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Design, synthesis and evaluation of novel triazole core based P-glycoprotein-mediated multidrug resistance reversal agents

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ABSTRACT

A novel series of triazol-*N*-ethyl-tetrahydroisoquinoline based compounds were designed and synthesized via click chemistry. Most of the synthesized compounds showed P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) reversal activities. Among them, compound **7** with little cytotoxicity towards GES-1 cells (IC_{50} >80 μM) and K562/A02 cells (IC_{50} >80 μM) exhibited more potency than verapamil (VRP) on increasing anticancer drug accumulation in K562/A02 cells. Moreover, compound **7** 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 **7** in reversing MDR revealed that it could remarkably increase the intracellular accumulation of both rhodamine-123 (Rh123) and adriamycin (ADM) in K562/A02 cells as well as inhibit their efflux from the cells. These results suggested that compound **7** showed more potency than the classical P-gp inhibitor VRP under the same conditions, which may be a promising P-gp-mediated MDR modulator for further development.

1. Introduction

Multidrug resistance (MDR) is a serious obstruction for successful tumor chemotherapy in the clinic.[1] It occurs due to the overexpression of membrane-associated transport proteins named ATP-binding cassette (ABC) family including P-glycoprotein (P-gp, ABCB1), the breast cancer-resistance protein (BCRP, ABCG2) and the multidrug resistance-associated protein 1 (MRP1, ABCC1), which has been recognized contributing to the decreased intracellular concentrations of chemotherapeutic drugs.[2] Among them, the most intensive study is focused on P-gp, which has the ability to transport a wide variety of structurally unrelated compounds out of tumor cells resulting in MDR.[3,4] Obviously, developing P-gp inhibitors to avoid P-gp-mediated MDR should be regarded as a potential strategy.[5] During the past three decades, considerable efforts have been made to suppress MDR and three generations of P-gp inhibitors have been developed such as Verapamil, Valspodar (PSC-833), dexverapamil and Tariquidar (XR9576).[6,7] However, all of these P-gp inhibitors suffer from toxicity or low selectivity or drug-drug interactions. Up to now, no P-gp inhibitors have been approved for clinical application.[8] Therefore, the exploration of potent P-gp inhibitors with high selectivity and low toxicity for cancer treatment remains a major goal in this field.

For designing novel multidrug resistance reversal agents, an approach we adopted was to connect chemical fragments, such as *N*-ethyl-tetrahydroisoquinoline and aromatic amides, which are frequently appeared in reported potent p-gp inhibitors based on a Triazole Core by click chemistry method (Fig. 1).[9–11] 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.[11] Click chemistry, commonly copper-(I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes, has been widely applied in drug discovery.[12] Based on the principles above, we synthesized and biologically evaluated compounds **1**–**19** as P-gp-mediated MDR reversal agents.

2. Chemistry

The synthetic routes of target compounds **1**–**19** are outlined in Scheme 1. Compound **a** was prepared starting from 2-nitrophenol and 3-bromo-prop-1-yne, which refluxed in the mixture of acetone and potassium carbonate for 4 h. Treatment of compound **a** with ammonium chloride in the presence of iron powder in 80% ethanol afforded compound **b** which was then reacted with freshly formed aromatic acyl chloride and triethylamine in dry dichloromethane to give compounds **1c**–**19c**. Compounds **d**–**f** were synthesized according to literature procedures with minor modification.[13–15] Subsequently, compounds **1c**–**19c** and compound **f** were treated with ascorbate sodium and copper sulfate in 75% methanol stirring

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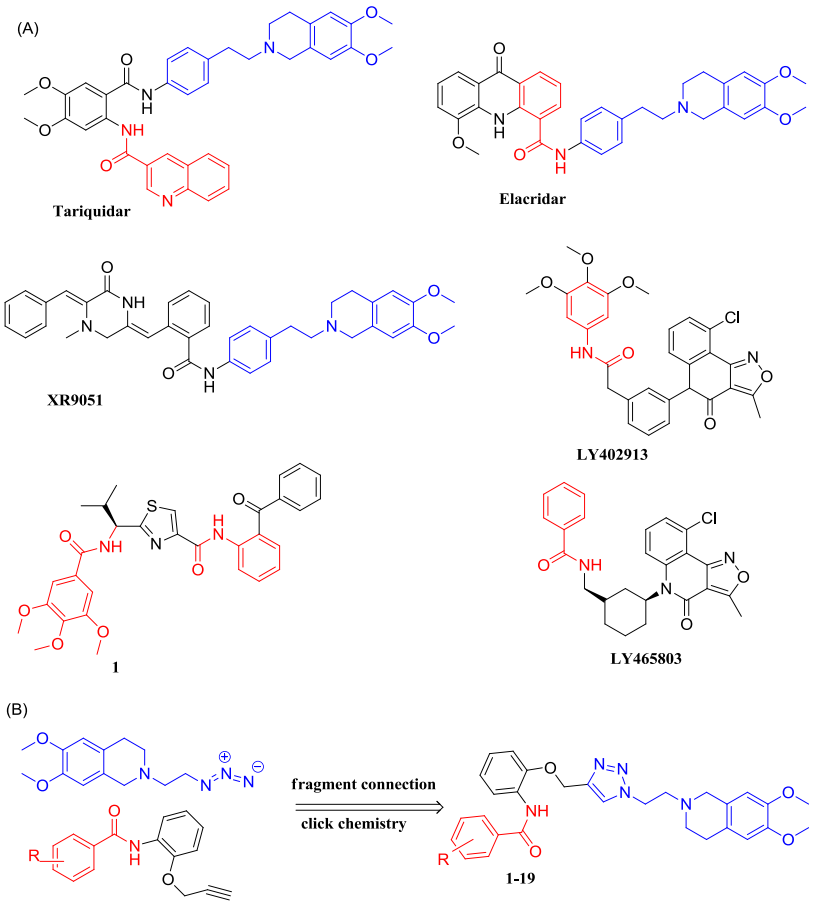
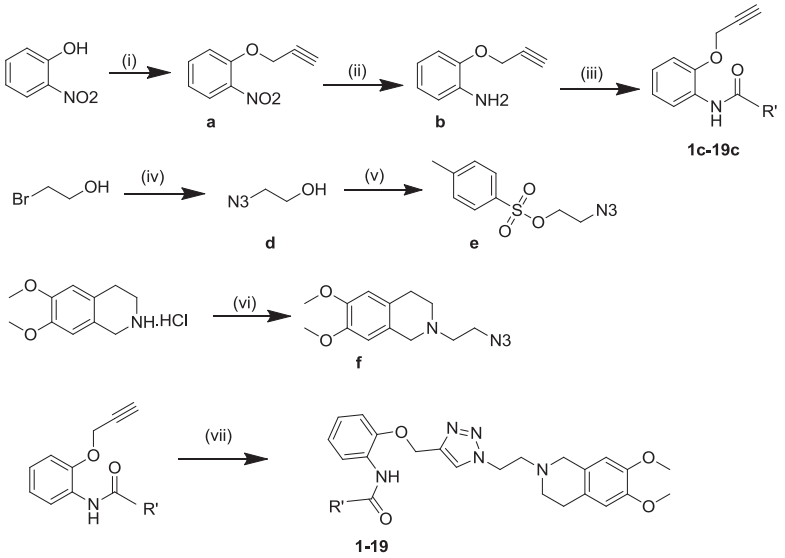


Figure 1. (A) Structures of the reported potent P-gp inhibitors containing chemical fragments such as *N*-ethyltetrahydroisoquinoline and aromatic amides. (B) Design of the target compounds **1**–**19**.



Scheme 1. Synthesis of the target compounds. Reagents and conditions: (i) 3-bromoprop-1-yne, K_2CO_3, acetone, reflux, 4 h; (ii) Fe/NH_4Cl, 80% EtOH, reflux, 5 h; (iii) aromatic acyl chlorides, TEA/DCM, rt, 24 h; (iv) NaN_3, water, 80 °C, 24 h; (v) TEA/DCM, TsCl, rt, 24 h; (vi) TEA/acetonitrile, 60 °C, 24 h; (vii) **1c**–**19c**, ascorbate sodium, $CuSO_4$, 75% CH_3OH, 24–48 h.

at room temperature for 24–48 h to provide compounds **1**–**19**. 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.

