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CARBONIC ANHYDRASE INHIBITORS: SYNTHESIS OF SCHIFF BASES OF HYDROXYBENZALDEHYDES WITH AROMATIC SULFONAMIDES AND THEIR REACTIONS WITH ARYLSULFONYL ISOCYANATES[#]

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Reaction of *o*- or *p*-hydroxybenzaldehydes with sulfanilamide, homosulfanilamide and *p*-(2-aminoethyl)- benzene-sulfonamide afforded several new Schiff bases which were subsequently derivatized at the phenolic hydroxy moiety by reaction with arylsulfonylisocyanates. The new arylsulfonylcarbamates obtained in this way possessed interesting inhibitory properties against three carbonic anhydrase (CA) isozymes, hCA I, hCA II and bCA IV (h = human, b = bovine isozyme). All these new derivatives, the simple Schiff bases and the arylsulfonylcarbamates obtained as outlined above, were more inhibitory against all isozymes as compared to the corresponding parent sulfonamide from which they were obtained. Generally, the *p*-hydroxybenzaldehyde derivatives were more active than the corresponding *ortho* isomers. An interesting behavior was evidenced for some of the *ortho*-substituted arylsulfonylcarbamato-sulfonamides, which showed higher affinities for the isozyme hCA I, as compared to hCA II and bCA IV (generally hCA I is 10–1000 less sensitive to "normal" sulfonamide inhibitors, such as acetazolamide, methazolamide or dorzolamide, as compared to hCA II). This make the new derivatives attractive leads for designing isozyme I-specific inhibitors.

Keywords: Carbonic anhydrase; Isozymes I, II, IV; Aromatic sulfonamides; Schiff bases; Arylsulfonyl isocyanate; Arylsulfonylcarbamates

[#] See ref. [1]

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INTRODUCTION

The 14 different carbonic anhydrase (CA, EC 4.2.1.1) isozymes described up to now in higher vertebrates, including humans (Table I), are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions, such as the lipogenesis, gluconeogenesis and ureagenesis among others.[2–6] Some of these isozymes are cytosolic (such as CA I, CA II, CA III, CA VII), others are membrane-bound (CA IV, CA IX, CA XII and CA XIV), CA V is present only in mitochondria, CA VI is secreted in saliva, whereas several acatalytic forms are also known (CA VIII, CA X and CA XI).[2–14]

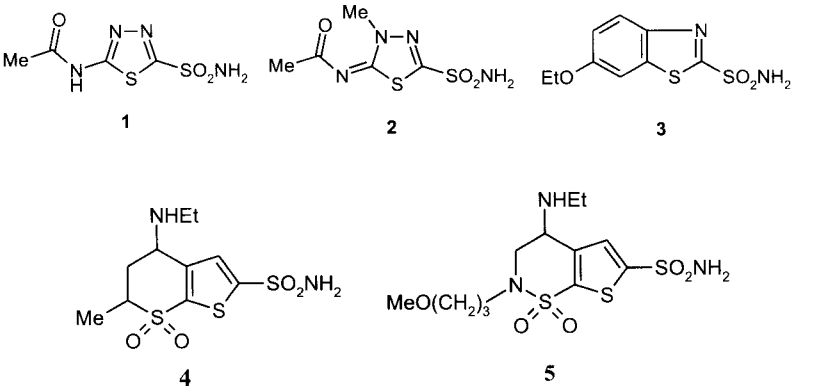
Recently, three novel membrane-bound CA isozymes, CA IX,[8] XII [11] and XIV,[12] (in addition to the previously reported "classical" one, CA IV),[13] have been isolated and characterized. Some of them were identified only in tumor cells, and little is known for the moment regarding the physiological consequences of their inhibition/activation.[11–14] Inhibition of CAs by aromatic/heterocyclic sulfonamides has been exploited clinically for more than 45 years in the treatment of a variety of diseases such as glaucoma,[4,15] epilepsy,[16] congestive heart failure,[4] mountain sickness,[17] gastric and duodenal ulcers,[18] or as diuretic agents.[19] Several clinical agents from this class include acetazolamide **1**, methazolamide **2** or ethoxzolamide **3**,[4] as well as

