FACILE SYNTHETIC METHODS FOR BIOFILM AND ANTIFUNGAL RESISTANCE

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Abstract

Eco-friendly and facile methods for nanoparticles synthesis with multi-faceted applications has revolutionized the field of modern medicine and technology (. Interestingly among the metallic nanoparticles, Silver nanoparticles (AgNPs) have a wide range of applications and are synthesized up to 500 tonnes each year for biomedical uses. Biogenic syntheses of NPs are strongly addressed to be cost-effective, nature-friendly, rapid and facile when compared to chemical and physical methods. Among the bio-based methods for the synthesis of NPs, plant extract mediated synthesis has gained immense attention and appreciation due to its non-toxic attributes. Fabrication of metallic NPs from microbial sources has its own advantages but the process of culturing and maintaining microbial cells is tedious and additional chemicals in the form of culturing media are indirectly involved in these methods.

Introduction

When compared with antibacterial research, little progress has been made in the development of new antifungal agents, which has been justified by the low occurrence of fungal infections. However, the current increase in incidence of fungal infections has led to aggressive research on new antifungal agents as evidenced by the rise in the number of publications since the 1960s (Maertens, 2004; Ngo et al., 2016). Another reason for the slow development of antifungal agents is the fact that fungi are eukaryotic, with a close evolutionary relationship with human hosts, which complicates the search for antifungal targets. Nonetheless, detailed knowledge regarding the structure, composition and biochemistry of fungal cells, in addition to various facets of fungal infections, has contributed to our understanding about the mechanism of action of many antifungal agents (Borgers, 1980; Kanafani and Perfect, 2008). Typically a long period of 8 to 10 years is required for an antifungal to be approved for clinical use. Reducing toxicity, enhancing bioavailability, improving the antifungal spectrum and combating resistance are efforts that are expected to increase the efficacy of the available antifungals. Indeed, elucidation of the mode of action of a potential antifungal compound can shorten the time from lead to candidate drug. Small antifungal molecules from natural products could represent structural templates for structure-activity relationship studies, thus providing more information to optimize potential new antifungal agents (Sheng and Zhang, 2011). Overall, new strategies regarding antifungal therapy, target identification and rational drug design technologies can significantly accelerate the process of new antifungal development, reducing the time to cure or providing better quality of life to patients.

Literature Review

K M Khandarkar et al (2013) A series of novel Mannich base and their hydrazone derivatives were synthesized by highly resourceful, chic, simple and green technique with exceptionally facile

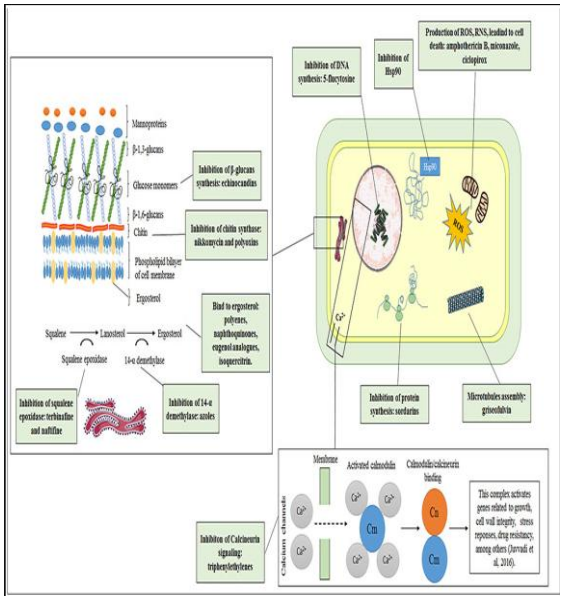
reaction conditions of one-pot, three component reaction with an array of biologically and pharmaceutically active novel heterocycles. The protocol offers a valuable alternative to known methods and will find applications in the field of green synthesis and antimicrobial study against pathogenic microbes, supporting the development of bioinformatical database of novel and derelict heterocycles. These data indicate their potential to become antifungal agents. The novel products were established by elemental, IR, mass spectroscopic and NMR analysis. The environmental advantages of the method include short reaction time, excellent yield, easy work-up, absence of extraction and chromatographic purification steps.

