J. Enzyme Inhibition, 1999, Vol. 15, pp. 23–46
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CARBONIC ANHYDRASE INHIBITORS. SYNTHESIS OF TOPICALLY EFFECTIVE INTRAOCULAR PRESSURE LOWERING AGENTS DERIVED FROM 5-(ω-AMINO-ALKYLCARBOXAMIDO)-1,3,4-THIA-DIAZOLE-2-SULFONAMIDE*

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(Received 12 February 1999)

Reaction of the acyl chlorides of phthalimido-glycine or phthalimido-β-alanine with 5-amino-1,3,4-thiadiazole-2-sulfonamide afforded after hydrazinolysis and deprotection of the phthalimido group the corresponding 5-(ω-aminoalkylcarboxamido)-1,3,4-thiadiazole-2-sulfonamides. Reaction of 5-(β-aminoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide with sulfonyl halides or acyl halides afforded a series of compounds possessing β-alkyl/arylsulfonyl/carbonyl-amidoethylcarboxamido moieties in the 5 position of the thiadiazole-2-sulfonamide ring. The new derivatives were efficient inhibitors of three carbonic anhydrase (CA) isozymes, CA I, II (cytosolic forms) and IV (membrane-bound form), but especially against CA II and CA IV (in nanomolar range), the two isozymes known to play an important role in aqueous humor secretion within the ciliary processes of the eye. Some of the synthesized inhibitors possessed good water solubility (as hydrochlorides or sodium salts) and were applied as 2% solutions directly into the eye of normotensive or glaucomatous albino rabbits. Very strong intraocular pressure (IOP) lowering was observed for many of them for prolonged periods of 1–2 h, and the

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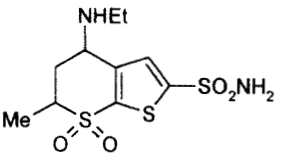
active drug was detected in eye tissues and fluids indicating that the antiglaucoma effect is due to CA inhibition within the eye.

Keywords: Carbonic anhydrase; Heterocyclic sulfonamides; 1,3,4-Thiadiazole-2-sulfonamide; Antiglaucoma drugs; Hydrochloride salts; Sodium salts

INTRODUCTION

Topically acting sulfonamide inhibitors of carbonic anhydrase (CA, EC 4.2.1.1) constitute an important clinical success in the treatment of glaucoma, a condition affecting increasingly large numbers of the aging population.[2–4] Two compounds of this type are presently used clinically, dorzolamide **1** (since 1995 in USA and Europe)[5] and brinzolamide **2**, recently approved for clinical use in USA.[6] The interest in pharmacological agents of this type fostered much synthetic work for the preparation and evaluation as enzyme inhibitors of a large number of aromatic and heterocyclic sulfonamides by our[7–10] and other research groups.[11–17] Although



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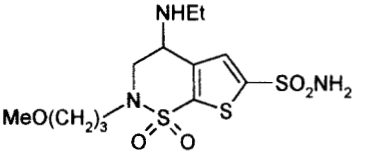

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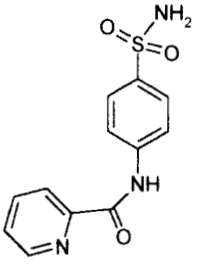

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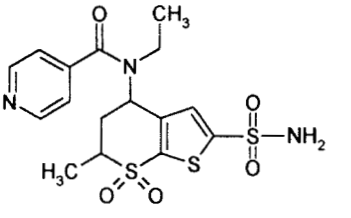

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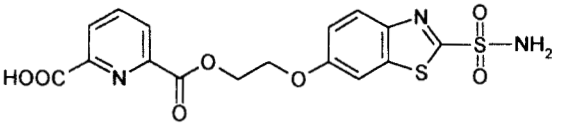

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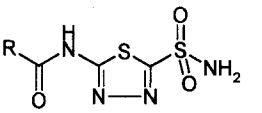
many high-affinity inhibitors were obtained in the previously mentioned works,[7–17] the main draw-back for the design of an efficient, clinically useful topical sulfonamide CA inhibitor was the lack of water solubility of the great majority of the unsubstituted sulfonamides. Thus, the two clinically used compounds, **1** and **2**, are generally employed as hydrochloride salts, but the relative low pH of such solutions led to unpleasant side effects for these drugs, which seem to be more accentuated in the case of dorzolamide than in that of brinzolamide.[18]

A different approach has been recently proposed by our group,[1,19] consisting in attaching water solubilizing moieties, such as pyridine-carboxamido-, pyridine-carboximido- or carboxy-pyridine-carboxamido-, to the classical aromatic/heterocyclic sulfonamide ring systems. The obtained compounds, of the type **3–5** possessed good water solubilities as hydrochloride, triflate or sodium carboxylate salts, with pH values in the range of **6–8**, strongly inhibited several CA isozymes, and acted as powerful intraocular pressure (IOP) lowering agents in experimental models of glaucoma.[1,19] This approach prompted us to examine other possibilities for introducing moieties with buffering properties into the structure of the CA inhibitors, and amino acid derivatives seemed to us among the most promising candidates for this purpose. However, very few such compounds have been reported up to the present time, although three 1,3,4-thiadiazole-2-sulfonamide derivatives of type **6–8** possessing glycylamido-, alanylamido- and phenylalanylamido moieties in the 5 position were reported by Blackburn's group,[17] and the γ-aminovaleric acid derivative **9** was synthesized and evaluated as an IOP lowering drug by Antonaroli *et al.*[20]

In this paper we report the synthesis of two 5-(ω-aminoalkylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide derivatives, namely the glycine derivative **6** as well as the corresponding β-alanyl compound **10**, which were obtained by a procedure alternative to that reported by Blackburn's



6: R = H_2NCH_2
7: R = $CH_3CH(NH_2)$-
8: R = $PhCH_2CH(NH_2)$-
9: R = $H_2N(CH_2)_4$
10: R = $H_2N(CH_2)_2$

group.[17] This included the acylation of 5-amino-1,3,4-thiadiazole-2-sulfonamide with the acyl chlorides of the phthalimido derivatives of the two amino acids, followed by deprotection by means of a hydrazinolysis reaction. Reaction of 5-(β-aminoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide **10** obtained in this way with alkyl/aryl sulfonyl halides or acyl halides afforded then a series of compounds substituted at the ω-aminoalkyl moiety. The new derivaties were assayed for inhibition of three physiologically relevant CA isozymes, i.e., hCA I, hCA II and bCA IV (h = human; b = bovine isozyme). High affinity inhibitors (in the nanomolar range) were found against isozymes II and IV. *In vivo* data, in normotensive and glaucomatous rabbits, proved that some of the most effective *in vitro* inhibitors strongly reduced IOP in the experimental animals, making such compounds attractive candidates for developing new generations of antiglaucoma drugs.

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 Nicolet ZDXFTIR or Perkin-Elmer 16PC FTIR spectrometers and ^{1}H-NMR spectra with a Varian 300CXP or Bruker AC200 spectrometers (200 MHz for ^{1}H and 50.3 MHz for ^{13}C) with DMSO-d_6 as solvent. Chemical shifts are reported as δ values relative to Me_4Si as internal standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and were ±0.4% of the theoretical values.

