



Discovery of sodium 6-[(5-chloro-2-[(4-chloro-2-fluorophenyl)methyl]oxy)phenyl)methyl]-2-pyridinecarboxylate (GSK269984A) an EP₁ receptor antagonist for the treatment of inflammatory pain

Adrian Hall*, Andy Billinton, Susan H. Brown, Anita Chowdhury, Nicholas M. Clayton, Gerard M. P. Giblin, Mairi Gibson, Paul A. Goldsmith, David N. Hurst, Alan Naylor, Caroline F. Peet, Tiziana Scoccitti, Alexander W. Wilson, Wendy Winchester

Neurosciences Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

ARTICLE INFO

Article history:

Received 18 February 2009

Revised 25 February 2009

Accepted 26 February 2009

Available online 3 March 2009

Keywords:

EP₁ antagonist

Inflammatory pain

Pain

CNS penetration

Microdose

ABSTRACT

We describe the medicinal chemistry programme that led to the identification of the EP₁ receptor antagonist GSK269984A (**8h**). GSK269984A was designed to overcome development issues encountered with previous EP₁ antagonists such as GW848687X and was found to display excellent activity in preclinical models of inflammatory pain. However, upon cross species pharmacokinetic profiling, GSK269984A was predicted to have suboptimal human pharmacokinetic and was thus progressed to a human micro-dose study.

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Prostaglandin E₂ (PGE₂) is a proinflammatory mediator, produced from arachidonic acid by the action of the cyclooxygenase (COX) enzymes and prostaglandin E₂ synthase (PGES).¹ The role of inhibitors of the COX enzymes in the treatment of pain is well established.² Recent preclinical data has also highlighted the role of PGE₂ in hyperalgesia via studies with PGES knockout (KO) mice³ and PGES inhibitors.⁴ PGE₂ mediates its physiological actions via the activation of four G-protein coupled receptors (GPCRs) known as EP_{1–4}.⁵ There is substantial literature evidence to link activation of the EP₁ receptor subtype to hyperalgesia,⁶ thus antagonism of the EP₁ receptor offers a novel approach for the treatment of inflammatory pain.⁷ In addition, recent reports have highlighted the potential of EP₁ antagonists in the treatment of visceral pain,⁸ overactive bladder (OAB)⁹ and cerebral ischemia.¹⁰ Thus novel EP₁ antagonists would have the potential to treat several diseases.

We have previously reported on our efforts to identify novel EP₁ receptor antagonists for the treatment of inflammatory pain. Thus we reported the discovery of the clinical candidate GW848687X (**1**)¹¹ and analogues such as GSK345931A (**2**)¹² and GW845706X (**3**)¹¹ (Fig. 1). We also reported on a novel methylene-linked pyrazole series as exemplified by GSK180100B (**4**).¹³

Several key issues were encountered with GW848687X (**1**) upon further investigation. In the first instance, when the compound was dosed in a 5 day rat joint pain study^{11,14} analysis of the blood exposures from animals in the drug treated groups revealed a marked decrease in exposure on days 4–5 relative to earlier time points (e.g., day 1). This was observed when AUC values

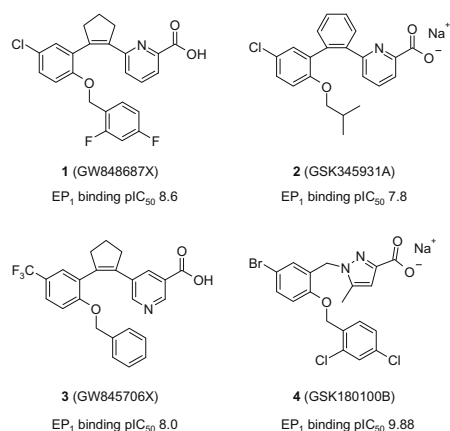


Figure 1. Key literature EP₁ receptor antagonists from GSK.

* Corresponding author. Tel.: +44 1279 643464.

