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Synthesis and Antifungal Activity of Novel Triazole Derivatives

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A series of novel azoles (**a**-**v**), which are analogues of fluconazole, have been designed and synthesized as potential antifungal agents by the click reaction. The click reaction approach toward the synthesis of novel 1,2,3-triazolyl linked triazole antifungal derivatives **a**-**v** was achieved by Cu(I)-catalyzed 1,3-dipolar cycloaddition of propargylated intermediate **5** with substituted azidomethyl benzene. In addition, the target compounds tested can increase antifungal activity.

Key words: Azole, Click reaction, Antifungal activity, CYP51

INTRODUCTION

Fungal infections have become an important complication and a major cause of morbidity and mortality in immunocompromised individuals suffering from tuberculosis, cancer, or AIDS and in organ transplant cases (Fridkin and Jarvis, 1996; Wingard and Leather, 2004). Several clinical drugs, such as azoles, amphotericin B, 5-fluorocytosine, and caspofungin, have been developed to reduce the impact of fungal diseases. Among those, azoles, especially triazole antifungal agents, have been used widely and efficiently. For example, fluconazole, voriconazole, and itraconazole (Fig. 1) presently play a leading role in the treatment of invasive fungal infections. Triazole antifungals (e.g. fluconazole and voriconazole), which act by inhibiting lanosterol cytochrome P450 14α-demethylase (CYP51), have now become the most rapidly expanding group of antifungal compounds. However, current antifungal therapy suffers from drug-related toxicity, severe drug resistance, non-optimal pharmacokinetics, and serious drug-drug interactions (Latgé, 1999; Steenbergen and Casadevall, 2000). That situation highlights the need for advent of safe, novel, and effective antifungal compounds. In addition, the structure-activity of the inhibition of CYP51 should be further studied.

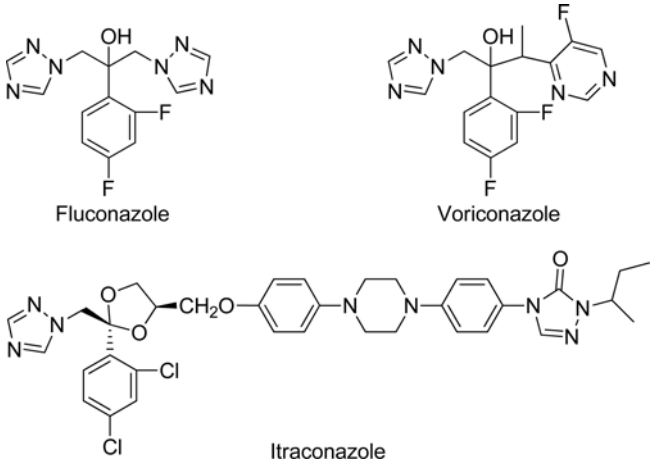

Fig. 1. Triazole antifungal agents used in clinical therapy

Azoles exert antifungal activity through inhibition of CYP51 by a mechanism in which the heterocyclic nitrogen (N-3 of imidazole or N-4 of 1,2,4-tiazole) binds to the sixth coordination of heme iron atom of the porphyrin in the substrate binding site of the enzyme (Trzaskos et al., 1986; Aoyama et al., 1987). Based on the structure of the active site of CYP51 and extensive investigation of the structure-activity relationships (SAR) of azole antifungals, it was found that the triazole ring, the difluorophenyl group, and the hydroxyl group were the pharmacophores of antifungal agents. The side chains located in the narrow hydrophobic

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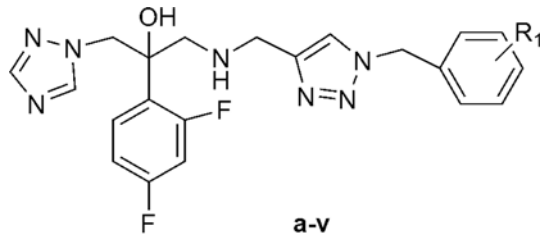


a: R_1 = 2-F
b: R_1 = 3-F
c: R_1 = 4-F
d: R_1 = 2-Cl
e: R_1 = 3-Cl
f: R_1 = 4-Cl
g: R_1 = 2-Br
h: R_1 = 3-Br
i: R_1 = 4-Br
j: R_1 = 2-CH_3
k: R_1 = 3-CH_3
l: R_1 = 4-CH_3
m: R_1 = 2-NO_2
n: R_1 = 3-NO_2
o: R_1 = 4-NO_2
p: R_1 = 2-CN
q: R_1 = 3-CN
r: R_1 = 4-CN
s: R_1 = H
t: R_1 = 4-OCH_3
u: R_1 = 2-Cl,6-Cl
v: R_1 = 4-$C(CH_3)_3$

Fig. 2. Generic structure of the designed fluconazole analogues

cleft were also found to be important (Fischer et al., 1991; Hope et al., 2007).

