

2,3-Diarylthiophenes as selective EP₁ receptor antagonists

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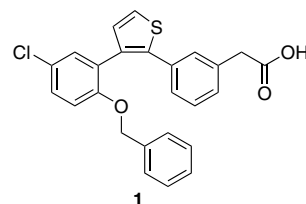
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Abstract—The synthesis and the EP₁ receptor binding affinity of 2,3-diarylthiophene derivatives are described. The evaluation of the structure–activity relationship (SAR) in this series led to the identification of compounds **4**, **7**, and **12a**, which exhibit high affinity for the human EP₁ receptor and a selectivity greater than 100-fold against the EP₂, EP₃, EP₄, DP, FP, and IP receptors and greater than 25-fold versus the TP receptor. These three antagonists present good pharmacokinetics in rats and significant differences in the way they are distributed in the brain.

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Prostanoids are important lipid mediators involved in a broad spectrum of physiological and pathophysiological events.¹ They exert their effects through specific interactions with prostanoid receptors, which are all members of the G-protein coupled receptor superfamily.² In particular, prostaglandin E₂ (PGE₂), the most abundant mammalian prostaglandin, elicits its effects primarily through interaction with four distinct receptors designated EP₁, EP₂, EP₃ and EP₄.³ Recent studies with EP₁ receptor knockout mice suggest that this receptor is involved in PGE₂-induced allodynia⁴ and in acute inflammatory pain.⁵ The involvement of the EP₁ receptor in pain is further supported by the demonstrated efficacy of the EP₁ selective antagonist ONO-8711 in rat models of allodynia,⁶ postoperative pain⁷, and neuropathic pain.⁸ Another EP₁ antagonist, ZD6416, is reported to attenuate secondary esophageal hyperalgesia in man.⁹ Further studies with knockout mice and the antagonists ONO-8711 and ONO-8713 suggest a role for the EP₁ receptor in colon¹⁰, and breast¹¹ carcinogenesis, blood pressure regulation,⁵ adaptive gastric cytoprotection,¹² and diabetic nephropathy.¹³ Previous reports from this laboratory described the SAR of various prostanoid analogs¹⁴ and of a family of dibenzazo-

cinone derivatives¹⁵ with the human EP₁ receptor. Previous work also permitted the identification of 2,3-diarylthiophene **1** as a prototypical EP₁ receptor antagonist.¹⁶ Compound **1** binds with good affinity to the EP₁ receptor ($K_i = 15$ nM) and is shifted 15-fold to a K_i of 220 nM in the presence of 2% human serum albumin (HSA). The selectivity ratio of **1** is at least 100-fold against the EP₂, EP₄, FP, and IP receptors. Its selectivity versus the EP₃, DP, and TP receptors is 67-, 11-, and 10-fold, respectively. While this antagonist presents an acceptable pharmacokinetic profile in rats ($F = 51\%$; $C_{max} = 18$ μ M at 0.5 h, 10 mg/kg P.O., $t_{1/2} = 3$ h), only a modest brain–blood ratio of 0.1 is measured in the same species.



In order to evaluate the in vivo pharmacology associated with EP₁ receptor antagonism, we wished to discover antagonists presenting a higher degree of selectivity over the other prostanoid receptors. We also wanted to increase the brain–blood ratio since it is

