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Discovery of 13-oxa prostaglandin analogs as antiglaucoma agents: Synthesis and biological activity

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ABSTRACT

FP-Class prostaglandin analogs have demonstrated utility for the treatment of glaucoma and ocular hypertension. A series of novel FP prostaglandin analogs was designed to optimize topical ocular activity and reduce ocular side-effects by replacing 13-carbon with oxygen. A facile synthesis was successfully developed for synthesis of the 13-oxa prostaglandins from the commercially available Corey aldehyde benzoate. Among the compounds synthesized, **AL-16082** was the most potent prostaglandin FP agonist in vitro. In a prostaglandin FP receptor-linked second-messenger assay, phosphoinositide (PI) turnover, it exhibited a potency value (EC_{50}) of 1.9 nM (78% max. response relative to fluprostenol). The isopropyl ester of **AL-16082**, compound **AL-16049**, significantly lowered intraocular pressure (IOP) in the ocular hypertensive monkey eyes by 30%. In the study of acute ocular irritation response in New Zealand albino rabbits, **AL-16049** produced lower incidence of hyperemia, swelling, and discharge than $PGF_{2\alpha}$ (1 μg), and a similar incidence of hyperemia, swelling, and discharge to latanoprost (1.8 μg). AL-16049 also produced no signs of ocular irritation or discomfort in the cat at the doses evaluated.

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1. Introduction

Glaucoma is a progressive disease which leads to optic nerve damage and loss of vision. The principal risk factor for the disease is elevated intraocular pressure (IOP). Elevated IOP is caused by a decrease in the rate aqueous humor exits the anterior chamber of the eye as opposed to being caused by an increase in the production of aqueous humor. Although the mechanisms that lead to reduced outflow are poorly understood, it is known that elevated IOP can be at least partially controlled by administering drugs which either reduce the production of aqueous humor within the eye, or increase the outflow of aqueous humor from the eye. FP prostaglandin agonists have been shown to effectively reduce IOP by increasing outflow of aqueous humor through the uveoscleral pathway.[1] Thus, there has been a great deal of interest in developing synthetic FP prostaglandin agonists with IOP-lowering efficacy. Certain prostaglandins and their pro-drugs, such as $PGF_{2\alpha}$ Fig. 1) isopropyl ester, reduce IOP in monkeys and in humans, but also cause conjunctival hyperemia and foreign-body sensation.[2] The development of pro-drugs of potent, selective synthetic prostaglandin FP receptor agonists as clinically effective IOP-lowering agents devoid of ocular side-effects has been an important advance in the treatment of glaucoma.[3] Agents latanoprost, bimatoprost, travaprost and bimatoprost[4] (Fig. 1), are the active ingredients of

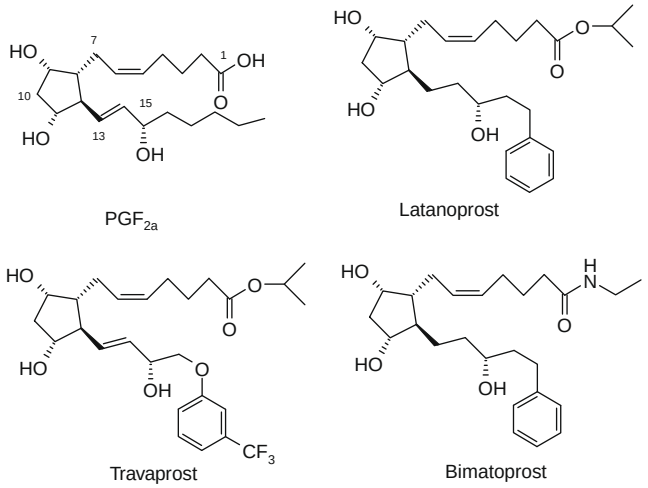

Figure 1. Structures of $PGF_{2\alpha}$, latanoprost, bimatoprost, travaprost and bimatoprost.

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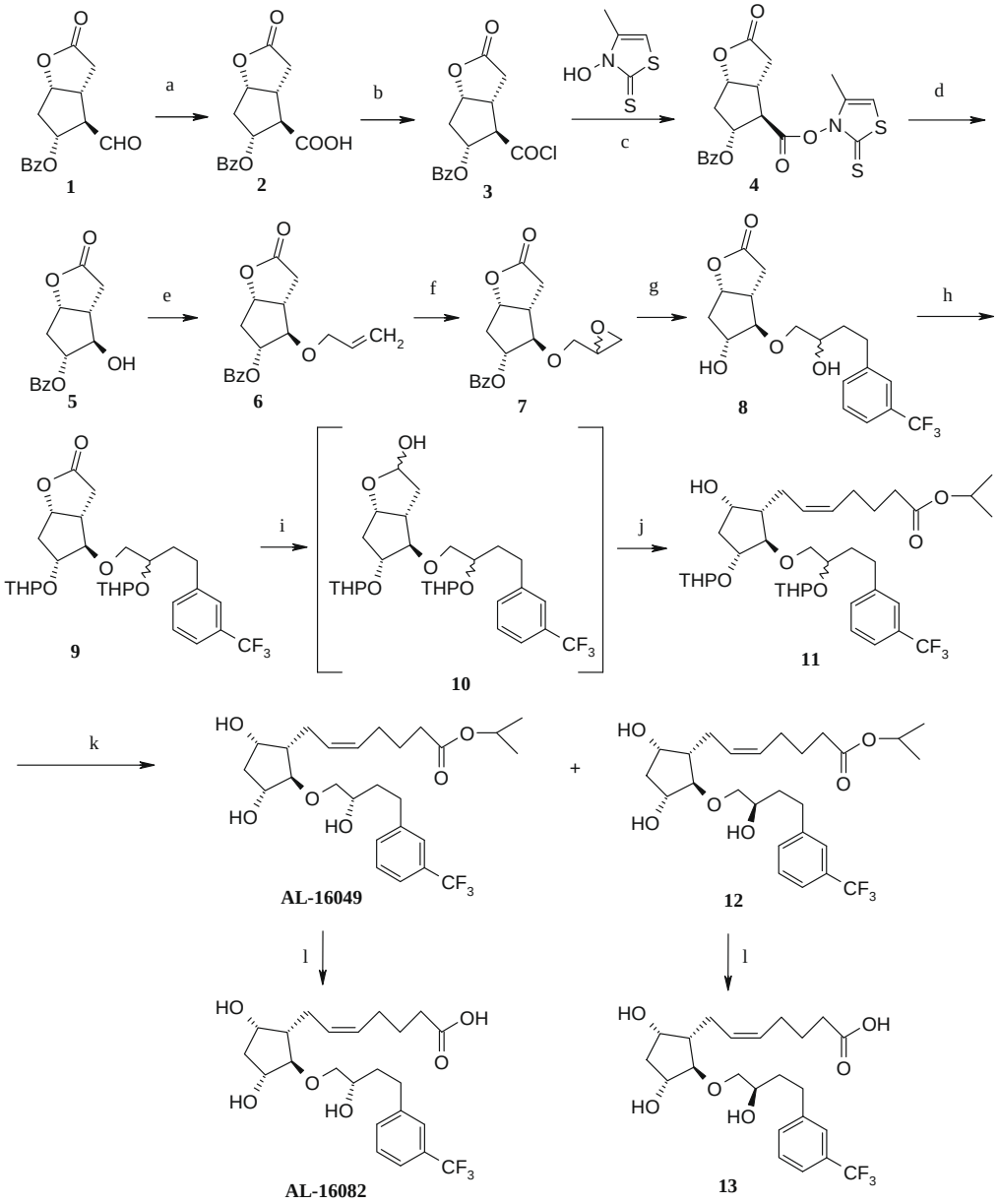
topically IOP-lowering medications. Their introduction has revolutionized the treatment of ocular hypertension and glaucoma.

