Editor’s note: This manuscript and its four first references were edited using US English, in track changes, focusing on language, grammar, and syntax, and ensuring technical accuracy. It has been formatted to the journal style and formatting requirements of *BMC Microbiology*.

Title page:

**Unraveling the importance of natural origin molecules in antifungal drug development through targeting the ergosterol biosynthesis pathway**

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**ABSTRACT**

Over the past decades, life-threatening fungal infections have dramatically increased, particularly among patients with hampered immune function. Fungal infections cause around 1.5 million deaths annually, superior to malaria and tuberculosis. Concerning high toxicity, narrow spectrum of activity, and drug resistance to current antifungals, there is an urgent need to discover novel leads from molecules of natural origin, especially those derived from plants and microorganisms, for antifungal drug discovery. Among antifungal drugs that have been introduced into the clinic, those affecting ergosterol biosynthesis remain superior to other classes of antifungals, and the vital role of ergosterol in fungal growth and development. Therefore, this review highlights current knowledge about available antifungal agents and antifungal drug discovery from compounds of a natural origin that affect ergosterol biosynthesis. Special attention is made to the fungal sterol C24-methyltransferase (SMT), a crucial enzyme in the ergosterol biosynthesis pathway, as a novel target for rational drug design.

**Keywords:** Ergosterol biosynthesis; Antifungal drug discovery; Natural compounds; Fungal infections.

# INTRODUCTION

The mortality rate of fungal infections worldwide is more than 1,500,000 cases yearly. This figure is higher than mortality due to HIV and malaria infections and equal to tuberculosis. The incidence of infections varies from person to person, and depending on each individual’s immune system, the level of exposure varies considerably [1, 2]. The current therapeutic methods for invasive fungal infections are limited compared to bacterial infections. Moreover, there are no reliable methods for treating many fungal diseases like candidiasis due to the resistance of the etiologic fungi to available antifungal drugs [2]. Regarding the high prevalence of fungal infections and the associated treatment problems, further efforts are needed to identify and detect novel antifungal drugs.

Currently, there are three antifungal drug groups used in the clinic, including polyenes, azoles, and echinocandins [3]. These antifungals target the cell membrane and cell wall components of molds and yeasts. However, they have limitations in treating invasive fungal infections, which have forced researchers to conduct further studies on producing new drugs [4] (Fig. 1). For example. the famous polyene i.e. amphotericin B has a minor function in the safe form of a liposome; azoles produce drug resistance, and echinocandins, which are prescribed only intravenously, are very expensive [4]. Concerning these issues, a series of specific natural compounds have been used recently to produce new antifungal drugs [4]. Although many of these alternative new natural compounds are not categorized as antifungal drugs, their antifungal effects have been demonstrated alone or synergically with other compounds, [5]. The α-bisabolol in chamomile interferes with zymosterol synthesis by a novel mode of action inhibiting 24-methyl transferase and preventing the foundation of fecosterol from zymosterol in ergosterol biosynthesis [6]. Many antifungal drugs have a natural origin as the phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, and quinines. The plants produce them during secondary metabolism or when a plant is injured. Microorganisms also can synthesize various groups of natural antifungal compounds [7].

**Challenges and novel approaches of antifungal drug development**

Nowadays, the development of novel antifungal drugs has overgrown increasingly. However, compared to the advances in new antibiotics that are used to treat bacterial infections, fundamental advances in fungal treatments are challenging due to the eukaryotic nature of fungi [2]. One of these challenges is the toxic effect of antifungal drugs on the host, besides the pathogen fungus. Thus, the three main classes of antifungal drugs are designed to be unique to fungi, and the biggest challenge in developing new drugs is the complexity of knowing the clinical effects of these drugs [8]. Other factors slowing the development of new drugs are a lack of sufficient scientific documentation, economic challenges, and rigorous monitoring of centers and government agencies [9]. Consequently, new creative solutions are needed to overcome these factors.

It has been shown that new antifungal drugs can be produced by targeting ergosterol biosynthesis at pathogenic fungi's cell membrane as well as cell wall components [10]. In fact, the mechanism of action of these drugs is to destroy or disable the enzymes and the compounds necessary for fungi’ survival present in their cell membrane or cell wall. One of the novel classes of antifungal drugs is enfumafungin, a natural suppressant of GS1 3-β glucan synthesis produced by an endophytic Hormonema species [11]. For the development of new antifungal drugs, finding specific compounds from a natural origin by high throughput screening is in progress (Fig. 2). One approach is the screening of chemical compounds, which are used to create mutations in pathogenic fungi. Then, the resistance to infection in a mutated fungus is evaluated using the wild type of the desired fungus. Assessing the lack of growth by increasing the sensitivity or specificity of the combination is another essential approach to antifungal drug discovery from natural sources [12, 13].

**Natural product-based antifungal drug discovery** Since the discovery of penicillin, the pharmaceutical industry has begun tremendous efforts to use natural compounds to make antibiotic drugs, especially in producing antifungal drugs. Natural compounds that inhibit cell wall synthesis are an essential class of antifungal drugs [4]. As shown in Fig. 3, antifungal drugs with a natural combination origin belong to two groups: a group that has an entirely natural origin directly extracted from plants or microorganisms through cultivation [5, 14, 15] and another group discovered by using metagenomics approaches.

Methods to investigate the genomic structure of natural compounds are also used to determine the best performance of drugs. The best fungi for genomic testing of drugs include *Saccharomyces cerevisiae,* *Candida albicans*, and *Aspergillus fumigatus* [16]. Unfortunately, despite the large number of antifungal compounds introduced in recent years, there is a minimal report on the mode of action of such antifungals. Fig. 4 shows the chemical structure of natural antifungal compounds with known mechanisms of action, of which echinocandins are examples of recently developed antifungals used in clinics. The other compounds are candidates to develop as novel drugs antifungal.

**Echinocandins:** Echinocandins, including caspofungin (Fig. 4-1), micafungin, and anidulafungin are a novel class of antifungal drugs that inhibit the glucan synthesis in the fungal cell wall of main pathogens i.e., *Aspergillus* and *Candida,* via inhibiting the enzyme 1,3-β glucan synthase. Due to β-glucan destruction, the resistance to osmotic forces is impaired, leading to fungal cell lysis. In addition, it has been shown that echinocandins improve host immune responses via exposing antigenic β-glucan epitopes that trigger host cellular recognition and inflammatory responses [REF].

## CONCLUDING REMARKS AND FUTURE PROSPECTS

The development and production of antifungal drugs have progressed markedly since the 1950s. With the spread of cryptococcosis disease worldwide, at that time, penicillin was the only known drug that was used for all infections. When amphotericin was discovered and developed, fungal treatments improved significantly. Presently, the growth trend of novel antifungal drugs is very slow compared to the rise in fungal infections. The reason for that is the low market demand, the low profits, and the opinion of drug manufacturing companies that these new drugs are more expensive than current drugs. Therefore, it is logical to research new antifungal drug compounds based on natural compounds that originated from nature at reasonable prices. This review further indicates that broad spectrum bio-active molecules by natural origin which target specific sites in the ergosterol biosynthesis pathway, such as α-bisabolol, are potential candidates for drug development against a wide array of fungi with minimum toxicity for the mammalian host. Further studies for drug discovery based on omics approaches are necessary to reduce the upcoming challenges in making novel antifungal drugs.

#### [Consider including the topics: “Competing interests”, "Funding”, and 'Authors' Contributions"].

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