TABLE I CA isozymes, their relative CO_2 hydrase activity and sub-cellular location

Isozyme	*Catalytic activity (CO_2 hydration)*	*Sub-cellular location*
CA I	low (10% of that of CA II)	cytosol
CA II	high	cytosol
CA III	very low (1% of that of CA II)	cytosol
CA IV	high	membrane-bound
CA V	moderate	mitochondria
CA VI	moderate	secreted into saliva
CA VII	high	cytosol
CA-RP VIII	acatalytic	probably cytosolic
CA IX*	active (probably low)	membrane-bound
CA-RP X	acatalytic	unknown
CA-RP XI	acatalytic	unknown
CA XII*	active (no quantitative data)	membrane-bound
CA XIII**	probably high	unknown
CA XIV	low	membrane-bound

* CA IX and CA XII are known to be tumor associated. **CA XIII has not been isolated as a protein but has been identified from EST derived from a mouse mammary gland cDNA library.[2a]

the recently introduced topical antiglaucoma drugs dorzolamide **4** and brinzolamide **5**.[20]



With so many new CA isozymes in addition to the abundant, ubiquitously spread classical ones (CA I, II and IV), present in a large variety of tissues/organs, it is of crucial importance to design novel types of inhibitors, either with specificity for certain isozymes, or with applications for specific pathophysiological processes. Thus, we have recently reported[21] a new class of potent sulfonamide CA inhibitors of the type R_2N-CSSNH-A-SO_2NH_2 (R = Me,Et; A = aromatic or heterocyclic ring) which showed very powerful tumor cell growth inhibitory properties *in vitro* and *in vivo*, against a variety of cancer types (GI_{50} of 10–70 nM, where GI_{50} is the molarity of inhibitor producing a 50% of tumor cell growth after 48 hours exposure to the drug).[21]

Another approach that we investigated for obtaining novel types of sulfonamide CA inhibitors, consisted in the preparation of Schiff bases of aromatic/heterocyclic aldehydes with benzenesulfonamide derivatives of type **6–8**, which led to more specific CA IV versus CA II, potent inhibitors.[22,23]

In other contributions from this laboratory[15,24–28] it was also shown that by attaching different "tails" to the molecules of aromatic/heterocyclic sulfonamides, it is possible to obtain potent CA inhibitors possessing the desired physico-chemical properties, such as enhanced water solubility (for their use as antiglaucoma drugs),[15,24–26] membrane impermeability (important for obtaining isozyme-specific inhibitors),[27,28] etc., all requisites of interest for the drug design of novel types of pharmacological agents. Similar or alternative approaches were also explored by Whiteside's and Jain's groups.[29,30] Here we combine the two approaches mentioned above, and

report the synthesis of novel types of Schiff bases, containing groups (at the aromatic aldehyde moiety) easily derivatizable by different reagents, leading thus to the possibility of preparing compounds with different physico-chemical properties. We chose two hydroxybenzaldehydes (*o*- and *p*-hydroxybenzaldehyde) for the preparation of novel Schiff bases of sulfonamides **6–8**, as it was previously shown[22,23] that this type of Schiff base possesses good CA inhibitory properties. The six new compounds obtained in this way were subsequently derivatized by reaction with five different arylsulfonyl isocyanates. The new compounds reported here were assayed for the inhibition of three CA isozymes, hCA I, hCA II and bCA IV (h = human, b = bovine isozyme), and showed inhibition constants in the range 10^{-8}–10^{-9} M (against isozymes II and IV) for the most active compounds.

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 Gemini 200 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.

