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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and bioactivity evaluation of novel azoles containing dithiocarbamate moieties

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Abstract A series of novel azoles, *N*-methyl-*N*-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)]-propyl-(1-substituted phenyl)-dithiocarbamate has been designed, synthesized, and screened for antifungal activity by minimum inhibitory concentration. Results of preliminary antifungal tests showed that most of the title compounds had good antifungal activities against nearly all the tested fungal pathogens, especially against the *Candida* species. The most surprising finding of this study is that compound **4a**, **4e**, and **4k** exhibited higher activities than fluconazole against *Aspergillus fumigatus*.

Keywords Azole · Synthesis · Antifungal activity · CACYP51

Introduction

Yeast pathogenic species (e.g., *Candida albicans*, *C. glabrata*) and filamentous pathogens (e.g., *Aspergillus fumigatus*) are generally regarded as the leading causes of invasive fungal infections. They lead to an increasing cause of morbidity and mortality in hospitalized cases, including human immunodeficiency virus infection, undergoing cancer chemotherapy or organ transplantation (Aly and Berger 1996; Wingard and Leather 2004; Hahn-Ast et al. 2010). At present, several structurally distinct antifungal drugs classified by targeting different cell components, are used in clinic, such as azoles, allylamines, polyenes, and echinocandins (Chapman et al. 2008; Kathiravan et al. 2012; Ostrosky-Zeichner et al. 2010). Azoles (e.g., fluconazole (FCZ), itraconazole (ICZ), and voriconazole (VCZ)) act by blocking the biosynthesis of ergosterol through inhibiting CYP51 through a mechanism in which the heterocyclic nitrogen atom (N-4 of triazole) binds to the heme iron atom (Abe et al. 2009). Allylamines (e.g., terbinafine (TRB) and butenafine) also act by blocking ergosterol biosynthesis, but through inhibiting the squalene oxidase (Nowosielski et al. 2011). Polyenes, such as amphotericin B (AMB), act as membrane-disrupting agents by binding to ergosterol (Janout et al. 2015). Echinocandins (e.g., Caspofungin and Micafungin) are antifungal drugs that inhibit the synthesis of glucan in the cell wall, via noncompetitive inhibition of the enzyme 1,3-β glucan synthase (Emrick et al. 2013). However, their clinical uses have been limited by the emergence of drug resistance, high risk of toxicity, insufficiencies in their antifungal activity and undesirable side effects.

Among the clinical antifungal agents, azoles were used widely and efficiently. However, the broad use of azoles has led to development of severe resistance, which significantly reduced their efficacy (Casalinuovo et al. 2004; Hoffman et al. 2000). So the discovery of novel azoles with higher activities, lower toxicity, and better pharmacokinetic properties is the best way to overcome resistance and develop effective therapies.

Azoles act by competitive inhibition cytochrome P450 14α demethylase (CYP51), a necessary enzyme in the

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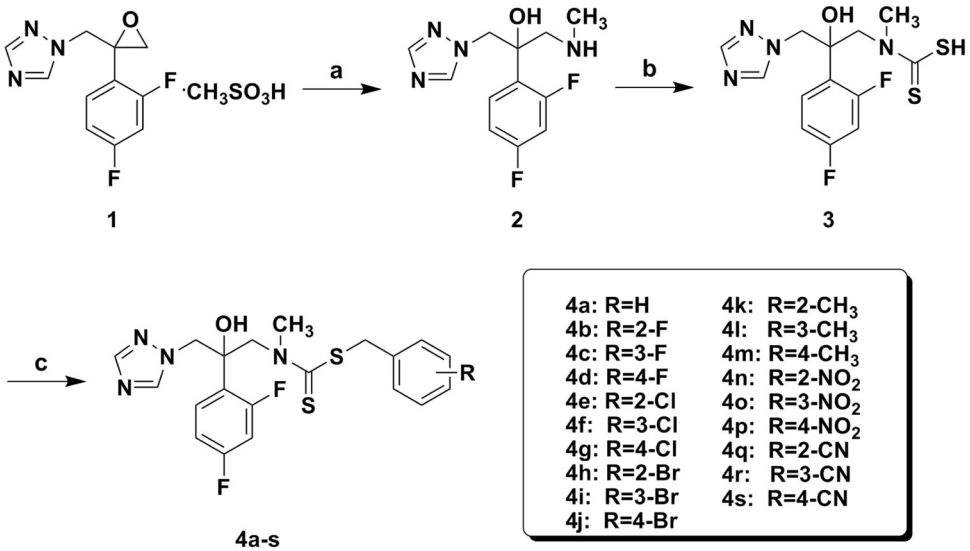

