J. Enzyme Inhibition, Vol. 12, pp. 175–190
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Amsterdam B.V. Published in The Netherlands
by Harwood Academic Publishers
Printed in India

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SULFENAMIDO-SULFONAMIDES AS INHIBITORS OF CARBONIC ANHYDRASE ISOZYMES I, II AND IV ☆

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(Received 20 January 1997)

Reaction of 2-nitrophenyl- and 4-nitrophenylsulfenyl-chlorides with aromatic/heterocyclic sulfonamides containing a free amino group afforded sulfenamido-sulfonamides, which were inhibitors of the zinc enzyme carbonic anhydrase (CA). Oxidation of these derivatives with potassium permanganate in acetone led to the corresponding bis-sulfonamides. Good inhibition of three CA isozymes (CA I, II and IV, respectively) was observed with some of the new compounds, the bis-sulfonamides being more active than the sulfenamido-sulfonamides. A possible *in vivo* transformation of the last type of compounds, leading to an omeprazole-like gastric acid secretion inhibitor is also discussed.

Keywords: Aromatic/heterocyclic sulfonamide; Carbonic anhydrase; Isozyme I, II, IV; Sulfenamide; Gastric acid secretion inhibition

INTRODUCTION

The eight carbonic anhydrase (CA, EC 4.2.1.1) isozymes isolated up to now in higher vertebrates,[2] are involved in critical physiological processes such as transport of CO_2 between metabolizing tissues and lungs,[3] secretion of electrolytes in many organs,[3,4] and signal transduction in a variety of cells.[5] Sulfonamide CA inhibitors are widely used drugs in the treatment or prevention of glaucoma,[3] gastro-duodenal ulcers,[4b] or diverse neurological

☆ See ref. 1.

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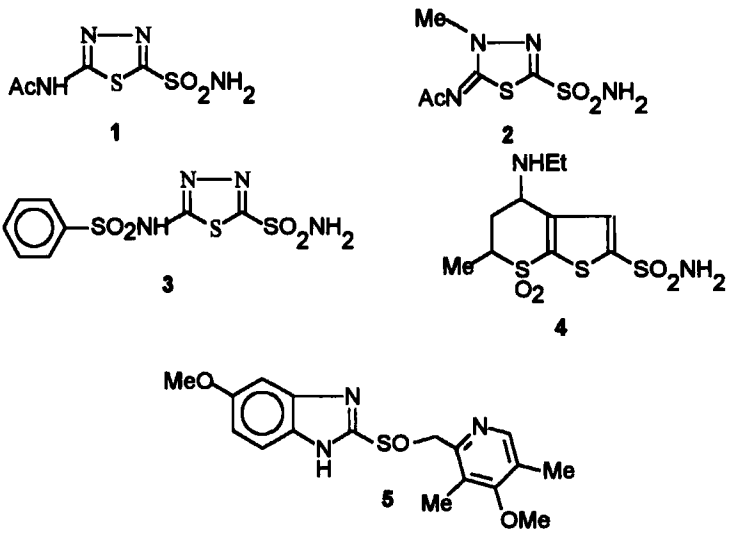
disorders[6] but recently they were shown to be useful for the inhibition of osteoclastic bone resorption[7] and thus as a new therapy for osteoporosis. Moreover, sulfonamide CA inhibitors, such as acetazolamide **1** were shown to be useful in the therapy of cancer in association with a lot of classical antineoplastic agents, such as carmustine, cisplatin or melphalan.[8a] Other sulfonamide inhibitors, such as methazolamide **2** were recently shown to inhibit the growth of human lymphoma cells in culture.[8b] CA inhibition by sulfonamides has as a consequence the acidification of the intratumoral environment, markedly decreasing tumor growth in experimental animals, but the definitive mechanism of action of the CA inhibitors in cancer therapy has not yet been fully clarified.[8,9] Recently a human tumor-associated protein (detectable only in cancerous cells), possessing a domain homologous to CA, and enzyme activity of the CA type (subsequently this protein was termed CA IX),[10] was also isolated. This indicates that studies on CA activity and its inhibition might be important for a better understanding of cell proliferation and transformation, as well as in the design of novel types of anti-cancer therapies.



