Drug repurposing strategies in the development of potential antifungal agents

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Abstract
The morbidity and mortality caused by invasive fungal infections are increasing across the globe due to developments in transplant surgery, the use of immunosuppressive agents, and the emergence of drug-resistant fungal strains, which has led to a challenge in terms of treatment due to the limitations of three classes of drugs. Hence, it is imperative to establish effective strategies to identify and design new antifungal drugs. Drug repurposing is a potential way of expanding the application of existing drugs. Recently, various existing drugs have been shown to be useful in the prevention and treatment of invasive fungi. In this review, we summarize the currently used antifungal agents. In addition, the most up-to-date information on the effectiveness of existing drugs with antifungal activity is discussed. Moreover, the antifungal mechanisms of existing drugs are highlighted. These data will provide valuable knowledge to stimulate further investigation and clinical application in this field.

Key points
- Conventional antifungal agents have limitations due to the occurrence of drug-resistant strains.
- Non-antifungal drugs act as antifungal agents in various ways toward different targets.
- Non-antifungal drugs with antifungal activity are demonstrated as effective antifungal strategies.

Keywords Drug repurposing · Antifungal therapy · Antifungal mechanism · Clinical application · Antifungal agent

Introduction
Fungal infection has become a significant event leading to over 1.5 million deaths annually worldwide (Deaguero et al. 2020). To date, the most common fungal infections related to human mortality and morbidity are caused by Cryptococcus, Candida, and Aspergillus (Boral et al. 2018). The impact of mycoses has increased, especially in patients with immunodeficiency disorders who have undergone transplant surgery, chemoradiotherapy, hemodialysis, or the treatment with immunosuppressive agents (Drgona et al. 2014). Hence, antifungal therapy represents a challenging problem for clinicians. In addition, the limited number of antifungal agents in the clinic can induce side effects and a great number of drug-resistant or multidrug-resistant strains have emerged. Candida auris, a multidrug-resistant fungus, has shown a global increase in recent years. Importantly, some of these infections are resistant to almost all current antifungal agents (Du et al. 2020). In

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New Delhi, it was reported that 15 COVID-19 patients had secondary candidiasis in the intensive care unit (ICU), two-thirds of which were caused by *C. auris*, and the mortality rate was up to 60 % (Chowdhary et al. 2020). *Lomentospora prolificans* is another classic fungus with intrinsic resistance to nearly all existing antifungal agents (Pellon et al. 2018). In *A. fumigatus*, the emergence of azole resistance has made the treatment of aspergillosis a global public health problem, especially in Australia, China, the USA, and parts of Europe (Meis et al. 2016). More than 25 % of *A. fumigatus* has been found to be resistant to the triazole antifungal agents in an ICU in the Netherlands (Lestrade et al. 2016). More concerning, patients with invasive aspergillosis caused by azole-resistant *A. fumigatus* have mortality rates ranging from 50 to 100 % (Lestrade et al. 2019).

Currently, the first-line antifungal agents for invasive fungal infections are amphotericin B, echinocandins, isavuconazole, itraconazole, posaconazole, and voriconazole (Zhao et al. 2016). However, due to the existence of toxicity and drug-resistant strains, the present antifungal options have become more restricted. A variety of approaches have been employed to conduct antifungal therapies, such as the synthesis of new substances, the use of extracts from organisms, changing of the administration methods or forms of old drugs to treat fungal diseases, and an association between known antifungal drugs and non-antifungal agents (Robbins et al. 2016). Moreover, drug repurposing is a potential strategy for the treatment of invasive fungal infections, owing to the excellent antifungal activity of these drugs. Several agents have recently been confirmed to serve as antifungal candidates in the treatment of mycoses. The purpose of this review is to present a series of known drugs that have been investigated for their application in the treatment of fungal infections. Firstly, the strategies, mechanisms, and challenges of current antifungal drugs are described. Secondly, the extensive application and antifungal mechanisms of drugs with antifungal activity that is used in the clinic to treat non-mycotic infections are highlighted.

**Current antifungal drugs used in clinics**

Since the first active antymycotic griseofulvin was recognized in 1939, a multitude of antifungal agents have been used clinically. Polymenes, azoles, echinocandins, and fluconosine are currently the main treatments for invasive fungal infections in clinical settings. In fungi, ergosterol, located in the cell membrane, regulates membrane structure permeability, mobility, and substance transportation by making direct linkages with the phospholipid membrane (Anderson et al. 2014). The representative polyene drug is amphotericin B, which can bind to ergosterol from lipid bilayers and form large and extramembranous aggregates (Anderson et al. 2014). These extramembranous aggregates lead to the formation of transmembranal pores, which can leak cellular components. This results in the death of pathogenic fungi (Anderson et al. 2014). As the “gold standard” for combating invasive fungal infections for decades, amphotericin B has a relatively broad spectrum of antifungal activity against yeasts and molds (Ostrosky-Zeichner et al. 2003). For instance, an investigation of 78 *Candida* sp. clinical strains showed that all examined free-living cells were susceptible to amphotericin B (Prazynska and Gospodarek 2014). The MIC90 of amphotericin B for common yeasts in clinical settings ranges from 0.25 to 2 μg/mL and is 1–4 μg/mL for clinically important molds (Ellis 2002). In addition, amphotericin B has been used as an alternative therapy for invasive aspergillosis (Patterson et al. 2016). In vitro, amphotericin B combined with caspofungin and voriconazole has synergistic anti-*Aspergillus* species effects (O’Shaughnessy et al. 2006), but these findings have not been validated in vivo (Baddley and Pappas 2005; Haidar and Singh 2018). Moreover, the clinical applications of amphotericin B have been limited due to toxicity, which includes nephrotoxicity and infusion-related reactions such as chest pain, dyspnea, hypoxia, flushing, and urticaria (Rodan et al. 2003). To resolve this problem, lipid formations of amphotericin B, including liposomal amphotericin B (LAmB), AmpB lipid complex (ABLC), and AmpB colloid dispersion (ABCD), were developed (Ostrosky-Zeichner et al. 2003). Toxicity was greatly reduced using these formulations; however, they are inefficient in penetrating certain tissues, such as the kidney, and thus fail to reach therapeutic concentrations (Smitherman 2016).

Due to the safety and wide availability, azoles (including fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole) are the most widely used antifungal agents. Azoles can be used against the majority of fungi, as they inhibit the cytochrome P450-dependent enzyme 14α demethylase (Cyp51) (Emami et al. 2017). Lanosterol conversion into ergosterol is blocked when azoles inhibit Cyp51. Azoles have excellent therapeutic effects on molds as well as yeasts (Meis et al. 2016; Patterson et al. 2016; Robbins et al. 2017). For example, in a randomized trial, voriconazole was used in therapy for invasive aspergillosis and achieved a 52.8 % curative rate (Haidar and Singh 2018). However, the extensive use of azoles has subsequently led to the emergence of acquired azole resistance, particularly in *Aspergillus* species (Wiederhold 2017). A resistance rate of 26 % was found in *A. fumigatus* culture–positive patients in the ICU (Paasen et al. 2016). Azoles can also bind to the human cytochrome P450 enzyme system (CYP450), leading to reduced antifungal efficiency (Wiederhold 2018).

