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Advancements in Antifungal Agents: Overcoming Resistance and Exploring New Targets

Chetan C. Turankar*[1], Jitendra A. Kubde[2], Pooja R. Hatwar[3], Ravindra L. Bakal[4]

[1,2,3,4]Shri Swami Samarth Institute of Pharmacy, At Parsodi, Dhamangaon Rly, Dist: Amaravati (444709) Maharashtra, India

Abstract:
Invasive fungal infections (IFIs) continue to pose significant morbidity and mortality risks, particularly among immunocompromised individuals. Despite recent advancements in antifungal treatment, the emergence of resistance and limitations of existing agents necessitate the development of novel therapeutic strategies. This review highlights the current state of antifungal agents, including polyenes, azoles, allylamines, and echinocandins, and explores new targets for antifungal therapy. The review studies the challenges associated with existing antifungal agents, such as toxicity, resistance, and limited spectrum of activity. Emerging targets for antifungal therapy, including fungal cell wall components, plasma membrane, DNA and topoisomerases, protein synthesis, and intermediary metabolism, are also discussed. The development of novel antifungal agents with improved efficacy, safety, and spectrum of activity is crucial to address the growing threat of IFIs.

Keyword: Invasive fungal infections, Antifungal agents, new targets

1. Introduction:

Invasive fungal infections (IFI) continue to be a major source of morbidity and mortality, even with recent advancements in antifungal treatment [1]. Numerous hematologic, oncologic, and rheumatologic disorders now have life-saving treatment options thanks to ongoing medical science advancements. A major side effect of treatment, immunosuppression, puts patients at risk for invasive fungal infections, which are becoming more common [2]. The range of activity, pharmacokinetic and pharmacodynamic characteristics, and side effect profiles of these agents vary. It has been demonstrated that therapeutic drug monitoring (TDM) improves the treatment of invasive fungal infections (IFIs), especially for specific antifungal medications with a limited therapeutic index or an unexpected pharmacokinetic profile and a well-defined exposure-response association [3].

Therapeutic alternatives have increased over the last few decades, and novel medicines like triazoles and echinocandins have replaced traditional antifungal medications (such amphotericin products) [4]. According to published estimates, the mortality rates for invasive candidiasis, disseminated cryptococcosis, and invasive aspergillosis are 30–40%, 20–30%, and a comparable number, respectively

[5]. In a Dutch intensive care unit, it was discovered that over 25% of A. fumigatus were resistant to the triazole antifungal drugs [6].

Because of the pathogen's aggressiveness and delayed diagnosis, treatment of invasive fungal infections in critically ill patients frequently ends in less than ideal outcomes. There are currently few antifungal medications on the market, and resistance development particularly in Aspergillus and Candida is a growing issue [7]. Only 15 medicines are now licensed for clinical usage, despite a 30% increase in the number of drugs available to treat fungal infections since 2000 [8]. There are over 300,000 species of fungi, which are eukaryotic organisms. Of these, 200 are parasitic, but only a small number of them have an impact on humans. Any of the 160 species of the genus Candida can cause candidiasis, a fungal illness that affects animals and can range from moderate subcutaneous infections to acute invasive infections [9]. Even if the present antifungal medications have made it possible to treat fungal infections, there is still need for development in therapeutic antifungal compounds due to factors including growing drug resistance (azoles, echinocandins), high toxicity (polyenes), and low oral availability (polyenes, echinocandins) [10].

2. Antifungal agents in clinical use

Currently, four main groups of systemic antifungal drugs are used in clinical settings: fluoropyrimidines, allylamines and thiocarbamates, azole derivatives, and polyene antibiotics (Table 1). The primary fungal sterol in the plasma membrane, ergosterol, is the target of the first three. Because P. carinii has cholesterol rather than ergosterol, which it may have obtained from its mammalian host, they are therefore useless against it [11].

Table 1. Mechanisms of action of some antifungal agents used clinically [11].

