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Structure–Activity Relationship of Biaryl Acylsulfonamide Analogues on the Human EP_3 Prostanoid Receptor

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Abstract—Potent and selective ligands for the human EP3 prostanoid receptor are described. Biaryl compounds bearing a tethered ortho substituted acidic moiety were identified as potent EP3 antagonists based on the SAR described herein. The binding affinity of key compounds on all eight human prostanoid receptors is reported.

In response to various extracellular stimuli, prostaglandins are rapidly generated through the consecutive action of the cyclo-oxygenases and distinct synthases on free arachidonic acid and exert their action in close proximity to the site of their synthesis. To date, eight prostanoid receptors have been cloned and characterized. These receptors are members of the growing class of G-protein-coupled receptors. PGE_2 will bind preferentially to the EP_1, EP_2, EP_3, and EP_4 receptors, PGD_2 to the DP and FP receptors, $PGF_{2\alpha}$ to the FP and EP_3 receptors, PGI_2 to the IP receptor and TXA_2 to the TP receptor.[1,2] PGE_2 binding to the EP_3 receptor has been found to play a key role in the regulation of ion transport, smooth muscle contraction of the GI tract, acid secretion, uterine contraction during fertilization and implantation, fever generation and PGE_2-mediated hyperalgesia. The EP_3 receptor has been detected in many organs such as the kidney, the gastrointestinal tract, the uterus and the brain.[3] The molecular characterization of these receptors has generated renewed interest in this field as the selectivity of prostanoid ligands can now be determined. Thus far, PGE_2 or prostanoid-like compounds have been utilized to study the pharmacological role of the EP_3 receptor.[4] Since these compounds behave as agonists and cross react with other prostanoid receptors, the search for selective EP_3 antagonists has become a critical issue in the pharmacological characterization of the EP_3 receptor.

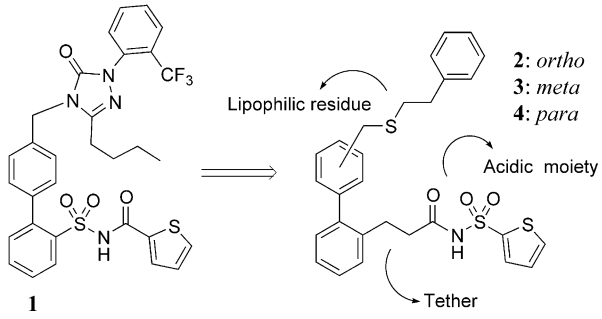

Figure 1. Lead optimization through combinatorial library.

All compounds described herein behave as full antagonist.

Our search for a selective EP_3 receptor ligand was initiated by screening a large number of compounds in our in vitro binding assays.[5] The biaryl acylsulfonamide[6] (**1**), an angiotensin II antagonist, was found to be a submicromolar ligand for the EP_4 receptor (Fig. 1, Table 1). The lead structure was explored by preparing a combinatorial library of 750 compounds leading to the identification of potent and selective EP_3 (**3** and **4**) and EP_1 (**2**) ligands. The resulting SAR clearly established that affinity for the EP_3 receptor, was improved by introducing an *ortho* substituted acidic moiety, consisting of a two carbon tethered acyl sulfonamide, relative to the biaryl scaffold.

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Table 1. Affinity of lead compounds on prostanoid E_2 receptors

Entry		[a]K_i (μM)			
		EP_1	EP_2	EP_3	EP_4
1		>10	>10	7.0	0.046
2	*ortho*[b]	0.083	3.8	0.51	1.5
3	*meta*[b]	0.82	2.0	0.027	1.0
4	*para*[b]	1.7	2.7	0.046	1.1

[a]K_i determinations are averages of at least two experiments.
[b]Position of phenethylthiomethyl ether moiety relative to biaryl bond.

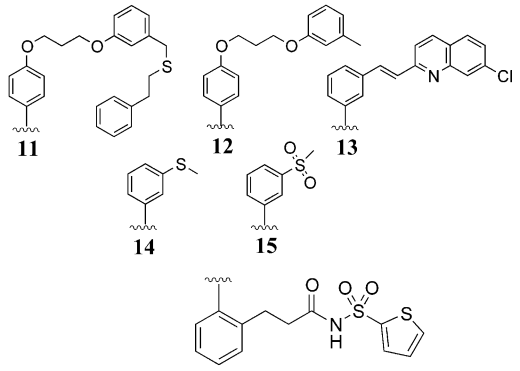
The nature of the acid group was firstly investigated. Acidic surrogates (Table 2) to the acylsulfonamide group, such as carboxylic acid (**5**), tetrazole (**6**), inverse acylsulfonamide (**7**) and sulfonimide (**8**) failed to improved potency on the EP_3 receptor. Interestingly, the introduction of a tetrazole moiety (**6**) modified the receptor specificity to give an EP_2 selective ligand. Attempt to modify the tether length, as exemplified by **9** and **10** did not improve selectivity nor potency toward the EP_3 receptor.

