Taylor & Francis
Taylor & Francis Group

Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: informahealthcare.com/journals/ienz20

Sulfapyridine-like benzenesulfonamide derivatives as inhibitors of carbonic anhydrase isoenzymes I, II and VI

Cemalettin Alp, Şeyda Özsoy, Nurdan Alcan Alp, Deryanur Erdem, Mehmet Serdar Gültekin, Ömer İrfan Küfrevioğlu, Murat Şentürk & Claudiu T. Supuran

To cite this article: Cemalettin Alp, Şeyda Özsoy, Nurdan Alcan Alp, Deryanur Erdem, Mehmet Serdar Gültekin, Ömer İrfan Küfrevioğlu, Murat Şentürk & Claudiu T. Supuran (2012) Sulfapyridine-like benzenesulfonamide derivatives as inhibitors of carbonic anhydrase isoenzymes I, II and VI, Journal of Enzyme Inhibition and Medicinal Chemistry, 27:6, 818-824, DOI: 10.3109/14756366.2011.617745

To link to this article: https://doi.org/10.3109/14756366.2011.617745

Published online: 22 Dec 2011.

Journal of Enzyme Inhibition and Medicinal Chemistry, 2012; 27(6): 818–824

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2011.617745

informa
healthcare

RESEARCH ARTICLE

Sulfapyridine-like benzenesulfonamide derivatives as inhibitors of carbonic anhydrase isoenzymes I, II and VI

Cemalettin Alp[1,2], Şeyda Özsoy[1], Nurdan Alcan Alp[1], Deryanur Erdem[1,3], Mehmet Serdar Gültekin[1], Ömer İrfan Küfrevioğlu[1], Murat Şentürk[1,4], and Claudiu T. Supuran[5]

[1]Ataturk University, Science Faculty, Department of Chemistry, Erzurum, Turkey, [2]Erzincan University, Çayırlı Vocational School, Erzincan, Turkey, [3]Aksaray University, Faculty of Arts and Science, Chemistry Department, Aksaray, Turkey, [4]Ağrı İbrahim Çeçen University, Science and Art Faculty, Chemistry Department, Ağrı, Turkey, [5]Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Sesto Fiorentino (Florence), Italy

Abstract
The inhibition of two human cytosolic carbonic anhydrase (hCA, EC 4.2.1.1) isozymes I, II and human serum isozyme VI, with a series of tosylited aromatic amine derivatives was investigated. The K_I ranges of compounds **1–14** and acetazolamide against hCA I ranged between 1.130 and- 448.2 μM, against hCA II between 0.103 and- 14.3 μM, and against hCA VI ranged between 0.340 and- 42.39 μM. Tosylited aromatic amine derivatives are thus interesting hCA I, II and VI inhibitors, and might be used as leads for generating enzyme inhibitors eventually targeting other isoforms which have not been assayed yet for their interactions with such agents.

Keywords: Carbonic anhydrase, inhibition, benzenesulfonamides

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) enzymes are involved in important physiological and pathological functions, such as pH and CO_2 homeostasis, respiration and transport of CO_2/HCO_3^- between metabolizing tissues and the lungs, ion secretion in different tissues/organs and biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis). Among the sixteen 16 isoenzymes, described up to now, CAI and CAII are present at high concentrations in the cytosol in erythrocytes, and CAII has the highest turnover rate of all the CAs[1–8]. Carbonic anhydrase VI (CA VI) is a secretory enzyme that was initially described in the ovine parotid gland, saliva and normal human serum[8].

The interactions between specific enzymes and various types of inhibitors are of great importance and corresponding investigations have become very popular in the recent years. These substances are sometimes useful in medicinal applications, whereas they have undesirable effects on both human metabolism and other organisms[9–21].

Carbonic anhydrase inhibitors or activators have several medical applications, such as in the treatment of glaucoma, as diuretics, in the management of several neurological disorders, including epilepsy, possibly in the treatment of Alzheimer's disease, whereas several agents are in clinical evaluations as antiobesity or antitumor drugs/diagnostic tools[3–7].

A class of derivatives which showed very promising applications among the various CAIs reported by Supuran's group in the last years, were the thioureas obtained from isothiocyanato sulfonamides (such as e.g., 4-isothiocyanatobenzenesulfonamide) and amines, hydrazines or amino acids[22–24]. Such compounds generally showed potent inhibitory activity against the cytosolic isozyme hCA II as well as the transmembrane, tumor-associated isozyme hCA IX, being thus interesting candidates for developing anti-glaucoma/anti-tumor therapies based on them[22–24].

Many sulfonamide derivatives have been widely used as pro-drugs or drugs. For instance, sulfadiazine is used

Address for Correspondence: Dr. Murat Şentürk, Ağrı İbrahim Çeçen University, Science and Art Faculty, Chemistry Department, 04100-Ağrı, Turkey. Tel: +90 442 2314414. Fax: +90 442 2314388. E-mail: senturkm36@gmail.com; msenturk@agri.edu.tr

(Received 03 August 2011; revised 22 August 2011; accepted 23 August 2011)

as an antibiotic, sulfapyridine is mainly used for treatment of bacterial infections, acetazolamide is mainly used anti-glaucoma agent.

