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CARBONIC ANHYDRASE INHIBITORS: INHIBITION OF ISOZYMES I, II AND IV BY SULFAMIDE AND SULFAMIC ACID DERIVATIVES*

ANDREA SCOZZAFAVA[a], MIRCEA D. BANCIU[b], ANGELA POPESCU[b] and CLAUDIU T. SUPURAN[a,†]

[a]*Università degli Studi di Firenze, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121 Firenze, Italy;*
[b]*Polytechnic University of Bucharest, Laboratory of Organic Chemistry, Polizu Str. 1, Bucharest, Roumania*

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Sulfamide and sulfamic acid are the simplest compounds containing the SO_2NH_2 moiety, responsible for binding to the Zn(II) ion within carbonic anhydrase (CA, EC 4.2.1.1) active site, and thus acting as inhibitors of the many CA isozymes presently known. Here we describe two novel classes of CA inhibitors obtained by derivatizations of the lead molecules mentioned above. The new compounds, possessing the general formula RSO_2NH-SO_2X (X = OH, NH_2), were obtained by reaction of sulfamide or sulfamic acid with alkyl/arylsulfonyl halides or arylsulfonyl isocyanates. A smaller series of derivatives has been obtained by reaction of aromatic aldehydes with sulfamide, leading to Schiff bases of the type $ArCH=NSO_2NH_2$. All the new compounds act as strong inhibitors of isozymes I, II and IV of carbonic anhydrase. Their mechanism of CA inhibition is also discussed based on electronic spectroscopic measurements on adducts with the Co(II)-substituted enzyme. These experiments led to the conclusion that the new inhibitors are directly coordinated (in a monodentate manner) to the metal ion within the enzyme active site, similarly to the classical inhibitors, the aromatic/heterocyclic sulfonamides.

Keywords: Carbonic anhydrase; Sulfamide; Sulfamic acid; Sulfonyl-sulfamide; Schiff base; Co(II)-substitution; Inhibition mechanism

* See Ref. [1].

† Corresponding author. Fax: +39-055-2757555; E-mail: cts@bio.chim.unifi.it.

INTRODUCTION

Sulfonamides are well known inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) and have been extensively investigated for their applications in the treatment of diverse diseases in clinical medicine.[1-3] The other important class of CA inhibitors is represented by metal-complexing inorganic anions,[4] such as cyanide, cyanate, thiocyanate, and hydrosulfide, which were first studied by the discoverers of this enzyme, Meldrum and Roughton.[5] Inhibitors of both types bind to the Zn(II) ion within the enzyme active site as anions, by substituting the metal-bound solvent molecule or increasing the coordination number of the metal.[4,6]

In a previous work,[4b] we showed that sulfamide $H_2NSO_2NH_2$ **A** and sulfamic acid $HOSO_2NH_2$ **B**, the simplest compounds containing the SO_2NH_2 moiety, responsible for binding to the Zn(II) ion within carbonic anhydrase active site, also act as inhibitors of the many CA isozymes presently known.[7] The two derivatives **A** and **B**, act as weak CA inhibitors (inhibition constants around 20–35 μM against hCA I, and of 80–100 μM against hCA II)[4b] but they coordinate to the metal ion in the enzyme active site, similarly to the classical inhibitors of the aromatic/heterocyclic sulfonamide type, as shown by electronic spectroscopic and ^{1}H-NMR studies on their adducts with Co(II)-substituted CA[4b] (Figure 1). Our studies also showed that in the

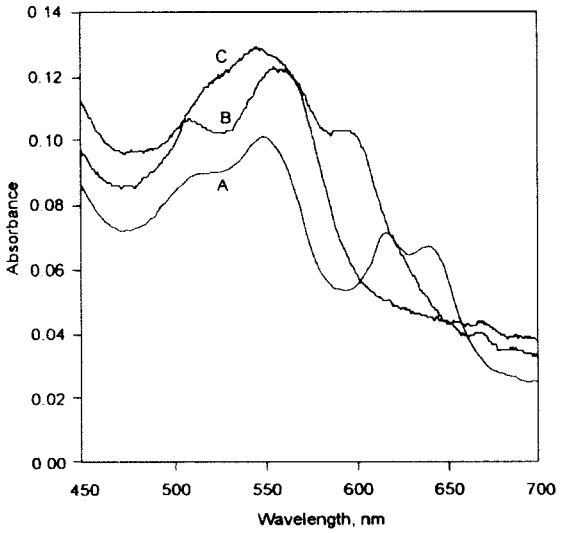
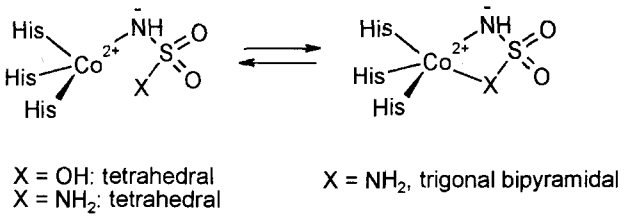


