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Structure–activity relationship studies of novel 3-oxazolidinedione-6-naphthyl-2-pyridinones as potent and orally bioavailable EP_3 receptor antagonists

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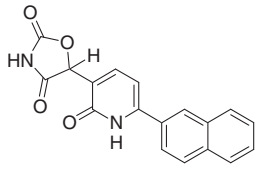
ABSTRACT

Multiple regions of the 3-oxazolidinedione-6-naphthyl-pyridinone series identified via high throughput screening were explored. SAR studies of these regions including the left-hand side oxazolidinedione moiety, α-substituent on the oxazolidinedione ring, central pyridinone core, and substituents on the central pyridinone core led to the discovery of potent EP_3 receptor antagonists such as compound **29** which possesses outstanding rat pharmacokinetic properties. Synthesis and SAR of these novel compounds and DMPK properties of representative compounds are discussed.

Prostaglandins (PGs), are produced from the cyclooxygenase-mediated metabolism of arachidonic acid. They are a subclass of prostanoids involved in many physiological and pathophysiological processes.[1,2] Five prostanoid family receptors are known: DP, EP, FP, IP and TP. Prostaglandin E_2 (PGE_2) is the natural ligand attributed to the activation of subtype receptors EP_1, EP_2, EP_3 and EP_4.[3] Studies on the EP_3 receptor have shown that it plays a key role in platelet aggregation and thrombosis,[4,5] fever generation,[6] hyperalgesia,[7] duodenal bicarbonate secretion and mucosal integrity.[8] Recently, PGE_2 and the EP_3 receptor have been linked to overactive bladder (OAB).[9–12] The potential of selectively inhibiting the EP_3 receptor to treat various inflammatory conditions has prompted us to pursue a potent and selective EP_3 antagonist.

A number of small molecule EP_3 antagonists have been reported.[3,13–26] Most recently, we have reported the discovery of 3-oxazolidinedione-6-aryl-pyridinones as potent, selective, and orally active EP_3 antagonists, which demonstrated robust in vivo activity in several OAB animal models.[27] Herein, we describe our detailed SAR studies and optimization of multiple regions of this chemical series that resulted in the identification of lead compounds such as compound **29**. We began the hit to lead optimization with the high-throughput screening (HTS) hit, compound **1** (Fig. 1), which had a functional pK_i (fpK_i)[28] of 6.7 in a human EP_3 fluorometric imaging plate reader (FLIPR) assay.[27,29]



1, Human EP_3 $fpK_i = 6.7$

Figure 1.

The α-substitution of the oxazolidinedione moiety was investigated first. A general synthetic approach for these analogs is shown in Scheme 1.[30] Commercially available 2-bromo-6-methoxypyridine (**2**) was coupled with 2-naphthalenylboronic acid, under Suzuki cross-coupling conditions yielding the biaryl compound **3**. The *ortho*-lithiation adduct of **3** was quenched with diethyl oxalate yielding the α-ketoester **4** as the major regioisomer with a regioselectivity of 20:1. Treatment of **4** with different Grignard reagents provided alcohols **5**. The oxazolidinedione ring was efficiently formed by the acylation of the α-hydroxyesters **5** with trichloro-

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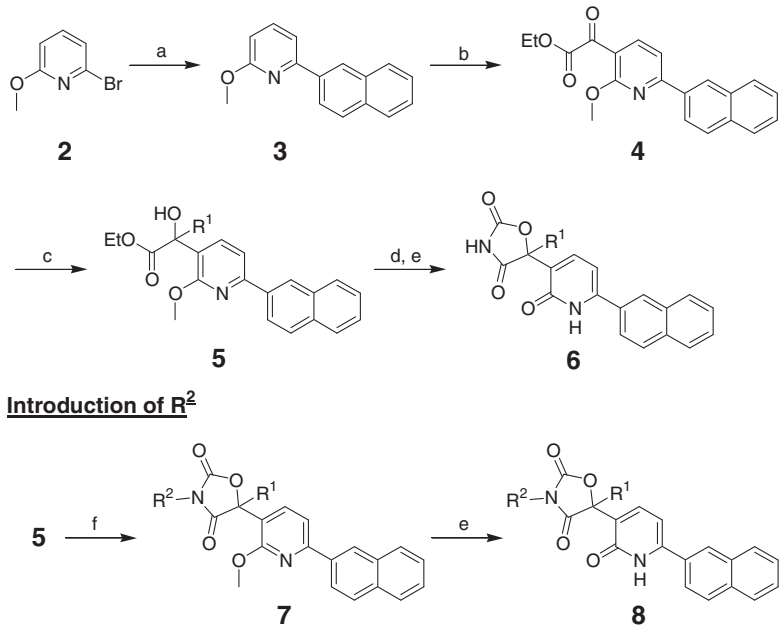
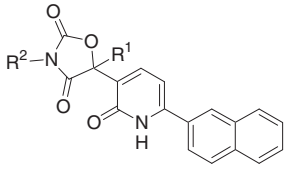


