J. Enzyme Inhibition, 1998, Vol. 13, pp. 419–442
Reprints available directly from the publisher
Photocopying permitted by license only

CARBONIC ANHYDRASE INHIBITORS: NOVEL COMPOUNDS CONTAINING S–NH MOIETIES: SULFENAMIDO-SULFONAMIDES, SULFENIMIDO-SULFONAMIDES AND THEIR INTERACTION WITH ISOZYMES I, II AND IV*

ANDREA SCOZZAFAVA and CLAUDIU T. SUPURAN†

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

(Received 11 March 1998)

Reaction of 2-nitrophenyl- and 4-nitrophenylsulfenyl chlorides with aromatic/heterocyclic sulfonamides/bis-sulfonamides containing a free amino, hydrazino or imino group afforded sulfenamido-sulfonamides, or sulfenimido-sulfonamides. Oxidation of these derivatives with potassium permanganate in acetone led to the corresponding bis-sulfonamides. The obtained compounds were assayed as inhibitors of the zinc enzyme carbonic anhydrase (CA), isozymes hCA I, hCA II (human cytosolic forms from red cells) and bCA IV (bovine membrane-associated form). Good inhibition of the three CA isozymes was observed with some of the new compounds, the bis-sulfonamides being more active than the sulfenamido-sulfonamides. Structure–active correlations for the new series of inhibitors are discussed. Some of the sulfenamido-sulfonamides (but not the corresponding bis-sulfonamides) showed topical intraocular pressure lowering effects when applied as a 2% solution directly into the rabbit eye.

Keywords: Aromatic/heterocyclic sulfonamide; Bis-sulfonamide; Carbonic anhydrase; Isozyme I, II, IV; Sulfenamide; Antiglaucoma agents

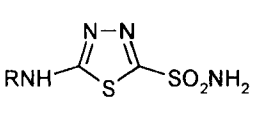
INTRODUCTION

Heterocyclic/aromatic sulfonamide derivatives possessing the general formula RSO_2NH_2 played a critical role in the development of several

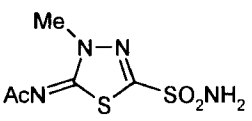
*See Ref. [1].

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

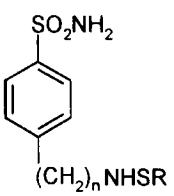
important classes of pharmacological agents,[2-7] such as the diuretics with saluretic action,[8,9] benzothiadiazine[10] and high-ceiling diuretics,[11] or the antiglaucoma drugs with carbonic anhydrase (CA) inhibitory action among others.[12,13] The prototype for all these drugs was constituted by acetazolamide **1a**, the first non-mercurial diuretic,[2,8] used for more than 45 years in clinical medicine as a diuretic,[8] antiglaucoma,[8,12] antiepileptic[14] and antiulcer compound.[15] It is still used nowadays, mainly as a diagnostic tool in NMR imaging,[16,17] and in many physiological studies.[18-20] The major biological action of acetazolamide and related sulfonamides such as benzolamide **1b** or methazolamide **2** is connected with the powerful inhibition of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1), for which at least eight isozymes are presently known in higher vertebrates.[4-8,12] The multitude of different isoforms present in diverse cellular compartments (in the cytosol the isozymes CA I, II, III and VII;[21] the isozyme CA IV is membrane bound,[22] whereas CA V is found in mitochondria[23] and CA VI secreted into the saliva[24]) or tissues (CA I is very abundant in blood red cells;[7,8] CA II is also present in red blood cells, in lower concentration as compared to CA I, but is also very abundant in secretory tissues within the gastro-intestinal tract, kidneys, pancreas, cerebrospinal fluid, eye;[6-8,12] CA III in the muscle[25] and CA IV in the plasma membranes, mainly in lungs, kidneys and cilliary pocesses of the eye[22,26]) makes this enzyme the target for designing many types of drugs or diagnostic tools, based on such sulfonamide inhibitors.[27-30] Thus, in addition to their well-known action as diuretic or systemic/topical antiglaucoma drugs, sulfonamides possessing CA inhibitory properties which might be developed as diagnostic tools in positron emission tomography (PET) have recently been reported by this group,[30] as well as some omeprazole-like compounds of type **3**, which by activation in acidic media are transformed into a potent sulfonamide CA inhibitor together with an agent able to inactivate H^+/K^+-ATP-ase by reaction with cysteine residues of this enzyme; in this way, the two key enzymes involved in gastric acid secretion are both inactivated.[28,31]



1 **a**: R = MeCO
b: R = $PhSO_2$



2



3
n = 0, 1, 2
R = substituted-phenyl

Compounds of type **3** mentioned above are sulfenamido-sulfonamides, obtained by reaction of sulfenyl chlorides with amino-containing aromatic/heterocyclic sulfonamides.[28] Their interesting CA inhibitory properties, as well as their potential use for novel types of pharmacological agents, prompted us to extend the previous studies[28] regarding the reaction of sulfenyl halides with sulfonamides. Here we report the preparation of novel compounds of type **3**, obtained by reaction of sulfenyl chlorides with 11 amino-sulfonamides/bis-sulfonamides (compounds **4**–**14**) and one imino-sulfonamide **15**. The obtained sulfenamido-sulfonamides **16**–**26** and **28**–**38**, as well as the sulfenimido-sulfonamides **27** and **39** were then oxidized to the corresponding bis-sulfonamides **40**–**63** with potassium permanganate in acetone. The new compounds reported here were characterized by standard procedures, assayed *in vitro* as inhibitors of three CA isozymes, CA I, II and IV (the physiologically most relevant isozymes[4–7]), and some of them were studied *in vivo* for their possible topical intraocular pressure (IOP) lowering properties. Interesting *in vitro* and *in vivo* activities were detected for some of the compounds belonging to the above mentioned series of CA inhibitors.

MATERIALS AND METHODS

Melting points were determined with a heating plate microscope and are not corrected. IR spectra were obtained from KBr pellets with a Perkin-Elmer 16PC FTIR spectrometer, whereas ^{1}H-NMR spectra were obtained 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 **4**, **12**, **13**, sulfanilamide, acetazolamide and methazolamide used in synthesis were commercially available (from Sigma, Acros or Aldrich). 4-Hydrazino-benzenesulfonamide **5** was prepared by diazotization of sulfanilamide followed by reduction of the diazonium salt with tin(II) chloride[32] Halogeno/dihalogeno-sulfanilamides **6**–**11** were prepared by halogenation of sulfanilamide as reported in the literature.[33] Compound **14** was obtained from 5-amino-1,3,4-thiadiazole-2-sulfonamide (from acetazolamide)[34] by acylation with the phthalimido-derivative of β-alanine, followed by hydrazinolysis,[35] and the imine **15** by deprotection of methazolamide with concentrated hydrochloric acid.[30] 2-Nitrobenzenesulfenyl chloride and triethylamine were from Acros, 4-nitrobenzenesulfenyl chloride from Aldrich. Acetonitrile (Merck) used as solvent in the synthesis was doubly

distilled and kept on molecular sieves in order to maintain it in anhydrous conditions. Acetone and potassium permanganate were from Merck.

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL2I (DE3) from the plasmids pACA/HCA I and pACA/HCA II as 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.*,[41] and its concentration determined by titration with ethoxzolamide.

Initial rates of 4-nitrophenyl acetate hydrolysis at 25°C catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm with a Cary 3 instrument interfaced with an IBM compatible PC.[42] Solutions of substrate (2×10^{-2}–1×10^{-6} M), were prepared in anhydrous acetonitrile. A molar absorption coefficient ε of $18{,}400\,M^{-1}\,cm^{-1}$ was used for the 4-nitrophenolate ion, 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 conducted 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 3.3 nM for hCA II, 10 nM for hCA I and 34 nM for bCA IV (this isozyme has a decreased esterase activity[43] and higher concentrations had to be used for the measurements).

Measurements of Tonometric IOP

Adult male New Zealand albino rabbits weighing 2–3 kg were used in the experiments, three animals being used for each inhibitor studied. The experimental procedures conform to the requirements of the Association for Research in Vision and Ophthalmology Resolution on the use of animals.

The rabbits were kept in individual cages with food and water provided *ad libitum*. The animals were maintained on a 12 h/12 h light/dark cycle in a temperature controlled room, at 22–26°C. Solutions of inhibitors (2%, by weight) were obtained in DMSO-water (2 : 3, v/v) due to the lower water solubility of some of these derivatives. Control experiments with DMSO, at the same concentration as that used for obtaining the inhibitor solutions, showed that it does not possess IOP lowering or increasing effects.

