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Structure–activity relationship studies on *ortho*-substituted cinnamic acids, a new class of selective EP_3 antagonists

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Abstract—A series of novel *ortho*-substituted cinnamic acids have been synthesized, and their binding activity and selectivity on the four prostaglandin E_2 receptors evaluated. Many of them are very potent and selective EP_3 antagonists (K_i 3–10 nM), while compound **9** is a very good and selective EP_2 agonist (K_i 8 nM). The biological profile of the EP_2 agonist **9** in vivo and the metabolic profile of selected EP_3 antagonists are also reported.

Prostaglandins (PGs) are generated from arachidonic acid in response to extracellular stimuli and are presumed to play an important role in a variety of physiological and pathophysiological processes in the body. The eight known human prostanoid receptors have been cloned, their sequences determined and the affinities and selectivities of many agonists and antagonists to these receptors have been measured.[1]

Prostaglandin E_2 (PGE_2) binds preferentially to the four receptors EP_1, EP_2, EP_3, and EP_4. Studies on EP_3 knockout mice and experiments with EP_3 agonists suggest that activation of the EP_3 receptor might play a key role in fever generation, hyperalgesia (exaggerated response to a normally painful stimulus), uterine contraction, gastric acid secretion, smooth muscle contraction of the GI tract, neurotransmitter release, and sodium/water reabsorption in kidney tubules.[2] Prior to our work, no EP_3 antagonists had been identified.[3] In order to fully characterize the biological role of this receptor, pharmacologically active and selective antagonists would be required. Herein, we wish to disclose the discovery of potent and selective EP_3 antagonists.

We have previously observed that minor modification of substituents or variation of the substitution pattern around the aromatic cores of ligands for the PG receptors can dramatically alter their EP selectivity profile.[4] Using this strategy, we hoped to obtain selective EP_3 antagonists by modifying the structure of known EP_1 antagonists of general structure **1**, where X and Y are linkers containing 2–3 and 0–2 atoms, respectively (Fig. 1).[5] A small number of compounds were synthesized and we rapidly found that a mixture of the two isomers **2a** and **3a** (Fig. 1 and Table 1) provided very good

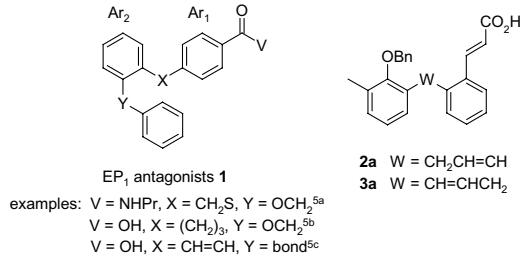

Figure 1.

Keywords: PGE_2; EP_3 receptor antagonist.
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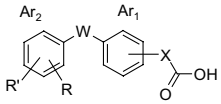
Table 1. Affinities (K_i μM)[a] of a series of cinnamic acids and their analogs for the different human prostanoid E_2 receptor subtypes and their stimulation/inhibition of *c*-AMP production (K_b μM) in HEK (EBNA) cells