Scheme 1. Synthesis of compounds **6** and **8**. Reagents and conditions: (a) 2-naphthalenylboronic acid, $PXPd_2$, K_2CO_3, MeOH, 60 °C; (b) *t*-BuLi, THF, −78 °C or MeLi, HN(*i*-Pr)$_2$ cat. THF, −78 °C, then added to $(CO_2Et)_2$, THF, −78 °C; (c) R^1-MgCl or R^1-MgBr, THF, −78 °C; (d) (i) $Cl_3CCONCO$, DCM, rt, (ii) 1 M K_2CO_3 (aq), reflux; (e) TMSCl, NaI, CH_3CN, rt or TMSI, CH_3CN, rt; (f) R^2NCO, NaH, THF, rt.

acetyl isocyanate followed by concominant cyclization upon treatment with aqueous potassium carbonate at reflux.[31] Demethylation of the 2-methoxypyridines provided 6-arylpyridinones **6**. To introduce a substituent in the oxazolidinedione nitrogen, the corresponding alkyl isocyanates were reacted with the intermediate **5** to directly afford the cyclization adducts **7** and, upon demethylation arylpyridinones **8** were obtained.

The results for α-substituted oxazolidinediones **6a–i** and **8a–b** are illustrated in Table 1. The replacement of the α-hydrogen (**1**) by a methyl group (**6a**) provided 10-fold boost in potency. The introduction of an isopropyl group (**6b**) further increased potency (fpK_i = 8.0). While its cyclopropyl analog **6c** was less potent, the less strained analogs such as **6d** and **6e** containing a respective cyclopentyl and cyclohexyl ring showed similar potency as **6b**. Phenyl and benzyl analogs **6f** and **6g**, respectively, showed similar results as methyl analog **6a**, while trifluoromethyl analog **6h** was near 10-fold less potent than **6a**. Interestingly, 4-substituted tetrahydropyran **6i** was near 30-fold less potent than its cyclohexyl analog **6e**. Substitution in the oxazolidinedione nitrogen (**8a–b**) proved to be unfavorable, showing a decrease in potency when increasing the bulkiness of the substituent.

Table 1
SAR of α-substituted oxazolidinediones **6a–i** and alkyl analogs **8a–b**

