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Pharmacological characteristics of AFP-168 (tafluprost), a new prostanoid FP receptor agonist, as an ocular hypotensive drug

Yasutaka Takagi[a], Tadashi Nakajima[a], Atsushi Shimazaki[a], Masaaki Kageyama[a], Takeshi Matsugi[a], Yasushi Matsumura[b], B'Ann T. Gabelt[c], Paul L. Kaufman[c], Hideaki Hara[a,*]

[a]*Glaucoma Group, Research and Development Center, Santen Pharmaceutical Co., Ltd, 8916-16, Takayama-cho, Ikoma 630-0101, Nara, Japan*
[b]*Fine Chemicals Group, Chemicals Company, Asahi Glass Co., Ltd, Tokyo, Japan*
[c]*Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, Madison, WI, USA*

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Abstract

To evaluate the pharmacological characteristics of AFP-168 (tafluprost), a new prostaglandin (PG) $F_{2\alpha}$ derivative, we examined its receptor-binding affinities, intraocular pressure (IOP)-lowering effect, effects on aqueous humor dynamics, and stimulating effect on melanogenesis. The receptor-binding profile for AFP-172, a carboxylic acid of AFP-168, was determined by measuring muscle contractions in an organ bath, inhibition of platelet aggregation, and competitive binding of a radio-labelled ligand. For the IOP-measurement study, ocular normotensive and laser-induced ocular hypertensive cynomolgus monkeys were used, and IOP was measured using a pneumatonograph. For the studies of aqueous humor dynamics, IOP (Goldmann applanation tonometry), fluorophotometry, two-level constant pressure perfusion, and isotope dilution and accumulation techniques were used in ocular normotensive monkeys. The melanin contents in the medium and in the cell bodies of cultured B16-F0 melanoma cells were measured. The affinity for the FP receptor shown by AFP-172 (K_i: 0·4 nM) was 12 times that of PhXA85 (K_i: 4·7 nM), a carboxylic acid of latanoprost. A single application of AFP-168 at 0·0025% significantly lowered IOP in both ocular normotensive and hypertensive monkeys (3·1 and 11·8 mmHg, respectively, $p < 0·01$) and latanoprost at 0·005% significantly lowered IOP (2·1 mmHg, $p < 0·01$ and 9·5 mmHg, $p = 0·059$, respectively). Once daily instillation of AFP-168 at 0·001, 0·0025, or 0·005% for 5 days in normotensive monkeys significantly reduced IOP not only for a few hours, but also at the drug-trough time 24 hr after application. Latanoprost at 0·005% also reduced IOP, but not at the drug-trough time. AFP-168 decreased IOP mainly by increasing uveoscleral outflow by 65% ($p < 0·05$) and, as sometimes seen with other prostanoids, also increased total outflow facility (33% increase, $p < 0·05$). In cultured B16-F0 melanoma cells, AFP-172 (100 μM) did not stimulate melanogenesis, but PhXA85 (100 μM) did. These findings indicate that AFP-168 has a high affinity for the prostanoid FP receptor, has potent IOP-lowering effects in both ocular normotensive and hypertensive monkeys that exceed those of latanoprost, and has less stimulating effect on melanogenesis in melanoma cells.

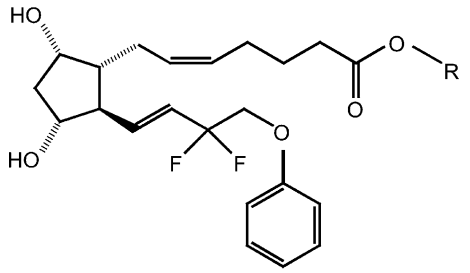
Keywords: AFP-168; tafluprost; prostaglandin derivatives; FP agonist; receptor binding; intraocular pressure; aqueous humor dynamics; melanogenesis; cynomolgus monkey