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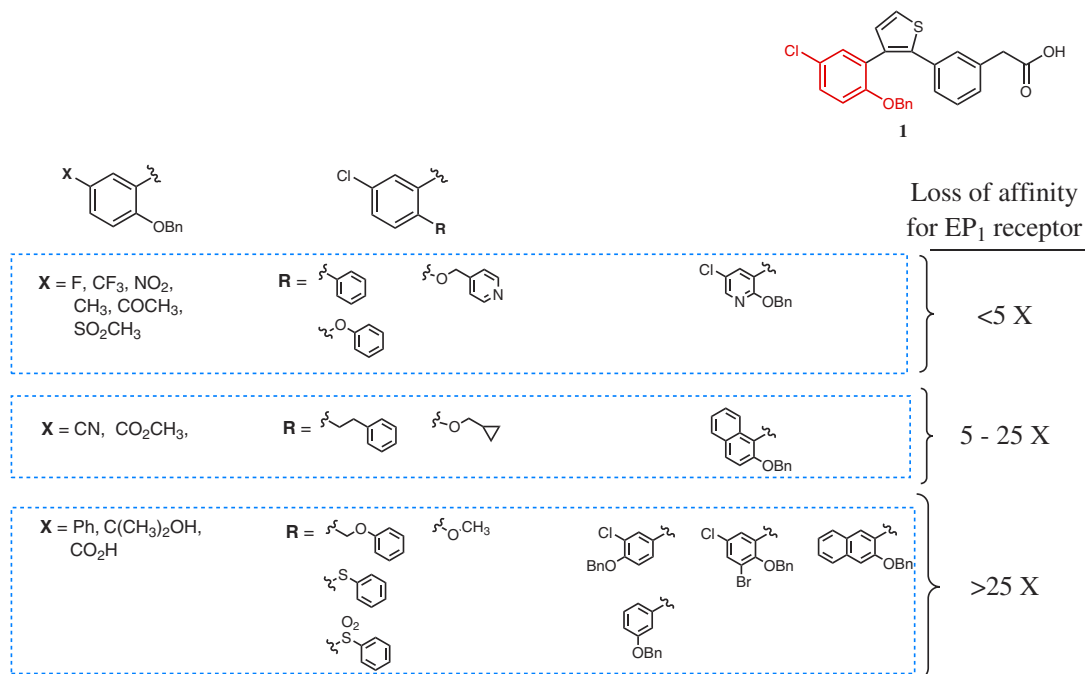


Figure 1. SAR overview at the 3-thienyl position.

probable that centrally mediated EP₁ agonism plays a role in certain types of pain.⁴ We now disclose the results of a SAR study directed toward the optimization of analogs of antagonist **1** in terms of EP₁ binding affinity, selectivity, functional activity, and pharmacokinetics.

Human prostanoid receptor binding affinities were determined as described previously by Abramovitz et al.¹⁷ The functional antagonist properties of compounds were evaluated using a cell-based assay.¹⁸

An extensive SAR study was conducted on the structure of compound **1**. Modifications made at the 3-thienyl position at best permitted preservation of the binding affinity for the EP₁ receptor (Fig. 1). Surrogates for the central thiophene ring were identified since small lipophilic rings such as cyclopentene and pyrrole are tolerated (Fig. 2). However, no significant effects on selectivity were observed as a result of these structural modifications. In contrast, modifications at the 2-thienyl position proved more fruitful. Three classes of deriva-

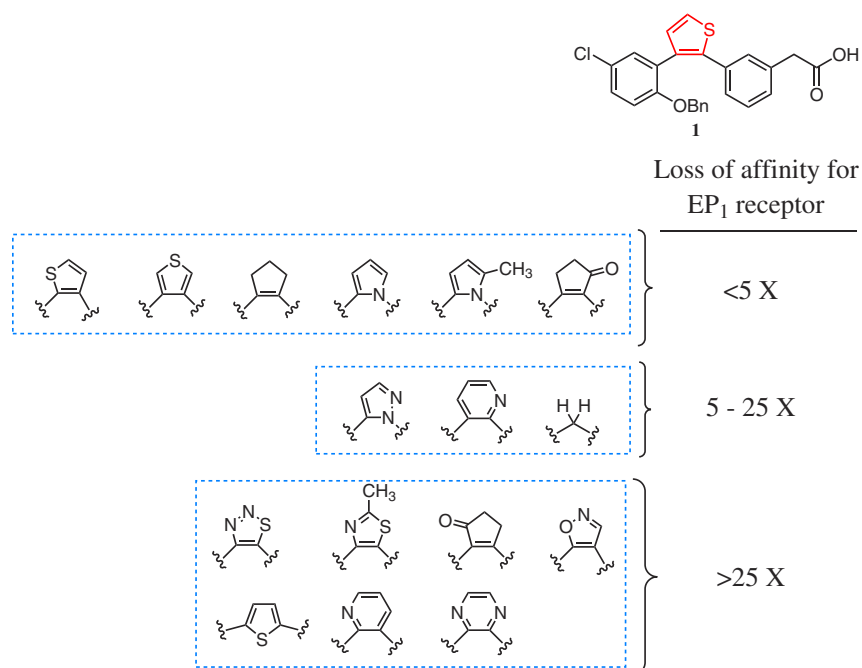
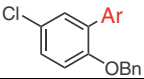
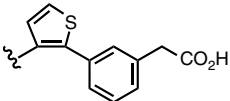
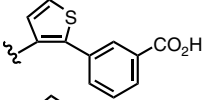
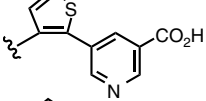
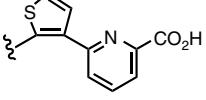


Figure 2. SAR overview at the central ring.

