebet, G., Ramadan, N., Dahl, A.M., Hsu, M.J., Bostian, K.A., Foor, F., 1993. Calcineurin-dependent growth of an FK506- and CsA-hypersensitive mutant of *Saccharomyces cerevisiae*. J. Gen. Microbiol. 139(12), 2973-2984.

Park, B.J., Wannemuehler, K.A., Marston, B.J., Govender, N., Pappas, P.G., Chiller, T.M., 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23(4), 525-530.

Park, S., Kelly, R., Kahn, J.N., Robles, J., Hsu, M.J., Register, E., Li, W., Vyas, V., Fan, H., Abruzzo, G., Flattery, A., Gill, C., Chrebet, G., Parent, S.A., Kurtz, M., Teppler, H., Douglas, C.M., Perlin, D.S., 2005. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical *Candida sp.* isolates. Antimicrob. Agents Chemother. 49(8), 3264-3273.

Peláez, F., Cabello, A., Platas, G., Díez, M.T., González del Val, A., Basilio, A., Martán, I., Vicente, F., Bills, G.E., Giacobbe, R.A., Schwartz, R.E., Onish, J.C., Meinz, M.S., Abruzzo, G.K., Flattery, A.M., Kong, L., Kurtz, M.B., 2000. The discovery of enfumafungin, a novel antifungal compound produced by an endophytic *Hormonema* species biological activity and taxonomy of the producing organisms. Syst. Appl. Microbiol. 23(3), 333-343.

Pérez, P., Cortés, J.C.G., Martín-García, R., Ribas, J.C., 2016. Overview of fission yeast septation. Cell. Microbiol. 18(9), 1201-1207.

Pérez, P., Ribas, J.C., 2004. Cell wall analysis. Methods 33(3), 245-251.

Pérez, P., Varona, R., García-Acha, I., Durán, A., 1981. Effect of papulacandin B and aculeacin A on β-(1,3)glucan-synthase from *Geotrichum lactis*. FEBS Lett. 129, 249-252.

Perfect, J.R., 2017. The antifungal pipeline: a reality check. Nat. Rev. Drug Discov. 16(9), 603-616.

Perlin, D.S., 2007. Resistance to echinocandin-class antifungal drugs. Drug Resist. Updat. 10(3), 121-130.

Perlin, D.S., Rautemaa-Richardson, R., Alastruey-Izquierdo, A., 2017. The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect. Dis. 17(12), e383-e392.

Pfaller, M.A., Diekema, D.J., Andes, D., Arendrup, M.C., Brown, S.D., Lockhart, S.R., Motyl, M., Perlin, D.S., 2011a. Clinical breakpoints for the echinocandins and *Candida*

revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. Drug Resist. Updat. 14(3), 164-176.

Pfaller, M.A., Diekema, D.J., Gibbs, D.L., Newell, V.A., Ellis, D., Tullio, V., Rodloff, A., Fu, W., Ling, T.A., Global Antifungal Surveillance, G., 2010. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J. Clin. Microbiol. 48(4), 1366-1377.

Pfaller, M.A., Duncanson, F., Messer, S.A., Moet, G.J., Jones, R.N., Castanheira, M., 2011b. In vitro activity of a novel broad-spectrum antifungal, E1210, tested against *Aspergillus* spp. determined by CLSI and EUCAST broth microdilution methods. Antimicrob. Agents Chemother. 55(11), 5155-5158.

Pfaller, M.A., Hata, K., Jones, R.N., Messer, S.A., Moet, G.J., Castanheira, M., 2011c. In vitro activity of a novel broad-spectrum antifungal, E1210, tested against *Candida* spp. as determined by CLSI broth microdilution method. Diagn. Microbiol. Infect. Dis. 71(2), 167-170.

Pfaller, M.A., Messer, S.A., Motyl, M.R., Jones, R.N., Castanheira, M., 2013a. Activity of MK-3118, a new oral glucan synthase inhibitor, tested against *Candida* spp. by two international methods (CLSI and EUCAST). J. Antimicrob. Chemother. 68(4), 858-863.

Pfaller, M.A., Messer, S.A., Motyl, M.R., Jones, R.N., Castanheira, M., 2013b. 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. 57(2), 1065-1068.

Pfaller, M.A., Messer, S.A., Rhomberg, P.R., Castanheira, M., 2017. Activity of a Long-Acting Echinocandin (CD101) and Seven Comparator Antifungal Agents Tested against a Global Collection of Contemporary Invasive Fungal Isolates in the SENTRY 2014 Antifungal Surveillance Program. Antimicrob. Agents Chemother. 61(3).

Pfaller, M.A., Messer, S.A., Rhomberg, P.R., Jones, R.N., Castanheira, M., 2016. Activity of a long-acting echinocandin, CD101, determined using CLSI and EUCAST reference methods, against *Candida* and *Aspergillus* spp., including echinocandin- and azole-resistant isolates. J. Antimicrob. Chemother. 71(10), 2868-2873.

Piotrowski, J.S., Okada, H., Lu, F., Li, S.C., Hinchman, L., Ranjan, A., Smith, D.L., Higbee, A.J., Ulbrich, A., Coon, J.J., Deshpande, R., Bukhman, Y.V., McIlwain, S., Ong, I.M., Myers, C.L., Boone, C., Landick, R., Ralph, J., Kabbage, M., Ohya, Y.,

2015. Plant-derived antifungal agent poacic acid targets β-1,3-glucan. Proc. Natl. Acad. Sci. U. S. A. 112(12), E1490-1497.

Plaine, A., Walker, L., Da Costa, G., Mora-Montes, H.M., McKinnon, A., Gow, N.A., Gaillardin, C., Munro, C.A., Richard, M.L., 2008. Functional analysis of *Candida albicans* GPI-anchored proteins: roles in cell wall integrity and caspofungin sensitivity. Fungal Genet. Biol. 45(10), 1404-1414.

Polvi, E.J., Averette, A.F., Lee, S.C., Kim, T., Bahn, Y.S., Veri, A.O., Robbins, N., Heitman, J., Cowen, L.E., 2016. Metal Chelation as a Powerful Strategy to Probe Cellular Circuitry Governing Fungal Drug Resistance and Morphogenesis. PLoS Genet. 12(10), e1006350.

Popolo, L., Gilardelli, D., Bonfante, P., Vai, M., 1997. Increase in chitin as an essential response to defects in assembly of cell wall polymers in the *ggp1*Δ mutant of *Saccharomyces cerevisiae*. J. Bacteriol. 179(2), 463-469.

Popolo, L., Gualtieri, T., Ragni, E., 2001. The yeast cell-wall salvage pathway. Med. Mycol. 39 Suppl 1, 111-121.

Ram, A.F., Brekelmans, S.S., Oehlen, L.J., Klis, F.M., 1995. Identification of two cell cycle regulated genes affecting the β1,3-glucan content of cell walls in *Saccharomyces cerevisiae*. FEBS Lett. 358(2), 165-170.

