Medicinal Chemistry

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Fluorinated Prostanoids: Development of Tafluprost, a New Anti-glaucoma Agent

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2.1 Introduction

2.1.1 Background

Prostanoids, consisting of prostaglandins (PGs) and thromboxanes (TXs), are members of the lipid mediators derived enzymatically from fatty acids. Arachidonic acid, a C_{20} essential fatty acid for most mammalians, is freed from the phospholipid molecule by phospholipase A_2, which cleaves off the fatty acid precursor. Prostanoids are produced in a wide variety of cells throughout the body from the sequential oxidation of arachidonic acid by cyclooxygenase, PG hydroperoxidase, and a series of prostaglandin synthases (Figure 2.1).

Prostanoids have extensive pharmacological actions in various tissues in order to maintain homeostasis [1]. They play an important role as local hormones that mediate inflammation, pain, fever, vasoconstriction or vasodilation, coagulation, calcium regulation, cell growth, and so on. The prostanoids have been thought to exert their multiple physiological actions via specific protein receptors on the surface of target cells. The prostanoid receptors were pharmacologically identified from the functional data and classified into DP, EP, FP, IP and TP receptors, which are specific for PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin), and TXA_2, respectively [2]. The recent molecular cloning of cDNAs that encode the prostanoid receptors has identified the receptor structures and confirmed

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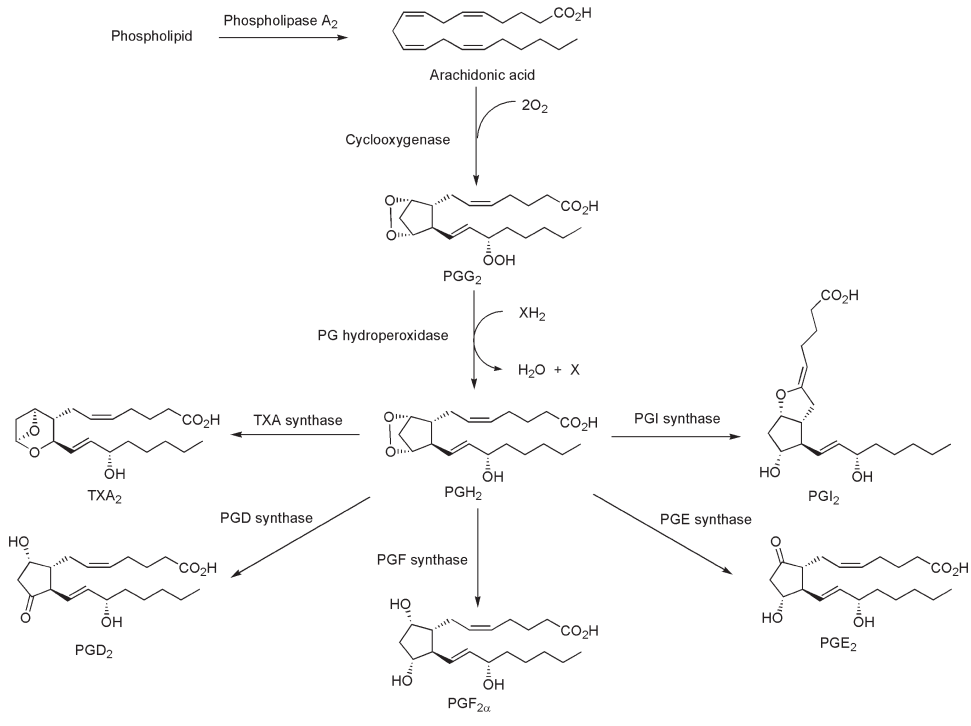


Figure 2.1 *Biosynthesis of prostanoids.*

the receptor classification including further subdivision for the EP receptor subtypes, EP1–EP4 [3, 4].

The prostanoid receptors belong to the G-protein-coupled rhodopsin-type receptors with seven transmembrane domains. They include an extracellular amino group terminus, an intracellular carboxyl group terminus, three extracellular loops, and three intracellular loops (Figure 2.2) [3]. The overall homology among the receptors is not high, though these receptors conserve important amino acid sequences in several regions, especially in the seventh transmembrane domain. It is proposed that the conserved arginine residue in the domain serves as the binding site for the terminal carboxyl group of the prostanoid molecules. Information obtained from studies on the receptor structures and properties has been utilized recently for computer-aided molecular modeling and structure-based drug design. The use of expressed receptors aids in screening of compounds for the specific receptor agonists or antagonists.

The properties of the prostanoid receptors, such as second messengers in signal transduction pathways, and localization in the eye are summarized in Table 2.1. Prostanoid receptors are widely distributed in the monkey and human eyes [5]. The expression and localization of the FP and EP receptor subtypes in the tissues was studied intensely by in-situ hybridization and immunohistochemistry to gain a better understanding of the ocular effects of the prostanoids and their analogues. This work suggests a wide distribution but differential expression of FP and EP receptor subtypes in human ocular tissues. The highest expression of FP receptor mRNA and protein was found in the corneal epithelium, ciliary