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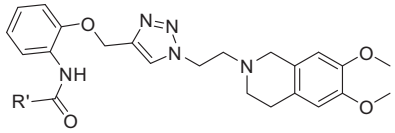
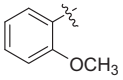
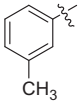
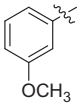
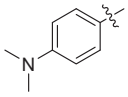
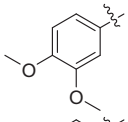
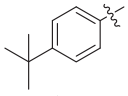
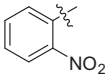
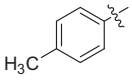
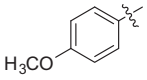
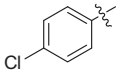
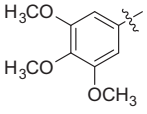
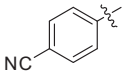
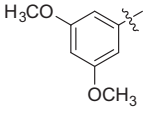
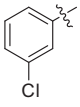
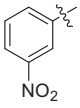
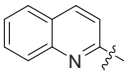
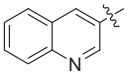
Table 1
Structures and ADM-resistance reversal activity of the target compounds **1**–**19** at 5 μM concentration in K562/A02 cells[a]



Compounds	R′	IC_{50} of ADM (μM)	RF
1		10.81 ± 1.45	5.0
2		6.75 ± 0.11	8.1
3		7.16 ± 0.18	7.6
4		9.71 ± 0.66	5.6
5		9.32 ± 0.67	5.8
6		6.45 ± 0.80	8.4
7		1.67 ± 0.48	32.5
8		15.63 ± 1.58	3.5
9		7.25 ± 0.31	7.5
10		6.25 ± 0.83	8.7
11		5.21 ± 0.29	10.4
12		3.23 ± 0.45	16.8
13		14.43 ± 0.03	3.6
14		13.85 ± 0.31	3.9
15		11.00 ± 1.06	4.9
16		15.07 ± 0.20	3.6
17		5.21 ± 0.52	10.4
18		8.89 ± 0.55	6.1
19		18.86 ± 1.65	2.9
Control[b]		54.22 ± 2.15	1
VRP		6.80 ± 0.45	8.7

[a] The IC_{50} value was determined after exposure to a series of ADM concentration with different target compounds at 5 μM using K562/A02 cells. Reversal fold (RF) refers to fold-change in drug sensitivity. RF = (IC_{50} without modulator)/($IC_{50}$with 5 μM 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. 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 human erythroleukemia adriamycin-selected 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.[16,17] The well-known classical P-gp inhibitor VRP was chose as a positive control. All compounds **1**–**19** and verapamil were assayed at 5 μM, a low concentration which could result in less than 10% cytotoxicity towards K562/A02 cells after 48 h incubation (data not shown). As the results summarized in Table 1, anticancer drug ADM alone displayed little inhibitory effect on the survival of K562/A02 cells (IC_{50} of 54.22 ± 2.15 μM). However, the combination treatment of ADM with target compounds or VRP increased the inhibitory effect in different degrees. It revealed that most of the target compounds exerted MDR reversal activities. Among them, compound **7** with a significantly decreased IC_{50} of ADM (1.67 ± 0.48 μM) showed the strongest reversal activity and its reversal fold (RF) was 32.5. 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.

3.1.2. Cytotoxicity assay

Based on the results above, the most potent compound **7** was selected for further study. The intrinsic cytotoxicity of the selected compound **7** against K562/A02 and normal human gastric epithelial cell strain-1 (GES-1) was examined by MTT assay. As shown in Figure 2, compound **7** exhibited little intrinsic cytotoxicity against either GES-1 (IC_{50} >80 μM) or K562/A02 cells (IC_{50} >80 μM). Particularly, concentrations of compound **7** ranging from 1.25 μM to 80 μM, showed more inhibitory effects on MDR K562/A02 cells than on normal GES-1 cells. It suggests that compound **7** may be more secure to normal cells comparing to tumor cells. Thus, compound **7**, which has little cytotoxicity to normal cells and P-gp overexpressing cell line, is a suitable candidate for the development of MDR reversal agents.

3.1.3. Chemo-sensitizing effect of target compounds

To further investigate the reversal potency and dose–response effects, we have determined the reversal activity of compound **7**

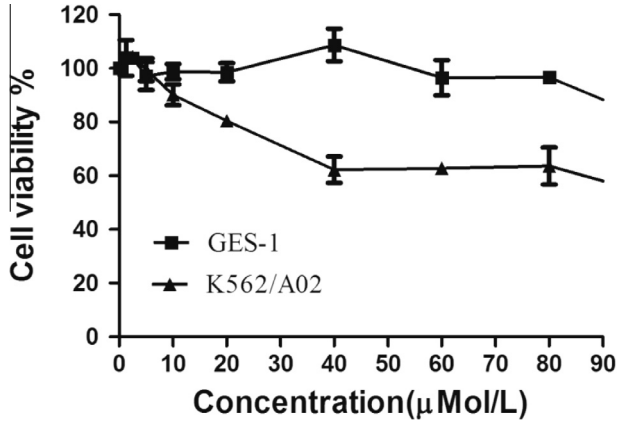


Figure 2. Effect of Compound **7** on the growth of K562/A02 cells and GES-1 cells. The cells were treated with various concentrations (0, 1.25, 2.5, 5, 10, 20, 40, 60, 80 μM) of compound **7** for 48 h. Viable cells were evaluated by MTT assay. Data are expressed as mean ± SD of three independent experiments.

Table 2
Sensitization of compound **7** on reversing MDR towards K562/A02 cells at different concentrations[a]

Compounds	IC_{50} of ADM (μM)	RF
None	54.93 ± 2.37	1
VRP, 2.5 μM	15.92 ± 1.17	3.4
Compound **7**, 2.5 μM	2.97 ± 0.16	18.5
Compound **7**, 1.25 μM	3.14 ± 0.30	17.4
Compound **7**, 0.625 μM	5.20 ± 0.35	10.5
Compound **7**, 0.31 μM	6.39 ± 0.41	8.6
Compound **7**, 0.156 μM	37.03 ± 3.4	1.5
Compound **7**, 0.078 μM	43.8 ± 0.16	1.3
Compound **7**, 0.04 μM	32.94 ± 1.04	1.6

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

at various other concentrations (2.5 μM, 1.25 μM, 0.625 μM, 0.31 μM, 0.156 μM, 0.078 μM, 0.04 μM) towards K562/A02 cells (P-gp-overexpression) by MTT assay, selecting VRP as positive controls.[18] As demonstrated in Table 2, VRP showed slight modulating activity at 2.5 μM, however, compound **7** showed apparent dose dependent activity and still exhibited potent MDR reversal activity (RF = 8.6) when the concentration decreased to 0.31 μmol/L. Additionally, the EC_{50} value of compound **7** was 147.7 ± 5.6 nM, which was calculated by GraphPad Prism 5.0 software from the dose–response curves. The results suggested that compound **7** had the significantly potential to enhance the sensitivity of P-gp-overexpressing cells to anticancer drug substrates in a dose-dependent manner.