Ram, A.F., Kapteyn, J.C., Montijn, R.C., Caro, L.H., Douwes, J.E., Baginsky, W., Mazur, P., van den Ende, H., Klis, F.M., 1998. Loss of the plasma membrane-bound protein Gas1p in *Saccharomyces cerevisiae* results in the release of β1,3-glucan into the medium and induces a compensation mechanism to ensure cell wall integrity. J. Bacteriol. 180(6), 1418-1424.

Rappleye, C.A., Eissenberg, L.G., Goldman, W.E., 2007. *Histoplasma capsulatum* α-(1,3)-glucan blocks innate immune recognition by the β-glucan receptor. Proc. Natl. Acad. Sci. U. S. A. 104(4), 1366-1370.

Rappleye, C.A., Engle, J.T., Goldman, W.E., 2004. RNA interference in *Histoplasma capsulatum* demonstrates a role for α-(1,3)-glucan in virulence. Mol. Microbiol. 53(1), 153-165.

Rappleye, C.A., Goldman, W.E., 2006. Defining virulence genes in the dimorphic fungi. Annu. Rev. Microbiol. 60, 281-303.

Reese, A.J., Doering, T.L., 2003. Cell wall α-1,3-glucan is required to anchor the *Cryptococcus neoformans* capsule. Mol. Microbiol. 50(4), 1401-1409.

Reese, A.J., Yoneda, A., Breger, J.A., Beauvais, A., Liu, H., Griffith, C.L., Bose, I., Kim, M.J., Skau, C., Yang, S., Sefko, J.A., Osumi, M., Latge, J.P., Mylonakis, E., Doering, T.L., 2007. Loss of cell wall α(1-3) glucan affects *Cryptococcus neoformans* from ultrastructure to virulence. Mol. Microbiol. 63(5), 1385-1398.

Revie, N.M., Iyer, K.R., Robbins, N., Cowen, L.E., 2018. Antifungal drug resistance: evolution, mechanisms and impact. Curr. Opin. Microbiol. 45, 70-76.

Ribas, J.C., Díaz, M., Durán, A., Pérez, P., 1991a. Isolation and characterization of *Schizosaccharomyces pombe* mutants defective in cell wall (1-3)β-D-glucan. J. Bacteriol. 173(11), 3456-3462.

Ribas, J.C., Durán, Á., Cortés, J.C.G., 2014. New Cell Wall-Affecting Antifungal Antibiotics, in: Villa, T.G., Veiga-Crespo, P. (Eds.), Antimicrobial Compounds. Springer Berlin Heidelberg, pp. 237-268.

Ribas, J.C., Roncero, C., Rico, H., Durán, A., 1991b. Characterization of a *Schizosaccharomyces pombe* morphological mutant altered in the galactomannan content. FEMS Microbiol. Lett. 79(2-3), 263-268.

Robbins, N., Caplan, T., Cowen, L.E., 2017. Molecular Evolution of Antifungal Drug Resistance. Annu. Rev. Microbiol. 71, 753-775.

Roberts, J.A., Gonzalez-Carranza, Z., 2007. Plant cell separation and adhesion. Blackwell, Oxford.

Rocha, E.M., Garcia-Effron, G., Park, S., Perlin, D.S., 2007. A Ser678Pro substitution in Fks1p confers resistance to echinocandin drugs in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. 51(11), 4174-4176.

Rodríguez-Tudela, J.L., Alastruey-Izquierdo, A., Gago, S., Cuenca-Estrella, M., León, C., Miro, J.M., Nuñez Boluda, A., Ruiz Camps, I., Sole, A., Denning, D.W., University of Manchester in association with the, L.p., 2015. Burden of serious fungal infections in Spain. Clin. Microbiol. Infect. 21(2), 183-189.

Roemer, T., Xu, D., Singh, S.B., Parish, C.A., Harris, G., Wang, H., Davies, J.E., Bills, G.F., 2011. Confronting the challenges of natural product-based antifungal discovery. Chem. Biol. 18(2), 148-164.

Roncero, C., 2002. The genetic complexity of chitin synthesis in fungi. Curr. Genet. 41(6), 367-378.

Roncero, C., Sánchez, Y., 2010. Cell separation and the maintenance of cell integrity during cytokinesis in yeast: the assembly of a septum. Yeast 27(8), 521-530.

Ruiz-Herrera, J., Elorza, M.V., Valentín, E., Sentandreu, R., 2006. Molecular organization of the cell wall of *Candida albicans* and its relation to pathogenicity. FEMS Yeast Res. 6(1), 14-29.

Sakai, K., Suga, T., Iwatsuki, M., Chinen, T., Nonaka, K., Usui, T., Asami, Y., Omura, S., Shiomi, K., 2018. Pestiocandin, a new papulacandin class antibiotic isolated from *Pestalotiopsis humus*. J. Antibiot. (Tokyo).

Samarasinghe, H., Xu, J., 2018. Hybrids and hybridization in the *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes. Infect. Genet. Evol. 66, 245-255.

San-Blas, G., San-Blas, F., Serrano, L.E., 1977. Host-parasite relationships in the yeastlike form of *Paracoccidioides brasiliensis* strain IVIC Pb9. Infect. Immun. 15(2), 343-346.

Sawistowska-Schroder, E.T., Kerridge, D., Perry, H., 1984. Echinocandin inhibition of 1,3-β-D-glucan synthase from *Candida albicans*. FEBS Lett. 173(1), 134-138.

Schmidt, M., Bowers, B., Varma, A., Roh, D.H., Cabib, E., 2002. In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. J. Cell Sci. 115(Pt 2), 293-302.

Sethi, K., Palani, S., Cortés, J.C.G., Sato, M., Sevugan, M., Ramos, M., Vijaykumar, S., Osumi, M., Naqvi, N.I., Ribas, J.C., Balasubramanian, M., 2016. A New Membrane Protein Sbg1 Links the Contractile Ring Apparatus and Septum Synthesis Machinery in Fission Yeast. PLoS Genet 12(10), e1006383.

Shahinian, S., Bussey, H., 2000. β-1,6-glucan synthesis in *Saccharomyces cerevisiae*. Mol. Microbiol. 35(3), 477-489.

Sharma, C., Kumar, N., Pandey, R., Meis, J.F., Chowdhary, A., 2016. Whole genome sequencing of emerging multidrug resistant *Candida auris* isolates in India demonstrates low genetic variation. New Microbes New Infect. 13, 77-82.

Shaw, J.A., Mol, P.C., Bowers, B., Silverman, S.J., Valdivieso, M.H., Durán, A., Cabib, E., 1991. The function of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. 114(1), 111-123.