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA, USA) as described by Maren's group.[44–46] The pressure readings were matched with two-point standard pressure measurements at least twice each day using a Digilab Calibration verifier. All IOP measurements were done by the same investigator with the same tonometer. One drop of 0.2% oxybuprocaine hydrochloride (novesine, Sandoz) diluted 1 : 1 with saline was instilled in each eye immediately before each set of pressure measurements. IOP was measured three times at each time interval, and the means reported. IOP was measured first immediately before drug administration, then at 30 min after the instillation of the pharmacological agent, and then each 30 min for a period of several hours. For all IOP experiments drug was administered to only one eye, leaving the contralateral eye as an untreated control. The ocular hypotensive activity is expressed as the average difference in IOP between the treated and control eye, in this way minimizing the diurnal, seasonal and interindividual variations commonly observed in the rabbit.[44–46] All data are expressed as mean ± SE, using a one-tailed *t*-test.

General Procedure for the Preparation of Compounds 16–39

One mM of sulfonamide **4–15** was dissolved/suspended in 50 mL of anhydrous acetonitrile or acetone and 147 μL (1.0 mM) of triethylamine were added dropwise. The reaction mixture was magnetically stirred at room temperature for 15 min, then 189 mg (1.0 mM) of 2- or 4-nitrobenzenesulfenyl chloride dissolved in 3 mL of anhydrous acetonitrile were added dropwise for a period of 30 min. The reaction mixture was stirred at room temperature for 3 h, when thin layer chromatography showed that the reaction was complete. After half of the solvent has been evaporated *in vacuo*, the reaction mixture was poured into 20 mL of cold water. The precipitated product was filtered and recrystallized from ethanol. Yields were in the range of 62–80%.

General Procedure for the Preparation of Compounds 40–63

One mM of sulfenamido-sulfonamide **16–39** was dissolved in 10 mL of acetone and an amount of saturated potassium permanganate solution in

acetone was added so as to ensure a small excess (in this case 1.1 mM) of oxidizing agent over the sulfenamide. The permanganate solution was obtained by stirring overnight an excess of solid $KMnO_4$ in acetone, and then filtration of the excess permanganate and precipitated MnO_2; the amount of permanganate contained was then determined by titration with a standardized oxalic acid solution. This reaction mixture was stirred at room temperature for 1 h. The excess potassium permanganate was then destroyed by adding a small amount of oxalic acid, the brown precipitate formed was filtered and discarded, and the clear acetone solution containing the sulfonylamido-sulfonamide **40–63** evaporated *in vacuo*. The obtained residue was recrystallized from ethanol–water or methanol–water. Yields were in the range of 35–69%.

***2-(2-Nitrobenzenesulfenylamido)-benzenesulfonamide* 16**

As yellow-orange crystals, m.p. 133–4°C, IR (KBr), cm^{-1}: 885, 956, 1090 and 1250 (NO_2), 1152 (SO_2^{sym}), 1310 (SO_2^{as}), 3270 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.12 (br s, 1H, NH); 6.54 (br s, 2H, NH_2); 7.01–7.62 (m, 8H, ArH from the two *ortho*-substituted phenylene moieties). Found: C: 44.1; H, 3.2; N, 12.8%. $C_{12}H_{11}N_3O_4S_2$ requires: C, 44.3; H, 3.4; N, 12.9%.

***4-(2-Nitrobenzenesulfenylhydrazido)-benzenesulfonamide* 17**

As yellow crystals, m.p. 212–4°C, IR (KBr), cm^{-1}: 739, 980 (N–N), 1040, 1085 and 1250 (NO_2), 1170 (SO_2^{sym}), 1339 (SO_2^{as}), 3280 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.48 (br s, 2H, NHNH); 6.57 (br s, 2H, NH_2); 7.08 (m, 4H AA′BB′, ArH from 1,4-phenylene); 7.05–7.73 (m, 4H, Ar H from *ortho*-substituted phenylene). Found: C: 44.2; H, 3.5; N, 14.5%. $C_{12}H_{12}N_4O_4S_2$ requires: C, 44.3; H, 3.4; N, 14.7%.

***3-Fluoro-4-(2-nitrobenzenesulfenylamido)-benzenesulfonamide* 18**

As orange-yellow crystals, m.p. 202–3°C, IR (KBr), cm^{-1}: 654, 749, 912, 1046, 1085 and 1250 (NO_2), 1151 (SO_2^{sym}), 1337 (SO_2^{as}), 1420, 3180 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.58 (br s, 1H, NH); 6.60 (br s, 2H, SO_2NH_2); 7.05–7.89 (m, 7H, ArH from *ortho*-substituted phenylene + ArH from the F-substituted ring). Found: C, 42.5; H, 3.0; N, 14.3%. $C_{12}H_{10}FN_3O_4S_2$ requires: C, 42.1; H, 3.2; N, 14.3%.

***3-Chloro-4-(2-nitrobenzenesulfenylamido)-benzenesulfonamide* 19**

As orange-yellow crystals, m.p. 225–7°C, IR (KBr), cm^{-1}: 648, 836, 951, 1040, 1085 and 1250 (NO_2), 1151 (SO_2^{sym}), 1330 (SO_2^{as}), 1420, 3180 (NH),

3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.68 (br s, 1H, NH); 6.60 (br s, 2H, SO_2NH_2); 7.05–7.86 (m, 7H, ArH from *ortho*-substituted phenylene + ArH from the Cl-substituted ring). Found: C, 41.2; H, 3.0; N, 14.0%. $C_{12}H_{10}ClN_3O_4S_2$ requires: C, 41.1; H, 3.1; N, 14.0%.

***3-Bromo-4-(2-nitrobenzenesulfenylamido)-benzenesulfonamide* 20**

As orange-yellow crystals, m.p. 237–8°C, IR (KBr), cm^{-1}: 712, 849, 925, 1040, 1080 and 1250 (NO_2), 1159 (SO_2^{sym}), 1352 (SO_2^{as}), 3180 (NH), 3340 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.52 (br s, 1H, NH); 6.61 (br s, 2H, SO_2NH_2); 7.05–7.92 (m, 7H, ArH from *ortho*-substituted phenylene + ArH from the Br-substituted ring). Found: C, 38.6; H, 3.0; N, 13.0%. $C_{12}H_{10}BrN_3O_4S_2$ requires: C, 38.7; H, 2.9; N, 13.1%.

***3,5-Dichloro-4-(2-nitrobenzenesulfenylamido)-benzenesulfonamide* 21**

As orange-yellow crystals, m.p. 244–5°C, IR (KBr), cm^{-1}: 630, 745, 909, 1046, 1085 and 1250 (NO_2), 1158 (SO_2^{sym}), 1360 (SO_2^{as}), 3180 (NH), 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.60 (br s, 1H, NH); 6.60 (br s, 2H, SO_2NH_2); 7.05–7.75 (m, 6H, ArH from *ortho*-substituted phenylene + ArH from dichlorosubstituted phenyl). Found: C, 39.5; H, 2.7; N, 13.3%. $C_{12}H_9Cl_2N_3O_4S_2$ requires: C, 39.2; H, 2.8; N, 13.3%.

***3,5-Dibromo-4-(2-nitrobenzenesulfenylamido)-benzenesulfonamide* 22**

As yellow crystals, m.p. 219–21°C, IR (KBr), cm^{-1}: 678, 884, 925, 1042, 1085 and 1250 (NO_2), 1155 (SO_2^{sym}), 1362 (SO_2^{as}), 3180 (NH), 3350 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.52 (br s, 1H, NH); 6.61 (br s, 2H, SO_2NH_2); 7.05–7.79 (m, 6H, ArH from *ortho*-substituted phenylene + ArH from dibromosubstituted phenyl). Found: C, 35.1; H, 2.7; N, 11.7%. $C_{12}H_9Br_2N_3O_4S_2$ requires: C, 35.0; H, 2.5; N, 11.9%.

***3,5-Diiodo-4-(2-nitrobenzenesulfenylamido)-benzenesulfonamide* 23**

As yellow crystals, m.p. 266–7°C (dec.), IR (KBr), cm^{-1}: 635, 704, 935, 1047, 1085 and 1250 (NO_2), 1158 (SO_2^{sym}), 1376 (SO_2^{as}), 1450, 3160 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.63 (br s, 1H, NH); 6.54 (br s, 2H, SO_2NH_2); 7.05–7.86 (m, 6H, ArH from *ortho*-substituted phenylene + ArH from diiodosubstituted phenyl). Found: C, 31.1; H, 2.3; N, 10.7%. $C_{12}H_9I_2N_3O_4S_2$ requires: C, 31.4; H, 2.3; N, 10.7%.

***6-Chloro-4-(2-nitrobenzenesulfenylamido)-1,3-benzenedisulfonamide* 24**

As yellow crystals, m.p. 240–2°C, IR (KBr), cm^{-1}: 657, 750, 778, 875, 945, 1060, 1085 and 1250 (NO_2), 1153 (SO_2^{sym}), 1346 (SO_2^{as}), 1446, 3165 (NH),

3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.70 (br s, 1H, NH); 7.05–7.49 (m, 4H, ArH from *ortho*-substituted phenylene); 7.40 (s, 1H, ArH); 7.57 (s, 1H, ArH); 7.65 (br s, 4H, 2 SO_2NH_2). Found: C, 39.4; H, 2.8; N, 13.9%. $C_{12}H_{11}ClN_4O_6S_3$ requires: C, 39.1; H, 3.1; N, 13.9%.