3.1.4. Duration of chemo-sensitizing effect of compound 7 toward ADM in K562/A02 cells

It is known that a relatively long duration of action with reversibility for a P-gp inhibitor is likely to be required for safe and effective therapy of P-gp-mediated MDR cancers.[19] Therefore, the duration of MDR reversal effect of compound **7** was evaluated according to the procedure previously described with minor modification.[19] Briefly, K562/A02 cells were incubated with 5 μM of compound **7**, VRP or vehicle for 24 h, then washed with growth medium. Various concentrations of ADM was then added to the culture at different time points (0, 6 or 12 h) after the removal of the compounds, and incubated for additional 48 h. The duration of the MDR-reversal activity of compound **7** or VRP was examined by MTT assay. As demonstrated in Table 3, the IC_{50}s of ADM

Table 3
Duration of MDR reversal in K562/A02 cells after incubation and washout of verapamil or compound **7**

Treatment schedule	IC_{50}/ADM (μM) (RF)[a]		
	Control	VRP (5 μM)	Compound **7** (5 μM)
No wash	80.8 ± 4.25 (1)	9.60 ± 2.23 (8.4)	2.16 ± 0.16 (37.4)
Wash, 0 h	nd[b]	40.80 ± 3.71 (1.9)	10.97 ± 1.53 (7.7)
Wash, 6 h	nd	>80	35.61 ± 2.30 (2.4)
Wash, 12 h	nd	>80	54.53 ± 1.30 (1.5)

[a] Numbers in parentheses, reversal fold (RF), RF = (IC_{50}without modulator)/($IC_{50}$with modulator). Each experiment was carried out two to three times, and the values were presented as the mean ± standard error of mean.
[b] nd: not determined.

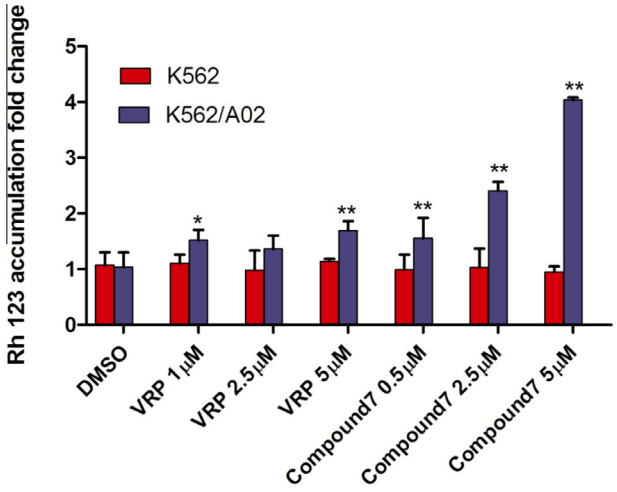


Figure 3. The effects of the target compounds on the intracellular accumulation of Rh123 (5 μM) in K562 and K562/A02 cells. Cells were pretreated with 0.5, 2.5, 5 μM of compound **7** or VRP for 60 min and then exposed to 5 μM of Rh123 for another 90 min. After treatment, the cells were washed twice with PBS and resuspended in medium. Rh123-associated mean fluorescence intensity was evaluated by flow cytometry. The Rh123 accumulation fold change values were identified by dividing the fluorescence intensity obtained from each measurement by that of control cells (0.1% DMSO) in each cell group, respectively. Data represent means ± SD of three independent experiments. $^{*}P$ <0.05, $^{**}P$ <0.01 versus untreated K562/A02 cells.

towards K562/A02 cells which pretreated with VRP and compound **7**, were 9.60 μM, 2.16 μM respectively (no wash group). The MDR-reversing effects of VRP (RF = 1.9) and compound **7** (RF = 7.7) decreased immediately after their removal from the medium. However, the reversal effect of VRP was disappeared after its removal from the medium for 6 h. In contrast, compound **7** showed reversal activity even after its removal from the medium for 12 h and the IC_{50} of ADM was 54.53 μM (RF = 1.5). These data indicated that compound **7** displayed potent MDR-reversing effect and persisted for longer time compared with the positive control VRP. The data also indicated that the chemo-sensitizing effect of compound **7** towards K562/A02 cells was reversible.

3.1.5. Effect of the target compounds on Rh123 accumulation

Furthermore, in order to investigate the mechanism of compound **7** in modulating the anticancer drug substrates accumulation level inside K562/A02 cells, the intracellular accumulation of rhodamine-123 (Rh123), a fluorescence substrate of P-gp, was assessed by monitoring its fluorescence intensity through flow cytometry assay. It is known that P-gp transporter, which overexpresses on the adriamycin-selected K562/A02 cells, can rapidly increase Rh123 efflux from cells and result in a decrease of Rh123 in the intracellular accumulation. As shown in Figure 3,

the classical P-gp inhibitor VRP was employed as a positive control. Once treated with compound **7** (5 μM), the P-gp-overexpressing K562/A02 cells exhibited significantly increased accumulation of Rh123. In comparison, both VRP and compound **7** showed no influence on the Rh123 accumulation fold change of ADM-sensitive K562 cells. When the K562/A02 cells were treated with compound **7** at various concentrations (0.5 μM, 2.5 μM, 5 μM), the retained amount of Rh123 was significantly increased in a dose-dependent manner (1.55, 2.40, 4.04). Moreover, the Rh123 accumulation fold change of compound **7** (5 μM) was 4.04, which was 2.4 times greater than that of VRP to K562/A02 cells at the same concentration. The results indicated that compound **7** exhibited dose-dependent effects on increasing accumulation of Rh123 towards K562/A02 cells. Additionally, compound **7** which was more potent than VRP in Rh123 accumulation might effectively block the drug efflux function of P-gp.

3.1.6. Effect of the target compound 7 on ADM accumulation

To validate our assumption, we evaluated the ADM accumulation, another fluorescence substrate of P-gp, towards K562/A02 cells by using fluorescence microscope. As shown in Figure 4, the sensitive K562 cells retained most of the red fluorescence ADM after 90-min incubation. On the contrary, there was little fluorescence of ADM being observed in P-gp overexpression K562/A02 cells (Fig. 4 Control). It occured due to the overexpression P-gp of K562/A02 cells could efficiently pump ADM out of the cells after a 90-min incubation. When K562/A02 cells were treated with compound **7** and VRP, the accumulation of ADM was significantly increased in a dose-dependent manner and the fluorescence intensity of K562/A02 cells treated with compound **7** was higher than that of VRP at the same concentrations.

3.1.7. Inhibitory effect of compound 7 on P-gp efflux function

In order to further verify the above presumption, the inhibitory effect of compound **7** on the efflux function of P-gp was assayed by detecting the retained intracellular Rh123 according to the method described in literatures with minor modification.[20,21] As demonstrated in Figure 5, the sensitive K562 cells retained most of the fluorescence Rh123, while the K562/A02 cells (P-gp overexpression) showed comparatively low intracellular level of Rh123. However, when treated with compound **7** or VRP, the Rh123 level in K562/A02 cells was obviously increased in a dose-dependent manner. Particularly, compound **7** was more potent than VRP at the same doses. Such as, the fluorescence intensity of Rh123 in