E-mail address: Adrian.2.hall@gsk.com (A. Hall).

were compared in addition to single time point concentrations. It is possible this effect on exposure is linked to a secondary observation that the mRNA of CYP2B1 and 2B2 was significantly upregulated in a 7 day rat toxicology study, where a similar decrease in exposure was observed. This effect on exposure posed an issue as it precluded the establishment of a suitable therapeutic index (TI). The situation became compounded when a potential back-up molecule, GSK345931A (**2**) also showed decreased exposure in the rat joint pain model of inflammatory pain on days 4 and 5. Both compounds were found to be unstable to light in solution, as has been described previously for related compounds,¹⁵ which posed a further issue.

We had found that analogues of GW848687X (**1**), such as GW845706X (**3**) which did not penetrate the central nervous system (CNS) failed to show efficacy in the rat joint pain model.¹¹ Thus, as part of a plan to identify further development candidates which addressed the issues identified with GW848687X (**1**) and GSK345931A (**2**) we sought to profile compounds of diverse chemical structures which demonstrated CNS penetration, efficacy in the rat joint pain model, but which did not show decreases in exposure. As a first step, we profiled compound **4** (GSK180100B, Fig. 1) in the joint pain model of inflammatory pain (this compound had previously shown efficacy in the CFA model of inflammatory pain (70% reversal of hypersensitivity, 1 h post-dose of 10 mg/kg po). In this study, compound **4** failed to show efficacy, which was attributed to its lack of CNS penetration (rat Br:Bl < 0.05). However, bioanalysis of blood samples from the joint pain study showed that the exposure had not decreased over the 5 day dosing schedule.

We had previously observed that picolinic acid derivatives, such as **1** and **2**, were able to penetrate the CNS in preclinical species and that this had also resulted in efficacy in the joint pain assay. Thus we sought to combine the positive attributes of compounds such as **1** and **4** by replacing the pyrazole acid of compounds such as **4** by a picolinic acid to give compounds of general structure **8**, Figure 2.

Scheme 1 depicts the synthesis of the methylene-linked picolinic acid derivatives. Selective benzylation of the phenolic group of 5-chloro-2-hydroxybenzyl alcohol (**5**) with 1 equiv of benzyl bromide, followed by reaction with phosphorous tribromide gave benzyl bromide **6**. Treatment of **6** with activated zinc¹⁶ followed by Negishi reaction¹⁷ with **9** formed the key carbon–carbon methylene linker. This unpurified intermediate was treated with sodium methane thiolate (debenzylation of the phenol and ester hydrolysis) and re-esterified to deliver phenol **7**. Alkylation of **7** followed by ester hydrolysis, as exemplified for derivative **8h**, delivered the target compounds (Scheme 1).

The SAR for derivatives **8a–n** is summarized in Table 1. Initial data for the unsubstituted benzyl derivative (**8a**) was disappointing¹⁸, and the mono-fluoro derivatives (**8b** and **8c**) offered little improvement. The 2,4-difluoro analogue (**8d**) provided an increase in affinity, but addition of a third fluorine atom (**8e**) was detrimental. Mono-chloro benzyl derivatives (**8f** and **8g**) proved marginally

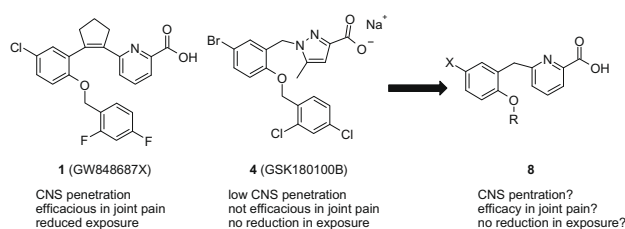
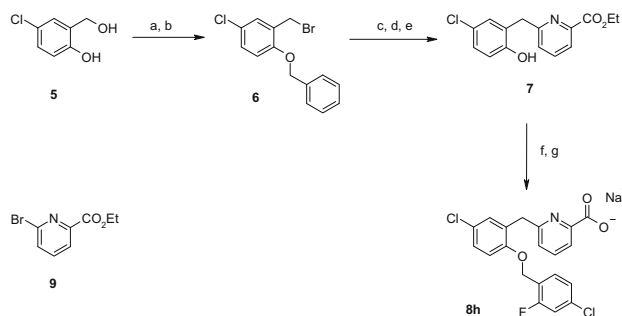


Figure 2. Medicinal chemistry strategy to combine the desirable attributes from compound **1** with compound **4** into a single molecule (**8**).



