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CARBONIC ANHYDRASE INHIBITORS. ARYLSULFONYLUREIDO- AND ARYLUREIDO-SUBSTITUTED AROMATIC AND HETEROCYCLIC SULFONAMIDES: TOWARDS SELECTIVE INHIBITORS OF CARBONIC ANHYDRASE ISOZYME I*

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Reaction of twenty aromatic/heterocyclic sulfonamides containing a free amino, imino, hydrazino or hydroxyl group, with tosyl isocyanate or 3,4-dichlorophenyl isocyanate afforded two series of derivatives containing arylsulfonylureido or diarylureido moieties in their molecule respectively. The new derivatives were assayed as inhibitors of three carbonic anhydrase (CA) isozymes, CA I, II (cytosolic forms) and IV (membrane-bound form). Potent inhibition was observed against all three isozymes but especially against CA I, which is generally 10–75 times less susceptible to inhibition by the classical sulfonamides in clinical use as compared to the other major red cell isozyme, CA II, or the membrane-bound one, CA IV. The derivatives obtained from tosyl isocyanate were generally more potent than the corresponding ones obtained from 3,4-dichlorophenyl isocyanate. This is the first reported example of selective inhibition of CA I and might lead to more selective drugs/diagnostic agents from this class of pharmacologically relevant compounds.

Keywords: Carbonic anhydrase; Aromatic, heterocyclic sulfonamides; Aryl, arylsulfonyl isocyanates; Isozyme-specific inhibitors; Isozyme-carbonic anhydrase I inhibitors

* See Ref. 1

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INTRODUCTION

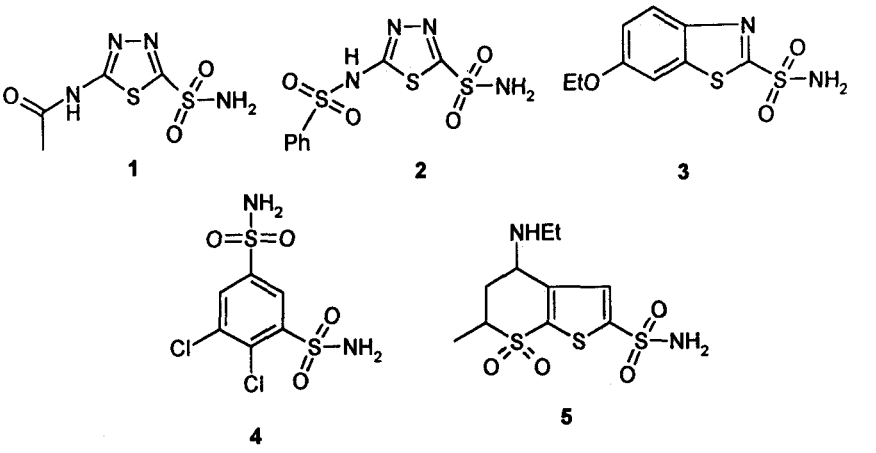
One of the most successful approaches to the discovery of new drugs and therapies is constituted by the inhibition of target enzymes with compounds acting as specific and tight-binding inhibitors.[2,3] The processes involved in these phenomena generally also bring new insights in important topics such as the role of binding in catalysis or molecular recognition, since the formation of the enzyme–substrate/inhibitor complexes is the critical step which greatly determines the success of this approach to drug design.[2–4]

Carbonic anhydrase (CA, EC 4.2.1.1), an enzyme playing a central role to both transport and metabolic processes involving CO_2 and bicarbonate, represents a special and fortunate case for drug design. This is mainly due to two factors: (i) the presence of different CA isozymes in a variety of tissues and organs, virtually in all living organisms, where they are involved in critical physiological processes (see later) and, (ii) the existence of strong, specific but unfortunately unselective inhibitors for many such isozymes.[4–6]

$$O=C=O + H_2O \Leftrightarrow HCO_3^- + H^+ \quad (1)$$

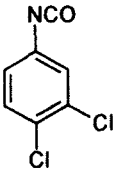
$$NH_3 + H^+ \rightarrow NH_4^+ \quad (2)$$

At least eight CA isozymes have been isolated up to now in a variety of tissues of higher vertebrates.[5,6] By catalyzing the reversible interconversion between the two chemical species mentioned above (reaction (1)) CA isozymes facilitate CO_2 transport out of the cell[6] in metabolically active tissues (such as the muscle cytoplasmic (CA I–III) and sarcolemmal (CA III)). The only membrane-bound isozyme known (CA IV), which is highly abundant in the kidneys and lungs, has been shown to possess an extracellular orientation of the active site, and to be critical in acidifying the outer boundary layer through the protons formed by CO_2 hydration according to equation (1).[5,6] This process further facilitates cellular ammonia transport by providing the H^+ ion for the NH_3 (equation (2)), so maintaining the transmembrane ammonia gradient.[5,7] The unique mitochondrial CA isozyme isolated (CA V) is known to supply bicarbonate/CO_2 for the initial reaction of gluconeogenesis and ureagenesis in many mammalian tissues,[7,8] as well as for pyruvate carboxylation in the *de novo* lipogenesis in adipocytes.[9] It is thus clear that different CA isozymes are involved in critical physiological processes, some of which were mentioned above.



Aromatic/heterocyclic sulfonamides with the general formula R-SO_2NH_2 are powerful inhibitors of CAs,[5,10–16] and some differences in affinity of these inhibitors for the different isozymes have been known for some time.[5] Thus, CA II is the most susceptible to inhibition by sulfonamides,[5] followed by CA IV and V,[6,9] whereas CA I has generally a lower affinity (10–100 times, as compared to CA II) for this type of inhibitor and a much larger one for the inorganic complexing anions, such as cyanide, cyanate, thiocyanate.[5,17–19] Finally, CA III is a sulfonamide-resistant isozyme,[20] being appreciably inhibited only at millimolar concentrations of inhibitor, whereas the other isozymes are inhibited at micromolar–nanomolar concentrations of sulfonamides such as acetazolamide **1**, benzolamide **2**, ethoxzolamide **3**, dichlorophenamide **4** or dorzolamide **5** which are all clinically used drugs.[21–24] The main application of such agents is as antiglaucoma drugs,[22–24] but they are also used as antiulcer,[21] diuretic,[25] or antiepileptic drugs[26] as well as diagnostic tools in NMR imaging.[27,28] Although many sulfonamide CA inhibitors possess high affinity for the major isozymes considered to play important physiological functions (such as CA I, CA II and CA IV),[5,7,8,11–16] the critical challenge for the design of novel pharmacological agents from this class is constituted by the lack of specificity of such compounds towards the different isozymes.[5,29,30] Among the eight mammalian isozymes described up to now, just the most widely spread isozymes, such as CA I, CA II and CA IV, have the most similar affinities to diverse sulfonamide inhibitors, although, as mentioned above, small differences between them do indeed exist.[5,30] This fact, as well as the physiological

importance and wide-spread distribution of these isozymes, prompted much research in our laboratory to find compounds which might discriminate between them.[10–16,29,31] In this paper we report a successful approach that led to compounds with higher affinity for CA I as compared to CA II and CA IV, sometimes even by a factor of 10 (although our compounds also appreciably inhibited isozymes II and IV). The new compounds have been prepared by reaction of 20 aromatic/heterocyclic sulfonamides containing free amino, imino, hydrazino or hydroxyl groups (**A**–**T**) with 3,4-dichlorophenyl isocyanate **6** or tosyl isocyanate **7**, leading to the corresponding urea or carbamic acid derivatives. The new compounds were characterized by standard procedures and assayed as inhibitors of three isozymes, human (h) hCA I and hCA II and bovine (b) bCA IV.