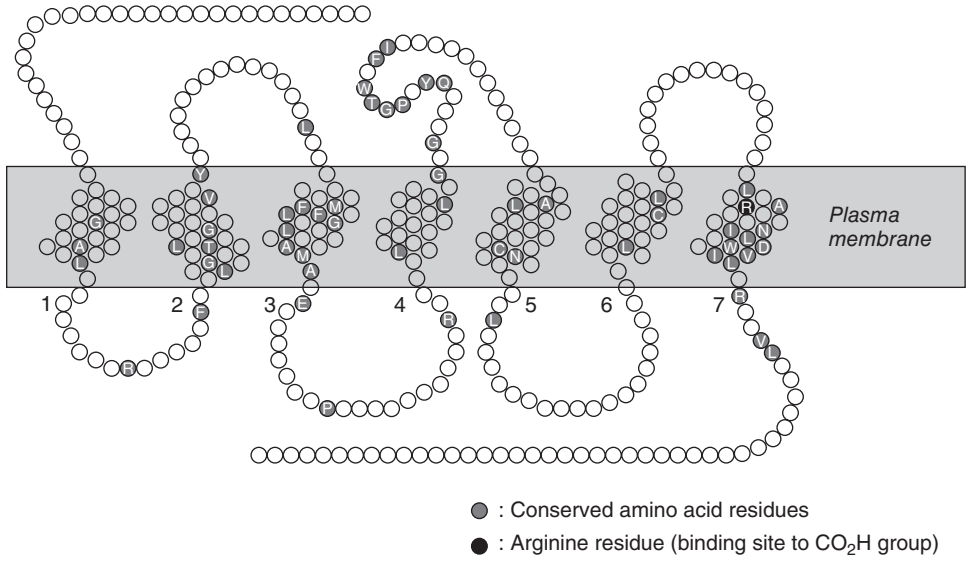


Figure 2.2 *Prostanoid receptor.*

Table 2.1 *Properties of prostanoid receptors*

PGs	Receptor	Signal transduction	Localization in the human eye
PGD_2	DP	cAMP ↑	Retina
PGE_2	EP_1	Ca^{2+}↑	Ciliary body, trabecular meshwork, retina, iris, lens, cornea, conjunctiva
	EP_2	cAMP ↑	Trabecular meshwork, cornea, choroid
	EP_3	cAMP ↓	Ciliary body, trabecular meshwork, retina, iris, cornea, conjunctiva
	EP_4	cAMP ↑	Ciliary body, trabecular meshwork, retina, iris, cornea, conjunctiva
$PGF_{2\alpha}$	FP	PI response	Ciliary body, trabecular meshwork, iris, cornea
PGI_2	IP	cAMP ↑, PI response	Trabecular meshwork
TXA_2	TP	PI response	Trabecular meshwork, corneal epithelium, ciliary processes, retina

PI response: phosphatidylinositol response.

epithelium, ciliary muscle, and iris muscles by immunohistochemistry [6]. In the trabecular meshwork, the gene expression of the EP2 receptor is more abundant than that of other receptors [7].

2.1.2 Fluorinated Prostanoids Research

It is well known that introduction of fluorine atoms into biologically active substances may lead to improvements in pharmacological properties and an increase in therapeutic

efficacy [8]. These advantageous pharmacological effects of fluorinated molecules are mainly derived from the following physicochemical characters of fluorine: (1) relatively small atomic size, (2) high carbon–fluorine bond energy, (3) high electronegativity, and (4) enhancement of lipophilicity.

The main problems with the use of natural prostanoids utilized as drugs have been perceived to be both chemical and metabolic instability, and separation of side-effects from the multiple physiological actions. In order to overcome these difficulties, chemical modifications of natural prostanoids have been studied extensively along with the development of new synthetic methodologies since the 1970s [9]. Taking advantage of the unique characteristics of fluorine, a large number of fluorinated prostanoids have also been reported (Figure 2.3) [10]. For example, fluprostenol, with a *m*-trifluoromethylphenoxy group in the ω-chain, emerged in 1974 was one of the first successfully marketed analogues with application as a potent luteolytic agent in veterinary medicine [11]. The compound is known as a selective FP receptor agonist and is widely used as a pharmacological tool. The strong inductive effect and enhancement in lipophilicity caused by the CF_3 group should contribute to improvement of the biological profile. The 16,16-difluoro-PGE_2 was reported in 1975 to be metabolically stabilized by 15-dehydrogenase inhibition of the degradation pathways *in vitro* [12]. The inhibition of enzymatic oxidation is explained by the destabilization effects of electron-withdrawing fluorine atoms causing a shift to the reduced form between the allyl alcohol and the enone in equilibrium. Fried *et al.* reported that 10,10-difluoro-13,14-dehydro-PGI_2 [13] and 10,10-difluoro-TXA_2 [14] showed an increase in the stability against hydrolysis in comparison with PGI_2 and TXA_2, respectively. Our group studied a 7,7-difluoro-PGI_2 derivative (AFP-07) for modification of the physical and physiological properties of natural PGI_2 by the inductive effects of fluorine atoms [15]. AFP-07 showed not only higher stability in aqueous media of at least 10 000

