Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 2397–2406

BIOORGANIC & MEDICINAL CHEMISTRY

Carbonic Anhydrase Inhibitors: Synthesis of Water-Soluble, Topically Effective Intraocular Pressure Lowering Aromatic/Heterocyclic Sulfonamides Containing 8-Quinoline-sulfonyl Moieties: Is the Tail More Important than the Ring?†

Joaquin Borras,[a] Andrea Scozzafava,[b] Luca Menabuoni,[c] Francesco Mincione,[d] Fabrizio Briganti,[b] Giovanna Mincione[b] and Claudiu T. Supuran[b,*]

[a]*Universidad de Valencia, Facultad de Farmacia, Departamento de Quimica Inorganica, Avda. Vincent Andres Estelles s/n, 46100-Burjassot, Valencia, Spain*

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

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

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

Received 13 March 1999; accepted 17 May 1999

Abstract—Reaction of 20 aromatic/heterocyclic sulfonamides containing a free amino, imino, hydrazino or hydroxyl group, with 8-quinoline-sulfonyl chloride afforded a series of water-soluble (as hydrochloride or triflate salts) compounds. The new derivatives were assayed as inhibitors of the zinc enzyme carbonic anhydrase (CA), and more precisely of three of its isozymes, CA I, II (cytosolic forms) and IV (membrane-bound form), involved in important physiological processes. Efficient inhibition was observed against all three isozymes, but especially against CA II (in nanomolar range), which is the isozyme known to play a critical role in aqueous humor secretion within the ciliary processes of the eye. Some of the best inhibitors synthesized were topically applied as 2% water solutions onto the eye of normotensive and glaucomatous albino rabbits, when strong and long-lasting intraocular pressure (IOP) lowering was observed with many of them. This result prompted us to reanalyze the synthetic work done by other groups for the design of water soluble, topically effective antiglaucoma sulfonamides. According to these researchers, the IOP lowering effect is due to the intrinsic nature of the specific heterocyclic sulfonamide considered, among which the thienothiopyran-2-sulfonamide derivatives represent the best studied case. Indeed, the first agents developed for such applications, such as dorzolamide, are derivatives of this ring system. In order to prove that the tail (in this case the 8-quinoline-sulfonyl moiety) conferring water solubility to a sulfonamide CA inhibitor is more important than the ring to which the sulfonamido group is grafted, we also prepared a dorzolamide derivative to which the 8-quinoline-sulfonyl moiety was attached. This new compound is quite water soluble as hydrochloride salt, behaves as a strong CA II inhibitor, and fared better than the parent molecule in lowering IOP in experimental animals. Thus, the tail conferring water solubility to such an enzyme inhibitor is more important for its topical activity as antiglaucoma drug than the heterocyclic/aromatic ring to which the sulfonamido moiety is grafted.

Introduction

The sulfonamides represent an important class of biologically active compounds, with at least five different classes of pharmacological agents that have been obtained from the sulfanilamide structure as lead, the derivative initially studied by Domagk[2] as the first modern chemotherapeutic drug. Indeed, the antibacterial sulfonamides[3] continue to play an important role in chemotherapy, alone or in combination with other drugs,[4] the sulfonamides that inhibit the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) possess many applications as diuretic, antiglaucoma or antiepileptic drugs among others,[5–8] the hypoglycemic sulfonamides are extensively used in the treatment of some forms of diabetes,[9] whereas the thiazides and high-ceiling diuretics might be considered as a fortunate development

Key words: Aromatic, heterocyclic sulfonamides; carbonic anhydrase; 8-quinoline-sulfonyl chloride; antiglaucoma drugs; hydrochloride salts; dorzolamide.

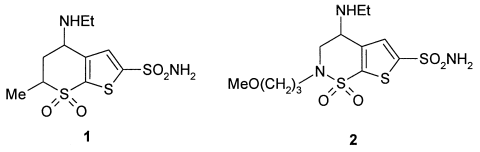
† This paper is part 73 of the series 'Carbonic Anhydrase Inhibitors'. For part 72, see ref 1.

* Corresponding author. Tel.: +39-055-275-7552; fax: +39-055-275-7555; e-mail: cts@bio.chim.unifi.it

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00190-X

of the CA inhibitors,[10] but these compounds possess a different pharmacological profile, independent of CA inhibition.[11,12] Finally, some antithyroid drugs have also been developed starting from the sulfonamide structure as lead molecule.[13]

The second class of the above mentioned pharmacological agents (i.e. the sulfonamides with CA inhibitory action) have thoroughly been investigated in the last 10 years, mainly in the search of a topically effective antiglaucoma drug.[14–29] The possibility of administering a sulfonamide via the topical route directly into the eye, although investigated in the 1950s,[30,31] has been totally unsuccessful, whereas the systemic administration, quite useful in lowering intraocular pressure (IOP), was generally accompanied by undesired side effects, due to CA inhibition in other tissues than the eye.[31] In 1983, Maren's group[14] postulated that a water-soluble sulfonamide, possessing a relatively balanced lipid solubility, and strong CA inhibitory properties, might be an effective IOP lowering drug via the topical route, but at that moment no inhibitors possessing such physicochemical properties existed, as the organic chemistry of this class of compounds remained relatively little studied. Water-soluble sulfonamide CA inhibitors started to be developed in several laboratories soon thereafter,[14–29] and in 1995 the first such pharmacological agent, dorzolamide **1** entered in clinical use in the USA and Europe.[32] A second compound, brinzolamide **2**, quite similar structurally with dorzolamide has also recently been approved for the topical treatment of glaucoma in the USA.[33]



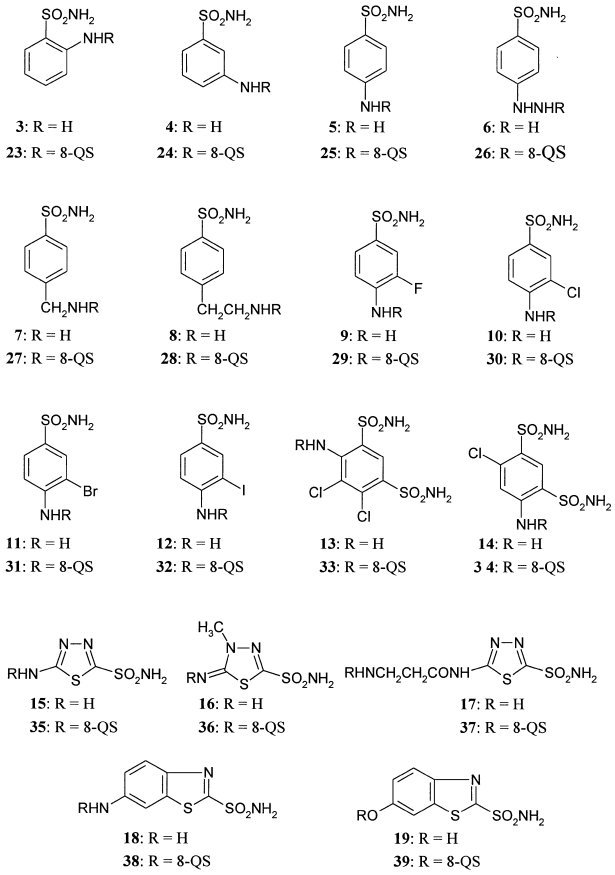
Thus, in a series of papers,[18,34–41] the Merck, Sharp and Dohme group has developed the synthesis of a large series of generally bicyclic heterocyclic sulfonamides (derivatives of benzothiazole-,[34,35] benzofuran-,[36,37] indole-,[38] benzo[*b*]-thiophene-,[39,40] thieno-thiopyran[18,40]), which were then tested as IOP lowering agents, and which led to the above mentioned drug (dorzolamide). Still, the greatest majority of the synthesized compounds proved to be potent allergens in vivo, since their sulfonamido group was nucleophilically displaced by reduced glutathione or other nucleophiles present in the reaction medium. More than that, the only compounds with acceptable water solubility proved to be hydrochlorides of amino-derivatives of the thienothiopyran-sulfonamides of dorzolamide type.[18,40] Obviously, the approach followed by this group was to explore as many as possible heterocyclic ring systems on which the sulfonamido moiety should be attached, and this approach was extremely beneficial for the chemistry of heterocyclic sulfonamides. Still, this approach seemed to us not a very fortunate one for the design of topically active IOP lowering agents, and we decided to explore the opposite one, i.e. to graft moieties that would ensure water solubility (as salts of a strong acid for instance) on the classical ring systems of the aromatic/heterocyclic sulfonamides possessing CA inhibitory properties.