Based on the above-discussed results and new discoveries (Ji et al., 2000), we have designed a series of novel azoles (Fig. 2) as fluconazole analogues that contain a triazole ring, a difluorophenyl group, a hydroxyl group, and a side chain with another triazole group. While those compounds contain all the essential pharmacophores, the introduction of an additional triazole group was expected to enhance the interaction between those molecules and CYP51 to potentially result in systemic antifungal compounds that are less likely to develop drug resistance. Moreover, we systematically altered the side chain structure, which is oriented to interact with the narrow hydrophobic cleft, to explore how it may further affect antifungal activity.

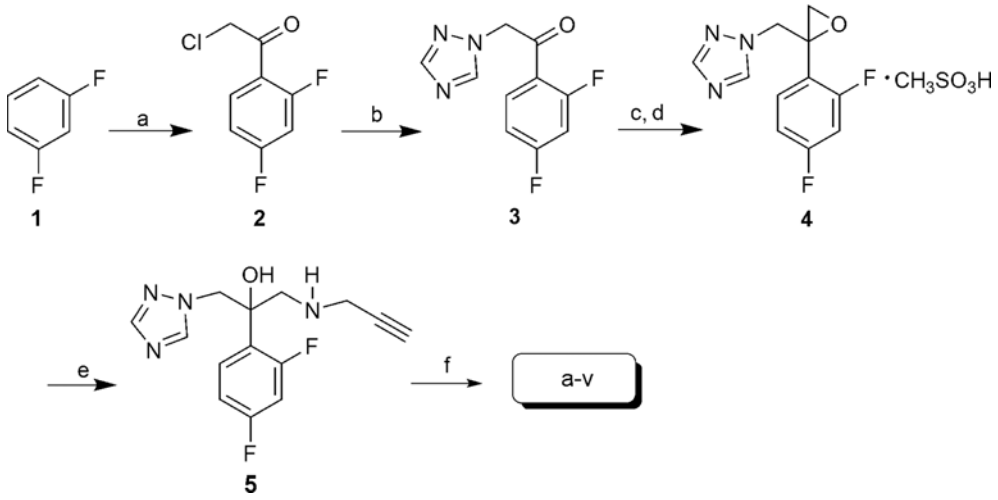
MATERIALS AND METHODS

^{1}H- and ^{13}C-NMR (Nuclear magnetic resonance) spectra were recorded in $CDCl_3$ unless otherwise indicated with a Bruker AC-300P spectrometer, using TMS (Tetramethylsilane) as internal standard. The HPLC-MS (High Performance Liquid Chromatography-Mass Spectrometry) were recorded on Agilent 1100 series LC-MS. The solvents and reagents were purchased from commercial vendors and were used either as received or dried prior to use as needed.

Compounds **a-v** were synthesized according to an efficient route based on the click reaction, as outlined in Scheme 1. After the key intermediate **4** was prepared by a reported procedure, compound **5** was obtained by ring-open reaction of oxirane **4** with alkyne propylamine. Synthesis of all the target triazole antifungal derivatives **a-v** was achieved using the Cu (I)-catalyzed Sharpless click chemistry (Zhang et al., 2006) approach from propargylated intermediate **5** and substituted azidomethyl benzene. The addition of Cu (I) catalyst strongly activates terminal acetylenes toward 1,3-dipole in organic azides, exclusively forming the 1,4-disubstituted regioisomer.

Synthesis of 1-(1H-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-alkyne propylamine-2-propanol (5)

A mixture of compound **4** (33.3 g, 0.10 mol), CH_3CH_2OH (500 mL), and Et_3N (50 mL), alkyne pro-



Scheme 1. Synthesis of the target compounds **a-v**. Conditions: (a) $ClCH_2COCl$, $AlCl_3$, 50°C, 5 h, 80%; (b) 1H-1,2,4-triazole, $NaHCO_3$, toluene, reflux, 5 h, 42%; (c) $(CH_3)_3SOI$, NaOH, centylmethylammonium bromide, toluene, 60°C, 3 h, 53%; (d) CH_3SO_3H, 0°C, 1 h, 89%; (e) Et_3N, alkyne propylamine, EtOH, reflux, 6 h, 81.5%; (f) NaN_3, substituted alkyne propylamine, DMSO/H_2O, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, rt, 12 h, 75-88%

pylamine (8.3 g, 0.15 mol) was stirred and refluxed for 6 h. The reaction was monitored by thin liquid chromatography (TLC). After filtration, the filtrate was evaporated under reduced pressure. Water was added to the residue, extracted with ethylacetate twice, combined with the organic layer, washed with saturated NaCl solution twice, dried over anhydrous Na_2SO_4, and evaporated to get compound **5** (23.8 g, 81.5%).

