Eur. J. Med. Chem. 34 (1999) 799–808

Original article

Carbonic anhydrase inhibitors – part 70[#]. Synthesis and ocular pharmacology of a new class of water-soluble, topically effective intraocular pressure lowering agents derived from nicotinic acid and aromatic/heterocyclic sulfonamides

Claudiu T. Supuran[a]*, Andrea Scozzafava[a], Luca Menabuoni[b], Francesco Mincione[c], Fabrizio Briganti[a], Giovanna Mincione[a]

[a]*Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121, Firenze, Italy*
[b]*Ospedale San Giovanni di Dio, S.O. Oculistica, Via Torregalli 3, I-50123, Firenze, Italy*
[c]*Università degli Studi, Institute of Ophthalmology, Viale Morgagni 85, I-50134, Firenze, Italy*

(Received 14 January 1999; revised 30 April 1999; accepted 6 May 1999)

Abstract – Reaction of twenty aromatic/heterocyclic sulfonamides containing a free amino, imino, hydrazino or hydroxyl group, with nicotinoyl chloride afforded a series of water-soluble (as hydrochloride or triflate salts) compounds. The new derivatives were assayed as inhibitors of three carbonic anhydrase (CA) isozymes, hCA I, hCA II (cytosolic forms) and bCA IV (membrane-bound form); h = human, b = bovine isozyme. Efficient inhibition was observed against all three isozymes, but especially against hCA II and bCA IV (in nanomolar range), two isozymes known to play a critical role in aqueous humour secretion within the ciliary processes of the eye. Some of the best 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. This result prompted us to re-analyse the synthetic work done by other groups for the design of water soluble, topically effective antiglaucoma sulfonamides. According to these researchers, the IOP lowering effect is due to the intrinsic nature of the specific heterocyclic sulfonamide considered, among which the thienothiopyran-2-sulfonamide derivatives represent the best studied case. Indeed, the first agents developed for such applications, such as dorzolamide, are derivatives of this ring system. In order to prove that the tail (in this case the nicotinoyl moiety) conferring water solubility to a sulfonamide CA inhibitor is critically important, similarly to the ring to which the sulfonamido group is grafted, we also prepared a dorzolamide derivative to which the nicotinoyl moiety was attached. 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 grafted. © 1999 Éditions scientifiques et médicales Elsevier SAS

carbonic anhydrase / aromatic, heterocyclic sulfonamides / nicotinoyl chloride / antiglaucoma drugs / hydrochloride salts / dorzolamide

1. 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 drugs among others [5–7], the hypoglycaemic 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], but these compounds possess a different pharmacological profile, independent of CA inhibition [10, 11]. Finally, some antithy-

[#] See [1].

*Correspondence and reprints

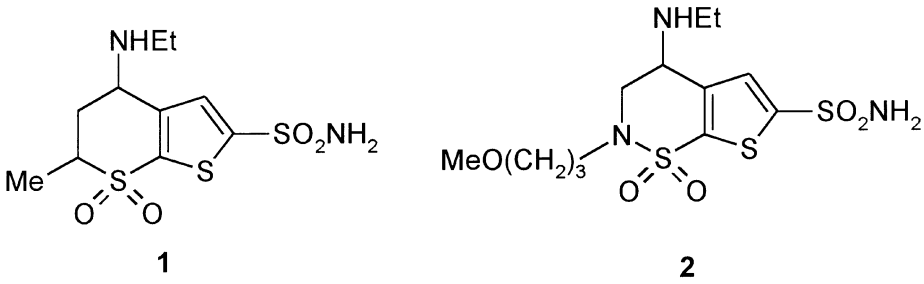

Figure 1. Structures of dorzolamide **1** and brinzolamide **2**.

roid drugs have also been developed starting from the sulfonamide structure as lead molecule [12].

The second class of the above mentioned pharmacological agents, ie., the sulfonamides with CA inhibitory action, have been thoroughly investigated in the last 10 years, mainly in the search for 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, but at that moment no inhibitors possessing such physico-chemical properties existed. They started to be developed in several laboratories soon thereafter [13–19], and in 1995 the first such pharmacological agent, dorzolamide **1** entered into clinical use in the USA and Europe [22]. A second compound, brinzolamide **2**, quite similar structurally to dorzolamide has also recently been approved for the topical treatment of glaucoma in the USA *(figure 1)* [23].