C. P. Kaushik (2017) A library of twenty five amide linked 1,4-disubstituted 1,2,3-triazoles have been prepared through a facile expeditious synthetic protocol involving Cu(I) mediated cyclization of N-(2-methylbut-3-yn-2-yl)aromatic amides and in situ generated 2-azido-N-substituted propanamides. Structures of newly synthesized compounds (5a–5y) were confirmed by analytical techniques, such as FTIR, 1H NMR, 13C NMR, and HRMS. In vitro antifungal activity was also examined against two fungal strains Candida albicans and Aspergillus niger by serial dilution method. The compounds 5 m and 5w exhibited appreciable potent activity.

Sumath et al (2019) In vitro antibacterial activity of the novel piperidone derivatives synthesised from chalcone in dry media under microwave irradiation through the Michael addition reaction has been represented. It's a comparative study of synthesizing compounds by conventional as well as non-conventional methods of specific greener process. Then the structures of newly synthesized compounds were characterized by FT-IR, UV-Vis, NMR (13C, 1 H) and GC-Mass spectrums were investigated. The in vitro antimicrobial activities were screened against the standard strains: Staphylococcus aureus, Streptococcus pyogenes and Bacillus subtilis as Gram positive, Escherichia coli and Pseudomonas aeruginosa as Gram negative. Nitrile group containing piperidone derivative 5d showed better antibacterial activity on Streptococcus pyogenes, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa than the reference drug Ciprofloxacin.

Antifungal Mechanism of Action

Although the commercially available antifungal agents to date have targets that are restricted to the plasma membrane and the cell wall, a certain diversity of targets has been discovered. To develop new therapies, recent studies have focused on the inhibition of fungal virulence factors. Some mechanisms of action are described below, and an overview is presented in Figure.



Ergosterol

Ergosterol is a lipid responsible for membrane fluidity and permeability and for the function of fungal integral membrane proteins; accordingly, this sterol is essential for cell viability. Several antifungals primarily target ergosterol, either by inhibiting its biosynthesis or by binding to it, causing formation of pores in the membrane.

Azole antifungals act by inhibiting ergosterol biosynthesis via the cytochrome P450 enzyme 14-α demethylase, which catalyzes the conversion of lanosterol to ergosterol. Azoles affect the integrity of fungal membranes, altering their morphology and inhibiting growth.

Cell Wall

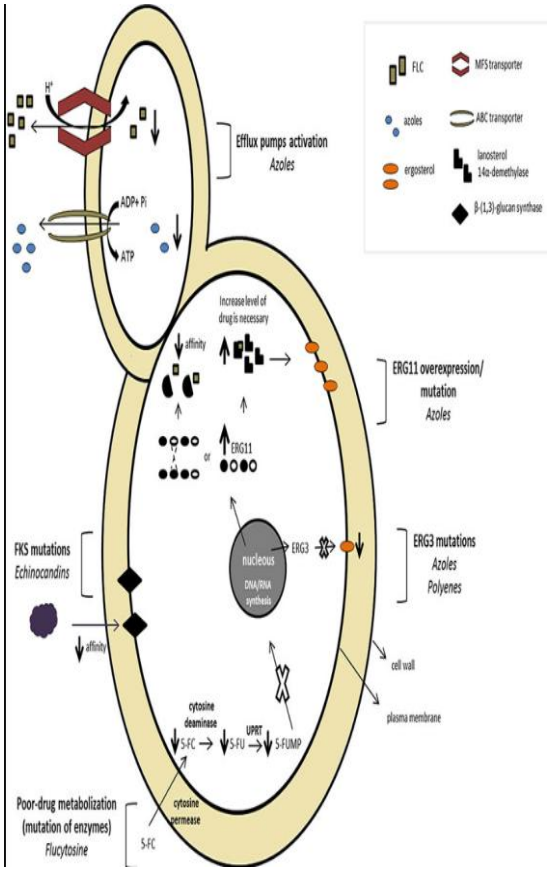
The fungal cell wall, which is primarily composed of chitin, glucans, mannans, and glycoproteins, is essential for adhesion and fungal pathogenesis and also serves as a protective barrier, limiting the access of molecules to the plasma membrane. The two main mechanisms of action of antifungals targeting the cell wall are related to the inhibition of chitin and β-glucan synthesis.

Inhibition of Nucleic Acid, Protein, and Microtubule Syntheses

Inhibition of nucleic acid synthesis is related to the action of 5-flucytosine, which is converted primarily to 5-fluorouracil by the enzyme cytosine deaminase and then to 5-fluorouridylic acid by UMP pyrophosphorylase. Although 5-flucytosine was synthesized in 1957, its antifungal property was not discovered until 1964, 7 years later. This acid can be incorporated into RNA, resulting in premature chain termination, thereby inhibiting DNA synthesis through effects on the enzyme thymidylate synthase.