***4,5-Dichloro-6-(2-nitrobenzenesulfenylamido)-1,3-benzenedisulfonamide* 25**

As yellow crystals, m.p. 250–1°C, IR (KBr), cm^{-1}: 624, 680, 754, 858, 952, 1085 and 1250 (NO_2), 1145 (SO_2^{sym}), 1380 (SO_2^{as}), 3170 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.62 (br s, 1H, NH); 7.05–7.49 (m, 4H, ArH from *ortho*-substituted phenylene); 7.54 (s, 1H, ArH); 7.68 (br s, 4H, 2 SO_2NH_2). Found: C, 37.8; H, 2.8; N, 13.1%. $C_{12}H_{10}Cl_2N_4O_6S_3$ requires: C, 37.9; H, 2.9; N, 13.5%.

***5-(2-Nitrobenzenesulfenylamidoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide* 26**

As yellow crystals, m.p. 239–41°C (dec.), IR (KBr), cm^{-1}: 631, 715, 970, 1030, 1085 and 1250 (NO_2), 1180 (SO_2^{sym}), 1320 (SO_2^{as}), 1490, 1540, 1740 (CONH), 3280 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.25–2.63 (m, 4H, CH_2CH_2); 5.08 (br s, 1H, SNH); 6.80 (br s, 3H, CONH + SO_2NH_2); 7.13–7.75 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 38.2; H, 2.9; N, 15.7%. $C_{11}H_{12}N_6O_5S_3$ requires: C, 38.2; H, 3.2; N, 16.1%.

***5-(2-Nitrobenzenesulfenylimido)-4-methyl-2-sulfonamide-δ^2-1,3,4-thiadiazoline* 27**

As yellow crystals, m.p. 201–3°C, IR (KBr), cm^{-1}: 875, 973, 1085 and 1250 (NO_2), 1130 (SO_2^{sym}), 1379 (SO_2^{as}), 1416, 1590, 3069 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.90 (s, 3H, N–Me); 7.20–7.61 (m, 4H, ArH); 8.15 (br s, 2H, SO_2NH_2). Found: C, 37.2; H, 2.9; N, 16.7%. $C_9H_9N_5O_4S_3$ requires: C, 36.9; H, 3.1; N, 16.9%.

***2-(4-Nitrobenzenesulfenylamido)-benzenesulfonamide* 28**

As pale yellow crystals, m.p. 181–3°C, IR (KBr), cm^{-1}: 851, 969, 1093 and 1250 (NO_2), 1127 (SO_2^{sym}), 1313 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.11 (br s, 1H, NH); 6.50 (br s, 2H, NH_2); 7.00–7.45 (m, 4H, ArH from the *ortho*-substituted phenylene); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene). Found: C, 44.4; H, 3.3; N, 12.5%. $C_{12}H_{11}N_3O_4S_2$ requires: C, 44.3; H, 3.3; N, 12.9%.

***4-(4-Nitrobenzenesulfenylhydrazido)-benzenesulfonamide* 29**

As yellow crystals, m.p. 223–4°C, IR (KBr), cm^{-1}: 694, 980 (N–N), 1045, 1082 and 1250 (NO_2), 1162 (SO_2^{sym}), 1344 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.42 (br s, 2H, NHNH); 6.68 (br s, 2H, NH_2); 7.08 (m, 4H, AA′BB′, ArH from 1,4-phenylene); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene). Found: C, 44.0; H, 3.4; N, 14.6%. $C_{12}H_{12}N_4O_4S_2$ requires: C, 44.3; H, 3.4; N, 14.7%.

***3-Fluoro-4-(4-nitrobenzenesulfenylamido)-benzenesulfonamide* 30**

As pale yellow crystals, m.p. 223–5°C, IR (KBr), cm^{-1}: 698, 880, 929, 1041, 1085 and 1250 (NO_2), 1150 (SO_2^{sym}), 1345 (SO_2^{as}), 1460, 3180 (NH), 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.81 (br s, 1H, NH); 6.69 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.45–7.93 (m, 3H, ArH). Found: C, 42.1; H, 3.3; N, 14.2%. $C_{12}H_{10}FN_3O_4S_2$ requires: C, 42.1; H, 3.2; N, 14.3%.

***3-Chloro-4-(4-nitrobenzenesulfenylamido)-benzenesulfonamide* 31**

As pale yellow crystals, m.p. 210–3°C, IR (KBr), cm^{-1}: 780, 854, 912, 1046, 1085 and 1250 (NO_2), 1145 (SO_2^{sym}), 1350 (SO_2^{as}), 3180 (NH), 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.61 (br s, 1H, NH); 6.60 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.25–7.91 (m, 3H, ArH). Found: C, 41.0; H, 3.4; N, 14.0%. $C_{12}H_{10}ClN_3O_4S_2$ requires: C, 41.1; H, 3.1; N, 14.0%.

***3-Bromo-4-(4-nitrobenzenesulfenylamido)-benzenesulfonamide* 32**

As pale yellow crystals, m.p. 237–9°C, IR (KBr), cm^{-1}: 651, 843, 957, 1084 and 1250 (NO_2), 1150 (SO_2^{sym}), 1346 (SO_2^{as}), 3180 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.52 (br s, 1H, NH); 6.61 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.30–7.87 (m, 3H, ArH). Found: C, 38.9; H, 3.0; N, 13.1%. $C_{12}H_{10}BrN_3O_4S_2$ requires: C, 38.7; H, 2.9; N, 13.1%.

***3,5-Dichloro-4-(4-nitrobenzenesulfenylamido)-benzenesulfonamide* 33**

As yellow crystals, m.p. 250–3°C, IR (KBr), cm^{-1}: 680, 854, 917, 1041, 1082 and 1254 (NO_2), 1171 (SO_2^{sym}), 1360 (SO_2^{as}), 3160 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.61 (br s, 1H, NH); 6.60 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.45 (s, 2H, ArH). Found: C, 39.5; H, 2.7; N, 13.3%. $C_{12}H_9Cl_2N_3O_4S_2$ requires: C, 39.2; H, 2.8; N, 13.3%.

***3,5-Dibromo-4-(4-nitrobenzenesulfenylamido)-benzenesulfonamide* 34**

As yellow crystals, m.p. 234–6°C, IR (KBr), cm^{-1}: 745, 828, 1029, 1087 and 1255 (NO_2), 1150 (SO_2^{sym}), 1371 (SO_2^{as}), 3170 (NH), 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.70 (br s, 1H, NH); 6.61 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.45 (s, 2H, ArH). Found: C, 35.0; H, 2.8; N, 11.5%. $C_{12}H_9Br_2N_3O_4S_2$ requires: C, 35.0; H, 2.5; N, 11.9%.

***3,5-Diiodo-4-(4-nitrobenzenesulfenylamido)-benzenesulfonamide* 35**

As yellow crystals, m.p. 266–7°C (dec.), IR (KBr), cm^{-1}: 697, 755, 881, 939, 1040, 1081 and 1250 (NO_2), 1145 (SO_2^{sym}), 1380 (SO_2^{as}), 1450, 3175 (NH), 3340 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.62 (br s, 1H, NH); 6.61 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.49 (s, 2H, ArH). Found: C, 31.5; H, 2.3; N, 10.4%. $C_{12}H_9I_2N_3O_4S_2$ requires: C, 31.4; H, 2.3; N, 10.7%.

***6-Chloro-4-(4-nitrobenzenesulfenylamido)-1,3-benzenedisulfonamide* 36**

As pale yellow crystals, m.p. 246–8°C, IR (KBr), cm^{-1}: 652, 839, 897, 1018, 1085 and 1250 (NO_2), 1159 (SO_2^{sym}), 1340 (SO_2^{as}), 1449, 3165 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.74 (br s, 1H, NH); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.40 (s, 1H, ArH); 7.50 (s, 1H, ArH); 7.59 (br s, 4H, 2 SO_2NH_2). Found: C, 39.3; H, 2.9; N, 13.9%. $C_{12}H_{11}ClN_4O_6S_3$ requires: C, 39.1; H, 3.1; N, 13.9%.

***4,5-Dichloro-6-(4-nitrobenzenesulfenylamido)-1,3-benzenedisulfonamide* 37**

As pale yellow crystals, m.p. 235–6°C, IR (KBr), cm^{-1}: 728, 839, 1061, 1080 and 1250 (NO_2), 1135 (SO_2^{sym}), 1382 (SO_2^{as}), 3160 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.67 (br s, 1H, NH); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.50 (s, 1H, ArH); 7.60 (br s, 4H, 2 SO_2NH_2). Found: C, 37.6; H, 2.9; N, 13.3%. $C_{12}H_{10}Cl_2N_4O_6S_3$ requires: C, 37.9; H, 2.9; N, 13.5%.