Thus, in a series of interesting papers [15, 24–29], the Merck, Sharp and Dohme group has developed the synthesis of a large series of generally bicyclic heterocyclic sulfonamides (derivatives of benzothiazole- [24]; benzofuran- [25]; indole- [26]; benzo[*b*]-thiophene- [27, 28]; thieno-thiopyran [15, 29], etc), which were then tested as IOP lowering agents, and which led to the above mentioned drug (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. More than that, the only compounds with acceptable water solubility proved to be hydrochlorides of amino-derivatives of the thienothiopyran-sulfonamides of the dorzolamide type [15, 29]. Obviously, the approach followed by this group was to explore as many heterocyclic rings as possible on which the sulfonamido moiety should be grafted, and this approach was extremely beneficial to the chemistry of heterocyclic sulfonamides. Still, this approach seemed to us not a very fortunate one for the design of topically active IOP lowering agents, and we decided to explore the opposite one, i.e., to graft 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 nicotinoyl chloride, which afforded a series of water-soluble (as hydrochloride or triflate salts) sulfonamides with strong CA inhibitory properties. Moreover, dorzolamide has been derivatized similarly, at its secondary amino group, and the obtained compound also possessed a good water solubility as the hydrochloride salt. The new compounds reported here were tested for the inhibition of three CA isozymes, hCA I, hCA II and bCA IV (h = human, b = bovine isozyme). Affinities in the nanomolar range were detected for some 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 intraocular pressure (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 other classes of sulfonamides than the thienothiopyran-sulfonamides and their derivatives.

2. Experimental protocols

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, whereas ^{1}H-NMR spectra were obtained with a Varian 300CXP apparatus in solvents specified in each case. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyser, and were ± 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 [33], whereas imine **16** by deprotection of methazolamide with concentrated hydrochloric acid [34]. The benzothiazole-2-sulfonamide derivatives **18–20** were prepared as described in ref. [35], whereas the alcohols **21** and **22** from the corresponding amines by diazotization followed by hydrolysis of the diazonium salts [31]. Dorzolamide **1** was prepared as described in the literature [36]. Nicotinoyl chloride hydrochloride, 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.

2.1. Chemistry

2.1.1. General procedure for the preparation of nicotinoyl derivatives of the aromatic/heterocyclic sulfonamides **23–43**

An amount of 10 mM sulfonamide **3–22** or **1** was dissolved/suspended in 50 mL of anhydrous acetonitrile or acetone and then treated with 0.178 g (10 mM) of nicotinoyl 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. By means of TLC, the conversion of all the sulfonamides to the corresponding nicotinoyl derivatives has been monitored. When the reaction was completed, the solvent was evaporated until a small volume of the reaction mixture was obtained. Generally the new compounds crystallized spontaneously by leaving the above mixture at 4 °C overnight. In some cases, the concentrated liquor obtained after the evaporation of the solvent was poured into 50 mL of cold water, then the reaction products precipitated and 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 and a methanolic HCl solution, in methanol as solvent. The hydrochlorides precipitated by leaving the above mixtures at 4 °C overnight. The hydrochlorides were analysed for the presence of Cl^- by potentiometric titrations. The obtained data were ± 0.5% of the theoretical values 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.

2.1.1.1. 2-(Nicotinoylamido)-benzenesulfonamide **23**

White crystals, m.p. 250–252 °C; IR (KBr), cm^{-1}: 1 140 (SO_2^{sym}), 1 290 (amide III), 1 370 (SO_2^{as}), 1 550 (amide II), 1 680 (amide I), 3 090 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.15–7.66 (m, 4H, ArH, 1,2-phenylene); 7.50 (br s, 2H, SO_2NH_2); 8.14 (br s, 1H, CONH); Anal., 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%.

2.1.1.2. 3-(Nicotinoylamido)-benzenesulfonamide **24**

White crystals, m.p. 265–266 °C (dec.); IR (KBr), cm^{-1}: 1 135 (SO_2^{sym}), 1 290 (amide III), 1 370 (SO_2^{as}), 1 570 (amide II), 1 690 (amide I), 3 080 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.10–7.50 (m, 4H, ArH, 1,3-phenylene); 7.56 (br s, 2H, SO_2NH_2); 8.11 (br s, 1H, CONH); Anal., 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%.