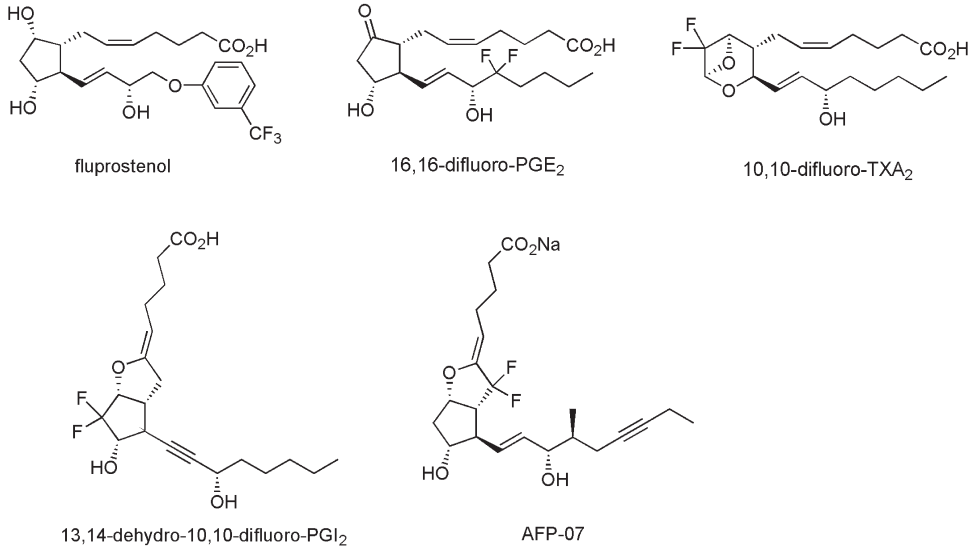


Figure 2.3 *Fluorinated prostanoids.*

times that of the natural compound, but also potent and selective affinity for the IP receptor [16]. These instances demonstrate the high potential of chemical modification of prostanoids with fluorine for lead discovery and optimization in drug development, if fluorine atoms can be introduced into the right positions of the prostanoid structure on the basis of rational drug design.

2.2 Therapy of Glaucoma

2.2.1 Glaucoma

Glaucoma is one of the most common but serious eye diseases and that can damage the optic nerve and result in loss of vision and blindness. There may be no symptoms in the early stages of the disease. A recent epidemiological survey of glaucoma conducted in Japan (the Tajimi study) showed that the prevalence of glaucoma in residents aged 40 years and older is about 5.0%, which is higher than that of previous surveys and demonstrates that the number of patients with glaucoma has been increasing in Japan [17]. Worldwide, it is the second leading cause of blindness, according to the World Health Organization.

It is thought that high pressure within the eye (intraocular OP) is the main cause of the optic nerve damage. Although elevated IOP is clearly a risk factor, other factors must also be involved because even people with normal levels of pressure can experience vision loss from glaucoma. The Tajimi study in Japan revealed that a substantial number of glaucoma patients are diagnosed as suffering normal tension glaucoma (NTG). The evidence suggests that in patients with NTG a 30% reduction in IOP can slow the rate of progressive visual field loss [18]. IOP can be lowered with medication, usually eye drops. There are several classes of drugs for treating glaucoma, with several medications in each class. The first-line therapy of glaucoma treatment is currently prostanoids, which will allow better flow of fluid within the eye. IOP-lowering eye drops, by acting on their respective receptors to decrease the secretion of aqueous humor such as β-blockers or α_2 agonists, also might be considered, although these may not be used in people with heart conditions, because they can affect cardiovascular and pulmonary functions.

2.2.2 Prostanoids in the Therapy of Glaucoma

Since the discovery in 1980s that $PGF_{2\alpha}$ reduces IOP in an animal model [19], extensive efforts have been devoted to developing FP receptor agonists as promising new antiglaucoma agents [20]. Most reported analogues are esters used as prodrugs that are rapidly hydrolyzed by corneal enzymes to the free acids to account for the ocular hypotensive effects by activation of the FP prostanoid receptor (Figure 2.4).

Unoprostone [21], a docosanoid, a structural analogue of an inactive biosynthetic metabolite of $PGF_{2\alpha}$,was developed by Ueno *et al.* and first marketed in Japan for the treatment of glaucoma in 1994. Clinical studies showed that in patients with mean baseline IOP of 23 mmHg, it lowered IOP by approximately 3–4 mmHg throughout the day. The recommended dosage is one drop in the affected eyes twice daily.

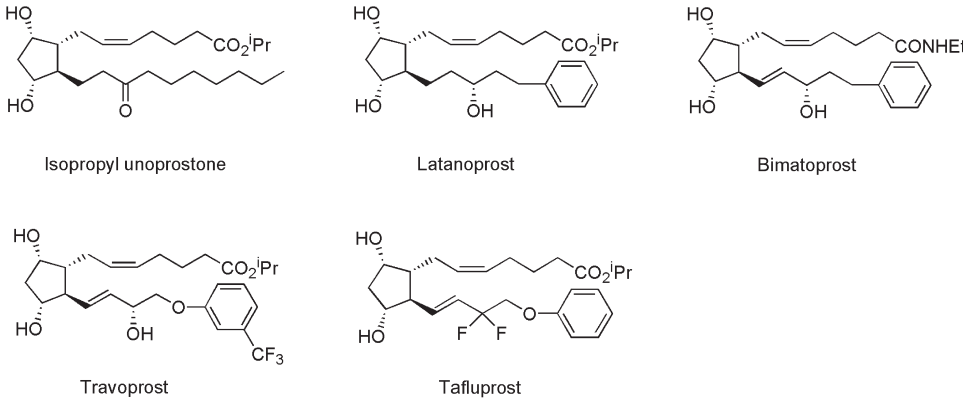


Figure 2.4 *Anti-glaucoma prostanoids.*

Stjernschantz *et al.* later developed latanoprost [22], which has potent IOP-reducing effects with topical administration once daily in the evening. Compared with a representative β-blocker, timolol, it demonstrated superior efficacy in clinical studies in reducing IOP by approximately 27–34% from baseline. Latanoprost is the FP receptor agonist most widely used worldwide as an anti-glaucoma drug.

