Eur. J. Med. Chem. 33 (1998) 83–93
© Elsevier, Paris

Carbonic anhydrase inhibitors – Part 49**: Synthesis of substituted ureido and thioureido derivatives of aromatic/heterocyclic sulfonamides with increased affinities for isozyme I

Claudiu T. Supuran[a]*, Andrea Scozzafava[a], Bogdan C. Jurca[b], Marc A. Ilies[b]

[a]*Università degli Studi di Firenze, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, 50121 Firenze, Italy*
[b]*University of Bucharest, TH Maren Laboratory of Bioorganic and Bioinorganic Chemistry, Bd. Republicii 13, 70346 Bucharest, Roumania*

(Received 11 June 1997; accepted 11 September 1997)

Abstract – Reaction of nine aromatic/heterocyclic sulfonamides containing a free amino group with aryl isocyanates/isothiocyanates or allyl isothiocyanate afforded the corresponding urea/thiourea derivatives, which were characterized by standard physico-chemical procedures and assayed as inhibitors of three isozymes of carbonic anhydrase (CA), i.e. hCA I, hCA II and bCA IV (h = human, b = bovine isozyme). Another series of compounds, 1,5-disubstituted-2-thiobiuret derivatives, were prepared by reaction of 3,4-dichlorophenyl isocyanate with thioureido-containing aromatic/heterocyclic sulfonamides. Good inhibition of all these three CA isozymes was observed with the new compounds, but an exciting finding was that the ureas/thioureas and especially the above-mentioned thiobiurets reported here have an increased affinity to the slow isozyme hCA I, generally less susceptible to inhibition by sulfonamides, as compared to the rapid isozymes hCA II and bCA IV. Some of the new compounds might constitute good lead molecules for developing more selective CA I inhibitors. © Elsevier, Paris

aromatic/heterocyclic sulfonamide / carbonic anhydrase / isozyme I, II, IV / isocyanate / isothiocyanate / 1,3-disubstituted (thio)ureas / 1,5-disubstituted-2-thiobiurets / isozyme-specific inhibitor

1. Introduction

Ten carbonic anhydrases (CAs, EC 4.2.1.1) or CA-like proteins (CA I–X) were isolated in mammals up to now [2, 3]. They generally catalyze a very simple but critical physiological reaction, the reversible hydration of carbon dioxide to bicarbonate [4]. Already in the 1950's, after the report of Mann and Keilin [5] that sulfanilamide is a specific inhibitor of this zinc enzyme, red cell CAs were a target for drug design. 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 [4–8], leading to the first such clinical agent, acetazolamide **1** (in 1956) [9], followed shortly thereafter by methazolamide 2 [9, 10] and dichlorophenamide **3** [11]. Compounds **1–3** are still used clinically for the treatment or prevention of a variety of disorders such as glaucoma [12], mountain sickness [13], or epilepsy [14, 15]. Their use as diuretics is relatively limited nowadays, except for dichlorophenamide **3** [16]. Still, this class of pharmacological agents has led to the development of two important types of diuretic drugs [4, 11, 17] the benzothiadiazines and the high-ceiling diuretics, widely used for the mobilization of edema fluids in a large number of disorders [16, 17] (see *figure 1*).

The major drawback of classical sulfonamide CA inhibitors of type **1–3** is their total lack of specificity due to indiscriminate inhibition of all isozymes (except for CA III, which is much less sensitive to this class of inhibitors [4]) in many tissues in which these are present [4, 18]. Still, this problem may be circumvented by designing organ-selective compounds, as well as isozyme-specific inhibitors [4, 18]. An important example from the first type of derivatives is represented by the topically active antiglaucoma agents [19, 20] recently introduced in clinical medicine with great success. Thus, dorzolamide **4**, a water-soluble

*Correspondence and reprints
**See [1]

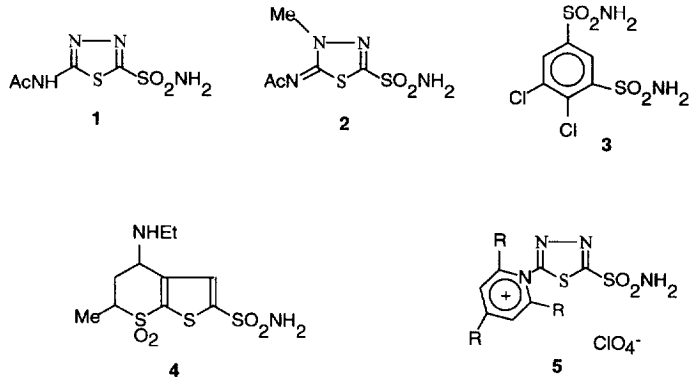

Figure 1.

sulfonamide, is highly effective in reducing elevated intraocular pressure in glaucomatous patients, after topical instillation directly into the eye, without the side effects of systematically administered inhibitors (of type **1–3** for instance), as only the enzyme within the cilliary processes of the eye is inhibited [19–24]. On the other hand, although few isozyme-specific sulfonamide inhibitors have been reported up to now [4] 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) [25–26]. Thus, in the search of inhibitors with higher affinity for diverse isozymes, a lot of aromatic/heterocyclic sulfonamides are permanently designed and synthesized in different laboratories [4, 25–32], which ultimately have led to interesting developments, such as the obtaining of a sulfonamide probe with nanomolar affinity to hCA II, used for fluorescence anisotropy detection of zinc with a CA-based biosensor [32] or the design of tight binding inhibitors of hCA II (and to a lesser degree hCA I-avid compounds too) by Whitesides' [33] and Burbaum's [34] groups. Recently this groups has reported [1] that aromatic sulfonamides or bis-sulfonamides possessing among others the unsubstituted ureido or thioureido moieties (H_2NCXNH–; X = O, S) attached to the aromatic ring, not only behave as very potent inhibitors against three isozymes, hCA I, hCA II and bCA IV (h = human, b = bovine isozyme), but possess an unexpectedly high affinity for the slow isozyme hCA I. Using as lead molecules such derivatives mentioned above, in this paper we extended the range of compounds of this type, reporting novel sulfonamides possessing aryl/allyl-substituted ureido/thioureido moieties in their molecule. The new inhibitors were characterized by standard procedures and were assayed for inhibition of the previously mentioned CA isozymes. Some compounds show very good hCA I inhibition profiles, although they equally act as powerful inhibitors for the other two isozymes.

2. Experimental protocols

2.1. Chemistry

Melting points were determined with a heating plate microscope and are not corrected; IR spectra were obtained in KBr pellets with a Perkin-Elmer 16PC FTIR spectrometer, whereas ^{1}H- and ^{13}C-NMR spectra with a Bruker 400XLP apparatus (working at 400 MHz for the proton spectra, and 100.61 MHz for the carbon spectra, respectively) in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Mass spectra were obtained on a MAT 310 spectrometer operating at 70 eV, with an electron emission of 100 mA and an ion source temperature of 150 °C. Elemental Analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and were ±0.4% of the theoretical values.

Sulfonamides **6–8** and **10–12** used in syntheses were commercially available (from Sigma, Acros or Aldrich) whereas compounds **9**, **13** and **14** were prepared as described in the literature [35–37]. Metanilamide **9** was prepared from 3-aminobenzene-sulfonyl fluoride hydrochloride (Acros) by treatment with excess aqueous ammonia. The dichlorosulfanilamide **13** was obtained by chlorination of sulfanilamide **6** with HCl–H_2O_2 [35, 36] whereas 5-amino-1,3,4-thiadiazole-2-sulfonamide **14** was prepared from acetazolamide **1** (Sigma) by deacetylation with concentrated HCl, as described in the literature [37]. All these compounds were recrystallized from ethanol–water (1:1, v/v). Phenyl isocyanate and isothiocyanate, 3,4-dichlorophenyl isocyanate, allyl isothiocyanate as well as other inorganic reagents and solvents were from Acros or Merck, and were used without further purification. Thioureidosulfonamides **51–59** were prepared as described previously [1, 38].

hCA I and hCA 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. [39] (the two plasmids were a gift from Prof. Sven Lindskog, Umeå University, Sweden). Cell growth conditions were those described by Lindskog's group [40] and enzymes were purified by affinity chromatography according to the method of Khalifah et al. [41]. Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 mM^{-1} cm^{-1} for CA I and 54 mM^{-1} cm^{-1} for CA II, respectively, based on M_r = 28.85 kDa for hCA I, and 29.30 kDa for hCA II, respectively [42, 43]. bCA IV was isolated from bovine lung microsomes as described by Maren et al., and its concentration has been determined by titration with ethoxzolamide [44].

