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Discovery of Novel P-Glycoprotein-Mediated Multidrug Resistance Inhibitors Bearing Triazole Core *via* Click Chemistry

Baomin Liu [1], Qianqian Qiu [1], Tianxiao Zhao [1], Lei Jiao [1], Jianyu Hou [1], Yunman Li [2], Hai Qian [1, *], Wenlong Huang [1, *]

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Abstract

A novel series of P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) inhibitors bearing a triazol-phenethyl-tetrahydroisoquinoline scaffold were designed and synthesized *via* click chemistry. Most of the synthesized compounds showed higher reversal activity than verapamil (VRP). Among them, the most potent compound **5** showed a comparable activity with the known potent P-gp inhibitor WK-X-34 with lower cytotoxicity (IC_{50}s > 100 μM). Compared with VRP, compound **5** exhibited more potency in increasing drug accumulation in K562/A02 MDR cells. Moreover, compound **5** persisted longer chemo-sensitizing effect (> 24 h) than VRP (< 6 h) with reversibility. Given the low intrinsic cytotoxicity and the potent reversal activity, compound **5** may represent a promising candidate for developing P-gp-mediated MDR inhibitor.

Keywords: P-glycoprotein; Multidrug resistance inhibitors; Click chemistry; Reversal activity

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Abbreviations: MDR (multidrug resistance**), P-gp (**P-glycoprotein**), VRP** (verapamil), **ADM** (Adriamycin), **FBS** (fetal bovine serum), **MTT** [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], **TEA** (triethylamine), **DCM** (dichloromethane), **Reversal fold** (RF).

Multidrug resistance (MDR) to chemotherapeutic agent is a major obstacle for successful cancer chemotherapy (1). Three major ATP-binding cassette (ABC) transporters confer the development of MDR, including P-glycoprotein (P-gp/ABCB1), breast cancer resistance protein (BCRP/ABCG2) and multidrug resistance protein 1 (MRP/ABCC1) (2). Among them, P-gp is the most extensively characterized drug transporter for MDR, which can actively expel a variety of different cytotoxic drugs out of the cells, resulting in an intracellular drug level below effective concentrations and the development of MDR (3). To circumvent MDR, combination of P-gp inhibitors and chemotherapeutic drugs is regarded as a potential strategy (4). Since the identity of VRP as the first P-gp mediated MDR inhibitor, great efforts have been made and three generations of P-gp inhibitors have been developed over the past three decades. The first-generation inhibitors such as verapamil, and cyclosporine A were limited by cardiotoxicity or other unacceptable toxicities. The second-generation inhibitors including dexverapamil, PSC833 had better pharmacologic profiles than the first-generation, but they significantly inhibited the metabolism of cytotoxic agents, thus leading to unacceptable toxicity. The third-generation inhibitors can specifically and potently inhibit P-gp function, but no satisfactory results of clinical trials have been obtained. To date, no P-gp inhibitor has been approved for clinical application (5). Thus, it is still urgent for development of potent P-gp inhibitors with less toxicity for cancer treatment.

It has been reported that the tetrahydroisoquinolinethyl-phenylamine based P-gp inhibitor WK-X-34 showed potent MDR reversal activity. However, a relative high cytotoxicity (IC_{50} < 10 μM) of WK-X-34 was observed (6). In the present study, in order to develop potent P-gp inhibitor with low toxicity, we chose WK-X-34 as a lead compound and adopted a scaffold hopping approach to obtain a novel series of P-gp-mediated MDR inhibitors with a triazol-phenethyl-tetrahydroisoquinoline scaffold (Figure 1). 1, 2, 3-triazole ring was introduced as a bioisostere of carboxamide group in the designed compounds by click chemistry. Click chemistry, which commonly employ a Cu (I)-catalyzed azide-alkyne cycloaddition (CuAAC), has been widely applied in drug discovery (7). Then the target compounds were evaluated their cytotoxicity in Adriamycin (ADM) sensitive cells and ADM insensitive cells followed by MDR reversal activity evaluation. The compounds (**3, 5, 8**) with low cytotoxic activity and potent reversal activity on cancer MDR were chosen to further evaluate their reversal potency at different concentrations. Finally, the most potent compound (**5**) was evaluated its effects on P-gp transport activity and duration of reversal effect.

Methods and Materials

General chemistry

All reagents and solvents were reagent grade and were used without further purification unless otherwise stated. All of the target compounds were analyzed by ^{1}H NMR and ^{13}C NMR (Bruker ACF-300Q, 300 MHz), MS (Hewlett–Packard, 1100 LC/MSD spectrometer) and Elemental analyses (CHN-O-Rapid instrument); Melting points were measured using a Mel-TEMP II melting point apparatus which was uncorrected. Thin-layer

chromatography (TLC) was performed on GF/UV 254 plates and the chromatograms were visualized under UV light at 254 and 365 nm. Compounds **d** and **e** were prepared as previously reported (8).

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

The mixture of 2-nitrophenol (11.2 g, 80 mmol), 3-bromoprop-1-yne (9.52 g, 80 mmol) and potassium carbonate (22.1 g, 160 mmol) in acetone (120 ml) was heated to reflux for 3 h. The reaction mixture was cooled to 0 °C followed by filtration and concentration to afford 5.41 g compound **a**. Yield: 99.3%. Yellow powder, m.p.: 66–68 °C.

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

The mixture of **a** (7.05 g, 40 mmol), ammonium chloride (10.7 g, 200 mmol) and iron powder (6.7 g, 120 mmol) in 80% ethanol (100 ml) was heated to reflux for 3.5 h. After cooling to room temperature, the mixture was adjusted with sodium carbonate to PH = 7~8, filtered with Celite and concentrated under vacuum to afford a brown residue. Dichloromethane (50 ml) was added into the residue and the resulted solution was washed by saturated aqueous Na_2CO_3 (30 ml) and brine (30 ml) in sequence. The organic layer was dried by anhydrous Na_2SO4. The solvent was evaporated to afford 4.80 g compound **b** as brown oil. Yield: 99.3%.