Sulfonamides (sulfanilamide **6**, homosulfanilamide **7** and *p*-(2-aminoethyl)-benzene-sulfonamide **8**), aromatic aldehydes **9a,b** and arylsulfonyl isocyanates **16a–e** used in synthesis were commercially available from Sigma, Aldrich or Fluka. Solvents 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.*[31] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group,[32] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[33] 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.[34,35] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[36]

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.[37] 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.[37] 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 0.1 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constant K_I was determined as described in reference.[37] Enzyme concentrations were 2.6 nM for CA II, 10 nM for CA I and 27 nM for CA IV (this isozyme has a decreased esterase activity and higher concentrations had to be used for the measurements).[13]

General Procedure for the Preparation of Schiff Bases 10–15

An amount of 10 mMol of sulfonamide **6–8** was dissolved in 40 mL of boiling methanol or ethanol and the required amount (10 mmol) of aldehyde **9** was added to the reaction mixture, together with a small amount of 4-toluenesulfonic acid as catalyst. Boiling was continued for 3–8 h, then a portion of the solvent was evaporated in vacuum, and by cooling crystals of Schiff bases **10–15** were obtained which were recrystallized from 96% ethanol or solvents specified in each case. Yields were generally high (see later in the text). Some of these compounds (such as **10**, **12**) have been reported previously by us.[22,23]

General Procedure for the Reaction of the Schiff Bases 10–15 with Arylsulfonyl Isocyanates

A variant of the method previously reported by us[28c] for the preparation of arylsulfonylureido sulfonamide CA inhibitors has been developed for the reaction of the Schiff bases **10–15** with arylsulfonyl isocyanates **16a-e**. An amount of 10 mM sulfonamide **10–15** was dissolved/suspended in 50 mL of

anhydrous acetonitrile and then treated with a solution obtained from 10 mM of the isocyanates **16** dissolved in 10 mL of the same solvent. The reaction was performed by magnetically stirring the reaction mixture at room temperature for 2–5 h (TLC control). 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, when the reaction products precipitated and were filtered. The prepared compounds were recrystallized from ethanol or ethanol-water (1:1, v/v). Yields were practically quantitative. All the new compounds were fully characterized by means of analytic and spectroscopic methods. Data for some representative derivatives of each type reported in the paper are provided below.

4-(2-Hydroxybenzylidene)-aminobenzenesulfonamide **10**: as pale yellow crystals, (yield 89%), m.p. 260–2°C. IR (KBr), cm^{-1}: 1120 (SO_2^{sym}), 1300 (SO_2^{as}), 1620 (C=N), 3300 (NH), 3550 (OH); ^{1}H-NMR (DMSO-d6), δ, ppm: 7.20 (br s, 2H, SO_2NH_2), 7.29 (d, 2H, H3 and H5 from 1,4-phenylene), 7.76 (d, 2H, ArH, H2 and H6 from 1,4-phenylene), 7.40–7.95 (m, 4H, ArH from *o*-HO-C_6H_4), 8.42 (s, 1H, C*H*=N) Found: C, 56.40; H, 4.56; N, 10.01. $C_{13}H_{12}N_2O_3S$ requires; C, 56.51; H, 4.38; N, 10.14%.

4-(2-Hydroxybenzylidene)-aminomethylbenzenesulfonamide **11**: as yellow crystals, (yield 74%), m.p. 234–5°C. IR (KBr), cm^{-1}: 1170 (SO_2^{sym}), 1365 (SO_2^{as}), 1620 (C=N), 3300 (NH), 3550 (OH), ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.70 (s, 2H, CH_2), 6.90 (br s, 2H, SO_2NH_2), 7.33 (d, 2H, H3 and H5 from 1,4-phenylene), 7.82 (d, 2H, ArH, H2 and H6 from 1,4-phenylene), 7.47–7.99 (m, 4H, ArH from *o*-HO-C_6H_4), 8.45 (s, 1H, C*H*=N). Found: C, 57.80; H, 4.78; N, 9.54. $C_{14}H_{14}N_2O_3S$ requires; C, 57.92; H, 4.86; N, 9.65%.

4-(2-Hydroxybenzylidene)-aminoethylbenzenesulfonamide **12**: as yellow crystals, (yield of 82%), m.p. 201–3°C. IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1350 (SO_2^{as}), 1620 (C=N), 3300 (NH + OH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.05 (t, 2H, αCH_2), 3.95 (t, 2H, βCH_2), 7.00 (br s, 2H, SO_2NH_2), 7.30 (d, 2H, H3 and H5 from phenylene), 7.80 (d, 2H, ArH, H2 and H6 from phenylene), 7.45–7.98 (m, 4H, ArH from *o*-HO-C_6H_4), 8.45 (s, 1H, C*H*=N). Found: C, 59.40; H, 5.56; N, 9.01. $C_{15}H_{16}N_2O_3S$ requires; C, 59.19; H, 5.30; N, 9.20%.

