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Design, synthesis and biological evaluation of LBM-A5 derivatives as potent P-glycoprotein-mediated multidrug resistance inhibitors

Yuxiang Wu[a,#], Miaobo Pan[a,#], Yuxuan Dai[a], Baomin Liu[a,b], Jian Cui[a], Wei Shi[a], Qianqian Qiu[a], Wenlong Huang [a,*] , Hai Qian [a,*]

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Abstract

A novel series of P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) inhibitors with triazol-N-phenethyl-tetrahydroisoquinoline or triazol-N-ethyl-tetrahydroisoquinoline scaffold were designed and synthesized via click chemistry. Most of the synthesized compounds showed higher reversal activity than verapamil (VRP). Among them, the most potent compound **4** showed a comparable activity with the known potent P-gp inhibitor WK-X-34 with lower cytotoxicity towards K562 cells (IC_{50} > 100μM). Compared with VRP, compound **4** exhibited more potency in increasing drug accumulation in K562/A02 MDR cells. Moreover, compound **4** could significantly reverse MDR in a dose-dependent manner and also persist longer chemo-sensitizing effect than VRP with reversibility. Further mechanism studies revealed that compound **4** could remarkably increase the intracellular accumulation of Adriamycin (ADM) in K562/A02 cells as well as inhibit rhodamine-123 (Rh123) efflux from the cells. These results suggested that compound **4** may represent a promising candidate for developing P-gp-mediated MDR inhibitors.

Key words: click chemistry, multidrug resistance inhibitors, P-glycoprotein, reversal activity

1. Introduction

The resistance of tumour cells against cytotoxic agents is a significant obstruction for successful tumor chemotherapy. As this resistance is structurally unrelated to cytostatics, it was named multidrug resistance (MDR), which is characterized by a decreased sensitivity to cytotoxic agents. In MDR tumour cells, various members of the ATP-binding cassette (ABC) family transporters, including P-glycoprotein (P-gp, ABCB1), the breast cancer-resistance protein (BCRP, ABCG2) and the multidrug resistance-associated protein 1 (MRP1,ABCC1), can simultaneously be overexpressed.[1] Among them, P-gp is the most extensive drug transporter , which has the ability to expel a wide variety of chemotherapeutic agents out of tumor cells resulting in MDR.[2,3] Obviously, it should be a potential strategy to develop P-gp inhibitors to suppress P-gp-mediated MDR.[4] In the past three decades, considerable efforts have been made to overcome MDR of tumor cells and have developed three generations of P-gp inhibitors such as Verapamil, Valspodar (PSC-833) and Tariquidar (XR9576).[5,6] Nevertheless, all of these P-gp inhibitors failed later on due to observed toxicity, low selectivity or pharmacokinetic interactions. Up to now, no P-gp inhibitors have been approved for clinical application.[7] Therefore, it is still exigent to develop new non-toxic and potent P-gp inhibitors with high

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selectivity for cancer treatment.

For designing novel multidrug resistance inhibitors, an approach we adopted was to reserve the chemical fragment, such as N-ethyl-tetrahydroisoquinoline and nitrogen heterocyclic ring, which are frequently appeared in reported potent P-gp inhibitors .[8-10] In the present study, LBM-A5 effectively reversed MDR (EC_{50} = 483.6 ± 81.7 nmol·L^{-1}) by inhibiting the function of P-gp, with relatively ideal pharmacokinetics and in a safe manner.[11,12] Thus, LBM-A5 could be served as a lead compound. The third generation P-gp inhibitors with a phenethyl-tetrahydroisoquinoline structure, such as Tariquidar and WK-X-34, contain multiple hydrophobic aromatic rings leading to the larger molecular weights, which go against their druggability. Therefore, the novel multidrug resistance inhibitors were designed to decrease the molecular weight through structural modification that the aromatic amide structures were replaced by the different aromatic rings (Fig.1). 1,2,3-triazole ring, a hydrophobic aromatic group, associating with biological targets through hydrogen bonding and dipole interactions was introduced to the designed compounds by click chemistry.[10] Click chemistry, commonly copper-(I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes, has been widely applied in drug discovery.[13] Based on the principles above, we synthesized and biologically evaluated compounds **1**–**25** as P-gp-mediated MDR inhibitors.

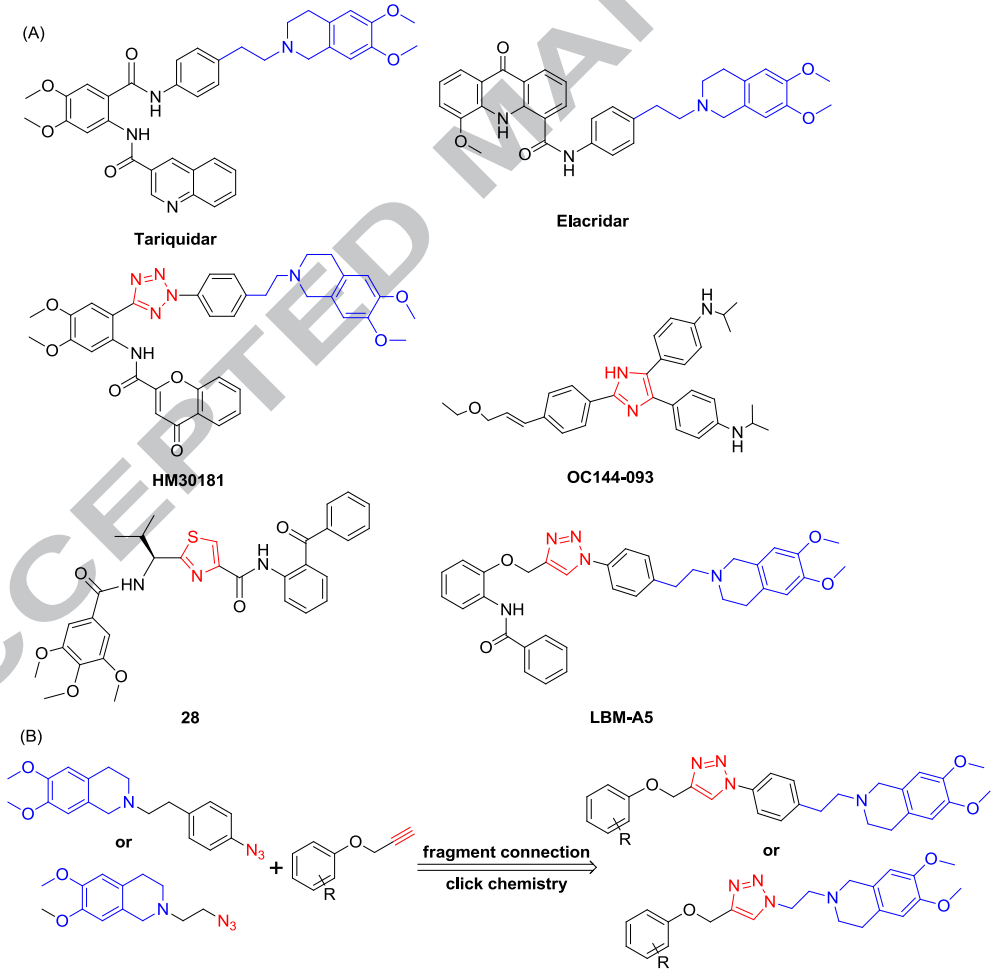
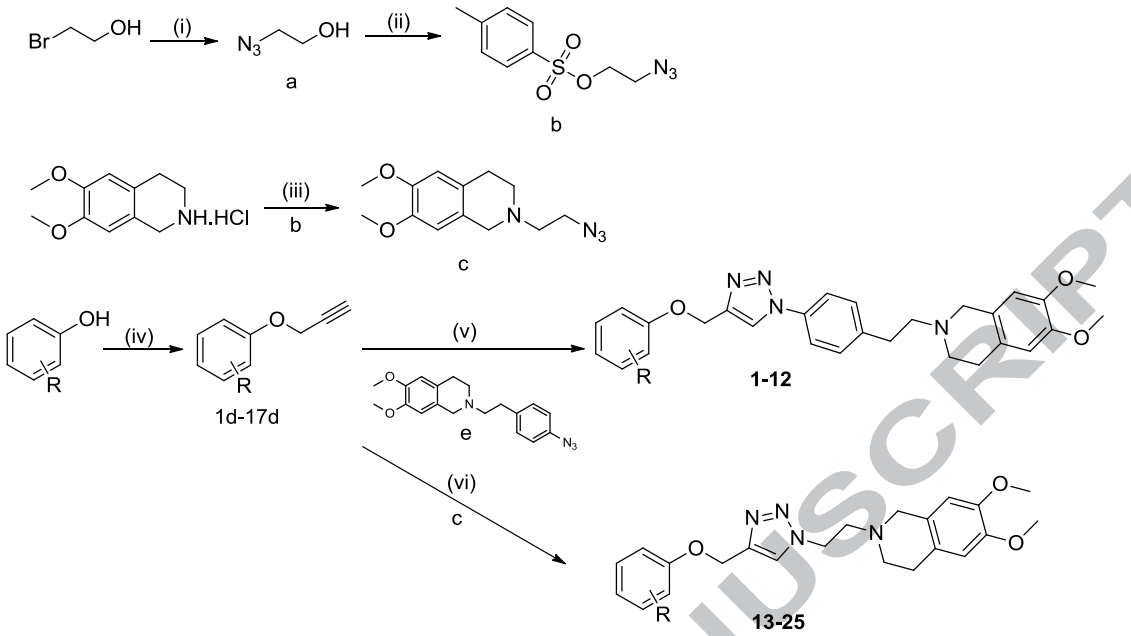


Figure 1. (A) Structures of the reported potent P-gp inhibitors containing chemical fragments such as N-ethyltetrahydroisoquinoline and nitrogen heterocyclic ring. (B) Design of the target compounds **1**–**25**.