Isozyme-specific or organ-selective CA inhibitors[11] are also desirable in order to improve the efficiency of clinically used inhibitors (such as acetazolamide **1**, methazolamide **2**, benzolamide **3** or sezolamide **4**) and for understanding the physiological function of some CA isozymes.[3,4] Although few compounds possess high specificity for diverse CA isozymes, recently some progress was achieved in the design of low molecular weight

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inhibitors with high affinity for the membrane-bound form CA IV,[12,13] as well as some tight-binding-CA I sulfonamides.[14]

CA together with H^+/K^+-ATP-ase are the key enzymes involved in gastric acid secretion,[4,5,15,16] and since high doses of acetazolamide were shown to be useful as a therapy for gastro-duodenal ulcers[4b,17] in this paper we propose a novel approach for a possible treatment of this disease, by combining inhibition of CA by sulfonamide inhibitors with inactivation of H^+/K^+-ATP-ase by sulfenyl derivatives. Here we report the synthesis of some sulfenyl-sulfonamide derivatives that in acidic media (for instance in the stomach) might be transformed to effective sulfonamide CA inhibitors and sulfenyl halides (or similarly reactive intermediates) that by reaction with H^+/K^+-ATP-ase, would inactivate this enzyme similarly to omeprazole **5**, the most potent known gastric acid secretion inhibitor.[18–20] In this way, the two *in vivo* sources of H^+ ions (i.e., generated by hydration of CO_2, a reaction catalyzed by CAs – reaction (1), as well as by ATP hydrolysis – reaction (2) – in the presence of H^+/K^+-ATP-ase) would be blocked, and a strong inhibition of acid production would be expected.[15a,b,19]

$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+ \quad (1)$$

$$ATP^{4-} + H_2O \Leftrightarrow ADP^{3-} + HPO_4^{2-} + H^+ \quad (2)$$

The new compounds reported here were characterized by standard chemical and spectroscopic methods and were assayed as CA inhibitors against three isozymes (CA I, II and IV). *In vitro* tests also showed that in acidic medium they undergo the type of transformation that constituted the hypothesis for their design, i.e., they lead to formation of a sulfonamide inhibitor, together with the sulfenyl halide that should subsequently sulfenylate and inactivate H^+/K^+-ATP-ase.

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.

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Sulfonamides used in synthesis were commercially available (from Sigma, Acros or Aldrich) except for 5-amino-1,3,4-thiadiazole-2-sulfonamide **8e** which was prepared from acetazolamide (Sigma) by deacetylation,[21] and methanilamide **8b** which was prepared from 3-aminobenzene-sulfonyl fluoride hydrochloride (Acros) by treatment with excess aqueous ammonia. Both compounds were recrystallized from ethanol–water (1 : 1, v/v). 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 BL21 (DE3) from the plasmids pACA/HCA I and pACA/HVA II described by Forsman *et al.*[22] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[23] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[24] 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.[25,26] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration has been determined by titration with ethoxzolamide.[27a]

Inhibitors were assayed by Maren's micromethod,[27] at 0°C, in the conditions of the E–I (enzyme–inhibitor) technique. Water saturated with 100% CO_2 (at 0°C) was used as substrate, as originally described by Maren *et al.*[27] 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] and dilutions up to 0.1 nM were done thereafter with distilled–deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–I complex.[27] In a special CO_2 bubbler cell 0.3 mL of distilled water was added, followed by 0.4 ml of phenol red indicator solution (1%) and (0.1 ml) of inhibitor + 0.1 mL of CA solution, preincubated as mentioned above). The CA concentrations were 1.5 nM for CA II, 210 nM for CA I and 3.5 nM for CA IV. The hydration reaction was initiated by addition of 0.1 mL of barbital buffer (pH 7.5), and the time to obtain a color change was recorded with a stopwatch. Enzyme specific activity in the presence and in the absence of inhibitors, as well as IC_{50} values (the mean of two determinations) were determined as described by Maren. The standard error of these measurements is around 5–10%.[3,27]

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General Procedure for the Preparation of Compounds 9–18

An amount of 10 mM sulfonamide **8a–e** was dissolved/suspended in 50 mL of anhydrous acetonitrile and 1.4 mL (10 mm) of triethylamine were added dropwise. The reaction mixture was magnetically stirred at room temperature for 15 min, then 1.89 g (10 mM) of sulfenyl chloride **6** or **7** dissolved in 10 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 by means of thin layer chromatography it was observed that the reaction was completed. After half of the solvent has been evaporated in vacuo, the reaction mixture was poured into 50 mL of cold water. The precipitated product was filtered and recrystallized from ethanol. Yields were in the range of 85–90%.

General Procedure for the Preparation of Compounds 19–28

5 mM of sulfenamido-sulfonamide **9–18** were dissolved in 50 mL of acetone and the required amount of saturated potassium permanganate solution in acetone was added (this solution was obtained by stirring overnight an excess of solid $KMnO_4$ in acetone, followed by filtration of the excess permanganate and precipitated MnO_2; the amount of permanganate contained was then assayed by titration with a standardized oxalic acid solution). This reaction mixture was stirred at room temperature for 1 h, the brown precipitate formed was filtered and discarded, and the clear acetone solution evaporated in vacuo. The residue was recrystallized from ethanol–water or methanol–water. Yields were in the range of 60–70%.