Echinocandins are a group of effective antifungal agents which, according to the current Infectious Society of America (IDSA) and European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) guidelines,
are useful for the primary treatment of invasive candidiasis (Chang et al. 2017). Anidulafungin, caspofungin, and micafungin are representatives of this group (Emri et al. 2013). Compared with the azole antifungal agents, echinocandins rarely cause resistance, have a good safety profile, have better clinical outcomes, and have been used for two decades. The mechanism of echinocandins mainly involves inhibition of cell wall synthesis by inhibiting β-1,3-D-glucan synthase, a key cell wall component of pathogenic fungi. Following treatment with echinocandins, the buried glucan cell wall architecture can be exposed, which induces abnormal morphology and growth limitation. The time-kill methodology of C. albicans biofilms, treated with caspofungin, displayed at least 99 % killing at physiological concentrations. Investigations in AIDS patients, who were unable to tolerate amphotericin B, have also shown that caspofungin is an efficient therapeutic option for azole-resistant Candida infections (Ramage et al. 2002; Garbino 2004). Anidulafungin is an excellent therapeutic choice against Aspergillus and Candida species, including those resistant to either fluconazole or amphotericin B. A literature review mentioned that it was a superior option to fluconazole in the treatment of oesophageal candidiasis and candidemia (Vazquez 2005). Micafungin also exhibits excellent activity on Candida spp. resistant to multiple azoles, and 8 μg/mL of micafungin demonstrated a 57 % antifungal rate in C. parapsilosis (Gil-Alonso et al. 2015). Nevertheless, probably due to the differences in cell wall composition or structure, this class is largely inactive in most filamentous fungi including Zygomycetes and Fusarium species (Perlin 2020). In addition, C. neoformans is naturally resistant to echinocandins, which are completely ineffective in treating cryptococcosis (Denning 2003). Additionally, due to their characteristic large molecular weight, low oral bioavailability, and limited absorption in the gastrointestinal tract, these drugs are only administered intravenously.

Flucytosine is also an important antifungal agent that inhibits the synthesis of DNA and RNA and is mainly used to treat cryptococcosis and candidiasis (Bennet 1977). Because of the poor efficacy of monotherapy due to the prevalence of intrinsically resistant strains (approximately 10 % C. albicans isolates) and the frequent development of resistance during treatment (Vermes et al. 2000), flucytosine is always used in combination with another antifungal agent (such as amphotericin B). These combination treatments have shown promising clinical effects. For example, 49 HIV-infected patients with cryptococcosis treated with combination flucytosine and amphotericin B were reported to have a higher survival rate than patients receiving amphotericin B alone (Chuck and Sande 1989). For candidiasis, several similar trials have shown that this combined regimen also has synergic activity against a variety of Candida spp. (Medoff et al. 1971; Montgomerie et al. 1975; Smego Jr et al. 1984). Currently, flucytosine combined with amphotericin B is the standard induction therapy for Cryptococcal and Candida meningitis, because it has lower rates of treatment failure and mortality than other alternative approaches (Bridges et al. 2017; Greene et al. 2020).

Azole resistance among Candida species, such as C. auris and Aspergillus species, as well as echinocandin resistance in C. glabrata is an alarming problem (Wiederhold 2017). In addition, other molds such as Scedosporium and Fusarium species have reduced susceptibility to clinically available antifungal drugs (Wiederhold 2017). Although amphotericin B is recognized to have excellent antifungal activity, there are still fungi, such as C. lusitaniae and A. terreus that show intrinsic resistance to amphotericin B (Arendrup et al. 2012). Consequently, the development of new antifungals is needed to resolve this issue.

Drug repurposing strategy

Compared with the development of new drugs, drug repurposing has several advantages, as mainly reflected in decreased cost and security risk (Cha et al. 2018). The average new drug development time savings has been estimated to reach 5–7 years, and the failure rate for problems related to safety or toxicity is less than 50 % (Ashburn and Thor 2004). Drug repurposing involves tapping into new applications for existing drugs and thus requires an innovative clinical in-depth investigation of the pharmacological mechanisms. For example, aspirin, a traditional drug with analgesic and antipyretic activity initially, and is now gradually being used in the prevention and treatment of multi-system diseases including cardiovascular disease, stroke, and digestive tract cancers (Theken and Grosser 2018; Bosetti et al. 2020; Burn et al. 2020; Johnston et al. 2020). Vitamin D is widely used to regulate calcium and phosphate metabolism for bone health (Ng et al. 2019). Recent studies have found that vitamin D deficiency is associated with an increased risk of cardiovascular disease, diabetes, hypercholesterolemia, and even COVID-19 (Kouvari et al. 2020; Meltzer et al. 2020). Metformin, originally used to treat diabetes, has so far more than a dozen potential new functions including the treatment and prevention of cancer, cardiovascular disease, and mental illness such as infantile autism and cognitive disorder (Tseng 2015; Dy et al. 2018; Shiers et al. 2018; Arrieta et al. 2019; Cheung et al. 2019; Lee et al. 2019; Mohan et al. 2019).

In addition, drug repurposing is also very significant in the development of new antifungal drugs, the effectiveness of existing drugs with antifungal activity have been associated with exciting non-antifungal agents chiefly consisting of antibacterial drugs, immunosuppressants, statins, antiarrhythmic drugs, antipsychotic drugs, antidepressant drugs, and non-steroidal anti-inflammatory drugs (NSAIDs).
Antibacterial drugs

