J. Enzyme Inhibition, 1997, Vol. 12, pp. 37–51
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NOVEL AROMATIC/HETEROCYCLIC SULFONAMIDES AND THEIR METAL COMPLEXES AS INHIBITORS OF CARBONIC ANHYDRASE ISOZYMES I, II AND IV#

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(Received 25 October 1996)

Reaction of five aromatic/heterocyclic sulfonamides containing free amino, groups with 5-nitro-α-2-toluenesultone and diethyl pyrocarbonate, respectively, afforded novel inhibitors of the zinc enzyme carbonic anhydrase (CA). Zn(II) complexes of the new sulfonamides were prepared. Excellent inhibition of three CA isozymes (CA I, II and IV respectively) were observed with some of the new sulfonamides, but especially with their Zn(II) complexes. Structure-activity correlations in this series of inhibitors are discussed.

Keywords: Aromatic/heterocyclic sulfonamide; carbonic anhydrase; isozyme I, II, IV.

INTRODUCTION

Inhibition of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) with sulfonamides[2,3] has important clinical applications in the treatment or prevention of many diseases, such as glaucoma[4], gastro-duodenal ulcers[5], acid-base disequilibria[6], epilepsy[7] and some other minor neurological disorders[8] among others.

Clinically used inhibitors such as acetazolamide **1**, methazolamide **2**, benzolamide **3**, dichlorophenamide **4** or ethoxzolamide **5** have had a firm place in medicine for more than 40 years[2,3,8,9], whereas dorzolamide **6** is a representative of the last generation CA inhibitors, recently introduced into therapy as topical antiglaucoma

See reference[1].

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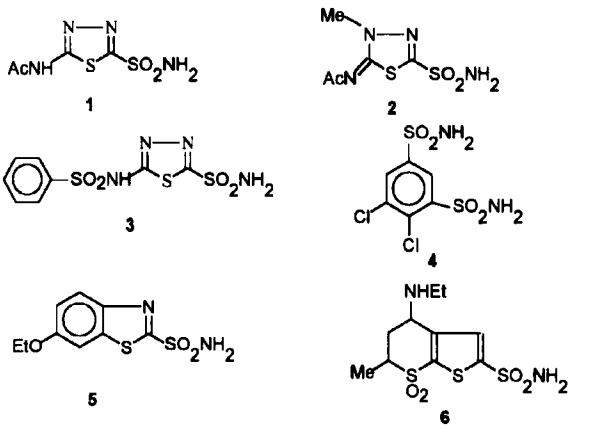


agents with great success.[5] Currently novel sulfonamides with diverse chemical structures are being synthesized and assayed as CA inhibitors[10,11], for two main reasons: (i) no compound presently used clinically showed selectivity for any of the nine CA isozymes described in higher vertebrates, including humans[12], and (ii) so far, except for the topical inhibitors used in the treatment of glaucoma mentioned above, no other compound showed specificity for organs or tissues in which these enzymes are present, although much synthetic and pharmacologic work has been done in order to develop inhibitors for use as selective cerebrovasodilators[13] or antiulcer agents.[14] Compounds which would satisfy conditions (i) and/or (ii) mentioned above would lead to drugs devoid of untoward side effects due to CA inhibition in tissues other than the target ones.

Recently a novel class of CA inhibitors was reported, i.e., the metal complexes of heterocyclic/aromatic sulfonamides[15–17] which generally show an increased inhibitory efficiency when compared to the parent sulfonamide from which they were prepared.[18] This is probably due to their involvement in two steps of the catalytic/inhibition mechanism of CA.[18,19] The presence of the metal ions in such inhibitors also leads to additional properties which can have pharmacological relevance: thus, Borras' group[20] reported some Cu(II) complexes of methazolamide and some of its congeners which showed promising anti-epileptic properties, whereas our group synthesized complexes of different sulfonamides **1–6** containing among others Pt(II) and Pd(II) ions, which showed good cytotoxic effects.[21]

In this paper we report the synthesis of a series of novel aromatic/heterocyclic sulfonamide CA inhibitors and their Zn(II) complexes and inhibition studies against three physiologically relevant[2,4] isozymes, CA I, II and IV. Qualitative structure-activity correlations for the new series of inhibitors are discussed.

MATERIALS AND METHODS

Melting points were determined with a heating plate microscope and are not corrected; IR spectra were obtained in KBr pellets with a Perkin-Elmer 16PC FTIR spectrometer, whereas ^{1}H-NMR spectra were determined on a Varian 300CXP apparatus in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Conductimetric measurements were done in DMF solutions, at 25°C (concentrations of 1 mM of complex) with a Fisher conductimeter. Elemental analyses were done by combustion for C,H,N with an automated Carlo Erba analyzer, and gravimetrically for the metal ions, and were $\pm 0.4\%$ of the theoretical values. Thermogravimetric measurements were done in air, at a heating rate of 10°C/min, with a Perkin Elmer 3600 thermobalance.

Synthesis

Sulfonamides used in synthesis were commercially available (from Sigma, Acros or Aldrich) except for 5-amino-1,3,4-thiadiazole-2-sulfonamide **8e** which was prepared from acetazolamide (Sigma) by deacetylation[37], and metanilamide **8b** which was prepared from 3-aminobenzene-sulfonyl fluoride hydrochloride (Acros) by treatment with excess aqueous ammonia. Both compounds were recrystallized from ethanol-water (1:1, v/v). Diethyl pyrocarbonate (ethoxyformic anhydride) **14** was from Acros. 2-Hydroxy-5-nitrotoluenesulfonic acid sultone **7** was prepared from 4-nitrophenol (Merck) as described in the literature.[26] Metal salts and solvents were analytical grade and were used without further purification.

