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Molecular docking, design, synthesis and antifungal activity study of novel triazole derivatives containing the 1,2,3-triazole group†

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A series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-[*N*-propyl-*N*-((1-substituted-1*H*-1,2,3-triazol-4-yl)methyl)amino]-2-propanols which are analogues of fluconazole, have been designed and synthesized 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 HR ESI MS. The MIC_{80} values indicate that the target compounds **1a–o** showed higher activities against nearly all the fungi tested to some extent except against *A. fum.* and *T. rub.* All of the target compounds exhibited higher activities against *C. alb.* SC5314 and *C. alb.* Y0109 than all of the six positive controls.

Introduction

During the past several decades, the number of invasive deep fungal infections has continued to increase, particularly in immunocompromised individuals such as patients undergoing organ transplantation, anticancer chemotherapy, or those suffering from AIDS.[1,2] Three major fungal infections, including candidosis, aspergillosis and cryptococcosis, are responsible for clinical infections in immunocompromised patients.[3,4] Currently, triazole agents (fluconazole (FCZ), itraconazole (ICZ), voriconazole (VCZ), and posaconazole) are the most frequently used antifungals in clinical use Fig. 1.[5] However, FCZ is not effective against invasive aspergillosis and has suffered from severe drug resistance.[6,7] This situation, thus, highlights the need for new triazole derivatives possessing broader antifungal spectra and higher therapeutic indexes.

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 the ergosterol biosynthesis.[8] In general, the active site of CYP51 for ligand binding can be divided into four subsites: a coordination bond with the iron in 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 the N-terminus of helix I.[9]

Some studies[10,11] have 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. We intended to alter the side chains to find potent systemic antifungal compounds with a broad antifungal spectrum and less potential to develop resistance.

To design the new antifungal compounds, firstly we did a molecular docking investigation, we constructed a 3D *Candida*

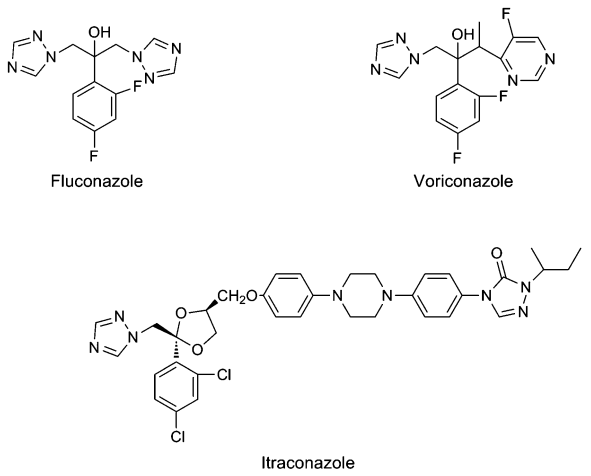

Fig. 1 Triazole antifungal agents used in clinical therapy.

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† Electronic supplementary information (ESI) available: ^{1}H NMR, ^{13}C NMR and HR ESI MS spectral data of compounds **1b–o**. See DOI: 10.1039/c3ra41310a

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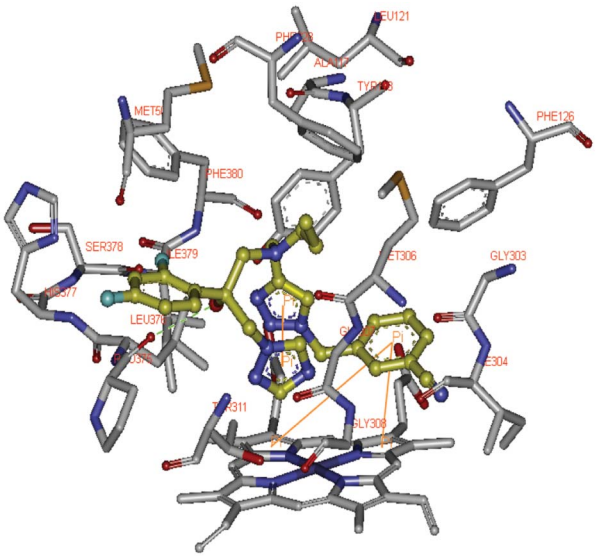

Fig. 2 Computed compound **1i** binding to the heme iron atom at the active site of CYP51.

