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Discovery and characterization of a potent and selective EP_4 receptor antagonist

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ABSTRACT

EP_4 is a prostaglandin E_2 receptor that is a target for potential anti-nociceptive therapy. Described herein is a class of amphoteric EP_4 antagonists which reverses PGE_2-induced suppression of TNFα production in human whole blood. From this class, a potent and highly bioavailable compound (**6**) has been selected for potential clinical studies. EP_4 binding and functional data, selectivity, and pharmacokinetic properties of this compound are included.

Prostaglandin E_2 (PGE_2) is a prostanoid implicated in a variety of disease states including inflammation, cancer,[1] and bronchitis.[2] PGE_2 operates through a family of rhodopsin-like 7TM G-protein coupled receptors known as EP_{1-4}.[3] The EP_4 receptor was first reported in 1994 by Lydford and McKechnie.[4] It has been demonstrated in rats that EP_4 is upregulated in dorsal root ganglia during CFA-induced peripheral inflammatory events, and that both administration of an EP_4 antagonist and knockdown of EP_4 decrease pain sensitivity in a thermal withdrawal latency model.[5] Therefore, EP_4 antagonism is hypothesized to constitute a potential pain management therapy for patients suffering from inflammatory conditions such as osteoarthritis.[6] Related therapeutic approaches include inhibition of COX-2[7] or *m*PGES-1;[8] however, selective EP_4 antagonism could carry reduced gastrointestinal risk by not directly interrupting PGE_2 synthesis, thereby enabling normal function of the EP_2 receptor.[9] Thus, widespread interest has led to the discovery of varied EP_4 antagonists.[10] Notably, CJ-023423 (**1**),[11] PGN-1531 (**2**),[12] and ER-886046 (**3**)[13] (Fig. 1) have been studied extensively.

PGE_2 (**4**, Fig. 2) is the endogenous ligand of EP_4. Like PGE_2, most known EP_4 antagonists contain a carboxylic acid or another functional group of similar acidity. Through our own studies, we discovered **5** (Table 1), which contained not only a carboxylic acid,

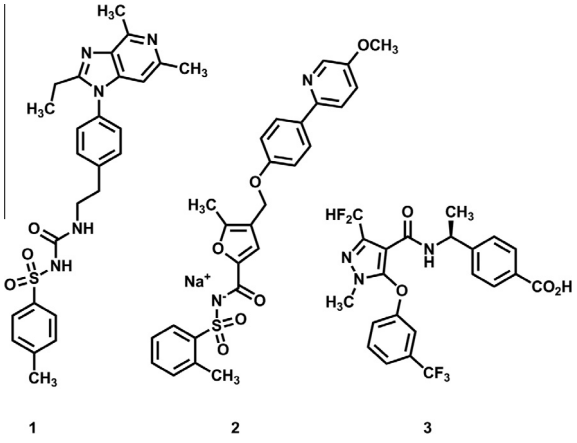

Figure 1. Structures of known EP_4 antagonists.

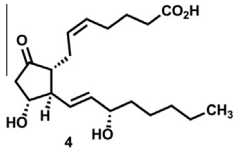

Figure 2. Prostaglandin E_2.

Abbreviations: CFA, complete Freund's adjuvant; CHO, Chinese hamster ovary.

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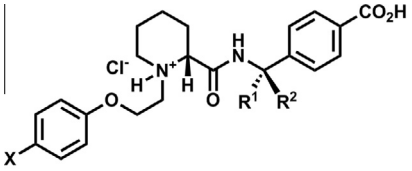
Table 1
SAR of (phenoxyethyl)piperidine EP_4 antagonists[a]



	X	R^1	R^2
5	H	H	H
6	H	CH_3	H
7	H	Et	H
8	H	H_2C-CH_2	
9	CN	CH_3	H
10	F	CH_3	H

Compd	MW of neutral form (g/mol)	*c*log*D* @ pH 7.4[b]	hEP_4 filter binding K_i[c]	hEP_4 filter binding IC_{50}[c]	hEP_4 antagonism functional IC_{50}[c]	HWB IC_{50}[c]
1	491.6	3.08	449 nM ×/÷ 1.85 (8)	689 nM ×/÷ 1.74 (8)	11.7 nM ×/÷ 1.98 (6)	1614 nM ×/÷ 2.13 (120)
5	382.4	0.30	126 nM ×/÷ 1.10 (3)	203 nM ×/÷ 1.19 (3)	18.1 nM ×/÷ 1.69 (3)	899 nM ×/÷ 2.73 (4)
6	396.5	0.73	40.6 nM ×/÷ 1.72 (12)	68.8 nM ×/÷ 1.64 (12)	5.62 nM ×/÷ 1.70 (10)	126 nM ×/÷ 2.26 (20)
7	410.5	1.24	129 nM ×/÷ 1.14 (3)	232 nM ×/÷ 1.06 (3)	14.4 nM ×/÷ 1.62 (3)	291 nM ×/÷ 1.88 (9)
8	408.5	0.89	44.8 nM ×/÷ 1.45 (2)	77.8 nM ×/÷ 1.22 (2)	5.42 nM ×/÷ 1.76 (5)	586 nM ×/÷ 1.14 (2)
9	421.5	0.80	372 nM ×/÷ 1.08 (3)	621 nM ×/÷ 1.07 (3)	35.8 nM ×/÷ 1.41 (2)	1209 nM ×/÷ 1.74 (2)
10	414.5	1.08	40.4 nM ×/÷ 1.43 (5)	71.8 nM ×/÷ 1.31 (5)	2.87 nM ×/÷ 1.66 (7)	121 nM ×/÷ 2.35 (11)

