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Synthesis and Bioactivity of Novel Adamantyl Derivatives as Potent MDR Reversal Agents

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Multidrug Resistance (MDR) is a type of resistance of tumor cells to various kinds of chemotherapeutic drugs which are structurally unrelated and also one of the major impediments to chemotherapeutic treatment of cancer.[1-4] It has been reported that 49 ATP-binding cassette (ABC) transporter genes are present in the human genome. Among them, over-expression of P-glycoprotein (P-gp), encoded by MDR1 gene is one of the major factors contributing to multidrug resistance which leads to barrier to successful chemotherapy.[5,6] Therefore the availability of safe and potent MDR reversal agents would be beneficial for clinical use. Reversal agents can act by binding to the membrane transport protein (P-gp), by inhibiting MDR's drug efflux capacity, or by suppressing expression of the MDR1 gene itself.[7] Although a number of P-gp inhibitors have been developed, there is currently no clinically useful drug that inhibits P-gp. In an attempt to find new and more effective MDR reversal agents, we previously identified adamantyl derivatives exhibiting more potent MDR reversal activity than verapamil, a well-known P-gp inhibitor, without considerable intrinsic cytotoxicity. Compounds **1-2** were identified utilizing high throughput image-based $DIOC_2$ efflux assays and anti-proliferation assays in a P-gp over-expressing MDR sarcoma cell line, MES-SA/DX5. Herein we describe a further structure-activity relationship study to find additional compounds in this series.[8,9]

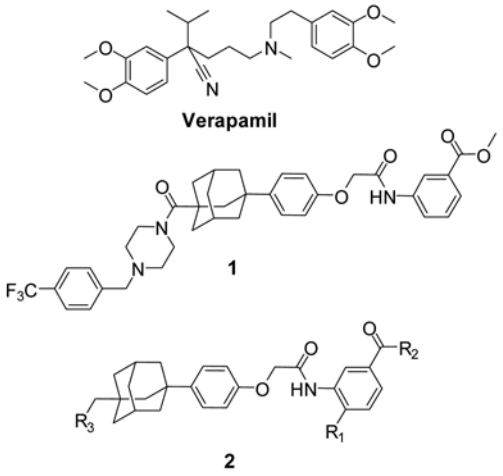
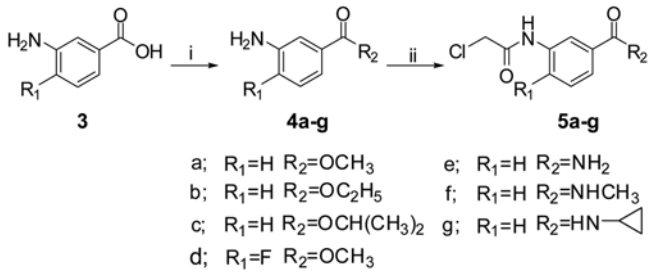


Figure 1

With the objective of investigating the effect of amine-based substituents on reversal activity, we synthesized a series of adamantyl derivatives **2a-t** that feature reductive amination of the adamantyl aldehyde derivatives as a key step. The synthetic procedures for **2a-t** are described in Schemes 1 to 3.