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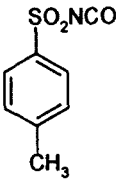

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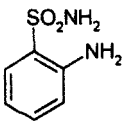

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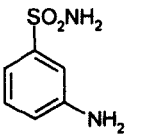

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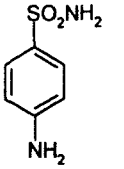

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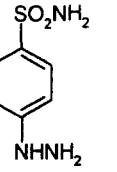

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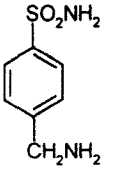

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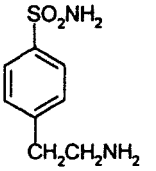

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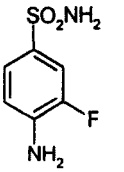

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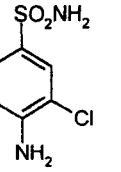

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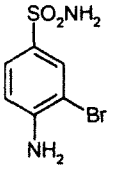

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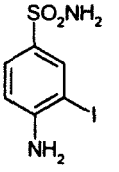

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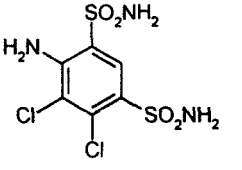

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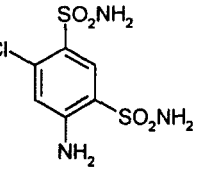

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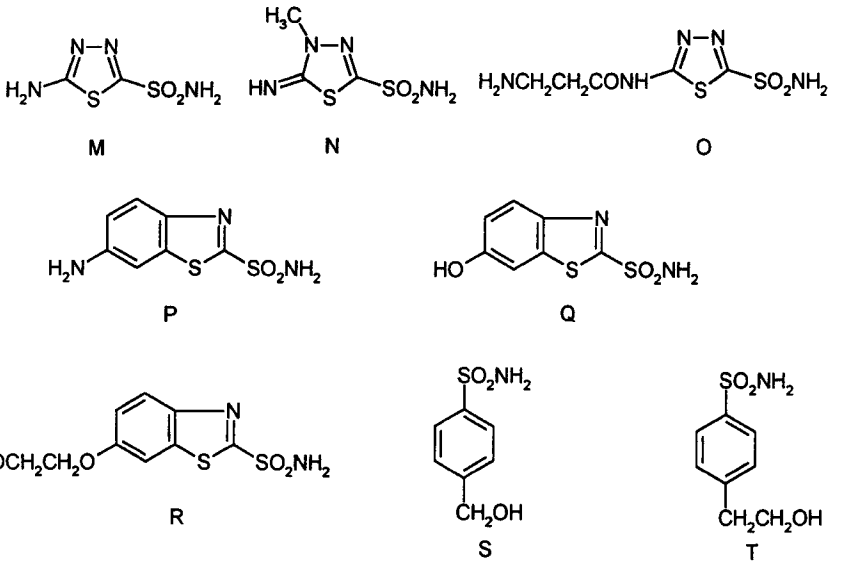


MATERIALS AND METHODS

Melting points were determined with a heating plate microscope and are not corrected. IR spectra were obtained in KBr pellets with a Perkin-Elmer 16PC FTIR spectrometer and ^{1}H-NMR spectra with a Varian 300CXP apparatus in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. 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 **A**–**T** used in synthesis were either commercially available compounds (from Sigma, Acros or Aldrich) or were prepared as described previously: 4-hydrazino-benzenesulfonamide **D** by diazotization of sulfanilamide followed by reduction of the diazonium salt with tin(II) chloride;[32] halogenosulfanilamides **G**–**J** by halogenation of sulfanilamide as reported in the literature;[33] compound **O** from 5-amino-1,3,4-thiadiazole-2-sulfonamide (obtained from acetazolamide)[34] by acylation with the phthalimido-derivative of β-alanine, followed by hydrazinolysis,[35a] imine **N** by deprotection of methazolamide with concentrated hydrochloric acid.[30] The benzothiazole-2-sulfonamide derivatives **P**–**R** were prepared as described in Ref. 35b, and the alcohols **S** and **T** from the corresponding amines by diazotization followed by hydrolysis of the diazonium salts.[35c] 3,4-Dichlorophenyl isocyanate **6**, tosyl isocyanate **7**, and triethylamine were from Acros. Acetonitrile (Merck) or other solvents used in the synthesis were double

distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/HCA I and pACA/HCA II described by Forsman *et al.*[36] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[37] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[38] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of $49\,mM^{-1}\,cm^{-1}$ for CA I and $54\,mM^{-1}\,cm^{-1}$ for CA II, respectively, based on $M_r = 28.85$ kDa for CA I, and 29.30 kDa for CA II, respectively.[39,40] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration has been determined by titration with ethoxzolamide.[41]

Initial rates of 4-nitrophenyl acetate hydrolysis catalysed by different CA isozymes at 25°C were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC.[42] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $2 \cdot 10^{-2}$ and $1 \cdot 10^{-6}$ M. A molar absorption coefficient ε of $18{,}400\,M^{-1}\,cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, under the conditions of the experiments (pH 7.40), as reported in the literature.[42] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations[4–6]) 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.[42] Enzyme concentrations were 3.5 nM for hCA II, 12 nM for hCA I and 36 nM for bCA IV (this isozyme has a decreased esterase activity[43] and higher concentrations had to be used for the measurements).

General Procedure for the Preparation of Arylsulfonylureas and Diaryl Ureas

An amount of 10 mM sulfonamide **A–T** was dissolved or suspended in 50 mL of anhydrous acetonitrile and then treated with a solution obtained from 10 mM of isocyanates **6** or **7** dissolved in 10 mL of the same solvent.

A small volume (100 μL) of triethylamine was added as catalyst. The reaction was performed by heating the mixture at reflux for 6 h when isocyanate **6** was used, or by magnetic stirring at room temperature when tosyl isocyanate **7** was used (no catalyst was used when working with this very reactive isocyanate). The conversion of all the sulfonamide to the corresponding urea or carbamic acid derivatives was monitored by TLC. When the reaction was completed, the solvent was evaporated until a small volume of the reaction mixture was obtained. Generally the new compounds crystallized spontaneously on standing 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 products precipitated and were filtered. The prepared compounds were recrystallized from ethanol or ethanol–water (1 : 1, v/v). Yields were in the range of 70–90% (higher for tosyl isocyanate than for 3,4-dichlorophenyl isocyanate, when working with the same parent sulfonamide). Compounds **6C**, **6E**, **6F** and **6M** were previously reported by this group,[31] and are not described in the present series of compounds, whereas **6N** could not be obtained (in the previously mentioned work or in the present one) since reaction of 3,4-dichlorophenyl isocyanate with the imine **N** only led to the formation of 1,2-bis-(3,4-dichlorophenyl)-urea, by a reaction mechanism explained by us previously.[31]

N^1-(2-Sulfamoylphenyl)-N^3-3,4-dichlorophenyl-urea **6A**, as white crystals, m.p. 247–248°C. IR (KBr), cm^{-1}: 760, 995, 1028, 1040, 1130 (SO_2^{sym}), 1350 (SO_2^{as}), 1405, 1520 (amide II), 1730 (CO), 3165 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15–7.69 (m, 4H, ArH, 1,2-phenylene); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.54 (br s, 2H, SO_2NH_2); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.94 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.30 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.67 (br s, 1H, $NHC_6H_3Cl_2$). Found: C, 43.40; H, 3.15; N, 11.50. $C_{13}H_{11}Cl_2N_3O_3S$ requires: C, 43.35; H, 3.08; N, 11.67%.

N^1-(3-Sulfamoylphenyl)-N^3-3,4-dichlorophenyl-urea **6B**, as white crystals, m.p. 273–274°C. IR (KBr), cm^{-1}: 707, 845, 1028, 1035, 1136 (SO_2^{sym}), 1360 (SO_2^{as}), 1400, 1520 (amide II), 1734 (CO), 3160 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10–7.50 (m, 4H, ArH, 1,3-phenylene); 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.56 (br s, 2H, SO_2NH_2); 7.64 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.95 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.30 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.64 (br s, 1H, $NHC_6H_3Cl_2$). Found: C, 43.23; H, 3.00; N, 11.45. $C_{13}H_{11}Cl_2N_3O_3S$ requires: C, 43.35; H, 3.08; N, 11.67%.

N^1-(4-Sulfamoylphenyl)-N^4-3,4-dichlorophenyl-semicarbazide **6D**, as white crystals, m.p. 269–270°C. IR (KBr), cm^{-1}: 771, 850, 990 (N–N), 1040, 1171 (SO_2^{sym}), 1350 (SO_2^{as}), 1410, 1518 (amide II), 1730 (CO), 3169

(NHCONH), 3300 (NH, NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.39 (m, AA′BB′, 4H, ArH, 1,4-phenylene); 7.42 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.57 (br s, 2H, SO_2NH_2); 7.62 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.90 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.37 (br s, 2H, $H_2NO_2SC_6H_4$-N*H*N*H*); 10.60 (br s, 1H, N*H*$C_6H_3Cl_2$). Found: C, 41.54; H, 2.97; N, 14.75. $C_{13}H_{12}Cl_2N_4O_3S$ requires: C, 41.61; H, 3.22; N, 14.93%.

N^1-(2-Fluoro-4-sulfamoyl-phenyl)-N^3-3,4-dichlorophenyl-urea **6G**, as white crystals, m.p. 241–243°C. IR (KBr), cm^{-1}: 654, 910, 1040, 1151 (SO_2^{sym}), 1337 (SO_2^{as}), 1415, 1515 (amide II), 1732 (CO), 3170 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.60 (br s, 2H, SO_2NH_2); 7.05–7.89 (m, 3H, ArH from the F-substituted ring); 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.64 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.97 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.30 (s, 1H, $H_2NO_2SC_6H_3FN$*H*); 10.66 (br s, 1H, N*H*$C_6H_3Cl_2$). Found: C, 41.50; H, 3.01; N, 11.03. $C_{13}H_{10}Cl_2FN_3O_3S$ requires: C, 41.29; H, 2.67; N, 11.11%.

