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Original article

Design, synthesis and structure–activity relationships of new triazole derivatives containing *N*-substituted phenoxypropylamino side chains

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ABSTRACT

The incidence of invasive fungal infections and resistance to antifungal agents is increasing dramatically. It is highly desirable to develop novel azoles with improved biological profiles. The structure–activity relationship (SAR) of the *N*-substitutions was investigated in this study. *In vitro* antifungal activities revealed that sterically large groups were not favored for the *N*-substitutions. The removal of the *N*-substitutions had little effect on the antifungal activity. Two compounds with free amine group (*i.e.* **9a** and **10a**) showed excellent activity with broad antifungal spectrum. The SAR results were supported by molecular docking and the *N*-substitutions were found to be important for the conformation of the side chains. The SAR and binding mode of the azoles are useful for further lead optimization.

1. Introduction

During the past two decades, the incidence of systemic fungal infections has been increasing dramatically due to an increasing number of immunocompromised hosts, such as patients undergoing organ transplants or anticancer chemotherapy and patients with AIDS. However, there is a lack of effective antifungal agents that can be used for life-threatening fungal infections. Clinically available antifungal agents include amphotericin B, 5-fluorocytosine, azoles (*e.g.* fluconazole and itraconazole), and echinocandins (*e.g.* caspofungin and micafungin).

Azoles are currently the most widely used agents in antifungal chemotherapy. They possess the antifungal activity by competitive inhibition of lanosterol 14α-demethylase (CYP51) [1]. Sterol δ^{22} desaturase (CYP61), a cytochrome P450 enzyme involved in the last step of ergosterol biosynthesis, has also been described as the second target for the azole antifungals [2,3]. Fluconazole (FLC) shows good antifungal activity and relatively low toxicity, which is used as the first-line agent in treating *Candida* infections [4]. However, FLC is not effective against invasive aspergillosis and has suffered severe drug resistance [5,6]. In comparison with FLC, itraconazole (ITR) has a broader antifungal spectrum and better toleration but its variable oral absorption and low bioavailability have hampered its clinical use. Several novel azole antifungal agents, such as voriconazole [7], posaconazole [8], ravuconazole [9] and albaconazole [10], are marketed or currently in the late stages of clinical trials.

Nowadays, fungal resistance caused by the broad use of azoles is becoming serious, which has significantly reduced the therapeutic efficacy of them. The mechanism of drug resistance includes mutation or abnormal expression of CYP51 [11,12], over expression of drug excretion genes (*e.g.* CDR1, CDR2 and MDR1) [13], the formation of biofilm changes [14], and so on. The severe resistance has led to an ongoing search for new azoles [15–22].

In our previous studies, new azoles with substituted phenoxypropylamino side chain (Fig. 1) were designed and synthesized. Most of them showed excellent *in vitro* antifungal activity with broad spectrum, representing promising leads for further optimization [23–25]. In order to get more information of structure–activity relationships (SARs), the influence of *N*-substituents on the antifungal activity was investigated in the present study (Fig. 1). Their *in vitro* antifungal activity in combination with binding modes obtained from molecular docking can provide useful information for further optimization.

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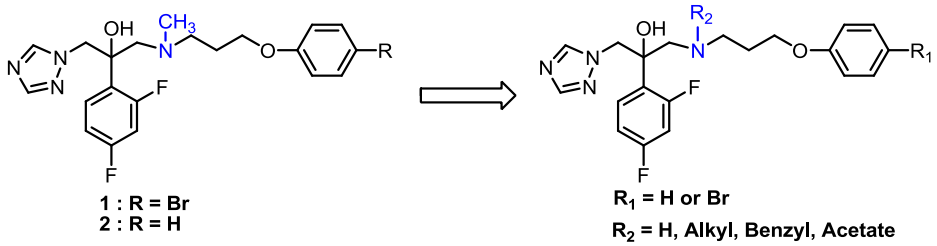


Fig. 1. Design rationale of the target compounds.

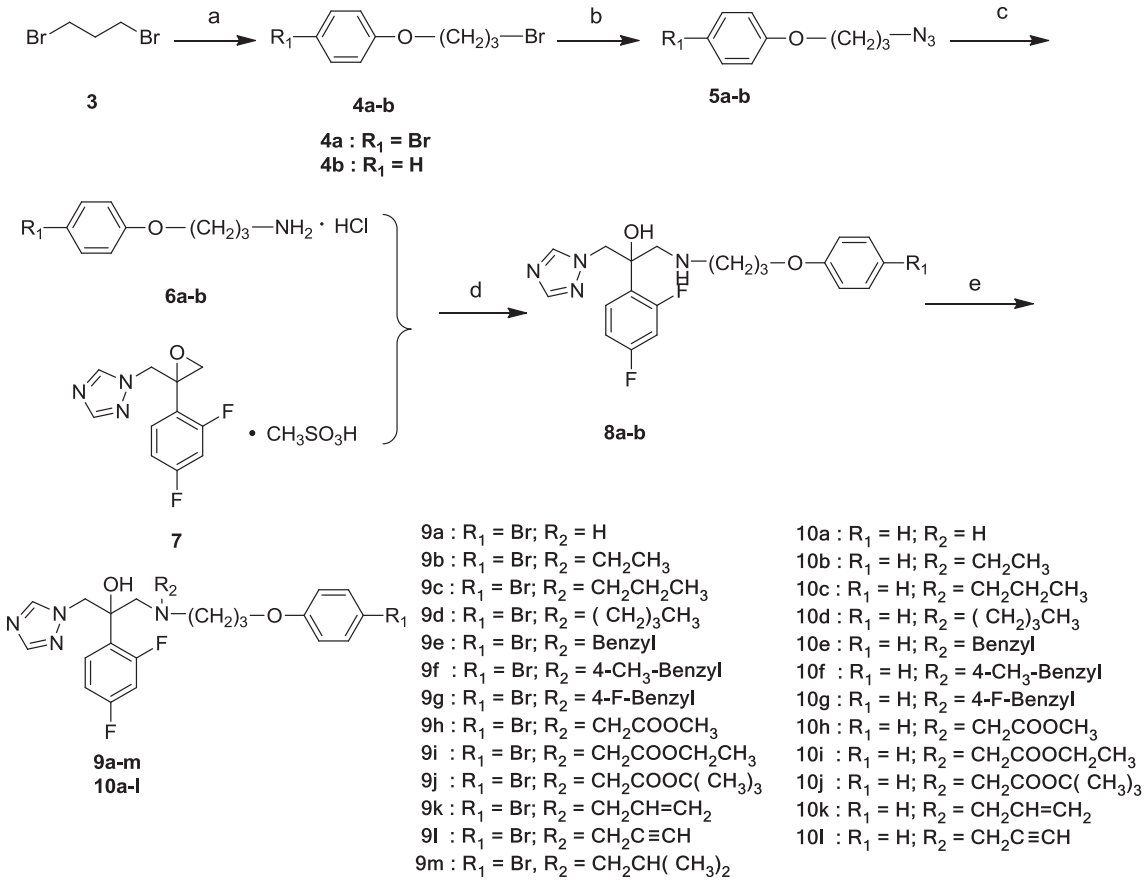
2. Chemistry

The chemical synthesis of the target compounds was outlined in Scheme 1. The oxirane intermediate **7** was synthesized by our reported procedure [26]. The phenoxypropylamino side chains **6a**, **b** were synthesized via three steps. Excess 1,3-dibromopropane was treated with phenols to give bromopropoxybenzene **4a**, **b**. Then, compounds **4a**, **b** reacted with NaN_3 in DMSO at room temperature to afford azides **5a**, **b**. The azide groups of compounds **5a**, **b** were reduced to amino groups in the presence of Ph_3P and MeOH. After treating with EtOAc saturated by hydrogen chloride, the side chains **6a**, **b** were obtained. Ring-open reactions were performed between compounds **6a**, **b** and oxirane **7** to give intermediates **8a**, **b**. In the presence of KI and K_2CO_3, various halogen substituted reagents were reacted with compounds **8a**, **b** to afford the target compounds **9a**–**m** and **10a**–**l**. All the target compounds were obtained as racemates.

