Phenyl-Substituted Prostaglandins: Potent and Selective Antiglaucoma Agents

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A series of phenyl-substituted analogues of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were prepared and evaluated for ocular hypotensive effect and side effects in different animal models. In addition, the activity of the analogues on FP receptors was studied in vitro. The results were compared with those of $PGF_{2\alpha}$ and its isopropyl ester. The phenyl-substituted $PGF_{2\alpha}$ analogues exhibited good intraocular pressure reducing effect, were more selective, and exhibited a much higher therapeutic index in the eye than $PGF_{2\alpha}$ or its isopropyl ester. The analogues exhibited high activity on FP receptors in a stereoselective manner for the 15$_\alpha$-hydroxyl group.

Introduction

Glaucoma, a potentially blinding eye disorder, is characterized by increased intraocular pressure (IOP), excavation of the optic nerve head, and gradual loss of visual field. Initial treatment usually involves topical application of muscarinic agonists, particularly pilocarpine, or adrenergic agonists or antagonists, e.g. epinephrine and timolol, respectively. If treatment with such topically applied drugs is not effective, systemic administration of carbonic anhydrase inhibitors or surgical intervention may be employed.

Recently, attention has been focused on prostaglandins (PG:s), primarily prostaglandin $F_{2\alpha}$ esters as IOP-lowering substances.[1,2,3] Several studies indicate that PG:s of the $F_{2\alpha}$ type reduce IOP by increasing uveoscleral outflow of aqueous humor.[4,5] This is a new principle of reducing IOP for therapeutic purpose.

$PGF_{2\alpha}$ isopropyl ester **1b** (Figure 1) significantly reduces IOP.[1,2,3] However, this substance is not suitable for therapeutic use due to side effects such as superficial irritation and vasodilation in the conjunctiva. Consequently, the therapeutic selectivity of $PGF_{2\alpha}$ in the eye is low. In an attempt to increase ocular selectivity, we have prepared a number of phenyl-substituted $PGF_{2\alpha}$ analogues and found that such analogues with modified omega chains containing an aromatic ring possess high selectivity and display good IOP-lowering activity. In this paper we report the synthesis and biological activity of two 17-phenyl-$PGF_{2\alpha}$ analogues, **3** and **5**, and their 15-epimers, **4** and **6** (Figure 1). The biologic activity of these analogues has been compared with that of $PGF_{2\alpha}$ and its 15-epimer.

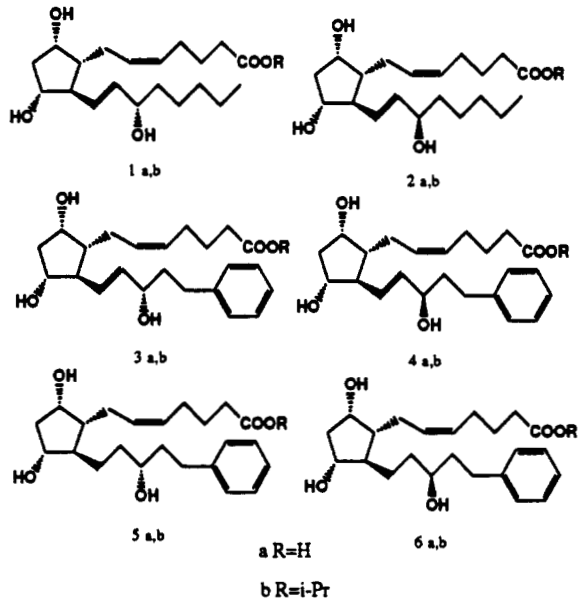
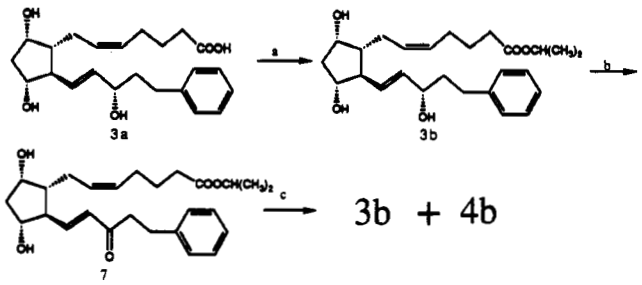


Figure 1. Structures of compounds referred to in the text by numbers.

Chemistry

Synthesis. 17-Phenyl-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester (**3b**) and (15*R*)-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester (**4b**) are prepared as shown in Scheme I. The commercially available compound **3** is esterified in acetone with isopropyl iodide in the presence of DBU[6] to yield **3b** which is oxidized in dioxane with DDQ[7,8] to give the enone **7**. The unsaturated ketone **7** is reduced in methanol with sodium borohydride in the presence of cerium chloride heptahydrate[9–11] to give the epimeric mixture of **3b** and **4b** which is then chromatographed on silica gel, using ethyl acetate, to achieve separation of the isomers **3b** and **4b**. Compound **2b** is prepared in a similar way using $PGF_{2\alpha}$.

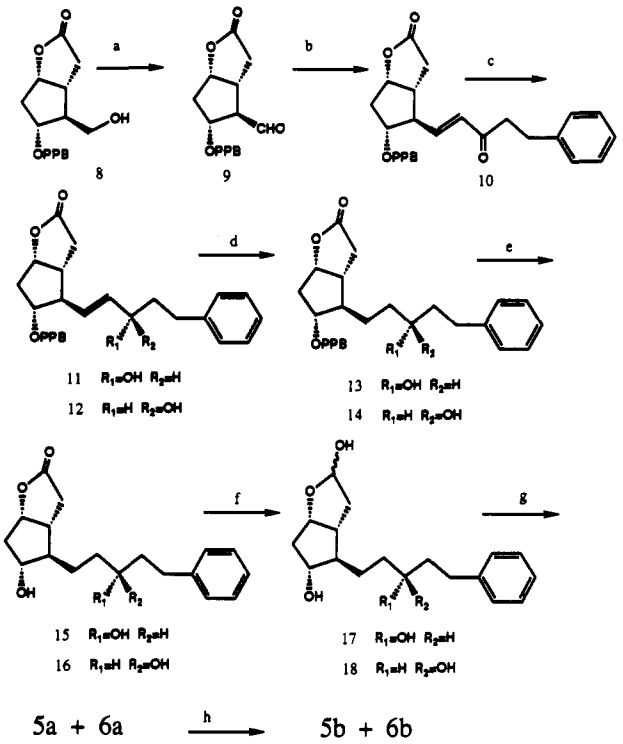
Scheme I[a]



[a] Reagents: (a) DBU, i-Pr I/acetone; (b) DDQ (3.0 equiv)/dioxane; (c) $CeCl_3 \cdot 7H_2O$, $NaBH_4$/methanol.