Class and compound	Route of administration	Mechanism of action
Polyenes Amphotericin B Nystatin	 Systemic Topical	Interact with ergosterol, thereby disrupting the cytoplasmic membrane
Azoles Miconazole Ketoconazole Itraconazole Fluconazole	 Topical Systemic Systemic Systemic	Interact with cytochrome P-450; inhibit C-14 demethylation of lanosterol, thereby causing ergosterol depletion and accumulation of aberrant sterols in the membran
Allylamines and thiocarbamates Naftifine Terbinafine Tolnaftate	 Topical Systemic topical	Inhibit oxidosqualene cyclase, thereby causing ergosterol depletion and accumulation of squalene oxides in the membrane
Morpholine, amorolfine	Topical	Inhibit sterol D 14 reductase and D 7 -D 8 isomerase; only the former is essential

Nucleoside analog, 5-FC	Systemic	Is deaminated to 5-FU, which (i) is converted to triphosphate and incorporated into RNA, thereby causing miscoding, and (ii) is converted to deoxynucleoside which inhibits thymidylate synthase and thereby DNA synthesis

2.1 Polyenes:

The many antifungal classes, including azoles and polyenes, target this variation in the sterol component. Candida and other opportunistic fungi have a well-established ergosterol biosynthesis pathway [9]. When used in conjunction with amphotericin B, the echinocandins (inhibitors of glucan synthesis) and nikkomycins (inhibitors of chitin production) are less hazardous than the polyenes [12]. Polyenes, azoles, and echinocandins are the three main groups of systemic antifungal agents [13]. The majority of chemotherapy medications, with the exception of azoles and polyenes, have a limited range of action, poor potency, and several harmful side effects [14].

In addition to the introduction of various formulations of invasive respiratory diseases that were restricted to three classes and routes of administration of previously existing agents, antifungal agents that have been available for treatment for more than 20 years have also seen the introduction of compounds made of polyenes, nucleoside analogs, and newer azoles with expanded antifungal azoles [15]. The only antifungals that are clinically utilized to treat invasive fungal infections are polyenes (like amphotericin B), azoles (like fluconazole and voriconazole), and echinocandins (like capsafungin and micafungin) [16]. Since the toxicities and drug-drug interactions linked to polyenes and azoles are not present, these antifungals suppression of a fungal specific target is beneficial [17].

Polyenes (amphotericin B), triazoles (fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole), echinocandins (caspofungin, micafungin, and anidulafungin), and flucytosine are among the antifungal medications that can be used to treat IFIs [3]. Polyenes are macrocyclic chemical compounds, also referred to as macrolides. Most of them are composed of a macrolactone ring with 20–40 carbons conjugated with a d-mycosimine group [5]. There are several formulations available for the treatment of systemic fungal infections. To reduce toxicity and increase acceptability, three lipid-based formulations have been created since the deoxycholate formulation was first created [2].

2.2 Azoles:

The most quickly growing class of antifungal chemicals are the azole derivatives, which were discovered in the late 1960s and are entirely synthetic. Depending on whether the five-membered azole ring has two or three nitrogens, they are categorized as either imidazoles or triazoles [11]. The most widely used antifungal medications in clinical settings are azoles. Because of their wide range of activity, they are widely utilized in the prevention and treatment of mycoses. Fungal growth and replication are inhibited by azoles because they block the cytochrome P450-dependent enzyme 14a-lanosterol demethylase (CYP51), which is encoded by the ERG11 gene and transforms lanosterol to ergosterol in the cell membrane [5].

Azoles inhibit Cyp51, which prevents lanosterol from being converted to ergosterol. Azoles are quite effective in treating both yeast and molds [6]. Although echinocandins with azoles or echinocandins and

amphotericin B have not been tested in human trials, animal research and a few case reports indicate that these combinations might work better [18].

