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Synthesis and antifungal activity of the novel triazole compounds†

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Shichong Yu,‡[a] Xiaoyun Chai,‡[a] Nan Wang,[ab] Hong Cui,[a] Qingjie Zhao,[a] Honggang Hu,[a] Yan Zou,[a] Qingyan Sun*[a] and Qiuye Wu*[a]

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A series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-substituted-2-propanols (**1a-o**) which are analogues of fluconazole, have been designed and synthesized for the first time by the click reaction on the basis of computational docking experiments to the active site of the cytochrome P450 14α-demethylase (CYP51). Their structures were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. The *in vitro* antifungal activities of all the target compounds were evaluated against eight human pathogenic fungi.

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Introduction

Fungal infections represent a serious problem for patients with immune systems compromised either by HIV infection or administration of immunosuppressive drugs during cancer therapy and organ transplantation.[1,2] Many microbes, including fungi those were previously thought to be nonpathogenic, have emerged as significant pathogens in immunosuppressed populations. Clinically, *Candidosis, Aspergillosis* and *Cryptococcosis* are three major fungal infections in immunocompromized patients.[3,4] Currently, triazole agents (fluconazole (FCZ), itraconazole (ICZ), voriconazole (VCZ) and posaconazole, Fig. 1) are the most frequently used antifungals in clinic.[5] However, FCZ is not effective against invasive *Aspergillosis* and has suffered severe drug resistance.[6,7] This has led to an interest to develop new triazole derivatives possessing broader antifungal spectra and higher therapeutic indexes for pathogens that affect patients with impaired immunity.

Azole antifungals act by competitive inhibition of CYP51, the enzyme that catalyzes the oxidative removal of the 14a-methyl group of lanosterol to give $\triangle^{14,15}$-desaturated intermediates in ergosterol biosynthesis.[8] 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.[9]

Some studies[10,11] had revealed a pharmacophore of antifungal triazoles, which contains a triazole ring linked to a dihalophenyl ring through a two carbon chain. In addition, the carbon alpha to the phenyl ring bears a hydroxyl group. Moreover the side chain located in the narrow hydrophobic cleft was also very important.[10] We intended to alter the side chain to find potent systemic antifungal compounds with a broad antifungal spectrum and less potential to develop resistance.

According to the above characteristics of target enzyme CYP51 and the previous research results,[12–17] we here designed a new series of 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4- difluorophenyl)-3-substituted-2-propanols (**1** Fig. 2) containing a triazole ring, a difluorophenyl group, a hydroxyl group and a side chain. In our design, We systematically altered the structure of FCZ as a platform and tried to insert a 1,2,3-triazole group into the side chain for the first time.

Compounds **1a-o** were synthesized according to a very efficient and straightforward synthetic route outlined in Scheme 1. After the key intermediate oxirane **5** was synthesized by a known procedure,[18] compound **6** was synthesized by ring-opening reaction of oxirane **5** with methylamine and then in the presence of KI and K_2CO_3 in acetonitrile at room temperature to obtain compound **7**. The target compounds were obtained for the first time using a click reaction[19] with various substituted benzyl azides.

The results of antifungal activities *in vitro* showed that all the 15 target compounds (**1a-o**) were active against nearly all fungi tested to some extent except against *Aspergillus fumigatus (A. fum.).* Most of the target compounds exhibited higher activities against *Candida albicans SC5314 (C. alb SC5314)* and *Candida albicans Y0109 (C. alb Y0109)* than all six positive controls. The MIC_{80} value of compound **1k** is 32 times lower than that of FCZ against *C. alb. SC5314 in vitro* (with the MIC_{80} value of 0.0156 μg mL^{-1}), and 64 times lower than that of FCZ against *Candida kefyr (C. kef.)* (with the MIC_{80} value of 0.0156 μg mL^{-1}). The MIC_{80} value of compound **1o** is 8 times lower than

[a]*Department of Organic Chemistry, College of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, People's Republic of China. E-mail: wuqy6439@sohu.com; sqy_2000@163.com; Fax: +86 21 81871225; Tel: +86 21 81871225*

[b]*College of Pharmacy, Yantai University, Qingquan Road 30, Yantai 264005, People's Republic of China*

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‡ Shichong Yu and Xiaoyun Chai contributed equally to this work.

