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Carbonic Anhydrase Inhibitors: Synthesis and Inhibition Against Isozymes I, II and IV of Topically Acting Antiglaucoma Sulfonamides Incorporating *cis*-5-Norbornene-*endo*-3-Carboxy-2-Carboxamido Moieties

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Sulfonamides incorporating *cis*-5-norbornene-*endo*-3-carboxy-2-carboxamido moieties in their molecules were prepared by reaction of *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride with aromatic/heterocyclic sulfonamides possessing free amino, hydrazino, or imino groups. Some of these compounds showed very good CA II and CA IV inhibitory properties, with affinities for the enzymes in the low nanomolar range. Some of the most active CA II inhibitors reported here have been formulated as aqueous solutions for topical administration as antiglaucoma agents in normotensive rabbits. Some of the derivatives incorporating *cis*-5-norbornene-*endo*-3-carboxy-2-carboxamido and aromatic sulfonamide moieties (as sodium salts) showed effective and longer lasting intraocular pressure (IOP) lowering as compared to dorzolamide, a widely used topical antiglaucoma drug. Compounds incorporating *cis*-5-norbornene-*endo*-2,3-carboximido moieties, although stronger *in vitro* CA inhibitors as compared to the corresponding *cis*-5-norbornene-*endo*-3-carboxy-2-carboxamido-derivatives, showed no topical IOP lowering properties, probably due to their very poor water solubility.

Keywords: Aromatic/heterocyclic sulfonamide, Carbonic anhydrase, Isozyme I, II, IV, Antiglaucoma drugs, Topical action, *cis*-5-norbornene-*endo*-2,3-dicarboxylic acid

INTRODUCTION

The various carbonic anhydrase (CA, EC 4.2.1.1) isozymes differ substantially in specific activity and tissue distribution, but the only known physiological reaction catalyzed by these zinc metalloenzymes is the reversible hydration of CO_2 to HCO_3^-.[1,2] Some CA forms (belonging to the α-CA family of the higher verebrates)[3] were

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also shown to catalyze several related reactions of unknown physiological significance, such as the hydrolysis of aromatic esters and the hydration of carbonyl compounds.[1,2] Because of the wide distribution of the CA isoforms (14 are known for the moment in higher vertebrates)[3] in different physiological systems, it is of particular interest to find compounds that regulate the activity of this enzyme such as CA inhibitors (CAIs)[1,2] and CA activators.[4] Among the first type of such compounds, the sulfonamide CAIs were shown to be useful as diuretics, or in the treatment and prevention of a variety of diseases such as glaucoma, epilepsy, congestive heart failure, mountain sickness, gastric and duodenal ulcers, neurological disorders or osteoporosis among others.[1,2]

Here we focus on the use of sulfonamide CAIs in the treatment of glaucoma.[5] In the past years CAIs such as acetazolamide, methazolamide, ethoxzolamide and dichlorophenamide were widely used systemic antiglaucoma drugs.[1,2,5,6,7] Their mechanism of action consists in inhibition of CA II present in ciliary processes of the eye, with the consequent reduction of bicarbonate and aqueous humour secretion and of elevated intraocular pressure (IOP) characteristic of this disease.[1,2] However, since CA II is present in many other tissues/organs, generally systemic CAIs possess many undesired side effects.[1,2,5,6,7] In order to avoid these effects, recently, topically effective CAIs have been developed.[1a,8,9] Two such drugs are clinically available: dorzolamide **1** (since 1995) and brinzolamide **2** (since 1999). Both drugs are applied topically as aqueous solutions/suspensions, alone or in combination with other agents (such as β-blockers, prostaglandin derivatives, etc) and produce a consistent and prolonged reduction of IOP.[1,8,9]

In this paper we report several new types of aromatic/heterocyclic sulfonamide CA inhibitors with potential use as topical antiglaucoma agents. An ideal antiglaucoma drug of this type should possess the following features: (i) it must be a strong CA II and CA IV inhibitor, in order to achieve a significant reduction of aqueous humor secretion,[1,8,9] (ii) it should possess a sufficiently high liposolubility in order to penetrate through the cornea, but this should eventually be balanced by an adequate hydrosolubility, in order to formulate it as an acceptable form for topical administration. Up to now, only dorzolamide **1** seems to possess such properties but many serious side effects have been reported after its administration.[10–13] Less eye stinging/reddening than with dorzolamide has been reported for brinzolamide **2**, which is applied as an aqueous suspension due to its reduced water solubility.[9] Thus, we considered the design of novel sulfonamides possessing a relatively balanced hydro-/lipo-solubility, that can be formulated either as solutions, or suspensions for topical administration. The drug design has been based on the "tail" strategy reported previously by our group,[14–19] which consists in attaching certain moieties that would induce the desired physico-chemical properties to the molecules of aromatic/heterocyclic sulfonamides possessing free amino/hydroxy groups. These moieties should induce in the newly obtained CA inhibitors, high affinity to the CA active site, acceptable water/lipid solubility and good penetrability through the biological membranes. The tails chosen for incorporation in the compounds reported here are of the norbornene-carboxyl-carboxamide/carboximide type, since such derivatives have not been investigated up until now and they could well lead to the desired pharmacological properties for the new CAIs.