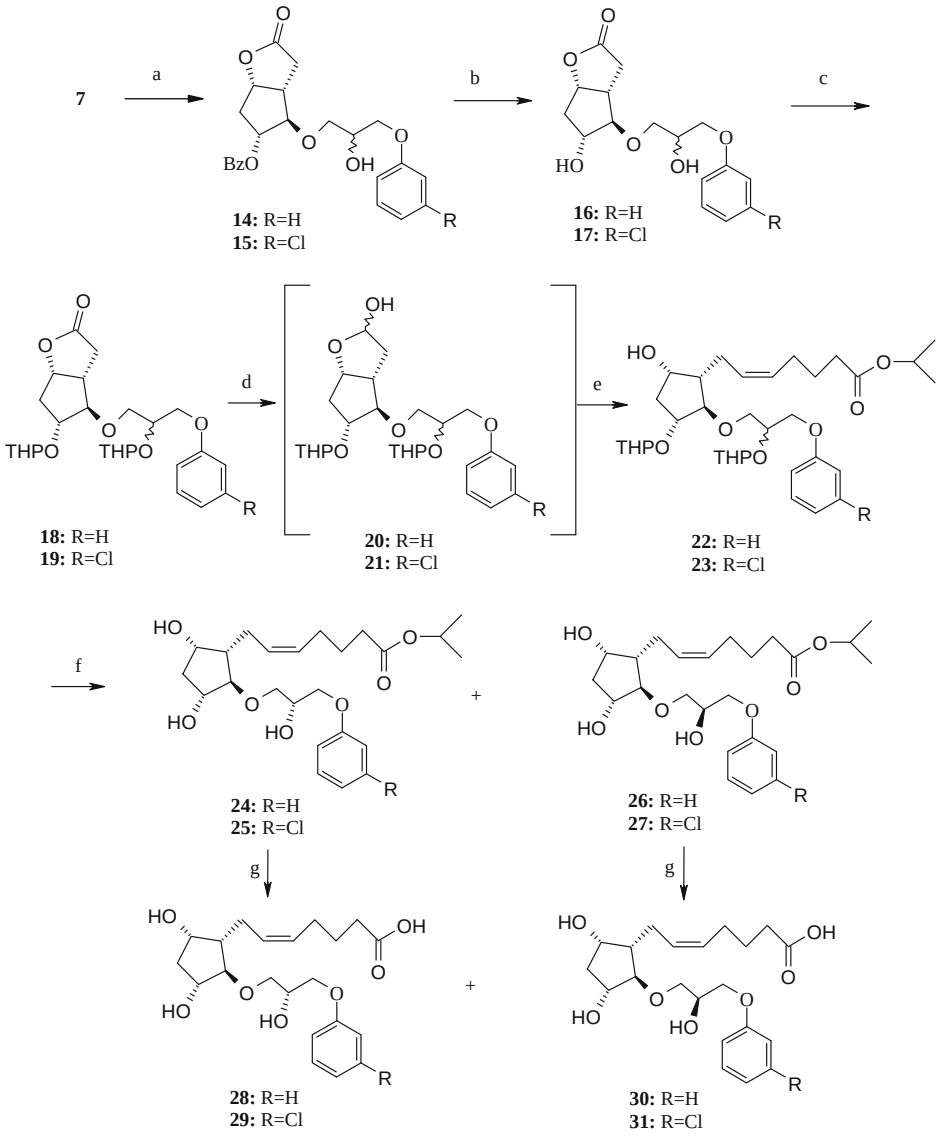
As part of our continuing research program targeted at the discovery of prostaglandin agonists that lower IOP with reduced side-effects, we wish to report the successful discovery of 13-oxa-prostaglandins for the treatment of glaucoma and ocular hypertension. This series was designed to optimize prostaglandin FP agonist topical ocular activity and reduce side effect by replacing 13-carbon with oxygen. This type of modification usually reduces the hydrophobicity of compounds, a property that is believed to be related to ocular side-effects. A facile synthetic route to 13-oxa prostaglandins was developed.

2. Chemistry

The primary synthetic target was to develop a facile route to the 13-oxa prostaglandins from commercially available chemicals avoiding a complicated total synthesis. The synthetic routes are described in Schemes 1 and 2. The tentative assignment of the stereochemistry at C15 was based on the well-documented difference in polarity between the 15α and 15β epimers.[5] Commercially available Corey aldehyde benzoate **1** was oxidized to carboxylic acid **2** by Jones reagent at 0 °C. Chlorination of **2** afforded acid chloride **3**. The crude product **3** was reacted with 3-hydroxy-4-methylthiazole-2(3*H*)-thione to yield the thiohydroxamate ester **4**. The nor-alcohol 5 was obtained in good yield by stirring the thiohydroxamate ester **4** with tris (phenylthio) antimony in dichloromethane in a flask open to air.[6] This synthetic route to intermediate **5** was an improvement over the previously reported methods.[7,8] Alkylation of alcohol **5** followed by epoxidation using MCPBA provided the epoxide **7**. Regiospecific ring-opening of the epoxide with a Grignard reagent *m*-trifluoromethyl-benzylmagnesium bromide made in situ gave a diastereomer mixture **8**.[9–11] Protection of the diol afforded the di-THP-ether **9**. Lactone **9** was treated with dii-



Scheme 1. Reagents and conditions: (a) Jones reagent, acetone, 0 °C, 20 min; (b) $SOCl_2$, 65 °C; (c) pyridine, CH_2Cl_2, rt, 1 h; (d) $(PhS)_3Sb$, CH_2Cl_2, rt, 16 h, (open flask); (e) allyl bromide, Ag_2O, DMF, rt, 48 h; (f) MCPBA, $NaHCO_3$, CH_2Cl_2, rt, 24 h; (g) *m*-trifluromethyl-benzylmagnesium bromide, CuI, ether, −78 °C, 6 h; (h) dihydropyran, *p*-TsOH, CH_2Cl_2, 0 °C, 30 min; (i) DIBAL-H, toluene, −78 °C, 1 h; (j) (1) $Ph_3P^+(CH_2)_4COOHBr^+$, $KOBu^t$, THF; (2) 2-iodopropane, DBU, rt, 16 h; (k) (1) CH_3COOH (65%), THF, 550 C, 1 h; (2) chiral separation by HPLC; (l) $LiOH.H_2O$, MeOH, H_2O, rt, 16 h.



Scheme 2. Reagents and conditions: (a) phenol or 3-chlorophenol, K_2CO_3, CH_3CN, 0 °C, 4 h; (b) K_2CO_3, MeOH, rt, 1.5 h; (c) dihydropyran, *p*-TsOH, CH_2Cl_2, 0 °C, 30 min; (d) DIBAL-H, toluene, −78 °C, 1 h; (e) (1) $Ph_3P^+(CH_2)_4COOHBr^+$, $KOBu^t$, THF; (2) 2-iodopropane, DBU, rt, 16 h; (f) (1) CH_3COOH (65%), THF, 55 °C, 1 h; (2) chiral separation by HPLC; (g) $LiOH{\cdot}H_2O$, MeOH, H_2O, rt, 16 h.

sobutylaluminum hydride (DIBAL-H) in toluene at −78 °C to give the lactol **10**. The Wittig reaction with (4-carboxybutyl)triphenylphosphonium bromide and potassium *tert*-butoxide in THF afforded the acid which was reacted without isolation with isopropyl iodide and DBU in acetone to give the ester **11**.[12] Cleavage of the THP ethers under mildly acidic conditions followed by chiral separation on HPLC gave the isopropyl esters **AL-16049** (15α) and **12** (15β). Hydrolysis using basic condition provided the corresponding free acids **AL-16082** and **13**.[13]

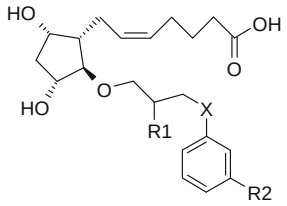
The 16-phenoxy analogues (Scheme 2) were prepared in a similar manner except for the ring-opening of the epoxide **7**. The epoxide was opened by the reaction with phenol or 3-chlorophenol in the presence of potassium carbonate in acetonitrile to afford the intermediates **14** and **15**[7] which were hydrolyzed to intermediates **16** and **17**.

