J. Enzyme Inhibition, 1998, Vol. 13, pp. 103–123
Reprints available directly from the publisher
Photocopying permitted by license only

CARBONIC ANHYDRASE INHIBITORS: UREIDO AND THIOUREIDO DERIVATIVES OF AROMATIC SULFONAMIDES POSSESSING INCREASED AFFINITIES FOR ISOZYME I. A NOVEL ROUTE TO 2,5-DISUBSTITUTED-1,3,4-THIADIAZOLES VIA THIOUREAS, AND THEIR INTERACTION WITH ISOZYMES I, II AND IV *

ANDREA SCOZZAFAVA and CLAUDIU T. SUPURAN †

Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121, Firenze, Italia

(Received 21 May 1997)

Reaction of 12 aromatic sulfonamides containing a free amino group with cyanate or thiocyanate in the presence of acid afforded the corresponding urea/thiourea derivatives which were assayed as inhibitors of three isozymes of carbonic anhydrase (CA), i.e., CA I, II and IV. Oxidation of the obtained thioureas with iodine in acidic medium afforded symmetrical 2,5-bis-(substituted-phenyl)-1,3,4-thiadiazole derivatives possessing sulfonamido groups on the aromatic ring, *via* a new synthesis of the heterocyclic moiety. Good inhibition of all these three CA isozymes was observed with the new compounds, but a novel finding was that the ureas/thioureas reported here had an increased affinity for the slow isozyme CA I, which generally is less sensitive to inhibition by sulfonamides when compared to the rapid isozymes CA II and IV. The disubstituted-1,3,4-thiadiazoles on the other hand were better inhibitors of CA II than CA IV and especially CA I, similarly to the large majority of aromatic/heterocyclic sulfonamides. Some of the new compounds could constitute good lead molecules for developing more selective CA I inhibitors.

Keywords: Aromatic sulfonamide; Carbonic anhydrase; Isozyme I, II, IV; Cyanate; Thiocyanate; 1,3,4-thiadiazole; (thio)ureas; Isozyme-specific inhibitor

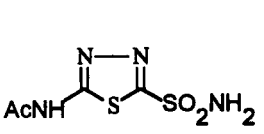
* Part 48. See Reference 1.

† Corresponding author. Fax: +39-55-2757555. Tel.: +39-55-222007.
E-mail: scoz@risc1.lrm.fi.cnr.it.

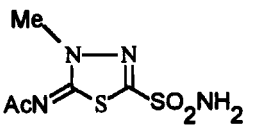
INTRODUCTION

The ten carbonic anhydrases (CAs, EC 4.2.1.1) or CA-like proteins (CA I–X) isolated in mammals up to the present time[2,3] have been a target for drug design since in the 1950s, after the report of Mann and Keilin[4] that sulfanilamide is a specific inhibitor of this zinc enzyme. Thus, in the search of non-mercurial diuretic agents, a large number of aromatic and heterocyclic sulfonamides possessing the general formula RSO_2NH_2 were synthesized and assayed as CA inhibitors,[5–7] leading to the first such clinical agent, acetazolamide **1** (in 1956),[8] followed shortly thereafter by methazolamide **2**[8,9] and dichlorophenamide **3**.[10] Compounds **1–3** are still used clinically for the treatment or prevention of a variety of disorders such as glaucoma,[11] mountain sickness,[12] and epilepsy,[13] their use as diuretics (excepting for dichlorophenamide) being relatively limited nowadays.[14] Still, this class of pharmacological agents have led to the development of two important types of diuretic drugs,[10,15,16] the benzothiadiazines and the high-ceiling diuretics, widely used for the mobilization of oedema fluids in a large number of disorders.[14,15]

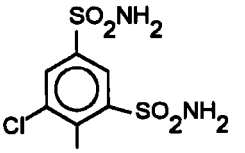
The major draw-back of classical sulfonamide CA inhibitors of the type **1–3**, is their total lack of specificity due to indiscriminate inhibition of all isozymes, in the many tissues in which these are present.[15,17] However, this problem may be circumvented by designing organ-selective compounds, as well as isozyme-specific inhibitors.[15,17] An important example from the first



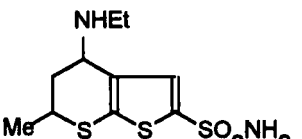
1



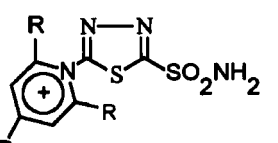
2



3



4

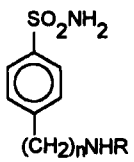


5

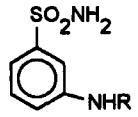
type of derivatives is represented by the topically active antiglaucoma agents, recently introduced in clinical medicine with great success. Thus, dorzolamide **4**, a water-soluble sulfonamide, is highly effective in reducing elevated intraocular pressure in glaucomatous patients, after topical instillation directly into the eye, without the side effects of systemically administered inhibitors (e.g. types **1**–**3**) since only the enzyme within the cilliary processes of the eye is inhibited.[18,19] On the other hand, although few isozyme-specific sulfonamide inhibitors have been reported up to the present time[15] a promising class of CA IV-specific inhibitors is represented by the positively-charged derivatives of type **5**, which are membrane-impermeant and inhibit only the membrane bound isozyme (CA IV) without affecting the cytosolic ones (CA I and II).[20]

Except for their interest as putative pharmacologic agents (for instance, although the use of sulfonamide inhibitors e.g. acetazolamide **1** as antiepileptics seemed to be obsolete,[13a] a recent study [13b] showed their efficiency in a lot of situations where other medication proved to be ineffective), CA inhibitors are also important for assessing the physiological role for some CA isozymes.[15,21] Thus, CA I is one of the most abundant proteins in human erythrocytes, where its concentration reaches 150 μM.[21] This isozyme is a slow reacting one, possessing only about 10% activity for CO_2 hydration of that of the perfectly evolved catalyst which is isozyme CA II (found in a concentration of about 20 μM in red blood cells, but highly abundant in a variety of H^+ or bicarbonate-secreting cells too).[8,21] Although a large number of artificial substrates have been investigated for CA I, its physiological role is still unknown, and one of the hypothesis made regarding its function was that it is an evolutionary accident.[22] Still, evolution rarely preserves unnecessary proteins for hundreds of millions of years (as the CA I gene is already present in primitive vertebrates)[2] and the best answer to the above-mentioned enigma is that the function of such an abundant protein has not yet been discovered. The same is true about the muscle isozyme CA III, possessing only 0.3% of the hydrase activity of CA II, and which is present at a concentration of 240 μM in skeletal muscle.[21,23] Inhibitors that would interact (*in vivo*) selectively with only one of the above-mentioned isozymes would be of considerable help in resolving some questions regarding their physiological role.

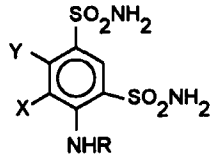
In this paper we report novel sulfonamide CA inhibitors containing ureido or thioureido moieties, prepared from 12 aromatic sulfonamides, **6**–**17**, containing a free amino group, by reaction with cyanate or thiocyanate respectively in acidic medium. Oxidation of the thiourea derivatives **30**–**39** with iodine in acidic medium afforded 2,5-disubstituted-1,3,4-thiadiazoles of



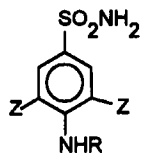
6: n = 0, R = H
7: n = 1, R = H
8: n = 2, R = H
18: n = 0, R = $CONH_2$
19: n = 1, R = $CONH_2$
20: n = 2, R = $CONH_2$
30: n = 0, R = $CSNH_2$
31: n = 1, R = $CSNH_2$
32: n = 2, R = $CSNH_2$



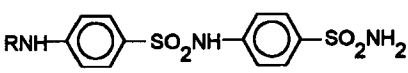
9: R = H
21: R = $CONH_2$
33: R = $CSNH_2$



