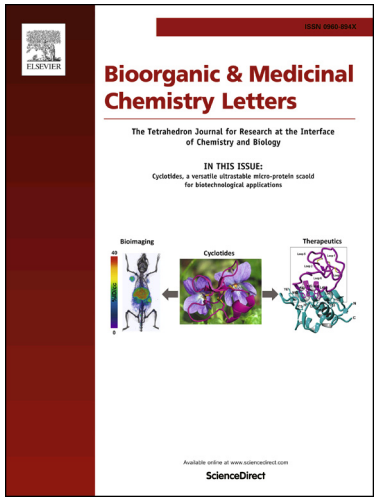
Accepted Manuscript



Discovery of {4-[4,9-bis(ethyloxy)-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl]-2-fluorophenyl}acetic acid (GSK726701A), a novel EP_4 receptor partial agonist for the treatment of pain

Mark P. Healy, Amanda C. Allan, Kristin Bailey, Andy Billinton, Iain P. Chessell, Nicholas M. Clayton, Gerard M. P. Giblin, Melanie A. Kay, Tarik Khaznadar, Anton D. Michel, Alan Naylor, Helen Price, David J. Spalding, David A. Stevens, Martin E. Swarbrick, Alexander W. Wilson

PII: S0960-894X(18)30296-8
DOI: https://doi.org/10.1016/j.bmcl.2018.03.091
Reference: BMCL 25745

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 10 January 2018
Revised Date: 29 March 2018
Accepted Date: 30 March 2018

Please cite this article as: Healy, M.P., Allan, A.C., Bailey, K., Billinton, A., Chessell, I.P., Clayton, N.M., M. P. Giblin, G., Kay, M.A., Khaznadar, T., Michel, A.D., Naylor, A., Price, H., Spalding, D.J., Stevens, D.A., Swarbrick, M.E., Wilson, A.W., Discovery of {4-[4,9-bis(ethyloxy)-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl]-2-fluorophenyl}acetic acid (GSK726701A), a novel EP_4 receptor partial agonist for the treatment of pain, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.03.091

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Discovery of {4-[4,9-bis(ethyloxy)-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl]-2-fluorophenyl}acetic acid (GSK726701A), a novel EP_4 receptor partial agonist for the treatment of pain

Mark P. Healy,* Amanda C. Allan, Kristin Bailey, Andy Billinton, Iain P. Chessell, Nicholas M. Clayton, Gerard M. P. Giblin, Melanie A. Kay, Tarik Khaznadar, Anton D. Michel, Alan Naylor, Helen Price, David J. Spalding, David A. Stevens, Martin E. Swarbrick, Alexander W. Wilson

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

Declarations of interest: none

This is where the receipt/accepted dates will go; Received Month XX, 2018; Accepted Month XX, 2018 [BMCL RECEIPT]

Abstract— A novel series of EP_4 agonists and antagonists have been identified, and then used to validate their potential in the treatment of inflammatory pain. This paper describes these novel ligands and their activity within a number of pre-clinical models of pain, ultimately leading to the identification of the EP_4 partial agonist GSK726701A.

Prostaglandin E2 (PGE_2) **1**, synthesised from arachidonic acid (AA) by the cyclooxygenase enzymes, is a ubiquitous mediator of mammalian physiology and pathophysiology.[1] Physiologically, PGE_2 contributes to the modulation of bone formation[2], gut homeostasis including pH control[3], renal function and blood pressure.[4] In addition PGE_2 is associated with the growth of certain cancers[5] and is a well characterised inflammatory mediator contributing to pain and inflammation.[6] To date, pharmacological intervention in the AA pathway has led to the discovery and commercialisation of NSAIDS and COX2 inhibitors.[7] However these drugs have a number of safety concerns associated with gastro-intestinal bleeding and damage and adverse cardiovascular events, respectively.[8]

The biological effects of PGE_2 are mediated by four G-protein coupled receptors designated $EP_{1\text{-}4}$.[9] Each of these receptors has characteristic distribution in various tissues and this feature, together with the paracrine nature of PGE_2, allows PGE_2 to play varied and sometimes opposing roles in the mammalian system. For example, PGE_2 is thought to act as a pro-inflammatory agent in the early stages of inflammation, while helping to promote inflammatory resolution at later time points[10] and these effects may be mediated by different receptor sub-types. Thus selectively targeting individual EP receptors offers the opportunity to intervene in pathophysiology with specificity and to potentially avoid toxicity.