3. Results and discussion

3.1. In vitro antifungal activity

The *in vitro* antifungal activity is shown in Table 1. Six important fungal pathogens were chosen for assaying. FLC and ITR were used as positive controls. The synthesized compounds showed moderate to excellent antifungal activity against the tested fungal pathogens. Several compounds, such as **9a**, **10a**, **10b** and **10k**, showed better antifungal activity than FLC. On the *Candida albicans* strain, compounds **9a**, **10a**, and **10b** displayed the highest activity with their MIC_{80} in the range of 0.0313–0.0625 μg/mL, which was more potent than FLC and ITR. In addition, compounds **9l**, **10k** and **10l** also showed comparable activity to FLC (MIC_{80} range: 0.25–0.5 μg/mL). Particularly, compounds **9a** and **10a** also displayed broad spectrum. Their inhibitory activity toward *Candida tropicalis* and *Candida krusei* ($MIC_{80} < 0.125$ μg/mL) was better than FLC and lead



Scheme 1. The synthetic route for target compounds **9a**–**10l**. Reagents and conditions: a. phenol, K_2CO_3, EtOH, reflux, 4 h, 75–82%; b. Sodium azide, DMSO, rt, 88–91%; c. Ph_3P, MeOH, reflux; HCl, EtOAc, rt, 44–56%; d. EtOH, $(Et)_3N$, reflux, 58–65%; e. K_2CO_3, KI, CH_3CN, reflux, 9 h, 21–58%.

Table 1
In vitro antifungal activity of the compounds (MIC_{80}, $\mu g \cdot mL^{-1}$).[a]

Compounds	*C. albicans*	*C. tropicalis*	*C. parapsilosis*	*C. neoformans*	*C. krusei*	*T. rubrum*
9a	0.0313	<0.125	0.5	1	<0.125	0.5
9b	0.5	1	2	1	4	2
9c	2	2	>64	4	4	8
9d	2	4	4	4	4	16
9e	2	8	32	16	4	4
9f	4	8	0.5	64	8	64
9g	4	8	2	32	8	16
9h	2	4	64	8	4	8
9i	4	16	16	32	32	16
9j	4	16	64	64	4	64
9k	2	1	16	4	2	8
9l	0.5	8	32	8	8	8
9m	4	8	8	16	4	8
10a	0.0625	<0.125	0.5	1	<0.125	2
10b	0.0313	0.5	2	1	1	1
10c	1	16	32	1	1	4
10d	2	8	16	4	1	8
10e	2	8	64	16	8	16
10f	8	8	8	4	4	16
10g	4	8	16	8	8	32
10h	2	8	>64	16	4	2
10i	2	8	>64	8	4	8
10j	4	8	>64	>64	8	64
10k	0.25	1	64	2	1	1
10l	0.25	4	8	4	1	4
1	0.5	2	1	1	2	2
2	0.0125	<0.125	0.125	1	0.5	1
FLC	0.5	1	1	2	4	1
ITR	0.125	<0.125	4	0.5	1	0.125

[a] Abbreviations: *C. albicans*, *Candida albicans*; *C. tropicalis*, *Candida tropicalis*; *C. parapsilosis*, *Candida parapsilosis*; *C. neoformans*, *Cryptococcus neoformans*; *C. krusei*, *Candida krusei*; *T. rubrum*, *Trichophyton rubrum*; FLC, Fluconazole; ITR, Itraconazole.

compound **1**, and comparable to that of ITR. In comparison with the activity against *C. albicans*, most of the compounds showed decreased inhibitory activity toward other *Candida* spp. The same trend was also observed for the activity against *Cryptococcus neoformans*. However, the activity of compounds **9a**, **b**, **10a**–**c** toward *C. neoformans* (MIC_{80} = 1 μg/mL) was comparable to the lead compounds (**1** and **2**) and superior to FLC. Moreover, all the compounds were also active against *Trichophyton rubrum* and compound **9a** (MIC_{80} = 0.5 μg/mL) showed better activity than the lead compounds and FLC.

3.2. Structure–activity relationships

In our previous studies, *N*-methyl group of the lead structure **2** could form hydrophobic interaction with the surrounding residues and maintain the suitable conformation of the side chain in the CYP51 active site [23–25]. The role of *N*-methyl side chains were also confirmed by other reports [15,16]. Compounds **9a** and **10a**, whose methyl group is replaced by hydrogen atom, showed better antifungal activity than FLC with broad antifungal spectrum. However, they were slightly less potent than lead compound **2** on the *C. albicans* strain, indicating the introduction of the methyl group could enhance the antifungal activity. On the contrary, compounds **9a** and **10a** showed better activity against *C. krusei* than the lead compounds, suggesting the difference between the CYP51 active sites of various fungal pathogens. When the *N*-methyl group of the lead compounds was replaced by the ethyl group (compounds **9b** and **10b**), the antifungal activity was decreased slightly apart from *C. albicans* and *T. rubrum*. Compounds with larger *N*-alkyl groups, such as propyl (compound **9c**), butyl (compound **9d**), and isobutyl (compound **9m**), showed significantly decreased antifungal activity. It is inferred that larger substituents might clash with the surrounding residues and disturb the side chain's suitable conformation in the CYP51 active site.

Wu's group reported that the introduction of *N*-allyl group and *N*-propargyl group would interact with the surrounding hydrophobic residues and enhance the antifungal activity [27,28]. In the present study, *N*-propargyl derivatives **9l** and **10l** showed good antifungal activity on the *C. albicans* strain, but their antifungal spectrum is relatively narrow. For *N*-allyl substituted compounds **9k** and **10k**, they only showed moderate antifungal activity. The difference in the influence of *N*-substitutions on the antifungal activity indicates that the same substitution might result in distinct conformational changes for the different types of side chains. When larger groups (*e.g.* benzyl and ester) were introduced, significant decrease of the antifungal activity was observed for compounds **9e**–**j** and **10e**–**j**.