Bimatoprost and travoprost are recently approved prostanoids with high efficacy in reducing IOP. The chemical structure of bimatoprost differs from that of latanoprost only in a double bond of the ω-chain and an ethylamide in the C-1 position. Although the classification of bimatoprost is still subject of controversy, it has been demonstrated as a "prostamide," a class of drugs distinct from PGs [23]. Travoprost is an isopropyl ester of a single enantiomer of fluprostenol, which was already known as a potent and selective FP receptor agonist [24]. A new synthetic route of travoprost from a tricyclic ketone with ring cleavage by the attack of a vinyl cuprate has recently been reported [25].

The aqueous humor flows out the eye mainly via the conventional route of the trabecular meshwork and Schlemm's canal. However, about 10–20% of outflow is via the uveoscleral (nonconventional) route whereby the aqueous humor passes between the ciliary muscle bundles and into the episcleral tissues, where it is reabsorbed into orbital blood vessels and drained via the conjunctival vessels. The IOP-lowering effect of these prostanoid drugs occurs predominantly through enhancement of uveoscleral outflow [26], although unoprostone and bimatoprost also increase flow via the trabecular meshwork to a lesser extent.

The prostanoids have been widely used for the treatment of ocular hypertension in many countries because they have good IOP-reducing effects without causing serious systemic side-effects. However, the drugs cause local adverse effects, such as hyperemia and iris/skin pigmentation [27]. Moreover, the existing ocular hypotensive drugs, even latanoprost, do not produce satisfactory IOP control in all patients. It is therefore hoped that a new-generation prostanoid having powerful and prolonged IOP-reducing efficacy together with improvement in ocular circulation, and causing fewer side-effects, will become available for patients with glaucoma.

2.3 Development of Tafluprost

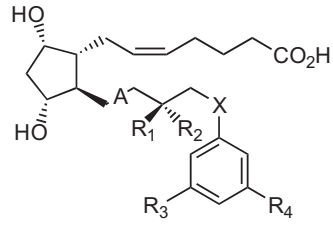
2.3.1 Screening and Discovery

Prostanoids are generally flexible molecules that change their conformation in response to changes in their environment through the intramolecular hydrogen bonding between the terminal carboxylic acid and the hydroxyl group at C-9, C-11, or C-15 [28]. In the drug–receptor complex, the prostanoids can adopt a preferred conformation through the forces involved ionic interactions and dipole–dipole interactions, including hydrogen bonding, between these functional groups and the corresponding amino acid residues of receptors [29]. If a specific position of the prostanoids is substituted by fluorine, it should affect not only the molecular conformation but also the drug–receptor complex through possible participation of the fluorine in the interactions.

The substitution of the hydroxyl group at C-15 of $PGF_{2\alpha}$ with fluorine atoms and the biological effects of the molecule has not been well-studied [30] because the 15-hydroxyl group is believed to be essential for pharmacological activity of PGs [31]. The carbon–fluorine bond (van der Waals radius = 1.47 Å) is nearly isosteric with the carbon–oxygen bond (van der Waals radius = 1.52 Å). Compared to the hydroxylated carbon, the fluorinated atom should be much more electronegative because of the strong electron-withdrawing effect of fluorine. In contrast to the hydroxyl group, the fluorine cannot be a donor in hydrogen bonding; it can be a weak acceptor for hydrogen bonding, although this is still a matter of controversy [32]. In addition, the enhancement of lipophilicity on introducing fluorine atoms in a position close to a rigid pharmacophore such as an aromatic functionality in the ω-chain may be an effective way to increase the specific affinity for a hydrophobic pocket of the receptors.

Our research group at Asahi Glass Co., Ltd. has collaborated with Santen Pharmaceutical Co., Ltd. to find a new FP receptor agonist having more potent IOP-reducing activity and weaker side-effects. We have recently discovered a 15-deoxy-15,15-difluoro-17,18,19,20-tetranor-16-phenoxy-$PGF_{2\alpha}$ isopropyl ester, tafluprost (AFP-168), which shows highly potent and selective affinity for the FP receptor [33]. We have synthesized newly designed $PGF_{2\alpha}$ derivatives and investigated their prostanoid FP receptor-mediated functional activities both *in vitro* and *in vivo*. A functional prostanoid FP-receptor-affinity assay was performed using iris sphincter muscle isolated from cat eyes, which predominantly expresses the prostanoid FP receptor. The results on constrictions induced by PG-derivatives are shown in Table 2.2. A carboxylic acid of latanoprost induced constriction with an EC_{50} value of 13.6 nM. In the functional FP receptor affinity assay, we found that 15-deoxy-15-fluoro-16-aryloxy-tetranor-$PGF_{2\alpha}$ derivatives (AFPs-159 and 120) caused strong constriction of the isolated cat iris sphincter [34]. This suggested that exchanging the 15-hydroxy group for fluorine preserved agonistic activities on FP receptor. In contrast, the diastereomers of these derivatives with the fluorine atom attached at C-15 showed much weaker binding affinities (data not shown). Interestingly, 15,15-difluorinated analogues – AFPs-164, 157, 162, and 172 – demonstrated much more potent agonistic activities than the monofluorinated derivatives. The introduction of a chlorine atom into the *meta*-position of the benzene ring of these difluorinated derivatives reduced the prostanoid FP-receptor functional activities. The 13,14-dihydro analogues AFP-164 and AFP-162 had a weaker affinities than the unsaturated ones, AFPs-157 and 172.