N^1-(2-Chloro-4-sulfamoyl-phenyl)-N^3-3,4-dichlorophenyl-urea **6H**, as white crystals, m.p. 258–259°C. IR (KBr), cm^{-1}: 648, 959, 1040, 1156 (SO_2^{sym}), 1334 (SO_2^{as}), 1415, 1519 (amide II), 1730 (CO), 3170 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.68 (br s, 2H, SO_2NH_2); 7.05–7.80 (m, 3H, ArH the 2-Cl-substituted ring); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.64 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.96 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.30 (s, 1H, $H_2NO_2SC_6H_3ClN$*H*); 10.66 (br s, 1H, N*H*$C_6H_3Cl_2$). Found: C, 39.29; H, 2.60; N, 10.50. $C_{13}H_{10}C_{13}N_3O_3S$ requires: C, 39.56; H, 2.55; N, 10.65%.

N^1-(2-Bromo-4-sulfamoyl-phenyl)-N^3-3,4-dichlorophenyl-urea **6I**, as white crystals, m.p. 255–257°C. IR (KBr), cm^{-1}: 712, 846, 1040, 1159 (SO_2^{sym}), 1352 (SO_2^{as}), 1415, 1521 (amide II), 1730 (CO), 3170 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.63 (br s, 2H, SO_2NH_2); 7.05–7.85 (m, 3H, ArH the 2-Br-substituted ring); 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.64 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.97 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.29 (s, 1H, $H_2NO_2SC_6H_3BrN$*H*); 10.67 (br s, 1H, N*H*$C_6H_3Cl_2$). Found: C, 35.24; H, 2.08; N, 9.34. $C_{13}H_{10}BrCl_2N_3O_3S$ requires: C, 35.56; H, 2.30; N, 9.57%.

N^1-(2-Iodo-4-sulfamoyl-phenyl)-N^3-3,4-dichlorophenyl-urea **6J**, as white crystals, m.p. 280–283°C. (dec.). IR (KBr), cm^{-1}: 759, 960, 1040, 1155 (SO_2^{sym}), 1361 (SO_2^{as}), 1410, 1520 (amide II), 1730 (CO), 3175 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.60 (br s, 2H, SO_2NH_2); 7.05–7.79 (m, 3H, ArH the 2-I-substituted ring); 7.40 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.63 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.97 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.27 (s, 1H, $H_2NO_2SC_6H_3IN$*H*); 10.64 (br s, 1H,

N*H*$C_6H_3Cl_2$). Found: C, 32.21; H, 1.90; N, 8.55. $C_{13}H_{10}Cl_2IN_3O_3S$ requires: C, 32.12; H, 2.07; N, 8.64%.

N^1-(5,6-Dichloro-2,4-disulfamoyl-phenyl)-N^3-3,4-dichlorophenyl-urea **6K**, as white crystals, m.p. 220–221°C. IR (KBr), cm^{-1}: 624, 670, 952, 1039, 1145 (SO_2^{sym}), 1380 (SO_2^{as}), 1410, 1520 (amide II), 1730 (CO), 3175 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.40 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.54 (s, 1H, ArH from the pentasubstituted benzene ring); 7.63 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.68 (br s, 4H, 2 SO_2NH_2); 7.96 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.35 (s, 1H, $(H_2NO_2S)_2C_6HCl_2NH$); 10.64 (br s, 1H, N*H*$C_6H_3Cl_2$). Found: C, 30.85; H, 1.86; N, 11.01. $C_{13}H_{10}Cl_4N_4O_5S_2$ requires: C, 30.73; H, 1.98; N, 11.02%.

N^1-(5-Chloro-2,4-disulfamoyl-phenyl)-N^3-3,4-dichlorophenyl-urea **6L**, as white crystals, m.p. 247–248°C. IR (KBr), cm^{-1}: 657, 780, 945, 1040, 1153 (SO_2^{sym}), 1349 (SO_2^{as}), 1415, 1520 (amide II), 1733 (CO), 3170 (NHCONH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.35 (s, 1H, ArH from disulfamoylphenyl); 7.40 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.57 (s, 1H, ArH from disulfamoylphenyl); 7.63 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.69 (br s, 4H, 2 SO_2NH_2); 7.95 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.34 (s, 1H, $(H_2NO_2S)_2C_6H_2ClNH$); 10.60 (br s, 1H, N*H*$C_6H_3Cl_2$); Found: C, 32.74; H, 2.48; N, 11.69. $C_{13}H_{11}Cl_3N_4O_5S_2$ requires: C, 32.96; H, 2.34; N, 11.83%.

N^1-[(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)ethylcarboxamido]-N^3-3,4-dichlorophenyl-urea **6O**, as white crystals, m.p. 290–294°C (dec.). IR (KBr), cm^{-1}: 710, 960, 1040, 1180 (SO_2^{sym}), 1320 (SO_2^{as}), 1490, 1520 and 1540 (amide II), 1730 and 1740 (CONH), 3280 and 3390 (NHCONH and NH_2), ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.25–2.60 (m, 4H, CH_2CH_2); 6.80 (br s, 3H, CONH + SO_2NH_2); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.94 (s, 1H, H-2 of 3,4-dichlorophenyl); 9.32 (s, 1H, urea N^1H); 10.67 (br s, 1H, urea N*H*$C_6H_3Cl_2$). Found: C, 32.62; H, 2.90; N, 19.07. $C_{12}H_{12}Cl_2N_6O_4S_2$ requires: C, 32.81; H, 2.75; N, 19.13%.

N^1-(2-Sulfamoyl-benzothiazol-6-yl)-N^3-3,4-dichlorophenyl-urea **6P**, as white crystals, m.p. 271–273°C (dec.). IR (KBr), cm^{-1}: 745, 870, 1040, 1160 (SO_2^{sym}), 1345 (SO_2^{as}), 1470, 1525 (amide II), 1740 (CONH); 3280 (NHCONH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.94 (dd, 1H, $J = 9$ Hz; $J = 3$ Hz, H-5 of benzothiazole); 7.10 (d, 1H, $J = 3$ Hz, H-7 of benzothiazole); 7.39 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.62 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.78 (d, 1H, $J = 9$ Hz, H-4 of benzothiazole); 7.96 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.08 (br s, 2H, SO_2NH_2); 9.30 (s, 1H, urea N^1H); 10.64 (br s, 1H, urea N*H*$C_6H_3Cl_2$). Found: C, 40.27; H, 2.69; N, 13.37. $C_{14}H_{10}Cl_2N_4O_3S_2$ requires: C, 40.30; H, 2.42; N, 13.43%.

O^1-(2-Sulfamoyl-benzothiazol-6-yl)-N-3,4-dichlorophenyl-carbamic acid **6Q**, as white crystals, m.p. 248–249°C. IR (KBr), cm^{-1}: 723, 935, 1030, 1160 (SO_2^{sym}), 1345 (SO_2^{as}), 1420, 1535 (amide II), 1770 (COO), 3300 (NHCOO); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.90 (dd, 1H, $J = 9$ Hz; $J = 3$ Hz, H-5 of benzothiazole); 7.11 (d, 1H, $J = 3$ Hz, H-7 of benzothiazole); 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.78 (d, 1H, $J = 9$ Hz, H-4 of benzothiazole); 7.95 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.10 (br s, 2H, SO_2NH_2); 10.75 (br s, 1H, urea N*H*$C_6H_3Cl_2$). Found: C, 40.29; H, 2.15; N, 10.00. $C_{14}H_9Cl_2N_3O_4S_2$ requires: C, 40.20; H, 2.17; N, 10.05%.

O^1-(2-Sulfamoyl-benzothiazol-6-oxyethyl)-N-3,4-dichlorophenyl-carbamic acid **6R**, as white crystals, m.p. 221–222°C. IR (KBr), cm^{-1}: 730, 845, 935, 1030, 1165 (SO_2^{sym}), 1355 (SO_2^{as}), 1425, 1535 (amide II), 1770 (COO), 3300 (NHCOO); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.89 (t, 3H, CH_2); 3.14 (t, 3H, CH_2); 6.95 (dd, 1H, $J = 9$ Hz; $J = 3$ Hz, H-5 of benzothiazole); 7.10 (d, 1H, $J = 3$ Hz, H-7 of benzothiazole); 7.38 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.62 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.79 (d, 1H, $J = 9$ Hz, H-4 of benzothiazole); 7.94 (s, 1H, H-2 of 3,4-dichlorophenyl); 8.14 (br s, 2H, SO_2NH_2); 10.75 (br s, 1H, urea N*H*$C_6H_3Cl_2$). Found: C, 41.28; H, 2.69; N, 8.79. $C_{16}H_{13}Cl_2N_3O_5S_2$ requires: C, 41.57; H, 2.83; N, 9.09%.

