MEDICINAL CHEMISTRY RESEARCH

REVIEW ARTICLE

Adenosine receptors as promising targets for the management of ocular diseases

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Abstract
The ocular drug discovery arena has undergone a significant improvement in the last few years culminating in the FDA approvals of 8 new drugs. However, despite a large number of drugs, generics, and combination products available, it remains an urgent need to find breakthrough strategies and therapies for tackling ocular diseases. Targeting the adenosinergic system may represent an innovative strategy for discovering new ocular therapeutics. This review focused on the recent advance in the field and described the numerous nucleoside and non-nucleoside modulators of the four adenosine receptors (ARs) used as potential tools or clinical drug candidates.

Keywords Adenosine receptors (A$_1$AR · A$_{2A}$AR · A$_{2B}$AR · A$_3$AR) · Ocular diseases · Glaucoma · Intraocular pressure · Dry eye · Retinal inflammation

Abbreviations

AC	adenylyl cyclase
AH	aqueous humor
ADME	Absorption, Distribution, Metabolism, and Excretion
AMD	age-related macular degeneration
ARs	adenosine receptors
CHF	congestive heart failure
DE	dry eye
DR	diabetic retinopathy
ERK	signal-regulated kinase
HCC	hepatocellular carcinoma
INL	the inner outer layer
IOP	intraocular pressure
IP3	inositol triphosphate
MMP-2	matrix metalloproteins
NASH	nonalcoholic steatohepatitis
NFL	nerve fiber layer
NPE	non-pigmented ciliary epithelial
NTPDase	ecto-nucleoside triphosphate diphosphohydrolase
ONH	optic nerve head
ONL	outer nuclear layer
PACG	primary angle-closure glaucoma
PD	Parkinson's disease
PLC	phospholipase C
POAG	primary open-angle glaucoma
RGCs	retinal ganglion cells
RNFL	retinal nerve fiber layer
ROCK	rho kinase
RPE	retinal pigment epithelium
SC	scleroderma
SNMS	servo-null micropipette system
TM	trabecular meshwork
VEGF	vascular endothelial growth factor.

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Introduction

The eye is a unique and complex organ considered the "window to the brain" and the disruption of any of its tissues can end up in ocular discomfort, visual impairment, or loss of vision. Its anatomical complexity makes extremely

difficult the understanding of disease pathogenesis and ophthalmic drug discovery challenging. With respect to other tissues of the human body, the eye owns several unique features. It is an immune-privileged site that actively restrains some immune and inflammatory responses due to the presence of the blood-retinal barrier (BRB). BRB is a physical barrier that allows solutes' movements across the vascular bed and affords a primordial defense from invaders. Moreover, some of his tissues are transparent and avascular, making the eye easy to reach clinically.

Recent data released by WHO reported that about 1 billion people suffer from vision impairment, and most of them are over 50 years [1]. It seems evident that the leading causes of visual impairment are age-related and include the following ocular diseases: glaucoma, dry eye (DE), retinal inflammation, age-related macular degeneration (AMD), and diabetic retinopathy (DR).

Glaucoma is an aetiologically complex optic neuropathy distinguished by the accelerated death of axons and retinal ganglion cells (RGCs). Regretfully, it is considered the second most frequent cause of irreversible blindness worldwide [2–4]. In 2014, Tham et al. have performed a systematic population-based meta-analysis study anticipating that the global glaucoma burden will grow from 76 million in 2020 to 112 million in 2040, mainly affecting people living in low-income countries such as Africa and Asia [5]. The disease covers a broad and complex group of optic neuropathies defined by a progressive loss of RGCs and associated alterations in the optic nerve head (ONH) and the retinal nerve fiber layer (RNFL), with resultant vision loss [6]. Even though the exact explanation of the etiopathogenesis is yet unclear, the elevation of intraocular pressure (IOP) represents the only known treatable risk factor [7]. Hence, IOP might be considered the pillar target in contemporary glaucoma management [8], and its reduction is deemed as the primary efficacy endpoint in almost all ongoing glaucoma clinical trials.

Primary angle-closure glaucoma (PACG) and primary open-angle glaucoma (POAG) are the two most common primary glaucoma types. Although they have some common characteristics, they have completely different etiopathogenesis, leading up to different pharmacological approaches. POAG is the most prevalent type of glaucoma in western Europe and the United States. On the contrary, PACG is highly frequent in China and other Asian countries.

A broad range of pharmacological and surgical options are available for glaucoma treatment, including eye drops and laser procedures. All are meant to protect the optic nerve by decreasing eye pressure. In spite of the full availability of different options, eye drops often represent the first choice for treating patients. Eye pressure can be safely controlled for many years using a combination of drugs and laser treatments. The mechanism of action by which the eye drops operate through is by helping the eye's fluid to drain off or by cutting down the fluid load produced by the eye. Drug treatments used for glaucoma management are ranked based on their active components, including carbonic anhydrase inhibitors, alpha agonists, beta-blockers, and prostaglandin analogs. Patients who demand more than one type of medication are often treated with a combination of different drugs. The older class of cholinergic agonists (such as carbachol and pilocarpine) represents the first class of drugs used to manage glaucoma and operates by lowering IOP through increasing aqueous humor (AH) outflow. However, due to their severe systemic side effects, they have now fallen into disuse. The brand-new FDA-approved rho kinase (ROCK) inhibitor, Rhopressa® (Netarsudil ophthalmic solution, Aerie Pharmaceuticals), reduces IOP by lowering the elevated pressure in patients with POAG or ocular hypertension. The drug exerts its pharmacological effect by increasing the outflow of AH or by the inhibition of norepinephrine transport along with reducing the episcleral venous pressure [9]. Just recently, the US FDA approved Roclatan® (a combination of latanoprost and netarsudil) to manage POAG [10]. The combination relies on the ability of netarsudil to loweringIOP complemented by the latanoprost-mediated increase of outflow. Vyzulta® (latanoprostene ophthalmic solution, LBN), licensed by Bausch & Lomb and FDA approved in 2017, is nitric oxide (NO)-donating PGF2α analog that demonstrated a high potential for lowering IOP [10].

The DE syndrome is often known by a variety of terms ranging from keratitis sicca, keratoconjunctivitis sicca (KCS), to dysfunctional tear syndrome. In DE the eye turns into dry either because there are not enough tears being produced or because there is an abnormal evaporation of tears. In an eye suffering from DE, the tears' content seems to be impaired with a lesser concentration of proteins, and one of the characteristic features of DE is ocular surface inflammation. Tear supplements represent the first-line treatments for mild dry eye disease (DED), whereas cyclosporine and Lifitegrast® result to be appropriate choices in patients with more severe symptoms [11].

Neuroinflammation, a feature of chronic neurodegenerative diseases, plays an essential role in several retinal degenerative diseases' pathophysiology [12]. The retina is a complex and highly organized tissue made of neuronal and non-neuronal cells. Neurons aside, the retina also contains endothelial cells, pericytes, and glial cells named astrocytes, microglia, and Müller cells. An intact BRB is essential in protecting neural tissues from harmful materials and maintaining the retina's neural functions. It is well documented that an inflammatory insult impacts negatively on the BRB's integrity. The immune cells respond to an insult by secretion of pro-inflammatory cytokines that contribute to

endothelial cell dysfunction, inducing retinal cell death, and BRB breakdown. It has been reported that a pro-inflammatory environment is a breeding ground for the onset of glaucoma and DR. Based on these findings, fighting the actions of pro-inflammatory cytokines has been proposed as an innovative therapeutic strategy for managing retinal inflammation [13].

AMD is the leading cause of severe and irreversible loss of vision in patients aged 60 or over [14]. This disease causes damage to the macula, the central part of the retina responsible for high-resolution vision, and is due to an anomaly (e.g., structural changes and a general decline of essential functions) of retinal pigment epithelium (RPE) [15]. Current known risk factors include age, people with a family history of AMD, smokers, ethnicity (high incidence in Caucasians), gender (high incidence in women), and race. Anti-vascular endothelial growth factor (VEGF) therapy is regarded as the first-line treatment for AMD and aims to reduce edema and neovascularization (new blood vessel growth). Anti-VEGF therapy has tremendously decreased the prevalence of AMD globally [16].

DR is a complication of diabetes mellitus that leads to vision loss through progressive damage in retinal blood vessels [17]. People of all ages who have had diabetes for more than 20 years have the maximum probability of developing DR. The recent therapeutic trend for managing DR foresees the intravitreal administration of anti-VEGF with the goal of improving vision with fewer side effects. On the other side, the laser therapy facilitates to stabilize the vision.

