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Full Paper

Synthesis, *In Vitro* Biological Evaluation, and Molecular Docking of New Triazoles as Potent Antifungal Agents

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Based on the structure of the active site of CYP51 and the structure–activity relationships of azole antifungal compounds that we designed in a previous study, a series of 1-{1-[2-(substitutedbenzyloxy)-ethyl]-1*H*-1,2,3-triazol-4-yl}-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols (**6a–n**) were designed and synthesized utilizing copper-catalyzed azide-alkyne cycloaddition. Preliminary antifungal tests against eight human pathogenic fungi *in vitro* showed that all the title compounds exhibited excellent antifungal activities with a broad spectrum *in vitro*. Molecular docking results indicated that the interaction between the title compounds and CYP51 comprised π–π interactions, hydrophobic interactions, and the narrow hydrophobic cleft.

Keywords: Antifungal activity / CYP51 / Molecular docking / SAR / Triazoles

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Introduction

Since the late 1960s, a drastic rise in the prevalence of fungal infections was observed, which currently represented a global health threat. This increasing incidence of infection is accompanied by the growing number of immunosuppression cases related to AIDS, cancer, old age, diabetes, cystic fibrosis, long-range antibiotics, bone marrow and organ transplants, and other invasive surgical procedures [1, 2]. In spite of extensive research dedicated to the development of new antifungal drugs, a limited number of available drugs can fight against invasive fungal infections. Among the four molecular classes of antifungal drugs (including fluoropyrimidines, polyenes, azoles, and echinocandins as shown in Fig. 1), azoles are by far the most commonly used drugs in clinical practice. Unfortunately, the broad use of azoles has led to the development of severe resistance, which significantly reduced their efficacy. So to discover novel and potent antifungal azoles is urgent and important to overcome the growing drug resistance [3].

In our previous research, we have designed highly potent azole derivatives with different C-(3) side chains [4–10]. Recently, we reported a series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-substituted-2-propanols as potent antifungal compounds shown in Fig. 2 [4]. Some of them showed higher activity against almost all fungi than fluconazole and amphotericin. The compound **5i** was docked into the active site of CACYP51 and the docking result (as shown in Fig. 2) revealed that the 1,2,3-triazole group in the side chain would generate π–π stacking interactions with Tyr118. Moreover, the substituted benzyl group could interact with a hydrophobic pocket formed by Ala114, Phe126, Gln142, and Phe145, and it could also generate π–π stacking interactions with Phe380. It was concluded that the incorporation of the 1,2,3-triazole group could improve antifungal activities.

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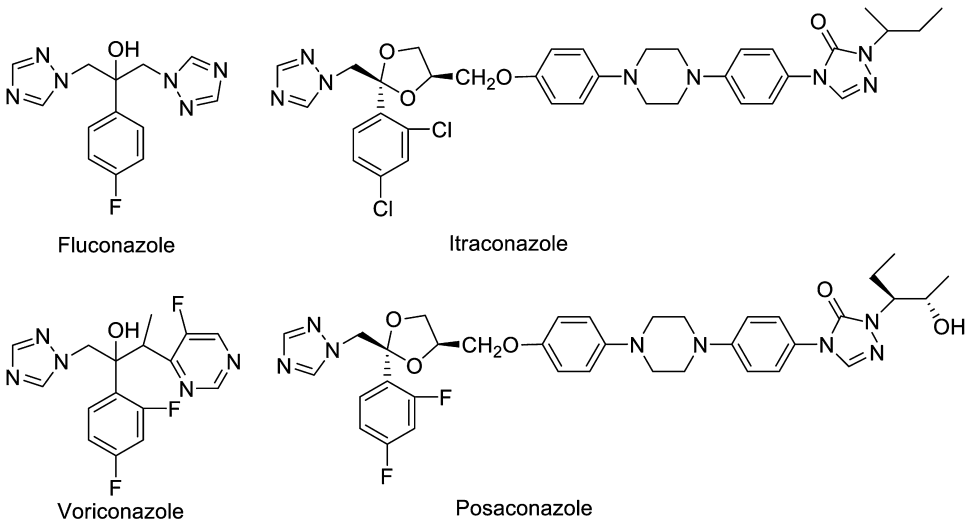


Figure 1. Triazole antifungal agents used in clinical therapy.

However, it can be observed that the computed geometry of **5i** in the CYP51 was so crowded especially in the C-(3) side chain, which could have an adverse influence on the interaction with the target. So in the current study, we focused on modifying the side chain with a substituted benzyl group and a longer linkage to the N-atom of the 1,2,3-triazole group, which could reduce the congestion with CYP51 to some extent. In addition, owing to the importance of the 1,2,3-triazole group moiety for the antifungal activity, we chose to retain the remaining part of **5i**. Specifically, a series of 1-{1-[2-(substitutedbenzyloxy)ethyl]-1*H*-1,2,3-triazol-4-yl}-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols (as shown in Fig. 3) were designed, synthesized, and evaluated for their antifungal activities to develop better antifungal compounds. Also, a docking study was conducted in an attempt to understand the mechanism of action of the compounds on the enzyme target.

Results and discussion

Chemistry

As a key intermediate of our designed compounds, compound **2** was synthesized by our reported procedure. In the presence of zinc dust in a mixed solvent (DMF/THF, 1:1, v/v, 20 mL), treatment of the intermediate **1** at 60°C for 7 h gave compound **2**. The target compounds were synthesized through three steps from the racemic compound **2** [11].

