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Discovery and SAR development of 2-(phenylamino) imidazolines as postacyclin receptor antagonists

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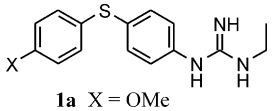
Abstract—On the basis of screening hits (**1a**,**b**), a series of selective, high affinity prostacyclin receptor antagonists was developed. The optimized lead compound **25d** [(4,5-dihydro-1*H*-imidazol-2-yl)-[4-(4-isopropoxybenzyl)phenyl]amine] had analgesic activity in the rat.

Considerable evidence indicates that prostaglandin I_2 (PGI_2 or prostacyclin) is a major mediator of inflammation and pain.[1] Prostacyclin is one of several prostanoids released upon tissue damage and causes sensitization or direct activation of sensory nerve endings. Prostaglandin E_2 has previously been assumed to be the major prostanoid involved in these processes, but recent evidence suggests that prostacyclin may play a more important role than has heretofore been recognized. For example, high concentrations of the prostacyclin metabolite 6-keto-$PGF_{1\alpha}$ have been found in inflammatory exudates, for example from synovial fluid from human arthritic knee joints[2] and from the peritoneal cavity fluid of mice injected with chemical irritants.[3] More compelling evidence for a major role of prostacyclin in inflammatory pain is the altered pain and inflammatory responses observed in transgenic mice lacking the prostacyclin receptor (IP receptor). In these IP receptor deficient animals, responses to pain and inflammation (acetic acid-induced writhing and carrageenan-induced edema) were reduced to levels observed when wild-type mice were treated with indomethacin.[4] Thus it can be hypothesized that an IP receptor antagonist could have therapeutic potential as an analgesic/anti-inflammatory agent with the important caveat that untoward cardiovascular and thrombotic side effects would not be induced. Herein we describe the discovery of the first-reported IP receptor antagonists.

Prostacyclin receptor affinity was determined by radioligand binding utilizing displacement of the high-affinity ligand [^{3}H]iloprost from prostacyclin receptors endogenously expressed in NG108-15 cells, a rodent neuroblastoma cell line. Initial screening was performed on a subset (460 compounds chosen for structural diversity) of the Syntex compound library. Fortuitously, arylguanidines **1a** and **1b** were found to have modest affinity with pK_i values of 6.6 and 6.2, respectively. Structure–activity relationship studies were then undertaken based on these leads with the goal of producing higher affinity ligands.



1a X = OMe

1b X = Cl

Modification of the guanidine moiety of **1a** and **1b** (Table 1) led to identification of the 2-(phenylamino) imidazolines **4a**,**b** and tetrahydropyrimidine **8** with improved affinity.[5] A degree of steric intolerance was noted by the reduced affinity of ligands **2** and **6**. In

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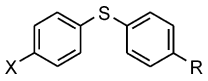
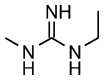
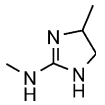
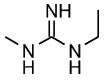
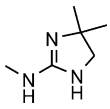
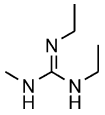
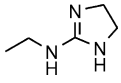
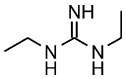
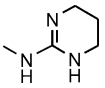
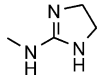
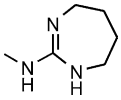
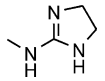
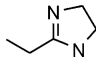
Table 1. Receptor bindings for compounds **1–16**



Compd	X	R	pK_i[a]	Compd	X	R	pK_i[a]	Compd	X	R	pK_i[a]
1a	OMe		6.6	**5**	OMe		7.2	**11**	OMe		5.9
1b	Cl		6.2	**6**	OMe		6.2	**12**	OMe		6.5
2	OMe		<5	**7**	Cl		6.3	**13**	OMe		6.7
3	Cl		<5	**8**	OMe		7.1	**14**	OMe		<5
4a	OMe		7.3	**9**	OMe		5.9	**15**	OMe		<5
4b	Cl		7.1	**10**	OMe		5.3	**16**	OMe		5.0

[a] Displacement of [^{3}H]iloprost in rodent NG-108-15 neuroblastoma cells, mean of two or more determinations ±0.1.

addition, the 2-(amino)imidazoline was clearly preferred over the methylene linked imidazoline group as in **10**. Other basic groups led to a significant decrease in affinity; therefore, the 2-(phenylamino)-imidazoline group was maintained for subsequent SAR development.

Compounds in Tables 2 and 3 were prepared by reaction of the requisite aniline (**17**) with 2-chloroimidazoline[6] as exemplified in Scheme 1. Transposition of the *para*-thiophenyl group in compound **4a** to the *ortho* and *meta* positions (**18** and **19**) led to a dramatic decrease in receptor affinity, as did constraint into the dibenzothiophene system (des-methoxy analogue **20**) (Table 2). Similarly, constraint of the imidazoline group into the tetrahydro-imidazo[2,1-*b*] ring system (**21**) resulted in loss of affinity.

