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Design, Synthesis, and Biological Evaluation of Novel 1, 2, 4-Triazole Derivatives as Antifungal Agent

Xiaoyun Chai[1,*], Shichong Yu[1,*], Yongwei Jiang[1], Yan Zou[1], Qiuye Wu[1], Dazhi Zhang[1], Yuanying Jiang[2], Yongbing Cao[2], and Qingyan Sun[1]

[1]Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, China and [2]Drug Research Center, School of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, China

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A series of novel 1, 2, 4-triazole derivatives (**9a**-**p**) have been designed and synthesized as the potential antifungal agents. All compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and LC-MS. Their antifungal activities against seven human pathogenic fungi were evaluated *in vitro* by measuring the minimal inhibitory concentrations. Most of the tested compounds were found to be more potent against *Candida albicans* than the control drug fluconazole.

Key words: Triazole, Synthesis, Antifungal activity, CACYP51, Molecular docking

INTRODUCTION

There has been tremendous increase in the frequency of fungal infections during the past four decades (Warnock, 2007). As we all know, *Candida albicans, Cryptococcus neoformans,* and *Aspergillus fumigatus* are the most dreadful human pathogens (Walsh et al., 2004; Vonberg and Gastmeier, 2006). And for the treatment of these infections, azoles (such as fluconazole and itraconazole) are available in clinical use due to their broad antifungal spectrum, high potency and low toxicity (Maertens, 2004). However, azoles' clinical uses have been limited by the emergence of drug resistance recently (Mayr and Lass-Flörl, 2011). Hence, it is now widely recognized that there is still a need to screen for safe and efficient chemotherapeutic agents with potent antifungal activities.

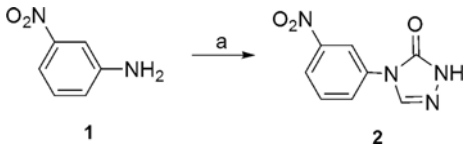
Azoles, which is used as the first choice of fungal infections, have been shown to inhibit lanosterol 14α-demethylase (CYP51), leading to block ergosterol synthesis, and thereby, fungal growth by binding in the active-site cavity of the enzyme and ligating the iron atom of the heme cofactor through a nitrogen atom of the azole (Trösken et al., 2006). From previous researches (Chai et al., 2009, 2011; Yu et al., 2010; Yan et al., 2011), we concluded that the triazole ring, the diurophenyl group and the hydroxyl group were very indispensable for the antifungal activity. However, the side chains were also very important to the effect of antifungal agent.

On the basis of these observations, we thought of an alteration of the side chains with a triazolone side chain. In this case, the N atom of the target triazole antifungal compounds may be coordinated to the iron atom of the heme, the diuorophenyl group may be located into the hydrophobic pocket, and the triazolone side chain may be interacted with the residues of the narrow hydrophobic cleft, according to our hypothesis. We hope the introduction of the side chains can help us find potent systemic antifungal agents with a broad antifungal spectrum and less potential to develop resistance.

MATERIALS AND METHODS

^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$,

*These authors contributed equally to this work.
Correspondence to: Qingyan Sun, Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, China
Tel, Fax: 86-21-8187-1228
E-mail: sqy_2000@163.com
Yongbing Cao, Drug Research Center, School of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, China
Tel, Fax: 86-21-8187-1275
E-mail: ybcao@vip.sina.com



Scheme 1. Synthesis of the Intermediate **2**. Conditions: (a) Methyl carbazate, Trimethoxymethane, CH_3ONa, CH_3OH, *p*-toluenesulfonic acid, reflux, 5 h.

unless otherwise indicated with a Bruker AC-300P spectrometer, using TMS as internal standard. The HPLC-MS were recorded on Agilent 1100 series LC-MS. The solvents and reagents were purchased from commercial vendors and were used as received or dried, prior to use as needed.

