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Carbonic Anhydrase Activity Modulators: Synthesis of Inhibitors and Activators Incorporating 2-substituted-thiazol-4-yl-methyl Scaffolds

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A small series of 2-[4-(4-substituted-phenylsulfonyl)-phenyl]-4-chloromethylthiazoles has been used as a scaffold for the preparation of carbonic anhydrase (CA) inhibitors and activators. For obtaining CA inhibitors, zinc-binding functions of the sulfamide and sulfamate type have been introduced into the molecules of these compounds, by reaction of the chloromethyl derivatives with sodium sulfamide/sodium sulfamate. For obtaining CA activators, the primary amino function has been introduced in these molecules by means of the Gabriel syntheses. The new sulfamide/sulfamates were effective CA II and CA IV inhibitors, but showed no inhibitory activity against isozyme I. The new amines on the other hand were much more effective CA I, II and IV activators compared to histamine, the lead compound used for their synthesis.

Keywords: Carbonic anhydrase; Thiazole; Sulfamide; Sulfamate; Amine; Histamine

INTRODUCTION

Carbonic anhydrase (CA, EC 4.2.1.1) inhibition by sulphanilamide, discovered a long time ago by Mann and Keilin,[1] was the beginning of a great scientific adventure that led to important drugs such as the antihypertensives of the benzothiadiazine and high-ceiling diuretic type,[2] the sulfonamides with CA inhibitory properties, which are not only used as antiglaucoma agents, but also as diagnostic tools and for the management of many other diseases,[3–5] some anti-thyroid drugs, the hypoglycemic sulfonamides[6] and ultimately to some novel types of anticancer agents.[7,8]

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Activation of this enzyme, although originally reported[9,10] simultaneously with the inhibition by the above mentioned sulfonamides, was a controversial issue for a long time, being retracted and re-reported several times,[11] before the first X-ray crystallographic structures of adducts of different activators with the human isozyme CA II were reported by this group.[12,13]

It is now clear that these versatile and widespread enzymes (14 different isozymes are known at present in higher vertebrates)[14] possess specific modulators for their activity, as both inhibitors and activators, and that such compounds may be important for the development of novel therapeutic agents.[4,11]

In this paper we extend our investigations to the design of both inhibitors[15–23] and activators[24–29] of CAs, choosing a heterocyclic scaffold (2-substituted-aryl-thiazol-4-yl)[30] on which grafted moieties were able to ensue affinity for the enzyme active site: sulfamide/sulfamates moieties for the design of new inhibitors, and amine moieties, for obtaining novel CA activators.

MATERIALS AND METHODS

Chemistry

Melting points were determined with a heating plate microscope and were not corrected. IR spectra were obtained in KBr pellets with a Carl Zeiss UR 20 spectrometer. ^{1}H-NMR spectra were obtained using a Varian Gemini apparatus operating at 300 MHz with d_6-DMSO as solvent. Chemical shifts are expressed as δ values (ppm) relative to Me_4Si as internal standard. Elemental analyses were done by combustion, for C, H, N, with an automated Carlo Erba analyzer and the results were found ±0.4% within the theoretical values. All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm thick precoated silica gel plates (E. Merck) eluted with MeOH:$CHCl_3$ (1:4 v/v). Derivatives **1a–c** used in the synthesis have been previously reported by Saramet *et al.*[25] Sulfamide, sulfamic acid, phthalimide and all other reagents and solvents were commercially available (from Acros or Sigma–Aldrich) and used without further purification.

General Procedure for the Preparation of Compounds 2a–c and 3a–c

Ten mM chloromethylthiazole **1a–c**[30] was suspended in 50 ml of anhydrous tetrahydrofuran (THF) and treated with the stoichiometric amount of sulfamide sodium salt (obtained from sulfamide and the stoichiometric amount of metallic sodium, in THF solvent). The reaction mixture was stirred at room temperature for 4 h, then heated at 50°C for 1 h (TLC control), the precipitated sodium chloride was filtered and the solvent evaporated in vacuum. The crude solid products **2a–c** thus obtained were recrystallized from ethanol–water (2:1, v/v). Similarly the sulfamates **3a–c** were obtained by using sodium sulfamate instead of sodium sulfamide.

