Received: 23 April 2019 | Revised: 11 July 2019 | Accepted: 17 July 2019

DOI: 10.1002/ardp.201900127

DPhG ARCH PHARM
Archiv der Pharmazie

FULL PAPER

Discovery to solve multidrug resistance: Design, synthesis, and biological evaluation of novel agents

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Funding information
The Foundation of Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection, Grant/Award Number: JKLBS2017011; National Natural Science Foundation of China, Grant/Award Numbers: 81872734, 81803353, 81872733

Abstract

Chemotherapy remains a pillar in the treatment and management of various cancers. However, multidrug resistance (MDR) becomes a severe problem after long-term administration of chemotherapy drugs. Overexpression of P-glycoprotein (P-gp) is a significant cause for tumor MDR. Therefore, P-gp inhibition is considered as an effective strategy to reverse MDR. A third-generation P-gp inhibitor tariquidar was selected as a lead compound, and a new series of triazol-*N*-ethyl tetrahydroisoquinoline based compounds were designed as novel P-gp inhibitors and synthesized through click chemistry. These compounds presented higher reversal activities than the positive-control verapamil (VRP). Among 18 compounds, compound **11** without cytotoxicity reversed MDR in a dose-dependent manner, with a persistent longer chemosensitizing effect and reversibility compared to others. Mechanism studies discovered that compound **11** could escalate the intracellular accumulation of rhodamine-123 and doxorubicin in K562/A02 cells as well as inhibit their efflux from cells. The results obtained suggest that compound **11** is more potent than VRP administered under the same conditions; it may be a potent and safe candidate for P-gp modulation for further development.

KEYWORDS

click chemistry, multidrug resistance, P-glycoprotein inhibitor, reversal activity

1 | INTRODUCTION

Cancer is a severe disease without a highly effective cure as of now. It usually arises from a single stem cell with the ability for self-renewal using intrinsic cellular properties and environmental factors. The success of anticancer chemotherapy is always hindered by drug resistance, which acts as a defensive mechanism that the tumor cells cultivate against chemotherapeutic drugs.[1] Multidrug resistance (MDR) is a type of acquired drug resistance to multiple classes of structurally and mechanistically unrelated anticancer drugs.[2] The resistance occurs due to overexpression of membrane-associated transport proteins known as ATP-binding cassette (ABC) transporters, which escalate efflux of chemotherapeutic drugs from tumor cells.[3] ABC transporters mainly consist of breast cancer (BCRP/ABCG2), multidrug resistance protein 1 (MRP/ABCC1) and P-glycoprotein (P-gp/ABCB1). Among them, P-gp is the most studied of the ABC transporters and plays an important role in drug efflux.[4,5]

P-gp is quantified in various crucial tissues and blood–tissue barriers, where it plays important physiological roles such as extrusion of exogenous and endogenous toxic agents that enter the body and direct of the secretion of lipophilic molecules.[6] However, P-gp is overexpressed in tumor cells as a result of regulation of the human gene expression of MDR1 that causes an accelerated efflux of the chemotherapeutic drugs, inducing classical multidrug resistance

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(MDR).[7] P-gp uses ATP hydrolysis for energy to pump drugs out of the cell and has various substrate specificities.[8] Coadministration of a P-gp inhibitor together with a primary chemotherapeutic agent is suggested as an effective approach to overcome MDR.[9] Over many decades, three generations of P-gp inhibitors have been developed: verapamil (first generation),[10] dexverapamil (second generation),[11] and tariquidar (third generation[12]; Figure 1). However, there is no P-gp inhibitor that has been approved for clinical use due to their poor potency, unsatisfactory toxicity, and low selectivity properties.[13]

For designing new MDR reversal agents, we selected tariquidar as the lead compound and designed the structure of triazole as bioisosteres to replace benzene, the amido bond was modified by the secondary amine. We linked various chemical structures with aromatic amides through the click chemistry method.[14] The 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 (Figure 2). Click chemistry, commonly copper(I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes, has been widely applied in drug discovery.[15] Applying the procedure illustrated, we synthesized and biologically evaluated compounds **1–18** as P-gp-mediated MDR reversal agents.[16]

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthetic routes of the target compounds **1–18** are delineated in Scheme 1. The detailed synthesis procedures are shown in Section 4 (Table 1Aa and 1Ab).

2.2 | Biological evaluation

2.2.1 | Cytotoxicity assay on K562 and K562/A02 cells

The cytotoxicity of the synthesized compounds towards K562 and doxorubicin (DOX)-resistant K562/A02 cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.[17,18] As shown in Table 2, most of the tested compounds showed no cytotoxicity to K562 and K562/A02 cells, their IC_{50} values were greater than 40 μM. Compounds **6**, **8**, **13**, VRP, and tariquidar show weak cytotoxicity to K562 cells. The IC_{50} value of DOX in resistant K562/A02 cells was almost 46-fold that in K562 cells.

2.2.2 | Effects of the target compounds on reversing DOX resistance in K562/A02 cells

According to the cytotoxicity assay results, the tested compound shows no cytotoxicity to K562 and K562/A02 cells at a concentration of 5 μM. The properties of the synthesized compounds on reversing DOX resistance towards human erythroleukemia adriamycin-resistant K562/A02 cells (P-gp overexpression) were tested by MTT assay at such a concentration. The results are shown in Table 3. Among them, compounds **6**, **10**, **11**, **12** show obvious reversal activity towards K562/A02 cells. And compound **11** is much better than the positive-control VRP and similar to tariquidar. The reversal fold (RF) value of compound **11** reached 18.7.

2.2.3 | Chemosensitizing effect of compound 11

Furthermore, we investigated compound **11** for its reversal potency and dose–response effects using different concentrations such as 20, 10, 5, 2.5, 1.25, 0.625, 0.31, 0.156, and 0.078 μM towards K562/A02 cells by MTT assay taking VRP as a positive control. As shown in Table 4, compound **11** displayed a dose-dependent reversal activity. Moreover, the EC_{50} value of compound **11** was 590.6 ± 3.7 nM (Figure 3), which was premeditated using GraphPad Prism 5.0 software from the dosage–response curves. The outcomes suggested that compound **11** had significant potential to develop the sensitivity of P-gp overexpressing cells to anticancer drug substrates in a dose-dependent manner.

