

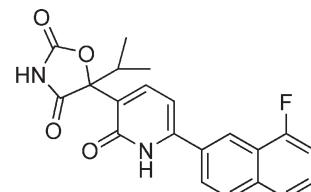
Novel 3-Oxazolidinedione-6-aryl-pyridinones as Potent, Selective, and Orally Active EP₃ Receptor Antagonists

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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 EP₃ 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 EP₃ antagonists are excellent tool compounds for investigating and validating potential therapeutic benefits from selectively inhibiting the EP₃ receptor.

KEYWORDS EP₃ receptor, novel, potent, selective, and orally active antagonists, 3-oxazolidinedione-6-aryl-pyridinones



human EP₃: fpK_i = 8.5
rat EP₃: 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 E₂ (PGE₂), PGF_{2α}, PG₁, PGD₂, and thromboxane A₂. 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, PGE₂ is the most widely produced and has a number of diverse actions.¹ Molecular cloning has revealed the existence of four different receptors for PGE₂, which accounts for the diverse effects of this prostanoid. These receptors are designated as EP₁, EP₂, EP₃, and EP₄ 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 EP₃ receptor antagonism could provide a new and effective treatment option for OAB. First, EP₃ receptor knockout (KO) mice demonstrated an enhanced bladder capacity as compared to wild-type (WT) controls. Infusion of PGE₂ or a selective EP₃ receptor agonist, GR63799, into WT mice induced bladder overactivity. These responses were absent in KO mice.⁵ Second, PGE₂ is synthesized in the bladder in

response to bladder distension, nerve stimulation, inflammatory mediators, and mechanical trauma.⁶ Elevated urinary PGE₂ has been associated with OAB in humans.^{7,8} Third, EP₃ 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 PGE₂ improve OAB symptoms.¹⁰

A number of small molecule EP₃ receptor antagonists have been reported recently.^{11–16} In this letter, we disclose a novel series of 3-oxazolidinedione-6-aryl-pyridinones as potent, selective, and orally active EP₃ 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 EP₃ fluorometric imaging plate reader (FLIPR) assay¹⁷ led to the identification of 3-oxazolidinedione-6-aryl-pyridinone **1** as an EP₃ antagonist with a functional pK_i (fpK_i) of 6.7 (Figure 1).^{18–20} The compound was subsequently tested in human EP₁ and rat EP₃ FLIPR assays and found to have good selectivity for EP₃ over EP₁ and good cross-species activity.²¹ In a rat pharmacokinetic (PK) study,²²

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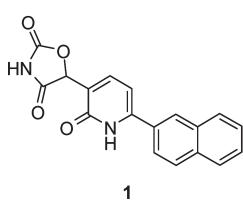
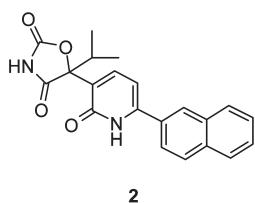


Figure 1. HTS hit 1.

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) (μ g·h/mL/mg/kg)	= 0.17
Oral F (%)	= 32