MATERIALS AND METHODS

Chemistry

Melting points were determined with a Boetius heating plate microscope and are not corrected: IR spectra were obtained in KBr pellets with a Carl Zeiss UR 20 spectrometer. ^{1}H-NMR spectra were obtained using a Varian Gemini 200 appar-

atus operating at 300 MHz in d_6-DMSO as solvent. Chemical shifts are expressed as δ values (ppm) relative to Me_4Si as internal standard. Elemental analyses were done by combustion, for C, H, N, with an automated Carlo Erba analyzer and the results were found $\pm$ 0.4% within the theoretical values. All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with MeOH:$CHCl_3$ (1:4 v/v). *cis*-5-Norbornene-*endo*-2,3-dicarboxylic anhydride and several sulfonamides used in the experiments (orthanilamide, metanilamide, sulfanilamide, homosulfanilamide (hydrochloride), 4-(2-aminoethyl)-benzene sulfonamide, acetazolamide, methazolamide and derivative **22**) were from Sigma-Aldrich (Milan, Italy). 4-Hydrazinobenzenesulfonamide (**6**) was prepared by diazotization of sulfanilamide followed by reduction of the diazonium salt with tin(II) chloride;[20] halogenosulfanilamides (**9–12**) by halogenation of sulfanilamide as reported in the literature;[21] compound (**17**) from 5-amino-1,3,4-thiadiazole-2-sulfonamide (obtained from acetazolamide)[22] by acylation with the phthalimido-derivative of β-alanine, followed by hydrazinolysis,[18] and the imine (**16**) by deprotection of methazolamide with concentrated hydrochloric acid.[23] The sulfanilyl substituted derivatives (**18–21**) were prepared as described in reference,[24] from the corresponding amino sulfonamides and 4-*N*-acetamidobenzenesulfonyl chloride, followed by deprotection of the 4-amino group with 10% HCl in EtOH.[24] Dorzolamide **1** was prepared as described in the literature.[25]

General Procedure for the Preparation of Compounds 3A–22A

The compounds were obtained by reaction of *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride with aromatic/heterocyclic sulfonamides in acetonitrile as solvent. An amount of 5 mmoles aminosulfonamide was suspended/dissolved in 20–30 ml anhydrous MeCN then a solution of 0.82 g (5 mmoles) of *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride dissolved in 3 ml MeCN was added dropwise during 10 min, together with 0.78 ml (0.56 g, 5.5 mmoles) triethylamine. The reaction was stirred overnight or until a reasonable conversion was reached (TLC control). The solvent was evaporated in vacuum and the resulting product was treated with 15–20 ml water acidified with 10% HCl. The crude solid product was filtered, washed with 5 ml water and air dried. Yields were in the range of 45–90%, depending on the reactivity of the aminosulfonamide. The obtained compounds were further purified by recrystallisation from the specified solvent or, in some cases, by column chromatography using silicagel 60 as stationary phase and eluted with MeOH/$CHCl_3$ gradients. The fractions containing the desired compound were collected, evaporated to dryness and the obtained product was recrystallized from solvents specified in each case.

General Procedure for the Preparation of Compounds 3B–22B

An amount of 0.82 g (5 mmoles) of *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride, 5 mmoles aminosulfonamide (**3–22**) and a catalytic amount of 4-toluenesulfonic acid (TsOH – 150–200 mg) were suspended in 200 mL of anhydrous toluene and heated at reflux under Dean-Stark conditions, until the calculated amount of water had separated (generally 4–6 h). The solvent was evaporated under vacuum and the residues obtained recrystallized from ethanol. Yields were in the range of 90–95%. Detailed spectroscopic and analytical data for some of the new derivatives prepared in this study are shown below.

