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A novel class of highly potent multidrug resistance reversal agents: Disubstituted adamantyl derivatives

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ABSTRACT

Novel disubstituted adamantyl derivatives were synthesized and evaluated in a P-glycoprotein dependent multidrug resistance cancer cell line. The hit to lead optimization provided potent MDR reversal agents. Some potent adamantyl derivatives were more than 10-fold more potent than verapamil without considerable intrinsic cytotoxicity. The 3-trifluorophenyl derivative **14f** did not affect the metabolism of CYP450 3A4, whereas most of MDR revertants had a weak inhibitory effect.

Multidrug resistance (MDR) disables many potent anticancer drugs and is primarily responsible for the failure of cancer chemotherapy.[1] Although many mechanisms have been reported, MDR results mainly from the overexpression of ATP-binding cassette (ABC) transporters, such as, ABCB1 (P-glycoprotein, P-gp), ABCC1-7 (multidrug resistant related protein 1-7, MRP1-7) and ABCG2 (breast cancer resistance protein, BCRP), which pump out a huge number of chemically unrelated anticancer agents. P-gp is considered to be more clinically significant than the other transporters, and therefore, is viewed as a therapeutic target for re-sensitizing multidrug resistant cancer cells to anticancer agents. Despite intensive efforts to develop P-gp modulators to overcome MDR, many candidates have been dropped because of their intrinsic toxicities and pharmacokinetic interactions. Some agents like Zosuquidar and Tariquidar are currently under clinical trials, but research efforts have not yielded a clinically useful drug as yet. Hence, the development of small molecule sensitizers remains a high-level priority to meet clinical needs. Improved clinical trial protocols utilizing functional imaging technology based on the use of ^{99m}Tc-sestamibi in conjunction with RNA or protein-based technologies have differentiated responders and non-responders with respect to ABC transporter inhibition.[2] Herein, we describe a novel class of highly potent MDR reversal compounds and structure–activity relationships.

We initially screened thousands of small molecules from an in-house chemical library by using an image-based high-throughput screening assay in a P-gp overexpressing MDR sarcoma cell line, MES-SA/DX5. A number of primary hits were obtained by image-based efflux assay using 3-ethyl-2-[3-(3-ethyl-2(3*H*)-benzooxazolylidene-1-propenyl) benzoxzolium iodide ($DiOC_2$)[3] in a 384-well format, and these findings were confirmed by cytotoxicity assays. Subsequently, the adamantyl compound (**10a**), which showed good activity at 5 μM, became a focus of attention (Fig. 1). The hit compound was re-synthesized and showed similar activity to verapamil (EC_{50} = 8.48 vs 7.87 μM, respectively), a well-known P-gp inhibitor. We next have carried out hit to lead optimization to increase the MDR reversal activities of hit compounds. We designed and synthesized the adamantyl derivatives described in Schemes 1 and 2 by incorporating the common features of P-gp inhibitors, that is, high hydrophobicity, and the presence of aromatic rings and protonable tertiary amines.

The chemistry employed to design the new compounds described here is shown in Schemes 1 and 2. Compound **2** was

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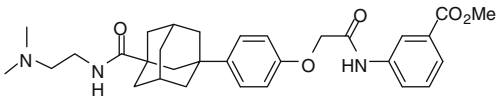