*2.2. General procedure for the preparation of ureas **15–32** and thioureas **33–50***

An amount of 10 mM sulfonamide **6–14** was dissolved/suspended in 50 mL of tetrahydrofuran and the solution was heated at refluxation for 20 min, then a solution obtained from 10 mM of aryl/allyl isocyanate/isothiocyanate dissolved in 5 mL of the same solvent was added dropwise and the obtained mixture was heated at reflux for 4–6 hours. A small volume (100 μL) of triethylamine was added as catalyst after the first half our of heating at reflux into the above mixture. By means of TLC the conversion of all the sulfonamide **6–14** to the corresponding urea/thiourea derivative has been monitored. When the reaction was completed, the solvent was evaporated until a small volume of the reaction mixture was obtained. Generally compounds **15–50** 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%.

*2.3. General procedure for the reaction of thiureido-sulfonamides **51–59** with 3,4-dichlorophenyl isocyanate **60***

An amount of 2 mM of thioureido sulfonamide **51–59** and the equimolar amount of 3,4-dichloro-phenyl isocyanate **60** were heated at reflux in 50 mL anhydrous DMF or acetonitrile, in the presence of 100 μL of triethylamine, for 15 h. The solvent was evaporated in vacuo and the obtained residue treated with 5 mL of 2 N aqueous HCl solution. The obtained precipitate was filtered and recrystallized from acetone/water (2:1, v/v). Yields in compounds **61–69** were around 50%.

*2.3.1. N^1-(4-Sulfamoylphenyl)-N^3-phenyl-urea **15***

White crystals, mp 232–234 °C, the compound is mentioned by Roth and Degering [45] but only its mp, without other physico-chemical/biochemical characterization reported; literature [45]: mp 231–233 °C. IR (KBr), cm^{-1}: 650, 773, 786, 820, 864, 989, 1028, 1043, 1146 (SO_2^{sym}), 1325 (SO_2^{as}), 1410, 1510 (amide II), 1730 (CO), 3165 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.39 (m, 9H, ArH, 1,4-phenylene + Ph); 7.55 (br s, 2H, SO_2NH_2); 9.33 (s, 1H, Ph–N*H*); 9.46 (s, 1H, N*H*); Anal. $C_{13}H_{13}N_3O_3S$ (C, H, N).

*2.3.2. N^1-(4-Sulfamoylphenylmethyl)-N^3-phenyl-urea **16***

White crystals, mp 210–212 °C, IR (KBr), cm^{-1}: 622, 671, 854, 881, 930, 983, 1020, 1035, 1151 (SO_2^{sym}), 1332 (SO_2^{as}), 1515 (amide II), 1730 (CO), 3165 (NHCONH); 3310 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CONHCH_2); 7.08–7.44 (m, 9H, ArH, phenylene + Ph); 7.46 (s, 2H, SO_2NH_2); 9.33 (s, 1H, Ph–N*H*); 9.40 (s, 1H, NH); Anal. $C_{14}H_{15}N_3O_3S$ (C, H, N).

*2.3.3. N^1-(4-Sulfamoylphenylethyl)-N^3-phenyl-urea **17***

White crystals, mp 232–234 °C; IR (KBr), cm^{-1}: 653, 826, 940, 1037, 1060, 1080, 1139 (SO_2^{sym}), 1332 (SO_2^{as}), 1516 (amide II), 1735 (CO), 3170 (NHCONH); 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); 6.97 (br s, 2H, SO_2NH_2); 7.05–7.54 (m, 9H, ArH, phenylene + Ph); 9.32 (s, 1H, Ph–N*H*); 9.40 (s, 1H, NH); Anal. $C_{15}H_{17}N_3O_3S$ (C, H, N).

*2.3.4. N^1-(3-Sulfamoylphenyl)-N^3-phenyl-urea **18***

White crystals, mp 226–228 °C IR (KBr), cm^{-1}: 627, 730, 809, 882, 975, 1010, 1040, 1134 (SO_2^{sym}), 1336 (SO_2^{as}), 1512 (amide II), 1730 (CO), 3160 (NHCONH); 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.12 (br s, 2H, + SO_2NH_2); the signal disappears by addition of D_2O into the NMR tube; 7.10–7.61 (m, 9H, ArH, 1,3-phenylene + Ph); 9.33 (s, 1H, Ph–N*H*); 9.47 (s, 1H, NH); Anal. $C_{13}H_{13}N_3O_3S$ (C, H, N).

*2.3.5. N^1-(2,4-Disulfamoylphenyl)-N^3-phenyl-urea **19***

White crystals, mp 262–265 °C; IR (KBr), cm^{-1}: 621, 715, 748, 775, 824, 869, 974, 1016, 1043, 1155 (SO_2^{sym}), 1344 (SO_2^{as}), 1414, 1515 (amide II), 1728 (CO), 3166 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10–7.58 (m, 8H, ArH); 7.60 (br s, 4H, 2 SO_2NH_2); 9.34 (s, 1H, Ph–N*H*); 9.65 (s, 1H, NH); Anal. $C_{13}H_{15}N_4O_5S_2$ (C, H, N).

*2.3.6. N^1-(2,4-Disulfamoyl-6-chlorophenyl)-N^3-phenyl-urea **20***

White crystals, mp 270–272 °C; IR (KBr), cm^{-1} : 627, 742, 786, 822, 884, 951, 1015, 1060, 1153 (SO_2^{sym}), 1344 (SO_2^{as}), 1470, 1520 (amide II), 1724 (CO), 3165 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10–7.53 (m, 7H, ArH); 7.63 (br s, 4H, 2 SO_2NH_2); 9.33 (s, 1H, Ph–N*H*); 9.84 (s, 1H, NH); Anal. $C_{13}H_{14}N_4O_5S_2Cl$ (C, H, N).

*2.3.7. N^1-(2,4-Disulfamoyl-5,6-dichlorophenyl)-N^3-phenyl-urea **21***

White crystals, mp 283–284 °C; IR (KBr), cm^{-1}: 604, 698, 754, 788, 839, 850, 952, 1076, 1155 (SO_2^{sym}), 1350 (SO_2^{as}), 1515 (amide II), 1724 (CO), 3170 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.12–7.50 (m, 6H, ArH); 7.58 (br s, 4H, 2 SO_2NH_2); 9.39 (s, 1H, Ph–N*H*); 9.88 (s, 1H, NH); Anal. $C_{13}H_{13}N_4O_5S_2Cl_2$ (C, H, N).

*2.3.8. N^1-(2,6-Dichloro-4-sulfamoylphenyl)-N^3-phenyl-urea **22***

White crystals, mp 244–245 °C; IR (KBr), cm^{-1}: 684, 731, 778, 853, 932, 1046, 1153 (SO_2^{sym}), 1337 (SO_2^{as}), 1460, 1515 (amide II), 1730 (CO), 3180 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.06–7.45 (s, 7H, ArH); 7.62 (br s, 2H, SO_2NH_2); 9.33 (s, 1H, Ph–N*H*); 9.49 (s, 1H, NH); Anal. $C_{13}H_{11}N_3O_3SCl_2$ (C, H, N).

*2.3.9. N^1-(2-Sulfonamido-1,3,4-thiadiazol-5-yl)-N^3-phenyl-urea **23***

White crystals, mp 255–257 °C, lit. [51] mp 255–258 °C. IR (KBr), cm^{-1}: 760, 1178 (SO_2^{sym}), 1343 (SO_2^{as}), 1545 (amide II), 1585, 1730 (CO), 2820, 3290 (NHCONH), 3380; ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10–7.80 (m, 5H, ArH); 8.43 (br s, 2H, SO_2NH_2); 9.33 (s, 1H, PhNH); 11.65 (br s, 1H, N*H*Ar); Anal. $C_9H_9N_5O_3S_2$ (C, H, N).

*2.3.10. N^1-(4-Sulfamoylphenyl)-N^3-3,4-dichlorophenyl-urea **24***

White crystals, mp 260–261 °C; IR (KBr), cm^{-1}: 658, 771, 790, 832, 875, 989, 1028, 1040, 1141 (SO_2^{sym}), 1330 (SO_2^{as}),

1410, 1517 (amide II), 1730 (CO), 3163 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.38 (m, AA'BB', 4H, ArH, 1,4-phenylene); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.55 (br s, 2H, SO_2NH_2); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.33 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.65 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{13}H_{11}N_3O_3SCl_2$ (C, H, N).

2.3.11. N^1-(4-Sulfamoylphenylmethyl)-N^3-3,4-dichlorophenyl-urea ***25***

White crystals, mp 241–243 °C; IR (KBr), cm^{-1}: 644, 685, 840, 882, 930, 985, 1040, 1150 (SO_2^{sym}), 1339 (SO_2^{as}), 1515 (amide II), 1720 (CO), 3170 (NHCONH); 3310 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, $CONHCH_2$); 7.08–7.41 (m, AA'BB', 4H, ArH, phenylene); 7.46 (s, 2H, SO_2NH_2) 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.89 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.30 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.64 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{12}H_{13}N_3O_3SCl_2$ (C, H, N).