2.1.1.3. 4-(Nicotinoylamido)-benzenesulfonamide **25**

White crystals, m.p. 280–281 °C (dec.); IR (KBr), cm^{-1}: 1 150 (SO_2^{sym}), 1 290 (amide III), 1 345 (SO_2^{as}), 1 560 (amide II), 1 690 (amide I), 3 060 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Anal., 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%.

2.1.1.4. 4-(Nicotinoylhydrazido)-benzenesulfonamide **26**

White crystals, m.p. 265–267 °C; IR (KBr), cm^{-1}: 980 (N–N), 1 150 (SO_2^{sym}), 1 290 (amide III), 1 365 (SO_2^{as}), 1 555 (amide II), 1 690 (amide I), 3 090 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.98 (m, 4H, ArH from nicotinoyl); δ_A 7.18, δ_B 7.71 (AA´BB´system, 4H, J_{AB} = 7.8 Hz, ArH from 4-sulfamoylphenyl); 7.59 (br s, 2H, SO_2NH_2); 8.06 (br s, 2H, CONHNH); Anal., 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%.

2.1.1.5. 4-(Nicotinoylamidomethyl)-benzenesulfonamide **27**

White crystals, m.p. 271–273 °C (dec.); IR (KBr), cm^{-1}: 1 170 (SO_2^{sym}), 1 290 (amide III), 1 372 (SO_2^{as}), 1 545 (amide II), 1 690 (amide I), 3 090 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Anal., 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%.

2.1.1.6. 4-(Nicotinoylamidoethyl)-benzenesulfonamide **28**

White crystals, m.p. 278–280 °C (dec.); IR (KBr), cm^{-1}: 1 150 (SO_2^{sym}), 1 290 (amide III), 1 359 (SO_2^{as}), 1 540 (amide II), 1 690 (amide I), 3 080 (NH); 3 360 (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); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Anal., 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%.

2.1.1.7. 3-Fluoro-4-(nicotinoylamido)-benzenesulfonamide **29**

White crystals, m.p. 234–235 °C. IR (KBr), cm^{-1}: 1 150 (SO_2^{sym}), 1 290 (amide III), 1 348 (SO_2^{as}), 1 550 (amide II), 1 680 (amide I), 3 060 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.60 (br s, 2H, SO_2NH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.05–7.89 (m, 3H, Ar H from the F-substituted ring); 8.15 (br s, 1H, CONH); Analysis, 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%.

2.1.1.8. 3-Chloro-4-(nicotinoylamido)-benzenesulfonamide **30**

White crystals, m.p. 238–239 °C. IR (KBr), cm^{-1}: 1 155 (SO_2^{sym}), 1 290 (amide III), 1 339 (SO_2^{as}), 1 550 (amide II), 1 690 (amide I), 3 090 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.70 (br s, 2H, SO_2NH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.05–7.76 (m, 3H, Ar H the 2-Cl-substituted ring); 8.15 (br s, 1H, CONH); Analysis, 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%.

2.1.1.9. 3-Bromo-4-(nicotinoylamido)-benzenesulfonamide **31**

White crystals, m.p. 230–232 °C. IR (KBr), cm^{-1}: 1 160 (SO_2^{sym}), 1 290 (amide III), 1 356 (SO_2^{as}), 1 540 (amide II), 1 690 (amide I), 3 060 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.65 (br s, 2H, SO_2NH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.05–7.86 (m, 3H, Ar H the 2-Br-substituted ring); 8.14 (br s, 1H, CONH); Analysis, 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%.

2.1.1.10. 3-Iodo-4-(nicotinoylamido)-benzenesulfonamide **32**

White crystals, m.p. 210–212 °C. IR (KBr), cm^{-1}: 1 145 (SO_2^{sym}), 1 290 (amide III), 1 360 (SO_2^{as}), 1 545 (amide II), 1 690 (amide I), 3 070 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.60 (br s, 2H, SO_2NH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.08–7.79 (m, 3H, Ar H the 2-I-substituted ring); 8.14 (br s, 1H, CONH); Analysis, 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%.

