Chapter 17
Glaucoma and the Applications of Carbonic Anhydrase Inhibitors

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Abstract Inhibition of carbonic anhydrase (CA, EC 4.2.1.1) has pharmacologic applications in the treatment of glaucoma, a disease affecting a large number of people and characterized by an elevated intraocular pressure (IOP). At least three isoforms, CA II, IV and XII are targeted by the sulfonamide inhibitors, some of which are clinically used drugs. Acetazolamide, methazolamide and dichlorophenamide are first generation CA inhibitors (CAIs) still used as systemic drugs for the management of this disease. Dorzolamide and brinzolamide represent the second generation inhibitors, being used topically, as eye drops, with less side effects compared to the first generation drugs. Third generation inhibitors have been developed by using the tail approach, but they did not reach the clinics yet. The most promising such derivatives are the sulfonamides incorporating either tails with nitric oxide releasing moieties or hybrid drugs possessing prostaglandin (PG) F agonist moieties in their molecules. Recently, the dithiocarbamates have also been described as CAIs possessing IOP lowering effects in animal models of glaucoma. CAIs are used alone or in combination with other drugs such as adrenergic agonist/antagonists, or PG analogs, being an important component of the antiglaucoma drugs armamentarium.

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1 Introduction

Glaucoma consists of a group of eye diseases showing a broad spectrum of clinical presentation and aetiologies, which lead to a permanent loss of visual function due to the damage of the optical nerve [1–5]. Several different types of glaucoma are known, and they are characterized by an elevated intraocular pressure (IOP) [1–4]. The source of this abnormal increase is related to a misfunctioning of tissues of the trabecular meshwork, located in the anterior chamber angle of the eye, between the cornea and the iris. Their role is to maintain a balanced pressure in the anterior chamber of the eye by allowing the outflow of aqueous humor [1–5]. Aqueous humor is a transparent liquid, rich in bicarbonate, which fills the region between the cornea and the lens [5–11]. It is continuously secreted by the ciliary body around the lens, and constantly flowing from the ciliary body to the anterior chamber [5–7]. From the anterior chamber the aqueous humor passes through the trabecular meshwork into the Schlemm canal and from it into a multitude of aqueous veins which eventually merge with the blood carrying veins [5–11]. Glaucoma is very often characterized by an excessive retention/secretion of aqueous humor in the anterior chamber, whose effect leads to a steep increase of the IOP to values as high as 25–30 mmHg [1–5].

Glaucoma has been classified in two different categories, the closed angle and the open angle glaucoma [1, 2]. In the first case the closure of the anterior chamber angle is observed as a result of a contact between the iris and the surface of the trabecular meshwork [1, 2]. Closure of this angle prevents the drainage of the aqueous humor. The open angle form is represented by any kind of glaucoma in which the above angle remains open, but the drainage of the aqueous humor is decreased [1, 2]. The exact reasons of such an altered flow remains largely unknown [1].

The general method for treating glaucoma consists in IOP reduction therapy, through pharmacology, laser therapy or surgical operation [1–4]. It should be noted that some patients with glaucomatous vision loss can show relatively low IOP, but they represent a minority of the glaucoma patients [1, 2]. Also in such cases, therapies that control IOP are recommended [1, 2]. Drug therapies effective for IOP reduction include both agents that decrease aqueous humor production and agents that increase the outflow facility. Such therapies can be applied topically (directly on the eye) or administered systemically [1–4].

