Furazan and furoxan sulfonamides are strong α-carbonic anhydrase inhibitors and potential antiglaucoma agents

Furazan and furoxan sulfonamides are strong α-carbonic anhydrase inhibitors and potential antiglaucoma agents

Konstantin Chegaev[a], Loretta Lazzarato[a], Yasinalli Tamboli[a], Andrea Scozzafava[b], Fabrizio Carta[b], Emanuela Masini[b], Roberta Fruttero[a,*] Claudiu T. Supuran,[b,c,*] Alberto Gasco[a]

[a]*Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, via Pietro Giuria 9, 10125 Torino, Italy.*
[b]*Università degli Studi di Firenze, Dipartimento di Chimica, Lab. Chimica Bioinorganica, Via della Lastruccia 3, 50019 Sesto Fiorentino (Florence), Italy.*
[c]*Università degli Studi di Firenze, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche e Nutraceutiche, Via Ugo Schiff 6, I-50019 Sesto Fiorentino (Florence), Italy.*

ABSTRACT

A series of furazan and furoxan sulfonamides were prepared and studied for their ability to inhibit human carbonic anhydrase (CA, EC 4.2.1.1) isoforms hCA I, hCA II, hCA IX, and hCA XII. The simple methyl substituted products **3**-**5** were potent inhibitors. Differing structural modifications of these leads had differing effects on potency and selectivity. In particular, products in which the sulfonamide group is separated from the hetero ring by a phenylene bridge retained high potency only on the hCA XII isoform. The sulfonamides **3**-**5** exerted intraocular pressure (IOP) lowering effects in vivo in hypertensive rabbits more efficiently than dorzolamide. Some other products (**39**-**42**), although less effective in vitro hCA II/XII inhibitors, also effectively lowered IOP in two different animal models of glaucoma.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are enzymes found in both eukaryotic and prokaryotic cells, which catalyze the conversion of carbon dioxide to bicarbonate and a proton (eq. 1).

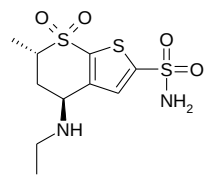
$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \quad \text{eq. 1)}$$

CAs evolved along at least five different lines, giving rise to the distinct α, β, γ, δ, and ζ enzyme families. In mammals, 13 CA isoenzymes and 3 acatalytic CA-related proteins (CARPs) with different subcellular localizations have been identified to date: CA I, CA II, CA III, CA VII, CA XIII are cytosolic, CA IV, CA IX, CA XII, CA XIV, CA XV (not expressed in humans) are membrane bound, CA VA, CA VB are mitochondrial, CA VI is a secreted enzyme, while CARP VIII, CARP X, CARP XI appear to be cytosolic proteins. These isoenzymes belong to the evolutional α-CA gene family, and are present in various tissues, including the eyes, kidneys, lungs, the gastrointestinal and reproductive tracts, and the CNS.[1,2] CAs are associated with many physiological processes related to respiration and the transport of CO_2/bicarbonate, pH and CO_2 homeostasis, electrolyte secretion in various tissues and organs, biosynthetic reactions, bone remodeling, and calcification. Inhibition of the CAs involved in these processes is an important target for the treatment of a number of disorders, including edema, glaucoma, obesity, osteoporosis, epilepsy, pain, and cancer.[3,4] Recently, CAs belonging not only to the α-family but also to the β-, γ-, δ-, and ζ- families have been characterized in several pathogens, including protozoa, bacteria, fungi, and parasites such as nematodes.[2] An increasing number of studies show that they are involved in the virulence and growth of all these agents.[5,6] These findings open exciting new perspectives for the use of CAIs in treating infections and infestations.

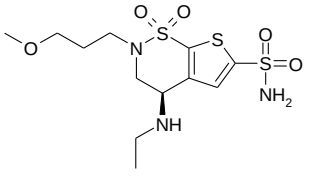
Most CAs are metalloenzymes containing a Zn^{2+} ion in the active site; primary sulfonamides are their classical inhibitors. Crystallographic studies of adducts between members of this class and several CA isoenzymes show that the nitrogen deprotonated sulfonamide moiety binds the catalytic Zn^{2+} ion and simultaneously interacts with the Thr199 residue through two hydrogen bonds.[3] Some sulfonamide inhibitors are heterocyclic compounds, in which the sulfonamide group is linked to the hetero ring, either directly or through appropriate spacers.[2] To our knowledge, no examples of 1,2,5-oxadiazole (furazan) sulfonamide derivatives or of the related 2-oxides (furoxans) have been reported thus far. We recently described a synthetic method to obtain these products, starting from the easily-accessible related phenylmercapto compounds.[7] The methyl and phenyl substituted compounds are water soluble products and their pKas fall in the 6.80 - 7.16 range.

This paper reports the characterization of some furazan and furoxan sulfonamides (Table 1) as inhibitors of human CA I and CA II, the two isoforms most widespread in organs and tissues, and as inhibitors of CA IX and CA XII, the two isoforms predominantly associated with and overexpressed in many tumors. It also looks at a series of their phenylogues and related derivatives. The results of a study aimed at evaluating the capacity of selected sulfonamides to lower intraocular

pressure (IOP) in hyper- and normo-tensive rabbits are also discussed. This in vivo study was designed in the light of the fact that most of the investigated sulfonamides, such as dorzolamide **1**, and brinzolamide **2** (see Chart), effectively inhibit CA II and CA XII, two isoforms associated with aqueous humor secretion in the eye, and well-established anti-glaucoma targets.[6]



1, Dorzolamide (Trusopt)



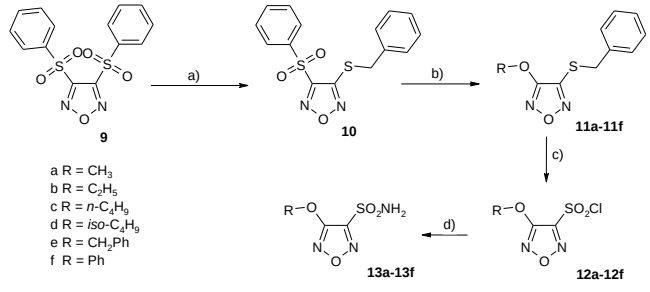
2, Brinzolamide (Azopt)

Chart.