O-(4-Sulfamoylphenylmethyl)-N-3,4-dichlorophenyl-carbamic acid **6S**, as white crystals, m.p. 211–213°C. IR (KBr), cm^{-1}: 654, 870, 982, 1040, 1150 SO_2^{sym}, 1340 SO_2^{as}, 1515 (amide II), 1770 (COO), 3170 (NHCONH), 3310 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CONHC*H*$_2$); 7.08–7.41 (m, AA′BB′, $J = 7.2$ Hz; 4H, ArH, phenylene); 7.37 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.49 (s, 2H, SO_2NH_2); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.92 (s, 1H, H-2 of 3,4-dichlorophenyl); 10.66 (br s, 1H, N*H*$C_6H_3Cl_2$). Found: C, 45.13; H, 3.12; N, 7.30. $C_{14}H_{12}Cl_2N_2O_4S$ requires: C, 44.81; H, 3.22; N, 7.47%.

O^1-(4-Sulfamoylphenylethyl)-N-3,4-dichlorophenyl-carbamic acid **6T**, as white crystals, m.p. 228–230°C. IR (KBr), cm^{-1}: 824, 960, 1030, 1059, 1146 (SO_2^{sym}), 1365 (SO_2^{as}), 1520 (amide II), 1760 (COO), 3180 (NHCOO), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.70 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 6.95 (br s, 2H, SO_2NH_2); 7.05–7.52 (m, AA′BB′, $J = 7.3$ Hz, 4H, ArH, phenylene); 7.41 (d, 1H, H-5 of 3,4-dichlorophenyl); 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl); 7.95 (s, 1H, H-2 of 3,4-dichlorophenyl); 10.70 (br s, 1H, N*H*$C_6H_3Cl_2$). Found: C, 46.51; H, 3.80; N, 6.97. $C_{15}H_{14}Cl_2N_2O_4S$ requires: C, 46.28; H, 3.63; N, 7.20%.

N^1-(2-Sulfamoylphenyl)-N^3-(4-toluenesulfonyl)-urea **7A**, as white crystals, m.p. 275–276°C. IR (KBr), cm^{-1}: 560, 700, 815, 1035, 1130 and 1150

(SO_2^{sym}), 1350 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1700 (CO), 3190 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); 7.15–7.69 (m, 4H, ArH, 1,2-phenylene); 7.54 (br s, 2H, SO_2NH_2); 9.30 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.60 (br s, 1H, $NHSO_2C_6H_4Me$). Found: C, 45.19; H, 4.20; N, 11.15. $C_{14}H_{15}N_3O_5S_2$ requires: C, 45.52; H, 4.09; N, 11.37%.

N^1-(3-Sulfamoylphenyl)-N^3-(4-toluenesulfonyl)-urea **7B**, as white crystals, m.p. 290–292°C. (dec.). IR (KBr), cm^{-1}: 775, 845, 1035, 1136 and 1150 (SO_2^{sym}), 1355 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1710 (CO), 3180 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); 7.10–7.50 (m, 4H, ArH, 1,3-phenylene); 7.56 (br s, 2H, SO_2NH_2); 9.30 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.64 (br s, 1H, $NHSO_2C_6H_4Me$). Found: C, 45.58; H, 3.93; N, 11.30. $C_{14}H_{15}N_3O_5S_2$ requires: C, 45.52; H, 4.09; N, 11.37%.

N^1-(4-Sulfamoylphenyl)-N^3-(4-toluenesulfonyl)-urea **7C**, as white crystals, m.p. 299–302°C (dec.). IR (KBr), cm^{-1}: 652, 770, 1040, 1139 and 1150 (SO_2^{sym}), 1325 and 1375 (SO_2^{as}), 1440, 1575 (amide II), 1710 (CO), 3180 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me), δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); δ_A 7.18, δ_B 7.75 (AA′BB′ system, 4H, $J_{AB} = 7.9$ Hz, ArH from 4-sulfamoylphenyl); 7.56 (br s, 2H, SO_2NH_2) 9.30 (s, 1H, $H_2NO_2SC_6H_4NH$); 10.64 (br s, 1H, $NHSO_2C_6H_4Me$). Found: C, 45.43; H, 4.11; N, 11.35. $C_{14}H_{15}N_3O_5S_2$ requires: C, 45.52; H, 4.09; N, 11.37%.

N^1-(4-Sulfamoylphenyl)-N^4-(4-toluenesulfonyl)-semicarbazide **7D**, as white crystals, m.p. 276–277°C. IR (KBr), cm^{-1}: 710, 830, 980 (N–N), 1040, 1171 and 1150 (SO_2^{sym}), 1350 and 1375 (SO_2^{as}), 1440, 1575 (amide II), 1700 (CO), 3190 (NHCONH); 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); 7.05–7.39 (m, AA′BB′, 4H, ArH, 1,4-phenylene); 7.57 (br s, 2H, SO_2NH_2); 9.37 (br s, 2H, $H_2NO_2SC_6H_4NHNH$); 10.60 (br s, 1H, $NHSO_2C_6H_4Me$). Found: C, 43.55; H, 4.16; N, 14.39. $C_{14}H_{16}N_4O_5S_2$ requires: C, 43.74; H, 4.20; N, 14.57%.

N^1-(4-Sulfamoylphenylmethyl)-N^3-(4-toluenesulfonyl)-urea **7E**, as white crystals, m.p. 284–285°C (dec.). IR (KBr), cm^{-1}: 712, 840, 1035, 1150 and 1176 (SO_2^{sym}), 1344 and 1375 (SO_2^{as}), 1440, 1575 (amide II), 1715 (CO), 3180 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 4.90 (s, 2H, CH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); δ_A 7.22, δ_B 7.79 (AA′BB′ system, 4H, $J_{AB} = 7.9$ Hz, ArH from 4-sulfamoylphenyl); 7.61 (br s, 2H, SO_2NH_2); 9.31 (s, 1H, $H_2NO_2SC_6H_4CH_2NH$);

10.72 (br s, 1H, N*H*$SO_2C_6H_4Me$). Found: C, 47.13; H, 5.08; N, 10.91. $C_{15}H_{17}N_3O_5S_2$ requires: C, 46.99; H, 4.97; N, 10.96%.

N^1-(4-Sulfamoylphenylethyl)-N^3-(4-toluenesulfonyl)-urea **7F**, as white crystals, m.p. 278–280°C (dec.). IR (KBr), cm^{-1}: 687, 794, 1040, 1137 and 1150 (SO_2^{sym}), 1351 and 1375 (SO_2^{as}), 1440, 1575 (amide II), 1715 (CO), 3180 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.70 (t, 2H, βCH_2 from the CH_2CH_2 bridge); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); δ_A 7.15, δ_B 7.63 (AA′BB′ system, 4H, $J_{AB} = 7.9$ Hz, ArH from 4-sulfamoylphenyl); 7.61 (br s, 2H, SO_2NH_2); 9.33 (s, 1H, $H_2NO_2SC_6H_4$-CH_2CH_2N*H*); 10.68 (br s, 1H, N*H*$SO_2C_6H_4Me$). Found: C, 48.49; H, 5.10; N, 10.40. $C_{16}H_{19}N_3O_5S_2$ requires: C, 48.35; H, 4.82; N, 10.57%.

N^1-(2-Fluoro-4-sulfamoyl-phenyl)-N^3-(4-toluenesulfonyl)-urea **7G**, as white crystals, m.p. 255–257°C. IR (KBr), cm^{-1}: 654, 910, 1040, 1151 and 1150 (SO_2^{sym}), 1342 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1710 (CO), 3190 (NHCONH); 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.60 (br s, 2H, SO_2NH_2); 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} =$ 7.4 Hz, ArH from tosyl); 7.05–7.89 (m, 3H, ArH from the F-substituted ring); 9.30 (s, 1H, $H_2NO_2SC_6H_3FN$*H*); 10.66 (br s, 1H, urea N*H*-$SO_2C_6H_4Me$). Found: C, 43.50; H, 3.60; N, 10.73. $C_{14}H_{14}FN_3O_5S_2$ requires: C, 43.41; H, 3.64; N, 10.85%.