FIGURE 1 Electronic spectra of Co(II)–hCA II (spectrum A) and its adducts with sulfamide (spectrum B) and sulfamic acid (spectrum C). In all experiments [E] = 0.4 mM, pH 7.2, [sulfamide] = 2.5 mM and [sulfamic acid] = 2.1 mM.



SCHEME 1 Coordination of Sulfamide (X=NH_2) and Sulfamic acid (X=OH) with Co(II)–hCA II.

adduct of Co(II)–hCA II with sulfamide **A** equilibria between tetra- and pentacoordinated Co(II) occurred, whereas for the binding of sulfamic acid **B** the usual tetrahedral geometry of the metal ion was seen (Scheme 1).[4b] The differences between the two types of spectra (Figure 1) reflect the different geometry of the metal ion when the inhibitor is bound within the active site of the enzyme.[4]

This data prompted us to consider sulfamide **A** and sulfamic acid **B** as interesting leads for obtaining novel types of CA inhibitors. The new compounds reported here, possessing the general formula RSO_2NH-SO_2X (X = OH, NH_2), were obtained by reaction of sulfamide or sulfamic acid with alkyl/arylsulfonyl halides or arylsulfonyl isocyanates. Another series of derivatives has been obtained by reaction of aromatic aldehydes with sulfamide, leading to Schiff bases of the type $ArCH=NSO_2NH_2$. Many of the new compounds showed high affinities for several CA isozymes, such as hCA I, hCA II and bCA IV (h = human; b = bovine isozyme).

MATERIALS AND METHODS

Melting points were determined on a heating plate microscope (not corrected), IR spectra as KBr pellets on a 400–4000 cm^{-1} Perkin-Elmer 16PC FTIR spectrometer, ^{1}H-NMR spectra an a Varian Gemini 300 apparatus (chemical shifts are expressed as δ values relative to Me_4Si as standard) and elemental analysis on a Carlo Erba Instrument CHNS Elemental Analyzer, Model 1106. All reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm precoated silica gel plates (E. Merck). Sulfamide, sulfamic acid, sulfonyl halides, arylsulfonyl isocyanates and aromatic aldehydes were commercially available products from Acros, Sigma or Aldrich. 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 an anhydrous condition.

General Procedure for the Preparation of Compounds (A,B) 1–38

An amount of 100 mg (1 mM) of sulfamide **A** or sulfamic acid **B** were dissolved/suspended in 50 mL of acetonitrile or acetone, then the stoichiometric amount of a 1 N NaOH solution was added (in order to form the sulfonamide monosodium salt) followed by 1 mM of alkyl/arylsulfonyl halide dissolved in a small amount of the same solvent. The mixture was stirred at room temperature for 4–12 h (TLC control). The solvent was evaporated *in vacuo* and the reaction products recrystallized from ethanol. Yields were in the range 85–95%.

General Procedure for the Preparation of Compounds (C,D) 39–42

An amount of 100 mg (1 mM) of sulfamide **A** or sulfamic acid monosodium salt **B** were dissolved/suspended in 50 mL of anhydrous acetonitrile or acetone, and the stoichiometric amount of arylsulfonyl isocyanate was added dropwise. The mixture was stirred at room temperature for 30 min, then the solvent was evaporated and the title compounds recrystallized from ethanol. Yields were quantitative.

General Procedure for the Preparation of Compounds E1–E11

An amount of 100 mg (1 mM) of sulfamide **A** and the stoichiometric amount of aromatic/heterocyclic aldehyde were suspended in 100 mL of anhydrous ethanol and refluxed for 4–6 h. The solvent was then evaporated *in vacuo* and the obtained Schiff bases recrystallized from ethanol. Yields were in the range 45–89%.