3.3. Binding mode of the azoles

Our group has constructed three-dimensional (3D) models of CYP51 from *C. albicans* (CACYP51) using homology modeling methods [29,30]. In this study, all the analogs were included in the molecular docking to clarify their binding modes with CACYP51 and provide information for further optimization. As shown in Table 2, compounds with smaller substituents such as **9a**, **9b**, **10a**, **10b** and **10l** have relatively higher *Fitness* scores, which are consistent with their antifungal activity. In contrast, weakly active compounds such as **9f**, **9j**, **10f** and **10g** also have relatively lower *Fitness* scores. The good correlation between antifungal activity and docking score indicates that a suitable substituent has a great impact on the antifungal activity. Fig. 2 shows the interaction between compound **9a** and the active site of CACYP51. The triazole ring of compound **9a** binds to the heme group through the formation of a coordination bond with the iron atom. The difluorophenyl group forms hydrophobic interactions with Phe126, Met306, and Tyr132. The phenoxypropylamino side chain, extended in the CYP51 channel, mainly forms hydrophobic and *Van*

Table 2
GOLD docking scores of the compounds.[a]

Compounds	*Fitness*	*S*(*hb_ext*)	*S*(*vdw_ext*)	*S*(*int*)
9a	73.05	10	50.51	−6.4
9b	72.24	10	53.4	−11.19
9c	64.79	10.12	55.37	−21.47
9d	63.65	10	52.08	−17.96
9e	63.01	10	47.93	−12.89
9f	56.48	8.99	48.11	−18.67
9g	55.02	10	40.97	−11.31
9h	61.62	9.64	50.23	−17.08
9i	62.56	9.46	50.99	−17.01
9j	33.91	0.12	41.31	−23.01
9k	71.71	10	53.16	−11.39
9l	71.42	8.56	52.09	−8.76
9m	59.53	10.12	52.33	−22.55
10a	68.95	10.01	46.81	−5.43
10b	71.63	10	52.38	−10.39
10c	63.14	10	50.37	−16.12
10d	62.96	10	53.87	−21.12
10e	58.46	10.22	45.58	−14.44
10f	49.87	9.95	45.94	−23.24
10g	49.9	10	42.98	−19.2
10h	60.84	4.01	51.05	−13.37
10i	62.64	0.24	54.8	−12.95
10j	26.26	1.5	33.22	−20.92
10k	73.76	10	54.11	−10.65
10l	68.19	10	51.26	−12.3
1	74.38	10	51.9	−6.98
2	70.55	10.05	49.01	−6.89

[a] Abbreviations: *Fitness*, the negative of the sum of the component energy terms; *S*(*hb_ext*), protein–ligand hydrogen bond energy; *S*(*vdw_ext*), protein–ligand van der Waals (vdw) energy; *S*(*int*), the sum of ligand internal vdw energy and ligand torsional strain energy.

der Waals interactions with the surrounding residues such as Tyr118, Leu121, Phe233, Phe380, and Tyr505. The pocket around the NH group (Tyr118, Leu121 and Thr122) is not large enough to accommodate the large substitutions (*e.g.* benzyl). It is worth noting that the oxygen atom in the side chain of lead compound **2** formed hydrogen-bonding interaction with Ser378 [23–25].

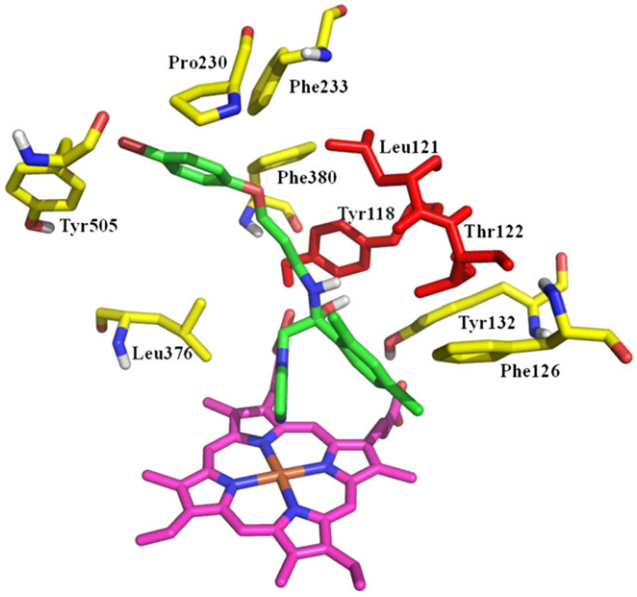


Fig. 2. The docking conformation of compound **9a** in the active site of CACYP51. Important residues involved in the binding site are shown. Residues that form the hydrophobic pocket near the nitrogen atom (Leu121, Thr122, and Tyr118) are colored red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

However, this hydrogen bond was lost for compound **9a**, which highlighted the importance of *N*-substitutions on the conformation of the side chains.

4. Conclusion

With an aim to get more SAR information for the new antifungal azoles with phenoxypropylamino side chains, the impact of *N*-substitutions on the antifungal activity was investigated. The *in vitro* antifungal activity assay indicates that the substituent has a great influence on the antifungal activity. A suitable substituent (*e.g.* the methyl group) could form hydrophobic interaction with the surrounding residues such as Tyr118, Leu121, and Thr122, and enhance the antifungal activity. A larger group is not tolerated at this position because it might crash with the active site residues. The removal of the *N*-substitution led to the slightly decrease of the antifungal activity against *C. albicans*. Interestingly, such azoles with free amine showed increased activity toward other *Candida* spp. Molecular docking studies revealed that the *N*-substitutions played an important role for the conformation of the side chain. Among the synthesized compounds, **9a** and **10a** showed excellent antifungal activity against all the tested pathogens, which are promising leads for further study. The obtained SAR and binding mode can provide useful information for lead optimization.

5. Experimental protocols

5.1. Pharmacology

In vitro antifungal activity was measured by means of the serial dilution method in 96-well microtest plates. Test fungal strains were obtained from the American Type Culture Collection (ATCC) or were clinical isolates. The determination of minimum inhibitory concentration (MIC) was performed according to the recommendations of National Committee for Clinical Laboratory Standards (NCCLS) with RPMI 1640 (Sigma) buffered with 0.165MMOPS (Sigma) as the test medium. The MIC_{80} value is defined as the lowest concentration of test compound that results in a culture with turbidity less than or equal to 80% inhibition relative to the growth of the control. Test compounds were dissolved in DMSO serially diluted in growth medium. The yeast strains were incubated at 35 °C, and the dermatophytes at 28 °C. Growth MIC_{80} was determined at 24 h for *Candida* spp., and at 72 h for *C. neoformans*.

5.2. General procedure for the synthesis of compounds

All reagents and solvents were reagent grade or were purified by standard methods before use. ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer with TMS as an internal standard and $CDCl_3$ as solvent. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. ESI mass spectra were performed on an API-3000 LC-MS spectrometer. Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within ±0.4%. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60G (Qindao Haiyang Chemical, China). Anhydrous solvent and reagents were all analytical pure and dried through routine protocols.

5.2.1. Chemical synthesis of 1-bromo-4-(3-bromopropoxy)benzene (*4a*)

1,3-Dibromopropane (21.09 g, 0.10 mol) was added dropwise to a stirred mixture of 4-bromophenol (8.65 g, 0.05 mol), K_2CO_3 (6.9 g, 0.05 mol) in 50 mL EtOH at 80 °C for 4 h. The solvent was removed

under reduced pressure, diluted with H_2O (70 mL) and extracted by EtOAc (80 mL × 3). The organic layer was separated, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (hexane) to give **4a** as transparent oil: 12.05 g (82.0%). ^{1}H NMR δ: 6.79–7.40 (m, 4H, Ar–H), 4.08 (t, 2H, J = 5.8 Hz, OC$\underline{H}_2$), 3.60 (t, 2H, J = 6.5 Hz, C$\underline{H}_2$Br), 2.28–2.36 (m, 2H, $CH_2C\underline{H}_2CH_2$). ESI-MS: 294.49 [M + 1]. The synthetic method for the compound **4b** was similar to the synthesis of compound **4a**.