General procedure for the target compounds (a-v)

A mixture of NaN_3 (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. Next, we added the compound **5** (175 mg, 0.6 mmol), sodium ascorbate (20 mg), $CuSO_4 \cdot 5H_2O$ (25 mg), and H_2O (1 mL) and stirred the mixture at room temperature for 2 h, then put the reaction solution into $NH_3 \cdot H_2O$, extracted with ethylacetate. Following that, the organic layer was acidificated with dilute hydrochloric acid, then the aqueous layer was adjusted to a pH of about 7 by saturation sodium bicarbonate, extracted with ethylacetate, washed with water, and dried with Na_2SO_4 concentrated in a vacuum to afford compound **a** (181 mg, 68%).

The target compounds **b-v** were synthesized by same procedure as compound **a.**

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl) methyl] amino}-2-propanol (a)

IR (KBr): 3350, 3284, 3080, 1635, 1550, 1173, 785; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.06 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 7.58 (1H, s, triazole-H), 6.70-7.36 (7H, m, Ar-H), 5.54 (2H, s, Ar-CH_2-), 4.51 (1H, d, J = 14.2 Hz, triazole-CH_2-), 4.41 (1H, d, J = 14.2 Hz, triazole-CH_2-), 3.63 (1H, d, J = 14.3 Hz, triazole-CH_2-), 3.47 (1H, d, J = 14.3 Hz, triazole-CH_2-), 2.73 (1H, d, J = 13.9 Hz, CH_2), 2.65 (1H, d, J = 13.9 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.67, 160.33, 153.52, 148.63, 143.13, 137.68, 133.64, 131.11, 129.35, 128.96, 128.74, 128.46, 127.42, 124.78, 110.65, 102.47, 65.10, 62.38, 59.51, 58.74, 51.54; LC-MS m/z: 444.1 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}F_3N_7O$: 443.1681; found: 443.1697.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (b)

IR (KBr): 3351, 3274, 3082, 1638, 1557, 1174, 783; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.09 (1H, s, triazole-H), 7.77 (1H, s, triazole-H), 7.68 (1H, s, triazole-H), 6.72-7.38 (7H, m, Ar-H), 5.57 (2H, s, Ar-CH_2-), 4.47 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.43 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.53 (1H, d, J = 14.2 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.2 Hz, triazole-CH_2-), 2.83 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.52, 160.91, 151.52, 148.23, 143.38, 137.74, 134.64, 131.17, 129.50, 129.21, 129.24, 128.46, 128.42, 125.78, 110.08, 102.26, 64.10, 62.29, 59.52, 58.34, 50.13; LC-MS m/z: 444.3 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}F_3N_7O$: 443.1681; found: 443.1695.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (c)

IR (KBr): 3353, 3281, 3078, 1637, 1559, 1164, 835; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.07 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 7.67 (1H, s, triazole-H), 6.71-7.44 (7H, m, Ar-H), 5.48 (2H, s, Ar-CH_2-), 4.46 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.43 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.53 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.42 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.87 (1H, d, J = 13.8 Hz, CH_2), 2.69 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 163.38, 160.76, 152.51, 148.61, 143.22, 137.64, 134.64, 131.17, 129.61, 129.44, 129.23, 128.56, 128.52, 124.78, 110.45, 101.26, 64.10, 62.38, 59.52, 57.34, 51.11; LC-MS m/z: 444.2 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}F_3N_7O$: 443.1681; found: 443.1703.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (d)

IR (KBr): 3363, 3273, 3079, 1643, 1567, 1176, 744; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.11 (1H, s, triazole-H), 7.76 (1H, s, triazole-H), 7.63 (1H, s, triazole-H), 6.71-7.46 (7H, m, Ar-H), 5.56 (2H, s, Ar-CH_2-), 4.45 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.32 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.54 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.84 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.42, 160.73, 151.32, 147.23, 143.46, 137.69, 134.74, 132.17, 129.56, 129.36, 129.21, 128.46, 128.32, 125.49, 111.08, 102.26, 64.11, 62.37, 59.42, 58.56, 51.12; LC-MS m/z: 460.1 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}ClF_2N_7O$: 459.1386; found: 459.1399.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(3-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (e)