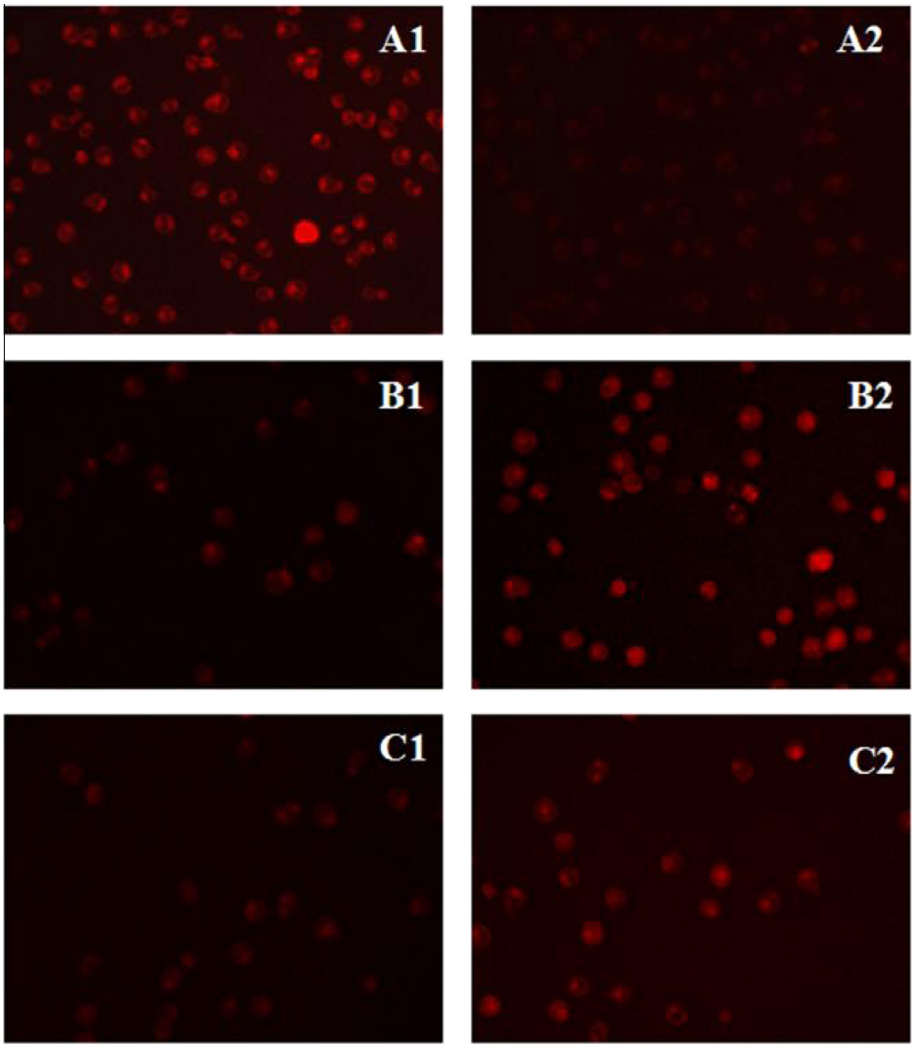


Figure 4. The effect of the target compounds on the intracellular accumulation of ADM (20 μM). K562 cells (A1) or K562/A02 cells (A2, B–C) were incubated without (control: A) or with compound **7** (B1: 0.5 μM, B2: 5 μM) or VRP (C1: 0.5 μM, C2: 5 μM) for 60 min, then treated with 20 μM ADM at 37 °C for another 90 min. After treatment, the cells were washed twice with PBS and centrifuged. The cells were then observed and photographed under a fluorescence microscope. VRP was used as a positive control and 0.1% DMSO was used as vehicle control.

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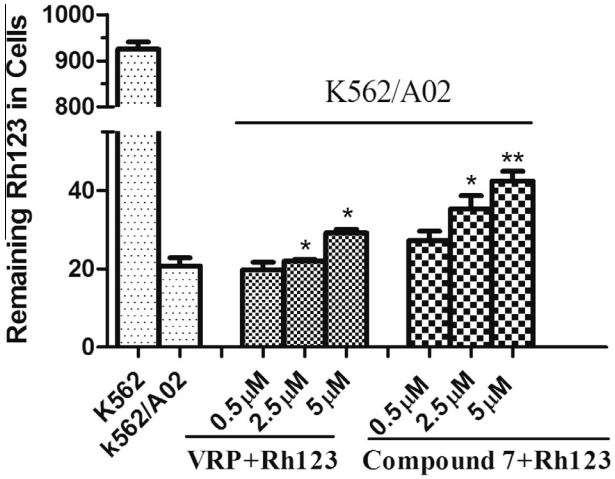

Figure 5. Inhibitory effect of compound **7** on the efflux of Rh123. K562 or K562/A02 cells were incubated with 5 μM Rh123 for 60 min before washing with ice PBS for three times. Then the cells were incubated with or without various concentrations of compound **7** or VRP (0.5, 2.5, 5 μM) for another 90 min. Afterwards the cells were washed twice in ice-cold PBS. The mean fluorescence intensity of retained intracellular Rh123 was estimated by flow cytometry. Data were expressed as means ± SD of two independent experiments. $^{*}P$ <0.05, $^{**}P$ <0.01 versus untreated K562/A02 cells.

K562/A02 cells treated with compound **7** was 42.5, which was significantly higher than that of VRP with 29.2 at the same dose of 5 μM. These results suggest that compound **7** could effectively block the drug efflux function of P-gp. What is more, the potency of compound **7** is much higher than the classical P-gp inhibitor VRP under the same conditions.

The results above verified our assumption that compound **7** was an effective P-gp inhibitor which had the potential to enhance the sensitivity of P-gp overexpressing drug-selected cell lines to anticancer drug substrates by effectively blocking the drug efflux function of P-gp and showed more potency than the classical P-gp inhibitor VRP under the same conditions.

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**–**19** 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 **2**, **6**, **7**, **10** and compound **12** which possessed electron-donating group like $-OCH_3$ and $-C(CH_3)_3$ showed higher MDR reversal activity than others. (b) The substitution position of electron-donating group in R′ has an influence on the reverse activity. Such as compounds **6**, **7**, **10** and compound **12**, which were substituted in para position, was more potent than in other positions. (c) The size of substituent in R′ is likely to affect MDR reversal activity. For example, compound **12** (RF = 16.8) containing 3,4,5-trimethoxy in R′ was more potent than compound 10 which was substituted by 4-methoxy. When 3,4,5-trimethoxy was replaced by 4-tertiary butyl, a bigger size substituent, the corresponding compound **7** showed the strongest activity (RF = 32.5). It suggested that the introduction of 4-tertiary butyl to the molecule of compound **7** could dramatically strengthen its binding affinity to P-gp, leading to more ADM accumulation in K562/A02 cells.

4. Conclusions

In summary, nineteen compounds containing triazol-*N*-ethyl-tetrahydroisoquinoline and aromatic amides were synthesized based on the click chemistry and evaluated in vitro as P-gp-mediated MDR reversal agents. Among them, compound **7** with low cytotoxicity could significantly reverse MDR in a dose-dependent manner and persist longer chemo-sensitizing effect than VRP with reversibility. Additionally, compound **7** could remarkably increase the intracellular accumulation of both Rh123 and ADM in K562/A02 cells as well as inhibit their efflux from the cells. These results suggest that compound **7** could effectively block the drug efflux function of P-gp and lead to increased drug accumulation in MDR cells. Therefore, compound **7** could be served as a promising candidate for the development of P-gp-mediated MDR-Reversal Agents.

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. Compounds **d** and **e** were prepared as previously described.[9,10] The starting compound 2-nitrophenol was commercially available.

5.2. Synthesis of 1-Nitro-2-(prop-2-yn-1-yloxy) benzene (a)

2-Nitrophenol (11.2 g, 80 mmol) and 3-bromo-prop-1-yne (6.4 ml, 80 mmol) was dissolved in acetone (120 mL) and added potassium carbonate (22.0 g, 160 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 compound **a** (14.1 g). Yield: 99.3%. Yellow powder, mp: 66–68 °C.

5.3. Synthesis of 2-(Prop-2-yn-1-yloxy) aniline (b)

The mixture of compound **a** (14.0 g, 80 mmol), ammonium chloride (21.4 g, 400 mmol) and iron powder (13.4 g, 240 mmol) in 80% ethanol (200 mL) was heated to reflux for 5 h. After cooling to room temperature, the mixture was adjusted with sodium carbonate to PH = 7–8, filtered with Celite, and evaporated in vacuo to afford a brown residue. Dichloromethane (50 mL) was added into the residue and the resulted solution was washed by saturated aqueous Na_2CO_3 (40 mL) and brine (40 mL) in sequence. The organic layer was dried by anhydrous Na_2SO_4. The solvent was evaporated to give corresponding compound **b** as brown oil (9.5 g). Yield: 82.3%.

5.4. General procedure for the preparation of 1c–19c

The freshly formed aromatic acyl chloride (5 mmol) dissolved in dry dichloromethane (25 ml) was added dropwise to a solution of compound **b** (5 mmol) and triethylamine (6 mmol) dissolved in dichloromethane (25 mL), keeping the temperature at 0 °C for 2 h, and then, the mixture was stirred at room temperature for 24 h. The reaction solution was washed by 1 N hydrochloric acid (3×30 mL), saturated aqueous Na_2CO_3 (3×30 mL), and brine (2×30 mL) in sequence. The organic layer was dried by anhydrous Na_2SO_4, then, filtered and evaporated to afford the desired product compound **1c**–**19c**.