3. Results and discussion

The carboxylic acid believed to be the pharmacologically active form was used in all in vitro studies. The compounds shown in Table 1 were tested for their binding affinity and functional efficacy to the FP prostaglandin receptor. The acids **AL-16082**, **13**, **28**, **29**, 30 and **31** were evaluated for their ability to stimulate FP receptor-linked phosphoinositide turnover in Swiss 3T3 mouse fibroblast cells (expressing an endogenous FP receptor), and to bind to FP receptor expressed in bovine corpus luteum, using recently published procedures.[14,15] The results from these studies are summarized in Table 1.

The 17-carbon-trifluoromethyl substituted phenyl analogs **AL-16082** and **13** had equal affinity and were more potent in the functional assay than $PGF_{2\alpha}$ and the carboxy acid hydrolysis product of latanoprost (latanoprost acid). The three fold difference in functional potency and the slightly higher affinity of **AL-16082** than **13** support the tentative assignment of the 15-OH of **AL-16082** as being in the alpha configuration. The glycol analogs **28**–**31** are less potent than the standards $PGF_{2\alpha}$ and latanoprost acid and are at least ten fold less potent than the 17-carbon-trifluoromethyl substituted phenyl analogs in the functional assay. The affinity of compounds **29**–**30** for the bovine FP receptor is very similar to each other and to the standards. Compound **28** has a lower affinity for

Table 1
FP receptor-mediated functional response and fp-receptor binding data



Compd	X	R1	R2	EC_{50} (nM)	Max. response (%)	K_i (nM)
AL-16082	CH_2	α-OH	CF_3	1.9 ± 0.3	78	72 ± 19
13	CH_2	β-OH	CF_3	5.7 ± 0.3	82	130 ± 34
28	O	α-OH	H	294 ± 69	67	120 ± 9
29	O	α-OH	Cl	52.4 ± 25.4	62	680 ± 27
30	O	β-OH	H	68.6 ± 13.1	67	87 ± 37
31	O	β-OH	Cl	62.2 ± 5.5	86	140 ± 63
$PGF_{2\alpha}$				24.5 ± 4.4	92	130 ± 6
Latanoprost acid				31.7 ± 4.1	85	98 ± 11

the bovine receptor despite its potency in the functional assay. The in vitro results with this narrow series indicate that the substitution of the 13-carbon with oxygen can result in potent prostaglandin analogs.

Compound **AL-16049**, an isopropyl ester of the most potent compound **AL-16082**, was used as a pro-drug in the in vivo evaluations for corneal penetration and delivery of the hydrolyzed carboxylic acid to the aqueous humor. Compound **AL-16049** was evaluated for side effect in the rabbit ocular irritation (ROI) model, for topical ocular potency in the cat pupil diameter (CPD) constriction model, and for IOP-lowering activity in the lasered ocular hypertensive monkey model.

A dose response (0.03–100 μg) for hyperemia was established for **AL-16049** (Table 2.). The Max_{15} (dose at which 15% incidence of hyperemia occurs) is approximately 1 μg. At all doses, **AL-16049** produced a lower incidence of hyperemia, swelling, and discharge than $PGF_{2\alpha}$ (1 μg) and produced less or at least no greater incidence of hyperemia, swelling, and discharge than latanoprost (1.8 μg).[16]

These data are qualitative, allowing comparison between compounds and between doses of the same compound. Results are reproducible. No statistical analysis was performed.

Published data suggest that FP prostaglandins produce a dose related miotic effect in the cat.[17] Topical ocular application of **AL-16049** produced a dose-dependent miotic response in the conscious cat (Table 3.). Data are Area under the Curve (AUC1-5) for group average pupil diameter change during one through 5 h after test compound instillation. **AL-16049** produced no signs of ocular irritation or discomfort in the cat at any dose tested. These data

Table 2
Dose response effect of **AL-16049** on ocular irritation in NZA rabbits*

AL-16049 (μg)	% Incidence hyperemia	% Incidence swelling	% Incidence discharge
0.03	13	23	5
0.1	13	38	3
0.3	8	25	5
1	5, 13	3, 30	3, 5
3	25	8	0
10	0, 23	8, 35	15, 8
30	73	45	0
Latanoprost, 1.8	45, 23, 20	40, 8, 30	10, 13, 20
$PGF_{2\alpha}$, 1	100, 100, 100	63, 45, 83	68, 55, 93

* More than one number indicates results from different studies.

Table 3
Effect of **AL-16049** on pupil diameter in the cat

Dose (μg)	AUC_{1-5}
0.1	3.12
0.3	6.57
1.0	10.2

are qualitative, allowing comparison between compounds and between doses of the same compound. Results are reproducible. No statistical analysis is performed.

AL-16049 produced a dose-dependent reduction in IOP (Fig. 2) in ocular hypertensive monkeys.[18] The lower dose, 1 μg, was marginally effective after the first instillation and did not show any increase in IOP reduction on b.i.d. treatment. The higher dose, 3 μg, produced a 22% reduction in IOP after the first instillation which was increased to 30% after dose 5 b.i.d. There was a sustained reduction in IOP of 21% evidenced at 16 h after dose 4 b.i.d. Contrary to literature reports with related compounds **18**, no transient ocular hypertensive effect was observed. The compound did not cause miosis in the monkeys and was well tolerated.

In conclusion, a series of 13-oxa prostaglandin analogues were synthesized using a facile synthesis we developed. This work demonstrates that these prostaglandins are potential candidates as ocular hypotensive agents for the treatment of glaucoma. Compound **AL-16082** is a very potent prostaglandin FP agonist in vitro (EC_{50} = 1.9 nM) and its ester as a pro-drug, compound **AL-16049**, significantly lowers IOP in the ocular hypertensive monkey by 30%. In the study of acute ocular irritation response in New Zealand albino rabbits, it produced less incidence of hyperemia, swelling, and discharge than $PGF_{2\alpha}$ (1 μg) and produced less or no greater incidence of hyperemia, swelling, and discharge than latanoprost (1.8 μg). AL-16049 also produced no signs of ocular irritation or discomfort in the cat at the doses evaluated. The further study is on the way.

4. Experimental

Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. ^1H NMR and ^{13}C NMR spectra were determined at 200 MHz with a Varian model VXR-200 spectrometer or at 600 MHz with a Brucker DRX-600. Low resolution mass spectra were acquired on a Finnegan TSQ 46 triple quadrupole mass spectrometer operating in the positive electrospray mode. High-resolution mass spectra were acquired in the FAB mode by Analytical Instrument Group in Raleigh, NC. Unless otherwise stated, all reactions without added water were run under a positive pressure of nitrogen. Concentration refers to removal of solvent in vacuo on a rotary evaporator. Reactions were monitored by TLC on E. Merck silica gel 60 F254 plates, with visualization by UV light, or phosphomolybdic acid. Column chromatographic purifications were performed under positive air flow using 230–400 mesh silica gel from E.M. Science. Chromatography solvents used were HPLC grade from E.M. Science.

