CARBONIC ANHYDRASE INHIBITORS. Part 59[1] INHIBITION OF CARBONIC ANHYDRASE ISOZYMES I, II AND IV WITH ARSANILIC ACID DERIVATIVES

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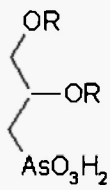
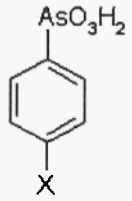
Abstract: Reaction of arsanilic acid with sulfonyl halides, sulfenyl halides, acyl chlorides, isocyanates, isothiocyanates and cyanamide afforded a series of derivatives possessing the general formula RNH-C_6H_4-AsO_3H_2, which were characterized by standard physico-chemical procedures. The new derivatives showed good inhibitory activity against three isozymes of carbonic anhydrase (CA), i.e., CA I, II and IV. Structure-activity correlations in the series of prepared enzyme inhibitors are discussed too.

Introduction

In addition to the inorganic complexing anions [2,3] and the unsubstituted sulfonamides possessing the general formula R-SO_2NH_2 [4-6], the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) has recently been shown to possess several other classes of potent inhibitors, such as the hydroxamic acids of the type RCONHOH [7,8], some heterocyclic mercaptans and their metal complexes [9-11], as well as some derivatives containing Ge(IV); P(V); As(III) and As(V) in their molecules [12-16]. In the series of investigated derivatives, the arsonolipids of type **1a,b** showed very good CA inhibitory properties [15] and this prompted us to investigated other derivatives possessing the arsonic acid moiety in their molecule. The most simple aromatic such derivative, benzenearsonic acid **2a** was thus the first to be investigated, since it is known that in the sulfonamide series of CA inhibitors, aromatic derivatives possessing the general formula Ar-SO_2NH_2 are 100-1000 times more potent inhibitors than the aliphatic derivatives R-SO_2NH_2 [4-6].



1a: R = H
1b: R = *n*-$C_{15}H_{31}CO$



2a: X = H
2b: X = NH_2

The interesting inhibitory properties of **2a**, reported here for the first time, led us to consider it as lead molecule for developing more potent CA inhibitors from this class of compounds. Thus, starting from arsanilic acid **2b**, a large series of 4-substituted-benzenearsonic acids of type **3-34** were prepared, by reaction of the amino-compound **2b** with sulfonyl halides, sulfenyl halides, acyl chlorides, organic and inorganic isocyanates/isothiocyanates as well as cyanamide. The new compounds were characterized by elemental analysis, IR and ^{1}H-NMR spectroscopy, and were assayed as inhibitors of three physiologically relevant CA isozymes, CA I, II and IV.

Materials and Methods

IR spectra were recorded on a Perkin-Elmer 16PC FTIR instrument, in the range 400-4000 cm^{-1}, in KBr pellets. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and gravimetrically for arsenic, and were 0.4% of the theoretical values. NMR spectra were recorded in DMSO-d_6 as solvent with a Varian CPX-300 instrument.

Arsonic acids **2a, b**; sulfonyl halides, sulfenyl halides; acyl chlorides; isocyanates and other reagents used for the preparation of the new derivatives described in the present paper were commercially available from Aldrich, Sigma or E. Merck, and were used without further purification.

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 (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group [17] and enzymes were purified by affinity chromatography according to the method of Khalifah et al. [18]. Enzyme

concentrations were determined spectrophotometrically at 280 nm, using 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 29.3 kDa for CA II, respectively [19]. CA IV was isolated from bovine lung microsomes [20].

Initial rates of 4-nitrophenyl acetate hydrolysis were monitored spectrophotometrically, at 400 nm and 25°C, with a Cary 3 apparatus interfaced with an IBM compatible PC by the method of Pocker and Stone [21]. Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 10^{-2} and 10^{-6} M. A molar absorption coefficient equal to 18,400 $M^{-1}.cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.80), as reported by Pocker and Stone [21]. Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor, and the values reported throughout the paper are the averages of such results. IC_{50} represents the molarity of inhibitor producing a 50% decrease of enzyme catalyzed hydrolysis of 4-nitrophenyl acetate.