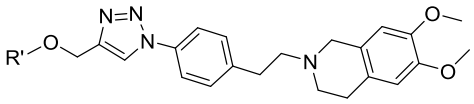
Scheme 1. Synthesis of the target compounds. Reagents and conditions: (i) NaN_3, water, 80°C, 24 h; (ii) TEA/DCM, TsCl, r.t, 24 h; (iii) 2-azidoethyl 4-methylbenzenesulfonate(b), TEA/acetonitrile, 60°C, 24 h; (iv) 3-bromoprop-1-yne, K_2CO_3, acetone, reflux, 4 h; (v) 2-(4-Azidophenethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (e), ascorbate sodium, $CuSO_4$, 75% CH_3OH, 24–48 h; (vi) 2-(2-azidoethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (c), ascorbate sodium, $CuSO_4$, 75% CH_3OH, 24–48 h.

2. Chemsitry

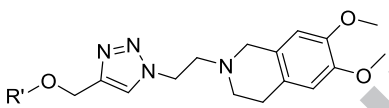
The synthetic routes of target compounds **1–25** are outlined in Scheme 1. Compounds **a–c** were synthesized according to literature procedures with minor modification.[14–16] Compounds **1d-17d** were prepared starting from the disparate aromatic phenols and 3-bromo-prop-1-yne, which refluxed in the mixture of acetone and potassium carbonate for 4 h. Subsequently, compound **c** or **e** and compounds **1d–17d** were treated with ascorbate sodium and copper sulfate in 75% methanol stirring at room temperature for 24–48 h to provide compounds **1–25**. Interestingly, some of the target compounds could precipitate from the reaction solution directly and were isolated with high purity. While others which could not precipitate directly were purified by column chromatography. The structures of target compounds obtained were listed in Table 1.

Table 1

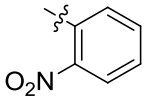
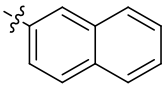
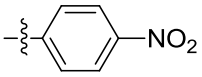
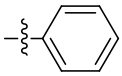
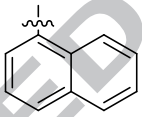
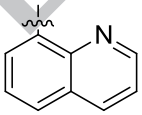
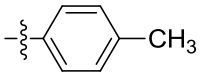
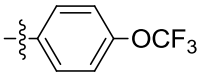
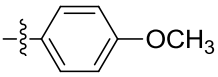
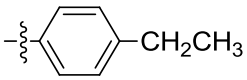
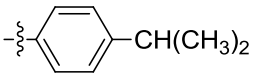
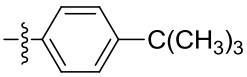
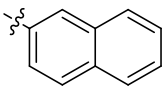
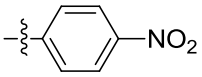
Structures and ADM-resistance reversal activity of the target compounds **1–25** at 5 μM concentration in K562/A02 cells[a]

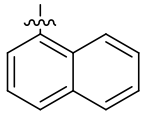
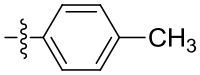
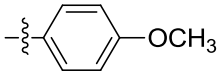
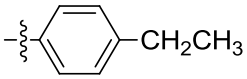
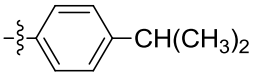
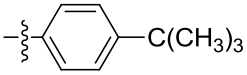
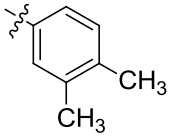
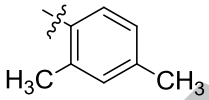
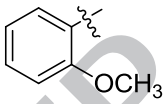
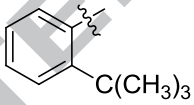
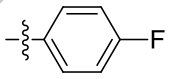


Compound 1-12



Compound 13-25

Compounds	R'	IC_{50} of ADM(μmol/L)	RF
1		5.11±0.32	7.4
2		4.48±0.17	8.5
3		7.02±0.23	5.4
4		1.21±0.18	31.4
5		1.81±0.13	21.0
6		3.79±0.08	10.0
7		6.53±0.11	5.8
8		6.45±0.31	5.9
9		10.96±0.42	3.5
10		4.17±0.07	9.1
11		4.15±0.12	9.2
12		4.11±0.06	9.3
13		10.58±0.55	3.6
14		12.35±0.61	3.1

15		6.68±0.33	5.7
16		6.17±0.56	6.2
17		11.22±0.79	3.4
18		6.23±0.65	6.1
19		4.95±0.29	7.7
20		1.86±0.15	20.4
21		4.17±0.38	9.1
22		8.35±0.48	4.6
23		9.35±0.56	4.1
24		3.98±0.41	9.6
25		14.08±1.06	2.7
Control[b]		38.02±1.65	1
WK-X-34		1.25±0.12	30.4
LBM-A5		1.65±0.22	23.0
VRP		8.50±0.45	4.5

[a] The IC_{50}s were determined after exposure to a series of ADM concentration with different target compounds at 5μM using K562/A02 cells. Reversal fold (RF) refers to fold-change in drug sensitivity. RF = (IC_{50} without modulator)/(IC_{50} with 5μM modulator).

[b] 0.1% DMSO was added as solvent control for testing the P-gp modulating activity.

3. Results and discussion

3.1. Biological evaluation

3.1.1. Cytotoxicity assay

To identify ideal P-gp inhibitors reversing MDR at non-toxic concentrations, the intrinsic cytotoxicity of the target compounds against parental sensitive K562 cells and their ADM-resistant sublines K562/A02 cells which overexpress P-gp (induced by ADM) was evaluated by MTT assay.

Anticancer drug adriamycin (ADM) and P-gp inhibitors VRP, WK-X-34 and the lead compound LBM-A5 were selected as controls. As shown in Table 2, VRP had weak cytotoxic effects toward K562 and K562/A02 cells with IC_{50} values 65.28 and 56.24μM, respectively. Nevertheless, WK-X-34 displayed a high level of toxicity toward K562 cells (IC_{50} of 19.56±1.32μM) but a weak toxicity toward K562/A02 cells (IC_{50} of 50.10 ±2.51μM). In contrast, LBM-A5 and all of the synthesized compounds showed no toxicity with IC_{50} values higher than 100μM to K562/A02 cells. Compound **6** bearing a quinoline ring and compound **12** with 4-tert-butylbenzene were found to exhibit high toxicity (IC_{50} < 30μM) in K562 cells. Compound **1** and **3** which both contain a nitro group in their formula showed weak cytotoxic effects (IC_{50} ≈ 50μM) toward K562 cells. With a trifluoromethoxy group at the para-position, compound **8** showed moderate cytotoxic effect (IC_{50} of 37.40±3.09μM). The other synthesized compounds showed no toxicity to K562 cells with IC_{50} values higher than 100μM. In addition, The cytotoxicity assays indicated that most of our compounds possess little cytotoxic effects in tested cell lines and are suitable candidates for the development of P-gp inhibitors.

Table 2

Cytotoxicity of the target compounds against K562 and K562/A02 cell lines[a]

Compounds	Cytotoxicity IC_{50} (μM)	
	K562	K562/A02
1	48.77±1.21	>100
2	>100	>100
3	49.22±1.61	>100
4	>100	>100
5	>100	>100
6	28.02±1.97	>100
7	>100	>100
8	37.40±3.09	>100
9	>100	>100
10	>100	>100
11	>100	>100
12	21.10±1.07	>100
13	>100	>100
14	>100	>100
15	>100	>100
16	>100	>100
17	>100	>100
18	>100	>100
19	>100	>100
20	>100	>100
21	>100	>100
22	>100	>100
23	>100	>100
24	>100	>100
25	>100	>100
VRP	65.28±3.54	56.24±2.12
WK-X-34	19.56±1.32	50.10±2.51

LBM-A5	>100	>100
ADM	6.23±0.28	38.02±1.65

[a]The IC_{50}s for the target compounds were determined by MTT method. Each experiment was carried out three times.

3.1.2. Effects of the target compounds on reversing ADM resistance in K562/A02 cells

The effects of the target compounds on reversing adriamycin (ADM) resistance towards K562/A02 cells (P-gp-overexpression) were preliminarily investigated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) method. [11, 17] The well-known classical P-gp inhibitor VRP, WK-X-34 and LBM-A5 were chosen as positive controls. All compounds **1–25** and the positive controls were assayed at 5μM, a low concentration which could result in less than 10% cytotoxicity towards K562/A02 cells after 48 h incubation. As the results summarized in Table 1, anticancer drug ADM alone displayed little inhibitory effect on the survival of K562/A02 cells (IC50 of 38.02±1.65μM). However, the combination treatment of ADM with target compounds or the positive controls increased the inhibitory effect in different degrees. It revealed that most of the target compounds exerted MDR reversal activities. Among them, compound **4** with a significantly decreased IC_{50} of ADM (1.21± 0.18 μM) showed the strongest reversal activity and its reversal fold (RF) was 31.4, which was close to WK-X-34 (RF=30.4) and a little higher than LBM-A5 (RF=23.0). In addition, some of the target compounds exhibited more active MDR reversal activity than the positive control VRP, when co-administered with ADM at the same condition.

3.1.3. Chemo-sensitizing effect of target compound

To further investigate the reversal potency and dose–response effects, we have determined the reversal activity of compound **4** at various other concentrations (2.5μM, 1.25μM, 0.625μM, 0.31μM, 0.156μM, 0.078μM) towards K562/A02 cells (P-gp-overexpression) by MTT assay, selecting VRP as positive controls.[18,19] As demonstrated in Table 3, VRP showed slight modulating activity at 2.5μM (RF = 1.9). However, compound **4** showed apparent dose dependent activity and still exhibited potent MDR reversal activity (RF = 7.2) when the concentration decreased to 0.31μmol/L. Additionally, the EC_{50} value of compound **4** was 192.6±34.3nM, which was calculated by GraphPad Prism 5.0 software from the dose–response curves. The results suggested that compound **4** had the significantly potential to enhance the sensitivity of P-gp-overexpressing cells to anticancer drug substrates in a dose-dependent manner.