2.2.4 | Duration of MDR reversal effect of compound 11 for DOX in K562/A02 cells

The desired P-gp inhibitor should possess a lasting effect over a certain period. The duration of the MDR reversal effect was accessed to verify the reversal activity of compound **11**. Data in Table 5 shows that the MDR reversal effect of positive-control VRP did not last more than 6 hr. However, compound **11** presented reversal activity even after its removal from the medium for 12 hr

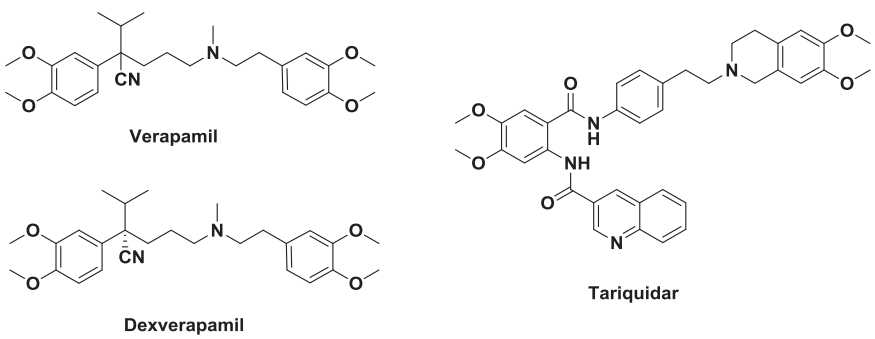


FIGURE 1 Structures of the known P-glycoprotein inhibitors

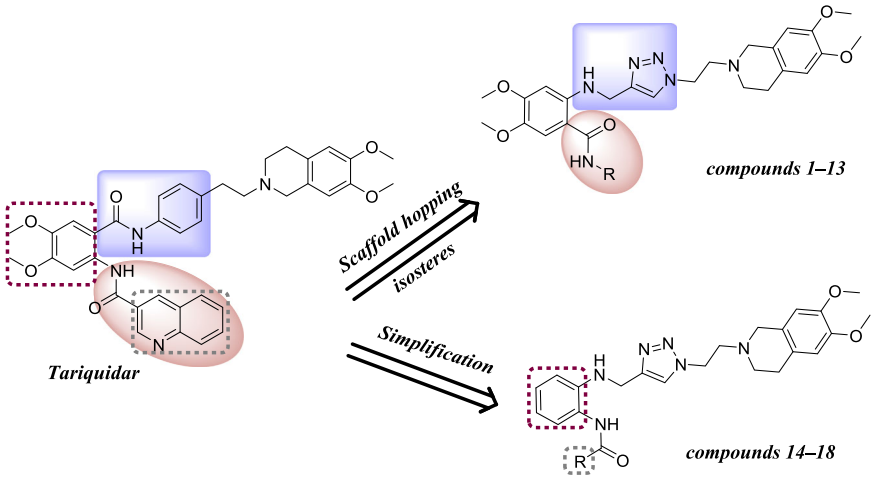


FIGURE 2 Design of the target compounds

and the IC_{50} was 8.65 ± 0.64 μM (RF = 2.96). These data illustrate that compound **11** displayed a potent MDR reversing effect and persevered for a longer time compared with the positive-control VRP.

2.2.5 | Effect of compound 11 on DOX accumulation in K562/A02 cells

To confirm our results, we assessed the accumulation of doxorubicin as a fluorescence substrate of P-gp in K562/A02 cells by fluorescence spectrophotometry.[19,20] As detailed in Figure 4, DOX accumulation in DOX-sensitive K562 cells is far more than in DOX-resistant K562/A02 cells. Compared with blank control, DOX accumulation increased in varying degrees after treatment with VRP or compound **11**. And even at the low concentration of 0.5 μM, compound **11** still increased DOX accumulation in K562/A02 cells. These data illustrated that compound **11** could inhibit the function of P-gp, causing DOX accumulation.

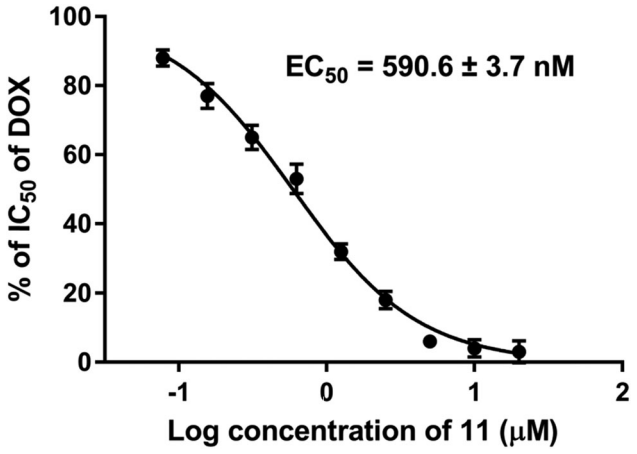


FIGURE 3 EC_{50} value of compound **11** in reversing doxorubicin (DOX)-resistance in K562/A02 cells. The percentage of DOX IC_{50} was charted with a log concentration of **11**, which is equal to (DOX IC_{50} in each modulator concentration/DOX IC_{50} without modulator) × 100%. Thus, EC_{50} can be noted for the modulator concentration, which can diminish the DOX IC_{50} by 50%

2.2.6 | Inhibitory effect of compound 11 on the P-gp efflux function

For further verification of our assumption, the inhibitory effect of compound **11** on the efflux function of P-gp was analyzed by spotting the reserved intracellular rhodamine-123 (Rh123), a fluorescent substrate of P-gp. As indicated in Figure 5, the fluorescence intensity of Rh123 in P-gp overexpressed K562/A02 cells decreased significantly over time. When 5 μM VRP or compound **11** was added in K562/A02 cells, the degree of fluorescence intensity increased, which denotes VRP or compound **11** helps inhibit efflux of Rh123 out of cells. At the same concentration, the effect of compound **11** was better than that of VRP.

2.3 | Structure–activity relationships

The study of structure–activity relationship suggests that various substituents in R of compounds **1–18** could affect MDR reversal

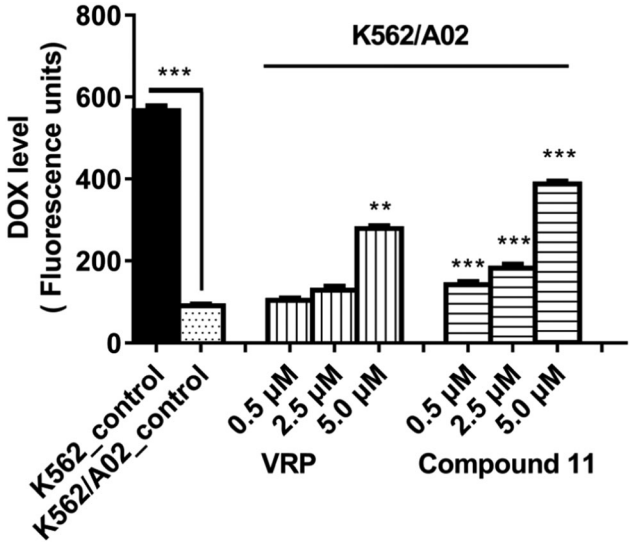


FIGURE 4 The effect of compound **11** on intracellular DOX accumulation in K562/A02 cells. The results are presented as the mean ± SD (standard deviation) for three independent experiments; $^{**}p < 0.01$, $^{*}p < 0.05$ relative to the negative control (K562/A02). DOX, doxorubicin; SD, standard deviation; VRP, verapamil