IR (KBr): 3357, 3248, 3047, 1629, 1565, 1169, 786; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.07 (1H, s, triazole-H), 7.68 (1H, s, triazole-H), 7.54 (1H, s, triazole-H), 6.77-7.41 (7H, m, Ar-H), 5.76 (2H, s, Ar-CH_2-), 4.48 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.31 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.54 (1H, d, J = 13.7 Hz, triazole-CH_2-), 3.41 (1H, d, J = 13.7 Hz, triazole-CH_2-), 2.76 (1H, d, J = 13.8 Hz,

CH_2), 2.73 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.57, 161.43, 154.32, 147.38, 143.47, 137.76, 134.54, 132.16, 129.56, 129.28, 129.19, 128.56, 127.32, 125.46, 111.48, 102.17, 64.12, 62.37, 59.36, 58.57, 53.14; LC-MS m/z: 460.1 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}ClF_2N_7O$: 459.1386; found: 459.1402.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (f)

IR (KBr): 3352, 3274, 3082, 1638, 1558, 1174, 843; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.09 (1H, s, triazole-H), 7.97 (1H, s, triazole-H), 7.68 (1H, s, triazole-H), 6.72-7.38 (7H, m, Ar-H), 5.57 (2H, s, Ar-CH_2-), 4.47 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.43 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.54 (1H, d, J = 14.2 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.2 Hz, triazole-CH_2-), 2.83 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.52, 160.91, 151.52, 149.23, 143.38, 137.74, 134.64, 131.17, 129.50, 129.21, 129.37, 128.46, 128.42, 125.78, 110.08, 102.26, 64.10, 62.29, 59.52, 58.54, 50.13; LC-MS m/z: 460.3 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}ClF_2N_7O$: 459.1386; found: 459.1403.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(2-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (g)

IR (KBr): 3373, 3268, 3071, 1643, 1567, 1175, 741; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.13 (1H, s, triazole-H), 7.76 (1H, s, triazole-H), 7.61 (1H, s, triazole-H), 6.73-7.46 (7H, m, Ar-H), 5.54 (2H, s, Ar-CH_2-), 4.45 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.32 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.44 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.84 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.42, 160.71, 151.32, 147.23, 144.46, 137.69, 134.71, 132.16, 129.56, 129.36, 129.22, 128.46, 128.37, 125.49, 111.18, 102.26, 64.11, 62.36, 59.42, 58.56, 52.12; LC-MS m/z: 504.4 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}BrF_2N_7O$: 503.0881; found: 503.0895.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(3-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (h)

IR (KBr): 3351, 3283, 3082, 1636, 1550, 1163, 786; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.16 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 7.58 (1H, s, triazole-H), 6.70-7.46 (7H, m, Ar-H), 5.54 (2H, s, Ar-CH_2-), 4.51 (1H, d, J = 14.2 Hz, triazole-CH_2-), 4.41 (1H, d, J = 14.2 Hz, triazole-CH_2-), 3.62 (1H, d, J = 14.3 Hz, triazole-CH_2-), 3.47 (1H, d, J = 14.3 Hz, triazole-CH_2-), 2.73 (1H, d, J = 13.9 Hz, CH_2), 2.64 (1H, d, J = 13.9 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.67, 160.32, 153.52, 148.65, 143.13, 137.58, 133.64, 131.21, 129.35, 128.96, 128.76, 128.46, 127.42, 124.76, 111.65, 102.47, 65.10, 62.37, 59.51, 58.74, 51.53; LC-MS m/z: 504.1 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}BrF_2N_7O$: 503.0881; found: 503.0898.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (i)

IR (KBr): 3361, 3262, 3076, 1643, 1561, 1176, 839; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.14 (1H, s, triazole-H), 7.86 (1H, s, triazole-H), 7.61 (1H, s, triazole-H), 6.71-7.41 (7H, m, Ar-H), 5.56 (2H, s, Ar-CH_2-), 4.47 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.32 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.54 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.83 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.37, 160.71, 151.32, 147.23, 143.46, 136.69, 134.71, 132.17, 129.56, 129.46, 129.21, 128.36, 128.12, 125.49, 111.17, 102.26, 64.11, 62.38, 59.42, 58.56, 52.14; LC-MS m/z: 504.5 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}BrF_2N_7O$: 503.0881; found: 503.0897.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (j)

