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CARBONIC ANHYDRASE INHIBITORS. WATER-SOLUBLE, TOPICALLY EFFECTIVE INTRAOCULAR PRESSURE LOWERING AGENTS DERIVED FROM ISONICOTINIC ACID AND AROMATIC/HETEROCYCLIC SULFONAMIDES: IS THE TAIL MORE IMPORTANT THAN THE RING?*

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Reaction of twenty aromatic/heterocyclic sulfonamides containing a free amino, imino, hydrazino or hydroxyl group, with isonicotinoyl chloride afforded a series of water-soluble compounds (as hydrochloride or triflate salts). The new derivatives were examined as inhibitors of three carbonic anhydrase (CA) isozymes, CA I, II (cytosolic forms) and IV (membrane-bound form). Efficient inhibition was observed against all three isozymes, but especially against CA II and CA IV (K_I in the nanomolar range), the two isozymes known to play a critical role in aqueous humor secretion within the ciliary processes of the eye. Some of the most potent inhibitors synthesized were applied as 2% water solutions directly into the eye of normotensive or glaucomatous albino rabbits. Very strong intraocular pressure (IOP) lowering was observed for many of them, and the active drug was detected in eye tissues and fluids. According to others the IOP lowering effect of topically effective antiglaucoma sulfonamides is due to the intrinsic nature of the specific heterocyclic sulfonamide considered, among which the thienothiopyran-2-sulfonamide derivatives represent the best studied case e.g. dorzolamide. In order to prove that the tail (in this case the isonicotinoyl moiety) conferring water solubility to a sulfonamide CA inhibitor is more important than the ring to which the sulfonamido group is grafted a

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dorzolamide derivative to which the isonicotinoyl moiety was attached was also prepared. This new compound is more water soluble than dorzolamide (as hydrochloride salt), behaves as a strong CA II inhibitor and acts similarly to the parent derivative in lowering IOP in experimental animals. Thus, it seems that the tail conferring water solubility is more important for topical activity as an antiglaucoma drug than the heterocyclic/aromatic ring to which the sulfonamido moiety is attached.

Keywords: Carbonic anhydrase; Aromatic; Heterocyclic sulfonamides; Isonicotinoyl chloride; Antiglaucoma drugs; Dorzolamide

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INTRODUCTION

The sulfonamides represent an important class of biologically active compounds, with at least five different classes of pharmacological agents that have been obtained from the sulfanilamide structure as lead, the derivative initially studied by Domagk[2] as the first modern chemotherapeutic drug. Indeed, the antibacterial sulfonamides[3] continue to play an important role in chemotherapy, alone or in combination with other drugs.[4] The sulfonamides that inhibit the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) possess many applications as diuretic, antiglaucoma or antiepileptic drug among others.[5-7] The hypoglycemic sulfonamides are extensively used in the treatment of some forms of diabetes,[8] whereas the thiazides and high-ceiling diuretics might be considered as a fortunate development of the CA inhibitors[9] although these compounds possess a different pharmacological profile, independent of CA inhibition.[10,11] Finally, some antithyroid drugs have also been developed commencing from the sulfonamide structure as lead molecule.[12]

The second class of the above mentioned pharmacological agents, i.e., the sulfonamides with CA inhibitory action, have thoroughly been investigated in the last 10 years, mainly in the search of a topically effective antiglaucoma drug.[13-19] The possibility of administering a sulfonamide via the topical route directly into the eye, although investigated in the 1950s,[20,21] has been totally unsuccessful, whereas the systemic administration, quite useful in lowering intraocular pressure (IOP), was generally accompanied by undesired side effects due to CA inhibition in other tissues than the eye.[21] In 1983, Maren's group[13] postulated that a water-soluble sulfonamide, also possessing a relatively balanced lipid solubility, would be an effective IOP lowering drug via the topical route. Inhibitors possessing such physicochemical properties were not known then but they started to be developed in several laboratories soon thereafter[13-19] and in 1995 the first such pharmacological agent, dorzolamide **1**, entered clinical use in USA and Europe.[22]

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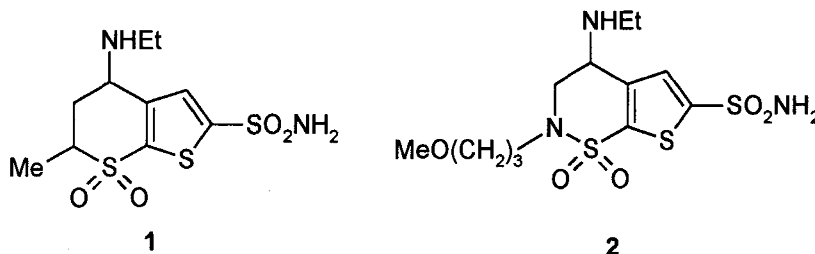
A second compound, brinzolamide **2**, quite structurally similar to dorzolamide has also recently been approved topical treatment of glaucoma in USA.[23]