As part of a wider investigation of the role of EP receptors in pain and inflammation, we initiated a programme to discover selective EP_4 ligands. We were particularly intrigued by seemingly conflicting roles of EP_4 in pain and inflammation. Studies have confirmed that PGE_2 acting on the EP_4 receptor can either stimulate or inhibit inflammation in concert with IFNγ.[11] Furthermore, EP_4 receptor knockout mice showed resistance to inflammation in a collagen induced arthritis model[12] and selective EP_4 antagonists show robust anti-inflammatory effects in vivo.[13] However, EP_4 agonists have also been reported to attenuate levels of the pro-inflammatory mediator TNFα in rats[14] and can potently suppress inflammation in an adjuvant induced arthritis model.[15]

The PGE_2 analogue ONO-4819CD (rivenprost) **2** has been in clinical development and was reported to have an

* Corresponding author present address: Novartis Institutes for BioMedical Research, 22 Windsor St, Cambridge, MA, 02139, USA. e-mail: mark.healy@novartis.com (M.P.Healy).

ACCEPTED MANUSCRIPT

improvement in histological scores in ulcerative colitis patients.[16] ONO-4819CD is the pro-drug of the EP_4 agonist ONO-AE1-437 **3** (Figure 1). Previously we reported the first non-prostanoid, selective EP_4 agonist CCI17464 **4**[17] as a result of an internal focussed screen. From the same naphtholactam template an EP_4 antagonist, GW627368X **5**[18] was identified. The sulphonyl urea EP_4 antagonist CJ-023,423 (grapipant, RQ-007, AAT-007) **6**,[19] has been reported as being effective for treating osteoarthritis (OA) pain in a phase 2 study in humans,[20] whilst recently been approved to control pain and inflammation associated with osteoarthritis in dogs.[21]

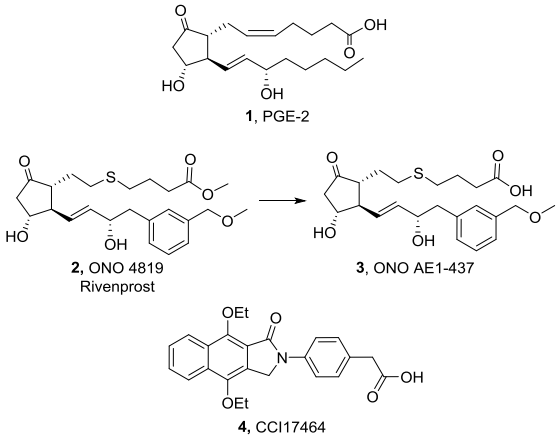


Figure 1 – Structures of selected EP_4 receptor agonists

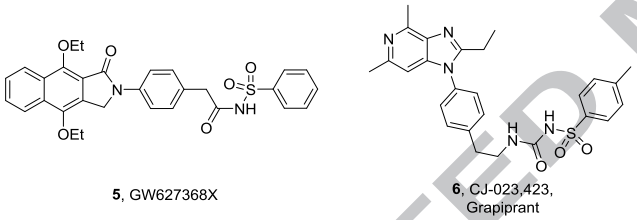
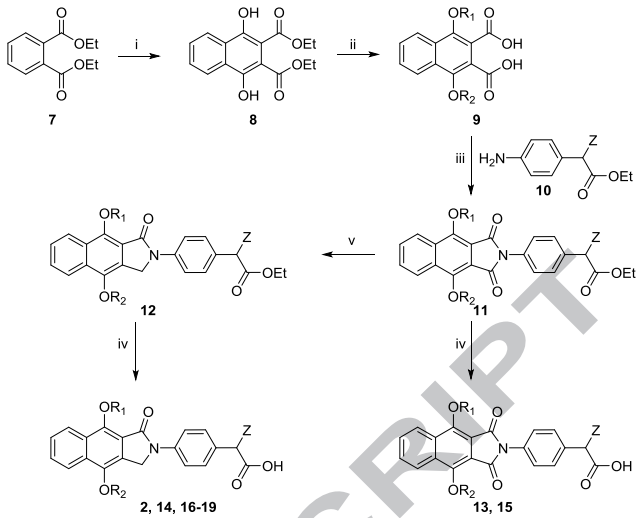


Figure 2 – Structures of selected EP_4 receptor antagonists

We were interested in exploring the biological profile of EP_4 ligands and as such set out to explore the SAR of the EP_4 partial agonist CCI17464. The reported potential for partial agonists to induce less receptor desensitisation[22] was attractive to us if we could demonstrate human whole blood activity and efficacy in preclinical models of pain.