10a

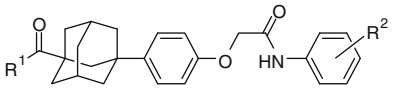
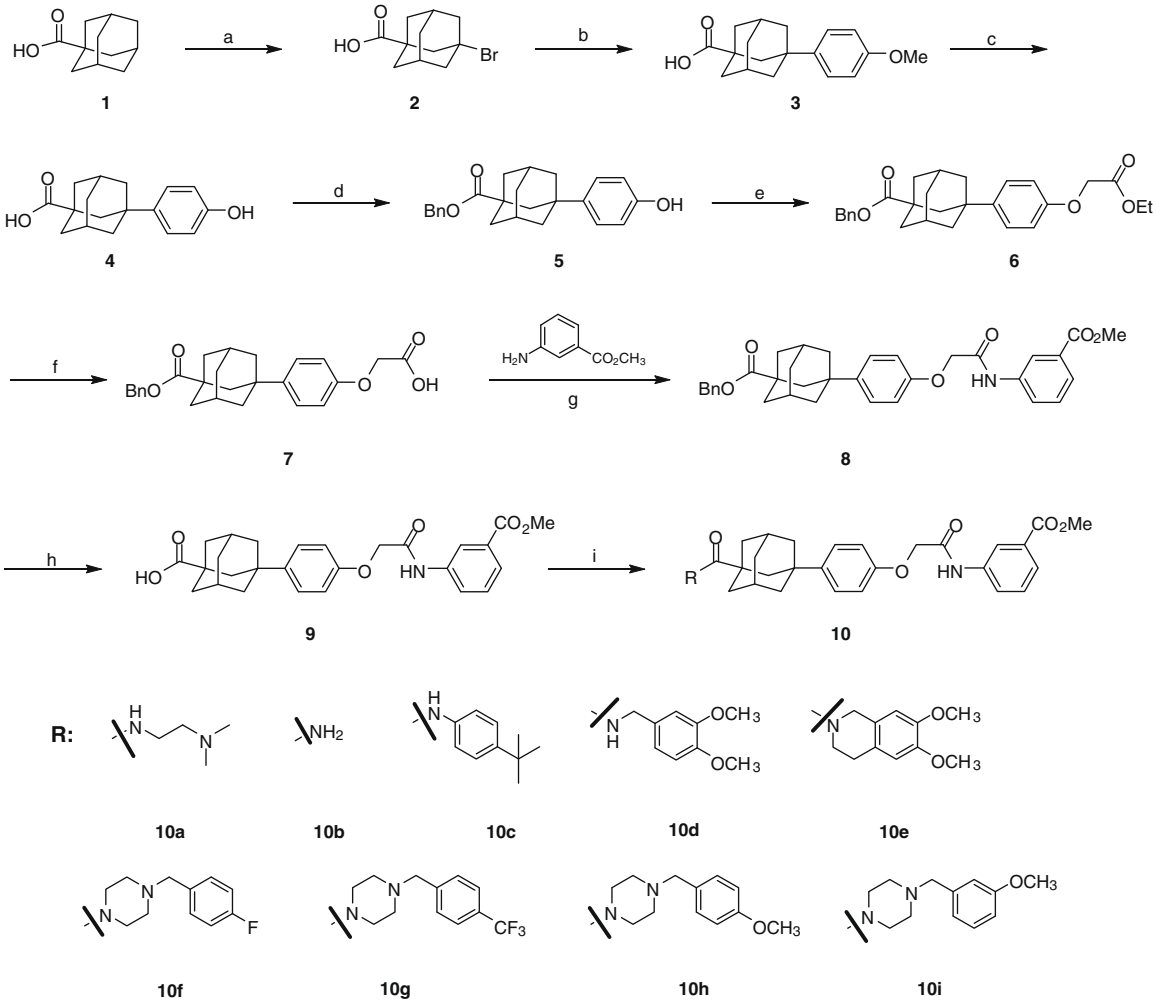


Figure 1. Hit molecule **10a** identified from HTS and the general structure of the MDR revertants prepared in this Letter.