Thus, in a series of very interesting papers,[15,24–29] the Merck, Sharp and Dohme group has described the synthesis of a large series of generally bicyclic heterocyclic sulfonamides (derivatives of benzothiazole-,[24] benzofuran-,[25] indole-,[26] benzo[*b*]-thiophene-,[27,28] thienothiopyran,[15,29] etc), which were tested as IOP lowering agents, and led to dorzolamide. Still, the greatest majority of the synthesized compounds proved to be potent allergens *in vivo*, since their sulfonamido group was nucleophilically displaced by reduced glutathione. Furthermore, the only compounds with acceptable water solubility proved to be the hydrochlorides of amino-derivatives of the thienothiopyran-sulfonamides of the dorzolamide type.[15,19] Obviously, the approach followed by this group was to explore as many possible heterocyclic rings on which the sulfonamido moiety could be grafted, and this approach developed the chemistry of heterocyclic sulfonamides. However, this approach seemed to us not a very fortunate one for the design of topically active IOP lowering agents, and we decided to explore an alternative one of grafting moieties that would ensure water solubility (as salts of a strong acid) on the classical ring systems of the aromatic/heterocyclic sulfonamides possessing CA inhibitory properties.

In this paper we report the reaction of twenty aromatic/heterocyclic sulfonamides containing a free amino, imino, hydrazino or hydroxyl group, with isonicotinoyl chloride, which afforded a series of water-soluble (as hydrochloride or triflate salts) sulfonamides with strong CA inhibitory properties. Moreover, dorzolamide has been similarly derivatized at its secondary amino group, and the obtained compound also possessed good water solubility as the hydrochloride salt. The new compounds reported here were tested for inhibition of three CA isozymes, hCA I, hCA II and bCA IV. Affinities in the nanomolar range were detected with some

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compounds for isozymes II and IV. The most active derivatives were assayed *in vivo* in normotensive and glaucomatous rabbits for their IOP lowering properties. Very strong IOP lowering was observed for many of them, and the active drug was detected in eye tissues and fluids. Our conclusion is that the water-solubilizing tail seems to be more important than the ring on which the sulfonamido moiety is grafted, and that topically active antiglaucoma drugs might be obtained from many classes of sulfonamides other than the thienothiopyran-sulfonamides and their derivatives.

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MATERIALS AND METHODS

Melting points were determined with a heating plate microscope and are not corrected. IR spectra were obtained in KBr pellets with a Perkin-Elmer 16PC FTIR spectrometer and ^{1}H-NMR spectra with a Varian 300CXP apparatus in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and were $\pm 0.4\%$ of the theoretical values.

Sulfonamides **3–22** used in synthesis were either commercially available compounds (from Sigma, Acros or Aldrich) or were prepared as described previously: 4-hydrazino-benzenesulfonamide **6** by diazotization of sulfanilamide followed by reduction of the diazonium salt with tin(II) chloride;[30] halogenosulfanilamides **9–12** by halogenation of sulfanilamide as reported in the literature;[31] compound **17** from 5-amino-1,3,4-thiadiazole-2-sulfonamide (obtained from acetazolamide)[32] by acylation with the phthalimido-derivative of β-alanine followed by hydrazinolysis,[33a] and imine **16** by deprotection of methazolamide with concentrated hydrochloric acid.[33b] The benzothiazole-2-sulfonamide derivatives **18–20** were prepared as described in Ref. 33c, whereas the alcohols **21** and **22** were obtained from the corresponding amines by diazotization followed by hydrolysis of the diazonium salts.[33c] Dorzolamide **1** was prepared as described in the literature.[34] Isonicotinoyl chloride, triflic acid and triethylamine were from Acros. Acetonitrile, acetone (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.*[35] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those

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described by Lindskog's group[36] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[37] 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.[38,39] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[40]

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.[41] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $2 \cdot 10^{-2}$ and $1 \cdot 10^{-6}$ 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.[41] 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.[41] 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[42] and higher concentrations had to be used for the measurements).