albicans CYP51 on the basis of work by Ji *et al.*[12] A likely binding mode of compound **1i** in the active site of CYP51 is proposed based on computational docking results (Fig. 2). Sheng *et al.*[10] reported that both the *R* and *S* isomers of the compounds interacted with CYP51 through a similar binding mode. However, the *R* isomer had a lower interaction energy with CYP51 than the *S* isomer, which indicated that the *R* isomer might have better antifungal activity than the *S* isomer. In our discussion, the docked conformations refer to the *R* configuration of the compounds. As usual, the N(4) atom of the 1,2,4-triazole moiety of **1i** coordinates with the heme Fe-atom, while the 2,4-difluorophenyl group in the designed compound could be placed into the hydrophobic pocket formed by Pro375, Leu376, His377, Ser378, Ile379 and Phe380. The 1,2,3-triazole group in the side chain would generate π–π stacking interactions with Tyr118. Finally, the substituted benzyl could interact with the hydrophobic pocket formed by Gly303, Met306, Gly307, Gly308 and Tyr311. In addition, the side chains were the pharmacophores, and they were oriented in the hydrophobic pocket. The side chains of the inhibitors were found to be very important.

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 (**1**, Fig. 3) containing a triazole ring, a difluorophenyl group, a hydroxyl group and a side chain. In our design, we systematically altered the structure of the fluconazole platform and inserted a 1,2,3-triazole group into the side chain.

Results and discussion

Chemistry

The target compounds **1a–o** were synthsized according to the very efficient and straightforward synthetic route outlined in

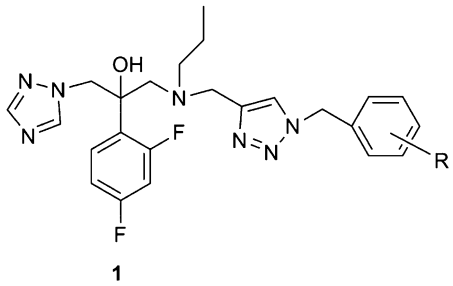

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Fig. 3 Generic structure of the designed fluconazole analogues.

Scheme 1. After the key intermediate oxirane **5** was synthesized by a known procedure,[13] compound **6** was synthesized by the ring-opening reaction of the oxirane **5** with propylamine, and then in the presence of KI and K_2CO_3 in acetonitrile at room temperature compound **7** was prepared. The target compounds were obtained by using a click reaction[14] with various substituted benzyl derivatives.

Pharmacology

The *in vitro* antifungal activities of all the target compounds were evaluated against eight human pathogenic fungi, *Candida albicans* SC5314 (*C. alb.* SC5314), *Cryptococcus neoformans* (*C. neo.*), *Candida parapsilosis* (*C. par.*), *Candida albicans* Y0109 (*C. alb.* Y0109), *Candida tropicalis* (*C. tro.*), *Trichophyton rubrum* (*T. rub.*), *Candida krusei* (*C. kru.*), *Aspergillus fumigatus* (*A. fum.*), which are often encountered clinically, and they were compared with itraconazole (ICZ), voriconazole (VCZ) and fluconazole (FCZ). *C. alb.* SC5314 and *C. neo.* were provided by Shanghai Changzheng Hospital; *C. par.*, *C. alb.* Y0109, *C. tro.*, *T. rub.*, *C. Kru.* 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. Fluconazole (FCZ), itraconazole (ICZ) and voriconazole (VCZ) served as the positive controls and 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 microtest plates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[15] The MIC_{80} was defined as the first well with an approximately 80% reduction in growth compared to the growth in 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. The growth MIC was determined at 24 h for *C. alb.* and at 72 h for *C. neo*. The results of the assays are summarized in Table 1. The data points are the mean of the replicates. All of our susceptibility tests were performed three times with each of the antifungal agents.

The *in vitro* antifungal activities are summarized in Table 1. The MIC values (in μg mL^{-1}) are presented against different pathogenic fungi, in comparison with ICZ, VCZ and FCZ.

The results of the antifungal activities *in vitro* showed that all of the target compounds were active against nearly all of the fungi tested to some extent except against *A. fum.* and *T. rub*.

