Pharmacology & Therapeutics 195 (2019) 21–38

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Antifungal drugs: New insights in research & development

André Moraes Nicola [a], Patrícia Albuquerque [b,d], Hugo Costa Paes [c], Larissa Fernandes [b,d], Fabricio F. Costa [e,f,g], Erika Seki Kioshima [h], Ana Karina Rodrigues Abadio [i], Anamélia Lorenzetti Bocca [j,*,1], Maria Sueli Felipe [e,j,**,1]

[a] Division of Pathology, University of Brasília Medical School, Brazil
[b] Faculty of Ceilândia, University of Brasília, Brazil
[c] Division of Clinical Medicine, University of Brasília Medical School, Brazil
[d] Graduate Programme in Microbial Biology, University of Brasília, Brazil
[e] Graduate Programme in Genomic Science and Biotechnology, Catholic University of Brasília, Brazil
[f] MATTER, Chicago, IL, USA
[g] Cancer Biology and Epigenomics Program, Ann & Robert Lurie Children's Hospital of Chicago Research Center, Northwestern University's Feinberg School of Medicine, Chicago, Illinois, USA
[h] Department of Clinical Analysis and Biomedicine, State University of Maringá, Paraná, Brazil
[i] School for Applied Social and Agricultural Sciences, State University of Mato Grosso, Nova Mutum Campus, Mato Grosso, Brazil
[j] Department of Cell Biology, Institute of Biological Sciences, University of Brasília, Brazil

ARTICLE INFO

Keywords:
Drug targets
Rational drug design
Essential genes
Antifungal immunotherapy
Drug discovery and development
Systemic mycoses

ABSTRACT

The need for better antifungal therapy is commonly accepted in view of the high mortality rates associated with systemic infections, the low number of available antifungal classes, their associated toxicity and the increasing number of infections caused by strains with natural or acquired resistance. The urgency to expand the range of therapeutic options for the treatment of fungal infections has led researchers in recent decades to seek alternative antifungal targets when compared to the conventional ones currently used. Although new potential targets are reported, translating the discoveries from bench to bedside is a long process and most of these drugs fail to reach the patients. In this review, we discuss the development of antifungal drugs focusing on the approach of drug repurposing and the search for novel drugs for classical targets, the most recently described gene targets for drug development, the possibilities of immunotherapy using antibodies, cytokines, therapeutic vaccines and antimicrobial peptides.

Contents

1. Introduction

In recent decades, advances in the medical field due to the discovery of new drugs and development of therapeutic protocols have promoted an increment in the lifespan of immunosuppressed patients such as those with HIV, cancer patients and transplant recipients. However, the increased immunocompromised population led to a dramatic expansion of human invasive mycosis cases, which has become a global public health problem. Currently, the approved therapeutic arsenal of

* Corresponding author at: Department of Cell Biology, Institute of Biology Sciences, Campus Darci Ribeiro, Asa Norte, University of Brasilia, Brasilia – DF CEP 70910-900, Brazil.
** Corresponding author at: Catholic University of Brasilia - SGAN 916 Módulo B W5 Norte, Brasilia – DF CEP 70790-160, Brazil.
E-mail addresses: albocca@unb.br (A.L. Bocca), msueli@ucb.br (M.S. Felipe).
[1] A.L.Bocca and M.S.Felipe share senior authorship of this article.

https://doi.org/10.1016/j.pharmthera.2018.10.008
0163-7258/

drugs for treatment of mycoses is restricted to a few antifungal drugs, and most of them have limitations such as low effectiveness and high toxicity. In addition, the fact that some fungal species are resistant to the available drugs is noted as an obstacle to treating those infections. Thus, researchers have been looking for ways to deal with these problems, since the identification of new antifungal compounds or new therapeutic protocols is an urgent need. This review will focus on and explore the main strategies and advances that have been reported and proposed by the academic, industrial and medical communities for the development of new therapeutic approaches for the proper management of fungal infections.

2. Drug development

2.1. Drugs against old pathways

Recent advances in compound screening and drug design have not only covered substances with activity against previously untargeted pathways, but also molecules that act on the same pathways as classical antifungals. Pathways that have already been successfully targeted by drugs in clinical use have the advantage of being "field-tested"; chemicals that are found to act against targets in them are likelier to be effective antifungals. Of note, several new classes of compounds have been reported against enzymes of the ergosterol biosynthetic pathway, including but not limited to the classical azole target, lanosterol 14α-demethylase. In this subtopic, we discuss recent reports of novel compounds with antifungal activity on ergosterol synthesis inhibition published in the past three years, which are: alkylated isoquinoline derivatives; aminopiperidine derivatives; pyranopyrimidines; and coumarin derivatives. Recent developments in the search for second-generation echinocandins with improved activity against fungal strains that evolved resistance to first-generation compounds will also be discussed. In this section, we also present investigational drugs that have been shown to be useful against systemic fungal pathogens, including strains resistant to clinically available azoles, and which inhibit enzymes of ergosterol or beta-glucan biosynthesis.

2.1.1. Erg 11 azole and Erg1 inhibitors

The only targets of the ergosterol biosynthetic pathway for which there are drugs used in systemic (as opposed to topical) therapy are lanosterol 14α-demethylase (Erg11 or Cyp51; EC 1.14.13.154), the target of azole antifungals; and squalene monooxygenase (Erg1; EC 1.14.14.17), the target of terbinafine. However, with the emergence of azole-resistant strains obtained by the overexpression of the enzyme, or by amino acid substitutions that remove inhibition by the drugs (Cowen et al., 2015), attention has turned to other enzymes of the same pathway, or to non-azole drugs that inhibit Erg11.

2.1.2. Erg11, non-azole inhibitors

Coumarins are widespread in plants and thought to act as antifungal chemical barriers. Accordingly, research on coumarin derivatives was first pursued with a view to the development of agricultural herbicides (Zhang et al., 2014). However, a recent work by Tiwari et al., 2017 resulted in the synthesis of derivatives with antimicrobial activity, one of which was a promising antifungal, although they presented higher Minimal Inhibitory Concentration (MIC) values (12 μg/mL against *Aspergillus niger* to 38 μg/mL against *A. fumigatus*). *In vitro* toxicity against HeLa cells was negative, and oral administration to mice resulted in no obvious toxicity or behavioural changes in the animals. Analysis of ergosterol synthesis intermediate accumulation suggested inhibition of Erg11 and *in silico* docking studies gave support to this observation.

2.1.3. Erg24 (Δ 14-sterol reductase) inhibitors

The second step in the conversion of lanosterol to ergosterol involves catalysis by this enzyme (Erg24; EC 1.3.1.70). In contrast with Erg11, it is not a component of cytochrome P450 in the mammalian liver, which suggests that drugs against this enzyme might not produce the adverse drug interactions often observed in azole drugs. Hata et al. (2010, 2010) identified new hit compounds from the aminopiperidin class that showed activity against fluconazole-resistant strains of *C. albicans, C. glabrata, C. krusei* and *A. fumigatus*, as well as against *C. neoformans*. *In vitro* MICs for effective compounds ranged from 0.063 (against fluconazole-resistant *C. albicans* MYA-573) to 16 μg/mL (against *A. fumigatus* ATCC 13073). The chemicals were shown to be fungistatic against *C. albicans*, and one of them was comparable in efficacy to fluconazole in a murine model of systemic candidiasis. Another class of compounds with activity against Erg24 is the isoquinoline derivatives. Synthesised by Krauss et al. (2014), they include hits with activity against azole-resistant *C. glabrata* (MIC of 2.5 μg/mL) and no toxicity against the human HL 60 cell line.

2.1.4. Erg2 (Δ 8,7-isomerase) inhibitors

This enzyme, Erg2, converts fecosterol to episterol in a branch of steroid synthesis that is not present in mammals. A topical antifungal, amorolfine, inhibits Erg2, but no compounds for systemic use are available. However, isoquinoline derivatives (Krauss et al., 2014) described above include hit compounds with activity against Erg2 instead of Erg24. One such compound had a MIC of 5 μg/mL against azole-resistant *C. glabrata* and no toxicity against HL60 cells.

2.1.5. Erg6 (Δ 24-sterol methyltransferase) inhibitors

Erg6 (EC 2.1.1.41), acts in conversion of lanosterol to eburicol, or of zymosterol to fecosterol, both of which reactions are exclusive to the fungal ergosterol biosynthetic pathway. Therefore, it has long been an object of interest for the development of antifungals. Recently, Erg6 inhibitors have been reported with antifungal activity *in vitro*. The phenylpropanoid α-bisabolol has been shown to inhibit the growth of *A. fumigatus* (Jahanshiri et al., 2017) and the sterol hydrazine, 22-hydrazone-imidazolin-2-yl-chol-5-ene-3β-ol, can inhibit growth of several fungi, including *Sporothrix* spp. (Borba-Santos et al., 2016) and, at sub-micromolar concentrations, *Paracoccidioides brasiliensis* (Visbal et al., 2011).

2.1.6. Other compounds that inhibit ergosterol synthesis

A few chemicals have shown antifungal activity coupled with ergosterol depletion *in vitro*, but a specific target enzyme has not been determined for them until now. Isoniazid derivatives have been reported (de Aguiar Cordeiro et al., 2014) as being active against *Histoplasma capsulatum*, including synergism with amphotericin B and suppression of metabolic activity of yeast embedded in biofilms. Raja Mohamed et al. (2014) reported that levofloxacin derivatives exhibit suppressive activity against *C. albicans* biofilms, an effect that was accompanied by a decrease in ergosterol content and in expression of ergosterol biosynthesis genes. Suresh et al. (2015) reported the synthesis of pyranopyrimidine compounds with fungicidal activity (at concentrations ranging from 7.8 to 62.5 μg/mL) against several pathogenic *Candida strains* by inhibition of the ergosterol pathway in an unspecified manner.

2.1.7. Fks (1,3-β-glucan synthesis) and chitin inhibition

The cell wall of fungal cells has also been widely exploited as a target for selective antifungal therapy (Song and Stevens, 2016; Gow et al., 2017; Chang et al., 2017). This unique structure is composed mainly of chitin and glucan polymers with a role in the protection of fungal cells against extracellular stress from the natural environmental and host immune response. The latest drug class approved for clinical use was the echinocandins, which block glucan biosynthesis (Chang et al., 2017). The three echinocandin antifungals, caspofungin, anidulafungin and micafungin, inhibit the activity of 1,3-β-glucan synthase (Fks; EC 2.4.1.34), an enzyme involved in the synthesis of the fungal cell wall. Today, they are the chosen therapy against disseminated candidiasis

due to the potent activities and low side effects (Sucher et al., 2009; Chang et al., 2017). They are also part of combination therapy against systemic aspergillosis (Jenks & Hoenigl, 2018). However, echinocandins require hospitalisation due to their poor bioavailability upon oral administration (Sucher et al., 2009; Chang et al., 2017), and there has been interest in developing drugs that act on the same enzyme but can be taken orally. To improve the pharmacological properties, two new inhibitors of glucan synthases are under evaluation, CD101 (or rezafungin or SP3025) from Cidara Therapeutics (USA), and SCY-078 from Scynexis (USA). Rezafungin is an echinocandin, so technically it does not constitute a new class against an old pathway, but it is a chemically more stable molecule than the first-generation drugs of its class, and is highly soluble in aqueous systems (Ong et al., 2016, 2017; Sofjan et al., 2018). It demonstrated low toxicity and long half-life in animal models, reducing the frequency of intravenous application to once a week (Lepak et al., 2017). The activity of rezafungin was evaluated on *C. albicans* and *A. fumigatus* systemic infections with effects comparable to those of anidulafungin and more potent than caspofungin (Ong et al., 2017; Lepak et al., 2017). It is currently the object of a phase II trial for candidemia and systemic candidiasis (trial #NCT02734862 at clinicaltrials.gov), and a phase III trial is scheduled for the same clinical conditions (#NCT03667690).

2.1.8. MK-3118/SCY-078

triterpenoid derived from the non-echinocandin natural antifungal, enfumafugin (Pfaller et al., 2013a), has shown broad *in vitro* activity (MIC ranging from 0.06 to 16 μg/mL) against *Candida* spp. (*C. albicans, C. glabrata, C. krusei, C. parapsilosis*), including azole- and echinocandin-resistant strains. Subsequent studies confirmed promising *in vivo* activity in a murine model of candidiasis (Lepak et al., 2015), against the emerging *Candida auris in vitro* (Larkin et al., 2017), against *Candida* biofilms (Marcos-Zambrano et al., 2017) and against *Aspergillus* spp. *in vitro* (CLSI 90% minimum effective concentration, ranging from 60 to 120 ng/mL; Pfaller et al., 2013b). Synergy with both azoles and amphotericin B (AmB) has been shown against *A. fumigatus in vitro* (Ghannoum et al., 2018).

2.1.9. SCY-078

SCY-078 is a different structure from the conventional echinocandins, derived from enfumafungin (Heasley et al., 2012; Onishi et al., 2000), which has finalized Phase 2 of clinical trials, with high rates of clinical cures in comparison to fluconazole and non-serious adverse effects. It is a safe drug with daily oral administration, as potent as caspofungin against *Candida* and *Aspergillus* species, including those resistant to azoles and echinocandins. This drug showed poor activity to treat *Mucor* and *Fusarium* infections (Jiménez-Ortigosa et al., 2014; Lepak et al., 2015; Schell et al., 2017; Ghannoum et al., 2018; Wiederhold et al., 2018).

2.1.10. Nikkomycins

comprise peptidyl nucleoside agents that competitively inhibit the chitin synthase (Ruiz-Herrera et al., 2003). Nikkomycins were isolated in the 1970s from *Streptomyces*, are active against some yeasts and thermal dimorphic fungal pathogens that cause systemic disease, but not against moulds. The preclinical and Phase 1 clinical studies showed that they are selective, with low toxicity and superior activity relative to fluconazole in animal models of systemic mycoses caused by dimorphs, both intravenously or orally (Hector et al., 1990; Shubitz et al., 2014). The short half-life due to structural instability has led to a delay in the availability of these compounds as clinical alternatives to treat fungal infections.

Of all the compounds described (Fig. 1), only SCY-078 represents a new class that has made the transition into clinical trials. Coumarins are the only other class that has moved beyond *in vitro* assays to animal toxicity studies, and their MICs may be too high for effective clinical use, at least without chemical optimisation. Nikkomycins, so far, are an unfulfilled promise. While, for the reasons outlined at the beginning of this section, finding new molecules against old targets is a shortcut to obtaining new drugs that are clinically useful, it is clear that the search for new antifungals needs to be broadened, as we discuss in the next sections.

2.2. New molecules against other targets

The development of the tools for functional genetic studies in association with new high-throughput molecular technologies was crucial for the generation of a long list of new candidates for antifungal targets (De Backer and Van Dijck, 2003). There is no doubt that in the coming years, this list will increase even more with large-scale projects. We know that there is a long and expensive road to validate an inhibitor of a new molecular target, including preclinical and clinical phases, until it reaches the market, however these new targets presents a new horizon for the development of a broader therapeutic arsenal against the increasingly fungal infections. From the list of promising antifungal targets, in this topic we will focus on new molecules against: glucosphingolipids biosynthesis, GPI anchoring protein, pyrimidine pathway, amino acids biosynthesis, glyoxylate cycle, siderophore, signaling pathways, transcriptional factors and histone deacetylases (Table 1). Some of them are already in preclinical or clinical trials, while others still require more effort for antifungal drug optimization, thus opening up new perspectives in the biotechnological industry applied to human health.

2.2.1. Glucosphingolipids biosynthesis

Glucosphingolipids are plasma membrane components regulating the replication and pathogenicity of several fungi. IPC synthase is a fungal exclusive enzyme required to produce inositol phosphatyl-ceramide (IPC) and Aureobasidin A is a cyclic depsipeptide inhibitor of this enzyme isolated from the *Aurebasidium pullulans* with potent antifungal activity (Takesako et al., 1993; Sugimoto et al., 2004). Aureobasidin A derivatives, Khafrefungin and Rustmicin have also been tested for therapeutic uses (Mandala et al., 1997; Mandala et al., 1998; Wuts et al., 2015). Recently, two studies found the acylhydrazone compounds BHBM [(E)-N′-(3-bromo-6-hydroxybenzylidene)-2-methylbenzohydrazide] selectively reduced fungal IPC and are well tolerated in animals with high potency against a broad spectrum of pathogenic fungi (Mor et al., 2015; Lazzarini et al., 2018).

2.2.2. GPI anchoring protein

For proper integrity and homeostasis of fungal cell wall, many mannosylated proteins are attached to it by Glycosylphosphatidylinositol (GPI) anchors (Komath et al., 2018). The inositol acyltransferase (Gwp1) catalyzes the fourth step of this pathway, and is the target of E1210 and Gepinacin. E1210 (now APX001, Amplyx Pharmaceutical, USA) showed high activity against *Candida* species and molds, with limited effect on *Mucor* infections (Miyazaki et al., 2011; Wiederhold et al., 2015; Schell et al., 2017). It has low toxicity, is well tolerated by oral administration and now is in Phase 1 clinical trials. Gepinacin is a phenoxyacetanilide still requiring structural optimization for *in vivo* pharmacological evaluation (McLellan et al., 2012).

2.2.3. Pyrimidine pathway

Among the orotomides compounds, F901318 (F2G, United Kingdom) was identified as a potent antifungal drug which targets the fungal dihydroorotate dehydrogenase on the *de novo* pyrimidine pathway in a very specific manner (Oliver et al., 2016). This drug showed low toxicity in mammal cells and good tolerability during Phase 1 of clinical studies. It now moved to Phase 2 for prophylactic use on acute myeloid leukemia patients (Kennedy et al., 2017; Kennedy et al., 2017; Du Pre et al., 2018).

