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Themed Section: Eicosanoids 35 years from the 1982 Nobel: where are we now?

REVIEW ARTICLE

Prostaglandin FP receptor antagonists: discovery, pharmacological characterization and therapeutic utility

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In contrast to the availability of potent and selective antagonists of several prostaglandin receptor types (including DP_1, DP_2, EP and TP receptors), there has been a paucity of well-characterized, selective FP receptor antagonists. The earliest ones included dimethyl amide and dimethyl amine derivatives of $PGF_{2\alpha}$, but these have failed to gain prominence. The fluorinated $PGF_{2\alpha}$ analogues, AL-8810 and AL-3138, were subsequently discovered as competitive and non-competitive FP receptor antagonists respectively. Non-prostanoid structures, such as the thiazolidinone AS604872, the D-amino acid-based oligopeptide PDC31 and its peptidomimic analogue PDC113.824 came next, but the latter two are allosteric inhibitors of FP receptor signalling. AL-8810 has a sub-micromolar *in vitro* potency and ≥2 log unit selectivity against most other PG receptors when tested in several cell- and tissue-based functional assays. Additionally, AL-8810 has demonstrated therapeutic efficacy as an FP receptor antagonist in animal models of stroke, traumatic brain injury, multiple sclerosis, allodynia and endometriosis. Consequently, it appears that AL-8810 has become the FP receptor antagonist of choice.

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Abbreviations

$[Ca^{2+}]_i$, intracellular Ca^{2+}; BK, bradykinin; CSD, cortical spreading depression; ECM, extracellular matrix; IOP, intraocular pressure; IPs, inositol phosphates; KA, kainic acid; K_i, equilibrium inhibition constant; OHT, ocular hypertension; PGA, PG agonist; POAG, primary open angle glaucoma

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Introduction

It is now well established that arachidonic acid (**AA**) formed by the action of phospholipase A_2 on phospholipids is a major substrate for future conversion by lipoxygenase to create leukotrienes and for **COX-1** and **COX-2** to form PGs *via* **PGG_2** and **PGH_2** (Corey and Snider, 1974; Coleman *et al.,* 1994). The five distinct bioactive lipids created by COX-1 and COX-2 include **PGD_2**, **PGE_2**, **$PGF_{2\alpha}$**, **PGI_2** and **TXA_2** (Coleman *et al.,* 1994). The biological actions of these cellular eicosanoids are mediated by separate **prostanoid receptors** whose names originate from these PGs and are known as DP, EP, **FP**, **IP** and **TP** receptors respectively (Coleman *et al.,* 1994; Alexander *et al.,* 2017a). Some of these major receptors also have sub-types such as **EP_1**, **EP_2**, **EP_3** and **EP_4** receptors. The heptahelical GPCRs associated with these PGs are embedded in the plasma membranes of the majority of the mammalian cells and transduce either an elevation (**DP_1**, EP_2, EP_4 and IP receptors) or reduction (EP_3-receptors) (Narumiya *et al.,* 1999) of cAMP. Activation of EP_1, FP and TXA_2 receptors by their cognate ligands results in the production of intracellular inositol phosphates (IPs) that in turn raise intracellular Ca^{2+} ($[Ca^{2+}]_i$) by releasing it from the endoplasmic reticulum of the cells (Narumiya *et al.,* 1999). These changes in $[Ca^{2+}]_i$ and cAMP then evoke downstream signal transduction culminating in the final biological activity of the eicosanoid (e.g. enzyme or hormone release, muscle contraction or relaxation) (Coleman *et al.,* 1994).

Progress in the PG field accelerated when organic chemists began synthesizing not only the endogenous PGs but also analogues and derivatives with arbitrary structural modifications, all in quantities sufficient for biological testing (Corey and Snider, 1974). Additionally, widespread adoption of new physico-chemical characterization techniques, such as nuclear magnetic resonance spectroscopy and mass spectrometry, enabled researchers to vouchsafe the compositions of what they were testing and to understand the metabolism of the PGs. Additional impetus for new discoveries in the PG arena was provided by the establishment of homogeneous cell cultures with expression of a single or multiple PG receptor sub-type(s), with analytical technologies measuring well-defined functional read-outs such as second messengers (e.g. Griffin *et al.,* 1997; 1998; 1999; Crider *et al.,* 1998; 2000; Crider and Sharif, 2001). These isolated primary cells from normal or diseased animal and human tissues ensured a degree of fidelity and replication of normal physiological and pathological states *in vitro* respectively. The addition of host cells engineered to express animal or human cloned receptors, which integrated with intracellular signalling machinery, allowed high throughput screening (e.g. Rocha *et al.,* 2016) to be undertaken to facilitate rapid discovery of agents able to activate or inhibit PG receptor-induced signalling. This technology has been further refined to permit screening and quantification of other phenotypic changes observable in living cells in culture (e.g. cell contraction) using high-content screening tools (Hu *et al.,* 2017). These and other techniques and assays (e.g. Maddox *et al.,* 1978; Stinger *et al.,* 1982; Crider *et al.,* 1998; Griffin *et al.,* 1999) allowed the discovery of suitable receptor-selective agonist and antagonists of PG receptors and their subtypes (Coleman *et al.,* 1994; Delaey and Van de Voorde, 1995; Narumiya *et al.,* 1999; Griffin *et al.,* 1999; Sharif *et al.,* 2000a,b; Alexander *et al.,* 2017a).

The first reported FP receptor antagonists

Two general properties are desired for a candidate antagonist useful for probing the role of receptor function in a biological system. The first is that the antagonist should exhibit a relatively high affinity and functional potency (IC_{50}s/K_is at least in low μM range; preferably nM). The second characteristic is at least 1-log-unit functional potency selectivity against other related PG receptors such that non-specific effects at other PG receptors or other off-targets can be avoided. This is very important because most of the endogenous PGs cross-react with each other's receptors; for example, $PGF_{2\alpha}$ has been reported as having more potent binding at the PG EP_3 receptor sub-type versus its cognate FP receptor (Sharif *et al.,* 2003a,b,c,d).

The first purported FP receptor antagonists were the N,N-dimethylamine and -amide derivatives of $PGF_{2\alpha}$, in which the C-1 carboxylic acid has been modified to a non-acidic function (see Table 1). These compounds were reported as competitive antagonists of the FP receptor that apparently blocked $PGF_{2\alpha}$-induced lobar arterial pressure increase in a canine *ex vivo* lung preparation, with IC_{50} values in the low micromolar range (Fitzpatrick *et al.,* 1978). The same group then reported on antagonism of the $PGF_{2\alpha}$-induced contraction in the gerbil isolated colon *ex vivo* (Maddox *et al.,* 1978) and rat pulmonary and systemic arterial pressure increase *in vivo* (Stinger *et al.,* 1982). It should be noted that the investigators included members from Professor E.J. Corey's laboratory, who had been a key player in advancing synthetic methodology and reporting *de novo* synthesis of endogenous PGs and a number of analogues (Corey and Snider, 1974). This highlights the key role that synthetic discoveries played in advancing molecular level understanding of PG pharmacology. Although these N,N-dimethylamine and -amide derivatives of $PGF_{2\alpha}$ appeared to show promise as potential FP receptor antagonist tools, only a few reports of the use of either compound have appeared in the non-patent literature (Arnould *et al.,* 2001). A contributing factor may include conflicting reports of no measureable $PGF_{2\alpha}$ – competitive binding (Anderson *et al.,* 1999) or functional antagonism (Sharif *et al.,* 2000a) at the FP receptor.

The discovery of AL-3138 and AL-8810 as FP receptor antagonists

Serendipity often results in new discoveries, but due vigilance and fast action on novel observations are also vital components. At the time our work in the PG arena began, we were primarily interested in finding new and improved FP-class PG agonists for the treatment of ocular hypertension (OHT) and primary open angle glaucoma (POAG; Weinreb and Khaw, 2004). One particular relatively selective FP-class PG analogue (FP-PGA) agonist of $PGF_{2\alpha}$, **latanoprost isopropyyl ester** (Xalatan), had been discovered and

Table 1

Some key FP receptor agonists and purported and *bona fide* FP receptor antagonists

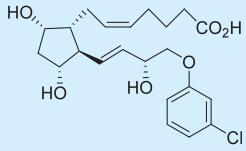
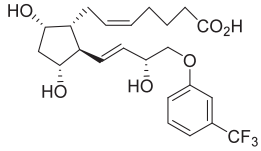
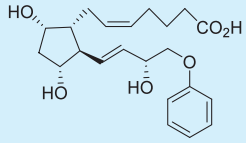
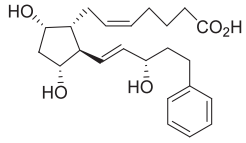
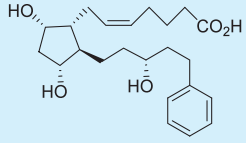
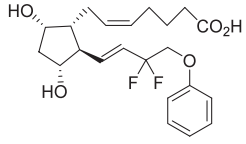
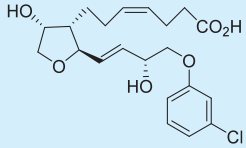
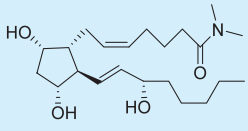
FP-R agonist name	Structure
Cloprostenol	
Fluprostenol (travoprost acid)	
16-Phenoxy-ω-tetranor-$PGF_{2\alpha}$	
17-Phenyl-ω-trinor-$PGF_{2\alpha}$ (bimatoprost acid)	
13,14-Dihydro-17-phenyl-ω-trinor-$PGF_{2\alpha}$ (latanoprost-free acid) (PhXA85)	
AFP-172 (tafluprost acid)	
AL-12182 acid (AL-12180)	
FP receptor antagonists name	**Structure**
$PGF_{2\alpha}$ dimethylamide	