5-Amino-1,3,4-thiadiazole-2-sulfonamide **11** was obtained from acetazolamide by deacetylation in the presence of concentrated hydrochloric acid.[21a] Acetazolamide, β-alanine, glycine, phthalic anhydride, thionyl chloride and hydrazine hydrate 100% were from E. Merck and were used without further purification. Phthalimido-glycine and phthalimido-β-alanine were prepared by the Gabriel method as reported previously,[21b] whereas the acyl halides **12** and **13** were obtained from the protected amino acid and $SOCl_2$ in refluxing benzene.[21b] Dorzolamide **1** used for the *in vivo* experiments was from Merck, Sharp and Dohme, or was prepared as described in the literature.[22] Alkyl/arylsulfonyl halides, acyl chlorides, sulfonic acid cyclic anhydrides and triethylamine were from Acros, Aldrich or E. Merck. Acetonitrile, acetone (E. Merck) or other solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described

by Forsman *et al.*[23] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[24] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[25] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of $49\,mM^{-1}\cdot cm^{-1}$ for CA I and $54\,mM^{-1}\cdot cm^{-1}$ for CA II, respectively, based on $M_r = 28.85$ kDa for CA I, and 29.30 kDa for CA II, respectively.[26,27] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration has been determined by titration with ethoxzolamide.[28]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically at 400 nm with a Cary 3 instrument interfaced with an IBM compatible PC.[29] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $2\cdot10^{-2}$ and $1\cdot10^{-6}$ M, working at 25°C. A molar absorption coefficient ε of $18{,}400\,M^{-1}\cdot cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis under the conditions of the experiments (pH 7.40), as reported in the literature.[29] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations) 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.[29] Enzyme concentrations were 3.5 nM for hCA II, 12 nM for hCA I and 36 nM for bCA IV (this isozyme has a decreased esterase activity[30] and higher concentrations had to be used for the measurements).

Preparation of 5-(*N*-Phthalimidomethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide 14 and 5-(2-*N*-Phthalimidoethyl-carboxamido)-1,3,4-thiadiazole-2-sulfonamide, 15

To a stirred and cooled (−10°C to −5°C) solution of 5.00 g ($2.77\cdot10^{-2}$ mol) of 5-amino-1,3,4-thiadiazole-2-sulfonamide **11** in 50 ml of dry pyridine, 6.21 g of **12** or 6.59 g of **13** ($2.77\cdot10^{-2}$ mol) dissolved in 25 ml of dry pyridine were added dropwise and the resulting solution stirred overnight and then refluxed for 3 h. The reaction mixture was poured into 250 ml of water and

ice and the white precipitates obtained were filtered, washed with 500 ml aqueous acetic acid solution (50%, v/v) and then with water. The crude products were recrystallized from *i*-PrOH to give 8.67 g ($2.36 \cdot 10^{-2}$ mol, 85%) of **14** or 9.35 g ($2.45 \cdot 10^{-2}$ mol, 88%) of **15** as white solids.

5-(N-Phthalimidomethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide, **14** m.p. 196–198°C (from *i*-PrOH). IR (KBr), cm^{-1}: 1089, 1180, 1198, 1329, 1375, 1536, 1650, 1701, 1770, 2736, 2919, 3000, 3146, 3233, 3323. ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.35 (s, 2H), 7.67–7.71 and 7.79–7.86 (m, 4H), 8.33 (s, 2H), 13.11 (s, 1H). ^{13}C-NMR (DMSO-d_6), δ, ppm: 47.44, 123.00, 131.57, 134.35, 157.84, 167.53, 169.97, 171.62. Found: C, 39.63; H, 2.09; N, 19.43. $C_{12}H_9N_5O_5S_2$ requires: C, 39.24; H, 2.47; N, 19.06%.

5-(2-N-Phthalimidomethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide, **15** m.p. 199–201°C (from *i*-PrOH). IR (KBr), cm^{-1}: 1180, 1198, 1329, 1375, 1536, 1650, 1701, 1770, 2736, 2919, 3000, 3146, 3233, 3323, ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.85–2.91 (t, 2H), 3.92–3.99 (t, 2H), 7.67–7.71 and 7.79–7.83 (m, 4H), 8.33 (s, 2H), 13.11 (s, 1H). ^{13}C-NMR (DMSO-d_6), δ, ppm: 33.58, 33.95, 123.00, 131.57, 134.35, 157.84, 167.53, 169.97, 171.62. Found: C, 40.55; H, 2.99; N, 18.45. $C_{13}H_{11}N_5O_5S_2$ requires: C, 40.94; H, 2.91; N, 18.36%.

Preparation of 5-Aminomethylcarboxamido-1,3,4-thiadiazole-2-sulfonamide, 6 and 5-(2-Aminoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide, 10

Compounds **14** (9.9 g, $2.7 \cdot 10^{-2}$ mol) or **15** (10.3 g, $2.7 \cdot 10^{-2}$ mol) was dissolved in 100 ml of ethanol and 7 ml ($1.4 \cdot 10^{-1}$ mol) of hydrazinium hydroxide was added. The mixture was refluxed for 5 h, and the solvent was evaporated *in vacuo* and 100 ml of *i*-PrOH was added. The precipitated phthalylhydrazide was filtered off and the free amines **6** (5.63 g, 88%) and **10** (5.76 g, 85%) were obtained after evaporation of the solvent (*in vacuo*).

5-Aminomethylcarboxamido-1,3,4-thiadiazole-2-sulfonamide, **6** m.p. 178–180°C (from *i*-PrOH); Ref. 17 cites m.p. 207–209°C (no characterization of the compound was published in Ref. 17). IR (KBr), cm^{-1}: 1180, 1285, 1329, 1375, 1536, 1650, 2736, 2919, 3000, 3146, 3233, 3346. ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.51 (s, 2H), 8.27 (s, 2H), 8.33 (s, 2H), 10.86 (s, 1H). ^{13}C-NMR (DMSO-d_6), δ, ppm: 46.04, 157.84, 164.31, 171.62. Found: C, 20.12; H, 2.99; N, 29.45. $C_4H_7N_5O_3S_2$ requires: C, 20.25; H, 2.97; N, 29.52%.

5-(2-Aminoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide, **10** m.p. 282–284°C (from *i*-PrOH). IR (KBr), cm^{-1}: 1180, 1198, 1375, 1539, 1650, 2910, 3000, 3160, 3238, 3354. ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H),

3.03 (t, 2H), 8.14 (s, 2H), 8.33 (s, 2H), 10.32 (s, 1H). ^{13}C-NMR (DMSO-d_6), δ ppm: 33.01, 34.90, 157.84, 167.82, 171.62. Found: C, 24.22; H, 3.99; N, 18.45. $C_5H_9N_5O_3S_2$ requires: C, 23.90; H, 3.61; N, 27.87%.

