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3-Urea-1-(phenylmethyl)-pyridones as novel, potent, and selective EP_3 receptor antagonists

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ABSTRACT

A series of 3-urea-1-(phenylmethyl)-pyridones was discovered as novel EP_3 antagonists via high-throughput screening and subsequent optimization. The synthesis, structure–activity relationships, and optimization of the initial hit that resulted in potent and selective EP_3 receptor antagonists such as **11g** are described.

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Prostaglandins (PGs) are synthesized from arachidonic acid in response to extracellular stimuli via the cyclooxygenase (COX1 and COX2) isozyme pathway. Once synthesized, PGs are immediately released from cells and act as hormones in the vicinity of the cells to maintain local homeostasis.[1] There are currently five known naturally occurring PGs including PGD_2, PGE_2, PGF_2, PGI_2, and thromboxane A_2 (TXA_2).[2] Their ability to affect various physiological and biological processes in the body depends solely on their binding to the distinct prostanoid receptors. Prostanoid receptors are G-protein coupled, rhodopsin type receptors with seven transmembrane domains.[3] The eight known prostanoid receptors are EP_{1-4}, DP, FP, IP, and TP. Prostaglandin E2 (PGE_2), a pro-inflammatory mediator,[4] binds preferentially to the four EP receptors, EP_{1-4}.[5] Studies with EP_3 knockout (KO) mice and EP_3 agonists have implicated that activation of the EP_3 receptor might play a key role in fever generation, uterine contraction, gastric acid secretion, smooth muscle contraction of GI tract, neurotransmitter release, and sodium/water reabsorption in kidney.[6]

Several small molecule EP_3 receptor antagonists have been reported in the literature.[7] Herein we describe the identification, synthesis, structure–activity relationships (SAR), functional activity, and initial physicochemical properties of a novel series of z3-urea-1-(phenylmethyl)-pyridones as potent and selective human EP_3 receptor antagonists.

High-throughput screening (HTS) of the corporate compound collection using a fluorometric imaging plate reader (FLIPR) assay (which measures the inhibition of PGE_2-induced $[Ca^{2+}]_i$-mobilization in U2OS (human osteosarcoma) cells transfected with a vector carrying full length human EP_3 cDNA) led to the identification of compound **1** as a hit with a functional pK_i (fpK_i) of 6.6 against human EP_3 receptor compound **1** also had good binding affinity to the human EP_3 receptor (Fig. 1).[8] To improve potency and selectivity, and establish tractable SAR, we focused on exploring the highlighted four regions of compound **1**: left-hand side (LHS) benzyl, middle pyridone, urea linker (UL), and right-hand side (RHS) phenyl moieties in Figure 1, and used the EP_3 FLIPR assay as the primary assay to support our SAR exploration.

SAR exploration of this chemical series was primarily achieved through the general synthetic route outlined in Scheme 1. Benzyl halides were used to alkylate 3-nitro-2(1*H*)-pyridinone under basic conditions. Using KOH powder in DMSO, the reaction was completed within 5 min to afford compound **4**. Reduction of the nitro group using tin(II) chloride in concentrated HCl gave the desired 3-amino-2(1*H*)-pyridone **5**. The urea was formed either by reacting **5** with commercially available isocyanates or with amines using triphosgene as the activating reagent. Intermediates **5** could also be reacted with sulfonyl chlorides, chloroformates, acid chlorides, or carboxylic acids in a similar fashion to form sulfonamides, carbamates, and amides. Using this versatile three-step synthetic route, multiple regions of this chemical series were explored.

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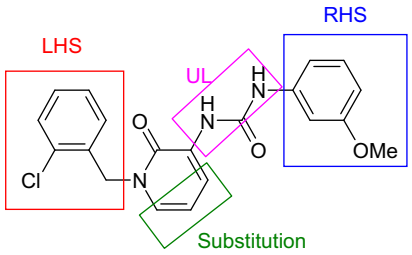


1

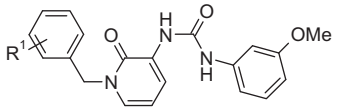
hEP_3 binding: pK_i = 7.8
hEP_3 FLIPR: fpK_i = 6.6
rEP_3 FLIPR: fpK_i = 6.3

Figure 1. HTS-hit compound **1**.