Synthesis of derivatives **3-34**

Methods A and B: 108 mg (5 mmol) of arsanilic acid suspended in 10 mL of acetonitrile were treated with 5 mmol of sulfonyl/sulfenyl chloride (method A) or fluoride (method B) dissolved in a small amount of anhydrous acetonitrile. The stoichiometric amount of triethylamine was added, and the mixture was stirred at 40°C for 4 hours (A) or at 60°C for 6 hours (B), then the solvent was evaporated *in vacuo* and the reaction mixture poured into 10 mL of water and ice. The precipitated sulfonylamido derivatives were recrystallized from ethanol-water (1:1, v/v).

Method C: 217 mg (10 mmol) of arsanilic acid and 0.84 mL (5 mmol) of triflic anhydride were suspended in 10 mL of acetone and magnetically stirred at 4°C for 15 hours. The solvent was then evaporated in vacuo, and the tan residue treated with 10 mL of cold water. The triflate salt of arsanilic acid being water-soluble has thus been separated from **5** by filtration. The latter compound was recrystallized from ethanol.

Method D: 108 mg (5 mmol) of arsanilic acid and 5 mmol of sulfobenzoic cyclic anhydride or tetrabromo-*O*-sulfobenzoic cyclic anhydride were heated at refluxation in 50 mL of anhydrous acetonitrile for 2 hours, with a small amount of *p*-toluenesulfonic acid added as catalyst. After evaporation of the solvent, the products **19, 20** were recrystallized from ethanol.

Method E: 108 mg (5 mmol) of arsanilic acid and 10 mmol of isocyanate or isothiocyanate were heated at refluxation in 50 mL of anhydrous acetonitrile for 2-10 hours, with a small amount (0.2 mL) of triethylamine added as catalyst. After evaporation of the solvent, the crude products were recrystallized from ethanol or methanol.

Method F: 108 mg (5 mmol) of arsanilic acid and 10 mmol of potassium cyanate or potassium thiocyanate or cyanamide, respectively, were heated at refluxation in 50 mL of anhydrous ethanol for 2-10 hours, with the stoichiometric amount (5 mmol) of HCl (solution 10 % in methanol) added. After evaporation of the solvent, the crude products were recrystallized from ethanol or methanol.

4-(N,N-Dimethylsulfamoylamido)-benzenearsonic acid **3**: tan crystals, mp 219-22 °C. IR (KBr), cm^{-1}: 1140 (SO_2^{sym}), 1339 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ ppm: 4.80 (s, 6H, Me_2N); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.06 (s, 1H, SO_2NH); Anal., found: C, 29.41; H, 4.30; As, 23.36; N, 8.39 %; $C_8H_{13}AsN_2O_5S$ requires: C, 29.64; H, 4.40; As, 23.11; N, 8.64 %.

4-Phenylmethylsulfonylamido-benzenearsonic acid **4**: tan crystals, mp 269-71 °C. IR: 1176 (SO_2^{sym}), 1360 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: 3.17 (s, 2H, Ph*CH*$_2$); 7.12 - 7.49 (m, 5H, ArH from Ph); δ_A 7.54, δ_B 7.83, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.11 (s, 1H, SO_2NH); Anal., found: C, 42.40; H, 3.62; As, 20.39; N, 8.39 %; $C_{13}H_{14}AsNO_5S$ requires: C, 42.06; H, 3.80; As, 20.18; N, 3.77 %.

4-Trifluoromethylsulfonylamido-benzenearsonic acid **5**: colorless crystals, mp 192-3 °C. IR: 1169 (SO_2^{sym}), 1351 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.35 (s, 1H, SO_2NH); Anal., found: C, 23.90; H, 2.21; As, 21.33; N, 3.95 %; $C_7H_7AsF_3NO_5S$ requires: C, 24.08; H, 2.02; As, 21.46; N, 4.01 %.

4-(4-Fluorophenylsulfonylamido)-benzenearsonic acid 6: colorless crystals, mp 198-9 °C. IR: 1171 (SO_2^{sym}), 1366 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: δ_A 7.04, δ_B 7.35, J_{AB} 7.4, AA'BB' system, 4H, p-F-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.05 (s, 1H, SO_2NH); Anal., found: C, 39.10; H, 2.69; As, 20.01; N, 3.50 %; $C_{12}H_{11}AsFNO_5S$ requires: C, 38.91; H, 2.96; As, 19.97; N, 3.73 %.