13,14-Dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester (**5b**) and (15*S*)-13,14-dihydro-17-phenyl-18,19,-20-trinor-$PGF_{2\alpha}$ isopropyl ester (**6b**) are prepared as shown in Scheme II. The primary alcohol of the bicyclic lactone **8**[12–14] is oxidized using the Pfitzner–Moffat method[15–17]

Scheme II[a]



[a] Reagents: (a) DCC, DMSO, H_3PO_4/DME; (b) $(CH_3O)_2PO$-$CH_2CO(CH_2)_2C_6H_5$, NaH/DME; (c) $CeCl_3 \cdot 7H_2O$, $NaBH_4$/MeOH; (d) Pd/C, 1 M NaOH/EtOH; (e) K_2CO_3/MeOH; (f) DIBAL/THF, −78 °C; (g) $Ph_3P(CH_2)_4COOH$, KOtBu/THF, −10 °C; (h) DBU, $ICH(CH_3)_2$/acetone, room temperature.

9 is reacted further with dimethyl (2-oxo-4-phenylbutyl)-phosphonate using the Wadsworth–Emmons method to give **10**.[18,19] The resulting crystalline α,β-unsaturated ketone was reduced stereoselectivity with lithium tri-*sec*-butylborohydride (Lithium Selectride)[20,21] at −120 °C to furnish a 7:3 mixture of 15*S*-isomer **11** and 15*R*-isomer **12**. Alternatively, sodium borohydride in the presence of cerium chloride heptahydrate in methanol at room temperature can be used to reduce the enone with lower selectivity. The epimers are separated by flash column chromatography on silica gel to give **11** as a solid (crystallized from ethanol) in 45% yield. This is reduced under hydrogen atmosphere in ethanol using Pd–C as catalyst in the presence of sodium hydroxide[22,23] to give compound **13** as an oil in quantitative yield. The phenylbenzoyl group is removed using potassium carbonate in methanol, affording compound **15** as an oil, which was purified by column chromatography on silica gel using EtOAc as eluent. The lactone **15** is treated with diisobutylaluminum hydride (DIBAL)[24,25] in dry THF at −78 °C to give the lactol **17** as a solid in good yield. The Wittig reaction[26] with (4-carboxybutyl)triphenylphosphonium bromide and potassium *tert*-butoxide in THF to afford the acid **5a**. This is further reacted without isolation with isopropyl iodide and DBU in acetone to give the ester **5b**, which is isolated by gradient flash column chromatography using first dichloromethane then ethanol in dichloromethane (5.0–7.5%) to give the desired product **5b** as an oil. Compound **6b** (Scheme II) is prepared in a similar way using the 15*R* isomer **12**.

Pharmacology

The compounds were tested for IOP-reducing effect

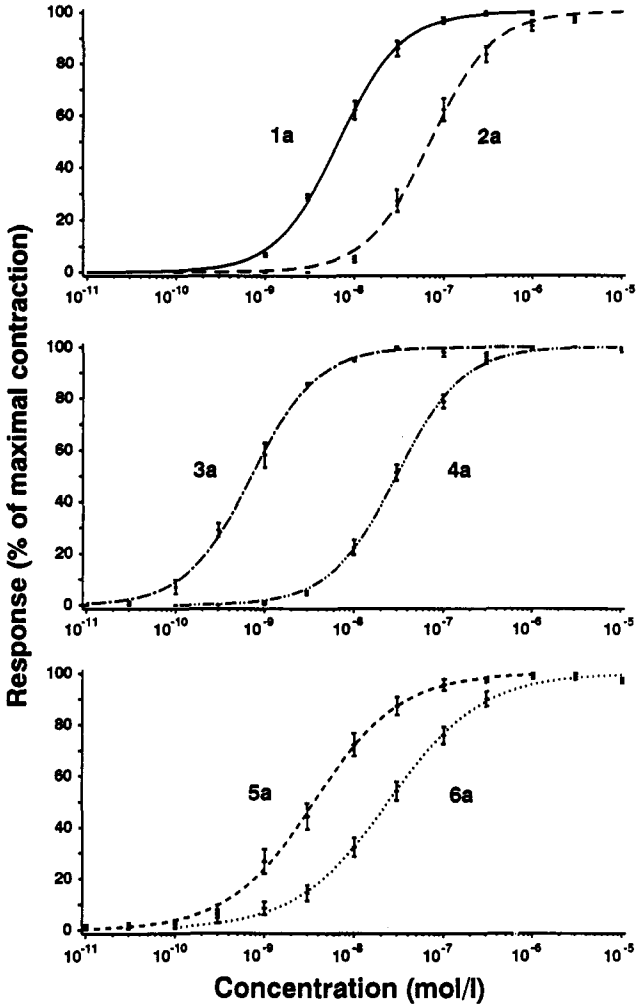


Figure 2. Effect of the test compounds on the cat iridial sphincter muscle. Each point represents the mean of four values obtained on four different preparations. Bars indicate SEM. The EC_{50} values for compounds **1a–6a** are 6.7×10^{-9}, 7.1×10^{-8}, 7.1×10^{-10}, 3.0×10^{-8}, 3.6×10^{-9}, and 2.4×10^{-8} M, respectively.

hyperemia. IOP was measured in cynomolgus monkeys. The cat eye was used as a model for ocular irritation. Conjunctival hyperemia (surface hyperemia of the eye) was evaluated in albino rabbits. The activity of the test compounds was also tested in a muscle bath using isolated cat iris sphincters. This muscle expresses predominantly FP receptors.[27]

Compound **3a** exerted the strongest contractile effect on the feline iris sphincter muscle with an $EC_{50} = 7.1 \times 10^{-10}$ M. Interestingly, this compound exhibited higher potency than the naturally occuring ligand (**1a**) (Figure 2). Inversion of the 15-hydroxyl group decreased the activity in all the analogues.