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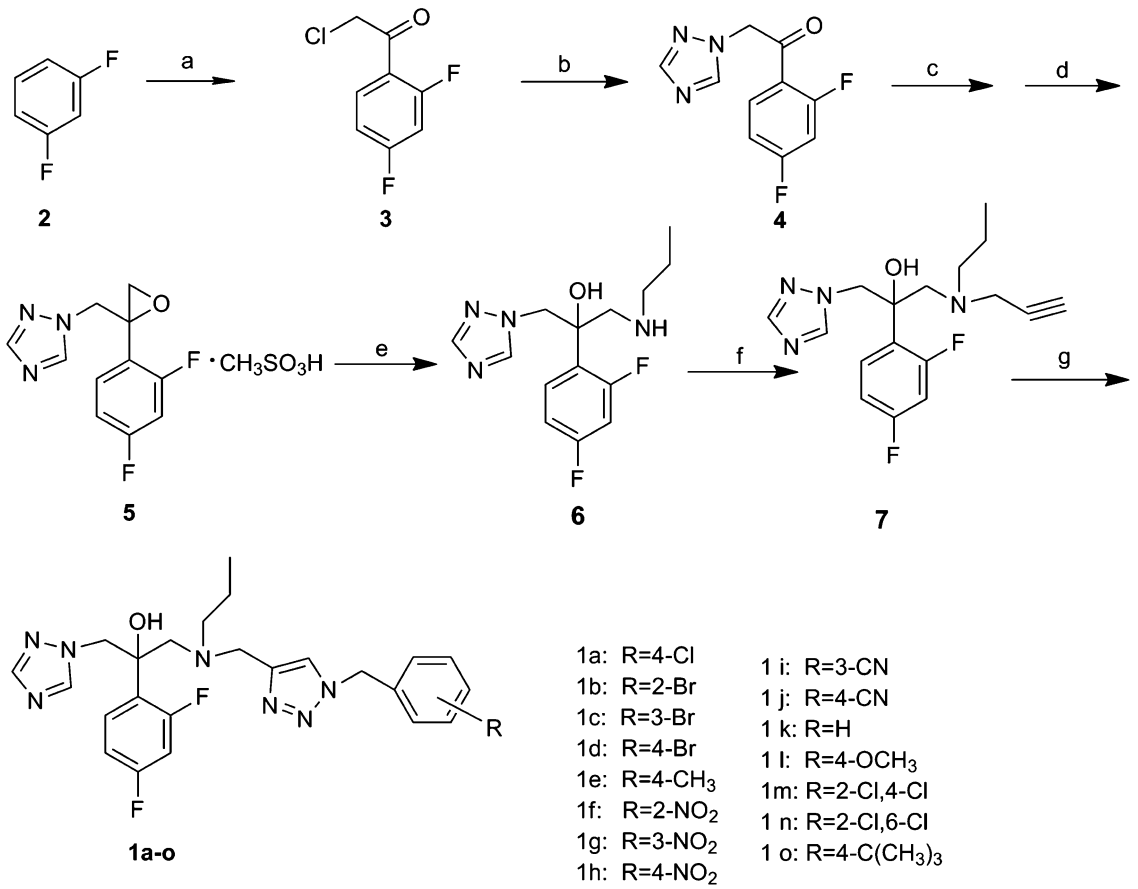

Scheme 1 Synthesis of the target compounds **1a–o**. Conditions: (a) $ClCH_2COCl$, $AlCl_3$, 50 °C, 5 h, in 80% yield; (b) $C_6H_5CH_3$, $NaHCO_3$, 1*H*-1,2,4-triazole, reflux, 5 h, in 42% yield; (c) $C_6H_5CH_3$, $(CH_3)_3SOI$, NaOH, cetylmethylammonium bromide, 60 °C, 3 h, in 86% yield; (d) CH_3SO_3H, 0 °C, 1 h, in 89% yield; (e) CH_3CH_2OH, Et_3N, propylamine, reflux, 6 h, in 80% yield; (f) CH_3CN, KI, K_2CO_3, propargyl bromide, rt, 5–6 h, in 70% yield; (g) NaN_3, substituted benzyl bromide, DMSO, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, rt, 12 h, in 60–70% yield.

All of the target compounds exhibited higher activities against *C. alb.* SC5314 and *C. alb.* Y0109 than all of the three positive controls. Obviously, the MIC_{80} value of compound **1i** is 128 times lower than that of FCZ against *C. alb.* Y0109 *in vitro* (with an MIC_{80} value of 0.0039 μg mL^{-1}), 16 times lower than that of ICZ against *C. alb.* Y0109 *in vitro*; the MIC_{80} values of

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

Compd	*C. alb.* SC5314	*C. alb.* Y0109	*C. neo.*	*A. fum.*	*T. rub.*	*C. tro.*	*C. pra.*	*C. kef.*
1a	4	0.0156	8	>64	4	4	<0.125	0.25
1b	2	0.0625	64	>64	4	8	0.5	0.25
1c	1	0.25	32	>64	1	32	4	0.25
1d	0.5	0.0625	8	>64	4	8	1	0.25
1e	1	0.0625	16	>64	4	8	1	16
1f	0.25	0.0625	32	>64	4	8	1	1
1g	2	0.0625	32	>64	8	16	2	0.25
1h	0.25	0.0156	16	>64	4	4	0.5	0.0625
1i	0.25	0.0039	32	64	4	16	2	1
1j	0.5	0.25	8	>64	4	1	4	0.0625
1k	<0.125	0.0156	8	>64	1	0.25	0.25	0.0625
1l	<0.125	0.25	8	>64	2	1	2	0.25
1m	0.5	0.25	4	>64	4	4	4	4
1n	<0.125	0.25	64	>64	4	4	0.5	0.25
1o	0.25	0.25	16	>64	4	4	1	1
ICZ	<0.0625	0.0625	0.125	2	0.0625	<0.0625	0.0625	0.0625
VCZ	32	<0.125	<0.125	<0.125	<0.125	<0.125	0.25	0.0039
FCZ	0.5	0.5	8	>64	2	<0.125	<0.125	1

