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Development of a Highly Selective EP2-receptor Agonist. Part 2: Identification of 16-Hydroxy-17,17-trimethylene 9β-chloro PGF Derivatives

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Abstract—Further chemical modification of **1a** and **2** was undertaken to identify a more chemically stable selective EP2-receptor agonist for development as a clinical candidate. 9β-Chloro PG analogues **4a–e** and **5a**, **c–e** were found to be potent and selective EP2-receptor agonists. Among them, the compound **4aLy**, which is a chemically stabilized lysine salt of **4a**, exhibited an excellent profile both in biological activities and physicochemical properties. The agonist **4aLy** was found to suppress uterine motility in anesthetized pregnant rats, while PGE_2 stimulated uterine motility. Structure–activity relationships (SARs) are discussed.

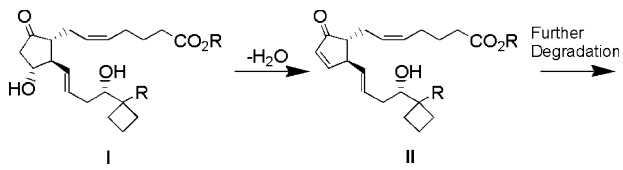
Introduction

The diverse biological activities of PGE_2 are considered to be a hybrid of the activities that are mediated by the four EP-receptor subtypes EP1, EP2, EP3 and EP4.[1] Of these, EP2-receptor[2] has been characterized as inducing relaxation of blood vessel, gastrointestinal tract, trachea and uterine smooth muscle,[3] and is suggested to play some role in the production and control of cytokines[4] and bone metabolism.[5] Development of a potent and selective EP2-receptor agonist is expected to be a promising approach to developing a therapeutically useful drug. In a preceding study,[6] we reported the identification of 16-hydroxy-17,17-trimethylene PGE_2 **1a** as a highly selective EP2-receptor agonist. Although **1a** could be a useful research tool for the EP2-receptor mediated pharmacological activities, its clinical utility is limited by a weak potency relative to that of PGE_2. Moreover it is a chemically unstable compound because it is well known that PGE derivatives **I** start to degrade with the initial conversion to the corresponding PGA derivatives **II** as shown in Scheme 1.

Since our final goal is to identify a highly selective EP2-receptor agonist and develop it as a clinical candidate, we focused on the identification of a more potent and chemically stable EP2-receptor agonist. Thus, synthesis and biological evaluation of PGF derivatives, which do not undergo the degradation reaction shown in Scheme 1, were continued based on the information obtained from the preceding experimental findings. We report here the identification and biological evaluation of a more potent and chemically stable EP2-receptor agonist. Structure–activity relationships (SARs) are also discussed.

Chemistry

9β-Chloro PGF analogues **4a–e** were synthesized from the corresponding 9-keto analogues **6a–e**[6] as shown in Scheme 3. Sequential reactions were: (1) stereoselective



I II

Scheme 1. Degradation pathway of PGE derivatives.

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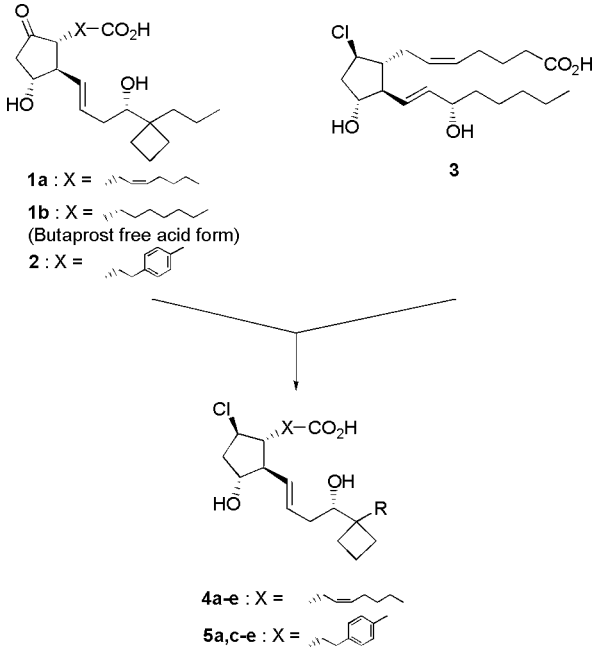
reduction of the 9-keto group of **6a–e** with lithium tri-*sec*-butylborohydride;[7] (2) acetylation with acetic anhydride; (3) deprotection of the *t*-butylsimethylsilyl (TBS) groups with aqueous hydrogen fluoride;[8] (4) separation of the diastereoisomer (16*R*-OH) by a silica gel column chromatography gave **8a–e**, respectively. Protection of the two hydroxy groups with *t*-butyldimethylsilyl trifluoromethanesulfonate followed by deacetylation with potassium carbonate in methanol gave **10a–e**, respectively. Tosylation of **10a–e** followed by chlorination with tetrabutylammonium chloride[9] and deprotection of the two TBS groups yielded **12a–e**. The corresponding $\Delta^{8,9}$ unsaturated side product was removed by column chromatography. Compounds **12a–e** were converted to **4a–e** by the alkaline hydrolysis, respectively. Compounds **5a** and **5c–e** were synthesized from **7a** and **7c–e**,[6] respectively, by the same procedure as described in the synthesis of **4a–e** from **6a–e**. The configuration of C-16 of **4a** was tentatively assigned to 16*S* and reconfirmed by the enantioselective synthesis, which will be presented in a subsequent report.

