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Preliminary communication

Synthesis and evaluation of novel 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-[(4-substitutedphenyl)-piperazin-1-yl]-propan-2-ols as antifungal agents

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Abstract

A series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-[(4-substitutedphenyl)-piperazin-1-yl]-propan-2-ols have been designed and synthesized on the basis of the structure–activity relationships and antimycotic mechanism of azole antifungal agents. Their structures were confirmed by elemental analysis, IR, MS, ^{1}H NMR and ^{13}C NMR. Results of preliminary antifungal tests against six human pathogenic fungi (*Candida albicans*, *Candida parapsilosis*, *Cryptococcus neoformans*, *Candida tropicalis*, inherently fluconazole-resistant *Candida krusei*, *Candida glabrata*) in vitro showed that all title compounds exhibited activity against fungi tested to some extent except against *C. tropicalis*. Compound **5b** showed higher activity against *C. albicans*, *C. parapsilosis* and *C. krusei* than fluconazole, and its MIC values were determined to be 0.5 μg/mL, 1 μg/mL and 4 μg/mL, respectively. Compound **5k** showed higher activities against *Torulopsis glabrata* than fluconazole (with the MIC value of 2 μg/mL). Compounds **5a**, **5c**, **5f**, **5g**, **5i** exhibited higher activities against *C. parapsilosis* than fluconazole (with the MIC values of 2 μg/mL, 2 μg/mL, 2 μg/mL, 1 μg/mL and 2 μg/mL, respectively).

Keywords: Triazole; Synthesis; Antifungal activity

1. Introduction

In the past two or three decades, 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 [1,2]. Triazole antifungals (e.g. fluconazole and itraconazole) which act by inhibiting cytochrome P450 14α-demethylase (CYP51), a key enzyme in the fungal ergosterol biosynthesis, now become the most rapidly expanding group of antifungal compounds. However, their clinical value has been limited by their relatively high risk of toxicity, the emergence of drug resistance, pharmacokinetic deficiencies, and/or insufficiencies in their antifungal activities. Despite recent developments [3–6], there is still a need for genuinely broad-spectrum and low-toxicity antifungal agents.

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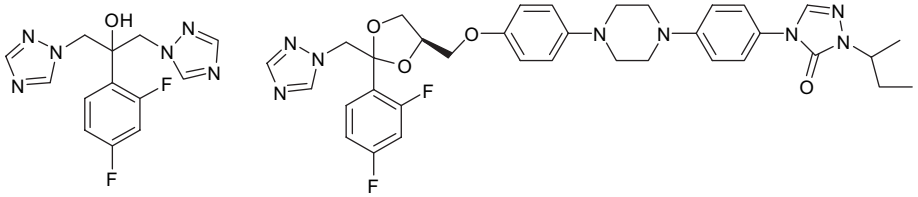


Fluconazole Itraconazole

Ji's [7] study indicated that the triazole ring in the parent structure of triazole antifungal agents was positioned perpendicularly to the porphyrin plane with a ring nitrogen atom coordinated to the heme iron and was of key importance for the antifungal activity. The halogenated phenyl group was deep in the same hydrophobic binding cleft in the active site of the target enzyme CYP51 and long chains of some antifungals such as itraconazole and ketoconazole surpassed the active site and interacted with residues in the substrate access channel.

We herein designed a novel series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-[(4-substitutedphenyl)-piperazin-1-yl]-propan-2-ols containing a triazole ring, a difluorogenated phenyl group and a long side chain, to find potent systemic antifungal agents that have a broad antifungal spectrum but with less potential to develop resistance. The 4-(4-substituted-phenyl) piperazine was chosen as side chains to adjust the physicochemical properties of the whole molecule to avoid the dissatisfying side effects and/or improve the pharmacokinetic and pharmacodynamic behavior. The structures of this series of compounds are shown in Scheme 1 and Table 1.

2. Chemistry

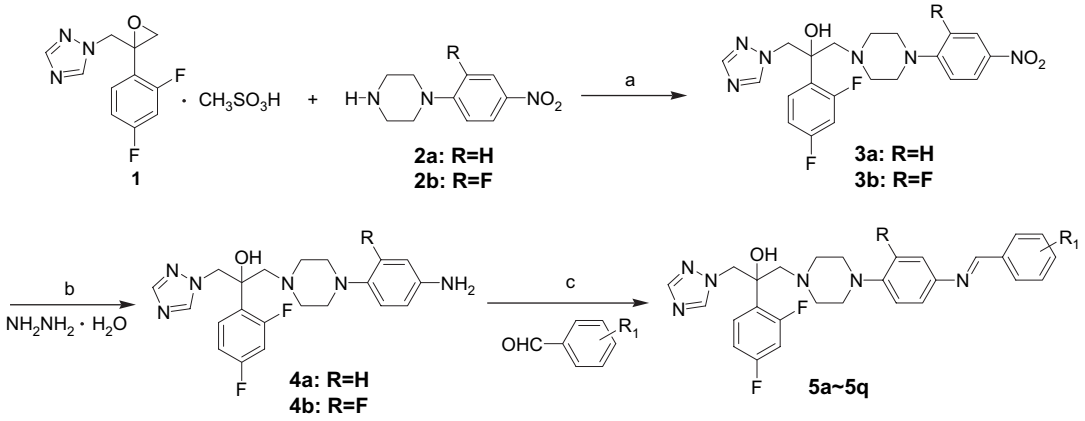
The general synthetic route of title compounds 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluoro-phenyl)-3-[(4-substitutedphenyl)-piperazin-1-yl]-propan-2-ols (**5a–5q**) is outlined in Scheme 1. The important intermediate oxirane **1**, compounds **2a** and **2b** were synthesized with known procedures [8–10].