*5.2.2. Chemical synthesis of 1-(3-azidopropoxy)-4-bromobenzene (**5a**)*

NaN_3 (0.42 g, 6.5 mmol) was added to a stirred solution of 1-bromo-4-(3-bromopropoxy)benzene (1.47 g, 5 mmol) in DMSO (25 mL). The mixture was stirred for 12 h at room temperature, diluted with H_2O (50 mL), and extracted by EtOAc (50 mL × 3). The organic layer was separated, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was white solid and reacted to the next step without further purification. The synthetic method for the compound **5b** was similar to the synthesis of compound **5a**.

*5.2.3. Chemical synthesis of 3-(4-bromophenoxy)propan-1-amine hydrochloride (**6a**)*

Ph_3P (1.77 g, 6.75 mmol) was added to a stirred solution of 1-(3-azidopropoxy)-4-bromobenzene in MeOH (30 mL). The mixture was refluxed for 5 h, and concentrated under reduced pressure. The residue was added 20 mL EtOAc saturated by hydrogen chloride. After filtration, compound **6a** was obtained as white solid powder (0.71 g). The total yield of two steps was 56%. ^{1}H NMR δ: 6.90–7.48 (m, 4H, Ar–H), 4.05 (t, 2H, J = 6.1 Hz, OC$\underline{H}_2$), 2.93 (t, 2H, J = 6.9 Hz, C$\underline{H}_2NH_2$), 2.01 (m, 2H, $CH_2C\underline{H}_2CH_2$). ESI-MS: 232.33 [M + 1]. The synthetic method for the compound **6b** was similar to the synthesis of compound **6a**.

*5.2.4. Chemical synthesis of 1-((3-(4-bromophenoxy)propyl) amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9a**)*

A solution of intermediate **7** (0.63 g, 1.9 mmol), **6a** (0.45 g, 1.8 mmol), and triethylamine (2 mL) in EtOH (20 mL) was heated to reflux for 8 h. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 100:2, v/v) to give **9a** as pale yellow oil, 0.55 g (65.1%). ^{1}H NMR δ: 8.27 (s, 1H, TriazC$_3$–H), 7.73 (s, 1H, TriazC$_5$–H), 6.84–7.43 (m, 7H, Ar–H), 4.55 (s, 2H, C$_1$–H), 3.93 (t, 2H, J = 5.9 Hz, OC$\underline{H}_2$), 3.29 (s, 2H, C$_3$–H), 2.63 (t, 2H, J = 6.9 Hz, NC$\underline{H}_2CH_2CH_2O$), 1.78 (m, 2H, $NCH_2C\underline{H}_2CH_2O$). ESI-MS: 469.66 [M + 2]. Anal. calcd. for $C_{20}H_{21}BrF_2N_4O_2$: C, 51.40; H, 4.53; N, 11.99. Found: C, 51.38; H, 4.52; N, 11.97. The synthetic method for the compound **10a** was similar to the synthesis of compound **9a**.

*5.2.5. Chemical synthesis of 1-((3-(4-bromophenoxy)propyl)(ethyl) amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9b**)*

A solution of chloroethane (0.045 g, 0.7 mmol), **9a** (0.18 g, 0.39 mmol), K_2CO_3 (0.15 g, 1.1 mmol), and KI (0.03 g, 0.18 mmol) in acetonitrile (15 mL) was heated to reflux for 8 h. The mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane:EtOAc = 2:1, v/v) to give **9b** as pale yellow oil, 0.09 g (46.6%). ^{1}H NMR δ: 8.13 (s, 1H, TriazC$_3$–H), 7.84 (s, 1H, TriazC$_5$–H), 6.69–7.56 (m, 7H, Ar–H), 4.55 (d, 1H, J = 14.1 Hz, C$_1$–Ha), 4.44 (d, 1H, J = 14.1 Hz, C$_1$–Hb), 3.78 (t, 2H, J = 5.9 Hz, OC$\underline{H}_2$), 3.13 (d, 1H, J = 13.8 Hz, C$_3$–Ha), 2.75 (d, 1H, J = 13.8 Hz, C$_3$–Hb), 2.45 (t, 2H, J = 6.9 Hz, NC$\underline{H}_2CH_2CH_2O$), 2.33 (m, 2H, NC$\underline{H}_2CH_3$), 1.68 (m, 2H, $NCH_2C\underline{H}_2CH_2O$), 0.79 (t, 3H, NCH$_2C\underline{H}_3$). ESI-MS: 497.51 [M + 2]. Anal. calcd. for $C_{22}H_{25}BrF_2N_4O_2$: C, 53.34; H, 5.09; N, 11.31. Found: C, 53.33; H, 5.10; N, 11.29. The synthetic method for the compounds **9c**–**m** and **10b**–**l** was similar to the synthesis of compound **9b**.

*5.2.6. 1-((3-(4-Bromophenoxy)propyl)(propyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9c**)*

Yellow oil: 0.09 g (43.6%). ^{1}H NMR δ: 8.16 (s, 1H, TriazC$_3$–H), 7.80 (s, 1H, TriazC$_5$–H), 6.69–7.59 (m, 7H, Ar–H), 4.56 (d, 1H, J = 14.4 Hz, C$_1$–Ha), 4.44 (d, 1H, J = 14.4 Hz, C$_1$–Hb), 3.78 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.13 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 2.77 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.45 (t, 2H, J = 7.2 Hz, NC$\underline{H}_2CH_2CH_2O$), 2.27 (t, 2H, NC$\underline{H}_2CH_2CH_3$), 1.69 (m, 2H, $NCH_2C\underline{H}_2CH_2O$), 1.31 (m, 2H, $NCH_2C\underline{H}_2CH_3$), 0.59 (t, 3H, $NCH_2CH_2C\underline{H}_3$). ESI-MS: 509.59 [M]. Anal. calcd. for $C_{23}H_{27}BrF_2N_4O_2$: C, 54.23; H, 5.34; N, 11.00. Found: C, 54.25; H, 5.33; N, 11.01.

*5.2.7. 1-((3-(4-Bromophenoxy)propyl)(butyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9d**)*

Brown solid: 0.11 g (42.8%). ^{1}H NMR δ: 8.15 (s, 1H, TriazC$_3$–H), 7.80 (s, 1H, TriazC$_5$–H), 6.69–7.55 (m, 7H, Ar–H), 4.54 (d, 1H, J = 14.3 Hz, C$_1$–Ha), 4.42 (d, 1H, J = 14.4 Hz, C$_1$–Hb), 3.78 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.13 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 2.77 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.44 (t, 2H, J = 7.5 Hz, NC$\underline{H}_2CH_2CH_2O$), 2.24 (t, 2H, NC$\underline{H}_2CH_2CH_2CH_3$), 1.68 (m, 2H, $NCH_2C\underline{H}_2CH_2O$), 1.10–1.20 (m, 4H, $NCH_2C\underline{H}_2C\underline{H}_2CH_3$), 0.78 (t, 3H, $NCH_2CH_2CH_2C\underline{H}_3$). ^{13}C NMR δ: 163.67, 161.69, 159.89, 157.97, 157.81, 150.92, 144.80, 132.24, 129.47, 126.70, 116.13, 112.93, 111.36, 104.19, 71.47, 65.75, 59.14, 56.31, 54.84, 51.84, 28.93, 26.69, 20.22, 13.78. ESI-MS: 525.61 [M + 2]. Anal. calcd. for $C_{24}H_{29}BrF_2N_4O_2$: C, 55.07; H, 5.58; N, 10.70. Found: C, 55.05; H, 5.57; N, 10.68.