2. Results and discussion

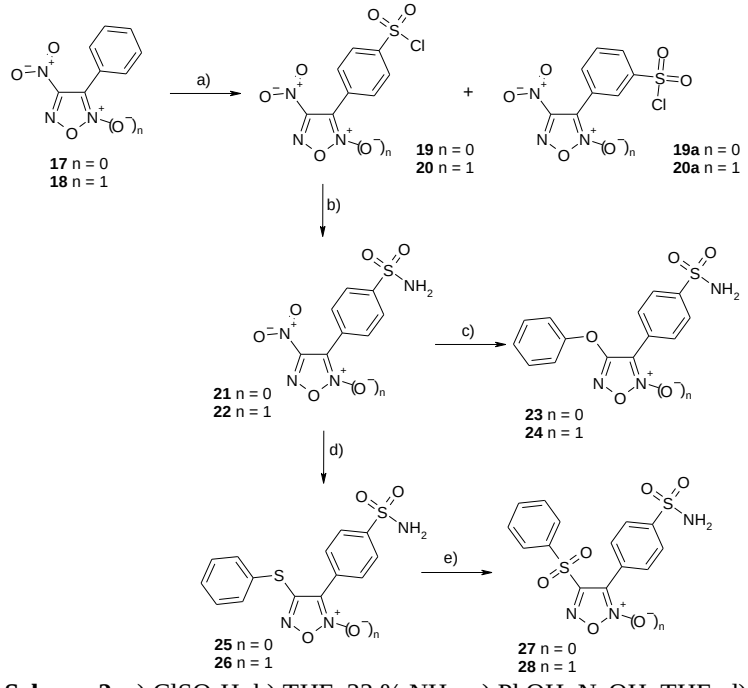
2.1. Chemistry

The synthesis of the simple methyl or phenyl substituted furazan and furoxan sulphonamides studied here (der.s **3**-**8**, Table 1) is described elsewhere.[7] The alkyloxy substituted furazansulfonamides **13a-f** were prepared by the procedure outlined in Scheme 1. The action of benzyl mercaptane on 3,4-diphenylsulfonylfurazan (**9**) dissolved in methylenchloride in the presence of triethylamine gave rise to the 3-benzylthio-4-phenylsulfonylfurazan (**10**) subsequently transformed into the related alkyloxy **11a-f** derivatives by reaction with sodium hydride and the appropriate alcohols. These intermediates were treated with chlorine in acetic acid solution to afford the corresponding sulphonyl chlorides **12a-f**, which yielded the expected sulfonamides when reacted with ammonia.



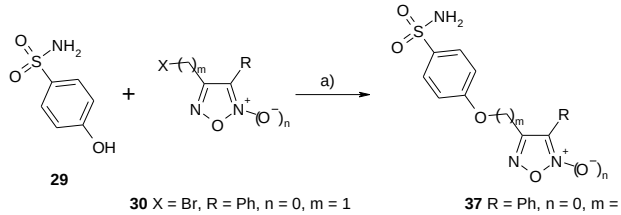
Scheme 1. a) $PhCH_2SH$, Et_3N, CH_2Cl_2; b) ROH, *t*-BuOK, THF; c) Cl_2, CH_3COOH; d) NH_3.

The three sulphonamides **14**-**16** (Table 1), which are phenylogues of **6**-**8**, are described elsewhere.[8] The substitution in **14** and **16** of nitro, phenoxy, phenylthio, and phenylsulfonyl groups for the phenyl ring gives rise to the related furazan and furoxan derivatives **21**-**28** respectively. The synthesis of these products is reported in Scheme 2. The action of chlorosulfonic acid on 4-nitro-3-phenylfurazan (**17**) and on the related furoxan **18** affords the corresponding chlorosulfonyl derivatives **19**, **20**, which are easily transformed by action of ammonia into the sulfonamides **21**, **22**. Nucleophilic displacement, under basic conditions, of the nitro group by phenol and thiophenol yields the final products **23**, **24** and **25**, **26**, respectively. Potassium permanganate oxidation of the latter two substances in acetic acid solution gives the expected target compounds **27**, **28.**



Scheme 2. a) $ClSO_3H$; b) THF, 33 % NH_3; c) PhOH, NaOH, THF; d) PhSH, NaOH, THF; e) $KMnO_4$, CH_3COOH.

Furazans **37**, **39**, **41**, in which the sulfonamide group is separated from the variously-substituted furazan ring by a phenoxymethyl bridge, were prepared by reaction under basic conditions of the 4-hydroxybenzenesulfonamide (**29**) with the appropriate bromomethyl derivatives **30**, **32**, **34** (Scheme 3). Similarly, the furoxans **38**, **40**, **42** were obtained from **31**, **33**, **35**. The two phenylsulfonylfurazanyloxy and 3-phenylsulfonylfuroxanyloxy para substituted benzenesulfonamides **43**, **44** were obtained from the corresponding furazan **9** and furoxan **36** respectively. Compounds **37**, **39**, **41**, **43** were previously used as intermediates for the preparation of a series of hypoglycemic products described in a paper [9] that gave no details of their preparation and structural characterization, which, conversely, are given here (see experimental).

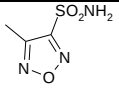
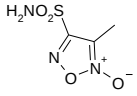
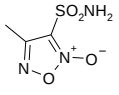
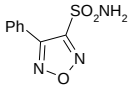
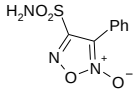
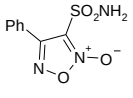
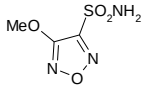
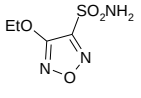
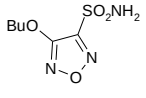
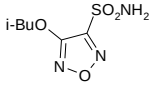
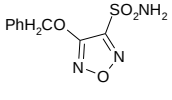
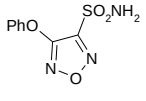


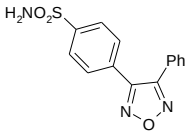
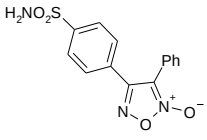
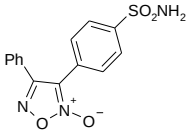
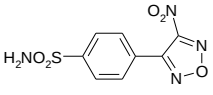
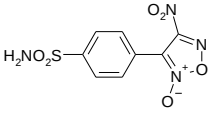
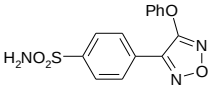
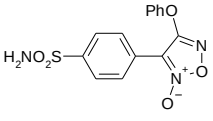
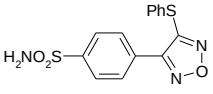
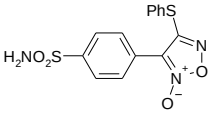
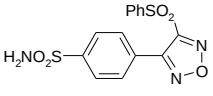
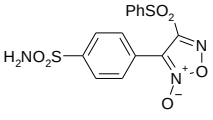
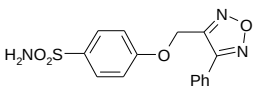
Scheme 3. a) NaOH, EtOH for R = Ph, $CONH_2$; Na_2CO_3, DMF for R = CN, SO_2Ph.

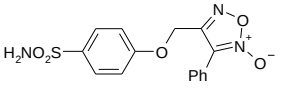
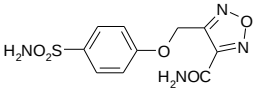
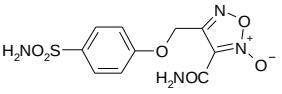
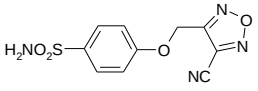
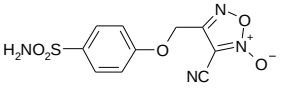
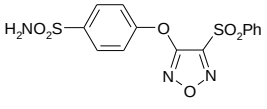
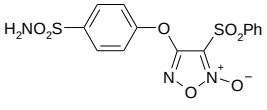
2.2 Carbonic anhydrase inhibition

The sulfonamide products described here were tested by a stopped flow assay10 for their ability to inhibit human (h) catalytically-active hCA I, hCA II, hCA IX, h CA XII isoforms. All the compounds were able to inhibit the four CA isoforms, but the strength of their inhibitory potency, evaluated as inhibition constant Ki (nM), depended on their structure (see Table 1).