4-[cis-5-Norbornene-endo-3-carboxy-2-carboxamido]-benzenesulfonamide, **5A** As white crystals, m.p. 245–6 °C (EtOH). IR (KBr), cm^{-1}: 1162 (SO_2^{sym}), 1335 (SO_2^{as}), 1585 (amide II), 1640 (amide I), 1760 (COOH); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz: 1.55 (s, 2H, CH_2 of norbornene), 3.21 (t, 2H, 2CH of norbornene), 3.70 (d, 2H,

CH–CH of norbornene), 6.24 (m, 2H, CH=CH of norbornene), 7.25 (s, 2H, SO_2NH_2), 7.78 (d, 2H, ArH, AA′BB′, 8.9), 7.91 (d, 2H, ArH, AA′BB′, 8.9), 10.03 (s, 1H, CONH), 11.15 (s, 1H, COOH). Found: C, 53.71; H, 5.12; N, 8.21. $C_{15}H_{16}N_2O_5S$ requires: C, 53.56; H, 4.79; N, 8.33%.

4-[cis-5-Norbornene-endo-3-carboxy-2-carboxamidomethyl]-benzenesulfonamide, **7A** As white crystals, m.p. 250–51 °C (MeOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1335 (SO_2^{as}), 1560 (amide II), 1630 (amide I), 1760 (COOH); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz: 1.59 (s, 2H, CH_2 of norbornene), 3.20 (t, 2H, 2CH of norbornene), 3.75 (d, 2H, CH–CH of norbornene), 4.49 (d, 2H, CONHCH_2; 6.1), 6.24 (m, 2H, CH=CH of norbornene), 7.34 (s, 2H, SO_2NH_2), 7.46 (d, 2H, ArH, AA′BB′, 8.3), 7.81 (d, 2H, ArH, AA′BB′, 8.3), 8.68 (t, 1H, CONH, 6.1), 11.03 (s, 1H, COOH). Found: C, 53.97; H, 5.01; N, 7.76. $C_{16}H_{18}N_2O_5S$ requires: C, 54.85; H, 5.18; N, 7.99%.

4-[cis-5-Norbornene-endo-3-carboxy-2-carboxamidoethyl]-benzenesulfonamide, **8A** As white crystals, m.p. 262–3 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1335 (SO_2^{as}), 1560 (amide II), 1610 (amide I); 1770 (COOH); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz: 1.59 (s, 2H, CH_2 of norbornene), 2.90 (t, 2H, 7.2), 3.21 (t, 2H, 2CH of norbornene), 3.47 (q, 2H, 6.5), 3.78 (d, 2H, CH–CH of norbornene), 6.21 (m, 2H, CH=CH of norbornene), 7.25 (s, 2H, SO_2NH_2), 7.42 (d, 2H, ArH, AA′BB′, 8.2), 7.75 (d, 2H, ArH, AA′BB′, 8.2), 8.08 (t, 1H, CONH, 5.7), 11.12 (s, 1H, COOH). Found: C, 56.14; H, 5.66; N, 7.32. $C_{17}H_{20}N_2O_5S$ requires: C, 56.03; H, 5.53; N, 7.69%.

5-[cis-5-Norbornene-endo-3-carboxy-2-carboxamido]-1,3,4-thiadiazole-2-sulfonamide, **15A** As white crystals, m.p. > 300 °C (EtOH-water, 1:1). IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1380 (SO_2^{as}), 1560 (amide II), 1615 (amide I), 1775 (COOH); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz: 1.59 (s, 2H, CH_2 of norbornene), 3.23 (t, 2H, 2CH of norbornene), 3.81 (d, 2H, CH–CH of norbornene), 6.25 (m, 2H, CH=CH of norbornene), 7.60 (s, 2H, SO_2NH_2), 8.25 (s, 1H, CONH), 11.10 (s, 1H, COOH). Found: C, 38.19; H, 3.62; N, 16.11. $C_{11}H_{12}N_4O_5S_2$ requires: C, 38.37; H, 3.51; N, 16.27%.

4-[cis-5-Norbornene-endo-2,3-dicarboximido]-benzenesulfonamide, **5B** As white crystals, m.p. 291–2 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1335 (SO_2^{as}), 1555 (amide II), 1620 (amide I); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz: 1.55 (s, 2H, CH_2 of norbornene), 3.20 (t, 2H, 2CH of norbornene), 3.72 (d, 2H, CH–CH of norbornene), 6.23 (m, 2H, CH=CH of norbornene), 7.25 (s, 2H, SO_2NH_2), 7.80 (d, 2H, ArH, AA′BB′, 8.9), 7.93 (d, 2H, ArH, AA′BB′, 8.9). Found: C, 56.70; H, 4.67; N, 8.52. $C_{15}H_{14}N_2O_4S$ requires: C, 56.59; H, 4.93; N, 8.80%.

