Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3813–3816

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure–Activity Relationship of Triaryl Propionic Acid Analogues on the Human EP_3 Prostanoid Receptor

Michel Gallant,* Michel Belley, Marie-Claude Carrière, Anne Chateauneuf, Danielle Denis, Nicolas Lachance, Sonia Lamontagne, Kathleen M. Metters, Nicole Sawyer, Deborah Slipetz, Jean François Truchon and Marc Labelle

Merck Frosst Centre for Therapeutic Research, PO Box 1005, Pointe Claire-Dorval, Québec, Canada H9R 4P8

Received 18 April 2003; revised 10 July 2003; accepted 28 July 2003

Abstract—Potent and selective ligands for the human EP_3 prostanoid receptor are described. Triaryl compounds bearing an *ortho*-substituted propionic acid moiety were identified as potent EP_3 antagonists based on the SAR described herein. The binding affinities of key compound on all eight human prostanoid receptors is reported.

The lipid mediator prostaglandins are generated from the enzymatic metabolism of free arachidonic acid through the consecutive action of the cyclo-oxygenases and distinct synthases. They exert their action in close proximity to the site of their synthesis by interacting with specific receptors. To date, eight prostanoid receptors from the growing class of G-protein-coupled receptors have been cloned and characterized. 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] The EP_3 receptor has been found to play a key role in duodenal bicarbonate secretion and mucosal integrity,[3] platelet aggregation and thrombosis,[4] fever generation[5] and PGE_2-mediated hyperalgesia.[6] Thus far, PGE_2 or prostanoid-like compounds,[7] acting as non-selective agonists and mice lacking specific receptor (knock-out)[8] have been utilized to study the pharmacological role of the EP_3 receptor. Therefore, the search for selective EP_3 antagonists has become a critical issue in the pharmacological characterization of the EP_3 receptor.

We previously described a series of biaryl acylsulfonamides[9] which behaves as full antagonists on the human EP_3 receptor. In the present communication, we wish to report the identification of a second series of EP_3 antagonists. Though structurally similar to the first series, the triaryl series differs mainly by the nature of the acidic moiety essential for potency. In the biaryl series, affinity for the targeted receptor was achieved by introducing an acylsulfonamide moiety as the acidic surrogate (e.g., **1**, Fig. 1). The corresponding carboxylic acid **2** being 64-fold less active (Table 1) on the EP_3 receptor.[10]

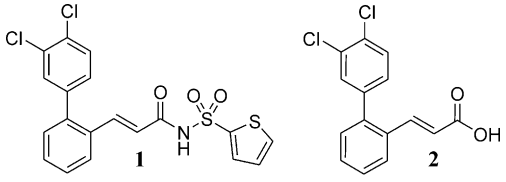


Figure 1. Biaryl EP_3 antagonists.

Because of the inherent instability of the acylsulfonamide moiety in rat plasma, though the introduction of a double bond as in **1** alleviated part of the problem, we initiated a search for potent carboxylic acid ligand on the EP_3 receptor. While conducting SAR on the non-selective EP ligand **3**,[11] (Scheme 1) we found that the introduction of a propenoic acid group led to the potent EP_3 selective antagonist **4**. Compound **4** was further investigated but will be the subject of a separate communication. Introduction of a phenyl group to rigidify the propenyl tether provided us our lead compound, the triaryl propenoic acid **5**.

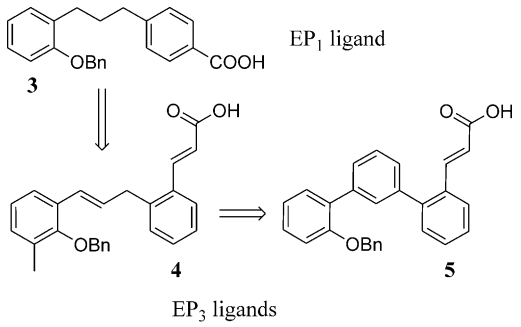
*Corresponding author. Tel.: +51-442-830-54; fax: +51-442-849-00; e-mail: michel_gallant@merck.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00794-7

Table 1. Affinity of lead compounds on prostanoid E_2 receptors

Compd	K_i (μM)[a]			
	EP_1	EP_2	EP_3	EP_4
1	8.1	10	0.025	3.7
2	10	7.5	1.6	>10
3	0.23	0.15	2.3	0.44
4	12	8.1	0.007	1.9
5	72	0.83	0.084	1.7

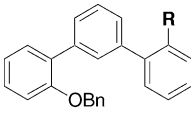
[a]K_i determinations are averages of at least two experiments.



