ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

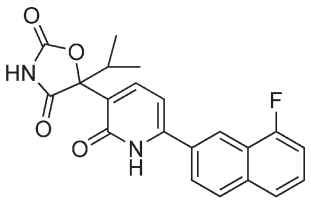
Novel 3-Oxazolidinedione-6-aryl-pyridinones as Potent, Selective, and Orally Active EP_3 Receptor Antagonists

Jian Jin,*,† Ángel Morales-Ramos, Patrick Eidam, John Mecom, Yue Li, Carl Brooks, Mark Hilfiker, David Zhang, Ning Wang, Dongchuan Shi, Pei-San Tseng, Karen Wheless, Brian Budzik, Karen Evans, Jon-Paul Jaworski, Jack Jugus, Lisa Leon, Charlene Wu, Mark Pullen, Bhumika Karamshi, Parvathi Rao, Emma Ward, Nicholas Laping, Christopher Evans, Colin Leach, Dennis Holt, Xin Su, Dwight Morrow, Harvey Fries, Kevin Thorneloe, and Richard Edwards

GlaxoSmithKline, 709 Swedeland Road, King of Prussia, Pennsylvania 19406

ABSTRACT High-throughput screening and subsequent optimization led to the discovery of novel 3-oxazolidinedione-6-aryl-pyridinones exemplified by compound **2** as potent and selective EP_3 antagonists with excellent pharmacokinetic properties. Compound **2** was orally active and showed robust in vivo activities in overactive bladder models. To address potential bioactivation liabilities of compound **2**, further optimization resulted in compounds **9** and **10**, which maintained excellent potency, selectivity, and pharmacokinetic properties and showed no bioactivation liability in glutathione trapping studies. These highly potent, selective, and orally active EP_3 antagonists are excellent tool compounds for investigating and validating potential therapeutic benefits from selectively inhibiting the EP_3 receptor.

KEYWORDS EP_3 receptor, novel, potent, selective, and orally active antagonists, 3-oxazolidinedione-6-aryl-pyridinones



human EP_3: fpK_i = 8.5
rat EP_3: fpK_i = 8.5
rat PK: Cl = 2.8 mL/min/kg
$T_{1/2}$ = 3.9 h
F = 93%
No GSH conjugates detected

Prostanoids are members of a family of biologically active lipids derived from arachidonic acid that include prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, PGI_2, PGD_2, and thromboxane A_2. Each of the five prostanoids has a distinct receptor class, named as EP, FP, IP, DP, and TP. Through their respective receptors, prostanoids mediate a wide array of physiological and pathophysiological processes including inflammation, pain, smooth muscle function, platelet function, neuronal function, and kidney function.[1,2] Among the prostanoids, PGE_2 is the most widely produced and has a number of diverse actions.[1] Molecular cloning has revealed the existence of four different receptors for PGE_2, which accounts for the diverse effects of this prostanoid. These receptors are designated as EP_1, EP_2, EP_3, and EP_4 and are encoded by distinct genes.[3]

Overactive bladder (OAB) is characterized by symptoms of increased voiding frequency and urgency. Although the exact cause remains unknown, both bladder smooth muscle excitability and increased bladder sensory nerve sensitivity have been proposed.[4] Several lines of evidence support that EP_3 receptor antagonism could provide a new and effective treatment option for OAB. First, EP_3 receptor knockout (KO) mice demonstrated an enhanced bladder capacity as compared to wild-type (WT) controls. Infusion of PGE_2 or a selective EP_3 receptor agonist, GR63799, into WT mice induced bladder overactivity. These responses were absent in KO mice.[5] Second, PGE_2 is synthesized in the bladder in response to bladder distension, nerve stimulation, inflammatory mediators, and mechanical trauma.[6] Elevated urinary PGE_2 has been associated with OAB in humans.[7,8] Third, EP_3 receptors are present in dorsal root ganglia and enhance the excitability of dorsal root ganglia neurons.[9] Finally, a number of small clinical studies have shown that cyclooxygenase (COX) inhibitors that function to reduce production of PGE_2 improve OAB symptoms.[10]

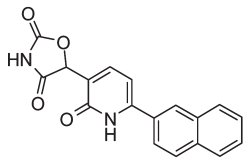
A number of small molecule EP_3 receptor antagonists have been reported recently.[11−16] In this letter, we disclose a novel series of 3-oxozolidinedione-6-aryl-pyridinones as potent, selective, and orally active EP_3 receptor antagonists, which demonstrated robust in vivo activities in several OAB animal models.