General Procedure for the Preparation of Alkyl/Arylsulfonylamido and Acylamido Derivatives, 16–46

An amount of 150 mg (1 mM) of sulfonamide **10** was dissolved/suspended in 50 ml of anhydrous acetonitrile or acetone and then treated with 1 mM of alkyl/arylsulfonyl chloride (method A) or arylsulfonyl fluoride (method B) or sulfonic acid cyclic anhydride (method C) or acyl chloride (method D) dissolved in a small amount (5–10 ml) of the same solvent. The stoichiometric amount (16 µl) of triethylamine was then added (except for method C) and the reaction mixture was magnetically stirred at 4°C for 4–10 h. The conversion of all the sulfonamide to the corresponding sulfonylated/acylated derivatives was monitored by TLC. When the reaction was complete, the mixture was evaporated to a small volume. The concentrated liquor was then poured into 50 ml of cold water, when the reaction products precipitated and were filtered off. The compounds obtained were recrystallized from ethanol or ethanol–water (1 : 1, v/v). Yields were in the range of 50–75%.

5-[2-(N,N-Dimethylaminosulfonylamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **16** as colorless crystals, m.p. 289–290°C; IR (KBr), cm^{-1}: 1140 and 1180 (SO_2^{sym}), 1285 (amide III), 1341 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 4.80 (s, 6H, Me_2N); 8.33 (s, 2H, SO_2NH_2, 8.79 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 23.29; H, 3.61; N, 23.18. $C_7H_{14}N_6O_5S_3$ requires: C, 23.46; H, 3.94; N, 23.45%.

5-[2-(Phenylmethylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **17** as colorless crystals, m.p. 255–256°C. IR (KBr), cm^{-1}: 1170 and 1180 (SO_2^{sym}), 1285 (amide III), 1362 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 3.23 (s, 2H, $PhCH_2$), 7.15–7.59 (m, 5H, ArH from Ph), 8.33 (s, 2H, SO_2NH_2), 9.21 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 35.59; H, 4.03; N, 17.20. $C_{12}H_{15}N_5O_5S_3$ requires: C, 35.55; H, 3.73; N, 17.27%.

5-[2-(Trifluoromethylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **18** as colorless crystals, m.p. 267–268°C (dec.). IR (KBr), cm^{-1}: 1162 and 1180 (SO_2^{sym}), 1285 (amide III), 1347 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 8.33 (s, 2H, SO_2NH_2), 9.85

(s, 1H, SO_2NH); 10.32 (s, 1H, CONH). Found: C, 18.95; H, 1.91; N, 18.12. $C_6H_8F_3N_5O_5S_3$ requires: C, 18.80; H, 2.10; N, 18.27%.

5-[2-(p-Fluorophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **19** as colorless crystals, m.p. 289–290°C. IR (KBr), cm^{-1}: 1165 and 1180 (SO_2^{sym}), 1285 (amide III), 1362 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.11–7.49 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-F-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.30 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 32.43; H, 2.90; N, 16.87. $C_{11}H_{12}FN_5O_5S_3$ requires: C, 32.27; H, 2.95; N, 17.10%.

5-[2-(p-Chlorophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **20** as colorless crystals, m.p. 282–284°C. IR (KBr), cm^{-1}: 1168 and 1180 (SO_2^{sym}), 1285 (amide III), 1363 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.10–7.56 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-Cl-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.30 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 31.19; H, 2.68; N, 16.38. $C_{11}H_{12}ClN_5O_5S_3$ requires: C, 31.02; H, 2.84; N, 16.44%.

5-[2-(p-Bromophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **21** as colorless crystals, m.p. 280–282°C. IR (KBr), cm^{-1}: 1171 and 1180 (SO_2^{sym}), 1285 (amide III), 1366 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.15–7.47 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-Br-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.25 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 28.25; H, 2.64; N, 14.60. $C_{11}H_{12}BrN_5O_5S_3$ requires: C, 28.09; H, 2.57; N, 14.89%.

5-[2-(p-Iodophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **22** as colorless crystals, m.p. 284–285°C. IR (KBr), cm^{-1}: 1180 and 1195 (SO_2^{sym}), 1285 (amide III), 1367 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.10–7.50 (m, AA′BB′, $J_{AB} = 7.5$ Hz, 4H, ArH, *p*-I-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.26 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 25.30; H, 2.52; N, 13.29. $C_{11}H_{12}IN_5O_5S_3$ requires: C, 25.54; H, 2.34; N, 13.54%.

5-[2-(p-Toluenesulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **23** as colorless crystals, m.p. 276–278°C. IR (KBr), cm^{-1}: 1152 and 1180 (SO_2^{sym}), 1285 (amide III), 1350 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me), 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.30–8.10 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-Me-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.21 (s, 1H,

SO_2NH), 10.32 (s, 1H, CONH). Found: C, 35.66; H, 3.95; N, 17.03. $C_{12}H_{15}N_5O_5S_3$ requires: C, 35.55; H, 3.73; N, 17.27%.

5-[2-(p-Nitrophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **24** as yellow crystals, m.p. 268–269°C. IR (KBr), cm^{-1}: 1150 and 1180 (SO_2^{sym}), 1285 (amide III), 1340 (NO_2), 1362 and 1375 (SO_2^{as}), 1510 (NO_2), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.08–7.89 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-O_2N-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.25 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 30.11; H, 2.54; N, 19.20. $C_{11}H_{12}N_6O_7S_3$ requires: C, 30.27; H, 2.77; N, 19.26%.

5-[2-(m-Nitrophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **25** as yellow crystals, m.p. 252–254°C. IR (KBr), cm^{-1}: 1150 and 1180 (SO_2^{sym}), 1285 (amide III), 1340 (NO_2), 1345 and 1375 (SO_2^{as}), 1515 (NO_2), 1536 (amide II), 1650 (amide I), 3065 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.08–7.70 (m, 4H, ArH, *m*-O_2N-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.15 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 30.43; H, 2.75; N, 19.08. $C_{11}H_{12}N_6O_7S_3$ requires: C, 30.27; H, 2.77; N, 19.26%.

5-[2-(o-Nitrophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **26** as yellow crystals, m.p. 281–282°C. IR (KBr), cm^{-1}: 1160 and 1180 (SO_2^{sym}), 1330 (NO_2), 1285 (amide III), 1364 and 1375 (SO_2^{as}), 1510 (NO_2), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.12–7.58 (m, 4H, ArH, *o*-O_2N-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.10 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 30.34; H, 2.97; N, 19.13. $C_{11}H_{12}N_6O_7S_3$ requires: C, 30.27; H, 2.77; N, 19.26%.

5-[2-(3-Nitro-4-chlorophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **27** as yellow crystals, m.p. 247–249°C. IR (KBr), cm^{-1}: 1155 and 1180 (SO_2^{sym}), 1285 (amide III), 1336 (NO_2), 1347 and 1375 (SO_2^{as}), 1510 (NO_2), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.09–7.69 (m, 3H, ArH), 8.33 (s, 2H, SO_2NH_2), 9.35 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 27.96; H, 2.72; N, 17.84. $C_{11}H_{11}ClN_6O_7S_3$ requires: C, 28.06; H, 2.35; N, 17.85%.

5-[2-(p-Acetylaminophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **28** as colorless crystals, m.p. 275–277°C. IR (KBr), cm^{-1}: 1154 and 1180 (SO_2^{sym}), 1285 (amide III), 1354 and 1375 (SO_2^{as}), 1530 and 1536 (amide II), 1650 and 1680 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.80 (s, 3H, Me from Ac), 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 6.21 (s, 1H, AcNH), 7.07–7.80 (m, AA′BB′, $J_{AB} = 7.4$ Hz,

4H, ArH, *p*-AcNH-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.24 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 34.82; H, 3.61; N, 18.69. $C_{13}H_{16}N_6O_6S_3$ requires: C, 34.81; H, 3.60; N, 18.74%.