2.1.1.11. 4,5-Dichloro-6-nicotinoylamido-benzene-1,3-disulfonamide **33**

White crystals, m.p. 267–268 °C. IR (KBr), cm^{-1}: 1 140 (SO_2^{sym}), 1 290 (amide III), 1 370 (SO_2^{as}), 1 550 (amide II), 1 690 (amide I), 3 080 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Analysis, 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%.

2.1.1.12. 6-Chloro-4-nicotinoylamido-benzene-1,3-disulfonamide **34**

White crystals, m.p. 290–294 °C (dec.). IR (KBr), cm^{-1}: 1 150 (SO_2^{sym}), 1 290 (amide III), 1 330 (SO_2^{as}), 1 540 (amide II), 1 680 (amide I), 3 060 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: δ_A 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Analysis, 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%.

2.1.1.13. 5-Nicotinoylamido-1,3,4-thiadiazol-2-sulfonamide **35**

White crystals, m.p. > 310 °C; IR (KBr), cm^{-1}: 1 180 (SO_2^{sym}), 1 295 (amide III), 1 340 (SO_2^{as}), 1 545 (amide II), 1 690 (amide I), 3 060 (NH), 3 375; ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.94 (br s, 2H, SO_2NH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 8.26 (br s, 1H, CONH); Anal., 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%.

2.1.1.14. 5-Nicotinoylimido-4-methyl-2-sulfonamido-δ^2-1,3,4-thiadiazoline **36**

White crystals, m.p. > 310 °C; IR (KBr), cm^{-1}: 1 182 (SO_2^{sym}), 1 290 (amide III), 1 366 (SO_2^{as}), 1 540 (amide

II), 1 690 (amide I), 3 080 (NH), 3 380 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.90 (s, 3H, Me); 6.96 (br s, 2H, SO_2NH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); Anal., 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%.

2.1.1.15. 5-(Nicotinoylamidoethylcarboxamido)-1,3,4-thiadiazol-2-sulfonamide **37**

White crystals, m.p. 287–289 °C (dec.), IR (KBr), cm^{-1}: 1 150 (SO_2^{sym}), 1 270 and 1 290 (amide III), 1 330 (SO_2^{as}), 1 450, 1 570 (amide II), 1 690 and 1 710 (amide I), 3 090 (NH); 3 360 (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); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 8.24 (br s, 1H, CONH from nicotinoylamido moiety); Analysis, 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%.

2.1.1.16. 6-Nicotinoylamido-benzothiazol-2-sulfonamide **38**

White crystals, m.p. 290–294 °C (dec.), IR (KBr), cm^{-1}: 1 165 (SO_2^{sym}), 1 290 (amide III), 1 344 (SO_2^{as}), 1 540 (amide II), 1 680 (amide I), 3 060 (NH); 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Analysis, 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%.

2.1.1.17. 6-Nicotinoyloxy-benzothiazol-2-sulfonamide **39**

White crystals, m.p. 281–283 °C (dec.), IR (KBr), cm^{-1}: 1 030 (CO–O), 1 160 (SO_2^{sym}), 1 350 (SO_2^{as}), 1 450, 1 775 (COO), 3 360 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Analysis, 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%.

2.1.1.18. 6-Nicotinoyloxyethyloxy-benzothiazol-2-sulfonamide **40**

White crystals, m.p. 245–246 °C, IR (KBr), cm^{-1}: 1 030 (CO–O), 1 175 (SO_2^{sym}), 1 341 (SO_2^{as}), 1 450, 1 770 (COO), 3 360 (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); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 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); Analysis, 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%.

2.1.1.19. 4-(Nicotinoyloxymethyl)-benzenesulfonamide **41**

White crystals, m.p. 244–246 °C; IR (KBr), cm^{-1}: 1 040 (CO–O), 1 155 (SO_2^{sym}), 1 325 (SO_2^{as}), 1 780 (COO), 3 310 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.90 (s, 2H, CONHCH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.08–7.41 (m, AA´BB´, J = 7.2 Hz; 4H, ArH, phenylene); 7.49 (s, 2H, SO_2NH_2); Anal., 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%.

