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Synthesis and Activity of Novel Indole Linked Triazole Derivatives as Antifungal Agents

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Systemic fungal diseases continue to be a significant problem in health care today. These infections are often induced by opportunistic causative fungi that are not normally pathogenic and commonly live in the patient’s body or are commonly found in the environment. The key opportunistic fungal pathogens were Candida albicans, Aspergillus fumigatus and Cryptococcus spp., which cause mucormycosis, a rapidly fatal infection especially in immunocompromised patients. Systems of fungal disease continue to be a significant problem in health care today. These infections are often induced by opportunistic causative fungi that are not normally pathogenic and commonly live in the patient’s body or are commonly found in the environment. The key opportunistic fungal pathogens were Candida albicans, Aspergillus fumigatus and Cryptococcus spp., which cause mucormycosis, a rapidly fatal infection especially in immunocompromised patients.

Theazole antifungals are currently the most widely used and studied class of antifungal agents. Azole compounds prevent the synthesis of ergosterol, a major component of fungal plasma membranes, by inhibiting the cytochrome P-450 dependent enzyme lanosterol demethylase. Their antifungal efficacy is attributed to their greater affinity for fungal P-450DM than for the mammalian enzyme. Theazole antifungals are classified as imidazoles or triazoles. Generally, use of imidazoles is limited to the treatment of superficial mycoses, whereas the triazoles have a broad range of applications in the treatment of both superficial and systemic infections because of their good affinity for fungal (rather than mammalian cytochrome P-450 enzymes).

Hence, novel triazole compounds are prepared with different substituents on 2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1H,1,2,4-triazol-1-yl)propyl moiety. The present study on the synthesis and antifungal activity of new voriconazole analogues are incorporated indole derivatives. A variety of indole derivatives with different substituents could exhibit the biological activities through different action and sometimes improve upon the activities. The presence of amino, cyano, halo and alkyl substituents of indoles was considerably important factor to affect their antifungal activity. Based on this speculation, indole-linked triazole derivatives were synthesized and were evaluated for their antifungal activity.

The synthesis of the key intermediate 1, as seen in Scheme 1, may be preceded by well optimized method. This compound has a chiral center, and the final compounds could be controlled according to the configuration of oxirane. Thus, (2R,3R)-2-(2,4-difluorophenyl)-3-(piperazin-1-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol (2) was prepared using R-lactate as a starting material. R-lactate was treated firstly with morpholine and then protected compound was reacted with phenylmagnesium bromide, followed by epoxydation (Corey’s reagents) to afford the epoxy ring. The triazole compound was deprotected with TsOH in MeOH, and then diols compound was mesylated with MsCl in pyridine. Nucleophilic substitution of mesylated group by means of NaOMe in MeOH with concomitant epoxide formation affords the desired intermediate 1. Finally piperazine compound 2 was prepared by reacting an oxirane compound with a piperazine in the presence of LiClO4.

The synthesis and functionalization of indoles was performed by well established classical methods. There are many approaches to the synthesis of indole derivatives which have been categorized into two main types, corresponding to two main sections: the de novo indole system construction from benzenoid precursors through cyclization reactions and the functionalization of performed indole rings as described in Scheme 2.

Scheme 1. Synthesis of (2R,3R)-2-(2,4-difluorophenyl)-3-(piperazin-1-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol (2). Reagents and conditions: (i) morpholine, reflux; 3,4-dihydro-2-pyridin, pyridinium-p-toluenesulfate, CH2Cl2, rt, 5 h, 50%; 1,3-difluorophenylmagnesium bromide, 20 °C → rt, overnight, 95%; (ii) TMSOJ, CH2Cl2, 70 °C, overnight, 75%; (iii) 1,2,4-triazole, K2CO3, 100 oC, 6 h, 96%; (iv) toluenesulfonic acid monohydrate, MeOH, rt, 1 h, 61%; MsCl, Pyridine, rt, 6 h, 99%; NaOMe, MeOH, 0 °C, 30 min, 81%; (v) piperazine, LiClO4, CH2CN, reflux, 50%.

