In patients with ocular hypertension or glaucoma, all treatments aim to lower intraocular pressure (IOP) by modulating aqueous humour (AH) production and/or uveoscleral and trabecular meshwork/Schlemm’s canal AH drainage. PG analogues are considered to be the ‘gold standard’ treatment and are the most frequently used IOP-lowering agents. Recent data support an important role for NO in regulating IOP. Thus, novel PG analogues carrying a NO-donating moiety were recently advanced. Latanoprostene bunod (LBN) and NCX 470, NO-donating derivatives of latanoprost and bimatoprost, respectively, are examples of such compounds. LBN ophthalmic solution, 0.024% (Vyzulta™), showed greater IOP-lowering efficacy compared with that of Xalatan® (latanoprost ophthalmic solution, 0.005%) or 0.5% timolol maleate in clinical settings. NCX 470 was found to be more effective than bimatoprost in animal models of ocular hypertension and glaucoma. Selective EP₂ receptor agonists (i.e. taprenepag isopropyl, omidenepag isopropyl and aganepag isopropyl) and non-selective prostanoid receptor agonists (i.e. ONO-9054, sepetaprost isopropyl) that concomitantly stimulate FP and EP₃ receptors have also been shown to hold promise as effective IOP-lowering agents.

**LINKED ARTICLES**
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**Abbreviations**
AH, aqueous humour; IOP, intraocular pressure; LBN, latanoprostene bunod; SC, Schlemm’s canal; TM, trabecular meshwork
Introduction

Prostaglandins elicit various biological effects including regulation of smooth muscles, inflammation and immune response. Pioneer studies characterizing the effects of PGs in ocular compartments mostly describe significant ocular irritation and an initial intraocular pressure (IOP) elevation following topical ocular dosing. Later studies, however, demonstrated that the topical administration of PGE$_2$ and PGE$_2$ to rabbits decreases IOP in a dose-dependent fashion, thus establishing, for the first time, that stimulation of the respective PGE$_2$ receptors (FP receptor) and PGE$_2$ receptors (mainly EP$_2$ and EP$_4$) could be novel approaches to effectively treat ocular hypertension and glaucomatous patients (Camras et al., 1977). These initial findings led several independent laboratories and worldwide pharmaceutical companies to devote large research efforts to the identification of potent and selective synthetic compounds that are able to stimulate FP receptors and, more recently, EP$_2$ and EP$_3$ receptors.

The first PGE$_2$, analogue, latanoprost, received Food and Drug Administration (FDA) approval in 1996 (Xalatan®, latanoprost ophthalmic solution, 0.005%) as the second-line treatment for patients with open-angle glaucoma who are intolerant or unresponsive to other IOP-lowering products.

Bimatoprost (Lumigan®, bimatoprost ophthalmic solution, 0.03%), travoprost (Travatan®, travoprost ophthalmic solution, 0.004%), tafluprost (Zioptan®, tafluprost ophthalmic solution, 0.0015%) and the partial agonist, unoprostone (Rescula®, unoprostone isopropyl ophthalmic solution 0.04%), were subsequently approved. Latanoprost, travoprost and tafluprost are ester prodrugs of their more active respective de-esterified free carboxylic acid derivatives. Controversy exists for bimatoprost since it is an amide derivative and less prone to release the respective derivatives. Controversy exists for bimatoprost since it is an α-analogue, bimatoprost (Lumigan®, bimatoprost ophthalmic solution, 0.024%), which is an NO-donating derivative of latanoprost, is the most advanced compound from this class. Similar technology is applied to the less advanced compound, NCX 470, which is an NO-donating derivative of bimatoprost.

Somewhat interesting are also EP$$_2$$ receptor-selective agonists, which have recently been reported to effectively lower IOP (Prasanna et al., 2011; Schachar et al., 2011; Alhara et al., 2017). From this class, the most advanced is omidenepag isopropyl (DE-117), which is being co-developed by Santen and Ube Industries and is currently in phase 3 clinical trials. Likewise, the EP$_3$/FP receptor agonist, ONO-9054, is currently being developed by Santen and ONO Pharmaceutical for the reduction of IOP in patients with ocular hypertension and glaucoma (Miller Ellis et al., 2017).