In this paper we report the reaction of 20 aromatic/heterocyclic sulfonamides containing a free amino, imino, hydrazino or hydroxyl group, with 8-quinoline-sulfonyl chloride, which afforded a series of water-soluble (as hydrochloride or triflate salts) sulfonamides with strong CA inhibitory properties. Moreover, dorzolamide has been derivatized similarly, at its secondary amino group, and the obtained compound also possessed good water solubility as hydrochloride salt. The new compounds reported here were tested for the inhibition of three CA isozymes, hCA I, hCA II and bCA IV (h = human, b = bovine isozymes). Affinities in the nanomolar range were detected for some compounds for isozymes II and IV. The most active derivatives were assayed in vivo in normotensive and glaucomatous rabbits, for their IOP lowering properties. Very strong IOP lowering was observed for many of them. Our conclusion is that the water-solubilizing tail seems to be more important for the topical effectiveness than the ring on which the sulfonamido moiety is grafted, and that topically-acting antiglaucoma drugs might be obtained from many other classes of sulfonamides than the thienothiopyran-sulfonamides and their derivatives.

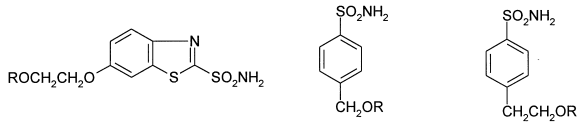
Results

Synthesis

Compounds prepared by reaction of 8-quinoline-sulfonyl chloride with aromatic/heterocyclic sulfonamides **1** and **3**–**22**, of type **23**–**43,** are shown below.



3: R = H; **23**: R = 8-QS
4: R = H; **24**: R = 8-QS
5: R = H; **25**: R = 8-QS
6: R = H; **26**: R = 8-QS
7: R = H; **27**: R = 8-QS
8: R = H; **28**: R = 8-QS
9: R = H; **29**: R = 8-QS
10: R = H; **30**: R = 8-QS
11: R = H; **31**: R = 8-QS
12: R = H; **32**: R = 8-QS
13: R = H; **33**: R = 8-QS
14: R = H; **3 4**: R = 8-QS
15: R = H; **35**: R = 8-QS
16: R = H; **36**: R = 8-QS
17: R = H; **37**: R = 8-QS
18: R = H; **38**: R = 8-QS
19: R = H; **39**: R = 8-QS

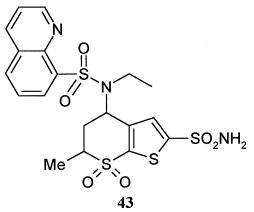


20: R = H
40: R = 8-QS

21: R = H
41: R = 8-QS

22: R = H
42: R = 8-QS

8-QS = 8-quinoline-sulfonyl



43

Non-exceptional routine synthetic procedures were employed for the reaction of amines/alcohols/phenols with sulfonyl halides, as reported previously by Wolin's[41] or our[22–29] groups.

Carbonic anhydrase inhibitory activity. Inhibition data against three CA isozymes, hCA I, hCA II and bCA IV with compounds **1**–**43** are shown in Table 1. The esterase activity of CA against 4-nitrophenyl acetate has been used in this assay (see Experimental for details).

Some physicochemical properties of the new compounds, relevant for their pharmacological activity, such as buffer solubility, chloroform-buffer partition coefficient, rate constant of transfer across the cornea (k_{in}) are shown in Table 2.

IOP measurements

In vivo IOP lowering data with some of the most active CA inhibitors reported here, in normotensive and glaucomatous rabbits, after topical administration of the drug, are shown in Tables 3 and 4, respectively.

The full time dependence of the IOP after dorzolamide and the some of the new compounds reported here in normotensive albino rabbits is shown in Figure 1.

Distribution of drugs in ocular fluids and tissues

Ex vivo distribution data of some active compound in ocular tissues and fluids after the topical administration in normotensive rabbits are shown in Table 5.

Discussion

Reaction of sulfonamides **3**–**22** or **1** with 8-quinoline-sulfonyl chloride afforded the new compounds **23**–**43**. The reaction has generally been performed in acetone or acetonitrile as solvents, in the presence of triethylamine as base, by the procedure previously reported by this and other groups[22–29,41] for arylsulfonyl halides and different nucleophiles. In the case of compounds **15** and **16** the above procedure led to very low yields of sulfonylated derivatives, and Schotten–Baumann conditions[29] had to be applied for obtaining **35** and **36** in good yields. Mention should be made that the reaction of 8-quinoline-sulfonyl chloride with this type of nucleophiles has not been studied previously. Only its reaction with ammonia has been investigated by us, and a Zn(II) complex of the obtained ligand (8-quinoline-sulfonamide) was characterized by means of X-ray crystallography.[28] Hydrochlorides of the new derivatives were then prepared by reacting the free bases **23**–**43** with a methanolic HCl solution. Similarly were obtained the triflate salts, by reaction of bases **23**–**43** with triflic acid in water as solvent. Aromatic sulfonamides such as sulfanilamide, metanilamide, which is known[20–29] that

Table 1. Carbonic anydrase (CA) inhibition data with dorzolamide **1**, the parent sulfonamides **3**–**22** and the new derivatives **23**–**43** reported in the present study, against isozymes I, II and IV

Inhibitor	K_I[a] (nM)		
	hCA I[b]	hCA II[b]	bCA IV[c]
Dorzolamide **1**	50,000	9	45
3	45,400	295	1310
4	25,000	240	2200
5	28,000	300	3000
6	78,500	320	3215
7	25,000	170	2800
8	21,000	160	2450
9	8300	60	180
10	9800	110	320
11	6500	40	66
12	6000	70	125
13	6100	28	175
14	8400	75	160
15	8600	60	540
16	9300	19	355
17	455	3	125
18	70	9	19
19	55	8	17
20	50	7	15
21	24,000	125	560
22	18,000	110	450
23	21,200	294	277
24	20,000	270	335
25	15,500	134	176
26	22,000	300	380
27	1240	75	124
28	1100	60	103
29	545	32	97
30	620	44	79
31	605	45	83
32	610	40	75
33	500	32	69
34	600	39	62
35	33	2	9
36	29	3	12
37	18	3	8
38	12	4	10
39	10	2	10
40	15	3	9
41	2150	70	125
42	2000	66	115
43	170	5	10

[a] Standard error for the determination of K_Is was of 5–10 % (from two different assays).
[b] Human (cloned) isozyme.
[c] Isolated from bovine lung microsomes.