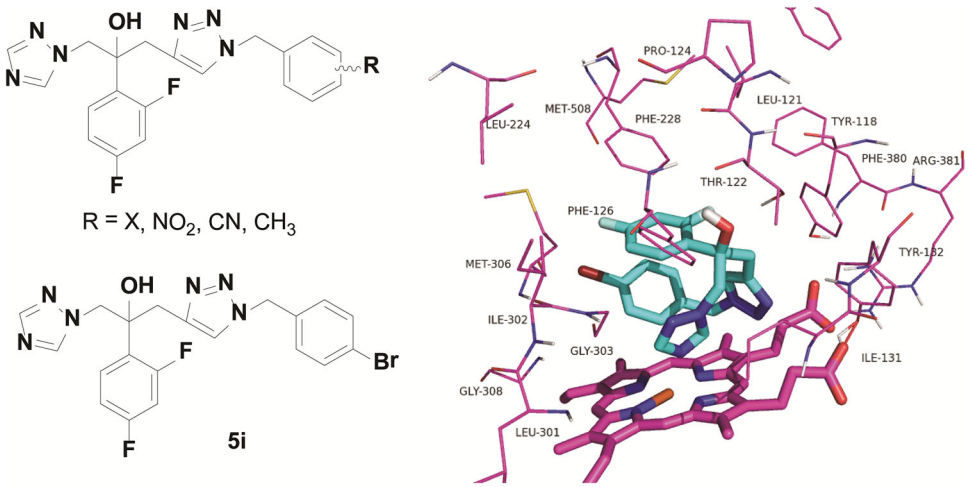


Figure 2. The structure of the compounds we have synthesized in the previous study and the computed binding geometry of **5i** in the active site of CYP51.

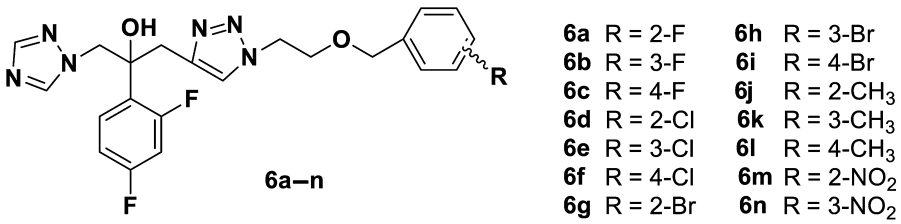


Figure 3. The structures of all the compounds we designed and synthesized.

Compound **3** was obtained utilizing Click reaction [12]. Compound **3** was reduced to give compound **4** in the presence of $LiAlH_4$ in dry THF. The target compounds **6a–n** were synthesized by treating compound **4** with substituted benzyl bromide in the presence of NaH at 0°C for 2 h (as shown in Scheme 1). All the target compounds were obtained as racemates.

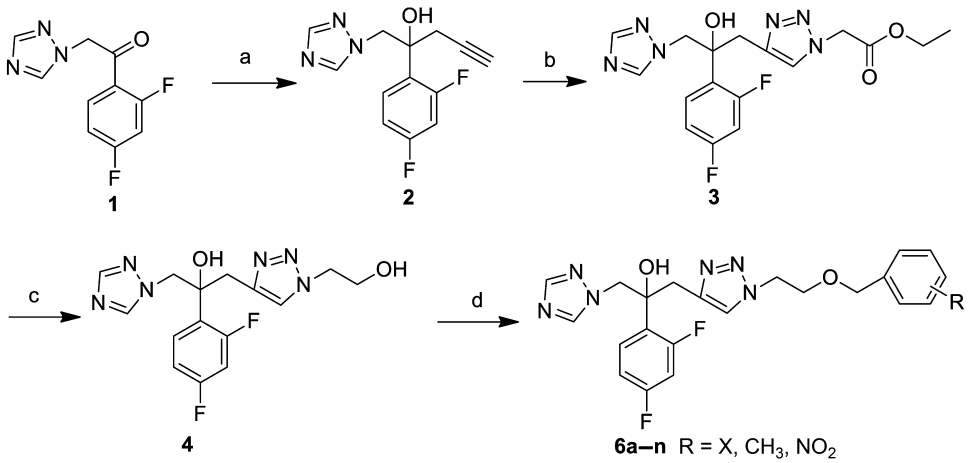
Biological activity

The results of assays are summarized in Table 1. It showed that the activities of most compounds were better than fluconazole and amphotericin B as well as itraconazole *in vitro* against tested clinical species of *Candida*. Compounds **6c**, **6f**, **6i**, and **6j** exhibited excellent activity against *C. albicans* and *C. parapsiosis* than all five positive controls. The MIC_{80} values of compounds **6c** and **6i** are 256 times lower than that of ICZ against *C. alb* SC5314 and *C. alb* Y0109 *in vitro* (with the MIC_{80} value of 0.0156 μg/mL), and 32 times lower than that of FCZ. The MIC_{80} value of compound **6i** is 128 times lower than that of FCZ against *C. par in vitro* (with the MIC_{80} value of 0.0156 μg/mL), and 256 times lower than that of ICZ. The MIC_{80} values of compounds **6b**, **6d**, **6g**, and **6h** are eight times lower than that of FCZ against *C. alb* SC5314 and *C. alb* Y0109 *in vitro* (with the MIC_{80} value of 0.0625 μg/mL), 16 times lower than that of AMB, and 64 times lower than that of ICZ. Compounds **6d**, **6f**, **6g**, and **6j** also exhibited higher activity against *C. par* than itraconazole, voriconazole, and fluconazole with the MIC_{80} value of 0.0625 μg/mL. Compounds **6b**, **6d**, and **6f–j** are worthy of further study, and compound **6i** is expected to be developed into a new antifungal drug.

