

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

Young Min Na

HanWha Pharma Co. Ltd., Yangji R&D center, 14F Gyunggi Biocenter, Iui Dong, Yeongtong-gu, Suwon, kyunggi-do, Korea. E-mail: youngminna@hotmail.com

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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.¹

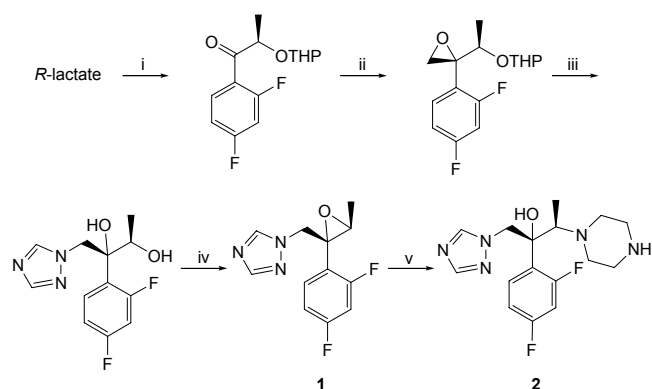
The azole antifungals are currently the most widely used and studied class of antifungal agents. Azole compounds prevent the synthesis of ergosterol, a major component of fungal plasma membranes, by inhibiting the cytochrome P-450 dependent enzyme lanosterol demethylase. Their antifungal efficacy is attributed to their greater affinity for fungal P-450_{DM} than for the mammalian enzyme. The azole 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).²

Hence, novel triazole compounds are prepared with different substituents on 2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl moiety.³ 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.⁴ This compound has a chiral center, and the final compounds could be controlled according to the configuration of oxirane. Thus, (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-(piperazin-1-yl)-1-(1*H*-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 hydroxy group was protected with tetrahydropyrane. The protected compound was reacted with phenylmagnesium bromide, followed by epoxydation (Corey's reagents) to afford the oxirane as a (2*R*,3*R*) and (2*R*,3*S*) 4:1 diastereomeric mixture. The desired (2*R*,3*R*)-isomer was isolated by crystallization. The

1,2,4-triazole group was introduced with opening 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.⁵ 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 (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-(piperazin-1-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (**2**). Reagents and conditions: (i) morpholine, reflux; 3,4-dihydro-2-pyran, pyridium-*p*-toluenesulfate, CH₂Cl₂, rt, 5 h, 50%; 1,3-difluorophenylmagnesium bromide, 20 °C → rt, overnight, 95%; (ii) TMSOI, CH₂Cl₂, 70 °C, overnight, 75%; (iii) 1,2,4-triazole, K₂CO₃, 100 °C, 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, CH₃CN, reflux, 50%



Scheme 2. Synthesis of indole derivatives

Table 1. *in vitro* antifungal activity of indole-conazole compounds **4a-y**

4	R ₁	MIC ₈₀ (μmol/mL) ^a						
		<i>C. albicans</i>				<i>C. krusei</i>	<i>C. neoformans</i>	<i>A. fumigatus</i>
		ATCC 64548	ATCC 36082	ATCC MYA-1003	ATCC 90028	ATCC 6258	ATCC 36556	ATCC 16424
a	H	1.07	1.07	274	0.54	34.3	> 274	137
b	4-F	0.52	0.52	132	0.52	33.04	> 529	132
c	4-Cl	0.50	0.50	63.88	0.50	31.94	> 511	63.88
d	4-tetrazole	59.86	59.86	239	59.86	> 239	> 239	> 239
e	5-F	0.52	0.52	66.04	0.26	16.51	> 264	33.02
f	5-Cl	0.25	0.25	63.88	0.25	7.98	> 256	31.94
g	5-Br	0.23	0.23	29.34	0.23	1.83	> 235	7.33
h	5-Me	0.52	0.26	66.59	0.26	4.16	> 266	66.59
i	5-NO ₂	0.24	0.24	62.56	0.24	3.91	> 250	7.82
j	5-MeO	0.5	0.5	129	0.5	8.06	> 258	32.22
k	5-N ₃	0.25	0.25	126	0.25	3.94	> 252	63.05
l	5-CN	4.07	2.03	260	2.03	65.10	> 260	65.10
m	5-CNOH	31.40	31.40	> 251	31.40	> 251	> 251	> 251
n	5-acrylic acid	119	119	> 239	119	> 239	> 239	> 239
o	6-F	0.52	0.26	264	0.52	16.51	> 264	33.02
p	6-Cl	0.25	0.25	31.94	0.25	1.2	> 256	1.2
q	6-N ₃	0.25	0.25	63.05	0.25	1.97	> 252	7.88
r	6-Me	0.26	0.26	66.59	0.26	4.16	> 266	16.65
s	6-CN	0.51	0.51	> 260	0.51	8.14	> 260	4.07
t	5,6-Cl	0.23	0.23	14.94	0.23	1.87	> 239	3.74
u	7-F	0.52	0.26	132	0.26	16.51	> 264	33.02
v	7-Cl	0.50	0.50	63.88	0.50	63.88	> 511	399
w	7-Me	0.26	0.26	66.59	0.26	4.16	> 266	16.65
x	7-CN	0.25	0.25	65.10	0.25	4.07	> 260	2.03
y	4,5,6,7-F	0.23	0.23	29.27	0.23	1.86	> 238	7.43
	Amphotericin B	0.54	0.54	0.54	0.54	1.08	> 69.26	2.16
	Fluconazole	0.82	0.82	> 418	0.41	104	> 418	418 - 836
	Itraconazole	0.18	0.18	> 181	0.18	0.18	> 363	0.18