We first explored the left-hand side (LHS) (R^1) benzyl moiety of the initial hit **1** while keeping the urea moiety and RHS constant. A number of compounds were prepared and evaluated in the EP_3 FLIPR assay (Table 1). It was observed that the position and nature of the substituents on the benzylic phenyl ring was important for EP_3 potency. In general, electron-withdrawing substituents, as in **7b**, **7c**, **7e**, **7f**, were preferred over electron-donating ones, as in **7d**, **7g**, **7h**. As for the position of substituents, it was found that electron-withdrawing substituents in the *ortho* position had better relative EP_3 potency than their *meta* and *para* counter parts, as in **1** versus **7f**, **7j**, and **7b** versus **7e**, **7i**. The best result was achieved by installing both chloro and fluoro on the *ortho* positions (**7o**), which improved the EP_3 potency by more than 10-fold over the initial hit **1**.

We next investigated variation of the RHS phenyl moiety R^2 while keeping other regions of **1** constant (Table 2). Repositioning the methoxy group from the *meta* position (**1**) to the *ortho* and the *para* position on the phenyl ring (**8f**, **8x**) led to a decline in EP_3 potency. It was found in general that substituents in the *para* position resulted in the loss of activity. Variation of the *ortho* position showed small substituents were tolerated, but no significant improvement of activity was observed, as in **8a, 8b**. Replacing the methoxy group with other polar groups resulted in loss of potency (**8l, 8p, 8q, 8r**) while replacement with electron-withdrawing groups such as fluoro, chloro, bromo, iodo, and cyano resulted in 10-fold reduction of EP_3 potency (**8g**, **8i**, **8j**, **8k**). Improvement of EP_3 potency was achieved when the methoxy group was replaced with smaller non-polar alkyl groups. In particular, both 3-ethyl (**8n**) and 3-isopropyl (**8o**) analogs showed more than three-fold improvement of EP_3 potency and were the best substituents at this RHS region.

Table 1
SAR of the left-hand side (LHS) benzyl moiety, compounds **1** and **7a–7o**



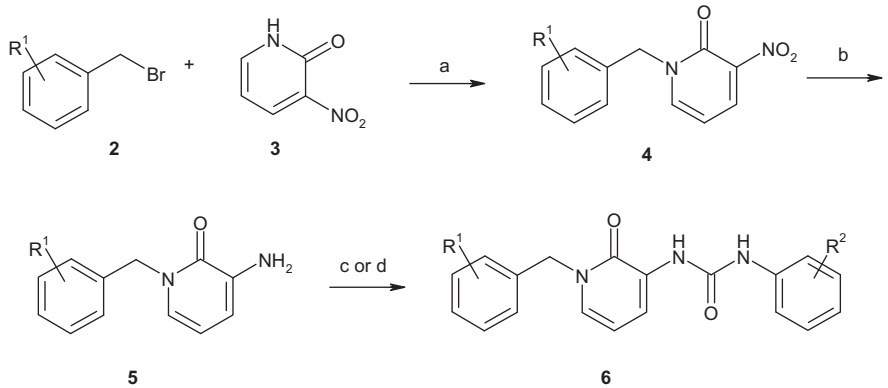
Compound	R^1	hEP_3 FLIPR[a] (fpK_i)
1	2-Cl	6.6
7a	H	6.0
7b	2-F	6.3
7c	2-Br	6.5
7d	2-Me	5.6
7e	3-F	6.0
7f	3-Cl	6.4
7g	3-Me	5.9
7h	3-MeO	5.7
7i	4-F	5.9
7j	4-Cl	6.0
7k	4-Me	5.5
7l	4-MeO	6.1
7m	2-Cl-4-F	6.4
7n	2-Cl-5-F	6.1
7o	2-Cl-6-F	7.6

[a] Mean of at least two determinations with standard deviation of <±0.3.

Changes and requirements at the urea linkage (UL) was subsequently explored (Table 3). It was found that both hydrogen bond donors on the urea moiety were critical to EP_3 activity (**1** vs **9a**). In addition, replacement of the urea with amides (**9b**, **9c**) resulted in the total loss of EP_3 potency. Thus, the original N,N′-disubstituted urea moiety was found to be optimal at this position.