5-[2-(p-Aminophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **29** as colorless crystals, m.p. 290–291°C (dec.). IR (KBr), cm^{-1}: 1152 and 1180 (SO_2^{sym}), 1285 (amide III), 1351 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH, NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 5.46 (s, 2H, H_2N-phenylene), 7.05–7.65 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-H_2N-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.19 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 32.50; H, 3.45; N, 20.49. $C_{11}H_{14}N_6O_5S_3$ requires: C, 32.51; H, 3.47; N, 20.68%.

5-[2-(m-Aminophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **30** as tan crystals, m.p. 279–280°C. IR (KBr), cm^{-1}: 1164 and 1180 (SO_2^{sym}), 1285 (amide III), 1355 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH, NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 5.22 (s, 2H, H_2N-phenylene), 7.21–7.59 (m, 4H, ArH, *m*-H_2N-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.89 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 32.33; H, 3.68; N, 20.54. $C_{11}H_{14}N_6O_5S_3$ requires: C, 32.51; H, 3.47; N, 20.68%.

5-[2-(o-Carboxyphenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **31** as colorless crystals, m.p. 293–294°C (dec.). IR (KBr), cm^{-1}: 1153 and 1180 (SO_2^{sym}), 1285 (amide III), 1352 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 1720 (COOH), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.15–7.67 (m, 4H, ArH, *o*-HOOC-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.89 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH), 10.39 (br s, 1H, COOH). Found: C, 33.42; H, 2.96; N, 16.02. $C_{12}H_{13}N_5O_7S_3$ requires: C, 33.10; H, 3.01; N, 16.08%.

5-[2-(m-Carboxyphenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **32** as colorless crystals, m.p. >300°C (dec.). IR (KBr), cm^{-1}: 1150 and 1180 (SO_2^{sym}), 1285 (amide III), 1350 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 1724 (COOH), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.09–7.93 (m, 4H, ArH, *m*-HOOC-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.91 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH), 10.53 (br s, 1H, COOH). Found: C, 33.11; H, 3.15; N, 16.01. $C_{12}H_{13}N_5O_7S_3$ requires: C, 33.10; H, 3.01; N, 16.08%.

5-[2-(p-Carboxyphenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **33** as colorless crystals, m.p. >300°C (dec.). IR (KBr), cm^{-1}: 1151 and 1180 (SO_2^{sym}), 1285 (amide III), 1353 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 1720 (COOH), 3060 (NH). ^{1}H-NMR

(DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.10–7.72 (m, 4H, AA'BB', $J_{AB} = 7.7$ Hz, *p*-HOOC-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.83 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH), 10.50 (br s, 1H, COOH). Found: C, 33.03; H, 3.27; N, 15.91. $C_{12}H_{13}N_5O_7S_3$ requires: C, 33.10; H, 3.01; N, 16.08%.

5-[2-(2-Carboxytetrabromophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **34** as colorless crystals, m.p. 239–241°C. IR (KBr), cm^{-1}: 1155 and 1180 (SO_2^{sym}), 1285 (amide III), 1370 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 1720 (COOH), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 8.33 (s, 2H, SO_2NH_2), 9.90 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH), 10.45 (br s, 1H, COOH). Found: C, 19.50; H, 1.43; N, 9.06. $C_{12}H_9Br_4N_5O_7S_3$ requires: C, 19.19; H, 1.21; N, 9.32%.

5-[2-(p-Methoxyphenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **35** as colorless crystals, m.p. 247–248°C. IR (KBr), cm^{-1}: 1163 and 1180 (SO_2^{sym}), 1285 (amide III), 1319 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 3.50 (s, 3H, MeO), 7.10–7.83 (m, AA'BB', $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-MeO-phenylene), 8.33 (s, 2H, SO_2NH_2), 9.12 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 34.56; H, 3.35; N, 16.57. $C_{12}H_{15}N_5O_6S_3$ requires: C, 34.20; H, 3.59; N, 16.62%.

5-[2-(2,4,6-Trimethylphenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **36** as colorless crystals, m.p. 223–225°C. IR (KBr), cm^{-1}: 1164 and 1180 (SO_2^{sym}), 1285 (amide III), 1324 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, 4-Me), 2.75 (s, 6H, 2-Me_2), 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.10–7.85 (m, 2H, ArH), 8.33 (s, 2H, SO_2NH_2), 9.09 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 38.64; H, 4.17; N, 16.08. $C_{14}H_{19}N_5O_5S_3$ requires: C, 38.79; H, 4.42; N, 16.15%.

5-[2-(4-Methoxy-3-aminophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **37** as colorless crystals, m.p. 273–274°C. IR (KBr), cm^{-1}: 1160 and 1180 (SO_2^{sym}), 1285 (amide III), 1339 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH), 3300 br (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 3.50 (s, 3H, MeO), 5.22 (s, 2H, H_2N-phenylene), 7.20–7.64 (m, 3H, ArH, trisubstituted-phenyl), 8.33 (s, 2H, SO_2NH_2), 9.11 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 32.98; H, 3.53; N, 19.15. $C_{12}H_{16}N_6O_6S_3$ requires: C, 33.02; H, 3.69; N, 19.25%.

5-[2-(2-Hydroxy-3,5-dichlorophenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **38** as tan crystals, m.p. 231–233°C. IR (KBr),

cm^{-1}: 1143 and 1180 (SO_2^{sym}), 1285 (amide III), 1336 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH + OH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 6.22 (br s, 1H, HO), 7.35 (s, 1H, ArH, 4H), 7.80 (s, 1H, ArH, 6H), 8.33 (s, 2H, SO_2NH_2), 9.27 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 27.56; H, 2.33; N, 14.48. $C_{11}H_{11}Cl_2N_5O_6S_3$ requires: C, 27.74; H, 2.33; N, 14.70%.

5-[2-(p-Dimethylaminophenylazo-4-phenylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **39** as yellow crystals, m.p. 232–234°C. IR (KBr), cm^{-1}: 1160 and 1180 (SO_2^{sym}), 1285 (amide III), 1318 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 3.10 (s, 6H, Me_2N), 7.08–8.10 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 8H, ArH), 8.33 (s, 2H, SO_2NH_2), 9.15 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 42.56; H, 4.50; N, 20.75. $C_{19}H_{22}N_8O_5S_3$ requires: C, 42.37; H, 4.12; N, 20.80%.

5-[2-(5-Dimethylamino-1-naphthalenesulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **40** as yellow crystals, m.p. 254–257°C. IR (KBr), cm^{-1}: 1167 and 1180 (SO_2^{sym}), 1285 (amide III), 1325 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 3.14 (s, 6H, Me_2N), 7.66–7.80 (m, 4H, H^2, H^3, H^6, H^7 from naphthalene), 7.96 (d, 1H, H^4 from the substituted naphthyl), 8.15 (m, 1H, H^8 from the substituted naphthyl), 8.33 (s, 2H, SO_2NH_2), 9.20 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 42.40; H, 4.23; N, 17.12. $C_{17}H_{20}N_6O_5S_3$ requires: C, 42.14; H, 4.16; N, 17.34%.