General Procedure for the Preparation of Isonicotinoyl Derivatives of the Aromatic/Heterocyclic Sulfonamides, 23–43

A 10 mM sulfonamide **1** or **3–22** was dissolved/suspended in 50 mL of anhydrous acetonitrile or acetone and then treated with 0.178 g (10 mM) of isonicotinoyl chloride hydrochloride. The stoichiometric amount (200 µL) of triethylamine was then added and the reaction mixture was magnetically stirred at 4°C for 4–10 h. The conversion of all the sulfonamide to the corresponding isonicotinoyl derivatives was monitored by TLC. When the

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reaction was completed, the solvent was evaporated down to a small volume. Generally the new compounds crystallized spontaneously by leaving this mixture at 4°C overnight. In some cases, the concentrated liquor was poured into 50 mL of cold water, when the reaction products precipitated and were filtered. The prepared compounds were recrystallized from ethanol or ethanol–water (1 : 1, v/v). Yields were in the range of 70–90%. Hydrochlorides of derivatives **23–43** were obtained from the free bases in methanol and a methanolic HCl solution. The hydrochlorides precipitated by leaving the above mixtures at 4°C overnight. The hydrochlorides were analyzed for the presence of Cl^- by potentiometric titrations. The obtained data were ±0.5% of the theoretical data calculated for the proposed formulas (data not shown). Triflate salts were similarly obtained from the free bases **23–43** and the stoichiometric amount of triflic acid, in water as solvent.

2-(Isonicotinoylamido)-benzenesulfonamide **23** as white crystals, m.p. 255–6°C; IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1290 (amide III), 1370 (SO_2^{as}), 1550 (amide II), 1680 (amide I), 3090 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 7.15–7.69 (m, 4H, ArH, 1,2-phenylene); 7.50 (br s, 2H, SO_2NH_2); 8.14 (br s, 1H, CONH). Found: C, 51.90; H, 4.10; N, 14.96. $C_{12}H_{11}N_3O_3S$ requires: C, 51.98; H, 4.00; N, 15.15%.

3-(Isonicotinoylamido)-benzenesulfonamide **24** as white crystals, m.p. 274–6°C (dec.); IR (KBr), cm^{-1}: 1135 (SO_2^{sym}), 1290 (amide III), 1370 (SO_2^{as}), 1570 (amide II), 1690 (amide I), 3080 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.15, δ_B 7.67 (AA′BB′system, 4H, J_{AB} = 7.4 Hz, ArH from isonicotinoyl); 7.10–7.50 (m, 4H, ArH, 1,3-phenylene); 7.56 (br s, 2H, SO_2NH_2); 8.11 (br s, 1H, CONH). Found: C, 51.78; H, 3.85; N, 15.07. $C_{12}H_{11}N_3O_3S$ requires: C, 51.98; H, 4.00; N, 15.15%.

4-(Isonicotinoylamido)-benzenesulfonamide **25** as white crystals, m.p. 287–9°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1290 (amide III), 1345 (SO_2^{as}), 1560 (amide II), 1690 (amide I), 3060 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); δ_A 7.18, δ_B 7.75 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from 4-sulfamoylphenyl); 7.56 (br s, 2H, SO_2NH_2); 8.19 (br s, 1H, CONH). Found: C, 51.67; H, 4.05; N, 14.88. $C_{12}H_{11}N_3O_3S$ requires: C, 51.98; H, 4.00; N, 15.15%.

4-(Isonicotinoylhydrazido)-benzenesulfonamide **26** as white crystals, m.p. 270–2°C; IR (KBr), cm^{-1}: 980 (N–N), 1150 (SO_2^{sym}), 1290 (amide III), 1365 (SO_2^{as}), 1555 (amide II), 1690 (amide I), 3090 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.14, δ_B 7.65 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 7.05–7.39 (m, AA′BB′, 4H, ArH, 1,4-phenylene);

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7.59 (br s, 2H, SO_2NH_2); 8.06 (br s, 2H, CONHNH). Found: C, 49.40; H, 4.16; N, 19.03. $C_{12}H_{12}N_4O_3S$ requires: C, 49.31; H, 4.14; N, 19.17%.

4-(Isonicotinoylamidomethyl)-benzenesulfonamide **27** as white crystals, m.p. 276–7°C (dec.); IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1290 (amide III), 1372 (SO_2^{as}), 1545 (amide II), 1690 (amide I), 3090 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CH_2); δ_A 7.16, δ_B 7.69 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); δ_A 7.22, δ_B 7.79 (AA′BB′-system, 4H, $J_{AB}=7.9$ Hz, ArH from 4-sulfamoylphenyl); 7.67 (br s, 2H, SO_2NH_2); 8.16 (br s, 1H, CONH). Found: C, 53.81; H, 4.78; N, 14.21. $C_{13}H_{13}N_3O_3S$ requires: C, 53.60; H, 4.50; N, 14.42%.

