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CARBONIC ANHYDRASE INHIBITORS. SCHIFF BASES OF SOME AROMATIC SULFONAMIDES AND THEIR METAL COMPLEXES: TOWARDS MORE SELECTIVE INHIBITORS OF CARBONIC ANHYDRASE ISOZYME IV*

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Reaction of three aromatic sulfonamides possessing a primary amino group, i.e., sulfanilamide, homosulfanilamide and *p*-aminoethyl-benzenesulfonamide with heterocyclic and aromatic aldehydes afforded a series of Schiff bases. Metal complexes of some of these Schiff bases with divalent transition ions such as Zn(II), Cu(II), Co(II) and Ni(II) have also been obtained. The new compounds were assayed as inhibitors of three isozymes of carbonic anhydrase (CA). Several of the new compounds showed a modest selectivity for the membrane-bound (bovine) isozyme CA IV (bCA IV) as compared to the cytosolic human isozymes hCA I and II, in contrast to classical inhibitors which generally possess a 17–33 times lower affinity for bCA IV. This greater selectivity toward bCA IV is due mainly to a slightly decreased potency against hCA II relative to classical inhibitors. However, metal complexes of these Schiff bases possessed an increased affinity for hCA II, being less inhibitory against bCA IV. The first type of compounds reported here (i.e., the Schiff bases of aromatic sulfonamides with heterocyclic aldehydes) might thus lead to the development of low molecular weight isozyme specific CA IV inhibitors. The difference in affinity for the three isozymes of the inhibitors reported by us here is tentatively explained on the basis of recent X-ray crystallographic studies of these isozymes and their adducts with substrates/inhibitors.

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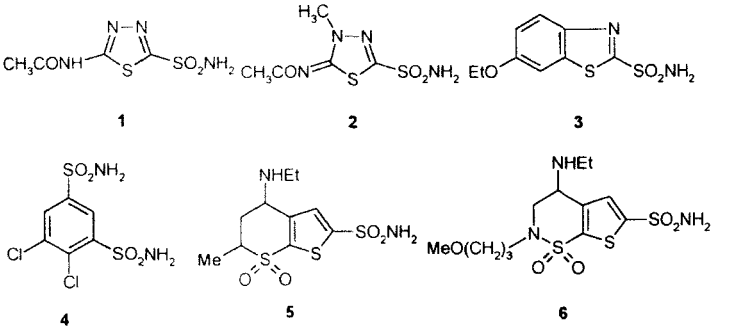
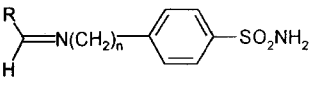
INTRODUCTION

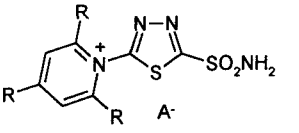
Unsubstituted sulfonamides with the general formula RSO_2NH_2 (R = aryl, heteroaryl or perhaloalkyl) are among the strongest inhibitors of the widely-spread zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1).[2–4] These inhibitors bind as sulfonamidate anions to the metal ion within the enzyme active site, sometimes with affinities in the nanomolar range.[3–6] Since CAs are involved in many physiologically important processes,[2–4] their inhibition has been exploited clinically for the management or prevention of a variety of diseases, such as: hypertension,[7,8] glaucoma,[9–11] gastric and duodenal ulcers,[12] mountain sickness,[13] etc. The main draw-back of the classical, clinically used inhibitors[7] such as acetazolamide **1**, methazolamide **2**, ethoxzolamide **3** or dichlorophenamide **4**, is related to their total lack of specificity for the different CA isozymes (at least eight different isozymes have been isolated up to now in humans and higher vertebrates)[3,4] present in high amounts in a variety of tissues or cell compartments.[14] Although small differences of affinity for sulfonamides between the different isozymes are presently known,[3,6,7,9] little progress has been achieved up to now for the design of isozyme-specific CA inhibitors. This problem is even more complicated considering the fact that just the two isozymes which seem to play the major physiological function among the CAs, i.e., CA II and CA IV, possess very similar affinities for many types of sulfonamide inhibitors (although, generally the cytosolic CA II is slightly more "sulfonamide-avid" as compared to the membrane-bound form, CA IV).[14,15] Even the topical antiglaucoma sulfonamides, recently introduced into clinical medicine, dorzolamide **5**[9] and brinzolamide **6**,[10,16] do not posses different selectivities, except that dorzolamide seems to have a very low affinity for isozyme I.[9]



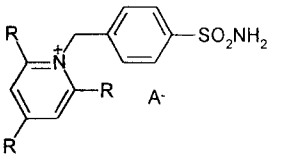
Taking into account the fact that an isozyme-specific CA inhibitor would probably act as a better drug, devoid of the side effects observed with sulfonamides of types **1–6**,[3,7–10] and might also lead to novel applications of this class of pharmacological agents (i.e. in NMR imaging or PET (positron emission tomography)[17]), a large program for designing novel classes of sulfonamides has been initiated in our laboratory.[18–20] Another interesting novel class of CA inhibitors is represented by the metal complexes of heterocyclic/aromatic sulfonamides reported by this group for the first time.[21–24]