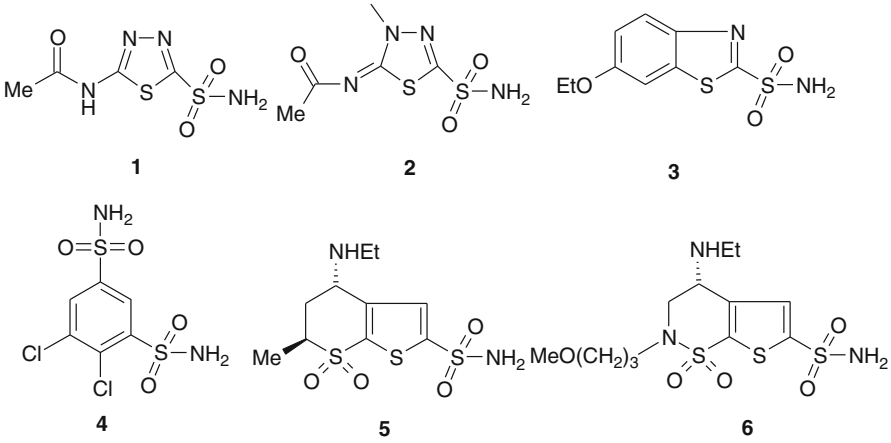
The relationship between glaucoma and carbonic anhydrase (CA, EC 4.2.1.1), the enzyme catalyzing the hydration of CO_2 to bicarbonate and protons [12–16] is rather straightforward and known for decades. The pioneering studies of Friedenwald [6], Kinsey [7] and Kinsey and Barany [8] on the chemistry and dynamics of aqueous humor showed that the main constituent of this secretion is sodium bicarbonate. The next step was the identification of CA, in the anterior uvea

of the eye by Wistrand [9], who demonstrated that it is this enzyme (present mainly in the ciliary processes) responsible for the bicarbonate secretion, as a consequence of the hydration reaction of carbon dioxide, with formation of bicarbonate (and obviously, protons). Becker [10] then showed that the sulfonamide CA inhibitor (CAI) acetazolamide, **1**, produced a drop of IOP in experimental animals and humans, whereas Kinsey and Reddy [11] proved that this phenomenon is due to a reduced bicarbonate secretion as a consequence of CA inhibition by the sulfonamide drug. This was the beginning of a novel treatment for glaucoma, already in the 1950s [17]. As this condition is affecting an increasing number of the population, also being the leading cause of blindness in the Western countries, it is essential to explore novel medications for its treatment [1–4]. In fact, CAIs constitute one of the main drug classes used for the treatment of glaucoma [12–17].

2 First Generation Inhibitors: Systemically Acting Drugs (Acetazolamide, Methazolamide, Dichlorophenamide)

Discovered in the 1950s by Roblin's group [18], the heterocyclic sulfonamides such as acetazolamide **1**, methazolamide **2** and ethoxzolamide **3** [19] as well as the aromatic compound dichlorophenamide **4**, represent the first generation of clinically used CAIs [17]. They are very strong (typically low nanomolar) inhibitors of most CA isoforms of the 15 ones presently known in humans (see Ref. [13] for details). Except ethoxzolamide which has fewer clinical applications, acetazolamide **1**, methazolamide **2** and dichlorophenamide **4** are systemically used CAIs, mainly as antiglaucoma drugs, even if they were discovered decades ago, and even if they have a range of side effects (see discussion later in the text) [5, 17].

Systemic inhibitors are useful in reducing elevated IOP characteristic of many glaucoma forms, as they represent the most efficient physiological treatment of glaucoma. Indeed by inhibiting the ciliary process enzymes (the sulfonamide susceptible isozymes CA II, CA IV and CA XII [5, 12, 17, 20]), a reduced rate of bicarbonate and aqueous humor secretion is achieved, which leads to a 25–30 % decrease of IOP [5, 17]. However, as mentioned above, these compounds are promiscuous, strong inhibitors of all CA isoforms, and the inhibition of various CA isozymes present in other tissues than the eye leads to an entire range of side effects [5, 12, 17]. The most prominent ones are: numbness and tingling of extremities, metallic taste, depression, fatigue, malaise, weight loss, decreased libido, gastrointestinal irritation, metabolic acidosis, renal calculi and transient myopia [5, 12, 17]. Indeed, all these compounds (**1**–**4**) indiscriminately inhibit most of the CA isozymes (such as CA I, CA II, CA IV, CA VA, VB, CA VII, XIII and XIV) abundant in other organs than the eye, such as blood, kidneys, lungs, gastrointestinal tract, CNS, etc. [5, 12, 17]. As a consequence there are limitations of their use due to patience compliance. However, acetazolamide and dichlorophenamide are even nowadays components of regimens for the treatment of refractory glaucoma, which does not respond to adrenergic antagonists, or prostaglandin (PG) analogs [1, 5].