Shaw, K.J., Schell, W.A., Covel, J., Duboc, G., Giamberardino, C., Kapoor, M., Moloney, M., Soltow, Q.A., Tenor, J.L., Toffaletti, D.L., Trzoss, M., Webb, P., Perfect, J.R., 2018. *In Vitro* and *In Vivo* Evaluation of APX001A/APX001 and Other Gwt1 Inhibitors against *Cryptococcus*. Antimicrob. Agents Chemother. 62(8).

Shematek, E.M., Braatz, J.A., Cabib, E., 1980. Biosynthesis of yeast cell wall. I. Preparation and properties of β(1-3)glucan synthetase. J. Biol. Chem. 255(3), 888-894.

Simpson, K.L., Chichester, C.O., Phaff, H.J., 1972. Carotenoid pigments of yeasts, in: Rose, A.H., Harrison, J.S. (Eds.), The Yeasts, Volume 2, Physiology and Biochemistry of Yeasts. Academic Press, New York, pp. 493-515.

Sipiczki, M., 2016. Visualization of Fission Yeast Cells by Transmission Electron Microscopy. Methods Mol. Biol. 1369, 97-111.

Staab, J.F., Kahn, J.N., Marr, K.A., 2010. Differential *Aspergillus lentulus* echinocandin susceptibilities are Fksp independent. Antimicrob. Agents Chemother. 54(12), 4992-4998.

Stevens, D.A., Clemons, K.V., Liu, M., 2011. Developing a vaccine against aspergillosis. Med. Mycol. 49 Suppl. 1, S170-176.

Stevens, D.A., White, T.C., Perlin, D.S., Selitrennikoff, C.P., 2005. Studies of the paradoxical effect of caspofungin at high drug concentrations. Diagn. Microbiol. Infect. Dis. 51(3), 173-178.

Strobel, G.A., Miller, R.V., Martínez-Miller, C., Condron, M.M., Teplow, D.B., Hess, W.M., 1999. Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis cf. quercina*. Microbiology 145 (Pt 8), 1919-1926.

Sugui, J.A., Kwon-Chung, K.J., Juvvadi, P.R., Latge, J.P., Steinbach, W.J., 2014. *Aspergillus fumigatus* and related species. Cold. Spring Harb. Perspect. Med. 5(2), a019786.

Taft, C.S., Selitrennikoff, C.P., 1988. LY121019 inhibits *Neurospora crassa* growth and (1-3)-β-D-glucan synthase. J. Antibiot. (Tokyo) 41(5), 697-701.

Takeshita, H., Watanabe, J., Kimura, Y., Kawakami, K., Takahashi, H., Takemura, M., Kitamura, A., Someya, K., Nakajima, R., 2010. Novel pyridobenzimidazole derivatives exhibiting antifungal activity by the inhibition of β-1,6-glucan synthesis. Bioorg. Med. Chem. Lett. 20(13), 3893-3896.

Thompson, J.R., Douglas, C.M., Li, W., Jue, C.K., Pramanik, B., Yuan, X., Rude, T.H., Toffaletti, D.L., Perfect, J.R., Kurtz, M., 1999. A glucan synthase *FKS1* homolog in *Cryptococcus neoformans* is single copy and encodes an essential function. J. Bacteriol. 181(2), 444-453.

Tobudic, S., Kratzer, C., Lassnigg, A., Graninger, W., Presterl, E., 2010. *In vitro* activity of antifungal combinations against *Candida albicans* biofilms. J. Antimicrob. Chemother. 65(2), 271-274.

Torosantucci, A., Bromuro, C., Chiani, P., De Bernardis, F., Berti, F., Galli, C., Norelli, F., Bellucci, C., Polonelli, L., Costantino, P., Rappuoli, R., Cassone, A., 2005. A novel glyco-conjugate vaccine against fungal pathogens. J. Exp. Med. 202(5), 597-606.

Traxler, P., Gruner, J., Auden, J.A., 1977. 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. (Tokyo) 30(4), 289-296.

Tsukahara, K., Hata, K., Nakamoto, K., Sagane, K., Watanabe, N.A., Kuromitsu, J., Kai, J., Tsuchiya, M., Ohba, F., Jigami, Y., Yoshimatsu, K., Nagasu, T., 2003. Medicinal genetics approach towards identifying the molecular target of a novel inhibitor of fungal cell wall assembly. Mol. Microbiol. 48(4), 1029-1042.

Umeda, K., Yoko-o, T., Nakayama, K., Suzuki, T., Jigami, Y., 2000. *Schizosaccharomyces pombe gmd3*$^{+}$/*alg11*$^{+}$ is a functional homologue of *Saccharomyces cerevisiae ALG11* which is involved in *N*-linked oligosaccharide synthesis. Yeast 16(14), 1261-1271.

Umemura, M., Okamoto, M., Nakayama, K., Sagane, K., Tsukahara, K., Hata, K., Jigami, Y., 2003. *GWT1* gene is required for inositol acylation of glycosylphosphatidylinositol anchors in yeast. J. Biol. Chem. 278(26), 23639-23647.

Umeyama, T., Kaneko, A., Watanabe, H., Hirai, A., Uehara, Y., Niimi, M., Azuma, M., 2006. Deletion of the CaBIG1 gene reduces β-1,6-glucan synthesis, filamentation, adhesion, and virulence in *Candida albicans*. Infect. Immun. 74(4), 2373-2381.

Urbina, J.M., Cortés, J.C.G., Palma, A., López, S.N., Zacchino, S.A., Enriz, R.D., Ribas, J.C., Kouznetzov, V.V., 2000. Inhibitors of the fungal cell wall. Synthesis of 4-aryl-4-N-arylamine-1-butenes and related compounds with inhibitory activities on β(1-3) glucan and chitin synthases. Bioorg. Med. Chem. 8(4), 691-698.

Valdivieso, M.H., Ferrario, L., Vai, M., Duran, A., Popolo, L., 2000. Chitin synthesis in a *gas1* mutant of *Saccharomyces cerevisiae*. J. Bacteriol. 182(17), 4752-4757.

van der Kaaden, M., Breukink, E., Pieters, R.J., 2012. Synthesis and antifungal properties of papulacandin derivatives. Beilstein J. Org. Chem. 8, 732-737.

Van Der Vaart, J.M., te Biesebeke, R., Chapman, J.W., Klis, F.M., Verrips, C.T., 1996. The β-1, 6-glucan containing side-chain of cell wall proteins of *Saccharomyces cerevisiae* is bound to the glycan core of the GPI moiety. FEMS Microbiol. Lett. 145(3), 401-407.

Vargas, M.L.Y., Castelli, M.V., Kouznetsov, V.V., Urbina G, J.M., López, S.N., Sortino, M., Enriz, R.D., Ribas, J.C., Zacchino, S., 2003. In vitro antifungal activity of new series of homoallylamines and related compounds with inhibitory properties of the synthesis of fungal cell wall polymers. Bioorg. Med. Chem. 11(7), 1531-1550.

