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Discovery of a novel series of nonacidic benzofuran EP_1 receptor antagonists

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ABSTRACT

We describe the discovery and optimization of a novel series of benzofuran EP_1 antagonists, leading to the identification of **26d**, a novel nonacidic EP_1 antagonist which demonstrated efficacy in preclinical models of chronic inflammatory pain.

There is significant interest in the identification of EP_1 receptor antagonists for the treatment of inflammatory pain.[1] Toward this end we have previously reported several classes of EP_1 receptor antagonists, for example, the development compound GW848687X (**1**)[2] and the related phenyl derivative GSK345931A (**2**)[3] We have also described the pyrrole series, exemplified by **3** and **4**,[4] and the methylene-linked pyrazole series, represented by **5** and **6**.[5] The latter examples demonstrated the utility of the isobutyl group (R = ^{i}Pr) as a replacement for the benzyl group (R = Ph), Figure 1. We have also described analogues of **5** and **6** where the carboxylic acid moiety was replaced by nonacidic groups such as amides, reversed amides and heterocycles.[6]

As part of an ongoing medicinal chemistry programme we were interested in identifying new lead series which may offer potential improvements over the compounds described in Figure 1, for example, increased affinity for the EP_1 receptor, increased metabolic stability or increased oral exposure.

During the course of our research activities we conducted small molecule X-ray crystallographic studies with several compounds. Analysis of these structures revealed that the alkoxy group of the left hand phenyl ring adopted a co-planar conformation with the phenyl ring as represented in Figure 2. As the solid state conformation may be a low energy conformation, we hypothesized that it could be mimicked by conformational constraint as outlined in Figure 2. In addition, the resultant benzofurans also have less rotatable bonds than the corresponding alkoxy analogues, a property which has been shown to have a beneficial effect on oral bioavailability.[7]

Initially, we targeted benzofuran derivatives where R = ^{i}Pr. The synthesis is outlined in Scheme 1. 2-Methyl-3-butyn-2-ol (**8**) was

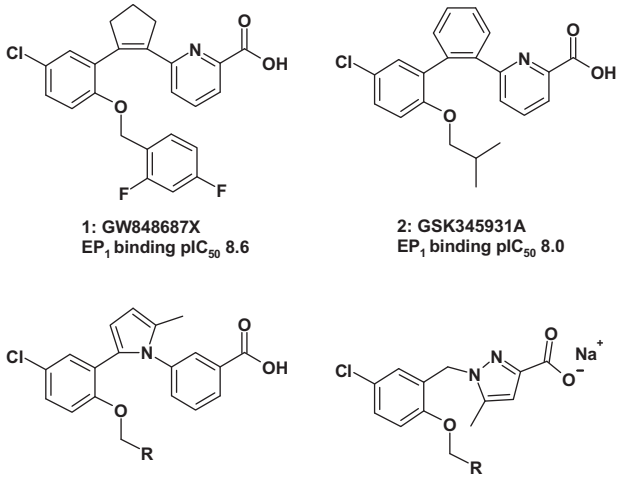

Figure 1. Selected GSK EP_1 receptor antagonists.