Synthetic routes of the intermediate **2** and the oxirane intermediate **6** are outlined in Scheme 1 and Scheme 2. The intermediate **2** was generated by using 3-Nitroaniline to react with Trimethoxymethane, Methyl carbazate and *p*-toluenesulfonic acid under basic medium (Krishnamurthy and James, 1998). According to a known procedure, the oxirane intermediate **6** was obtained by Corey-Chaykovsky epoxidation in the presence of trimethylsulfoxonium iodide and NaOH (Richardson, 1983).

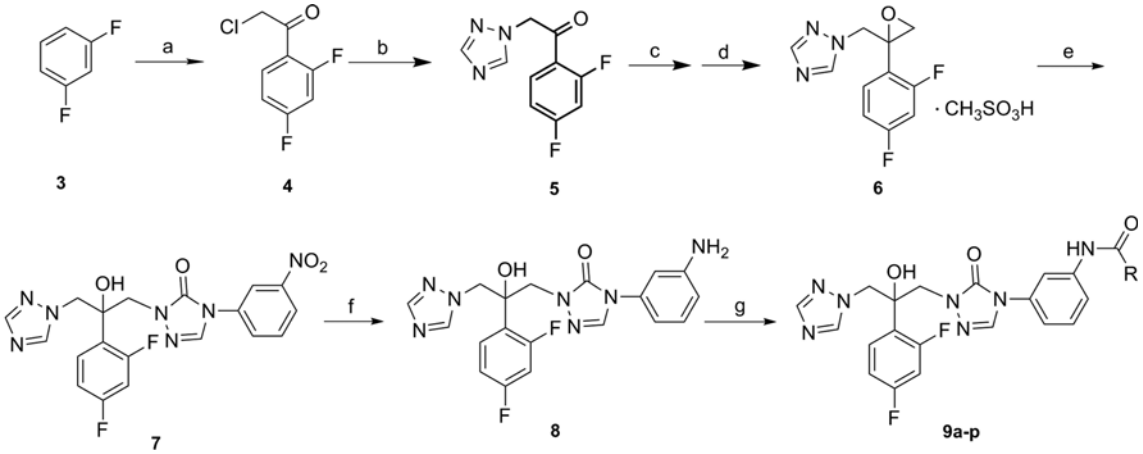
The synthetic route of compounds **9a**-**p** was outlined in Scheme 2. In the presence of potassium carbonate in DMF, treatment of the oxirane intermediate **6** with the corresponding intermediate **2** at 80°C for 6 h gave compound **7**. Reduction of compound **7** with H_2, originated compound **8**, which by reacting with the corresponding carboxylic acid in the presence of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI) and 4-dimethyl-aminopyridine (DMAP) at room temperature for 6 h led to the synthesis of compounds **9a**-**p** (Scheme 2). All the target compounds were obtained as racemates.

Synthesis of the intermediate 4-(3-nitrophenyl)-1H-1, 2, 4-triazol-5(4H)-one (2)

Methyl carbazate (3.90 g, 0.043 mol) and Trimethoxymethane (4.60 g, 0.043 mol) were added to a solution of 3-Nitroaniline (5.70 g, 0.041 mol) in methanol (150 mL). After the reaction mixture was refluxed for 2 h, 25% sodium methoxide (3.50 g, 0.064 mol) was added dropwise to the mixture which continued to reflux for additional 3 h. The resulting reaction mixture was concentrated under reduced pressure, then added 200 mL water to the syrup and adjusted the pH value to 1 with concentrated hydrochloric acid. Filtration of the latter mixture gave the corresponding intermediate **2** (5.51 g, 65%).

Synthesis of 1-(2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl) -4-(3-nitrophenyl)-1H-1, 2, 4-triazol-5(4H)-one (7)

The oxirane intermediate **6** (5.00 g, 0.015 mol) and corresponding intermediate **2** (2.10 g, 0.010 mol) were added to a suspension of potassium carbonate in DMF. Then the reaction mixture was refluxed for 6 h. The reaction was monitored by TLC. 5-fold amount water was added to the mixture, which was extracted with ethyl acetate. The combined organic layer was washed with saturated brine (80 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was crystallized from ethanol (5.65 g, 85%).