Scheme 1. *Reagents and conditions*: i) Diethylsuccinate, NaOEt, EtOH; ii) (a) R_1I, NaOEt, EtOH then R_2I, NaOEt, EtOH; (b) NaOH, EtOH; iii) AcOH; iv) NaOH, EtOH; v) Zn, AcOH or i) $NaBH_4$, then ii) Et_3SiH, TFA.

The synthesis of CCI17464 and analogues is shown in **Scheme 1**. Diethyl phthalate **7** was treated under basic conditions with diethyl succinate to give the naphthylene diol **8**. Diol **8** was then alkylated, followed by saponification to yield the bis-acids **9**. Condensation with ethyl (4-amino phenyl) acetate **10** yielded the phthalimides **11**, which were then hydrolysed to give carboxylic acids **13** and **15**. Alternatively reduction of the imides **11** with Zn/AcOH or a two-step protocol with $NaBH_4$ followed by Et_3SiH/TFA,[23] gave the lactam analogues **12**, which were then subsequently hydrolysed to give carboxylic acids **2**, **14**, and **16-19**.

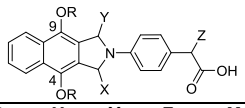
Table 1 summarises the early SAR in the series. The phthalimide analogue **13**, shows equipotency, but higher intrinsic activity than the lactam partial agonist **2** in a functional cAMP accumulation assay,[24] which is a consistent observation between these two functionalities. Earlier studies have suggested the carboxylic acid functionality on PGE_2 forms a key interaction with a conserved arginine residue within TMVII of all prostanoid receptors.[24] Indeed, the para-acetic acid is the optimum position for EP_4 agonist potency, and introduction of a methyl group next to the carboxylic acid is detrimental to agonist potency (compound **14**) highlighting the sensitivity of this part of the molecule for EP_4 agonism.

Investigation into the size of the alkyl substituent on oxygen atoms at the 4 and 9 positions of the naphthylene ring showed the ethyl and *iso*-propyl groups to offer the best potency (compounds **13** and **17**). Increasing the size of these groups beyond a butyl group leads to a loss in potency (compound **19**).

Removal of both oxygen atoms on the imide ring resulted in a weak EP_4 partial agonist (compound **20**) suggesting the carbonyl group has an important role in the agonist mode of action.

Table 1 – SAR of compounds **2-20**

ACCEPTED MANUSCRIPT



Compound	R	X	Y	Z	cAMP pEC_{50} [a]	IA[b]	pKi[c]
CCI17464, 2	Et	H,H	=O	H	7.7	61%	7.1
13	Et	=O	=O	H	7.6	90%	6.8
14	Et	H,H	=O	Me	<6	-	6.7
15	Me	=O	=O	H	6.7	69%	NT
16	nPr	H,H	=O	H	6.9	74%	6.2
17	iPr	H,H	=O	H	7.4	87%	6.4
18	nBu	H,H	=O	H	6.7	90%	5.7
19	nHex	H,H	=O	H	<6	-	<5.2
20	Et	H,H	H,H	H	6.6	43%	6.2

[a] functional cAMP accumulation assay, values are a mean of at least 2 experiments; [b] intrinsic activity compared to the full agonist PGE_2; [c] radioligand binding assay, values are a mean of at least 2 experiments; NT not tested

Selected compounds were assessed in human recombinant CYP450 assays and it was found the analogues had low potential for CYP450 inhibition with inhibitory activities (IC_{50}) typically in excess of 10 µM (e.g. Compound **13** CYP1A2 IC_{50} 65µM, CYP2C19 IC_{50} >100µM, CYP2C9 IC_{50} >100µM, CYP2D6 IC_{50} 42µM, CYP3A4 (DEF) IC_{50} >100µM, CYP3A4 (7BQ) IC_{50} >100µM). Furthermore all compounds tested had low intrinsic clearance in rat and human microsomes (<0.5mL/min/g tissue). The rat in-vivo pharmacokinetics were assessed for selected analogues by intravenous administration and are summarised in **Table 2**.