4-(4-Hydroxybenzylidene)-aminobenzenesulfonamide **13**: as pale yellowish crystals, (yield of 89%), m.p. 288–9°C. IR (KBr), cm^{-1}: 1120 (SO_2^{sym}), 1310 (SO_2^{as}), 1620 (C=N), 3300 (NH), 3500 (OH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.24 (br s, 2H, SO_2NH_2), 7.32 (d, 4H, H3 and H5 from the two 1,4-phenylene moieties), 7.82 (d, 4H, ArH, H2 and H6 from the two

1,4-phenylenes), 8.39 (s, 1H, C*H*=N). Found: C, 56.63; H, 4.41; N, 10.12. $C_{13}H_{12}N_2O_3S$ requires; C, 56.51; H, 4.38; N, 10.14%.

4-(4-Hydroxybenzylidene)-aminomethylbenzenesulfonamide **14**: as pale yellow crystals, (yield of 77%), m.p. 223–5°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1360 (SO_2^{as}), 1620 (C=N), 3300 (NH), 3500 (OH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.68 (s, 2H, CH_2), 6.96 (br s, 2H, SO_2NH_2), 7.33 (d, 4H, H3 and H5 from the two 1,4-phenylene moieties), 7.84 (d, 4H, ArH, H2 and H6 from the two 1,4-phenylenes), 8.40 (s,1H, C*H*=N). Found: C, 57.89; H, 4.95; N, 9.56. $C_{14}H_{14}N_2O_3S$ requires; C, 57.92; H, 4.86; N, 9.65%.

4-(4-Hydroxybenzylidene)-aminoethylbenzenesulfonamide **15**: as pale tan crystals, (yield of 63%), m.p. 176–7°C. IR (KBr), cm^{-1}: 1130 (SO_2^{sym}), 1355 (SO_2^{as}), 1620 (C=N), 3300 (NH), 3500 (OH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.07 (t, 2H, αCH_2), 3.90 (t, 2H, βCH_2), 7.00 (br s, 2H, SO_2NH_2), 7.31 (d, 4H, H3 and H5 from the two 1,4-phenylene moieties), 7.78 (d, 4H, ArH, H2 and H6 from the two 1,4-phenylenes), 8.41 (s,1H, C*H*=N). Found: C, 59.50; H, 5.07; N, 9.00. $C_{15}H_{16}N_2O_3S$ requires; C, 59.19; H, 5.30; N, 9.20%.

4-[2-(Phenylsulfamoylcarboxy)-benzylidene]aminobenzenesulfonamide **17**: as white crystals, m.p. 289–91°C (dec.). IR (KBr), cm^{-1}: 1121 and 1170 (SO_2^{sym}), 1290 (amide III), 1300 and 1380 (SO_2^{as}), 1540 (amide II), 1624 (C=N), 1675 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.20 (br s, 2H, SO_2NH_2), 7.04–7.98 (m, 13H, ArH, from Ph + $C_6H_4SO_2NH_2$ + *o*-substituted-C_6H_4), 8.45 (s,1H, C*H*=N), 10.13 (s, 1H, SO_2N*H*-CO). Found: C, 52.40; H, 3.56; N, 8.85. $C_{20}H_{17}N_3O_6S_2$ requires; C, 52.28; H, 3.73; N, 9.14%.

4-[4-(Phenylsulfamoylcarboxy)-benzylidene]-aminomethylbenzenesulfonamide **21**: as white crystals m.p. > 300°C. IR (KBr), cm^{-1}: 1150 and 1165 (SO_2^{sym}), 1280 (amide III), 1310 and 1370 (SO_2^{as}), 1540 (amide II), 1627 (C=N), 1670 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.71 (s, 2H, CH_2), 6.99 (br s, 2H, SO_2NH_2), 7.00–7.75 (m, 13H, ArH, from Ph + $C_6H_4SO_2NH_2$ + *p*-substituted-C_6H_4), 8.40 (s,1H, C*H*=N), 10.04 (s, 1H, SO_2N*H*-CO). Found: C, 53.39; H, 3.92; N, 8.64. $C_{21}H_{19}N_3O_6S_2$ requires; C, 53.27; H, 4.04; N, 8.87%.