The ocular therapeutic field has achieved significant improvements in the last decade, as evidenced by the FDA approval of Lifitegrast® (an integrin antagonist) for DE, Brolucizumab® (a recombinant humanized antibody fragment) for wet AMD, Roclatan® (a combination product), Vyzulta® (a NO-donating PGF2α analog), and Rhopressa® (ROCK inhibitor) for glaucoma, Luxturna® (gene therapy) for retinitis pigmentosa and Dextenza® (a corticosteroid) for ocular inflammation. However, despite all the efforts to develop new drugs, generics, and innovative therapies, the successful management of ocular diseases still lingers as an unmet medical need.

Among the multitude of transmitters/modulators present in the eye, nucleosides and nucleotides emerge as peculiar molecules able to regulate many physiopathological and biochemical processes.

For this reason, the purinergic signaling pathways are increasingly becoming recognized in ophthalmology as a potential target for pharmaceuticals. In particular, the adenosine signaling pathway, including its sub-receptor biology, is increasingly being investigated to develop new ophthalmic drugs. Different categories of agonists and antagonists of various subtypes of G protein-coupled receptors such as adenosine receptors (ARs) and P2Y receptors (P2YRs), and ATP-gated P2X receptor ion channels (P2XRs) are currently under investigation as potential agents for the treatment of DE, ocular inflammation, and glaucoma [18–20]. Three out of four subtypes of ARs (A_1, A_{2A}, and A_3, respectively) have extensively been exploited to treat eye disorders, yielding some clinical candidates [21–23].

It is also worth mentioning that extracellular concentrations of dinucleoside polyphosphates are significantly increased in pathological states, activating P2YRs and P2XRs throughout the eye. P2YRs and P2XRs have multiple functions in the eye, and their modulation has been explored for ophthalmic drug discovery purposes. P2Y2R and P2Y6R agonists lower IOP, whereas the P2X7R antagonists thwart the ATP-induced neuronal apoptosis in the retina. For the above reasons, the modulators of the "purinome" present in the eye might represent a valuable source of new therapeutic options for ocular diseases. In this context, this review provides an overview of recent ocular drug discovery literature covering this emerging and dynamic field.

Rationale for treating ocular diseases via adenosine receptor agonists and antagonists

The endogenous nucleoside modulator adenosine is continuously produced both at the intracellular and extracellular levels. It has a very short half-life (~1.5 s), and it is quickly metabolized to inosine and hypoxanthine. In the CNS, adenosine controls synaptic activity, neurotransmitter release, and neuronal excitability, acting as a neuromodulator. It may also act as an intracellular messenger embroiled in pathological and physiological processes (e.g., inflammation). Low extracellular levels of adenosine (in the nanomolar level) are deemed as positive signs of cellular homeostasis, as usually occurs during physiological conditions. In response to cellular stress or pathological conditions (e.g., ischemia or tissue hypoxia), the adenosine levels considerably increase, reaching the micromolar range [24]. If we compared the normotensive individuals with ocular hypertensive patients, it could easily be observed that the adenosine levels in AH are significantly boosted [25, 26].

The biological functions of adenosine are mediated by activation of four different G-coupled ARs: A_1AR, $A_{2A}AR$, $A_{2B}AR$, and A_3AR, respectively. All of them are implicated in inhibiting and stimulating cyclic AMP (cAMP) production in several manners. By giving details, A_1AR and A_3AR down-regulate cAMP levels inhibiting adenylyl cyclase (AC), whereas $A_{2A}AR$ and $A_{2B}AR$ activate AC, boosting cAMP production.

Fig. 1 Chemical structures of compounds 1-6
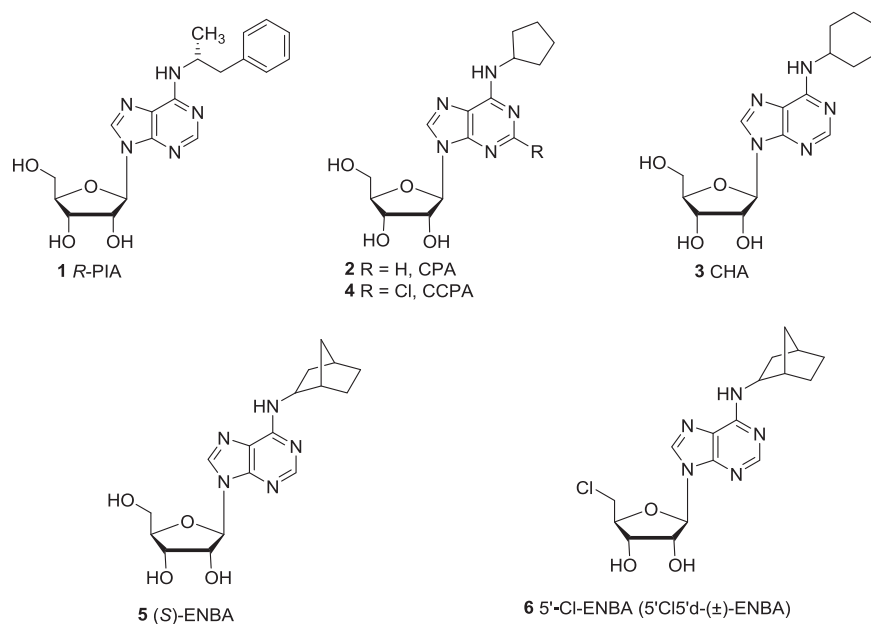
Besides being widely distributed in the whole body, ARs are worthy of special mention in numerous ocular tissues such as the ciliary body, trabecular meshwork (TM), scleroderma (SC), and retina [27]. Activation or inactivation of ARs crashes onto AH formation, outflow, and as a consequence on IOP homeostasis. Furthermore, ARs are implicated in retinal function neuronal, deeply affecting neuronal survival and blood flow [25]. For the above reasons, the modulation of ARs has been explored for several decades as an insightful strategy for treating ocular diseases. Nowadays, there is a readily-available arsenal of potent agonists and antagonists associated with each of the four AR subtypes. However, cautiousness shall be used when they are applied to pharmacological experiments due to the selectivities that are not completely unequivocal for each AR subtype [19, 20]. For example, the A_1AR agonist R-phenyl isopropyl adenosine (R-PIA, **1**, Fig. 1) causes reductions in body temperature and heart rate in mice which are dependent on the presence of the A_3 subtype. Indeed, genetic knockout of the A_3AR corroborates this hypothesis and prevents some of these effects [19, 26].

A_1AR agonists

The A_1AR was the first AR to be cloned and is widely expressed in the brain and peripheral tissues (e.g., kidney, liver, heart, smooth muscle, fat cells, and eye) [28]. Just recently A_1AR has been well characterized in the retina of Rhesus monkey and has been localized on the outer plexiform layer (OPL), in the RPE, in the ganglion cell layer (GCL), and on photoreceptors [29]. A_1AR plays an essential role in inflammation, and in the brain the activation of A_1AR weakens the ischemic neuronal

impairment [30]. Retinal ischemia phenomenon is attributable to glaucoma, diabetes, central artery occlusion, and retinopathy and always ends up with vision loss. The activation of A_1AR by selective agonists protects against ischemic impairment, probably through a mechanism that involves Müller cells. Unfortunately, this protection depends on ischemia duration and is not effective in prolonged ischemia. Another effect of A_1AR activation is the attenuation of the damage caused by N-methyl-D-aspartate (NMDA). Adenosine acts as a neuromodulator of RGCs by inhibiting the glutamate-induced calcium influx through the A_1AR activation. In addition, adenosine binds to A_1AR present on the TM, increasing the AH outflow through secretion of matrix metalloproteins (MMP-2) [31]. This process has the effect of lowering IOP [32].

Adenosine is the primary endogenous agonist of the A_1AR, whereas countless A_1AR agonists with every disparate modification at ribose moiety and the adenine N^6 and C2 positions have been disclosed in the last two decades [33–40]. Before the discovery of the A_3 subtype, several agonists have been identified as selective for A_1AR (Fig. 1) since it has been defined their A_1AR selectivity based on their affinity at the rat A_1AR vs. $A_{2A}AR$ [41]. However, a comparison of N^6-cyclopentyladenosine (CPA, **2**) and N^6-cyclohexyladenosine (CHA, **3**) binding affinities at the four human ARs showed only a limited A_1AR selectivity compared to the A_3AR [20]. Later on, the A_1AR full agonist 2-chloro-N^6-cyclopentyladenosine (CCPA, **4**, Fig. 1) showed lower than full efficacy at the A_3AR, acting as a partial agonist or an antagonist [42] and (S)-ENBA (**5**, Fig. 1) emerged as an extremely selective A_1AR agonist (> 1000-fold) respect to rat and human A_3AR [43].