2.1.1.20. 4-(Nicotinoyloxyethyl)-benzenesulfonamide **42**

White crystals, m.p. 240–243 °C. IR (KBr), cm^{-1}: 1 040 (CO–O), 1 157 (SO_2^{sym}), 1 332 (SO_2^{as}), 1 760 (COO), 3 300 (NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.10 (t, 2H, αCH_2 from the CH_2CH_2 bridge); 3.70 (t, 2H, βCH_2 from the CH_2CH_2 bridge); 6.95 (br s, 2H, SO_2NH_2); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 7.05–7.52 (m, AA´BB´, J = 7.3 Hz, 4H, ArH, phenylene); Anal., 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%.

2.1.1.21. 5,6-Dihydro-4-[N-nicotinoyl-(ethylamido)]-6-methyl-4H-thieno-[2,3-b]thiopyran-2-sulfonamide 7,7-dioxide **43**

White crystals, m.p. 270–272 °C; IR (KBr), cm^{-1}: 1 135 (SO_2^{sym}), 1 290 (amide III), 1 345 (SO_2^{as}), 1 545 (amide II), 1 680 (amide I), 3 366 (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); 7.05–7.98 (m, 4H, ArH from nicotinoyl); 8.03 (s, 1H, CH, ArH from thienyl); 8.25 (br s, 2H, SO_2NH_2); Anal., 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%.

2.2. Pharmacology

2.2.1. Enzyme assay

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. [37] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group [38] and enzymes were purified by affinity chromatography according to the method of Khalifah et al. [39]. Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 mM^{-1}.cm^{-1} for CA I and 54 mM^{-1}.cm^{-1} for CA II, respectively, based on M_r = 28.85 kDa for CA I, and 29.30 kDa for CA II, respectively [40, 41]. CA IV was isolated from bovine lung microsomes as described by

Maren et al., and its concentration has been determined by titration with ethoxzolamide [42].

Initial rates of 4-nitrophenyl acetate hydrolysis catalysed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC [43]. Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2×10^{-2} and 1×10^{-6} M, working at 25 °C. A molar absorption coefficient ε of 18 400 $M^{-1}.cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.40), as reported in the literature [43]. 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 [43]. 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 [44] and higher concentrations had to be used for the measurements).

2.2.2. Measurement of tonometric IOP

Adult male New Zealand albino rabbits weighing 3–3.5 kg were used in the experiments (three animals were 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: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 [45, 46]. The pressure readings were matched with two-point standard pressure measurements at least twice each day using a Digilab calibration verifier. All IOP measurements were done by the same investigator with the same tonometer. One drop of 0.2% oxybuprocaine hydrochloride (novesine, Sandoz) diluted 1:1 with saline was instilled in each eye immediately before each set of pressure measurements. IOP was measured three times at each time interval, and the means reported. IOP was measured first, immediately before drug administration, then at 30 min after the instillation of the pharmacological agent, and then every 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 [45, 46]. All data are expressed as mean ± SE, using a one-tailed *t* test. Ocular hypertension was elicited in the right eye of albino rabbits by the injection of α-chymotrypsin (from Sigma) as described by Melena et al. [48]. The IOP of operated animals was checked after approximately four weeks, and animals with an elevated pressure of 30–36 mm Hg were used at least one month after the injection of α-chymotrypsin.

2.2.3. Drug distribution in ocular fluids and tissues

The general procedure of Maren's group has been followed [45, 46]. The animals were killed with an intracardiac air injection. Aqueous humour (both posterior and anterior chamber fluids) were 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, then 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 [45, 46].

3. Results

Compounds prepared by reaction of nicotinoyl chloride with aromatic/heterocyclic sulfonamides, of type **23–43**, are shown below *(figure 2)*. Inhibition data against three CA isozymes, hCA I, hCA II and bCA IV with compounds **1–43** are presented in *table I*. In vivo IOP