7: R = aryl; heteroaryl

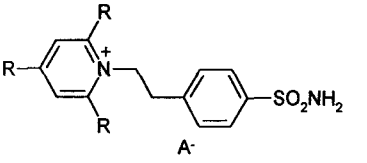


8: R = alkyl; aryl



9: R = alkyl; aryl

A = ClO_4



10: R = alkyl; aryl

Among the many sulfonamides synthesized up to now by this and other groups, the only derivatives which showed some specificity for the membrane-bound isozyme CA IV were some Schiff bases of type **7**[18] and the cationic sulfonamides of type **8–10**,[25] which being membrane-impermeant, showed a very good specificity for CA IV *in vivo* (although they appreciably inhibited the cytosolic isozymes CA I and II).[25] Practically the only derivatives possessing intrinsically an enhanced affinity for CA IV were the Schiff bases **7**, derived from aromatic sulfonamides and aromatic/heterocyclic aldehydes.[18] Thus, in this paper we extend our previous studies[18] in the synthesis and evaluation as CA inhibitors of novel Schiff bases obtained from sulfanilamide, homosulfanilamide and *p*-aminoethyl-benzenesulfonamide and aromatic/heterocyclic aldehydes. Since metal complexes of such derivatives have not been obtained previously, we also prepared a series of such derivatives containing ligands obtained from *N*-methylimidazole-2-carboxaldehyde and the previously mentioned sulfonamides, and metal ions such as Zn(II), Cu(II), Co(II) and Ni(II). The new compounds reported here were characterized by standard procedures and assayed as inhibitor of three CA

isozymes: hCA I, hCA II and bCA IV. As with the previously mentioned Schiff bases,[18] good affinities for bCA IV were evidenced for the new sulfonamides but not for their metal complexes (which act as better CA II inhibitors, as compared to the parent ligands from which they were obtained).

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 and ^{1}H-NMR spectra with a Varian 300CXP apparatus in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Electronic spectra of the metal complexes were obtained by the diffuse reflectance technique in MgO as reference, with a Perkin-Elmer Lambda 15 apparatus, in the range 200–900 cm^{-1}. Elemental analyses were done by combustion for C, H, N, with an automated Carlo Erba analyzer, or gravimetrically for the metal ions, and were $\pm 0.4\%$ of the theoretical values. Thermogravimetric (TG) measurements were done in air, at a heating rate of 10°C/min, with a Perkin-Elmer 3600 thermobalance.

Sulfonamides (sulfanilamide, homosulfanilamide and *p*-(2-aminoethyl)-benzene-sulfonamide) and aromatic/heterocyclic aldehydes used in synthesis were commercially available from Sigma, Acros or Aldrich. Solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions. Metal salts used for the preparation of the coordination compounds were analytical grade from E. Merck.

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.*[26] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[27] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[28] 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, based on $M_r =$ 28.85 kDa for CA I and 29.30 kDa for CA II.[29,30] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[15]

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.[31] 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.[31] Non-enzymic 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.[31] Enzyme concentrations were 3 nM for CA II, 12 nM for (CA I and 39 nM for CA IV (this isozyme has a decreased esterase activity and higher concentrations had to be used for the measurements).[32]

General Procedure for the Preparation of Schiff Bases 11–28

An amount of 10 mmol of sulfonamide (sulfanilamide, homosulfanilamide or *p*-(2-aminoethyl)-benzenesulfonamide) was dissolved in 40 mL of boiling methanol and the required amount (10 mmol) of aldehyde was added to the reaction mixture. Boiling was continued for 3–8 h, then a portion of the solvent was evaporated in vacuum, and on cooling crystals of Schiff bases **11–28** were obtained which were recrystallized from 96% ethanol or solvents specified in other cases. Yields were generally high (see later in the text).

Preparation of Coordination Compounds 29–36

An amount of 2 mmol of sulfonamide ligand **19** or **28** was suspended/dissolved in 15 ml of anhydrous acetonitrile under gentle reflux. When all the sulfonamide was in solution, 5 mL of an acetonitrile solution of $MCl_2 \cdot nH_2O$ (M = Zn(II), Cu(II), Co(II) or Ni(II)) was added and heating continued for an other 2 h. The precipitated complexes were filtered and air dried, whereas the acetonitrile solutions were kept in order to allow for the formation of monocrystals suitable for X-ray diffraction experiments. Unfortunately only microcrystalline powders could be obtained, which could not be used for structural determination. In fact this is the usual technical problem related to the thorough characterization of this type of metal complexes.[21–24]

N^4-(2-Pyrrolylidene) Sulfanilamide **11**

As pale yellow crystals (yield 75%), m.p. 160°C. IR (KBr), cm^{-1}: 1120 (SO_2^{sym}), 1300 (SO_2^{as}), 1540, 1590 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.20 (m, 2H, H3 and H4 of pyrrol); 6.80 (m, 1H, H5 of pyrrol); 7.60 (s, 2H, SO_2NH_2); 7.00–7.70 (d, 2H, H2 and H6 from phenylene); 8.00 (d, 2H, H3 and H5 from phenylene); 8.40 (s, 1H, C*H*=N); 8.90 (s, 1H, NH from pyrrol); ^{13}C-NMR: 109.20 (CH from pyrrolyl); 118.70 (CH); 121.39 (CH from pyrrolyl); 127.54 (CH from phenylene); 129.94 (C_q); 130.51 (CH); 140.16 (C_q); 152.71 (CH from the Schiff base moiety); 155.50 (C_q). Found: C, 53.34; H, 4.19; N, 16.77. $C_{11}H_{11}N_3O_2S$ requires; C, 53.0; H, 4.45; N, 16.86%.

N^4-(2-Thienylidene) Sulfanilamide **12**

As yellow crystals (yield 68%), m.p. 196–198°C. IR (KBr), cm^{-1}: 1130 (SO_2^{sym}), 1310 (SO_2^{as}), 1560, 1600 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10–7.60 (m, 5H, ArH from thiophene + SO_2NH_2); 7.00–7.70 (d, 2H, H2 and H6 from phenylene); 8.10 (d, 2H, H3 and H5 from phenylene); 8.90 (s, 1H, C*H*=N); ^{13}C-NMR: 113.50 (CH from thienyl); 119.90 (CH); 125.30 (CH from thienyl); 127.58 (CH from phenylene); 129.94 (C_q); 130.50 (CH); 140.16 (C_q); 156.24 (CH from the Schiff base moiety); 155.80 (C_q). Found: C, 49.55; H, 4.02; N, 10.24. $C_{11}H_{10}N_3O_2S_2$ requires; C, 49.61; H, 3.78; N, 10.52%.