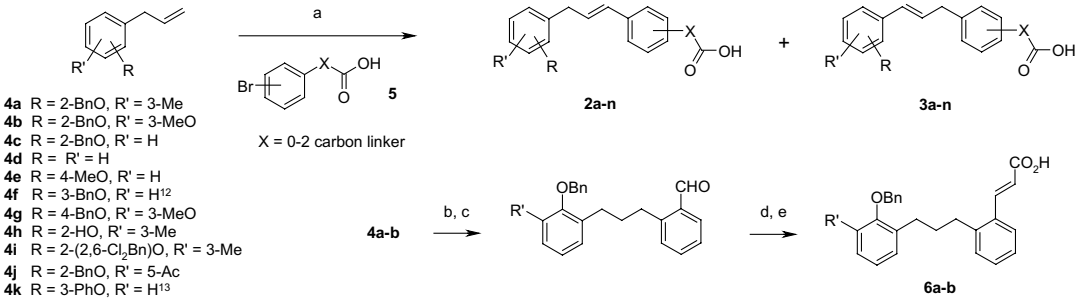


Compd	R	R′	W	Ar_1 Substitution	X	EP_1	EP_2	$EP_{3\text{-III}}$	EP_4	*c*-AMP assay
2a/3a	2-BnO	3-Me	CH=CHCH$_2$[b]	*ortho*	CH=CH	19	0.86	0.009	2.0	0.032
2a	2-BnO	3-Me	CH$_2$CH=CH	*ortho*	CH=CH	>15	0.55	0.013	1.2	0.023
3a	2-BnO	3-Me	CH=CHCH$_2$	*ortho*	CH=CH	12	8.1	0.007	1.9	0.017
2b/3b	2-BnO	3-MeO	CH=CHCH$_2$[b]	*ortho*	CH=CH	16	5.3	0.011	1.8	0.020
2c/3c	2-BnO	H	CH=CHCH$_2$[b]	*ortho*	CH=CH	24	0.68	0.025	1.0	0.050
2d/3d	H	H	CH=CHCH$_2$[b]	*ortho*	CH=CH	>100	1.4	0.10	4.4	
2e/3e	4-MeO	H	CH=CHCH$_2$[b]	*ortho*	CH=CH	>100	2.5	0.068	6.2	
2f/3f[12]	3-BnO	H	CH=CHCH$_2$[b]	*ortho*	CH=CH	14	0.018	0.050	0.45	0.062
2g/3g	4-BnO	3-MeO	CH=CHCH$_2$[b]	*ortho*	CH=CH	>20	0.75	0.008	0.61	0.006
3h	2-HO	3-Me	CH=CHCH$_2$	*ortho*	CH=CH	68	7.3	0.010	1.6	0.028
2i/3i	2-BnO	3-Me	CH=CHCH$_2$[b]	*meta*	CH=CH	21	0.63	2.0	3.1	
2j/3j	2-BnO	3-Me	CH=CHCH$_2$[b]	*para*	CH=CH	>30	1.7	2.9	5.5	
2k/3k[14]	2-BnO	3-Me	CH=CHCH$_2$[b]	*ortho*	—	16	7.1	2.7	5.7	
2l/3l	2-BnO	3-Me	CH=CHCH$_2$[b]	*ortho*	CH$_2$	21	2.9	3.4	4.3	
2m/3m	2-BnO	5-Ac	CH=CHCH$_2$[b]	*ortho*	CH=CH	2.8	8.7	0.26	0.83	
2n/3n[13]	3-PhO	H	CH=CHCH$_2$[b]	*ortho*	CH=CH	>18	0.11	0.060	0.59	
3o	2-(4-FBn)O	3-Me	CH=CHCH$_2$	*ortho*	CH=CH	17	4.1	0.007	2.4	0.004
3p	2-(2,6-Cl$_2$Bn)O	3-Me	CH=CHCH$_2$	*ortho*	CH=CH	6.7	5.4	0.003	0.62	0.006
6a	2-BnO	3-Me	(CH$_2$)$_3$	*ortho*	CH=CH	>10	0.38	0.013	2.9	0.038
6b	2-BnO	3-MeO	(CH$_2$)$_3$	*ortho*	CH=CH	>9	1.1	0.019	4.3	0.029
6c	2-BnO	3-Me	(CH$_2$)$_3$	*ortho*	(CH$_2$)$_2$	23	0.94	0.065	0.44	
7, 8	2-BnO	3-Me	(1,2-*c*-Pr)CH$_2$[c]	*ortho*	CH=CH	>9	0.99	0.041	1.7	0.059
9	2-BnO	H	CH=CH	*ortho*	CH=CH	45	0.008	1.4	2.8	0.003[d]
10/11	2-(2,6-Cl$_2$Bn)O	3-(HOCH$_2$)	CH=CHCH$_2$[e]	*ortho*	CH=CH	7.1	2.5	0.013	0.74	
12	2-(2,6-Cl$_2$Bn)O	3-Me	CH=CHCHOH	*ortho*	CH=CH	3.0	2.1	0.010	0.93	0.030
13	2-(2,6-Cl$_2$Bn)O	3-Me	CHOHCH=CH	*ortho*	CH=CH	13	3.9	0.009	1.0	0.030