2.3.12. N^1-(4-Sulfamoylphenylethyl)-N^3-3,4-dichlorophenyl-urea ***26***

White crystals, mp 254–256 °C. IR (KBr), cm^{-1}: 714, 889, 980, 1030, 1085, 1142 (SO_2^{sym}), 1339 (SO_2^{as}), 1510 (amide II), 1730 (CO), 3178 (NHCONH); 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); 6.89 (br s, 2H, SO_2NH_2); 7.05–7.52 (m, AA'BB', 4H, ArH, phenylene); 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.34 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.67 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{13}H_{15}N_3O_3SCl_2$ (C, H, N).

2.3.13. N^1-(3-Sulfamoylphenyl)-N^3-3,4-dichlorophenyl-urea ***27***

White crystals, mp 258–259 °C IR (KBr), cm^{-1}: 733, 840, 960, 1040, 1045, 1139 (SO_2^{sym}), 1343 (SO_2^{as}), 1517 (amide II), 1730 (CO), 3170 (NHCONH); 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.12 (br s, 2H, + SO_2NH_2); the signal disappears by addition of D_2O into the NMR tube; 7.08–7.45 (m, 4H, ArH, 1,3-phenylene); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.61 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.33 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.60 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{13}H_{11}N_3O_3SCl_2$ (C, H, N).

2.3.14. N^1-(2,4-Disulfamoylphenyl)-N^3-3,4-dichlorophenyl-urea ***28***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 690, 715, 790, 837, 995, 1017, 1040, 1155 (SO_2^{sym}), 1347 (SO_2^{as}), 1515 (amide II), 1728 (CO), 3165 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.26 (d, 2H, ArH from 2,4-disulfamoylphenyl); 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.50 (s, 1H, ArH from 2,4-disulfamoylphenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.68 (br s, 4H, 2 SO_2NH_2); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.43 (s, 1H, $(H_2NO_2S)_2C_6H_3NH$); 10.75 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{13}H_{13}N_4O_5S_2Cl_2$ (C, H, N).

2.3.15. N^1-(2,4-Disulfamoyl-6-chlorophenyl)-N^3-3,4-dichlorophenyl-urea ***29***

White crystals, mp 292–294 °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: 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.40 (s, 1H, ArH from disulfamoyl-chlorophenyl); 7.50 (s, 1H, ArH from disulfamoyl-chlorophenyl); 7.58 (br s, 4H, 2 SO_2NH_2); 7.64 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.39 (s, 1H, $(H_2NO_2S)_2ClC_6H_2NH$); 10.71 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{13}H_{12}N_4O_5S_2Cl_3$ (C, H, N).

2.3.16. N^1-(2,4-Disulfamoyl-5,6-dichlorophenyl)-N^3-3,4-dichlorophenyl-urea ***30***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 654, 702, 754, 790, 850, 958, 1060, 1156 (SO_2^{sym}), 1360 (SO_2^{as}), 1515 (amide II), 1734 (CO), 3170 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.50 (s, 1H, ArH from disulfamoyldichlorophenyl); 7.59 (br s, 4H, 2 SO_2NH_2); 7.66 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.99 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.47 (s, 1H, $(H_2NO_2S)_2Cl_2C_6HNH$); 10.76 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{13}H_{11}N_4O_5S_2Cl_4$ (C, H, N).

2.3.17. N^1-(2,6-Dichloro-4-sulfamoylphenyl)-N^3-3,4-dichlorophenyl-urea ***31***

White crystals, mp 264–265 °C. IR (KBr), cm^{-1}: 680, 734, 798, 880, 902, 1046, 1152 (SO_2^{sym}), 1336 (SO_2^{as}), 1420, 1517 (amide II), 1730 (CO), 3180 (NHCONH); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.45 (s, 2H, ArH from 2,6-dichlorophenyl); 7.60 (br s, 2H, SO_2NH_2); 7.63 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.30 (s, 1H, $H_2NO_2SCl_2C_6H_2NH$); 10.69 (br s, 1H, $NHC_6H_3Cl_2$); Anal. $C_{13}H_9N_3O_3SCl_4$ (C, H, N).

2.3.18. N^1-(2-Sulfonamido-1,3,4-thiadiazol-5-yl)-N^3-3,4-dichlorophenyl-urea ***32***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 768, 983, 1176 (SO_2^{sym}), 1344 (SO_2^{as}), 1545 (amide II), 1585, 1730 (CO), 2820, 3290 (NHCONH), 3380; ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.63 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.43 (br s, 2H, SO_2NH_2); 10.48 (br s, 1H, $NHC_6H_3Cl_2$); 11.52 (s, 1H, H_2NO_2S-1,3,4-thiadiazole-N*H*); Anal. $C_9H_7N_5O_3S_2Cl_2$ (C, H, N).

2.3.19. N^1-(4-Sulfamoylphenyl)-N^3-phenyl-thiourea ***33***

White crystals, mp 201–203 °C, the compound is mentioned by Roth and Degering [45], but without physico-chemical/biochemical characterization; lit. [45] mp 190–191 °C. IR (KBr), cm^{-1}: 725 830, 864, 993, 1043 (thioamide III), 1146 (SO_2^{sym}), 1327 (SO_2^{as}), 1410, 1539 (thioamide I), 3289 (NHCSNH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.70 and 6.94 (br s, 2H, NHCSNH); 7.20–7.80 (m, 9H, ArH, 1,4-phenylene + Ph); 7.55 (br s, 2H, SO_2NH_2); the last signal disapears by addition of D_2O into the NMR tube. Anal. $C_{13}H_{13}N_3O_2S_2$ (C, H, N).

2.3.20. N^1-(4-Sulfamoylphenylmethyl)-N^3-phenyl-thiourea ***34***

White crystals, mp 218–220 °C, IR (KBr), cm^{-1}: 638, 671, 931, 983, 1040 (thioamide III), 1150 (SO_2^{sym}), 1332 (SO_2^{as}), 1430, 1539 (thioamide I), 3289 (NHCSNH), 3320 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, $CONHCH_2$); 6.87 and 6.93 (br s, 2H, NHCSNH); 7.21–7.80 (m, 9H, ArH, phenylene + Ph); 7.46 (s, 2H, SO_2NH_2); the last signal disappears by addition of D_2O into the NMR tube. Anal. $C_{14}H_{15}N_3O_2S_2$ (C, H, N).

2.3.21. N^1-(4-Sulfamoylphenylethyl)-N^3-phenyl-thiourea ***35***

White crystals, mp 189–181 °C, IR (KBr), cm^{-1}: 653, 884, 1037 (thioamide III), 1139 (SO_2^{sym}), 1339 (SO_2^{as}), 1430, 1539 (thioamide I), 3295 (NHCSNH), 3330 (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$_2$CH$_2$ bridge); 6.90 and 6.95 (br s, 2H, NHCSNH); 7.15–7.76 (m, 9H, ArH, phenylene + Ph); 7.45 (br s, 2H, SO$_2$NH$_2$) the last signal disappears by addition of D$_2$O into the NMR tube. Anal. $C_{15}H_{17}N_3O_2S_2$ (C, H, N).

2.3.22. N^1-(3-Sulfamoylphenyl)-N^3-phenyl-thiourea ***36***

White crystals, mp 200–202 °C IR (KBr), cm^{-1}: 621, 840, 1020 (thioamide III), 1134 (SO$_2^{sym}$), 1343 (SO$_2^{as}$), 1438, 1543 (thioamide I), 3295 (NHCSNH), 3350 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.90 and 6.96 (br s, 2H, NHCSNH); 7.18–7.81 (m, 9H, ArH, 1,3-phenylene + Ph); 7.72 (br s, 2H, + SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{13}H_{13}N_3O_2S_2$ (C, H, N).

2.3.23. N^1-(2,4-Disulfamoylphenyl)-N^3-phenyl-thiourea ***37***

White crystals, mp 228–229 °C; IR (KBr), cm^{-1}: 669, 795, 817, 863, 1030 (thioamide III), 1155 (SO$_2^{sym}$), 1345 (SO$_2^{as}$), 1438, 1541 (thioamide I), 3295 (NHCSNH), 3330 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.75 and 6.92 (br s, 2H, NHCSNH); 7.20–7.80 (m, 8H, ArH); 7.60 (br s, 4H, 2 SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{13}H_{15}N_4O_4S_3$ (C, H, N).