Varona, R., Pérez, P., Durán, A., 1983. Effect of papulacandin B on β-glucan synthesis in *Schizosaccharomyces pombe*. FEMS Microbiol. Lett. 20, 243-247.

Verma, D.P., 2001. Cytokinesis and building of the cell plate in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 751-784.

Verweij, P.E., Snelders, E., Kema, G.H., Mellado, E., Melchers, W.J., 2009. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? Lancet Infect. Dis. 9(12), 789-795.

Vicente, M.F., Basilio, A., Cabello, A., Peláez, F., 2003. Microbial natural products as a source of antifungals. Clin. Microbiol. Infect. 9(1), 15-32.

Vink, E., Rodriguez-Suarez, R.J., Gerard-Vincent, M., Ribas, J.C., de Nobel, H., van den Ende, H., Durán, A., Klis, F.M., Bussey, H., 2004. An *in vitro* assay for (1-6)-β-D-glucan synthesis in *Saccharomyces cerevisiae*. Yeast 21(13), 1121-1131.

Vos, A., Dekker, N., Distel, B., Leunissen, J.A., Hochstenbach, F., 2007. Role of the synthase domain of Ags1p in cell wall α-glucan biosynthesis in fission yeast. J. Biol. Chem. 282(26), 18969-18979.

Vossen, J.H., Muller, W.H., Lipke, P.N., Klis, F.M., 1997. Restrictive glycosylphosphatidylinositol anchor synthesis in *cwh6*/*gpi3* yeast cells causes aberrant biogenesis of cell wall proteins. J. Bacteriol. 179(7), 2202-2209.

Walker, L., Sood, P., Lenardon, M.D., Milne, G., Olson, J., Jensen, G., Wolf, J., Casadevall, A., Adler-Moore, J., Gow, N.A.R., 2018. The Viscoelastic Properties of the Fungal Cell Wall Allow Traffic of AmBisome as Intact Liposome Vesicles. MBio 9(1).

Walker, L.A., Gow, N.A., Munro, C.A., 2010. Fungal echinocandin resistance. Fungal Genet. Biol. 47(2), 117-126.

Walker, L.A., Munro, C.A., de Bruijn, I., Lenardon, M.D., McKinnon, A., Gow, N.A., 2008. Stimulation of chitin synthesis rescues *Candida albicans* from echinocandins. PLoS Pathog. 4(4), e1000040.

Walker, S.S., Xu, Y., Triantafyllou, I., Waldman, M.F., Mendrick, C., Brown, N., Mann, P., Chau, A., Patel, R., Bauman, N., Norris, C., Antonacci, B., Gurnani, M., Cacciapuoti, A., McNicholas, P.M., Wainhaus, S., Herr, R.J., Kuang, R., Aslanian, R.G., Ting, P.C., Black, T.A., 2011. Discovery of a novel class of orally active antifungal β-1,3-D-glucan synthase inhibitors. Antimicrob. Agents Chemother. 55(11), 5099-5106.

Walther, P., Müller, M., Schweingruber, M.E., 1984. The ultrastructure of the cell surface and plasma membrane of exponential and stationary phase cells of *Schizosaccharomyces pombe*, grown in different media. Arch. Microbiol. 137(2), 128-134.

Walther, P., Schweingruber, A., Müller, M., Schweingruber, M.E., 1988. Morphological organization of glycoprotein containing cell surface structures in yeast. Journal of Ultrastructure and Molecular Structure Research 101(2), 123-136.

Wang, Z.A., Li, L.X., Doering, T.L., 2018. Unraveling synthesis of the cryptococcal cell wall and capsule. Glycobiology 28(10), 719-730.

Warn, P.A., Sharp, A., Morrissey, G., Denning, D.W., 2005. Activity of aminocandin (IP960) compared with amphotericin B and fluconazole in a neutropenic murine model of disseminated infection caused by a fluconazole-resistant strain of *Candida tropicalis*. J. Antimicrob. Chemother. 56(3), 590-593.

Warn, P.A., Sharp, A., Morrissey, G., Denning, D.W., 2010. Activity of aminocandin (IP960; HMR3270) compared with amphotericin B, itraconazole, caspofungin and micafungin in neutropenic murine models of disseminated infection caused by itraconazole-susceptible and -resistant strains of *Aspergillus fumigatus*. Int. J. Antimicrob. Agents 35(2), 146-151.

Watanabe, N.A., Miyazaki, M., Horii, T., Sagane, K., Tsukahara, K., Hata, K., 2012. E1210, a new broad-spectrum antifungal, suppresses *Candida albicans* hyphal growth through inhibition of glycosylphosphatidylinositol biosynthesis. Antimicrob. Agents Chemother. 56(2), 960-971.

Wiederhold, N.P., 2007. Attenuation of echinocandin activity at elevated concentrations: a review of the paradoxical effect. Curr. Opin. Infect. Dis. 20(6), 574-578.

Wiederhold, N.P., 2018. The antifungal arsenal: alternative drugs and future targets. Int. J. Antimicrob. Agents 51(3), 333-339.

Wiederhold, N.P., Kontoyiannis, D.P., Prince, R.A., Lewis, R.E., 2005. Attenuation of the activity of Caspofungin at high concentrations against *Candida albicans*: possible role of cell wall integrity and calcineurin pathways. Antimicrob. Agents Chemother. 49(12), 5146-5148.

Wiedman, J.M., Fabre, A.L., Taron, B.W., Taron, C.H., Orlean, P., 2007. *In vivo* characterization of the GPI assembly defect in yeast mcd4-174 mutants and bypass of the Mcd4p-dependent step in *mcd4*Δ cells. FEMS Yeast Res. 7(1), 78-83.

Willer, T., Brandl, M., Sipiczki, M., Strahl, S., 2005. Protein O-mannosylation is crucial for cell wall integrity, septation and viability in fission yeast. Mol. Microbiol. 57(1), 156-170.

Wisplinghoff, H., Bischoff, T., Tallent, S.M., Seifert, H., Wenzel, R.P., Edmond, M.B., 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. 39(3), 309-317.

Yamaguchi, H., Hiratani, T., Baba, M., Osumi, M., 1985. Effect of aculeacin A, a wall-active antibiotic, on synthesis of the yeast cell wall. Microbiol. Immunol. 29(7), 609-623.

Yoshimi, A., Miyazawa, K., Abe, K., 2017. Function and Biosynthesis of Cell Wall α-1,3-Glucan in Fungi. J Fungi (Basel) 3(4).

Yoshimi, A., Sano, M., Inaba, A., Kokubun, Y., Fujioka, T., Mizutani, O., Hagiwara, D., Fujikawa, T., Nishimura, M., Yano, S., Kasahara, S., Shimizu, K., Yamaguchi, M., Kawakami, K., Abe, K., 2013. Functional analysis of the α-1,3-glucan synthase genes *agsA* and *agsB* in *Aspergillus nidulans*: agsB is the major α-1,3-glucan synthase in this fungus. PLoS One 8(1), e54893.