Compounds **1b**, **3b**, and **5b** effectively reduced IOP in monkeys while the 15-epimers had much weaker effects (Table I). All compounds, in particular **1b**, **3b**, and **5b**, decreased the pupil diameter of the cat, while in monkeys a slight increase in pupil diameter was observed with some of the phenyl-substituted compounds.

None of the phenyl-substituted analogues (compounds **3b–6b**) caused irritation, whereas **1b** and **2b** had a clear-cut irritating effect on feline eyes (Table I). While all analogues caused some conjunctival hyperemia, this side effect was much less pronounced with the phenyl-substituted analogues, as compared to the isopropyl ester of

Table I. Maximum Reduction of the Intraocular Pressure in Normotensive Monkeys and Maximum Irritation of Cat Eyes after Topical Administration of 3 μg of Compounds **1b–6b** (n = 6)

compound no.	maximum reduction of IOP in monkeys (mmHg)[a]	maximum irritation of cat eyes (arbitrary units)[a]
1b	2.5 ± 0.3***	2.5 ± 0.0***
2b	0.7 ± 0.5	2.3 ± 0.1***
3b	2.9 ± 1.0*	0.2 ± 0.2[b]
4b	1.9 ± 1.0	0.0 ± 0.0[b]
5b	2.6 ± 0.4**	0.0 ± 0.0
6b	1.0 ± 0.2**	0.0 ± 0.0

[a] (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$ (matched pair t-test). [b] Dose = 5 μg in cats.

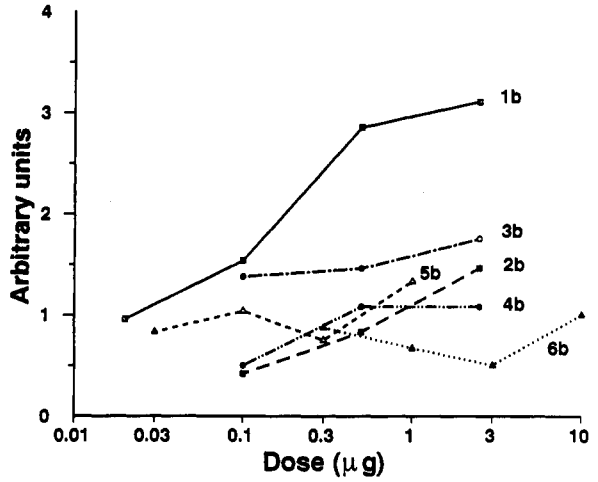


Figure 3. Maximum difference in hyperemia (experimental eye–control eye) of rabbit eyes treated with the test compounds. Each point represents the mean derived from six animals.

Conclusions

Replacement of the C_{18-20} fragment in $PGF_{2\alpha}$ and its 13,14-dihydro derivative with a phenyl group affords analogues with unique properties, in that they exert ocular hypotensive effects with greatly reduced side effects when applied topically to the eye. The 13,14-dihydro derivative **5b** is a nearly ideal ocular hypotensive compound that lacks significant irritative and conjunctival hyperemic side effects while retaining full ocular hypotensive potency in primates. We speculate that the phenyl ring imposes a conformational change on the omega chain that enables discrimination between yet undefined prostaglandin receptor subtypes.

Experimental Section

Chemistry. All chemical reagents were commercially available. Melting points were determined in open glass capillaries on a Buchi apparatus. Flash column chromatography was performed on silica gel 60, 230–400 mesh (E. Merck). Thin-layer chromatography (TLC) was carried out on silica gel 60 F245 glass plates and visualized by spraying the plate with 3% cupric acetate in 15% phosphoric acid or 5% phosphomolybdic acid in ethanol, followed by heating. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD, using tetramethylsilane as an internal standard, with a JEOL-200 or a Varian-500 instrument. Chemical shifts are expressed as δ values. All the spectra were in accordance with the assigned structures. Specific rotations were measured with a Perkin-Elmer 241 polarimeter. Analytical HPLC was performed on a Nucleosil C_{18} column (7 μm, 250 × 4 mm) in a gradient system using phosphate buffer pH 2.5/CH_3CN as the mobile phase, flow rate 1.8 mL/min. Preparative HPLC was at a flow rate of 15 mL/min. Spectrophotometric detection was performed at 200 nm in both cases. Elemental analyses were performed by Mikrokemi AB, Uppsala and were within 0.4% of the calculated value.