4.1. 6-exo-Carboxylicacid-7-endo-benzoyloxy-2-oxabicyclo[3.3.0]octan-3-one (2)

Corey aldehyde benzoate (15 g, 54.7 mmol) was dissolved in 150 mL of acetone and cooled to 0 °C. Jones reagent was added dropwise with stirring until the brown color did not change to green (25 mL). The mixture was stirred at 0 °C for another 15 min. Isopropanol (30 mL) was added to the reaction mixture. The resulting mixture was filtered through a celite funnel and the filtrate was concentrated. The residue was dissolved in ethyl

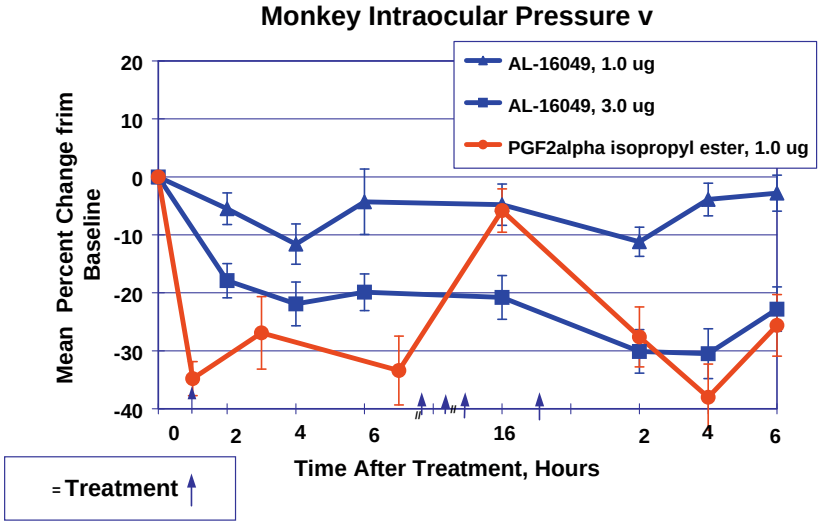


Figure 2. Effect of **AL-16049** on intraocular pressure in ocular hypertensive cynomolgus monkey eyes: (a) formulations administered 1 × 30 μL, OD topical at 0900 and 1600 h on days 1 and 2; at 0900 h on day 3. $N = 9$, drug; $N = 5$, control, (b) OD lasered (hypertensive), and (c) $p < 0.001$.

acetate and washed with water, saturated NaCl, dried over $MgSO_4$, filtered and concentrated. The residue was crystallized in ethyl acetate and hexane (5:1). White crystals of **2 (13.4 g)** were obtained. MS *m*/*z* calcd for $C_{15}H_{15}O_6$ $(M+H)^+$ 291, found 291.

4.2. 7-α-Benzoyloxy-*cis*-oxabicyclo[3.3.0]octan-3-one-6-carboxy-4-methylthiazole-2(3*H*)-thion (4)

Compound **2** (10 g, 34.47 mmol) in 50 mL of thionyl chloride was gently refluxed for 3 h and then concentrated to chloride **3**. It was dissolved in 30 mL of CH_2Cl_2 and then added to a solution of 3-hydroxy-4-methylthiazole-2(3*H*)-thione (3.56 g, 24.1 mmol) in 30 mL of CH_2Cl_2 in dry pyridine (4.2 mL, 51.7 mmol). The resulting mixture was stirred at room temperature for 1 h. The solvent was evaporated and the residue was put in a solution comprised of 300 ml of ethyl acetate and 100 mL of hexane. The white crystals formed were washed with ethyl acetate and hexane (3:1) and dried in vacuum to yield thiohydroxamate ester **4** (10.1 g). MS *m*/*z* calcd for $C_{19}H_{18}O_6NS_2$ $(M+H)^+$ 420, found 420.

4.3. 6β-Hydroxy-7α-benzoyloxy-cis-2-oxabicyclo[3.3.0]octan-3-one (5)

Antimony triphenylsulphide (2 g, 4.45 mmol) was added to a solution of **4** (0.97 g, 2.23 mmol) in 8 mL of CH_2Cl_2. The mixture was stirred in an open flask at room temperature for 16 h. The white solid was filtered and the solution was evaporated to dryness. Purification of the residue by flash chromatography using ethyl acetate and hexane (1:1) afforded the desired nor-alcohol **5** (0.4 g). ^{1}H NMR ($CDCl_3$) δ 7.99–7.95 (m, 2H), 7.62–7.54 (m, 1H), 7.48–7.27 (m, 2H), 5.32–5.20 (m, 2H), 4.25 (s, 1H), 3.06–2.36 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 176.6 (C), 166.5 (C), 113.6 (CH), 129.7 (CH), 128.7 (C), 128.6 (CH), 84.1 (CH), 81.9 (CH), 81.1 (CH), 45.7 (CH), 36.4 (CH_2), 33.5 (CH_2). MS *m*/*z* calcd for $C_{14}H_{15}O_5$ $(M+H)^+$ 263, found 263.

4.4. 6β-Allyloxy-7α-benzoyloxy-cis-2-oxabicyclo[3.3.0]octan-3-one (6)

Compound **5** (0.4 g, 1.53 mmol), allyl bromide (1 mL, 12.2 mmol) and silver(I) oxide (1.1 g, 4.6 mmol) were stirred in 8 mL of dry DMF at room temperature for 48 h. Water (2 mL) was added and the mixture was filtered through a celite coated funnel, and washed with ethyl acetate. The filtrate was extracted with ethyl acetate. The combined organic extracts were washed with a saturated NaCl solution and dried over anhydrous $MgSO_4$. Concentration of the combined extracts under reduced pressure, and chromatography of the residue on silica gel, eluting with 40% ethyl acetate in hexane, gave 0.36 g of **6** as light yellow oil. ^{1}H NMR ($CDCl_3$) δ 8.01–7.95 (m, 2H), 7.61–7.54 (m, 1H), 7.48–7.41 (m, 2H), 6.00–5.80 (m, 1H), 5.50–5.14 (m, 4H), 4.25–4.15 (m, 1H), 3.12–2.86 (m, 4H), 2.56–2.36 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 176.7 (C), 165.7 (C) 133.9 (CH), 133.5 (CH), 129.7 (CH), 129.6 (C), 128.6 (CH), 117.7 (C), 88.7 (CH), 84.8 (CH), 77.9 (CH), 70.7 (CH_2), 44.5 (CH), 36.5 (CH_2), 33.7 (CH2). MS *m*/*z* calcd for $C_{17}H_{19}O_5Na$ $(M+Na)^+$ 325, found 325.

4.5. 6β-Glycidoxy-7α-benzoyloxy-*cis*-2-oxabicyclo[3.3.0]octan-3-one (7)

The allyl ether **6** (0.36 g, 1.19 mmol) was stirred with *m*-chloroperoxybenzoic acid (0.62 g, 3.56 mmol) and sodium hydrogen carbonate (0.36 g, 4.3 mmol) in 10 mL of CH_2Cl_2 at 0 °C. The mixture was warmed slowly to room temperature and stirred for 24 h. The reaction mixture was washed with saturated aqueous sodium sulphite, saturated sodium hydrogen carbonate, water and brine, and dried over anhydrous $MgSO_4$. Evaporation of solvent afforded the crude product, which was chromatographed on a silica gel column, eluting with 60% ethyl acetate in hexanes to provide the epoxide **7** as oil 0.12 g. ^{1}H NMR ($CDCl_3$) δ 7.99–7.94 (m, 2H), 7.61–7.54 (m, 1H), 7.48–7.40 (m, 2H), 5.48 (s, 1H), 5.27–5.22 (m, 1H), 4.10–3.91 (m, 2H), 3.64–3.46 (1H), 3.17–2.80 (m, 4H), 2.68–2.42 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 176.5 (C), 165.7 (C), 133.5 (CH), 129.7 (CH), 129.6 (C), 128.5 (CH), 89.7 (CH), 84.6 (CH), 77.7 (CH), 71.0 (CH_2), 70.2 (CH_2), 50.6 (CH), 44.3 (CH), 44.1 (CH_2), 36.8(CH2), 33.6 (CH_2). MS *m*/*z* calcd for $C_{17}H_{19}O_6$ $(M+H)^+$ 340, found 340.

4.6. 6β-[2αβ-Hydroxy-4-(3-trifluoromethyl)phenyl-butoxy]-7α-hydroxy-*cis*-2-oxabicyclo[3.3.0]-octan-3-one (8)

Copper(I) iodide (8.3 g, 43.38 mmol) was stirred in dry diethyl ether (60 mL) at −30 °C under N_2. A solution of *m*-trifluoromethylbenzylmagnesium bromide (86.7 mmol) made in situ from Mg (2.1 g, 86.7 mmol) and *m*-trifluoromethylbenzyl bromide (13.3 mL, 86.7 mmol) in 20 mL of ether was added dropwise and

the mixture was stirred at −30 °C for 15 min. The mixture was cooled to −78 °C, and the epoxide **7** (2.3 g, 7.23 mmol) in ether (30 mL) was added dropwise. The resulting mixture was stirred at −78 °C for 6 h, after which saturated ammonium chloride (15 mL) was added and the mixture warmed to room temperature and stirred for 15 min. The two layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a colorless oil, which was chromatographed on a silica gel column, eluting with ethyl acetate to afford **8** (0.56 g). MS *m*/*z* calcd for $C_{18}H_{21}O_5F_3$ $(M+NH_4)^+$ 392, found 392.