N^4-(5-Methyl-2-thienylidene) Sulfanilamide **13**

As yellow crystals (yield 63%), m.p. 225–227°C. IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1310 (SO_2^{as}), 1570, 1600 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me); 7.00 (d, 2H, ArH from thiophene); 7.25–8.05 (m, 6H, arH from phenylene + SO_2N_2); 8.90 (s, 1H, C*H*=N); ^{13}C-NMR: 24.79 (Me); 113.40 (CH from thienyl); 125.30 (CH from thienyl); 127.58 (CH from phenylene); 129.94 (C_q); 130.50 (CH); 135.57 (C_q); 140.16 (C_q); 157.81 (CH from the Schiff base moiety); 155.37 (C_q). Found: C, 51.60; H, 4.40; N, 10.00. $C_{12}H_{12}N_3O_2S_2$ requires; C, 51.41; H, 4.31; N, 9.99%.

N^4-(Imidazol-2-ylidene) Sulfanilamide **14**

As white crystals (yield 43%), m.p. 220°C. IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1300 (SO_2^{as}), 1590, 1600 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.80–8.00 (m, 8H, ArH from the phenylene + imidazole + SO_2NH_2); 8.50 (s, 1H, C*H*=N); 9.10 (s, 1H, N*H* from imidazole). Found: C, 48.15; H, 3.90; N, 22.18. $C_{10}H_{10}N_4O_2S$ requires; C, 47.99; H, 4.03; N, 22.39%.

***N^4-(2,4,6-Trichloropyrimidine-5-ylidene) Sulfanilamide* 15**

As orange crystals (yield 30%), m.p. 250°C. IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1310 (SO_2^{as}), 1550, 1600 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.60 (br s, 2H, SO_2NH_2); 7.70 (d, 2H, H2 and H6 from phenylene); 8.10 (d, 2H, H3 and H5 from phenylene); 8.90 (s, 1H, C*H*=N). Found: C, 35.97; H, 2.14; N, 15.13. $C_{11}H_7Cl_3N_4O_2S$ requires; C, 36.14; H, 1.93; N, 15.32%.

***4-(2-Pyrrolylidene) Homosulfanilamide* 16**

As tan crystals (yield 60%), m.p. 167°C. IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1320 (SO_2^{as}), 1630 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.70 (s, 2H, CH_2); 6.15 (m, 2H, H3 and H4 of pyrrol); 6.50 (m, 1H, H5 of pyrrol); 6.90 (s, 2H, SO_2NH_2); 7.50 (d, 2H, H2 and H6 from phenylene); 7.85 (d, 2H, H3 and H5 from phenylene); 8.30 (s, 1H, C*H*=N); 8.90 (s, 1H, NH from pyrrol). Found: C, 55.03; H, 5.10; N, 15.79. $C_{12}H_{13}N_3O_2S$ requires; C, 54.74; H, 4.98; N, 15.96%.

***4-(N-Methyl-2-pyrrolylidene) Homosulfanilamide* 17**

As white crystals (yield 81%), m.p. 122°C. IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1315 (SO_2^{as}), 1620 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.90 (s, 3H, Me); 4.75 (s, 2H, CH_2); 6.10 (m, 2H, H3 and H4 of pyrrol); 6.50 (m, 1H, H5 of pyrrol); 6.90 (s, 2H, SO_2NH_2); 7.40 (d, 2H, H2 and H6 from phenylene); 7.80 (d, 2H, H3 and H5 from phenylene); 8.35 (s, 1H, C*H*=N). Found: C, 56.21; H, 5.57; N, 14.90. $C_{13}H_{15}N_3O_2S$ requires; C, 56.30; H, 5.45: N, 15.15%.

***4-(5-Methyl-2-thienylidene) Homosulfanilamide* 18**

As yellow crystals (yield 54%), m.p. 156°C. IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1340 (SO_2^{as}), 1580 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.35 (s, 3H, Me); 4.70 (s, 2H, CH_2); 6.86–7.20 (m, 4H, ArH from thiophene + SO_2NH_2); 7.35 (d, 2H, H2 and H6 from phenylene); 8.00 (d, 2H, H3 and H5 from phenylene); 8.50 (s, 1H, C*H*=N). Found: C, 51.78; H, 4.12; N, 9.85. $C_{12}H_{12}N_2O_2S_2$ requires; C, 51.41; H, 4.31; N, 9.99%.

***4-(2-Pyrrolylidene) Aminoethylbenzenesulfonamide* 20**

As white crystals (yield 88%), m.p. 162°C. IR (KBr), cm^{-1}: 1155 (SO_2^{sym}), 1310 (SO_2^{as}), 1630 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.00 (t, 2H, αCH_2); 3.80 (t, 2H, βCH_2); 6.20 (m, 2H, H3, H4 of pyrrol); 6.40 (m, 1H, H5 of pyrrol); 6.90 (br s, 2H, SO_2NH_2); 7.30 (d, 2H, H3 and H5 from phenylene); 7.90 (d, 2H, ArH, H2 and H6 from phenylene); 8.10 (s, 1H, C*H*=N).

Found: C, 56.25; H, 5.50; N, 15.00. $C_{13}H_{15}N_3O_2S$ requires; C, 56.30; H, 5.45; N, 15.25%.

***4-(N-Methyl-2-pyrrolylidene) Aminoethylbenzenesulfonamide* 21**

As white crystals (yield 83%), m.p. 172°C. IR (KBr), cm^{-1}: 1130 (SO_2^{sym}), 1300 (SO_2^{as}), 1610 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me); 3.00 (t, 2H, αCH_2); 3.70 (t, 2H, βCH_2); 6.05 (m, 2H, H3, H4 of pyrrol); 6.50 (m, 1H, H5 of pyrrol); 6.95 (br s, 2H, SO_2NH_2); 7.60 (d, 2H, H3 and H5 from phenylene); 7.80 (d, 2H, ArH, H2 and H6 from phenylene); 8.15 (s, 1H, C*H*=N). Found: C, 57.90; H, 5.90; N, 14.35. $C_{14}H_{17}N_3O_2S$ requires; C, 57.71; H, 5.88; N, 14.42%.