Prostanoid receptors in the eye

Nine prostanoid receptors localized in the cell membrane and nuclear envelope have been described, namely, the FP receptor (most potent endogenous ligand: PGE$_2$); EP$_1$, EP$_2$, EP$_3$ and EP$_4$ receptors (most potent endogenous ligand: PGE$_2$); DP$_1$ and DP$_2$ receptors (most potent endogenous ligand: PGD$_2$); IP receptor (most potent endogenous ligand: PGI$_2$); and TP receptor (most potent endogenous ligand: TXA$_2$) (Alexander et al., 2017a). In addition, there is pharmacological evidence for the existence of an as yet unidentified specific prostanamide receptor (Woodward et al., 2008). The molecular structure of all the prostanoid receptors identified is typical of that for GPCRs and consists of seven α-helical transmembrane domains, three loops and an amino terminus lining in the extracellular compartment and three loops and a carboxyl terminus intracellularly.

In ocular tissues, only FP and EP$_{1-4}$ receptors have been demonstrated in most compartments. The EP$_{1-4}$ and FP receptors are mostly localized in uveoscleral tissues where they are found in ciliary body and sclera. In these tissues, FP receptors seem the most abundant and are specifically localized in the circular portion of the ciliary muscle (Schlötzter-Schrehardt et al., 2002). The FP and EP$_{1-4}$ receptors are also found within the TM and SC (Anthony et al., 1998; Schlötzer-Schrehardt et al., 2002). In the TM, the gene expression of the EP$_2$ receptor is the most abundant, followed by FP, EP$_1$ and EP$_4$ receptors with EP$_3$ receptors having the lowest levels. EP$_1$ receptor staining is predominantly found in TM cells and in cells lining the SC, while EP$_2$ receptors seem mainly to be localized within the wall and periphery of the SC. EP$_3$ and EP$_4$ receptors are present throughout the entire meshwork. FP receptor protein expression is found in the outer portion of the TM and in SC endothelial cells, collector channels and aqueous vein (Kamphuis et al., 2001).
In more superficial structures, EP_1 receptor protein is found in the epithelia of the cornea and in the conjunctiva. Likewise, EP_2 receptor labelling is also prominent in the corneal epithelium and choriocapillaris, while EP_3 and EP_4 receptors are primarily observed in the corneal endothelium and keratocytes, as well as in conjunctival and iridal stroma cells. The FP receptor protein is mainly present in the corneal epithelium (Schlötzer-Schrehardt et al., 2002).

**NO signalling in the eye**

Since the discovery of NO in 1987, research on this molecule has expanded in many directions. Most recently, NO and its second messenger cGMP were shown to be important for multiple functions in the eye including IOP homeostasis (Cavet et al., 2014; Aliancy et al., 2017; Muenster et al., 2017) and blood flow modulation (Schmidl et al., 2013). Under physiological conditions, NO is synthesized by the constitutive NOS isoforms, neuronal NOS (nNOS) and endothelial NOS (eNOS) that catalyse the oxidation of L-arginine to form NO and L-citrulline in a Ca^{2+}/calmodulin-dependent manner. NO produced in low nM concentrations by nNOS and eNOS activates soluble GCs, thereby leading to cGMP formation and activation of protein kinases that ultimately produce biological functions (Ignarro, 1991). Other NO-mediated activities may occur through cGMP-independent pathways such as post-translational modification of proteins by S-nitrosylation or transcriptional modulation of protein synthesis via regulation of the transcription factor NF-xB (Colasanti and Suzuki, 2000). In pathological conditions, where inflammation or ischaemia is prominent, NO is produced by a third NOS isoform, namely, the inducible NOS (iNOS); iNOS works in a Ca^{2+}-independent manner and generates large amounts of NO (μM range) that are generally considered detrimental to exposed tissues.

While nNOS is mainly found in ciliary processes and in nerve endings, intense nNOS expression is found in the anterior segment of the eye, not only in the vascular endothelium and perivascular nerve fibres but also in the non-pigmented epithelium of ciliary processes, ciliary muscle, TM, SC and collecting channels (Nathanson and McKee, 1995). These structures efficiently respond to NO and play a pivotal role in regulating AH dynamics and IOP homeostasis in physiological and in pathological conditions such as ocular hypertension and glaucoma (Aliancy et al., 2017).