General Procedure for the Preparation of Compounds 5a–c

Ten mM chloromethylthiazole **1a–c**[30] and the corresponding amount of potassium phthalimide were suspended in 50 ml of anhydrous acetonitrile. The reaction mixture was refluxed for 3 h, the precipitated KCl filtered and discarded, and after evaporation of the solvent, the crude products **4a–c** were used directly in the deprotection step. For this purpose, the crude derivatives **4a–c** were dissolved in 100 ml of ethanol and treated with the stoichiometric amount of hydrazine hydrate. The mixture was boiled for 3 h (TLC control) until all the phthalimide was converted to the free amine and phthalylhydrazide. Half of the solvent was then evaporated in vacuum, and after cooling,

the precipitated phthalylhydrazide was filtered and discarded and the ethanolic solution evaporated to dryness, to afford the crude amines **5a–c**. These derivatives were purified by preparative HPLC (^{18}C reversed-phase μ-Bondapack or Dynamax-60A (25 mm × 250 mm) columns (90% acetonitrile/8% methanol/2% water, 30 ml min^{-1}).

2-[4-(Phenylsulfonyl)-phenyl]-thiazole-4-yl-methyl-sulfamide, **2a**: as colorless crystals, mp 176–177°C; IR (KBr), cm^{-1}: 1125 and 1160 (SO_2^{sym}), 1323 and 1355 (SO_2^{as}), 3065 and 3190 (NH). ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 3.70 (s, 2H, CH_2), 7.28 (s, 1H, ArH, H-5 of thiazole), 7.56 (m, 3H, ArH, 5H of Ph), 7.99 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.07 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 7.95 (m, 2H, ArH from Ph).Found: C, 46.59; H, 3.70; N, 10.25%. $C_{16}H_{15}N_3O_4S_3$ requires: C, 46.93; H, 3.69; N, 10.26%.

2-[4-(4-Chlorophenylsulfonyl)-phenyl]-thiazole-4-yl-methyl-sulfamide, **2b**: as colorless crystals, mp 209–210°C; IR (KBr), cm^{-1}: 1125 and 1160 (SO_2^{sym}), 1320 and 1357 (SO_2^{as}), 3060 and 3190 (NH). ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 3.73 (s, 2H, CH_2), 7.27 (s, 1H, ArH, H-5 of thiazole), 7.38 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.80 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.89 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.00 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole). Found: C, 43.36; H, 3.45; N, 9.37%. $C_{16}H_{14}ClN_3O_4S_3$ requires: C, 43.29; H, 3.18; N, 9.46%.

2-[4-(4-Bromophenylsulfonyl)-phenyl]-thiazole-4-yl-methyl-sulfamide, **2c**: as colorless crystals, mp 193–194°C; IR (KBr), cm^{-1}: 1125 and 1163 (SO_2^{sym}), 1320 and 1359 (SO_2^{as}), 3065 and 3190 (NH). ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 3.73 (s, 2H, CH_2), 7.30 (s, 1H, ArH, H-5 of thiazole), 7.49 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.88 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.96 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.08 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole). Found: C, 39.13; H, 2.76; N, 8.43%. $C_{16}H_{14}BrN_3O_4S_3$ requires: C, 39.35; H, 2.89; N, 8.60%.

2-[4-(Phenylsulfonyl)-phenyl]-thiazole-4-yl-methyl-sulfamate, **3a**: as colorless crystals, mp 155–156°C; IR (KBr), cm^{-1}: 1121 and 1160 (SO_2^{sym}), 1320 and 1358 (SO_2^{as}), 3065 (NH). ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 3.74 (s, 2H, CH_2), 7.27 (s, 1H, ArH, H-5 of thiazole), 7.56 (m, 3H, ArH, 5H of Ph), 7.99 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.08 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 7.95 (m, 2H, ArH from Ph). Found: C, 46.65; H, 3.29; N, 6.72%. $C_{16}H_{14}N_2O_5S_3$ requires: C, 46.82; H, 3.44; N, 6.82%.

