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Synthesis and Activity of Novel 1-Halogenobenzylindole Linked Triazole Derivatives as Antifungal Agents

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Fungal infection became an important complication and a major cause of morbidity and mortality in immunocompromised individuals suffering from tuberculosis, cancer or AIDS and in organ transplant cases.[1] 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. Triazole antifungals which act by inhibiting lanosterol cytochrome P-450 14 α-demethylase (CYP51) have now become the most rapidly expanding group of antifungal compounds. These drugs are orally active, show a broad-spectrum against most yeasts and filamentous fungi, and are relatively nontoxic. However, the increase of opportunistic fungal infections and emergence of azole resistance strains have intensified the work of medicinal chemists to develop new antifungal drugs.[2]

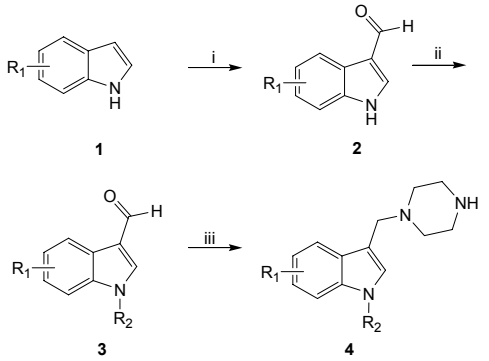
Hence, novel triazole compounds are prepared with different substituents on (1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl moiety. The present study on the synthesis and antifungal activity of new voriconazole analogues which are incorporated indole derivatives. A variety of indole derivatives with different substituents could exhibit the biological activities through different action and sometimes improve upon the activities. For example, the synthesis and antifungal activity of 1-halogenobenzyl-3-imidazolylmethylindole derivatives showing various degrees of antifungal against *Candida albicans* and *Aspergillus fumigatus* strains was repored.[3] The presence of halogen and halogenobenzyl group on the indole ring was considerably important factor to affect their antifungal activity. Based on this speculation, indoleconazole derivatives were synthesized and were evaluated their antifungal activity.

The synthesis of indole moiety was proceded by general methods as shown in Scheme 1. 5-,6- or 7-substituted indoles **1** were formylated with dimethylformamide using phosphorus oxychloride as a catalyst. The yield of aldehyde **2** is nearly quantitative, and the product is obtained in a state of high purity. 1-Substituted indole derivatives **3** were obtained by the nucleophilic substitution of 3-formyl-1-*H* indole **2** with appropriate substituted benzyl bromides(or chlorides) or alkyl chlorides in the presence of the base such as NaH and K_2CO_3. 3-Piperazinylmethyl indole compounds **4** were obtained using reductive amination conditions.

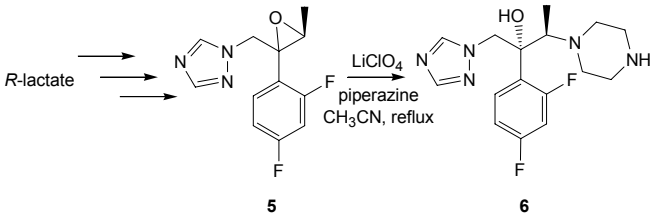
The optically active key intermediate (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-methyl-2-(1*H*-1,2,4-triazol-1-yl)methyloxirane **5** was derived from *R*-lactate with a well known method.[5] The piperazine compound **6** was prepared by reacting an oxirane compound **5** with a piperazine in the presence of $LiClO_4$ in a 50% yield.

Having obtained the key intermediate (**5** or **6**), the next steps was to couple it with a number of substituted indoles (**3** or **4**) as shown in Scheme 3. The piperazine compound **6** was reacted with various formylindoles (**3**) in presence of $NaBH(OAc)_3$ in dichloromethane at room temperature. Alternatively, the oxirane key compound **5** on reaction with 3-piperazinylmethylindoles (**4**) in the presence of lithium perchlorate produces the final compounds.

All the newly synthesized compounds **7-39** was assayed for



Scheme 1. Synthesis of 3-piperazinylmethyl-1*H*-indole derivatives. Reagents and conditions: i) $POCl_3$, DMF; ii) NaH, R_2-X, DMSO; iii) $NaBH(OAc)_3$, piperazine, AcOH, DCM

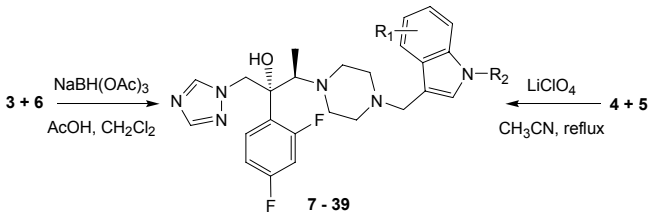


Scheme 2. Synthesis of (2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-(piperazin-1-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (**6**).