Results and Discussion

To block the dehydration reaction of the β-hydroxy ketone moiety of the PGE analogues **I** described in Scheme 1, 9β-chloro PGF analogues[10] **4a–e**, **5a** and **5c–e** were synthesized and biologically evaluated. The 9β-chloro PGF analogue, which is illustrated in Schering's nocloprost[11] and DP-receptor agonist,[9,12] has been reported to be chemically stable. In our preliminary study, the 9-deoxy-9β-chloro PGF_2 **3**[10] exhibited a high EP2-receptor affinity (Table 1). As such, 9β-chloro PGF analogue was expected to show a further increased EP2-receptor affinity. As demonstrated in Scheme 2, chemical modification began with structural hybridization of

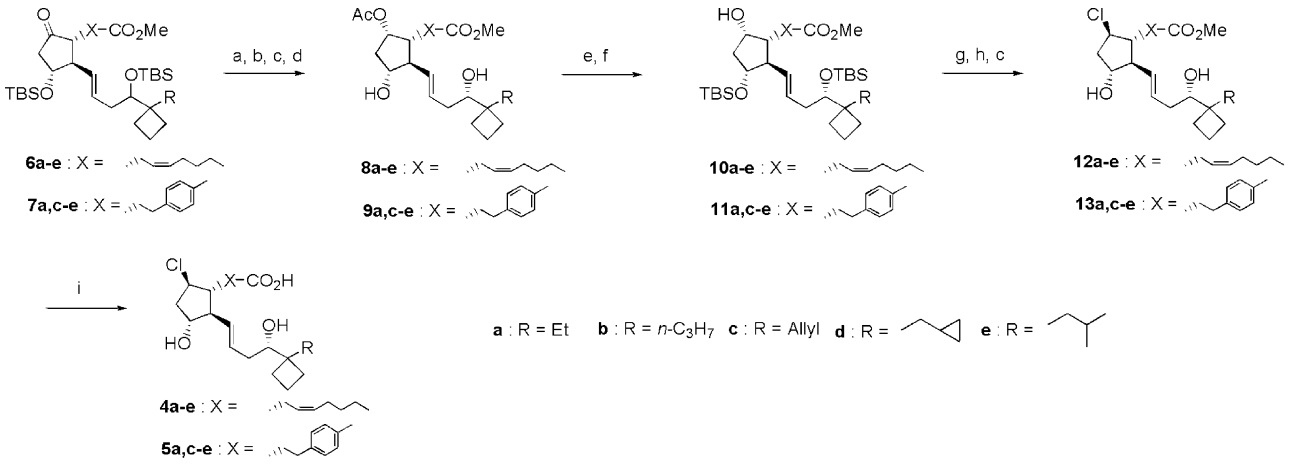


Scheme 2. Molecular design of 9β-chloro PGF analogues **4a–e, 5a** and **5c–e**.

1a, **2** and **3**. A series of 9β-chloro PGF analogues was evaluated for mouse (m) EP-receptor and human (h) IP-receptor affinities, and mEP2-receptor agonist activity as reported previously.[6] Two series of 9β-chloro PGF analogues, **4a–e**, **5a** and **5c–e** were identified. Of these, **4a** exhibited the most promising profiles both in the in vitro and in vivo studies. Compound **4a** was further evaluated for its ability to suppress uterine motility in anesthetized pregnant rats after intravenous administration.

Previously,[6] we succeeded in identifying a series of 16-hydroxy-17,17-trimethylene PGE_2 analogues, which demonstrated highly selective EP2-receptor agonist activity. On the basis of the reported SARs, **4a–e, 5a** and **5c–e** were synthesized and biologically evaluated (Table 2). All of these compounds showed only weak affinity to the hIP-receptor up to 10^4 μM. 9β-Chloro-16-hydroxy-17,17-trimethylene-ω-nor-PGF_2 **4a** exhibited a promising profile both in EP2-receptor selectivity and agonist activity. Homologation of the ω-chain of **4a** provided **4b** with slightly less subtype selectivity with regards to the EP3- and EP4-subtypes, while their potencies both in the EP2-receptor affinity (K_i) and agonist activity (EC_{50}) were slightly reduced. Replacement of the *n*-propyl moiety of **4b** with an allyl moiety afforded **4c** an increase both in the EP2-receptor affinity and agonist activity, while its EP1-receptor selectivity was reduced relative to **4b**. The EP2-receptor affinity was maximized in the 16-cyclopropylmethyl derivative **4d**, while its EP4-receptor affinity increased relative to **4a–c** and its potent EC_{50} value was retained. Replacement of the 16-cyclopropylmethyl moiety of **4d** with an isobutyl moiety afforded **4e** a 10-fold less potent EC_{50} value relative to **4d**. The EP1-receptor affinity of **4e** was reduced compared with **4d** while its EP4-receptor affinity was restored.