1. Introduction

AFP-168 (tafluprost), 1-methylethyl (5Z)-7-[(1R,2R, 3R,5S)-2-[(1E)-3,3-difluoro-4-phenoxy -1-butenyl]-3,5-dihydroxycyclopentyl]-5-heptenoate and AFP-172, a carboxylic acid of AFP-168, are newly synthesized prostaglandin (PG) $F_{2\alpha}$ analogues (Fig. 1). AFP-168 is under development as an ocular hypotensive drug in the USA, Europe, and Japan. It is a pro-drug ester that facilitates corneal penetration and allows delivery of the active carboxylic acid form (AFP-172) to the aqueous humor, a similar situation to that seen with $PGF_{2\alpha}$-isopropyl ester and other ocular hypotensive derivatives of $PGF_{2\alpha}$, such as latanoprost.

Glaucoma is a major cause of blindness worldwide, and many ocular hypotensive drugs are in existence. For over 20 years, β-blockers such as timolol were the first-line therapy.

* Corresponding author. Dr Hideaki Hara, Glaucoma Group, Research and Development Center, Santen Pharmaceutical Co., Ltd, 8916-16, Takayama-cho, Ikoma 630-0101, Nara, Japan.
E-mail address: hideaki.hara@santen.co.jp (H. Hara).



R = isopropyl : AFP-168
R = H : AFP-172

Fig. 1. Chemical structures of AFP-168 and AFP-172.

Then, after the discovery of the intraocular pressure (IOP)-lowering effects of PGs (Camras and Bito, 1981), several PG-derivatives such as isopropyl unoprostone (Taniguchi et al., 1996), latanoprost (Stjernschantz et al., 1995; Camras et al., 1996), bimatoprost (Woodward et al., 2001), and travoprost (Sharif et al., 1999; Hellberg et al., 2001) were launched into the market as anti-glaucoma drugs. The IOP-lowering effect of latanoprost has been found to be greater than that of timolol in a number of clinical comparative studies (Alm, 1995; Mishima et al., 1996; Watson et al., 1996; Alm et al., 2000). Recently, because of its potent IOP-lowering effects and fewer general side effects, latanoprost has been used as first-line therapy in many countries, including the USA and Japan.

Prostanoid FP-receptor agonists have potent IOP-lowering efficacy, and effectively no general side effects. However, PG-related ocular hypotensive drugs do have local side effects, such as pigmentation of the iris (Wistrand et al., 1997; Yamamoto and Kitazawa, 1997; Sherwood and Brandt, 2001; Netland et al., 2001), palpebra or/and periocular skin (Wand et al., 2001), and abnormal eyelash growth (trichiasis) (Johnstone, 1997). Furthermore, the existing PG-related ocular hypotensive drugs, including latanoprost, do not produce satisfactory IOP control in all patients. We therefore tried to find a new candidate as an ocular hypotensive drug that exceeds latanoprost in terms of IOP-lowering efficacy, and has weaker local side effects.

In this study, in a comparison with latanoprost, we examined the binding affinity of AFP-172 for recombinant human prostanoid FP receptors, the binding selectivity of AFP-172, the IOP-lowering effects of AFP-168 in ocular normotensive and laser-induced ocular hypertensive monkeys, the effects of AFP-168 on aqueous humor dynamics in ocular normotensive monkeys, and the effects of AFP-172 on melanogenesis in cultured B16-F0 melanoma cells.

2. Materials and methods

2.1. Materials

AFP-168 and AFP-172 were synthesized at the laboratories of Asahi Glass Co. Ltd (Tokyo, Japan). Latanoprost, unoprostone isopropyl, and $PGF_{2\alpha}$-isopropyl ester were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Latanoprost ophthalmic solution (Xalatan®) was purchased from Pharmacia K. K. (Tokyo, Japan), pilocarpine hydrochloride from Sigma-Aldrich Corp. (St Louis, MO, USA), and [^{3}H] $PGF_{2\alpha}$ (200 μCi/μl) from Amersham Biosciences Corp. (Piscataway, NJ, USA). [^{125}I] and [^{131}I] were obtained from PerkinElmer Life Sciences, Inc. (Boston, MA, USA). Timolol-gel forming ophthalmic solution (Timoptol-XE®) was a product of Santen Pharmaceutical Co. Ltd (Osaka, Japan). PhXA85 and unoprostone (carboxylic acids of latanoprost and unoprostone isopropyl, respectively) were prepared at the laboratories of Santen Pharmaceutical Co. Ltd. Because latanoprost, unoprostone isopropyl, and AFP-168 are pro-drug esters, and each is converted to its respective acid by the esterase in the cornea, the carboxylic acid forms were used in vitro, while the ester forms were used in vivo.