The lipophilic residue was examined next. As demonstrated by the selected examples in Table 3, large lipophilic group are well tolerated. Compound, such as **11**, bearing a long lipophilic chain exhibit high affinity for the EP_3 receptor but are found to be metabolically unstable. The truncated analogue **12** and the quinoline **13**, though metabolically more robust, are less potent on the EP_3 receptor. The relatively small methylthioether **14** is surprisingly only 7.5-fold less potent than **11** while the corresponding polar methylsulfone **15** analogue is a poor ligand. Following characterization of the major metabolites of **11**, we discovered that the acylsulfonamide hydrolysis, was the major degradation pathway

In fact, these acylsulfonamides derivatives of phenylpropionic acid are readily cleaved in an ex vivo rat plasma assay. Compounds **12** and **13** were incubated for 1.5 h in rat plasma at 37 °C. This resulted in partial hydrolysis to their corresponding inactive carboxylic acid in 20 and 66%, respectively. To alleviate this problem we prepared a small number of compounds in which the acylsulfonamide moiety was sterically hindered or electronically enriched. Compounds **16** and **17** (Table 4) in which an α-methyl groups were introduced, are stable in rat plasma. However, they show a 6-fold reduction in affinity for the EP_3 receptor. The introduction of an oxygen atom (**18**) resulted in a rat plasma stable compound with a 2.2-fold shift in affinity. In contrast, the corresponding nitrogen analogue **19** is readily cleaved in rat plasma.

Ultimately, the metabolic instability of the phenylpropionic acylsulfonamide class of compounds was solved by introducing a double bond in the tether. The cinnamic acylsulfonamide **20** (Table 5) is stable in rat plasma and is equipotent to its saturated analogue **13** on the EP_3 receptor with K_i of 0.11 and 0.12 μM,

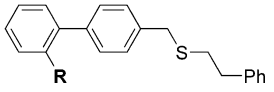
Table 3. SAR on the lipophilic residue



Entry	[a]K_i (μM)			
	EP_1	EP_2	EP_3	EP_4
11	13	4.9	0.016	7.1
12	4.6	2.9	0.053	1.6
13	49	16	0.12	19
14	27	31	0.24	25
15	>100	>100	5.9	>100

[a]K_i determinations are averages of at least two experiments.

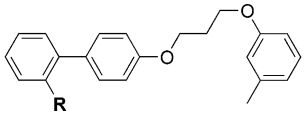
Table 2. SAR on the acidic moiety; acylsulfonamide surrogates and tether length



Entry	R	[a]K_i (μM)			
		EP_1	EP_2	EP_3	EP_4
5	$(CH_2)_2COOH$	8.6	0.14	0.74	4.4
6	$(CH_2)_2$-tetrazole	4.9	0.063	1.0	2.6
7	$(CH_2)_2SO_2NHCOTh$[b]	2.8	2.5	0.48	0.69
8	$(CH_2)_2SO_2NHSO_2Th$[b]	7.5	3.0	0.61	1.1
9	$CH_2CONHSO_2Th$[b]	3.7	0.59	0.33	0.85
10	$(CH_2)_3CONHSO_2Th$[b]	3.0	1.8	0.20	0.70

[a]K_i determinations are averages of at least two experiments.
[b]Th = 2-thienyl.

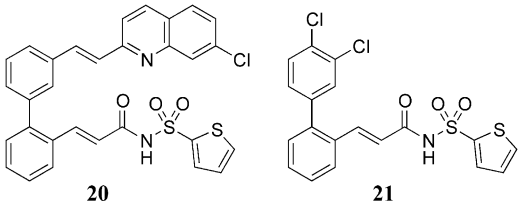
Table 4. SAR on the metabolic stability of the acylsulfonamide moiety[b]



Entry	R	[a]K_i (μM)			
		EP_1	EP_2	EP_3	EP_4
16	$CH_2CH(CH_3)CONHSO_2Th$	5.8	2.9	0.34	1.1
17	$CH_2C(CH_3)_2CONHSO_2Th$	1.8	2.9	0.44	1.0
18	$CH_2OCONHSO_2Th$	2.8	2.1	0.12	0.73
19	$CH_2NHCONHSO_2Th$	15	8.6	0.15	2.9

[a]K_i determinations are averages of at least two experiments.
[b]Th = 2-thienyl.

