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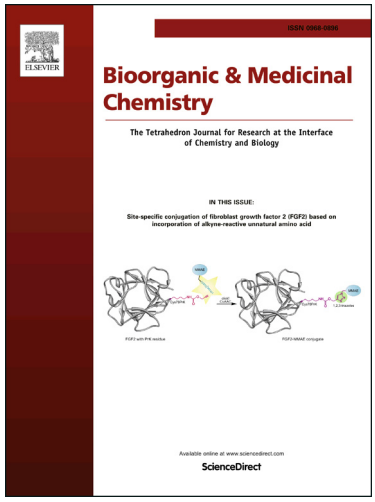

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Synthesis and biological evaluation of JL-A7 derivatives as potent ABCB1 inhibitors

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Abstract

Cancer chemotherapy failure is often due to the overexpression of ATP-binding cassette (ABC) transporters (particularly ABCB1), resulting in a variety of structurally and pharmacologically unrelated drugs efflux. The multidrug resistance (MDR) phenomenon could be reversed by ABCB1 inhibitors. Now, JL-A7 as the lead compound based on a triazol-N-ethyl-tetrahydroisoquinoline scaffold, 18 compounds were designed and synthesized. Substitution in para positions yielded high activities toward ABCB1. Moreover, compound **5** could effectively block the drug efflux function of ABCB1 and increase the accumulation of anti-cancer drugs to achieve effective treatment concentration in MDR cells.

Key words: ABCB1 inhibitors, MDR, p-glycoprotein, cancer resistance.

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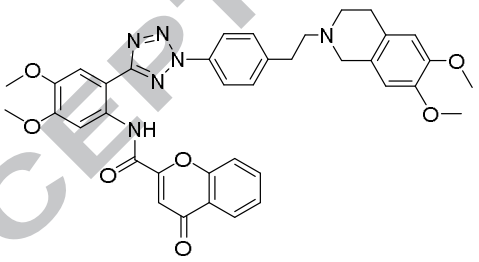
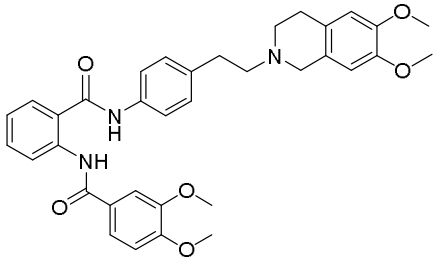
1. Introduction

The Multidrug resistance (MDR) is cross-resistant to structurally and pharmacologically distinct class of drugs, remaining one of impediment for tumor chemotherapy in clinic.[1] In general, MDR-mediated xenobiotic efflux is mainly related to the over-expression of membrane-associated transport proteins named ATP-binding cassette (ABC) transport proteins.[2-4] These transport proteins, especially ABCB1(P-glycoprotein/P-gp), ABCC1 (multidrug resistance protein 1/MRP) and ABCG2(breast cancer resistance protein /BCRP) are believed to expel a wide variety of anti-tumor drugs out of tumor cells, leading to the emergence of MDR.[5, 6] In recent years, the importance of ABCB1 in cancer chemotherapy is widely recognized.[5]

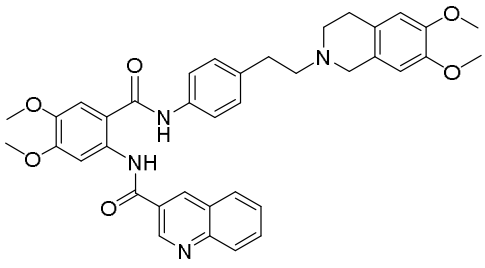
During the past decades, numerous ABCB1 inhibitors have been discovered in a continuing quest to reverse MDR in tumor.[7-9] Unfortunately , none of above compounds has been approved present in clinic application.[10] The failure may be attributed to many reasons with example implicated in cytotoxicity , influence on P450 enzymes, and adverse drug interaction.[6, 10, 11] Actually , some third-generation ABCB1 inhibitors, like HM30181,[12] zosuquidar (LY335979),[13] and WK-X-34,[8, 9] have shown potent activity and remarkable promise, even tariquidar (XR9576) (Figure.1) have reached phase III clinical trials.[6, 14] As many deficiencies of the existing ABCB1 inhibitors, there is a need to develop more effective and selective inhibitors of the protein.



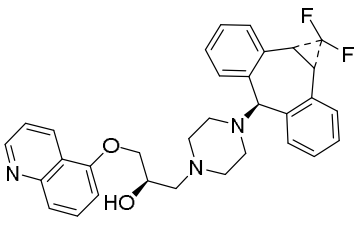
HM30181



WK-X-34



Tariquidar



Zosuquidar

Figure 1. Structures of third generation p-gp inhibitors: HM30181, WK-X-34, Tariquidar and Zosuquidar.

We have reported the synthesis and biological evaluation of various substituted triazol-N-ethyl-tetrahydroisoquinoline that proved to be even more potent inhibitors of ABCB1.[15] Moreover, compound JL-A7 exhibited better potency than verapamil (VRP) on increasing anticancer drug accumulation in K562/A02 cells. It could significantly reverse MDR in a dose-dependent manner and also persist longer chemo-sensitizing effect than VRP. Further mechanism studies revealed that compound JL-A7 could inhibit the efflux function of P-gp. In drug design, the strategy of amide reversal is always adopted to improve the biological activity of the compound. To find more promising candidates for MDR cancer treatment, with JL-A7 as the leading compound, 18 new compounds were synthesized by the optimization of amide inversion (**Figure.2**). And then more comprehensive activity evaluation was performed, consist of toxicity assessment, MDR reversal activity in vitro, and effects on ABCB1.