Scheme 2. Synthesis of indole derivatives
Indoles 3a-y were on reaction with formaldehyde in the presence of AcOH in ethanol at 0 °C furnished (2\text{R},3\text{R})-2-(2,4-difluorophenyl)-3-[4-(indol-3-ylmethyl)piperazin-1-yl]-1-(1H-1,2,4-triazol-1-yl)butan-2-ol 4a-y.

All the newly synthesized compounds 4a-y was assayed for their antifungal activity against C. albicans, C. neoformans, A. fumigatus and C. krusei strains and their MIC\textsubscript{80} values are summarized in Table 1. The growth inhibition test for drug evaluation against C. albicans, C. neoformans, A. fumigatus and C. krusei was carried out by the method based on the National Committee for Clinical Laboratory Standards M27-A and M38-A. Amphotericin B, fluconazole and itraconazole were used as positive controls.\textsuperscript{6}

The MIC\textsubscript{80} values indicate that nearly all the indole derivatives showed excellent antifungal activities against the five yeast Candida species including C. albicans and C. krusei. Noticeably, more than half of 25 compounds showed higher activity against C. albicans than the tested two conventional drugs such as amphotericin B and fluconazole. These result suggested that introduction of an indole moiety to the triazole pharmacophore strongly enhanced the antifungal activity of these analogs against Candida species. However, C. neoformans was found to be much less sensitive to these indole derivatives.

Compared to their strong activities against yeast species, the MIC\textsubscript{80} values indicate that the activities of these indole derivatives against mold fungi are much lower. Only two compounds (4p, 4x) showed comparable activities against A. fumigatus (MIC\textsubscript{80} = 1.2 and 2.16 μmol/mL, respectively). The incompetent activity of these indole derivatives against A. fumigatus was not a surprise because it has been known that this mold species possesses an intrinsic mechanism resistant to triazole antifungals.

Pharmacomodulation aimed at evaluating the influence of (i) the nature and position of halogen group(s) fixed at the in-
dole ring and (ii) a diverse group(s) at C5 of indole.

SAR resulting from introducing of an halogen group (R1) were explored in the subseries of 4-, 5-, 6- and 7-position on the indole ring. Replacing hydrogen by halogen exerted a favorable effect. 4-Cl (4c), 5-Cl (4f), 6-Cl (4p) and 7-Cl (4v) were 4-fold as active as 4a and the substituted indole derivatives 4b-y were generally more potent than unsubstituted indole compound 4a. Interestingly, the most potent antifungal activity against C. albicans is obtained with halogenated indole derivatives and the antifungal activity against A. fumigatus showed higher activity of this series of compounds against fungi shows a high selectivity of action against C. albicans. Compounds 4a and 4y were prepared from their corresponding indole derivatives 3a-d, 3f-y according to the same protocol as described for compound 4e.

Synthesis of (2R,3R)-2-(2,4-difluorophenyl)-3-(5,6-dichloro-1H-indol-3-ylmethyl)-piperazin-1-yl)-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol (4f). A mixture of (2R,3S)-2-(2,4-difluorophenyl)-3-methyl-1-(1H-1,2,4-triazol-1-yl)methyloxirane (1) (2.0 g, 8.0 mmol), piperase (686 mg, 20 mmol) and lithium perchlorate (508 mg, 12 mmol) in acetonitrile (30 mL) was heated under reflux for 48h. The solvent was removed under reduced pressure, the residue was treated with crushed ice and extracted with ethyl acetate (3 × 30 mL). The combined organic extract was washed with water, brine, dried (Na2SO4) and concentrated to give the desired compound (2) as thick viscous gum (3.0 g, 78%). 1H NMR (CDCl3) δ 0.97 (d, 3H, CH3), 2.32-2.42 (m, 2H, CH2), 2.66-2.90 (m, 8H, 3x CH2, CH, OH), 4.36 (d, J = 14.0 Hz, 1H, CH2), 4.57 (d, J = 14.0 Hz, 1H, CH2), 6.68-6.83 (m, 2H, Ar-H), 7.42-7.55 (m, 1H, Ar-H), 7.78 (s, 1H), 8.0 (s, 1H); FAB-MS m/z 337.3 (Calcd. for C16H16F2N4O: 337.3). 