Scheme 1. Identification of the lead compound **5**.

As exemplified in Table 2, the acid moiety was investigated first. The propanoic acid analogue **6** was found to be equipotent to the lead compound **5**. A two-carbon tether between the acid moiety and the triaryl scaffold was, within the limited number of analogues prepared, optimum for affinity on the EP_3 receptor (**6** vs **7**–**9**). The corresponding acylsulfonamide **10**, though more potent, was metabolically unstable as previously disclosed.[9]

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

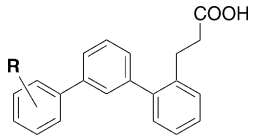


Entry	R	K_i (μM)[a]			
		EP_1	EP_2	EP_3	EP_4
6	$(CH_2)_2COOH$	8.3	3.7	0.055	1.8
7	CH_2COOH	9.3	3.6	1.7	1.1
8	$(CH_2)_3COOH$	11.9	3.5	0.59	2.0
9	$CH_2CH(CH_3)COOH$	8.3	3.6	0.82	1.2
10	$(CH)_2CONHSO_2Th$[b]	2.6	1.6	0.016	1.7

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

The presence of an *ortho*-substituted lipophilic tail on the triaryl scaffold was found to be essential for affinity to the EP_3 receptor. Removing or displacing the benzyloxy group to the *para* position, as in **11** and **13**, respectively (Table 3), led to dramatic loss in affinity (>14-fold) compared to compound **6**. Increasing the lipophilicity by introducing two chlorine atoms as in **14**, improved the affinity for the EP_3 receptor by 2-fold over **6** but with a reduction in selectivity over the EP_1 receptor.

Table 3. SAR on the lipophilic residue



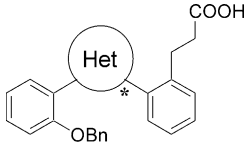
Entry	R	K_i (μM)[a]			
		EP_1	EP_2	EP_3	EP_4
11	H	34	8.1	0.75	6.0
12	$2\text{-}CH_2OPh$	9.5	2.9	0.089	1.0
13	4-OBn	>97	>97	1.5	21
14	$2\text{-}OBn(2,6\text{-}Cl_2)$	1.1	4.6	0.021	1.1

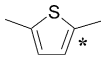
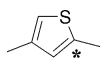
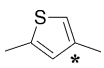
[a]K_i determinations are averages of at least two experiments.

The nature of the triaryl scaffold was also explored. Three disubstituted thiophenes **15**, **16**, and **17** were prepared and were found to have very distinct affinities (Table 4) for the EP_3 receptor. Interestingly, the 2-5-disubstituted thiophene **15** was found to be 22-fold less potent than the corresponding 2,4- (**16**) and 3,5- (**17**) disubstituted analogues. We were unable to identify significant geometrical differences between analogues **15**, **16**, and **17** as determined by gas-phase energy minimization.[12] Several stable conformers were examined and were found to be superimposable for all three analogues. Differences in the spatial orientation of the substituents on the thiophene ring or potential electrostatic interaction with the sulfur atom could not explain the differences in affinity for these analogues based on our calculations.

Following optimization of the lipophilic scaffold, we re-examined the SAR around the acidic moiety following the observation, in a closely related series, that substitution at the β position of the propionic chain was allowed without loss of affinity for the EP_3 receptor. The β-methyl analogue **19** is equipotent to the non-substituted analogue **17** (Table 5), whereas the α-methyl **18** is a poor ligand of the EP_3 receptor. The introduction of a heteroatom in the linker is also allowed as

Table 4. SAR on the triaryl scaffold



Entry	R	K_i (μM)[a]			
		EP_1	EP_2	EP_3	EP_4
15		27	4.1	0.52	1.1
16		17.5	2.9	0.027	0.76
17		17.8	2.5	0.023	0.65