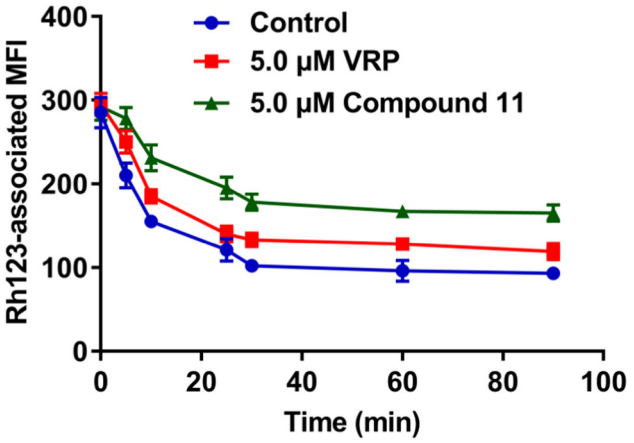


FIGURE 5 Effect of compound **11** on efflux of Rh123 from K562/A02. Each data point is presented as mean ± SD for three independent experiments. MFI, mean fluorescence intensity; Rh123, rhodamine-123; SD, standard deviation; VRP, verapamil

activities on K562/A02 cells. The electron-donating group in R can be of great importance for the activity. The preferred compound **11** possesses three methoxy groups, which contribute a lot to its good potency. Comparing compounds **1**–**13** with compounds **14**–**18**, the activity of the latter group is obviously worse than the former, which demonstrates 3,4-dimethoxy on the benzene ring can increase the reversal activity because more interaction with P-gp has occurred.

3 | CONCLUSION

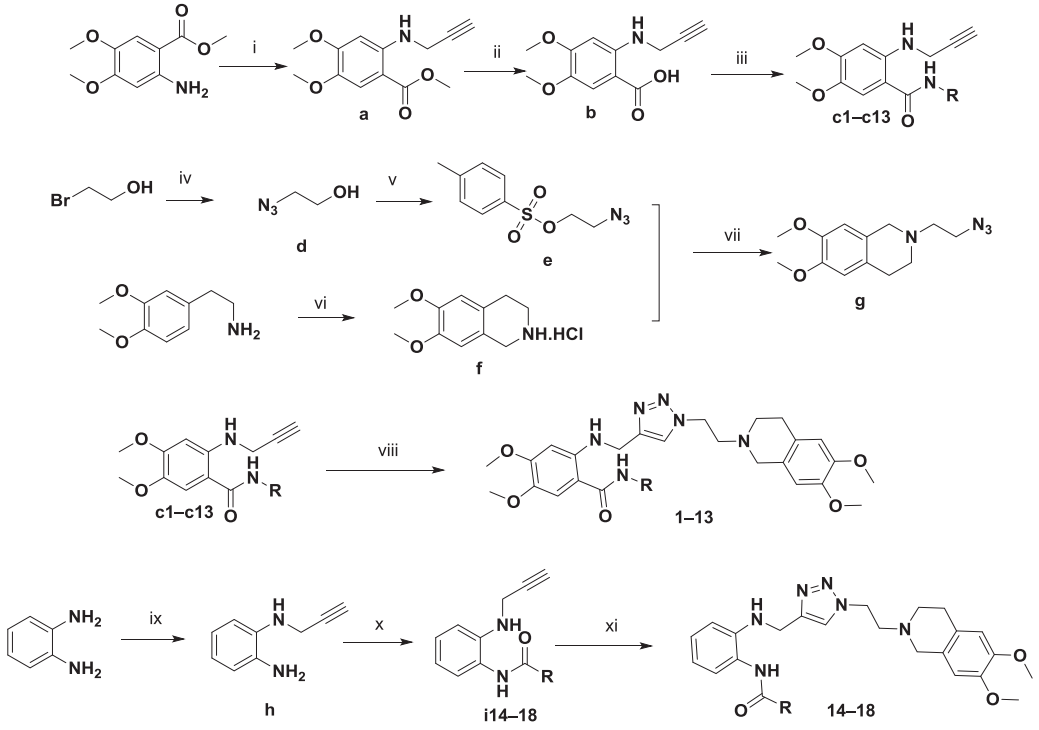
In this study, a new series of novel P-gp inhibitors has been designed, synthesized and evaluated for biological activity. Results demonstrated that a few of them possess a certain MDR reversal activity. In particular, among them, compound **11** shows a potent effect compared with the positive control. The EC_{50} value of compound **11** is 590.6 ± 3.7 nM. Compound **11** shows no obvious cytotoxicity towards K562 and K562/A02 cells. The MDR reversal effect of compound **11** could last over a certain period, longer than that of VRP. Compound **11** shows remarkable potency in increased accumulation of doxorubicin in K562/A02 cells. And it also obviously decreases P-gp-mediated efflux of Rh123 out of cells. In conclusion, compound **11** should be appropriate as a potential P-gp modulator for further study.

4 | EXPERIMENTAL

4.1 | Chemistry

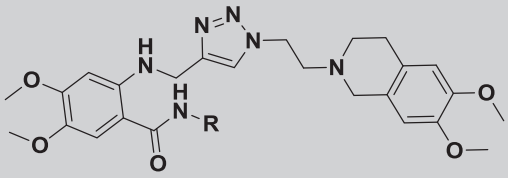
4.1.1 | General

The structures of the synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR spectroscopy (Bruker ACF-300Q, 300 MHz), with Me4Si as an internal standard and dimethyl sulfoxide (DMSO-d_6) as a solvent. Melting points were taken on an RY-1 melting-point apparatus. Purification by column chromatography was carried out



SCHEME 1 Synthesis of the target compounds. Reagents and conditions: (i) 3-bromoprop-1-yne, K_2CO_3, acetonitrile, 80°C, reflux, 6 hr; (ii) LiOH, 80% MeOH, 64°C, 1 hr; (iii) RNH_2, EDCI/HOBt, DCM r.t., 3–5 hr; (iv) NaN_3, water, 80°C, 24 hr; (v) TEA/DCM, TsCl, r.t., 24 hr; (vi) paraformaldehyde, EtOH, HCl, 78°C, reflux, 5 hr; (vii) TEA, acetonitrile, 60°C, 24 hr; (viii) 1.0 eq. g, ascorbate sodium, $CuSO_4$, 75% MeOH, 24–48 hr; (ix) 3-bromoprop-1-yne, K_2CO_3, acetonitrile, 80°C, reflux, 6 hr; (x) RCOOH, EDCI/HOBt, DCM r.t., 3–5 hr; (xi) 1.0 eq. g, ascorbate sodium, $CuSO_4$, 75% CH_3OH, 24–48 hr. r.t., room temperature