The discovery of antibacterial drugs has been the greatest invention of medical science in reducing morbidity and mortality. Traditionally, antibacterial drugs were commonly used as medical treatments for infectious diseases caused by gram-negative bacteria, gram-positive bacteria, mycoplasma, chlamydia, rickettsia, as well as spirochaetes. Antibacterial drugs with antifungal activity mainly include tetracyclines (e.g., demeclocycline, doxycycline, minocycline, and tigecycline), aminoglycosides (e.g., gentamicin, neomycin, paromomycin, ribostamycin, streptomycin, and tobramycin), macrolides (e.g., azithromycin and clarithromycin), quinolone polypeptides (e.g., ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, norfloxacin, and trovafloxacin), polypeptides (e.g., polymyxin B), and others such as linezolid and rifampicin. Many studies in vitro and in animal models have revealed that antibacterial drugs have broad-spectrum antifungal activity (Table 1). In general, they are commonly used alone or in combination to regulate the gene expression levels of adhesion, hypha, or biofilm formation, to decrease extracellular glycans and cell surface hydrophobicity, and even to inhibit efflux pump activity. In Fusarium spp., tobramycin combined with amphotericin B and voriconazole has an 80 % and 76 % synergistic effect, respectively, in increasing the permeability of the cell wall and cell membrane (Venturini et al. 2016). Minocycline, a tetracycline, has been verified to exhibit broad-spectrum antifungal activity against A. fumigatus (Venturini et al. 1977; Shi et al. 2010; Loreto et al. 2014; Gao et al. 2016). Minocycline, a tetracycline, has been verified to exhibit broad-spectrum antifungal activity against A. fumigatus, A. flavus, Fusarium solani, and F. oxysporum with MICs in the range of 0.125–4 μg/mL. In addition, a series of studies on the antifungal activity of minocycline combined with antifungal agents (amphotericin B, fluconazole, itraconazole, posaconazole, and voriconazole) have been reported (Lew et al. 1977; Shi et al. 2010; Loreto et al. 2014; Gao et al. 2020). Similar findings have also been confirmed for polymyxin B which mainly combines with ketoconazole, micafungin, and amphotericin B in C. albicans to alter the permeability of the cell membrane (Moneib 1995). Moreover, polymyxin B can bind anionic lipids on the fungal membrane, both alone, with the MIC100 range from 8 to 256 μg/mL, and combined with fluconazole for Fusarium spp., C. neoformans, Rhizopus oryzae, and A. fumigatus, which destroys the membrane integrity (Zhai et al. 2010; Venturini et al. 2016). Many animal models have also been used to verify the antifungal activity of antibacterial agents. In the murine model of candidiasis, a combination of cefoperazone–sulbactam, colistin, or meropenem with caspofungin has been found to result in lower fungal burden in the kidneys, than that after treatment with caspofungin alone (Ozcan et al. 2006; Zeidler et al. 2013; Keçeli et al. 2014). Histopathological results indicated that the degree of inflammation was 25 % less in the group treated with caspofungin and meropenem than caspofungin monotherapy (Ozcan et al. 2006). In mice, quinolones combined with fluconazole and amphotericin B also have synergistic effects against invasive candidiasis (Sugar et al. 1997; Sasaki et al. 2000). β-Lactam derivatives show broad-spectrum antifungal activity in vitro against Candida spp. and Aspergillus spp. (Gowri et al. 2016; O’Driscoll et al. 2008; Mohamadzadeh et al. 2020). However, studies have indicated that β-lactam antibiotics are unable to inhibit the growth of Candida species, and amoxicillin can increase the virulence of Candida krusei and Candida tropicalis in Caenorhabditis elegans (Aguiar Cordeiro et al. 2018).

Biofilms represent one of the major virulence factors in pathogenic fungi, which develop on the surfaces of stents, shunts, prostheses, implants, endotracheal tubes, pacemakers, and various types of catheters (Sardi Jde et al. 2014). Compared to planktonic cells, the interior biofilm cells display severe resistance to a wide variety of clinical antifungal agents. A small subset of yeast cells in C. albicans biofilms were found to be highly resistant to amphotericin B. This resistance was independent of the upregulation of efflux pumps and cell membrane composition (Sardi Jde et al. 2014). When combined with clarithromycin, the permeability barrier of the biofilm matrix was altered, which resulted in increased penetration of amphotericin B (Del Pozo et al. 2011). In addition, doxycycline, tigecycline, and rifampicin were also found to enhance the activity of amphotericin B to suppress biofilm formation (Lew et al. 1977; El-Azizi 2007; Miceli et al. 2009; Ku et al. 2010; Del Pozo et al. 2011; Venturini et al. 2016; Fernández-Rivero et al. 2017).