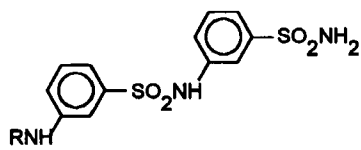
10: X = Y = R = H
11: X = R = H, Y = Cl
12: X = Y = Cl, R = H
22: X = Y = H, R = $CONH_2$
23: X = H, Y = Cl, R = $CONH_2$
24: X = Y = Cl, R = $CONH_2$
34: X = Y = H, R = $CSNH_2$
35: X = H, Y = Cl, R = $CSNH_2$
36: X = Y = Cl, R = $CSNH_2$



13: Z = Cl, R = H
14: Z = Br, R = H
15: Z = I, R = H
25: Z = Cl, R = $CONH_2$
26: Z = Br, R = $CONH_2$
27: Z = I, R = $CONH_2$
37: Z = Cl, R = $CSNH_2$
38: Z = Br, R = $CSNH_2$
39: Z = I, R = $CSNH_2$



16: R = H
28: R = $CONH_2$
40: R = $CSNH_2$



17: R = H
29: R = $CONH_2$
41: R = $CSNH_2$

the type **42**–**51**, bearing two sulfonamido moieties in their molecule, *via* a new synthesis of this ring system.

All the prepared derivatives were assayed as CA inhibitors against three isozymes, CA I, II and IV. The great majority of them showed good inhibitory activity against all three isozymes, but certain ureas/thioureas had

larger affinities for isozyme CA I compared to CA II and CA IV, which constitutes a novel observation, since this is generally an isozyme with lower affinity for this type of inhibitor when compared to the other two previously mentioned. The bis-sulfonamides derived from 1,3,4-thiadiazole on the other hand possessed a highly increased affinity for CA II, similarly to the classical inhibitors acetazolamide, methazolamide, etc., and lower affinities for CA IV and especially CA I.

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- and ^{13}C-NMR spectra with a Varian 300CXP apparatus (^{1}H, 300 MHz; ^{13}C, 75.57 MHz) in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and were $\pm 0.4\%$ of the theoretical values.

Sulfonamides **6**–**8** and **10**–**12** used in synthesis were commercially available (from Sigma, Acros or Aldrich) whereas compounds **9** and **13**–**17** were prepared as described in the literature.[24] Metanilamide **9** was prepared from 3-aminobenzene-sulfonyl fluoride hydrochloride (Acros) by treatment with excess aqueous ammonia. The dihalogenosulfanilamides **13**–**15** were obtained by halogenation of sulfanilamide **6**,[24c] whereas sulfanilyl-sulfanilamide **16** and metanilyl-metanilamide **17** were prepared by arylsulfonylation of sulfanilamide and metanilamide, respectively, with the corresponding sulfonyl halide.[24a,b,d] All these compounds were recrystallized from ethanol–water (1 : 1, v/v). Sodium cyanate and sodium thiocyanate, iodine as well as other inorganic reagents and solvents were from Acros or Merck, and were used without further purification.

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 (H = human isozyme) described by Forsman *et al.*[25] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[26] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[27] 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.[28,29] CA IV was isolated from bovine lung

microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[30]

Inhibitors were assayed by Maren's micromethod,[31] at 0°C, in the conditions of the E–I (enzyme–inhibitor) technique. Water saturated with 100% CO_2 (at 0°C) was used as substrate, as originally described by Maren *et al.*[31] Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO for compounds possessing poor water solubility (DMSO was not inhibitory at the concentrations used in these experiments) 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.[27] 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 the enzyme–inhibitor solution (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, 18 nM for CA I and 3.8 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 color 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.[31] The standard error of this measurements is around 5–10%.[31]

General Procedure for the Preparation of Ureas (18–29) and Thioureas (30–41)

10 mM of the sulfonamide **6–17** was dissolved or suspended in 50 mL of ethanol and the required amount of aqueous 37% HCl solution was added to form the hydrochloride salt. The solution was heated at reflux for 20 min when a solution obtained from 12 mM (80 mg) NaCNO or 12 mM (105 mg) NaSCN, respectively, dissolved in 5 mL of water was added and the resulting mixture was heated at reflux for 4–6 h. The conversion of all the sulfonamide **6–17** to the corresponding urea/thiourea derivative was monitored by TLC. The solvent was then evaporated to a small volume. Generally, compounds **18–41** crystallized spontaneously by leaving the above mixture at 4°C overnight. In some cases, the concentrated liquor obtained after the evaporation of the solvent was poured into 50 mL of cold water, when the reaction product precipitated and was filtered. The prepared compounds were recrystallized from ethanol or ethanol–water (1 : 1, v/v). Yields were in the range 70–80%.

General Procedure for the Synthesis of 1,3,4-thiadiazoles (42–51)

10 mMoles of the thiourea **30–39** were dissolved in 10 mL of 37% aqueous HCl solution and 25 mL of ethanol. The highly acidic solution was treated dropwise with the stoichiometric amount (10 mM) of an aqueous-alcoholic solution of KI_3 for 30 min, when a pale yellow precipitate was obtained (an intense elemental sulfur odour was noticed). The precipitate was filtered off, thoroughly washed with water until neutral and purified by repeated dissolution in 1 N NaOH solution, filtration (to remove the insoluble elemental sulfur) and reprecipitation with 4 N HCl solution. As for many bis-sulfonamides,[32,33] the disodium salts of the obtained compounds **42–51** have a good water solubility (unlike the free sulfonamides which are poorly water-soluble) but are also almost insoluble in the large majority of organic solvents, except for DMSO and DMF. This is the reason why the prepared compounds were not recrystallized, but were purified by repeatedly transforming them into the disodium salt followed by reprecipitation with acid, as described above. The elemental sulfur obtained from these purification processes could be isolated with a yield of 75–85% (based on the starting thioureas), whereas the corresponding yield for the thiadiazoles was 57–78%.

4-Ureido-benzenesulfonamide **18** as white crystals, m.p. 178°–9°C. The compound is mentioned by Beasley *et al.*,[7] but no m.p. or any other physicochemical data is described in their study; lit.[24a,34] m.p. 172°–3°C and 208°–9°C. IR (KBr), cm^{-1}: 659, 778, 786, 810, 864, 989, 1028, 1043, 1146 (SO_2^{sym}), 1325 (SO_2^{as}), 1410, 1515 (amide II), 1700 (CO), 3163 (NHCONH), 3300 (NH_2): ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.70 (br s, 3H, $NHCONH_2$); 7.05 (m, AA′BB′, 4H, ArH, 1,4-phenylene); 7.55 (br s, 2H, SO_2NH_2). Found: C, 39.0; H, 4.0; N, 19.2. $C_7H_9N_3O_3S$ requires: C, 39.0; H, 4.1; N, 19.5%.

4-(Ureidomethyl)-benzenesulfonamide **19** as white crystals, m.p. 176°–7°C. IR (KBr), cm^{-1}: 638, 671, 850, 888, 931, 983, 1020, 1035, 1150 (SO_2^{sym}), 1332 (SO_2^{as}), 1515 (amide II), 1700 (CO), 3170 (CONH), 3310 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CONHCH_2); 5.87 (br s, 3H, $NHCONH_2$); 7.08 (m, AA′BB′, 4H, ArH, phenylene); 7.46 (s, 2H, SO_2NH_2). Found: C, 42.0; H, 4.8; N, 18.0. $C_8H_{11}N_3O_3S$ requires: C, 41.9; H, 4.8; N, 18.3%.

4-(Ureidoethyl)-benzenesulfonamide **20** as white crystals, m.p. 166°–7°C. IR (KBr), cm^{-1}: 653, 884, 940, 1030, 1056, 1080, 1139 (SO_2^{sym}), 1338 (SO_2^{as}), 1515 (amide II), 1690 (CO), 3178 (CONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.90 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 5.90 (br s, 3H, $NHCONH_2$); 6.85

(br s, 2H, SO_2NH_2); 7.05 (m, AA′BB′, 4H, ArH, phenylene). Found: C, 44.2; H, 5.2; N, 17.2. $C_9H_{13}N_3O_3S$ requires: C, 44.4; H, 5.3; N, 17.2%.