Table 3

Sensitization of compound **4** on reversing MDR towards K562/A02 cells at different concentrations[a]

Compounds	IC_{50} of ADM (μM)	RF
None	38.02±1.65	1
VRP, 2.5μM	19.84±0.82	1.9
Compound **4**, 2.5μM	1.87±0.31	20.3
Compound **4**, 1.25μM	2.25±0.32	16.9
Compound **4**, 0.625μM	3.36±0.28	11.3
Compound **4**, 0.31μM	5.25±0.25	7.2
Compound **4**, 0.156μM	16.98±0.82	2.2
Compound **4**, 0.078μM	23.45±1.04	1.6

[a] Reversal fold (RF), RF = (IC_{50} without modulator)/(IC_{50} with modulator). Each experiment was carried out three times, and the values were presented as the mean±standard error of mean.

3.1.4. Effect of compound 4 on ADM accumulation

It is known that ADM is a fluorescent substrate of P-gp and can be used to monitor drug accumulation in cells. In order to investigate the mechanism of compound **4** in modulating the anticancer drug substrates accumulation level inside P-gp-overexpressing K562/A02 cells, the intracellular accumulation of ADM was assessed by monitoring its fluorescence intensity through flow cytometry assay. The classical P-gp inhibitor VRP was chosen as a positive control. As shown in Figure 2, the level of ADM in K562 cells was about 4.5-fold higher than that of K562/A02 cells in the absence of P-gp inhibitors. As probably P-gp can pump ADM out of the cells, it leads to a lower ADM level in K562/A02 cells. Once treated with compound **4** (5μM), the retained amount of ADM in the K562/A02 cells significantly increased. When the K562/A02 cells were treated with compound **4** at various concentrations (0.5μM, 2.5μM, 5μM), the accumulation of ADM was remarkably increased in a dose-dependent manner. Additionally, the intracellular ADM level of compound **4** (5μM) was 1280.5±35.62, which was 1.5 times higher than that of VRP in K562/A02 cells at the same dose. The results suggested that compound **4** was more potent than VRP in inhibiting the drug efflux function of P-gp.

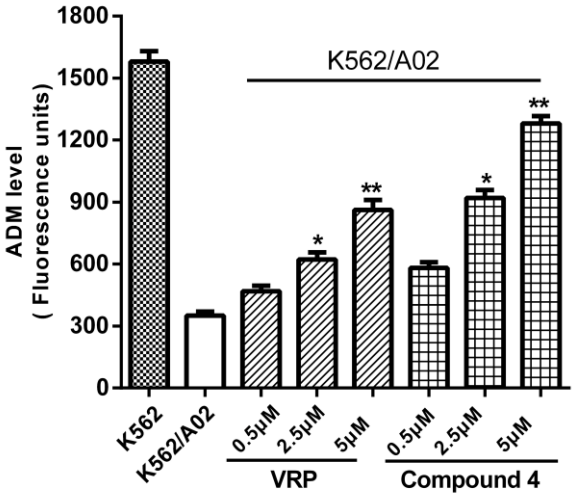


Figure 2. The effect of compound **4** on intracellular ADM accumulation in K562/A02 cells. 0.1% DMSO was used as vehicle control and VRP was chosen as a positive control. Different concentrations of compound **4** and VRP were pre-incubated with cells for 60 min. Then 20μM ADM was added into every well and incubated for 90 min, washed three times with PBS at 4°C. Data were expressed as means±SD of two independent experiments. **P* < 0.05, ** *P* < 0.01 relative to the negative control (K562/A02).

3.1.5. Inhibitory effect of compound 4 on Rh123 efflux function

In order to further supplement the above assumption, the inhibitory effect of compound **4** on the P-gp efflux function was assayed by detecting the retained intracellular Rh123, another fluorescence substrate of P-gp, according to the method described in literatures with minor modification.[20,21] The results from the Rh123 efflux assay showed that 5μM of compound **4** or VRP inhibited Rh123 efflux from K562/A02 cells, and the inhibitory effect of compound **4** was obviously better than VRP over the 90 min interval (Fig. 3). The rate constant (K) of efflux in compound **4**-treated K562/A02 cells was 0.0020±0.00053s^{-1}, which is lower than the value for the control group (0.0253±0.0069 s^{-1}) and the VRP-treated group (0.0085±0.0012 s^{-1}). The calculated data for K were shown as the mean±SD for 3

independent tests. These results suggest that the efflux function of P-gp on anticancer drugs, such as ADM, could be effectively blocked by compound **4**. Moreover, the potency of compound **4** is much higher than the classical P-gp inhibitor VRP under the same conditions.

The results above verified our presumption that compound **4** was an effective P-gp inhibitor which possessed the ability to enhance the sensitivity of anticancer drugs to P-gp overexpressing drug-selected cell lines by effectively blocking the drug efflux function of P-gp and showed higher potency than the classical P-gp inhibitor VRP under the same conditions.

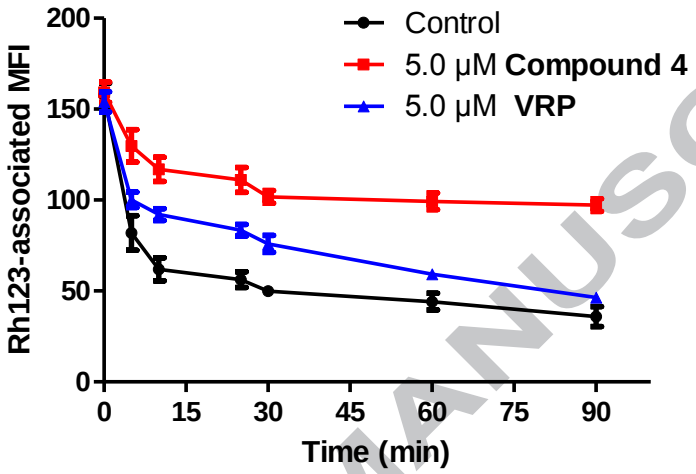


Figure 3. Effect of compound **4** and verapamil (VRP) on the efflux of rhodamine 123 (Rh123) in K562/A02 cells. K562/A02 cells were incubated in 5μM of Rh123 at 37 °C for 60 min, washed 3 times with phosphate-buffered saline at 4 °C, and then respectively incubated with or without 5μM of compound **4** or VRP at 37 °C for 0,5, 10, 25, 30, 60 and 90 min. The results were shown as the mean±SD for 3 independent tests.

3.2. Structure–activity relationships

The analysis of structure–activity relationships (SARs) according to the results in Table 1 indicates that different substituents in R' of compounds **1**–**25** could affect MDR reversal activities towards K562/A02 cells: (a) Electron-donating groups in R' might be beneficial for the MDR reversal activity. For example, compounds **11**, **19** and compound **12**, **20** which possessed electron-donating group like $–CH(CH_3)_2$ and $–C(CH_3)_3$ showed higher MDR reversal activity than VRP. However, compound **9**, **17** and **23**, which was substituted by $–OCH_3$, showed lower MDR reversal activity than VRP. In addition, electron-withdrawing groups in R' represented poor MDR reversal activity , such as compound **14**, **25**. (b) The substitution position of electron-donating group in R' has an influence on the reverse activity. Such as compound **20**, which was substituted in para-position, was more potent than compound **24** in ortho-position. (c) The size of substituent in R' is likely to affect MDR reversal activity. For example, compound **20** (RF = 20.4) containing 4-tert-butyl in R' was more potent than compound **16**, **18**, **19** which were substituted by other alkyls. It suggested that the introduction of 4-tertiary butyl to the molecule of compound **20** could dramatically strengthen its binding affinity to P-gp, leading to more ADM accumulation in K562/A02 cells. (d) Interestingly, compound **4** and compound **20** bearing the close molecular weight (compound **4**: 470.56, compound **20**: 450.57) showed the best MDR reversal activity among the twenty-five target compounds, whose IC_{50} were respectively 1.21±0.18μmol/L and 1.86±0.15μmol/L and close to the lead compound LBM-A5 (IC_{50}=1.65±0.22μmol/L). Compounds with triazol-N-phenethyl-tetrahydroisoquinoline (**1**–**12**) were

generally more potent than compounds with triazol-N-ethyl-tetrahydroisoquinoline (**13**–**25**) with the same substituent R', and compound **4** showed the strongest activity (RF = 31.4).

4. Conclusions

In summary, twenty-five compounds containing triazol-N-phenethyl-tetrahydroisoquinoline or triazol-N-ethyl-tetrahydroisoquinoline were synthesized based on the click chemistry and evaluated in vitro as P-gp-mediated MDR reversal agents. Among them, compound **4** with low cytotoxicity could significantly reverse MDR in a dose-dependent manner and persist longer chemo-sensitizing effect than VRP with reversibility. Additionally, compound **4** could remarkably increase the intracellular accumulation of ADM in K562/A02 cells as well as inhibit Rh123 efflux from the cells. These results suggest that compound **4** could effectively block the drug efflux function of P-gp and lead to increased drug accumulation in MDR cells. Therefore, compound **4** might be a promising candidate to develop P-gp-mediated MDR reversal modulator in cancer chemotherapy.