2.3.24. N^1-(2,4-Disulfamoyl-6-chlorophenyl)-N^3-phenyl-thiourea ***38***

White crystals, mp 221–224 °C; IR (KBr), cm^{-1}: 657, 750, 877, 956, 1015, 1040 (thioamide III), 1153 (SO$_2^{sym}$), 1346 (SO$_2^{as}$), 1446, 1547 (thioamide I), 3290 (NHCSNH), 3360 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.77 and 6.86 (br s, 2H, NHCSNH); 7.20–7.75 (m, 7H, ArH); 7.58 (br s, 4H, 2 SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{13}H_{15}N_4O_4S_3Cl$ (C, H, N).

2.3.25. N^1-(2,4-Disulfamoyl-5,6-dichloro phenyl)-N^3-phenyl-thiourea ***39***

White crystals, mp 236–238 °C; IR (KBr), cm^{-1}: 604, 698, 788, 839, 952, 1030 (thioamide III), 1155 (SO$_2^{sym}$), 1350 (SO$_2^{as}$), 1443, 1547 (thioamide I), 3295 (NHCSNH), 3330 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.82 and 6.90 (br s, 2H, NHCSNH); 7.20–7.70 (m, 6H, ArH); 7.59 (br s, 4H, 2 SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{13}H_{14}N_4O_4S_3Cl_2$ (C, H, N).

2.3.26. N^1-(2,6-Dichloro-4-sulfamoylphenyl)-N^3-phenyl-thiourea ***40***

White crystals, mp 130–133 °C; IR (KBr), cm^{-1}: 734, 798, 880, 1038 (thioamide III), 1155 (SO$_2^{sym}$), 1330 (SO$_2^{as}$), 1420, 1540 (thioamide I), 3295 (NHCSNH), 3330 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.68 and 6.89 (br s, 2H, NHCSNH); 7.18–7.75 (m, 7H, ArH); 7.60 (br s, 2H, SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{13}H_{11}N_3O_2S_2Cl_2$ (C, H, N).

2.3.27. N^1-(2-Sulfonamido-1,3,4-thiadiazol-5-yl)-N^3-phenyl-thiourea ***41***

White crystals, mp 154–156 °C; IR (KBr), cm^{-1}: 721, 886, 1030 (thioamide III), 1175 (SO$_2^{sym}$), 1343 (SO$_2^{as}$), 1420, 1438, 1541 (thioamide I), 1640 (C=N), 3295 (NHCSNH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15–7.68 (m, 5H, ArH); 7.88 and 8.09 (br s, 2H, NHCSNH); 8.43 (br s, 2H, SO$_2$NH$_2$); this signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_9H_9N_5O_2S_3$ (C, H, N).

2.3.28. N^1-(4-Sulfamoylphenyl)-N^3-allyl-thiourea ***42***

White crystals, mp 191–193 °C, lit [45] mp 189–190 °C. IR (KBr), cm^{-1}: 659, 810, 864, 989, 1028, 1040 (thioamide III), 1146 (SO$_2^{sym}$), 1325 (SO$_2^{as}$), 1410, 1547 (thioamide I), 3298 (NHCSNH), 3360 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNH*CH$_2$*); 5.60–5.97 (m, 3H, CH=CH$_2$); 6.70 and 6.82 (br s, 2H, NHCSNH); 7.05–7.46 (m, AA'BB', 4H, ArH, 1,4-phenylene); 7.55 (br s, 2H, SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{10}H_{13}N_3O_2S_2$ (C, H, N).

2.3.29. N^1-(4-Sulfamoylphenylmethyl)-N^3-allyl-thiourea ***43***

White crystals, mp 167–169 °C, IR (KBr), cm^{-1}: 638, 888, 937, 983, 1030 (thioamide III), 1150 (SO$_2^{sym}$), 1332 (SO$_2^{as}$), 1540 (thioamide I), 3294 (NHCSNH), 3330 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNH*CH$_2$* from allyl); 4.90 (s, 2H, CSNH*CH$_2$* from phenylmethyl); 5.60–5.97 (m, 3H, CH=CH$_2$); 6.87 and 6.95 (br s, 2H, NHCSNH); 7.08–7.53 (m, AA'BB', 4H, ArH, phenylene); 7.46 (s, 2H, SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{11}H_{15}N_3O_2S_2$ (C, H, N).

2.3.30. N^1-(4-Sulfamoylphenylethyl)-N^3-allyl-thiourea ***44***

White crystals, mp 144–146 °C, IR (KBr), cm^{-1}: 653, 884, 1035 (thioamide III), 1080, 1139 (SO$_2^{sym}$), 1338 (SO$_2^{as}$), 1415 1547 (thioamide I), 3298 (NHCSNH), 3350 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH$_2$ from the CH$_2$CH$_2$ bridge); 3.90 (t, 2H, βCH$_2$ from the CH$_2$CH$_2$ bridge); 4.45–4.60 (m, 2H, CSNH*CH$_2$* from allyl); 5.60–5.97 (m, 3H, CH=CH$_2$); 6.90 and 6.96 (br s, 2H, NHCSNH); 7.05–7.60 (m, AA'BB', 4H, ArH, phenylene); 7.85 (br s, 2H, SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{12}H_{17}N_3O_2S_2$ (C, H, N).

2.3.31. N^1-(3-Sulfamoylphenyl)-N^3-allyl-thiourea ***45***

White crystals, mp 159–161 °C IR (KBr), cm^{-1}: 621, 739, 840, 975, 1045 (thioamide III), 1134 (SO$_2^{sym}$), 1333 (SO$_2^{as}$), 1545 (thioamide I), 3290 (NHCSNH), 3330 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNH*CH$_2$*); 5.60–5.97 (m, 3H, CH=CH$_2$); 6.69 and 6.90 (br s, 2H, NHCSNH); 7.12 (br s, 2H, + SO$_2$NH$_2$); the signal disappears by addition of D$_2$O into the NMR tube; 7.08–7.59 (m, 4H, ArH, 1,3-phenylene). Anal. $C_{10}H_{13}N_3O_2S_2$ (C, H, N).

2.3.32. N^1-(2,4-Disulfamoylphenyl)-N^3-allyl-thiourea ***46***

White crystals, mp 218–219 °C; IR (KBr), cm^{-1}: 629, 713, 827, 863, 991, 1043 (thioamide III), 1155 (SO$_2^{sym}$), 1342 (SO$_2^{as}$), 1414, 1540 (thioamide I), 3295 (NHCSNH), 3325 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNH*CH$_2$*); 5.60–5.97 (m, 3H, CH=CH$_2$); 6.75 and 6.88 (br s, 2H, NHCSNH); 7.26 (d, 2H, ArH); 7.50 (s, 1H, ArH); 7.60 (br s, 4H, 2 SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{10}H_{14}N_4O_5S_2$ (C, H, N).

2.3.33. N^1-(2,4-Disulfamoyl-6-chlorophenyl)-N^3-allyl-thiourea ***47***

White crystals, mp 225–227 °C; IR (KBr), cm^{-1}: 657, 778, 832, 945, 1040 (thioamide III), 1153 (SO$_2^{sym}$), 1346 (SO$_2^{as}$), 1446, 1548 (thioamide I), 3290 (NHCSNH), 3340 (NH$_2$); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNH*CH$_2$*); 5.60–5.97 (m, 3H, CH=CH$_2$); 6.77 and 6.90 (br s, 2H, NHCSNH); 7.40 (s, 1H, ArH); 7.53 (s, 1H, ArH); 7.66 (br s, 4H, 2 SO$_2$NH$_2$); the last signal disappears by addition of D$_2$O into the NMR tube; Anal. $C_{10}H_{13}N_4O_5S_2Cl$ (C, H, N).

2.3.34. N^1-(2,4-Disulfamoyl-5,6-dichloro phenyl)-N^3-allyl-thiourea ***48***

White crystals, mp 231–234 °C; IR (KBr), cm^{-1}: 698, 754, 850, 952, 1044 (thioamide III), 1155 (SO$_2^{sym}$), 1350 (SO$_2^{as}$),

1547 (thioamide I), 3290 (NHCSNH), 3325 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNHCH_2); 5.60–5.97 (m, 3H, $CH{=}CH_2$); 6.82 and 7.00 (br s, 2H, NHCSNH); 7.50 (s, 1H, ArH); 7.58 (br s, 4H, 2 SO_2NH_2); the last signal disappears by addition of D_2O into the NMR tube; Anal. $C_{10}H_{12}N_4O_5S_2Cl_2$ (C, H, N).

