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2,4,5-Trisubstituted Imidazoles: Novel Nontoxic Modulators of P-glycoprotein Mediated Multidrug Resistance. Part 1[1]

Sepehr Sarshar,* Chengzhi Zhang,[†] Edmund J. Moran,[‡] Sonja Krane,[§] Jennifer C. Rodarte, Khalid D. Benbatoul,[¶] Ross Dixon and Adnan M. M. Mjalli[‖]

Ontogen Corporation, 6451 El Camino, Real Carlsbad, CA 92009, USA

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Abstract—*N*-4,5-Di-(4-dialkylamino)phenyl imidazoles (**A**) are potent modulators of P-glycoprotein mediated multidrug resistance. This manuscript describes the discovery and lead optimization of this novel class of inhibitors.

The emergence of tumor cell resistance to chemotherapy and subsequent patient relapse is a major hurdle in the treatment of cancer.[2] Resistance may occur either at the initial presentation of the disease (intrinsic resistance) or at the time of relapse (acquired resistance). Both phenomena, commonly referred to as multidrug resistance (MDR), are often associated with the over-expression of P-glycoprotein (Pgp), a transmembrane ATP-dependent efflux pump.[3] Pgp actively extrudes a wide variety of structurally unrelated compounds, including a full range of antineoplastic drugs. The level of expression of Pgp correlates directly with the degree of resistance.[4] This is evidenced by reduced membrane permeability and increased removal of drug from the cell resulting in overall lower intracellular drug accumulation. Another ATP-dependent membrane efflux pump, the product of the MRP gene, has also been implicated in the MDR phenomenon,[5] as have other ATP-dependent and enzymatic mechanisms.

To date, a number of compounds have been identified that can partially or sometimes completely resensitize resistant tumor cells.[2a] However, the clinical toxicity associated with these agents has limited their use. Simultaneously, a lack of structural information on the substrate and ATP binding sites of Pgp has hampered rational drug design in this field.

We now wish to report the successful application of combinatorial chemistry to the discovery and initial lead optimization of a novel class of potent and nontoxic modulators of Pgp mediated MDR. Previously, we described the first solid-phase synthesis of imidazoles using functionalized Wang resin (Fig. 1).[6] This methodology provides an efficient route to large libraries of tri- and tetra-substituted imidazoles. In this manner, high purity material may be prepared rapidly and used directly in a variety of biological assays.

The exploratory imidazole library for the MDR project was designed around structural characteristics of known Pgp substrates and modulators: hydrophobic compounds with multiple amine groups. As such, a number of hydrophobic aldehydes, amines and 1,2-diaryldiones with Lewis basic substituents (dialkyl amines, methoxy) were chosen as initial reagents. Following our published protocol,[6] a 500-member library was prepared and screened in various MDR potentiation assays using the CEM/VLB1000 cell line.[7] To our delight, the screens uncovered a number of active compounds. Structure–activity relationships (SARs) outlined a specific

*Corresponding author. Present address: Pfizer Global, San Diego, CA 92121, USA. Tel.: +1-858-622-7506; fax: +1-858-678-8277; e-mail: sep.sarshar@agouron.com

[†]Present address: Bayer Corporation, Pharmaceuticals Division, West Haven, CT 06516, USA.

[‡]Present address: Advanced Medicine, South San Francisco, CA 94080, USA.

[§]Present address: Columbia University, Department of Chemistry, New York, NY 10027, USA.

[¶]Present address: Ligand Pharmaceuticals, La Jolla, CA 92121, USA.

[‖]Present address: TransTech Pharma, High Point, NC 27265, USA.

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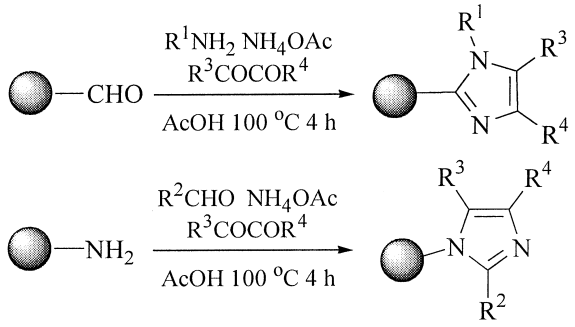
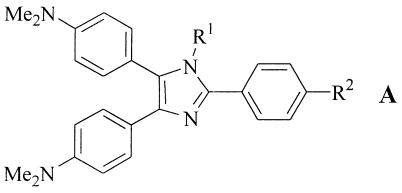


Figure 1. Solid-phase synthesis of tetra- and tri-substituted imidazoles.