Scheme 1. Reagents and conditions: (a) PhCH₂Br, K₂CO₃, Me₂CO, reflux, 2 h; (b) PBr₃, CH₂Cl₂, –10 °C to rt (82%, two steps); (c) activated zinc, THF, then **9**, Pd(PPh₃)₄, THF, rt; (d) NaOMe, DMF, 100 °C; (e) concn H₂SO₄, EtOH, reflux (36%, three steps); (f) 4-chloro-2-fluorobenzyl bromide, K₂CO₃, Me₂CO, 50 °C (79%); (g) EtOH, 2 M NaOH, 90 °C (93%).

Table 1
Structure–activity relationships of derivatives **8a–n**

Compds	R	EP ₁ binding ^a pIC ₅₀
8a	Bn	6.8 ± 0.2
8b	2-F-Bn	7.1 ± 0.2
8c	4-F-Bn	7.0 ± 0.1
8d	2,4-diF-Bn	7.6 ± 0.1
8e	2,4,6-triF-Bn	7.1 ± 0.2
8f	2-Cl-Bn	7.4 ± 0.1
8g	4-Cl Bn	7.4 ± 0.1
8h	2-F,4-Cl-Bn	7.9 ± 0.2
8i	2-Cl,4-F-Bn	7.9 ± 0.1
8j	2,4-diClBn	8.0 ± 0.1
8k	<i>i</i> -Bu	7.4 ± 0.1
8l	CH ₂ cyhex	7.0 ± 0.1
8m	CH ₂ cyptent	7.5 ± 0.1
8n	CH ₂ cyprop	6.7 ± 0.0

^a Data from [³H]-PGE₂ binding assay in CHO cells overexpressing the human EP₁ receptor, values are the mean of at least three experiments with standard deviations.

higher in affinity than their fluoro counterparts (**8b** and **8c**, respectively). Highest affinity was achieved with the chloro-fluoro benzyl derivatives (**8h** and **8i**) and the dichloro derivative (**8j**). These three compounds also showed good in vitro metabolic stability (intrinsic clearance values of <0.5 mL/min/g liver in both rat and human liver microsomes for **8h** and **8j**, which equates to <15% turnover after 30 min incubation and slightly higher values for **8i** (<1.5 mL/min/g liver).¹⁹

The alkyl analogues (**8k–n**) displayed sub-optimal affinity, and furthermore, **8m** displayed higher in vitro metabolic instability (CLi 10.0 mL/min/g liver in human liver microsomes).

On the basis of the data in Table 1 compounds **8h–j** were selected for further profiling. All three compounds were found to be functional antagonists (Table 2) as measured by their ability to block PGE₂-mediated intracellular Ca²⁺ mobilization in CHO cells recombinantly overexpressing the human EP₁ receptor. Furthermore, in a functional assay, compound **8h** was found to cause a concentration-dependent rightward shift of the PGE₂ dose–response curve and Schild analysis²⁰ showed it to be a competitive antagonist with pA₂ 8.1 ± 0.3, slope 1.0. As expected, the dichloro analogue **8j** was found to be the most lipophilic of the three com-

Table 2Functional antagonism data, measured logD data and CYP450 inhibition data for compounds **8h–j**

Compds	EP ₁ ^a FLIPR pK _i	logD ^b	CYP450 IC ₅₀ ^c (μM)
8h	8.1 ± 0.5	2.1	20 (1A2), >100 (2C19), 8 (2C9), >100 (2D6), no data @ 3A4
8i	7.0 ± 0.6	2.0	61 (1A2), 28 (2C19), 15 (2C9), >100 (2D6), 35 (3A4 DEF), >100 (3A4 PPR)
8j	7.6 ± 0.8	2.6	32 (1A2), >50 (2C19), 4.8 (2C9), >50 (2D6), no data @ 3A4

^a Data from PGE₂-mediated Ca²⁺ mobilization assay in CHO cells overexpressing the human EP₁ receptor, values are the mean of at least three experiments with standard deviations.

^b Measured data at pH 7.4.

^c Inhibition of metabolism of fluorometric substrates using Gentest protocol, single experiment.²¹

pounds, and this subtle increase in lipophilicity could be the cause of the increase in CYP inhibition.