17-Phenyl-18,19,20-trinor-$PGF_{2\alpha}$ Isopropyl Ester (3b). DBU (0.37 g, 2.43 mmol) was added dropwise to a stirred solution of **3a** (0.40 g, 0.81 mmol) in acetone (16 mL) at 0 °C. The mixture was allowed to warm to room temperature whereupon isopropyl iodide (0.41 g, 2.43 mmol) was added dropwise. After being stirred for 10 h (TLC monitoring), the reaction was quenched with water, the mixture was extracted with EtOAc (50 mL), and the extract was washed with brine (20 mL), citric acid 3% (30 mL), and finally sodium hydrogen carbonate 5% (2 × 20 mL). After drying with anhydrous sodium sulfate, the solvent was removed in vacuo and the residual oil was chromatographed on silica gel using EtOAc as eluent. This afforded 0.34 g (80%) of the title compound as a colorless oil: R_f = 0.24 (EtOAc); $[\alpha]^{20}_D$ = +33.49° (c = 0.74, CH_3CN); ^1H NMR ($CDCl_3$) δ 1.2 (δ, 6 H $(CH_3)_2$), 1.46 (m, 1 H), 1.64 (m, 2 H), 1.74–1.78 (dd, 1 H), 1.85 (m, 2 H), 1.78–1.9 (m, 2 H), 2.08 (m, 2 H), 2.23 (t, 2 H), 2.24 (m, 2 H), 2.27 (m, 1 H), 2.3 (m, 1 H), 2.62–2.72 (m, 2 H), 3.9 (m, 1 H), 4.07 (t, 1 H), 4.13 (m, 1 H), 4.9 (m, 1 H), 5.43–5.59 (m, 4 H), 7.1–7.3 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 173.37, 141.9, 128.3, 125.7, 134.6 (C db), 132.03 (C db), 129.6 (C db), 129.04 (C db), 78.01, 72.83, 71.50, 67.55, 55.6, 42.9, 38.70, 33.90, 31.70, 26.50, 25.50, 24.80, 21.76, 21.75.

17-Phenyl-15R-18,19,20-trinor-$PGF_{2\alpha}$ Isopropyl Ester (4b). To a stirred solution of **3b** (0.24 g, 0.55 mmol) in dioxane (8.0 mL) was added portionwise DDQ (0.51 g, 2.2 mmol) whereupon the mixture became brown. After being stirred at room temperature for 24 h (TLC monitoring), the mixture was filtered, the precipitate was washed with ether (3 × 50 mL), and the combined organic phase was washed with water (2 × 20 mL), 1 M NaOH (3 × 30 mL), and brine (2 × 20 mL). Drying with sodium sulfate and concentrating in vacuo gave an oily residue which after chromatography on silica gel using ether as eluent gave 180 mg (76%) of the enone 7. This was dissolved in dichloromethane (5 mL) and added dropwise to a suspension of sodium borohydride (0.01 g, 0.25 mmol) and cerium chloride heptahydrate (0.05 g, 0.13 mmol) in methanol–CH_2Cl_2 2:1 (12 mL). After 30 min, the mixture was quenched with saturated ammonium chloride, extracted with EtOAc (2 × 30 mL), and dried with sodium sulfate. The solvent was removed in vacuo, and the residue was chromatographed on silica gel, achieving separation of the isomers **3b** 67.5 mg (28%) and **4b** 98.5 mg (41%) which was obtained as a colorless oil R_f = 0.32 (EtOAc); $[\alpha]^{20}_D$ = +42.32° (15 mL) (c = 2.1, CH_3CN); ^1H NMR ($CDCl_3$) δ 1.2 (δ, 6 H), 1.46 (m, 1 H), 1.65 (m, 2 H), 1.74–1.78 (dd, 1 H), 1.85 (m, 2 H), 1.78–1.90 (m, 2 H), 2.15 (m, 2 H), 2.25 (t, 2 H), 2.64–2.77 (m, 2 H), 3.93 (m, 1 H), 4.15 (t, 1 H), 4.16 (m, 1 H), 5.0 (m, 1 H), 5.39–5.44 (m, 2 H), 5.61–5.65 (m, 2 H), 7.1–7.3 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 173.37, 141.9, 128.3, 125.7, 134.6 (Cdb), 132.03 (Cdb), 129.6 (Cdb), 129.04 (Cdb), 78.01, 72.83, 71.50, 67.55, 55.6, 42.9, 38.70, 33.90, 31.70, 26.50, 25.50, 24.80, 21.76, 21.75.

Dimethyl (2-Oxo-4-phenylbutyl)phosphonate. To a stirred suspension of sodium hydride (7.20 g, 300 mmol) previously washed with n-pentane in dry THF (250 mL) at room temperature was added dropwise a solution of dimethyl (2-oxopropyl)-phosphonate (47.5 g, 285.9 mmol) in THF (110 mL). The reaction mixture was stirred for 2 h, then cooled in an ice bath and treated with a solution of n-BuLi (22 g, 340 mmol) in hexane, causing a dark brown solution to be formed. Stirring was continued for 2 h at 0 °C, followed by dropwise addition of benzyl bromide (53.8 g, 314.5 mmol) in THF (50 mL). The reaction mixture was gradually warmed to room temperature and after 3 h (TLC monitoring), it was quenched with 1 M HCl (20 mL). The mixture was poured into ice-water (200 mL) and extracted with $CHCl_3$ (2 × 150 mL), the organic layers were collected, washed with brine (150 mL), and chromatographed on silica gel using CH_2Cl_2 and EtOAc successively as eluent, furnishing 43.4 g (68%) of a slightly yellow oil: R_f = 0.23 (EtOAc–acetone 1:1), ^1H NMR ($CDCl_3$) δ 2.9 (m, 4 H, $(CH_2)_2$), 3.04–3.14 (d, 2 H), 3.69–3.79 (d,

(1*S*,5*R*,6*R*,7*R*)-6-Formyl-7-[(4-phenylbenzoyl)oxy]-2-oxabicyclo[3.3.0]octan-3-one (9). To a solution of the alcohol **8** (15.0 g, 42.56 mmol) in DME (100 mL), cooled to 18 °C, were added dicyclohexylcarbodiimide (DCC) (26.3 g, 127.69 mmol) and phosphoric acid (1.43 mL, 21.28 mmol). The temperature of the reaction mixture was kept below 25 °C for 30 min. The reaction mixture was stirred at room temperature for additional 2 h (TLC monitoring) and the precipitate was removed by filtration and washed with ether (2 × 50 mL). The combined organic layer was washed with water (50 mL) and brine (2 × 50 mL), the aqueous solution was extracted with ether (100 mL), and the organic layers were collected and dried with sodium sulfate, filtered, and used directly for the next step: TLC R_f = 0.42 (silica gel, EtOAc–toluene 2:1).