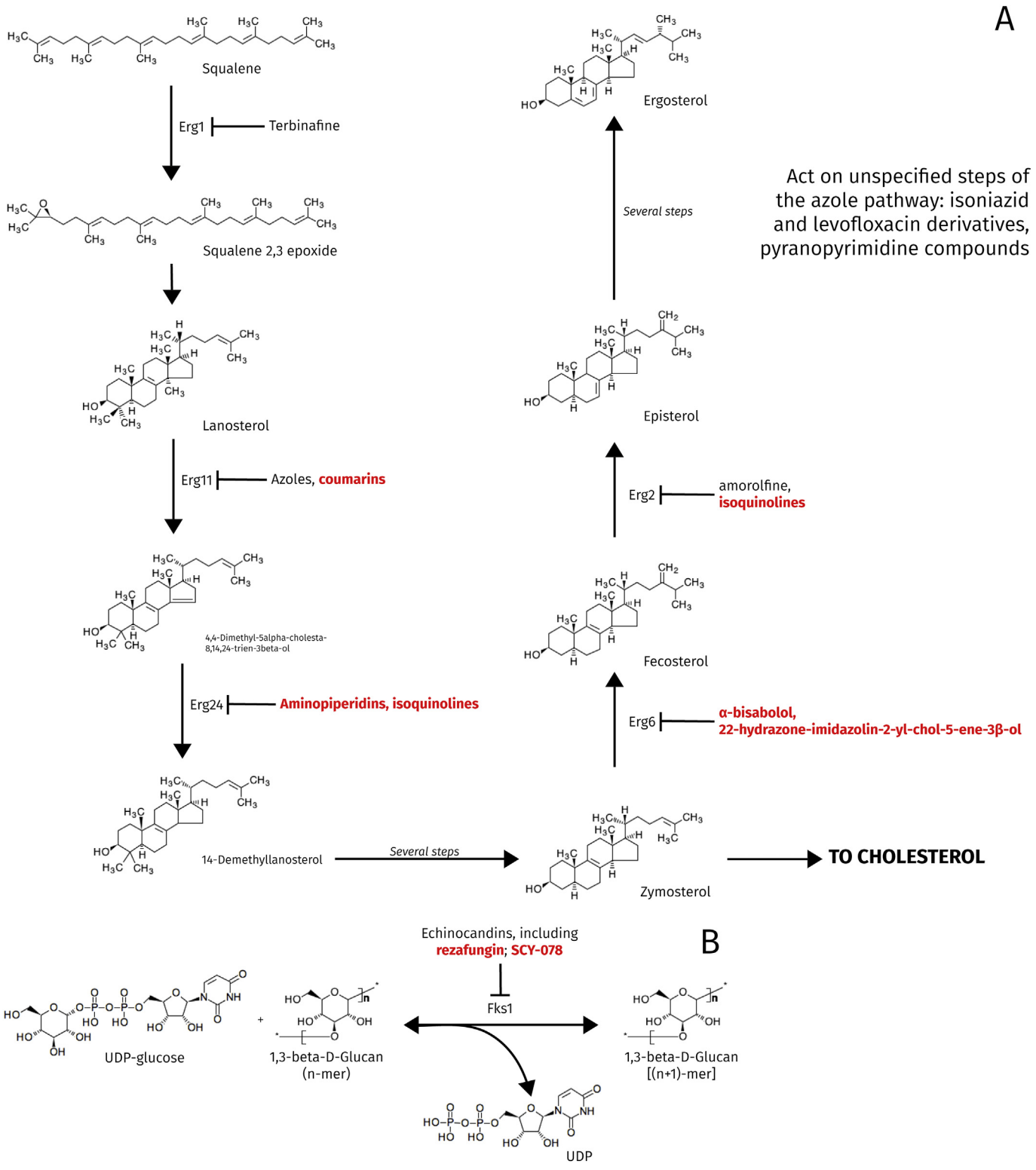

Fig. 1. New classes, old targets. (A) The ergosterol synthesis pathway, in simplified form to highlight the steps that are catalysed by enzymes targeted by investigational compounds (red). Drugs in clinical use are shown in black. The second reaction catalysed by Erg6, from lanosterol to eburicol, is not shown. (B) The reaction catalysed by Fks1 and the agents that act on it

2.2.4. Amino acids biosynthesis

Acetohydroxyacid synthase (AHAS) is the first enzyme of the branched-chain amino acids synthesis not available in human cells (McCourt and Duggleby, 2006). Two different studies found several sulfonamide molecules against this enzyme with a broad spectrum of antifungal action *in vitro* and low cytotoxicity (Lee et al., 2013; Richie et al., 2013); however, none advanced to pre-clinical studies.

2.2.5. Glyoxylate cycle

The glyoxylate cycle enables fungal cells to adapt and survive under limited host nutrient conditions. It allows the use of two carbon sources

Table 1
New molecules against other targets

Cellular function	Target	Drugs/Inhibitors	Clinical studies for fungal infections	Company/Reference
Glucosphingolipids GPI anchoring protein	IPC synthase	Aureobasidin A and derivatives	-	AureoGen Biosciences/Merck & Co
		Khafrefungin	-	Takesako et al. (1993); Sugimoto et al. (2004)
		Rustmicin	-	Mandala et al. (1998) Wuts et al. (2015)
	Inositol phosphatyl-ceramide	BHBM	-	Mor et al. (2015); Lazzarini et al. (2018)
	Inositol acyltransferase (GWP1)	E1210 (APX001)	Phase I (NCT03333005[a] – under development, NCT02957929[a] and NCT02956499[a] - concluded)	Amplyx Pharmaceutical
		Gepinacin	Phase II (NCT0360470[a]- planned)	McLellan et al. (2012)
			-	
Pyrimidine pathway	Dihydroorotate dehydrogenase	F901318 (Olorofim)	Phase I (NCT03340597[a], NCT02680808[a], NCT02730442[a], NCT02142153[a] - concluded) Phase II (NCT03583164[a] - under development)	F2G
Amino acids biosynthesis	Acetohydroxyacid synthase	Sulfonamide molecules	-	Lee et al. (2013); Richie et al. (2013)
Glyoxylate cycle	Isocitrate lyase (ICL)	Caffeic acid (CAFF), Rosmarinic acid (ROS), and Apigenin (API)	-	Cheah et al., 2014
		5-Hydroxyindole-type alkaloids	-	Lee et al. (2009)
		Mohangamide A and B	-	Bae et al. (2015)
Siderophore	Unknown	ASP2397 (VL-2397)	Phase II (NCT03327727[a] - under development)	Vical
Signaling pathways	MAP kinase High-osmolarity glycerol (HOG1)	Fludioxonil	-	Kojima et al. (2006); Dinér et al. (2011)
	Phosphoinositide-dependent kinase 1 (PDK1)	KP-372-1	-	Baxter et al. (2011); Koselny et al. (2016)
		UCN-01 (7-Hydroxystaurosporine)	Clinical trials for other diseases	
		OSU-03012	Clinical trials for other diseases	
	Sphingosine 1-phosphate (S1P)	FTY720 (Fingolimod)	Clinical trials for other diseases, FDA approved for Multiple Sclerosis	Hagihara et al. (2013)
Transcriptional factors	Sterol uptake control protein 2 (Upc2)	VB00075177, VB00075853 and VB00075845 (Venenum)	-	Gallo-Ebert et al. (2014)
Heterochromatin remodelation	Histone Deacetylase (Hos2)	MGCD290	Phase II (NCT01497223[a]- concluded)	MethylGene

[a] ClinicalTrials.gov Identifier (https://clinicaltrials.gov/)

bypassing the CO_2-generating steps of the TCA cycle (Lorenz and Fink, 2002; Dunn et al., 2009). Isocitrate lyase (ICL1) is essential for fungal virulence and is not present in the human genome. Many inhibitors of ICL1 have high activity against different fungal species (Lee et al., 2009; Cheah et al., 2014; Bae et al., 2015) opened new perspectives in the design of new antifungal drugs targeting this metabolic pathway.

2.2.6. Siderophore

ASP2397 (VL-2397, Vical, USA) is a cyclic hexapeptide isolated from *Acremonium persicinum* structurally similar to ferrichrome, a hydroxamate siderophore, with activity against yeasts and molds (Nakamura et al., 2017). Although its specific cellular target is unknown, it is transported by siderophore protein (Sit1) not available in human cells. It is already in a Phase 2 clinical trial for treatment of invasive aspergillosis in acute leukemia patients and recipients of allogeneic hematopoietic cell transplant.

2.2.7. Signaling pathways

Fungal pathogens have co-opted the eukaryotic conserved signaling pathways to adapt to the environment in order to survive even inside the host. The slight differences of fungal components in relation to human cells are attractive for antifungal drug development (Chauhan and Calderone, 2008). Fludioxonil constitutively activates HOG1 (MAP kinase High-osmolarity glycerol) provoking lethal effects on fungal cells (Dinér et al., 2011; Kojima et al., 2006). The Pdk1 (phosphoinositide-dependent kinase 1) is the target of KP-372-1, UCN-01, OSU-03012 (Baxter et al., 2011; Koselny et al., 2016). The calcium signaling pathway has also attracted attention as this cation serves as a second messenger on different cascades and is required for many biological functions inside the fungal cells. FTY720, a sphingosine 1-phosphate (S1P) receptor modulator, induces an influx of calcium across the membrane, resulting in growth impairment of the fungal cells (Hagihara et al., 2013).

2.2.8. Transcriptional factors

As transcriptional factors are master regulators of several genes involved in a variety of cellular functions, recent studies have shown that inhibition of these molecules provokes a huge disturbance of genetic expression of the fungal cells culminating in cell death. High-throughput screening for inhibitors of the Upc2, a sterol uptake control protein 2 that is activated in response to azole treatment, successfully identified several molecules that may be used as precursors for designing new antifungal drugs (Gallo-Ebert et al., 2014).

2.2.9. Histone deacetylases

The last strategy to be discussed in this section is the use of epigenetic inhibitors to modulate the gene expression of fungal pathogens (Lamoth et al., 2015; Kmetzsch, 2015). It is already applied in cancer therapy, to target histone deacetylases (HDACs), which are responsible for removing acetyl groups from histones and some other proteins, generally down-regulating transcription and gene expression. Those proteins play a pivotal role in the modulation of virulence traits, drug resistance and morphogenesis of fungal pathogens (Zachi et al., 2010; Brandão et al., 2015 and Brandão et al., 2018). A recent work employing MGCD290, a HDAC inhibitor, showed synergism with commonly used azoles indicating the potential use as an alternative antifungal therapy (Pfaller et al., 2009).

2.3. Essential genes/proteins as drug targets

Essential genes are absolutely required for organism survival and are therefore considered the foundation of life. The identification of those genes in several microorganisms has been used as a promising strategy

for searching new antimicrobial targets in both bacteria and fungi. With the rapid advance of molecular biology and bioinformatics techniques, several genomes were sequenced and accurate analyses of huge amounts of information can be implemented to improve the identification of new targets genes. Essential genes, such as FSK1 (encoding 1,3-β-D-glucan synthase) and ERG11 (encoding 14α-demethylase), are drug targets used in current medical practice for the treatment of invasive fungal infections. However, the available antifungal drugs present limitations such as toxicity, low selectivity and antifungal resistance (Wiederhold, 2018).

Comparative genomic approaches have contributed to the identification of genes that are highly conserved within fungal species, and thus, may represent genes/proteins that participate in key processes (Liu et al., 2006; Abadio et al., 2011). The comparative approach is based on the hypothesis that different genomes share a common ancestor, and nucleotide sequences in each organism are the result of evolution, resulting in a possible maintenance of conserved domains in proteins indispensable to organism survival (te Velthuis & Bagowski, 2008; Abadio et al., 2011; Lu et al., 2014; Fan et al., 2017). The identification of essential genes in several microorganisms has been used as a promising strategy for seeking new antimicrobial targets in bacteria (Boyd et al., 2015) and fungi (Becker et al., 2010; Carr et al., 2010; Ianiri and Idnurm, 2015).

Fungal agents involved in invasive fungal infections such as *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., *Paracoccidioides brasiliensis* and *P. lutzii* are associated with high risk of mortality. Essential genes for some of those fungi have been confirmed using mutant screening with different strategies, such as: (i) antisense-based gene inactivation (De Backer et al., 2001); (ii) gene replacement and conditional expression (Roemer et al., 2003; Hu et al., 2007; Becker et al., 2010); (iii) parasexual genetics coupled with transposon mutagenesis (Carr et al., 2010); (iv) insertional mutagenesis using *Agrobacterium*-mediated transformation (Ianiri et al., 2017).

De Backer et al. (2001) used antisense RNA inhibition and promoter interference to obtain gene suppression in *C. albicans* enabling them to identify 85 genes critical for growth. In addition, molecular genetic approaches introducing targeted gene mutations allowed the identification of essential genes in *C. albicans* (Becker et al. 2010). Using 177 tetracycline-regulated promoter based conditional mutants in an immunocompetent murine infection model of systemic candidiasis, 102 genes were defined as essential for growth, virulence and survival in the host. In addition, the construction of conditional mutants with replacement of one allele and the expression control of the remaining allele allowed the confirmation by phenotypic analysis of 567 essential genes for *C. albicans* growth, encoding proteins involved in a broad spectrum of basic cellular processes (Roemer et al., 2003). Essential genes were also identified in *Aspergillus fumigatus,* by gene replacement and conditional expression strategy, using a nitrogen-regulated promoter, and 35 of them were validated by *in vivo* phenotypic analyses (Hu et al. 2007). More recently, Carr et al. (2010) employed the parasexual cycle to construct diploid isolates and, coupled with transposon-based mutagenesis, this allowed the identification of 20 essential genes of *A. fumigatus* and 96 loci that are critical for pathogen viability. Strategies to identify essential genes in *C. neoformans* by insertional mutagenesis using *Agrobacterium tumefaciens*-mediated transformation (*At*MT) was described by Ianiri & Idnurm (2015) and Ianiri et al. (2017). This last study altered the transcription of genes adjacent to the insertion of T-DNA molecules, inserting the GAL7 promoter as close as possible to one border of the T-DNA, allowing the identification of 6 essential genes for *C. neoformans*.

Some other groups have shown the identification of essential gene targets, such as: histone deacetylases and UDP-N-acetylglucosamine pyrophosphorylase from *A. fumigatus* (Fang et al., 2013; Bauer et al., 2016); molecular chaperone, Kar2/BiP of *C. neoformans* (Jung et al., 2013); ROM2 of *C. glabrata* and *C. albicans* (Kanno et al., 2015); γ-glutamyl cysteine synthase of *C. glabrata* (Yadav et al., 2011); thioredoxin reductase and alfa-1,2-mannosyltransferase of *P. lutzii* (Abadio et al., 2011). Using comparative genomic strategy, based on *in silico* analyses and data mining, our group has selected 57 orthologs genes in the pathogens: *C. albicans*, *A. fumigatus*, *Blastomyces dermatitidis*, *P. brasiliensis*, *P. lutzii*, *Coccidioides immitis*, *C. neoformans* and *Histoplasma capsulatum* as potential drug targets. Of those, 10 genes were present in the aforementioned pathogenic fungi and absent in the human. We focused on *trr1* and *kre2* genes, encoding thioredoxin reductase and alfa-1,2-manosyltransferase, respectively, to perform molecular modeling and virtual screening, in order to select new antifungal compounds (Felipe et al., 2005; Abadio et al., 2015). The construction of three-dimensional models of proteins key to fungal survival, such as *C. albicans* and *P. lutzii*, enabled the selection of new small compounds against those enzymes (Abadio et al., 2015; Godoy et al., 2016; Salci et al., 2017), offering new perspectives on technological development and innovation of drug discovery (see section 1.4).

A total of 275 essential genes required for fungal survival in different pathogenic fungal were identified by the authors cited in this section (Table 2). The genes listed in Table 1 were categorized according to their Gene Ontology (GO) classes and significantly enriched were deduced by hypergeometric tests (GO terms with p-value < 0.05) (Table 3). Despite the differences in the methodologies to test gene essentiality in those fungi as well the number of evaluated genes, few categories are commonly enriched among few of those species: sterol and terpenoid biosynthesis, cell wall architecture and mitochondrial functions.

Several strategies can be used aiming at the development of new antifungal drugs. In this context, the identification and knowledge of essential genes required for fungi survival represents a possibility to achieving this development.

2.4. Rational antifungal drug design

Rational drug design has enabled the application of computational tools for drug discovery as cost-effective alternatives to traditional experimental protocols. *In silico* methods are important tools for antifungal development and can considerably accelerate this process, because they reduce the number of compounds that need testing in order to identify early hits (Lionta et al., 2014; Kontoyianni, 2017).

Molecular docking has been extensively applied in virtual screening of small molecules in chemical libraries (Life Chemicals, Zinc, ChEMBL, ChemBridge among others) for drug discovery and hit-to-lead optimization. Inhibitory compounds (hits) with potential antifungal action have been discovered and tests *in vitro* and/or *in vivo* have shown their effective action against several fungal species (Abadio et al., 2015; Salci et al., 2017). Inhibition tests with proven hits validate *in silico* strategies and constitute an advance in the discovery of new drugs and they have also contributed in obtaining small molecules with promising antifungal activity. As mentioned before, two of the targets described by Abadio et al., (2011) have already been successfully explored: thioredoxin reductase (TRR1) and α-1,2-mannosyltransferase (KRE2). Three inhibitors of thioredoxin reductase were selected by virtual screening with promising antifungal activity against *Paracoccidioides* spp. (Abadio et al., 2015). More recently, Kioshima et al. (unpublished data) extended the antifungal spectrum of thioredoxin reductase inhibitors by two new compounds with promising antifungal activity against *Candida* spp. and *Cryptococcus* spp. A promising hit molecule was selected as KRE2 inhibitor by virtual screening (Alves et al., unpublished data) and Salci et al. (2017) proved their antifungal activity against different *Candida* species, especially *C. parapsilosis*. Two other small molecules selected by virtual screening against the enzyme homoserine dehydrogenase of *Paracoccidioides brasiliensis* exhibited promising antifungal activity against fungi of this genus (Bagatin et al., 2017). Tripathi & Khan (2018) used a different approach with clustering

Table 2
Essential genes in pathogenic fungi identified by the authors cited in the Section 2.3.