Further profiling of compound **8h** using an alternative enzyme source confirmed the low potential for CYP450 inhibition, Table 3.

Based on these results, compounds **8h** and **8i** were progressed to the rat CFA model of inflammatory pain. Compounds were dosed orally at doses of 1, 3 and 10 mg/kg, 23 h after intraplantar administration of the adjuvant. Analgesia was assessed 1 h post-dose of compound by the weight-bearing protocol. Compound **8h** demonstrated an ED₅₀ of 2.6 mg/kg (po), with the 10 mg/kg dose showing equivalent reversal of hypersensitivity to the standard (GW855454X, 30 mg/kg po). On the other hand, compound **8i** gave an ED₅₀ of 5.6 mg/kg (po) but did not show reversal of hypersensitivity equivalent to the standard (GW855454X, 30 mg/kg po). Bioanalysis from these studies showed both compounds penetrated the CNS to a similar degree and that the Br:Bl ratio for each compound was constant over the dose range investigated, Table 4.

On the basis of this data, compound **8h** was selected for progression to the rat joint pain model of inflammatory pain (dosed at 3 and 10 mg/kg orally *b.i.d.* for 5 days) where it demonstrated full reversal of hypersensitivity at 10 mg/kg (equivalent to rofecoxib) and an ED₅₀ of ~3 mg/kg. Bioanalysis again confirmed similar CNS penetration to the CFA study (Br:Bl 0.41 ± 0.08 at 10 mg/kg and Br:Bl 0.34 ± 0.03 at 3 mg/kg, 1 h post-final dose). Pleasingly,

Table 3CYP450 (Cypex) inhibition data for compound **8h**^a

CYP isoform	1A2	2C19	2C9	2D6	3A4
IC ₅₀ (μM)	51 ± 9	85 ± 19	20 ± 2	≥ 100	≥ 100

^a Inhibition of metabolism of fluorometric substrates using Cypex protocol, values are the mean of three experiments ± standard deviation.

Table 4Summary of rat CFA data for compounds **8h** and **8i**

Compds	Dose (mg/kg) p.o.	ED ₅₀ (mg/kg) p.o.	Blood concn ^a (nM)	Brain concn ^b (nM)	Br:Bl ^c
8h	1	2.6	144 ± 44	60 ± 12	0.37 ± 0.04
	3		415 ± 106	175 ± 30	0.37 ± 0.03
	10		1512 ± 355	501 ± 65	0.39 ± 0.05
8i	1	5.6	346 ± 114	93 ± 29	0.31 ± 0.03
	3		836 ± 161	256 ± 29	0.30 ± 0.02
	10		3013 ± 841	1117 ± 59	0.31 ± 0.03

^a Values are the mean from seven animals.

^b Values are the mean from three animals.

^c Values are the mean from three animals where the blood and brain samples are from the same animal.

Table 5CNS penetration for compound **8h** in the mouse, rat, and landrace pig

Species	Blood concn (nM)	Brain concn (nM)	Br:Bl
Mouse ^a	199 ± 12	56 ± 10	0.28 ± 0.05
Rat ^b	955 ± 72	225 ± 25	0.24 ± 0.04
Rat ^c	1798 ± 7	374 ± 32	0.21 ± 0.02
Landrace pig ^d	2304	627	0.27

^a Steady-state infusion (12 h) of 5 mL/kg/h to achieve target dose of 0.5 mg/kg/h, data are the mean from three animals.

^b Steady-state (12 h) infusion of 5 mL/kg/h to achieve target dose of 0.5 mg/kg/h, data are the mean from three animals.

^c 1 h infusion.

^d 1 h infusion, single animal.

no reduction in exposure was observed throughout the course of the study (from a satellite study group dosed at 10 mg/kg *b.i.d.* for 5 days blood concentrations were 2.393 ± 0.552 μM 1 h post-dose on day 1 and 2.607 ± 0.286 μM 1 h post-dose on day 5). This was later confirmed in rat 7 and 28 day toxicological studies (data not shown).

The CNS penetration of compound **8h** was assessed in several species, Table 5, and found to be consistent across the species investigated.

The pharmacokinetics of compound **8h** were assessed in the rat, dog and cynomolgus monkey, data are summarized in Table 6.