General procedure for the preparation of 1c-16c

To the solution of compound **b** (5 mmol) and triethylamine (6 mmol) in dichloromethane (20 ml), aromatic acyl chloride (5 mmol) in dichloromethane (15 ml) was added at 0 °C, and then the mixture was stirred at room temperature for 24 h. The reaction solution was washed by 3% hydrochloric acid (2 × 20 ml), saturated aqueous Na_2CO_3 (20 ml) and brine (20 ml) in sequence. The organic layer was dried by anhydrous Na_2SO4. The solvent was evaporated to afford the desire product **1c-16c**.

Synthesis of 2-(4-Azidophenethyl)-6, 7-dimethoxy-1, 2, 3, 4-tetrahydroisoquinoline (f)

Compound **e** (5.62 g, 18 mmol) was dissolved in 50% acetic acid (40 ml). To this solution, sodium nitrite (1.74 g, 25.2 mmol) was slowly added at -5-0 °C within 30 min. The solution was vigorous stirred at 0-5 °C for 50 min. Sodium azide (1.76 g, 27.0 mmol) was batch added into the reaction mixture at 0-5 °C. The resulting solution was stirred at 0-5 °C for 1 h followed by diluting with ice water (200 ml) and extracting with EtOAc (3 × 100 ml). The combined organic layer was washed with water (3 × 150 ml), saturated aqueous $NaHCO_3$ (150 ml × 3) and brine (100 ml), dried over anhydrous Na_2SO_4, filtered and concentrated to afford 5.28 g pink solid. Yield 86.7%, m.p.: 68–70 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.29 (d, J = 8.3 Hz, 2H, ArH), 7.02 (d, J = 8.3 Hz, 2H, ArH), 3.70, 3.69 (2s, 6H, 2 × OCH_3), 3.52 (s, 2H, $ArCH_2N$), 2.83-2.78 (m, 2H, CH_2), 2.67-2.50 (m, 6H, 3 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 147.1, 146.8, 137.5, 136.8, 130.1, 126.5, 125.8, 118.8, 111.7, 109.9, 59.2, 55.4, 55.0, 50.4, 32.1, 28.2.

General procedure for the preparation of 1-16

To the solution of **1c-16c** (1 mmol) and **f** (1 mmol) in 75% methanol (40 ml), ascorbate sodium (30 mg) and $CuSO_4$ (10 mg) were added respectively. The reaction solution was stirred at room temperature for 24-48 h. Then the mixture was filtered to give the desire product with high purity.

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

Yield 80.2%; White powder; mp: 104−105 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.30 (s, 1H, CONH), 8.82 (s, 1H, NCH=C), 7.86 (d, *J* = 6.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.3 Hz, 2H, ArH), 7.56 (d, *J* = 8.4 Hz, 2H, ArH), 7.48-7.45 (m, 3H, ArH), 7.35 (d, *J* = 8.1 Hz, 1H, ArH), 7.18 (dd, *J* = 7.0, 7.5 Hz, 1H, ArH), 7.04-7.00 (m, 2H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.33 (s, 2H , OCH_2), 3.80 (s, 3H, OCH_3), 3.73-3.69 (m, 9H, 3 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.90-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.3, 151.6, 149.9, 148.3, 147.1, 146.8, 144.0, 141.5, 134.5, 130.0, 127.8, 126.6, 126.5, 125.8, 125.2, 123.7, 122.4, 121.1, 120.6, 119.9, 113.7, 111.7, 110.9, 110.5, 109.9, 62.5, 58.9, 55.5, 55.4, 55.3, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 650.6 ($[M + H]^+$); Anal. calcd. For $C_{36}H_{37}N_5O_5$: C, 68.40; H, 6.05; N, 10.78 %; Found: C, 68.45; H, 6.11; N, 10.69 %.

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

Yield 64.5%; White powder; mp: 134−136 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.53 (s, 1H, CONH), 9.01 (s, 1H, NCH=C), 8.55 (d, *J* = 7.1 Hz, 1H, ArH), 8.08 (d, *J* = 6.5 Hz, 1H, ArH), 7.81 (d, *J* = 8.3 Hz, 2H, ArH), 7.54-7.48 (m, 3H, ArH), 7.36 (d, *J* = 7.9 Hz, 1H, ArH), 7.16-7.09 (m, 3H, ArH), 7.01 (dd, *J* = 7.7, 7.5 Hz, 1H, ArH), 6.65, 6.63 (2s, 2H, ArH), 5.37 (s, 2H , OCH_2), 3.69 (s, 6H, 2 × OCH_3), 3.53 (s, 5H, OCH_3, $ArCH_2N$), 2.94-2.71(m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 162.0, 157.0, 147.1, 146.8, 143.1, 141.6, 134.5, 133.5, 131.3, 130.0, 128.0, 126.5, 125.8, 123.6, 123.5, 121.1, 120.8, 120.1, 119.5, 112.4, 111.9, 111.7, 109.9, 61.6, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2; MS (70 eV) m/z: 620.8 ($[M + H]^+$); Anal. calcd. For $C_{36}H_{37}N_5O_5$: C, 69.77; H, 6.02; N, 11.30 %; Found: C, 69.81; H, 6.06; N, 11.33 %.

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

Yield 71.2%; White powder; mp: 126−128 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.44 (s, 1H, CONH), 8.82 (s, 1H, NCH=C), 7.85 (d, *J* = 7.3 Hz, 1H, ArH), 7.72 (d, *J* = 8.3 Hz, 2H, ArH), 7.53-7.34 (m, 6H, ArH), 7.20 (dd, *J* = 7.2, 7.6 Hz, 1H, ArH), 7.11 (dd, *J* = 1.7, 8.04 Hz, 1H, ArH), 7.02 (dd, *J* = 7.6, 7.6 Hz, 1H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.33 (s, 2H , OCH_2), 3.73-3.69 (m, 9H, 3 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.92-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.6, 159.2, 150.0, 147.1, 146.9, 144.0, 141.5, 135.9, 134.5, 130.0, 129.6, 127.5, 126.6, 125.9,125.6, 123.9,122.4, 121.1, 119.9, 199.5, 117.5, 113.7, 112.4, 111.8, 109.9, 62.5, 58.9, 55.4, 55.1, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 620.6 ($[M + H]^+$); Anal. calcd. For $C_{36}H_{37}N_5O_5$: C, 69.77; H, 6.02; N, 11.30 %; Found: C, 69.71; H, 6.13; N, 11.23 %.