IR (KBr): 3374, 3257, 3074, 1652, 1568, 1176, 741; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.15 (1H, s, triazole-H), 7.73 (1H, s, triazole-H), 7.61 (1H, s, triazole-H), 6.71-7.46 (7H, m, Ar-H), 5.54 (2H, s, Ar-CH_2-), 4.46 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.31 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.44 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.37 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.84 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2), 2.37 (3H, s, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 163.41, 161.71, 151.48, 147.21, 144.45, 137.79, 135.71, 132.16, 129.57, 129.36, 129.02, 128.46, 128.36, 125.49, 111.17, 103.26, 64.15, 62.34, 59.42, 58.57, 52.22, 20.93; LC-MS m/z: 440.2 $(M+H)^+$; HRMS (m/z): calcd. for $C_{22}H_{23}F_2N_7O$: 439.1932; found: 439.1951.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (k)

IR (KBr): 3374, 3268, 3071, 1642, 1567, 1185, 776; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.03 (1H, s, triazole-H), 7.76 (1H, s, triazole-H), 7.61 (1H, s, triazole-H), 6.73-7.56 (7H, m, Ar-H), 5.54 (2H, s, Ar-CH_2-), 4.45 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.32 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.43 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.40 (1H, d, J = 14.1Hz, triazole-CH_2-), 2.84 (1H, d, J =

13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2), 2.35 (3H, s, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.41, 160.71, 151.32, 147.23, 144.56, 137.69, 134.71, 132.16, 129.57, 129.36, 129.32, 128.46, 128.37, 125.49, 111.28, 102.26, 64.13, 62.36, 59.42, 58.56, 52.12, 21.92; LC-MS m/z: 440.7 $(M+H)^+$; HRMS (m/z): calcd. for $C_{22}H_{23}F_2N_7O$: 439.1932; found: 439.1955.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (l)

IR (KBr): 3374, 3268, 3071, 1653, 1567, 1175, 832; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.13 (1H, s, triazole-H), 7.75 (1H, s, triazole-H), 7.61 (1H, s, triazole-H), 6.73-7.46 (7H, m, Ar-H), 5.53 (2H, s, Ar-CH_2-), 4.45 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.32 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.54 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.84 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2), 2.33 (3H, s, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.42, 160.77, 151.32, 147.13, 144.46, 137.69, 134.75, 132.16, 129.56, 129.46, 129.22, 128.46, 128.38, 125.49, 111.38, 102.26, 64.12, 62.36, 59.42, 58.56, 52.32, 20.62; LC-MS m/z: 440.4 $(M+H)^+$; HRMS (m/z): calcd. for $C_{22}H_{23}F_2N_7O$: 439.1932; found: 439.1948.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(2-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (m)

IR (KBr): 3351, 3274, 3080, 1633, 1551, 1159, 784; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.16 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 7.58 (1H, s, triazole-H), 6.70-7.26 (7H, m, Ar-H), 5.54 (2H, s, Ar-CH_2-), 4.53 (1H, d, J = 14.2 Hz, triazole-CH_2-), 4.41 (1H, d, J = 14.2 Hz, triazole-CH_2-), 3.61 (1H, d, J = 14.3 Hz, triazole-CH_2-), 3.47 (1H, d, J = 14.3 Hz, triazole-CH_2-), 2.73 (1H, d, J = 13.9 Hz, CH_2), 2.65 (1H, d, J = 13.9 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.77, 160.43, 153.52, 148.63, 142.13, 137.68, 133.64, 131.36, 129.35, 128.97, 128.74, 128.41, 127.42, 124.78, 110.45, 102.42, 65.10, 62.37, 59.51, 58.72, 53.54; LC-MS m/z: 471.5 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}F_2N_8O_3$: 470.1626; found: 470.1641.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(3-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (n)

IR (KBr): 3359, 3248, 3057, 1628, 1565, 1167, 785; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.17 (1H, s, triazole-H), 7.69 (1H, s, triazole-H), 7.54 (1H, s, triazole-H), 6.77-7.46 (7H, m, Ar-H), 5.76 (2H, s, Ar-CH_2-), 4.38 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.21 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.54 (1H, d, J = 13.7 Hz, triazole-CH_2-), 3.41 (1H, d, J = 13.7 Hz, triazole-CH_2-), 2.78 (1H, d, J = 13.8 Hz, CH_2), 2.73 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.58, 161.43, 154.32, 147.48, 143.47, 137.76, 134.55, 132.16, 129.57, 129.28, 129.18, 128.52, 127.31, 125.46, 111.48, 102.27, 64.12, 62.47, 59.31, 58.57, 53.13; LC-MS m/z: 471.1 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}F_2N_8O_3$: 470.1626; found: 470.1641.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (o)

