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Original article

Design, synthesis and antifungal activity of novel triazole derivatives containing substituted 1,2,3-triazole-piperdine side chains

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ABSTRACT

Due to increasing incidence of invasive fungal infections and severe drug resistance to triazole antifungal agents, a series of novel antifungal triazoles with substituted triazole-piperidine side chains were designed and synthesized. Most of the target compounds showed good inhibitory activity against a variety of clinically important fungal pathogens. In particular, compounds **8t** and **8v** were highly active against *Candida albicans* and *Cryptococcus neoformans* with MIC values in the range of 0.125 μg/mL to 0.0125 μg/mL. They represent promising leads for the development of new generation of triazole antifungal agents. Molecular docking studies revealed that the target compounds interacted with CACYP51 mainly through hydrophobic and *Van der Waals* interactions.

1. Introduction

The incidence of invasive fungal infections (IFIs) has significantly increased over the past two decades due to increasing number of organ transplant recipients and stem cell transplantation, broad use of aggressive chemotherapy and prevalence of human immunodeficiency virus (HIV) infection [1]. Candidosis, aspergillosis, and cryptococcosis represent the three most common IFIs, whose mortality rate is high (*e.g.* about 50% for candidosis and nearly 100% for invasive aspergillosis). However, effective and safe antifungal agents are very limited. Clinically available antifungal agents (Fig. 1) for IFIs mainly include amphotericin B, triazoles, echinocandins, and 5-fluorocytosine [2,3]. Among them, triazole antifungal agents (*i.e.* fluconazole, voriconazole, itraconazole and posaconazole) are widely used as the first-line antifungal therapy. However, broad use of them has caused severe drug resistance [4]. Thus, the development of new generation of triazole antifungal agents is urgently needed. New triazoles with improved pharmacological and pharmacokinetic profiles are emerging rapidly [5–9]. For example, isavuconazole and albaconazole are under late stages of clinical evaluations [10].

Triazole antifungal agents act by inhibition of fungal lanosterol 14α-demethylase (**CYP51**), which is a key enzyme in ergosterol biosynthesis. The crystal structure of fungal CYP51 has not been solved up to date. In our previous studies, we built three-dimensional models of CYP51 from *Candida albicans* (CACYP51), *Cryptococcus neoformans* (CNCYP51), and *Aspergillus fumigatus* (AFCYP51) by homology modeling [11–13]. Binding modes of triazole antifungal agents were investigated by molecular docking and molecular dynamics simulations [11,14,15]. Guided by the results from molecular modeling, a number of new triazoles were rationally designed and synthesized by our group [15–24]. Among them, triazoles with benzyloxypiperidinyl side chains showed good antifungal activity with a broad spectrum (Fig. 1) [24]. In order to investigate their structure-activity relationship (SAR) and discover highly active antifungal lead compound, herein a series of triazole-piperidine derivatives were designed and synthesized. Most of the target compounds showed excellent antifungal activity against a variety of fungal pathogens.

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2. Chemistry

The oxirane intermediate **5** was synthesized according to our previously reported procedure (Scheme 1) [15]. As a key intermediate of the designed triazoles, the azide compound **7** was synthesized by the NaN_3 displacement reaction with mesylate of intermediate **6** [25]. The target compounds were obtained by click reaction of intermediate **7** with various alkynes in $H_2O/^tBuOH$ in the presence of $CuSO_4.5H_2O$ and sodium ascorbate.

3. Microbiology

In vitro antifungal activity was measured according to the protocols from National Committee for Clinical Laboratory Standards (NCCLS). Serial dilution method in 96-well microtest plate was used to determine the minimum inhibitory concentration (MIC) of the target compounds [15]. Tested fungal strains were obtained from the ATCC or clinical isolates. Briefly, the MIC value was defined as the lowest concentration of tested compounds that resulted in a culture with turbidity less than or equal to 80% inhibition when compared with the growth of the control. Tested compounds were dissolved in DMSO serially diluted in growth medium. The yeasts were incubated at 35 °C and the mold and dermatophytes at 28 °C. Growth MIC was determined at 24 h for *Candida* species, at 72 h for *C. neoformans*, and at 7 days for *A. fumigatus*.

4. Results and discussion

4.1. Design rationale

Triazole, difluorophenyl and tertiary alcohol are essential pharmacophore for triazole antifungal agents. Most of the structural optimization studies were focused on variation of the C3-side chain. In our previous studies, new triazoles containing benzyloxypiperidinyl side chain were designed by structure-based lead fusion, which showed excellent antifungal activity [24]. Thus, it is highly desirable to obtain more SAR information for this promising class of antifungal lead compound. Click reaction is an efficient approach to the synthesis of drug-like molecules which has been widely applied in drug discovery [26]. Herein, 1,2,3-triazole was used to replace the benzyl alcohol group and various alkyl or aromatic groups were also introduced to form additional interactions with CYP51 (Fig. 2).

4.2. In vitro antifungal activity

In vitro antifungal activity of the target compounds is listed in Table 1. Fluconazole and itraconazole were used as reference drugs. In general, most of the target compounds showed good inhibitory activity against the tested fungal pathogens, especially for *C. albicans* and *C. neoformans*. There are thirteen compounds (**8a**, **8b**, **8c**, **8e**, **8l**, **8q**–**x**) that showed better inhibitory activity against *C. albicans* than fluconazole and itraconazole (MIC range: 0.125 μg/mL ~ 0.0125 μg/mL). Particularly, compounds **8t** (MIC = 0.0625 μg/mL) and **8v** (MIC = 0.0125 μg/mL) were highly active *C. albicans* inhibitors. In contrast, the target compounds as well as positive drugs showed decreased inhibitory activity against other *Candida* species including *Candida parapsilosis* and *Candida glabrata*. Even-though, three compounds (**8e**, **8t**, **8x**) still revealed good activity with MIC values in the range of 0.25 μg/mL to 0.5 μg/mL. On the *C. neoformans* strain, ten compounds (**8b**, **8e**, **8l**, **8q**, **8r**, **8s**, **8t**, **8v**, **8w** and **8x**) were more active (MIC range: 0.25 μg/mL ~ 0.5 μg/mL) than fluconazole and itraconazole. Fluconazole is inactive against *A. fumigatus* (MIC > 64 μg/mL), while several target compounds showed moderate activity (MIC range: 8 μg/mL ~ 32 μg/mL). However, all of them were less active than itraconazole (MIC = 1 μg/mL). Moreover, several compounds also showed good activity against dermatophytes *Trichophyton rubrum* and *Microsporum gypseum*. For example, the MIC values of compounds **8e**, **8p**, **8s**, **8v**, **8w** and **8x** against *T. rubrum* were in the range of 0.25 μg/mL to 0.5 μg/mL, whose activity was comparable or superior to that of itraconazole and fluconazole. Among the synthesized triazole derivatives, compounds **8e**, **8t**, **8v** and **8x** were highly active with a broad spectrum.