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Table 1

(Continued)

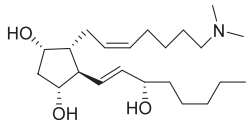
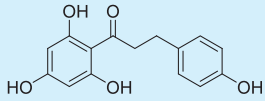
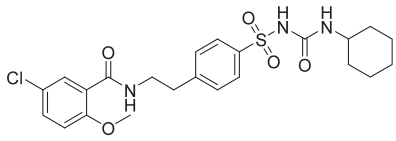
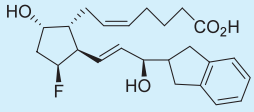
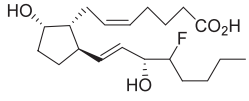
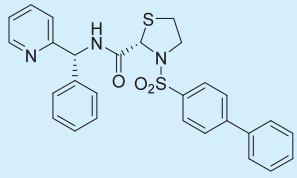
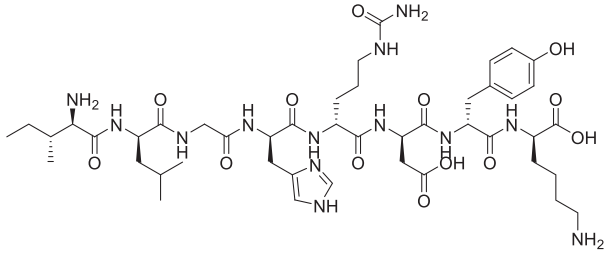
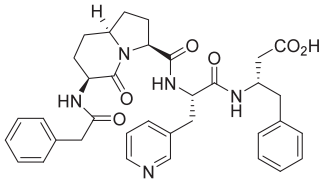
FP receptor antagonists name	Structure
$PGF_{2\alpha}$ dimethylamine	
Phloretin	
Glibenclamide	
AL-8810	
AL-3138	
AS604872	
THG-113.31; apparently equiv. to PDC31; a D-oligopeptide of sequence D-isoleucyl-D-leucylglycyl-D-histidyl-N5-(aminocarbonyl)-D-ornithyl-D-aspartyl-D-tyrosyl-D-lysine	
PDC113.824	

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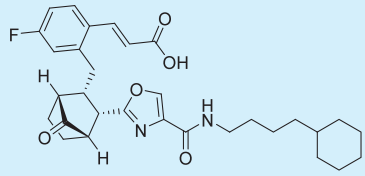
Table 1

(Continued)

FP receptor antagonists name	Structure
AGN 211377	

reported on (Stjernschantz, 2001). Our medicinal chemists at Alcon (Discovery Research) had been synthesizing numerous analogues of $PGF_{2\alpha}$ for the same purpose, and these had been screened in a battery of PGreceptor radioligand binding assays (Sharif and Davis, 2002; Sharif and Xu, 2004; Sharif *et al.,* 1998, 1999; 2000b, 2002a,b, 2003a), cell-based functional assays (with second messenger readouts; e.g. Crider *et al.,* 1998, 2000; Crider and Sharif, 2001; Griffin *et al.,* 1997, 1998, 1999; Kelly *et al.,* 2003; Sharif *et al.,* 1998, 1999, 2000a,b, 2001, 2002a,b,c,d, 2003a,b,c, 2004), rat uterine (Sharif, 2008) and in feline iris sphincter muscle (Sharif *et al.,* 2008) contraction assays. During such screening of compounds in mouse Swiss 3T3 fibroblasts (Griffin *et al.,* 1997) and in rat aortic smooth muscle cells (A7r5; Griffin *et al.,* 1998) in particular, it was noticed that unlike many reference FP-PG agonists (e.g. **cloprostenol** and **fluprostenol**) that produced full functional responses akin to those of $PGF_{2\alpha}$, two newly synthesized analogues of $PGF_{2\alpha}$, AL-3138 ((Z)-7-((1R,2R,5S)-2-((3R,E)-4-fluoro-3-hydroxyoct-1-en-1-yl)-5-hydroxycyclopentyl) hept-5-enoic acid) and **AL-8810** ((Z)-7-((1R,2R,3S,5S)-2-((R,E)-3-(2,3-dihydro-1H-inden-2-yl)-3-hydroxyprop-1-en-1-yl)-3-fluoro-5-hydroxycyclopentyl)hept-5-enoic acid; Table 1) exhibited a relatively low *in vitro* intrinsic activity (efficacy, E_{max}; only 19% E_{max} for AL-8810; E_{max} of 33–37% for AL-3138) (Griffin *et al.,* 1999; Sharif *et al.,* 2000a; Figures 1A). Since these compounds did not meet the selection criteria and desired pharmacological characteristics for our OHT/POAG programme, they were removed from the screening funnels. However, having established a multitude of receptor binding assays and functional screening assays for various PG receptors and some of their sub-types for our drug discovery research programmes (Griffin *et al.,* 1997, 1998; Crider *et al.,* 1998, 2000; Sharif *et al.,* 1998, 1999, 2000a,b, 2001, 2002a,b,c,d, 2003a,b,c; Crider and Sharif, 2001; Kelly *et al.,* 2003), the unavailability of suitable FP receptor antagonists was recalled. It was at that point that the recently ascertained pharmacological properties of AL-8810 (Griffin *et al.,* 1999; Sharif *et al.,* 2000a) and AL-3138 (Sharif *et al.,* 2000a) became very important, since pharmacology had taught us that very low-efficacy partial agonists could behave as 'antagonists' under certain experimental conditions (Stephenson, 1956; Kenakin, 1997). These compounds were quickly tested as potential antagonists. [^{3}H]-myo-inositol-loaded A7r5 cells were pretreated with different concentrations of AL-8810 or AL-3138 for 15 min and then exposed to a sub-maximal concentration of a selective FP receptor full agonist, fluprostenol (100 nM), and the total water-soluble [^{3}H]-IPs allowed to accumulate in the extracellular medium over an hour as a result of FP receptor activation. The total [^{3}H]-IPs were isolated by ion-exchange chromatography and quantified by β-scintillation spectroscopy (Griffin *et al.,* 1997, 1998; Sharif *et al.,* 1998). It became obvious that both AL-3138 and AL-8810 antagonized the effects of fluprostenol in a concentration-dependent manner (Figure 1B; Griffin *et al.,* 1999; Sharif *et al.,* 2000a). These experiments were repeated several times, and a number of other purported FP receptor blockers were tested simultaneously. Additional experiments included testing AL-3138 and AL-8810 against selective agonists of other PG receptors and non-PG receptors in order to determine their relative selectivities for the FP receptor. Both compounds were found to be relatively selective for the FP receptor (Sharif *et al.,* 2000a, 2003a; Table 2). More detailed pharmacological studies involving the use of Schild-analysis (Arunlakshana and Schild, 1959) were performed, and it was determined that while AL-3138 appeared to have non-competitive antagonist properties (Sharif *et al.,* 2000a), AL-8810 was a competitive antagonist (Griffin *et al.,* 1999; Sharif *et al.,* 2000a, 2003a) being able to produce a rightward-shift of the concentration–response curves of FP receptor agonists without diminishing the maximal effect of the agonists (Arunlakshana and Schild, 1959; Figure 1C, D). Since AL-8810 exhibited a lower efficacy as a partial agonist compared to AL-3138 and was also a competitive antagonist, it was subsequently out-licensed to Sigma/Research Biochemicals Inc. and Cayman Chemicals in order that other researchers could verify our findings and start using AL-8810 as a pharmacological tool. Consequently, there have been over 70 reports published (per Embase/Medline/PubMed searches) in which our original observations of FP receptor antagonist properties of AL-8810 were confirmed in a variety of *in vitro* and *ex vivo* assay systems. Furthermore, the uses of AL-8810 have been expanded to show antagonist behaviour in animal models of various diseases and to investigate other physiological systems involving endogenous PGs or exogenously administered FP receptor agonists. Some selected key applications of AL-8810 in biomedical research will now be discussed below.