Table 1. Modulation of Pgp mediated multidrug resistance in CEM/VLB 1000 cells

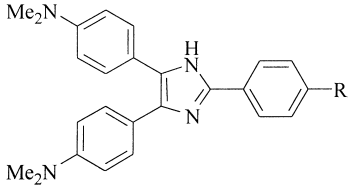


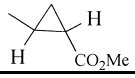
A

Compound	R^1	$R^{2,a}$	ED_{50}, μM[b]
1	H	CO_2H	60
2	H	CO_2CH_3	0.6
3	*n*-Hexyl	CO_2H	2
4	*n*-Hexyl	CO_2CH_3	6
5	H	$CH{=}CHCO_2H$	10
6	H	$CH{=}CHCO_2CH_3$	0.3
7	H	OH	5
8	Phenethyl	OH	1
9	Phenethyl	CO_2H	10
10	Phenethyl	$CH{=}CHCO_2H$	20

[a]Entries **5**, **6**, and **10** have the *E* configuration.
[b]ED_{50} is defined as the compound concentration that causes 50% inhibition of cell growth.

Table 2. Modulation of Pgp mediated multidrug resistance in CEM/VLB 1000 cells



Compound	R^a	ED_{50}, μM[b]
6	$CH{=}CHCO_2CH_3$	0.30
15	$CH_2CH_2CO_2CH_3$	1.00
16	CH=CHCN	0.60
17	$CH{=}CHCN_2OMe$	0.13
18	$CH{=}CHCH_2OEt$	0.08
19	$CH_2CH_2CH_2OMe$	0.30
20	$CH_2CH_2CH_2CH_2OMe$	1.10
21		0.31

[a]Entries **6**, **16**, **17**, and **18** have the *E* configuration.
[b]ED_{50} is defined as the compound concentration that causes 50% inhibition of cell growth.

trend: weakly acidic (CO_2H) or neutral polar substituents (OH) at the 2-position combined with Lewis basic functional groups (Me_2N, OMe) at the 4- and 5-positions of the imidazole ring were necessary for activity (Table 1).

Preliminary solution-phase SAR optimization also revealed that the lack of substitution at the 1-position and a cinnamic methyl ester at the 2-position increased activity dramatically (Table 1, entries **2**, **4**, and **6**). Armed with this knowledge, a number of ester analogues of **6** were prepared [Et (**11**), *i*-Pr (**12**), *tert*-Bu (**13**), $PhCH_2$- (**14**)] but no change in activity was observed.

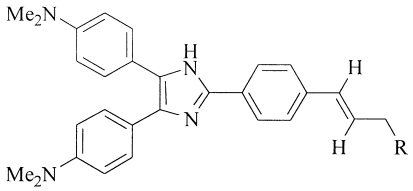
Although initial in vitro metabolism suggested that these compounds had some degree of metabolic stability,[8] ester hydrolysis was deemed an unnecessary liability. To this end, a number of ester-free analogues were prepared and in fact the allylic ethyl ether stood out as indicated by its higher potency and acceptable oral bioavailability (Table 2, entry **18**).[9]

Replacement of the ethyl ether moiety of **18** with other functional groups resulted in no gain or loss of potency (Table 3).

These modulators are at least an order of magnitude more potent than Verapamil against a variety of resistant cell lines (Table 4).[10] In fact, compound **18** completely resensitizes both cell lines even in the presence of Taxol.

In conclusion, we have described the successful application of combinatorial chemistry to the discovery and optimization of novel, potent and nontoxic[11] modulators of Pgp dependent multidrug resistance.[12]

Table 3. Modulation of Pgp mediated multidrug resistance in CEM/VLB 1000 cells



Compound	R	ED_{50}, μM[a]
18	OEt	0.08
22	OBu	0.11
23	OCH_2CH_2OMe	0.09
24	OCH_2Ph	0.30
25	OPh	0.45
26	$OC(O)NEt_2$	0.88
27	OC(O)Bu	0.48
28	NMe_2	1.30
29	*N*-Piperidine	0.96
30	*N*-Morpholine	0.32

[a]ED_{50} is defined as the compound concentration that causes 50% inhibition of cell growth.

