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MINIREVIEW

The Future of Antifungal Drug Therapy: Novel Compounds and Targets

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ABSTRACT Fungal infections are a universal problem and are routinely associated with high morbidity and mortality rates in immunocompromised patients. Existing therapies comprise five different classes of antifungal agents, four of which target the synthesis of ergosterol and cell wall glucans. However, the currently available antifungals have many limitations, including poor oral bioavailability, narrow therapeutic indices, and emerging drug resistance resulting from their use, thus making it essential to investigate the development of novel drugs which can overcome these limitations and add to the antifungal armamentarium. Advances have been made in antifungal drug discovery research and development over the past few years as evidenced by the presence of several new compounds currently in various stages of development. In the following minireview, we provide a comprehensive summary of compounds aimed at one or more novel molecular targets. We also briefly describe potential pathways relevant for fungal pathogenesis that can be considered for drug development in the near future.

KEYWORDS acylhydrazones, antifungal agents, arylamidine, calcineurin, drug therapy, new targets, nikkomycin, olorofilm, sphingolipids, threalose

An estimated 1.7 billion individuals suffer from fungal infections worldwide (1, 2). Fungal infections that are pathologically relevant can be categorized into two main types: superficial fungal infections and invasive fungal infections (3). Superficial infections affect the skin, mucous membranes, and keratinous tissues, causing ailments such as thrush, oropharyngeal candidiasis, and dermatophyte infections. Invasive fungal infections are more life-threatening and affect sterile areas of the body such as the bloodstream, organs (lungs, liver, and kidneys), and the central nervous system (3, 4). Fungal infections can affect immunocompetent and immunocompromised individuals; however, the severity of invasive fungal infections in persons having an underlying disease, immunocompromised individuals undergoing organ transplant or chemotherapy, or patients with HIV/AIDS or autoimmune diseases is concerning (3–5) as such infections result in approximately 1.7 million deaths per year (1, 6, 7).

Of 5 million known fungal species, 300 are known to cause diseases in humans (8, 9); of these, 20 infect humans frequently (8). Examples include *Candida albicans*, *Candida auris*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Malassezia furfur*, *Blastomyces dermatitidis*, *Sporothrix* spp., *Fusarium*, and *Scedosporium* (4, 5, 8, 10).

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Globally, invasive fungal infections of aspergillosis account for 300,000 cases per year, candidiasis accounts for 750,000 cases, and cryptococcosis (in AIDS patients) accounts for 223,000 cases. Mortality rates are estimated to be 30% to 90%, 10% to 75%, and 20% to 70% for aspergillosis, candidiasis, and cryptococcosis, respectively (6, 7, 11, 12).

There are currently 5 structural classes of antifungal drugs being used to treat infections—polyenes, azoles, allylamines, pyrimidines, and echinocandins (2, 4–6). Polyenes (e.g., amphotericin B) bind ergosterol on the surface of the fungus, altering the permeability of the cell membrane (13, 14). They have potent fungicidal activity against *Aspergillus* spp., *Cryptococcus* spp., *Candida* spp., and other fungi. Azoles target lanosterol 14α-demethylase enzymatic activity, thus decreasing ergosterol content in fungi. Most azoles are fungistatic although they can behave as a fungicidal in certain molds, such as *Aspergillus* spp. Echinocandins target 1,3-β-glucan synthase activity, thus altering cell wall organization. Echinocandins are fungicidal against *Candida* spp. and fungistatic against *Aspergillus*, and they have no activity against *Cryptococcus* spp. Pyrimidines disrupt DNA and RNA biosynthesis by interfering with pyrimidine metabolism. This class is fungistatic against *Cryptococcus* spp. and against *Candida* spp. when used in conjunction with polyenes and with azoles, respectively (6, 7, 15, 16). Allylamines act by attenuating an enzyme (squalene epoxidase) of the ergosterol synthesis pathway (17, 18). They are fungicidal against dermatophytes and are fungistatic against *C. albicans* (19).