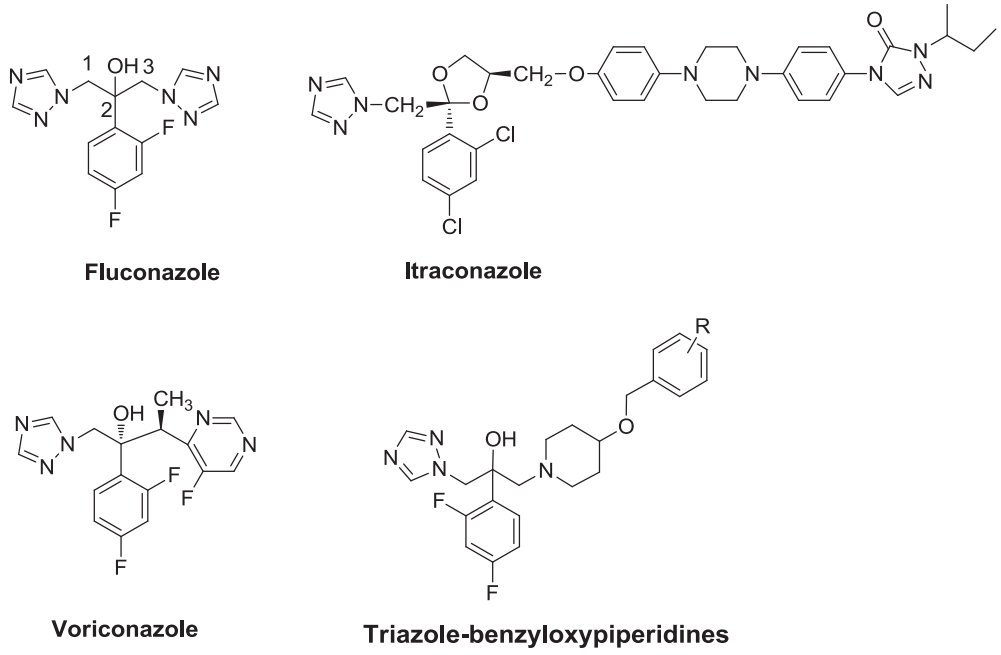
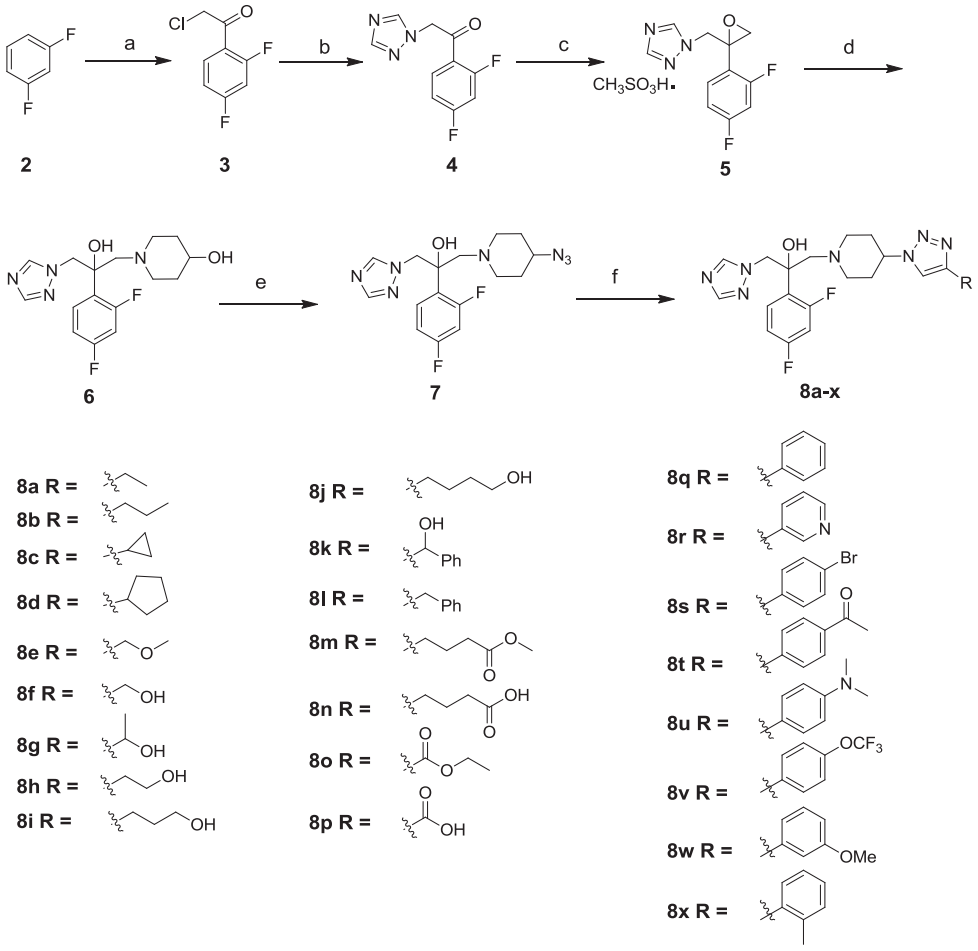


Fig. 1. Chemical structures triazole antifungal agents and lead compounds.



Scheme 1. a. $ClCH_2COCl$, $AlCl_3$, CH_2Cl_2, 40 °C, 3 h, 50%; b. triazole, K_2CO_3, CH_2Cl_2, r.t., 24 h, 70.0%; c. $(CH_3)_3SOI$, NaOH, toluene, 60 °C, 3 h, 62.3%; d. piperidin-4-ol, Et_3N, EtOH, 78 °C, 12 h, 78.5%; e. 1) MsCl, Et_3N, DCM, rt, 2 h; 2) NaN_3, DMF, 40 °C, 6 h, 76.2%; f. R—≡, $CuSO_4.5H_2O$, Sodium ascorbate, Et_3N, $H_2O/^tBuOH$, 50 °C, 12 h, 51.2–87.3%.

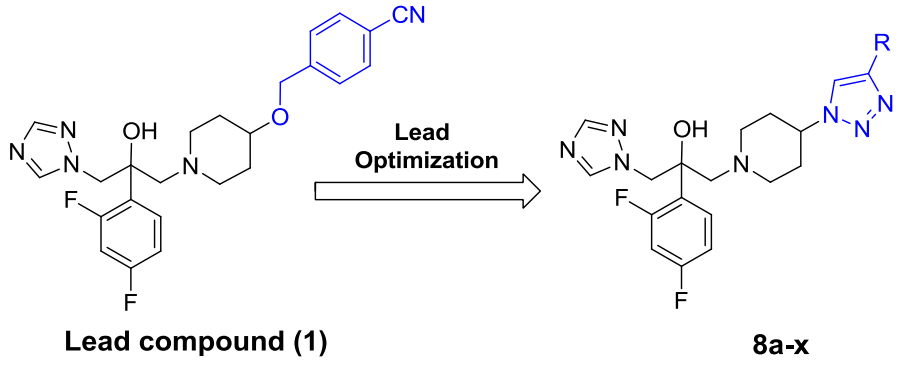


Fig. 2. Design rationale of the target compounds.