General procedure for the preparation of compounds 9–13 (Scheme 1)

2.15 g (10 mMoles) of sultone **7** was dissolved in 30 mL of anhydrous acetonitrile and 10 mMoles of sulfonamide **8a–e** added. The reaction mixture was magnetically stirred at room temperature for 24–48 h, when the reaction was complete as shown by TLC. After removal of solvent, the reaction product was recrystallized from ethanol. Yields were in the range of 85–95%.

General procedure for the preparation of compounds 15–19 (Scheme 2)

10 mMoles of sulfonamide **8a–e** was suspended in 50 mL of anhydrous acetonitrile and 1.62 g (1.47 mL = 10 mMoles) of diethyl pyrocarbonate were added. The mixture was magnetically stirred at 4°C for 5 h and then stirred at room temperature overnight. The solvent was evaporated *in vacuo*, and the reaction product was recrystallized from ethanol. Yields were in the range of 79–94%.

General procedure for the preparation of complexes 20–29

5 mMoles of sulfonamide **9–13** or **15–19**, were dissolved in the theoretical amount of 2 N NAOH solution required for preparation of the monosulfonamidate salt. 2.5 mMoles of $ZnCl_2.2H_2O$ dissolved in 10 mL water were then added and the mixture was magnetically stirred at room temperature for 3 h. The precipitated complexes were filtered, abundantly washed with water and air dried. They were not recrystallized due to poor solubility in the usual organic solvents (except DMSO or DMF). All the complexes melted with decomposition at a temperature > 320°C.

4-(2-Hydroxy-5-nitro-α-toluenesulfonylamido)-benzenesulfonamide **9**, white crystals, m.p. 211–3°C, IR (KBr), cm^{-1}: 715, 748, 796, 810, 1030, 1140 and 1178 (SO_2^{sym}), 1330 (SO_2^{as}, 3057 (OH); 3280 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.95 (s, 2H, CH_2SO_2); 6.45 (br s, 3H, NH_2 + NH); 7.05 (m, AA′ BB′, 4H, ArH from phenylene); 7.44 (d, 1H, ArH from trisubstituted phenyl); 8.35 (d, 2H, ArH from trisubstituted phenyl). Found: C: 40.4; H, 3.1; N, 10.5. $C_{13}H_{13}N_3O_7S_2$ requires: C: 40.3; H, 3.3; N, 10.8%.

3-(2-Hydroxy-5-nitro-α-toluenesulfonylamido)-benzenesulfonamide **10**, white crystals, m.p. 202°C, IR (KBr), cm^{-1}: 632, 738, 822, 989, 1041, 1090, 1148 and 1181 (SO_2^{sym}.), 1310 (SO_2^{as}), 3057 (OH); 3280 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.99 (s, 2H, CH_2SO_2); 6.40 (br s, 3H, NH_2 + NH); 7.10–7.58 (m, 4H, ArH from 1,3-phenylene); 7.40 (d, 1H, ArH from trisubstituted phenyl); 8.38 (d, 2H, ArH from trisubstituted phenyl). Found: C: 40.3; H, 3.0; N, 10.6. $C_{13}H_{13}N_3O_7S_2$ requires: C: 40.3; H, 3.3; N, 10.8%.

4-(2-Hydroxy-5-nitro-α-toluenesulfonylamidomethyl)-benzenesulfonamide **11**, white crystals, m.p. 191–2°C, IR (KBr), cm^{-1} 710, 745, 797, 810, 1034, 1152 and 1170 (SO_2^{sym}), 1320 (SO_2^{as}), 3057 (OH); 3280 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.88 (s, 2H, SO_2NHCH_2); 4.97 (s, 2H, CH_2SO_2); 5.80 (br s, 2H, NH_2); 6.84 (br s, 1H, NH); 7.05(m, AA′BB′, 4H, ArH from phenylene); 7.49 (d, 1H, ArH from trisubstituted phenyl); 8.42 (d, 2H, ArH from trisubstituted phenyl). Found: C, 41.8; H 3.9; N, 10.2. $C_{14}H_{15}N_3O_7S_2$ requires: C, 41.9; H, 3.7; N, 10.4%.

4-(2-Hydroxy-5-nitro-α-toluenesulfonylamidoethyl)-benzenesulfolnamide **12**, white crystals, m.p, 154–6°C, IR (KBr), cm^{-1}: 682, 840, 888, 932, 1031, 1092, 1155 and 1169 (SO_2^{sym}), 1325 (SO_2^{as}), 3055 (OH); 3280 and 3360 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2); 3.70 (t, 2H, βCH_2); 4.95 (s, 2H, CH_2SO_2); 5.38 (br s, 2H, NH_2); 6.69 (br s, 1H, NH); 7.05 (m, AA′BB′, 4H, ArH from phenylene); 7.44 (d, 1H, ArH from trisubstituted phenyl); 8.35 (d, 2H,

ArH from trisubstituted phenyl). Found: C, 43.0; H, 3.8; N, 10.2. $C_{15}H_{17}N_3O_7S_2$ requires: C, 43.3; H, 4.1; N, 10.1%.

5–(2–Hydroxy-5-nitro-α-toluenesulfonylamido)–1,3,4–thiadiazole-2-sulfonamide **13**, white crystals, m.p. 261–4°C (dec.), IR (KBr), cm^{-1}: 553, 651, 710, 970, 1030, 1155 and 1180 (SO_2^{sym}), 1320 (SO_2^{as}), 1487, 1530, 3055 (OH); 3280 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.98 (s, 2H, CH_2SO_2); 6.10 (br s, 2H, NH_2); 6.80 (br s, 1H, NH); 7.52 (d, 1H, ArH from trisubstituted phenyl); 8.40 (d, 2H, ArH from trisubstituted phenyl). Found: C, 27.2; H, 1.9; N, 17.3. $C_9H_9N_5O_7S_3$ requires: C, 27.3; H, 2.2; N, 17.7%.