Our groups recently investigated the interaction of CA isozymes I, II with salicylic acid derivatives, some antioxidant phenolic compounds, some purine analogue drugs, organic nitrates, and etc.[9–13,25,26]. We would like to extend these earlier investigations to some benzenesulfonamide derivatives in order to discover novel powerful CAIs which might have implications in medicine.

In this work, toward the discovery of CA inhibitors, we synthesized benzenesulfonamide derivatives. Compounds were evaluated for their ability to inhibit human CA-I, II and VI. Inhibition is reported as K_I (μM) and the results are the average of at least three independent experiments.

Materials and methods

Chemicals

All chemicals and solvents are commercially available and were used after distillation or treatment with drying agents. Column chromatography (CC): silica gel (SiO_2; 60 mesh, Merck-Darmstadt, Germany). Preparative thick layer chromatography (PLC): 1 mm of SiO_2 60 PF (Merck) on glass plates. Mp: cap. melting-point apparatus (BUCHI 530 - Flawil, Switzerland); uncorrected. IR Spectra: solns. in 0.1 mm cells with a Mattson 1000 FT-IR spectrophotometer (Cambrige, England). ^{1}H- and ^{13}C-NMR spectra: 200 (50) and 400 (100)-MHz Varian spectrometer (Danbury, USA); δ in ppm; Me_4Si as the internal standard. Elemental analyses: Leco CHNS-932 apparatus (Michigan, USA).

General procedure for the synthesis of N-aryl-ptoluenesulfonamides

To a mixture of aryl amine derivative (10 mmol) and pyridine (10 mL, 120 mmol) in dichloromethane (50 mL) was added slowly p-toluenesulfonyl chloride (2.29 g, 12 mol, 1.2 eq.). The reaction mixture was stirred at room temperature for 16 h[27]. The reaction mixture was washed with 5% HCl. Then the organic layer was washed with $NaHCO_3$ solution and dried over sodium sulfate, filtered, and evaporated to afford a residue. Purification of the residue by silica gel column chromatography with ethylacetate/hexane provided the corresponding N-aryl-*p*-toluenesulfonamide 2–11 in 67–100% yields.

Synthesis of 4-methyl-N-p-tolylbenzenesulfonamide (2):

In Quantitative yield, Compound **2** was recrystallized from CH_2Cl_2-hexane solution. **M.P.: 97–99°C**. ^{1}H -NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.36 (s, 3H in NH-Ts), 7.06 (s, 1H, -NH), 7.10 (d, 2H, *J* = 8.23 Hz, AA' part of AA'BB' system in NH-Ts), 7.22 (m, 5H in Ph), 7.68 (d, 2H, *J* = 8.23 Hz, BB' part of AA'BB' system in NH-Ts). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 21.8, 121.7, 125.4, 127.5, 129.5, 129.9, 136.0, 136.8, 144.1. IR (KBr, cm^{-1}): 3257, 1599, 1496, 1412, 1336, 1303, 1159, 1092. Anal. calcd. for $C_{13}H_{13}NO_2S$ (247.31) C, 63.13; H, 5.30; N, 5.66; S, 12.97. Found: C, 62.90; H, 5.40; N, 5.64; S, 12.72.

Synthesis of 4-Methyl-N-(naphthalen-2-yl) benzenesulfonamide (3)

In %98 yield, Compound **3** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 153–155°oC. ^{1}H -NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.32 (s, 3H in NH-Ts), 7.14 (d, 2H, *J* = 8.24 Hz, AA' part of AA'BB' system in NH-Ts), 7.25–7.46 (m, 4H in Naph.), 7.64 (d, 2H, *J* = 8.24 Hz, BB' part of AA'BB' system in NH-Ts), 7.70 (d, 1H, *J* = 6.95 Hz in Naph.), 7.79 (m, 1H in Naph.), 7.85 (d, 1H, *J* = 8.05 Hz in Naph.). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 21.7, 121.7, 122.9, 125.6, 126.5, 126.8, 127.4, 127.6, 128.6, 129.1, 129.7, 131.7, 134.5, 136.7, 144.0. IR (KBr, cm^{--1}): 3264, 1597, 1454, 1410, 1344, 1314, 1160, 1091. Anal. calcd. for $C_{17}H_{15}NO_2S$ (297.37) C, 68.66; H, 5.08; N, 4.71; S, 10.78. Found: C, 68.46; H, 5.20; N, 4.72; S, 10.89.

Synthesis of 4-Methyl-N-(2,4,6-trimethyl-phenyl)-benzenesulfonamide (4)

In %95 yield, Compound **4** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 166–168°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 1.98 (s, 6H in Ph-CH_3), 2.24 (s, 3H in Ph-CH_3) 2.41 (s, 3H in NH-Ts), 5.92 (brs, 1H, -NH), 6.81(s, 2H in Ph-CH_3), 7.24 (d, 2H, *J* = 8.23 Hz, AA' part of AA'BB' system in NH-Ts), 7.60 (d, 2H, *J* = 8.23 Hz, BB' part of AA'BB' system in NH-Ts). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 18.8, 21.1, 21.7, 127.5, 129.7, 129.8, 130.2, 137.7, 137.8, 138.2, 143.7. IR (KBr, cm^{-1}): 3282, 1597, 1481, 1400, 1328, 1289, 1161, 1091. Anal. calcd. for $C_{16}H_{19}NO_2S$ (289.39) C, 66.41; H, 6.62; N, 4.84; S, 11.08. Found: C, 66.24; H, 6.80; N, 4.85; S, 10.97.