4-[2-(p-Fluorophenylsulfamoylcarboxy)-benzylidene]-aminomethylbenzenesulfonamide **24**: as white crystals m.p. > 300°C. IR (KBr), cm^{-1}: 1160 and 1175 (SO_2^{sym}), 1290 (amide III), 1310 and 1360 (SO_2^{as}), 1540 (amide II), 1624 (C=N), 1675 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.70 (s, 2H, CH_2), 6.98 (br s, 2H, SO_2NH_2), 7.00–7.91 (m, 12H, ArH, from *p*-FC_6H_4 + $C_6H_4SO_2NH_2$ + *o*-substituted-C_6H_4), 8.40 (s, 1H, C*H*=N), 10.13 (s, 1H, SO_2N*H*-CO). Found: C, 51.50; H, 3.76; N, 8.31. $C_{21}H_{18}FN_3O_6S_2$ requires; C, 51.32; H, 3.68; N, 8.55%.

4-[4-(p-Fluorophenylsulfamoylcarboxy)-benzylidene]-aminoethylbenzenesulfonamide **28**: as tan crystals, m.p. 280–1°C. IR (KBr), cm^{-1}: 1130 and 1175 (SO_2^{sym}), 1290 (amide III), 1340 and 1360 (SO_2^{as}), 1540 (amide II), 1620 (C=N), 1675 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.05 (t, 2H, αCH_2), 3.90 (t, 2H, βCH_2), 7.04 (br s, 2H, SO_2NH_2), 7.00–7.96 (m, 12H, ArH, from *p*-$FC_6H_4 + C_6H_4SO_2NH_2$ + *p*-substituted-C_6H_4), 8.41 (s, 1H, C*H*=N), 10.24 (s, 1H, SO_2NH-CO). Found: C, 52.08; H, 4.13; N, 8.13. $C_{22}H_{20}FN_3O_6S_2$ requires; C, 52.27; H, 3.99; N, 8.31%.

4-[2-(p-Chlorophenylsulfamoylcarboxy)benzylidene]aminobenzenesulfonamide **29**: as white crystals, m.p. 299–300°C (dec.), IR (KBr), cm^{-1}: 1120 and 1175 (SO_2^{sym}), 1290 (amide III), 1310 and 1365 (SO_2^{as}), 1540 (amide II), 1620 (C=N), 1675 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05 – 7.90 (m, 12H, ArH, from *p*-$ClC_6H_4 + C_6H_4SO_2NH_2$ + *o*-substituted-C_6H_4), 7.23 (br s, 2H, SO_2NH_2), 8.44 (s,1H, C*H*=N), 10.11 (s, 1H, SO_2NH-CO). Found: C, 48.42; H, 3.39; N, 8.30. $C_{20}H_{16}ClN_3O_6S_2$ requires; C, 48.63; H, 3.27; N, 8.51%.

4-[4-(Chlorophenylsulfamoylcarboxy)benzylidene]aminomethylbenzenesulfonamide **33**: as white crystals m.p. > 300°C. IR (KBr), cm^{-1}: 1160 and 1185 (SO_2^{sym}), 1290 (amide III), 1350 and 1365 (SO_2^{as}), 1540 (amide II), 1620 (C=N), 1670 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.73 (s, 2H, CH_2), 6.97 (br s, 2H, SO_2NH_2), 7.01 – 7.86 (m, 12H, ArH, from *p*-$ClC_6H_4 + C_6H_4SO_2NH_2$ + *p*-substituted-C_6H_4), 8.39 (s,1H, C*H*=N), 10.10 (s, 1H, SO_2NH-CO). Found: C, 49.80; H, 3.56; N, 8.11. $C_{21}H_{18}ClN_3O_6S_2$ requires; C, 49.65; H, 3.57; N, 8.27%.