4.7. 6β-[2αβ-Tetrahydro-2*H*-pyran-2-yl)oxy-4-(3-trifluoromethyl)phenyl-butoxy]-7α-hydroxy-*cis*-2- oxabicyclo-[3.3.0]octan-3-one (9)

3,4-Dihydro-2*H*-pyran (0.54 mL, 5.88 mmol) was added to a solution of 8 (0.55 g, 1.47 mmol) in CH_2Cl_2 (25 mL) and the mixture was cooled to 0 °C. *p*-Toluenesulfonic acid monohydrate (28 mg, 0.15 mmol) was added and the resulting mixture was stirred at 0 °C for 30 min and then quenched by addition of triethylamine (0.03 mL). Evaporation of solvent afforded the crude product which was chromatographed on silica gel (ethyl acetate–hexanes, 1:2) to provide the title compound **9** as oil 0.75 g. MS *m*/*z* calcd for $C_{28}H_{37}O_7F_3$ $(M+NH_4)^+$ 560, found 560.

4.8. 5*Z*-(9*S*,11*R*,15*RS*)-13-Oxa-17-(3-trifluoromethyl)phenyl]-9-hydroxy-11,15-bis(tetrahydro-2*H*-pyran-2-yl)oxy-17,18,19,20-tetranor-5-prostadienoic acid isopropyl ester (11)

To a solution of **9** (0.75 g, 1.38 mmol) in 6 mL of toluene at −78 °C, was added dropwise a 1.5 M solution of DIBAL-H in toluene (1.2 mL, 1.8 mmol). The resulting mixture was stirred at −78 °C for 1 h. Methanol (3 mL) was added to quench the reaction. Saturated ammonium chloride (10 mL) and ethyl acetate (10 mL) were added and the mixture was stirred at room temperature until two layers were separated. The aqueous layer was separated and was extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a white semi-solid **10** (0.75 g), which was used for next step without further purification.

To a suspension of (4-carboxybutyl)triphenylphosphonium bromide (1.53 g, 3.45 mmol) in THF (7 mL) at 0 °C (bath temperature) was added a 1 M solution of potassium *tert*-butoxide in THF (6.9 mL, 6.9 mmol). After stirring 30 min at 0 °C for 10 min at room temperature, a solution of the crude product **10** (0.75 g, 1.38 mmol) in THF (12 mL) was added dropwise under N_2, and the reaction was stirred at room temperature for 1 h. Saturated ammonium chloride (15 mL) and ethyl acetate (15 mL) were added. The aqueous layer was treated with 2 N HCl to adjust the pH to 5 and was then extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a white semi-solid. This was dissolved in acetone (15 mL), the solution was cooled to 0 °C (bath temperature), and DBU (1.3 mL, 8.28 mmol) was added. After stirring 20 min at room temperature, isopropyl iodide (0.7 ml, 6.9 mmol) was added and the reaction was warmed to room temperature and stirred overnight. Saturated ammonium chloride (15 mL) and ethyl acetate (15 mL) were added. The aqueous layer was separated and was extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a colorless oil, which was chromatographed on a silica gel column, eluting with 30% ethyl acetate in hexane, to afford **11** (0.6 g). MS *m*/*z* calcd for $C_{36}H_{53}O_8F_3$ $(M+NH_4)^+$ 688, found 688.

4.9. 5*Z*-(9*S*,11*R*,15*S*)-13-Oxa-17-(3-trifluoromethyl)phenyl-9,11,15-trihydroxy-18,19,20-trinor-5-prostadienoic acid isopropyl ester (AL-16049)

Compound **11** (0.6 g, 0.89 mmol) was stirred in 5 mL of MeOH, 0.5 mL of water and 5 drop of 12 N HCl at 0 °C for 15 min at room temperature for 2 h. Ethyl acetate (10 mL) and saturated $NaHCO_3$ (10 mL) were added. The two layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to an oil residue. The crude product was chromatographed on a silica gel column eluting with ethyl acetate followed by HPLC chiral separation to afford **AL-16049** (0.11 g) and **12** (0.095 g).

^1H NMR ($CDCl_3$) δ 7.45–7.27 (m, 4H), 5.48–5.40 (m, 2H), 5.06–4.93 (m, 1H), 4.21–411 (m, 2H), 3.65–3.62 (m, 1H), 3.57–3.50 (m, 3H), 2.37–1.64 (m, 15H), 1.24–1.21 (d, J = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 173.59 (C), 142.84 (C), 131.89 (CH), 130.33 (C), 129.94 (CH), 128.87 (CH), 125.14 (CH), 125.07 (CH), 122.77 (CH), 122.70 (CH), 93.04 (CH), 77.53 (CH), 75.03 (CH_2), 73.82 (CH), 69.96 (CH), 68.11 (CH), 51.77 (CH), 41.62 (CH_2), 34.93 (CH_2), 34.33 (CH_2), 31.91 (CH_2), 26.95 (CH_2), 26.16 (CH_2), 25.22 (CH_2), 22.14 (CH_3). MS (ESI) *m*/*z* calcd for $C_{26}H_{37}O_6F_3$ $(M+H)^+$ 503, found 503, HRMS calcd for $C_{26}H_{37}O_6F_3$ $(M+H)^+$ 503.5778, found 503.2625.

4.10. 5*Z*-(9*S*,11*R*,15*R*)-13-Oxa-17-(3-trifluoromethyl)phenyl-9,11,15-trihydroxy-18,19,20-trinor-5-prostadienoic acid isopropyl ester (12)

Colorless oil. ^1H NMR ($CDCl_3$) δ 7.45–7.27 (m, 4H), 5.49–5.41 (m, 2H), 5.06–4.94 (m, 1H), 4.23–410 (m, 2H), 3.71–3.56 (m, 3H), 3.42–4.37 (m, 1H), 2.86–1.65 (m, 15H), 1.24–1.21 (d, J = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 173.59 (C), 142.84 (C), 131.90 (CH), 129.99 (C), 128.91 (CH), 128.76 (CH), 125.18 (CH), 125.10 (CH), 122.80 (CH), 122.72 (CH), 93.15 (CH), 77.06 (CH), 74.76 (CH_2), 73.70 (CH), 69.66 (CH), 67.78 (CH), 51.63 (CH), 41.36 (CH_2), 34.47 (CH_2), 33.98 (CH_2), 31.56 (CH_2), 26.61 (CH_2), 25.94 (CH_2), 24.87 (CH_2), 21.82 (CH_3). MS (ESI) *m*/*z* calcd for $C_{26}H_{37}O_6F_3$ $(M+H)^+$ 503, found 503, HRMS calcd for $C_{26}H_{37}O_6F_3$ $(M+H)^+$ 503.5778, found 503.2621.