4-(2-Nitrobenzenesulfenylamido)-benzenesulfonamide **9**, pale yellow crystals, m.p. 185–7°C, IR (KBr), cm^{-1}: 735, 751, 776, 838, 1030, 1080 and 1250 (NO_2), 1172 (SO_2^{sym}), 1331 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.19 (br s, 1H, NH); 6.55 (br s, 2H, NH_2); 7.08 (m, AA′BB′, 4H, ArH from phenylene); 7.29–7.73 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 44.4; H, 3.1; N, 12.5. $C_{12}H_{11}N_3O_4S_2$ requires: C, 44.3; H, 3.3; N, 12.9%.

3-(2-Nitrobenzenesulfenylamido)-benzenesulfonamide **10**, yellow crystals, m.p. 194–6°C, IR (KBr), cm^{-1}: 632, 741, 828, 995, 1090 and 1250 (NO_2), 1181 (SO_2^{sym}), 1314 (SO_2^{as}), 3270 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.07 (br s, 1H, NH); 6.40 (br s, 2H, NH_2); 7.10–7.59 (m, 4H, ArH from 1,3-phenylene); 7.29–7.73 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 44.3; H, 3.0; N, 12.6. $C_{12}H_{11}N_3O_4S_2$ requires: C, 44.3; H, 3.3; N, 12.9%.

4-(2-Nitrobenzenesulfenylamidomethyl)-benzenesulfonamide **11**, yellow crystals, m.p. 210–2°C, IR (KBr), cm^{-1}: 725, 749, 783, 818, 1084 and 1249

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(NO_2), 1171 (SO_2^{sym}), 1320 (SO_2^{as}), 3280 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.89 (s, 2H, SNH*CH*$_2$); 5.17 (br s, 1H, NH); 5.85 (br s, 2H, NH_2); 7.05 (m, AA′BB′, 4H, ArH from phenylene); 7.23–7.67 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 45.8; H, 3.9; N, 12.2. $C_{13}H_{13}N_3O_4S_2$ requires: C, 46.0; H, 3.8; N, 12.3%.

4-(2-Nitrobenzenesulfenylamidoethyl)-benzenesulfonamide **12**, pale yellow crystals, m.p. 187–9°C, IR (KBr), cm^{-1}: 662, 849, 888, 957, 1051, 1082 and 1250 (NO_2), 1169 (SO_2^{sym}), 1325 (SO_2^{as}), 3280 and 3360 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2); 3.70 (t, 2H, βCH_2); 5.05 (br s, 1H, NH); 5.58 (br s, 2H, NH_2); 7.06 (m, AA′BB′, 4H, ArH from phenylene); 7.29–7.70 (m, 4H, Ar H from *ortho*-substituted phenyl). Found: C, 47.3; H, 3.8; N, 11.8. $C_{14}H_{15}N_3O_4S_2$ requires: C, 47.6; H, 4.2; N, 11.9%.

5-(2-Nitrobenzenesulfenylamido)-1,3,4-thiadiazole-2-sulfonamide **13**, yellow crystals, m.p. 239–41°C (dec.), IR(KBr), cm^{-1}: 559, 631, 715, 970, 1030, 1085 and 1250 (NO_2), 1180 (SO_2^{sym}), 1320 (SO_2^{as}), 1490, 1540, 3280 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.08 (br s, 1H, NH); 6.20 (br s, 2H, NH_2); 7.23–7.75 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 28.2; H, 2.9; N, 20.7. $C_8H_9N_5O_4S_3$ requires: C, 28.6; H, 2.6; N, 20.9%.

4-(4-Nitrobenzenesulfenylamido)-benzenesulfonamide **14**, yellow crystals, m.p. 212–3°C, IR (KBr), cm^{-1}: 708, 725, 810, 839, 1050, 1075 and 1250 (NO_2), 1173 (SO_2^{sym}), 1336 (SO_2^{as}), 3230 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.12 (br s, 1H, NH); 6.71 (br s, 2H, NH_2); 7.08 (m, AA′BB′, 4H, ArH from phenylene-sulfonamido); 7.15 (m, AA′BB′, 4H, ArH from nitro-phenylene). Found: C, 44.2; H, 3.0; N, 12.9. $C_{12}H_{11}N_3O_4S_2$ requires: C, 44.3; H, 3.3; N, 12.9%.

3-(4-Nitrobenzenesulfenylamido)-benzenesulfonamide **15**, yellow crystals, m.p. 209–11°C, IR (KBr), cm^{-1}: 669, 753, 832, 990, 1080 and 1250 (NO_2), 1181 (SO_2^{sym}), 1315 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.10 (br s, 1H, NH); 6.40 (br s, 2H, NH_2); 7.10–7.59 (m, 4H, ArH from 1,3-phenylene); 7.15 (m, AA′BB′, 4H, ArH from nitro-phenylene). Found: C, 44.1; H, 3.4; N, 12.7. $C_{12}H_{11}N_3O_4S_2$ requires: C, 44.3; H, 3.3; N, 12.9%.