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

Yield 69.3%; White powder; mp: 147−149 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.28 (s, 1H, CONH), 8.80 (s, 1H, NCH=C), 7.92 (d, *J* = 8.8 Hz, 2H, ArH), 7.84 (d, *J* = 7.9 Hz, 2H, ArH), 7.72 (d, *J* = 8.4 Hz, 2H, ArH), 7.47 (d, *J* = 8.4 Hz, 1H, ArH), 7.34 (d, *J* = 8.2 Hz, 1H, ArH), 7.17 (m, 1H, ArH), 7.03-7.00 (m, 3H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.34 (s, 2H , OCH_2), 3.81 (s, 3H, OCH_3), 3.69 (s, 6H, 2 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.93-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.3, 161.9, 149.8, 147.1, 146.8, 144.0, 141.4, 134.5, 129.9, 129.2, 127.9, 126.6,126.5, 125.8, 125.2, 123.7, 122.4, 121.1, 119.9, 113.8, 113.7, 111.7, 109.9, 62.6, 58.9, 55.4, 55.3, 55.0, 50.4, 32.2, 28.2; MS (70 eV) m/z: 620.6 ([M + H]$^+$); Anal. calcd. For $C_{36}H_{37}N_5O_5$: C, 69.77; H, 6.02; N, 11.30; Found: C, 69.75; H, 6.10; N, 11.41 %.

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

Yield 60.3%; White powder; mp: 133−135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.47 (s, 1H, CONH), 8.82 (s, 1H, NCH=C), 7.96 (d, *J* = 7.2 Hz, 2H, ArH), 7.87(d, *J* = 7.6 Hz, 1H, ArH), 7.72(d, *J* = 8.0 Hz, 2H, ArH), 7.57-7.45 (m, 5H, ArH), 7.36 (d, *J* = 8.0 Hz, 1H, ArH), 7.20(dd, *J* = 7.2, 7.6 Hz, 1H, ArH), 7.03(dd, *J* = 7.5, 7.4 Hz, 1H), 6.65, 6.63 (2s, 2H, ArH), 5.35(s, 2H , OCH_2), 3.69(s, 6H, 2 × OCH_3), 3.55(s, 2H, $ArCH_2N$), 2.90-2.71(m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.9, 150.1, 147.1, 146.8, 144.1, 141.5, 134.5, 131.6, 130.0, 128.5, 127.6, 127.4, 126.6, 125.9, 125.5, 124.0, 122.4, 121.2, 119.9, 113.8, 111.8, 109.9, 62.5, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 590.6 ([M + H]$^+$); Anal. calcd. For $C_{35}H_{35}N_5O_4$: C, 71.29; H, 5.98; N, 11.88 %; Found: C, 71.32; H, 6.02; N, 11.92 %.

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

Yield 59.6% ; White solid; mp: 110−112 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.39 (s, 1H, CONH), 8.83 (s, 1H, NCH=C), 7.83 (d, *J* = 7.8 Hz, 1H, ArH), 7.72 (d, *J* = 7.5 Hz, 2H, ArH), 7.48 (d, *J* = 7.6 Hz, 2H, ArH), 7.35 (d, *J* = 8.1 Hz, 1H, ArH), 7.20 (dd, *J* = 7.6, 7.8 Hz, 1H, ArH), 7.04-7.00 (m, 3H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.33 (s, 2H , OCH_2), 3.72-3.69 (m, 12H, 4 × OCH_3), 3.54 (s, 2H, $ArCH_2N$), 2.91-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.4, 160.4, 150.1, 147.1, 146.8, 144.0, 141.5, 136.6, 134.5, 129.9, 127.4, 126.6, 125.9, 125.5, 123.8, 122.4, 121.1, 119.9, 113.6, 111.8, 109.9, 105.2, 103.5, 62.5, 58.9, 55.4, 55.3, 55.0, 32.2, 28.2; ESI-MS m/z: 650.4 ([M + H]$^+$); Anal. calcd. For $C_{36}H_{37}N_5O_5$: C, 68.40; H, 6.05; N, 10.78 %; Found: C, 68.55; H, 6.16; N, 10.79 %.

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

Yield 61.0%; White powder; mp: 108−110 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.41 (s, 1H, CONH), 8.84 (s, 1H, NCH=C), 7.88 (d, *J* = 7.6, 1H, ArH), 7.74-7.71 (m, 4H, ArH), 7.47 (d, *J* = 7.8 Hz, 2H, ArH), 7.35 (m, 3H,

ArH), 7.20 (dd, *J* = 7.2, 7.2 Hz, 1H, ArH), 7.03 (dd, *J* = 7.4, 7.3 Hz, 1H, ArH), 6.65, 6.63 (2s, 2H, ArH), 5.35 (s, 2H , OCH_2), 3.69 (s, 6H, 2 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.91-2.70 (m, 8H, 4 × CH_2), 2.09 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.9, 149.9, 147.1, 146.8, 141.5, 137.8, 134.5, 134.4, 132.1, 130.0, 128.3, 127.8, 127.7, 126.5, 125.8, 125.4, 124.5, 123.6, 122.4, 121.1, 119.8, 113.7, 111.7, 109.9, 62.6, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2, 20.7; ESI-MS m/z: 604.7 ([M + H]$^+$); Anal. calcd. For $C_{36}H_{37}N_5O_5$: C, 71.62; H, 6.18; N, 11.60 %; Found: C, 71.56; H, 6.13; N, 11.71 %.