The same chemical modification as described above was applied to the 1,6-inter-*p*-phenylene analogues to give **5a** and **5c–e**. Compound **5a** exhibited EP2-receptor selectivity with several times less potent affinity than those of **4a–e**, while its EC_{50} value was markedly reduced relative to those of **4a–e**. The 16-allyl analogue **5c** demonstrated more potent EP2-receptor affinity and agonist activity than **5a** with good subtype selectivity. The 16-cyclopropylmethyl analogue **5d** demonstrated more potent EP2-receptor affinity and agonist activity than **5a** and **5c**, while its EP1- and EP4-receptor affinities increased. The EP2-receptor affinity of the 17-isobutyl analogue **5e** was as potent as that of **5a**, while its agonist activity was less potent. The EP2-receptor affinities of **5a** and **5c–e** were nearly 3-fold less potent than those of the corresponding PGF_2 analogues **4a** and **4c–e**, respectively, while their EC_{50} values were more than 100-fold less potent than those of **4a** and **4c–e**. With regards to the two types of α-chain, SAR of 9β-chloro analogues was different from that of 9-keto analogues.[6] 9β-Chloro analogues demonstrated a relatively big difference in their EC_{50} values up to their structural change in the α-chain, although 9-keto analogues demonstrated a relatively small difference in their EC_{50} values.[6] In the case of compound **5**, the flexibility of the α-chain was limited compared with that of **4**. Thus, the conformational



Scheme 3. Synthesis of 9β-chloro analogues **4a–e**, **5a** and **5c–e.** Reagent: (a) lithium tri-*sec*-butylborohydride, THF, −78 °C; (b) Ac_2O, DMAP, pyridine; (c) aqueous HF, MeCN; (d) separation; (e) TBSOTf, 2,6-lutidine, CH_2Cl_2; (f) K_2CO_3, MeOH; (g) TsCl, pyridine; (h) *n*-Bu_4NCl, toluene; (i) aqueous NaOH, MeOH.

change in the 5-membered ring that was caused by transformation of C-9 from sp^2 to sp^3 reduced the EP2 agonist activity of **5**.

The compound **4a** displaced [^{3}H]PGE_2, bound to the membrane of CHO cells expressing the mEP2- and hEP2 receptors with K_i values of 2.2 and 0.74 nM, respectively. The EP2-receptor affinity was nearly 10-fold higher than that of PGE_2, and 150- and 300-fold higher than those of AH-13205[13] and butaprost,[14] respectively. Compound **4a** exhibited a much lower affinity for the other EP subtypes (mEP1, mEP3α and EP4) as well as prostanoid receptors (mFP, hTP and hIP) other than the mDP-receptor. The affinity of **4a** for the mDP-receptor was at least 15-fold lower than for the mEP2-receptor. Similar findings were confirmed by functional studies based on increases in intracellular cAMP production and Ca^{2+} levels. As shown in Figure 1, **4a** stimulated cAMP production in mEP2-receptor expressing cells with an EC_{50} value of 2.8 nM. Its potency was comparable to that of PGE_2, and nearly 10-fold more potent than that of butaprost free acid form **1b**. Compound **4a** showed much lower agonist activity to the other prostanoid receptor subtypes. The details of the biological profiles of **4a** will be described in a subsequent report.

The modifications resulted in the discovery of highly selective EP2-receptor agonists **4a–e**, **5a** and **5c–e**. The in vivo activities of the test compounds **1a**, **1b**, **4a, 4c** and **5c** were evaluated using anesthetized pregnant rats (on days 18–20 of pregnancy: $n = 5$–6). Whereas PGE_2 stimulated uterine motility,[15] **1a**, **1b**, **4a, 4c** and **5c** suppressed uterine motility (Table 3). Intravenous administration of **4a** and **4c** showed significant suppression of uterine motility in a dose-dependent manner. Increased potency in the suppression of uterine motility by **4a** was observed relative to those of 9-keto analogues **1a** and **1b**, while the interphenylene analogue **5c** demonstrated much less potency. Complete elimination of side effects such as hypotension could not be accomplished, although the hypotensive effects tended to decrease by reducing the affinity for the IP-receptor compared with that of **1b**. Consequently, the hypotension caused by **1a**, **4a** and **4b** was considered to be one of the effects inherent in an EP2-receptor agonist. Based on its highly selective EP2-receptor affinity, potent agonist activity and in vivo effect on spontaneous uterine motility in late term pregnant rats, compound **4a** was selected for further evaluation.

Although 9β-chloro PGF_2 **4a** was expected to be chemically stable, it demonstrated a similar degree of instability to the 9-keto analogues **1a–b**. However, it was found to be markedly stabilized by forming a salt of **4a** with a base such as lysine. The physicochemical properties of the lysine salt **4aLy** in handling and water-solubility, etc. were excellent. As shown in Table 4, both of the compounds **1a** and **4a** were viscous oils, and unstable at ambient temperatures. According to the

Table 1. The K_i values of PGE_2 and **3**

Compd	Binding K_i (nM)			
	mEP1	mEP2	mEP3	mEP4
PGE_2	18	38	5.0	3.1
3	0.3	1.4	0.8	0.2

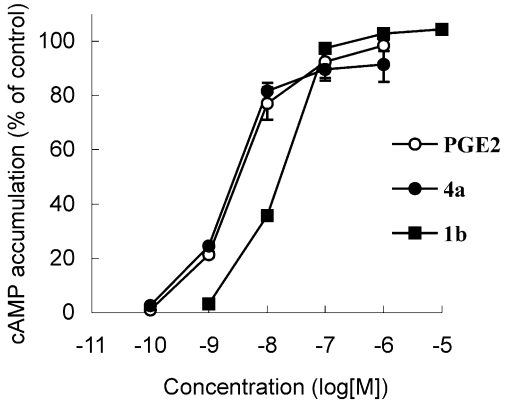
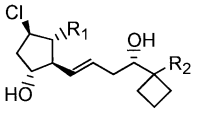


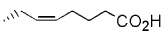
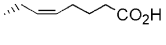
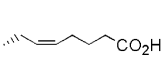
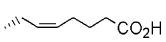
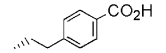
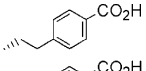
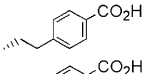
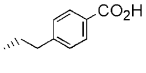
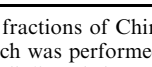
Figure 1. Effect of **PGE_2**, **1b** and **4a** on cAMP production in CHO cells expressing mouse EP2-receptors.

