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From Natural products to therapeutically important antifungals

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Abstract

Although, there has been a significant progress in the field of pharmaceuticals and drug designing in the recent past, yet there is a vacuum in the development of broad spectrum antifungal drugs. Those drugs that are at our disposal are not free from side effects having several risks and associated toxicities on prolonged usage. Therefore, there has been an ever escalating demand of devising new drugs that are preferably natural and free from side effects. The current article deals with the most recent natural antifungals, their salient features and their targets in order to provide the readers a clear understanding of the importance of the natural drugs and to provide the motivation so as to devise new and effective analogs of these drugs thus helping us combat the fungal diseases in a better way.

Key words: natural, antifungal, diseases, chitin synthase inhibitors

Introduction

Each year over a billion people come in contact with the fungal infections and the evidences suggest that the rate is ever escalating (1, 2, 3, 4). Fungi are eukaryotic organisms, having the ability to infect any part of the human body, ranging from superficial infections of the skin to the disseminated life threatening diseases. Some fungi like *Cryptococcus neoformans* not known to cause serious diseases in the past, are now posing new challenges to human health. This change in scenario can be attributed to the increased number of immunocompromised patients (people suffering from AIDS, undergoing chemotherapy or organ transplant etc.), use of central venous catheters and increased use of broad spectrum antibiotics (5).

Prior to 1970, medical science claimed of treating the fungal infections by and large with the medications in hand. Therefore, there were hardly any new antifungals devised apart from flucytosine in 1964 and the polyenes nystatin and amphotericin B in 1950's. Only in the early 1970's came the majority of the azoles which are currently being used against the life threatening fungal diseases To add to the misery, the currently used antifungals have side effects like the problem of nephrotoxicity with amphotericin B (6) and growing resistant against the azoles (7), drug – drug interactions and fungistatic mode of action.

Therefore, it is the need of the hour to develop new antifungals which should preferably be natural and having broad spectrum. Pharmaceutical giants are striving hard to focus their research towards the nature based products. The increasing role of natural products in the drug discovery has stemmed from the diverse structure and the complex carbon skeleton of natural products. Moreover, the natural product based drugs show far more drug likeliness and biological friendliness in comparison to the synthetic drugs as they have been elaborated within living systems (8, 9). This can be emphasized by a report published in the Journal of Natural Products stating that 70 % of the drugs that have been developed in the past 25

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years in United States are based on the natural products (10). In the current article the various nature based antifungals have been divided on the basis of their respective targets and have been explained accordingly. A brief outline of this article has been explained in Figure 1.

Types of natural antifungals -
1,3 beta glucan synthase inhibitors

FR901469 : FR901469, known to be a 1,3 β glucan synthase inhibitor has been isolated from an unidentified fungus no. 11243 in the form of a hydrochloride salt (11). It is an echinocandin derivative having high water solubility. The MIC of this natural inhibitor has been calculated to be 0.63 and 0.16 µg/ml against *Candida albicans* and *Aspergillus fumigatus* respectively using the broth micro-dilution method. These MICs give a clear idea about the high antifungal activity of this product. It has a limitation in the form of its high haemolytic behaviour which is lesser than the amphotericin B but higher that echinocandin B (5). So, efforts are being made to design its analogs in order to address this shortcoming.

FR901379 : FR901379 is another echinocandin derivative which has been isolated from *Coleophoma empedri* F-11899. It is a cyclic hexapeptide, highly water soluble due to the presence of a sufonate moiety (12, 13). It targets 1, 3 β glucan synthase, having an IC_{50} value of 0.7 ug/ml in comparison to 2.6 ug/ml of echinocandin B against *C.albicans*.However, this compound could not prolong the survival of a model infected with aspergillosis and the MLC associated was 62 ug/ml indicating the need to form its analogs (5).