3. Bioassays of antifungal activities

The in vitro minimal inhibitory concentrations (MICs) of the title compounds were determined by the microbroth dilution method according to the methods defined by the National Committee for Clinical Laboratory Standards [11]. Fungi strains for testing: *Candida albicans*, *Candida parapsilosis*, *Cryptococcus neoformans*, *Candida tropicalis*, *Candida krusei*, *Torulopsis glabrata* were provided through Shanghai Changhai Hospital. *C. krusei* and *C. parapsilosis* are ATCC standard strains, others are clinic isolates. *C. krusei* (ATCC6258) and *C. parapsilosis* (ATCC22019) were quality-controlled strains, and tested in each assay. Fluconazole (FLC) and itraconazole (ICZ) obtained from their respective manufacturers served as the positive control. The drug MIC_{80} was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well. 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 *Cr. neoformans*.

4. Results and discussion

The in vitro antifungal activities of all title compounds were evaluated against six human pathogenic fungi (*C. albicans*, *C. parapsilosis*, *Cr. neoformans*, *C. tropicalis*, inherently fluconazole-resistant *C. krusei*, *Candida glabrata*) which are



Scheme 1. Synthetic route to the title compounds. Conditions: (a) CH_3CH_2OH, Et_3N, 80 °C, 5 h; (b) Raney Ni, $NH_2NH_2 \cdot H_2O$, CH_3CH_2OH, 8 0 °C, 3.5 h; (c) HAc, reflux, 0.5–4 h.

Table 1
Some characteristics of the title compounds

Compound	R	R_1	Yield (%)	Mp (°C)
5a	H	4-Fluoro	85	184–186
5b	H	4-Chloro	87	194–196
5c	H	4-Bromo	83	190–192
5d	H	2,4-Dichloro	93	195–196
5e	H	3,4-Dichloro	85	176–178
5f	H	4-Methyl	88	174–176
5g	H	4-Hydroxy	70	204–206
5h	H	2-Hydroxy-3-methoxy	76	171–173
5i	H	3,4-Methylenedioxy	72	180–182
5j	H	4-Benzyloxy	68	184–185
5k	H	4-(3-Chloro-benzyloxy)	66	169–171
5l	H	4-Nitro	80	195–197
5m	H	3-Nitro	81	185–186
5n	F	4-Fluoro	76	166–168
5o	F	4-Chloro	75	192–193
5p	F	4-Bromo	72	188–190
5q	F	4-Methyl	74	186–188

often encountered clinically and are summarized in Table 2. The MIC values (in μg/mL) against different pathogenic fungi, in comparison with fluconazole (FLC) and itraconazole (ICZ), are given.

The results of antifungal activities in vitro showed that the title compounds were active against nearly all fungi tested to some extent except against *C. tropicalis*. Among the compounds tested, Compound **5b** showed higher activity against *C. albicans*, *C. parapsilosis* and *C. krusei* than fluconazole, and its MIC values were determined to be 0.5 μg/mL, 1 μg/mL and 4 μg/mL, respectively. Compounds **5a**, **5c**, **5f**, **5g**, **5i** exhibited higher activities against *C. parapsilosis* than fluconazole (with the MIC values of 2 μg/mL, 2 μg/mL, 2 μg/mL, 1 μg/mL and 2 μg/mL, respectively). Especially, the MIC value of compound **5b** is 4 times lower than that of fluconazole against *C. parapsilosis* in vitro. Compounds **5f**, **5g**, **5i**, **5l** showed higher activities against inherently fluconazole-resistant *C. krusei* than fluconazole (with the same MIC values of 4 μg/mL). Compounds **5a** and **5g** showed higher activities against *Cr. neoformans* than fluconazole (with the same MIC values of 4 μg/mL). Compound **5k** showed higher activities against *T. glabrata* than fluconazole (with the MIC values of 2 μg/mL).