Table 2 – Rat *iv* pharmacokinetics (1mg/kg) for selected analogues

Compound	CLb (mL/min/kg)	Vss (L/kg)	$t_{1/2}$ (h)
CCI17464, **2**	0.6[#]	0.30	8.4
13	0.2*	0.28	19.9*

[#]dosed orally in 10% DMSO/water with pH adjustment to aid solubility; dosed orally in 5% (w/v) glucose/2% DMSO (v/v)/water

Whereas compounds **2** and **13** had low clearance in rat, the imide **13** showed considerably greater stability in-vivo and hence had a much extended half-life. This was a common feature of the imides within the series and few were progressed further due to their long half lives. CCI17464 **2** was progressed to the Freunds complete adjuvant (FCA) acute rat model of inflammatory pain and shows full reversal of the hypersensitivity,[25] with an ED_{50} of 1.5mg/kg (corresponding to a total blood concentration of ~5-11uM), and we were keen to identify compounds with greater potency in this model. Importantly, the anti-hyperalgesic effect of CCI17464 **2** is completely reversed when pre-challenged with the selective EP_4 antagonist GW627368X **5**, confirming the anti-hyperalgesic effect is driven by the selective EP_4 agonist (**Figure 3**).

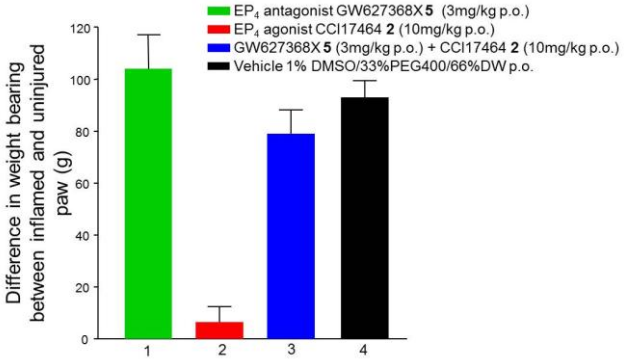
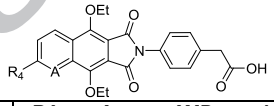


Figure 3 – The effect of selective EP_4 antagonist GW627368X **5** (3mg/kg p.o.; green bar 1); selective EP_4 agonist CCI17464 **2** (10mg/kg p.o.; red bar 2); selective EP_4 agonist CCI17464 **2** (10mg/kg) pre-challenged with selective EP_4 antagonist GW627368X **5** (3mg/kg) (blue bar 3) on FCA-induced hypersensitivity (weight bearing) versus vehicle (1% DMSO/33% PEG400/66% distilled water p.o.; black bar 4).

Introduction of substitution onto the benzene ring of the phthalimide was achieved by using the appropriately substituted diethyl phthalate in the condensation with diethyl succinate. The fluoro or methyl analogues (**Table 3**, compounds **21** and **22** respectively) showed good agonist potency. Aza-analogue **23** showed no EP_4 agonist effects, further emphasising the sensitivity within the template for EP_4 agonism. Full agonist imide **22**, demonstrated a long half-life in rat ($t_{1/2}$ = 31.7h) (as observed with other imides e.g. **13**) and was progressed to the FCA model, however similar potency versus CCI17464 was observed (76% reversal of FCA induced hypersensitivity @10mg/kg), demonstrating there was no apparent advantage for a compound with a full agonist profile.

Table 3 – SAR of Phthalimide Ring.



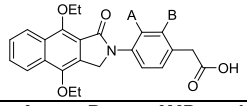
Compound	R4	A	cAMP pEC_{50} [a]	IA[b]	pKi[c]
21	F	C	7.1	72%	NT
22	Me	C	7.7	112%	6.8
23	H	N	<6	-	7.5

[a] functional cAMP accumulation assay, values are a mean of at least 2 experiments; [b] intrinsic activity compared to the full agonist PGE_2; [c] radioligand binding assay, values are a mean of at least 2 experiments; NT not tested

It is interesting to note that in certain compounds that lost EP_4 agonist activity, binding affinity was sometimes maintained (e.g. compounds **14** and **23** display pKi's of 6.7 and 7.5 in a radioligand binding assay with ^{3}H[PGE2]) demonstrating the sensitive structural requirements for agonist activity.

We next turned our attention to the phenylacetic acid ring. Substituted phenyl acetic acids were condensed with the bis-carboxylic acid **9**. **Table 4** summarises the SAR. Substituents ortho to the lactam nitrogen generally gave an increase in the in-vitro potency (Compounds **24**-**27** and **29**). In contrast, only a F substituent was tolerated in the ortho position to the acetic acid (see compounds **30**-**32**).

