

Article

Synthesis and Antifungal Activity of Novel Triazole Compounds Containing Piperazine Moiety

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Abstract: Design and synthesis of triazole library antifungal agents having piperazine side chains, analogues to fluconazole were documented. The synthesis highlighted utilization of the click chemistry on the basis of the active site of the cytochrome P450 14 α -demethylase (CYP51). Their structures were characterized by ¹H-NMR, ¹³C-NMR, MS and IR. The influences of piperazine moiety on *in vitro* antifungal activities of all the target compounds were evaluated against eight human pathogenic fungi.

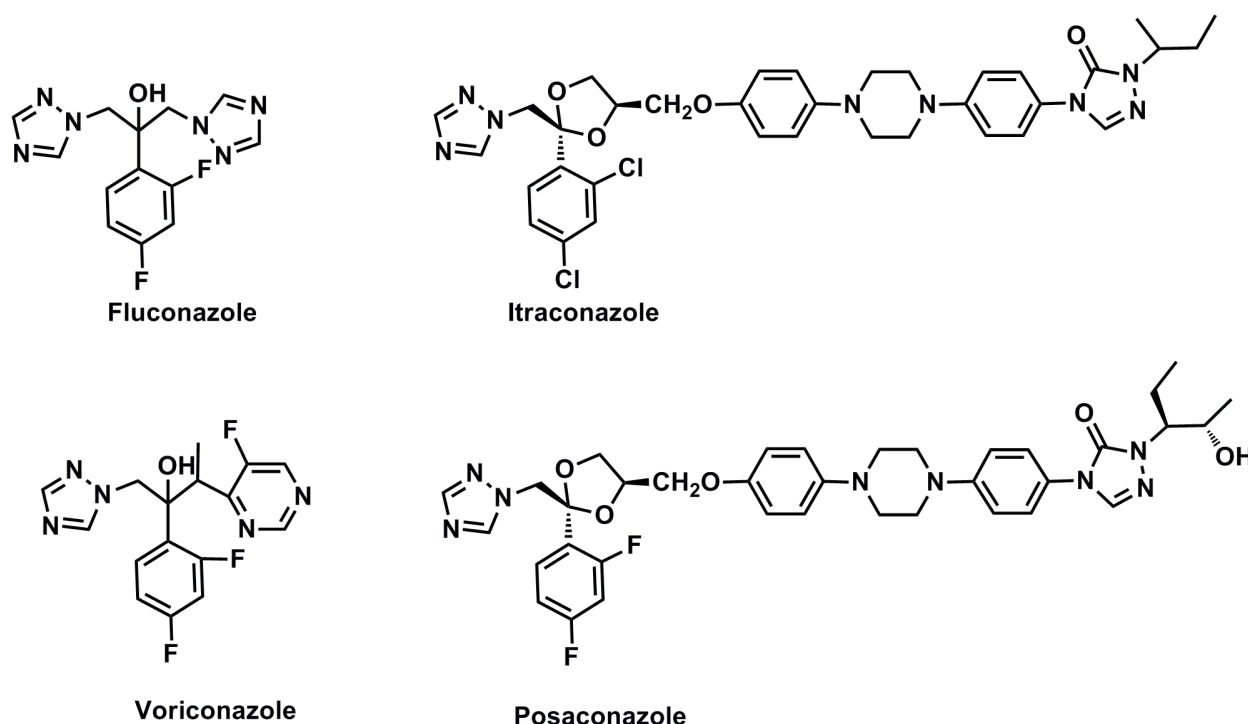
Keywords: antifungal; triazole; synthesis; piperazine

1. Introduction

Triazole compounds have been specially paid increasing attention because of their extensively medicinal applications in antimicrobial agent's particularly antifungal therapy, and a large number of

predominant triazole drugs have been successfully developed and prevalently used in the treatment of various microbial infections for many years [1]. Azoles (fluconazole, itraconazole, voriconazole, and posaconazole, Figure 1) are important antifungal drugs for the treatment of invasive fungal infections (IFIs), which continue to be a major cause of morbidity and mortality in immunocompromised or severely ill patients [2]. However, fluconazole is not effective against invasive aspergillosis and has suffered severe drug resistance [3,4]. The increasing frequency of fungal infections and development of resistance to the current treatment highlight the need for development of new triazole derivatives possessing broader antifungal spectra and higher therapeutic indexes.

