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2,3-Diarylthiophenes as selective EP_1 receptor antagonists

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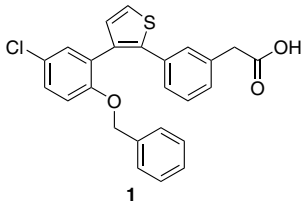
Abstract—The synthesis and the EP_1 receptor binding affinity of 2,3-diarylthiophene derivatives are described. The evaluation of the structure–activity relationship (SAR) in this series led to the identification of compounds **4**, **7**, and **12a**, which exhibit high affinity for the human EP_1 receptor and a selectivity greater than 100-fold against the EP_2, EP_3, EP_4, 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.

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 E_2 (PGE_2), the most abundant mammalian prostaglandin, elicits its effects primarily through interaction with four distinct receptors designated EP_1, EP_2, EP_3 and EP_4.[3] Recent studies with EP_1 receptor knockout mice suggest that this receptor is involved in PGE_2-induced allodynia[4] and in acute inflammatory pain.[5] The involvement of the EP_1 receptor in pain is further supported by the demonstrated efficacy of the EP_1 selective antagonist ONO-8711 in rat models of allodynia,[6] postoperative pain[7], and neuropathic pain.[8] Another EP_1 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 EP_1 receptor in colon[10], and breast[11] carcinogenesis, blood pressure regulation,[5] adaptive gastric cytoprotection,[12] and diabetic nephropathy.[13] Previous reports from this laboratory described the SAR of various prostanoid analogs[14] and of a family of dibenzazocinone derivatives[15] with the human EP_1 receptor. Previous work also permitted the identification of 2,3-diarylthiophene **1** as a prototypical EP_1 receptor antagonist.[16] Compound **1** binds with good affinity to the EP_1 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 EP_2, EP_4, FP, and IP receptors. Its selectivity versus the EP_3, 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 μM 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.



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In order to evaluate the in vivo pharmacology associated with EP_1 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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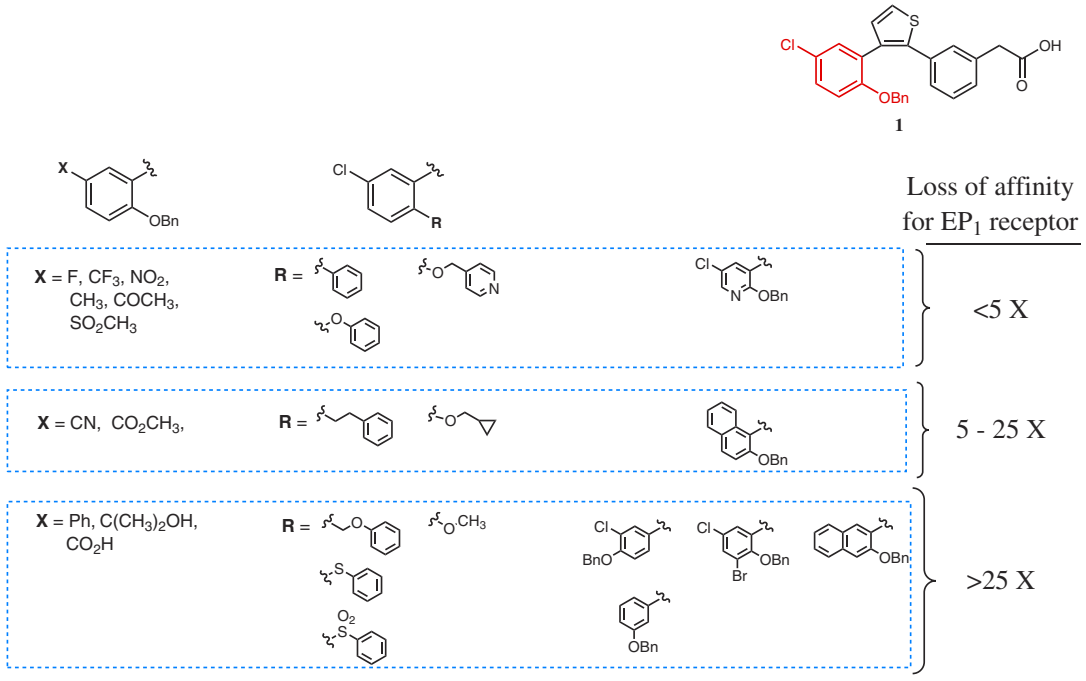


Figure 1. SAR overview at the 3-thienyl position.

probable that centrally mediated EP_1 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 EP_1 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 EP_1 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-