Although agents in these classes are effectively used as treatments today, there are some drawbacks to their use. Overuse, long treatment courses, and environmental exposure of azoles, polyenes, and echinocandins in the past decade have resulted in drug resistance (4). There is a high prevalence of *Candida* resistance to azoles and echinocandins. According to the 2019 Antibiotic Resistance Threats in the United States report generated by the CDC, there were 34,800 cases of infection and 1,700 deaths caused by drug-resistant *Candida* spp. Azole resistance is likely attributable to the drug being fungistatic in nature, creating a selection pressure leading to resistance, while resistance to echinocandins is relatively recent and has emerged due to the overuse of the drug in the past decade. *Aspergillus* and *Cryptococcus* also display azole resistance (7). Drug resistance arises from a reduced intracellular accumulation of the drug, decreased affinity between the drug target and drug, or a counteraction of the effect of a drug (15, 20). In addition to drug resistance, the polyenes and echinocandins have been shown to be highly toxic with a narrow therapeutic index which is confounded by poor (and variable) oral availability (5, 20). The use of extended-spectrum triazoles, posaconazole, and voriconazole is restricted by considerable drug-drug interactions, variable bioavailability, acute adverse events, and emergence of resistance (21).

With the large numbers of fungal infections, mortality rates associated with invasive fungal infections, and shortcomings of currently used antifungals, there is an ever-increasing need to discover new drugs with an improved range of properties. Only two antifungal drugs have been approved since the start of the 21st century: (i) caspofungin, the first echinocandin to be approved for use (in 2001) (echinocandins were the latest class of antifungals to be discovered in 1970 [2]); and (ii) isavuconazole, a triazole effective against dimorphic fungi, yeast, and molds (approved for use in 2015 (21, 22).

There are a variety of approaches to antifungal drug discovery. Such approaches can take the form of either a whole-cell-based or growth-based assay, where optical density as an indication of cell growth is utilized (23). Usually, protocols from the Clinical and laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are used to determine *in vitro* susceptibility against a variety of fungal pathogens (24). Another approach could be by targeting specific pathways in fungi. Advantages of this approach include minimal risk of toxicity and also utility in helping to identify unique classes of compounds (23). Libraries of compounds used to screen utilizing these approaches could include synthetic, semisynthetic, or natural product libraries (24). They could also be compounds

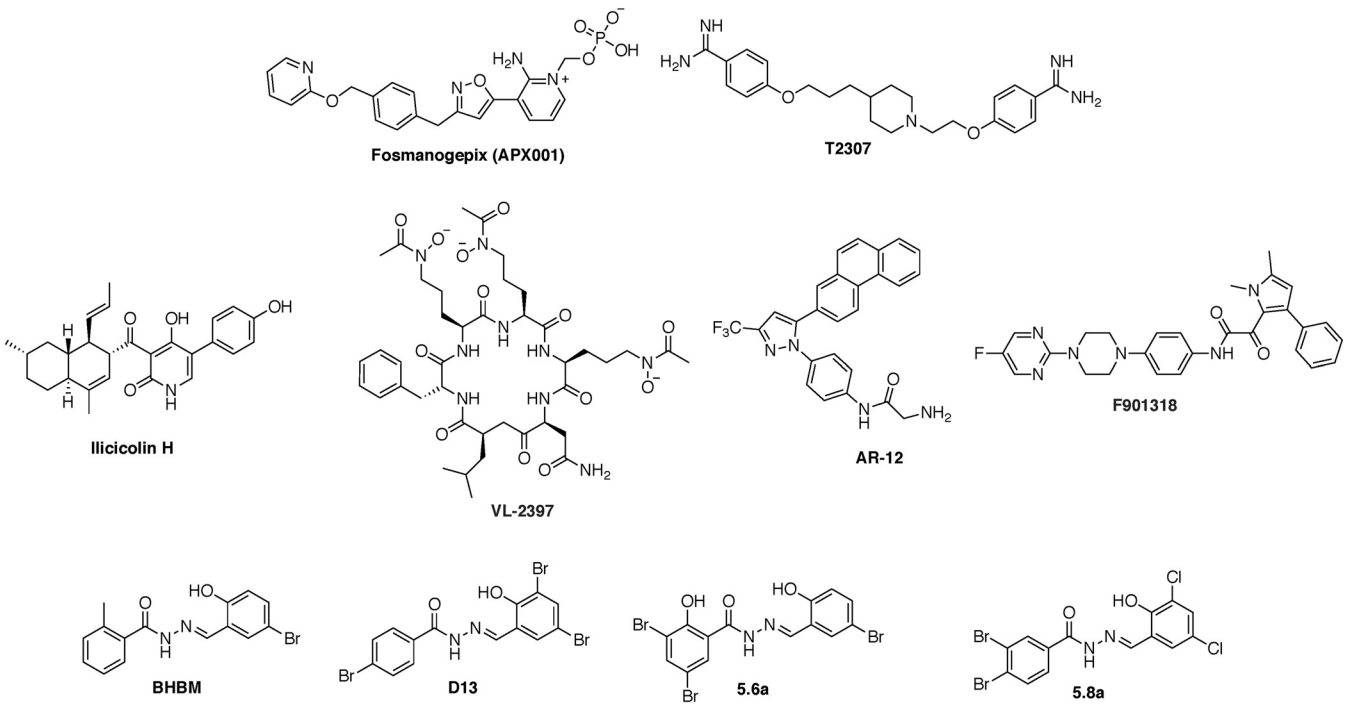

FIG 1 Structures of the antifungal agents in development.