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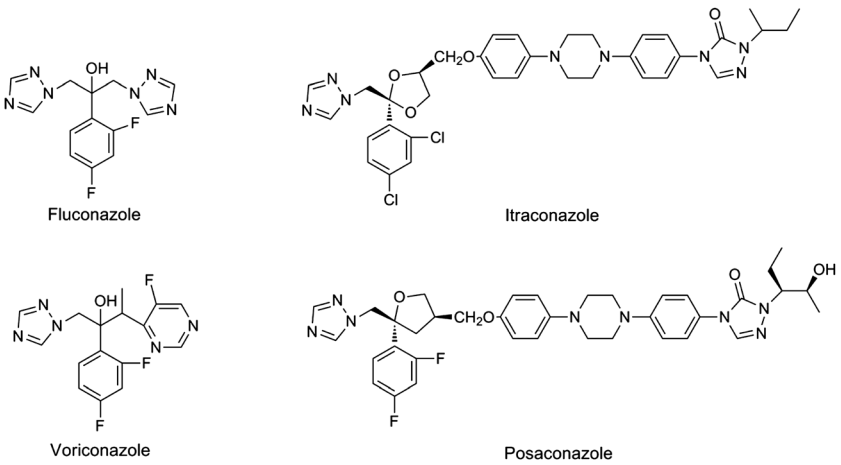


Fig. 1 Triazole antifungal agents used in clinical therapy.

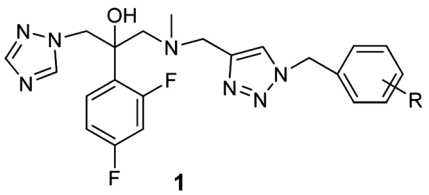
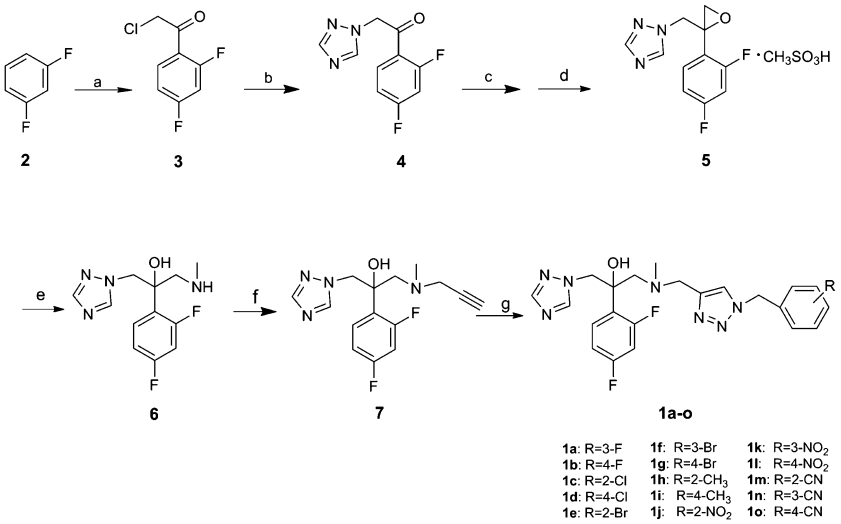


Fig. 2 Generic structure of the designed fluconazole analogues.

that of FCZ against *C. alb. SC5314 in vitro* (with the MIC_{80} value of 0.0625 μg mL^{-1}), and 16 times lower than that of FCZ against *C. kef. in vitro* (with the MIC_{80} value of 0.0625 μg mL^{-1}). and 32 times lower than that of FCZ against *Trichophyton rubrum (T. rub.) in vitro* (with the MIC_{80} value of 0.0625 μg mL^{-1}). The MIC_{80} value of compound **1o** is the same as ICZ against all 8 fungi test except *A. fum.* Compounds **1k** and **1o** are worthy of further study, and are expected to be developed into new antifungal drugs.