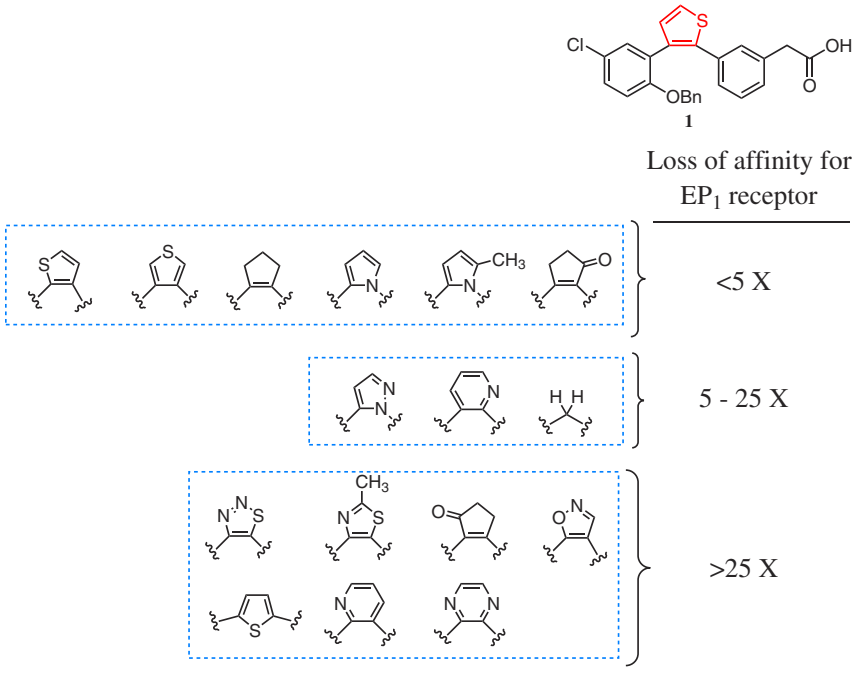
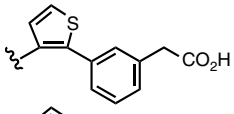
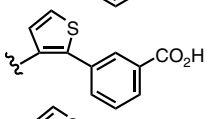
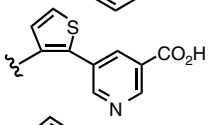
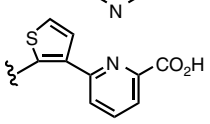


Figure 2. SAR overview at the central ring.

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 EP_1 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 EP_1 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 EP_2, EP_3, EP_4, DP, FP, and IP receptors and 25-fold versus the TP receptor. Antagonist **4** binds with high affinity to the rat EP_1 receptor (K_i = 12 nM) and, in contrast to other benzoic acid analogs, 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.

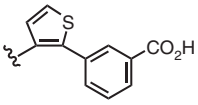
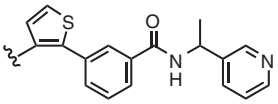
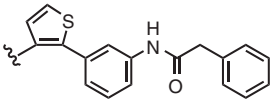
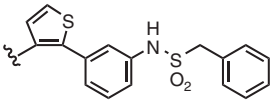
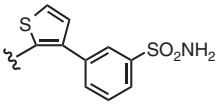
A second class of analogs, the trifluoromethylketone hydrates was discovered by preparing carboxylic acid surrogates. For example, in the nicotinic acid series, the carboxylic acid moiety found in antagonist **3** was replaced by various functional groups (Table 2). While substitution by a bis(trifluoromethyl)methanol (**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 EP_1 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 EP_2, EP_3, EP_4, 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

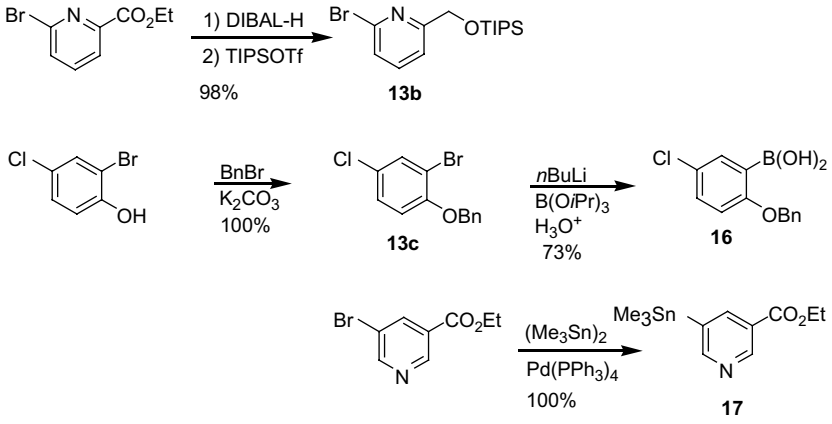
Each K_i value is an average of at least three experiments.

functional EP_1 cell-based assay. Also, antagonist **7** shows good pharmacokinetics in rats ($F = 42\%$; $C_{max} = 5.5$ μM at 6 h, 20 mg/kg P.O., $t_{1/2} = 2$ h) and a higher brain–blood ratio of 1.