HPLC analysis, the percentage of compound **1a** remaining after storage at ambient temperatures for 8 weeks was 8%. The compound **4a** showed low stability (after 1 month at 60 °C, the percentage of compound remaining was 21%) while the sodium salt **4aNa** showed quite good stability at 60 °C. However **4aNa** was still not good enough to be developed as a clinical candidate because it was hygroscopically very unstable. The lysine salt **4aLy** demonstrated excellent physicochemical properties such as stability, solubility in water and descriptive features. The percentage remaining of **4aLy** after storage at 60 °C for one month was 100%, and its solubility in water was more than 100 mg. Additionally, **4aLy** was a good crystalline product with a melting point of 166–168 °C.

In summary, the highly selective EP2-receptor agonist **4aLy** was identified by chemical modification of the chemical lead **1a**, which was derived from butaprost. Using compound **4aLy**, its biological activities, which are mediated by the EP2-receptor, were investigated regarding uterine contractile activity. The selective EP2-receptor agonist **4aLy** was found to suppress uterine motility in anesthetized pregnant rats, while PGE_2 sti-

Table 2. Biological evaluation of **4a–e**, **5a** and **5c–e**



Compd	R_1	R_2	Binding K_i (nM)				EC_{50} EC_{50} (nM)[b]
			mEP1	mEP2	mEP3	mEP4	EP2
4a			$>10^4$	3.3	$>10^4$	6100	3.8
4b			$>10^4$	4.2	2800	3300	6.0
4c			1300	1.7	2700	2300	1.8
4d			1100	0.9	2700	400	2.5
4e			740	2.7	5200	2600	21
5a			2100	10	$>10^4$	$>10^4$	310
5c			$>10^4$	5.1	$>10^4$	$>10^4$	240
5d			660	3.0	$>10^4$	1100	180
5e			$>10^4$	10	$>10^4$	2900	790

[a]Using membrane fractions of Chinese hamster ovary (CHO) cells expressing prostanoid receptors, K_i values were determined by the competitive binding assay, which was performed according to the method of Kiriyama et al.[17] 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 regards to the subtype-receptor agonist activity, EC_{50} values were determined based on the effects of the test compounds on the increases in intracellular cAMP production in the mouse (m) EP2 receptor.

Table 3. Pharmacological effects of selective EP2-receptor agonists in rat

Compd	Uterine activity[a]		Hypotensive effect	
	ED_{50} (μg/kg iv)	Relative potency (**1b** = 1.0)	ΔBP (maximal response, mmHg)	Relative potency (**1b** = 1.0)
PGE_2	Stimulate[b]	—	—	—
1a	274.7	1.7	22.8 (1000)[c]	0.57
1b	455.2	1.0	39.8 (1000)	1.0
4a	32.9	13.8	24.4 (100)	0.61
4c	33.5	13.6	19.2 (100)	0.48
5c	>300	<1.5	15.8 (300)	—

[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-minute period before and after commencement of the administration, and post-dose uterine activity was calculated as percentage of inhibition to pre-dose uterine activity.
[b]PGE_2 stimulated uterine motility at >1.8 μg/kg.[15]
[c]Hypotensive effects (ΔBP) showed maximal efficacy at roughly 3-fold dosage of ED_{50} values of the uterine activity. Numbers in parentheses indicate the dose of maximal hypotensive response (μg/kg iv).

Table 4. Physicochemical properties of EP2-receptor agonists

Compd	Stability[a] (%)	Solubility (mg/mL of H_2O)	Description	Mp (°C)
1a	8[b]	N.T.[d]	Viscous oil	—
4a	21[c]	N.T.	Viscous oil	—
4aNa	97[c]	>10	Hygroscopic amorphous	—
4aLy	100[c]	>100	Colorless crystal	166–168

[a]Percentage of the remaining test compound was determined by HPLC analysis.
[b]Percentage after 8 weeks at ambient temperatures.
[c]Percentage after 1 month at 60 °C.
[d]Not tested.

mulated uterine motility. Other biological activities of **4aLy** are being investigated in our laboratory.

Experimental

General directions

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. All ^{1}H NMR spectra were obtained using a Varian Gemini-200, VXR-200s or Mercury300 spectrometer. Mass spectra were obtained on a Hitachi M1200H, JEOL JMS-DX303HF or PerSeptive Voyager Elite spectrometer. IR spectra were measured on a Perkin–Elmer FT-IR 1760X or Jasco FT/IR-430 spectrometer. Elemental analyses for carbon, hydrogen, nitrogen and sulfur were carried out on a Perkin–Elmer PE2400 SeriesII CHNS/O analyzer. Optical rotations were measured using a Jasco DIP-1000 polarimeter. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm) or Wako Gel C200]. Thin layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F_{254}).