4.3. Structure–activity relationships

Compounds with ethyl (**8a**), propyl (**8b**) and cyclopropyl (**8c**) substitutions on the 1,2,3-triazole ring showed broad-spectrum antifungal activity. In contrast, the cyclopentyl derivative **8d** was less active. Moreover, all the hydroxylalkyl derivatives (**8f**–**8k**) showed significantly decreased antifungal activity. Most of them were inactive against *C. parapsilosis*, *C. neoformans*, *A. fumigatus*, *T. rubrum* and *M. gypseum*. Interestingly, the methylation of compound **8f** led to obvious improvement of the antifungal activity. Compound **8e** is one of the most active compounds in this series. For the compounds with ester and carboxylic acid substitutions, the butyrate (**8m**) and butyric acid (**8n**) derivatives were more potent than the corresponding formate (**8o**) and formic acid (**8p**) derivatives. All the aromatic derivatives **8q**-**8x** were proven to be excellent fungal inhibitors. The replacement of the phenyl group of compound **8q** by a pyridinyl group (**8r**) resulted in slight increase of the activity against *C. neoformans*, but its inhibitory activity against dermatophytes was reduced. For the substitutions on the phenyl ring of compound **8q**, the introduction of 4-acetyl (**8t**, MIC = 0.0625 μg/mL) and 4-trifluoromethoxy (**8v**, MIC = 0.0125 μg/mL) group was favorable for the anti-*C. albicans* activity. In contrast,

Table 1
In vitro antifungal activities of the target compounds (MIC_{80}, μg/mL).[a]

Compd.	*C. alb.*	*C. par.*	*C. neo.*	*C. gla.*	*A. fum.*	*T. rub*	*M. gyp*
8a	0.125	1	1	0.5	64	1	8
8b	0.125	1	0.5	0.25	16	1	4
8c	0.125	1	1	0.25	64	2	8
8d	0.5	8	8	4	>64	16	64
8e	0.125	0.5	0.25	0.25	16	0.5	2
8f	8	>64	64	32	>64	>64	>64
8g	8	>64	64	16	>64	>64	>64
8h	4	>64	>64	16	>64	>64	>64
8i	8	>64	>64	16	>64	>64	>64
8j	4	32	32	8	>64	64	>64
8k	2	8	32	8	16	32	>64
8l	0.125	1	0.5	1	32	1	4
8m	0.25	4	4	2	64	4	8
8n	0.5	8	4	4	>64	4	32
8o	8	>64	>64	32	>64	>64	>64
8p	4	64	16	8	>64	>64	>64
8q	0.125	1	0.25	1	>64	0.5	4
8r	0.125	1	0.125	1	64	4	16
8s	0.125	1	0.125	0.5	>64	0.25	2
8t	0.0625	0.5	0.125	0.5	64	1	2
8u	0.25	2	1	2	8	2	4
8v	0.0125	2	0.125	0.5	8	0.5	4
8w	0.125	4	0.125	2	>64	4	32
8x	0.125	0.5	0.25	0.5	>64	0.5	64
ITZ	1	4	2	1	1	0.5	2
FCZ	0.25	2	1	1	>64	8	32

[a] Abbreviations: *C. alb. Candida albicans*; *C. par. Candida parapsilosis*; *C. neo. Cryptococcus neoformans*; *C. gla. Candida glabrata*; *A. fum. Aspergillus fumigatus*; *T. rub. Trichophyton rubrum*; *M. gyp. Microsporum gypseum*; FCZ: Fluconazole; ITZ: Itraconazole.

the addition of 4-*N*,*N*-dimethyl group (**8u**) led to slight decrease of the antifungal activity. Moreover, the addition of the 2-methyl substitution (**8x**) had positive effects on inhibitory activity against *C. parapsilosis* and *C. glabrata*.

4.4. Binding mode of compounds *8t* and *8v* with the active site of CACYP51

In order to investigate the binding mode of the designed triazoles, two highly active compounds, **8t** and **8v**, were docked into the active site of CACYP51. As shown in Fig. 3, the triazole ring and difluorophenyl group of compounds **8t** and **8v** formed coordination bond and hydrophobic interactions with heme group (Fe ion) and the hydrophobic pocket (Phe126, Ile131 and Tyr132), respectively. The side chains of the two compounds were extended into the S4 pocket [13,27] of the active site of CACYP51. The triazoyl and piperidinyl groups interacted with Leu376, Met508, Leu121, Phe380 and Tyr118 through hydrophobic and *Van der Waals* interactions. The substitutions on the 1,2,3-triazole ring were located into a hydrophobic pocket lined with Tyr505, Phe233, Pro230 and Tyr64. The magnitude of hydrophobic interactions of various substitutions with this pocket played an important role for the binding affinity and antifungal activity. For example, compounds with hydrophilic hydroxyl group (**8f**–**8k**) at this site showed weak antifungal activity.

5. Conclusion

In summary, a series of novel antifungal triazoles with substituted triazole-piperidine side chains were designed and synthesized. Several compounds showed excellent antifungal activity against a variety of clinically important fungal pathogens. Molecular docking studies revealed that the target compounds interacted with CACYP51 mainly through hydrophobic and *Van der Waals* interactions. Compounds **8e**, **8t**, **8v** and **8x** were identified as highly active fungal inhibitors, which represent promising lead compounds for the development of new generation of triazole antifungal agents. Further pharmacological and pharmacokinetic evaluation of them is in progress.

6. Experimental section

6.1. General procedure for the synthesis of compounds

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 spectrometer with TMS as an internal standard. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. High-resolution mass spectrometry data were collected on a Kratos Concept mass spectrometer. Infrared spectra (IR) were recorded on a Brucker Vector II

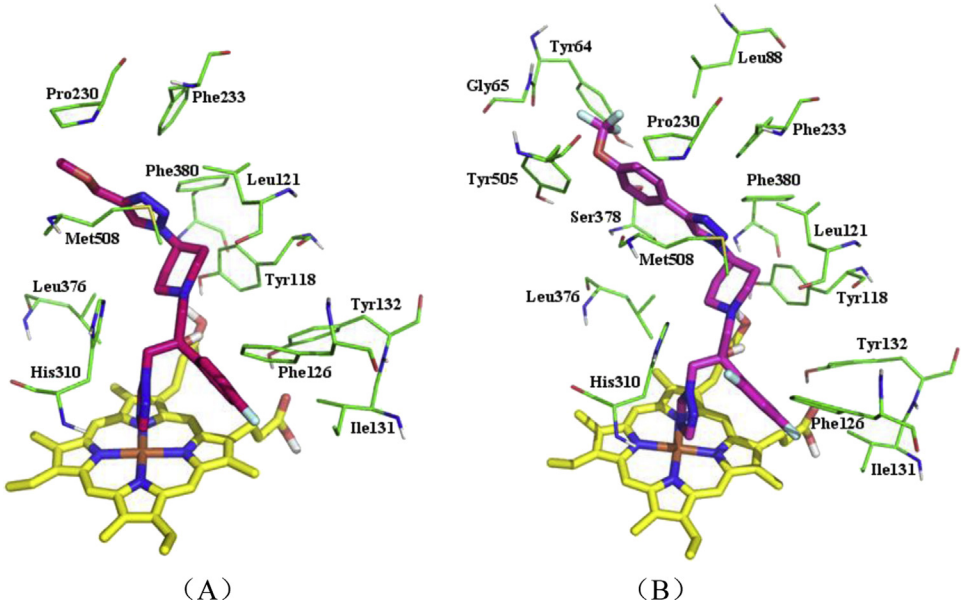


Fig. 3. The binding mode of compounds **8t** (A) and **8v** (B) in the active site of CACYP51.

instrument. Melting points were carried out on OptiMelt MPA100. TLC analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60 G (Qingdao Haiyang Chemical, China). Commercial solvents were used without any pretreatment.

