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# Comparison between two classes of selective $EP_3$ antagonists and their biological activities

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**Abstract**—Two different series of very potent and selective $EP_3$ antagonists have been reported: a novel series of ortho-substituted cinnamic acids [Belley, M., Gallant, M., Roy, B., Houde, K., Lachance, N., Labelle, M., Trimble, L., Chauret, N., Li, C., Sawyer, N., Tremblay, N., Lamontagne, S., Carrière, M.-C., Denis, D., Greig, G. M., Slipetz, D., Metters, K. M., Gordon, R., Chan, C. C., Zamboni, R. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 527] and the acylsulfonamides of *ortho*-(arylmethyl)cinnamates. [(a) Juteau, H., Gareau, Y., Labelle, M., Sturino, C. F., Sawyer, N., Tremblay, N., Lamontagne, S., Carrière, M.-C., Denis, D., Metters, K. M. *Bioorg. Med. Chem.* **2001**, *9*, 1977; (b) Juteau, H., Gareau, Y., Labelle, M., Lamontagne, S., Tremblay, N., Carrière, M.-C., Denis, D., Sawyer, N., Metters, K. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 747] The structural differences between the two series, along with their biological activity in vivo, in vitro, and metabolism, are analyzed. Some of those compounds, including hybrids containing the best structural features of both series, possess $K_i$ as low as 0.6 nM on the $EP_3$ receptor.


Previously, our group had disclosed two novel series of potent and selective $EP_3$ antagonists which might be potentially useful as analgesics and for the treatment of inflammatory condition such as in arthritis.[1] They were consisting of ortho-substituted cinnamic acids **1**–**3**[1] (Table 1) and acylsulfonamides derived from *ortho*-(arylmethyl)cinnamic acids such as compounds **7b**–**c**[2] (Table 2). These two classes of compounds were discovered using two very distinct approaches: compounds **1**–**3** were found by varying the substitution pattern around the general structure of $EP_1$ antagonists, while **7a**–**c** were discovered by SAR studies starting from a lead compound found by screening our sample collection.[2b] Comparing their structures, three differences can be noted: (1) the styrene group in **1** is replaced by a naphthalene, (2) a carboxylic acid is converted to an acylsulfonamide, and (3) the presence of an additional benzyl ether substituent on compounds **1**–**3**. Herein, we wish to report the effect of each of these modifications on the biological activity of the compounds, along with the biological profile of hybrids containing the best structural features from each series.

In the *ortho*-(arylmethyl)cinnamate series, it was reported that the acylsulfonamides **7b**–**c** were 20 to 60 times more potent than the parent cinnamic acid **7a** (Table 2).[2] To determine whether the same boost in activity could be applied to the cinnamic acids **1**–**3**, a small number of compounds having potencies ranging from 9 to 126 nM on the $EP_3$ receptor were derivatized to their corresponding thiophenesulfonamides.[3] These compounds were synthesized using reactions (g–h) in Scheme 1 or via a coupling reaction with the cinnamic acid in the presence of EDCI.[2a,4]

Table 1 shows the potency of all these acylsulfonamides on the different $PGE_2$ receptors compared with their parent cinnamic acids. The data for the best compound

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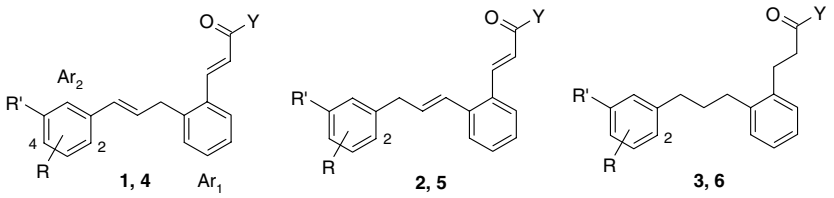
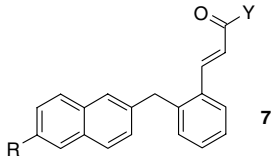
**Table 1.** Affinities ($K_i$, μM)[a] of a series of cinnamic acids and their acylsulfonamide analogs to the different prostanoid $E_2$ receptor subtypes