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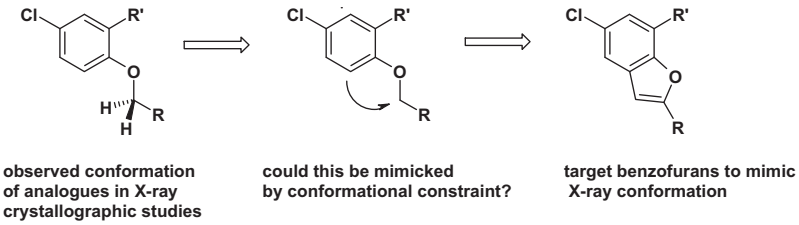
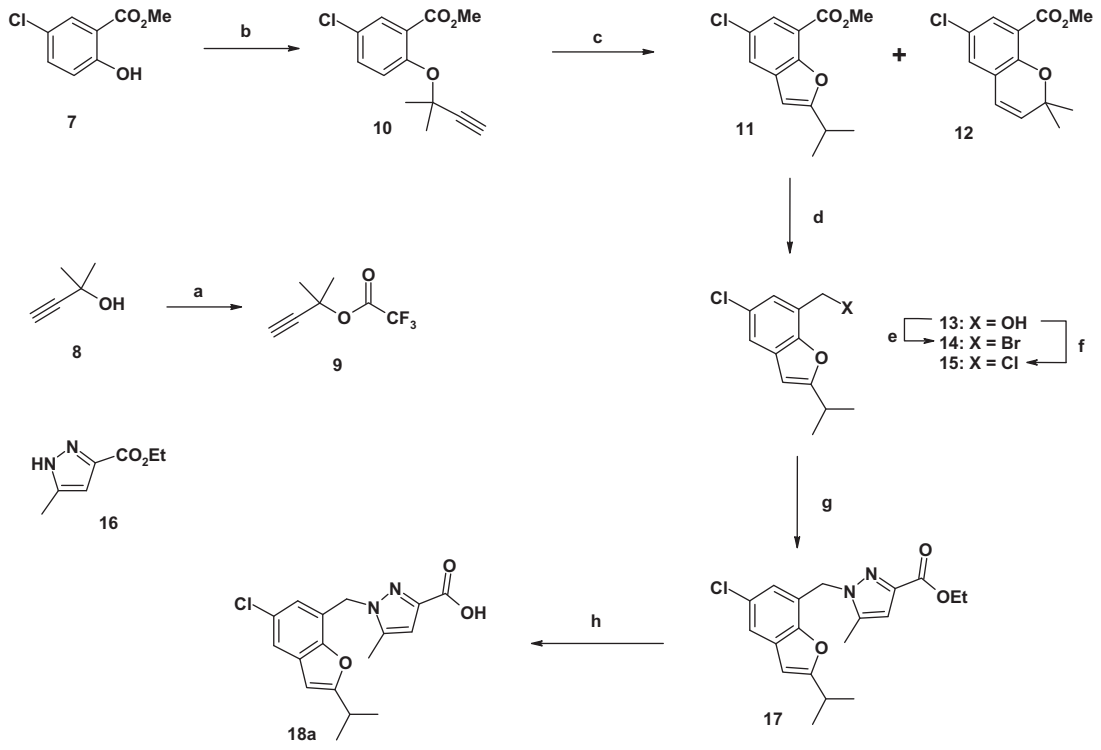


Figure 2. Selected GSK EP_1 receptor antagonists.



Scheme 1. Reagents and conditions: (a) TFAA, DBU, MeCN, −10 to 0 °C; (b) **9**, DBU, MeCN, $CuCl_2$, −10 to 0 °C; (c) $PhNEt_2$, CsF, 180 °C, 50%; (d) 1 M $LiAlH_4$ in THF, THF, rt; (e) PBr_3, hexane/CH_2Cl_2 (1:1), rt; (f) $SOCl_2$, CH_2Cl_2, rt; (g) **16**, **14**, DMF, K_2CO_3, rt, 59%; (h) NaOH, EtOH, rt.

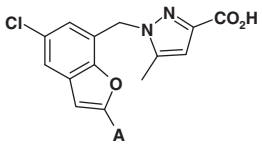
converted to the corresponding trifluoroacetate derivative **9** and treated in situ with phenol **7** in the presence of copper(II) chloride to give derivative **10**. Acetylene **10** underwent thermal rearrangement in *N,N*-diethylaniline to give a separable mixture of **11** and **12**, the former being the major product.[8] Ester reduction gave the corresponding alcohol **13** which was converted to a suitable leaving group and displaced with pyrazole derivative **16** to give two regioisomers (approximately 2:1 when X = Br), of which **17** was the major product (59%). The yield of the undesired regioisomer was 32% and the isomers where easily separated by column chromatography. Ester hydrolysis of **17** thus furnished **18a** (Scheme 1).[9]

We were pleased to find that compound **18a** showed good affinity for the EP_1 receptor in our [^{3}H]-PGE_2 binding assay, Table 1.[9] Testing in a functional assay (FLIPR)[9] confirmed that compound **18a** was a potent EP_1 antagonist, FLIPR pK_i 9.4 ± 0.1 (n = 3) with good selectivity over the EP_3 receptor subtype, EP_3 FLIPR pK_i 6.0 (n = 1).