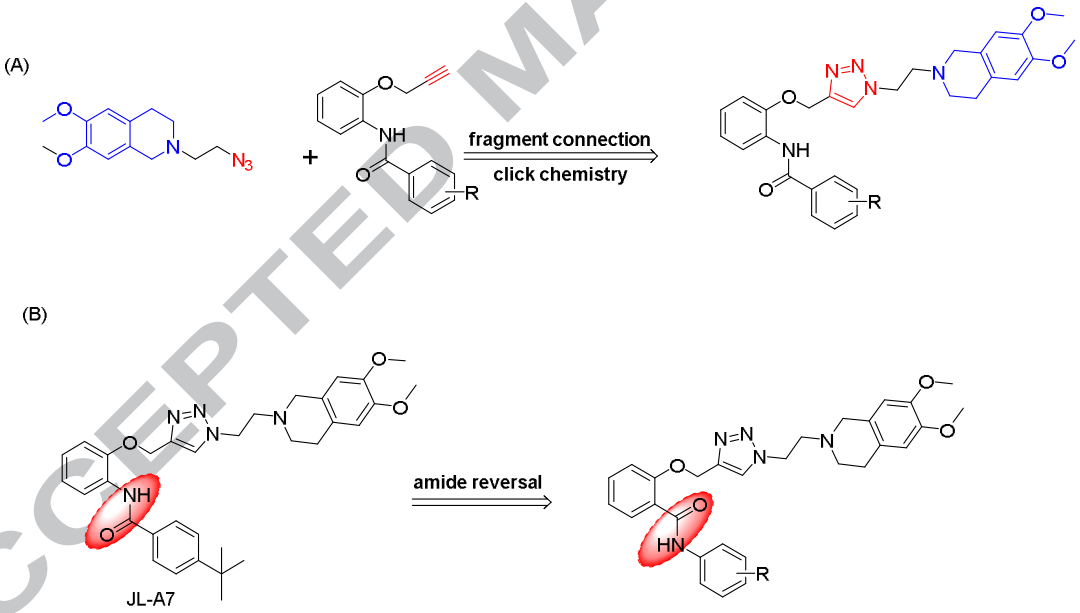
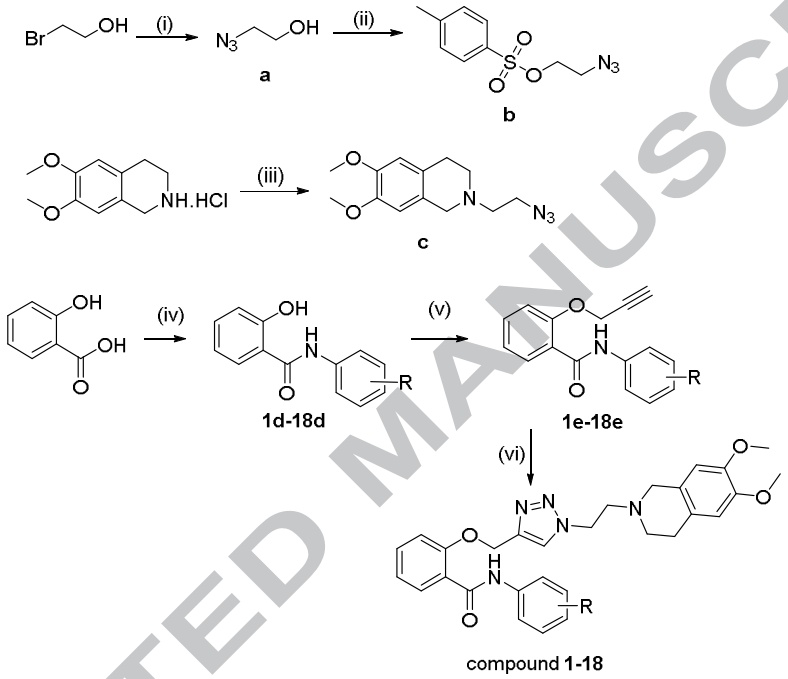


Figure 2. (A) Previous design of JL-A serious compounds. (B) Structures of JL-A7 and Design of the target compounds **1–18**.

2. Chemistry

Synthesis of target compounds **1–18** are outlined in **Scheme 1** Compounds **a–c** were synthesized according to literature procedures with minor modification. Compounds **1d-18d** were

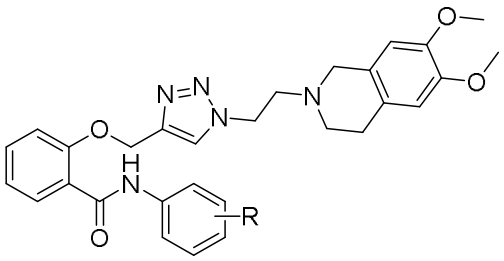
prepared by amide condensation , starting from the disparate Aromatic amines and salicylic acid. Compounds **1e-18e** were prepared by **1d-18d** and 3-Bromo-1-propyne, which refluxed in the mixture of acetonitrile with potassium carbonate for 6 h. Subsequently, compound c and compounds **1e–18e** were treated with sodium ascorbate and copper sulfate in 75% methanol stirring at room temperature for 24–48 h to provide compounds **1–18**. The structures of target compounds obtained were listed in **Table 1**.



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) TEA/acetonitrile, 60°C, 24h; (iv) aromatic amines, EDCI/HOBT, DCM r.t.; (v) 3-bromoprop-1-yne, K_2CO_3, acetone, reflux, 4 h; (vi) 2-(4-Azidophenethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**c**), ascorbate sodium, $CuSO_4$, 75% CH_3OH, 24–48 h.

Table 1 Structures and ADM-resistance reversal activity of the target compounds **1–18** at 5 μM concentration in K562/A02 cells[a]



Compounds	R	IC_{50} of ADM(μmol/L)	RF
1	3-CH_3	12.16±0.60	8.0
2	4-CH_3	7.65±1.06	12.6
3	4-OCH_3	8.38 ± 1.63	11.6
4	3,4-OCH_3	7.82 ± 0.45	12.4
5	4-$C(CH_3)_3$	2.89 ± 0.43	33.5
6	4-Cl	6.93 ± 0.73	14.0
7	3-OCH_3	8.73±1.18	11.1
8	3-Cl	22.82 ± 1.95	4.3
9	2-CH_3	21.91 ± 3.19	4.4
10	H	25.52 ± 1.50	3.8
11	3,4,5-OCH3	31.92 ± 3.10	3.0
12	4-F	8.29 ± 1.22	11.7
13	4-OCF_3	4.52 ± 1.49	21.4
14	4-$CH_2CH_2CH_2CH_3$	6.25 ± 0.91	15.5
15	4-NO_2	21.07 ± 1.66	4.6
16	3-NO_2	27.69 ± 0.20	3.5
17	2-NO_2	26.92 ± 1.58	3.6
18	3-OCH_3	17.31 ± 0.66	5.6
JL-A7	-	3.42±1.12	28.2
ADM	-	96.91 ± 12.24	1.0
tariquidar	-	1.97 ± 0.23	49.1
VRP	-	17.55 ± 1.32	5.5

[a] The IC_{50} value was 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).

3. Biological evaluation

3.1 Reversal of MDR in K562/A02 cells

The activity of the compounds in reversing multidrug resistance in tumors toward K562/A02 cells (ABCB1-overexpression) were preliminarily evaluated by the 3-(4, 5-dimethyl-2-thia-zolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) method.[16] We chose Verapamil (VRP) and tariquidar as positive controls，and tested all of compounds' intrinsic toxity towards K562/A02 cells. As the results summarized in **Table 1**, control group with Adriamycin (ADM) alone displayed little inhibitory effect (IC_{50} of 96.91 ± 12.24 μM). However, the combination groups, ADM with target compounds increased the inhibitory effect in different degrees. It revealed that most of the compounds showed significant MDR reversal activity. Among them, compound 5 showed the best potent as reversal fold (RF) was up to 31.4, superior to JL-A7 (RF = 28.2), tariquidar (RF = 49.1), VRP (RF = 5.5).

3.1.1 Chemo-sensitizing effect

To further investigate compound **5** activity on reversing MDR, we evaluated its therapeutic effect and safety by testing the half-maximal effective concentration (EC_{50}) and therapeutic index (TI).