2-[4-(4-Chlorophenylsulfonyl)-phenyl]-thiazole-4-yl-methyl-sulfamate, **3b**: as colorless crystals, mp 179–180°C; IR (KBr), cm^{-1}: 1120 and 1160 (SO_2^{sym}), 1324 and 1360 (SO_2^{as}), 3065 (NH). ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 3.75 (s, 2H, CH_2), 7.26 (s, 1H, ArH, H-5 of thiazole), 7.38 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.80 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.87 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.01 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole). Found: C, 43.47; H, 2.70; N, 6.18%. $C_{16}H_{13}ClN_2O_5S_3$ requires: C, 43.19; H, 2.95; N, 6.30%.

2-[4-(4-Bromophenylsulfonyl)-phenyl]-thiazole-4-yl-methyl-sulfamate, **3c**: as colorless crystals, mp 180–181°C; IR (KBr), cm^{-1}: 1122 and 1160 (SO_2^{sym}), 1320 and 1361 (SO_2^{as}), 3065 (NH). ^{1}H-NMR (DMSO-d$_6$), δ, ppm: 3.74 (s, 2H, CH_2), 7.31 (s, 1H, ArH, H-5 of thiazole), 7.49 (d, 2H,

AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.88 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.95 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.09 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole). Found: C, 39.44; H, 2.89; N, 5.56%. $C_{16}H_{13}BrN_2O_5S_3$ requires: C, 39.27; H, 2.68; N, 5.72%.

2-[4-(Phenylsulfonyl)-phenyl]-4-aminomethyl-thiazole, **5a**: as colorless crystals, mp 168–169°C; IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1320 (SO_2^{as}), 3065 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.70 (s, 2H, CH_2), 7.27 (s, 1H, ArH, H-5 of thiazole), 7.56 (m, 3H, ArH, 5H of Ph), 7.99 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.08 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 7.95 (m, 2H, ArH from Ph). Found: C, 58.45; H, 3.98; N, 8.44%. $C_{16}H_{14}N_2O_2S_2$ requires: C, 58.16; H, 4.27; N, 8.48%.

2-[4-(4-Chlorophenylsulfonyl)-phenyl]-4-aminomethyl-thiazole, **5b**: as colorless crystals, mp 171–173°C; IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1320 (SO_2^{as}), 3065 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.71 (s, 2H, CH_2), 7.29 (s, 1H, ArH, H-5 of thiazole), 7.38 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.80 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.87 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.01 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole). Found: C, 52.51; H, 3.83; N, 7.55%. $C_{16}H_{13}ClN_2O_2S_2$ requires: C, 52.67; H, 3.59; N, 7.68%.

2-[4-(4-Bromophenylsulfonyl)-phenyl]-4-aminomethyl-thiazole, **5c**: as colorless crystals, mp 166–167°C; IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1320 (SO_2^{as}), 3065 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.76 (s, 2H, CH_2), 7.30 (s, 1H, ArH, H-5 of thiazole), 7.48 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.89 (d, 2H, AA′BB′, J_{AB} = 8.6 Hz, 4H, ArH, phenylene from the X–$C_6H_4SO_2$), 7.95 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole), 8.09 (d, 2H, AA′BB′, J_{AB} = 8.7 Hz, 4H, ArH, phenylene from the ring adjacent to the thiazole). Found: C, 46.87; H, 3.33; N, 6.72%. $C_{16}H_{13}BrN_2O_2S_2$ requires: C, 46.95; H, 3.20; N, 6.84%.

Biochemistry

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described by Lidskog *et al.*[26] (the two plasmids were a gift from Prof Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group,[32] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[32] Enzyme concentrations were determined spectrophotometrically at 280 nm, using 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.[34,35] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[36]

Initial rates of 4-nitrophenylacetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC.[37] 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, under the experimental conditions (pH 7.40), as reported in the literature.[37] 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. Prior to assay, the inhibitor and enzyme solutions were preincubated together for 10 min at room temperature in order to allow the formation of the E–I complex. The inhibition constant K_I was determined as described by Pocker and Stone.[32] Enzyme concentrations were 3.0 nM for hCA II, 12 nM for hCA I and 33 nM for bCA IV.