4-(4-Chlorophenylsulfonylamido)-benzenearsonic acid **7**: colorless crystals, mp 210-2 °C. IR: 1175 (SO_2^{sym}), 1367 (SO_2^{as}), 3065 (NH); ^{1}H-NMR: δ_A 7.06, δ_B 7.37, J_{AB} 7.4, AA'BB' system, 4H, p-Cl-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.06 (s, 1H, SO_2NH); Anal., found: C, 39.05; H, 2.75; As, 19.05; N, 3.55 %; $C_{12}H_{11}AsClNO_5S$ requires: C, 38.80; H, 2.83; As, 19.13; N, 3.58 %.

4-(4-Bromophenylsulfonylamido)-benzenearsonic acid **8**: colorless crystals, mp 213-5 °C. IR: 1179 (SO_2^{sym}), 1376 (SO_2^{as}), 3065 (NH); ^{1}H-NMR: δ_A 7.03, δ_B 7.38, J_{AB} 7.4, AA'BB' system, 4H, p-Br-phenylene); δ_A 7.53, δ_B 7.85, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.05 (s, 1H, SO_2NH); Anal., found: C, 32.90; H, 2.15; As, 17.00; N, 3.06 %; $C_{12}H_{11}AsBrNO_5S$ requires: C, 33.05; H, 2.54; As, 17.18; N, 3.21 %.

4-(4-Iodophenylsulfonylamido)-benzenearsonic acid **9**: colorless crystals, mp 229-31 °C. IR: 1185 (SO_2^{sym}), 1380 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: δ_A 7.10, δ_B 7.40, J_{AB} 7.4, AA'BB' system, 4H, p-I-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.09 (s, 1H, SO_2NH); Anal., found: C, 29.55; H, 2.40; As, 15.30; N, 2.80 %; $C_{12}H_{11}AsINO_5S$ requires: C, 29.83; H, 2.30; As, 15.51; N, 2.90 %.

4-p-Tosylamido-benzenearsonic acid **10**: tan crystals, mp 188-9 °C. IR: 1165 (SO_2^{sym}), 1350 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: 2.50 (s, 3H, Me from tosyl); δ_A 7.06, δ_B 7.39, J_{AB} 7.4, AA'BB' system, 4H, p-Me-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.05 (s, 1H, SO_2NH); Anal., found: C, 31.95; H, 4.05; As, 20.00; N, 3.60 %; $C_{13}H_{14}AsNO_5S$ requires: C, 42.06; H, 3.80; As, 20.18; N, 3.77 %.

4-(4-Nitrophenylsulfonylamido)-benzenearsonic acid **11**: yellow crystals, mp 213-5 °C. IR: 1150 (SO_2^{sym}), 1366 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: δ_A 7.04, δ_B 7.34, J_{AB} 7.4, AA'BB' system, 4H, p-O_2N-phenylene); δ_A 7.54, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.10 (s, 1H, SO_2NH); Anal., found: C, 35.90; H, 2.60; As, 18.90; N, 6.60 %; $C_{12}H_{11}AsN_2O_7S$ requires: C, 35.83; H, 2.76; As, 18.63; N, 6.96 %.

4-(3-Nitrophenylsulfonylamido)-benzenearsonic acid **12**: yellow crystals, mp 208-9 °C. IR: 1152 (SO_2^{sym}), 1374 (SO_2^{as}), 3065 (NH); ^{1}H-NMR: 7.08 – 7.50 (m, 4H, ArH, *m*-O_2N-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.10 (s, 1H, SO_2NH); Anal., found: C, 35.75; H, 2.65; As, 18.45; N, 6.80 %; $C_{12}H_{11}AsN_2O_7S$ requires: C, 35.83; H, 2.76; As, 18.63; N, 6.96 %.

4-(2-Nitrophenylsulfonylamido)- benzenearsonic acid **13**: yellow crystals, mp 193-5 °C. IR: 1152 (SO_2^{sym}), 1369 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: 7.02 – 7.49 (m, 4H, ArH, *o*-O_2N-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.07 (s, 1H, SO_2NH); Anal., found: C, 35.80; H, 2.70; As, 18.50; N, 6.90 %; $C_{12}H_{11}AsN_2O_7S$ requires: C, 35.83; H, 2.76; As, 18.63; N, 6.96 %.

4-(3-Chloro-4-nitrophenylsulfonylamido)-benzenearsonic acid **14**: yellow crystals, mp 199-9 °C. IR: 1157 (SO_2^{sym}), 1369 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: 7.08 – 7.67 (m, 3H, ArH, 3-Cl-4-O_2N-phenyl); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.11 (s, 1H, SO_2NH); Anal., found: C, 32.96; H, 2.20; As, 17.00; N, 6.40 %; $C_{12}H_{10}AsClN_2O_7S$ requires: C, 33.01; H, 2.31; As, 17.16; N, 6.42 %.