which have already been approved for other indications, an approach known as drug repurposing (24). The pursuit of natural or synthetic compounds as antifungal drugs has its own set of advantages. Natural products are highly complex, and they help us access chemical space that might be difficult to achieve with synthetic compounds (25). They also offer a good source for semisynthetic derivatives (26). Advantages of synthetic compounds include certainty of purity in terms of compounds not being mixtures of isomers, ease of large-scale synthesis, and availability of a large number of libraries for initial screening.

Antifungal drug discovery is challenging as fungal pathogens use much the same eukaryotic machinery as humans, thus reducing the number of pathogen-specific targets. Therefore, it is essential to identify biochemical mechanisms unique to fungi as drug discovery targets in order to develop the next generation(s) of antifungal therapies. An antifungal should ideally have the following properties: (i) minimal or manageable toxicities/side effects, providing a wide therapeutic index; (ii) pharmaceutical properties commensurate with multiple routes of delivery; (iii) activity corresponding to fungus-specific primary pathways and targets; (iv) effects that are preferably fungicidal; (v) a broad spectrum of activity against a range of fungi (7, 10). Development of a drug with all these properties will be arduous. In this minireview, we address the metabolic and signaling pathways that are unique to fungi and/or regulate virulence and which constitute promising targets for drug development.

AGENTS THAT TARGET FUNGAL CELL WALL SYNTHESIS

Fosmanogepix (APX001). Fosmanogepix (Fig. 1) is a small-molecule antifungal developed by Amplyx Pharmaceuticals. It is an *N*-phosphonooxymethyl prodrug of APX001A, which targets the Gwt1 enzyme that catalyzes one of the early steps in the glycosylphosphatidylinositol (GPI)-anchored biosynthesis pathway (Fig. 2) (27, 28). Glycosylphosphatidylinositol (GPI)-anchored proteins are found in eukaryotic organisms, playing a crucial role in fungal adhesion to the host cells. Inhibition of Gwt1 prevents proper localization of mannoproteins, which are essential for cell wall integrity and fungal growth (27, 29). Fosmanogepix inhibited the growth of yeasts such as