3 Second Generation Inhibitors: Topically Acting Sulfonamides (Dorzolamide and Brinzolamide)

The idea to administer topically, directly into the eye the sulfonamide CAI was already addressed by Becker in the 1950s [10]. This and other studies involving the clinically used compounds **1**–**4** which were administered as suspensions into the eye of experimental animals, only gave negative results, being concluded that sulfonamide CAIs were effective as antiglaucoma drugs only via the systemic route [10, 17]. The lack of efficiency of the first generation sulfonamide CAIs via the topical route was due to the fact that the drug was unable to arrive at the ciliary processes where CAs are present [5, 21]. The inadequate drug penetrability through the cornea was ascrivable to the inappropriate physico-chemical properties for such a route of administration of the sulfonamides **1**–**4**.

In 1983, in a seminal paper Maren's group [21] postulated that a water-soluble sulfonamide, possessing a relatively balanced lipid solubility (in order to be able to penetrate through the cornea) as well as strong enough CA inhibitory properties, would be an effective IOP lowering drug via the topical route, but at that moment no inhibitors possessing such properties existed, as the bio-organic chemistry of this class of compounds was rather unexplored at that time [19]. Water-soluble sulfonamide CAIs started to be developed in several laboratories soon thereafter, and by 1995 the first such pharmacological agent, dorzolamide **5** has been launched for clinical use by Merck, as 2 % eye drops [22, 23]. A second, structurally related compound, brinzolamide **6** (discovered at Alcon Laboratories), has then been approved for the topical treatment of glaucoma in 1999 [24]. These two compounds are still the only topically acting CAIs in clinical use at this moment.

Dorzolamide **5** and brinzolamide **6** are nanomolar CA II/CA XII inhibitors [13, 22], possess a good water solubility, are sufficiently liposoluble to penetrate through the cornea, and may be administered topically, directly into the eye, as a

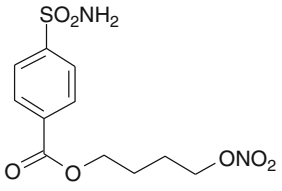
2 % water solution (of the dorzolamide hydrochloride salt) or as 1 % suspension (as the brinzolamide hydrochloride salt) 2–3 times a day [5, 22, 24]. The two drugs are effective in reducing IOP and show fewer side effects as compared to the systemically applied drugs. The observed common side effects include stinging, burning or reddening of the eye, blurred vision, pruritus, bitter taste [5, 22, 24]. All but the last are probably due to the fact that dorzolamide (the best studied topical CAI) is the salt of a weak base with a very strong acid, so that the pH of the drug solution is rather acidic (generally around 5.5). The last side effect mentioned above is probably due to drug-laden lachrymal fluid draining into the oropharynx and inhibition of CA present in the saliva (CA VI) and the taste buds (CA II and CA VI), with the consequent accumulation of bicarbonate, and was observed both with systemic as well as topical CAIs [5, 12]. Brinzolamide produces less stinging but more blurred vision as compared to dorzolamide [5, 24]. Unfortunately, dorzolamide showed some more serious side effects, such as contact allergy, nephrolithiasis, anorexia, depression and dementia and irreversible corneal decompensation in patients who already presented corneal problems [25–28]. Thus, even if dorzolamide and brinzolamide represent indeed a major progress in the fight against glaucoma with therapies based on CAIs, novel types of topically effective inhibitors belonging to this class of pharmacological agents are still needed.