***5-(4-Nitrobenzenesulfenylamidoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide* 38**

As pale yellow crystals, m.p. 225–6°C (dec.), IR (KBr), cm^{-1}: 639, 844, 975, 1020, 1083 and 1250 (NO_2), 1180 (SO_2^{sym}), 1320 (SO_2^{as}), 1484, 1540, 1740 (CONH), 3280 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.25–2.66 (m, 4H, CH_2CH_2); 5.12 (br s, 1H, SNH); 6.85 (br s, 3H, CONH + SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-substituted

phenylene). Found: C, 38.1; H, 3.3; N, 15.9%. $C_{11}H_{12}N_6O_5S_3$ requires: C, 38.2; H, 3.2; N, 16.1%.

***5-(4-Nitrobenzenesulfenylamido)-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline* 39**

As pale yellow crystals, m.p. 206–7°C (dec.), IR (KBr), cm^{-1}: 620, 686, 839, 1085 and 1250 (NO_2), 1175 (SO_2^{sym}), 1361 (SO_2^{as}), 1426, 1595, 3067 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.90 (s, 3H, N–Me); 7.20–7.60 (m, 4H, AA′BB′, ArH); 8.18 (br s, 2H, SO_2NH_2). Found: C, 37.0; H, 2.8; N, 16.8%. $C_9H_9N_5O_4S_3$ requires: C, 36.9; H, 3.1; N, 16.9%.

***2-(2-Nitrobenzenesulfonylamido)-benzenesulfonamide* 40**

As yellow crystals, m.p. 179–81°C, IR (KBr), cm^{-1}: 853, 906, 1090 and 1250 (NO_2), 1148 and 1154 (SO_2^{sym}), 1310 and 1364 (SO_2^{as}), 3270 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.90 (br s, 2H, NH_2); 7.01–7.69 (m, 8H, ArH from the two *ortho*-substituted phenylene moieties); 8.29 (br s, 1H, NH). Found: C, 40.2; H, 3.0; N, 11.6%. $C_{12}H_{11}N_3O_6S_2$ requires: C, 40.3; H, 3.0; N, 11.7%.

***4-(2-Nitrobenzenesulfonylhydrazido)-benzenesulfonamide* 41**

As yellow crystals, m.p. 229–31°C, IR (KBr), cm^{-1}: 787, 983 (N–N), 1054, 1085 and 1250 (NO_2), 1125 and 1170 (SO_2^{sym}), 1339 and 1368 (SO_2^{as}), 3290 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.97 (br s, 2H, NH_2); 7.08 (m, 4H, AA′BB′, ArH from 1,4-phenylene); 7.05–7.73 (m, 4H, ArH from *ortho*-substituted phenylene); 8.08 (br s, 2H, NHNH). Found: C, 36.2; H, 3.1; N, 16.5%. $C_{12}H_{12}N_4O_6S_2$ requires: C, 36.2; H, 3.0; N, 16.6%.

***3-Fluoro-4-(2-nitrobenzenesulfonylamido)-benzenesulfonamide* 42**

As yellow crystals, m.p. 235–6°C, IR (KBr), cm^{-1}: 690, 783, 927, 1026, 1079 and 1253 (NO_2), 1128 and 1151 (SO_2^{sym}), 1337 and 1370 (SO_2^{as}), 1490, 3180 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.85 (br s, 2H, SO_2NH_2); 7.05–7.83 (m, 7H, ArH from *ortho*-substituted phenylene + ArH from the F-substituted ring); 8.15 (br s, 1H, NH). Found: C, 35.5; H, 3.0; N, 15.9%. $C_{12}H_{10}FN_3O_6S_2$ requires: C, 35.3; H, 2.9; N, 16.1%.

***3-Chloro-4-(2-nitrobenzenesulfonylamido)-benzenesulfonamide* 43**

As yellow crystals, m.p. 244–5°C, IR (KBr), cm^{-1}: 723, 869, 919, 1040, 1085 and 1254 (NO_2), 1132 and 1155 (SO_2^{sym}), 1330 and 1368 (SO_2^{as}), 1440, 3180 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.83 (br s, 2H, SO_2NH_2);

7.05–7.89 (m, 7H, ArH from *ortho*-substituted phenylene + ArH from the Cl-substituted ring); 8.18 (br s, 1H, NH). Found: C, 35.1; H, 3.0; N, 16.0%. $C_{12}H_{10}ClN_3O_6S_2$ requires: C, 35.0; H, 2.9; N, 16.0%.

***3-Bromo-4-(2-nitrobenzenesulfonylamido)-benzenesulfonamide* 44**

As yellow crystals, m.p. 252–3°C, IR (KBr), cm^{-1}: 738, 872, 954, 1044, 1080 and 1250 (NO_2), 1127 and 1155 (SO_2^{sym}), 1352 and 1374 (SO_2^{as}), 3180 (NH), 3340 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.84 (br s, 2H, SO_2NH_2); 7.05–7.92 (m, 7H, ArH from *ortho*-substituted phenylene + ArH from the Br-substituted ring); 8.12 (br s, 1H, NH). Found: C, 34.3; H, 3.0; N, 15.3%. $C_{12}H_{10}BrN_3O_6S_2$ requires: C, 34.2; H, 2.8; N, 15.6%.

***3,5-Dichloro-4-(2-nitrobenzenesulfonylamido)-benzenesulfonamide* 45**

As yellow crystals, m.p. 249–51°C, IR (KBr), cm^{-1}: 685, 759, 946, 1040, 1082 and 1250 (NO_2), 1130 and 1158 (SO_2^{sym}), 1360 and 1379 (SO_2^{as}), 3180 (NH), 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.92 (br s, 2H, SO_2NH_2); 7.05–7.79 (m, 6H, ArH from *ortho*-substituted phenylene + ArH from dichlorosubstituted phenyl); 8.16 (br s, 1H, NH). Found: C, 34.3; H, 2.7; N, 15.3%. $C_{12}H_9Cl_2N_3O_6S_2$ requires: C, 34.4; H, 2.7; N, 15.7%.

***3,5-Dibromo-4-(2-nitrobenzenesulfonylamido)-benzenesulfonamide* 46**

As yellow crystals, m.p. 252–4°C, IR (KBr), cm^{-1}: 653, 749, 951, 1040, 1085 and 1250 (NO_2), 1126 and 1155 (SO_2^{sym}), 1362 and 1378 (SO_2^{as}), 3180 (NH), 3350 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.91 (br s, 2H, SO_2NH_2); 7.05–7.76 (m, 6H, ArH from *ortho*-substituted phenylene + ArH from dibromo-substituted phenyl); 8.21 (br s, 1H, NH). Found: C, 33.1; H, 2.5; N, 14.7%. $C_{12}H_9Br_2N_3O_6S_2$ requires: C, 32.9; H, 2.6; N, 15.0%.

***3,5-Diiodo-4-(2-nitrobenzenesulfonylamido)-benzenesulfonamide* 47**

As yellow crystals, m.p. 275–7°C (dec.), IR (KBr), cm^{-1}: 651, 702, 869, 1040, 1085 and 1250 (NO_2), 1131 and 1158 (SO_2^{sym}), 1376 (SO_2^{as}), 1450, 3180 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.88 (br s, 2H, SO_2NH_2); 7.05–7.89 (m, 6H, ArH from *ortho*-substituted phenylene + ArH from diiodosubstituted phenyl); 8.13 (br s, 1H, NH). Found: C, 31.3; H, 2.3; N, 14.4%. $C_{12}H_9I_2N_3O_6S_2$ requires: C, 31.4; H, 2.5; N, 14.4%.

***6-Chloro-4-(2-nitrobenzenesulfonylamido)-1,3-benzenesulfonamide* 48**

As yellow crystals, m.p. 248–9°C, IR (KBr), cm^{-1}: 625, 736, 789, 851, 1050, 1085 and 1250 (NO_2), 1129 and 1153 (SO_2^{sym}), 1346 and 1375 (SO_2^{as}), 1450,

3170 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.46 (m, 4H, ArH from *ortho*-substituted phenylene); 7.40 (s, 1H, ArH); 7.59 (s, 1H, ArH); 7.69 (br s, 4H, 2 SO_2NH_2); 8.17 (br s, 1H, NH). Found: C, 34.4; H, 2.8; N, 15.5%. $C_{12}H_{11}ClN_4O_8S_3$ requires: C, 34.4; H, 2.8; N, 15.8%.