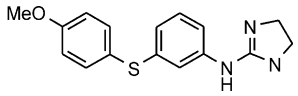
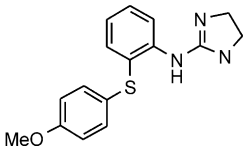
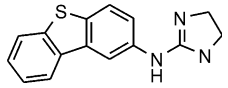
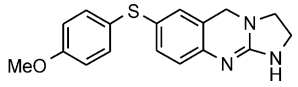
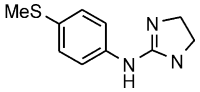
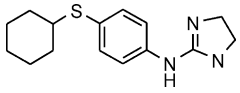
The importance of the distal phenyl ring in **4** was emphasized by the low affinity of analogues **22** and **23** (Table 2). Variation of the substituent pattern in this phenyl ring indicated that, at least for a methoxy group, the para position was optimal (Table 3). Although para-alkoxy groups resulted in the highest affinity, a number of other para substituents groups were tolerated.

The *iso*-propoxy derivative **4j**, with a pK_i of 7.5 at the prostacyclin receptor was evaluated to assess whether this prototypical compound was a functional antagonist, and hence a suitable candidate for in vivo evaluation. The prostacyclin receptor is a G-protein coupled receptor that is positively linked to adenylyl cyclase;[7] therefore, functional antagonism can be determined using a classical cyclic AMP (cAMP) assay. When tested for antagonism of carbaprostacyclin-stimulated cAMP accumulation in human SH-SY5Y neuroblastoma cells, **4j** was indeed found to be a competitive antagonist with a pK_B of 8.1. However, the moderate affinity of this compound, coupled with poor oral bioavailability due to slow absorption and high first-pass metabolism precluded further development.

Continued SAR development indicated that a modest increase in affinity was obtained when the link between the phenyl rings in prototype **4a** was changed from sulfur to carbon as in bibenzyl analogue **25a** (Table 4). This prompted synthesis[8] (Scheme 2) of a series of related ethers which were tested for functional antagonism (Table 5). Increased activity generally correlated with the size of the ether moiety and antagonists with pK_B values in the nanomolar range were obtained. These were subsequently differentiated on the basis of in-vitro metabolic and in-vivo pharmacokinetic parameters and the *iso*-propoxy derivative **25d** was determined to have the best overall profile. This compound had an oral bioavailability of 85% in the dog with a $T_{1/2}$ of 8 h.

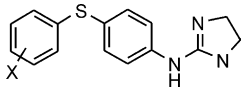
Selectivity of **25d** over other receptors was evaluated through several protocols. PGE_2 stimulates cAMP formation through the EP_2 (and EP_4) receptor; however, **25d** failed to inhibit this response in SH-SY5Y cells (pK_B <5) indicating that this compound is not an $EP_{2,4}$ receptor antagonist. Similarly, **25d** did not display

Table 2. IP receptor binding for compounds **18–23**

Compd		pK_i[a]
18		5.4
19		6.2
20		5.5
21		< 5
22		< 5
23		5.5

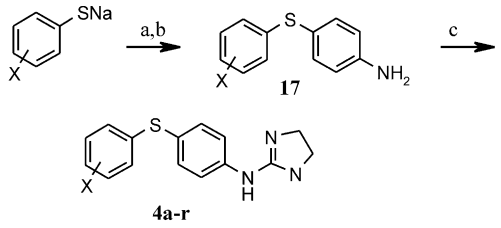
[a] Displacement of [^{3}H]iloprost in rodent NG-108-15 neuroblastoma cells, mean of two or more determinations ±0.1.

Table 3. Receptor binding for compounds **4a–r**

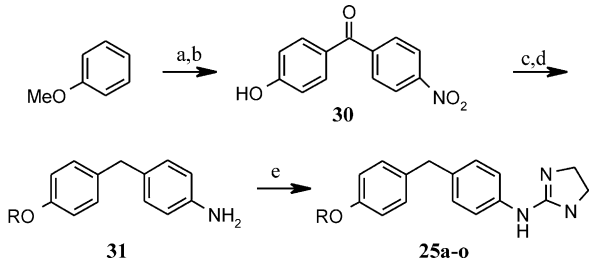


Compd	X	pK_i[a]
4a	4-OMe	7.3
4b	4-Cl	7.1
4c	2,6-Cl_2	6.2
4d	H	6.1
4e	2-OMe	5.4
4f	3-OMe	6.4
4g	2,5-(OMe)	6.1
4h	3,4-(OCH_2CH_2O)	6.2
4i	4-OEt	7.4
4j	4-*O*-*i*-Pr	7.5
4k	4-Me	7.0
4l	4-CF_3	7.3
4m	4-*i*-Pr	6.6
4n	4-*t*-Bu	6.4
4r	4-CO_2Et	7.3
4p	4-CO_2H	< 5
4o	4-NO_2	6.5
4q	4-$MeSO_2NH$	7.1

[a] Displacement of [^{3}H]iloprost in rodent NG-108-15 neuroblastoma cells, mean of two or more determinations ±0.1.