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compounds **1h**, **1j**, and **1k** are 16 times lower than that of FCZ against *C. kef. in vitro* (with an MIC_{80} value of 0.0625 μg mL^{-1}). The MIC_{80} value of compounds **1a** and **1k** are 32 times lower than that of FCZ against *C. alb.* Y0109 *in vitro* (with an MIC_{80} value of 0.0156 μg mL^{-1}).

Conclusions

In summary, a novel series of antifungal agents have been designed and synthesized. *In vitro* antifungal activity assays indicate that most of the compounds showed moderate antifungal activities against both systemic pathogenic fungi and dermatophytes. The activity did not differ significantly in terms of the strength of the electron-withdrawing or donating groups substituted on the benzene ring. The disubstituted activity was not as good as the monosubstituted activity. Several of the compounds showed high *in vitro* antifungal activity with a broad spectrum, and are valuable for further evaluation.

Experimental part

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 the internal standard. High resolution electron spray ionization mass spectra (HR ESI MS) were recorded 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-propylamino-2-propanol (6)

A mixture of compound **5** (33.3 g, 0.10 mol), CH_3CH_2OH (500 mL), Et_3N (50 mL), and propylamine (8.85 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** (23.4 g, 79%).

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

A mixture of compound **6** (5.92 g, 0.02 mol), propargyl bromide (4.72 g, 0.04 mol), KI (332 mg, 0.002 mol), K_2CO_3 (6.90 g, 0.05 mol), and CH_3CN (200 mL) was stirred at room temperature for 6 h. The reaction was monitored by TLC. After the reaction, the solid was filtrated off, washed with CH_3CN, and the filtrate was concentrated in a vacuum. Column chromatography of the residue afforded compound **7** as a white solid (4.14 g, 62%).

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

A mixture of NaN_3 (100 mg, 1.4 mmol), 4-Cl benzyl bromide (200 mg, 1.2 mmol), and DMSO (15 mL) was stirred at room temperature for 6 h. Then compound **7** (200 mg, 0.6 mmol), sodium ascorbate (20 mg), $CuSO_4{\cdot}5H_2O$ (25 mg), and H_2O(1 mL) were added, the solution was stirred at room temperature for another 2 h. The reaction was monitored by TLC. The reaction solution was then added to 10 mL $NH_3{\cdot}H_2O$, and extracted with ethyl acetate. The organic layer was acidified with dilute hydrochloric acid, then the aqueous layer was adjusted to around pH 7 using saturated sodium bicarbonate solution, and extracted with ethyl acetate, the organic layer was washed with water and brine, and dried with Na_2SO_4. Concentration in vacuum afforded compound **1a** (201 mg, 67%). Mp: 94.9–96.4 °C; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.09 (1 H, s, triazole–H), 7.77 (1 H, s, triazole–H), 7.19–7.60 (5 H, m, Ar–H), 7.04 (1 H, s, triazole–H), 6.73–6.81 (2 H, m, Ar–H), 5.50 (2 H, s, Ar–CH_2–), 5.46 (1 H, s, OH), 4.52 (1 H, d, J = 13.8 Hz, triazole–CH_2–), 4.39 (1 H, d, J = 14.1 Hz, triazole–CH_2–), 3.61 (1 H, d, J = 14.4 Hz, triazole–CH_2–), 3.54 (1 H, d, J = 12.9 Hz, triazole–CH_2–), 3.15 (1 H, d, J = 13.8 Hz, –CH_2–), 2.70 (1 H, d, J = 13.8 Hz, –CH_2–), 2.11–2.29 (2 H, m, $NCH^*_2CH_2CH_3$), 1.24–1.30 (2 H, m, $NCH_2CH^*_2CH_3$), 0.65–0.70 (3 H, m, $NCH_2CH_2CH^*_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.7, 160.8, 152.7, 146.5, 135.6, 134.1, 131.7, 131.6, 128.4, 124.8, 123.8, 113.3, 105.9, 74.1, 74.0, 60.0, 60.0, 59.3, 58.0, 57.9, 55.3, 51.2, 22.1, 13.1; HR ESI MS: calcd for $C_{24}H_{26}ClF_2N_7O$, $[M + H]^+$ m/z: 502.1928; found: 502.1938.

The target compounds **1b–o** were synthesized by the same synthetic procedure that was used for compound **1a**.

Acknowledgements

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