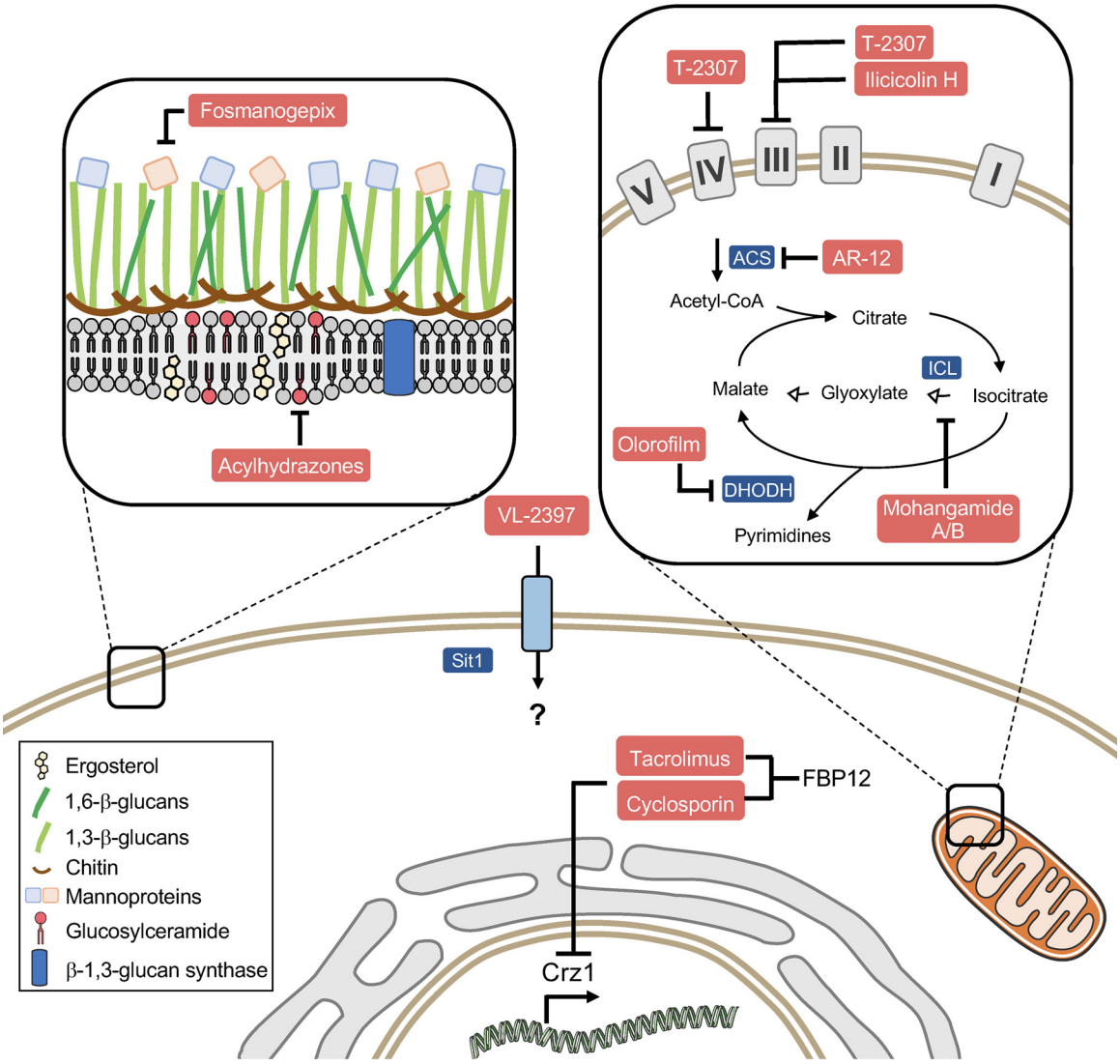

FIG 2 New antifungal drugs and targets. Acylhydrazones impair the production of glucosylceramide. T-2307 and ilicicolin H act by inhibiting the mitochondrial respiratory chain complexes. AR-12, olorofilm, and mohangamides target metabolism-related enzymes. Tacrolimus and cyclosporine inhibit the fungal calcineurin (Crz1) pathway. ACS, acetyl-CoA synthetase.

Candida spp. and *C. neoformans*, as well as filamentous fungi such as *A. fumigatus*, *Fusarium solani*, *Scedosporium prolificans*, and *Pseudallescheria boydii* (30). In addition, fosmanogepix prevented the inositol acylation of GPI in *C. albicans* and *A. fumigatus*, but not in human cells, suggesting that the compound is selective toward fungal cells (29). In mouse models, data from experiments performed with APX001 and APX001A displayed high rates of survival and reduced CFU levels of fungi in lung, kidney, and brain (27). Fosmanogepix was well tolerated when administered orally or intravenously in clinical phase 1 studies and was given fast-track status by the US FDA in September 2019 for seven invasive fungal infections, including candidiasis, aspergillosis, scedosporiosis, fusariosis, mucormycosis, cryptococcosis, and coccidioidomycosis (31). It is currently in phase 2 clinical trial for invasive candidiasis (31).

Nikkomycin Z. Nikkomycin is a pyrimidine nucleoside isolated from *Streptomyces tendae* (32). It inhibits the synthesis of chitin, an essential component of fungal cell wall, by competitively inhibiting chitin synthase and thus septation and causing osmotic stress to the fungal cell (32, 33). Since chitin is absent in mammalian cells, it makes an excellent antifungal target and in turn renders nikkomycin devoid of

cytotoxic effects (33). Coccidiomycosis, also known as valley fever, is caused by *Coccidioides posadasii* or *Coccidioides immitis* (34). Investigators at the University of Arizona who were involved in the development of nikkomycin Z for treatment of pulmonary coccidiomycosis reported in 2014 that preparations were being made for a phase 2 clinical trial (35), although there have been no updates since.

INHIBITORS OF THE FUNCTION OF MITOCHONDRIA

Mitochondria represent the powerhouse of eukaryotic cells, producing most of the cellular ATP pool through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. The roles of mitochondria in energy metabolism include the synthesis of amino acids and phospholipids, which, in addition to respiration, govern processes such as senescence, virulence, and antifungal drug resistance (36, 37). Although the mitochondrial genomes of fungi and humans share high similarity, fungus-specific proteins (such as yeast Nuo1 and Nuo2) constitute promising targets for the development of selective antifungals (38). To our knowledge, the following two inhibitors of the fungal mitochondria have been described so far.