3-Ureido-benzenesulfonamide **21** as white crystals, m.p. 199°–202°C. IR (KBr), cm^{-1}: 621, 739, 840, 975, 1020, 1045, 1134 (SO_2^{sym}), 1333 (SO_2^{as}), 1517 (amide II), 1690 (CO), 3170 (CONH), 3330 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.90 (br s, 3H, $NHCONH_2$); 7.12 (br s, 2H, + SO_2NH_2) – the signals disappear by addition of D_2O into the NMR tube; 7.08–7.51 (m, 4H, ArH, 1,3-phenylene). Found: C, 38.9; H, 4.0; N, 19.3. $C_7H_9N_3O_3S$ requires: C, 39.0; H, 4.1; N, 19.5 %.

4-Ureido-1,3-benzenedisulfonamide **22** as white crystals, m.p. 218°–9°C. IR (KBr), cm^{-1}: 629, 713, 748, 795, 817, 863, 991, 1010, 1049, 1155 (SO_2^{sym}), 1342 (SO_2^{as}), 1414, 1515 (amide II), 1694 (CO), 3166 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.75 (br s, 3H, $NHCONH_2$); 7.26 (d, 2H, ArH); 7.50 (s, 1H, ArH); 7.60 (br s, 4H, 2 SO_2NH_2). Found: C, 28.3; H, 3.1; N, 19.2. $C_7H_{10}N_4O_5S_2$ requires: C, 28.5; H, 3.4; N, 19.0%.

6-Chloro-4-ureido-1,3-benzenedisulfonamide **23** as white crystals, m.p. 210°–2°C. IR (KBr), cm^{-1}: 657, 750, 778, 832, 877, 945, 1015, 1080, 1153 (SO_2^{sym}), 1346 (SO_2^{as}), 1446, 1520 (amide II), 1696 (CO), 3165 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.77 (br s, 3H, $NHCONH_2$); 7.40 (s, 1H, ArH); 7.50 (s, 1H, ArH); 7.58 (br s, 4H, 2 SO_2NH_2). Found: C, 25.4; H, 2.8; N, 16.9. $C_7H_9N_4O_5S_2Cl$ requires: C, 25.5; H, 2.7; N, 17.0%.

5,6-Dichloro-4-ureido-1,3-benzenedisulfonamide **24** as white crystals, m.p. 200°–3°C. IR (KBr), cm^{-1}: 604, 698, 754, 788, 839, 850, 952, 1076, 1155 (SO_2^{sym}), 1350 (SO_2^{as}), 1515 (amide II), 1704 (CO), 3170 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.82 (br s, 3H, $NHCONH_2$); 7.50 (S, 1H, ArH); 7.58 (br s, 4H, 2 SO_2NH_2). Found: C, 23.0; H, 1.8; N, 15.2. $C_7H_8N_4O_5S_2Cl_2$ requires: C, 23.1; H, 2.2; N, 15.4%.

3,5-Dichloro-4-ureido-benzenesulfonamide **25** as white crystals, m.p. 210°–3°C. IR (KBr), cm^{-1}: 680, 734, 798, 880, 902, 1046, 1155 (SO_2^{sym}), 1330 (SO_2^{as}), 1420, 1517 (amide II), 1700 (CO), 3180 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.68 (br s, 3H, ($NHCONH_2$); 7.45 (s, 2H, ArH); 7.60 (br s, 2H, SO_2NH_2). Found: C, 29.5; H, 2.0; N, 14.6. $C_7H_7N_3Cl_2O_3S$ requires: C, 29.5; H, 2.4; N, 14.7%.

3,5-Dibromo-4-ureido-benzenesulfonamide **26** as white crystals, m.p. 219°–21°C. IR (KBr), cm^{-1}: 678, 740, 812, 884, 925, 1042, 1155 (SO_2^{sym}), 1332 (SO_2^{as}), 1420, 1515 (amide II), 1690 (CO), 3180 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.72 (br s, 3H, $NHCONH_2$); 7.45

(S, 2H, ArH); 7.61 (br s, 2H, SO_2NH_2). Found: C, 22.2; H, 1.8; N, 11.2. $C_7H_7N_3Br_2O_3S$ requires: C, 22.5; H, 1.8; N, 11.2%.

3,5-Diiodo-4-ureido-benzenesulfonamide **27** as white crystals, m.p. 226°–7°C. IR (KBr), cm^{-1}: 643, 704, 780, 868, 935, 1043, 1155 (SO_2^{sym}), 1330 (SO_2^{as}), 1450, 1515 (amide II), 1690 (CO), 3180 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.66 (br s, 3H, $NHCONH_2$); 7.45 (s, 2H, ArH); 7.61 (br s, 2H, SO_2NH_2). Found: C, 17.6; H, 1.8; N, 9.2. $C_7H_7N_3I_2O_3S$ requires: C, 17.9; H, 1.5; N, 9.0%.

4-[4-(Ureido-benzenesulfonylamido)]-benzenesulfonamide **28** as white crystals, m.p. 158°–9°C. IR (KBr), cm^{-1}: 660, 713, 771, 790, 818, 995, 1044, 1093, 1125 and 1146 (SO_2^{sym}), 1325 and 1368 (SO_2^{as}), 1450, 1515 (amide II), 1700 (CO), 3165 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.70 (br s, 3H, $NHCONH_2$); 7.05 (m, AA′BB′, 8H, ArH from the two 1,4-phenylene moieties); 7.49 (br s, 3H, $SO_2NH + SO_2NH_2$). Found: C, 42.0; H, 3.8; N, 15.2. $C_{13}H_{14}N_4O_5S_2$ requires: C, 42.1; H, 3.8; N, 15.1%.

3-[3-(Ureido-benzenesulfonylamido)]-benzenesulfonamide **29** as white crystals, m.p. 133°–6°C. IR (KBr), cm^{-1}: 609, 664, 735, 778, 882, 905, 1054, 1090, 1129 and 1155 (SO_2^{sym}), 1337 and 1365 (SO_2^{as}), 1452, 1520 (amide II), 1694 (CO), 3165 (NHCONH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.75 (br s, 3H, $NHCONH_2$); 7.10–7.51 (m, 8H, ArH, 1,3-phenylene from the two moieties); 7.64 (br s, 3H, $SO_2NH + SO_2NH_2$). Found: C, 42.3; H, 3.6; N, 15.0. $C_{13}H_{14}N_4O_5S_2$ requires: C, 42.1; H, 3.8; N, 15.1%.

4-Thioureido-benzenesulfonamide **30** as pale yellow crystals, m.p. 205°–7°C. The compound is mentioned by Beasley *et al.*,[7] but no m.p. or any other physico-chemical/biochemical characterization are presented in their study; lit.[35] m.p. 205°–6°C. IR (KBr), cm^{-1}: 775, 786, 834, 887, 926, 1039 (thioamide III), 1150 (SO_2^{sym}), 1320 (SO_2^{as}), 1406, 1538 (C=S, thioamide I), 1592, 3284 (NHCSNH), 3390 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.50 (br s, 3H, $NHCSNH_2$); 7.02 (m, AA′BB′, 4H, ArH, 1,4-phenylene); 7.58 (br s, 2H, SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.3; 119.5; 130.4; 131.8; 141.2 (C=S). Found: C, 36.1; H, 3.5; N, 18.2. $C_7H_9N_3O_2S_2$ requires: C, 36.4; H, 3.9; N, 18.2%.