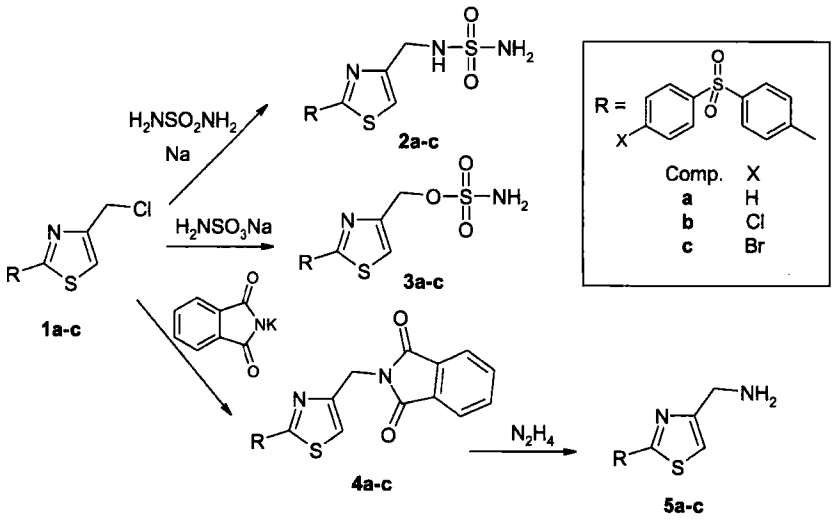
RESULTS AND DISCUSSION

Other than the classical CA inhibitors of the aromatic/heterocyclic sulfonamide type (R–SO_2NH_2), few compounds with high affinity for the enzyme, possessing different zinc-binding functions, were reported.[4,5] Among them, Maren's group[33] showed that sulfamates of the general formula R–O(CH_2)$_n$O–SO_2NH_2 (**6**) possess good CA inhibitory properties, probably coordinating to the Zn(II) ion, through the sulfamate nitrogen atom of the enzyme, similar to the aromatic sulfonamides. Our group[34] also reported recently some sulfonylated sulfamides possessing the general formula R–SO_2NHSO_2-NH_2 (**7**), which were very effective inhibitors of several isozymes, such as CA I, CA II and CA IV. Thus, such studies were important because they enlarged the classes of CA inhibitors available, from the well known aromatic/heterocyclic sulfonamides to aliphatic sulfonamides and compounds with a modified sulfonamide moiety, among which the sulfamates/sulfamides were the first examples. Furthermore, some compounds of type **6** and **7** showed affinity to CA II in the low nanomolar range, as well as good water solubility, and were formulated as eye drops.[37,38] They also showed moderate intraocular pressure (IOP) lowering in normotensive rabbits, but were not further evaluated for clinical development, probably due to their modest efficacy compared to dorzolamide, the clinically used topically effective sulfonamide.[4,5] Anyhow, such derivatives may possess different inhibition patterns against different isozymes, and this is the reason why we have investigated several other compounds related to **6** and **7**, which were obtained as outlined in Scheme 1.

The chloromethylthiazoles **1a–c** were treated with sulfamide sodium salt or sodium sulfamate in anhydrous conditions, leading to the facile replacement of the halogen atom by the zinc-binding functions of the sulfamide/sulfamate type (compounds **2a–c** and **3a–c**, respectively, Scheme 1).

Since CA activators should possess moieties able to shuttle protons between the active site and the reaction medium,[11] the primary amine group was introduced in the above mentioned 2-substituted-thiazole scaffold, by the well known Gabriel procedure involving potassium phthalimide, followed by deprotection with hydrazine (Scheme 1). The obvious lead molecule for activators **5a–c** was histamine, one of the simplest and best studied among such compounds, for which the X-ray structure (its adduct with the isozyme hCA II) is available.[11,12]

It must also be mentioned that the 2-subtituted-thiazol-4-yl-methyl scaffolds present in inhibitors/activators reported here were chosen for several reasons: (i) QSAR studies on both CA activators[40] and CA inhibitors[41–44] showed that the best biological activity was correlated with compounds possessing an elongated molecule in the direction of the axis passing through the Zn(II) ion of the enzyme, the inhibitor/activator enzyme-binding function and the long axis of the modulator molecule itself; (ii) the presence of heteroatoms in the molecule enables the inhibitor/activator able to interact with amino acid residues at the entrance (rim) of the active site cavity was shown by the authors[15–19,27,28] and by Whitesides' group,[45,46] leading to an enhanced affinity of the inhibitor (activator) for the enzyme.