The reversal activity of compound **5** was determined at various concentrations (5.0 μM, 2.5 μM, 1.0μM, 0.5 μM, 0.25 μM, 0.1μM, 0.05 μM) towards K562/A02 cells by MTT assay, selecting VRP as the positive control. As demonstrated in **Table 2**, VRP showed slight modulating activity at 5.0 μM (RF = 2.3). However, compound **5** showed apparent dose dependent activity and still exhibited potent MDR reversal activity (RF = 3.9) when the concentration decreased to 0.5μM. Additionally, the EC_{50} value of compound **5** was 256.2±6.2 nM, which was calculated by GraphPad Prism 6.0 software from the dose response curve as showed in **Figure 3**. The results suggested that compound **5** possessed the significantly potential to enhance the sensitivity of ABCB1-overexpressing cells to anticancer drug in a dose-dependent manner, at Non-toxic concentration.

Table 2 Percentage of IC_{50} of ADM and RF in different concentrations of compound **5** in

K562/A02cells.

Compounds	IC_{50} of ADM (μM)	RF
None	43.75 ± 1.65	1
VRP, 5.0 μM	19.84 ± 0.82	2.3
Compound **5**, 5.0 μM	1.27 ± 0.07	24.1
Compound **5,** 2.5 μM	3.33 ± 0.50	13.5
Compound **5**, 1.0 μM	6.02 ± 0.52	7.5
Compound **5**, 0.5 μM	12.52 ± 1.08	3.9
Compound **5**, 0.25 μM	18.39 ± 0.86	2.4
Compound **5**, 0.1 μM	34.90 ± 2.10	1.6
Compound **5**, 0.05 μM	37.18 ± 2.37	1.4
Compound **5**, 0.025 μM	37.21 ± 2.21	1.4

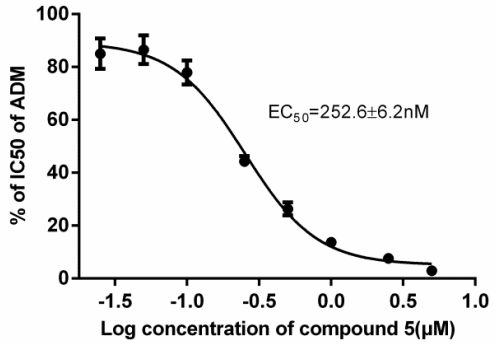


Figure 3. Dose response curve for the compound 5 in K562/A02 cells.

Various concentrations of target compound (0.025, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, or 5.0 μM) and ADM were added to 96-well plates. The cells were incubated for 48 h. The absorption values were measured with a Microplate Reader at 570 nm. Data were analyzed with GraphPad Prism 6.0 software and are the mean ± SD for 3 independent tests.

3.1.2 Duration of reversal effects

It is known that a relatively long duration of action with reversibility for an ABCB1 inhibitor is required for safe and effective therapy of ABCB1-mediated MDR. Therefore, the duration of MDR reversal effect of compound **5** was evaluated according to the procedure previously described with minor modification.[15] VRP was chosen as the control. As demonstrated in **Table 3**, the IC_{50}s of ADM towards K562/A02 cells which pretreated with VRP and compound **5**, were

10.73 μM, 1.26 μM respectively (no wash group). The MDR- reversing effects of VRP (RF = 1.6) and compound **5** (RF = 11.83) 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 **5** showed reversal activity even after its removal from the medium for 24 h and the IC_{50} of ADM was 10.32 μM (RF = 4.93). These data indicated that compound **5** displayed potent MDR-reversing effect which could persist for longer time compared with the positive control VRP.

Table 3 Duration of MDR reversal in K562/A02 cells after incubation and washout of verapamil, tariquidar and compound **5**

Treatment schedule	IC_{50}/ADM [μM] (RF)			
	Control	VRP	Tariquidar	Compound 5
No wash	51.34 ± 5.1 (1.0)	10.73 ± 2.1 (4.98)	1.60 ± 0.2 (31.98)	1.26± 0.2 (40.34)
wash, 0 h	nd	31.28 ± 2.77 (1.6)	3.02 ± 0.2 (16.86)	4.33. ± 0.3 (11.83)
wash, 6 h	nd	nd	4.97 ± 1.0 (10.37)	6.32 ± 0.6 (8.16)
wash, 12 h	nd	nd	8.28 ± 0.5 (6.10)	10.32 ± 1.2 (4.93)
wash, 24 h	nd	nd	14.39 ± 0.7 (3.55)	17.21 ± 3.4 (2.96)

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 ± SD
nd: not determined.

3.2 Cytotoxicity assay

Since most potent molecular failure due to toxicity within the therapeutic concentration range,[6] the intrinsic cytotoxicity of 18 compounds against parental sensitive K562 cells and K562/A02 cells (ADM-resistant sub-lines) were evaluated by MTT assay. Chemotherapy drug ADM and ABCB1 inhibitors VRP and tariquidar were selected as controls.

As shown in **Table 4**, VRP had weak cytotoxic effects toward K562 and K562/A02 cells with IC_{50} values 61.23 and 47.34M, respectively. Nevertheless, tariquidar displayed a high level of toxicity toward K562 cells (IC_{50} of 31.56 μM) and K562/A02 cells (IC_{50} of 27.19 μM). In contrast,

all of the synthesized compounds showed no toxicity with IC_{50} values higher than 100 μM to K562/A02 cells. Compound **3** and **8** were found to exhibit quite high toxicity (IC_{50} < 30μM) in K562 cells.

Table 4 Cytotoxicity of the target compounds against K562 and K562/A02 cell lines

Compounds	Cytotoxicity IC_{50} (μM)	
	K562	K562/A02
1	>100	>100
2	>100	>100
3	79.24±5.61	>100
4	>100	>100
5	>100	>100
6	>100	>100
7	>100	>100
8	87.40±3.09	>100
9	>100	>100
10	>100	>100
11	>100	>100
12	>100	>100
13	>100	>100
14	>100	>100
15	>100	>100
16	>100	>100
17	>100	>100
18	>100	>100
JL-A7	>100	>100
VRP	65.28 ± 3.54	56.24 ± 2.12
tariquidar	31.56 ± 2.32	27.19 ± 1.41
ADM	4.29 ± 2.28	96.91 ± 12.24

[a] The IC_{50}s for the target compounds were determined by MTT method. Each experiment was carried out 3 times.