To explain the results, we proposed a likely binding mode for **1k** to the active site of CYP51 based on computational docking results (Fig. 3). As usual, the triazole interacts with iron of the heme group, while the 2,4-difluorophenyl group in the designed compound could be placed into the hydrophobic pocket formed by Leu121, Phe126, Phe228, Met306, Gly307 and Gly308. The 3-nitrobenzyl of the side chain would generate π–π stacking interactions with the Phe380. The side chain incorporated to adjust the overall physical-chemical properties of the molecules and orientation of aromatic rings. It would also be oriented to interact with a hydrophobic pocket formed by Gly65, Met92, Ala117, Tyr118, Pro375, Leu376, His377, Ser378, Ile379 and Met508.

In addition, the side chains were the pharmacophores, and the spatial orientations of the pharmacophores were just



Scheme 1 Synthesis of the target compounds **1a-o** *Conditions*: (a) $ClCH_2COCl$, $AlCl_3$, 50 °C, 5 h, 80%; (b) 1*H*-1,2,4-triazole, $NaHCO_3$, toluene, reflux, 5 h, 42%; (c) $(CH_3)_3SOI$, NaOH, cetylmethylammonium bromide, toluene, 60 °C, 3 h, 53%; (d) CH_3SO_3H, 0 °C, 1 h, 89%; (e) Et_3N, methylamine, EtOH, reflux, 6 h, 80%; (f) propargyl bromide, KI, K_2CO_3, CH_3CN, rt, 5–6 h, 70%; (g) NaN_3, substituted benzyl bromide, DMSO, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, rt, 12 h, 60–70%.

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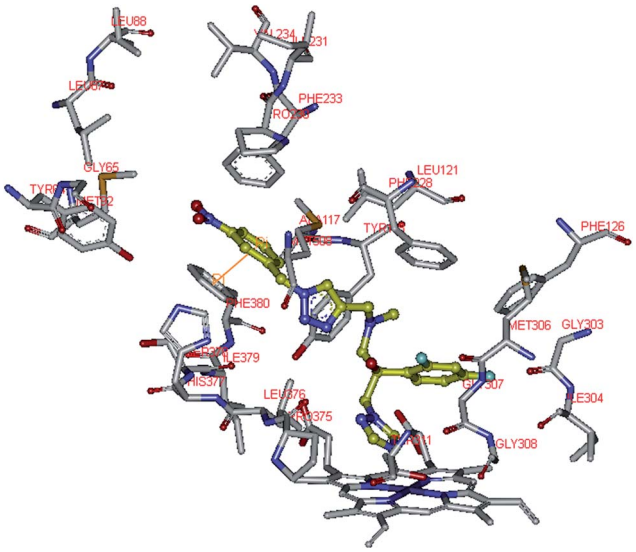

Fig. 3 Computed binding geometry of the new inhibitor **1k** in the active site of CYP51.

oriented in the hydrophobic pocket. They played a role in adjusting the physico-chemical properties of the whole molecule to avoid some dissatisfying side effects and improve their pharmacokinetic and pharmacodynamic behavior.

In conclusion, an efficient method using the click reaction has been developed for the synthesis of a diverse series of novel triazole derivatives. All the target compounds are reported for the first time. Results of preliminary antifungal tests against eight human pathogenic fungi *in vitro* showed that these analogs exhibited excellent activities with broad spectrum. The obtained results indicated that for antifungal activity of these novel triazole derivatives it is very helpful to introduce the 1,2,3-triazole group and the substituted benzyl as side chains. The research has led to the discovery of a series of compounds for further optimization.

Experimental

Pharmacology

The *in vitro* antifungal activities of all the target compounds were evaluated against eight human pathogenic fungi, *C. alb.SC5314, Cryptococcus neoformans (C. neo.), Candida parapsilosis (C. par.), C. alb.Y0109, Candida tropicalis (C. tro.), T. rub., C. kef., A. fum.*, which are often encountered clinically, and were compared with ICZ, terbinafine (TRB), ketoconazole (KCZ), amphotericin B (AMB), VCZ and FCZ. *C. alb. SC5314* and *C. neo.* were provided by Shanghai Changzheng Hospital; *C. par., C. alb. Y0109, C. tro., T. rub., C. kef. and A. fum.* were provided by Shanghai Changhai Hospital. *C. alb. SC5314* and *C. neo.* were purchased from ATCC, and other strains were clinic isolates. FCZ, ICZ, KCZ, VCZ, AMB and TRB served as the positive control were obtained from their respective manufacturers.