All compounds reported here were characterized by elemental analysis, IR and ^{1}H-NMR spectroscopy. Representative data for one compound of each series is provided below.

N,N-Dimethylsulfamoyl-sulfamide, **A7** as colorless crystals, m.p. 224–5°C. IR (KBr), cm^{-1}: 1140 and 1149 (SO_2^{sym}), 1337 and 1356 (SO_2^{as}), 3060 (NH, NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.80 (s, 6H, Me_2N), 8.85 (br s, 3H, $SO_2NH + SO_2NH_2$). Found, C, 12.09; H, 4.63; N, 20.54. $C_2H_9N_3O_4S_2$ requires C, 11.82; H, 4.46; N, 20.68%.

4-Fluorophenylsulfonyl-sulfamide, **A10** as colorless crystals, m.p. 287–9°C. IR (KBr), cm^{-1}: 1150 and 1171 (SO_2^{sym}), 1354 and 1360 (SO_2^{as}), 3060 (NH, NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10–7.55 (m, AA′BB′, 4H, $J_{AB} = 7.3$ Hz, ArH, *p*-F-phenylene), 9.210 (br s, 3H, $SO_2NH + SO_2NH_2$). Found, C, 28.50; H, 2.46; N, 10.87. $C_6H_7FN_2O_4S_2$ requires: C, 28.34; H, 2.78; N, 11.02%.

4-Nitrophenylsulfonyl-sulfamic acid, **B15** as yellow crystals, m.p. 238–9°C. IR (KBr), cm^{-1}: 1150 and 1167 (SO_2^{sym}), 1340 (NO_2), 1355 and 1365 (SO_2^{as}), 1510 (NO_2), 3065 (NH), 3300 (OH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.09–7.84 (m, AA′BB′, 4H, $J_{AB} = 7.2$ Hz, ArH, *p*-O_2N-phenylene), 10.12 (br s, 1H, SO_2NH). Found, C, 25.80; H, 2.51; N, 9.76. $C_6H_6N_2O_7S_2$ requires: C, 25.52; H, 2.14; N, 9.92%.

Pentafluorophenylsulfonyl-sulfamic acid, **B22** as colorless crystals, m.p. 171–2°C. IR (KBr), cm^{-1}: 1148 and 1162 (SO_2^{sym}), 1336 and 1369 (SO_2^{as}), 3060 (NH), 3300 (OH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 10.75 (s, 1H, SO_2NH). Found, C, 22.00; H, 0.48; N, 4.18. $C_6H_2F_5NO_5S_2$ requires: C, 22.02; H, 0.62; N, 4.28%.

N-(4-Tosylamidocarbonyl)-sulfamide, **C41** as colorless crystals, m.p. 289–90°C (dec.). IR (KBr), cm^{-1}: 1125 and 1163 (SO_2^{sym}), 1290 (amide III), 1342 and 1355 (SO_2^{as}), 1540 (amide II), 1683 (amide I), 3065 and 3190 (NH), 3500 (OH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me from tosyl), 7.05–7.62 (m, AA′BB′, 4H, $J_{AB} = 7.3$ Hz, ArH, phenylene from tosyl), 7.94 (br s, 3H, $SO_2NH + SO_2NH_2$), 8.31 (s, 1H, CONH). Found: C, 32.50; H, 3.70; N, 14.25. $C_8H_{11}N_3O_5S_2$ requires: C, 32.76; H, 3.78; N, 14.33%.

N-(4-Fluorophenylsulfonylamidocarbonyl)-sulfamic acid, **D39** as colorless crystals, m.p. 263–4°C (dec.). IR (KBr), cm^{-1}: 1120 and 1155 (SO_2^{sym}), 1293 (amide III), 1344 and 1365 (SO_2^{as}), 1540 (amide II), 1681 (amide I), 3065 and 3190 (NH), 3300 (OH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.12–7.86 (m, AA′BB′, 4H, $J_{AB} = 7.4$ Hz, ArH, phenylene), 8.05 (br s, 3H, $SO_2NH + SO_2NH_2$), 8.38 (s, 1H, CONH). Found: C, 28.50; H, 2.54; N, 9.23. $C_7H_7FN_2O_6S_2$ requires: C, 28.19; H, 2.37; N, 9.39%.