IR (KBr): 3351, 3284, 3087, 1637, 1556, 1174, 847; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.02 (1H, s, triazole-H), 7.77 (1H, s, triazole-H), 7.58 (1H, s, triazole-H), 6.71-7.38 (7H, m, Ar-H), 5.57 (2H, s, Ar-CH_2-), 4.46 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.43 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.53 (1H, d, J = 14.2 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.2 Hz, triazole-CH_2-), 2.81 (1H, d, J = 13.8 Hz, CH_2), 2.74 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 163.51, 160.78, 151.52, 149.24, 143.38, 138.74, 134.64, 131.29, 129.51, 129.26, 129.17, 128.46, 128.32, 125.71, 110.48, 102.21, 64.10, 62.29, 58.52, 58.54, 51.14; LC-MS m/z: 471.4 $(M + H)^+$; HRMS (m/z): calcd. for $C_{21}H_{20}F_2N_8O_3$: 470.1626; found: 470.1645.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(2-cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (p)

IR (KBr): 3374, 3278, 3072, 1637, 1576, 1173, 744; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.12 (1H, s, triazole-H), 7.75 (1H, s, triazole-H), 7.61 (1H, s, triazole-H), 6.71-7.46 (7H, m, Ar-H), 5.54 (2H, s, Ar-CH_2-), 4.46 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.32 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.42 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.84 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.41, 160.71, 151.32, 147.53, 144.46, 137.61, 134.71, 132.16, 129.56, 129.35, 129.22, 128.46, 128.31, 125.47, 111.18, 101.26, 64.11, 62.36, 59.42, 58.53, 52.15; LC-MS m/z: 451.1 $(M+H)^+$; HRMS (m/z): calcd. for $C_{22}H_{20}F_2N_8O$: 450.1728; found: 450.1745.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(3-cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (q)

IR (KBr): 3356, 3247, 3049, 1626, 1564, 1169, 785; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.17 (1H, s, triazole-H), 7.68 (1H, s, triazole-H), 7.54 (1H, s, triazole-H), 6.76-7.41 (7H, m, Ar-H), 5.76 (2H, s, Ar-CH_2-), 4.47 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.31 (1H, d, J = 14.4 Hz,

triazole-CH_2-), 3.55 (1H, d, J = 13.7 Hz, triazole-CH_2-), 3.41 (1H, d, J = 13.7 Hz, triazole-CH_2-), 2.76 (1H, d, J = 13.8 Hz, CH_2), 2.72 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.58, 161.53, 154.32, 147.38, 143.57, 137.76, 134.58, 132.16, 129.56, 129.38, 129.19, 128.54, 127.31, 125.46, 111.48, 102.17, 64.12, 62.39, 59.36, 58.57, 52.17; LC-MS m/z: 451.3 $(M+H)^+$; HRMS (m/z): calcd. for $C_{22}H_{20}F_2N_8O$: 450.1728; found: 450.1742.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (r)

IR (KBr): 3352, 3287, 3078, 1647, 1559, 1164, 833; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.06 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 7.67 (1H, s, triazole-H), 6.71-7.54 (7H, m, Ar-H), 5.48 (2H, s, Ar-CH_2-), 4.46 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.41 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.53 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.42 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.86 (1H, d, J = 13.8 Hz, CH_2), 2.69 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.31, 160.75, 152.51, 148.62, 143.22, 137.64, 134.63, 131.17, 129.61, 129.44, 129.21, 128.56, 128.52, 124.79, 110.45, 102.26, 64.10, 62.37, 59.52, 57.34, 52.14; LC-MS m/z: 451.4 $(M+H)^+$; HRMS (m/z): calcd. for $C_{22}H_{20}F_2N_8O$: 450.1728; found: 450.1743.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amino}-2-propanol (s)

IR (KBr): 3361, 3262, 3076, 1643, 1561, 1176; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.13 (1H, s, triazole-H), 7.81 (1H, s, triazole-H), 7.56 (1H, s, triazole-H), 6.74-7.31 (8H, m, Ar-H), 5.53 (2H, s, Ar-CH_2-), 4.45 (1H, d, J = 13.9 Hz, triazole-CH_2-), 4.31 (1H, d, J = 13.9 Hz, triazole-CH_2-), 3.54 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.45 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.83 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.32, 160.57, 151.49, 147.21, 143.56, 136.61, 134.74, 132.38, 129.51, 129.38, 129.24, 128.36, 127.12, 125.36, 110.17, 102.25, 64.12, 62.49, 59.41, 58.57, 53.28; LC-MS m/z: 426.5 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{21}F_2N_7O$: 425.1776; found: 425.1790.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (t)

