Discovery of Highly Potent Multidrug Resistance (MDR) Reversal Agents: Aminosulfonylaryl Isoxazole Derivatives

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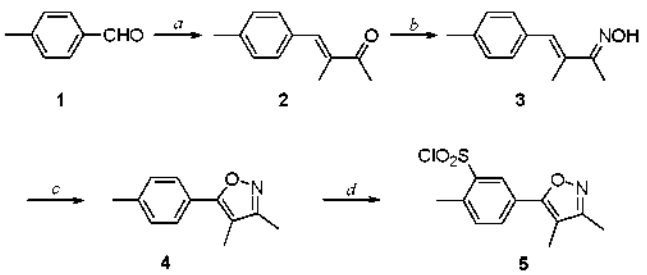
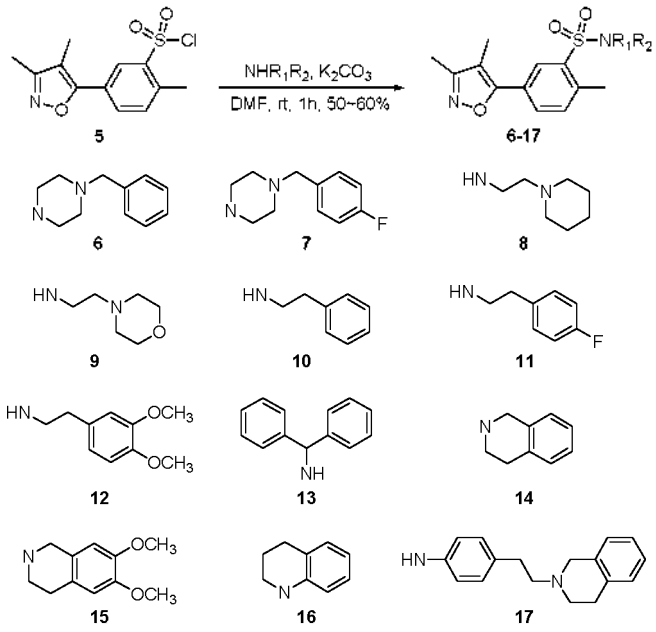
Multidrug resistance (MDR) resulting in chemotherapy failure has become a major cancer treatment issue, and of the many reported mechanisms, ATP-binding cassette (ABC) transporters are predominantly responsible for MDR.[1] The overexpression of P-glycoprotein (P-gp), an ATP-binding cassette (ABC) transporter, has been demonstrated not only to be primarily involved in the incapacitation of a variety of widely used anticancer agents, such as, paclitaxel (Taxol), doxorubicin, and vinorelbine, but also is observed much more frequently than other ABC transporters. However, despite the developments of a number of P-gp inhibitors to overcome MDR, no drug is currently clinically available. A number of drug candidates have been dropped because of critical limitations, such as, intrinsic toxicity and pharmacokinetic interactions. Furthermore, few accurate methods are available for identifying patients that might benefit from MDR inhibition. However, advanced functional imaging technology using ^{99m}Tc-sestamibi enables cancer patients responsive to P-glycoprotein modulation to be identified.[2] This advance and the hitherto unmet clinical need create a need for a large pool of novel drug candidates with high potency and selectivity. Herein, we report on a new class of highly potent MDR reversal compounds.

The aldol condensation of *p*-tolualdehyde with 2-butanone in the presence of 4 M HCl in 1,4-dioxane provided α,β-unsaturated ketone **2**, which was readily converted into oxime **3** by hydroxylamine. Following treatment with MnO_2 afforded the desired isoxazole **4**.[3] Mono-chlorosulphonylation[4] of isoxazole **4** gave the key intermediate **5**, and the coupling of **5** with appropriate amine fragments provided the desired sulfonamide derivatives (Scheme 2).



Scheme 1. Reagents and condition: (*a*) 2-butanone, 4M HCl in 1,4-dioxane, rt, overnight, 90% (*b*) NH_2OH, NaOAc, H_2O/EtOH, reflux, 2h, 63% (*c*) MnO_2, $CHCl_3$, reflux, 3h, 56% (*d*) chlorosulfonic acid, 55-60 °C, 9h, 66%.

The abilities of compounds to reverse MDR were examined using highly resistant human sarcoma MES-SA/DX5 cells, a P-gp overexpressing cancer cell line. The reversal activities of all synthesized compounds are summarized in table 1. The activities were evaluated with IC_{50} values of Taxol enhanced by co-treatment with 1 μM and 5 μM of each compound. The IC_{50} value of taxol against MES-SA/DX5 was 7.5 μM, although taxol usually has an IC_{50} value in the 1-10 nanomolar level against MES-SA, a non-MDR cancer cell line. Analogs bearing either the piperidino ethyl group (**8**) or the morpholino ethyl group (**9**), which lack aromatic rings, were inactive. On the other hand, most analogs with an aromatic group showed reversal activity. Benzyl piperazine **6** and fluorobenzyl piperazine **7** were too toxic at 5 μM, and inactive and moderately active at 1 μM, respectively. Compounds **10** and **12** were as active as verapamil,[5] a well-known P-gp inhibitor. In particular, 5 μM of compound **10** made MDR cancer cells 52-fold more sensitive to Taxol. Furthermore, fluorination (**11**) improved reversal activity, but also slightly increased intrinsic cytotoxicity. 1,2,3,4-tetrahydroisoquinolone, a cyclic form of phenethylamine was introduced to make the flexible phenethyl side chain more rigid.