Table 5. Affinity of compounds **21** on all eight prostanoid receptors



20 **21**

	[a]K_i (μM)							
Entry	EP_1	EP_2	EP_3	EP_4	DP	FP	IP	TP
20	41	70	0.11	28				
21	8.2	27	0.025	3.7	0.83	17	7.3	0.77

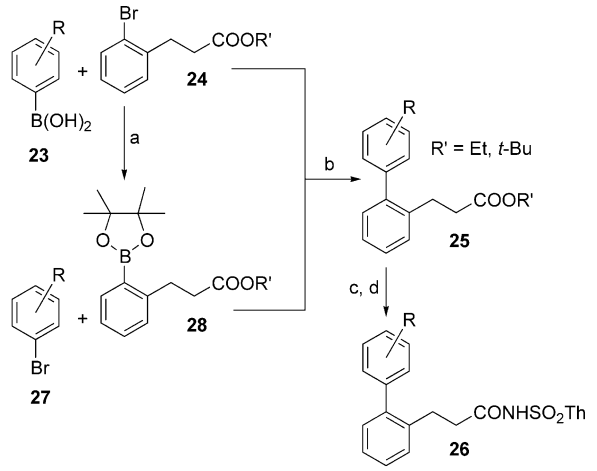
[a]K_i determinations are averages of at least two experiments.

respectively. Following this observation we pursued the SAR of the lipophilic residue. Previous results had shown that the presence of a large lipophilic group was not essential for potency on the EP_3 receptor (e.g., **14**, Table 3). Consequently, we identified the 3,4-dichlorophenyl analogue **21**, with a K_i of 25 nM on the EP_3 receptor and an acceptable selectivity profile (>30-fold) against all eight prostanoid receptors. Compound **21** behaves as a full antagonist with a K_b of 130 nM in our EP_3 functional assay.[7]

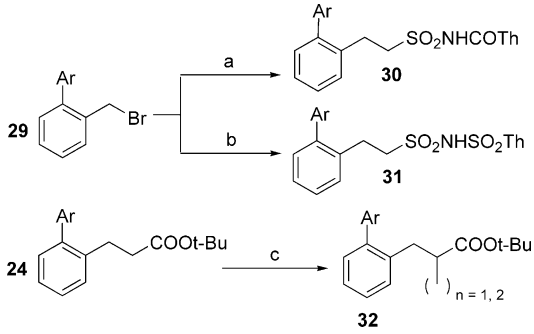
Lead optimization through combinatorial library is a powerful tool to initiate SAR. It allowed us to address the spatial characteristics of a good ligand for the EP_3 receptor. Conventional SAR allowed the optimization of the binding affinity of our antagonists but more importantly to address the metabolic stability of this series of compounds. We ultimately identified the potent and selective ligand **21** which pharmacologic properties will be disclosed elsewhere.

Generally, biaryl acylsulfonamide are prepared in three steps (Scheme 1). Suzuki coupling between a properly substituted phenylboronic acid (**23**) and the corresponding 2-bromophenyl propionate (**24**) affords the biaryl ester **25**. Subsequent deprotection followed by an EDCI/DMAP coupling[8] with a sulfonamine generates the desired acylsulfonamide **26**. Occasionally, the boronic acid **23** could not be prepared or was not commercially available. In such cases, the pinacol-boronate **28** was prepared from the corresponding aryl bromide **24** by a palladium catalyzed coupling with pinacol diboron ester followed by a Suzuki coupling with the aryl bromide **26**. The latter procedure can not be performed on the phenyl cinnamic analogues because of addition of the pinacol diboron ester on the electron poor double bond.