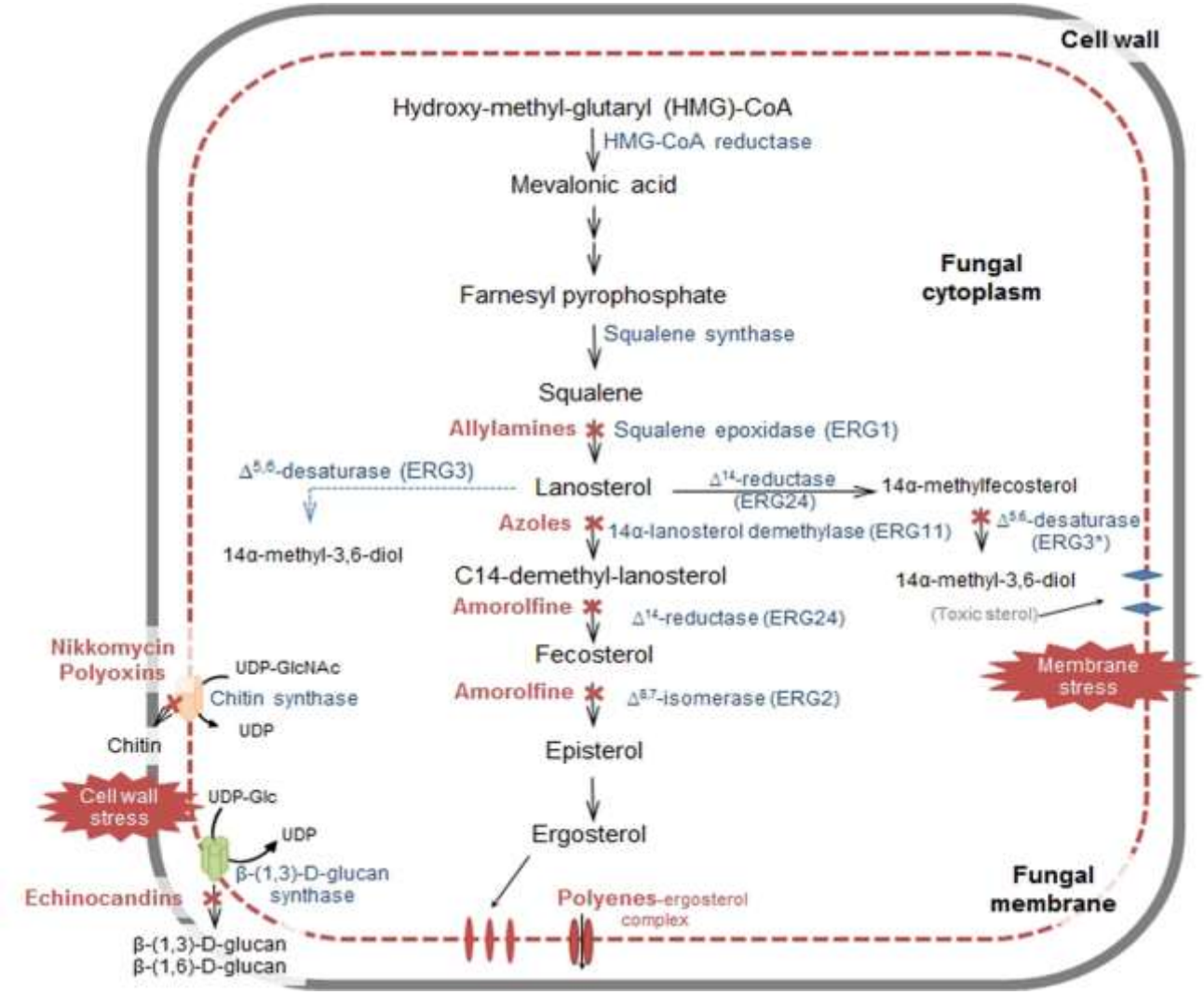

Figure 1: Antifungal agents targets [6].

2.3 Allylamines and thiocarbamates:

One thiocarbamate, tolnaftate, and two allylamine antifungal drugs, terbinafine and naftifine, are now in clinical use [11]. Because they are reversible, non-competitive inhibitors of squalene epoxidase (ERG1), these synthetic fungicidal drugs prevent the formation of ergosterol. The transformation of squalene into 2,3-squalene epoxide is catalyzed by this enzyme [5].

2.4 Morpholines:

Apart from amorolfine, which is applied topically to treat nail infections, the morpholines, which were identified in the 1970s, are entirely synthetic and classified as agricultural fungicides [11]. Two enzymes involved in ergosterol production, D7-D8 isomerase (ERG2) and the D14-reductase (ERG24), are inhibited by the synthetic water-soluble morpholine derivative amorolfine (Fig. 2) (Fig. 1). Amorolfine has both fungistatic and fungicidal activity in vitro and is applied topically to treat nail infections [5]. The polyene antibiotics, azole derivatives, allylamines and thiocarbamates, morpholines, and nucleoside

analogs are the five main types of systemic antifungal agents that are now being used in clinical settings [12].

