Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2145–2155

BIOORGANIC & MEDICINAL CHEMISTRY

Carbonic Anhydrase Inhibitors: Sulfonamides Incorporating Furan-, Thiophene- and Pyrrole-carboxamido Groups Possess Strong Topical Intraocular Pressure Lowering Properties as Aqueous Suspensions

Monica Ilies,[a] Claudiu T. Supuran,[b,*] Andrea Scozzafava,[b] Angela Casini,[b] Francesco Mincione,[c] Luca Menabuoni,[d] Miron T. Caproiu,[e] Maria Maganu[e] and Mircea D. Banciu[f]

[a]*University of Agricultural Sciences and Veterinary Medicine, Faculty of Biotechnologies, Department of Chemistry, B-dul Marasti 59, 71331-Bucharest, Romania*

[b]*Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121, Florence, Italy*

[c]*Università degli Studi, Istituto Oculistico, Viale Morgagni 85, I-50134 Florence, Italy*

[d]*Ospedale San Giovanni di Dio, S. O. Oculistica, Via Torregalli 3, I-50123, Florence, Italy*

[e]*'C.D. Nenitzescu' Institute of Organic Chemistry, Splaiul Independentei 202B, Bucharest, Romania*

[f]*Polytechnic University, Department of Organic Chemistry, Splaiul Independentei 313, Bucharest, Romania*

Received 3 April 2000; accepted 31 May 2000

Abstract—Important physiological and physio-pathological functions are played by several carbonic anhydrase (CA, EC 4.2.1.1) isozymes, which are strongly inhibited by aromatic and heterocyclic sulfonamides. Here we report several new types of such sulfonamides, incorporating furan-, thiophene- and pyrrole-carboxamide moieties in their molecules. Some of these compounds showed very good CA II and CA IV inhibitory properties, with affinities for the enzymes in the low nanomolar range. Due to their relatively low water solubility, some of the most active CA II inhibitors reported here have been formulated as aqueous suspension for topical administration as antiglaucoma agents, in normotensive and glaucomatous rabbits. The derivatives incorporating furan- and pyrrole-carboxamide moieties (but not the corresponding thiophene-substituted derivatives), showed effective and long-lasting intraocular pressure (IOP) lowering both in normotensive as well as glaucomatous animals, with potencies superior to dorzolamide and brinzolamide, the two available topically acting sulfonamide drugs. This is the first example of non-water soluble sulfonamides that significantly lower IOP, being thus similar with the recently introduced drug brinzolamide, which belongs to a completely different chemical family of antiglaucoma sulfonamides.

Introduction

At least 14 different isozymes of the zinc protein carbonic anhydrase (CA, EC 4.2.1.1.) have been reported up to now in higher vertebrates.[1–3] By catalyzing the reversible carbon dioxide hydration to bicarbonate, some of these enzymes participate in crucial physiological processes in these organisms, such as: transport of CO_2/HCO_3^- between metabolizing tissues and lungs,[4] pH homeostasis,[5] cellular signal transduction,[5b] secretion of electrolytes in different tissues/organs (e.g. aqueous humor in the ciliary processes,[5a,6] pancreatic[7] and gastric juice[7] formation, cerebrospinal fluid secretion,[5a,7] urinary acid production in the proximal tubule of the kidney[7]), as well as some biosynthetic reactions such as the lipogenesis in adypocites[8] or gluconeogenesis and ureagenesis in the liver/kidneys.[9] CA inhibition may ensue interesting medical applications for the prevention or treatment of disorders caused by an excessive activity of these enzymes. The inhibitory ability of aromatic/heterocyclic sulfonamides against α-CAs has been extensively studied and periodically reviewed[3,5a,10] during the last 50 years. The continuous development of research in this field is motivated by the practical applications of sulfonamide CA inhibitors. Such compounds have been used first as diuretics,[11] or afterwards in the therapy of acid-base disequilibria,[5,12] epilepsy,[13] mountain sickness,[14] gastroduodenal ulcers,[7,15] glaucoma,[16,17] congestive heart failure,[16] osteoporosis.[18] Recently, some new approaches

*Corresponding author. Tel.: +39-055-275-7552; fax: +39-055-2757555; e-mail: cts@bio.chim.unifi.it

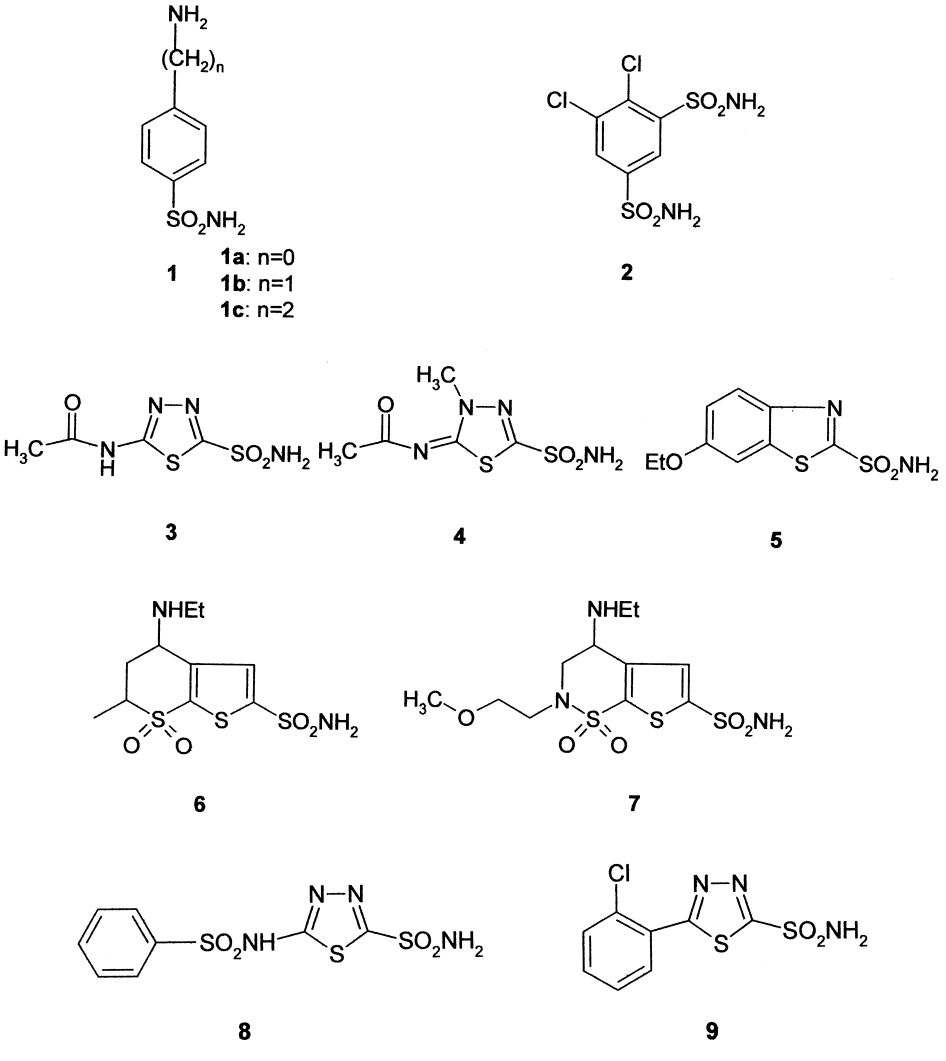
0968-0896/00/$ - see front matter
PII: S0968-0896(00)00143-7

for the therapy of cancer[19] or for designing novel diagnostic tools in positron emission tomography (PET) and magnetic resonance imaging (MRI) have been reported for some compounds belonging to this class of pharmacological agents.[20,21]

Aromatic sulfonamide CA inhibitors such as sulfanilamide[3,5a,22] **1a** or dichlorophenamide[11a] **2** have been used both as drugs as well as leads for designing other biologically active compounds (such as the thiazides and high-ceiling diuretics[11a,16a,23] or antithyroid drugs[24]). Among the heterocyclic sulfonamides, acetazolamide[25] **3**, methazolamide[26] **4**, and ethoxzolamide[27] **5** have been widely used clinically for more than 45 years, mainly as systemic antiglaucoma drugs. More recently, dorzolamide[28,29] **6** and the structurally related brinzolamide[30] **7** have been approved as topical antiglaucoma agents. Benzolamide **8** and chlorzolamide **9** — sulfonamides containing both aromatic and heterocyclic rings — are also representatives of this class of CA inhibitors, and possess interesting applications both for the design of efficient new antiglaucoma agents[31] or for PET-based diagnostic tools.[32] All the clinically available CA inhibitors[3,33] possess some relevant side effects, due to their lack of selectivity towards different CA isozymes or the organs/tissues in which these are present.[3,34–37]Therefore, the synthesis and assay as CA inhibitors of novel aromatic/heterocyclic sulfonamide derivatives still represents an interesting challenge in order to discover isozyme-specific or, at least, organ-selective such pharmacological agents, devoid of severe side effects.