***4,5-Dichloro-6-(2-nitrobenzenesulfonylamido)-1,3-benzenesulfonamide* 49**

As yellow crystals, m.p. 281–3°C (dec.), IR (KBr), cm^{-1}: 641, 736, 883, 1085 and 1250 (NO_2), 1126 and 1145 (SO_2^{sym}), 1366 and 1380 (SO_2^{as}), 3170 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.49 (m, 4H, ArH from *ortho*-substituted phenylene); 7.58 (s, 1H, ArH); 7.72 (br s, 4H, 2 SO_2NH_2); 8.12 (br s, 1H, NH). Found: C, 33.8; H, 2.8; N, 15.4%. $C_{12}H_{10}Cl_2N_4O_8S_3$ requires: C, 33.8; H, 2.7; N, 15.5%.

***5-(2-Nitrobenzenesulfonylamidoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide* 50**

As yellow crystals, m.p. 255–7°C (dec.), IR (KBr), cm^{-1}: 719, 822, 953, 1030, 1085 and 1250 (NO_2), 1132 and 1180 (SO_2^{sym}), 1320 and 1369 (SO_2^{as}), 1490, 1540, 1740 (CONH), 3280 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.25–2.66 (m, 4H, CH_2CH_2); 6.84 (br s, 3H, CONH + SO_2NH_2); 7.13–7.70 (m, 4H, ArH from *ortho*-substituted phenyl); 8.09 (br s, 1H, SO_2NH). Found: C, 34.2; H, 2.8; N, 15.7%. $C_{12}H_{12}N_6O_7S_3$ requires: C, 34.5; H, 2.9; N, 15.8%.

***5-(2-Nitrobenzenesulfonylamido)-4-methyl-2-sulfonamide-δ^2-1,3,4-thiadiazoline* 51**

As pale yellow crystals, m.p. 231–3°C, IR (KBr), cm^{-1}: 640, 745, 871, 933, 1133, 1170, 1286, 1364, 1416, 1590, 3063; ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.89 (s, 3H, N–Me); 7.20–7.61 (m, 4H, ArH); 8.20 (br s, 2H, SO_2NH_2). Found: C, 33.8; H, 3.1; N, 15.4%. $C_9H_9N_5O_6S_3$ requires: C, 33.9; H, 2.8; N, 15.5%.

***2-(4-Nitrobenzenesulfonylamido)-benzenesulfonamide* 52**

As yellow crystals, m.p. 232–3°C, IR (KBr), cm^{-1}: 650, 823, 969, 1079 and 1250 (NO_2), 1124 and 1139 (SO_2^{sym}), 1313 and 1366 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.86 (br s, 2H, NH_2); 7.00–7.45 (m, 4H, ArH) from the *ortho*-substituted phenylene); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 8.11 (br s, 1H, NH). Found: C, 40.4; H, 3.1; N, 11.7%. $C_{12}H_{11}N_3O_6S_2$ requires: C, 40.3; H, 3.0; N, 11.7%.

***4-(4-Nitrobenzenesulfonylhydrazido)-benzenesulfonamide* 53**

As yellow crystals, m.p. 239–41°C, IR (KBr), cm^{-1}: 727, 980 (N–N), 1056, 1080 and 1250 (NO_2), 1135 and 1162 (SO_2^{sym}), 1344 and 1381 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.97 (br s, 2H, NH_2); 7.10 (m, 4H, AA′BB′, ArH from 1,4-phenylene); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.92 (br s, 2H, NHNH). Found: C, 36.0; H, 3.3; N, 16.4%. $C_{12}H_{12}N_4O_6S_2$ requires: C, 36.2; H, 3.0; N, 16.6%.

***3-Fluoro-4-(4-nitrobenzenesulfonylamido)-benzenesulfonamide* 54**

As yellow crystals, m.p. 244–5°C, IR (KBr), cm^{-1}: 713, 809, 954, 1048, 1081 and 1250 (NO_2), 1132 and 1150 (SO_2^{sym}), 1345 and 1382 (SO_2^{as}), 1460 and 3180 (NH), 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.91 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.41–7.97 (m, 3H, ArH); 8.10 (br s, 1H, NH). Found: C, 35.3; H, 3.1; N, 15.8%. $C_{12}H_{10}FN_3O_6S_2$ requires: C, 35.3; H, 2.9; N, 16.1%.

***3-Chloro-4-(4-nitrobenzenesulfonylamido)-benzenesulfonamide* 55**

As yellow crystals, m.p. 245–7°C, IR (KBr), cm^{-1}: 638, 829, 920, 1041, 1085 and 1250 (NO_2), 1135 and 1145 (SO_2^{sym}), 1350 and 1386 (SO_2^{as}), 3180 (NH), 3335 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.92 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.24–7.86 (m, 3H, ArH); 8.07 (br s, 1H, NH); Found: C, 35.0; H, 2.6; N, 15.9%. $C_{12}H_{10}ClN_3O_6S_2$ requires: C, 35.0; H, 2.9; N, 16.0%.

***3-Bromo-4-(4-nitrobenzenesulfonylamido)-benzenesulfonamide* 56**

As yellow crystals, m.p. 271–4°C (dec.), IR (KBr), cm^{-1}: 720, 836, 889, 1080 and 1250 (NO_2), 1134 and 1150 (SO_2^{sym}), 1346 and 1380 (SO_2^{as}), 3180 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.94 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.32–7.89 (m, 3H, ArH); 8.12 (br s, 1H, NH); Found: C, 34.4; H, 2.9; N, 15.5%. $C_{12}H_{10}BrN_3O_6S_2$ requires: C, 34.2; H, 2.8; N, 15.6%.

***3,5-Dichloro-4-(4-nitrobenzenesulfonylamido)-benzenesulfonamide* 57**

As yellow crystals, m.p. 285–7°C (dec.), IR (KBr), cm^{-1}: 724, 840, 975, 1049, 1085 and 1250 (NO_2), 1130 and 1171 (SO_2^{sym}), 1360 and 1385 (SO_2^{as}), 3160 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.89 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.44 (s, 2H,

ArH); 8.11 (br s, 1H, NH). Found: C, 34.4; H, 2.5; N, 15.5%. $C_{12}H_9Cl_2N_3O_6S_2$ requires: C, 34.4; H, 2.7; N, 15.7%.

***3,5-Dibromo-4-(4-nitrobenzenesulfonylamido)-benzenesulfonamide* 58**

As yellow crystals, m.p. 266–7°C, IR (KBr), cm^{-1}: 689, 750, 1045, 1028 and 1250 (NO_2), 1130 and 1150 (SO_2^{sym}), 1371 and 1386 (SO_2^{as}), 3160 (NH), 3330 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.94 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.46 (s, 2H, ArH); 8.13 (br s, 1H, NH). Found: C, 32.9; H, 2.5; N, 14.9%. $C_{12}H_9Br_2N_3O_6S_2$ requires: C, 32.9; H, 2.6; N, 15.0%.

***3,5-Diiodo-4-(4-nitrobenzenesulfonylamido)-benzenesulfonamide* 59**

As yellow crystals, m.p. 279–80°C (dec.), IR (KBr), cm^{-1}: 721, 796, 850, 1040, 1080 and 1250 (NO_2), 1129 and 1145 (SG_2^{sym}), 1370 and 1388 (SO_2^{as}), 1450, 3170 (NH), 3340 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.84 (br s, 2H, SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.46 (s, 2H, ArH); 8.14 (br s, 1H, NH). Found: C, 31.2; H, 2.5; N, 14.1%. $C_{12}H_9I_2N_3O_6S_2$ requires: C, 31.4; H, 2.5; N, 14.4%.

***6-Chloro-4-(4-nitrobenzenesulfonylamido)-1,3-benzenedisulfonamide* 60**

As yellow crystals, m.p. 267–8°C (dec.), IR (KBr), cm^{-1}: 710, 805, 897, 1018, 1085 and 1250 (NO_2), 1134 and 1159 (SO_2^{sym}), 1340 and 1377 (SO_2^{as}), 3160 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.44 (s, 1H, ArH); 7.53 (s, 1H, ArH); 7.68 (br s, 4H, 2 SO_2NH_2); 8.08 (br s, 1H, NH). Found: C, 34.7; H, 2.6; N, 15.8%. $C_{12}H_{11}ClN_4O_8S_3$ requires: C, 34.4; H, 2.8; N, 15.8%.

***4,5-Dichloro-6-(4-nitrobenzenesulfonylamido)-1,3-benzenedisulfonamide* 61**

As yellow crystals, m.p. 256–8°C, IR (KBr), cm^{-1}: 669, 895, 1046, 1080 and 1250 (NO_2), 1129 and 1138 (SO_2^{sym}), 1371 and 1386 (SO_2^{as}), 3160 (NH), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15 (m, 4H, AA′BB′, ArH from *p*-nitro-phenylene); 7.50 (s, 1H, ArH); 7.62 (br s, 4H, 2 SO_2NH_2); 8.10 (br s, 1H, NH); Found: C, 33.6; H, 2.6; N, 15.4%. $C_{12}H_{10}Cl_2N_4O_8S_3$ requires: C, 33.8; H, 2.7; N, 15.5%.