3.3 Effects on ABCB1

Investigation of the inhibitory activity of the test compounds against ABCB1 was performed with K562/A02 cell line. As ADM and Rhodamine 123 (Rh123) are fluorescent dye and substrate of ABCB1, an increase of fluorescence by intracellular accumulation can be correlated to the inhibition effects on ABCB1 of the compound.[17]

3.3.1 ADM accumulation

Firstly, in order to investigate the mechanism of compound **5** in modulating the anticancer drug

substrates accumulation level inside K562/A02 cells. The mean fluorescence intensity (MFI) of retained intracellular DOX was estimated by BD FACSCalibur flow cytometer through the FL2 tunnel. The classical P-gp inhibitor VRP was chosen as the positive control. As the results shown in **Figure 4**, K562/A02 cells treated with compound **5**, the MFI of cells increased much more than treated with VRP at the same concentration. The result indicated that compound **5** was more potent than VRP in DOX accumulation in K562/A02 cells.[17, 18]

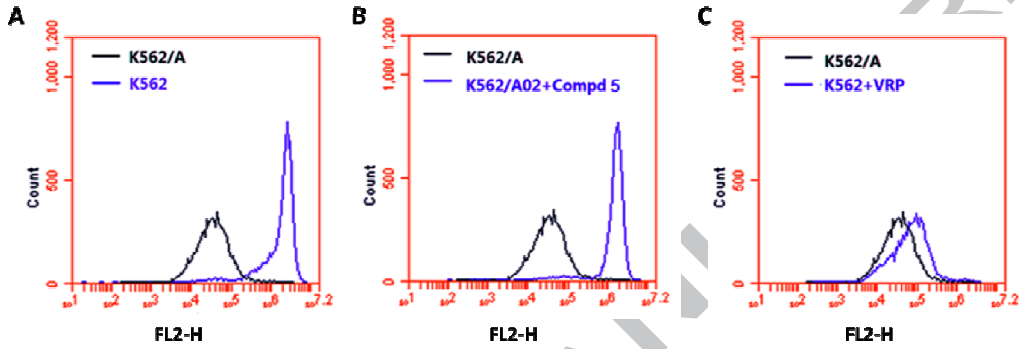


Figure 4. The effect of target compounds on the DOX accumulation in K562 or K562/A02 cells. K562 or K562/A02 Cells were incubated with 20μM Rh123 without or with compound 5 or VRP (5μM) for 150 min at 37℃ before washing with ice PBS for three times. The MFI of retained intracellular DOX was measured by BD FACSCalibur flow cytometer through the FL2 tunnel. The shift of the histogram to the right indicates an increase in intracellular accumulation of DOX due to the inhibition of P-gp. (A: K562 and K562/A02 without modulator; B: K562/A without modulator vs K562/A02 with 5μM compound 5; C: K562/A without modulator vs K562/A02 with 5μM VRP)

Furthermore, when incubated with modulators at various concentrations, the MFI of DOX accumulated in K562/A02 cells showed dose-effect dependence. As shown in **Figure 5**, in blank groups，the level of ADM in K562 cells was about 4.5-fold higher than that of K562/A02 cells in the absence of ABCB1 inhibitors. And groups treated with ABCB1 modulators (such as VRP or compound **5**) showed increasing concentration than blank in K562/A02 cells. Once treated with compound **5** (2.5 μM), 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 (0.5, 2.5, 5 μM), the accumulation of ADM was remarkably increased in a dose-dependent manner. Additionally, the intracellular ADM level of compound **5** (5 μM) was 1.4 times higher than that of VRP in K562/A02 cells at the same dose. The results suggested that ABCB1 can pump ADM out of the cells which leads to a lower ADM level in K562/A02 cells，and compound **5** was more

potent than VRP in inhibiting the drug efflux function of ABCB1.

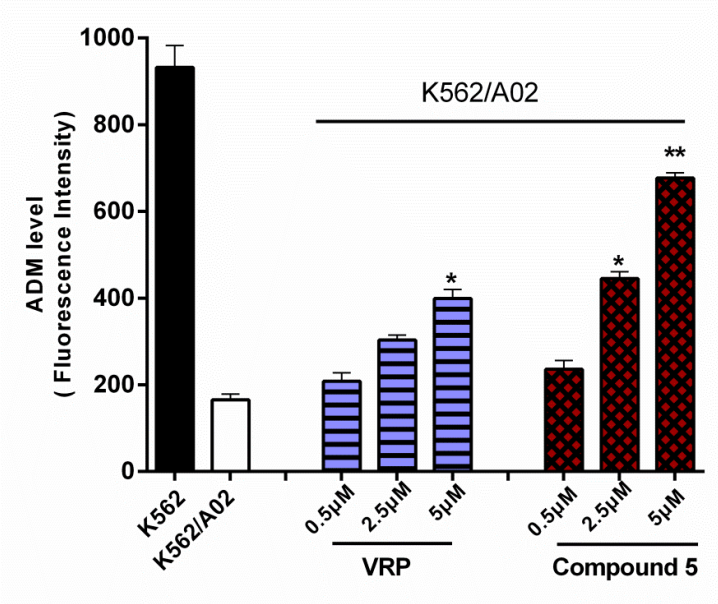


Figure 5. Effect of compound **5** and verapamil (VRP) on ADM intracellular accumulation in K562/A02 cells.

*, $P < 0.05$, **, $P < 0.01$ compared with 5 the group of K562/A02 cells without modulators. Data were analyzed with GraphPad Prism 6.0 software and are the mean ± SD for 3 independent tests.

3.3.2 Inhibition on Rh123 efflux

Furthermore, another ABCB1 substrate with fluorescent Rh123[19] was chosen to further assess effects on ABCB1 by compound **5**. As shown in **Figure 6.**, both compound **5** and VRP can inhibit Rh123 efflux from K562/A02 cells, but the inhibitory effect of compound **5** was obviously better than that of VRP over 90 min interval on the same condition. The rate constant (K) of efflux in compound-treated K562/A02 cells was 0.0067±0.0095 s^{-1}, which was lower than the value of both the Vehicle control group (0.0231±0.0058 s^{-1}) and the VRP-treated group (0.0092±0.0035 s^{-1}).

These results above verified our presumption that compound **5** was an effective ABCB1 inhibitor by blocking the drug efflux function of ABCB1. Moreover, the potency of compound **5** is much higher than the classical ABCB1 inhibitor VRP under the same conditions.

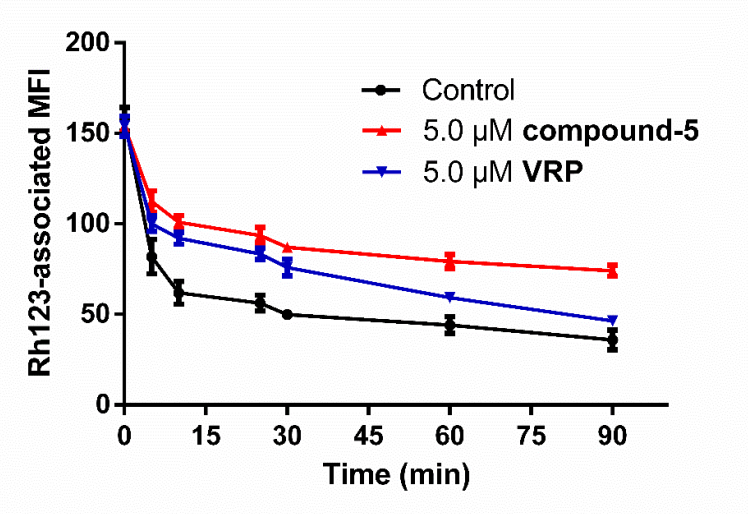


Figure 6. The effect of compound **5** and verapamil (VRP) on the efflux of rhodamine 123 (Rh123) in K562/A02 cells. Data were analyzed with GraphPad Prism 6.0 software and are the mean ± SD for 3 independent tests.