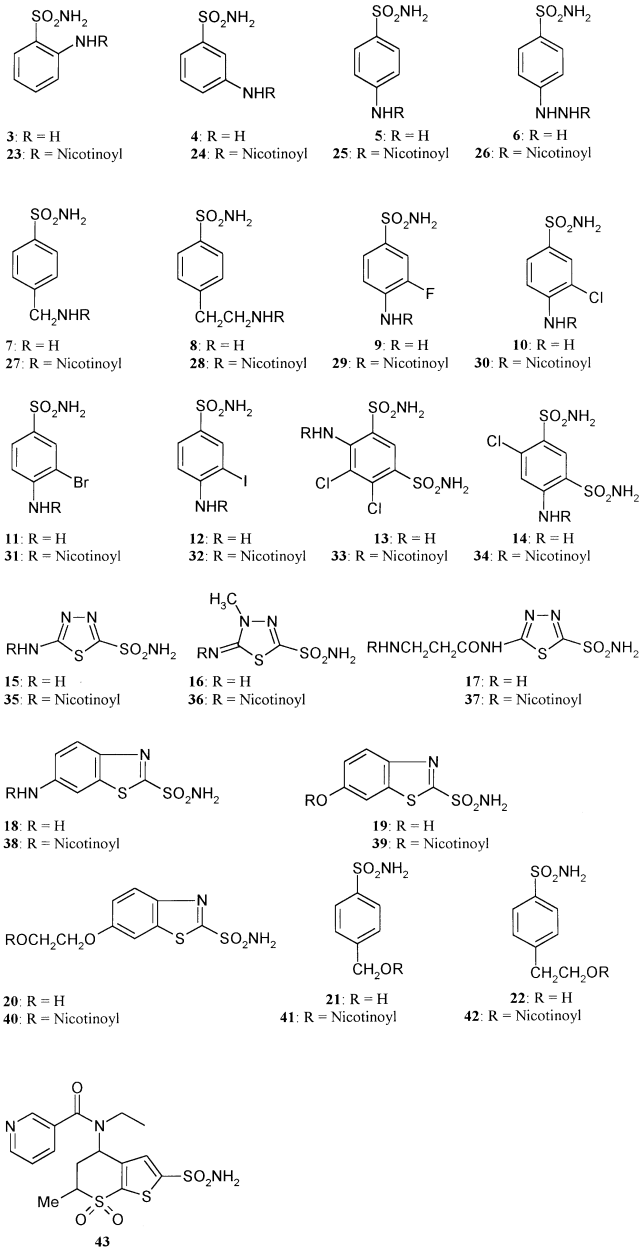

Figure 2. Structure of derivatives **3–43**.

lowering data with some of the most active CA inhibitors reported here, in normotensive and glaucomatous rabbits, after topical administration of the drug, are shown in *tables II* and *III*, respectively. Ex vivo distribution data of compound **35** in ocular tissues and fluids after the topical administration in normotensive rabbits, are shown in *table IV*.

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[a]	bCA IV[b]
Dorzolamide **1**	50 000	9	45
Brinzolamide[c] **2**	–	3.2	45.3
3	45 400	295	1 310
4	25 000	240	2 200
5	28 000	300	3 000
6	78 500	320	3 215
7	25 000	170	2 800
8	21 000	160	2 450
9	8 300	60	180
10	9 800	110	320
11	6 500	40	66
12	6 000	70	125
13	6 100	28	175
14	8 400	75	160
15	8 600	60	540
16	9 300	19	355
17	455	3	125
18	70	9	19
19	55	8	17
20	50	7	15
21	24 000	125	560
22	18 000	110	450
23	21 800	310	570
24	20 500	285	310
25	16 000	142	165
26	23 200	324	400
27	1 200	79	115
28	1 100	66	102
29	547	38	70
30	630	49	78
31	650	45	80
32	650	42	69
33	540	36	77
34	628	39	65
35	36	2	9
36	28	3	11
37	21	5	12
38	13	5	19
39	9	3	8
40	9	2	7
41	2 160	79	140
42	2 100	64	116
43	2 000	5	11

*Standard error for the determination of K_I-s was of 5–10% (from two different assays). [a]Human (cloned) isozyme; [b]Isolated from bovine lung microsomes; [c]From [47].

4. Discussion

Reaction of sulfonamides **3–22** or **1** with nicotinoyl chloride afforded a series of new compounds of type

Table II. Fall of IOP of normotensive rabbits (20.1 ± 2.0 mm Hg), after treatment with one drop (50 μL) of a solution 2 % of CA inhibitor (as hydrochloride salt, with the pH value shown below) directly into the eye, at 30, 60 and 90 min after administration.