High-throughput screening (HTS) of the corporate compound collection using a human EP_3 fluorometric imaging plate reader (FLIPR) assay[17] led to the identification of 3-oxazolidinedione-6-aryl-pyridinone **1** as an EP_3 antagonist with a functional pK_i (fpK_i) of 6.7 (Figure 1).[18−20] The compound was subsequently tested in human EP_1 and rat EP_3 FLIPR assays and found to have good selectivity for EP_3 over EP_1 and good cross-species activity.[21] In a rat pharmacokinetic (PK) study,[22]

Received Date: April 15, 2010
Accepted Date: May 6, 2010
Published on Web Date: May 14, 2010

DOI: 10.1021/ml100077x | *ACS Med. Chem. Lett.* **2010**, *1*, 316–320

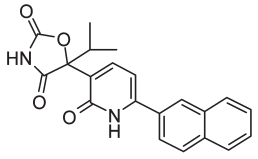
ACS Medicinal Chemistry Letters
pubs.acs.org/acsmedchemlett



1

Human EP_3 fpK_i	= 6.7
Human EP_1 fpK_i	< 5.3
Rat EP_3 fpK_i	= 6.5
Rat PK	
$T_{1/2}$ (h, iv)	= 5.1
Cl (mL/min/kg)	= 31
DNAUC (po) (μg•h/mL/mg/kg)	= 0.17
Oral F (%)	= 32

Figure 1. HTS hit **1**.



2

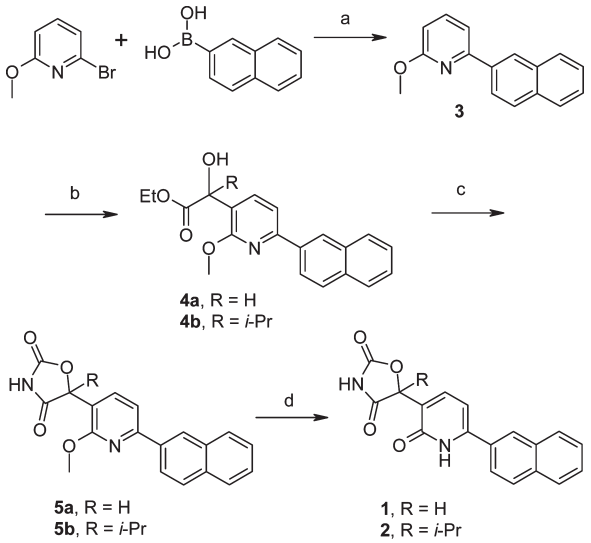
Potency	
hEP_3 FLIPR fpK_i	= 8.0
hEP_3 binding pK_i	= 8.3
rEP_3 FLIPR fpK_i	= 8.0
dEP_3 FLIPR fpK_i	= 7.7
Selectivity	
EP_1, EP_2, EP_4, FP, & DP pIC_{50}	< 4.3
COX1 & COX2 pIC_{50}	< 4.0
Rat PK & Developability	
$T_{1/2}$ (h, iv)	= 4.0
Cl (mL/min/kg)	= 2.6
DNAUC (po) (μg•h/mL/mg/kg)	= 5.5
Oral F (%)	= 83
CYP450 pIC_{50}	< 4.3
hERG binding pIC_{50}	< 4.3
Solubility (μM)	= 193

Figure 2. Profiles of pyridinone **2**.

pyridinone **1** had a moderate to high clearance, good half-life, low dose-normalized area under curve (DNAUC) via oral administration (po), and moderate oral bioavailability.