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

Compound	Solubility[a] mM	Log P [b]	$k_{in}\times10^3$ (h^{-1})[c]	
			Cornea intact	No epithelium
1 (Dorzolamide)	60[d]	2.0[e]	3.0	5.2
35	70[e]	0.395	2.8	7.9
36	85[e]	0.490	3.4	8.5
37	69[e]	0.356	3.0	8.0
43	54[e]	1.924	3.9	6.3

[a] Solubility in pH 7.40 buffer, at 25°C.
[b] Chloroform–buffer partition coefficient.
[c] Determined as described in ref 13, 58, 59.
[d] As hydrochloride, at pH 5.8, from ref 56.
[e] As hydrochloride salts.

Table 3. Fall of IOP of normotensive rabbits (23.1 ± 2.5 mm Hg), after treatment with one drop (50 μL) solution 2% of CA inhibitor (as hydrochloride salt, with the pH value shown below) directly into the eye, at 30, 60, and 90 min after administration

Inhibitor	pH	ΔIOP (mm Hg)[a]			
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
1 (Dorzolamide)	5.5	0	2.2 ± 0.10	4.1 ± 0.15	2.7 ± 0.08
35	5.0	0	4.7 ± 0.12	9.8 ± 0.15	7.2 ± 0.13
36	5.5	0	5.5 ± 0.10	11.0 ± 0.14	14.1 ± 0.10
37	5.5	0	4.8 ± 0.10	8.2 ± 0.13	7.0 ± 0.12
39	5.9	0	2.0 ± 0.05	4.4 ± 0.10	4.7 ± 0.09
40	5.5	0	2.4 ± 0.05	4.7 ± 0.11	3.9 ± 0.10
43	5.5	0	2.5 ± 0.06	4.4 ± 0.10	3.8 ± 0.12

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

Table 4. Fall of IOP of glaucomatous rabbits (33.6 ± 2.2 mm Hg), after treatment with one drop (50 μL) solution 2% of CA inhibitor (as hydrochloride salt, with the pH value shown below) directly into the eye, at 30, 60, and 90 min after administration

Inhibitor	pH	ΔIOP (mm Hg)[a]			
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
1	5.5	0	3.6 ± 0.20	6.7 ± 0.30	4.2 ± 0.15
35	5.0	0	9.0 ± 0.10	15.5 ± 0.15	19.0 ± 0.12
36	5.5	0	7.4 ± 0.13	12.8 ± 0.11	20.3 ± 0.15
43	5.5	0	4.8 ± 0.10	6.0 ± 0.10	7.9 ± 0.15

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

would lead to relatively weaker CA inhibitors (as compared to the heterocyclic derivatives) were also included in this study, in order to prove that the approach proposed by us here is a general one, being possible to apply it for the design of very efficient as well as weak enzyme inhibitors.

The obtained salts possessed good water solubility, generally in the range of 2–5% (by weight), that is, in the range of 50–80 mM (Table 2). Triflates and hydrochlorides possessed quite similar water solubilities (data not shown), so that the largest majority of studies with the cationic derivatives has been performed with hydrochlorides. The pH of such solution was in the

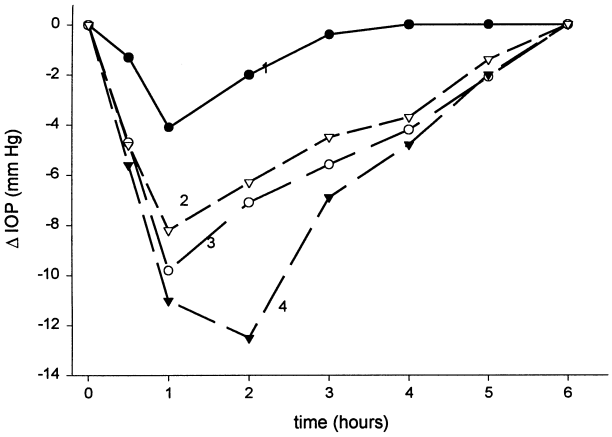


Figure 1. Effect of topically administered sulfonamide inhibitors (2% water solutions) on the intraocular pressure (IOP) of normotensive albino rabbits. Curve 1: dorzolamide **1** (hydrochloride salt, pH 5.5); curve 2: compound **37** (hydrochloride salt, pH 5.50); curve 3: compound **35** (as hydrochloride salt, pH 5.0); curve 4: compound **36** (as hydrochloride salt, pH 5.5).

Table 5. Ocular tissue concentrations (μM) after one and two hours, following corneal application of one drop (50 μL) of 2% solution of compounds **35** and **37** in normotensive albino rabbits

Compound	Time (h)	Drug concentration (μM)		
		Cornea	Aqueous humor	Ciliary process
35	1 h	156 ± 7	274 ± 12	50 ± 3
	2 h	49 ± 5	36 ± 3	13 ± 1
37	1 h	163 ± 6	280 ± 10	47 ± 5
	2 h	52 ± 5	46 ± 4	17 ± 2

range of 5.0-6.0, making them appropriate for topical application directly into the eye. As seen from the data of Table 2, some of the newly obtained compounds for which detailed pharmacological data were obtained, possessed a relatively moderate lipid solubility, similarly or slightly less than that of dorzolamide **1**. In fact, Maren[13] noted in his classical review that one of the conditions needed for a sulfonamide to act as an effective IOP lowering agent is to possess a modest lipid solubility (attributable to its unionized form), accompanied by a good water solubility (conferred by the presence of ionizable groups of appropriate pK_a).[13] As seen from data of Table 2, the compounds reported here possessed just this type of physicochemical properties. Their accession rates across the cornea were thus of the same order of magnitude as those of dorzolamide.

Compounds **3**–**43** were characterized by standard chemical and physical methods that confirmed their structure (see Experimental for details) and were assayed for the inhibition of isozymes hCA I, hCA II and bCA IV (Table 1).

Inhibition data against three CA isozymes, hCA I, hCA II and bCA IV with the new derivatives (Table 1) prove that the 8-quinoline-sulfonyl-derivatives **23**–**43** reported here generally behave as strong inhibitors, with greatly

increased efficiencies as compared to the parent compounds from which they were prepared (the sulfonamides **3**–**22**). The efficiency of the obtained inhibitor generally varied in the following way, based on the parent sulfonamide from which it was prepared: the derivative of *p*-hydrazino-benzenesulfonamide **26** < the orthanilamide **23** ≅ the metanilamide **24** < the sulfanilamide **25** < the homosulfanilamides **27** < the *p*-aminoethyl-benzenesulfonamides **28** < the 1,3-benzene-disulfonamides **33** and **34** ≅ the halogeno-substituted sulfanilamides **29**–**32** < the 1,3,4-thiadiazole-2-sulfonamides **35** and **37** ≅ 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide **36** ≅ the dorzolamide derivative **43** < the benzothiazole-2-sulfonamides **38**–**40**. All three CA isozymes investigated here were susceptible to inhibition with this type of sulfonamides, with hCA II and bCA IV the most inhibitable, followed by hCA I, generally less susceptible to inhibition as compared to the first two isozymes.