The *in vitro* minimal inhibitory concentrations (MICs) of the compounds were determined by the micro-broth dilution method in 96-well microtestplates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[20] The MIC_{80} 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 results of assays are summarized in Table 1. The data points form the mean of replicates. All of our susceptibility tests were performed three times by each antifungal agent.

Molecular docking

In our studies, we constructed a 3D model of *Candida albicans* CYP51 on the basis of Ji *et al.*[21] All the molecular modeling calculations were performed using SYBYL 6.9 version. And the structures of the compounds were assigned with Gasteiger-Hückle partial atomic charges. Energy minimization was performed using the Tripos force field, Powell optimization method, and MAXIMIN2 minimizer with a convergence criterion 0.001 kcal mol^{-1} Å. Simulated annealing was then performed. The system was heated to 1000 K for 1.0 ps and then annealed to 250 K for 1.5 ps. The annealing function was exponential; 50 such cycles of annealing were run and the resulting 50 conformers were optimized using methods described above. The lowest energy conformation was selected. All the other parameters were default value.

General procedures

Melting points were measured on a Yamato MP-21 melting-point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ unless otherwise indicated with a Bruker AC- 300P spectrometer or a Bruker Avance II 600 spectrometer, using TMS as internal standard. High resolution electron spray ionization mass spectra (HR ESI MS) were performed on an Agilent 6538 Q-TOF mass spectrometer. The solvents and reagents were used as received or dried prior to use as needed.

1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-methylamino)-2-propanol (6)

A mixture of compound **5** (33.3 g, 0.10 mol), CH_3CH_2OH (500 mL) and Et_3N (50 mL), methylamine (4.7 g, 0.15 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 ethyl acetate twice, the organic layers were combined, washed with saturated NaCl solution twice, dried over anhydrous Na_2SO_4 and evaporated to get compound **6** (ref. 22) (21.0 g, 79%).

1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-methyl-*N*-propargyl amino)-2- propanol (7)

A mixture of compound **6** (2.68 g, 0.01 mol), propargyl bromide (2.36 g, 0.02 mol), KI (166 mg, 0.001 mol), K_2CO_3 (3.45 g, 0.025 mol), and CH_3CN (100 mL) was stirred at room temperature for 6 h. The reaction was monitored by TLC. After reaction, filtrated

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Table 1 Antifungal activities of the target compounds *in vitro* (MIC_{80}, μg mL^{-1})

Compd	R	*C. alb SC5314*	*C. alb Y0109*	*C. kef*	*C. neo*	*T. rub*	*C. tro*	*C. par*	*A. fum*
1a	3 F	0.25	0.25	64	2	16	0.5	0.5	>64
1b	4 F	1	0.5	16	1	4	0.25	0.25	>64
1c	2-Cl	0.25	0.25	16	0.25	4	1	0.25	>64
1d	4-Cl	0.25	<0.125	0.25	1	4	1	0.25	>64
1e	2-Br	0.25	0.25	0.0625	0.25	0.25	0.25	0.25	>64
1f	3-Br	0.25	<0.125	0.25	0.25	1	0.25	0.0625	>64
1g	4-Br	0.25	<0.125	1	1	4	0.25	0.25	>64
1h	2-CH_3	0.25	0.25	0.25	0.25	4	0.25	0.0625	>64
1i	4-CH_3	0.25	<0.125	0.25	1	1	1	0.0625	>64
1j	2-NO_2	0.25	<0.125	0.25	1	4	1	0.25	>64
1k	3-NO_2	0.0156	<0.125	0.0156	0.25	0.0625	0.0625	0.0625	>64
1l	4-NO_2	0.25	0.25	0.25	0.25	1	0.25	0.25	>64
1m	2-CN	0.25	<0.125	0.25	0.25	1	0.25	0.0625	>64
1n	3-CN	1	0.5	1	4	1	1	0.25	>64
1o	4-CN	0.0625	<0.0125	0.0625	0.25	0.0625	0.0625	0.0156	>64
ICZ	—	<0.0625	0.0625	0.0625	0.125	0.0625	<0.0625	0.0625	2
TBR	—	16	2	0.0625	8	<0.125	<0.125	<0.125	0.25
KCZ	—	<0.125	<0.125	0.0625	0.5	<0.125	<0.125	<0.125	0.125
AMB	—	8	4	0.25	4	0.125	0.25	1	32
VCZ	—	32	<0.125	0.0039	<0.125	<0.125	<0.125	0.25	<0.125
FCZ	—	0.5	0.5	1	8	2	<0.125	<0.125	>64