2.3.35. N^1-(2,6-Dichloro-4-sulfamoylphenyl)-N^3-phenyl-thiourea ***49***

White crystals, mp 198–200 °C; IR (KBr), cm^{-1}: 680, 734, 880, 902, 1040 (thioamide III), 1155 (SO_2^{sym}), 1330 (SO_2^{as}), 1420, 1547 (thioamide I), 3298 (NHCSNH), 3350 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNHCH_2); 5.60–5.97 (m, 3H, $CH{=}CH_2$); 6.68 and 6.87 (br s, 2H, NHCSNH); 7.45 (s, 2H, ArH); 7.60 (br s, 2H, SO_2NH_2); the last signal disappears by addition of D_2O into the NMR tube; Anal. $C_{10}H_{11}N_3O_2S_2Cl_2$ (C, H, N).

2.3.36. N^1-(2-Sulfonamido-1,3,4-thiadiazol-5-yl)-N^3-allyl-thiourea ***50***

White crystals, mp 212–213 °C; IR (KBr), cm^{-1}: 680, 1038 (thioamide III), 1145 (SO_2^{sym}), 1370 (SO_2^{as}), 1547 (thioamide I), 1640 (C=N); 3298 (NHCSNH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNHCH_2); 5.60–5.97 (m, 3H, $CH{=}CH_2$); 8.45 (br s 2H, SO_2NH_2); the last signal disappears by addition of D_2O into the NMR tube; Anal. $C_6H_9N_5O_3S_2$ (C, H, N).

2.3.37. N^1-(4-Sulfamoylphenyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***61***

White crystals, mp < 300 °C; IR (KBr), cm^{-1}: 615, 775, 790, 832, 875, 989, 1028, 1043 (thioamide III), 1141 (SO_2^{sym}), 1330 (SO_2^{as}), 1410, 1510 (amide II), 1539 (thioamide I); 1738 (CO), 3160 (NHCONHCSNH); 3350 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.40 (m, AA'BB', 4H, ArH, 1,4-phenylene); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.59 (br s, 2H, SO_2NH_2); 7.68 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.62 (s, 1H, CON*H*CS); 9.35 (s, 1H, $H_2NO_2SC_6H_4N$*H*); 10.62 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{15}H_{14}N_4O_3S_2Cl_2$ (C, H, N).

2.3.38. N^1-(4-Sulfamoylphenylmethyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***62***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 640,710, 850, 882, 930, 985, 1040 (thioamide III), 1152 (SO_2^{sym}), 1342 (SO_2^{as}), 1515 (amide II), 1539 (thioamide I), 1725 (CO), 3170 (NHCONHCSNH); 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CONHCH_2); 7.08–7.45 (m, AA'BB', 4H, ArH, phenylene); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.52 (s, 2H, SO_2NH_2); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.89 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.60 (s, 1H, CON*H*CS); 9.30 (s, 1H, $H_2NO_2SC_6H_4N$*H*); 10.64 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{16}H_{16}N_4O_3S_2Cl_2$ (C, H, N).

2.3.39. N^1-(4-Sulfamoylphenylethyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***63***

White crystals, mp 298–303 °C. IR (KBr), cm^{-1}: 625, 894, 950, 1030, 1045 (thioamide III), 1085, 1145 (SO_2^{sym}), 1333 (SO_2^{as}), 1510 (amide II), 1541 (thioamide I), 1730 (CO), 3178 (NHCONHCS); 3360 (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); 6.94 (br s, 2H, SO_2NH_2); 7.05–7.45 (m, AA'BB', 4H, ArH, phenylene); 7.38 (d, 1H, H-5 of 3,4–dichlorophenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.65 (s, 1H, CON*H*CS); 9.32 (s, 1H, $H_2NO_2SC_6H_4N$*H*); 10.60 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{17}H_{18}N_4O_3S_2Cl_2$ (C, H, N).

2.3.40. N^1-(3-Sulfamoylphenyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***64***

White crystals, mp > 300 °C IR (KBr), cm^{-1}: 752, 864, 980, 1040 (thioamide III), 1139 (SO_2^{sym}), 1340 (SO_2^{as}), 1517 (amide II), 1540 (thioamide I), 1733 (CO), 3160 (NHCONHCSNH); 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.12 (br s, 2H, + SO_2NH_2); the signal disappears by addition of D_2O into the NMR tube; 7.08–7.44 (m, 4H, ArH, 1,3-phenylene); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.64 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.66 (s, 1H, CON*H*CS); 9.33 (s, 1H, $H_2NO_2SC_6H_4N$*H*); 10.62 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{15}H_{14}N_4O_3S_2Cl_2$ (C, H, N).

2.3.41. N^1-(2,4-Disulfamoylphenyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***65***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 643, 759, 790, 875, 995, 1017, 1040 (thioamide III), 1150 (SO_2^{sym}), 1349 (SO_2^{as}), 1515 (amide II), 1540 (thioamide I), 1730 (CO), 3165 (NHCONHCSNH); 3350 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.26 (d, 2H, ArH from 2,4-disulfamoylphenyl); 7.40 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.53 (s, 1H, ArH from 2,4-disulfamoylphenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.73 (br s, 4H, 2 SO_2NH_2); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.70 (s, 1H, CON*H*CS); 9.40 (s, 1H, $(H_2NO_2S)_2C_6H_3N$*H*); 10.75 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{14}H_{13}N_5O_5S_3Cl_2$ (C, H, N).

2.3.42. N^1-(2,4-Disulfamoyl-6-chlorophenyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***66***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 671, 759, 780, 873, 945, 1015, 1040 (thioamide III), 1150 (SO_2^{sym}), 1347 (SO_2^{as}), 1446, 1520 (amide II), 1540 (thioamide I), 1705 (CO), 3165 (NHCONHCSNH); 3350 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.44 (s, 1H, ArH from disulfamoyl-chlorophenyl); 7.53 (s, 1H, ArH from disulfamoyl-chlorophenyl); 7.60 (br s, 4H, 2 SO_2NH_2); 7.64 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.64 (s, 1H, CON*H*CS); 9.39 (s, 1H, $(H_2NO_2S)_2ClC_6H_2N$*H*); 10.78 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{14}H_{12}N_5O_5S_3Cl_3$ (C, H, N).

2.3.43. N^1-(2,4-Disulfamoyl-5,6-dichlorophenyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***67***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 710, 781, 830, 958, 1040 (thioamide III), 1155 (SO_2^{sym}), 1360 (SO_2^{as}), 1515 (amide II), 1540 (thioamide I), 1730 (CO), 3160 (NHCONHCSNH); 3340 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.50 (s, 1H, ArH from disulfamoyldichlorophenyl); 7.61 (br s, 4H, 2 SO_2NH_2); 7.68 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.95 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.68 (s, 1H, CON*H*CS); 9.47 (s, 1H, $(H_2NO_2S)_2Cl_2C_6HN$*H*); 10.70 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{14}H_{11}N_5O_5S_3Cl_4$ (C, H, N).

2.3.44. N^1-(2,6-Dichloro-4-sulfamoylphenyl)-N^5-3,4-dichlorophenyl-2-thiobiuret ***68***

White crystals, mp > 300 °C. IR (KBr), cm^{-1}: 655, 739, 786, 843, 902, 1042 (thioamide III), 1158 (SO_2^{sym}), 1341 (SO_2^{as}), 1420, 1517 (amide II), 1540 (thioamide I), 1733 (CO), 3160 (NHCONHCSNH); 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.37 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.45 (s, 2H, ArH from 2,6-dichlorophenyl); 7.60 (br s, 2H, SO_2NH_2); 7.68 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.91 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.67 (s, 1H, CON*H*CS); 9.39 (s, 1H, $H_2NO_2SCl_2C_6H_2N$*H*); 10.68 (br s, 1H, N*H*$C_6H_3Cl_2$); Anal. $C_{14}H_{10}N_4O_3S_2Cl_4$ (C, H, N).

*2.3.45. N^1-(2-Sulfonamido-1,3,4-thiadiazol-5-yl)-N^5-3,4-dichlorophenyl-2-thiobiuret **69***

White crystals, mp > 300 °C; IR (KBr), cm^{-1}: 685, 780, 935, 1044 (thioamide III), 1175 (SO_2^{sym}), 1344 (SO_2^{as}), 1545 (amide II), 1550 (thioamide I), 1585, 1730 (CO), 2810, 3290 (NHCONHCSNH), 3360; ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.35 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.63 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.91 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.43 (br s, 2H, SO_2NH_2); 10.12 (s, 1H, CON*H*CS); 10.40 (br s, 1H, N*H*$C_6H_3Cl_2$); 11.56 (s, 1H, H_2NO_2S-1,3,4-thiadiazole-N*H*); Anal. $C_{10}H_8N_6O_3S_3Cl_2$ (C, H, N).