2.2. Receptor-binding assays

2.2.1. Prostanoid FP receptor

For the prostanoid FP receptor-binding study, we used a clonal cell line that stably expresses the recombinant human prostanoid FP receptor (Abramovitz et al., 1994). The radioligand-binding assay was performed according to the methods of Abramovitz et al. (1994) and Graves et al. (1995). In this conventional filtration assay, membranes expressing the receptor are incubated with a fixed concentration of the radioligand and increasing concentrations of the test compounds. After reaching equilibrium, the radioligand bound to the membranes (bound) is separated from the unbound (free) radioligand by filtration through glass-fiber filters. The amount of bound radioactivity is determined by liquid scintillation spectrometry (LS5000TD; Beckman Coulter Inc., Fullerton, CA, USA), and the amount of bound radioactivity is plotted as a function of the logarithm of the concentration of the competing test compound. Increasing concentrations of the competing test compound decrease the binding of the radioligand according to the laws of mass action, and the affinity (IC_{50}) of the test compound for the receptor can be determined by non-linear regression analysis according to

$$B = \text{non-specific binding} + \frac{(\text{total binding} - \text{non-specific binding})}{1 + 10^{\log[C] - \log(IC_{50})}}$$

where B equals the amount of radioligand-binding at a given concentration of test compound [C], total binding equals the amount of radioligand-binding in the absence of test compound, and non-specific binding equals the residual amount of radioligand-binding after complete displacement of specific binding by a saturating concentration of the test compound.

2.2.2. Prostanoid DP, EP_1, EP_2, EP_3, IP, and TP receptors

Binding affinity for the prostanoid DP receptor was determined by measuring the inhibition of the adenosine diphosphate (ADP)-induced aggregation of rabbit platelets. Platelet-rich plasma was prepared from freshly obtained rabbit blood, and platelet aggregation was induced by 10 μM ADP (Eglen and Whiting, 1989). Binding affinities for the prostanoid EP_1, EP_2, and EP_3 receptors were determined by measuring muscle contractions in an organ bath. The muscles, which were attached to an isometric transducer, were maintained at 37°C in aerated (95% O_2, 5% CO_2) Krebs–Hensleit solution. For the prostanoid EP_1 receptor, contraction of the guinea pig ileum was induced by 1 μM AFP-172 (Eglen and Whiting, 1989), and the contraction induced by AFP-172 was expressed as a percentage of the maximal response to 10 μM acetylcholine. For the prostanoid EP_2 receptor, contraction of the guinea pig trachea was induced by 1 μM carbachol, then inhibited by 1 μM AFP-172. For the prostanoid EP_3 receptor, twitch contractions of the guinea pig vas deferens were induced by electrical stimulation (1 min interval, 5 sec trains of pulses at maximal voltage; pulse width, 1 msec; frequency, 5 Hz), then inhibited by AFP-172 (Savage et al., 1993). The binding studies for prostanoid IP and TP receptors were performed by a contract facility (Cerep, France) with standard binding procedures.

2.2.3. Other receptors and transporters

The binding studies for other receptors and transporters listed in Table 3 were performed by another contract facility (Daiichikagaku Pure Chemicals Co. Ltd, Toukaimura, Japan) with standard binding procedures.