Thus, antibacterial drugs have potential antifungal value due to their good antifungal activity. However, human health is based on the balance of microbiota (Limon et al. 2017; Liu et al. 2020). If antibacterial drugs are approved for the treatment of invasive fungal infections, many problems still need to be considered. For instance, antibiotic treatment will reduce the composition of colonizing microbiota (Sam et al. 2017). Moreover, many antimicrobial drugs can promote fungal growth and enhance fungal pathogenicity indirectly by disrupting the microbiome and eliminating anaerobic bacteria, which might inhibit fungi, especially in the gut (Sam et al. 2017). Our previous investigation (unpublished article) also showed that the antifungal applications of antibiotics interfere with the homeostasis of symbiotic bacteria and fungi in the body. Moreover, dysbiosis of microbiota is responsible for the occurrence of many other diseases in humans such as cardiovascular, cancer, allergy, and the microbiota also affect the human immune system and the synthesis of nutrients (Thomas et al. 2017; Ran et al. 2020). In addition, the pharmacokinetics of antibiotics with antifungal activity in vivo require further investigation.
| Antibiotics | Fungus | In vitro (MIC: μg/mL) | In vivo | In combination (synergistic effects) | Relevant molecular mechanism                                                                                                                                                                                                 | Ref. |
|-------------|--------|-----------------------|---------|------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Tobramycin  | Fusarium spp. | > 64 | - | VRC, AmB | Probably increases permeability of the cell wall and cell membrane. | Venturini et al. (2016) |
| Gentamicin  | Resistant-azole C. albicans | > 512 | G. mellonella | FLC | (I) Suppresses overexpression of the efflux pump. (II) Reduces phospholipase activity of resistant C. albicans. | Lu et al. (2018) |
| Clarithromycin | C. tropicalis | - | - | AmB, ANI | - | Femández-Rivero et al. (2017) |
| | C. parapsilosis, C. glabrata, C. albicans | No effects | - | AmB | - | Del Pozo et al. (2011) |
| | Pythium insidiosum | 0.25–8 | - | - | - | Loreto et al. (2014) |
| Azithromycin | Pythium insidiosum | 1–16 | - | - | - | Loreto et al. (2014) |
| Norfloxacin | C. albicans | - | - | MIA | - | Moneib (1995) |
| Levofloxacin | C. albicans, A. fumigatus | + | - | AmB, CAS | Probably inhibits fungal DNA replication by binding to fungal topoisomerase. | Stergiopoulou et al. (2009) |
| Gatifloxacin | Candida spp. | + | - | - | - | Ozdek et al. (2006) |
| Moxifloxacin | C. albicans | + | - | Liposomal AmB, CAS | Probably inhibits fungal DNA replication by binding to fungal topoisomerase. | Ozdek et al. (2006); Stergiopoulou et al. (2009); Deren et al. (2010) |
| Ciprofloxacin | C. albicans | + | - | AmB | Probably inhibits fungal DNA replication by binding to C. albicans topoisomerase. | Stergiopoulou et al. (2009) |
| | A. fumigatus | + | - | AmB, CAS, ARC | - | - | |
| Trovafloxacin | C. albicans | No effects | Murine | FLC, AmB | - | Sugar et al. (1997) |
| | C. tropicalis, C. neoformans | No effects | - | FLC, AmB | - | - | |
| Tetracycline | C. albicans | 320–2560 | G. mellonella | AmB, FLC | - | Lew et al. (1977); Gu et al. (2018) |
| Demeclocycline | C. albicans | 640 | - | AmB | - | Lew et al. (1977) |
| Doxyeclycline | C. parapsilosis, C. krusei, C. glabrata | No effects | - | AmB | Probably inhibits protein synthesis. | El-Azizi (2007) |
| | C. albicans | 640–1280 | G. mellonella | AmB, FLC, CAS | (I) Inhibits FLC-inducible efflux pump gene overexpression. (II) Disturbs calcium homeostasis. (III) Disturbs iron homeostasis. Disturbs calcium homeostasis. | Lew et al. (1977); Miceli et al. (2009); Fiori and Van Dijck (2012); Gao et al. (2013); Gao et al. (2014); Gu et al. (2018) |
| Minocycline | Resistant-FLC C. albicans | 256–512 | - | AmB, FLC | - | Lew et al. (1977); Shi et al. (2010) |
| | A. fumigatus | 0.125–4 | G. mellonella | ITR, VRC, POS | Probably interferes with the balance of cellular electrolytes and loss of mitochondrial function. | Loreto et al. (2014); Gao et al. (2020) |
| | A. flavus, F. solani, F. oxysporum | 0.125–4 | - | ITR, VRC, POS | - | - | |
| Antibiotics | Fungus                    | In vitro (MIC: μg/mL) | In vivo | In combination (synergistic effects) | Relevant molecular mechanism                                                                                                                                                                                                 | Ref.                                      |
|-------------|--------------------------|-----------------------|---------|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| Tigecycline | C. albicans              | 2048 -                | AmB, FLC, CAS | -                                   | Ku et al. (2010)                                                                                                                                                                                                             |
|             | Fusarium spp.            | 0.25–4 -              | VRC, AmB     | Inhibits the synthesis of protein.   | Loreto et al. (2014); Venturini et al. (2016)                                                                                                                                                                               |
| Polymyxin B | C. albicans              | -                     | AmB, KET, MIA | -                                   | Moneib (1995)                                                                                                                                                                                                             |
|             | Fusarium spp.            | 4–16 -                | VRC, AmB     | Probably disturbs the synthesis of ergosterol. | Venturini et al. (2016)                                                                                                                                                                                                      |
|             | C. neoformans            | 8–256 -               | FLC         | Probably through binding anionic lipids on fungal membrane and destroys membrane integrity. | Zhai et al. (2010)                                                                                                                                                                                                         |
|             | Rhizopus oryzae          | 32 -                  | -           | AmB                                 | Moneib (1995)                                                                                                                                                                                                             |
|              | A. fumigatus              | 28-56 -               | -           | AmB                                 | Moneib (1995)                                                                                                                                                                                                             |
| Rifampicin  | C. tropicalis            | -                     | AmB, ANI    | -                                   | Femández-Rivero et al. (2017)                                                                                                                                                                                                  |
|             | C. parapsilosis, C. glabrata, C. albicans, C. krusei | No effects - | AmB | Probably disturbs RNA synthesis in the presence of AmB. | El-Azizi (2007); Loreto et al. (2014)                                                                                                                                                                                          |
| Linezolid   | Pythium insidiosum       | 1–32 -                | -           | Inhibits protein synthesis.          | Loreto et al. (2014)                                                                                                                                                                                                         |
|             | C. neoformans            | > 64 -                | AmB         | -                                   | Rossato et al. (2015)                                                                                                                                                                                                       |
|             | C. albicans              | > 512 -               | FLC, ITR, VRC | -                                   | Lu et al. (2019)                                                                                                                                                                                                             |

Note: VRC, voriconazole; AmB, amphotericin B; FLC, fluconazole; ITR, itraconazole; CTZ, clotrimazole; POS, posaconazole; ANI, anidulafungin; MIA, miconazol; CAS, caspofungin; KET, ketoconazole; - , no studies were mentioned in the corresponding references; +, the drug has antifungal effect, but no specific data in the corresponding references.
Table 2  Summary of immunosuppressant drugs with antifungal activity

| Immunosuppressants | Fungus                  | In vitro (MIC: \( \mu g/mL \)) | In vivo | In combination (synergistic effects) | Relevant molecular mechanism                                                                 | Ref.                                                                 |
|---------------------|-------------------------|---------------------------------|---------|-------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| Cyclosporine        | *Aspergillus* spp.       | 1–25                            | -       | CAS, ISA                            | Inhibits the calcineurin pathways.                                                              | Marchetti et al. (2000a, 2000b); Kontoyiannis et al. (2003)          |
|                     | *C. albicans*            | >10 Rat                         | FLC, VRC, CAS, AmB | ISA                                 | Inhibits the expression of genes related to hyphal development, adhesion, biofilm formation, and drug transporter in *C. albicans* (Jia et al. 2016). | Uppuluri et al. (2008); Shinde et al. (2012); Schwarz et al. (2019) |
|                     | *Rhizopus* spp., *Lichtheimia* spp., *Mucor* spp., *Rhizomucor* spp. | 1–16                            | -       | ISA                                 |                                                                                               |                                                                      |
| Tacrolimus          | *Trichosporon asahii*    | > 64                            | -       | AmB, CAS                            | Inhibits the activity of calcineurin pathway.                                                  | Kubiça et al. (2016)                                                 |
|                     | *Aspergillus* spp.       | 0.25–16 Mice                    | CAS     |                                     |                                                                                               | High and Washburn (1997); Steinbach et al. (2004); Kubiça et al. (2016); Schwarz and Dannaoui (2020) |
|                     | *Fusarium* spp.          | 1–40                            | -       | CAS                                 |                                                                                               | Shalit et al. (2009)                                                 |
|                     | *C. albicans*            | No effects.                     | FLC, ITR, VRC |                                     |                                                                                               | Uppuluri et al. (2008); Sun et al. (2008)                             |
|                     | *C. dubliniensis*        | > 4                             | -       | CAS, FLC, POS                      |                                                                                               | Zhang et al. (2012)                                                  |
|                     | *Rhizopus* spp., *Lichtheimia* spp., *Mucor* spp., *Rhizomucor* spp. | 1–8                             | -       | ISA                                 |                                                                                               | Schwarz et al. (2019)                                                |
| Pimecrolimus        | *Malassezia* spp.        | 16–64                           | -       | -                                   |                                                                                               | Sugita et al. (2006)                                                 |
| Rapamycin           | *C. albicans*            | < 0.09 - 100                    | -       | -                                   | Inhibits the TOR pathways via FKBP12-Rapa complex.                                              | Cruz et al. (2001)                                                   |
|                     | *C. neoformans*          | 0.39 - > 100                    | -       | -                                   |                                                                                               | Cruz et al. (2001)                                                   |
|                     | *Mucor* spp.             | 8 *G. mellonella*               | ISA     |                                     |                                                                                               | Bastidas et al. 2012; Schwarz et al. (2019)                              |
|                     | *Rhizopus* spp., *Lichtheimia* spp., *Rhizomucor* spp. | 8                               | ISA     |                                     |                                                                                               | Schwarz et al. (2019)                                                |
|                     | *Phycomyces blakesleeanus* | 6.3-200                        | CAS, ISA |                                     |                                                                                               | Bastidas et al. (2012)                                               |
|                     | *Aspergillus* spp.       | 16 Mice                         | ISA     |                                     |                                                                                               | High and Washburn (1997); Kontoyiannis et al. (2003); Schwarz and Dannaoui (2020) |
| Mycophenolic acid   | *C. neoformans*          | 30 Nematode                     | AmB     | -                                   | Disrupts de novo GTP biosynthesis.                                                              | Morrow et al. (2012); Banerjee et al. (2014)                         |
|                     | *C. albicans*            | 0.25                            | -       | -                                   |                                                                                               | Köhler et al. (2005)                                                 |
| Mizonbine           | *C. albicans*            | -                               | -       | -                                   | Disrupt de novo GTP biosynthesis.                                                               | Köhler et al. (2005)                                                 |
Immunosuppressants