In this paper we report several new types of aromatic/heterocyclic sulfonamide CA inhibitors with potential use as topical antiglaucoma agents. An ideal antiglaucoma drug of this type should possess the following features: (i) it must be a strong CA II and CA IV inhibitor, in order to achieve a significant reduction of aqueous humor secretion; (ii) it should posses a high enough liposolubility in order to penetrate through the cornea, but this should eventually be balanced by an acceptable hydrosolubility, in order to formulate it in acceptable form for the topical administration.[34–40]

Up to now, only dorzolamide **6** seemed to possess such properties,[17] but this drug is not very effective, and many serious side effects have been reported after its administration.[41–44] Less eye stinging/reddening than with dorzolamide has been reported with brinzolamide



7,[30] which is applied as an aqueous suspension, due to its reduced water solubility. Thus, we thought to design such sulfonamides, possessing a relatively balanced hydro-/lipo-solubility, that can be formulated either as solutions, or suspensions for the topical administration. The drug design has been based on the 'tail' strategy reported previously by our group,[37,38] which consists in attaching certain moieties that would induce the desired physico-chemical properties to the molecules of aromatic/heterocyclic sulfonamides possessing free amino/hydroxy groups. These moieties should induce among others high affinity to the CA active site, acceptable water/lipid solubility, and good penetrability through the biological membranes, to the molecules of the newly obtained CA inhibitors. The tails chosen to be incorporated in the compounds reported here are of the heterocyclic-carboxamide type, since such derivatives were little investigated up to now, and they may be easily fine-tuned for obtaining both compounds with an increased water- as well as lipid solubility.

Results

The new compounds **A1**–**A7**, **B1**–**B9** and **C1**–**C9**, incorporating pyrrole-, furan- and thiophene-carboxamide moieties, respectively, reported here, are shown in Scheme 1, and were prepared by non-exceptional procedures, related to those previously reported by Whiteside's[39,40] and our groups.[37,38]

Inhibition data against three CA isozymes (hCA I, hCA II and bCA IV; h = human, b = bovine isozyme) with the new compounds reported here and standard inhibitors are shown in Table 1.

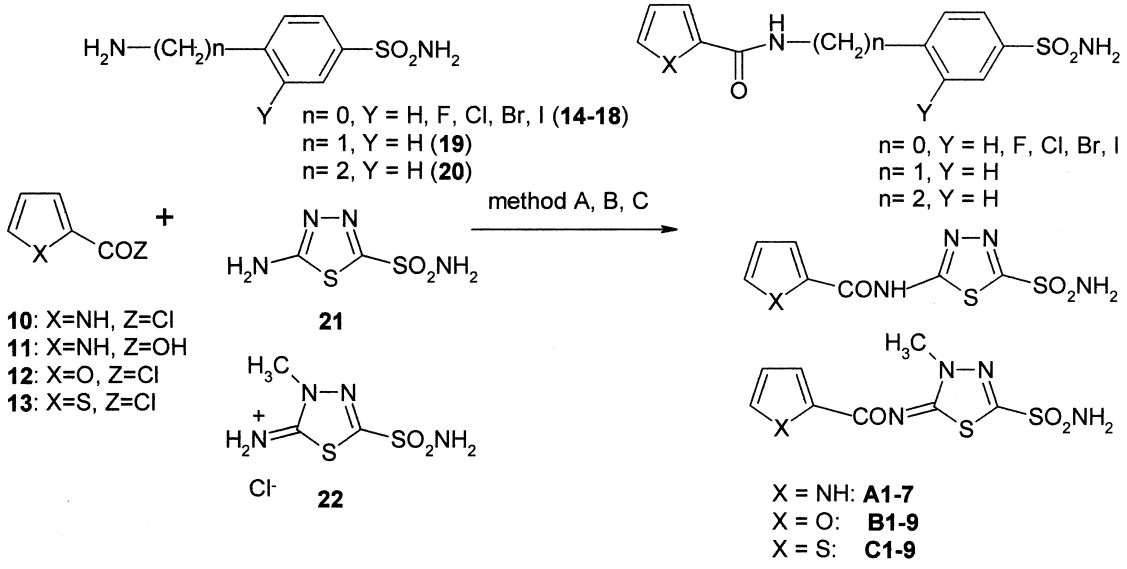
Some physico-chemical properties of several highly active CA inhibitors reported in the paper (as well as data for some standard compounds) are shown in Table 2.

In vivo IOP lowering after topical administration of some of the new sulfonamides as well as dorzolamide **6** (as standard), in normotensive rabbits, are shown in Table 3 and Figure 1, whereas data of glaucomatous rabbits are shown in Table 4.

Discussion

Three related series of compounds, **A1**–**A7**, **B1**–**B9** and **C1**–**C9**, incorporating pyrrole-, furan- and thiophene-carboxamide moieties, respectively, were prepared as shown in Scheme 1. For the furan and thiophene derivatives, classical acylation procedures involving the acyl chlorides (method A) led to good results. In the case of the pyrrole carboxylic acid derivatives (which are highly sensitive to acidic medium), two different procedures were employed: a peptide-like coupling with some aminosulfonamides, in the presence of diisopropylcarbodiimide (method B),[37,38] or the reaction of in situ generated pyrrole-2-carboxylic acid chloride with the above mentioned aminosulfonamides (method C). Mention should be made that paradoxically, very few sulfonamide CA inhibitors incorporating heterocyclic-carboxamido moieties have been reported up to now, although several thousand different sulfonamides have been synthesized in the search of topically acting antiglaucoma sulfonamides in the last 15 years.[35–40]

Inhibition data with the new compounds, the parent sulfonamides and standard inhibitors (Table 1) showed the following: (i) the new compounds **A1**–**A7**, **B1**–**B9** and **C1**–**C9** showed increased affinities for the three investigated CA isozymes, as compared to the corresponding parent compounds from which they were prepared (the sulfonamides **14**–**22**); (ii) the affinities of the new inhibitors generally varied in the following way, based on the parent sulfonamide from which they were prepared: the derivative of sulfanilamide < the homosulfanilamides < the *p*-



Scheme 1. Method A: Z=Cl, Net_3/MeCN, 0 °C; Method B: Z=OH, DIPCD/DMAP/dioxane, rt (X=NH); Method C: Z=OH, first $SOCl_2$/Py, 1 h 30′, rt then aminosulfonamide (X=NH).

Table 1. CA inhibition data with standard inhibitors, the parent sulfonamides **14**–**22** and the new derivatives reported in the present study, against isozymes I, II and IV[c]

Inhibitor	K_I^* (nM)		
	hCA I[a]	hCA II[a]	bCA IV[b]
Acetazolamide **1**	250	12	70
Methazolamide **2**	50	14	36
Dorzolamide **6**	50,000	9	45
14	28,000	300	3000
15	8300	60	180
16	9800	110	320
17	6500	40	66
18	6000	70	125
19	25,000	170	2800
20	21,000	160	2450
21	8600	60	540
22	9300	19	355
A1	25,000	105	160
A2	500	10	41
A3	550	21	56
A4	1000	36	80
A5	850	21	52
A6	350	8	40
A7	320	7	36
B1	15,000	95	140
B2	445	10	37
B3	510	17	45
B4	520	30	55
B5	550	23	48
B6	960	31	49
B7	800	20	47
B8	280	6	32
B9	290	6	30
C1	11,500	62	103
C2	365	8	23
C3	240	7	15
C4	140	12	21
C5	120	9	17
C6	540	16	49
C7	270	12	47
C8	280	3	15
C9	290	4	19

[a]Human (cloned) isozyme.
[b]Isolated from bovine lung microsomes.
[c]Data from ref 30 Standard error for the determination of K_I-s was of 10–20% (from 3 different assays).

Table 2. Solubility, chloroform-buffer partition coefficients and in vitro corneal permeability of some sulfonamide CA inhibitors

Compound	Solubility[a] nM	Log P[b]	$k_{in}\times10^3$ (h^{-1})[c]	
			Cornea intact	No epithelium
1 (acetazolamide)[d]	3.2	0.001	0.37	7.0
2 (methazolamide)[d]	12	0.06	1.90	13
6 (dorzolamide)	60[e]	2.0[e]	3.0	5.2
A2	5.0	1.39	4.0	4.6
A6	5.8	0.0008	0.6	1.9
A7	9.2	0.536	2.8	6.0
B8	2.4	0.005	0.9	3.8
B9	7.5	0.633	4.1	7.6
C2	0.05	0.44	4.7	8.5
C8	0.04	0.033	4.8	11.5
C9	0.1	0.750	5.0	10.2

[a]Solubility in pH 7.40 buffer, at 25 °C.
[b]Chloroform-buffer partition coefficient.
[c]Determined as described in ref[56,57]
[d]Data from ref[37]
[e]As hydrochloride, at pH 5.8, from ref[17]

Table 3. Fall of IOP of normotensive rabbits (20±3 mm Hg), after treatment with one drop (50 μL) 2% water suspension/solution (for dorzolamide **6**) of CA inhibitor directly into the eye, at 30, 60 and 90 min after administration

Inhibitor	pH	ΔIOP (mm Hg)*			
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
6[a]	5.5	0	2±0.20	4±0.30	3±0.25
A2	7.5	0	8±0.40	2±0.25	2±0.20
A6	7.5	0	0	0	2±0.15
A7	7.0	0	4±0.30	8±0.40	5±0.20
B5	7.5	0	0	3±0.10	5±0.15
B8	7.5	0	0	0	7±0.30
B9	7.5	0	7±0.25	9±0.45	9±0.35
C2	7.5	0	0	0	0.5
C8	7.5	0	0	0	0
C9	7.5	0	0	0	0

[a]As HCl salt, in solution. *$\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; Mean±SE ($n=3$).

aminoethyl-benzenesulfonamides ≅the bromo/iodo-substituted sulfanilamides < the the chloro/fluoro-substituted sulfanilamides < the 1,3,4-thiadiazole-2-sulfonamides ≅4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide; (iii) based on the carboxylic acid from which they were obtained, the thiophene carboxylic acid derivatives were more effective CA inhibitors as compared to the corresponding furan carboxylic acid derivatives, which in turn were slightly more active than the corresponding pyrrole carboxamido derivatives; (iv) all three CA isozymes investigated here were susceptible to inhibition with this type of sulfonamide, with hCA II and bCA IV the most sensitive, whereas hCA I was generally less susceptible to inhibition as compared to the first two isozymes.