TABLE 1A Structures of targeted compounds **1–13**



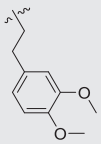
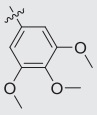
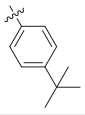
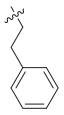
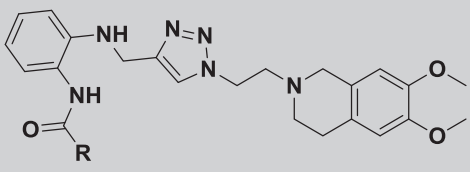
Compound	R	Compound	R
1		8	
2		9	
3		10	
4		11	
5		12	
6		13	
7			

TABLE 1B Structures of targeted compounds **14–18**



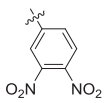
Compound	R	Compound	R
14		17	
15		18	
16			

TABLE 2 Cytotoxicity of compounds towards K562 and K562/A02 cell lines

Compounds	Cytotoxicity IC_{50} (μM)	
	K562	K562/A02
1	48.77 ± 1.21	>100
2	>100	>100
3	45.22 ± 1.33	>100
4	>100	>100
5	>100	>100
6	32.02 ± 1.25	>100
7	>100	>100
8	40.31 ± 3.05	>100
9	>100	>100
10	>100	>100
11	>100	>100
12	>100	>100
13	26.10 ± 1.53	>100
14	>100	>100
15	>100	>100
16	>100	>100
17	>100	>100
18	>100	>100
VRP	32.24 ± 2.89	35.13 ± 2.05
Tariquidar	20.78 ± 1.44	33.11 ± 3.66
DOX	0.53 ± 0.06	24.31 ± 0.86

Abbreviations: DOX, doxorubicin; RF, reversal fold; VRP, verapamil.

over silica gel (100–200 or 200–300 mesh); the reactions were monitored by thin-layer chromatography on GF/UV254 plates and were visualized using UV light at 254 or 365 nm. The electrospray ionization mass spectrometry (ESI–MS) data were recorded on Waters ACQUITY UPLC systems with mass (Waters, Milford, MA). All chemical reagents were obtained from commercial sources and used without purification unless otherwise indicated.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | General procedure for preparation of compounds 1–18

The synthetic routes to target compounds **1–18** are shown in Scheme 1. For compounds **1–13**, first, methyl 2-amino-4,5-dimethoxybenzoate and 3-bromo-prop-1-yne were refluxed in a mixture of acetonitrile and potassium carbonate for 6 hr to afford compound **a**. Second, compound **a** was reacted with lithium hydroxide in 75% methanol to get compound **b**, which was then treated with the substituted amine (DIEA) in dry dichloromethane, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBT) to give compounds **c1–c13**. 2-Bromoethanol and sodium azide in water were stirred at 80°C for 24 hr, and then

TABLE 3 DOX resistance reversal activity of the target compounds **1–18** at 5 μM concentration in K562/A02 cells

Compound (5.0 μM)	IC_{50}/DOX (μM)	RF	Compound (5.0 μM)	IC_{50}/DOX (μM)	RF
1	26.13 ± 0.56	0.93	**12**	3.72 ± 0.24	6.53
2	19.32 ± 0.18	1.26	**13**	16.26 ± 0.25	1.50
3	18.85 ± 0.65	1.29	**14**	15.63 ± 0.14	1.56
4	39.85 ± 0.84	0.61	**15**	10.35 ± 0.39	2.35
5	14.82 ± 0.45	1.64	**16**	9.25 ± 0.26	2.63
6	4.23 ± 0.39	5.60	**17**	18.20 ± 0.33	1.34
7	22.92 ± 0.48	1.06	**18**	9.33 ± 0.86	2.61
8	7.51 ± 0.36	3.24	VRP	5.23 ± 0.26	4.66
9	6.22 ± 0.33	3.91	Tariquidar	1.42 ± 0.15	17.12
10	5.12 ± 0.25	4.75	Control	24.31 ± 0.86	1
11	1.30 ± 0.35	18.7			

Abbreviations: DOX, doxorubicin; RF, reversal fold; VRP, verapamil.

TABLE 4 Sensitization of compound **11** on reversing MDR toward K562/A02 cells at different concentrations

Compound	IC_{50} of DOX (μM)	RF
None	24.31 ± 0.86	1
VRP, 5 μM	5.23 ± 0.26	4.66
Compound **11**, 20 μM	0.73 ± 0.29	33.30
Compound **11**, 10 μM	0.97 ± 0.14	25.06
Compound **11**, 5 μM	1.42 ± 0.15	17.12
Compound **11**, 2.5 μM	4.37 ± 0.34	5.56
Compound **11**, 1.25 μM	7.78 ± 0.74	3.12
Compound **11**, 0.625 μM	12.88 ± 0.31	1.89
Compound **11**, 0.31 μM	15.80 ± 0.29	1.54
Compound **11**, 0.156 μM	18.71 ± 0.53	1.30
Compound **11**, 0.078 μM	21.39 ± 0.28	1.14

Abbreviations: DOX, doxorubicin; MDR, multidrug resistance; RF, reversal fold.

extracted with ethyl acetate. The solvent was removed under vacuum to get compound **d**. Compound **d** was reacted with 4-toluenesulfonylchloride and triethylamine in dry dichloromethane to get compound **e**. 3,4-Dimethoxyphenethylamine, paraformaldehyde, and absolute ethanol were firstly stirred at room temperature for 3 hr, the mixture was adjusted to pH = 2 with concentrated hydrochloric acid, then refluxed for 4 hr, and then cooled down to afford compound **f**. To a solution of compound **e** in dry acetonitrile and triethylamine, compound **f** was added for 24 h-reflux. Column chromatography on a silica gel column (EtOAc/PE 3:2 to EtOAc/MeOH 16:1) was used to give compound **g**.[16,21,22] For compounds **14–18**, *o*-phenylenediamine and 3-bromo-prop-1-yne were refluxed in a mixture of acetonitrile and potassium carbonate for 6 hr to afford compound **h**, which was then treated with the substituted benzoic acid (DIEA) in dry dichloromethane, followed by EDCI and HOBT to give compounds **i14–i18**. To the solution of **c1–13** or **i14–18** (1 mmol) and intermediates **g** (1 mmol) in 75% methanol (40 ml), ascorbate sodium (30 mg), and $CuSO_4$ (10 mg) were added for stirring at room temperature for 24–48 hr. The crude product was purified by chromatography on silica gel utilizing a mixture of chloroform and acetone (80:1, v/v) as eluent to furnish the desired target compounds **1–18**.