5-[2-(1-Naphthalenesulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **41** as white crystals, m.p. 271–272°C. IR (KBr), cm^{-1}: 1161 and 1180 (SO_2^{sym}), 1285 (amide III), 1357 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.66–7.79 (m, 4H, H^2, H^3, H^6, H^7 from naphthalene), 7.94 (m, 1H, H^5 or H^8 from the substituted naphthyl), 7.98 (d, 1H, H^4 from the substituted naphthyl), 8.16 (m, 1H, H^5 or H^8 from the substituted naphthyl), 8.33 (s, 2H, SO_2NH_2), 9.20 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 40.69; H, 3.30; N, 15.79. $C_{15}H_{15}N_5O_5S_3$ requires: C, 40.81; H, 3.42; N, 15.86%.

5-[2-(2-Naphthalenesulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **42** as white crystals, m.p. 269–270°C. IR (KBr), cm^{-1}: 1158 and 1180 (SO_2^{sym}), 1285 (amide III), 1360 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.66–7.74 (m, 2H, H^6, H^7 from naphthalene), 7.94 (m, 1H, H^5 or H^8 from the substituted naphthyl), 7.96 (d, 1H, H^3 or H^4

from the substituted naphthyl), 8.20 (d, 1H, H^4 or H^3 from the substituted naphthyl), 8.33 (s, 1H, H^1 of the substituted naphthyl), 8.35 (dd, $J = 9.7$ Hz, 2.3, 1H, H^8 or H^5 from the substituted naphthyl), 8.33 (s, 2H, SO_2NH_2), 9.21 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 40.98; H, 3.35; N, 15.76. $C_{15}H_{15}N_5O_5S_3$ requires: C, 40.81; H, 3.42; N, 15.86%.

5-[2-(2-Thienylsulfonamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **43** as colorless crystals, m.p. > 300°C. IR (KBr), cm^{-1}: 1152 and 1180 (SO_2^{sym}), 1285 (amide III), 1350 and 1375 (SO_2^{as}), 1536 (amide II), 1650 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 6.28 (dd, $J = 3.8$ Hz, $J' = 2.5$ Hz, 1H, from thienyl), 6.88 (dd, $J = 3.8$ Hz, $J' = 1.8$ Hz, 1H, from thienyl), 7.16 (dd, $J = 2.5$ Hz, $J' = 1.8$ Hz, 1H, from thienyl), 8.33 (s, 2H, SO_2NH_2), 9.76 (s, 1H, SO_2NH), 10.32 (s, 1H, CONH). Found: C, 27.09; H, 2.46; N, 17.66. $C_9H_{11}N_5O_5S_4$ requires: C, 27.20; H, 2.79; N, 17.62%.

5-[2-(2,4-Dichlorobenzoylamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **44** as white crystals, m.p. 250–252°C. IR (KBr), cm^{-1}: 1180 (SO_2^{sym}), 1285 (amide III), 1375 (SO_2^{as}), 1536 and 1550 (amide II), 1650 and 1680 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.05–7.64 (m, 3H, ArH), 8.33 (s, 2H, SO_2NH^2), 9.50 (s, 1H, ArCONH), 10.32 (s, 1H, CH_2CONH). Found: C, 33.70; H, 2.45; N, 16.47. $C_{12}H_{11}Cl_2N_5O_4S_2$ requires: C, 33.97; H, 2.61 N, 16.51%.

5-[2-(2-Thienylcarboxamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **45** as colorless crystals, m.p. 278–279°C. IR (KBr), cm^{-1}: 1180 (SO_2^{sym}), 1280 and 1285 (amide III), 1375 (SO_2^{as}), 1525 and 1540 (amide II), 1650 and 1675 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 6.20 (dd, $J = 3.7$ Hz, $J' = 2.4$ Hz, 1H, from thienyl), 6.85 (dd, $J = 3.7$ Hz, $J' = 1.8$ Hz, 1H, from thienyl), 7.14 (dd, $J = 2.4$ Hz, $J' = 1.8$ Hz, 1H, from thienyl), 8.24 (s, 1H, thienylCONH), 8.33 (s, 2H, SO_2NH_2), 10.32 (s, 1H, CH_2CONH). Found: C, 33.45; H, 2.79; N, 19.23. $C_{10}H_{11}N_5O_4S_3$ requires: C, 33.23; H, 3.07; N, 19.38%.

5-[2-(N,N-Diphenylaminocarboxamido)-ethylcarboxamido]-1,3,4-thiadiazole-2-sulfonamide, **46** as colorless crystals, m.p. 234–235°C. IR (KBr), cm^{-1}: 1180 (SO_2^{sym}), 1274 and 1285 (amide III), 1375 (SO_2^{as}), 1535 and 1540 (amide II), 1650 and 1670 (amide I), 3060 (NH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.77 (t, 2H, CH_2CO), 3.03 (t, 2H, NCH_2), 7.20–7.85 (m, 10 H, 2 Ph), 7.87 (s, 1H, Ph_2NCONH), 8.33 (s, 2H, SO_2NH_2), 10.32 (s, 1H, CH_2CONH). Found: C, 48.56; H, 4.30; N, 18.57. $C_{18}H_{18}N_6O_4S_2$ requires: C, 48.42; H, 4.06; N, 18.82%.

Measurement of Tonometric IOP

Adult male New Zealand albino rabbits weighting 3–3.5 kg were used in the experiments. Three animals were used for each inhibitor studied. The experimental procedures conform to those 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 : 12 h light/dark cycle in a temperature controlled room, at 22–26°C. Solutions of inhibitors (2% as hydrochlorides, by weight) were obtained in distilled-deionized water. The pH of these solutions was around pH 5.50–8.00.

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA, USA) as described by Maren' group.[31,32] 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 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.[31,32] All data are expressed as mean ± SE, using a one-tailed *t* test. Ocular hypertension was elicited in the albino rabbits by injection of α-chymotrypsin (from Sigma) into the posterior chamber of the eye, as described by Sugrue *et al.*[32b]

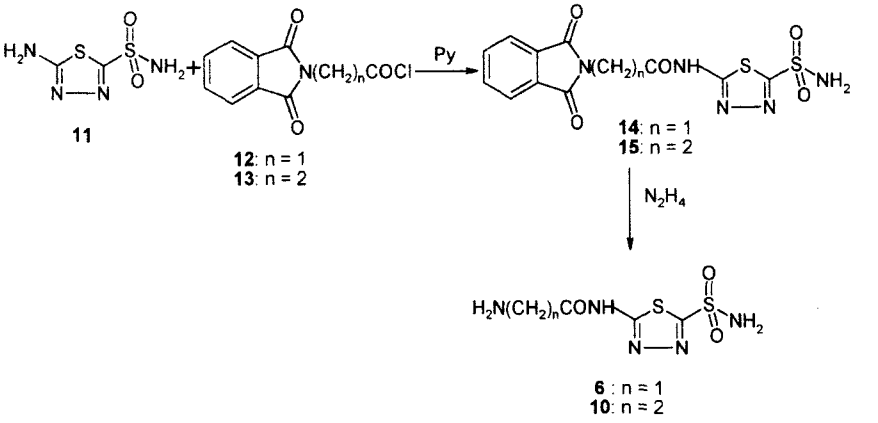
Drug Distribution in Ocular Fluids and Tissues