Table 2.2 *Functional assay of PG derivatives on FP receptor*[a]



Compounds	A	X	R^1	R^2	R^3	R^4	EC_{50} (nM)
Latanoprost acid form	Single bond	CH_2	H	OH	H	H	13.6
AFP-159	Single bond	O	H	F	H	H	6.6
AFP-120	Double bond	O	H	F	Cl	Cl	37.9
AFP-164	Single bond	O	F	F	H	Cl	9.4
AFP-157	Double bond	O	F	F	H	Cl	1.9
AFP-162	Single bond	O	F	F	H	H	2.4
AFP-172	Double bond	O	F	F	H	H	0.6

[a] Constriction effects of PG derivatives on cat iris sphincters.

Overall, AFP-172, the active carboxylic acid form of tafluprost, displayed the most potent activity.

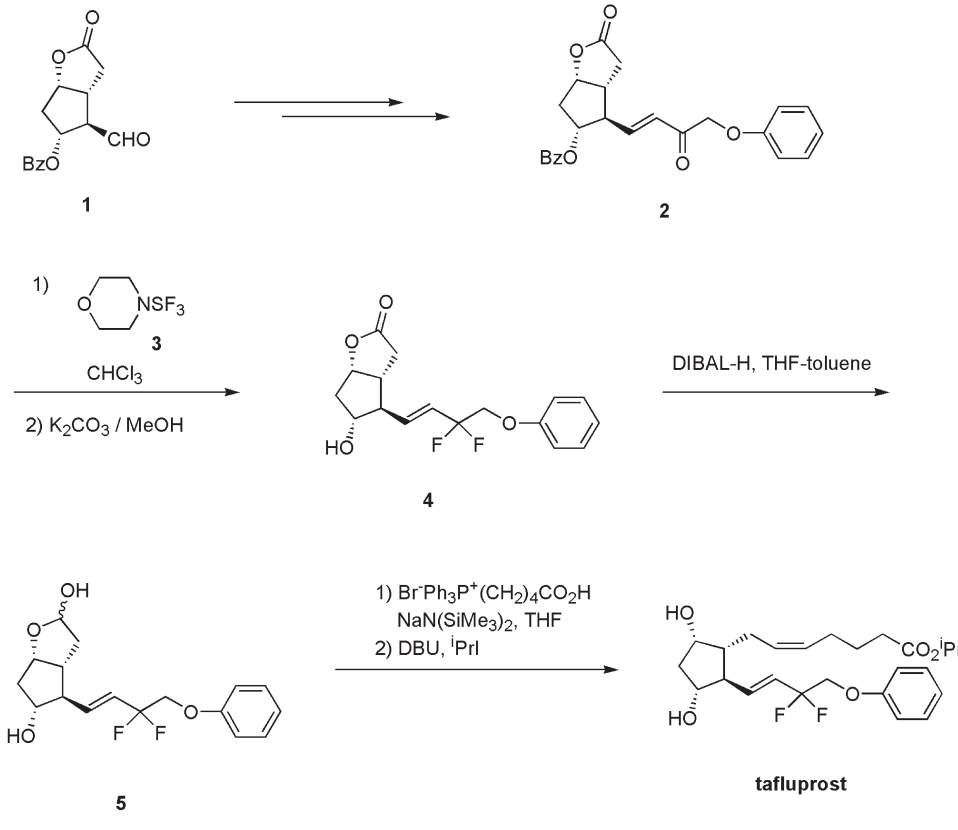
2.3.2 Synthesis of Tafluprost

A synthetic route for tafluprost is shown in Scheme 2.1 [33]. The synthesis was started from the Corey aldehyde **1**, which was converted to enone **2** by Horner–Emmons reaction. Since a general method to prepare allyl difluorides from enones had not been reported, we studied the fluorination reaction. It was found that the reaction of the enone **2** with morpholinosulfur trifluoride **3** and successive deprotection gave the desired geminal difluoride **4** in good yield. Reduction of the lactone **4** with diisobutylaluminum hydride in THF–toluene at −78 °C afforded the lactol **5**. The Wittig reactions of the lactol **5** with the ylide prepared from 4-carboxybutyltriphenylphosphonium bromide with various bases yielded the 15-deoxy-15,15-difluoro-$PGF_{2\alpha}$ derivative as a mixture of 5*Z* and 5*E* isomers. The Wittig reaction using sodium bis(trimethylsilyl)amide as base gave the best result for stereoselectivity (5*Z*/5*E* = 99/1). The esterification of the crude acid treated with isopropyl iodide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded the desired 15-deoxy-15,15-difluoro-$PGF_{2\alpha}$ derivative (tafluprost).