Molecular docking

To explain the results, we proposed a hypothetical binding mode for **6i** to the active site of CYP51 based on computational docking results (Fig. 4). The docking results revealed that the compound binds to the active site of CACYP51 through the formation of a coordination bond with Fe of the heme group. The difluorophenyl group was located in the hydrophobic binding cleft lined with Phe126, Leu224, Phe228, and Met508. The long side chain of the compound **6i** formed hydrophobic and van der Waals interactions with surrounding hydrophobic residues such as Tyr118, Thr122, Ile131, Tyr132, Phe380, and Arg381. Furthermore, the substituted benzyl could generate π–π stacking interactions with Tyr118.

Compared with the computed geometry of **5i**, the interaction of **6i** with CYP41 was obviously better. A longer linkage between substituted benzyl group with the N-atom of



Scheme 1. Synthetic routes of the target compounds. Reagents and conditions: (a) Zn, propargyl bromide, DMF/THF, 60°C, 6 h, 95%; (b) NaN_3, ethyl bromoacetate, sodium ascorbate, $CuSO_4$, DMSO, 76%; (c) $LiAlH_4$, THF, 95%; (d) NaH, substituted benzyl bromide, ice bath, 2 h, 65–78%.

Table 1. ***In vitro*** **antifungal activities of the target compounds.**

Compound no.	R	MIC_{80} (μg/mL)[a]							
		C. alb Y0109	*C. alb* SC5314	*C. par*	*C. kru*	*M. gyp*	*C. neo*	*T. nru*	*A. fumi*
6a	2-F	0.125	0.125	0.25	0.25	16	4	0.5	>64
6b	3-F	0.0625	0.0625	0.125	0.25	0.0625	0.5	0.25	>64
6c	4-F	0.0156	0.0156	0.125	0.25	0.125	2	0.5	>64
6d	2-Cl	0.0625	0.0625	0.0625	0.25	0.125	1	0.125	>64
6e	3-Cl	0.125	0.125	0.25	0.5	64	8	0.5	>64
6f	4-Cl	0.0156	0.0625	0.0625	0.125	0.125	0.5	0.125	>64
6g	2-Br	0.0625	0.0625	0.0625	0.0625	0.0156	2	0.25	>64
6h	3-Br	0.25	0.25	1	1	32	8	0.5	>64
6i	4-Br	0.0156	0.0156	0.0156	0.0625	0.125	0.25	0.25	>64
6j	2-CH_3	0.0156	0.0625	0.0625	0.125	0.0625	4	0.125	>64
6k	3-CH_3	0.0625	0.0625	0.125	0.125	0.125	0.5	0.125	>64
6l	4-CH_3	0.0625	0.0156	0.0625	0.0625	1	0.5	0.25	>64
6m	2-NO_2	0.25	0.25	1	0.5	32	16	2	>64
6n	3-NO_2	0.25	0.5	1	1	64	16	0.5	>64
ICZ		4	4	4	0.0625	0.0625	2	0.5	8
KCZ	–	0.0625	0.0625	0.25	0.0039	0.00097	0.5	0.25	4
AMB	–	1	2	1	1	0.25	2	1	4
VCZ	–	0.0625	0.125	0.25	0.0039	0.0039	0.25	0.125	4
FCZ	–	0. 5	0.5	2	1	4	4	1	>64

C. alb., *Candida albicans*; *C. par.*, *Candida parasilosis*; *C. kru.*, *Candida krusei*; *C. neo.*, *Cryptococcus neoformans*; *M. gyp.*, *Microsporumgypseum*; *T. nru.*, *Trichophytonrubrum*; *A. fumi.*, *Aspergillus fumigatus*; ICZ, itraconazole; KCZ, ketoconazole; VCZ, voriconazole; AMB, amphotericin B; FCZ, fluconazole.
[a]Minimum inhibitory concentration for 80% inhibition of growth.

the 1,2,3-triazole group could reduce the congestion of the title compounds in the CYP41, and a π–π stacking interaction formed between substituted benzyl group with Tyr118 was also beneficial to the better antifungal activities of this series of compounds. We can believe that the substituted benzyl group in C-(3) side chain was significant to the activities when it was accompanied with a suited linkage. In addition, they played an essential role in adjusting the physicochemical