4 Third Generation Sulfonamide Inhibitors

An approach was reported [29] for obtaining novel types of sulfonamide CAIs with good hydrosolubility and IOP lowering effects, which has been nominated the “tail approach”. It consists in attaching water-solubilizing functionalities to the molecules of aromatic/heterocyclic sulfonamides incorporating derivatizable moieties of the amino, imino or hydroxyl type. Such moieties included, among others, pyridine-carboximido; carboxypyridine-carboxamido, quinolinesulfonamido; picolinoyl, isonicotinoyl, perfluoroalkyl/arylsulfonyl-, as well as amino acyl groups, whereas ring systems which have been derivatized by using the above mentioned moieties included: 2-; 3- or 4-aminobenzenesulfonamides; 4-(ω-aminoalkyl)-benzenesulfonamides; 3-halogeno-substituted-sulfanilamides; 1,3-benzene-disulfonamides; 1,3,4-thiadiazole-2-sulfonamides; benzothiazole-2-sulfonamides as well as thienothiopyran-2-sulfonamides, and were chosen in such a way as to demonstrate that the proposed approach was a general one [29–32]. Compounds prepared by the tail approach showed 2–3 times more effective topical IOP lowering effects in rabbits as compared to dorzolamide **5** [29–32]. They possessed good water solubility (as hydrochlorides, triflates or trifloroacetates), inhibition in the low nanomolar range against hCA II and IV, good penetrability through the cornea, and very good IOP lowering properties in both normotensive and glaucomatous rabbits (widespread animal models of glaucoma) [29–32]. What is more important, this effect lasted for a prolonged period of time as compared to the similar effect of dorzolamide [29–32]. These promising compounds were

not developed for clinical use mainly because the company which acquired rights on them was incorporated in another one, which was not interested in this type of applications.

The tail approach proved however to be a general and versatile modality to obtain a wide range of CAIs belonging to several classes of compounds, the sulfonamides being just one particular case (reviewed in Refs. [13, 33–35]).



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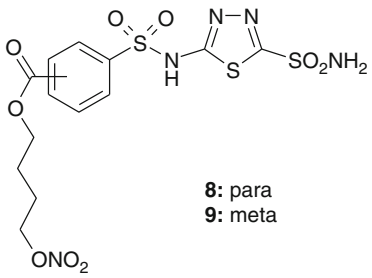
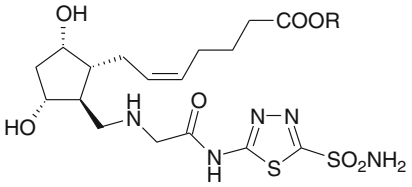


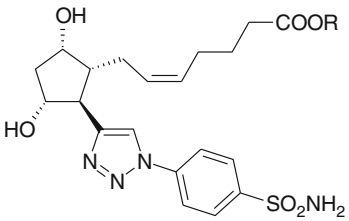
8: para
9: meta

An interesting development of the tail approach consisted in designing CAIs incorporating nitrate ester moieties, which led to hybrid drugs possessing a sulfonamide and an NO-donating moiety pharmacophore in their molecule [36–38]. Nitric oxide (NO), a radical gas produced by the enzyme nitric oxide synthase (NOS), is involved in vasodilation, aqueous humour outflow within the eye, local modulation of ocular blood flow and retinal ganglion cells death apoptosis [36–38]. It appeared thus of interest to combine these two pharmacophores, a sulfonamide CAI, and a moiety able to donate NO, in the molecule of the same compound [36–38]. In this way, a large number of sulfonamides with NO-donating properties were reported in the last several years, among which those of types **7**–**9** were among the most interesting ones [36–39]. Several aromatic/heterocyclic sulfonamide scaffolds have been used to synthesize compounds incorporating NO-donating moieties of the nitrate ester type [36–38]. Some of these compounds showed effective in vitro inhibition of the target isoforms involved in glaucoma (in the low nanomolar range) i.e., hCA II, IV and XII, and the X-ray crystal structure of some of them bound to the dominant isoform hCA II also revealed factors associated with this marked inhibitory activity (see Chap. 15 in this book) [36–38]. More importantly, in an animal model of ocular hypertension, one of these new compounds (derivative **7**), was twice more effective than dorzolamide **5** in reducing elevated IOP characteristic of this disease, anticipating its potential for the treatment of glaucoma [37]. A detailed pharmacologic study of **7** was recently reported [38]. Chronic administration of **7** as 2 % eyedrops to glaucomatous albino rabbits resulted in an important reduction in IOP (of 45–50 %) already after the first week of treatment, with a regular decreasing trend during the treatment period [38]. This reduction was much higher than that observed when dorzolamide at 2 % was administered in the same animal model and with an identical administration schedule [38]. Furthermore, in the ophthalmic artery of the treated rabbits, both systolic and diastolic velocities,