4-(Thioureidomethyl)-benzenesulfonamide **31** as yellow crystals, m.p. 188°–9°C. IR (KBr), cm^{-1}: 690, 771, 794, 842, 885, 921, 1040 (thioamide III), 1150 (SO_2^{sym}), 1332 (SO_2^{as}), 1410, 1539 (thioamide I), 1602, 3289 (NHCSNH), 3390 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, $CSNHCH_2$); 5.54 (br s, 3H, $NHCSNH_2$); 7.04 (m, AA′BB′, 4H, ArH, 1,4-phenylene); 7.50 (br s, 2H, SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 48.7

(CH_2); 118.0; 119.4; 130.5; 131.8; 140.9 (C=S). Found: C, 38.9; H, 4.5; N, 17.2. $C_8H_{11}N_3O_2S_2$ requires: C, 39.1; H, 4.4; N, 17.1%.

4-(Thioureidoethyl)-benzenesulfonamide **32** as pale yellow crystals, m.p. 175°–6°C. IR (KBr), cm^{-1}: 697, 737, 782, 840, 883, 918, 1042 (thioamide III), 1140 (SO_2^{sym}), 1337 (SO_2^{as}), 1418, 1538 (thioamide I), 3295 (NHCSNH), 3390 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2 from the CH_2CH_2bridge); 3.90 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 5.65 (br s, 3H, $NHCSNH_2$); 7.09 (m, AA′BB′, 4H, ArH, 1,4-phenylene); 7.50 (br s, 2H, SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 39.5 and 48.7 (CH_2); 118.7; 119.5; 130.9; 131.9; 141.2 (C=S). Found: C, 41.8; H, 5.0; N, 16.2. $C_9H_{13}N_3O_2S_2$ requires: C, 41.7; H, 5.0; N, 16.2%.

3-Thioureido-benzenesulfonamide **33** as pale yellow crystals, m.p. 180°–2°C. IR (KBr), cm^{-1}: 750, 778, 849, 874, 926, 1039 (thioamide III), 1135 (SO_2^{sym}), 1332 (SO_2^{as}), 1409, 1539 (thioamide I), 1595, 3290 (NHCSNH), 3390 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.50 (br s, 3H, $NHCSNH_2$); 7.10–7.49 (m, 4H, ArH, 1,3-phenylene); 7.56 (br s, 2H, SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.8; 129.5; 130.4; 131.8; 134.7; 142.2 (C=S). Found: C, 36.1; H, 3.5; N, 18.2. $C_7H_9N_3O_2S_2$ requires: C, 36.4; H, 3.9; N, 18.2%.

4-Thioureido-1,3-benzenedisulfonamide **34** as pale yellow crystals, m.p. 227°–8°C. IR (KBr), cm^{-1}: 632, 743, 780, 795, 836, 869, 980, 1025, 1041 (thioamide III), 1155 (SO_2^{sym}), 1345 (SO_2^{as}), 1404, 1539 (thioamide I), 1595, 3185 (NHCSNH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.80 (br s, 3H, $NHCSNH_2$); 7.29 (d, 2H, ArH); 7.50 (s, 1H, ArH); 7.62 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.4; 125.7; 129.0; 130.6; 131.9; 134.8; 142.5 (C=S). Found: C, 27.0; H, 2.8; N, 18.2. $C_7H_{10}N_4O_4S_3$ requires: C, 27.0; H, 3.2; N, 18.0%.

6-Chloro-4-ureido-1,3-benzenedisulfonamide **35** as yellow crystals, m.p. 221°–2°C. IR (KBr), cm^{-1}: 665, 754, 787, 822, 871, 953, 1015, 1040 (thioamide III), 1153 (SO_2^{sym}), 1348 (SO_2^{as}), 1446, 1543 (thioamide I), 1595, 3185 (NHCSNH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.86 (br s, 3H, $NHCSNH_2$); 7.42 (S, 1H, ArH); 7.50 (s, 1H, ArH); 7.59 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.9, 125.2; 129.0; 130.6; 134.8; 137.9; 142.5 (C=S). Found: C, 24.0; H, 2.8; N, 16.2. $C_7H_9N_4O_4S_3Cl$ requires: C, 24.3; H, 2.6; N, 16.2%.

5,6-Dichloro-4-thioureido-1,3-benzenedisulfonamide **36** as white crystals, m.p. 242°–3°C. IR (KBr), cm^{-1}: 656, 759, 780, 841, 855, 928, 1042 (thioamide III), 1155 (SO_2^{sym}), 1353 (SO_2^{as}), 1545 (thioamide I), 1588, 3185 (NHCSNH), 3300 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.77 (br s, 3H,

$NHCSNH_2$); 7.50 (s, 1H, ArH) 7.59 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.4; 125.5; 129.4; 131.9; 134.8; 135.2; 142.5 (C=S). Found: C, 22.0; H, 1.8; N, 14.4. $C_7H_8N_4O_4S_3Cl_2$ requires: C, 22.1; H, 2.1; N, 14.7%.

3,5-Dichloro-4-thioureido-benzenesulfonamide **37** as pale yellow crystals, m.p. 201°–3°C. IR (KBr), cm^{-1}: 688, 722, 795, 905, 1040 (thioamide III), 1154 (SO_2^{sym}), 1325 (SO_2^{as}), 1410, 1539 (thioamide I), 1594, 3280 (NHCSNH), 3385 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.50 (br s, 3H, $NHCSNH_2$); 7.45 (s, 2H, ArH); 7.62 (br s, 2H, SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 124.5; 126.1; 131.4; 132.0; 141.9 (C=S). Found: C, 27.8; H, 2.0; N, 13.9. $C_7H_7N_3Cl_2O_2S_2$ requires: C, 28.0; H, 2.3; N, 14.0%.

3,5-Dibromo-4-thioureido-benzenesulfonamide **38** as pale yellow crystals, m.p. 225°–9°C. IR (KBr), cm^{-1}: 654, 710, 729, 790, 945, 1042 (thioamide III), 1157 (SO_2^{sym}), 1325 (SO_2^{as}), 1410, 1542 (thioamide I), 1590, 3284 (NHCSNH), 3390 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.54 (br s, 3H, $NHCSNH_2$); 7.40 (s, 2H, ArH); 7.65 (br s, 2H, SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 125.8; 126.0; 132.4; 132.9; 142.4; (C=S). Found: C, 21.8; H, 2.0; N, 10.9. $C_7H_7N_3Br_2O_2S_2$ requires: C, 21.6; H, 1.8; N, 10.8%.

3,5-Diiodo-4-thioureido-benzenesulfonamide **39** as pale yellow crystals, m.p. 244°–7°C. IR (KBr), cm^{-1}: 605, 663, 760, 794, 958, 1040 (thioamide III), 1158 (SO_2^{sym}), 1334 (SO_2^{as}), 1430, 1541 (thioamide I), 1594, 3280 (NHCSNH), 3395 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.59 (br s, 3H, $NHCSNH_2$); 7.43 (s, 2H ArH); 7.66 (br s, 2H, SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 125.4; 126.9; 133.4; 135.0; 142.7 (C=S). Found: C, 17.5; H, 1.0; N, 8.9. $C_7H_7N_3I_2O_2S_2$ requires: C, 17.4; H, 1.4; N, 8.7%.

4-[4-(Thioureido-benzenesulfonylamido)]-benzenesulfonamide **40** as yellow crystals, m.p. 150°–1°C. IR (KBr), cm^{-1}: 642, 701, 738, 775, 827, 989, 1044 (thioamide III), 1095, 1125 and 1150 (SO_2^{sym}), 1332 and 1369 (SO_2^{as}), 1450, 1541 (thioamide I), 1590, 3288 (NHCSNH), 3360 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.61 (br s, 3H, $NHCSNH_2$); 7.05 (m, AA′BB′, 8H, ArH from the two 1,4-phenylene moieties); 7.42 (br s, 3H, SO_2NH + SO_2NH_2). Found: C, 40.0; H, 3.8; N, 14.2. $C_{13}H_{14}N_4O_4S_3$ requires: C, 40.4; H, 3.6; N, 14.5%.