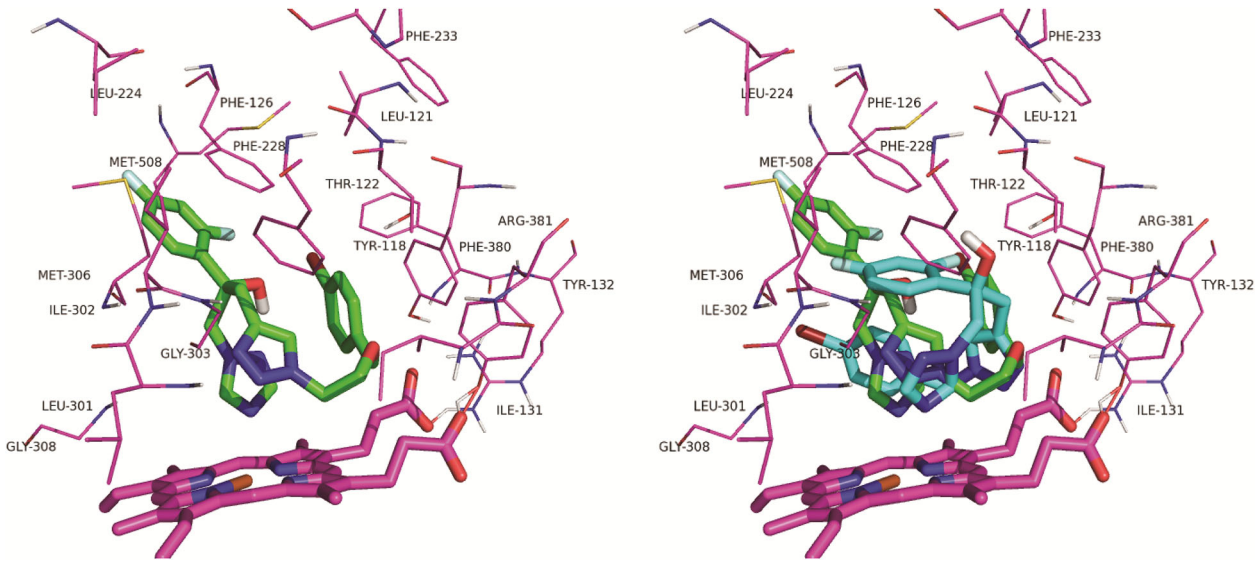


Figure 4. Computed binding geometry of the new inhibitor **6i** in the active site of CYP51 and superimposed geometry of **5i** and **6i** in CYP51 (**5i** shown in blue stick and **6i** shown in green stick).

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properties of the whole molecule to avoid side effects and improve antifungal activities.

Conclusion

In conclusion, a series of novel triazole antifungal agents were synthesized and their antifungal activities were screened for eight human pathogenic fungi. Some compounds showed improved antifungal activities *in vitro* especially against *Candida* species than positive control. This research helps us to discover compound **6i** to be worthy of further optimization. The obtained results indicated that for antifungal activity of these novel triazole derivatives, it is very helpful to introduce the 1,2,3-triazolyl group and substituted benzyl groups as side chains.

Experimental

Chemistry

General

Melting points were measured on a Yamato MP-21 melting-point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 unless otherwise indicated with a Bruker AC-300P or AC-500P spectrometer, using TMS as an internal standard. ESI mass spectra were performed on an API-3000 LC-MS spectrometer. Elemental analysis was undertaken with an MOD 1106 analyzer (Carlo Erba, Milan, Italy) at the Analysis Center of Shanghai Institute of Pharmaceutical Industry. Column chromatography was carried out on silica gel (300–400 mesh). The solvents and reagents were used as received or dried prior to use as needed. All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Detection was effected by examination under UV light.

Synthesis of the key intermediates

*1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-pent-4-yn-2-ol (**2**)*

The compound (**1**) (20 mmol) and propargyl bromide (40 mmol) were dissolved in a mixed solvent (DMF/THF, 1:1, 20 mL). Zinc dust (60 mmol, washed with 2% HCl, water, and dried in vacuum) was added to this well-stirred solution. The exothermic reaction brought itself to reflux after 2–5 min. The whole reaction mixture was stirred for 7 h at 60°C until TLC indicated that the reaction was finished. 4 M HCl was added to the mixture, extracted with ethyl acetate twice. The combined organic layer was washed with water several times until the organic layer was adjusted to pH about 7, dried over anhydrous Na_2SO_4 and evaporated to get compound **2**.

*Ethyl-2-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-1H-1,2,3-triazol-1-yl)acetate (**3**)*

A mixture of NaN_3 (860 mg, 14.4 mmol), ethyl bromoacetate (2 g, 12 mmol), and DMSO (50 mL) was stirred at room temperature for 6 h. Then the compound **2** (3.2 g, 12 mmol), sodium ascorbate (200 mg), $CuSO_4{\cdot}5H_2O$ (200 mg) and H_2O (5 mL) were added to the reaction and stirred at room temperature for 2 h. After that, the reaction solution was poured into $NH_3{\cdot}H_2O$, extracted with ethyl acetate, and the organic layer was acidated with dilute hydrochloric acid. The aqueous layer was adjusted pH about 7 by saturation sodium bicarbonate and extracted with ethyl acetate. The combined organic layer was washed with water, dried with anhydrous Na_2SO_4 and concentrated in a vacuum to afford compound **3**.

*2-(2,4-Difluorophenyl)-1-(1-(2-hydroxyethyl)-1H-1,2,3-triazol-4-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**4**)*

$LiAlH_4$ (200 mg, 5.2 mmol) was added to the solution of compound **3** (2 g, 0.008 mmol) in THF (30 mL) slowly under ice condition. The mixture was stirred at room temperature until the reaction was completed. $MgSO_4$ was added to remove $LiAlH_4$. The precipitate was removed by filtration and the filtrate was concentrated to dryness under reduced pressure to get the compound **4** as white solid.