5. Experimental section

5.1 General chemistry

All reagents were reagent grade and all solvents were dried by standards methods before using. Column chromatography was carried out on silica gel or alumina (200–300 mesh). Melting points were measured using a Mel-TEMP II melting point apparatus, which was uncorrected. All of the target compounds were analyzed by ^{1}H NMR, ^{13}C NMR (Bruker ACF-300Q, 300 MHz), MS (1100 LC/ MSD spectrometer; Hewlett–Packard) and elemental analyses (CHN-O-Rapid instrument); Thin-layer chromatography (TLC) was performed on GF/UV 254 plates and the chromato-grams were visualized under UV light at 254 and 365 nm. Compound **e** was prepared as previously described.[11]

5.2. Synthesis of 2-azidoethanol (a)

To a 100mL round bottom flask was added 2-bromoethanol (5.3 g, 42.4mmol) and sodium azide (5.5 g, 84.6 mmol) in water (50mL). The mixture was stirred at 80°C for 24 h, and then, cooled to room temperature. The solution was extracted with ethyl acetate (4 × 30 mL) and the organic layer was dried with sodium sulfate overnight, then, filtered. After the removal of the solvent under vacuum, compound **a** was obtained as a crude pale yellow liquid (3.3g, yield: 90.5%), which was used in the next step directly. ^{1}H NMR (300 MHz, DMSO-d_6): δ= 3.79-3.76 (t, J = 5.1 Hz, 2H, O$\underline{CH_2}CH_2N_3$), 3.47-3.44 (t, J = 5.1 Hz, 2H, $OCH_2\underline{CH_2}N_3$), 2.02 (s, 1H, OH).

5.3. Synthesis of 2-azidoethyl 4-methylbenzenesulfonate (b)

To the solution of 2-azidoethanol (3.33 g, 37.9 mmol) and triethylamine (10mL, 72.1 mmol) in dry dichloromethane (25 mL), 4-toluenesulfonylchloride (6.2 g, 32.5 mmol) in dry dichloromethane (25 mL) was added drop-wise under constant stirring at 0°C and then the mixture was stirred at room temperature for 24 h. The reaction mixture was washed with 1 N HCl (3 × 40 mL) and 1 N $NaHCO_3$ (3 × 40 mL) and brine (3 × 40 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using a mixture of ethyl acetate/ petroleum ether (1:15, v/v) as eluent to afford the compound **b** (4.13 g). As colorless liquid, yield: 52.6%. ^{1}H NMR (300 MHz, DMSO-d_6): δ= 7.81 (d, J = 8.3 Hz, 2H, ArH), 7.50 (d, J = 8.0 Hz, 2H, ArH), 4.16 (t, J=6.1Hz, 2H, OCH_2), 3.56 (t, J = 5.1 Hz, 2H, CH_2), 2.42 (s, 3H, CH_3).

5.4. Synthesis of 2-(2-azidoethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (c)

To a solution of 2-azidoethyl-4-methylbenzenesulfonate (4.1 g, 17 mmol) in dry acetonitrile (50

mL) and triethylamine (5.0 mL, 36 mmol), 7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (3.9 g, 17 mmol) was added. The mixture was refluxed for 24 h, evaporated in vacuo, and chromatographed on a silica gel column (EtOAc/PE 3/2 to EtOAc/MeOH 16/1, v/v) to give compound **c**(3.1 g). As pale yellow solid, yield: 68.7%.^{1}H NMR (300 MHz, $CDCl_3$): δ= 6.61 (s, 1H, ArH), 6.53 (s, 1H, ArH), 3.85 (s, 6H, 2×OCH_3), 3.64 (s, 2H, $ArNCH_2$), 3.52 (t, J = 6.1Hz, 2H, $ArCH_2$), 2.80 (dd, 6H, J = 13.3, 6.1Hz, 3×CH_2).

5.5. General procedure for the preparation of 1d–17d

The aromatic phenol (3mmol) and 3-bromo-prop-1-yne (0.25 ml, 3.2 mmol) was dissolved in acetone (15 mL) and added potassium carbonate (1.0 g, 7.3 mmol), then the mixture was heated to reflux for 4 h. Then the reaction mixture was cooled to room temperature followed by filtration and evaporated in vacuo to get the desired product compound **1d–17d**.

5.5.1. 1-nitro-2-prop-2-ynyloxy-benzene (1d)

Yield 82.6%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.80 (1H, dd, J = 7.8, 1.2 Hz, ArH), 7.53 (1H, td, J = 7.8, 1.8 Hz, ArH), 7.23 (1H, d, J = 7.8Hz, ArH), 7.08-7.03 (1H, m), 4.81 (2H, d, J = 2.4 Hz, OCH_2), 2.58 (1H, t, J = 2.4 Hz, C≡CH).

5.5.2. 2-prop-2-ynyloxy-naphthalene (2d)

Yield 87.9%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.61-7.45 (m, 2H, ArH), 7.38-7.23 (m, 5H, ArH), 4.91 (d, J = 2.4 Hz, 2H, OCH_2), 2.65 (t, J = 2.4 Hz, 1H, C≡CH).

5.5.3. 1-nitro-4-prop-2-ynyloxy-benzene (3d)

Yield 86.0%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 8.39 (d, J = 8Hz, 2H, ArH), 7.36 (d, J = 8 Hz, 2H, ArH), 4.92 (d, J = 2.4 Hz, 2H, OCH_2), 2.69 (t, J = 2.4 Hz, 1H, C≡CH).

5.5.4. prop-2-ynyloxy-benzene (4d)

Yield 85.5%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.30-7.27 (m, 2H, ArH), 7.20-7.15 (m, 3H, ArH), 4.80 (d, J = 2.4 Hz, 2H, OCH_2), 2.61 (t, J = 2.4Hz, 1H, C≡CH).

5.5.5. 1-prop-2-ynyloxy-naphthalene (5d)

Yield 86.8%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 8.29 (m, 1H, ArH), 7.82 (m, 1H, ArH), 7.55-7.48 (m, 3H, ArH), 7.40 (t, J = 8.0 Hz, 1H, ArH), 4.91 (d, J = 2.5 Hz, 2H, OCH_2), 2.57 (q, J = 2.5 Hz, 1H, C≡CH).

5.5.6. 8-prop-2-ynyloxy-quinoline (6d)

Yield 72.8%; brown solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 8.82 (d, 1H, J = 4.1 Hz), 7.97 (d, 1H, J = 8.3 Hz), 7.37-7.26(m, 3H, ArH), 7.14 (d, 1H, J = 7.2 Hz, ArH), 4.92 (s, 2H, OCH_2), 2.44 (t, J = 2.5 Hz, 1H, C≡CH).

5.5.7. 1-methyl-4-prop-2-ynyloxy-benzene (7d)

Yield 88.8%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.10 (d, J = 8.5 Hz, 2H, ArH), 6.86 (d, J = 8.5 Hz, 2H, ArH), 4.66 (d, J = 2.5 Hz, 2H, OCH_2), 2.50 (t, J = 2.5 Hz, 1H, C≡CH), 2.29 (s, 3H, CH_3).

5.5.8. 1-prop-2-ynyloxy-4-trifluoromethoxy-benzene (8d)

Yield 72.6%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.17- 6.94 (m, 4H, ArH), 4.68 (d, J = 2.4 Hz, 2H, OCH_2), 2.53(t, J = 2.4 Hz, 1H, C ≡ CH).

5.5.9. 1-methoxy-4-prop-2-ynyloxy-benzene (9d)

Yield 88.8%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 6.93 (d, J = 9.0 Hz, 2H, ArH), 6.85 (d, J = 9.0 Hz, 2H, ArH), 4.64 (d, J = 2.5 Hz, 2H, OCH_2), 3.78 (s, 3H, OCH_3), 2.51 (t, J = 2.5 Hz, 1H, C≡CH).

5.5.10. 1-ethyl-4-prop-2-ynyloxy-benzene (10d)

Yield 86.3%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.17 (d, J = 8.7 Hz, 2H, ArH), 6.95 (d, J = 8.7 Hz, 2H, ArH), 4.70(d, J = 2.4 Hz, 2H, OCH_2), 2.65 (q, J = 7.6 Hz, 2H, $\underline{CH_2}CH_3$), 2.54 (t, J = 2.4 Hz, 1H, C≡CH), 1.26 (t, J = 7.6 Hz, 3H, $CH_2\underline{CH_3}$).

5.5.11. 1-isopropyl-4-prop-2-ynyloxy-benzene (11d)

Yield 80.3%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.13 (d, J = 8.4 Hz, 2H, ArH), 6.92 (d, J = 8.5 Hz, 2H, ArH), 4.68(d, J = 2.4 Hz, 2H, OCH_2), 2.84 – 2.79 (m, 1H, –$\underline{CH}$ $(CH_3)_2$), 2.52 (t, J = 2.4 Hz, 1H, C≡CH), 1.18 (d, J = 6.9 Hz, 6H, –CH $(\underline{CH_3})_2$).

5.5.12. 1-(tert-butyl)-4-prop-2-ynyloxy-benzene (12d)

Yield 83.0%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.27 (d, J = 7.4 Hz, 2H, ArH), 6.92 (d, J = 7.5 Hz, 2H, ArH), 4.65 (s, 2H, OCH_2), 2.54 (t, J = 2.4 Hz, 1H, C≡CH), 1.23 (s, 9H, $–C(CH_3)_3$).

5.5.13. 1,2-dimethyl-4-prop-2-ynyloxy-benzene (13d)

Yield 87.3%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.05 (d, J = 8.1 Hz, 1H, ArH), 6.77 (d, J = 2.7 Hz, 1H, ArH), 6.72 (dd, J = 8.1, 2.7 Hz, 1H, ArH), 4.65(d, J = 2.4 Hz, 2H, OCH_2), 2.49 (t, J = 2.4 Hz, 1H, C≡CH), 2.24 (s, 3H, CH_3), 2.19 (s, 3H, CH_3).