5. Experimental part

Melting points were measured on a Yamato MP-21 melting-point apparatus and are uncorrected. Infrared spectra were recorded in potassium bromide disks on a HITACHI 270-50 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-*d*6 unless otherwise indicated with a Bruker AC-300P spectrometer, using TMS as internal standard. Elemental analysis was undertaken with an Italian MOD 1106 analyzer at the Analysis Center of Shanghai Institute of Pharmaceutical Industry. The solvents and reagents were used as received or dried prior to use as needed.

*5.1. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(4-nitro-phenyl)-piperazin-1-yl]-propan-2-ol (**3a**)*

To a stirred mixture of 1-[2-(2,4-difluorophenyl)-2,3-epoxypropyl]-1*H*-1,2,4-triazole methanesulfonate **1** (1.65 g, 0.005 mol), CH_3CH_2OH (30 mL) and $N(C_2H_5)_3$ (3 mL), 1-(4-nitro-phenyl)-piperazine (**2a**, 1.3 g, 0.006 mol) was added and was heated at 70–80 °C for 5 h. The reaction was monitored by TLC. After filtration, the filtrate was evaporated under reduced pressure. Water (30 mL) was added to the residue, which was then extracted with ethyl acetate (80 mL × 3). The extract was washed with saturated NaCl solution (20 mL × 3), dried over anhydrous Na_2SO_4 and evaporated. The residue was crystallized from DMF/H_2O to afford **3a** (1.78 g, 80.3%). Mp 186–187 °C [lit. [12] 186–187 °C].

Table 2
Antifungal activities of the title compounds in vitro (MIC_{80}, μg/mL)

Compound	*C. albicans*	*C. parapsilosis* ATCC22019	*Cr. neoformans*	*C. tropicalis*	*C. krusei* ATCC6258	*T. glabrata*
5a	64	2	4	>64	16	>64
5b	0.5	1	32	>64	4	>64
5c	64	2	32	>64	8	>64
5d	>64	8	64	>64	8	8
5e	1	8	>64	>64	16	>64
5f	1	2	>64	>64	4	16
5g	32	1	4	>64	4	8
5h	>64	4	64	>64	16	16
5i	16	2	32	>64	4	16
5j	64	>64	16	>64	8	32
5k	>64	16	64	>64	32	2
5l	>64	4	>64	>64	4	8
5m	64	4	16	>64	8	4
5n	4	32	8	>64	8	8
5o	16	16	32	>64	16	32
5p	32	8	32	>64	8	16
5q	16	32	8	>64	16	16
FLC	1	4	32	64	8	8
ICZ	0.25	0.25	2	32	2	1

*5.2. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(2-fluoro-4-nitro-phenyl)-piperazin-1-yl]-propan-2-ol (**3b**)*

The reaction was run similarly to that used to synthesize **3a**. A white solid was obtained, and it was crystallized from DMF/H_2O to afford **3b** in 78% yield, mp 166–167 °C. ^{1}H NMR (DMSO-d_6): 6.80–7.96 (6H, m, Ar-H), 7.81, 8.13 (2H, ss, triazole-H), 4.52–4.61 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.50–3.18 (8H, m, piperazine-H), 2.73–3.17 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). IR (KBr): 3200, 2940, 2893, 1605, 1514, 1338, 1257, 1136, 918 cm^{-1}. EIMS, *m/z*: 463 (M + 1).

*5.3. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(4-amino-phenyl)-piperazin-1-yl]-propan-2-ol (**4a**)*

To a stirred mixture of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(4-nitro-phenyl)-piperazin-1-yl]-propan-2-ol (2.22 g, 0.005 mol), CH_3CH_2OH (20 mL) and 85% hydrazine hydrate (8 mL), freshly prepared Raney Ni (0.5 g) was added and heated at 70–80 °C for 3 h. The reaction was monitored by TLC. After filtration, the filtrate was evaporated under reduced pressure. Water (20 mL) was added to the residue, which was then extracted with ethyl acetate (60 mL × 3). The extract was washed with saturated NaCl solution (20 mL × 3), dried over anhydrous Na_2SO_4 and evaporated. The residue was crystallized from ethyl acetate to afford **4a** (1.45 g, 70.1%). Mp 129–130 °C [lit. [12] 129–130 °C].

*5.4. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(2-fluoro-4-amino-phenyl)-piperazin-1-yl]-propan-2-ol (**4b**)*

The reaction was run similarly to that used to synthesize **4a**. A white solid was obtained, and it was recrystallized from ethyl acetate to afford **4b** in 75% yield, mp 134–135 °C.^{1}H NMR (DMSO-d_6): 6.35–7.56 (6H, m, Ar-H), 7.79, 8.15 (2H, ss, triazole-H), 4.52–4.54 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.49–2.84 (8H, m, piperazine-H), 2.69–3.14 (2H, dd, $J = 17$ Hz, CH_2-piperazines-), 5.28 (1H, s, OH). IR (KBr): 3404, 3212, 3117, 3053, 2950, 2835, 1616, 1514, 1274, 1139, 965 cm^{-1}. EIMS, *m/z*: 433 (M + 1).