Table 1. Data on the inhibition of hCA I, II (cytosolic) and IX, XII (transmembrane, tumor-associated isoforms) by a stopped-flow CO_2 hydrase assay of compounds **3**-**8**, **13a**-**f**, **14**-**16**, **21**-**28**, **37**-**44**.

		Ki (nM)			
Cpd	**Formula**	**hCA I**	**hCA II**	**hCA IX**	**hCA XII**
3		9.0	8.6	7.9	5.9
4		11.0	4.7	7.3	8.2
5		10.1	4.2	6.5	7.6
6		11.1	10.5	6.3	7.9
7		41.5	38.2	7.1	9.9
8		56.5	49.3	7.4	7.5
13a		15.9	6.4	5.8	26.4
13b		12.4	5.9	6.6	6.6
13c		8.5	5.5	4.8	23.7
13d		16.0	5.3	3.9	7.1
13e		19.8	5.1	5.8	7.9
13f		21.1	7.2	6.3	6.3

14		620	51.2	62.9	6.5
15		667	56.9	62.8	8.3
16		563	49.5	58.0	7.1
21		492	358	54.7	77.8
22		591	479	54.5	82.3
23		214	75.9	71.1	6.1
24		498	204	63.9	6.3
25		244	103	57.6	3.9
26		262	113	5037	3.8
27		303	135	63.6	6.2
28		282	64.1	34.5	6.0
37		387	340	110	70.3

38		354	285	47.9	72.5
39		337	146	49.8	28.4
40		229	113	32.3	30.1
41		101	50	10.8	5.6
42		106	53	11.3	5.9
43		9.5	8.9	7.3	3.1
44		103	9.5	6.4	2.9

The simple methyl-substituted compounds **3-5** are potent inhibitors of all four CA isoforms, since their Kis fall in the low nanomolar range (4.2-11). The substitution of alkoxy moieties for the methyl group in **3**, to give **13a-f**, does not substantially modify this pattern; there is only a slight fall in the activity of **13e**,**f** on the hCA I isoform, and in the activity of **13a**,**c** on hCA XII isoform. The phenyl substituted sulfonamides **6-8** behave similarly to the analogue methyl derivatives, the only difference being that the two furoxans **7**, **8** are less active on both the hCA I and hCA II isoforms (Ki range 41.5-56.5).

This preference for the hCA IX and hCA XII isoforms is interesting, since both isoforms are overexpressed in many tumor species.[11,12] The sulfonamides **14-16** that are phenylogues of **6-8** retain strong inhibitory activity on the hCA XII isoform (Ki = 6.5-8.3), while they display moderate (58.4-62.9) or weak (563-667) activity, respectively, on the hCA II/hCA IX and hCA I isoforms. The substitution in **14** and **16** of phenoxy, phenylthio, phenylsolfonyl groups for the phenyl ring does not substantially modify this picture, since compounds **23-28** are quite selective and potent inhibitors of the hCA XII, and display moderate or weak potency on the remaining isoforms. Interestingly, the presence of the nitro group as substituent at the hetero ring (**21** and **22**) switches off the selective action on the hCA XII isoform. The suppression of the phenylogy (cyclic vinylogy) in **14** and **15**, following separation of the benzensulfonamide moiety from the heterocycle system through an oxymethyl bridge, gives rise to **37** and **38**, respectively, in which the strong inhibitory action on hCA XII shown by the leads is reduced. This action is maintained in the cyano analogues **41**, **42**, but not in the related amides **39**, **40**. Finally, the separation of the benzensulfonamide moiety by an oxy bridge in **27** and **28** gives rise to **43** and **44**, respectively, which show strong inhibitory potency on all the hCA isoforms considered (Ki = 2.9-9.5), with the sole exception of the furoxan **44** on hCA I (Ki = 103).

2.3. IOP lowering studies

One of the principal therapeutic applications of CAIs is in treating glaucoma, one of major causes of visual impairment worldwide.[13] Current medical therapy of open-angle glaucoma seeks to decrease the elevated IOP resulting from increased production of aqueous humor by the ciliary body, and/or decreased aqueous outflow through trabecular meshwork and the canal of Schlemm or uveo-scleral route. Humor dynamics are principally controlled by β-adrenergic receptors present on the ciliary epithelium involved in aqueous production, and by prostaglandin PGF2α receptors, which control uveo-scleral drainage.[14] Also significantly involved in humor dynamics are the carbonic anhydrases (CAs), in particular the CA II isoform, localized to both the pigmented and the non-pigmented ciliary epithelium, as well as in the anterior uvea of eye;

they control bicarbonate and aqueous humor secretion rates.[2] CAIs, such as dorzolamide **1**, and brinzolamide **2** (see Chart), are currently used to reduce IOP in open-angle glaucoma therapy.[14] The three methyl substituted furazan and furoxan sulfonamides **3**-**5** were chosen for the in vivo IOP lowering studies since they are freely soluble in water and trigger potent hCA II/XII inhibition; dorzolamide was taken as reference compound. Furoxan **5** possesses NO-dependent vasodilating properties, as does its isomer **4**, although to a much lesser extent.[7] NO release might bring additional beneficial effects on the IOP lowering properties of these products.[15,16,17]

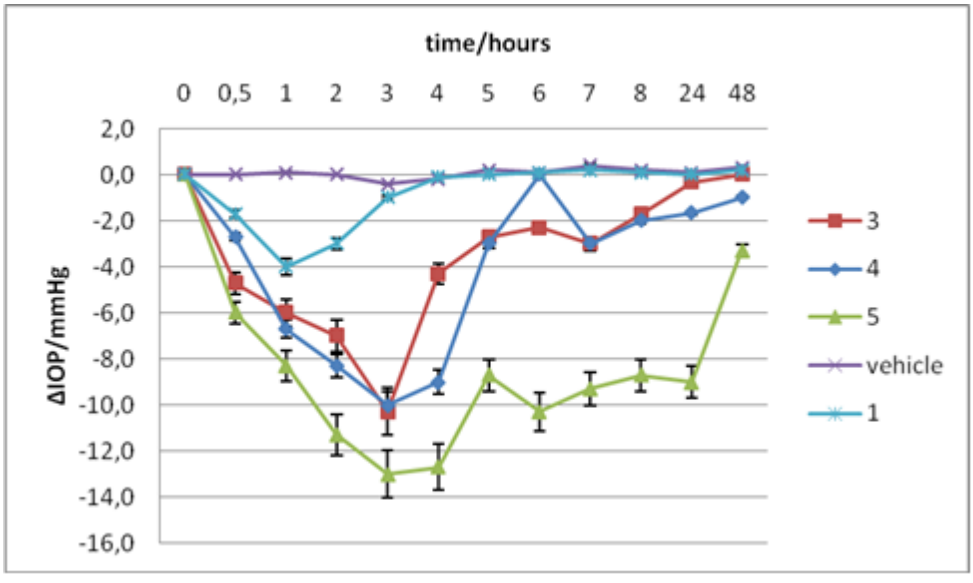


Figure **1**. ΔIOP (mmHg) versus time (hours), in carbomer-induced ocular hypertension in rabbit eyes treated with 50 µL of 2 % solution of compounds **3**-**5**, dorzolamide **1** at 2% as standard drug and vehicle. Errors were within 10−15% of the reported IOP values (from three different measurements for each of the four animals in the study group) and were statistically significant (p = 0.045 by the Student's t test).

