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Discovery of G Protein-Biased EP2 Receptor Agonists

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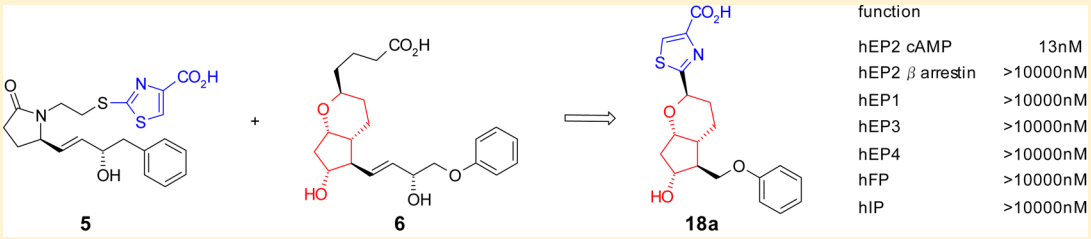
S *Supporting Information*



ABSTRACT: To identify G protein-biased and highly subtype-selective EP2 receptor agonists, a series of bicyclic prostaglandin analogues were designed and synthesized. Structural hybridization of EP2/4 dual agonist **5** and prostacyclin analogue **6**, followed by simplification of the ω chain enabled us to discover novel EP2 agonists with a unique prostacyclin-like scaffold. Further optimization of the ω chain was performed to improve EP2 agonist activity and subtype selectivity. Phenoxy derivative **18a** showed potent agonist activity and excellent subtype selectivity. Furthermore, a series of compounds were identified as G protein-biased EP2 receptor agonists. These are the first examples of biased ligands of prostanoid receptors.

KEYWORDS: *Prostaglandin, EP2, agonist, biased ligand, structure−functional selectivity relationship*

Prostaglandin E_2 (PGE_2) is an oxidative metabolite of arachidonic acid that exerts a wide variety of biological actions through four receptor subtypes, EP1−EP4, in various tissues. The EP2 receptor has been characterized by relaxation of blood vessels.[1] Furthermore, EP2 receptor plays important roles in cytokine production and bone metabolism.[2,3] It has also been reported that activation of EP2 receptor led to neuroprotective effects in ischemic stroke models.[4−8] EP2 receptor receives a lot of attention as a therapeutic target for various diseases.

A number of EP2 agonists have previously been reported.[9−15] The PGE_2 analogue, butaprost (**1**), is well-known as a selective EP2 agonist and is widely used as a chemical tool compound in many studies on pharmacological activities mediated by EP2 receptor (Figure 1). In previous studies, we developed the highly selective and chemically stable EP2 agonist, **2**,[10] which is a good tool compound for EP2 receptor. A number of nonprostanoid scaffolds of EP2 agonists have also been reported to show potent EP2 agonist activity (for example, PF-4217329 **3**[13] and **4**[15]). In recent studies by Pfizer, PF-4217329 **3**, an isopropyl ester, showed remarkable intraocular pressure lowering effects in primary open-angle glaucoma and ocular hypertension.[16] To date, however, there is no EP2 agonist that is approved for clinical use. Although the true reasons for the suspension of clinical trials of EP2 agonists are not clear, we assume that a variety of biological actions induced by EP2 agonists caused crucial side effects for clinical use.

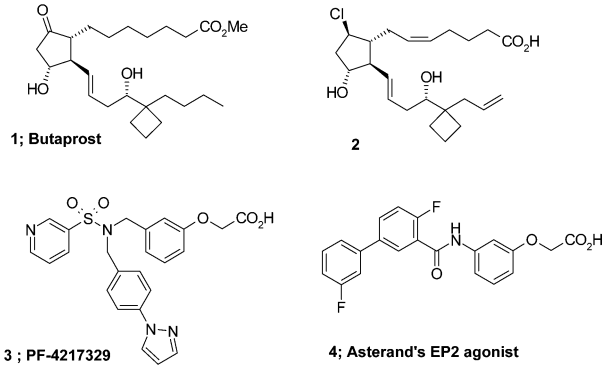


Figure 1. Reported EP2 agonists.