N^1-(2-Chloro-4-sulfamoyl-phenyl)-N^3-(4-toluenesulfonyl)-urea **7H**, as white crystals, m.p. 264–266°C. IR (KBr), cm^{-1}: 855, 1040, 1156 and 1150 (SO_2^{sym}), 1335 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1700 (CO), 3190 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 6.68 (br s, 2H, SO_2NH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} =$ 7.4 Hz, ArH from tosyl); 7.05–7.80 (m, 3H, ArH from the 2-Cl-substituted ring); 9.30 (s, 1H, $H_2NO_2SC_6H_3ClN$*H*); 10.66 (br s, 1H, urea N*H*SO_2-C_6H_4Me). Found: C, 42.42; H, 3.30; N, 10.09. $C_{14}H_{14}ClN_3O_5S_2$ requires: C, 42.64; H, 3.49; N, 10.40%.

N^1-(2-Bromo-4-sulfamoyl-phenyl)-N^3-(4-toluenesulfonyl)-urea **7I**, as white crystals, m.p. 260–263°C. IR (KBr), cm^{-1}: 720, 1040, 1159 and 1150 (SO_2^{sym}), 1351 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1700 (CO), 3190 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 6.63 (br s, 2H, SO_2NH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} =$ 7.4 Hz, ArH from tosyl); 7.05–7.85 (m, 3H, ArH from the 2-Br-substituted ring); 9.29 (s, 1H, $H_2NO_2SC_6H_3BrN$*H*); 10.67 (br s, 1H, urea N*H*SO_2-C_6H_4Me). Found: C, 37.28; H, 3.04; N, 9.10. $C_{14}H_{14}BrN_3O_5S_2$ requires: C, 37.51; H, 3.15; N, 9.37%.

N^1-(2-Iodo-4-sulfamoyl-phenyl)-N^3-(4-toluenesulfonyl)-urea **7J**, as white crystals, m.p. 288–290°C (dec.). IR (KBr), cm^{-1}: 752, 960, 1040, 1145 and

1150 (SO_2^{sym}), 1345 and 1377 (SO_2^{as}), 1450, 1575 (amide II), 1710 (CO), 3190 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 6.60 (br s, 2H, SO_2NH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB}=7.4$ Hz, ArH from tosyl); 7.05–7.79 (m, 3H, ArH from the 2-I-substituted ring); 9.27 (s, 1H, $H_2NO_2SC_6H_3IN\mathit{H}$); 10.64 (br s, 1H, $N\mathit{H}SO_2C_6H_4Me$). Found: C, 34.12; H, 3.01; N, 8.30. $C_{14}H_{14}IN_3O_5S_2$ requires: C, 33.95; H, 2.85; N, 8.48%.

N^1-(5,6-Dichloro-2,4-disulfamoyl-phenyl)-N^3-(4-toluenesulfonyl)-urea **7K**, as white crystals, m.p. 237–239°C. IR (KBr), cm^{-1}: 675, 928, 1040, 1145 and 1150 (SO_2^{sym}), 1370 and 1375 (SO_2^{as}), 1454, 1570 (amide II), 1720 (CO), 3180 (NHCONH); 3360 (NH_2), ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB}=7.4$ Hz, ArH from tosyl); 7.54 (s, 1H, ArH from the pentasubstituted benzene ring); 7.68 (br s, 4H, 2 SO_2NH_2); 9.35 (s, 1H, $(H_2NO_2S)_2C_6HCl_2N\mathit{H}$); 10.64 (br s, 1H, $N\mathit{H}SO_2C_6H_4Me$). Found: C, 32.68; H, 2.81; N, 10.61. $C_{14}H_{14}Cl_2N_4O_7S_3$ requires: C, 32.50; H, 2.73; N, 10.83%.

N^1-(5-Chloro-2,4-disulfamoyl-phenyl)-N^3-(4-toluenesulfonyl)-urea **7L**, as white crystals, m.p. 249–250°C. IR (KBr), cm^{-1}: 675, 740, 1040, 1153 and 1150 (SO_2^{sym}), 1350 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1720 (CO), 3190 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB}=7.4$ Hz, ArH from tosyl); 7.35 (s, 1H, ArH from disulfamoylphenyl); 7.57 (s, 1H, ArH from disulfamoylphenyl); 7.69 (br s, 4H, 2 SO_2NH_2); 9.34 (s, 1H, $(H_2NO_2S)_2C_6H_2ClN\mathit{H}$); 10.60 (br s, 1H, $N\mathit{H}SO_2C_6H_4Me$). Found: C, 34.54; H, 2.98; N, 11.49. $C_{14}H_{15}ClN_4O_7S_3$ requires: C, 34.82; H, 3.13; N, 11.60%.

N^1-(2-Sulfonamido-1,3,4-thiadiazol-5-yl)-N^3-(4-toulenesulfonyl)-urea **7M**, as white crystals, m.p. > 320°C. IR (KBr), cm^{-1}: 765, 989, 1150 and 1177 (SO_2^{sym}), 1344 and 1375 (SO_2^{as}), 1545 (amide II), 1585, 1730 (CO), 3290 (NHCONH), 3380; ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 6.88 (br s, 2H, SO_2NH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB}=7.4$ Hz, ArH from tosyl); 10.79 (br s, 1H, urea $N\mathit{H}SO_2C_6H_4Me$); 11.55 (s, 1H, H_2NO_2S-1,3,4-thiadiazole-NH). Found: C, 32.10;, H, 3.05; N, 18.45. $C_{10}H_{11}N_5O_5S_3$ requires: C, 31.82; H, 2.94; N, 18.56%.

N^1-(4-Methyl-2-sulfonamido-δ^2-1,3,4-thiadiazolin-5-yl)-N^3-(4-toluenesulfonyl)-urea **7N**, as white crystals, m.p. > 310°C. IR (KBr), cm^{-1}: 640, 835, 957, 1150 and 1184 (SO_2^{sym}), 1361 and 1375 (SO_2^{as}), 1540 (amide II), 1585, 1730 (CO), 3280 (NHCONH), 3380; ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 3.90 (s, 3H, N–Me); 6.92 (br s, 2H, SO_2NH_2); δ_A 7.06, δ_B 7.47 (AA′BB′ system, 4H, $J_{AB}=7.4$ Hz, ArH from tosyl); 10.80 (br s, 1H, urea $N\mathit{H}SO_2C_6H_4Me$); 11.53 (s, 1H, H_2NO_2S-1,3,4-thiadiazoline-NH). Found: C, 33.98; H, 3.19; N, 17.76. $C_{11}H_{13}N_5O_5S_3$ requires: C, 33.75; H, 3.35; N, 17.89%.

N^1-[(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)ethylcarboxamido]-N^3-(4-toluenesulfonyl)-urea **7O**, as white crystals, m.p. 299–303°C (dec.). IR (KBr), cm^{-1}: 700, 834, 1040, 1150 and 1180 (SO_2^{sym}), 1330 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1710 (CO), 3190 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.25–2.60 (m, 4H, CH_2CH_2); 2.40 (s, 3H, Me); 6.80 (br s, 3H, CONH + SO_2NH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); 9.32 (s, 1H, urea N^1H); 10.67 (br s, 1H, urea $NHSO_2C_6H_4Me$). Found: C, 34.62; H, 3.91; N, 18.70. $C_{13}H_{16}N_6O_6S_3$ requires: C, 34.81; H, 3.60; N, 18.74%.

N^1-(2-Sulfamoyl-benzothiazol-6-yl)-N^3-(4-toluenesulfonyl)-urea **7P**, as white crystals, m.p. 279–281°C (dec.). IR (KBr), cm^{-1}: 655, 850, 1040, 1150 and 1165 (SO_2^{sym}), 1345 and 1377 (SO_2^{as}), 1450, 1570 (amide II), 1720 (CO), 3190 (NHCONH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); 6.94 (dd, 1H, $J = 9$ Hz; $J = 3$ Hz, H-5 of benzothiazole); 7.10 (d, 1H, $J = 3$ Hz, H-7 of benzothiazole); 7.78 (d, 1H, $J = 9$ Hz, H-4 of benzothiazole); 8.08 (br s, 2H, SO_2NH_2); 9.30 (s, 1H, urea N^1H); 10.64 (br s, 1H, urea $NHSO_2C_6H_4Me$). Found: C, 42.28; H, 2.99; N, 13.07. $C_{15}H_{14}N_4O_5S_3$ requires: C, 42.24; H, 3.31; N, 13.14%.

O-(2-Sulfamoyl-benzothiazol-6-yl)-N-(4-toluenesulfonyl)-carbamic acid **7Q**, as white crystals, m.p. 280–282°C, IR (KBr), cm^{-1}: 846, 931, 1030, 1150 and 1160 (SO_2^{sym}), 1350 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1775 (COO), 3300 (NHCOO); 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); 6.90 (dd, 1H, $J = 9$ Hz; $J = 3$ Hz, H-5 of benzothiazole); 7.11 (d, 1H, $J = 3$ Hz, H-7 of benzothiazole); 7.78 (d, 1H, $J = 9$ Hz, H-4 of benzothiazole); 8.10 (br s, 2H, SO_2NH_2); 10.75 (br s, 1H, $NHSO_2C_6H_4Me$). Found: C, 42.20; H, 2.95; N, 9.77. $C_{15}H_{13}N_3O_6S_3$ requires: C, 42.15; H, 3.07; N, 9.83%.