Investigation of the SAR at the 2-position of the benzofurans, Table 1, revealed that a range of alkyl groups could be introduced, with iso-propyl and cyclohexyl resulting in the highest affinity.

We next sought to investigate benzofuran replacements with the goal of lowering the lipophilicity of the series. Results from this investigation are summarized in Table 2. Disappointingly, all derivatives showed a significant decrease in affinity. Surprisingly, the

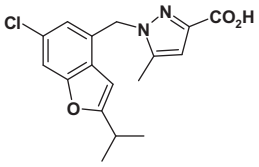
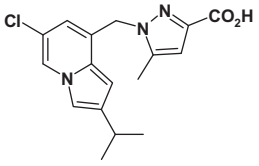
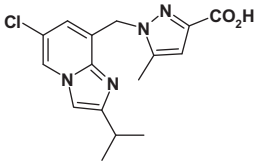
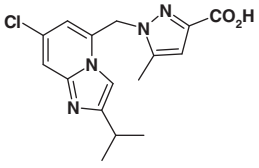
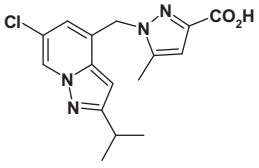
Table 1
SAR of C-2 alkyl benzofurans[a]



Compd.	A	EP_1 binding pIC_{50}[b]
18a	^{i}Pr	8.3[c]
18b	cPr	7.3
18c	Pr	7.6
18d	Cyhex	8.4
18e	*t*-Bu	7.2

[a] Data from [^{3}H]-GW875240X binding assay.
[b] Values are means of at least three experiments.
[c] Data from [^{3}H]-PGE_2 binding assay.

Table 2
SAR of bicyclic benzofurans replacements[a]

Compd.	Structure	EP_1 binding pIC_{50}
19a		6.6[b]
19b		6.9[c]
19c		6.3[c]
19d		<6[c]
19e		<6[b]

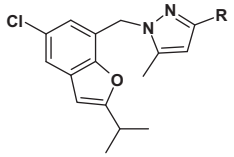
[a] Values are means of at least three experiments.
[b] Data from [^{3}H]-GW875240X binding assay.
[c] Data from [^{3}H]-PGE_2 binding assay.

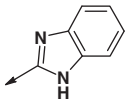
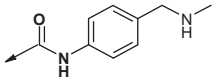
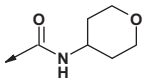
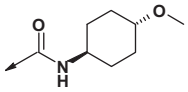
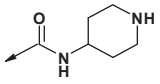
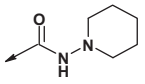
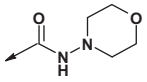
regioisomeric benzofuran **19a** also displayed a 50-fold decrease in activity. More polar heterocycles (**19c**–**e**) showed very weak affinity.

Retaining the iso-propyl group in the 2-postion of the benzofuran ring, we investigated several replacements for the carboxylic acid, Table 3.