were significantly reduced in eyes treated with the hybrid drug **7** in comparison to dorzolamide **5**, suggesting thus a beneficial effect of this class of CAIs on the blood supply to the optic nerve (in addition to the IOP reduction), which was not observed with dorzolamide or brinzolamide [38]. Thus, the sulfonamides incorporating NO-donating moieties represent good candidates for the next generation topically acting antiglaucoma agents: they may exert beneficial activity both by reducing elevated IOP and by supplying more blood (and thus oxygen) to the optic nerve.



10a: R = i-Pr
10b: R = H



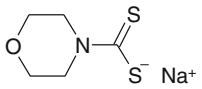
11a: R = Me
11b: R = H

Very recently [39], this hybrid drugs approach has been also used for obtaining compounds incorporating sulfonamide and PG moieties in the same molecule. The rationale of having two pharmacophores with well-known antiglaucoma effects, i.e., the aromatic/heterocyclic sulfonamide one associated with CA inhibition (and whence reduced bicarbonate secretion in the aqueous humor), and the PG F one associated with increased outflow of the aqueous humor, has led to molecules of types **10** and **11** [39]. Both heterocyclic (1,3,4-thiadiazole-2-sulfonamide, such as **10a,b**) and aromatic (benzenesulfonamides, such as **11a,b**) derivatives have been reported. They incorporate, in addition to the aromatic/heterocyclic sulfonamide moiety, the $PGF_{2\alpha}$ moiety, occurring in eicosanoid such as latanoprost, which are effective antiglaucoma drugs [5]. The most interesting compound seems to be **11b** which showed an inhibition constants of around 10 nM against hCA II and was also a good PG F receptor (FP) agonist (EC_{50} of 5.7 nM) [39]. Although the ocular permeability of this (and related) hybrid drugs was good, no in vivo IOP lowering data with these derivatives were reported so far. However, the hybrid drug approach is a valid one for obtaining CAIs with additional biological activity, which can add on to the efficacy of such agents as antiglaucoma drugs.

5 Dithiocarbamates as Topically Acting Antiglaucoma CAIs

Although the sulfonamides dominated the drug design landscape of CAIs for many years, recently, new chemotypes emerged that interact with these enzymes by a similar or different inhibition mechanism as the sulfonamides [13, 33–35]. Among them, the dithiocarbamates (DTCs) are undoubtedly the most interesting

ones [40–43]. These compounds have been rationally discovered as CAIs after our report of trithiocarbonate (CS_3^{2-}) as an interesting (milli – micromolar) inhibitor [44]. In the X-ray crystal structure of this inorganic anion bound to CA II, it has been observed a monodentate coordination of the inhibitor via one sulfur atom to the zinc ion from the enzyme active site, and a hydrogen bond in which another sulfur and the OH of Thr199 were involved [44]. Thus, the CS_2^- was discovered as a new zinc-binding group (ZBG) for generating CAIs. As DTCs are simple compounds that incorporate this new ZBG, a rather large series of such compounds was prepared and evaluated for their inhibitory activity against several mammalian, fungal and bacterial CAs [40–43]. Several low nanomolar and subnanomolar CAIs were thus detected against all these enzymes [40–43]. X-ray crystal structures were also reported for several DTCs complexed to hCA II. Many DTCs inhibited hCA II in the low nanomolar range. Their binding mode to the enzyme is identical to that of trithiocarbonate, i.e. with one sulfur of the CS_2^- moiety coordinated to the metal ion, but the organic scaffold present in the DTC was observed to make extensive contacts with many amino acid residues from the active site, which explained the wide range of inhibitory power of these derivatives (from the subnanomolar to the micromolar, for the entire series of around 30 DTC reported so far [40–43]). Interestingly, the highly water soluble morpholine DTC **12** was also very effective in vivo as an antiglaucoma agent when administered topically, directly into the eye of hypertensive rabbits [41], a widely used animal model of glaucoma [38]. Although few such compounds were investigated up until now in detail, the DTCs, being readily available, easy to synthesize and with an excellent water solubility, may have a firm place in the antiglaucoma drugs armamentarium.