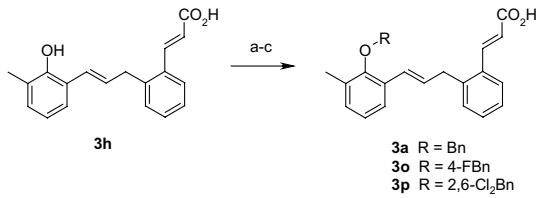
[a] K_i determinations are averages based on at least two experiments.
[b] Mixture of regioisomers where W is also CH$_2$CH=CH.
[c] Mixture of regioisomers where W is also CH$_2$-(1,2-*c*-Pr); *c*-Pr is a cyclopropyl group.
[d] Stimulation of *c*-AMP production.
[e] Mixture of regioisomers where W is CH=CHCH$_2$ for **10** and CH$_2$CH=CH for **11**.

and selective antagonism (9 nM) on the EP_3 receptor subtype. With this lead in hand, additional analogs were prepared and their in vitro activity assessed.

Most of the compounds in this study were synthesized[6] starting from the allylbenzenes **4**, which can be prepared in >80% yield by benzylation of the known phenols with NaH and benzyl bromide in DMF at room temperature. A Heck reaction[7] between **4** and a bromobenzoic, phenylacetic, phenylpropanoic, or cinnamic acid **5** gave a mixture of allylic isomers **2** and **3**, which could easily be purified by a simple trituration in ether/hexane (Scheme 1). We were able to separate the two regioisomers **2a** and **3a** using HPLC techniques only to find that their difference of activity on the EP_3 receptor was only 2-fold (vide infra). We thus decided to test the remaining analogs as mixtures in order to obtain a rapid estimate of the structure–activity relationship (SAR).[8] Later, it



Scheme 1. Reagents and conditions: (a) Pd(OAc)$_2$, LiCl, LiOAc, Bu$_4$NCl, DMF, 100 °C, 16 h,[7] 50–85% yield; (b) 9-BBN; (c) PdCl$_2$ (dppf), 2-bromobenzaldehyde, K$_3$PO$_4$, DMF, 50 °C, 9–16 h,[9] 50–72% yield; (d) Ph$_3$P=CHCO$_2$Me, toluene, 80 °C, 10 h, 82–91% yield; (e) NaOH, MeOH/THF/H$_2$O, 60–98% yield.



Scheme 2. Reagents and conditions: (a) NaH, MeI, DMF, rt, 2 h, 94% yield; (b) NaH, BnBr, DMF, 0 °C, then rt, 1 h, >75% yield; (c) NaOH, MeOH/THF/water, >75% yield.

was found that the major isomer **3h** could be obtained in 48% yield after purification by crystallization. Benzylation of its phenol group afforded the more active isomer **3** (Scheme 2).

Compounds **6a** and **6b** were prepared using a slightly different approach. Addition of 9-BBN to the allylbenzene **4** gave a *B*-alkyl-9-BBN derivative, which could not be cross-coupled[9] with 2-bromocinnamic acid in good yield. It was thus reacted with *o*-bromobenzaldehyde to produce an intermediate aldehyde, which was then subjected to a Wittig–Horner reaction followed by hydrolysis of the ester (Scheme 1). Compound **6c** was prepared via the catalytic hydrogenation of the mixture of compounds **2a** and **3a** over $(Ph_3P)_3RhCl$ in MeOH–EtOAc 1:1. The mixture of compounds **7** and **8**, which contains a cyclopropane unit replacing the double bond in analogs **2a** and **3a**, was prepared in six steps with an overall yield of 71% as follows: (1) Heck reaction between the allylbenzene **4a** and ethyl 2-bromobenzoate (as in Scheme 1, reaction (a)), (2) cyclopropanation of the double bond by sequential treatments with diazomethane and $Pd(OAc)_2$,[10] (3) reduction of the ester to the alcohol with DIBAL-H (THF, −72 °C, then −40 °C for 5 min), (4) oxidation of the alcohol with MnO_2 (EtOAc, rt, 6 h), and (5) Wittig–Horner reaction on the aldehyde followed by hydrolysis of the ester (as in Scheme 1, reactions (d and e)).