Figure 1. Triazole antifungal agents used in clinical therapy.



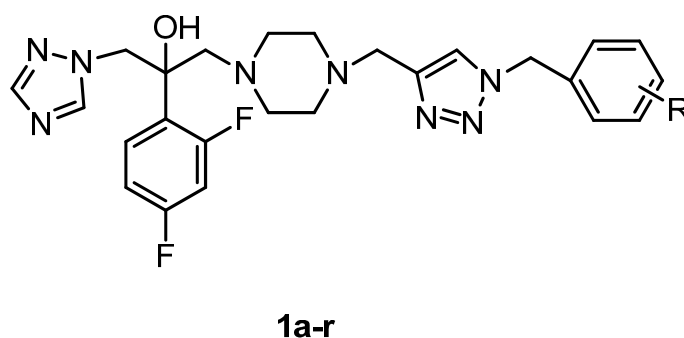
Azole antifungals act by competitive inhibition of CYP51, the enzyme that catalyzes the oxidative removal of the 14α -methyl group of lanosterol to give $\Delta^{14,15}$ -desaturated intermediates in ergosterol biosynthesis [5]. In general, the active site of CYP51 for ligand binding can be divided into four subsites: a coordination bond with iron of the heme group, the hydrophilic H-bonding region, the hydrophobic region, and the narrow hydrophobic cleft formed by the residues in the helix B'-meander 1 loop and N-terminus of helix I [6]. These compounds target the biosynthesis of ergosterol by inhibiting the cytochrome P450-dependent lanosterol 14α -demethylase (Erg11p, CYP51), encoded by the ERG11 gene, resulting in accumulation of toxic methylsterols in membranes that may culminate in fungi static effect or fungal death [7].

Literature precedents [8,9] revealed a pharmacophore of antifungal triazoles, which contained a triazole ring linking to a dihalophenyl ring through a two carbon chain. In addition, the carbon alpha to the phenyl ring bears a hydroxyl group. Itraconazole and posaconazole which containing the group of piperazine. We intended to alter the side chains to find potent systemic antifungal compounds with a broad antifungal spectrum and less potential to develop resistance. In our previous works [10–16],

many studies on the structure-activity relationships (SAR) of antifungal azoles have been developed, and these studies have led to new compounds endowed with better biological and pharmacological properties.

According to the above results, we designed a series of 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-substituted-2-propanols (**1a–r** Figure 2) containing a triazole ring, a difluorophenyl group, a hydroxyl group and a side chain containing piperazine group. In our design, we systematically altered the structure of fluconazole as a platform and tried to insert the 1,2,3-triazole group into the side chain.

Figure 2. Generic structure of the designed fluconazole analogues.



2. Results and Discussion

2.1. Chemistry

Target compounds **1a–r** were synthesized according to a very efficient and straightforward synthetic route outlined in Scheme 1. Compound **3** was synthesized by ring-open reaction of oxirane **2** with *N*-Boc-piperazine and simultaneous Boc-deprotection was accomplished by treatment with F₃CCOOH to furnish compound **4**. *N*-alkylation was effective in the presence of KI, K₂CO₃ and propargyl bromide in acetonitrile at room temperature to secure compound **5**. The target compounds were achieved by using click reaction [17] with variously substituted benzyl azides.