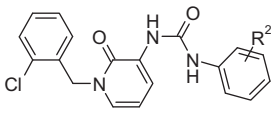
Substitutions on the central pyridone ring were studied by installing small substituents on the ring (Table 4). Unsubstituted pyridone rings remained the most potent while compounds **10d–10g** showed that small substituents at the 5- and 6-positions retained some potency. Substitutions at the 4-position produced inactive compounds (**10a**, **10b**).

Having explored the four regions of defined in Figure 1 and identified the potency-enhancing moieties, we then turned our attentions to combining potency-enhancing moieties into single molecules to further improve potency and prepared the compounds listed in Table 5. We were pleased to find that, EP_3 potency improvement was indeed achieved when combining potency-enhancing groups. For example, compound **11g** with 2-chloro-6-fluoro on the left-hand side benzyl ring (R^1) and 3-ethyl on the right-hand side phenyl ring (R^2) showed an EP_3 FLIPR fpK_i of 8.2, a more than 25-fold potency increase comparing to the initial hit.



Scheme 1. Reagents and conditions: (a) KOH (powder), DMSO, rt, 5 min; (b) $SnCl_2$, HCl, rt; (c) R^2NCO, DCM, rt; (d) Aryl–NH_2, triphosgene, DIEA, DCM, rt.

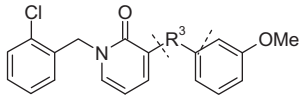
Table 2
SAR of the right-hand side (RHS) phenyl moiety, compounds **1** and **8a–8x**

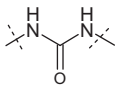
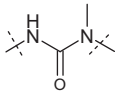
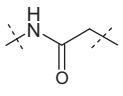


Compound	R^2	hEP$_3$ FLIPR[a] (fpK_i)
1	3-OMe	6.8
8a	2-F	6.8
8b	2-Cl	6.9
8c	2-I	5.3
8d	2-Me	6.1
8e	2-Et	5.4
8f	2-OMe	6.2
8g	3-F	5.8
8i	3-Cl	6.0
8j	3-Br	6.0
8k	3-I	6.4
8l	3-CN	<4.6
8m	3-Me	6.6
8n	3-Et	7.3
8o	3-Isopropyl	7.2
8p	3-Benzyl	<4.6
8q	3-CH_2COOH	<4.6
8r	3-CH_2COOCH_3	<4.6
8s	3-$COCH_3$	<4.6
8t	4-Cl	<4.6
8u	4-Me	5.5
8v	4-Et	<4.6
8w	4-CF_3	<4.6
8x	4-OMe	<4.6

[a] Mean of at least two determinations with standard deviation of <±0.3.

Table 3
SAR of the urea moiety, compounds **1** and **9a–9c**



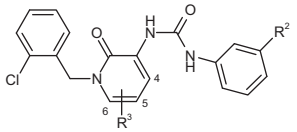
Compound	R^3	hEP$_3$ FLIPR[a] (fpK_i)
1		6.8
9a		<4.6
9b		<4.6
9c		<4.6

[a] Mean of at least two determinations with standard deviation of <±0.3.

Key compounds in the series were then evaluated for selectivity, cross-species activities, hERG and CYP450 inhibition, and in vivo PK properties.

With regards to selectivity, compound **8n**, for example, was found to be highly selective in a CEREP screen of 50 receptors,

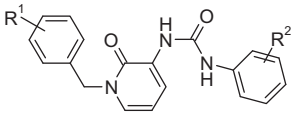
Table 4
SAR of substitutions on pyridone ring, compounds **1**, **8n** and **10a–10g**



Compound	R^2	R^3	hEP$_3$ FLIPR[a] (fpK_i)
1	MeO	H	6.8
8n	Et	H	7.3
10a	Et	4-Me	<4.6
10b	MeO	5-Me	5.9
10d	Et	5-Me	6.7
10e	Et	5-Cl	6.3
10f	MeO	6-Me	5.9
10g	Et	6-Me	6.9

[a] Mean of at least two determinations with standard deviation of <±0.3.