Recently, biased ligands have received a fair amount of attention in drug discovery[17−22] because they have the potential to suppress on-target adverse effects and enhance efficacy. In addition to G protein signaling, G protein-coupled receptors

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(GPCRs) can activate other distinct signaling pathways, like β arrestin-mediated signaling. GPCR-biased ligands are compounds that selectively stimulate either G protein or β arrestin pathways. A number of studies have been performed to investigate the biological roles of G protein- and β arrestin-mediated EP2 receptor signaling. In the brain, EP2 receptor modulates beneficial neuroprotective effects in acute models of excitotoxicity through G protein-mediated cAMP-PKA signaling.[4,5,23,24] Conversely, activation of β arrestin-mediated EP2 receptor signaling led to deleterious effects, like tumorigenesis and angiogenesis.[25−27] Therefore, we hypothesized that G protein-biased ligands of EP2 receptor have the potential to be next generation EP2 agonists that will overcome the clinical problems of previously reported EP2 agonists.

To the best of our knowledge, there is no report of biased ligands of prostanoid receptors. Moreover, we performed screening of our in-house EP2 agonists and failed to identify G protein-biased agonists. As the compounds we evaluated have a similar structure to PGE_2, we aimed to discover G protein-biased EP2 agonists by design and investigation of a new scaffold. In this report, we describe our discovery of novel, highly selective EP2 agonists with a unique bicyclic scaffold, which were identified as G protein-biased EP2 agonists. The functional selectivity and signaling bias of the compounds are also discussed.

First, to identify novel subtype selective EP2 agonists with a new scaffold, we focused on EP2/EP4 dual agonist **5**. In our previous study,[28] a thiazole group of **5** was one of the key substructures to increase EP2 agonist activity. Introduction of a thiazole group into various reported scaffolds seemed to contribute to the development of novel and potent EP2 agonists. Chemically stable prostacyclin analogue **6**,[29] which has been reported by the Upjohn group in the 1970s, showed very weak EP2 agonist activity (EC_{50} = 8900 nM). We designed and synthesized compound **7** with a bicyclic scaffold by hybridization of **6** and the thiazole moiety of **5** (Figure 2). The resulting **7** exhibited remarkably potent EP2 agonist activity as we expected, however, it also showed potent agonist activity toward the other receptor subtypes, especially EP1 and EP3 (Table 1). To increase the subtype selectivity, we next focused on the ω chain of **7**.

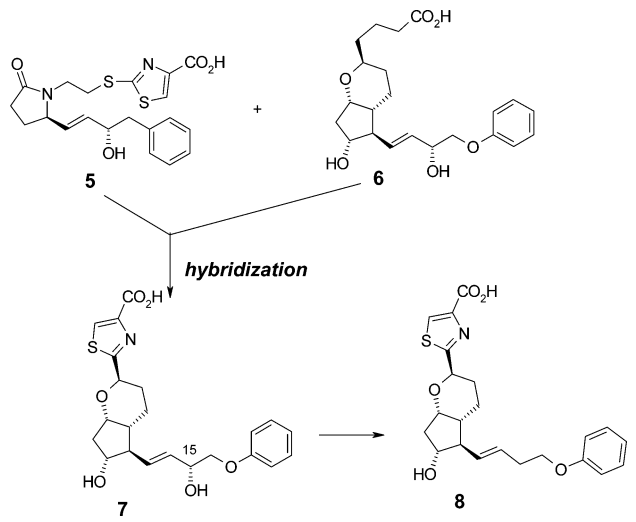

Figure 2. Design of novel EP2 agonists with unique bicyclic scaffold.