4. Conclusion and discussion

In summary, 18 compounds were designed on the base of amide reversal and evaluated in vitro as ABCB1-mediated MDR reversal agents. The reversal of the amide bond keeps the activity of the compound maintained or even enhanced. Among them, compound **5** is a potent MDR reverse agent showing a dose-dependent manner well below the toxic concentration. Additionally, compound **5** could effectively block the drug efflux function of ABCB1 to increase the accumulation of anti-cancer drugs to achieve effective treatment concentration in MDR cells. Therefore, compound **5** could be served as a promising candidate for the development of ABCB1-mediated MDR-Reversal agents.

According to data in **Table 1** above, the structure and activity relationship can be derived: The Electronic effect of the substituents at the aniline moiety play a minor role. Substitution in para positions yielded high activities. For example, compounds **5**, **6**, **13** and **14** with substitution in para positions showed higher MDR reversal activity than others, applicable in JL-A7. However, no distinct trend for the electron withdrawing substitutions was found, such as compound **15**. In particular, the benzene ring para-substituted compounds show great difference in reversal activity. Among the para-substituted compound, compound **14** (RF=15.5) with n-butyl substituted has a better activity than the methyl (compound **2**, RF=12.6), methoxy (compound **3**, RF=11.6) and

chlorine (compound **6**, RF=14.0) substituted compounds but is weaker than the t-butyl substituted compound (compound **5**, RF=33.5). This difference may be related to the steric hindrance at the active site, and may also be related to the binding force of the compound to the hydrophobic pocket, which is worth discussing further.

To analyze and further characterize the interaction between target compound and P-gp, the most promoting compound **5** was chosen to docking with a model of P-gp (PDB code: 3G60) As shown in **Figure 7**, the interacting mode of compound **5** revealed the structure occupied a small hydrophobic pocket of Tyr_{-949} and PHE_{-339}.

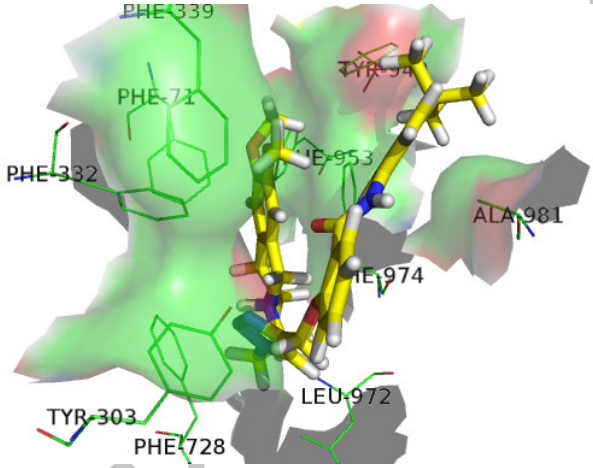


Figure 7. Docking interaction of compound 5 with P-gp (PDB ID: 3G60).

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); Thin-layer chromatography (TLC) was performed on GF/UV 254 plates and the chromatograms were visualized under UV light at 254 and 365 nm. Compound **e** was prepared as previously described.

5.2. Synthesis of 2-azidoethanol (a)

To a 100 mL round bottom flask was added 2-bromoethanol (5.3 g, 42.4 mmol) and sodium azide (5.5 g, 84.6 mmol) in water (50 mL). The mixture was stirred at 80℃ 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 ppm: 3.78 (t, J = 5.1 Hz, 2H, $OCH_2CH_2N_3$), 3.45 (t, J = 5.1 Hz, 2H, $OCH_2CH_2N_3$), 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 (10 mL, 72.1 mmol) in dry dichloromethane (25 mL), 4-toluenesulfonylchloride (6.2 g, 32.5 mmol) in dry dichloromethane (25 mL) was added dropwise under constant stirring at 0℃ 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 1N $NaHCO_3$ (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%

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 ppm: 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 = 6.1 Hz, 2H, $ArCH_2$), 2.80 (dd, 6H, J = 13.3, 6.1 Hz, 3×CH_2).

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

To a solution of Salicylic acid (2 mmol) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (2.2 mmol) in dichloromethane (30 mL) ,then aromatic amine (2 mmol) was added , then the solution was stirred at temperature for 3h. Water (80 ml) added, and 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 (8:1, v/v) as eluent to give the desire white solid product compounds.

5.5. General procedure for the preparation of 1e–18e

To a solution of **1d-18d** (1 mmol) and 3-bromo-prop-1-yne (1.1 mmol) in acetonitrile (15 mL), potassium carbonate (3 mmol) was added, then the mixture was heated to reflux for 6 h. Then the reaction mixture was cooled to room temperature followed by filtration and evaporated in vacuo to get the desired product compound **1e–18e**.

5.6. General procedure for the preparation of compounds 1–18

To the solution of **1e–18e** (1 mmol) and **c** (1 mmol) in 75% methanol (20 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. The solution was evaporated in vacuo and 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, Yeild: 38.5%-71.7%.

5.6.1

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(m-tolyl)benzamid (1)

Yield 75.9%; mp: 127-129℃; pale yellow powder; ^{1}H NMR(300 MHz, DMSO-d_6,) δ ppm: 10.11 (s, 1H, CONH), 8.24 (s, 1H, NCH=C), 7.77 (d, J = 6.6Hz, 1H, ArH), 7.45 (m, 4H, ArH), 7.16 (m, 2H, ArH), 6.89 (d, J = 7.4Hz, 1H,ArH), 6.61 (s, 1H, ArH), 6.54 (s, 1H, ArH), 5.36 (s, 2H, OCH_2), 4.58 (t, J = 5.8Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2×OCH_3), 3.49 (s, 2H, $ArCH_2N$), 2.86 (t, J = 6.0Hz, 2H, NCH_2), 2.62 (s, 4H, 2×CH_2), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO, 75 MHz) δ ppm:21.1, 28.0, 47.1, 50.0, 54.8, 55.4, 56.6, 62.2, 109.8,111.7, 113.6, 116.5, 119.9, 121.2, 123.9, 124.2, 124.8, 125.7, 126.1, 128.5, 130.4, 132.4,137.9, 138.8, 141.9, 146.8, 147.1, 155.5, 163.5; 528.4 ; ESI-HRMS m/z calcd for $C_{30}H_{33}N_5O_4$ $[M+Na]^+$ 550.2425, found 550.2420.