Optimization of this promising hit to improve potency and PK properties led to the discovery of compound **2**, which possesses an isopropyl group at the 5-position of the oxazolidinedione ring. Pyridinone **2** had excellent potency in human EP_3 FLIPR assay with an fpK_i of 8.0, which is about 20-fold more potent as compared to compound **1** (Figure 2). Compound **2** was also tested in a human EP_3 binding assay[23] and was found to have high binding affinity (pK_i = 8.3), consistent with its potency (fpK_i = 8.0) in the FLIPR assay. In addition, pyridinone **2** was very potent in rat (fpK_i = 8.0) and dog (fpK_i = 7.7) EP_3 FLIPR assays,[21] demonstrating excellent cross-species activities. Compound **2** had excellent selectivity for EP_3 over other EP and prostanoid receptors. It was inactive in human EP_1 FLIPR,[21] EP_2 LANCE,[21] EP_4 binding,[23] DP binding,[23] and FP FLIPR[21] assays. Because COX inhibitors function to reduce production of PGE_2, this promising lead was evaluated in human COX1 and COX2 enzyme inhibitory assays and rat COX1 and COX2 whole blood assays to assess its selectivity for EP_3 over COX1 and COX2. We were pleased to find that pyridinone **2** was inactive in the COX assays.[24] Pyridinone **2** was next evaluated in a rat PK study[22] and found to have a low clearance (Cl = 2.6 mL/min/kg), good half-life ($T_{1/2}$ = 4.0 h), and high oral exposure as demonstrated by excellent oral DNAUC (5.5 μg h/mL/mg/kg) and oral bioavailability (F = 83%). In addition to excellent PK parameters, **2** had good developability properties. For example, **2** was inactive against all five common cytochrome P450 (CYP450) isozymes (1A2, 2C19, 2C9, 2D6, and 3A4) and in the hERG binding assay and had good aqueous solubility (193 μM). The combination of high potency with excellent cross-species activities, selectivity, and rat PK properties makes pyridinone **2** an outstanding tool compound for in vivo studies.

Scheme 1. Synthesis of Pyridinones **1** and **2**[a]



[a] Reagents and conditions: (a) $PXPd_2$, K_2CO_3, MeOH, 60 °C, 89%. (b) (i) *t*-BuLi, THF, −78 °C; (ii) ethyl 3-methyl-2-oxobutyrate or ethyl 2-oxoacetate, THF, −78 °C to room temperature (**4a**, 30%; **4b**, 62%). (c) (i) Trichloroacetyl isocyanate, CH_2Cl_2, room temperature; (ii) 2 M K_2CO_3 aqueous solution, reflux (**5a**, 80%; **5b**, 48%). (d) TMSCl, NaI or TMSI, CH_3CN, room temperature (**1**, 49%; **2**, 58%).

The synthesis of pyridinones **1** and **2** is outlined in Scheme 1. Suzuki coupling of commercially available 2-bromo-6-methoxypyridine with 2-naphthyl boronic acid yielded methoxypyridine **3**, which was then deprotonated and added to α-keto esters to afford α-hydroxy esters **4a** and **4b**. Oxozolidinediones **5a** and **5b** were prepared from α-hydroxy esters **4a** and **4b** via a one-pot two-step sequence using trichloroacetyl isocyanate.[25] Demethylation of methoxypyridines **5a** and **5b** resulted in the desired pyridinones **1** and **2** in good overall yields.