Table 1. *In vitro* antifungal activity of indole-conazole compounds **7-39**

Compound			MIC_{80} (μg/mL)						
			C. albicans				*C. krusei*	*C. neoformans*	*A. fumigatus*
No	R_1	R_2	ATCC 64548	ATCC36082	ATCC MYA-1003	ATCC 90028	ATCC 6258	ATCC 36556	ATCC 16424
7	H	H	0.5	0.5	128	0.25	16	> 128	64
8	H	4-F-Ph	0.125	0.125	4	0.125	0.5	> 128	1
9	H	4-F-Bz	0.125	8	16	2	1	> 128	16
10	H	2,4-F-Bz	0.25	0.125	8	0.125	0.5	> 128	4
11	H	4-Cl-Bz	0.125	0.25	8	0.125	1	> 128	16
12	H	2,4-Cl-Bz	0.25	0.25	4	0.125	1	> 128	16
13	H	3-CF_3-Bz	0.125	0.125	16	0.125	1	> 128	8
14	H	4-CF_3-Bz	0.125	0.125	64	0.125	0.5	> 128	2
15	H	sec-butyl	0.125	8	32	4	2	> 128	32
16	H	methylallyl	0.125	16	32	2	2	> 128	32
17	H	propan-2-ol	8	4	> 128	4	128	> 128	> 128
18	5-F	4-F-Bz	0.125	8	16	2	1	> 128	16
19	5-F	2,4-F-Bz	0.5	0.125	8	0.125	0.5	> 128	8
20	5-F	4-Cl-Bz	0.125	0.125	8	0.125	1	> 128	16
21	5-F	2,4-Cl-Bz	0.125	0.125	> 128	0.125	1	> 128	8
22	5-F	3-CF_3-Bz	0.125	0.125	> 128	0.125	1	> 128	8
23	5-F	4-CF_3-Bz	0.125	0.125	> 128	0.125	1	> 128	8
24	5-F	methylallyl	0.125	4	> 128	1	>128	> 128	64
25	5-F	propan-2-ol	2	2	> 128	2	64	> 128	> 128
26	5-Cl	4-F-Bz	0.125	4	16	2	1	> 128	8
27	5-Cl	2,4-F-Bz	0.125	0.125	2	0.125	0.5	> 128	2
28	5-Cl	4-Cl-Bz	0.125	0.125	> 128	0.125	1	> 128	8
29	5-Cl	2,4-Cl-Bz	0.125	0.125	> 128	0.125	1	> 128	8
30	5-Cl	propan-2-ol	1	1	64	1	16	> 128	128
31	5-CN	2,4-F-Bz	1	1	128	1	32	> 128	32
32	6-Cl	2,4-F-Bz	0.125	0.125	8	0.125	0.5	> 128	8
33	6-Cl	2,4-Cl-Bz	0.125	0.125	> 128	0.125	2	> 128	8
34	6-Cl	4-CF_3-Bz	0.125	0.125	> 128	0.125	1	> 128	4
35	6-Cl	methylallyl	0.125	0.125	8	0.125	1	> 128	8
36	6-Cl	4-F-Ph	0.125	0.125	> 128	0.125	0.5	> 128	1
37	6-N_3	2,4-F-Bz	0.125	0.125	> 128	0.125	1	> 128	4
38	7-CN	2,4-F-Bz	0.125	0.125	2	0.125	0.25	> 128	1
39	7-CN	2,4-Cl-Bz	0.25	0.25	> 128	0.25	0.5	> 128	2
Amphotericine B			0.125	0.25	0.125	0.125	0.5	> 32	0.5
Fluconazole			0.25	0.25	> 128	0.125	32	> 128	128
Itraconazole			0.125	0.125	> 128	0.125	0.125	> 128	0.125

Bz = Benzyl, Ph = Phenyl



Scheme 3. Synthesis of targeted compounds **X**-XX

their antifungal activity against *C. albicans*, *C. neoformans*, *A. fumigatus* and *C. krusei* strains and their MIC_{80}s 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 NCCLS.[6] Amphotericin B, fluconazole and itraconazole were used as positive controls. The MIC_{80} values indicate that nearly all the halogenobenzylindole derivatives showed excellent antifungal activities against the five yeast *Candida* species including *C. albicans* and *C. krusei* but no activity was found in compounds **15**, **16**, **17**, **24**, **25**, **30**, **31**.