4-[cis-5-Norbornene-endo-2,3-dicarboximidomethyl]-benzenesulfonamide, **7B** As white crystals, m.p. 289–90 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1350 (SO_2^{as}), 1560 (amide II), 1620 (amide I); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz: 1.54 (s, 2H, CH_2 of norbornene), 3.21 (t, 2H, 2CH of norbornene), 3.70 (d, 2H, CH–CH of norbornene), 4.48 (s, 2H, CONHCH_2), 6.24 (m, 2H, CH=CH of norbornene), 7.37 (s, 2H, SO_2NH_2), 7.48 (d, 2H, ArH, AA′BB′, 8.3), 7.80 (d, 2H, ArH, AA′BB′, 8.3). Found: C, 57.60; H, 5.13; N, 8.24. $C_{16}H_{16}N_2O_4S$ requires: C, 57.82; H, 4.85; N, 8.43%.

4-[cis-5-Norbornene-endo-2,3-dicarboximidoethyl]-benzenesulfonamide, **8B** As white crystals, m.p. > 300 °C (EtOH). IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1350 (SO_2^{as}), 1540 (amide II), 1600 (amide I); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz: 1.55 (s, 2H, CH_2 of norbornene), 2.90 (t, 2H, 7.2), 3.22 (t, 2H, 2CH of norbornene), 3.50 (q, 2H, 6.5), 3.77 (d, 2H, CH–CH of norbornene), 6.24 (m, 2H, CH=CH of norbornene), 7.28 (s, 2H, SO_2NH_2), 7.46 (d, 2H, ArH, AA′BB′, 8.2), 7.77 (d, 2H, ArH, AA′BB′, 8.2). Found: C, 59.15; H, 5.13; N, 7.96. $C_{17}H_{18}N_2O_4S$ requires: C, 58.94; H, 5.24; N, 8.09%.

5-[cis-5-Norbornene-endo-2,3-dicarboximido]-1,3,4-thiadiazole-2-sulfonamide **15B,** As white crystals, m.p. > 300 °C (EtOH-water, 1:1). IR (KBr), cm^{-1}: 1173 (SO_2^{sym}), 1360 (SO_2^{as}), 1540 (amide II), 1600 (amide I); ^{1}H-NMR (d_6-DMSO), δ, ppm; J, Hz:

1.56 (s, 2H, CH_2 of norbornene), 3.18 (t, 2H, 2CH of norbornene), 3.79 (d, 2H, CH–CH of norbornene), 6.25 (m, 2H, CH=CH of norbornene), 7.54 (s, 2H, SO_2NH_2). Found: C, 40.54; H, 3.33; N, 17.10. $C_{11}H_{10}N_4O_4S_2$ requires: C, 40.48; H, 3.09; N, 17.17%.

Biochemistry and Pharmacology

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 Lindskog *et al.*[26] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group,[27] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[28] 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.[29,30] CA IV was isolated from bovine lung microsomes as described by Maren *et al.* and its concentration was determined by titration with ethoxzolamide.[31]

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.[32] 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 $M^{-1} \cdot cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis under the conditions of the experiments (pH 7.40), as reported in the literature.[32] 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.[32] Enzyme concentrations were 3.1 nM for hCA II, 13 nM for hCA I and 33 nM for bCA IV.

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 conformed 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%, by weight) were obtained in distilled deionized water. The pH of these solutions was in the range pH 7.0–7.5.

IOP Measurements

IOP was measured using a Digilab 30R pneumatonometer (BioRad, Cambridge, MA, USA) as described by Maren's group.[33,34] 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 minutes for a period of 4–6 h. 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 inter-individual variations commonly observed in the rabbit.[33–35] All data are expressed as mean ± SE, using a one-tailed t test.

Determination of water (buffer) solubility

A standard solution was prepared by dissolving a precisely weighted amount (generally 1 mg) of inhibitor in 10 mL of methanol. The UV absorption maximum of each compound was determined (with a Cary 3 spectrophotometer) with dilutution of the solution (with MeOH) as necessary. A saturated solution of each compound was then prepared by stirring magnetically a small volume of 0.039 M phosphate buffer (pH 7.4) in the presence of excess inhibitor for 3 h. The obtained saturated solution was filtered to remove solid compound through a Millipore 0.45 μm filter and scanned by UV at the wavelength of the absorption maximum previously determined. Total solubility was determined by the relationship: $C' = A'C/A$, where C = concentration of standard solution (mg/mL); A = absorbance of standard solution; A′ = absorbance of the saturated solution; C′ = concentration of the saturated solution (mg/mL).[21b]

Transcorneal penetration of drugs

The method of Maren *et al.*[36] with the modifications of Pierce's group[37] (for the HPLC assay of sulfonamides) was used. Excised rabbit corneas with either intact or denuded epithelium were used in these experiments. The pH was 7.4 and the exposed area was 1.2 cm^2. Concentrations of drug (40–2000 μM) were placed in the epithelial chamber and samples of fluid were collected from the endothelial chamber at different intervals during a period of 4 h. Both chambers contained 6 mL. Drugs present in these fluids were assayed by the HPLC method of Pierce *et al.*[37] The results of the drug analyses were used to calculate the rate constant of transfer across the cornea (k_{in}). As described by Pierce[37] this value was determined using equation (1):

$$k_{in} \cdot (\times 10^3\ hr^{-1}) = [drug]_{endo}/[drug]_{epi} \times 60/t \times 1000 \quad (1)$$

where $[drug]_{endo}$ = concentration of drug on endothelial side, $[drug]_{epi}$ = concentration of drug on epithelial side and t = time (in min).

