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

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Abstract—Solution-phase combinatorial chemistry was applied to the optimization and development of clinical candidate OC144-093 (**22**), a novel and nontoxic modulator of P-glycoprotein mediated multidrug resistance.

P-glycoprotein (Pgp) is a high molecular weight head-to-tail dimeric protein that was originally isolated from mutant Chinese hamster ovary cells in 1976.[2] Each monomer consists of multiple transmembrane domains and an ATP binding site.[3] Pgp is a normal constituent of several tissues (e.g. intestinal, renal, and blood–brain barrier) and plays a vital role in the clearance of structurally unrelated toxic agents from the body via an active efflux mechanism.[4] Unfortunately, this critical property of Pgp is a leading cause of fatality in cancer patients whose tumor cells display intrinsic or acquired immunity against a wide range of chemotherapies. Dubbed multidrug resistance or MDR, clinical oncologists were the first to describe this syndrome which is often associated with the over-expression of Pgp in cancer cells.[5]

Over the last decade, the development of in vitro MDR modulators has been remarkably successful, yet to date no agent has displayed significant clinical efficacy.[6] This is largely due either to the inherent toxicity of these compounds or to the heightened toxicity of the antineoplastic agents when co-administered with the modulator.

In the preceding article, we described a novel class of 2,4,5-trisubstituted imidazoles (**A**) with potent Pgp inhibitory profile.[7] We now wish to report the successful application of solution-phase combinatorial chemistry to the development of the clinical candidate OC144-093 (**22**), a nontoxic modulator of Pgp mediated MDR.[8] The structure–activity relationships (SARs) of the 1- and 2-positions of the imidazole core were explored and optimized using solid-phase combinatorial chemistry.[7] In short, imidazoles with structures **C** and **D** showed the highest potency. Although ester metabolism of imidazoles **C** was not very significant, imidazoles **D** proved more stable. However, pharmacokinetic measurements indicated that the Me_2N groups in both classes were rapidly metabolized. *N*-Demethylation and *N*-oxide formation are common metabolites of tertiary amines, a problem that may be circumvented through the introduction of bulky secondary amines. Our strategy for the optimization of these amine functionalities is summarized in Figure 1. Due to the limited availability of 1,2-aryldiones, we decided to employ a solution-phase approach in which various amine groups could be introduced consecutively via nucleophilic displacement on difluorobenzil.

This route proved most desirable since a large number of symmetrical and unsymmetrical imidazoles could be generated efficiently. Tables 1 and 2 summarize the activities of a number of these compounds.[9] When the nucleophile was Me_2NH, a demethylated by-product **B** formed in 10–15% during the cyclo-dehydration step. This fortuitous result allowed us to explore the activity

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of potential first pass metabolites. To our delight, imidazoles **B** showed both improved potency and metabolic stability (Tables 1, 2, and 3). Subsequently, other analogues of **5** were prepared using CH_3NH_2–HCl salt. For imidazoles **B** increasing the bulk of the tertiary amine substituent proved detrimental to the activity (Table 1, entries **5** to **8**). The best results were obtained when both amine substituents were secondary (Table 1, entries **11** and **12**; Table 2 entries **19**, **20**, and **22**). Replacement of one of the amino substituents with a fluorine did not increase the metabolic stability of this class (Table 3, entries **19**, **21**, and **22**).

Given the excellent potency and half-life of **22** (OC144-093), we next turned our attention to improving the oral bioavailability of these compounds. We initially focused our efforts on reducing the molecular weight of the modulators. Figure 2 summarizes our strategy for the synthesis of 2,5-disubstituted imidazoles **E**.

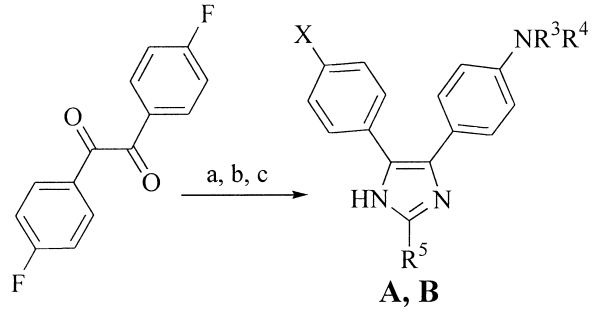
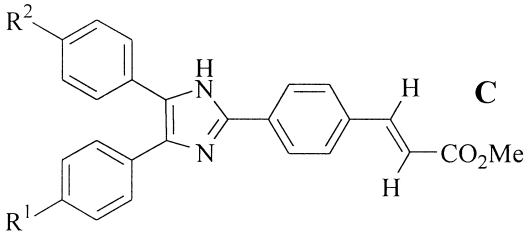


Figure 1. Solution-phase synthesis of tri-substituted imidazoles **A** and **B**. (a) R^1R^2NH, K_2CO_3, DMSO, 90 °C; (b) R^3R^4NH, K_2CO_3, DMSO, 90 °C; (c) R^5CHO, NH_4OAc, AcOH, 100 °C. **A:** $X = R^1R^2N$ (80%); **B:** $X = R^1NH$ (10–15%).