*Synthesis of the title compounds **6a–n***

General procedure: NaH (25 mg, 1.1 mmol) was added to the solution of compound **4** (350 mg, 1 mmol) in dry DCM (20 mL) slowly under ice condition. After 0.5 h, 2-fluorobenzyl bromide was added to the mixture. The mixture was stirred at ice condition for 2 h. DCM (20 mL) was added. Then methanol was added dropwise to remove the remaining NaH. The mixture was washed by brine, and the solvent was removed at reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (1:1) as eluent.

*1-(1-(2-((2-Fluorobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6a**)*

The product **6a** was obtained as a white solid. Mp: 64.4–66.2°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.27 (1H, s, 1,2,4-triazole-H), 7.75 (1H, s, 1,2,4-triazole-H), 7.58 (1H, s, 1,2,3-triazole-H), 7.27–6.77 (7H, m, Ar-H), 5.98 (1H, s, OH), 4.69–4.48 (2H, dd, $J = 14.4$ Hz, 1,2,4-triazole-$\underline{CH_2}$), 4.44 (2H, s, $\underline{CH_2}CH_2O$), 4.40 (2H, s, Ar$\underline{CH_2}$O), 3.74–3.71 (2H, t, $CH_2\underline{CH_2}O$), 3.35–3.10 (2H, dd, $J = 15.0$ Hz, 1,2,3-triazole-$\underline{CH_2}$C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 161.53, 159.58, 151.01, 145.35, 141.90, 130.57, 130.54, 130.35, 130.30, 130.24, 124.80, 124.77, 124.37, 115.68, 115.51, 104.29, 74.18, 68.85, 66.05, 57.32, 49.61, 35.01; ESI-MS (*m/z*): 459.2 (M+H); Anal. calcd. for $C_{22}H_{21}F_3N_6O_2$: C, 57.64; H, 4.62; N, 18.33. Found, C, 57.65; H, 4.59; N, 18.41.

*1-(1-(2-((3-Fluorobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6b**)*

The product **6b** was obtained as a white solid. Mp: 112.5–114.1°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.27 (1H, s, 1,2,4-triazole-H), 7.75 (1H, s, 1,2,4-triazole-H), 7.60 (1H, s,

1,2,3-triazole-H), 7.35–6.77 (7H, m, Ar-H), 5.98 (1H, s, OH), 4.69–4.49 (2H, dd, $J = 14.1$ Hz, 1,2,4-triazole-$\underline{CH_2}$), 4.46–4.43 (2H, t, $\underline{CH_2}CH_2O$), 4.40 (2H, s, Ar$\underline{CH_2}$O), 3.72–3.69 (2H, t, $CH_2\underline{CH_2}O$), 3.38–3.10 (2H, dd, $J = 15.3$ Hz, 1,2,3-triazole-$\underline{CH_2}$C-(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 163.97, 161.66, 150.99, 145.32, 141.90, 141.45, 130.70, 130.63, 130.41, 126.90, 124.42, 123.57, 114.64, 114.24, 110.99, 104.39, 74.16, 71.37, 68.75, 57.29, 49.65, 34.98; ESI-MS (*m/z*): 459.2 (M+H); Anal. calcd. for $C_{22}H_{21}F_3N_6O_2$: C, 57.64; H, 4.62; N, 18.33. Found, C, 57.58; H, 4.69; N, 18.43.

*1-(1-(2-((4-Fluorobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6c**)*

The product **6c** was obtained as a white solid. Mp: 79.7–81.1°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.37 (1H, s, 1,2,4-triazole-H), 7.74 (1H, s, 1,2,4-triazole-H), 7.61 (1H, s, 1,2,3-triazole-H), 7.33–6.72 (7H, m, Ar-H), 6.24 (1H, s, OH), 4.71–4.55 (2H, dd, $J = 14.1$ Hz, 1,2,4-triazole-CH_2), 4.44–4.40 (2H, t, $\underline{CH_2}CH_2O$), 4.35 (2H, s, Ar$\underline{CH_2}$O), 3.69–3.66 (2H, t, $CH_2\underline{CH_2}O$), 3.40–3.11 (2H, dd, $J = 15.0$ Hz, 1,2,3-triazole-$\underline{CH_2}$C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 162.97, 161.04, 158.33, 150.94, 141.99, 134.62, 130.55, 130.51, 130.44, 130.03, 129.97, 124.40, 115.55, 115.38, 110.93, 104.99, 74.16, 71.46, 68.53, 57.20, 49.65, 34.97; ESI-MS (*m/z*): 459.2 (M+H); Anal. calcd. for $C_{22}H_{21}F_3N_6O_2$: C, 57.64; H, 4.62; N, 18.33. Found, C, 57.49; H, 4.58; N, 18.40.