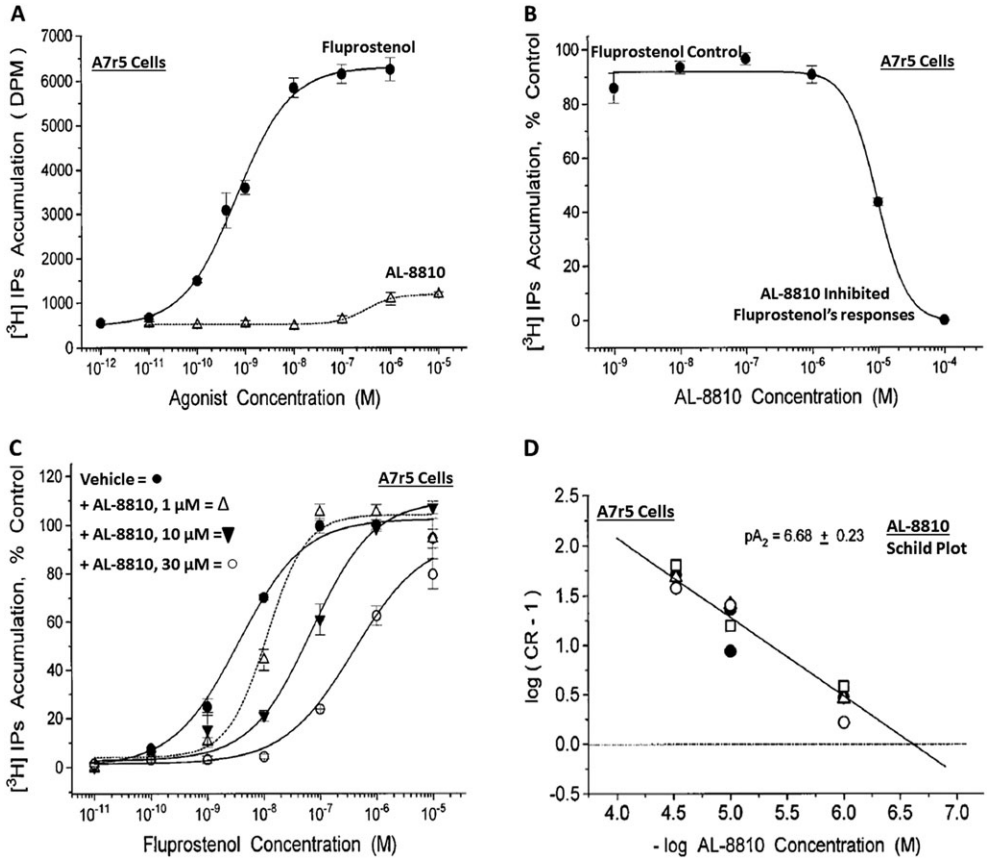


Figure 1

Pharmacological characterization of AL-8810 in rat aortic smooth muscle cells (A7r5). (A) depicts the ability of AL-8810 to stimulate the production of [^{3}H]-IPs as an agonist compared to the full FP receptor PG agonist, fluprostenol. It is clear that AL-8810 is a very weak low intrinsic activity partial agonist relative to fluprostenol. (B) Shows how increasing concentrations of AL-8810 are able to antagonize the agonist activity of fluprostenol. (C) Depicts the ability of AL-8810 (1–30 μM) to rightward-shift the concentration–response curves of the agonist PG, fluprostenol, in a concentration-dependent manner, without reducing the overall maximal agonist effects of fluprostenol (Schild analysis). (D) Illustrates a Schild plot of the transformed data from (C). Here, the cumulative pA_2 values obtained are shown (all modified from Griffin *et al.,* 1999).

Table 2

FP receptor antagonist and purported antagonist, affinity and/or functional potencies from various assay systems

	Antagonist receptor binding affinity or functional potency parameters at prostanoid receptors (IC_{50} or K_i or K_b, nM)							
Compound	**DP**	**EP_1**	**EP_2**	**EP_3**	**EP_4**	**FP**	**IP**	**TP**
AL-8810	>30 000	nd	>30 000	nd	>30 000	285–426 (competitive)	>100 000	>100 000
AL-3138	>100 000	nd	>100 000	nd	>100 000	86;182–296 (non-competitive)	>10 000	nd
AS604872	>10 000	>10 000	650	>10 000	>10 000	35–323 (undefined)	>10 000	>10 000
AGN-211377	49 ± 16	266 ± 67	nd	5008 ± 2571	117 ± 29	61 ± 16 (apparently non-competitive)	nd	11 ± 6
Phloretin	~4250	nd	~4229	nd	nd	1400–5248 (non-competitive)	2119	3383
THG-113.31 (PDC31)	–	–	–	–	–	~30 (non-competitive)	–	–

AL-8810 antagonizes *in vitro* ocular actions of FP receptor agonists

Ocular hypertension (OHT) and primary open angle glaucoma (POAG) adversely affect vision in millions of patients making POAG the most prevalent form of glaucoma and the second leading cause of blindness worldwide (Weinreb and Khaw, 2004; Tham *et al.,* 2014). OHT is caused by the accumulation of excess aqueous humour (AQH) within the anterior chamber of the eye due to poor or incomplete drainage from the trabecular meshwork (TM) into the Schlemm's canal and into the episcleral veinous circulation (Ritch, 2014; Weinreb *et al.,* 2016). A number of FP receptor agonist prodrugs including isopropyl esters of $PGF_{2\alpha}$, **latanoprost-free acid** (Latanoprost; Xalatan®) (Stjernschantz, 2001), (+)-fluprostenol (**travoprost**; Travatan®) (Hellberg *et al.,* 2002), **tafluprost** (Taflutan®; Takagi *et al.,* 2004) and the amide of **bimatoprost-free acid** (**bimatoprost**; Lumigan®) (Woodward *et al.,* 2011) (see Table 1) lower intraocular pressure (IOP) in ocular hypertensive monkeys and humans (Weinreb *et al.,* 2016; Sharif, 2017). The PG-like **unoprostone isopropyl ester** has also been introduced into clinical practice as an ocular hypotensive (Toris *et al.,* 2004), but it has a low potency and is a medium-level partial agonist at the FP receptor (Sharif *et al.,* 2003a,b,c,d). The major effect of these drugs is mediated *via* the FP receptors located in the ciliary muscle and scleral tissues (Sharif *et al.,* 2003a,b,c,d; Husain *et al.,* 2005) where extracellular matrix (ECM) is digested, thereby enhancing the drainage of AQH *via* the uveoscleral pathway and thus lowering IOP (Weinreb *et al.,* 2002; Weinreb and Lindsey, 2002). However, since functionally active FP receptors are located on human TM (h-TM) cells (Sharif *et al.,* 2003c), it has been shown that FP receptor PGAs also promote some TM outflow of the AQH (Toris *et al.,* 2004; Lim *et al.,* 2008). Due to their extraordinary efficacy at lowering and controlling IOP, FP receptor PGAs are now first-line therapeutics used clinically to treat OHT and POAG (Weinreb *et al.,* 2016; Sharif, 2017, 2018).

Even though it was shown that bimatoprost gets hydrolysed to its free acid *in vitro* in the presence of ocular tissue enzymes (Maxey *et al.,* 2002; Sharif *et al.,* 2002c; Davies *et al.,* 2003; Hellberg *et al.,* 2003) and also when topical ocularly applied to cause IOP lowering in animals and in humans (Camras *et al.,* 2004, 2008; Faulkner *et al.,* 2010), one research group has invoked an enigmatic '**prostamide** receptor' to explain the mechanism of action of this amide compound (Woodward *et al.,* 2001; Sharif and Klimko, 2009). Thus, it was considered important to use AL-8810 and check whether it would interact with the FP receptor at the binding site level and then whether it would block the accumulation of [^{3}H]-IPs and $[Ca^{2+}]_i$ mobilization responses induced by bimatoprost. It was shown that indeed bimatoprost (and many other FP receptor agonists) competed for specific **[^{3}H]-$PGF_{2\alpha}$** and [^{3}H]-AL-5848 (travoprost acid) binding (Sharif *et al.,* 1998, 1999) to FP receptors in homogenates of bovine corpus luteum (Sharif *et al.,* 2001, 2003a,b,c,d). Bimatoprost, along with several other FP receptor agonists, was then shown to stimulate $[Ca^{2+}]_i$ mobilization and activate MAPK *via* the FP receptor located on a variety of cell types expressing either endogenous FP receptors [human ciliary muscle (h-CM; Sharif *et al.,* 2003b), h-TM cells (Sharif *et al.,* 2003c), A7r5 rat aortic smooth muscle cells (Kelly *et al.,* 2003) and Swiss 3T3 fibroblasts (Sharif *et al.,* 2001; Kelly and Sharif, 2003)] or HEK-host cells expressing the human cloned ciliary body FP receptor (Kunapuli *et al.,* 1997; Sharif *et al.,* 2003c,d). The inhibition of these various functional responses induced by bimatoprost and the other FP receptor agonists by AL-8810, in an apparent agonist-independent manner, confirmed that all these FP-class PGs were binding to and activating the same classical FP receptor (Sharif *et al.,* 2002a,b,c,d, 2003a,b,c,d). Thus, for instance, AL-8810 exhibited a similar apparent FP agonist-independent antagonist potency against the ocular FP receptor cloned from a human ciliary body library (Sharif *et al.,* 2003c,d). The pooled antagonist potencies of AL-8810 were: mouse 3T3 cell K_i 0.2 ± 0.06 μM; rat A7r5 cell K_i = 0.4 ± 0.1 μM; human cloned ciliary body-derived FP receptor K_i = 1.9 ± 0.3 M; h-TM cell K_i = 2.6 ± 0.5 μM; h-CM cell K_i = 5.7 μM; using a variety of FP agonists including fluprostenol, travoprost acid, unoprostone (free acid), bimatoprost and bimatoprost-free acid (Figure 2).