*5.5. General procedure for the preparation of 1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(substitutedbenzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5a–5q**)*

To a stirred mixture of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(4-amino-phenyl)-piperazin-1-yl]-propan-2-ol (**4a**, 0.001 mol) or 1-(1*H*-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(2-fluoro-4-amino-phenyl)-piperazin-1-yl]-propan-2-ol (**4b**, 0.001 mol) and CH_3COOH (10 mL) substituted benzaldehyde (0.001 mol) was dropwise added and refluxed for 0.5–4 h. The reaction was monitored by TLC. After filtration, the filtrate was evaporated under reduced pressure. The residue was recrystallized from ethanol to afford the title compounds **5a–5q**.

The title compounds were characterized as follows.

*5.5.1. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(4-fluoro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5a**)*

^{1}H NMR (DMSO-d_6): δ 8.42 (1H, s, N=CH), 6.78–7.87 (11H, m, Ar-H), 7.79, 8.12 (2H, ss, triazole-H), 4.50–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.49–3.10 (8H, m, piperazine-H), 2.73–3.14 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 165.5, 163.9, 163.8, 163.5, 161.9, 161.8, 160.0, 158.1, 158.0, 156.1, 151.2, 149.6, 144.7, 143.8, 133.0, 130.5, 130.4, 129.4, 126.2, 126.1, 122.0, 116.5, 116.0, 115.8, 111.7, 111.6, 104.6, 104.4, 104.1, 77.3, 77.1, 76.8, 72.4, 62.4, 56.3, 54.3, 51.0, 49.3, 48.3. IR (KBr): 3130, 2943, 2878, 2829, 1619, 1508, 1233 cm^{-1}. EIMS, *m/z*: 521 (M + 1). Anal. calcd for $C_{28}H_{27}F_3N_6O$: C, 64.60; H, 5.23; N, 16.14. Found: C, 64.30; H, 5.15; N, 15.72.

*5.5.2. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(4-chloro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5b**)*

^{1}H NMR (DMSO-d_6): δ 8.42 (1H, s, N=CH), 6.78–7.83 (11H, m, Ar-H), 7.78, 8.12 (2H, ss, triazole-H), 4.50–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.48–3.10 (8H, m, piperazine-H), 2.73–3.14 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 163.8, 161.9, 159.9, 158.1, 158.0, 156.0, 151.2, 149.8, 144.7, 143.6, 136.8, 135.1, 129.6, 129.5, 129.4, 129.0, 126.1, 122.1, 116.5, 111.8, 111.6, 104.6, 104.4, 104.2, 77.3, 77.0, 76.8, 72.3, 62.4, 56.3, 54.3, 49.3. IR (KBr): 3128, 2955, 2877, 2830, 1619, 1506, 1233, 1141, 829 cm^{-1}. EIMS, *m/z*: 537 (M + 1). Anal. calcd for $C_{28}H_{27}ClF_2N_6O$: C, 62.63; H, 5.07; N, 15.65. Found: C, 62.76; H, 5.05; N, 15.52.

*5.5.3. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(4-bromo-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5c**)*

^{1}H NMR (DMSO-d_6): δ 8.41 (1H, s, N=CH), 6.78–7.73 (11H, m, Ar-H), 7.79, 8.12 (2H, ss, triazole-H), 4.50–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.49–3.10 (8H, m, piperazine-H), 2.73–3.14 (2H, dd, $J = 17$ Hz, CH_2-piperazine-), 5.13 (1H, s, OH). ^{13}C NMR (DMSO-d_6): δ 163.8, 161.8, 160.0, 158.1, 156.0, 151.2, 149.8, 144.7, 143.5, 135.5, 132.0, 129.8, 129.4, 126.2, 126.1, 125.3, 122.1, 116.4, 111.8, 111.6, 104.6, 104.4, 104.2, 77.3, 77.0, 76.8, 72.3, 62.4, 56.2, 54.3, 49.3. IR (KBr): 3320, 3128, 3049, 2948, 2877, 2828, 1618, 1506, 1233, 1140, 1010, 825 cm^{-1}. EIMS, *m/z*: 583 (M + 1). Anal. calcd for $C_{28}H_{27}BrF_2N_6O$: C, 57.84; H, 4.68; N, 14.45. Found: C, 57.81; H, 4.66; N, 14.29.

