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Design and Synthesis of a Highly Selective EP4-Receptor Agonist. Part 1: 3,7-DithiaPG Derivatives with High Selectivity

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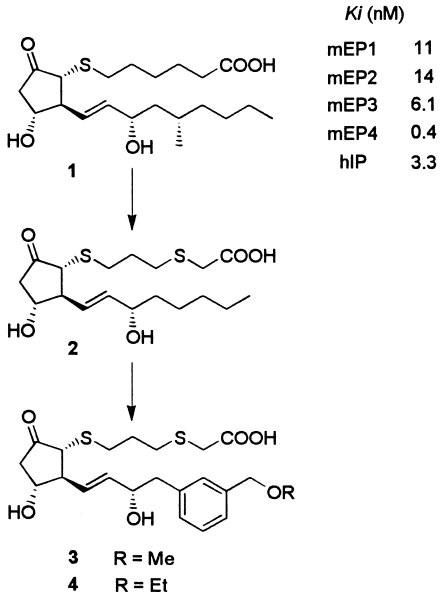
Abstract—A series of 3,7-dithiaPGE$_1$ analogues **3**, **4**, **11**, **16** and **19** were identified as highly selective EP4-receptor agonists starting from the chemical modification of 7-thiaPGE$_1$ analogue **1**. EP4-receptor selectivity and agonist activity were maximized in **3** and **4**.

Introduction

Prostaglandins (PG: PGE_2, PGD_2, $PGF_2\alpha$, PGI_2 and TXA_2) are the oxidative metabolites of arachidonic acid by cyclooxygenase and have been known to be produced in a multitude of significant physiological processes. Much attention has been paid to their receptor levels since the discovery and characterization of PG receptors by Coleman et al.[1] Receptors for the prostanoids have been classified into five classes, EP, FP, IP, DP and TP.[2] Recently, it was reported that the EP receptor can be classified into four subtypes, EP1, EP2, EP3 and EP4, each of which mediates different effects in various tissues and cells.[1] Of these, the EP4-receptor subtype, which is located in thymus, lung, heart, kidney, bone, womb and other organs, has been characterized with relaxation of the saphenous vein of a pig and a dog, and the jugular vein of a rabbit.[3] The biological effects have been considered to coordinate with an enhancement of the intracellular c-AMP concentration. Identification of a highly selective EP4-receptor agonist, which demonstrates a selective agonist activity without causing side effects such as constriction of the uterus or diarrhea, is an attractive approach to develop a clinically useful drug. Some of the PG congeners have been used as probes for the EP4-receptor ligands[4] although their subtype selectivity was poor. We report here the design and synthesis of highly selective EP4-receptor agonists **3** and **4** (Scheme 1), and their biological evaluation. Their structure–activity relationships are also discussed.



Scheme 1. Discovery of 3,7-dithiaPGs **3** and **4** as highly selective EP4-receptor.

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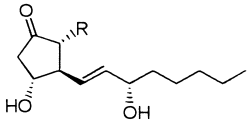
Results and Discussion

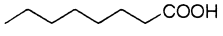
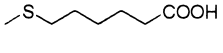
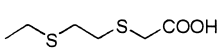
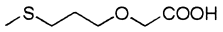
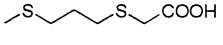
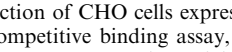
In the course of a screening program of our prostaglandin (PG) library, one of the PG congeners **1** possessing the 7-thia α side chain was found to exhibit a potent EP4-receptor affinity ($K_i = 0.4$ nM) although it showed non-selective affinity also to other PGE-receptor subtypes (EP1, EP2 and EP3). In 1985, 17(*S*),20-dimethyl-7-thiaPGE$_1$ **1**, the slight EP4-receptor selectivity of which is demonstrated in Scheme 1, was reported by Teijin's coworkers.[5] A much more selective EP4-receptor agonist alternative to the reported one **1** has been needed to develop the agonist as a clinically useful drug because of the diversity of the biological activities of PGs. According to the information obtained by the biological evaluation of 7-thiaPGE$_1$ **5**, our chemical modification was started with the introduction of another sulfur atom into the α chain of **5**. Compound **2** exhibited excellent selectivity and potent agonist activity while **6** demonstrated high affinity to both the EP3- and EP4-receptors and much reduced EP4-receptor agonist activity relative to **2**. Replacement of the sulfur atom at position-3 of **2** with an oxygen atom (**8**) increased the affinity to the EP3- and IP-receptor while its affinity to EP4-receptor and its potent agonist activity were maintained. Shifting the sulfur atom of position-7 of **2** to position-6 afforded **7** with 40-fold less potent affinity to the EP4-receptor while its affinity to EP1- and EP3-receptors was restored. A marked reduction in the EP4-receptor agonist activity was caused by this modification. The potency of the EP4-receptor agonist activity was very much influenced by the position of the newly introduced sulfur atom.

As described in Table 1, excellent EC_{50} values were obtained in **2**, **5** and **8** while 40-fold less potency and 195-fold less potency were observed in the 4,7-dithia and 3,6-dithia derivatives **6** and **7**, respectively. The important role of the 7-thia moiety that is played in the potency of the agonist activity of **2** was clearly disclosed by the marked reduction in the agonist activity of **7**. Especially for its reduced EP3-receptor affinity, the important role of the 3-thia moiety of **2** was also disclosed by the comparison with the reduced receptor selectivity of **1**, **5** and **8**. As such, the 3,7-dithia α chain was identified as the most optimized α chain. Next, our attention was paid to the chemical modification of the ω chain of 3,7-dithia PGE$_1$.