As mentioned above, it is now well accepted that the FP class of PGs exert their ocular hypotensive activity by releasing a variety of MMPs (mostly **MMP-1** (Hinz *et al.,* 2005) and **MMP-3**) that digest ECM within ciliary muscle bundles and create an enlarged uveoscleral pathway for the AQH to drain from the anterior chamber of the eye (Weinreb and Lindsey, 2002). This process is usually a slow and protracted one since the synthesis and secretion of the MMPs takes several hours (Weinreb and Lindsey, 2002). In a study to unravel the acute cellular and molecular mechanism of action of FP-PGAs in h-CM cells, Husain *et al.* (2005) showed that AL-8810 was able to block the $PGF_{2\alpha}$-mediated secretion of **MMP-2**. These data strongly suggested that MMP-2 probably mediates the early phase of IOP lowering *via* a PKC- and ERK-dependent mechanism, while other MMPs (e.g. MMP-3) are responsible for the long-term ocular hypotensive activity of FP receptor agonists. In a similar vein, it was shown that AL-8810 concentration-dependently inhibited $[Ca^{2+}]_i$ mobilization and also blocked MAPK activity stimulated by a variety of FP receptor PGAs (including bimatoprost and its free acid) in h-CM cells (Sharif *et al.,* 2003b). Furthermore, Romano and Lograno (2007), showed that in isolated human CM segments, the free acids of travoprost and latanoprost, as well as the intact amide compound (bimatoprost), evoked strong contractile activity. AL-8810 competitively antagonized the contractions induced by all these PGs (Romano and Lograno, 2007), indicating once again that bimatoprost's actions are indistinguishable from other *bona fide* FP receptor PGAs that activate the FP receptor either as intact esters or amides or by the more active moieties produced after their hydrolysis to the free acid species. To further elaborate such findings, it was shown that, as expected, a phospholipase C inhibitor prevented contractions induced by all the latter compounds (Romano and Lograno, 2007) as well, indicating that these agents share the same downstream signal transduction mechanism known to be associated with FP receptors (Narumiya *et al.,* 1999; Sharif *et al.,* 2003a,b,c,d). These results further helped explain the molecular mechanisms of action of FP receptor agonists in key cell types (CM and TM) heavily involved in AQH dynamics and ultimately in reducing IOP *in vivo*.

One possible pathological event leading to OHT/POAG, and perhaps other forms of glaucoma, is the slow but progressive

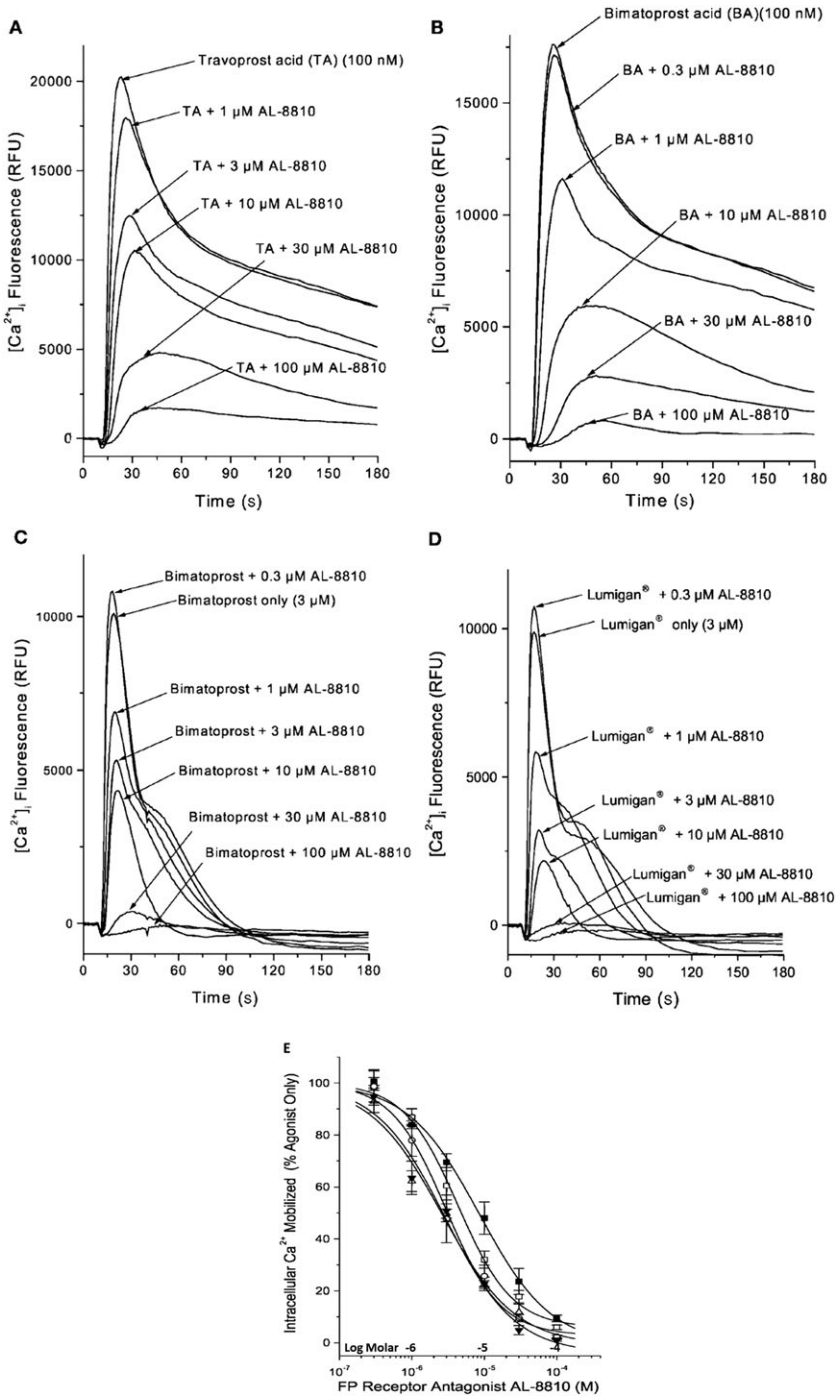


Figure 2

The ability of AL-8810 to antagonize the signal transduction process in rat aortic smooth muscle A7r5 cells mediated by various FP receptor PGAs is shown. (A) and (B) Illustrate the $[Ca^{2+}]_i$ mobilization induced by free acids of travoprost (TA) and bimatoprost (BA) in the absence and presence of increasing concentrations of the FP receptor antagonist, AL-8810. Note the reduction in $[Ca^{2+}]_i$ mobilization evoked by the agonists in the presence of AL-8810. (C) and (D) Show how AL-8810 also antagonized $[Ca^{2+}]_i$ mobilization induced by the commercially available bimatoprost (amide) and the clinically used version of bimatoprost (Lumigan®). (E) The cumulative concentration–response curves showing the inhibitory effects of AL-8810 on FP receptor PG agonists-stimulated generation of $[Ca^{2+}]_i$ (all adapted and modified from Sharif *et al.,* 2001, 2002d, 2003a,d).

ill-health and subsequent loss of phagocytic/autophagic activity of TM cells located within the AQH drainage pathway of the anterior chamber of the eye (Weinreb *et al.*, 2014). Oxidative stress caused by local ischaemia/hypoxia leading to a decline in ATP, an energy source, in the TM cells, and in other important cell types within the visual axis [e.g. retinal ganglion cells (RGCs)], could be responsible for causing the eventual demise of TM and RGCs (Thomas *et al.,* 2000;

Weinreb *et al.*, 2014; Sharif, 2017, 2018). Thus, it was of some significance that Yu *et al.* (2009) demonstrated a protective effect of various FP receptor PGAs in isolated human TM cells subjected to oxidative stress *via* exposure to hydrogen peroxide. Importantly, they showed that AL-8810 and two other purported FP receptor antagonists ($PGF_{2\alpha}$ dimethyl amine and $PGF_{2\alpha}$ dimethyl amide) reversed such protection afforded by travoprost, latanoprost and bimatoprost (Yu *et al.*, 2009), thereby strongly supporting the involvement of FP receptors in mediating these effects of FP-PGAs (Sharif *et al.*, 2003c). Once again, these data underscored the probability that bimatoprost behaves just like other FP receptor agonists. These *in vitro* data were further supported by the observations that whilst all the FP receptor PGA prodrugs tested (e.g. travoprost and bimatoprost) reduced IOP in wild-type mice, this ocular hypotensive activity was absent in mice where the FP receptor had been genetically deleted (Crowston *et al.*, 2005; Ota *et al.*, 2005).

Functionally active FP receptors were identified and pharmacologically characterized in h-TM cells using a variety of FP receptor agonists (Sharif *et al.*, 2003c). Significantly, it was demonstrated that AL-8810 concentration-dependently antagonized the actions of these PG agonists, including those of bimatoprost and its free acid (Sharif *et al.*, 2003c). Additional studies revealed that both free acids of latanoprost and travoprost increased h-TM cell membrane potential which could also be blocked by AL-8810 (Cuppoletti *et al.*, 2007). Likewise, other researchers showed that cultured h-TM cells respond to $PGF_{2\alpha}$ and fluprostenol by suppressing endothelin-induced $[Ca^{2+}]_i$ mobilization and contraction (Thieme *et al.*, 2006), activities that were sensitive to the FP receptor antagonists, AL-8810 and $PGF_{2\alpha}$ dimethyl amide. The overall significance of the latter studies extended to the hypothesis that perhaps one way FP receptor PGAs exert their therapeutic effects in OHT/POAG is by suppressing the deleterious actions of locally produced endothelin, a peptide that is elevated in glaucomatous situations in animal models of OHT and in OHT/POAG patients (Choritz *et al.*, 2012).

A subpopulation of myofibroblastic cells in TM impart important contractile property that is important for cell volume changes (Dismuke *et al.*, 2009), the release of local MMPs (Yang *et al.*, 2016) and for affecting closely associated Schlemm's canal cells, which enhance conventional outflow of AQH to reduce IOP. In studying the cellular actions of FP receptor and EP_2 receptor agonists in h-TM cells, Kalouche *et al.* (2016) showed that latanoprost-free acid prevented collagen accumulation and contracted TM cells, both processes being antagonized by AL-8810. In contrast, the EP_2 receptor agonist, butaprost, relaxed TM cells and reduced collagen deposition induced by **TGFβ2**. These actions of latanoprost-free acid in h-TM cells appeared to involve the induction of the accumulation of calcipressin, a regulator of calcineurin, by utilization of both intracellular and extracellular Ca^{2+}, an action that was also strongly blocked by AL-8810 (Fautsch *et al.*, 2011).