4-(Ethoxycarbonylamido)-benzenesulfonamide **15**, white crystals, m.p. 189–90°C, IR (KBr), cm^{-1}. 654, 712, 736, 810, 822, 989, 1020, 1043, 1139 (SO_2^{sym}), 1328 (SO_2^{as}), 1700 (CO), 3168 (CONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.20 (t, 3H, CH_3CH_2); 4.83 (q, 2H, CH_3CH_2); 5.90 (br s, 3H, CONH + SO_2NH_2); 7.05 (m, AA′BB′, 4H, ArH, 1,4-phenylene). Found: C, 44.0; H, 4.8; N, 11.2 $C_9H_{12}N_2O_4S$ requires: C, 44.2; H, 4.9; N, 11.4%.

3-(Ethoxycarbonylamido)-benzenesulfonamide **16**, white crystals, m.p. 193–50°C, IR (KBr), cm^{-1}: 616, 719, 870, 995, 1021, 1047, 1134 (SO_2^{sym}), 1332 (SO_2^{as}), 1700 (CO), 3170 (CONH); 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.21 (t, 3H, CH_3CH_2; 4.85 (q, 2H, CH_3CH_2); 6.06 (br s, 3H, CONH + SO_2NH_2); 7.00–7.57 (m, 4H, ArH, 1,3-phenylene). Found: C, 44.3; H, 4.9; N, 11.0. $C_9H_{12}N_2O_4S$ requires: C, 44.2; H, 4.9; N, 11.4%.

4-(Ethoxycarbonylamidomethyl)-benzenesulfonamide **17**, white crystals, m.p. 177–9°C, IR (KBr), cm^{-1}: 630, 678, 820, 888, 931, 983, 1020, 1035, 1154 (SO_2^{sym}), 1327 (SO_2^{as}), 1700 (CO), 3170 (CONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.20 (t, 3H, CH_3CH_2); 4.83 (q, 2H, CH_3CH_2); 4.90 (s, 2H, $CONHCH_2$); 5.97 (br s, 3H, CONH + SO_2NH_2); 7.08 (m, AA′BB′, 4H, ArH). Found: C, 46.5; H, 5.8; N, 11.0. $C_{10}H_{14}N_2O_4S$ requires: C, 46.5; H, 5.4; N, 10.8%.

4-(Ethoxycarbonylamidoethyl)-benzenesulfonaimide **18**, white crystals, m.p. 176–8°C, IR (KBr), cm^{-1}: 655, 946, 1030, 1050, 1080, 1139 (SO_2^{sym}, 1340 (SO_2^{as}), 1700 (CO), 3170 (CONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2 from the CH_2CH_2). 3.20(t, 3H, CH_3CH_2); 3.90 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 4.85 (q, 2H, CH_3CH_2); 4.90 (s, 2H, $CONHCH_2$); 6.15 (br s, 3H, CONH + SO_2NH_2); 7.06 (m, AA′BB′, 4H, ArH, phenylene). Found: C, 48.2; H, 5.8; N, 10.2. $C_{11}H_{16}N_2O_4S$ requires: C, 48.5; H, 5.8; N, 10.3%.

5-(Ethoxycarbonylamido)-1,3,4-thiadiazole-2-sulfonamide **19**, white crystals, m.p. 209–10°C, IR (KBr), cm^{-1}: 559, 617, 710, 930, 981, 1030, 1180 (SO_2^{sym}),

1320 (SO_2^{as}), 1485, 1550, 3055 (OH); 1700 (CO), 3160 (CONH); 3370 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.20 (t, 3H, CH_3CH$_2$); 4.80 (q, 2H, CH$_3$CH_2); 6.10 (br s, 3H, CONH + NH_2). Found: C, 24.0; H, 3.0; N, 22.2. $C_5H_8N_4O_4S_2$ requires: C, 23.8; H, 3.1; N, 22.2%.

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/HCA I and pACA/HCA II described by Forsman *et al.*[38] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group[39], and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[40] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1}.cm^{-1}$ for CA I and 54 $mM^{-1}.cm^{-1}$ for CA II, respectively, based on M_r = 28.85 kDa for CA I, and 29.3 kDa for CA II, respectively.[41,42]

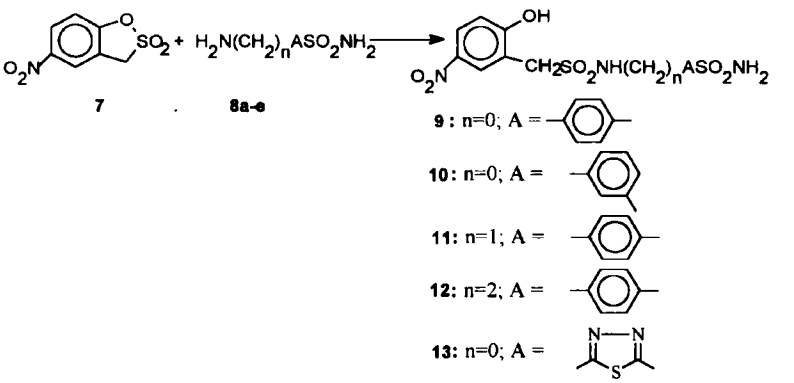
Inhibitors were assayed by Maren's micromethod[36], at 0°C, under the conditions of the E-I (enzyme-inhibitor) technique. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations)[2,3] and dilutions up to 0.1 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.[36] In a special CO_2 bubbler cell 0.3 mL of distilled water was added, followed by 0.4 mL of phenol red indicator solution (1%) and (0.1 mL of inhibitor + 0.1 mL of CA solution, preincubated as mentioned above). The CA concentrations were 1.5 nM for CA II, 210 nM for CA I and 3.5 nM for CA IV. The hydration reaction was initiated by addition of 0.1 mL of barbital buffer (pH 7.5), and the time to obtain a colour change was recorded with a stopwatch. Enzyme specific activity in the presence and in the absence of inhibitors, as well as IC_{50} values (the mean of two determinations) were determined as described by Maren. The standard error of this measurement is c. 5–10%.[3,36]