5.5. Synthesis of 2-azidoethanol (d)

To a 100 mL round bottom flask was added 2-bromoethanol (3.4 g, 27 mmol) and sodium azide (3.1 g, 47.7 mmol) in water (40 mL). 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 **d** was obtained as a crude pale yellow liquid (2.74 g), which was used in the next step directly.

5.6. Synthesis of 2-azidoethyl 4-methylbenzenesulfonate (e)

To the solution of 2-azidoethanol (2.74 g, 24 mmol) and triethylamine (8.4 mL, 60 mmol) in dry dichloromethane (20 mL), 4-toluenesulfonylchloride (4.0 g, 21 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/pet ether (1:15, v/v) as eluent to afford the compound **e** (3.1 g). As colorless liquid, yield: 56.3%.

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

To a solution of 2-azidoethyl-4-methylbenzenesulfonate (3.1 g, 13 mmol) in dry acetonitrile (50 mL) and triethylamine (2.8 mL, 21 mmol), 7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (2.5 g, 11 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) to give compound **f** (2.0 g).As pale yellow solid, yield:70%.

5.8. General procedure for the preparation of compounds 1–19

To a 100 mL round bottom flask was added **1c**–**19c** (1 mmol) and **f** (1 mmol) in 75% methanol (30 mL), ascorbate sodium (23 mg) and $CuSO_4$ (7.5 mg) were added, respectively. The reaction solution was stirred at room temperature for 24–48 h. Some of the reaction solutions precipitated white solid, 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 **1**–**19**.

5.8.1. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)benzamide (1)

Yield 58.8%; white powder; mp: 114–116 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.40 (s, 1H, CONH), 8.18 (s, 1H, NCH═C), 7.92 (d, *J* = 7.1 Hz, 2H, ArH), 7.82 (d, *J* = 7.4 Hz, 1H, ArH), 7.53 (m, 3H, ArH), 7.29 (d, *J* = 7.8 Hz, 1H, ArH), 7.14 (dd, *J* = 7.4 Hz, 7.4 Hz, 1H, ArH), 6.99 (dd, *J* = 7.4 Hz, 7.4 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.56 (s, 1H, ArH), 5.24 (s, 2H, OCH_2), 4.55 (t, *J* = 6.0 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, –OCH_3), 3.67 (s, 3H, –OCH_3), 3.50 (s, 2H, $ArCH_2N$), 2.85 (t, *J* = 6.1 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2). ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.6, 109.8, 111.8, 113.9, 121.0, 123.8, 124.5, 125.4, 125.7, 126.2, 127.3, 127.6, 128.5, 131.6, 134.5, 142.6, 146.9, 147.1, 150.0, 164.8; ESI-MS *m*/*z*: 514.4 $[M+H]^+$, 536.3 $[M+Na]^+$; Anal. Calcd for $C_{29}H_{31}N_5O_4$: C, 67.82; H, 6.08; N, 13.64. Found: C, 67.69; H, 6.04; N, 13.70.

5.8.2. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-2-methoxybenzamide (2)

Yield 31.5%; white powder; mp: 180–182 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.51 (s, 1H, CONH), 8.64 (dd, *J* = 6.8 Hz, 4.0 Hz, 1H, ArH), 8.25 (dd, *J* = 7.8 Hz, 1.7 Hz, 1H, ArH), 7.85 (s, 1H, NCH═C), 7.42 (ddd, *J* = 7.6 Hz, 1.6 Hz, 1.6 Hz, 1H, ArH), 7.05 (m, 4H, ArH), 6.80 (d, *J* = 8.2 Hz, 1H, ArH), 6.56 (s, 1H, ArH), 6.45 (s, 1H, ArH), 5.30 (s, 2H, OCH_2), 4.67 (s, 2H, $CHNCH_2$), 3.83 (s, 3H, –OCH_3), 3.80 (s, 3H, –OCH_3), 3.66 (m, 5H, $ArCH_2N$, –OCH_3), 3.12 (m, 2H, NCH_2), 2.80 (m, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 46.9, 49.9, 55.1, 55.5, 56.5, 61.6, 109.9, 111.7, 112.1, 119.3, 120.9, 121.0, 123.5, 125.6, 125.8, 126.3, 127.9, 131.3, 133.4, 141.7, 146.9, 147.0, 147.3, 157.0, 162.0; ESI-MS *m*/*z*: 544.3 $[M+H]^+$; Anal. Calcd for $C_{30}H_{33}N_5O_5$: C, 66.28; H, 6.12; N, 12.88. Found: C, 66.33; H, 6.15; N, 12.70.

5.8.3. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-3-methylbenzamide (3)

Yield 19.2%; pale yellow powder; mp: 106–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.61 (s, 1H, CONH), 8.47 (dd, *J* = 5.0 Hz, 4.5 Hz, 1H, ArH), 7.84 (s, 1H, NCH═C), 7.72 (s, 1H, ArH), 7.65 (d, *J* = 6.1 Hz, 1H, ArH), 7.35 (m, 2H, ArH), 7.06 (m, 3H, ArH), 6.56 (s, 1H, ArH), 6.45 (s, 1H, ArH), 5.30 (s, 2H, OCH_2), 4.70 (s, 2H, $CHNCH_2$), 3.86 (s, 3H, –OCH_3), 3.83 (s, 3H, –OCH_3), 3.66 (s, 2H, $ArCH_2N$), 3.15 (s, 2H, NCH_2), 2.81 (s, 4H, 2 × CH_2), 2.42 (s, 3H, –CH3); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 20.9, 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.8, 109.9, 111.8, 114.0, 121.1, 123.4, 124.4, 124.5, 125.2, 125.7, 126.2, 127.8, 127.8, 128.4, 132.2, 134.5, 137.9, 142.6, 146.9, 147.2, 149.8, 164.8; ESI-MS *m*/*z*: 528.3 $[M+H]^+$, 550.6 $[M+Na]^+$; Anal. Calcd for $C_{30}H_{33}N_5O_4$: C, 68.29; H, 6.30; N, 13.27. Found: C, 68.18; H, 6.27; N, 13.31.

5.8.4. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-3-methoxybenzamide (4)

Yield 18.5%; white powder; mp: 115–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.62 (s, 1H, CONH), 8.47 (dd, *J* = 5.1 Hz, 4.3 Hz, 1H, ArH), 7.78 (s, 1H, NCH═C), 7.38 (m, 3H, ArH), 7.06 (m, 4H, ArH), 6.56 (s, 1H, ArH), 6.46 (s, 1H, ArH), 5.30 (s, 2H, OCH_2), 4.58 (t, *J* = 4.2 Hz, 2H, $CHNCH_2$), 3.86 (s, 3H, –OCH_3), 3.84 (s, 3H, –OCH_3), 3.81 (s, 3H, –OCH_3), 3.64 (s, 2H, $ArCH_2N$), 3.01 (s, 2H, NCH_2), 2.74 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 27.9, 47.1, 50.0, 54.8, 55.3, 55.4, 56.6, 62.6, 109.9, 111.8, 112.4, 113.8, 117.5, 119.4, 121.0, 123.9, 124.5, 125.5, 125.7, 126.2, 127.5, 129.7, 135.9, 142.6, 146.9, 147.2, 150.0, 159.3, 164.5; ESI-MS *m*/*z*: 544.4 $[M+H]^+$, 566.1 $[M+Na]^+$; Anal. Calcd for $C_{30}H_{33}N_5O_5$: C, 66.28; H, 6.12; N, 12.88. Found: C, 66.25; H, 6.14; N, 12.81.