Compound **8h** displayed moderate blood clearance in the rat and dog and high clearance in the monkey which is reflected in the half-life for each species. The bioavailability appeared to be limited by first pass hepatic extraction for each species. This data is supported by the permeability data, (MDCKII-MDR1²³ Pgp assay; Pexact²⁴ A–B = 700 nm/s, efflux ratio = 0.9) and solubility data (all data at 0.5 h time point; H₂O: 890 μg/mL, FaSSIF: 60 μg/mL, FeSSIF: 358 μg/mL).

In vitro metabolic stability profiling of compound **8h** showed that it was stable across species, Table 7, which thus highlighted a clear discrepancy between in vitro and in vivo metabolic stability, particularly in the dog and cynomolgus monkey. As this data posed difficulties in the prediction of human pharmacokinetics, further work was undertaken with rat, monkey and human hepatocytes and S9 fraction to include phase 2 pathways in the clearance deter-

Table 6Summary of in vivo pharmacokinetic data for compound **8h**, values are the mean from three animals ± standard deviation

Species	CL _B ^a (mL/min/kg)	V _{ss} ^a (L/kg)	t _{1/2} ^a (h)	F _{po} ^b (%)
Rat ^c	19 ± 2	2.1 ± 0.3	2.3 ± 0.5	94 ± 26
Dog ^d	18 ± 2	0.6 ± 0.1	1.1 ± 0.2	39 ± 9
Cyno ^e	41 ± 2	0.6 ± 0.2	0.6 ± 0.5	7 ± 4

^a Intravenous administration, 1 h infusion of 1 mg/kg dose.

^b Oral administration of 3 mg/kg dose, vehicle = 1% (w/v) methylcellulose.

^c Male Sprague–Dawley rats.

^d Beagle dog.

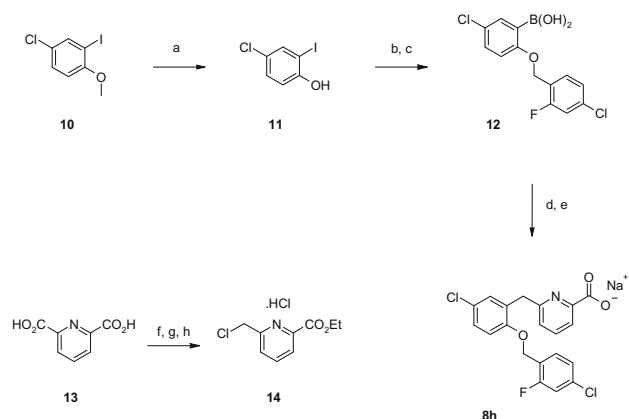
^e Cynomolgus monkey.

Table 7Summary of in vitro metabolic stability (intrinsic clearance, mL/min/g liver) data for compound **8h** in different hepatic fractions

Fraction	Mouse	Rat	Dog	Monkey	Human
Microsomes	<0.5	≤0.7	<0.5	<0.5	<0.5
Hepatocytes	Not tested	2.1 ^a	Not tested	4.8, ^a 2.7 ^b	3.7, ^a 0.7 ^b
S9	Not tested	≤1.0	≤1.0	≤1.0	≤1.0

^a Cell density 0.15–0.2 × 10⁶ cells/mL.

^b Cell density 0.54–0.7 × 10⁶ cells/mL.



Scheme 2. Reagents and conditions: (a) BBr_3 , CH_2Cl_2 , -78°C to rt (99%); (b) 4-chloro-2-fluorobenzyl bromide, K_2CO_3 , Me_2CO , reflux, 2 h (98%); (c) $i\text{-PrMgCl}$, THF, B(OMe)_3 , -40°C to rt then 2 M HCl (90–93%); (d) $\text{Pd(PPh}_3)_4$, K_2CO_3 , **13**, PhMe/EtOH (1:1), 90°C , 2 h (51%); (e) EtOH, 2 M NaOH, reflux to rt (96%); (f) EtOH, concn H_2SO_4 , reflux (95%); (g) NaBH_4 , EtOH, rt (72–83%); (h) SOCl_2 , CH_2Cl_2 (100%).