Immunosuppressants are another example of drug repurposing due to their antifungal activity. It is known that immunosuppressants mainly include calcineurin inhibitors (e.g., cyclosporine; pimecrolimus; and tacrolimus, FK506), target of rapamycin inhibitors (e.g., rapamycin), anti-metabolic agents (e.g., mizoribine, MZP; mycophenolic acid, MPA), and glucocorticoids (e.g., budesonide, dexamethasone, and hydrocortisone). Initially, rapamycin was found to be an antifungal agent and inhibited yeasts including *C. albicans*, *dermatophytes*, *Microsporum gypseum*, and *Trichophyton granulosum* (Vézina et al. 1975). Rapamycin, probably acting on the recombinant FK506 binding protein (FKBP) complex, showed activity on clinical isolates of *Candida* in vitro, on the basis of a randomized controlled trial of rapamycin versus candididin, nystatin, and amphotericin B. The results demonstrated that rapamycin has antifungal activity superior to that of the other tested compounds (Sehgal et al. 1975). The immunosuppressants found to have antifungal ability are briefly summarized in Table 2.

Inosine monophosphate dehydrogenase (IMPDH) is a crucial enzyme in de novo guanine nucleotide biosynthesis and plays an important role in the rapid proliferation of cells (Cuny et al. 2017). A recent investigation found that benzo[b]thiophene 1,1-dioxide, an IMPDH inhibitor, can weaken the virulence of *C. neoformans* or kill it entirely (Kummari et al. 2018). In addition, MPA and MZP, other types of IMPDH inhibitors, were confirmed to have significant antifungal effects in *C. albicans* and *C. neoformans* by disrupting de novo GTP biosynthesis (Köhler et al. 2005; Morrow et al. 2012; Banerjee et al. 2014). Ribavirin, an antiviral agent, is also a type of IMPDA inhibitor. Interestingly, it was found that ribavirin displayed potent antifungal activity in *C. albicans* in monotherapy or in combination with fluconazole, itraconazole, and posaconazole in vitro and in vivo. The antifungal mechanism involves disruption of vacuolar function and the reduction of extracellular phospholipase activity (Yousfi et al. 2019; Zhang et al. 2020).

Calcineurin, a conserved serine-threonine specific phosphatase, consists of a catalytic subunit and a regulatory subunit (Bandyopadhyay et al. 2004; Sun et al. 2016). It is one of the important mediators in calcium signals and is involved in hyphal/mycelium formation in *C. neoformans* and *A. fumigatus*, and virulence in *C. tropicalis* (Zhang et al. 2012; Chen et al. 2014). Calcineurin inhibitors (cyclosporin A and FK506) have been shown to improve the survival of mice infected with *A. fumigatus* (High and Washburn 1997). Clinical experience has also suggested that calcineurin inhibitors (cyclosporin A and FK506) can decrease the mortality of invasive *Aspergillus* by forming protein-drug complexes (Torre-Cisneros et al. 1991; Singh et al. 2003). Moreover, cyclosporin A and FK506 combined with fluconazole have

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**Table 2**

| Immunosuppressants | Fungus | In vitro (MIC, \(\mu g/mL\)) | In vivo | Relevant molecular mechanism (synergistic effects) | Ref. |
|--------------------|--------|-------------------------------|---------|-----------------------------------------------|------|
| Dexamethasone      |        |                               |         | Inhibits the drug efflux pump and reduces the activity of extracellular phospholipases. | Sun et al. (2017) |
| Budesonide         |        |                               |         | (I) Inhibits the function of drug transporters. (II) Reduces the activity of extracellular phospholipases and the formation of biofilm. (III) Promotes apoptosis by the accumulation of ROS. | Li et al. (2016) |
| Hydrocortisone     |        |                               |         |                                              | Ramondenc et al. (1998) |

| Immunosuppressants | Fungus | MIC (\(\mu g/mL\)) | Ref. |
|--------------------|--------|-----------------|------|
| Dexamethasone      |        | Resistant-azole C. albicans | Note: VRC: voriconazole; AMB: amphotericin B; FLC: fluconazole; ITR: itraconazole; POS: posaconazole; CAS: caspofungin; ISA: isavuconazole; -, no studies were mentioned in the corresponding references |
| Budesonide         |        | Resistant-azole C. albicans | |
| Hydrocortisone     |        | Resistant-azole A. fumigatus | |