*5.2.8. 1-(Benzyl(3-(4-bromophenoxy)propyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9e**)*

Yellow oil: 0.12 g (57.8%). ^{1}H NMR δ: 8.05 (s, 1H, TriazC$_3$–H), 7.75 (s, 1H, TriazC$_5$–H), 6.64–7.59 (m, 12H, Ar–H), 4.48 (d, 1H, J = 14.0 Hz, C$_1$–Ha), 4.37 (d, 1H, J = 14.0 Hz, C$_1$–Hb), 3.72 (t, 2H, J = 5.6 Hz, OC$\underline{H}_2$), 3.50 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.33 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.19 (d, 1H, J = 13.9 Hz, C$_3$–Ha), 2.86 (d, 1H, J = 13.9 Hz, C$_3$–Hb), 2.50 (t, 2H, J = 7.1 Hz, NC$\underline{H}_2CH_2CH_2O$), 1.71 (m, 2H, $NCH_2C\underline{H}_2CH_2O$). ESI-MS: 557.63 [M]. Anal. calcd. for $C_{27}H_{27}BrF_2N_4O_2$: C, 58.18; H, 4.88; N, 10.05. Found: C, 58.17; H, 4.89; N, 10.04.

*5.2.9. 1-((3-(4-Bromophenoxy)propyl)(4-methylbenzyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9f**)*

Yellow oil: 0.08 g (48.2%). ^{1}H NMR δ: 8.06 (s, 1H, TriazC$_3$–H), 7.75 (s, 1H, TriazC$_5$–H), 6.63–7.56 (m, 11H, Ar–H), 4.47 (d, 1H, J = 14.0 Hz, C$_1$–Ha), 4.38 (d, 1H, J = 14.0 Hz, C$_1$–Hb), 3.71 (t, 2H, J = 5.8 Hz, OC$\underline{H}_2$), 3.43 (d, 1H, J = 13.2 Hz, NC$\underline{H}_2$Ar), 3.30 (d, 1H, J = 13.2 Hz, NC$\underline{H}_2$Ar), 3.18 (d, 1H, J = 13.8 Hz, C$_3$–Ha), 2.85 (d, 1H, J = 13.8 Hz, C$_3$–Hb), 2.47 (t, 2H, J = 7.5 Hz, NC$\underline{H}_2CH_2CH_2O$), 2.30 (s, 3H, C$\underline{H}_3$), 1.70 (m, 2H, $NCH_2C\underline{H}_2CH_2O$). ESI-MS: 571.36 [M]. Anal. calcd. for $C_{28}H_{29}BrF_2N_4O_2$: C, 58.85; H, 5.12; N, 9.80. Found: C, 58.86; H, 5.13; N, 9.81.

*5.2.10. 1-((3-(4-Bromophenoxy)propyl)(4-fluorobenzyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9g**)*

Pale yellow solid: 0.13 g (47.1%). ^{1}H NMR δ: 8.06 (s, 1H, TriazC$_3$–H), 7.76 (s, 1H, TriazC$_5$–H), 6.64–7.56 (m, 11H, Ar–H), 4.50 (d, 1H, J = 14.0 Hz, C$_1$–Ha), 4.39 (d, 1H, J = 14.0 Hz, C$_1$–Hb), 3.72 (t, 2H, J = 5.8 Hz, OC$\underline{H}_2$), 3.44 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.31 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.18 (d, 1H, J = 13.8 Hz, C$_3$–Ha), 2.84 (d, 1H, J = 13.8 Hz, C$_3$–Hb), 2.50 (t, 2H, J = 7.1 Hz, NC$\underline{H}_2CH_2CH_2O$), 1.70 (m, 2H, $NCH_2C\underline{H}_2CH_2O$). ESI-MS: 575.68 [M]. Anal. calcd. for $C_{27}H_{26}BrF_3N_4O_2$: C, 56.36; H, 4.55; N, 9.74. Found: C, 56.35; H, 4.54; N, 9.76.

*5.2.11. Methyl 2-((3-(4-bromophenoxy)propyl)(2-(2,4-difluorophenyl)-2-hydroxyl-3-(1H-1,2,4-triazol-1-yl)propyl)amino) acetate (**9h**)*

Brown oil: 0.14 g (45.8%). ^{1}H NMR δ: 8.11 (s, 1H, TriazC$_3$–H), 7.76 (s, 1H, TriazC$_5$–H), 6.68–7.54 (m, 7H, Ar–H), 4.56 (d, 1H, J = 14.3 Hz, C$_1$–Ha), 4.49 (d, 1H, J = 14.4 Hz, C$_1$–Hb), 3.79 (t, 2H, J = 6.0 Hz, OC$\underline{H}_2$), 3.66 (s, 1H, OC$\underline{H}_3$) 3.44 (d, 1H, J = 13.7 Hz, C$_3$–Ha), 3.25 (s, 2H, NC$\underline{H}_2$CO), 2.83 (d, 1H, J = 13.7 Hz, C$_3$–Hb), 2.79 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.56 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.68 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ESI-MS: 539.70 [M]. Anal. calcd. for C$_{23}$H$_{25}$BrF$_2$N$_4$O$_4$: C, 51.22; H, 4.67; N, 10.39. Found: C, 51.20; H, 4.68; N, 10.41.

*5.2.12. Ethyl 2-((3-(4-bromophenoxy)propyl)(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)amino) acetate (**9i**)*

Yellow oil: 0.09 g (45.3%). ^{1}H NMR δ: 8.14 (s, 1H, TriazC$_3$–H), 7.77 (s, 1H, TriazC$_5$–H), 6.68–7.59 (m, 7H, Ar–H), 4.57 (d, 1H, J = 14.3 Hz, C$_1$–Ha), 4.50 (d, 1H, J = 14.3 Hz, C$_1$–Hb), 4.12 (m, 2H, COOC$\underline{H}_2$CH$_3$), 3.80 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.45 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 3.22 (s, 2H, NC$\underline{H}_2$CO), 2.80 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.53 (t, 2H, J = 6.7 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.70 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 1.24 (t, 3H, CH$_2$C$\underline{H}_3$). ESI-MS: 552.05 [M − 1]. Anal. calcd. for C$_{24}$H$_{27}$BrF$_2$N$_4$O$_4$: C, 52.09; H, 4.92; N, 10.12. Found: C, 52.08; H, 4.91; N, 10.11.