Pyridinone **2** was first evaluated in a GR63799-induced OAB model in conscious, spontaneously hypertensive rats (SHR).[26] As shown in Figure 3, intraduodenal (id) pretreatment with **2** at doses of 3 and 30 mg/kg (p = 0.04 and p = 0.01 vs GR63799 + vehicle, two-way RM ANOVA) significantly inhibited the GR63799-induced decrease in bladder capacity (as measured by average voided volume), while 0.3 mg/kg of **2** was found to be ineffective. Compound **2** was next tested in a PGE_2-induced OAB model in the conscious SHR.[5] Intravesical infusion of PGE_2 (120 μM) led to reduced bladder capacity (Figure 4). Pretreatment with **2** (30 mg/kg, id) (p = 0.006 vs PGE_2 + vehicle, two-way RM ANOVA) significantly inhibited the bladder capacity decrease caused by the PGE_2 infusion. Compound **2** was also evaluated in an acetic acid-induced OAB model in conscious SHR.[5] Similar to PGE_2, intravesical infusion of acetic acid (0.25%) also resulted in a bladder capacity decrease (Figure 5).[5] This bladder capacity decrease was significantly inhibited by id

DOI: 10.1021/ml100077x | ACS Med. Chem. Lett. 2010, 1, 316–320

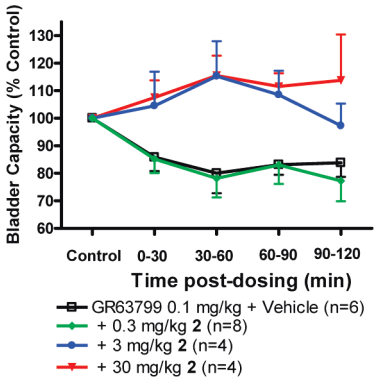


Figure 3. Effect of id administration of **2** on the GR63799-induced decrease in conscious SHR bladder capacity.

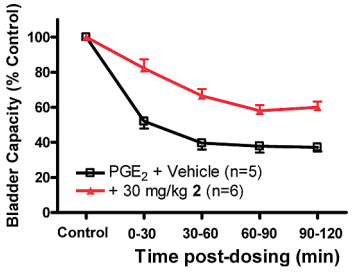


Figure 4. Effect of id administration of **2** on the PGE_2-induced decrease in conscious SHR bladder capacity.

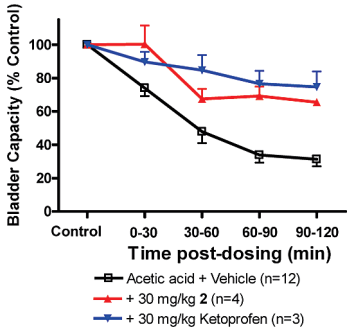


Figure 5. Effect of id administration of **2** on the acetic acid-induced decrease in conscious SHR bladder capacity. Comparison to the COX inhibitor ketoprofen.

pretreatment of **2** at 30 mg/kg ($p = 0.002$ vs acetic acid + vehicle, two-way RM ANOVA), an effect similar to that observed with the COX inhibitor ketoprofen.

In addition, compound **2** was evaluated in a bladder rhythmic contraction model in anesthetized Sprague−Dawley (SD) rats.[23] Intravenous (iv) administration of compound **2** at 3 and 10 mg/kg ($p < 0.05$ vs vehicle for both doses, two-way ANOVA) significantly and dose dependently inhibited the bladder rhythmic contraction induced by intravesical infusion of saline (Figure 6). The robust in vivo activities in several OAB models indicate that this novel and selective EP_3 receptor antagonist series could potentially lead to effective therapeutic agents for treating OAB.

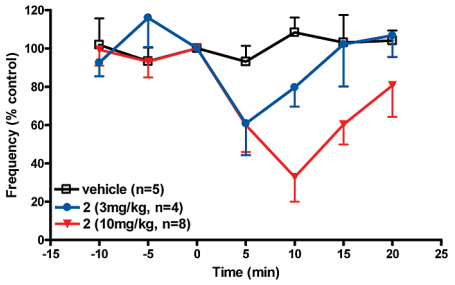


Figure 6. Effect of iv administration of **2** on bladder rhythmic contraction in anesthetized SD rats.