*2.4. Reaction of 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline **70** with 3,4-dichlorophenyl isocyanate **60***

An amount of 0.49 g (5 mM) of **70** was suspended in 50 mL of anhydrous acetonitrile or THF and the required amount (0.95 g = 10 mM) of 3,4-dichlorophenyl isocyanate **60**, dissolved in 10 mL of the same solvent was added under argon. The experiment has been done with or without catalyst (triethylamine, 100 μL), leading basically to the same results. The reaction mixture was refluxed for 5–10 h, the solvent has been evaporated in vacuo and the obtained oil was recrystallized from acetone–water (1:2, v/v). Only 1,3-bis(3,4-dichlorophenyl)urea **74** could be isolated with a yield of 37–40 %. White crystals, mp 285–289 °C; ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.40 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.62 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.33 (s, 2H, NHCONH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 118.1; 119.6; 123.8; 129.8; 130.1; 131.6; 139.8; 152.6 (C=O); MS: 355 (M + 5); 353 (M + 3); 351 (100%; MH+); 349 (80%; M – 2); 348 (5%; M – 3); 315 (23%; M – Cl); 281 (15%; M – 2Cl).

2.5. Pharmacology

Initial rates of 4-nitrophenyl acetate hydrolysis catalysed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC [46]. Substrate solutions were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2×10^{-2} and 1×10^{-6} M, working at 25 °C. A molar absorption coefficient ε of 18400 M^{-1} cm^{-1} was used for the 4-nitrophenolate formed by hydrolysis, under experiment conditions (pH 7.40) as reported in the literature [46]. Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the means of such results. Stock inhibitor solutions (1 mM) were prepared in distilled–deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations [4]) 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 [46]. Enzyme concentrations were 3 nM for hCA II, 12 nM for hCA I and 39 nM for bCA IV (this isozyme has a decreased esterase activity [47] and higher concentrations had to be used for the measurements).

3. Results

Reaction of aromatic/heterocyclic sulfonamides **6–14**, containing a free amino group, with phenyl isocyanate, 3,4-dichlorophenyl isocyanate, phenyl isothiocyanate and allyl isothiocyanate afforded the substituted-urea/thiourea derivatives **15–50** (*table I*) (see *figure 2*).

Reaction of thioureido-containing sulfonamides **51–59** with 3,4-dichloro-phenyl isocyanate 60 afforded the new 1,5-disubstituted-2-thio-biurets **61–69** (*table II*).

Compounds **15–50** and **61–69** were characterized by standard chemical and physical methods that confirmed their structure (see Experimental protocols for details) and were assayed for the inhibition of isozymes hCA I, hCA II and bCA IV (*tables I* and *II*). Inhibition data against the three isozymes with standard CA inhibitors are shown in *table III*.

4. Discussion

4.1. Chemistry

Reactions of alkyl/aryl-isocyanates and isothiocyanates with active hydrogen compounds, and especially with amines [48, 49] have been thoroughly investigated due to the applications of such derivatives in the field of polymers [48], insecticides [48, 49] or pharmacological agents [35, 50]. Their reactions with aromatic sulfonamides containing free amino groups have also been investigated in the search for more effective antibacterial agents [35, 47, 50]. Thus, N^1-(4-sulfamoylphenyl)-N^3-phenyl-urea **15**, as well as the corresponding thiourea **33** and the allyl-substituted compound **42** have been reported by Roth and Degering [45], these compounds acting as effective antibacterial agents. In the heterocyclic series, only recently Katritzky's group [51, 52] investigated the reaction of 5-amino-1,3,4-thiadiazole-2-sulfonamide **14** and of 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline **70** with alkyl/aryl isocyanates/isothiocyanates, in the search for topically effective antiglaucoma sulfonamide CA inhibitors. We have also recently investigated the reaction of aromatic sulfonamides containing amino moieties, such as **6–14**, with inorganic cyanate (NCO^-) and thiocyanate (SCN^-), obtaining a large series of sulfonamides possessing ureido and thioureido groups, which showed very good CA inhibitory properties [1] (see *figure 3*).

The high affinity of some of the previously reported ureido/thioureido-sulfonamides of type **71**, **72** [1] for isozyme hCA I prompted us to investigate the synthesis and CA inhibitory properties of novel types of such derivatives, possessing bulkier groups as substituents of the N-1 atom of the ureido/thioureido moiety. Based on recent QSAR calculations on heterocyclic/aromatic sulfonamides possessing CA inhibitory properties, it was documented that the longer the molecule of the inhibitor on one of its axes, the stronger also its affinity for the enzyme [26, 53,

Table I. Biological activity data of sulfonamide CA inhibitors **6–50** prepared in the present study ($K_I - s$, the mean of two different assays, was obtained by the esterasic method of Pocker and Stone [46]).

Compound	K_I (nM)		
	hCA I[a]	hCA II[a]	bCA IV[b]
6[c]	2800	300	3000
7[d]	2500	170	2800
8[e]	2100	180	2450
9[f]	2500	240	2200
10	1700	110	240
11	840	75	160
12	900	100	340
13	1700	220	1200
14	860	60	540
15	1200	240	460
16	800	105	140
17	430	75	150
18	1500	150	210
19	620	25	45
20	900	100	230
21	650	29	74
22	180	62	75
23	5	4	7
24	20	9	10
25	12	7	10
26	12	13	20
27	10	8	11
28	42	37	40
29	47	10	40
30	49	10	21
31	18	20	24
32	3	6	8
33	410	19	24
34	266	12	16
35	50	53	70
36	340	60	120
37	100	110	170
38	55	74	109
39	60	85	124
40	17	32	41
41	4	12	25
42	400	130	170
43	300	90	140
44	95	80	105
45	360	250	390
46	220	90	100
47	72	58	89
48	28	30	64
49	10	18	35
50	6	9	28

[a]Human (cloned) isozyme; [b]isolated from bovine lung microsomes; [c]sulfanilamide; [d]homosulfanilamide; [e]*p*-aminoethylbenzene sulfonamide; [f]metanilamide.

54]. Thus, phenyl, 3,4-dichlorophenyl and allyl moieties were attached at this end of the molecule. Reaction of sulfonamides **6–14** with phenyl isocyanate, 3,4-dichlorophenyl isocyanate, phenyl isothiocyanate and allyl isothiocyanate afforded the substituted urea/thiourea derivatives **15–50** (*table I*). Mention should be made that even if Katritzky et al. [51] reported that the reaction of 5-amino-1,3,4-thiadiazole-2-sulfonamide with alkyl or aryl isothiocyanates did not lead to the corresponding thioureas, in our case we were able to prepare two such derivatives, **41** and **50**. The reaction of the aromatic sulfonamides **6–13** on the other hand with isocyanates or isothiocyanated occurred without problems [45].

As it is documented that isocyanates/isothiocyanates also react with amides, imides, urea or thiourea derivatives [48–50, 55, 56], we investigated the reaction of 3,4-dichlorophenyl isocyanate **60** with the thioureido-substituted sulfonamides **51–59** (*figure 4*). This was primarily due to the fact that this isocyanate generally led to the strongest hCA I inhibitors in the series investigated by us here (see later in the text). The prepared 1,5-disubstituted-2-thiobiurets **61–69** were indeed among the strongest CA inhibitors in the series of synthesized compounds (see later in the text).

An unexpected finding also emerged during these experiments. The reaction of 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline **70** with 3,4-dichlorophenyl isocyanate **60** has been investigated, in order to prepare the derivative **73** (*figure 5*), as it is documented that sulfonamides derived from this ring system behave as very potent CA inhibitors and also possess an increased lipophilicity, making them interesting candidates for certain pharmacological investigations [4, 52, 57].