*5.2.13. tert-Butyl-2-((3-(4-bromophenoxy)propyl)(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)amino) acetate (**9j**)*

Yellow oil: 0.08 g (43.7%). ^{1}H NMR δ: 8.22 (s, 1H, TriazC$_3$–H), 7.72 (s, 1H, TriazC$_5$–H), 6.76–7.42 (m, 7H, Ar–H), 4.51 (s, 2H, C$_1$–2H), 3.71 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.22 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 3.15 (s, 2H, NC$\underline{H}_2$CO), 2.97 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.58(t, 2H, J = 6.7 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.62 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 1.36 (s, 9H, C(C$\underline{H}_3$)$_3$). ESI-MS: 581.46 [M]. Anal. calcd. for C$_{26}$H$_{31}$BrF$_2$N$_4$O$_4$: C, 53.71; H, 5.37; N, 9.64. Found: C, 53.68; H, 5.35; N, 9.62.

*5.2.14. 1-(Allyl(3-(4-bromophenoxy)propyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9k**)*

Brown oil: 0.13 g (40.2%). ^{1}H NMR δ: 8.13 (s, 1H, TriazC$_3$–H), 7.78 (s, 1H, TriazC$_5$–H), 6.69–7.54 (m, 7H, Ar–H), 5.62 (m, 1H, NCH$_2$C$\underline{H}$=CH$_2$), 5.06 (d, 1H, J = 10.2 Hz, NCH$_2$CH=C$\underline{H}_2$), 4.98 (d, 1H, J = 17.3 Hz, NCH$_2$CH=C$\underline{H}_2$), 4.54 (d, 1H, J = 14.2 Hz, C$_1$–Ha), 4.45 (d, 1H, J = 14.2 Hz, C$_1$–Hb), 3.78 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.13 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 2.96 (m, 1H, NC$\underline{H}_2$CH=CH$_2$), 2.86 (m, 1H, NC$\underline{H}_2$CH=CH$_2$), 2.78 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.50 (t, 2H, J = 6.7 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.70 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ESI-MS: 507.65 [M]. Anal. calcd. for C$_{23}$H$_{25}$BrF$_2$N$_4$O$_2$: C, 54.45; H, 4.97; N, 11.04. Found: C, 54.44; H, 4.96; N, 11.06.

*5.2.15. 1-((3-(4-Bromophenoxy)propyl)(prop-2-yn-1-yl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9l**)*

Pale green oil: 0.07 g (33.6%). ^{1}H NMR δ: 8.06 (s, 1H, TriazC$_3$–H), 7.79 (s, 1H, TriazC$_5$–H), 6.71–7.51 (m, 8H, Ar–H), 4.56 (d, 1H, J = 14.3 Hz, C$_1$–Ha), 4.50 (d, 1H, J = 14.3 Hz, C$_1$–Hb), 3.82 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.30 (d, 1H, J = 13.9 Hz, NC$\underline{H}_2$C≡CH), 3.21 (d, 1H, J = 13.9 Hz, NC$\underline{H}_2$C≡CH), 3.12 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 2.79 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.65 (s, 2H, NCH$_2$C≡C$\underline{H}$), 2.53 (t, 2H, J = 6.7 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.74 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ^{13}C NMR δ: 163.79, 161.75, 159.85, 157.93, 157.74, 151.18, 144.59, 132.27, 129.55, 125.57, 116.18, 113.01, 111.55, 104.27, 77.94, 73.30, 73.12, 65.60, 58.79, 55.92, 51.94, 43.49, 27.06. ESI-MS: 505.71 [M]. Anal. calcd. for C$_{23}$H$_{23}$BrF$_2$N$_4$O$_2$: C, 54.66; H, 4.59; N, 11.09. Found: C, 54.65; H, 4.59; N, 11.07.

*5.2.16. 1-((3-(4-Bromophenoxy)propyl)(isobutyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9m**)*

Yellow oil: 0.05 g (21.2%). ^{1}H NMR δ: 8.13 (s, 1H, TriazC$_3$–H), 7.89 (s, 1H, TriazC$_5$–H), 6.13–7.38 (m, 7H, Ar–H), 4.41–4.58 (dd, 2H, J = 14.1 Hz, C$_1$–H), 3.92 (t, 2H, J = 5.9 Hz, OC$\underline{H}_2$), 3.54–3.58 (d, 1H, J = 11.4 Hz, C$_3$–Ha), 3.32–3.36 (d, 1H, J = 11.4 Hz, C$_3$–Hb), 3.39–3.42 (m, 1H, C$\underline{H}_2$CH(CH$_3$)$_2$), 3.24–3.27 (m, 1H, C$\underline{H}_2$CH(CH$_3$)$_2$), 2.82–2.86 (t, 2H, J = 6.9 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.91–1.95 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 1.86–1.92 (m, 1H, CH$_2$C$\underline{H}$(CH$_3$)$_2$), 0.86–0.93 (m, 6H, CH(C$\underline{H}_3$)$_2$). ESI-MS: 523.65 [M]. Anal. calcd. for C$_{24}$H$_{29}$BrF$_2$N$_4$O$_2$: C, 55.07; H, 5.58; N, 10.70. Found: C, 55.04; H, 5.56; N, 10.72.

*5.2.17. 2-(2,4-Difluorophenyl)-1-((3-phenoxypropyl)amino)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10a**)*

Yellow oil: 0.16 g (58.0%). ^{1}H NMR δ: 8.27 (s, 1H, TriazC$_3$–H), 7.73 (s, 1H, TriazC$_5$–H), 6.84–7.43 (m, 7H, Ar–H), 4.55 (s, 2H, C$_1$–2H), 3.93 (t, 2H, J = 5.9 Hz, OC$\underline{H}_2$), 3.29 (s, 2H, C$_3$–2H), 2.63 (t, 2H, J = 6.9 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.78 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ^{13}C NMR δ: 163.78, 161.79, 159.95, 158.60, 157.98, 151.24, 144.67, 129.93, 129.43, 124.89, 120.85, 114.35, 111.51, 104.22, 73.33, 65.95, 55.93, 54.37, 47.38, 29.40. ESI-MS: 389.80 [M + 1]. Anal. calcd. for C$_{20}$H$_{22}$F$_2$N$_4$O$_2$: C, 61.85; H, 5.71; N, 14.42. Found: C, 61.83; H, 5.69; N, 14.43.

*5.2.18. 2-(2,4-Difluorophenyl)-1-(ethyl(3-phenoxypropyl)amino)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10b**)*

Yellow oil: 0.08 g (51.3%). ^{1}H NMR δ: 8.06 (s, 1H, TriazC$_3$–H), 7.89 (s, 1H, TriazC$_5$–H), 6.79–7.77 (m, 8H, Ar–H), 4.52 (d, 1H, J = 14.1 Hz, C$_1$–Ha), 4.46 (d, 1H, J = 14.1 Hz, C$_1$–Hb), 3.83 (t, 2H, J = 5.9 Hz, OC$\underline{H}_2$), 3.12 (d, 1H, J = 13.8 Hz, C$_3$–Ha), 2.77 (d, 1H, J = 13.8 Hz, C$_3$–Hb), 2.48 (t, 2H, J = 6.9 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.38 (m, 2H, NC$\underline{H}_2$CH$_3$), 1.71(m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 0.88 (t, 3H, J = 6.7 Hz, NCH$_2$C$\underline{H}_3$). ESI-MS: 417.65 [M + 1]. Anal. calcd. for C$_{22}$H$_{26}$F$_2$N$_4$O$_2$: C, 63.45; H, 6.29; N, 13.45. Found: C, 63.43; H, 6.27; N, 13.44.