Scheme 2. Synthesis of the target compounds **9a**-**p**. 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) Intermediate **2**, DMF, K_2CO_3, 80°C, 6 h, 85%; (f) Pd/C, H_2, rt, 4 h, 95%; (g) RCOOH, EDCI/DMAP, CH_2Cl_2, rt, 6 h, 70-80%.

Synthesis of 4-(3-aminophenyl)-1-(2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl) propyl)-1H-1, 2, 4-triazol-5(4H)-one (8)

Compound **7** (5.65 g, 0.013 mol) was reduced by catalytic hydrogenation with H_2 and 10% Pd/C (0.20 g) in 20 mL methanol to get compound **8** (5.10 g, 95%).

General procedure for the target compounds (9a-p)

Substituted Carboxylic Acids (0.0015 mol), EDCI (0.20 g, 0.0013 mol) and DMAP (0.10 g, 0.0008 mol) was added to a solution of compound **8** (0.413 g, 0.001 mol) in CH_2Cl_2 (30 mL). Then the reaction mixture was stirred at room temperature for 6 h. The mixture was concentrated under reduced pressure, and extracted with ethyl acetate. The combined organic layer was washed with 1 N HCl (80 mL), saturated $NaHCO_3$ (80 mL) and saturated brine (80 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a crude product which was crystallized from ethanol.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4 (5H)-yl}phenyl)-2-fluorobenzamide (9a)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.45 (s, 1H, NH), 8.19 (1H, s, triazole-H), 7.94 (1H, s, triazole-H), 6.78-7.81 (12H, m, Ar-H), 5.75 (1H, s, OH), 4.71 (2H, s, triazole-CH_2-), 4.18-4.57 (2H, dd, J = 14.7 Hz, -CH_2-triazolone); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.9, 161.0, 159.8, 158.5, 151.7, 150.2, 146.9, 143.2, 136.1, 133.7, 132.9, 131.4, 131.3, 130.0, 129.5, 126.8, 125.3, 123.5, 118.4, 115.6, 113.7, 110.3, 104.7, 62.5, 62.0, 61.1; LC-MS (ESI), m/z Calcd. for $C_{26}H_{20}F_3N_7O_3$, 535.2, found $[M+1]^+$ 536.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-3-fluorobenzamide (9b)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.46 (s, 1H, NH), 8.18 (1H, s, triazole-H), 7.95 (1H, s, triazole-H), 6.79-7.82 (12H, m, Ar-H), 5.71 (1H, s, OH), 4.72 (2H, s, triazole-CH_2-), 4.18-4.58 (2H, dd, J = 14.7 Hz, -CH_2-triazolone); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 165.2, 161.4, 159.6, 158.2, 151.3, 150.5, 146.6, 143.2, 136.1, 133.5, 132.2, 131.4, 131.0, 130.1, 129.3, 126.6, 125.5, 123.8, 118.2, 115.2, 113.5, 110.9, 104.0, 62.4, 62.1, 61.0; LC-MS (ESI), m/z Calcd. for $C_{26}H_{20}F_3N_7O_3$, 535.2, found $[M+1]^+$ 536.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-3-chlorobenzamide (9c)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.72 (s, 1H, NH), 8.41 (1H, s, triazole-H), 7.94 (1H, s, triazole-H), 6.76-7.83 (12H, m, Ar-H), 5.71 (1H, s, OH), 4.76 (2H, s, triazole-CH_2-), 4.21-4.52 (2H, dd, J = 14.7 Hz, -CH_2-triazolone); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.5, 160.7, 159.2, 158.5, 151.4, 150.2, 146.2, 143.4, 136.5, 133.6, 132.4, 131.6, 131.0, 130.5, 129.2, 126.8, 125.0, 123.3, 118.5, 115.2, 113.6, 110.1, 104.5, 63.4, 62.2, 61.6; LC-MS (ESI), m/z Calcd. for $C_{26}H_{20}ClF_2N_7O_3$, 551.1, found $[M+1]^+$ 552.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-4-chlorobenzamide (9d)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.46 (s, 1H, NH), 8.17 (1H, s, triazole-H), 7.95 (1H, s, triazole-H), 6.78-7.81 (12H, m, Ar-H), 5.71 (1H, s, OH), 4.71 (2H, s, triazole-CH_2-), 4.17-4.59 (2H, dd, J = 14.7 Hz, -CH_2-triazolone); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.2, 161.0, 159.4, 158.7, 151.7, 150.6, 146.4, 143.5, 136.3, 133.7, 132.5, 131.3, 131.0, 130.2, 129.4, 126.6, 125.3, 123.4, 118.8, 115.9, 113.5, 110.6, 104.7, 62.2, 61.9, 61.0; LC-MS (ESI), m/z Calcd. for $C_{26}H_{20}ClF_2N_7O_3$, 551.1, found $[M+1]^+$ 552.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-4-methylbenzamide (9e)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.43 (s, 1H, NH), 8.27 (1H, s, triazole-H), 7.94 (1H, s, triazole-H), 6.78-7.84 (12H, m, Ar-H), 5.74 (1H, s, OH), 4.73 (2H, s, triazole-CH_2-), 4.18-4.56 (2H, dd, J = 14.4 Hz, -CH_2-triazolone), 3.85 (3H, s, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 165.3, 162.0, 159.3, 158.5, 152.1, 150.6, 146.4, 143.5, 136.3, 133.6, 132.8, 131.5, 131.2, 130.1, 129.6, 126.8, 125.5, 123.4, 118.8, 115.8, 113.4, 110.6, 104.5, 62.2, 61.6, 61.0, 18.5; LC-MS (ESI), m/z Calcd. for $C_{27}H_{23}F_2N_7O_3$, 531.2, found $[M+1]^+$ 532.3.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-3, 4-dimethylbenzamide (9f)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.41 (s, 1H, NH), 8.35 (1H, s, triazole-H), 7.96 (1H, s, triazole-H), 6.75-7.84 (11H, m, Ar-H), 5.68 (1H, s, OH), 4.75 (2H, s, triazole-CH_2-), 4.20-4.54 (2H, dd, J = 14.7 Hz, -CH_2-triazolone), 2.30 (6H, m, 2×CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.5, 161.8, 159.5, 158.3, 151.8, 150.9, 146.6, 143.3, 136.5, 133.7, 132.9, 131.5, 131.1, 130.3, 129.6, 126.7, 125.5, 123.8, 118.6, 115.5, 113.4, 110.8, 104.7, 62.4, 62.0, 61.2, 18.7, 18.5; LC-MS (ESI), m/z Calcd. for $C_{28}H_{25}F_2N_7O_3$, 545.2, found $[M+1]^+$ 546.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-4-methoxybenzamide (9g)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.27 (s, 1H, NH), 8.15