RESULTS AND DISCUSSION

One of the most important structural requirement regarding sulfonamides with CA inhibitory properties is that the SO_2NH_2 moiety present in their molecule must be unsubstituted in order to obtain potent inhibitors.[2,3,22] Thus, much of the synthetic work on preparing inhibitors was concentrated on exploring different ring systems as well as the substitution patterns that would confer strong affinity for the enzyme, together with the desired pharmacological properties such as the

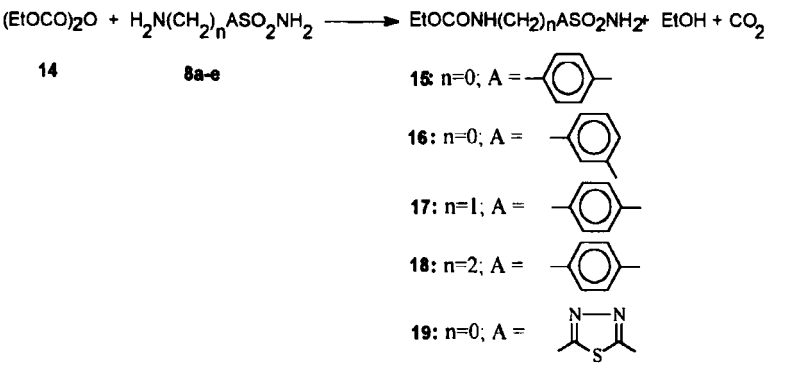
lack of toxicity and side effects.[2,8,22] Among the many compounds synthesized, the 1,3,4-thiadiazole-2-sulfonamide derivatives[23,24] (such as **1–3**) and the simple benzene-sulfonamide derivatives[24] led to strong and non-toxic inhibitors.

The clinical success of acetazolamide and methazolamide prompted intense research on these ring systems and a large number of 5-acylamido- and 5-arylsulfonylamido- derivatives of 1,3,4-thiadiazole-2-sulfonamide and 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide were prepared and assayed as CA inhibitors.[24,25] All approaches for preparing such inhibitors used the acylation/arylsulfonylation of 5-amino-1,3,4-thiadiazole-2-sulfonamide (or the corresponding imino-thiadiazoline) with acyl/aryl sulfonyl halides.[24,25] Few variations on these theme were done, and this is one of the reasons why we used the reaction of 2-hydroxy-5-nitrotoluenesulfonic acid sultone **7** with amino-containing aromatic/heterocyclic sulfonamides **8a–e** (Scheme 1). It is interesting to note that sultone **7** is a non-physiological substrate of CA, which hydrolyzes it to the corresponding sulfonic acid.[26] On the other hand, although many acylated/arylsulfonylated derivatives of sulfanilamide **8a** were reported in the search of antibacterial sulfonamides[27], such compounds have been rarely tested as CA inhibitors.[2] Recently the interest in aromatic sulfonamides was revived after the report by the Whitesides group[28] of the high potency of inhibitors towards CA I.



Scheme 1

Another approach by which novel inhibitors were prepared in the present work, consisted of the reaction of the amino-sulfonamides **8a–e** with diethyl pyrocarbonate **14** (Scheme 2). Although several alkyloxycarbonylamido derivatives of 5-amino-1,3,4-thiadiazole-2-sulfonamide have been reported[24,25], no systematic study was done regarding the reaction of anhydrides of type **14** with sulfonamides, and the previously reported compounds have not been characterized.[24,25]



Scheme 2

The main reason why inhibitors of type **9–13** and **15–19** were prepared was that the presence of side chains containing phenolic OH, or EtOCONH moieties, should lead to compounds with different solubilities and acid-base properties, and possibly a different affinity for CAs. We hypothesized that such inhibitors would have a greater affinity for certain CA isozymes when compared to simple sulfonamides of type **8** since it has been previously observed that the most efficient inhibitors possessed moieties inducing good lipid solubility as well as

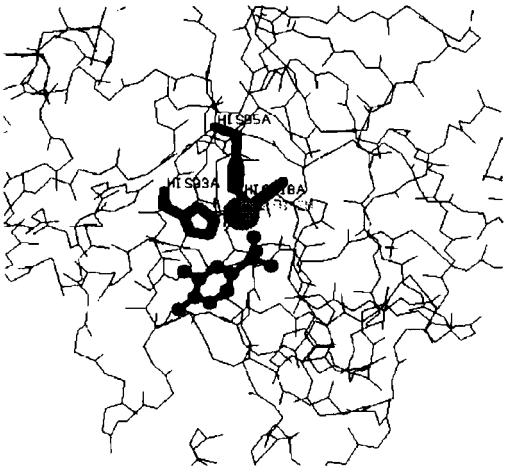


FIGURE 1 Binding of 3-acetoxymercury-4-amino-benzenesulfonamide to human CA II.[30] The three histidine ligands of the Zn(II) ion (central sphere) are evidenced together with the inhibitor molecule, which is directly coordinated to Zn(II) by means of the SO_2NH moiety. The figure was generated using X-ray crystallographic data from Brookhaven Protein Database (file code 3ca2) with the programme RasMol for Windows (version 2.6), available ***via*** Internet.

TABLE I Zn(II) complexes **20–29** prepared from the conjugate base of sulfonamides **9–13** and **15–19** and their elemental analysis data. L stands for the sulfonamide deprotonated species of ligands **9–13, 15–19**.