O-(2-Sulfamoyl-benzothiazol-6-oxyethyl)-N-(4-toluenesulfonyl)-carbamic acid **7R**, as white crystals, m.p. 254–255°C. IR (KBr), cm^{-1}: 650, 894, 955, 1030, 1150 and 1175 (SO_2^{sym}), 1348 and 1375 (SO_2^{as}), 1450, 1770 (COO), 3300 (NHCOO), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 2.89 (t, 3H, CH_2); 3.14 (t, 3H, CH_2); δ_A, 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, from tosyl); 6.95 (dd, 1H, $J = 9$ Hz; $J = 3$ Hz, H-5 of benzothiazole); 7.10 (d, 1H, $J = 3$ Hz, H-7 of benzothiazole); 7.79 (d, 1H, $J =$ 9 Hz, H-4 of benzothiazole); 8.14 (br s, 2H, SO_2NH_2); 10.75 (br s, 1H, urea $NHSO_2C_6H_4Me$). Found: C, 43.21; H, 3.90; N, 8.79. $C_{17}H_{17}N_3O_7S_3$ requires: C, 43.30; H, 3.63; N, 8.91%.

O-(4-Sulfamoylphenylmethyl)-N-(4-toluenesulfonyl)-carbamic acid **7S**, as white crystals, m.p. 240–243°C. IR (KBr), cm^{-1}: 724, 840, 935, 1040, 1150

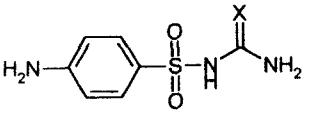
and 1155 (SO_2^{sym}), 1325 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1770 (COO), 3170 (NHCO), 3310 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 4.90 (s, 2H, CONHCH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl); 7.08–7.41 (m, AA′BB′, $J = 7.2$ Hz; 4H, ArH, phenylene); 7.49 (s, 2H, SO_2NH_2); 10.66 (br s, 1H, NHSO$_2$C$_6$H$_4$Me). Found: C, 47.19; H, 5.56; N, 4.98. $C_{15}H_{16}N_2O_6S_2$ requires: C 47.35; H, 5.30; N, 5.26%.

O-(4-Sulfamoylphenyl)-N-(4-toluenesulfonyl)-carbamic acid **7T**, as white crystals, m.p. 264–267°C. IR (KBr), cm^{-1}: 840, 936, 1030, 1146 and 1153 (SO_2^{sym}), 1326 and 1375 (SO_2^{as}), 1450, 1570 (amide II), 1760 (COO), 3180 (NHCOO); 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.40 (s, 3H, Me); 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.70 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 6.95 (br s, 2H, SO_2NH_2); δ_A 7.06, δ_B 7.46 (AA′BB′ system, 4H, $J_{AB} = 7.4$ Hz, ArH from tosyl), 7.05–7.52 (m, AA′BB′, $J = 7.3$ Hz, 4H, ArH, phenylene); 10.70 (br s, 1H, NHSO$_2$C$_6$H$_4$Me). Found: C, 47.95; H, 4.67; N, 6.81. $C_{16}H_{18}N_2O_6S_2$ requires: C, 48.23; H, 4.55; N, 7.03%.

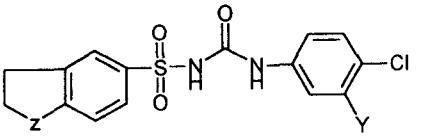
RESULTS AND DISCUSSION

The reactions of aryl isocyanates[44] and arylsulfonyl isocyanates[45] with active hydrogen containing compounds, such as amines or alcohols/phenols, have thoroughly been investigated due to the many applications of the obtained derivatives as polymers (plastics), insecticides or biologically active substances with potential clinical use.[44–46] Thus, in the search of more active antibacterial sulfonamides, a large number of arylsulfonyl ureas[47a,b] or thioureas[47c] containing sulfanilamide moieties have been prepared and assayed for biological activity.[46,47] Sulfanilylurea **8** and sulfanilylthiourea **9** were found to possess excellent antimycotic, antibacterial and anti-*Mycobacterium tuberculosi* activity.[46,47] More recently, the interest in these classes of compounds has been revived by the report of the strong anticancer properties of some arylsulfonylureas by researchers at Eli Lilly,[48] with derivatives of type **10**–**13** highly active in preliminary clinical studies against a variety of tumors.[49] Although the precise mechanism of action of these new anticancer compounds is presently unknown, it is assumed that their cytotoxicity may be a consequence of the uncoupling of mitochondria,[50] where high concentrations of a CA are also present.[7c,8] However, up to the present time studies have not been made to determine whether such anticancer compounds interfere with CA activity, although we hypothesize a strong CA V inhibition as very probable. Hopefully, these compounds will

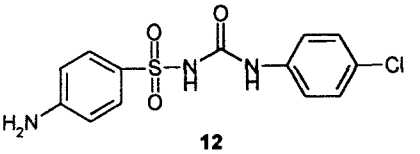
lead to effective new types of anticancer drugs in the near future.[48]



8: X = O
9: X = S



10: Y = H, Z = CH_2 - sulofenur
11: Y = Cl, Z = O - LY295501



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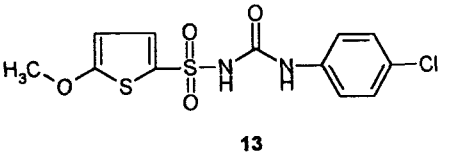
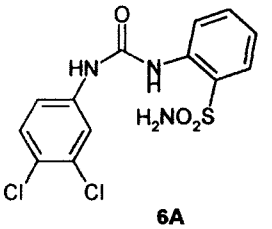
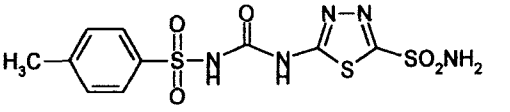


13

Only recently the reactions of aryl isocyanates or isothiocyanates with amino-containing sulfonamides was applied to the preparation of CA inhibitors by this group.[31] A series of compounds was obtained by reaction of phenyl isocyanate, 3,4-dichlorophenyl isocyanate, phenyl isothiocyanate and allyl isothiocyanate with aromatic/heterocyclic sulfonamides of type **C**, **E**, **F**, **M** and **N**. The exciting finding was that some of those compounds strongly inhibited isozyme CA I (in addition to the sulfonamide-avid isozymes II and IV) with affinities sometimes in the nanomolar range. The most interesting compounds prepared in the previous study[31] were those derived from 3,4-dichlorophenyl isocyanate **6**, so that here we extend the investigations to include more sulfonamides (compounds **A**–**T**) as well as derivatives of an arylsulfonyl isocyanate (tosyl isocyanate **7**), in an attempt to obtain a lead molecule for a selective CA I inhibitor. For simplicity the new derivatives prepared in the present study are designated with a number followed by a letter: the number specifies the isocyanate and the letter the parent sulfonamide from which the compounds were prepared e.g. **6A** is the urea derivative obtained from orthanilamide **A** and 3,4-dichlorophenyl isocyanate **6**; **7M** is the arylsulfonylurea obtained from 5-amino-1,3,4-thiadiazole-2-sulfonamide **M** and tosyl isocyanate **7** (see structures below).



6A



7M

Compounds (**6A–T**)–(**7A–T**) were characterized by standard chemical and physical methods that confirmed their structure (see Materials and Methods for details) and were assayed inhibitors of isozymes hCA I, hCA II and bCA IV (Table I).