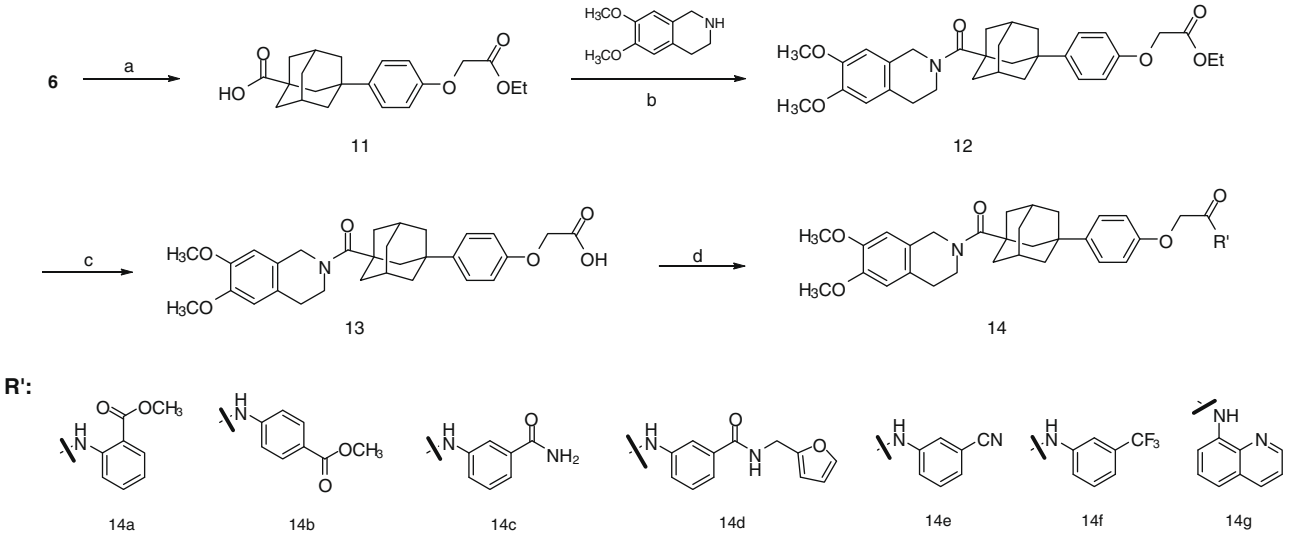
synthesized by brominating 1-adamantane carboxylic acid **1**, and the corresponding intermediate **2** was subjected to Friedel–Crafts alkylation with anisole to obtain compound **3**. Deprotection of the phenolic –OH of **3** via the cleavage of its methyl ether under Lewis acid conditions (BBr_3)[4] and the subsequent selective protection of **4** with benzyl bromide under basic conditions afforded **5**, which was subsequently alkylated with ethyl chloroacetate to produce **6** in excellent yields. Compound **7** was obtained by saponifying **6** with LiOH, and subsequent coupling with methyl 3-aminobenzoate produced the amide **8** in good yield. The precursor for the synthesis of the target adamantyl motifs (**10a–i)** was generated by removing the benzyl group of **8** by catalytic hydrogenation in the presence of palladium on carbon. Compound **9** has a free carboxylic group, which is capable of reacting with amines. For compounds **10a** and **b** coupling was achieved by using coupling agents, that is, EDC or PyBOP in the presence of an organic base, whereas the other amide coupled products **10c–i** were readily synthesized in the presence of PPAA in good yields.[5] Based on synthetic profile of **10e**, we replaced the ester moiety of the aryl ring in **10e** with different amides, as shown in Scheme 2. Hydrogenolysis of the benzyl group of **6** furnished **11**, which was further coupled with 6,7-dimethoxy-1,2,3,4-dihydroisoquinoline to give **12**.

Subsequently, base mediated hydrolysis of **11** provided the free acid **13**. Finally, PPAA-mediated coupling with various commercially available amines led to the corresponding desired amides **14a–g**. Furthermore, saponification of the methyl ester intermediate provided the corresponding *meta*-substituted benzoic acid on the B-ring of the target compound, which was subsequently coupled with furfurylamine to afford **14d**.