Scheme 1 Synthetic routes of the target compounds. Reagents and conditions: **a** CH_3NH_2, EtOH, triethylamine, reflux, 5 h, 82%; **b** CS_2, EtOH, triethylamine, 0 °C, 0.5 h; **c** substituted benzyl bromide, EtOH, triethylamine, rt, 4 h, 61–71%

biosynthesis of ergosterol which is the primary membrane sterol in fungi (Georgopapadakou and Walsh 1996; de Pauw and Picazo 2008). The hydrophilic H-bonding region, the hydrophobic region, and the narrow hydrophobic cleft formed the three subsites of the active site of CYP51 for ligand binding (Sheng et al. 2004). And azoles could bind to the active site of CYP51 through their pharmacophores. Dithiocarbamates have attracted considerable attention recently, in view of its variety of biological activities. Some studies have revealed that dithiocarbamates agents had antifungal and anti-tumor effects (Ferreira et al. 2012; Zou et al. 2014; Conaway et al. 2002; Spitz et al. 2000; Callaway et al. 2004; Ronconi et al. 2006; Cao et al. 2005; Hou et al. 2006). In order to develop new type of antifungal azoles, the triazole key segments, which included 2,4-difluorobenzene, 1,2,4-triazole, and tertiary hydroxyl, were incorporated with the dithiocarbamate moieties. Therefore, a series of novel azoles containing dithiocarbamate moieties were designed, synthesized, and evaluated for their antifungal activities in our studies. The dithiocarbamate moieties of the novel compounds, which make a big difference to the classical antifungal drug FCZ, break the symmetry of the molecule and significantly increase the molecular complexity.

Materials and methods

Chemistry

General chemicals were of the best grade available, supplied by Shanghai Titan technology Co. Ltd., J&K chemical LTD and were used without additional purification. Thin-layer chromatographys (TLCs) were performed on silica gel plates HSGF254 plates (0.2 ± 0.03 mm, analytical; Yantai Huanghai, China). Compounds were visualized by ultraviolet light (Yukang, Shanghai, China). Flash chromatography was carried out on silica gel (300–400 mesh; Qingdao Ocean Chemicals, China). Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AC-300P spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) in $CDCl_3$ solution. δ values (s = singlet, d = doublet, dd = doublet doublet, t = triplet and m = multiplet) are given in p.p.m. relative to tetramethylsilane. ESI mass spectra were performed on an API-3000 LC-MS spectrometer.

The general synthetic methodology for the preparation of target compounds (**4a–s**) is outlined in Scheme 1. As a key intermediate of our designed compounds, compound **1** was synthesized through the reported procedure (Richardson 1983). Compound **1** was allowed to react with methylamine in the presence of triethylamine in ethanol to formed compound **2**. To a stirred mixture of compound **2** and carbon disulfide in the presence of triethylamine in ethanol, we afforded compound **3**. Then the target compounds (**4a–s**) were synthesized and the good yield was obtained when the reaction was performed in ethanol in the presence of compound **3** and substituted benzyl bromide.

*Synthesis of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-pent-4-yn-2-ol (2)*

The compound **1** (20.0 g, 0.06 mmol), methylamine (20 mL), and triethylamine (30 mL) were dissolved in ethanol (300 mL). The reaction was stirred and reflux for 5 h. The solvent was evaporated under reduce pressure. The mixture was extracted with DCM, the organic layers were washed with 10% citric acid solution, saturated sodium carbonate solution and saturated sodium chloride solution, then dried over anhydrous $NaSO_4$, filtered, and concentrated in

vacuum. The residue was purified by silica gel column chromatography to afford compound **2** (13.2 g, 82%).