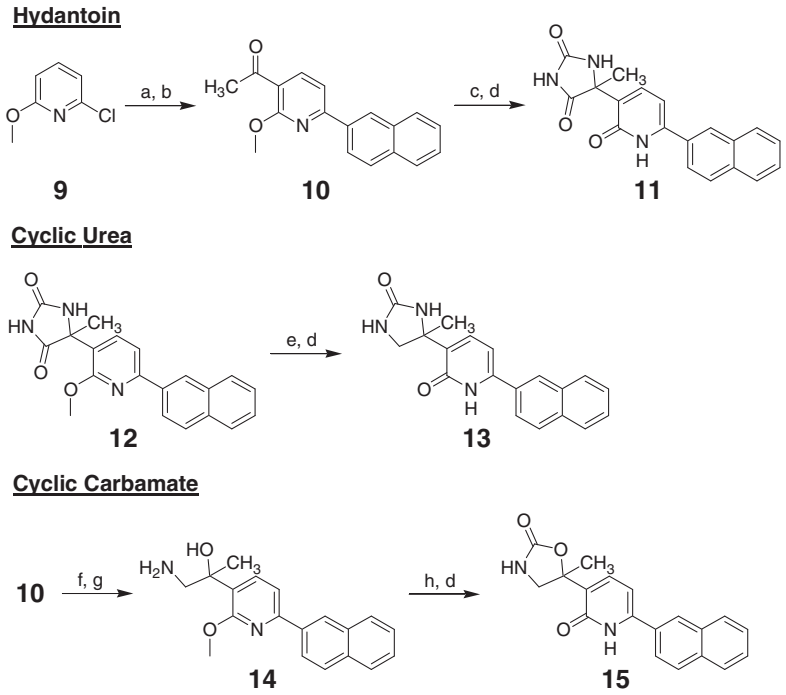


Compounds	R^1	R^2	hEP$_3$ FLIPR fpK_i
1	H	H	6.7
6a	CH_3	H	7.7
6b	*i*-Pr	H	8.0
6c	*c*-Pr	H	7.3
6d	*c*-Pentyl	H	8.1
6e	*c*-Hexyl	H	8.1
6f	Ph	H	7.5
6g	Bn	H	7.8
6h	CF_3	H	6.9
6i	4-Tetrahydropyran	H	6.7
8a	CH_3	CH_3	6.3
8b	CH_3	*i*-Pr	5.8

After investigating the α-substituent of the oxazolidinedione moiety, we turned our attention to exploring other heterocyclic rings at the C_3 of the pyridine ring. The syntheses of the studied analogs are shown in Scheme 2. Commercially available 2-chloro-6-methoxypyridine **9** was ortho-lithiated, quenched with an acetyl Weinreb amide and coupled with 2-naphthalenylboronic acid yielding the methyl ketone **10** as the major regioisomer again with 20:1 regioselectivity. This ketone was exposed to ammonium carbonate and potassium cyanide providing the hydantoin pyridine intermediate which upon demethylation afforded the hydantoin pyridinone **11**. The cyclic urea analog **13** was obtained by chemoselective reduction of hydantoin **12** followed by demethylation. To obtain the oxazolidinone **15**, the methyl ketone **10** was treated with TMSCN and the corresponding product was reduced to the amino alcohol **14**. Treatment of the amino alcohol with phosgene produced the oxazolidinone pyridine adduct which was subsequently demethylated to afford **15**.

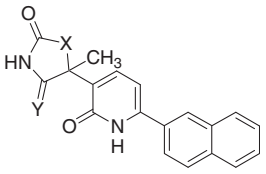
Our findings on oxazolidinedione replacements **11**, **13** and **15** are shown in Table 2. Hydantoin **11** showed a 15-fold decrease in potency compared with oxazolidinedione **6a**. Interestingly, cyclic urea **13** and oxazolidinone **15** were only slightly less potent compared to oxazolidinedione **6a**. Since the oxazolidinedione moiety was the most potent left-hand side group identified to date, we have held it constant during subsequent SAR studies.

The 2-pyridinone core was investigated next. Synthesis of analogs **18**, **19**, **22**, **25** and **26** are outlined in Scheme 3. Compound **17** was prepared by selective lithium halogen exchange and quenched with ethyl pyruvate of bromoanisole **16** followed by the oxazolidinedione ring formation. Suzuki cross-coupling of chloroanisole **17** yielded the methoxyphenyl analog **18** which upon treatment with boron tribromide afforded the phenol **19**. Selective lithium halogen exchange of 5-bromo-2-chloropyridine **20** followed by quenching



Scheme 2. Synthesis of compounds **11, 13** and **15**. Reagents and conditions: (a) *t*-BuLi, THF, −78 °C, then added to $CH_3CON(CH_3)OMe$, THF, −78 °C; (b) 2-naphthalenylboronic acid, $PXPd_2$, K_2CO_3, MeOH, 100 °C, μW, 5 min.; (c) $(NH_4)_2CO_3$, KCN, EtOH/H_2O (1:1), 75 °C; (d) TMSCl, NaI, CH_3CN, rt (e) $AlCl_3$, LAH, THF, 0 °C–rt; (f) TMSCN, NEt_3 (g) LAH, Et_2O, 0 °C–rt; (h) $COCl_2$, NEt_3, Et_2O.

Table 2
SAR of oxazolidinedione replacements **11**, **13** and **15**



Compounds	X	Y	hEP$_3$ FLIPR fpK_i
6a	O	O	7.7
11	NH	O	6.5
13	NH	H_2	7.4
15	O	H_2	7.1

with diethyl oxalate produced the α-ketoester intermediate, which was treated with *i*-PrMgCl. The resulting hydroxyester was cyclized to afford the oxazolidinedione **21**. Palladium catalyzed cross-coupling of **21** with 2-naphthalenylboronic acid produced the pyridine analog **22**. The synthesis of the 4-pyridinone **25** started from the Suzuki cross-coupling of chloropyridine **23** followed by bromination to yield the intermediate **24** as the only isomer observed. The oxazolidinedione ring was installed as previously described and subsequent demethylation afforded **25**. *N*-methyl pyridinone **26** was prepared by methylation of the corresponding pyridinone followed by deprotection of the 4-methoxybenzyl group.

