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Novel therapies for glaucoma: a patent review (2013-2019)

Paolo Guglielmi[1], Simone Carradori[2], Cristina Campestre[2] and Giovanna Poce[1]

[1]Department of Chemistry and Technologies of Drugs, Sapienza University of Rome, Rome, Italy

[2]Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy

Corresponding author:

Simone Carradori,

Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy

Email: simone.carradori@unich.it

Abstract

Introduction: Glaucoma is one of the main leading causes of irreversible blindness in the word. The treatment of this disease relies on the use of drugs able to reduce/control the intraocular pressure (IOP), one of the main risk factors for glaucoma. Current therapies are based on the use of compounds belonging to well-established categories (prostaglandin analogues, β-adrenergic blockers, α-adrenergic agonists, carbonic anhydrase inhibitors, Rho kinase inhibitors and cholinergic agonists). However, even if they are effective in reducing IOP, important side effects impair patient compliance, accounting for the necessity of novel therapy approaches. Therefore, new targets are emerging as alternative and more complete routes to fight glaucoma disease.

Areas covered: This review provides a comprehensive update on the development state of innovative strategies against glaucoma describing results, administration routes, pharmaceutical compositions, structures, and SARs as well as the related shortcomings within the 2013-2019 range.

Expert opinion: New innovative pharmacological targets have been explored in the last six years, allowing a broader therapeutic arsenal against glaucoma and IOP-related pathologies. The endocannabinoid system and FAAH inhibitors were the most investigated from a medicinal chemistry point of view.

Key words: glaucoma, melatonin receptor agonists, adenosine receptor, FAAH inhibitors, endocannabinoids, RNA interference

Article highlights

- Novel drugs acting upon new targets are studied alone or in combination with well-established therapies.
- In the last six years, most of the patents concern the endocannabinoid system to prevent/treat glaucoma.
- A large plethora of FAAH inhibitors featured by the carbamate moiety are disclosed.
- Pharmaceutical compositions are licensed after demonstrating their efficacy in *in vivo* models with respect to conventional therapies.

1. Introduction

Glaucoma represents one of the worldwide diseases along with cataracts, responsible for irreversible blindness. It consists of a collection of neuropathies, whose biological bases are still not completely understood. The several different types of glaucoma share the demise of retinal ganglion cells, neurons having their cell body in the inner retina, with their long axons forming the optic nerve [1]. The degeneration of these neurons leads to a series of morphological alterations with the most prominent one consisting in the "cupping" of the optic disc (damage of the optic nerve) with permanent loss of visual function.

Glaucoma diseases are characterized (often but not always) by an elevated intraocular pressure (IOP), due to the malfunctioning of the systems designated to maintain the correct amount of aqueous humor (AH) in the anterior chamber of the eye [2]. The production and secretion of aqueous humor are performed by ciliary body, while the outflow is guaranteed by the trabecular meshwork. The fluids collected from the trabecular meshwork pass into the Schlemm's canal and from it into a series of aqueous veins, that merge with the blood carrying veins. The imbalance between the production and drainage of AH leads to excessive IOP [3].

Depending on the mechanism bringing to the raise of IOP, glaucoma can be classified into two main categories named "closed-angle" and "open-angle". In the closed-angle glaucoma the site for the aqueous drainage is "mechanically" hampered as a result of a contact between the iris and the surface of the trabecular meshwork. On the contrary, the open-angle glaucoma is characterized by the absence of this "mechanical" block, while presenting an increased resistance to AH outflow through the trabecular meshwork.

In the past, the excessive intraocular pressure (over 21 mmHg) was considered as the unique trigger mechanism for the onset of glaucoma and, currently, is considered the main risk factor. However, a certain number of the patients (especially in Asian countries), showing primary open-angle glaucoma, did not exhibit pathological intraocular pressure (normal-tension glaucoma), accounting for the existence of other factors (vascular, genetic, anatomical, and immunological) playing an important role in its etiology [4,5]. Nevertheless, the therapy of glaucoma relies on the use of drugs able to reduce IOP that is, to date, the only clinical modifiable target for treatment and prevention of glaucomatous states. This goal can be reached with pharmacological therapy, laser treatment and incisional surgery. Nowadays, the medications used to decrease IOP act either by increasing the outflow facility or by reducing the aqueous humor production; they can be applied topically or administered orally. These compounds belong to different drug

classes: prostaglandin analogues (latanoprost, travoprost, tafluprost), β-adrenergic blockers (timolol, levobunolol, betaxolol), α-adrenergic agonists (brimonidine, apracloridine), carbonic anhydrase inhibitors (dorzolamide, brinzolamide) [6,7] and cholinergic agonist (pilocarpine, carbachol) [8,9].

The use of Rho Kinase (ROCK) inhibitors as valid therapeutic agents in ophthalmology is currently emerging, not only for glaucoma, but also for Fuchs' endothelial dystrophy, diabetic retinopathy and several chronic diseases in ophthalmology. The analysis of several databases demonstrated that inhibition of Rho kinase significantly contributes to decrease IOP in the glaucomatous eye, healing the damaged corneal endothelium and the optic neurons. These drugs also act directly on the neurons in the central visual pathway, limiting the RGC apoptosis [10]. Recently, two new drugs were approved: Ripasudil (in Japan) and Rhopressa (in USA). The latter has been improved in association with latanoprost (Rocklatan). The main side effect associated to these drugs is conjunctival hyperemia in certain formulations [11].

Despite their efficacy in lowering the IOP, some important adverse effects, exhibited at local as well as at systemic level, have been observed. For example, prostaglandin analogues can induce conjunctival hyperemia, lengthening and darkening of eyelashes, and macular edema. β-adrenergic blockers provoke locally ocular irritation and dry eyes, while can cause bradycardia and hypertension at systemic level and for these reasons are contraindicated in patients with asthma, bradycardia and chronic pulmonary obstructive disease. α-Adrenergic agonists induce ocular irritation and dry eyes along with fairly important systemic side effects involving the central nervous system. Cholinergic agents (e.g., pilocarpine) cause fixed pupils and induce myopia and cataracts.

In the light of the above, the searching for novel targets affecting IOP and playing neuroprotective role could be a breakthrough in the glaucoma treatment, avoiding side effects of the currently available drugs [12]. Adenosine system is one of the new emerging targets for glaucoma treatment [13]. Adenosine (Figure 1, a) can interact with four different receptors called A_1, A_{2A}, A_{2B} and A_3, belonging to the G protein-coupled receptor family which mediate different responses, depending on the G protein coupled type. While A_1 and A_3 are coupled with G_i-proteins (inducing the inhibition of adenylyl cyclase, then reducing cAMP concentration), A_{2A} and A_{2B} are coupled with G_s-proteins (mediating the activation of adenylyl cyclase, then increasing the cAMP concentration).