*1-(1-(2-((2-Chlorobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6d**)*

The product **6d** was obtained as a white solid. Mp: 87.7–88.6°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.27 (1H, s, 1,2,4-triazole-H), 7.75 (1H, s, 1,2,4-triazole-H), 7.61 (1H, s, 1,2,3-triazole-H), 7.43–6.72 (7H, m, Ar-H), 5.97 (1H, s, OH), 4.69–4.49 (2H, dd, $J = 14.4$ Hz, 1,2,4-triazole-CH_2), 4.49–4.46 (4H, t, Ar$\underline{CH_2}$O, $\underline{CH_2}CH_2O$), 3.80–3.77 (2H, t, $CH_2\underline{CH_2}O$), 3.39–3.11 (2H, dd, $J = 14.7$ Hz, 1,2,3-triazole-$\underline{CH_2}$C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.26, 158.29, 151.01, 145.35, 141.92, 135.84, 132.39, 130.54, 129.69, 129.60, 129.57, 127.58, 126.30, 124.43, 110.98, 105.72, 74.18, 69.52, 69.11, 57.32, 49.67, 35.02; ESI-MS (*m/z*): 475.1 (M+H); Anal. calcd. for $C_{22}H_{21}ClF_2N_6O_2$: C, 55.64; H, 4.46; N, 17.70. Found, C, 55.72; H, 4.53; N, 17.68.

*1-(1-(2-((3-Chlorobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6e**)*

The product **6e** was obtained as a white solid. Mp: 139.7–140.7°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.24 (1H, s, 1,2,4-triazole-H), 7.83 (1H, s, 1,2,4-triazole-H), 7.38 (1H, s, 1,2,3-triazole-H), 7.40–6.66 (7H, m, Ar-H), 5.45 (1H, s, OH), 4.76–4.56 (2H, dd, $J = 14.1$ Hz, 1,2,4-triazole-CH_2), 4.51–4.45 (2H, t, $\underline{CH_2}CH_2O$), 4.39 (2H, s, Ar$\underline{CH_2}$O), 3.76–3.73 (2H, t, $CH_2\underline{CH_2}O$), 3.51–3.14 (2H, dd, $J = 15.0$ Hz, 1,2,3-triazole-$\underline{CH_2}$C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 161.23, 158.49, 151.37, 141.91, 141.07, 133.45, 130.60, 130.43, 127.84, 127.44, 126.27, 126.20, 126.10, 124.41, 110.99, 104.39, 74.16, 71.2, 68.76, 57.33, 49.63, 34.99; δ ESI-MS (*m/z*): 475.2 (M+H); Anal. calcd. for $C_{22}H_{21}ClF_2N_6O_2$: C, 55.64; H, 4.46; N, 17.70. Found, C, 55.58; H, 4.55; N, 17.61.

*1-(1-(2-((4-Chlorobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6f**)*

The product **6f** was obtained as a white solid. Mp: 105.9–106.6°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.26 (1H, s, 1,2,4-triazole-H), 7.74 (1H, s, 1,2,4-triazole-H), 7.58 (1H, s, 1,2,3-triazole-H), 7.36–6.73 (7H, m, Ar-H), 5.97 (1H, s, OH), 4.68–4.48 (2H, dd, $J = 14.4$ Hz, 1,2,4-triazole-CH_2), 4.44–4.41 (2H, t, $\underline{CH_2}CH_2O$), 4.36 (2H, s, Ar$\underline{CH_2}$O), 3.70–3.67 (2H, t, $CH_2\underline{CH_2}O$), 3.39–3.10 (2H, dd, $J = 15.0$ Hz, 1,2,3-triazole-$\underline{CH_2}$C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 161.08, 158.28, 151.01, 145.34, 141.90, 137.48, 132.47, 130.42, 130.30, 129.61, 128.67, 126.17, 126.07, 124.40, 111.02, 104.76, 74.17, 71.35, 68.65, 57.33, 49.65, 35.00; ESI-MS (*m/z*): 475.1 (M+H); Anal. calcd. for $C_{22}H_{21}ClF_2N_6O_2$: C, 55.64; H, 4.46; N, 17.70. Found, C, 55.66; H, 4.39; N, 17.80.

*1-(1-(2-((2-Bromobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6g**)*

The product **6g** was obtained as a white solid. Mp: 87.6–89.2°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.26 (1H, s, 1,2,4-triazole-H), 7.74 (1H, s, 1,2,4-triazole-H), 7.58 (1H, s, 1,2,3-triazole-H), 7.56–6.71 (7H, m, Ar-H), 5.96 (1H, s, OH), 4.68–4.48 (2H, dd, $J = 14.1$ Hz, 1,2,4-triazole-CH_2), 4.48–4.45 (2H, t, $\underline{CH_2}CH_2O$), 4.43 (2H, s, Ar$\underline{CH_2}$O), 3.81–3.79 (2H, t, $CH_2\underline{CH_2}O$), 3.38–3.10 (2H, dd, $J = 15.0$ Hz, 1,2,3-triazole-$\underline{CH_2}$C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 161.07, 158.28, 151.00, 145.33, 141.90, 137.40, 132.78, 130.72, 129.94, 129.64, 128.12, 126.41, 124.44, 122.49, 110.99, 107.05, 74.16, 71.76, 69.11, 57.30, 49.68, 35.01; ESI-MS (*m/z*): 519.3 (M+H); Anal. calcd. for $C_{22}H_{21}BrF_2N_6O_2$: C, 50.88; H, 4.08; N, 16.18. Found, C, 50.77; H, 4.13; N, 16.12.