*5.2.19. 2-(2,4-Difluorophenyl)-1-((3-phenoxypropyl)(propyl) amino)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10c**)*

Brown oil: 0.12 g (45.4%). ^{1}H NMR δ: 8.12 (s, 1H, TriazC$_3$–H), 7.78 (s, 1H, TriazC$_5$–H), 6.76–7.54 (m, 8H, Ar–H), 4.52 (d, 1H, J = 14.2 Hz, C$_1$–Ha), 4.44 (d, 1H, J = 14.2 Hz, C$_1$–Hb), 3.82 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.11 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 2.76 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.47 (t, 2H, J = 7.2 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.25 (t, 2H, NC$\underline{H}_2$CH$_2$CH$_3$), 1.70 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 1.24 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_3$), 0.72 (t, 3H, J = 7.0 Hz, NCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR δ: 163.65, 161.65, 159.90, 158.64, 157.88, 150.87, 144.76, 129.38, 126.63, 120.79, 120.45, 114.46, 104.20, 71.57, 65.34, 59.32, 57.00, 56.39, 51.97, 29.65, 27.09, 20.01, 11.48. ESI-MS: 429.35 [M − 1]. Anal. calcd. for C$_{23}$H$_{28}$F$_2$N$_4$O$_2$: C, 64.17; H, 6.56; N, 13.01. Found: C, 64.15; H, 6.54; N, 13.00.

*5.2.20. 1-(Butyl(3-phenoxypropyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10d**)*

Brown oil: 0.09 g (45.4%). ^{1}H NMR δ: 8.12 (s, 1H, TriazC$_3$–H), 7.78 (s, 1H, TriazC$_5$–H), 6.76–7.54 (m, 8H, Ar–H), 4.53 (d, 1H, J = 14.1 Hz, C$_1$–Ha), 4.43 (d, 1H, J = 14.1 Hz, C$_1$–Hb), 3.83 (t, 2H, J = 5.9 Hz, OCH$_2$), 3.12 (d, 1H, J = 14.1 Hz, C$_3$–Ha), 2.78 (d, 1H, J = 14.1 Hz, C$_3$–Hb), 2.45 (t, 2H, J = 7.5 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.29 (t, 2H, NC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 1.69 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 1.10–1.20 (m, 4H, NCH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_3$), 0.80 (t, 3H, NCH$_2$CH$_2$CH$_2$C$\underline{H}_3$). ESI-MS: 443.41 [M − 1]. Anal. calcd. for C$_{24}$H$_{30}$F$_2$N$_4$O$_2$: C, 64.85; H, 6.80; N, 12.60. Found: C, 64.83; H, 6.79; N, 12.58.

*5.2.21. 1-(Benzyl(3-phenoxypropyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10e**)*

Pale yellow oil: 0.15 g (55.8%). ^{1}H NMR δ: 8.03 (s, 1H, TriazC$_3$–H), 7.73 (s, 1H, TriazC$_5$–H), 6.74–7.29 (m, 13H, Ar–H), 4.46 (d, 1H, J = 14.1 Hz, C$_1$–Ha), 4.37 (d, 1H, J = 14.1 Hz, C$_1$–Hb), 3.78 (t, 2H,

J = 5.6 Hz, OC$\underline{H}_2$), 3.52 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.34 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.19 (d, 1H, J = 13.8 Hz, C_3–Ha), 2.87 (d, 1H, J = 13.8 Hz, C_3–Hb), 2.52 (t, 2H, J = 6.5 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.72 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ^{13}C NMR δ: 163.66, 161.67, 159.80, 158.56, 157.83, 150.90, 144.65, 137.74, 129.41, 129.32 128.89, 128.47, 126.25, 120.79, 114.36, 111.48, 104.19, 72.30, 65.50, 59.53, 58.60, 56.18, 52.13, 26.65. ESI-MS: 479.50 [M + 1]. Anal. calcd. for $C_{27}H_{28}F_2N_4O_2$: C, 67.77; H, 5.90; N, 11.71. Found: C, 67.75; H, 5.88; N, 11.73.

*5.2.22. 2-(2,4-Difluorophenyl)-1-((4-methylbenzyl)(3-phenoxypropyl)amino)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10f**)*

Yellow oil: 0.14 g (46.3%). ^{1}H NMR δ: 8.06 (s, 1H, TriazC$_3$–H), 7.76 (s, 1H, TriazC$_5$–H), 6.74–7.53 (m, 12H, Ar–H), 4.46 (d, 1H, J = 14.0 Hz, C_1–Ha), 4.39 (d, 1H, J = 14.0 Hz, C_1–Hb), 3.78 (t, 2H, J = 5.6 Hz, OC$\underline{H}_2$), 3.46 (d, 1H, J = 13.3 Hz, NC$\underline{H}_2$Ar), 3.32 (d, 1H, J = 13.3 Hz, NC$\underline{H}_2$Ar), 3.18 (d, 1H, J = 13.9 Hz, C_3–Ha), 2.86 (d, 1H, J = 13.9 Hz, C_3–Hb), 2.50 (t, 2H, J = 7.1 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.30 (s, 3H, Ar–C$\underline{H}_3$), 1.71 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ESI-MS: 493.54 [M + 1]. Anal. calcd. for $C_{28}H_{30}F_2N_4O_2$: C, 68.28; H, 6.14; N, 11.37. Found: C, 68.30; H, 6.13; N, 11.38.

*5.2.23. 2-(2,4-Difluorophenyl)-1-((4-fluorobenzyl)(3-phenoxypropyl)amino)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10g**)*

Pale brown solid: 0.16 g (45.9%). ^{1}H NMR δ: 8.05 (s, 1H, TriazC$_3$–H), 7.75 (s, 1H, TriazC$_5$–H), 6.75–7.56 (m, 12H, Ar–H), 4.49 (d, 1H, J = 14.0 Hz, C_1–Ha), 4.39 (d, 1H, J = 14.0 Hz, C_1–Hb), 3.78 (t, 2H, J = 5.6 Hz, OC$\underline{H}_2$), 3.44 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.34 (d, 1H, J = 13.4 Hz, NC$\underline{H}_2$Ar), 3.18 (d, 1H, J = 13.9 Hz, C_3–Ha), 2.85 (d, 1H, J = 13.9 Hz, C_3–Hb), 2.50 (t, 2H, J = 7.1 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.71 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ESI-MS: 497.63 [M + 1]. Anal. calcd. for $C_{27}H_{27}F_3N_4O_2$: C, 65.31; H, 5.48; N, 11.28. Found: C, 65.30; H, 5.46; N, 11.26.