4-(Isonicotinoylamidoethyl)-benzenesulfonamide **28** as white crystals, m.p. 284–5°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1290 (amide III), 1359 (SO_2^{as}), 1540 (amide II), 1690 (amide I), 3080 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.70 (t, 2H, βCH_2 from the CH_2CH_2 bridge); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); δ_A 7.15, δ_B 7.62 (AA′BB′-system, 4H, $J_{AB}=7.9$ Hz, ArH from 4-sulfamoylphenyl); 7.67 (br s, 2H, SO_2NH_2); 8.17 (br s, 1H, CONH). Found: C, 55.40; H, 5.03; N, 13.45. $C_{14}H_{15}N_3O_3S$ requires: C, 55.07; H, 4.95; N, 13.76%.

3-Fluoro-4-(isonicotinoylamido)-benzenesulfonamide **29** as white crystals, m.p. 235–6°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1290 (amide III), 1348 (SO_2^{as}), 1550 (amide II), 1680 (amide I), 3060 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.60 (br s, 2H, SO_2NH_2); δ_A 7.12, δ_B 7.65 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); 7.05–7.89 (m, 3H, Ar H, from the F-substituted ring); 8.15 (br s, 1H, CONH). Found: C, 48.54; H, 3.61; N, 14.07. $C_{12}H_{10}FN_3O_3S$ requires: C, 48.81; H, 3.41; N, 14.23%.

3-Chloro-4-(isonicotinoylamido)-benzenesulfonamide **30** as white crystals, m.p. 240–3°C; IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1290 (amide III), 1339 (SO_2^{as}), 1550 (amide II), 1690 (amide I), 3090 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.70 (br s, 2H, SO_2NH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); 7.05–7.76 (m, 3H, Ar H the 2-Cl-substituted ring); 8.15 (br s, 1H, CONH). Found: C, 46.27; H, 3.37; N, 13.39. $C_{12}H_{10}ClN_3O_3S$ requires: C, 46.23; H, 3.23; N, 13.48%.

3-Bromo-4-(isonicotinoylamido)-benzenesulfonamide **31** as white crystals, m.p. 233–5°C; IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1290 (amide III), 1356 (SO_2^{as}), 1540 (amide II), 1690 (amide I), 3060 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.65 (br s, 2H, SO_2NH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); 7.05–7.86 (m, 3H, Ar H

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the 2-Br-substituted ring); 8.14 (br s, 1H, CONH). Found: C, 40.55; H, 3.00; N, 11.50. $C_{12}H_{10}BrN_3O_3S$ requires: C, 40.46; H, 2.83; N, 11.80%.

3-Iodo-4-(isonicotinoylamido)-benzenesulfonamide **32** as white crystals, m.p. 208–9°C (dec.); IR (KBr), cm^{-1}: 1145 (SO_2^{sym}), 1290 (amide III), 1360 (SO_2^{as}), 1545 (amide II), 1690 (amide I), 3070 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.60 (br s, 2H, SO_2NH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); 7.08–7.79 (m, 3H, Ar H the 2-I-substituted ring); 8.14 (br s, 1H, CONH). Found: C, 35.87; H, 2.43; N, 10.36. $C_{12}H_{10}IN_3O_3S$ requires: C, 35.75; H, 2.50; N, 10.42%.

4,5-Dichloro-6-isonicotinoylamido-benzene-1,3-disulfonamide **33** as white crystals, m.p. 271–3°C; IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1290 (amide III), 1370 (SO_2^{as}), 1550 (amide II), 1690 (amide I), 3080 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); 7.54 (s, 1H, ArH from the pentasubstituted benzene ring); 7.68 (br s, 4H, 2 SO_2NH_2); 8.10 (br s, 1H, CONH). Found: C, 33.69; H, 2.40; N, 13.08. $C_{12}H_{10}Cl_2N_4O_5S_2$ requires: C, 33.89; H, 2.37; N, 13.17%.

6-Chloro-4-isonicotinoylamido-benzene-1,3-disulfonamide **34** as white crystals, m.p. 293–5°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1290 (amide III), 1330 (SO_2^{as}), 1540 (amide II), 1680 (amide I), 3060 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); 7.35 (s, 1H, ArH from disulfamoylphenyl); 7.59 (s, 1H, ArH from disulfamoylphenyl); 7.75 (br s, 4H, 2 SO_2NH_2); 8.14 (br s, 1H, CONH). Found: C, 36.59; H, 2.90; N, 14.21. $C_{12}H_{11}ClN_4O_5S_2$ requires: C, 36.88; H, 2.84; N, 14.34%.