The promising in vitro CA inhibitory activity as well as other physico-chemical properties (Table 2) for some of the newly prepared compounds prompted us to investigate their effect in vivo, on the intraocular pressure (IOP), after topical application directly into the eye, in normotensive and glaucomatous rabbits (frequently used as an animal model of glaucoma) of suspensions of these CA inhibitors.[35–37]Mention should be made that in contrast to dorzolamide **6**, the compounds reported here do not possess a good enough water solubility in order to formulate them as 2% solutions. This is in fact also the case of brinzolamide **7**, the recently approved new topical CA inhibitor. Thus, we used 2% water suspensions of the new compounds for the in vivo experiments discussed shortly.

The compounds selected for in vivo studies were among the most active in vitro inhibitors against hCA II and bCA IV, in the prepared series, such as: **A2, A6, A7, B5, B8, B9, C2, C8** and **C9**. The following should be noted regarding data shown in Tables 2–4: (i) the compounds incorporating thiophene-carboxamide moieties (such as **C2**, **C8** and **C9**) are ineffective as topical IOP lowering agents, although they were the best CA II and CA IV inhibitors in the new series; (ii) the compounds incorporating

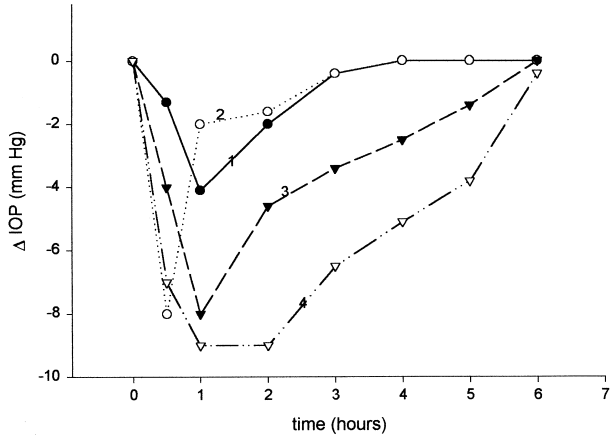


Figure 1. Effect of topically administered sulfonamide inhibitors (2% water solutions/suspensions) on the IOP of normotensive albino rabbits. Curve 1: dorzolamide **1** (hydrochloride salt, pH 5.5; solution); curve 2: compound **A2** (suspension, pH 7.50); curve 3: compound **A7** (suspension, pH 7.0), curve 4: compound **B9** (suspension, pH 7.50).

Table 4. Fall of IOP of glaucomatous rabbits (36±2 mm Hg) after treatment with one drop (50 μL) 2% suspension of sulfonamide directly into the eye, at 30, 60 and 90 min after administration

		ΔIOP (mm Hg)[a*]			
Inhibitor	pH	$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
A2	7.5	0	10±0.35	10.5±0.30	8.5±0.40
A7	7.0	0	7.5±0.50	11±0.25	15±0.20
B9	7.5	0	9.5±0.35	14±0.20	16±0.35

[a*] $\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; Mean±SE ($n=3$).

pyrrole-carboxamide- and furan-carboxamide moieties (such as **A2**, **A6**, **A7**, **B5**, **B8**, **B9**) are show topical activity in lowering IOP, after administration as aqueous suspensions. Some of these derivatives, such as **A6**, **B5** and **B8** show relatively modest activity, of the same order of magnitude (or slightly less) as that of dorzolamide **6**, used as standard in these measurements (brinzolamide **7**, although already available clinically in USA, has not been approved yet in Europe and we could not include it in our experiments). Other compounds, such as **A7** and **B9** showed very effective IOP lowering after topical administration, being 2–4 times more effective than dorzoalmide, both at 30, 60 and 90 min after administration. Furthermore, their efficacy is much more prolonged in time, as compared to that of dorzolamide, as observed from the data of Figure 1. Practically IOP returns at basal values 2 h after administration of dorzolamide **6**, whereas the same effect is observed after 6 h with the most active compound in this series, which is **B9**. Returning now to the structure–activity relationship, some very interesting facts emerged during this research. Thus, one may observe that the most active compounds in lowering IOP in this series were those possessing the following three features: (1) strong CA II (and CA IV) inhibitory properties, (2) moderate water and lipid solubilities; and (3) good accession rates through the cornea. If only one of these three factors is absent, the corresponding compound is a failure from the point of view of topical IOP lowering. Thus, the thiophene-carboxamide derivatives investigated here were the most effective in vitro CA inhibitors in the three reported subseries, were liposoluble enough so that they had the best accession rates through the cornea, but due to their virtually zero water solubility, they did not show at all any topical action. In fact, these compounds are quite similar to methazolamide, acetazolamide or ethoxzolamide, which are all three ineffective topically, due to the same problem encountered above, i.e. absence of appropriate water solubility. The thiadiazole-sulfonamide derivatives **A6** and **B8** act as potent CA inhibitors, have a low (but acceptable) water solubility, but being very polar (log P of the same order of magnitude as that of acetazolamide or lower), their access through the cornea is problematic (see k_{in} values in Table 2), so that they only showed modest IOP lowering effects, which appeared after 90 min or longer periods from administration (data not shown). In contrast to these compounds, the closely related thiadiazolines **A7** and **B9** (possessing an additional methyl group as compared to the previously mentioned thiadiazoles **A6** and **B8**) possess all the three factors mentioned above in the right measure: they are strong CA inhibitors (similarly to the thiadiazoles), possess a low (but acceptable) water solubility, balanced by a modest lipid solubility (augmented as compared to that of the corresponding thiadiazoles, due to the presence of the additional methyl moieties), so that their accession rates through the cornea are of the same magnitude (or better) than those of dorzolamide. This seems to be the reason why these compounds act as more efficient IOP lowering agents than the standard drug **6**. The other compound studied in some detail (**A2**) offers another examples that may validate our 'theory' exposed above. Thus, **A2** is very efficient in lowering IOP at early periods after administration, but then the IOP lowering vanishes rapidly. We consider this to be due to the relatively high liposolubility of this compound, which helps a lot its penetration through the cornea, but at the same time assures its easy diffusibility into the blood (where high amounts of CA I and CA II are present), so that the drug may be washed out from the ciliary processes too rapidly.

The data of Table 4 show that these compounds are highly active IOP lowering agents also in the glaucomatous rabbit, again with **B9** acting very efficiently, followed by **A7** and **A2**.

Conclusion

We report here some novel aromatic/heterocyclic sulfonamides incorporating pyrrole-, furan- and thiophene-carboxamide moieties in their molecules. Many of the new compounds showed low nanomolar affinities for isozymes CA II and CA IV, being slightly less effective in inhibiting isozyme CA I. The compounds which showed strong CA II (and CA IV) inhibitory properties, correlated with moderate (but not insignificant) water and lipid solubilities; as well as good accession rates through the cornea, effectively lowered IOP in normotensive and glaucomatous rabbits, when administered as 2% water suspensions directly into the eye. Furthermore,

such compounds showed a prolonged duration of action as compared to the clinically used drug dorzolamide, administered as 2% solution. As far as we know this is one of the first studies which fine-tunes the in vivo properties of topically acting sulfonamide CA inhibitors, based on the physico-chemical properties of the obtained derivatives, allowing thus for a rationale drug design of more effective antiglaucoma therapies.

Experimental

Chemistry

Melting points were determined with a Boetius heating plate microscope and are not corrected; IR spectra were obtained in KBr pellets with a Carl Zeiss UR 20 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian Gemini 300BB apparatus operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, in d_6-DMSO as solvent. Chemical shifts are expressed as δ values (ppm) relative to Me_4Si as internal standard. Attributions were done by means of chemical shifts, peak integration, COSY (^{1}H-^{1}H), HETCOR (^{1}H-^{13}C), attached proton test (APT), model spectra and selective deuteration. Elemental analyses were done by combustion, for C, H, N, with an automated Carlo Erba analyzer and the results were found±0.4% within the theoretical values.

All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with MeOH:$CHCl_3$ 1:4 v/v. 2-Pyrrole-carboxylic acid, homosulfanilamide (hydrochloride) and 4-(2-aminoethyl)benzenesulfonamide were from Sigma-Aldrich (Milan, Italy). 2-Thiophene- and 2-furancarboxylic acid chlorides, sulfanilamide, as well as pyridine, acetonitrile and other solvents used in syntheses were from E. Merck (Darmstadt, Germany). 5-Amino-1,3,4-thiadiazole-2-sulfonamide was obtained from acetazolamide (Sigma) by deprotection with concentrated hydrochloric acid followed by neutralization, using a previously reported procedure.[41,43] 5-Imino-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide hydrochloride was similarly prepared from methazolamide (Sigma). Halogenosulfonamides were prepared by literature procedures,[45] optimized in our laboratory. Preparative column chromatography was performed on silica gel 60 (0.063–0.200 mm)-from Merck-eluted with MeOH:$CHCl_3$ 1:9 v/v.