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

Yield 77.3%; White solid; mp: 90−92 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.36 (s, 1H, CONH), 8.81 (s, 1H, NCH=C), 7.89-7.84 (m, 3H, ArH), 7.72 (d, *J* = 8.2 Hz, 2H, ArH), 7.47 (d, *J* = 8.2 Hz, 2H, ArH), 7.37-7.27 (m, 3H, ArH), 7.18 (dd, J = 7.6, 7.6 Hz, 1H), 7.02 (dd, J = 7.6, 7.5 Hz, 1H, ArH), 6.65, 6.63 (2s, 2H, ArH), 5.35(s, 2H , OCH_2), 3.69 (s, 6H, 2 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.91-2.70 (m, 8H, 4 × CH_2), 2.35 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.7, 149.8, 147.1, 146.8, 144.0, 141.6, 141.5, 134.5, 131.6, 129.9, 129.0, 127.8, 127.3, 126.5, 125.8, 125.3, 123.6, 122.4, 121.1, 119.9, 113.8, 111.7, 109.9, 62.59, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2, 20.9; ESI-MS m/z: 604.7 ([M + H]$^+$); Anal. calcd. For $C_{36}H_{37}N_5O_5$: C, 71.62; H, 6.18; N, 11.60 %; Found: C, 71.56; H, 6.13; N, 11.71 %.

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

Yield 65.1%; White powder; mp: 144−145 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.28 (s, 1 H, CONH), 8.83 (s, 1 H, NCH=C), 7.90 (d, *J* = 7.7 Hz, 1 H, ArH), 7.72 (d, *J* = 8.0 Hz, 2 H, ArH), 7.48 (d, *J* = 8.0 Hz, 2 H, ArH), 7.36-7.16 (m, 5 H, ArH), 7.02 (t, *J* = 7.4 Hz, 1 H, ArH), 6.88 (d, *J* = 6.9 Hz, 1 H, ArH), 6.65, 6.63 (2s, 2 H, ArH), 5.33 (s, 2H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3), 3.55 (s, 2 H, $ArCH_2N$), 2.85-2.70 (m, 14H, $2CH_3$, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 165.4, 150.2, 149.5, 147.1, 146.8, 143.9, 141.4, 135.2, 134.5, 129.9, 129.0, 127.7, 126.5, 125.8, 125.1, 123.1, 122.4, 121.1, 119.8, 115.2, 114.7, 113.4, 111.7, 110.5, 109.9, 62.4, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 633.7 ([M + H]$^+$); Anal. calcd. For $C_{37}H_{40}N_6O_4$: C, 70.23; H, 6.37; N, 13.28 %; Found: C, 70.29; H, 6.31; N, 13.33 %.

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

Yield 52.1%; Yellow powder; mp: 164−166 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.95 (s, 1H, CONH), 8.81 (s, 1H, NCH=C), 8.11 (d, *J* = 8.0 Hz, 1H, ArH), 7.88 (d, *J* = 7.7 Hz, 1H, ArH), 7.82-7.70 (m, 5H, ArH), 7.48 (d, *J* = 8.3 Hz, 2H, ArH), 7.34 (d, *J* = 8.1 Hz, 1H, ArH), 7.20 (dd, *J* = 7.4, 7.6 Hz, 1H, ArH), 7.03 (dd, *J* = 7.4, 7.3 Hz, 1H, ArH), 5.31 (s, 2H , OCH_2), 3.69 (s, 6H, 2 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.93-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.4, 149.8, 147.1, 146.8, 146.4, 143.9, 134.5, 133.8, 132.8, 130.6, 130.2, 130.0, 129.1, 127.0, 126.5, 125.8, 125.7, 124.0, 123.9, 122.6, 120.9, 119.9, 118.8, 113.6, 111.7, 109.9, 62.2, 58.9, 55.5,

55.4, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 633.7 ([M + H]$^+$); Anal. calcd. For $C_{37}H_{40}N_6O_4$: C, 66.23; H, 5.40; N, 13.24; Found: C, 66.31; H, 5.45; N, 13.31 %.

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

Yield 49.2%; White powder; mp: 112−114 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.36 (s, 1H, CONH), 8.86 (s, 1H, NCH=C), 7.90-7.85 (m, 3 H, ArH), 7.75 (d, *J* = 8.0 Hz, 2 H, ArH), 7.47 (d, *J* = 8.1 Hz, 4 H, ArH), 7.35 (d, *J* = 7.9 Hz, 1 H, ArH), 7.19 (t, *J* = 7.5 Hz, 1 H, ArH), 7.02 (t, *J* = 7.5 Hz, 1 H, ArH), 6.65, 6.63 (2s, 2 H, ArH), 5.35 (s, 2H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3), 3.55 (s, 2 H, $ArCH_2N$), 2.90-2.70 (m, 8 H, 4 × CH_2), 1.27 (s, 9 H, 3 × CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.7, 154.4, 149.8, 147.0, 146.8, 144.1, 141.4, 134.5, 131.7, 130.0, 127.7, 127.2, 125.8, 125.2, 123.5, 122.4, 121.1, 119.8, 113.7, 111.6, 109.8, 62.5, 58.9, 55.3, 55.0, 34.5, 32.2, 30.8, 28.2; ESI-MS m/z: 646.7 ([M + H]$^+$); Anal. calcd. For $C_{39}H_{43}N_5O_4$: C, 72.53; H, 6.71; N, 10.84 %; Found: C, 72.46; H, 6.75; N, 10.79 %.

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

Yield 62.2%; White powder; mp: 76−78 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.60 (s, 1H, CONH), 8.79 (s, 1H, NCH=C), 7.96 (d, *J* = 8.4 Hz, 2H, ArH), 7.77-7.70 (m, 3 H, ArH), 7.55 (d, *J* = 8.4 Hz, 2 H, ArH), 7.47 (d, *J* = 8.3 Hz, 2 H, ArH), 7.36 (d, *J* = 7.9 Hz, 1 H, ArH), 7.22 (t, *J* = 6.8 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1 H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.33 (s, 2H, OCH_2), 3.69 (s, 6 H, 2 × OCH_3), 3.54 (s, 2 H, $ArCH_2N$), 2.90-2.70 (m, 8 H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.0, 150.4, 147.1, 146.8, 144.0, 141.4, 136.4, 134.5, 133.2, 129.9, 129.3, 128.4, 127.3, 126.5, 125.8, 124.6, 122.4, 121.1, 119.8, 113.8, 111.7, 109.8, 62.4, 58.9, 55.4, 55.0, 50.3, 32.2, 28.2; ESI-MS m/z: 625.1 ([M + H]$^+$); Anal. calcd. For $C_{35}H_{34}ClN_5O_4$: C, 67.35; H, 5.49; Cl, 5.68; N, 11.22 %; Found: C, 67.32; H, 5.40; Cl, 5.61; N, 11.18 %.