Synthesis of 4-Methyl-N-o-tolyl-benzenesulfonamide (5)

In %96 yield, Compound **5** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 102–104°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.01 (s, 3H in Ph-CH_3),2.38 (s, 3H in NH-Ts), 6.60 (brs, 1H, -NH), 7.06 (m, 2H (*ortho, para*) in Ph-CH_3), 7.12 (m, 2H (*meta*) in Ph-CH_3), 7.21 (d, 2H, *J* = 8.26 Hz, AA' part of AA'BB' system in NH-Ts), 7.31 (d, 1H, *J* = 8.06 Hz (*meta*) in Ph-CH_3), 7.62 (d, 2H, *J* = 8.26 Hz, BB' part of AA'BB' system in NH-Ts). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 17.8, 21.7, 124.6, 126.4, 127.1, 127.4, 129.8, 131.0, 131.6, 134.8, 137.0, 144.0. IR (KBr, cm^{-1}): 3278, 1598, 1495, 1403, 1330, 1290, 1162, 1092. Anal. calcd. for $C_{14}H_{15}NO_2S$ (261.34) C, 64.34; H, 5.79; N, 5.36; S, 12.27. Found: C, 64.18; H, 5.84; N, 5.34; S, 12.25.

Synthesis of N-(2-cyanophenyl)-4-methylbenzenesulfonamide (6)

In %67 yield, Compound **6** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 129–131°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.38 (s, 3H in NH-Ts), 7.17 (m, 1H (*meta*) in Ph-CN), 7.25 (d, 2H, *J* = 8.42 Hz, AA' part of AA'BB' system in NH-Ts), 7.46 (dd, 1H (*meta*) in Ph-CN, *J* = 7.7, 1.5 Hz), 7.52 (brdt, 1H(*para*) in Ph-CN, *J* = 8.8, 1.5 Hz), 7.70 (d, 2H, *J* = 8.42 Hz, BB' part of AA'BB'

system in NH-Ts), 7.72 (d, 1H(*ortho*) in Ph-CN, $J = 8.80$ Hz). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 21.8, 104.5, 115.9, 121.9, 125.4, 127.6, 130.2, 133.0, 134.4, 135.7, 139.6, 145.0. IR (KBr, cm^{-1}): 3241, 2230, 1599, 1493, 1455, 1413, 1342, 1163, 1091. Anal. calcd. for $C_{14}H_{12}N_2O_2S$ (272.32) C, 61.75; H, 4.44; N, 10.29; S, 11.77. Found: C, 61.64; H, 4.54; N, 10.36; S, 11.61.

Synthesis of 4-Methyl-N-(2-nitrophenyl) benzenesulfonamide (7)

In %75 yield, Compound **7** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 108–110°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.37 (s, 3H in NH-Ts), 7.14 (ddd, 1H (*meta*) in Ph-NO_2, $J = 8.4$, 7.3, 1.5 Hz), 7.25 (d, 2H, $J = 8.2$ Hz, AA' part of AA'BB' system in NH-Ts), 7.57 (ddd, 1H (*para*) in Ph-NO_2, $J = 8.4$, 7.3, 1.5 Hz), 7.72 (d, 2H, $J = 8.2$ Hz, BB' part of AA'BB' system in NH-Ts), 7.83 (dd, 1H (*meta*) in Ph-CN, $J = 8.4$, 1.5 Hz), 8.09 (dd, 1H (*ortho*) in Ph-CN, $J = 8.4$, 1.5 Hz), 9.84 (brs, 1H, -NH). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 21.8, 121.2, 124.0, 126.4, 127.5 (2C), 130.2, 134.2, 135.9, 136.1, 145.0. IR (KBr, cm^{-1}): 3286, 1610, 1529, 1487, 1393, 1348, 1275, 1169, 1090. Anal. calcd. for $C_{13}H_{12}N_2O_4S$ (292.31) C, 53.42; H, 4.14; N, 9.58; S, 10.97. Found: C, 53.31; H, 4.17; N, 9.56; S, 10.90.

Synthesis of 4-Methyl-N-p-tolyl-benzenesulfonamide (8)

In Quantitative yield, Compound **8** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 115–117°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.24 (s, 3H in Ph-CH_3), 2.35 (s, 3H in NH-Ts), 6.89 (brs, 1H, -NH), 7.00 (d, 2H, $J = 4.21$ Hz, AA' part of AA'BB' system (*ortho*) in Ph-CH_3), 7.18 (d, 2H, $J = 8.23$ Hz, AA' part of AA'BB' system in NH-Ts), 7.21 (d, 2H, $J = 4.21$ Hz, BB' part of AA'BB' system (*meta*) in Ph-CH_3), 7.67 (d, 2H, $J = 8.23$ Hz, BB' part of AA'BB' system in NH-Ts). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 21.0, 21.7, 122.4, 127.5, 129.8, 130.0, 134.1, 135.4, 136.4, 143.9. IR (KBr, cm^{-1}): 3258, 1598, 1511, 1450, 1393, 1331, 1160, 1092. Anal. calcd. for $C_{14}H_{15}NO_2S$ (261.34) C, 64.34; H, 5.79; N, 5.36; S, 12.27. Found: C, 64.12; H, 5.86; N, 5.38; S, 12.35.