Results in Table 3 indicated that it was possible to incorporate a weakly basic centre to the compounds, compound **20g** retained reasonable affinity whilst displaying modest solubility. Compound **20g** was profiled further, a summary of the data generated is listed in Table 4. The compound was found to have good in vitro metabolic stability, poor aqueous solubility, but moderate solubility in fed state simulated intestinal fluid. Compound **20g** was also found to be highly permeable in MDCK cells and was not a Pgp substrate (efflux ratio 0.9). Based on this data, compound **20g** was profiled in the rat CFA model of inflammatory pain[10] where it demonstrated an oral ED_{50} of 14.7 mg/kg, with a dose of 30 mg/kg showing comparable reversal of hypersensitivity to the standard (celecoxib at a dose of 10 mg/kg po). Disappointingly, compound **20g** was found to have low oral bioavailability in the rat, which we attributed to its high blood clearance, Table 4. The intrinsic clearance in rat liver microsomes predicts low to moderate metabolic stability for **20g**, however in vivo the clearance is high, equal to or in excess of liver blood flow. The high in vivo clearance may be suggestive of non-cytochrome P450 metabolism also occurring. Despite the high in vivo clearance, bioanalysis from the CFA study revealed that compound **20g** exhibited high blood concentrations 1 h post-dose; 3 mg/kg blood concn 1.945 μM ($n = 7$), 10 mg/kg blood concn 5.614 μM ($n = 7$), 30 mg/kg blood concn 10.166 μM ($n = 7$). The

Table 3
SAR of various novel carboxylic acid replacements[a]



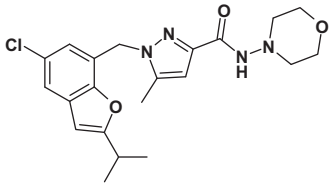
Compd.	R	EP_1 binding pIC_{50}[b]
20a		7.3
20b		7.2
20c		7.2
20d		[c]6.5
20e		6.1
20f		7.0
20g		7.3

[a] Values are means of at least three experiments.
[b] Data from [^{3}H]-PGE_2 binding assay.
[c] Data from [^{3}H]-GW875240X binding assay.

CNS penetration was also good (as measured from the 30 mg/kg dose group); brain concn 11.315 μM ($n = 3$) with a blood concn of 10.818 μM ($n = 3$) for the corresponding rats, giving a Br:Bl of 1.05. Free fractions in blood and brain tissue were not determined for this compound but are likely to be low in line with that of close analogues such as **26a** and **26d**, vide infra. The high blood concentrations do not exclude the fact that the efficacy is mediated by a metabolite, however in a separate study compound **20g** was dosed orally in the rat CFA model at 5 mg/kg (in 1% methylcellulose, as before) and efficacy was assessed at 1 and 3 h post-dose. At this dose, there was a small but significant reversal of hypersensitivity at the 1 h time point but no effect at the 3 hour time point supporting the fact that the efficacy is mediated by the parent compound, but not excluding a contribution from the 2-isopropenyl metabolite, vide infra, which is likely to have EP_1 activity. The blood and brain concentrations of **20g** 3 h post-dose were 0.694 and 0.804 μM giving a Br:Bl of 1.15.

To further optimize this series and improve the in vivo metabolic stability, a study was conducted to assess the sites of metabolism and the vulnerability of this compound to form reactive metabolites. One of the major sites of metabolism was found to be the iso-propyl group in the 2-position of the benzofuran which appeared to undergo oxidation and elimination. In order to address this liability we sought to replace the iso-propyl group with a phenyl group.

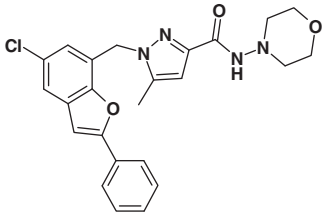
Table 4
Summary of data for compound **20g**



Metabolic stability[a]	Human CL_i (mL/min/g liver)	1.1
	Rat CL_i (mL/min/g liver)	2.8
Solubility[b] (1 h)	Water (μg/mL)	0
	SGF (μg/mL)	1
	FaSSIF (μg/mL)	5
	FeSSIF (μg/mL)	71
Lipophilicity[c]	Log *D* (pH 7.4)	2.6
Permeability[d]	Pexact	671 nm/sec
	Efflux ratio	0.9
In vivo activity[e] (CFA)	Doses 3,10 and 30 mg/kg	ED_{50} 14.7 mg/kg
Rat pharmacokinetics	CL_b[f] (mL/min/kg)	85 ± 8
	V_{ss}[f] (L/kg)	3.0 ± 0.3
	$t_{½}$[f] (h)	0.5 ± 1
	F_{po}[g]	19 ± 6%
	$t_{½}$[g] (h)	1.7 ± 0.2