Table 4. Sensitization of various MDR cell lines[a]

Compound	MCF7/ADR[b]		MES-SA/DX5[c]	
	Vinblastine	Taxol	Doxorubicin	Taxol
Wild type	0.025	0.025	0.11	0.009
No modulator	1	11.9	6.8	4.6
Verapamil	0.251	2.5	3	2.5
12	0.028	0.15	0.34	0.13
18	0.01	0.036	0.089	0.024

[a]Numbers refer to ED_{50} of antitumor agents in μg/mL. ED_{50} is defined as the compound concentration that causes 50% inhibition of cell growth.
[b]MCF7/ADR is a MCF7 (human breast carcinoma) derived cell line that overexpresses Pgp and is selected for Doxorubicin resistance.
[c]MES-SA/DX5 is a MES-SA (human uterine carcinoma) derived cell line that overexpresses Pgp and is selected for Doxorubicin resistance.

References and Notes

1. Part 1 in the series. For additional data related to this work see: (a) Mjalli, A. M. M.; Sarshar, S. U.S. Patent 5,700,826, 1997; *Chem. Abstr.* **1998**, *126*, 8263. (b) Mjalli, A. M. M.; Sarshar, S. U.S. Patent 5,756,527, 1998; *Chem. Abstr.* **1998**, *126*, 352632. (c) Mjalli, A. M. M.; Zhang, C. U.S. Patent 5,840,721, 1998; *Chem. Abstr.* **1998**, *126*, 774230.
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3. Juranka, P. F.; Zastawny, R. L.; Ling, V. *FASEB J.* **1989**, *3*, 2583.
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6. Sarshar, S.; Siev, D.; Mjalli, A. M. M. *Tetrahedron Lett.* **1996**, *37*, 835. For detailed experimental protocols see this reference and ref 1.
7. MDR potentiation assays were carried out using CEM/VLB1000 cells in the presence of Vinblastine (5 μg/mL) and compound (0.01 to 50 μM). Verapamil was used as the reference standard. CEM/VLB1000 is a CEM (human lymphoma) derived cell line that overexpresses Pgp and is selected for Vinblastine resistance. For detailed in vitro protocols see ref 1 as well as Newman, M. J.; Rodarte, J. C.; Benbatoul, K. D.; Romano, S. J.; Zhang, C.; Krane, S.; Moran, E. J.; Uyeda, R. T.; Dixon, R.; Guns, E. S.; Mayer, L. D. *Cancer Res.* **2000**, *60*, 2964.
8. Results of liver microsomal incubation for **12** in various species. Numbers in parentheses represent the amount of metabolized compound after 2 h: rat (23%), dog (11%), human (11%).
9. Results of liver microsomal incubation for **18** in various species. Numbers in parentheses represent the amount of metabolized compound after 2 h: rat (14%), dog (20%), human (8%). Pharmacokinetic parameters (5 mg/kg, iv; 10 mg/kg, po): $T_{1/2}$(dog) = 30 min; %F (dog) = 35.
10. MDR sensitization assays were carried in the presence of Verapamil (1 μM), **12** (1 μM), and **18** (1 μM). The concentrations of the cytotoxic agents were varied from 0.01 to 30 μg/mL. For detailed in vitro protocols see ref 7.
11. The compounds evaluated in Tables 1–4 did not show inherent toxicity at the concentrations tested. For details see ref 7.
12. Standard experimental procedure—solution phase: The dione (3.04 mmol) and aldehyde (4.56 mol) were placed in 5.85 mL of acetic acid. In a separate flask, NH_4OAc (30.4 mmol) was taken up in 1.75 mL of acetic acid. Both flasks were placed in a pre-heated 140 °C oil bath. As soon as the solids in the flasks dissolved, the NH_4OAc solution was poured into the mixture of aldehyde and dione. The resulting solution was kept at 140 °C for another 40 min. It was then cooled to room temperature, the pH of the solution was adjusted to 0.8 using 3.0 M HCl. The solution was extracted with Et_2O (5×) to remove any unreacted aldehyde and dione. The aqueous layer was then taken to pH 8.0 with 3.0 N NaOH and was extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and the solvent was removed to yield the product.