The immediate-early gene **Nur77** (also called **NGFI-B**, TR3 or *NR4A1*) encodes an orphan nuclear receptor that is up-regulated by growth factors and which is involved in cell differentiation and proliferation and perhaps in cellular protection. $PGF_{2\alpha}$ strongly induced Nur77 expression in h-CM and h-TM cells, indicating that endogenous PGs acting on the FP receptor impart a beneficial effect to maintain the health of these cell types. The up-regulation of Nur77 induced by $PGF_{2\alpha}$ in these cells was effectively inhibited by AL-8810 (Liang *et al.*, 2004).

Pathological neovascularization underlies the action of several types of prostanoids in causing ocular diseases. Indeed, it has been shown that $PGF_{2\alpha}$ is elevated in retinal Muller glial cells and microvascular cells subjected to disease-relevant stimuli such as ischaemia/hypoxia as in oxygen-induced retinopathy of prematurity (Barnett *et al.*, 2010; Hu *et al.*, 2017). Accordingly, Savage *et al.* (2011) showed that latanoprost induced a significant elevation in the secretion of **VEGF** from isolated Muller cells and increased proliferation of human retinal microvascular endothelial cells that could be blocked by AL-8810. These data suggested that FP receptor antagonists may have clinical utility as anti-angiogenic agents, but such findings need additional corroboration and extension.

Numerous PGs modulate contractility of smooth muscle of various ocular tissues (CM, TM and iris sphincter muscle), blood vessels, pulmonary tissues and mammalian uterus (Woodward *et al.*, 2011). AL-8810 has proven useful in delineating the PG receptors involved in these effects of PGs. Thus, latanoprost, travoprost and AL-12182-free acid (**AL-12180**; Table 1) potently contracted isolated porcine ciliary arteries, and these effects were effectively antagonized by AL-8810 (Sharif *et al.*, 2006; Vysniauskiene *et al.*, 2006). Holmgaard and Bek (2010) demonstrated a similar FP receptor inhibition of $PGF_{2\alpha}$-induced contraction by AL-8810 in porcine retinal arterioles mounted in organ baths. In a contrasting study, hypoxia-induced relaxation of the same porcine tissues was shown to be mediated by nitric oxide and EP_4 receptors (but not FP receptors) since **GW627368** (an EP_4 antagonist) blocked the contraction but AL-8810 did not (Hansen *et al.*, 2015).

Non-ocular *in vitro* utility of the FP receptor antagonist, AL-8810

The involvement of PGs in cardiac tissue and coronary arteries function/dysfunction is well documented (Zhang *et al.*, 2010). In an additional study, Zhang *et al.* (2017) recently tried to define the PG receptors involved in the secretion of atrial natriuretic peptide (Bai *et al.*, 2009) and the spontaneous contraction of this cardiac tissue in isolated perfused rat atria. Since both these activities were blocked by perfusion of **AH-6809** (a DP receptor antagonist; Coleman *et al.*, 1994) and AL-8810 into the atria, they concluded that endogenously released PGD_2 and $PGF_{2\alpha}$ were most likely involved. Likewise, since cardiac fibrosis is characterized by collagen I and III deposition in the myocardium, Ding *et al.* (2012) recently demonstrated that AL-8810 could prevent this feature of $PGF_{2\alpha}$ and thus suggested a potential clinical utility of AL-8810 in preventing cardiac fibrosis. This was important since the fibrosis induced by activation of the FP receptor was independent of the fibrotic cytokine, **TGFβ1** (Ding *et al.*, 2012). Moreover, Hara *et al.* (2009) showed that the muscarinic receptor-mediated positive ionotropic response of mouse isolated left atrium was actually mediated by

endogenously released PGs, including $PGF_{2\alpha}$, since amongst other PG receptor antagonists, AL-8810 significantly blunted the response to carbachol. In another related study, it was shown that while $PGF_{2\alpha}$ significantly decreased the chronotropic response to cholinergic stimulation of isolated atria from endotoxin-treated rats (Nikoui *et al.*, 2015), AL-8810 or **indomethacin** was able to counteract and reverse this phenomenon.

As mentioned above, a variety of PGs influence venous and arterial blood vessel contractility and/or relaxation in the ocular system. In endotoxin-treated porcine coronary arteries, the **bradykinin (BK) B_1 receptor** agonist, **des-Arg^9-BK**, caused endothelium-independent contractions of the tissue that were inhibited by COX-2 inhibitors and a TP receptor antagonist but neither by a COX-1 inhibitor (**aspirin**) nor by DP/EP (AH-6809)-, FP (AL-8810)- and IP (**RO1138452**) receptor antagonists (More *et al.*, 2014a,b). However, since AL-8810 potently and competitively antagonized the contractile effects of free acids of latanoprost, bimatoprost and $PGF_{2\alpha}$ in isolated rings of human umbilical vein, the involvement of FP receptors in causing these responses was confirmed (Errasti *et al.*, 2009). Likewise, AL-8810 proved useful in ascribing an FP receptor-mediated vasoconstrictor effect of endogenously released $PGF_{2\alpha}$ in freshly regenerated rat femoral arteries in a rat model of atherosclerosis (Hirao *et al.*, 2008).

The pulmonary system is also a target of endogenous PGs under normal and disease conditions. Airways smooth muscle of various species is contracted by growth factors such as EGF, platelet-derived growth factor and insulin (Schaafsma *et al.*, 2005, 2007), isoprostanes (Paredes *et al.*, 2007), thromboxanes (Hernandez and Janssen, 2011) and PGE_2 (Säfholm *et al.*, 2015). In the bovine trachealis, it appears that E-ring isoprostanes augment the cholinergic neurotransmission *via* an FP receptor-mediated mechanism since AL-8810 significantly inhibited the response evoked by 15-E_{2t}–isoprostane (Paredes *et al.*, 2007). Similarly, since guinea pig airway smooth muscle contractions induced by insulin were abolished by AL-8810, the authors concluded that endogenously released $PGF_{2\alpha}$ plays a major role in airways constriction (Schaafsma *et al.*, 2007). However, in the other aforementioned studies (Hernandez and Janssen, 2011; Säfholm *et al.*, 2015), AL-8810 proved helpful in ruling out the involvement of FP receptors.

Due to the ubiquity of endogenous PGs in the mammalian body, these agents have numerous other functions that can be classified as physiological or pathological depending on the nature of the tissue and the prevailing circumstances. In many instances, it is either PGE_2 and/or $PGF_{2\alpha}$ that are the benefactors or culprits. Isolated uterus strips from mice responded strongly to exogenously added $PGF_{2\alpha}$ and 17-phenyl-$PGF_{2\alpha}$ (bimatoprost-free acid) (Hutchinson *et al.*, 2003). The contractile activity of both FP receptor agonists was competitively antagonized by AL-8810 since it produced rightward shifts of the concentration–response curves to both agonists (Hutchinson *et al.*, 2003). Basal tone recordings of rat uterus and full contractions of this tissue can also be achieved using a variety of FP receptor agonists (e.g. $PGF_{2\alpha}$, latanoprost-free acid and cloprostenol) (Sharif, 2008). The rat uterus appeared to be uniquely sensitive to these PGs, since fine gradations of both potency and efficacy of FP receptor agonists was observed and their agonist actions fully antagonized by AL-8810 (Sharif, 2008).

In concordance with the heterogeneous distribution of various PG receptors in the gastrointestinal tract, PGE_2, $PGF_{2\alpha}$ and TXA_2 enhance chloride secretion in human and mouse colon *in vitro* (see Collins *et al.*, 2009). However, while PGD_2 stimulates chloride release in the guinea pig, it inhibits this function in the rat colon. $PGF_{2\alpha}$ also stimulates chloride secretion in porcine small intestine and increases rat, guinea pig and human colonic smooth muscle contractility (see Collins *et al.*, 2009). The effects of $PGF_{2\alpha}$ on chloride secretion were mediated in a cAMP-dependent fashion in isolated human colon, and AL-8810 effectively antagonized these effects (IC_{50} = 190 nM) (Collins *et al.*, 2009). Since $PGF_{2\alpha}$ concentration is raised in Crohn's disease, and FP receptor levels are strikingly elevated in colorectal carcinoma tumors (see Collins *et al.*, 2009), the pharmacological and clinical utility of FP receptor antagonists to combat or down-regulate these intestinal disorders appears warranted.

The discovery of the phenomenon of receptor dimerization and crosstalk between heterologous receptors has opened potential new avenues for novel drug discovery and treatment of diseases (Rozenfeld and Devi, 2010). Such allosteric functional interactions amongst FP receptors and **angiotensin-II** (AT) receptors (**AT_1 sub-type**) by formation of receptor dimers have been recently described (Goupil *et al.*, 2013, 2015). Here, AL-8810 (FP receptor antagonist) and **AS604872** (allosteric inhibitor; see above) produced trans-inhibition of IPs production in A7r5 cells and antagonized mouse aortic contraction induced by either angiontensin II or $PGF_{2\alpha}$ (Goupil *et al.*, 2015). Thus, AL-8810 was able to apparently enter the FP receptor binding pocket to block the activity of the FP receptor and the co-joined AT_1 receptor within the heterodimer (Sleno *et al.*, 2017).