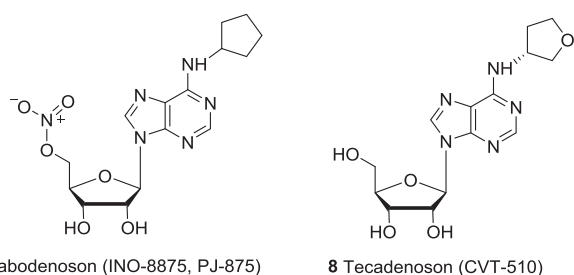**7** Trabodenoson (INO-8875, PJ-875)   **8** Tecadenoson (CVT-510)

Fig. 2 Chemical structures of trabodenoson (**7**) and tecadenoson (**8**)

However, among the most selective A_1AR agonists deserve particular mention 5′-chloro-5′-deoxy-N^6-(±)-endo-norborn-2-yl-adenosine (5′Cl5′d-(±)-ENBA or 5′-Cl-ENBA, **6**), developed in our laboratory, which had exceptionally high A_1AR affinity and selectivity versus the other AR subtypes [44, 45].

Trabodenoson (INO-8875, PJ-875, **7**, Fig. 2), the 5′-nitrate ester derived from CPA, was developed using a 3D structure and rational drug design techniques to create adenosine mimetic with a high affinity and selectivity for the A_1AR ($K_i = 0.97$ nM, partial agonist). Based on trabodenoson's high selectivity, a consistent reduction in IOP has been observed without the transient IOP increase associated with other less specific compounds that interact with other AR subtypes [21, 46]. Based on this finding, it reached the clinical trials in 2015 as an ophthalmic formulation for ocular hypertension (OHT) and POAG [47] and advanced in the phase 3 trial (ClinicalTrials.gov Identifier: NCT02565173). Unfortunately, the development was discontinued in 2017 due to failure to achieve its primary endpoint to better reduce IOP compared to placebo [23]. Despite not yet published, an important study of phase 2 (NCT02829996) reported the combination of trabodenoson (two different doses 3.0% and 6.0%) and latanoprost (0.005% or 0.0025%) in patients affected by OHT or POAG. Unfortunately, no significant clinical outcomes have been observed with the combination, and in 2018, Inotek, after merging with Rocket Pharma, decided to stop all the development activities.

The potent and selective A_1AR full agonist Tecadenoson (CVT-510, **8**, Fig. 2) has been evaluated in a phase 3 trial to terminate induced paroxysmal supraventricular tachycardia (PSVT) without showing the typical side effects caused by stimulation of other AR subtypes. Nevertheless, as other adenosine A_1AR agonists (capadenoson and selodenoson), has not fared well in cardiovascular disease, and it was discontinued in 2009.

5′-Cl-ENBA (**6**, Fig. 1) is a potent and selective A_1AR full agonist. It binds to the human A_1AR (hA_1AR) with an affinity at the subnanomolar level ($K_i = 0.5$ nM), showing a relevant selectivity versus the hA_3AR (2530-fold) [45]. It is also able to penetrate the blood-brain barrier efficiently [48].

As recently reported by us, 5′-Cl-ENBA can reduce pain in the formalin test or prevent mechanical allodynia and thermal hyperalgesia in the spared nerve injury (SNI) model of neuropathic pain in mice, without exerting any drawbacks on motor coordination and blood pressure [49, 50]. It has also been reported that it can decrease l-DOPA-induced dyskinesia and locomotor activity in a mice model of Parkinson's disease (PD) [51], as well as the harmaline-induced tremor in rats [48].

The advantage of 5′-Cl-ENBA over the other A_1AR agonists lies in the fact that at effective doses, it does not affect either motor coordination, blood pressure, or heart rate, corroborating the hypothesis that it is scarcely active on the peripheral A_1AR. Ossowska et al. has recently investigated 5′-Cl-ENBA as a potential antipsychotic agent [52]. The authors reported that the hyperlocomotion induced by either amphetamine (1 mg/kg sc) or MK-801 (dizocilpine) (0.3 mg/kg ip) was inhibited by 5′-Cl-ENBA (0.1 mg/kg ip). A selective A_1AR antagonist entirely reverted the effect.

5′-Cl-ENBA has never been studied in experimental models of glaucoma and ocular hypertension. However, given its high selectivity at A_1AR and the distinctive feature to not generate cardiovascular drawbacks, it should be taken into consideration for further studies.

Non-nucleoside A_1AR partial agonists

As a consequence of the crucial involvement of A_1 agonists in heart rate and blood pressure regulation, the therapy for glaucoma or ocular hypertension built on A_1AR agonists suffers from significant limitations [53]. For this reason, several non-nucleoside A_1AR partial agonists have been developed [54]. The rationale for using A_1AR partial agonists is based on avoiding receptor desensitization and on achieving a specific tissue selectivity of the effects without generating drawbacks [55, 56].

In 2012 Bayer HealthCare Pharmaceuticals patented a series of dicyanopyridine derivatives as potential therapeutic agents for the treatment and/or prophylaxis of glaucoma and ocular hypertension. As the outcome, the authors found out that some dicyanopyridine derivatives, and specifically capadenoson (BAY68-4986, **9**, Fig. 3), lower IOP after topical eye application without affecting hemodynamics and the cardiovascular system [57]. Capadenoson (**9**) has been in the clinical evaluation (phase 2) in patients with persistent or permanent atrial fibrillation (AF) and angina pectoris (NCT00568945 and NCT00518921). However, **9** was withdrawn when it failed to show heart rate reduction for patients with atrial fibrillation. Just recently, it has been reported that **9** also activated human $A_{2A}AR$, even though the affinities at A_1AR and $A_{2A}AR$ were not entirely comparable [58].

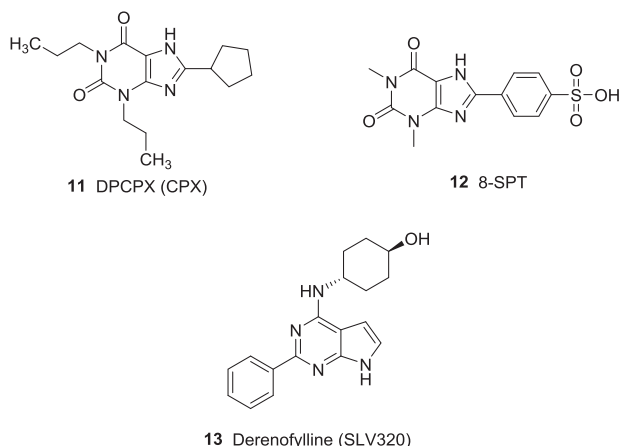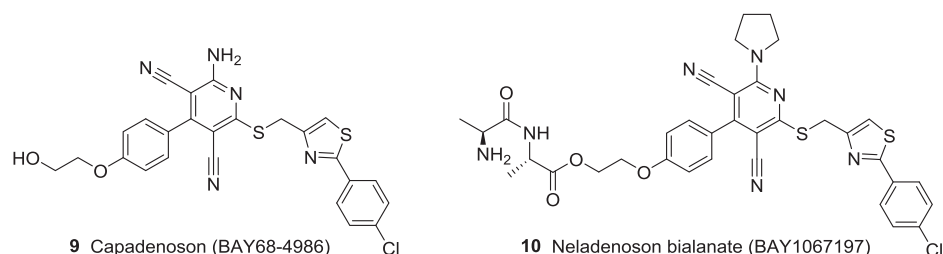Fig. 3 Chemical structures of capadenoson (9) and neladenoson bialanate (10)

9 Capadenoson (BAY68-4986)

10 Neladenoson bialanate (BAY1067197)

11 DPCPX (CPX)

12 8-SPT

13 Derenofylline (SLV320)

Fig. 4 Chemical structures of DPCPX (11), 8-SPT (12), and derenofylline (13)

Neladenoson bialanate (BAY1067197, **10**, Fig. 3), a dipeptide ester prodrug of neladenoson, has been clinically evaluated by Bayer HealthCare Pharmaceuticals in patients with chronic heart failure (NCT03098979). Unfortunately, the expected beneficial cardiac effects have not been proven while it has been observed a consistent impairment in renal function [59]. For these reasons, in 2020, Bayer decided to do not further support the development of **10**, and the drug has been discontinued (NCT04320771).

A₁AR antagonists

While the activation of A_1AR lowers IOP in rabbits and monkeys ([60]; Tian et al., 1997), the selective action of A_1AR antagonists such as DPCPX (**11**), 8-(p-sulfophenyl) theophylline (8-SPT, **12**), and derenofylline (SLV320, **13**, Fig. 4) raises IOP instead [61]. In a pioneering work aimed to evaluate the role of AR activation in epinephrine-induced changes in ocular function, Crosson et al. reported that 8-SPT (**12**) enhanced initial hypertension by inhibiting the epinephrine-induced reduction in IOP [62]. On the other hand, the A_1AR antagonists DPCPX diminished recovery after retinal ischemia in rats, while an $A_{2A}AR$ antagonist considerably protected the retinal function and structure even with long-lasting ischemia [63].

The pyrrolo-pyrimidine derivative **13** has never been studied as potential drug candidate for ocular diseases.