(2-Thienylidene) sulfamide **E1** as pale yellow crystals m.p. 112°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1340 (SO_2^{as}), 1560, 1560–1600 (C=N). ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.45 (br s, 2H, SO_2NH_2), 7.20–8.20 (m, 3H, ArH from thiophene), 9.00 (s, 1H, CH=N). Found: C, 31.55; H, 3.12; N, 14.62. $C_5H_6N_2O_2S_2$ requires; C, 31.57; H, 3.18; N, 14.73%.

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 Lindskog *et al.*[8] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group,[9] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[10] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1} \cdot cm^{-1}$ for CA I and 54 $mM^{-1} \cdot cm^{-1}$ for CA II, respectively, based on $M_r = 28.85$ kDa for CA I, and 29.30 kDa for CA II, respectively.[11,12] CA IV was isolated from bovine lung microsomes as

described by Maren *et al.* and its concentration has been determined by titration with ethoxzolamide.[13]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC.[14] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $2 \cdot 10^{-2}$ and $1 \cdot 10^{-6}$ M, working at 25°C. A molar absorption coefficient ε of 18,400 $M^{-1} \cdot cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis under the conditions of the experiments (pH 7.40), as reported in the literature.[14] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in distilled–deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.01 nM were done thereafter with distilled–deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constant K_I was determined as described by Pocker and Stone.[14] Enzyme concentrations were 3.0 nM for hCA II, 9.8 nM for hCA I and 33 nM for bCA IV (this isozyme has a decreased esterase activity[14b] and higher concentrations had to be used for the measurements). Cobalt(II)–hCA II was prepared as described in the literature by removing zinc from the native enzyme with pyridine-2,6-dicarboxylic acid, followed by dialysis against metal-free Tris–H_2SO_4 buffer and then addition of the stoichiometric amount of Co(II) salt.[15]

RESULTS AND DISCUSSION

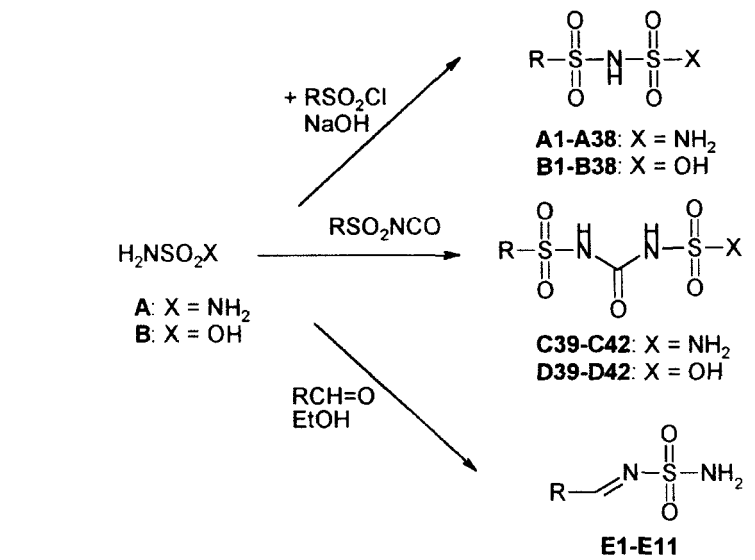
The new compounds reported here were prepared as shown in Scheme 2. Reaction of sulfamide or sulfamic acid with alkyl/arylsulfonyl halides under Schotten–Baumann conditions[16,17] afforded a large series of sulfonylated derivatives of types **A1–A38** and **B1–B38** (Table I).

Reaction of the two leads mentioned above with arylsulfonyl isocyanates[18] afforded on the other hand the new derivatives of type **C39–C42** and **D39–D42** (Scheme 2 and Table I). Finally, condensation of sulfamide with aromatic/heterocyclic aldehydes afforded the Schiff bases[19] of type **E1–E11** – Scheme 2 and Table II. The corresponding sulfamic acid Schiff bases could not be obtained in sufficiently pure state and are not described here.