*5.5.4. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(2,4-dichloro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5d**)*

^{1}H NMR (DMSO-d_6): δ 8.82 (1H, s, N=CH), 6.90–8.12 (10H, m, Ar-H), 7.74, 8.28 (2H, ss, triazole-H), 5.64 (1H, s, OH), 4.60 (2H, s, triazole-CH_2-), 2.48–3.06 (8H, m,

piperazine-H), 2.73–2.95 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 163.9, 161.8, 160.0, 158.1, 158.0, 152.5, 151.2, 150.1, 144.7, 143.3, 137.0, 136.1, 132.3, 129.6, 129.4, 129.2, 127.6, 126.1, 122.4, 116.3, 111.8, 111.6, 104.6, 104.4, 104.2, 77.3, 77.3, 76.8, 72.3, 62.4, 56.3, 54.3, 49.1. IR (KBr): 3070, 3051, 2944, 2881, 2836, 1614, 1587, 1511, 1384, 1269, 1141, 831 cm^{-1}. EIMS, *m/z*: 572 (M + 1). Anal. calcd for $C_{28}H_{26}Cl_2F_2N_6O$: C, 58.85; H, 4.59; N, 14.71. Found: C, 58.84; H, 4.62; N, 14.83.

5.5.5. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(3,4-dichloro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5e**)

^{1}H NMR (DMSO-d_6): δ 8.42 (1H, s, N=CH), 6.82–8.02 (10H, m, Ar-H), 7.83, 8.17 (2H, ss, triazole-H), 4.55–4.64 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.53–3.15 (8H, m, piperazine-H), 2.62–3.18 (2H, dd, $J = 16.5$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 163.8, 161.9, 161.8, 158.1, 158.0, 154.3, 151.2, 150.0, 144.7, 143.0, 136.7, 134.7, 133.2, 130.7, 129.9, 129.4, 127.5, 126.2, 122.2, 116.6, 116.4, 111.8, 111.6, 104.6, 104.4, 104.2, 77.3, 77.0, 76.8, 72.4, 62.4, 56.2, 54.3, 49.1. IR (KBr): 3361, 3131, 2953, 2881, 2828, 1618, 1507, 1243, 1168, 967, 821, 698, 579 cm^{-1}. EIMS, *m/z*: 571 (M^+). Anal. calcd for $C_{28}H_{26}Cl_2F_2N_6O$: C, 58.85; H, 4.59; N, 14.71. Found: C, 58.89; H, 4.58; N, 14.58.

5.5.6. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(4-methyl-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5f**)

^{1}H NMR (DMSO-d_6): δ 8.42 (1H, s, N=CH), 6.78–7.76 (11H, m, Ar-H), 7.79, 8.12 (2H, ss, triazole-H), 4.50–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.49–3.10 (8H, m, piperazine-H), 2.72–3.13 (2H, dd, $J = 17$ Hz, CH_2-piperazine-), 2.39 (3H, s, $-CH_3$). ^{13}C NMR (DMSO-d_6): δ 163.9, 163.8, 158.1, 157.9, 151.2, 149.4, 144.7, 144.4, 141.3, 134.1, 129.5, 128.5, 126.2, 126.1, 122.0, 116.8, 116.6, 111.7, 111.6, 104.6, 104.4, 104.1, 77.3, 77.0, 76.8, 72.3, 62.4, 56.3, 54.4, 51.1, 49.4, 21.6. IR (KBr): 3407, 3138, 2939, 2881, 2823, 1619, 1498, 1233, 1141, 967, 824 cm^{-1}. EIMS, *m/z*: 517 (M + 1). Anal. calcd for $C_{29}H_{30}F_2N_6O$: C, 67.43; H, 5.85; N, 16.27. Found: C, 67.51; H, 5.87; N, 16.04.

5.5.7. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(4-hydroxy-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5g**)

^{1}H NMR (DMSO-d_6): δ 9.30 (1H, s, OH), 8.35 (1H, s, N=CH), 6.79–7.71 (11H, m, Ar-H), 7.74, 8.16 (2H, ss, triazole-H), 4.58 (2H, s, triazole-CH_2-), 2.55–3.07 (8H, m, piperazine-H), 2.75–3.11 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 160.7, 160.6, 160.0, 159.9, 156.5, 150.4, 149.1, 144.8, 142.9, 130.0, 129.8, 129.7, 127.9, 126.2, 121.6, 115.6, 115.5, 110.7, 110.6, 103.9, 103.6, 103.4, 74.5, 74.4, 63.4, 55.6, 54.0, 48.3. IR (KBr): 3122, 2950, 2877, 2821, 1607, 1514, 1233, 1136, 966, 827, 678 cm^{-1}. EIMS, *m/z*: 519 (M + 1). Anal. calcd for $C_{28}H_{28}F_2N_6O_2$: C, 64.85; H, 5.44; N, 16.21. Found: C, 64.83; H, 5.48; N, 16.04.