4-[2-(o-Toluenesulfamoylcarboxy)benzylidene]aminomethylbenzenesulfonamide **36**: as white crystals m.p. > 300°C. IR (KBr), cm^{-1}: 1123 and 1170 (SO_2^{sym}), 1290 (amide III), 1350 and 1370 (SO_2^{as}), 1540 (amide II), 1620 (C=N), 1680 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.62 (s, 3H, Me from *o*-tosyl), 4.71 (s, 2H, CH_2), 6.93 (br s, 2H, SO_2NH_2), 7.06 – 7.99 (m, 12H, ArH, from *o*-$MeC_6H_4 + C_6H_4SO_2NH_2$ + *o*-substituted-C_6H_4), 8.45 (s,1H, C*H*=N), 9.88 (s, 1H, SO_2NH-CO). Found: C, 54.41; H, 4.28; N, 8.43. $C_{22}H_{21}N_3O_6S_2$ requires; C, 54.20; H, 4.34; N, 8.62%.

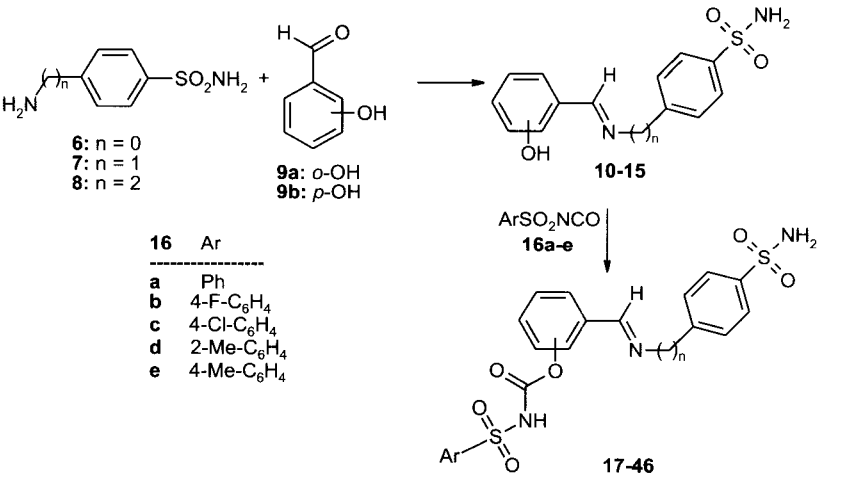
4-[4-(o-Toluenesulfamoylcarboxy)benzylidene]aminomethylbenzenesulfonamide **39**: as white crystals m.p. > 300°C. IR (KBr), cm^{-1}: 1125 and 1160 (SO_2^{sym}), 1290 (amide III), 1350 and 1365 (SO_2^{as}), 1540 (amide II), 1620 (C=N), 1680 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.60 (s, 3H, Me from *o*-tosyl), 4.68 (s, 2H, CH_2), 6.96 (br s, 2H, SO_2NH_2), 7.02–8.98 (m, 12H, ArH, from *o*-$MeC_6H_4 + C_6H_4SO_2NH_2$ + *p*-substituted-C_6H_4), 8.41 (s,1H, C*H*=N), 9.96 (s, 1H, SO_2NH-CO). Found: C, 54.17; H, 4.65; N, 8.59. $C_{22}H_{21}N_3O_6S_2$ requires; C, 54.20; H, 4.34; N, 8.62%.

4-[2-(p-Toluenesulfamoylcarboxy)benzylidene]aminobenzenesulfonamide **41**: as white crystals, m.p. 277–8°C, IR (KBr), cm^{-1}: 1120 and 1160 (SO_2^{sym}), 1290 (amide III), 1300 and 1360 (SO_2^{as}), 1540 (amide II), 1620 (C=N), 1675 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me from *p*-tosyl), 7.15 (br s, 2H, SO_2NH_2), 7.07–7.93 (m, 12H, ArH, from *p*-MeC_6H_4 + $C_6H_4SO_2NH_2$ + *o*-substituted-C_6H_4), 8.43 (s,1H, C*H*=N), 9.90 (s, 1H, SO_2NH-CO). Found: C, 53.40; H, 4.12; N, 8.71. $C_{20}H_{17}N_3O_6S_2$ requires; C, 53.27; H, 4.04; N, 8.87%.