4-(4-Acetylaminophenylsulfonylamido)-benzenearsonic acid **15**: colorless crystals, mp 275-6 °C. IR: 1155 (SO_2^{sym}), 1296 (amide III), 1350 (SO_2^{as}), 1533 (amide II); 1680 (amide I); 3066 (NH); ^{1}H-NMR: 1.80 (s, 3H, Me from Ac); δ_A 7.02, δ_B 7.39, J_{AB} 7.4, AA'BB' system, 4H, p-AcN-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.08 (s, 1H, SO_2NH); Anal., found: C, 39.80; H, 2.96; As, 19.20; N, 7.10 %; $C_{14}H_{15}AsN_2O_6S$ requires: C, 39.81; H, 3.34; As, 19.10; N, 7.14 %.

4-(4-Aminophenylsulfonylamido)-benzenearsonic acid **16**: colorless crystals, mp 239-42 °C. IR: 1155 (SO_2^{sym}), 1347 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: 5.42 (s, 2H, H_2N-phenylene); δ_A 7.05, δ_B 7.38, J_{AB} 7.4, AA'BB' system, 4H, p-H_2N-phenylene); δ_A 7.51, δ_B 7.83, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.11 (s, 1H, SO_2NH. Anal., found: C, 38.95; H, 3.30; As, 19.98; N, 7.40 %; $C_{12}H_{13}AsN_2O_5S$ requires: C, 38.83; H, 3.26; As, 20.18; N, 7.55 %.

4-(3-Aminophenylsulfonylamido)-benzenearsonic acid **17**: tan crystals, mp 227-9 °C. IR: 1172 (SO_2^{sym}), 1364 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: 5.11 (s, 2H, H_2N-phenylene) 7.21 - 7.45 (m, 4H, ArH, *m*-H_2N-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.05 (s, 1H, SO_2NH); Anal., found: C, 38.66; H, 3.20; As, 20.45; N, 7.50 %; $C_{12}H_{13}AsN_2O_5S$ requires: C, 38.83; H, 3.26; As, 20.18; N, 7.55 %.

4-(Pentafluorolphenylsulfonylamido-benzenearsonic acid **18**: white crystals, mp 150-3 °C (dec). IR: 1148 (SO_2^{sym}), 1330 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.38 (s, 1H, SO_2NH); Anal., found: C, 35.40; H, 2.21; As, 18.25; N, 4.98 %; $C_{12}H_7AsF_5NO_5S$ requires: C, 35.27; H, 2.22; As, 18.33; N, 5.14 %.

4-(2-Carboxyphenylsulfonylamido)-benzenearsonic acid **19**: tan crystals, mp 201-2 °C. IR: 1153 (SO_2^{sym}), 1355 (SO_2^{as}), 1720 (COOH); 3065 (NH); ^{1}H-NMR: 7.15 - 7.43 (m, 4H, ArH, *o*-HOOC-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.05 (s, 1H, SO_2NH); 10.15 (br s, 1H, COOH); Anal., found: C, 38.95; H, 3.10; As, 19.50; N, 5.40 %; $C_{13}H_{12}AsNO_7S$ requires: C, 38.92; H, 3.01; As, 19.42; N, 5.45 %.

4-(2-Carboxytetrabromophenylsulfonylamido)-benzenearsonic acid **20**: tan crystals, mp 187-9 °C (dec). IR: 1158 (SO_2^{sym}), 1371 (SO_2^{as}), 1720 (COOH); 3060 (NH); ^{1}H-NMR: δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB'

system, 4H, p-phenylene); 8.09 (s, 1H, SO_2NH); 10.40 (br s, 1H, COOH); Anal., found: C, 27.45; H, 1.84; As, 13.55; N, 3.80 %; $C_{13}H_8AsBr_4NO_7S$ requires: C, 27.62; H, 1.76; As, 13.78; N, 3.87 %.