***4-(2-Thienylidene) Aminoethylbenzenesulfonamide* 22**

As yellow crystals (yield 91%), m.p. 205–207°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1325 (SO_2^{as}), 1630 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.00 (t, 2H, αCH_2); 3.80 (t, 2H, βCH_2); 7.00–7.30 (m, 5H, ArH from thiophene + SO_2NH_2); 7.40 (d, 2H, H3 and H5 from phenylene); 7.70 (d, 2H, ArH, H2 and H6 from phenylene); 8.46 (s, 1H, C*H*=N). Found: C, 52.86; H, 4.98; N, 9.40. $C_{13}H_{14}N_2O_2S_2$ requires; C, 53.04; H, 4.79; N, 9.52%.

***4-(5-Methyl-2-thienylidene) Aminoethylbenzenesulfonamide* 23**

As yellow crystals (yield 66%), m.p. 118°C. IR (KBr), cm^{-1}: 1125 (SO_2^{sym}), 1300 (SO_2^{as}), 1605 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me); 3.00 (t, 2H, αCH_2); 3.90 (t, 2H, βCH_2); 6.90 (m, 2M, ArH from thiophene); 7.20 (br s, 2H, SO_2NH_2); 7.40 (d, 2H, H3 and H5 from phenylene); 7.80 (d, 2H, ArH, H2 and H6 from phenylene); 8.30 (s, 1H, C*H*=N). Found: C, 54.68; H, 5.14; N, 8.90. $C_{14}H_{16}N_2O_2S_2$ requires; C, 54.52; H, 5.23; N, 9.08%.

***4-(4-Pyridylidene) Aminoethylbenzenesulfonamide* 24**

As pale yellow crystals (yield 67%), m.p. 155–157°C. IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1315 (SO_2^{as}), 1590 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.05 (t, 2H, αCH_2); 3.95 (t, 2H, βCH_2); 7.30 (br s, 2H, SO_2NH_2); 7.50–8.70 (m, 8H, ArH from phenylene and pyridyl); 8.80 (s, 1H, C*H*=N). Found: C, 58.03; H, 5.60; N, 14.47. $C_{14}H_{15}N_3O_2S$ requires; C, 58.11; H, 5.23; N, 14.52%.

***4-(Imidazol-2-ylidene) Aminoethylbenzenesulfonamide* 25**

As white crystals (yield 81%), m.p. 223°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1300 (SO_2^{as}), 1640 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.05 (t, 2H, αCH_2); 3.95 (t, 2H, βCH_2); 6.80 (m, 2H, imidazole); 7.10 (br s, 2H, SO_2NH_2); 7.30 (d, 2H, H3 and H5 from phenylene); 7.80 (d, 2H, ArH, H2

and H6 from phenylene); 8.50 (s, 1H, C*H*=N); 9.10 (s, 1H, N*H* from imidazole). Found: C, 51.55; H, 4.89; N, 20.10. $C_{12}H_{14}N_4O_2S$ requires; C, 51.78; H, 5.07; N, 20.13%.

4-(3,4,5-Trimethoxybenzylidene) Aminoethylbenzenesulfonamide **26**

As white crystals (yield 69%), m.p. 188–189°C. IR (KBr), cm^{-1}: 1120 (C–OMe), 1150 (SO_2^{sym}), 1320 (SO_2^{as}), 1630 (C=N); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.05 (t, 2H, αCH_2); 3.70 (s, 3H, 4-MeO); 3.80 (s, 6H, 3,5-MeO); 3.95 (t, 2H, βCH_2); 7.10 (br s, 2H, SO_2NH_2); 7.30 (d, 2H, H3 and H5 from phenylene); 7.80 (d, 2H, ArH, H2 and H6 from phenylene); 7.49–8.20 (s, 2H, ArH from C_6H_2); 8.50 (s, 1H, C*H*=N). Found: C, 57.36; H, 6.02; N, 7.13. $C_{18}H_{22}N_2O_5S$ requires; C, 57.13; H, 5.86; N, 7.40%

4-(2-Hydroxybenzylidene) Aminoethylbenzenesulfonamide **27**

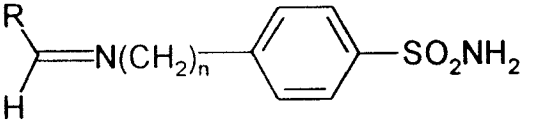
As yellow crystals (yield 82%), m.p. 201–203°C IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1350 (SO_2^{as}), 1620 (C=N), 3300 (NH + OH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.05 (t, 2H, δCH_2); 3.95 (t, 2H, βCH_2); 7.00 (br s, 2H, SO_2NH_2); 7.30 (d, 2H, H3 and H5 from phenylene); 7.80 (d, 2H, ArH, H2 and H6 from phenylene); 7.45–7.98 (m, 4H, ArH from *o*-HO-C_6H_4); 8.45 (s, 1H, C*H*=N). Found C, 59.40; H, 5.56; N, 9.01. $C_{15}H_{16}N_2O_3S$ requires; C, 59.19; H, 5.30; N, 9.20%.

RESULTS AND DISCUSSION

The Schiff bases **11–28** prepared from sulfanilamide, homosulfanilamide or *p*-(2-aminoethyl)-benzenesulfonamide and aromatic/heterocyclic aldehydes reported in the present study and their CA inhibitory properties against isozymes I, II and IV are shown in Table I. Two compounds (**19** and **28**) have previously been reported by us,[18c] but were included in the present study for two reasons: (i) they were among the best CA IV inhibitors, possessing a stronger affinity for this isozyme as compared to the sulfonamide-avid CA II and, (ii) they possess a donor system (the Schiff base moiety nitrogen atom and the imidazolic N-3 atom) which make them very interesting candidates for the preparation of metal complexes.