Partition coefficient determinations

Chloroform-buffer partition coefficients were obtained by equilibrating the test compound between chloroform and 0.1-ionic strength pH 7.4 phosphate buffer. The concentration in each phase was determined by UV spectrophotometry or HPLC.[21b,37]

RESULTS AND DISCUSSION

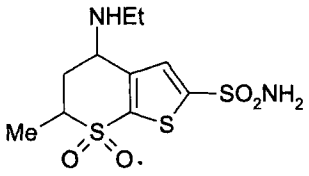
Reaction of *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride (**23**) with aromatic/heterocyclic sulfonamides of type (**3–22**) was performed with two variants: (i) at room temperature, in acetonitrile as solvent, when sulfonamides incorporating *cis*-5-norbornene-*endo*-3-carboxy-2-carboxamido moieties of type (**3A–22A**) were obtained, and (ii) in toluene as solvent under reflux, in anhydrous conditions, when sulfonamides incorporating *cis*-5-norbornene-*endo*-2,3-dicarboximido moieties were obtained, of type (**3B–22B**) (Scheme 1).

Inhibition data with the new compounds, the parent sulfonamides and standard inhibitors (Table I) showed the following relationships: (i) the new compounds (**3A–22A**) and (**3B–22B**) reported here were generally better CAIs as compared to the parent sulfonamides from which they were prepared; (ii) compounds of

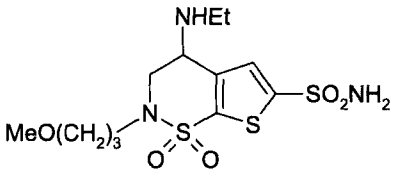
the **B** series were slightly more inhibitory than the corresponding compounds of the **A** series; (iii) the main parameter influencing CA inhibitory potency was the nature of the aromatic/heterocyclic ring to which the *cis*-5-norbornene-*endo*-2,3-dicarboxylic acid moiety had been grafted. Thus, aromatic sulfonamides were generally less inhibitory than heterocyclic derivatives; furthermore, compounds possessing an elongated molecule (such as **15A,B**; **17A,B**; **18A,B–22A,B** among others) were the most effective enzyme inhibitors, as explained theoretically by us in previous works using QSAR calculations.[24] Anyhow, potency varied in the following manner, based on the nature of the aromatic/heterocyclic sulfonamide moiety present in these new derivatives: 4-hydrazinobenzenesulfonamides (**6**) < metanilamides (**4**) < orthanilamides (**3**) < sulfanilamides (**5**) < the halogeno-substituted sulfanilamides (**9–12**) ≅ the benzene-1,3-disulfonamides (**13** and **14**) ≅ the homosulfanilamides (**7**) < the *p*-aminoethylbenzenesulfonamides (**8**) < the 1,3,4-thiadiazole-2-sulfonamides (**15** and **17**) ≅ 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide (**16**) ≅ the pyrimidine-substituted sulfanilamides of type (**22**) < the sulfanilyl-sulfanilamides (**20**) and the sulfanilyl-metanilamides (**21**) < the aminobenzolamide derivatives (**18** and **19**); (iv) isozyme hCA II was the most susceptible to inhibition, followed by bCA IV, whereas hCA I was the least prone to inhibition by these sulfonamides. This particular affinity of different CA isozymes for sulfonamide CAIs is well documented.[1]

The promising *in vitro* CA inhibitory activity, as well as other physico-chemical properties (Table II), for some of the newly prepared compounds prompted us to investigate their effect *in vivo* on intraocular pressure (IOP), after topical application directly into the eye in normotensive rabbits (frequently used as an animal model of glaucoma).[14–16,18] The data obtained are shown in Table III.