TABLE 5 Duration of MDR reversal in K562/A02 cell after incubation and washout of VRP or compound **11**

Treatment schedule	IC_{50}/DOX (μM) (RF)		
	Control	VRP (5 μM)	Compound 11 (5 μM)
No wash	25.59 ± 1.87 (1.00)	3.55 ± 0.61 (7.21)	1.31 ± 0.12 (19.53)
Wash 0 hr	nd[b]	12.54 ± 1.93 (2.04)	2.35 ± 0.17 (10.89)
Wash 6 hr	nd	nd	5.31 ± 0.34 (4.82)
Wash 12 hr	nd	nd	8.65 ± 0.64 (2.96)

Abbreviations: DOX, doxorubicin; MDR, multidrug resistance; RF, reversal fold; VRP, verapamil.

*N-Benzyl-2-(((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxybenzamide (**1**)*

Yield: 75.3%; brown powder; mp: 145–147°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.70 (s, 1H), 8.42 (s, 1H), 8.00 (s, 1H), 7.41–7.17 (m, 6H), 6.60 (d, J = 12.3 Hz, 2H), 6.41 (s, 1H), 4.54 (s, 2H), 4.40 (s, 4H), 3.71 (d, J = 13.7 Hz, 12H), 3.49 (s, 2H), 2.88 (s, 2H), 2.64 (s, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 167.25, 151.82, 147.22, 147.18, 145.32, 142.51, 141.04, 138.30, 128.75, 127.56, 127.28, 127.17, 126.31, 121.32, 114.18, 112.52, 111.32, 111.01, 101.01, 56.35, 56.17, 56.08, 56.00, 55.99, 54.58, 50.81, 48.90, 44.82, 36.64, and 28.77. ESI–MS m/z: 587.1 $[M+H]^+$. Anal. calcd. for $C_{32}H_{38}N_6O_5$: C, 65.51; H, 6.53; N, 14.32; Found: C, 65.54; H, 6.51; N, 14.34.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-N-(3,4-dimethoxyphenethyl)-4,5-dimethoxybenzamide (**2**)*

Yield: 72.8%; brown powder; mp: 142–143°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.33 (t, J = 5.3 Hz, 1H), 8.20 (d, J = 4.5 Hz, 1H), 8.02 (s,

1H), 7.15 (s, 1H), 6.91–6.79 (m, 2H), 6.74 (d, J = 8.1 Hz, 1H), 6.60 (d, J = 11.3 Hz, 2H), 6.40 (s, 1H), 4.55 (t, J = 6.0 Hz, 2H), 4.39 (d, J = 5.3 Hz, 2H), 3.86–3.65 (m, 19H), 3.54 (s, 2H), 2.89 (t, J = 6.0 Hz, 2H), 2.74 (t, J = 7.3 Hz, 2H), 2.66 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 167.57, 152.12, 149.20, 147.89, 147.19, 147.14, 145.42, 142.81, 141.02, 132.23, 127.27, 126.55, 121.33, 121.31, 114.33, 112.47, 112.29, 112.20, 111.54, 111.29, 101.04, 56.37, 56.16, 56.01, 55.99, 55.97, 55.91, 54.59, 50.65, 48.86, 40.67, 36.64, 34.74, and 28.78. ESI–MS m/z: 661.3 $[M+H]^+$. Anal. calcd. for $C_{35}H_{44}N_6O_7$: C, 63.62; H, 6.71; N, 12.72; Found: C, 63.54; H, 6.65; N, 12.70.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxy-N-(4-methoxyphenyl)benzamide (**3**)*

Yield: 64.5%; brown powder; mp: 153–156°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.70 (s, 1H), 8.09 (t, J = 5.4 Hz, 1H), 8.02 (s, 1H), 7.52 (d, J = 8.9 Hz, 2H), 7.33 (s, 1H), 6.90 (d, J = 8.9 Hz, 2H), 6.59 (d, J = 10.3 Hz, 2H), 6.44 (s, 1H), 4.54 (t, J = 6.0 Hz, 2H), 4.42 (d, J = 5.3 Hz, 2H), 3.75 (d, J = 7.0 Hz, 9H), 3.67 (s, 6H), 3.52 (s, 2 H), 2.88 (t, J = 6.0 Hz, 2H), 2.64 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.11, 156.59, 152.02, 147.22, 147.18, 145.27, 143.19, 141.01, 133.57, 127.17, 126.60, 122.97, 121.32, 114.96, 114.34, 112.52, 111.63, 111.01, 101.07, 56.35, 56.16, 56.00, 55.99, 55.35, 54.58, 50.65, 48.86, 36.64, and 28.76. ESI–MS m/z: 663.3 $[M+H]^+$. Anal. calcd. for $C_{32}H_{38}N_6O_6$: C, 63.77; H, 6.36; N, 13.94; Found: C, 63.74; H, 6.32; N, 13.89.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-N-isobutyl-4,5-dimethoxybenzamide (**4**)*

Yield: 58.4%; brown powder; mp: 156–158°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.31 (t, J = 5.3 Hz, 1H), 8.14 (t, J = 5.5 Hz, 1H), 8.01 (s, 1H), 7.21 (s, 1H), 6.60 (d, J = 13.3 Hz, 2H), 6.39 (s, 1H), 4.55 (t, J = 6.0 Hz, 2H), 4.38 (d, J = 5.2 Hz, 2H), 3.71 (d, J = 11.0 Hz, 12H), 3.53 (s, 2H), 3.00 (t, J = 6.2 Hz, 2H), 2.89 (t, J = 6.0 Hz, 2H), 2.65 (s, 2H), 1.81 (dt, J = 13.3, 6.6 Hz, 1H), 0.87 (d, J = 6.6 Hz, 8H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 167.81, 151.82, 147.23, 147.18, 145.39, 142.57, 141.04, 127.25, 126.35, 121.33, 114.18, 112.52, 111.28, 111.02, 101.02, 56.38, 56.18, 56.08, 56.06, 56.04, 54.58, 50.86, 48.90, 47.69, 36.64, 28.79, 28.23, and 20.12. ESI–MS m/z: 553.5 $[M+H]^+$. Anal. calcd. for $C_{29}H_{40}N_6O_5$: C, 63.02; H, 7.30; N, 15.21; Found: C, 63.10; H, 7.35; N, 15.17.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxy-N-phenylbenzamide (**5**)*

Yield: 72.5%; brown powder; mp: 142–144°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.81 (s, 1H), 8.05 (d, J = 8.9 Hz, 2H), 7.63 (d, J = 7.8 Hz, 2H), 7.40–7.23 (m, 3H), 7.07 (t, J = 7.2 Hz, 1H), 6.59 (d, J = 9.8 Hz, 2H), 6.46 (s, 1H), 4.48 (2d, J = 5.3 Hz, 4H), 3.82–3.59 (m, 12H), 3.52 (s, 2H), 2.87 (d, J = 5.6 Hz, 2H), 2.64 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.11, 152.02, 147.22, 147.18, 145.27, 143.19, 141.04, 138.68, 128.80, 127.27, 126.35, 124.13, 121.33, 121.11, 114.80, 112.52, 111.63, 111.02, 101.07, 56.37, 56.17, 56.08, 56.03, 56.02, 54.58, 50.81, 48.90, 36.64, and 28.77. ESI–MS m/z: 573.6 $[M+H]^+$. Anal. calcd. for $C_{31}H_{36}N_6O_5$: C, 65.02; H, 6.34; N, 14.68; Found: C, 65.09; H, 6.28; N, 14.65.