2.3.3 Pharmacology of Tafluprost

2.3.3.1 Prostanoid Receptor Affinities

The affinity of the corresponding carboxylic acid of tafluprost for the recombinant human FP receptor expressed in clonal cells was 0.4 nM, which was 12 times and 1700 times



Scheme 2.1 *Synthesis of tafluprost.*

Table 2.3 *Affinities of prostanoids for the human prostanoid FP receptor*

Compound (acid form)	K_i (nM)	Ratio (tafluprost = 1)
Tafluprost	0.40	1
Latanoprost	4.7	12
Unoprostone	680	1700

higher than those of latanoprost and isopropyl unoprostone, respectively (Table 2.3) [35]. It should be noted that substitution of difluoro-moiety for the hydroxyl group at C-15 of $PGF_{2\alpha}$ derivatives increases binding to the FP receptor to such a large extent because the hydroxyl group was thought to be indispensable to exhibit biological activity [31].

Since the acid form of tafluprost did not show significant affinities for other prostanoid receptors, the drug proved to be a highly selective compound [35]. Compared with the other prostanoids [26b, 36], tafluprost is regarded as one of the most selective prostanoid FP receptor agonists.

2.3.3.2 IOP-Reducing Effects

Tafluprost has a potent IOP-reducing effect in animal models. For example, the maximal IOP reduction achieved with tafluprost at 0.0025% was greater than with latanoprost at 0.005% in both laser-induced glaucomatous and ocular normotensive monkeys [35]. The effects of tafluprost and latanoprost on IOP reduction in conscious ocular normotensive monkeys are indicated in Figure 2.5. The peak time for the IOP reduction induced by tafluprost was 6–8 h after its application, similar to that of latanoprost. The duration of the IOP reduction seen with tafluprost was greater than that seen with latanoprost. Once-daily applications of tafluprost led to progressive increases in the daily maximal IOP reduction and in the IOP reduction at the trough time-point (just before the next application), while these effects at the trough time-point were not observed with latanoprost in the monkey study. These results indicate that the IOP-lowering effect of tafluprost is stronger and more continuous than that of latanoprost.

The mechanism underlying the IOP-lowering effect of tafluprost was investigated in ocular normotensive monkeys (Table 2.4) [35]. The methods used in this study were validated by their ability to reveal the effects of positive controls, such as timolol, $PGF_{2\alpha}$-isopropyl ester, and pilocarpine. Tafluprost decreased the flow to blood (FTB, conventional outflow) and increased the uveoscleral outflow. The effect of tafluprost on aqueous humor formation (AHF) was similar by the different methods, increases of 10% by fluorophotometry (not significant) and 14% by isotope perfusion ($p < 0.05$). Compared with the increase in uveoscleral outflow, this increase in AHF is relatively small. Thus, tafluprost may affect AHF slightly, as do the other $PGF_{2\alpha}$ analogues. Tafluprost also decreased FTB and the mechanism may due to rerouting of flow to the uveoscleral pathway. Thus, the primary mechanism underlying the IOP-reducing effect of tafluprost is via an increase in uveoscleral outflow, as with other PG derivatives [26, 37].

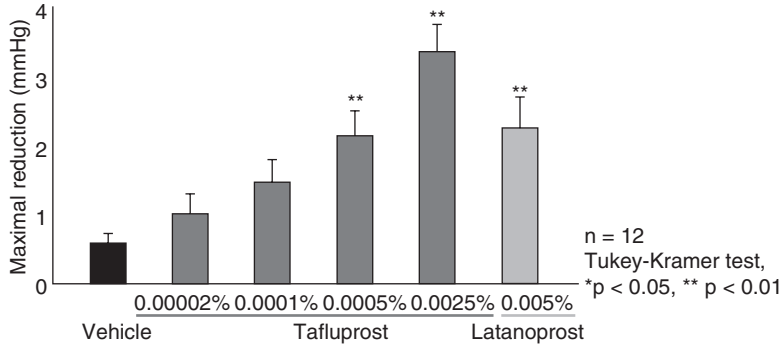


Figure 2.5 *Effects of tafluprost and latanoprost on maximal reduction of intraocular pressure (IOP) in conscious ocular normotensive monkeys.*
*(*Source: *Reprinted from Takagi, Y., Nakajima, T., Shimazaki, A.,* et al. *Pharmacological characteristics of AFP-168 (tafluprost), a new prostanoid FP receptor agonist, as an ocular hypotensive drug. Exp. Eye Res., (2004)* **78***, 767–776, with permission from Elsevier)*

Table 2.4 *Effects of tafluprost on aqueous humor dynamics in anesthetized ocular normotensive monkeys*