Table 1. Subtype Selectivity of Initial Lead Compounds

	EC_{50} (nM)[a]					
cmpd	hEP1	hEP2	hEP3	hEP4	hFP	hIP
5	N.T.[b]	5.6	3000	0.5	N.T.	N.T.
6	N.T.	8900	N.T.	4600	N.T.	47
7	1.4	7.9	0.8	33	32	11
8	160	3.9	260	1900	380	2500

[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments. [b]N.T.: Not tested.

Because all the natural prostanoids (for example, PGE_2, PGI_2, and $PGF_{2\alpha}$) have a hydroxyl group at a particular position in the ω chain, the hydroxyl group is supposed to be a crucial moiety for exerting agonist activity toward PG receptors. However, a number of nonprostanoid scaffolds of EP2 agonists without a hydroxyl group have been reported to show potent EP2 agonist activity (for example, **3**[13] and **4**[15]). We hypothesized that removal of the 15-hydroxyl group from compound **7** would be effective for decreasing the agonist activity toward all of the receptor subtypes except for EP2. As expected, the dehydroxylated derivative **8** dramatically improved the subtype selectivity without any loss of EP2 agonist activity. As a result of the preliminary modification, compound **8** was identified as an initial lead compound that is a highly selective EP2 agonist.

All test compounds in Tables 1−3 were synthesized as outlined in Schemes 1.

Syntheses of compounds **8** and **11a,b** are outlined in Scheme 1A. Oxidation of the common intermediate **9**, followed by the Julia−Kocienski reaction with reagent **19** or **20** gave compound **10a** or **10b**. Hydrolysis provided compounds **8** and **11a**. Reduction of the double bond of **10b**, followed by hydrolysis gave compound **11b**.

Syntheses of compounds **11c,d** are outlined in Scheme 1B. Oxidation of the common intermediate **9**, followed by the Julia−Kocienski reaction using reagent **21** gave compound **12**. Deprotection of the TBS group, followed by a Mitsunobu reaction gave compound **13**. Hydrolysis provided compound **11c**. Reduction of the double bond of **13** and hydrolysis gave compound **11d**.

Synthesis of compound **11e** is outlined in Scheme 1C. Hydrolysis of **14**, esterification and protection of the hydroxyl group by a THP moiety, followed by deprotection of the TBDPS gave alcohol **15**. The resulting alcohol **15** was treated with Dess−Martin reagent to give an aldehyde, which was transformed to a vinyl ether by treatment with a phosphoylide. Acidic hydrolysis of the vinyl ether gave compound **16**. Acetylation of the hydroxy group, followed by reduction of the aldehyde gave compound **17**. Introduction of a phenoxy group by the Mitsunobu reaction and hydrolysis provided compound **11e**.

Syntheses of compounds **18a−n** were started from commercially available phenols as outlined in Scheme 1D. Phenol was introduced into **9** by the Mitsunobu reaction, and the product was hydrolyzed under basic conditions to give **18a**. Compounds **18b−n** were synthesized in a similar manner using the corresponding phenols.

Chemical modification of the ω chain was performed to further improve subtype selectivity of the initial lead compound **8**. As described in Table 2, **11a−e** and **18a** were synthesized to adjust the length between the cyclopentane scaffold and the