T-2307. The arylamidine T-2307 exhibits fungicidal activity *in vitro* against *Candida*, *Aspergillus*, and *Cryptococcus* spp., preventing disseminated infection in mice (39, 40). This compound is efficiently internalized by *C. albicans* cells through polyamine transporters, which does not seem to occur in rat hepatocytes (41, 42). Once internalized into the fungal cell, T-2307 inhibits mainly complexes III and IV of the respiratory chain, disrupting the mitochondrial membrane potential (Fig. 2) (43, 44). Interestingly, only a minimal effect on rat mitochondria was observed, highlighting this compound's potential to act as a selective inhibitor (44). Initially studied by the Fujifilm Toyama Chemical Co., T-2307 was licensed to Appili Therapeutics in November 2019, where it was renamed ATI-2307. The compound was well tolerated in human phase 1 studies and is currently under preclinical analysis prior phase 2, expected for 2021 (https://www.appilitherapeutics.com/ati-2307).

Ilicicolin H. Ilicicolin H is a polyketide that was isolated from the fungus *Cylindrocladium ilicicola* and shows activity against *Cryptococcus*, *Candida*, and *Aspergillus* spp. (45). The mechanism of action of this compound involves the inhibition of the mitochondrial cytochrome bc_1 reductase (50% inhibitory concentration [IC_{50}], 2 to 3 ng/ml) (Fig. 2) (45–47). In the animal model, ilicicolin H reduced the fungal burden in mice infected with *C. albicans* and *C. neoformans* (45), exhibiting a low affinity toward rat mitochondria (48). Together, these observations highlight the potential of ilicicolin as an efficacious and selective antifungal.

OTHER/UNKNOWN

AR-12. The celecoxib derivative AR-12 (Arno Therapeutics Inc.) was developed as a protein kinase inhibitor (PKI) and was initially used as an anticancer agent in phase I trial (ClinicalTrials registration no. NCT00978523). As protein kinases share structural and functional similarities across distinct organisms, PKI compounds were also screened for antibacterial and antifungal activity (49, 50). AR-12 inhibits the growth of several fungi, including yeasts such as *Candida* and *C. neoformans* and filamentous species such as *A. fumigatus* (51). The most interesting features of AR-12 involve its potent activity against dimorphic fungi and molds that are notoriously challenging in clinical settings, such as *Scedosporium* and *Rhizopus oryzae* (51). In addition, combination therapy using AR-12 and fluconazole reduced the fungal burden in brain in a mouse model of cryptococcosis (51). Although initially characterized as a PDK1 inhibitor, AR-12 does not inhibit the kinase ortholog in *C. neoformans* (52). Apparently, the antifungal activity of AR-12 involves dual mechanisms of action: (i) targeting of fungal acetyl coenzyme A (acetyl-CoA) synthetase, which catalyzes the production of acetyl-CoA from acetate and CoA (Fig. 2), regulating the histone acetylation and carbon metabolism (52), and (ii) downregulation of the host chaperones, modulating the immune response (8, 53). The future progress of AR-12 in clinical trials for antifungal therapy remains uncertain, as Arno Therapeutics declared bankruptcy in 2017 (2).

Acylhydrazones. Aromatic acylhydrazones BHBM and D2 were identified as inhibitors of fungal sphingolipid synthesis through screening of a commercially available library (54, 55). Further analysis of BHBM and D2 derivatives led to the identification of a more potent compound, D13, that was highly active *in vitro* and performed better than BHBM in *in vivo* models of cryptococcosis, candidiasis, and pulmonary aspergillosis (56). Based on the structures of BHBM, D2, and D13, a novel library of ~300 aromatic acylhydrazones was designed and synthesized. Further study resulted in the identification of 5 compounds which are potent, fungicidal, and highly selective toward fungi, with selectivity index values of >500 (57). Among the 5 lead compounds, SB-AF-1002 was tested in mouse models of a variety of invasive fungal infections and was found to outperform the current standard of care (58).

VL-2397. VL-2397 (formerly termed ASP2397) is a cyclic hexapeptide isolated from the fungus *Acremonium persicinum* (59), potentially representing a novel class of antifungals with a unique mode of action. This compound chelates aluminum and is structurally related to ferrichrome, a low-molecular-weight siderophore (2, 59). Supplementation of the culture media with 0.03 mM iron increased the VL-2397 MIC for *A. fumigatus* from 1 mg/liter to 2 mg/liter (60). Similarly, the addition of bathophenanthroline disulfonate (BPS), an iron chelator, reduced the VL-2397 MIC from 1 mg/liter to 0.06 mg/liter. Together, these observations suggest that the compounds' activity is affected by iron availability (60). In addition, the replacement of the Al in VL-2397 structure for Fe, generating the compound AS2488053, impaired antifungal activity (59). It was recently reported that iron abundance regulates expression of Sit1, the siderophore transporter that promotes VL-2397 internalization in the fungal cell (60). In fact, *A. fumigatus* cells lacking Sit1 were resistant to VL-2397 (63). Furthermore, expression of *A. fumigatus* Sit1 renders the intrinsically resistant species *Saccharomyces cerevisiae* susceptible to VL-2397 (63). Despite the requirement of Sit1 for VL-2397 uptake by the fungal cell, the intracellular target of the compound remains to be elucidated (Fig. 2).