6.1.1. 1-(4-Azidopiperidin-1-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl) propan-2-ol (**7**)

Methanesulfonyl chloride (1.88 g, 1.1 eq) was added dropwise to a solution of compound **6** (5.07 g, 15 mmol) and triethylamine (1.1 mL, 4 eq) in anhydrous CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was stirred for 1.5 h at this temperature. Then, water (30 mL) was added to quench the reaction and the mixture was extracted with CH_2Cl_2 for 3 times. After drying and evaporation, the crude product was used in the next step without further purification. The above intermediate was dissolved in DMF (30 mL) and was added sodium azide (2.93 g, 3 eq). The reaction solution was stirred at 40 °C for 6 h. Then, water (20 mL) and CH_2Cl_2 (50 mL) was added and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (eluents:hexane:EtOAc = 2:1, v/v) to afford compound **7** as yellow oil (3.93 g, 76.2% yield of two steps). ^{1}H NMR (400 MHz, $CDCl_3$)δ: δ8.16 (s, 1H), 7.81 (s, 1H), 7.45–7.66 (m, 1H), 6.73–6.97 (m, 2H), 5.19 (brs, 1H), 4.44–4.61 (m, 2H), 3.38 (brs, 1H), 3.08 (d, J = 13.55 Hz, 1H), 2.68 (d, J = 13.80 Hz, 1H), 2.51–2.60 (m, 1H), 2.41–2.51 (m, 1H), 2.27–2.38 (m, 1H), 2.18 (t, J = 9.41 Hz, 1H), 1.70–1.91 (m, 2H), 1.45–1.60 (m, 2H). MS (ESI) m/z: 363.9 (M+1).

6.1.2. 2-(2,4-Difluorophenyl)-1-(4-(4-ethyl-1H-1,2,3-triazol-1-yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (***8a***)

To a solution of compound **7** (40 mg, 0.11 mmol) and but-1-yne (8 mg, 1.3 equiv) in *tert*-butyl alcohol (1 mL) was added a mixture of $CuSO_4.5H_2O$ (20 mol %) and sodium ascorbate (40 mol %) in H_2O (0.5 mL). The reaction mixture was stirred at 50 °C overnight. Then, water (10 mL) was added and was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with water (10 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash chromatography using EtOAc/heptane as eluents (1:5) to give the target compound as a white solid: 20 mg, yield 43.3%, 53.0–55.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ: 1.26 (t, J = 7.53 Hz, 3H), 1.81–2.04 (m, 3H), 2.08–2.15 (m, 1H), 2.21–2.30 (m, 1H), 2.46–2.59 (m, 2H), 2.73 (q, J = 7.45 Hz, 4H), 3.12 (d, J = 13.55 Hz, 1H), 4.29–4.38 (m, 1H), 4.48–4.60 (m, 2H), 6.76–6.87 (m, 2H), 7.24 (s, 1H), 7.52–7.61 (m, 1H), 7.80 (s, 1H), 8.14 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.31, 159.52, 150.54, 147.82, 143.90, 129.28, 125.45, 117.97, 111.85, 103.56, 72.47, 62.24, 57.34, 56.60, 54.55, 52.34, 32.48, 31.53, 25.62, 12.78. IR (KBr) 3256, 2843, 2360, 1617, 1540, 1500, 1420, 1395, 1255, 1201, 1152, 1034, 835, 812, 632 cm^{-1}. HRMS calcd for $C_{20}H_{25}F_2N_7O$ $[M+H]^+$: 418.2445, found: 418.2448.

The synthetic procedure for compounds **8b**–**x** was similar to the synthesis of compound **8a**.

6.1.3. 2-(2,4-Difluorophenyl)-1-(4-(4-propyl-1H-1,2,3-triazol-1-yl) piperidin-1-yl)- 3-(1H-1,2,4-triazol-1-yl)propan-2-ol (***8b***)

White solid: 26 mg, yield 47.8%, 50.0–51.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (t, J = 7.40 Hz, 3H), 1.63–1.71 (m, 2H), 1.82–2.05 (m, 3H), 2.08–2.16 (m, 1H), 2.25 (td, J = 11.80, 2.26 Hz, 1H), 2.45–2.60 (m, 2H), 2.61–2.79 (m, 4H), 3.12 (d, J = 13.55 Hz, 1H), 4.27–4.39 (m, 1H), 4.46–4.64 (m, 2H), 6.68–6.92 (m, 2H), 7.23 (s, 1H), 7.47–7.62 (m, 1H), 7.80 (s, 1H), 8.14 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.35, 159.22, 150.85, 147.43, 143.34, 130.02, 125.78, 118.03, 112.12, 103.43, 72.57, 62.27, 57.45, 56.22, 54.34, 52.44, 32.86, 32.28, 31.23, 24.36, 13.98. IR (KBr) 3312, 2825, 2798, 2361, 1615, 1534, 1499, 1450, 1268, 1200, 1143, 1056, 1021, 858, 810, 766, 698, 670, 622 cm^{-1}. HRMS calcd for $C_{21}H_{27}F_2N_7O$ $[M+H]^+$: 432.2521, found: 432.2519.

6.1.4. 1-(4-(4-Cyclopropyl-1H-1,2,3-triazol-1-yl)piperidin-1-yl)-2-(2,4- difluorophenyl) -3-(1H-1,2,4-triazol-1-yl)propan-2-ol (***8c***)

Colorless oil: 35 mg, yield 45.3%. ^{1}H NMR (400 MHz, $CDCl_3$)δ: 0.78–0.87 (m, 2H), 0.88–0.98 (m, 2H), 1.24–1.26 (m, 1H), 1.83–1.99 (m, 3H), 2.06–2.13 (m, 1H), 2.25 (td, J = 11.80, 2.26 Hz, 1H), 2.45–2.60 (m, 2H), 2.68–2.77 (m, 2H), 3.11 (d, J = 13.55 Hz, 1H), 3.72 (q, J = 7.03 Hz, 1H), 4.25–4.38 (m, 1H), 4.47–4.61 (m, 2H), 6.76–6.89 (m, 2H), 7.19 (s, 1H), 7.56 (td, J = 9.03, 6.78 Hz, 1H), 7.80 (s, 1H), 8.14 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.44, 159.85, 152.44, 151.23, 144.58, 129.46, 125.46, 117.53, 111.67, 104.55, 72.67, 62.24, 57.34, 56.46, 54.56, 52.79, 33.38, 32.63, 10.22, 9.58. IR (KBr) 3276, 2874, 2812, 2360, 1622, 1540, 1500, 1462, 1420, 1285, 1223, 1145, 1065, 986, 856, 732, 688, 650, 594 cm^{-1}. HRMS calcd for $C_{21}H_{25}F_2N_7O$ $[M+H]^+$: 430.2056, found: 430.2053.

6.1.5. 1-(4-(4-Cyclopentyl-1H-1,2,3-triazol-1-yl)piperidin-1-yl)-2-(2,4- difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (***8d***)

White solid: 80 mg, yield 60.3%, 58.0–60.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.13 (s, 1H), 7.77 (s, 1H), 7.46–7.60 (m, 1H), 7.22 (s, 1H), 6.71–6.87 (m, 2H), 5.12 (s, 1H), 4.44–4.63 (m, 2H), 4.22–4.36 (m, 1H), 3.03–3.18 (m, 2H), 2.65–2.77 (m, 2H), 2.40–2.53 (m, 2H), 2.17–2.29 (m, 1H), 1.80–2.14 (m, 6H), 1.49–1.78 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.32, 159.72, 152.43, 151.07, 144.68, 129.36, 125.88, 117.33, 111.60, 104.31, 72.37, 62.04, 57.09, 56.10, 54.35, 52.89, 36.76, 33.18, 32.83, 32.33, 25.11. IR (KBr) 3128, 2954, 2868, 2808, 2359, 1617, 1547, 1499, 1452, 1421, 1383, 1272, 1206, 1138, 1050, 1011, 965, 850, 818, 724, 679, 652 cm^{-1}. HRMS calcd for $C_{23}H_{29}F_2N_7O$ $[M+H]^+$: 458.2474, found: 458.2483.