Synthesis of N-(4-ethylphenyl)-4-methylbenzenesulfonamide (9)

In %98 yield, Compound **9** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 90–92°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 1.17 (t, 3H, $J = 7.7$ Hz in Ph-C_2H_5), 2.37 (s, 3H in NH-Ts), 2.55 (q, 2H, $J = 7.7$ Hz in Ph-C_2H_5), 6.76 (brs, 1H, -NH), 6.98 (d, 2H, $J = 8.6$ Hz, AA' part of AA'BB' system (*ortho*) in Ph-C_2H_5), 7.05 (d, 2H, $J = 8.60$ Hz, BB' part of AA'BB' system (*meta*) in Ph-C_2H_5), 7.21 (d, 2H, $J = 8.05$ Hz, AA' part of AA'BB' system in NH-Ts), 7.64 (d, 2H, $J = 8.05$ Hz, BB' part of AA'BB' system in NH-Ts). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 15.6, 21.7, 28.4, 122.5, 127.5, 128.8, 129.8, 134.2, 136.5, 141.9, 143.9. IR (KBr, cm^{-1}): 3259, 1598, 1512, 1457, 1397, 1333, 1160, 1092. Anal. calcd. for $C_{15}H_{17}NO_2S$ (275.37) C, 65.43; H, 6.22; N, 5.09; S, 11.64. Found: C, 65.29; H, 6.20; N, 5.12; S, 11.65.

Synthesis of N-(4-cyanophenyl)-4-methylbenzenesulfonamide (10)

In %72 yield, Compound **10** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 177–179°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.39 (s, 3H in NH-Ts), 7.18 (d, 2H, $J = 9.0$ Hz, AA' part of AA'BB' system (*meta*) in Ph-CN), 7.27 (d, 2H, $J = 8.2$ Hz, AA' part of AA'BB' system in NH-Ts), 7.51 (d, 2H, $J = 9.0$ Hz, BB' part of AA'BB' system (*ortho*) in Ph-CN), 7.76 (d, 2H, $J = 8.2$ Hz, BB' part of AA'BB' system in NH-Ts). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 21.8, 107.9, 118.7, 119.5, 127.5, 130.2, 133.8, 135.8, 141.3, 145.0. IR (KBr, cm^{-1}): 3237, 2226, 1607, 1508, 1462, 1402, 1342, 1159, 1090. Anal. calcd. for $C_{14}H_{12}N_2O_2S$ (272.32) C, 61.75; H, 4.44; N, 10.29; S, 11.77. Found: C, 61.63; H, 4.46; N, 10.28; S, 11.70.

Synthesis of 4-Methyl-N-(4-nitrophenyl) benzenesulfonamide (11)

In %71 yield, Compound **11** was recrystallized from CH_2Cl_2-hexane solution. M.P.: 181–183°C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): δ = 2.40 (s, 3H in NH-Ts), 7.20 (d, 2H, $J = 9.2$ Hz, AA' part of AA'BB' system (*meta*) in Ph-NO_2), 7.29 (d, 2H, $J = 8.3$ Hz, AA' part of AA'BB' system in NH-Ts), 7.77 (d, 2H, $J = 8.3$ Hz, BB' part of AA'BB' system in NH-Ts), 8.12 (d, 2H, $J = 9.2$ Hz, BB' part of AA'BB' system (*meta*) in Ph-CN). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 21.8, 118.9, 125.6, 127.5, 130.3, 135.8, 142.9, 145. IR (KBr, cm^{-1}): 3254, 1596, 1520, 1497, 1342, 1296, 1161, 1090. Anal. calcd. for $C_{13}H_{12}N_2O_4S$ (292.31) C, 53.42; H, 4.14; N, 9.58; S, 10.97. Found: C, 53.27; H, 4.16; N, 9.63; S, 11.03.

Purification of carbonic anhydrase isozymes from human blood by affinity chromatography

Purification of hCA I and hCA II were previously described: hCA I and hCA II in[18]. Serum were purified from fresh human blood obtained from the Blood Center of the Research Hospital at Atatürk University. The blood samples were centrifuged at 5000 rpm for 15 min and precipitant were removed. The serum was isolated. The pH of the hemolysate was adjusted to 8.7 with solid Tris[9–11]. Sepharose-4B-aniline-sulfanylamide affinity column equilibrated with 25 mM Tris-HCl/0.1M Na_2SO_4 (pH 8.7). The affinity gel was washed with 25 mM Tris-HCl/22 mM Na_2SO_4 (pH 8.7). The human carbonic anhydrase (hCA-VI) isozyme were eluted with 0.25 M H_2NSO_3H/25 mM Na_2HPO_4 (pH = 6.7). All procedures were performed at 4°C[9–12].