The general procedure of Maren's group has been followed.[31,32] The animals were killed with an intracardiac injection. Aqueous humor (both posterior and anterior chamber fluids) was withdrawn, then the cornea and anterior uvea (iris plus attached ciliary body) were dissected, rinsed well with water, blotted, weighed and put into 1–2 ml of water. For isolation of the ciliary processes, intact anterior uvea rings were placed on a parafilm covered piece of polystyrene foam in a Petri dish. The tissue was wetted with

normal saline and dissected under a microscope, when ciliary processes were liberated from their attachment to the iris, cut, weighed and put in 0.5 ml of distilled water. The tissue from 4 eyes (average weight of 8 mg/eye) was pooled for drug analysis. Samples were bioled for 5 min (in order to denature CA, and free drug from the E–I complex), diluted and then incubated with a known amount of enzyme. The activity of the free enzyme and in the presence of the inhibitor were determined as described above. A calibration curve has been used in order to determine the fractional inhibition in the different tissues, as described in refs. 31,32 Alternatively, the amount of inhibitor in the eye tissues was also determined by an HPLC method, as reported previously by us.[7a]

RESULTS AND DISCUSSION

The starting materials in the preparation of the new 1,3,4-thiadiazole derivatives included glycine and β-alanine, which were converted to the corresponding *N*-phthalimido derivatives by reaction with phthalic anhydride in refluxing toluene.[21b] The corresponding acyl chlorides **12** and **13** were then obtained by reaction of the above-mentioned *N*-protected amino acids with $SOCl_2$ in benzene. Acylation of 5-amino-1,3,4-thiadiazole-2-sulfonamide **11** with **12** and **13** in the presence of pyridine afforded the new compounds **14** and **15** respectively. Deprotection of the *N*-phthalimido group by hydrazinolysis afforded then compounds possessing free amino groups **6** and **10** (Scheme 1). It should be noted that our synthetic strategy is completely different from that of Blackburn *et al.*[17] or Antonaroli *et al.*[20] who reported the only other four amino acid derivatives of 1,3,4-thiadiazole-2-sulfonamide, of type **6–9**, mentioned in the introductory section. A special mention must be made regarding **6**, which was prepared by the first group[17] from the *N*-carbobenzyloxycarbonyl derivative of glycine (Boc-Gly) by condensation with **11** in the presence of isobutyl chloroformate. The protecting group could not then be removed by catalytic hydrogenolysis but it seems that it was removed by treatment with hydrogen bromide in glacial acetic acid. This method has several disadvantages compared to our method: (i) the isobutyl chloroformate also participates in the acylation of amine **11**, and appreciable amounts of 5-isobutyloxycarboxamido-1,3,4-thiadiazole-2-sulfonamide were formed as secondary product,[17] (ii) the deprotection of the Boc-derivative of **6** is a complicated process, and the strong acidic medium in which it is performed might also hydrolyze the acyl bond between the thiadiazole ring and the aminoacyl moiety.[21] Our strategy shown in



SCHEME 1 Synthesis of compounds **6** and **10**.

Scheme 1 avoids these complications, whereas hydrazinolysis used to remove the phthalimido protecting group did not affect the bond between the thiadiazole ring and the aminoacyl moiety, which is very sensitive to acid hydrolysis, but is stable in the presence of bases even during prolonged heating.[21a]

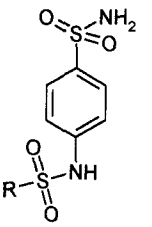
In the IR spectra of the prepared derivatives **12–15** the following main features were observed: (i) the strong –COCl vibration at $\nu = 1769\,cm^{-1}$ in the acyl chlorides **12** and **13**; (ii) the strong –CO–N–CO– vibrations at $\nu = 1701$ and $1770\,cm^{-1}$ in the compounds possessing the *N*-phthalimido protecting groups (**14**, **15**); (iii) the presence of amino bands at $\nu = 3346\,cm^{-1}$ superposed with the sulfonamido vibration bands at $\nu = 3233$ and $3323\,cm^{-1}$ in the IR spectra of compounds **6** and **10**; (iv) the presence of the intense amide bands at $\nu = 3000$, 1650 and $1536\,cm^{-1}$ in the IR spectra of compounds **6** and **10**; (v) the very intense sulfonamido vibrations at 1180 and $1375\,cm^{-1}$ in the IR spectra of compounds **6** and **10**.

In the ^{1}H-NMR spectra of the pre0pared derivatives the following main features were evidenced: (i) the ethylenic protons of β-alanine derivatives **13**, **15**, **10** showed important variations in the different compounds obtained. Thus the CH_2CO protons resonated as a triplet at $\delta = 2.72–2.79$ in the acyl chloride **13**, whereas in the amide derivative **15**, they were shifted to $\delta = 2.85–2.91$ ppm. The CH_2N protons were seen as a triplet at $\delta = 3.92–3.99$ ppm, whereas in the deprotected amino derivative **10**, they appeared at $\delta = 3.02–3.04$ ppm; (ii) the methylenic protons of the glycine derivatives **12**, **14**, **6**, appeared as a singlet at $\delta = 4.02$ in the acyl chloride **12**, at $\delta = 3.35$ in the *N*-protected derivative **14** and at $\delta = 2.51$ in the amino derivative **6**; (iii) the aromatic protons of the phthalimido group appeared as multiplets at

$\delta = 7.67-7.71$ and $\delta = 7.79-7.83$ in the *N*-protected derivatives **12–15**; (iv) the sulfonamide protons H_2NSO_2- resonated as a sharp singlet at $\delta = 8.06$ ppm for the starting derivative **11** and were shifted to $\delta = 8.33$ ppm for the functionalized derivatives **14**, **15**, **6** and **10**; (v) the amide proton $-HN-CO$ gave rise to a sharp singlet at $\delta = 13.11$ ppm for the *N*-protected derivatives **14**, **15** and at $\delta = 10.86$ and $\delta = 10.32$ for the deprotected amino derivatives **6** and **10**, respectively; (vi) after hydrazinolysis the amino protons $-NH_2$ appeared as a singlet at $\delta = 8.27$ (**6**) and $\delta = 8.14$ (**10**). In the ^{13}C-NMR spectra of the 1,3,4-thiadiazole derivatives **1** and **14**, **15**, **6** and **10** the thiadiazole carbons appeared at $\delta = 157.84$ ($C-NH_2$) and $\delta = 171.62$ ppm (CSO_2NH_2) and did not vary in the diversely substituted compounds reported here. The amide carbon (*C*ONH) resonated at $\delta = 169.96$ ppm for the compounds **14** and **15**, whereas it was shifted to $\delta = 164.31$ ppm for the derivative **6** and to $\delta = 167.82$ ppm for derivative **10**.