The promising in vitro CA inhibitory activity of some of the newly prepared compounds prompted us to investigate their effect in vivo, on the IOP, after topical application directly into the eye, in normotensive and glaucomatous rabbits, frequently used as an animal model of glaucoma.[3–18,32,33] Some of these results are shown in Tables 3 and 4.

The compounds selected for in vivo studies were among the most active inhibitors against hCA II and IV, in the prepared series, such as **35**–**40**, and **43**. The following facts should be noted regarding the data of Tables 3 and 4. Some of the new compounds assayed in vivo, such as **35**–**37**, and **43**, showed much more effective IOP lowering effects as compared to dorzolamide **1**, both after 30 min from the administration of the inhibitor within the rabbit eye, as well as at other times (1, 1.5 and 2 h, respectively), in normotensive as well as glaucomatous animals. A second group of inhibitors, such as **39** and **40**, showed IOP lowering effects of the same order of magnitude as those of dorzolamide, both after half an hour or longer periods after the administration. Mention should be made that the pH of the solutions administered in these experiments was in the range of 5.0–5.9 for all inhibitors used.

But one of the most interesting findings is that IOP remains low for longer periods (3–6 h) after the topical administration of the new type of compounds reported in this paper, as compared to the standard drug dorzolamide (Fig. 1). As seen from Figure 1, compounds such as **35**, **36**, or **37** possessed maximal IOP lowering effects at 1 h after administration, similarly to dorzolamide. The main difference between them was that the new compounds acted as much more potent IOP lowering agents, and that at 3 or 4 h after administration (when the effects of dorzolamide completely vanished) they still diminished eye pressure appreciably (4.8–6.2 mm Hg). IOP generally returned at the baseline values after 5–6 h after administration of the drug. Thus, all these derivatives are longer lasting IOP lowering agents as compared to the clinically available drug dorzolamide.

The above findings also apply for the glaucomatous rabbits experiments (Table 4) but the IOP are much more important as compared to those of normotensive rabbits. Thus, IOP reductions of around 9 mm Hg were generally observed after 30 min, whereas at longer periods, these amounted to 12–20 mm Hg. The long-lasting effect mentioned above has also been evidenced for the glaucomatous rabbit experiments (data not shown).

As seen from the data of Table 5 (obtained ex vivo in normotensive rabbits after the topical administration of two of the most active topical inhibitors in the prepared series, i.e. compounds **35** and **37**) at 1 and 2 h after topical administration of the drug, high levels of inhibitors were found in the cornea, aqueous humor and ciliary processes. Based on the inhibition constant of these compounds (2 nM for CA II for **35**, and 3 nM for CA II for **37**, respectively), the fractional inhibition estimated in these tissues/fluids is of 99.5–99.9%, indicating the fact that the IOP decrease is indeed due to CA inhibition.

Conclusion

We report here a general approach for the preparation of water-soluble, topically effective antiglaucoma sulfonamides, by attaching water-solubilizing moieties (such as 8-quinoline-sulfonyl) to well-known aromatic/heterocyclic sulfonamides. By applying simple chemical reactions between a heterocyclic sulfonyl chloride with aromatic/heterocyclic sulfonamides containing free amino, imino, hydrazino or hydroxy groups, a series of new compounds was prepared. Ring systems which have been derivatized by the above mentioned procedure included: 2-; 3- or 4-aminobenzenesulfonamides; 4-(ω-aminoalkyl)-benzene-sulfonamides; 3-halogeno-substituted-sulfanilamides; 1,3-benzene-disulfonamides; 1,3,4-thiadiazole-2-sulfonamides; benzothiazole-2-sulfonamides as well as thienothiopyran-2-sulfonamides among others, and were chosen in such a way as to prove that the proposed approach is a general one. The obtained compounds formed water-soluble salts by reaction with strong acids, such as hydrochloric or trifluoromethanesulfonic acid, with protonation of the pyridine nitrogen atom. These salts possessed good water solubility, in the range of 2–5%, whereas their lipid solubility, hydrophobicity (Log P) as well as accession rates across the cornea were those appropriate for acting as efficient topical IOP lowering agents. Many of the reported inhibitors possessed affinities in the nanomolar range for isozymes hCA II and bCA IV, acting as effective enzyme inhibitors in vitro. Some of the most active inhibitors strongly lowered IOP pressure in normotensive and glaucomatous rabbits, showing a highly prolonged duration of action as compared to dorzolamide. The new compounds reported here might lead to the development of more efficient and inexpensive antiglaucoma drugs.

Experimental

Melting points: heating plate microscope (not corrected); IR spectra: KBr pellets, 400–4000 cm^{-1} Perkin–Elmer

16PC FT-IR spectrometer; ^{1}H NMR spectra: Varian 300CXP apparatus (chemical shifts are expressed as δ values relative to Me_4Si as standard); Elemental analysis: Carlo Erba Instrument CHNS Elemental Analyser, Model 1106. Analytical and preparative HPLC was performed on a reversed-phase C_{18} Bondapack column, with a Beckman EM-1760 instrument. Sulfonamides **3**–**22** used in synthesis were either commercially available compounds (from Sigma, Acros or Aldrich) or were prepared as described previously: 4-hydrazino-benzenesulfonamide **6** by diazotization of sulfanilamide followed by reduction of the diazonium salt with tin(II) chloride;[42] halogenosulfanilamides **9**–**12** by halogenation of sulfanilamide as reported in the literature;[43] compound **17** from 5-amino-1,3,4-thiadiazole-2-sulfonamide (obtained from acetazolamide)[44] by acylation with the phthalimido-derivative of β-alanine, followed by hydrazinolysis,[45] whereas imine **16** by deprotection of methazolamide with concentrated hydrochloric acid.[45] The benzothiazole-2-sulfonamide derivatives **18**–**20** were prepared as described in ref 17, whereas the alcohols **21** and **22** from the corresponding amines by diazotization followed by hydrolysis of the diazonium salts.[45] Dorzolamide **1** was from Merck or was prepared as described in the literature.[46] 8-Quinoline-sulfonyl chloride, triflic acid and triethylamine were from Sigma Chemical Co. Acetonitrile, acetone (E. Merck) or other solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

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 Forsman et al.[47] (the two plasmids were a gift from Professor Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[48] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[49] 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.[50,51] CA IV was isolated from bovine lung microsomes as described by Maren et al., and its concentration has been determined by titration with ethoxzolamide.[52]

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.[53] 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.[53] 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 (for all CA isozymes used). The inhibition constant K_I was determined as described by Pocker and Stone.[53] Enzyme concentrations were 3.5 nM for hCA II, 12 nM for hCA I and 36 nM for bCA IV (this isozyme has a decreased esterase activity[54] and higher concentrations had to be used for the measurements).