minations, Table 7. The metabolic stability in hepatocytes and S9 again failed to predict the in vivo metabolic stability in the rat and monkey and did not therefore help understand this mismatch. As a result of this, compound **8h** was progressed to a human microdose study in order to assess the human pharmacokinetics.²⁵

It was found that the synthetic route in Scheme 1 was not suitable to support large scale synthesis of **8**. Thus, an alternative route employing a Suzuki reaction as the key step was developed, Scheme 2, and was used to supply more than 100 g of compound. Commercially available 4-chloro-2-iodoanisole (**10**) was demethylated with boron tribromide to give the corresponding phenol **11** in high yield. Alkylation of the phenol and subsequent halogen-metal exchange using Knochel's conditions²⁶ followed by reaction with trimethylborate gave boronic acid **12**. Suzuki–Miyaura coupling²⁷ of **12** with the chloromethylpyridine derivative **14** delivered the ester in moderate yield, which underwent ester hydrolysis to give **8h** as the sodium salt.²⁸ Intermediate **14** was prepared from diacid **13** as outlined in Scheme 2 and was used without purification.

The selectivity of compound **8h** was profiled at a number of prostaglandin and thromboxane targets (EP_2 pIC_{50} 5.8, EP_3 FLIPR pK_i 5.9, EP_4 pIC_{50} < 5, FP pIC_{50} < 5, IP pIC_{50} < 6, TP pK_i 8.0, COX-1 pIC_{50} < 4.5, COX-2 pIC_{50} < 4) where it showed good selectivity except for the thromboxane A_2 (TP) receptor. No data was generated against the DP_1 or DP_2 (CRTH2) receptors, however, in general compounds from this programme were found to be inactive at the DP_1 receptor, for example, GW848687X displayed >400-fold selectivity over the DP_1 receptor.¹¹ When screened against a panel of 50 receptors, enzymes and ion channels (Cerep, France) at $1\ \mu\text{M}$, compound **8h** showed no significant activity (inhibition $\leq 30\%$). Compound **8h** had a low risk of QT interval prolongation, mediated by blockade of the hERG channel, dofetilide binding pIC_{50} < 4.5 (IC_{50} > $32\ \mu\text{M}$).

In summary, we have described the identification of compound **8h** (GSK269984A) which overcame the major issues that precluded development of previous EP_1 antagonists. Compound **8h** was selected as a development candidate for the treatment of inflammatory pain and was progressed to a human microdose study. Full details of the in vivo biological profile and the human microdose PK data will be the subject of future publications.

Acknowledgements

The authors thank Helen Hooper, Lee Abberley, Alison Gilbert, Karen Davies, Barry Passingham, Teresa Heslop, Sadhia Mahmood,

Lindsay Mulligan, Shiyam Mohamed for DMPK data, Alan K. Bristow for solubility data and Thomas G. Hayhow for assistance with large scale chemical synthesis.