IR (KBr): 3363, 3252, 3074, 1642, 1567, 1176, 837; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.14 (1H, s, triazole-H), 7.86 (1H, s, triazole-H), 7.71 (1H, s, triazole-H), 6.72-7.41 (7H, m, Ar-H), 5.56 (2H, s, Ar-CH_2-), 4.37 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.31 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.73 (3H, s, -OCH_3), 3.54 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.83 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.48, 160.72, 151.32, 147.27, 143.46, 136.69, 134.71, 132.15, 129.56, 129.43, 129.21, 128.37, 128.12, 125.49, 111.17, 101.26, 64.11, 62.35, 59.42, 58.46, 56.3, 52.13; LC-MS m/z: 456.7 $(M+H)^+$; HRMS (m/z): calcd. for $C_{22}H_{23}F_2N_7O_2$: 455.1881; found: 455.1898.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(2,6-dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (u)

IR (KBr): 3358, 3263, 3075, 1641, 1562, 1178, 876, 834; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.15 (1H, s, triazole-H), 7.83 (1H, s, triazole-H), 7.61 (1H, s, triazole-H), 6.73-7.41 (6H, m, Ar-H), 5.56 (2H, s, Ar-CH_2-), 4.45 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.32 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.53 (1H, d, J = 14.1 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.1 Hz, triazole-CH_2-), 2.81 (1H, d, J = 13.8 Hz, CH_2), 2.75 (1H, d, J = 13.8 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.45, 161.71, 151.32, 147.20, 143.46, 136.69, 134.01, 132.17, 129.56, 129.37, 129.21, 128.36, 128.12, 125.48, 111.17, 101.26, 64.11, 62.36, 59.42, 58.47, 52.24; LC-MS m/z: 494.3 $(M+H)^+$; HRMS (m/z): calcd. for $C_{21}H_{19}Cl_2F_2N_7O$: 493.0996; found: 493.1011.

1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{N-[(1-(4-tertbutylbenzyl)-1H-1,2,3-triazol-4-yl)methyl] amino}-2-propanol (v)

IR (KBr): 3352, 3284, 3086, 1637, 1556, 1175, 845; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.12 (1H, s, triazole-H), 7.83 (1H, s, triazole-H), 7.58 (1H, s, triazole-H), 6.72-7.38 (7H, m, Ar-H), 5.57 (2H, s, Ar-CH_2-), 4.47 (1H, d, J = 14.4 Hz, triazole-CH_2-), 4.43 (1H, d, J = 14.4 Hz, triazole-CH_2-), 3.53 (1H, d, J = 14.2 Hz, triazole-CH_2-), 3.41 (1H, d, J = 14.2 Hz, triazole-CH_2-), 2.82 (1H, d, J = 13.8 Hz, CH_2), 2.74 (1H, d, J = 13.8 Hz, CH_2), 1.29-1.33 (9H, m, 3-CH3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.47, 160.59, 151.51, 149.24, 143.48, 138.74, 134.64, 131.26, 129.51, 129.36, 129.17, 128.47, 128.32, 125.72, 110.48, 102.24, 64.10, 62.29, 58.32, 58.54, 51.17, 34.83, 31.52, 31.56, 31.54; LC-MS m/z: 482.2 $(M+H)^+$; HRMS (m/z): calcd. for $C_{25}H_{29}F_2N_7O$: 481.2402; found: 481.2425.

Antifungal activity

The *in vitro* antifungal activities of all target compounds were evaluated against eight human pathogenic fungi, *Candida albicans* SC5314 and Y0109, *Cryptococcus neoformans*, *Candida parapsilosis*, *Candida tropicalis*, *Trichophyton rubrum*, *Candida kefyr*, and

Aspergillus fumigatus, which are frequently encountered in clinic. The results were compared with positive controls itraconazole, ketoconazole, voriconazole, and fluconazole. *C. albicans SC5314* and *C. neoformans*, purchased from ATCC (American type culture collection), were provided by Shanghai Changzheng Hospital; *C. parapsilosis*, *C. albicans* Y0109, *C. tropicalis*, *T. rubrum*, *C. kefyr*, and *A. fumigatus*, which are clinic isolates, were provided by Shanghai Changhai Hospital. Fluconazole, itraconazole, ketoconazole, and voriconazole, which served as the positive controls, were obtained from their respective manufacturers. The *in vitro* minimal inhibitory concentrations (MICs) of the title 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, 2002). 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. albicans* and at 72 h for *C. neoformans*. The results of assays are summarized in Table I. Those data are the mean of two replicate tests performed with each antifungal agent.