5.6.2

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(ptolyl)benzamide (2)

Yield 56.9%; mp: 167-168℃; pale yellow powder; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.09 (s, 1H, CONH), 8.23 (s, 1H, NCH=C), 7.76(d, J = 7.6Hz, 1H, ArH), 7.50 (m, 3H, ArH), 7.37 (d, J

= 8.8Hz, 1H, ArH), 7.10 (m, 3H, ArH), 6.62 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.35 (s, 2H, OCH_2), 4.58 (t, *J* = 5.9Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2×OCH_3), 3.50 (s, 2H, $ArCH_2N$), 2.86 (t, *J* = 5.9Hz, 2H, NCH_2), 2.63 (s, 4H, 2×CH_2), 2.25 (s, 3H, CH_3); ^{13}C NMR (DMSO, 75 MHz)δ ppm: 20.4, 28.0, 47.0, 50.0, 54.8, 55.4, 56.7, 62.2, 109.7,111.7, 113.6, 119.3, 121.1, 124.1, 124.7, 125.7, 126.1, 129.1, 130.3, 132.3, 132.4, 136.4,142.0, 146.8, 147.1, 155.5, 163.4; ESI-HRMS m/z calcd for $C_{30}H_{33}N_5O_4$ $[M+Na]^+$ 550.2425, found 550.2422.

5.6.3

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(4-methoxyphenyl)benzamide (3)

Yield 46.0%; pale yellow powder; mp: 172-173℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.07 (s, 1H, CONH), 8.23 (s, 1H, NCH=C), 7.75(d, *J* = 6.8Hz, 1H, ArH), 7.50 (m, 3H, ArH), 7.37 (d, *J* = 7.8Hz, 1H, ArH), 7.10 (dd, *J* = 6.8Hz ,6.8Hz, 1H, ArH), 6.88 (d, *J* = 7.9Hz, 2H, ArH),6.62 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.35 (s, 2H, OCH2), 4.58 (t, *J* = 5.9Hz, 2H, $CHNCH_2$), 3.73(s, 3H, OCH_3), 3.67(s, 6H, 2×OCH_3), 3.49 (s, 2H, $ArCH_2N$), 2.89 (s, 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, 55.1, 55.4, 56.7, 62.3, 109.8,111.7, 113.6, 113.8, 120.8, 121.1, 124.2, 124.7, 125.7, 126.2, 130.3, 132.1, 132.2, 142.1,146.8, 147.1, 155.4, 163.2; ESI-HRMS m/z calcd for $C_{30}H_{33}N_5O_5$ $[M+Na]^+$ 566.2374, found 566.2380.

5.6.4

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3,4-dimethoxyphenyl)benzamide (4)

Yield 59.3%; pale yellow powder; mp:118-120℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.11 (s, 1H, CONH), 8.24 (s, 1H, NCH=C), 7.82(d, *J* = 7.2Hz, 1H, ArH), 7.50(m, 1H, ArH), 7.37 (m, 2H, ArH), 7.23 (d, J = 8.3Hz ,1H, ArH), 7.11 (m, 1H, ArH),6.89(d, *J* = 8.5Hz, 1H, ArH), 6.62 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.36 (s, 2H, OCH_2), 4.58 (s, 2H, $CHNCH_2$), 3.73(m, 12H, OCH_3), 3.48 (s, 2H, $ArCH_2N$), 2.86 (s, 2H, NCH_2), 2.62 (s, 4H, 2×CH_2);^{13}C NMR (DMSO, 75 MHz) δ ppm:27.9, 47.1, 50.0, 54.8, 55.4, 55.7, 56.7, 62.2, 104.5,109.8, 111.2, 111.7, 111.9, 113.5, 121.2, 123.7, 124.6, 125.7, 126.1, 130.4, 132.4, 132.5,141.9, 145.0, 146.8, 147.1, 148.6, 155.5, 163.0; ESI-HRMS m/z calcd for $C_{31}H_{35}N_5O_6$ $[M+Na]^+$ 596.2480, found 596.2480.

5.6.5

N-(4-(tert-butyl)phenyl)-2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzamide (5)

Yield 61.4%; pale yellow powder; mp: 150-152℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.10 (s, 1H, CONH), 8.24 (s, 1H, NCH=C), 7.78(dd, *J* = 7.6, 1.3Hz, 1H, ArH), 7.52 (m, 3H, ArH), 7.35 (m, 3H, ArH), 7.10 (dd, 1H, *J*= 7.4, 7.4Hz, ArH), 6.62 (s, 1H, ArH), 6.53 (s, 1H, ArH), 5.35 (s, 2H, OCH_2), 4.59 (t, *J* =5.9Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.48 (s, 2H, CH_2N), 2.88 (t, *J* =5.5Hz, 2H, NCH_2), 2.63 (s, 4H, 2 × CH_2), 1.27 (s, 9H, 3×CH_3); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 8.0, 31.1, 34.0, 47.1, 50.0, 54.8, 55.4, 56.7, 62.3,109.8, 111.8, 113.6, 119.2, 121.2, 124.0, 124.7, 125.3, 125.7, 126.2, 130.4, 132.4, 136.3,142.0, 145.8, 146.9, 147.2, 155.5, 163.4; ESI-HRMS m/z calcd for $C_{33}H_{39}N_5O_4$ $[M+Na]^+$ 592.2894, found 592.2896.

5.6.6

N-(4-chlorophenyl)-2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzamide (6)

Yield 70.8%; pale yellow powder; mp: 165-166℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.32 (s, 1H, CONH), 8.22 (s, 1H, NCH=C), 7.73(m, 3H, ArH), 7.51 (ddd, *J* = 8.4, 8.4, 1.3Hz, 1H, ArH), 7.37 (m, 3H, ArH), 7.10 (dd, *J*= 7.4, 7.4 Hz, 1H, ArH), 6.62 (s, 1H, ArH), 6.56 (s, 1H, ArH), 5.34 (s, 2H, OCH_2), 4.58(t, *J* = 5.9Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2×OCH_3), 3.50 (s, 2H, $ArCH_2N$), 2.85 (t, *J* = 5.8Hz, 2H, NCH_2), 2.63 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 28.5. 47.6, 50.5, 55.3, 55.9, 57.2, 62.8, 110.3,112.2, 114.1, 121.4, 121.6, 124.7, 125.1, 126.2, 126.7, 127.5, 129.1, 130.7, 132.9, 138.4, 142.6, 147.4, 147.7, 155.9, 164.6; ESI-HRMS m/z calcd for $C_{29}H_{30}ClN_5O_4$ $[M+Na]^+$ 570.1879 , found 570.1880.

5.6.7

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-methoxyphenyl)benzamide (7)

Yield 64.4%; pale yellow powder; mp: 127-129℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.19 (s, 1H, CONH), 8.24 (s, 1H, NCH=C), 7.78(d, *J* = 6.5Hz, 1H, ArH), 7.52 (dd, *J* = 7.4, 7.4 Hz, 1H, ArH), 7.37 (m, 2H, ArH), 7.21(m, 2H, ArH), 7.11 (dd, *J* = 7.4, 7.4 Hz, 1H, ArH), 6.65 (m, 1H, ArH), 6.62 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.35 (s, 2H, OCH_2), 4.58 (t, *J* = 5.5 Hz, 2H, $CHNCH_2$),

3.75(s, 3H, 6H, 2×OCH_3), 3.49 (s, 2H, $ArCH_2N$), 2.86 (s, 2H, NCH_2), 2.50 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 28.5, 47.6, 50.5, 55.3, 55.5, 55.9, 57.1, 62.7, 105.7, 109.5, 110.3, 112.2, 114.1, 121.7, 124.4, 125.2, 126.2, 126.7, 130.0, 131.0, 133.0, 140.5, 147.4, 147.6, 156.0, 160.0, 164.2; ESI-HRMS m/z calcd for $C_{30}H_{33}N_5O_5$ $[M+Na]^+$ 566.2374, found 566.2382.