However, compound **2** may have potential bioactivation liabilities as evidenced by the findings in glutathione (GSH) trapping studies: NADPH-dependent GSH conjugates were observed when rat liver S9 fractions were used, while no GSH conjugates were observed when using human liver S9 fractions (Table 1).[21] It was hypothesized that the unsubstituted naphthyl moiety of compound **2** could be oxidized to form reactive metabolites. To overcome the potential bioactivation liabilities, compounds **8**−**10** were prepared using a new synthetic strategy that introduces the right-hand side aryl group closer to the end of the reaction sequence (Scheme 2). Commercially available 2-chloro-6-methoxypyridine was converted to α-hydroxy ester **6**, which was subsequently converted to oxozolidinedione **7** using chemistry similar to that outlined in Scheme 1. Suzuki coupling of chloropyridine **7** with commercially available (1-methyl-1*H*-indol-5-yl)boronic acid, 3,4-dimethylphenyl boronic acid, or boronic acid **12**, followed by deprotection, afforded the desired pyridinones **8**, **9**, and **10**. Boronic acid **12** was prepared from 1-fluoro-naphth-7-ol **11**,[27] which was synthesized from commercially available 8-amino- naphth-2-ol in three steps.

Compounds **8**−**10** had excellent potency in human and rat EP_3 FLIPR assays[21] (Table 1). In particular, 8-fluoronaphthyl analog **10** was slightly more potent than unsubstituted naphthyl compound **2**. In rat PK studies,[22] compounds **8**−**10** displayed excellent PK parameters−low clearance, good half-life, and high oral exposure as demonstrated by excellent oral DNAUC and oral bioavailability, similar to compound **2**. In addition, compounds **8**−**10** were inactive in EP_1, EP_2, EP_4, DP, TP, FP, COX1, and COX2 assays−demonstrating excellent selectivity for EP_3 over other EP and prostanoid receptors. In GSH trapping studies,[21] no NADPH-dependent GSH conjugates were detected for compounds **9** and **10** when using either rat or human liver S9 fractions. No GSH conjugates were detected in the absence of NADPH as well. Compound **8** was not metabolized by either rat or human liver S9 fractions in the same experiment. These findings indicated that the unsubstituted naphthyl moiety of compound **2** was indeed responsible for the potential bioactivation liabilities, and such potential liabilities could be mitigated by replacing the unsubstituted naphthyl group with other aryl groups (**8** and **9**) or a properly substituted naphthyl group (**10**).

In conclusion, a novel series of 3-oxozolidinedione-6-aryl-pyridinones exemplified by compound **2** was discovered as potent and selective EP_3 receptor antagonists with excellent

DOI: 10.1021/ml100077x | *ACS Med. Chem. Lett.* **2010**, *1*, 316–320

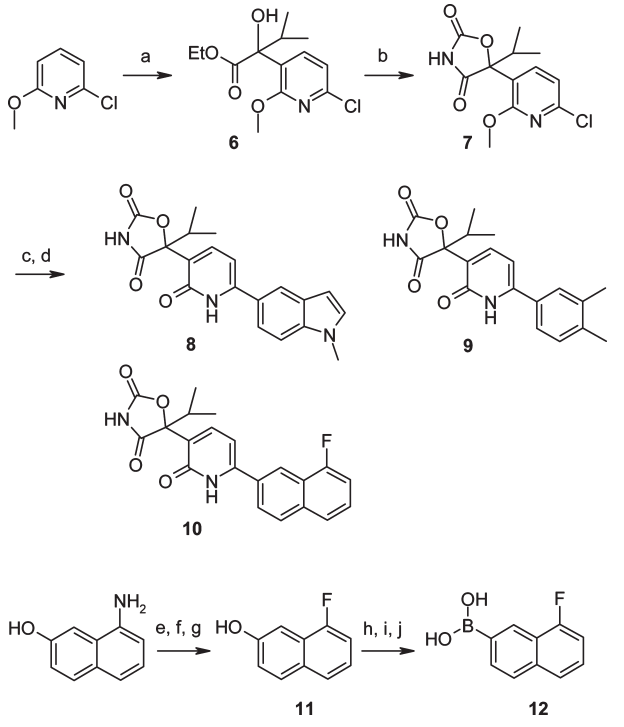
ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