(1H, s, triazole-H), 7.97 (1H, s, triazole-H), 6.79-7.86 (12H, m, Ar-H), 5.82 (1H, s, OH), 4.74 (2H, s, triazole-CH_2-), 4.19-4.60 (2H, dd, J = 14.7 Hz, -CH_2-triazolone), 3.87 (3H, s, OCH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 165.0, 162.2, 159.4, 158.5, 151.9, 150.6, 146.4, 143.4, 136.6, 133.2, 132.9, 131.8, 131.2, 130.4, 129.0, 126.5, 125.3, 123.8, 118.4, 115.5, 113.5, 110.7, 104.4, 62.7, 62.1, 61.4, 55.6; LC-MS (ESI), m/z Calcd. for $C_{27}H_{23}F_2N_7O_4$, 547.2, found $[M+1]^+$ 548.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-4-ethylbenzamide (9h)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.45 (s, 1H, NH), 8.24 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 6.80-7.82 (12H, m, Ar-H), 5.76 (1H, s, OH), 4.71 (2H, s, triazole-CH_2-), 4.16-4.55 (2H, dd, J = 14.7 Hz, -CH_2-triazolone), 2.60 (2H, q, CH_2), 1.25 (3H, t, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 165.0, 161.9, 159.5, 158.2, 152.7, 150.7, 146.5, 143.6, 136.1, 134.2, 133.5, 131.8, 131.3, 130.6, 129.2, 126.8, 125.5, 123.7, 118.6, 115.0, 113.5, 110.2, 104.5, 62.7, 62.1, 61.1, 27.2, 15.2; LC-MS (ESI), m/z Calcd. for $C_{28}H_{25}F_2N_7O_3$, 545.2, found $[M+1]^+$ 546.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-4-isopropylbenzamide (9i)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.47 (s, 1H, NH), 8.21 (1H, s, triazole-H), 7.96 (1H, s, triazole-H), 6.78-7.80 (12H, m, Ar-H), 5.72 (1H, s, OH), 4.71 (2H, s, triazole-CH_2-), 4.17-4.59 (2H, dd, J = 14.7 Hz, -CH_2-triazolone), 2.87 (1H, m, CH), 1.20 (6H, s, 2×CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.6, 161.4, 159.3, 158.1, 152.4, 150.6, 146.2, 143.4, 136.7, 134.5, 133.3, 131.6, 131.1, 130.5, 129.6, 126.4, 125.2, 123.5, 118.3, 115.8, 113.1, 110.2, 104.6, 62.3, 61.8, 61.2, 33.5, 23.2, 23.0; LC-MS (ESI), m/z Calcd. for $C_{29}H_{27}F_2N_7O_3$, 559.2, found $[M+1]^+$ 560.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-4-n-butylbenzamide (9j)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.36 (s, 1H, NH), 8.22 (1H, s, triazole-H), 7.96 (1H, s, triazole-H), 5.81-7.81 (12H, m, Ar-H), 5.81 (1H, s, OH), 4.72 (2H, s, triazole-CH_2-), 4.18-4.58 (2H, dd, J = 15 Hz, -CH_2-triazolone), 2.67 (2H, t, Ar-CH_2), 1.56 (2H, m, $\underline{CH_2}CH_2CH_3$), 1.25 (2H, m, $CH_2\underline{CH_2}CH_3$), 0.89 (3H, t, $CH_2CH_2\underline{CH_3}$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 165.1, 161.6, 159.8, 158.0, 152.7, 150.5, 146.3, 143.5, 136.4, 134.5, 133.1, 131.8, 131.5, 130.2, 129.6, 126.5, 125.2, 123.6, 118.5, 115.2, 113.4, 110.5, 104.5, 62.4, 62.0, 61.4, 33.4, 31.7, 23.2, 14.2; LC-MS (ESI), m/z Calcd. for $C_{30}H_{29}F_2N_7O_3$, 573.2, found $[M+1]^+$ 574.3.