2.3. IOP measurement

For the studies on ocular normotensive monkeys, 10 adult male cynomolgus monkeys, each weighing 4·7–7·4 kg (Keari Co. Ltd, Osaka, Japan), were used for the single-application experiment, and another 10 adult monkeys, each weighing 4·5–8·1 kg (Animal Care Co. Ltd, Tokyo, Japan), were used for a repeated-application study. Only animals with normal eyes (in terms of anterior chamber, angles, IOP, ocular media, and optic nerve heads) were included in these studies. For the ocular hypertensive monkey study, 12 adult cynomolgus monkeys, each weighing 5·2–8·1 kg (Keari Co. Ltd, Osaka, Japan), were used. Elevated IOP was produced in the monkeys according to a previous report (Lee et al., 1985), with a minor modification. Briefly, ocular hypertension was induced in the left eye by photocoagulating the trabecular meshwork using an argon laser (Ultima 2000; Coherent-Japan, Tokyo, Japan), applying 150 burns around 360 degrees twice with a 1-week interval. The present study was carried out more than 4 weeks after the last photocoagulation. If the IOP in the photocoagulated eye was not 5 mmHg greater than that in the non-photocoagulated fellow eye, the animal was not used for this study.

Before we measured IOP, all monkeys were trained for restraint in a monkey chair (CL-4535; Primate Products, Miami, FL, USA) and for measurements of IOP. For IOP measurements, the monkey was kept in a sitting position in the monkey chair, and the IOP was measured with a pneumatonograph (Model Classic 30R; Soran Ophthalmic, Jacksonville, FL, USA) without any general anesthesia or sedation. For corneal anesthesia, 0·4% oxybuprocain solution (Santen Pharmaceutical Co. Ltd, Osaka, Japan) was topically applied prior to IOP measurement. Drugs (20 μl) were applied to one eye in each monkey, with the contralateral eye remaining untreated. In the normotensive monkey study (single application), IOP was measured just before and at 2, 4, 6, 8, and 24 hr after drug application. In the normotensive monkey study (repeated application), IOP was measured just before and at 4, 6, 8, and 24 hr on days 1, 3, and 5 of the drug-application protocol. In the ocular hypertensive monkey study, drugs (20 μl) were applied to the left eye with elevated IOP in each monkey, at 9–10 a.m. IOP was measured just before and at 2, 4, 6, 8, 12, and 24 hr after drug application. A masked observer (F. N.) measured IOP in all experiments. A crossover design was applied to these studies; each animal contributed to all groups.

Prior to aqueous humor dynamics studies, IOP responses to AFP-168 (4 × 5 μl of 0·005%), timolol (2 μl containing 5 μg timolol) and $PGF_{2\alpha}$-isopropyl ester (2 × 5 μl of 0·02%) were confirmed in monkeys anaesthetized with ketamine HCl (10 mg kg^{-1}, i.m.) using a minified Goldmann applanation tonometer (Kaufman and Davis, 1980).

2.4. Aqueous humor dynamics assays

For the aqueous humor dynamics study, we used a total of 28 adult male and female cynomolgus monkeys, each weighing 2·5–6·0 kg (the majority from Covance Inc., Madison, WI, USA; two from the Coulston Foundation, Almagordo, NM, USA; one born at the University of Wisconsin, Madison, WI, USA). Aqueous humor formation (AHF) was measured in eight monkeys (i.m. ketamine anesthesia, 10 mg kg^{-1} initial supplemented by 5 mg kg^{-1} as needed) by fluorophotometry (Gabelt et al., 1994); total outflow facility was measured in the same eight monkeys plus four additional ones (i.m. ketamine (10 mg kg^{-1}) followed by i.v. pentobarbital (15 mg kg^{-1} initial, supplemented with 5–10 mg kg^{-1} as needed)) using two-level constant pressure perfusion (Bárány, 1964; Gabelt et al., 1991); uveoscleral outflow and trabecular outflow were measured in the same 12 monkeys by means of isotope accumulation (Sperber and Bill, 1984; Gabelt et al., 2003) prior to outflow facility measurements on the same day. Only animals with normal eyes (in terms of anterior chamber, angles, IOP, ocular media, and optic nerve heads) were used. In these studies, 4 × 5 μl of 0·005% AFP-168 was applied once daily for 3–5 days; 5 μg timolol (2 μl of timolol-gel forming solution diluted with saline) was applied once daily for 3 days; 2 × 5 μl of 0·02%