*1-(1-(2-((3-Bromobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6h**)*

The product **6h** was obtained as a white solid. Mp: 137.9–139.4°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.27 (1H, s, 1,2,4-triazole-H), 7.75 (1H, s, 1,2,4-triazole-H), 7.60 (1H, s, 1,2,3-triazole-H), 7.47–6.74 (7H, m, Ar-H), 5.97 (1H, s, OH), 4.69–4.49 (2H, dd, $J = 14.1$Hz, 1,2,4-triazole-CH_2), 4.46–4.43 (2H, t, $\underline{CH_2}CH_2O$), 4.38 (2H, s, Ar$\underline{CH_2}$O), 3.72–3.69 (2H, t, $CH_2\underline{CH_2}O$), 3.39–3.11 (2H, dd, $J = 15.0$ Hz, 1,2,3-triazole-$\underline{CH_2}$C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.51, 158.87, 151.01, 145.34, 141.91, 141.34, 130.91, 130.77, 130.37, 126.69, 126.24, 126.09, 124.41, 122.07, 111.00, 105.49, 74.18, 71.25, 68.77, 57.29, 49.63, 35.03; ESI-MS (*m/z*): 519.2 (M+H); Anal. calcd. for $C_{22}H_{21}BrF_2N_6O_2$: C, 50.88; H, 4.08; N, 16.18. Found, C, 50.80; H, 4.15; N, 16.11.

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*1-(1-(2-((4-Bromobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6i**)*

The product **6i** was obtained as a white solid. Mp: 97.4–98.2°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.27 (1H, s, 1,2,4-triazole-H), 7.75 (1H, s, 1,2,4-triazole-H), 7.59 (1H, s, 1,2,3-triazole-H), 7.51–6.74 (7H, m, Ar-H), 5.98 (1H, s, OH), 4.69–4.49 (2H, dd, J = 14.4 Hz, 1,2,4-triazole-CH_2), 4.45–4.42 (2H, t, CH_2CH_2O), 4.39 (2H, s, ArCH_2O), 3.71–3.67 (2H, t, CH_2CH_2O), 3.39–3.11 (2H, dd, J = 15.0 Hz, 1,2,3-triazole-CH_2C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.77, 158.30, 151.03, 145.35, 137.91, 131.60, 130.43, 130.39, 130.33, 129.95, 126.18, 126.10, 124.41, 121.00, 111.02, 105.72, 74.18, 71.39, 68.66, 57.31, 49.65, 34.98; ESI-MS (*m/z*): 519.2 (M+H); Anal. calcd. for $C_{22}H_{21}BrF_2N_6O_2$: C, 50.88; H, 4.08; N, 16.18. Found, C, 50.81; H, 4.11; N, 16.08.

*1-(1-(2-((2-Methylbenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6j**)*

The product **6j** was obtained as a white solid. Mp: 95.4–96.6°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.14 (1H, s, 1,2,4-triazole-H), 7.81 (1H, s, 1,2,4-triazole-H), 7.36 (1H, s, 1,2,3-triazole-H), 7.42–6.65 (7H, m, Ar-H), 5.48 (1H, s, OH), 4.72–4.53 (2H, dd, J = 14.1 Hz, 1,2,4-triazole-CH_2), 4.47–4.43 (4H, t, CH_2CH_2O, ArCH_2O), 3.77–3.73 (2H, t, CH_2CH_2O), 3.48–3.12 (2H, dd, J = 15.0 Hz, 1,2,3-triazole-CH_2C(OH), 2.22 (3H, s, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.72, 158.62, 151.01, 145.34, 141.85, 136.72, 136.31, 130.49, 130.35, 128.57, 128.09, 126.19, 125.98, 124.35, 112.19, 105.48, 74.14, 70.84, 68.68, 57.33, 49.73, 34.96, 18.66; ESI-MS (*m/z*): 455.3 (M+H); Anal. calcd. for $C_{23}H_{24}F_2N_6O_2$: C, 60.78; H, 5.32; N, 18.49. Found, C, 60.71; H, 5.25; N, 18.53.

*1-(1-(2-((3-Methylbenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6k**)*

The product **6k** was obtained as a white solid. Mp: 110.5–111.8°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.26 (1H, s, 1,2,4-triazole-H), 7.75 (1H, s, 1,2,4-triazole-H), 7.59 (1H, s, 1,2,3-triazole-H), 7.20–6.76 (7H, m, Ar-H), 5.99 (1H, s, OH), 4.69–4.49 (2H, dd, J = 14.4 Hz, 1,2,4-triazole-CH_2), 4.44–4.41 (2H, t, CH_2CH_2O), 4.33 (2H, s, ArCH_2O), 3.69–3.66 (2H, t, CH_2CH_2O), 3.39–3.11 (2H, dd, J = 15.0 Hz, 1,2,3-triazole-CH_2C(OH), 2.25 (3H, s, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.29, 158.27, 151.00, 145.32, 141.86, 138.30, 137.81, 130.40, 128.58, 128.47, 126.14, 126.04, 124.96, 124.41, 111.00, 105.93, 74.16, 72.28, 68.53, 57.30, 49.69, 35.03, 21.41; ESI-MS (*m/z*): 455.3 (M+H); Anal. calcd. for $C_{23}H_{24}F_2N_6O_2$: C, 60.78; H, 5.32; N, 18.49. Found, C, 60.77; H, 5.26; N, 18.56.