4-(4-Nitrobenzenesulfenylamidomethyl)-benzenesulfonamide **16**, yellow crystals, m.p. 219–20°C, IR (KBr), cm^{-1}: 705, 736, 780, 838, 1080 and 1247 (NO_2), 1171 (SO_2^{sym}), 1324 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.93 (s, 2H, SNH*CH*$_2$); 5.15 (br s, 1H, NH); 5.82 (br s, 2H, NH_2); 7.05 (m, AA′BB′, 4H, ArH from phenylene-sulfonamido); 7.15 (m, AA′BB′, 4H, ArH from nitro-phenylene). Found: C, 46.2; H, 3.8; N, 12.3. $C_{13}H_{13}N_3O_4S_2$ requires: C, 46.0; H, 3.8; N, 12.3%.

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4-(4-Nitrobenzenesulfenylamidoethyl)-benzenesulfonamide **17**, yellow crystals, m.p. 198–9°C, IR (KBr), cm^{-1}: 659, 732, 856, 889, 974, 1060, 1087 and 1250 (NO_2), 1169 (SO_2^{sym}), 1321 (SO_2^{as}), 3270 and 3360 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.12 (t, 2H, αCH_2); 3.71 (t, 2H, βCH_2); 5.10 (br s, 1H, NH); 5.61 (br s, 2H, NH_2); 7.07 (m, AA′BB′, 4H, ArH from phenylene-sulfonamido); 7.15 (m, AA′BB′, 4H, ArH from nitro-phenylene). Found: C, 47.5; H, 4.0; N, 11.5. $C_{14}H_{15}N_3O_4S_2$ requires: C, 47.6; H, 4.2; N, 11.9%.

5-(4-Nitrobenzenesulfenylamido)-1,3,4-thiadiazole-2-sulfonamide **18**, yellow crystals, m.p. 251–4°C (dec.), IR (KBr), cm^{-1}: 612, 638, 710, 966, 1034, 1081 and 1250 (NO_2), 1179 (SO_2^{sym}), 1320 (SO_2^{as}), 1470, 1540, 3275 and 3380 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.10 (br s, 1H, NH); 6.26 (br s, 2H, NH_2); 7.15 (m, AA′BB′, 4H, ArH from phenylene). Found: C, 28.5; H, 2.6; N, 20.7. $C_8H_9N_5O_4S_3$ requires: C, 28.6; H, 2.6; N, 20.9%.

4-(2-Nitrobenzenesulfonylamido)-benzenesulfonamide **19**, pale yellow crystals, m.p. 218–9°C, IR (KBr), cm^{-1}: 662, 745, 853, 1030, 1078 and 1250 (NO_2), 1139 and 1172 (SO_2^{sym}), 1335 (SO_2^{as}), 3290 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.75 (br s, 3H, NH + NH_2); 7.05 (m, AA′BB′, 4H, ArH from phenylene); 7.32–7.64 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 40.0; H, 3.1; N, 11.5. $C_{12}H_{11}N_3O_6S_2$ requires: C, 40.3; H, 3.0; N, 11.7%.

3-(2-Nitrobenzenesulfonylamido)-benzenesulfonamide **20**, pale yellow crystals, m.p. 201–4°C. IR (KBr), cm^{-1}: 685, 717, 810, 943, 1080 and 1250 (NO_2), 1144 and 1181 (SO_2^{sym}), 1314 (SO_2^{as}), 3275 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.50 (br s, 3H, NH_2 + NH); 7.15–7.50 (m, 4H, ArH from 1, 3-phenylene); 7.33–7.78 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 40.3; H, 3.2; N, 11.6. $C_{12}H_{11}N_3O_6S_2$ requires: C, 40.3; H, 3.0; N, 11.7%.

4-(2-Nitrobenzenesulfonylamidomethyl)-benzenesulfonamide **21**, white crystals, m.p. 228–9°C, IR (KBr), cm^{-1}: 694, 725, 789, 798, 885, 1080 and 1255 (NO_2), 1150 and 1171 (SO_2^{sym}), 1323 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.95 (s, 2H, SO_2NHCH_2); 6.28 (br s, 3H, NH_2 + NH); 7.05 (m, AA′BB′, 4H, ArH from phenylene); 7.25–7.70 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 41.8; H, 3.6; N, 11.2. $C_{13}H_{13}N_3O_6S_2$ requires: C, 42.0; H, 3.5; N, 11.3%.

4-(2-Nitrobenzenesulfonylamidoethyl)-benzenesulfonamide **22**, white crystals, m.p. 218–9°C, IR (KBr), cm^{-1}: 691, 735, 844, 873, 975, 1039, 1080 and 1250 (NO_2), 1154 and 1169 (SO_2^{sym}), 1328 (SO_2^{as}), 3260 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.12 (t, 2H, αCH_2); 3.77 (t, 2H,

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βCH_2); 6.74 (br s, 3H, NH_2+NH); 7.08 (m, AA'BB', 4H, ArH from phenylene); 7.26–7.61 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 43.5; H, 3.8; N, 10.8. $C_{14}H_{15}N_3O_6S_2$ requires: C, 43.6; H, 3.8; N, 10.9%.