Molecular Mechanisms of Antifungal Resistance

The widespread use of antifungal agents and the limited arsenal associated with the increased number of opportunistic infections have resulted in the progression of resistance to available drugs. The antifungal resistance mechanism may occur through different conditions such as a decrease in the effective drug concentration, changes or overexpression of the drug targets, and metabolic bypasses. Below Figure depicts an overview of several antifungal resistance mechanisms described for Candida spp.



Molecular Mechanisms of Resistance to Azoles

Azole resistance includes the following mechanisms: (1) activation of efflux pumps, (2) qualitative changes in the target enzyme, (3) quantitative changes caused by over expression of ERG11, and (4) alterations in cell wall composition.

Activation of Efflux Pumps

Reduction in intracellular antifungal accumulation in Candida spp. is a consequence of the overexpression of membrane-associated transporters acting as multidrug efflux pumps (Prasad and Rawal, 2014). Two main classes of transporters are described as being involved in this resistance mechanism. The superfamily of ATP-binding cassette (ABC) proteins comprises the primary activity, hydrolyzing ATP to provide energy to drive the efflux of drugs. Transporters belonging to the major facilitator superfamily (MFS) constitute the secondary activity; these pumps utilize a proton electrochemical gradient across the plasma membrane to extrude substrates

Biofilm and Antifungal Resistance

The ability of many fungi to form biofilms is one of the reasons for antifungal drug resistance. Many medically important fungi are described as biofilm-forming organisms, such as Candida spp. Pneumocystis spp, Coccidioides spp., Zygomycetes, Malassezia spp., Trichosporon spp., Cryptococcus, Histoplasma capsulatum, Trichophyton spp and Paracoccidioides spp.

Biofilms are highly structured and complex microbial communities embedded in a self-produced extracellular matrix (ECM) that attach to a wide range of surfaces and. Several factors contribute to initial surface attachment, such as pH, temperature, osmolarity, flow of the surrounding biologic medium, host immune factors and even the presence of antimicrobial agents.

Fungal biofilm formation occurs through a sequential process including planktonic cell adhesion to an appropriate substratum, colonization, ECM production, biofilm maturation, and dispersion. Despite these specific characteristics, all types of fungal biofilms have distinct properties from planktonic yeast cells and increase antifungal drug resistance up to 1000-fold. Indeed, several studies have shown the inefficacy of antifungal therapy against different fungal biofilms.

Multiple other biofilm-specific factors contribute simultaneously to the resistance of yeasts to antifungal drugs, including cell density, quorum sensing, efflux pump activity, persister cells, ECM presence, stress responses and overexpression of drug targets. The cell density is an important factor that contributes to the antifungal resistance of biofilms. However, some studies show that this is not a biofilm-specific resistance mechanism because a similar trend was observed for planktonic cells. The efficacy of different azoles, AmB and caspofungin on planktonic cells at densities similar to those found in biofilms. The susceptibility of dissociated biofilm cells was similar to that of planktonic cells at the same cell density, and this susceptibility decreased as the density of the cells increased. The density-dependent susceptibility of planktonic or biofilm for ketoconazole and 5-FC. The similar drug resistance results by increasing the inoculum sizes of Aspergillus species, supporting the idea that the physical density of the cells influences antifungal agent activity.

New Antifungal Formulations and New Antifungal Drug Structure Modification

Two different strategies have been developed to increase the therapeutic index of antifungal agents: chemical modifications and/or elaboration of new formulations of antifungal agents to obtain less toxic derivatives. Theoretical and experimental studies on the mechanism of action of AmB and its derivatives were

performed. Two generations of derivatives were developed. First-generation compounds are modified at the carboxyl group, which improves selective toxicity based on disturbance of the hydrogen bond network in complex with sterols. Second-generation compounds have introduction of a bulky substituent, resulting in an appropriate steric hindrance effect that disturbs interaction with cholesterol but not with ergosterol, leading to improved selective toxicity. Among second-generation derivatives, N-methyl-N-D-fructosyl AMB methyl ester (MFAME) is considered the most interesting compound because it is able to form water-soluble salts, has a broad antifungal spectrum, and lower toxicity than AMB toward animal cells in in vitro and in vivo experiments.