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-2-phenylacetamide (9k)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.35 (s, 1H, NH), 8.20 (1H, s, triazole-H), 7.96 (1H, s, triazole-H), 5.81-7.81 (13H, m, Ar-H), 5.83 (1H, s, OH), 4.72 (2H, s, triazole-CH_2-), 4.19-4.57 (2H, dd, J = 15 Hz, -CH_2-triazolone), 3.71 (2H, s, Ar-CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.7, 160.5, 159.2, 158.3, 152.6, 150.5, 146.8, 143.3, 136.7, 134.6, 133.3, 131.5, 130.9, 130.4, 129.5, 126.4, 125.3, 123.5, 118.2, 115.3, 113.0, 110.8, 104.4, 62.0, 61.7, 61.0, 43.6; LC-MS (ESI), m/z Calcd. for $C_{27}H_{23}F_2N_7O_3$, 531.2, found $[M+1]^+$ 532.2.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-pivalamide (9l)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.41 (s, 1H, NH), 8.17 (1H, s, triazole-H), 7.92 (1H, s, triazole-H), 6.78-7.88 (8H, m, Ar-H), 5.78 (1H, s, OH), 4.77 (2H, s, triazole-CH_2-), 4.20-4.59 (2H, dd, J = 14.7 Hz, -CH_2-triazolone), 1.28 (9H, s, 3×CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 176.7, 163.2, 158.3, 152.0, 150.5, 146.5, 143.7, 138.6, 133.3, 131.5, 130.4, 129.5, 123.5, 118.2, 115.3, 110.5, 104.2, 62.7, 62.4, 61.3, 35.6, 28.2; LC-MS (ESI), m/z Calcd. for $C_{24}H_{25}F_2N_7O_3$, 497.2, found $[M+1]^+$ 498.3.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-hexanamide (9m)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.34 (s, 1H, NH), 7.97 (1H, s, triazole-H), 7.85 (1H, s, triazole-H), 6.77-7.61 (8H, m, Ar-H), 5.92 (1H, s, OH), 4.75 (2H, s, triazole-CH_2-), 4.19-4.57 (2H, dd, J = 15 Hz, -CH_2-triazolone), 2.32-2.37 (2H, t, $\underline{CH_2}CH_2CH_2CH_3$), 1.65 (2H, m, $CH_2\underline{CH_2}CH_2$ CH_3), 1.24 (5H, m, $CH_2CH_2\underline{CH_2CH_3}$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 177.2, 163.7, 158.5, 152.2, 150.8, 146.1, 143.3, 138.9, 133.7, 131.4, 130.5, 129.8, 123.2, 118.8, 115.6, 110.2, 104.0, 62.4, 62.0, 61.0, 35.3, 32.7, 28.0, 22.7, 14.2; LC-MS (ESI), m/z Calcd. for $C_{24}H_{25}F_2N_7O_3$, 497.2, found $[M+1]^+$ 498.3.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-heptanamide (9n)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.24 (s, 1H, NH), 8.20 (1H, s, triazole-H), 7.84 (1H, s, triazole-H), 6.75-7.82 (8H, m, Ar-H), 5.58 (1H, s, OH), 4.72 (2H, s, triazole-CH_2-), 4.17-4.57 (2H, dd, J = 14.7 Hz, -CH_2-triazolone), 2.31 (2H, t, $\underline{CH_2}$ $CH_2CH_2CH_3$), 1.67 (2H, m, $CH_2\underline{CH_2}CH_2$ CH_3), 1.24 (5H, m, $CH_2CH_2\underline{CH_2CH_3}$); ^{13}C-NMR (75 MHz, $CDCl_3$):