Zeidler, U., Bougnoux, M.E., Lupan, A., Helynck, O., Doyen, A., Garcia, Z., Sertour, N., Clavaud, C., Munier-Lehmann, H., Saveanu, C., d'Enfert, C., 2013. Synergy of the antibiotic colistin with echinocandin antifungals in *Candida* species. J. Antimicrob. Chemother. 68(6), 1285-1296.

Zeng, R., Li, M., Chen, Q., Wang, L., Zhan, P., Wang, C., Lv, G., Shen, Y., Liu, W., 2014. *In vitro* analyses of mild heat stress in combination with antifungal agents against *Aspergillus fumigatus* biofilm. Antimicrob. Agents Chemother. 58(3), 1443-1450.

Zhao, Y., Lee, M.H., Paderu, P., Lee, A., Jiménez-Ortigosa, C., Park, S., Mansbach, R.S., Shaw, K.J., Perlin, D.S., 2018. Significantly Improved Pharmacokinetics Enhances *In Vivo* Efficacy of APX001 against Echinocandin- and Multidrug-Resistant *Candida* Isolates in a Mouse Model of Invasive Candidiasis. Antimicrob. Agents Chemother. 62(10).

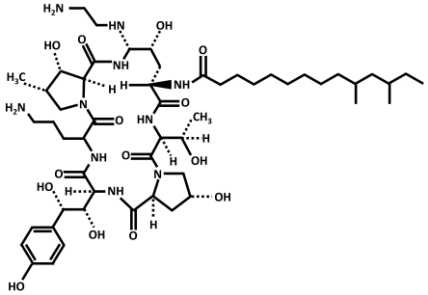
Zhao, Y., Perez, W.B., Jiménez-Ortigosa, C., Hough, G., Locke, J.B., Ong, V., Bartizal, K., Perlin, D.S., 2016. CD101: a novel long-acting echinocandin. Cell. Microbiol. 18(9), 1308-1316.

Table 1. Estimated burden of the main life-threatening fungal diseases

Disease (most common species)	Estimated number of affected individuals per year	Estimated mortality rate
Aspergillosis (*Aspergillus fumigatus*[#1])	Invasive aspergillosis >300,000[2,3]	~30% mortality if treated in HIC -in AIDS[2] ~50% non-AIDS, in HIC[2]
	Chronic pulmonary aspergillosis >3,000,000[2,3]	~15-40% mortality in HIC[2] ~15% mortality in the developed world[2]
Blastomycosis (*Blastomyces dermatidis*)	~3,000[3,4]	<2-68%[4]
Candidiasis (*Candida albicans** [3])	Invasive candidiasis >750,000[2,3]	~40% mortality treated[2]
Coccidioidomycosis (*Coccidioides immitis*)	~25,000[3,4]	<1-70%[4]
Cryptococcosis (*Cryptococcus neoformans*[+5])	>1,000,000[4,6]	15-70%[4,6,7]
Histoplasmosis (*Histoplasma capsulatum*)	~100,000[2,3]	15-30%, if diagnosed and treated[2]
Paracoccidioidomycosis (*Paracoccidioides brasiliensis*)	~4,000[3,4]	5-27%[4]
Pneumocystis pneumonia (*Pneumocystis jirovecii*)	~500,000 in AIDS and non-AIDS[3,4]	20-80%[4]
Sporotrichosis (*Sporothrix schenckii*)	>40,000[2]	No data

HIC: High-income countries; AIDS: Acquired Immunodeficiency Syndrome;
[#]Other *Aspergillus* isolates: *A. flavus, A. niger, A. terreus, A. nidulans*
*Other *Candida* isolates: *C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, C. auris*
[+]Other *Cryptococcus* isolates: *C. gattii*
[1](Sugui et al., 2014)
[2](GAFFI)
[3](Bongomin et al., 2017)
[4](Brown et al., 2012a)
[5](Samarasinghe and Xu, 2018)
[6](Park et al., 2009)
[7](Bratton et al., 2012)

Table 2. Current commercial availability of echinocandin derivatives approved for therapeutic use

Compound, commercial name, owner company and chemical structure	Available commercial suppliers
Caspofungin (Cancidas®, Merck Sharp & Dohme, MSD). 	Abyntek Biopharma http://buscador.abyntek.com/?term=caspofung AMS Biotechnology http://www.amsbio.com/search-results.aspx?value=caspofungin BioVision https://www.biovision.com/caspofungin-acetate.html BOC Sciences https://www.bocsci.com/search?q=caspofungin EMELCA Bioscience http://www.emelcabio.com/productdisplay/caspofungin-acetate LifeSpan BioSciences https://www.lsbio.com/biochemicals/caspofungin-acetate-cas-179463-17-3-ls-h3150/3150 MyBioSource https://www.mybiosource.com/products.php?keywords=caspofungin Selleck Chemicals http://www.selleckchem.com/products/caspofungin-acetate.html Sigma-Aldrich (now fused to Merck) https://www.sigmaaldrich.com/catalog/search?term=caspofungin&interface=All&N=0&mode=match%20partialmax&lang=es&region=ES&focus=product Tebu-bio https://www.tebu-bio.com/search/?contexte=&autocomplete=0&type_contexte=0&search=caspofungin

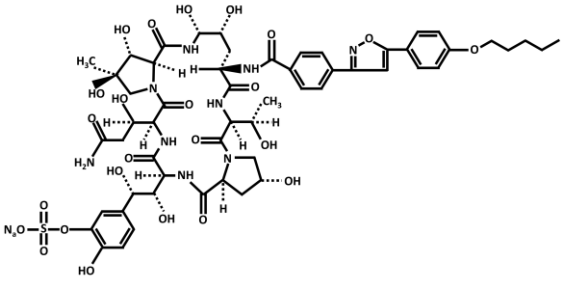
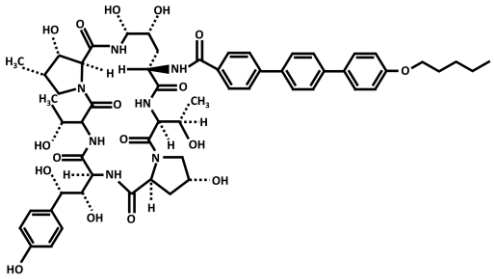
Micafungin (Mycamine®, Astellas Pharma). 	Abyntek Biopharma http://buscador.abyntek.com/?term=micafung AMS Biotechnology http://www.amsbio.com/search-results.aspx?value=micafungin BioVision https://www.biovision.com/micafungin-sodium.html BOC Sciences https://www.bocsci.com/search?q=micafungin EMELCA Bioscience http://www.emelcabio.com/productdisplay/micafungin-sodium LifeSpan BioSciences (*shipment within the USA only*) https://www.lsbio.com/biochemicals/micafungin-sodium-salt-cas-208538-73-2-ls-h8670/8670 MyBioSource https://www.mybiosource.com/products.php?keywords=micafungin Selleck Chemicals http://www.selleckchem.com/products/micafungin-sodium.html Tebu-bio https://www.tebu-bio.com/search/?contexte=&autocomplete=0&type_contexte=0&search=micafungin
Anidulafungin (Eraxis® or Ecalta®, Pfizer). 	Abyntek Biopharma http://buscador.abyntek.com/?term=anidulafung BOC Sciences https://www.bocsci.com/search?q=anidulafungin LifeSpan BioSciences https://www.lsbio.com/search?q=anidulafungin&sid=098t97dm7w MyBioSource https://www.mybiosource.com/prods/Inhibitor/Anidulafungin/datasheet.php?products_id=578661 Selleck Chemicals http://www.selleckchem.com/products/anidulafungin-ly303366.html Sigma-Aldrich (now fused to Merck) https://www.sigmaaldrich.com/catalog/product/sigma/sml2288?lang=es&region=ES Tebu-bio https://www.tebu-bio.com/search/?contexte=&autocomplete=0&type_contexte=0&search=anidulafungin

