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Design, Synthesis and Evaluation of a Novel Series of Inhibitors Reversing P-Glycoprotein-Mediated Multidrug Resistance

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Abstract

Multidrug resistance (MDR) is still the main barrier to attaining effective results with chemotherapy. Discovery of new chemo-reversal agents is needed to overcome MDR. Our study focused on a better way to obtain novel drugs with triazole rings that have an MDR-

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reversal ability through click chemistry. Among 20 developed compounds, compound **19** had a minimal cytotoxic effect compared to tariquidar and verapamil (VRP) and showed a higher reversal activity than VRP through increased accumulation in K562/A02 cells. Compound **19** also played an important role in the P-gp efflux function of intracellular Rh123 and doxorubicin (DOX) accumulation in K562/A02 cells. Moreover, compound **19** exhibited a long lifetime of approximately 24 h. These results indicated that compound **19** is a potential lead compound for the design of new drugs to overcome cancer MDR.

***Keywords*:** MDR; P-gp inhibitor; Click chemistry; Reversal activity.

Introduction

Cancer has devastated the lives of many people worldwide, and multidrug resistance (MDR) is mainly accountable for the failure of chemotherapy in over 90% of metastatic cancer patients. Whether acquired during treatment or inherent with the disease being refractory to chemotherapy from the outset, [1-4] MDR is caused by reduced apoptosis, altered drug metabolism or DNA damage repair mechanisms and the active efflux of drugs by ATP-binding cassette (ABC) transporters. Overexpression of P-glycoprotein (P-gp) in cancer cells is the main aetiological reason for the enhancement of MDR.[5, 6]

P-glycoprotein (P-gp) is the primary human ABC drug transporter. It utilizes ATP hydrolysis for energy to pump drugs out of cells and has diverse substrate specificities. [7, 8] Co-administration of a P-gp inhibitor together with the primary chemotherapeutic agent is recommended as an effective strategy to overcome MDR. [9] Currently, three generations of P-gp inhibitors have been developed, with the first generation being VRP and cyclosporine, the second generation consisting of dexverapamil, valspodar (PSC 833), [10, 11] and the third generation being tariquidar[12] and WK-X-34[13] (**Figure 1**). However, a series of side effects due to high toxicity, low specificity and drug interactions have limited the development of first- and second-generation inhibitors. Third-generation MDR inhibitors have attracted attention due to their high potency and specificity.[4] To date, no P-gp inhibitors have been approved for therapeutic application.[14] Therefore, it is still imperative to develop novel P-gp inhibitors with better safety and higher activities for use against cancer MDR.

In this paper, we sought to develop potent P-gp inhibitors though structure modification by the introduction of a triazole ring to a potent third-generation P-gp inhibitor, tariquidar. In the experiments 1H-1,2,3-triazole rings were introduced as a bioisostere of the carboxamide group in the designed compounds through click chemistry methods commonly applied in drug discovery and amide reversal.[15, 16] Finally, we obtained a novel series of P-gp inhibitors with a triazol-phenethyl-tetrahydroisoquinoline scaffold (**Figure 2**). All of the target compounds were evaluated for cytotoxicity and the ability to reverse P-gp-mediated MDR against doxorubicin (DOX). Among the active compounds, compound **19** had low cytotoxic activity and potent reversal activity on resistant cancer cells, and this compound was chosen for further evaluation at different concentrations. Additionally, the in-depth studies demonstrated that compound **19** had an extraordinary inhibition effect on P-gp transport activity and persistent duration of its reversal effect, which indicates promising potential.