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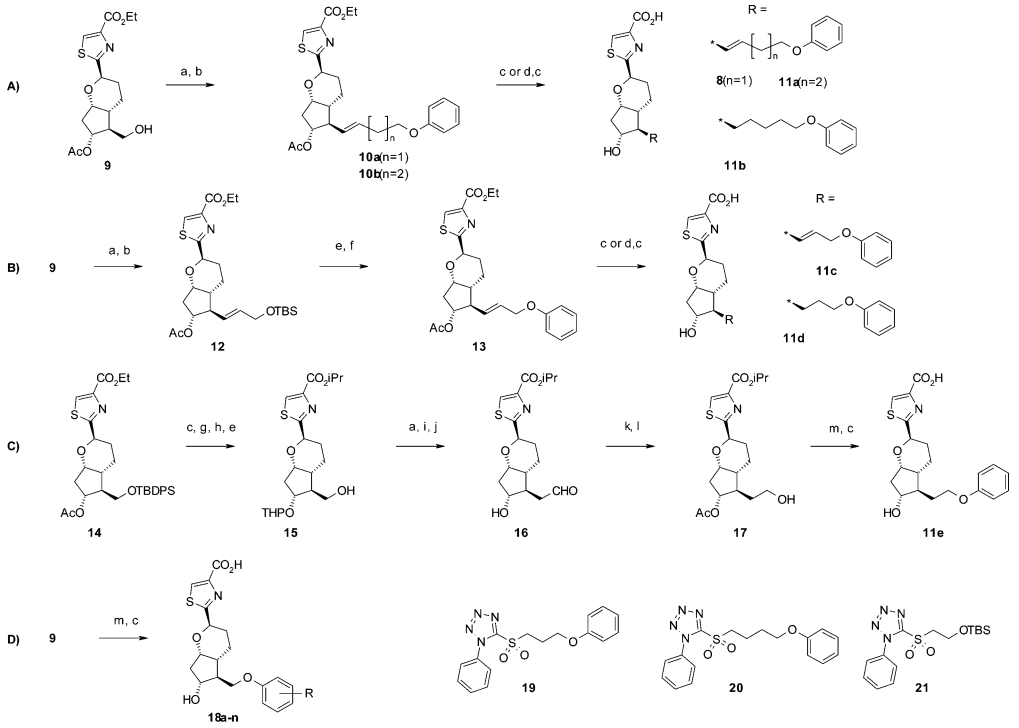
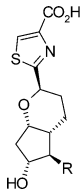
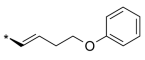
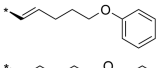
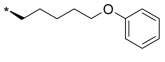
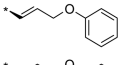
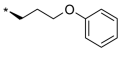
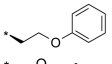
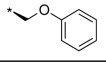
Scheme 1. Syntheses of Compounds 8, 11a−e, and 18a−n[a]



[a]Reagents and conditions: (a) Dess−Martin periodinane, CH_2Cl_2, 0 °C, 77%; (b) **19**, **20**, or **21**, KHMDS, DME, 0 °C, 18−66%; (c) 2 mol/L NaOHaq, DME, MeOH, rt, 56−96%; (d) $TsNHNH_2$, NaOAc, EtOH, H_2O, 80 °C, 55−71%, (e) TBAF, THF, rt, 96%; (f) DEAD, Ph_3P, THF, rt, 82%; (g) *i*-PrI, K_2CO_3, DMF, rt, 54%; (h) PPTS, CH_2Cl_2, DHP, rt; (i) (methoxymethyl)triphenylphospine chrolide, KO*t*Bu, THF, rt, 64%; (j) TsOH, acetone, H_2O, rt, 78%; (k) Ac_2O, Py, rt, 82%; (l) $NaBH_4$, THF, rt, 61%; (m) phenol analogues, TMAD, Bu_3P, THF, rt, 61−92%. Syntheses of common intermediate **9** and Julia−Kocienski reagents **19**−**21** are shown in the Supporting Information.