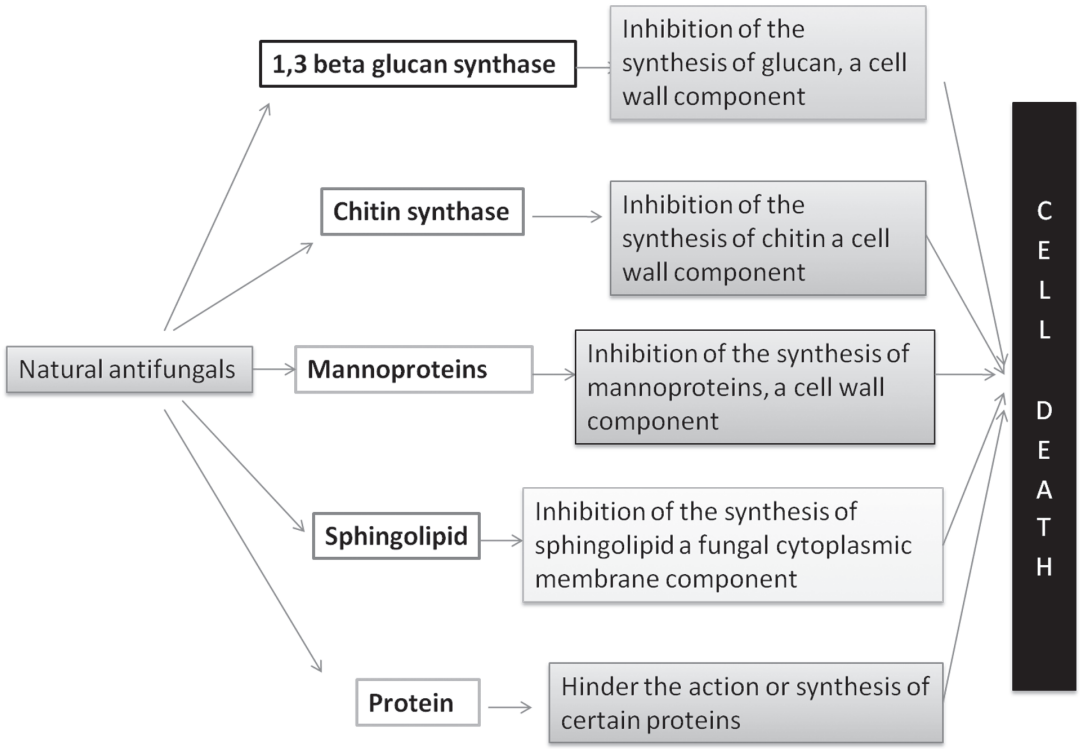

Fig.1: Action of natural antifungals on various cellular components of the fungi leading to cell death

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Caspofungin : Caspofungin is the first of its class echinocandins which got the approval from Food and Drug Administration (FDA), USA. It is semi-synthetically derived from a fermentation product of the fungus *Glarea lozoyensis*. It is known to target the synthesis of 1,3 β glucan which is an integral component of the fungal cell wall thus causing an osmotic imbalance inside the fungal cell. Caspofungin in a head to head comparison was at par to the liposomal amphotericin B in case of neutropenic patients, amphotericin B deoxycholate in case of treatment of invasive candidiasis and far better than amphotericin B and similar to fluconazole in case of tolerability profile thus providing a viable alternate to liposomal amphotericin B, amphotericin deoxycholate and fluconazole at the same time (14).

Micafungin : Micafungin is the second drug of class echinocandin. It is a semi-synthetic drug which is formed by the chemical modification of the fermentation product of *Coleophoma empetri*. Micafungin is administered intravenously and targets the synthesis of 1-3 β glucan. Literature suggests that micafungin is similar to caspofungin in many respects like identical *in vitro* activity against *Candida albicans*, non-albicans species of *Candida*, and *Aspergillus* species (15). A favourable tolerability profile has been observed in case of this drug, no drug to drug interactions have been observed. Moreover, it does not need adjustment for renal or hepatic insufficiency (16).

Chitin synthase (Chs) inhibitors

Nikkomycin Z : Nikkomycin Z is a competitive inhibitor of chitin synthases in fungi. It is an antifungal peptide that has been produced as a by-product of *Streptomyces tendae* fermentation (17). Reports suggest that in case of *Saccharomyces cerevisiae*, nikkomycin inhibits only Chs 1 and 3 but not 2 whereas in case of *C.albicans* it inhibits all the 3 Chs having different IC_{50} for each of them. It has been concluded through studies conducted in different growth mediums that nikkomycin inhibits the formation of the septum and chitin by inhibiting the chitin synthases in a medium dependent manner (18).