*5.2.24. Methyl 2-((2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl) propyl)(3-phenoxypropyl)amino)acetate (**10h**)*

Pale yellow oil: 0.09 g (50.3%). ^{1}H NMR δ: 8.08 (s, 1H, TriazC$_3$–H), 7.74 (s, 1H, TriazC$_5$–H), 6.75–7.53 (m, 8H, Ar–H), 4.54 (d, 1H, J = 14.3 Hz, C_1–Ha), 4.51 (d, 1H, J = 14.3 Hz, C_1–Hb), 4.12 (m, 2H, COOC$\underline{H}_2$CH$_3$), 3.83 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.66 (s, 3H, OC$\underline{H}_3$), 3.44 (d, 1H, J = 14.1 Hz, C_3–Ha), 3.28 (s, 2H, NC$\underline{H}_2$CO), 2.86 (d, 1H, J = 14.1 Hz, C_3–Hb), 2.76 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.62 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.70 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ^{13}C NMR δ: 172.29, 163.73, 161.74, 159.78, 158.57, 157.82, 150.95, 144.63, 129.97, 129.44, 125.39, 120.83, 114.35, 111.45, 104.08, 73.62, 64.97, 61.26, 56.48, 55.91, 53.44, 51.85, 27.41. ESI-MS: 461.52 [M + 1]. Anal. calcd. for $C_{23}H_{26}F_2N_4O_4$: C, 59.99; H, 5.69; N, 12.17. Found: C, 59.98; H, 5.71; N, 12.15.

*5.2.25. Ethyl 2-((2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl) propyl)(3-phenoxypropyl)amino)acetate (**10i**)*

Pale yellow oil: 0.13 g (46.9%). ^{1}H NMR δ: 8.09 (s, 1H, TriazC$_3$–H), 7.74 (s, 1H, TriazC$_5$–H), 6.74–7.54 (m, 8H, Ar–H), 4.54 (d, 1H, J = 14.3 Hz, C_1–Ha), 4.51 (d, 1H, J = 14.3 Hz, C_1–Hb), 4.12 (m, 2H, COOC$\underline{H}_2$CH$_3$), 3.82 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.42 (d, 1H, J = 14.1 Hz, C_3–Ha), 3.23 (s, 2H, NC$\underline{H}_2$CO), 2.82 (d, 1H, J = 14.1 Hz, C_3–Hb), 2.72 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.58 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.68 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 1.23 (t, 3H, CH$_2$C$\underline{H}_3$). ESI-MS: 475.76 [M + 1]. Anal. calcd. for $C_{24}H_{28}F_2N_4O_4$: C, 60.75; H, 5.95; N, 11.81. Found: C, 60.76; H, 5.94; N, 11.82.

*5.2.26. tert-Butyl 2-((2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl) propyl)(3-phenoxypropyl)amino)acetate (**10j**)*

Pale yellow solid: 0.15 g (50.1%). ^{1}H NMR δ: 8.12 (s, 1H, TriazC$_3$–H), 7.74 (s, 1H, TriazC$_5$–H), 6.75–7.57 (m, 8H, Ar–H), 4.54 (d, 1H, J = 14.1 Hz, C_1–Ha), 4.48 (d, 1H, J = 14.1 Hz, C_1–Hb), 3.82 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.43 (d, 1H, J = 14.1 Hz, C_3–Ha), 3.11 (s, 2H, NC$\underline{H}_2$CO), 2.78 (d, 1H, J = 14.1 Hz, C_3–Hb), 2.70 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 2.50 (br, 1H, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.68 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O), 1.42 (s, 9H, C(C$\underline{H}_3$)$_3$). ESI-MS: 503.58 [M + 1]. Anal. calcd. for $C_{26}H_{32}F_2N_4O_4$: C, 62.14; H, 6.42; N, 11.15. Found: C, 62.13; H, 6.45; N, 11.14.

*5.2.27. 1-(Allyl(3-phenoxypropyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10k**)*

Yellow oil: 0.07 g (36.8%). ^{1}H NMR δ: 8.11 (s, 1H, TriazC$_3$–H), 7.77 (s, 1H, TriazC$_5$–H), 6.77–7.54 (m, 8H, Ar–H), 5.63 (m, 1H, NCH$_2$C$\underline{H}$=CH$_2$), 5.06 (d, 1H, J = 9.6 Hz, NCH$_2$CH=C$\underline{H}_2$), 4.98 (d, 1H, J = 17.3 Hz, NCH$_2$CH=C$\underline{H}_2$), 4.52 (d, 1H, J = 14.2 Hz, C_1–Ha), 4.46 (d, 1H, J = 14.2 Hz, C_1–Hb), 3.83 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.13 (d, 1H, J = 14.1 Hz, C_3–Ha), 2.98 (m, 1H, NC$\underline{H}_2$CH=CH$_2$), 2.87 (m, 1H, NC$\underline{H}_2$CH=CH$_2$), 2.78 (d, 1H, J = 14.1 Hz, C_3–Hb), 2.52 (t, 2H, J = 6.7 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.72 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ESI-MS: 427.51 [M – 1]. Anal. calcd. for $C_{23}H_{26}F_2N_4O_2$: C, 64.47; H, 6.12; N, 13.08. Found: C, 64.49; H, 6.11; N, 13.06.

*5.2.28. 2-(2,4-Difluorophenyl)-1-((3-phenoxypropyl)(prop-2-yn-1-yl)amino)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10l**)*

Brown oil: 0.09 g (35.6%). ^{1}H NMR δ: 8.23 (s, 1H, TriazC$_3$–H), 7.73 (s, 1H, TriazC$_5$–H), 6.80–7.37 (m, 8H, Ar–H), 4.52 (s, 2H, C_1–2H), 3.72 (t, 2H, J = 5.7 Hz, OC$\underline{H}_2$), 3.40 (d, 1H, J = 13.9 Hz, NC$\underline{H}_2$C≡CH), 3.27 (d, 1H, J = 13.9 Hz, NC$\underline{H}_2$C≡CH), 3.04 (d, 1H, J = 14.1 Hz, C_3–Ha), 2.82 (d, 1H, J = 14.1 Hz, C_3–Hb), 2.64 (s, 1H, NCH$_2$C≡C$\underline{H}$), 2.52 (t, 2H, J = 6.7 Hz, NC$\underline{H}_2$CH$_2$CH$_2$O), 1.68 (m, 2H, NCH$_2$C$\underline{H}_2$CH$_2$O). ESI-MS: 427.73 [M + 1]. Anal. calcd. for $C_{23}H_{24}F_2N_4O_2$: C, 64.78; H, 5.67; N, 13.14. Found: C, 64.75; H, 5.65; N, 13.13.

6. Molecular docking

The 3D structures of the synthesized compounds were built by the builder module within Sybyl-X 1.2 software package. The R isomers were selected for molecular docking [26]. The active site was defined the same with our previous reports [24,31,32]. Before docking, we firstly tested the ability of Gold 4.1.2 [33] software to reproduce the binding pose of the ligands in the crystal structures of the CYP51 family. Twelve crystal structures of CYP51 in complex with various ligands (PDB codes: 1EA1, 1E9X, 2W09, 2W0A, 2W0B, 2WX2, 2WUZ, 3GW9, 3K1O, 3L4D, 3I3K, 3LD6) [34–40] were used as the test set and all the ligands were docked with default parameters. The results indicated that Gold software with Goldscore function showed satisfying accuracy with the average RMSD of 1.37 Å. Further optimization of the docking parameters did not yield better results. Therefore, Gold 4.1.2 with default parameters was used for molecular docking. The docking conformation was selected according to the docking score and visual inspection. MVD 4.3.0 [41] was also used to re-dock the compounds to confirm the robustness of the docking pose.

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