TABLE I CA inhibition data for standard inhibitors **1–5**, the parent sulfonamides **A–T** and the new derivatives reported in the present study against isozymes I, II and IV

Inhibitor	K_I* (nM)		
	hCA I[a]	*hCA II*[a]	*bCA IV*[b]
Acetazolamide **1**	900	12	220
Benzolamide **2**	15	9	12
Ethoxzolamide **3**	25	8	13
Dichlorophenamide **4**	1200	38	380
Dorzolamide **5**	50000	9	45
A	45400	295	1310
B	25000	240	2200
C	28000	300	3000
D	78500	320	3215
E	25000	170	2800
F	21000	160	2450
G	8300	60	180
H	9800	110	320
I	6500	40	66
J	6000	70	125
K	6100	28	175
L	8400	75	160
M	8600	60	540
N	9300	19	355
O	455	3	125
P	70	9	19
Q	55	8	17
R	50	7	15
S	24000	125	560
T	18000	110	450
6A	5000	250	900
6B	120	80	110
6C[c]	20	9	10
6D	20	12	24
6E[c]	12	7	10
6F[c]	12	13	20
6G	10	13	15
6H	25	20	35
6I	14	16	21
6J	18	17	24
6K	10	13	19
6L	29	18	45
6M[c]	360	2	9
6O	8	3	12
6P	5	6	9
6Q	6	6	8
6R	5	7	10
6S	24	21	38

TABLE I (*Continued*)

Inhibitor	K_I* (nM)		
	hCA I[a]	*hCA II*[a]	*bCA IV*[b]
6T	21	20	35
7A	2000	85	180
7B	9	10	13
7C	12	13	24
7D	16	16	31
7E	8	8	25
7F	7	12	23
7G	7	9	14
7H	15	11	27
7I	10	12	26
7J	15	15	30
7K	8	12	14
7L	22	13	20
7M	3	4	12
7N	5	6	14
7O	4	5	11
7P	3	4	5
7Q	5	6	8
7R	5	8	10
7S	21	25	40
7T	20	24	36

*Standard error for the determination of K_I was 5–10% (from 2 different assays).
[a]Human (cloned) isozyme.
[b]Isolated from bovine lung microsomes.
[c]From Ref. [31].

Inhibition data against three CA isozymes, hCA I, hCA II and bCA IV with the new derivatives (Table I) shows that the substituted-ureido/arylsulfonylureido sulfonamides generally behave as strong inhibitors and have increased potency as compared to the parent compounds from which they were prepared (the amino-sulfonamides **A**–**T**). The potency of the obtained inhibitor generally varied in the following manner, based on the parent sulfonamide from which it was prepared: *p*-hydrazino-benzenesulfonamides **D** < sulfanilamides **C** < orthanilamides **A** ≅ metanilamides **B** < homosulfanilamides **E** < *p*-aminoethyl-benzenesulfonamides **F** < 1,3-benzenedisulfonamides **K** and **L** ≅ halogeno-substituted sulfanilamides **G**–**J** < 1,3,4-thiadiazoline-2-sulfonamides **M** and **O** ≅ 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide **N** < benzothiazole-2-sulfonamides **P**–**R** derivatives. Based on the isocyanate, the order was: $C_6H_3Cl_2NCO$ < TsNCO. This means that the best inhibitors (against all isozymes) were those obtained from tosyl isocyanate and heterocyclic derivatives, such as 1,3,4-thiadiazole or benzothiazole sulfonamides. All three CA isozymes investigated here were susceptible to inhibition with this type of sulfonamide, but again the interesting finding was the increased affinity for hCA I by some of these

inhibitors, comparable with those previously mentioned by our group.[31] As seen from, data of Table I, generally hCA II is the most susceptible to inhibition with aromatic/heterocyclic sulfonamides of type **A**–**T**, followed by bCA IV, whereas hCA I has a much lower affinity for this class of inhibitors. These statements are well illustrated by the case of the classical inhibitors of type **1**–**5**, which generally have the ratios K_I (hCA I)/K_I (hCA II) $\cong$ 1.67–75, and K_I (bCA IV)/K_I (hCA II) $\cong$ 1.33–18. This means that hCA I is 1.67–75 times less susceptible to inhibition by acetazolamide and its congeners, whereas bCA IV only 1.33–18 times less susceptible (mention should be made that the low figures for the above ratios refer to benzolamide; otherwise, for other types of classical sulfonamide inhibitors, the affinity for hCA I is generally 5–75 less as compared to that for hCA II, etc.). A very interesting case is dorzolamide **4**, the new representative of topically effective antiglaucoma sulfonamides discovered at Merck, Sharp & Dohme[24] which is 5500 times less effective as an inhibitor of hCA I compared to hCA II. A notable exception is also represented by benzolamide **2**, which has the highest hCA I affinity among the classical inhibitors,[51] with the lowest ratio K_I(hCA 1)/K_I(hCA II) $\cong$ 1.66 (but this is an atypical behavior for a classical sulfonamide inhibitor).[51] With the compounds reported here by us, there are instances of derivatives possessing an inhibition ratio K_I(hCA I)/K_I(hCA II) $\cong$ 1 (e.g. **6H**, **6J**, **6L**, **6Q**, **6T**, **7D**, **7E**, **7J**), whereas others have the above mentioned ratio less than 1 (e.g. **6G**, **6I**, **6K**, **6P**, **6R**, **7B**, **7C**, **7F**, **7G**, **7I**, **7K**, **7M**–**7T**). As far as we know, this is the first report (except for our preliminary communication)[31] of sulfonamides that act as better inhibitors of isozyme I as compared to the "sulfonamide-avid" isozymes II and IV. We do not claim that we have obtained hCA I-specific inhibitors, since the compounds synthesized inhibit to a large extent the other two isozymes. However, our results are a promising start towards the design of high affinity and entirely isozyme I-specific CA inhibitors.

References

[1] This paper is Part 66 of the series. "Carbonic anhydrase inhibitors". Preceding part of the series: Supuran, C.T., Mincione, F., Scozzafava, A. *et al.* (1999) *Eur. J. Med. Chem.*, **34**, in press.

[2] M. Sandler and H.J. Smith (Eds.) (1994) *Design of Enzyme Inhibitors as Drugs*, Vols. 1 and 2. Oxford University Press; Oxford.

[3] R.B. Silverman (1992) *The Organic Chemistry of Drug Design and Drug Action.* Academic Press; San Diego.

[4] (a) F. Briganti, S. Mangani, P. Orioli, A. Scozzafava, G. Vernaglione and C.T. Supuran (1997) *Biochemistry*, **36**, 10384–10392; (b) F. Briganti, S. Mangani, P. Orioli, A. Scozzafava, G. Vernaglione and C.T. Supuran (1998) *Biochemistry*, **37**, in press; (c) M. Barboiu, C.T. Supuran, A. Scozzafava, F. Briganti, C. Luca, G. Popescu, L. Cot and N. Hovnanian (1997) *Liebigs Ann./Recueil*, 1853–1859.

