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Design and Synthesis of a Highly Selective EP2-Receptor Agonist

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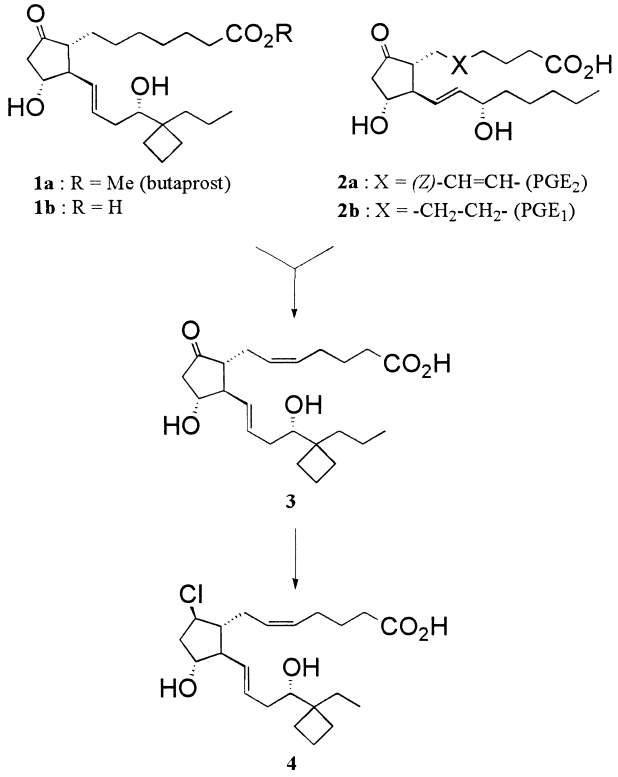
Abstract—EP2-receptor selective agonist **3** was identified by the structural hybridization of butaprost **1a** and PGE_2 **2a**. Based on this information, a chemically more stabilized **4** was discovered as another highly selective EP2-receptor agonist, iv administration of which to anesthetized rats suppressed uterine motility, while PGE_2 **2a** stimulated uterine motility. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Prostanoid receptors are members of the G-protein coupled receptor superfamily. Recently, eight prostanoid receptors were cloned and characterized.[1,2] The study of the receptor level of prostanoids has resulted in renewed interest in the field because the identification of a subtype of selective ligands might enable the development of a clinically useful drug without side effects such as hypotension, diarrhea or uterine contractions. In fact, therapeutic application of most of the launched prostanoids is limited because of their poorly selective agonist activity.[3] Receptors of PGE_2 have been classified into four subtypes EP1, EP2, EP3 and EP4.[1] The diverse biological activities of PGE_2 have been considered to be expressed as a hybrid of the activities which mediate these four EP-receptor subtypes. Among them, the EP2-receptor subtype[4,5] has been characterized with a relaxation of blood vessels, the gastrointestinal tract, the trachea and uterine smooth muscle[6] and has been suggested to play an important role in the production and control of cytokines[7] and bone metabolism.[8] Development of a highly selective EP2-receptor agonist has been expected as one of the attractive approaches to develop a therapeutically useful drug, such as a tocolytic. There is no known report of a selective EP2-receptor agonist which demonstrates a selective agonist activity in an in vivo study. We report here the design and synthesis of a highly selective EP2-receptor agonist **4** (L-lysine salt) that is currently under clinical trial (phase I).



Scheme 1. Discovery of highly selective EP2-receptor agonists **3** and **4**.

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Results and Discussion

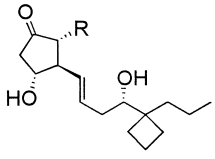
In 1986, 16-hydroxy-17,17-trimethylene-PGE_1 methyl ester (butaprost **1a**) possessing a structurally unique ω chain was reported as a selective EP2-receptor agonist.[9,10] According to our assay, its free form **1b**, which is metabolically produced, demonstrated affinity also to the IP receptor and the IP agonist activity was unexpectedly potent for its K_i value (Table 1). Moreover, the EP2 agonist activity of **1b** was nearly 10-fold less potent than that of PGE_2. Since a more selective and potent EP2-receptor agonist would be expected to be a clinically useful agent, our research of an EP2-receptor agonist was started.