Table 1. Benzoic acid analogs

	Ar	EP ₁ K _i (nM)	EP ₁ 2% HSA K _i (nM) (shift)	Functional assay K _b (nM)	TP K _i (nM) (selectivity)	Rat PK half-life (h)
1		15	220 (15X)	66	150 (10X)	3
2		4	70 (18X)	7	100 (25X)	<0.5
3		3	90 (30X)	4	230 (75X)	<0.5
4		6	280 (45X)	1	140 (25X)	4

Each K_i value is an average of at least three experiments.

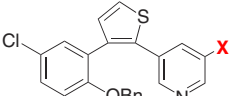
tives were identified with improved binding affinity, selectivity and/or pharmacokinetic profiles.

First is a class of benzoic acid analogs (Table 1). The benzoic acid derivative **2** and the nicotinic acid **3** both show higher affinity for the EP₁ receptor in the binding assay compared to the phenylacetic acid analog **1**. This behavior is translated in the functional assay where antagonists **2** and **3** are 10- to 16-fold more potent than phenylacetic acid **1**. Unfortunately, these two compounds are plagued with short half-lives in rats (*t*_{1/2} < 0.5 h). The best representative of this class is picolinic acid derivative **4**, which binds with high affinity to the human EP₁ receptor (K_i = 6 nM) and is shifted 45-fold to a K_i of 280 nM in the presence of 2% HSA. Picolinic acid **4** is very potent in the functional assay with a K_b of 1 nM. The selectivity ratio of antagonist **4** is at least 100-fold against the EP₂, EP₃, EP₄, DP, FP, and IP receptors and 25-fold versus the TP receptor. Antagonist **4** binds with high affinity to the rat EP₁ receptor (K_i = 12 nM) and, in contrast to other benzoic acid ana-

logs, shows good pharmacokinetics in the same species (*F* = 73%; C_{max} = 20 μM at 4 h, 10 mg/kg P.O., *t*_{1/2} = 4 h). The brain–blood ratio (0.1) is modest for this compound.

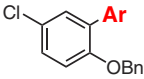
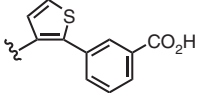
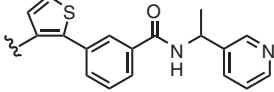
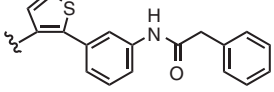
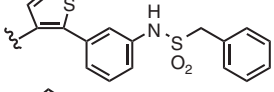
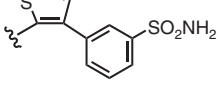
A second class of analogs, the trifluoromethylketone hydrates was discovered by preparing carboxylic acid surrogates. For example, in the nicotinic acid series, the carboxylic acid moiety found in antagonist **3** was replaced by various functional groups (Table 2). While substitution by a bis(trifluoromethyl)methanol (**5**) or a cyanohydrin (**8**) function was detrimental to binding affinity, equipotent ligands were obtained by replacing the carboxylic acid group by a 2,2,2-trifluoroethan-1-ol (**6**) or a trifluoromethylketone hydrate moiety (**7**). This last analog binds with high affinity to the human EP₁ receptor (K_i = 4 nM), is not shifted in presence of 2% HSA (K_i = 5 nM) and presents a selectivity greater than 100-fold against the EP₂, EP₃, EP₄, DP, FP, and IP receptors. Its selectivity versus the TP receptor is 55-fold. Compound **7** is potent (K_b = 70 nM) in the

Table 2. Trifluoromethylketone hydrate as a carboxylic acid bioisostere

	X	EP ₁ K _i (nM)	EP ₁ 2% HSA K _i (nM) (shift)	Functional assay K _b (nM)	TP K _i (nM) (selectivity)	Rat PK Half-life (h)
3		3	90 (30X)	4	230 (75X)	<0.5
5		260	—	—	200 (0.8X)	—
6		3	6 (2X)	—	120 (40X)	1
7		4	5 (1X)	70	220 (55X)	2
8		23	160 (7X)	—	360 (15X)	—

Each K_i value is an average of at least three experiments.