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

Yield 66.4%; White powder; mp: 101-103 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.68 (s, 1H, CONH), 8.82 (s, 1H, NCH=C), 7.96-7.88 (m, 2H, ArH), 7.74-7.71 (m, 3 H, ArH), 7.62 (d, *J* = 7.5 Hz, 1 H, ArH), 7.54-7.46 (m, 3 H, ArH), 7.36 (d, *J* = 7.8 Hz, 1 H, ArH), 7.23 (t, *J* = 7.5 Hz, 1 H, ArH), 7.03 (t, *J* = 7.5 Hz, 1H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.33 (s, 2H, OCH_2), 3.69 (s, 6H, 2 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.90-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.7, 150.6, 147.1, 146.8, 144.0, 141.4, 136.5, 134.5, 133.2, 131.3, 130.3, 129.9, 127.3, 127.1, 126.5, 126.1, 126.0, 125.8, 124.8, 122.4, 121.0, 119.9, 113.8, 111.7, 109.9, 62.5, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 625.1 ([M + H]$^+$); Anal. calcd. For $C_{35}H_{34}ClN_5O_4$: C, 67.35; H, 5.49; Cl, 5.68; N, 11.22 %; Found: C, 67.41; H, 5.53; Cl, 5.64; N, 11.17 %.

N-(2-((1-(4-(2-(6, 7-dimethoxy-3, 4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-1*H*-1, 2, 3-triazol-4-yl)methoxy)phenyl)-4-fluorobenzamide (14)

Yield 70.2%; White powder; mp: 162−164 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.53 (s, 1H, CONH), 8.80 (s, 1H, NCH=C), 8.03 (dd, *J* = 5.6, 8.4 Hz, 2H, ArH), 7.79-7.71(m, 3H, ArH), 7.47 (d, *J* = 8.1 Hz, 2H, ArH), 7.37-7.29 (m, 3H, ArH), 7.21 (dd, J = 7.3, 7.6 Hz, 1H, ArH), 7.02 (dd, J = 7.6, 7.4 Hz, 1H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.34 (s, 2H, OCH_2), 3.70 (s, 6H, 2 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.90-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 165.7, 163.9, 162.4, 150.4, 147.1, 146.8, 144.0, 141.5, 134.5, 130.9, 130.2, 130.0, 127.5, 126.5, 125.8, 125.7, 124.5, 122.4, 121.1, 119.9, 115.5, 115.2, 113.8, 111.7, 109.9, 62.4, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 608.7 ([M + H]$^+$); Anal. calcd. For $C_{35}H_{34}FN_5O_4$: C, 69.18; H, 5.64; F, 3.13; N, 11.52 %; Found: C, 69.31; H, 5.73; F, 3.21; N, 11.49 %.

N-(2-((1-(4-(2-(6, 7-dimethoxy-3, 4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenyl)-1*H*-1, 2, 3-triazol-4-yl)methoxy)phenyl)-3, 4, 5-trimethoxybenzamide (15)

Yield 77.3%; White powder; mp: 136−138 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.43 (s, 1H, CONH), 8.85 (s, 1H, NCH=C), 7.84 (d, *J* = 6.8 Hz, 1H, ArH), 7.73 (d, *J* = 8.4 Hz, 2H, ArH), 7.48 (d, *J* = 8.4 Hz, 2H, ArH), 7.37 (d, *J* = 8.0 Hz, 2H, ArH), 7.23-7.19 (m, 3H, ArH), 7.04 (dd, *J* = 7.7, 7.5 Hz, 1H, ArH), 6.65, 6.63 (2s, 2H, ArH), 5.32 (s, 2H, OCH_2), 3.75 (s, 6H, 2 × OCH_3), 3.70 (s, 9H, 3 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.93-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.3, 152.6, 150.1, 147.1, 146.8, 143.9, 141.5, 140.2, 134.5, 130.0 129.6, 127.5, 126.5, 125.8, 125.5, 124.0, 122.5, 121.1, 119.8, 113.6, 111.7, 109.9, 104.8, 62.4, 60.0, 58.9, 55.8, 55.4, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 680.8 ([M + H]$^+$); Anal. calcd. For $C_{38}H_{41}N_5O_7$: C, 67.14; H, 6.08; N, 10.30 %; Found: C, 67.18; H, 6.12; N, 10.35 %.

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

Yield 71.3%; White powder; mp: 150−152 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.80 (s, 1H, CONH), 8.79 (s, 1H, NCH=C), 8.10 (d, *J* = 8.1Hz, 2H, ArH), 7.96 (d, *J* = 8.1 Hz, 2H, ArH), 7.76-7.70 (m, 3H, ArH), 7.47 (d, *J* = 8.2 Hz, 2H, ArH), 7.38 (d, *J* = 8.1 Hz, 1H, ArH), 7.24 (dd, J = 7.4, 7.4 Hz, ArH), 7.04 (dd, J = 7.6, 7.5 Hz, 1H, ArH), 6.65, 6.62 (2s, 2H, ArH), 5.34 (s, 2H, OCH_2), 3.70 (s, 6H, 2 × OCH_3), 3.55 (s, 2H, $ArCH_2N$), 2.90-2.70 (m, 8H, 4 × CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.8, 150.7, 147.1, 146.8, 143.9, 141.4, 138.5, 134.5, 132.4, 129.9, 128.3, 127.0, 126.5, 126.2, 125.8, 124.9, 122.4, 121.1, 119.9, 118.2, 113.9, 111.7, 109.9, 62.4, 58.9, 55.4, 55.0, 50.4, 32.2, 28.2; ESI-MS m/z: 615.7 ([M + H]$^+$); Anal. calcd. For $C_{38}H_{41}N_5O_7$: C, 70.34; H, 5.58; N, 13.67 %; Found: C, 70.41; H, 5.53; N, 13.71 %.