5.5.14. 2,4-dimethyl-1-prop-2-ynyloxy-benzene (14d)

Yield 77.9%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 6.93 (q, J = 8.0 Hz, 3H, ArH), 4.68 (s, 2H, OCH_2), 2.50 (t, J = 2.4 Hz, 1H, C≡CH), 2.18 (s, 3H, $–CH_3$), 2.04 (s, 3H, $–CH_3$).

5.5.15. 1-methoxy-2-prop-2-ynyloxy-benzene (15d)

Yield 72.9%; pale yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.09 (d, J = 7.6 Hz, 1H, ArH), 6.98 – 6.82 (m, 3H, ArH), 4.68 (s, 2H, OCH_2), 3.69 (s, 3H, OCH_3), 2.58 (t, J = 2.5 Hz, 1H, C≡CH).

5.5.16. 1-(tert-butyl)-2-prop-2-ynyloxy-benzene (16d)

Yield 73.1 %; white powder; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 7.31 – 7.05 (m, 3H, ArH), 6.87 (d, J = 6.1 Hz, 1H, ArH), 4.72 (s, 2H, OCH_2), 2.60 (t, J = 2.5 Hz, 1H, C≡CH), 1.21 (s, 9H, $–C(CH_3)_3$).

5.5.17. 1-fluoro-4-prop-2-ynyloxy-benzene (17d)

Yield 88.0%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.02-6.94 (m, 4H, ArH), 4.66 (d, J = 2.5 Hz, 2H, OCH_2), 2.52 (t, J = 2.5 Hz, 1H, C≡CH).

5.6. General procedure for the preparation of compounds 1–12

To the solution of **1d–12d** (1 mmol) and **e**(1 mmol) in 75% methanol (40 mL), ascorbate sodium (30 mg) and $CuSO_4$ (10 mg) were added, respectively. The reaction solution was stirred at room temperature for 24–48 h. Then, the mixture was filtered to give the desire product with high purity.

5.6.1.

6,7-dimethoxy-2-(4-(4-((2-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline (1)

Yield 45.5%; pale yellow powder; mp: 96-98°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.91 (s, 1H, -CH=C-), 7.88 (d, J = 7.6 Hz, 1 H, ArH), 7.81 (d, J = 8.0 Hz, 2 H, ArH), 7.72-7.61 (m, 2H, ArH), 7.49 (d, J = 7.8 Hz, 2 H, ArH), 7.16 (dd, J =7.5, 7.5 Hz, 1 H, ArH), 6.65, 6.63 (2s, 2 H, ArH), 5.44 (s, 2H, OCH_2), 3.69 (s, 6 H, 2×OCH_3), 3.55 (s, 2 H, $ArCH_2N$), 2.91-2.70 (m, 8 H, 4 × CH_2). ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 28.2, 32.3, 50.4, 55.0, 55.4, 59.0, 62.4, 109.9, 111.7, 115.6, 120.0, 121.0, 123.1, 124.9, 125.9, 126.6, 130.0, 134.3, 134.5, 139.8, 141.6, 142.7, 146.9, 147.1, 150.5; ESI-MS m/z: 516.5 ($[M + H]^+$); Anal. calcd. For $C_{28}H_{29}N_5O_5$: C, 65.23; H, 5.67; N, 13.58; Found: C, 65.28; H, 5.65; N, 13.55.

5.6.2.

6,7-dimethoxy-2-(4-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetra

hydroisoquinoline (2)

Yield 41.9%; pale yellow powder; mp: 162−164 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.94 (s, 1H, -CH=C-), 7.85-7.80 (m, 5 H, ArH), 7.55 (s, 1 H, ArH), 7.49-7.64 (m, 3 H, ArH), 7.36 (t, J = 7.5 Hz, 1 H, ArH), 7.23 (dd, J = 7.5, 1.8 Hz, 1 H, ArH), 6.65, 6.62 (2×s, 2 H, ArH), 5.35 (s, 2 H, OCH_2), 3.69 (s, 6 H, 2×OCH_3), 3.54 (s, 2 H, $ArCH_2N$), 2.90-2.69 (m, 8 H, 4×CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.2, 32.2, 50.4, 55.0, 55.4, 58.9, 61.1, 107.2, 109.9, 111.7, 118.6, 120.0, 122.8, 123.7, 125.8, 126.4, 126.5,126.7, 127.4,128.6, 129.3, 130.0, 134.1, 134.6, 141.4, 143.6, 146.8, 147.1, 155.8; ESI-MS m/z: 521.8 ([M + H]$^+$); Anal. calcd. For $C_{32}H_{32}N_4O_3$: C, 73.82; H, 6.20; N, 10.76; Found: C, 73.88; H, 6.18; N, 10.73.

5.6.3.

6,7-dimethoxy-2-(4-(4-((4-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline (3)

Yield 52.4%; pale yellow powder; mp: 132-135°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.94 (s, 1H, -CH=C-), 8.24 (d, J = 9.2 Hz, 2H, ArH), 8.07 (d, J = 9.2 Hz, 2H, ArH), 7.81 (d, J = 8.3 Hz, 2H, ArH), 7.49 (d, J = 8.3 Hz, 2H, ArH), 7.31(d, J = 9.0 Hz, 2H), 6.66(s, 1H, ArH), 6.63(s, 1H, ArH), 5.42(s, 2H, OCH_2), 3.69(s, 6H, 2×OCH_3), 3.56(s, 2H, $ArCH_2N$), 2.92-2.71(m, 8H, 4×CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.2, 32.2, 50.4, 55.0, 55.4, 58.9, 61.8, 109.9, 111.7, 115.3, 120.1, 123.1, 125.9, 130.0, 134.5, 141.1, 141.6, 142.8, 146.8, 147.1, 163.2; ESI-MS m/z: 516.8 ([M + H]$^+$); Anal. calcd. For $C_{28}H_{29}N_5O_5$: C, 65.23; H, 5.67; N, 13.58; Found: C, 65.20; H, 5.69; N, 13.56.

5.6.4.

6,7-dimethoxy-2-(4-(4-(phenoxymethyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline (4)

Yield 42.5%; pale yellow powder; mp: 118-120°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.91 (s, 1H, -CH=C-), 7.81 (d, J = 8.4 Hz, 2H, ArH), 7.50 (d, J = 8.4Hz, 2H, ArH), 7.49 (d, J = 8.3 Hz, 2H, ArH),7.32 (dd, J = 8.1, 8.1Hz, 2H, ArH), 7.07 (d, J = 8.1Hz, 2H, ArH), 6.97 (dd, J = 7.3, 7.3 Hz, 1H), 6.66 (s,1H, ArH, ArH), 6.63 (s, 1H, ArH, ArH), 5.23 (s, 2H, OCH_2), 3.70 (s, 6H, 2×OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.92-2.71 (m, 8H, 4×CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.2, 32.3, 50.5, 55.0, 55.4, 59.0, 60.9, 109.9, 111.7, 114.7, 120.0, 120.9, 122.7, 125.8, 126.6, 129.5, 130.0, 134.6, 141.5, 143.8, 146.8, 147.1, 157.9; ESI-MS m/z: 471.2 ([M + H]$^+$); Anal. calcd. For $C_{28}H_{30}N_4O_3$: C, 71.47; H, 6.43; N, 11.91; Found: C, 71.42; H, 6.44; N, 11.93.

5.6.5.

6,7-dimethoxy-2-(4-(4-((naphthalen-1-yloxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline (5)

Yield 38.5%; pale yellow powder; mp: 130-132°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 9.02(s, 1H, -CH=C-), 8.20 (d, J = 7.9 Hz, 1H, ArH), 7.89-7.84 (m, 3H, ArH), 7.54-7.43 (m, 6H, ArH), 7.23 (d, J = 7.3 Hz, 1H, ArH), 6.66 (s, 1H, ArH), 6.63 (s, 1H, ArH), 5.43 (s, 2H, OCH_2), 3.70 (s, 6H, 2×OCH_3), 3.55(s, 2H, $ArCH_2N$), 2.92-2.71(m, 8H, 4×CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.3, 32.3, 50.4, 55.0, 55.4, 59.0, 61.7, 105.8, 109.9, 111.7, 120.0, 120.4, 121.7, 122.6, 124.9, 125.3, 125.8, 126.1, 126.5, 126.6, 127.4, 130.0, 134.0, 134.6, 141.5, 143.9, 1468.8, 147.1, 153.4; ESI-MS m/z: 522.2 ([M + H]$^+$); Anal. calcd. For $C_{32}H_{32}N_4O_3$: C, 73.82; H, 6.20; N, 10.76; Found: C, 73.87; H, 6.19; N, 10.74.

5.6.6.

8-((1-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)phenyl)-1H-1,2,3-triazol-4-yl)methoxy)quinoline (6)

Yield 38.5%; pale yellow powder; mp: 100-102°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.98 (s, 1 H, ArH), 8.84 (s, 1 H, NCH=C), 8.32 (d, J = 8.1 Hz, 1H, ArH), 7.84 (d, J = 8.2 Hz, 2 H, ArH), 7.55-7.44 (m, 6 H, ArH), 6.65, 6.63 (2s, 2 H, ArH), 5.44 (s, 2 H, OCH_2), 3.70 (s, 6 H, 2 × OCH_3), 3.54 (s, 2 H, ArCH2N), 2.91-2.70 (m, 8 H, 4 × CH2); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 28.2, 32.3, 50.4, 55.0, 55.4, 59.0, 61.8, 109.9, 110.2, 111.7, 120.1, 120.2, 121.8, 123.1, 125.9, 126.6, 126.7, 129.1, 130.0, 134.6, 135.8, 139.7, 141.5, 143.6, 146.9, 147.1, 149.0, 153.8; ESI-MS m/z: 523.0 ([M + H]$^+$); Anal. calcd. For $C_{31}H_{31}N_5O_3$: C, 71.38; H, 5.99; N, 13.43; Found: C, 71.33; H, 6.01; N, 13.45.