3-[3-(Thioureido-benzenesulfonylamido)]-benezenesulfonamide **41** as yellow crystals, m.p. 136°–9°C. IR (KBr), cm^{-1}: 640, 695, 718, 756, 874, 980, 1041 (thioamide III), 1122 and 1145 (SO_2^{sym}), 1330 and 1369 (SO_2^{as}), 1455, 1541 (thioamide I), 1591, 3290 (NHCSNH), 3360 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.55 (br s, 3H, $NHCSNH_2$); 7.10–7.51 (m, 8H, ArH

from the two 1,3-phenylene moieties); 7.60 (br s, 3H, $SO_2NH + SO_2NH_2$). Found: C, 40.3; H, 3.5; N, 14.4. $C_{13}H_{14}N_4O_4S_3$ requires: C, 40.4; H, 3.6; N, 14.5%.

2,5-Bis-(4-sulfamoyl-phenylamino)-1,3,4-thiadiazole **42** as white crystals, m.p. 203°–6°C, lit.[33] m.p. 203°–6°C. IR (KBr), cm^{-1}; 630, 771, 788, 846, 894, 1154 (SO_2^{sym}), 1325 (SO_2^{as}), 1386, 1595, 1612 (C=N thiadiazole), 1646, 3367 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.64 (s, 2H, 2 NH); 7.09 (m, AA′BB′, 8H, ArH from the two 1,4-phenylene moieties); 7.61 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.5; 119.3; 131.0; 131.6; 161.5 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 39.2; H, 3.1; N, 19.7. $C_{14}H_{14}N_6O_4S_3$ requires: C, 39.4; H, 3.3; N, 19.7%.

2,5-Bis-(4-sulfamoyl-phenylaminomethyl)-1,3,4-thiadiazole **43** as tan crystals, m.p. 188°–91°C. IR (KBr), cm^{-1}: 633, 682, 757, 790, 851, 894, 1152 (SO_2^{sym}), 1329 (SO_2^{as}), 1380, 1595, 1610 (C=N thiadiazole), 1645, 3370 ($NH + NH_2$) ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.70 (s, 2H, 2 NH); 4.90 (s, 2H, $NHCH_2$); 7.08 (m, AA′BB′, 8H, ArH from the two 1,4-phenylene moieties); 7.64 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 49.9 (CH_2); 118.8; 119.4; 131.2; 131.6; 161.6 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 42.2; H, 3.8; N, 18.7. $C_{16}H_{18}N_6O_4S_3$ requires: C, 42.3; H, 3.9; N, 18.5%.

2,5-Bis-(4-sulfamoyl-phenylaminoethyl)-1,3,4-thiadiazole **44** as tan crystals, m.p. 165°–7°C. IR (KBr), cm^{-1}: 624, 672, 713, 757, 798, 819, 869, 925, 1150 (SO_2^{sym}), 1332 (SO_2^{as}), 1377, 1590, 1610 (C=N thiadiazole), 1657, 3375 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, PPM: 2.65 (s, 2H, 2 NH); 3.12 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.90 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 7.05 (m, AA′BB′, 8H, ArH from the two 1,4-phenylene moieties); 7.60 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 44.7 and 49.5 (CH_2); 118.0; 119.5; 131.8; 132.7; 161.8 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 49.9; H, 4.1; N, 17.1. $C_{20}H_{22}N_6O_4S_3$ requires: C, 49.8; H, 4.5; N, 17.4%.

2,5-Bis-(3-sulfamoyl-phenylamino)-1,3,4-thiadiazole **45** as tan crystals, m.p. 195°–6°C. IR (KBr), cm^{-1}: 623, 691, 775, 798, 834, 880, 919, 1155 (SO_2^{sym}), 1335 (SO_2^{as}), 1399, 1590, 1619 (C=N thiadiazole), 3378 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.60 (s, 2H, 2 NH); 7.10–7.48 (M, 8H, ArH from the two 1,3-phenylene moieties); 7.57 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 117.6, 118.0; 119.6; 131.5; 131.9; 161.0 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 39.5; H, 3.2; N, 19.5. $C_{14}H_{14}N_6O_4S_3$ requires: C, 39.4; H, 3.3; N, 19.7%.

2,5-Bis-[(2,4-disulfamoyl-phenyl)amino]-1,3,4-thiadiazole **46** as white crystals, m.p. 195°–8°C. IR (KBr), cm^{-1}: 684, 712, 758, 840, 897, 990, 1149 (SO_2^{sym}), 1338 (SO_2^{as}), 1386, 1590, 1613 (C=N thiadiazole), 3365 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.69 (s, 2H, 2 NH); 7.30 (d, 4H, ArH); 7.50 (s, 2H, ArH); 7.61 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.5; 125.8; 130.0; 130.6; 132.8; 161.4 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 28.5; H, 3.0; N, 19.0. $C_{14}H_{16}N_8O_8S_5$ requires: C, 28.7; H, 2.7; N, 19.1%.

2,5-Bis-[(6-chloro-2,4-disulfamoyl-phenyl)amino]-1,3,4-thiadiazole **47** as white crystals, m.p. 238°–9°C. IR (KBr), cm^{-1}: 680, 732, 758, 844, 895, 990, 1018, 1150 (SO_2^{sym}), 1342 (SO_2^{as}), 1382, 1590, 1610 (C=N thiadiazole), 3360 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.66 (s, 2H, 2 NH); 7.30 (s, 2H, ArH); 7.52 (s, 2H, ArH); 7.61 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.5; 125.4; 130.1; 130.9; 134.6; 161.7 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 25.2; H, 2.1; N, 18.7. $C_{14}H_{14}N_8O_8S_5Cl_2$ requires: C, 25.5; H, 2.1; N, 17.0%.

2,5-Bis-[(5,6-dichloro-2,4-disulfamoyl-phenyl)amino]-1,3,4-thiadiazole **48** as white crystals, m.p. 213°–5°C. IR (KBr), cm^{-1}: 660, 753, 781, 844, 895, 950, 1018, 1152 (SO_2^{sym}), 1340 (SO_2^{as}), 1385, 1590, 1615 (C=N thiadiazole), 3360 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.66 (s, 2H, 2 NH); 7.51 (s, 2H, ArH); 7.60 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.3; 125.4; 130.8; 132.5; 134.6; 135.7; 161.6 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 23.2; H, 1.5; N, 15.2. $C_{14}H_{12}N_8O_8S_5Cl_4$ requires: C, 23.0; H, 1.6; N, 15.3%.

2,5-Bis-(4-sulfamoyl-2,6-dichlorophenylamino)-1,3,4-thiadiazole **49** as white crystals, m.p. 208°–211°C, lit.[33] m.p. 208°–211°C. IR (KBr), cm^{-1}: 675, 695, 724, 789, 890, 1154 (SO_2^{sym}), 1327 (SO_2^{as}), 1402, 1597, 1619 (C=N thiadiazole), 1647, 3369 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.76 (s, 2H, 2 NH) 7.43 (s, 4H, ArH); 7.65 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 124.8; 125.7; 132.9; 133.2; 163.5 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 33.6; H, 2.3; N, 16.7. $C_{14}H_{12}N_6Cl_2O_4S_3$ requires: C, 33.9; H, 2.4; N, 16.9%.

2,5-Bis-(4-sulfamoyl-2,6-dibromophenylamino)-1,3,4-thiadiazole **50** as white crystals, m.p. 224°–7°C. IR (KBr), cm^{-1}: 670, 696, 754, 790, 849, 1154 (SO_2^{sym}), 1329 (SO_2^{as}), 1410, 1596, 1612 (C=N thiadiazole), 1648, 3370 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.78 (s, 2H, 2 NH); 7.45 (s, 4H, ArH); 7.64 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 124.9; 125.6; 133.0; 133.2; 163.4 (the two equivalent carbons of 1,3,4-thiadiazole). Found:

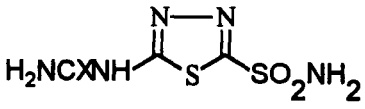
C, 28.6; H, 2.1; N, 14.2. $C_{14}H_{12}N_6Br_2O_4S_3$ requires: C, 28.7; H, 2.0; N, 14.3%.