Aldehydes used for the preparation of the new derivatives **11–28** were generally substituted benzaldehydes or heterocyclic derivatives, as in the previous work.[18] The main difference was that heterocyclic aldehydes were predominant in this study, since it had previously been observed that the Schiff bases obtained from them had the highest CA IV affinity.[18]

TABLE I Schiff bases **11–28** and some of their metal complexes **29–36** prepared in the present study and their biological activity against isozymes hCA I, hCA II and bCA IV (K_I is the mean of two different assays using the esterase method)[31]



11–28

Comp.	*n*	*R*	K_I (nM)		
			hCA I[a]	hCA II[a]	bCA IV[b]
Sulfanilamide		—	28000	300	3000
Homosulfanilamide		—	25000	170	2800
p-aminoethylbenzene sulfonamide		—	21000	160	2450
11	0	Pyrrol-2-yl	1400	103	115
12	0	Thien-2-yl	890	54	55
13	0	5-Me-thien-2-yl	900	49	40
14	0	Imidazol-2-yl	660	22	12
16	0	2,4,6-Trichloropyrimidine-5-yl	800	30	40
16	1	Pyrrol-2-yl	1200	65	74
17	1	*N*-Me-pyrrol-2-yl	1100	60	67
18	1	5-Me-thien-2-yl	860	45	41
19	1	1-*N*-Me-imidazol-2-yl[c]	25	5	4
20	2	Pyrrol-2-yl	920	56	54
21	2	*N*-Me-pyrrol-2-yl	900	43	40
22	2	Thien-2-yl	840	40	38
23	2	5-Me-thien-2-yl	780	39	34
24	2	4-Pyridyl	180	15	12
25	2	Imidazol-2-yl	620	12	10
26	2	3,4,5-trimethoxyphenyl	550	9	9
27	2	2-Hydroxyphenyl	420	14	13
28	2	1-*N*-Me-imidazol-2-yl[c]	21	4	3
29	—	—	20	1	2
30	—	—	7	0.5	1
31	—	—	12	2	3
32	—	—	13	3	3
33	—	—	15	0.8	2
34	—	—	5	0.4	1.5
35	—	—	9	1.5	2
36	—	—	10	0.7	1.8

[a]Human (cloned) isozyme. [b]Isolated from bovine lung microsomes. [c]Prepared as described in Ref. [18c].

Since metal complexes of heterocyclic sulfonamides have recently been shown[21–24] to possess strong CA inhibitory properties, and in some cases topical intraocular pressure lowering effects in animal models of glaucoma,[11a,21d] it appeared of interest to synthesize some metal complexes of the Schiff bases reported here. Several derivatives prepared in the present or previous studies[18] possess donor systems (such as imidazole, *N*-methyl-imidazole or pyrrole moieties) which make them very attractive candidates

TABLE II Metal complexes containing sulfonamides **19** (L) and **28** (L′) as ligands and their elemental analysis data

No.	*Complex*	*Yield* (%)	*Analysis* (*calculated*/*found*)			
			%M[a]	%C[b]	%H[b]	%N[b]
29	$[ZnL_2Cl_2]$	78	9.38/9.50	41.57/41.31	4.04/4.30	16.17/16.12
30	$[CuL_2Cl_2]$	69	9.18/9.10	41.66/41.34	4.05/4.33	16.20/16.05
31	$[CoL_2Cl_2]$	84	8.58/8.29	41.94/41.80	4.07/4.19	16.31/16.15
32	$[NiL_2Cl_2]$	91	8.55/8.21	41.96/41.75	4.07/4.25	16.31/16.30
33	$[ZnL'_2Cl_2]$	75	9.01/9.27	43.29/43.10	4.44/4.67	15.54/15.30
34	$[CuL'_2Cl_2]$	87	8.82/8.70	43.38/43.15	4.44/4.30	15.57/15.45
35	$[CoL'_2Cl_2]$	76	8.24/8.10	43.66/43.81	4.47/4.58	15.67/15.49
36	$[NiL'_2Cl_2]$	79	8.21/8.36	43.67/43.49	4.47/4.35	15.67/15.55

[a]By gravimetry. [b]By combustion.

for the binding of metal ions. In addition to the heteroatom from the aldehyde moiety of the Schiff base, it is expected that the nitrogen atom of the CH=N moiety would also participate in binding such metal ions. In order to test this hypothesis, the strong and relatively specific CA IV inhibitors **19** and **28** were chosen as ligands for the preparation of Zn(II), Cu(II), Co(II) and Ni(II) complexes (Table II). It should be noted that unlike other ligands investigated in previous studies as metal complexing sulfonamides with CA inhibitory properties,[11,21–24] the two compounds investigated here act as neutral ligands, not in the deprotonated state (see later in the text). Practically the new derivatives reported here (**29–36**) are the first non-chelate metal complexes of sulfonamide CA inhibitors. In fact these complexes have been prepared in the absence of deprotonating agents (NaOH, NH_3 or organic amines) in order to favor the formation of the Schiff bases as neutral ligands. Working in the presence of bases leads to the deprotonation of the sulfonamido moiety of the ligand, which thereafter generally interacts with the metal ion(s) present in the coordination compound.[11,21–24]

The new complexes **29–36** were characterized by elemental and thermogravimetric (TG) analyses (Table II) and spectral data that allowed us to assign the coordination mode of ligands L and L′ (Table III) in these complexes.