(16*S*)-9-*O*-Acetyl-15-deoxy-16-hydroxy-17,17-trimethylene PGF$_{2\alpha}$ methyl ester (8b). To a stirred solution of **6b** (740 mg, 1.17 mmol) in 20 mL of THF was added lithium tri-*sec*-butylborohydride (1.0 M in THF, 1.76 mL, 1.76 mmol) at −78 °C under an argon atmosphere, and stirring continued for 30 min. The reaction was quenched with 1 mL of 30% aqueous hydrogen peroxide and allowed to warm up to 0 °C. The reaction mixture was extracted with EtOAc. The organic layer was washed with aqueous HCl, then brine, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel (10% EtOAc/*n*-hexane) to yield the 9α-hydroxy derivative (558 mg, 75%) as a colorless oil. TLC R_f 0.35 (*n*-hexane/EtOAc, 9/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.60–5.10 (m, 4H), 4.15–3.90 (m, 2H), 3.66 (s, 3H), 3.55 (t, J = 5 Hz, 1H), 2.70–2.50 (m, 1H), 2.40–1.20 (m, 24H), 1.00–0.80 (m, 21H), 0.10–0.00 (m, 12H).

A solution of the 9α-hydroxy derivative (518 mg, 0.813 mmol), acetic anhydride (0.15 mL, 1.62 mmol), 4-(dimethylamino)pyridine (3 mg) and 1 mL of pyridine was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc and washed with water. The organic layer was washed with aqueous HCl water and brine, dried over magnesium sulfate and concentrated in vacuo to give a crude 9α-acetoxy derivative as an oil.

To a stirred solution of above-described crude 9α-acetoxy derivative and 10 mL of acetonitrile, 0.5 mL of 48% aqueous HF was added at 0 °C and stirring continued for 1.5 h at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography (Lobar® pre-packed column, size B, 60% EtOAc/*n*-hexane) to give a less polar product (16*R*–OH, 142 mg, 39%) and a more polar product (16*S*–OH, **8b**, 148 mg, 40%). Less polar product; Colorless oil; TLC R_f 0.30 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.66 (ddd, J = 15.0, 7.8, 6.0 Hz, 1H), 5.45–5.30 (m, 3H), 5.15–5.05 (m, 1H), 4.00–3.85 (m, 1H), 3.67 (s, 3H), 3.55 (dd, J = 10.0, 2.4 Hz, 1H), 2.58–2.40 (m, 1H), 2.40–1.30 (m, 23H), 2.31 (t, J = 7.4 Hz, 2H), 2.06 (s, 3H), 0.94 (t, J = 7.2 Hz, 3H). More polar product **8b**; Colorless oil; TLC R_f = 0.23 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.65 (ddd, J = 14.8, 8.0, 6.2 Hz, 1H), 5.43–5.25 (m, 3H), 5.15–5.05 (m, 1H), 3.95–3.82 (m, 1H), 3.67 (s, 3H), 3.55 (dd, J = 10.0, 2.4 Hz, 1H), 2.60–2.40 (m, 1H), 2.40–1.20 (m, 23H), 2.30 (t, J = 7.4 Hz, 2H), 2.06 (s, 3H), 0.94 (t, J = 6.7 Hz, 3H).

(16*S*)-11-*O*-(*t*-Butyldimethylsilyl)-15-deoxy-16-(*t*-butyldimethylsilyloxy)-17,17-trimethylenePGF$_{2\alpha}$ methyl ester (10b). To a stirred solution of **8b** (148 mg, 0.329 mmol) and 2,6-lutidine (0.18 mL, 1.58 mmol) in methylene chloride (3 mL) was added dropwise *t*-butyldimethylsilyl trifluoromethanesulfonate (0.22 mL, 0.948 mmol) at 0 °C under argon atmosphere. After stirring for 1 h at room temperature, the reaction was quenched with saturated aqueous sodium bicarbonate. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over magnesium sulfate and concentrated in vacuo. The residue was dissolved in methanol (2 mL) after the addition of potassium carbonate (87 mg, 0.632 mmol), then the suspension was stirred for 2 h at 40 °C. After cooling in an ice-bath, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with water, then brine, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromato-

graphy on silica gel to yield **10b** (202 mg, 96%) as a colorless oil. TLC R_f = 0.35 (*n*-hexane/EtOAc, 9/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.60–5.10 (m, 4H), 4.15–3.90 (m, 2H), 3.66 (s, 3H), 3.55 (t, *J* = 5 Hz, 1H), 2.70–2.50 (m, 1H), 2.40-1.20 (m, 24H), 1.00–0.80 (m, 21H), 0.10–0.00 (m, 12H).

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylenePGF_2 methyl ester (12b). To a stirred solution of **10b** (137 mg, 0.215 mmol) in pyridine (1 mL) was added *p*-toluenesulfonyl chloride (435 mg, 2.28 mmol) at room temperature under argon atmosphere. After stirring for 12 h at room temperature, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with aqueous HCl, water, then brine, dried over magnesium sulfate and concentrated in vacuo to give a tosylate as an oil. To a stirred solution of the tosylate in toluene (5 mL) was added tetrabutylammonium chloride (598 mg, 2.15 mmol). The reaction mixture was stirred for 6 h at 50 °C under an argon atmosphere. After cooling in an ice-bath, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over magnesium sulfate and concentrated in vacuo to give a crude product. To a stirred solution of this crude product and 5 mL of acetonitrile, 0.25 mL of 48% aqueous HF was added at 0 °C. The reaction mixture was stirred for 1.5 h at room temperature, and then poured into saturated aqueous sodium bicarbonate–EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography (Lobar® pre-packed column, size B, 5% 2-propanol/toluene) to remove an eliminated product ($\Delta^{8,9}$ unsaturated product) to yield **12b** (62 mg, 67%) as a colorless oil. TLC R_f = 0.26 (*n*-hexane/EtOAc, 2/1); MS (APCI, Pos, 20V) *m*/*z* 409 $(M+H-H_2O)^+$, 391 $(M+H-2H_2O)^+$, 355 $(M+H-2H_2O-HCl)^+$; IR (neat) 3369, 2954, 1739, 1436, 1222, 1071, 968 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.58 (ddd, *J* = 15.0, 8.2, 5.6 Hz, 1H), 5.50-5.32 (m, 3H), 4.18-3.95 (m, 2H), 3.67 (s, 3H), 3.53 (dd, *J* = 10.4, 2.2 Hz, 1H), 2.76 (br, 1H), 2.40–1.20 (m, 23H), 2.33 (t, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 6.8 Hz, 3H).