Ocular hypertensive patients were shown to have reduced NO formation compared with healthy subjects (Nathanson and McKee, 1995), and exogenously administered NO was shown to lower IOP in these patients (Nathanson and McKee, 1995). Moreover, recent genetic studies showed that polymorphisms in eNOS are associated with a higher risk of glaucoma (Kang et al., 2010). Further confirming the importance of NO signalling in regulating IOP, eNOS-deficient mice (Lei et al., 2015) or animals with impaired GC activity (Buys et al., 2014; Muenster et al., 2017) were shown to have higher IOP compared with their respective wild-type littermates.

**PG analogues as ocular hypotensive agents**

Early studies analysing the effects of PG analogues in the eye reported substantial irritation and IOP elevation following topical dosing. However, later work demonstrated that the administration of low doses of PGF_2α consistently lowered IOP after an initial transient increase (Camras et al., 1977). More recently, the introduction of knockout animals for various prostanoid receptors has been pivotal in the documentation of the specific contribution of each receptor subtype to IOP regulation. As a result of these studies, various PG analogues have been discovered that either act by stimulating FP receptors (below referred to as first-generation PG analogues), carry residues that are able to either activate other prostanoid receptors (i.e. EP_2 and EP_3) or are processed into two active metabolites each able to activate independent signalling pathways (below referred as modified PG analogues).

**First-generation PG analogues**

Unoprostone isopropyl and latanoprost, an isopropyl ester analogue of PGF_2α (Figure 1A–C), were initially approved by the FDA for the treatment of elevated IOP in patients...
with open-angle glaucoma or ocular hypertension who are intolerant of other IOP-lowering medications or insufficiently responsive to other IOP-lowering agents (Rescula, unoprostone isopropyl ophthalmic solution, 0.15%, and Xalatan, latanoprost ophthalmic solution, 0.005%) followed several years later by bimatoprost (Figure 1F) (Lumigan, bimatoprost ophthalmic solution, 0.03%), travoprost (Figure 1D) (Travatan, travoprost ophthalmic solution, 0.004%) and, more recently, tafluprost (Figure 1E) (Zioptan, tafluprost ophthalmic solution, 0.0015%). Latanoprost, travoprost and tafluprost are prostanoid analogue ester prodrugs with similar modes of action. These compounds are cleaved by tissue esterases to the respective free acid carboxylic derivative and bind, albeit with different affinity and selectivity, to FP receptors (Nakajima et al., 2003). The free carboxylic acid form of tafluprost also binds with slightly less affinity to the EP₃ receptor subtype (ICₛ₀ = 67 nM) (Takagi et al., 2004; Ota et al., 2007).

Bimatoprost is the amide of 17-phenyl-PGF₂α and has been classified by some as a prodrug because low concentrations of the respective free acid, which are sufficient to stimulate the FP receptors, are found in AH following its topical ocular application (Faulkner et al., 2010) and by others as a compound acting on an unidentified prostamide receptor having a unique pharmacological profile (Woodward et al., 2008).

All these compounds produce a similar increase in uveoscleral outflow in animal models of ocular hypertension and glaucoma, with the exception of rabbits in which anatomical constraints make the uveoscleral outflow pathway less functional than in other species. These compounds also have been shown repeatedly to be highly effective IOP-lowering agents in ocular hypertensive and normotensive glaucomatous patients (Alm and Nilsson, 2009; Winkler and Fautsch, 2014). However, the pharmacodynamic efficacy of this class of molecules in patients seems to be less at nighttime because of physiological changes in uveoscleral outflow during the night (Orzalesi et al., 2006; Gulati et al., 2012).