Comp. No.	*Ligand*	*Formula*	*Yield*	*Analysis, (calculated/found)*			
			%	%M[a]	%C[b]	%H[b]	%N[b]
20	**9**[c]	$[ZnL_2]$	76	7.8/7.5	37.2/37.5	2.8/3.1	10.0/9.7
21	**10**[c]	$[ZnL_2]$	79	7.8/7.9	37.2/37.1	2.8/3.0	10.0/9.9
22	**11**[c]	$[ZnL_2]$	86	7.5/7.0	38.8/38.9	3.2/3.1	9.7/9.3
23	**12**[c]	$[ZnL_2]$	56	7.3/7.4	40.3/40.7	3.5/3.5	9.4/9.3
24	**13**[c]	$[ZnL_2]$	95	7.6/7.3	25.3/25.2	1.8/1.7	16.4/16.0
25	**15**[d]	$[ZnL_2(H_2O)_2]$	89	11.1/11.0	36.7/36.5	4.2/3.8	9.5/9.5
26	**16**[d]	$[ZnL_2(H_2O)_2]$	68	11.1/10.8	36.7/36.5	4.2/4.1	9.5/9.3
27	**17**[d]	$[ZnL_2(H_2O)_2]$	77	10.6/10.5	39.0/39.1	4.8/4.5	9.1/8.8
28	**18**[d]	$[ZnL_2(H_2O)_2]$	91	10.1/10.0	41.0/41.1	5.2/5.5	8.7/8.5
29	**19**[d]	$[ZnL_2]$	58	11.5/11.5	21.1/20.9	2.4/2.1	19.7/19.6

[a] By gravimetry; [b] By combustion; [c] Ligand deprotonated at the SO_2NH moiety; [d] Ligand deprotonated at the SO_2NH_2 moiety.

groups that promote water solubility.[2–4,10] This has been correlated with the special architecture of the CA active site, which comprises a hydrophobic half and a highly hydrophilic opposite part, a feature unique to this enzyme.[29] A simplified representation for the binding of an aromatic sulfonamide inhibitor (3-acetoxymercuri-4-aminobenzenesulfonamide) to human CA II is shown in Figure 1.[30]

Another design aspect was that sulfonamides of the type reported here would possess a diverse donor system for complexation of metal ions, as compared to the clinically used inhibitors for which metal complexes have been reported.[17,18]

The new compounds **9–13** and **15–19** were characterized by elemental analysis, IR and ^{1}H-NMR spectroscopy. They were also used (as the corresponding monosodium salts) for the preparation of metal complexes containing Zn(II) ions. The reported complexes together with their elemental analysis data are shown in Table I.

Complexes **20–29** were also characterized by IR, ^{1}H-NMR, conductimetric and thermogravimetric (TG) data (Table II), in order to assign their structure.

The followling features were seen in the IR spectra of complexes **20–29**: (i) the shift of the sulfonamido vibrations by 20–40 cm^{-1} towards lower wavenumbers in the spectra of the complexes, as compared to the corresponding bands in the spectra of the ligands. This shift was not seen for the second symmetric SO_2 vibration – the one around 1170–1180 cm^{-1} – in complexes of the bis-sulfonamides **9–13**. Practically in this case only one symmetric vibration undergoes such a shift, as already documented in complexes of benzolamide reported by this group.[31] This

type of behaviour is well documented for other sulfonamide complexes, proving the involvement of this moiety in the interaction with the metal ions[15–20], (ii) the NH and NH_2 vibrations are greatly reduced in intensity in the spectra of complexes, as compared to the spectra of the corresponding ligands, but they are not considerably shifted to lower wavenumbers (data not shown), (iii) for complexes **20–24** the phenolic OH vibration, which for the ligands **9–13** appeared at 3055–3057 cm^{-1}, is shifted to 2980–2990 cm^{-1} (data not shown), suggesting the participation of this group in coordination of the metal ions too, and (iv) for complexes **25–29**, no shifts of the amido band from 1700 cm^{-1} are seen in the spectra of the complexes (data not shown), suggesting that this moiety does not participate in coordination.

In the ^{1}H-NMR spectra of complexes **20–29**, no major modifications are seen as compared to the corresponding spectra of the sulfonamides from which they were prepared (data not shown). Still, in the case of derivatives **20–24**, the SO_2NH_2 protons appeared as well-resolved broad singlets, whereas no SO_2NH protons were seen in these spectra (Table II). For complexes **25–29**, the signals of the SO_2NH and CONH protons are not well resolved, a fact already noted previously for other Zn(II) complexes with monodentate sulfonamido ligands interacting with the metal ions by means of this moiety.[32]

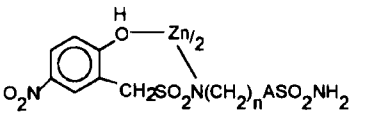
TG analysis showed the presence of two coordinated water molecules (lost between 130–175°C) in complexes **25–28**. The other compounds underwent weight losses with decomposition at much higher temperatures, obviously due to the oxidation of the organic moiety (data not shown). Finally, all the new complexes reported here (as well as the sulfonamides from which they were derived – data not shown) behave as non-electrolytes in DMSO at room temperature (Table II).

TABLE II Spectroscopic, thermogravimetric and conductimetric data for complexes **20–29**.

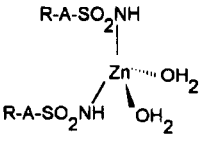
Comp.	*IR Spectra*[a], cm^{-1} $\nu(SO_2)^s$	$\nu(SO_2)^{as}$	*^{1}H-NMR Spectra*[b] SO_2NH_2, δ (ppm)	*TG analysis*[c] calc./found	*Conductimetry*[d] Λ_M ($\Omega^{-1} \times cm^2 \times mol^{-1}$)
20	1118; 1170	1296	6.40 (2H)	e	18
21	1125; 1181	1280	6.35 (2H)	e	15
22	1123; 1165	1286	5.80 (2H)	e	17
23	1126; 1165	1302	5.35 (2H)	e	17
24	1124; 1175	1294	6.10 (2H)	e	10
25	1109	1292	f	6.1/5.8[g]	15
26	1107	1306	f	6.1/5.9[g]	21
27	1127	1298	f	5.8/5.4[g]	22
28	1112	1318	f	5.6/5.5[g]	11
29	1140	1297	f	e	14

[a] In KBr; [b] In DMSO-d_6; [c] Weight loss between 130–175°C; [d] 10^{-3} M solution, in DMF, at 25°C; [e] No weight loss seen under 250°C; [f] No clear signal of the SO_2NH_2 protons evidenced; [g] Corresponding to two coordinated water molecules.