References and notes

- (a) Kassuya, C. A. L.; Ferreira, J.; Claudino, R. F.; Calixto, J. B. *Br. J. Pharmacol.* **2007**, *150*, 727; (b) Minami, T.; Uda, R.; Horiguchi, S.; Ito, S.; Hyodo, M.; Hayashi, M. *Pain* **1994**, *57*, 217.
- (a) Fitzgerald, G. A. C. *Nat. Rev. Drug Disc.* **2003**, *2*, 879; (b) Huntjens, D. R. H.; Danhof, M.; Della Pasqua, O. E. *Rheumatology* **2005**, *44*, 846; (c) Bianchi, M.; Brogini, M. *Drugs* **2003**, *63*, 37; (d) Zhang, Y.; Shaffer, A.; Portanova, J.; Seibert, K.; Isakson, P. C. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1069.
- (a) Trebino, C. E.; Stock, J. L.; Gibbons, C. P.; Naiman, B. M.; Wachtmann, T. S.; Umland, J. P.; Pandher, K.; Lapointe, J.-M.; Saha, S.; Roach, M. L.; Carter, D.; Thomas, N. A.; Durtschi, B. A.; McNeish, J. D.; Hambor, J. E.; Jakobsson, P.-J.; Carty, T. J.; Peez, J. R.; Audoly, L. P. *Proc. Nat. Acad. Sci.* **2003**, *100*, 9044; (b) Kamei, D.; Yamakawa, K.; Takegoshi, Y.; Mikami-Nakanishi, M.; Nakatani, Y.; Oh-ishi, S.; Yasui, H.; Azuma, Y.; Hirasawa, N.; Ohuchi, K.; Kawaguchi, H.; Ishikawa, Y.; Ishii, T.; Uematsu, S.; Akira, S.; Murakami, M.; Kudo, I. *J. Biol. Chem.* **2004**, *279*, 33684.
- (a) Xu, D.; Rowland, S. E.; Clark, P.; Giroux, A.; Côté, B.; Guiral, S.; Salem, M.; Ducharme, Y.; Friesen, R. W.; Méthot, N.; Mancini, J.; Audoly, L.; Riendeau, D. *J. Pharm. Expt. Ther.* **2008**, *326*, 754; (b) Friesen, R. W.; Mancini, J. A. *J. Med. Chem.* **2008**, *51*, 1059.
- (a) Coleman, R. A.; Smith, W. L.; Narumiya, S. *Pharmacol. Rev.* **1994**, *46*, 205; (b) Narumiya, S.; Sugimoto, Y.; Ushikubi, F. *Physiol. Rev.* **1999**, *79*, 1193; (c) Coleman, R. A.; Kennedy, I.; Humphrey, P. P. A.; Bunce, K.; Lumley, P. In *Comprehensive Medicinal Chemistry*; Pergamon: Oxford, UK, 1990; Vol. 3.
- (a) Tober, K. L.; Wilgus, T. A.; Kusewitt, D. F.; Thomas-Ahner, J. M.; Maruyama, T.; Oberyzy, T. M. *J. Invest. Dermatol.* **2006**, *126*, 205; (b) Omote, K.; Kawamata, T.; Nakayama, Y.; Kawamata, M.; Hazama, K.; Namiki, A. *Anesth. Analg.* **2001**, *92*, 233; (c) Kawahara, H.; Sakamoto, A.; Takeda, A.; Onodera, H.; Imaki, J.; Ogawa, R. *Anesth. Analg.* **2001**, *93*, 1012; (d) Omote, K.; Yamamoto, H.; Kawamata, T.; Nakayama, Y.; Namiki, A. *Anesth. Analg.* **2002**, *95*, 1708; (e) Minami, T.; Nakano, H.; Kobayashi, T.; Sugimoto, Y.; Ushikubi, F.; Ichikawa, A.; Narumiya, S.; Ito, S. *Br. J. Pharmacol.* **2001**, *133*, 438; (f) Mebane, H.; Turnbach, M. E.; Randich, A. *J. Pain* **2003**, *4*, 392.
- Hall, A.; Billinton, A.; Giblin, G. M. P. *Curr. Opin. Drug Disc. Dev.* **2007**, *10*, 597.
- Sarkar, S.; Hobson, A. R.; Hughes, A.; Grewcott, J.; Woolf, C. J.; Thompson, D. G.; Aziz, Q. *Gastroenterology* **2003**, *124*, '8.
- (a) Lee, T.; Hedlund, P.; Newgreen, D.; Andersson, K.-E. *J. Urol.* **2007**, *177*, 1562; (b) Schröder, A.; Newgreen, D.; Andersson, K.-E. *J. Urol.* **2004**, *172*, 1166.
- (a) Kawano, T.; Anrather, J.; Zhou, P.; Park, L.; Wang, G.; Frys, K. A.; Kunz, A.; Cho, S.; Orío, M.; Iadecola, C. *Nat. Med.* **2006**, *122*, 225; (b) Ahmad, A. S.; Saleem, S.; Ahmad, M.; Doré, S. *Toxicol. Sci.* **2006**, *89*, 265.
- Giblin, G. M. P.; Bit, R. A.; Brown, S. H.; Chaignot, H. M.; Chowdhury, A.; Chessell, I. P.; Clayton, N. M.; Coleman, T.; Hall, A.; Hammond, B.; Hurst, D. N.; Michel, A. D.; Naylor, A.; Novelli, R.; Scoccitti, T.; Spalding, D.; Tang, S. P.; Wilson, A. W.; Wilson, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 385–389.