5-Isonicotinoylamido-1,3,4-thiadiazol-2-sulfonamide **35** as white crystals, m.p. > 310°C; IR (KBr), cm^{-1}: 1180 (SO_2^{sym}), 1295 (amide III), 1340 (SO_2^{as}), 1545 (amide II), 1690 (amide I), 3060 (NH), 3375 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.94 (br s, 2H, SO_2NH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl); 8.26 (br s, 1H, CONH). Found: C, 33.60; H, 2.60; N, 24.45. $C_8H_7N_5O_3S_2$ requires: C, 33.68; H, 2.47; N, 24.55%.

5-Isonicotinoylimido-4-methyl-2-sulfonamide-δ^2-1,3,4-thiadiazoline **36** as white crystals, m.p. > 310°C; IR (KBr), cm^{-1}: 118 (SO_2^{sym}), 1290 (amide III), 1366 (SO_2^{as}), 1540 (amide II), 1690 (amide I), 3080 (NH), 3380 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.90 (s, 3H, Me); 6.96 (br s, 2H, SO_2NH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, $J_{AB}=7.9$ Hz, ArH from isonicotinoyl). Found: C, 35.99; H, 3.12; N, 23.25. $C_9H_9N_5O_3S_2$ requires: C, 36.11; H, 3.03; N, 23.40%.

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5-(Isonicotinoylamidoethylcarboxamido)-1,3,4-thiadiazol-2-sulfonamide **37** as white crystals, m.p. 290–3°C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1270 and 1290 (amide III), 1330 (SO_2^{as}), 1450, 1570 (amide II), 1690 and 1710 (amide I), 3090 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.25–2.60 (m, 4H, CH_2CH_2); 6.88 (br s, 3H, CONH + SO_2NH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 8.24 (br s, 1H, CONH from isonicotinoylamido moiety). Found: C, 37.15; H, 3.19; N, 23.46. $C_{11}H_{12}N_6O_4S_2$ requires: C, 37.07; H, 3.39; N, 23.58%.

6-Isonicotinoylamido-benzothiazol-2-sulfonamide **38** as white crystals, m.p. 295–8°C (dec.); IR (KBr), cm^{-1}: 1165 (SO_2^{sym}), 1290 (amide III), 1344 (SO_2^{as}), 1540 (amide II), 1680 (amide I), 3060 (NH), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 6.94 (dd, 1H, J = 9 Hz; J = 3 Hz, H-5 of benzothiazole); 7.10 (d, 1H, J = 3 Hz;, H-7 of benzothiazole); 7.78 (d, 1H, J = 9 Hz, H-4 of benzothiazole); 8.10 (br s, 2H, SO_2NH_2); 8.18 (br s, 1H, CONH). Found: C, 46.85; H, 2.94; N, 16.47. $C_{13}H_{10}N_4O_3S_2$ requires: C, 46.70; H, 3.01; N, 16.76%.

6-Isonicotinoyloxy-benzothiazol-2-sulfonamide **39** as white crystals, m.p. 286–8°C (dec.); IR (KBr), cm^{-1}: 1030 (CO–O), 1160 (SO_2^{sym}), 1350 (SO_2^{as}), 1450, 1775 (COO), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.13, δ_B 7.66 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 6.90 (dd, 1H, J = 9 Hz; J = 3 Hz, H-5 of benzothiazole); 7.11 (d, 1H, J = 3 Hz;, H-7 of benzothiazole); 7.79 (d, 1H, J = 9 Hz, H-4 of benzothiazole); 8.10 (br s, 2H, SO_2NH_2). Found: C, 46.40; H, 2.90; N, 12.38. $C_{13}H_9N_3O_4S_2$ requires: C, 46.56; H, 2.71; N, 12.53%.

6-Isonicotinoyloxyethyloxy-benzothiazol-2-sulfonamide **40** as white crystals, m.p. 247–9°C; IR (KBr), cm^{-1}: 1030 (CO–O), 1175 (SO_2^{sym}), 1341 (SO_2^{as}), 1450, 1770 (COO), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.89 (t, 3H, CH_2); 3.14 (t, 3H, CH_2); 6.95 (dd, 1H, J = 9 Hz; J = 3 Hz, H-5 of benzothiazole); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 7.10 (d, 1H, J = 3 Hz, H-7 of benzothiazole); 7.79 (d, 1H, J = 9 Hz, H-4 of benzothiazole); 8.15 (br s, 2H, SO_2NH_2). Found: C, 47.58; H, 3.66; N, 10.89. $C_{15}H_{13}N_3O_5S_2$ requires: C, 47.49; H, 3.45; N, 11.07%.