Several molecular mechanisms have been proposed for their IOP-lowering effectiveness. Remodelling of the extra-cellular matrix within the ciliary muscle and sclera via direct activation of the FP receptors or indirectly, by de novo synthesis of an endogenous PG via PLA₂ stimulation, is the most commonly described mechanism involved in their IOP lowering effects. Stimulation of FP receptors induces an increase in metalloproteinase (MMP) enzymes (i.e. MMP1, MMP2 and MMP3) in the ciliary muscle, as well as in the surrounding tissues. MMPs dissolve collagenase types I and III within the connective tissue promoting specific rearrangements of the ciliary muscle and sclera that favour a more efficient uveoscleral drainage of the AH from the eye.

Less consistent are reports suggesting an effect of this class of molecules on TM/SC outflow. While it seems that all PG analogues result in a marginal yet consistent increase in TM/SC facility in the human eye, animal studies, with the exception of those in canine and primate models, have shown either no change or an increase depending on the specific PG analogue, the animal species or the experimental design. The mechanism of action for this effect may differ from that involved in the regulation of uveoscleral outflow. For example, travoprost was reported to affect TM contractility and conventional outflow by decreasing the secretion of endothelin-1 (Thieme et al., 2006), while latanoprost and bimatoprost were shown to promote the release of endogenous NO (Chen et al., 2005).

**Modified PG analogues**

**Novel NO-donating PGs with dual mechanism of action.** PGF₂α analogues and NO provide robust IOP-lowering activity by concomitantly activating two independent mechanisms: uveoscleral outflow and TM/SC conventional outflow facility as a result of FP receptor activation and soluble GC/cGMP stimulation in target tissues respectively (Figure 2). Moreover, enhanced vasodilatation, anti-inflammatory effects and antiplatelet activity have been attributed to NO making this class of molecules particularly interesting as impaired ocular blood flow and inflammation seem to also contribute to glaucoma progression (Resch et al., 2009; Costa et al., 2014). Various NO-donating PGs have been described in recent years for their enhanced IOP-lowering activity in animal models of ocular hypertension and glaucoma compared with standard of care treatments (Nong et al., 2010; Impagnatiello et al., 2011; Krauss et al., 2011; Impagnatiello et al., 2015). Among others, LBN, a compound discovered at Nicox and later licensed by Bausch + Lomb, has been advanced through phase 3 and recently received FDA approval for the reduction of IOP in patients with open-angle glaucoma and ocular hypertension (Vyzulta™, LBN ophthalmic solution, 0.024%). NCX 470 is a follow-up compound fully owned by Nicox and for which an Investigational New Drug application is expected to be submitted during 2018.

Vyzulta™ (latanoprostene bunod ophthalmic solution, 0.024%). LBN is an NO-donating PGF₂α analogue (Figure 3A) that is rapidly metabolized in situ by esterases into latanoprost free carboxyl acid and the NO-donating moiety, butanediol mononitrate (BDMN). BDMN is further metabolized to 1,4-butanediol and NO (Krauss et al., 2011). Latanoprost acid reduces IOP by increasing AH outflow primarily through the uveoscleral pathway (unconventional route), whereas NO facilitates AH outflow through the TM and SC pathways (conventional route) (Cavet et al., 2015; Garcia et al., 2016; Cavet and DeCory, 2017).

LBN reduced IOP to a greater degree than equimolar concentrations of latanoprost in various experimental animal models of ocular hypertension and glaucoma (Krauss et al., 2011). Furthermore, LBN ophthalmic solution, 0.024%, was shown to be more effective at reducing IOP compared with latanoprost ophthalmic solution, 0.005%, in a phase 2 dose-ranging study in patients with open-angle glaucoma or ocular hypertension (Table 1) (VOYAGER study, Weinreb et al., 2015). Moreover, a phase 2, open-label, randomized trial (CONSTELLATION study) found that, compared with timolol maleate ophthalmic solution, 0.5%, LBN 0.024% resulted in similar diurnal efficacy and a greater reduction in nocturnal IOP in patients with open-angle glaucoma or ocular hypertension (Table 1) (Liu et al., 2016; Kaufman, 2017).