Note: VRC, voriconazole; AMB, amphotericin B; FLC, fluconazole; ITR, itraconazole; POS, posaconazole; CAS, caspofungin; ISA, isavuconazole; -, no studies were mentioned in the corresponding references.
| Statins   | Fungus               | In vitro (MIC: μg/mL) | In vivo | In combination (synergistic effects) | Relevant molecular mechanism                                                                 | Ref.                                                                 |
|----------|----------------------|-----------------------|---------|-------------------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| Lovastatin | Paecilomyces variotii | 64                    | -       | -                                   | -                                                                                            | Chamilos et al. (2006); Nyilasi et al. (2010); Zhou et al. (2018)    |
|           | Rhizopus oryzae       | 128                   | -       | -                                   | -                                                                                            |                                                                      |
|           | C. albicans           | 50–64                 | -       | FLC, ITR                           | Inhibits ergosterol synthesis.                                                              |                                                                      |
|           | C. glabrata           | 128                   | -       | FLC                                | -                                                                                            |                                                                      |
|           | A. fumigatus          | 25                    | -       | FLC                                | -                                                                                            |                                                                      |
|           | Zygomycetes           | 32–56                 | -       | VRC                                | -                                                                                            |                                                                      |
| Simvastatin | C. glabrata           | 16–32                 | -       | -                                   | (I) Inhibits ergosterol synthesis; (II) Causes the loss of mtDNA.                            | Chamilos et al. (2006); Nyilasi et al. (2010); Westermeyer and Macreadie (2007) |
|           | C. albicans           | 8                     | -       | MIA                                | -                                                                                            | Nyilasi et al. (2010)                                               |
|           | C. utilis             | 200                   | -       | MIA                                | Inhibits ergosterol synthesis.                                                              | Cabral et al. (2013)                                                |
|           | Cryptococcus spp.     | 62.5–1000             | -       | AmB, ITR, FLC                      | Inhibits ergosterol synthesis.                                                              | Silva et al. (2020)                                                 |
|           | Saccharomyces cerevisiae | 40                | -       | CTZ, ITR, MIA                      | Inhibits ergosterol synthesis.                                                              | Cabral et al. (2013)                                                |
|           | Paecilomyces variotii | 8                     | -       | -                                   | -                                                                                            | Nyilasi et al. (2010)                                               |
|           | Rhizopus oryzae       | 64                    | -       | -                                   | -                                                                                            | Nyilasi et al. (2010)                                               |
|           | A. fumigatus          | 6.25                  | -       | FLC                                | -                                                                                            | Nyilasi et al. (2010)                                               |
| Pravastatin | C. albicans           | No effects.           | Mice    | FLC                                | Inhibits famesol production.                                                               | Nash et al. (2002)                                                  |
| Atorvastatin | C. albicans           | 16–256                | G. mellonella | MIA                          | Inhibits ergosterol synthesis.                                                              | Nyilasi et al. (2010); Ajdidi et al. (2019); Esfahani et al. (2019) |
|           | C. glabrata           | 4–64                  | -       | FLC                                | -                                                                                            | Nyilasi et al. (2010); Lima et al. (2019)                           |
|           | C. utilis             | 200                   | -       | -                                   | -                                                                                            | Silva et al. (2020)                                                 |
|           | C. krusei             | 8                     | -       | -                                   | -                                                                                            | Esfahani et al. (2019)                                              |
|           | C. kefyr              | 4-16                  | -       | -                                   | -                                                                                            | Esfahani et al. (2019)                                              |
|           | Cryptococcus gatti    | ≥ 256                 | Mice    | FLC                                | (I) Inhibits ergosterol synthesis; (II) Induces the production of ROS.                     | Chin et al. (1997); Ribeiro et al. (2017)                           |
|           | Saccharomyces cerevisiae | 40                | -       | CTZ, ITR, MIA                      | -                                                                                            | Cabral et al. (2013)                                                |
|           | C. stellatoidea       | 16                    | -       | -                                   | -                                                                                            | Esfahani et al. (2019)                                              |
|           | Rhizopus oryzae       | 32                    | -       | ITR, KET                           | -                                                                                            | Nyilasi et al. (2010)                                               |
|           | Paecilomyces variotii | 32                    | -       | -                                   | -                                                                                            | Nyilasi et al. (2010)                                               |
|           | Aspergillus flavus    | > 128                 | -       | ITR                                | (I) Stimulates oxidative stress response; (II) Inhibits ergosterol synthesis.               | Nyilasi et al. (2010); Ajdidi et al. (2020)                         |
|           | A. fumigatus          | 64                    | -       | ITR, FLC, MIA, KET                 | (I) Stimulates oxidative stress response; (II) Inhibits ergosterol synthesis.               | Nyilasi et al. (2010); Lima et al. (2019)                           |
| Fluvastatin | C. albicans           | 25                    | No effects in mice | FLC                          | -                                                                                            | Nyilasi et al. (2010); Lima et al. (2019)                           |
| Statins | Fungus          | In vitro (MIC: μg/mL) | In vivo | In combination (synergistic effects) | Relevant molecular mechanism                  | Ref.                              |
|---------|-----------------|-----------------------|---------|-------------------------------------|-----------------------------------------------|---------------------------------------|
|         |                 |                       |         |                                     |                                               |                                      |
| C. glabrata | 32–64          | -                     | -       | -                                   |                                               | Nyilasi et al. (2010); Lima et al. (2019) |
| C. tropicalis | 128            | -                     | -       | -                                   |                                               | Lima et al. (2019)                      |
| C. dubliniensis | 16–512      | -                     | -       | -                                   |                                               | Lima et al. (2019)                      |
| Cryptococcus spp. | 64–>128  | -                     | ITR, FLC| -                                   |                                               | Chin et al. (1997)                       |
| Paecilomyces variotii | 25         | -                     | -       | -                                   |                                               | Nyilasi et al. (2010)                   |
| Rhizopus oryzae | 2–3.125        | -                     | KET, ITR| -                                   |                                               | Nyilasi et al. (2010)                   |
| A. fumigatus | 2              | -                     | -       | -                                   |                                               | Nyilasi et al. (2010)                   |
| A. flavus | 128             | -                     | KET, MIA, ITR| -                                |                                               | Nyilasi et al. (2010)                   |
| Rosuvastatin | C. albicans | 8–128                | -       | MIA                                |                                               | Nyilasi et al. (2010); Lima et al. (2019) |
| C. glabrata | 32–128         | -                     | -       | -                                   |                                               | Nyilasi et al. (2010); Lima et al. (2019) |
| C. utilis | 200             | -                     | MIA | -                                   |                                               | Cabral et al. (2013)                     |
| Paecilomyces variotii | 32         | -                     | -       | -                                   |                                               | Nyilasi et al. (2010)                   |
| Saccharomyces cerevisiae | 40    | -                     | CTZ, ITR, MIA| -                                |                                               | Cabral et al. (2013)                     |
| Rhizopus oryzae | >128        | -                     | KET, ITR| -                                   |                                               | Nyilasi et al. (2010)                   |
| A. fumigatus | 128            | -                     | ITR | -                                   |                                               | Nyilasi et al. (2010)                   |
| A. flavus | >128            | -                     | -       | -                                   |                                               | Nyilasi et al. (2010)                   |
| Pitavastatin | C. albicans, C. glabrata, C. auris | 8 | Caenorhabditis elegans | ITR, VRC, FLC | -                           |                                               | Eldesouky et al. (2020)                  |