General procedure for the preparation of compounds

The compounds were obtained from the corresponding hetharylcarboxylic acid chlorides, commercially available for thiophene and furan, using classical acylation procedures[26] in acetonitrile as solvent (method A). In the case of pyrrol carboxylic acid — very sensitive to acidic medium, two procedures were used: peptide-like coupling using carbodiimides (DIPCD/DMAP), which works well with aminosulfonamides with an aliphatic amino group (method B) and coupling of the corresponding pyrroyl chloride, generated in situ (by treating the pyrrol-2-carboxylic acid with $SOCl_2$ in anhydrous pyridine) with amino(halogeno)sulfonamides (method C).

Method A. An amount of 5 mmoles aminosulfonamide was suspended/dissolved in 20–30 mL anhydrous MeCN and 0.78 mL (0.56 g, 5.5 mmoles) triethylamine was added under stirring. The mixture was cooled to 0–5 °C, then a solution of 5 mmoles furoyl/thienyl chloride dissolved in 3 mL MeCN was added dropwise during 10 min (immediately, a white precipitate appeared). The reaction was stirred overnight or until a reasonable conversion was reached (TLC control). The solvent was evaporated in vacuum and the resulted product was treated with 15–20 mL water. The crude solid product was filtered, washed with 2 mL water and air dried. Yields were in the range of 35–90%, depending on the reactivity of the aminosulfonamide. The obtained compounds were further purified by recrystallisation from the specified solvent or, in some cases, by column chromatography, using silicagel 60 as stationary phase, eluted with MeOH/$CHCl_3$ gradients. The fractions containing the desired compound were collected, evaporated to dryness and the obtained product was recrystallized from solvents specified in each case.

Method B. Five mmoles of aminosulfonamide, dissolved in 30 mL anhydrous dioxane were treated, under stirring, with 0.78 mL (0.63 g, 5 mmoles) diisopropylcarbodiimide (DIPCD) and 0.06 g (0.5 mmoles) dimethylaminopyridine (DMAP); the reaction mixture was kept under argon and 0.56 g (5 mmoles) of pyrrol-2-carboxylic acid was added. The obtained homogeneous colorless solution turned to orange-cream and in around 20 min a precipitate appeared. The stirring was continued overnight, then the precipitated diisopropylurea (DIPU) was filtered, washed with a small volume of dioxane and discarded. The combined dioxanic solutions were subsequently evaporated to a small volume and treated with 20 mL of ethyl acetate, for complete precipitation of DIPU (vide infra). After filtering, the precipitate (DIPU) was triturated with 10 mL aqueous NaOH (10%) refiltered, then discarded. The aqueous solution was brought to pH = 1.5 with 20% aqueous HCl. The precipitated crude product was filtered, washed with 2 mL water and dried. An additional amount of crude product was obtained by extracting twice the previous ethyl acetate mother liquor with 10 mL 10% aqueous NaOH, separating the aqueous layer, treating it with 20% aqueous HCl and filtering, washing and drying the resulting precipitate. Total yields were in the range of 45–55%. The pure products were obtained after column chromatography, using a similar procedure as in method A.

Method C. An amount of 0.56 g (5 mmoles) of pyrrol-2-carboxylic acid was dissolved under stirring in 10 mL of anhydrous pyridine, then 0.4 mL (0.654 g, 5.5 mmoles) freshly distilled thionyl cloride was added dropwise. After the flask was septum stoppered and flushed with argon, three drops of dimethylformamide were added with a syringe needle, left afterwards into the septum. The generation of pyrroyl chloride was completed in one hour (TLC control); meanwhile a precipitate of pyridinium chloride was formed. The reaction mixture was treated with the stoechiometric amount (5 mmoles) of the corresponding aminosulfonamide/aminosulfonamide hydrochloride and stirred under inert atmosphere

until a reasonable conversion was obtained (TLC control). The solvent was then evaporated in vacuum and the resulted glue was treated with 15–20 mL of water, when the crude product precipitated. This was filtered, washed with 2 mL water and air-dried. Yields were in the range of 15–55%, depending on the reactivity of the aminosulfonamide. The pure products were obtained as described in method A.

4-(Pyrrole-2-carboxamido)benzenesulfonamide, A1, (method C). White crystals, mp 297–9 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1330 (SO_2^{as}), 1585 (amide II), 1640 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.19 (m, 1H, H4-pyrrole, 3.7, 2.3, 1.4 etc.), 7.01 (m, 1H, H3-pyrrole, 2.7, 1.5 etc.), 7.12 (m, 1H, H5-pyrrole, 2.4, 1.4 etc.), 7.23 (s, 2H, SO_2NH_2), 7.78 (d, 2H, AA′BB′, 8.9), 7.91 (d, 2H, AA′BB′, 8.9), 10.03 (s, 1H, NHCO), 11.72 (s, 1H, H1-pyrrole); ^{13}C NMR (d_6-DMSO), δ, ppm: 109.07 (C4-pyrrole), 112.00 (C3-pyrrole), 119.20 (C2/C3-Ph), 123.18 (C5-pyrrole), 125.60 (C2-pyrrole), 126.5 (C3/C2-Ph), 137.92 (C1/C4-Ph), 142.39 (C4/C1-Ph), 159.27 (CONH). Anal. ($C_{11}H_{11}N_3SO_3$) C, H, N, S.

4-(Pyrrole-2-carboxamido)-3-fluorobenzenesulfonamide, A2, (method C). White crystals, mp 284–6 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1320 (SO_2^{as}), 1600 (amide II), 1650 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.20 (m, 1H, H4-pyrrole, 3.7, 2.4, 1.4 etc.), 7.01 (m, 1H, H3-pyrrole, 3.7, 2.1 etc.), 7.10 (m, 1H, H5-pyrrole, 2.2, 1.5 etc.), 7.46 (s, 2H, SO_2NH_2), 7.65 (ddd, 1H, H2-Ph, 6.1, 2.0), 7.68 (dtr, 1H, H6-Ph, 8.4, 2.0), 7.92 (ddtr, 1H, H5-Ph, 8.3, 4.1, 1.6), 9.84 (s, 1H, NHCO), 11.79 (s, 1H, H1-pyrrole); ^{13}C NMR (DMSO-d_6), δ, ppm; $J_{C,F}$, Hz: 109.24 (C4-pyrrole), 112.59 (C3-pyrrole), 113.40 (d, C2-Ph, 22.9), 121.13 (C5-Ph), 123.28 (C6-Ph), 125.12 (C2-pyrrole), 125.98 (C5-pyrrole), 129.39 (d, C4-Ph, 11.8), 140.81 (d, C1-Ph, 6.2), 153.89 (d, C3-Ph, 249.9), 159.01 (CONH). Anal. ($C_{11}H_{10}N_3SO_3F$) C, H, N, S.

4-(Pyrrole-2-carboxamido)-3-chlorobenzenesulfonamide, A3, (method C). White crystals, mp 254–7 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1320 (SO_2^{as}), 1580 (amide II), 1660 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.20 (dtr, 1H, H4-pyrrole, 3.7, 2.3), 7.01 (dtr, 1H, H5-pyrrole, 2.6, 1.4), 7.09 (ddd, 1H, H3-pyrrole, 3.8, 2.4, 1.4), 7.49 (s, 2H, SO_2NH_2), 7.78 (dd, 1H, H6-Ph, 8.4, 2.1), 7.89 (d, 1H, H5-Ph, 8.5), 7.92 (d, 1H, H2-Ph, 2.1), 9.64 (s, 1H, NHCO), 11.80 (s, 1H, H1-pyrrole); ^{13}C NMR (DMSO-d_6), δ, ppm: 109.25 (C3-pyrrole), 112.33 (C2-pyrrole), 123.33 (C6-Ph), 124.82 (C4-pyrrole), 125.08 (C4-Ph), 126.82 (C5-Ph), 127.09 (C2-Ph), 127.79 (C1-pyrrole), 138.14 (C1-Ph), 141.39 (C3-Ph), 158.95 (CONH). Anal. ($C_{11}H_{10}N_3SO_3Cl$) C, H, N, S.

4-(Pyrrole-2-carboxamidomethyl)benzenesulfonamide, A4, (method B). White crystals, mp 256–8,5°C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1330 (SO_2^{as}), 1560 (amide II), 1630 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 4.49 (d, 2H, 6.0), 6.10 (m, 1H, H4-pyrrole, 3.7, 2.3 etc.), 6.83 (m, 1H, H3-pyrrole, 3.7, 1.5 etc.), 6.87 (m, 1H, H5-pyrrole, 2.7, 1.4 etc.), 7.31 (s, 2H, SO_2NH_2), 7.46 (d, 2H, AA′BB′, 8.3), 7.78 (d, 2H, AA′BB′, 8.3), 8.62 (t, 1H, NHCO, 6.1), 11.47 (s, 1H, H1-pyrrole); ^{13}C NMR (DMSO-d_6), δ, ppm: 41.58 (CH_2), 108.64 (C4-pyrrole), 110.14 (C3-pyrrole), 125.68 (C2/C3-Ph), 121.56 (C5-pyrrole), 125.94 (C2-pyrrole), 127.41 (C3/C2-Ph), 142.53(C1/C4-Ph), 144.19 (C4/C1-Ph), 160.75 (CONH). Anal. ($C_{12}H_{13}N_3SO_3$) C, H, N, S.