It has recently been reported by this group[33–36] that alkyl/arylsulfonylamido derivatives of aromatic/heterocyclic sulfonamides of type **47**, **48** act as very strong inhibitors of several CA isozymes, their potency increasing with the length of the R moiety of the secondary sulfonamido group. This finding was later explained theoretically by means of two QSAR studies of such derivatives.[35,36] It thus appeared of interest to try to derivatize some of the amino acid derivatives of 1,3,4-thiadiazole-2-sulfonamide reported here, of types **6** or **10**, by the same approach. Finally, compound **10** has been chosen for further derivatization as it is 3-fold more potent as a hCA II inhibitor than **6** (see later than discussion on inhibition data). Thus, reaction of 5-(β-aminoethylcarboxamido)-1,3,4-thiadiazole-2-sulfonamide **10** with alkyl/arylsulfonyl halides, sulfobenzonic cyclic anhydrides or acyl chlorides afforded the new compounds **16–46** shown in Table I.

The sulfonylation/acylation reaction of **10** was best achieved in acetone or acetonitrile as solvents, in the presence of triethylamine as base, similar to the corresponding reactions of the aromatic sulfonamides such as



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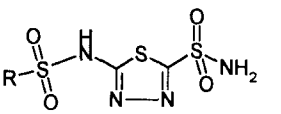

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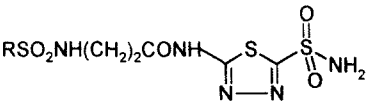
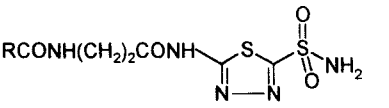
sulfanilamide, homosulfanilamide, etc., leading to derivatives of type **47** previously reported by this group.[35,36] However, Schotten–Baumann conditions also generally led to good yields of the desired products **16–46**.[33] Derivatives **16–46** prepared by the above-mentioned procedure contained alkylsulfonyl-, dimethylaminosulfonyl- or halogeno-, alkyl-, methoxy-, amino-, carboxy- and nitro- substituted-phenylsulfonyl moieties, and were chosen in order to obtain derivates with different physico-chemical

TABLE I Synthesized β-alkyl/arylsulfonylamidoethylcarboxamido **16–43** and β-acylamidoethyl-carboxamido derivatives **44–46**



16–43



44–46

Compound	*R*	*Synthesis method*	*Yield*
16	Me_2N-	A	53
17	$PhCH_2-$	B	69
18	CF_3-	A	50
19	*p*-F–C_6H_4-	A	64
20	*p*-Cl–C_6H_4-	A	70
21	*p*-Br–C_6H_4-	A	72
22	*p*-I–C_6H_4-	A	75
23	*p*-CH_3–C_6H_4-	A	61
24	*p*-O_2N–C_6H_4-	A	57
25	*m*-O_2N–C_6H_4-	A	60
26	*o*-O_2N–C_6H_4-	A	54
27	4-Cl-3-O_2N–C_6H_3-	A	62
28	*p*-AcNH–C_6H_4-	A	74
29	*p*-H_2N–C_6H_4-	B	71
30	*m*-H_2N–C_6H_4-	B	66
31	*o*-HOOC–C_6H_4-	C	75
32	*m*-HOOC–C_6H_4-	A	51
33	*p*-HOOC–C_6H_4-	A	55
34	*o*HOOC–C_6Br_4-	C	75
35	*p*-CH_3O–C_6H_4-	A	55
36	2,4,6-$(CH_3)_3$–C_6H_2-	A	50
37	4-CH_3O-3-H_2N–C_6H_3-	A	56
38	2-HO-3, 5-Cl_2–C_6H_2-	A	54
39	4-Me_2N–C_6H_4–N=N–C_6H_4-	A	71
40	5-Dimethylamino-1-naphthyl-	A	62
41	1-Naphthyl	A	70
42	2-Naphthyl	A	80
43	2-Thienyl	A	75
44	2,4-$Cl_2C_6H_3-$	D	69
45	2-Thienyl	D	70
46	Ph_2N	D	62

A – **10** + RSO_2Cl; B – **10** + RSO_2F; C – **10** + sulfobenzoic cyclic anhydride; D – **10** + RCOCl.

properties (i.e., enhanced lipophilicity or conversely, enhanced water solubility, etc.) but also groups that would allow an easy derivatization, which might be important for the IOP lowering properties of such enzyme inhibitors. Thus, groups that would allow formation of water-soluble salts, such as hydrochlorides (in derivatives of type **29**, **30**, **37**) or sodium salts (of the RSO_2NH or COOH moieties, in derivatives such as **24–28**; **31–34**, etc) were obtained, in order to prepare their water-soluble sulfonamide CA inbibitors used for the *in vivo* experiments. Such salts have been obtained generally *in situ*, by adding the required amount of acid (HCl) or base (NaOH) solution to a water suspension of the desired inhibitor. The pH of such solutions was then brought to pH 5.0–8.5, in order to avoid undesired topical side effects due to the extreme acidity/basicity of the employed inhibitor solution.

Inhibition data with sulfonamides **6–46** and standard CA inhibitors (**1** and **2**) against isozymes I, II and IV, are shown in Table II.

An interesting finding which emerged from the above data was that the β-aminoethylcarboxamido derivative **10** is 3-fold more efficient as a CA II inhibitor than the glycine derivative **6**, and in turn, these two compounds are both much more active compared to the γ-aminovaleric acid derivative **9** reported by Antonaroli *et al.*[20] Against the other two isozymes investigated the differences between **6** and **10** are not so important, although the former compound is slightly less active than the latter. The two phthalimido derivatives **14** and **15** are, on the other hand, less active than the corresponding unprotected compounds **6** and **10**, respectively. As seen from data in Table II, it is clear that the whole class of 5-alkyl/arylsulfonylamidoethylcarboxamido-1,3,4-thiadizole-2-sulfonamides are very potent inhibitors of all three CA isozymes investigated. The most susceptible isozyme to inhibition was again hCA II, followed by bCA IV and then hCA I. However, in contrast to dorzolamide **1**, the compounds reported by us have a much greater affinity for hCA I. Very good inhibition profiles, with affinities in the nanomolar range were observed both for hCA II and bCA IV, but without reaching any isozyme-selectivity as reported previously by us for some other classes of inhibitors. Some degree of selectivity is however observed.[7a,8] Still in the case of CA IV, some kind of specificity might be expected *in vivo*, since at physiological pH values, these inhibitors should be highly dissociated (due to the relatively low pK_a value of the RSO_2NH moiety)[33] and thus membrane-impermeable when they would interfere principally with the membrane-bound isozyme, CA IV. Substitution patterns leading to very powerful inhibitors included such moieties as acetamido, nitro, halogeno, amino and carboxy attached to the phenyl group of these molecules. Obviously, these groups participate in supplementary interactions

with amino acid residues at the entrance of the enzyme active site assuring an enhanced stability to the enzyme–inhibitor adducts.