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6 Conclusions

CAIs are widely used in the treatment of glaucoma, both systemically and as topically-acting agents. Three such drugs, acetazolamide, methazolamide and dichlorophenamide, are the first generation, systemic sulfonamides, in clinical use since the 1950s, and even if they possess a range of side effects they are still useful for the treatment of refractory forms of the disease. The newer, topically acting agents dorzolamide and brinzolamide, are used as eye drops and show fewer systemic side effects compared to acetazolamide or dichlorophenamide, but they show many ocular side effects. Both drugs are used alone or in combination with β-blockers or PG F2 receptor agonists. However, no new drugs from this pharmacological class appeared for more than 15 years. Several interesting approaches have been however reported, which led to many interesting compounds

which were evaluated in animal models of the disease. Among them, the tail approach led to a high number of very effective sulfonamide CAIs, many of which with excellent water solubility, good activity against the target enzymes, and excellent preliminary activity in several animal models of glaucoma. Among these approaches, the hybrid drug one seems to have led to highly interesting compounds incorporating sulfonamides and NO-donating moieties on one hand, or sulfonamides and Prostaglandin F receptor agonist on the other hand. Such compounds, possessing a dual mode of action, were active in animal models of the disease. Finally, the dithiocarbamates have been reported as a new class of CAIs, and some representatives were also showed to possess significant antiglaucoma activity in animal models. Considering the lack of innovation in the ophthalmology field for the last 15 years, it would be desirable that some of these interesting compounds may arrive to clinics soon.

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References

1. Quigley HA (2011) Glaucoma. Lancet 377:1367–1377
2. Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90:151–156
3. Mincione F, Menabuoni L, Supuran CT (2004) Clinical applications of the carbonic anhydrase inhibitors in ophthalmology. In: Supuran CT, Scozzafava A, Conway J (eds) Carbonic anhydrase – its inhibitors and activators. CRC Press, Boca Raton, pp 243–254
4. Mincione F, Scozzafava A, Supuran CT (2009) Antiglaucoma carbonic anhydrase inhibitors as ophthalomologic drugs. In: Supuran CT, Winum JY (eds) Drug design of zinc-enzyme inhibitors: functional, structural, and disease applications. Wiley, Hoboken, pp 139–154
5. Carta F, Supuran CT, Scozzafava A (2012) Novel therapies for glaucoma: a patent review 2007–2011. Expert Opin Ther Pat 22:79–88
6. Friedenwald JS (1949) The formation of the intraocular fluid. Am J Ophthalmol 32:9–27
7. Kinsey VE (1953) Comparative chemistry of of aqueous humor in posterior and anterior chambers of rabbit eye. Arch Ophthalmol 50:401–417
8. Kinsey VE, Barany E (1949) The rate flow of aqueous humor. II. Derivation of rate of flow and its physiologic significance. Am J Ophthalmol 32:189–202
9. Wistrand PJ (1951) Carbonic anhydrase in the anterior uvea of the rabbit. Acta Physiol Scand 24:144–148
10. Becker B (1955) The mechanism of the fall in intraocular pressure by the carbonic anhydrase inhibitor Diamox. Am J Ophthalmol 39:177–183
11. Kinsey VE, Reddy DVN (1959) Turnover of total carbon dioxide in aqueous humors and the effect thereon of acetazolamide. Arch Ophthalmol 62:78–83
12. Supuran CT, Scozzafava A (2000) Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opin Ther Pat 10:575–600
13. Supuran CT (2008) Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nature Rev Drug Discov 7:168–181
14. Supuran CT (2010) Carbonic anhydrase inhibitors. Bioorg Med Chem Lett 20:3467–3474
15. Supuran CT (2010) Carbonic anhydrase inhibition/activation: trip of a scientist around the world in the search of novel chemotypes and drug targets. Curr Pharm Des 16:3233–3245