4-(Pyrrole-2-carboxamidoethyl)benzenesulfonamide, A5, (method B). White crystals, mp 263–6 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1330 (SO_2^{as}), 1560 (amide II), 1610 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 2.90 (t, 2H, 7.2), 3.47 (q, 2H, 6.5), 6.07 (m, 1H, H4-pyrrole, 3.6, 2.5, 2.2 etc.), 6.73 (m, 1H, H3-pyrrole, 3.7, 1.5 etc.), 6.84 (m, 1H, H5-pyrrole, 2.6, 1.4 etc.), 7.29 (s, 2H, SO_2NH_2), 7.42 (d, 2H, AA′BB′, 8.2), 7.75 (d, 2H, AA′BB′, 8.2), 8.08 (t, 1H, NHCO, 5.7), 11.39 (s, 1H, H1-pyrrole); ^{13}C NMR (DMSO-d_6), δ, ppm: 35.14 (CH_2-Ph), 39.70, (N-CH_2), 108.50 (C4-pyrrole), 109.77 (C3-pyrrole), 125.68 (C2/C3-Ph), 121.23 (C5-pyrrole), 126.25 (C2-pyrrole), 129.14 (C3/C2-Ph), 141.99 (C1/C4-Ph), 143.89 (C4/C1-Ph), 160.66 (CONH). Anal. ($C_{13}H_{15}N_3SO_3$) C, H, N, S.

5-(Pyrrole-2-carboxamido)-1,3,4-thiadiazole-2-sulfonamide, A6, (method C). Dark yellow crystals, mp 291–4 °C (MeOH). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1320 (SO_2^{as}), 1580 (amide II), 1650 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.26 (dt, 1H, H4-pyrrole, 3.9, 2.3 etc.), 7.16 (m, 1H, H3-pyrrole, 3.9, 1.5 etc.), 7.43 (m, 1H, H5-pyrrole, 2.5, 1.4 etc.), 8.31 (s, 2H, SO_2NH_2), 12.12 (s, 1H, H1-pyrrole), 13.07 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 110.11 (C4-pyrrole), 115.11 (C3-pyrrole), 125.69 (C5-pyrrole), 122.95 (C2-pyrrole), 158.07 (CONH), 161.93 (C-thiadiazole), 164.22 (C-thiadiazole). Anal. ($C_7H_7N_5S_2O_3$) C, H, N, S.

5-(Pyrrole-2-carboximido)-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide, A7, (method C). White crystals, mp 294–7 °C (MeOH). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1325 (SO_2^{as}), 1520 (amide II), 1600 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.22 (dt, 1H, H4-pyrrole, 3.7, 2.3 etc.), 6.99 (m, 1H, H3-pyrrole, 3.8, 1.6, 0.5 etc.), 7.07 (m, 1H, H5-pyrrole, 2.6, 1.6 etc.), 8.44 (s, 2H, SO_2NH_2), 11.84 (s, 1H, H1-pyrrole); ^{13}C NMR (DMSO-d_6), δ, ppm: 37.72 (CH_3), 109.60 (C4-pyrrole), 114.99 (C3-pyrrole), 123.89 (C5-pyrrole), 128.55 (C2-pyrrole), 157.09 (CONH), 163.45 (C-thiadiazole), 166.52 (C-thiadiazole). Anal. ($C_8H_9N_5S_2O_3$) C, H, N, S.

4-(Furan-2-carboxamido)benzenesulfonamide, B1, (method A). White crystals, mp 283–4 °C (MeOH). IR (KBr), cm^{-1}: 1180 (SO_2^{sym}), 1320 (SO_2^{as}), 1555 (amide II), 1660 (amide I); ^1H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.72 (dd, 1H, H4-furan, 3.5, 1.7), 7.40 (d, 1H, H3- furan, 3.6), 7.96 (d, 1H, H5-furan, 1.7), 7.29 (s, 2H, SO_2NH_2), 7.80 (d, 2H, AA′BB′, 8.8), 7.94 (d, 2H, AA′BB′, 8.8), 10.51 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 112.40 (C4-furan), 115.60 (C3-furan), 119.96 (C2/C3-Ph), 146.26 (C5-furan), 147.12 (C2-furan), 126.64 (C3/C2-Ph), 138.83 (C1/C4-Ph), 141.64 (C4/C1-Ph), 156.22 (CONH). Anal. ($C_{11}H_{10}N_2SO_4$) C, H, N, S.

4-(Furan-2-carboxamido)-3-fluorobenzenesulfonamide, B2, (method A). White crystals, mp 239–41.5 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1340 (SO_2^{as}), 1600 (amide

II), 1670 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.73 (dd, 1H, H4-furan, 3.5, 1.7), 7.40 (ddd, 1H, H3-furan, 3.5, 1.8, 0.4), 7.49 (s, 2H, SO_2NH_2), 7.67 (dd, 1H, H2-Ph, 5.8, 2.0), 7.70 (dd, 1H, H6-Ph, 8.3, 1.9), 7.88 (ddd, 1H, H5-Ph, 8.3, 7.4, 4.7), 7.98 (dd, 1H, H5-furan, 0.7, 1.7), 10.23 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm; $J_{C,F}$, Hz: 112.34 (C4-furan), 113.48 (d, C2-Ph, 23.16), 115.74 (C3-furan), 121.95 (d, C5-Ph, 3.1), 126.5 (C6-Ph), 128.44 (d, C4-Ph, 11.8), 141.81 (d, C1-Ph, 6.0), 146.32 (C5-furan), 146.71 (C2-furan), 154.30 (d, C3-Ph, 251.3), 156.2 (CONH). Anal. ($C_{11}H_9N_2SO_4F$) C, H, N, S.

4-(Furan-2-carboxamido)-3-chlorobenzenesulfonamide, B3, (method A). White crystals, mp 224–5 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1310 (SO_2^{as}), 1575 (amide II), 1660 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.74 (dd, 1H, H4-furan, 3.5, 1.7), 7.39 (d, 1H, H3-furan, 3.5), 7.52 (s, 2H, SO_2NH_2), 7.81 (dd, 1H, H6-Ph, 8.5, 2.1), 7.93 (dd, 1H, H5-Ph, 8.5, 0.9), 7.95 (dd, 1H, H2-Ph, 2.1, 0.9), 7.99 (dd, 1H, H5- furan, 1.7, 0.7), 10.02 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 112.49 (C4-furan), 115.84 (C3-furan), 125.01 (C6-Ph), 126.87 (C5-Ph), 127.06 (C2-Ph), 128.14 (C4-Ph), 137.35 (C1-Ph), 142.08 (C3-Ph), 146.35 (C5-furan), 146.73 (C2-furan), 156.14 (CONH). Anal. ($C_{11}H_9N_2SO_4Cl$) C, H, N, S.

4-(Furan-2-carboxamido)-3-bromobenzenesulfonamide, B4, (method A). White crystals, mp 216–8°C (MeOH). IR (KBr), cm^{-1}: 1165 (SO_2^{sym}), 1310 (SO_2^{as}), 1585 (amide II), 1650 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.74 (dd, 1H, H4-furan, 3.5, 1.7), 7.39 (dd, 1H, H3-furan, 3.5, 0.7), 7.56 (vbrs, 2H, SO_2NH_2), 7.84 (dd, 1H, H6-Ph, 8.5, 2.0), 7.91 (d, 1H, H5-Ph, 8.4), 7.99 (dd, 1H, H5-furan, 1.7, 0.7), 8.10 (d, 1H, H2-Ph, 2.0), 9.80 (vbrs, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 112.49 (C4-furan), 115.76 (C3-furan), 118.66 (C4-Ph), 125.55 (C6-Ph), 127.11 (C5-Ph), 129.91 (C2-Ph), 138.71 (C1-Ph), 142.26 (C3-Ph), 146.30 (C5-furan), 146.76 (C2-furan), 156.02 (CONH). Anal. ($C_{11}H_9N_2SO_4Br$) C, H, N, S.

4-(Furan-2-carboxamido)-3-iodobenzenesulfonamide, B5, (method A). White crystals, mp 205–6°C (MeOH). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1330 (SO_2^{as}), 1570 (amide II), 1650 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.74 (dd, 1H, H4-furan, 3.5, 1.8), 7.37 (d, 1H, H3-furan, 3.5), 7.48 (s, 2H, SO_2NH_2), 7.76 (d, 1H, H5-Ph, 8.4), 7.85 (dd, 1H, H6-Ph, 8.4, 2.1), 7.99 (dd, 1H, H5-furan, 1.8, 0.9), 8.29 (d, 1H, H2-Ph, 2.1), 9.92 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 96.98 (C4-Ph), 112.49 (C4-furan), 115.58 (C3- furan), 126.15 (C6-Ph), 126.84 (C5-Ph), 136.03 (C2-Ph), 141.97 (C1-Ph), 142.26 (C3-Ph), 146.23 (C5-furan), 146.99 (C2-furan), 156.09 (CONH). Anal. ($C_{11}H_9N_2SO_4I$) C, H, N, S.