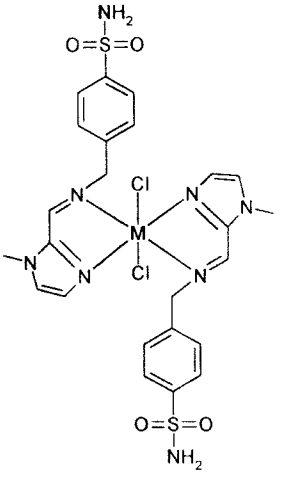
The electronic spectroscopic data for the Cu(II), Co(II) and Ni(II) derivatives (Table III), provided evidence for the presence of octahedral metal ions in the prepared complexes.[34–36] IR data (Table III) on the other hand proved that the donor system of the two ligands is indeed the expected one i.e., the N-3 imidazole atom and the Schiff base moiety nitrogen. In this way, five-membered, strainless rings are formed by binding of the metal ions. Practically, the CH=N vibration is shifted by 45–70 cm^{-1} towards lower wavenumbers in the IR spectra of the complexes as compared to the same band in the spectra of the parent ligands (Table III), whereas other IR

TABLE III IR and electronic spectroscopic data for ligands **19** (L) and **28** (L′) and their metal complexes **29–36**

Compound	*IR spectra*[a] cm^{-1}			*Electronic spectra*[b]
	$\nu(SO_2)^s$	$\nu(SO_2)^{as}$	$\nu(C{=}N)$	*Wavenumber* (ν, cm^{-1})
19	1150	1325	1645	—
29	1150	1325	1600	—
30	1150	1325	1600	16,500–16,850[c]
31	1150	1325	1600	8,200; 15,350; 20,800; 25,740
32	1150	1325	1595	9,100; 12,300; 16,000; 26,400
28	1145	1320	1645	—
33	1145	1320	1585	—
34	1145	1320	1575	16,500–16,850[c]
35	1145	1320	1575	8,250; 15,390; 20,900; 25,750
36	1145	1320	1585	9,000; 12,400; 16,000; 26,450

[a]In KBr. [b]In MgO as standard, by the diffuse reflectance technique. [c]Large, structureless band.

bands (e.g. the very intense sulfonamide vibrations) are unchanged in the spectra of the ligands and their metal derivatives (Table III). For the two Zn(II) complexes **29** and **33**, we also propose an octahedral geometry of Zn(II), similarly to that of the other metal ions present in these new complexes. It should be noted that the IR spectra of the Zn(II) complexes are quite similar to those of the other prepared derivatives. TG analysis data showed no weight loss under 250°C, proving that water was not present in the coordination sphere of the prepared complexes (data not shown). A schematic structure for the obtained compounds is shown below for derivatives **29–32**, but the same type of structure would be also valid for the metal complexes of ligand L′ (**33–36**).



29-32 (M = Zn(II); Cu(II); Co(II); Ni(II))

The following observations can be made about inhibition of isozymes CA I, II and IV with the obtained Schiff bases **11–28** (Table I): (i) potency in inhibiting all three isozymes generally increased with *n*, with the *p*-aminoethylbenzenesulfonamide derivatives being more inhibitory than the homosulfanilamide derivatives, which in turn were better inhibitors than the sulfanilamides bearing the same substitution pattern (compare also with data from Ref. [18] where a larger series of sulfanilamide Schiff bases were assayed for the inhibition of these isozymes). This result is in agreement with data inferred from X-ray crystallographic and QSAR studies of this group,[33] (ii) as shown in the previous studies,[18] the nature of group R (from the aromatic/heterocyclic aldehyde used to prepare the Schiff bases) greatly influenced CA inhibitory properties. Again derivatives of heterocyclic aldehydes were generally slightly more active than the aromatic ones.[18] For the two benzaldehyde derivatives reported here, an effective inhibitor was the one containing the 3,4,5-trimethoxyphenyl moiety (in the previous studies[18] a much larger series of aromatic aldehyde derivatives was obtained, and the structure–activity relationship is discussed there), (iii) all the Schiff bases were at least one order of magnitude more active CA IV inhibitors as when compared to the parent sulfonamides from which they were derived, (iv) the most interesting feature of the new inhibitors is in some cases their slight discrimination in selectivity towards CA IV as compared to CA II, a fact first seen for the previously reported Schiff bases.[18] Thus, several compounds reported here, such as **13**, **14**, **18–25**, **27** and **28**, possess higher affinities for CA IV as compared to CA II. This constitutes an encouraging result for the prospect of obtaining more selective, isozyme-specific CA IV inhibitors. It should also be mentioned that some of these compounds (**19**, **25**, **26** or **28**) are very strong inhibitors, with potencies comparable to those of acetazolamide for CA II, making them attractive candidates for further pharmacological evaluation.

Metal complexes **29–36** on the other hand act as very potent inhibitors against all three investigated isozymes. Their potency is enhanced as compared to the parent sulfonamides from which they are derived, a feature shared with all the metal complexes of sulfonamide CA inhibitors reported up to now.[21–24] Still, the unexpected and relatively undesired feature of the obtained metal complexes was that they possess an enhanced affinity towards hCA II and not bCA IV, in contrast to the ligands **19** and **28** which showed some preference for binding to the latter isozyme. The most effective inhibitors were those containing Cu(II) and Zn(II) ions, but the differences in activity between these compounds and the Co(II) and Ni(II)-containing derivatives are not so important (Table I).

The above differences in affinity for the different isozymes by the new inhibitors reported in this paper, and the fact that the X-ray crystallographic structure of the adduct of murine (m) mCA IV with sulfonamide **6** (brinzolamide) has been recently reported,[16] prompted us to compare the tridimensional structure and active site architecture for the three CA isozymes (CA I, II and IV) used in the present work (bovine and murine CA IV possess similar kinetic and inhibition susceptibility properties)[32] in order to explain their affinity for the sulfonamide inhibitors reported by us here.