4-(Isonicotinoyloxymethyl)-benzenesulfonamide **41** as white crystals, m.p. 251–2°C; IR (KBr), cm^{-1}: 1040 (CO–O), 1155 (SO_2^{sym}), 1325 (SO_2^{as}), 1780 (COO), 3310 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CONHCH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 7.08–7.41 (m, AA′BB′, 4H, J = 7.2 Hz, ArH, phenylene); 7.49 (s, 2H, SO_2NH_2). Found: C, 53.19; H, 4.21; N, 9.37. $C_{13}H_{12}N_2O_4S$ requires: C, 53.42; H, 4.14; N, 9.58%.

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4-(Isonicotinoyloxyethyl)-benzenesulfonamide **42** as white crystals, m.p. 244–6°C; IR (KBr), cm^{-1}: 1040 (CO–O), 1157 (SO_2^{sym}), 1332 (SO_2^{as}), 1760 (COO), 3300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.70 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 6.95 (br s, 2H, SO_2NH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 7.05–7.52 (m, AA′BB′, J = 7.3 Hz, 4H, ArH, phenylene). Found: C, 54.95; H, 4.67; N, 8.97. $C_{14}H_{14}N_2O_4S$ requires: C, 54.89; H, 4.61; N, 9.14%.

5,6-Dihydro-4-[N-isonicotinoyl-(ethylamido)]-6-methyl-4H-thieno-[2,3-b] thiopyran-2-sulfonamide 7,7-dioxide **43** as white crystals, m.p. 281–2°C; IR (KBr), cm^{-1}: 1133 (SO_2^{sym}), 1290 (amide III), 1345 (SO_2^{as}), 1545 (amide II), 1680 (amide I), 3360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.29 (d, 3H, Me); 1.39 (t, 3H, Me from ethyl); 2.55 (m, 1H, CH); 2.80 (m, 1H, CH); 3.05–3.20 (m, 2H, CH_2 from ethyl); 4.37 (m, 2H, CH_2); δ_A 7.13, δ_B 7.68 (AA′BB′system, 4H, J_{AB} = 7.9 Hz, ArH from isonicotinoyl); 8.03 (s, 1H, CH, ArH from thienyl); 8.25 (br s, 2H, SO_2NH_2). Found: C, 44.57; H, 4.39; N, 9.66. $C_{16}H_{19}N_3O_5S_3$ requires: C, 44.74; H, 4.46; N, 9.78%.

Measurement of Tonometric IOP

Adult male New Zealand albino rabbits weighing 3–3.5 kg were used in the experiments with three animals being used for each inhibitor studied. The experimental procedures conform to 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 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 5.50–6.40.

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA, USA) as described by Maren's group.[43,44] 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,

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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.[43,44] All data are expressed as mean±SE, using a one-tailed *t* test.

Drug Distribution in Ocular Fluids and Tissues

The general procedure of Maren's group was followed.[43,44] 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 boiled 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. 43,44.

RESULTS AND DISCUSSION

Reaction of sulfonamides **1** or **3–22** with isonicotinoyl chloride afforded the new compounds **23–43**. The reaction was generally performed in acetone or acetonitrile as solvents, in the presence of triethylamine as base. In the case of compounds **15** and **16** the above procedure led to very low yields of isonicotinoylamido derivatives, and Schotten–Baumaun conditions were applied for obtaining **35** and **36** in good yields. Hydrochlorides of the new derivatives were then prepared by reacting the free bases **23–43** with a methanolic HCl solution. The triflate salts were similarly obtained by reaction of bases **23–43** with triflic acid in water as solvent. These salts possess a

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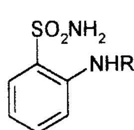
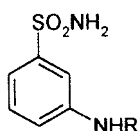
very good water solubility, generally in the range of 3–5% (data not shown). The pH of such solution was generally around 5.5–6.0, making them appropriate for topical application directly into the eye.

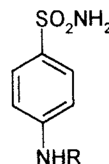
Compounds **3–43** were characterized and assayed for inhibition of isozymes hCA I, hCA II and bCA IV (Table I).