5-(2-Nitrobenzenesulfonylamido)-1,3,4-thiadiazole-2-sulfonamide **23**, pale yellow crystals, m.p. 239–41°C (dec.), IR (KBr), cm^{-1}: 590, 637, 685, 719, 971, 1050, 1085 and 1250 (NO_2), 1153 and 1180 (SO_2^{sym}), 1327 (SO_2^{as}), 1495, 1540, 3260 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10 (br s, 3H, NH_2+NH); 7.28–7.65 (m, 4H, ArH from *ortho*-substituted phenyl). Found: C, 26.2; H, 2.3; N, 18.7. $C_8H_9N_5O_6S_3$ requires: C, 26.1; H, 2.4; N, 19.0%.

4-(4-Nitrobenzenesulfonylamido)-benzenesulfonamide **24**, pale yellow crystals, m.p. 229–33°C, IR (KBr), cm^{-1}: 701, 755, 818, 894, 1045, 1078 and 1250 (NO_2), 1139 and 1173 (SO_2^{sym}), 1335 (SO_2^{as}), 3260 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.00 (br s, 3H, NH_2+NH); 7.09 (m, AA'BB', 4H, ArH from phenylene-sulfonamido); 7.21 (m, AA'BB', 4H, ArH from nitro-phenylene). Analysis, found: C, 40.4; H, 3.0, N, 11.5%. $C_{12}H_{11}N_3O_6S_2$ requires: C, 40.3; H, 3.0; N, 11.7%.

3-(4-Nitrobenzenesulfonylamido)-benzenesulfonamide **25**, pale yellow crystals, m.p. 235–7°C, IR (KBr), cm^{-1}: 690, 725, 778, 821, 970, 1085 and 1250 (NO_2), 1150 and 1182 (SO_2^{sym}), 1319 (SO_2^{as}), 3290 and 3400 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1H, 6.90 (br s, 3H, NH_2+NH); 7.10–7.55 (m, 4H, ArH from 1,3-phenylene); 7.19 (m, AA'BB', 4H, ArH from nitro-phenylene). Found: C, 40.1; H, 3.4; N, 11.7. $C_{12}H_{11}N_3O_6S_2$ requires: C, 40.3; H, 3.0; N, 11.7%.

4-(4-Nitrobenzenesulfonylamidomethyl)-benzenesulfonamide **26**, white crystals, m.p. 249–51°C, IR (KBr), cm^{-1}: 661, 715, 762, 820, 884, 1075 and 1249 (NO_2), 1151 and 1173 (SO_2^{sym}), 1325 (SO_2^{as}), 3270 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, SO_2NHCH_2); 6.86 (br s, 3H, NH_2+NH); 7.05 (m, AA'BB', 4H, ArH from phenylene-sulfonamido); 7.23 (m, AA'BB', 4H, ArH from nitro-phenylene). Found: C, 42.2; H, 3.6; N, 11.3. $C_{13}H_{13}N_3O_6S_2$ requires: C, 42.0; H, 3.5; N, 11.3%.

4-(4-Nitrobenzenesulfonylamidoethyl)-benzenesulfonamide **27**, white crystals, m.p. 237–40°C, IR (KBr), cm^{-1}: 690, 728, 779, 860, 898, 970, 1045, 1080 and 1255 (NO_2), 1150 and 1171 (SO_2^{sym}), 1328 (SO_2^{as}), 3270 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.15 (t, 2H, αCH_2); 3.71 (t, 2H, βCH_2); 6.86 (br s, 3H, NH_2+NH); 7.06 (m, AA'BB', 4H, ArH from phenylene-sulfonamido); 7.21 (m, AA'BB', 4H, ArH from nitro-phenylene). Found: C, 43.5; H, 3.9; N, 11.0. $C_{14}H_{15}N_3O_6S_2$ requires: C, 43.6; H, 3.8; N, 10.9%.