[a] All compounds prepared as a single stereoisomer. Absolute stereochemistry as depicted.
[b] Calculated using Marvin and calculator plugin freeware (www.chemaxon.com, ChemAxon Kft, Budapest, Hungary).
[c] Expressed as geometric mean times or divided by the geometric sample standard deviation, with number of determinations in parentheses.

but also a (phenoxyethyl)piperidine.[14] The latter moiety, which contains a basic amine, is not found in any other currently known class of EP_4 antagonists.[15]

Compounds were compared to known EP_4 antagonist **1** in a set of three assays. Affinity for the receptor was tested using a filter binding assay with [^{3}H]PGE_2 as the radioligand in recombinant HEK293 cells stably expressing the human EP_4 receptor.[16] A PGE_2-stimulated cAMP antagonist assay in HEK293 cells was used to determine functional activity.[17] Then, the compounds were evaluated for their ability to reverse the inhibition of PGE_2 on TNFα production (a known downstream effect of activating the EP_4 receptor) in LPS-stimulated human whole blood (HWB).[18] In each of these assays, **5** compared favorably to **1**. Additionally, the molecular weight and *c*log*D* of **5** were both lower than the corresponding properties of **1**. At neutral pH, **5** was predicted to exist predominantly as a zwitterion (acid pK_a 4.0, amine pK_a 7.5).[19] For indications such as osteoarthritic pain, rapid absorption could be a desirable factor because it would theoretically result in quick onset. To the extent that its amphoteric character could provide diverse formulation options to facilitate absorption,[20] **5** was judged a worthy starting point for SAR optimization.

Incorporation of a benzylic methyl group (**6**) increased the potency in all three of the above assays; notably, the IC_{50} in HWB was an order of magnitude lower than that of **1**. Homologation of the benzylic substituent (**7**) did not improve the potency, nor did incorporation of a second substituent in the form of a 1,1-cyclopropane (**8**). Addition of a cyano substituent at the *para* position of the phenoxy ring (**9**) had a detrimental effect on potency; however, 4-fluoro analogue **10** performed similarly to **6** in all three assays. Although **6** and **10** had similar potency profiles, **6** was chosen for further characterization due to its slightly lower molecular weight and lipophilicity.

Compound **6** was tested in filter binding assays to assess its selectivity against the other known EP receptors (Table 2). No detectable binding was observed with either EP_1 or EP_3. Although binding was observed with EP_2, it was approximately 25-fold weaker than the EP_4 binding of **6**.[16] At a 1 μM concentration in cAMP EP_2 functional assays in human recombinant CHO cells, measurable activity was observed in antagonist mode, but not in agonist mode. Thus, **6** was found to be a relatively weak EP_2 antagonist as well as a potent EP_4 antagonist. With the expectation that the observed selectivity would be sufficient to obviate significant competing pharmacology, **6** was characterized further.

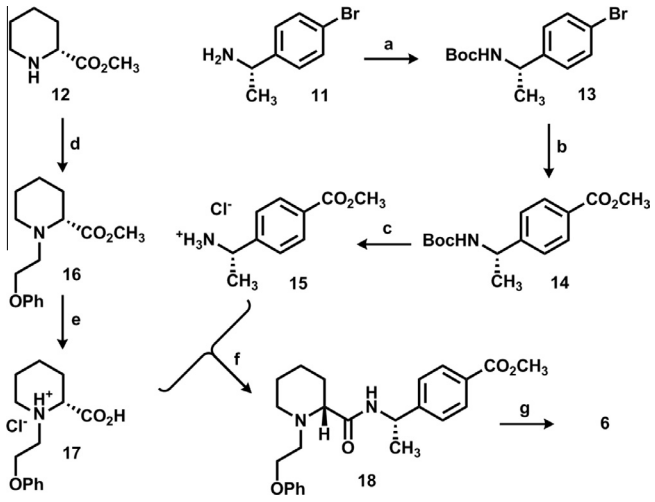
Table 2
In vitro properties of **6**

Assay	Results[a]
hEP_1 filter binding	K_i >17.5 μM, IC_{50} >25 μM (3)
hEP_2 filter binding	K_i = 1.21 μM ×/÷ 2.32, IC_{50} = 1.63 μM ×/÷ 2.31 (6)
hEP_3 filter binding	K_i >12.7 μM, IC_{50} >25 μM (5)
CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP3A4 inhibition	IC_{50} >10 μM for all

[a] Expressed as geometric mean times or divided by the geometric sample standard deviation, with number of determinations in parentheses.