Table 2. Optimization of the *ω* Chain for Functional and Subtype Selectivity



cmpd	R	hEP2 G protein (cAMP) EC_{50}(nM)[a]	hEP2 G protein (cAMP) E_{max}(%)[b]	hEP2 β arrestin EC_{50}(nM)[a]	hEP2 β arrestin E_{max}(%)	hEP1 EC_{50}(nM)[a]	hEP3 EC_{50}(nM)[a]	hEP4 EC_{50}(nM)[a]	hIP EC_{50}(nM)[a]	hFP EC_{50}(nM)[a]
	Prostaglandin E_2	1.9	105	346	107	3.7	2.5	7.5	347	250
8		3.9	98	>10,000	38	160	260	1900	2500	380
11a		13	91	>10,000	42	970	360	>10,000	>10,000	8500
11b		28	105	>10,000	35	N.T.[c]	N.T.	N.T.	N.T.	N.T.
11c		3.8	96	>10,000	11	700	7600	>10,000	>10,000	>10,000
11d		10	107	>10,000	10	4200	5800	>10,000	>10,000	>10,000
11e		1.4	77	>10,000	22	97	1400	>10,000	>10,000	>10,000
18a[d]		13	118	>10,000	28	>10,000	>10,000	>10,000	>10,000	>10,000

[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments. [b]All E_{max} were normalized to PGE_2 results. [c]N.T.; Not tested. [d]Concentration−response data is shown in Figure S2 (Supporting Information).

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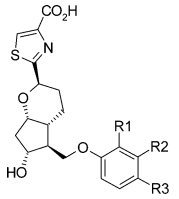
Table 3. Structure−Functional Selectivity Relationship Study of Phenoxy Derivatives



				hEP2			
				G protein (cAMP)		β arrestin	
cmpd	R1	R2	R3	EC_{50} (nM)[a]	E_{max} (%)[b]	EC_{50} (nM)[a]	E_{max} (%)
prostaglandin E_2				1.9	105	346	107
18a	H	H	H	13	118	>10,000	28
18b	Cl	H	H	3.3	95	203	78
18c	CF_3	H	H	0.5	119	11	121
18d	F	H	H	23	59	>10,000	38
18e	H	Me	H	3.6	100	>10,000	38
18f	H	Cl	H	6.5	65	>10,000	27
18g	H	OCF_3	H	0.9	119	69	79
18h	H	CF_3	H	0.9	112	9	62
18i	H	F	H	5.4	94	>10,000	35
18j	H	H	Me	27	85	>10,000	20
18k[c]	H	H	Cl	3.9	96	>10,000	12
18l	H	H	OCF_3	14	110	4500	57
18m	H	H	CF_3	13	103	>10,000	23
18n	H	H	F	7	99	>10,000	22

[a]Assay protocols are provided in the Supporting Information. EC_{50} values represent the mean of two experiments. [b]All E_{max} were normalized to PGE_2 results. [c]Concentration−response data is shown in Figure S2 (Supporting Information).

phenoxy moiety, and to investigate the effect of the double bond of the ω chain.

Compound **11a**, which has a longer linker relative to **8**, demonstrated improved subtype selectivity to EP4 and FP receptors, while it showed a 3.3-fold decreased EP2 agonist activity. Conversely, **11c** with a shorter linker relative to **8** showed potent EP2 agonist activity and improved subtype selectivity. Reduction of the double bond of **11a** and **11c** gave **11b** and **11d** with 2.2- and 2.6-fold decreases in EP2 agonist activity, respectively. Compound **11e**, with a shorter linker relative to **11c**, showed the most potent EP2 agonist activity; however, it also had a potent EP1 agonist activity. The shortest ω chain derivative **18a** exhibited an excellent selectivity to all other receptor subtypes with favorable G protein activity.

We next investigated the functional selectivity of the newly identified EP2 agonists **8**, **11a−e**, and **18a**. The compounds were evaluated by the EP2-mediated β arrestin recruitment Path Hunter assay[30] (DiscoveRX), to determine their functional selectivity. Surprisingly, none of the compounds exerted full agonist activity toward β arrestin recruitment at 10 μM, that is, these compounds were identified as G protein-biased EP2 agonists (see Table 2). To our knowledge, these are the first examples of biased ligands of prostanoid receptors.