4-(4-Methoxyphenylsulfonylamido)-benzenearsonic acid **21**: white crystals, mp 188-9 °C. IR: 1167 (SO_2^{sym}), 1350 (SO_2^{as}), 3060 (NH); ^{1}H-NMR: 3.50 (s, 3H, Me); δ_A 7.02, δ_B 7.39, J_{AB} 7.4, AA'BB' system, 4H, p-MeO-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.09 (s, 1H, SO_2NH); Anal., found: C, 39.75; H, 3.00; As, 20.10; N, 5.50 %; $C_{13}H_{14}AsNO_6S$ requires: C, 39.64; H, 3.33; As, 19.78; N, 5.55 %.

4-(2,4,6-Trimethylphenylsulfonylamido)-benzenearsonic acid **22**: tan crystals, mp 165-6 °C. IR: 1165 (SO_2^{sym}), 1355 (SO_2^{as}), 3065 (NH); ^{1}H-NMR: 2.50 (s, 3H, 4-Me); 2.71 (s, 6H, 2,6-Me_2); 7.35 (s, 2H, ArH, 3,5-H from mesityl); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 8.05 (s, 1H, SO_2NH); Anal., found: C, 42.15; H, 4.00; As, 19.30; N, 5.50 %; $C_{15}H_{18}AsNO_5S$ requires: C, 42.14; H, 3.80; As, 19.47; N, 5.46 %.

4-(N,N-Diphenylcarbamoylamido)-benzenearsonic acid **23**: white crystals, mp 198-9 °C. IR: 1295 (amide III); 1520 (amide II); 1680 (amide I); 3060 (NH); ^{1}H-NMR: 6.61 (br s, 1H, CONH); 7.18 - 7.43 (m, 10H, ArH from 2 Ph); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 49.70; H, 3.98; As, 19.75; N, 4.70 %; $C_{19}H_{17}AsN_2O_4$ requires: C, 49.84; H, 4.18; As, 19.84; N, 4.95 %.

4-(Isonicotinoylamido)- benzenearsonic acid **24**: white crystals, mp 190-1 °C. IR: 1290 (amide III); 1540 (amide II); 1680 (amide I); 3060 (NH); ^{1}H-NMR: δ_A 7.12, δ_B 7.61, J_{AB} 7.9, AA'BB' system, 4H, *iso*-nicotinoyl); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 7.98 (s, 1H, CONH); Anal., found: C, 45.97; H, 3.84; As, 21.55; N, 5.36 %; $C_{12}H_{11}AsN_2O_4$ requires: C, 46.08; H, 3.96; As, 21.56; N, 5.37 %.

4-(Nicotinoylamido)-benzenearsonic acid **25**: white crystals, mp 178-81 °C. IR: 1293 (amide III); 1540 (amide II); 1675 (amide I); 3060 (NH); ^{1}H-NMR: 7.02 – 7.57 (m, 4H, ArH from nicotinoyl); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); 7.98 (s, 1H, CONH); Anal., found: C, 46.10; H, 3.90; As, 21.35; N, 5.40 %; $C_{12}H_{11}AsN_2O_4$ requires: C, 46.08; H, 3.96; As, 21.56; N, 5.37 %.

4-(2,4-Dichlorophenylcarboxamido)-benzenearsonic acid **26**: white crystals, mp 177-9 °C. IR: 1300 (amide III); 1540 (amide II); 1700 (amide I); 3060 (NH); ^{1}H-NMR: 7.05 – 7.64 (m, 3H, ArH); 7.90 (s, 1H, CONH); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 42.54; H, 3.55; As, 19.00; N, 3.40 %; $C_{13}H_{10}AsCl_2NO_4$ requires: C, 42.61; H, 3.58; As, 18.98; N, 3.55 %.

4-(3,4-Dichlorophenylureido)-benzenearsonic acid **27**: white crystals, mp 218-9 °C. IR: 1290 (amide III), 1550 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR: 5.23 (s, 2H, HN-CO-NH); 7.25 - 7.39 (m, 3H, ArH, dichlorophenyl); δ_A 7.50, δ_B 7.87, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 46.50; H, 3.50; As, 18.34; N, 5.70 %; $C_{13}H_{11}AsCl_2N_2O_4$ requires: C, 46.40; H, 3.81; As, 18.47; N, 5.76 %.