All these receptors are expressed, although with different distribution, in the tissues involved in the secretion and absorption of AH. The ciliary epithelium, devoted to the

production of AH, expresses all the four subtypes, although A_1 and A_3 receptors seem to be the main responsible of IOP control. Trabecular meshwork expresses mainly A_1, A_{2A} and A_3, while all subtypes were detected in the Schlemm's canal cells [14]. Due to the distribution of the adenosine receptors in the different eye tissues, the precise modulation of their activity using agonists or antagonists could be exploited to control IOP. In particular, the activation of A_1 receptors in the trabecular meshwork and in the ciliary body reduces the outflow resistance and diminishes the aqueous secretion, respectively, with beneficial effect for IOP. The activation of A_{2A} receptors located at the Schlemm's canal could elicit either the increase or reduction of IOP. However, A_{2A} agonists (as well as A_1 ones) can ameliorate the blood flow across the retina and optic nerve head (optic disc), reducing vascular resistance and working as neuroprotectant. The activation of A_3 receptors in the ciliary body triggers fluid release, affecting negatively IOP, while the use of antagonists, which selectively bind to A_3 receptor, is advantageous in order to diminish IOP.

Trabodenoson is a first-in-class, selective, adenosine-based compound targeting A_1. In a preliminary Phase 1 dose escalation study in 60 healthy adult volunteers, it was shown to be safe and well tolerated up to 3.2 mg per eye with no detectable systemic effects (14 days of twice-daily topical monocular application). It lowers IOP by augmenting the primary outflow pathway for aqueous humor through the trabecular meshwork [15]. Moreover, in a successive multicenter, double-masked, randomized, placebo-controlled Phase 2 study, patients with ocular hypertension or primary open-angle glaucoma received unilateral topical twice-daily trabodenoson up to 28 days. Ocular and systemic safety and tolerability were assessed. Trabodenoson was well tolerated with no clinically ocular or systemic side effects and it provided a dose-dependent IOP reduction from diurnal baseline ranging from -3.5 to -5.0 mmHg with a mean change of -4.1 mmHg at the highest dose tested. This effect was also improved with longer treatment time [16].

Another non-classical target for the treatment of glaucoma is the endocannabinoid system (ECS), that includes different receptors and endocannabinoids [17]. These components have been observed in the tissues of the eye taking part in the AH flow, accounting for their role in controlling IOP. In particular, the endocannabinoids anandamide (AE, Figure 1, b) and 2-arachidonoylglycerol (2-AG, Figure 1, c), found in both the anterior and posterior ocular tissues, can bind to both cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2). The expression of these receptors is quite different with CB_1 mainly located in the ciliary body, trabecular meshwork, Schlemm's canal and retina. CB_2 localization is not well

defined, although it seems to be present in the retina and the anterior eye. Along with endocannabinoid receptors, some non-cannabinoid ones expressed in various portions of the eye can be stimulated by AE and 2-AG, participating in the IOP modulation. In particular, transient receptor potential type vanilloid 1 (TRPV1) can be activated by AE, whereas GPR18, a cannabinoid-related receptor, is triggered by a metabolite of AE (*N*-arachidonoyl glycine) [18].

The activity of endocannabinoid system is controlled through the balance between production and degradation of endocannabinoids. The synthesis of AE and 2-AG is accomplished from arachidonic acid-containing phospholipids. The degradation of AE is controlled mainly by fatty acid amide hydrolase (FAAH) found in the ciliary body and some retinal cells, and at lesser extent by *N*-acylethanolamine-hydrolysing acid amidase (NAAA). On the contrary, 2-AG is degraded by monoacylglicerol lipase (MAG-L) and α,β-hydrolase domain containing 6 (ABHD6). Furthermore, both the endocannabinoids can be substrates of the cyclooxygenase-2 (COX-2) producing prostaglandin-ethanolamides and prostaglandin-glyceryl ester from AE and 2-AG, respectively. In the presence of ocular pathology, this complex pathway undergoes deviations from homeostasis. In particular, the reduction of endocannabinoid tone seems to be the basis for different pathologies, including glaucoma; the increase of the endocannabinoid tone seems to be beneficial for IOP eliciting neuroprotective effects [19,20]. Two compounds, Δ^9-tetrahydrocannabinol (Δ^9-THC) and WIN 55,212-2 (Figure 1, d and e), affecting endocannabinoid system demonstrated to be effective in reducing IOP. While Δ^9-THC can bind indiscriminately to both CB_1 and CB_2 receptors, WIN 55,212-2 is a selective CB_1 agonist. However, it is postulated that also the activity of Δ^9-THC is mainly mediated by the activation of CB_1, being this receptor located both in ciliary body, where the AH is produced, and in the trabecular meshwork, where the draining of the fluid takes part. Moreover, it is also supposed that the hypotensive effect of CB_1 activation could be mediated by β-adrenergic receptors [21].

As assessed above, the enhancement of the endocannabinoid tone elicits neuroprotective effects and improves IOP. One of the strategies to adopt to obtain this outcome is the decrease of AE metabolism, using FAAH inhibitors [22]. This strategy appeared to be effective in reducing both IOP and the damage on retinal ganglion cells, assuring neuroprotective activity. FAAH also metabolizes other fatty acid ethanolamines found in the eye tissues where they play roles for the IOP regulation. FAAH increased activity was

observed in some glaucomatous conditions, accounting for the lowering of the endocannabinoid tone and so enforcing the glaucomatous state [23].

The neurohormone melatonin (Figure 1, f) also plays an important role in IOP control and retinal physiology [24]. This molecule is produced starting from the amino acid tryptophan mainly by the pineal gland, with the highest concentrations at night; however, its production can also take place in the retina, although at lesser extent. The effects of melatonin are mediated mainly by the receptors named MT_1 and MT_2, coupled to the G_i/cAMP pathway. These receptors are located in the retina, ciliary body, sclera and cornea, and participate in IOP regulation. The melatonin receptor MT_3 (also described as quinone reductase II cytosolic enzyme, albeit controversy is ongoing about the real identity if this receptor in the eye tissues), appears to be positively linked to the same signal transduction of MT_1 and MT_2, also contributing to the control of IOP inside the eye [25]. Data coming from experimental animal models displayed as the concentration of melatonin in the retina of glaucomatous rats is highly reduced, accounting for the use of MTs agonist in order to restore the physiological activity of this system. Melatonin is able to effectively reduce IOP after topical administration [26]; furthermore, it also works as an effective antioxidant in the retina exhibiting radical scavenger activity. Administration of melatonin was also related with effects caused on the adrenergic system with the down-regulation of β_2-adrenergic receptor and the up-regulation of α_{2A}-adrenergic receptor, both involved in the production and outflow of AH [27].