To investigate the structure−functional selectivity relationship[20] and improve G protein agonist activity, we performed further optimization of compound **18a**. As demonstrated in Table 3 (**18b−d**), introduction of steric hindering substituents to the *ortho* position on the phenyl moiety improved the β arrestin activity, and the electron nature of the *ortho* substituents had a small effect on its functional selectivity. Introduction of 2-Cl substituent to the phenyl moiety afforded **18b**, which showed a 3.9-fold increase in G protein activity, and it dramatically increased β arrestin recruitment. Compound **18c**, which has a 2-CF_3 substituent on the phenyl moiety, also increased the G protein activity and showed a more than 900-fold increase in β arrestin recruitment. Conversely, compound **18d**, which possesses a 2-F substituent, showed a partial G protein activity without any change in β arrestin recruitment.

As shown in Table 3 (**18e−i**), introduction of *meta* substituents into the phenyl moiety generally improved G protein activity. Additionally, steric hindrance of *meta* substituents on the phenyl moiety significantly affected the functional selectivity, that is, bulky substituents enhanced β arrestin recruitment. Compound **18e**, which possesses a 3-Me substituent on the phenyl moiety, was 3.6-fold more potent in G protein activity without increasing β arrestin activity. Introduction of a 3-Cl substituent gave **18f**, which retained both G protein and β arrestin activity relative to **18a**. However, introduction of 3-OCF_3 and 3-CF_3 substituents gave **18g** and **18h**, respectively, both of which showed a 14-fold increase in G protein activity compared with **18a**. Additionally, **18g** and **18h** showed dramatically increased β arrestin activity (144-fold increase for **18g** and 1111-fold increase for **18h**). Compound **18i** with a 3-F substituent retained both G protein and β arrestin activity relative to **18a**.

Introduction of *para* substituents into the phenyl moiety had little effect on the functional selectivity, namely, all five substituent derivatives were found to be G protein-biased EP2 agonists (see Table3, **18j−n**). Introduction of a 4-Me moiety (**18j**) slightly decreased G protein activity with no effect on β arrestin recruitment. 4-Cl derivative **18k** showed a 3.3-fold more potent G protein activity without an increase in β arrestin activity. Compound **18l**, possessing bulky substituents (OCF_3) at the *para* position, showed moderate G protein activity and very weak β arrestin recruitment. In contrast to the *ortho* or *meta* position, introduction of a CF_3 group into the *para*

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position of the phenyl moiety (**18m**) surprisingly lost the β arrestin activity. Compound **18n**, possessing a less hindered fluoride at the *para* position, showed similar profiles to **18a**.

Overall, steric hindrance of the *ortho* and *meta* positions on the phenyl moiety dramatically enhanced β arrestin recruitment and changed the functional selectivity, though the electron characteristics of the substituents did not show any significant difference in functional selectivity among the analogues. These structure−activity relationship studies suggest that the functional selectivity is easily controlled by small chemical modifications of the phenyl moiety.

To confirm the G protein-biased agonism of our EP2 agonists, lead compound **18a** and **18k**, which was the most potent G protein activity in *para* substituents derivatives, were evaluated in an equimolar comparison[31] of G protein and β arrestin responses (see Figure S1 in Supporting Information). Both compounds showed markedly less β arrestin activity with equivalent G protein activity relative to PGE_2, this result indicates **18a**, and a series of compounds are G protein-biased agonists of EP2 receptor.

In summary, we designed a novel EP2 agonist **8** by hybridization of the thiazole moiety and the bicyclic scaffold mimicking prostacyclins. Simplification of the ω chain enabled us to discover the highly selective EP2 phenoxy derivative **18a**, which was identified as a G protein-biased EP2 agonist. The substituents on the phenyl group of **18a** play an important role in modulating the functional selectivity. Further optimization of phenoxy analogues, including the structure−functional selectivity relationship, will be performed in future studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00455.

Experimental procedures of compounds, characterization data, and conditions of the biological assays (PDF)

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Notes

The authors declare no competing financial interest.

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