4.11. 5*Z*-(9*S*,11*R*,15*S*)-13-Oxa-17-(3-trifluoromethyl)phenyl-9,11,15-trihydroxy-18,19,20-trinor-5-prostadienoic acid (AL-16082)

A mixture of **AL-16049** (25 mg, 0.05 mmol), lithium hydroxide monohydrate (21 mg, 0.5 mmol), methanol (1 ml) and water (0.1 mL) was stirred at room temperature overnight. Saturated KH_2PO_4 solution was added to adjust the pH to 6 and the mixture was extracted with ethyl acetate (3 × 3 mL). The combined organic layer was washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a colorless oil, which was chromatographed on a short silica gel column, eluting with 5% methanol in ethyl acetate, to afford **AL-16082** (12.5 mg) as a colorless oil. ^1H NMR (CD_3OD) δ 7.51–7.45 (m, 4H), 5.51–5.49 (m, 1H), 5.37–5.35 (m, 1H), 4.09 (s, 1H), 4.0 (s, 1H), 3.66–3.64 (m, 2H), 3.53–3.49 (m, 2H), 2.90–2.87 (m, 1H), 2.73–2.70 (m, 1H), 2.29–2.25 (m, 5H), 2.22–2.11 (m, 2H), 1.80–1.74 (m, 3H), 1.65–1,62 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 177.79 (C), 145.01 (C), 133.36 (CH), 131.76 (C), 130.45 (CH), 130.39 (CH), 130.14 (CH), 126.10 (CH), 124.92.1 (CH), 123.64 (CH), 92.72 (CH), 77.40 (CH), 75.86 (CH_2), 71.89 (CH), 70.88 (CH), 51.06 (CH), 47.90 (CH), 42.23 (CH_2), 36.58 (CH_2), 34.57 (CH_2), 32.50

(CH_2), 27.65 (CH_2), 26.14 (CH_2), 26.07 (CH_2). MS (ESI) *m*/*z* calcd for $C_{23}H_{31}O_6F_3$ $(M+H)^+$ 461, found 461, HRMS calcd for $C_{23}H_{31}O_6F_3$ $(M+H)^+$ 461.4974, found 461.2150.

4.12. 5*Z*-(9*S*,11*R*,15*R*)-13-Oxa-17-(3-trifluoromethyl)phenyl-9,11,15-trihydroxy-18,19,20-trinor-5-prostadienoic acid (13)

Colorless oil. ^1H NMR (CD_3OD) δ 7.51–7.45 (m, 4H), 5.51–5.49 (m, 1H), 5.37–5.35 (m, 1H), 4.09 (s, 1H), 4.0 (s, 1H), 3.69–3.65 (m, 1H), 3.63–3.61 (m, 1H), 3.55–3.53 (m, 1H), 3.49–3.47 (m, 1H), 2.90–2.87 (m, 1H), 2.73–2.70 (m, 1H), 2.31–2.25 (m, 5H), 2.12–2.09 (m, 2H), 1.85–1.83 (m, 1H), 1.73–1.71 (m, 2H), 1.64–1.60 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 177.80 (C), 145.01 (C), 133.36 (CH), 131.75 (C), 130.50 (CH), 130.36 (CH), 130.15(CH), 126.72 (C), 126.09, 123.64 (CH), 91.21 (CH), 76.00 (CH), 74.15 (CH_2), 70.43 (CH), 69.33 (CH), 49.63 (CH), 47.87 (CH), 40.81 (CH_2), 35.05 (CH_2), 31.21 (CH_2), 26.62 (CH_2), 24.82 (CH_2), 24.63 (CH_2). MS (ESI) *m*/*z* calcd for $C_{23}H_{31}O_6F_3$ $(M+H)^+$ 461, found 461, HRMS calcd for $C_{23}H_{31}O_6F_3$ $(M+H)^+$ 461.4974, found 461.21457.

4.13. 6β-[2αβ-Hydroxy-3-(3-chloro)phenoxy-propoxy]-7-α-benzoyloxy-*cis*-2-oxabicyclo[3.3.0]octan-3-one (15)

To a solution of **7** (0.1 g, 0.314 mmol) in 2 mL of acetonitrile, 3-chlorophenol (0.07 ml, 0.63 mmol) and potassium carbonate (0.13 g, 0.95 mmol) were added and the mixture was refluxed for 4 h. After cooling, the mixture was filtered and the filtrate was concentrated. The residue was chromatographed on a silica gel column eluting with ethyl acetate–hexanes (1:1) to provide the title compound **7** as yellowish oil 0.09 g. MS *m*/*z* calcd for $C_{23}H_{23}O_7ClNa$ $(M+Na)^+$ 469, found 469.

4.14. 6β-[2αβ-Hydroxy-3-(3-chloro)phenoxy-propoxy]-7α-hydroxy-*cis*-2-oxabicyclo[3.3.0]octan-3-one (17)

Potassium carbonate (0.03 g, 0.2 mmol) was added to a solution of **15** (0.09 g, 0.2 mmol) in 2 mL of methanol. The resulting mixture was stirred at room temperature for 1.5 h. Ethyl acetate (2 mL) and 1 N HCl (1 mL) were added. The aqueous layer was separated and extracted with ethyl acetate (3 × 3 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and saturated NaCl solutions and dried over anhydrous $MgSO_4$. Evaporation of solvent afforded crude product which was chromatographed on silica gel (ethyl acetate–hexanes, 5:1) to provide the title compound **17** as yellowish oil 0.075 g. MS *m*/*z* calcd for $C_{16}H_{19}O_6ClNa$ $(M+Na)^+$ 365, found 365.

4.15. 6-[2-Tetrahydro-2*H*-pyran-2-yl)oxy-3-(3-chloro)phenoxy-propoxy]-7-tetrahydro-2*H*-pyran-2-yl)oxy-cis-2-oxabicyclo[3.3.0]octan-3-one (19)

3,4-Dihydro-2*H*-pyran (0.08 ml, 0.85 mmol) was added to a solution of **17** (0.075 g, 0.211 mmol) in CH_2Cl_2 (1 mL) and the mixture was cooled to 0 °C. *p*-Toluenesulfonic acid monohydrate (2 mg, 0.01 mmol) was added and the resulting mixture was stirred at 0 °C for 30 min and then quenched by addition of saturated $NaHCO_3$ (1 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 3 mL). The combined organic extracts were washed with a saturated NaCl solution and dried over anhydrous $MgSO_4$. Evaporation of solvent afforded the crude product which was chromatographed on silica gel (ethyl acetate–hexanes, 1:2) to provide the title compound **19** as oil 0.1 g. MS *m*/*z* calcd for $C_{26}H_{35}O_8ClNa$ $(M+Na)^+$ 533, found 533.

4.16. 5*Z*-(9*S*,11*R*,15*RS*)-13-Oxa-16-(3-chloro)phenoxy-propoxy]-9-hydroxy-11,15-bis(tetrahydro-2*H*-pyran-2-yl)oxy-17,18,19,20-tetranor-5-prostadienoic acid isopropyl ester (23)

To a solution of **19** (0.1 g, 0.2 mmol) in 1 mL of toluene at −78 °C, was added dropwise a 1.5 M solution of DIBAL-H in toluene (0.18 mL, 0.26 mmol). The resulting mixture was stirred at −78 °C for 1 h. Methanol (0.5 mL) was added to quench the reaction. Saturated ammonium chloride (2 mL) and ethyl acetate (3 mL) were added, the mixture was extracted with ethyl acetate (3 × 3 mL). The combined organic extracts were washed with a saturated NaCl solution and dried over anhydrous $MgSO_4$, filtered and concentrated to a white semi-solid (0.1 g), which was used for next step without further purification.

To a suspension of (4-carboxybutyl)triphenylphosphonium bromide (0.22 g, 0.5 mmol) in THF (1 mL) at 0 °C (bath temperature) was added a 1 M solution of potassium *tert*-butoxide in THF (1 mL, 1 mmol). After 40 min, a solution of the crude product above (0.1 g, 0.2 mmol) in THF (2 mL) was added dropwise under N_2, and the reaction was stirred at room temperature for 1 h. Saturated ammonium chloride (2 mL) and ethyl acetate (3 mL) were added. The aqueous layer was treated with 2 N HCl to adjust the pH to 5 and was then extracted with ethyl acetate (3 × 3 mL). The combined organic layer was washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a white semi-solid. This was dissolved in acetone (2 ml), the solution was cooled to 0 °C (bath temperature), and DBU (0.2 mL, 1.2 mmol) was added. After 20 min isopropyl iodide was added (0.1 mL, 1.0 mmol) and the reaction was warmed to room temperature and stirred overnight. Saturated ammonium chloride (2 mL) and ethyl acetate (3 mL) were added, the mixture was extracted with ethyl acetate (3 × 3 mL). The combined organic extracts were washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a colorless oil, which was chromatographed on a silica gel column, eluting with 30% ethyl acetate in hexane to afford 23 (0.065 g). MS *m*/*z* calcd for $C_{34}H_{51}O_9ClNa$ $(M+Na)^+$, 661, found 661.