However, it is in phase 2 clinical trials by Abbvie (previously Solvay Pharmaceuticals and Abbott) for the treatment of congestive heart failure (CHF) and renal failure (NCT00568009). Although it has completed a phase 2 trial, no recent developments have been reported.

Taken together, those data support the idea that A_1AR antagonists hold few promises as ocular drug candidates, given the fact they increase IOP in mice and rabbits [25].

A₂AAR agonists

The human $A_{2A}AR$ was identified in 1992 and has been localized in the retina in several places, including GCL, outer nuclear layer (ONL), and the inner outer layer (INL) [64]. $A_{2A}AR$ has also been detected in microglia and Müller cells [65]. In the last decade, $A_{2A}AR$ has been extensively explored as a potential therapeutic target for lowering IOP or treating retinal disorders. Recently, it has been emphasized that its role played in neuroinflammation, and the modulation of its activity can shield the retinal cells from an inflammatory insult [13, 66].

$A_{2A}AR$ agonists and antagonists have also been widely investigated as ocular disease therapeutics, with particular reference to glaucoma [67]. Selective $A_{2A}AR$ agonists are typically amended at the C2 position and modified at the C5′ of the ribose ring.

A shortlist of $A_{2A}AR$ agonists that have demonstrated to lower IOP and to be useful for the treatment of glaucoma included: OPA-6566 (structure not disclosed), CGS-21680 (**14**), 2-O-Ado (**15**), 2-CN-Ado (**16**), and ATL-313 (**17**) (Fig. 5).

Otsuka Pharmaceutical Co., Ltd. (Otsuka) and Acucela, Inc. in September 2010 signed a co-development and co-commercialization agreement for the development of OPA-6566 for the management of POAG or ocular hypertension. OPA-6566 went through phase 1 and 2 clinical trials (NCT01410188) due to its ability to lowering IOP by enhancing AH outflow via the TM. The trial has been recently completed, but no results have yet been released. Although this Otsuka Pharmaceutical decided to end up its participation in further development activities (e.g., glaucoma) for OPA-6566, exercising its contractual right to terminate the agreement with Acucela. Acucela is now called Kubota Vision and in 2019, announced to have

Fig. 5 Chemical structures of CGS21680 (**14**), 2-O-Ado (**15**), 2-CN-Ado (**16**), ATL-313 (**17**) and apadenoson (**18**)

14 CGS-21680

15 2-O-Ado

16 2-CN-Ado

17 ATL-313 (Evodenoson)

18 Apadenoson (ATL-146-e)
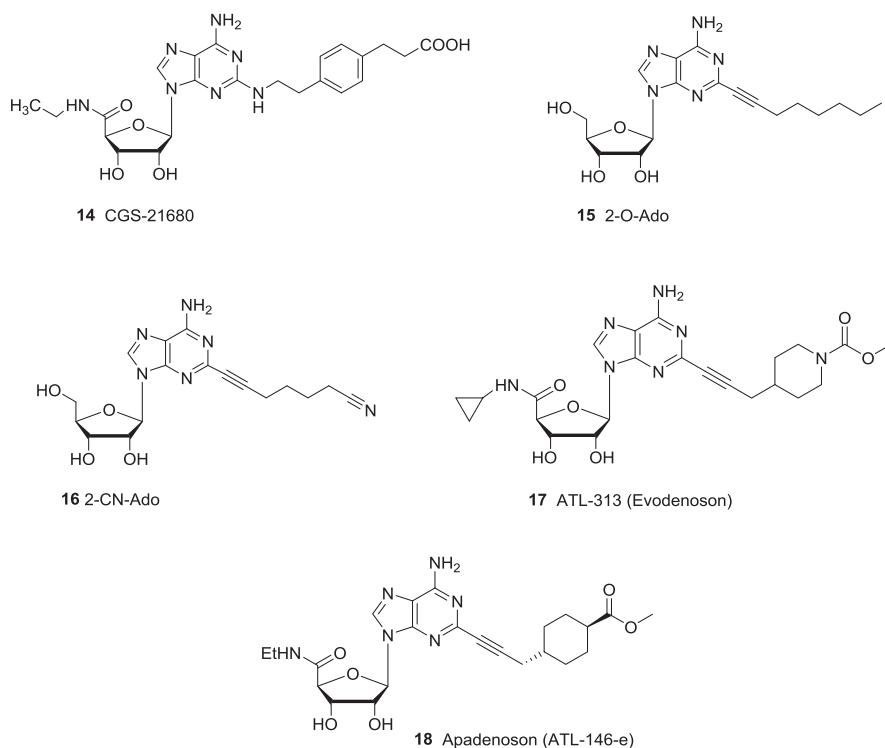
discontinued the phase 1 and 2 for managing glaucoma and ocular hypertension in the US.

In 2005 Konno et al. conducted a study with CGS21680 (**14**) and the 2-alkynyladenosine derivatives 2-O-Ado (**15**), and 2-CN-Ado (**16**) in order to clarify the mechanism underlying the changing of IOP observed in rabbits [67]. The results showed that **15** and **16** decreased the IOP significantly in a concentration-dependent manner, whereas the relatively selective $A_{2A}AR$ agonist **14** slightly decreased IOP. Also, **15** and **16** remarkably increased cAMP levels in the AH after 60 min of their administration. The authors stated that the observed IOP decreasing could be linked to increasing of cAMP levels induced by **15** and **16**, mediated by a rise in outflow facility.

Evodenoson (ATL-313, DE-112, **17**, Fig. 5), a more stable urethane-containing analog of apadenoson (**18**, Fig. 5), has been developed by Santen Pharmaceutical, capitalizing on its property of lowering IOP [23]. In 2012 Santen completed a phase 1/2 clinical trial investigating the safety and efficacy of ATL-313 in lowering IOP in patients with POAG or ocular hypertension (NCT01279083). The study results have been submitted to ClinicalTrials.gov but are not yet publicly available.

Over the past two decades, Pfizer developed an extensive series of N^6-(2,2-diphenylethyl)-adenosine analogs acting as potent and selective $A_{2A}AR$ agonists, and UK-432097 (**19**, Fig. 6) has emerged as the best-in-class [68]. It has been clinically developed in phase 1 for asthma and phase 2 for chronic obstructive pulmonary disease (COPD,
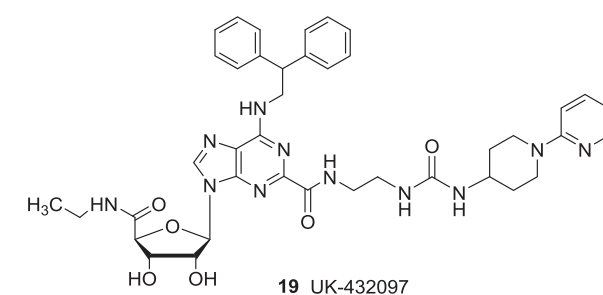
19 UK-432097

Fig. 6 Chemical structure of UK-432097 (**19**)

NCT00430300), failing on both. It was discontinued in 2008 for lacking efficacy [69].

Despite all efforts, the potentiality of $A_{2A}AR$ agonists for the treatment of ocular diseases is uncertain since they have revealed both unfavorable and favorable effects in preclinical and clinical studies [19, 20].

It is worth mentioning that $A_{2A}AR$ agonists had an anti-inflammatory role in RGCs subjected to elevated pressure [70]. As reported by Ahmad et al., the administration of CGS-21680 (**14**) significantly attenuated the expression of inflammatory mediators (e.g., ICAM-1, IL-6, and TNFα) and cell death markers in a mouse model of traumatic optic neuropathy (TON) [66]. TON is one of the significant visual repercussions of severe head trauma. The anti-inflammatory effect of **14** is mediated by blocking extracellular signal-regulated kinase (ERK) activation and further cytokine release in TON. The role played by $A_{2A}AR$ in TON has also
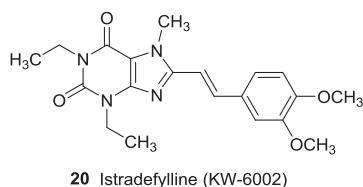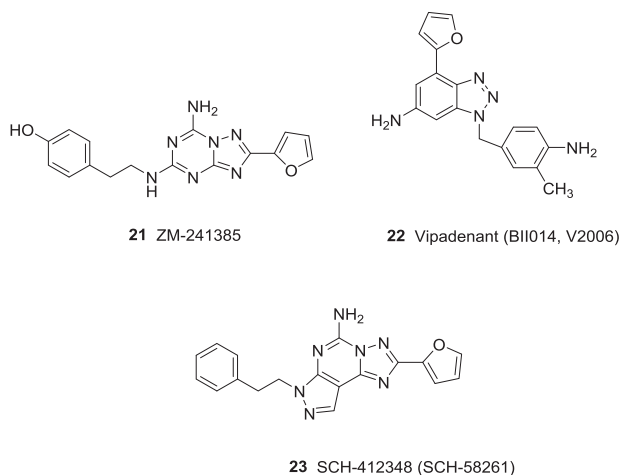
Fig. 7 Chemical structure of istradefylline (KW-6002) (20)

Fig. 8 Chemical structures of ZM-241385 (21), vipadenant (BII014, V2006, 22), and SCH-412348 (SCH-58261, 23)

Fig. 9 Chemical structures of tozadenant (SYN-115, 24), and 25

been evaluated in $A_{2A}AR$ knockout mice, uncovering its potential as a therapeutic target in TON. Given that inflammation is a key player in the pathogenesis of glaucoma [71–73], $A_{2A}AR$ agonists might be helpful. Another crucial function that they have shown, together with A_1AR agonists, is to reduce vascular resistance and increase blood flow to the retina and ONH [25].