2. Chemistry

The synthetic routes to target compounds **1-20** are shown in **Scheme 1**. First, methyl 2-amino-4,5-dimethoxybenzoate and 3-bromo-prop-1-yne were refluxed in a mixture of DMF and potassium carbonate for 6 h 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 (CH_2Cl_2), followed by EDCI and HOBT to give compounds $\mathbf{c_1}$-$\mathbf{c_{20}}$. Compounds **d-f** were synthesized according to literature procedures with slight modifications. [16, 17] Compounds $\mathbf{c_1}$-$\mathbf{c_{20}}$ and compound **f** were treated with sodium ascorbate and copper sulfate in 75% methanol with stirring at room temperature for 24-48 h to provide compounds **1-20**. All compounds were purified by column chromatography. The structures of the target compounds obtained are listed in **Scheme 1**.

3. Results and discussion

3.1. Biological evaluation

3.1.1. Determination of the Intrinsic Cytotoxicity via MTT Assay.

A 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was used to evaluate the cytotoxicity of the newly synthesized compounds against human erythroleukaemia K562 cells and DOX-resistant K562/A02 cells. The chemotherapy drugs DOX, VRP and tariquidar were selected as controls. As shown in **Table 1**, VRP possessed weak cytotoxic effects towards K562 and K562/A02 cells with IC_{50} values of **70.13** and **61.51** μM, respectively. Tariquidar showed a high level of toxicity towards K562 cells (IC_{50} of **30.77** μM) and K562/A02 cells (IC_{50} of **28.8 μM**). In contrast, all of the synthesized compounds displayed no toxicity, with IC_{50} values higher than 100 μM for both types of cells (K562/A02 and K562).

3.1.2 Effects of the target compounds on reversal of DOX resistance in K562/A02 cells.

Based on the cytotoxicity testing experiment, all of the target compounds were found to have low cytotoxicity in the tested cell lines. To evaluate the activity of the target compounds, we tested their effects on reversal of DOX resistance in P-gp-overexpressing drug-resistant leukaemic K562/A02 cells by the MTT method. [18, 19] We selected verapamil (VRP) and tariquidar as positive controls. Compounds **1–20** and the positive controls were tested at 4 μM. The IC_{50} values of DOX combined with individual compounds against each tumour cell line are depicted in **Table 2**. The anticancer drug DOX alone demonstrated a slight effect on K562/A02 cells (half-maximal inhibitory concentration IC_{50} = **35.9±2.1** μM). However, co-administration of DOX and target compounds, VRP or tariquidar led to an increase of the inhibitory effects on K562/A02 cells to different extents, suggesting that all of the test compounds could reverse DOX resistance. Compound **19** showed the most potent reversal fold (RF = **31.2**), which was higher than that of VRP (RF = **8.2**). Therefore, compound **19** may represent a promising candidate for developing a P-gp-mediated MDR inhibitor.

3.2. Structure–activity relationships

Analysis of the structure-activity relationships (SARs) was based on the results in **Table 2**. Different substituents in the R groups of compounds 1-20 could affect the MDR reversal activities in K562/A02 cells. Compound **1** showed the least activity among the twenty compounds, and it can be concluded that the absence of a benzene ring led to a decrease in activity. Meanwhile, the presence of a benzene ring produced increases in efficiencies, such as that seen with compound **2.** It was also observed that compounds bearing electron-donating groups in R showed better MDR reversal activities than those with electron-withdrawing groups. For example, compounds **3**, **4**, **5** and **6**, which possessed electron-donating groups like $-CH_2CH_3$, $-CH(CH_3)_2$, $-CH_3$ and $-C(CH_3)_3$, showed higher MDR reversal activities. Additionally, the size of the substituent in the R group is likely to affect the MDR reversal activity. For example, compound **6**, containing a 4-tert-butyl group in R, was more potent than compounds **3**, **4** and **5**, which had other alkyl substituents. Interestingly, in spite of having the same molecular weight (**704, 8**) and the same alkyl substituent position, the activity of compounds **6** and **7** showed a slight difference produced from the arrangement of the carbon chain in which the 4-tertiary butyl group of compound **6** was more active than the n-butyl group of compound **7**.