4.17. 5*Z*-(9*S*,11*R*,15*R*)-13-Oxa-16-phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid isopropyl ester (24)

Colorless oil. ^1H NMR of 24 ($CDCl_3$) δ 7.32–7.24 (m, 2H), 6.99–6.88 (m, 3H), 5.44–5.41 (m, 2H), 5.02–4.96 (m, 1H), 4.16–3.64 (m, 8H), 2.40–1.64 (m, 11H), 1.24–1.21 (d, J = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 173.55 (C), 158.51 (C), 130.22 (CH), 129.84 (CH), 129.29 (CH), 121.45 (CH), 114.90 (CH), 93.39 (CH), 77.41 (CH), 73.70 (CH), 71.83 (CH_2), 69.84 (CH), 69.14 (CH_2), 68.09 (CH), 51.74 (CH), 41.54 (CH_2), 34.36 (CH_2), 26.96 (CH_2), 26.16 (CH_2), 25.22 (CH_2), 22.17 (CH_3). MS (ESI) *m*/*z* calcd for $C_{24}H_{36}O_7$ $(M+1)^+$ 437, found 437.

4.18. 5*Z*-(9*S*,11*R*,15*R*)-13-Oxa-16-(3-chloro)phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid isopropyl ester (25)

Compound **23** (65 mg, 0.1 mmol) was stirred in 1 ml of 65% acetic acid and 1 mL of THF at 55 °C for 1.5 h. The solvent was evaporated and the residue was dried under vacuum. The crude product was chromatographed on a silica gel column eluting with 80% ethyl acetate in hexane and followed by HPLC chiral separation to afford **25** (20 mg). ^1H NMR of 25 ($CDCl_3$) δ 7.20–7.12 (m, 1H), 7.08–6.85 (m, 2H), 6.75–6.70 (m, 1H), 5.40–5.31 (m, 2H), 4.95–4.89 (m, 1H), 4.13–3.57 (m, 8H), 2.33–1.57 (m, 11H), 1.17–1.14 (d, J = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 173.57 (C), 159.27 (C), 134.84 (C), 130.22 (CH), 129.90 (CH), 128.85 (CH), 121.24 (CH), 114.96

(CH), 113.03 (CH), 93.04 (CH), 77.08 (CH), 73.42 (CH), 71.28 (CH_2), 69.29 (CH), 69.12 (CH_2), 67.88 (CH), 51.41 (CH), 41.23 (CH_2), 34.0 (CH_2), 26.61 (CH_2), 25.82 (CH_2), 24.86 (CH_2), 21.82 (CH_3). MS (ESI) *m*/*z* calcd for $C_{24}H_{35}O_7Cl$ $(M+1)^+$ 471, found 471.

4.19. 5*Z*-(9*S*,11*R*,15*S*)-13-Oxa-16-phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid isopropyl ester (26)

Colorless oil. ^{1}H NMR of 26 ($CDCl_3$) δ 7.32–7.24 (m, 2H), 6.99–6.89 (m, 3H), 5.47–5.30 (m, 2H), 5.06–4.93 (m, 1H), 4.16–3.63 (m, 8H), 2.40–1.64 (m, 11H), 1.24–1.21 (d, *J* = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 173.59 (C), 158.56 (C), 130.22 (CH), 129.83 (CH), 129.28 (CH), 121.43 (CH), 114.90 (CH), 93.49 (CH), 77.37 (CH), 73.64 (CH), 71.78 (CH2), 69.77 (CH), 69.18 (CH2), 68.10 (CH), 51.69 (CH), 41.50 (CH_2), 34.36 (CH_2), 26.96 (CH_2), 26.15 (CH_2), 25.22 (CH_2), 22.17 (CH_3). MS (ESI) *m*/*z* calcd for $C_{24}H_{36}O_7$ $(M+1)^+$ 437, found 437.

4.20. 5*Z*-(9*S*,11*R*,15*S*)-13-Oxa-16-(3-chloro)phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid isopropyl ester (27)

Colorless oil. ^{1}H NMR of 27 ($CDCl_3$) δ 7.27–7.24 (m, 1H), 7.19–6.92 (m, 2H), 6.83–6.78 (m, 1H), 5.44–5.30 (m, 2H), 5.03–4.96 (m, 1H), 4.13–3.63 (m, 8H), 2.40–1.64 (m, 11H), 1.24–1.21 (d, *J* = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 173.60 (C), 159.30 (C), 134.85 (C), 130.22 (CH), 129.89 (CH), 128.86 (CH), 121.24 (CH), 114.98 (CH), 113.04 (CH), 93.15 (CH), 76.97 (CH), 73.92 (CH), 71.27 (CH_2), 69.20 (CH), 69.09 (CH_2), 67.73 (CH), 51.27 (CH), 41.11 (CH_2), 33.95 (CH_2), 26.55 (CH_2), 25.74 (CH_2), 24.81 (CH_2), 21.76 (CH_3). MS (ESI), *m*/*z* calcd for $C_{24}H_{35}O_7Cl$ $(M+1)^+$ 471, found 471.

4.21. 5*Z*-(9*S*,11*R*,15*R*)-13-Oxa-16-phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid (28)

Colorless oil. ^{1}NMR of 28 ($CDCl_3$) δ 7.47–7.39 (m, 2H), 7.13–7.05 (m, 3H), 5.70–5.48 (m, 2H), 4.29–3.71 (m, 8H), 2.50–1.76 (m, 11H). ^{13}C NMR ($CDCl_3$) δ 173.50 (C), 160.42 (C), 130.87 (CH), 130.74 (CH), 122.27 (CH), 116.02 (CH), 93.31 (CH), 77.79 (CH), 72.88 (CH_2), 72.38 (CH), 70.93 (CH_2), 70.79 (CH), 51.52 (CH), 42.65 (CH_2), 35.00 (CH_2), 28.05 (CH_2), 26.50 (CH_2). MS (APCI) *m*/*z* calcd for $C_{21}H_{30}O_7$ $(M+H)^+$ 395, found 395.

4.22. 5*Z*-(9*S*,11*R*,15*R*)-13-Oxa-16-(3-chloro)phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid (29)

A mixture of **25** (18 mg, 0.04 mmol), lithium hydroxide monohydrate (17 mg, 0.4 mmol), methanol (1 mL) and water (0.1 mL) was stirred at room temperature overnight. Saturated KH_2PO_4 solution was added to adjust the pH to 6 and the mixture was extracted with ethyl acetate (3 × 3 mL). The combined organic layer was washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated to a colorless oil, which was chromatographed on a short silica gel column, eluting with 5% methanol in ethyl acetate, to afford **29** (8 mg). 600 mz ^{1}H NMR ($CDCl_3$)) δ 7.25–7.22 (m, 1H), 6.97 (s, 1H), 6.93–6.92 (d, 1H, *J* = 6Hz), 6.89–6.88 (d, 1H, *J* = 6 Hz), 5.49–5.47 (m, 1H), 5.34–5.33 (m, 1H), 4.09–3.98 (m, 5H), 3.78–3.77 (m, 1H), 3.67–3.65 (m, 1H), 3.57–3.55 (m, 1H), 2.28–2.23 (m, 5H), 2.10–2.09 (m, 2H), 1.63–1.61 (m, 1H), 1.62–1.61 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 178.10(C), 161.33 (C), 135.88 (C), 131.57 (CH), 130.59 (CH), 130.24 (CH), 121.91 (CH), 116.01 (CH), 114.22 (CH), 92.89 (CH), 77.38 (CH), 72.26 (CH_2), 71.94 (CH), 70.80 (CH_2), 70.31 (CH), 51.09 (CH), 42.25 (CH_2), 35.14 (CH_2), 27.74 (CH_2), 26.536 (CH_2), 26.10 (CH_2). MS (ESI) *m*/*z* calcd for $C_{21}H_{29}O_7Cl$ $(M+1)^+$ 429, found 429.