Table 3. Amide and sulfonamide derivatives

	Ar	EP ₁ K _i (nM)	EP ₁ 2% HSA K _i (nM) (shift)	Functional assay K _b (nM)	TP K _i (nM) (selectivity)	Rat PK half-life (h)
2		4	70 (18X)	7	100 (25X)	<0.5
9		9	17 (2X)	97	1200 (130X)	<1
10		4	5 (1X)	—	>21,000 (>5000X)	<1
11		52	72 (1.4X)	—	>21,000 (>400X)	<1
12a		8	18 (2X)	100	390 (50X)	2

Each K_i value is an average of at least three experiments.

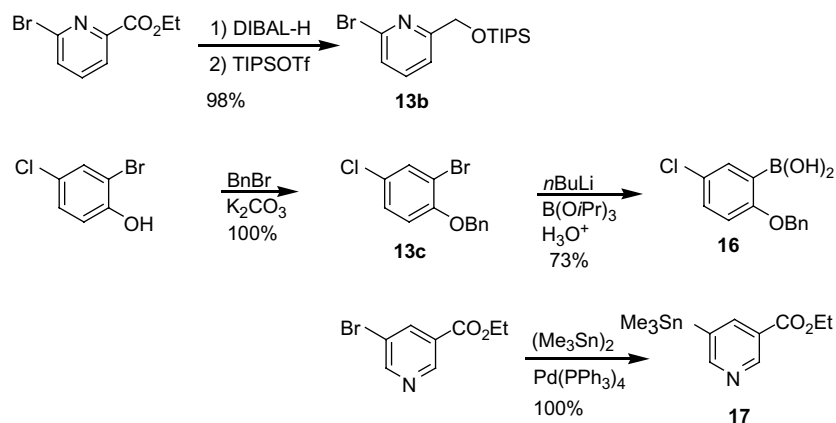
functional EP₁ cell-based assay. Also, antagonist **7** shows good pharmacokinetics in rats ($F = 42\%$; $C_{\max} = 5.5 \mu\text{M}$ at 6 h, 20 mg/kg P.O., $t_{1/2} = 2$ h) and a higher brain–blood ratio of 1.

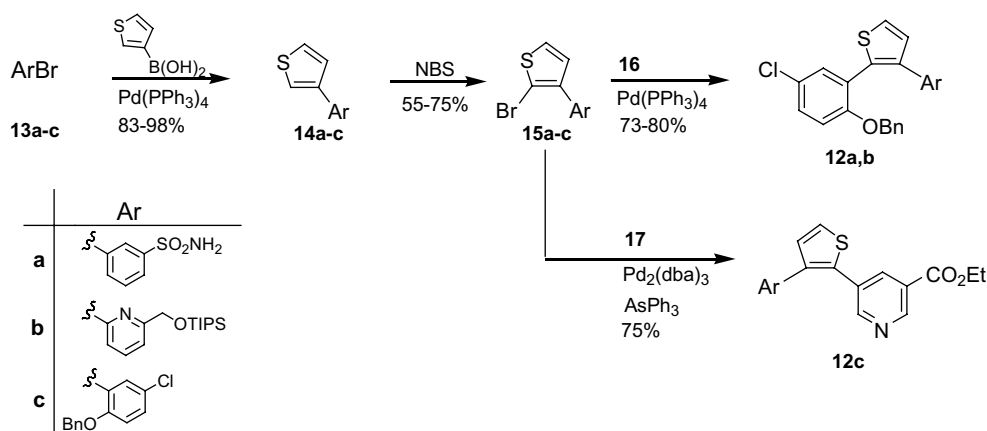
Benzamide and sulfonamide analogs, such as compounds **9–12**, form the third class identified (Table 3). The benzamides **9** and **10** are less protein shifted and more selective than their carboxylic acid counterparts, but formulation difficulties due to insolubility and poor pharmacokinetics reduced their utility. Sulfonamide **12a** binds with high affinity to the human EP₁ receptor ($K_i = 8$ nM) and is shifted only 2-fold to a K_i of 18 nM in the presence of 2% HSA. The selectivity ratio of **12a** is at least 100-fold against the EP₂, EP₃, EP₄, DP, FP, and IP receptors and 50-fold versus the TP receptor. Compound **12a** is potent ($K_b = 100$ nM) in the functional EP₁ cell-based assay. Antagonist **12a** is modestly

bioavailable in rats but presents an acceptable half-life ($F = 9\%$; $C_{\max} = 0.4 \mu\text{M}$ at 30 min, 20 mg/kg P.O., $t_{1/2} = 2$ h) and a high brain–blood ratio of 7.