Utility and therapeutic effects of AL-8810 in animal models of disease

As is often the case, *in vitro* observations of compound activity is not always replicated in animal models *in vivo*. This is not necessarily unexpected since compounds can be easily pronounced inactive or non-efficacious due to limitations of adequately delivering the agents to the target tissues/cells at an effective concentration. Such problems are further exacerbated by potential rapid enzymatic degradation of the compound administered or by sequestration by non-target cells by active uptake mechanisms and/or potential extrusion of the compound from the target or adjacent cells by P-glycoprotein multidrug transporter proteins. Nevertheless, it is imperative that a potential drug candidate exhibits the necessary pharmacological and potentially therapeutic effect in animal models of disease for which the compound has been synthesized. Accordingly, it would appear that AL-8810 has met such expectations in a number of *in vivo* studies that will be discussed below.

An area where AL-8810, as an FP receptor antagonist, has made a significant impact is in the CNS-related studies. Mechanical allodynia induced by intrathecal administration of $PGF_{2\alpha}$ and ATP is mediated *via* a capsaicin-insensitive primary afferent pathway within the spinal cord (Kunori *et al.*, 2009;

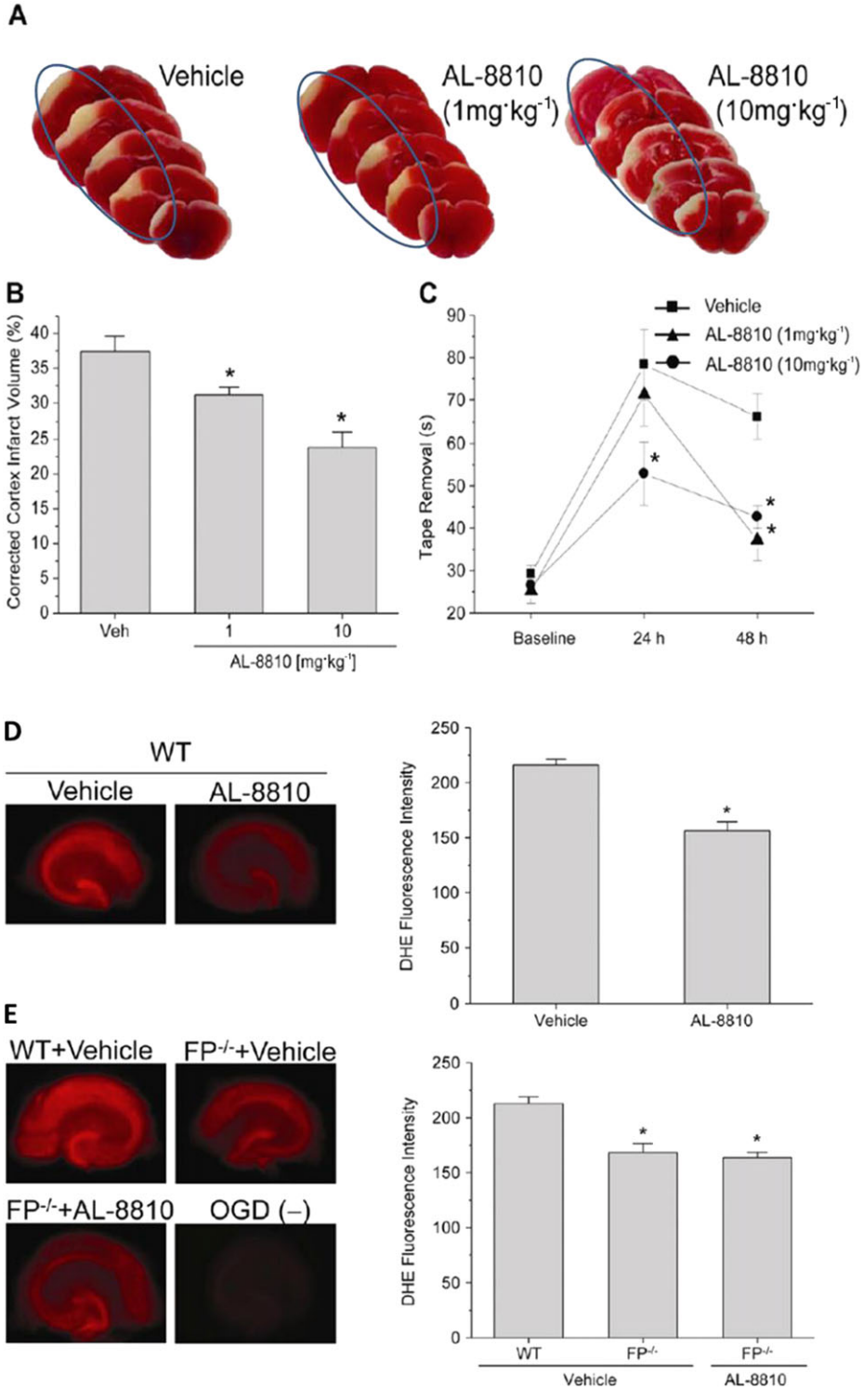


Figure 3

Protective effects of AL-8810 in experimental brain traumatic injury-induced changes in mouse brain cortex and in isolated hippocampal slices subjected to oxygen-glucose-deprivation (OGD). (A–C) Depict how systemically dosed AL-8810 (1 and 10 mg·kg^{-1}) was able to reduce the cortical infarct size relative to that of vehicle-treated mice in an animal model of stroke (A and B). A corresponding faster recovery from neurological dysfunctions was observed in mice treated with AL-8810 (reduced time for mice to remove a sticky tape from their paws; AL-8810-treated versus vehicle-treated mice; *$P < 0.05$; $n = 10$–13 mice). (D) Illustrates how AL-8810 (10 μM) was able to reduce the formation of ROS and thus protect mouse hippocampal slices [obtained from wild-type (WT) mice] from the detrimental effects of OGD *in vitro*. As can be seen in (E), OGD-induced ROS formation was significantly lower in hippocampal slices from FP−/− mice than in those obtained from WT mice. AL-8810 had no additional effect on slices from FP−/− mice. *$P < 0.05$ ($n = 20$–22 slices) (all figures were adapted and modified from Kim *et al.,* 2012).

Suzuki-yamamoto *et al.,* 2009). In FP receptor knockout mice, neither **αβ-methylene ATP** nor $PGF_{2\alpha}$ elicited allodynia. However, since AL-8810 blocked the pain response induced by intrathecal αβ-methylene ATP in naive wild-type mice, the authors concluded that activation of the **P2X2/3** receptors to cause allodynia is ultimately transduced *via* the FP receptors that are co-located with P2X2/3 receptors in the spinal cord (Kunori *et al.,* 2009). Extending these observations, Gatta *et al*. (2012) showed that direct spinal administration of $PGF_{2\alpha}$ into healthy mice excited nociceptive neurons and this activity was also abolished by AL-8810.

Brain damage caused by ischaemic insults, such as during stroke, and by traumatic/mechanical injury are debilitating disorders of the brain. In a mouse model of stroke, induced by permanent middle cerebral artery occlusion, pretreatment with i.v. AL-8810 significantly reduced the cerebral cortical infarct volume (Figure 3A–C), thereby providing protection against this ischaemic insult (Kim *et al.,* 2012). Commensurate with this structural protection, mice also displayed a much greater recovery of behavioural responses as a functional outcome. Both the cortical protection and functional results were confirmed in mice whose FP receptors had been knocked-out (Figure 3B). Interestingly, these authors also demonstrated these neuroprotective effects of AL-8810 *in vitro* using mouse hippocampal slices and cultured hippocampal neurons that were subjected to an oxygen–glucose-deprivation paradigm (Kim *et al.,* 2012). Mechanistically, AL-8810 reduced the production of reactive oxygen species and abolished the pathological elevation of excess $[Ca^{2+}]_i$ in cortical neurons exposed to the latter ischaemic challenge (Kim *et al.,* 2012) (Figure 3B).

It is well known that one major cause of brain damage and ensuing death, especially in elderly people who tend to fall more easily and injure themselves, is traumatic brain injury (TBI). This is characterized by an initial contusion, haematoma, subarachnoid haemorrhage and diffuse injury to the axons of brain neurons resulting in subsequent neuronal demise. The secondary phase of TBI is neuronal inflammation/oedema coupled with oxidative stress and Ca^{2+} overloading due to excitotoxicity and death of neurons. Endogenous production and release of pro-inflammatory PGs during and after TBI has been supported by observations of elevated AA release and enhanced COX-2 activity (Glushakov *et al.,* 2013a,b). Furthermore, elevated COX-2 has been reported in ischaemic neonatal and adult human brain, and in an experimental mouse models of TBI, Glushakov *et al*. (2013a,b) showed that a post-TBI i.p. injection of AL-8810 significantly reduced hippocampal swelling and improved neurological deficit scores 1 and 2 days post-insult (Figure 4A, B). They concluded that pharmacological blockade of the FP receptor may represent a novel means to blunt and lower the risk of brain damage after acute physical TBI.

$PGF_{2\alpha}$ can be formed by constitutively expressed PGF synthase in the myelin sheath and by isolated cultured oligodendrocytes, and it is believed that inflammation of the myelin sheath of nerves involves an up-regulation of $PGF_{2\alpha}$ production. This information indicates PGs are involved in the demyelination process and thus in the aetiology of multiple sclerosis (MS) (Iwasa *et al.,* 2014). In support of this hypothesis, in a rodent model of MS induced by dietary intake of cuprizone, i.c.v. administration of AL-8810 attenuated cytokine expression in the corpus callosum, prevented brain glial activation, attenuated the demyelination process and helped improve the motor function of the mice (Iwasa *et al.,* 2014) (Figure 4). Therefore, pharmacological antagonism of the FP receptor appears beneficial for reducing the pathological signs and symptoms associated with experimentally-induced MS. Whether these observations can be translated to the human MS patients remains to be determined, but FP receptor antagonists such as AL-8810 hold some promise in this regard.