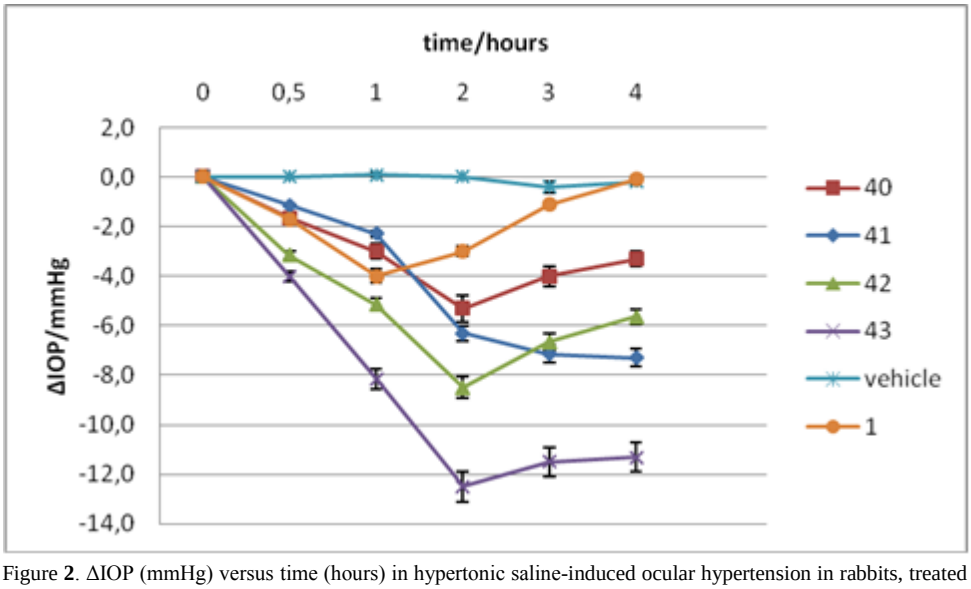


Figure **2**. ΔIOP (mmHg) versus time (hours) in hypertonic saline-induced ocular hypertension in rabbits, treated with 50 µL of 2 % solution of compounds **39**-**42**, dorzolamide **1** at 2% as standard drug and vehicle. Errors were within 10−15% of the reported IOP values (from three different measurements for each of the four animals in the study group) and were statistically significant (p = 0.045 by the Student's t test).

In hypertensive rabbits (eye pressure of around 35-42 mm Hg, generated as reported in ref. 15) compounds **3**-**5**, and dorzolamide **1** as standard drug, were investigated in a chronic administration schedule, and the IOP measured for 48 hours (Fig. 1). Dorzolamide was moderately effective for the first 2-3 hours post-administration, whereas the sulfonamides **3**-**5** investigated here showed much stronger IOP lowering activities. At the peak, a 10-13.5 mm Hg decrease of IOP was observed, which was usually achieved at 3-4 hours post administration. For **5**, this effect was present also at 8 and 24 h post-administration, making it a very interesting eye-drop drug candidate (Fig. 1).

Although the sulfonamides **39**-**42** were less effective hCA II/XII inhibitors than the methyl derivatives **3**-**5** discussed above, their good water solubility, combined with the possibility to formulate them as 2 % eye drops, prompted us to investigate their IOP lowering effects in another animal model of glaucoma, i.e., a normotensive rabbit model.[17] As seen from Fig. 2, dorzolamide **1** is rather ineffective as an IOP lowering agent in this model, with only a moderate effect, about 4 mm Hg, at 1 h post-administration. By contrast the sulfonamides **39**-**42** showed much stronger IOP lowering effects than the standard drug. Products **39** and **40**, which were the weakest hCA II/XII inhibitors of the four compounds studied here, showed IOP lowering in the 5-8 mm Hg range, peaking at 2 h post-administration. The stronger hCA II/XII inhibitors **41** and **42** showed a more significant effect, lowering IOP by some 8-12 mm Hg, with maximal effect at 2 h post-administration (Fig. 2).

3. Conclusions

Methylfurazan and methylfuroxan sulfonamides were found to be potent inhibitors of the human hCA I, hCA II, hCA IX, hCA XII isoforms; the analogue phenyl derivatives retain high inhibitory activity, particularly on the hCA IX, and hCA XII isoforms. Structural modification of these leads gave rise to products with modulated activity and selectivity. In particular, differently-substituted phenylogues of **3** and **5** showed high potency, particularly on the hCA XII isoform. The finding that some of the sulfonamides investigated here possess stronger IOP lowering activity than dorzolamide, when tested in rabbits, indicates that these products, and more in general the class of furazan and furoxan sulfonamide derivatives, deserve additional study.

4. Experimental protocols

4.1 Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300, at 300 and 75 MHz respectively, using $SiMe_4$ as internal standard. Low resolution mass spectra were recorded with a Finnigan- Mat TSQ-700. Melting points were determined with a capillary apparatus (Büchi 540). Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230×400 mesh ASTM); PE stands for 40-60 petroleum ether. The progress of the reactions was followed by thin layer chromatography (TLC) on 5 cm × 20 cm plates with a layer thickness of 0.2 mm. Anhydrous magnesium sulfate was used as drying agent for the organic phases. Organic solvents were removed under vacuum at 30 °C. Elemental analyses (C, H, N) were performed by Section de Pharmacie, Service de Microanalyse (Geneva) and REDOX (Monza): the results are within 0.4% of theoretical values. Compounds **3**-**8**,[7] **9**,[18] **14**-**16**,[8] **17**, [19] **18**,[19] **29**,[20] **32**,[21] **33**,[22] **34**,[21] **35**,[23] and **36**[24] were obtained as described elsewhere.

3-(Benzylsulfanyl)-4-(phenylsulfonyl)-furazan (10). To a solution of 3,4-bis-(phenylsulfonyl)-1,2,5-oxadiazol (1.05 g, 3.00 mmol) and triethylamine (0.45 mL, 3.2 mmol) in CH_2Cl_2 (25 mL), benzylmercaptane (0.35 mL, 3.0 mmol) was added and the reaction mixture was stirred at room temperature (rt) overnight. The following day, the reaction mixture was diluted with CH_2Cl_2 (25 mL) and the organic phase was washed with H_2O, HCl 1N solution and $NaHCO_3$ saturated solution, after which it was dried and the solvent evaporated. The resulting white solid was crystallized from EtOH to give the title compound. Yield 91%; Mp 88-89°C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 4.39 (s, 2H, CH_2); 7.27-7.41 (m, 5H; $CH_2C_6H_5$); 7.61 (t, 2H), 7.74 (t, 1H), 8.08 (d, 2H) ($SO_2C_6H_5$); ^{13}C-NMR ($CDCl_3$) δ: 37.0, 128.2, 128.8, 129.2, 129.7, 134.6, 135.5, 137.9, 151.5, 155.2, 163.1. MS EI: 332 (M^+).