*N-(4-Bromophenyl)-2-(((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxybenzamide (**6**)*

Yield: 70.5%; brown powder; mp: 149–151°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.92 (s, 1H), 8.05 (d, J = 8.5 Hz, 2H), 7.57 (2d, 7.7 Hz, 4H), 7.34 (s, 1H), 6.59 (d, J = 9.0 Hz, 2H), 6.46 (s, 1H), 4.49 (m, 4H), 3.96–3.56 (m, 12H), 3.52 (s, 2H), 2.88 (s, 2H), 2.64 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.11, 152.04, 147.22, 147.18, 145.27, 143.20, 141.04, 137.29, 131.50, 127.17, 126.60, 123.62, 121.32, 118.15, 114.80, 112.50, 111.66, 111.00, 101.20, 56.34, 56.16, 56.08, 56.00, 55.99, 54.58, 50.81, 48.86, 36.64, and 28.76. ESI–MS m/z: 652.4 $[M+H]^+$. Anal. calcd. for $C_{31}H_{35}BrN_6O_5$: C, 57.15; H, 5.41; N, 12.90; Found: C, 57.19; H, 5.38; N, 12.84.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxy-N-phenethylbenzamide (**7**)*

Yield: 62.3%; brown powder; mp: 156–157°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.32 (d, J = 5.0 Hz, 1H), 8.23 (s, 1H), 8.02 (s, 1H), 7.24 (m, 6H), 6.60 (d, J = 10.8 Hz, 2H), 6.40 (s, 1H), 4.55 (t, J = 5.5 Hz, 2H), 4.39 (d, J = 5.0 Hz, 2H), 3.71 (d, J = 15.4 Hz, 12H), 3.54 (s, 2H), 3.45–3.37 (m, 2H), 2.85 (dt, J = 14.9, 6.4 Hz, 4H), 2.66 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 167.57, 151.82, 147.22, 147.18, 145.32, 142.51, 141.01, 138.74, 128.94, 128.76, 127.17, 126.79, 126.60, 121.32, 114.45, 112.52, 111.32, 111.01, 101.01, 56.35, 56.16, 56.00, 55.99, 54.58, 50.65, 48.90, 40.82, 36.64, 34.78, and 28.76. ESI–MS m/z: 601.5 $[M+H]^+$. Anal. calcd. for $C_{33}H_{40}N_6O_5$: C, 65.98; H, 6.71; N, 13.99; Found: C, 65.94; H, 6.74; N, 13.91.

*N-(4-Chlorophenyl)-2-(((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxybenzamide (**8**)*

Yield: 71.2%; brown powder; mp: 142–144°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.94 (s, 1H), 8.19–8.02 (m, 2H), 7.85 (s, 1H), 7.60 (d, J = 8.2 Hz, 1H), 7.41–7.32 (m, 2H), 7.12 (d, J = 7.9 Hz, 1H), 6.59 (d, J = 8.6 Hz, 2H), 6.47 (s, 1H), 4.55 (t, J = 5.8 Hz, 2H), 4.45 (d, J = 5.1 Hz, 2H), 3.82–3.64 (m, 12H), 3.53 (s, 2H), 2.89 (t, J = 5.8 Hz, 2H), 2.65 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.11, 152.02, 147.22, 147.18, 145.27, 143.19, 141.04, 137.07, 128.62, 127.84, 127.17, 126.60, 122.50, 121.32, 114.80, 112.52, 111.63, 111.01, 101.07, 56.35, 56.16, 56.08, 56.00, 55.99, 54.58, 50.81, 48.86, 36.64, and 28.76. ESI–MS m/z: 607.1 $[M+H]^+$. Anal. calcd. for $C_{31}H_{35}ClN_6O_5$: C, 61.33; H, 5.81; N, 13.84; Found: C, 61.38; H, 5.74; Cl, 5.81; N, 13.79.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-N-(4-fluorophenyl)-4,5-dimethoxybenzamide (**9**)*

Yield: 59.3%; brown powder; mp: 150–151°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.86 (s, 1H), 8.05 (d, J = 8.0 Hz, 2H), 7.64 (dd, J = 8.6,

5.2 Hz, 2H), 7.34 (s, 1H), 7.16 (t, *J* = 8.8 Hz, 2H), 6.60 (d, *J* = 10.3 Hz, 2H), 6.46 (s, 1H), 4.55 (t, *J* = 5.7 Hz, 2H), 4.43 (d, *J* = 5.0 Hz, 2H), 3.73 (dd, *J* = 16.8, 9.4 Hz, 12H), 3.53 (s, 2H), 2.89 (t, *J* = 5.8 Hz, 2H), 2.65 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.11, 160.53, 158.51, 152.02, 147.22, 147.18, 145.27, 143.19, 141.04, 135.74, 135.71, 127.17, 126.31, 123.07, 123.01, 121.32, 115.75, 115.59, 115.00, 112.52, 111.63, 111.01, 101.07, 56.35, 56.17, 56.08, 56.00, 55.99, 54.58, 50.81, 48.90, 36.64, and 28.76. ESI–MS *m/z*: 591.5 $[M+H]^+$. Anal. calcd. for $C_{31}H_{35}FN_6O_5$: C, 63.04; H, 5.97; N, 14.23; Found: C, 63.08; H, 5.94; N, 14.27.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxy-N-(o-tolyl)benzamide (**10**)*

Yield: 68.1%; brown powder; mp: 147–149°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.77 (s, 1H), 8.06 (d, *J* = 18.1 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 4.8 Hz, 3H), 6.69–6.53 (m, 2H), 6.45 (s, 1H), 4.62–4.34 (m, 4H), 3.84–3.59 (m, 12H), 3.52 (s, 2H), 3.36 (s, 3H), 2.88 (s, 2H), 2.64 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.11, 152.02, 147.22, 147.18, 145.14, 143.19, 141.04, 135.08, 133.00, 129.67, 127.96, 127.17, 126.31, 124.53, 121.32, 120.24, 114.90, 112.52, 111.63, 111.01, 101.07, 56.35, 56.17, 56.08, 56.00, 55.99, 54.58, 50.81, 48.90, 36.64, 28.77, and 17.76. ESI–MS *m/z*: 587.6 $[M+H]^+$. Anal. calcd. for $C_{32}H_{38}N_6O_5$: C, 65.51; H, 6.53; N, 14.32; Found: C, 65.47; H, 6.51; N, 14.28.