***5-(4-Nitrobenzenesulfonylamidoethlycarboxamido)-1,3,4-thiadiazole-2-sulfonamide* 62**

As yellow crystals, m.p. 269–71°C (dec.), IR (KBr), cm^{-1}: 771, 860, 971, 1085 and 1250 (NO_2), 1132 and 1180 (SO_2^{sym}), 1320 and 1378 (SO_2^{as}), 1540,

1740 (CONH), 3280 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.25–2.68 (m, 4H, CH_2CH_2); 6.92 (br s, 3H, CONH + SO_2NH_2); 7.15 (m, 4H, AA′BB′, ArH from *p*-substituted phenylene); 8.10 (br s, 1H, SO_2NH). Found: C, 34.6; H, 2.7; N, 15.8%. $C_{11}H_{12}N_6O_7S_3$ requires: C, 34.5; H, 2.9; N, 15.8%.

***5-(4-Nitrobenzenesulfonylamido)-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline* 63**

As pale yellow crystals, m.p. 236–8°C (dec.), IR (KBr), cm^{-1}: 634, 697, 750, 815, 893, 1132, 1175, 1298, 1361, 1426, 1595, 3067; ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.90 (s, 3H, N–Me); 7.50–8.02 (m, 4H, AA′BB′, ArH); 8.18 (br s, 2H, SO_2NH_2). Found: C, 33.7; H, 3.0; N, 15.2%. $C_9H_9N_5O_6S_3$ requires: C, 33.9; H, 2.8; N, 15.5%.

RESULTS AND DISCUSSION

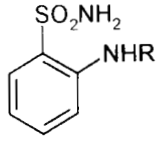
Although arylsulfenyl halides such as 2-nitro- or 4-nitrobenzenesulfenyl chlorides were extensively used as blocking reagents of amino groups in oligopeptide synthesis,[47–49] their reaction with sulfonamides has only recently been investigated by this group,[28] when several sulfenamido-sulfonamides of type **3**, possessing interesting CA inhibitory properties have been obtained. In this paper we report an extension of the previous work,[28] including more derivatives in the study. Thus, diverse aromatic/heterocyclic sulfonamides possessing amino/hydrazino/imino groups in their molecule were chosen in order to obtain different structural variants that we considered important for their influence upon the enzyme inhibitory properties of the new sulfenamido-sulfonamides. The compounds included in the study, which had been scarcely investigated previously for their interaction with different CA isozymes, such as orthanilamide **4**, 4-hydrazino-benzenesulfonamide **5**, different mono-/dihalogenated sulfanilamides of type 6–**11**, as well as benzene-1,3-disulfonamide derivatives of type **12**, **13** are shown below. The two heterocyclic derivatives **14** and **15** included in the present study on the other hand belong to a class of sulfonamides which have been investigated much more, since two widely used clinical inhibitors, acetazolamide **1a** and methazolamide **2**, respectively, are structurally related to them.

Thus, reaction of amino-/hydrazino-/imino-sulfonamides **4**–**15** with the above mentioned sulfenyl chlorides by the procedure previously described[28] yielded the sulfenamido-sulfonamides **16**–**39**. The latter compounds were

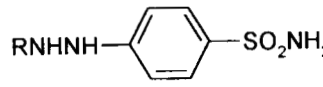
then oxidized with potassium permanganate in acetone to the corresponding bis-sulfonamides **40**–**63**.

CA inhibition data with the obtained compounds and standard sulfonamide inhibitors are in Table I.

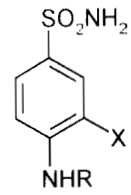
As seen from the inhibition data, the sulfenylamido-sulfonamides **16**–**39** and the bis-sulfonamides **40**–**63** behave as stronger inhibitors against all three investigated isozymes, as compared to the corresponding sulfonamides of type **4**–**15** from which they were prepared. The efficiency generally increases in the following direction: amino-/imino-sulfonamides < sulfenamido-sulfonamides < bis-sulfonamides, for a given starting amino-sulfonamide



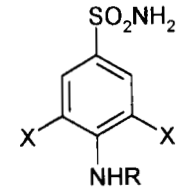
4: R = H
16: R = 2-O_2N-C_6H_4S
28: R = 4-O_2N-C_6H_4S
40: R = 2-O_2N-$C_6H_4SO_2$
52: R = 4-O_2N-$C_6H_4SO_2$



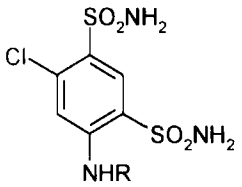
5: R = H
17: R = 2-O_2N-C_6H_4S
29: R = 4-O_2N-C_6H_4S
41: R = 2-O_2N-$C_6H_4SO_2$
53: R = 4-O_2N-$C_6H_4SO_2$



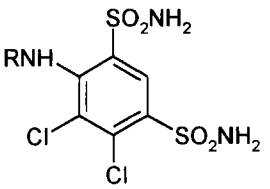
6: R = H; X = F
7: R = H; X = Cl
8: R = H; X = Br
18: R = 2-O_2N-C_6H_4S; X = F
19: R = 2-O_2N-C_6H_4S; X = Cl
20: R = 2-O_2N-C_6H_4S; X = Br
30: R = 4-O_2N-C_6H_4S; X = F
31: R = 4-O_2N-C_6H_4S; X = Cl
32: R = 4-O_2N-C_6H_4S; X = Br
42: R = 2-O_2N-$C_6H_4SO_2$; X = F
43: R = 2-O_2N-$C_6H_4SO_2$; X = Cl
44: R = 2-O_2N-$C_6H_4SO_2$; X = Br
54: R = 4-O_2N-$C_6H_4SO_2$; X = F
55: R = 4-O_2N-$C_6H_4SO_2$; X = Cl
56: R = 4-O_2N-$C_6H_4SO_2$; X = Br



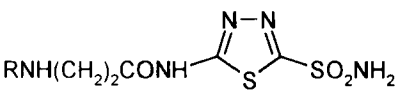
9: R = H; X = Cl
10: R = H; X = Br
11: R = H; X = I
21: R = 2-O_2N-C_6H_4S; X = Cl
22: R = 2-O_2N-C_6H_4S; X = Br
23: R = 2-O_2N-C_6H_4S; X = I
33: R = 4-O_2N-C_6H_4S; X = Cl
34: R = 4-O_2N-C_6H_4S; X = Br
35: R = 4-O_2N-C_6H_4S; X = I
45: R = 2-O_2N-$C_6H_4SO_2$; X = Cl
46: R = 2-O_2N-$C_6H_4SO_2$; X = Br
47: R = 2-O_2N-$C_6H_4SO_2$; X = I
57: R = 4-O_2N-$C_6H_4SO_2$; X = Cl
58: R = 4-O_2N-$C_6H_4SO_2$; X = Br
59: R = 4-O_2N-$C_6H_4SO_2$; X = I



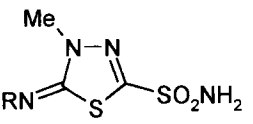
12: R = H
24: R = 2-O_2N-C_6H_4S
36: R = 4-O_2N-C_6H_4S
48: R = 2-O_2N-$C_6H_4SO_2$
60: R = 4-O_2N-$C_6H_4SO_2$



13: R = H
25: R = 2-O_2N-C_6H_4S
37: R = 4-O_2N-C_6H_4S
49: R = 2-O_2N-$C_6H_4SO_2$
61: R = 4-O_2N-$C_6H_4SO_2$



14: R = H
26: R = 2-O_2N-C_6H_4S
38: R = 4-O_2N-C_6H_4S
50: R = 2-O_2N-$C_6H_4SO_2$
62: R = 4-O_2N-$C_6H_4SO_2$



15: R = H
27: R = 2-O_2N-C_6H_4S
39: R = 4-O_2N-C_6H_4S
51: R = 2-O_2N-$C_6H_4SO_2$
63: R = 4-O_2N-$C_6H_4SO_2$

and its corresponding derivatives. 4-Nitrophenyl-derivatives were generally more active than the corresponding 2-nitrophenyl compounds, both for sulfenamides as well as bis-sulfonamides. The orthanilamide **4** as well as 4-hydrazino-benzenesulfonamide **5** derivatives were generally the least active of the whole series of prepared inhibitors, with potencies comparable to those of sulfanilamide (4-aminobenzenesulfonamide), the first compound for which CA inhibitory properties were found.[50] The presence of halogeno atom(s) in the aromatic ring of the newly prepared sulfonamides increased inhibitory power with the mono-substituted compounds generally more active than the dihalogeno-sulfonamides. For the compounds derived from **9**–**11**, inhibitory power increased from the dichloro- to the dibromo- derivatives, but was slightly diminished for the di-iodo compounds (probably due to steric hindrance caused by the two bulky iodine substituents). In the case of the monohalogeno-compounds (derived from **6**–**8**) the monofluoro- and the monobromo-derivatives possessed similar inhibitory properties, and were generally more active than the corresponding dichloro compounds against all three investigated isozymes. For the benzene-1,3-disulfonamide