SCHEME 1

The new compounds reported in this paper were assayed for their interaction with the CA isozymes I, II and IV (Tables I and II). It is seen that the sulfamide and sulfamate derivatives **2** and **3** act as efficient CA II and CA IV inhibitors, whereas they do not inhibit isozyme I, which is a rather unexpected discovery and difficult to explain at present. These compounds are slightly less effective CA II inhibitors than acetazolamide (a clinically used sulfonamide), possessing inhibition constants in the range of 16–33 nM (against 12 nM for acetazolamide). On the other hand, some derivatives **2** and **3** act as slightly better CA IV inhibitors than acetazolamide (inhibition constants in the range of 50–87 nM, against 70 nM for acetazolamide). Inhibition of both these isozymes increased with an increase in the molecular weight of the inhibitor, with the bromo-derivatives being more active than the chloro-derivatives, which in turn were more inhibitory than the parent, unsubstituted sulfamide/sulfamate **2a**/**3a** (Scheme 2).

CA activation data (Table II) showed the three amines **5a–c** to be much more effective CA I, II and IV activators compared to the standard compound histamine. Thus, activation constants

TABLE I CA inhibition data with compounds **2a–c** and **3a–c** against isozymes I, II and IV. Data for the standard inhibitor acetazolamide are also presented

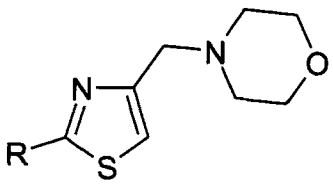
Inhibitor	K_I* (nM) hCA II†	bCA IV‡
Acetazolamide	250	12	70
2a	>1000	28	83
2b	>1000	24	65
2c	>1000	16	50
3a	>1000	33	87
3b	>1000	30	66
3c	>1000	21	54

*Mean from at least three determinations by the esterase method.[32] Standard error was in the range of 5–10%.
†Human cloned isozyme.
‡Purified from bovine lung microsomes.[31]

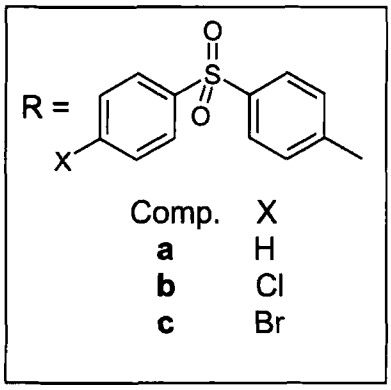
TABLE II CA isozymes I, II and IV activation with the standard activator histamine and the new derivatives **5a–c**

Number	X	K_A* (μM) hCA II†	bCA IV‡
Histamine		2	125	41
5a	H	0.35	18	5
5b	Cl	0.33	18	3
5c	Br	0.30	16	2

*K_A represents the activation constant, being defined similar to the inhibition constant, as the equilibrium constant for the process. EA↔E + A, where EA represents the enzyme–activator adduct, E the free enzyme and A the free activator in solution).[27,28] Mean from at least three determinations by the esterase method.[37] Standard error was in the range of 5–10%.
†Human cloned isozyme.
‡Purified from bovine lung microsomes.[36]



8a-c



SCHEME 2

of 0.30–0.35 μM against hCA I (compared to an activation constant of 2 μM for histamine), 16–18 μM against hCA II (125 μM for histamine), and 2–5 μM against bCA IV (compared to 41 μM for histamine), were observed. The best activation was again correlated with an increase in the molecular weight of the activator molecule, similar to the trend observed for the CA inhibition data with derivatives **2** and **3**. It must also be mentioned that some structurally related morpholine derivatives, of types **8a–c**, reported earlier,[30] were also assayed for their CA activation data, but they were completely ineffective. Thus, it is obvious that the CA activatory properties are completely abolished by substitution of the amino moiety in compounds **5**, and that the bulkier derivatives **6** do not bind within the CA active site, probably due to steric impairment.