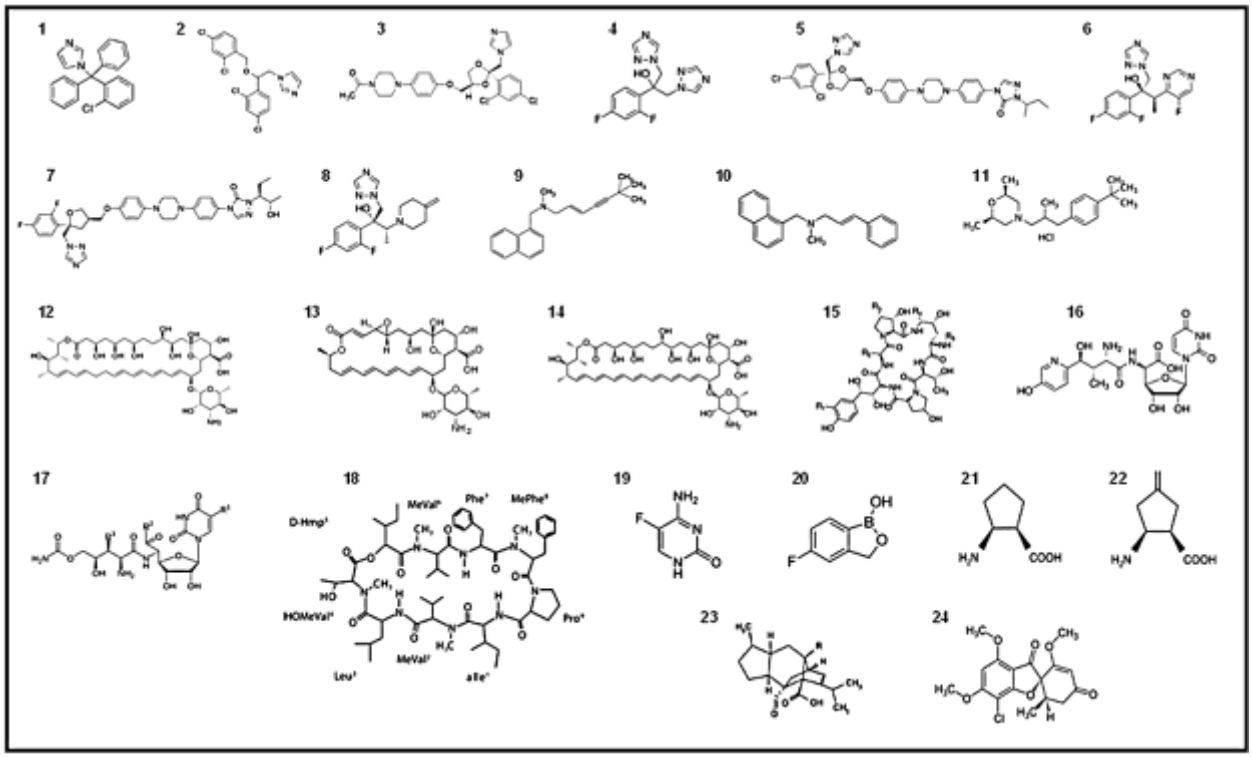

Figure 2. Antifungal agents structure. 1 Clotrimazole, 2 Miconazole, 3 Ketoconazole, 4 Fluconazole, 5 Itraconazole, 6 Voriconazole, 7 Posaconazole, 8 Efinaconazole, 9Terbinafine, 10 Naftifine, 11 Amorolfine, 12 Nystatin, 13 Natamycin, 14 Amphotericin B, 15 Echinocandins, 16 Nikkomycin, 17 Polyoxins, 18 Aureobasidin A 19 Flucytosine, 20Tavaborole, 21 Cispentacin, 22 Icofungipen, 23 Sordarins, 24 Griseofulvin [5].

2.5 Flucytosine:

Additionally, 5-FC is utilized as a single drug to treat urinary tract mycoses and chromoblastomycosis, where its concentrations can be up to 100 times greater than those in serum [11]. In these circumstances, the additional use of flucytosine plays a part [19]. Although flucytosine is mostly effective against yeasts, it must be used in conjunction with other medications to prevent resistance that results from cytosine permease and cytosine deaminase mutations, which reduce the drug's importation and conversion to its active form [20].

