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1,5-Biaryl pyrrole derivatives as EP_1 receptor antagonists: Structure–activity relationships of 4- and 5-substituted benzoic acid derivatives

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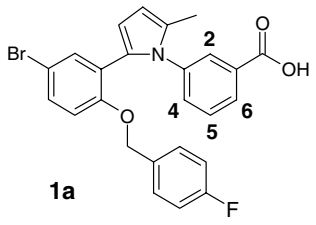
Abstract—This paper details the SAR of 1,5-biaryl pyrrole derivatives with substituents in the 2-, 4-, and 5-positions of the benzoic acid group as EP_1 receptor antagonists. Substitution at the 2-position was poorly tolerated, whereas only fluorine was tolerated at the 4-position. In contrast, a range of substituents at the 5-position were discovered which enhanced the in vitro affinity and led to compounds with promising oral exposure. Three derivatives showed efficacy in a preclinical model of inflammatory pain when dosed orally to rats.

Prostaglandin E_2 (PGE_2) exerts its physiological action by the activation of four 7-transmembrane receptor subtypes, known as EP_{1-4}.[1] Studies with EP_1 receptor knock-out (KO) mice and EP_1 receptor antagonists have shown that many of the pro-inflammatory actions of PGE_2 are mediated by the EP_1 receptor subtype. For example, KO mice have implicated the EP_1 receptor subtype in the sensation of PGE_2-mediated inflammatory pain.[2] In conjunction with this, EP_1 receptor antagonists have shown efficacy in preclinical models of postoperative pain,[3] neuropathic pain[4] and allodynia.[5] Recent reports from our laboratories have shown that EP_1 antagonists are analgesic in preclinical models of inflammatory pain.[6]

Several EP_1 receptor antagonists have been reported in the literature.[7] We have recently disclosed the structure–activity relationships (SAR), in vivo rat pharmacokinetic data and preclinical efficacy data of a series of 1,5-biaryl pyrrole EP_1 receptor antagonists, such as **1a** (Fig. 1).[6]

Compound **1a**[6] showed good in vitro affinity at the recombinant human EP_1 receptor with an IC_{50} of 6.3 nM in a [^{3}H]-PGE_2 binding assay in CHO cell membranes[6] and was found to be a competitive antagonist with a pA_2 of 9.1. Rat in vivo pharmacokinetic data showed **1a** to have moderate clearance, whilst data from the established complete Freund's adjuvant (CFA or FCA) model of inflammatory pain[6] showed **1a** to have an ED_{50} of 9.2 mg/kg when dosed orally to rats.



EP_1 binding pIC_{50} = 8.2
EP_1 pA_2 = 9.1

rat pharmacokinetics
CLb = 42 mL/min/kg
Vss = 0.7 L/kg

Figure 1. Profile of lead compound **1a**.

Keywords: EP_1 antagonist; Pyrrole; Pain; Established FCA.
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Herein we describe further SAR from this series where the substitution of the benzoic acid moiety at the 2-, 4- and 5-positions was investigated. In addition, we report the in vivo pharmacokinetic and efficacy data for key analogues.

Compound affinities were determined using a [^{3}H]-PGE_2 binding assay at the recombinant human EP_1 receptor stably expressed in CHO cell membranes.[7,8]

It was very quickly established that substitution at the 2-position generally proved detrimental to EP_1 activity (data not shown).

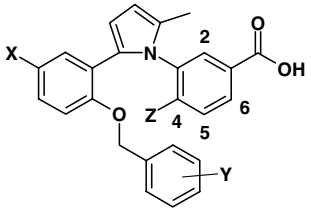
In the 4-position addition of a fluorine atom was tolerated, **1b**–**d**, although this did not increase affinity. Larger halogens such as chlorine, **1e**–**f**, resulted in a marked decrease in affinity indicating that substitution at this position may be sterically limited or that increased bulk at this position may give rise to an unfavourable conformation. Introduction of an electron-donating group, such as methoxy (**1g**) or ethoxy (**1h**), was also found to be detrimental, Table 1.

Substitution at the 5-position proved more fruitful. Electron-withdrawing groups such as Cl, Br and CF_3, **2a**–**f**, were well tolerated but did not increase EP_1 affinity, Table 2. Neither substitution of the benzyl group (Y) nor modification of the X-group (Cl to Br) had a significant effect on EP_1 activity, Table 2.

Further investigation of this position showed that it was possible to add electron-donating groups, such as NH_2, **3a**–**c**, Table 3. It is noteworthy that the 2,4-difluorobenzyl group significantly increased affinity, compare **3b** with **3c**, Table 3.

We also found that the amino group could be acylated to give the acetamides **3d**–**k**. In this series alternative X-groups were investigated on the left-hand side of the molecule such as F, Cl, Br, I and CF_3. Derivatives **3g**–**i** reveal that fluorination of the benzyl group resulted in a stepwise increase in activity with the addition of each fluorine atom.