6.1.6. 2-(2,4-Difluorophenyl)-1-(4-(4-(methoxymethyl)-1H-1,2,3-triazol-1-yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (8e)

Colorless oil: 40 mg, yield 51.0%. ^{1}H NMR (400 MHz, CD_3OD)δ:δ 8.38 (s, 1H), 8.00 (s, 1H), 7.78 (s, 1H), 7.46–7.59 (m, J = 6.78, 8.78, 8.78 Hz, 1H), 6.96 (ddd, J = 2.51, 9.10, 11.73 Hz, 1H), 6.88 (dt, J = 2.26, 8.41 Hz, 1H), 4.60–4.78 (m, 2H), 4.52 (s, 2H), 4.41–4.50 (m, 1H), 3.37 (s, 3H), 3.07 (d, J = 13.80 Hz, 1H), 2.95 (d, J = 11.80 Hz, 1H), 2.88 (d, J = 14.05 Hz, 1H), 2.70–2.80 (m, 1H), 2.50–2.61 (m, 1H), 2.33–2.44 (m, 1H), 2.06–2.19 (m, 2H), 1.93–2.05 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 162.20, 159.60, 149.68, 144.67, 144.03, 129.52, 125.46, 121.81, 110.72, 103.50, 73.73, 64.86, 62.76, 57.90, 56.96, 55.98, 53.98, 53.15, 32.22, 31.95. IR (KBr) 3406, 2932, 2817, 2359, 1618, 1499, 1455, 1421, 1338, 1272, 1138, 1100, 1050, 1010, 965, 852, 819, 791, 723, 679, 619, 601 cm^{-1}. HRMS calcd for $C_{20}H_{25}F_2N_7O_2$ $[M+H]^+$: 434.2269, found: 434.2263.

6.1.7. 2-(2,4-Difluorophenyl)-1-(4-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (***8f***)

White solid: 20 mg, yield 33.3%, 68.5–69.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.30 (s, 1H), 7.95 (s, 1H), 7.74 (s, 1H), 7.34–7.46 (m, 1H), 7.16 (ddd, J = 2.26, 9.29, 11.80 Hz, 1H), 6.96 (dt, J = 2.26, 8.53 Hz, 1H), 5.69 (br s, 1H), 5.12 (t, J = 5.65 Hz, 1H), 4.57 (s, 2H), 4.46 (d, J = 5.52 Hz, 2H), 4.28–4.40 (m, 1H), 2.90 (d, J = 13.80 Hz, 1H), 2.84 (d, J = 11.80 Hz, 1H), 2.69–2.79 (m, 2H), 2.27–2.37 (m, 2H), 1.81–1.91 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.45, 159.72, 148.25, 142.88, 142.05, 130.15, 124.85, 121.80, 110.95, 102.40, 72.93, 64.66, 54.86, 56.28, 53.65, 52.26, 54.20, 31.82, 31.55. IR (KBr) 3405, 2928, 2822, 2360, 2030, 1499, 1422, 1386, 1272,

1206, 1137, 1102, 1056, 1010, 966, 872, 822, 791, 728 cm^{-1}. HRMS calcd for $C_{19}H_{23}F_2N_7O_2$ $[M+H]^+$: 420.1954, found: 420.1963.

*6.1.8. 2-(2,4-Difluorophenyl)-1-(4-(4-(1-hydroxyethyl)-1H-1,2,3-triazol-1-yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8g**)*

White solid: 46 mg, yield 50.5%, 64.0–65.0 °C. ^1H NMR (400 MHz, CD_3OD) δ: 1.53 (d, J = 6.53 Hz, 3H), 1.96–2.15 (m, 4H), 2.39 (td, J = 11.29, 3.51 Hz, 1H), 2.55 (td, J = 11.36, 3.14 Hz, 1H), 2.75 (d, J = 11.80 Hz, 1H), 2.88 (d, J = 13.80 Hz, 1H), 2.95 (d, J = 11.80 Hz, 1H), 3.07 (d, J = 13.80 Hz, 1H), 4.37–4.50 (m, 1H), 4.62–4.76 (m, 2H), 4.94–5.01 (m, 1H), 6.83–6.92 (m, 1H), 6.96 (ddd, J = 11.67, 9.03, 2.38 Hz, 1H), 7.48–7.58 (m, 1H), 7.78 (s, 1H), 7.89 (s, 1H), 8.38 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 162.21, 159.46, 152.01, 149.72, 144.71, 129.55, 126.86, 119.35, 110.74, 103.51, 73.72, 62.76, 62.26, 57.86, 55.99, 53.92, 53.19, 32.28, 32.00, 22.25. IR (KBr) 3405, 2926, 2853, 2360, 2025, 1709, 1618, 1500, 1421, 1384, 1272, 1206, 1138, 1102, 1011, 965, 892, 851, 808, 723, 679 cm^{-1}. HRMS calcd for $C_{20}H_{25}F_2N_7O_2$ $[M+H]^+$: 434.2305, found: 434.2312.

*6.1.9. 2-(2,4-Difluorophenyl)-1-(4-(4-(2-hydroxyethyl)-1H-1,2,3-triazol-1-yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8h**)*

Gray solid: 60 mg, yield 56.2%, 63.0–64.0 °C. ^1H NMR (400 MHz, CD_3OD) δ: 1.93–2.14 (m, 4H), 2.34–2.44 (m, 1H), 2.54 (td, J = 11.17, 3.76 Hz, 1H), 2.75 (d, J = 11.80 Hz, 1H), 2.85–2.97 (m, 4H), 3.07 (d, J = 13.80 Hz, 1H), 3.80 (t, J = 6.65 Hz, 2H), 4.42 (tt, J = 10.45, 5.24 Hz, 1H), 4.64–4.78 (m, 2H), 6.88 (td, J = 8.41, 2.26 Hz, 1H), 6.96 (ddd, J = 11.80, 9.03, 2.26 Hz, 1H), 7.47–7.57 (m, 1H), 7.79 (d, J = 10.04 Hz, 2H), 8.38 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 162.20, 162.26, 152.09, 147.09, 131.98, 128.20, 123.09, 113.15, 105.88, 76.14, 65.13, 63.04, 60.15, 58.34, 56.30, 55.59, 34.62, 34.35, 30.89. IR (KBr) 3310, 3113, 2957, 2855, 2803, 2360, 2026, 1615, 1515, 1498, 1420, 1384, 1306, 1274, 1140, 1098, 1051, 996, 965, 860, 805, 777, 737, 680, 655, 625, 588, 533 cm^{-1}. HRMS calcd for $C_{20}H_{25}F_2N_7O_2$ $[M+H]^+$: 434.2318, found: 434.2323.