General procedure for the synthesis of 4-alkyl/aryloxy-furazan-3-sulfonamides (13a-f): To a solution of the corresponding alcohol/phenol (10.0 mmol) in dry THF (15 mL), under positive N_2 pressure, *t*-BuOK (0.67 g, 6.0 mmol) was added and the reaction mixture stirred at rt until it became transparent (c.a. 15 min). Then **10** (6.0 mmol) was added in one portion and stirring was continued for 1h. The reaction mixture was then poured into H_2O (50 mL) and extracted with Et_2O (2×50 mL). The organic phase was washed with brine, dried and evaporated. The resulting oil was dissolved in AcOH (15 mL) and gaseous chlorine was bubbled through the solution for 1h. The resulting mixture was poured into H_2O (50 mL) and extracted with PE (2×50 mL). The organic phase was washed with brine, dried and evaporated. The residue was cooled in an ice-bath and treated with NH_3 conc.sol. (2 mL) at 0°C for 30 min. The mixture was then diluted with HCl 4N sol. (10 mL) and extracted with EtOAc (2×25 mL). The organic phase was washed with brine, dried and evaporated. The resulting product was purified as described.

4-Methoxyfurazan-3-sulfonamide (13a). The resulting oil was purified by flash chromatography (eluent 95/5 v/v CH_2Cl_2/EtOAc). Yield 68%; Mp 71-72 °C (CCl_4 / 1,2-dichloroetane). ^{1}H-NMR (DMSO-d_6) δ: 4.14 (s, 3H, CH_3); 8.67 (s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6) δ: 60.4, 150.0, 161.8. MS EI: 179 (M^+).

4-Ethoxyfurazan-3-sulfonamide (13b). The resulting oil was purified by flash chromatography (eluent 8/2 v/v PE/EtOAc). Yield 52%; Mp 66-67.5 C (CCl_4 / 1,2-dichloroethane). ^{1}H-NMR (DMSO-d_6) δ: 1.42 (t, 3H, CH_3); 4.48 (q, 2H, CH_2); 8.67 (s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6) δ: 14.0, 69.6, 150.1, 160.8. MS EI: 193 (M^+).

4-Butoxyfurazan-3-sulfonamide (13c). The resulting oil was purified by flash chromatography (eluent 9/1 v/v PE/EtOAc). Yield 50%; Mp 85-86 °C (CCl_4). ^{1}H-NMR (DMSO-d_6) δ: 0.92 (t, 3H, CH_3); 1.38-1.50 (m, 2H, CH_2); 1.74-1.83 (m, 2H, CH_2); 4.43 (t, 2H, CH_2); 8.66 (s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6) δ: 13.3, 18.2, 29.9, 73.3, 150.1, 161.0. MS CI: 222 ($M+H^+$).

4-(2-Methylpropoxy)-furazan-3-sulfonamide (13d). The resulting oil was purified by flash chromatography (eluent 95/5 v/v CH_2Cl_2/EtOAc). Yield 52%; Mp 96.5-97.5 °C (CCl_4). ^{1}H-NMR (DMSO-d_6) δ: 0.98 (d, 6H, 2CH_3); 2.07-2.20 (m, 1H, C*H*); 4.21 (d, 2H, CH_2); 8.66 (s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6) δ: 18.3, 27.2, 79.0, 150.1, 161.1. MS CI: 222 ($M+H^+$).

4-Benzyloxyfurazan-3-sulfonamide (13e). The resulting solid was crystallized from H_2O. Yield 32%; Mp 126-127.5 °C (H_2O). ^{1}H-NMR (DMSO-d_6) δ: 5.50 (s, 2H, CH_2); 7.38-7.54 (m, 5H, C_6H_5) 8.70 (s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6) δ: 74.4, 128.4, 128.5, 128.9, 134.3, 150.1, 160.7. MS CI: 256 ($M+H^+$).

4-Phenoxyfurazan-3-sulfonamide (13f). The resulting solid was crystallized from H_2O. Yield 14%; Mp 143.5-145 °C (H_2O). ^{1}H-NMR (DMSO-d_6) δ: 7.34-7.61 (m, 5H, C_6H_5) 8.84 (s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6) δ: 119.4, 126.6, 130.2, 150.7, 153.7, 160.6. MS EI: 241 (M^+).

4-(4-Nitrofurazan-3-yl)benzensulfonamide (21). To a stirred solution of $ClSO_3H$ (100 mL) at 0 °C, compound **17** (10.00 g; 52.0 mmol) was added portionwise. When the addition was complete, the mixture was allowed to reach rt, and stirred for 4 h. The mixture was then added slowly to ice and the resulting solution was extracted with CH_2Cl_2. The collected organic layers were washed with H_2O and brine, dried, and evaporated under reduced pressure. The mixture of the resulting meta- and para-regioisomers was resolved by flash chromatography (PE/EtOAc 90/10 v/v) to obtain the first eluted compound **19** as a white solid with 13% yield, and the second eluted meta-isomer **19a** as a white solid, with 53% yield. Compound **19** (2.89 g, 10.0 mmol) was immediately dissolved in dry THF (50 ml), and 32 % NH_3 (11 mL) was added. After 30 min the reaction was complete and the solvent was evaporated under reduced pressure. The solid thus obtained was taken up with H_2O and extracted with EtOAc; the organic layers were washed with brine, dried, and evaporated under reduced pressure. The crude product was crystallized from PE/EtOAC without heating to give the title compound as a white solid, with 86 % yield. M.p. 141.5-142.5 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.61 (s, 2H, NH_2), 8.04 (m, 4H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 125.8, 130.1, 146.6, 149.8, 159.8. MS EI: 270 (M^+).

4-(4-Nitrofuroxan-3-yl)benzensulfonamide (22). Benzensulfonylchloride **20a** was prepared from **18** by action of $ClSO_3H$, following the procedure described above, with 34% yield. The mixture of meta and para regioisomers was resolved by flash chromatography (PE/EtOAc 90/10 v/v) to obtain the first eluted **20**, as a white solid, 34% yield, and the second eluted meta isomer **20a** as a white solid, 16% yield. Compound **20** (3.05 g, 10.0 mmol) was immediately dissolved in dry THF (100 mL), and 32 % NH_3 (16 mL) was added. After 30 min the reaction was complete and the solvent was evaporated under reduced pressure. The solid thus obtained was taken up with H_2O and extracted with EtOAc; the organic layers were washed with brine, dried, and evaporated under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 70/30 v/v) to give the title compound as a yellow solid, 67 % yield. M.p. 138-139 °C (PE/EtOAc). ^{1}H-NMR (DMSO-d_6) δ: 7.57 (s, 2H, NH_2), 7.94 (d, 2H, C_6H_4), 8.03(d, 2H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 111.3, 124.8, 126.8, 131.7, 147.3, 159.7. MS EI: 286 (M^+).

General procedure for the synthesis of compounds 23-26: to a solution of the appropriate nitro derivative (2.0 mmol) in acetone (8 mL), stirred at r.t., a solution of phenol or thiophenol (1.3 eq) and NaOH 6M (1.3 eq) in H_2O (10 mL) was slowly added. After 6 h the acetone was evaporated and the residue was taken up with H_2O; the white solid thus obtained was filtered to give the desired compound.