5.8.5. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-4-(dimethylamino)benzamide (5)

Yield 71.9%; white powder; mp: 143–144 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.20 (s, 1H, CONH), 8.18 (s, 1H, NCH═C), 7.89 (d, *J* = 7.0 Hz, 1H, ArH), 7.28 (dd, *J* = 7.9 Hz, 7.9 Hz, 2H, ArH), 7.13 (m, 3H, ArH), 7.00(dd, *J* = 7.5 Hz, 7.5 Hz, 1H, ArH), 6.90 (d, *J* = 7.4 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.23 (s, 2H, OCH_2), 4.55 (t, *J* = 5.8 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, –OCH_3), 3.67(s, 3H, –OCH_3), 3.50 (s, 2H, $ArCH_2N$), 2.92(s, 6H, 2×CH_3), 2.85 (t, *J* = 5.9 Hz, 2H, NCH_2), 2.61 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.5, 109.9,

110.6, 111.8, 113.6, 114.6, 115.3, 121.0, 123.0, 124.6, 125.0, 125.7, 126.2, 127.8, 129.1, 135.2, 142.5, 146.9, 147.2, 149.4, 150.3, 165.3; ESI-MS *m*/*z*: 557.4 $[M+H]^+$, 579.2 $[M+Na]^+$; Anal. Calcd For $C_{31}H_{36}N_6O_4$: C, 66.89; H, 6.52; N, 15.10. Found: C, 66.79; H, 6.55; N, 15.15.

5.8.6. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-3,4-dimethoxybenzamide (6)

Yield 34.9%; white powder; mp: 146–148 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.26 (s, 1H, CONH), 8.16 (s, 1H, NCH═C), 7.81 (d, *J* = 7.2 Hz, 1H, ArH), 7.54 (dd, *J* = 8.3 Hz, 7.9 Hz, 1H, ArH), 7.48 (s, 1H, ArH), 7.28 (d, *J* = 7.8 Hz, 1H, ArH), 7.13 (dd, *J* = 6.7 Hz, 6.7 Hz, 1H, ArH), 7.05 (d, *J* = 8.4 Hz, 1H, ArH), 6.98 (dd, *J* = 7.3 Hz, 7.3 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.23 (s, 2H, OCH_2), 4.54 (t, *J* = 5.9 Hz, 2H, $CHNCH_2$), 3.82 (s, 3H, $-OCH_3$), 3.81 (s, 3H, $-OCH_3$), 3.68 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$),3.49 (s, 2H, $ArCH_2N$), 2.85 (t, *J* = 5.8 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 27.9, 47.1, 50.0, 54.8, 55.4, 55.5, 55.6, 56.6, 62.6, 109.8, 110.5, 110.5, 111.0, 111.7, 113.9, 120.6, 121.1, 123.6, 124.5, 125.1, 125.7, 126.2, 126.7, 127.9, 142.6, 146.9, 147.1, 148.4, 149.8, 151.6, 164.2; ESI-MS *m*/*z*: 574.4 $[M+H]^+$, 596.6 $[M+Na]^+$; Anal. Calcd for $C_{31}H_{35}N_5O_6$: C, 64.91; H, 6.15; N, 12.21. Found: C, 64.80; H, 6.17; N, 12.25.

5.8.7. 4-(*tert*-Butyl)-*N*-(2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2, 3-triazol-4-yl)methoxy)phenyl)benzamide (7)

Yield 52.7%; white powder; mp: 126–128 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.30 (s, 1H, CONH), 8.18 (s, 1H, NCH═C), 7.86 (d, *J* = 8.1 Hz, 3H, ArH), 7.52 (d, *J* = 8.2 Hz, 2H, ArH), 7.28 (d, *J* = 8.0 Hz, 1H, ArH), 7.13 (dd, *J* = 7.0 Hz, 7.0 Hz, 1H, ArH), 6.98 (dd, *J* = 7.6 Hz, 7.6 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.54 (s, 1H, ArH), 5.24 (s, 2H, OCH_2), 4.55 (t, *J* = 5.3 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, $-OCH_3$), 3.66 (s, 3H, $-OCH_3$), 3.49 (s, 2H, $ArCH_2N$), 2.85 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2), 1.31 (s, 9H, 3×CH_3); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 30.9, 34.6, 47.1, 50.0, 54.8, 55.4, 56.6, 62.6, 109.8, 111.7, 113.8, 121.0, 123.4, 124.5, 125.2, 125.3, 125.7, 126.1, 127.2, 127.7, 131.7, 142.6, 146.9, 147.1, 147.1, 149.7, 154.5, 164.6; ESI-MS *m*/*z*: 570.2 $[M+H]^+$, 592.5 $[M+Na]^+$; Anal. Calcd for $C_{33}H_{39}N_5O_4$: C, 69.57; H, 6.90; N, 12.29. Found: C, 69.62; H, 6.88; N, 12.33.

5.8.8. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-2-nitrobenzamide (8)

Yield 71.7%; yellow powder; mp: 149–152 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.91 (s, 1H, CONH), 8.15 (s, 1H, NCH═C), 8.12 (d, *J* = 8.0 Hz, 1H, ArH), 7.75 (m, 4H, ArH), 7.25 (d, *J* = 8.1 Hz, 1H, ArH), 7.13 (dd, *J* = 7.2 Hz, 7.2 Hz, 1H, ArH), 6.98 (dd, *J* = 7.6 Hz, 7.6 Hz, 1H, ArH), 6.62 (s, 1H, ArH), 6.56 (s, 1H, ArH), 5.21 (s, 2H, OCH_2), 4.55 (t, *J* = 5.7 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, $-OCH_3$), 3.67 (s, 3H, $-OCH_3$), 3.51 (s, 2H, $ArCH_2N$), 2.87 (t, *J* = 5.8 Hz, 2H, NCH_2), 2.63 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.3, 109.9, 111.8, 113.6, 120.8, 123.9, 124.1, 124.7, 125.6, 125.7, 126.2, 127.1, 129.2, 130.7, 132.8, 133.9, 142.5, 146.5, 146.9, 147.2, 149.8, 164.4; ESI-MS *m*/*z*: 559.3 $[M+H]^+$, 581.1 $[M+Na]^+$; Anal. Calcd for $C_{29}H_{30}N_6O_6$: C, 62.36; H, 5.41; N, 15.05. Found: C, 62.42; H, 5.44; N, 15.01.

5.8.9. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-4-methylbenzamide (9)

Yield 38.0%; pale yellow powder; mp: 122–124 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.31 (s, 1H, CONH), 8.18 (s, 1H, NCH═C), 7.83 (d, *J* = 8.0 Hz, 3H, ArH), 7.30 (dd, *J* = 8.0 Hz, 8.0 Hz, 3H, ArH), 7.13 (dd, *J* = 6.9 Hz, 6.9 Hz, 1H, ArH), 6.98 (dd, *J* = 7.4 Hz, 7.4 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.24 (s, 2H, OCH_2), 4.55 (t, *J* = 5.9 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, $-OCH_3$), 3.67 (s, 3H, $-OCH_3$), 3.50 (s, 2H, $ArCH_2N$), 2.85 (t, *J* = 5.9 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2), 2.37 (s, 3H, $-CH_3$); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 20.9, 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.7, 109.8, 111.7, 113.9, 121.0, 123.5, 124.5, 125.2, 125.7, 126.2, 127.3, 127.8, 129.0, 131.7, 141.6, 142.6, 146.9, 147.2, 149.8, 164.6; ESI-MS *m*/*z*: 528.4 $[M+H]^+$, 550.3 $[M+Na]^+$; Anal. Calcd for $C_{30}H_{33}N_5O_4$: C, 68.29; H, 6.30; N, 13.27. Found: C, 68.33; H, 6.28; N, 13.25.

5.8.10. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-4-methoxybenzamide (10)

Yield 74.1%; white powder; mp: 166–168 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.24 (s, 1H, CONH), 8.17 (s, 1H, NCH═C), 7.92 (d, *J* = 8.7 Hz, 2H, ArH), 7.83 (d, *J* = 7.1 Hz, 1H, ArH), 7.28 (d, *J* = 8.0 Hz, 1H, ArH), 7.12 (dd, *J* = 7.1 Hz, 7.1 Hz, 1H, ArH), 7.00 (m, 3H, ArH), 6.61 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.25 (s, 2H, OCH_2), 4.55 (t, *J* = 5.8 Hz, 2H, $CHNCH_2$), 3.83 (s, 3H, $-OCH_3$), 3.68 (s, 6H, 2 × $-OCH_3$), 3.50 (s, 2H, $ArCH_2N$), 2.85 (s, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.3, 55.4, 56.6, 62.7, 109.8, 111.7, 113.7, 113.8, 121.0, 123.6, 124.5, 125.1, 125.7, 126.2, 126.6, 127.9, 129.2, 146.9, 147.1, 149.8, 161.9, 164.2; ESI-MS *m*/*z*: 544.3 $[M+H]^+$, 566.2 $[M+Na]^+$; Anal. Calcd For $C_{30}H_{33}N_5O_5$: C, 66.28; H, 6.12; N, 12.88. Found: C, 66.30; H, 6.11; N, 12.86.