[a] See Ref. 11 for details.
[b] Solubility of solid material in each fluid determined at several time points, only 1 h time point quoted. See Ref. 12 for details, SGF (simulated gastric fluid), FaSSIF (fasted state simulated intestinal fluid), FeSSIF (fed state simulated intestinal fluid).
[c] Shake flask method.
[d] MDCK cells.
[e] Efficacy at 1 h post-dose (oral).
[f] 1 mg/kg intravenous dose, compound dissolved in 2% (v/v) DMSO then added to 0.9% (w/v) saline containing 10% (w/v) Kleptose.
[g] 3 mg/kg oral dose, vehicle = 1% (w/v) methylcelluose aq.

Table 5
Summary of data for compound **26a**

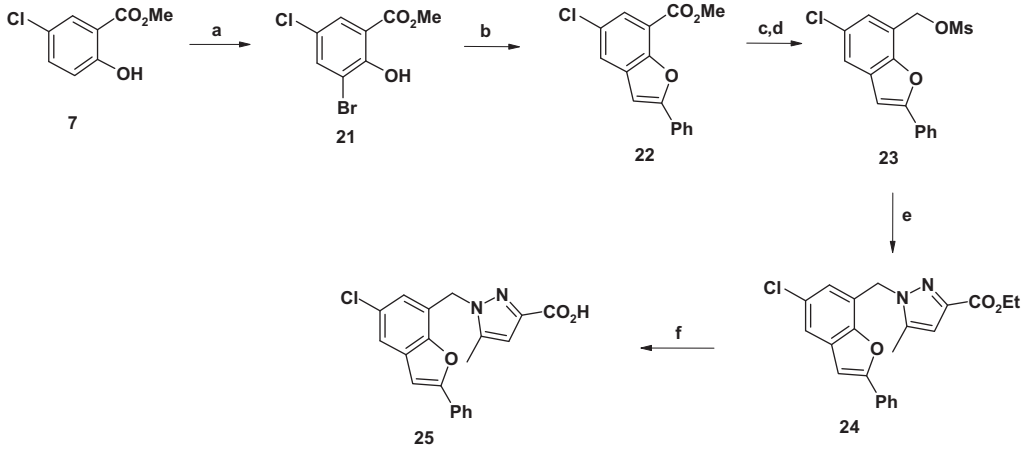


EP_1 binding affinity	pIC_{50}	8.0
Metabolic stability[a]	Human CL_i (mL/min/g liver)	1.1
	Rat CL_i (mL/min/g liver)	1.3
Solubility[b] (1 h)	Water (μg/mL)	171
	SGF (μg/mL)	227
	FaSSIF (μg/mL)	382
	FeSSIF (μg/mL)	0
Lipophilicity[c]	Log D (pH 7.4)	3.0
In vivo activity[d] (CFA)	Dose 10 mg/kg	49% reversal
Rat pharmacokinetics	CL_b[e] (mL/min/kg)	22
	V_{ss}[e] (L/kg)	3.5
	$t_{½}$[e] (h)	2.1

[a] See Ref. 11 for details.
[b] Solubility of solid material in each fluid determined at several time points, only 1 h time point quoted. See Ref. 12 for details, SGF (simulated gastric fluid), FaSSIF (fasted state simulated intestinal fluid), FeSSIF (fed state simulated intestinal fluid).
[c] Shake flask method.
[d] Efficacy at 1 h post-dose (oral).
[e] 1 mg/kg intravenous dose, compound dissolved in 2% (v/v) DMSO then added to 0.9% (w/v) saline containing 10% (w/v) kleptose.