Esterase activity assay

Carbonic anhydrase activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25°C using a spectrophotometer (CHEBIOS UV-VIS) according to the method described by Verpoorte et al.[28]. The inhibitory effects of compounds **1**–**14** and **AZA** were examined. All compounds were tested in triplicate at each concentration used. Control cuvette activity in the

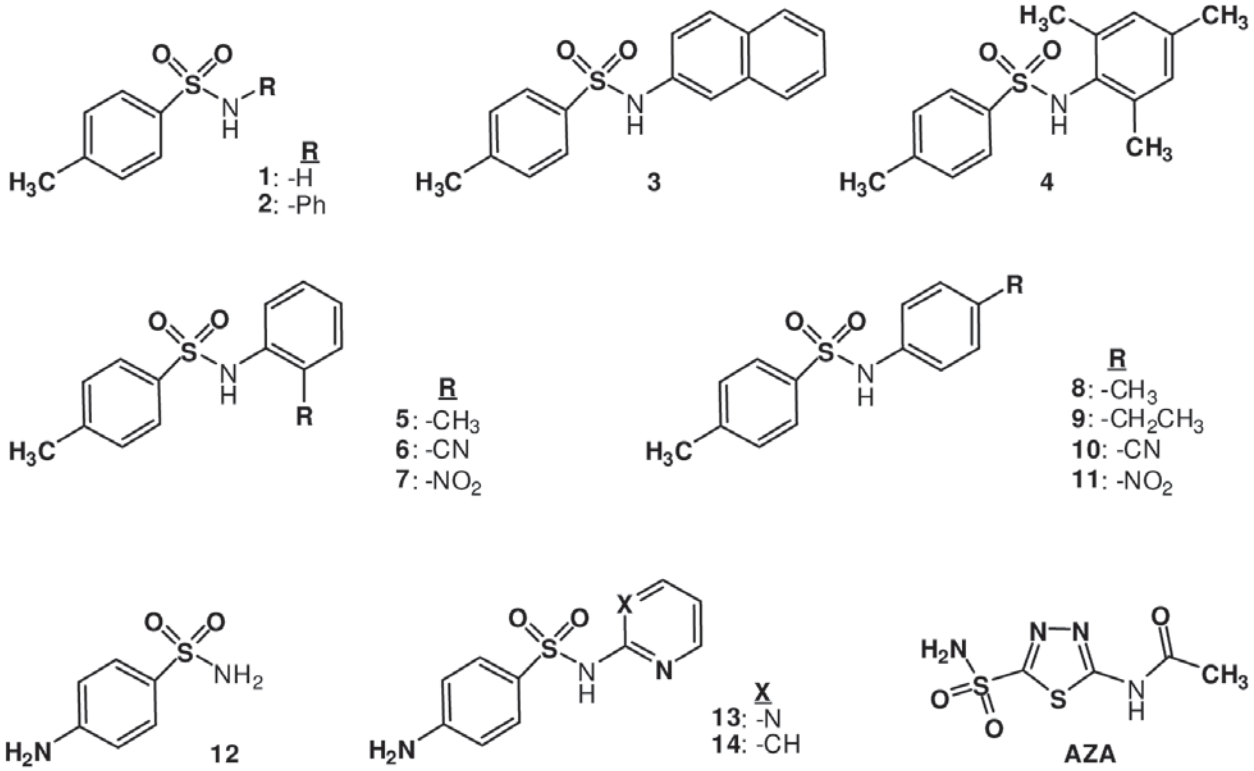

Figure 1. Structures of the compounds.

absence of inhibitor was taken as 100%. For each inhibitor an Activity%- [Inhibitor] graph was drawn. To determine K_I values, three different inhibitor concentrations were tested;. In these experiments, 4-nitrophenylacetate was used as substrate at five different concentrations (0.15–0.75 mM). The Lineweaver-Burk curves were drawn[29]. Regression analysis graphs were drawn for IC_{50} using inhibition % values by a statistical package (SPSS-for windows; version 10.0) on a computer (Student's *t*-test; *n*: 3).

Protein determination

Protein during the purification steps was determined spectrophotometrically at 595 nm according to the Bradford method, using bovine serum albumin as the Standard[30].

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was performed after purification of the enzymes. It was carried out in 10% and 3% acrylamide for the running and the stacking gel, respectively, containing 0.1% SDS according to Laemmli[31].

Results and discussion

Benzenesulfonamide derivatives (**1**–**14**) investigated for the inhibition of CA I, II and CA VI isozymes. Compounds **1**, **12**–**14** and AZA are clinically used compounds (Figure. 1). We (commercially available) have also been assayed compound (**1**, **12**–**14**), since no such data are available in the literature.

Recently, Supuran's group investigated the interactions of some methoxy-benzenesulfonamide derivatives and some of its substituted derivatives with isozymes, CA I, II and IX[32], evidencing some low micromolar/submicromolar inhibitors as well as the possibility to design isozyme selective CAIs. Indeed, the inhibition profile of various isozymes with this class of agents is very variable, with inhibition constants ranging from the millimolar to the submicromolar range for some methoxy-benzenesulfonamide or acid hydroxamide derivatives[32]. It appeared thus of interest to extend the previous studies[7,9–14], including in this research some benzenesulfonamides with clinical applications, sulfapyridine **13**, sulfadiazine **14**, as well as some of its substituted derivatives incorporating amino, cyano, methyl and nitro moieties as substituents at the aromatic ring in different positions.