Cell lines

Human leukemia cell line K562 and its adriamycin-selected Pgp-overexpressing subline K562/A02 were kindly provided by Dr. Yan-Yan Zhang (Department of Physiology, China Pharmaceutical University, Nanjing, China).

The cell lines were grown in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and incubated at 37 °C in a humidified incubator with 5% CO_2 in air growth. To maintain MDR phenotype, 1 mg/ml adriamycin was added to K562/A02 cultures and maintained in drug-free medium for 2 weeks before used. All experiments were performed with cells in exponential growth. To maintain MDR phenotype, 1 mg/ml adriamycin was added to K562/A02 cultures and maintained in drug-free medium for 2 weeks before used.

Chemicals for biological evaluation

Adriamycin (ADM), verapamil (VRP), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and DMSO were purchased from Sigma-Aldrich (St. Louis, MO, USA). WK-X-34 with high purity was synthesized by our laboratory before. All other chemicals were molecular biology grade and obtained from Sigma-Aldrich or Thermo Fisher Scientific (Waltham, MA, USA).

Cytotoxicity assay

K562 and K562/A02 cells were grown in 96-well micro-titer 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, 10 μM or other concentrations of the target compounds were added into the wells followed by various concentrations of ADM. And the exponentially growing cancer cells were incubated for 48 h in an atmosphere of 95% air with 5% CO_2 at 37 °C. Then, MTT was added directly to the cells. After additional incubation for 4 h at 37 °C, the absorbance at 570 nm was read on a microplate reader (Thermo, USA) (9). The IC_{50} values of the compounds for cytotoxicity were calculated by GraphPad Prism 5.0 software from the dose–response curves.

Accumulation of ADM

ADM accumulation assay was performed according to the reported procedures with minor modification (10). In brief, 2×10^5 cells of K562 and K562/A02 were incubated with 20 μM ADM and different concentrations of compound **5** for 2.5 h at 37 °C. 0.1% DMSO was used as a negative control. VRP and WK-X-34 were used as positive controls. After incubation, the cells were washed with cold PBS and lysed with lysis buffer (0.75 M HCl, 0.2% Triton-X100 in isopropanol). The fluorescence level of ADM in the lysate was determined by fluorescence spectrophotometer (RF-5301 PC, SHIMADZU) using an excitation and an emission wavelength pair of 460 and 610 nm. Accumulation was expressed as ADM fluorescent intensity (FI) per 2×10^5 cells. All incubations were carried out in triplicate and three independent experiments were performed.

Duration of MDR reversal

The experiment was performed according to the reported procedures with minor modification (11). Briefly, 1×10^4 cells per well were plated in 96-well plates and cultured over-night, followed by incubation for 24 h with or without 10 μM of compound **5,** VRP or WK-X-34 before being washed 0 or 3 times with growth medium. Then, the cells were incubated for 0, 6, 12, or 24 h before the addition of various concentrations of ADM or vehicle, and the cells were incubated for an additional 48 h. MTT was added and absorbance at 570 nm was measured on

a microplate reader (Thermo, USA). The IC_{50} values of the compounds for cytotoxicity were calculated by GraphPad Prism 5.0 software from the dose–response curves.

Results and discussion

Chemistry

The synthesis of the designed compounds **1-16** is depicted in Scheme 1. Table 1 demonstrates the structures of the synthesized compounds. The starting material 2-nitrophenol was treated with 3-bromoprop-1-yne in acetone at the presence of potassium carbonate to give **a** in high yield. Reduction of **a** with iron afforded amine **b**, which in turn reacted with different aromatic acyl chlorides to form **1c-16c**. The intermediate product **d** was obtained by alkylation of the corresponding tetrahydroisoquinoline with 1-(2 bromoethyl)-4-nitrobenzene followed by catalytic reduction of the nitro group yielding aniline **e**. Azide **f** was obtained by treating **e** with $NaNO_2$ followed by NaN_3 in 50% acetic acid. The intermediates **1c-16c** reacted smoothly with azide **f** at room temperature in the presence of catalytic amount of copper sulfate and sodium ascorbate in methanol and water, giving the target compounds **1-16**. Aqueous condition (H_2O/MeOH, 1:3) facilitated precipitation of the products, which were isolated with high purity.

Cytotoxicity of the target compounds

In order to identify ideal P-gp inhibitors reversing MDR at non-toxic concentrations, the intrinsic cytotoxicity of the target compounds against parental sensitive K562 cells and their ADM-resistant sublines K562/A02 cells which overexpress P-gp (induced by ADM) was evaluated by MTT assay. Anticancer drug Adriamycin (ADM) and P-gp inhibitors VRP and WK-X-34 were selected as controls. As shown in Table 2, K562/A02 (IC_{50} for ADM of 96.42 ± 3.40 μM) displayed about 205.1-fold greater resistance than K562 cells (IC_{50} of 0.47 ± 0.03 μM). VRP had weak cytotoxic effects toward K562 and K562/A02 cells with IC_{50} values 62.2 μM and 57.2 μM, respectively. Nevertheless, WK-X-34 displayed a high level of toxicity toward K562 cells (IC_{50} of 9.56 ± 1.32 μM) but a weak toxicity toward K562/A02 cells (IC_{50} of 50.10 ± 2.51 μM). In contrast, the majority of the synthesized compounds showed no toxicity with IC_{50} values higher than 100 μM to both cell lines. Compound **1** which has 3, 4-dimethoxylphenyl substitute also exiting in WK-X-34 was found to exhibit high toxicity (IC_{50} < 10 μM) in both cell lines. However, compounds bearing single methoxy (OMe) group at position 2, 3 or 4, two OMe at positions 3, 5 and three OMe at positions 3, 4, 5 of the phenyl moiety showed very weak cytotoxic effects, suggesting that the number and positions of OMe groups may have a play role in the increased cytotoxicity. The cytotoxicity assays indicated that most of our compounds possess little cytotoxic effects in tested cell lines and are suitable candidates for the development of P-gp inhibitors.