TABLE I CA inhibition data with the sulfamides **A1–A38**, **C39–C42** and the sulfamic acid derivatives **B1–B38**, **D39–D42**. **A1–A38**, R–$SO_2NHSO_2NH_2$; **B1–B38**, R–SO_2NHSO_2OH: **C39–C42**, R–$SO_2NHCONHSO_2NH_2$; **D39–D42**, R–$SO_2NHCONHSO_2OH$

R	*Compound*	[a]K_I (μM)			*Compound*	[a]K_I (μM)		
		hCA I	*hCA* II	*bCA* IV		*hCA* I	*hCA* II	*bCA* IV
Sulfamide **A**		35	82	110		—	—	—
Sulfamic acid **B**		—	—	—		21	97	125
Me	**A1**	23	34	95	**B1**	20	54	106
n-Pr	**A2**	20	33	78	**B2**	18	52	97
CF_3	**A3**	11	21	50	**B3**	10	23	76
CCl_3	**A4**	12	22	51	**B4**	11	21	71
n-C_4F_9–	**A5**	3	12	42	**B5**	2	11	45
n-C_8F_{17}	**A6**	1.8	7	35	**B6**	1.1	8	36
Me_2N–	**A7**	33	76	75	**B7**	18	62	77
C_6H_5–	**A8**	16	26	64	**B8**	17	41	70
$PhCH_2$–	**A9**	15	25	53	**B9**	15	37	54
4-F–C_6H_4–	**A10**	12	13	40	**B10**	14	33	36
4-Cl–C_6H_4–	**A11**	11	12	36	**B11**	12	30	45
4-Br–C_6H_4–	**A12**	10	11	35	**B12**	12	24	41
4-I–C_6H_4–	**A13**	9	11	34	**B13**	10	20	30
4-CH_3–C_6H_4–	**A14**	13	14	35	**B13**	12	22	45
4-O_2N–C_6H_4–	**A15**	0.1	0.4	3	**B15**	1.4	1.5	6
3-O_2N–C_6H_4–	**A16**	0.2	0.5	4	**B16**	1.6	1.9	5
2-O_2N–C_6H_4–	**A17**	0.9	1.2	4	**B17**	1.7	2.0	6
3-Cl–4–O_2N–C_6H_3–	**A18**	0.09	0.3	2	**B18**	1.1	2.0	3
4-AcNH–C_6H_4–	**A19**	0.07	0.2	1.5	**B19**	1.0	1.1	2
4-BocNH–C_6H_4–	**A20**	0.05	0.1	1.2	**B20**	0.9	1.2	2
3-BocNH–C_6H_4–	**A21**	0.04	0.1	1.1	**B21**	0.8	1.0	1.6
C_6F_5–	**A22**	0.003	0.01	0.8	**B22**	0.05	1.2	1.8
3-CF_3–C_6H_4	**A23**	0.06	0.2	1.5	**B23**	0.9	1.6	2.6
2,5-$Cl_2C_6H_3$	**A24**	0.01	0.1	0.9	**B24**	0.9	1.5	3.2
4-CH_3O–C_6H_4–	**A25**	3	12	21	**B25**	4	17	22
2,4,6-$(CH_3)_3$–C_6H_2–	**A26**	0.8	2.1	5	**B26**	1.2	1.7	3.9
2,4,6-(*i*-Pr)$_3$–C_6H_2–	**A27**	0.4	1.5	3	**B27**	1.0	1.2	3.5
4-MeO-3-BocNH–C_6H_3–	**A28**	0.03	0.1	1.2	**B28**	0.7	0.8	2.4
2-HO–3,5-Cl_2–C_6H_2–	**A29**	0.3	0.5	2.1	**B29**	0.8	0.9	2.1
3-HOOC–C_6H_4–	**A30**	0.006	0.01	0.7	**B30**	0.07	0.8	1.0
4-HOOC–C_6H_4–	**A31**	0.008	0.01	0.5	**B31**	0.06	0.7	0.9
4-Ac–C_6H_4–	**A32**	1.8	3.6	11	**B32**	2.5	3.6	10
1-Naphthyl	**A33**	1.5	3.2	9	**B33**	3	8	11
2-Naphthyl	**A34**	1.2	3.4	9	**B34**	4	9	10
5-Me_2N-1-naphthyl–	**A35**	2.0	4.1	8	**B35**	3	10	12
2-Thienyl	**A36**	2.4	3.6	7	**B33**	4	6	11
Quinoline-8-yl	**A37**	2.0	3.5	8	**B37**	5	7	10
Camphor-10-sulfonyl	**A38**	2.2	3.3	6	**B38**	5	7	9
4-F–C_6H_4–	**C39**	0.006	0.02	0.3	**D39**	0.08	0.9	1.2
4-Cl–C_6H_4–	**C40**	0.004	0.02	0.4	**D40**	0.05	0.8	1.1
4-CH_3–C_6H_4–	**C41**	0.010	0.03	0.6	**D41**	0.06	1.1	1.4
2-CH_3–C_6H_4–	**C42**	0.008	0.03	0.3	**D42**	0.05	0.7	0.9