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-PGF_2 (4b). To a stirred solution of **12b** (45 mg, 0.110 mmol) in methanol (3 mL), 2 N sodium hydroxide (1 mL) was added at room temperature. After stirring for 1 h, the reaction mixture was acidified with aqueous hydrochloric acid and extracted with EtOAc. The organic layer was washed with water, then brine, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel to yield **4b** (44 mg, quant.) as a pale yellow oil. TLC R_f 0.31 (*n*-hexane/EtOAc/AcOH, 3/2/0.05); MS (APCI, Neg, 20V) *m*/*z* 411 (M−H)−; IR (neat) 3369, 2931, 1709, 1435, 1249, 1070, 968 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.58 (ddd, *J* = 15.4, 7.6, 5.4 Hz, 1H), 5.55–5.35 (m, 3H), 4.20–4.00 (m, 2H), 4.00–3.00 (br, 3H), 3.57 (dd, *J* = 10.2, 2.2 Hz, 1H), 2.40–1.20 (m, 22H), 2.36 (t, *J* = 6.9 Hz, 2H), 0.94 (t, *J* = 6.8 Hz, 3H). Compounds **4a**, **4c**–**e**, **5a** and **5c**–**e** were synthesized from **6a**, **6c**–**e**, **7a** and **7c**–**e**, respectively, according to the same procedure described above.

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-ω-norPGF_2 (4a). Pale yellow viscous oil; optical rotation $[\alpha]_D^{25} = -24.4$ (*c* 1.00, EtOH); TLC R_f = 0.33 (EtOAc/*n*-hexane/AcOH, 60/30/1); IR (neat) 3351, 2936, 1709, 1432, 1243, 1069, 968, 866 cm^{-1}; MS (FAB, Neg) *m*/*z* 397 (M−H)−, 361 $(M-H-HCl)^-$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.56 (ddd, *J* = 15.4, 8.2, 5.2 Hz, 1H), 5.55–5.30 (m, 3H), 5.60–5.00 (br, 3H), 4.20–3.96 (m, 2H), 3.56 (dd, *J* = 10.2, 2.0 Hz, 1H), 2.42–1.30 (m, 20H), 2.35 (t, *J* = 7.0 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H).

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-19,20-didehydroPGF_2 (4c). Pale yellow viscous oil; TLC R_f 0.74 (*n*-hexane/EtOAc/AcOH, 1/3/0.05); MS (APCI, Neg, 20V) *m*/*z* 409 $(M-H)^-$; IR (neat) 3363, 2936, 1709, 1639, 1435, 1245, 1072, 996, 969, 914, 734 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.94 (ddt, *J* = 17.1, 10.2, 7.2 Hz, 1H), 5.56 (ddd, *J* = 15.3, 7.8, 6.0 Hz, 1H), 5.50–5.36 (m, 3H), 5.17–5.07 (m, 2H), 4.11 (q, *J* = 7.2 Hz, 1H), 4.04 (m, 1H), 3.57 (dd, *J* = 10.2, 2.1 Hz, 1H), 2.40–1.63 (m, 25H).

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-ω-dinor-18-cyclopropylPGF_2 (4d). Colorless viscous oil; TLC R_f 0.31 (*n*-hexane/AcOEt/AcOH, 3/2/0.05); IR (neat) 3367, 2932, 1708, 1433, 1248, 1019, 969 cm^{-1}; MS (FAB, Pos) *m*/*z* 425 $(M+H)^+$, 407 $(M+H-H_2O)^+$, 389 $(M+H-2H_2O)^+$, 353 $(M+H-2H_2O-HCl)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.60 (ddd, *J* = 15.4, 7.6, 5.4 Hz, 1H), 5.55–5.35 (m, 3H), 4.20–3.98 (m, 2H), 4.20–3.00 (br, 3H), 3.71 (dd, *J* = 10.4, 2.2 Hz, 1H), 2.40–1.60 (m, 8H), 2.36 (t, *J* = 6.9 Hz, 2H), 1.51 (dd, *J* = 14.2, 6.8 Hz, 1H), 1.37 (dd, *J* = 14.2, 6.2 Hz, 1H), 0.90–0.65 (m, 1H), 0.57–0.45 (m, 2H), 0.15–0.05 (m, 2H).