In an in vitro microsomal *m*PGES-1 assay, **6** displayed no inhibition up to 62.5 μM. Therefore, by not interrupting PGE_2 synthesis, **6** was expected to exhibit distinct pharmacology from an *m*PGES-1 inhibitor. Antagonist **6** was tested in vitro against several CYP enzymes (Table 2) and found to have no inhibitory activity up to 10 μM. It was thus concluded that the risk of drug-drug interactions with **6** was low. Additionally, **6** was tested at 10 μM against a panel of 13 receptors, four ion channels, one transporter, and one enzyme, and showed no significant activity on any of the targets.[21] Against hERG in a [^{3}H]-astemizole binding assay, **6** had no activity up to 100 μM.

A synthesis of **6** was achieved from commercially available chiral building blocks **11** and **12** as shown in Scheme 1.[22] Protection of the amine of **11** gave **13**, which was subjected to palladium-catalyzed carbonylation. The resulting ester **14** was deprotected to give the amine coupling partner **15**. Alkylation of the amine of **12** yielded **16**, which was saponified to reveal carboxylic acid **17**. BOP-promoted coupling of **17** and **15** provided amide **18** as a single diastereomer, which was saponified and acidified to give hydrochloride salt **6**.



Scheme 1. Synthesis of **6**. Reagents and conditions: (a) Boc_2O, Et_3N, CH_2Cl_2, 0 °C, 99%; (b) CO (105 psi), $Pd(OAc)_2$, dppf, CH_3OH, Et_3N, CH_3CN, 85 °C, 72%; (c) HCl, 1,4-dioxane, 97%; (d) β-bromophenetole, K_2CO_3, DMF, 100 °C, 64%; (e) NaOH(aq), THF, 65 °C, then HCl(aq), 81%; (f) BOP, DMF, Et_3N, rt, 86%; (g) NaOH(aq), CH_3OH, THF, then HCl, 1,4-dioxane, 66%.

Compound **6** was soluble in FasSIF and FedSIF to concentrations greater than 2.0 mg/mL, and had a solubility of 1.32 mg/mL in SGF. Correspondingly, in vivo ADME studies revealed that **6** was rapidly absorbed and highly bioavailable as the hydrochloride salt. In dog (Fig. 3), the t_{max} was 15 min, and the plasma concentration curve after oral dosing was of a biphasic nature that closely resembled that seen after IV dosing. These observations indicated that absorption was very rapid. The bioavailability calculated from a 10 mg/kg PO dose and a 1 mg/kg IV dose was 81% ×/÷ 1.20 ($n = 3$), and the clearance was 5.2 mL/(min kg) ×/÷ 1.15 ($n = 3$). After a 10 mg/kg PO dose, the plasma concentration of **6** remained above 1 μM in all subjects for at least 12 h and above 100 nM for at least 24 h.

The combination of the ex vivo HWB IC_{50} and the plasma concentrations observed over the time course of oral dosing in dog suggested that **6** could be a viable compound to test EP_4 antagonism as a method of treating inflammatory pain in the clinic.

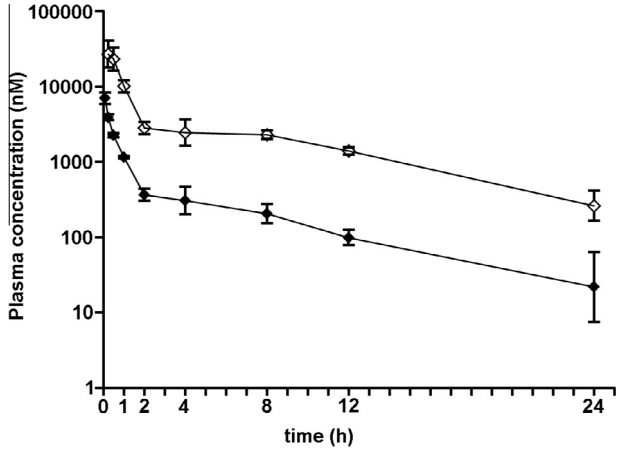


Figure 3. Pharmacokinetics of **6** in beagle dog after 10 mg/kg oral dosing ($n = 3$, open diamonds) and 1 mg/kg IV dosing ($n = 3$, filled diamonds). Diamonds represent geometric mean values, and error bars represent geometric sample standard deviations.

Based in part on its potency, selectivity, oral bioavailability, and duration of exposure, **6** was selected for further studies and eventually chosen for potential clinical development.

In conclusion, a series of EP_4 antagonists was identified which contained both acidic and basic functionality. SAR optimization led to **6**, which displayed an IC_{50} of 126 nM ×/÷ 2.26 for inhibition of PGE_2-induced TNFα reduction in an ex vivo LPS-stimulated human whole blood assay. In vitro assays indicated that **6** was selective for EP_4 versus other EP receptors, *m*PGES-1, and a panel of other targets. In dog, **6** was rapidly absorbed, highly bioavailable, and slowly cleared. These observations contributed to the selection of **6** as a clinical candidate.

Acknowledgments

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Supplementary data

Supplementary data (CEREP panel data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.05.091.

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