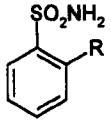
The data from the above Tables clearly shows that a close correlation exists between the CA inhibitory properties, physico-chemical properties and IOP lowering effects of the new sulfonamides reported here. Thus, only the compounds of the **A** series (**3A–22A**) possessed IOP lowering properties after topical administration, although they were generally slightly weaker CAIs as compared to the corresponding compounds of the **B** (**3B–22B**) series (Table I). The **B** type derivatives were totally inactive when administered as aqueous suspensions (due to their very low water solubility they could not be formulated as aqueous solutions) (solubility data not shown, but of the order of magnitude of $< 0.1\%$ by weight). On the other hand, among the compounds of the **A** series, it was unexpectedly observed that a very strong hCA II inhibitor, such as (**15A**) (K_I of 10 nM against isozyme II ≡ dorzolamide **1**), had worse IOP lowering properties as compared to a medium potency hCA II inhibitor, such as **3A** (K_I of 120 nM). It was immediately noted that the main difference in physico-chemical properties between the two compounds (**15A**) and (**3A**) resides in their very different liposolubilities, with (**15A**) being very poorly liposoluble ($\log P = 0.005$), whereas the latter derivative, (**3A**), possessing an acceptable liposolubility ($\log P = 1.12$). As a consequence, (**15A**) only poorly penetrates through the cornea (for inhibiting the ciliary processes CA II), whereas (**3A**), although much less effective against the enzyme, due to its favored penetration leads to a more effective IOP reduction. It should also be mentioned that these two compounds are less effective IOP lowering agents as compared to dorzolamide **1**, the clinically used CAI (Table III). More effective IOP lowering was observed with other compounds of the **A** series described here, such as (**7A**), (**8A**) and (**18A**) (Table III). These derivatives were more effective than dorzolamide (except for **7A** at 30 min) or the previously mentioned sulfonamides (**3A**), (**15A**), and this is probably due to their balanced hydro- and liposolubility, which leads to a good corneal penetration. At longer periods of time after administration (4–6 hours), the effect of



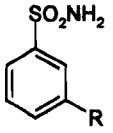
1



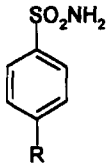
2



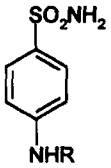
3: R = NH_2
3A: R = 2,3-NC
3B: R = 2,3-NI



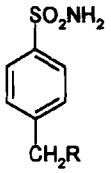
4: R = NH_2
4A: R = 2,3-NC
4B: R = 2,3-NI



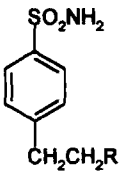
5: R = NH_2
5A: R = 2,3-NC
5B: R = 2,3-NI



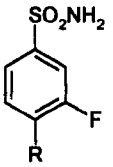
6: R = NH_2
6A: R = 2,3-NC
6B: R = 2,3-NI



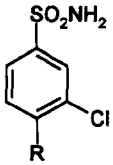
7: R = NH_2
7A: R = 2,3-NC
7B: R = 2,3-NI



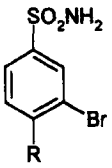
8: R = NH_2
8A: R = 2,3-NC
8B: R = 2,3-NI



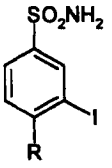
9: R = NH_2
9A: R = 2,3-NC
9B: R = 2,3-NI



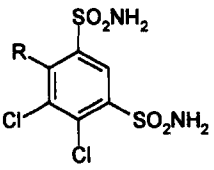
10: R = NH_2
10A: R = 2,3-NC
10B: R = 2,3-NI



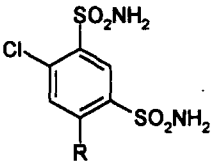
11: R = NH_2
11A: R = 2,3-NC
11B: R = 2,3-NI



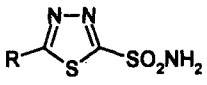
12: R = NH_2
12A: R = 2,3-NC
12B: R = 2,3-NI



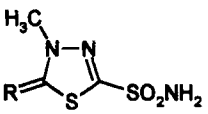
13: R = NH_2
13A: R = 2,3-NC
13B: R = 2,3-NI



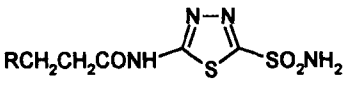
14: R = NH_2
14A: R = 2,3-NC
14B: R = 2,3-NI