2,5-Bis-(4-sulfamoyl-2,6-diiodophenylamino)-1,3,4-thiadiazole **51** as white crystals, m.p. 238°–9°C. IR (KBr), cm^{-1}: 618, 667, 699, 724, 793, 913, 1155 (SO_2^{sym}), 1330 (SO_2^{as}), 1411, 1596, 1610 (C=N thiadiazole), 1652, 3370 ($NH + NH_2$). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.80 (s, 2H, 2 NH); 7.45 (s, 4H, ArH); 7.66 (br s, 4H, 2 SO_2NH_2); ^{13}C-NMR (DMSO-d_6), δ, ppm: 125.5; 125.9; 133.8; 134.7; 163.5 (the two equivalent carbons of 1,3,4-thiadiazole). Found: C, 24.6; H, 1.3; N, 12.2. $C_{14}H_{12}N_6I_2O_4S_3$ requires: C, 24.7; H, 1.7; N, 12.4%.

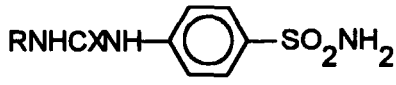
RESULTS AND DISCUSSION

Except for 4-ureido-benzenesulfonamide **18**[34] and the corresponding thioureido analogue **30**,[35] previously reported at the beginning of research in the field of antibacterial sulfonamides, and prepared from sulfanilamide **6** and cyanate or thiocyanate, respectively, the reaction of other sulfonamides possessing amino groups in their molecule with these reagents has not been investigated. Only recently such derivatives (**30**)[33] as well as the heterocyclic urea **52**[36] and the corresponding thiourea **53**[33] have been reinvestigated or prepared by this[33] and Katritzky's groups,[36] in the search for isozyme-specific or antiglaucoma agents with CA inhibitory properties. The reaction of sulfanilamide **6** with alkyl-/aryl-isocyanates or isothiocyanates for the preparation of compounds of type **54** and **55** has been reported by Roth and Degering,[37] but such sulfonamides have not been assayed as CA inhibitors. It thus appeared of interest to prepare and assay as CA inhibitors a large series of such urea-/thiourea derivatives, mainly because in a preliminary communication from this laboratory,[33] as well as in the study of Katritzky *et al.*,[36] many of the compounds bearing these moieties showed promising activities as enzyme inhibitors. It also became of interest to enlarge the series of amino-sulfonamides included in such studies to bis-sulfonamides, as well as halogeno-containing mono- or bis-sulfonamides, since such compounds show interesting biological activity connected to CA inhibition.[10,16,38]

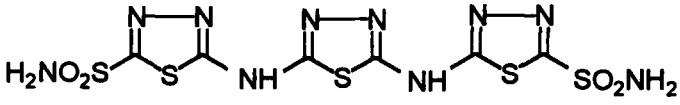
Reaction of the hydrochloride salts of sulfonamides **6**–**17**, prepared *in situ* from the corresponding sulfonamide and the required amount of aqueous concentrated HCl solution, with sodium cyanate afforded the ureas **18**–**29**, whereas the corresponding reaction with sodium thiocyanate, led to the thioureas **30**–**41**. These synthesis occurred with high yields (70–80%).



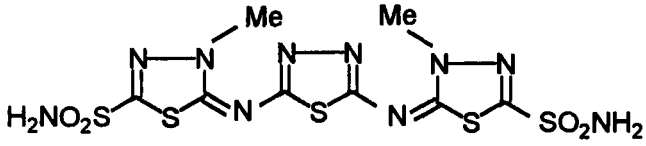
52: X = O
53: X = S



54: X = O, R = alkyl
55: X = S, R = alkyl, aryl.



56

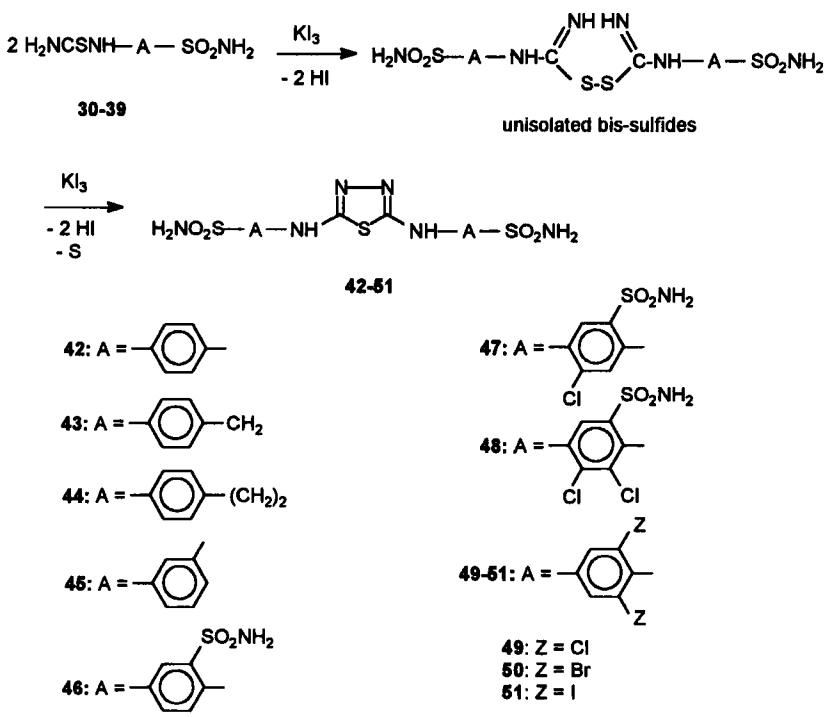


57

In the IR spectra of the synthesized derivatives **18**–**41**, in addition to the intense sulfonamido vibrations (already present in the spectra of the starting materials **6**–**17**; data not shown), the strongest bands evidenced were those due to the ureido- and the thioureido moieties, respectively. Thus, for ureas **18**–**29**, the amide I bands (C=O) appeared at 1690–1705 cm^{-1}, whereas the amide II band at 1515–1520 cm^{-1}. In the case of the thioureas **30**–**41**, the thioamide I band (C=S) was seen around 1540 cm^{-1}, the thioamide III around 1040 cm^{-1}, whereas the thioamide II band was generally overlapped by the intense SO_2 vibrations, in the region 1320–1360 cm^{-1}. In the ^{1}H-NMR spectra of compounds **18**–**41** the SO_2NH_2 and $NHCXNH_2$ protons appeared as broad singlets that readily disappeared when D_2O was added due to fast exchange with the solvent. The other signals for protons present in these compounds appeared in their normal ranges (see Material and Methods for details). In the ^{13}C-NMR spectra of thioureas **30**–**39**, the C=S carbon showed a resonance around 142.5 ppm, whereas the aromatic carbons resonated in their normal ranges.