The EP_2 selective agonist **9** was prepared via a Heck coupling between 1-(benzyloxy)-2-vinylbenzene[11] and 2-bromocinnamic acid in 67% yield (as in Scheme 1, reaction (a)). The mixture containing the metabolite **10** (W is $CH{=}CHCH_2$) and its isomer **11** was prepared in 42% overall yield in four steps as follows: (1) Claisen rearrangement of 2-allyloxybenzaldehyde to 3-allyl-2-hydroxybenzaldehyde (1,2-dichlorobenzene, reflux 16 h), (2) benzylation of the phenol (as in Scheme 2), (3) reduction of the aldehyde with DIBAL-H (THF, −10 °C, 15 min), and (4) palladium catalyzed cross-coupling with 2-bromocinnamic acid (as in Scheme 1). Finally, compounds **12** and **13** were prepared from acetoxylation of the mixture **2p/3p** using SeO_2 (AcOH, reflux, 20 min, 92% yield),[15] followed by hydrolysis of the acetate (AcOH–water 1:1, reflux, 45 min, 31% yield) and HPLC separation.

Table 1 shows the affinity of several analogs of the lead mixture **2a/3a** on the different EP receptors. The *ortho*-substituted cinnamic acid portion of the molecule is critical for antagonism at the EP_3 receptor. Moving the $CH{=}CHCO_2H$ substituent to the *meta* or *para* position (mixtures **2i/3i** and **2j/3j**), as well as diminishing its chain length to give benzoic or phenylacetic acids (mixtures **2k/3k** and **2l/3l**), reduced the K_i on the EP_3 receptor by at least 200-fold. Reduction of the cinnamic acid to the phenylpropanoic acid also decreased the activity 5-fold (**6c** vs **6a**).

The length and the nature of the linker W between the benzene rings Ar_1 and Ar_2 are also very important. Reducing it by one carbon unit dramatically changed the selectivity pattern to give the very potent and selective EP_2 agonist **9**. The two isomers **2a** and **3a** were also separated by HPLC and the isomer **3a** was found to be twofold more active on the EP_3 receptor and more selective against the EP_2 receptor than **2a**. Finally, reduction or cyclopropanation of the double bond afforded derivatives that were twofold or fivefold less potent on the EP_3 receptor, respectively. These compounds were also less selective toward EP_2 (**6a** vs **3a** and **7/8** vs **2a/3a**).

The substitution pattern around the second benzene ring Ar_2 does not have a pronounced effect on the selectivity or the activity of the compounds. For example, while removal of the benzyl ether gave a less active mixture (compounds **2d/3d** vs **2c/3c**, 4-fold decrease), changing its position around the ring did not significantly alter the activity on the EP_3 receptor (compounds **2g/3g** vs **2b/3b** and **2f/3f** vs **2c/3c**), even though **2f/3f** binds to the EP_2 receptor. Replacement of the benzyloxy by a methoxy or phenoxy group gave slightly less potent compounds (**2e/3e** and **2n/3n** vs **2f/3f**), while the smaller hydroxy substituent was found to be equipotent (compounds **2h/3h** vs **2a/3a**). Addition of a methyl or a methoxy group in position 3 provided a slight increase in activity (compounds **2a/3a** and **2b/3b** vs **2c/3c**), while addition of an electron withdrawing group such as an acetyl in position 5 (compounds **2m/3m** vs **2c/3c**) afforded a mixture of isomers with 10-fold loss in potency. Finally, halogen substituents on the benzyl ether are well tolerated (compound **3o** vs **3a**), with the 2,6-dichloro substitution giving the best EP_3 analog in the series (compound **3p**).