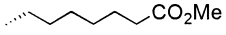
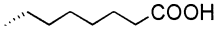
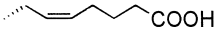
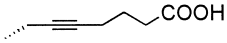
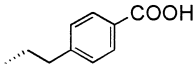
The uniquely designed ω chain of butaprost **1a** was considered to be an attractive entity to start our drug design for a selective EP2-receptor agonist. PGE_2 showed low affinity to the IP-receptor at 10^4 nM, while PGE_1 showed moderate affinity ($K_i = 150$ nM) to the receptor and very potent IP-receptor agonist activity. Based on the information described above, chemical modifications of the α chain of **1b** were made to produce a highly selective EP2-receptor agonist. Structural hybridization of **1b** and PGE_2 **2a** led us to prepare and evaluate **3**[11] which was found to exhibit an excellent EP2-receptor selectivity and agonist activity at the concentration of 43 nM as shown in Table 1. An interphenylene derivative **6** was also discovered to exhibit high EP2-receptor selectivity and excellent agonist activity. The modification described above resulted in the discovery of **3** and **6** in which complete removal of the IP-receptor agonist activity was accomplished. As such, introduction of a (*Z*) double bond or aromatic ring into the α chain of **1b** dramatically improved EP2-receptor selectivity. Replacement of the double bond of **3** with a triple bond appeared not to be an exception while its EP2-receptor affinity was markedly reduced.

Further optimization of the cycloalkyl moiety at the C17 position of **3** was attempted. As demonstrated in Table 2, the EP2-receptor selectivity of **7** and **8** was nicely retained in this chemical modification while the in vitro potency tended to decrease by a larger 17-cycloalkyl derivative. The in vitro activity was maximized in the cyclobutyl derivative **3**. Compound **10**,[11] a C16-epimer of **3**, demonstrated a more decreased EP2-receptor affinity and agonist activity than **3**.

Optimization of the alkyl chain at C17 was also attempted as shown in Table 2. The binding affinities of **11** and **12** were similar to that of **3** while their EP2-receptor selectivities were less than that of **3**. The smaller C17-ethyl derivative **9** tended to show better EP2-receptor selectivity. The agonist activity was maximized in **9**. Compound **12**, possessing the 17-isobutyl moiety, restored both the EP2-receptor selectivity and agonist activity.

Table 1. Optimization of the α chain



Compound	R	Binding K_i(nM)[a]					EC_{50}(nM)[b]	
		mEP1	mEP2	mEP3	mEP4	hIP	mEP2	hIP
1a (butaprost)		$>10^4$	2400	$>10^4$	$>10^4$	$>10^4$	33	37
1b		$>10^4$	73	$>10^4$	$>10^4$	870	32	25
3		$>10^4$	92	$>10^4$	$>10^4$	$>10^4$	43	$>10^4$
5		$>10^4$	1100	$>10^4$	$>10^4$	N.T.[c]	N.T.	N.T.
6		$>10^4$	25	$>10^4$	$>10^4$	$>10^4$	54	$>10^4$
2a	PGE_2	18	38	5.0	3.1	$>10^4$	2.1	260
2b	PGE_1	100	87	5.0	3.3	150	2.6	1.8

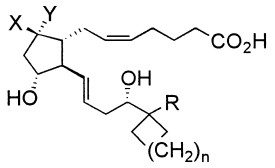
[a]Using membrane fractions of CHO cells expressing the prostanoid receptors, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al.[3] with some modifications. When the test compound did not displace binding of radioligands by 50% even at a concentration of 10^4 nM, the K_i value was not determined (expressed $>10^4$).
[b]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 cAMP production in each receptor, the mouse (m) EP2 receptor or human (h) IP-receptor.
[c]N.T., not tested.

Our final goal was to develop a chemically stable EP2-receptor agonist as a clinically useful drug. However, PGE derivatives including the compounds described above cause self-degradation starting from its initial conversion to the corresponding PGA derivatives. To block such a degradation pathway, the C9-carbonyl moiety of these PGE derivatives had to be chemically modified. Removal of the carbonyl oxygen of **9** produced **14** with a marked loss of agonist activity while the binding affinity (K_i value) was maintained. Introduction of a 9β-chloro group[12,13] into **14** provided **4** with a marked increase in both the EP2-receptor selectivity and the agonist activity, while the 9α-chloro derivative **15** exhibited less potency both in the binding affinity and agonist activity relative to **4**. This modification resulted in the discovery of a highly selective EP2-receptor agonist **4**.