4-(4-Phenoxyfurazan-3-yl)benzensulfonamide (23). Yield 86 %. Mp 184-184.5 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.36-7.42 (m, 1H, C_6H_5), 7.62-7.64 (m, 6H, C_6H_5 + NH_2), 8.08 (d, 2H, C_6H_4), 8.25(d, 2H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 119.9, 126.6, 126.7, 127.3, 128.5, 130.3, 145.6, 146.4, 154.2, 163.3. MS EI: 317 (M^+).

3-(4-Phenoxyfuroxan-3-yl)benzensulfonamide (24). Yield 84 %. Mp 178-179.5 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.38-7.40 (m, 1H, C_6H_5), 7.54-7.59 (m, 6H, C_6H_5 + NH_2), 8.08 (d, 2H, C_6H_4), 8.25(d, 2H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 108.1, 120.4, 125.2, 126.4, 126.7, 127.4, 130.3, 145.7, 152.7, 162.2. MS EI: 333 (M^+).

4-(4-Phenylthiofurazan-3-yl)benzensulfonamide (25). Yield 93 %. Mp 158-158.5 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.45-7.61 (m, 7H, C_6H_5 + NH_2), 8.02 (m, 4H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 126.6, 127.5, 127.9, 129.2, 129.8, 130.1, 133.0, 146.4, 151.5, 152.7. MS EI: 333 (M^+).

3-(4-Phenylthiofuroxan-3-yl)benzensulfonamide (26). Yield 91 %. Mp 184-185 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.44-7.66 (m, 5H, C_6H_5), 7.61 (s, 2H, NH_2), 8.03 (m, 4H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 114.4, 125.2, 126.3, 126.6, 128.8, 129.8, 130.0, 133.0, 145.9, 154.2. MS EI: 349 (M^+).

General procedure for the synthesis of compounds 27-28: to a solution of the appropriate thio derivative (3 mmol) in AcOH (10-40 mL) stirred at 0 °C, $KMnO_4$ (3 eq) was added portionwise. The mixture was stirred at r.t until completion of the reaction, after which water and $Na_2S_2O_3$ were added portionwise to turn the mixture white. The white solid thus obtained was filtered to give the desired compound.

4-(4-Phenylsulfonylfurazan-3-yl)benzensulfonamide (27). Yield 94 %. Mp 191.5-192 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.61 (s, 2H, NH_2), 7.72-8.05 (m, 9H, C_6H_5 + C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 126.0, 126.3, 129.0, 130.2, 130.5, 136.3, 137.0, 146.8, 151.6, 156.5. MS EI: 365 (M^+).

3-(4-Phenylsulfonylfuroxan-3-yl)benzensulfonamide (28). Yield 85 %. Mp 209-213 dec. °C (iPrOH). ^{1}H-NMR (DMSO-d_6) δ: 7.47-8.01 (m, 11H, C_6H_5 + C_6H_4 + NH_2). ^{13}C-NMR (DMSO-d_6) δ: 112.6, 124.1, 126.1, 129.2, 130.1, 130.5, 135.8, 136.3, 146.4, 158.5. MS EI: 381 (M^+).

General procedure for the synthesis of compounds 30-31: to a solution of the appropriate alcohol (0.52 mmol) in DMF (5 mL) stirred at 0 °C, $SOBr_2$ (1.5 eq) was added and the mixture was stirred at rt for 1h. The mixture was then poured into H_2O (30 mL) and extracted with EtOAc (3×10 mL). The organic layers were washed with $NaHCO_3$ (3×10 mL), brine (20 mL), dried, filtered and evaporated under reduced pressure The crude product was purified by flash chromatography. Chromatographic eluents and yields of the products were as follows.

4-Bromomethyl-3-phenylfurazan (30)

Eluent (PE/EtOAc 90/10 v/v); yield 85 %. ^{1}H-NMR ($CDCl_3$) δ: 4.67 (s, 2H, C*H*$_2$), 7.54-7.59 (m, 3H, C_6H_5), 7.84-7.86 (m, 2H, C_6H_5), . ^{13}C-NMR ($CDCl_3$) δ: 17.5, 125.4, 128.7, 129.8, 131.4, 151.1, 153.9.

4-Bromomethyl-3-phenylfuroxan (31)

Eluent (PE/EtOAc 85/15 v/v); yield 97 %. ^{1}H-NMR ($CDCl_3$) δ: 4.53 (s, 2H, C*H*$_2$), 7.59-7.46 (m, 3H, C_6H_5), 7.83-7.74 (m, 2H, C_6H_5). ^{13}C-NMR ($CDCl_3$) δ: 19.8, 114.0, 122.2, 127.8, 129.6, 131.2, 154.0.

General procedure for the synthesis of compounds 37-40: to a solution of **29** (0.69 g, 4.0 mmol) in ethanol (25 mL), 1N NaOH (2.7 mL, 0.7 eq) plus the appropriate bromo derivative (4.0 mmol) were added; the resulting mixture was then stirred at 50 °C for 6 hours. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (3×50 mL). The organic layers were dried, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography. Chromatographic eluents and yields of the products were as follows.

4-(3-Phenylfurazan-4-yl-methoxy)benzenesulfonamide (37)

Eluent (PE/EtOAc 65/35 v/v); yield: 50%; Mp 175 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 5.68 (s, 2H, -OC*H*$_2$-), 7.19 (d, 2H, C_6H_4), 7.27 (s, 2H, N*H*$_2$), 7.54-7.62 (m, 3H, C_6H_5), 7.80 (d, 2H, C_6H_4), 7.84-7.86 (m, 2H, C_6H_5). ^{13}C-NMR (DMSO-d_6) δ: 59.3, 114.9, 124.6, 127.6, 127.6, 128.2, 129.1, 131.0, 137.3, 150.1, 153.8, 159.4. MS CI: 332 ($M+H^+$).

4-(3-Phenylfuroxan-4-yl-methoxy)benzenesulfonamide (38)

Eluent (PE/EtOAc 80/20 v/v); yield: 45%; Mp 204 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 5.55 (s, 2H, -OC*H*$_2$-), 7.20 (d, 2H, C_6H_4), 7.28 (s, 2H, -N*H*$_2$), 7.57-7.62 (m, 3H, C_6H_5), 7.76 (d, 2H, C_6H_4), 7.83-7.87 (m, 2H, C_6H_5). ^{13}C-NMR (DMSO-d_6) δ: 61.2, 114.3, 115.0, 121.9, 127.8, 129.0, 130.8, 137.3, 153.7, 159.3. MS CI: 348 ($M+H^+$).

4-(3-Carbamoylfurazan-4-yl-methoxy)benzenesulfonamide (39)

Eluent (CH_2Cl_2/EtOAc 80/20 v/v); yield: 59%; Mp 183 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 5.61 (s, 2H, -OC*H*$_2$-), 7.20 (d, 2H, C_6H_4), 7.24 (s, 2H, -SO_2N*H*$_2$), 7.76 (d, 2H, C_6H_4), 8.23 (s br, 1H, -CON*H*$_2$), 8.61 (s br, 1H, -CON*H*$_2$). ^{13}C-NMR (DMSO-d_6) δ: 59.8, 114.8, 127.6, 137.0, 149.2, 152.0, 158.1, 159.7. MS CI: 299 ($M+H^+$).