4-[4-(Tosylsulfonylureido)]-benzenearsonic acid **28**: colorless crystals, mp 248-9 °C. IR: 1150 (SO_2^{sym}), 1290 (amide III), 1360 (SO_2^{as}), 1566 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR: 2.50 (s, 3H, Me from tosyl); 5.20 (br s, 2H, HN-CO-NH); δ_A 7.02, δ_B 7.38, J_{AB} 7.4, AA'BB' system, 4H, p-Me-phenylene); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 46.86; H, 3.95; As, 18.05; N, 5.65 %; $C_{14}H_{15}AsN_2O_6S$ requires: C, 47.03; H, 4.11; As, 18.34; N, 5.71 %.

N^1-(4-Arsonylphenyl)-N^3-allyl-thiourea **29**, white crystals, mp 193-4 °C. IR: 1040 (thioamide III), 1547 (thioamide I), 3298 (NHCSNH); ^{1}H-NMR: 4.45 - 4.60 (m, 2H, CSNHCH_2); 5.60 - 5.97 (m, 3H, CH=CH_2); 6.70 and 6.82 (br s, 2H, NHCSNH); δ_A 7.06, δ_B 7.37, J_{AB} 7.4, AA'BB' system, 4H, p-Cl-phenylene); Anal., found: C, 46.90; H, 4.25; As, 19.75; N, 6.10 %; $C_{10}H_{13}AsN_2O_3S$ requires: C, 46.86; H, 4.29; As, 19.93; N, 6.21 %.

4-(4-Nitrobenzenesulfenylamido)-benzenearsonic acid **30**, yellow crystals, m.p. 212-3 °C, IR: 1075 and 1250 (NO_2), 1490, 1585 (C=C); 3230 (NH); ^{1}H-NMR: 5.12 (br s, 1H, NH); δ_A 7.07, δ_B 7.37, J_{AB} 7.3, AA'BB' system, 4H, p-O_2N-phenylene); δ_A 7.51, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 37.15; H, 2.70; As, 19.90; N, 7.10 %; $C_{12}H_{11}AsN_2O_5S$ requires: C, 37.32; H, 2.87; As, 19.90; N, 7.25 %.

4-(2-Nitrobenzenesulfenylamido)-benzenearsonic acid **31**, pale yellow crystals, m.p. 175-7 °C, IR: 1080 and 1250 (NO_2), 1490, 1585 (C=C); 3260 (NH); ^{1}H-NMR: 5.19 (br s, 1H, NH); 7.29 - 7.53 (m, 4H, Ar H from *ortho*-substituted phenyl); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 37.40; H, 2.80; As, 19.95; N, 7.20 %; $C_{12}H_{11}AsN_2O_5S$ requires: C, 37.32; H, 2.87; As, 19.90; N, 7.25 %.

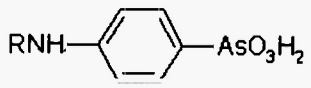
4-(Ureido)-benzenearsonic acid **32**, colorless crystals, m.p. 273-4 °C, IR: 1155 (SO_2^{sym}), 1290 (amide III), 1380 (SO_2^{as}), 1560 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR: 5.20 (br s, 3H, H_2N-CO-NH); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 46.05; H, 3.95; As, 21.00; N, 6.40 %; $C_7H_9AsN_2O_4$ requires: C, 45.95; H, 4.14; As, 20.97; N, 6.55 %.

4-(Thioureido)-benzenearsonic acid **33**, white crystals, mp 189-91 °C. IR: 1040 (thioamide III), 1540 (thioamide I), 3280 (NHCSNH); ^{1}H-NMR: 6.77 (br s, 3H, H_2NCSNH); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 45.30; H, 3.92; As, 20.70; N, 6.30 %; $C_7H_9AsN_2O_3S$ requires: C, 45.27; H, 4.08; As, 20.66; N, 6.44 %.
4-(Guanidino)-benzenearsonic acid **34**, white crystals, mp 189-91 °C. IR: 1035, 1545, 1710, 3260; ^{1}H-NMR: 6.20 - 6.59 (br s, 4H, $H_2NC(=NH)NH$-); δ_A 7.52, δ_B 7.86, J_{AB} 8.5, AA'BB' system, 4H, p-phenylene); Anal., found: C, 46.15; H, 4.10; As, 21.00; N, 7.70 %; $C_7H_{10}AsN_3O_3$ requires: C, 45.99; H, 4.24; As, 20.99; N, 7.85 %.

Results and Discussion

The new compounds **3-34** prepared by reaction of arsanilic acid **2b** with sulfonyl and sulfenyl halides, acyl chlorides, isocyanates, isothiocyanates and cyanamide are shown in Table I, together with the synthetic procedure used. Basically the methods previously devised [22, 23] for the preparation of sulfonylamido derivatives of aminoglutethimide or 2-aminophenoxathiine have been also employed in the present work.