4-(Furan-2-carboxamidomethyl)benzenesulfonamide, B6, (method A). White crystals, mp 214–7 °C (MeOH). IR (KBr), cm^{-1}: 1185 (SO_2^{sym}), 1320 (SO_2^{as}), 1530 (amide II), 1635 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 4.48 (d, 1H, 6.0), 6.63 (dd, 1H, H4-furan, 3.4, 1.7 etc.), 7.14 (d, 1H, H3-furan, 3.4), 7.85 (d, 1H, H5-furan, 1.8), 7.31 (s, 2H, SO_2NH_2), 7.46 (d, 2H, AA′BB′, 8.3), 7.77 (d, 2H, AA′BB′, 8.3), 9.04 (t, 1H, NHCO, 6.1); ^{13}C NMR (DMSO-d_6), δ, ppm: 41.66 (CH_2), 111.98 (C4-furan), 113.79 (C3-furan), 125.78 (C2/C3-Ph), 127.58 (C3/C2-Ph), 142.66 (C1/C4 Ph), 143.62 (C4/C1-Ph), 145.29 (C5-furan), 147.69 (C2-furan), 157.97 (CONH). Anal. ($C_{12}H_{12}N_2SO_4$) C, H, N, S.

4-(Furan-2-carboxamidoethyl)benzenesulfonamide, B7, (method A). White crystals, mp 231–2.5 °C (MeOH). IR (KBr), cm^{-1}: 1165 (SO_2^{sym}), 1300 (SO_2^{as}), 1535 (amide II), 1640 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 2.91 (t, 2H, 7.1), 3.49 (q, 2H, 6.7), 6.61 (dd, 1H, H4-furan, 3.4, 1.7 etc.), 7.08 (d, 1H, H3-furan, 3.5), 7.82 (d, 1H, H5-furan, 1.6), 7.32 (s, 2H, SO_2NH_2), 7.43 (d, 2H, AA′BB′, 8.2), 7.76 (d, 2H, AA′BB′, 8.2), 8.49 (t, 1H, NHCO, 5.6); ^{13}C NMR (DMSO-d_6), δ, ppm: 34.85 (CH_2-Ph), 39.70 (N-CH_2), 111.91 (C4-furan), 113.38 (C3-furan), 125.79 (C2/C3-Ph), 129.22 (C3/C2-Ph), 142.09 (C1/C4-Ph), 143.71 (C4/C1-Ph), 145.03 (C5-furan), 147.93 (C2-furan), 157.85 (CONH). Anal. ($C_{13}H_{14}N_2SO_4$) C, H, N, S.

5-(Furan-2-carboxamido)-1,3,4-thiadiazole-2-sulfonamide, B8, (method A). White crystals, mp 299–300 °C (MeOH). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1320 (SO_2^{as}), 1580 (amide II), 1680 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.78 (dd, 1H, H4-furan, 3.6, 1.7 etc.), 7.77 (d, 1H, H3-furan, 3.6), 8.07 (d, 1H, H5-furan, 1.7), 8.38 (s, 2H, SO_2NH_2), 13.53 (br s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 112.77 (C4-furan), 118.38 (C3-furan), 144.83 (C2-furan), 148.32 (C5-furan), 156.00 (CONH), 161.57 (C-thiadiazole), 164.75 (C-thiadiazole). Anal. ($C_7H_6N_4S_2O_4$) C, H, N, S.

5-(Furan-2-carboxamido)-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide, B9, (method A). White crystals, mp 28–7 °C (MeOH). IR (KBr), cm^{-1}: 1175 (SO_2^{sym}), 1325 (SO_2^{as}), 1510 (amide II), 1600 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 6.71 (m, 1H, H4-furan, 3.4, 1.7 etc.), 7.42 (d, 1H, H3-furan, 3.4), 7.97 (br s, 1H, H5-furan), 8.51 (s, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6), δ, ppm: 112.37 (C4-furan), 117.79 (C3-furan), 147.34 (C5-furan), 150.01 (C2-furan), 157.94 (CONH), 164.91 (C-thiadiazole), 164.94 (C-thiadiazole). Anal. ($C_8H_8N_4S_2O_4$) C, H, N, S.

4-(Thiophene-2-carboxamido)benzenesulfonamide, C1, (method A). White crystals, mp 284–6 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1320 (SO_2^{as}), 1585 (amide II), 1650 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 7.25 (dd, 1H, H4-thiophene, 5.0, 3.8), 7.89 (dd, 1H, H3-thiophene, 1.1 5.0), 8.08 (dd, 1H, H5-thiophene, 3.8, 1.1), 7.30 (s, 2H, SO_2NH_2), 7.82 (d, 2H, AA′BB′, 8.9), 7.92 (d, 2H, AA′BB′, 8.9), 10.54 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 119.90 (C4-thiophene), 126.67 (C2/C3-Ph), 128.26 (C3/C2-Ph), 129.77 (C3-thiophene), 132.61 (C5-thiophene), 138.79 (C2-thiophene), 139.49 (C1/C4-Ph), 141.80 (C4/C1-Ph), 160.28 (CONH). Anal. ($C_{11}H_{10}N_2S_2O_3$) C, H, N, S.

4-(Thiophene-2-carboxamido)-3-fluorobenzenesulfonamide, C2, (method A). White crystals, mp 237–9 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1340 (SO_2^{as}), 1600 (amide II), 1650 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 7.25 (dd, 1H, H4-thiophene, 5.0, 3.8), 7.50 (s, 2H, SO_2NH_2), 7.68 (dd, 1H, H2-Ph, 6.7, 2.0), 7.71 (dd, 1H,

H6-Ph, 8.3, 2.0), 7.85 (ddd, 1H, H5-Ph, 8.3, 7.1, 3.3), 7.91 (dd, 1H, H3-thiophene, 5.0, 1.1), 8.06 (dd, 1H, H5-thiophene, 3.8, 1.1), 10.40 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm; $J_{C,F}$, Hz: 113.55 (d, C2-Ph, 23.1), 121.97 (C5-Ph), 126.85 (C6-Ph), 128.30 (C4-thiophene), 128.70 (d, C4-Ph, 12.2), 130.21 (C3-thiophene), 132.65 (C5-thiophene), 138.50 (C2-thiophene), 141.87 (d, C1-Ph, 5.3), 154.42 (d, C3-Ph, 250.9), 160.02 (CONH). Anal. ($C_{11}H_9N_2S_2O_3F$) C, H, N, S.

4-(Thiophene-2-carboxamido)-3-chlorobenzenesulfonamide, C3, (method A). White crystals, mp 222–3 °C (MeOH). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1320 (SO_2^{as}), 1580 (amide II), 1660 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 7.25 (dd, 1H, H4-thiophene, 5.0, 3.8), 7.54 (brs, 2H, SO_2NH_2), 7.81 (m, 2H (H2 + H6)-Ph, 1.5, 0.9 etc.), 7.91 (dd, 1H, H3-thiophene, 5.0, 1.1), 7.95 (dd, 1H, H5-Ph, 1.5, 0.9), 8.05 (dd, 1H, H5-thiophene, 3.8, 1.1), 10.26 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 124.89 (C6-Ph), 126.91 (C5-Ph), 128.30 (C2-Ph), 128.35 (C4-thiophene), 129.13 (C4-Ph), 130.03 (C3-thiophene), 132.58 (C5-thiophene), 137.72 (C1-Ph), 138.56 (C2-thiophene), 142.39 (C3-Ph), 160.01 (CONH). Anal. ($C_{11}H_9N_2S_2O_3Cl$) C, H, N, S.

4-(Thiophene-2-carboxamido)-3-bromobenzenesulfonamide, C4, (method A). White slightly pink crystals, mp 206–7 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1310 (SO_2^{as}), 1580 (amide II), 1650 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 7.26 (dd, 1H, H4-thiophene, 5.0, 3.7), 7.54 (brs, 2H, SO_2NH_2), 7.77 (d, 1H, H5-Ph, 8.4), 7.86 (dd, 1H, H6-Ph, 8.4, 2.0), 7.91 (dd, 1H, H3-thiophene, 5.0, 1.2), 8.04 (dd, 1H, H5-thiophene, 3.8, 1.2), 8.11 (d, 1H, H2-Ph, 2.0), 10.26 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 119.95 (C4-Ph), 125.46 (C6-Ph), 128.30 (C5-Ph), 128.75 (C4- hiophene), 129.93 (C2-Ph), 129.98 (C3-thiophene), 132.52 (C5-thiophene), 138.61 (C2-thiophene), 139.17 (C1-Ph), 142.77 (C3-Ph), 159.96 (CONH). Anal. ($C_{11}H_9N_2S_2O_3Br$) C, H, N, S.

4-(Thiophene-2-carboxamido)-3-iodobenzenesulfonamide, C5, (method A). White slightly pink crystals, mp 232.5–4 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1335 (SO_2^{as}), 1580 (amide II), 1650 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 7.26 (dd, 1H, H4-thiophene, 5.0, 3.7), 7.51 (s, 2H, SO_2NH_2), 7.65 (d, 1H, H5-Ph, 8.4), 7.87 (dd, 1H, H6-Ph, 8.4, 2.1), 7.90 (dd, 1H, H3-thiophene, 5.0, 1.1), 8.03 (dd, 1H, H5-thiophene, 3.7, 1.1), 8.32 (d, 1H, H2-Ph, 2.0), 10.21 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 98.12 (C4-Ph), 126.12 (C6-Ph), 128.32 (C4-thiophene), 129.79 (C3-thiophene), 132.38 (C5-thiophene), 136.02 (C5-Ph), 136.06 (C2-Ph), 138.87 (C2-thiophene), 142.53 (C1-Ph), 142.96 (C3-Ph), 159.96 (CONH). Anal. ($C_{11}H_9N_2S_2O_3I$) C, H, N, S.