The most phenylindole or benzylindole compounds (**8-14**, **18-23**, **26-29**, **31-34** and **36-39**) have significant inhibitory activity on *C. albicans* strains with inhibitory zone similar to that of itraconazole (125 μg/mL). On the drug-resistant-*C. albicans* ATCC MYA-1003 strain, 2,4-difluorobenzylindole derivatives **10**, **19**, **27**, **32** and **38** demonstrated an potential activity with inhibitory zone range between 8 and 2 μg/mL, respectively

compared to that of Amphotericine B (0.125 μg/mL). The significant activity was found for compounds **8**, **10**, **19**, **27**, **32**, **38**. The presence of 2,4-difluorobenzyl group on 1-position of indole moiety showed better activity than compounds with mono/dichlorobenzyl or mono-fluorobenzyl substituents on 1-position of indole, and a unsubstituted compound **7**. The compounds **8**, **10**, **19**, **27**, **32**, **38** showed potential activity due the presence of 4-fluorophenyl or 2,4-difluorobenzyl at 1-position of the indole ring.

The MIC of indole compounds **7**-**39** against mold fungi were compared with that of itraconazole. The result showed that compound **8** (MIC = 1 μg/mL) and **36** (MIC = 1 μg/mL) had less active than that of itraconazole, but had comparable antifungal profile with amphotericine B against *A. fumigatus* ATCC16424. However, the MIC values of tested compounds also demonstrated that antifungal activity was decreased considerably when the hydrogen atom at 1-position of indole structure were replaced by an alkyl chain and alcohol group such as sec-butyl (**15**), methylallyl (**16**, **24**, **35**) and propan-2-ol (**17**, **25**, **30**).

The substituents (R_1:F, Cl, CN, N_3) on the 5,6,7-position of the indole ring appear to contribute partially to antifungal potency. Interestingly, replacing the hydrogen with a cyano group at the 7-position of indole ring resulted in a promising antifungal activity.

In conclusion, we have synthesized a series of novel substituted indole-conazole compounds. The antifungal profile of these compounds indicated that 2,4-difluorobenzylindole compounds **10**, **19**, **27**, **32**, **37**, **38** and 4-fluorophenylindole compounds **8**, **36** have potent antifungal activity. Among these promising antifungal candidates, (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-{4-[1-(4-fluorophenyl)-1*H*-indol-3-ylmethyl]-piperazin-1-yl}-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (**8**) and (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-{4-[7-cyano-1-(2,4-difluorobenzyl)-1*H*-indol-3-ylmethyl]-piperazin-1-yl}-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (**38**) showed better antifungal activity than that of the clinically prevalent antifungal drug fluconazole against *C. albicans* ATCC64548, ATCC36082, ATCC90028 and the drug-resistant *C. albicans* ATCC MYA-1003.

The observed antifungal activity was believed to be exerted through specific target because the introduction of the substituent groups on the indole caused differences in the antifungal activity. Therefore, the modification of these compounds is must go on being investigated.

Experimental Section

Synthesis of 1-(2,4-difluorobenzyl)-3-((piperazin-1-yl)methyl)-1*H*-indole. 1-(2,4-difluorobenzyl)-3-formylindole was prepared according to the method described in *Eur. J. Med. Chem*. **2003**, *38*, 75. To a solution of 1-(2,4-difluorobenzyl)-3-formylindole (1.0 g, 3.7 mmol) and piperazine (637 mg, 7.4 mmol) in dichloromethane, acetic acid was added and the stirred for 30 min. To the mixture, $NaBH(OAc)_3$ (3.1 g, 14.8 mmol) was added with vigorous stirring and then the resulting mixture was stirred for 24 h. The mixture was extracted with dichloromethane (3 × 20 mL). The combined organic extract was washed with water, brine, dried (Na_2SO_4) and concentrated to give the desired piperazinyl compound in yields of 48%. ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 1H), 3.01 (t, 4H, CH_2), 3.27 (t, 4H, CH_2), 3.77 (s, 2H, CH_2), 5.23 (s, 2H, CH_2), 7.11 (m, 1H, Ph-H), 7.30 (m, 4H, Ph-H and indole ring), 7.66 (m, 1H, Ph-H), 8.1 (s, 1H, CH); FAB-MS *m*/*z* 341.1 (Calcd. for $C_{20}H_{21}F_2N_3$: 341.4).