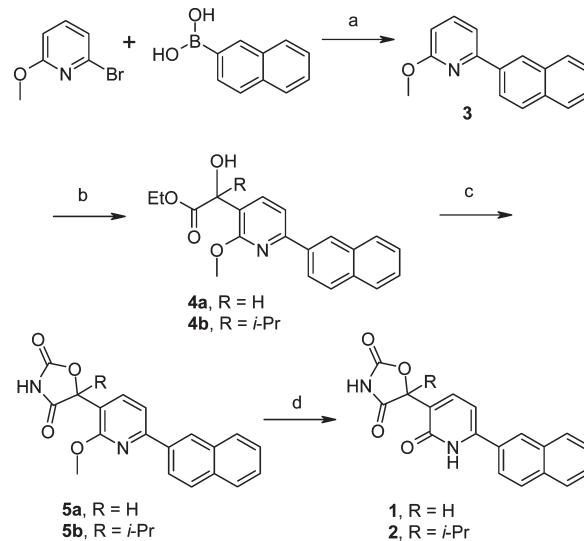
Potency	
hEP ₃ FLIPR fpK _i	= 8.0
hEP ₃ binding pK _i	= 8.3
rEP ₃ FLIPR fpK _i	= 8.0
dEP ₃ FLIPR fpK _i	= 7.7
Selectivity	
EP ₁ , EP ₂ , EP ₄ , FP, & DP pIC ₅₀	< 4.3
COX1 & COX2 pIC ₅₀	< 4.0
Rat PK & Developability	
T _{1/2} (h, iv)	= 4.0
Cl (mL/min/kg)	= 2.6
DNAUC (po) (μ g·h/mL/mg/kg)	= 5.5
Oral F (%)	= 83
CYP450 pIC ₅₀	< 4.3
hERG binding pIC ₅₀	< 4.3
Solubility (μ M)	= 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 EP₃ 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 EP₃ binding assay²³ 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) EP₃ FLIPR assays,²¹ demonstrating excellent cross-species activities. Compound **2** had excellent selectivity for EP₃ over other EP and prostanoid receptors. It was inactive in human EP₁ FLIPR,²¹ EP₂ LANCE,²¹ EP₄ binding,²³ DP binding,²³ and FP FLIPR²¹ assays. Because COX inhibitors function to reduce production of PGE₂, 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 EP₃ 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 study²² 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 μ g 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,

Scheme 1. Synthesis of Pyridinones **1** and **2**^a



^a Reagents and conditions: (a) PXPd₂, K₂CO₃, 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, CH₂Cl₂, room temperature; (ii) 2 M K₂CO₃ aqueous solution, reflux (5a, 80%; 5b, 48%). (d) TMSCl, NaI or TMSI, CH₃CN, room temperature (1, 49%; 2, 58%).

2C9, 2D6, and 3A4) and in the hERG binding assay and had good aqueous solubility (193 μ M). 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.

The synthesis of pyridinones **1** and **2** is outlined in Scheme 1. Suzuki coupling of commercially available 2-bromo-6-methoxy-pyridine 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**. Oxazolidinediones **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 PGE₂-induced OAB model in the conscious SHR.⁵ Intravesical infusion of PGE₂ (120 μ M) led to reduced bladder capacity (Figure 4). Pretreatment with **2** (30 mg/kg, id) (p = 0.006 vs PGE₂ + vehicle, two-way RM ANOVA) significantly inhibited the bladder capacity decrease caused by the PGE₂ infusion. Compound **2** was also evaluated in an acetic acid-induced OAB model in conscious SHR.⁵ Similar to PGE₂, 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

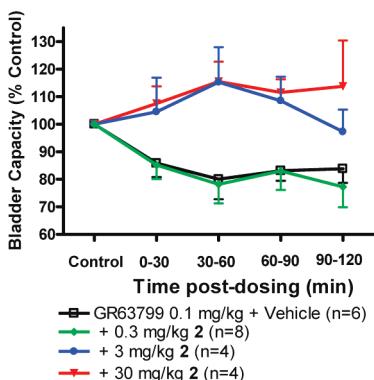


Figure 3. Effect of id administration of **2** on the GR63799-induced decrease in conscious SHR bladder capacity.

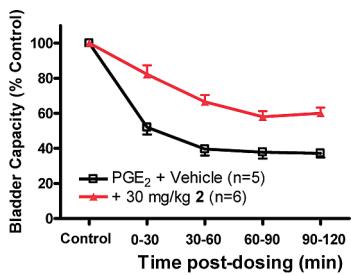


Figure 4. Effect of id administration of **2** on the PGE₂-induced decrease in conscious SHR bladder capacity.

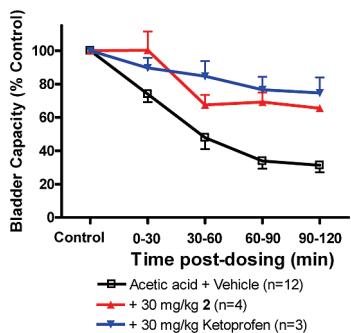


Figure 5. Effect of id administration of **2** on the acetic acid-induced decrease in conscious SHR bladder capacity. Comparison to the COX inhibitor ketoprofen.