Small-interfering RNAs (siRNAs) are gaining importance as promising tools for therapy of ocular diseases. This type of therapeutic approach is based on the use of little sequences of RNA which interfere with the expression of a specific gene, leading to the loss of the properties that characterize the disease. siRNAs are produced from the cleaving of long pieces of double-stranded RNA, which activate the RNAi post-transcriptional gene-silencing pathway. The presence of siRNA in the cytoplasm of the cell induces its incorporation into a protein complex, called RNA-inducing silencing complex (RISC), containing an ATP helicase and an endonuclease 2 activity. The activated RISC complex is able to bind to and destroy mRNA complementary to the siRNA, abolishing the expression of the selected protein. Therefore, siRNA approach can be useful to target all the main actors (for example β_2-adreoceptors), participating in the glaucoma film, modulating their expression and influencing either the AH secretion or its drainage [28].

As previously stated, glaucoma is a multifactorial optic neuropathy leading to the death of retinal ganglion cells and their axons. The control and health maintenance of these

neurons are guaranteed by a series of glia cells, called Müller cells. They regulate the extracellular environment, affecting glutamate and ions level and buffering oxidative stress. The latter is relevant for the damages that could induce in the neurons, eliciting their demise. Most of the compounds used for the alternative targets just seen possess also anti-oxidant and neuroprotective effects, paving the way for a multi-target approach for glaucoma treatment.

2. Adenosine receptor inhibitors

Palobiofarma S.L. patented heterocyclic compounds not based on the adenosine scaffold [29]. The inventors proposed the synthesis and characterization of 52 novel 2-amido-1,3-thiazoles as selective inhibitors of A_3 subreceptor to provide pharmaceutical compositions useful for the treatment of adenosine-based conditions (Figure 2). The pharmacological activity was determined in an adenosine receptor subtypes (human A_1 and A_3) competition radioligand binding assay. K_i A_3 values for the derivatives ranged from 10 to 99 nM, whereas K_i A_1 values spanned from 34 to 1000 nM, thus indicating a strong subtype selectivity.

Two other patents show adenosine-based analogues, proposing 15 compounds acting as selective A_1 agonists [30] and 241 compounds as selective A_3 antagonists [31]. The former disclosed agonist compounds useful to treat/reduce or prevent retinal ganglion cell damage or to provide ocular neuroprotection. Each retinal ganglion cell has an axion extending into the brain (the optic nerve). Any injury to these cells and their consequent loss lead to vision distortion and blindness. Among the main causes, a crucial role is exerted by glaucoma and, as a common therapy, the neuroprotective role sustained by topical administration of 0.2% brimonidine solution (an α_2 agonist) is well-established with respect to timolol [32,33]. Unfortunately, low compliance, due to multiple dosing and several side effects, required new and alternative approaches to treat this disease. Some adenosine derivatives were synthesized and fully characterized. Then, they were tested *in vitro* for the selective modulation of A_1 over A_{2A} and A_3 adenosine receptors (Figure 3) and in transfected CHO cells (radioligand binding experiments).

All the adenosine derivatives showed agonist activity in the nanomolar or sub-nanomolar range ($0.73 < K_i$ A_1 (nM) < 10.6). Cyclopentyl and cyclohexyl rings were preferred with respect to tetrahydrofuran-3-yl or bicyclo[2.2.1]heptan-2-yl at N6 of purine core. The introduction of a chlorine atom at C2 was favorable only if the sugar was unsubstituted. The primary alcohol at C5' could be derivatized as NO_2 or SO_3Na ester. Compound **1**, a

picomolar A_1 agonist endowed with the highest selectivity, was further studied in an *in vivo* murine model of ocular ischemic injury in comparison to brimonidine. Histological analyses revealed that some regions of the retina displayed a thinning degree less pronounced after treatment with brimonidine and compound **1**, whereas brimonidine was less effective in neuroprotection and against the ischemic insult with respect to compound **1**.

The second patent disclosed the synthesis, characterization and biological activity of a large number of heteroaryl compounds linked to tetrahydrothiophen-3,4-diol, tetrahydrofuran-3,4-diol and cyclopentane-1,2-diol rings as selective and potent A_3 adenosine receptor antagonists (Figure 4).

The compounds can be used alone or in combination therapy. In the binding affinity assay in CHO and HEK-293 cell membrane homogenates, most of them displayed potent (nanomolar) and selective (over A_1 and A_{2A}) antagonism *in vitro* and induced a strong IOP reduction *in vivo* (New Zealand white rabbits).

3. Melatonin receptor (MT) agonists

The pineal neurohormone melatonin (5-methoxy-*N*-acetyltryptamine, Figure 1, f), produced and secreted according to the circadian rhythm, has been related to a huge therapeutic potential due to the presence of melatonin receptors (MT_1-MT_3) in the eye and intraocular tissues [34]. Contradictory reports revealed that melatonin could both raise and reduce IOP [35,36] based on the administration route and the animal model differences.

Several analogues were investigated for their ocular hypotensive effect because of the peculiar receptor subtype affinity or the alternative binding to other targets involved in the modulation of IOP [37]. Their efficacy was also shown to be correlated to the clinical profile of the patient (hypertensive or normotensive condition, glaucomatous or not-glaucomatous eye). The most promising melatonin analogue is agomelatine (*N*-[2-(7-methoxynaphthalen-1-yl)ethyl]acetamide, Figure 5), currently marketed as a safe anti-depressant agent [38], which acts as a potent MT_1 and MT_2 agonist [39] and a non-selective 5-HT_{2C} inhibitor [40]. Studies on its bioactivity on New Zealand white rabbits, both in normotensive and hypertensive conditions [26,27], as well in a pilot prospective study (patients with primary open angle glaucoma) [41] revealed that (*i*) it reduced IOP, both in normotensive and hypertensive conditions, through the activation of MT_2 and MT_3 melatonin receptors; (*ii*) its action was partially reversed by MT_2 and MT_3 antagonists, but not by α_1-adrenergic receptor antagonists; (*iii*) with respect to melatonin and 5-MCA-NAT (Figure 4) it was more potent in normotensive conditions, being only better than melatonin in the hypertensive

model; (*iv*) its pharmacological effects were comparable to those of commercially available latanoprost and brimonidine, but inferior to dorzolamide and timolol in reducing IOP in normotensive conditions; (*v*) in a hypertensive animal model (Trendelemburg position) its biological action was more pronounced and statistically comparable to dorzolamide and timolol.