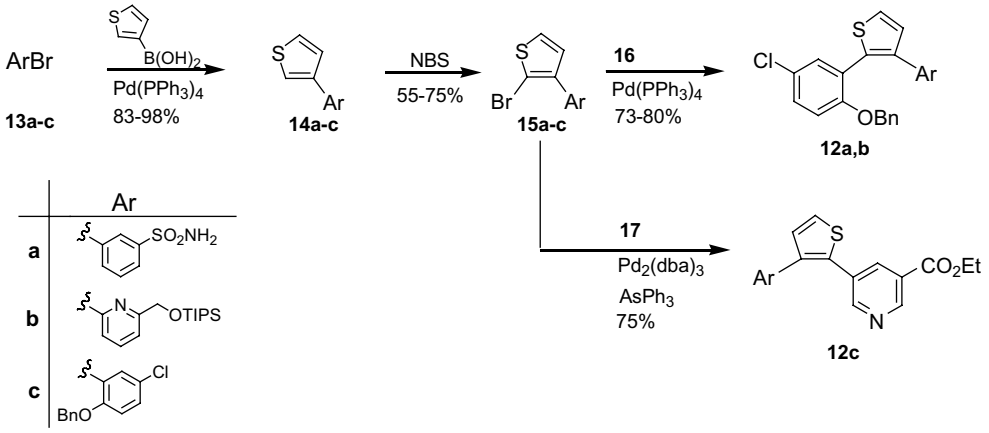
Benzamide and sulfonamide analogs, such as compounds **9**–**12**, form the third class identified (Table 3). The benzamides **9** and **10** are less protein shifted and more selective than their carboxylic acid counterparts, but formulation difficulties due to insolubility and poor pharmacokinetics reduced their utility. Sulfonamide **12a** binds with high affinity to the human EP_1 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 EP_2, EP_3, EP_4, DP, FP, and IP receptors and 50-fold versus the TP receptor. Compound **12a** is potent ($K_b = 100$ nM) in the functional EP_1 cell-based assay. Antagonist **12a** is modestly bioavailable in rats but presents an acceptable half-life ($F = 9\%$; $C_{max} = 0.4$ μM 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.

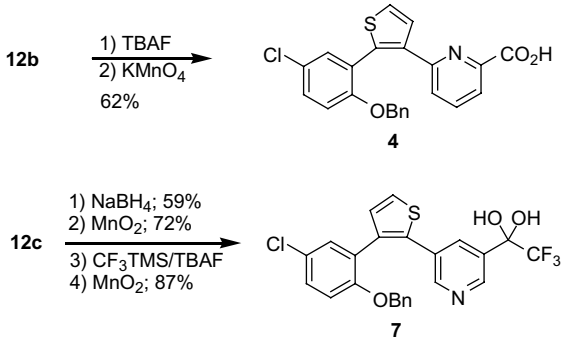


Scheme 2. Palladium-catalyzed coupling reactions.

tinate 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-thiopheneboronic 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 EP_1 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 EP_1 receptor ($K_i < 10$ nM) and a selectivity greater than 100-fold against the EP_2, EP_3, EP_4, 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 EP_1 antagonism. These results will be reported in due course.

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18. HEK cells were grown in DMEM, 10% (v/v) heat inactivated fetal bovine serum, 100 units/ml penicillin-G and 100 μg/ml streptomycin sulfate, 1 mM sodium pyruvate, 0.1 mg /ml hygromycin B, and 500 μg/ml G418 in a humidified atmosphere at 37 °C, 6% CO_2. One day prior to fluorescent imaging plate reader (FLIPR) functional assay, hEP_1 stably transfected HEK293 cells were seeded at 35,000 cells/well. Agonist (PGE_2) and antagonists were prepared in DMSO at 200× concentration and stored at −20 °C. For the FLIPR assay, agonist and antagonist drug plates were thawed and diluted 10-fold to 20× in Hanks Balanced Salt Solution (HBSS) containing 20 mM HEPES, pH 7.4 (HBSS/HEPES). FLIPR no wash dye (Molecular Probes R8033) was diluted in HBSS/ HEPES. Confluent cell monolayers were washed twice in HBSS/ HEPES buffer. HBSS/HEPES buffer and FLIPR dye were added to cell monolayers and cells were incubated for 1 h at 37 °C in the presence of CO_2 to enable dye loading. The plates containing the dye loaded cells, diluted antagonists and agonist were placed in the FLIPR. Addition of compound to cells and fluorescence reading were performed automatically by the FLIPR. Fluorescence was monitored for 15 min (10 min antagonist preincubation followed by 5 min agonist stimulation). For each antagonist tested, a dose response curve (from 0.001 to 30 μM) was first generated for PGE_2. Three additional dose response curves were generated in which cells were preincubated in the presence of fixed concentrations of antagonist for 10 min followed by challenge with increasing concentrations of PGE_2.