The purification of human erythrocyte CA I and II isoenzymes were performed using simple single-step method by Sepharose-4B-anilin-sulfanilamide affinity gel chromatography[18]. We report here the first study on the inhibitory effects of benzenesulfonamides (**1**–**14**) and **AZA** on the CA esterase activity. Data of Table 1 show the following regarding inhibition of hCA-I, II and VI with compounds **1**–**15**:

(i) Against the slow cytosolic isozyme hCA I, sulfonamides **2**–**5** and **9** behave as weak inhibitors, with K_I values in the range of 148.27–448.2 μM. Isoform hCA I was moderately inhibited by sulfonamides **1**, **6**, **8**, **12**–**15** reported here. Thus, several derivatives, such as **1, 6** and **8**, showed medium inhibitory activity, with inhibition

Table 1. hCA I, II and VI inhibition data with new sythetizedsynthesized sulfanilamine derivatives **1-12,** sulfapyridine (**13**), sulfadiazine (**14**) and acetazolamide (**15**), by an esterase assay with 4-nitrophenylacetate as substrate.

	K_I (μμM)		
Compound	hCA I	hCA II	hCA VI
4-Methylbenzenesulfonamide (**1**)	22.120	0.715	18.970
4-Methyl-N-phenylbenzenesulfonamide (**2**)	210.3	14.3	13.44
4-Methyl-N-(naphthalen-2-yl)benzenesulfonamide (**3**)	322.0	0.612	42.39
4-Methyl-N-(2,4,6-trimethyl-phenyl)-benzenesulfonamide (**4**)	249.12	0.425	32.18
4-Methyl-N-o-tolyl-benzenesulfonamide (**5**)	148.27	0.407	29.58
N-(2-cyanophenyl)-4-methylbenzenesulfonamide (**6**)	18.24	0.213	8.430
4-Methyl-N-(2-nitrophenyl) benzenesulfonamide (**7**)	5.380	0.103	4.710
4-Methyl-N-p-tolyl-benzenesulfonamide (**8**)	50.02	1.015	24.28
N-(4-ethylphenyl)-4-methylbenzenesulfonamide (**9**)	448.2	0.977	26.53
N-(4-cyanophenyl)-4-methylbenzenesulfonamide (**10**)	1.480	0.120	1.230
4-Methyl-N-(4-nitrophenyl) benzenesulfonamide (**11**)	1.130	0.139	1.112
Benzenesulfonamide (**12**)	42.87	0.628	1.214
4-Amino-N-(pyridin-2-yl) benzenesulfonamide (Sulfapyridine) (**13**)	26.19	0.341	11.63
4-Amino-N-(pyrimidin-2-yl) benzenesulfonamide (Sulfadiazine) (**14**)	28.38	0.276	15.52
Acetazolamide (**AZA**) (**15**)	36.2	0.37	0.340

constants in the range of 18.24–50.02 μM, in the same range as the clinically used compounds sulfapyridine (**13**), sulfadiazine (**14**) and acetazolamide (**15**) (K_I of 26.19–36.2 μM for both compounds against this isoform, Table 1). The remaining sulfonamides were more effective hCA I inhibitors as compared to derivatives discussed above, with K_I-s in the range of 1.130–5.380 μM. Obviously, both the aromatic sulfonamide head as well as the aryl/hetaryl-acetyl moiety influence the biological activity of these hCA I inhibitors[6]. It may be observed that efficient inhibitors incorporate both sulfanilamide, 4-Methyl-N-(4-nitrophenyl) benzenesulfonamide and N-(4-cyanophenyl)-4-methylbenzenesulfonamide. Except for the five less active compounds mentioned above (**2-5** and **9**) which incorporate methylbenzenesulfonamide (**1** or **8**), cyanophenyl (**6**), 4-amino-pyridin (**13**) or amino-pyrimidin (**14**) moiety, the remaining compounds showed a quite compact behavior of moderately-efficient hCA I inhibitors. Thus, benzenesulfonamides lead to significant hCA I inhibition, but all these compounds possess K_I-s >0.100 μM, being thus only moderately active. Kinetic investigations (Lineweaver Burk plots, data not shown) indicate that similarly to sulfonamides and inorganic anions[33–38], all the investigated compounds act as noncompetitive inhibitors with 4-NPA as substrate, i.e., they bind in different regions of the active site cavity as compared to the substrate. However, the binding site of 4-NPA itself is unknown, but it is presumed to be in the same region as that of CO_2, the physiological substrate of this enzyme[39].

(ii) A rather strong activity of these compounds has been observed also for the inhibition of the rapid cytosolic isozyme hCA-II (Table 1). Indeed, again the same one compound (**2**) showed weaker hCA II inhibitory activity, with K_I in the range of 14.3 μM, whereas compounscompounds **7**, **8** behave as moderate inhibitors (K_I-s in the range of 0.977–1.015 μM). It should be noted that the three clinically used compounds (**13, 14** and AZA) and **1**, **3–6**, **9–12** show much more potent hCA II inhibitory activity, with K_I-s in the range of 0.103–0.715 μM (Table 1). Theses finding clearly illustrate that a very big variation in the structure of a CAI (such as the presence of an additional CN, NO_2 and CH_3 moiety, in this case) may have drastic consequences for the enzyme inhibitory activity and selectivity profile against various isozymes of such derivatives. Thus, except for the one less active compound **2**, all derivatives showed moderate or powerful hCA-II inhibitory activity (Table 1).

(iii) Sulfapyridine **13** and some of its congeners such as **1–9**, and **14** are also weak inhibitors of the secreted isozyme hCA VI, with K_I-s of 4.710–42.39 μM. However, again the compounds **10–12** are medium potency inhibitors (K_I of 1.112–1.230 μM), and **AZA** (**15**) show a higher affinity for this isozyme, with inhibition constant in the range of 0.340 μM (Table 1).

