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Title: Antifungal evaluation and molecular docking studies on new azole derivatives against *Candida* spp.

ABSTRACT

This study includes the synthesis of some novel 1,2,3-triazole derivatives and evaluation of their biological properties against *Candida* cells. In the first scheme of synthesis, an efficient one pot three component stepwise approach using azide-chalcone oxidative cycloaddition and post-triazolearylation has been followed to get new *N*-2-aryl-substituted-1,2,3-triazole derivatives (**2a-j**) under mild conditions with moderate yields. Ten different 1,2,3-triazole derivatives were synthesized and confirmed on the basis of FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral analyses. These compounds did not showed any significant inhibition of *Candida* cells as the MIC values of all the compounds were more than 1 mg/mL. However, haemolytic assay showed less toxicity profile of these compounds, but the compounds of this series were not proceed further on the basis of their anticandidal activity.

In second scheme, we used ten naturally bioactive scaffolds (**1a-j**) with most of them having promisingantimicrobial activities and synthesised their novel 1,2,3-triazole derivatives (**3a-j**). Initially, the eight precursor compounds (**1a-h**) were converted to their respective alkyne (**2a-h**) followed byaddition of benzyl azide, freshly prepared by the reaction of benzyl bromide with sodium azide to give 1,2,3 triazole derivatives (**3a-h**)were obtained using [3+2] azide-alkyne cycloaddition strategy. The result of *in vitro*anticandidal activity performed against three different strainsof *Candida* showed that compound **3e**was found superior to fluconazole (FLC) with IC_{50}values of 0.04 µg/mL against *C. albicans* (ATCC 90028), 12.02 µg/mL against *C. glabrata*(ATCC 90030), and 3.60µg/mL against *C. tropicalis* (ATCC 750).Structurally compound **3e** was different from other synthesized 1,2,3-triazoles by having quinoline ring instead of benzene in its structure. To examine the role of quinoline ring (especially the nitrogen atom) in the inhibitory potential of **3e**, we synthesized two more 1,2,3 triazole derivatives (**3i** and **3j**) starting from 5-Cl,8-hydroxy quinoline (**1i**) and naphthol (**1j**) using the same procedure. The results of *in vitro*anticandidal activities of **3i**

and **3j** indicated that quinoline ring plays important role in the inhibition of *Candida* cells. Compounds **3e** and **3i** also showed good inhibition of clinically isolated *Candida* strains including fluconazole resistance strains with IC_{50}values range from 1.9 to 4.1 μg/mL and 32.8 to 47.1 μg/mL, respectively. The cytotoxicity of all the synthesized compounds was determined on VERO cell line by MTT assay which indicated their non-toxic nature. The toxicity of lead compounds **3e** and **3i** was also determined by haemolytic assay which again confirmed their non-toxic behaviour. Therefore on the basis of these preliminary results, we selected **3e** and **3i** as lead inhibitors and explored their further biological potential as anticandidal agents. The ADME predictions of all the compounds were predicted by QikProp version 3.2 using Schrödinger software suggested the drug like properties of these synthesized compounds.

Growth, virulence attributes (proteinase and phodpholipase secretion, yeast to hyphal transition), H+ ATPase activity, ergosterol biosynthesis and morphology of *Candida* were examined under the effect of lead inhibitors **3e** and **3i**. Growth curve, disk diffusion and time kill curve analysis showed fungicidal and fungistatic nature of compounds **3e** and **3i**, respectively. Secretion of hydrolytic enzymes, mainly proteinases and phospholipases, decreased considerably in the presence of two lead inhibitory compounds, indicating towards their interference in fungal virulence. H^+ ATPase is an important transporter protein and an emerging antifungal therapeutic target which involves in the intracellular pH maintenance and nutrient uptake in *Candida*. About 50-70% inhibition in the H+ ATPase activity was found when different strains of *Candida* exposed to 100 μg/mL concentration of lead inhibitors **3e** or **3i**. The intracellular pH was also acidified by the presence of these inhibitors which also supported their inhibitory effect on H^+ ATPase activity. TEM analysis of *Candida* cells exposed to the lead inhibitors (**3e** and **3i**) clearly showed morphological changes and intracellular damage as their possible mode of action. A preliminary mechanistic study carried out on the two most effective compounds (**3e** and **3i)** revealed inhibition of ergosterol biosynthesis thereby causing the cells to lose their integrity and viability. The inhibitor**3e** was found as potent inhibitor of antioxidant defence system in *C. albicans.* An *in silico* analysis of **3e** and **3i**binding to a modeled *C. albican*s CYP51 showed critical H-bond interactions with the important active site residues indicating the basis of their anti-*Candida* role.

Keywords: 1,2,3, triazoles, *Candida*, cytotoxicity, anticandidal, docking