To our greatest surprise, the only compound that was isolated from the above-mentioned reaction mixture was 1,3-bis(3,4-dichlorophenyl)urea **74**, which has been unambiguously characterized by means of ^{1}H-NMR, ^{13}C-NMR spectroscopy and MS data (see Experimental protocols for details). The only explanation we propose for this anomaly is the following: it has been reported [58] that symmetrical carbodiimides **75** can be prepared from isocyanates by a disproportionation reaction, in the presence of suitable catalysts, such as the phospholene-oxide **76** or the phospholane-oxide **77** (*figure 6*). Considering that 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline **70** may act as such a catalyst, it is probable that the carbodiimide **75** (R = 3,4-dichlorophenyl) is the oily intermediate separated after the reaction, which when recrystallized in aqueous solvents by reaction with water leads to the symmetrical urea derivative **74**. Thus, it seems that 5-imino-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline may act as a very good catalyst for the transformation of isocyanates into carbodiimides

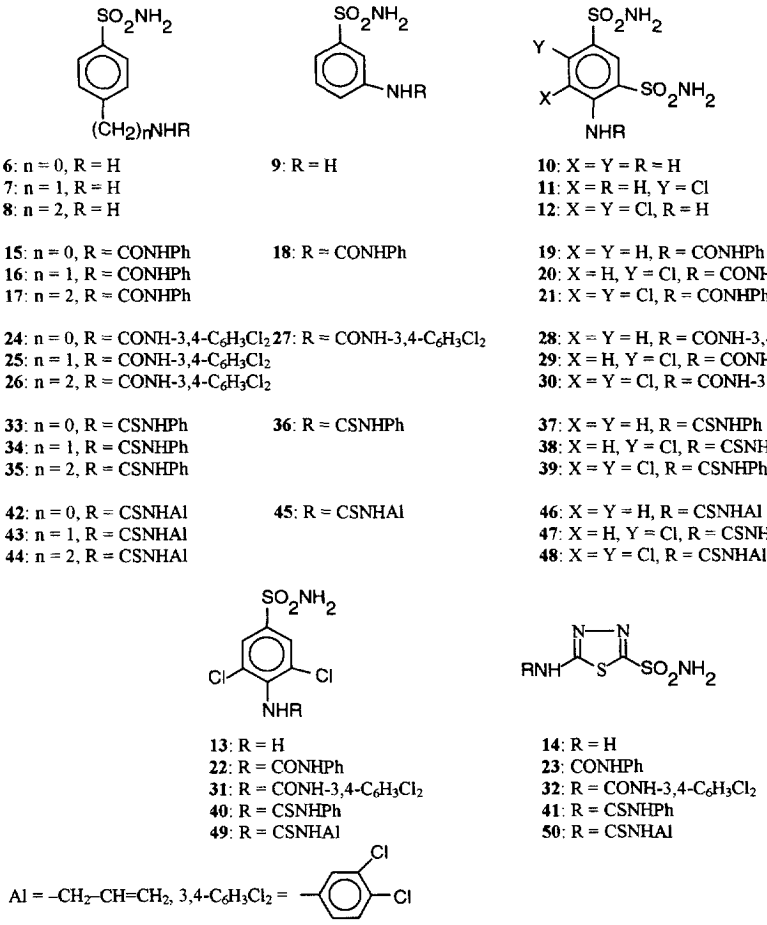

Figure 2.

Table II. Biological activity data of sulfonamides **61–69** prepared in the present study ($K_I - s$, the mean of two different assays, was obtained by the esterasic method of Pocker and Stone [46]).

Compound	K_I (nM)		
	hCA I[a]	hCA II[a]	bCA IV[b]
61	12	20	30
62	8	9	26
63	7	10	24
64	10	13	29
65	16	19	24
66	54	75	102
67	47	59	78
68	10	18	21
69	3	6	28

[a]Human (cloned) isozyme; [b]isolated from bovine lung microsomes.

Table III. CA inhibition data with standard sulfonamide inhibitors **1–4** ($K_I - s$, the mean of two different assays, was obtained by the esterasic method of Pocker and Stone [46]).

Inhibitor	K_I (nM)		
	hCA I[a]	hCA II[a]	bCA IV[b]
Acetazolamide **1**	900	12	220
Methazolamide **2**	780	14	240
Dichlorophenamide **3**	1200	38	380
Dorzolamide **4**	> 50000	9	43

[a]Human (cloned) isozymes; [b]from bovine lung microsomes.

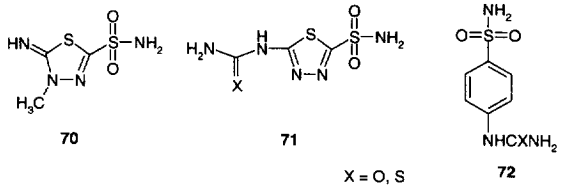

Figure 3.

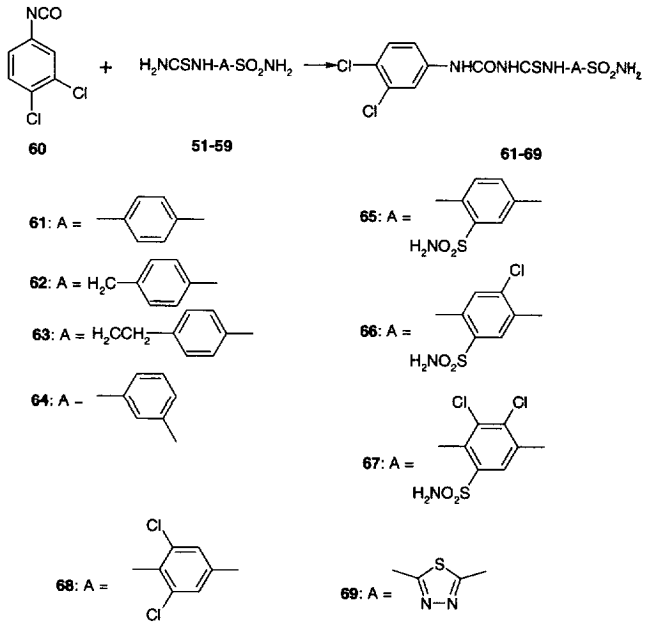

Figure 4.

under much milder conditions as those reported in the literature [58, 59].

It is interesting to note that Katritzky's group [52] reported that imine **70** reacts normally with alkyl/aryl isocyanates, leading to the normal reaction products, the ureido-imino-thiadiazoline-sulfonamides (three compounds were reported, which were obtained from **70** and methyl, phenyl and 4-chlorophenyl isocyanate, respectively). In our case, this type of products could not be isolated, probably due to the different reactivity of the aryl isocyanate used in our experiments.

Inhibition data against three CA isozymes, hCA I, hCA II and bCA IV with the new derivatives **15–50** and **61–69**, shown in *tables I* and *II*, prove that the substituted ureido/thioureido sulfonamides generally behave as strong inhibitors, with increased efficiencies as compared to the parent compounds from which they were prepared (the amino-sulfonamides **6–14**),

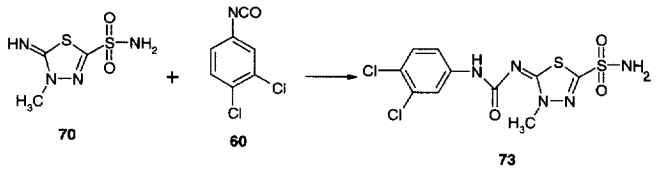

Figure 5.

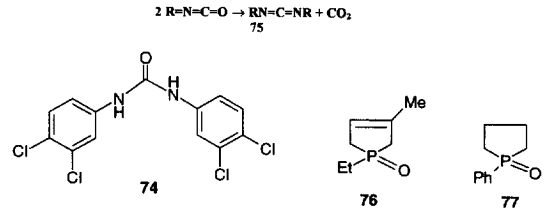

Figure 6.

but also as compared to the unsubstituted ureido/thioureido analogues previously reported by our group [1] (data not shown). The efficiency of the obtained inhibitor varied in the following way, based on the parent sulfonamide from which it was prepared:

sulfanilamides < homosulfanilamides ~ metanilamides < *p*-aminoethyl-benzenesulfonamides < 1,3-benzene-disulfonamides < 3,5-dichloro-sulfanilamides < 1,3,4-thiadiazole-2-sulfonamide derivatives.

Based on the isocyanate/isothiocyanate, the order generally was: $C_6H_3Cl_2NCO$ > PhNCS ~ allyl-NCS > PhNCO. Of course, exceptions from the above rule were evidenced. The strongest inhibitors were the 1,5-disubstituted-2-thiobiuret derivatives **61–69**, confirming again the QSAR discovery about the length of a CA inhibitor molecule [26, 53, 54]. All three CA isozymes investigated here were susceptible to inhibition with this type of sulfonamides, but the interesting finding was the increased affinity for hCA I of some of these inhibitors. As seen from the data in *tables I–III*, generally hCA II is the most susceptible to inhibition with aromatic/heterocyclic sulfonamides, followed by bCA IV, whereas hCA I has a much lower affinity for this class of inhibitors. These statements are well illustrated for the case of the classical inhibitors of type **1–3** (*table III*), with the ratios K_I(hCA I)/K_I(hCA II) ~ 40–75, and K_I(bCA IV)/K_I(hCA II) ~ 18. This means that hCA I is 40–75 less susceptible to be inhibited with acetazolamide and its congeners, whereas bCA IV is only 18 times less susceptible. A very interesting case is dorzolamide **4**, the new representative of topically effective antiglaucoma sulfonamides discovered at Merck [19, 60]

which is 5500 times less effective as hCA I inhibitor compared to hCA II. In the case of the compounds reported here by us, there are cases of derivatives possessing an inhibition ratio K_I(hCA I)/K_I(hCA II) ~ 1 (such as **23–25**, **28**, **44** and **47**), whereas others have the above-mentioned ratio smaller than 1 (such as **26**, **31**, **32**, **35**, **37–41**, **48–50** and **61–69**). As far as we know, this is the first report of sulfonamide CA inhibitors that act as better inhibitors of isozyme I as compared to the 'sulfonamide-avid' isozymes II and IV. We did not claim that we have obtained hCA I-specific inhibitors, since the compounds synthesized inhibit to a large extent the other two isozymes. Still, our results are a promising start for the design of high-affinity and hopefully isozyme I-specific CA inhibitors.