2.2. Pharmacology

The *in vitro* antifungal activities of all the target compounds were evaluated against eight human pathogenic fungi, *Candida albicans* 14053 (*C.alb.14053*), *Candida albicans* 20352 (*C.alb.20352*), *Candida parapsilosis* (*C. Par.*), *Cryptococcus neoformans* (*C.neo.*), *Candida glabrata* (*C. gla.*), *Aspergillus fumigates* (*A.fum.*), *Trichophyton rubrum* (*T.rub.*), *Microsporium gypseum* (*M. gyp.*), which are often encountered clinically, and were compared with itraconazole (ICZ), voriconazole (VCZ) and fluconazole (FCZ). All of the above eight human pathogenic fungi were provided by Shanghai Changzheng Hospital; Fluconazole (FCZ), itraconazole (ICZ) and voriconazole (VCZ) served as the positive control and were obtained from their respective manufacturers.

Scheme 1. Synthesis of the target compounds **1a–r**. Conditions: (a) Et₃N, *N*-Boc-piperazine, EtOH, reflux, 6 h, 80%; (b) CF₃COOH, room temperature, 1 h, 87%; (c) propargyl bromide, KI, K₂CO₃, CH₃CN, room temperature, 5–6 h, 72%; (d) NaN₃, substituted benzyl bromide, DMSO, CuSO₄·5H₂O, sodium ascorbate, rt, 12 h, 60%–70%.

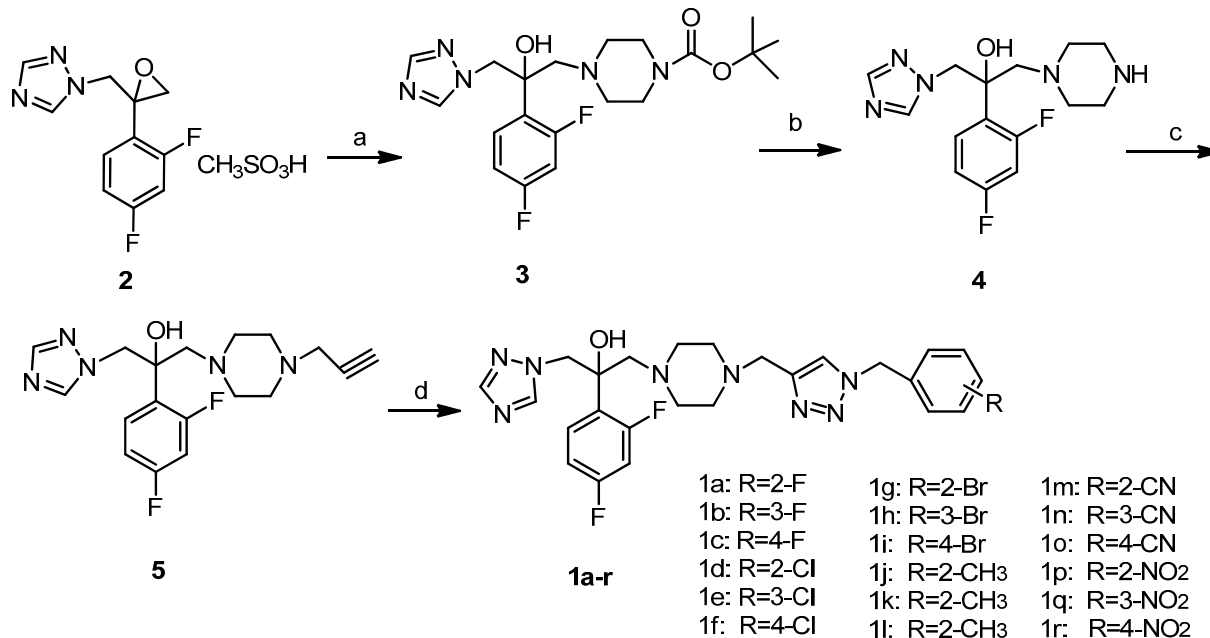


Table 1. Antifungal activities of the target compounds *in vitro* (MIC₈₀ µg/mL).