4.23. 5*Z*-(9*S*,11*R*,15*S*)-13-Oxa-16-phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid (30)

Colorless oil. ^{1}NMR of 28 ($CDCl_3$) δ 7.52–7.44 (m, 2H), 7.17–7.09 (m, 3H), 5.75–5.53 (m, 2H), 4.33–3.76 (m, 8H), 2.55–1.85 (m, 11H). ^{13}C NMR ($CDCl_3$) δ 173.50 (C), 160.42 (C), 130.39 (CH), 130.26 (CH), 121.78 (CH), 115.53 (CH), 92.78 (CH), 77.32 (CH), 72.29 (CH_2), 71.90 (CH), 70.31 (CH), 70.14 (CH_2), 51.04 (CH), 42.14 (CH_2), 34.47 (CH_2), 27.56 (CH_2), 26.05 (CH_2). MS (APCI) *m*/*z* calcd for $C_{21}H_{30}O_7$ $(M+H)^+$ 395, found 395.

4.24. 5*Z*-(9*S*,11*R*,15*S*)-13-Oxa-16-(3-chloro)phenoxy-propoxy]-9,11,15-trihydroxy-17,18,19,20-tetranor-5-prostadienoic acid (31)

Colorless oil. 600 mz ^{1}H NMR ($CDCl_3$) δ 7.25–7.22 (m, 1H), 6.97 (s, 1H), 6.94–6.92 (d, 1H, *J* = 12 Hz), 6.89–6.87 (d, 1H, *J* = 12 Hz), 5.53–5.46 (m, 1H), 5.37–5.30 (m, 1H), 4.09–3.96 (m, 5H), 3.83–3.80 (m, 1H), 3.69–3.66 (m, 1H), 3.56–3.54 (m, 1H), 2.29–2.24 (m, 5H), 2.11–2.09 (m, 2H), 1.64–1.63 (m, 1H), 1.62–1.61 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 178.16(C), 161.30 (C), 135.88 (C), 131.55 (CH), 130.52 (CH), 130.27 (CH), 121.90 (CH), 116.0 (CH), 114.20 (CH), 92.85 (CH), 77.38 (CH), 72.22 (CH_2), 71.94 (CH), 70.62 (CH_2), 70.19 (CH), 51.09 (CH), 42.22 (CH_2), 34.87 (CH_2), 27.69 (CH_2), 26.52 (CH_2), 26.10 (CH_2). MS (ESI) *m*/*z* calcd for $C_{21}H_{29}O_7Cl$ $(M+1)^+$ 429, found 429.

4.25. FP Receptor binding assay

Washed total particulate bovine corpus luteum membranes (20 mg wet weight/mL final concentration) were incubated with [^{3}H]$PGF_{2\alpha}$ (1–2 nM final) in Krebs buffer (pH 7.4) for 2 h at 23 °C in a total volume of 500 μL as previously described.[15] Non-specific binding was defined with 10 μM unlabeled $PGF_{2\alpha}$ or cloprostenol. The assays were terminated by rapid vacuum filtration (using Whatman GF/B glass fiber filter previously soaked in 0.3% polyethyleneimine) and the filter-bound radioactivity determined by liquid scintillation spectrometry on a beta-counter. The equilibrium concentration of the test prostaglandin required to displace [^{3}H]$PGF_{2\alpha}$ from the FP-receptor by 50% (K_i) was determined from several experiments. The K_i value is inversely related to the affinity of the compound for the FP-receptor.

4.26. FP Receptor mediated phosphoinositide (PI) turnover assays

Total [^{3}H]Inositol phosphates ([^{3}H]-IPs) produced by agonist-mediated activation of phospholipase C were quantified by previously published procedures using mouse Swiss 3T3 fibroblasts.[15] In brief, confluent Swiss 3T3 cells were exposed to 1.0-1.5 μCi of [^{3}H]-myo-inositol in 0.5 mL DMEM culture medium for 24-30 h at 37 °C in order to label the cell membranes. Then the cells were rinsed once with DMEM/F-12 containing 10 mM LiCl, and the agonist stimulation experiment was performed in 0.5 mL of the same medium to facilitate the accumulation of [^{3}H]-IPs following PI hydrolysis. Cells were exposed to the agonist or vehicle solvent for 60 min at 37 °C, followed by aspiration of the medium and immediate addition of 1 mL of ice-cold 0.1 M formic acid. The cell lysates (0.9 mL) were loaded on columns containing 1 mL AG 1-X8 anion exchange resin in the formate form. Unincorporated [^{3}H]-myo-inositol was removed by washing with 10 mL of distilled water and discarded. The total [^{3}H]-IPs were collected into scintillation vials by washing the columns with 8 mL of 50 mM ammo-

nium formate and 4 mL of 1.2 M ammonium formate made in 0.1 M formic acid. The radioactivity associated with the total [^{3}H]-IPs was determined by scintillation counting on a beta-counter. Concentration-response curves from several experiments were used to determine the potency (EC_{50}) and relative intrinsic activity (efficacy, E_{max}) for each compound.

4.27. Data analyses

The original data (dpm) from the different ligand binding and second messenger assay experiments were analyzed using a non-linear, iterative curve-fitting computer program.[14,15]

4.28. Formulation for in vivo studies

Prostaglandins were formulated in a trimethamine/boric acid buffered vehicle containing 4.6% mannitol, HCO-40 (at a ratio of 10–1 with the active ingredient, but not less than 0.1%), 0.01% EDTA, and 0.01% benzalkonium chloride, pH 7.4.

4.29. Acute ocular irritation response in New Zealand albino rabbits

A method was devised to grossly compare ocular irritation potential of prostaglandin FP agonists using the New Zealand albino (NZA) rabbit.[16,17] The eyes were examined microscopically using a Topcon SL-6E or SL-7E slit-lamp The eyes scored for conjunctival hyperemia, conjunctival swelling, and ocular discharge according to the Hackett and McDonald Scoring System.[16] Test article was instilled in one 30 μL aliquot to both eyes of each of five rabbits. Post-dose evaluations were made at 1, 2, 3, and 5 h. The percent of eyes with score +2 or greater was calculated for each parameter at each time point. The percent of eyes with score +2 or greater for all post-dose time points combined and reported as study percent (%) incidence. This method is used to qualitatively rank the irritation potential of FP prostaglandins and data are not analyzed statistically.

4.30. Acute miotic response in normotensive cats

Animals were restrained in cat bags in rooms with uniform lighting. Pupil diameter (PD) was measured horizontally across the width of the pupil using a hand-held micrometer. After baseline PD measurements, test item was instilled in one 30 μL aliquot to one eye of each animal ($N = 6$). Vehicle was instilled in all contralateral eyes. Subsequent PD measurements were made at 0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 h post-dose. The area under the curve (AUC) was calculated for time (1 through 5 h) after dosing vs. PD. This method is used to qualitatively rank the potency of FP prostaglandins and data are not analyzed statistically.

4.31. Acute intraocular pressure (IOP) response in ocular hypertensive monkeys

Animals were minimally restrained in specially designed monkey chairs and had been trained to accept eyedrops and applanation tonometry without chemical restraint.[18] All right eyes were hypertensive as a result of argon laser trabeculoplasty. Left eyes were normal and untreated. Intraocular pressure (IOP) was measured with an Alcon Applanation Pneumatonometer after light corneal anesthesia with 0.1% proparacaine. Following each measurement, residual anesthetic was washed out with saline. Test item was administered in one 30 μL aliquot topically to the lasered (ocular hypertensive) eyes of eight or nine monkeys. Doses 1, 3, and 5 were instilled at approximately 0900 h on three consecutive days; doses 2 and 4 at 1630 h on days 1 and 2. Vehicle was administered on the same schedule to the lasered eyes of five or six other animals. IOP measurements were taken at baseline, at selected intervals (up to 7 h) after doses 1 and 5 and 16 h after dose 4. The percent change of IOP from baseline was determined for each animal for every IOP measurement. Group means and standard error of the mean (SEM) were calculated for each time point. Student's t-test and ANOVA were used to analyze data. Significance levels: $p < 0.05$; $\alpha = 0.05$. All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

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