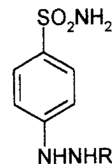
3: R = H
23: R = isonicotinoyl



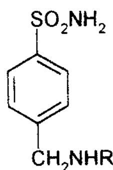
4: R = H
24: R = isonicotinoyl



5: R = H
25: R = isonicotinoyl

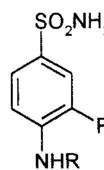


6: R = H
26: R = isonicotinoyl

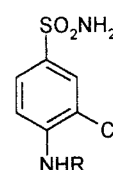


7: R = H
27: R = isonicotinoyl

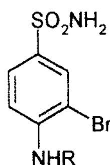
8: R = H
28: R = isonicotinoyl



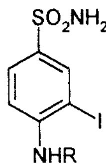
9: R = H
29: R = isonicotinoyl



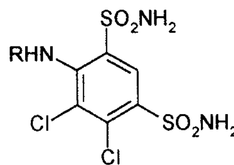
10: R = H
30: R = isonicotinoyl



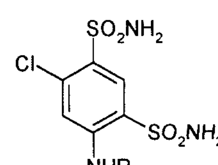
11: R = H
31: R = isonicotinoyl



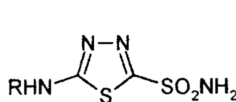
12: R = H
32: R = isonicotinoyl



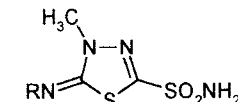
13: R = H
33: R = isonicotinoyl



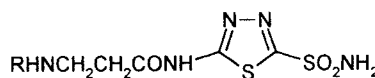
14: R = H
34: R = isonicotinoyl



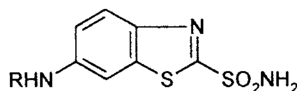
15: R = H
35: R = isonicotinoyl



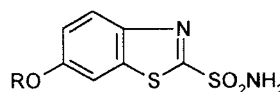
16: R = H
36: R = isonicotinoyl



17: R = H
37: R = isonicotinoyl



18: R = H
38: R = isonicotinoyl



19: R = H
39: R = isonicotinoyl

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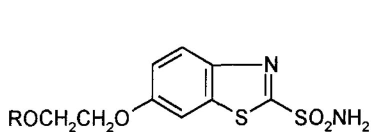
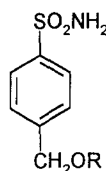
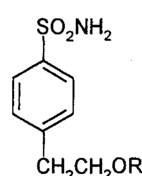


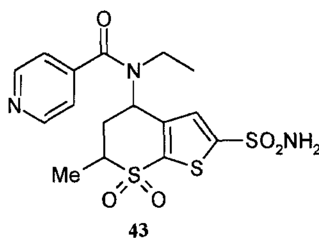




20: R = H
40: R = isonicotinoyl

21: R = H
41: R = isonicotinoyl

22: R = H
42: R = isonicotinoyl



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Inhibition data against three CA isozymes, hCA I, hCA II and bCA IV with the new derivatives (Table I) prove that the isonicotinoylamido-sulfonamides **23–43** reported here generally behave as strong inhibitors, with greatly increased potency when compared to the parent compounds from which they were prepared (the sulfonamides **3–22**). The potency of the obtained inhibitor generally varied in the following way, based on the parent sulfonamide from which it was prepared: the derivative of *p*-hydrazino-benzenesulfonamide **26** < the orthanilamide **23** ≅ the metanilamide **24** < the sulfanilamide **25** < the homosulfanilamides **27** < the *p*-aminoethyl-benzene-sulfonamides **28** < the 1,3-benzene-disulfonamides **33** and **34** ≅ the halogeno-substituted sulfanilamides **29–32** < the 1,3,4-thiadiazole-2-sulfonamides **35** and **37** ≅ 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide **36** ≅ the dorzolamide derivative **43** < the benzothiazole-2-sulfonamides **38–40**. All three CA isozymes investigated here were susceptible to inhibition with this type of sulfonamide, with hCA II and bCA IV the most susceptible with hCA I generally less susceptible to inhibition.

The promising *in vitro* CA inhibitory activity of some of the newly prepared compounds prompted us to investigate their effect *in vivo* on the IOP, after topical application directly into the eye, in normotensive and glaucomatous rabbits, frequently used as an animal model of glaucoma.[13–15,22,23,43] Some of these results are shown in Tables II and III.

The inhibitors selected for *in vivo* studies were among the most active against hCA II and bCA IV, in the prepared series, such as compounds **35–40**, and **43**. Tables II and III show that some of the new compounds assayed

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TABLE I CA inhibition data with standard inhibitors **1–2**, the parent sulfonamides **3–22** and the new derivatives **23–43** reported in the present study, against isozymes I, II and IV

Inhibitor	K_I* (nM)		
	hCA I[a]	*hCA II*[b]	*bCA IV*[b]
Dorzolamide **1**	50000	9	45
Brinzolamide[c] **2**	—	3.2	45.3
3	45400	295	1310
4	25000	240	2200
5	28000	300	3000
6	78500	320	3215
7	25000	170	2800
8	21000	160	2450
9	8300	60	180
10	9800	110	320
11	6500	40	66
12	6000	70	125
13	6100	28	175
14	8400	75	160
15	8600	60	540
16	9300	19	355
17	455	3	125
18	70	9	19
19	55	8	17
20	50	7	15
21	24000	125	560
22	18000	110	450
23	21000	290	270
24	20000	280	290
25	15000	130	150
26	23000	320	280
27	1200	78	109
28	1000	64	97
29	540	31	66
30	600	40	75
31	650	42	77
32	650	39	62
33	500	30	73
34	620	37	59
35	33	2	8
36	25	3	10
37	19	4	9
38	10	5	11
39	9	2	10
40	8	1	8
41	2100	76	120
42	2000	60	110
43	160	4	10

*Standard error for the determination of K_Is was of 5–10% (from 2 different assays).
[a]Human (cloned) isozyme.
[b]Isolated from bovine lung microsomes.
[c]Data from Ref. 45.