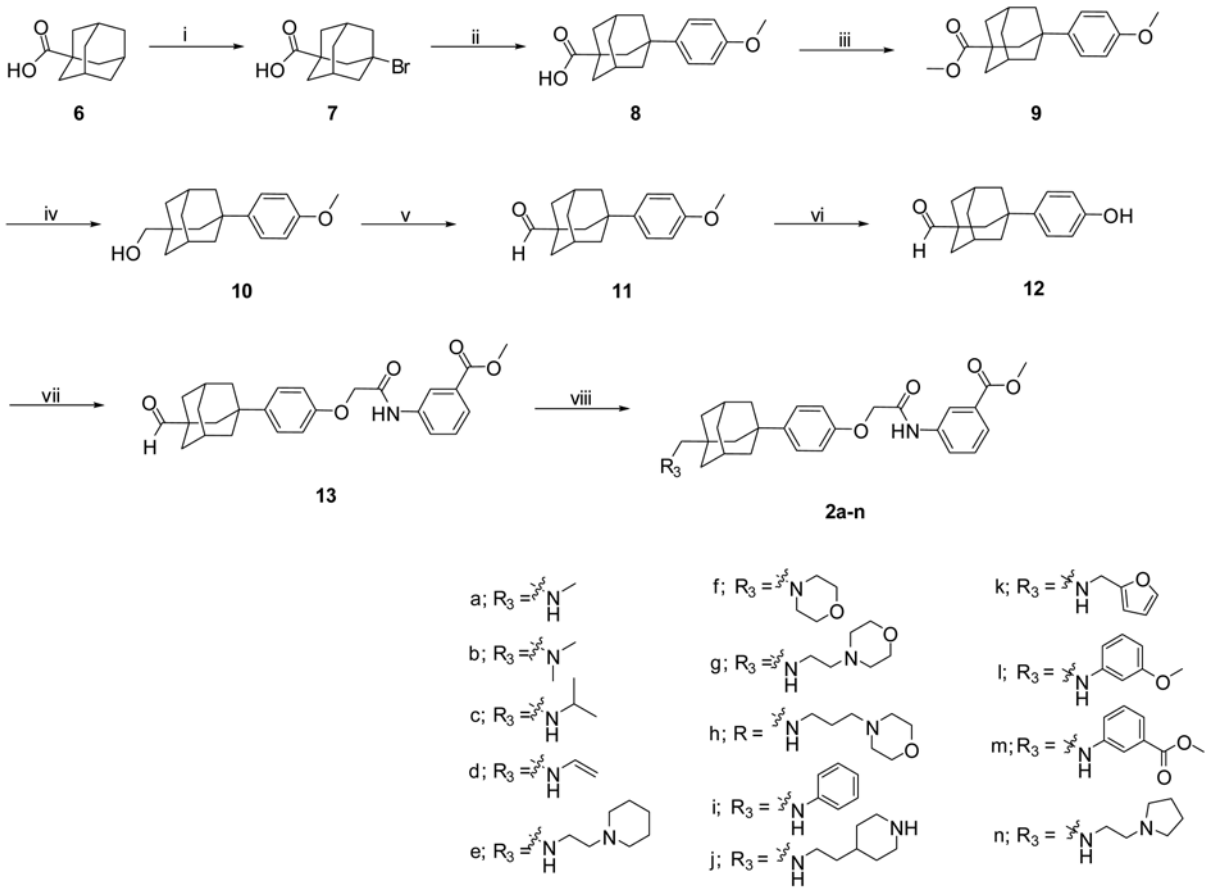
Synthesis of a new series of adamantyl derivatives (**2a-t**) were carried out starting from commercially available 3-amino benzoic acid **3** (Scheme 1). The corresponding esters (**4a-d**) and amides (**4e-g**) of 3-amino benzoic acid were prepared in the next step. Further alkylation with chloroacetyl chloride resulted in the alkylated derivatives (**5a-g**). Bromination of 1-adamantane carboxylic acid **6** with bromine gave an intermediate **7**, which was followed by Friedel-Crafts alkylation with anisole to provide compound **8**, as shown in Scheme 2. Esterification of **8** resulted in compound **9**, which was reacted with $LiAlH_4$ to give an alcohol **10**. The aldehyde **11** was then obtained by oxidation of alcohol **10**. Demethylation of **11** was carried out by using BBr_3 to afford a phenol compound **12**, which was utilized as a key intermediate to prepare the final compounds. Adamantyl aldehyde **13**, a precursor for the synthesis of the target adamantyl motifs (**2a-n**), was generated by reacting **12** with methyl 3-(2-chloroacetamido) benzoate **5a**. In similar fashion, the precursor **14o-t** was generated by reacting **12** with **5b-g** for the synthesis of derivatives **2o-t**. Finally the reductive amination reaction of **13** or **14o-t** with a variety of amine resulted in P-gp related MDR modulators **2a-t**.

Reversal activity of these derivatives was evaluated in

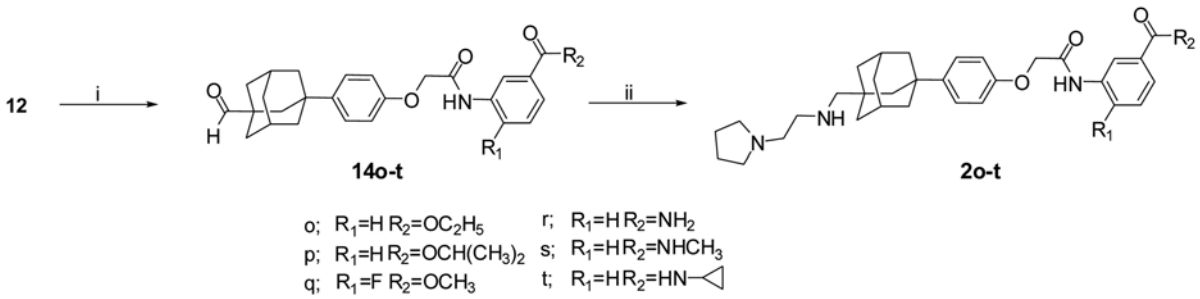


Scheme 1. Reagents and Conditions; (i) R_2-H, $SOCl_2$, 0 °C to 90 °C, 4 h for **4a-d** or EDC·HCl, HOBt, DIPEA, DMF, rt, 8 h for **4e-g** (ii) 2-Chloroacetyl chloride, Triethylamine, CH_2Cl_2, rt, 12 h.

[a]These authors contributed equally to this work.



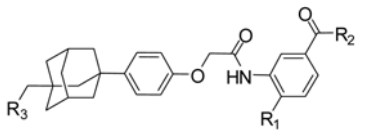
Scheme 2. Reagents and Conditions: (i)Br_2, $AlCl_3$, −5 °C to rt, 24 h; (ii) $AlCl_3$, Anisole, −10 °C to rt, 24 h; (iii) $SOCl_2$, MeOH, 0 °C to 90 °C, 4 h; (iv) $LiAlH_4$,THF, 0 °C to rt, 2 h; (v) PCC, CH_2Cl_2, 0 °C to rt, 1 h; (vi) BBr_3, CH_2Cl_2, 0 °C, 1 h; (vii) K_2CO_3, Cs_2CO_3, **5a**, DMF, 60 °C, 18 h; (viii) R_3H, $NaCNBH_3$, $ZnCl_2$, MeOH, rt, 12 h.