C. krusei's natural resistance to the common medication fluconazole and decreased sensitivity to other medications like flucytosine and amphotericin B served as the driving force for this selection [14]. Flucytosine, an analog of pyrimidine Novel Compositions of It has been shown that inhibiting DNA synthesis limits its application due to the toxicity of antifungal agents and the quick emergence of resistance when used alone [15]. For invasive candidiasis, there is insufficient preclinical evidence to support an exposure-response association with 5-FC. With concentrations over the organism's MIC for 25–45% of the dosing period, Time > MIC (T > MIC) was determined to be the best predictor of success in these models [3].

3. New targets for antifungal agents:

- Fungal cell wall
- Plasma membrane
- DNA and topoisomerases
- Protein synthesis
- Intermediary metabolism
- Other cellular functions
- Virulence factors

3.1 Fungal cell wall:

Before the peptide can reach the membrane, it must interact with a variety of charged compounds found in the fungal cell wall. As a result, many antimicrobial peptides are probably rendered ineffective by the cell membrane, which acts as a major barrier to peptide penetration. In fact, resistance to short antifungal peptides can be mediated by proteins and mannosylated glycoproteins found in the fungal cell wall [21]. Glucan and mannan polymers are among the many components of this extracellular matrix that are also found in fungal cell walls [22]. These include substances that target well-known targets, like 1,3-D-glucan in the fungal cell wall and ergosterol in the cell membrane but have been altered to circumvent the drawbacks of the polyenes, triazoles, and echinocandins that are already on the market [17].

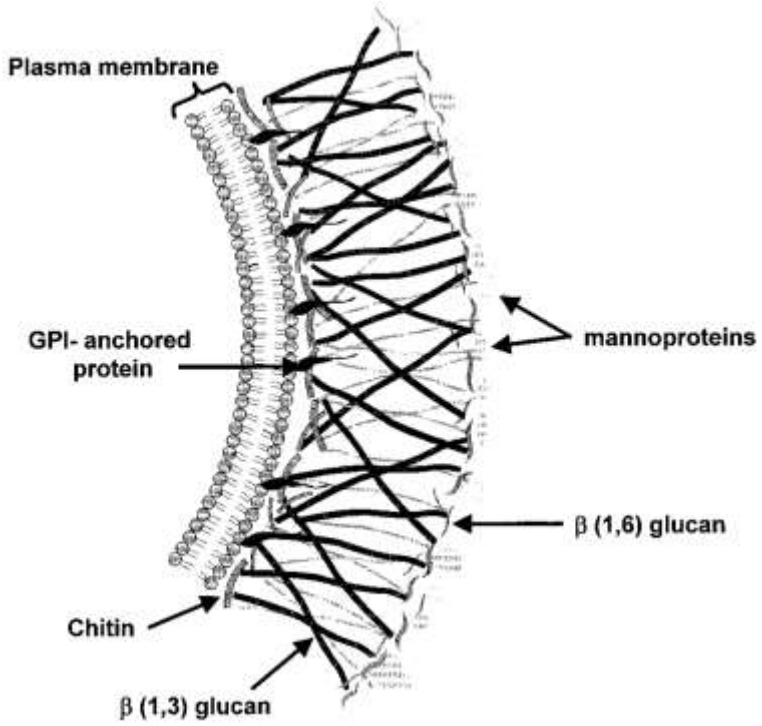

Figure 3: Schematic of fungal cell wall. GPI, glycophosphatidylinosi-tol [23]

Chitin: Chitin is a linear homo-polymer of N-acetylglucosamine (GlcNAc) residues that are b-(1,4)-linked. It is produced on the plasma membrane's cytoplasmic surface, extruded as microfibrils perpendicular to the cell surface, and crystallized as a -chitin (the poly-GlcNAc chains run anti-parallel) outside the cell by means of strong hydrogen bonds [11]. For example, chitin, (1–3)b-D-glucan, (1,6)b-glucans, lipids, and peptides contained in a protein matrix make up the walls of Neurospora crassa and Candida albicans [23].

Inhibiting chitin synthesis is a viable tactic since chitin is a second crucial element of the fungal cell wall that is not present in mammalian cells [15].