5.6.7.

6,7-dimethoxy-2-(4-(4-((p-tolyloxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline (7)

Yield 62.0%; pale yellow powder; mp: 122-124°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.86 (s, 1 H, NCH=C), 7.80 (d, J = 8.0 Hz, 2 H, ArH), 7.47 (d, J = 8.0 Hz, 2 H, ArH), 7.10 (d, J = 8.0 Hz, 2 H, ArH), 6.96 (d, J = 8.1 Hz, 2 H, ArH), 6.65, 6.62 (2s, 2 H, ArH), 5.17 (s, 2 H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3), 3.54 (s, 2 H, $ArCH_2N$), 2.90-2.70 (m, 8 H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 20.0, 28.3, 32.3, 50.4, 55.0, 55.4, 59.0, 61.0, 109.9, 111.7, 114.5, 120.0, 122.6, 125.9, 126.6, 129.6, 129.8, 130.0, 134.6, 141.4, 143.9, 146.9, 147.1, 155.9; ESI-MS m/z: 485.3 ([M + H]$^+$); Anal. calcd. For $C_{29}H_{32}N_4O_3$: C, 71.88; H, 6.66; N, 11.56; Found: C, 71.82; H, 6.68; N, 11.58.

5.6.8.

6,7-dimethoxy-2-(4-(4-((4-(trifluoromethoxy)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline (8)

Yield 54.2%; pale yellow powder; mp: 90-92°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.90 (s, 1 H, NCH=C), 7.80 (d, J = 8.0 Hz, 2 H, ArH), 7.47 (d, J = 8.0 Hz, 2 H, ArH), 7.23 (d, J = 8.9 Hz, 2 H, ArH), 7.17 (d, J = 8.9 Hz, 2 H, ArH), 6.64, 6.62 (2s, 2 H, ArH), 5.25 (s, 2 H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3), 3.55 (s, 2 H, $ArCH_2N$), 2.90-2.70 (m, 8 H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 28.3,32.3, 50.4, 55.0, 55.4, 59.0, 61.5, 109.9, 111.7, 116.0, 116.3, 118.4, 120.0, 121.8, 122.3, 122.5, 122.8, 125.9, 126.5, 130.0, 134.6, 141.5, 142.0, 143.4, 146.9, 147.1,156.8; ESI-MS m/z: 555.2 ([M + H]$^+$); Anal. calcd. For $C_{29}H_{29}F_3N_4O_4$: C, 62.81; H, 5.27; F, 10.28; N, 10.10; Found: C, 62.88; H, 5.25; F, 10.31; N, 10.08.

5.6.9.

6,7-dimethoxy-2-(4-(4-((4-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-1,2,3,4-tetrahydroisoquinoline (9)

Yield 40.2%; pale yellow powder; mp: 136-138°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.88 (s, 1 H, NCH=C), 7.83 (d, J = 7.0 Hz, 2 H, ArH), 7.51 (d, J = 7.0 Hz, 2 H, ArH), 7.04 (d, J = 7.8 Hz, 2 H, ArH), 6.91 (d, J = 7.7 Hz, 2 H, ArH), 6.68, 6.66 (2s, 2 H, ArH), 5.18 (s, 2 H, OCH_2), 3.73 (s, 6 H, 2 × OCH_3), 3.58 (s, 2 H, $ArCH_2N$), 2.93-2.74 (m, 8 H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 28.3, 32.3, 50.4, 55.0, 55.2, 55.4, 59.0, 61.5, 109.9, 111.7, 114.6, 115.7, 120.0, 122.6, 125.9, 126.6, 130.0, 134.6, 141.4, 144.0, 146.9, 147.1, 152.0, 153.6; ESI-MS m/z: 501.4 ([M + H]$^+$); Anal. calcd. For $C_{29}H_{32}N_4O_4$: C, 69.58; H, 6.44; N, 11.19; Found: C, 69.52; H, 6.46; N, 11.21.

5.6.10.

2-(4-(4-((4-ethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (10)

Yield 50.2%; yellow powder; mp: 114-116°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.88 (s, 1 H, NCH=C), 7.81 (d, J = 8.1 Hz, 2 H, ArH), 7.47 (d, J = 8.1Hz, 2 H, ArH), 7.13 (d, J = 8.1 Hz, 2 H, ArH), 6.98 (d, J = 8.3 Hz, 2 H, ArH), 6.65, 6.63 (2s, 2 H, ArH), 5.18 (s, 2 H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3),

3.54 (s, 2 H, $ArCH_2N$), 2.90-2.70 (m, 8 H, 4 × CH_2), 2.57-2.52 (m, 2 H, $\underline{CH_2}CH_3$), 1.14 (t, J = 7.5 Hz, 3 H, $CH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 15.8, 28.3, 32.3, 50.4, 55.0, 55.4, 59.0, 61.0, 109.9, 111.7, 114.5, 120.0, 122.6, 125.9, 126.6, 128.6, 130.0, 134.6, 136.1, 141.4, 143.9, 146.9, 147.1, 156.0; ESI-MS m/z: 499.3 ($[M + H]^+$); Anal. calcd. For $C_{30}H_{34}N_4O_3$: C, 72.26; H, 6.87; N, 11.24; Found: C, 72.30; H, 6.89; N, 11.23.

5.6.11.

2-(4-(4-((4-isopropylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (11)

Yield 19.5%; pale yellow powder; mp: 113-115°C; ^1H NMR (300 MHz, DMSO-d_6): δ= 8.88 (s, 1 H, NCH=C), 7.81 (d, J = 8.2 Hz, 2 H, ArH), 7.48 (d, J = 8.2 Hz, 2 H, ArH), 7.16 (d, J = 8.4 Hz, 2 H, ArH), 6.98 (d, J = 8.4 Hz, 2 H, ArH), 6.65, 6.63 (2s, 2 H, ArH), 5.18 (s, 2 H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3), 3.54 (s, 2 H, $ArCH_2N$), 2.93-2.70 (m, 9 H, 4 × CH2, CH), 1.1 (d, J = 6.8 Hz, 6 H, 2 × CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 24.0, 28.3, 32.3, 32.5, 50.4, 55.0, 55.4, 59.0, 61.0, 109.9, 111.7, 114.5, 120.0, 122.6, 125.9, 126.6, 127.1, 130.0, 134.6, 140.8, 141.4, 143.9, 146.9, 147.1, 156.1; ESI-MS m/z: 513.4($[M + H]^+$); Anal. calcd. For $C_{31}H_{36}N_4O_3$: C, 72.63; H, 7.08; N, 10.93; Found: C, 72.68; H, 7.10; N, 10.96.

5.6.12.

2-(4-(4-((4-(tert-butyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)phenethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (12)

Yield 38.0%; yellow powder; mp: 118-120°C; ^1H NMR (300 MHz, DMSO-d_6): δ= 8.89 (s, 1 H, NCH=C), 7.81 (d, J = 8.3 Hz, 2 H, ArH), 7.48 (d, J = 8.3 Hz, 2 H, ArH), 7.31 (d, J = 8.6 Hz, 2 H, ArH), 6.98 (d, J = 8.6 Hz, 2 H, ArH), 6.65, 6.63 (2s, 2 H, ArH), 5.19 (s, 2H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3), 3.54 (s, 2 H, $ArCH_2N$), 2.93-2.70 (m, 8 H, 4 × CH_2), 1.25 (s, 9 H, 3 × CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.3, 31.3, 32.3, 33.7, 55.0, 55.4, 59.0, 61.0, 109.9, 111.7, 114.1, 120.0, 122.6, 125.9, 126.1, 126.6, 130.0, 134.6, 141.4, 143.1, 143.9, 146.9, 147.1, 155.7; ESI-MS m/z: 527.3 ($[M + H]^+$); Anal. calcd. For $C_{32}H_{38}N_4O_3$: C, 72.98; H, 7.27; N, 10.64; Found: C, 72.93; H, 7.28; N, 10.67.

5.7. General procedure for the preparation of compounds 13–25

To the solution of **2d, 3d, 5d, 7d, 9d–17d** (1 mmol) and **c**(1 mmol) in 75% methanol (40 mL), ascorbate sodium (30 mg) and $CuSO_4$ (10 mg) were added, respectively. The reaction solution was stirred at room temperature for 24–48 h. Some of the reaction solutions precipitated white solid (compound **13** and **17**), the others none. The solution with precipitate appeared filtered directly and the solution without precipitate appeared evaporated in vacuo, extracted with dichloromethane. Then, the organic layer was dried by anhydrous Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by silica gel column chromatography using a mixture of ethyl acetate/ methanol (15:1, v/v) as eluent to give the desire white solid product compounds **13–25**.

5.7.1.

6,7-dimethoxy-2-(2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (13)

Yield 24.6%; white powder; mp: 142–144°C ; ^1H NMR (300 MHz, DMSO-d_6): δ= 8.28 (s, 1H, NCH=C), 7.89 – 7.72 (m, 3H, ArH), 7.54 – 7.42 (m, 2H, ArH), 7.39 – 7.31 (m, 1H, ArH), 7.17 (dd, J = 9.0, 2.5 Hz, 1H, ArH), 6.62 (s, 1H, ArH), 6.58 (s, 1H, ArH), 5.25 (s, 2H, OCH_2), 4.59 (t, J = 6.1 Hz, 2H, $CHNCH_2$), 3.69 (s, 6H, 2 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.91 (t, J = 6.1 Hz, 2H, NCH_2), 2.65 (s, 4H, 2 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.3, 32.1, 50.4, 55.1, 55.6, 58.9, 61.1, 107.3, 109.9, 111.7, 118.5, 120.0, 122.8, 123.8, 125.7, 126.4, 127.4, 128.6, 129.3, 130.1, 134.5, 141.4, 143.6, 146.7,

155.7; ESI-MS m/z: 445.1 ([M + H]$^+$); Anal. calcd. For $C_{26}H_{28}N_4O_3$: C, 70.25; H, 6.35; N, 12.60; Found: C, 70.30; H, 6.33; N, 12.58.