δ 176.5, 164.0, 158.3, 152.7, 150.5, 146.4, 143.7, 138.5, 133.6, 131.8, 130.1, 129.5, 123.5, 118.8, 115.3, 110.8, 104.1, 62.6, 62.1, 61.3, 36.5, 33.5, 29.3, 24.4, 22.5, 13.9; LC-MS (ESI), m/z Calcd. for $C_{25}H_{27}F_2N_7O_3$, 511.2, found $[M+1]^+$ 512.3.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-octanamide (9o)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.35 (s, 1H, NH), 8.28 (1H, s, triazole-H), 7.86 (1H, s, triazole-H), 6.79-7.82 (8H, m, Ar-H), 5.92 (1H, s, OH), 4.74 (2H, s, triazole-CH_2-), 4.19-4.59 (2H, dd, J = 15Hz, -CH_2-triazolone), 2.34 (2H, t, $\underline{CH_2}CH_2$ $CH_2CH_2CH_2CH_3$), 1.67 (2H, m, $CH_2\underline{CH_2}CH_2$ CH_2 CH_2CH_3), 1.30 (6H, m, $CH_2CH_2\underline{CH_2CH_2}$ $\underline{CH_2}CH_3$), 0.88 (3H, t, $CH_2CH_2CH_2CH_2CH_2$ $\underline{CH_3}$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 176.3, 164.2, 158.4, 152.4, 150.3, 146.8, 143.4, 138.7, 133.6, 131.6, 130.4, 129.3, 123.8, 118.5, 115.7, 110.6, 104.6, 62.7, 62.3, 61.0, 36.9, 33.3, 29.5, 25.6, 24.0, 21.4, 13.5; LC-MS (ESI), m/z Calcd. for $C_{26}H_{29}F_2N_7O_3$, 525.2, found $[M+1]^+$ 526.3.