The substitution position of the electron-donating group in R also influences the reversal activity, as in the effect seen with compounds **4** and **5**, which showed that substitution in the ortho position produced a more potent reversal effect than that of VRP. Moreover, compounds **8**, **9** and **10**, which were substituted by $-OCH_3$ in ortho, meta, and para positions showed lower MDR reversal activities than VRP. In addition, compound **11**, containing a 4,5-dimethoxy group in R, was more potent than compound **12**, which contained a 3,4,5-trimethoxy group. This difference may be related to the increased molecular weight. Compounds **13**, **14**, **15** and **16** with electron-withdrawing groups displayed less MDR reversal activity, and compound **17**, which was substituted by $-OCF_3$, presented poor MDR reversal activity.

The absence of substituent groups on the benzene ring produced increases in efficiencies such as those seen with compounds **18** and **19** (IC_{50}s = 1.76 and 1.51 μM), in which compound **19** showed the best activity. Structurally, compound **19** has a -CH2CH2 linker between the carbonyl group and R, but surprisingly, compound **18** with a single $-CH_2$ linker and

compound **20** with two methoxy groups on the benzene ring resulted in lower IC_{50} values of 2.34 and 1.92 μM, respectively, compared with that of compound **19**.

To investigate and further characterize the interaction between the target compound and P-gp, compound **19** was selected for a docking study with a model of P-gp (PDB code: 3G60). As shown in **Figure 3**, the interaction mode of compound **19** occurred in a small hydrophobic pocket produced by Tyr-949 and Phe-339.

3.1.3. Chemo-sensitizing effect of target compounds

Based on the above results, we chose the most potent compound **19** for further investigation of reversal potency and dose–response effects. We examined the reversal activity of compound **19** at numerous other concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156 and 0.078 μM) in K562/A02 cells by an MTT assay, for which we selected VRP as a positive control. [13] The results are summarized in **Table 3**. VRP displayed a slight modulating activity at 5.0 μM (RF = **14.7**). However, compound **19** showed apparent dose-dependent activity and still displayed potent MDR reversal activity (RF = **38.3**) when the concentration was decreased to 2.5 μM. Additionally, the EC_{50} value of compound **19** was 306±8.6 nM, which was calculated by GraphPad Prism 6.0 software from the dose-response curve as shown in **Figure 4**. The results suggested that compound **19** possessed significant potential to improve the sensitivity of P-gp-overexpression cells to anticancer drug substrates in a dose-dependent manner.

3.1.4. Duration of the MDR reversal effect of compound 19 for DOX in K562/A02 cells

It is obvious that an ideal P-gp inhibitor should have a relatively long duration of reversal action for safe and effective therapy of P-gp-mediated cancer MDR. [20] The assay was performed as reported previously. [11] We selected VRP as a positive control to determine the duration of the MDR reversal effect of compound **19**. The duration time results are displayed in **Table 4**. The reversal activity of 5.0 μM VRP totally disappeared after its removal from the medium, indicating that the action of VRP appeared to persist no more than 6 h. In contrast, a 5.0 μM concentration of compound **19** exhibited reversal activity even after its removal from the medium for 24 h, and the IC_{50} of DOX was 33.67 μM (RF = 1.78). These

data suggested that compound **19** revealed potent MDR-reversing effects that could persist for a longer time compared with those of the positive control, VRP.

3.1.5. Effect of target compound 19 on DOX accumulation

To confirm our assumption, we assessed the accumulation of DOX, a fluorescent substrate of P-gp, in K562/A02 cells by using laser confocal fluorescence microscopy. The classical P-gp inhibitor VRP was selected as a positive control, as shown in **Figure 5.** Sensitive K562 cells maintained most of the red fluorescence of DOX after incubation. In contrast, there was no fluorescence of DOX observed in P-gp overexpressing K562/A02 cells in the absence of P-gp inhibitors. This effect may have been due to the ability of P-gp to pump DOX out of the cells. When K562/A02 cells were treated with compound **19** and VRP, the accumulation of DOX was significantly increased in a dose-dependent manner, and the fluorescence intensity of K562/A02 cells treated with compound **19** was higher than that of cells treated with VRP at the same conditions. Therefore, the results indicated that compound **19** was more potent than VRP in inhibiting the drug efflux function of P-gp.