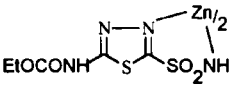
From all this data it can be concluded that sulfonamides **9–13** interact with the Zn(II) ions in a 2:1 molar ratio, in deprotonated form at the SO_2NH moiety, by means of the sulfonamido nitrogen and the phenolic oxygen, acting as bidentate ligands. Zn(II) ions are in this way in their predilect, tetrahedral geometry. Sulfonamides **15–18** on the other hand, probably act as monodentate ligands, coordinating the Zn(II) ions by means of the deprotonated SO_2NH^- group. Two water molecule are also coordinated to the metal ion, which is probably again in tetrahedral geometry. Compound **19** has a coordination chemistry similar to that of acetazolamide, acting bidentately in its Zn(II) complexes, through the sulfonamido nitrogen (deprotonated SO_2NH_2 moiety) and the endocyclic thiadiazole nitrogen N-3). A schematic representation of the structure of complexes **20–29** is shown below.



20-24



25-28



29

Inhibition data with the newly synthesized compounds as well as standard CA inhibitors against isozymes CA I, II and IV are shown in Table III.

As seen from the data of Table III, a large difference exists between the three investigated CA isozymes in their sensitivity to sulfonamide inhibitors, as described earlier by Maren's group.[33] Thus, generally CA I is a sulfonamide-resistant isozyme, being inhibited basically only by micromolar concentrations of heterocyclic sulfonamides (the most potent of such inhibitors). In contrast, CA II is the most susceptible to inhibition by aromatic/heterocyclic sulfonamides. Some representatives of this class show excellent inhibitory properties in the nanomolar range (see Table III, acetazolamide **1** and methazolamide **2**). CA IV on the other hand, an isozyme involved in many critical secretory processes[33,34], is generally 17–33 times less sensitive to the clinically used CA inhibitors.[33] Only recently we reported[1,10b] the first sulfonamide inhibitors with equal affinity for the cytosolic (CA II) and membrane bound (CA IV) isozymes.

Aromatic sulfonamides on the other hand, such as sulfanilamide **8a** and some of its derivatives **8b–d**, are much weaker inhibitors as compared to the

TABLE III Biological activity data of sulfonamide CA inhibitors and their Zn(II) complexes (IC_{50} – the mean of two different assays – represents the molarity of inhibitor producing a 50% decrease of enzyme specific activity for the CO_2 hydration reaction, by Maren's micromethod[36]).

Compound	*IC_{50} (nM)*		
	CA I[a]	*CA II*[a]	*CA IV*[b]
1 (acetazolamide)	200	7	120
2 (methazolamide)	10	9	145
8a (sulfanilamide)	2800	300	3000
8b (metanilamide)	2500	240	2200
8c (homosulfanilamide)	2500	170	2800
8d (*p*-aminoethylbenzene sulfonamide)	2100	180	2450
8e (aminothiadiazolesulfonamide)	280	30	190
9	1100	25	420
10	1000	14	360
11	640	12	180
12	800	10	140
13	9	0.8	24
15	1500	20	48
16	2800	8	120
17	240	13	150
18	230	9	90
19	200	9	85
20	580	21	350
21	640	13	100
22	490	5	65
23	360	2	53
24	15	0.5	12
25	885	19	37
26	1800	6	105
27	265	7	110
28	107	4	10
29	89	2	10

[a] Human (cloned) isozyme; [b] Isolated from bovine lung microsomes (ref.[33]).

heterocyclic compounds **1,2, 8e**, a feature accounted for by both the effect of the aromatic/heterocyclic moiety on the acidity of the sulfonamido protons, as well as on sterical effects. Presumably, heteroatoms present in the heterocyclic derivatives stabilize in a supplementary manner the enzyme-inhibitor adducts, due to electrostatic interactions/hydrogen bond networks with amino acid residues at the active site. It is interesting to note that metanilamide, a sulfonamide not previously tested for CA inhibition, is slightly more active than its isomer sulfanilamide, the first sulfonamide for which CA inhibitory properties were reported.[35]

The newly synthesized sulfonamides **9–13** and **15–19** are all more effective in inhibiting these three CA isozymes when compared to the parent sulfonamides from which they were prepared. The compounds bearing the

2-hydroxy-5-nitro-toluenesulfonylamido moieties (**9–13**) are slightly more active than the corresponding ones bearing the ethoxycarbonylamido groups (**15–19**). The Zn(II) complexes of the new sulfonamides, **20–29**, are much more active than the corresponding ligands from which they were prepared, with the notable exception of **24** which is less active than **13** in inhibiting CA I, but has a "normal" behaviour against the other two isozymes. No explanation for this phenomenon is available at the present moment. For all three isozymes, activity generally increased with sulfanilamide derivatives < homosulfanilamide < *p*-aminoethylbenzene-sulfonamide < thiadiazole sulfonamide derivatives (the most active compounds). The metanilamide derivatives were on the other hand more active than the corresponding sulfanilamides, and sometimes even more than those of the homosulfanilamides (compare **16** and **17** against CA II and CA IV for instance).