5.8.11. 4-Chloro-*N*-(2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)benzamide (11)

Yield 40.0%; yellow powder; mp: 150–153 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.54 (s, 1H, CONH), 8.16 (s, 1H, NCH═C), 7.95 (d, *J* = 8.4 Hz, 2H, ArH), 7.74 (d, *J* = 7.5 Hz, 1H, ArH), 7.58 (d, *J* = 8.4 Hz, 2H, ArH), 7.28 (d, *J* = 8.1 Hz, 1H, ArH), 7.16 (dd, *J* = 7.1 Hz, 7.1 Hz, 1H, ArH), 7.00 (dd, *J* = 7.5 Hz, 7.5 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.23 (s, 2H, OCH_2), 4.55 (t, *J* = 5.7 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 × $-OCH_3$), 3.50 (s, 2H, $ArCH_2N$), 2.85 (t, *J* = 5.9 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.7, 109.9, 111.8, 114.0, 121.0, 124.5, 125.7, 126.2, 127.4, 128.5, 129.4, 133.3, 136.4, 142.7, 146.9, 147.2, 150.4, 164.0; ESI-MS *m*/*z*: 548.3 $[M+H]^+$, 570.2 $[M+Na]^+$; Anal. Calcd for $C_{29}H_{30}ClN_5O_4$: C, 63.56; H, 5.52; Cl, 6.47; N, 12.78. Found: C, 63.49; H, 5.53; Cl, 6.49; N, 12.77.

5.8.12. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)-3,4,5-trimethoxybenzamide (12)

Yield 66.3%; white powder; mp: 124–126 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.36 (s, 1H, CONH), 8.16 (s, 1H, NCH═C), 7.78 (d, *J* = 7.4 Hz, 1H, ArH), 7.30 (d, *J* = 8.0 Hz, 1H, ArH), 7.23 (s, 2H, ArH), 7.16 (dd, *J* = 7.6 Hz, 7.6 Hz, 1H, ArH), 7.00 (dd, *J* = 7.7 Hz, 7.7 Hz, 1H, ArH), 6.60 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.22 (s, 2H, OCH_2), 4.54 (t, *J* = 5.7 Hz, 2H, $CHNCH_2$), 3.83 (s, 6H, 2 × $-OCH_3$), 3.68 (m, 9H, 3 × $-OCH_3$), 3.49 (s, 2H, $ArCH_2N$), 2.85 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.61 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 27.9, 47.1, 50.0, 54.8, 55.4, 55.9, 56.6, 60.1, 62.6, 104.8, 109.8, 111.7, 113.8, 121.1, 123.9, 124.6, 125.4, 125.7, 126.2, 127.6, 129.7, 129.7, 142.5, 146.9, 147.2, 150.1, 152.7, 164.2; ESI-MS *m*/*z*: 604.3 $[M+H]^+$, 626.1 $[M+Na]^+$; Anal. Calcd For $C_{32}H_{37}N_5O_7$: C, 63.67; H, 6.18; N, 11.60. Found: C, 63.71; H, 6.19; N, 11.65.

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5.8.13. 4-Cyano-*N*-(2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)benzamide (13)

Yield 63.2%; white powder; mp: 154–156 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.75 (s, 1H, CONH), 8.15 (s, 1H, NCH=C), 8.07 (d, J = 8.2 Hz, 2H, ArH), 7.99 (d, J = 8.2 Hz, 2H, ArH), 7.71 (d, J = 7.5 Hz, 1H, ArH), 7.30 (d, J = 8.0 Hz, 1H, ArH), 7.18 (dd, J = 6.9 Hz, 6.9 Hz, 1H, ArH), 7.00 (dd, J = 7.4 Hz, 7.4 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.56 (s, 1H, ArH), 5.23 (s, 2H, OCH_2), 4.55 (t, J = 5.9 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, $-OCH_3$), 3.67 (s, 3H, $-OCH_3$), 3.50 (s, 2H, $ArCH_2N$), 2.86 (t, J = 5.9 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.6, 109.8, 111.7, 113.8, 114.0, 118.3, 121.0, 124.5, 124.9, 125.7, 126.2, 127.0, 128.3, 132.5, 138.5, 142.6, 146.9, 147.2, 150.7, 163.7; ESI-MS m/z: 539.4 $[M+H]^+$, 561.1 $[M+Na]^+$; Anal. Calcd for $C_{30}H_{30}N_6O_4$: C, 66.90; H, 5.61; N, 15.60. Found: C, 66.83; H, 5.63; N, 15.67.

5.8.14. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-3,5-dimethoxybenzamide (14)

Yield 69.8%; white powder; mp: 122–124 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.35 (s, 1H, CONH), 8.16 (s, 1H, NCH=C), 7.78 (d, J = 7.3 Hz, 1H, ArH), 7.29 (d, J = 8.0 Hz, 1H, ArH), 7.15 (dd, J = 7.1 Hz, 7.1 Hz, 1H, ArH), 7.06 (s, 2H, ArH), 6.99 (dd, J = 7.5 Hz, 7.5 Hz, 1H, ArH), 6.70 (s, 1H, ArH), 6.60 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.22 (s, 2H, OCH_2), 4.54 (t, J = 5.7 Hz, 2H, $CHNCH_2$), 3.80 (s, 6H, 2 × $-OCH_3$), 3.68 (s, 3H, $-OCH_3$), 3.67(s, 3H, $-OCH_3$), 3.49 (s, 2H, $ArCH_2N$), 2.85 (t, J = 5.8 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.5, 103.6, 105.2, 109.8, 111.7, 113.7, 121.0, 124.0, 124.6, 125.5, 125.7, 126.2, 127.4, 136.6, 142.6, 146.9, 147.1, 150.1,160.4, 164.4; ESI-MS m/z: 574.4 $[M+H]^+$, 596.7 $[M+Na]^+$; Anal. Calcd for $C_{31}H_{35}N_5O_6$: C, 64.91; H, 6.15; N, 12.21. Found: C, 64.83; H, 6.11; N, 12.24.

5.8.15. 3-Chloro-*N*-(2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)benzamide (15)

Yield 73.0%; pale yellow powder; mp: 150–152 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.54 (s, 1H, CONH), 8.15 (s, 1H, NCH=C), 7.94 (d, J = 8.4 Hz, 2H, ArH), 7.74 (d, J = 7.5 Hz, 1H, ArH), 7.57 (d, J = 8.4 Hz, 2H, ArH), 7.29 (d, J = 8.0 Hz, 1H, ArH), 7.15 (dd, J = 7.6 Hz, 7.6 Hz, 1H, ArH), 7.00 (dd, J = 7.6 Hz, 7.6 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.56 (s, 1H, ArH), 5.23 (s, 2H, OCH_2), 4.55 (t, J = 5.9 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, $-OCH_3$), 3.67 (s, 3H, $-OCH_3$), 3.50 (s, 2H, $ArCH_2N$), 2.85 (t, J = 5.7 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.6, 109.8, 111.7, 114.0, 121.0, 124.5, 125.7, 125.8, 126.2, 127.4, 128.5, 129.4, 133.2, 136.4, 142.6, 146.9, 147.2, 150.4, 163.9; ESI-MS m/z: 548.4 $[M+H]^+$, 570.4 $[M+Na]^+$; Anal. Calcd for $C_{29}H_{30}ClN_5O_4$: C, 63.56; H, 5.52; Cl, 6.47; N, 12.78. Found: C, 63.62; H, 5.51; Cl, 6.50; N, 12.74.