The compounds selected for *in vivo* studies were among the most active *in vitro* inhibitors against hCA II and bCA IV in the prepared series i.e. **10**, **24**, **28**, **30**, **37** and **44**. The data of Table III shows that some of the new

TABLE II CA inhibition data for the standard inhibitors **1–2**, and the new derivatives **6–46** against isozymes I, II and IV

Inhibitor	K_I^* (nM)		
	hCA I[a]	*hCA II*[a]	*bCA IV*[b]
Dorzolamide **1**	50000	9	45
Brinzolamide[c] **2**	—	3.2	45.3
6	460	10	155
9[d]	—	440	—
10	455	3.0	125
14	540	20	220
15	525	10	200
16	420	3.1	115
17	325	2.0	109
18	210	2.3	120
19	300	1.9	110
20	300	1.8	120
21	280	1.4	106
22	260	1.2	121
23	410	2.8	105
24	125	0.75	100
25	170	1.6	98
26	210	1.9	107
27	190	1.9	109
28	155	0.8	90
29	250	1.7	95
30	240	2.5	69
31	180	1.1	45
32	170	0.9	70
33	200	0.8	66
34	150	1.3	105
35	230	3.0	120
36	320	4.5	109
37	100	2.0	58
38	440	3.1	66
39	390	2.4	75
40	375	3.2	77
41	350	3.9	82
42	400	3.0	73
43	320	1.4	59
44	230	2.0	62
45	250	3.0	100
46	390	3.3	123

*Error for the determination of K_I values was of 5–10% (from 2 different assays);
[a]Human (cloned) isozyme.
[b]Isolated from bovine lung microsomes.
[c]Data from Ref. 6b
[d]Data from Ref. 20

compounds assayed *in vivo*, such as **24**, **28**, and **44**, showed IOP lowering effects generally of the same order of magnitude as those of dorzolamide **1**. Thus, after half or one hour, these were around 2.2–3.8 and 4.0–5.5 mmHg, respectively, both for dorzolamide as well as for the new derivatives. An important difference between the two groups of drugs appeared at longer periods after the administration since unlike dorzolamide, where it action diminishes to an IOP lowering of 2.7 mmHg after 90 min, the new compounds maintained a much more effective IOP lowering i.e. 6.0–6.6 mmHg. A second group of inhibitors, such as **10**, **30** and **37**, showed much more effective IOP lowering effects compared to dorzolamide **1**, both after 30 min from the administration of the inhibitor within the rabbit eye, as well as at longer times (1, 1.5 and 2 h respectively). Thus, after 30 min, the IOP lowering was in the range of 4.3–4.5 mmHg with the new compounds mentioned above and only 2.2 mmHg with dorzolamide. One hour after administration the new compounds generally were at least twice doubly as

TABLE III Fall of IOP of normotensive rabbits (initial IOP of 23 ± 3 mmHg), after treatment with one drop (50 μL) of a 2% solution of CA inhibitor (as hydrochloride or sodium salt, at the pH value shown) directly into the eye, at 30, 60 and 90 min after administration

Inhibitor	*pH*	ΔIOP (mmHg)*			
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
1[a] (dorzolamide)	5.5	0	2.2 ± 0.10	4.1 ± 0.15	2.7 ± 0.08
10[a]	7.0	0	4.5 ± 0.14	10.1 ± 0.15	7.9 ± 0.13
24[b]	7.5	0	2.3 ± 0.11	4.0 ± 0.20	6.4 ± 0.17
28[b]	9.0	0	3.8 ± 0.13	5.5 ± 0.13	6.0 ± 0.10
30[a]	6.0	0	4.3 ± 0.10	9.5 ± 0.12	8.0 ± 0.12
31[b]	8.0	0	4.4 ± 0.12	10.5 ± 0.10	4.5 ± 0.15
37[a]	5.5	0	4.4 ± 0.05	8.1 ± 0.10	8.5 ± 0.08
44[b]	8.5	0	2.5 ± 0.06	4.0 ± 0.09	6.6 ± 0.11

*$\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; Mean ± average spread ($n=3$).
[a]As hydrochloride. [b]As sodium salt.

TABLE IV Fall of IOP of glaucomatous rabbits, after treatment with one drop (50 μl) of a 2% solution of CA inhibitor (as hydrochloride salt, at the pH value shown) directly into the eye, at 30, 60 and 90 min after administration. Initial IOP of untreated eyes was in the range of 32–36 mmHg

Inhibitor	*pH*	ΔIOP (mmHg)*			
		$t=0$	$t=30$ min	$t=60$ min	$t=90$ min
10[a]	6.0	0	9.5 ± 0.14	19.0 ± 0.20	11.4 ± 0.15
30[a]	6.0	0	7.9 ± 0.10	15.3 ± 0.15	16.0 ± 0.12
37[a]	6.0	0	7.8 ± 0.10	10.3 ± 0.09	11.5 ± 0.15
44[b]	8.5	0	7.0 ± 0.13	12.7 ± 0.12	10.6 ± 0.11

*$\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; Mean ± average spread ($n=3$).
[a]As hydrochloride. [b]As sodium salt.

the clinically used drug **1** (8.1–10.1 mmHg for the new derivatives, versus 4.1 mmHg for dorzolamide) and this strong effect was maintained after another half an hour (whereas it was halved for **1**, where the pressure decreased to 2.7 mmHg after 90 min). Both cationic compounds (hydrochlorides, such as **10**, **30**, **37**), as well as anionic derivatives (as sodium salts, such as **24**, **28** or **44**), were equally active as IOP lowering agents. Sulfonamido-sulfonamides as well as carboxamido-sulfonamides possessed similar IOP lowering properties.

The above findings also applied to the glaucomatous rabbits experiments (Table IV) but the IOPs were much more enhanced as compared to those for normotensive rabbits. Thus, IOP reductions of around 7.8–9.5 mmHg were generally observed after 30 min whereas at longer periods these increased to 10–19 mmHg. No important differences between the cationic and anionic inhibitors were observed. The pH of the solutions used in these experiments varied between pH 5.5 and 9.0, and was primarily the pH obtained by dissolving the corresponding compounds in the required amount of water. It is well-known that pH may influence the ocular penetration of the drugs,[31,32] but this influence is relatively small (0.5–0.8 mmHg)[31,32] as compared to the IOP lowering due to the CA inhibition *per se*. Thus, we did not consider it necessary to bring all media to the same pH value in this preliminary (non-pharmacological) study. The detailed pharmacological profile of this new class of IOP lowering agents will be published elsewhere.

Table V shows *ex vivo* data obtained in normotensive rabbits after the topical administration of two of the most active topical inhibitors in the prepared series, i.e., compounds **10** and **30**. It can be observed that at one and two hours after topical administration of the drug, high levels of inhibitors were found in the cornea, aqueous humor and ciliary processes. Based on the inhibition constant of these compound (3 nM for CA II for **10**, and 2.5 nM for CA II for **30**, respectively), the fractional inhibition estimated in

TABLE V Ocular tissue drug concentrations (μM) after one and two hours following corneal application of one drop (50 μl) of a 2% solution of compounds **10** and **30** (as hydrochlorides) in normotensive albino rabbits

Inhibitor	*Time* (h)	*Drug concentration* (μM)*		
		Cornea	*Aqueous humor*	*Ciliary process*
10	1	150 ± 5	275 ± 10	56 ± 3
(HCl)	2	49 ± 3	44 ± 3	17 ± 1
30	1	141 ± 4	266 ± 8	49 ± 2
(HCl)	2	47 ± 4	40 ± 2	16 ± 2

*Mean ± standard deviation ($n = 3$).

these tissues/fluids is of 99.5–99.9%, indicating the fact that the IOP decrease is indeed due to CA inhibition.

Acknowledgments

This research was financed in part by the EU grant ERB CIPDCT 940051.

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