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TABLE II Fall of IOP in normotensive rabbits, after treatment with one drop (50 μL) of a solution (2%) of CA inhibitor (as hydrochloride salt, at the indicated pH value directly into the eye, at 30, 60 and 90 min after administration

Inhibitor	*pH*	ΔIOP (mmHg)*,†			
		$t=0$ min	$t=30$ min	$t=60$ min	$t=90$ min
1 (dorzolamide)	5.5	0	2.2 ± 0.10	4.1 ± 0.15	2.7 ± 0.08
35	5.0	0	4.5 ± 0.14	10 ± 0.15	7.5 ± 0.13
36	5.5	0	5.9 ± 0.10	11.2 ± 0.10	13.1 ± 0.15
37	5.5	0	5.1 ± 0.10	8.1 ± 0.10	8.0 ± 0.07
39	5.9	0	2.1 ± 0.05	4.5 ± 0.13	4.5 ± 0.10
40	5.5	0	2.4 ± 0.05	4.1 ± 0.07	3.5 ± 0.08
43	5.5	0	2.5 ± 0.06	4.0 ± 0.09	6.9 ± 0.11

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

TABLE III Fall of IOP in glaucomatous rabbits, after treatment with one drop (50 μL) of a solution (2%) of CA inhibitor (as hydrochloride salt, at the indicated pH value 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)*,†			
		$t=0$ min	$t=30$ min	$t=60$ min	$t=90$ min
1 (dorzolamide)	5.5	0	4.3 ± 0.12	7.1 ± 0.13	5.6 ± 0.11
35	5.0	0	9.2 ± 0.10	14.5 ± 0.20	19.5 ± 0.15
36	5.5	0	7.8 ± 0.10	12.2 ± 0.10	20.0 ± 0.12
43	5.5	0	4.8 ± 0.10	6.3 ± 0.09	10.5 ± 0.13

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

TABLE IV Ocular tissue concentrations (μM) after 1 and 2 h following corneal application of one drop (50 μL) of a solution (2%) of compound **35**. HCl in normotensive albino rabbits

Time (h)	*Drug concentration* (μM)*		
	Cornea	*Aqueous humor*	*Ciliary process*
1	150 ± 5	283 ± 10	51 ± 3
2	47 ± 4	39 ± 3	10 ± 1

*Mean ± standard deviation ($n=3$).

in vivo, such as **35**, **36**, **37** and **43**, showed much more effective IOP lowering effects when compared to dorzolamide **1**, both after 30 min from the administration of the inhibitor within the rabbit eye, as well as at other times (1, 1.5 and 2 h, respectively), in normotensive as well as glaucomatous animals. A second group of inhibitors, such as **39** and **40**, showed IOP lowering effects of the same order of magnitude as those of dorzolamide, both after half an hour or longer periods after administration. Mention should be made that the pH of the solutions administered in these experiments was in the range of pH 5.0–5.9 for all inhibitors used.

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Table IV shows the drug distribution in ocular fluids and tissues of normotensive rabbits after the topical administration of compound **35**, one of the most active topical inhibitors in the prepared series.

It is seen from the above data that at 1 and 2 h after topical administration of the drug, high levels of **35** were found in the cornea, aqueous humor and ciliary processes. Based on the inhibition constant of this compound (2 nM for CA II, and 8 nM for CA IV, respectively), the fractional inhibition estimated in these tissues/fluids is 99.5–99.9% indicating that the IOP decrease is indeed due to CA inhibition.[43,44]

In conclusion, we report here a general approach for the preparation of water-soluble, topically effective antiglaucoma sulfonamides, by attaching water-solubilizing moieties (such as isonicotinoyl) to well-known aromatic/heterocyclic sulfonamides. The new compounds reported here may lead to the development of more efficient antiglaucoma drugs.

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[1] This paper is Part 69 of the series. *Carbonic anhydrase inhibitors*. Preceding part of the series: P.A. Salvadori, C.T. Supuran, A. Scozzafava *et al.* (1998) *Eur. J. Med. Chem.*, **33**, in press.
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