TABLE 1 Biological activity data for sulfonamides prepared in the present study as inhibitors of CA

Compound	*hCA* I[a]	IC_{50} (nM) *hCA* II[a]	*bCA* IV[b]
1a (acetazolamide)	900	12	220
1b (benzolamide)	15	9	12
2 (methazolamide)	780	14	240
Sulfanilamide	2800	300	3000
4	4540	295	1310
5	7850	320	3215
6	830	60	180
7	980	110	320
8	650	40	66
9	1700	220	1200
10	1380	150	850
11	1420	180	870
12	840	75	160
13	610	28	175
14	455	3	125
15	930	19	355
16	4110	230	1200
17	5330	260	1390
18	665	20	102
19	755	72	215
20	605	31	60
21	1450	194	670
22	1080	108	535
23	780	53	241
24	630	61	113
25	505	17	32
26	325	0.8	6.6
27	880	0.7	5.3
28	3785	150	1120
29	5120	205	1140
30	585	17	87
31	695	69	64
32	590	28	55
33	1285	158	495
34	975	69	384
35	635	49	240
36	260	38	68
37	380	17	30
38	330	0.6	2.5
39	815	0.6	4.4
40	1680	117	395
41	1655	196	640
42	324	17	93
43	540	57	148
44	390	16	34
45	675	52	127
46	540	19	66
47	290	18	95
48	375	15	28
49	370	9	18
50	185	0.2	3.2
51	655	0.1	4.6

TABLE I (*Continued*)

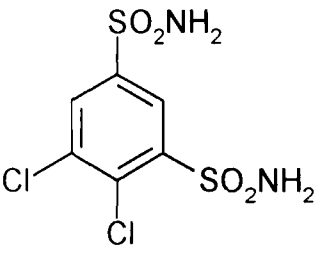
Compound	*hCA* I[a]	IC_{50} (nM) *hCA* II[a]	*bCA* IV[b]
52	1620	105	260
53	1395	160	550
54	237	16	84
55	535	54	129
56	375	10	26
57	620	17	95
58	370	15	25
59	185	16	84
60	98	11	31
61	350	9	16
62	180	0.2	2.3
63	370	0.1	4.5

[a]Human (cloned) isozyme.
[b]Isolated from bovine lung microsomes.[26]
IC_{50} is the mean of two different assays and represents the molarity of inhibitor producing a 50% decrease of enzyme specific activity for the *p*-nitrophenyl acetate hydrolysis reaction.[42]

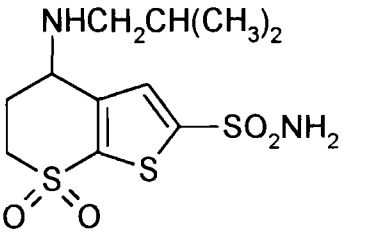
derivatives **24**, **25** and **36**, **37**, the most active were the pentasubstituted derivatives (obtained from **13** as starting material) and not the tetrasubstituted compounds (obtained from **12** as starting material), although the converse might have been expected, due to steric hindrance reasons. Probably the additional chlorine atom present in the compounds derived from **13** ensures a further stabilization of the enzyme–inhibitor complex, in a similar manner to dichlorophenamide **64** (3,4-dichloro-benzene-1,3-disulfonamide), a clinically used diuretic possessing very strong CA inhibitory properties.[4–7] Since X-ray crystallographic data on adducts of CA with benzene-1,3-disulfonamide derivatives are not available at present, this result cannot be rationalized in more detail. The most active inhibitors in the whole series were those derived from the heterocyclic sulfonamides **14** and **15**. Thus, both the sulfenamido-sulfonamides as well as the bis-sulfonamides derived from these two compounds were very strong inhibitors, with potencies 10–12 times greater (against hCA II and bCA IV) than those of the clinically used inhibitors such as acetozolamide or methazolamide.

The susceptibility to inhibition of the three isozymes, investigated with the compounds reported here was: hCA I $\ll$ bCA IV $<$ hCA II. Thus, the classical response of these isozymes was observed again, with hCA II and bCA IV being very susceptible to inhibition with these sulfonamides, whereas hCA I was more resistant to this class of inhibitors.[4–7]

Since isozymes **II** and **IV** were shown to be critical in processes connected with aqueous humor secretion within cilliary processes of the eye,[8,13] and their inhibition represents an important antiglaucoma therapy,[13,44–46] some



64



65

of the most active inhibitors obtained in the present study were investigated for their capacity to act as topical IOP lowering agents (Table II). The clinically used topical sulfonamide dorzolamide[13] **65** has also been included in the study for comparison.

The following should be noted regarding the IOP lowering effects of the investigated sulfonamides: (i) none of the parent sulfonamides **4**–**15** show IOP lowering properties (data not shown, excepting for **14** which belongs to a new class of less investigated CA inhibitors),[35] (ii) among the investigated compounds, the sulfenamido-sulfonamides (such as **26**, **27**, **38**, **39**) do show important IOP lowering properties, in contrast to the corresponding bis-sulfonamides (such as **50**, **51**, **62** and **63**), which, although being stronger CA inhibitors, show extremely modest IOP lowering effects, (iii) among the detected topically active derivatives **27** and **39** show comparable activity with dorzolamide at 30 min after administration, but are better IOP lowering agents at longer times after administration, and their effect seems

TABLE II IOP after treatment with one drop (50 μL) of a 2% solution of CA inhibitor directly into the rabbit eye, at 30, 60 and 90 min after administration. The clinically used inhibitor dorzolamide was also included in the experiment

Inhibitor	ΔIOP (mmHg)*			
	$t = 0$	$t = 30$ min	$t = 60$ min	$t = 90$ min
5 (dorzolamide)	0	2.2 ± 0.10	4.1 ± 0.15	2.7 ± 0.08
14	0	0	0	0
26	0	0	2.5 ± 0.05	3.9 ± 0.06
27	0	2.1 ± 0.10	5.5 ± 0.08	4.4 ± 0.05
38	0	0	3.5 ± 0.07	4.0 ± 0.08
39	0	2.1 ± 0.08	6.3 ± 0.10	6.5 ± 0.10
50	0	0	0	0.6 ± 0.05
51	0	0	0	0.9 ± 0.10
62	0	0	0	0.5 ± 0.04
63	0	0	0	0.9 ± 0.07

* $\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; mean ± average spread ($n = 3$).

to be prolonged where compared to that of dorzolamide (Table II). On the other hand, the other two compounds, **26** and **38**, had no effect at 30 min after the administration, acted as less effective IOP lowering agents than dorzolamide at one hour, but reached a lower pressure than the clinically used agent at 90 min after administration. Thus, this is a nice example supporting our opinion of how an extremely small structural variation in the molecule of a CA inhibitor drastically changes biological activity. Practically, in this specific case, the presence of two additional oxygen atoms (in the second sulfonamido moiety) of compounds **50**, **51**, **62** and **63** (as compared to the parent sulfenamido-sulfonamides) completely abolishes the IOP lowering properties of these compounds. It is difficult to explain this result at the present time, but we consider that the oxidation of the sulfenamido moiety leads to compounds with a too large a hydrophilicity and a diminished lipophilicity, whereas for the sulfenamido-sulfonamides the right balance of these two parameters has been achieved, so that the penetrability to the enzyme in the target organ (the cilliary processes) was assured. Although the bis-sulfonamides were stronger inhibitors than the corresponding sulfenamido-sulfonamides, the above-mentioned 'defect' in some of their physico-chemical properties completely impairs their ability to act topically as IOP lowering agents.