$A_{2A}AR$ antagonists

Over the years, several $A_{2A}AR$ antagonists have been synthesized, and if we would divide them by relying on their chemical structure, they can be split into xanthine and nonxanthine derivatives [74]. The xanthine derivative istradefylline (KW6002, 20, Fig. 7), developed by investigators at Kyowa Hakko Kirin, has been one of the first potent $A_{2A}AR$ antagonists reported. Recently Boia et al. Boia et al. [75], reported that the blockade of $A_{2A}AR$ might represent a therapeutic option for the treatment of retinal ischemic diseases (e.g., transient retinal ischemia). For this reason, they evaluated KW6002 against the damage induced by ischemia-reperfusion (I–R). Its oral administration after inducing ischemia reduces microglia reactivity and inflammatory response considerably, affording retina protection. This seminal work has laid the basis for further studies on.
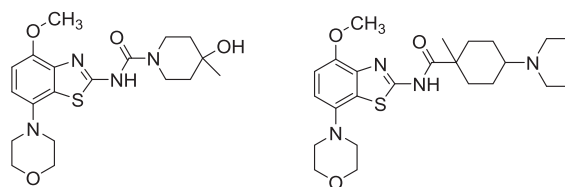
$A_{2A}AR$ antagonists as potential tools for managing retinal ischemic or neurodegenerative diseases. After being approved in Japan in 2013 to treat PD, istradefylline was launched in 2019 in the US (product name: NOURIANZ™) as an add-on therapy to levodopa/carbidopa in adults who are experiencing off episodes. The approval was based on its success in 4 clinical studies that included around 1200 participants. In January 2020, Kyowa Kirin Co., Ltd. announced that its marketing authorization application (MAA), as an adjunctive treatment for PD, had been validated by the European Medicines Agency (EMA).

Highly potent $A_{2A}AR$ antagonists of the new generation are represented by triazolotriazine ZM-241385 (21, Fig. 8), the triazolopyrimidine vipadenant (BII014, V2006, 22), and the pyrazolotriazolopyrimidine SCH-412348 (SCH-58261, 23). The highly potent and selective $A_{2A}AR$ antagonist, ZM-241385 (21), also binds to the human $A_{2B}AR$ with moderate affinity, and its structure in complex with thermostabilised $A_{2A}AR$ adenosine has been reported [76]. 21 also behaves as an inverse agonist [77]. Recently Liu et al. reported the effect of ZM-241385 on microglial activation in experimental glaucoma [78]. The authors showed that ZM-241385 might downregulate the activation of pro-inflammatory cytokines and microglia under the conditions of a high concentration of glutamate and chronic ocular hypertension. They assume this could be one of the plausible mechanisms that protected RGCs in experimental glaucoma. Surprisingly, 21 failed to protect the RGCs, presumably because they own long axons that have to deal with more challenges.

Vipadenant (BII014, V2006, 22), initially discovered by Juno Therapeutics and then acquired by Vernalis and Ligand Pharmaceuticals, is a selective $A_{2A}AR$ antagonist developed as an oral drug for the treatment of PD (phase 2) but later discontinued due to safety concerns [74, 79]. Vipadenant was then licensed out to RedoxTherapies because of its high potential to disrupt an immunosuppressive mechanism of tumor protection [80]. When used in combination with other anticancer drugs, vipadenant is able to generate an improved efficacy for immunotherapies targeting different cancers. Vipadenant is currently in preclinical trials for cancer in the US.

The potent and selective $A_{2A}AR$ antagonist SCH-412348 (SCH-58261, **23**) has been initially identified and developed by Schering-Plough as a potential treatment for neurological conditions such as depression and PD. Given that **23** suffered from poor solubility and was not active when orally dosed, it has been discontinued after having completed phase 2. Starting from the observation that the blockade of $A_{2A}AR$ prevents the neuroinflammatory response and can improve recovery of retinal function after exposure to elevated hydrostatic pressure (EHP), Madeira et al. investigated SCH-412348 [73, 81]. Their results showed that **23** attenuated the release of pro-inflammatory cytokines and the microglia reactivity. Moreover, it prevented ischemia-reperfusion (I-R)-induced cell death resulting from the excessive raising of IOP, by attenuating the neuroinflammatory response. The authors concluded by suggesting that the blockade of $A_{2A}AR$ may play an important role in the management of retinal neurodegenerative diseases such as glaucoma and ischemic diseases.

Over the last decades, academia and the pharma industry have made significant efforts toward the discovery of potent and selective $A_{2A}AR$ antagonists, even if the achievement of the optimal $A_{2A}AR$ antagonist with adequate physico-chemical and pharmacological properties still remains a challenge.

The benzothiazole-based scaffold tozadenant (SYN-115, **24**, Fig. 9), developed by Hoffmann-La Roche, represents a potent and selective $A_{2A}AR$ antagonist with the desired Absorption, Distribution, Metabolism, and Excretion (ADME) properties [82, 83]. **24** has been then developed for the treatment of PD by the Finnish company Biotie Therapies, which was acquired by Acorda Therapeutics in 2016. At the end of 2017, Acorda Therapeutics announced that they discontinued the development of tozadenant for the treatment of PD (phase 2, NCT02453386) and liver disorders (phase 1, NCT03212313), due to serious adverse events (e.g., agranulocytosis) and safety concerns. As of yet, no preclinical or clinical studies are ongoing or have been carried out to explore the potential use of tozadenant for the treatment of glaucoma or ocular diseases.

Inspired by the scaffold of **24** and with the goal to discover a potent and selective series of $A_{2A}AR$ antagonists with advantageous ADME properties, Basu et al. reported a new series of 2-substituted benzothiazole and thiazolo[5,4-c]pyridine derivatives [84]. Compound **25** emerged as a potent and selective $A_{2A}AR$ antagonist with desirable drug-like properties (e.g., high oral bioavailability), making it a promising drug candidate for treating PD, cancer, and ocular diseases.

To sum up, $A_{2A}AR$ agonists and antagonists showed multiple activities which seem to be in conflict with each other, and the current evidence that $A_{2A}AR$ agonists may be useful on the management of glaucoma is not yet convincing.

$A_{2B}AR$ agonists and antagonists

The $A_{2B}AR$ has been cloned in 1992, and among the 4 adenosine subtypes remains the least studied, mostly because few selective ligands are available [85].

The evidence of $A_{2B}AR$ in the anterior ocular segment of bovine corneal endothelium has been for the first time reported by Blazynski, highlighting that $A_{2B}AR$ is present and functional in the retina [86]. Subsequently, other researchers have identified the presence of $A_{2B}AR$ in Müller cells and in retinal pigment epithelial cells [87, 88].

The $A_{2B}AR$ is implicated in inflammation in several tissues (e.g., lung, kidney, and heart) and has a critical role in hypoxic conditions and brain inflammation (e.g., Alzheimer's disease) [89–92]. So far, very little is known about the role of the $A_{2B}AR$ in the eye. What is widely recognized is that $A_{2B}AR$ plays an important role in similar types of inflammation but occurring in different tissues, even though discrepancies between anti-inflammatory and pro-inflammatory effects have been recorded [93].

The activation of $A_{2B}AR$ by adenosine in retinal endothelial cells triggers the extracellular ERK pathway and boosts the levels of VEGF under hypoxic conditions [94, 95]. Considering that $A_{2B}AR$ is expressed in human RPE, its activation resulted in the inhibition of phagocytosis of outer photoreceptor segments [96]. On the other hand, the advantages or disadvantages of blocking $A_{2B}AR$ in the eye have not yet been reported.

Since the involvement of $A_{2B}AR$ in neuroinflammation is starting to be uncovered, it is reasonable to assume that in the next years, the role of $A_{2B}AR$ in the retina under pathological conditions will be better explained. Such a new scenario will open up the opportunity to explore different therapeutic options based on $A_{2B}AR$ ligands.