The results of the *in vivo* administration of agomelatine in rabbits were anticipated in a patent [42] disclosing some pharmaceutical formulations to prevent or reduce diseases characterized by high intraocular pressure. A 21% IOP reduction was evidenced at 180 min after administration with a long-lasting effect till 360 min.

Tasimelteon (*N*-(((1*R*,2*R*)-2-(2,3-dihydro-1-benzofuran-4-yl)cyclopropyl)methyl)propanamide, Figure 6) is a selective MT_1/MT_2 agonist clinically approved for the treatment of non-24-hour sleep-wake disorder. Recently, it was also licensed for the treatment of glaucoma trying to overcome its extensive CYP-mediated oxidative metabolism (especially at C2 position) [43]. The inventors patented a large plethora (286) of mono- and poly-deuterated analogues, which could limit the formation of degradation products due to the deuterium kinetic isotope effect. The derivatives were synthesized, fully characterized and tested by incubation with liver microsomal enzymes, human cytochrome P450 enzymes and monoamine oxidases (both A and B isoforms). The metabolites were studied by means of HPLC-MS/MS, along with the analysis of the pharmacokinetic data and melatonergic receptor binding affinity. This patent proposed the better stability of the deuterated analogues limiting oxidative metabolism of the parent compound and allowing the reduction of side effects, an improved and sustained plasma concentration, and increased clinical efficacy alone or in combination therapy.

4. Endocannabinoid system and glaucoma treatment

Two patents show THC-containing cannabis extract or pure Δ^9-THC in formulations to decrease IOP. The first patent disclosed a composition comprising cannabis extract and melatonin useful for the treatment or prevention of a large plethora of diseases including glaucoma [44]. Cannabis extract is characterized by the presence of numerous cannabinoids, terpenes and flavonoids in a specific ratio, depending on the optimization of the extraction conditions (e.g., temperature) and plant species (*C. sativa*, *C. indica* and *C. ruderalis*), as previously reported [45,46]. Melatonin was present in a concentration ranging from 0.05 to 1.6% by weight.

The second patent analyzed *in vivo* (knockout mice) the dual activation of CB1 receptor and GPR18-based signaling system in the mammalian eye [47]. The inventors demonstrated that Δ^9-THC could lower IOP, the main hallmark and therapeutic target of glaucoma, of -30% up to 8-hour treatment by means of the activation not only of CB1 receptor, but also through the interaction with GPR18 system. This pharmacological behavior was shown to reduce IOP in a sex-dependent manner, thus providing an alternative tool to manage glaucoma in male subjects. Different formulations were disclosed along with their proper administration way and excipients to improve corneal transit and bioavailability. The dual activation was due to the presence of Δ^9-THC alone or in combination with monoacylglycerol lipase (MAGL) blockers (2-arachidonoyl glycerol, 2-AG) or GPR18 activators such as *N*-arachidonoylglycine (NAGly), abnormal cannabidiol (abnCBD), O1602 and O1918.

Another patent analyzed in-depth several Δ^9-THC-containing formulations to provide ocular neuroprotection and IOP lowering effects [48]. The inventors investigated emulsions and microemulsions to deliver acceptable amounts of cannabinoids (THC ranging from 0.005 to 0.5%) varying the type and quantity of oils and surfactants. A particular attention was devoted to the analysis of viscosity or chemical-physical stability under normal and stressed conditions of temperature up to 12 months (e.g., freeze-thaw cycle), microbiological stability, interactions with the packaging and presence of antioxidants or vitamins. Observations from formulation stability studies attested that homogenization speed and time decreased the particle size of the emulsions, whereas surfactant concentration did not alter it.

In vivo studies were performed (female C57BL/6J mice) in comparison to timolol maleate 0.5%. The IOP reduction in conscious and anesthetized animals was about -30% at 2-6 h, comparable to the reference drug and without the presence of dangerous side effects. Other parameters were positively affected also after repeated dosing studies in animals registering a lowered AH production, an increased aqueous outflow facility, a strong neuroprotective effect against mouse retinal ischemia/reperfusion damage, and a limited episcleral venous pressure. The results opened a new scenario in the field of effective IOP-lowering agents because of the multifaceted effects exerted by this ophthalmic emulsion in contrast to the single mode of action of current monotherapies.

5. FAAH inhibitors

One patent explored a large number of novel triazolyl carbamates as inhibitors of FAAH [49]. These small molecules belong to the class of *O*-alkylcarbamates with improved chemical and hydrolytic stability with respect to esters of *O*-arylcarbamic acids due to the lower acidity of aliphatic alcohols. Forty-four compounds were synthesized, characterized and tested *in vitro* against rat FAHH enzyme by a radiometric assay and against human FAAH by a fluorescent assay in a HEK-293 FAAH-1 overexpressing stable cell line. The general structure and the two most active compounds were proposed in Table 1. Moreover, some formulations containing them were disclosed and tested *in vivo* (mice) to assess the FAAH inhibition in the brain and in the liver after intraperitoneal administration.

All the compounds displayed potent inhibitory activity against both isozymes in the nanomolar or low micromolar range and reliable SAR studies can be extrapolated. In general, the cycle should be a triazolylmethyl, the alkyl portion must be composed of one methylene unit (n= 1 2), the aryl pendant could be directly connected to or spaced from the triazole (m= 0 1 2). Substituents on the aryl ring were preferred in the meta or para position (OCH_3 OH, CN Cl, F, CH_3, $CONH_2$ CF_3, COOH). The aryl ring should not be substituted with pyridin-3-yl or naphth-2-yl rings. The best FAAH inhibitors were compounds **3** and **4**. In particular, compound **3** presented a molecular doubling of the triazolyl portion and a methoxyl substituent in the meta position of the benzyl pendant.

In the search of new *O*-arylcarbamate-based FAAH inhibitors, another patent disclosed the biological activity of 13 compounds incorporating fluorine atoms or difluoromethoxyl groups to improve solubility and gastro-intestinal absorption [50]. They were synthesized and fully characterized also in terms of pharmaceutical compositions, aqueous solubility and pharmacokinetic parameters. They were tested against rat and human FAAH enzymes (Table 2). With respect to the previous *O*-alkylcarbamates, these derivatives better inhibited the rat isoform of FAAH.

Another patent investigated the synthesis, characterization and formulations of three imidazole-based FAAH inhibitors [51], as reported in Table 3, alone or in combination with anandamide (the natural substrate of FAAH enzyme acting as both cannabinoid and vanilloid receptors agonist). Imidazole-based carboxamides were previously reported to inhibit FAAH [52].

The compounds displayed the ability to modulate the activity of FAAH with relatively high peripheral activity (liver brain) after oral administration *in vivo* (mice) and to be metabolically stable in mouse and human liver microsomes, both in the presence or not of NADPH. Improved data were obtained with an *N*-substituted piperidine as a lateral chain.