3.1.6. Inhibitory effect of compound 19 on the P-gp efflux function

Furthermore, to confirm whether compound **19** could reverse the P-gp-mediated drug efflux function, we selected rhodamine-123 (Rh123) as another fluorescent substrate of P-gp, which was photographed via laser confocal fluorescence microscopy. As demonstrated in **Figure 6**, the classical P-gp inhibitor VRP was chosen as a positive control. The sensitive K562 cells maintained most of the fluorescence of Rh123, while the K562/A02 cells (P-gp overexpression) did not show fluorescence of Rh123. In comparison, the fluorescence intensity of Rh123 in K562/A02 cells was obviously increased in a dose-dependent manner after treatment with compound **19** or VRP, but cells treated with compound **19** exhibited a much higher level of Rh123 fluorescence than those treated with VRP at the same doses. These results supported our presumption that compound **19** exhibited a stronger inhibitory effect on the P-gp-mediated drug efflux function than the classical P-gp inhibitor VRP under the same conditions.

4. Conclusions

In summary, we selected tariquidar as a lead compound, from which twenty compounds were newly designed and synthesized based on click chemistry. Our studies permitted us to identify a new compound as a highly potent P-gp modulator in cancer cells. Based on the results obtained, compound **19** exhibited a higher reversal activity than VRP through its increased accumulation in K562/A02 cells and by blocking its efflux from the cells, producing a much longer chemosensitizing effect (>24 h) than the known P-gp inhibitor VRP (<6 h) with reversibility. Moreover, the cytotoxic effects of compound **19** were negligible against all tested cell lines compared with those of tariquidar and VRP. Therefore, compound **19** can be considered promising for the development of P-gp-mediated MDR reversal agents.

5. Experimental section

5.1. Chemistry

The structures of the target 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) and $CDCl_3$ as the solvents. MS was performed on an 1100 LC/MSD spectrometer (Hewlett–Packard). Reactions were monitored by thin layer chromatography (TLC) on GF/UV 254 plates, and the chromatograms were visualized under UV light at 254 and 365 nm. After separation from the aqueous layers, the organic solvents were dried over anhydrous sodium sulfate. All reagents were of reagent grade, and all solvents were dried by standard 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 and are uncorrected. Compounds **d**-**f** were synthesized as previously described.[16, 17] The starting compound methyl 2-amino-4,5-dimethoxybenzoate was commercially available.

The physical characteristics, MS, 1H-NMR and 13C-NMR data for all target compounds, are reported in the **Supporting Information**.

5.2. Cytotoxicity assay

First, 1×104 K562 and K562/A02 cells were seeded into 96-well plates in RPMI-1640 and incubated for 24 h. In the assay of cytotoxicity, a graded dose of compounds diluted with medium were added into the wells. In the assay of drug resistant modulation, 4 μM concentrations of the target compounds were added into the wells, followed by numerous concentrations of DOX. The cells were incubated for another 48 h in an atmosphere of 95% air with 5% CO2 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 using GraphPad Prism 6.0 software from the dose–response curves.

5.3. MTT assay

K562/A02 cells were incubated in RPMI 1640 medium supplemented with 10% foetal bovine serum at 37 °C in a 5% CO2 humidified atmosphere. K562/A02 cells were seeded into 96-well plates at 1×10^4 cells per well. After 24 h of incubation, cells were treated with various concentrations of DOX in the absence or presence of target compounds for 48 h in an atmosphere of 95% air with 5% CO2 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 using GraphPad Prism 6.0 software from the dose–response curves. Experiments were repeated three times independently.