*General procedure for the preparation of the compounds **4a–s***

A mixture of compound **2** (160 mg, 1.4 mmol) and triethylamine (3 mL) was stirred in ethanol (15 mL) at 0 °C for 10 min. Then carbon disulfide (0.5 mL, 0.76 mmol) was added in the reaction and stirred at 0 °C for 20 min. After that substituted benzyl bromide (2.1 mmol) was added, the reaction was stirred for 4 h at room temperature until TLC indicating the reaction finished. Oil compound was received after the solvent evaporated under reduce pressure. The residue was purified by silica gel column chromatography to afford **4a–s**.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-(1-benzyl)-dithiocarbamate **(4a)** Yield: 70%; m.p.: 93–94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, s, triazole-H), 7.85 (1H, s, triazole-H), 6.84–7.60 (8H, m, Ar–H), 5.64 (1H, s, OH), 4.71 (2H, s, SCH_2), 4.54 (1H, d, $J = 14.2$ Hz, triazole-H), 4.34 (1H, d, $J = 14.2$ Hz, triazole-H), 3.17 (1 H, d, $J = 14.3$ Hz, NCH_2), 3.12 (3H, s, CH_3), 2.95 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.8 (C = S), 161.4 (Ar–2F), 161.3 (Ar–4F), 151.1 (triazole-3C), 144.4 (triazole-5C), 135.2, 130.3, 130.3, 130.2, 130.1, 129.3, 128.6, 127.7, 111.8, 104.2, 77.4, 77.0, 76.6, 43.1 (Ar–CH_2), 42.3 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{20}F_2N_4OS_2$ 434.10, Found, 435.11$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(2-fluorobenzyl)]-dithiocarbamate **(4b)** Yield: 66%; m.p.: 93–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, s, triazole-H), 7.83 (1H, s, triazole-H), 6.84–7.69 (7H, m, Ar–H), 5.60 (1H, br, OH), 4.73 (2H, s, SCH_2), 4.60 (1H, d, $J = 14.2$ Hz, triazole-H), 4.39 (1H, d, $J = 14.2$ Hz, triazole-H), 3.15 (1H, d, $J = 14.3$ Hz, NCH_2), 3.15 (3H, s, CH_3), 2.93 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.4 (C = S), 161.5 (Ar–2F), 161.3 (C–F), 159.4 (Ar–4F), 151.4 (triazole-3C), 144.7 (triazole-5C), 135.1, 130.5, 130.4, 130.1, 129.5, 128.4, 127.1, 111.5, 104.2, 77.6, 77.2, 76.3, 43.4 (Ar–CH_2), 42.8 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_3N_4OS_2$ 452.10, Found, 453.10 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(3-fluorobenzyl)]-dithiocarbamate **(4c)** Yield: 65%; m.p.: 94–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (1H, s, triazole-H), 7.86 (1H, s, triazole-H), 6.76–7.58 (7H, m, Ar–H), 4.73 (2H, s, SCH_2), 4.55 (1H, d, $J = 14.2$ Hz, triazole-H), 4.34 (1H, d, $J = 14.2$ Hz, triazole-H), 3.21 (1H, d, $J = 14.3$ Hz, NCH_2), 3.13 (3H, s, CH_3), 2.98(1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.5 (C = S), 161.3 (Ar–2F), 161.1 (C–F), 158.9 (Ar–4F), 151.7 (triazole-3C), 144.4 (triazole-5C), 135.3, 130.7, 130.4, 130.0, 129.1, 128.3, 127.4, 111.7, 104.6, 77.5, 77.2, 76.4, 43.5 (Ar–CH_2), 42.7 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_3N_4OS_2$ 452.10, Found, 453.10 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(4-fluorobenzyl)]-dithiocarbamate **(4d)** Yield: 66%; m.p.: 93–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (1H, s, triazole-H), 7.86 (1H, s, triazole-H), 6.85–7.59 (7H, m, Ar–H), 4.73 (2H, s, SCH_2), 4.51 (1H, d, $J = 14.2$ Hz, triazole-H), 4.39 (1H, d, $J = 14.2$ Hz, triazole-H), 3.23 (1H, d, $J = 14.2$ Hz, NCH_2), 3.15 (3H, s, CH_3), 2.98 (1H, d, $J = 14.2$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.5 (C = S), 161.7 (Ar–2F), 161.5 (C–F), 159.0 (Ar–4F), 151.5 (triazole-3C), 144.6 (triazole-5C), 135.4, 130.5, 130.3, 130.2, 129.5, 128.7, 127.2, 111.6, 104.2, 77.7, 77.2, 76.3, 43.5 (Ar–CH_2), 42.7 