$PGF_{2\alpha}$-isopropyl ester was applied twice daily for 4 or 5 days to eight monkeys different from the previous 12; 100 μg/10 μl pilocarpine HCl was applied intracamerally to yet another group of eight different monkeys.

The rate of AHF was determined in ketamine anaesthetized monkeys using a Coherent scanning ocular fluorophotometer (Fluorotron Master, Palo Alto, CA, USA). Fluorescein (10% fluorescite for injection, Alcon Laboratories, Inc., Fort Worth, TX, USA) was administered topically on the afternoon preceding the fluorophotometry experiments (usually five 2 μl drops of 5% solution diluted with 0·1 M sodium phosphate buffer, pH 7·4) 5 min after one drop of 0·5% proparacaine hydrochloride (Bausch and Lomb Pharmaceuticals, Inc., Tampa, FL, USA). This kept the corneal fluorescein concentration at 200 ng ml^{-1} or more during the measurement period. On the experimental day, scans were recorded in each eye every hour for 5 hr.

Isotopic determination of AHF was performed in pentobarbital anaesthetized monkeys beginning 3 hr after the last dose of AFP-168 (fourth or fifth dose of once daily treatments) or $PGF_{2\alpha}$-isopropyl ester (seventh or ninth dose of twice daily treatments) by circulating [^{125}I] or [^{131}I]monkey albumin through the anterior chamber and measuring the dilution of label with newly formed aqueous (by counting a portion of the circuit every 5 min over a 2 hr period in a well detector/multichannel analyzer system, (ASA-100 NaI multichannel analyzer, Genie-2000 Spectroscopy Software, Canberra Industries, Inc., Meriden, CT, USA). Flow through the trabecular meshwork was determined by taking blood samples every 10 min from a femoral artery and counting them in a gamma counter (COBRA model D5003, Packard Instrument Co., Downers Grove, IL, USA). Uveoscleral outflow was calculated as the difference between AHF and trabecular outflow.

Total outflow facility was determined in pentobarbital anaesthetized monkeys by two-level constant pressure perfusion of the anterior chamber with Bárány's mock aqueous humor, correcting for the internal resistance of the perfusion apparatus as appropriate (Bárány, 1964). For AFP-168 and $PGF_{2\alpha}$-isopropyl ester studies, total outflow facility was determined for approximately 45 min at the conclusion of isotope studies and were completed within hours 5–6 after the fourth or fifth dose of once daily treatments with AFP-168 or after the seventh or ninth dose of twice daily treatments with $PGF_{2\alpha}$-isopropyl ester. For pilocarpine, the anterior chambers of both eyes were cannulated with a branched needle (with one branch connected to a reservoir and the other to a pressure transducer). Baseline facility measurements were taken for 35–45 min. Following baseline measurements, a bolus injection of 10 μl containing 100 μg pilocarpine HCl was made into the inflow tubing of one eye, Bárány's into the opposite eye tubing. Following a 5-min wash in period with the reservoirs elevated, cold air was blown on the corneas to mix the anterior chamber contents by convection. Post-drug outflow facility was then determined for another 35–45 min. Facility was calculated by successive averaging (Bárány, 1964).