(1*S*,5*R*,6*R*,7*R*)-6-(3-Oxo-5-phenyl-1*E*-pentenyl)-7-[(4-phenylbenzoyl)oxy]-2-oxabicyclo[3.3.0]octan-3-one (10). To a stirred suspension of NaH (3.08 g, 128.3 mmol), prewashed with *n*-pentane, in DME (150 mL) under nitrogen was added dropwise dimethyl (2-oxo-4-phenylbutyl)phosphonate (28.52 g, 113.3 mmol) in DME (100 mL) and stirred vigorously for 1 h at room temperature. The mixture was then cooled to −10 °C, and a solution of the crude aldehyde **9** was added dropwise. After 30 min at 0 °C and 2 h at room temperature (TLC monitoring), the reaction mixture was neutralized with AcOH, the solvent was removed, and to the residue was added EtOAc (200 mL). The solution was washed with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate. The solvent was removed in vacuo, the oil was stirred with ether (100 mL) and the resulting white precipitate was filtered off and washed with cold ether, giving a white crystalline substance: mp 134–135.5 °C; yield 24.0 g (58.5%); $[\alpha]^{20}_D$ = −116° (*c* = 1.26, CH_3CN); ^1H NMR ($CDCl_3$) δ 2.9 (m, 8 H), 5.1 (t, 1 H), 5.3 (q, 1 H), 6.2 (d, 1 H), 6.7 (dd, 1 H), 7.1–7.6 (m, 10 H), 8.1 (d, 4 H); ^{13}C NMR ($CDCl_3$) δ 198.49 (CH=CH*C*O), 175.68 (C_6H_4CO), 146.19, 142.95 (*C*H=CHCO), 140.79, 139.75, 131.36 (CH=*C*HCO), 130.17, 128.89, 128.46, 128.34, 128.31, 128.21, 127.87, 127.22, 127.17, 126.15, 83.11, 78.50, 54.07, 42.56, 42.44, 37.80, 34.90, 29.96. Anal. ($C_{31}H_{28}O_5$) C, H.

(1*S*,5*R*,6*R*,7*R*)-6-[(3*S*)-3-Hydroxy-5-phenyl-1-pentenyl]-7-[(4-phenylbenzoyl)oxy]-2-oxabicyclo[3.3.0]octan-3-one (11). To a stirred solution of lithium tri-*sec*-butylborohydride (Lithium Selectride) (5.14 g, 27.05 mmol) in THF–ether 1:2 (60 mL) at −130 °C under nitrogen was added dropwise a solution of the enone **10** (13.0 g, 27.05 mmol) in THF (20 mL) cooled to −75/−78 °C. The reaction mixture was stirred for 1 h (TLC monitoring) and then quenched with saturated ammonium chloride. The temperature was raised to 0 °C, water (20 mL) was added, and the mixture was diluted with EtOAc (80 mL). The organic layer was separated and washed with brine, dried on anhydrous sodium sulfate, concentrated in vacuo, and chromatographed twice on silica gel using toluene–EtOAc 2:1 and 1:1 successively as eluent, furnishing **11** as a white crystalline product: mp 108–110 °C; yield 6.8 g (52%); R_f = 0.33 (silica gel, EtOAc–toluene 2:1); $[\alpha]^{20}_D$ = −101.59° (*c* = 0.69, CH_3CN); ^1H NMR ($CDCl_3$) δ 1.84 (m, 2 H), 2.25 (dq, 1 H), 2.5 (dd, 1 H), 2.60–2.90 (m, 6 H), 4.14 (m, C*H*OH), 5.06 (m, 1 H), 5.27 (q, 1 H), 5.59–5.72 (m, CH=CH), 7.10–7.28 (m, 6 H), 7.38–7.48 (m, 2 H), 7.58–7.67 (m, 4 H), 8.02–8.08 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 176.24 (C_6H_4C=O), 165.89 (lactone C=O), 146.05, 141.50, 139.85, 136.02 (CH=*C*H), 130.14, 128.89 (*C*H=CH), 128.71, 128.35, 128.21, 128.18, 125.92, 83.19 (CH_2*C*HOCO), 78.99 (C_6H_4COOCH), 71.39 (CH=CH*C*HOH), 54.03, 42.72, 38.67, 37.58, 34.89, 31.56. Anal. ($C_{31}H_{30}O_5$) C, H.

(1*S*,5*R*,6*R*,7*R*)-6-[(3*R*)-3-Hydroxy-5-phenyl-1-pentenyl]-7-[(4-phenylbenzoyl)oxy]-2-oxabicyclo[3.3.0]octan-3-one (12). The preparation of this compound was achieved by chromatographic separation of the isomers obtained by following the procedure described for the preparation of compound **11**. This gave a colorless oil: yield 4.3 g (33.1%); R_f = 0.25 (silica gel, EtOAc–toluene 2:1); $[\alpha]^{20}_D$ = −109.29° (*c* = 0.7, CH_3CN); ^1H NMR ($CDCl_3$) δ 1.85 (m, 2 H), 2.24 (dq, 1 H), 2.52 (dd, 1 H), 2.58–2.90 (m, 6 H), 4.12 (m, C*H*OH), 5.04 (m, 1 H), 5.26 (q, 1 H), 5.56–5.72 (m, CH=CH), 7.10–7.28 (m, 6 H), 7.38–7.48 (m, 2 H), 7.58–7.67 (m, 4 H), 8.02–8.08 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 176.27 (C_6H_4C=O), 165.83 (lactone C=O), 146.01, 141.46, 139.79, 136.16 71.53 (CH=CH*C*HOH), 54.04, 42.66, 38.71, 37.55, 34.83, 31.57. Anal. ($C_{31}H_{30}O_5$) C, H.