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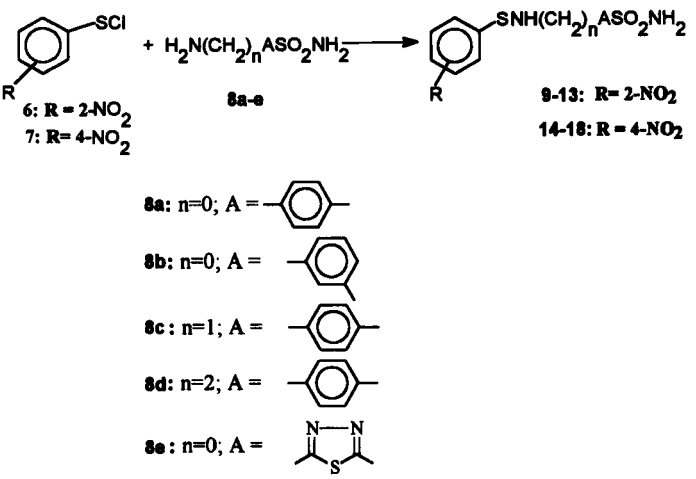
5-(4-Nitrobenzenesulfonylamido)-1,3,4-thiadiazole-2-sulfonamide **28**, white crystals, m.p. 278–9°C (dec.), IR (KBr), cm^{-1}: 625, 674, 754, 970, 1045, 1078 and 1250 (NO_2), 1150 and 1180 (SO_2^{sym}), 1326 (SO_2^{as}) 1460, 1545, 3280 and 3390 (NH and NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.03 (br s, 3H, NH_2 + NH); 7.28 (m, AA′BB′, 4H, ArH from phenylene). Found: C, 26.2; H, 2.4; N, 18.8. $C_8H_9N_5O_6S_3$ requires: C, 26.1; H, 2.4; N, 19.0%.

RESULTS AND DISCUSSION

Although arylsulfenyl halides such as the nitrosubstituted derivatives **6** and **7** are extensively used reagents for oligo- and polypeptide synthesis, their reaction with amino-sulfonamides of type **8** has not been investigated previously.[28–30] In Scheme 1, the synthesis of arylsulfenyl-sulfonamides **9–18**, by reaction of the above mentioned reagents, is shown. The new derivatives have been characterized by elemental analysis, IR and ^{1}H-NMR spectroscopy which confirmed the proposed structure for the new derivatives (see Materials and Methods for details).



SCHEME 1

The presence of the arylsulfenamido moiety in the derivatives **9–18** is not only of interest for their possible transformation in acidic medium into a sulfonamide CA inhibitor plus a compound able to modify H^+/K^+-ATPase, but also as precursors for other types of CA inhibitors. Thus, recently the X-ray crystallographic structure of the adduct of human CA II with aminobenzolamide (the 4-amino-derivative of compound **3**) has been

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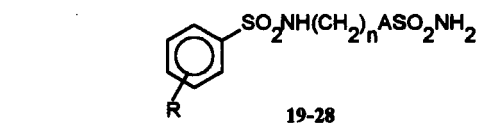
reported.[31] Aminobenzolamide is not only one of the most potent inhibitors of isozyme CA II (with a K_I of 1 nM)[32,33] but it is also one of the sulfonamides with the highest affinity for CA III, a sulfonamide-resistant isozyme[11] (with a K_I of 3.7 μM).[33] The above-mentioned X-ray crystallographic study as well as molecular modeling studies for the binding of this inhibitor to CA III,[31] showed that it is the second arylsulfonamido moiety ($X\text{-}C_6H_4\text{-}SO_2NH\text{-}$) which is important for the tight binding to the enzyme as well as for the differences of affinity of this inhibitor to diverse isozymes, as compared to other sulfonamides, such as acetazolamide **1**, methazolamide **2** or sezolamide **4** (all acting as very good CA II inhibitors and weak CA III inhibitors).[3,4] As seen from Figure 1, generated from the X-ray crystallographic coordinates kindly provided by Prof. A. Liljas, Drs. A. Svensson and J. Vidgren (University of Lund, Sweden), the high affinity of aminobenzolamide can be explained by the supplementary hydrogen bonds formed between the oxygen atoms of the second SO_2NH moiety and active site residues Leu 198 and Gln 92, as well as the edge-to-face arrangement of the amino-phenyl ring of the inhibitor and the phenyl ring of active site residue Phe 131,[31] which is very favorable for protein-ligand interactions.[34,35]

Thus, inhibitors possessing this type of substitution ($Aryl\text{-}SO_2NH\text{-}aryl'\text{-}SO_2NH_2$) might constitute interesting lead molecules for obtaining isozyme-specific inhibitors, and this is the main reason that prompted us to prepare compounds **19–28**, by oxidation of the sulfenamido-sulfonamides **9–18** with potassium permanganate in acetone.[13c] These new compounds have also been characterized by elemental analysis and spectroscopic measurements that confirmed their structure (see Materials and Methods).