It has previously been shown by us[37] that the high catalytic efficiency of the most active isozyme, hCA II, is due to unique feature of its active site: the presence of a histidine cluster, consisting of the residues: His 64 – two conformations; His 4 – two conformations, His 3, His 10, His 15, His 17. This cluster extends from the interior of the active site (His 64) to its entrance (His 4 and His 3) upto the surface of the protein (in the proximity of the active site entrance) and it probably constitutes a very appropriate "channel" for efficiently transferring protons from the active site to the reaction medium, as well as the binding of amphipatic compounds, such as the sulfonamide inhibitors, since such compounds generally possess at least a charged atom in their molecule, i.e., the SO_2NH^- anion. In the low activity isozyme hCA I, on the other hand, which also binds sulfonamide inhibitors but with a 10–100 times lower affinity, such a cluster does not exit.[37] Moreover, the pathways for the proton transfer are somehow bifurcated and divergent as the four histidines present within the active site, i.e., His 64, 67, 200 and 243 (excepting, of course, for the three Zn(II) ligands, which in all isozymes are His 94, 96 and 119) are placed at bifurcating positions. These four histidines in hCA I (His 64, His 67, His 200 and His 243) are rather buried in the active site so that, probably, the proton transfer cannot be as efficient as the one assisted by the histidine cluster present in hCA II and inhibitors are probably unable to interact with them, as documented by crystallographic studies of adducts of hCA I with sulfonamides and anion inhibitors.[38] In this context, we suggest a new hypothesis for explaining the differences of affinity of the discussed CA isozymes for the sulfonamide inhibitors, based on the interaction of inhibitors with the structural elements of the active site mentioned above. Indeed, for example in hCA II the amino acid residue in position 4 is His, whereas in hCA I it is an Asp. This residue, situated just at the entrance within the active site, is a constituent of the histidine cluster of isozyme II. The negative charge of the carboxylate group of the Asp residue in hCA I probably influences its interaction with sulfonamide or complex inhibitors of the type described by us here. In the case of mCA IV only one histidine residue is present within the active site, His 64,

which as in hCA II, plays a critical role in catalysis as proton shuttle residue between the active site and the environment. But the most characteristic feature of the active site of this isozyme is related to the presence of four cysteine residues, which form two disulfide bonds, situated at the entrance within the active site cavity (Cys 6–Cys 11G, and Cys 23–Cys 203, respectively). These residues occupy practically the same region of the active site as the histidine cluster in hCA II, and we consider this as the most relevant aspect explaining the difference in affinity for sulfonamide inhibitors of the two isozymes. It is thus highly probable that Schiff bases of the type reported here or in previous papers[18] possess certain structural features that favor their binding to this membrane-bound isozyme, whereas slightly decreasing their affinity for the cytosolic rapid isozyme, hCA II. Only in this way can we explain the relatively enhanced affinity of many Schiff bases for bCA IV as compared to hCA II. In the case of the metal complexes this feature seems to be lost, as they are stronger inhibitors of hCA II, but our hypothesis also explains this behavior. Thus, it is well known that histidine residues in proteins possess a high affinity for binding metal ions. In fact Liljas' group[39] reported the X-ray crystal structure of Hg^{2+} bound to His 64 in hCA II. Since this isozyme is the only one possessing a large histidine cluster of the three investigated by us here, it is quite probable that the moieties of the six residues forming the cluster contribute in an important manner to the binding of metal complexes of aromatic sulfonamides of the type reported by us here. One can thus explain why these complex inhibitors bind stronger to CA II and not to CA IV, in contrast to the sulfonamide ligands from which they were obtained.

In conclusion, in the present paper we report novel Schiff bases obtained from aromatic sulfonamides and heterocyclic/aromatic aldehydes, some of which possess a slightly increased affinity in binding to the membrane-bound isozyme bCA IV, as compared to the cytosolic one hCA II. Some metal complexes of these Schiff bases on the other hand do not show this desired feature, but bind stronger to the sulfonamide avid isozyme hCA II. A hypothesis is made in order to explain this binding.

References

[1] This paper is part 67 of the series "Carbonic anhydrase inhibitors". Preceding part: A. Scozzafava and C.T. Supuran (1998) *J. Enz. Inhib.* (in press).
[2] R.P. Henry (1996) *Annu. Rev. Physiol.*, **58**, 523–538.
[3] C.T. Supuran (1994) In *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism*, I. Puscas (Ed.) pp. 29–111. Helicon; Timisoara.
[4] D. Hewett-Emmett and R.E. Tashian (1996) *Mol. Phylogenet. Evol.*, **5**, 50–77.
[5] T. Mann and D. Keilin (1940) *Nature* (*hond.*), **146**, 164–165.