In conclusion, we report here a new versatile scaffold that may be successfully used for the design of modulators of CA activity: both inhibitors and activators of three CA isozymes have been obtained by attaching zinc-binding functions of the sulfamide/sulfamate type or amino groups that afford the possibility to shuttle protons between the active site and the environment. This scaffold might also be very useful for the design of other enzyme inhibitors, such as metalloprotease inhibitors.[47]

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References

[1] Mann, T. and Keilin, D. (1940), *Nature* **164**, 146–147.
[2] Maren, T.H. (1976), *Annu. Rev. Pharmacol. Toxicol.* **16**, 309–325.
[3] Maren, T.H. (1967), *Physiol. Rev.* **47**, 595–782.
[4] Supuran, C.T. and Scozzafava, A. (2000), *Exp. Opin. Ther. Patents* **10**, 575–600.
[5] Supuran, C.T. and Scozzafava, A. (2001), *Curr. Med. Chem.*.
[6] Drew, J. (2000), *Science* **287**, 1960–1964.
[7] Owa, T. and Nagasu, T. (2000), *Exp. Opin. Ther. Patents* **10**, 1725–1740.
[8] Supuran, C.T., Briganti, F., Tilli, S., Chegwidden, W.R. and Scozzafava, A. (2001), *Bioorg. Med. Chem.* **9**, 703–714.
[9] Leiner, M. (1940), *Naturwiss* **28**, 316–317.
[10] Leiner, M. and Leiner, G. (1941), *Naturwiss* **29**, 195–197.
[11] Supuran, C.T. and Scozzafava, A. (2000) "Activation of carbonic anhydrase isozymes", In: Chegwidden, W.R., Carter, N. and Edwards, Y, eds, The Carbonic Anhydrases—New Horizons (Birkhäuser, Basel, Switzerland), pp 197–219.
[12] Briganti, F., Mangani, S., Orioli, P., Scozzafava, A., Vernaglione, G. and Supuran, C.T. (1997), *Biochemistry* **36**, 10384–10392.
[13] Briganti, F., Iaconi, V., Mangani, S., Orioli, P., Scozzafava, A., Vernaglione, G. and Supuran, C.T. (1998), *Inorg. Chim. Acta* **275–276**, 295–300.
[14] Hewett-Emmett, D. (2000) "Evolution and distribution of the carbonic anhydrase gene families", In: Chegwidden, W.R, Edwards, Y. and Carter, N., eds, The Carbonic Anhydrases—New Horizons (Birkhäuser, Basel, Switzerland), pp 29–78.
[15] Scozzafava, A., Menabuoni, L., Mincione, F., Briganti, F., Mincione, G. and Supuran, C.T. (1999), *J. Med. Chem.* **42**, 2641–2650.
[16] Scozzafava, A., Briganti, F., Mincione, G., Menabuoni, L., Mincione, F. and Supuran, C.T. (1999), *J. Med. Chem.* **42**, 3690–3700.
[17] Scozzafava, A., Briganti, F., Ilies, M.A. and Supuran, C.T. (2000), *J. Med. Chem.* **43**, 292–300.