The in vivo activities of the test compounds **3**, **4** and a butaprost free form **1b** were evaluated using anesthetized pregnant rats (on days 18–20 of pregnancy; $n = 5$–6). In this test, PGE_2 **2a** stimulated uterine motility,[14] while **3**, **4** and **1b** suppressed uterine motility (Table 3). Intravenous administration of **4** caused significant suppression of uterine motility in a dose-dependent manner. Increased potency in the suppression of the uterine motility of **4** was observed relative to that of **1b**. Complete removal of the side effects such as hypotension could not be accomplished although their hypotensive effect tended to decrease by their reduced affinity to the IP-receptor compared with that of **1b**. As a result, the hypotension caused by **3** and **4** were estimated to be one of the effects inherent in the EP2-receptor agonist. Based on the highly selective EP2-receptor affinity, potent agonist activity and the in vivo effect on spontaneous uterine motility in late term pregnant rats, compound **4** was selected for further clinical evaluation.

In summary, a highly selective EP2-receptor agonist **4**, whose lysine salt[15] was selected for further clinical evaluation, was identified by the chemical modification of butaprost **1a** (Scheme 1). Using compound **4**, the biological activities, which mediate EP2-receptor, will be disclosed soon. This is the first report of a clinical candidate as a highly selective EP2-receptor agonist. Full details including chemistry will be reported in the following full paper which will be submitted to *Bioorg. Med. Chem.*

Table 2. Optimization of the substituent of position-9 and ω chain



Compound	R	*n*	X, Y	Binding K_i(nM)[a]					EC_{50}(nM)[b]
				mEP1	mEP2	mEP3	mEP4	hIP	mEP2
7	n-C_3H_7	2	X = Y = O	$>10^4$	370	$>10^4$	$>10^4$	$>10^4$	580
8	n-C_3H_7	3	X = Y = O	$>10^4$	3300	$>10^4$	$>10^4$	$>10^4$	$>10^4$
9	C_2H_5	1	X = Y = O	$>10^4$	30	$>10^4$	$>10^4$	$>10^4$	11
10 (16-epimer of **3**)	n-C_3H_7	1	X = Y = O	$>10^4$	330	1200	$>10^4$	$>10^4$	220
11	n-C_4H_9	1	X = Y = O	780	43	2000	$>10^4$	$>10^4$	71
12	n-C_5H_{11}	1	X = Y = O	1600	20	830	2100	$>10^4$	130
13	i-C_4H_9	1	X = Y = O	$>10^4$	40	$>10^4$	$>10^4$	$>10^4$	45
14	C_2H_5	1	X = Y = H	5100	11	$>10^4$	$>10^4$	$>10^4$	1400
4	C_2H_5	1	X = Cl Y = H	3400	2.2[c]	$>10^4$	$>10^4$	$>10^4$	2.8
15	C_2H_5	1	X = H Y = Cl	$>10^4$	13	$>10^4$	$>10^4$	$>10^4$	39

[a]Using membrane fractions of CHO cells expressing the prostanoid receptors, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al.[3] with some modifications. When the test compound did not displace binding of radioligands by 50% even at a concentration of 10^4 nM, the K_i value was not determined (expressed $>10^4$).
[b]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 cAMP production in the mouse (m) EP2 receptor.
[c]Compound **4** bound to human EP2-receptor with a K_i value of 0.74 nM.

Table 3. Pharmacological effects of selective EP2-receptor agonist in rats

Compounds	Suppression of uterine motility[ab] ED_{50} (μg/kg iv)	Hypotensive effect maximal response[c] ($n = 5$–6, Δ mmHg)
1b	455.2	39.8
3	274.7	22.8
4	32.9	24.4

[a]The test compounds were intravenously administrated to anesthetized pregnant rats (on days 18–20 of pregnancy, $n = 5$–6). Uterine motility was evaluated according to the Montevideo method.[16] Uterine activity was calculated from the uterine motility for the 5-min period before and after commencement of the administration, and post-dose uterine activity was calculated as the percentage of inhibition to pre-dose uterine activity.
[b]PGE_2 stimulated uterine motility at >1.8 μg/kg iv.[14]
[c]Maximal response of the hypotensive effect of **1b**, **3** and **4** were observed at doses of 1000, 1000 and 300 μg/kg iv, respectively.

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