Table 4 – Further Modifications to the Phenyl Ring



Compound	A	B	cAMP pEC_{50} [a]	IA[b]	FCA[c] reversal
24	F	H	8.4	72%	5% @ 3mg/kg
25	Cl	H	8.3	73%	14% @ 3mg/kg
26	Br	H	8.3	91%	47% @ 10mg/kg
27	Me	H	8.1	78%	19% @ 3mg/kg
28	CF_3	H	7.3	73%	NT
29	MeO	H	8.0	68%	NT
30	H	F	7.4	67%	80% @ 3mg/kg
31	H	Cl	<6	-	NT

ACCEPTED MANUSCRIPT

32	H	Me	<6	-	NT

[a] functional cAMP accumulation assay, values are a mean of at least 2 experiments; [b] intrinsic activity compared to the full agonist PGE_2; [c] % reversal of FCA induced hypersensitivity as measured via weight bearing 1hr post dose. Compounds dosed orally in 1% methyl cellulose. NT not tested.

Selected compounds were tested in the FCA model to determine their in-vivo potencies. Surprisingly, the compounds which had increased in-vitro potency (Compounds **24**-**27**) showed a poor response in-vivo, whereas compound **30** showed an excellent reversal of the FCA induced hypersensitivity at 3mg/kg. Compound **30**, GSK726701A was further investigated in this model and was shown to have an ED_{50} of 0.2mg/kg (corresponding to a total blood concentration at this dose of 0.6uM), which compares well to the gold standard COX-2 inhibitor celecoxib (full reversal of FCA induced hypersensitivity at 10mg/kg). This represents >10 fold increase in potency of the lead compound CCI17474, **2**.

GSK726701A has high selectivity (>100-fold) against a set of other prostaglandin receptors ($EP_{1\text{-}3}$, DP_1, FP, IP, TP see **Table 5**) and no significant activity against a wider panel of targets. It demonstrates EP_4 agonist activity with similar potency and intrinsic activity (pEC_{50} 8.2 (I.A. 68 %)) in a human whole blood (HWB) assay on the inhibition of LPS-mediated TNFαinduction (**Figure 4**), supporting the potent anti-inflammatory potential of EP_4 agonists.[14,15]

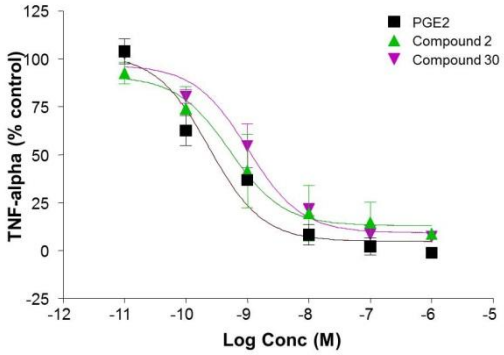


Figure 4 – The effect of PGE_2 (black), compound **2** (green) and compound **30** (purple) on the inhibition of LPS-mediated TNF-alpha induction in human whole blood.

GSK726701A has low human CYP inhibition potential and low intrinsic clearance across species. In addition, it displays good in-vivo pharmacokinetics with low blood clearance and good oral bioavailability in rat (CLb 1 mL/min/kg, $t_{1/2}$ 6 h, Fpo 112 %), dog (CLb 4 mL/min/kg, $t_{1/2}$ 8 h, Fpo 69 %) and cynomolgus monkey (CLb 4 mL/min/kg, $t_{1/2}$ 5-10 h, Fpo 50 %). GSK726701 has high plasma protein binding across species (rat 99.8%, dog 99.1%, human 99.9%) and is non-CNS penetrant (rat brain:blood ratio <0.05:1).