*1-(1-(2-((4-Methylbenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6l**)*

The product **6l** was obtained as a white solid. Mp: 116.8–117.9°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.28 (1H, s, 1,2,4-triazole-H), 7.76 (1H, s, 1,2,4-triazole-H), 7.59 (1H, s, 1,2,3-triazole-H), 7.24–6.74 (7H, m, Ar-H), 5.98 (1H, s, OH), 4.70–4.49 (2H, dd, J = 14.1 Hz, 1,2,4-triazole-CH_2), 4.44–4.40 (2H, t, CH_2CH_2O), 4.33 (2H, s, ArCH_2O), 3.68–3.65 (2H, t, CH_2CH_2O), 3.42–3.11 (2H, dd, J = 15.0 Hz, 1,2,3-triazole-CH_2C-(OH), 2.23 (3H, s, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.25, 158.28, 151.00, 145.33, 141.87, 137.12, 135.32, 130.41, 130.33, 129.24, 128.00, 126.17, 126.07, 124.38, 112.19, 105.48, 74.17, 72.16, 68.40, 57.32, 49.70, 35.01, 21.20; ESI-MS (*m/z*): 455.3 (M+H); Anal. calcd. for $C_{23}H_{24}F_2N_6O_2$: C, 60.78; H, 5.32; N, 18.49. Found, C, 60.69; H, 5.37; N, 18.55.

*1-(1-(2-((2-Nitrobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6m**)*

The product **6m** was obtained as a white solid. Mp: 129.6–130.5°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.26 (1H, s, 1,2,4-triazole-H), 7.74 (1H, s, 1,2,4-triazole-H), 7.61 (1H, s, 1,2,3-triazole-H), 8.05–6.71 (7H, m, Ar-H), 5.96 (1H, s, OH), 4.75 (2H, s, ArCH_2O), 4.68–4.48 (2H, dd, J = 14.1 Hz, 1,2,4-triazole-CH_2), 4.47–4.46 (2H, t, CH_2CH_2O), 3.80–3.76 (2H, t, CH_2CH_2O), 3.39–3.11 (2H, dd, J = 14.7 Hz, 1,2,3-triazole-CH_2C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.25, 158.28, 151.01, 147.59, 145.34, 141.95, 134.29, 130.37, 129.04, 128.95, 126.19, 126.06, 124.98, 124.46, 110.97, 106.16, 74.17, 69.33, 68.92, 57.31, 49.61, 35.03; ESI-MS (*m/z*): 486.1 (M+H); Anal. calcd. for $C_{22}H_{21}F_2N_7O_4$: C, 54.43; H, 4.36; N, 20.20. Found, C, 54.35; H, 4.46; N, 20.13.

*1-(1-(2-((3-Nitrobenzyl)oxy)ethyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**6n**)*

The product **6n** was obtained as a white solid. Mp: 138.3–139.8°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 8.26 (1H, s, 1,2,4-triazole-H), 7.74 (1H, s, 1,2,4-triazole-H), 7.61 (1H, s, 1,2,3-triazole-H), 8.15–6.72 (7H, m, Ar-H), 5.96 (1H, s, OH), 4.68–4.52 (2H, dd, J = 4.4 Hz, 1,2,4-triazole-CH_2), 4.57–4.46 (4H, m, ArCH_2O, CH_2CH_2O), 3.78–3.75 (2H, t, CH_2CH_2O), 3.39–3.11 (2H, dd, J = 15.0 Hz, 1,2,3-triazole-CH_2C(OH); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): δ 160.69, 158.22, 150.99, 148.21, 145.31, 141.93, 140.90, 134.18, 130.28, 126.16, 126.09, 124.40, 122.85, 122.12, 110.96, 106.60, 74.16, 70.90, 68.94, 57.30, 49.59, 34.98; ESI-MS (*m/z*): 486.1 (M+H); Anal. calcd. for $C_{22}H_{21}F_2N_7O_4$: C, 54.43; H, 4.36; N, 20.20. Found, C, 54.37; H, 4.40; N, 20.25

Pharmacology

The *in vitro* antifungal activities of all title compounds were evaluated against eight human pathogenic fungi, *Candida albicans*, *Candida parasilosis*, *Candida krusei*, *Cryptococcus neoformans*, *Microsporum gypseum*, *Trichophyton rubrum*, *Aspergillus fumigates*, which are often encountered clinically, and were compared with fluconazole, itraconazole, voriconazole, amphotericin B. *Candida albicans* (ATCCSC5314) and *Cryptococcus neoformans* were provided by Shanghai Changzheng Hospital; *Candida albicans* (Y0109), *Candida parapsilosis*, *Candida krusei*, *Trichophyton rubrum*, *Microsporum gypseum*, and *Aspergillus fumigatus* were provided by Shanghai Changhai Hospital. *Candida albicans* and *Cryptococcus*

neoformans were purchased from ATCC, and other strains were clinic isolates. *Candida albicans* (ATCCSC5314) and *Cryptococcus neoformans* were used as the quality-controlled strains, and tested in each assay. Fluconazole (FCZ), ketoconazole (KCZ), itraconazole (ICZ), voriconazole (VCZ), and amphotericin B (AMB) 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) [1]. 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 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 data are the mean of three replicate tests with each antifungal compound [13].

Molecular docking

In our studies, we constructed a 3D model of *Candida albicans* CYP51 on the basis of Ji et al. All the molecular modeling calculations were performed using SYBYL 6.9 version, and the structures of the compounds were assigned with Gasteiger–Hückel partial atomic charges. Energy minimization was performed using the Tripos force field, Powell optimization method, and MAXIMIN 2 minimizer with a convergence criterion 0.001 kcal/mol 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 values.

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