Synthesis

The synthesis of these antagonists relied heavily on Stille and Suzuki coupling reactions. The preparation of the required building blocks is presented in Scheme 1. Reduction of ethyl 6-bromo-2-pyridinecarboxylate with diisobutylaluminum hydride in tetrahydrofuran followed by treatment with triisopropylsilyl trifluoromethanesulfonate afforded silyl ether **13b** in 98% yield. Benzyl ether **13c** was obtained quantitatively by treatment of 2-bromo-4-chlorophenol with benzyl bromide. Subsequent addition of *n*-butyllithium followed by triisopropyl borate afforded boronic acid **16** in 73% yield. Palladium-catalyzed stannylation of ethyl 5-bromonico-

**Scheme 1.** Preparation of building blocks.

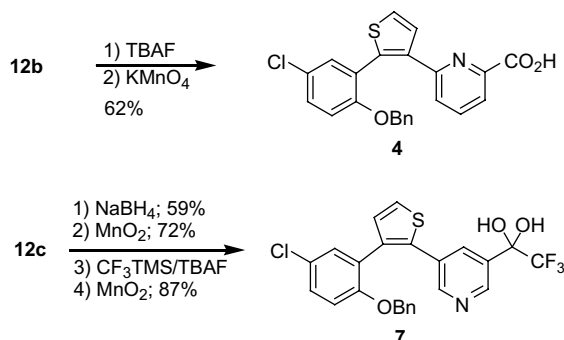


Scheme 2. Palladium-catalyzed coupling reactions.

tinane resulted in the quantitative production of arylstannane **17**.

The elaboration of the tricyclic 2,3-diarylthiophenes is shown in Scheme 2. First, Suzuki coupling between the appropriate aryl bromides **13a–c** and 3-thiophenylboronic acid afforded the corresponding 3-arylthiophenes **14a–c**, which upon treatment with *N*-bromosuccinimide, yielded the 2-bromo-3-arylthiophenes **15a–c**. Further Suzuki coupling reactions between intermediates **15a** and **b** with boronic acid **16** afforded 2,3-diarylthiophenes **12a** and **b**. Alternatively, Stille coupling between bromothiophene **15c** and 3-pyridylstannane **17** gave access to analog **12c**.

The final transformations leading to antagonists **4** and **7** are shown in Scheme 3. Deprotection of silyl ether **12b** with tetrabutylammonium fluoride (TBAF) followed by oxidation of the resulting primary alcohol with potassium permanganate afforded antagonist **4** in 62% yield. Reduction of nicotinate derivative **12c** with sodium borohydride followed by oxidation with manganese oxide afforded the corresponding aldehyde. Reaction of this intermediate with (trifluoromethyl)trimethylsilane and TBAF followed by a further oxidative treatment with manganese oxide gave access to trifluoromethylketone hydrate **7**.



Scheme 3. Synthesis of antagonists **4** and **7**.

In conclusion, we have optimized a novel series of potent EP₁ receptor antagonists. The results of the SAR study in this series led to the identification of antagonists **4**, **7**, and **12a**, which exhibit high affinity for the human EP₁ receptor ($K_i < 10$ nM) and a selectivity greater than 100-fold against the EP₂, EP₃, EP₄, DP, FP, and IP receptors and greater than 25-fold versus the TP receptor. These three antagonists present good pharmacokinetics in rats. Interestingly, antagonists **4**, **7**, and **12a** show significant differences in the way they are distributed in rats with brain to blood ratios of 0.1, 1, and 7, respectively. The overall profiles of these antagonists are complementary and appropriate for the pharmacological evaluation of EP₁ antagonism. These results will be reported in due course.

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