In order to enhance water solubility, the same inventors developed urea-based FAAH inhibitors for the treatment of glaucoma [53]. The inventors synthesized and fully characterized 39 compounds and their formulations to further test them by an *in vitro*/*ex vivo* protocol in male NMRI mice to assess the inhibitory activity, the aqueous solubility and the metabolic stability against human liver microsomes in presence/absence of NADPH. These new derivatives showed promising solubility profiles (14 mg/mL) suitable for ocular instillation, but were largely metabolized by CYP enzymes. The FAAH inhibitory activity is reported in Table 4 at 0.1 mg/Kg, po. The compounds displayed the ability to modulate the activity of FAAH with relatively high peripheral activity (liver brain) after oral administration in *in vivo* (mice) as reported for the previous ones and their general structure. The nature and type of the organic or inorganic acid for salt formation were important for the biological activity and the improved aqueous solubility. The R substituent (amide, urea, sulfonamide, sulfamate, or sulfamide) was preferred in the meta position with respect to the para, whereas the alkyl spacer followed the order: n= 1 2. The aryl could be substituted (especially in the meta position) with OCH_3 OH Hal or changed to a benzodioxol-5-yl ring.

6. RNA interference, anti-sense oligonucleotides and CRISPR-Cas9 gene editing strategies

Ribonucleic acid interference (RNAi) pathways are evolutionary conserved post-transcriptional gene silencing mechanisms first discovered in the nematode worm *Caenorhabditis elegans* in 1998 [54]. RNAi pathways, which were also shown to occur in mammalian cells [55], are triggered by the generation of small RNA fragments from long pieces of double-stranded RNA that are able to target and cleave complementary mRNA and halting of protein synthesis. Given the ease of both production and direct drug delivery, RNAi pathways have garnered considerable attention for ophthalmic use [28]. Moreover, the fact that the eye is a relatively isolated tissue compartment provides several advantages to the use of siRNA-based therapies. Indeed, local delivery to the eye limits systemic exposure and reduces the amount of compound needed.

Sylentis S.A. has developed a chemically synthesized double-stranded siRNAs (SYL040012, bamosiran) targeting the β-adrenergic receptor, as an IOP lowering target [56-58]. SYL040012 has undergone Ph1 and Ph2 clinical evaluation. The aim of Ph2 evaluation was to assess the tolerability and IOP lowering effect of three different doses of SYL040012 eye drops administered once a day over a period of 14 days to subjects with

increased IOP or glaucoma. The dose of 300 µg/eye/day afforded a statistically significant reduction in IOP at day 14 when compared to placebo. Bamosiran was very well tolerated with only a 14.6% of the patients reporting an adverse event; most of these events (80%) were of mild intensity [59,60]. Arrowhead Pharmaceuticals Inc. developed interfering RNAs that silence connexin 43 (Cx43) mRNA expression in the eye. Cx43 expression has been related to AH production [61], thus silencing Cx43 mRNA expression should result in the lowering of intraocular pressure in patients with IOP-related conditions. The siRNA of the invention is a double-stranded nucleic acid molecule comprising two nucleotide strands, each strand having from 19 to about 28 nucleotides. These siRNAs caused a dramatic reduction in Cx43 protein expression, determined by western blot using an anti-Cx43 antibody, at the 10 and 1 nM siRNA concentrations. Of the four siRNAs tested, siCx43 #3 appeared to be the most potent, causing a greater level of knock-down at the 0.1 nM concentration than the other three siRNAs [62]. Arrowhead Pharmaceuticals Inc. also developed siRNA for inhibition of expression of hypoxia-inducible factor-1α (HIF-1α), the protein encoded by HIF1A mRNA. Higher expression of HIF-1α in glaucomatous human donor eyes was noticed, suggesting the importance of hypoxia signaling mechanism in the pathogenesis of glaucoma [63]. The invention comprises an effective amount of interfering RNA having a length of 19 to 49 nucleotides and a pharmaceutically acceptable carrier, the interfering RNA comprising a sense nucleotide strand, an antisense nucleotide strand, and a region of at least near-perfect contiguous complementarity of at least 19 nucleotides. Each of the four HIF1A siRNAs reported in the patent reduced HIF-1α protein expression significantly at 10 nM relative to the control siRNAs. However, siHIF1A #3 and siHIF1A #6 also silenced HIF-1α protein expression significantly at 0.1 nM, indicating that these HIF1A siRNAs are particularly effective relative to siHIF1A #1 and siHIF1A #5. Finally, Arrowhead Pharmaceuticals Inc. developed interfering RNAs that target Frizzled Related Protein-1 (FRP-1) mRNA, thus interfering with the Wnt signaling pathway and preventing a cascade of events related to glaucoma and pre-glaucoma cellular activity. One double-stranded FRP1-siRNA showed significant inhibition of FRP1 mRNA at day 1 (22%) and at day 3 (32%) [64]. Humphries and coworkers developed RNAi inducing agents, that result in production of an siRNA or shRNA to target junction proteins expressed in the tight junction complex joining Schlemm's canal endothelial cells (SCEC) in the eye, for the opening of these tight junctions. It has been demonstrated that IOP is due to elevated resistance to AH outflow, and that resistance is modulated by a synergistic hydrodynamic interaction between juxtacanalicular tissue and Schlemm's canal

endothelium [65]. The siRNA developed down-regulates selected tight junction (TJ) components of endothelial cells lining the inner wall of Schlemm's canal such as Claudin-11, Tricellulin and Zo-1 increasing the paracellular spaces between these cells, facilitating the flow of AH across the inner wall into the SC [66].

One of the major challenges of developing an RNAi therapeutic is to develop an efficient delivery into the cell type of interest. RXi Pharmaceuticals Inc. developed a class of small, hydrophobic, asymmetric RNAi compounds. These compounds, termed "self-delivering rxRNAs" (sd-rxRNA®), have a small duplex region of <15 base pairs, containing a fully phosphorothioated single-stranded tail. sd-rxRNA® readily enters cells and tissues without the requirement for a delivery vehicle. When comparing sd-rxRNA compounds with stabilized siRNAs *in vitro* (in ARPE-19 cells) and *in vivo* (intravitreal injection in mouse and rabbit eyes), treatment with sd-rxRNAs resulted in uniform cellular uptake, while no detectable cellular uptake was observed with stabilized siRNAs either *in vitro* or *in vivo*. Moreover, both *in vitro* and *in vivo* delivery of sd-rxRNAs resulted in a significant reduction of the targeted mRNA levels (CTGF and TGFβ) [67]. The invention provides interfering RNA molecule-ligand conjugates, wherein the ligand can bind to a low-density lipoprotein receptor (LDLR) or LDLR family member. The invention also provides methods of using the conjugates for delivering an interfering RNA molecule into a cell *in vitro* or *in vivo*. In one aspect, an interfering RNA molecule-ligand conjugate of the invention can be used to deliver an interfering RNA molecule to an eye of a patient.