Beasley *et al.*[7] reported a compound obtained by oxidation of 4-thioureido-benzenesulfonamide **30** with elemental iodine, but the structure of the obtained sulfonamide (possessing strong CA inhibitory properties) could not be assigned.[7] This report prompted us to reinvestigate the above synthesis, and in a short note we reported[33] that the compound probably obtained in the above work was the 1,3,4-thiadiazole derivative **42** which has been fully characterized by us. In the same study, the tris-thiadiazole **56** and the corresponding thiadiazoline **57** have also been reported.[33] Some of these compounds, such as **56** and **57** showed excellent CA inhibitory properties.[33]

These inhibitory properties, and the fact that the synthesis by which compounds **42, 56** and **57** have been prepared seems to represent a novel route to symmetrical 2,5-disubstituted-1,3,4-thiadiazole derivatives *via* thioureas, prompted us to enlarge the series of compounds prepared in this way. In the present work we report the preparation of thiadiazoles **42–51**, obtained from the thioureas **30–39**, by the sequence of reactions shown in Scheme 1, previously applied by us for obtaining compounds **42, 56** and **57**.[33]



SCHEME 1

Treatment of the thioureas **30–39** with one equivalent of alcoholic KI_3 solution ($KI + I_2$) in the presence of excess HCl afforded the 2,5-disubstituted-1,3,4-thiadiazoles **42–51**. Presumably bis-sulfides of the type shown in Scheme 1 are the intermediates of these oxidation reactions, but no attempts were made to isolate them, as their stability would be rather low.[39] They are formed by oxidation of the isothiourea tautomers of compounds **30–39** with 0.5 equivalents of iodine. Further oxidation of the bis-sulfide intermediates with the remaining 0.5 equivalents of iodine, and the concomitant extrusion of elemental sulfur leads to the thiadiazoles **42–51**. The driving force of the above processes is probably the high aromaticity of the 1,3,4-thiadiazole ring finally obtained.[33,39] Sulfur formed as a by-product in the above reactions could be isolated with yields of 75–85% (based on the initial thiourea – data not shown). Spectral data confirmed the formation of the heterocyclic ring in derivatives **42–51**. Thus, in the IR spectra of these compounds the ν(C=N) vibrations of the 1,3,4-thiadiazole were seen around 1610–1620 cm^{-1}, whereas the thioamide bands disappeared from their spectra (see Experimental part for details). In the ^{13}C-NMR spectrum of the 2,5-disubstituted 1,3,4-thiadiazoles the C=S signal (at around 140–143 ppm in the thioureas used as starting materials in the synthesis) was absent, whereas thiadiazole carbon signals appeared at 160–163 ppm, as for related compounds reported in the literature (the two carbons of the heterocyclic ring are equivalent and only one signal was observed).[33,36,39] All these data confirm that the procedure described by us here is an easy route to 2,5-disubstituted-1,3,4-thiadiazoles.

Inhibition data with the compounds prepared in the present study as well as standard CA inhibitors are shown in Table I, against three CA isozymes, CA I, II and IV.

As seen from the above data, the ureas **18–29** and the thioureas **30–41** behave as stronger inhibitors against all three investigated isozymes, when compared to the corresponding sulfonamides from which they were prepared of type **6–17**. Thioureas are more active than the corresponding urea derivatives, without exception. The presence of halogeno atom(s) substituting the aromatic ring in the molecules of these sulfonamides is beneficial for the increased inhibitory power of the obtained ureas/thioureas. For derivatives **25–27** and **37–39**, inhibitory power increases from the dichloro- to the dibromo- derivatives, and diminishes for the diiodo compounds which are the most ineffective in this sub-series. This is probably due to steric hindrance in the case of the diiodo derivatives, containing two bulky idoine atoms as substituents of the benzene nucleus. For the bis-sulfonamides **22–24** and **34–36**, the trend is already diverse, as the new derivatives are only 2–3 times less

TABLE I Biological activity of sulfonamide CA inhibitors **6–51** and standard inhibitors **1**, **3** and **4**

Compound	*IC_{50}* (nM)[c]		
	CA I[a]	*CA II*[a]	*CA IV*[b]
1 (acetazolamide)	200	7	120
3 (dichlorophenamide)	550	30	90
4 (dorzolamide)	$>5.10^6$	2	35
6 (sulfanilamide)	2800	300	3000
7 (homosulfanilamide)	2500	170	2800
8 (p-aminoethylbenzene sulfonamide)	2100	180	2450
9 (metanilamide)	2500	240	2200
10	1700	110	240
11	840	75	160
12	900	100	340
13	1700	220	1200
14	1200	150	580
15	1800	310	450
16	90	11	40
17	90	15	50
18	2200	150	300
19	1250	90	450
20	900	100	250
21	400	220	290
22	180	85	170
23	150	60	120
24	150	65	110
25	190	95	130
26	100	90	110
27	220	200	230
28	8	7	30
29	9	10	40
30	1100	100	210
31	400	35	200
32	340	70	180
33	280	190	250
34	65	75	160
35	50	55	130
36	70	60	100
37	100	110	170
38	45	70	130
39	120	100	245
40	3	8	20
41	4	10	25
42	400	130	170
43	300	90	150
44	95	60	85
45	570	150	320
46	820	9	100
47	250	5	80
48	280	10	130
49	90	10	45
50	110	6	50
51	40	12	25

[a]Human (cloned) isozyme; [b]Isolated from bovine lung microsomes. [c]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.[31]

active against HCA I, as compared to HCA II. The strongest inhibitors were the derivatives of sulfanilyl-sulfanilamide **28** and **40**, as well as metanilyl-metanilamide, **29** and **41**. Already the two parent sulfanilyl-sulfonamides **16** and **17** have high affinities for all three CA isozymes investigated here, similarly with other sulfonylamido-sulfonamides reported by this[40] or other groups.[41] Their ureido and thioureido derivatives behave as very potent inhibitors, mainly against HCA I, to which they bind with inhibition constants in the nanomolar range, similarly to the clinically effective inhibitors, such as acetazolamide or dorzolamide which bind in the similar way to HCA II. One should note the tremendous difference in affinity for HCA I of dorzolamide, the last CA inhibitor introduced into clinical medicine, as compared to other sulfonamides investigated here, and especially with the high affinity CA I compounds **28**, **29**, **40** and **41**. This is the most striking feature of some of the prepared derivatives reported here, i.e., their enhanced affinity for CA I, an isozyme generally considered much more resistant to sulfonamide inhibition as compared to CA II and to a lesser extent CA IV. As there are no CA I-specific inhibitors, and as the function of this isozyme is unknown, as outlined in the introductory section, such compounds might be used as leads for developing isozyme-specific inhibitors.

By comparing inhibition data against the first two CA isozymes for the "classical" sulfonamides **1**–**9** of Table I, it can be seen that HCA II is generally 10–30 times easier to inhibit than HCA I. Almost the same situation is maintained for the halogeno-containing derivatives and the bis-sulfonamides **10**–**15**, but the two sulfanylil-sulfonamides **16** and **17** have already an improved efficiency towards CA I. This trend is much more increased in the case of ureas and especially thioureas, with several compounds such as **28**, **40** and **41** behaving as stronger CA I inhibitors. However all of them appreciably inhibit CA II and CA IV too. Thus, the first condition in order to obtain an isozyme-specific inhibitor for CA I has been attained. The derivatives mentioned above have nanomolar affinities for CA I but selectivity has not been realized as these sulfonamides possess high affinities for other isozymes. These are promising results in the search for isozyme-specific inhibitors, and some of the prepared derivatives might constitute a lead for developing better inhibitors.

The 1,3,4-thiadiazole-bis-sulfonamides **42**–**51** on the other hand possess good inhibitory properties against all three isozymes, but mainly HCA II, with a pattern similar to that of the classical inhibitor, i.e., no discrimination for CA I, and a much stronger affinity to CA II, followed by CA IV.

In conclusion, the present study presents the synthesis and characterization of ureido-/thioureido-substituted aromatic sulfonamides, as well as a

novel route to symmetrical 2,5-disubstituted-1,3,4-thiadiazoles *via* thioureas. Some of the obtained compounds possess higher affinity to HCA I as compared to the sulfonamide sensitive isozyme II, making them interesting lead molecules for the development of CA I-specific inhibitors.

Acknowledgments

Thanks are addressed to Prof. Sven Lindskog (Umea University, Sweden) for the gift of the two CA plasmids and to Mr. Marc A. Ilies (University of Bucharest) for technical assistance with the preparation of some halogeno-sulfanilamides.