A_3AR agonists

A_3AR is the last receptor to be cloned [97] and is involved in a variety of different intracellular signaling pathways, ranging from inositol triphosphate (IP3), intracellular calcium (Ca^{+2}), and Gq protein coupling activating phospholipase C (PLC), or inhibition of AC activity through Gi coupling [98]. The pioneering studies conducted in the retina did not detect the presence of A_3AR, but subsequently, the receptor has been identified in the RGCs. Then Beach et al. confirmed the presence of A_3AR in RPE, RGCs, and nerve fiber layer (NFL) by investigating the distribution of ARs in the retina of Rhesus monkeys [29]. Recently has emerged the role of A_3AR as a modulator of inflammation, thus making it an appealing target for treating inflammatory diseases (e.g., osteoarthritis) [99]. Regarding ocular inflammation, A_3AR agonists have received much attention to treating DE syndrome and uveitis [100]. Even

Fig. 10 Chemical structures of
IB-MECA (CF101, **26**), Cl-IB-
MECA (CF102, **27**), MRS-3558
(**28**), and MRS-3630 (**29**)

26 IB-MECA (CF101) R = H
27 Cl-IB-MECA (CF102) R = Cl   **28** MRS-3558   **29** MRS-3630
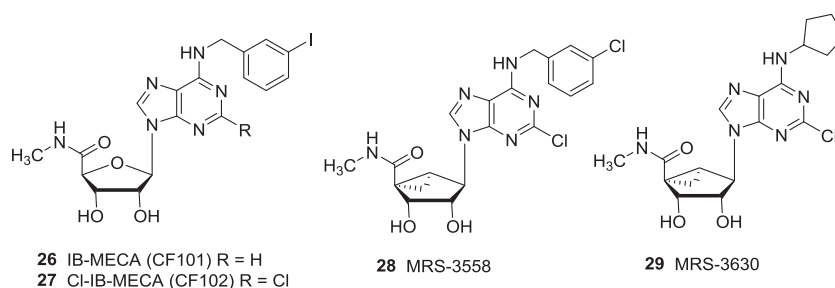
the influence of A₃AR activation on glaucoma, DR, and ischemic diseases has been extensively surveyed. The damage to the NFL surrounding RGCs, followed by RGC apoptotic death induced by the ATP-gated P2X7R ion channel activation, is the main feature of DR and glaucoma [101]. In chronic glaucoma, ATP is released from astrocytes of the optic nerve and triggered P2X7R resulting in apoptotic cell death of RGCs in the retina [19]. During homeostasis perturbation, cells release ATP that acts as an inflammasome activator, leading to pro-inflammatory mediators' release. Elevated ATP levels have been found in different animal models of glaucoma, and in the retina, ATP is released by Müller cells, astrocytes, and microglia. As reported by Lu et al., in the extracellular environment, ATP can interact with P2XRs or be hydrolyzed into adenosine by the enzymatic activity of ecto-nucleoside triphosphate diphosphohydrolases (NTPDases) [102]. An unbalanced activity of NTPDase-1, might convert more ATP into adenosine, increasing the chance of activating ARs. Indeed, Rodriguez-Neves et al. reported that in the experimental models of glaucoma, the adenosine levels are highly enhanced [103].

A readily checked risk factor of glaucoma is considered to be the elevated IOP, which is believed to be the result of reduced AH outflow from the anterior segment. However, the role of A₃AR on lowering/increasing IOP is still controversial. It is well documented that the treatment with A₃AR antagonists reduced IOP in mice as well as in A₃AR knockout mice experimental models, but not all reported studies on A₃AR converge on the same conclusions [25, 104, 105]. For example, the case of IB-MECA (CF101, piclodenoson, **26**, Fig. 10), an A₃AR agonist, is emblematic because, during the clinical trials for the treatment of DE syndrome, it has been found to lower IOP [106]. This surprising result could be due to unintentional cross-activation of A₁AR [107] or be linked to the chronic administration of **26** that led to lower IOP via the pharmacological mechanism known as "effect inversion" already seen for other ARs ligands and theorized by Jacobson et al. [108].

The "inversion effect" can better clarify why the pharmacological effect of an AR ligand administered in acute can be entirely reversed if it is administered chronically

[109]. Since glaucoma therapy is administered chronically, the "inversion effect" should be considered when deciding to undertake a trial that involves AR ligands.

The activation of A₃AR preserves RGC cells and restrains the rise in calcium levels caused by the stimulation of glutamate receptors [110]. In glaucoma and DR, the overactivation of glutamate receptors is responsible for raising of intracellular calcium concentration, which consequently ends up in cell death. Since RGCs express A₃AR, the protective effect may rise up from the direct action of an A₃AR agonist with those cells [88]. Galvao et al. showed that A₃AR activation lowered the retinal apoptotic cell death and boosted the RGC survival rate [111]. In addition, the same authors reported that the activation of A₃AR preserves the retina from retinal ischemia-reperfusion (I-R) injury, from damage induced by partial optic nerve transection, and excitotoxic-induced cell death.

26 is an orally bioavailable small molecule with a favorable therapeutic index, which is being tested in several phase 2 studies by the Israeli biopharmaceutical company Can-Fite (NCT00349466 and NCT01235234). In a phase 2 study completed in 2011 to treat plaque psoriasis, **26** successfully met all its endpoints, significantly improving the disease symptoms (NCT00428974). In a phase 2/3 study the data showed a significant clinical activity from week 16 up to 32 with the 2 mg dose daily (NCT01265667) [112], and in 2018 it entered a phase 3 study for the treatment of moderate-to-severe psoriasis (NCT03168256). Similarly, phase 2 studies demonstrated the efficacy of **26** given as a monotherapy for the treatment of rheumatoid arthritis. A phase 3 study is still active and is aimed to compare **26** to methotrexate as a first-line treatment of rheumatoid arthritis (NCT02647762).

During an early unsuccessful trial of **26** for DE syndrome (NCT01235234), researchers from OphthaliX, a subsidiary company of Can-Fite, have noted a modest reduction of IOP in patients obtaining the drug. The IOP reduction was further investigated and was found to be 0.92 mmHg at a dose of 1 mg of **26** twice daily [106]. Then OphthaliX conducted a phase 2 safety and efficacy clinical trial of orally administered **26** in patients with elevated IOP and glaucoma (NCT01033422) [113]. Unfortunately, during the 16 weeks of treatment with **26** (1 or 2 mg twice daily) of patients with

Fig. 11 Chemical structures of P2X7R antagonists MRS-2540 (**30**), and JNJ-47965567 (**31**)

30 MRS-2540   **31** JNJ-47965567
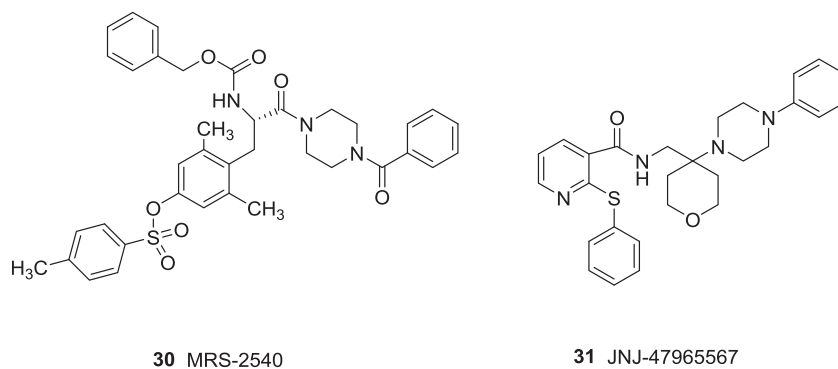
elevated IOP, no statistically significant differences were found between the placebo group and drug-treated group in the primary endpoint of lowering IOP.

Considering that **26** failed to meet its primary endpoint, OphthaliX has declared that they do not see any immediate path forward in glaucoma. The mechanistic limitation of using A₃AR agonists to treat glaucoma may lie in the desensitization of ARs. For this reason, nucleoside prodrugs of A₃AR agonists have been explored for their action on lowering IOP [114, 115].

26 also induced an anti-inflammatory effect in experimental autoimmune uveitis induced by retinal antigen interphotoreceptor retinoid-binding protein [116]. A phase 2 study to assess the safety and efficacy of **26** to subjects with uveitis has been planned in 2013 (NCT01905124), but later Can-Fite decided to withdraw.

Given that **26** has a full-blown anti-viral effect already protected by a US patent (US7589075), Can-Fite, in collaboration with the Lewis Katz School of Medicine at Temple University (Philadelphia), has recently started a study on the anti-viral activity of **26** on COVID-19 viral load. At the beginning of September 2020, Can-Fite received approval from the US FDA for use **26** in a phase 2 study to treat COVID-19 patients with moderate symptoms (NCT04333472).