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



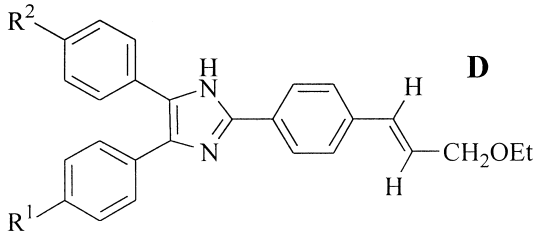
Compound	R^1	R^2	ED_{50}, μM[b]
1	Me_2N	Me_2N	0.30
2	Et_2N	Et_2N	0.57
3	Pyrrolidine	Pyrrolidine	0.37
4	$(Allyl)_2N$	$(Allyl)_2N$	5.50
5	Me_2N	MeNH	0.13
6	Et_2N	MeNH	0.07
7	Pr_2N	MeNH	0.18
8	Bu_2N	MeNH	2.10
9	Me_2N	NH_2	0.59
10	Et_2N	NH_2	0.31
11	MeNH	MeNH	0.20
12	*i*-PrNH	MeNH	0.12

[a]CEM/VLB1000 is a CEM (human lymphoma) derived cell line that overexpresses Pgp and is selected for Vinblastine resistance.
[b]ED_{50} is defined as the compound concentration that causes 50% inhibition of cell growth.

The 2,5-disubstituted imidazoles **F** show similar SAR trends as the 2,4,5-trisubstituted imidazoles **C** and **D** but have lower in vitro potency (Table 4). Hence, further optimization was not pursued and compound **22** was chosen as the initial clinical development candidate.

Pharmacokinetic studies in healthy male volunteers (400 mg/oral gave) revealed 3–5 μM concentrations of **22** (OC144-093) in the plasma after 3–4 h. These plasma levels are significantly higher than the 1 μM concentration required for full reversal of Pgp mediated MDR in preclinical models. Virtually no adverse side effects or evidence of CNS toxicity was observed. When administered with a fatty meal, a significant increase in the oral bioavailability of **22** was noted as compared to the fasted state. Based on previous intravenous data, the oral bioavailability of **22** in man is estimated to be greater than 60% with food and its terminal half-life at around 22 h. The latter suggests that once- or twice-a-day oral dosing may be sufficient to maintain therapeutic levels of the modulator. In a second placebo controlled study, the tolerability and safety of three sequential escalating doses of 200, 300, or 400 mg of **22** were evaluated. Healthy male volunteers were dosed in parallel groups

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



Compound	R^1	R^2	ED_{50}, μM[a]
13	Me_2N	Me_2N	0.08
14	Et_2N	Et_2N	0.26
15	*N*-Morpholine	*N*-Morpholine	0.22
16	MeNH	Me_2N	0.11
17	MeNH	Et_2N	0.06
18	MeNH	*N*-Pyrolidine	0.15
19	MeNH	*i*-PrNH	0.05
20	EtNH	*i*-PrNH	0.05
21	F	*i*-PrNH	0.16
22	*i*-PrNH	*i*-PrNH	0.05

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

Table 3. Dog pharmacokinetic parameters for selected imidazoles[a]

Compound	R^1	R^2	$t_{1/2}$, h	F%
13	Me_2N	Me_2N	0.5	35
19	MeNH	*i*-PrNH	1.35	36
21	F	*i*-PrNH	1.27	45
22	*i*-PrNH	*i*-PrNH	2.79	41

[a]Dosage: 5 mg/kg, iv; 10 mg/kg, po

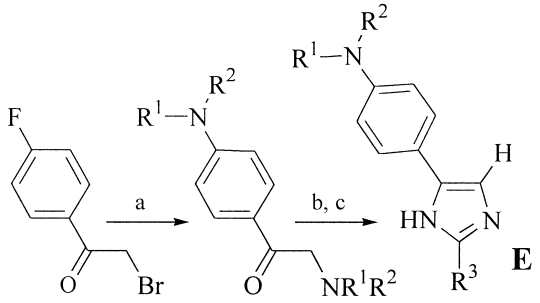
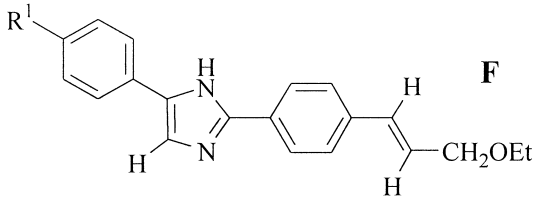


Figure 2. Synthesis of 2,5-disubstituted imidazoles **E**. (a) R^1R^2NH, K_2CO_3, DMSO, 90 °C (85–95%); (b) $Hg(OAc)_2$ (50–60%); (c) NH_3, R^3CHO, MeOH, 23 °C (40–50%).

Table 4. Modulation of Pgp mediated multidrug resistance in CEM/VLB 1000 cells



Compound	R^1	ED_{50}, μM[a]
23	Et_2N	0.3
24	Pr_2N	0.28
25	Morpholine	0.37
26	*i*-PrNH	0.34
27	*i*-BuNH	0.25

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

at 6, 12, and 24 h intervals, respectively. OC144-093 was well tolerated at all dose levels and was free of any serious side effects. Currently, **22** is also being evaluated for the enhancement of oral bioavailability of certain therapeutic agents (e.g. paclitaxel and saquinavir) that are substrates for Pgp in the gut.

References and Notes

1. Part 2 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.* **1997**, *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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8. Details of the biological experiments have been published separately, see: 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.
9. 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. The compounds evaluated in Tables 1, 2, and 4 did not show inherent toxicity at the concentrations tested. For detailed in vitro protocols see ref 8.