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_3N_4OS_2$ 452.10, Found, 453.10 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(2-chlorobenzy)]-dithiocarbamate **(4e)** Yield: 61%; m.p.: 94–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (1H, s, triazole-H), 7.86 (1H, s, triazole-H), 6.84–7.73 (7H, m, Ar–H), 4.68 (2H, s, SCH_2), 4.48 (1H, d, $J = 14.2$ Hz, triazole-H), 4.30 (1H, d, $J = 14.2$ Hz, triazole-H), 3.22 (1H, d, $J = 14.3$ Hz, NCH_2), 3.17 (3H, s, CH_3), 3.00 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.1 (C = S), 160.0 (Ar–2F), 158.2 (Ar–4F), 151.3 (triazole-3C), 144.5 (triazole-5C), 135.3, 132.8, 130.7, 130.5, 130.2, 129.7, 128.3, 127.0, 111.5, 104.4, 77.8, 77.4, 76.3, 43.4 (Ar–CH_2), 42.5 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2ClN_4OS_2$ 468.07, Found, 469.10 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(3-chlorobenzy)]-dithiocarbamate **(4f)** Yield: 64%; m.p.: 94–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (1H, s, triazole-H), 7.94 (1H, s, triazole-H), 6.88–7.85 (7H, m, Ar–H), 4.67 (2H, s, SCH_2), 4.44 (1H, d, $J = 14.2$ Hz, triazole-H), 4.26 (1H, d, $J = 14.2$ Hz, triazole-H), 3.25 (1H, d, $J = 14.3$ Hz, NCH_2), 3.18 (3H, s, CH_3), 3.04 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.9 (C = S), 160.2 (Ar–2F), 158.6 (Ar–4F), 151.4 (triazole-3C), 144.6 (triazole-5C), 135.5, 132.7, 130.7, 130.6, 130.3, 130.1, 128.5, 127.1, 111.8, 104.6, 77.2, 76.6, 76.0, 43.5 (Ar–CH_2), 42.7 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2ClN_4OS_2$ 468.07, Found, 469.09 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(4-chlorobenzy)]-dithiocarbamate **(4g)** Yield: 63%; m.p.: 94–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (1H, s, triazole-H), 7.85 (1H, s, triazole-H), 6.85–7.69 (7H, m, Ar–H), 4.68 (2H, s, SCH_2), 4.38 (1H, d, $J = 14.2$ Hz, triazole-H), 4.20 (1H, d, $J = 14.2$ Hz, triazole-H), 3.32 (1H, d, $J = 14.2$ Hz, NCH_2), 3.18 (3H, s, CH_3), 3.08 (1H, d, $J = 14.2$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.0 (C = S), 160.3 (Ar–2F), 158.8 (Ar–4F), 151.5 (triazole-3C), 144.8 (triazole-5C), 135.4, 132.8, 130.5, 130.4, 130.2, 129.9, 128.6, 127.4, 111.7, 104.6, 77.7, 77.2, 76.4, 43.6 (Ar–CH_2), 42.7 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2ClN_4OS_2$ 468.07, Found, 469.06 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(2-brorobenzyl)]-dithiocarbamate **(4h)** Yield: 67%; m.p.: 95–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, s, triazole-H), 7.85 (1H, s, triazole-H), 6.85–7.38 (7H, m, Ar–H), 4.81 (2H, s, SCH_2), 4.50 (1H, d, $J = 14.2$ Hz, triazole-H), 4.37 (1H, d, $J = 14.2$ Hz, triazole-H), 3.32 (1H, d, $J = 14.3$ Hz, NCH_2), 3.18 (3H, s, CH_3), 2.97(1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.2 (C = S), 160.5 (Ar–2F), 158.5 (Ar–4F), 151.4 (triazole-3C), 145.3 (triazole-5C), 136.0, 132.5, 131.1, 130.8, 130.5, 130.1, 128.6, 127.8, 112.1, 104.5, 78.0, 77.5, 76.7, 43.8 (Ar–CH_2), 43.1 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2BrN_4OS_2$ 514.01, Found, 515.02 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(3-brorobenzyl)]-dithiocarbamate **(4i)** Yield: 64%; m.p.: 97–98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, s, triazole-H), 7.85 (1H, s, triazole-H), 6.84–7.43 (7H, m, Ar–H), 4.68 (2H, s, SCH_2), 4.58 (1H, d, $J = 14.2$ Hz, triazole-H), 4.38 (1H, d, $J = 14.2$ Hz, triazole-H), 3.32 (1H, d, $J = 14.2$ Hz, NCH_2), 3.17 (3H, s, CH_3), 2.95 (1H, d, $J = 14.2$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.3 (C = S), 160.5 (Ar–2F), 158.6 (Ar–4F), 151.5 (triazole-3C), 145.2 (triazole-5C), 136.3, 132.7, 130.9, 130.7, 130.5, 130.2, 128.7, 127.7, 112.4, 104.6, 77.9, 77.5, 76.6, 43.6 (Ar–CH_2), 43.2 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2BrN_4OS_2$ 514.01, Found, 515.02$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(4-brorobenzyl)]-dithiocarbamate **(4j)** Yield: 64%; m.p.: 94–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, s, triazole-H), 7.87 (1H, s, triazole-H), 6.84–7.69 (7H, m, Ar–H), 4.67 (2H, s, SCH_2), 4.53 (1H, d, $J = 14.2$ Hz, triazole-H), 4.39 (1H, d, $J = 14.2$ Hz, triazole-H), 3.35 (1H, d, $J = 14.3$ Hz, NCH_2), 3.17 (3H, s, CH_3), 2.96 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.3 (C = S), 160.4 (Ar–2F), 158.5 (Ar–4F), 151.6 (triazole-3C), 145.6 (triazole-5C), 136.1, 132.7, 131.3, 130.7, 130.5, 130.4, 128.7, 127.5, 112.1, 104.6, 77.8, 77.3, 76.4, 43.5 (Ar–CH_2), 42.9 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2BrN_4OS_2$ 514.01, Found, 515.03$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(2-methylbenzyl)]-dithiocarbamate **(4k)** Yield: 61%; m.p.: 95–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (1H, s, triazole-H), 7.86 (1H, s, triazole-H), 6.85–7.60 (7H, m, Ar–H), 4.62 (2H, s, SCH_2), 4.49 (1H, d, $J = 14.2$ Hz, triazole-H), 4.27 (1H, d, $J = 14.2$ Hz, triazole-H), 3.38 (1H, d, $J = 14.3$ Hz, NCH_2), 3.29 (3H, s, CH_3), 3.08 (1H, d, $J = 14.3$ Hz, NCH_2), 2.33 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.5 (C = S), 160.7 (Ar–2F), 158.8 (Ar–4F), 151.4 (triazole-3C), 145.5 (triazole-5C), 140.2, 136.3, 132.4, 131.2, 130.4, 129.7 128.3, 127.6, 112.2, 104.6, 77.5, 77.1, 76.6, 43.7 (Ar–CH_2), 42.5 (CH_3), 17.8 (Ar–CH_3); MS (ESI) *m/z* calcd. For $C_{21}H_{22}F_2N_4OS_2$ 448.12, Found, 449.12$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(3-methylbenzyl)]-dithiocarbamate **(4l)** Yield: 68%; m.p.: 98–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, s, triazole-H), 7.85 (1H, s, triazole-H), 6.85–7.70 (7H, m, Ar–H), 4.70 (2H, s, SCH_2), 4.48 (1H, d, $J = 14.2$ Hz, triazole-H), 4.35 (1H, d, $J = 14.2$ Hz, triazole-H), 3.28 (1H, d, $J = 14.3$ Hz, NCH_2), 3.17 (3H, s, CH_3), 2.99 (1H, d, $J = 14.3$ Hz, NCH_2), 2.34 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.4 (C = S), 160.5 (Ar–2F), 158.6 (Ar–4F), 151.7 (triazole-3C), 145.3 (triazole-5C), 140.7, 136.2, 132.4, 131.5, 130.2, 129.5 128.4, 127.3, 112.1, 104.6, 77.5, 77.0, 76.3, 43.5 (Ar–CH_2), 42.0 (CH_3), 17.7 (Ar–CH_3); MS (ESI) *m/z* calcd. For $C_{21}H_{22}F_2N_4OS_2$ 448.12, Found, 449.12$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(4-methylbenzyl)]-dithiocarbamate **(4m)** Yield: 62%; m.p.: 96–98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (1H, s, triazole-H), 7.84 (1H, s, triazole-H), 6.84–7.60 (7H, m, Ar–H), 4.66 (2H, s, SCH_2), 4.48 (1H, d, $J = 14.2$ Hz, triazole-H), 4.39 (1H, d, $J = 14.2$ Hz, triazole-H), 3.25 (1H, d, $J = 14.3$ Hz, NCH_2), 3.18 (3H, s, CH_3), 2.98 (1H, d, $J = 14.3$ Hz, NCH_2), 2.33 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.0 (C = S), 161.3 (Ar–2F), 157.2 (Ar–4F), 150.8 (triazole-3C), 144.3 (triazole-5C), 140.5, 137.5, 131.9, 130.4, 130.2, 130.1, 129.4, 129.3, 111.7, 104.2, 77.5, 77.1, 76.6, 43.0 (Ar-CH_2), 42.3 (CH_3), 17.7 (Ar-CH_3); MS (ESI) *m/z* calcd. For $C_{21}H_{22}F_2N_4OS_2$ 448.12, Found, 449.14$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(2-nitrobenzyl)]-dithiocarbamate **(4n)** Yield: 64%; m.p.: 112–114 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (1H, s, triazole-H), 7.96 (1H, s, triazole-H), 