The ability of all synthesized compounds to reverse MDR was determined using a P-gp overexpressing human sarcoma cell line, MES-SA/DX5, in the presence of 100 nM Taxol (Table 1). Furthermore, the MDR reversing activity of selected compounds was measured by reversal index, the ratio of the IC_{50} for Taxol only divided by the IC_{50} for Taxol in combination with test compounds in the



Scheme 1. Reagents and conditions: (a) $AlCl_3$, Br_2, −5 °C to rt, 93%; (b) anisole, $AlCl_3$, −10 °C to rt, 94%; (c) BBr_3, −10 °C to rt, 84%; (d) BnBr, $KHCO_3$, 40 °C, 85%; (e) ethylchloroacetate, K_2CO_3, rt, 85%; (f) LiOH, THF/H_2O, 97%; (g) PyBOP, DMAP, rt, 72%; (h) H_2,Pd/C, 94%; (i) EDC, DIPEA, HOBt, rt, 55% for **10a** or PyBOP, DMAP, rt, 94% for **10b**; or PPAA, Et_3N, DCM, rt for **10c–10i** (28–70%).



Scheme 2. Reagents and conditions: (a) Pd/C, MeOH, 99%; (b) PPAA, Et_3N, NaCN, rt, 66%; (c) LiOH, THF/H_2O, 97%; (d) PPAA, Et_3N, NaCN, rt for **14a–c** (32–40%) and **14e–f** (42–50%); or (i) PPAA, Et_3N, NaCN, methyl 3-amino benzoate, rt, (ii) LiOH, THF/H_2O, rt, (iii) EDC, DIPEA, HOBt, furfurylamine, rt for **14d** (76%).

Table 1
Reversal activities of synthesized compounds in MES-SA/DX5 sarcoma cells

Compound	EC_{50}[a] (μM, 100 nM Taxol)	Fold increase	Intrinsic cytotoxicity GI_{50}[b] (μM)
Taxol only	—	—	7.54
Verapamil	7.87	1.0	>20
8	>15	—	>20
10a	8.48	0.9	>20
10b	7.78	1.0	14.3
10c	>15	—	>20
10d	2.74	2.9	>20
10e	1.13	7.0	>20
10f	1.58	5.0	>20
10g	0.58	13.6	>20
10h	0.74	10.6	14.0
10i	1.10	7.2	13.2
14a	1.10	7.2	>20
14b	1.24	6.4	14.3
14c	>15	—	16.3
14d	10.76	0.7	>20
14e	0.84	9.4	>20
14f	0.71	11.1	12.1
14g	0.83	9.5	>20

[a] EC_{50} values were determined in the presence of 100 nM Taxol.[10]
[b] GI_{50} values are the intrinsic cytotoxicities of test compounds.

Table 2
Effects of adamantyl derivatives with a more potent effect than verapamil on Taxol cytotoxicity in MES-SA/DX5 sarcoma cells[a]

Compound	Concd (μM)	IC_{50} of Taxol (nM)	Reversal index[b]
Taxol only		7538	1
Verapamil	5	257	29
	1	>2500	—
10d	5	34	222
	1	1253	6.0
10e	5	9	836
	1	611	12.3
10f	5	9	836
	1	917	8.2
10g	5	8	942
	1	94	80.2
10h	5	3	>1000
	1	167	45.1
10i	5	3	>1000
	1	610	12.4
14a	5	4	>1000
	1	740	10.2
14b	5	5	>1000
	1	1083	7.0
14e	5	13	580
	1	200	37.7
14f	5	7	>1000
	1	107	70.5
14g	5	5	>1000
	1	216	34.9

[a] IC_{50} values of taxol were obtained in the presence or absence of 1 or 5 μM of the test compound.
[b] Reversal index is defined as the quotient of the IC_{50} of Taxol in resistant cells and the IC_{50} of Taxol in the presence of a modulator.