The APOLLO (Weinreb et al., 2016) and LUNAR (Medeiros et al., 2016) studies were nearly identically designed phase 3 safety and efficacy studies of LBN 0.024% in patients with open-angle glaucoma or ocular hypertension. Each study...
consisted of a 3 month active (timolol) comparator, doublemasked efficacy phase followed by a 9 month (APOLLO) or 3month (LUNAR) open-label safety extension (Table 1). At the conclusion of the 3 month phase of the studies, pooled data from the studies showed significantly lower IOP in the LBN group compared with the timolol group at all time points (least squares mean IOP in the study eye ranged from 17.8 to 18.9 mmHg and from 19.0 to 19.7 mmHg, in the LBN 0.024% QD group and timolol 0.5% BID group, respectively) (Table 1). Furthermore, the proportion of patients reaching an IOP of ≤18 mmHg was greater in the LBN group compared with the timolol group (Medeiros et al., 2016; Weinreb et al., 2016; Kaufman, 2017). Pooled results from the APOLLO and LUNAR open-label safety extension phases demonstrated that the IOP reduction reached with LBN 0.024% was maintained up to 12 months, with no apparent loss of activity over time (Vittitow et al., 2016). In all studies, the compound was well tolerated; the percentages of patients experiencing treatment-related adverse events, primarily eye irritation and conjunctival hyperaemia, were similar in the LBN- and timolol-treated groups. However, as might be expected for a

**Figure 2**
Diagram describing the dual mode of action of the NO-donating derivative of latanoprost, LBN. AH, produced by the ciliary body, exits the eye through the TM/SC, also known as the conventional pathway (blue arrows), and the uveoscleral pathway, also known as the unconventional pathway (green arrows). The balance between AH production and outflow via the conventional (pressure-dependent) and unconventional pathway determines IOP. PG analogues mostly affect the uveoscleral, unconventional outflow while NO increases the TM/SC, conventional outflow pathway.

**Figure 3**
Chemical structures of (A) LBN and (B) NCX 470.
Table 1
Summary of clinical studies performed with Vyzulta™ (LBN ophthalmic solution, 0.024%)

<table>
<thead>
<tr>
<th>Trial features (ID)</th>
<th>Phase subjects (n)</th>
<th>Design</th>
<th>Duration</th>
<th>Arms</th>
<th>Dosage</th>
<th>Results Mean change in IOP in mmHg (±SD, where noted)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kronus® (NCT01895985)</td>
<td>I (n = 24)</td>
<td>Single-centre, controlled, open-label</td>
<td>14 days</td>
<td>LBN 0.024% qPM</td>
<td>3.6 ± 0.8, treated eye 3.5 ± 0.9, fellow eye</td>
<td>Araie et al. (2015)</td>
<td></td>
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<tr>
<td>VOYAGER®b (NCT01223378)</td>
<td>II (n = 413)</td>
<td>Multicentre, randomized, controlled, investigator-masked, dose-ranging</td>
<td>28 days</td>
<td>LBN 0.006% qPM</td>
<td>6.8, day 7; 7.6, day 14; and 7.8, day 28</td>
<td>Weinreb et al. (2015)</td>
<td></td>
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<tr>
<td>LAT 0.005% qPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.012% qPM</td>
<td>7.7, day 7; 7.9, day 14; and 8.3, day 28</td>
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<td></td>
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<td></td>
<td></td>
<td>0.024% qPM</td>
<td>8.3, day 7; 8.9, day 14; and 9.0, day 28</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.040% qPM</td>
<td>8.5, day 7; 8.6, day 14; and 8.9, day 28</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LAT 0.005% qPM</td>
<td>7.3, day 7; 7.7, day 14; and 7.8, day 28</td>
<td></td>
</tr>
<tr>
<td>CONSTELLATIONc (NCT1707381)</td>
<td>II (n = 25)</td>
<td>Single-centre, randomized, controlled, open-label, crossover</td>
<td>8 weeks, crossover at 4 weeks</td>
<td>LBN 0.024% qPM</td>
<td>1.1–1.2, diurnal/wake</td>
<td>Liu et al. (2016)</td>
<td></td>
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<tr>
<td>TIM 0.5% BID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3 ± 3.0, nocturnal/sleep</td>
<td></td>
<td></td>
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<tr>
<td>APOLLOd (NCT01749904)</td>
<td>III (n = 420)</td>
<td>Multicentre, randomized, controlled, double-masked</td>
<td>3 months</td>
<td>LBN 0.024% qPM</td>
<td>1.2, 1.4 and 1.1, week 2</td>
<td>Weinreb et al. (2016)</td>
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<tr>
<td>TIM 0.5% BID</td>
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<td>1.0, 1.3 and 1.3, week 6</td>
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<td>1.0, 1.3 and 1.3, 3 months</td>
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<tr>
<td>LUNARd (NCT01749930)</td>
<td>III (n = 420)</td>
<td>Multicentre, randomized, controlled, double-masked</td>
<td>3 months</td>
<td>LBN 0.024% qPM</td>
<td>0.4, 0.8 and 0.7, week 2</td>
<td>Medeiros et al. (2016)</td>
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<tr>
<td>TIM 0.5% BID</td>
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<td></td>
<td></td>
<td>0.9, 0.8 and 1.0 week 6</td>
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<td></td>
<td>0.9, 1.3 and 1.3, 3 months</td>
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<tr>
<td>JUPITERe (NCT01895972)</td>
<td>III (n = 130)</td>
<td>Multicentre, open-label</td>
<td>1 year</td>
<td>LBN 0.024% qPM</td>
<td>4.3, 4 weeks 5.3, 1 year</td>
<td>Kawase et al. (2016)</td>
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</table>