6.84–7.81 (7H, m, Ar–H), 4.83 (2H, s, SCH_2), 4.56 (1H, d, $J = 14.2$ Hz, triazole-H), 4.38 (1H, d, $J = 14.2$ Hz, triazole-H), 3.33 (1H, d, $J = 14.3$ Hz, NCH_2), 3.16 (3H, s, CH_3), 3.02 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.7 (C = S), 161.5 (Ar–2F), 157.8 (Ar–4F), 150.4 (triazole-3C), 147.5 (Ar–NO_2), 144.5 (triazole-5C), 137.0, 131.5, 130.7, 130.5, 130.4, 129.8, 128.5, 111.4, 104.5, 77.7, 77.2, 76.5, 43.2 (Ar–CH_2), 42.4 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2N_5O_3S_2$ 479.09, Found, 480.10$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl))-propyl-[1-(3-nitrobenzyl)]-dithiocarbamate **(4o)** Yield: 71%; m.p.: 108–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (1H, s, triazole-H), 7.95 (1H, s, triazole-H), 6.83–7.84 (7H, m, Ar–H), 4.79 (2H, s, SCH_2), 4.61 (1H, d, $J = 14.2$ Hz, triazole-H), 4.38 (1H, d, $J = 14.2$ Hz, triazole-H), 3.35 (1H, d, $J = 14.3$ Hz, NCH_2), 3.18 (3H, s, CH_3), 3.05 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6 (C = S), 161.4 (Ar–2F), 157.8 (Ar–4F), 150.5 (triazole-3C), 147.6 (Ar–NO_2), 144.4 (triazole-5C), 136.8, 131.2, 130.7, 130.4, 130.3, 129.9, 128.4, 111.6, 104.4, 77.8, 77.2, 76.6, 43.1 (Ar–CH_2), 42.5 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2N_5O_3S_2$ 479.09, Found, 480.11$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(4-nitrobenzyl)]-dithiocarbamate **(4p)** Yield: 63%; m.p.: 110–111 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (1H, s, triazole-H), 8.01 (1H, s, triazole-H), 6.66–7.83 (7H, m, Ar–H), 4.85 (2H, s, SCH_2), 4.64 (1H, d, $J = 14.2$ Hz, triazole-H), 4.39 (1H, d, $J = 14.2$ Hz, triazole-H), 3.35 (1H, d, $J = 14.3$ Hz, NCH_2), 3.17 (3H, s, CH_3), 3.06 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.3 (C = S), 161.3 (Ar–2F), 157.2 (Ar–4F), 150.6 (triazole-3C), 147.2 (Ar–NO_2), 144.3 (triazole-5C), 137.1, 131.8, 130.7, 130.5, 130.3, 129.5, 128.7, 111.8, 104.3, 77.5, 77.0, 76.6, 43.2 (Ar–CH_2), 41.5 (CH_3); MS (ESI) *m/z* calcd. For $C_{20}H_{19}F_2N_5O_3S_2$ 479.09, Found, 480.09$[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(2-cyanobenzyl)]-dithiocarbamate **(4q)** Yield: 64%; m.p.: 102–114 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (1H, s, triazole-H),7.83 (1H, s, triazole-H), 6.73–7.61 (7H, m, Ar–H), 4.85 (2H, s, SCH_2), 4.56 (1H, d, $J = 14.2$ Hz, triazole-H), 4.33 (1H, d, $J = 14.2$ Hz, triazole-H), 3.37 (1H, d, $J = 14.3$ Hz, NCH_2), 3.18 (3H, s, CH_3), 3.02 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.4 (C = S), 161.5 (Ar–2F), 157.4 (Ar–4F), 150.8 (triazole-3C), 144.5 (triazole-5C), 137.4, 131.5, 130.6, 130.4, 130.1, 128.8, 128.7, 128.4, 112.3, 111.4, 104.5, 77.2, 76.8, 76.3, 42.8 (Ar–CH_2), 41.7 (CH_3); MS (ESI) *m/z* calcd. For $C_{21}H_{19}F_2N_5OS_2$ 459.10, Found, 460.10 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(3-cyanobenzyl)]-dithiocarbamate **(4r)** Yield: 67%; m.p.: 103–105 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (1H, s, triazole-H),7.84 (1H, s, triazole-H), 6.83–7.76 (7H, m, Ar–H), 4.84 (2H, s, SCH_2), 4.55 (1H, d, $J = 14.2$ Hz, triazole-H), 4.27 (1H, d, $J = 14.2$ Hz, triazole-H), 3.36 (1H, d, $J = 14.3$ Hz, NCH_2), 3.17 (3H, s, CH_3), 3.06 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.5 (C = S), 161.7 (Ar–2F), 157.6 (Ar–4F), 151.2 (triazole-3C), 144.4 (triazole-5C), 137.8, 131.2, 130.2, 130.1, 130.0, 129.3, 128.7, 128.4, 112.6, 111.5, 104.3, 77.5, 77.0, 76.2, 42.4 (Ar–CH_2), 41.4 (CH_3); MS (ESI) *m/z* calcd. For $C_{21}H_{19}F_2N_5OS_2$ 459.10, Found, 460.12 $[M + H]^+$.