(16*S*)-9-deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-19-methylPGF_2 (4e). Colorless viscous oil; TLC R_f = 0.34 (*n*-hexane/AcOEt/AcOH, 3/2/0.05); IR (neat) 3367, 2952, 1708, 1435, 1251, 1074, 969 cm^{-1}; MS (FAB, Pos) *m*/*z* = 427 $(M+H)^+$, 391 $(M+H-2H_2O)^+$, 355 $(M+H-2H_2O-HCl)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.60 (ddd, *J* = 15.4, 8.2, 5.6 Hz, 1H), 5.55–5.35 (m, 3H), 4.20–3.98 (m, 2H), 4.20–3.00 (br, 3H), 3.65 (dd, *J* = 10.2, 2.2 Hz, 1H), 2.40–1.65 (m, 19H), 2.36 (t, *J* = 7.1 Hz, 2H), 1.55 (dd, *J* = 14.2, 6.6 Hz, 1H), 1.33 (dd, *J* = 14.2, 6.2 Hz, 1H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H).

(1*R*,2*R*,3*R*,4*R*)-1-Chloro-2-[2-(4-carboxyphenyl)ethyl]-3-[(1*E*,4*S*)-4-hydroxy-5,5-trimethylene-1-heptenoyl]-4-hydroxycyclopentane (5a). Colorless viscous oil; TLC R_f = 0.14 (*n*-hexane/AcOEt, 1/1); MS (APCI, Neg, 20V) *m*/*z* 419 (M−H)−; IR (neat) 3391, 2931, 1694, 1611, 1575, 1421, 1278, 1179, 1074 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 18.0 Hz, 2H), 5.58 (ddd, *J* = 15.4, 8.4, 6.6 Hz, 1H), 5.40 (dd, *J* = 15.4, 8.2 Hz, 1H), 4.40–3.00 (br, 3H), 4.18–4.03 (m,

2H), 3.56 (dd, $J=10.2$, 2.2 Hz, 1H), 2.79 (t, $J=7.6$ Hz,2H), 2.38–1.30 (m, 16H), 0.91 (t, $J=7.8$ Hz, 3H).

(1*R*,2*R*,3*R*,4*R*)-1-Chloro-2-[2-(4-carboxyphenyl)ethyl]-3-[(1*E*,4*S*)-4-hydroxy-5,5-trimethylene-1,7-octadienoyl]-4-hydroxycyclopentane (5c). Colorless viscous oil; TLC R_f 0.15 (*n*-hexane/AcOEt, 1/1); MS (APCI, Neg, 20V) *m/z* 431 $(M-H)^-$; IR (neat) 3390, 2930, 1695, 1640, 1611, 1575, 1422, 1279, 1179, 1078 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.00 (d, $J=8.0$ Hz, 2H), 7.27 (d, $J=18.0$ Hz, 2H), 5.93 (ddt, $J=17.4$, 10.0, 7.2 Hz, 1H), 5.65–5.50 (m, 1H), 5.38 (dd, $J=15.4$, 8.4 Hz, 1H), 5.15–5.06 (m, 2H), 4.80–3.60 (br, 3H), 4.18–4.00 (m, 2H), 3.55 (bd, $J=8.4$ Hz, 1H), 2.78 (t, $J=7.6$ Hz, 2H), 2.41–1.60 (m, 16H).

(1*R*,2*R*,3*R*,4*R*)-1-Chloro-2-[2-(4-carboxyphenyl)ethyl]-3-[(1*E*,4*S*)-4-hydroxy-5,5-trimethylene-6-cyclopropyl-1-hexenoyl]-4-hydroxycyclopentane (5d). Colorless viscous oil; TLC R_f 0.26 (*n*-hexane/EtOAc, 1/2); MS (APCI, Neg. 20V) *m/z* 445 $(M-H)^-$, 409 $(M-H-HCl)-$; IR (neat) 3369, 2930, 1694, 1611, 1423, 1284, 1179, 1083, 1019, 970, 909, 858, 734 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.01 (d, $J=8.0$ Hz, 2H), 7.28 (d, $J=8.0$ Hz, 2H), 5.57 (m, 1H), 5.39 (m, 1H), 4.38 (br, 3H), 4.10 (m, 2H), 3.70 (bd, $J=8.4$ Hz, 1H), 2.79 (t, $J=7.6$ Hz, 2H), 2.40–1.62 (m, 14H), 1.52 (dd, $J=14$, 6.6 Hz, 1H), 1.36 (dd, $J=14$, 6.4 Hz, 1H), 0.78 (m, 1H), 0.49 (m, 2H), 0.10 (m, 2H).

(1*R*,2*R*,3*R*,4*R*)-1-Chloro-2-[2-(4-carboxyphenyl)ethyl]-3-[(1*E*,4*S*)-4-hydroxy-5,5-trimethylene-7-methyl-1-octenoyl]-4-hydroxycyclopentane (5e). Colorless viscous oil; TLC R_f 0.33 (*n*-hexane/EtOAc, 1/2); MS (APCI, Neg. 20V) *m/z* 447 $(M-H)^-$, 411 $(M-H-HCl)^-$; IR (neat) 3368, 2952, 1695, 1611, 1422, 1315, 1283, 1179, 1080, 970, 909, 859, 735 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.00 (d, $J=8.2$ Hz, 2H), 7.27 (d, $J=8.2$ Hz, 2H), 5.60 (ddd, $J=15$, 8.8, 5.2 Hz, 1H), 5.42 (dd, $J=15$, 7.8 Hz, 1H), 4.12 (m, 2H), 3.69 (br, 3H), 3.63 (dd, $J=10$, 1.8 Hz, 1H), 2.79 (t, $J=7.9$ Hz, 2H), 2.38–1.62 (m, 15H), 1.54 (dd, $J=14$, 6.8 Hz, 1H), 1.33 (dd, $J=14$, 6.4 Hz, 1H), 0.91 (d, $J=6.6$ Hz, 3H), 0.90 (d, $J=6.6$ Hz, 3H).