off the solid, washed with CH_3CN, the filtrate was concentrated in a vacuum. Column chromatography of the residue afforded compound **7** as a brown oil (1.9 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.13 (1H, s, triazole-H), 7.78 (1H, s, triazole-H), 7.58-7.50 (1H, m, Ar-H), 6.84-6.74 (2H, m, Ar-H), 4.54 (2H, s, CH_2), 3.22-3.07 (2H, m, triazole-CH_2), 2.73 (1H, d, J = 12.0 Hz, CH_2), 2.21-2.19 (2H, m, CH_2), 2.17 (3H, s, NCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 152.9, 146.5, 131.4, 113.4, 106.1, 79.8, 75.3, 74.5, 62.0, 58.0, 49.1, 45.4; HR ESI MS: calcd. for $C_{15}H_{17}F_2N_4O$ $[M + H]^+$ *m/z*: 307.1365; found: 307.1362.

General procedure for the preparation of the compounds 1a–o

A mixture of NaN_3 (100 mg, 1.4 mmol), 3-fluorobenzyl bromide (200 mg, 1.2 mmol) and DMSO (15 mL) was stirred at room temperature for 6 h. Then was added the compound **7** (184 mg, 0.6 mmol), sodium ascorbate (20 mg), $CuSO_4 \cdot 5H_2O$ (25 mg), H_2O (1 mL), was stirred at room temperature for 2 h, then put the reaction solution into $NH_3 \cdot H_2O$, extracted with ethyl-acetate, the organic layer was acidificated with dilute hydrochloric acid, then the aqueous layer was adjusted pH about 7.0 by saturation sodium bicarbonate, extracted with ethyl acetate, washed with water, dried with Na_2SO_4. concentrated in a vacuum to afford compound **1a** (186 mg, 68%). White powder, Mp: 86.6–88.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10 (1H, s, triazole-H), 7.77 (1H, s, triazole-H), 7.54-7.62 (1H, m, Ar-H), 7.33-7.40 (1H, m, Ar-H), 7.14 (1H, s, triazole-H), 6.79-7.10 (3H, m, Ar-H), 6.73-6.78 (2H, m, Ar-H), 5.54 (2H, s, Ar–CH_2–), 4.53 (1H, d, J = 13.8 Hz, triazole-CH_2), 4.42 (1H, d, J = 13.8 Hz, triazole-CH_2), 3.65 (1H, d, J = 13.8 Hz, triazole-CH_2), 3.56 (1H, d, J = 14.4 Hz, triazole-CH_2), 3.03 (1H, d, J = 13.8 Hz, CH_2), 2.72 (1H, d, J = 13.5 Hz, CH_2), 2.13 (3H, s, NCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.7, 159.2, 150.8, 144.6, 137.0, 130.7, 129.6, 126.0, 123.5, 122.2, 115.9, 115.7, 115.1, 114.8, 111.5, 104.1, 72.6, 60.3, 56.1, 56.0, 53.4, 44.1; HR ESI MS: calcd. for $C_{22}H_{23}F_3N_7O$ $[M + H]^+$ *m/z*: 458.1911; found: 458.1918.

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

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