4-(3-Carbamoylfuroxan-4-yl-methoxy)benzenesulfonamide (40)

Eluent (CH_2Cl_2/EtOAc 80/20 v/v); yield: 61%.;Mp. 226.5 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 5.51 (s, 2H, -OC*H*$_2$-), 7.22 (d, 2H, C_6H_4), 7.26 (s, 2H, N*H*$_2$), 7.76 (d, 2H, C_6H_4), 7.82 (s br, 1H, -CON*H*$_2$), 8.50 (s br, 1H, -CON*H*$_2$). ^{13}C-NMR (DMSO-d_6) δ: 61.3, 110.4, 114.8, 127.6, 134.4, 137.0, 154.9, 159.7. MS CI: 315 ($M+H^+$).

General procedure for the preparation of 41-44: to a solution of **29** (0.9 g, 5.2 mmol) in DMF (20 mL), Na_2CO_3 (0.55 g, 1 eq) plus a solution of bromo derivative or bisphenysulfonyl derivative (5.2 mmol) in DMF (10 mL) were added; the resulting mixture was then stirred at rt for 4 hours. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (3×50 mL). The organic layers were dried, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography Chromatographic eluents and yields of the products were as follows.

4-(3-Cyanofurazan-4-yl-methoxy) benzenesulfonamide (41)

Eluent (PE/EtOAc 65/35 v/v); yield: 43%; Mp 116 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 5.71 (s, 2H, -OC*H*$_2$-), 7.25-7.28 (m, 4H, C_6H_4 + N*H*$_2$), 7.81 (d, 2H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 59.6, 107.7, 114.8, 127.7, 133.0, 137.6, 154.0. 159.2. MS CI: 281 ($M+H^+$).

4-(3-Cyanofuroxan-4-yl-methoxy)benzenesulfonamide (42)

Eluent (PE/EtOAc 65/35 v/v); yield: 52%; Mp 149 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 5.52 (s, 2H, -OC*H*$_2$-), 7.20-7.29 (m, 4H, C_6H_4+ N*H*$_2$), 7.81 (d, 2H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 60.8, 98.1, 106.1, 114.9, 127.7, 137.6, 154.6, 159.2. MS CI: 297 ($M+H^+$).

4-(3-Phenylsulfonylfurazan-4-yloxy)benzensulfonamide (43)

Eluent (PE/EtOAc 80/20 v/v); yield: 62%; Mp 155 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 7.50 (s, 2H, N*H*$_2$), 7.63 (d, 2H, C_6H_4), 7.80 (t, 2H, C_6H_5), 7.92-7.96 (m, 3H, C_6H_5), 8.16 (d, 2H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 119.7, 128.0, 128.7, 130.2, 136.3, 136.8, 142.2, 149.6, 155.4, 160.0. MS CI: 382 ($M+H^+$).

4-(3-Phenylsulfonylfuroxan-4-yloxy)benzenesulfonamide (44)

Eluent (PE/EtOAc 65/35 v/v); yield: 29%; Mp 173 °C (EtOH /H_2O 1/1 v/v). ^{1}H-NMR (DMSO-d_6) δ: 7.48 (s, 2H, N*H*$_2$), 7.64 (d, 2H, C_6H_4), 7.76 (d, 2H, C_6H_5), 7.90-7.95 (m, 3H, C_6H_5), 8.02 (d, 2H, C_6H_4). ^{13}C-NMR (DMSO d_6) δ: 111.3, 119.9, 127.9, 128.5, 129.9, 136.2, 136.6, 142.0, 154.8, 157.7. MS CI: 398 ($M+H^+$).

4.2 CA inhibition

An Applied Photophysics stopped-flow instrument was used for assaying CA-catalysed CO_2 hydration activity.[10] Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.4) and 20 mM $NaBF_4$ (to maintain constant ionic strength), monitoring the initial rates of CA-catalyzed CO_2 hydration reaction for a period of 10-100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5-10% of the reaction were

used to determine the initial velocity. The uncatalyzed rates were determined similarly, and subtracted from the total observed rates. Stock solutions of inhibitor (10 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were prepared thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to the assay, in order to allow for formation of the E-I complex. The inhibition constants were calculated by non-linear least-squares methods using PRISM 3 and the Prusoff-Cheng equation, as reported elsewhere;[17] figures reported are means from at least three different determinations. All CAs were recombinant proteins obtained as reported earlier by these groups.[25-29]

4.3 IOP-lowering studies in hypertonic saline-induced IOP raise in rabbit

Male white New Zealand rabbits weighing 2-2.5 Kg were used in these studies. Animal were anaesthetized with Zoletil (Tiletamine chloride plus Zolazepam chloride, 3 mg/ kg b.w., i.m.) and injected with 0.1 mL hypertonic saline solution (5% in distilled water) into the vitreous of both eyes. IOP was determined using a tonometer (Tono-pen Avia Tonometer, Reichhert Inc. Depew, NY 14043, USA) prior to hypertonic saline injection (basal) and 0.5, 1.0, 1.5, 3, and 6 h thereafter. Vehicle (phosphate buffer 7.00 plus DMSO 2%) or drugs were instilled immediately after the injection of hypertonic saline. Eyes were randomly assigned to different groups. Vehicle or drug (0.50 mL) was directly instilled into the conjunctival pocket at the desired doses (usually 2 %).[17]

4.4 IOP-lowering studies in carbomer-induced IOP raise in rabbit

Adult male New Zealand Albino rabbits weighing 2-2.5 Kg were employed in this study. The animals were utilized in groups of eight for each specific treatment. The experimental procedures conformed to the tenets of the Declaration of Helsinki and the Guidelines for the Care and Use of Laboratory Animals, as adopted and promulgated by the U.S. National Institute of Health, and were conducted after authorization under the Italian regulations on the protection of animals used for experimental and other scientific purposes (DM 116/1992) as well as with the European Union Regulations (OJ of ECL 358/1, 12/12/1986); the experimental protocol was approved by the Animal Care Committee of the University of Florence (Florence, Italy). The rabbits were kept in individual cages; food and water were provided *ad libitum*. The animals were identified with a tattoo on the ear, numbered consecutively, and maintained on a 12-12h light/dark cycle in a temperature controlled room (22°-23°C). All selected animals were examined before the beginning of the study and were determined to be normal at ophthalmic and general health examinations. Glaucoma was induced by injection of 0.1 mL 0.25% carbomer (Siccafluid, FarMila - THEA Pharmaceuticals) into the anterior eye-chamber bilaterally, in rabbits anesthetized with tiletamine and zolazepam (Zoletil 100, 0.05 mg/Kg b.w.) plus xilazine (Xilor 2%, 0.05 ml/Kg b.w.) i.m., by the procedure described elsewhere.[17] IOP was measured before carbomer injection and after 1, 2 and 4 hours on the first day, then thrice daily until stabilization, and every 24 hours thereafter. All rabbits treated with carbomer presented a net increase in IOP. One drop of 0.2% oxybuprocaine hydrochloride (Novesine, Sandoz) diluted 1:1 with sterile saline was instilled in each eye immediately before each set of pressure measurements. IOP was measured using a Tono-Pen XL tonometer (Medtronic Solan, USA) as described elsewhere.[17] The pressure readings were matched with two-point standard pressure measurements at 1, 2, 4 and 8 hours after instillation of the drug, and once daily thereafter, using a Digilab calibration verifier. All IOP measurements were done by the same investigators using the same tonometer. As soon as a stable IOP increase was achieved, the animals were treated with the study drugs. The drugs' efficacy at lowering IOP was evaluated after drug administration throughout a four hour period, as follows: before and immediately after administration, then 30, 60, 90, 120, 240 and 300 minutes after drug administration. The treatment was applied in three animals for each drug, in one eye, and compared to the contralateral eye, treated with vehicle alone. A group of four non-glaucomatous rabbits was treated with the study drugs and used as control. At the end of the experiments, the animals were killed with a lethal dose of Pentothal (Abbott S.p.A., Campoverde di Aprilia, LT).