After many attempts, 16-phenyl-ω-tetranorPGE$_1$ derivative **9**[6] demonstrated good EP4-receptor selectivity while its agonist activity was still inadequate to evaluate its pharmacological effects (Table 2). A methyl group was introduced into the *ortho*, *meta* and *para* positions of the phenyl moiety of **9**. The *m*-methyl derivative **11** afforded the best result with regard to both the EP4-receptor selectivity and agonist activity. The corresponding methoxy derivatives **13**, **14** and **15** were also prepared and their EP4-receptor selectivity to the EP3-receptor was retained (K_iEP3/K_iEP4 = 30–50), while the agonist activity of **13** was decreased relative to **9**. However, the agonist activity of the *m*-methoxy derivative **14** was 13-fold less potent than the corresponding *m*-methyl derivative **11**. Both of the *meta* derivatives **11** and **14** demonstrated the best EP4-receptor selectivity to the EP3-receptor. Based on the above described findings, another series of 16-(*m*-substituted)phenyl-ω-tetranor-3,7-dithiaPGE$_1$ derivatives were prepared. Of these, the 16-(*m*-methoxymethyl)phenyl derivatives **3** exhibited excellent results both in the EP4-receptor affinity (K_iEP3/K_iEP4 = 120) and the agonist activity (EC_{50} 3.1 nM). The corresponding 16-(*m*-methoxyethyl)phenyl

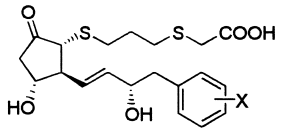
Table 1. Optimization of α chain



Compound	R	Binding K_i (nM)					EC_{50} (nM)
		mEP1	mEP2	mEP3	mEP4	hIP	mEP4
PGE$_1$		22	41	5.0	3.3	150	2.5
5		120	100	4.5	0.7	870	3.7
6		610	1200	4.4	3.2	$>10^4$	170
7		50	1000	3.5	28	$>10^4$	7800
8		1200	150	11	2.3	510	7.7
2		610	280	220	0.7	$>10^4$	4.3

Using membrane fraction of CHO cells expressing the prostanoid receptors, the mouse (m) EP-receptor or human (h) IP-receptor, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al. with some modifications.[9] With regard to the subtype-receptor agonist activity, EC_{50} values were determined based on the effect of the test compounds on the increase in the intracellular c-AMP production in the EP4-receptor.

Table 2. Optimization of ω chain of 3,7-dithiaPGE$_1$



Compound	X	Binding K_i (nM)					EC_{50} (nM)
		mEP1	mEP2	mEP3	mEP4	hIP	mEP4
9	H	2600	3900	130	7.0	$>10^4$	34
10	*o*-Me	$>10^4$	2500	140	22	$>10^4$	420
11	*m*-Me	$>10^4$	760	100	1.9	$>10^4$	2.8
12	*p*-Me	$>10^4$	1200	72	7.3	$>10^4$	270
13	*o*-OMe	$>10^4$	2600	1100	32	$>10^4$	580
14	*m*-OMe	$>10^4$	$>10^4$	510	9.9	$>10^4$	37
15	*p*-OMe	3100	1500	220	7.1	$>10^4$	32
3	*m*-CH_2OMe	$>10^4$	2100	1200	9.7	$>10^4$	3.1
16	*m*-$(CH_2)_2OMe$	$>10^4$	3100	3000	8.5	$>10^4$	12
17	*m*-$(CH_2)_3OMe$	$>10^4$	$>10^4$	$>10^4$	100	$>10^4$	44
18	*m*-CH_2SMe	$>10^4$	5300	430	6.0	$>10^4$	4.8
4	*m*-CH_2OEt	$>10^4$	$>10^4$	4500	9.4	$>10^4$	2.5
19	*m*-CH_2OPr	$>10^4$	7700	3000	5.2	$>10^4$	10

and 16-(*m*-methoxypropyl)phenyl derivatives **16** and **17** were prepared to optimize the chain length between the phenyl moiety and the methoxy moiety. The EP4-receptor selectivity of **16** was slightly improved while its agonist activity was 4-fold less potent than that of **3**. Both the EP4-receptor affinity and agonist activity of **17** were markedly decreased relative to those of **3**. Replacement of the oxygen atom of the methoxy group of **3** with a sulfur atom provided **18** with less selectivity (K_iEP3/K_iEP4 = 70) while the agonist activity was almost retained. Compounds **4** and **19** were prepared for further optimization of the alkoxy moiety of **3**. Both the selectivity (K_iEP3/K_iEP4 = 450) and the agonist activity (EC_{50} 2.5 nM) were improved in the 16-(*m*-ethoxymethyl)phenyl derivative **4** compared with those of **3**. The EP4-receptor selectivity (K_iEP2/K_iEP4 and K_iEP3/K_iEP4) of **19** was also improved while its agonist activity tended to decrease.

Loss of the proton at position-8 of PGE$_1$ occurs readily in both acidic and basic conditions.[7] Epimerization at position-8 may lead to the formation of 8-epi-PGE$_1$. The newly identified 7-thia derivatives reported here were not an exception. All the 7-thiaPGs described here were biologically evaluated as a mixture of 8*R*- and 8*S*-epimers because separate evaluation of both the epimers after their isolation exhibited similar biological results according to our internal data.[8]

In summary, we have discovered a new series of EP4-receptor selective agonists which contain the 3,7-dithia α chain and the 16-(*m*-substituted)phenyl ω chain. A number of the 16-phenyl-3,7-dithiaPGEs, most notably **3**, **4**, **11**, **16** and **19**, were excellent EP4-receptor agonists. The findings from the present study will be used in the discovery of the first clinical candidate which will be reported in the following full paper.

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