resistant cells (Table 2). Taxol had an IC_{50} value in micromolar range against MES-SA/DX5 (IC_{50} = 7.54 μM), whereas Taxol usually has an IC_{50} value at the single-digit nanomolar level against MES-SA, a sensitive wild-type cancer cell line. Table 1 summarizes reversal activity of adamantyl derivatives. The hit to lead optimization was commenced with diversifying R^1. The removal of the dimethylaminoethyl group (**10b**) was well tolerated, but caused intrinsic cytotoxicity against the MDR cells (GI_{50} = 14.3 μM). The ester **8** did not show any reversal activity, indicating that amide group at R_1 is important for this activity. Incorporation of an aromatic ring at the R^1 position significantly increased activity, whereas the introduction of 4-*t*-butylaniline (**10c**) led to a loss of activity. Reversal activities of **10d–10i** were more potent than that of verapamil, an established MDR-reversing agent. A comparison of **10e** and **10d** revealed that a more lipophilic and conformationally rigid substituent was slightly more effective. Furthermore, introduction of substituted piperazine significantly improved reversal activity. Best reversal activity was observed for the trifluorobenzyl-substituted compound **10g** (EC_{50} = 0.58 μM), which was about 14 times more potent than verapamil.[6] In addition, it was found that 5 μM of compound **10g** almost completely restored the cytotoxicity of Taxol (Table 2), and that 1 μM of **10g** made the resistant cancer cells about 80-fold more sensitive to Taxol. A number of derivatives modified at the R^2 position were also synthesized to investigate structure–activity relationships (SAR). Transposition of the *meta*-ester group (**10e**) to the *ortho* (**14a**) or *para* (**14b**) positions did not affect reversal activity, whereas replacement of the ester with an amide group (**14c**) resulted in a loss of activity and an increase in cytotoxicity. The furfuryl amido derivative **14d** displayed weaker activity than verapamil, but the nitrile **14e** and trifluoromethyl **14f** derivatives (EC_{50} = 0.84, 0.71 μM, respectively)

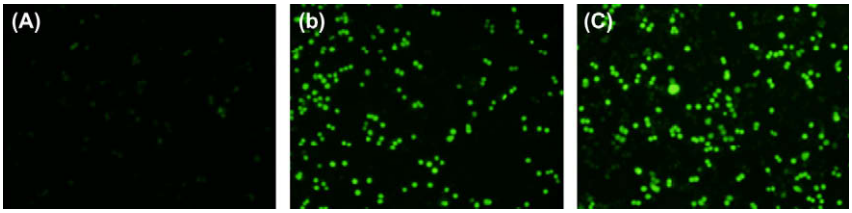


Figure 2. Inhibition of $DIOC_2$ efflux from MES-SA/DX5 cells by an adamantyl derivative **10h**. The cells were treated with fluorescent P-gp substrate $DiOC_2$, incubated for 1 h and washed three times with PBS. (A) Control, (B) 5 μM verapamil, (C) 1 μM **10h**.

Table 3
CYP3A4 inhibition assay findings for compounds **10g–i**, **14a**, and **14e–g**

Compound	IC_{50}[a] (μM)
10g	2.03
10h	0.89
10i	0.82
14a	3.79
14e	>5.00
14f	No inhibition
14g	2.84
ketoconazole	0.004

[a] IC_{50} values against human CYP3A4 were determined using a CYP3A4/BFC high-throughput inhibitor screening kit purchased from BD Gentest.

had higher activity than the corresponding ester **10e**. On the other hand, weak cytotoxicity was observed for **14f**[7] (GI_{50} = 12.1 μM). Five micromolar of the quinoline **14g** also completely restored the cytotoxic effect of Taxol. As illustrated in Table 2, selected derivatives completely restored the cytotoxic effect of Taxol against the resistant cancer cells at 5 μM, while verapamil only partially enhanced the effect of Taxol at this concentration. $DiOC_2$ efflux assay images indicate that the reversal activities of these adamantyl derivatives resulted from P-gp inhibition (Fig. 2).

With regard to the development of MDR reversal agents, pharmacokinetic interactions have been considered as an important factor, because MDR modulators can change the pharmacokinetic properties of anticancer drugs by inhibition of CYP450 system.[1] Over half of all drugs are metabolized by CYP3A4, which is one of the most important human metabolic enzymes, and the inhibition of CYP3A4 has been reported to alter the plasma pharmacokinetics of anticancer agents, and to be able to lead to unpredictable side effects. In the present Letter, we evaluated the inhibitory activities of selected compounds on CYP3A4 (Table 3).