- Hall, A.; Brown, S. H.; Budd, C.; Clayton, N. M.; Giblin, G. M. P.; Goldsmith, P.; Hayhow, T. G.; Hurst, D. N.; Naylor, A.; Rawlings, D. A.; Scoccitti, T.; Wilson, A. W.; Winchester, W. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 497.
- McKeown, S. C.; Hall, A.; Giblin, G. M. P.; Lorthioir, O.; Blunt, R.; Lewell, X. Q.; Wilson, R. J.; Brown, S. H.; Chowdhury, A.; Coleman, T.; Watson, S. P.; Chessell, I. P.; Pipe, A.; Clayton, N.; Goldsmith, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4767.
- Wilson, A. W.; Medhurst, S. J.; Dixon, C. I.; Bontoft, N. C.; Winyard, L. A.; Brackenborough, K. T.; De Alba, J.; Clarke, C. J.; Gunthorpe, M. J.; Hicks, G. A.; Bountra, C.; McQueen, D. S.; Chessell, I. P. *Eur. J. Pain* **2006**, *10*, 537.
- (a) Mao, B.; Abraham, A.; Ge, Z.; Ellison, D. K.; Hartman, R.; Prabu, S. V.; Reamer, R. A.; Wyvratt, J. *J. Pharmaceut. Biomed.* **2002**, *28*, 1101; (b) Hartman, R.; Abraham, A.; Clausen, A.; Mao, B.; Crocker, L. S.; Ge, Z. *J. Liq. Chromatogr. R. T.* **2003**, *26*, 2551.
- Knochel, P.; Yeh, C. M. P.; Berk, S. C.; Talbert, J. J. *Org. Chem.* **1988**, *53*, 2390.
- Knochel, P.; Singer, R. D. *Chem. Rev.* **1993**, *93*, 2117.
- Hall, A.; Bit, R. A.; Brown, S. H.; Chowdhury, A.; Giblin, G. M. P.; Hurst, D. N.; Kilford, I. R.; Lewell, X.; Naylor, A.; Scoccitti, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1592.
- Clarke, S. E.; Jeffrey, P. *Xenobiotica* **2001**, *31*, 591.
- For Schild analysis, the effect of various single concentrations of antagonist on PGE_2 concentration response curves were observed in a luciferase reporter assay. Antagonist concentrations between the pIC_{50} value and maximum response were incubated with a concentration range of PGE_2 and cells containing the human EP_1 receptor and an NFAT reporter gene for 4 h at 37°C , 5% CO_2 and 95% humidity. Following incubation, reporter gene activity was assessed by addition of a luciferin substrate and the subsequent light response measured in a luminescence counter, as an indication of EP_1 function. For further details see: (a) Schild, H. O. *Br. J. Pharmacol. Chemother.* **1949**, *4*, 277; (b) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48.
- Crespi, C. L.; Miller, V. P.; Penman, B. W. *Anal. Biochem.* **1997**, *248*, 188.
- Hall, A.; Brown, S. H.; Chessell, I. P.; Chowdhury, A.; Clayton, N. M.; Coleman, T.; Giblin, G. M. P.; Hammond, B.; Healy, M. P.; Johnson, M. R.; Metcalf, A.; Michel, A. D.; Naylor, A.; Novelli, R.; Spalding, D. J.; Sweeting, J.; Winyard, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 916.

23. Keogh, J. P.; Kunta, J. R. *Eur. J. Pharm. Sci.* **2006**, 27, 543.
24. Tran, T. T.; Mital, A.; Gales, T.; Maleeff, B.; Aldinger, T.; Polli, J. W.; Aryton, A.; Ellens, H.; Bentz, J. J. *J. Pharm. Sci.* **2004**, 93, 2108.
25. Details of the full pharmacological profile and human pharmacokinetic data will be published elsewhere.
26. Knochel, P.; Dohle, W.; Gommermann, N.; Kniesel, F. F.; Kopp, F.; Korn, T.; Sapountzis, I.; Vu, V. A. *Angew. Chem., Int. Ed.* **2003**, 42, 4302.
27. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, 95, 2457.
28. Full experimental details and characterising data have been published, see: Giblin, G. M. P.; Hall, A.; Hurst, D. N.; Rawlings, D. A.; Scoccitti, T. PCT patent application WO2006066968A1, 2006.