[5] (a) D. Hewett-Emmett and R.E. Tashian (1996) *Mol. Phylogenet. Evol.*, **5**, 50–77; (b) C.T. Supuran (1994) "Carbonic anhydrase inhibitors", In *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism*, (Puscas, I., Ed.) pp. 29–111. Helicon; Timisoara.
[6] R.P. Henry (1996) *Annu. Rev. Physiol.*, **58**, 523–538.
[7] (a) C.A. Winkler, A.M. Kittelberger, and G.J. Schwartz (1997) *Am. J. Physiol.*, **272**, F551–F560; (b) T.H. Maren, C.W. Conroy, G.C. Wynns and D.R. Godman (1997) *J. Pharmacol. Exp. Ther.*, **280**, 98–104; (c) S.J. Dodgson and K. Cherian (1990) *Arch. Biochem. Biophys.*, **282**, 1–7.
[8] S.J. Dodgson (1987) *J. Appl. Physiol.*, **63**, 2134–2141.
[9] S.A. Hazen, A. Waheed, W.S. Sly, K.F. LaNoue and C.J. Lynch (1996) *FASEB J.* **10**, 481–490.
[10] C.T. Supuran, C.W. Conroy and T.H. Maren (1996) *Eur. J. Med. Chem.*, **31**, 843–846.
[11] C.T. Supuran, A. Nicolae and A. Popescu (1996) *Eur. J. Med. Chem.*, **31**, 431–438.
[12] C.T. Supuran, A. Popescu, M. Ilisiu, A. Costandache and M.D. Banciu (1996) *Eur. J. Med. Chem.*, **31**, 439–448.
[13] (a) C.T. Supuran, F. Briganti and A. Scozzafava (1997) *J. Enz. Inhib.*, **12**, 175–190; (b) C.T. Supuran, C.W. Conroy and T.H. Maren (1997) *Proteins*, **27**, 272–278.
[14] (a) F. Briganti, R. Pierattelli, A. Scozzafava and C.T. Supuran (1996) *Eur. J. Med. Chem.*, **31**, 1001–1010; (b) C.T. Supuran and B.W. Clare (1995) *Eur. J. Med. Chem.*, **30**, 687–696.
[15] (a) C.T. Supuran and A. Scozzafava (1997) *J. Enz. Inhib.*, **12**, 37–51; (b) G. Mincione, A. Scozzafava and C.T. Supuran (1997) *Metal Based Drugs*, **4**, 27–34; (c) A. Scozzafava and C.T. Supuran (1997) *Metal Based Drugs*, **4**, 19–26.
[16] C.T. Supuran, A. Scozzafava, A. Popeseu, R. Bobes-Tureac, A. Banciu, A. Creanga, G. Bobes-Tureac and M.D. Banciu (1997) *Eur. J. Med. Chem.*, **32**; 445–452.
[17] I. Bertini, F. Briganti and A. Scozzafava (1995) Zinc proteins. In *Handbook of Metal – Ligand Interactions of Biological Fluids* (Vol. 1), (Berthon, G. Ed.), pp. 175–191. M Dekker; New York.
[18] I. Bertini, G. Canti, C. Luchinat and A. Scozzafava (1978) *J. Am. Chem. Soc.*, **100**, 4873–4877.
[19] I. Bertini, C. Luchinat and A. Scozzafava (1982) *Struct. Bonding*, **48**, 45–92.
[20] C. Tu, S.R. Paranawithana, D.A. Jewell, S.M. Tanhauser, P.V. LoGrasso, G.C. Wynns, P.J. Laipis and D.N. Silverman (1990) *Biochemistry*, **29**, 6400–6405.
[21] I. Puscas and C.T. Supuran (1996) Farmacologia clinica da ulcer peptica. In *Aparelho Digestivo*. (J. Coelho, Ed.), pp. 1704–1734. Rio de Janeiro; MEDSI.
[22] (a) C.T. Supuran, F. Mincione, A. Scozzafava, F. Briganti, G. Mincione and M.A. Ilies (1998) *Eur. J. Med. Chem.*, **33**; 247–254; (b) A. Scozzafava and C.T. Supuran (1998) *J. Enz. Inhib.*, **13**; 419–442.
[23] F. Mincione, L. Menabuoni, F. Briganti, G. Mincione, A. Scozzafava and C.T. Supuran (1998) *J. Enz. Inhib.*, **13**; 267–284.
[24] (a) M.F. Sugrue (1996) *J. Ocul. Pharmacol. Ther.*, **12**; 363–376; (b) L.H. Silver (1998) *Am. J. Ophthalmol.*, **126**; 400–408.
[25] I.M. Weiner (1990) Diuretics and other agents employed in the mobilization of edema fluids. In *The Pharmacological Basis of Therapeutics*, 8th edn. (A.G. Gilman, T.W. Rall, A.S. Nies and P. Taylor Eds.), pp. 713–732. Pergamon Press; New York.
[26] W.G. Reiss (1996) *Ann. Pharmacother.*, **30**, 514–519.
[27] M. Stoll, G.F. Hamann, V. Jost, U.A. Bompotti, R. Fitridge and K. Schimrigk (1996) *J. Neuroimaging*, **6**, 144–149.
[28] R.L. Levine, P.A. Tursky, W.D. Turnipseed and T. Grist (1996) *J. Neuroimaging*, **6**, 126–130.
[29] (a) C.T. Supuran, G. Manole, A. Dinculescu, A. Schiketanz, M.D. Gheorghiu, I. Puscas and A.T. Balaban (1992) *J. Pharm. Sci.*, **81**, 716–719; (b) C.T. Supuran, A. Scozzafava, M.A. Ilies, B. Iorga, T. Cristea, F. Briganti, F. Chiraleu and M.D. Banciu (1998) *Eur. J. Med. Chem.*, **33**, 577–595.
[30] B.W. Clare and C.T. Supuran (1997) *Eur. J. Med. Chem.*, **32**, 311–319.
[31] C.T. Supuran, A. Scozzafava, B.C. Jurca and M.A. Ilies (1998) *Eur. J. Med. Chem.*, **33**, 83–93.

[32] G.B. Crippa and S. Maffei (1941) *Gazz. Chim. Ital.*, **71**, 97–99.
[33] (a) J.V. Scudi (1937) *J. Am. Chem. Soc.*, **59**, 1480–1483; (b) M.A. Ilies, A. Scozzafava and C.T. Supuran (1999) *Eur. J. Med. Chem.*, **34**, in press; (c) E. Cingolani (1948) *Gazz. Chim. Ital.*, **78**, 275–282; (d) H. Bauer (1939) *J. Am. Chem. Soc.*, **61**, 613–616.
[34] A. Jitianu, M.A. Ilies, A. Scozzafava and C.T. Supuran (1997) *Main Group Met. Chem.*, **20**, 147–153.
[35] (a) M. Barboiu, A. Scozzafava and C.T. Supuran (1999), *J. Enzyme Inhib.*, in press; (b) J. Korman (1958) *J. Org. Chem.*, **23**, 1768–1771; R.D. Scoenwald, M.G. Eller, J.A. Dixson and C.F. Barfknecht (1984). *J. Med. Chem.*, **27**, 810–812; (c) A. Scozzafava and C.T. Supuran (1998), manuscript in preparation.
[36] C. Forsman, G. Behravan, A. Osterman and B.H. Jonsson (1988) *Acta Chem. Scand.*, **B42**, 314–318.
[37] G. Behravan, P. Jonasson, B.H. Jonsson and S. Lindskog (1991) *Eur. J. Biochem.*, **198**, 589–592.
[38] R.G. Khalifah, D.J. Strader, S.H. Bryant and S.M. Gibson (1977) *Biochemistry*, **16**, 2241–2247.
[39] P.O. Nyman and S. Lindskog (1964) *Biochim. Biophys. Acta*, **85**, 141–151.
[40] L.E. Henderson, D. Henriksson and P.O. Nyman (1976) *J. Biol. Chem.*, **251**, 5457–5463.
[41] T.H. Maren, G.C. Wynns and P.J. Wistrand (1993) *Mol. Pharmacol.*, **44**, 901–906.
[42] Y. Pocker and J.T. Stone (1967) *Biochemistry*, **6**, 668–678.
[43] T.T. Baird, A. Waheed, T. Okuyama, W.S. Sly and C.A. Fierke (1997) *Biochemistry*, **36**, 2669–2678.
[44] (a) J.H. Saunders and R.J. Slocombe (1948) *Chem. Rev.*, **43**, 203–218; (b) R.G. Arnold, J.A. Nelson and J.J. Verbanc (1957) *Chem. Rev.*, **57**, 47–76.
[45] (a) H. Ulrich (1965) *Chem. Rev.*, **65**, 369–376 (and references cited therein); (b) A. Vigroux, M. Bergon, C. Bergonzi and P. Tisnès (1994) *J. Am. Chem. Soc.*, **116**, 11787–11796.
[46] F. Kurzer (1952) *Chem. Rev.*, **50**, 1–46 (and references cited therein).
[47] (a) E.H. Cox and S.M. Raymond (1941) *J. Am. Chem. Soc.*, **63**, 300–301; (b) J.S. Roth and E.F. Degering (1945) *J. Am. Chem. Soc.*, **67**, 126–128; (c) F. Bergmann (1946) *J. Am. Chem. Soc.*, **68**, 761–764.
[48] (a) J.J. Howbert, C.S. Grossman, T.A. Crowell, B.J. Rieder, R.W. Harper, K.E. Kramer, E.V. Tao, J. Aikins, G.A. Poore and S.M. Rinzel (1990) *J. Med. Chem.*, **33**, 2393–2407; (b) F. Mohamadi, M.M. Spees and G.B. Grindey (1992) *J. Med. Chem.*, **35**, 3012–3016; (c) J.E. Toth, G.B. Grindey, W.J. Ehlhardt, J.E. Ray, G.B. Boder, J.R. Bewley, K.K. Klingerman, S.B. Gates, S.M. Rinzel, R.M. Schultz, L.C. Weir and J.F. Worzalla (1997) *J. Med. Chem.*, **40**, 1018–1025.
[49] (a) W.J. Ehlhardt, J.M. Woodland, J.F. Worzalla, J.R. Bewley, G.B. Grindey, G.C. Todd, J.E. Toth and J.J. Howbert (1992) *Chem. Res. Toxicol.*, **5**, 667–673; (b) C. Kim, W.C. MacKellar, N.M. Cho, S.R. Byrn and D.J. Morre (1997) *Biochim. Biophys. Acta*, **13**, 171–181; (c) P.J. Houghton, P.J. Cheshire, L. Myers, L. Lutz, J. Toth, G.B. Grindey and J.A. Houghton (1995) *Anticancer Drugs*, **6**, 317–323.
[50] (a) G.F. Rush, S.M. Rinzel, G. Boder, R.A. Heim, J.E. Toth and G.D. Ponsler (1992) *Biochem. Pharmacol.*, **44**, 2387–2394; (b) J. Sosinski, J.H. Thakar, G.S. Germain, P. Dias, F.C. Harwood, J.F. Kuttesch and P.J. Houghton (1994) *Mol. Pharmacol.*, **45**, 962–970; (c) P.J. Houghton and J.A. Houghton (1996) *Invest. New Drugs*, **14**, 271–280.
[51] C.T. Supuran, M.A. Ilies and A. Scozzafava (1998) *Eur. J. Med. Chem.*, **33**, 739–751.