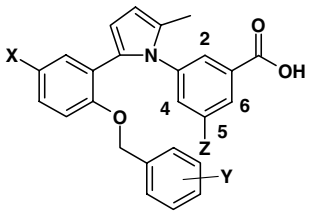
Table 1. In vitro EP_1 binding data for 4-substituted derivatives **1a**–**h**



Compound	X	Y	Z	Binding pIC_{50}[a]
1a	Br	4-F	H	8.2 ± 0.1
1b	Cl	2,4-DiF	F	8.2 ± 0.1
1c	CF_3	H	F	8.2 ± 0.2
1d	Br	4-F	F	8.2 ± 0.1
1e	Cl	4-F	Cl	6.5 ± 0.0
1f	Cl	2,4-DiF	Cl	6.8 ± 0.1
1g	Cl	H	MeO	6.6 ± 0.1
1h	Cl	H	EtO	6.9 ± 0.1

[a] Mean of at least three experiments.

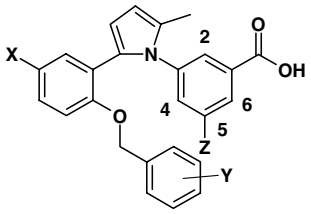
Table 2. In vitro EP_1 binding data of 5-halogenated and 5-CF_3 derivatives **2a**–**f**

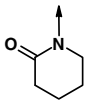
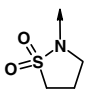


Compound	X	Y	Z	Binding pIC_{50}[a]
2a	Cl	2,4-DiF	Cl	8.4 ± 0.0
2b	Br	2,4-DiF	Cl	8.4 ± 0.1
2c	Cl	H	Br	8.3 ± 0.2
2d	Cl	2,4-DiF	Br	8.3 ± 0.1
2e	Cl	2,4-DiF	CF_3	8.0 ± 0.2
2f	Br	2,4-DiF	CF_3	7.9 ± 0.1

[a] Mean of at least three experiments.

Table 3. In vitro EP_1 binding data for compounds **3a**–**r**



Compound	X	Y	Z	Binding pIC_{50}[a]
3a	Cl	H	NH_2	8.4 ± 0.2
3b	Br	H	NH_2	8.5 ± 0.0
3c	Br	2,4-DiF	NH_2	9.3 ± 0.4
3d	F	4-F	NHAc	8.4 ± 0.1
3e	Cl	H	NHAc	8.7 ± 0.1
3f	Cl	4-F	NHAc	8.6 ± 0.2
3g	Br	H	NHAc	8.9 ± 0.1
3h	Br	4-F	NHAc	9.1 ± 0.1
3i	Br	2,4-DiF	NHAc	9.4 ± 0.1
3j	I	2,4-DiF	NHAc	8.8 ± 0.2
3k	CF_3	H	NHAc	8.9 ± 0.1
3l	Cl	4-F	$NHSO_2Me$	8.8 ± 0.2
3m	Br	2,4-DiF	MeNAc	8.5 ± 0.2
3n	Br	2,4-DiF		8.7 ± 0.2
3o	Br	2,4-DiF		8.9 ± 0.1
3p	Cl	H		8.3 ± 0.1
3q	CF_3	4-F		8.1 ± 0.1
3r	Cl	H	$NHCO_2Me$	8.3 ± 0.1

[a] Mean of at least three experiments.

It was found that the acetamide could be replaced by a methylsulfonamide, compare **3f** with **3l**, with little impact on affinity. Methylation of the acetamide was possible, **3m**, although this resulted in approximately 10-fold loss in affinity, implying that the acetamide may be acting as a hydrogen bond donor (HBD). Based on this result we also investigated the preparation of the γ-lactams **3n** and the δ-lactam **3o**, both of which showed similar affinity to the methyl acetamide **3m**. The cyclic sulfonamides **3p–q** were slightly less active than the corresponding lactams. Finally, replacement of the acetamide by a methyl carbamate, **3e** versus **3r**, resulted in a moderate decrease in activity.

The oral exposure of several analogues was assessed in the rat, Table 4. Compounds **1b** and **1d**, with a fluorine atom in the 4-position of the benzoic acid, and **2c**, with the 5-Br substituent, did not show sufficient exposure, defined as a dose-normalised area under the curve (AUC) of $\leqslant$10 min kg/L. The amino derivative **3c** also showed poor exposure, however, the acetylated analogues **3d–f**, **3i** and **3k** generally showed good exposure, with the exception of **3e** and **3k**. Replacement of the acetamide by a methylsulfonamide **3l** (compare **3l** with the corresponding acetamide **3f**) abolished exposure. Similarly, the lactams **3n** and **3o** showed low exposure, below the limit of quantification of the assay.

Table 4. In vivo rat pharmacokinetic data for selected compounds[a]

Compound	AUC (o-t)/dose (min kg/L)	T_{max} (h)	C_{max} (μM)
1b	4 ± 1	0.5	0.23 ± 0.06
1d	6 ± 1	0.5	0.23 ± 0.04
2c	6 ± 1	0.5	0.31 ± 0.07
3c	<1	0.5	0.05 ± 0.02
3d	60 ± 8	0.5	2.95 ± 0.91
3e	8 ± 1	0.5	0.58 ± 0.16
3f	20 ± 19	0.5	2.08 ± 2.14
3i	21 ± 12	0.5	1.41 ± 0.78
3k	n.d.	0.7	0.02 ± 0.01
3l	n.d.	n.d.	n.q.
3n	n.d.	n.d.	n.q.
3o	n.d.	n.d.	n.q.