Note: VRC, voriconazole; AmB, amphotericin B; FLC, fluconazole; ITR, itraconazole; CTZ, clotrimazole; ANI, anidulafungin; MIA, micafungin; -, no relevant studies were mentioned in the corresponding references.
highly synergistic effects on *C. albicans* biofilms both *in vitro* and in rat venous catheter biofilm models (Uppuluri et al. 2008). Glucocorticoids, such as hydrocortisone, budesonide, and dexamethasone, are immunosuppressants with antifungal activity (Ramondene et al. 1998; Li et al. 2016; Sun et al. 2017). However, the antifungal activity of glucocorticoids remains controversial. For instance, an investigation found that hydrocortisone enhanced the growth of *Aspergillus* spp. (Ng et al. 1994). Betamethasone, one of the glucocorticoids, is also capable of promoting hyphal formation, stimulating extracellular phospholipase production, and decreasing the anti-*C. albicans* activity of amphotericin B and nystatin (Jakab et al. 2015). Moreover, an in vivo investigation also found that glucocorticoids increased fungal burden in the gastrointestinal tract in rats (Myerowitz 1981) and enhanced the frequency of fungal translocation in mice (Maraki et al. 1999).

Thus, the practical application of glucocorticoids in fungal infections requires further investigation.

Immunosuppressant-treated fungal cells show phenotypes consistent with inhibition of planktonic cell growth, morphological transformation, and biofilm formation (Uppuluri et al. 2008; Jia et al. 2016). However, at present, the exact mechanisms of immunosuppressant synergism with various antifungal drugs have not been delineated. In particular, the TOR as a representative target may be a promising antifungal approach. To date, there are no relevant fungal-specific inhibitors available (Cordeiro et al. 2014). On the other hand, the use of immunosuppressants can inhibit the host immune response, which increases the risk of fungal infection. According to a large number of in vitro antifungal experiments, immunosuppressants do have potent antifungal effects. However, there are no clinical studies to demonstrate whether immunosuppressants have antifungal effects in humans.

**Statins**

Statins are firstly known as lipid-lowering and cholesterol-lowering drugs as they inhibit HMG-CoA reductase (an essential enzyme in cholesterol biosynthesis) (Esfahani et al. 2019) and are classified according to their hydrophobicity into hydrophilic statins (pravastatin and rosuvastatin) and lipophilic statins (atorvastatin, cerivastatin, fluvastatin, lovastatin, pitavastatin, and simvastatin). It has been confirmed that statins exert a broad spectrum of anti-fungal effects on *Candida* spp., *Aspergillus* spp., and Zygomycetes (Callegari et al. 2010). Table 3 shows a brief summary of the statins with antifungal ability. The antifungal mechanism of statins is focused primarily on the biofilm. For example, the changes in the main components of the biofilm or genes associated with fungal biofilm formation following monotherapy or/and synergism of antifungal agents have been verified. Inexplicably, antifungal activity findings are inconsistent. For instance, pravastatin has synergistic effects with fluconazole by inhibiting farnesol production against *C. albicans* (Tashiro et al. 2012), and in contrast, no synergy was found between pravastatin and fluconazole in vitro in another investigation (Nash et al. 2002). In addition, a study even reported that pravastatin did not inhibit the growth of *Candida* spp. (Brilhante et al. 2015). The reason for this may be due to differences in fungi strains and different methodology. These contradictory findings require clarification in further investigations.

**Antiarhythmic drugs**

Antiarhythmic drugs, which are used in the prevention and treatment of tachycardia, bradycardia, or arrhythmia, include sodium channel antagonists, β-receptor blockers, potassium channel blockers (PCRs), and calcium channel blockers (CCBs). Trials have shown that amiodarone, PCRs, and CCBs exhibit favorable antifungal activity when administered alone or combined with conventional antifungals. CC Bs, as the name suggests, prevent calcium ions from entering cells and maintain metabolic processes. Verapamil (verapamil hydrochloride), a phenylalkylamine CCB, mainly combats *C. albicans* by affecting hyphal development, adhesion, gastrointestinal colonization, or increasing strain susceptibility to oxidative stress (Yu et al. 2014a, 2014b). In addition, verapamil enhances the antifungal activity of tunicamycin or fluconazole against *C. albicans* during biofilm formation and preformed biofilms (Yu et al. 2013). In *A. fumigatus*, the drug efflux pump was blocked and ergosterol content was decreased following treatment with verapamil and itraconazole simultaneously (Zeng et al. 2019). Other CC Bs, such as diltiazem, nicardipine, and nifedipine, have shown antifungal activity against *C. albicans*, *C. glabrata*, Ascomycetous, and Mucoralean fungi, either alone or in combination with antifungals (Bulatova and Darwish 2008; Homa et al. 2017; Alnajjar et al. 2018). Amiodarone is known to block potassium, sodium, and calcium channels and is commonly used to treat and prevent tachyarrhythmia (Kodama et al. 1999; Nattel and Singh 1999; Dorian 2000). In pathogens, amiodarone mainly disrupted calcium homeostasis to elicit high levels of cytoplasmic calcium, leading to cell death in *Cryptococcus* spp., *Aspergillus* spp., *Fusarium oxysporum*, *C. albicans*, *C. tropicalis*, and *Saccharomyces cerevisiae* (Courchesne 2002; Afeltra et al. 2004; Courchesne et al. 2009; Knorre et al. 2009; Gamarra et al. 2010; Bagar and Benčina 2012; Silva et al. 2013). In addition, amiodarone displayed potent fungicidal effects at a low dose combined with fluconazole and miconazole (Gupta et al. 2003). Hence, calcium channels may be potential targets in the therapy of fungal-related infections.
**Antipsychotic drugs**