Acknowledgements

We are extremely grateful to BASF AG (Ludwigshafen, Germany), and especially to Drs. T. Grote, R. Müller and C. Bussche for helpful discussions. The symmetrical disubstituted urea nature of compound **74** has been suggested to us by the above-mentioned scientists. Prof. S. Lindskog (Umeå University, Sweden) is thanked for the gift of the two plasmids encoding the CA I and II genes.

References

[1] Preceding part of this series: Scozzafava A., Supuran C.T., submitted for publication in J. Enzyme Inhib.
[2] Hewett-Emmett D., Tashian R.E., Mol. Phylogenet. Evol. 5 (1996) 50–77.
[3] Lakkis M.M., Bergenhem N.C., Tashian R.E., Biochem. Biophys. Res. Commun. 226 (1996) 268–272.
[4] Supuran C.T., in: Puscas I. (Ed.), Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism, Helicon, Timisoara, 1994, pp. 29–111.
[5] Mann T., Keilin D., Nature 146 (1940) 164–165.
[6] Roblin R.O., Clapp J.W., J. Am. Chem. Soc. 72 (1950) 4890–4892.
[7] Miller W.H., Dessert A.M., Roblin R.O., J. Am. Chem. Soc. 72 (1950) 4893–4896.
[8] Beasley Y.M., Overell B.G., Petrow V., Stephenson O., J. Pharm. Pharmacol. 10 (1958) 696–705.
[9] Maren T.H., Physiol. Rev. 47 (1967) 595–782.
[10] Sisson G.M., Maren T.H., Federation Proc. 15 (1956) 484–490.
[11] Beyer K.H., Baer J.E., Pharmacol. Rev. 13 (1961) 517–562.
[12] Maren T.H., in: BotrÈ F., Gros G., Storey B.T. (Eds.), Carbonic Anhydrase – From Biochemistry and Genetics to Physiology and Clinical Medicine, VCH, Weinheim, 1991, pp. 186–207.
[13] Larson E.B., Roach R.C., Schoene R.B., Hornbein T.F., J. Am. Med. Ass. 248 (1982) 328–332.
[14] Woodbury D.M., in: Glaser G.H., Penry J.K., Woodbury D.M. (Eds.), Antiepileptic Drugs: Mechanisms of Action, Raven, New York, 1980, pp. 617– 633.
[15] Reiss W.G., Oles K.S., Ann. Pharmacother. 30 (1996) 514–519.
[16] Weiner I.M., in: Gilman A.G., Rall T.W., Nies A.S., Taylor P. (Eds.), The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, New York, 1990, pp. 713–732.
[17] Supuran C.T., Conroy C.W., Maren T.H., Eur. J. Med. Chem. 31 (1996) 843–846.
[18] Lindskog S., Wistrand P.J., in: Sandler M.J., Smith H.J. (Eds.), Design of Enzyme Inhibitors as Drugs, Oxford University Press, 1987, pp. 698–723.
[19] Sugrue M.F., J. Ocul. Pharmacol. Ther. 12 (1996) 363–376.
[20] Hartenbaum D., Clin. Ther. 18 (1996) 460–465.
[21] Maus T.L., Larsson L.I., McLaren J.W., Brubaker R.F., Arch. Ophthalmol. 115 (1997) 45–49.
[22] Heijl A., Strahlman E., Sverisson T., Brinchman-Hansen O., Pustjarvi T., Tipping R., Ophthalmology 104 (1997) 137–142.
[23] Maren T.H., Conroy C.W., Wynns G.C., Levy N.S., J. Ocul. Pharmacol. Ther. 13 (1997) 23–30.
[24] Schoenwald R.D., Deshpande G.S., Rethwisch D.G., Barfknecht C.F., J. Ocul. Pharmacol. Ther. 13 (1997) 41–59.
[25] Supuran C.T., Manole G., Dinculescu A., Schiketanz A., Gheorghiu M.D., Puscas I., Balaban A.T., J. Pharm. Sci 81 (1992) 716–719.
[26] Supuran C.T., Clare B.W., Eur. J. Med. Chem. 30 (1995) 687–696.
[27] Supuran C.T., Nicolae A., Popescu A., Eur. J. Med. Chem. 31 (1996) 431–438.
[28] Supuran C.T., Popescu A., Ilisiu M. et al., Eur. J. Med. Chem. 31 (1996) 439–448.
[29] Scozzafava A., Supuran C.T., Metal Based Drugs 4 (1997) 19–26.
[30] Mincione G., Scozzafava A., Supuran C.T., Metal Based Drugs 4 (1997) 27–34.
[31] Supuran C.T., Scozzafava A., J. Enzyme. Inhib. 12 (1997) 37–51.
[32] Elbaum D., Nair S.K., Patchan M.W., Thompson R.B., Christianson D.W., J. Am. Chem. Soc. 118 (1996) 8381–8387.
[33] Jain A., Whitesides G.M., Alexander R.S., Christianson D.W., J. Med. Chem. 37 (1994) 2100–2105.
[34] Burbaum N.J., Ohlmeyer M.H.J., Reader J.C. et al., Proc. Natl. Acad. Sci. USA 92 (1995) 6027–6031.
[35] Northey E.H., The sulfonamides and allied compounds, Reinhold, New York, 1948, pp. 3–339.
[36] Scudi J.V., J. Am. Chem. Soc. 59 (1937) 1480–1483.
[37] Jitianu A., Ilies M.A., Scozzafava A., Supuran C.T., Main Group Met. Chem. 20 (1997) 151–156.
[38] Supuran C.T., Rev. Roum. Chim. 40 (1995) 643–651.
[39] Forsman C., Behravan G., Osterman A., Jonsson B.H., Acta. Chem. Scand. B42 (1988) 314–318.
[40] Behravan G., Jonasson P., Jonsson B.H., Lindskog S., Eur. J. Biochem. 198 (1991) 589–592.
[41] Khalifah R.G., Strader D.J., Bryant S.H., Gibson S.M., Biochemistry 16 (1977) 2241–2247.
[42] Nyman P.O., Lindskog S., Biochim. Biophys. Acta 85 (1964) 141–151.
[43] Henderson L.E., Henriksson D., Nyman P.O., J. Biol. Chem. 251 (1976) 5457–5463.
[44] Maren T.H., Wynns G.C., Wistrand P.J., Mol. Pharmacol. 44 (1993) 901–906.
[45] Roth J.S., Degering E.F., J. Am. Chem. Soc. 67 (1945) 126–128.
[46] Pocker Y., Stone J.T., Biochemistry 6 (1967) 668–678.
[47] Baird T.T., Waheed A., Okuyama T., Sly W.S., Fierke C.A., Biochemistry 36 (1997) 2669–2678.
[48] Saunders J.H., Slocombe R.J., Chem. Rev. 43 (1948) 203–218.
[49] Arnold R.G., Nelson J.A., Verbanc J.J., Chem. Rev 57 (1957) 47–75.
[50] Kurzer F., Chem. Rev. 50 (1952) 1–46.
[51] Katritzky A.R., Caster K.C., Maren T.H., Conroy C.W., Bar-Ilan A., J. Med. Chem. 30 (1987) 2058–2062.
[52] Maren T.H., Bar-Ilan A., Caster K.C., Katritzky A.R., J. Pharmacol. Exp. Ther. 241 (1987) 56–63.
[53] Maren T.H., Clare B.W., Supuran C.T., Roum. Chem. Quart. Rev. 2 (1994) 259–282.
[54] Supuran C.T., Clare B.W., accepted for publication in Eur. J. Med. Chem.
[55] Kurzer F., Chem. Rev. 56 (1956) 96–197.
[56] Hargreaves F., Pritchard J.G., Dave H.R., Chem. Rev. 70 (1970) 439–469.
[57] Supuran C.T., Ilies M.A., Scozzafava A., accepted for publication in Eur. J. Med. Chem.
[58] Kurzer F., Douraghi-Zadeh K., Chem. Rev. 67 (1967) 107–139.
[59] Campbell T.W., Monagle J.J., J. Am. Chem. Soc. 84 (1962) 1493–1495.
[60] Baldwin J.J., Ponticello G.S., Anderson G.S et al., J. Med. Chem. 32 (1989) 2510–2513.