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RESEARCH ARTICLE

Synthesis and evaluation of sulfonamide-bearing thiazole as carbonic anhydrase isoforms hCA I and hCA II

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Abstract

Sulfonamide-bearing thiazole compounds were synthesized and their inhibitory effects on the activity of purified human carbonic anhydrase I and II were evaluated. Human carbonic anhydrase isoenzymes (hCA-I and hCA-II) were purified from erythrocyte cells by affinity chromatography. The inhibitory effects of the 12 synthesized sulfonamide (**5a**–**l**) on the hydratase and esterase activities of these isoenzymes (hCA-I and hCA-II) were studied *in vitro*. In relation to these activities, the inhibition equilibrium constants (*Ki*) were determined. The results showed that all the synthesized compounds inhibited the CA isoenzyme activity. Among them **5b** was found to be the most active ($IC_{50} = 0.35\ \mu M$; *Ki*: 0.33 μM) for hCA I and hCA II.

Keywords

Carbonic anhydrase, enzyme inhibitor, sulfonamide, thiazole

History

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Introduction

Heterocyclic moieties, such as indoles, thiazolidine-4-ones, pyrazoles, piperazines, pyridines, etc., always have considerable attention due to their pharmacological activities[1,2]. Heterocycles bearing one or more nitrogen and sulfur atoms have received more attention recently. Among the most frequently encountered heterocyclic compounds, thiazole and its derivatives play an important role in nature. The heterocyclic compounds have broad applications including the treatment of hypertension, bacterial and HIV infections, allergies and as antibiotics and ligands for estrogen receptors[3,4]. Beside, these compounds have used as inhibitors against fructose 1,6-bisphosphatase[5], tumor-associated carbonic anhydrase isoforms hCA IX and hCA XII[6], sphingosine kinase[7] and evaluation of *in vitro* anticancer activity[8].

The carbonic anhydrases (CAs, EC 4.2.1.1) are superfamily of metalloenzymes present in Archaea, prokaryotes and eukaryotes, and in all life kingdoms with five genetically distinct families described in various organisms. Evolutionarily unrelated gene families are α-, β-, δ-, γ-, ζ-CAs[9]. α-, β-, δ CAs contain zinc metal at the active site. α-CAs are found in vertebrates, eubacteria, algae and cytoplasm of green plants and the β-CAs are predominantly found in eubacteria, algae and chloroplasts of both mono- as well as dicotyledons[10–12]. γ-CAs may have Fe(II) at the active site and mainly are found in Archaea, some eubacteria and plants. Cd(II) and Zn(II) ions are equally effective for catalysis in ζ-CAs (diatoms encode). Three His residues coordinate the metal ion in the α-, δ-, γ-class enzymes or one His and two Cys residues coordinate it with the fourth ligand being a water molecule/hydroxide ion in the β- and ζ-CAs[9,13].

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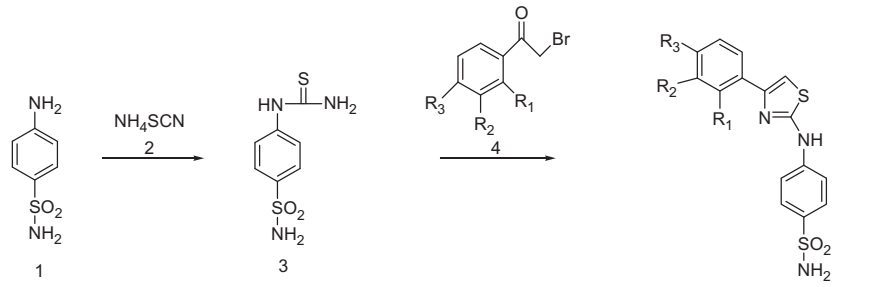
Until now, 15 human CA (hCA) isoforms have been identified that exhibit significant differences in catalytic activity, subcelluler localization and tissue expression. Some isoforms are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), two are mitochondrial (CA VA and CAVB), others are membrane bound (CA IV, CA IX, CA XII and CA XIV), and one is secreted in saliva (CA VI). It has been reported that the CA XV isoform is not expressed in humans or in other primates, but it is abundant in rodents and other vertebrates[14,15].