In addition to the excellent profile of GSK726701A in the FCA acute rat model of inflammatory pain, when dosed twice daily for 5 days in a chronic rat model of joint pain, it demonstrates potent activity with an ED_{50} of 0.2mg/kg and full reversal at 1mg/kg which is equivalent to the clinical gold standard celecoxib (30mg/kg) (**Figure 5**).[26]

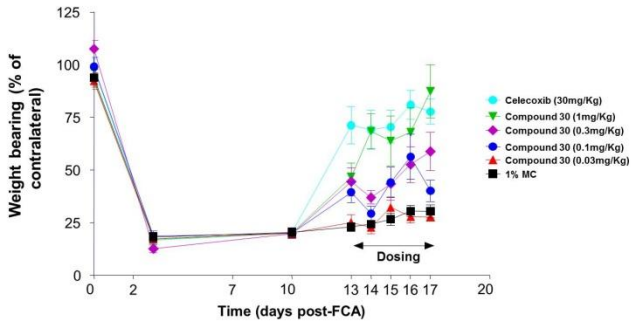


Figure 5 – The effect of compound **30** (0.03-1mg/kg p.o. b.i.d. x5 days) on FCA-induced chronic joint hypersensitivity (weight bearing) versus vehicle (methylcellulose) and positive control (celecoxib, 30mg/kg p.o.).

GSK726701A was also examined in the chronic constriction injury (CCI) model of neuropathic pain[27] where it was dosed twice daily for 8 days and demonstrated a time-dependant, full reversal of CCI-induced mechanical allodynia at 3mg/kg, equivalent to the clinical gold standard gabapentin (30mg/kg).

Table 5 – Profile of GSK726701A

EP_4 pEC_{50} cAMP acc	7.4 (67%)
EP_4 pEC_{50} HWB	8.2 (68%)
EP_1 pIC_{50}	<5
EP_2 agonism	14% activation @ 1uM
EP_3 pIC_{50}	<5
DP_1 pIC_{50}	<4.5
FP pIC_{50}	<5
IP agonism	11% activation at 1uM
IP antagonism	31% inhibition at 1uM
TP pIC_{50}	<5
S9 stability (r, d, h)	<1ml/min/g liver
P450 inhibition	>100 μM (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (DEF), CYP3A4(7BQ))
CLb (r,d,c)	1, 4, 4mL/min/kg
$t_{1/2}$(r,d,c)	6, 8, 5-10 h
FCA reversal of hypersensitivity ED_{50}	0.22mg/kg
Joint Pain reversal of hypersensitivity ED_{50}	0.23mg/kg
CCI reversal of mechanical allodynia	Full reversal @ 3mg/kg

In summary starting from the screening hit CCI17464 **2**, the discovery of a highly selective and potent EP_4 partial agonist **30**, {4-[4,9-bis(ethyloxy)-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl]-2-fluorophenyl}acetic acid (GSK726701A), has been described which demonstrates robust activity in a range of pre-clinical models of pain. The data shows this mechanism has the potential to offer an alternative therapeutic approach in the treatment of pain and inflammatory conditions and supported the selection of GSK726701A for further investigation.

Acknowledgements
We would like to acknowledge Ann Walker, Sue Roomans, Andy Mason and V Weller for the first synthesis of selected compounds in Table 1, Anita Chowdhury and Davina Mitchell for generation of EP_1, EP_3, and FP selectivity data, and Simon Peace for assistance in manuscript preparation.

References and notes

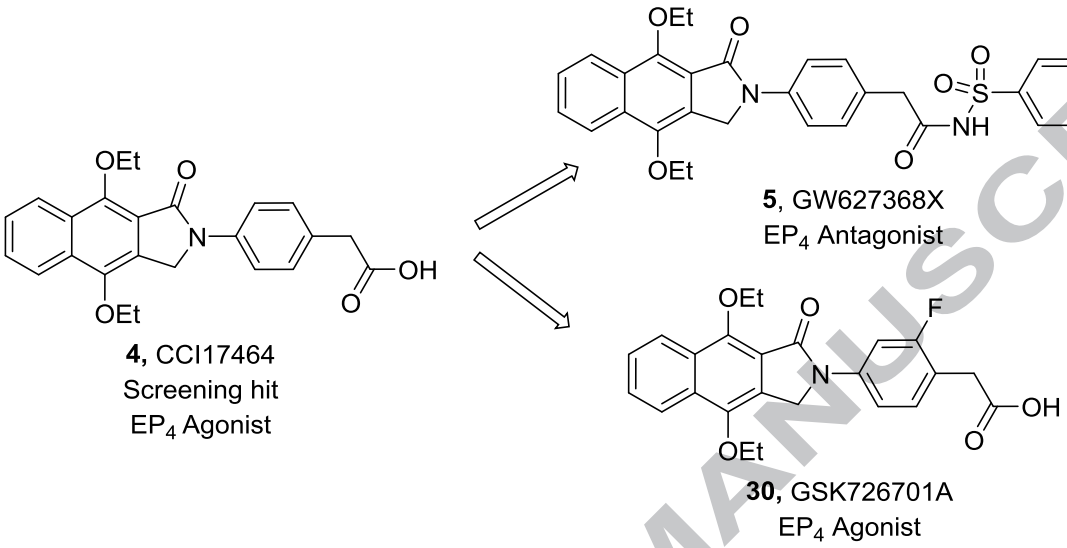
1. Furuyashiki, T.; Narumiya, S. *Curr. Opin. Pharmacol.* ***2009****, 9(1), 31.*
2. Hikiji, H; Takato, T.; Shimizu, T.; Ishii, S. *Prog. Lipid Res.* ***2008****, 47(2), 107.*
3. Dey, I.; Lejeune, M.; Chadee, K. *Br. J. Pharmacol.* ***2006****, 149(6), 611.*
4. Hao, C.; Breyer, M. D. *Annu. Rev. Physiol.* ***2008****, 70*