Table 1. Profile of 3-Oxazolidinedione-6-aryl-pyridinones **8**−**10**

	EP_3 FLIPR fpK_i		rat PK parameters				NADPH-dependent GSH conjugates	
compound	human	rat	$T_{1/2}$ (h, iv)	Cl (mL/min/kg)	DNAUC (po) (μg h/mL/mg/kg)	oral F (%)	human liver S9 fractions	rat liver S9 fractions
1	6.7	6.5	5.1	31	0.17	32	not tested	not tested
2	8.0	8.0	4.0	2.6	5.5	83	not detected	detected
8	8.2	8.1	4.2	4.5	3.0	80	no compound turnover	
9	7.7	7.9	2.8	6.3	1.8	68	not detected	not detected
10	8.5	8.5	3.9	2.8	5.6	93	not detected	not detected

Scheme 2. Synthesis of Pyridinones **8**−**10**[a]



[a] Conditions and reagents: (a) (i) *t*-BuLi, THF, −78 °C; (ii) ethyl 3-methyl-2-oxobutyrate, THF, −78 °C to room temperature, 73%. (b) (i) Trichloroacetyl isocyanate, CH_2Cl_2, room temperature; (ii) 2 M K_2CO_3 aqueous solution, reflux, 94%. (c) Corresponding boronic acids, Suzuki coupling conditions (see the Supporting Information). (d) TMSCl, NaI, or TMSI, CH_3CN, room temperature (**8**, 27%; **9**, 48%; and **10**, 64%). (e) NaH, MeI, DMF, 0 °C to room temperature, 90%. (f) $NaNO_2$, HCl, HBF_4, H_2O, 51%. (g) BBr_3, CH_2Cl_2, 0 °C to room temperature, 100%. (h) Tf_2O, K_3PO_4, toluene, H_2O, 0 °C to room temperature, 91%. (i) $PdCl_2$(dppf), dppf, KOAc, bis-(pinacolato)diborane, dioxane, 80 °C, 70%. (j) $NaIO_4$, HCl, THF, H_2O, 77%.

rat PK parameters and broad cross-species activity. Optimization of this series resulted in compounds **8**−**10** that maintained excellent potency, selectivity, and PK properties and mitigated potential bioactivation liabilities. As illustrated by robust in vivo activities of **2** in several OAB animal models, these novel compounds are potentially useful therapeutic agents for treating OAB. In addition, these highly potent, selective, and orally bioavailable compounds are valuable tools for investigating and validating other potential therapeutic benefits resulting from selective EP_3 inhibition.

SUPPORTING INFORMATION AVAILABLE

Synthetic procedures and characterization data for all compounds; procedures for human, rat, and dog EP_3, EP_1, EP_2, and FP assays; and GSH trapping studies. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *To whom correspondence should be addressed. Tel: 919-843-8459. Fax: 919-843-8465. E-mail: jianjin@unc.edu.

Present Addresses: †Center for Integrative Chemical Biology and Drug Discovery, Division of Medicinal Chemistry & Natural Products, Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.

ACKNOWLEDGMENT

We thank Minghui Wang for NMR, Bill Leavens for HR-MS, and Jeff Guss, Mike Fisher, Kalindi Vaidya, Elizabeth Davenport, Jim Fornwald, and Evanson Agyeman for assay support.

ABBREVIATIONS

PGE_2, prostaglandin E_2; OAB, overactive bladder; KO, knockout; WT, wild-type; COX, cyclooxygenase; HTS, high-throughput screening; FLIPR, fluorometric imaging plate reader; fpK_i, functional pK_i; PK, pharmacokinetic; DNAUC, dose-normalized area under curve; CYP450, cytochrome P450; SHR, spontaneously hypertensive rats; GSH, glutathione.

REFERENCES

(1) Narumiya, S.; Sugimoto, Y.; Ushikubi, F. Prostanoid receptors: Structures, properties, and functions. *Physiol. Rev.* **1999,** *79*, 1193–1226.