5.4. Duration of the MDR reversal

First, $1\sim2\times10^4$ K562/A02 cells per well were plated into 96-well plates and cultured overnight, then the cells were incubated for another 24 h with or without compound 19, VRP or tariquidar at the concentration of 5 μM before being washed 0 or 3 times with growth medium. Then, the cells were incubated for 0, 6, 12, or 24 h before addition of various concentrations of DOX or the vehicle. The incubation was continued for 48 h prior to MTT analysis. The IC_{50} values of the compounds for cytotoxicity were calculated by GraphPad Prism 6.0 software from the dose-response curves.

5.5. Accumulation of DOX

A laser confocal fluorescence microscope (FV 1000, Olympus, Japan) was used for in vitro cell fluorescence imaging. K562 and K562/A02 cells were seeded into a laser co-focusing dish at 1.5 $\times 10^5$ cells/well. Various concentrations of compound 19 and VRP (10 μM, 2 μM, and 0.2 μM) were preincubated with the cells for 60 min. Then, DOX was added into every well and incubated for 90 min. The cells were fixed using 4% paraformaldehyde. The DOX accumulation level was measured in photographs taken using a laser confocal fluorescence microscope.

5.6. Rhodamine123 efflux assay

A laser confocal fluorescence microscope (FV 1000, Olympus, Japan) was used for in vitro cell fluorescence imaging. K562 or K562/A02 cells were seeded into a laser co-focusing dish at 1.5 $\times 10^5$ cells/well and incubated with 5 μM Rh123 for 60 min before washing with ice-cold PBS three times. Then, the cells were incubated with or without different concentrations of compound 19 or VRP (10, 2, 0.2 μM) for another 90 min. The cells were fixed using 4% paraformaldehyde (MeCHO). The mean fluorescence intensity of the maintained intracellular Rh123 was assessed using a laser confocal fluorescence microscope.

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Figure 1. Structures of verapamil, dexverapamil, tariquidar and WK-X-34

Figure 2. Design of the target compounds.

Figure 3. Docking interaction of compound 19 with P-gp (PDB ID: 3G60)

Figure 4.EC_{50} for compound **19** in K562/A02 cells

Figure 5.The effects of the target compounds on the DOX accumulation in K562 or K562/A02 cells which were detected and photographed under a laser confocal fluorescence microscope. A1: K562 cells (DOX alone); A2: K562/A02 cells (DOX alone); B1-B3: K562/A02 cells DOX + numerous concentrations

of compound **19** (B1: 10 μM, B2: 2 μM, B3: 0.2 μM); C1-C3: K562/A02 cells DOX + numerous concentrations of VRP (C1: 10 μM, C2: 2 μM, C3: 0.2 μM).

Figure 6. The effects of the target compounds on the P-gp efflux function on intracellular Rh123 in K562 or K562/A02 cells which were detected and photographed under a laser confocal fluorescence microscope. D: K562 cells (Rh123 alone); D1: K562/A02 cells (Rh123 alone); E1-E3: K562/A02 cells Rh123 + numerous concentrations of compound **19** (E1: 10 μM, E2: 2 μM, E3: 0.2 μM); F1-F3: K562/A02 cells Rh123 + numerous concentrations of VRP (F1: 10 μM, F2: 2 μM, F3: 0.2 μM).

Scheme 1: Synthesis of the target compounds. Reagents and conditions: (i) 3-bromoprop-1-yne, K2CO3, DMF, reflux, 6 h; (ii) LiOH, 75% MeOH reflux, 3 h; (iii) substituted amine, HOBt, DIEA, DCM, EDCI, rt, 24 h; (iv) K2CO3, acetonitrile, reflux, 17 h; (v) H2/Pd/C, DCM/EtOH, rt, 24 h; (vi) NaNO2, 50% AcOH, 0–5 °C, 30 min; NaN3, 0–5 °C, 50 min; (vii), $\mathbf{c_1}$-$\mathbf{c_{20}}$ sodium ascorbate, CuSO4, 75% CH3OH, 24– 48 h.