| Compound | R | R′ | Y | $EP_1$ | $EP_2$ | $EP_{3\text{-III}}$ | $EP_4$ |
|---|---|---|---|---|---|---|---|
| **1a**, **2a** | 2-BnO | Me | OH | 19 | 0.86 | 0.009 | 2.0 |
| **4a**, **5a** | 2-BnO | Me | $NHSO_2$-2-thienyl | 2.3 | 2.1 | 0.010 | 0.96 |
| **1b**, **2b** | H | H | OH | >100 | 1.4 | 0.10 | 4.4 |
| **4b**, **5b** | H | H | $NHSO_2$-2-thienyl | 8.5 | 8.0 | 0.005 | 1.5 |
| **1c**, **2c** | 4-MeO | H | OH | >100 | 2.5 | 0.068 | 6.2 |
| **4c**, **5c** | 4-MeO | H | $NHSO_2$-2-thienyl | 2.6 | 6.5 | 0.0024 | 1.0 |
| **1d**, **2d** | 4-BnO | MeO | OH | >20 | 0.75 | 0.008 | 0.61 |
| **4d**, **5d** | 4-BnO | MeO | $NHSO_2$-2-thienyl | 1.5 | 8.7 | 0.0016 | 0.84 |
| **1e** | 2-(2,6-$Cl_2$Bn)O | Me | OH | 6.7 | 5.4 | 0.003 | 0.62 |
| **3** | 2-BnO | Me | OH | 23 | 0.94 | 0.065 | 0.44 |
| **6** | 2-BnO | Me | $NHSO_2$-2-thienyl | 6.7 | 1.6 | 0.056 | 1.0 |

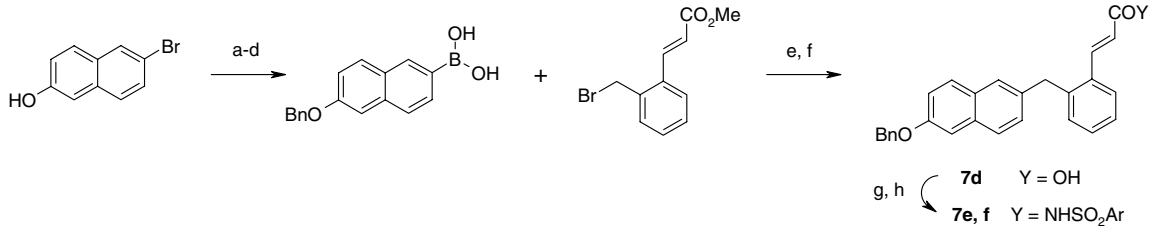
[a] $K_i$ determinations are averages based on at least two experiments.

**Table 2.** Affinities ($K_i$, μM)[a] of a series of (naphthylmethyl)cinnamic acids and their acylsulfonamide analogs to the different prostanoid $E_2$ receptor subtypes




| Compound | R | Y | $EP_1$ | $EP_2$ | $EP_{3\text{-III}}$ | $EP_{3\text{-III}}$ (0.05% HSA) | $EP_4$ |
|---|---|---|---|---|---|---|---|
| **7a** | H | OH | >100 | 12 | 0.047 | 0.40 | 13 |
| **7b** | H | $NHSO_2$-2-thienyl | 9.6 | 8.6 | 0.0013 | 0.28 | 1.3 |
| **7c** | H | $NHSO_2$-(2-MeO-5-BrPh) | 39 | >50 | 0.0006 | 0.0069 | 0.89 |
| **7d** | BnO | OH | >100 | >40 | 0.0032 | 0.046 | >15 |
| **7e** | BnO | $NHSO_2$-2-thienyl | 3.2 | 8.0 | 0.0006 | 0.0024 | 1.1 |
| **7f** | BnO | $NHSO_2$-(2-MeO-5-BrPh) | >100 | >100 | 0.0009 | 0.0007 | 8.0 |

[a] $K_i$ determinations are averages based on at least two experiments.