2.5. Melanogenesis assay

B16-F0 melanoma cells were purchased from Dainippon Pharmaceutical Co. Ltd (Osaka, Japan). Melanin contents were measured as in previous reports (Siegrist and Eberle, 1986; Kosano et al., 1995). Briefly, fourth- or fifth-passage B16-F0 melanoma cells (ATCC No. CRL6322; 5×10^3 cells/well) were seeded in a 24-well plate (Asahi Techno Glass Corp., Funabashi, Japan) in culture medium (Dulbecco's modified Eagles medium containing 10% fetal bovine serum and 50 μg ml^{-1} gentamicin). Starting the day after seeding, the cells were cultured for 4 days in culture medium with or without drug (AFP-172 or PhXA85). The culture medium was collected, and the cells dissolved in lysis buffer (0·1N NaOH solution containing 10% Triton X-100) with 10-sec sonication. The melanin content of the medium and cell lysates was measured by absorbance at 490 and 415 nm, respectively.

2.6. Statistical analysis

The following statistical analyses were performed. For the IOP measurement study, the maximal reduction in IOP was used for statistical analysis. A Student's *t*-test was carried out following an *F*-test. Dunnett's test and the Tukey–Kramer test were carried out following a one-way analysis of variance. For the aqueous humor dynamics study, a two-tailed paired *t*-test was carried out on the values obtained for treated/control ratios. $p < 0{\cdot}05$ was taken as statistically significant.

3. Results

3.1. Receptor-binding assays

3.1.1. Prostanoid FP receptor

Prior to the FP binding study, we confirmed the expression of prostanoid FP receptors in the clonal cells. Immunofluorescence microscopy using an anti-FP receptor antibody, examination of the accumulation of inositol phosphates induced by fluprostenol, and radioligand-binding competition curve analysis of $PGF_{2\alpha}$ to [^{3}H]$PGF_{2\alpha}$ revealed that the clonal cell line used in this study did indeed express human FP receptors (data not shown). AFP-172 showed a high affinity for the human prostanoid FP receptor, with an EC_{50} of 0·53 nM. The affinity of AFP-172 was 12 times that of PhXA85 and 1700 times that of unoprostone (Table 1). AFP-172 produced full displacement of specific [^{3}H]$PGF_{2\alpha}$ binding and competed in a manner consistent with a simple bimolecular reaction (Hill slope ~1), as did PhXA85 and unoprostone.

Table 1
Fifty percent inhibition concentration (IC_{50}) and affinities of AFP-172, PhXA85, and unoprostone for the human prostanoid FP receptor

Compounds	IC_{50} (nM)	K_i (nM)	Potency ratio (K_i value of AFP-172 = 1)
AFP-172	0·53 ± 0·18	0·4	1
PhXA85	6·3 ± 1·3	4·7	12
Unoprostone	900 ± 55	680	1700

Affinity for the prostanoid FP receptor was determined by competitive binding to radiolabelled prostaglandin $F_{2\alpha}$. The 50% inhibition concentration (IC_{50}) and K_i values were calculated from the following equations:

$$B = \text{non-specific binding} + \frac{(\text{total binding} - \text{non-specific binding})}{1 + 10^{\log[C] - \log(IC_{50})}};$$

$$K_i = \frac{IC_{50}}{1 + [\text{radioligand}]/K_d};$$

Data represent the mean ± S.E.M. from far to five experiments; B; amount of radioligand binding; C; concentration of test compound; K_i; equilibrium inhibition constant; K_d; equilibrium dissociation constant.

3.1.2. Prostanoid DP, EP_1, EP_2, EP_3, IP, and TP receptors

The inhibitory or stimulatory effects of AFP-172 on the binding or pharmacological responses of prostanoid receptors are shown in Table 2. AFP-172 at 1 μM did not show evidence of inhibitory or stimulatory effects on any receptor binding, except that of the prostanoid EP_3 receptor. The IC_{50} of AFP-172 for the prostanoid EP_3 receptor was 67 nM, and the binding affinity of AFP-172 was 126 times weaker for this receptor than for the prostanoid FP receptor (see Tables 1 and 2). PGD_2 (DP), PGE_2 (EP_1), butaprost (EP_2), 17-phenyl trinol-PGE_2 (EP_3), iloprost (IP) and U44069 (TP) were used as positive controls in each experiment.