*6.1.10. 3-(1-(1-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl) piperidin-4-yl)-1H-1,2,3-triazol-4-yl)propan-1-ol (**8i**)*

Yellow oil: 30 mg, yield 36.2%. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.13 (s, 1H), 7.79 (s, 1H), 7.55 (dt, J = 6.65, 8.97 Hz, 1H), 7.20–7.34 (m, 1H), 6.69–6.89 (m, 2H), 5.09 (br, s, 1H), 4.45–4.60 (m, 2H), 4.23–4.37 (m, 1H), 3.68 (t, J = 6.02 Hz, 2H), 3.10 (d, J = 13.55 Hz, 1H), 2.79 (t, J = 7.40 Hz, 2H), 2.69–2.75 (m, 2H), 2.46–2.58 (m, 2H), 2.25 (dt, J = 2.13, 11.73 Hz, 1H), 1.84–2.12 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.24, 159.69, 151.16, 147.30, 144.72, 129.38, 125.83, 118.70, 111.75, 104.38, 72.43, 62.03, 61.88, 57.24, 56.11, 54.35, 52.94, 32.83, 32.35, 31.92, 22.17. IR (KBr) 3405, 2932, 2857, 2360, 2341, 2025, 1617, 1551, 1499, 1420, 1384, 1272, 1207, 1137, 1056, 965, 851, 816, 723, 679, 654, 619, 590 cm^{-1}. HRMS calcd for $C_{21}H_{27}F_2N_7O_2$ $[M+H]^+$: 448.2267, found: 448.2259.

*6.1.11. 4-(1-(1-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl) piperidin-4-yl)-1H-1,2,3-triazol-4-yl)butan-1-ol (**8j**)*

Yellow oil: 56 mg, yield 54.6%. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.60–1.67 (m, 2H), 1.72–1.80 (m, 2H), 1.82–2.17 (m, 6H), 2.26 (td, J = 11.80, 2.76 Hz, 1H), 2.48–2.60 (m, 2H), 2.68–2.81 (m, 4H), 3.13 (dd, J = 13.55, 1.25 Hz, 1H), 3.68 (t, J = 6.40 Hz, 2H), 4.29–4.39 (m, 1H), 4.50–4.60 (m, 2H), 6.77–6.87 (m, 2H), 7.26 (s, 1H), 7.53–7.62 (m, 1H), 7.81 (s, 1H), 8.15 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.33, 159.71, 151.14, 147.80, 144.70, 129.28, 125.77, 118.43, 111.63, 104.36, 72.36, 62.40, 62.03, 57.16, 56.09, 54.36, 52.94, 32.84, 32.36, 32.10, 25.52, 25.28. IR (KBr) 3381, 2936, 2861, 2359, 2026, 1708, 1616, 1550, 1499, 1421, 1384, 1272, 1208, 1138, 995, 965, 852, 817, 723, 679, 655, 620, 590, 514 cm^{-1}. HRMS calcd for $C_{22}H_{29}F_2N_7O_2$ $[M+H]^+$: 462.2424, found: 462.2421.

*6.1.12. 2-(2,4-Difluorophenyl)-1-(4-(4-(hydroxy(phenyl)methyl)-1H-1,2,3-triazol-1 -yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl) propan-2-ol (**8k**)*

White solid: 50 mg, yield 62.1%, 65.0–67.0 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.11 (s, 1H), 7.75 (s, 1H), 7.48–7.58 (m, 1H), 7.39–7.46 (m, 2H), 7.27–7.38 (m, 3H), 7.20 (s, 1H), 6.74–6.84 (m, 2H), 6.00 (s, 1H), 4.96–5.16 (m, 1H), 4.45–4.56 (m, 2H), 4.22–4.34 (m, 1H), 3.82 (br. s., 1H), 3.08 (d, J = 13.55 Hz, 1H), 2.68 (d, J = 13.55 Hz, 2H), 2.41–2.56 (m, 2H), 2.15–2.26 (m, 1H), 1.81–2.08 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.08, 159.64, 151.18, 144.69, 141.97, 129.37, 128.59, 127.97, 126.35, 125.82, 119.93, 111.80, 104.37, 72.43, 69.07, 62.02, 57.46, 56.02, 54.30, 52.88, 32.76, 32.25. IR (KBr) 3423, 2923, 2360, 2026, 1618, 1498, 1420, 1384, 1272, 1137, 1047, 1013, 965, 850, 679, 619, 561 cm^{-1}. HRMS calcd for $C_{25}H_{27}F_2N_7O_2$ $[M+H]^+$: 496.2167, found: 496.2255.

*6.1.13. 1-(4-(4-Benzyl-1H-1,2,3-triazol-1-yl)piperidin-1-yl)-2-(2,4-difluorophenyl) -3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8l**)*

White solid: 42 mg, yield 50.0%, 70.0–71.5 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.39–8.44 (m, 2H), 8.24 (s, 1H), 8.14 (s, 1H), 7.81 (s, 1H), 7.56–7.65 (m, 2H), 7.46–7.54 (m, 2H), 6.78–6.89 (m, 2H), 5.01 (br, s, 1H), 4.53–4.67 (m, 2H), 4.45–4.52 (m, 1H), 3.13 (d, J = 13.55 Hz, 1H), 2.73–2.85 (m, 2H), 2.54–2.64 (m, 2H), 2.25–2.36 (m, 1H), 2.22 (d, J = 12.30 Hz, 1H), 2.03–2.15 (m, 2H), 1.89–2.01 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.18, 159.60, 150.48, 146.21, 140.66, 128.27, 127.39, 127.27, 126.54, 126.22, 118.24, 112.30, 105.70, 71.72, 61.62, 57.46, 56.02, 54.30, 52.88, 33.24, 32.76, 32.25. IR (KBr) 3288, 2964, 2817, 2361, 2028, 1736, 1609, 1499, 1422, 1378, 1135, 1041, 965, 850, 799, 770, 728, 677, 588, 531 cm^{-1}. HRMS calcd for $C_{25}H_{27}F_2N_7O$ $[M+H]^+$: 480.2341, found: 480.2344.

*6.1.14. Methyl 4-(1-(1-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl) propyl) piperidin-4-yl)-1H-1,2,3-triazol-4-yl) butanoate (**8m**)*

Yellow oil: 30 mg, yield 33.2%. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.90–2.02 (m, 4H), 2.03–2.17 (m, 2H), 2.23–2.31 (m, 1H), 2.38 (t, J = 7.40 Hz, 2H), 2.49–2.60 (m, 2H), 2.69–2.79 (m, 4H), 3.13 (d, J = 13.55 Hz, 1H), 3.67 (s, 3H), 4.29–4.39 (m, 1H), 4.50–4.61 (m, 2H), 6.79–6.88 (m, 2H), 7.28–7.30 (m, 1H), 7.53–7.62 (m, 1H), 7.81 (s, 1H), 8.16 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.85, 162.44, 159.25, 151.88, 148.12, 144.32, 129.50, 126.02, 119.10, 112.05, 105.18, 72.78, 61.85, 57.26, 56.72, 54.65, 52.92, 51.79, 32.85, 32.41, 31.92, 28.72, 20.24. HRMS calcd for $C_{23}H_{29}F_2N_7O_3$ $[M+H]^+$: 490.2158, found: 490.2162.