Cl-IB-MECA (CF102, namodenoson, **27**, Fig. 10), has been initially originated in the laboratory of Prof. Kenneth A. Jacobson at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and then developed by Can-Fite for the treatment of liver conditions, such as nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC) [117]. The safety of **27** has been extensively demonstrated in several preclinical studies and in phase 1/2 clinical studies, proving to be safe and effective (NCT00790218 and NCT02927314). In 2012, **27** has achieved the orphan drug status from the FDA for the treatment of HCC [118]. **27** is currently available in Israel to treat liver cancer patients through compassionate use when all available treatment options have been exhausted. Can-Fite has recently reached an agreement with the FDA and

EMA to begin a more extensive phase 3 clinical trial to treat liver cancer.

Elevation of IOP induces impairment and apoptotic death of RGCs, and A₃AR agonists such as **27** and MRS-3558 (**28**, Fig. 10) preserve RGCs from apoptosis induced by P2X7R activation (e.g., ATP or other nucleotides). The P2X7R overexpression in RGCs is tied with other factors (e.g., increased MAPK and caspase-3 proteins in the retina), implying a pro-apoptotic mechanism in RGC death induced by elevated IOP. Recently, Sakamoto et al. reported that P2X7R activation is involved in the mechanism of NMDA-induced retinal injury, leading to glaucomatous RGC loss [119]. The treatment with **27** decreased the number of cells encountering apoptosis and, at the same time, enhancing the RGCs survival [111]. This work has proven for the first time that **27** is neuroprotective to the retina. A dual A₁/A₃ agonist, MRS-3630 (**29**, Fig. 10), was also efficacious in protecting RGCs from cell death [19].

On the other hand, the treatment with P2X7R antagonists, such as MRS-2540 (**30**, Fig. 11) and JNJ-47965567 (**31**), prevents neuronal apoptosis in the retina induced by hypoxia or ATP [101, 120].

A₃AR antagonists

As stated before, A₃AR has a pivotal role in IOP regulation, even though there are concerns about agonists and antagonists of A₃AR that might be useful in treating the same pathologic conditions (e.g., glaucoma). Usually, topically applied A₃AR agonists increased IOP, whereas A₃AR antagonists decreased IOP in mice. The selective activation of A₃AR can increase AH, and consequently increase IOP, by a mechanism that involves the activation of Cl⁻ channels in the non-pigmented ciliary epithelial (NPE) cells [121, 122]. Studies on A₃AR knockout mice have corroborated this observation. Avila et al. reported that the baseline IOP in A₃AR knockout mice was significantly lower than that in wild-type ones, and A₃AR agonists or antagonists did not affect at all IOP [105].

Fig. 12 Chemical structures of A$_3$AR antagonists **32**-**41**
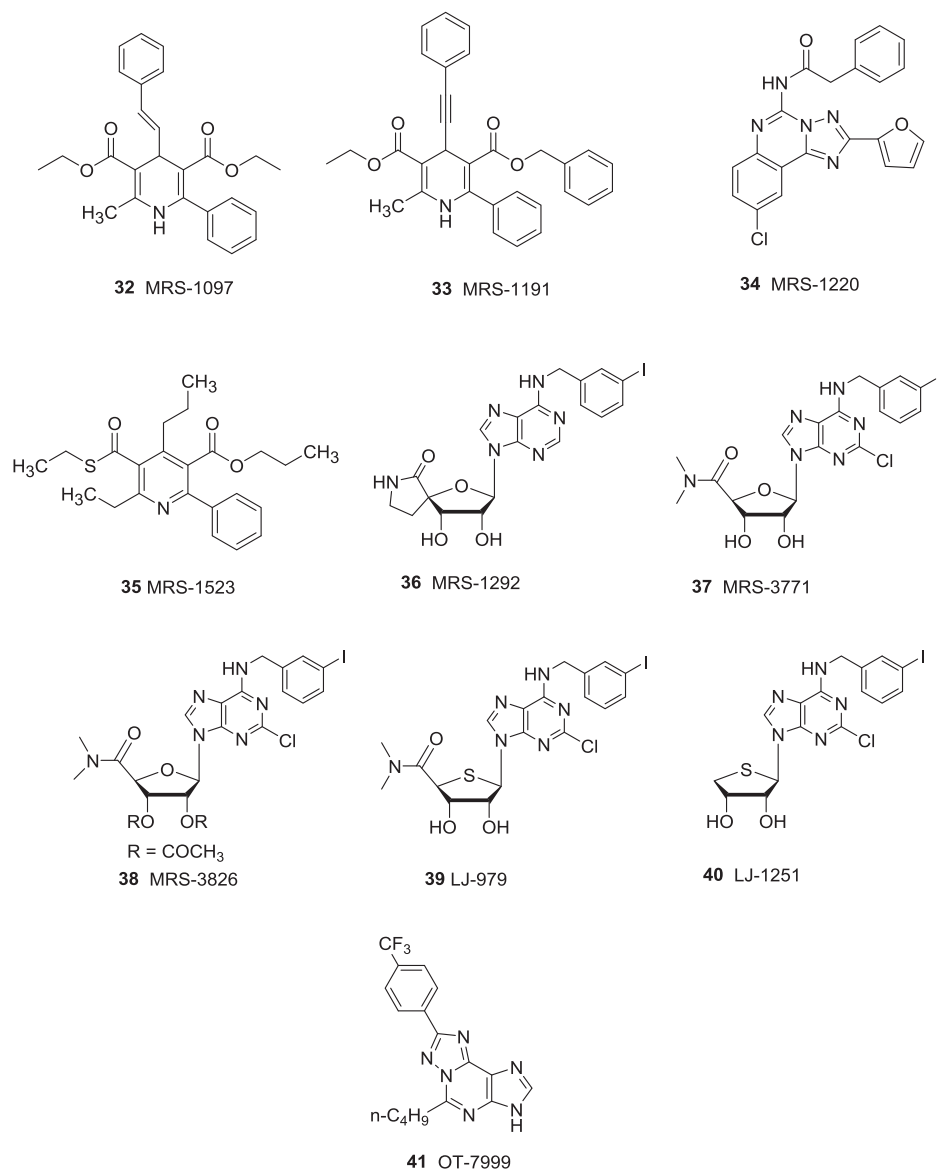
As a reasonable interconnection to clinical glaucoma, A$_3$AR is upregulated by an order of magnitude in the non-pigmented ciliary epithelium in the pseudoexfoliation syndrome, the largest cause of secondary open-angle glaucoma. Considering the ability of the A$_3$AR to act as a regulator of chloride transport in epithelial cells, its upregulation may have an additional influence on AH secretion and hence on increasing IOP [123]. This finding has opened the way to the development of selective A$_3$AR antagonists for the treatment of glaucoma. To date, the A$_3$AR antagonists tested for their effects on lowering IOP consisted of: the 6-phenyl-1,4-dihydropyridine derivatives MRS-1097 (**32**, Fig. 12) and MRS-1191 (**33**), the nonxanthine heterocyclic derivative MRS-1220 (**34**), the pyridine derivative MRS 1523 (**35**), the nucleoside-based antagonists MRS-1292 (**36**), MRS-3771 (**37**), MRS3826 (**38**), LJ-979 (**39**), and LJ-

1251 (**40**), the triazolopurine derivative OT-7999 (**41**), PBF-677 (structure not disclosed), and ACN-1052 (structure not disclosed).

Civan et al. [124], have patented compounds **32**-**35** as part of a method aimed to decrease IOP by administrating an A$_3$AR antagonist. As reported before, adenosine and A$_3$AR agonists cause isotonic cell shrinkage by activating Cl$^-$ channels in the basolateral membranes of NPE cells, accelerating the rate of AH formation and increasing IOP. A$_3$AR antagonists such as **32**-**35** prevent Cl$^-$ outflow and decrease AH production in NPE cells [125].

The IOP responses to A$_3$AR antagonists were monitored in A$_3$-knockout (A$_3$AR$^-$/$^-$) and control mice (A$_3$AR$^+$/$^+$) [105]. The highly selective A$_3$AR antagonist **33** did not impact IOP in A$_3$-knockout mice but lowered it in control mice (by 7.0 ± 0.9 mmHg). Instead, **35**,

structurally different to **33**, reduced baseline IOP in A_3-knockout mice by 3.6 ± 0.6 mmHg, but reduced the IOP in the control mice to a more considerable extent (by 9.8 ± 1.1 mmHg). Such a phenomenon might be explained by the relative selectivity of **35** for A_3ARs over A_{2A}AR with respect to **33**, which raises the possibility that **33** may have cross-occupied A_{2A}AR, leading to a decrease of IOP.

Investigation of A_3AR antagonists has often been limited by their species variability, whereas A_3AR agonists usually display high affinity across species. This problem has been brilliantly overcome by modifying the highly specific, cross-species agonist IB-MECA, providing a pool of A_3AR antagonists (compounds **36-40**) able to act across species.