RESULTS

In our approach to synthesize title compounds, we have performed the click reaction to connect the key intermediate **5** containing the terminal alkynyl and the substituted azidomethyl benzene in the presence of Cu (I)-catalyst in DMSO at room temperature to yield sufficient amounts of the title compounds.

The results of Table I clearly show that the *in vitro* antifungal activities of all the target compounds **a**-**v** were active against nearly all fungi tested, except for *A. fumigatus*. Among the compounds tested, **b**, **c**, **e**, **i**, **p** showed higher activity against *C. albicans* SC5314, *C. albicans* Y0109, *C. parapsilosis*, and *C. kefyr* than

Table I. Antifungal activities of the target compounds *in vitro*

Compound No.	MIC[a] (μg/mL)							
	C. alb SC5314	*C. alb* Y0109	*C. neo*	*T. nru*	*C. tro*	*C. pra*	*C. kef*	*A. fum*
a	0.0625	0.25	16	4	1	0.25	1	>64
b	0.0625	<0.125	0.0156	1	0.0625	0.0625	0.0625	>64
c	0.0156	<0.125	1	0.25	0.0625	0.0625	0.0625	>64
d	0.0625	0.5	16	4	0.0156	0.25	1	>64
e	0.0156	<0.125	0.25	0	0.0625	0.0625	0.25	64
f	0.25	2	4	4	1	0.25	16	>64
g	1	1	16	16	1	1	4	>64
h	0.25	0.5	16	4	1	1	1	>64
i	0.25	<0.125	4	4	1	0.25	1	>64
j	0.25	1	4	16	1	1	4	>64
k	0.0156	0.25	1	4	1	0.25	1	>64
l	0.25	0.5	>64	4	1	0.0625	4	>64
m	0.25	2	>64	4	1	4	4	>64
n	1	1	16	4	4	4	4	>64
o	0.0156	1	16	16	4	1	4	>64
p	0.0625	0.25	4	1	0.25	0.25	0.25	>64
q	0.0625	<0.125	1	0.25	0.0625	0.25	0.0156	>64
r	1	1	16	16	4	1	1	>64
s	0.0156	1	16	16	1	1	1	32
t	0.0625	1	16	4	1	1	1	16
u	0.00097	1	16	4	16	1	1	32
v	0.0156	1	1	4	4	0.25	0.25	32
ICZ	<0.0625	0.0625	0.125	0.0625	<0.0625	0.0625	0.0625	2
KCZ	<0.125	<0.125	0.5	<0.125	<0.125	<0.125	0.0625	0.125
VCZ	32	<0.125	<0.125	<0.125	<0.125	0.25	0.0039	<0.125
FCZ	0.5	0.5	8	2	<0.125	<0.125	1	>64

[a]Minimum inhibitory concentration for 80% inhibition of growth

FCZ. Most of the target compounds exhibited higher activities against *C. albicans* SC5314 than all four positive controls. In particular, the MIC values of compounds **c**, **e**, **k**, **s**, and **v** were 32 times lower than that of FCZ against *C. albicans* SC5314. The MIC values of compounds **b**, **c**, **e**, **i**, and **q** were 40 times lower than that of FCZ against *C. albicans* Y0109, and the MIC value of compound **q** was 64 times lower than that of FCZ against *C. kefyr*.

From those results, we may state that differences between the activities of the compounds were due to different substituents such as F, Br, Cl, and CN over the benzene ring. The substituted positions were also very important for antifungal activity. The hydrophobic group is favorable at position 3 or position 4 of the terminal phenyl group, possibly because of the additional hydrophobic interaction with CYP51.

DISCUSSION

In this work, we found the click reaction to be a facile method to incorporate a hydrophobic substitution onto the side chain of azole. We were able to place a phenyl group onto the side chain of azole via the 1,2,3-triazole. The resulting material suggests self-assembly behavior in both water and DMSO. In addition to its simplicity and mild reaction conditions, that method provides a wide range of inserting 1,2,3-triazoles at excellent yields with high regioselectivity in a single step operation.

On the basis of the active site of CYP51, a series of novel azoles with side chains including a 1,2,3-triazole group and a substituted-phenyl group were designed and synthesized. The side chains interacted with CYP51 through hydrophobic and van der Waals reactions. The *in vitro* antifungal activity assay indicated that the new azoles show good activity against important fungal pathogens. Interestingly, the compounds were also active against an azole-resistant clinical strain. The research has led to the discovery of a series of compounds for further optimization.

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