^aValues are means of three experiments.

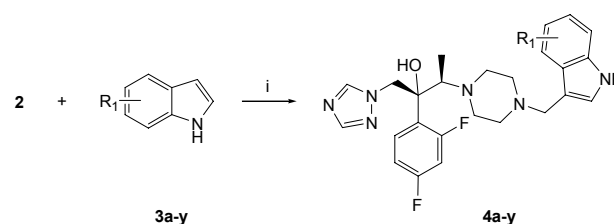
Indoles **3a-y** were on reaction with formaldehyde in the presence of AcOH in ethanol at 0 °C furnished (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-[4-(indol-3-ylmethyl) piperazin-1-yl]-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol **4a-y**.

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 MIC₈₀s 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.⁶

The MIC₈₀ 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 MIC₈₀ 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* (MIC₈₀ = 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.⁷

Pharmacomodulation aimed at evaluating the influence of (i) the nature and position of halogen group(s) fixed at the in-



Scheme 3. Synthesis of indole-conazole compounds. Reagents and conditions: i) HCHO, AcOH, ethanol, 0 °C → 30 - 40 °C, 12 h, 60 - 82%

dole ring and (ii) a diverse group(s) at C5 of indole.

SAR resulting from introducing of an halogen group (R_1) 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* and *C. krusei* were prominent. In contrast, these indole compounds did not show significant antifungal activity against *C. neoformans* as positive control exhibited poor activities.

Of the 11 halogenated indole derivatives, multi-halogenated compounds (**4t**, **4y**) (MIC₈₀ = 0.23 - 29.27 μ mol/mL against *C. albicans*, 1.87 and 1.86 μ mol/mL against *C. krusei*, 3.74 and 7.43 μ mol/mL against *A. fumigatus*, respectively) are effective against both *Candida* and mold species. Their broad antifungal spectra are comparable to that of amphotericin B, fluconazole and itraconazole. This result suggests that higher lipophilicity given by the halogens group may contribute to the antifungal activity against yeast fungi.

The halogen-moieties of substituents (R_1 :F, Cl, Br) on the 5-position of the indole ring appear to contribute partially to antifungal potency. However, replacing the halogen groups with a heteroatom on the 5-position of indole ring resulted in poor activities. These results indicated that bulky substituents deteriorated the antifungal activity of these triazole analogs against yeast fungi.

The cyano group at the 5, 6, 7-position of the indole ring also appear to contribute to antifungal activity. Especially, the promising results were obtained with compounds **4s**, **4x** (MIC = 0.25 - 0.51 μ mol/mL against *C. albicans* and 2.03 - 4.07 μ mol/mL against *A. fumigatus*) which have cyano group at the 6- and 7-position of the indole ring.

These data demonstrate that the position and the presence of halogen/cyano group on the indole ring increase the *in vitro* activity against fungi by comparison with other substituents.

In conclusion, a novel class of antifungal agents was synthesized by a short synthetic sequence and in good to excellent yields. Some of the indole derivatives exhibited good antifungal activity against *C. albicans* and two of the compounds (**4t**, **4y**) exhibited excellent anti-*Candida* activity. Analysis of the activity of this series of compounds against fungi shows a high selectivity of action against *C. albicans*. These compounds seem to be selective of the cytochrome P450-dependent 14 α -lanosterol demethylase (P-450_{DM}) of *C. albicans*. These observations will be very useful in designing newer molecules in the pursuit of more effective antifungal agents.

Experimental Section

All melting points were measured with Büchi melting point B-545 and are uncorrected. ¹H NMR spectra were recorded on a Bruker AC-250 instrument (250 MHz) using DMSO-*d*₆ or CDCl₃ with TMS. *R*-Lactate and other reagents were purchased from Aldrich Chemical Co.

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 (2). A mixture of (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-methyl-2-(1*H*-1,2,4-triazol-1-yl)methyloxirane (**1**) (2.0 g, 8.0 mmol), piperazine (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 C₁₆H₂₁F₂N₅O: 337.38).