Specie	Essential genes
C. albicans	*DPB2, HEM3, HIS1, PRS1, PSA1, SEC14, SEC4, YPT1, MIG1, MNN9, KRE9, SEC20, PHR2, PRA1, ECM31, RIB2, ARO7, BIO2, ERG13, CCA1, NUG1, EBP2, UTP10, YEF3, ERG27, FAS1, FAS2, EFT2, SAS10, SUI2, ESF2, UTP6, URA5, ARO2, NOP9, SEC6, ERD2, ERG11, ERG9, PMA1, URA7, VTI1, TRS23, PUP1, SCL1, RET3, GPI16, RHO1, CHS2, NPL6, PRE1, TIM44, MRPL6, TIM50, RSC2, BRN1, MCD1, TBF1, YSH1, CLP1, TIM23, MRPL23, RPO41, MRPL10, SPC97, DBF4, TFB1, PAP1, PRP8, SLU7, TAF6, CSL4, TEM1, ARC35, YTM1, SRM1, BDP1, PFS2, EPL1, TFG1, CFT2, MEX67, BRL1, NUP192, YNL313C, ERG1, 18S rRNA, 25S rRNA, 5S rRNA, Mitochondrial 15S rRNA[a], RPS7, RPL37, RPL2[a], RPS21[a], RPL16[a], EFB1, TEF3, TEF4[a], TUF1[a], ABP1[a], YDR388w/RVS167[a], RNR1, YAE1, TRA1[a], SAP45[c], FAL1[a], MEG1[a], ENO1, SHA3[a], SDH2[a], HXT6[a], TPI1[a], KGD2[a], ARS1/2, SAM2, RPS102/RPS1, RAD18[a], HOL1[a], NDE1[a], OST4[a], PSP2[a], ECM21[a], MAA[d], CBR1[a,b], CIT[e], ERG7, CDC28* and *ARG5,6*
C. neoformans	*BOT1, BYR4, FOL1, HYM1, HRB1, KEI1, MGM101, MRPL31, MRPS18, NAM9, RSM18, SEN54, SFI1, TIM54, BUR6, CDC14, IDI1, MRPL37, NOP56, PRE3, RPL17* and *KAR2/BIP*
A. fumigatus	*UAP1, RPDA, PRO1[a], LYS1[a], LYS2[a], MET22[a], TFP1[a], NUP188[a], ACT1[a], GIM5[a], FMN1[a], ZWF1[a], IQG1[a], HHT1[a], CDC54[a], RFC5[a], POL3[a], RFC4[a], MOT1[a], RAD3[a], TOA1[a], HFI1[a], RRN11[a], SRP68[a], APS1[a], YDL058W/USO1[a], COP1[a], YIP1[a], PEP12[a], BCP1[a], COG1[a], SEC24[a], SRP RNA[a], MRD1[a], URB2[a], RRP46[a], RRP45[a], RPS17A[a], RRP12[a], RRP5[a], RPL22A[a], MAK11[a], RPL11A[a], SQT1[a], LCB1[a], ATP17[a], YNL213C[a], MRPL49[a], IMP1[a], NAM2[a], MRPL40[a], ATP11[a], PDI1[a], GPI2[a], GPI18[a], DOA1[a], PCH1[b], Cul4B[b], SFC9[b], SSR4[b], CDC27[b], HIS3, LYS4, LYS9, MET16, MET2, TRP5, DIB1[a], RSE1[a], RRD2[a], KOG1[a], CDS1, HEM15, IPP1, SPE2, PRI1, SEC31, SLY1, BRX1, ESF1, NOB1* and *NOP4*
C. glabrata	*GSH-1*
C. albicans and *C. glabrata*	*ROM2*
A. fumigatus and *C. albicans*	*NSP1, CCT8[a], RAM2, ERG20[a], MMM1[a], ALG7[a], GNA1[a], ESS1[a], PTA1[a], GSP1[a], GUS1[a], GRS1[a], CDC60[a], ARO1, GFA1, OLE1, TUB1, ERG10, ERG12, GCD6, PAB1, TIF35, KRR1* and *LUC7*
C. albicans and *C. neoformans*	*ERG8, FBA1, GUA1, MVD1* and *CET1*
A. fumigatus, *C. albicans* and *C. neoformans*	*TRL1* and *NOC3*
C. albicans, *P. brasiliensis*, *P. lutzii*, *B. dermatitidis*, *C. immitis*, *C. neoformans* and *H. capsulatum*	*CHS1[f], RIM8[f], KRE6[f], ERG6[f]* and *NMT1/KRE2[f]*
A. fumigatus, *P. brasiliensis*, *P. lutzii*, *B. dermatitidis*, *C. immitis*, *C. neoformans* and *H. capsulatum*	*TRR1[f]*
A. fumigatus, *C. albicans*, *P. brasiliensis*, *P. lutzii*, *B. dermatitidis*, *C. immitis*, *C. neoformans* and *H. capsulatum*	*FKS1[f], AUR1[f], TOM40[f]* and *MAK5[f]*

[a] *Saccharomyces cerevisiae* name.
[b] *Schizosaccharomyces pombe* name.
[c] *Homo sapiens* name.
[d] *Escherichia coli* name.
[e] *Candida tropicalis* name.
[f] Genes identified experimentally and by comparative genomic analysis.

and virtual screening to identify potential inhibitors of *C. albicans* fungus with specific nuclear target (Ask1 subunit of the Dam1 complex).

Ligand-based virtual screening (LBVS) uses known active ligands as a template to search for other active ligands, whereas chemically similar compounds would have similar biological activities (Maggiora et al., 2014). Although this is not always true, this methodology has also been applied successfully to identify promising compounds. Gidaro et al., (2016) selected three coumarin derivatives with anti-*Candida* activity similar to fluconazole (MIC ranging from 2-8 μg/mL). Singh et al. (2016) identified two new non-azole CYP51 inhibitors with antifungal activity against *Candida* spp and *Aspergillus niger*.

Another approach for small molecule identification consists of obtaining a series of chemical derivatives that will be evaluated for antifungal activity and the ability to bind to a certain target, by molecular docking. Several groups have focused on finding new inhibitors of fungal lanosterol 14–demethylase. In this regard, two thiazolin-4-one derivatives were tested against several *Candida* species, and were shown to be more active than fluconazole (MIC 0.015ug/mL to *C. albicans*, *C. parapsilosis* and *C. krusei*) (Stana et al., 2017). A series of triazole–thiazolidinedione derivatives, from molecular hybridization of fluconazole and rosiglitazone, were able to select four compounds with excellent activity against *C. albicans* (MIC_{80} = 0.029–0.15 mm). These compounds also showed activity against *C. glabrata*, *C. parapsilosis* and *C. neoformans* (Wu et al., 2014).

Pyridine and oxadiazole rings were found to be important for the activity of new imidazole derivatives, whereas the presence of different substituents on the phenyl ring of the 1,3,4-oxadiazole unit played a major role in deciding their potent antifungal activities (MIC ranged 2 to 6 μg/mL) (Wani et al., 2015). Three compounds of oxadiazole-thiadiazole hybrid, bearing a nitro group, showed significant antifungal activity against four *Candida* species, with MIC range of 0.78–3.12 μg/mL (Levent et al., 2017). Five compounds from a series of 3-benzoyl imidazon [1,2-a] pyrimidines showed promising antifungal activity against seven *Candida* species, with MIC values lower than fluconazole, range of 0.0312–0.5μg/mL (Gómez-García et al., 2018). Three benzothiazole derivatives showed excellent antifungal activities, with MIC values in the range of 0.125-2 ug/mL, against a variety of clinically relevant fungal pathogens, such as *C. albicans*, *C. neoformans* and *A. fumigatus* (Zhao et al., 2016). Taken together, the results obtained by these groups have shown some promising hit compounds for the development of new antifungal agents and the effectiveness of *in silico* approaches in antifungal drug discovery.

2.5. Drug repurposing

Drug repositioning or repurposing approaches allow new indications for previously approved drugs that are marketed for other conditions. This approach offers many benefits over *de novo* drug development. For the pharmaceutical market, the pharmacokinetic and pharmacodynamic profiles previously established provide acceleration with reduced costs in the development pipeline (Oprea & Mestres, 2012). While the time required for a new drug to reach the market ranges from 10 to 17 years, a drug identified by repositioning takes from 3 to 10 years, with a greater approval rate. About 25% of repurposed candidates move on from Clinical Phase II to approval (Hodos et al. 2016). Additionally, the stability, large-scale synthesis and manufacturing issues are already known. This approach also has been a viable alternative to the discovery of new drugs against neglected diseases (Ferreira & Andricopulo, 2016). However, one important limitation is the cost for clinical trials and it may be difficult with the absence of a patentable drug.

The most advanced studies are for sertraline, an anti-depressive agent that has been reported to be fungicidal against *C. neoformans* (Zhai et al., 2012; Rhein et al., 2016). Spitzer et al. (2014) selected the antidepressant sertraline, by systematic screens against different fungal pathogens with sub-concentrations of fluconazole. This fungicidal combination was effective against all species tested, including drug-resistant clinical isolates of *Candida*, and in an *in vivo* model of

Table 3
GO terms enrichment with the essential genes cited in the section 2.3.

Specie	GO ID	Hit	Description	Category	p-value
C. albicans	GO:0036180	11	Filamentous growth of a population of unicellular organisms in response to biotic stimulus	Biological process	0,00428
	GO:0006696	8	Ergosterol biosynthetic process	Biological process	0,01671
	GO:0005934	4	Cellular bud tip	Cellular component	0,01820
	GO:0035690	13	Cellular response to drug	Biological process	0,02681
	GO:0030150	3	Protein import into mitochondrial matrix	Biological process	0,04964
	GO:0008299	3	Isoprenoid biosynthetic process	Biological process	0,04964
	GO:0019287	3	Isopentenyl diphosphate biosynthetic process, mevalonate pathway	Biological process	0,04964
	GO:0006031	3	Chitin biosynthetic process	Biological process	0,04964
	GO:0008144	3	Drug binding	Molecular function	0,04964
A. fumigatus	GO:0019878	5	Lysine biosynthetic process via aminoadipic acid	Biological process	0,00134
	GO:0003735	5	Structural constituent of ribosome	Molecular function	0,00628
	GO:0006048	3	UDP-N-acetylglucosamine biosynthetic process	Biological process	0,01901
	GO:0006412	3	Translation	Biological process	0,01901
C. neoformans	GO:0003735	6	Structural constituent of ribosome	Molecular function	0,00113
	GO:0032543	5	Mitochondrial translation	Biological process	0,00197
	GO:0005763	3	Mitochondrial small ribosomal subunit	Molecular function	0,00423
	GO:0019287	2	Isopentenyl diphosphate biosynthetic process, mevalonate pathway	Biological process	0,01102
	GO:0030148	2	Sphingolipid biosynthetic process	Biological process	0,03078
	GO:0016126	2	Sterol biosynthetic process	Biological process	0,03078
	GO:0008360	2	Regulation of cell shape	Biological process	0,03078
Dimorphic fungi	GO:0005741	2	Mitochondrial outer membrane	Cellular component	0,01569
	GO:0031505	2	Fungal-type cell wall organization	Biological process	0,03660

C. neoformans infection in *Galleria mellonella*. Infections that affect the central nervous system (CNS) are a great challenge because of the blood-brain barrier. The fact that sertraline acts on CNS receptors suggests a therapeutic success of this combination in the treatment of fungal meningoencephalitis. Zhai et al., (2012) showed sertraline antifungal potential against several clinical isolates of *C. neoformans* (MIC 2-6μg/mL), and the majority of these isolates showed synergistic effect with fluconazole. These authors also proved *in vivo* antifungal activity in a murine model of cryptococcosis since sertraline reduces the fungal burden alone or in combination with fluconazole. Treviño-Rangel et al., 2016 presented that MIC values of sertraline can range between 1 and 8 μg/ml (MIC90= 4 μg/ml) against several clinical isolates from *C. neoformans*. Corroborating the results found by Zhai et al., (2012), for *in vivo* treatment, the effective sertraline dose was 15mg/kg, with significant reduction of the fungal burden in the brain and spleen in murine model of cryptococcosis. These promising results led to the first clinical study to determine whether adjunctive sertraline therapy with Amphotericin B and fluconazole will lead to improved survival (NCT01802385). The patients treated with sertraline had faster cryptococcal CSF clearance and lower relapse rates than those reported in the past (Rhein et al., 2016).

The *in vitro* antifungal activity of statins has been frequently reported. The best antifungal activity has been described against yeast. In general, synthetic statins are more active than natural ones (Galgóczy et al., 2011). Although these compounds are currently used to treat hypercholesterolaemia, they inhibit the activity of fungal HMG-CoA reductase (Lorenz et al., 1990), which is upstream of the ergosterol biosynthesis pathway. In clinical practice, it has also been reported that the administration of statins to hospitalized patients reduced the risk of postoperative infectious complications (Ma et al.,2015) and decreased cultures positive for *Candida* species in diabetic patients (Spanakis et al., 2010). However, *in vivo* studies that prove this activity are scarce. Atorvastatin (ATO) was tested as an adjuvant to control infections caused by *C. gattii*. In an animal model, infected mice treated with ATO+FLC showed increased survival and better clinical outcome (Ribeiro et al., 2017).

Auranofin, an FDA-approved drug for the treatment of rheumatoid arthritis, inhibits growth of relevant pathogenic fungi, with different MIC values: *C. albicans* (0.25–1μg/ml), *C. parapsilosis* (1 – ≥16μg/ml), *C. glabrata* (1–16μg/ml), *C. neoformans* (1–2μg/ml), *A. fumigatus* (2 – 4 μg/ml), *S. apiospermum* (1– 4 μg/ml) and *L. prolificans* (2 – 8 μg/ml) (Wiederhold et al., 2018). Considering the auranofin properties, including oral bioavailability, clinical safety, potent broad-spectrum fungicidal activity, and the ability to cross the blood-brain barrier, this compound present advantageous qualities to be repurposed as an antifungal agent. The *in vivo* antifungal activity of auranofin against *C. neoformans* was promising significantly reducing the fungal load in infected *Caenorhabditis elegans* (Thangamani et al., 2017).

Non-steroidal anti-inflammatory drugs, including aspirin and ibuprofen, are commonly used to ameliorate fever and other symptoms of different illness. However, a new indication as alternative drugs to control *Cryptococcus* growth has been proposed (Ogundeji et al., 2016). Some studies have demonstrated anti-biofilm activity of aspirin against *C. albicans*. This effect was observed both with the aspirin alone (Alem et al., 2004) and with it combined with a conventional antifungal (Rosato et al., 2016). Additionally, ibuprofen has also shown promising antifungal activity against *Candida* species, especially when associated with fluconazole (Pina-Vaz et al., 2005). Some studies have shown the ability of this drug to reverse resistance related to efflux activity in some clinical isolates from *Candida* species (Pina-Vaz et al., 2005, Sharma et al., 2015). Ibuprofen as a repurposed drug was able to reduce kidney burden when combined with fluconazole in the murine model of systemic *Candida* infection (Costa-de-Oliveira et al., 2015).

Among the compounds originally described as anti-parasitic several classes have been described with antifungal activity: Chloroquine, Quinacrine, Oxyclozanide and benzimidazoles. Chloroquine was also found to be effective against the yeasts *C. albicans* (Shinde et al., 2013), *Histoplasma capsulatum* (Newman et al., 1994) and *C. neoformans* (Khan et al., 2005). The anthelmintic oxyclozanide used in clinical veterinary showed antifungal activity against both sensitive and resistant clinical isolates of *Candida* species, with MIC values range 16 – 32 μg/mL. The authors propose a mechanism of action by uncoupling the mitochondrial electron transport from oxidative phosphorylation and disturbing the mitochondrial membrane potential (Pic et al., 2018). Flubendazole, a benzimidazole previously described as an antiparasitic drug, showed potent *in vitro* activity against *Cryptococcus neoformans*, with a modal MIC 0.06 μg/mL. In the murine model, the maximal dose of flubendazole (12 mg/kg of body weight/day) orally resulted in 2 log10CFU/g reduction in fungal burden as compared with control (Nixon et al., 2018). In addition, flubendazole was equally effective against fluconazole-resistant isolates (Truong et al., 2018).

Compound DM262 was identified in the Malaria Box (Medicines for Malaria Ventures, Geneva, Switzerland) with significant antifungal activity against *C. neoformans* (16- to 32-fold-higher activity than

fluconazole), *Lomentospora prolificans*, *C. gattii* and *C. albicans* (Jung et al., 2018). NSC319726, a thiosemicarbazone compound, was selected from the cancer therapeutic agent library (~3000 compounds) which was screened for activity against a panel of pathogenic fungi including *Candida* species, *Aspergillus fumigatus*, and *Cryptococcus neoformans*, with MIC values ranging from 0.1–2.0μg/ml for these fungi. Strikingly, NSC319726 was also highly inhibitory in multidrug-resistant isolates of *Candida* species (Sun et al., 2017).

The antimycobacterial drug clofazimine, which targets membrane lipids, was shown to act synergistically with caspofungin and posaconazole against C. *albicans* and *A. fumigatus*. This drug dramatically improved the efficacy of caspofungin in non-*albicans Candida*. Combination therapy with clofazimine and caspofungin reversed a lethal *C. albicans* infection in the invertebrate host *G. mellonella*. However, in a mouse model of Candidemia, the drug combination was not effective in controlling the infection (Robbins et al., 2015).

Thus, the great challenge of new antifungal development is the eukaryotic nature of those pathogens, sharing metabolic pathways with their hosts. This results in difficulties in identifying targets that provide sufficient fungal selectivity (Butts et al., 2017). However, regardless of the limited repertoire of antifungal agents, there are abundant approved medications that may exhibit or enhance antifungal activity at reduced costs, increasing the possibility of new therapeutic options, as summarized in Fig. 2.

3. Biopharmaceuticals as new therapies

3.1. Monoclonal antibodies

Monoclonal antibodies are relatively recent tools for the treatment of infectious diseases. Whereas other areas of Medicine started using monoclonal antibodies in the late 1980's and 1990's, the first anti-infective antibody to reach the market was palivizumab in 1998. Other antimicrobial drugs based on monoclonal antibodies would only be FDA-approved fourteen years later. In the last six years the number of FDA-approved monoclonal antibodies to treat infectious diseases has increased to five. None of these, however, are used to treat diseases caused by fungi.

One of the reasons why monoclonal antibodies are becoming so useful to treat infectious diseases is because they can have great specificity for their targets. Given the similarities in the physiology between fungi and humans, this characteristic could result in drugs with a better safety profile in comparison with those that are routinely used today. A second argument in favor of the development of antifungal monoclonal antibodies is that these molecules can modulate and enhance the host's immune response to fungi. Most systemic mycoses are opportunistic, affecting immunocompromised people such as those with AIDS, neutropenia, hematological cancers and use of immunosuppressants. Moreover, in several of these diseases much of the damage to host tissues is a result of overt inflammation. Thus, the immunomodulation conferred by monoclonal antibodies can also treat invasive mycoses by altering the immune dysfunction that renders the host susceptible to infection and dampening the immune-mediated tissue injury. On the other hand, targets for monoclonal antibodies are largely restricted to antigens that are secreted or present on the cell surface, which limits the number of available drug targets.