(1*S*,5*R*,6*R*,7*R*)-6-[(3*R*)-3-Hydroxy-5-phenyl-1-pentyl)-7-[(4-phenylbenzoyl)oxy]-2-oxabicyclo[3.3.0]octan-3-one (13). To a suspension of 10% Pd/C (0.4 g) in 1 M NaOH (1.0 mL, 0.4 mmol) and ethanol (15 mL) was added a solution of **11** (1.94 g, 4.01 mmol) in ethanol (6.0 mL). The solution was stirred under hydrogen atmosphere for 6 h (TLC monitoring) and quenched with 1 M HCl. The catalyst was removed by filtration through a Celite pad, washed with ethanol absolute (20 mL). The solvent was removed in vacuo. The resulting oil was dissolved in EtOAc (110 mL) and washed with brine 15% (30 mL). The water phase was washed with EtOAc (30 mL). The combined organic extracts were dried with sodium sulfate and filtered. The solvent was removed in vacuo giving **13** as a colorless oil. Chromatography on silica gel using EtOAc as eluent yielded 1.51 g (78.4%): R_f = 0.44 (silica gel, EtOAc); $[\alpha]^{20}_D$ = −69.62° (*c* = 0.8, CH_3CN); ^1H NMR ($CDCl_3$) δ 1.40 (m, 1 H), 1.60 (m, 4 H), 1.78 (m, 2 H), 2.15 (m, 1 H), 2.40 (m, 2 H), 2.50 (dd, 1 H), 2.63 (m, 2 H), 2.78 (m, 1 H), 2.90 (m, 1 H), 3.14 (m, C*H*OH), 5.08 (m, 1 H), 5.28 (q, 1 H), 7.18 (m, 3 H), 7.26 (m, 2 H), 7.38 (m, 1 H), 7.45 (m, 2 H), 7.60 (m, 2 H), 7.67 (m, 2 H), 8.50 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 176.81 (C_6H_4C=O), 165.86 (lactone C=O), 145.91, 141.72, 139.85, 130.12, 128.85, 128.41, 128.35, 128.32, 128.11, 127.21, 127.12, 125.89, 84.44 (CH_2CHOCO), 80.13 (C_6H_4COOCH), 70.88 (CH_2*C*HOH), 52.67, 43.57, 39.07, 37.80, 36.28, 35.12, 32.00, 29.37. Anal. ($C_{31}H_{32}O_5$) C, H.

(1*S*,5*R*,6*R*,7*R*)-6-[(3*S*)-3-Hydroxy-5-phenyl-1-pentyl]-7-[(4-phenylbenzoyl)oxy]-2-oxabicyclo[3.3.0]octan-3-one (14). This was prepared as compound **13** from the lactone **12** giving a colorless oil yield 1.25 g (65%). The compound was used directly for the next step, R_f = 0.47 (silica gel EtOAc).

(1*S*,5*R*,6*R*,7*R*)-6-[(3*R*)-3-Hydroxy-5-phenyl-1-pentyl]-7(*R*)-hydroxy-2-oxabicyclo[3.3.0]octan-3-one (15). To a solution of the lactone **13** (1.6 g, 3.1 mmol) in methanol (15 mL) was added potassium carbonate (0.28 g, 1.98 mmol) and the mixture stirred at ambient temperature for 6 h (TLC monitoring). The mixture was neutralized with 1 N HCl and extracted with EtOAc (2 × 30 mL). The organic phase was dried on anhydrous sodium sulfate and evaporated to dryness. The crude product was chromatographed (silica gel EtOAc–acetone 1:1). The title compound **15** was obtained as a colorless oil: yield 0.77 g (85%); R_f = 0.19 (EtOAc); $[\alpha]^{20}_D$ = −17.60° (*c* = 0.34, CH_3CN); ^1H NMR ($CDCl_3$) δ 1.28 (m, 1 H), 1.54 (m, 3 H), 1.78 (m, 3 H), 2.15 (m, 1 H), 2.28 (m, 1 H), 2.46 (m, 1 H), 2.51 (m, 1 H), 2.67 (m, 1 H), 2.78 (m, 2 H), 3.60 (m, CH_2C*H*OHCH$_2$), 3.97 (m, C*H*OH), 4.92 (m, C*H*OC=O), 7.18 (m, 3 H), 7.28 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 177.55 (lactone C=O), 141.81, 128.42, 128.33, 125.89, 83.88 (CH_2CHOCO), 77.46 (CH_2*C*HOH), 71.28 (CH_2CHOHCH$_2$), 53.94, 43.22, 40.52, 39.08, 35.96, 35.20, 32.03, 28.95. Anal. ($C_{18}H_{24}O_4$) C, H.

(1*S*,5*R*,6*R*,7*R*)-6-[(3*S*)-3-Hydroxy-5-phenyl-1-pentyl]-7(*R*)-hydroxy-2-oxabicyclo[3.3.0]octan-3-one (16). Prepared as compound **15** from the lactone **14**. The product was chromatographed on silica gel using ethyl acetate as eluent. This gave a colorless oil: yield 0.75 g (83.6%); $[\alpha]^{20}_D$ = −8.26° (*c* = 0.79, CH_3CN); R_f = 0.18 (EtOAc); ^1H NMR ($CDCl_3$) δ 1.40 (m, 1 H), 1.54 (m, 2 H), 1.78 (m, 3 H), 2.15 (m, 1 H), 2.28 (m, 1 H), 2.48 (m, 2 H), 2.66 (m, 1 H), 2.78 (m, 2 H), 3.60 (m, CH_2C*H*OHCH$_2$), 3.97 (m, C*H*OH), 4.92 (m, C*H*OC=O), 7.18 (m, 3 H), 7.28 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 177.60 (lactone C=O), 141.82, 128.41, 128.32, 125.87, 83.90 (CH_2CHOCO), 77.27 (CH_2*C*HOH), 71.06 (CH_2CHOHCH$_2$), 53.54, 43.01, 40.47, 39.16, 35.92, 34.90, 32.00, 28.80. Anal. ($C_{18}H_{24}O_4$) C, H.