*6.1.15. 4-(1-(1-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl) piperidin-4-yl)-1H-1,2,3-triazol-4-yl)butanoic acid (**8n**)*

Yellow oil: 40 mg, yield 45.0%. ^1H NMR (400 MHz, CD_3OD) δ: 1.97–2.12 (m, 2H), 2.38–2.71 (m, 6H), 2.89–2.98 (m, 2H), 3.61 (br, s, 2H), 3.85–4.29 (m, 3H), 5.03–5.22 (m, 3H), 7.00–7.09 (m, 1H), 7.18 (ddd, J = 11.73, 8.97, 2.38 Hz, 1H), 7.52–7.64 (m, 1H), 8.54 (br, s, 1H), 8.74 (s, 1H), 9.86 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 178.65, 162.74, 159.33, 151.51, 148.02, 144.65, 123.22, 126.32, 119.52, 112.19, 106.08, 72.08, 61.05, 58.13, 56.14, 54.35, 51.89, 32.47, 33.02, 31.54, 28.99, 21.79. HRMS calcd for $C_{22}H_{27}F_2N_7O_3$ $[M+H]^+$: 476.2216, found: 462.2230.

*6.1.16. Ethyl 1-(1-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl) propyl) piperidin-4-yl)-1H-1,2,3-triazole-4-carboxylate (**8o**)*

Colorless oil: 36 mg, yield 52.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12 (s, 1H), 8.05 (s, 1H), 7.79 (s, 1H), 7.52–7.59 (m, 1H), 6.77–6.85 (m, 2H), 5.01 (br, s, 1H), 4.49–4.59 (m, 2H), 4.37–4.44 (m, 3H), 3.11 (d, J = 13.80 Hz, 1H), 2.70–2.79 (m, 2H), 2.52–2.59 (m, 2H), 2.23–2.31 (m, 1H), 2.13–2.19 (m, 1H), 1.98–2.06 (m, 2H), 1.90 (dd, J = 3.51, 12.05 Hz, 1H), 1.38 (t, J = 7.15 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.82, 159.64, 151.21, 144.70, 140.06, 129.35, 125.77, 125.60, 111.64, 104.37, 72.62, 61.99, 61.31, 57.80, 55.94, 54.15, 52.76, 32.76, 32.24, 14.29. HRMS calcd for $C_{21}H_{25}F_2N_7O_3$ $[M+H]^+$: 462.2060, found: 462.2057.

*6.1.17. 1-(1-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl) piperidin-4-yl)-1H-1,2,3-triazole-4-carboxylic acid (**8p**)*

White solid: 51 mg, yield 56.2%, 80.5–82.0 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 9.81 (s, 1H), 8.54–8.81 (m, 2H), 7.52–7.69 (m, 1H), 7.19 (t, J = 9.41 Hz, 1H), 6.94–7.12 (m, 1H), 5.08–5.16 (m, 1H), 5.02 (br s, 1H), 4.91 (br s, 1H), 3.86–4.35 (m, 3H), 3.54 (d, J = 11.29 Hz, 1H), 3.69 (br, s, 1H), 3.37 (s, 1H), 2.35–2.70 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ 166.45, 161.35, 159.24, 150.88, 141.70, 139.86, 129.55, 122.45, 121.50, 110.25, 103.85, 71.62, 61.65, 55.80, 56.25, 53.18, 52.21, 32.56, 32.25. HRMS calcd for $C_{19}H_{21}F_2N_7O_3$ $[M+H]^+$: 434.1747, found: 434.1749.

*6.1.18. 2-(2,4-Difluorophenyl)-1-(4-(4-phenyl-1H-1,2,3-triazol-1-yl)piperidin-1-yl) -3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8q**)*

White solid: 43 mg, yield 46.5%, 85.0–86.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17 (s, 1H), 7.80–7.85 (m, 3H), 7.75 (s, 1H), 7.57–7.64 (m, 1H), 7.41–7.47 (m, 2H), 7.32–7.38 (m, 1H), 6.82–6.89 (m, 2H), 5.11 (br. s., 1H), 4.53–4.63 (m, 2H), 4.42–4.49 (m, 1H), 3.16 (d, J = 13.55 Hz, 1H), 2.75–2.83 (m, 2H), 2.56–2.64 (m, 2H), 2.28–2.36 (m, 1H), 1.97–2.23 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.12, 159.66, 151.58, 146.05, 144.28, 131.25, 128.87, 127.55, 126.54, 125.22, 123.03, 116.88, 110.99, 105.26, 72.30, 62.80, 56.20, 55.95, 54.16, 52.45, 32.41, 32.02. IR (KBr) 3128, 2962, 2814, 2359, 2232, 2025, 1730, 1617, 1500, 1421, 1379, 1207, 1137, 1039, 965, 851, 802, 777, 736, 723, 679, 620, 590, 531 cm^{-1}. HRMS calcd for $C_{24}H_{25}F_2N_7O$ $[M+H]^+$: 466.2161, found: 466.2183.

*6.1.19. 2-(2,4-Difluorophenyl)-1-(4-(4-(pyridin-3-yl)-1H-1,2,3-triazol-1-yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8r**)*

White solid: 52 mg, yield 43.6%, 98.0–100.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.97 (br s, 1H), 8.58 (br s, 1H), 8.20 (d, J = 7.78 Hz, 1H), 8.15 (s, 1H), 7.82 (s, 2H), 7.55–7.63 (m, 1H), 7.38 (dd, J = 4.89, 7.65 Hz, 1H), 6.80–6.88 (m, 2H), 4.57 (q, J = 14.31 Hz, 2H), 4.41–4.50 (m, 1H), 3.15 (d, J = 13.55 Hz, 1H), 2.72–2.84 (m, 2H), 2.55–2.65 (m, 2H), 2.31 (t, J = 11.04 Hz, 1H), 1.95–2.23 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.30, 159.78, 151.20, 149.23, 146.93, 144.72, 132.99, 129.42, 126.75, 125.73, 117.85, 111.74, 104.37, 72.56, 62.07, 57.68, 56.02, 54.29, 52.91, 32.85, 32.37. IR (KBr) 3423, 2955, 2360, 2026, 1618, 1499, 1420, 1384, 1272, 1138, 1272, 1138, 966, 851, 808, 709, 679, 620 cm^{-1}. HRMS calcd for $C_{23}H_{24}F_2N_8O$ $[M+H]^+$: 467.2181, found: 467.2190.

*6.1.20. 1-(4-(4-(4-Bromophenyl)-1H-1,2,3-triazol-1-yl)piperidin-1-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8s**)*

White solid: 60 mg, yield 62.0%, 88.0–90.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (s, 1H), 7.80 (s, 1H), 7.72 (s, 1H), 7.66 (d, J = 8.53 Hz, 2H), 7.48–7.61 (m, 3H), 6.76–6.88 (m, 2H), 5.07 (br s, 1H), 4.48–4.62 (m, 2H), 4.34–4.46 (m, 1H), 3.12 (d, J = 13.55 Hz, 1H), 2.68–2.82 (m, 2H), 2.50–2.62 (m, 2H), 2.28 (t, J = 10.92 Hz, 1H), 1.90–2.21 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.02, 159.38, 151.21, 146.53, 144.70, 131.98, 129.77, 127.14, 125.82, 122.03, 117.44, 111.70, 104.36, 72.51, 62.35, 57.50, 56.03, 54.26, 52.89, 32.82, 32.35. IR (KBr) 3529, 3403, 3114, 3098, 3077, 2961, 2938, 2857, 2810, 1615, 1495, 1477, 1241, 1305, 1272, 1210, 1139, 1084, 1069, 1011, 995, 964, 876, 849, 826, 736, 719, 719, 678, 653, 622, 607, 530, 510, 471 cm^{-1}. HRMS calcd for $C_{24}H_{24}BrF_2N_7O$ $[M+H]^+$: 544.1875, found: 544.1872.