The first – and so far only – antifungal drug based on monoclonal antibodies to reach late-stage clinical development is efungumab. Previously called Mycograb, it was a recombinant fragment of a human monoclonal antibody that recognized the *C. albicans* molecular chaperone HSP90 (Matthews et al., 1995). It was shown to be protective in animal candidiasis models (Matthews et al., 1995) and to have direct antifungal activity against several *Candida* species, as well as strong synergy with amphotericin B (Matthews et al., 2003). A Phase III clinical

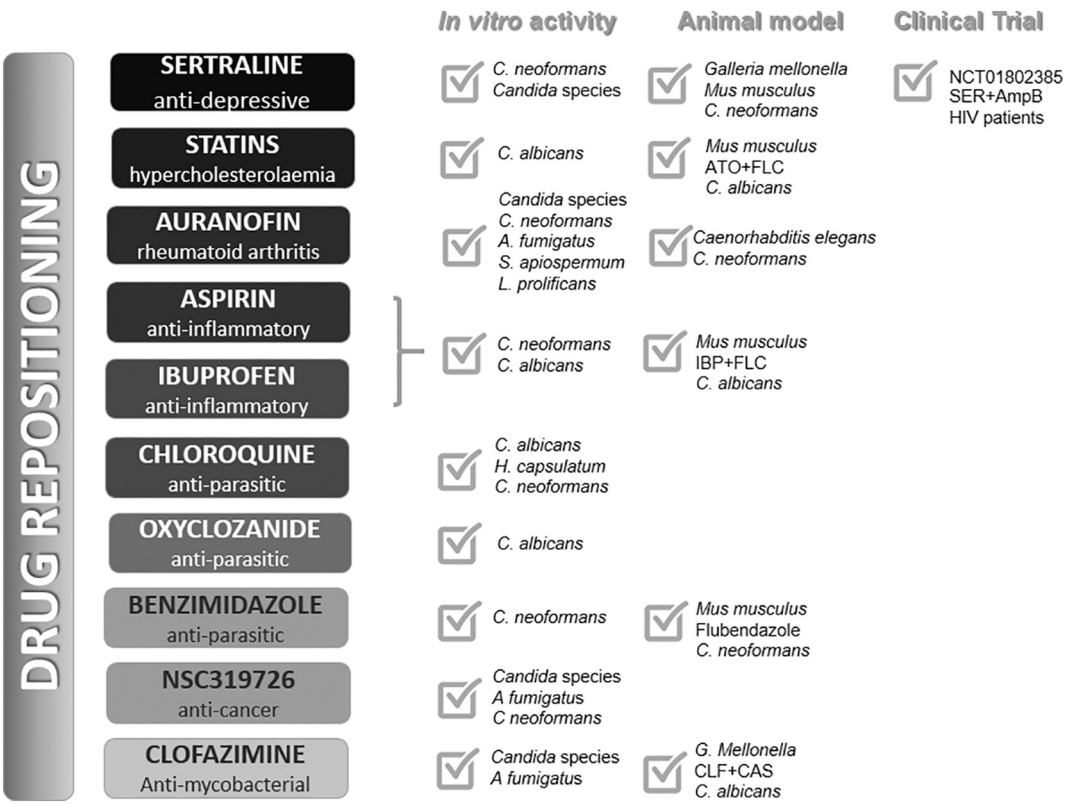

Fig. 2. Drug repositioning for fungal infection treatment. Ten drug options already available on the market that have promising antifungal activity. Among those options, the one that is most advanced in the development stages is sertraline, which in 2015 started a phase III randomized trial in HIV-infected adults with cryptococcal meningitis. ATO (Atorvastatin), FLC (fluconoazole), SER (sertraline), AmpB (Amphotericin B), IBP (Ibuprofen), CLF (Clofazimine), CAS (caspofungin).

trial with invasive candidiasis patients also had encouraging results (Pachl et al., 2006). However, the European Medicines Agency (EMA) refused twice, in 2006 and 2007, to grant marketing authorization to efungumab due to concerns with production and purification of the recombinant antibody fragment. No further development of efungumab seems likely after its synergistic effect with amphotericin B was shown to be non-specific (Louie et al., 2011).

The only other antifungal antibody to reach clinical development was 18B7, which targets the *Cryptococcus neoformans* capsule polysaccharide glucuronoxylomannan (GXM). In a Phase I Trial, this murine monoclonal antibody was administered to HIV-positive patients with a history of adequately treated cryptococcal meningitis (Larsen et al., 2005). No further clinical studies were performed, but this antibody became an important research tool to study cryptococcal host-pathogen interaction that is widely shared by the group that developed it with the research community. Despite being the most used antibody to *C. neoformans* GXM, it is not the only and not even the first one to have been developed. The first one, called E1, was generated by immunizing mice with purified capsular polysaccharide (Dromer et al., 1987). This same antibody was later shown to be protective in animal models of cryptococcosis when administered alone (Dromer et al., 1987), and also to boost the therapeutic effect of amphotericin B (Dromer and Charreire, 1991). Almost simultaneously, another group also generated three monoclonal antibodies to the cryptococcal capsule by immunizing mice with polysaccharide coupled to sheep red blood cells (Eckert and Kozel, 1987). Isotype switch variants were later generated and tested *in vitro* (Schlageter and Kozel, 1990) and in murine models of candidiasis (Sanford et al., 1990), showing a relative therapeutic efficacy.

Another group obtained many other monoclonal antibodies from mice infected with *C. neoformans* cells (Casadevall and Scharff, 1991) and immunized with polysaccharide-protein conjugates, which made the capsule material more immunogenic (Casadevall et al., 1992). The large number of monoclonal antibodies made against the *C. neoformans* capsule by several research groups led to dozens of publications describing in detail the mechanisms by which monoclonal antibodies protect the host and can thus work as antifungal agents. Casadevall and Pirofski classified these mechanisms into three large groups: 1) direct effects on the fungal cell targeted by the antibody; 2) immunomodulation and enhancement of the immune response to fungi; 3) neutralization of fungal components that are toxic to the host (Casadevall and Pirofski, 2012).

Many other monoclonal antibodies have shown promising results against fungi in pre-clinical tests. Some of these have the potential to become broad-spectrum antifungals, such a monoclonal antibody (2G8) to beta glucans that is protective in animal models of systemic candidiasis, vaginal candidiasis and invasive aspergillosis (Torosantucci et al., 2005) as well as cryptococcosis (Rachini et al., 2007). The variable domain sequences from this antibody were used to generate a chimeric human/murine IgG by heterologous expression in plants, and this chimeric antibody was shown to bind β-glucans and protect in systemic and vulvovaginal candidiasis animal models (Capodicasa et al., 2011). A chimeric human IgA1 using 2G8 variable sequences was also produced in plants, and successfully inhibited *C. albicans* adherence (Capodicasa et al., 2017). Another series of broad-spectrum antibodies was raised against fungal melanin (Rosas et al., 2000), and these antibodies were protective in mice infected with *C. neoformans* (Rosas et al., 2001).

Numerous antibodies that are specific for antigens present in one or a few related fungal species have also been generated, as exemplified below:

- *Candida albicans*: an antibody raised against the cell wall bound mannoproteins and had direct fungicidal activity (Moragues et al., 2003); it was also protective in mice infected systemically with *C. albicans* (Sevilla et al., 2006).
- *Aspergillus fumigatus*: also raised against the fungal cell wall, the monoclonal antibody A9 bound to the cell wall, had direct fungicidal effects and was very effective in murine aspergillosis models (Chaturvedi et al., 2005).
- *Paracoccidioides brasiliensis*: an antibody to gp43, an immunodominant antigen from this dimorphic fungus, was protective in vitro and in vivo (Buissa-Filho et al., 2008).
- *Histoplasma capsulatum*: immunization of mice with *H. capsulatum* yeast cells led to the generation of an antibody that binds to a histone-like protein present in the fungal cell wall; passive immunization with this antibody resulted in decreased disease intensity and prolonged survival in murine models of histoplasmosis (Nosanchuk et al., 2003).

Despite the large number of monoclonal antibodies with promising *in vitro* and *in vivo* efficacy against pathogenic fungi, no drugs of this class are currently in clinical development. A large share of the literature on antifungal antibodies is more than a decade old, reflecting a certain loss of momentum in terms of drug development. This could change, however, due to three forces: 1) a large body of knowledge has accumulated on immunoglobulin anti-infective mechanisms of action, which could lead to rational design of more effective drugs; 2) novel technologies such as engineering of glycans and constant chains can create antibodies that are safer and more effective; 3) a drastic decrease in monoclonal antibody manufacturing costs that resulted from the expiration of patents and incremental increases in industrial scale productivity.

3.2. Cytokine immunotherapy

Cytokines are molecules that mediate cellular communication, which is necessary for proper immunity. An immunocompromised host frequently underlies systemic mycoses and affects cytokine signaling, so a different immunotherapeutic approach involves using cytokines to induce protective immune responses.

Recombinant macrophage colony-stimulating factor (M-CSF), which has been in clinical use to treat neutropenia for a long time (Lyman et al., 2010), should be an important strategy for immunotherapy in systemic mycoses. For recipients of allogeneic hematopoietic stem-cell transplantation, treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF) has shown a better response to transplantation-related mortality, cumulative mortality and invasive fungal disease-related mortality in phase IV clinical trial (Wan et al., 2015 - NCT01232504). These results support the hypothesis that prophylactic therapy during neutropenia with GM-CSF decreases the time a patient endures neutropenia and reduces an important risk factor for invasive aspergillosis and candidemia. However more clinical trials must be done, because another study with an experimental model using granulocyte colony-stimulating factor (G-CSF) showed a different result. The infusion of G-CSF is associated not only with increased neutrophil bactericidal activity but also with T helper lymphocyte (Th) 2 rather than Th1 stimulation, and in that study the efficient adaptive immune response to fungal infection was reduced (Valente et al., 2002).

The cytokine pattern changes according to the clinical form of paracoccidioidomycosis patients, among whom the patients with asymptomatic, juvenile and adult forms presented a predominance of Th1, Th2 and Th17 response, respectively (de Castro et al., 2013). Considering an efficient adaptive immune response, the production of interferon gamma (IFN-γ) and induction of cell-mediated immunity to some fungal infection is of critical importance in host defense. IFN-γ is an important cytokine produced by Th1 cellular immunity, and it is beneficial for fungal clearance in *Cryptococcus neoformans* meningoencephalitis (CM) and *Paracoccidioides brasiliensis* infection.

A randomized controlled trial of IFN-γ treatment in HIV-associated cryptococcal meningitis has shown that although the fungal burden

decreased after the addition of short-course IFN-γ to standard treatment, with no side effects, mortality was the same among the groups (Jarvis et al., 2012). Fungal growth is only one aspect of the disease, although inflammatory response could influence the pathology. The presence of IFN-γ-producing CD4 T cells in the central nervous system (CNS) increased the *C. neoformans*-clearance, albeit with high mortality of mice. Depletion of these cells decreases IFN-γ production and fungal clearance, although it prevents the development of clinical symptoms and mortality (Neal et al., 2017). In the same way, the experimental treatment with rIL-12 of mice infected with *P. brasiliensis* showed a decreased fungal burden with an increase in IFN-γ production, during a high pulmonary inflammation (Arruda et al., 2002). These findings suggest that although CD4 T- producing IFN-γ is necessary for fungal control, it concurrently contributes to clinical symptoms and disease. Therefore, more clinical trials must be done to understand what the complex influence of cytokine therapy could induce in patients with different forms of fungal disease.

3.3. Vaccines

The balance of host resistance and the limitation of damage caused by fungal infection are very important for the development of novel therapeutic strategies such as vaccines. Most invasive infections occur as a result of altered immune status, so immune dysregulation is one important aspect to be considered when we analyze the immunopathology in fungal disease (reviewed by Lionakis & Levitz, 2018). It is important to consider that vaccines for fungal infections have not been licensed yet, and there are only vaccine candidates in pre-clinical studies or in clinical trials (Fig. 3).

Considering prophylactic vaccines, one peptide that has shown interesting results is P10, a T-cell epitope of the 43 kDa glycoprotein (gp43). Immunization of mice experimentally infected with *Paracoccidioides brasiliensis* increased the activity of TCD4+ cells and improved host immunity (Taborda et al., 1998). The immunization with P10 combined with antifungal drugs improved the survival of anergic-infected mice, representing a powerful adjuvant therapy to chemotherapy (Munõz et al., 2014). Host immunity can be improved when a chimera of P10 and hepatitis B virus-derived particle (VLP), as an antigen carrier, is used in an immunoprophylaxis assay (Holanda et al., 2017).

Other proteins, such as Als3p, an adhesion of *Candida albicans*, induced a rapid production of anti-Als3p antibodies and enhanced Th1 and Th17 cell response (Schmidt et al., 2012). The NDV-3A (rAls3) and NDV-3 (rAls3 with 6-His tag) antigens are in phase 1b and phase 2a, respectively, to preventing recurrent vulvovaginal candidiasis (RVVC) (NCT01926028).

One strategy to produce an effective vaccine delivery system is the use of conserved components of the cell wall, such as β-glucan. A panfungal vaccine using this carbohydrate of *Saccharomyces cerevisiae* induced a protective immune response to several fungi (Liu et al., 2011), as confirmed against coccidioidomycosis (Clemons et al., 2015) and aspergillosis (Clemons et al., 2014), in a murine model, which provides the basis for development of a pan-fungal vaccine. *S. cerevisiae* can also be a suitable vehicle for immunization, whilst expressing specific proteins of fungus, such as gp43 (Assis-Marques et al., 2015). In the same way, β-glucan-containing *C. neoformans* alkaline particles showed a significant reduction of lung damage and robust Th1 and Th17-biased CD4 T cell activation when hosts were infected experimentally with *C. neoformans* or *C. gatti* (Specht et al., 2015).

Immunization of mice with a *C. neoformans* strain engineered to produce an immune response enhancer, such as Th1-type cytokine gamma interferon (IFN-γ), could be a good strategy for a new vaccine. In a *C. neoformans* murine experimental infection, there was complete eradication of fungi during the second challenge, and hosts showed a robust Th1 protective response (Wormley et al., 2007).

Most prophylactic vaccines obtain their maximal efficacy in immunocompetent hosts, so their application can be used for individuals before an immunosuppressive situation, such as prior to treatment with

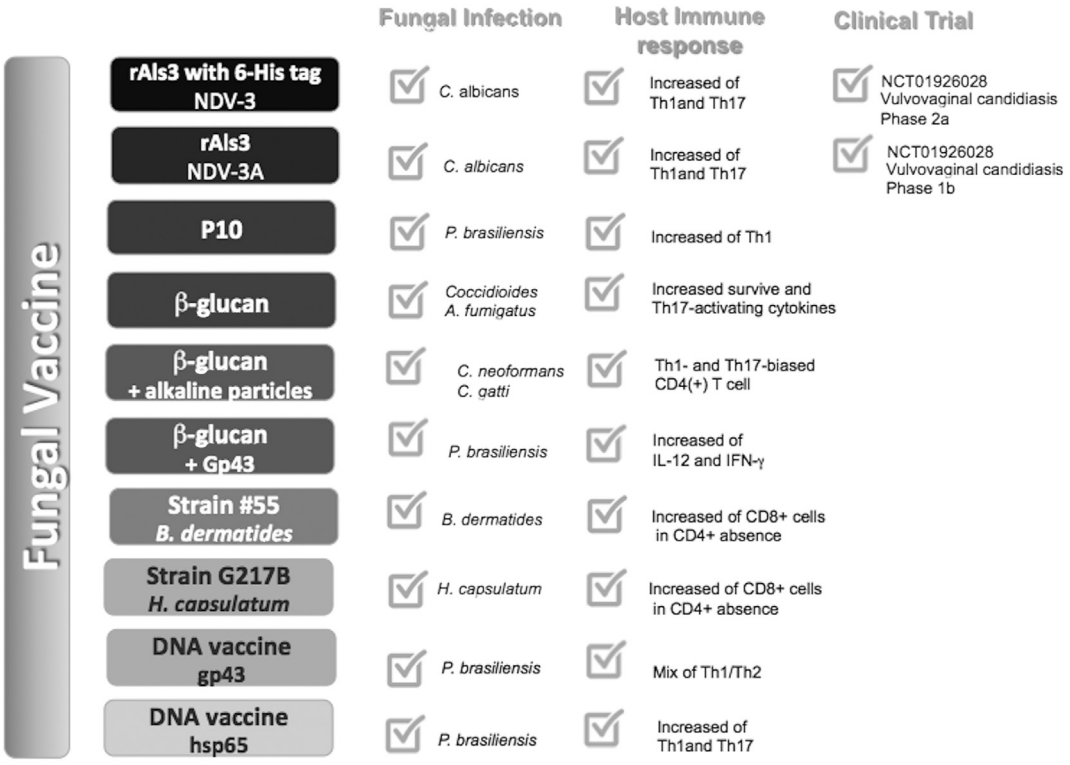

Fig. 3. Fungal Vaccine. Fungal vaccines developed in murine model and in clinical trials for candidiasis, cryptococcosis, aspergillosis, paracoccidoidomycosis, coccoidoidomycosis, histoplasmosis and blastomycosis. The improvement of host immune response is indicated too.

immunosuppressive drugs. However, an immunocompromised situation is not easy to predict. Prophylactic vaccines were developed for immunocompromised mice, before experimental infection with *Blastomyces dermatitides* or *Hystoplasma capsulatum*, inducing long-lasting memory CD8+ T cells in the absence of CD4+ T cells (Wüthrich et al., 2003). The antifungal CD8+ T cells produce IL-17A, which is indispensable to augmenting adaptive immunity, demonstrating that residual elements of a compromised immune system can be effectively activated (Nanjappa et al., 2012).

Another alternative strategy for the immunocompromised individual is the development of therapeutic vaccines, which stimulate the host immune response after fungal infection. One possibility is the enhancement of dendritic cells (DC) through P10-pulsed DC, which induced a protective response against *P. brasiliensis* in immunosuppressed mice. The therapeutic efficacy of DC was augmented by association with trimethoprim-sulfamethoxazole treatment, with a Th1 immune response and reduced tissue damage (Silva et al., 2017).

As a therapeutic vaccine, DNA vaccines have been shown to be a good strategy. The immunization of mice with plasmid DNA containing Gp43 of *P. brasiliensis* was able to induce a specific humoral immune response with a mixed Th1/Th2 cellular immune response in experimental murine infection. The immunized mice showed decreased fungal colony forming units (CFUs) in the lung and reduced dissemination to other organs (Pinto et al., 2000). This therapeutic function was also observed when mice experimentally infected with *P. brasiliensis* were treated with a plasmid containing P10 and IL-12 (Rittner et al., 2012) or a DNAP10 mixture with a TLR-5-engaging-molecule, i.e. flagellin (Braga et al., 2009). Lung architecture was restored, and the fungus was eradicated, besides activating the prevailing Th1-type immune response.

Another strategy is the use of heat-shock proteins, which are molecules used as immunomodulators in different types of diseases. The gene of HSP65 from *Mycobacterium leprae* has shown prophylactic (Ribeiro et al., 2009) and therapeutic (Ribeiro et al., 2010) activity in an experimental model of Paracoccidioidomycosis. Immunization with DNAhsp65 induced an increase in Th1-type cytokine levels and a fall in fungal burdens associated with a reduction in collagen deposition and lung remodeling. DNAhsp65 has also been used as an adjuvant in experimental chromoblastomycosis treatment, and it has shown a higher reduction in the fungal burden than antifungal drugs alone (Siqueira et al., 2013). Despite all the approaches studied, the challenge of conducting rationally designed clinical trials using these vaccines still remains.

3.4. Antifungal peptides

Antimicrobial peptides (AMPs) are evolutionary conserved molecules acting on the innate immune response of organisms as diverse as prokaryotes, plants and humans (Brown and Hancock, 2006; Giuliani et al., 2007; Pasupuleti et al., 2012). These small cationic molecules are known for their role in innate immune response by acting both by directly killing microbial pathogens and/or indirectly by modulation of the host immune system, or activation of secondary host defenses (Nijnik and Hancock, 2009; Steinstraesser et al., 2011). They are generally defined as peptides of less than 100 amino acid residues, rich in hydrophobic and positively charged amino acid residues such as lysine and arginine and they fold into amphipathic conformations that are crucial to their interaction with microbial membranes.