16. Supuran CT, Scozzafava A, Casini A (2003) Carbonic anhydrase inhibitors. Med Res Rev 23:146–189
17. Maren TH (1967) Carbonic anhydrase: chemistry, physiology and inhibition. Physiol Rev 47:595–781
18. Miller WH, Dessert AM, Roblin RO Jr (1950) Heterocyclic sulfonamides as carbonic anhydrase inhibitors. J Am Chem Soc 72:4893–4896
19. Supuran CT, Scozzafava A, Casini A (2004) Development of sulfonamide carbonic anhydrase inhibitors (CAIs). In: Supuran CT, Scozzafava A, Conway J (eds) Carbonic anhydrase – its inhibitors and activators. CRC Press, Boca Raton, pp 67–147
20. Liao SY, Ivanov S, Ivanova A, Ghosh S, Cote MA, Keefe K, Coca-Prados M, Stanbridge EJ, Lerman MI (2003) Expression of cell surface transmembrane carbonic anhydrase genes CA9 and CA12 in the human eye: overexpression of CA12 (CAXII) in glaucoma. J Med Genet 40:257–261
21. Maren TH, Jankowska L, Sanyal G, Edelhauser HF (1983) The transcorneal permeability of sulfonamide carbonic anhydrase innhibitors and their effect on aqueous humor secretion. Exp Eye Res 36:457–480
22. Sugrue MF (2000) Pharmacological and ocular hypotensive properties of topical carbonic anhydrase inhibitors. Prog Ret Eye Res 19:87–112
23. Maus TL, Larsson LI, McLaren JW, Brubaker RF (1997) Comparison of dorzolamide and acetazolamide as suppresors of aqueous humor flow in humans. Arch Ophthalmol 115:45–49
24. Silver LH (2000) Dose–response evaluation of the ocular hypotensive effect of brinzolamide ophthalmic suspension (Azopt). Brinzolamide dose–response study group. Surv Ophthalmol 44(suppl 2):147–153
25. Aalto-Korte K (1998) Contact allergy to dorzolamide eyedrops. Contact Dermatitis 39:206
26. Carlsen J, Durcan J, Swartz M, Crandall A (1999) Nephrolithiasis with dorzolamide. Arch Ophthalmol 117:1087–1088
27. Thoe Schwartzenberg GW, Trop GE (1999) Anorexia, depression and dementia induced by dorzolamide eyedrops (Trusopt). Can J Ophthalmol 34:93–94
28. Konowal A, Morrison JC, Brown SV, Cooke DL, Maguire LJ, Verdier DV (1999) Irreversible corneal decompensation in patients treated with topical dorzolamide. Am J Ophthalmol 127:403–406
29. Scozzafava A, Menabuoni L, Mincione F, Briganti F, Mincione G, Supuran CT (1999) Carbonic anhydrase inhibitors: synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/heterocyclic sulfonamides containing cationic or anionic moieties: is the tail more important than the ring*?* J Med Chem 42:2641–2650
30. Scozzafava A, Briganti F, Mincione G, Menabuoni L, Mincione F, Supuran CT (1999) Carbonic anhydrase inhibitors. Synthesis of water-soluble, amino acyl/dipeptidyl sulfonamides possessing long lasting-intraocular pressure lowering properties via the topical route. J Med Chem 42:3690–3700
31. Scozzafava A, Menabuoni L, Mincione F, Briganti F, Mincione G, Supuran CT (2000) Carbonic anhydrase inhibitors: perfluoroalkyl/aryl-substituted derivatives of aromatic/heterocyclic sulfonamides as topical intraocular pressure lowering agents with prolonged duration of action. J Med Chem 43:4542–4551
32. Scozzafava A, Menabuoni L, Mincione F, Supuran CT (2002) Carbonic anhydrase inhibitors. A general approach for the preparation of water soluble sulfonamides incorporating polyamino-polycarboxylate tails and of their metal complexes possessing long lasting, topical intraocular pressure lowering properties. J Med Chem 45:1466–1476
33. Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone G (2012) Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem Rev 112:4421–4468
34. Supuran CT (2011) Carbonic anhydrase inhibitors and activators for novel therapeutic applications. Future Med Chem 3:1165–1180
35. Supuran CT (2012) Structure-based drug discovery of carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 27:759–772