5.6.8

N-(3-chlorophenyl)-2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzamide (8)

Yield 68.6%; pale yellow powder; mp: 127-130℃; ^1H NMR(300 MHz, DMSO-d_6) δ ppm: 10.33 (s, 1H, CONH), 8.20 (s, 1H, NCH=C), 7.86 (s, 1H, ArH), 7.72(d, *J* = 6.4 Hz , 1H, ArH), 7.52 (m, 2H, ArH), 7.34 (m, 2H, ArH),7.12 (m, 2H, ArH), 6.61 (s, 1H, ArH), 6.54 (s, 1H, ArH), 5.34 (s, 2H, OCH2), 4.57 (s, 2H, $CHNCH_2$), 3.67 (s, 6H, 2×OCH_3), 3.48 (s, 2H, $ArCH_2N$), 2.85 (s, 2H, NCH_2), 2.62 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 27.9, 47.1, 49.9, 54.8, 55.4, 56.6, 62.2, 109.8, 111.7, 113.6, 117.8, 118.8, 121.1, 123.2, 124.0, 124.7, 125.7, 126.1, 130.2, 130.3, 132.5, 133.1, 140.3, 146.8, 147.1, 155.5, 164.2 ; ESI-HRMS m/z calcd for $C_{29}H_{30}ClN_5O_4$ $[M+Na]^+$ 570.1879, found 570.1882.

5.6.9

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(o-tolyl)benzamide (9)

Yield 56.9%; pale yellow powder; mp: 131-133℃; ^1H NMR(300 MHz, DMSO-d_6) δ ppm: 9.69 (s, 1H, CONH), 8.28 (s, 1H, NCH=C), 7.98(d, *J* = 7.6 Hz, 1H, ArH), 7.90 (d, *J* = 7.9 Hz, 1H, ArH), 7.55 (dd, *J* = 7.0 Hz, 7.0 Hz, 1H, ArH), 7.44 (d, *J* = 8.2 Hz, 1H, ArH), 7.10 (m, 4H, ArH), 6.62 (s, 1H, ArH), 6.56 (s, 1H, ArH), 5.41 (s, 2H, OCH_2), 4.58 (t, *J* = 5.7 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2×OCH_3), 3.50 (s, 2H, $ArCH_2N$), 2.86 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.62 (s, 4H, 2×CH_2), 1.91 (s, 3H, CH_3); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 28.0, 47.0, 50.0, 54.8, 55.4, 56.6, 62.1, 109.8, 111.7, 113.5, 121.2, 122.7, 124.3, 125.4, 125.7, 126.0, 126.1, 129.2, 130.1, 131.1, 132.8, 136.4, 141.4, 146.8, 147.1, 155.7, 162.9; ESI-HRMS m/z calcd for $C_{30}H_{33}N_5O_4$ $[M+Na]^+$ 550.2425, found 550.2426.

5.6.10

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-phenylbenzamide (10)

Yield 60.2%; pale yellow powder; mp: 143-145℃ ;^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.19(s, 1H, CONH), 8.56 (s, 1H, NCH=C), 7.77 (d, *J* = 7.2 Hz, 1H, ArH), 7.65 (d, *J* = 7.8 Hz, 2H, ArH), 7.51 (dd, *J* = 7.2 Hz, 7.5 Hz,1H, ArH), 7.34 (m, 4H, ArH), 7.08 (dd, *J* = 7.5 Hz, 7.2Hz, 2H, ArH), 6.62 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.35 (s, 2H, OCH_2), 4.57(t, *J* = 5.7 Hz, 2H, $CHNCH_2$), 3.68 (s, 6H, 2 ×OCH_3), 3.49 (s, 2H, $ArCH_2N$), 2.85 (s, 2H, NCH_2), 2.63 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 27.9, 47.0, 49.9, 54.8, 55.4, 56.6, 62.2, 109.8, 111.7, 113.5.0, 119.3, 121.1, 123.4, 124.1, 124.7, 125.7, 126.1, 128.7, 130.3, 132.3, 138.9, 142.0, 146.8, 147.1, 155.4, 163.6; ESI-HRMS m/z calcd for $C_{29}H_{31}N_5O_4$ $[M+Na]^+$ 536.2268, found 536.2268.

5.6.11

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3,4,5-trimethoxyphenyl)benzamide (11)

Yield 56.2%; yellow powder; mp: 66-68℃; ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm: 10.19 (s, 1H, CONH), 8.24 (s, 1H, NCH=C), 7.85(d, *J* = 7.2 Hz, 1H, ArH), 7.54 (dd, *J* =7.2 Hz, 7.2 Hz, 1H, ArH), 7.37 (d, *J* = 8.1 Hz,1H, ArH), 7.12 (m, 3H, ArH), 6.61 (s, 1H, ArH), 6.55 (s, 1H, ArH), 5.37 (s, 2H, OCH_2),4.58 (s, 2H, $CHNCH_2$), 3.76 (m, 6H, 2×OCH_3), 3.65(s, 6H, 2×OCH_3), 3.49 (s, 3H, OCH_3), 3.36 (s, 2H, $ArCH_2N$), 2.86 (s, 2H, NCH_2), 2.62 (s, 4H, 2×CH_2); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 27.9, 47.1, 49.9, 54.8, 55.4, 55.7, 56.6, 60.0, 62.2,97.2, 109.8, 111.7, 113.5, 121.3, 123.4, 124.7, 125.7, 126.1, 130.5, 132.7, 135.0, 141.9,152.7, 163.0; ESI-HRMS m/z calcd for $C_{32}H_{37}N_5O_7$ $[M+Na]^+$ 626.2585, found 626.2587.

5.6.12

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(4-fluorophenyl)benzamide (12)

Yield 46.0%, pale yellow powder mp: 143-145℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.13 (s, 1H, CONH), 8.01 (s, 1H, NCH=C), 7.70 (s, 4H, NCH=C), 7.28 (d, *J* = 6.8 Hz, 1H, ArH), 7.23 – 7.05 (m, 2H, ArH), 6.83 (d, *J* = 7.3 Hz, 1H, ArH), 6.74 – 6.45 (m, 3H, ArH), 4.52 (s, 2H, $CHNCH_2$), 4.40 (d, *J* = 2.1 Hz, 2H), 3.67 (s, 6H, 2×OCH_3), 3.51 (s, 2H, $ArCH_2N$), 2.87 (s, 2H,

NCH_2), 2.64 (s, 4H, 2×CH_2); ^{13}C NMR (75 MHz, DMSO) δ ppm: 167.87, 148.74, 147.19, 146.92, 144.56, 135.37, 132.66, 128.89, 126.29, 125.80, 123.04, 122.46, 115.76, 115.16, 114.80, 111.84, 111.50, 109.96, 56.71, 55.46, 54.82, 50.10, 47.04, 38.17, 28.04; ESI-HRMS m/z calcd for $C_{29}H_{30}FN_5O_4$ $[M+Na]^+$ 554.2174, found 554.2179.