General procedure for the preparation of 8-quinoline-sulfonyl derivatives of the aromatic/heterocyclic sulfonamides, 23–43

An amount of 1 mM sulfonamide **3**–**22** or **1** was dissolved/suspended in 25 mL of anhydrous acetonitrile or acetone and then treated with 0.227 g (1 mM) of 8-quinoline-sulfonyl chloride. The stoichiometric amount (20 μL) of triethylamine was then added and the reaction mixture was magnetically stirred at 4°C for 4–10 h. By means of TLC the conversion of all the sulfonamide to the corresponding 8-quinoline-sulfonyl derivatives has been monitored. When the reaction was completed, the solvent was evaporated until a small volume of the reaction mixture was obtained. Generally the new compounds crystallized spontaneously by leaving the above mixture at 4°C overnight. In some cases, the concentrated liquor obtained after the evaporation of the solvent was poured into 50 mL of cold water, when the reaction products precipitated and were filtered. The prepared compounds were recrystallized from ethanol or ethanol:water (1:1, v/v). Yields were in the range of 70–90%. Hydrochlorides of derivatives **23**–**43** were obtained from the free bases and a methanolic HCl solution, in methanol as solvent. The hydrochlorides precipitated by leaving the above mixtures at 4°C overnight. The hydrochlorides were analyzed for the presence of Cl^- by potentiometric titrations. The obtained data were ±0.5% of the theoretical data calculated for the proposed formulas (data not shown). Triflate salts were similarly obtained from the free bases **23**–**43** and the stoichiometric amount of triflic acid, in water as solvent.

2-(8-Quinoline-sulfonylamido)-benzenesulfonamide 23. White crystals, mp 265–266°C; IR (KBr), cm^{-1}: 1140 and 1166 (SO_2^{sym}), 1320 and 1370 (SO_2^{as}), 3090 (NH); 3360 (NH_2). ^{1}H NMR (DMSO-d_6) δ 7.15–7.69 (m, 4H, ArH, 1,2-phenylene), 7.50 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.21 (br s, 1H, SO_2NH). $C_{15}H_{13}N_3O_4S_2$ (363.42) calcd C, 45.58; H, 3.61; N, 11.56; found: C, 45.80; H, 4.00; N, 11.48.

3-(8-Quinoline-sulfonylamido)-benzenesulfonamide 24. White crystals, mp 279–280°C (dec.); IR (KBr), cm^{-1}: 1135 and 1166 (SO_2^{sym}), 1320 and 1370 (SO_2^{as}), 3080 (NH); 3360 (NH_2). ^{1}H NMR (DMSO-d_6) δ 7.10–7.50

(m, 4H, ArH, 1,3-phenylene), 7.56 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.15 (br s, 1H, SO_2NH). $C_{15}H_{13}N_3O_4S_2$ (363.42) calcd C, 45.58; H, 3.61; N, 11.56; found: C, 45.43; H, 3.74; N, 11.36.

4-(8-Quinoline-sulfonylamido)-benzenesulfonamide 25. White crystals, mp 289–291°C (dec.); IR (KBr), cm^{-1}: 1150 and 1166 (SO_2^{sym}), 1320 and 1345 (SO_2^{as}), 3060 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ, δ_A 7.18, δ_B 7.75 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from 4-sulfamoylphenyl), 7.56 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.19 (br s, 1H, SO_2NH). $C_{15}H_{13}N_3O_4S_2$ (363.42); calcd C, 45.58; H, 3.61; N, 11.56; found: C, 45.54; H, 3.60; N, 11.42.

4-(8-Quinoline-sulfonylhydrazido)-benzenesulfonamide 26. White crystals, mp 283–284°C; IR (KBr), cm^{-1}: 980 (N–N), 1150 and 1166 (SO_2^{sym}), 1320 and 1365 (SO_2^{as}), 3090 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 7.05–7.39 (m, AA′BB′, 4H, ArH, 1,4-phenylene), 7.59 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.36 (br, 2H, SO_2NHNH). $C_{15}H_{14}N_4O_4S_2$ (378.43); calcd C, 47.61; H, 3.73; N, 14.81; found: C, 47.76; H, 4.05; N, 14.80.

4-(8-Quinoline-sulfonylamidomethyl)-benzenesulfonamide 27. White crystals, mp 281–282°C (dec.); IR (KBr), cm^{-1}: 1166 and 1175 (SO_2^{sym}), 1320 and 1372 (SO_2^{as}), 3090 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 4.90 (s, 2H, CH_2), δ_A 7.22, δ_B 7.79 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from 4-sulfamoylphenyl), 7.67 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.26 (br s, 1H, SO_2NH). $C_{16}H_{15}N_3O_4S_2$ (377.44); calcd C, 50.92; H, 4.01; N, 11.13; found: C, 50.87; H, 4.12; N, 11.04.

4-(8-Quinoline-sulfonylamidoethyl)-benzenesulfonamide 28. White crystals, mp 291–293°C (dec.); IR (KBr), cm^{-1}: 1150 and 1166 (SO_2^{sym}), 1320 and 1359 (SO_2^{as}), 3080 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge), 3.70 (t, 2H, βCH_2 from the CH_2CH_2 bridge), δ_A 7.15, δ_B 7.62 (AA′BB′ system, 4H, $J_{AB}=7.9$ Hz, ArH from 4-sulfamoylphenyl), 7.67 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.25 (br s, 1H, SO_2NH). $C_{17}H_{17}N_3O_4S_2$ (391.47); calcd C, 52.16; H, 4.38; N, 10.73; found: C, 52.40; H, 4.31; N, 10.65.

3-Fluoro-4-(8-quinoline-sulfonylamido)-benzenesulfonamide 29. White crystals, mp 255–256°C. IR (KBr), cm^{-1}: 1150 and 1166 (SO_2^{sym}), 1320 and 1348 (SO_2^{as}), 3060 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 6.60 (br s, 2H, SO_2NH_2), 7.05–7.89 (m, 3H, ArH from the F-substituted ring), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.25 (br s, 1H, SO_2NH). $C_{15}H_{12}FN_3O_4S_2$ (381.41); calcd C, 47.24; H, 3.17; N, 11.02; found: C, 46.97; H, 3.30; N, 10.85.

3-Chloro-4-(8-quinoline-sulfonylamido)-benzenesulfonamide 30. White crystals, mp 260–261°C. IR (KBr), cm^{-1}: 1155 and 1166 (SO_2^{sym}), 1320 and 1339 (SO_2^{as}), 3090 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 6.70 (br s, 2H, SO_2NH_2), 7.05–7.76 (m, 3H, ArH the 2-Cl-substituted ring), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.28 (br s, 1H, SO_2NH). $C_{15}H_{12}ClN_3O_4S_2$ (397.86); calcd C, 45.28; H, 3.04; N, 10.56; found: C, 45.11; H, 3.32; N, 10.51.