VL-2397 inhibits the *in vitro* growth of *Aspergillus* spp., *Fusarium solani*, *Candida glabrata*, and *C. neoformans* (61). The compound also kills *A. fumigatus* conidia and prevents hyphal elongation in germlings (61). Potent and fungicidal activity of VL-2397, especially against *Aspergillus*, was also observed *in vivo*, as the drug increased survival rates in mouse and silkworm larva models of aspergillosis (61, 62). VL-2397 was well tolerated by healthy volunteers, successfully passing the phase I trials as a treatment for invasive aspergillosis (2, 60). Unfortunately, phase II studies using VL-2397 were discontinued for financial reasons.

Olorofilm. Another promising class of antifungals includes the orotomide F901318 (olorofilm; F2G Ltd.), which targets the dihydroorotate dehydrogenase (DHODH) enzyme, involved in *de novo* pyrimidine biosynthesis (Fig. 2) (63). Pyrimidines act as structural precursors of molecules required for the synthesis of DNA/RNA, cell wall, and phospholipids (64), playing a crucial role in fungal virulence (65, 66). Although the DHODH enzyme is also found in mammals, F901318 affinity toward the human enzyme was 2,000-fold lower, suggesting that the drug inhibits specifically the fungal protein (63). F901318 showed potent activity against molds, such as *Aspergillus* species, and dimorphic fungi, such as *H. capsulatum*, *B. dermatitidis*, *C. immitis*, and *Paracoccidioides brasiliensis* (63, 67). Potential limitations in the clinical use of orotomides include the lack of activity against yeasts (such as *Candida* and *Cryptococcus*) (63). F901318 improved the rate of survival in a murine model of invasive aspergilosis, possibly through preventing *A. fumigatus* germination and hyphal extension (63, 68). Pharmacokinetic (PK) studies of F901318 showed good tissue distribution in mice, and the drug is being studied in phase IIb trials for the treatment of invasive infections caused by *Aspergillus*, *Scedosporium*, and other resistant species (ClinicalTrials registration no.03583164).

FUNGAL PATHWAYS AS PROMISING TARGETS FOR DRUG DEVELOPMENT

The glyoxylate cycle. The glyoxylate cycle corresponds to an anaplerotic route of the tricarboxylic acid (TCA) cycle, bypassing the reactions that generate CO_2 and allowing the use of two-carbon compounds as carbon sources for gluconeogenesis (69, 70).

This metabolic pathway includes five enzymatic reactions; two of them, catalyzed by isocitrate lyase (ICL) and malate synthase (MS), are unique to this cycle, while the remaining three (citrate synthase, aconitase, and malate dehydrogenase) are shared with the TCA cycle (71). Interestingly, *C. albicans* cells lacking *ICL1*, the isocitrate lyase-encoding gene, were avirulent in mice (70). In addition, phagocytosis of *C. albicans* and *P. brasiliensis* induced glyoxylate cycle-related genes, suggesting that this pathway plays an important role in fungal survival inside the macrophages (72, 73). Besides its relevance to fungal pathogenesis, the glyoxylate cycle is not observed in the mammalian host and therefore constitutes a promising target for selective antifungals (74).

Mohangamides A and B were isolated from *Streptomyces* sp. and shown to inhibit *C. albicans* ICL (75). Additionally, mohangamide A impaired the growth of *C. albicans in vitro* when acetate but not glucose was used as a carbon source (75). The efficacy of these compounds in the treatment of a murine model of candidiasis and their pharmacokinetics properties remain to be further elucidated.