[6] C.T. Supuran, C.W. Conroy and T.H. Maren (1996) *Eur. J. Med. Chem.*, **31**, 843–846.
[7] T.M. Maren (1967) *Physiol. Rev.*, **47**, 595–782.
[8] I.M. Weiner (1990) In *The Pharmocological Basis of Therapeutics*, 8th edition, A.G. Gilman, T.W. Rall, A.S. Nies and P. Taylor (Eds.) pp. 713–732. Pergamon Press; New York.
[9] M.F. Sugrue (1996) *J. Ocul. Pharmocol. Ther.*, **12**, 363–376.
[10] L.H. Silver (1998) *Am. J. Ophthamol.*, **126**, 400–408.
[11] (a) C.T. Supuran, F. Mincione, A. Scozzafava, F. Briganti, G. Mincione and M.A. Ilies (1998) *Eur. J. Med. Chem.*, **33**, 247–254; (b) F. Mincione, L. Menabuoni, F. Briganti, G. Mincione, A. Scozzafava and C.T. Supuran (1998) *J. Enz. Inhib.*, **13**, 267–284.
[12] I. Puscas and C.T. Supuran (1996) In *Aparelho Digestivo*, J. Coelho (Ed.) pp. 1704–1734. MEDSI; Rio de Janeiro.
[13] E.B. Larson, R.C. Roach, R.B. Schoene and T.F. Hornbein (1982) *J. Am. Med. Assoc.*, **248**, 328–332.
[14] P.J. Wistrand and A. Lindquist (1991) In *Carbonic Anhydrase – From Biochemistry and Genetics to Physiology and Clinical Medicine*, F. Botrè, G. Gros and B.T. Storey (Eds.) pp. 352–378. VCH; New York.
[15] T.H. Maren, G.C. Wynns and P.J. Wistrand (1993) *Mol. Pharmacol.*, **44**, 901–906.
[16] T. Stams, Y. Chen, Boriack-Sjodin, J.D. Hurt, J. Liao, J.A. May, T. Dean, P. Laipis, D.N. Silverman and D.W. Christianson (1998) *Protein Sci.*, **7**, 556–563.
[17] C.T. Supuran, M.A. Ilies and A. Scozzafava (1998) *Eur. J. Med. Chem.*, **33**, 739–752.
[18] (a) C.T. Supuran, A. Nicolae and A. Popescu (1996) *Eur. J. Med. Chem.*, **31**, 431–438; (b) C.T. Supuran, A. Popescu, M. Ilisiu, A. Costandache and M.D. Banciu (1996) *Eur. J. Med. Chem.*, **31**, 439–448; (c) C.T. Supuran, A. Scozzafava, A. Popescu, R. Bobes-Tureac, A. Creanga, A. Creanga, G. Bobes-Tureac and M.D. Banciu (1997) *Eur. J. Med. Chem.*, **32**, 445–452.
[19] (a) C.T. Supuran, F. Briganti and A. Scozzafava (1997) *J. Enz. Inhib.*, **12**, 175–190; (b) C.T. Supuran, C.W. Conroy and T.H. Maren (1997) *Proteins*, **27**, 272–278.
[20] (a) F. Briganti, R. Pierattelli, A. Scozzafava and C.T. Supuran (1996) *Eur. J. Med. Chem.*, **31**, 1001–1010; (b) C.T. Supuran and B.W. Clare (1995) *Eur. J. Med. Chem.*, **30**, 687–696.
[21] (a) C.T. Supuran and A. Scozzafava (1997) *J. Enz. Inhib.*, **12**, 37–51; (b) G. Mincione, A. Scozzafava and C.T. Supuran (1997) *Metal Based Drugs*, **4**, 27–34; (c) A. Scozzafava and C.T. Supuran (1997) *Metal Based Drugs*, **4**, 19–26; (d) C.T. Supuran, A. Scozzafava, I. Saramet and M.D. Banciu (1998) *J. Enz. Inhib.*, **13**, 177–194.
[22] (a) S.L. Sumalan, J. Casanova, G. Alzuet, J. Borras, A. Castiñeiras and C.T. Supuran (1996) *J. Inorg. Biochem.*, **62**, 31–39; (b) C.T. Supuran (1995) *Metal Based Drugs*, **2**, 323–330; (c) J. Borras, T. Cristea and C.T. Supuran (1996), *Main Group Met. Chem.*, **19**, 339–346.
[23] G. Alzuet, S. Ferrer, J. Borras and C.T. Supuran (1994) *Roum. Chem. Quart. Rev.*, **2**, 283–300.
[24] (a) J. Borras, J. Casanova, T. Cristea, A. Gheorghe, A. Scozzafava, C.T. Supuran and V. Tudor (1996) *Metal Based Drugs*, **3**, 143–148; (b) C.T. Supuran, G. Manole and M. Andruh (1993) *J. Inorg. Biochem.*, **49**, 97–103.
[25] (a) C.T. Supuran, G. Manole, A. Dinculescu, A. Schiketanz, M.D. Gheorghiu, I. Puscas and A.T. Balaban (1992) *J. Pharm. Sci.*, **81**, 716–719; (b) C.T. Supuran, A. Scozzafava, M.A. Ilies, B. Iorga, T. Cristea, F. Briganti, F. Chiraleu and M.D. Banciu (1998) *Eur. J. Med. Chem.*, **33**, 577–595.
[26] C. Forsman, G. Behravan, A. Osterman and B.M. Jonsson (1988) *Acta Chem. Scand.*, **B42**, 314–318.
[27] G. Behravan, P. Jonasson, B.M. Jonsson and S. Lindskog (1991) *Eur. J. Biochem.*, **198**, 589–592.
[28] R.G. Khalifah, D.J. Strader, S.H. Bryant and S.M. Gibson (1977) *Biochemistry*, **16**, 2241–2247.
[29] P.O. Nyman and S. Lindskog (1964) *Biochim. Biophys. Acta*, **85**, 141–151.
[30] L.E. Henderson, D. Henriksson and P.O. Nyman (1976) *J. Biol. Chem.*, **251**, 5457–5463.
[31] Y. Pocker and J.T. Stone (1967) *Biochemistry*, **6**, 668–678.
[32] T.T. Baird, A. Waheed, T. Okuyama, W.S. Sly and C.A. Fierke (1997) *Biochemistry*, **36**, 2669–2678.

[33] C.T. Supuran and B.W. Clare (1998) *Eur. J. Med. Chem.*, **33**, 489–500.
[34] (a) F. Briganti, A. Scozzafava and C.T. Supuran (1997) *Eur. J. Med. Chem.*, **32**, 901–910; (b) G. Alzuet, J. Casanova, J. Borras, S. Garcia-Granda, A. Gutiérrez-Rodriguez and C.T. Supuran (1998) *Inorg. Chim. Acta*, **273**, 334–338.
[35] P. Borja, G. Alzuet, J. Server-Carriò, J. Borras and C.T. Supuran (1998) *Main Group Met. Chem.*, **21**, 279–292.
[36] L. Sacconi, F. Mani and A. Bencini (1987) In *Comprehensive Coordination Chemistry*, G. Wilkinson, R. Gillard and J. McCleverty (Eds.) Vol. 5, pp. 1–347. Pergamon Press; Oxford.
[37] F. Briganti, S. Mangani, P. Orioli, A. Scozzafava, G. Vernaglione and C.T. Supuran (1997) *Biochemistry*, **36**, 10384–10392.
[38] S. Chakravarti and K.K. Kannan (1994) *J. Mol. Biol.*, **243**, 298–309.
[39] A.E. Eriksson and A. Liljas (1991) In *The Carbonic Anhydrases*, S.J. Dodgson, R.E. Tashian, G. Gros, N.D. Carter (Eds.) pp. 33–48. Plenum; New York.