Other advanced techniques that are under development are anti-sense oligonucleotides (ASO) and the areas of CRISPR-Cas9 gene editing [68]. One of the earliest reports describes an adenovirus-5 viral vector to deliver CRISPR-Cas9 to lower IOP in a mouse model of primary open angle glaucoma (POAG) (Tg-MYOCY437H). Researchers achieved statistically lower IOP in the animals treated with adenovirus delivered CRISPR-Cas9 targeting the mutated myocilin protein for 2 months after the initial injection [68,69]. Editas Medicine Inc. patented a CRISPR-Cas-related method for treating POAG, but no data on the therapeutic efficacy are reported in the patent [70]. Isarna Therapeutics Gmbh developed ISTH0036, an antisense oligonucleotide selectively targeting TGF-β_2 administered as an intravitreal injection [71]. ISTH0036 completed Ph1 clinical trial. Single-dose ISTH0036 administration of 67.5 µg or 225 µg resulted in IOP values persistently lower than 10 mmHg over the three-month postoperative observation period [72].

7. Conclusion

Glaucoma is a multifactorial disease characterized by a progressive optic neuropathy, visual impairment and retinal ganglionic cells death. The intraocular pressure is the foremost risk factor. Despite the presence of a wide arsenal of therapeutic options, a deep knowledge of the patho-physiology of this disease prompted the research toward alternative targets to be exploited. In this review we have summarized the most recent patents as well the literature survey on these new targets (melatonin receptor agonists, adenosine receptor modulators FAAH inhibitors, endocannabinoids, and RNA interference). Among the above described candidates, clinical studies have been exhaustively performed only on trabodenoson (a strongly selective A_1 agonist) reporting a promising efficacy with limited local and systemic side effects. Unfortunately, a trabodenoson ophthalmic suspension by Inotek Pharmaceuticals failed to achieve the primary endpoint of superiority in the reduction of intraocular pressure in a pivotal phase 3 trial (MATrX-1). Summarizing our data, we can conclude that the endocannabinoid system and FAAH inhibitors were the most investigated as innovative tools to manage glaucoma and its clinical manifestations.

8. Expert opinion

Glaucoma is a challenging chronic disease for both patients and physicians. It requires more than one drug to reduce the intraocular pressure at the physiologically benign level. Over time, patients may loss adherence to multiple topical administrations or become refractory to therapeutic compounds. Fortunately, the available arsenal to struggle with glaucoma has been improving rapidly with particular emphasis on derivatives displaying multiple pharmacological mechanisms and acting toward the trabecular meshwork (TM)/Schlemm's canal/conventional outflow pathway (rho kinase inhibitors and nitric oxide donating compounds) [73]. The new FDA-approved drugs reduce the outflow resistance offering additivity and complementary to current therapeutics. Alternative therapies offer long-term IOP lowering action or allow the patient to take advantage of innovative delivery systems [74] to reduce long lasting topical drop self-administration (gene therapy and stem cell strategies) [75,76]. The alternative approaches described in this short review were validated in *in vitro* and *in vivo* animal models, thus representing the best-in-class strategies to follow in order to maintain functional vision outcome in patients. Their molecular mechanisms have been elucidated and some candidates have been exploited in pharmaceutical compositions to assess the enhancements with respect to the generally accepted approaches [77]. Among the ones proposed in this review, adenosine receptor

modulators (trabodenoson) and endocannabinoid system agents (dronabinol, WIN 55,212-2) were the most investigated, despite a large amount of FAAH inhibitors were designed and synthesized. From a medicinal chemistry point of view, the inventors not only aimed at improving the efficacy, but also the metabolic stability of their chemical scaffolds (usually based on the carbamate function). All these targets have been studied for their efficacy alone or in combination with other agents (e.g., FAAH and MAGL inhibitors [78]) to better achieve the therapeutic goal.

Conversely, in our opinion, little interest has been focused on the future of glaucoma therapy related to the specificity of each individual patient (e.g., genetic make-up), Toll-like receptors (TLRs) crucial for the production of inflammatory cytokines and up-regulated by elevated IOP in human glaucomatous eye [79], neuroinflammation [80], and the beneficial neuroprotective impact on the outflow channel and to the optic nerve of the dietary antioxidant components such as polyunsaturated fatty acids, *Ginkgo biloba* leaf or bilberry extract, and other plant polyphenols [81]. Recently, the promising results of a multicenter, controlled trial corroborated the neuroprotective effects of Coqun®, an ophthalmic solution of Coenzyme q10 and Vitamin E, in patients affected by primary open-angle glaucoma, registered in previous pre-clinical studies and small non-controlled clinical trials both *in vitro* and *in vivo* [82].

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

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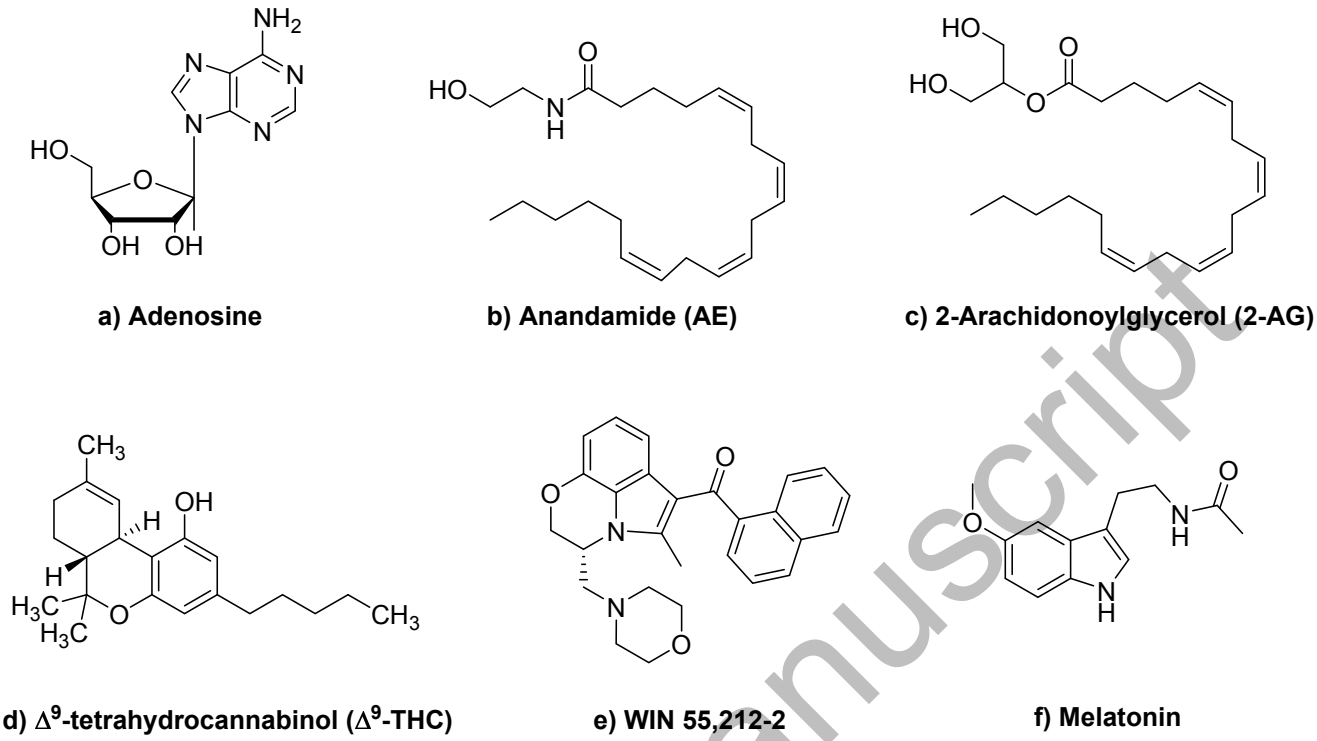