5.7.2.

6,7-dimethoxy-2-(2-(4-((4-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline(14)

Yield 70.6%; white powder; mp: 113–115°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.25 (s, 1H, NCH=C), 7.14 – 7.07 (m, 2H, ArH), 7.04 – 6.98 (m, 2H, ArH), 6.65 (s, 1H, ArH), 6.56 (s, 1H, ArH), 5.15 (s, 2H, OCH_2), 4.58 (t, J = 6.0 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × OCH_3), 3.53 (s, 2H, $ArCH_2N$), 2. 90 (t, J = 6.0 Hz, 2H, NCH_2), 2.66 (s, 4H, 2 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.2, 32.2, 50.3, 54.9, 55.4, 58.8, 61.8, 109.9, 111.7, 115.3, 120.0, 123.1, 125.7, 130.0, 134.3, 141.1, 142.7, 146.5, 147.0, 162.0; ESI-MS m/z: 444.0([M + H]$^+$); Anal. calcd. For $C_{22}H_{25}N_5O_5$: 60.13; H, 5.73; N, 15.94; Found: C, 60.10; H, 5.74; N, 15.95.

5.7.3.

6,7-dimethoxy-2-(2-(4-((naphthalen-1-yloxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (15)

Yield 61.5%; pale yellow powder; mp: 110-112°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.33 (s, 1H, NCH=C), 8.07 (d, J = 7.7 Hz, 1H, ArH), 7.86 (d, J = 7.5 Hz, 1H, ArH), 7.56 – 7.36 (m, 4H, ArH), 7.15 (d, J = 7.4 Hz, 1H, ArH), 6.61 (dd, J = 13.6, 2.8 Hz, 2H, ArH), 5.32 (d, J = 2.7 Hz, 2H, OCH_2), 4.59 (s, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.92 (s, 2H, NCH_2), 2.66 (s, 4H, 2 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.3, 32.3, 50.3, 55.0, 55.6, 59.1, 61.5, 105.7, 109.7, 111.7, 120.3, 121.6, 122.5, 124.8, 125.1, 125.7, 126.5, 127.3, 130.1, 134.5, 141.3, 143.8, 146.8, 153.2; ESI-MS m/z: 445.0 ([M + H]$^+$); Anal. calcd. For $C_{26}H_{28}N_4O_3$: C, 70.25; H, 6.35; N, 12.60; Found: C, 70.29; H, 6.34; N, 12.58.

5.7.4

6,7-dimethoxy-2-(2-(4-((p-tolyloxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (16)

Yield 62.0%; pale yellow powder; mp: 108-110°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.19 (s, 1H, NCH=C), 7.06 (d, J = 8.3 Hz, 2H, ArH), 6.89 (d, J = 8.5 Hz, 2H, ArH), 6.65 (s, 1H, ArH), 6.59 (s, 1H, ArH), 5.07 (s, 2H, OCH_2), 4.57 (t, J = 5.9 Hz, 2H, $CHNCH_2$), 3.69 (s, 6H, 2 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.94 – 2.84 (m, 2H, NCH_2), 2.67 (s, 4H, 2 × CH_2), 2.22 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 20.1, 28.2, 32.1, 50.4, 55.0, 55.4, 59.0, 60.9, 109.9, 111.7, 114.5, 119.8, 122.5, 125.8, 126.5, 129.8, 134.7, 141.3, 143.8, 146.8, 155.7; ESI-MS m/z: 409.1 ([M + H]$^+$); Anal. calcd. For $C_{23}H_{28}N_4O_3$: C, 67.63; H, 6.91; N, 13.72; Found: C, 67.59; H, 6.90; N, 13.74.

5.7.5

6,7-dimethoxy-2-(2-(4-((4-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (17)

Yield 23.3%; pale yellow powder; mp: 118-121°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.18 (s, 1H, NCH=C), 6.94 (d, J = 9.1 Hz, 2H, ArH), 6.83 (d, J = 9.1 Hz, 2H, ArH), 6.64 (s, 1H, ArH), 6.59 (s, 1H, ArH), 5.04 (s, 2H, OCH_2), 4.57 (t, J = 6.0 Hz, 2H, $CHNCH_2$), 3.68 (s, 9H, 3× OCH_3), 3.53 (s, 2H, $ArCH_2N$), 2.90 (t, J = 5.6 Hz, 2H, NCH_2), 2.66 (s, 4H, 2 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.2, 32.4, 50.4, 55.0, 55.2, 55.4, 59.1, 61.3, 109.9, 111.8, 114.6, 115.7, 120.0, 122.8, 125.9, 126.7, 130.0, 134.6, 141.5, 144.0, 146.9, 153.8; ESI-MS m/z: 425.1 ([M + H]$^+$); Anal. calcd. For $C_{23}H_{28}N_4O_4$: C, 65.08; H, 6.65; N, 13.20; Found: C, 65.12; H, 6.64; N, 13.18.

5.7.6.

2-(2-(4-((4-ethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroiso quinoline (18)

Yield 47.3%; pale yellow powder; mp: 106–108°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.20 (s, 1H, NCH=C), 7.10 (d, J = 8.3 Hz, 2H, ArH), 6.92 (d, J = 8.3 Hz, 2H, ArH), 6.62 (d, J = 16.3 Hz, 2H, ArH), 5.08 (s, 2H, OCH_2), 4.64–4.47 (m, 2H, $CHNCH_2$), 3.69 (s, 6H, 2 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.94–2.83 (m, 2H, NCH_2), 2.67 (s, 4H, 2 × CH_2), 2.56–2.52 (m, 2H,–$\underline{CH_2}CH_3$), 1.14 (t, J = 7.5 Hz, 3H,–$CH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 15.8, 28.5, 32.4, 50.5, 55.3, 55.9, 57.2, 61.5, 110.3, 112.2, 116.5, 121.1, 124.6, 125.1, 126.7, 128.7, 130.6, 134.7, 140.8, 143.7, 147.9, 156.8; ESI-MS m/z: 423.1 ($[M + H]^+$); Anal. calcd. For $C_{24}H_{30}N_4O_3$: C, 68.22; H, 7.16; N, 13.26; Found: C, 68.18; H, 7.17; N, 13.28.

5.7.7.

2-(2-(4-((4-isopropylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahyd roisoquinoline (19)

Yield 70.4%; pale yellow powder; mp: 102–105°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.20 (s, 1H, NCH=C), 7.13 (d, J = 8.4 Hz, 2H, ArH), 6.92 (d, J = 8.5 Hz, 2H, ArH), 6.64 (s, 1H, ArH), 6.59 (s, 1H, ArH), 5.08 (s, 2H, OCH_2), 4.57 (t, J = 5.7 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.89 (dd, J = 12.3, 6.6 Hz, 2H, NCH_2), 2.84 – 2.79 (m, 1H, –$\underline{CH}(CH_3)_2$), 2.67 (s, 4H, 2 × CH_2), 1.16 (d, J = 6.9 Hz, 6H, –$CH(CH_3)_2$); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 23.8, 28.2, 32.2, 32.5, 50.4, 55.0, 55.4, 59.0, 60.9, 109.9, 111.7, 114.4, 120.0, 122.5, 125.8, 126.5, 127.2, 130.1, 134.6, 140.7, 143.8, 147.1, 156.2; ESI-MS m/z: 437.1 ($[M + H]^+$); Anal. calcd. For $C_{25}H_{32}N_4O_3$: C, 68.78; H, 7.39; N, 12.83; Found: C, 68.82; H, 7.38; N, 12.81.

5.7.8.

2-(2-(4-((4-(tert-butyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahy droisoquinoline (20)

Yield 74.4%; white powder; mp: 108–111°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.20 (s, 1H, NCH=C), 7.27 (d, J = 7.4 Hz, 2H, ArH), 6.92 (d, J = 7.5 Hz, 2H, ArH), 6.64 (s, 1H, ArH), 6.59 (s, 1H, ArH), 5.08 (s, 2H, OCH_2), 4.57 (t, J = 5.8 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.90 (s, 2H, NCH_2), 2.66 (s, 4H, 2 × CH_2), 1.23 (s, 9H, –$C(CH_3)_3$); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 28.3. 31.2, 32.3, 33.8, 55.0, 55.4, 59.1, 61.0, 109.9, 111.7, 114.4, 120.0, 122.6, 125.9, 126.0, 126.7, 130.0, 134.6, 141.4, 143.9, 147.0, 155.8; ESI-MS m/z: 451.2 ($[M + H]^+$); Anal. calcd. For $C_{26}H_{34}N_4O_3$: C, 69.31; H, 7.61; N, 12.43; Found: C, 69.26; H, 7.62; N, 12.45.

5.7.9.

2-(2-(4-((3,4-dimethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahy droisoquinoline (21)

Yield 68.7%; pale yellow powder; mp: 110–112°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.18 (s, 1H, NCH=C), 7.00 (d, J = 8.3 Hz, 1H, ArH), 6.80 (d, J = 2.4 Hz, 1H, ArH), 6.72 (dd, J = 8.2, 2.6 Hz, 1H, ArH), 6.64 (s, 1H, ArH), 6.59 (s, 1H, ArH), 5.05 (s, 2H, OCH_2), 4.57 (t, J = 6.1 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × OCH_3), 3.53 (s, 2H, $ArCH_2N$), 2.90 (t, J = 6.2 Hz, 2H, NCH_2), 2.66 (s, 4H, 2 × CH_2), 2.16 (s, 3H, –CH_3), 2.12 (s, 3H, –CH_3); ^{13}C NMR (75 MHz, DMSO-d_6 , δ ppm): 18.9, 20.1, 28.5, 32.4, 50.5, 55.3, 55.9, 57.2, 61.5, 110.3, 112.1, 116.5, 121.1, 124.6, 125.1, 126.2, 128.7, 130.6, 137.7, 140.8, 143.7, 147.9, 156.7; ESI-MS m/z: 423.1 ($[M + H]^+$); Anal. calcd. For $C_{24}H_{30}N_4O_3$: C, 68.22; H, 7.16; N, 13.26; Found: C, 68.18; H, 7.17; N, 13.28.