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

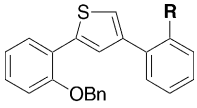
exemplified by the amino acetic acid **21**. Ultimately, the 2-phenyl cyclopropane carboxylic acid **20** was found to be the most potent EP_3 antagonist discovered within the scope of the SAR described herein. Consequently, we identified the antagonist **20**, with a K_i of 3 nM on the EP_3 receptor and an acceptable selectivity profile (>40-fold) against all eight prostanoid receptors (Table 6). The ligand **20** behaves as a full antagonist with a K_b of 5.8 nM in our EP_3 functional assay.[13]

We have therefore identified a second generation of ligands for the EP_3 human receptor. The new series of triaryl carboxylic acids originated from integrating structural features of two separate series which ultimately led to the identification of compound **20**.

In general, triaryl propionic acids were prepared in two consecutive palladium catalyzed cross-coupling reactions. Triphenyl analogues (Scheme 2) were synthesized in a straight-forward manner by a Suzuki–Miura coupling between a properly substituted phenylboronic acid (**22**) and 1,3-dibromobenzene. The resulting biaryl bromide was converted to the corresponding boronic acid by lithium–halogen exchange at low temperature followed by addition of triisopropyl boronate and acidic workup. A subsequent coupling between the resulting boronic acid **23** and 2-bromophenyl propionate **24** afforded, following deprotection, the desired triaryl propionic acid **26**. The latter can be converted to the corresponding acylsulfonamide **27** using an EDCI/DMAP coupling[7] reaction with a sulfonamide.

The two types of disubstituted thiophene analogues (**16** and **17**) were prepared independently by inverting the

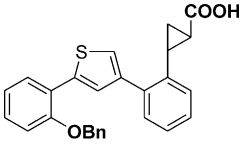
Table 5. SAR on the acidic moiety



Entry	R	K_i (μM)[a]			
		EP_1	EP_2	EP_3	EP_4
18	$CH_2CH(CH_3)COOH$	13	5.5	0.62	0.98
19	$CH(CH_3)CH_2COOH$	35	4.6	0.035	1.1
20	c-$(C_3H_4)COOH$	48	2.3	0.003	0.3
21	$NHCH_2COOH$	85	2.5	0.052	0.46

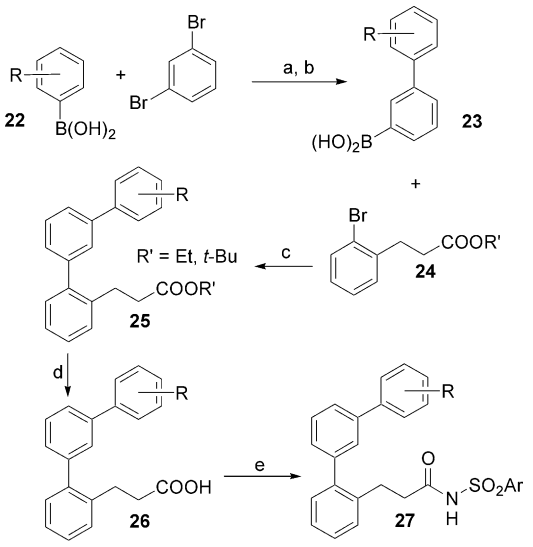
[a]K_i determinations are averages of at least two experiments.

Table 6. Affinity of compounds **21** on all eight prostanoid receptors



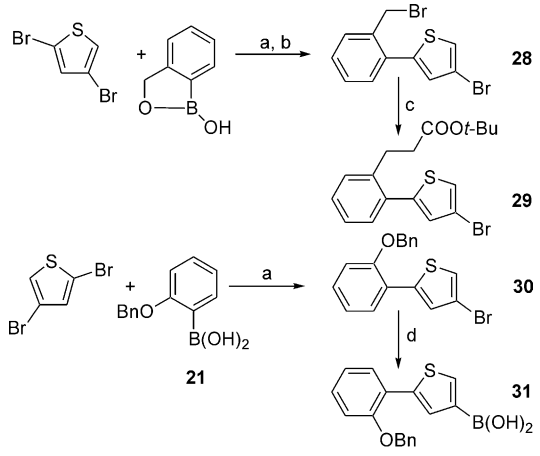
K_i (μM)[a]							
EP_1	EP_2	EP_3	EP_4	DP	FP	IP	TP
47	2.3	0.003	0.30	0.12	22	0.71	0.40