Mention should be made that a very strong CA I inhibitor was discovered i.e., compound **9**, which inhibits this isozyme better than methazolamide and has a 20 times greater affinity than acetazolamide. This compound might constitute a lead for obtaining high affinity CA I inhibitors, but unfortunately the other two isozymes are also greatly inhibited by it, so that isozyme-specificity is not attained. The best inhibition of CA IV on the other hand was obtained with some Zn(II) complexes, such as **24, 28, 29**. Although the crystal structure of this isozyme has not yet been reported, probably its active site is quite different from that of CA II, being thus able to accomodate diverse inhibitors. It should be noted that although we have previously hypothesized[15–19] that inhibition with coordination compounds is achieved after dissociation of the complex in dilute solution, and binding of both anions and cations to different binding sites on the enzyme, it seems that an additional mechanism is effective for some complexed inhibitors and some CA isozymes, i.e., direct inhibition by the undissociated complex, which binds at the entrance of the active site, presumably coordinating to a histidine cluster present in some isozymes.[17e]

In conclusion, this work describes the synthesis of novel structural types of sulfonamide CA inhibitors, of their Zn(II) complexes, as well as inhibition studies with isozymes CA I, II and IV. A very different behaviour was seen for the three isozymes towards these inhibitors, constituting a promising start for the search for isozyme-specific inhibitors targetted mainly to CA I.

Acknowledgements

This research was financed by the EU grant ERB CIPDCT 940051. Thanks are addressed to Prof. Sven Lindskog, Umea University, Sweden, for the gift of the two plasmids expressing CA I and CA II.

References

[1] This paper is part 44 of the series Carbonic anhydrase inhibitors. For Part 43 see: Supuran, C.T., Scozzafava, A., Popescu, A., Bobes-Tureac, R., Banciu, A., Creanga, A., Bobes-Tureac, G. and Banciu, M.D., *Eur. J. Med. Chem.*, in press.

[2] Supuran, C.T. (1994). In *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism*, (Puscas, I., Ed) pp. 29–111. Helicon, Timisoara.

[3] Maren, T.H. (1967). *Pharmacol. Rev.*, **47**, 595–782.

[4] Maren, T.H. (1995). *J. Glaucoma*, **4**, 49–62.

[5] Puscas, I., Supuran, C.T. (1996). In *Aparelho Digestivo*, (Coelho, J., Ed.) pp. 1704–1734. MEDSI, Rio de Janeiro.

[6] Swenson, E.R. and Maren, T.H. (1978). *Respir. Physiol.*, **35**, 129–159.

[7] Woodbury, D.M. (1980). In *Antiepileptic Drugs: Mechanisms of Action* (G.H. Glaser, J.K. Penry and D.M. Woodbury Eds.) pp. 617–633. Raven, New York.

[8] Lindskog, S. and Wistrand, P.J. (1987). In *Design of Enzyme Inhibitors as Drugs* (Sandler, M. and Smith, H.J., Eds.) pp. 698–723. Oxford University Press.

[9] Weiner, I.M. (1990). In *The Pharmacological Basis of Therapeutics* (A.G. Gilman, T.W. Rall, A.S. Nies and P. Taylor Eds.) 8th Edition, pp. 713–732. Pergamon Press, New York.

[10] (a) Supuran, C.T. and Clare, B.W. (1995). *Eur. J. Med. Chem.*, **30**, 687–696; (b) Supuran, C.T., Nicolae, A. and Popescu, A. (1996). *Eur. J. Med. Chem.*, **31**, 431–438; (c) Supuran, C.T., Popescu, A., Ilisiu, M., Costandache, A. and Banciu, M.D. (1996). *Eur. J. Med. Chem.*, **31**, 439–447; (d) Supuran, C.T., Conroy, C.W. and Maren, T.H. (1996). *Eur. J. Med. Chem.*, **31**, 843–846; (e) Briganti, F., Pierattelli, R., Scozzafava, A. and Supuran, C.T. (1996). *Eur. J. Med. Chem.*, **31**, 843–846.

[11] Boriack, P.A., Christianson, D.W., Kingery-Wood, J. and Whitesides, G.M. (1995). *J. Med. Chem.*, **38**, 2286–2291.

[12] Hewett-Emmett, D. and Tashian, R.E. (1996). *Mol. Phylogenet. Evol.*, **5**, 50–77.

[13] (a) Cross, P.E., Gadsby, B., Holland, G.F. and McLamore, W.M. (1978). *J. Med. Chem.*, **21**, 845–849; (b) Barnish, I.T., Cross, P.E., Dickinson, R.P., Gadsby, B., Parry, M.J., Randall, M.J. and Sinclair, I.W. (1980). *J. Med. Chem.*, **23**, 117–121; (c) Barnish, I.T., Cross, P.E., Dickinson, R.P., Parry, M.J. and Randall, M.J. (1981). *J. Med. Chem.*, **24**, 959–964.

[14] (a) Supuran, C.T., Manole, G., Dinculescu, A., Schiketanz, A., Gheorghiu, M.D., Puscas, I. and Balaban, A.T. (1992). *J. Pharm. Sci.*, **81**, 716–719; (b) Supuran, C.T., Balaban, A.T., Gheorghiu, M.D., Schiketanz, A., Dinculescu, A. and Puscas, I. (1990). *Rev. Roum. Chim.*, **35**, 399–405; (c) Supuran, C.T., Manole, G., Schiketanz, A., Gheorghiu, M.D. and Puscas, I. (1991). *Rev. Roum. Chim.*, **36**, 251–255; (d) Supuran, C.T., Popescu, A. and Banciu, M.D. (1992). *Rev. Roum. Chim.*, **37**, 289–297.

[15] (a) Supuran, C.T., Stefan, R., Manole, G., Puscas, I. and Andruh, M. (1991). *Rev. Roum. Chim.*, **36**, 1175–1179; (b) Supuran, C.T., Manole, G. and Andruh, M. (1993). *J. Inorg. Biochem.*, **49**, 97–103; (c) Supuran, C.T. (1992). *Rev. Roum. Chim.*, **37**, 849–855.