**Scheme 1.** Reagents and conditions: (a) NaH, DMF, $PhCH_2Br$, 0 °C to rt, 1 h; (b) BuLi, THF, −78 °C; (c) B(Oi-Pr)$_3$, rt, 16 h; (d) HCl, 81% yield (four steps); (e) $Pd(Ph_3P)_4$, aq $Na_2CO_3$, toluene, 100 °C, 4 h, 73% yield; (f) NaOH, MeOH/THF/water, 90% yield; (g) $(COCl)_2$, DMF, $CH_2Cl_2$; (h) $ArSO_2NH_2$, $Et_3N$, $CH_2Cl_2$/THF 1:1, 30–60% yield.

in the cinnamic acid series **1e** are also given for comparison. In general, very potent and selective $EP_3$ antagonists were obtained when the acylsulfonamide derivatives were prepared. The potency improvement could be correlated with the position and/or absence of a benzyl ether substituent on the aromatic ring $Ar_2$. In the absence of that substituent (**4b**/**5b** and **4c**/**5c** vs **1b**/**2b** and **1c**/**2c**) the increase of activity is equivalent to what is found in the *ortho*-(arylmethyl)cinnamate series (compounds **7a**–**b**, Table 2). In the presence of a benzyl ether group in position 2 (**1a**/**2a** and **3** vs **4**/**5a** and **6**), equipotent acylsulfonamides were obtained.

The boost of potency observed with the thiophenesulfonamide substitution is not additive with the effect of introducing a benzyl ether in position 2 of $Ar_2$.

On the other hand, incorporating a benzyl ether in position 4 gives acylsulfonamide analogs which are 5 times more active than their corresponding parent acids (**4d/5d** vs **1d/2d**). This result led us to believe that the addition of a benzyl ether group in position 6 of the (naphthylmethyl)cinnamic acid and acylsulfonamide series might improve their potency as well. Compounds **7d–f** were thus prepared as described in Scheme 1, with the key step involving a palladium catalyzed cross-coupling reaction between methyl (bromomethyl)cinnamate and a naphthaleneboronic acid.[2a]

Table 2 shows the effect of adding that benzyl ether group on the activity of the (naphthylmethyl)cinnamates as selective $EP_3$ antagonists. The most striking result is the 15-fold improvement in activity observed with the cinnamic acid **7d** (vs **7a**), which brings its potency to a level that is closer to the acylsulfonamide derivatives. However, addition of the benzyl group on the acylsulfonamides **7b** and **7c** gives compounds that are equipotent (**7e** and **7f**).

Finally, a comparison of the $K_i$ of the compounds **1b/2b**, **4b/5b**, **1d/2d**, and **4d/5d** with their respective analogs **7a**, **7b**, **7d**, and **7e** shows that the modification of the styryl group of the former compounds to a naphthalene unit is giving approximatively a 3-fold improvement of the potency on the $EP_3$ receptor, along with an appreciable boost of selectivity.

In our functional assay measuring the inhibition of c-AMP production in HEK (EBNA) cells[5] (Table 3), compounds **1a/2a**, **1e**, and **7** all behave as full $EP_3$ antagonists.

Metabolism studies of the $EP_3$ selective antagonists **7** demonstrate that they are more stable in vitro than compounds **1**–**2**.[1] After incubations with rat hepatocytes[6] for 3 h, 43% of **7b** and 30% of **7c** were recovered intact, compared with 8% for the mixture of compounds **1a** and **2a**. With rat liver microsomes,[6] **7b** was 35% metabolized after a typical 1-h incubation, while compound **1a** was 65% metabolized.[1] The major metabolites of **7b** and **7c** were isolated and partially identified by HPLC/MS and NMR. The major metabolic pathways are: (1) hydroxylation of the methylene linker, (2) oxidation of the phenyl group to a phenol, (3) oxidation of the naphthalene moiety to an *ortho*-dihydronaphthalenediol, and (4) hydrolysis of the sulfonamide to the cinnamic acid **7a**. Demethylation of the methoxyphenyl portion of the molecule was also observed for **7c**. In human hepatocytes, however, the metabolism of **7b** and **7c** is reduced and almost exclusively limited to the hydrolysis of the acylsulfonamide to the cinnamic acid **7a** (45% for **7b** and 36% for **7c**, with the remainder being the parent drug).