Replacement of the 2-pyridinone core with the methoxy phenyl group (**18**) resulted in a detrimental 100-fold potency loss compared with the parent compound **6a** (Table 3). A similar result was observed with the phenol analog **19**. The pyridine core such as in compound **22** abolished the antagonist activity versus the EP_3 receptor. Interestingly, 4-pyridinone analog **25** was close to 100-fold less potent compared to its parent compound **6b**. Finally,

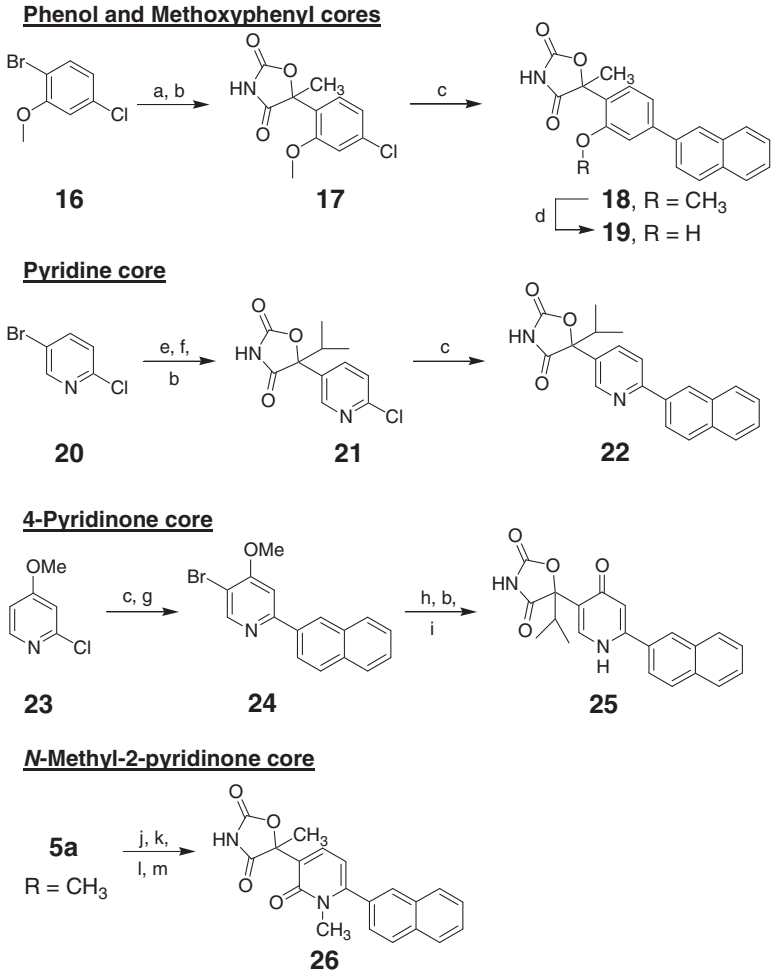
Table 3
Pyridinone core SAR studies

Compounds	hEP$_3$ FLIPR fpK_i
18	5.5
19	5.9
22	<4.6
25	6.1
26	<4.6

N-methylation of the 2-pyridinone nitrogen (**26**) resulted in complete lost of its activity against the receptor. Therefore, it can be concluded that the 2-pyridinone moiety is the optimal core for this chemical series.