Synthesis of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-[4-(1-(2,4-difluorobenzyl)-5-fluoro-1*H*-indol-3-yl)methyl]-piperazin-1-yl}-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (19). To a solution of 1-(2,4-difluorobenzyl)-5-fluoro-3-formylindole (0.5 g, 1.7 mmol) and (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-(piperazin-1-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (**6**) (1.2 g, 3.4 mmol) in dichloromethane, acetic acid was added and the stirred for 30 min. To the mixture, $NaBH(OAc)_3$ (1.44 g , 6.8 mmol) was added with vigorous stirring and then the resulting mixture was stirred for 24 h. The mixture was extracted with dichloromethane (3 × 20 mL). The combined organic extract was washed with water, brine, dried (Na_2SO_4) and concentrated to give the desired piperazinyl compound in yields of 48%. ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 3H, CH_3), 2.30 (s, 1H, OH), 2.81 (m, 8H, CH_2), 3.53 (m, 1H, CH), 3.89 (s, 2H, CH_2), 4.21 (d, 1H, J = 14.0 Hz, CH_2), 4.48 (d, 1H, J = 14.0 Hz, CH_2), 5.44 (s, 2H, CH_2), 6.72 (s, 1H, C-2 indole), 7.01-7.42 (m, 9H, Ar-H), 8.08 (s, 1H), 8.33 (s, 1H); FAB-MS *m*/*z* 610.2 (Calcd. for $C_{32}H_{31}F_5N_6O$: 610.62).

Synthesis of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-{4-[1-(2,4-difluorobenzyl)-6-chloro-1*H*-indol-3-yl]methyl)-piperazin-1-yl}-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (32). A mixture of (2*R*,3*S*)-2-(2,4difluorophenyl)-3-methyl-2-(1*H*-1,2,4-triazol-1-yl)methyloxirane (**5**) (2.0 g, 7.5 mmol), 6-chloro-1-(2,4-difluorobenzyl)-3-piperazinylmethyl-1*H*-indole (5.6 g, 15 mmol) and lithium perchlorate (2.4 g, 22.5 mmol) in acetonitrile (100 mL) was heated under reflux for 24 h. The solvent was removed under reduced pressure, the residue was treated with crushed ice and extracted with ethyl acetate (3 × 20 mL). The combined organic extract was washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on silica gel (dichloromethane : ethanol = 1 : 1, v/v) to give (2*R*,3*R*)-**32** in yields of 65%. ^{1}H NMR ($CDCl_3$) δ 1.01 (d, 3H, CH_3), 2.35 (s, 1H, OH), 2.64 (m, 8H, CH_2), 3.31 (m, 1H, CH), 3.72 (s, 2H, CH_2), 4.29 (d, 1H, J = 14.0 Hz, CH_2), 4.55 (d, 1H, J = 14.0 Hz, CH_2), 5.55 (s, 2H, CH_2), 6.63 (s, 1H, C-2 indole), 7.11-7.52 (m, 9H, Ar-H), 8.15 (s, 1H), 8.26 (s, 1H); FAB-MS *m*/*z* 626.2 (Calcd. for $C_{32}H_{31}ClF_4N_6O$: 626.22).

Evaluation of *In vitro* Antifungal Activity. Seven strains of *Candida albican* (ATCC 64548, ATCC 26310, ATCC 90028, ATCC MYA-1003), *Cryptococcus neoformans* (ATCC 24065), *Candida krusei* (ATCC 6258), and *Asregillus fumigatus* (ATCC 16424) were used. These 7 strains have been generally used to evaluate anti-fungal activity. These strains were obtained from ATCC and subcultured in the laboratory and used for experiments. The prepared test article was serially diluted with RPMI 1640 medium to make final concentration range of 0.488 - 500 mg/mL in sterilized microtubes. Fungal strains used this study were inoculated on YM agar, Potato dextrose agar and Sabouraud's agars. The inoculated agar plates were incubated for enough days. The spore suspensions of *Cryptococcus neoformans* and *Candida krusei* strains were prepared in 0.85% saline (the other strains in saline with 0.2% tween 20), and were measured absorbance and transmittance at 530 nm. The spore sus-

pension 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 $1 \times 10^3 - 5 \times 10^3$ 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 $0.4 \times 10^4 - 5 \times 10^4$ 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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