Figure 1. Chemical structures of adenosine, AE, 2-AG, Δ^9-THC, WIN 55,212-2 and melatonin.

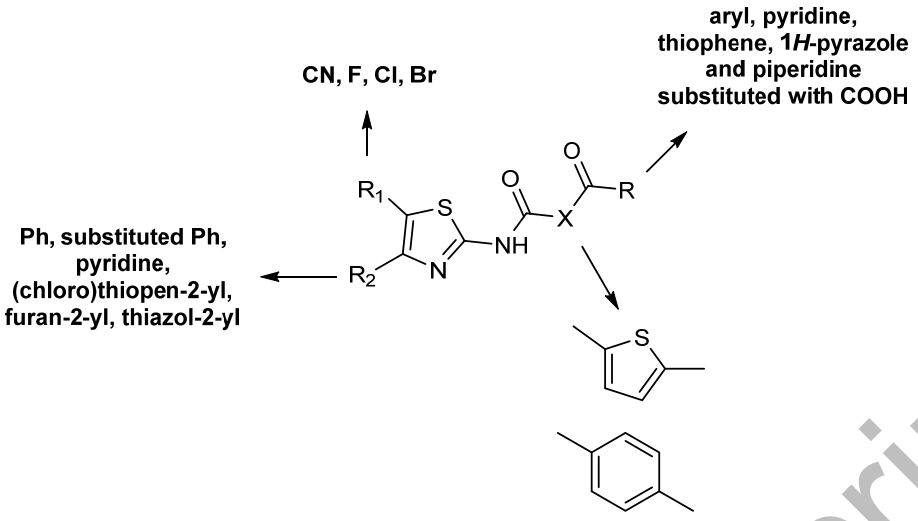


Figure 2. 2-Amido-1,3-thiazoles as modulators of A_3 receptor.

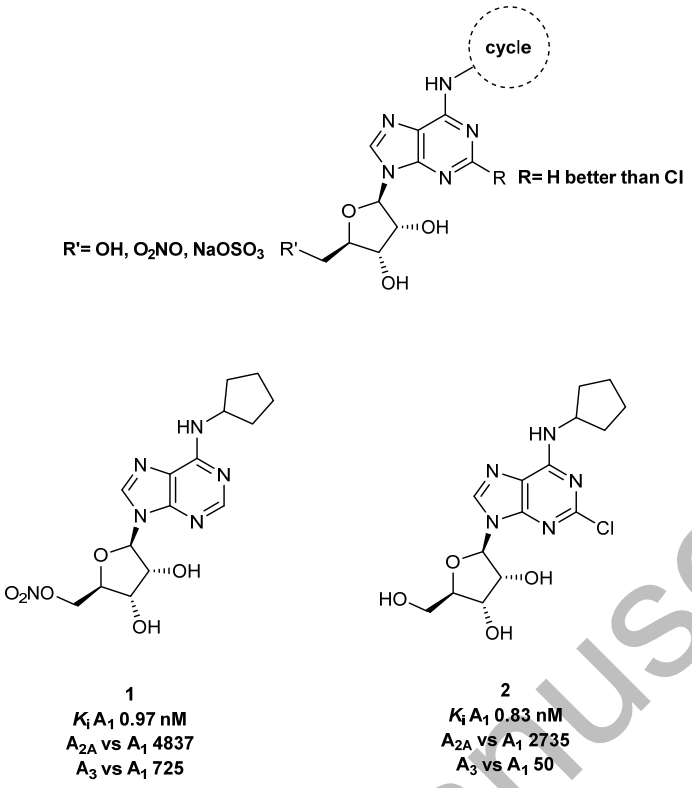


Figure 3. Selective adenosine-based agonists of A_1R.

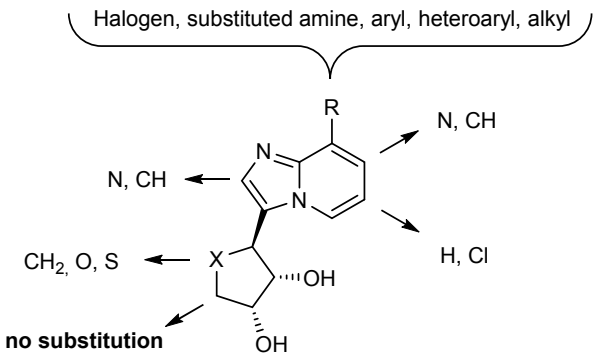


Figure 4. Chemical modifications carried out on the heterocyclic scaffold developed for the A_3 receptor antagonism.

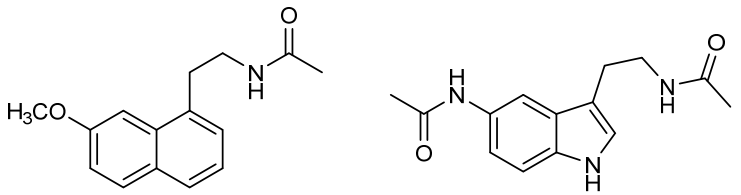


Figure 5. Melatonin analogues tested for their ability to reduce IOP.