Compd	<i>C. alb.14053</i>	<i>C. alb. 20352</i>	<i>C. par.</i>	<i>C. neo.</i>	<i>C. gla.</i>	<i>A. fum.</i>	<i>T. rub.</i>	<i>M. gyp.</i>
1a	2	8	4	8	>64	>64	8	4
1b	1	4	4	4	4	>64	4	16
1c	1	2	8	2	4	>64	8	8
1d	0.25	0.5	1	1	16	>64	2	8
1e	1	1	1	1	16	>64	1	4
1f	0.5	0.5	1	1	>64	>64	1	1
1g	0.5	0.5	1	1	16	>64	1	0.5
1h	0.5	0.5	1	0.5	32	>64	1	0.5
1i	0.25	0.5	0.5	0.5	>64	>64	1	0.5
1j	1	2	4	4	>64	>64	4	0.25
1k	64	64	4	4	>64	>64	4	0.25
1l	1	4	4	0.5	64	>64	1	0.25
1m	4	16	16	16	>64	>64	16	16
1n	8	8	16	8	>64	>64	16	4
1o	8	8	16	8	>64	>64	16	2
1p	2	4	8	4	>64	>64	4	2
1q	1	8	8	8	>64	>64	4	2
1r	2	8	8	4	64	>64	4	0.25
ICZ	0.0625	0.0625	0.03125	0.0156	0.5	2	0.125	4
VCZ	0.03125	0.0625	0.03125	0.0156	0.5	0.25	0.0625	0.25
FCZ	1	0.5	0.5	0.5	2	>64	4	64

Abbreviations: *C. alb.14053*, *Candida albicans14053*; *C. alb.20352*, *Candida albicans20352*; *C. par.*, *Candida parapsilosis*; *C. neo.*, *Cryptococcus neoformans*; *C. gla.*, *Candida glabrata*; *A. fum.*, *Aspergillusfumigates*; *T. rub.*, *Trichophytonrubrum*; *M. gyp.*, *Microsporium gypseum*. ICZ, Itraconazole; VCZ, Voriconazole; FCZ, Fluconazole.

The *in vitro* minimal inhibitory concentrations (MICs) of the compounds were determined by the micro-broth dilution method in 96-well microtest plates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS) [18]. The MIC₈₀ was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well. For assays, the title compounds to be tested were dissolved in dimethyl sulfoxide (DMSO), serially diluted in growth medium, inoculated and incubated at 35 °C. Growth MIC was determined at 24 h for *C. alb.* and at 72 h for *C. neo.* The *in vitro* antifungal activities are summarized in Table 1. The MIC values (in µg/mL) are presented against different pathogenic fungi, in comparison with ICZ, VCZ and FCZ.

The results of antifungal activities *in vitro* showed that all the 18 target compounds (**1a–r**) were active against nearly all fungi tested to some extent except against *A. fum.* and *C. gla.* The MIC₈₀ value of compound **1d** and **1i** is 4 times lower than that of FCZ against *C. alb. 14053 in vitro* (with the MIC₈₀ value of 0.25 µg/mL). The MIC₈₀ value of compound **1j**, **1k**, **1l** and **1r** is 128 times lower than that of FCZ against *M. gyp. in vitro*, and the same as VCZ against *M. gyp. in vitro* (with the MIC₈₀ value of 0.25 µg/mL). However, most of the target compounds' antifungal activities were not as good as the ICZ and VCZ.

3. Experimental Section

3.1. General Procedures

¹H and spectra were recorded in CDCl₃ unless otherwise indicated with a Bruker AC-300P spectrometer, using TMS as internal standard. ESI mass spectra were performed on an API-3000 LC-MS spectrometer. The solvents and reagents were used as received or dried prior to use as needed.

Tert-butyl-4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)piperazine-1-carboxylate (3): A mixture of compound **2** (16.65 g, 0.05 mol), CH₃CH₂OH (300 mL) and Et₃N (25 mL), *N*-Boc-piperazine (13.96 g, 0.075 mol) was stirred and refluxed for 6 h. The reaction was monitored by TLC. After filtration, the filtrate was evaporated under reduced pressure. Water was added to the residue, extracted with ethylacetate twice, combine the organic layer, washed with saturated NaHCO₃ and NaCl solution twice, dried over anhydrous Na₂SO₄ and evaporated to get compound **3** (16.93 g, 80%).