Experiments/treatments (*n*)	Control (contralateral eye)	Treayed eye	Ratio of treated/control
Fluorophotometry for aqueous humor formation (AHF, μl/min)			
Baseline (8)	1.49 ± 0.14	1.43 ± 0.12	0.97 ± 0.03
Tafluprost (8)	1.73 ± 0.13	1.88 ± 0.12	1.10 ± 0.04
Baseline (8)	1.55 ± 0.14	1.64 ± 0.17	1.06 ± 0.05
Timolol-gel (8)	1.39 ± 0.15	1.06 ± 0.10	0.77 ± 0.02**
Isotope perfusion for AHF (μl/min), flow to blood (FTB, μl/min), and uveoscleral outflow (Fu, μl/min)			
AHF tafluprost (12)	1.54 ± 0.12	1.73 ± 0.15	1.14 ± 0.06*
FTB tafluprost (12)	0.78 ± 0.16	0.61 ± 0.14	0.78 ± 0.06**
Fu tafluprost (10)	0.92 ± 0.17	1.22 ± 0.14	1.65 ± 0.24*
AHF $PGF_{2\alpha}$-ie (8)	1.45 ± 0.17	1.54 ± 0.19	1.11 ± 0.14
FTB $PGF_{2\alpha}$-ie (8)	0.43 ± 0.12	0.14 ± 0.03	0.41 ± 0.08**
Fu $PGF_{2\alpha}$-ie (8)	1.01 ± 0.22	1.40 ± 0.20	2.31 ± 0.99
Two-level constant-pressure perfusion for total outflow facility (μl/min/mmHg)			
Tafluprost (12)	0.45 ± 0.08	0.57 ± 0.11	1.33 ± 0.13*
$PGF_{2\alpha}$-ie (8)	0.60 ± 0.10	0.58 ± 0.09	1.15 ± 0.23
Pilocarpine (8)	0.83 ± 0.16	2.23 ± 0.40	2.84 ± 0.33**

$PGF_{2\alpha}$-ie: prostaglandin $F_{2\alpha}$-isopropyl ester.
Data represent the mean ± SEM. For ratio values, $*p < 0.05$,
$**p < 0.01$ for difference from 1.0 (two-tailed paired *t*-test).
Source: Reprinted from Ref. 35, Copyright (2004) with permission from Elsevier Limited.

2.3.3.3 IOP-Lowering Effects in Prostanoid Receptor-Deficient Mice

Ota *et al.* reported the IOP-lowering effects of tafluprost in wild-type mice [38] and prostanoid receptor-deficient mice [39], topically administered by a microneedle method. The IOP-lowering effect of tafluprost was compared with that of latanoprost in ddY mice over a 24 h period. By area-under-the-curve analysis, tafluprost was more effective in reducing mouse IOP, and its ocular hypotensive effect lasted longer than that of latanoprost [38]. In B6 mice, both tafluprost and latanoprost lowered IOP in a dose-dependent manner from 1 to 6 h after administration, but the magnitude of IOP reduction induced by tafluprost was significantly greater than that induced by latanoprost. The more effective IOP reduction of tafluprost may be the result of its higher affinity for FP receptor. In EP1KO and EP2KO mice, there was no significant difference in IOP reduction induced by tafluprost and latanoprost as compared with B6 mice. Although tafluprost and latanoprost significantly lowered IOP in EP3KO mice, the magnitude of IOP reduction was significantly less than the effect in B6 mice. The EP3 receptor may play a role in IOP reduction induced by tafluprost and latanoprost. In FPKO mice, tafluprost and latanoprost had no obvious IOP reduction. These results suggest that tafluprost lowers IOP and produces endogenous PG via mainly prostanoid FP receptor, and the endogenous PG may lower IOP via prostanoid EP3 receptor, similarly to the findings with travoprost, bimatoprost, and unoprostone in a previous study [40].

2.3.3.4 Increase of Ocular Blood Flow

Tafluprost significantly increases retinal blood flow and blood velocity in animal models. The improvement of ocular blood flow is thought to be relevant in glaucoma therapy, especially for normal-tension glaucoma patients since it is assumed that optic nerve damage is involved not only in mechanical compression caused by IOP but also in impairment of ocular blood flow.

The effects of tafluprost on IOP and retinal blood flow (RBF) were studied in adult cats [41]. A single drop of tafluprost was placed in one eye and IOP, vessel diameter, blood velocity, and RBF were measured simultaneously by laser Doppler velocimetry. Measurements carried out at 30 and 60 min after dosing showed 16.1% and 21.0% IOP reduction, respectively, as well as 1% and 2.4% reduction in mean vessel diameter, respectively. The mean blood velocity increases were 17.4% and 13.7%, respectively, and the mean RBF increases were 20.7% and 18.8%, respectively, 30 and 60 min after dosing.

Another study aimed to evaluate and compare the effect of tafluprost, latanoprost, and travoprost on optic nerve head (ONH) blood flow in rabbits [42]. A quantitative index of blood flow, squared blur rate (SBR), was determined with the laser speckle method, when 50 µl of 0.0015% tafluprost, 0.005% latanoprost, or 0.004% travoprost were topically administrated once a daily for 28 days. After 28 days' administration of tafluprost, latanoprost, and travoprost, the trough SBR values became 111.9 ± 3.9%, 107.2 ± 4.3% and 106.7 ± 3.5%, respectively, compared with the value before administration. Sixty minutes after final administration on day 28, the SBR value with tafluprost, latanoprost, and travoprost become 116.1 ± 3.5%, 106.1 ± 3.0%, and 104.2 ± 3.7%, respectively, compared with the value before administration. These results indicate that topical administrations of these compounds stably increase the ONH blood flow in rabbits. The magnitude of increase in ONH blood flow produced by tafluprost was greater than that of latanoprost or travoprost.

2.3.3.5 Protective Effect of Tafluprost on Glutamate-Induced Cytotoxicity

The protective effect of tafluprost on the cytotoxicity and intracellular Ca^{2+} increase induced by l-glutamate (Glu) using primary cultures obtained from the fetal rat retina has been reported [43]. Tafluprost acid form significantly prevented Glu-induced cytotoxicity in a concentration-dependent manner of more than 10 nM. However, latanoprost acid form did not show any effect on Glu-induced cytotoxicity. Tafluprost acid form showed the cell protective effect on Glu-induced cytotoxicity through the inhibition of intracellular Ca^{2+} increase in retinal cells.