Inverse acylsulfonamides such as **30** and sulfonimides **31** were prepared in a similar fashion as exemplified in Scheme 2. This was achieved by displacement of the benzylic bromide **29** by the dianion generated from the deprotonation of *N*-(thiophene-2-carbonyl) methanesulfonamide or *N*-(thiophene-2-sulfonyl) methanesulfonamide using 2 equiv of *n*-BuLi at low temperature.[9]



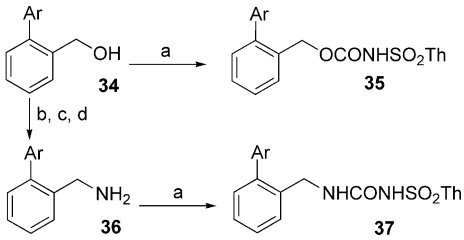
Scheme 1. (a) $PdCl_2(dppf)_2$, pinacoldiborane, KOAc, 80 °C; (b) $Pd(PPh_3)_4$, Na_2CO_3, DME, 80 °C; (c) TFA/CH_2Cl_2 for *t*-Bu, LiOH/MeOH/THF for Et; (d) EDCI, DMAP, thiophene sulfonamine, DMF.



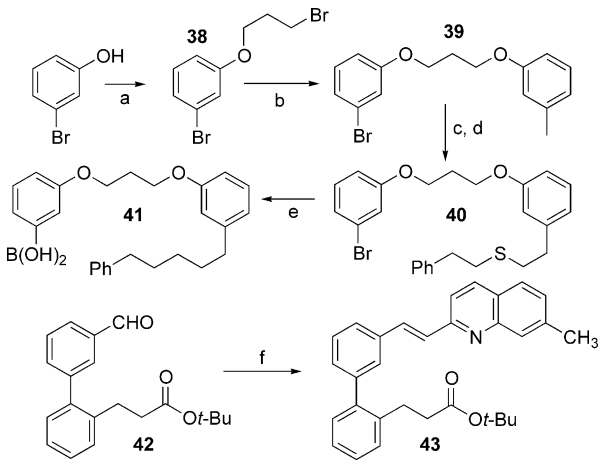
Scheme 2. (a) (i) *N*-(Thiophene-2-carbonyl) methanesulfonamide/*n*-BuLi, THF, −78 °C, (ii) benzyl bromide −78 °C to rt; (b) (i) *N*-(thiophene-2-sulfonyl) methanesulfonamide/*n*-BuLi, THF, −78 °C (ii) benzyl bromide −78 °C to rt; (c) (i) cyclohexyl-*iso*propyl amine/*n*-BuLi, THF, −78 °C, (ii) MeI −78 °C to rt.

The mono α-methyl substituted analogue **32** were prepared (Scheme 2) by first, formation of the enolate of the *tert*-butyl ester **24** using the lithium salt of cyclohexyl-isopropyl amine[10] followed by addition of methyl iodide. The dimethyl analogue was synthesized by repeating the procedure.

The carbamate sulfonamide **35** and urea sulfonamide **37** were synthesized starting from the benzyl alcohol **34** (Scheme 3). Addition of thiophene sulfonyl-isocyanate[11] to **34** led to the desired compound. The alcohol **34** was



Scheme 3. (a) Thiophene sulfonyl-isocyanate, Et_2O, rt; (b) AcOH/HBr, heat; (c) potasium phthalimide, DMF, rt; (d) hydrazine/EtOH, reflux.



Scheme 4. (a) (i) NaH, DMF, (ii) $BrCH_2CH_2CH_2OH$, (iii) AcOH/HBr, heat; (b) 3-methylphenol, NaH, DMF; (c) NBS, AIBN, CCl_4; (d) $PhCH_2CH_2SH$, NaH, DMF; (e) *n*-BuLi, −78 °C, $B(Oi\text{-}Pr)_3$, −78 °C to rt, then HCl; (f) (7-chloro-quinolin-2-ylmethyl) triphenylphosphonium bromide, *t*-BuOK, THF.

converted in three steps to the corresponding benzylic amine **36**. Formation of the benzyl bromide (AcOH/HBr) followed by nucleophilic displacement with the potassium phthalimide and finally, deprotection using hydrazine gave the amine **36**. Addition of thiophene sulfonyl-isocyanate[12] afforded the desired urea sulfonamide.

The aryl boronic acid **41** bearing the lipophilic chain was prepared by addition of 3-bromopropanol on the sodium salt of 3-bromophenol followed by a bromination to give **38**. Displacement with 3-methylphenol, radical bromination and displacement using the sodium salt 2-phenyl ethanethiol gave the aryl bromide **40**. Lithium halogen exchange at low temperature followed by addition of tri-isopropyl boronate and subsequent acid work up led to the desired boronic acid. The quinoline ester **43** was prepared by a Wittig reaction using the ylide generated form (7-chloro-quinolin-2-ylmethyl) triphenylphosphonium bromide and potassium *tert*-butoxide followed by addition of the aldehyde **42** (Scheme 4).

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