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

The chemo-sensitizing effects of the target compounds on the MDR phenotype have been investigated preliminarily in K562/A02 cells at 10 μM concentration, except compound **1** which exhibited high cytotoxicity (IC_{50} < 10 μM). Briefly, the cytotoxicity of ADM against ADM-resistant cells was evaluated in the presence or absence of 10 μM of the target compounds by MTT assay, selecting VRP and WK-X-34 as positive controls. As

shown in Table 3, anticancer drug ADM alone displayed little inhibitory effect on the survival of K562/A02 cells (IC_{50} of 96.42 ± 3.40 μM). However, combination treatment with ADM and the target compounds, VRP or WK-X-34 led to the increase of inhibitory effect to various extents, suggesting that all the tested compounds possess MDR reversal activity. Moreover, most of the target compounds displayed more potent MDR reversal activity than the classical P-gp inhibitor VRP when co-administered with ADM at the same condition. Notably, compounds **3, 5** and **8** showed the most potent efficacy and their reversal fold (RF) were 6.1, 7.7 and 7.4-fold of that of VRP, respectively. When compared with WK-X-34, similar reversal activity was observed and the ratios of RF between the three compounds and WK-X-34 were 0.9, 1.2 and 1.1, respectively.

To further differentiate reversal potency and investigate the dose response effects, we have determined the reversal activity of the most active compounds (**3, 5** and **8**) as well as VRP and WK-X-34 at other concentrations (12). As demonstrated in Table 4, VRP showed no significant modulating activity at 2.5 and 1.0 μM, and compound **5** showed more potent activity compared with compound **3** and **8** at tested concentrations. Moreover, compound **5** exhibited similar potency with WK-X-34 at different concentrations. The results indicated that compound **5** has the highest activity among the synthesized compounds.

Accumulation of ADM

It is known that ADM is a fluorescent substrate of P-gp and can be used to monitor drug accumulation in cells (13). Thus, we selected ADM as a fluorescent probe to investigate if the modulation of P-gp-mediated drug resistance by compound **5** is associated with a concomitant increase in ADM accumulation. The level of ADM was measured by spectrofluorometry according to a previously described method with minor modification. The P-gp inhibitor VRP and WK-X-34 were chosen as positive controls. As shown in Figure 2, the level of ADM accumulation in K562 cells was about 4.3-fold higher than that of K562/A02 cells in the absence of P-gp inhibitors. This is probably due to P-gp can pump ADM out of the cells, leading to a lower intracellular ADM level. The ADM accumulation in K562/A02 cells treated with compound **5** was significantly increased in a dose-dependent manner. Compared with VRP, treatment of K562/A02 cells with compound **5** led to a higher level of ADM accumulation at the same dose, suggesting that compound **5** is more potent than VRP in inhibiting the ADM transport activity of P-gp. Moreover, compound **5** and WK-X-34 demonstrated similar potency in increasing accumulation of ADM in K562/A02 cells at same conditions.

Duration of MDR reversal effect of compound 5 toward ADM in K562/A02 cells

A relatively long duration of action with reversibility for a P-gp inhibitor likely to be required for safe and effective therapy of P-gp-mediated MDR cancers (14). Therefore, we selected the most active compound **5** to evaluate its duration of MDR reversal effect, using VRP and WK-X-34 as positive controls. The experiment was carried out as previous report (14). Table 5 demonstrates that the MDR-reversing effects of VRP, WK-X-34 and compound **5** increased after preincubation with K562/A02 cells, the IC_{50}s of ADM were 23.9, 1.6 and 1.9 μM (No wash group). The MDR-reversing effect of VRP disappeared immediately after its removal from the medium. In contrast, the reversal effects of compound **5** and WK-X-34 were significant right after they were removed, the IC_{50}s of ADM were 4.7 and 3.0 μM, respectively. Moreover, compound **5** showed significant

reversal activity even at 24 h after exposure. The data also indicated that the MDR-reversing effect of compound **5** was reversible after a 24-h washout.

Conclusions and Future Directions

In summary, a novel class of potent P-gp-mediated MDR inhibitors with low cytotoxicity were designed, synthesized and evaluated for MDR reversal activity. The most potent compound **5** exhibited a comparable activity with the potent P-gp inhibitor WK-X-34 to reverse K562/A02 cells to ADM. It directly inhibited ADM transport activity of P-gp and increased the intracellular drug accumulation to restore ADM sensitivity. Moreover, compound **5** has no cytotoxic effect against all tested cell lines (IC_{50}s > 100 μM). Additionally, it inhibits P-gp-mediated MDR for a relatively long duration (> 24 h) with reversibility. Therefore, compound **5** might be a promising candidate to develop P-gp-mediated MDR reversal modulator in cancer chemotherapy.

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Figure 1. Structure of WK-X-34 and design of the target compounds.

Figure 2. Effect of compound **5** on intracellular ADM accumulation in K562/A02 cells. 0.1% DMSO was used as negative control. VRP and WK-X-34 were chosen as positive controls. N = 2 independent experiments. The results are presented as the mean ± standard error of mean: (**) $P < 0.01$, (***) $P < 0.001$ relative to the negative control (K562/A02).

Table 1. Structures of the synthesized compounds.

Table 2. Cytotoxicity of the target compounds against K562 and K562/A02 cell lines [a].

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

Table 3. ADM-resistance reversal activity of the target compounds at 10 μM concentration in K562/A02 cells [a].

[a] The IC_{50} value was determined after exposure to a series of ADM concentration with different target compounds at 10 μM using K562/A02 cells. Reversal fold (RF) refers to fold-change in drug sensitivity. RF = (IC_{50} without modulator)/(IC_{50} with 10 μM modulator). [b] 0.1% DMSO was added as solvent control for testing the P-gp modulating activity. [c] nd: not determined.

Table 4. Sensitization of K562/A02 cells by target compounds at different concentrations [a].

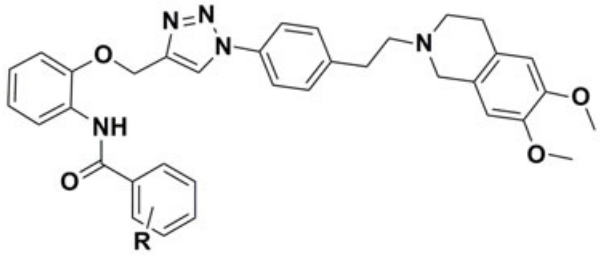
[a] Numbers in parentheses, Reversal fold (RF), RF = (IC_{50} without modulator)/(IC_{50} with 10 μM modulator). Each experiment was carried out two to three times, and the values were presented as the mean ± standard error of mean.