19-28

R= 2-NO2

19: n=0; A =

20: n=0; A =

21: n=1; A =

22: n=2; A =

23: n=0; A =

R = 4-NO2

24: n=0; A =

25: n=0; A =

26: n=1; A =

27: n=2; A =

28: n=0; A =

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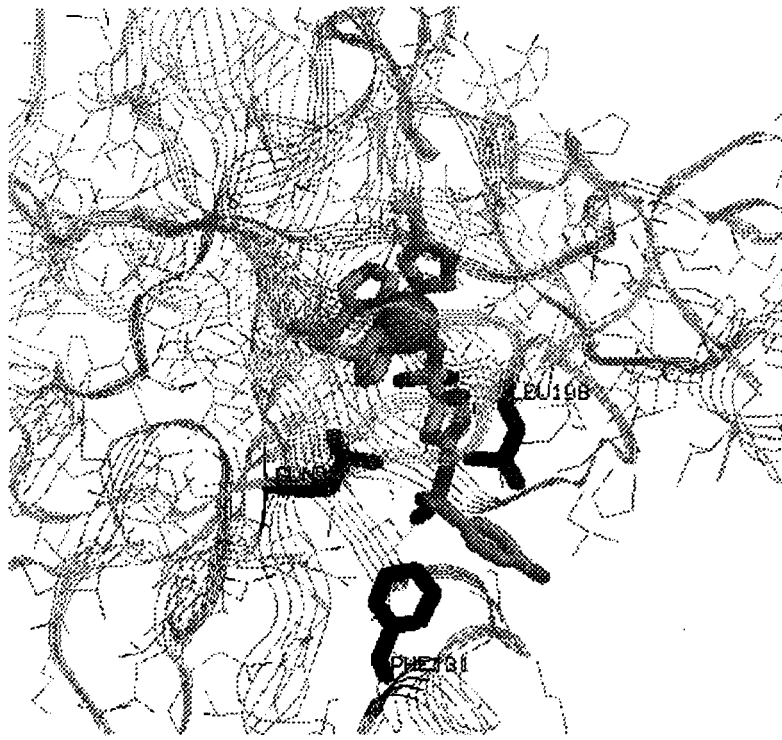

FIGURE 1 Human CA II – aminobenzolamide adduct: the Zn(II) ion and its three histidine ligands (His 94, 96, 119) as well as residues Leu 198, Gln 92 and Phe 131, important for inhibitor tight binding, are shown. The figure was generated from the X-ray crystallographic data of Vidgren *et al.* (ref.[31]) by using the program RasWin Molecular Graphics Version 2.6 (by R. Sayle).

It is interesting to note that some CA inhibitors of type **23** and **28**, i.e., 5-arylsulfonamido-1,3,4-thiadiazole-2-sulfonamides have been reported.[36] It was observed that the presence of the second SO_2NH moiety leads to improved inhibitory activity.[36] This improvement was explained as due to the acidification induced by the second sulfonamido moiety upon the SO_2NH_2 group, which should favor the binding (in the ionized state) to the Zn(II) ion within the enzyme active site.[36] As seen from the recent X-ray crystallographic data,[31] the explanation is much more complex, the improved inhibition being due to more subtle molecular interactions between the inhibitor and the enzyme molecules, in addition to the above mentioned acidification effect.

Inhibition data against three CA isozymes, CA I, II and IV, with the newly prepared compounds **9–28** as well as standard inhibitors of type **1–3** and **8** are shown in Table I.

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TABLE I Biological activity data of sulfonamide CA inhibitors **9–28** prepared in the present study and standard inhibitors **1–3** and **8**

Compound	IC_{50} (nM)		
	CA I†	CA II†	CA IV‡
1 (acetazolamide)	200	7	120
2 (methazolamide)	10	9	145
3 (benzolamide)	2	1	35
8a (sulfanilamide)	2800	300	3000
8b (methanilamide)	2500	240	2200
8c (homosulfanilamide)	2500	170	2800
8d (p-aminoethylbenzene sulfonamide)	2100	180	2450
8e (aminothiadiazolesulfonamide)	280	30	190
9	170	18	80
10	190	17	60
11	140	11	60
12	90	10	40
13	7	0.9	22
14	120	16	110
15	150	13	45
16	110	8	40
17	90	8	50
18	9	0.5	10
19	90	10	45
20	110	10	50
21	40	3	20
22	40	2	17
23	3	0.4	5
24	65	5	63
25	85	9	32
26	82	6	10
27	25	5	10
28	2	0.1	3

†Human (cloned) isozyme;
‡Isolated from bovine lung microsomes (ref.[27a]).
IC_{50} – the mean of two different assays – represents the molarity of inhibitor producing a 50% decrease of enzyme specific activity for the CO_2 hydration reaction, by Maren's micromethod.[27]

The following features were observed for inhibition by these new sulfonamides: (i) the strongest inhibition was observed with the bis-sulfonamides **19–24**, which are better inhibitors for all three isozymes as compared to the corresponding sulfenamido-sulfonamides **9–18**, which in turn are themselves better inhibitors than the parent amino-sulfonamides **8a–e**, (ii) the 4-nitrophenyl-substituted compounds were generally slightly more active than the corresponding 2-nitro-substituted derivatives, (iii) the heterocyclic derivatives **13, 18, 23** and **28** were the most potent inhibitors; for the aromatic derivatives, the inhibitory power increases with the number of aliphatic carbon atoms (*n* in formulas **9–28**). For the series of compounds with n = 0, the sulfanilamide derivatives were generally more inhibitory than the corresponding methanilamides and, (iv) isozyme CA II was the

