Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2033–2035

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of a Highly Selective EP4-Receptor Agonist. Part 2: 5-Thia and 9β-HaloPG Derivatives with Improved Stability

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Received 5 April 2001; accepted 19 May 2001

Abstract—Further chemical modification to identify more chemically stabilized EP4-receptor selective agonists was continued. As a result, a further two EP4-receptor selective agonists 5-thiaPGE$_1$ **2a**, **10** and 9β-chloroPGF$_2$ analogue **11** were discovered.

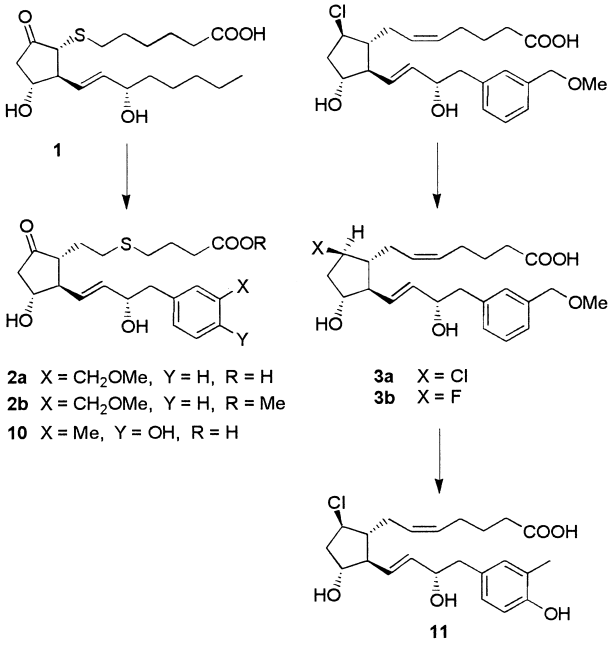
Introduction

In a preceding communication, we reported 3,7-dithia PGE derivatives derived from **1** as highly selective EP4-receptor agonists. Among them, 16-(*m*-methoxymethyl)phenyl-ω-tetranor-3,7-dithiaPGE$_1$ was found to demonstrate the most potent and selective agonist activity. However, PGE derivatives have been well-known to cause a self-degradation starting from its conversion to the corresponding PGA derivatives. In addition, 3,7-dithiaPGE derivatives were found to cause an easy epimerization at position-8[1] (prostaglandin numbering).

Since our final goal is to develop a chemically stable EP4-receptor agonist for clinical use, further chemical modification to block the self-degradation pathway and the easy epimerization at position-8 was continued. We focused our attention on the preparation and biological evaluation of other thiaPGE congeners **2a**–**b** and **10** to inhibit the epimerization, and 9-halo congeners **3a**–**b**, **11** and **13** to block the dehydration of the β-hydroxyketone moiety. We report here the identification of a highly selective EP4-receptor agonist 5-thiaPG congener **2a** whose methyl ester **2b** is currently under clinical trial (Phase I). 9-HaloPG derivatives **3a**–**b**, another series of selective EP4-receptor agonists, are also reported (Scheme 1).

Results and Discussion

As shown in Table 1, our chemical modification was further continued for the improvement of the chemical instability of 3,7-dithiaPGE$_1$ derived from the instability of the 7-thia moiety. Removal of the 7-thia moiety of



Scheme 1. Discovery of 5-thiaPGEs **2**, **10** and 9-haloPGs **3a**–**b**, **11** as highly selective EP4-receptor agonists.

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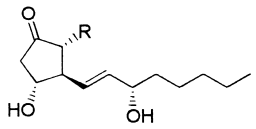
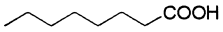
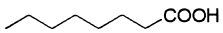
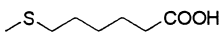
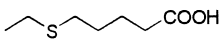
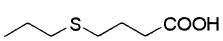
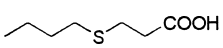
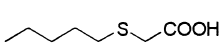
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00365-1

Table 1. Discovery of 5-thia α chain



Compound	R	Binding K_i (nM)					EC_{50} (nM)
		mEP1	mEP2	mEP3	mEP4	hIP	mEP4
4		6.0	22	5.0	3.1	$>10^4$	3.6
5		22	41	5.0	3.3	150	2.5
1		120	100	4.5	0.7	870	3.7
6		95	91	2.0	8.7	1400	1000
7		52	75	1.9	0.5	750	3.6
8		27	340	3.7	11	920	1000
9		200	49	1.2	2.1	$>10^4$	46

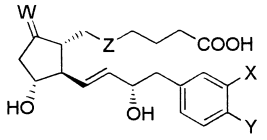
Using membrane fractions of CHO cells expressing the prostanoid receptors, the mouse (m) EP-receptor or human (h) IP-receptor, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al. with some modifications.[8] With regard to the subtype-receptor 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.

3,7-dithiaPGE$_1$ afforded **9** with marked reduction in the EP4-receptor selectivity.[2] Successive replacement of the methylene moiety of PGE$_1$ with a sulfur atom afforded **6**, **7** and **8**, respectively.[3] Their potent affinity to both the EP3- and EP4-receptors was retained in such a chemical modification while the potent agonist activity was maintained only in the 5-thia derivative **7**. As such, position-5 was a newly identified sulfur acceptable position for the EP4 receptor affinity, and 5-thiaPGE$_1$ **7** with its potent EC_{50} value (3.6 nM) was selected as a chemical lead for further optimization because of its greater EP4-receptor selectivity to the EP3-receptor (K_iEP3/K_iEP4 = 4) compared with those of **6**, **8** and **9** (K_iEP3/K_iEP4 <1). Next, our attention was focused on the optimization of the ω chain.