[a]K_I values were obtained from Dixon plots using a linear regression program from at least three different assays. hCA I and hCA II are human (cloned) isozymes whereas bCA IV was isolated from bovine lung microsomes.



SCHEME 2 Synthesis of Schiff bases **E1–E11.**

TABLE II CA inhibition data for the sulfamide Schiff bases **E1–E11** ($R-CH=N-SO_2NH_2$) against isozymes I, II and IV

Comp. **E**	*R*	K_I (μM)*		
		hCA I[a]	*hCA* II[b]	*bCA* IV[b]
1	thien-2-yl	8	0.05	12
2	5-Me–thien-2-yl	9	0.04	11
3	N-Me–pyrrol-2-yl	10	0.07	17
4	Ph	20	0.06	13
5	4-Me–C_6H_4–	21	0.05	12
6	4-MeO–C_6H_4–	13	0.04	14
7	3,4,5-$(MeO)_3$–C_6H_2–	9	0.02	10
8	4-Me_2N–C_6H_4–	8	0.02	8
9	4-AcNH–C_6H_4–	7	0.03	11
10	4-O_2N–C_6H_4–	18	1.5	12
11	3-O_2N–C_6H_4–	12	1.4	10

*K_I values were obtained from Dixon plots using a linear regression program from at least three different assays. [a]Human (cloned) isozyme. [b]Isolated from bovine lung microsomes.

Inhibition data against three CA isozymes (hCA I, hCA II and bCA IV) for the new compounds reported here are shown in Tables I and II. From these data it can be seen that derivatization of the two lead molecules (which both act as weak CA inhibitors) by means of sulfonylation or conversion to the Schiff bases, increases inhibitory properties for the new derivatives. The

following observations were made regarding the inhibitory properties of the obtained compounds: (i) the aliphatic derivatives of types **A1–A7** and **B1–B7** behaved as moderately weak inhibitors, with the sulfamic acids slightly better than the corresponding sulfamides. An interesting finding was the fact that hCA I was the most prone of the three isozymes to be inhibited by these compounds (generally it is hCA II a sulfonamide-avid isozyme, possessing a 10–100 times higher affinity for aromatic/heterocyclic sulfonamide inhibitors as compared to hCA I),[1–4] (ii) equally moderate inhibitors which are aromatic derivatives, containing moieties such as phenyl, halogenosubstituted-phenyl, etc., such as **A8–A13** and **B8–B13** among others. Here, the sulfamides are more inhibitory than the corresponding sulfamic acids, and again hCA I is very sensitive to this type of compound, (iii) another group of compounds containing moieties such as nitrophenyl (**A15–A18** and **B15–B18**), acetamido-phenyl and *tert*-butyloxycarbonylamido-phenyl (**A19–A21**, **A28** and **B19–B21**, **B28**) as well as 3- and 4-carboxyphenyl (**A30**, **A31** and **B30**, **B31**) behave as very efficient CA inhibitors, with inhibition constants in the 10^{-8} M range for hCA I and 10^{-7} M against hCA II. Again the sulfamides are more effective than the corresponding sulfamic acids, (iv) inhibitors such as **(A,B) 25**, **A32–A38** and **B32–B38**, as well as the Schiff bases **E1–E11**, behave as moderately weak to effective inhibitors against all three isozymes. A special feature of the Schiff bases is that they are the only compounds reported here possessing a much higher hCA II affinity as compared to hCA I (similarly to the classical aromatic/heterocyclic sulfonamides),[1–4] (v) very effective inhibitors (in the 3–10 nM range against hCA I and around 10–30 nM against hCA II) are the pentafluorophenylsulfonyl derivatives **A22** (and slightly less potent is the corresponding sulfamic acid **B22**) and the urea derivatives **C39–C42** and **D39–D42**, obtained via the arylsulfonyl isocyanates. In this small subseries, the best inhibitors were those containing halogen atoms (fluorine and chlorine in the *para* position) but the number of cases studied is too limited to afford more insight into the SAR, (vi) for the Schiff bases **E1–E11**, best substitution patterns included the presence of heterocyclic, 3,4,5-trimethoxyphenyl, 4-dimethylamino-phenyl or 4-acetamidophenyl moieties among others.