4-[4-(p-Toluenesulfamoylcarboxy)benzylidene]aminoethylbenzenesulfonamide **46**: as white crystals, m.p. 285–6°C. IR (KBr), cm^{-1}: 1130 and 1160 (SO_2^{sym}), 1290 (amide III), 1350 and 1370 (SO_2^{as}), 1540 (amide II), 1620 (C=N), 1675 (amide I), 3300 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.04 (t, 2H, αCH_2), 3.89 (t, 2H, βCH_2), 7.00 (br s, 2H, SO_2NH_2), 7.05–7.89 (m, 12H, ArH, from *p*-MeC_6H_4 + $C_6H_4SO_2NH_2$ + *p*-substituted-C_6H_4), 8.41 (s,1H, C*H*=N), 9.94 (s, 1H, SO_2NH-CO). Found: C, 55.04; H, 4.45; N, 8.13. $C_{23}H_{23}N_3O_6S_2$ requires; C, 55.08; H, 4.62; N, 8.38%.

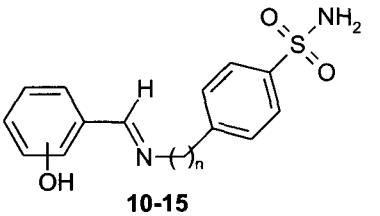
RESULTS AND DISCUSSION

The Schiff bases **10–15** prepared from sulfanilamide, homosulfanilamide or *p*-(2-aminoethyl)-benzenesulfonamide and *o*-/*p*-hydroxybenzaldehydes,

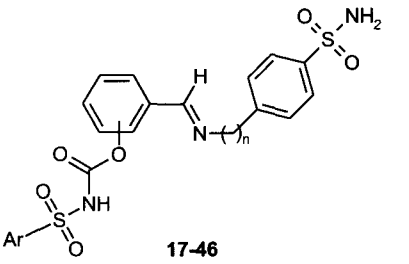


SCHEME 1 Synthesis of compounds under study.

TABLE II CA inhibition data with standard inhibitors **1–4**, the parent sulfonamides **6–8** and the new derivatives reported in the present study, against isozymes I, II and IV



10-15



17-46

Inhibitor	*n*	*OH (OR) group*	*X*	*hCA I* (μM)	K_I *hCA II*[a] (× 10^8M)	*bCA IV*[b] (× 10^8M)
1 Acetazolamide		–	–	0.2	0.7	12
2 Methazolamide		–	–	0.1	0.9	14
3 Ethoxzolamide		–	–	0.025	0.5	1.3
4 Dorzolamide		–	–	50	0.9	4.5
6 Sulfanilamide		–	–	28	30	30
7		–	–	25	17	28
8	–	–	21	16	24	
10	0	*o*	–	19	22	34
11	1	*o*	–	13	19	24
12	2	*o*	–	11	13	18
13	0	*p*	–	10	12	15
14	1	*p*	–	8	7	14
15	2	*p*	–	6	6	15
17	0	*o*	H	12	22	29
18	1	*o*	H	11	19	25
19	2	*o*	H	7	13	15
20	0	*p*	H	8	8	10
21	1	*p*	H	4	3	7
22	2	*p*	H	2.6	1.5	5
23	0	*o*	4-F	11	23	28
24	1	*o*	4-F	10	19	25
25	2	*o*	4-F	8	13	16
26	0	*p*	4-F	8	9	10
27	1	*p*	4-F	5	2.9	6
28	2	*p*	4-F	2.5	1.6	5
29	0	*o*	4-Cl	8	21	23
30	1	*o*	4-Cl	7	19	22
31	2	*o*	4-Cl	6	10	16
32	0	*p*	4-Cl	6	6	7
33	1	*p*	4-Cl	5	1.9	6
34	2	*p*	4-Cl	2.2	1.5	1.8
35	0	*o*	2-Me	10	20	24
36	1	*o*	2-Me	9	18	20
37	2	*o*	2-Me	8	13	15
38	0	*p*	2-Me	8	6	8
39	1	*p*	2-Me	7	1.2	5
40	2	*p*	2-Me	4	1.5	5
41	0	*o*	4-Me	3	15	21