The calcineurin pathway. Calcineurin is a Ca^{2+}/calmodulin-activated protein phosphatase, conserved from fungi to mammals (76). The calcineurin pathway is the target of tacrolimus (FK506) and cyclosporine (CsA), which are widely used as immunosuppressive agents that prevent graft rejection. These drugs bind to the corresponding immunophilins (FK506-FKBP12 and CsA-CypA) and impair the access of phosphatase substrates to calcineurin, ultimately inhibiting T-cell proliferation. In pathogenic fungi, the calcineurin pathway plays a pivotal role in growth and virulence (77–79). Targeting of calcineurin for antifungal drug development has been restricted by the immunomodulatory effects that the drugs exert in the host. An attempt to circumvent this limitation included the synthesis of FK506 antagonists, permeative with respect to mammalian cells but not fungal cells, which likely minimize the immunosuppression while retaining their antifungal properties (80). The structural characterization of the FK506-FKBP12 complex in fungi also shed light on regions that differ from their mammalian counterparts, allowing the development of the APX879 compound (81). APX879 efficiently reduced fungal burden in a murine model of cryptococcosis, improving survival (81). In addition, this compound showed reduced immunosuppressive activity in comparison to the parental drug, FK506 (81). These new findings pave the way for the design of selective and efficacious inhibitors of the fungal calcineurin.

Hsp90. Hsp90 is a conserved chaperone which regulates the function and stability of several client proteins, including its downstream effector calcineurin (82). In pathogenic fungi, Hsp90 mediates stress responses, virulence, and drug resistance (83–85). Hsp90 inhibitors improved fluconazole efficacy and prevented a lethal *C. albicans* infection (86), highlighting the therapeutic potential of molecules which target Hsp90. In fact, patients with invasive candidiasis who received an antibody against Hsp90 (efungumab [Mycograb; NeuTec Pharma/Novartis]) along with amphotericin B showed a better clinical response than those who were on amphotericin B monotherapy (87). Marketing authorization for Mycograb was denied in November 2006 due to quality concerns. A modified version of Mycograb, named Mycograb C28Y, was further developed but unfortunately was not as efficacious as the original formulation (88). Geldamycin and its derivatives, which target Hsp90, have been used for anticancer therapy; however, their use was restricted as they show host toxicity (86, 89). Novel inhibitors with reduced toxicity toward mammalian cells, enabling the development of Hsp90 inhibitors which retain antifungal activity and display increased selectivity, were previously described (90).

The trehalose pathway. The disaccharide trehalose is an energy storage molecule and serves as a source of carbon (91). This sugar also functions as a stress protectant, preventing protein degradation and preserving the cell membrane structure under stress conditions (92, 93). Trehalose synthesis is linked to the glycolytic pathway, as the first step of trehalose production involves the conversion of glucose-6-phosphate to trehalose-6-phosphate by trehalose 6-phosphate synthase 1 (Tps1) (94). Next, trehalose 6-phosphate phosphatase (Tps2) generates trehalose from trehalose-6-phosphate. In *Cryptococcus*, deletion of *TPS1* or *TPS2* genes impaired growth at 37°C and rendered

cells avirulent (95, 96). Interestingly, disruption of *TPS2* was followed by the accumulation of the toxic intermediate trehalose 6-phosphate, causing fungal cell death (96). These observations suggest that targeting of the trehalose pathway, especially Tps2, might compromise fungal viability and virulence. In addition, mammalian cells lack trehalose synthesis, indicating that Tps2 inhibitors might act as selective antifungals.

Sphingolipid pathway. Along with sterols, sphingolipids such as glucosylceramide (GlcCer), inositol phosphorylceramide (IPC), and mannosylinositol phosphorylceramide (MIPC) are major constituents of fungal lipid rafts (97). These molecules play crucial roles in fungal growth, differentiation, and virulence (98). Sphingolipid synthesis starts in the endoplasmic reticulum, with the condensation of serine and palmitoyl-CoA. This reaction produces 3-keto dihydrosphingosine and is catalyzed by the enzyme serine palmitoyltransferase (SPT), targeted by myriocin and serine (99, 100). The use of myriocin in antifungal therapy depends on the development of selective derivatives, as the compound also targets mammalian SPT (101). Another promising step in sphingolipid production to be targeted by novel compounds includes the synthesis of ceramide. Deletion of the ceramide synthase (CerS)-encoding gene renders *C. neoformans* cells avirulent (102), indicating that CerS is an important regulator of fungal pathogenesis. Fumonisin and australifungin were previously described to inhibit CerS (103) but showed a limited spectrum of activity and poor selectivity (104). Inhibitors of CerS might exhibit a dual mechanism of action, by depleting the pool of complex sphingolipids and leading to the accumulation of toxic intermediates. Therefore, the development of compounds which target the fungal but not the mammalian CerS might give rise to a novel class of potent antifungals.