Reduced cerebral blood (oligaemia) can be caused by ischaemic events due to a variety of vascular abnormalities including atherosclerosis, vasospasm and aberrant vasoconstriction. Such persistent cortical oligaemia has been implicated in the development of cortical spreading depression (CSD) in humans. In an experimental model of CSD using urethane-anaesthetized rats, Gariepy *et al*. (2017) demonstrated that long-lasting oligaemia consists of two phases; the initial phase involves activation of COX-1 and induction of TXA_2, while the later phase is mediated by $PGF_{2\alpha}$ derived from stimulation of COX-2 activity. In correspondence with these results, AL-8810 was able to prevent only the second phase of oligaemia and thus enhance cerebral blood flow. These findings provide an insight into possible treatment of CSD induced by pathologically reduced brain blood flow and suggest that in order to overcome the majority of the blood-flow deficit in CSD patients, a combination therapy approach using TXA_2 and FP receptor antagonists may be necessary.

Early studies focused on epileptogenic aspects of endogenous and exogenously administered PGs and COX-1 and COX-2 inhibitors in relation to kainic acid (**KA**)-induced seizures. Inhibitors of PG synthesis or AL-8810 administered intracisternally (i.c.) prior to induction of seizures exacerbated the condition of these animals by potentiating the seizure activity (Kim *et al.,* 2008). However, i.c. injection of $PGF_{2\alpha}$, but not PGD_2 or PGE_2, alleviated KA-induced seizures, thereby suggesting the involvement of FP receptors in the beneficial effects of endogenously released FP-PGAs in combating epilepsy. However, such findings need to be confirmed by other researchers.

There is a high degree of complexity surrounding the aetiology and progression of endometriosis, an oestrogen-driven disease. One important recent finding concerns the up-regulation of $PGF_{2\alpha}$ synthesizing enzymes in eutopic and ectopic endometria of women suffering from endometriosis (Ahmad *et al*., 2015). Following up on these observations, the same authors developed a heterologous animal model of endometriosis by implanting human endometrial tissue into the peritoneal cavity of nude mice. Ahmad *et al*. (2015) subsequently showed that administration of AL-8810 resulted in a marked reduction in the number and size of the endometriotic lesions by inhibiting biomarkers of inflammation, cell proliferation, angiogenesis and tissue remodelling and by readjusting the levels of pro- and anti-apoptotic factors. Interestingly, in a more detailed study using the same animal model as above, Ahmad *et al*. (2015) demonstrated that an exogenously administered FP receptor agonist, fluprostenol, elevated all the aforementioned biomarkers. In contrast, AL-8810 caused a highly statistically significant reduction of secreted **MMP-9** and

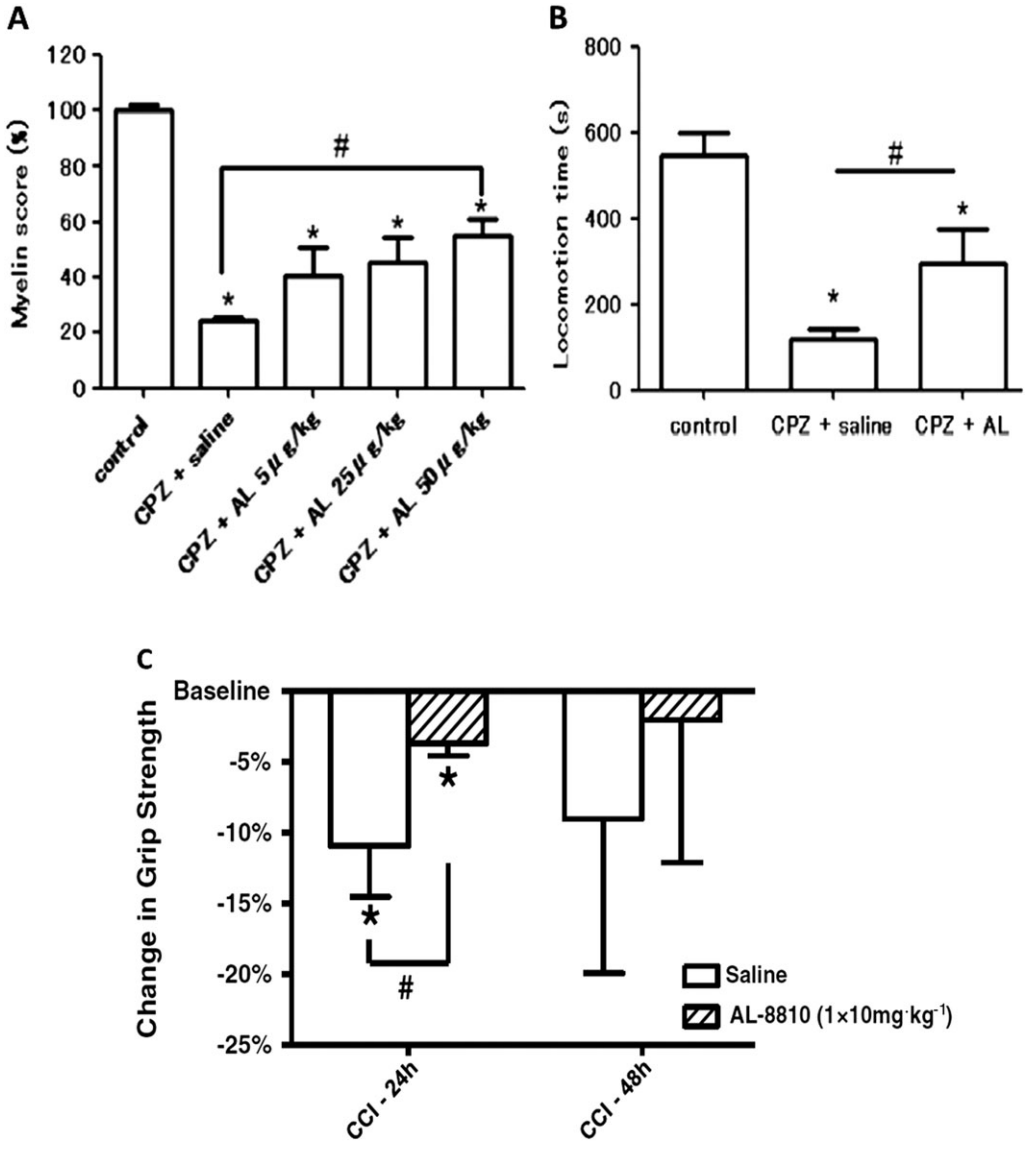


Figure 4

Protective effects of AL-8810 in an animal model of MS and in a mouse model of TBI. (A) and (B) demonstrate how AL-8810 (AL) was able to reduce the loss of myelin (A) and thus help retain motor function (B) in a cuprizone (CPZ)-induced mouse model of MS; (*$P < 0.05$ vs. control; #$P < 0.05$ vs. CPZ + saline; modified from Iwasa *et al.,* 2014). (C) Shows the protective effects of AL-8810 in a controlled cortical impact (CCI) traumatic injury model in mice. Note the improvement of grip strength in mice who received AL-8810 versus the control saline group at 24 h post CCI (*$P < 0.05$ vs. control group; modified from Glushakov *et al.,* 2013a,b).

VEGF and reduced the levels of cell proliferation and capillary formation nuclear antigen and vonWillebrand factor immunoreactivity in the endometriotic tissue (Ahmad *et al.,* 2015). These various beneficial effects of AL-8810 are summarized in Table 3.

Utility of other FP receptor antagonists in biological studies

An antioxidant and anti-inflammatory flavonoid compound, phloretin (de Oliveira, 2016), was historically dubbed as an FP receptor antagonist (Kitanaka *et al.,* 1993). Likewise, the sulfonylurea anti-diabetic agent, **glibenclamide**, was also claimed to block PG-induced tissue contractions *via* the FP receptor (Delaey and Van de Voorde, 1995). However, even though glibenclamide and **tolbutamide** competed for [^{3}H]-$PGF_{2\alpha}$ binding to FP receptors and they exhibited very low affinity, phloretin inexplicably potentiated the binding of this radioligand (Sharif *et al.,* 2000a). All these latter compounds very weakly inhibited the generation of second messengers induced by $PGF_{2\alpha}$ in rat aortic smooth muscle cells but were also weak antagonists at DP, EP and **vasopressin receptors** (Sharif *et al.,* 2000a). Thus, there appears to be little evidence supporting the true FP receptor antagonist properties of phloretin and glibenclamide.