(2) Breyer, R. M.; Bagdassarian, C. K.; Myers, S. A.; Breyer, M. D. Prostanoid receptors: Subtypes and signaling. *Annu. Rev. Pharmacol. Toxicol.* **2001,** *41*, 661–690.

(3) Sugimoto, Y.; Narumiya, S. Prostaglandin E receptors. *J. Biol. Chem.* **2007,** *282*, 11613–11617.

(4) Steers, W. D. Pathophysiology of overactive bladder and urge urinary incontinence. *Rev. Urol.* **2002,** *4* (Suppl. 4), S7–S18.

(5) McCafferty, G. P.; Misajet, B. A.; Laping, N. J.; Edwards, R. M.; Thorneloe, K. S. Enhanced bladder capacity and reduced prostaglandin E2-mediated bladder hyperactivity in EP3 receptor knockout mice. *Am. J. Physiol. Renal Physiol.* **2008,** *295*, F507–F514.

(6) Maggi, C. A. Prostanoids as local modulators of reflex micturition. *Pharmacol. Res.* **1992,** *25*, 13–20.

DOI: 10.1021/ml100077x | *ACS Med. Chem. Lett.* **2010,** *1*, 316–320

(7) Kim, J. C.; Park, E. Y.; Hong, S. H.; Seo, S. I.; Park, Y. H.; Hwang, T. K. Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. *Int. J. Urol.* **2005,** *12*, 875–880.
(8) Kim, J. C.; Park, E. Y.; Seo, S. I.; Park, Y. H.; Hwang, T. K. Nerve growth factor and prostaglandins in the urine of female patients with overactive bladder. *J. Urol.* **2006,** *175*, 1773–1776.
(9) Wang, C.; Li, G. W.; Huang, L. Y. Prostaglandin E2 potentiation of P2 × 3 receptor mediated currents in dorsal root ganglion neurons. *Mol. Pain* **2007,** *3*, 22.
(10) Wibberley, A. Overactive bladder: Targeting prostaglandins in sensory pathways. *Drug Discovery Today: Ther. Strategies* **2005,** *2*, 7–13.
(11) Juteau, H.; Gareau, Y.; Labelle, M.; Sturino, C. F.; Sawyer, N.; Tremblay, N.; Lamontagne, S.; Carriere, M. C.; Denis, D.; Metters, K. M. Structure-activity relationship of cinnamic acylsulfonamide analogues on the human EP3 prostanoid receptor. *Bioorg. Med. Chem.* **2001,** *9*, 1977–1984.
(12) Gallant, M.; Carriere, M. C.; Chateauneuf, A.; Denis, D.; Gareau, Y.; Godbout, C.; Greig, G.; Juteau, H.; Lachance, N.; Lacombe, P.; Lamontagne, S.; Metters, K. M.; Rochette, C.; Ruel, R.; Slipetz, D.; Sawyer, N.; Tremblay, N.; Labelle, M. Structure-activity relationship of biaryl acylsulfonamide analogues on the human EP(3) prostanoid receptor. *Bioorg. Med. Chem. Lett.* **2002,** *12*, 2583–2586.
(13) Belley, M.; Chan, C. C.; Gareau, Y.; Gallant, M.; Juteau, H.; Houde, K.; Lachance, N.; Labelle, M.; Sawyer, N.; Tremblay, N.; Lamontagne, S.; Carriere, M. C.; Denis, D.; Greig, G. M.; Slipetz, D.; Gordon, R.; Chauret, N.; Li, C.; Zamboni, R. J.; Metters, K. M. Comparison between two classes of selective EP(3) antagonists and their biological activities. *Bioorg. Med. Chem. Lett.* **2006,** *16*, 5639–5642.
(14) Zhou, N.; Zeller, W.; Krohn, M.; Anderson, H.; Zhang, J.; Onua, E.; Kiselyov, A. S.; Ramirez, J.; Halldorsdottir, G.; Andresson, T.; Gurney, M. E.; Singh, J. 3,4-Disubstituted indole acylsulfonamides: A novel series of potent and selective human EP3 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 123–126.