Table 1. Cytotoxicity of compounds towards K562 and K562/A02 cell lines [a]

Table 2.DOX-resistance reversal activity of the target compounds **1–20** at 4 μM concentration in K562/A02 cells [a]

Table 3. Effect of compound 19 on reversal of MDR in K562/A02 cells at different concentrations [a]

Table 4. Duration of MDR reversal in K562/A02 cells after incubation and washout of verapamil, tariquidar and compound **19**

Conflicts of interest

There are no conflicts to declare.

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Table 1. Cytotoxicity of compounds towards K562 and K562/A02 cell lines [a]

Compounds	Cytotoxicity IC_{50} (μM)		Compounds	Cytotoxicity IC_{50} (μM)	
	K562	K562/A02		K562	K562/A02
1	>100	>100	**13**	>100	>100
2	>100	>100	**14**	>100	>100
3	>100	>100	**15**	>100	>100
4	>100	>100	**16**	>100	>100
5	>100	>100	**17**	>100	>100
6	>100	>100	**18**	>100	>100
7	>100	>100	**19**	>100	>100
8	>100	>100	**20**	>100	>100
9	>100	>100	**VRP**	70.13±6.4	61.51±3.4
10	>100	>100	**Tariquidar**	30.77±1.94	28.8 ±1.2
11	>100	>100	**DOX**	3.092±2.1	107.3±1.9
12	>100	>100			

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

Table 2.DOX-resistance reversal activity of the target compounds **1–20** at 4 μM concentration in K562/A02 cells [a]

Compounds	IC_{50} of DOX (μM)	RF	Compounds	IC_{50} of DOX (μM)	RF
1	7.12±1.4	5	**13**	4.59±1.05	7.8
2	1.76±0.45	20.4	**14**	2.67±1.14	13.4
3	2.27±0.97	15.7	**15**	3.17±1.5	11.3
4	2.42±0.2	14.8	**16**	6.324±1.5	5.6
5	2.00±0.7	17.9	**17**	6.96±1.01	5.1
6	1.73±0.5	20.7	**18**	2.33±0.8	15.3
7	5.17±1.4	6.9	**19**	1.15±0.2	31.2
8	4.63±1.4	7.7	**20**	1.92±0.6	18.7
9	4.96±0.9	7.2	**Tariquidar**	0.96±0.3	37.2
10	4.70±1.3	7.6	**VRP**	4.35±1.3	8.2
11	2.20±0.58	16.2	**Control** [b]	35.92±2.1	1
12	3.34±0.8	10.7			

Note: [a] The IC_{50} value was determined after exposure to a series of DOX concentrations combined with the different target compounds at 4.0 μM using K562/A02 cells; reversal fold (RF, fold-change in drug sensitivity) = (IC_{50} without inhibitor/(IC_{50} with inhibitor). [b]0.1% DMSO was added as a solvent control. Data were analysed with GraphPad Prism 6.0 software and are presented as the mean ± SD of three independent tests.

Table 3.Effect of compound **19** on reversal of MDR in K562/A02 cells at different concentrations [a]

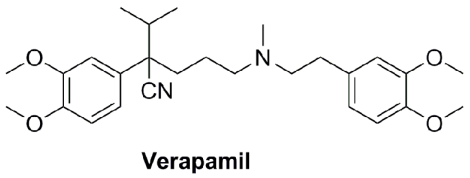
Compounds	**IC_{50} of DOX (μM)**	**RF**
None	107.3±3.2	1
VRP, 5 μM	7.31±1.2	14.7
Compound **19**, 10 μM	1.39±0.9	77.2
Compound **19**, 5 μM	1.67±0.4	64.3
Compound **19**, 2.5 μM	2.8±0.8	38.3
Compound **19**, 1.25 μM	20.45±1.6	5.2
Compound **19**, 0.625 μM	25.83±2.2	4.15
Compound **19**, 0.3125 μM	71.99±2.7	1.4
Compound **19**, 0.156 μM	49.37±1.6	2.17
Compound **19**, 0.078 μM	104.56±3.2	1.02