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

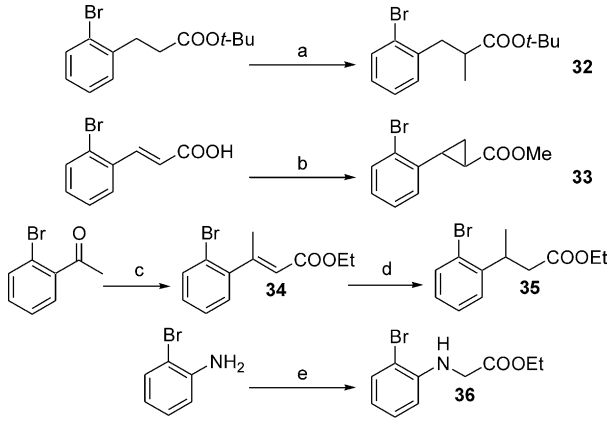


Scheme 2. (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, 80 °C; (b) *n*-BuLi, −78 °C, $B(Oi\text{-}Pr)_3$, −78 °C to rt, then HCl; (c) $Pd(PPh_3)_4$, Na_2CO_3, DME, 80 °C; (d) TFA/CH_2Cl_2 for *t*-Bu, LiOH/MeOH/THF for Et; (e) EDCI, DMAP, thiophene sulfonamine, DMF.

sequence of the two coupling reactions (Scheme 3). Compound **16**, which has the phenyl bearing the acidic moiety located at the 2 position of the thiophene ring, is prepared in the following manner. A Suzuki–Miura coupling between 2,4-dibromothiophene and the commercially available 2-hydroxymethylphenyl boronic acid gave almost exclusively (>10:1) the [2-(4-bromo-thiophen-2-yl)-phenyl]-methanol adduct, which was converted to the corresponding benzyl bromide **28**. The regioselectivity of the coupling reaction (2- vs 4-aryl) was determine by NOE experiments on the benzyl alcohol intermediate. Addition of the bromide **28** to the enolate of *tert*-butyl acetate, generated using a hindered base, afforded the desired ester **29**. Subsequent coupling to 2-benzyloxyphenyl boronic acid provided, after hydrolysis, the desired acid **16**. Similarly, compound **17** was synthesized by a Suzuki–Miura coupling between 2-benzyloxyphenyl boronic acid and 2,4-dibromothiophene to afford the



Scheme 3. (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, 80 °C; (b) HBr, AcOH, 110 °C; (c) (i) *t*-butyl acetate/cyclohexyl-*iso*propyl amine/*n*-BuLi, THF, −78 °C; (ii) add **28**, −78 to 0 °C; (d) *n*-BuLi, −78 °C, $B(Oi\text{-}Pr)_3$, −78 °C to rt, then HCl.



Scheme 4. (a) (i) cyclohexyl-*iso*propyl amine /*n*-BuLi, THF, −78 °C (ii) MeI-78 °C to rt; (b) CH_2N_2, $Pd(OAc)_2$, Et_2O; (c) (i) Triethyl phosphonoacetate/NaH, THF, 0 °C (ii) add XX, 0 °C–rt; (d) $PhSO_2NHNH_2$, Tol, reflux; (e) $BrCH_2COOEt$, Net_3, DMAP, EtOH, reflux.

2-(2-benzyloxy-phenyl)-4-bromo-thiophene adduct **30**. The latter was converted to the corresponding boronic acid **31** using previously described conditions. Finally, the boronic acid was coupled to methyl 2-bromophenyl-propionate to produce, after saponification, the desired analogue **17**.

The syntheses of the functionalized aryl ester intermediates are described in Scheme 4. The α-methyl ester **32** was prepared by addition of MeI to the enolate of *t*-butyl 2-bromophenylpropionate generated using an hindered base at low temperature. The cyclopropane ester **33** was prepared by a palladium acetate catalyzed cyclopropanation of 2-bromo cinnamic acid using diazomethane. The β-methyl ester analogue **35** can be obtained in two steps from 2-bromo acetophenone. Horner–Emmons reaction using triethyl phosphonoacetate followed by reduction of the resulting alkene **34** with phenyl sulfonylhydrazide in refluxing toluene gave the desired ester **35**. Finally, the amino acid **36** was prepared by alkylation of the corresponding aniline with ethyl bromoacetate.