References

[1] This paper is part 58 of the series "Carbonic Anhydrase Inhibitors": Preceding part: Supuran, C.T. and Clare, B.W. (1998) *Eur. J. Med. Chem.*, **33**, in press.
[2] (a) Roblin, R.O. and Clapp, J.W. (1950) *J. Am. Chem. Soc.*, **72**, 4890–4892; (b) Miller, W.H., Dessert, A.M. and Roblin, R.O. (1950) *J. Am. Chem. Soc.*, **72**, 4893–4896.
[3] (a) Vaughan, J.R., Eichler, J.A. and Anderson, G.W. (1956) *J. Org. Chem.*, **21**, 700–701; (b) Young, R.W., Wood, K.H., Vaughan, J.R. and Anderson, G.W. (1956) *J. Am. Chem. Soc.*, **78**, 4649–4654.
[4] Supuran, C.T. (1994) Carbonic anhydrase inbitors, in *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism.* (Puscas, I. Ed.), pp. 29–111. Helicon: Timisoara.
[5] Supuran, C.T. (1993) *Roum. Chem. Quart. Rev.*, **1**, 77–116.
[6] Lindskog, S. and Wistrand, P.J. (1987) Inhibition of carbonic anhydrase, in *Design of Enzyme Inhibitors as Drugs*, (Sandler, M.J. and Smith, H.J. Eds.), pp. 698–723. Oxford Univ. Press, Oxford.
[7] Wistrand, P.J. and Lindqvist, A. (1991) Design of carbonic anhydrase inhibitors and the relationship betwen the pharmacodynamics and pharmacokinetics of acetazolamide, in *Carbonic Anhydrase – From Biochemistry and Genetics to Physiology and Clinical Medicine* (Botré, F., Gros, G. and Storey, B.T. Eds.), pp. 352–374. VCH; Weinheim.
[8] Maren, T.H. (1967) *Physiol. Rev.*, **47**, 595–782.
[9] Supuran, C.T., Conroy, C.W. and Maren, T.H. (1996) *Eur. J. Med. Chem.*, **31**, 843–846.
[10] Bayer, K.H. and Baer, J.E. (1961) *Pharmacol. Rev.*, **13**, 517–562.
[11] Maren, T.H. (1976) *Annu. Rev. Pharmacol. Toxicol.*, **16**, 309–327.
[12] Maren, T.H. (1976) *J. Glaucoma*, **4**, 49–62.
[13] Sugrue, M.F. (1996) *J. Ocul. Pharmacol. Ther.*, **12**, 363–376.

[14] Reiss, W.G. and Oles, K.S. (1996) *Ann. Pharmacother.*, **30**, 514–519.
[15] Puscas, I. and Supuran, C.T. (1996) Farmacologia clinica da ulcera peptica in *Aparelho Digestivo* (Coelho, J. Ed.), pp. 1704–1734. MEDSI; Rio de Janeiro.
[16] Stoll, M., Hamann, G.F., Jost, V., Bompotti, U.A., Fitridge, R. and Schimrigk, K. (1996) *J. Neuroimaging*, **6**, 144–149.
[17] Levine, R.L., Tursky, P.A., Turnipseed, W.D. and Grist, T. (1996) *J. Neuroimaging*, **6**, 126–130.
[18] Maren, T.H., Jankowska, L., Sanyal, G. and Edelhauser, H.F. (1983) *Exp. Eye Res.*, **36**, 457–480.
[19] Tinker, J.P., Coulson, R. and Weiner, I.M. (1981) *J. Pharmacol Exp. Ther.*, **218**, 600–607.
[20] Swenson, E.R. and Maren, T.H. *Respir. Physiol.*, **35**, 129–159.
[21] Hewett-Emmett, D. and Tashian, R.E. (1996) *Mol. Phylogenet. Evol.*, **5**, 50–77.
[22] Sly, W.S., Zhu, X.L. and Sato, S. (1991) CA IV from human lung and kidney in *Carbonic Anhydrase From Biochemistry and Genetics to Physiology and Clinical Medicine* (Botré, F., Gros, G., Storey, B.T. Eds.), pp. 226–231. VCH; Weinheim.
[23] Boriack-Sjodin, P.A., Heck, R.W., Laipis, P.J., Silverman, D.N. and Christianson, D.W. (1995) *Proc. Nat. Acad. Sci. USA*, **92**, 10949–10953.
[24] Fernley, R.T. (1991) Secreted carbonic anhydrases in *Carbonic Anhydrase – From Biochemistry and Genetics to Physiology and Clinical Medicine* (Botré, F., Gros, G. and Storey, B.T. Eds.), pp. 178–185. VCH; Weinheim.
[25] Tu, C. and Silverman, D.N. (1985) *Biochemistry*, **24**, 5881–5887.
[26] Hazen, S.A., Waheed, A., Sly, W.S., LaNoue, K.F. and Lynch, C.J. (1996) *FASEB J.*, **10**, 481–490.
[27] (a) Supuran, C.T. and Scozzafava, A. (1997) *J. Enz. Inhib.*, **12**, 37–51; (b) Scozzafava, A. and Supuran, C.T. (1998) *J. Enz. Inhib.*, **13**, 103–123.
[28] Supuran, C.T., Briganti, F. and Scozzafava, A. (1997) *J. Enz. Inhib.*, **12**, 175–190.
[29] (a) Supuran, C.T., Popescu, A., Ilisiu, M., Costandache, A. and Banciu, M.D. (1996) *Eur. J. Med. Chem.*, **31**, 439–447; (b) Supuran, C.T., Scozzafava, A., Popescu, A., Bobes-Tureac, R., Banciu, A., Creanga, A., Bobes-Tureac, G. and Banciu, M.D. (1997) *Eur. J. Med. Chem.*, **32**, 445–452; (c) Supuran, C.T., Scozzafava, A., Jurca, B.C. and Ilies, M.A. (1998) *Eur. J. Med. Chem.*, **33**, 83–93.
[30] Supuran, C.T., Ilies, M.A. and Scozzafava, A. (1998) *Eur. J. Med. Chem.*, **33**, in press.
[31] (a) Sachs, G., Prinz, C., Loo, D., Bamberg, K., Besancon, M. and Shin, J.M. (1994) *Yale J. Biol. Med.*, **67**, 81–95; (b) Sachs, G., Shin, J.M., Briving, C., Wallmark, B. and Hersey, S. (1995) *Annu. Rev. Pharmacol. Toxicol.*, **35**, 277–305; (c) Hirschowitz, B.I., Keeling, D., Lewin, M., Okabe, S., Parsons, M., Sewing, K., Wallmark, B. and Sachs, G. (1995) *Ann. N.Y. Acad. Sci.*, **671**, 204–216.
[32] Crippa, G.B. and Maffei, S. (1941) *Gazz. Chim. Ital.*, **71**, 97–99.
[33] (a) Scudi, J.V. (1937) *J. Am. Chem. Soc.*, **59**, 1480–1483; (b) Bauer, H. (1939) *J. Am. Chem. Soc.*, **61**, 613–616; (c) Ilies, M.A., Scozzafava, A. and Supuran, C.T. (1998) *Eur. J. Med. Chem.*, **33**, in press; (d) Cingolani, E. (1948) *Gazz. Chim. Ital.*, **78**, 275–282.
[34] Jitianu, A., Ilies, M.A., Scozzafava, A. and Supuran, C.T. (1997) *Main Group Met. Chem.*, **20**, 147–153.
[35] Barboiu, M., Scozzafava, A. and Supuran, C.T. (1998), manuscript in preparation.
[36] Forsman, C., Behravan, G., Osterman, A. and Jonsson, B.H. (1988) *Acta Chem. Scand.*, **B42**, 314–318.
[37] Behravan, G., Jonasson, P., Jonsson, B.H. and Lindskog, S. (1991) *Eur. J. Biochem.*, **198**, 589–592.
[38] Khalifah, R.G., Strader, D.J., Bryant, S.H. and Gibson, S.M. (1977) *Biochemistry*, **16**, 2241–2247.
[39] Nyman, P.O. and Lindskog, S. (1964) *Biochim. Biophys. Acta*, **85**, 141–151.
[40] Henderson, L.E., Henriksson, D. and Nyman, P.O. (1976) *J. Biol. Chem.*, **251**, 5457–5463.
[41] Maren, T.H., Wynns, G.C. and Wistrand, P.J. (1993) *Mol. Pharmacol.*, **44**, 901–906.
[42] Pocker, Y. and Stone, J.T. (1967) *Biochemistry*, **6**, 668–678.
[43] Baird, T.T., Waheed, A., Okuyama, T., Sly, W.S. and Fierke, C.A. (1997) *Biochemistry*, **36**, 2669–2678.
[44] Maren, T.H., Bar-Ilan, A., Conroy, C.W. and Brechue, W.F. (1990) *Exp. Eye Res.*, **50**, 27–36.

[45] Maren, T.H., Brechue, W.F. and Bar-Ilan, A. (1992) *Exp. Eye Res.*, **55**, 73–79.
[46] Brechue, W.F. and Maren, T.H. (1993) *Invest. Ophthalmol. Vis. Sci.*, **34**, 2581–2587.
[47] (a) Goerdeler, J. and Holst, A. (1959) *Angew. Chem.*, **71**, 775–788; (b) Gordon, E.M., Ondetti, M.A., Pluscek, J., Cimarusti, C.M., Bonner, D.P. and Sykes, R.B. (1982) *J. Am. Chem. Soc.*, **104**, 6053–6059.
[48] Capozzi, G., Modena, G. and Pasquato, L. (1990) Chemistry of sulphenyl halides and sulphenamides, in *The Chemistry of Sulphenic Acids and Their Derivatives*, (Patai, S. Ed.), pp. 403–516. Wiley; Chichester.
[49] Craine, L. and Raban, M. (1989) *Chem. Rev.*, **89**, 689–712.
[50] Mann, T. and Keilin, D. (1940) *Nature* (London), **164**, 146–147.