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

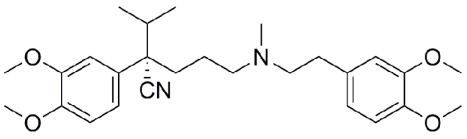
Table 4. Duration of MDR reversal in K562/A02 cells after incubation and washout of verapamil, tariquidar and compound **19**

Treatment Schedule	IC_{50}/DOX [μM] (RF)			
	Control (5 μM)	VRP (5 μM)	Compound19 (5μM)	Tariquidar (5 μM)
No wash	60.22±4.5(1)	6.54± 0.5(9.2)	2.45 ±0.2 (24.57)	1.47± 0.11(40.96)
Wash, 0 h	nd	33.6± 0.32(1.79)	5.32± 0.1(11.31)	3.43± 0.3(17.55)
Wash, 6 h	nd	nd	9.98± 0.32(6.03)	6.44 ± 0.21(9.35)
Wash, 12 h	nd	nd	21.99± 0.1(2.73)	13.45± 0.1(4.47)
Wash, 24 h	nd	nd	33.67± 0.42(1.78)	19.67± 0.32(3.06)

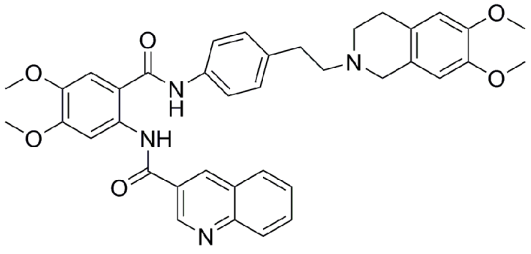
Numbers in parentheses indicate the reversal fold (RF), RF = (IC_{50} without modulator)/(IC_{50} with modulator). Each experiment was carried out two to three times, and the values are displayed as the mean ± SD
nd: not identified.



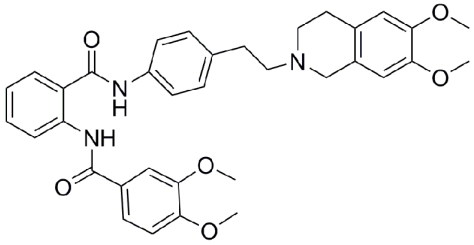
Verapamil



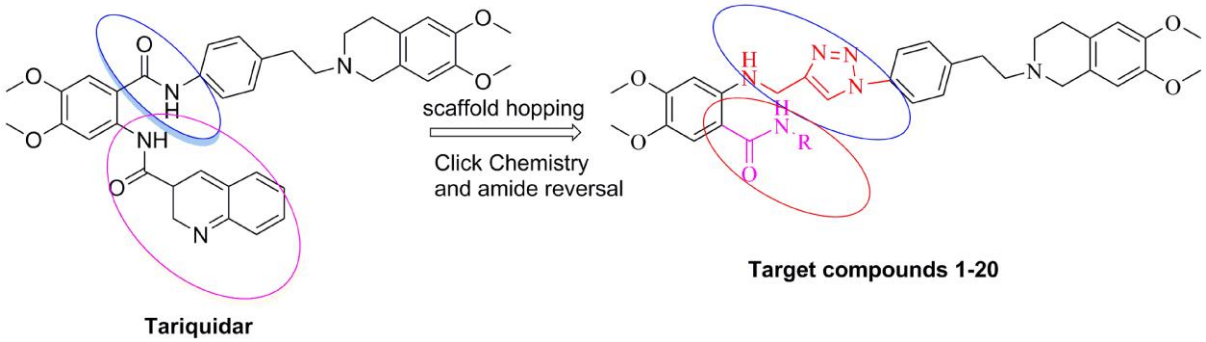
Dexverapamil



Tariquidar



WK-X-34



Tariquidar

Target compounds 1-20