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

Yield: 58.6%; brown powder; mp: 148–150°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.74 (s, 1H), 8.03 (d, *J* = 4.1 Hz, 2H), 7.32 (s, 1H), 7.07 (d, *J* = 3.9 Hz, 2H), 6.66–6.54 (m, 2H), 6.47 (s, 1H), 4.60–4.39 (m, 4H), 3.83–3.69 (m, 12H), 3.52 (s, 2H), 2.88 (s, 2H), 2.63 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.45, 154.18, 152.33, 147.19, 147.14, 145.23, 143.25, 141.02, 135.33, 133.24, 127.27, 126.55, 121.31, 114.81, 112.47, 111.60, 111.54, 101.28, 101.09, 60.78, 56.37, 56.34, 56.16, 56.01, 55.99, 54.59, 50.65, 48.86, 36.64, and 28.78. ESI–MS *m/z*: 663.6 $[M+H]^+$. Anal. calcd. for $C_{34}H_{42}N_6O_8$: C, 61.62; H, 6.39; N, 12.68; Found: C, 61.58; H, 6.35; N, 12.67.

*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)methyl)amino)-4,5-dimethoxybenzamide (**12**)*

Yield: 71.1%; brown powder; mp: 158–159°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.53 (s, 1H), 8.23 (d, *J* = 5.3 Hz, 1H), 8.02 (s, 1H), 7.43 (s, 1H), 7.31–7.11 (m, 4H), 6.60 (d, *J* = 12.6 Hz, 2H), 6.46 (s, 1H), 4.54 (t, *J* = 6.0 Hz, 2H), 4.41 (d, *J* = 5.2 Hz, 2H), 3.78–3.67 (m, 12H), 3.53 (s, 2H), 2.88 (t, *J* = 6.0 Hz, 2H), 2.64 (s, 4H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.11, 152.14, 147.21, 147.17, 146.10, 145.47, 143.24, 141.02, 136.98, 127.27, 126.55, 125.55, 121.31, 120.51, 114.81, 112.47, 111.61, 111.01, 101.09, 56.35, 56.16, 56.00, 55.99, 54.59, 50.65, 48.86, 36.64, 34.55, 31.20, and 28.78. ESI–MS *m/z*: 629.6 $[M+H]^+$. Anal. calcd. for $C_{35}H_{44}N_6O_5$: C, 66.86; H, 7.05; N, 13.37; Found: C, 66.81; H, 7.10; N, 13.34.

*2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-4,5-dimethoxy-N-(2-methoxyphenyl)benzamide (**13**)*

Yield: 69.3%; brown powder; mp: 146–148°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.25 (s, 1H), 8.19–7.92 (m, 2H), 7.57 (d, *J* = 6.3 Hz, 1H), 7.35 (d, *J* = 5.4 Hz, 1H), 7.19–6.86 (m, 3H), 6.68–6.52 (m, 2H), 6.45 (d, *J* = 5.4 Hz, 1H), 4.64–4.36 (m, 4H), 3.98–3.63 (m, 15H), 3.52 (s, 2H), 2.86 (d, *J* = 5.2 Hz, 2H), 2.64 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.33, 152.02, 150.67, 147.22, 147.18, 145.14, 143.19, 141.01, 128.26, 127.17, 126.60, 125.56, 123.00, 121.32, 121.31, 114.98, 114.37, 112.52, 111.61, 111.01, 101.00, 56.35, 56.16, 56.00, 55.99, 55.69, 54.58, 50.65, 48.86, 36.64, and 28.76. ESI–MS *m/z*: 603.5 $[M+H]^+$. Anal. calcd. for $C_{32}H_{38}N_6O_6$: C, 63.77; H, 6.36; N, 13.94; Found: C, 63.75; H, 6.34; N, 13.98.

*N-(2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)phenyl)-3,4-dinitrobenzamide (**14**)*

Yield: 73.2%; brown powder; mp: 151–153°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 10.35 (s, 1H), 9.19 (s, 2H), 9.01 (s, 1H), 7.94 (s, 1H), 7.15 (d, *J* = 7.4 Hz, 1H), 7.11–6.99 (m, 1H), 6.85–6.51 (m, 4H), 5.88 (s, 1H), 4.51 (s, 2H), 4.37 (d, *J* = 4.6 Hz, 2H), 3.70 (d, *J* = 17.6 Hz, 6H), 3.51 (s, 2H), 2.86 (s, 2H), 2.63 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.45, 147.22, 147.18, 144.30, 142.68, 141.01, 138.81, 137.96, 131.00, 128.18, 127.17, 126.71, 126.60, 125.92, 123.72, 122.39, 121.44, 121.32, 115.78, 112.52, 111.01, 56.16, 56.00, 55.99, 54.58, 50.65, 48.90, 36.52, and 28.76. ESI–MS *m/z*: 603.5 $[M+H]^+$. Anal. calcd. for $C_{29}H_{30}N_8O_7$: C, 57.80; H, 5.02; N, 18.60; Found: C, 57.73; H, 5.06; N, 18.54.

*N-(2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)phenyl)-4-isocyanobenzamide (**15**)*

Yield: 68.4%; brown powder; mp: 155–157°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.90 (s, 1H), 8.13 (d, *J* = 8.0 Hz, 2H), 8.00 (d, *J* = 7.9 Hz, 2H), 7.92 (s, 1H), 7.14 (d, *J* = 7.4 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.68–6.58 (m, 2H), 6.56 (s, 1H), 5.69 (s, 1H), 4.52 (d, *J* = 5.4 Hz, 2H), 4.34 (d, *J* = 5.5 Hz, 2H), 3.68 (d, *J* = 4.3 Hz, 6H), 3.50 (s, 2H), 2.85 (t, *J* = 5.4 Hz, 2H), 2.62 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.56, 165.47, 147.23, 147.18, 141.03, 138.01, 137.98, 128.46, 128.12, 127.25, 126.35, 126.23, 124.30, 123.73, 122.37, 121.49, 121.33, 115.79, 112.52, 111.02, 56.08, 56.07, 56.03, 54.58, 50.81, 48.90, 36.52, and 28.80. ESI–MS *m/z*: 538.5 $[M+H]^+$. Anal. calcd. for $C_{30}H_{31}N_7O_3$: C, 67.02; H, 5.81; N, 18.24; Found: C, 67.08; H, 5.84; N, 18.26.