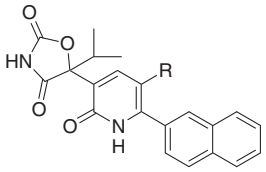
We next explored the 5-substituent at the 2-pyridinone ring. Synthesis of compounds **28a–b**, **29**, **30**, and **33** is depicted in Scheme 4. The intermediate **7b** was halogenated by either NCS or NBS and, after subsequent demethylation, analogs **28a–b** were obtained. Methyl and phenyl analogs **29** and **30**, respectively, were obtained by the corresponding Negishi or Suzuki-Miyaura cross-couplings. The trifluoromethyl analog **33** was obtained by the chemistry previously discussed using compound **31** as the key starting material.[27]

Table 4 illustrates the SAR trends on C_5 substitution at the 2-pyridinone ring. Introduction of a halo group such as chloro (**28a**) and bromo (**28b**) at C_5 displayed over 5-fold potency decrease. In addition, a phenyl or trifluoromethyl group at C_5, compounds **30** and **33** respectively, had detrimental effects on hEP$_3$ potency. On the other hand, 5-methyl analog **29** was equal to or slightly more potent than the parent compound **6b**. We next evaluated compounds **28a** and **29** in rat PK studies and were pleased to find that both compounds had outstanding rat PK properties including very low clearance, long half life, and excellent oral bioavailability (Table 4).[32] Despite being a sensitive area in respect of hEP$_3$ potency, substitution at the C_5 of the 2-pyridinone ring offered an improvement in an already good PK profile by further lowering the clearance and increasing the half life.



Scheme 3. Synthesis of compounds **18**, **19**, **22**, **25** and **26**. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then added to ethyl pyruvate, THF, -78 °C; b) i. $Cl_3CCONCO$, DCM, r.t., ii. 1M K_2CO_3 (aq), reflux; (c) 2-naphthalenylboronic acid, $Pd(OAc)_2$, PCy_3, 1,4-Dioxane/H_2O (6:1), K_2CO_3, 150 °C, μW, 20 min.; (d) BBr_3, DCM, rt; (e) *n*-BuLi, THF, −78 °C, then added to $(CO_2Et)_2$, THF, −78 °C; (f) *i*-PrMgCl, THF, −78 °C–0 °C; (g) NBS, TFA (cat), CH_3CN, 55 °C; (h) *n*-BuLi, THF, −78 °C, then added to ethyl 3-methyl-2-oxobutyrate, THF, −78 °C; (i) NaSMe, DMF, 80 °C; (j) PMBNCO, DBU, DCM, rt; (k) TMSCl, NaI, CH_3CN, rt; (l) NaH, MeI, DMF, 80 °C; (m) CAN, DCM, rt.

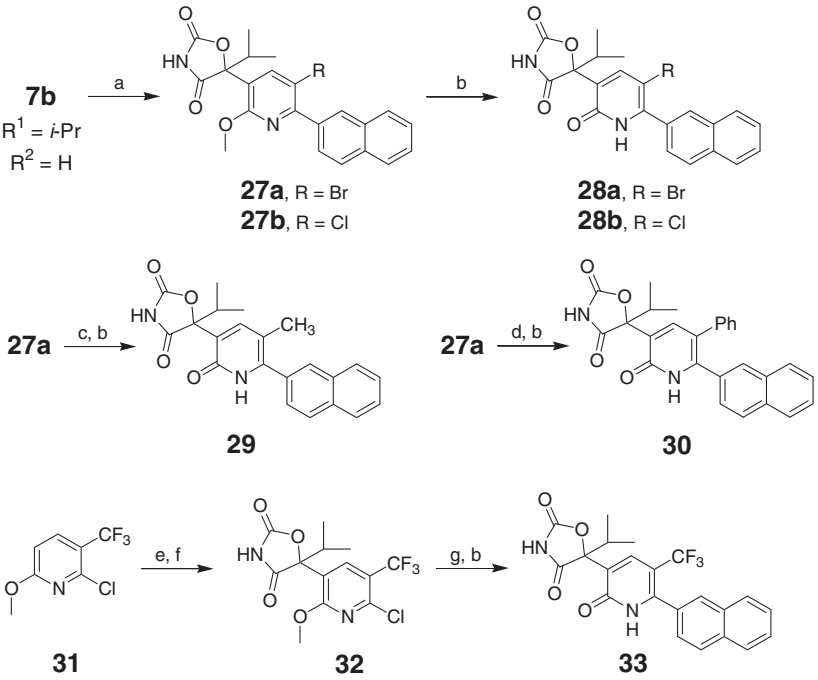
Table 4
SAR and rat PK properties of 5-substituted 2-pyridinones



Compds	R	hEP$_3$ FLIPR fpK_i	Rat PK[a]			
			Cl (mL/min/kg)	$T_{1/2}$ (h)	DNAUC_PO (μg.h/mL/mg/kg)	Oral F (%)
6b	H	8.0	5.1	3.5	4.4	100
28a	Cl	7.3	1.2	5.3	12.9	86
28b	Br	7.4	n/a			
29	CH_3	8.2	1.4	6.6	13.5	100
30	Ph	5.6	n/a			
33	CF_3	6.2	n/a			