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 EP₃ receptor antagonist series could potentially lead to effective therapeutic agents for treating OAB.

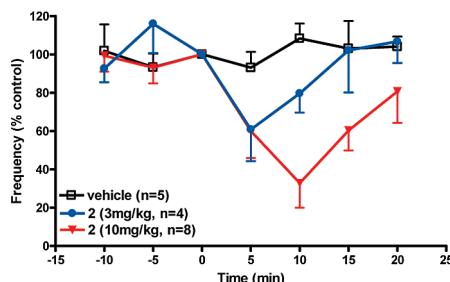


Figure 6. Effect of iv administration of **2** on bladder rhythmic contraction in anesthetized SD rats.

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 oxazolidinedione **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.

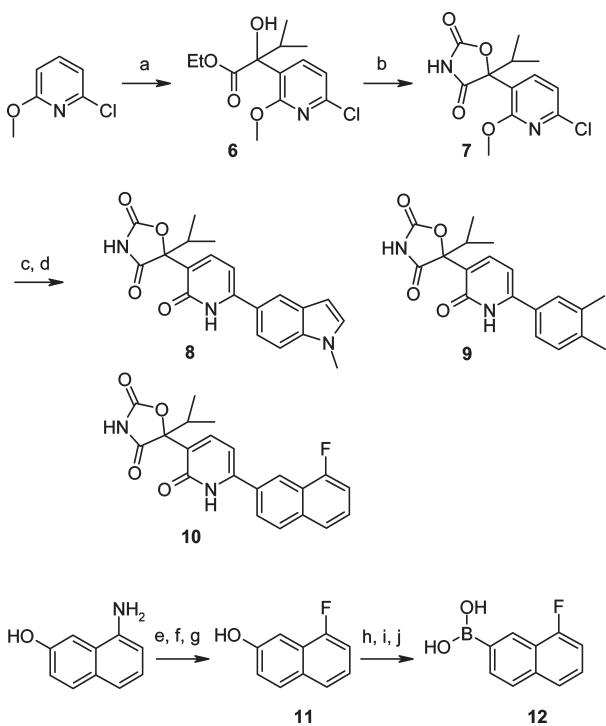
Compounds **8–10** had excellent potency in human and rat EP₃ FLIPR assays²¹ (Table 1). In particular, 8-fluoronaphthyl analog **10** was slightly more potent than unsubstituted naphthyl compound **2**. In rat PK studies,²² compounds **8–10** displayed excellent PK parameters—low clearance, good half-life, 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 EP₁, EP₂, EP₄, DP, TP, FP, COX1, and COX2 assays—demonstrating excellent selectivity for EP₃ 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-oxazolidinedione-6-arylpyridinones exemplified by compound **2** was discovered as potent and selective EP₃ receptor antagonists with excellent

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

compound	EP ₃ FLIPR fpK _i		rat PK parameters				NADPH-dependent GSH conjugates	
	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, CH₂Cl₂, room temperature; (ii) 2 M K₂CO₃ aqueous solution, reflux, 94%. (c) Corresponding boronic acids, Suzuki coupling conditions (see the Supporting Information). (d) TMSCl, NaI, or TMSI, CH₃CN, room temperature (8, 27%; 9, 48%; and 10, 64%). (e) NaH, MeI, DMF, 0 °C to room temperature, 90%. (f) NaNO₂, HCl, HBF₄, H₂O, 51%. (g) BBr₃, CH₂Cl₂, 0 °C to room temperature, 100%. (h) Tf₂O, K₃PO₄, toluene, H₂O, 0 °C to room temperature, 91%. (i) PdCl₂(dppf), dppf, KOAc, bis-(pinacolato)diborane, dioxane, 80 °C, 70%. (j) NaIO₄, HCl, THF, H₂O, 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 EP₃ inhibition.

SUPPORTING INFORMATION AVAILABLE Synthetic procedures and characterization data for all compounds; procedures for human, rat, and dog EP₃, EP₁, EP₂, and FP assays; and GSH trapping studies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ABBREVIATIONS PGE₂, prostaglandin E₂; 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.

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- (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 <±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).
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