Although most AMP studies focus on their activity on bacterial cells, the number of natural or synthetic AMPs presenting antifungal activity is increasing (Blondelle and Lohner, 2000; Ciociola et al., 2016). AMPs, which were shown to primarily act on fungal cells, have a greater therapeutic potential considering the need for molecules that produce less damage to patient's microbiota (Marcos et al., 2012; Matejuk et al., 2010). Most of the described antifungal peptides were studied against *Candida albicans*, but many of them also act in several other human and plant fungal pathogens, including other *Candida* species, *Aspergillus* spp, *Cryptococcus neoformans*, *Sporothrix schenckii* and thermodimorphic fungi (Ciociola et al., 2016; Datta et al., 2016; Galvez et al., 1993; Lupetti et al., 2008; Pushpanathan et al., 2012; Silva et al., 2011).

Among the many families of antifungal peptides, we have cecropins, magainins, dermaseptins, cathelicidins, defensins and peptides derived from other proteins, such as lactoferrin and the bactericidal-permeabilizing protein (BPI) (De Lucca and Walsh, 1999; Lakshmaiah Narayana and Chen, 2015; Matejuk et al., 2010; Zasloff, 1987). Histatins are one of the most studied antifungal peptides, and current peptides in clinical trials are derived from them. They are linear cationic peptides isolated from human saliva (Oppenheim et al., 1988). These peptides present strong and specific activity against fungi, including *Candida* spp, *Cryptococcus neoformans* and *Aspergillus fumigatus* (Marcos et al., 2012; Matejuk et al., 2010; Oppenheim et al., 1988; Tsai and Bobek, 1997). Other important group is defensins, small disulfide-stabilized antimicrobials found in fungi, animals and plants. Defensins were shown to present direct antimicrobial activity against *Candida albicans* and also immunomodulatory activities (Alcouloumre et al., 1993; Marcos et al., 2012; Vylkova et al., 2007). Extensive reviews from different groups about members of different classes of antifungal peptides are available (Ciociola et al., 2016; Duncan and O'Neil, 2013; Hegedüs and Marx, 2013; Matejuk et al., 2010) (Table 4).

3.4.1. Antifungal peptides as potential therapeutics

The main challenges for the clinical use of antifungal peptides involve the time and cost requirements associated with the lower therapeutic index of antifungal compounds. Despite the large number of identified AMPs showing potent antifungal activity *in vitro* and in animal models, only a few have undergone clinical trials and an even smaller group were able to proceed on this pathway showing safety and *in vivo* efficacy (Duncan and O'Neil, 2013; Lakshmaiah Narayana and Chen, 2015).

The main drawbacks threatening the clinical development and use of AMPs are their high costs of synthesis, screening and production, systemic and local toxicity, reduced activity under physiological conditions such as salt concentration, the presence of serum or pH sensitivity, susceptibility to proteolysis, pharmacokinetic and pharmacodynamics issues, possibility of sensitization and allergy after repeated applications, inhibited diffusive transport across membranes and their high molecular weight. Additionally, many AMPs showing potent antifungal activity *in vitro* can only act in animal models at very high concentrations, many times very close to the toxic doses for mammalians or they could not be proven safe or efficient in clinical use (Gordon et al., 2005; Greber and Dawgul, 2017).

Regarding the costs of AMP production, the values can be 5 to 20 times higher than the ones for the synthesis of small-molecule drugs (Giuliani et al., 2007). The strategies to decrease these costs and efficiently scale up production of those molecules include peptide size reduction, and new methodologies for *de novo* synthesis, such as hybrid synthesis methodologies based on the combination of solid and solution-phase synthesis. (Duncan and O'Neil, 2013; Kang et al., 2014).

Issues regarding their stability and toxicity drove the development of antifungal peptides mostly for topical applications, which are a safer and easier option. The main strategies to overcome the instability of antifungal peptides under physiological conditions have involved chemical modifications of those molecules such as amidation, acetylation, cyclization and the introduction of D or non-natural amino acids. In addition to those, modifications in their amphipathic balance and reduction in their content of cationic residues have been shown to help to increase their half-lives (Duncan and O'Neil, 2013; Greber and Dawgul, 2017; Kang et al., 2014). Changes in the overall hydrophobicity of AMPs have been one of the main strategies used and have been shown to reduce their hemolytic and cytotoxic effects on host cells.

Other strategies include the development of better delivery systems to selectively target AMPs, such as nanoencapsulation, adsorption to nanocarries, liposomal formulations, PEGylation and use of other drug delivery systems (Mahlapuu et al., 2016).

3.4.2. Antifungal peptides in clinical trials

Among the current AMPs in clinical trials are NP213 (Novexatin), NP339 (Novamycin), VL-2397 and PAC-113 (P-113). Phase 1 and Phase 2 studies indicate that NP213 (novexatin), a cyclic cationic peptide is safe and effective for treatment of onychomycosis caused by dermatophytes and non-dermatophyte fungi. Novamycin (NP339) is a poly-arginin-based cationic peptide in pre-clinical development for topical treatment of *Candida* spp and *Aspergillus* spp infections and has also been shown to be active against *Cryptococcus* spp and *Trichosporum* spp (Greber and Dawgul, 2017). It has been formulated for inhalation use and it is the first peptide designed for treatment of systemic and mucocutaneous diseases. VL-2397 is in phase 2 clinical trials for treatment of invasive fungal infections, especially aspergillosis. Finally, there is PAC-113 (or P-113), a 12-residue peptide derived from histatin, which is currently in Phase 2 clinical trials for topical treatment of oral candidiasis in HIV patients. Results from Phase 1 and Phase 2 clinical trials indicate that PAC-113 can be used in the treatment of fungal gingivitis. This antifungal peptide acts causing membrane permeabilization, ROS production after interactions with fungal mitochondria and changes in cytokine production. Additionally, PAC-113 is active against both planktonic and biofilm cells (Greber and Dawgul, 2017; Mishra et al., 2017,Gordon et al., 2005; Kang et al., 2014). Other antifungal peptides had reached clinical trials but did not succeed such as hLF1-11, a human-lactoferrin-derived peptide

In conclusion, a cheaper, less toxic, more stable and less prone to degradation antifungal peptide suitable for clinical use is still a long way off. However antifungal peptides can still be considered promising new molecules for antifungal therapy. The accumulated knowledge of their mechanisms of action, and the solution of current problems such as high costs and toxicity will probably bring more of these molecules to clinical use not only for topical application, but also for systemic treatments, alone or in combination with available conventional antifungals.

4. Concluding remarks and future prospects

This review focused on the main existing antifungal drugs for clinical use and new molecules in research and development. Enzymes belonging to the ergosterol pathway biosynthesis and fungal cell wall, especially involving 1,3-β-glucan and chitin production, are the main targets of commonly antifungal medicines today. Besides this, new small molecules are also in development against known targets involving GPI-anchor enzymes, sphingolipids and sterols, cell signaling molecules, metabolic pathway of pyrimidines, amino acids, iron and carbohydrates (glyoxylate cycle), and others

Table 4
Current antifungal peptides in clinical trials.

Antifungal peptide	Description	Indication	Preclinical	Phase I	Phase II	Phase III
PAC-113 (P-113)	AMP derived from a histatin protein found in saliva. (Pacgen)	Oral candidiasis in HIV seropositive patients	Completed	Completed	Completed	
NP213 (Novexatin)	A cyclic cationic peptide. (Novabiotics)	Fungal nail infections caused by dermatophytes and non-dermatophyte fungi	Completed	Completed	Phase IIa completed	
VL-2397 (ASP2397)	Cyclic hexapeptide that chelates aluminum ions (Vical)	invasive fungal infections, especially aspergillosis	Completed	completed	Recently started	
CZEN-002	A synthetic octapeptide, derived from alpha-Melanocyte-Stimulating Hormone (a-MSH) (Zengen)	Vulvovaginal *C. albicans* infections	Completed	Completed	Completed	
hLF1–11	Human lactoferricin based 11 I/II stem cell (AM-Pharma)	Candidemia and other fungal infections associated with Hematopoietic Stem Cell Transplantation	Completed	Completed	Withdrawn	
NP339 (Novamycin)	A poly-arginin based cationic peptide (Novabiotics)	Topical treatment of *Candida* spp and *Aspergillus* spp infections.	Late pre-clinical			
HB1275 (Cecropin mimetic)	Lipohexapeptide active against yeast and filamentous fungi. (Helix BioMedix)	*Trichophyton* infections	Ongoing			
V13K	α-helical antimicrobial peptide. V681 analog, containing D amino acids. (BioAMPS International)	Candidemia, aspergillosis and other fungal infections	Ongoing			

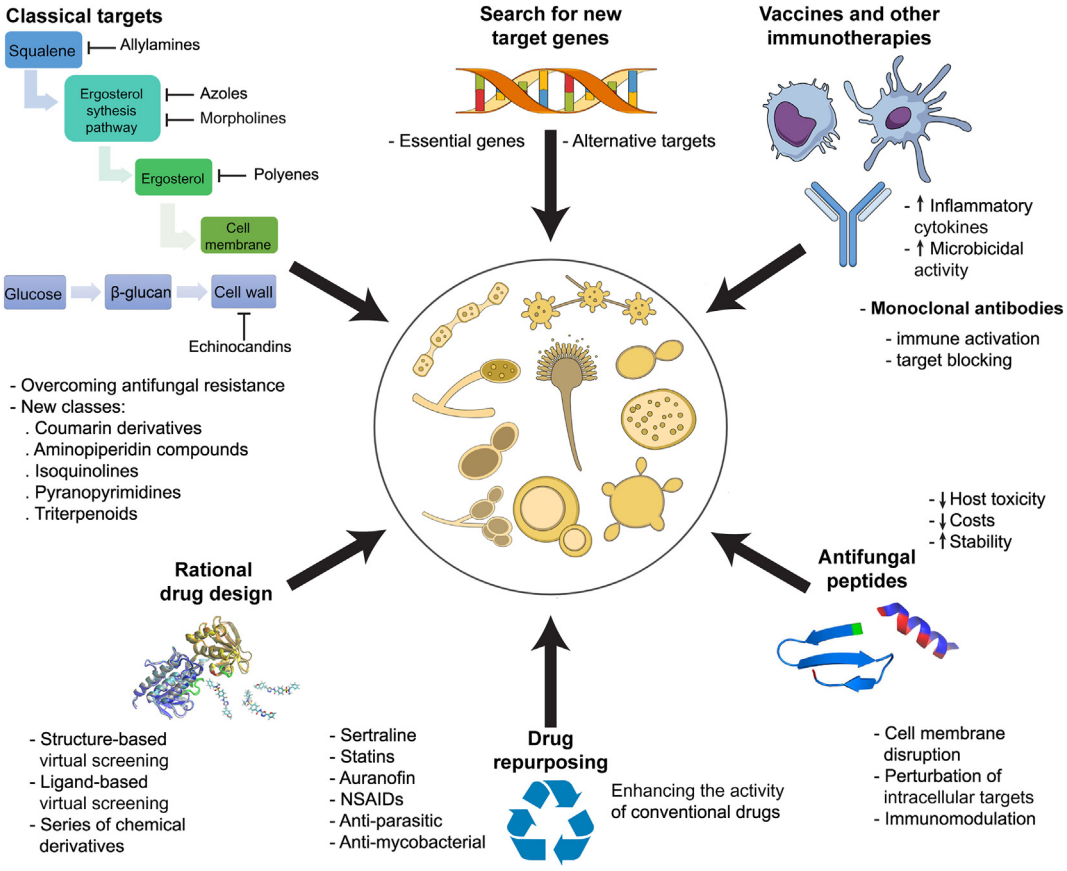

Fig. 4. Summary. The main strategies, which could be used in order to identify new molecules, including biopharmaceuticals, for research and development of new antifungal drugs.

related to the cellular machinery. Recently, molecular genetic approaches (targeted gene mutations, antisense RNA inhibition, insertional mutagenesis, gene replacement and conditional expression) and comparative genomic strategies have contributed to the identification of new essential genes/proteins as drug targets. Rational antifungal drug design against those targets is possible based on molecular docking and virtual screening of small molecules from chemical libraries (Life Chemicals, Zinc, ChEMBL, ChemBridge among others) for drug discovery and hit-to-lead optimization. Inhibitory compounds (hits) of enzymes/proteins, codified by those essential genes with potential antifungal action, have been discovered and tested *in vitro* and/or *in vivo,* showing effective action against several fungal species. In addition, drug repurposing approaches have explored old molecules used for many other diseases, and found they had antifungal activity and were potentially applicable for therapeutic mycosis treatment. The possibility of developing biopharmaceuticals as new antifungal therapies opened a new strategy that can act as a counterpart in treating fungal infection, especially considering host-pathogen interactions and their control. Monoclonal antibodies, cytokine immunotherapy, vaccines and antifungal peptides are now in the pipeline of research and development of new antifungal drugs. Despite the several strategies described in this review, the most important is always to focus on the patient, who needs support and medications that can control the infection and improve quality of life, without facing issues related to the resistance and adverse effects of antifungal drugs currently on the market. Fig. 4 shows all topics discussed in this review, summing up the main strategies, which could be used in order to identify new molecules, including biopharmaceuticals, for research and development of new antifungal drugs, considering the need for better antifungal therapy of systemic infections.

Conflict of interest statement

The authors declare that are no conflicts of interest.

Acknowledgement

The authors would like to thank Dr. Marcus de Melo Teixeira, for his support with the Gene Ontology (GO) assignments. This work was financially supported through a grant from the Pronex-FAPDF/CNPq; CNPq-Rede Pró-Centro-Oeste de Pesquisa; Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF) and Conselho Nacional de Pesquisa (CNPq).

References

Abadio, A. K. R., Kioshima, E. S., Teixeira, M. M., Martins, N. F., Maigret, B., & Felipe, M. S. S. (2011). Comparative genomics allowed the identification of drug targets against human fungal pathogens. *BMC Genomics*, 12. https://doi.org/10.1186/1471-2164-12-75.

Abadio, A. K. R., Kioshima, E. S., Leroux, V., Martins, N. F., Maigret, B., & Felipe, M. S. S. (2015). Identification of new antifungal compounds targeting thioredoxin reductase of *Paracoccidioides* Genus. *PLOS One 10*, e0142926.

Alcouloumre, M. S., Ghannoum, M. A., Ibrahim, A. S., Selsted, M. E., & Edwards, J. E. (1993). Fungicidal properties of defensin NP-1 and activity against Cryptococcus neoformans in vitro. *Antimicrob. Agents Chemother. 37*, 2628–2632.

Alem, M. A. S., & Douglas, L. J. (2004). Effects of aspirin and other nonsteroidal anti-inflammatory drugs on biofilms and planktonic cells of *Candida albicans*. *Antimicrob. Agents Chemother. 48*, 41–47.

Arruda, C., Franco, M. F., Kashino, S. S., Nascimento, F. R. F., Fazioli, R., Vaz, C. A. C., ... Calich, V. L. G. (2002). Interleukin-12 Protects Mice against Disseminated Infection Caused by *Paracoccidioides brasiliensis* but Enhances Pulmonary Inflammation. *Clin. Immunol. 103*, 185–195.

Assis-Marques, M. A., Oliveira, A. F., Ruas, L. P., dos Reis, T. F., Roque-Barreira, M. C., & Coelho, P. S. R. (2015). *Saccharomyces cerevisiae* Expressing Gp43 Protects Mice against *Paracoccidioides brasiliensis* Infection. *PLOS ONE 10*, e0120201.

Bae, M., Kim, H., Moon, K., Nam, S. -J., Shin, J., Oh, K. -B., & Oh, D. -C. (2015). Mohangamides A and B, New Dilactone-Tethered Pseudo-Dimeric Peptides Inhibiting *Candida albicans* Isocitrate Lyase. *Organic Lett. 17*, 712–715.

Bagatin, M. C., Pimentel, A. L., Biavatti, D. C., Basso, E. A., Kioshima, E. S., Seixas, F. A. V., & Gauze, G. (2017). Targeting the Homoserine Dehydrogenase of *Paracoccidioides* Species for Treatment of Systemic Fungal Infections. *Antimicrobial Agents and Chemotherapy 61*, e00165.

Bauer, I., Varadarajan, D., Pidroni, A., Gross, S., Vergeiner, S., Faber, B., ... Graessle, S. (2016). A Class 1 Histone Deacetylase with Potential as an Antifungal Target. *mBio 7*, e00831.

Baxter, B. K., DiDone, L., Ogu, D., Schor, S., & Krysan, D. J. (2011). Identification, *in Vitro* Activity and Mode of Action of Phosphoinositide-Dependent-1 Kinase Inhibitors as Antifungal Molecules. *ACS Chemical Biology 6*, 502–510.

Becker, J. M., Kauffman, S. J., Hauser, M., Huang, L., Lin, M., Sillaots, S., ... Roemer, T. (2010). Pathway analysis of *Candida albicans* survival and virulence determinants in a murine infection model. *Proceedings of the National Academy of Sciences 107*, 22044–22049.

Blondelle, S. E., & Lohner, K. (2000). Combinatorial libraries: A tool to design antimicrobial and antifungal peptide analogues having lytic specificities for structure-activity relationship studies. *Biopolymers 55*, 74–87.

Borba-Santos, L. P., Visbal, G., Gagini, T., Rodrigues, A. M., de Camargo, Z. P., Lopes-Bezerra, L. M., ... Rozental, S. (2016). Δ24-Sterol Methyltransferase Plays an Important Role in the Growth and Development of *Sporothrix schenckii* and *Sporothrix brasiliensis*. *Frontiers in Microbiology 7*.

Boyd, E. F., Carpenter, M. R., Chowdhury, N., Cohen, A. L., Haines-Menges, B. L., Kalburge, S. S., ... Whitaker, W. B. (2015). Post-Genomic Analysis of Members of the Family Vibrionaceae. *Microbiological Spectrum 3*(5).

Braga, C. J. M., Rittner, G. M. G., Munoz Henao, J. E., Teixeira, A. F., Massis, L. M., Sbrogio-Almeida, M. E., ... Ferreira, L. C. S. (2009). *Paracoccidioides brasiliensis* Vaccine Formulations Based on the gp43-Derived P10 Sequence and the Salmonella enterica FliC Flagellin. *Infection and Immunity 77*, 1700–1707.

Brandão, F. A., Derengowski, L. S., Albuquerque, P., Nicola, A. M., Silva-Pereira, I., & Poças-Fonseca, M. J. (2015). Histone deacetylases inhibitors effects on *Cryptococcus neoformans* major virulence phenotypes. *Virulence 6*, 618–630.

Brandão, F., Esher, S. K., Ost, K. S., Pianalto, K., Nichols, C. B., Fernandes, L., ... Alspaugh, J. A. (2018). HDAC genes play distinct and redundant roles in *Cryptococcus neoformans* virulence. *Scientific Reports 8*.

Brown, K. L., & Hancock, R. E. (2006). Cationic host defense (antimicrobial) peptides. *Current Opinion in Immunology 18*, 24–30.