LAT, latanoprost; TIM, timolol maleate; qPM, every evening; BID, twice a day.

aData reported refer to IOP changes from baseline (mean ± SD) over 24 h in study and fellow-treated eyes.

bData reported refer to least mean changes from baseline diurnal IOP at day 4, day 14 and day 28 study visits.

cData reported refer to IOP changes between treatments during diurnal/wake period taken in sitting and supine conditions (reported as range differences) or nocturnal/sleep period taken in supine condition (reported as mean difference ± SD).

dData reported refer to IOP changes between treatments recorded during different visits (week 2, week 6 and 3 months) at various time points (8:00 h, 12:00 h and 16:00 h).

eData reported refer to IOP changes from baseline.
PG analogue-containing molecule, the percentage of patients with moderate to severe hyperaemia tended to be greater in patients treated with LBN compared with those treated with timolol 0.5% (Medeiros et al., 2016; Weinreb et al., 2016; Kaufman, 2017).

Factors other than IOP seem to be involved in glaucoma progression. Among others, defective ocular perfusion pressure has been reported in patients with glaucoma (Tielsch et al., 1995; Resch et al., 2009; Costa et al., 2014). Of interest, in a crossover trial in patients with early open-angle glaucoma or ocular hypertension, LBN improved ocular perfusion pressure to a greater extent than timolol (Liu et al., 2016). This effect is particularly important in light of the well-established effect of NO on vascular reactivity. Although additional work is needed, these findings suggest that Vyzulta™ treatment might ultimately result in a protective effect on the head of the optic nerve, which is independent of the reduction in IOP.

NCX 470. NCX 470 is a new molecular entity composed of the prostamide bimatoprost that is structurally related to PGF₂α, with the hydroxyl group in position 15 esterified with the NO-donating moiety 6-(nitrooxy)hexanoic acid (Figure 3B).

Nonclinical pharmacological studies have demonstrated that the IOP-lowering efficacy of NCX 470 is greater than that of equimolar doses of bimatoprost in well-established animal models of glaucoma and ocular hypertension. In particular, NCX 470 was shown to lower IOP in transient ocular hypertensive rabbits (previously shown to respond poorly to FP receptor agonists, Borghi et al., 2010) probably by inducing the release of NO (Impagnatiello et al., 2015). Additionally, NCX 470 0.042% lowered IOP more effectively than equimolar doses of bimatoprost (0.03%) in ocular normotensive dogs (Figure 4A, Impagnatiello et al., 2015) and in laser-induced ocular hypertensive non-human primates (Figure 4B, Impagnatiello et al., 2015). This compound holds promise to become the best in class as it adds NO-mediated efficacy to that of bimatoprost, whose Lumigan 0.03% formulation was considered to be the most efficacious PG analogue among those approved to date.