(16*S*)-9-Deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-ω-norPGF$_2$ sodium salt (4aNa). To a stirred solution of **4a** (2.33 g, 5 84 mmol) in 1,4-dioxane (10 mL) was added aqueous 1 N sodium hydroxide (5.84 mL). The reaction mixture was stirred for 10 min, concentrated in vacuo and lyophilized to yield the sodium salt **4aNa** (2.39 g, 97%) as a white amorphous. MS (FAB, Pos.) *m/z* 421 $(M+H)^+$; IR (neat) 3398, 2937, 1562, 1407, 1074 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 6.00–4.00 (br, 2H), 5.60 (ddd, $J=15.4$, 8.0, 5.8 Hz, 1H), 5.51–5.23 (m, 3H), 4.09 (q, $J=7.3$ Hz, 1H), 3.87 (q, $J=6.5$Hz, 1H), 3.36 (dd, $J=9.2$, 2.8 Hz, 1H), 2.30–1.20 (m, 22H), 0.85 (t, $J=7.3$ Hz, 3H).

(16*S*)-9-deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-ω-norPGF$_2$ L-lysine salt (4aLy). To a stirred solution of **4a** (45.9 g, 110 mmol) in 460 mL of ethanol was added L-lysine (16.0 g, 110 mmol). To the resulting suspension, 1600 mL of ethanol was added. The mixture was heated at 80 °C until the precipitates dissolved, then insoluble substances were removed by filtration. After cooling, 5 L of EtOAc was added to the filtrate and the resulting precipitates were collected by filtration and dried in vacuo to yield the L-lysine salt **4aLy** (54.6 g, 91%) as a colorless crystal. Mp 166–168 °C (dec.); Optical rotation $[\alpha]_D^{25}=-20.4$ (*c* 1.00, H_2O); MS (FAB, Pos.) *m/z* 545 $(M+H)^+$, 421 $(M-Lys+Na)^+$; IR (KBr) 3424, 2934, 1618, 1542, 1406, 1307, 965 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 5.70–5.30 (m, 4H), 4.02 (q, $J=7.1$ Hz, 2H), 3.58–3.45 (m, 2H), 2.92 (t, $J=7.3$ Hz, 2H), 2.40–1.30 (m, 28H), 0.92 (t, $J=7.5$ Hz, 3H). Anal. calcd for $C_{28}H_{49}ClN_2O_6$; C, 61.69; H, 9.06; N, 5.14; found; C, 61.72; H, 9.21; N, 5.21.

Prostanoid EP receptor binding assay

Membranes from CHO cells expressing the prostanoid receptors were incubated with radioligand (2.5 nM of $[^3H]PGE_2$) and the test compounds at various concentrations in assay buffer (10 mM Kpi (KH_2PO_4, KOH; pH 6.0), 1 mM EDTA and 0.1 mM NaCl). Incubation was carried out at 25 °C for 60 min except for EP1-receptor (20 min). The incubation was terminated by filtration through Whatman GF/B filters. The filters were then washed with ice-cold buffer [10 mM Kpi (KH_2PO_4, KOH; pH 6.0), 0.1 mM NaCl], and the radioactivity on the filter was measured in 6mL of liquid scintillation (ACSII) mixture with a liquid scintillation counter. Nonspecific binding was determined by incubation of 10 μM unlabeled PGE_2 with assay buffer.

Measurement of cAMP production

CHO cells expressing EP2-receptor were cultured in 24-well plates (1×10^5 cells/well). After 2 days, the media were removed and cells were washed with 500 μL of Minimum Essential Medium (MEM) and preincubated for 10 min in 450 μL of assay buffer (MEM containing 1 mM of IBMX, 1% of BSA) at 37 °C. Then reaction was started with the addition of each test compound in 50 μL of assay buffer. After incubation for 10 min at 37 °C, the reaction was terminated by addition of 500 μL of ice-cold 10% trichloroacetic acid. The cAMP production was measured by radioimmunoassay using a cAMP assay kit (Amersham).

Evaluation of uterine motility suppression activity of EP2 agonists

Fed pregnant rats were anesthetized with urethane (1.5 g/5 mL/kg, sc) and fixed in the dorsal position. A midline incision was made in the lower abdomen and a small incision was made near the cervical area of the right or left uterine horn, and a balloon catheter (Okamoto Medical Industry, for rats) was inserted between the uterine wall and the amnion. After suturing the abdominal incision, the intraballoon pressure was loaded to approximately 5–15 mmHg. A catheter (POLYETHYLENE TUBE SP10, Natsume Seisakusho) was placed into the femoral vein for the administration of the test compound. Uterine motility was recorded on a recticoder (WR3320 or WR3701, GRAPHTEC) via a pressure transducer (Life Kit DX-360, Nihon Kohden

Corp.) and a strain pressure amplifier (AP-601G, Nihon Kohden Corp.). After spontaneous uterine motility was kept at a stable level, each experiment was started. Briefly, the vehicle and then the test compound were intravenously administered successively starting from lower doses at 10-minute or larger intervals. Before each dosing, it was confirmed that uterine motility was kept stable for at least 5 min.

Uterine motility was evaluated according to the Montevideo method.[16] In the intravenous injection study, uterine activity was calculated for 5-min periods before and after administration of the test compound, and expressed as a percentage relative to pre-treatment values: Uterine activity (% of pre-value) = (5-min post-dose uterine activity/5-min pre-dose uterine activity)×100.

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