Buissa-Filho, R., Puccia, R., Marques, A. F., Pinto, F. A., Munoz, J. E., Nosanchuk, J. D., ... Taborda, C. P. (2008). The Monoclonal Antibody against the Major Diagnostic Antigen of *Paracoccidioides brasiliensis* Mediates Immune Protection in Infected BALB/c Mice Challenged Intratracheally with the Fungus. *Infection and Immunity 76*, 3321–3328.

Butts, A., Palmer, G. E., & Rogers, P. D. (2017). Antifungal adjuvants: Preserving and extending the antifungal arsenal. *Virulence 8*, 198–210.

Capodicasa, C., Chiani, P., Bromuro, C., De Bernardis, F., Catellani, M., Palma, A. S., ... Torosantucci, A. (2011). Plant production of anti-β-glucan antibodies for immunotherapy of fungal infections in humans: Plant antibodies for human antifungal therapy. *Plant Biotechnology Journal 9*, 776–787.

Capodicasa, C., Catellani, M., Moscetti, I., Bromuro, C., Chiani, P., Torosantucci, A., & Benvenuto, E. (2017). Comparative analysis of plant-produced, recombinant dimeric IgA against cell wall β-glucan of pathogenic fungi. *Biotechnology and Bioengineering 114*, 2729–2738.

Carr, P. D., Tuckwell, D., Hey, P. M., Simon, L., d'Enfert, C., Birch, M., ... Bromley, M. J. (2010). The Transposon impala Is Activated by Low Temperatures: Use of a Controlled Transposition System To Identify Genes Critical for Viability of *Aspergillus fumigatus*. *Eukaryotic Cell 9*, 438–448.

Casadevall, A., & Pirofski, L. (2012). Immunoglobulins in Defense, Pathogenesis, and Therapy of Fungal Diseases. *Cell Host & Microbe 11*, 447–456.

Casadevall, A., & Sharff, D. (1991). The mouse antibody response to infection with Cryptococcus neoformans: VH and VL usage in polysaccharide binding antibodies. *Journal of Experimental Medicine 174*, 151–160.

Casadevall, A., Mukherjee, J., Devi, S. J. N., Schneerson, R., Robbins, J. B., & Scharff, M. D. (1992). Antibodies Elicited by a *Cryptococcus neoformans* -Tetanus Toxoid Conjugate Vaccine Have the Same Specificity as Those Elicited in Infection. *The Journal of Infectious Diseases 165*, 1086–1093.

Chang, C. C., Slavin, M. A., & Chen, S. C. -A. (2017). New developments and directions in the clinical application of the echinocandins. *Archives of Toxicology 91*, 1613–1621.

Chaturvedi, A. K., Kavishwar, A., Shiva Keshava, G. B., & Shukla, P. K. (2005). Monoclonal Immunoglobulin G1 Directed against *Aspergillus fumigatus* Cell Wall Glycoprotein Protects against Experimental Murine Aspergillosis. *Clinical and Vaccine Immunology 12*, 1063–1068.

Chauhan, N., & Calderone, R. (2008). Two-Component Signal Transduction Proteins as Potential Drug Targets in Medically Important Fungi. *Infection and Immunity 76*, 4795–4803.

Cheah, H. -L., Lim, V., & Sandai, D. (2014). Inhibitors of the Glyoxylate Cycle Enzyme ICL1 in *Candida albicans* for Potential Use as Antifungal Agents. *PLoS ONE 9*, e95951.

Ciociola, T., Giovati, L., Conti, S., Magliani, W., Santinoli, C., & Polonelli, L. (2016). Natural and synthetic peptides with antifungal activity. *Future Medicinal Chemistry 8*, 1413–1433.

Clemons, K. V., Danielson, M. E., Michel, K. S., Liu, M., Ottoson, N. C., Leonardo, S. M., ... Stevens, D. A. (2014). Whole glucan particles as a vaccine against murine aspergillosis. *Journal of Medical Microbiology 63*, 1750–1759.

Clemons, K. V., Antonysamy, M. A., Danielson, M. E., Michel, K. S., Martinez, M., Chen, V., & Stevens, D. A. (2015). Whole glucan particles as a vaccine against systemic coccidioidomycosis. *J Med Microbiol. 64*, 1237–1243.

Costa-de-Oliveira, S., Miranda, I. M., Silva-Dias, A., Silva, A. P., Rodrigues, A. G., & Pina-Vaz, C. (2015). Ibuprofen Potentiates the *In Vivo* Antifungal Activity of Fluconazole against *Candida albicans* Murine Infection. *Antimicrobial Agents and Chemotherapy 59*, 4289–4292.

Cowen, L. E., Sanglard, D., Howard, S. J., Rogers, P. D., & Perlin, D. S. (2015). Mechanisms of Antifungal Drug Resistance. *Cold Spring Harbor Perspectives in Medicine 5*, a019752.

Datta, A., Yadav, V., Ghosh, A., Choi, J., Bhattacharyya, D., Kar, R. K., ... Bhunia, A. (2016). Mode of Action of a Designed Antimicrobial Peptide: High Potency against *Cryptococcus neoformans*. *Biophysical Journal 111*, 1724–1737.

de Aguiar Cordeiro, R., de Farias Marques, F. J., de Aguiar Cordeiro, R., da Silva, M. R., Donato Maia Malaquias, A., Silva de Melo, C. V., ... Costa Sidrim, J. J. (2014). Synthesis and Antifungal Activity In Vitro of Isoniazid Derivatives against *Histoplasma capsulatum var. capsulatum*. *Antimicrobial Agents and Chemotherapy 58*, 2504–2511.

De Backer, M. D., & Van Dijck, P. (2003). Progress in functional genomics approaches to antifungal drug target discovery. *Trends in Microbiology 11*, 470–478.

De Backer, M. D., Nelissen, B., Logghe, M., Viaene, J., Loonen, I., Vandoninck, S., ... Luyten, W. H. M. L. (2001). An antisense-based functional genomics approach for identification of genes critical for growth of *Candida albicans*. *Nature Biotechnology 19*, 235–241.

de Castro, L. F., Ferreira, M. C., da Silva, R. M., Blotta, M. H., Longhi, L. N. A., & Mamoni, R. L. (2013). Characterization of the immune response in human paracoccidioidomycosis. *Journal of Infection 67*, 470–485.

De Lucca, A. J., & Walsh, T. J. (1999). Antifungal peptides: novel therapeutic compounds against emerging pathogens. *Antimicrob. Agents Chemother. 43*, 1–11.

Dinér, P., Veide Vilg, J., Kjellén, J., Migdal, I., Andersson, T., Gebbia, M., ... Grøtli, M. (2011). Design, Synthesis, and Characterization of a Highly Effective Hog1 Inhibitor: A Powerful Tool for Analyzing MAP Kinase Signaling in Yeast. *PLoS ONE 6*, e20012.

Dromer, F., & Charreire, J. (1991). Improved Amphotericin B Activity by a Monoclonal Anti-Cryptococcus neoformans Antibody: Study during Murine Cryptococcosis and Mechanisms of Action. *Journal of Infectious Diseases 163*, 1114–1120.

Dromer, F., Charreire, J., Contrepois, A., Carbon, C., & Yeni, P. (1987). Protection of mice against experimental cryptococcosis by anti-*Cryptococcus neoformans* monoclonal antibody. *Infectious Immunology 55*, 749–752.

Dromer, F., Salamero, J., Contrepois, A., Carbon, C., & Yeni, P. (1987). Production, characterization, and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. *Infectious Immunology 55*, 742–748.

Du, P., Beckmann, S., Almeida, M. C., Sibley, G. E. M., Law, D., Brand, A. C., ... Oliver, J. D. (2018). Effect of the Novel Antifungal Drug F901318 (Olorofim) on Growth and Viability of *Aspergillus fumigatus*. *Antimicrobial Agents Chemotherapy. 62*, 8.

Duncan, V. M. S., & O'Neil, D. A. (2013). Commercialization of antifungal peptides. *Fungal Biology Reviews 26*, 156–165.

Dunn, M. F., Ramirez-Trujillo, J. A., & Hernandez-Lucas, I. (2009). Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. *Microbiology 155*, 3166–3175.

Eckert, T. F., & Kozel, T. R. (1987). Production and characterization of monoclonal antibodies specific for *Cryptococcus neoformans* capsular polysaccharide. *Infect. Immun. 55*, 1895–1899.

Fan, Y., Tang, X., Hu, X., Wu, W., & Ping, Q. (2017). Prediction of essential proteins based on subcellular localization and gene expression correlation. *BMC Bioinformatics*, 18.

Fang, W., Du, T., Raimi, O. G., Hurtado-Guerrero, R., Urbaniak, M. D., Ibrahim, A. F. M., ... van Aalten, D. M. F. (2013). Genetic and structural validation of *Aspergillus fumigatus* UDP- *N* -acetylglucosamine pyrophosphorylase as an antifungal target: Structural and genetic validation of *Af* UAP1. *Molecular Microbiology 89*, 479–493.

Felipe, M. S. S., Andrade, R. V., Arraes, F. B. M., Nicola, A. M., Maranhão, A. Q., Torres, F. A. G., ... Brígido, M. M. (2005). Transcriptional Profiles of the Human Pathogenic Fungus *Paracoccidioides brasiliensis* in Mycelium and Yeast Cells. *Journal of Biological Chemistry 280*, 24706–24714.

Ferreira, L. G., & Andricopulo, A. D. (2016). Drug repositioning approaches to parasitic diseases: a medicinal chemistry perspective. *Drug Discovery Today 21*, 1699–1710.

Galgóczy, L., Nyilasi, I., Papp, T., & Vágvölgyi, C. (2011). Statins as antifungal agents. *World Journal of Clinical Infectious Diseases 1*, 4.

Gallo-Ebert, C., Donigan, M., Stroke, I. L., Swanson, R. N., Manners, M. T., Francisco, J., ... Nickels, J. T. (2014). Novel Antifungal Drug Discovery Based on Targeting Pathways Regulating the Fungus-Conserved Upc2 Transcription Factor. *Antimicrobial Agents and Chemotherapy 58*, 258–266.

Gálvez, A., Maqueda, M., Martínez-Bueno, M., Lebbadi, M., & Valdivia, E. (1993). Isolation and physico-chemical characterization of an antifungal and antibacterial peptide produced by *Bacillus licheniformis* A12. *Applied Microbiology and Biotechnology 39*, 438–442.

Ghannoum, M., Long, L., Larkin, E. L., Isham, N., Sherif, R., Borroto-Esoda, K., ... Angulo, D. (2018). Evaluation of the Antifungal Activity of the Novel Oral Glucan Synthase Inhibitor SCY-078, Singly and in Combination, for the Treatment of Invasive Aspergillosis. *Antimicrobial Agents and Chemotherapy 62*, e00244.

Gidaro, M. C., Alcaro, S., Secci, D., Rivanera, D., Mollica, A., Agamennone, M., ... Carradori, S. (2016). Identification of new anti- *Candida* compounds by ligand-based pharmacophore virtual screening. *Journal of Enzyme Inhibition and Medicinal Chemistry 31*, 1703–1706.

Giuliani, A., Pirri, G., & Nicoletto, S. (2007). Antimicrobial peptides: an overview of a promising class of therapeutics. *Open Life Sciences 2*, 1–33.

Godoy, J. S. R., Kioshima, É. S., Abadio, A. K. R., Felipe, M. S. S., de Freitas, S. M., & Svidzinski, T. I. E. (2016). Structural and functional characterization of the recombinant thioredoxin reductase from *Candida albicans* as a potential target for vaccine and drug design. *Applied Microbiology and Biotechnology 100*, 4015–4025.

Gómez-García, O., Andrade-Pavón, D., Campos-Aldrete, E., Ballinas-Indilí, R., Méndez-Tenorio, A., Villa-Tanaca, L., & Álvarez-Toledano, C. (2018). Synthesis, Molecular Docking, and Antimycotic Evaluation of Some 3-Acyl Imidazo[1,2-a]pyrimidines. *Molecules 23*, 599.

Gordon, Y. J., Romanowski, E. G., & McDermott, A. M. (2005). A Review of Antimicrobial Peptides and Their Therapeutic Potential as Anti-Infective Drugs. *Current Eye Research 30*, 505–515.

Gow, N. A. R., Latge, J. -P., & Munro, C. A. (2017). The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiology Spectrum 5*.

Greber, E., & Dawgul, K. (2017). Antimicrobial Peptides Under Clinical Trials. *Current Topics in Medicinal Chemistry 17*, 620–628.

Hagihara, K., Kita, A., Mizukura, A., Yao, M., Kitai, Y., Kunoh, T., ... Sugiura, R. (2013). Fingolimod (FTY720) Stimulates Ca2+/Calcineurin Signaling in Fission Yeast. *PLoS ONE 8*, e81907.

Hata, M., Ishii, Y., Watanabe, E., Uoto, K., Kobayashi, S., Yoshida, K. -I., ... Ando, A. (2010). Inhibition of ergosterol synthesis by novel antifungal compounds targeting C-14 reductase. *Medical Mycology 48*, 613–621.

Hata, M., Yoshida, K., Ishii, C., Otani, T., & Ando, A. (2010). In Vitro and in Vivo Antifungal Activities of Aminopiperidine Derivatives, Novel Ergosterol Synthesis Inhibitors. *Biological & Pharmaceutical Bulletin 33*, 473–476.

Heasley, B. H., Pacofsky, G. J., Mamai, A., Liu, H., Nelson, K., Coti, G., ... Kahn, J. N. (2012). Synthesis and biological evaluation of antifungal derivatives of enfumafungin as orally bioavailable inhibitors of β-1,3-glucan synthase. *Bioorganic & Medicinal Chemistry Letters 22*, 6811–6816.

Hector, R. F., Zimmer, B. L., & Pappagianis, D. (1990). Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis. *Antimicrobial Agents and Chemotherapy 34*, 587–593.

Hegedüs, N., & Marx, F. (2013). Antifungal proteins: More than antimicrobials? *Fungal Biology Reviews 26*, 132–145.

Hodos, R. A., Kidd, B. A., Shameer, K., Readhead, B. P., & Dudley, J. T. (2016). *In silico* methods for drug repurposing and pharmacology: Computational approaches to drug repurposing and pharmacology. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine 8*, 186–210.

Holanda, R. A., Muñoz, J. E., Dias, L. S., Silva, L. B. R., Santos, J. R. A., Pagliari, S., ... Bruña-Romero, O. (2017). Recombinant vaccines of a CD4+ T-cell epitope promote efficient control of *Paracoccidioides brasiliensis* burden by restraining primary organ infection. *PLOS Neglected Tropical Diseases 11*, e0005927.

Hu, W., Sillaots, S., Lemieux, S., Davison, J., Kauffman, S., Breton, A., ... Roemer, T. (2007). Essential Gene Identification and Drug Target Prioritization in *Aspergillus fumigatus*. *PLoS Pathogens 3*, e24.

Ianiri, G., & Idnurm, A. (2015). Essential Gene Discovery in the Basidiomycete *Cryptococcus neoformans* for Antifungal Drug Target Prioritization. *mBio 6*, e02334.

Ianiri, G., Boyce, K. J., & Idnurm, A. (2017). Isolation of conditional mutations in genes essential for viability of *Cryptococcus neoformans*. *Current Genetics 63*, 519–530.

Jahanshiri, Z., Shams-Ghahfarokhi, M., Asghari-Paskiabi, F., Saghiri, R., & Razzaghi-Abyaneh, M. (2017). α-Bisabolol inhibits *Aspergillus fumigatus* Af239 growth via affecting microsomal Δ24-sterol methyltransferase as a crucial enzyme in ergosterol biosynthesis pathway. *World Journal of Microbiology and Biotechnology 33*.

Jarvis, J. N., Meintjes, G., Rebe, K., Williams, G. N., Bicanic, T., Williams, A., ... Harrison, T. S. (2012). Adjunctive interferon-γ immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS 26*, 1105–1113.

Jenks, J. D., & Hoenigl, M. (2018). Treatment of Aspergillosis. *Journal of Fungi (Basel) 4*(3), E98.

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. *Antimicrobial Agents and Chemotherapy 58*, 1248–1251.

Jung, K. -W., Kang, H. A., & Bahn, Y. -S. (2013). Essential Roles of the Kar2/BiP Molecular Chaperone Downstream of the UPR Pathway in *Cryptococcus neoformans*. *PLoS ONE 8*, e58956.

Jung, E. H., Meyers, D. J., Bosch, J., & Casadevall, A. (2018). Novel Antifungal Compounds Discovered in Medicines for Malaria Venture's Malaria Box. *mSphere 3*, e00537.

Kang, S. -J., Park, S. J., Mishig-Ochir, T., & Lee, B. -J. (2014). Antimicrobial peptides: therapeutic potentials. *Expert Review of Anti-infective Therapy 12*, 1477–1486.

Kanno, T., Takekawa, D., & Miyakawa, Y. (2015). Analysis of the essentiality of *ROM2* genes in the pathogenic yeasts *Candida glabrata* and *Candida albicans* using temperature-sensitive mutants. *Journal of Applied Microbiology 118*, 851–863.

Kennedy, T., Allen, G., Steiner, J., Heep, M., & Birch, M. (2017). Assessment of the duration of infusion on the tolerability and repeat dose pharmacokinetics of F901318 in healthy volunteers. *ECCMID 2017, Vienna, Austria.*

Kennedy, T., Allen, G., Steiner, J., Heep, M., Oliver, J., Sibley, G., et al. (2017). *Multiple dose pharmacokinetics of an immediate-release tablet formulation of F901318 in healthy male and female subjects.* Vienna: ECCMID.

Khan, M. A., Jabeen, R., Nasti, T. H., & Mohammad, O. (2005). Enhanced anticryptococcal activity of chloroquine in phosphatidylserine-containing liposomes in a murine model. *Journal of Antimicrobial Chemotherapy 55*, 223–228.

Kmetzsch, L. (2015). Histone deacetylases: Targets for antifungal drug development. *Virulence 6*, 535–536.

Kojima, K., Bahn, Y. S., & Heitman, J. (2006). Calcineurin, Mpk1 and Hog1 MAPK pathways independently control fludioxonil antifungal sensitivity in Cryptococcus neoformans. *Microbiology 152*, 591–604.

Komath, S. S., Singh, S. L., Pratyusha, V. A., & Sah, S. K. (2018). Generating anchors only to lose them: The unusual story of glycosylphosphatidylinositol anchor biosynthesis and remodeling in yeast and fungi: GPI BIOSYNTHESIS IN YEAST AND FUNGI. *IUBMB Life 70*, 355–383.

Kontoyianni, M. (2017). Docking and Virtual Screening in Drug Discovery. In I. M. Lazar, M. Kontoyianni, & A. C. Lazar (Eds.), *Proteomics for Drug Discovery* (pp. 255–266). New York, NY: Springer New York.

Koselny, K., Green, J., DiDone, L., Halterman, J. P., Fothergill, A. W., Wiederhold, N. P., ... Krysan, D. J. (2016). The Celecoxib Derivative AR-12 Has Broad-Spectrum Antifungal Activity In Vitro and Improves the Activity of Fluconazole in a Murine Model of Cryptococcosis. *Antimicrobial Agents Chemotherapy. 60*, 7115–7127.