(1*S*,5*R*,6*R*,7*R*)-6-[(3*R*)-3-Hydroxy-5-phenyl-1-pentyl]-7-hydroxy-2-oxabicyclo[3.3.0]octan-3-one (17). A solution of diisobutylaluminium hydride (DIBAL) (1.53 g, 10.78 mmol) in dry toluene (6.2 mL) was added dropwise to a stirred solution of the lactone **15** (0.82 g, 2.70 mmol) in dry THF (22 mL) at −72/−80 °C. After 1 h (TLC monitoring), the reaction mixture was quenched with methanol (5 mL) and was warmed to room temperature followed by addition of water (50 mL) and 1 M HCl (50 mL), and extracted with EtOAc (2 × 50 mL). The organic phase was dried with sodium sulfate and filtered, and triethyl-

using EtOAc and EtOAc–acetone 1:1, respectively, as eluent to give a white crystalline product: yield 0.54 g (65%); mp 102–104 °C; R_f = 0.31 (EtOAc–acetone 1:1); $[\alpha]^{20}_D$ = −22.08° (c = 0.18, THF); ^{1}H NMR (CD_3OD) δ 1.24 (m, 1 H), 1.48 (m, 2 H), 1.58 (m, 2 H), 1.72 (m, 2 H), 1.94 (m, 1 H), 2.04 (m, 1 H), 2.26 (m, 2 H), 2.64 (m, 1 H), 2.77 (m, 1 H), 3.54 (m, 1 H), 3.74 (m, 1 H), 4.42–4.56 (dq, 1 H), 5.38–5.54 (dd, 1 H), 7.12–7.26 (m, 5 H); ^{13}C NMR (CD_3OD) δ 144.19, 129.88, 129.78, 127.15, 102.39, 101.57, 84.79, 81.97, 79.93, 79.73, 72.52, 55.39, 55.19, 47.30, 44.0, 42.59, 42.22, 41.98, 40.75, 40.70, 36.97, 33.52, 31.49, 30.62. Anal. ($C_{18}H_{26}O_4$) C, H.

(1*S*,5*R*,6*R*,7*R*)-6-[(3*S*)-3-Hydroxy-5-phenyl-1-pentyl]-7(*R*)-hydroxy-2-oxabicyclo[3.3.0]octan-3-one (18). Prepared as compound 17 from the lactone 16. The product was chromatographed on silica gel using ethyl acetate and ethyl acetate–acetone 1:1 as eluent. This gave a colorless oil: yield 0.34 g (83%); $[\alpha]^{20}_D$ = −13.23° (c = 0.4, CH_3CN); R_f = 0.32 (EtOAc–acetone 1:1); ^{1}H NMR ($CDCl_3$) δ 1.32–1.68 (m, 4 H), 1.74 (m, 2 H), 1.96 (m, 1 H), 2.12 (m, 2 H), 2.14–2.34 (m, 4 H), 2.64 (m, 1 H), 2.66 (m, 1 H), 2.78 (m, 1 H), 3.80–3.94 (dq, 1 H), 4.55–4.66 (m, 1 H), 5.46–5.64 (dd, 1 H), 7.15–7.20 (m, 3 H), 7.25–7.29 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 142.01, 128.37, 128.35, 125.79, 100.99, 99.92, 85.97, 81.87, 79.63, 79.02, 71.03, 70.94, 54.66, 54.21, 47.71, 46.16, 42.49, 41.28, 41.17, 40.29, 39.14, 39.10, 35.26, 35.20, 32.07, 32.05, 30.78, 29.29, 29.25. Anal. ($C_{18}H_{26}O_4$) C, H.

13,14-Dihydro-17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$ (5a). To a stirred suspension of (4-carboxybutyl)triphenylphosphonium bromide (2.32 g, 5.22 mmol) in THF (20 mL) under nitrogen at 0–5 °C was added potassium *tert*-butoxide (1.18 g, 10.44 mmol) and the mixture stirred for 30 min at room temperature. To the resultant red-orange solution of ylide at −15/−10 °C was added the lactol 17 (0.4 g, 1.30 mmol) in THF (5 mL), and the mixture was stirred for 3–4 h (TLC monitoring). The reaction mixture was diluted with water (20 mL) and washed with ether (4 × 40 mL). The water layer was acidified with 5% citric acid to pH 4 and extracted with EtOAc (2 × 40 mL). The organic phase was washed with brine (30 mL), dried on sodium sulfate, and filtered. The solvent was removed in vacuo, and the slurry **5a** was used directly without isolation for the next step, R_f = 0.27 (EtOAc).

(15*S*)-13,14-Dihydro-17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$ (6a). Prepared as compound **5a** from the lactol 18. The product was used directly without isolation for the next step.

13,14-Dihydro-17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$ Isopropyl Ester (5b). DBU (1.2 g, 7.98 mmol) was added dropwise to a stirred solution of the crude product 15 (0.52 g, 1.32 mmol) in acetone (15 mL) at 0 °C. The mixture was allowed to warm to room temperature when isopropyl iodide (1.13 g, 6.70 mmol) was added dropwise. After 4 h (TLC monitoring), the mixture was diluted with EtOAc (100 mL), washed with brine (30 mL), citric acid 3% (2 × 25 mL), and sodium hydrogen carbonate 5% (2 × 25 mL), and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel using a gradient elution with dichloromethane, dichloromethane–ethanol 10:0.5, and dichloromethane–ethanol 10:0.75 successively. This afforded a colorless oil: yield 0.19 g (38%); R_f = 0.32 (EtOAc); $[\alpha]^{20}_D$ = +31.57° (c = 0.91, CH_3CN); ^{1}H NMR ($CDCl_3$) δ 1.2 (δ, 6 H), 1.32–1.42 (m, 3 H), 1.62 (m, 2 H), 1.69 (m, 2 H), 1.79 (m, 2 H), 2.12 (m, 2 H), 2.22–2.33 (m, 2 H), 2.28 (t, 2 H), 2.45 (d, 1 H), 2.65–2.78 (dm, 2 H), 3.65 (m, $CH_2CHOHCH_2$), 3.94 (m, CH_2CHOH), 4.16 (m, CH_2CHOH), 5.0 (sept, 1 H), 5.38 (m, db), 5.47 (m, db), 7.19–7.27 (dm, Ar); ^{13}C NMR ($CDCl_3$) δ 173.46 (C=O), 142.84, 128.4, 125.8, 129.6 (C5), 129.3 (C6), 78.8 (C11), 74.79 (C9), 71.33 (C15), 67.65, 53.91 (C12), 51.94 (C8), 42.57 (C10), 39.10 (C16), 35.84 (C14), 34.07 (C2), 32.14 (C17), 29.69 (C13), 26.97 (C7), 26.67 (C4), 24.96 (C3), 21.86.