N-(3-{1-[2-(2, 4-difluorophenyl)-2-hydroxy-3-(1H-1, 2, 4-triazol-1-yl)propyl]-5-oxo-1H-1, 2, 4-triazol-4(5H)-yl}phenyl)-nonanamide (9p)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.34 (s, 1H, NH), 8.26 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 6.77-7.81 (8H, m, Ar-H), 5.92 (1H, s, OH), 4.73 (2H, s, triazole-CH_2-), 4.20-4.57 (2H, dd, J = 15 Hz, -CH_2-triazolone), 2.39 (2H, t, $\underline{CH_2}CH_2$ $CH_2CH_2CH_2CH_2CH_3$), 1.67 (2H, m, $CH_2\underline{CH_2}CH_2CH_2CH_2CH_2CH_3$), 1.29 (8H, m, CH_2CH_2 $\underline{CH_2CH_2CH_2CH_2}CH_3$), 0.86 (3H, t, CH_2CH_2 $CH_2CH_2CH_2$ $CH_2\underline{CH_3}$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 176.1, 164.2, 158.5, 152.3, 150.6, 146.8, 143.4, 138.2, 133.4, 131.5, 130.1, 129.2, 123.4, 118.6, 115.8, 110.5, 104.3, 62.6, 62.0, 61.1, 36.2, 33.3, 29.7, 24.7, 24.0, 22.2, 13.4; LC-MS (ESI), m/z Calcd. for $C_{27}H_{31}F_2N_7O_3$, 539.3, found $[M+1]^+$ 540.3.

Antifungal activity

The *in vitro* antifungal activities of all the target compounds were evaluated against seven human pathogenic fungi, *Candida albican SC5314* and *Y0109, Cryptococcus neoformans, Candida parapsilosis, Candida tropicalis, Trichophyton rubrum,* and *Candida kefyr,* which are frequently encountered in clinic. The results were compared with positive controls itraconazole, voriconazole and fluconazole. *C. albican SC5314* and *C. neoformans*, purchased from ATCC, were provided by Shanghai Changzheng Hospital; *C. parapsilosis, C. albican Y0109, C. tropicalis, T. rubrum* and *C. kefyr*, which are clinic isolates, were provided by Shanghai Changhai Hospital. Fluconazole, itraconazole and voriconazole 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. albican* and at 72 h for *C. neoformans*. The results of assays are summarized in Table I. These data are the mean of the three replicate tests performed with each antifungal agent.

RESULTS

In our research, the intermediate **2** was synthesized via a 'one-pot' high-temperature refluxing strategy, using methanol as the medium. Apparently, the former method (Hrebabecky and Beranek, 1985) needs three steps, while ours is a one step procedure. It is more succinct and efficient.