3. Conclusions

Benzenesulfonamide derivatives **1–14** used in this study affect the activity of CA isozymes due to the presence of the different functional groups (CH_3, CH_2CH_3, CN, NO_2 and NH_2) moieties present in their aromatic scaffold. It has been determined in our study that compounds **1–14** are effective inhibitors of hCA-II compared to AZA, which is used as reference inhibitor for carbonic anhydrase. Our findings here indicate thus the well-known class of possible CAIs of interest, sulfonamides/sulfamates/sulfamides. Indeed, some benzenesulfonamide derivatives investigated here showed effective CA I, II and VI inhibitory activity, in the low micromolar range, by the esterase method which usually gives K_I-s an order of magnitude higher as compared to the CO_2 hydrase assay.[13] Although benzenesulfonamide derivatives used in this study has previously synthesized, there was not such detailed spectroscopic data about these substances in previous works.

Also, most of these derivatives could not be obtained in one-step with high yields. These findings point out that substituted benzenesulfonamide derivatives may be used as leads for generating potent CAIs eventually targeting other isoforms which have not been assayed yet for their interactions with such agents.

Declaration of interest

This study was financed by Turkish Republic Prime Ministry State Planning Organization (DPT), (project no: 2010K120440) and Agri Ibrahim Cecen University Scientific Research Council, (project no: Agri BAP-2010/K-10) for (MS).

References

1. Ozensoy O, Arslan O, Sinan SO. A new method for purification of carbonic anhydrase isozymes by affinity chromatography. Biochemistry Mosc 2004;69:216–219.
2. Supuran CT. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–181.
3. Hilvo M, Baranauskiene L, Salzano AM, Scaloni A, Matulis D, Innocenti A et al. Biochemical characterization of CA IX, one of the most active carbonic anhydrase isozymes. J Biol Chem 2008;283:27799–27809.
4. Supuran CT, Scozzafava A, Conway J. Carbonic Anhydrase-Its Inhibitors and Activators. CRC Press: Boca Raton, New York, London, 2004. pp. 1–363.
5. Winum JY, Rami M, Scozzafava A, Montero JL, Supuran C. Carbonic anhydrase IX: A new druggable target for the design of antitumor agents. Med Res Rev 2008;28:445–463.
6. Güzel O, Innocenti A, Scozzafava A, Salman A, Parkkila S, Hilvo M et al. Carbonic anhydrase inhibitors: Synthesis and inhibition studies against mammalian isoforms I-XV with a series of 2-(hydrazinocarbonyl)-3-substituted-phenyl-1H-indole-5-sulfonamides. Bioorg Med Chem 2008;16:9113–9120.
7. Durdagi S, Sentürk M, Ekinci D, Balaydin HT, Göksu S, Küfrevioglu ÖI et al. Kinetic and docking studies of phenol-based inhibitors of carbonic anhydrase isoforms I, II, IX and XII evidence a new binding mode within the enzyme active site. Bioorg Med Chem 2011;19:1381–1389.
8. Kivelä J, Parkkila S, Waheed A, Parkkila AK, Sly WS, Rajaniemi H. Secretory carbonic anhydrase isoenzyme (CA VI) in human serum. Clin Chem 1997;43:2318–2322.
9. Sentürk M, Ekinci D, Göksu S, Supuran CT. Effects of dopaminergic compounds on carbonic anhydrase isozymes I, II, and VI. J Enzyme Inhib Med Chem 2011. (doi: 10.3109/14756366.2011.591290).
10. Ekinci D, Cavdar H, Talaz O, Sentürk M, Supuran CT. NO-releasing esters show carbonic anhydrase inhibitory action against human isoforms I and II. Bioorg Med Chem 2010;18:3559–3563.
11. Ekinci D, Ceyhun SB, Sentürk M, Erdem D, Küfrevioglu OI, Supuran CT. Characterization and anions inhibition studies of an a-carbonic anhydrase from the teleost fish Dicentrarchus labrax. Bioorg Med Chem 2011;19:744–748.
12. Alp C, Ekinci D, Gültekin MS, Sentürk M, Sahin E, Küfrevioglu OI. A novel and one-pot synthesis of new 1-tosyl pyrrol-2-one derivatives and analysis of carbonic anhydrase inhibitory potencies. Bioorg Med Chem 2010;18:4468–4474.
13. Ekinci D, Ceyhun SB, Aksakal E, Erdogan O. IGF and GH mRNA levels are suppressed upon exposure to micromolar concentrations of cobalt and zinc in rainbow trout white muscle. Comp Biochem Physiol C Toxicol Pharmacol 2011;153:336–341.
14. Ceyhun SB, Sentürk M, Ekinci D, Erdogan O, Ciltas A, Kocaman EM. δmethrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout. Comp Biochem Physiol C Toxicol Pharmacol 2010;152:215–223.
15. Erdogan O, Ceyhun SB, Ekinci D, Aksakal E. Impact of δmethrin exposure on mRNA expression levels of metallothionein A, B and cytochrome P450 1A in rainbow trout muscles. Gene 2011;484:13–17.
16. Ekinci D, Sentürk M, Beydemir S, Küfrevioglu OI, Supuran CT. An alternative purification method for human serum paraoxonase 1 and its interactions with sulfonamides. Chem Biol Drug Des 2010;76:552–558.
17. Aksakal E, Ceyhun SB, Erdogan O, Ekinci D. Acute and long-term genotoxicity of δmethrin to insulin-like growth factors and growth hormone in rainbow trout. Comp Biochem Physiol C Toxicol Pharmacol 2010;152:451–455.
18. Ceyhun SB, Aksakal E, Ekinci D, Erdogan O, Beydemir S. Influence of cobalt and zinc exposure on mRNA expression profiles of metallothionein and cytocrome P450 in rainbow trout. Biol Trace Elem Res. Doi: 10.1007/s12011-011-9068-z.
19. Cakmak R, Durdagi S, Ekinci D, Sentürk M, Topal G. Design, synthesis and biological evaluation of novel nitroaromatic compounds as potent glutathione reductase inhibitors. Bioorg Med Chem Lett 2011;21:5398–5402.
20. Aksakal E, Ekinci D, Erdogan O, Beydemir S, Alim Z, Ceyhun SB. Increasing stocking density causes inhibition of metabolic-antioxidant enzymes and elevates mRNA levels of heat shock protein 70 in rainbow trout. Livest Sci 2011. Doi:10.1016/j.livsci.2011.07.006.
21. Siktar E, Ekinci D, Siktar E, Beydemir S, Gulcin I, Gunay M. Protective role of L-carnitine supplementation against exhaustive exercise-induced oxidative stress in rats. Eur J Pharmacol 2011. Doi: 10.1016/j.ejphar.2011.07.032.
22. Casini A, Scozzafava A, Mincione F, Menabuoni L, Ilies MA, Supuran CT. Carbonic anhydrase inhibitors: Water-soluble 4-sulfamoylphenylthioureas as topical intraocular pressure-lowering agents with long-lasting effects. J Med Chem 2000;43:4884–4892.
23. Cecchi A, Winum JY, Innocenti A, Vullo D, Montero JL, Scozzafava A et al. Carbonic anhydrase inhibitors: Synthesis and inhibition of cytosolic/tumor-associated carbonic anhydrase isozymes I, II, and IX with sulfonamides derived from 4-isothiocyanato-benzolamide. Bioorg Med Chem Lett 2004;14:5775–5780.
24. Innocenti A, Casini A, Alcaro MC, Papini AM, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors: The first on-resin screening of a 4-sulfamoylphenylthiourea library. J Med Chem 2004;47:5224–5229.
25. Balaydin HT, Durdagi S, Ekinci D, Senturk M, Goksu S, Menzek A. Inhibition of human carbonic anhydrase isozymes I, II and VI with a series of bisphenol, metoxy and bromophenol compounds. J Enzym Inhib Med Ch 2011. Doi:10.3109/14756366.2011.596836.
26. Senturk M, Kufrevioglu OI. *In vitro* effects of some nucleoside analogue drugs on enzyme activities of carbonic anhydrase isozymes I and II from human erythrocytes. Hacettepe J Biol Chem 2009;37:289–294.
27. Park KK, Lee JJ, Ryu J. Photo-Fries rearrangement of N-arylsulfonamides to aminoaryl sulfone derivatives. Tetrahedron 2003;59:7651–7659.
28. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. J Biol Chem 1967;242:4221–4229.
29. Lineweaver H, Burk D. The determination of enzyme dissocation constants. J Am Chem Soc 1934;56:658–666.
30. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–254.
31. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–685.
32. Nuti E, Orlandini E, Nencetti S, Rossello A, Innocenti A, Scozzafava A et al. Carbonic anhydrase and matrix metalloproteinase inhibitors. Inhibition of human tumor-associated isozymes IX and cytosolic isozyme I and II with sulfonylated hydroxamates. Bioorg Med Chem 2007;15:2298–2311.
33. Briganti F, Pierattelli R, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Part 37. Novel classes of isozymes I and II inhibitors and their mechanism of action. Kinetic and