3-Bromo-4-(8-quinoline-sulfonylamido)-benzenesulfonamide 31. White crystals, mp 241–243°C. IR (KBr), cm^{-1}: 1151 and 1166 (SO_2^{sym}), 1320 and 1356 (SO_2^{as}), 3060 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 6.65 (br s, 2H, SO_2NH_2), 7.05–7.86 (m, 3H, ArH the 2-Br-substituted ring), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.24 (br s, 1H, SO_2NH). $C_{15}H_{12}BrN_3O_4S_2$ (442.31); calcd C, 40.73; H, 2.73; N, 9.50; found: C, 40.95; H, 2.54; N, 9.50.

3-Iodo-4-(8-quinoline-sulfonylamido)-benzenesulfonamide 32. White crystals, mp 224–226°C (dec.). IR (KBr), cm^{-1}: 1145 and 1166 (SO_2^{sym}), 1320 and 1360 (SO_2^{as}), 3070 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 6.60 (br s, 2H, SO_2NH_2), 7.08–7.70 (m, 3H, Ar H the 2-I-substituted ring), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.24 (br s, 1H, SO_2NH). $C_{15}H_{12}IN_3O_4S_2$ (489.31); calcd C, 36.82; H, 2.47; N, 8.59; found: C, 36.91; H, 2.35; N, 8.35.

4,5-Dichloro-6-(8-quinoline-sulfonylamido)-benzene-1,3-disulfonamide 33. White crystals, mp 277–278°C. IR (KBr), cm^{-1}: 1140 and 1166 (SO_2^{sym}), 1320 and 1370 (SO_2^{as}), 3080 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 7.54 (s, 1H, ArH from the pentasubstituted benzene ring), 7.60 (br s, 4H, 2 SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.30 (br s, 1H, SO_2NH). $C_{15}H_{12}Cl_2N_4O_6S_3$ (511.38) calcd C, 35.23; H, 2.37; N, 10.96; found: C, 35.19; H, 2.31; N, 10.88.

6-Chloro-4-(8-quinoline-sulfonylamido)-benzene-1,3-disulfonamide 34. White crystals, mp 290–292°C (dec.). IR (KBr), cm^{-1}: 1150 and 1166 (SO_2^{sym}), 1320 and 1334 (SO_2^{as}), 3060 (NH); 3360 (NH_2). ^1H NMR (DMSO-d_6) δ 7.35 (s, 1H, ArH from disulfamoylphenyl), 7.59 (s, 1H, ArH from disulfamoylphenyl), 7.75 (br s, 4H, 2 SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.33 (br s, 1H, SO_2NH). $C_{15}H_{13}ClN_4O_6S_3$ (476.94); calcd C, 37.78; H, 2.75; N, 11.75; found: C, 37.90; H, 2.67; N, 11.69.

5-(8-Quinoline-sulfonylamido)-1,3,4-thiadiazol-2-sulfonamide 35. White crystals, mp >300°C; IR (KBr), cm^{-1}:

1166 and 1180 (SO_2^{sym}), 1320 and 1340 (SO_2^{as}), 3060 (NH), 3375. ^{1}H NMR (DMSO-d_6) δ 6.94 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.45 (br s, 1H, SO_2NH). $C_{11}H_9N_5O_4S_3$ (371.42) calcd C, 35.57; H, 2.44; N, 18.86; found: C, 35.29; H, 2.60; N, 18.74.

5-(8-Quinoline-sulfonylimido)-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline 36. White crystals, mp >300°C; IR (KBr), cm^{-1}: 1166 and 1182 (SO_2^{sym}), 1320 and 1366 (SO_2^{as}), 3080 (NH), 3380 (NH_2). ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H, Me), 6.96 (br s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline). $C_{12}H_{11}N_5O_4S_3$ (385.44) calcd C, 37.39; H, 2.88; N, 18.17; found: C, 37.32; H, 2.79; N, 18.05.

5-(8-Quinoline-sulfonylamidoethylcarboxamido)-1,3,4-thiadiazol-2-sulfonamide 37. White crystals, mp 295-297°C (dec.), IR (KBr), cm^{-1}: 1150 and 1166 (SO_2^{sym}), 1290 (amide III), 1320 and 1334 (SO_2^{as}), 1570 (amide II), 1710 (amide I), 3090 (NH); 3360 (NH_2). ^{1}H NMR (DMSO-d_6) δ 2.25–2.60 (m, 4H, CH_2CH_2), 6.88 (br s, 3H, CONH + SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.44 (br s, 1H, SO_2NH). $C_{14}H_{14}N_6O_5S_3$ (442.50); calcd C, 38.00; H, 3.19; N, 18.99; found: C, 37.96; H, 2.94; N, 18.70.

6-(8-Quinoline-sulfonylamido)-benzothiazol-2-sulfonamide 38. White crystals, mp >300°C; IR (KBr), cm^{-1}: 1154 and 1166 (SO_2^{sym}), 1320 and 1344 (SO_2^{as}), 3060 (NH); 3360 (NH_2). ^{1}H NMR (DMSO-d_6) δ 6.94 (dd, 1H, J=9, J=3 Hz, H-5 of benzothiazole), 7.10 (d, 1H, J=3 Hz, H-7 of benzothiazole), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.78 (d, 1H, J=9 Hz, H-4 of benzothiazole), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.22 (br s, 2H, SO_2NH_2), 8.49 (br s, 1H, SO_2NH). $C_{16}H_{12}N_4O_4S_3$ (420.49); calcd C, 45.70; H, 2.88; N, 13.32; found: C, 45.90; H, 2.65; N, 13.09.

6-(8-Quinoline-sulfonyloxy)-benzothiazol-2-sulfonamide 39. White crystals, mp >300°C, IR (KBr), cm^{-1}: 1160 and 1174 (SO_2^{sym}), 1333 and 1350 (SO_2^{as}), 1450, 3360 (NH_2). ^{1}H NMR (DMSO-d_6) δ 6.90 (dd, 1H, J=9, J=3 Hz, H-5 of benzothiazole), 7.11 (d, 1H, J=3 Hz, H-7 of benzothiazole), 7.79 (d, 1H, J=9 Hz, H-4 of benzothiazole), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.19 (br s, 2H, SO_2NH_2). $C_{16}H_{11}N_3O_5S_3$ (421.48) calcd C, 45.60; H, 2.63; N, 9.97; found: C, 45.54; H, 2.71; N, 9.69.

6-(8-Quinoline-sulfonyloxyethyloxy)-benzothiazol-2-sulfonamide 40. White crystals, mp 278–279°C, IR (KBr), cm^{-1}: 1170 and 1184 (SO_2^{sym}), 1332 and 1345 (SO_2^{as}), 1450, 3360 (NH_2). ^{1}H NMR (DMSO-d_6) δ 2.89 (t, 3H, CH_2), 3.14 (t, 3H, CH_2), 6.95 (dd, 1H, J=9, J=3 Hz, H-5 of benzothiazole), 7.10 (d, 1H, J=3 Hz, H-7 of benzothiazole), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.79 (d, 1H, J=9 Hz, H-4 of benzothiazole), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.18 (br s, 2H, SO_2NH_2). $C_{18}H_{15}N_3O_6S_3$ (465.53); calcd C, 46.44; H, 3.25; N, 9.03; found: C, 46.28; H, 3.50; N, 9.00.