However, it remains to be seen whether the IOP-lowering effects of NCX 470 observed in animal models will be confirmed in patients.

**EP₂ and EP₃/FP receptor agonists.** In recent years, several attempts have been made to find compounds affecting IOP by modulating PG signalling on multiple receptor subtypes including EP₂ or concomitantly FP and EP₃ receptors. Several of these compounds are currently in clinical development.

PF-04217329 (taprenepag isopropyl), DE-117 (omidenepeg isopropyl) and AGN 210961 (aganepag isopropyl). Recent studies have defined EP₂ receptors as an attractive target for compounds intended for treatment of open-angle glaucoma and ocular hypertension. Prasanna et al. (2011) reported the IOP-lowering effects of **PF-04217329** (taprenepag isopropyl, Figure 5A), a prodrug of the respective carboxylic free acid (CP-544326) in rabbit, dog and non-human primate models of ocular hypertension and glaucoma. CP-544326 was demonstrated to be a highly selective and potent (IC₅₀ = 10 nM) EP₂ receptor agonist that is found in AH after topical ocular dosing of PF-04217329. This latter compound, tested at concentrations ranging between 0.0025 and 0.03%
agonists with little or no activity on EP4 receptors (Kirihara et al., 2015). DE-117 was also shown to be sufficiently safe in healthy volunteers when administered to subjects at 0.002% to warrant further clinical evaluation (Aihara et al., 2017). In a randomized, investigator-masked, active-controlled multicentre phase 3 trial in Japanese patients comparing DE-117 versus latanoprost 0.005%, the compound was shown to be similarly effective, although the IOP change from baseline in mean diurnal IOP for DE-117 and latanoprost was 0.63 mmHg in favour of latanoprost (Lu et al., 2018). Attempts are also being made to use it in long-term intracameral delivery devices (Kim et al., 2016). The compound was also shown to be effective in Xalatan non-responsive patients (Ropo et al., 2018).

AGN-210961 (Figure 5C) is likely to be an EP2 agonist and has been formulated as a proprietary ophthalmic solution by Allergan. A sustained-release formulation is also being developed as AGN-210669. This compound showed similar IOP lowering activity as that of Lumigan 0.03% but produced a high occurrence of adverse events, including increased corneal thickness as has been reported for other EP2 receptor agonists (Yanochko et al., 2014).

ONO-9054. ONO-9054 (DE-127, sepetaprost) is an isopropyl ester derivative of the respective biologically active free acid ONO-AG-367 (Figure 5D). Once processed in a biologically active environment by local esterases, the resulting carboxylic acid derivative activates EP3 and FP receptors (Yamane et al., 2015). ONO-9054 has nanomolar affinity (16.8 nM) for the FP receptor while it lacks any meaningful binding affinity for most other PG receptor subtypes excluding the EP3 receptor. Conversely, ONO-AG-367 is a more potent FP agonist compared with ONO-9054 ($K_i = 0.72$ nM) and, albeit with low potency, and binds the EP3 receptor subtype with a $K_i$ of 25 nM (Yamane et al., 2015).

In animal studies, ONO-9054 was shown to lower IOP more effectively than other more selective FP agonists (Yamane et al., 2015). Several studies tested this compound in healthy volunteers, as well as in ocular hypertensive and normotensive glaucoma patients (Harris et al., 2016). The compound was generally found to be well tolerated, and when compared with latanoprost in phase 2b studies, ONO-9054 achieved a slightly greater diurnal mean IOP reduction (−7.2 and −6.6 mmHg, for ONO-9054 and latanoprost, respectively) (Miller Ellis et al., 2017).

### Discussion and conclusion

PG analogues have been the most widely used agents to lower IOP in patients with open-angle glaucoma and ocular hypertension because of their proven efficacy in most patients, long duration of action and relative safety profile.

The first generation of PG analogues (i.e. unoprostone, latanoprost, travoprost, bimatoprost and tafluprost) primarily lowers IOP by increasing AH drainage via the uveoscleral outflow pathway with minor, if any, effect on the conventional, TM/SC pressure-dependent pathway that accounts for about 60–80% of normal AH drainage from the eye (Gabelt and Kaufman, 2005; Alm and Nilsson, 2009; Winkler and Fautsch, 2014).