[16] (a) Supuran, C.T. (1993). *Rev. Roum. Chim.*, **38**, 229–236; (b) Supuran, C.T. (1993). *Roum. Chem. Quart. Rev.*, **1**, 77–116; (c) Sumalan, S.L., Casanova, J., Alzuet, G., Borras, J., Castiñeiras, A. and Supuran, C.T. (1996). *J. Inorg. Biochem.*, **62**, 31–39.

[17] (a) Supuran, C.T. and Almajan, G.L. (1995). *Main Group Met. Chem.*, **18**, 347–351; (b) Supuran, C.T. (1995). *Metal Based Drugs*, **2**, 327–330; (c) Supuran, C.T. (1995). *Metal Based Drugs*, **2**, 331–336; (d) Borras, J., Cristea, T. and Supuran, C.T. (1996). *Main Group Met. Chem.*, **19**, 339–346; (e) Borras, J., Casanova, J., Cristea, T., Gheorghe, A., Scozzafava, A., Supuran, C.T. and Tudor, V. (1996). *Metal Based Drugs*, **3**, 143–148.

[18] Alzuet, G., Ferrer, S., Borras, J. and Supuran, C.T. (1994). *Roum. Chem. Quart. Rev.*, **2**, 283–300.

[19] Luca, C., Barboiu, M. and Supuran, C.T. (1991). *Rev. Roum. Chim.*, **36**, 1169–1173.

[20] (a) Alzuet, G., Casanova, J., Ramirez, J.A., Borras, J. and Carugo, O. (1995). *J. Inorg. Biochem.*, **57**, 219–234; (b) Pedregosa, J.C., Casanova, J., Alzuet, G., Borras, J., Garcia-Granda, S., Diaz, M.R. Gutierrez-Rodriguez, A. (1995). *Inorg. Chim. Acta*, **232**, 117–124.

[21] Andruh, M., Cristurean, E., Stefan, R. and Supuran, C.T. (1991). *Rev. Roum. Chim.*, **36**, 727–732.

[22] Maren, T.H. (1987). *Drug Dev. Res.*, **10**, 255–276.

[23] (a) Roblin, R.O. and Clapp, J.W. (1950). *J. Am. Chem. Soc.*, **72**, 4890–4892; (b) Miller, W.H., Dessert, A.M. and Roblin, R.O. (1950). *J. Am. Chem. Soc.*, **72**, 4893–4896; (c) Petrow, V., Stephenson, O., Thomas, A.J. and Wild, A.M. (1958). *J. Chem. Soc.*, 1508–1513.
[24] Beasley, Y.M., Overell, B.G., Petrow, V. and Stephenson, O. (1958). *J. Pharm. Pharmacol.*, **10**, 696–705.
[25] (a) Vaughan, J.R., Eichler, J.A. and Anderson, G.W. (1956). *J. Org. Chem.*, **21**, 700–705; (b) Young, R.W., Wood, K.H., Eichler, J.A., Vaughan, J.R. and Anderson, G.W. (1956). *J. Am. Chem. Soc.*, **78**, 4649–4654.
[26] Kaiser, E.T. and Lo, K.W. (1969). *J. Am. Chem. Soc.*, **91**, 4912–4918.
[27] (a) Bauer, H. (1939). *J. Am. Chem. Soc.*, **61**, 613–616; (b) Miller, E., Rock, H.J. and Moore, M.L. (1939). *J. Am. Chem. Soc.*, **61**, 1198–1200; (c) Kollof, H.G. and Hunter, J.H. (1940). *J. Am. Chem. Soc.*, **62**, 1646–1647; (d) Sprague, J.M., McBurney, L.F. and Kissinger, L.W. (1940). *J. Am. Chem. Soc.*, **62**, 1714–1716; (e) Finkelstein, J. (1944). *J. Am. Chem. Soc.*, **66**, 407–408.
[28] Jain, A., Whitesides, G.M., Alexander, R.S. and Christianson, D.W. (1994). *J. Med. Chem.*, **37**, 2100–2105.
[29] Vidgren, J., Svensson, A. and Liljas, A. (1993). *Int. J. Biol. Macromol.*, **15**, 97–100.
[30] Chakravarty, S. and Kannan, K.K. (1994). *J. Mol. Biol.*, **243**, 298–309.
[31] Borras, J., Casanova, J., Alzuet, G. and Supuran, C.T. *Inorg. Chim. Acta*, in press.
[32] Casanova, J. (1995). Ph.D. Thesis, University of Valencia, Spain.
[33] Maren, T.H., Wynns, G.C. and Wistrand, P.J. (1993). *Mol. Pharmacol.*, **44**, 901–906.
[34] Sly, W.S., Sato, S. and Zhu, X.L. (1991). *Clin. Biochem.*, **24**, 311–318.
[35] Mann, T. and Keilin, D. (1940). *Nature (Lond.)*, **164**, 146–147.
[36] Maren, T.H. (1960). *J. Pharmacol. Exp. Ther.*, **130**, 24–29.
[37] Le Bar, D., Luthra, S.K., Pike, V.W. and Swenson, E.R. (1988). *Appl. Radiat. Isot.*, **39**, 671–675.
[38] Forsman, C., Behravan, G., Osterman, A. and Jonsson, B.H. (1988). *Acta Chem. Scand.*, **B42**, 314–318.
[39] Behravan, G., Jonasson, P., Jonsson, B.H. and Lindskog, S. (1991). *Eur. J. Biochem.*, **198**, 589–592.
[40] Khalifah, R.G., Strader, D.J., Bryant, S.H. and Gibson, S.M. (1977). *Biochemistry*, **16**, 2241–2247.
[41] Nyman, P.O. and Lindskog, S. (1964). *Biochim. Biophys. Acta*, **85**, 141–151.
[42] Henderson, L.E., Henriksson, D. and Nyman, P.O. (1976). *J. Biol. Chem.*, **251**, 5457–5463.