(15*S*)-13,14-Dihydro-17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$ Isopropyl Ester (6b). Prepared as compound **5b** from **6a**: yield 34%; R_f = 0.32 (EtOAc); $[\alpha]^{20}_D$ = +42.3° (c = 2.1, CH_3CN); ^{1}H NMR ($CDCl_3$) δ 1.2 (δ, 6 H), 1.42 (m, 1 H), 1.54–1.65 (m, 6 H), 1.79 (m, 2 H), 1.9 (t, 2 H), 2.12 (m, 2 H), 2.25–2.35 (dm, 2 H), 2.45 (d, 1 H), 2.65–2.75 (dm, 2 H), 3.65 (m, $CH_2CHOHCH_2$), 3.87 (m, CH_2CHOH), 4.16 (m, CH_2CHOH), 5.0 (sept, 1 H), 5.38 (m, db), 5.47 (m, db), 7.19–7.27 (dm, Ar); ^{13}C NMR ($CDCl_3$) δ 173.46 (C=O), 142.1, 128.4, 125.82, 129.6 (C5), 129.3 (C6), 78.68 (C11),

Pharmacology. The analogues were used as free acids[28] in the in vitro tests and as isopropyl esters in the in vivo tests to enhance penetration into the eye.[29] The compounds were dissolved in a vehicle containing 0.9% sodium chloride and 0.5% polysorbate 80 for the in vivo experiments. The amount of drug used in various experiments corresponds to free acid equivalents. For the in vitro experiments the test substances were dissolved in 0.6 M $NaHCO_3$ solution titrated to pH = 7.4 using 1 M NaH_2PO_4 to a final concentration of 0.1 mol/L. Further dilutions were made with 0.9% NaCl.

IOP, Pupil Diameter, and Irritation in the Cat Eye. Domestic female cats weighing 2–3 kg and specially trained for IOP measurements were used. One eye of each animal was topically treated with the drug (drop size 20 μL) and the contralateral eye received the vehicle solution. The intraocular pressure was measured using a pneumatonometer (Digilab Modular One, Bio-Rad) under local anaesthesia with oxibuprocain. The horizontal diameter of the pupil was measured with a millimeter ruler under constant illumination conditions. Measurements of IOP and pupil diameter were performed before drug treatment and 1, 3, 6, and 23 h after treatment. The ocular irritation was evaluated from the behavior of the animals, in particular the degree of lid closure during the first hour after drug treatment. An arbitrary scale from 0 to 3 was used, 0 indicating absence of irritation and 3 complete closure of the lids.

IOP and Pupil Diameter in the Monkey Eye. Cynomolgus monkeys specially trained for IOP measurements were used. The animals were sedated with ketamine (2–3 mg/kg body weight) for transportation from the animal housing facility to the laboratory and placed in specially designed chairs. Oxibuprocain was used for local anaesthesia. IOP was measured with a pneumatonometer (Digilab Modular One) calibrated for monkey eyes[30] and the pupil diameter was measured with a millimeter ruler. The drug was applied to one eye (drop size 10 μL) and the contralateral eye received the vehicle solution. Measurements were performed before treatment and 1, 2, 4, and 6 h after treatment.

Conjunctival Hyperemia in the Rabbit. Albino rabbits (New Zealand White, 2–3 kg) were used for evaluation of conjunctival hyperemia. Color photographs of the drug-treated and control eyes were taken using a camera equipped with a Medical Nikkor lens (magnification 2X) before treatment, and 1, 2, 3, and 4 h after treatment. The photographs were used for semiquantitative evaluation of conjunctival hyperemia using an arbitrary scale from 0 to 5 (0 = totally pale conjunctiva, 1 = vessels normal, 2 = mild hyperemia, 3 = moderate hyperemia, 4 = severe hyperemia, 5 = severe hyperemia with chemosis).

Contraction of Cat Iris Sphincter in Vitro. Functional receptor studies were performed using iris sphincter muscles from cat eyes. The eyes were either used directly after enucleation or stored in ice-cold saline overnight. The iris sphincter muscles were prepared, cut in halves, and mounted in thermostated (37 °C) tissue baths with oxygenated modified Kreb's solution containing indomethacin (2.8 × 10^{-6} M), atropine (10^{-7} M), and propranolol (10^{-6} M). A resting tension of 150 mg was applied, and the contractile force was measured after cumulative dosing of prostaglandin analogues in free acid form. The interval between doses was approximately 5–10 min, which was the time required to reach a stable level of contraction. Force transducers for measurement of isometric contraction (Grass FT30C) connected to a polygraph (Grass Model 7) were used for registration of the response. For each tissue sample the maximal response was normalized to 100%. The mean response from four different preparations was calculated, and concentration–effect curves were fitted to the data using the following equation:

$$\text{Resp} = \frac{100}{1 + (10^{\log E}/10^{\log C})^D}$$

Resp = response in percent, log E = log EC_{50}, log C = log concentration, D = Hill coefficient.

Statistics. Results are given as arithmetic means ± standard

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