Glaucoma is a progressive neuropathy characterized by loss of the visual field resulting from neuronal cell death [44]. These results suggest that tafluprost is an effective therapy for glaucoma to prevent the retinal cell damage in addition to its effects of lowering IOP and increasing the activity of ocular blood flow.

2.3.3.6 Melanogenesis

In long-term clinical use, prostanoids are known to cause iris pigmentation as a characteristic side-effect; this has been observed in 5–15% of patients treated [27]. In cultured melanoma cells, a carboxylic acid of latanoprost has been reported to increase melanogenesis [45]. However, a carboxylic acid of tafluprost did not have the stimulatory effects

on melanin content in cultured B16-F10 melanoma cells [34, 35]. The melanogenesis-promoting effects of latanoprost acid and tafluprost acid *in vitro* are compared in Figure 2.6. This finding implies that the application of tafluprost may cause less iris pigmentation than that of latanoprost.

2.3.4 Pharmacokinetics and Metabolism

To evaluate the distribution and metabolism of [^{3}H]tafluprost in ocular tissues and to study the IOP-lowering effects of the major metabolites of tafluprost, single ocular doses of [^{3}H]tafluprost were administered to male/female cynomolgus monkeys (1 μg/eye for tissue distribution studies and 10 μg/eye for metabolic studies) [46]. Tafluprost was rapidly absorbed into ocular tissues and subsequently entered the systemic circulation. The highest concentrations of radioactivity were observed in the bulbar conjunctiva and the palpebral conjunctiva (323 and 180 ng-eq/g, respectively) at 0.083 h after administration, and in the cornea (784 ng-eq/g) at 0.25 h after administration. Nonvolatile radioactivity in plasma peaked (0.907 ng-eq/g) at 0.083 h after administration and then declined steadily. Three major metabolites shown in Figure 2.7, a carboxylic acid of tafluprost (AFP-172), 1,2-

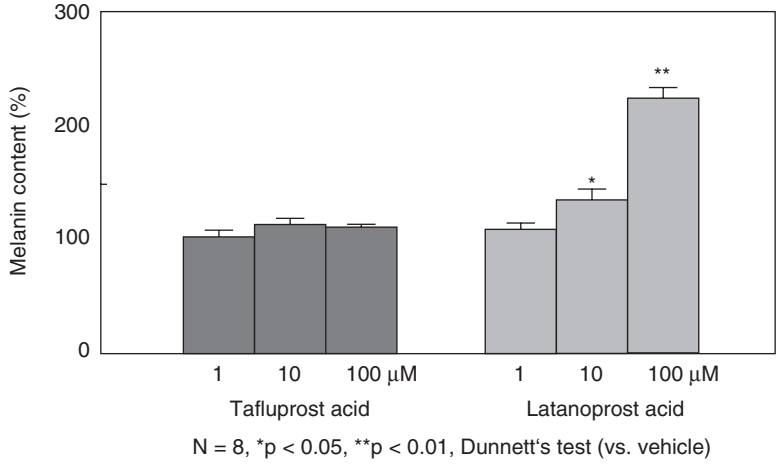


Figure 2.6 *Effects of tafluprost acid and latanoprost acid on melanin contents of cultured B16-F10 melanoma cells.*

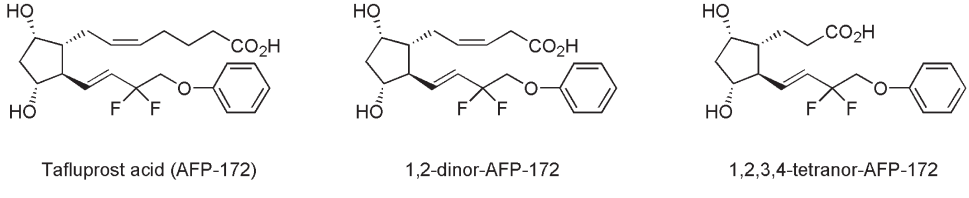


Figure 2.7 *Metabolites of tafluprost.*

dinor-AFP-172, and 1,2,3,4-tetranor-AFP-172, accounted for most of the radioactivity in the aqueous humor and other ocular tissues. AFP-172 was demonstrated to be the most abundant and the only pharmacologically active metabolite in ocular tissues. A small amount of tafluprost was detected in the ciliary body, cornea, and iris.

2.4 Conclusion

A novel 15,15-difluorinated prostanoid, tafluprost was discovered as a highly potent and selective prostanoid FP receptor agonist. Tafluprost demonstrates powerful and prolonged IOP-lowering effects in animal models. The maximal IOP reduction achieved with tafluprost was greater than that with latanoprost in both normotensive and glaucomatous monkeys. Tafluprost showed significantly increasing efficacy of ocular blood flow and protective effect on glutamate-induced cytotoxicity. In its pharmacological characteristics, tafluprost may be superior to latanoprost: potent IOP-reducing efficacy, effective increase in ocular blood flow, and weak melanogenetic side-effect. Tafluprost has completed clinical trials, and new drug applications for tafluprost have been filed in Japan and the EU. Tafluprost is expected to become a new-generation prostanoid FP agonist that strongly reduces IOP and effectively improves ocular circulation in patients with glaucoma.

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