The *in vitro* antifungal activities of all the target compounds were listed in Table I. The MIC_{80} values of all the target compounds were determined against seven important fungal pathogens and compared with itraconazole, voriconazole and fluconazole. The assay indicated that all the synthesized compounds showed moderate to excellent activity against all the tested fungal pathogens, except *C. neoformans*. The MIC_{80} value of compound **9a** was 32 times lower than that of FCZ against *C. albicans.* Most of the target compounds **9a-p** showed higher activities against *C. parasilosis* than the standard reference drug FCZ. Additionally, compound **9b** and **9e** exhibited excellent activities with an MIC_{80} value of 0.03125 μg/mL against *C. parasilosis* versus the MIC_{80} value of ICZ, which is only 0.125 μg/mL. All the title compounds, except for compound **9j** exhibited equivalent antifungal activities of FCZ against *C. kefyr* with the MIC_{80} value of 1 μg/mL. Besides, compounds **9m**, **9n**, **9o** and **9p**, which contained longer alkyl side chains showed poor activity against all the tested fungi with the MIC_{80} value in the range of 1-64 μg/mL.

DISCUSSION

In our paper, a series of novel triazole antifungal agents containing a triazolone side chain were successfully designed and synthesized. We used a 'one-

Table I. Antifungal activities of the target compounds *in vitro* (MIC_{80}, μg/mL)

Compd .	R	*C. alb*		*C. neo*	*C. par*	*C. kef*	*C. tro*	*T. rub*
		Y0109	SC5314					
9a	2-FC_6H_4	0.125	0.25	>64	0.0625	1	1	1
9b	3-FC_6H_4	1	1	>64	0.03125	1	1	8
9c	3-ClC_6H_4	1	1	>64	0.125	1	1	1
9d	4-ClC_6H_4	0.5	1	>64	0.125	1	1	1
9e	4-$CH_3C_6H_4$	1	1	>64	0.03125	1	1	8
9f	3,4-$CH_3C_6H_4$	1	1	>64	0.125	1	1	1
9g	4-$OCH_3C_6H_4$	1	1	>64	0.125	1	1	1
9h	4-$CH_2CH_3C_6H_4$	4	4	>64	0.5	1	>64	1
9i	4-$CH(CH_3)_2C_6H_4$	1	4	>64	4	1	>64	1
9j	4-$(CH_2)_3CH_3C_6H_4$	1	1	16	0.25	4	0.5	1
9k	$CH_2C_6H_5$	32	8	>64	16	1	>64	>64
9l	$C(CH_3)_3$	0.5	1	>64	0.125	1	1	1
9m	$(CH_2)_4CH_3$	32	>64	16	>64	1	>64	4
9n	$(CH_2)_5CH_3$	>64	>64	16	32	1	>64	16
9o	$(CH_2)_6CH_3$	>64	>64	>64	>64	1	>64	8
9p	$(CH_2)_7CH_3$	>64	>64	16	>64	1	>64	1
ICZ		0.25	0.125	0. 25	0.125	0. 5	0. 25	0. 25
VCZ		0.0625	0.0625	0.0078	0.0039	0.125	0.0156	0.0156
FCZ		4	4	4	0.25	1	1	1

C. alb, Candida albicans; *C. tro*, *Candida tropicalis*; *C. neo*, *Cryptococcus neoformans*; *C. par*, *Candida parapsilosis*; *C. kef*, *Candida kefyr*; *T. rub*, *Trichophyton rubrum*; ICZ, itraconazole; VCZ, voriconazole; FCZ, fluconazole.

pot' method to synthesize the side chains and also acquired a higher yield.

The antifungal activities of the target compounds were screened for seven human pathogenic fungi. *In vitro* antifungal activity assay indicated that most of these compounds showed moderate to excellent antifungal activities than the reference drug FCZ. Among them, the compounds **9a**-**j** with a substituted phenyl side chain present higher antifungal activities. However, the other compounds **9l**-**p** with longer or shorter alkyl side chains suffer a loss of antifungal activity. It indicates that bulky substituents are not beneficial to the antifungal activity of those fluconazole analogs. The compounds with 3-substituted phenyl, which was directly linked to the triazolone showed less activity than the compounds with 2-substituted phenyl (Jiang et al., 2011a, 2011b). It may attribute to the side chain's spatial configuration. The antifungal activity may be more excellent as the side chain becoming longer and straighter. All these observations will be helpful in designing newer antifungal agents to further our research efforts.

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