5.5.8. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(2-hydroxy-3-methoxy-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5h**)

^{1}H NMR (DMSO-d_6): δ 13.85 (1H, s, OH), 8.58 (1H, s, N=CH), 6.78–7.61 (10H, m, Ar-H), 7.79, 8.12 (2H, ss, triazole-H), 4.51–4.60 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.48–3.09 (8H, m, piperazine-H), 2.73–3.10 (2H, dd, $J = 17$ Hz, CH_2-piperazine-), 3.92 (3H, s, $-OCH_3$). ^{13}C NMR (DMSO-d_6): δ 163.8, 161.8, 159.9, 159.5, 157.9, 151.4, 151.2, 150.1, 148.5, 144.7, 140.0, 129.4, 129.3, 126.0, 123.5, 122.1, 119.4, 118.4, 116.4, 114.4, 111.8, 111.6, 104.6, 104.4, 104.2, 77.3, 77.0, 76.8, 72.4, 62.4, 56.2, 54.3, 49.1. IR (KBr): 3383, 3120, 3058, 2934, 2884, 2821, 1615, 1509, 1466, 1242, 1139, 966, 828, 735 cm^{-1}. EIMS, *m/z*: 549 (M + 1). Anal. calcd for $C_{29}H_{30}F_2N_6O_3$: C, 63.49; H, 5.51; N, 15.32. Found: C, 63.37; H, 5.45; N, 15.28.

5.5.9. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(benzo[1,3]dioxol-5-yl-methylene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5i**)

^{1}H NMR (DMSO-d_6): δ 8.33 (1H, s, N=CH), 6.78–7.60 (10H, m, Ar-H), 7.78, 8.12 (2H, ss, triazole-H), 4.50–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.48–3.10 (8H, m, piperazine-H), 2.72–3.13 (2H, dd, $J = 17.5$ Hz, CH_2-piperazine-), 6.00–6.01 (2H, d, O–CH_2–O). ^{13}C NMR (DMSO-d_6): δ 163.9, 161.8, 158.1, 158.0, 156.9, 151.2, 150.2, 149.3, 148.4, 144.7, 144.2, 131.6, 129.4, 129.3, 126.2, 126.1, 125.2, 121.9, 116.6, 111.7, 111.6, 108.2, 106.8, 104.6, 104.3, 104.1, 101.5, 77.3, 77.0, 76.8, 72.3, 72.2, 62.4, 56.3, 56.2, 54.4, 49.4. IR (KBr): 3418, 3118, 2938, 2888, 2826, 2777, 1620, 1507, 1446, 1242, 1140, 1036, 928, 830, 675, 613 cm^{-1}. EIMS, *m/z*: 547 (M + 1). Anal. calcd for $C_{29}H_{28}F_2N_6O_3$: C, 63.73; H, 5.16; N, 15.38. Found: C, 63.87; H, 5.19; N, 15.48.

5.5.10. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(4-benzyloxy-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5j**)

^{1}H NMR (DMSO-d_6): δ 8.39 (1H, s, N=CH), 6.79–7.80 (16H, m, Ar-H), 7.75, 8.15 (2H, ss, triazole-H), 4.57 (2H, s, triazole-CH_2-), 2.54–3.07 (8H, m, piperazine-H), 2.75–3.11 (2H, dd, $J = 17$ Hz, CH_2-piperazine-), 5.12 (2H, s, O–CH_2–), 5.20 (1H, s, OH). ^{13}C NMR (DMSO-d_6): δ 163.8, 161.8, 161.1, 158.0, 157.9, 157.2, 151.2, 149.3, 144.7, 144.5, 136.6, 130.2, 128.6, 128.1, 127.5, 126.1, 121.9, 116.6, 116.4, 115.1, 111.7, 111.6, 104.6, 104.3, 104.1, 78.4, 77.3, 77.0, 76.8, 72.3, 72.2, 70.1, 62.4, 56.3, 54.4, 49.5. IR (KBr): 3318, 3128, 3033, 2826, 1604, 1510, 1236, 1012, 834, 733 cm^{-1}. EIMS, *m/z*: 609 (M + 1). Anal. calcd for $C_{35}H_{34}F_2N_6O_2$: C, 69.06; H, 5.63; N, 13.81. Found: C, 69.26; H, 5.61; N, 13.61.

*5.5.11. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-[4-(4-{[4-(3-chloro-benzyloxy)-benzylidene]-amino}-phenyl)-piperazin-1-yl]-propan-2-ol (**5k**)*

^{1}H NMR (DMSO-d_6): δ 8.38 (1H, s, N=CH), 6.78–7.86 (15H, m, Ar-H), 7.81, 8.13 (2H, ss, triazole-H), 4.50–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.48–3.07 (8H, m, piperazine-H), 2.72–3.13 (2H, dd, $J = 17$ Hz, CH_2-piperazine-), 5.07–5.10 (2H, d, O–CH_2–). ^{13}C NMR (DMSO-d_6): δ 163.9, 161.0, 160.7, 158.7, 158.1, 157.1, 151.2, 150.0, 149.3, 144.7, 144.4, 142.5, 138.7, 134.6, 130.2, 129.9, 128.2, 127.4, 125.3, 121.9, 121.8, 116.6, 115.0, 111.7, 111.6, 104.6, 104.3, 104.1, 78.3, 77.3, 77.0, 76.8, 72.3, 69.2, 62.4, 56.3, 54.4, 49.5. IR (KBr): 3129, 3040, 2914, 2823, 1604, 1510, 1238, 1012, 832 cm^{-1}. EIMS, *m/z*: 643 (M + 1). Anal. calcd for $C_{35}H_{33}ClF_2N_6O_2$: C, 65.36; H, 5.17; N, 13.07. Found: C, 65.18; H, 5.00; N, 12.51.