Polyoxins : Polyoxins, produced by *Streptomyces cacaoi,* are a class of nucleoside antibiotics having nitrogen as a part of their heterocyclic ring. It targets the chitin synthase in order to block the production of chitin. Polyoxins have reportedly shown different activities against different organisms (19, 20, 21, 22). Polyoxin D is known to be fungistatic against *C.albicans* at concentrations as high as 500 to 2000 mg/ml and is also know to be a growth inhibitor in case of *C.neoformans* (23).

Mannoprotein synthesis inhibitor

Pradimicin/benanomycin family: Pradimicin and benanomycin are a new class of antifungals that have been isolated from actinomycetes. The primary character of these antifungals is that they possess a benzonathalene quinine skeleton substituted with an amino acid and a disaccharide side chain. This wide spectrum antifungal has a calcium dependent mode of action in which it forms a complex with the saccharide moiety present on the cell surface thus causing a leakage through the cell membrane and ultimately leading to death of the fungi (24).

Sphingolipid synthesis inhibitor : Lipoxamycin and hydroxylipoxamycin isolated from *Streptomyces sp*. have been reported to inhibit the fungal growth in case of various fungi but have a note worthy effect against *C.neoformans* and *C.albicans.* Although negative effect was shown by *Aspergillus fumigatus* against lipoxamycin tests but all the other filamentous fungi showed the inhibition during the disc diffusion tests (25, 26). Apart from the said compounds, viridofungin has also shown to inhibit the fungal growth (27). Further investigation showed that all these compounds inhibited serine palmitoyl transferase, an important enzyme in the course of formation of sphingolipid (28, 29, 30).

Another key enzyme that is responsible for the formation of ceramide during the synthesis of sphingolipid is ceramide synthase. Researchers have shown that a group of mycotoxins termed as fumonisins, primarily produced by *Fusarium verticillioides* and *Fusarium*

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proliferatum (31) are responsible for the inhibition of this enzyme. Although, there are evidences that show that fumonisin B1 has the potential to inhibit the sphingolipid synthesis *in-vitro* but altogether due to the low penetrance power, fumonisins are not considered as good therapeutic agents (32, 33). Apart from fumonisins, australifungin isolated from *Sporormiella australis* is the first non sphingosine based inhibitor having a unique combination of α-diketone and β-ketoaldehyde. Although australifungin has shown MIC of 1mg/L or less than all the organisms it has been tested against yet it has a hindrance for therapeutic use as it hampers the ceramide synthesis in HepG2 cells (30).

Likewise, a third enzyme, inositol phosphorylceramide (IPC) synthase catalyzes the transfer of phosphoinositol from phosphatidylinositol to ceramide to form IPC. Aureobasidins specially aureobasidin A has been known to produce high antifungal inhibitions against *Candida* species but are not effective against *Aspergillus* species the reason still being unknown (34, 30).

Protein synthesis inhibitors : A lot of protein synthesis inhibitors which can prove to be of therapeutic importance have come up in the recent past. Sordarin, isolated from the fermentation of *Sordaria araneosa* shows high degree of activity against *Candida albicans, C.tropicalis, C. kefyr and Cryptococcus neoformans,* but showed negative effect against *C. krusei, C. glabrata and C. parapsilosis*. It shows a high level of specificity against elongation factor 2 (EF2) (35, 36).

Similarly, Zofimarin has shown potency against *C.albicans, S.cervisiae, C. neoformans* and *Aspergillus* species also. A different antifungalcompound BE 31405 has a unique tricyclicstructure with a sugar and is reported to be active against *C. albicans, C. glabrata* and *C.neoformans* (30, 37).

Conclusion

The success of the natural products in the drug discovery is due to the biological compatibility and drug likeliness of the natural compounds as compared to the synthetic compounds. The countries which are rich in biological diversity have a great scope of utilization of their natural products in the formulation of effective drugs. But, it is necessary to use controlled scientific methodologies without disturbing the biodiversity.

In the recent past, after the failure of the alternate drug discovery strategy, the science of drug discovery has changed its course back to the utilization of the natural resources. It is the need of the hour that the present day technology for instance smart screening methods, robotic separation with structural analysis, metabolic engineering, bioinformatics and synthetic biology should lend a co-operative hand in developing the nature based drugs thus culminating the ill effects of the synthetic drugs and more efficiently fighting the war against mycological diseases.

Conflict of Interest : None

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