Table 3. Current cell wall antifungals in clinical development. Information was obtained from the website ClinicalTrials.gov (https://www.clinicaltrials.gov/ct2/home) from the U.S. National Library of Medicine (National Institutes of Health, USA)

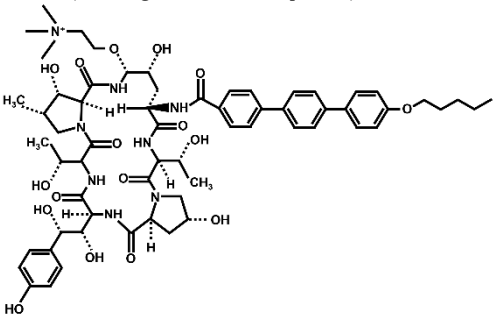
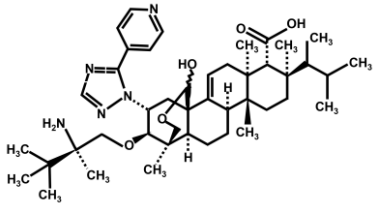
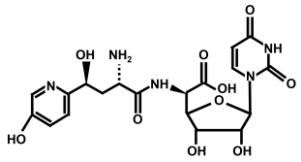
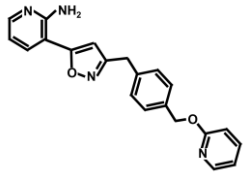
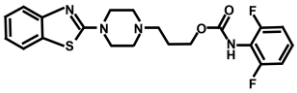
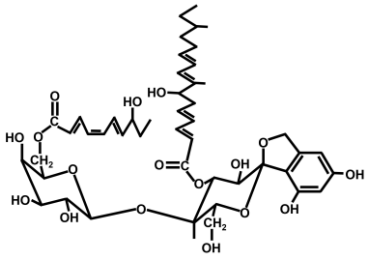
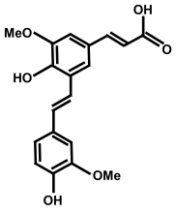
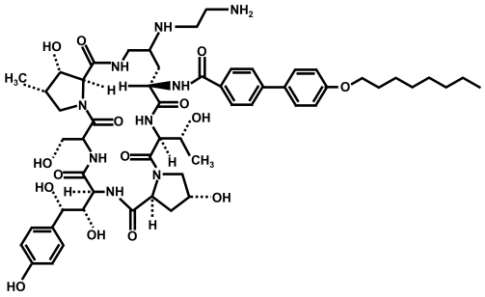
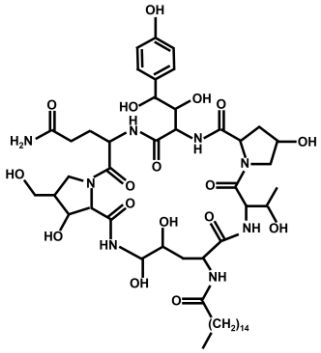
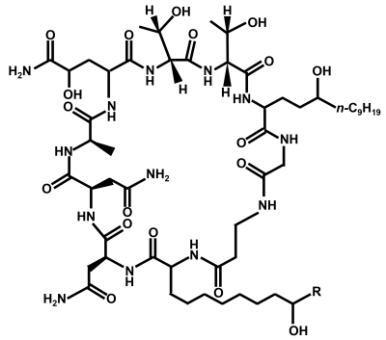
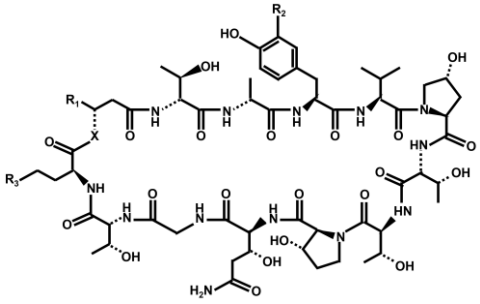
Compound, commercial name, owner company and chemical structure	Mechanism of action	Current state of clinical development
CD101 (rezafungin, Cidara Therapeutics). 	Inhibition of the synthesis of (1-3)β-D-glucan .	Phase 2. ID: NCT02734862 Candidemia, mycosis, fungal infection, fungemia, invasive candidiasis. Actual study start date: July 2016. Phase 3. ID: NCT03667690 Candidemia, mycosis, fungal infection, invasive candidiasis. Estimated study start date: October 2018.
SCY-078 / MK338 (ibrexafungerp, Scynexis). 	Inhibition of the synthesis of (1-3)β-D-glucan .	Phase 2. ID: NCT03672292 Invasive Pulmonary Aspergillosis Estimated study start date: October 2018. Phase 3. ID: NCT03363841 Candidiasis, invasive candidemia. Actual study start date: November 2017. Phase 3. NCT03059992 Invasive candidiasis, mucocutaneous candidiasis. Actual study start date: April 2017.
Nikkomycin Z (University of Arizona). 	Inhibition of the synthesis of chitin.	Phase 2. ID: NCT00614666 Coccidioidomycosis. Terminated due to the lack of funding and recruitment challenges.
APX001 / E1211 (Amplyx Pharmaceuticals). 	Inhibition of acyltransferase Gwt1 and thus of glycosylphosphatidylinositol (GPI) synthesis.	Phase 2. ID: NCT03604705 Candidemia. Estimated study start date: October 2018.