Krauss, J., Müller, C., Kießling, J., Richter, S., Staudacher, V., & Bracher, F. (2014). Synthesis and Biological Evaluation of Novel *N* -Alkyl Tetra- and Decahydroisoquinolines: Novel Antifungals that Target Ergosterol Biosynthesis: Antifungal *N* -Alkyl Tetra- and Decahydroisoquinolines. *Archiv der Pharmazie 347*, 283–290.

Lakshmaiah Narayana, J., & Chen, J. -Y. (2015). Antimicrobial peptides: Possible anti-infective agents. *Peptides 72*, 88–94.

Lamoth, F., Juvvadi, P. R., & Steinbach, W. J. (2015). Histone deacetylase inhibition as an alternative strategy against invasive aspergillosis. *Frontiers in Microbiology 6*.

Larkin, E., Hager, C., Chandra, J., Mukherjee, P. K., Retuerto, M., Salem, I., ... Ghannoum, M. (2017). The Emerging Pathogen Candida auris: Growth Phenotype, Virulence Factors, Activity of Antifungals, and Effect of SCY-078, a Novel Glucan Synthesis Inhibitor, on Growth Morphology and Biofilm Formation. *Antimicrobial Agents and Chemotherapy 61*, e02396.

Larsen, R. A., Pappas, P. G., Perfect, J., Aberg, J. A., Casadevall, A., Cloud, G. A., ... Dismukes, W. E. (2005). Phase I Evaluation of the Safety and Pharmacokinetics of Murine-Derived Anticryptococcal Antibody 18B7 in Subjects with Treated Cryptococcal Meningitis. *Antimicrobial Agents and Chemotherapy 49*, 952–958.

Lazzarini, C., Haranahalli, K., Rieger, R., Ananthula, H. K., Desai, P. B., Ashbaugh, A., ... Del Poeta, M. (2018). Acylhydrazones as Antifungal Agents Targeting the Synthesis of Fungal Sphingolipids. *Antimicrobial Agents and Chemotherapy 62*, e00156.

Lee, H. -S., Yoon, K. -M., Han, Y. -R., Lee, K. J., Chung, S. -C., Kim, T. -I., ... Oh, K. -B. (2009). 5-Hydroxyindole-type alkaloids, as *Candida albicans* isocitrate lyase inhibitors, from the tropical sponge Hyrtios sp. *Bioorganic & Medicinal Chemistry Letters 19*, 1051–1053.

Lee, Y. -T., Cui, C. -J., Chow, E. W. L., Pue, N., Lonhienne, T., Wang, J. -G., ... Guddat, L. W. (2013). Sulfonylureas Have Antifungal Activity and Are Potent Inhibitors of *Candida albicans* Acetohydroxyacid Synthase. *Journal of Medicinal Chemistry 56*, 210–219.

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. *Antimicrobial Agents and Chemotherapy 59*, 1265–1272.

Lepak, A. J., Zhao, M., VanScoy, B., Ambrose, P. G., & Andes, D. R. (2017). Pharmacodynamics of a Long-Acting Echinocandin, CD101, in a Neutropenic Invasive-Candidiasis Murine Model Using an Extended-Interval Dosing Design. *Antimicrobial Agents and Chemotherapy 62*, e02154.

Levent, S., Kaya Çavuşoğlu, B., Sağlık, B., Osmaniye, D., Acar Çevik, U., Atlı, Ö., ... Kaplancıklı, Z. (2017). Synthesis of Oxadiazole-Thiadiazole Hybrids and Their Anticandidal Activity. *Molecules 22*, 2004.

Lionakis, M. S., & Levitz, S. M. (2018). Host Control of Fungal Infections: Lessons from Basic Studies and Human Cohorts. *Annual Review of Immunology 36*, 157–191.

Lionta, E., Spyrou, G., Vassilatis, D., & Cournia, Z. (2014). Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances. *Current Topics in Medicinal Chemistry 14*, 1923–1938.

Liu, M., Healy, M. D., Dougherty, B. A., Esposito, K. M., Maurice, T. C., Mazzucco, C. E., ... Wang, Y. -K. (2006). Conserved Fungal Genes as Potential Targets for Broad-Spectrum Antifungal Drug Discovery. *Eukaryotic Cell 5*, 638–649.

Liu, M., Clemons, K. V., Bigos, M., Medovarska, I., Brummer, E., & Stevens, D. A. (2011). Immune responses induced by heat killed *Saccharomyces cerevisiae*: A vaccine against fungal infection. *Vaccine 29*, 1745–1753.

Lorenz, M. C., & Fink, G. R. (2002). Life and Death in a Macrophage: Role of the Glyoxylate Cycle in Virulence. *Eukaryotic Cell 1*, 657–662.

Lorenz, R. T., & Parks, L. W. (1990). Effects of lovastatin (mevinolin) on sterol levels and on activity of azoles in *Saccharomyces cerevisiae*. *Antimicrobial Agents and Chemotherapy 34*, 1660–1665.

Louie, A., Stein, D. S., Zack, J. Z., Liu, W., Conde, H., Fregeau, C., ... Drusano, G. L. (2011). Dose Range Evaluation of Mycograb C28Y Variant, a Human Recombinant Antibody Fragment to Heat Shock Protein 90, in Combination with Amphotericin B-Desoxycholate for Treatment of Murine Systemic Candidiasis. *Antimicrobial Agents and Chemotherapy 55*, 3295–3304.

Lu, Y., Deng, J., Rhodes, J. C., Lu, H., & Lu, L. J. (2014). Predicting essential genes for identifying potential drug targets in *Aspergillus fumigatus*. *Computational Biology and Chemistry 50*, 29–40.

Lupetti, A., Dissel, J. T., Brouwer, C. P. J. M., & Nibbering, P. H. (2008). Human antimicrobial peptides' antifungal activity against *Aspergillus fumigatus*. *European Journal of Clinical Microbiology & Infectious Diseases 27*, 1125–1129.

Lyman, G. H., Dale, D. C., Wolff, D. A., Culakova, E., Poniewierski, M. S., Kuderer, N. M., & Crawford, J. (2010). Acute Myeloid Leukemia or Myelodysplastic Syndrome in Randomized Controlled Clinical Trials of Cancer Chemotherapy With Granulocyte Colony-Stimulating Factor: A Systematic Review. *Journal of Clinical Oncology 28*, 2914–2924.

Ma, B. -X., Li, H., Li, J. -S., & Wu, S. -S. (2015). Effect of statins on preventing infectious complications after surgery: Systematic review and meta-analysis. *Journal of International Medical Research 43*, 610–618.

Maggiora, G. M., & Bajorath, J. (2014). Chemical space networks: a powerful new paradigm for the description of chemical space. *Journal of Computer-Aided Molecular Design 28*, 795–802.

Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016). Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. *Frontiers in Cellular and Infection Microbiology 6*.

Mandala, S. M., Thornton, R. A., Rosenbach, M., Milligan, J., Garcia-Calvo, M., Bull, H. G., & Kurtz, M. B. (1997). Khafrefungin, a Novel Inhibitor of Sphingolipid Synthesis. *Journal of Biological Chemistry 272*, 32709–32714 https://doi.org/10.1074/jbc.272.51.32709.

Mandala, S. M., Thornton, R. A., Milligan, J., Rosenbach, M., Garcia-Calvo, M., Bull, H. G., ... Kurtz, M. B. (1998). Rustmicin, a Potent Antifungal Agent, Inhibits Sphingolipid Synthesis at Inositol Phosphoceramide Synthase. *Journal of Biological Chemistry 273*, 14942–14949.

Marcos, J. F., Gandía, M., Harries, E., Carmona, L., & Muñoz, A. (2012). Antifungal Peptides: Exploiting Non-Lytic Mechanisms and Cell Penetration Properties. In K. Rajasekaran, J. W. Cary, J. M. Jaynes, & E. Montesinos (Eds.), *Small Wonders: Peptides for Disease Control* (pp. 337–357). Washington, DC: American Chemical Society https://doi.org/10.1021/bk-2012-1095.ch016.

Marcos-Zambrano, L. J., Gómez-Perosanz, M., Escribano, P., Bouza, E., & Guinea, J. (2017). The novel oral glucan synthase inhibitor SCY-078 shows in vitro activity against sessile and planktonic *Candida spp*. *Journal of Antimicrobial Chemotherapy 72*, 1969–1976 https://doi.org/10.1093/jac/dkx010.

Matejuk, A., Leng, Q., Begum, M. D., Woodle, M. C., Scaria, P., Chou, S. -T., & Mixson, A. J. (2010). Peptide-based antifungal therapies against emerging infections. *Drugs of the Future 35*, 197.

Matthews, R., Hodgetts, S., & Burnie, J. (1995). Preliminary Assessment of a Human Recombinant Antibody Fragment to hsp90 in Murine Invasive Candidiasis. *Journal of Infectious Diseases 171*, 1668–1671.

Matthews, R. C., Rigg, G., Hodgetts, S., Carter, T., Chapman, C., Gregory, C., ... Burnie, J. (2003). Preclinical Assessment of the Efficacy of Mycograb, a Human Recombinant Antibody against Fungal HSP90. *Antimicrobial Agents and Chemotherapy 47*, 2208–2216.

McCourt, J. A., & Duggleby, R. G. (2006). Acetohydroxyacid synthase and its role in the biosynthetic pathway for branched-chain amino acids. *Amino Acids 31*, 173–210.

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 Chemical Biology 7*, 1520–1528.

Mishra, B., Reiling, S., Zarena, D., & Wang, G. (2017). Host defense antimicrobial peptides as antibiotics: design and application strategies. *Current Opinion in Chemical Biology 38*, 87–96.

Miyazaki, M., Horii, T., Hata, K., Watanabe, N., Nakamoto, K., Tanaka, K., ... Asada, M. (2011). *In Vitro* Activity of E1210, a Novel Antifungal, against Clinically Important Yeasts and Molds. *Antimicrobial Agents and Chemotherapy 55*, 4652–4658.

Mor, V., Rella, A., Farnoud, A.M., Singh, A., Munshi, M., Bryan, A., Naseem, S., Konopka, J.B., Ojima, I., Bullesbach, E., Ashbaugh, A., Linke, M.J., Cushion, M., Collins, M., Ananthula, H.K., Sallans, L., Desai, P.B., Wiederhold, N.P., Fothergill, A.W., Kirkpatrick, W.R., Patterson, T., Wong, L.H., Sinha, S., Giaever, G., Nislow, C., Flaherty, P., Pan, X., Cesar, G.V., de Melo Tavares, P., Frases, S., Miranda, K., Rodrigues, M.L., Luberto, C., Nimrichter, L., Del Poeta, M., 2015. Identification of a New Class of Antifungals Targeting the Synthesis of Fungal Sphingolipids. mBio 6, e00647-15.

Moragues, M. D., Omaetxebarria, M. J., Elguezabal, N., Sevilla, M. J., Conti, S., Polonelli, L., & Ponton, J. (2003). A Monoclonal Antibody Directed against a Candida albicans Cell Wall Mannoprotein Exerts Three Anti-*C. albicans* Activities. *Infection and Immunity 71*, 5273–5279.

Muñoz, J. E., Luft, V. D., Amorim, J., Magalhães, A., Thomaz, L., Nosanchuk, J. D., ... Taborda, C. P. (2014). Immunization with P10 Peptide Increases Specific Immunity and Protects Immunosuppressed BALB/c Mice Infected with Virulent Yeasts of *Paracoccidioides brasiliensis*. *Mycopathologia 178*, 177–188.

Nakamura, I., Yoshimura, S., Masaki, T., Takase, S., Ohsumi, K., Hashimoto, M., ... Fujie, A. (2017). ASP2397: a novel antifungal agent produced by Acremonium persicinum MF-347833. *The Journal of Antibiotics 70*, 45–51.

Nanjappa, S. G., Heninger, E., Wüthrich, M., Gasper, D. J., & Klein, B. S. (2012). Tc17 Cells Mediate Vaccine Immunity against Lethal Fungal Pneumonia in Immune Deficient Hosts Lacking CD4+ T Cells. *PLoS Pathogens 8*, e1002771.

Neal, L.M., Xing, E., Xu, J., Kolbe, J.L., Osterholzer, J.J., Segal, B.M., Williamson, P.R., Olszewski, M.A., 2017. CD4 $^{+}$ T Cells Orchestrate Lethal Immune Pathology despite Fungal Clearance during *Cryptococcus neoformans* Meningoencephalitis. mBio 8, e01415-17.

Newman, S. L., Gootee, L., Brunner, G., & Deepe, G. S. (1994). Chloroquine induces human macrophage killing of Histoplasma capsulatum by limiting the availability of intracellular iron and is therapeutic in a murine model of histoplasmosis. *Journal of Clinical Investigation 93*, 1422–1429.

Nijnik, A., & Hancock, R. (2009). Host defence peptides: antimicrobial and immunomodulatory activity and potential applications for tackling antibiotic-resistant infections. *Emerging Health Threats Journal 2*, 7078.

Nixon, G. L., McEntee, L., Johnson, A., Farrington, N., Whalley, S., Livermore, J., ... Hope, W. (2018). Repurposing and Reformulation of the Antiparasitic Agent Flubendazole for Treatment of Cryptococcal Meningoencephalitis. *a Neglected Fungal Disease. Antimicrobial Agents and Chemotherapy 62*, e01909–e01917.

Nosanchuk, J. D., Steenbergen, J. N., Shi, L., Deepe, G. S., & Casadevall, A. (2003). Antibodies to a cell surface histone-like protein protect against *Histoplasma capsulatum*. *Journal of Clinical Investigation 112*, 1164–1175.

Ogundeji, A. O., Pohl, C. H., & Sebolai, O. M. (2016). Repurposing of Aspirin and Ibuprofen as Candidate Anti-Cryptococcus Drugs. *Antimicrobial Agents and Chemotherapy 60*, 4799–4808.

Oliver, J. D., Sibley, G. E. M., Beckmann, N., Dobb, K. S., Slater, M. J., McEntee, L., ... Birch, M. (2016). F901318 represents a novel class of antifungal drug that inhibits dihydroorotate dehydrogenase. *Proceedings of the National Academy of Sciences 113*, 12809–12814.

Ong, V., Hough, G., Schlosser, M., Bartizal, K., Balkovec, J. M., James, K. D., & Krishnan, B. R. (2016). Preclinical Evaluation of the Stability, Safety, and Efficacy of CD101, a Novel Echinocandin. *Antimicrobial Agents and Chemotherapy 60*, 6872–6879.

Ong, V., James, K. D., Smith, S., & Krishnan, B. R. (2017). Pharmacokinetics of the Novel Echinocandin CD101 in Multiple Animal Species. *Antimicrobial Agents and Chemotherapy 61*, e01626.

Onishi, J., Meinz, M., Thompson, J., Curotto, J., Dreikorn, S., Rosenbach, M., ... Kurtz, M. B. (2000). Discovery of Novel Antifungal (1,3)-beta -D-Glucan Synthase Inhibitors. *Antimicrobial Agents and Chemotherapy 44*, 368–377.

Oppenheim, F. G., Xu, T., McMillian, F. M., Levitz, S. M., Diamond, R. D., Offner, G. D., & Troxler, R. F. (1988). Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. *J. Biol. Chem. 263*, 7472–7477.

Oprea, T. I., & Mestres, J. (2012). Drug Repurposing: Far Beyond New Targets for Old Drugs. *The AAPS Journal 14*, 759–763.

Pachl, J., Svoboda, P., Jacobs, F., Vandewoude, K., van der Hoven, B., Spronk, P., ... Matthews, R. (2006). A Randomized, Blinded, Multicenter Trial of Lipid-Associated Amphotericin B Alone versus in Combination with an Antibody-Based Inhibitor of Heat Shock Protein 90 in Patients with Invasive Candidiasis. *Clinical Infectious Diseases 42*, 1404–1413.

Pasupuleti, M., Schmidtchen, A., & Malmsten, M. (2012). Antimicrobial peptides: key components of the innate immune system. *Critical Reviews in Biotechnology 32*, 143–171.

Pfaller, M. A., Messer, S. A., Georgopapadakou, N., Martell, L. A., Besterman, J. M., & Diekema, D. J. (2009). Activity of MGCD290, a Hos2 Histone Deacetylase Inhibitor, in Combination with Azole Antifungals against Opportunistic Fungal Pathogens. *Journal of Clinical Microbiology 47*, 3797–3804.

Pfaller, M. A., Messer, S. A., Motyl, M. R., Jones, R. N., & Castanheira, M. (2013a). In Vitro Activity of a New Oral Glucan Synthase Inhibitor (MK-3118) Tested against Aspergillus spp. by CLSI and EUCAST Broth Microdilution Methods. *Antimicrobial Agents and Chemotherapy 57*, 1065–1068.

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

Pic, E., Burgain, A., & Sellam, A. (2018). Repurposing the anthelminthic salicylanilide oxyclozanide against susceptible and clinical resistant *Candida albicans* strains. *Medical Mycology*. [Epub ahead of print].

Pina-Vaz, C., Rodrigues, A. G., Costa-de-Oliveira, S., Ricardo, E., & Mårdh, P. -A. (2005). Potent synergic effect between ibuprofen and azoles on Candida resulting from blockade of efflux pumps as determined by FUN-1 staining and flow cytometry. *Journal of Antimicrobial Chemotherapy 56*, 678–685.

Pinto, A. R., Puccia, R., Diniz, S. N., Franco, M. F., & Travassos, L. R. (2000). DNA-based vaccination against murine paracoccidioidomycosis using the gp43 gene from *Paracoccidioides brasiliensis*. *Vaccine 18*, 3050–3058.

Pushpanathan, M., Rajendhran, J., Jayashree, S., Sundarakrishnan, B., Jayachandran, S., & Gunasekaran, P. (2012). Identification of a Novel Antifungal Peptide with Chitin-Binding Property from Marine Metagenome. *Protein & Peptide Letters 19*, 1289–1296.

Rachini, A., Pietrella, D., Lupo, P., Torosantucci, A., Chiani, P., Bromuro, C., ... Vecchiarelli, A. (2007). An Anti- -Glucan Monoclonal Antibody Inhibits Growth and Capsule Formation of *Cryptococcus neoformans In Vitro* and Exerts Therapeutic, Anticryptococcal Activity *In Vivo*. *Infection and Immunity 75*, 5085–5094.

Raja Mohamed, B. S., Subramanian, M., & Shunmugiah, K. P. (2014). Inhibition of *Candida albicans* virulence factors by novel levofloxacin derivatives. *Applied Microbiology and Biotechnology 98*, 6775–6785.

Rhein, J., Morawski, B. M., Hullsiek, K. H., Nabeta, H. W., Kiggundu, R., Tugume, L., ... Boulware, D. R. (2016). Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study. *The Lancet Infectious Diseases 16*, 809–818.