*5.5.12. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(4-nitro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5l**)*

^{1}H NMR (DMSO-d_6): δ 8.55 (1H, s, N=CH), 6.79–8.29 (11H, m, Ar-H), 7.79, 8.12 (2H, ss, triazole-H), 4.51–4.60 (2H, dd, $J = 17.5$ Hz, triazole-CH_2-), 2.49–3.11 (8H, m, piperazine-H), 2.73–3.14 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 163.9, 163.8, 161.8, 160.0, 158.1, 153.8, 151.2, 150.5, 148.9, 144.7, 142.4, 142.2, 129.4, 129.0, 126.1, 126.0, 124.0, 122.5, 116.2, 111.8, 111.6, 104.6, 104.4, 104.2, 77.3, 77.0, 76.8, 72.4, 62.4, 56.2, 54.3, 48.9. IR (KBr): 3130, 3075, 2951, 2881, 2830, 1619, 1509, 1341, 1234, 1139, 854 cm^{-1}. EIMS, *m/z*: 548 (M + 1). Anal. calcd for $C_{28}H_{27}F_2N_7O_3$: C, 61.42; H, 4.97; N, 17.91. Found: C, 61.50; H, 4.95; N, 17.76.

*5.5.13. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{4-[(3-nitro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5m**)*

^{1}H NMR (DMSO-d_6): δ 8.69 (1H, s, N=CH), 6.79–8.27 (11H, m, Ar-H), 8.13, 8.55 (2H, ss, triazole-H), 4.51–4.60 (2H, dd, $J = 17.5$ Hz, triazole-CH_2-), 2.50–3.11 (8H, m, piperazine-H), 2.74–3.14 (2H, dd, $J = 16.5$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 163.9, 163.8, 161.9, 161.8, 160.0, 158.1, 153.9, 151.2, 150.3, 148.8, 144.7, 142.5, 138.4, 133.7, 129.7, 129.4, 126.0, 125.0, 123.2, 122.4, 116.5, 116.3, 111.8, 111.6, 104.6, 104.4, 104.2, 77.3, 77.0, 76.8, 72.4, 62.4, 56.2, 54.3, 49.0. IR (KBr): 3181, 3117, 3069, 2975, 2877, 2830, 1613, 1529, 1349, 1235, 964, 819, 674 cm^{-1}. EIMS, *m/z*: 548 (M + 1). Anal. calcd for $C_{28}H_{27}F_2N_7O_3$: C, 61.42; H, 4.97; N, 17.91. Found: C, 61.22; H, 4.96; N, 17.89.

*5.5.14. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{2-fluoro-4-[(4-fluoro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5n**)*

^{1}H NMR (DMSO-d_6): δ 8.39 (1H, s, N=CH), 6.83–7.87 (10H, m, Ar-H), 7.81, 8.16 (2H, ss, triazole-H), 4.54–4.60 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.53–3.00 (8H, m, piperazine-H), 2.71–3.14 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 165.3, 163.3, 158.6, 156.4, 154.4, 150.9, 145.7, 145.6, 145.3, 138.6, 138.5, 133.2, 131.2, 131.1, 130.2, 119.6, 118.6, 116.3, 116.2, 111.2, 109.2, 109.0, 104.1, 103.9, 75.1, 75.0, 64.0, 56.1, 54.6, 50.6. IR (KBr): 3396, 3134, 2830, 1615, 1510, 1249, 1139, 966, 834, 680 cm^{-1}. EIMS, *m/z*: 539 (M + 1). Anal. calcd for $C_{28}H_{26}F_4N_6O$: C, 62.45; H, 4.87; N, 15.61. Found: C, 62.35; H, 4.87; N, 15.39.

*5.5.15. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{2-fluoro-4-[(4-chloro-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5o**)*

^{1}H NMR (DMSO-d_6): δ 8.39 (1H, s, N=CH), 6.82–7.80 (10H, m, Ar-H), 7.81, 8.16 (2H, ss, triazole-H), 4.51–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.52–2.99 (8H, m, piperazine-H), 2.73–3.17 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 167.7, 160.5, 157.9, 155.7, 153.8, 150.2, 144.8, 144.6, 138.1, 135.6, 134.8, 129.8, 129.6, 128.6, 119.0, 118.0, 110.5, 110.4, 108.6, 108.4, 103.4, 74.4, 63.3, 55.8, 55.6, 53.9, 50.2, 50.0. IR (KBr): 3394, 3135, 2828, 1615, 1508, 1249, 1139, 966, 825, 679 cm^{-1}. EIMS, *m/z*: 555 (M + 1). Anal. calcd for $C_{28}H_{26}ClF_3N_6O$: C, 60.60; H, 4.72; N, 15.14. Found: C, 60.33; H, 4.90; N, 14.96.