Glucan: Long coiling chains of b-(1,3)-linked residues with sporadic side chains containing b-(1,6)-linkages make up glucans, which are glucose homo polymers. At least two functional components make up the enzyme b-(1,3)-glucan synthase, which catalyzes the polymerization: a regulatory component that binds GTP and a catalytic component that works on the UDP-glucose substrate [11]. Clinical investigations of patients with invasive candidiasis have confirmed that these drugs are efficient antifungal agents with minimal collateral harm in mammalian cells because they target a glucan-rich cell wall, which is not present in mammalian cells [20]. The product information for capsafungin does not identify any contraindicated medications since it inhibits the synthesis of β (1, 3)-D-glucan rather than the cytochrome P450 system [24].

Organ sterilization and C. ne-survival are not effectively inhibited by capsafungin. In an experimental model of dis-1,3-β-D-glucan linkages in the cell wall, ravuconazole prolonged survival, a characteristic that might be due to fewerval and reduced fungal burden [15].

Mannoproteins: Additionally, echinocandins have immunomodulatory properties. By breaking down the mannoproteins in the fungal cell wall, b-glucan is exposed [8]. The cell wall's inner layer, which is enriched in β (1-3)-glucan, is obscured by a layer of highly glycosylated mannoproteins (mannan), which prevents the pattern recognition receptor dectin-1 from binding and recognizing it [10]. Their free carboxyl group first complexes with the saccharide component of cell surface mannoproteins in a calcium-dependent manner, which is how they work against fungi [1].

3.2 Plasma membrane:

This innovation is predicated on the idea that the fungal attachment to a cell's plasma membrane (epithelium and endothelium) through contact with adhesion molecules (AM) is the initial stage of a fungal infection process, making the fungal infection harmful [25]. Through the hydrophobic tryptophan residues, which are found inside a trough and encircled by positivelycharged areas, indolicid binds and interact with fungal plasma membranes [21]. According to the SAplasma membraneR, the key structural characteristics of 3-acylindoles for the activities were the 4- or 6-methyl and 3-acetyl or propionyl groups. 4-methyl-3-propionylindole in particular [26]. The echinocandins' target, b-1,3-glucan synthase, is found in the fungal plasma membrane and has a catalytic subunit called Fks1 that is controlled by the GTP-binding protein Rho [22].

3.3 Ergosterol synthesis:

These include microtubule inhibitors, inhibitors of DNA function and topoisomerases (like pentamidine), inhibitors of ergosterol synthesis, and inhibitors of amino acid synthesis (like cispentacin) [12]. Fungal ergosterol production is impacted by this antifungal agent's inhibition of the squalene epoxidase enzyme [24]. Since fungal sterols differ structurally from their mammalian counterparts and their production has been thoroughly researched, the majority of logical drug design efforts have concentrated on them [11].

3.4 DNA and topoisomerases:

Agarose gel electrophoresis was initially applied to distinguish between distinct DNA topoisomers in 1975 [27]. For DNA to conduct transcription, replication, repair, and chromosomal segregation, topoisomerases I and II regulate its topological state. One enzyme that is universally necessary is topoisomerase II [11].

3.5 Protein synthesis:

Numerous adverse effects result from this, such as structural alterations in the cell wall or metabolic (phospholipid, protein synthesis, cell regulation) abnormalities. Interactions between drugs can happen

[28]. Rest in peace. RIPs are RNA N-glycosidases that depurinate rRNA, which causes ribosome damage and stops protein production [23]. Grizeofulvin possesses anti-inflammatory qualities in addition to antifungal ones. In fibroblasts, glieofulvin suppresses protein synthesis, glycosaminoglycan secretion, and proliferation [29].

Conclusion:

The development of novel antifungal agents is crucial to overcome the limitations of existing therapies and address the growing threat of invasive fungal infections. The exploration of new targets, such as fungal cell wall components, plasma membrane, DNA and topoisomerases, and protein synthesis, offers promising opportunities for the development of effective and safe antifungal agents. Understanding the mechanisms of resistance and developing strategies to overcome them is essential to improve treatment outcomes. The use of combination therapy, adjunctive therapy, and immunotherapy may also enhance the efficacy of antifungal treatment. Ultimately, a comprehensive approach to antifungal therapy, incorporating novel agents, new targets, and innovative treatment strategies, is necessary to reduce the morbidity and mortality associated with invasive fungal infections. By advancing our understanding of antifungal agents and developing more effective treatment options, we can improve patient outcomes and mitigate the impact of IFIs.

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