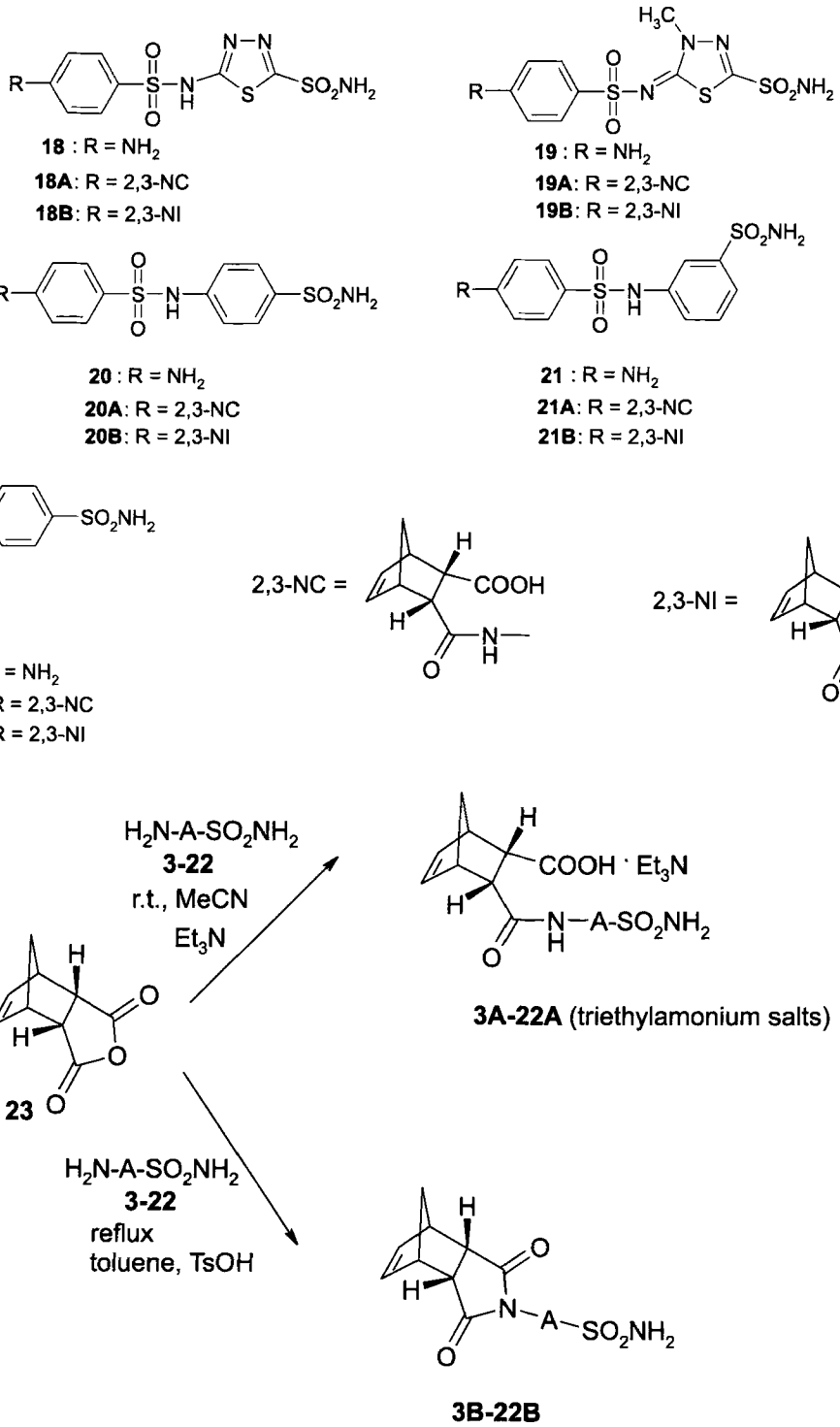
15: R = NH_2
15A: R = 2,3-NC
15B: R = 2,3-NI



16: R = NH
16A: R = 2,3-NC



17: R = NH_2
17A: R = 2,3-NC
17B: R = 2,3-NI



SCHEME 1 Synthesis of (**3A–22A**) and (**3B–22B**).

TABLE I CA inhibition data with the standard, clinically used inhibitors (**1**) and (**2**) and the new derivatives (**3A–22A**) reported in the present study, against isozymes I, II and IV. Data in parenthesis represent the inhibition constants of the corresponding parent sulfonamides (**3–22**)