References

[1] This paper is Part 48 of the series "Carbonic Anhydrase Inhibitors". Part 47: Supuran, C.T. and Clare, B.W. (1997). *Eur. J. Med. Chem.*, in press.
[2] Hewett-Emmett, D. and Tashian, F.E. (1996) *Mol. Phylogenet. Evol.*, **5**, 50–77.
[3] Lakkis, M.M., Bergenhem, N.C. and Tashian, R.E. (1996). *Biochem. Biophys. Res. Commun.*, **226**, 268–272.
[4] Mann, T. and Keilin, D. (1940). *Nature (hand.)*, **146**, 164–165.
[5] 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.
[6] a) Vaughan, J.R., Eichler, J.A. and Anderson, G.W. (1956). *J. Org. Chem.*, **21**, 700–701. b) Young, R.W., Wood, K.H., Vaughan, J.R. and Anderson, G.W. (1956). *J. Am. Chem. Soc.*, **78**, 4649–4654.
[7] Beasley, Y.M., Overell, B.G., Petrow, V. and Stephenson, O. (1958). *J. Pharm. Pharmacol.*, **10**, 696–705.
[8] Maren, T.H. (1967). *Physiol. Rev.*, **47**, 595–782.
[9] Sisson, G.M. and Maren, T.H. (1956). *Fed. Proc.*, **15**, 484–490.
[10] Beyer, K.H. and Baer, J.E. (1961). *Pharmacol. Rev.*, **13**, 517–562.
[11] a) Maren, T.H. (1991). In *Carbonic Anhydrase – From Biochemistry and Genetics to Physiology and Clinical Medicine* (F. Botré, G. Gros and B.T. Storey Eds.), pp. 186–207. VCH; Weinheim. b) Maren, T.H. (1995). *J. Glaucoma*, **4**, 49–62.
[12] Larson, E.B., Roach, R.C., Schoene, R.B. and Hornbein, T.F. (1982). *J. Am. Med. Ass.*, **248**, 328–332.
[13] a) 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. b) Reiss, W.G. and Oles, K.S. (1996). *Ann. Pharmacother.*, **30**, 514–519.
[14] 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.
[15] Supuran, C.T. (1994). In *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism* (I. Puscas Ed.) pp. 29–111. Helicon; Timisoara.
[16] Supuran, C.T., Conroy, C.W. and Maren, T.H. (1996). *Eur. J. Med. Chem.*, **31**, 843–846.
[17] a) Lindskog, S. and Wistrand, P.J. (1987). In *Design of Enzyme Inhibitors as Drugs* (M.J. Sandler and H.J. Smith Eds.), pp. 698–723. Oxford Univ. Press. b) Scozzafava, A. and Supuran, C.T. (1997). *Metal Based Drugs*, **4**, 19–26. c) Mincione, G., Scozzafava, A. and Supuran, C.T. (1997). *Metal Based Drugs*, **4**, 27–34.
[18] a) Sugrue, M.F. (1996). *J. Ocul. Pharmacol. Ther.*, **12**, 363–376. b) Hartenbaum, D. (1996). *Clin. Ther.*, **18**, 460–465.

[19] a) Maus, T.L., Larsson, L.I., McLaren, J.W. and Brubaker, R.F. (1997). *Arch. Ophthalmol.*, **115**, 45–49. b) Heijl, A., Strahlman, E., Sverisson, T., Brinchman-Hansen, O., Pustjarvi, T. and Tipping, R. (1997). *Ophthalmology*, **104**, 137–142. c) Maren, T.H., Conroy, C.W., Wynns, G.C. and Levy, N.S. (1997). *J. Ocul. Pharmacol. Ther.*, **13**, 23–30. d) Schoenwald, R.D., Deshpande, G.S., Rethwisch, D.G. and Barfknecht, C.F. (1997). *J. Ocul. Pharmacol. Ther.*, **13**, 41–59.
[20] a) Supuran, C.T., Balaban, A.T., Gheorghiu, M.D., Schiketanz, A., Dinculescu, A. and Puscas, I. (1990). *Rev. Roum. Chim.*, **35**, 399–405. b) 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. c) Supuran, C.T. and Clare, B.W. (1995). *Eur. J. Med. Chem.*, **30**, 687–696. d) Ilies, M.A., Iorga, B., Cristea, T., Banciu, M.D., Chiraleu, F., Scozzafava, A. and Supuran, C.T. (1997). *Eur. J. Med. Chem.*, in press. e) Supuran, C.T. and Scozzafava, A. (1997). *J. Enz. Inhib.*, **12**, 37–51.
[21] Wistrand, P.J. and Lindqvist, A. (1991). In *Carbonic Anhydrase – From Biochemistry and Genetics to Physiology and Clinical Medicine*, (F. Botré, G. Gros and B.T. Storey Eds.), pp. 352–374. VCH; Weinheim.
[22] Chapman, S.K. and Maren, T.H. (1978). *Biochem. Biophys. Acta*, **527**, 272–276.
[23] Jeffery, S., Edwards, Y. and Carter, N. (1980). *Biochem. Genetics*, **18**, 843–849.
[24] a) Northey, E.H. (1948). *The sulfonamides and allied compounds*, pp. 3–339. Reinhold; New York. b) Crossley, M.L., Northey, E.H. and Hultquist, M.E. (1938). *J. Am. Chem. Soc.*, **60**, 2222–2224. c) Scudi, J.V. (1937). *J. Am. Chem. Soc.*, 59, 1480–1483. d) Bauer, H. (1939). *J. Am. Chem. Soc.*, **61**, 613–616.
[25] Forsman, C., Behravan, G., Osterman, A. and Jonsson, B.H. (1988). *Acta. Chem. Scand.*, **B42**, 314–318.
[26] Behravan, G., Jonasson, P., Jonsson, B.H. and Lindskog, S. (1991). *Eur. J. Biochem.*, **198**, 589–592.
[27] Khalifah, R.G., Strader, D.J., Bryant, S.H. and Gibson, S.M. (1977). *Biochemistry*, **16**, 2241–2247.
[28] Nyman, P.O. and Lindskog, S. (1964). *Biochim. Biophys. Acta*, **85**, 141–151.
[29] Henderson, L.E., Henriksson, D. and Nyman, P.O. (1976). *J. Biol. Chem.*, **251**, 5457–5463.
[30] Maren, T.H., Wynns, G.C. and Wistrand, P.J. (1993). *Mol. Pharmacol.*, **44**, 901–906.
[31] Maren, T.H. (1960). *J. Pharmacol. Exp. Ther.*, **130**, 24–29.
[32] Supuran, C.T., Manole, G., Schiketanz, A., Gheorghiu, M.D. and Puscas, I. (1991). *Rev. Roum. Chim.*, **36**, 251–255.
[33] Supuran, C.T. (1995). *Rev. Roum. Chim.*, **40**, 643–651.
[34] Cox, E.H. (1940). *J. Am. Chem. Soc.*, **62**, 743–4.
[35] Walker, J. (1940). *J. Chem. Soc.*, 1304–1307.
[36] Katritzky, A.R., Caster, K.C., Maren, T.H., Conroy, C.W. and Bar-Ilan, A. (1987). *J. Med. Chem.*, **30**, 2058–2062.
[37] Roth, J.S. and Degering, E.F. (1945). *J. Am. Chem. Soc.*, **67**, 126–128.
[38] a) Boggiano, B.G., Condon, S., Davies, M.T., Jackman, G.B., Overell, B.G., Petrow, V., Stephenson, O. and Wild, A.M. (1960). *J. Pharm. Pharmacol.*, **12**, 419–425. b) Novello, F.C. and Sprague, J.M. (1957). *J. Am. Chem. Soc.*, **79**, 2028–2029. c) Maren, T.H. and Wiley, C.E. (1968). *J. Med. Chem.*, **11**, 228–232.
[39] Kornis, G. (1984). In *Comprehensive Heterocyclic Chemistry* (A.R. Katritzky Ed.), Vol. 6, part 4B, pp. 545–578. Pergamon; New York.
[40] Supuran, C.T., Briganti, F. and Scozzafava, A. (1997). *J. Enz. Inhib.*, **12**, 175–190.
[41] Vidgren, J., Svensson, A. and Liljas, A. (1993). *Int. J. Biol. Macromol.*, **15**, 97–100.