4-(8-Quinoline-sulfonyloxymethyl)-benzenesulfonamide 41. White crystals, mp 259–261°C; IR (KBr), cm^{-1}: 1155 and 1176 (SO_2^{sym}), 1325 and 1334 (SO_2^{as}), 3310 (NH_2). ^{1}H NMR (DMSO-d_6) δ 4.90 (s, 2H, CONHCH_2), 7.08–7.41 (m, AA′BB′, J=7.2 Hz; 4H, ArH, phenylene), 7.49 (s, 2H, SO_2NH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline). $C_{16}H_{14}N_2O_5S_2$ (378.43) calcd C, 50.78; H, 3.73; N, 7.40; found: C, 50.54; H, 3.62; N, 7.25.

4-(8-Quinoline-sulfonyloxyethyl)-benzenesulfonamide 42. White crystals, mp 254–255°C. IR (KBr), cm^{-1}: 1157 and 1176 (SO_2^{sym}), 1321 and 1332 (SO_2^{as}), 3300 (NH_2). ^{1}H NMR (DMSO-d_6) δ 3.10 (t, 2H, αCH$_2$ from the CH_2CH_2 bridge), 3.70 (t, 2H, βCH$_2$ from the CH_2CH_2 bridge), 6.95 (br s, 2H, SO_2NH_2), 7.05–7.52 (m, AA′BB′, J=7.3 Hz, 4H, ArH, phenylene), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline). $C_{17}H_{16}N_2O_5S_2$ (392.46) calcd C, 52.03; H, 4.11; N, 7.14; found: C, 52.31; H, 4.24; N, 7.08.

5,6-Dihydro-4-[*N*-8-quinoline-sulfonylamido-(ethylamido)]-6-methyl-4H-thieno-[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide 43. White crystals, mp >300°C; IR (KBr), cm^{-1}: 1133 and 1166 (SO_2^{sym}), 1320 and 1345 (SO_2^{as}), 3360 (NH_2). ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 3H, Me), 1.39 (t, 3H, Me from ethyl), 2.55 (m, 1H, CH), 2.80 (m, 1H, CH), 3.05–3.20 (m, 2H, CH_2 from ethyl), 4.37 (m, 2H, CH_2), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from quinoline), 7.99 (d, 1H, H^4 from quinoline), 8.10 (d, 1H, H^5 from quinoline), 8.03 (s, 1H, CH, ArH from thienyl), 8.25 (br s, 2H, SO_2NH_2). $C_{19}H_{21}N_3O_6S_4$ (515.65) calcd C, 44.26; H, 4.10; N, 8.15; found: C, 44.39; H, 4.41; N, 8.10.

Measurement of tonometric IOP

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. Solutions of inhibitors (2%, by weight, as hydrochlorides, triflates or sodium carboxylates) were obtained in distilled deionized water. The pH of these solutions was in the range of 5.5–8.4.

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA) as described by Maren's group.[55,56] 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 interindividual variations commonly observed in the rabbit.[55,56] 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 by Sugrue et al.[57] The IOP of operated animals was checked after approximately 4 weeks, and animals with an elevated pressure of 30–35 mm Hg were used at least 1 month after the injection of α-chymotrypsin.

Drug distribution in ocular fluids and tissues

The general procedure of Maren's group has been followed.[55,56] The animals were killed with an intracardiac air injection. Aqueous humor (both posterior and anterior chamber fluids) were withdrawn. Then, the cornea and anterior uvea (iris plus attached ciliary body) were dissected, rinsed well with water, blotted, weighed and put into 1–2 mL of water. For isolation of the ciliary processes, intact anterior uvea rings were placed on a parafilm covered piece of polystyrene foam in a Petri dish. The tissue has been wetted with normal saline and dissected under a microscope, when ciliary processes were liberated from their attachment to the iris, cut, weighed and put in 0.5 mL of distilled water. The tissue from four eyes (average weight of 8 mg/eye) was pooled for drug analysis. Samples were boiled for 5 min (in order to denature CA, and free drug from the E–I complex), diluted and then incubated with a known amount of enzyme. The activity of the free enzyme and in the presence of the inhibitor were determined as described above. A calibration curve has been used in order to determine the fractional inhibition in the different tissues, as described in refs 55 and 56.

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).[29]

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.[7,8,29]

Transcorneal penetration of drugs

The method of Maren et al.[14] with the modifications of Pierce's group[58,59] (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.,[58,59] or enzymatically.[7,8,14] The results of the drug analyses were used to calculate the rate constant of transfer across the cornea (k_{in}). As described by Pierce,[58,59] 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 Drs. M. A. Ilies and M. Barboiu for expert technical assistance.

References

1. Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.* **1999**, *42*, 2641.
2. Domagk, G. *Dt. Med. Wocheschr.* **1935**, *61*, 250.
3. Northey E. H. *The Sulfonamides and Allied Compounds*; Reinhold: New York, 1948; pp 1–267.
4. Mandell, G. L.; Sande, M. A. In *The Pharmacological Basis of Therapeutics*, 8th Edition; Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., Eds.; Pergamon: New York, 1990; pp 1047–1064.
5. 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.
6. Weiner, I. M. In *The Pharmacological Basis of Therapeutics*, 8th Edition; Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., Eds., Pergamon: New York, 1990; pp 713–732.