ACCEPTED MANUSCRIPT

357.
5. (a) Greenhough, A.; Smartt, H. J. M.; Moore, A. E.; Roberts, H. R.; Williams, A. C.; Paraskeva, C.; Kaidi, A. *Carcinogenesis* ***2009***, *30(3), 377*; (b) Parida, S.; Parekh, A.; Dey, G.; Ghosh, S. C.; Mandal, M. *Cancer Biol. Ther.* ***2015*** *16(6) 922.*
6. Matsuoka, T.; Narumiya, S. *Inflammation Regener.* ***2008****, 28(5), 423.*
7. Flowers, R. J. *Nat. Rev. Drug Discovery* ***2003****, 2, 179.*
8. FitzGerald G. A. *Nat. Rev. Drug Discovery* ***2003****, 879.*
9. Sugimoto, Y.; Narumiya, *J. Biol. Chem.* ***2007****, 282(16), 11613.*
10. Calder, P. C. *Biochimie* ***2009****, 91(6), 791.*
11. Mathieu, M.; Lord-Dufour, S.; Bernier, V.; Boie, Y.; Burch, J.D.; Clark, P.; Denis, D.; Han, Y.; Mortimer, J.R.; Therien, A.G. *Eur. J. Immunol.* ***2008****, 38(7), 1900.*
12. McCoy, J.M.; Wicks, J.R.; Audoly, L.P. *J. Clin. Invest.* ***2002****, 110(5), 651.*
13. (a) Clark, P.; Rowland, S.E.; Denis, D.; Mathieu, M.C.; Stocco, R.; Poirier, H.; Burch, J.; Han, Y.; Audoly, L.; Therien, A.G.; Xu, D. *J. Pharmacol. Exp. Ther.* ***2008****, 325(2), 425*; (b) Colucci, J.; Boyd, M.; Berthelette, C.; Chiasson, J.F.; Wang, Z.; Ducharme, Y.; Friesen, R.; Wrona, M.; Levesque, J.F.; Denis, D.; *Bioorg. Med. Chem. Lett.* ***2010****, 20(12), 3760*; (c) Blanco, M.; Vetman, T.; Chandrasekhar, S.; Fisher, M.J.; Harvey, A.; Kuklish, S.L.; Chambers, M.; Lin, C.; Mudra, D.; Oskins, J.; Wang, X.; Yu, X.; Warshawsky, A.M. *Bioorg. Med. Chem. Lett.* ***2016****, 26, 2303.*
14. Kambe, T.; Maruyama, T.; Nakano, M.; Yamaura, Y.; Shono, T.; Seki, A.; Sakata, K.; Maruyama, T.; Nakai, H.; Toda, M.; *Chem. Pharm. Bull.* ***2011****, 59 (12), 1523*.
15. Omote, K.; Kawamata, T.; Nakayama, Y.; Yamamoto, H.; Kawamata, M.; Namiki, A *Anesthesiology* ***2002****, 97(1), 170.*
16. Nakase, H.; Fujiyama, Y.; Oshitani, N.; Oga, T.; Nonomura, K.; Matsuoka, T.; Esaki, Y.; Murayama, T.; Teramukai, S.; Chiba, T.; Narumiya, S. *Inflammatory Bowel Dis.* ***2010****, 16(5), 731-3.*
17. (a) Clayton, N.M.; Collins, S.D.; Foord, S.M.; Giblin, G.M.P. WO2001010426A2; (b) Giblin, G.M.P.; Wilson, R.; Foord, S.; Swarbrick, M.; Walker, A.; Bamford, M.; Roomans, S.; Mason, A.; Miller, N.; Jones, H.; Shanahan, S.; Rasmussen, S.; Smith, L.; Spalding, D.; Ranshaw, L.; Fenwick, R.; Ancliff, R.; Saez, V.; Frye, S.; Stratton, S.; Lewell, X.; Green, R. *Abstracts of Papers*, 224th National Meeting of the American Chemical Society, Boston, MA, Aug 18-22, 2002.
18. (a) Giblin, G.M.P.; Frye, S.V.; Roomans, S.; WO2002050033A1; (b) Wilson, R.J.; Giblin, G.M.P.; Roomans, S.; Rhodes, S.A.; Cartwright, K.A.; Shield, V.J.; Brown, J.; Wise, A.; Chowdhury, J.; Pritchard, S.; Coote, J.; Noel, L.S.; Kenakin, T.; Burns-Kurtis, C.L.; Morrison, V.; Gray, D.W.; Giles, H. *Br. J. Pharmacol.* ***2006****, 148(3), 326.*