Scheme 2. Synthesis of sulfonamide derivatives.

Table 1. MDR reversal activity of compounds **6-17**

MDR modulator	μM	IC_{50} (nM) of Taxol[a]	Fold increase	Intrinsic Cytotoxicity (% viability)
No modulator	0	7500	1	> 95%
Verapamil	5	196	38	85%
	1	~2500	-	> 95%
6	5	ND[b]	-	44%
	1	> 2500	-	
7	5	ND	-	37%
	1	880	8.5	76%
8	5	> 2500	-	> 95%
9	5	> 2500	-	> 95%
10	5	143	52	88%
	1	> 2500	-	> 95%
11	5	39	192	64%
	1	1770	4.2	> 95%
12	5	170	44	87%
	1	1607	4.7	> 95%
13	5	296	25	90%
	1	> 2500	-	> 95%
14	5	206	36	> 95%
	1	1650	4.5	> 95%
15	5	ND	-	70%
	1	647	12	85%
16	5	> 2500	-	84%
17	5	< 10	> 750	87%
	1	32	234	> 95%
	0.5	200	37.5	> 95%

[a]IC_{50} values represent mean of three separate experiments made in triplicate. [b]ND, not determined (due to high toxicity or no activity at a lower concentration).

Compared to compound **10**, rigidification of the phenethyl side chain (**14**) lowered intrinsic cytotoxicity and maintained reversal activity. Dimethoxy tetrahydroisoquinoline **15** at 5 μM was toxic, but at 1 μM displayed weak toxicity and good reversal activity. We next tried to introduce the common side chain of highly active P-gp inhibitors into this scaffold. Compound **17** completely reversed the potency of Taxol at 5 μM. The IC_{50} of Taxol against resistant cells was found to be < 10 nM in the presence of 5 μM of compound **17**, which was almost the same as its IC_{50} against the sensitive cell line, MES-SA. To compare directly the isoxazoles with verapamil, we determined their EC_{50} values in the presence of 100 nM Taxol (Table 2).[6] Compounds **10**, **12**, and **14** were found to have inhibitory effects that were slightly better than verapamil. Compound **15** was 3.2-fold better than verapamil, but had GI_{50} value of 12.1 μM. Compound **17** was 10-fold better than verapamil, and displayed acceptable cytotoxicity (GI_{50} = 17.4 μM *versus* EC_{50} = 0.68 μM).

In summary, we developed highly potent MDR reversing small molecules without intrinsic cytotoxicity. Compound **17** showed excellent reversal activity, which was about 10-fold better than verapamil. Further manipulation of these isoxazoles may lead to the developments of MDR modulators with clinical potential.

Table 2. EC_{50} of selected compounds and Verapamil[a]

MDR modulator	EC_{50} (100 nM Taxol)[b] (μM)	Fold increase	Intrinsic cytotoxicity GI_{50}[c]
10	5.72	1.2	> 20
12	6.43	1.1	12.5
14	6.45	1.1	> 20
15	2.22	3.2	12.1
17	0.68	10.4	17.4
Verapamil	7.10	1.0	> 20

[a]Data are the mean of three independent experiments. [b]EC_{50} values were determined in the presence of 100 nM Taxol in MES-SA/DX5 cells. [c]GI_{50} (μM) was evaluated in the absence of Taxol in MES-SA/DX5 cell.

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6. Spectral data for **17**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.06 (s, 3H) 2.25 (s, 3H), 2.66 (m, 5H), 2.68-2.87 (m, 6H), 3.64 (s, 2H), 6.92-6.99 (m, 3H), 7.06-7.10 (m, 5H), 7.37 (d, 1H, *J* = 8.07 Hz), 7.75 (dd, 1H, *J* = 2.01, 8.04 Hz), 8.19 (d, 1H, *J* = 1.65 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.00, 10.16, 20.41, 29.02, 33.21, 50.91, 56.03, 59.83, 110.13, 121.83, 125.64, 126.17, 126.55, 127.06, 127.74, 128.65, 129.74, 130.71, 133.25, 133.91, 134.18, 134.55, 138.09, 138.23, 144.51; LRMS (FAB) *m/z* 502 $(M+H)^+$; HRMS (FAB) calcd for $C_{29}H_{32}N_3O_3S$ $(M+H)^+$ 502.2164, found 502.2164.
7. **MDR Inhibition Assay.** The multiple drug resistant cell line MES-SA/DX5 was purchased from ATCC (Manassas, VA, USA). The cells were plated in 96-well microtiter plates at 5×10^3 cells/well in media with and without 100 nM Taxol. Then isoxazole modulators were added at escalating concentrations ranging from 0.16 to 20 μM to the cells. The plates were incubated for 60 hrs. The cell viability was assessed using CCK-8 (Dojindo).