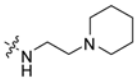
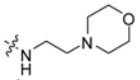
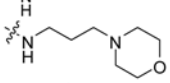
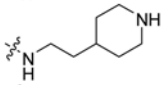
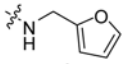
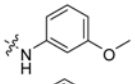
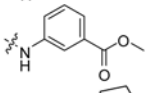
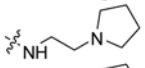
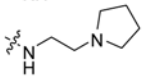
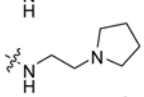
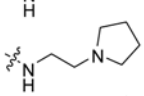
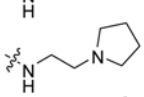
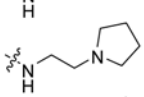
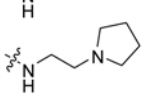


Scheme 3. Reagents and Conditions; (i) K_2CO_3, Cs_2CO_3, **5b-g**, DMF, 60 °C, 18 h; (ii) 2-(pyrrolidin-1-yl)ethanamine, $NaCNBH_3$, $ZnCl_2$, MeOH, rt, 12 h.

MES-SA/DX5 cells. EC_{50} values of the synthesized compounds were measured in the presence of 100 nM taxol. Some of these derivatives showed more potent MDR reversal activity than the well-known P-gp inhibitor, verapamil. Compound **2n** with a pyrrolidinyl ethyl amino moiety displayed an EC_{50} value of 0.66 μM, which is 13.5 fold more potent than verapamil (EC_{50} = 0.66 *vs* 8.94 μM, respectively) as shown in Table 1. Most alkyl amine derivatives were slightly more potent than verapamil. However, aniline analogues (**2i**, **2l** and **2m**) resulted in a marked loss of activity. Compound **2m** bearing an electron withdrawing group showed complete loss of reversal activity. Reversal activity of morpholine derivatives (**2f-h**) is prone to decrease with increasing chain length. A further SAR study has been carried out with the synthesis of adamantyl derivatives **2o-t**, keeping R_3 constant and introducing several substituents at either R_1 or R_2. Unfortunately, none of the derivatives have better IC_{50} values compared to **2n**. Interestingly, a cyclopropyl amino compound (**2t**) was found to be over 9 fold more potent than verapamil, while replacement of the ester group with an amide (**2r** and **2s**) led to no measurable activity. These results suggest that moderate hydrophobicity, size of the side chain and an amine with a proton seem to be essential to retain reversal activity.

Table 1. Structure-activity relationship of adamantyl analogues



No.	Structure			EC_{50}, μM[a] (100 nM taxol)	Fold increase
	R_1	R_2	R_3		
2a	H	OCH_3		11.7	0.8
2b	H	OCH_3		2.42	3.7
2c	H	OCH_3		4.22	2.1
2d	H	OCH_3		4.18	2.1
2e	H	OCH_3		4.7	1.9
2f	H	OCH_3		4.29	2.1
2g	H	OCH_3		6.56	1.4
2h	H	OCH_3		7.91	1.1
2i	H	OCH_3		14.53	0.6
2j	H	OCH_3		6.4	1.4
2k	H	OCH_3		4.83	1.9
2l	H	OCH_3		10.0	0.9
2m	H	OCH_3		>20	-
2n	H	OCH_3		0.66	13.5
2o	H	OC_2H_5		1.65	5.4
2p	H	$OCH(CH_3)_2$		7.03	1.3
2q	F	OCH_3		4.48	2.0
2r	H	NH_2		>20	-
2s	H	$NHCH_3$		>20	-
2t	H			11.33	0.8
Verapamil				8.94	1

[a]EC_{50} values were determined in the presence of 100 nM Taxol. The cells were then treated with varying concentrations of a test compound in the presence or absence of 100 nM Taxol for 60 hrs. Then, cell survival was assayed using Cell Counting Kit-8 (dojindo).

In summary, we identified potent MDR-reversing adamantyl compounds through a structure-activity relationship study. Compound **2n** showed excellent reversal activity, which was about 13-fold more potent than verapamil. Further investigation may lead to the developments of clinically valuable MDR modulators.

Experimental Section

General Procedure for Reductive Amination Reaction (2a-t). To a stirred solution of the adamantyl aldehyde (**13** for **2a-n**, and **14o-t** for **2o-t**, respectively) (1 equiv), and amines (3.0 equiv) in methanol (10 volumes) at room temperature was added a solution of sodium cyanoborohydride (1 M) (1 equiv) and zinc chloride (0.5 M) (1 equiv) in methanol (5 mL). The resulting solution was stirred at room temperature for 8 hours. After most of the methanol was removed by evaporation under reduced pressure, the residue was purified by column chromatography to obtain the product (**2a-t**). The spectral data can be found in the supporting information.

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References

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