19. Nakao, K.; Murase, A.; Ohshiro, H.; Okumura, T.; Taniguchi, K.; Murata, Y.; Masuda, M.; Kato, T.; Okumura, Y.; Takada, J. *J. Pharmacol. Exp. Ther.* ***2007****, 322(2), 686.*
20. AskAt Inc. unpublished data "AAT-007 is a novel prostaglandin EP4 antagonist that has exhibited robust pre-clinical and clinical analgesic efficacy in chronic inflammatory pain. In a 4-week Phase-2 osteoarthritis pain study, AAT-007 75 mg BID exhibited equivalent efficacy to Naproxen 500 mg BID, with a significant GI safety advantage." http://askat-inc.com/news/?article=2016 Accessed 1 January 2018.
21. Rausch-Derra, L.; Huebner, M.; Wofford, J.; Rhodes, L. *J. Vet. Intern. Med.* ***2016****, 30, 756.*
22. Clark, R. B.; Knoll. B. J.; Barber, R.; *Trends in Pharmacol. Sci.* ***1999****, 20(7), 279-286.*
23. Hughes, I.; Nolan, W.P.; Raphael, R.A.J.; *Chem. Soc. Perkin Trans. 1* ***1990****, 9, 2475.*
24. Ungrin, M. D.; Carrière, M. C.; Denis, D.; Lamontagne, S.; Sawyer, N.; Stocco, R.; Tremblay, N.; Metters, K. M.; Abramovitz, M. *Mol. Pharmacol.* ***2001****, 59, 1446* and ref therein.
25. (a) Iadarola, M.J.; Douglass, J.; Civelli, O.; Naranjo, J.R. *Brain Res.* ***1988****, 455, 205*; (b) Hay, C.H.; Trevethick, M.A.; Wheeldon, A.; Browers, J.S.; De Belleroche, J.S. *Neuroscience* ***1997****, 78, 843*.
26. 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.*
27. Woodhams, P. L.; MacDonald, R.E.; Collins, S.D.; Chessell, I.P.; Day, N. C. *Eur. J. Pain* ***2007****, 11(6), 605.*

ACCEPTED MANUSCRIPT

Graphical abstract:



Abstract— A novel series of EP_4 agonists and antagonists have been identified, and then used to validate their potential in the treatment of inflammatory pain. This paper describes these novel ligands and their activity within a number of pre-clinical models of pain, ultimately leading to the identification of the EP_4 partial agonist GSK726701A.

Keywords: EP_4 receptor agonist, EP_4 agonist, Prostaglandin E2, PGE2, Prostaglandin E2 receptor 4, EP_4, pain, arthritis, inflammation, anti-inflammatory, anti-hyperalgesic, neuropathic pain.

∗ Corresponding author present address: Novartis Institutes for BioMedical Research, 22 Windsor St, Cambridge, MA, 02139, USA. e-mail: mark.healy@novartis.com (M.P.Healy).

ACCEPTED MANUSCRIPT

- A novel series of selective and potent EP4 receptor partial agonists identified
- GSK726701A has good PK in rat, dog and monkey
- Robust activity in a range of animal models of inflammatory and neuropathic pain

ACCEPTED MANUSCRIPT