3.1.3. Other receptors and transporters

As shown in Table 3, the inhibitory effects of AFP-172 at 1 μM on a variety of receptor and transporter bindings were all less than 15%.

Table 2
Receptor-binding affinities or functional activities of AFP-172 to prostanoid DP, EP_1, EP_2, EP_3, IP, and TP receptors

Prostanoid receptors	Assays	EC_{50} or IC_{50} (nM)
DP	Inhibition of ADP-induced aggregation in rabbit platelets	>1000
EP_1	Contraction in guinea pig ileum	>1000
EP_2	Inhibition of carbachol-induced contraction in guinea pig trachea muscle	>1000
EP_3	Inhibition of electrical stimulation-induced contraction in guinea pig vas deferens	67
IP	Inhibition of [^{3}H]iloprost binding in human platelets	>1000
TP	Inhibition of [^{3}H]SQ29548 binding in human platelet	>1000

Data are averaged from two or four experiments.

Table 3
Inhibitory effects of AFP-172 on various receptors and transporters

Receptors/ transporters	Radioligand/assay	Inhibition (%) (1 μM AFP-172)
Adenosine A_1	[^{3}H]dipropylcyclopentylxanthine	<15
Adenosine A_{2a}	[^{3}H]CGS21680	<15
Adrenergic α_1 (non-selective)	[^{3}H]Prazosin	<15
Adrenergic α_2 (non-selective)	[^{3}H]RX821002	<15
Adrenergic β_1	[^{3}H]CGP12177	<15
Angiotensin AT_1	[^{125}I]Angiotensin II	<15
Benzodiazepine	[^{3}H]Flunitrazepam	<15
Bradykinin B_2	[^{3}H]Bradykinin	<15
Cannabinoid CB_1	[^{3}H]CP55940	<15
Cannabinoid CB_2	[^{3}H]WIN55212-2	<15
Cholecystokinin CCK_A	[^{3}H]L-364718	<15
Dopamine D_1	[^{3}H]SCH23390	<15
Dopamine D_2	[^{3}H]Spiperone	<15
Dopamine transporter	[^{3}H]WIN35428	<15
Endothelin ET_A	[^{125}I]Endothelin-1	<15
γ-Aminobutyric acid $(GABA)_A$	[^{3}H]Flunitrazepam	<15
Glutamate (non-selective)	[^{3}H]Glutamic acid	<15
Histamine H_1	[^{3}H]Pyrilamine	<15
Melanocortin MC_4	[^{3}H]4-Norleucine, 7-D-phenylalanine-α-melanocyte stimulating hormone	<15
Muscarinic (non-selective)	[^{3}H]Quinuclidinyl benzilate	<15
Neurokinin NK_1	[^{3}H]Substance P	<15
Neuropeptide Y_1	[^{125}I]Peptide YY	<15
Neuropeptide Y_2	[^{125}I]Peptide YY	<15
Norepinephrine transporter	[^{3}H]Nisoxetine	<15
Nicotinic Ni	[^{3}H]Epibatidine	<15
Opiate (non-selective)	[^{3}H]Naloxone	<15
Orphanin ORL_1	[^{3}H]Nociceptin	<15
Serotonin $5HT_1$ (non-selective)	[^{3}H]Serotonin	<15
Serotonin transporter	[^{3}H]Imipramine	<15
Sigma (σ)	[^{3}H]1,3,-Di-*o*-tolylguanidine	<15
Vasopressin V_{1b}	[^{3}H]Arg-Vasopressin	<15
Vasopressin V_2	[^{3}H]Arg-Vasopressin	<15

Data are averaged from two experiments.

3.2. Effects of a single application of AFP-168 on intraocular pressure in conscious normotensive monkeys

Fig. 2 shows the maximum IOP reductions of a single application of either AFP-168 or latanoprost in conscious ocular normotensive monkeys. The maximal IOP reductions with AFP-168 (0·00002–0·0025%) were dose-dependent, and significance was reached at doses of 0·0005 and 0·0025%. The maximal IOP reduction seen with AFP-168

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