Of these, **14f** did not inhibit CYP3A4 like third-generation MDR modulators, that is, Tariquidar. Compound **14e** exhibited a very weak inhibitory effect (IC_{50} >5 μM). In this series, electron withdrawing groups seemingly attenuate interaction of modulators with CYP3A4. Although the activities of **10h** and **10i** toward CYP3A4 were stronger than the other compounds, their activities were more than 200-fold weaker than that of ketoconazole. The expressional level and activity of CYP3A4 is known to depend on tumoral tissue type and on genetic polymorphisms, which contribute to inter-individual variations.[8] The inhibitions of both P-gp and drug metabolism could be advantageous during the treatment of MDR in specific types of cancers, in which CYP3A4 is expressed and co-localized with P-gp. In such a case, patients might also benefit from this type of dual inhibition as long as the modulators do not have unexpected intrinsic toxicity. Therefore, dual inhibitors, such as, **10g** and **14g**, as well as P-gp selective inhibitors like **14f** might be of therapeutic interest in terms of the personalizing cancer therapy.[8,9]

In summary, we synthesized and evaluated a novel class of disubstituted adamantane-based MDR reversal agents. Hit to lead optimization led to the elucidation of SAR for both terminal side chains. Many of the adamantyl derivatives produced were found to have a reversal activity greater than verapamil, and restore completely cytotoxicity of Taxol against the MDR cancer cells at 5 μM. The EC_{50} value of **10g** displayed 14-fold increase versus verapamil without intrinsic cytotoxicity. On the other hand, **14f** did not affect CYP3A4 at all, and thus, it is unlikely to influence the plasma pharmacokinetics of anticancer agents. SAR studies on reversal activity and CYP3A4 would provide useful information for the design and development of more selective and potent MDR reversal agents. The present work suggests that this new class of highly potent and selective compounds deserves further investigation.

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6. Spectral data for **10g**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.38 (1H, s), 8.07 (1H, s), 8.00–8.03 (1H, m), 7.83 (1H, d, J = 7.8 Hz), 7.56–7.60 (2H, m), 7.43–7.48 (3H, m), 7.33 (2H, d, J = 8.7 Hz), 6.96 (2H, d, J = 8.7 Hz), 4.61 (2H, s), 3.93 (3H, s), 3.71 (4H, m), 3.55 (2H, s), 2.43 (4H, m), 2.25 (2H, m), 2.00–2.09 (6H, m), 1.87 (4H, m), 1.73 (2H, m); MS (EI) m/z 689 (M^+); HRMS (EI^+) m/z calcd for $C_{39}H_{42}F_3N_3O_5$ 689.3077, found 689.3077.
7. Spectral data for **14f**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.41 (s, 1H), 7.89 (s, 1H), 7.83 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.36 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 6.60 (m, 2H), 4.71 (s, 2H), 4.62 (s, 2H), 3.91 (t, J = 5.1 Hz, 2H), 3.85 (s, 6H), 2.80 (t, J = 5.1 Hz, 2H), 2.28 (b, 2H), 1.63–2.15 (m, 12H); MS (EI) m/z 648 (M^+); HRMS (EI^+) m/z calcd for $C_{37}H_{39}F_3N_2O_5$ 648.2811, found 648.2811.
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10. MDR reversal assay. The ability of compounds to potentiate the cytotoxicity of Taxol was evaluated in MES-SA/DX5 cells, which was obtained from ATCC. Cells were plated in 96-well plates at 1.2×10^4 cells/well in 100 μl of medium and incubated for 24 h at 37 °C. The cells were then treated with varying concentrations of a test compound in the presence or absence of 100 nM Taxol for 60 h. Then, cell survival was assayed using Cell Counting Kit-8 (dojindo). Reversal of MDR is indicated if the compound enhances the toxicity of Taxol against MES-SA/DX5 cells.