36. Steele RM, Batugo MR, Benedini F, Biondi S, Borghi V, Carzaniga L, Impagnatiello F, Miglietta D, Chong WK, Rajapakse R, Cecchi A, Temperini C, Supuran CT (2009) Nitric oxide-donating carbonic anhydrase inhibitors for the treatment of open-angle glaucoma. Bioorg Med Chem Lett 19:6565–6570
37. Mincione F, Benedini F, Biondi S, Cecchi A, Temperini C, Formicola G, Pacileo I, Scozzafava A, Masini E, Supuran CT (2011) Synthesis and crystallographic analysis of new sulfonamides incorporating NO-donating moieties with potent antiglaucoma action. Bioorg Med Chem Lett 21:3216–3221
38. Fabrizi F, Mincione F, Somma T, Scozzafava G, Galassi F, Masini E, Impagnatiello F, Supuran CT (2012) A new approach to antiglaucoma drugs: carbonic anhydrase inhibitors with or without NO donating moieties. Mechanism of action and preliminary pharmacology. J Enzyme Inhib Med Chem 27:138–147
39. Long DD, Frieman B, Hegde SS, Hill CM, Jiang L, Kintz S, Marquess DG, Purkey H, Shaw JP, Steinfeld T, Wilson MS, Wrench K (2013) A Multivalent approach towards linked dual-pharmacology prostaglandin F receptor agonist/carbonic anhydrase-II inhibitors for the treatment of glaucoma. Bioorg Med Chem Lett 23:939–943
40. Carta F, Aggarwal M, Maresca A, Scozzafava A, McKenna R, Supuran CT (2012) Dithiocarbamates: a new class of carbonic anhydrase inhibitors. *Crystallographic and kinetic investigations*. Chem Commun (Cambridge) 48:1868–1870
41. Carta F, Aggarwal M, Maresca A, Scozzafava A, McKenna R, Masini E, Supuran CT (2012) Dithiocarbamates strongly inhibit carbonic anhydrases and show antiglaucoma action in vivo. J Med Chem 55:1721–1730
42. Monti SM, Maresca A, Viparelli F, Carta F, De Simone G, Mühlschlegel FA, Scozzafava A, Supuran CT (2012) Dithiocarbamates strongly inhibit the beta-class fungal carbonic anhydrases from *Cryptococcus neoformans, Candida albicans* and *Candida glabrata*. Bioorg Med Chem Lett 22:859–862
43. Maresca A, Carta F, Vullo D, Supuran CT (2013) Dithiocarbamates strongly inhibit the beta-class carbonic anhydrases from *Mycobacterium tuberculosis*. J Enzyme Inhib Med Chem 28:407–411
44. Temperini C, Scozzafava A, Supuran CT (2010) Carbonic anhydrase inhibitors. X-Ray crystal studies of the carbonic anhydrase II – trithiocarbonate adduct – an inhibitor mimicking the sulfonamide and urea binding to the enzyme. Bioorg Med Chem Lett 20:474–478