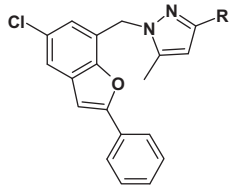
Our initial route to prepare the 2-alkyl benzofurans, Scheme 1, was clearly unsuitable for the preparation of 2-aryl benzofurans. As a result, a new route was devised, Scheme 2. Starting from phenol **7**, bromination using *N*-bromosuccinimide afforded **21**. Bromide **21** was subjected to a modified Castro reaction[13] to provide the benzofuran intermediate **22** in one step. Subsequent reduction of ester **22**, and coversion of the intermediate alcohol to a suitable leaving group gave mesylate **23** in good yield. The mesylate was reacted with commercially available pyrazole **16** to give **24** as a mixture of regiosiomers which were easily separated using column chromatography. Finally, ester hydrolysis of **24** gave carboxylic acid **25**.[14]

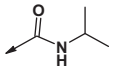
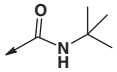
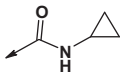
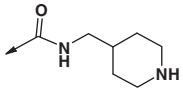
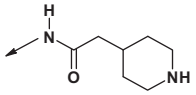
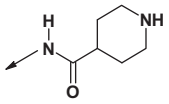
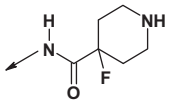
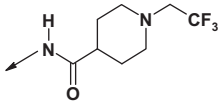
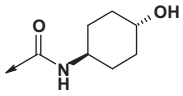
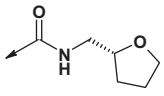
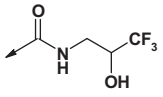
Gratifyingly, this structural change, to give **26a**, Table 5, resulted in increased affinity, EP_1 binding pIC_{50} 8.0, maintained in vitro metabolic stability and improved in vivo metabolic stability (rat CL_b 22 mL/min/kg), supporting the hypothesis that the isopropyl group of **20g** was a major site of metabolism. Surprisingly,



Scheme 2. Reagents and conditions: (a) NBS, DMF, rt; (b) phenylacetylene, CuI, $PdCl_2(PPh_3)_2$, Et_3N, DMF, 75 °C; (c) 1 M $LiAlH_4$ in THF, THF 0 °C; (d) MsCl, Et_3N, DCM, 0 °C; (e) **16**, DMF, K_2CO_3, rt; (h) NaOH, EtOH, 50 °C.

Table 6
SAR of various novel carboxylic acid replacements[a]

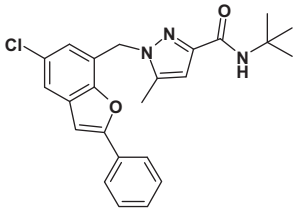


Compd.	R	EP_1 binding[b] pIC_{50}
25	CO_2H	8.7
26b		8.0
26c		8.3
26d		8.0
26e		8.3
26f		7.7
26g		7.2
26h		7.0
26i		7.4
26j		7.6
26k		8.1
26l		8.2
26m		7.6

[a] Data from [^{3}H]-GW875240X binding assay.
[b] Values are means of at least three experiments.

despite the increased lipophilicity (log *D* 3.0 @ pH 7.4) this analogue displayed higher solubility in all media tested apart from FaSSIF. When tested in the rat CFA model of inflammatory pain,[10] compound **26a** showed moderate reversal of hypersensitivity (using a weight bearing readout) following an oral dose of 10 mg/kg. Bioanalysis indicated good exposure in both blood and brain (blood concn 2.340 μM, $n = 6$, brain concn 3.969 μM, $n = 3$, 1 h post-dose, which equates to a Br:Bl of 1.70, $n = 3$).[15] In blood

Table 7
Summary of data for compound **26d**



EP_1 binding affinity	pIC_{50}	8.0
Lipophilicity[a]	Log D (pH 7.4)	2.9
In vivo activity[b] (CFA)	Doses 1, 3, 10 mg/kg	ED_{50} 5 mg/kg
Rat pharmacokinetics	CL_b[c] (mL/min/kg)	28
	V_{ss}[c] (L/kg)	4.3
	$t_{½}$[c] (h)	4.2

[a] Shake flask method.
[b] Efficacy at 1 h post-dose (oral).
[c] 1 mg/kg intravenous dose, compound dissolved in 2% (v/v) DMSO then added to 0.9% (w/v) saline containing 10% (w/v) kleptose.