Synthesis of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-[4-(5-fluoro-1*H*-indol-3-ylmethyl)-piperazin-1-yl]-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (4e). To a mixed solution of formaldehyde (144 μ L) and acetic acid (214 μ L) in ethanol was added (2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-(piperazin-1-yl)-1-(1*H*-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 (2*R*,3*R*)-**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: 484.52).

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 (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-[4-(5,6-dichloro-1*H*-indol-3-ylmethyl)-piperazin-1-yl]-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol (4t). yields : 66%; ¹H NMR (CDCl₃) δ 1.12 (d, 3H, CH₃), 2.21-2.67 (m, 9H, CH₂, OH), 3.31 (s, 1H, CH), 3.32 (d, 2H, CH₂), 4.01 (d, *J* = 14.0 Hz, 1H, CH₂), 4.34 (d, *J* = 14.0 Hz, 1H, CH₂), 6.66-6.74 (m, 3H), 7.07-7.11 (m, 2H), 8.22 (s, 1H), 8.24 (s, 1H), 10.1 (s, 1H); FT-IR (KBr): 3180, 2750, 1728, 1626, 894; FAB-MS *m/z* 534.1 (Calcd. for C₂₅H₂₆Cl₂F₂N₆O: 534.15).

Evaluation of *in vitro* antifungal activity. Seven strains of *Candida albicans* (ATCC64548, ATCC 26310, ATCC 90028, ATCC MYA-1003), *Cryptococcus neoformans* (ATCC 24065), *Candida krusei* (ATCC 6258), and *Aspergillus 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 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 $1 \times 10^3 - 5 \times 10^3$ 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 μ L of alamarblue (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

1. (a) Fridkin, S. K.; Jarvis, W. R. *Clin. Microbiol. Rev.* **1996**, *9*, 499. (b) Farowski, F.; Vehreschild, J. J.; Cornely, O. A. *Future Microbiol.* **2007**, *2*, 231.
2. Aoyama, Y.; Yoshida, Y.; Sato, R. *J. Biol. Chem.* **1984**, *259*, 1661.
3. (a) Sun, Q.; Xu, J.; Cao, Y.; Zhang, W.; Wu, Q.; Zhang, D.; Zhang, J.; Zhao, H.; Jiang, Y. *Eur. J. Med. Chem.* **2007**, *42*, 1226. (b) Uchida, T.; Kagoshima, Y.; Konosu, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2013.
4. (a) Singh, I. P.; Sidhu, I.; Palak, B. U.S. Patent 6,133,485,2000. (b) Konosu, T.; Miyaoka, T.; Tajima, Y.; Oida, S. *Chem. Pharm. Bull.* **1991**, *39*, 2241. (c) Tasaka, A.; Tamura, N.; Matsushita, Y.; Kitazaki, T.; Hayashi, R.; Okonogi, K.; Itoh, K. *Chem. Pharm. Bull.* **1995**, *43*, 441. (d) Tasaka, A.; Tamura, N.; Matsushita, Y.; Teranishi, K.; Hayashi, R.; Okonogi, K.; Itoh, K. *Chem. Pharm. Bull.* **1993**, *41*, 1035.
5. (a) Ezquerro, J.; Pedregal, C.; Lamas, C.; Barluenga, J.; Pérez, M.; García-Martín, M. A.; González, J. M. *J. Org. Chem.* **1996**, *61*, 5804. (b) Tsuji, Y.; Kotachi, S.; Huh, K. T.; Watanabe, Y. *J. Org. Chem.* **1990**, *55*, 580. (c) Fujita, K.; Yamamoto, K.; Yamaguchi, R. *Org. Lett.* **2002**, *4*, 2691. (d) Carbone, A.; Zamora, E. G.; Beugelmans, R.; Roussi, G. *Tetrahedron Lett.* **1998**, *39*, 4467.
6. (a) Galgiani, J.; Bartlett, M.; Ghannoum, M.; Espinel-Ingroff, A.; Lancaster, A.; Odds, F. *NCCLS* **1995**, *15*, 129. (b) Liao, R. S.; Robert, Rennie, P.; Talbot, J. A. *Antimicrob. Agents Chemother.* **2002**, *46*, 3236. (c) Davey, K. G.; Szekely, A.; Johnson, E. M.; Warnock, D. W. *Antimicrob. Agents Chemother.* **1998**, *42*, 439.
7. Mellado, E.; Garcia-Effron, G.; Alcázar-Fuoli, L.; Melchers, W. J. G.; Verweij, P. E.; Cuenca-Estrella, M.; Rodriguez-Tudela, J. L. *Antimicrob. Agents Chemother.* **2007**, *51*, 1897.