Inhibitor	pH	ΔIOP (mm Hg)*			
		t = 0	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	5.5	0	5.4 ± 0.12	10.9 ± 0.11	12.5 ± 0.17
36	5.8	0	6.2 ± 0.10	12.4 ± 0.14	14.1 ± 0.12
37	5.5	0	5.1 ± 0.12	8.1 ± 0.11	8.6 ± 0.12
39	5.7	0	2.1 ± 0.05	4.0 ± 0.10	4.5 ± 0.10
40	5.5	0	2.4 ± 0.05	4.3 ± 0.11	3.5 ± 0.08
43	5.5	0	2.5 ± 0.06	4.5 ± 019	7.0 ± 0.14

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

23–43. The reaction was generally performed in acetone or acetonitrile as solvent, in the presence of triethylamine as base. In the case of compounds **15** and **16**, the above procedure led to very low yields of nicotinoylamido derivatives, and Schotten-Baumann 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. Similarly were obtained the triflate salts, by reaction of bases **23–43** with triflic acid in water as solvent. These salts possess a very good water solubility, generally in the range of 3–5% (data not shown). The pH of such solutions were generally around 5.5–6.0, making them appropriate for topical application directly into the eye.

Compounds **3–43** were characterized by standard chemical and physical methods that confirmed their structure (see Experimental protocols for details) and were assayed for the inhibition of isozymes hCA I, hCA II and bCA IV *(table I)*.

Inhibition data against the three CA isozymes, hCA I, hCA II and bCA IV with the new derivatives *(table I)* prove that the nicotinoylamido-sulfonamides **23–43** reported here generally behave as strong inhibitors, with greatly increased efficiencies as compared to the parent compounds from which they were prepared (the sulfonamides **3–22**). The efficiency 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-benzenesulfonamides **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 inhibitable, followed by hCA I, generally less susceptible to inhibition as compared to the first two isozymes.

The promising in vitro CA inhibitory activity of some of the newly prepared compounds prompted us to investigate their effect in vivo, on the intraocular pressure (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, 45]. 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 IV, in the prepared series, such as compounds **35–40**, and **43**. The following

Table III. Fall of IOP of glaucomatous rabbits (34.1 ± 2.0 mm Hg), after treatment with one drop (50 μL) of a solution 2 % of CA inhibitor (as hydrochloride salt, with the pH value shown below) directly into the eye, at 30, 60 and 90 min after administration.

Inhibitor	pH	ΔIOP (mm Hg)*			
		t = 0	t = 30 min	t = 60 min	t = 90 min
1	5.5	0	4.3 ± 0.25	7.1 ± 0.30	5.0 ± 0.25
35	5.5	0	10.3 ± 0.20	15.2 ± 0.20	19.1 ± 0.15
36	5.8	0	8.5 ± 0.10	13.2 ± 0.20	20.4 ± 0.20
43	5.5	0	4.8 ± 0.10	6.6 ± 0.10	11.6 ± 0.15

* $\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; 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 2 % solution of the compound **35** in normotensive albino rabbits.

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

* Mean ± standard deviation (n = 3).

facts should be noted regarding the data of *tables II* and *III*. Some of the new compounds assayed in vivo, such as **35**, **36**, **37** and **43**, showed much more effective IOP lowering effects as 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 the administration. Mention should be made that the pH of the solutions administered in these experiments was in the range of 5.0–5.9 for all inhibitors used.

In *table IV*, the drug distribution in ocular fluids and tissues of normotensive rabbits is shown, after the topical administration of one of the most active topical inhibitors in the prepared series, i.e., compound **35**.

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 humour and ciliary processes. Based on the inhibition constant of this compound (2 nM for CA II, and 9 nM for CA IV, respectively), the fractional inhibition estimated in these tissues/fluids is of 99.5–99.9%, proving the fact that the IOP decrease is indeed due to CA inhibition [45, 46].

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 might lead to the development of more efficient antiglaucoma drugs.

Acknowledgements

This research was financed by the EU grant ERB CIPDCT 940051. Thanks are addressed to Drs M.A. Ilies and M. Barboiu for expert technical assistance with the preparation of several sulfonamide intermediates used in this work.

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