42	1	*o*	4-Me	4	16	20
43	2	*o*	4-Me	4	11	12
44	0	*p*	4-Me	3	4	7
45	1	*p*	4-Me	4	0.6	5
46	2	*p*	4-Me	2	0.5	4

o = ortho; *p* = para derivative; [a] Human (cloned) isozymes; [b] From bovine lung microsomes.

reported in the present study, with their CA inhibitory properties against isozymes I, II and IV, are shown in Table II. By their reaction with the arylsulfonyl isocyanates **16a-b**, the new derivatives **17–46** have also been synthesized (Scheme 1) and assayed for inhibition of the three CA isozymes mentioned above (Table II).

The reaction of arylsulfonyl isocyanates[28c,38] with active hydrogen-containing compounds, such as amines or alcohols/phenols, has thoroughly been investigated due to the many possible applications of the obtained derivatives as polymers (plastics), insecticides or biologically active substances with potential pharmacological use.[28c,38–40]

Here (Scheme 1), this reaction proceeded smoothly at the phenolic OH moiety, leading practically to quantitative yields to the arylsulfonylcarbamates **17–46**. The new compounds reported here (**10–15** and **17–46**) were characterized by elemental analysis (± 0.4% of the theoretical data calculated for the proposed formulas) and spectroscopic methods that confirmed their structures.

Enzyme inhibition data in Table II show the following features of these new sulfonamide CA inhibitors: (i) the *p*-aminoethylbenzenesulfonamide derivatives were more active than the corresponding homosulfanilamide derivatives, which in turn were better inhibitors than the sulfanilamides bearing the same substitution pattern, in agreement with data obtained for the previously reported Schiff bases of aromatic sulfonamides,[22,23] and QSAR studies of our laboratories.[41] This behavior was evidenced both for the simple derivatives of type **10–15**, as well as for the arylsulfonylcarbamates of type **17–46** (Table II). It should also be noted that all the new derivatives **10–15** and **17–46** were stronger inhibitors than the corresponding parent sulfonamides **6–8** from which they were obtained; (ii) without exception, the salicylaldehyde Schiff bases were less active than their *para*-isomers; (iii) regarding the substitution pattern at the arylsulfonylcarbamate moiety, activity decreased in the following way: 4-Me > 2-Me ≅ (4-Cl > 4-F) > H. Thus, compound (**46**) prepared from *p*-tosyl isocyanate, *p*-hydroxybenzaldehyde and 4-(2-aminoethyl)-benzene sulfonamide was the best inhibitor in the entire series of new derivatives reported here; (iv) an interesting feature of some of the new inhibitors (which was

already been present in some other compounds reported previously by us)[28c,42] is that they tended to slightly discriminate in selectivity towards CA I as compared to CA II and CA IV. Thus, several compounds such as **17–19, 24–26, 29–31, 36–38** and **42–46** possessed higher affinities for hCA I as compared to hCA II and bCA IV (Table II). This constitutes an encouraging result for obtaining more selective, isozyme-specific CA I inhibitors. In the case of the parent Schiff bases from which the latter were prepared (**10–15**), the same trend in activity/selectivity against the different investigated CA isozymes was observed, but differences in affinity were not so important. In contrast to the above-mentioned compounds, the *p*-hydroxybenzaldehyde Schiff bases generally possessed the usual,[3,4,23–28] "normal" affinity for the different isozymes investigated here, with the highest affinity for CA II, followed by CA IV ≅ CA I.

In conclusion, in the present paper we report novel Schiff bases obtained by derivatization of some hydroxybenzaldehyde derivatives. Some of the new derivatives reported here possess a slightly increased affinity in binding to the slow isozyme hCA I versus the membrane bound isozyme bCA IV and the cytosolic, very rapid one, hCA II. This is another small step on the presumably long road of obtaining isozyme-specific CA inhibitors.

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