In order to assess the inhibition mechanism with the new types of inhibitors reported here, the electronic spectra of Co(II)–hCA II and its adducts with different compounds synthesized in this work were obtained. The electronic spectral data (Table III) indicate that sulfamic acid **B** and the majority of its derivatives **B1–B42** bind to the Co(II) ion in the enzyme active site giving rise to a pseudotetrahedral geometry, similarly to the unsubstituted sulfonamides of the aromatic/heterocyclic type.[4] Such adducts are

TABLE III Spectral data (in the range 400–750 nm) for adducts of Co(II) hCA II with inhibitors reported here, as well as acetazolamide and thiocyanate (for comparison). Enzyme concentrations were in the range 0.1–0.4 mM, at the pH values specified in each case. Inhibitors concentrations were in the range of 0.1–2 mM

Adduct	*pH*	*Band position* (nm) *and molar absorptivity* ($M^{-1} \cdot cm^{-1}$)
Pure enzyme[a]	6.0	520 (180); 550 (250); 616.5 (135); 640 (100)
Pure enzyme[a]	7.2	520 (280); 550 (380); 616.5 (280); 640 (260)
Acetazolamide[a]	7.2	518 (390); 549 (220); 574 (530); 595 sh (500)
Thiocyanate[b]	8.0	465 (100); 529 sh (90); 571 (100); 689 (9)
A (sulfamide)	7.2	518 (210); 550 (270); 600 (215)
B (sulfamic acid)	7.2	545 (300); 550 (330); 600 sh (240)
A15	7.2	518 (320); 550 (210); 574 (380); 600 sh (380)
B15	8.0	517 (310); 554 (210); 600 sh (325)
A18	7.2	515 (325); 549 (270); 596 (320)
B18	8.0	515 (230); 552 (215); 574 (230); 598 (240)
A22	7.2	517 (355); 550 (375); 568 (360); 598 (340)
B22	7.2	518 (375); 551 (370); 600 (250)
C40	7.2	519 (310); 550 (380); 600 (245)
D40	7.2	518 (300); 549 (360); 600 (220)
E1	7.2	518 (350); 550 (230); 600 sh (235)
E7	7.2	515 (360); 548 (210); 600 sh (230)

[a]Tetrahedral Co(II); [b]Trigonal bipyramidal Co(II).[4]

characterized by intense spectra with molar absorbances above $300\,M^{-1} \cdot cm^{-1}$,[4] and the pseudotetrahedral geometry has been confirmed by X-ray crystallographic data for some of these complexes.[20,21] Probably the binding to the metal ion occurs as the $H_2N-SO_3^-$ anion for **A** and the $ArSO_2N^-SO_3H/ArSO_2NHSO_3^-$ anions for **B1–B42**. Sulfamide **A** possesses an electronic spectra which is indicative of the existence of equilibria between tetra- and pentacoordinated species (Scheme 1).[4] But in contrast to the lead molecule **A**, its derivatives **A1–A42** bind to the Co(II) ion in the normal tetrahedral geometry, monodentately, probably as anions of the type $ArSO_2N^-SO_2NH_2$. The Schiff bases **E1–E11** also probably bind in deprotonated form, monodentately, to the metal ion within the CA active site (Table III).

In conclusion, we report here two novel classes of CA inhibitors, obtained from sulfamide and sulfamic acid as lead molecules. Some of these derivatives show an unexpectedly higher affinity for hCA I as compared to hCA II, and might thus lead after their optimization to isozyme-specific enzyme inhibitors.

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[1] This paper is part 85 of the series. Preceding part: A. Scozzafava, F. Briganti, M.A. Ilies and C.T. Supuran (1999) *J. Enz. Inhib.* (in press).

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