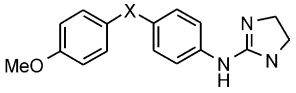


Scheme 1. (a) 4-Chloronitrobenzene, DMF, 120 °C; (b) $SnCl_2$, EtOH, reflux; (c) 2-chloroimidazoline, THF, heat.



Scheme 2. (a) Nitrobenzoyl chloride, CS_2, $AlCl_3$, rt; (b) HBr, HOAc, reflux; (c) RX, K_2CO_3, DMF, 60–70 °C; (d) H_2, 10% Pd/C, EtOH, HCl; (e) 2-chloroimidazoline, *i*-PrOH, reflux.

Table 4. IP receptor binding for compounds **26–31**



Compd	X	pK_i[a]
5a	S	7.3
24	O	6.8
25a	CH_2	7.6
27	CO	6.5
26	SO_2	6.8
28	NMe	6.3
29	(bond)	6.0

[a] Displacement of [^{3}H]iloprost in rodent NG-108-15 neuroblastoma cells, mean of two or more determinations ±0.1.

Table 5. IP receptor antagonism for compounds **25a–o**

Compd	R	pK_B[a]	SEM
25a	Me	7.7	0.2
25b	Et	8.2	0.2
25c	*n*-Pr	8.8[b]	
25d	*i*-Pr	8.8	0.1
25e	*n*-Bu	9.0[b]	
25f	*i*-Bu	8.8	0.3
25g	*n*-Hexyl	8.2	0.1
25h	CH_2Ph	7.7[b]	
25i	CH_2CF_3	8.7	0.1
25j	CH_2CH_2OH	8.0	0.2
25k	CH_2CH_2OMe	8.0	0.2
25l	Cyclopentyl	8.9	0.1
25m	Cyclohexyl	8.6	0.2
25n	4-Tetrahydropyranyl	8.4[b]	
25o	4-Tetrahydropyranylmethyl	8.5	0.2

[a] Antagonism of carbaprostacylin-stimulated c-AMP accumulation in human SH-SY5Y neuroblastoma cells, mean of two or more determinations ±SEM unless otherwise noted.
[b] $n = 1$.

significant affinity for cloned human EP_1, EP_3, FP, and TP receptors as determined by radioligand binding. In a standard profiling panel, this compound did not display significant affinity for 30 other receptors and ion channels with the exception of the human α_{2A} receptor (pK_i 6.5) and, perhaps not suprisingly given its chemical structure, the imidazoline I_2 binding site (pK_i 8.7). The potential pharmacological ramifications of the latter result are not clear.[9]

Thus **25d** was considered to be a sufficiently selective, bioavailable prostacyclin receptor antagonist to warrant in vivo testing in analgesic assays. In the rat acetic acid-induced writhing assay, **25d** caused a significant reduction in mean number of writhes over a dose range of 1–10 mg/kg, ip with an ED_{50} of 4 mg/kg. The effect of **25d** on carrageenan-induced paw hyperalgesia in the rat was evaluated and a significant reduction in mean withdrawal pressure was observed over the dose range of 0.3–10 mg/kg, iv (ED_{50} 2.8 mg/kg) and 1–100 mg/kg po (ED_{50} 18 mg/kg). Interestingly, **25d** had no effect on bleeding time in the mouse at a dose of 100 mg/kg, po, nor were there significant cardiovascular effects in the rat at analgesic doses.

A priori, the structures of these guanidine containing prostacyclin receptor antagonists appear rather unusual compared to the structure of the native ligand prostacyclin which contains a carboxylic acid group as a primary structural feature and recognition element for receptor binding.[10] The molecular biology of prostanoid receptors is well described[7] and all prostanoid receptors sequenced thus far, including the human prostacyclin receptor,[11] contain an arginine in the seventh transmembrane domain (TM7) which is proposed to be the carboxylic acid binding site. In the human prostacyclin receptor, this arginine is at position 279 in the second helical loop of TM7.[12] We hypothesized that the basic ligands bound to the aspartic acid residue at position 288, which is below Arg-279 in TM7 (i.e., closer to the intracellular side) in the fourth helical loop. Site-directed mutagenesis of the human prostacyclin receptor wherein Asp-288 was replaced with serine provided confirmation of this hypothesis. In the D288S mutant receptor, binding of the basic ligands **25f** and **25l** was decreased by three orders of magnitude, whereas [^{3}H]iloprost binding was not affected.[13] Thus, it would appear that binding of these guanidine containing ligands at Asp-288 interferes with binding of prostacyclin, the primary locus of which is presumed to be at Arg-279. The nature of this antagonism remains unclear although allosteric modulation could be hypothesized.

In conclusion, relatively simple structural modification of the screening hits **1a**,**b** led to a two-log order improvement in prostacyclin receptor affinity. The high affinity antagonist **25d** demonstrated analgesic activity in the rat and would appear to have sufficient selectivity and bioavailability for determining the therapeutic potential of this class of agents.

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13. Ilopost: $pK_I = 8.5$ (wild-type) and 8.1 (mutant), **25f**: $pK_I = 8.7$ (wild-type) and 5.7 (mutant), **25l**: $pK_I = 8.7$ (wild-type) and 5.5 (mutant).