Table 5. Duration of MDR reversal in K562/A02 cells after incubation and washout of P-gp inhibitor [a]. [a] Numbers in parentheses, reversal fold (IC_{50} without modulator)/(IC_{50} with modulator). Each experiment was carried out two to three times, and the values were presented as the mean ± standard error of mean.

Scheme1: Synthesis of the target compounds. Reagents and conditions: (i) 3-bromoprop-1-yne, K_2CO_3, acetone, reflux, 3h; (ii) Fe/NH_4Cl, 80% EtOH, reflux, 2.5 h; (iii) aromatic acyl chlorides, TEA/DCM, r.t., 24 h; (iv)

K_2CO_3, acetonitrile, reflux, 17 h; (vi) H_2/Pd/C, DCM/EtOH, r.t., 24 h; (vi) $NaNO_2$, 50% AcOH, 0 °C~5 °C, 30 min; NaN_3, 0 °C~5 °C, 50 min; (vii) **1c-16c**, ascorbate sodium, $CuSO_4$, 75% CH_3OH, 24h~48h.

Table 1: Structures of the synthesized compounds.



Compounds	R
1	3,4- OCH_3
2	2-OCH_3
3	3- OCH_3
4	4- OCH_3
5	H
6	3,5- OCH_3
7	3-CH_3
8	4- CH_3
9	4-$N(CH_3)_2$
10	2-NO_2
11	4-$C(CH_3)_3$
12	3-Cl
13	4-Cl
14	4-F
15	3,4,5-OCH_3
16	4-CN

Table 2: Cytotoxicity of the target compounds against K562 and K562/A02 cell lines [a]

Compounds	Cytotoxicity IC_{50} (μM)	
	K562	**K562/A02**
1	6.37 ± 1.21	8.34 ± 2.11
2	>100	>100
3	>100	>100
4	>100	>100
5	>100	>100
6	>100	>100
7	>100	>100
8	>100	>100
9	>100	>100
10	>100	>100
11	>100	53.33 ± 3.32
12	>100	>100
13	>100	>100
14	>100	>100
15	>100	>100
16	>100	>100
VRP	62.2 ± 3.24	57.2 ± 4.12
WK-X-34	9.56 ± 1.32	50.10 ± 2.51
ADM	0.47 ± 0.03	96.42 ± 3.40

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

Table 3: ADM-resistance reversal activity of the target compounds at 10 μM concentration in K562/A02 cells [a]

compd	IC_{50} (μM)	RF	compd	IC_{50} (μM)	RF
1	nd [c]	nd [c]	**11**	8.4 ± 0.7	11.4
2	7.2 ± 0.5	13.4	**12**	7.3 ± 0.4	13.2
3	4.2 ± 0.3	23.0	**13**	12.0 ± 1.0	8.0
4	11.7 ± 1.3	8.2	**14**	13.2 ± 0.9	7.3
5	3.3 ± 0.3	29.2	**15**	8.5 ± 0.6	11.3
6	10.1 ± 0.2	9.5	**16**	9.3 ± 0.8	10.3
7	9.0 ± 0.4	10.7	VRP	25.6 ± 2.4	3.8
8	3.4 ± 0.2	28.0	WK-X-34	3.9 ± 0.4	24.8
9	7.5 ± 0.4	12.9	Control [b]	96.4 ± 3.4	1.0
10	10.8 ± 1.4	8.9			

[a] The IC_{50} value was determined after exposure to a series of ADM concentration with different target compounds at 10 μM using K562/A02 cells. Reversal fold (RF) refers to fold-change in drug sensitivity. RF= (IC_{50} without modulator)/(IC_{50} with 10 μM modulator). [b] 0.1% DMSO was added as solvent control for testing the P-gp modulating activity. [c] nd: not determined.

Table 4: Sensitization of K562/A02 cells by target compounds at different concentrations [a]

compd	IC_{50} of ADM (μM)	compd	IC_{50} of ADM (μM)
None	96.4 ± 3.4 (1.0)	compd. **5**, 2.5μM	4.7 ± 0.2 (20.5)
VRP, 2.5μM	> 60	compd. **5**, 1μM	5.4 ± 0.4 (17.8)
VRP,1μM	> 60	compd. **5**, 0.5μM	8.1 ± 0.4 (12.0)
WK-X-34, 2.5μM	4.1 ± 0.1 (23.7)	compd. **8**, 2.5μM	6.4 ± 0.5 (15.1)
WK-X-34, 1μM	4.8 ± 0.2 (20.1)	compd. **8**, 1μM	10.3 ± 0.7 (9.4)
WK-X-34, 0.5μM	6.7 ± 0.5 (14.4)	compd. **8**, 0.5μM	24.5 ± 1.8 (3.9)
compd. **3**, 2.5μM	5.6 ± 0.4 (17.2)		
compd. **3**, 1μM	14.7 ± 1.3 (6.6)		
compd. **3**, 0.5μM	22.0 ± 1.1 (4.4)		

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

Table 5: Duration of MDR reversal in K562/A02 cells after incubation and washout of P-gp inhibitor [a]

Treatment schedule	IC_{50}/ADM [μM] (RF) [b]			
	Control	VRP	WK-X-34	Compound **5**
No wash	94.3 ± 3.9 (1.0)	23.9 ± 1.4 (3.9)	1.6 ± 0.2 (58.7)	1.9 ± 0.1 (48.6)
Wash, 0 h	nd	> 60	3.0 ± 0.2 (31.3)	4.7 ± 0.3 (20.1)
Wash, 6 h	nd	nd	4.3 ± 0.2 (21.7)	14.0 ± 0.8 (6.7)
Wash, 12 h	nd	nd	6.7 ± 0.5 (14.0)	18.2 ± 1.4 (5.2)
Wash, 24 h	nd	nd	8.2 ± 0.7 (11.6)	45.4 ± 3.4 (2.1)

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