4-(Thiophene-2-carboxamidomethyl)benzenesulfonamide, C6, (method A). Cream crystals, mp 223–5 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1310 (SO_2^{as}), 1550 (amide II), 1620 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 4.51 (d, 1H, 6.0), 7.17 (dd, 1H, H4-thiophene, 4.9, 3.7), 7.78 (dd, 1H, H3-thiophene, 4.9, 1.0), 7.82 (dd, 1H, H5-thiophene, 3.7, 1.0), 7.33 (s, 2H, SO_2NH_2), 7.49 (d, 2H, AA′BB′, 8.3), 7.80 (d, 2H, AA′BB′, 8.3), 9.15 (t, 1H, NHCO, 5.8); ^{13}C NMR (DMSO-d_6), δ, ppm: 42.23 (CH_2), 125.82 (C2/C3-Ph), 127.59 (C3/C2-Ph), 128.05 (C4-thiophene), 128.39 (C3-thiophene), 131.10 (C5-thiophene), 139.60 (C1/C4-Ph), 142.70 (C2-thiophene), 143.61 (C4/C1-Ph), 161.32 (CONH). Anal. ($C_{12}H_{12}N_2S_2O_3$) C, H, N, S.

4-(Thiophene-2-carboxamidoethyl)benzenesulfonamide, C7, (method A). White crystals, mp 239–40.5 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1330 (SO_2^{as}), 1550 (amide II), 1610 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 2.91 (t, 2H, 7.2), 3.49 (q, 2H, 6.4), 7.13 (dd, 1H, H4-thiophene, 5.0, 3.7), 7.73 (dd, 1H, H3-thiophene, 5.0, 1.2), 7.71 (dd, 1H, H5-thiophene, 3.8, 1.2), 7.31 (s, 2H, SO_2NH_2), 7.43 (d, 2H, AA′BB′, 8.3), 7.75 (d, 2H, AA′BB′, 8.1), 8.62 (t, 1H, NHCO, 5.7); ^{13}C NMR (DMSO-d_6), δ, ppm: 34.85 (CH_2-Ph), 40.22 (N-CH_2), 125.77 (C4-thiophene), 127.92 (C2/C3-Ph), 129.20 (C3/C2-Ph), 130.68 (C3-thiophene), 130.72 (C5-thiophene), 139.99 (C1/C4-Ph), 142.06 (C2-thiophene), 143.7 1 (C4/C1-Ph), 161.16 (CONH). Anal. ($C_{13}H_{14}N_2S_2O_3$) C, H, N, S.

5-(Thiophene-2-carboxamido)-1,3,4-thiadiazole-2-sulfonamide, C8, (method A). White crystals, mp 272–5 °C (EtOH). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1310 (SO_2^{as}), 1540 (amide II), 1650 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 7.30 (dd, 1H, H4-thiophene, 5.0, 3.9), 8.06 (dd, 1H, H3-thiophene, 5.0, 1.1), 8.33 (dd, 1H, H5-thiophene, 3.8, 1.1), 8.38 (s, 2H, SO_2NH_2), 13.62 (br s, 1H, NHCO); ^{13}C NMR (DMSO-d_6), δ, ppm: 128.92 (C4-thiophene), 132.34 (C3-thiophene), 135.13 (C5-thiophene), 135.80 (C2-thiophene), 160.13 (CONH), 162.01 (C-thiadiazole), 164.71 (C-thiadiazole). Anal. ($C_7H_6N_4S_3O_3$) C, H, N, S.

5-(Thiophene-2-carboxamido)-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide, C9, (method A). White crystals, mp 279–81 °C (EtOH). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1325 (SO_2^{as}), 1520 (amide II), 1580 (amide I); ^{1}H NMR (d_6-DMSO), δ, ppm; *J*, Hz: 7.23 (dd, 1H, H4-thiophene, 5.0, 3.7), 7.91 (dd, 1H, H3-thiophene, 5.0, 1.2), 7.93 (dd, 1H, H5-thiophene, 3.7, 1.2), 8.54 (s, 2H, SO_2NH_2); ^{13}C NMR (DMSO-d_6), δ, ppm: 128.54 (C4-thiophene), 132.63 (C3-thiophene), 133.59 (C5-thiophene), 140.73 (C2-thiophene), 158.10 (CONH), 164.94 (C-thiadiazole), 168.57 (C-thiadiazole). Anal. ($C_8H_8N_4S_3O_3$) C, H, N, S.

Pharmacology

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described by Lidskog et al.[46] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group,[47] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[48] Enzyme concentrations were determined spectrophotometrically at 280 nM, utilizing a molar absorptivity of 49 mM^{-1} cm^{-1} for CA I and 54 mM^{-1} cm^{-1} for CA II, respectively, based on M_r = 28.85 kDa for CA I, and 29.30 kDa for CA II, respectively.[49,50] CA IV was isolated from bovine lung microsomes as described by Maren et al, and its concentration has been determined by titration with ethoxzolamide.[51]

Initial rates of 4-nitrophenyl acetate hydrolysis catalysed by different CA isozymes were monitored spectrophotometrically, at 400 nM, with a Cary 3 instrument interfaced with an IBM compatible PC.[52] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2.10^{-2} and 1.10^{-6} M, working at 25 °C. A molar absorption coefficient ϵ of 18,400 $M^{-1}\,cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.40), as reported in the literature.[52] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.01 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constant K_I was determined as described by Pocker and Stone.[52] Enzyme concentrations were 3.1 nM for hCA II, 13 nM for hCA I and 33 nM for bCA IV.

Adult male New Zealand albino rabbits weighing 3–3.5 kg were used in the experiments (three animals were used for each inhibitor studied). The experimental procedures conform to the Association for Research in Vision and Ophthalmology Resolution on the use of animals. The rabbits were kept in individual cages with food and water provided ad libitum. The animals were maintained on a 12 h: 12 h light/dark cycle in a temperature controlled room, at 22–26 °C. Suspensions of inhibitors (2%, by weight) were obtained in distilled deionized water. The pH of these solutions was in the range of 7.0–7.5.

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA, USA) as described by Maren's group.[53,54] The pressure readings were matched with two-point standard pressure measurements at least twice each day using a Digilab Calibration verifier. All IOP measurements were done by the same investigator with the same tonometer. One drop of 0.2% oxybuprocaine hydrochloride (novesine, Sandoz) diluted 1:1 with saline was instilled in each eye immediately before each set of pressure measurements. IOP was measured three times at each time interval, and the means reported. IOP was measured first immediately before drug administration, then at 30 min after the instillation of the pharmacological agent, and then each 30 min for a period of 4–6 h. For all IOP experiments drug was administered to only one eye, leaving the contralateral eye as an untreated control. The ocular hypotensive activity is expressed as the average difference in IOP between the treated and control eye, in this way minimizing the diurnal, seasonal and inter-individual variations commonly observed in the rabbit.[53,54] All data are expressed as mean±SE, using a one-tailed t test.

Ocular hypertension was elicited in the right eye of albino rabbits by the injection of α-chymotrypsin (from Sigma) as described in ref[55] The IOP of operated animals was checked after approximately four weeks, and animals with an elevated pressure of 30–35 mm Hg were used at least one month after the injection of α-chymotrypsin.

Determination of water (buffer) solubility: A standard solution was prepared by dissolving a precisely weighted amount (generally 1 mg) of inhibitor in 10 mL of methanol. The UV absorption maximum of each compound has been determined (with a Cary 3 spectrophotometer) eventually diluting the solution (with MeOH) as necessary. A saturated solution of each compound was then prepared by stirring magnetically a small volume of 0.039 M phosphate buffer (pH 7.4) in the presence of excess inhibitor for 3 h. The obtained saturated solution was filtered in order to remove solid compound through a Millipore 0.45 μM filter and scanned by UV at the wavelength of the absorption maximum previously determined. Total solubility was determined by the relationship: $C' = A'C/A$, where C = concentration of standard solution (mg/mL); A = absorbance of standard solution; A′ = absorbance of the saturated solution; C′ = concentration of the saturated solution (mg/mL).[32]

Partition coefficient determinations: Chloroform–buffer partition coefficients were obtained by equilibrating the test compound between chloroform and 0.1-ionic strength pH 7.4 phosphate buffer. The concentration in each phase was determined by UV spectrophotometry or HPLC.[37]

Transcorneal penetration of drugs: The method of Maren et al.[56] with the modifications of Pierce's group[57] (for the HPLC assay of sulfonamides) have been used. Excised rabbit corneas with either intact or denuded epithelium were used in these experiments. The pH was 7.4 and exposed area was of 1.2 cm^2. Concentrations of drug of 40–2000 μM were placed in the epithelial chamber and samples of fluid were collected from the endothelial chamber at different intervals, up to 4 h. Both chambers contained 6 mL. Drugs present in these fluids were assayed both by the HPLC method of Pierce et al.,[57] or enzymatically.[37] The results of the drug analyses were used to calculate the rate constant of transfer across the cornea (k_{in}). As described by Pierce,[57] this value was determined by using the formula:

$$k_{in}(\times 10^3\ h^{-1}) = [drug]_{endo}/[drug]_{epi} \times 60/t \times 1000$$

where $[drug]_{endo}$ = concentration of drug on endothelial side; $[drug]_{epi}$ = concentration of drug on epithelial side; t = time (in min).