Antipsychotic drugs include benzamides, butyrophenones, dibenzoazepine, phenothiazines, and thioxanthene. Phenothiazines are the first generation of antipsychotics and are mainly used to treat schizophrenia and mania. In addition, phenothiazines possess multiple effects such as altering the metabolism of cyclic nucleotides, modifying the structure of membranes, binding to calmodulin and they participate in many intracellular responses (Kim et al. 2019), which may explain the antifungal action of phenothiazines (Wood and Nugent 1985, 1985; Rossato et al. 2016). Chlorpromazine and trifluoperazine are representative phenothiazines. Both block the central dopamine D_2 receptor to improve symptoms in mentally ill patients. They have excellent activity against *Candida* spp. and *C. neoformans* either alone or in combination with ketoconazole and amphotericin B (Wood and Nugent 1985; Ben-Gigi et al. 1988; Sharma et al. 2001; Galgóczy et al. 2011; Rossato et al. 2016). Trifluoperazine also has fungicidal effects on *C. neoformans*, especially on melanized cells (Wang and Casadevall 1996). In addition, chlorpromazine and trifluoperazine have fungicidal effects on Zygomyces when concentrations reach 25–200 μg/mL. Moreover, it has synergistic effects with amphotericin B (Galgóczy et al. 2009). The minimum fungicidal concentration of chlorpromazine and trifluoperazine in *Aspergillus* spp., *Scedosporium*, and *Pseudallescheria* ranged between 10 and 64 μg/mL (Vitale et al. 2007; Homa et al. 2015). Flunarizine is a difluorinated derivative of piperazine as well as a potent CCB. It has the same structure as phenothiazines. Flunarizine also exhibits broad-spectrum antifungal effects against *Candida* spp., *Cryptococcus* spp., and *Zygosaccharomyces* spp. alone or jointly with ketoconazole in vitro probably by inhibiting calmodulin activity and increasing the penetration of ketoconazole through cell walls (Krajewska-Kulak and Niczyporuk 1993).

Compared to the traditional antifungal drugs, the main advantage of phenothiazines is that they can cross the blood-brain barrier and improve bioavailability. Moreover, the levels achievable in the brain with antipsychotic therapeutic doses range from 50 to 100 μg/mL; however, the range in plasma is only between 0.5 and 1 μg/mL (Homa et al. 2015).

**Antidepressant drugs**

There are currently many types of antidepressant drugs used in clinics. These mainly include monoamine oxidase inhibitors (e.g., phenelzine), tricyclic (e.g., amitriptyline and doxepin), tetracyclic (e.g., maprotiline), and selective serotonin reuptake inhibitors (SSRIs) (e.g., fluoxetine and paroxetine). Of these, SSRIs are first-line antidepressant drugs with low side effects. They not only have anti-depression activity but also have anti-anxiety activity (Lamresse et al. 2017). SSRIs effectively inhibit the uptake of serotonin by neurons from synaptic spaces. This increases the availability of this neurotransmitter...
in these spaces and improves emotional states. As such, SSRIs can be used to treat depressive mental disorders (Bandelow et al. 2017). Encouragingly, SSRIs have been shown to have positive antifungal activity in various studies. An antifungal experiment showed that fluoxetine could kill some azole-resistant *Candida* sp. strains in vitro with or without fluconazole. Moreover, they also improved the survival rate of *Galleria mellonella*. The antifungal mechanism involves inhibition of extracellular phospholipase activity by down-regulated *SAP1–4* genes in resistant *C. albicans* (Oliveira et al. 2014; Gu et al. 2016). The *SAP* genes encode secreted aspartyl proteinases (*SAP*), which are key virulence factors and play an important role in the growth, development, and pathogenicity of *Candida* spp. (Naglik et al. 2003). In addition, fluoxetine exhibited synergistic effects against *C. albicans* biofilms and relieved oral candidiasis in infected mice when combined with caspofungin (Jiang et al. 2020). Sertraline, another type of SSRI, also showed positive antifungal activity alone or in combination with antifungal agents. It can reduce the fungal burden, improve survival rate, and impair tissue damage in mice and *G. mellonella* (Treviño-Rangel Rde et al. 2016; Treviño-Rangel et al. 2019). Similar results showed that sertraline had fungistatic or fungicidal effects in *Candida* spp., *Coccidioides immitis*, *C. neoformans*, *Trichosporon asahii*, and *A. fumigatus* (Cong et al. 2016; Paul et al. 2016; Treviño-Rangel Rde et al. 2016; Oliveira et al. 2018; Treviño-Rangel et al. 2019; Gowri et al. 2020).

**Non-steroidal anti-inflammatory drugs**

NSAIDs act mainly by inhibiting the activity of cyclooxygenase to reduce the production of prostaglandins (PGs) and thus have antipyretic, analgesic, anti-inflammatory, and other functions (Fitzpatrick 2004). NSAIDs, especially aspirin, etodolac, diclofenac, celecoxib, nimesulide, ibuprofen, meloxicam, ketoprofen, tenoxicam, and ketorolac exhibited favorable anti-*C. albicans* effects by inhibiting the synthesis of fungal PGs, which play an important role in biofilm development, adhesion, and morphogenesis in *C. albicans* (Alem and Douglas 2004; Abdelmegeed and Shaaban 2013). In addition, most of these drugs had synergistic or additive (ketorolac) activity with fluconazole against *C. albicans* (Abdelmegeed and Shaaban 2013). Subsequently, the antifungal mechanisms of aspirin and ibuprofen have become clearer. They can, by activating the high-osmolarity glycerol pathway, induce the accumulation of reactive oxygen species (ROS) and then simultaneously damage the integrity of cell membranes leading to the death of *Cryptococcus* cells (Ogundjei et al. 2016). In addition, aspirin and ibuprofen combined with fluconazole, caspofungin, and amphotericin B have effects on fungi (Ogundjei et al. 2016; Yang et al. 2016). Ibuprofen also has anti-*Sporothrix* activity singly (median MIC of 256 μg/mL) or in combination with antifungal agents including amphotericin B, itraconazole, and terbinafine (Borba-Santos et al. 2021). Diclofenac sodium can downregulate the expression of *Ef-1* gene, which is involved in cellular RNA transport, cell cycle, and apoptosis (Alves et al. 2015), thus resulting in a reduction in the formation of *A. fumigatus* filaments (Nargesi and Rezaie 2018).

**Conclusion**

In this review, we summarized the antifungal effects of a number of non-antifungal agents. In addition, some antitumor agents such as miltefosine (Wu et al. 2020), tamoxifen (Hai et al. 2019), methotrexate (Yang et al. 2019), and antiepileptic drugs (Kathwate et al. 2015) have been reported to have antifungal effects. The use of drug repurposing strategies in the discovery of novel antifungal agents is a revelation in the identification of new antifungal drugs through structural readjustment. With regard to their related antifungal targets, there are still many antifungal mechanisms of the above-mentioned drugs which are unclear (Fig. 1). To reveal the precise targets, further investigations should be performed using transcriptome analysis and molecular techniques, which will lay the foundation for the development of novel antifungal drugs for example using target design. In addition, these antifungal experiments have only focused on either in vitro studies or animal model experiments. There is not enough clinical evidence to prove their practical use in the clinic. Moreover, many factors, such as changes in medium composition will perhaps lead to different or completely opposite results. In in vivo studies, differences between animal models or homogeneous animal models and differences in pharmacokinetic and pharmacodynamic parameters of compounds in these models, and the effects of host-derived serum and, cellular factors should be clarified. Hence, it is essential to use systematic and standard research approaches and to collect more clinical data to evaluate the antifungal effectiveness of these agents.

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