Table 3

Utility and therapeutic actions of AL-8810 in various animal models of disease

Disease/disorder	Naive animal studies or experimentally-induced disorder/disease	Therapeutic effect of AL-8810	Reference
Allodynia (Pain)	Spinal injection of $PGF_{2\alpha}$ caused nociceptive neuronal excitation in mice.	AL-8810 blocked the pain-producing signalling of $PGF_{2\alpha}$.	Gatta *et al.*, 2012
	Allodynia was induced in mice by ATP or $PGF_{2\alpha}$.	AL-8810 abolished the pain induced by both agents.	Kunori *et al.*, 2009
Stroke	Middle cerebral artery occlusion in mouse model of stroke (cerebral ischaemia).	AL-8810 significantly reduced the cortical infarct volume and accelerated the recovery of behavioural function.	Kim *et al.*, 2012
	Mouse hippocampal neurons and slices subjected to oxygen–glucose-deprivation (hypoxia/aglycaemia).	AL-8810 inhibited the generation of free radicals (reactive oxygen species) and prevented excess $[Ca^{2+}]_i$.	Kim *et al.*, 2012
TBI	Controlled mechanical brain injury was induced in mice that caused contusion, subarachnoid haemorrhage and diffuse brain axonal injuries, followed by neuronal inflammation/oedema, oxidative stress and neuronal excitotoxicity. Elevated COX-2 activity and production of PGs was observed.	I.p. administration of AL-8810 (post-brain injury) decreased hippocampal swelling and accelerated neurological and behavioural recovery in mice.	Glushakov *et al.*, 2013a,b
	Ischaemic neonatal and adult human brains display the same features as above.	Mechanistically, AL-8810 blunted pro-inflammatory effects of released PGs and prevented Ca^{2+}-overloading and death of neurons/dendrites and axons.	Glushakov *et al.*, 2013a,b
Oligaemia (reduced cerebral blood)	Aberrant cerebro-vasoconstriction, vasospasm and atherosclerosis can reduce the amount of blood reaching the cerebral cortex. This causes ischaemia and results in CSD in humans. In a rat model of CSD, oligaemia was induced that exhibited two-phases. Phase-1 involved COX-1 activation with TXA_2 being released, while phase-2 was mediated by released $PGF_{2\alpha}$.	AL-8810 potently and effectively prevented the development of 2^{nd} phase of oligaemia and thus increased cerebral blood-flow thereby reducing the structural and functional cerebral damage.	Gariepy *et al.*, 2017
MS	Dietary intake of cuprizone, a copper chelator, in mice produced signs and symptoms akin to human MS pathology.	I.c.v. injection of AL-8810 abolished brain glial activation and cytokine production, reduced demyelination, and accelerated recovery of motor function in the mice.	Iwasa *et al.*, 2014
Endometriosis	$PGF_{2\alpha}$ is involved in eutopic and ectopic endometriosis in women that causes cramping and pain. Samples of human diseased endometrial tissue implanted in peritoneal cavity of nude mice capitulated the human disease (blood and cells accumulating in pelvic cavity and causing inflammation, swelling, pain and tissue scaring).	AL-8810 administration to the mice (with the implanted human diseased endometrial tissue) significantly decreased the number and size of the lesions by inhibiting inflammation, cell proliferation / angiogenesis, and by also reducing pro-apoptotic factors.	Ahmad *et al.*, 2015
	Exogenous administration of an FP agonist, fluprostenol, exacerbated the situation in the above mouse model.	AL-8810 reduced levels of secreted MMP-9 and VEGF (pro-angiogenic factors), with resultant decreased cell proliferation and capillary formation.	Ahmad *et al.*, 2015

There have been a few other attempts to discover FP receptor antagonists beyond AL-8810. Such discovery efforts led to the identification of a non-PG small molecule (**AS604872**) that had selective activity against the FP receptor (Chollet *et al.*, 2007; Cirillo *et al.*, 2007; Fukuda *et al.*, 2014). These authors showed that AS604872 binds well to the FP receptor (K_i = 35–323 nM), a little less weakly to EP_2 receptors (K_i = 650 nM) and much less weakly to the other PG receptors (K_i > 10 μM) (Cirillo *et al.*, 2007; Table 2) rendering it selective for the FP receptor. In functional studies, AS604872 inhibited $PGF_{2\alpha}$-induced [^{3}H]-IPs production and rat uterine contraction *in vivo* after i.v. infusion into pregnant female rats near term (Chollet *et al.*, 2007; Cirillo *et al.*, 2007). Despite its apparent high potency and receptor selectivity, it is curious that AS604872 has not been utilized as much as AL-8810. One possible reason could be that it is not commercially available. However, a more pertinent issue could be the side effects associated with AS604872. One such example concerns the exacerbation of intracranial aneurysm and aortic lesions observed in hypertensive rats that received AS604872 (Fukuda *et al.*, 2014). In rats rendered hypertensive by salt-loading, an infusion of AS604872 significantly accelerated the degeneration of the aorta and cerebral artery due to an up-regulation of genes for pro-inflammatory mediators and due to increased infiltration of macrophages (Fukuda *et al.*, 2014). It would have been much more informative if the authors had also tested AL-8810 in this rat hypertension model in order to determine if the deleterious effects of AS604872 were a compound-specific effect or a class effect of FP receptor antagonists.

As mentioned above, endogenous $PGF_{2\alpha}$ contracts myometrium and is heavily involved in labour and birth in mammals (Deaver *et al.*, 2015). During a search for agents that would prevent pre-term labour, Peri *et al*. (2002) found an octapeptide ligand (THG113; Ile-Leu-Gly-His-Arg-Asp-Tyr-Lys) that apparently interacted with FP receptors (Table 2) and which inhibited the generation of intracellular IPs in cells expressing FP receptors (IC_{50} = 27 nM). However, this blockade of the FP receptor seems to have been not directly at the FP receptor binding site since THG113 failed to displace [^{3}H]-$PGF_{2\alpha}$ binding from human-cloned FP receptors expressed in HEK-293 cells (Peri *et al.*, 2002). As far as it can be determined, a full receptor binding or functional profile of this compound against other PG receptors was not performed. When tested against $PGF_{2\alpha}$-induced contractions of retinal microvascular tissue, THG113 behaved as a non-competitive antagonist, but it did not affect EP_1 or TP receptor-mediated responses. Contractions of murine and porcine myometrial strips isolated from non-pregnant animals were largely blocked by THG113, and it delayed preterm delivery of fetuses of pregnant mice when an endotoxin was used to induce preterm labour (Peri *et al.*, 2002; Hirst *et al.*, 2005). However, the exact nature of the inhibition of exogenous and endogenous $PGF_{2\alpha}$-induced functional activities by THG113 remains undefined but appears to be beyond the extracellular components of the FP receptor protein. Nevertheless, perhaps in an attempt to enhance the FP receptor antagonist potency and/or selectivity of the parent peptide, various derivatives of THG113 were synthesized (Chemtob and Peri, 2006; Peri *et al.*, 2006). However, another THG113-derived peptidic agent (THG-113.31) exhibited minimal affinity for the FP receptor $PGF_{2\alpha}$ binding pocket, like the parent molecule (Chemtob and Peri, 2006; Peri *et al.*, 2006). THG113.31 insurmountably antagonized the contraction of pig retinal blood vessels evoked by $PGF_{2\alpha}$ but lacked the ability to block $PGF_{2\alpha}$-induced contractions of isolated pregnant human myometrium; and it only weakly inhibited sheep myometrial contractile activity caused by $PGF_{2\alpha}$ (Friel *et al.*, 2005). Curiously, THG113.31 (also apparently known as PDC31; Böttcher *et al.*, 2014) exerted a relaxant effect on both oxytocin-evoked and spontaneous contractions of human myometrial tissue *in vitro*, indicating the action of this compound is non-selective (Friel *et al.*, 2005). However, Doheny *et al*. (2007) demonstrated that THG113.31 apparently increased the opening of a Ca^{2+}-dependent large-conductance K^+-channel in human myometrial cells and caused the subsequent relaxation of this tissue. Despite the uncertainty of the exact mechanism of action of PDC31 (THG113.31), its incomplete pharmacological profile and the potential for significant side effects it could cause, the effects of i.v. administration of the latter compound were tested in a small clinical trial. Böttcher *et al*. (2014) showed that PDC31was capable of decreasing the strength and duration of uterine contractions *in vivo* and that it reduced the pain associated with excessive uterine contractility in women suffering from this disorder. As far as we know, no follow-up studies with this compound have been reported to-date. However, a small peptide based on the N-terminal region of the second extracellular loop of the FP receptor (PDC113.824) appeared to antagonize smooth muscle contractions elicited by $PGF_{2\alpha}$ by an undefined mechanism (Peri *et al.*, 2006). Regardless, Goupil *et al*. (2010) proceeded to show that PDC113.824 behaves as an allosteric inhibitor that blocks the $PGF_{2\alpha}$-induced $G\alpha_{12}$-dependent **Rho kinase**/ROCK signalling pathway and, in this manner, inhibited both endotoxin- and $PGF_{2\alpha}$-stimulated preterm labour and delayed normal parturition in mice. Further studies with these peptide and allied peptidomimetics are required to ascertain their future utility as tools for studying the actions of FP receptor-mediated functions/dysfunctions.

A small molecule compound (AGN211377) capable of binding to the FP receptor (K_i = 61 nM) and suppressing the $[Ca^{2+}]_i$-mobilization elicited by 17-phenyl-$PGF_{2\alpha}$ *via* the human cloned FP receptor was recently described (Wang *et al.*, 2016; Tables 1 and 2). Unfortunately, this agent was not selective for the FP receptor since it potently and efficaciously inhibited the signal transduction and functional responses mediated by TP, DP_1, EP_4, EP_1 and **DP_2** receptor agonists in a variety of cells in culture and in animal studies (Wang *et al.*, 2016). Perhaps analogues of this compound may be more FP receptor-selective, and such synthetic and pharmacological studies are much warranted.

Conclusions

The authors hope that the present discourse on the discovery, characterization and utility of purported and *bona fide* FP receptor antagonists, albeit of low affinity and potency, spurs on and inspires others to seek and find better next-generation antagonists. Such novel compounds are eagerly awaited.

Nomenclature of targets and ligands

Key proteins targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c).

Conflict of interest

The authors declare no conflicts of interest. They only wished to gather and summarize pertinent public domain information on the subject matter and to disseminate this to the scientific community, thereby promoting scientific exchange. The ultimate aim is to help foster and enhance further biomedical research on the role of PGs in health and disease and to find suitable remedies for the diseases discussed in this review.

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