[18] Borras, J., Scozzafava, A., Menabuoni, L., Mincione, F., Briganti, F., Mincione, G. and Supuran, C.T. (1999), *Bioorg. Med. Chem.* **7**, 2397–2406.
[19] Ilies, M., Supuran, C.T., Scozzafava, A., Casini, A., Mincione, F., Menabuoni, L., Caproiu, M.T., Maganu, M. and Banciu, M.D. (2000), *Bioorg. Med. Chem.* **8**, 2145–2155.
[20] Barboiu, M., Supuran, C.T., Menabuoni, L., Scozzafava, A., Mincione, F., Briganti, F. and Mincione, G. (1999), *J. Enz. Inhib.* **15**, 23–46.
[21] Scozzafava, A., Menabuoni, L., Mincione, F., Briganti, F., Mincione, G. and Supuran, C.T. (2000), *J. Med. Chem.* **43**, 4542–4551.
[22] Casini, A., Scozzafava, A., Mincione, F., Menabuoni, L., Ilies, M.A. and Supuran, C.T. (2000), *J. Med. Chem.* **43**, 4884–4892.
[23] Scozzafava, A., Menabuoni, L., Mincione, F., Mincione, G. and Supuran, C.T. (2001), *Bioorg. Med. Chem. Lett.* **11**, 575–582.
[24] Supuran, C.T. and Scozzafava, A. (2000), *J. Enz. Inhib.* **15**, 471–486.
[25] Scozzafava, A. and Supuran, C.T. (2000), *Eur. J. Pharm. Sci.* **10**, 29–41.
[26] Supuran, C.T. and Scozzafava, A. (1999), *Bioorg. Med. Chem.* **7**, 2915–2924.
[27] Scozzafava, A., Iorga, B. and Supuran, C.T. (2000), *J. Enz. Inhib.* **15**, 139–161.
[28] Scozzafava, A. and Supuran, C.T. (2000), *Eur. J. Med. Chem.* **35**, 31–39.
[29] Briganti, F., Scozzafava, A. and Supuran, C.T. (1999), *Bioorg. Med. Chem.Lett.* **9**, 2043–2048.
[30] Saramet, I., Draghici, C., Loloiu, T. and Banciu, M.D. (1999), *Rev. Roum. Chim.* **44**, 319–326.
[31] Lindskog, S., Behravan, G., Engstrand, C., Forsman, C., Jonsson, B.H., Liang, Z., Ren, X. and Xue, Y. (1991) In: Botrè, F., Gros, G. and Storey, B.T., eds, Carbonic anhydrase—From Biochemistry and Genetics to Physiology and Clinical Medicine (VCH, Weinheim), pp 1–13.
[32] Behravan, G., Jonsson, B.H. and Lindskog, S. (1990), *Eur. J. Biochem.* **190**, 351–357.
[33] Khalifah, R.G., Strader, D.J., Bryant, S.H. and Gibson, S.M. (1977), *Biochemistry* **16**, 2241–2247.
[34] Lindskog, S. and Coleman, J.E. (1964), *Proc. Natl. Acad Sci. USA* **70**, 2505–2508.
[35] Steiner, H., Jonsson, B.H. and Lindskog, S. (1975), *Eur. J. Biochem.* **59**, 253–259.
[36] Maren, T.H., Wynns, G.C. and Wistrand, P.J. (1993), *Mol. Pharmacol.* **44**, 901–906.
[37] Pocker, Y. and Stone, J.T. (1967), *Biochemistry* **6**, 668–678.
[38] Lo, Y.S., Nolan, J.C., Maren, T.H., Welstead, W.J., Gripshover, D.F. and Shamblee, D.A. (1992), *J. Med. Chem.* **35**, 4790–4794.
[39] Scozzafava, A., Banciu, M.D., Popescu, A. and Supuran, C.T. (2000), *J. Enz. Inhib.* **15**, 443–453.
[40] Clare, B.W. and Supuran, C.T. (1994), *J. Pharm. Sci.* **83**, 768–773.
[41] Supuran, C.T. and Clare, B.W. (1995), *Eur. J. Med. Chem.* **30**, 687–696.
[42] Supuran, C.T. and Clare, B.W. (1998), *Eur. J. Med. Chem.* **33**, 489–500.
[43] Supuran, C.T. and Clare, B.W. (1999), *Eur. J. Med. Chem.* **34**, 41–50.
[44] Clare, B.W. and Supuran, C.T. (1999), *Eur. J. Med. Chem.* **34**, 463–474.
[45] Boriack, P.A., Christianson, D.W., Kingery-Wood, J. and Whitesides, G.M. (1995), *J. Med. Chem.* **38**, 2286–2293.
[46] Gao, J.M., Cheng, X.H., Chen, R.D., Sigal, G.B., Bruce, J.E., Schwartz, B.L., Hofstadler, S.A., Anderson, G.A., Smith, R.D. and Whitesides, G.M. (1996), *J. Med. Chem.* **39**, 1949–1957.
[47] Supuran, C.T., Scozzafava, A. and Mastrolorenzo, A. (2001), *Exp. Opin. Ther. Patents* **11**, 221–259.