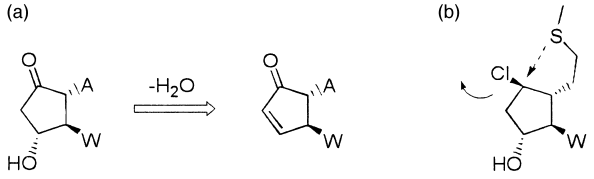
As demonstrated in Table 2, the 5-thia congener **2a**, possessing the most optimized ω chain 16-(*m*-methoxymethyl)phenyl moiety for the EP4-receptor agonist, exhibits potent agonist activity (EC_{50} = 1.6 nM). Another 5-thia congener **10**, possessing the most optimized 16-(3,4-disubstituted)phenyl moiety as the ω chain, was also identified as another excellent EP4-receptor selective agonist although its agonist activity was less potent than that of **2a**. To block the self-degradation pathway (Scheme 2a), which is characteristic of PGE derivatives, was another matter of concern. For this reason, 9β-halo derivatives were synthesized.[4] Of these, 9β-chloroPGF$_2$ derivatives **3a** and **11**, possessing the ω chains optimized for the EP4-receptor, exhibited the EP4-receptor selectivity as was expected, while the selectivity of **11** was much better than that of **3a**. 9β-FluoroPGF$_2$ derivative **3b** also demonstrated EP4-receptor selectivity while its selectivity was determined for the EP4-receptor and was slightly improved with respect to the affinity for EP3-receptor. The 16-(3-methyl-4-hydroxy)phenyl derivative **11** was more potent than the 16-(*m*-methoxymethyl)phenyl derivatives **3a** and **3b** in the agonist activity. The 9β-chloroPGF$_1$ derivative **12** more improved EP4-receptor selectivity compared with the 9β-chloroPGF$_2$ derivatives **3a**, **3b** and **11**, while its agonist activity was much less than those of the latter group. Structural hybridization of **2a** and **3b** provided **13** with nearly 10-fold less potent affinity to the EP4-receptor while its agonist activity was 20- to 40-fold less potent than those of **2a** and **3b**. As a result, the above-described chemical modification resulted in the discovery of **2a** which is a highly selective EP4-receptor agonist with a chemically more stable structure compared with the easily enolizable 3,7-dithiaPGE$_1$, reported previously. Synthesis of 9β-chloro-5-thiaPGF$_1$ was also attempted. However, it could not be isolated for the biological evaluation because of its instability which was estimated to be ascribed to the presumed ring closure reaction by the nucleophilic attack of the 9β-chloro group by the sulfur atom at position-5 (Scheme 2b).

Among the tested compounds, 16-(*m*-methoxymethyl)phenyl-5-thiaPGE$_1$ was identified as a most excellent EP4-receptor selective agonist. The EP4-receptor selectivity of **2a** to the EP3-receptor was discovered to be further improved in the evaluation of its agonist activity (EP3/EP4: 56/0.7→400/1.6). For the sake of its additional chemical stabilization, **2a** was converted to the corresponding methyl ester **2b** which demonstrated nearly identical effects to those of **2a** in the several in vivo studies, while their in vitro evaluation gave different K_i values and EC_{50} values. According to

Table 2. Optimization of the ω chain and discovery of 9β-haloPG derivatives



Compound	W	Z	X	Y	Binding K_i (nM)					EC_{50} (nM)
					mEP1	mEP2	mEP3	mEP4	hIP	mEP4
2a	O	$-CH_2-S-$	CH_2OMe	H	$>10^4$	620	56	0.7	$>10^4$	1.6
10	O	$-CH_2-S-$	Me	OH	$>10^4$	7400	2900	4.9	$>10^4$	20
3a		–CH=CH–	CH_2OMe	H	980	12	92	0.5	$>10^4$	3.3
11		–CH=CH–	Me	OH	3100	290	2200	7.7	$>10^4$	44
3b		–CH=CH–	CH_2OMe	H	1700	58	94	3.4	$>10^4$	14
12		$-CH_2-CH_2$	CH_2OMe	H	5400	370	740	2.7	$>10^4$	160
13		$-CH_2-S-$	CH_2OMe	H	$>10^4$	3100	1500	32	$>10^4$	270



Scheme 2.

our internal data, **2b** exhibited nearly 600-fold less potent EP4-receptor affinity and a nearly 10-fold less potent EC_{50} value than those of **2a**.

The effect of **2b** on the LPS-induced change of TNF-α and IL-10 levels in the plasma of rats was investigated.[5] Increased production of the plasma TNF-α after the intravenous administration of LPS (1 μg/kg) was significantly suppressed by the intravenous infusion of **2b** (10, 30, 100 and 300 ng/kg/min) in a dose-dependent manner. The plasma IL-10 level was augmented by the intravenous infusion of **2b** (30, 100 and 300 ng/kg/min) in a dose-dependent manner. Subcutaneous administration of **2b** (30 and 100 μg/kg) improved the indices of hepatitis induced by *Propionibacterium acnes*/LPS or GalN/LPS.[5] Regarding the uterine activity which mediates the EP3-receptor, **2b** stimulated uterine motility at >300 μg/kg while PGE_2 stimulated it at >1.8 μg/kg.[6]

In summary, we have discovered another series of highly selective EP4-receptor agonists possessing the 5-thia α chain. A number of the derivatives **2a**, **3a**–**b**, **10** and **11**, which contain 9-keto and 9β-halo moieties, were excellent EP4-receptor selective agonists. Based on the findings from this study, **2a** demonstrated the most attractive profile after biological evaluation. The corresponding methyl ester **2b**,[7] which demonstrated nearly identical biological effects to those of **2a** in the in vivo studies, was selected as the first clinical candidate in this field. Full details including chemistry will be reported in the following full papers.

References and Notes

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