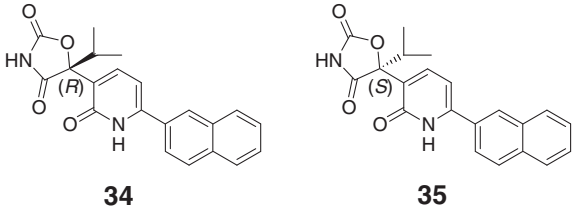
[a] DMPK properties are averaged values ($n = 3$) from an *i.v./p.o.* study in Sprague–Dawley rats dosed at 2 mg/kg (*p.o.*) and 1 mg/kg (*i.v.*).

The stereochemistry of the stereogenic carbon present in the oxazolidinedione moiety was also investigated. The enantiomers of compound **6b** were separated by chiral high-performance liquid chromatography (HPLC) yielding **34** and **35**.[33] The absolute configuration of the enantiomers was established by *vibrational circular dichroism* (VCD). As shown in Table 5, the *S* enantiomer (**35**) was



Scheme 4. Synthesis of compounds **28**–**30** and **33**. Reagents and conditions: (a) NBS, CH_3CN/MeOH/TFA, 60 °C or NCS, CH_3CN/MeOH / TFA, 60 °C; (b) TMSCl, NaI, CH_3CN, rt; (c) $Zn(CH_3)_2$, $PdCl_2(dppf)*CH_2Cl_2$, Dioxane, 80 °C; (d) $PhB(OH)_2$, $PdCl_2(dppf)*CH_2Cl_2$, Dioxane/H_2O, K_2CO_3, 160 °C, μW, 20 min; (e) *t*-BuLi, THF, −78 °C, then added to ethyl 3-methyl-2-oxobutyrate, THF, −78 °C; (f) (i) $Cl_3CCONCO$, DCM, rt, (ii) 1 M K_2CO_3 (aq), reflux; (g) 2-naphthalenylboronic acid, $PdCl_2(dppf)*CH_2Cl_2$, Dioxane/H_2O, K_2CO_3, 160 °C, μW, 20 min.

Table 5
Potency of enantiomers **34** and **35**



34 **35**

Compounds	Stereochemistry	hEP$_3$ FLIPR fpK_i
34	*R*	7.7
35	*S*	8.1

only slightly more potent than the *R* enantiomer (**34**)—indicating that the absolute configuration is not critical for the compounds binding to the EP_3 receptor.

The right-hand side naphthyl region of this chemical series was also explored and the discovery of several naphthyl replacements that maintain high potency and excellent rat PK properties and mitigate potential bioactivation liabilities was reported previously.[27] In addition, this 3-oxazolidinedione-6-aryl-pyridinone series exemplified by compound **6b** was very potent against rat and dog EP_3 receptors and had excellent selectivity for EP_3 over EP_1, EP_2, EP_4, DP, FP, COX1, and COX2.[27]

In summary, we have extensively explored multiple regions of the HTS hit **1**, an 3-oxazolidinedione-6-aryl-pyridinone, and observed the following SAR trends: (1) the α-substituent at the oxazolidinedione ring can be tuned to obtained high potency; (2) replacement of the oxazolidinedione for other heterocycles decreases potency in general; (3) the 2-pyridinone core is optimal; and (4) the C_5 substituent at the pyridinone ring can be beneficial for further improving DMPK properties while maintaining excellent potency. Our hit to lead optimization resulted in the discovery of highly potent and orally bioavailable EP_3 receptor antagonists such as **29**, which is a valuable tool for the biomedical research community to further identify and validate potential therapeutic benefits of selective EP_3 inhibition.

Acknowledgements

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28. fpK_i calculation is detailed in supporting information of Ref. 27.
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30. For an alternative synthetic approach see Ref. 27.
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32. All studies were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.
33. The enantiomers **34** and **35** were separated using the following chiral HPLC conditions: Chiralpac AS column (2 × 25 cm); 100% MeOH, 1 mL/min elution; dual UV detector (220 nm and 254 nM); R_t = 3.74 min (peak 1); R_t = 6.37 min (peak 2).