Synthesis of (2R,3R)-2-(2,4-difluorophenyl)-3-(5-fluoro-1H-indol-3-ylmethyl)-piperazin-1-yl)-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol (4e). A mixed solution of formaldehyde (144 μL) and acetic acid (214 μL) in ethanol was added (2R,3R)-2-(2,4-Difluorophenyl)-3-(piperazin-1-yl)-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol (2) (624 mg) at 0°C. The mixture was stirred for 1 hour, then 5-fluoroindole (250 mg) was added. The resulting mixture was stirred for 12 h at 30 - 40°C, and worked up (AcOEt; potassium hydroxide solution, brine). The residue was purified by chromatography on silica gel (dichloromethane : ethanol = 1 : 1, v/v) to give (2R,3R)-4e in yields of 72%. 1H NMR (CDCl3) δ 1.07 (d, 3H, CH3), 2.40-2.47 (m, 2H, CH2), 2.70-2.90 (m, 8H, 3x CH2, CH, OH), 4.31 (d, J = 14.0 Hz, 1H, CH2), 4.50 (d, J = 14.0 Hz, 1H, CH2), 6.68-6.85 (m, 4H, 7.22-7.33 (m, 2H), 8.10 (s, 1H), 8.13 (s, 1H), 10.2 (s, 1H); FT-IR (KBr): 3211, 2766, 1752, 1602, 934; FAB-MS m/z 484.5 (Calcd. for C25H27F3N6O: 484.5). 

Compounds 4a-d, 4f-y were prepared from their corresponding indole derivatives 3a-d, 3f-y according to the same protocol as described for compound 4e.

Synthesis of (2R,3R)-2-(2,4-difluorophenyl)-3-(5,6-dichloro-1H-indol-3-ylmethyl)-piperazin-1-yl)-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol (4f). A mixture of 5-fluoroindole (250 mg) was heated under reflux for 48h. The solvent was removed under reduced pressure, the residue was treated with crushed ice and extracted with ethyl acetate (3 × 30 mL). The combined organic extract was washed with water, brine, dried (Na2SO4) and concentrated to give the desired compound (2) as thick viscous gum (3.0 g, 78%). 1H NMR (CDCl3) δ 0.97 (d, 3H, CH3), 2.32-2.42 (m, 2H, CH2), 2.66-2.90 (m, 8H, 3x CH2, CH, OH), 4.36 (d, J = 14.0 Hz, 1H, CH2), 4.57 (d, J = 14.0 Hz, 1H, CH2), 6.68-6.83 (m, 2H, Ar-H), 7.42-7.55 (m, 1H, Ar-H), 7.78 (s, 1H), 8.0 (s, 1H); FAB-MS m/z 337.3 (Calcd. for C16H16F2N4O: 337.3).
(the other strains in saline with 0.2% tween 20), and were measured absorbance and transmittance at 530 nm. The spore suspension of Fungal strains were adjusted to 0.108 of absorbance and was 1:50 diluted with RPMI 1640 medium. Then another 1:20 dilution was made to have $1 \times 10^7 - 5 \times 10^7$ CFU/mL. The spore suspensions of the other strains were adjusted with sterile saline to 80 - 82% of transmittance and were 1:50 diluted with RPMI 1640 medium to make $0.4 \times 10^7 - 5 \times 10^7$ CFU/mL.

Autoclaved 96-well microplate was prepared in advance, and 0.1 mL of series of dilutions of the test article and 0.1 mL of spore suspensions were mixed in each well of the microplate to have concentration range of 0.244 - 250 mg/mL. And then 25 μL of alarmarblue (Biosource) was added into the well. Two wells were assigned for each concentration level to make duplicates. After 24 hour and 72 hour of incubation, the absorbance bands of the contents of the well were measured with Microplate reader (SOFTmax PRO, Molecular Devices) and observations with the naked eye were also referred. The results were presented as Minimum Inhibitory Concentration (MIC). The concentration of each test strains, at which the growth was inhibited by 80% compared to the negative control, was determined.

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References