Acknowledgments

This work was financed in part by two EU projects, Metoxia and Dynano.
This work was partially supported by a grant of University of Torino to R. F.

References and notes

1. Supuran C.T. Carbonic anhydrases: catalytic and inhibition mechanisms, distribution and physiological roles. In *Carbonic Anhydrase-Its Inhibitors and Activators;* Scozzafava, A., Conway, J., Supuran, C., Eds; CRC Press: Florida, 2004; pp 1-24.
2. Supuran C. T. *Nat. Rev. Drug Discov*. **2008**, *7*, 168-181, and references therein.
3. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C.T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421-4468.
4. Neri, D.; Supuran, C.T. *Nat. Rev. Drug Discov*. **2011**, *10*, 767-777
5. Supuran, C.T. *Curr. Pharm. Design* **2010**, *16*, 3233-3245, and references therein reported.
6. Scozzafava, A.; Supuran, C.T. Glaucoma and the applications of carbonic anhydrase inhibitors, in *Carbonic Anhydrase: Mechanism, regulation, Links to Disease, and Industrial Applications*, McKenna, R.; Frost, S. Eds., Springer Verlag, Heidelberg, **2014**, pp. 349-359.
7. Chegaev, K.; Rolando, B.; Guglielmo, S.; Fruttero, R.; Gasco, A. *J. Heterocyclic Chem.* **2009**,*46*, 866-872.
8. Del Grosso, E.; Boschi, D.; Lazzarato, L.; Cena, C.; Di Stilo, A.; Fruttero, R.; Moro, S.; Gasco, A. *Chem. Biodi*vers. **2005**, *2*, 886-900.

9. Tamboli,Y.; Lazzarato, L.; Marini, E.; Guglielmo,S.; Novelli, M.; Beffy, P.; Masiello, P.; Fruttero, R.; Gasco, A. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3810-3815.
10. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561-2573.
11. Thiry, A. Dogne, J.-M.; Masereel B.; Supuran, C. *Trends Pharmacol. Sci.* **2006**, *27*, 566-573.
12. Guler O.O.; De Simone, G.; Supuran, C.T. *Curr. Med. Chem.* **2010**,*17*,1516-1526.
13. Resnikoff, S.; Pascolini, D.; Etya'ale D.; Kocur, I.; Pararajasegaram, R.; Pokharel, G.P.; Mariotti, S.P. *B. World Health Organ.* **2004**, *82*, 844-851.
14. Henderer, J.D.; Rapuano, C.J. Ocular Pharmacology. In *Goodman & Gilman's, The Pharmacological Basis of Therapeutic*, 11th ed.; Brunton, L.; Lazo, J. S.; Parker, K. L., Eds.; McGraw-Hill: New York, **2006**; pp 1707-1737.
15. Steele, R. M.; Benedini, F.; Biondi, S.; Borghi, V.; Carzaniga, L.; Impagnatiello, F.; Miglietta, D.; Chong, W. K. M.; Rajapakse, R.; Cecchi, A.; Temperini, C.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6565-6570.
16. Chiroli, V.; Batugo, M.R.; Biondi, S.; Bonfanti, A.; Brambilla, S.; Gale, D.C.; Li, L.; Miglietta, D.; Nicoli, F.; Prasanna, G.A.; Ronchetti, D.; Vernier, W.F.; Chong, W.K.M. *Bioorg. Med. Chem. Lett.* **2009**, *19, 2785-2788*
17. Fabrizi, F.; Mincione, F.; Somma, T.; Scozzafava, G.; Galassi, F.; Masini, E.; Impagnatiello, F.; Supuran, C.T. *J. Enzym. Inhib. Med. Ch.* **2012**, *27*, 138-147.
18. Boschi, D.; Di Stilo, A.; Cena, C.; Lolli, M. L.; Fruttero, R.; Gasco, A. *Pharm. Res.* **1997**, *14*, 1750-1758.
19. Calvino, R.; Mortarini, V.; Gasco, A.; Sanfilippo, A.; Ricciardi, M. L. *Eur. J. Med. Chem.* **1980**, *15*, 485-487.
20. Schirrmacher, R.; Weber, M.; Schmitz, A.; Shiue, C. Y.; Alavi, A. A.; Feilen, P.; Schneider, S.; Kann, P.; Rosch, F. *J. Labelled Compd. Rad.* **2002**, *45*, 763-774.
21. Bertinaria, M.; Galli, U.; Sorba, G.; Fruttero, R.; Gasco, A.; Brenciaglia, M. I.; Scaltrito, M. M.; Dubini, F. *Drug Dev. Res.* **2003**, *60*, 225-239.
22. Di Stilo, A.; Visentin, S.; Cena, C.; Gasco, A. M.; Ermondi, G.; Gasco, A. *J. Med. Chem.* **1998**, *41*, 5393-5401.
23. Rolando, B.; Cena, C.; Caron, G.; Marini, E.; Grosa, G.; Fruttero, R.; Gasco, A. *Med. Chem. Res.* **2002**, *11*, 322-332.
24. Farrar, W. W. *J. Chem. Soc.* **1964**, 904-906
25. Wagner, J. M.; Avvaru, B. S.; Robbins, A. H.; Scozzafava, A.; Supuran, C. T.; McKenna, R. *Bioorg. Med. Chem.*, **2010**, *18*, 4873-4878.
26. Pacchiano, F.; Aggarwal, M.; Avvaru, B. S.; Robbins, A. H.; Scozzafava, A.; McKenna, R.; Supuran, C. T. *Chem. Commun. (Camb).* **2010**, *46*, 8371-8373.
27. Köhler, K.; Hillebrecht, A.; Wischeler, J. S.; Innocenti, A.; Heine, A.; Supuran, C. T.; Klebe, G. *Angew. Chem., Int. Ed.*, **2007**, *46*, 7697-7699.
28. De Simone, G.; Vitale, R. M.; Di Fiore, A.; Pedone, C.; Scozzafava, A.; Montero, J. L.; Winum, J. Y.; Supuran, C. T. *J. Med. Chem.* **2006**, *49*, 5544-5551.
29. Biswas, S.; Aggarwal, M.; Guzel, O.; Scozzafava, A.; McKenna, R.; Supuran, C. T., *Bioorg. Med. Chem.* **2011**, *19*, 3732-3738.