Table 4. Experimental cell wall antifungals under development	
Compound, commercial name, owner company and chemical structure	**Mechanism of action**
Pyridazinone derivatives (Schering-Plough Corporation and Albany Molecular Research). 	Inhibition of the synthesis of (1-3)β-D-glucan.
Papulacandins B (Ciba Geigy, now fused to Novartis). 	Papulacandin family. Inhibition of the synthesis of (1-3)β-D-glucan.
Poacic acid (Wisconsin Alumni Research Foundation). 	Inhibition of the synthesis of (1-3)β-D-glucan.
Aminocandin (IP960, HMR3270, NXL201; Aventis Pharma). 	Echinocandin family. Inhibition of the synthesis of (1-3)β-D-glucan.
Cryptocandin (HMV Corporation). 	Echinocandin family. Inhibition of the synthesis of (1-3)β-D-glucan.
Arborcandins (A, B, C, D, E, F; Daiichi Sankyo).	Inhibition of the synthesis of (1-3)β-D-glucan.

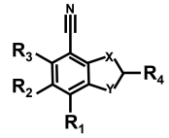


Aerothricin 3 / FR901469 (Chugai Pharmaceutical and Basilea Pharmaceutica).



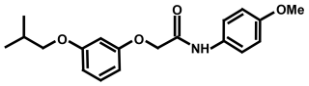
Inhibition of the synthesis of (1-3)β-D-glucan.

Bicyclic heteroaryl ring derivatives (D75-4590, D11-2040, D21-6076; Daiichi Sankyo).



Inhibition of the synthesis of (1-6)β-D-glucan.

Gepinacin (Whitehead Institute for Biomedical Research).



Inhibition of acyltransferase Gwt1 and thus glycosylphosphatidylinositol (GPI) synthesis.

Figure 1. **Structure and composition of the cell wall in different fungal species.**

The schemes show the main components of the cell wall in each fungal model. Most cell walls have a common inner layer composed of an alkali-insoluble core of branched (1-3)β-D-glucan, (1-6)β-D-glucan and chitin, with many species having also (1-3)α-D-glucan and a few lacking chitin. In *Aspergillus fumigatus* and *Aspergillus niger* the (1-3)α-D-glucan is located in different cell wall layers: in the inner wall core, interconnected with chitin and β-glucan; and in the outer wall region interacting with glycoproteins. The composition of the attached outer layer of glycoproteins is more diverse between fungal species. In the yeasts *Saccharomyces cerevisiae* and *Candida albicans*, this outer layer is enriched with mannosylated glycoproteins, while the glycoproteins of filamentous *A. fumigatus* and fission yeast *Schizosaccharomyces pombe* contain galactomannans instead of mannans. Basidiomycetous yeast

Cryptococcus neoformans has a differential external capsule structure that is composed of glucuronoxylomannan and a lower amount of glucuronoxylomannogalactan. This fungus also has a layer of melanin, whose exact localization is currently unknown. In other fungi, such as dimorphic *Histoplasma capsulatum* and *Blastomyces dermatitidis*, the layer of (1-3)α-D-glucan shields the efficient recognition of (1-3)β-D-glucan by the immune system of the host (figure adapted from Erwig and Gow, 2016).

Figure 2. Targets of cell wall antifungals.

Illustration showing the cell wall enzymes and synthesized cell wall components specifically inhibited by the available cell wall antifungals. A schematic representation of the cell wall architecture is also shown. The synthesis of linear chains of both (1-3)β-D-glucan and chitin starts in the plasma membrane by the corresponding (1-3)β-D-glucan and chitin synthases. Then, cell wall remodeling glucanosyltransferases form branched (1-3)β-D-glucan (green), which is cross-linked to (1-6)β-D-glucan (red), to chitin (blue) or to glycoproteins (black). Most glycoproteins are covalently attached to (1-6)β-D-glucan via a glycosylphosphatidylinositol (GPI) anchor. Also proteins with internal repeats (PIR) are covalently connected to branched (1-3)β-D-glucan through an alkali-soluble bond. Commercial echinocandins (caspofungin, micafungin and anidulafungin), drugs undergoing clinical trials (echinocandin CD101 and enfumafungin derivative SCY-078) and other antifungal compounds under development bind and inhibit the (1-3)β-D-glucan synthase (green), except poacic acid (asterisk) that apparently binds to the (1-3)β-D-glucan polysaccharide but also inhibits the (1-3)β-D-glucan synthase. Nikkomycin Z targets the synthesis of chitin by inhibiting the chitin synthase (blue). The family of bicyclic heteroaryl ring derivatives blocks the synthesis of (1-6)β-D-glucan (red). Finally, APX001 and gepinacin drugs target the acyltransferase Gwt1 in the endoplasmic reticulum (no depicted), blocking the synthesis of GPI and the consequent attachment of essential GPI-linked proteins to the outer cell wall layer (black).

Figure 3. Mechanisms of fungal resistance to echinocandins.

Scheme showing the adaptive mechanisms of echinocandin resistance via alteration of the target enzyme (1-3)β-D-glucan synthase and/or activation of cellular stress responses. Mutations in the three highly conserved hotspot regions of the *FKS* genes, encoding the (1-3)β-D-glucan synthase catalytic subunit, increase target resistance to

echinocandin drugs (left). The exposure of cells to echinocandins damages the cell wall, inducing multiple stress regulation pathways and the activation of compensatory mechanisms that lead to the overproduction of cell wall chitin and the paradoxical effect of fungal resistance adaptation to echinocandins (right). This adaptation can in last instance also favor the development of spontaneous resistance mutations.