Acknowledgements

The authors would like to thank Dr. Renata Oballa for proofreading this manuscript.

References and Notes

1. Abramovitz, M.; Adam, A.; Boie, Y.; Godbout, C.; Lamontagne, S.; Rochette, C.; Sawyer, N.; Tremblay, N. M.; Belley, M.; Gallant, M.; Dufresne, C.; Gareau, Y.; Ruel, R.; Juteau, H.; Labelle, M.; Ouimet, N.; Metters, K. M. *Biochim. Biophys. Acta* **2000**, *1483*, 285.
2. Kiryiama, A.; Ushikubi, F.; Kobayashi, T.; Hirata, M.; Sugimoto, Y.; Narumiya, S. *Br. J. Pharmacol.* **1997**, *122*, 217.
3. Takeuchi, K.; Ukawa, S.; Kato, S.; Furukawa, O.; Araki, H.; Sugimoto, Y.; Ichikawa, A.; Ushikubi, F.; Narumiya, S. *Gastroenterology* **1999**, *117*, 1128.
4. Fabre, J. E.; Nguyen, M. T.; Athirakul, K.; Coggins, K.; McNeish, J. D.; Autin, S.; Parise, L. K.; FitzGerald, G. A.; Coffman, T. M.; Koller, B. H. *J. Clin. Invest.* **2001**, *107*, 603.
5. Ushikubi, F.; Segi, E.; Sugimoto, Y.; Matsuoka, T.; Kobayashi, T.; Hizaki, H.; Tuboi, K.; Katsuyama, M.; Ichikawa, A.; Tanaka, T.; Yoshida, N.; Narumiya, S. *Nature* **1998**, *395*, 281.
6. Minami, T.; Nakano, H.; Kobayashi, T.; Sugimoto, Y.; Ushikubi, F.; Ichikawa, A.; Narumiya, S.; Ito, S. *Br. J. Pharmacol.* **2001**, *133*, 438.
7. Shimazaki, Y.; Kameo, K.; Tanami, T.; Tanaka, H.; Ono, N.; Kiuchi, Y.; Okamoto, S.; Sato, F.; Ichikawa, A. *Bioorg. Med. Chem.* **2000**, *8*, 353.
8. Ushikubi, F.; Sugimoto, Y.; Ichikawa, A.; Narumirya, S. *Jpn. J. Pharmacol.* **2000**, *83*, 279.
9. Gallant, M.; Carrière, M. C.; Chateauneuf, D.; Denis, D.; Gareau, Y.; Godbout, C.; Greig, G.; Juteau, H.; Lachance, N.; Lacombe, P.; Lamontagne, S.; Metters, K. M.; Rochette, C.; Ruel, R.; Slipetz, D.; Sawyer, N.; Tremblay, N.; Labelle, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2583.
10. See ref 1 for stable expression of prostanoid receptors in the human embryonic kidney (HEK) 293 cell line and also for prostanoid receptor binding assays. The EP_3-III subtype was used for the binding assay.
11. Breault, G.A.; Oldfield, J.; Tucker, H.; Warner, P. World Patent WO9606822, 1996; *Chem. Abstr.* **1996**, *125*, 86305
12. Conformational analysis using the MMFFs force field and DFT B3LYP with a G6-31* basis set was performed.
13. EP_3 antagonist assays were performed in human erythroleukemia cells (ATCC HEL 92.1.7) that endogenously express the EP3 receptor. cAMP accumulation assays were performed in a total of 0.2 mL HBSS containing 100 μM RO-20174 and 15 μM Forskolin. **21** ($0–3\times10^{-6}$ M) was added to the incubation mixture in DMSO (0.5% final) and pre-incubated with the cells (2×10^5 cells per incubation) for 10 min at 37 °C. The reaction was initiated with the EP_3 selective agonist sulprostone (Biomol) ($0–3\times10^{-5}$ M in 0.5% DMSO), and incubated at 37 °C for10 min. The reaction was terminated by boiling the samples for 3 min and cAMP was measured by cAMP SPA assay (Amersham).