Once produced, ceramides are transported to the Golgi apparatus, where synthesis of complex sphingolipids occurs. The transfer of a phosphoinositol from phosphatidylinositol to phytoceramide leads to the production of IPC, through the activity of IPC synthase (105). Aureobasidin and khafrefungin inhibit IPC synthase at nanomolar concentrations (106), showing activity against *C. neoformans* and *C. albicans* (107–109). Aureobasidin is also well tolerated in animals, efficiently treating invasive candidiasis (107). Aureogen Biosciences developed 58 novel derivatives from aureobasidin with improved potency against *A. fumigatus*, which were licensed to Merck in 2015.

Other pathways. To efficiently colonize the host, fungal cells must sense and adapt to the challenges imposed by the physiological conditions. Stress-responsive pathways in fungi include the cyclic AMP signaling pathway, protein kinase C (PKC)/Mpk1 mitogen-activated protein kinase pathway and the high-osmolarity glycerol (HOG) pathway (110). In *C. albicans* and *C. neoformans*, the disturbance of the HOG pathway attenuated virulence in mice, highlighting the relevance of this cascade for fungal pathogenesis and its potential as an antifungal target (111–113). A variety of antifungal compounds, including ambutricins and phenylpyrroles (as fludioxonil), targeted the HOG pathway, leading to the accumulation of glycerol and fatty acids (114). The disbalance in the osmoregulation led to the leakage of intracellular content and, ultimately, cell death (115). Conversely, the antifungal cercosporamide inhibited selectively fungal Pkc1 (116), which has a central role in cell wall biosynthesis and remodeling (117). The loss of Pkc1 function was accompanied by cell lysis (118), indicating that pharmacological targeting of the Pkc1 might lead to the synthesis of fungicidal drugs.

REPURPOSING OF EXISTING DRUGS

Recently, in a continued effort to find new antifungal agents, drug repurposing was widely undertaken. This involves identifying new uses for drugs that had previously been approved by the U.S. FDA for different conditions (119). The advantages of this strategy over conventional drug discovery include lower risk of failure, especially in terms of safety, and shorter time frame for the development of the drug (120). Some of the drugs that were previously identified/used for other indications were repurposed to treat fungal infections.

Sertraline. X. Lin and coworkers at Texas A&M University screened the Johns Hopkins clinical compound library and found that sertraline displayed modest inhibitory

activity against *Aspergillus nidulans* (121). Further analysis of sertraline against *Aspergillus* and *Candida* species showed that its MIC against these fungi was much higher than the serum concentration of sertraline that can be achieved therapeutically (121). However, sertraline was found to be fungicidal against *Cryptococcus* at concentrations of $<10\,\mu$g/ml and previous PK data in rats and dogs showed that its concentration in cerebrospinal fluid was 20-to-40-fold higher than its serum concentration. In an *in vivo* model of cryptococcosis, sertraline was found to reduce the fungal burden in the brain (121). In order to understand the antifungal mechanism of sertraline, X. Lin and coworkers screened a whole-genome deletion collection of *Saccharomyces cerevisiae* isolates and found that sertraline perturbs translation and, in turn, protein synthesis (121).

Tamoxifen. Tamoxifen is a drug that is generally used to treat breast cancer and also as a protective adjuvant in women who have high risk of developing breast cancer (122). Its anticancer properties are known to be mediated by estrogen receptor antagonism and also by oxidative stress on breast cancer cells (123, 124). In 1989, Wiseman, Cannon, and Arnstein reported inhibitory effect of tamoxifen against *Saccharomyces cerevisiae* (125). Tamoxifen was found to be fungicidal against *C. albicans* at 15 to 20 μM concentration, whereas its MIC against *C. neoformans* and other *Candida* species was 8 to 64 g/ml (122). Tamoxifen was also effective in a murine model of candidiasis at 200 mg/kg of body weight. Although the exact mechanism by which tamoxifen exerts antifungal activity is not well known, inhibition of some components of the calcium-calcineurin pathway and inhibition of the calmodulin site are two methods proposed by researchers (122).

SUMMARY

Despite the increased mortality caused by fungal infections in the past decade, only minor advances in antifungal therapy have been reported during this decade. In fact, most of the drugs that were recently approved or that are currently in development consist of derivatives of azoles and echinocandins. Several compounds highlighted here inhibited fungus-specific proteins/pathways, exhibiting low toxicity toward mammalian cells and good pharmacological properties with a broader spectrum of activity than current antifungals. As only a small fraction of compounds undergoing clinical studies will be approved and released into the market, it is imperative that more antifungal compounds are identified and thoroughly explored in the coming years.

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