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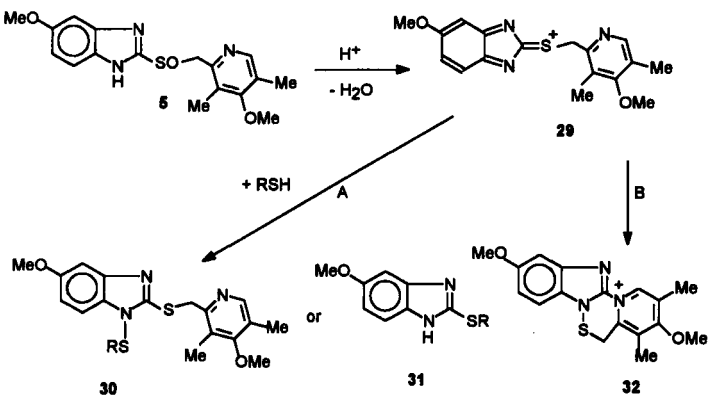
most sensitive to these new inhibitors, followed by CA IV, whereas CA I was the most resistant. Some tight-binding CA I inhibitors were obtained, such as **13**, **23** and **28**. Furthermore, some of the new compounds, such as **18**, **22**, **23**, **26–28** behave as much more efficient CA IV inhibitors as compared to the classical, clinically used compounds **1–3**.

As mentioned in the introductory section, the new compounds reported here were prepared in order to design a novel type of gastric acid secretion inhibitor, that should combine CA inhibitory properties with H^+/K^+-ATP-ase inactivation effects of the omeprazole type. Omeprazole, **5**, is in fact a prodrug that in an acidic millieu within the stomach undergoes the chemical transformations shown in Scheme 2.[18,19]



SCHEME 2

The active species formed from omeprazole are presumably **29** and/or **32**.[18] The first step of this transformations consists in protonation of the sulfoxide moiety of **5** and elimination of water, with the generation of the sulfenium ion key intermediate **29** via a Pumerer-like reaction.[18b] This sulfenium ion reacts subsequently with cysteine residues from the enzyme active site, leading either to sulfenamides of type **30**, or to mercaptobenzimidazoles of type **31**.[18] It seems that the first type of derivative is the one most probably formed, involving two cysteine residues (RSH in Scheme 2) of the H^+/K^+-ATP-ase, Cys 813 (or 822) and Cys 892.[18–20] The region of the protein containing these amino acid residues is situated in a membrane-spanning segment, accessible from the acidic face of the pump. Alternatively, a reaction product of type **32** has also been isolated when omeprazole was treated with acid in the absence of mercapto-derivatives. The cyclic sulfenamide **32** may also react subsequently with Cys residues in the protein.[18–20]

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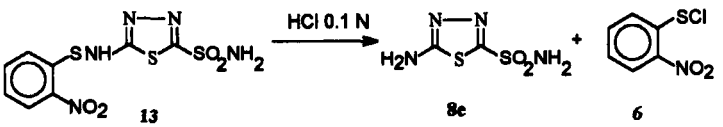
Since the nitrophenyl-sulfenyl moiety used as protecting group in oligo- and polypeptide synthesis is removable in acidic medium,[28–30] we tested whether some of the CA inhibitors **9–18** prepared in the present study could be transformed into reactive species similar to intermediates **29** and **32** formed from omeprazole in the presence of acid, that should subsequently be able to react with H^+/K^+-ATP-ase.

Thus, treatment of **13** with an aqueous 0.1 N HCl solution at room temperature for 15 min, in a biphasic system also containing diethyl ether, led to the generation of the *o*-nitrophenylsulfenyl chloride **6**, which was extracted into the ethereal phase (Scheme 3). The obtained compound was spectroscopically and chromatographically identical to an authentic sample from Acros.



SCHEME 3

Our hypothesis is that the sulfenyl chloride generated from the CA inhibitor reported by us here, reacts with the solvent exposed Cys residues in H^+/K^+-ATP-ase, similarly to the omeprazole intermediates mentioned above, inactivating in this way the enzyme and decreasing the gastric acid secretion. Preliminary data from this laboratory showed that sulfenyl chlorides react readily with cysteine and glutathione in conditions similar to those described above (data not shown; Supuran, CT *et al.*, manuscript in preparation). Combined with the CA inhibitory effect caused by the sulfonamide **8e** generated in the reaction of Scheme 3, such a putative pharmacological agent should constitute a potent gastric acid inhibitor. Work is in progress in this laboratory to evaluate the effect of compounds of the type described in this paper upon H^+/K^+-ATP-ase.

Acknowledgements

This research was financed by the EU grant ERB CIPDCT 940051. Thanks are addressed to Prof. Sven Lindskog, Umea University, Sweden, for the gift of the two plasmids expressing CA I and CA II, to Prof. Anders Liljas and Drs. Anders Svensson and Jukka Vidgren, Lund University, Sweden for the X-ray coordinates of the aminobenzolamide-HCA II adduct.

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