7. Supuran, C. T.; Scozzafava, A.; Ilies, M. A.; Iorga, B.; Cristea, T.; Briganti, F.; Chiraleu, F.; Banciu, M. D. *Eur. J. Med. Chem.* **1998**, *33*, 577.
8. Supuran, C. T.; Mincione, F.; Scozzafava, A.; Briganti, F.; Mincione, G.; Ilies, M. A. *Eur. J. Med. Chem.* **1998**, *33*, 247.
9. Boyd, A. E. *Diabetes* **1988**, *37*, 847.
10. Beyer, K. H.; Baer, J. E. *Pharmacol. Rev.* **1961**, *13*, 517.
11. Maren, T. H. *Drug Dev. Res.* **1987**, *10*, 255.
12. Supuran, C. T.; Conroy, C. W.; Maren, T. H. *Eur. J. Med. Chem.* **1996**, *31*, 843.
13. Maren, T. H. *J. Glaucoma* **1995**, *4*, 49.
14. Maren, T. H.; Jankowska, L.; Edelhauser, G. F.; Sanyal, G. *Exp. Eye Res.* **1983**, *36*, 457.
15. Katritzky, A. R.; Caster, K. C.; Maren, T. H.; Conroy, C. W.; Bar-Ilan, A. *J. Med. Chem.* **1987**, *30*, 2058.
16. Eller, M. G.; Schoenwald, R. D.; Dixson, J. A.; Segarra, T.; Barfknecht, C. F. *J. Pharm. Sci.* **1985**, *74*, 155.
17. Antonaroli, S.; Bianco, A.; Brufani, M.; Cellai, L.; Lo Baido, G.; Potier, E.; Bonomi, L.; Perfetti, S.; Fiaschi, A. I.; Segre, G. *J. Med. Chem.* **1992**, *35*, 2697.
18. Ponticello, G. S.; Freedman, M. B.; Habecker, C. N.; Lyle, P. A.; Schwam, H.; Varga, S. L.; Christy, M. E.; Randall, W. C.; Baldwin, J. J. *J. Med. Chem.* **1987**, *30*, 591.
19. Jain, A.; Whitesides, G. M.; Alexander, R. S.; Christianson, D. W. *J. Med. Chem.* **1994**, *37*, 2100.
20. Supuran, C. T.; Popescu, A.; Ilisiu, M.; Costandache, A.; Banciu, M. D. *Eur. J. Med. Chem.* **1996**, *31*, 439.
21. Supuran, C. T.; Scozzafava, A.; Popescu, A.; Bobes-Tureac, R.; Banciu, A.; Creanga, A.; Bobes-Tureac, G.; Banciu, M. D. *Eur. J. Med. Chem.* **1997**, *32*, 445.
22. Supuran, C. T.; Briganti, F.; Scozzafava, A. *J. Enzyme Inhib.* **1997**, *12*, 175.
23. Supuran, C. T.; Conroy, C. W.; Maren, T. H. *Proteins* **1997**, *27*, 272.
24. Briganti, F.; Pierattelli, R.; Scozzafava, A.; Supuran, C. T. *Eur. J. Med. Chem.* **1996**, *31*, 1001.
25. Supuran, C. T.; Clare, B. W. *Eur. J. Med. Chem.* **1999**, *34*, 41.
26. Supuran, C. T.; Scozzafava, A. *J. Enzyme Inhib.* **1997**, *12*, 37.
27. Mincione, G.; Scozzafava, A.; Supuran, C. T. *Metal Based Drugs* **1997**, *4*, 27.
28. Sumalan, S. L.; Casanova, J.; Alzuet, G.; Borras, J.; Castineiras, A.; Supuran, C. T. *J. Inorg. Biochem.* **1996**, *62*, 31.
29. Supuran, C. T.; Ilies, M. A.; Scozzafava, A. *Eur. J. Med. Chem.* **1998**, *33*, 739.
30. Becker, B. *Am. J. Ophthalmol.* **1955**, *39*, 177.
31. Maren, T. H. *Physiol. Rev.* **1967**, *47*, 595.
32. Ponticello, G. S.; Sugrue, M. F.; Plazonnet, B.; Durand-Cavagna, G. *Pharm. Biotechnol.* **1998**, *11*, 555.
33. Silver, L. H. *Am. J. Ophthalmol.* **1998**, *126*, 400.
34. Woltersdorf, W.; Schwam, H.; Bicking, J. B.; Brown, S. L.; deSolms, S. J.; Fishman, D. R.; Graham, S. L.; Gautheron, P. D.; Hoffman, J. M.; Larson, R. D.; Lee, W. S.; Michelson, S. R.; Robb, C. M.; Share, C. N.; Shepard, K. L.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Strohmeyer, K. M.; Sugrue, M. F.; Viader, M. P. *J. Med. Chem.* **1989**, *32*, 2486.
35. Graham, S. L.; Shepard, K. L.; Anderson, P. S.; Baldwin, J. J.; Best, D. B.; Christy, M. E.; Freedman, M. B.; Gautheron, P.; Habecker, C. N.; Hoffman, J. M.; Lyle, P. A.; Michelson, S. R.; Ponticello, G. S.; Robb, C. M.; Schwam, H.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Strohmaier, K. M.; Sugrue, M. F.; Varga, S. L. *J. Med. Chem.* **1989**, *32*, 2548.
36. Graham, S. L.; Hoffman, J. M.; Gautheron, P.; Michelson, S. R.; Scholz, T. H.; Schwam, H.; Shepard, K. L.; Smith, A. M.; Sondey, J. M.; Sugrue, M. F.; Smith, R. L. *J. Med. Chem.* **1990**, *33*, 749.
37. Hartmann, G. D.; Halczenko, W. *Heterocycles* **1989**, *29*, 1943.
38. Prugh, J. D.; Hartmann, G. D.; Mallorga, P. J.; McKeever, B. M.; Michelson, S. R.; Murcko, M. A.; Schwam, H.; Smith, R. L.; Sondey, J. M.; Springer, J. B.; Sugrue, M. F. *J. Med. Chem.* **1991**, *34*, 1805.
39. Hartmann, G. D.; Halczenko, W.; Prugh, J. D.; Smith, R. L.; Sugrue, M. F.; Mallorga, P. J.; Michelson, S. R.; Randall, W. C.; Schwam, H.; Sondey, J. M. *J. Med. Chem.* **1992**, *35*, 3027.
40. 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. B.; Gautheron, P.; Grove, J.; Mallorga, P.; Viader, M. P.; McKeever, B. M.; Navia, M. A. *J. Med. Chem.* **1989**, *32*, 2510.
41. Wolin, R.; Connolly, M.; Afonso, A.; Hey, J. A.; She, H.; Rivelli, M. A.; Williams, S. M.; West, R. E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2157.
42. Crippa, G. B.; Maffei, S. *Gazz. Chim. Ital.* **1941**, *71*, 97.
43. Cingolani, E. *Gazz. Chim. Ital.* **1948**, *78*, 275.
44. Jitianu, A.; Ilies, M. A.; Scozzafava, A.; Supuran, C. T. *Main Group Met. Chem.* **1997**, *20*, 147.
45. Barboiu, M.; Supuran, C. T.; Menabuoni, L.; Scozzafava, A.; Mincione, F.; Briganti, F.; Mincione, G. *J. Enzyme Inhib.* **1999**, in press.
46. Blacklock, T. J.; Sohar, P.; Butcher, J. W.; Lamanec, T.; Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 1672.
47. 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*; Botré, F.; Gros, G.; Storey, B. T., Eds.; VCH: Weinheim, 1991; pp 1–13.
48. Behravan, G.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1990**, *190*, 351.
49. Khalifah, R. G.; Strader, D. J.; Bryant, S. H.; Gibson, S. M. *Biochemistry* **1977**, *16*, 2241.
50. Lindskog, S.; Coleman, J. E. *Proc. Natl. Acad Sci. USA* **1964**, *70*, 2505.
51. Steiner, H.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1975**, *59*, 253.
52. Maren, T. H.; Wynns, G. C.; Wistrand, P. J. *Mol. Pharmacol.* **1993**, *44*, 901.
53. Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668.
54. Baird, T. T.; Waheed, A.; Okuyama, T.; Sly, W. S.; Fierke, C. A. *Biochemistry* **1997**, *36*, 2669.
55. Maren, T. H.; Brechue, W. F.; Bar-Ilan, A. *Exp. Eye Res.* **1992**, *55*, 73.
56. Brechue, W. F.; Maren, T. H. *Invest. Ophthalmol. Vis. Sci.* **1993**, *34*, 2581.
57. Sugrue, M. F.; Gautheron, P.; Mallorga, P.; Nolan, T. E.; Graham, S. L.; Schwam, H.; Shepard, K. L.; Smith, R. L. *Br. J. Pharmacol.* **1990**, *99*, 59.
58. Pierce, W. M. Jr.; Sharir, M.; Waite, K. J.; Chen, D.; Kaysinger, K. K. *Proc. Soc. Exp. Biol. Med.* **1993**, *203*, 360.
59. Sharir, M.; Pierce, W. M. Jr.; Chen, D.; Zimmerman, T. J. *Exp. Eye Res.* **1994**, *58*, 107.