In our functional assays measuring stimulation (for EP_2) or inhibition (for EP_3) of *c*-AMP production in HEK (EBNA) cells,[16] the compounds of Table 1 behave as full EP_3 antagonists, except for **9**, which behaves as a full EP_2 agonist. The EP_3 antagonistic activity is proportional to the affinity on the receptor. The selected EP_3 antagonists described in Table 2 are also selective against the other prostanoid receptors (K_i for the IP, TP, FP, and DP receptors >0.5 μM) and they displayed excellent pharmacokinetics in rats. Metabolism studies on the EP_3 selective antagonist **3a**, carried out with rat liver microsomes supplemented with NADPH, indicated that the compound was 65% metabolized after a typical 1 h incubation.[17] The major metabolites were isolated and characterized by HPLC/MS and NMR.[17] The major metabolic pathways are, in order of decreasing importance: (1) oxidation at the allylic position to give an allylic alcohol, (2) oxidation of the methyl group to produce an hydroxymethyl substituent, (3) oxidation

Table 2. Pharmacokinetics of the EP_2 agonist **9** and the EP_3 antagonists **2a/3a** and **3p** in rats

	2a/3a[a]	**3p**	**9**
Bioavailability (%)[b]	97	74	17
Clearance (mL/min/Kg)	10	5.8	16
C_{max} (μM)	22 (30 min)	25 (30 min)	1.7 (15 min)
$t_{1/2}$	2 h	2 h	2 h

[a] Ratio **2a**:**3a** 1:1.3.

[b] Compounds dosed as their sodium salts at 10 mg/kg PO in 0.5% methocel and 5 mg/kg IV in 5% dextrose, except for **9** where 25% β-cyclodextrin was used as vehicle IV and PO, in male Sprague–Dawley rats weighing 300–400 g.

of both the methyl group and the allylic position, and (4) cleavage of the benzyl group to yield the phenol **3h**. The structures of the major metabolites were also confirmed by synthesizing compounds **10**–**13**, from which **10** and **12** were found to be metabolites of **3p**, and their affinities to the PGE_2 receptors are reported in Table 1. All of these metabolites are EP_3 selective antagonists and only slightly less potent than **3p**, suggesting that they may also contribute to the in vivo activity.

The EP_2 agonist **9** was also quite selective against the other prostanoid receptors (K_i for the IP, DP, and FP receptors >0.7 μM), with the exception of TP (K_i 0.030 μM). Its pharmacokinetic profile is reported in Table 2. When dosed PO in 1% methocel, the EP_2 agonist **9** inhibited carrageenan-induced rat paw edema[18] in a dose-dependent manner with an ED_{50} of 3.3 mg/kg PO. In a carrageenan-induced hyperalgesia model,[18] **9** was active when given IV (ED_{50} 4.5 mg/kg), but was inactive when dosed PO (ED_{50} > 30 mg/kg) in rats, probably due to its low bioavailability.

In summary, we have provided examples demonstrating that changing the substitution pattern around the aromatic cores of known EP_1 antagonists can dramatically alter the selectivity toward prostanoid receptors. We have also described the synthesis, biological activity, pharmacokinetic profile and metabolism of a new series of *ortho*-substituted cinnamic acids. Compound **9** is a very good EP_2 agonist and shows promising anti-inflammatory and analgesic properties. Compounds **3a** and **3p** are very potent and selective EP_3 antagonists and their pharmacological properties in vivo will be discussed in a future publication.

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11. 1-(Benzyloxy)-2-vinylbenzene was prepared from a Wittig reaction on 2-benzyloxybenzaldehyde with $MePPh_3^+Br^-$ and BuLi in THF (0 °C, then rt 2 h, 98% yield).
12. Compound **4f** was prepared in 56% yield from the allylation of 3-(benzyloxy)bromobenzene with $allylSnBu_3$ (1.2 equiv), Ph_3P (2 equiv), LiCl (4 equiv) and $(Ph_3P)_2PdCl_2$ (0.4 equiv) in DMF at reflux for 2 h.
13. Reaction of sodium phenoxide with 1,3-dibromobenzene (5 equiv) in the presence of Cu_2O (0.5 equiv) in DMF at reflux for 4 h gave 1-bromo-3-phenoxybenzene, which was allylated[12] at 100 °C for 3 h to produce **4k** (81% yield).
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