N-methyl-N-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)]-propyl-[1-(4-cyanobenzyl)]-dithiocarbamate **(4s)** Yield: 64%; m.p.: 101–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (1H, s, triazole-H),7.85 (1H, s, triazole-H), 6.84–7.66 (7H, m, Ar–H), 4.75 (2H, s, SCH_2), 4.52 (1H, d, $J = 14.2$ Hz, triazole-H), 4.31 (1H, d, $J = 14.2$ Hz, triazole-H), 3.32 (1H, d, $J = 14.3$ Hz, NCH_2), 3.14 (3H, s, CH_3), 3.00 (1H, d, $J = 14.3$ Hz, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6 (C = S), 161.6 (Ar–2F), 157.4 (Ar–4F), 150.7 (triazole-3C), 144.6 (triazole-5C), 137.2, 131.0, 130.7, 130.5, 130.1, 128.9, 128.5, 128.2, 112.5, 111.3, 104.7, 77.3, 77.0, 76.2, 42.5 (Ar–CH_2), 41.4 (CH_3); MS (ESI) *m/z* calcd. For $C_{21}H_{19}F_2N_5OS_2$ 459.10, Found, 460.10 $[M + H]^+$.

Biological evaluation

Minimum inhibitory concentration values were determined using the broth microdilution method in 96-well micro test plates as recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2002). The MIC_{80} was read following the OD at 630 nm at the lowest compound concentration causing 80% growth inhibition compared to the control. For the assays, the title compounds to be tested were dissolved in dimethyl sulfoxide, serially diluted in growth medium, inoculated, and incubated at 35 °C for 48 h. The growth MIC was determined at 24 h for *C. albicans* and at 72 h for *Cryptococcus neoformans*. One of the *C. albicans* strains was from the American Type Culture Collection. The clinical isolates, including

Table 1 Antifungal activities of the title compounds in vitro (MIC_{80}, μg/mL)