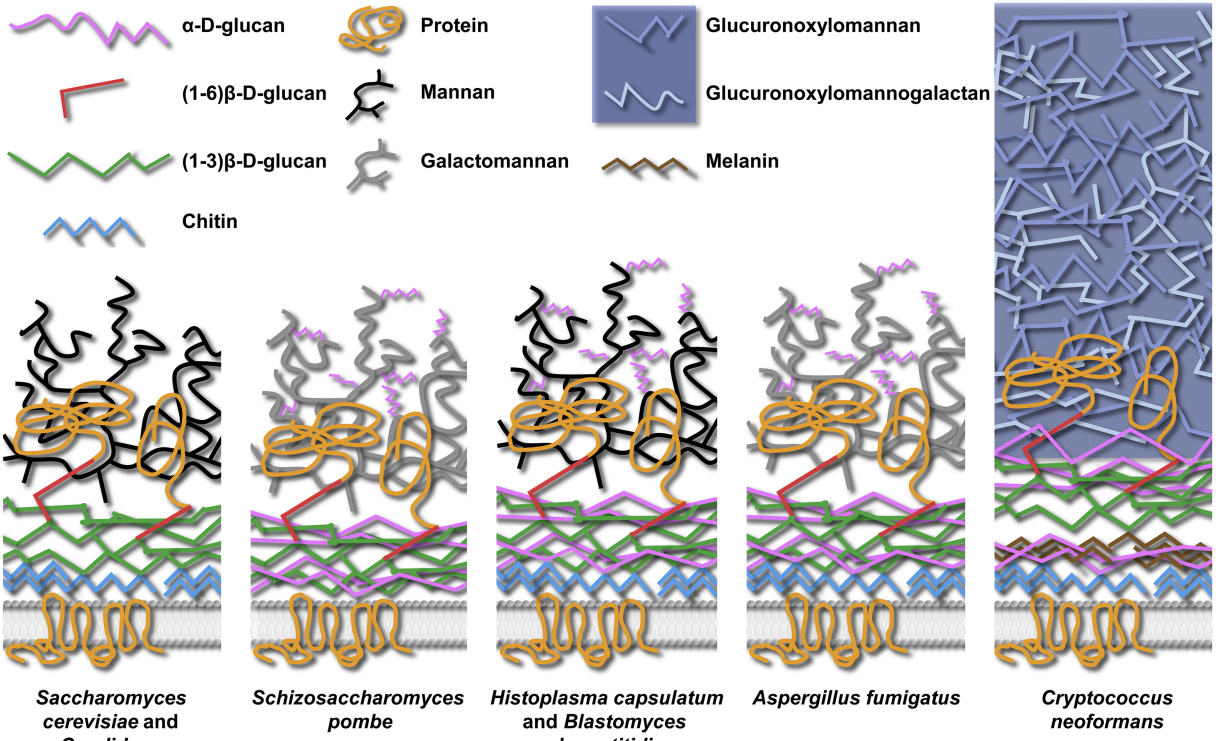

α-D-glucan
(1-6)β-D-glucan
(1-3)β-D-glucan
Chitin
Protein
Mannan
Galactomannan
Glucuronoxylomannan
Glucuronoxylomannogalactan
Melanin
Saccharomyces cerevisiae and Candida sp
Schizosaccharomyces pombe
Histoplasma capsulatum and Blastomyces dermatitidis
Aspergillus fumigatus
Cryptococcus neoformans

Figure 1

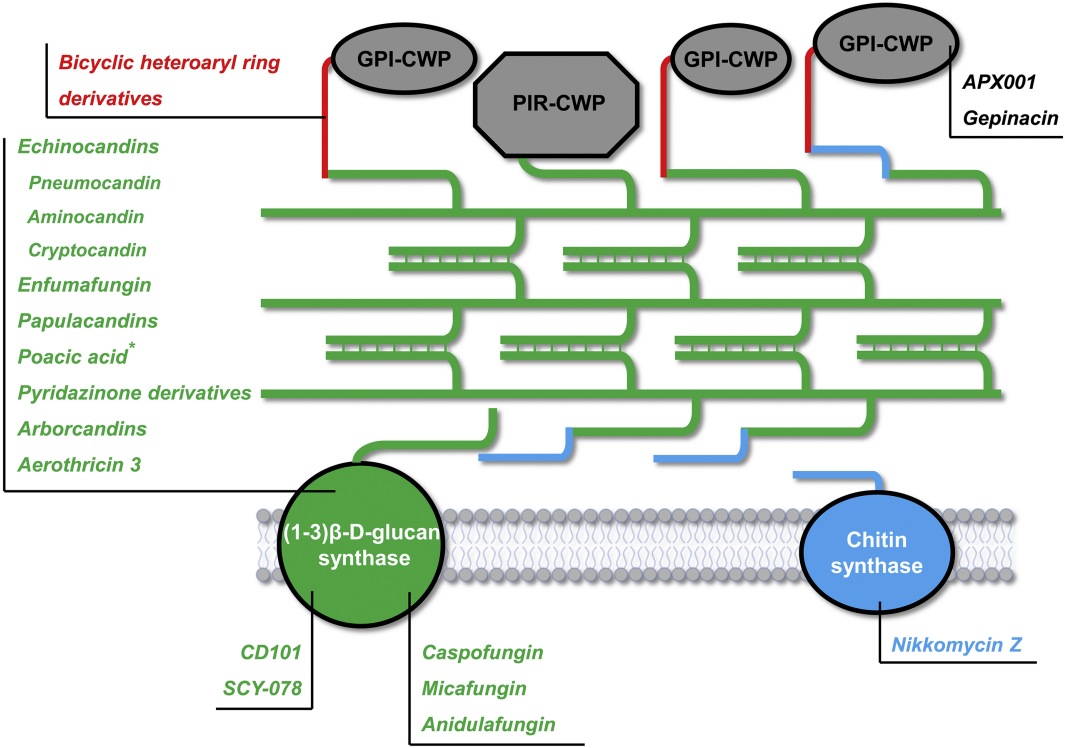
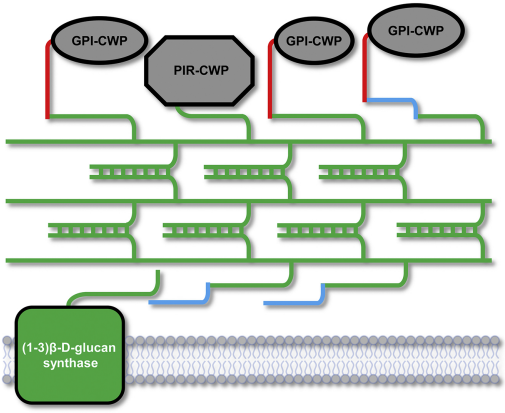
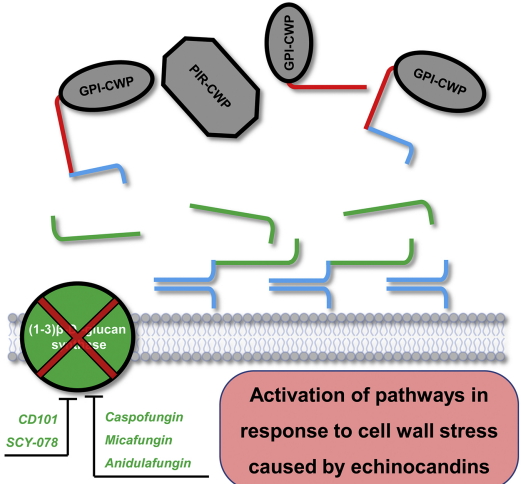

Bicyclic heteroaryl ring derivatives
GPI-CWP
PIR-CWP
GPI-CWP
GPI-CWP
APX001
Gepinacin
Echinocandins
Pneumocandin
Aminocandin
Cryptocandin
Enfumafungin
Papulacandins
Poacic acid*
Pyridazinone derivatives
Arborcandins
Aerothricin 3
(1-3)β-D-glucan synthase
Chitin synthase
CD101
SCY-078
Caspofungin
Micafungin
Anidulafungin
Nikkomycin Z

Figure 2



Reduction of echinocandins binding through point mutations in the three hot spots of resistance of the (1-3)β-D-glucan synthase



Activation of pathways in response to cell wall stress caused by echinocandins

Overproduction of cell wall chitin and resistance adaptation to echinocandins

Figure 3