*6.1.21. 1-(4-(1-(1-(2-(2,4-Difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl) propyl)piperidin-4-yl)-1H-1,2,3-triazol-4-yl)phenyl) ethanone (**8t**)*

White solid: 52 mg, yield 56.5%, 95.5–97.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: δ8.80 (s, 1H), 8.30 (s, 1H), 7.95–8.03 (m, 4H), 7.75 (s, 1H), 7.37–7.45 (m, 1H), 7.14–7.21 (m, 1H), 6.97 (dt, J = 2.26, 8.41 Hz, 1H), 5.72 (s, 1H), 4.58 (s, 2H), 4.40–4.49 (m, 1H), 2.85–2.97 (m, 2H), 2.80 (d, J = 11.80 Hz, 1H), 2.73 (d, J = 13.80 Hz, 1H), 2.58 (s, 3H), 2.36 (t, J = 11.29 Hz, 2H), 1.83–2.01 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.60, 159.85, 151.26, 146.48, 144.74136.55, 135.01, 129.31, 129.05, 125.76, 118.36, 111.85, 104.41, 72.58, 62.10, 57.63, 56.04, 54.29, 52.93, 32.88, 32.40, 26.63. IR (KBr) 3544, 3405, 3083, 2963, 2940, 2859, 2812, 2359, 1679, 1613, 1508, 1494, 1422, 1309, 1273, 1211, 1141, 1012, 963, 875, 848, 835, 799, 774, 687, 17, 594, 536 cm^{-1}. HRMS calcd for $C_{26}H_{27}F_2N_7O_2$ $[M+H]^+$: 508.2567, found: 508.2573.

*6.1.22. 2-(2,4-Difluorophenyl)-1-(4-(4-(4-(dimethylamino) phenyl)-1H-1,2,3-triazol -1-yl)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8u**)*

Brown solid: 30 mg, yield 30.4%, 92.0–93.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17 (s, 1H), 7.83 (s, 1H), 7.69 (d, J = 8.78 Hz, 2H), 7.61 (s, 2H), 6.81–6.91 (m, 2H), 6.78 (d, J = 8.53 Hz, 2H), 4.52–4.63 (m, 2H), 4.36–4.46 (m, 1H), 3.15 (d, J = 13.80 Hz, 1H), 3.01 (s, 6H), 2.73–2.84 (m, 2H), 2.53–2.67 (m, 2H), 2.30 (t, J = 10.79 Hz, 1H), 1.96–2.22 (m, 4H). HRMS calcd for $C_{26}H_{30}F_2N_8O$ $[M+H]^+$: 509.2583, found: 509.2582.

*6.1.23. 2-(2,4-Difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-3-(4-(4-(4-(trifluoromethoxy) phenyl)-1H-1,2,3-triazol-1-yl)piperidin-1-yl) propan-2-ol (**8v**)*

White solid: 53 mg, yield 58.0%, 86.0–88.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.69 (s, 1H), 8.30 (s, 1H), 7.94 (d, J = 8.78 Hz, 2H), 7.75 (s, 1H), 7.37–7.47 (m, 3H), 7.13–7.21 (m, 1H), 6.92–7.03 (m, 1H), 5.71 (s, 1H), 4.52–4.62 (m, 2H), 4.37–4.49 (m, 1H), 2.85–2.96 (m, 2H), 2.79 (d, J = 11.54 Hz, 1H), 2.72 (d, J = 13.55 Hz, 1H), 2.35 (t, J = 11.04 Hz, 2H), 1.83–2.01 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.54, 159.48, 151.21, 148.91, 146.31, 129.30, 126.98, 125.85, 121.36, 117.52, 111.62, 104.37, 72.44, 62.02, 57.52, 56.04, 54.28, 52.87, 32.84, 32.36. IR (KBr) 3415, 3134, 3072, 3052, 2498, 2927, 2824, 2360, 2089, 1618, 1498, 1455, 1419, 1378, 1329, 1255, 1224, 1172, 1140, 1112, 1015, 995, 876, 823, 783, 736, 678, 622 cm^{-1}. HRMS calcd for $C_{25}H_{24}F_5N_7O_2$ $[M+H]^+$: 550.2214, found: 550.2217.

*6.1.24. 2-(2,4-Difluorophenyl)-1-(4-(4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl) piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8w**)*

White solid: 36 mg, yield 52.8%, 89.5–90.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.96–2.24 (m, 4H), 2.32 (t, J = 11.29 Hz, 1H), 2.53–2.66 (m, 2H), 2.72–2.85 (m, 2H), 3.17 (d, J = 13.05 Hz, 1H), 3.89 (s, 3H), 4.45 (br, s, 1H), 4.53-4.64 (m, 2H), 6.81–6.92 (m, 3H), 7.30–7.37 (m, 2H), 7.45 (d, J = 1.76 Hz, 1H), 7.57–7.64 (m, 1H), 7.74 (s, 1H), 7.84 (s, 1H), 8.18 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.06, 159.76, 151.20, 147.45, 144.75, 131.86, 129.70, 129.39, 125.82, 118.03, 117.60, 114.26, 111.75, 110.73, 72.43, 62.06, 57.45, 56.07, 55.36, 54.34,

52.94, 32.86, 32.38. IR (KBr) 3405, 2963, 2360, 2026, 1618, 1500, 1457, 1420, 1384, 1284, 1270, 1138, 1049, 959, 874, 775, 682, 618, 518 cm^{-1}. HRMS calcd for $C_{25}H_{27}F_2N_7O_2$ $[M+H]^+$: 496.2267, found: 496.2276.

*6.1.25. 2-(2,4-Difluorophenyl)-1-(4-(4-(o-tolyl)-1H-1,2,3-triazol-1-yl)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8x**)*

White solid: 45 mg, yield 57.2%, 84.0–84.5 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.18 (s, 1H), 7.84 (s, 1H), 7.70–7.78 (m, 1H), 7.55–7.67 (m, 2H), 7.28 (s, 3H), 6.79–6.93 (m, 2H), 5.12 (br, s, 1H), 4.52–4.65 (m, 2H), 4.41–4.51 (m, 1H), 3.16 (d, J = 13.55 Hz, 1H), 2.73–2.85 (m, 2H), 2.55–2.67 (m, 2H), 2.48 (s, 3H), 2.28–2.38 (m, 1H), 2.03 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.55, 159.35, 151.77, 145.95, 144.68, 131.31, 130.88, 127.99, 127.25, 126.95, 126.76, 125.35, 123.13, 116.95, 111.24, 105.55, 72.36, 62.15, 57.20, 56.24, 54.66, 53.05, 32.88, 32.42, 20.26. IR (KBr) 3129, 2952, 2360, 2352, 2236, 1729, 1616, 1499, 1408, 1380, 1217, 966, 852, 781, 681, 679, 585 cm^{-1}. HRMS calcd for $C_{25}H_{27}F_2N_7O$ $[M+H]^+$: 480.2318, found: 480.2324.

6.2. Molecular docking

Homology model of CACYP51 were obtained from our previous studies [12]. GOLD [28] was used for molecular docking and the docking parameters were defined the same with our previous reports [29].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.079.

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