Table 5
hEP$_3$ potency of some key combination compounds, compounds **1** and **11a–11g**



Compound	R^1	R^2	hEP$_3$ FLIPR[a] (fpK_i)
1	2-Cl	3-MeO	6.8
11a	3-CF_3	3-Et	6.7
11b	2-Cl, 4-F	3-Et	6.9
11c	2-F	3-Et	7.0
11d	2-Cl	3-*i*-Pr	7.2
11e	2-Cl	3-Et	7.3
11f	2-Cl, 6-F	3-MeO	7.6
11g	2-Cl, 6-F	3-Et	8.2

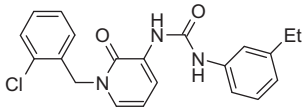
[a] Mean of at least two determinations with standard deviation of <±0.3.

transporters, and ion channels (<23% inhibition at 1 μM against all 50 targets in the panel) and showed at least 100-fold selectivity versus a number of 7TM receptors including other prostanoid receptors, such as hEP$_1$, hEP$_2$, hPE$_4$, hDP, hFP, and hTP. It was found that the potency of **8n** at the rat receptors was comparable to that for the human receptor with similar selectivity against rat EP$_1$ receptor. Additionally, since prostanoid synthesis is dependent upon the oxidative metabolism of arachidonic acid, COX1/2 activity was assayed. Compound **8n** was inactive in COX1 and COX2 enzyme inhibitory assays. Moreover, no agonist activity was observed for compounds in this series at the human EP$_3$ receptor as exemplified by compound **8n** (Table 6).

Compound **8n** also had no hERG liability (dofetilide binding pK_i <5), though it showed some inhibition against P450 isozyme 1A2 (pK_i = 5.7) and was clean against other major isozyme. Interestingly, no 1A2 inhibition was observed in more potent analog **11f** (IC_{50}s: 1A2 > 25 μM; 2C19 = 4.5 μM, 2C9 = 3.7 μM; 2D6 > 25 μM; 3A4 > 25 μM).

The compounds in the series generally exhibited good artificial membrane permeability (e.g., 220 nm/s for **11f**; 550 nm/s for **1**), but low aqueous solubility. In a rat pharmacokinetic (PK) study (dosed at 1.2 mg/kg iv and 2.0 mg/kg po), compound **1** demonstrated moderate clearance (Clb = 24 mL/min/kg) with a half life of 1.3 h, volume of distribution (V_{dss}) of 3.4 L/kg and oral bioavailability (F) of 27%. The initial developability data suggests that this series constitutes a reasonable starting point for further lead optimization.

Table 6
In vitro selectivity data for compound **8n**[9]



Selectivity[a] (pK_i)	hEP_1	hEP_2	hEP_4	hDP	hFP
	<5	<5	<5	<5	<5
	hTP	COX1	COX2	Rat-EP_3[b]	hEP_3 EC_{50}
	<5	<4	<4	6.8	<5

[a] Values are a mean of at least two determinations with a standard deviation of <±0.3 log units.
[b] Functional pK_i.

As shown in Figure 1 and Table 6, the series have excellent cross-species activities. For example, both the initial hit **1** and compound **8n** had fpK_i of 6.3 and 6.8 against rat EP_3 receptors.

In summary, exploration of a novel series of 3-urea-1-(phenylmethyl)-pyridones identified from high-throughput screening led to the discovery of potent and selective human EP_3 antagonists exemplified by compound **11g** ($fpK_i = 8.2$). The series possesses excellent functional activity, selectivity, and good initial developability properties.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.137.

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8. (a) Functional activity was determined by measuring mobilization of intracellular calcium on a fluorometric imaging plate reader (FLIPR) as described in detail in Ref. 6f; (b) $fpK_i = IC_{50}/(1+[A]/EC_{50})$, assuming slope = 1, where [A] = ligand concentration, EC_{50} = ligand concentration at 50% activation; IC_{50} = compd potency in antagonist mode.
9. Selectivity screening for compound **8n** was conducted using radioligand binding assays for the hEP_1, hEP_2, hEP_4, and hTP receptors as detailed in Ref. 6f. Binding studies for the hIP and hFP receptors were completed by Cerep (Redmond, WA). COX1 and COX2 inhibition was measured using human whole blood in ELISA assay format as described in detail in the following references: (a) Warner, T. D.; Giuliano, F.; Vojnovic, I.; Bukasa, A.; Mitchell, J. A.; Vane, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7563; (b) Patrignani, P.; Panara, M. R.; Greco, A.; Fusco, O.; Natoli, C.; Iacobelli, S.; Cipollone, F.; Ganci, A.; Creminon, C.; Maclouf, J.; Patrono, C. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1705.