Several selective and non-selective agonists of EP2 receptors are currently under development. All these compounds are being developed as the ester prodrug of their respective more active free carboxylic acid to enhance corneal penetration after topical application. Preclinical and clinical studies performed to date are encouraging; however, since this class of compounds appears to affect only uveoscleral outflow, it seems unlikely that they would perform better than any of the PG analogues already available. Some compounds under development were reported to have other activity in addition to acting as an FP receptor agonist (Schachar et al., 2011).
making them of potential interest as alternative treatments for patients insufficiently responsive to FP receptor agonists.

More recently, dual-acting PG analogues were identified, namely, NO-donating PGs. These compounds affect both the uveoscleral (PG mediated) and TM/SC (NO mediated) outflow pathways (Cavet et al., 2015; Cavet and DeCory, 2017). Furthermore, given the well-known activity of NO on blood flow, these compounds may be expected to also improve ocular blood flow and optic nerve head oxygenation, which are otherwise impaired in glaucomatous patients (Resch et al., 2009; Costa et al., 2014).

Vyzulta™ (LBN ophthalmic solution, 0.024%), a NO-donating derivative of latanoprost approved by the FDA for the reduction of IOP in patients with ocular hypertension and glaucoma in November 2017, is the most advanced example from this class. Several clinical trials clearly demonstrate that LBN lowers IOP more than latanoprost (Xalatan), either directly in the VOYAGER trial or indirectly by comparing the approved range of IOP reduction for Xalatan (6–8 mmHg) with that shown in the APOLLO and LUNAR trials (7.5–9.1 mmHg). LBN acts by releasing latanoprost acid and NO that simultaneously activate FP receptors and the NO/soluble GC/cGMP signalling pathway, thereby increasing both uveoscleral and conventional outflow (Krauss et al., 2011; Cavet et al., 2015; Cavet and DeCory, 2017). Interestingly, LBN also increases ocular perfusion pressure in patients with open-angle glaucoma or ocular hypertension (Liu et al., 2016) likely by virtue of NO release. More rigorous long-term, randomized, placebo-controlled clinical studies using indicators of patient’s disease-related visual impairments as primary outcome are needed for a better understanding of the neuroprotective ability of LBN in addition to its IOP-lowering effects.

NCX 470 is a NO-donating derivative of bimatoprost (Impagnatiello et al., 2015) holding greater expectations compared with LBN as it works, similarly to LBN, by activating bimatoprost-mediated uveoscleral outflow and NO-mediated conventional outflow. However, bimatoprost is considered the most effective PG analogue (Aptel et al., 2008; Quaranta et al., 2013): the added value of NO on top of that of bimatoprost may ultimately result in even greater IOP lowering with NCX 470 than with LBN. Initial exploratory findings in animal models of glaucoma and ocular hypertension support this concept. More direct confirmation will, however, only come after testing in human subjects.

In conclusion, the field of PG analogues for reducing IOP in patients with glaucoma and ocular hypertension has greatly expanded since their initial introduction in ophthalmic practice. Modified PGs analogues employ multiple mechanisms of action to maximize the IOP-lowering effects, targeting both outflow pathways (i.e. conventional and uveoscleral pathways). Furthermore, these compounds hold promise as they may also target mechanisms other than IOP reduction that are thought to play prominent roles in the pathogenesis of glaucoma.

Nomenclature of targets and ligands

Key protein 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).

Conflict of interest

While the studies reported were performed, F.I., E.B., N.A. and S.B. were employed at Nicox Research Institute, Italy, while B.D. and M.V.W.B. were employed at Nicox SA, France, and Nicox Ophthalmics, Inc., USA, respectively. A. C.K. was employed at Silver Pharma Consulting and was a consultant of Nicox.

References


F Impagnatiello et al.


Liu F, Aihara M, Kawata H, Iwata A, Odani-Kawabata N, Shams NK (2018). A phase 3 trial comparing omeniprog isopropyl 0.002% with latanoprost 0.005% in primary open-angle glaucoma and ocular hypertension: the AYAME study. ARVO E-abstract 31235 - A0076.