1-(1H-1,2,4-Triazole-1-yl)-2-(2,4-difluorophenyl)-3-(piperazin-1-yl)-2-propanol (4): A mixture of compound **3** (4.23 g, 0.01 mol), Trifluoroacetic acid (11.4 g, 0.1 mol) was stirred at room temperature for 30 min. The reaction was monitored by TLC. After reaction, most of the trifluoroacetic acid was removed in a vacuum. Column chromatography of the residue afforded compound **4** as yellow oil (DCM/CH₃OH, 20:1–10:1, 2.81 g, 87%).

1-(1H-1,2,4-Triazole-1-yl)-2-(2,4-difluorophenyl)-3-(4-(prop-2-yn-1-yl)piperazin-1-yl)-2-propanol (5): A mixture of compound **4** (3.23 g, 0.01 mol), propargyl bromide (2.36 g, 0.02 mol), KI (166 mg, 0.001 mol), K₂CO₃ (3.45 g, 0.025 mol), and CH₃CN (50 mL) was stirred at room temperature for 6 h. The reaction was monitored by TLC. After reaction, we filtrated off the solid and washed with CH₃CN. The filtrate was concentrated in a vacuum. Column chromatography of the residue afforded compound **5** as a brown oil (DCM/CH₃OH, 20:1–10:1, 2.60 g, 72%).

3.2. General Procedure for the Preparation of the Compounds **1a–r**

A mixture of NaN₃ (100 mg, 1.4 mmol), 2-fluorobenzyl bromide (200 mg, 1.2 mmol) and DMSO (15 mL) was stirred at room temperature for 6 h. Then the compound **5** (217 mg, 0.6 mmol) was added, sodium ascorbate (20 mg), CuSO₄·5H₂O (25 mg) and H₂O (1 mL) was added, then stirred at room temperature for another 2 h. The reaction was monitored by TLC, then NH₃·H₂O was added carefully and extracted with ethylacetate. The organic layer was acidificated with dilute hydrochloric acid, then the aqueous layer was adjusted pH about 7.0 by saturation sodium bicarbonate, then extracted with ethylacetate, washed with water, NaHCO₃ and NaCl, and dried with Na₂SO₄. It was then concentrated in a vacuum. Column chromatography of the residue afforded compound **1a** as yellow oil. (212 mg, 69%). ¹H-NMR (300 MHz, CDCl₃) δ: 8.17 (s, 1H), 7.81 (s, 1H), 7.58–7.55 (m, 2H), 7.46–7.34 (m, 2H), 7.32–7.27 (m, 1H), 7.19–7.09 (m, 1H), 6.85–6.78 (m, 2H), 5.58 (s, 2H), 4.53 (s, 2H), 3.62 (s, 2H), 3.10–2.63 (m, 2H), 2.41–2.27 (m, 8H); ¹³C NMR (75 MHz, CDCl₃) δ: 163.7, 158.7, 151.3, 147.5, 144.1, 139.1, 132.5, 130.1, 129.9, 129.8, 129.7, 127.7, 124.3, 123.7, 119.1, 118.5, 114.1, 111.4, 104.1, 71.7, 62.1, 56.3, 54.1, 53.1, 51.3; IR(KBr): 3380, 2923, 2821, 2100, 1615, 1498, 1458, 1273, 1137, 1048, 1015, 966, 852, 675, 616, 515 cm⁻¹; ESI-MS, *m/z* calcd. for C₂₅H₂₇F₃N₈O 512.2, found [M+H]⁺ 513.5.

The target compounds **1b–r** were synthesized by the same operation procedure of the compound **1a**.

4. Conclusions

In summary, a novel series of antifungal agents have been designed and synthesized by chemical methods. *In vitro* antifungal activity assay indicated that most of the compounds showed antifungal activities against both systemic pathogenic fungi. Several compounds show high *in vitro* antifungal activity with a broad spectrum, which were valuable for further evaluation.

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Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/19/8/11333/s1>.

Author Contributions

Yanwei Wang and Kehan Xu contributed equally to this work.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds are available from the authors.

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