spectroscopic investigations on the native and cobalt-substituted enzymes. Eur J Med Chem 1996;31:1001–1010.
34. Sentürk M, Gülçin I, Dastan A, Küfrevioglu OI, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of antioxidant phenols. Bioorg Med Chem 2009;17:3207–3211.
35. Balaydin HT, Soyut H, Ekinci D, Goksu S, Beydemir S, Menzek A, Sahin E. Synthesis and carbonic anhydrase inhibitory properties of novel bromophenols including natural products. J Enzym Inhib Med Ch 2011. Doi: 10.3109/14756366.2011.574131.
36. Sly WS, Hu PY. Human carbonic anhydrases and carbonic anhydrase deficiencies. Annu Rev Biochem 1995;64:375–401.
37. Pastorekova S, Parkkila S, Pastorek J, Supuran CT. Carbonic anhydrases: Current state of the art, therapeutic applications and future prospects. J Enzyme Inhib Med Chem 2004;19:199–229.
38. Ceyhun SB, Sentürk M, Yerlikaya E, Erdogan O, Küfrevioglu OI, Ekinci D. Purification and characterization of carbonic anhydrase from the teleost fish Dicentrarchus labrax (European seabass) liver and toxicological effects of metals on enzyme activity. Environ Toxicol Pharmacol 2011;32:69–74.
39. Nair SK, Ludwig PA, Christianson DW. Two-site binding of phenol in the active site of human carbonic anhydrase II: Structural implications for substrate association. J Am Chem Soc 1994;116:3659–3660.