But even with their enhanced metabolic stability in vitro, the plasma levels and anti-inflammatory activity of compounds **7** are similar to compounds **1a/2a** and **1e** (Table 3) in adult rats. They possess similar bioavailabilities, clearances, and $C_{max}$, with **7** showing a slightly longer half-life. However, in the young rats used in the hyperalgesia model, the plasma levels of compounds **7** are higher. When dosed po in 1% methocel, **7b** and **7c**, but not **7d**, inhibited carrageenan-induced rat paw edema[7] in a dose-dependent manner, but their effect on carrageenan-induced hyperalgesia[7] is very variable, with $ED_{50}$ ranging from 3.3 to >30 mg/kg. Their analgesic activity seems to be not related to their potency in vitro, nor to their plasma levels at the time of the assessment of hyperalgesia (1 h post-dosing; 6th row of Table 3).

We have published data in the past that supported the hypothesis stating that serum protein binding could decrease the effectiveness of a compound in vivo.[8] Addition of human serum albumin (HSA) in the enzymatic assay can help assess this effect. Most of the compounds of Table 2 showed a variable decrease of potency in the presence of HSA. However, this loss of potency is greatly decreased for analogs containing the benzyl ether substituent (**7d–f**). Yet, even if the activity of **7f** is not modified by HSA, it is still inactive in hyperalgesia. We have analyzed other parameters such as their binding affinities to the other prostanoid receptors[9] and their CSF and brain levels in vivo, but we could not find any good correlations.

**Table 3.** Inhibition of c-AMP production and biological profile of selected $EP_3$ antagonists in rats[a]

| | 1a/2a | 1e | 7a | 7b | 7c | 7d | 7f |
|---|---|---|---|---|---|---|---|
| c-AMP assay, $K_b$ (nM) | 32 | 5.8 | | 3.3 | 0.6 | | 0.7 |
| Bioavailability[b] (%) | 97 | 74 | | 144 | 72 | 59 | |
| Clearance[b] (ml/min) | 10 | 16 | | 12 | 1.0 | 3.1 | |
| $t_{1/2}$[b] (2–6 h) | 2 h | 2 h | | 3 h | 4 h | 3 h | |
| $C_{max}$[b] (μM) | 22 (30 min) | 25 (30 min) | | 21 (30 min) | 50 (1 h) | 28 (30 min) | |
| Rat paw edema ($ED_{50}$, mg/kg) | 0.9 | 5.1 | | 1.2 | 3.3 | >10 | |
| Concentration at 1 h post-dosing[c] (μM) | 1.4[d] | 2.5 | 5.4 | 5.0 | 32 | 11[e] | 8 |
| Rat paw hyperalgesia ($ED_{50}$, mg/kg) | 20 | 5.8 | 30 | 6.2 | 3.3 | 4.6 | >30 |

[a] Compounds dosed as their sodium salts.
[b] Compounds dosed at 10 mg/kg po in 0.5% methocel (except for **7d** in 25% β-cyclodextrin) and 5 mg/kg iv in 5% dextrose or 25% β-cyclodextrin in male Sprague–Dawley rats weighing 300–400 g.
[c] Compounds dosed at 10 mg/kg po in 0.5% methocel in the 100 g rats used for the hyperalgesia assay.
[d] Sum of 1.2 μM for **1a** and 0.24 μM for **2a**; ratio of **1a/2a** given po was 1.3:1.
[e] Dosed at 5 mg/kg po.

Several splice variants of the $EP_3$ receptor, defined by different C-terminal cytoplasmic tails, have been discovered.[10] Their localization in the body and their physiologic significance remain uncertain. The antagonists described here may bind in a different manner to these splice variants and it is also possible that only binding to a specific splice variant may be effective for the reduction of fever and/or hyperalgesia.

In summary, we have compared here the activity and biological profile in vivo of two different series of very potent and selective $EP_3$ antagonists: the *ortho*-substituted cinnamic acids **1**–**3** and the acylsulfonamides of *ortho*-(arylmethyl)cinnamates **7**. Compounds **1a/2a**, **1e**, and **7b**–**c** all showed good anti-inflammatory properties, as measured by the rat paw edema assay. Curiously, the analgesic activity of **1a/2a**, **1e**, and **7a**–**f** varies a lot from one compound to the next and seems not to be proportional to their potency on the $EP_3$ receptor, nor to their plasma concentration. Further studies will be needed to really understand why very potent and selective $EP_3$ antagonists are not always efficacious in the rat paw hyperalgesia model.

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