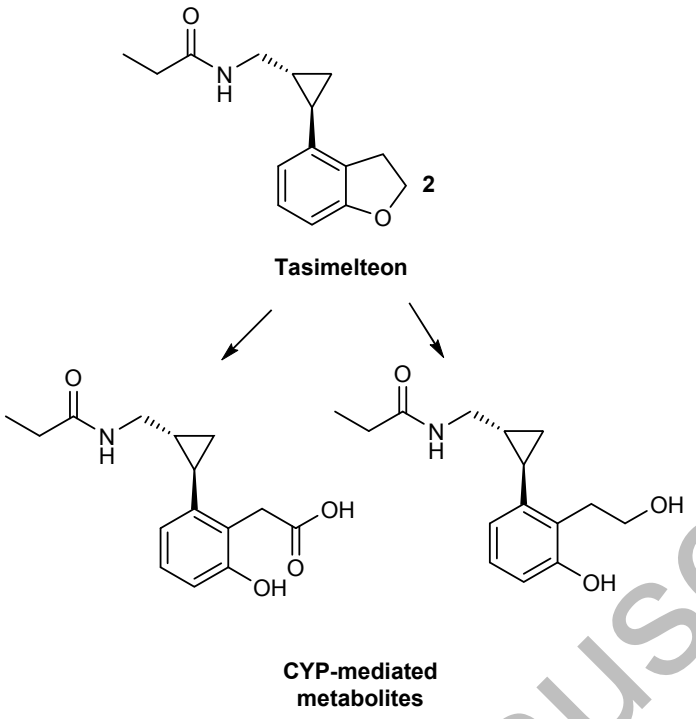
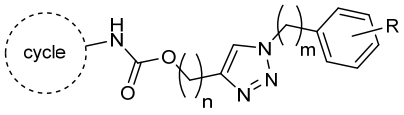


Figure 6. Tasimelteon and its metabolites.

Table 1. General structure of *O*-alkylcarbamates as FAAH inhibitors and the two most potent derivatives.



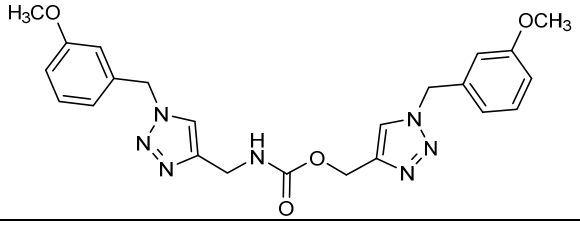
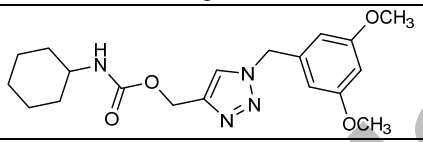
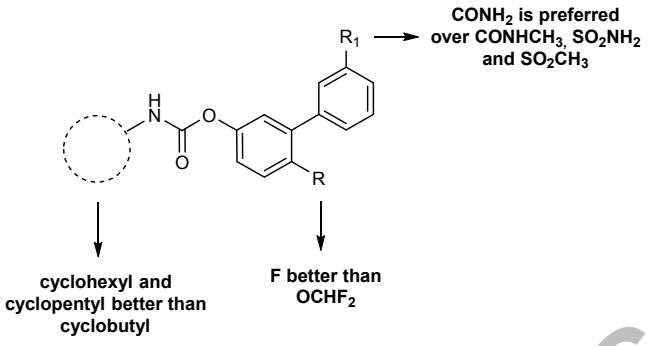
Compound	Structure	IC_{50} rFAAH (μM)	IC_{50} hFAAH-1 (μM)
3		0.004	0.004
4		0.011	0.004

Table 2. SAR studies regarding *O*-arylcarbamates as FAAH inhibitors and examples of the most promising derivatives.



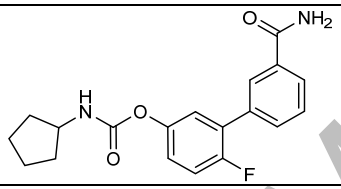
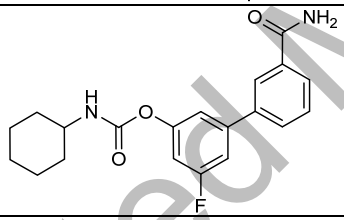
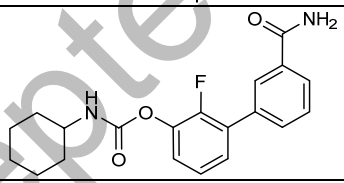
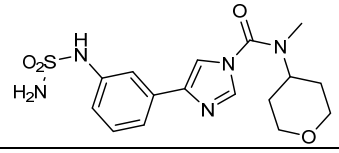
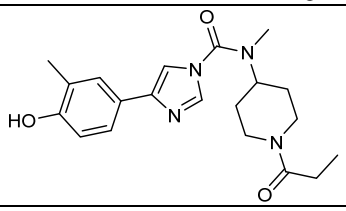
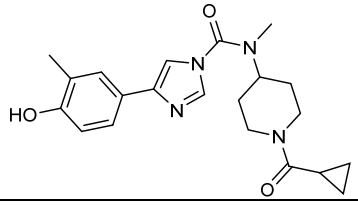
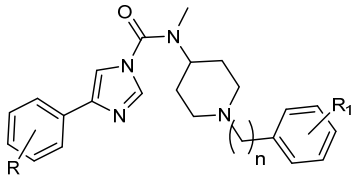
Compound	Structure	IC_{50} rFAAH (µM)	IC_{50} hFAAH-1 (µM)
5		2.9	0.84
6		0.23	0.87
7		1.9	0.89

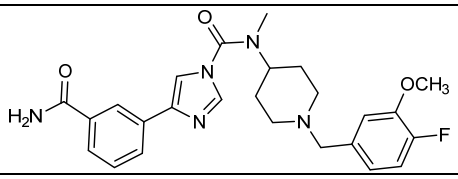
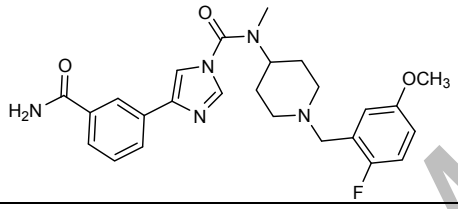
Table 3. The best imidazole-based carboxamide FAAH inhibitors.

Compound	Structure	FAAH activity % (brain)[a]	FAAH activity % (liver)[a]	Peripheral selectivity
8		117	4.9	0.042
9		81.7	1.5	0.018
10		91.7	1.8	0.020

[a]at 3 mg/Kg, po, after 8 h.

Table 4. General structure of urea-based derivatives as water-soluble FAAH inhibitors and the two most potent compounds.



Compound	Structure	Salified with	FAAH activity % (brain)[a]	FAAH activity % (liver)[a]
11		HCl	87.7	13.1
12		HCl	96.0	12.8