Compd.	*C. albicansATCC14053*	*C. albicansSCZ20352*	*C. parapsilosis90018*	*C. glabrataY10b*	*C. neoformans20322*	*T. rubrum*	*M. gypseum3627*	*A. fumigatus*
4a	0.125	0.125	0.125	0.25	0.125	0.25	0.125	2
4b	0.125	0.125	0.125	0.25	0.125	0.5	0.125	>64
4c	0.125	0.125	0.125	0.25	0.125	0.5	0.125	16
4d	0.125	0.125	0.125	0.125	0.125	0.25	0.125	16
4e	0.125	0.125	0.125	0.25	0.125	1	0.125	4
4f	0.125	0.125	0.125	0.5	0.125	1	0.125	16
4g	0.125	0.125	0.125	0.125	0.5	1	0.25	>64
4h	0.125	0.5	0.125	0.5	0.125	2	1	>64
4i	0.125	0.125	0.125	0.25	0.125	2	1	>64
4j	0.125	0.125	0.125	0.25	0.25	2	0.5	>64
4k	0.125	0.125	0.125	0.25	1	1	0.5	4
4l	0.125	0.125	0.125	0.125	1	0.5	0.5	>64
4m	0.125	0.125	0.125	0.125	0.125	0.125	0.5	>64
4n	0.125	1	0.125	1	0.125	8	1	>64
4o	0.125	0.125	0.125	0.125	4	4	0.5	>64
4p	0.125	0.125	0.125	0.125	0.125	8	64	>64
4q	0.125	2	0.125	8	0.125	64	1	>64
4r	0.125	0.125	0.125	2	4	32	0.5	>64
4s	0.125	0.125	0.125	0.25	0.125	32	0.25	>64
FCZ	1	0.5	0.5	2	0.5	4	64	64
ICZ	0.0625	0.0625	0.0313	0.5	0.0156	0.125	4	2
VCZ	0.0313	0.0625	0.0313	0.5	0.0156	0.0625	0.25	0.25
TRB	4	0.25	0.5	4	0.125	0.125	0.25	0.25
AMB	0.5	0.5	0.5	0.25	0.5	0.5	0.25	0.5

C. neoformans, *C. parapsilosis*, *C. glabrata*, *Trichophyton rubrum*, *Microsporum gypseum*, *A. fumigates* and the other *C. albicans* strain, were from the Shanghai Changzheng Hospital. FCZ, ICZ, VCZ, TRB and AMB were obtained from their respective manufacturers as positive controls (NCCLS 2002; Li et al. 2014).

Results and discussion

The title compounds **4a–s** were evaluated for their in vitro antifungal activities against eight human pathogenic fungi summarized in Table 1. The results of in vitro antifungal activities showed that all the synthesized compounds **4a–s** showed good activity against nearly all the tested fungal pathogens.

According to some clinical statistics, *C. albicans* is the most frequent agent of candidiasis. However, non-albicans *Candida* species, including *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, and *C. krusei*, account for a substantial proportion of clinical isolates collected worldwide (Papon et al. 2013). Obviously, near all the target compounds of our study show higher inhibitory activity against *C. albicans* and *C. parapsilosis* than FCZ, TRB, and AMB. Compound **4d**, **4g**, **4l**, **4m**, **4o**, and **4p** still significantly reduced the *C. glabrata* growth rate by 16-fold, 4-fold, 4-fold, 32-fold, and 2-fold, as compared to the positive control, respectively. Compounds **4a–f**, **4h**, **4i**, **4m**, **4n**, **4p**, **4q**, and **4s** (with MIC_{80} values of 0.125 μg/mL) are 4-fold more potent against *C. neoformans* than FCZ (with an MIC_{80} value of 0.5 μg/mL). Besides, compound **4m** showed the same activity (with the MIC_{80} value of 0.125 μg /mL) as that of ICZ against *T. rubrum*, and was 32-fold more potent than FCZ. The antifungal activity of compounds **4a–f** against *M. gypseum* was better than all the positive controls.

Fungal resistance has got worse over the past few decades, especially the *A. fumigates*. Survey has revealed that *A. fumigates* possesses an intrinsic mechanism resistant to triazole antifungal agents, especially to FCZ (Mellado et al. 2007). Fortunately for us, we have found that some compounds exhibit good activity against *A. fumigates*. Compound **4a** significantly reduced the *A. fumigates* growth rate at 2 μg/mL concentration, and the ICZ also did so at 2 μg/mL concentrations. The MIC_{80} value of compound **4e** and **4k** is 16 times lower than that of FCZ against *A. fumigates*.

Conclusions

In summary, a new type of antifungal azoles derivatives with dithiocarbamate side chains were synthesized and evaluated as potential antifungal agents. In vitro antifungal activities of all the compounds were evaluated against eight human pathogenic fungi encountered clinically. The results fully indicated that for antifungal activities of these novel azole derivatives, it is helpful to introduce the dithiocarbamate moieties as side chains for enhancing the antifungal activities of these title compounds against *Candida* species. Above all, compound **4a** exhibited the same activities against *A. fumigates* as ICZ. Given the interesting antifungal activities of these compounds, further studies should be launched.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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