The conformationally constrained ligand MRS-1292 (**36**, Fig. 12), a selective A_3AR antagonist in both the human and the rat, reduces mouse IOP and inhibits A_3AR-mediated shrinkage of cultured human NPE cells [104]. When applied topically, **36** generates a maximum reduction in IOP of 4.4 ± 0.8 mmHg, making it a drug of choice for additional animal testing to fully understand the role of A_3AR in increasing/decreasing IOP.

Of the four A_3AR antagonists (**37-40**) studied by Wang et al., obtained after modification of Cl-IB-MECA (**27**), all of them reduced measured IOP after topical administration (Wang et al., 2010). The 4 A_3AR antagonists' effect on mouse IOP was measured in two different manners: invasively (by the Servo-Null Micropipette System, SNMS) and non-invasively (by Pneumotonometry). The measurements of IOP by SNMS tonometry demonstrate the functional efficacy of **37-40** in the mouse, whereas their effect on the adenosine-triggered shrinkage of native bovine NPE cells showed that the antagonists are efficacious against bovine, as well as human and mouse A_3ARs.

The 5′-N,N-dimethyluronamide derivative MRS-3771 (**37**) [126] reduced IOP of 3.0 ± 1.1 mmHg and significantly inhibited adenosine-produced cell shrinkage of native bovine NPE, monitored by measuring cell area [127, 128].

The di-acetyl ester derivative of MRS-3771 (**37**), MRS-3826 (**38**) [115], lowered IOP measured by invasively SNMS tonometry of 4.0 ± 0.8 mmHg, but not reduced IOP measured by pneumotonome.

LJ-979 (**39**), an analog of Cl-IB-MECA (**27**) obtained by concomitant addition of a methyl group to the 5′-uronamide and 4′-thio substitution, reduced invasive-measured IOP of 4.2 ± 1.2 mmHg at a concentration of $250 \mu M$.

The only tested nucleoside-based derivative that lowered IOP at a concentration of $250 \mu M$ by SNMS tonometry (1.5 ± 0.6 mmHg) as well as by pneumotonometry (4.2 ± 0.7 mmHg) has been LJ-1251 (**40**) [129]. **40** has been synthesized starting from **39** by removing the 5′-uronamide moiety, with the aim to eliminate H-bonding at that position. Its moderate hydrophobicity (cLog $P = 2.25$) allowed

it to permeate the cornea remarkably quickly, although the underlying mechanism is still unknown and requires further study.

The triazolopurine derivative OT-7999 (**41**, Fig. 12), developed by Otsuka Pharmaceutical (Japan), has been studied to treat glaucoma. **41** was applied topically to one eye in cynomolgus monkeys with normal IOP. The results indicated that **41** lowered IOP of 2-3 mmHg after 2 and 4 hours of topical application, and no ophthalmologic side effects had been detected [24, 130].

PBF-677 (structure not disclosed), a selective and potent gastrointestinal (GI)-restricted A_3AR antagonist, has been developed by Palobiofarma, a Spanish biotechnology company, and has recently positively completed a "first in human" phase 1 study to assess safety and tolerability in healthy volunteers (NCT02639975). The drug is intended to treat glaucoma, ocular hypertension, and eye diseases and has already received the Spanish Regulatory Agency's approval.

PBF-677 is also in development as a "first-in-class" oral treatment for inflammatory bowel and is currently ongoing a proof of concept (POC) phase 2 study for assessing safety and efficacy in ulcerative colitis patients (NCT03773952).

Acorn Biomedical has suggested using ACN-1052 (structure not disclosed) for glaucoma treatment. ACN-1052 is an orally bioavailable A_3AR antagonist with a single-digit (nM) affinity for both the rat and human A_3AR. Its pharmacodynamics has been extensively studied in preclinical models. ACN-1052 reduced IOP after topical administration, and with a chronic administration, it generated a prolonged effect in reducing IOP. It is worth noting to mention that the IOP reduction obtained with ACN-1052 is 2-fold lower than that obtained with first-line drugs such as brimonidine tartrate (Alphagan®) or latanoprost (Xalatan®).

Summing up, it should be pointed out that all tested A_3AR antagonists are in the preclinical stage, except for PBF-677 [113].

A_1AR agonists/A_3AR antagonists

Given the wide distribution of AR subtypes in all mammal tissues, an innovative strategy that allows avoiding the side effects represents a good starting point for selective AR ligands' therapeutic development. For this reason, the emerging and alternative approach, called polypharmacology, has been gaining increasing attention in the last decade. Polypharmacology consists of modulating multiple targets related to only one disease pathway with a single drug. In this respect, a drug that hits multiple sensitive targets (multitarget drug) offers the potential for higher efficacy and curtails drawbacks that generally arise from the use of combination therapies [131, 132]. Furthermore, a
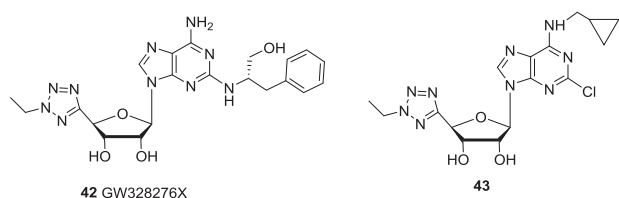
Fig. 13 Chemical structures of GW328276X (**42**) and **43**

multitarget drug has the benefit of following only one metabolic pattern and pharmacokinetic, which instead represent a significant constraint of the combination therapy.

In the AR arena, several examples of dual-acting ligands have been reported. A dual $A_{2A}AR$ agonist and A_3AR antagonist, called GW328267X (**42**, Fig. 13), has been designed by Glaxo as an anti-inflammatory agent [133]. Despite its full-blown anti-inflammatory efficacy in animal models, **42** failed to show efficacy in a clinical trial for allergic rhinitis and asthma (NCT01640990). Side effects directly linked to its agonist activity at A_1AR (i.e., tachycardia and hypotension) have limited its clinical development. Later on, Hou et al. synthesized a new series of dual-acting hA_2AR agonists and hA_3AR antagonists with the aim to be developed for the treatment of asthma and inflammatory diseases [134]. Recently, Jacobson et al. have hypothesized that a dual A_1AR antagonist and $A_{2A}AR$ agonist may have a beneficial impact on the treatment of asthma [135].

By relying on the dual-acting ligand approach, in 2015, we reported the first highly potent dual-acting hA_1AR agonists and hA_3AR antagonists [136]. Combining a 5′-C-ethyltetrazol-2-yl moiety with the appropriate N^6-substitution in adenosine derivatives, an increased affinity versus both hA_1AR and hA_3AR (at the subnanomolar range) was achieved while remaining agonists at hA_1AR and antagonists at hA_3AR. The follow-up paper aimed to extend the series of 5′-C-(ethyltetrazol-2-yl)adenosine and 2-chloroadenosine derivatives, modifying the substituent in the N^6-position of the adenine ring [137]. This study demonstrated for the first time that all dual-acting N^6-substituted 5′-C-ethyl-tetrazolyl adenosine derivatives act as antagonists at human A_3AR but as agonists at the rat A_3AR, highlighting the importance of species differences for both affinity and efficacy at A_3AR. Additionally, this new series has allowed us to disclose N^6-cyclopropylmethyl-5′-C-(ethyltetrazol-2-yl)-2-chloroadenosine (**43**), a very potent dual-acting A_1/A_3 agonist in rat and A_1AR agonist/A_3AR antagonist in human ARs.

Moreover, this new series of compounds may represent a set of pharmacological tools for in vivo studies with the aim of investigating the advantages of dual-acting A_1AR agonists and A_3AR antagonists in several diseases. A single

molecule with one pharmacokinetic profile, activating one signaling pathway while blocking another one, could potentially be a promising candidate to reduce elevated IOP and have the capability to be used in the treatment of glaucoma.

This approach had previously been attempted by Inotek Pharmaceuticals, who have patented the use of combination therapy based on an A_1AR agonist (i.e., trabodenoson) and an A_3AR antagonist for the treatment of diseases and conditions caused by elevated IOP [138]. The results of the in vivo administration of the combination therapy showed a substantial decrease in IOP during the entire observation period (~6 h).

Conclusions

In the past two decades, a significant medicinal chemistry campaign around the ARs in the ocular therapeutic field has been conducted. The activation or blockage of ARs is involved in the modulation of IOP, retinal function, AH formation, neuroprotection, and blood flow. Data from preclinical and clinical trials are extremely encouraging and shed some light on the role of ARs in ocular diseases. Although it has been highlighted a puzzling and contradictory scenario of modulation of purinoma in the eye, the ARs ligands hold promise for future ocular therapeutics. However, a better understanding of their exact contribution to glaucoma or other ocular diseases is a crucial step in order to future design new therapeutics built on ARs agonists or antagonists.

Compliance with ethical standards

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