(15) Zhou, N.; Zeller, W.; Zhang, J.; Onua, E.; Kiselyov, A. S.; Ramirez, J.; Palsdottir, G.; Halldorsdottir, G.; Andresson, T.; Gurney, M. E.; Singh, J. 3-Acrylamide-4-aryloxyindoles: Synthesis, biological evaluation and metabolic stability of potent and selective EP(3) receptor antagonists. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 1528–1531.
(16) Singh, J.; Zeller, W.; Zhou, N.; Hategen, G.; Mishra, R.; Polozov, A.; Yu, P.; Onua, E.; Zhang, J.; Zembower, D.; Kiselyov, A.; Ramirez, J. L.; Sigthorsson, G.; Bjornsson, J. M.; Thorsteinsdottir, M.; Andresson, T.; Bjarnadottir, M.; Magnusson, O.; Fabre, J. E.; Stefansson, K.; Gurney, M. E. Antagonists of the EP3 receptor for prostaglandin E2 are novel antiplatelet agents that do not prolong bleeding. *ACS Chem. Biol.* **2009,** *4*, 115–126.
(17) Measuring inhibition of PGE_2-induced $[Ca^{2+}]_i$ mobilization in U2OS (human osteosarcoma) cells transfected with a vector carrying the full length of human EP_3 cDNA. For assay details, see the Supporting Information.
(18) fpK_i calculations are detailed in the Supporting Information.
(19) The biological assay results in this paper are a mean of at least two determinations with a standard deviation of $< \pm 0.3$.
(20) Compounds reported in this paper are all racemic.
(21) For assay details, see the Supporting Information.
(22) PK parameters are averaged values from studies using Sprague−Dawley rats ($n = 3$) dosed at 1 mg/kg (iv) and 2 mg/kg (po).
(23) Su, X.; Leon, L. A.; Wu, C. W.; Morrow, D. M.; Jaworski, J. P.; Hieble, J. P.; Lashinger, E. S.; Jin, J.; Edwards, R. M.; Laping, N. J. Modulation of bladder function by prostaglandin EP3 receptors in the central nervous system. *Am. J. Physiol. Renal Physiol.* **2008,** *295*, F984–F994.
(24) Wibberley, A.; McCafferty, G. P.; Evans, C.; Edwards, R. M.; Hieble, J. P. Dual, but not selective, COX-1 and COX-2 inhibitors, attenuate acetic acid-evoked bladder irritation in the anaesthetised female cat. *Br. J. Pharmacol.* **2006,** *148*, 154–161.
(25) Li, Y. H.; Zhang, L.; Tseng, P.-S.; Zhang, Y.; Jin, Y.; Shen, J.; Jin, J. A facile one-pot synthesis of 3-unsubstituted-2,4-oxazolidinediones via in situ generation of carbamates from [alpha]-hydroxyesters using trichloroacetyl isocyanate. *Tetrahedron Lett.* **2009,** *50*, 790–792.
(26) Jugus, M. J.; Jaworski, J. P.; Patra, P. B.; Jin, J.; Morrow, D. M.; Laping, N. J.; Edwards, R. M.; Thorneloe, K. S. Dual modulation of urinary bladder activity and urine flow by prostanoid EP_3 receptors in the conscious rat. *Br. J. Pharmacol.* **2009,** *158*, 372–381.
(27) Mewshaw, R. E.; Edsall, R. J.; Yang, C.; Manas, E. S.; Xu, Z. B.; Henderson, R. A.; Keith, J. C.; Harris, H. A. ERβ ligands. 3. Exploiting two binding orientations of the 2-phenylnaphthalene scaffold to achieve ERβ selectivity. *J. Med. Chem.* **2005,** *48*, 3953–3979.

DOI: 10.1021/ml100077x | *ACS Med. Chem. Lett.* **2010,** *1*, 316–320