Ribeiro, A. M., Bocca, A. L., Amaral, A. C., Faccioli, L. H., Galetti, F. C. S., Zárate-Bladés, C. R., ... Felipe, M. S. S. (2009). DNAhsp65 vaccination induces protection in mice against *Paracoccidioides brasiliensis* infection. *Vaccine 27*, 606–613.

Ribeiro, A. M., Bocca, A. L., Amaral, A. C., Souza, A. C. C. O., Faccioli, L. H., Coelho-Castelo, A. A. M., ... Felipe, M. S. S. (2010). HSP65 DNA as therapeutic strategy to treat experimental paracoccidioidomycosis. *Vaccine 28*, 1528–1534.

Ribeiro, N. d. Q., Costa, M. C., Magalhães, T. F. F., Carneiro, H. C. S., Oliveira, L. V., Fontes, A. C. L., ... Santos, D. A. (2017). Atorvastatin as a promising anticryptococcal agent. *International Journal of Antimicrobial Agents 49*, 695–702.

Richie, D. L., Thompson, K. V., Studer, C., Prindle, V. C., Aust, T., Riedl, R., ... Hoepfner, D. (2013). Identification and Evaluation of Novel Acetolactate Synthase Inhibitors as Antifungal Agents. *Antimicrobial Agents and Chemotherapy 57*, 2272–2280.

Rittner, G. M. G., Muñoz, J. E., Marques, A. F., Nosanchuk, J. D., Taborda, C. P., & Travassos, L. R. (2012). Therapeutic DNA Vaccine Encoding Peptide P10 against Experimental Paracoccidioidomycosis. *PLoS Neglected Tropical Diseases 6*, e1519.

Robbins, N., Spitzer, M., Yu, T., Cerone, R. P., Averette, A. K., Bahn, Y. -S., ... Wright, G. D. (2015). An Antifungal Combination Matrix Identifies a Rich Pool of Adjuvant Molecules that Enhance Drug Activity against Diverse Fungal Pathogens. *Cell Reports 13*, 1481–1492.

Roemer, T., Jiang, B., Davison, J., Ketela, T., Veillette, K., Breton, A., ... Bussey, H. (2003). Large-scale essential gene identification in *Candida albicans* and applications to antifungal drug discovery: *C. albicans* essential gene identification and antifungal drug discovery. *Molecular Microbiology 50*, 167–181.

Rosas, A. L., Nosanchuk, J. D., Feldmesser, M., Cox, G. M., McDade, H. C., & Casadevall, A. (2000). Synthesis of Polymerized Melanin by *Cryptococcus neoformans* in Infected Rodents. *Infection and Immunity 68*, 2845–2853.

Rosas, A. L., Nosanchuk, J. D., & Casadevall, A. (2001). Passive Immunization with Melanin-Binding Monoclonal Antibodies Prolongs Survival of Mice with Lethal *Cryptococcus neoformans* Infection. *Infection and Immunity 69*, 3410–3412.

Rosato, A., Catalano, A., Carocci, A., Carrieri, A., Carone, A., Caggiano, G., ... Montagna, M. T. (2016). In vitro interactions between anidulafungin and nonsteroidal anti-inflammatory drugs on biofilms of Candida spp. *Bioorganic & Medicinal Chemistry 24*, 1002–1005.

Ruiz-Herrera, J., & San-Blas, G. (2003). Chitin Synthesis as a Target for Antifungal Drugs. *Current Drug Target -Infectious Disorders 3*, 77–91.

Salci, T. P., Negri, M., Abadio, R., Bonfim-Mendonça, P., Capoci, I., Caparroz-Assef, S. M., ... Svidzinski, T. I. (2017). A new small-molecule KRE2 inhibitor against invasive *Candida parapsilosis* infection. *Future Microbiology 12*, 1283–1295.

Sanford, J. E., Lupan, D. M., Schlageter, A. M., & Kozel, T. R. (1990). Passive immunization against *Cryptococcus neoformans* with an isotype-switch family of monoclonal antibodies reactive with cryptococcal polysaccharide. *Infect. Immun. 58*, 1919–1923.

Schell, W. A., Jones, A. M., Borroto-Esoda, K., & Alexander, B. D. (2017). Antifungal Activity of SCY-078 and Standard Antifungal Agents against 178 Clinical Isolates of Resistant and Susceptible *Candida* Species. *Antimicrobial Agents and Chemotherapy 61*, e01102–e01117.

Schlageter, A. M., & Kozel, T. R. (1990). Opsonization of Cryptococcus neoformans by a family of isotype-switch variant antibodies specific for the capsular polysaccharide. *Infect. Immun. 58*, 1914–1918.

Schmidt, C. S., White, C. J., Ibrahim, A. S., Filler, S. G., Fu, Y., Yeaman, M. R., ... Hennessey, J. P., Jr. (2012). NDV-3, a recombinant alum-adjuvanted vaccine for *Candida* and *Staphylococcus aureus*, is safe and immunogenic in healthy adults. *Vaccine 30*, 7594–7600.

Sevilla, M. J., Robledo, B., Rementeria, A., Moragues, M. D., & Ponton, J. (2006). A Fungicidal Monoclonal Antibody Protects against Murine Invasive Candidiasis. *Infection and Immunity 74*, 3042–3045.

Sharma, M., Biswas, D., Kotwal, A., Thakuria, B., Kakati, B., Chauhan, S. B., & Patras, A. (2015). Ibuprofen-Mediated Reversal of Fluconazole Resistance in Clinical Isolates of *Candida*. *Journal of Clinical and Diagnostic Research 9*(1), DC20–DC22. https://doi.org/10.7860/JCDR/2015/10094.5494.

Shinde, R. B., Rajput, S. B., Raut, J. S., & Karuppayil, S. M. (2013). An in vitro repositioning study reveals antifungal potential of chloroquine to inhibit growth and morphogenesis in *Candida albicans*. *The Journal of General and Applied Microbiology 59*, 167–170.

Shubitz, L. F., Trinh, H. T., Perrill, R. H., Thompson, C. M., Hanan, N. J., Galgiani, J. N., & Nix, D. E. (2014). Modeling Nikkomycin Z Dosing and Pharmacology in Murine Pulmonary Coccidioidomycosis Preparatory to Phase 2 Clinical Trials. *Journal of Infectious Diseases 209*, 1949–1954.

Silva, F. D., Rossi, D. C. P., Martinez, L. R., Frases, S., Fonseca, F. L., Campos, C. B. L., ... Daffre, S. (2011). Effects of microplusin, a copper-chelating antimicrobial peptide, against *Cryptococcus neoformans*. *FEMS Microbiology Letters 324*, 64–72.

Silva, L. B. R., Dias, L. S., Rittner, G. M. G., Muñoz, J. E., Souza, A. C. O., Nosanchuk, J. D., ... Taborda, C. P. (2017). Dendritic Cells Primed with *Paracoccidioides brasiliensis* Peptide P10 Are Therapeutic in Immunosuppressed Mice with Paracoccidioidomycosis. *Frontiers in Microbiology 8*.

Singh, A., Paliwal, S. K., Sharma, M., Mittal, A., Sharma, S., & Sharma, J. P. (2016). In silico and in vitro screening to identify structurally diverse non-azole CYP51 inhibitors as potent antifungal agent. *Journal of Molecular Graphics and Modelling 63*, 1–7.

Siqueira, I. M., Ribeiro, A. M., de Medeiros Nóbrega, Y. K., Simon, K. S., Souza, A. C. O., Jerônimo, M. S., ... Bocca, A. L. (2013). DNA-hsp65 Vaccine as Therapeutic Strategy to Treat Experimental Chromoblastomycosis Caused by *Fonsecaea Pedrosoi*. *Mycopathologia 175*, 463–475.

Sofjan, A. K., Mitchell, A., Shah, D. N., Nguyen, T., Sim, M., Trojcak, A., ... Garey, K. W. (2018). Rezafungin (CD101), a next generation echinocandin. A systematic literature review and assessment of possible place in therapy. *Journal of Global Antimicrobial Resistance. 14*, 58–64. https://doi.org/10.1016/j.jgar.2018.02.013.

Song, J. C., & Stevens, D. A. (2016). Caspofungin: Pharmacodynamics, pharmacokinetics, clinical uses and treatment outcomes. *Critical Reviews in Microbiology 42*, 813–846.

Spanakis, E. K., Kourkoumpetis, T. K., Livanis, G., Peleg, A. Y., & Mylonakis, E. (2010). Statin Therapy and Decreased Incidence of Positive *Candida* Cultures Among Patients With Type 2 Diabetes Mellitus Undergoing Gastrointestinal Surgery. *Mayo Clinic Proceedings 85*, 1073–1079.

Specht, C.A., Lee, C.K., Huang, H., Tipper, D.J., Shen, Z.T., Lodge, J.K., Leszyk, J., Ostroff, G.R., Levitz, S.M., 2015. Protection against Experimental Cryptococcosis following Vaccination with Glucan Particles Containing *Cryptococcus* Alkaline Extracts. mBio 6, e01905-15.

Spitzer, M., Griffiths, E., Blakely, K. M., Wildenhain, J., Ejim, L., Rossi, L., ... Wright, G. D. (2014). Cross-species discovery of syncretic drug combinations that potentiate the antifungal fluconazole. *Molecular Systems Biology 7*, 499. https://doi.org/10.1038/msb.2011.31.

Stana, A., Vodnar, D., Tamaian, R., Pîrnău, A., Vlase, L., Ionuț, I., ... Tiperciuc, B. (2017). Design, Synthesis and Antifungal Activity Evaluation of New Thiazolin-4-ones as Potential Lanosterol 14α-Demethylase Inhibitors. *International Journal of Molecular Sciences 18*, 177.

Steinstraesser, L., Kraneburg, U., Jacobsen, F., & Al-Benna, S. (2011). Host defense peptides and their antimicrobial-immunomodulatory duality. *Immunobiology 216*, 322–333.

Sucher, A. J., Chahine, E. B., & Balcer, H. E. (2009). Echinocandins: The Newest Class of Antifungals. *Annals of Pharmacotherapy 43*, 1647–1657.

Sugimoto, Y., Sakoh, H., & Yamada, K. (2004). IPC Synthase as a Useful Target for Antifungal Drugs. *Current Drug Target -Infectious Disorders 4*, 311–322.

Sun, N., Li, D., Zhang, Y., Killeen, K., Groutas, W., & Calderone, R. (2017). Repurposing an inhibitor of ribosomal biogenesis with broad anti-fungal activity. *Scientific Reports 7*.

Suresh, L., Poornachandra, Y., Kanakaraju, S., Ganesh Kumar, C., & Chandramouli, G. V. P. (2015). One-pot three-component domino protocol for the synthesis of novel pyrano[2,3-d]pyrimidines as antimicrobial and anti-biofilm agents. *Organic & Biomolecular Chemistry 13*, 7294–7306.

Taborda, C. P., Juliano, M. A., Puccia, R., Franco, M., & Travassos, L. R. (1998). Mapping of the T-cell epitope in the major 43-kilodalton glycoprotein of *Paracoccidioides brasiliensis*, which induces a Th-1 response protective against fungal infection in BALB/c mice. *Infect. Immun. 66*, 786–793.

Takesako, K., Kuroda, H., Inoue, T., Haruna, F., Yoshikawa, Y., Kato, I., ... Yamaguchi, H. (1993). Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic. *The Journal of Antibiotics 46*, 1414–1420.

te Velthuis, A., & Bagowski, C. (2008). Linking Fold, Function and Phylogeny: A Comparative Genomics View on Protein (Domain) Evolution. *Current Genomics 9*, 88–96.

Thangamani, S., Maland, M., Mohammad, H., Pascuzzi, P. E., Avramova, L., Koehler, C. M., ... Seleem, M. N. (2017). Repurposing Approach Identifies Auranofin with Broad Spectrum Antifungal Activity That Targets Mia40-Erv1 Pathway. *Frontiers in Cellular and Infection Microbiology 7*.

Tiwari, S., Seijas, J., Vazquez-Tato, M., Sarkate, A., Karnik, K., & Nikalje, A. (2017). Facile Synthesis of Novel Coumarin Derivatives, Antimicrobial Analysis, Enzyme Assay, Docking Study, ADMET Prediction and Toxicity Study. *Molecules 22*, 1172.

Torosantucci, A., Bromuro, C., Chiani, P., De Bernardis, F., Berti, F., Galli, C., ... Cassone, A. (2005). A novel glyco-conjugate vaccine against fungal pathogens. *The Journal of Experimental Medicine 202*, 597–606.

Treviño-Rangel, R. d. J., Villanueva-Lozano, H., Hernández-Rodríguez, P., Martínez-Reséndez, M. F., García-Juárez, J., Rodríguez-Rocha, H., & González, G. M. (2016). Activity of sertraline against *Cryptococcus neoformans* : *in vitro* and *in vivo* assays. *Medical Mycology 54*, 280–286.

Tripathi, H., & Khan, F. (2018). Identification of potential inhibitors against nuclear Dam1 complex subunit Ask1 of *Candida albicans* using virtual screening and MD simulations. *Computational Biology and Chemistry 72*, 33–44.

Truong, M., Monahan, L. G., Carter, D. A., & Charles, I. G. (2018). Repurposing drugs to fast-track therapeutic agents for the treatment of cryptococcosis. *PeerJ 6*, e4761.

Tsai, H., & Bobek, L. A. (1997). Human salivary histatin-5 exerts potent fungicidal activity against *Cryptococcus neoformans*. *Biochimica et Biophysica Acta (BBA) - General Subjects 1336*, 367–369.

Valente, J. F., Alexander, J. W., Li, B. -G., Noel, J. G., Custer, D. A., Ogle, J. D., & Ogle, C. K. (2002). Effect Of In vivo Infusion of Granulocyte Colony-Stimulating Factor on Immune Function. *Shock 17*, 23–29.

Visbal, G., San-Blas, G., Maldonado, A., Álvarez-Aular, Á., Capparelli, M. V., & Murgich, J. (2011). Synthesis, in vitro antifungal activity and mechanism of action of four sterol hydrazone analogues against the dimorphic fungus *Paracoccidioides brasiliensis*. *Steroids 76*, 1069–1081.

Vylkova, S., Nayyar, N., Li, W., & Edgerton, M. (2007). Human-Defensins Kill *Candida albicans* in an Energy-Dependent and Salt-Sensitive Manner without Causing Membrane Disruption. *Antimicrobial Agents and Chemotherapy 51*, 154–161.

Wan, L., Zhang, Y., Lai, Y., Jiang, M., Song, Y., Zhou, J., ... Wang, C. (2015). Effect of Granulocyte-Macrophage Colony-Stimulating Factor on Prevention and Treatment of Invasive Fungal Disease in Recipients of Allogeneic Stem-Cell Transplantation: A Prospective Multicenter Randomized Phase IV Trial. *Journal of Clinical Oncology 33*, 3999–4006.

Wani, M. Y., Ahmad, A., Shiekh, R. A., Al-Ghamdi, K. J., & Sobral, A. J. F. N. (2015). Imidazole clubbed 1,3,4-oxadiazole derivatives as potential antifungal agents. *Bioorganic & Medicinal Chemistry 23*, 4172–4180.

Wiederhold, N. P. (2018). The antifungal arsenal: alternative drugs and future targets. *International Journal of Antimicrobial Agents 51*, 333–339.

Wiederhold, N. P., Najvar, L. K., Fothergill, A. W., McCarthy, D. I., Bocanegra, R., Olivo, M., ... Patterson, T. F. (2015). The Investigational Agent E1210 Is Effective in Treatment of Experimental Invasive Candidiasis Caused by Resistant *Candida albicans*. *Antimicrobial Agents and Chemotherapy 59*, 690–692.

Wiederhold, N. P., Najvar, L. K., Jaramillo, R., Olivo, M., Pizzini, J., Catano, G., & Patterson, T. F. (2018). Oral glucan synthase inhibitor SCY-078 is effective in an experimental murine model of invasive candidiasis caused by WT and echinocandin-resistant Candida glabrata. *Journal of Antimicrobial Chemotherapy 73*, 448–451.

Wormley, F. L., Perfect, J. R., Steele, C., & Cox, G. M. (2007). Protection against Cryptococcosis by Using a Murine Gamma Interferon-Producing *Cryptococcus neoformans* Strain. *Infection and Immunity 75*, 1453–1462.

Wu, S., Zhang, Y., He, X., Che, X., Wang, S., Liu, Y., ... Sheng, C. (2014). From Antidiabetic to Antifungal: Discovery of Highly Potent Triazole-Thiazolidinedione Hybrids as Novel Antifungal Agents. *ChemMedChem 9*, 2639–2646.

Wüthrich, M., Filutowicz, H. I., Warner, T., Deepe, G. S., & Klein, B. S. (2003). Vaccine Immunity to Pathogenic Fungi Overcomes the Requirement for CD4 Help in Exogenous Antigen Presentation to CD8 $^{+}$ T Cells: Implications for Vaccine Development in Immune-deficient Hosts. *The Journal of Experimental Medicine 197*, 1405–1416.

Wuts, P. G. M., Simons, L. J., Metzger, B. P., Sterling, R. C., Slightom, J. L., & Elhammer, A. P. (2015). Generation of Broad-Spectrum Antifungal Drug Candidates from the Natural Product Compound Aureobasidin A. *ACS Medicinal Chemistry Letters 6*, 645–649.

Yadav, A. K., Desai, P. R., Rai, M. N., Kaur, R., Ganesan, K., & Bachhawat, A. K. (2011). Glutathione biosynthesis in the yeast pathogens *Candida glabrata* and *Candida albicans*: essential in *C. glabrata*, and essential for virulence in *C. albicans*. *Microbiology 157*, 484–495.

Zacchi, L. F., Schulz, W. L., & Davis, D. A. (2010). HOS2 and HDA1 Encode Histone Deacetylases with Opposing Roles in *Candida albicans* Morphogenesis. *PLoS ONE 5*, e12171.

Zasloff, M. (1987). Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proceedings of the National Academy of Sciences 84*, 5449–5453.

Zhai, B., Wu, C., Wang, L., Sachs, M. S., & Lin, X. (2012). The Antidepressant Sertraline Provides a Promising Therapeutic Option for Neurotropic Cryptococcal Infections. *Antimicrobial Agents and Chemotherapy 56*, 3758–3766.

Zhang, R., Xu, Z., Yin, W., Liu, P., & Zhang, W. (2014). Microwave-Assisted Synthesis and Antifungal Activities of Polysubstituted Furo[3,2-c]chromen-4-ones and 7,8,9,10-Tetrahydro-6 *H* -benzofuro[3,2-c]chromen-6-ones. *Synthetic Communications 44*, 3257–3263.

Zhao, S., Zhao, L., Zhang, X., Liu, C., Hao, C., Xie, H., ... Cheng, M. (2016). Design, synthesis, and structure-activity relationship studies of benzothiazole derivatives as antifungal agents. *European Journal of Medicinal Chemistry 123*, 514–522.