and brain tissue binding assays[16] **26a** exhibits high binding with 0.662% free in blood and 0.170% free in brain, that is, Bl Fu 0.0066, Br Fu 0.0017, which results in a calculated free blood concn of 15 nM and free brain concn of 7 nM, which are in line with the binding IC_{50} (10 nM). Following this encouraging data, compound **26a** was profiled in the joint pain model of chronic inflammatory pain[17] at doses of 1, 3, 10 and 30 mg/kg po b.i.d.. Disappointingly, a statistically significant reversal ($p < 0.001$) was only observed in the 30 mg/kg dose group. Thus, although we had made a significant improvement of optimizing the pharmacokinetic parameters of the series, the in vivo efficacy required improvement.

Toward this end, we opted to make further amide analogues, data summarized in Table 6. A selection of neutral amides (**26b**–**e**) showed good affinity. Various groups were assessed in the amide or reversed amide series to try to lower the lipophilicity of the series either via the introduction of a basic amine (**26f**–**j**) or polarity (**26k**–**m**). It was found that basic amines were relatively well tolerated in this region of the molecule, however it appeared that affinity could be increased by decreasing the amine pK_a, compare **26h** with **26i** and **26j**.

A range of compounds were assessed in the rat CFA model of inflammatory pain[10] (oral dose of 10 mg/kg in 1% methylcellulose, efficacy reading 1 h postdose, when blood and brain samples were also collected) to screen for efficacy and exposure in both blood and brain. Disappointingly, most compounds displayed low efficacy, which could generally be attributed to low exposure. However, compound **26d**, displayed promising efficacy in this screen (95% reversal of hypersensitivity, as measured by weight bearing, at 10 mg/kg po) and good CNS penetration (blood concn = 381 nM, brain concn = 112 nM, Br:Bl 0.3). Based on this data, the compound was progressed to a dose–response study in CFA-treated rats (doses 1, 3 and 10 mg/kg po) where it gave an ED_{50} of 5 mg/kg. Bioanalysis of the 10 mg/kg dose group showed similar blood exposure to the previous study (blood concn = 493 nM) but higher brain exposure (brain concn = 407nM) which resulted in a Br:Bl of 0.8. The slightly higher Br:Bl in the second experiment is within the range of variability of these assays (<3-fold variation), the same strain and sex of rat and administration vehicle were used in both studies. In blood and brain tissue binding assays[16] **26d** exhibits high binding with 0.028% free in blood and 0.017% free in brain, which results in calculated free blood and free brain concentrations considerably lower than the in vitro binding IC_{50} value of this

compound. The reason for this is unknown, however, there are likely to be errors in the measurements of the free fractions in both tissues with such lipophilic compounds. It is also feasible that the affinity at the rat receptor is higher than that at the human receptor. However without an assessment of in vivo/ex vivo receptor binding it is not possible to say whether the concentrations achieved in these studies were sufficient to block the receptor or whether the effect is on-target. Assessment in the joint pain model of chronic inflammatory pain[17] (at doses of 0.3, 1, 3 and 10 mg/kg po b.i.d.) produced a dose-related reversal of hypersensitivity with a calculated ED_{50} of ~0.6 mg/kg and a corresponding calculated blood concentration of 0.06 μM. Full reversal of hypersensitivity was achieved at 10 mg/kg. Profiling of the compound in a rat PK study demonstrated good metabolic stability with satisfactory half-life, Table 7.

In conclusion we have described the identification of a new series of EP_1 antagonists which enriches the chemical tools available for the investigation of EP_1 pharmacology. We have also described the use of metabolite identification in lead optimisation which resulted in compounds such as **26a** and **26d**, which, to the best of our knowledge, are the first nonacidic EP_1 antagonists to demonstrate efficacy in preclinical models of chronic inflammatory pain.

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15. Note the brain concentrations were measured in only three rats, mean brain concentration = 3.969 μM. The average blood concentration from the same three rats was 2.339 μM. Thus, this equates to a Br:Bl of 1.70, $n = 3$.
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