Table I: Arsanilic acid derivatives **3-34** prepared in the present study and their synthesis method.

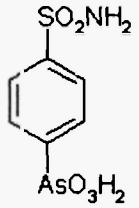


3-34

Compound	R	Yield (%)	Synthesis method
3	Me_2NSO_2	54	A
4	$PhCH_2SO_2$	59	B
5	CF_3SO_2	66	C
6	*p*-F-C_6H_4-SO_2	62	A
7	*p*-Cl-C_6H_4-SO_2	76	A
8	*p*-Br-C_6H_4-SO_2	78	A
9	*p*-I-C_6H_4-SO_2	84	A
10	*p*-CH_3-C_6H_4-SO_2	69	A
11	*p*-O_2N-C_6H_4-SO_2	74	A
12	*m*-O_2N-C_6H_4-SO_2	55	A
13	*o*-O_2N-C_6H_4-SO_2	38	A
14	3-Cl-4-O_2N-C_6H_3-SO_2	59	A
15	*p*-AcNH-C_6H_4-SO_2	92	A
16	*p*-H_2N-C_6H_4-SO_2	96	B
17	*m*-H_2N-C_6H_4-SO_2	39	B
18	C_6F_5-SO_2	82	A
19	*o*-HOOC-C_6H_4-SO_2	93	D
20	o-HOOC-C_6Br_4-SO_2	81	D
21	*p*-CH_3O-C_6H_4-SO_2	64	A
22	2,4,6-$(CH_3)_3$-C_6H_2-SO_2	69	A
23	Ph_2N-CO	95	A
24	isonicotinoyl	66	A
25	nicotinoyl	57	A
26	2,4-$Cl_2C_6H_3CO$	58	A
27	3,4-$Cl_2C_6H_3NHCO$	91	E
28	p-Me-$C_6H_4SO_2NHCO$	97	E
29	CH_2=$CHCH_2NHCS$	39	E
30	*p*-O_2N-C_6H_4-S	55	A
31	*o*-O_2N-C_6H_4-S	40	A
32	H_2N-CO	78	F
33	H_2N-CS	81	F
34	H_2N-C(=NH)-	75	F

A – Arsanilic acid + RSO_2Cl (or RCOCl or RSCl) ; B - Arsanilic acid + RSO_2F; C - Arsanilic acid + triflic anhydride; D - Arsanilic acid + sulfobenzoic cyclic anhydride; E - Arsanilic acid + RNCO (or RNCS); F - Arsanilic acid + KCNO (or KSCN or cyanamide).

Mention should be made that some As(V) derivatives containing sulfonamido- or substituted-sulfonamido moieties in their molecule, such as **35-37**, have been prepared in the search of more efficient antibacterial sulfonamides [24,25], but such compounds have never been investigated for their interaction with CA.



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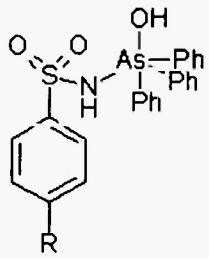

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(R = Me; AcNH; NH_2)

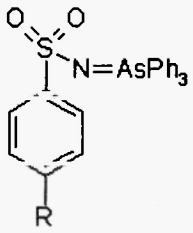

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Table II. CA inhibition data with the benzenarsonic acid derivatives **2-34** against isozymes CA I, II and IV.

No	Inhibitor	K_I (M)	
	hCA I[a]	hCA II[a]	bCA IV[b]
2a	620	120	240
2b	600	109	210
3	340	35	44
4	250	28	70
5	90	4	19
6	250	18	36
7	280	20	42
8	360	24	41
9	400	25	45
10	375	35	43
11	220	10	14
12	215	9	11
13	210	9	10
14	185	7	12
15	250	12	28
16	340	75	140
17	375	89	130
18	22	3	13
19	250	33	69
20	390	58	190
21	185	20	38
22	420	66	125
23	140	17	40
24	90	24	50
25	105	36	53
26	120	37	64
27	95	5	11
28	85	5	9
29	190	41	89
30	255	43	125
31	240	42	140
32	335	59	165
33	280	36	90
34	290	36	120

[a] Human (cloned) isozymes; [b] From bovine muscle; [c] From bovine lung microsomes.