5.6.13

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

Yield 68.6%; pale yellow powder; mp: 127-129℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.25 (s, 1H, CONH), 8.02 (s, 1H, NCH=C), 7.82 (d, *J* = 7.4 Hz, 2H, ArH), 7.78 – 7.65 (m, 2H, ArH), 7.33 (d, *J* = 7.8 Hz, 3H, ArH), 6.84 (d, *J* = 7.9 Hz, 1H, ArH), 6.67 (t, *J* = 6.9 Hz, 1H, ArH), 6.59 (d, *J* = 11.1 Hz, 2H, ArH), 4.53 (s, 2H, $CHNCH_2$), 4.40 (s, 2H), 3.68 (s, 6H, 2×OCH_3), 3.52 (s, 2H, $ArCH_2N$)), 2.88 (s, 2H, NCH_2), 2.65 (s, 4H, 2×CH_2). ^{13}C NMR (75 MHz, DMSO) δ ppm: 168.05, 148.74, 147.09, 146.80, 144.49, 138.30, 132.86, 129.02, 126.19, 125.70, 123.10, 121.86, 121.36, 115.48, 114.73, 111.59, 109.75 (s), 56.74 (s), 55.36, 54.81, 50.08, 46.97, 38.10, 28.04; ESI-HRMS m/z calcd for $C_{30}H_{30}F_3N_5O_5$ $[M+Na]^+$ 620.2091, found 620.2097.

5.6.14

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(4-nitrophenyl)benzamide (14)

Yield 68.6%; pale yellow powder; mp: 123-125℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 10.10 (s, 1H, CONH), 8.24 (s, 1H, NCH=C), 7.78 dd, *J* = 7.6, 1.3Hz, 1H, ArH), 7.52 (m, 3H, ArH), 7.35 (m, 3H, ArH), 7.10 (dd, 1H, *J* = 7.4, 7.4Hz, ArH), 6.62 (s, 1H, ArH), 6.53 (s, 1H, ArH), 5.35 (s, 2H, OCH_2), 4.59 (t, *J* =5.9Hz, 2H, $CHNCH_2$), 3.68 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.48 (s, 2H, CH_2N), 2.88 (t, *J* =5.5Hz, 2H, NCH_2); ^{13}C NMR (DMSO, 75 MHz) δ ppm: 14.0,21.1, 28.0, 47.1, 50.0, 54.8, 55.4, 56.7, 62.3,109.8, 111.8, 113.6, 119.2, 121.2, 124.0, 124.7, 125.3, 125.7, 126.2, 130.4, 132.4, 136.3,142.0, 145.8, 146.9, 147.2, 155.5, 163.4; ESI-HRMS m/z calcd for $C_{33}H_{39}N_5O_4$ $[M+Na]^+$ 592.2894, found 592.2895.

5.6.15

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(ptolyl)benzamide (15)

Yield 68.6%; pale yellow powder; mp:117-118℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 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-HRMS m/z calcd for $C_{29}H_{31}N_6O_6$ $[M+Na]^+$ 581.2119, found 581.2114.

5.6.16

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-nitrophenyl)benzamide (16)

Yield 53.8%; pale yellow powder; mp: 120-122℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 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* = 5.6Hz, 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-HRMS m/z calcd for $C_{29}H_{31}N_6O_6$ $[M+Na]^+$ 581.2119, found 581.2118.

5.6.17

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(2-nitrophenyl)benzamide (17)

Yield 71.7%; pale yellow powder; mp: 119-122℃; ^{1}H NMR(300 MHz, DMSO-d_6) δ ppm: 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, OCH3), 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-HRMS m/z calcd for $C_{29}H_{31}N_6O_6$ $[M+Na]^+$ 581.2119, found 581.2110.

5.6.18

2-((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-methoxyphenyl)benzamide (18)

Yield 38.5%; pale yellow powder; mp:115-118℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 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, NCH2), 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-HRMS m/z calcd for $C_{30}H_{33}N_5O_5$ $[M+Na]^+$ 566.2374, found 566.2372.

6. Biological assays

6.1. Cytotoxicity assay

The exponentially growing K562 and K562/A02 cells were grown in 96-well plates at 1×10^4 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 cells were incubated for another 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 6.0 software from the dose–response curves.

6.2. 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 were seeded in 96-well plates at 1×10^4 cells per well. 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 6.0 software from the dose–response curves. Experiments were conducted in triplicates and repeated three times independently.

6.3. Adriamycin intracellular accumulation

K562 and K562/A02 cells were seeded into 24-well plates at 1.5×10^5 /well. Different concentrations of compound **5** and VRP were pre-incubated with cells for 60 min. Then 20μM ADM was added into each 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 was measured by fluorescence spectrophotometer. Data were expressed as means ± SD of three independent experiments.

6.4. 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 **5** or VRP (0.5, 2.5, 5μM) for another 90 min. Afterwards the cells were washed twice with ice-cold PBS. The mean fluorescence intensity of retained intracellular Rh123 was estimated by BD FACSCalibur flow cytometer.

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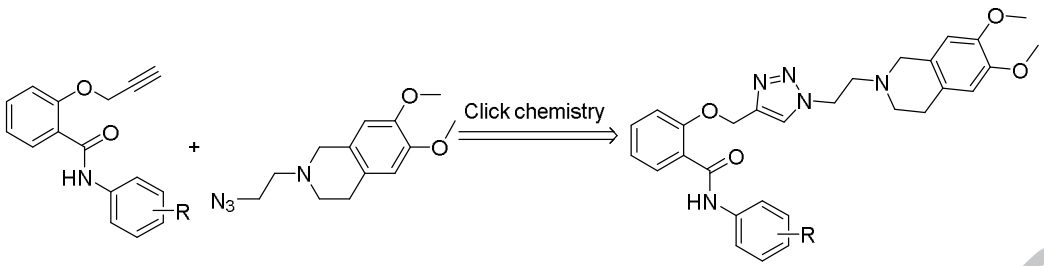
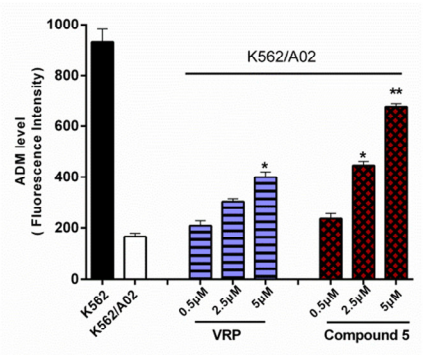


Compound **5**, R=4-C$(CH_3)_3$



Effect of compound **5** on intracellular ADM

- High potency, EC_{50} = 256.2±6.2nM;
- Low cytotoxity, with high therapeutic index (TI >780);
- Long activity duration, >24h;
- Significant increase of ADM cellular concentration ;
- Inhibition of Rh123 efflux