[a] Mean of three experiments. Compounds administered orally in 1% aqueous methylcellulose at 3 mg/kg. n.d., not determined; n.q., not quantifiable.

Table 5. In vivo rat pain data from FCA model of inflammatory pain[a]

Compound	% reversal of hypersensitivity at 5 mg/kg[a]	ED_{50}[a,b] (mg/kg)
3d	55	n/t
3f	94	n/t
3i	72	1.1

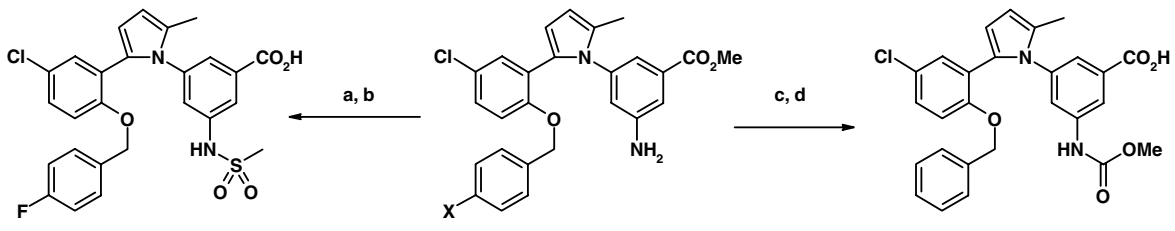
[a] Compounds administered orally in 1% aqueous methylcellulose at doses of 1, 3 and 10 mg/kg or at a single dose of 5 mg/kg. Pain readout taken 1 h post-dose of test compound.
[b] n/t, not tested.

Compounds which showed acceptable oral exposure were progressed to the established FCA model of inflammatory pain.[6] Compounds were either assayed at a single dose of 5 mg/kg or in dose–response format, Table 5. The *N*-acetyl derivatives **3f** and **3i** performed well when dosed at 5 mg/kg, whereas **3d** showed disappointing results despite its excellent exposure. On the basis of these results and its excellent CYP450 profile (IC_{50} values all $\geqslant$18 μM),[9] **3i** was profiled in a dose–response assay, and showed considerably improved efficacy relative to **1a**, with an ED_{50} of 1.1 mg/kg, Table 5.

In a functional Ca^{2+} mobilization assay (FLIPR using recombinant human receptor in CHO cells) **3i** displayed a pK_i of 10.0 ± 0.1 (n = 3) at the EP_1 receptor, a pK_i of 6.0 at the EP_3 receptor and at the TP receptor a pIC_{50} of 8.6 and pK_b of 9.0. At the EP_4 receptor (binding assay) **3i** had a pIC_{50} of 6.8. No activity was observed at the IP or EP_2 receptors (pIC_{50} < 6). Thus **3i** demonstrates approximately 400-fold selectivity over EP_4 (in terms of binding affinity) and about 10,000-fold selectivity over EP_3 but is essentially equipotent at the TP receptor in terms of functional antagonism.

The compounds described herein were prepared by Paal–Knorr condensation[10] of the requisite 1,4-diketone[6,8] with an appropriately functionalized aniline as described previously.[6] Full experimental procedures and characterizing data for key compounds have been described.[8] The anilines were commercially available or were prepared by literature procedures. Generally, if the aniline was not readily available it could be prepared by reduction of the analogous nitro derivative.

Sulfonamide **3l** was prepared by reaction of the methyl ester **4a** with MsCl under standard conditions (DCM-pyridine, DMAP) followed by basic hydrolysis of the ester. Hydrolysis of ester **4b**, under microwave conditions,



Scheme 1. Reagents and conditions: (a) DCM-pyridine, **4a**, MsCl, 76%; (b) 2 M NaOH, MeOH, reflux, then AcOH, 94%; (c) **4b**, 2 M NaOH, EtOH, microwave, 100 °C, 2 min; (d) DCM-pyridine, DMAP, $ClCO_2Me$, 42% for 2 steps.

followed by reaction with methyl chloroformate gave carbamate **3r**, Scheme 1.

In summary, we have investigated substitution of benzoic acid in the 2-, 3- and 4-positions. Substitution of the 2-position proved detrimental, whereas substitution of the 3-position by fluorine was tolerated but did not improve EP_1 affinity. It was found that the 5-position could tolerate a variety of functional groups, particularly noteworthy were the acetamide and sulfonamide groups which led to the identification of compounds with sub-nanomolar affinity. Three of the 5-acetamido derivatives demonstrated good exposure upon oral dosing. The same compounds displayed efficacy in the established FCA preclinical model of inflammatory pain when dosed orally at 5 mg/kg. Compound **3i** also demonstrated an ED_{50} of 1.1 mg/kg when dosed orally.

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