5.8.16. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-3-nitrobenzamide (16)

Yield 53.8%; yellow powder; mp: 150–152 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.94 (s, 1H, CONH), 8.73 (s, 1H, NCH=C), 8.40 (m, 2H, ArH), 8.18 (s, 1H, ArH), 7.80 (dd, J = 8.0 Hz, 8.0 Hz, 1H, ArH), 7.69 (d, J = 7.4 Hz, 1H, ArH), 7.31 (d, J = 7.9 Hz, 1H, ArH), 7.20 (dd, J = 8.0 Hz, 8.0 Hz, 1H, ArH), 7.01 (dd, J = 7.3 Hz, 7.3 Hz, 1H, ArH), 6.60 (s, 1H, ArH), 6.54 (s, 1H, ArH), 5.23 (s, 2H, OCH_2), 4.55 (t, J = 5.9 Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, $-OCH_3$), 3.67 (s, 3H, $-OCH_3$), 3.49 (s, 2H, $ArCH_2N$), 2.85 (t, J = 5.6 Hz, 2H, NCH_2), 2.61 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.6, 109.8, 111.7, 113.9, 120.9, 122.4, 124.5, 125.2, 125.7, 126.0, 126.1, 126.3, 126.9, 130.2, 133.9, 136.0, 142.6, 146.9, 147.1, 147.7, 150.9, 163.1; ESI-MS m/z: 559.3 $[M+H]^+$, 581.3 $[M+Na]^+$; Anal. Calcd For $C_{29}H_{30}N_6O_6$: C, 62.36; H, 5.41; N, 15.05. Found: C, 66.27; H, 5.40; N, 15.02.

5.8.17. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)quinoline-2-carboxamide (17)

Yield 35.5%; white powder; mp: 179–184 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 10.84 (s, 1H, CONH), 8.64 (d, J = 8.4 Hz, 1H, ArH), 8.47 (d, J = 7.5 Hz, 1H, ArH), 8.36 (s, 1H, NCH=C), 8.26 (d, J = 8.4 Hz, 1H, ArH), 8.12 (d, J = 8.4 Hz, 1H, ArH), 7.90 (s, 2H, ArH), 7.75 (m, 1H, ArH), 7.35 (d, J = 7.9 Hz, 1H, ArH), 7.09 (m, 2H, ArH), 6.60 (s, 1H, ArH), 6.54 (s, 1H, ArH), 5.36 (s, 2H, OCH_2), 4.61 (t, J = 5.5 Hz, 2H, $CHNCH_2$), 3.66 (s, 3H, $-OCH_3$), 3.64 (s, 3H, $-OCH_3$), 3.47 (s, 2H, $ArCH_2N$), 2.90 (t, J = 5.5 Hz, 2H, NCH_2), 2.50 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 27.9, 47.2, 50.0, 54.8, 55.4, 56.7, 62.4, 109.7, 111.6, 112.7, 118.1, 118.6, 121.3, 124.1, 124.9, 125.6, 126.1, 127.3, 128.1, 128.5, 129.0, 130.8, 138.6, 142.2, 147.3, 149.2, 161.1; ESI-MS m/z: 565.3 $[M+H]^+$, 587.1 $[M+Na]^+$; Anal. Calcd for $C_{32}H_{32}N_6O_4$: C, 68.07; H, 5.71; N, 14.88. Found: C, 67.88; H, 5.73; N, 14.91.

5.8.18. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)quinoline-3-carboxamide (18)

Yield 30.1%; white powder; mp: 148–150 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.39 (s, 1H, CCHN), 9.01 (s, 1H, CONH), 8.25 (s, 1H, ArH), 8.25 (s, 1H, NCH=C), 8.20 (m, 3H, ArH), 7.96 (ddd, J = 7.0 Hz, 7.0 Hz, 1.3 Hz, 1H, ArH), 7.85 (m, 2H, ArH), 7.38 (d, J = 7.3 Hz, 1H, ArH), 7.25 (ddd, J = 7.6 Hz, 7.6 Hz, 1.3 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.32 (s, 2H, OCH_2), 4.59 (t, J = 6.1 Hz, 2H, $CHNCH_2$), 3.73 (s, 3H, $-OCH_3$), 3.70 (s, 3H, $-OCH_3$), 3.50 (s, 2H, $ArCH_2N$), 2.88 (t, J = 6.1 Hz, 2H, NCH_2), 2.58 (m, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 27.9, 47.1, 49.9, 54.8, 55.4, 56.6, 62.8, 72.0,109.76, 111.7, 114.1, 121.1, 124.5, 124.6, 125.6, 126.0, 126.1, 126.5, 127.3, 127.5, 128.7, 129.2, 131.4, 135.9, 142.7, 146.8, 147.1, 148.5, 148.9, 150.6, 163.8; ESI-MS m/z: 565.4 $[M+H]^+$, 587.3 $[M+Na]^+$; Anal. Calcd for $C_{32}H_{32}N_6O_4$: C, 68.07; H, 5.71; N, 14.88. Found: C, 68.21; H, 5.73; N, 14.83.

5.8.19. *N*-(2-((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)nicotinamide (19)

Yield 58.3%; white powder; mp: 187–190 °C; ^{1}H NMR(300 MHz, DMSO-d_6): δ = 9.76 (s, 1H, CONH), 8.34 (d, J = 7.7 Hz, 1H, ArH), 8.22 (s, 1H, NCH=C), 7.79 (d, J = 6.9 Hz, 1H, ArH), 7.64 (m, 3H, ArH), 7.35 (d, J = 7.7 Hz, 1H, ArH), 7.23 (ddd, J = 8.0 Hz, 8.0 Hz, 1.3 Hz, 1H, ArH)), 7.05 (dd, J = 7.3 Hz, 7.3 Hz, 1H, ArH), 6.67 (s, 1H, ArH), 6.61 (s, 1H, ArH), 5.29 (s, 2H, OCH_2), 4.59 (t, J = 5.8 Hz, 2H, $CHNCH_2$), 3.74 (s, 3H, $-OCH_3$), 3.73 (s, 3H, $-OCH_3$), 3.55 (s, 2H, $ArCH_2N$), 2.91 (s, 2H, NCH_2), 2.67 (s, 4H, 2 × CH_2); ^{13}C NMR (DMSO, 75 MHz, δ ppm): 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.6, 109.8, 111.7, 113.9, 120.9, 124.6, 124.8, 125.7, 126.0, 126.2, 127.1, 134.9, 142.6, 146.9, 147.1, 150.6, 164.0; ESI-MS m/z: 515.4 $[M+H]^+$, 537.4 $[M+Na]^+$; Anal. Calcd for $C_{28}H_{30}N_6O_4$: C, 65.35; H, 5.88; N, 16.33. Found: C, 65.47; H, 5.83; N, 16.30.

5.9. Biological assays

5.9.1. 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 (1∼2 × 10^4 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.

5.9.2. Duration of the MDR reversal

$1\sim2 \times 10^4$ K562/A02 cells per well were plated in 96-well plates and cultured overnight, then the cells were incubated for another 24 h with or without compound **7**, VRP or PBS at the concentration of 5 μM before being washed 0 or 3 times with growth medium. Then, the cells were incubated for 0, 6 or 12 h before the addition of various concentrations of ADM or vehicle. The incubation was continued for 48 h prior to the MTT analysis. The IC_{50} values of the compounds for cytotoxicity were calculated by GraphPad Prism 5.0 software from the dose–response curves.

5.9.3. Flow cytometry

5.9.3.1. Accumulation of Rh123 and ADM. K562 and K562/A02 cells were seeded into 24-well plates at 1.5×10^5/well. Different concentrations of compound **7** and VRP were pre-incubated with cells for 60 min. Then Rh123 or ADM were 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 Rh123 was estimated by BD FACSCalibur flow cytometer. The ADM accumulation level was showed in photographs taking by using fluorescence microscope.

5.9.4. Rhodamine123 efflux assay

K562 or K562/A02 cells were seeded into 24-well plates at 1.5×10^5/well and incubated with 5 μM Rh123 for 60 min before washing with ice PBS for three times. Then the cells were incubated with or without various concentrations of compound **7** or VRP (0.5, 2.5, 5 μM) 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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