Elemental analysis and spectroscopic data of compounds **3-34** confirmed their structure (see Materials ad Methods for details). Thus, in the IR spectra of the new compounds the intense sulfonamido vibrations were evidenced (in the region 1140-1185 cm^{-1} for the symmetrical band, ad 1340-1380 cm^{-1} for the antisymmetrical one, respectively), which were absent in the spectrum of the original compound **2b**, used for their synthesis (data not shown). In the ^{1}H-NMR spectra of these compounds the four aromatic protons of the arsanilic acid moiety have been detected as an AA'BB' multiplet in the range of 7.55 – 7.99 ppm (, J_{AB} = 7.4 – 7.5 Hz) in all these derivatives, whereas the protons belonging to the „R‰ moiety appeared in their normal ranges (see Materials and Methods for details). The SO_2NH proton appeared as a broad singlet at 8.05 – 8.10 ppm, in the compounds containing this moiety.

CA inhibition data with the prepared compounds **3-34** as well as the original benzenearsonic acids **2a, b** are shown in Table II. The following facts should be noted regarding data of Table II: (i) benzenearsonic acid **2a** and its 4-amino-substituted derivative **2b** behave as weak inhibitors against all three investigated isozymes, with hCA II the most susceptible to inhibition, followed by bCA IV and hCA I the less susceptible. In this respect, arsonic acids seem to be very similar to the aromatic/heterocyclic sulfonamides which have the same type of affinity for these CA isozymes [4-6]; (ii) increasing the dimension of the group substituting the 4 position of benzenearsonic acid derivatives (the „R‰ moiety of compounds **3-34**), greatly enhances the CA inhibitory properties of the obtained derivatives, the nature of the substituting R group being an important parameter in controlling the efficiency as enzyme inhibitor of the corresponding derivative; (iii) best inhibitory activity against all isozymes (with the same trend noted above at point (i)) was connected with the presence of such groups as: trifluoromethylsulfonyl (compound **5**); nitro- and chloro-nitro-phenylsulfonylamido (compounds **11-14**); perfluorophenylsulfonylamido (compound **18**, the most effective inhibitor in the whole series) or substituted-aryl-/arylsulfonylureido such as in the two ureas **27** and **28**. All these derivatives bahave as good inhibitors, with potencies comparable to those of the unsubstituted benzenesulfonamide derivatives (such as sulfanilamide or benzenesulfonamide) some of which possess clinical applications [4-6,26,27]. A large group of the newly prepared derivatives, such as compounds containing *p*-halogenophenylsulfonylamido moieties (6-9), the *p*-acetamidophenyl-substituted compound **15**, the *p*-methoxy-substituted one **21**, the N,N-diphenyl-urea derivative **23** or the isonicotinoylamido derivative **24**, behave as moderate inhibitors, with inhibition constants in the range of 12-25 M against hCA II or 28-50 M against bCA IV. These compounds are much less effective against hCA I, with inhibition constants around 250 – 400 M. The most ineffective inhibitors in the prepared series contained the following groups as substituents in the 4 position of the benznearsonic acid: dimethylaminosulfamoyl; benzylsulfonylamido; tosyl; *p*- or *m*-aminophenylsulfonylamido; *o*-carboxyphenylsulfonylamido or tetrabromo-*o*-carboxyphenyl-sulfonylamido; mesitylamido, nicotinoylamido, asd well as ureido, thioureido or guanidino. These compounds were only slightly more active against all three isozymes as compared to the arsanilic acid from which they were obtained. It is thus clear that small structural variations in a molecule of such an enzyme inhibitor, dramatically change the biological activity of the prepared derivatives. Particularly, the nature of groups substituting the 4 position in benzenesulfonamide derivatives has previously been shown to strongly influence the CA inhibitory properties of the corresponding compounds [28], and it seems that this is also valid for the arsanilic acid derivatives reported here.

In conclusion, we report here a novel class of inhibitors of the zinc enzyme carbonic anhydrase. The 4-substituted-benzenearsonic acid derivatives obtained in the present study showed a large variety of biological activity, some of them behaving as strong inhibitors possessing the same affinity as the sulfonamide inhibitors with clinical applications, others being weak inhibitors against isozymes I, II and IV. The present study might reveal new insights in the search of isozyme-specific CA inhibitors, with possible applications as fungicides or plant growth inhibitors.

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