*2-Chloro-N-(2-(((1-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)phenyl)benzamide (**16**)*

Yield: 63.2%; brown powder; mp: 148–150°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.76 (s, 1H), 7.96 (d, 1H), 7.68–7.57 (m, 1H), 7.56–7.37

(m, 3H), 7.30 (d, J = 7.6 Hz, 1H), 7.03 (d, J = 6.8 Hz, 1H), 6.76 (d, J = 7.8 Hz, 1H), 6.71–6.48 (m, 3H), 4.64–4.26 (m, 4H), 3.84–3.58 (m, 6H), 3.51 (s, 2H), 2.86 (s, 2H), 2.63 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.88, 147.23, 147.18, 141.04, 137.98, 134.27, 132.02, 131.92, 130.01, 129.52, 127.75, 127.25, 126.49, 126.35, 123.70, 122.35, 121.49, 121.33, 115.79, 112.52, 111.02, 56.08, 56.07, 56.03, 54.58, 50.86, 48.90, 36.52, and 28.93. ESI–MS m/z: 548.1 $[M+H]^+$. Anal. calcd. for $C_{29}H_{31}ClN_6O_3$: C, 63.67; H, 5.71; N, 15.36; Found: C, 63.64; H, 5.73; N, 15.34.

*N-(2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)phenyl)-2-methylbenzamide (**17**)*
Yield: 74.1%; brown powder; mp: 156–157°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.62 (s, 1H), 7.99 (d, J = 4.2 Hz, 1H), 7.55 (s, 1H), 7.47–7.20 (m, 4H), 7.03 (s, 1H), 6.77 (s, 1H), 6.70–6.52 (m, 3H), 4.63–4.29 (m, 4H), 3.68 (d, J = 4.0 Hz, 6H), 3.51 (s, 2H), 2.87 (d, J = 3.8 Hz, 2H), 2.63 (s, 4H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.92, 147.23, 147.18, 141.04, 137.98, 136.56, 133.71, 129.99, 129.21, 128.35, 127.75, 127.17, 126.31, 125.89, 123.73, 122.34, 121.49, 121.33, 115.79, 112.52, 111.02, 56.08, 56.05, 56.02, 54.58, 50.81, 48.90, 36.52, 28.96, and 19.98. ESI–MS m/z: 527.6 $[M+H]^+$. Anal. calcd. for $C_{30}H_{34}N_6O_3$: C, 68.42; H, 6.51; N, 15.96; Found: C, 68.45; H, 6.53; N, 15.92.

*N-(2-(((1-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)phenyl)-2-nitrobenzamide (**18**)*
Yield: 59.8%; brown powder; mp: 148–149°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.97 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.00–7.81 (m, 3H), 7.81–7.69 (m, 1H), 7.29 (d, J = 7.7 Hz, 1H), 7.09–6.95 (m, 1H), 6.75 (d, J = 8.1 Hz, 1H), 6.67–6.44 (m, 3H), 4.61–4.26 (m, 4H), 3.68 (d, J = 4.2 Hz, 6H), 3.50 (s, 2H), 2.86 (s, 2H), 2.56 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.68, 147.23, 147.18, 146.05, 141.03, 137.97, 131.37, 130.66, 130.59, 129.52, 127.75, 127.25, 126.35, 124.01, 123.71, 122.37, 121.44, 121.33, 115.79, 112.52, 111.02, 56.08, 56.04, 56.01, 54.58, 50.86, 48.90, 36.52, and 28.79. ESI–MS m/z: 558.6 $[M+H]^+$. Anal. calcd. for $C_{29}H_{31}N_7O_5$: C, 62.47; H, 5.60; N, 17.58; Found: C, 62.44; H, 5.64; N, 17.56.

4.2 | Biological evaluation

4.2.1 | Cytotoxicity assay

Initially, 1×10^4 K562 and K562/A02 cells were seeded into 96-well plates in RPMI-1640 and incubated for 24 hr. In the assay for cytotoxicity, a graded dose of the compounds diluted with the medium were added into cells. In the assay for drug-resistant modulation, 4 μM concentrations of the target compounds were added into cells, followed by the numerous concentrations of DOX. The cells were incubated for another 48 hr 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 hr at 37°C, the optical density (OD) at 490 nm was read on a microplate reader (Thermo Fisher Scientific). The IC_{50} value of the compounds for cytotoxicity was calculated using GraphPad Prism 6.0 software from the dose–response curves.

4.2.2 | Reversing DOX resistance assay

K562 and K562/A02 cells (1×10^5) were seeded into 96-well plates in RPMI-1640 and incubated for 24 hr. The target compound was prepared as 5 mM DMSO stocks, the cells were incubated in the presence of DOX with or without P-gp inhibitors for 48 hr. Then, MTT was added directly to the cells. After additional incubation for 4 hr at 37°C, the absorbance at 490 nm was read on a microplate reader (Thermo Fisher Scientific). The IC_{50} values of the compounds for cytotoxicity were calculated from the dose–response curves by using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA). Cell inhibition rate = 1 − (average OD of experimental group/average OD of the control group) × 100%. Reversal fold (RF) = IC_{50}A/IC_{50}B. IC_{50}A refers to IC_{50} of doxorubicin towards K562/A02 cells with no reversal agents existing. IC_{50}B refers to IC_{50} of doxorubicin towards K562/A02 cells with reversal agents existing.

4.2.3 | Duration of MDR reversal effect

K562/A02 cells (1×10^5 per well) were placed into 96-well plates in RPMI-1640 and incubated overnight. Then, the cells were incubated for 24 hr with compound **11** or VRP or none at the concentration of 5 μM being washed 0 or three times with growth medium. Then the cells were incubated for 0, 6, 12 or 24 hr before the addition of different concentrations of DOX or the vehicle. The incubation continued for 48 hr before MTT analysis. The IC_{50} values of the compounds for cytotoxicity were calculated by GraphPad Prism 6.0, software from dose–response curves.

4.2.4 | DOX accumulation on K562/A02 cells

K562 and K562/A02 cells (1×10^5) were incubated with 20.0 μM DOX and different concentrations of the tested compound and VRP for 150 min at 37°C, with 0.1% DMSO as a negative control. After incubation, the cells were washed with cold PBS and lysed with lysis buffer (0.75 M HCl, 0.2% Triton-X 100 in 2-propanol). The fluorescence level of DOX in the lysate was measured by using a fluorescence spectrophotometer (RF-5301 PC, Shimadzu) using an excitation and an emission wavelength pair of 460 and 587 nm.

4.2.5 | Rhodamine-123 efflux assay

K562/A02 cells (1×10^5) were incubated with medium containing 5 μM Rh123 at 37°C for 90 min, washed three times with Rh123-free medium, and then incubated in the presence or absence of different concentrations of compound **11**, VRP, at 37°C for 5, 10, 25, 30, 60, and 90 min, respectively. The mean fluorescence intensity (MFI) of the retained Rh123 in per 10,000 cells was measured by flow cytometry. Graphs were plotted of cell-associated MFI against time.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (Nos. 81872733, 81872733, and 81803353) and the Foundation of Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection (No. JKLBS2017011).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Qiu Q, Shi W, Zhao S, et al. Discovery to solve multidrug resistance: Design, synthesis, and biological evaluation of novel agents. *Arch Pharm Chem Life Sci*. 2019;e1900127. https://doi.org/10.1002/ardp.201900127