Despite the huge effort made to decrease fungal resistance and the toxicity of AmB, the development of rational chemical modification of known antifungal agents was insufficient to solve these problems. Thus, new delivery systems have been evaluated to reach this goal.

Since 1990, nanostructured systems have been studied as carriers of antifungal agents. Clinically, the intravenous dosage form of AmB-deoxycholate has adverse effects, mainly nephrotoxicity. The synthesis of AmB analogs such as AmB esters or a preparation including an AmB lipid complex, AmB colloidal dispersion, liposomal AmB and intralipid AmB have been generated to improve the therapeutic index and lower toxicity. In addition, other delivery systems, such as carriers based on solid and nanostructure lipids, synthetic and natural polymers, inorganic and metal nanostructure lipids, dendrimers, silica, and carbon materials, have been pursued. These delivery systems are able to improve bioavailability and reduce toxicity and present specificity for target tissues; however, there is an associated high cost of production. Recent work shows that the composition of nanoparticles used as delivery vehicles is fundamental for increasing antifungal activity. The conjugate system with AmB and metal nanoparticles that displayed synergistic antifungal activity due to the antimicrobial property of silver against C. albicans and C. reported synergistic activity for the combination of polyenes and magnetic nanoparticles. This bio-active nano-sized formulation exhibited enhanced efficiency against two clinical isolates of Candida species in planktonic and biofilm states.

Conclusion

Despite the increasing number of reports regarding advances in antifungal therapy, the number of cases of infection and antifungal resistance are still alarmingly high, and control of antifungal disease is far from being achieved. Important new advances have been made in the discovery of antimicrobial fungal targets; however, many years are necessary from discovery to clinical use. Because of this, improving existing molecules and developing new formulations and alternative therapy for prevention and treatment are important for treating fungal infections and increasing treatment options and quality of life.

References

1. *C. P. Kaushik (2017) Facile expeditious one-pot synthesis and antifungal evaluation of disubstituted 1,2,3-triazole with two amide linkages, An International Journal for Rapid Communication of Synthetic Organic Chemistry, Volume 47, 2017 - Issue 23.*
2. *Sumath et al (2019) A Facile Solid-State Synthesis And Antimicrobial Activity Studies On Some Piperidone Derivatives, International Research Journal Of Pharmacy, ISSN 2230 – 8407, 10 (10).*

3. *Nepolraj A, Pitchai P, Mani P. One-pot Synthesis of Pyrono [2, 3] Quinoline via the Tandem Cyclization of Algar-FlynOyamanda Reactions. Org Chem Res 2019; 5(2): 167-173. 4. Mohammad Asif. Biological Potential and Chemical Properties of Pyridine and Piperidine Fused Pyridazine Compounds: Pyridopyridazine a Versatile Nucleus. Asian Journal of Chemistry and Pharmaceutical Sciences 2016; 1(1): 29-35.*
4. *Liu XH, Weng JQ, Wang BL, Li YH, Tan CX, Li ZM (2014) Microwave-assisted synthesis of novel fluorinated 1,2,4-triazole derivatives, and study of their biological activity. Res Chem Intermed 40:2605–2612.*
5. *Zhang LJ, Yang MY, Sun ZH, Tan CX, Weng JQ, Wu HK, Liu XH (2014) Syn‑thesis and antifungal activity of 1,3,4-thiadiazole derivatives containing pyridine group. Lett Drug Des Discov 11:1107–1111.*
6. *Anderson, T. M., Clay, M. C., Cioffi, A. G., Diaz, K. A., Hisao, G. S., Tuttle, M. D., et al. (2014). Amphotericin forms an extramembranous and fungicidal sterol sponge. Nat. Chem. Biol. 10, 400–406. doi: 10.1038/nchembio.1496.*
7. *Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M., and Mylonakis, E. (2007). Antifungal chemical compounds identified using a C. elegans pathogenicity assay. PLoS Pathog. 3:e18. doi: 10.1371/journal.ppat.0030018.*
8. *Chandra, J., Patel, J. D., Li, J., Zhou, G., Mukherjee, P. K., McCormick, T. S., et al. (2005). Modification of surface properties of biomaterials influences the ability of Candida albicans to form biofilms. Appl. Environ. Microbiol. 71, 8795–8801. doi: 10.1128/AEM.71.12.8795-8801.2005.*