*5.5.16. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{2-fluoro-4-[(4-bromo-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5p**)*

^{1}H NMR (DMSO-d_6): δ 8.37 (1H, s, N=CH), 6.82–7.80 (10H, m, Ar-H), 7.80, 8.15 (2H, ss, triazole-H), 4.51–4.59 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.51–2.99 (8H, m, piperazine-H), 2.72–3.16 (2H, dd, $J = 17$ Hz, CH_2-piperazine-). ^{13}C NMR (DMSO-d_6): δ 168.0, 162.6, 158.3, 155.8, 153.9, 150.4, 144.9, 144.8, 135.1, 131.8, 130.2, 129.7, 124.7, 119.1, 118.4, 110.7, 110.5, 108.7, 103.9, 103.7, 74.5, 63.4, 55.6, 54.1, 54.0, 50.3, 50.0. IR (KBr): 3395, 3135, 2946, 2826, 1615, 1507, 1248, 1139, 966, 822 cm^{-1}. EIMS, *m/z*: 599 (M + 1). Anal. calcd for $C_{28}H_{26}BrF_3N_6O$: C, 56.10; H, 4.37; N, 14.02. Found: C, 55.81; H, 4.45; N, 13.95.

*5.5.17. 1-(1H-1,2,4-Triazol-1-yl)-2-(2,4-difluorophenyl)-3-(4-{2-fluoro-4-[(4-methyl-benzylidene)-amino]-phenyl}-piperazin-1-yl)-propan-2-ol (**5q**)*

^{1}H NMR (DMSO-d_6): δ 8.38 (1H, s, N=CH), 6.81–7.76 (10H, m, Ar-H), 7.80, 8.16 (2H, ss, triazole-H), 4.51–4.58 (2H, dd, $J = 18$ Hz, triazole-CH_2-), 2.41–2.98 (8H, m, piperazine-H), 2.73–3.17 (2H, dd, $J = 17$ Hz, CH_2-piperazine-), 2.41 (3H, s, $-CH_3$). ^{13}C NMR (DMSO-d_6): δ 163.0, 161.0, 159.8, 156.4, 154.4, 150.9, 146.1, 145.3, 141.7, 138.4, 138.3, 133.9, 130.2, 129.8, 129.0, 119.6, 118.4, 111.2, 111.0, 109.1, 109.0, 104.3, 104.1, 75.1, 75.0, 64.0, 56.4, 56.1, 54.6, 50.8, 50.6, 24.2. IR (KBr): 3387, 3136, 2946, 2885, 2821, 1614, 1497, 1249, 1139, 966, 819, 679 cm^{-1}. EIMS, *m/z*: 535 (M + 1). Anal. calcd for $C_{29}H_{29}F_3N_6O$: C, 65.16; H, 5.47; N, 15.72. Found: C, 65.04; H, 5.60; N, 15.09.

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References

[1] S.K. Fridkin, W.R. Jarvis, Clin. Microbiol. Rev. 9 (1996) 499–511.
[2] J.R. Wingard, H. Leather, Biol. Blood Marrow Transplant. 10 (2004) 73–90.
[3] P. Kale, L.B. Johnson, Drugs Today 41 (2005) 91–105.
[4] P.H. Chandrasekar, E. Manavathu, Drugs Today 37 (2001) 135–148.
[5] A. Chen, J.D. Sobel, Expert Opin. Emerg. Drugs 10 (2005) 21–33.
[6] S. Sundriyal, R.K. Sharma, R. Jain, Curr. Med. Chem. 13 (2006) 1321–1335.
[7] H. Ji, W. Zhang, Y. Zhou, M. Zhang, J. Zhu, Y. Song, J. Lu, J. Med. Chem. 43 (2000) 2493–2505.
[8] K. Richardson, US Patent 4,404,216, 1983.
[9] J. Yang, L. Liu, X. Wang, J. Lu, C. Sun, H. Zhang, Y. Zhou, Z. Zhang, Yiyao Gongye 5 (1984) 1–4.
[10] D.K. Hutchinson, M.R. Barbachyn, S.J. Brickner, R.B. Gammill, M.V. Patel, US Patent 8,804,32, 1996.
[11] National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts Approved standard. Document M27-A2, National Committee for Clinical Laboratory Standards, Wayne, PA, 2002.
[12] Q. Sun, C. Liu, Q. Wu, D. Zhang, Y. Cao, Y. Jiang, H. Zhao, Zhongguo Yaowu Huaxue Zazhi 14 (2004) 14–18.