5.7.10.

2-(2-(4-((2,4-dimethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahy

droisoquinoline (22)

Yield 78.1%; pale yellow powder; mp: 112–114°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.18 (s, 1H, NCH=C), 6.93 (q, J = 8.0 Hz, 3H, ArH), 6.64 (s, 1H, ArH), 6.58 (s, 1H, ArH), 5.07 (s, 2H, OCH_2), 4.57 (t, J = 6.1 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × OCH_3), 3.53 (s, 2H, $ArCH_2N$), 2.90 (t, J = 6.1 Hz, 2H, NCH_2), 2.66 (s, 4H, 2 × CH_2), 2.18 (s, 3H, –CH_3), 2.04 (s, 3H, –CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 16.4, 20.5, 28.6, 32.5, 50.5, 55.3, 55.9, 57.1, 62.1, 110.3, 112.2, 116.4, 121.1, 125.0, 126.2, 126.7, 127.4, 129.6, 131.6, 137.2, 140.7, 143.5, 147.6, 154.4; ESI-MS m/z: 423.1 ($[M + H]^+$). Anal. calcd. For $C_{24}H_{30}N_4O_3$: C, 68.22; H, 7.16; N, 13.26; Found: C, 68.25; H, 7.15; N, 13.28.

5.7.11.

6,7-dimethoxy-2-(2-(4-((2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (23)

Yield 67.4%; pale yellow powder; mp: 116–119°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.20 (s, 1H, NCH=C), 7.09 (d, J = 7.6 Hz, 1H, ArH), 6.98 – 6.82 (m, 3H, ArH), 6.65 (s, 1H, ArH), 6.59 (s, 1H, ArH), 5.08 (s, 2H, OCH_2), 4.58 (t, J = 5.8 Hz, 2H, $CHNCH_2$), 3.69 (d, J = 5.4 Hz, 9H, 3 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.90 (t, J = 5.9 Hz, 2H, NCH_2), 2.67 (s, 4H, 2 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.3, 32.4, 50.4, 55.0, 55.3, 55.5, 59.1, 61.4, 109.9, 111.7, 114.6, 115.7, 120.0, 122.8, 125.9, 126.7, 129.9, 134.6, 141.5, 144.0, 146.9, 153.8; ESI-MS m/z: 425.1 ($[M + H]^+$). Anal. calcd. For $C_{23}H_{28}N_4O_4$: C, 65.08; H, 6.65; N, 13.20; Found: C, 65.13; H, 6.64; N, 13.17.

5.7.12.

2-(2-(4-((2-(tert-butyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (24)

Yield 66.5 %; white powder; mp: 120–122°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.18 (s, 1H, NCH=C), 7.31 – 7.05 (m, 3H, ArH), 6.87 (d, J = 6.1 Hz, 1H, ArH), 6.63 (s, 1H, ArH), 6.57 (s, 1H, ArH), 5.13 (s, 2H, OCH_2), 4.60 (t, J = 5.8 Hz, 2H,$CHNCH_2$), 3.62 (s, 6H, 2 × OCH_3), 3.52 (s, 2H，$ArCH_2N$), 2.88 (s, 2H, NCH_2), 2.65 (s, 4H, 2 × CH_2), 1.21 (s, 9H, –$C(CH_3)_3$); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.3. 31.2, 32.3, 33.8, 55.0, 55.4, 59.1, 61.0, 109.9, 111.7, 114.4, 120.0, 122.6, 125.9, 126.0, 126.7, 130.0, 134.6, 141.4, 143.9, 147.0, 155.8; ESI-MS m/z: 451.1 ($[M + H]^+$); Anal. calcd. For $C_{26}H_{34}N_4O_3$: C, 69.31; H, 7.61; N, 12.43; Found: C, 69.35; H, 7.59; N, 12.40.

5.7.13.

2-(2-(4-((4-fluorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (25)

Yield 38.1%; pale yellow powder; mp: 111–113°C; ^{1}H NMR (300 MHz, DMSO-d_6):δ= 8.23 (s, 1H, NCH=C), 7.14 – 7.06 (m, 2H, ArH), 7.04 – 6.98 (m, 2H, ArH), 6.64 (s, 1H, ArH), 6.58 (s, 1H, ArH), 5.09 (s, 2H, OCH_2), 4.57 (t, J = 6.0 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × OCH_3), 3.53 (s, 2H, $ArCH_2N$), 2.89 (t, J = 6.0 Hz, 2H, NCH_2), 2.66 (s, 4H, 2 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 28.2, 32.2, 50.3, 54.9, 55.4, 58.8, 61.8, 109.9, 111.6, 115.2, 120.0, 123.1, 125.7, 130.0, 134.3, 141.1, 142.7, 146.5, 147.0, 158.7; ESI-MS m/z: 413.0 ($[M + H]^+$), 435.1($[M + Na]^+$); Anal. calcd. For $C_{22}H_{25}FN_4O_3$: C, 64.06; H, 6.11; F, 4.61; N, 13.58; Found: C, 64.12; H, 6.09; F, 4.57; N, 13.60.

5.8. Biological assays

5.8.1. Cytotoxicity assay

K562 and K562/A02 cells were grown in 96-well microtiter plates at 1~2×10^4 cells per well and incubated for 24 h. In the assay of cytotoxic evaluation, a graded dose of compounds diluted with medium were added into the wells. In the assay of drug resistant modulation, 5μM of the target compounds were added into the wells followed by various concentrations of ADM. And the

exponentially growing cancer cells were incubated for 48 h in an atmosphere of 95% air with 5% CO_2 at 37 °C. Then, MTT was added directly to the cells. After additional incubation for 4 h at 37 °C, the absorbance at 570 nm was read on a microplate reader (Thermo Fisher Scientific). The IC_{50} values of the compounds for cytotoxicity were calculated by GraphPad Prism 5.0 software from the dose–response curves.

5.8.2. MTT assay

K562/A02 cells were incubated in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C in a 5% CO_2 humidified atmosphere. K562/A02 cells (cells per well) were seeded in 96-well plates. After 24 h incubation, cells were treated with various concentrations of ADM in absence or presence of target compounds for 48 h in an atmosphere of 95% air with 5% CO_2 at 37 °C. Then, MTT was added directly to the cells. After additional incubation for 4 h at 37°C, the absorbance at 570 nm was read on a microplate reader (Thermo Fisher Scientific). The IC_{50} values of the compounds for cytotoxicity were calculated by GraphPad Prism 5.0 software from the dose–response curves. Experiments were conducted in triplicates and repeated three times independently.

5.8.3. Adriamycin intracellular accumulation

K562 and K562/A02 cells were seeded into 24-well plates at 1.5×10^5 /well. Different concentrations of compound **4** and VRP were pre-incubated with cells for 60 min. Then 20μM ADM was added into every well and incubated for 90 min, washed three times with PBS at 4°C for intracellular mean fluorescence intensity (MFI) analysis. The mean fluorescence intensity of retained intracellular ADM was estimated by BD FACSCalibur flow cytometer.

5.8.4. Rhodamine123 efflux assay

K562 or K562/A02 cells were seeded into 24-well plates at 1.5×10^5/well and incubated with 5μM Rh123 for 60 min before washing with ice PBS for three times. Then the cells were incubated with or without various concentrations of compound **4** or VRP (0.5, 2.5, 5μM) for another 90 min. Afterwards the cells were washed twice in ice-cold PBS. The mean fluorescence intensity of retained intracellular Rh123 was estimated by BD FACSCalibur flow cytometer.

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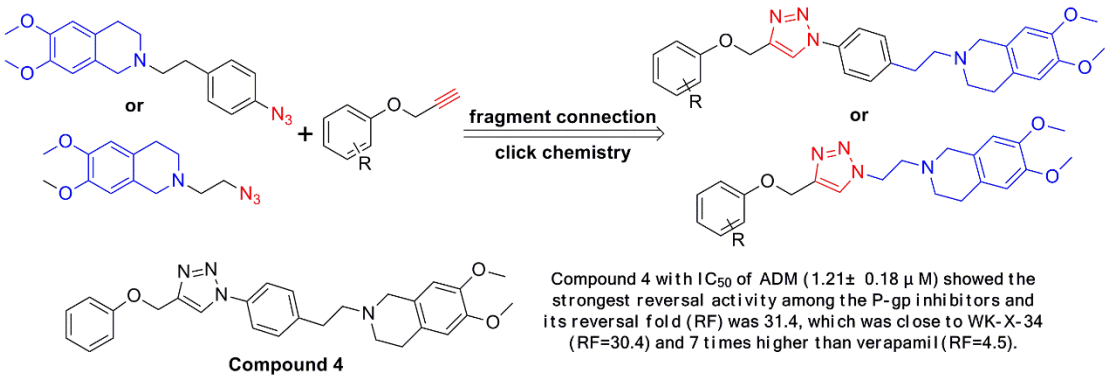
Graphical abstract



Compound 4

Compound 4 with IC_{50} of ADM (1.21± 0.18 μ M) showed the strongest reversal activity among the P-gp inhibitors and its reversal fold (RF) was 31.4, which was close to WK-X-34 (RF=30.4) and 7 times higher than verapamil (RF=4.5).