Inhibitor		K_I* (nM)		
		hCA I[a]	hCA II[a]	bCA IV[b]
Dorzolamide	**1**	50000	9	45
Brinzolamide	**2**	nt	3	45
	3A	9000 (45400)	120 (295)	150 (1310)
	3B	7200 (45400)	75 (295)	100 (1310)
	4A	11500 (25000)	185 (240)	340 (2200)
	4B	10000 (25000)	150 (240)	310 (2200)
	5A	9600 (28000)	36 (300)	100 (3000)
	5B	9500 (28000)	32 (300)	87 (3000)
	6A	13000 (78500)	450 (320)	680 (3200)
	6B	10700 (78500)	390 (320)	520 (3200)
	7A	645 (25000)	26 (170)	38 (2800)
	7B	450 (25000)	20 (170)	27 (2800)
	8A	375 (21000)	13 (160)	26 (2500)
	8B	350 (21000)	10 (160)	21 (2500)
	9A	700 (8300)	28 (60)	86 (180)
	9B	675 (8300)	22 (60)	70 (180)
	10A	715 (9800)	35 (110)	89 (320)
	10B	700 (9800)	27 (110)	75 (320)
	11A	480 (6500)	26 (40)	59 (66)
	11B	435 (6500)	21 (40)	40 (66)
	12A	340 (6000)	28 (70)	110 (125)
	12B	325 (6000)	19 (70)	105 (125)
	13A	520 (6100)	21 (28)	110 (175)
	13B	490 (6100)	18 (28)	91 (175)
	14A	545 (8400)	33 (75)	120 (160)
	14B	510 (8400)	27 (75)	100 (160)
	15A	195 (8600)	10 (60)	34 (540)
	15B	150 (8600)	8 (60)	25 (540)
	16A	200 (9300)	12 (19)	38 (355)
	16B	185 (9300)	11 (19)	33 (355)
	17A	150 (455)	9 (3)	23 (125)
	17B	140 (455)	8 (3)	16 (125)
	18A	5.5 (6)	1.5 (2)	4.0 (5)
	18B	4.1 (6)	1.2 (2)	3.3 (5)
	19A	1.0 (1.2)	0.5 (0.6)	0.7 (0.8)
	19B	1.1 (1.2)	0.4 (0.6)	0.6 (0.8)
	20A	27 (42)	5 (6)	13 (50)
	20B	21 (42)	3 (6)	9 (50)
	21A	32 (44)	6 (9)	11 (53)
	21B	24 (44)	4 (9)	8 (53)
	22A	55 (690)	9 (12)	43 (150)
	22B	43 (690)	5 (12)	13 (150)

*Mean from at least three determinations by the esterase method.[32] Standard error was in the range of 5–10%; nt = not tested. [a] Human cloned isozyme. [b] Purified from bovine lung microsomes.[31]

TABLE II Solubility, chloroform-buffer partition coefficients and *in vitro* corneal permeability of some sulfonamide CA inhibitors

Compound	Solubility[a] mM	Log P[b] –	$k_{in} \times 10^3$ (hr^{-1})[c]	
			Cornea intact	No epithelium
1 (dorzolamide)	60[d]	2.0[e]	3.0	5.2
3A	47[e]	1.12	2.1	4.9
7A	43[e]	1.58	2.5	5.9
8A	52[e]	1.75	3.9	5.8
15A	39[e]	0.005	1.1	3.6
18A	36[e]	0.86	3.1	5.5

[a] Solubility in pH 7.40 buffer, at 25 °C. [b] Chloroform-buffer partition coefficient. [c] Determined as described in Ref. 37. [d] As hydrochloride, at pH 5.8, from Ref. 8. [e] As monosodium salts (at the COOH moiety).

TABLE III Fall of IOP of normotensive rabbits (20 ± 3 mm Hg), after treatment with one drop (50 µL) 2% aqueous solution of CA inhibitor 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	2 ± 0.20	4 ± 0.30	3 ± 0.25
3A	7.5	0	0	4 ± 0.25	2 ± 0.20
7A	7.5	0	0	5 ± 0.30	5 ± 0.15
8A	7.5	0	5 ± 0.30	6 ± 0.40	5 ± 0.20
15A	7.5	0	0	2 ± 0.10	0
18A	7.5	0	3 ± 0.20	6 ± 0.25	5 ± 0.30

* $\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$; Mean ± SE ($n = 3$). As HCl salt, in solution. All the other inhibitors were monosodium carboxylate salts.

dorzolamide on the IOP lowering is practically nonexistent, whereas compounds such as (**8A**) and (**18A**) brought about IOP lowering of 3.5–4.0 mm Hg at 4 hours, and 1.5–2.0 mm Hg at 5 hours, with IOP returning to baseline values generally after 6 hours. Thus, the advantage of these new compounds over dorzolamide is that they induce a longer lasting IOP lowering effect, and the pH of their solutions is in the neutral range (7.0–7.5); these compounds are sodium salts of a carboxylic acid, whereas the dorzolamide solutions have an acidic pH of 5.5, which provokes unpleasant stinging/burning eye effects and patient non-compliance with such a therapy.

In conclusion, we describe here a series of potent CA inhibitors, incorporating *cis*-5-norbor-

nene-*endo*-2,3-dicarboxylic acid moieties in their molecules. Some of the best CA II inhibitors obtained were tested *in vivo* for their IOP lowering properties. Several of the new compounds showed activities comparable to dorzolamide, a clinically used topical sulfonamide for the treatment of glaucoma and ocular hypertension. Their improvement over dorzolamide resides in the possibility of formulating such solutions at neutral – slightly basic pH values (7.0–7.5 pH units), which would presumably avoid the unpleasant side effects of dorzolamide due to its acidic pH, as well as their longer lasting IOP lowering effects.

Acknowledgments

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