Acknowledgements

This research was financed by the EU grant ERB CIPDCT 940051. Thanks are addressed to Dr. M. A. Ilies for expert technical assistance.

References and Notes

1. This paper is Part 96 of the series "Carbonic Anhydrase Inhibitors". Part 95: Scozzafava, A., Supuran, C. T. *J. Enz. Inhib.*, **2000**, in press.

2. Hewett-Emmett, D. In *Carbonic Anhydrase — New Horizons*; Chegwidden, W.R., Edwards, Y., Carter, N., Eds.; Birkhauser: Basel, **2000**, in press.
3. Supuran, C. T. In *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism*; Puscas, I., Ed.; Helicon: Timisoara (Roumania), 1994; pp 29–111.
4. Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Patents* **2000**, *10*, 575.
5. Maren, T. H. *Ann. Rev. Physiol.* **1988**, *B50*, 717.
6. Maren, T. H. *J. Glaucoma* **1995**, *4*, 49.
7. Maren, T. H. *Physiol. Rev.* **1967**, *47*, 595.
8. Hazen, S. A.; Waheed, A.; Sly, W. S.; LaNoue, K. F.; Lynch, C. J. *FASEB J.* **1996**, *10*, 481.
9. Dodgson, S. J. *J. Appl. Physiol* **1987**, *63*, 2134.
10. Liljas, A.; Håkansson, K.; Jonsson, B. H.; Xue, Y. *Eur. J. Biochem.* **1994**, *219*, 1.
11. Weiner, I. M. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S. and Taylor, P., Eds.; Pergamon: New York, 1990; pp 713–732.
12. Lindskog, S. *Pharmacol. Ther.* **1997**, *74*, 1.
13. Chufan, E. E.; Pedregosa, J. C.; Baldini, O. N.; Bruno-Blanch, L. *Farmaco* **1999**, *54*, 838.
14. Bernhard, W. N.; Schalik, L. M.; Delaney, P. A.; Bernhard, T. M.; Barnas, G. M. *Aviat. Space Environ. Med.* **1998**, *69*, 883.
15. Puscas, I.; Supuran, C. T. In *Aparelho Digestivo*; Coelho, J., Eds., MEDSI, Rio de Janeiro, 1996 pp 1704.
16. Maren, T. H.; Conroy, C. W.; Wynns, G. C.; Levy, N. S. *J. Ocul. Pharmacol. Ther.* **1997**, *13*, 23.
17. Sugrue, M. F. *Progr. Ret. Eye Res.* **2000**, *19*, 87.
18. Laitalo, T.; Vaananen, K. *J. Bone Miner. Res.* **1993**, *8*, 119.
19. Supuran, C. T.; Briganti, F., Tilli, S.; Chegwidden, W. R.; Scozzafava, A. *J. Med. Chem.* **2000**, *43*, in press.
20. Grossmann, W. M.; Koeberle, B. *Cerebrovasc. Dis* **2000**, *10*, 65.
21. Schiefer, U.; Skalej, M.; Kolb, R.; Grodd, W.; Fahle, M.; Herzog, H. *Ger. J. Ophthalmol* **1996**, *5*, 109.
22. Keilin, D.; Mann, T. *Biochem. J.* **1940**, *34*, 1163.
23. Supuran, C. T.; Conroy, C. W.; Maren, T. H. *Eur. J. Med. Chem.* **1996**, *31*, 843.
24. Maren, T. H. *Annu. Rev. Pharmacol. Toxicol* **1976**, *16*, 309.
25. Maren, T. H.; Mayer, E.; Wadsworth, B. C. *Bull. John Hopkins Hosp* **1954**, *95*, 199 .
26. (a) Vaughan, J. R.; Eichler, J. A.; Anderson, G. W. *J. Org. Chem.*, **1956**, *21*, 700; (b) Young, R. W.; Wood, K. H.; Vaughan, J. R.; Anderson, G. W. *J. Am. Chem. Soc.*, **1956**, *78*, 4649.
27. Korman, J. *J. Org. Chem.* **1958**, *23*, 1768.
28. Baldwin, J. J.; Ponticello, G. S.; Anderson, G. S.; Christy, M. E.; Murcko, M. A.; Randall, W. C.; Schwam, H.; Sugrue, M. F.; Springer, J. P.; Gautheron, P.; Grove, J.; Mallorga, P.; Viader, M. P.; McKeever, B. M.; Navia, M. A. *J. Med. Chem.* **1989**, *32*, 2510.
29. Ponticello, G. S.; Sugrue, M. F.; Plazonnet, B.; Durand-Cavagna, G. *Pharm. Biotechnol* **1998**, *11*, 555.
30. Silver, L. H. *Surv. Ophthalmol* **2000**, *44* (Suppl. 2), 147.
31. Supuran, C. T.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G. *Metal Based Drugs* **1999**, *6*, 67.
32. (a) Renzi, G.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 673; (b) Supuran, C. T., Ilies, M. A. and Scozzafava, A. *Eur. J. Med. Chem.* **1998**, *33*, 739.
33. Supuran, C. T.; Clare, B. W. *Eur. J. Med. Chem.* **1995**, *30*, 687.
34. Supuran, C. T. *Roum. Chem. Quart. Rev.* **1993**, *1*, 77.
35. Maren, T. H. In *Carbonic Anhydrase-From Biochemistry and Genetics to Physiology and Clinical Medicine*; Botrè, F., Gros, G., Storey, B. T., Eds., VCH, Weinheim, 1991, pp 186–207.
36. Briganti, F.; Mangani, S.; Orioli, P.; Scozzafava, A.; Vernaglione, G.; Supuran, C. T. *Biochemistry* **1997**, *36*, 10384.
37. (a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.*, **1999**, *42*, 2641; (b) Borras, J., Scozzafava, A., Menabuoni, L., Mincione, F., Briganti, F., Mincione, G., Supuran, C. T. *Bioorg. Med. Chem.* **1999**, *7*, 2397.
38. (a) Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. *J. Med. Chem.*, **1999**, *42*, 3690; (b) Scozzafava, A.; Briganti, F.; Ilies, M. A.; Supuran, C. T. *J. Med. Chem.*, **2000**, *43*, 292.
39. Gao, J. M.; Qiao, S.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 2292.
40. Gao, J. M.; Cheng, X. H.; Chen, R. D.; Sigal, G. B.; Bruce, J. E.; Schwartz, B. L.; Hofstadler, S. A.; Anderson, G. A.; Smith, R. D.; Whitesides, G. M. *J. Med. Chem.* **1996**, *39*, 1949.
41. Aalto-Korte, K. *Contact Dermatitis* **1998**, *39*, 206.
42. Carlsen, J.; Durcan, J.; Swartz, M.; Crandall, A. *Arch. Ophthalmol* **1999**, *117*, 1087.
43. Thoe Schwartzenberg, G. W.; Trope, G. E. *Can. J. Ophthalmol* **1999**, *34*, 93.
44. Konowal, A.; Morrison, J. C.; Brown, S. V.; Cooke, D. I.; Maguire, L. J.; Verdier, D. V.; Fraunfelder, F. T.; Dennis, R. F.; Epstein, R. J. *Am. J. Ophthalmol* **1999**, *127*, 403.
45. Cingolani, E. *Gazz. Chim. Ital* **1948**, *78*, 275.
46. Lindskog, S.; Behravan, G.; Engstrand, C.; Forsman, C.; Jonsson, B. H.; Liang, Z.; Ren, X.; Xue, Y. In *Carbonic Anhydrase-From Biochemistry and Genetics to Physiology and Clinical Medicine*, Botrè, F., Gros, G., Storey, B. T. Eds., VCH, Weinheim, 1991, pp 1–13.
47. Behravan, G.; Jonsson, B. H.; Lindskog, S. *Eur.-J.Biochem.* **1990**, *190*, 351.
48. Khalifah, R. G.; Strader, D. J.; Bryant, S. H.; Gibson, S. M. *Biochemistry* **1977**, *16*, 2241.
49. Lindskog, S.; Coleman, J. E. *Proc. Natl. Acad. Sci. USA* **1964**, *70*, 2505.
50. Steiner, H.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1975**, *59*, 253.
51. Maren, T. H.; Wynns, G. C.; Wistrand, P. J. *Mol. Pharmacol.* **1993**, *44*, 901.
52. Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668.
53. Maren, T. H.; Brechue, W. F.; Bar-Ilan, A. *Exp. Eye Res.* **1992**, *55*, 73.
54. Brechue, W. F.; Maren, T. H. *Invest. Ophthalmol. Vis. Sci.* **1993**, *34*, 2581.
55. Melena, J.; Santafe, J.; Segarra-Domenech, J.; Puras, G. *J. Ocul. Pharmacol. Ther.* **1999**, *15*, 19.
56. Maren, T. H.; Jankowska, L.; Edelhauser, G. F.; Sanyal, G. *Exp. Eye. Res.* **1983**, *36*, 457.
57. Pierce, W. M. Jr.; Sharir, M.; Waite, K. J.; Chen, D.; Kaysinger, K. K. *Proc. Soc. Exp. Biol. Med.* **1993**, *203*, 360.