These enzymes catalyze a very simple but critically important physiological reaction, rapid interconversion of carbon dioxide and water into protons and bicarbonate ions, in many physiological/pathological processes open up widespread opportunities for the development of diverse, specific inhibitors for clinical applications[16,17]. In addition to this physiological reaction, the reversible hydration of CO_2 to bicarbonate, CAs catalyze several other reactions, such as the hydration of cyanate or cyanamide to carbamic acid, or urea, the aldehyde hydration to gem-diols, the hydrolysis of carboxylic, or sulfonic and some others[18,19].

The importance of sulfonamide compounds in medicinal chemistry cannot be ignored and the compounds are well-known pharmaceutical agents and have gained much attention due to their diverse biological activities in pharmaceutical as well as in agricultural areas[20]. The sulfonamide compounds have a number of biological activities, such as antibacterial[21], anti-inflammatory[22], antitumor[23], anticancer[24] and carbonic anhydrase inhibitory functions[25].

Carbonic anhydrase inhibitors or activators have many medical applications, such as in the treatment of glaucoma, in the management of neurological disorders, in the treatment of Alzheimer's disease. Acetazolamide (AAZ), dorzalamide (DZA) and brinzolamide (BRZ) are sulfonamide derivatives and used in the treatment of glaucoma[26–28]. In this study, 12 sulfonamide-bearing thiazole compounds were synthesized and their inhibitory

Scheme 1. Synthesis of sulfonamide-bearing thiazole compounds.



	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k	5l
R1	H	H	H	H	H	H	H	H	NO_2	H	Cl	H
R2	H	H	H	H	H	H	H	H	H	NO_2	H	OH
R3	H	NO_2	C_N	F	Cl	OCH_3	CH_3	OH	H	H	Cl	OH

effects on the activity of purified human carbonic anhydrase (hCA) I and II were evaluated.

Materials and methods

The compounds were prepared from a mixture of sulfanilamide-substituted thiourea and α-haloacetophenone derivatives in DMF-Ethanol (1:1 volume) by heating for 5 h at 80 °C, as shown in Scheme 1. The prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis.

General procedure

Melting points were taken on a Yanagimoto Barnstead Electrothermal (Surrey, UK) micromelting point apparatus and are uncorrected. IR spectra were measured on a SHIMADZU Prestige-21 (200VCE) (Kyoto, Japan) spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured on spectrometer at Varian Infinity Plus 300 and at 75 Hz (Palo Alto, CA). ^{1}H and ^{13}C chemical shifts are referenced to the internal deuteranated solvent. The elemental analysis was carried out with a Leco CHNS-932 (St. Joseph, MI) instrument. All chemicals were purchased from Merck (Darmstadt, Germany), Alfa Aesar (Ward Hill, MA) and Sigma-Aldrich (Taufkirchen, Germany).

General procedure for the synthesis of sulfanilamide substituted thiourea

Sulfanilamide (20 mmol) was dissolved in a mixture of conc. HCl (5 ml) and water (15 ml) by heating. The solution was cooled and ammonium thiocyanate (26.3 mmol) in 15 ml water was added slowly, and then the mixture was refluxed for 12 h at 100 °C. Then the reaction was completed, cooled and filtered off. The product was crystallized with or through aqueous ethanol.

General procedure for the synthesis of sulfonamide-bearing thiazole derivatives (5a–l)

α-Haloacetophenone derivatives (1 mmol) and sulfanilamide-substituted thiourea (1 mmol) were dissolved in DMF (6 ml) and 6 ml of ethanol was added to the mixture. Then, it was heated for 5 h at 80 °C, after the reaction was completed. The mixture was cooled to room temperature and poured into ice cold water. It was then filtered and crystallized through acetone. ^{1}H NMR and ^{13}C NMR spectra of **5a**, **5h** and **5l** have been found to be consistent with the literature[5].

4–(4-Phenylthiazol-2-ylamino)benzene sulfonamide (5a)

Yield 85% (0.281 g), m.p. 243–44 °C; IR (KBr, ν, cm^{-1}): 3328 (NH), 3307 (NH_2), 1602 (C=) 1310 and 1150 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.5 (1H, s, –NH), 7.90 (2H, d), 7.80 (2H, d), 7.80 (2H, d), 7.52 (2H, t), 7.38 (1H, t), 7.18 (1H, s, thiazole ring), 7.10 (2H, s, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 162.9, 151.0, 144.5, 136.3, 134.9, 129.0, 128.1, 127.6, 126.2, 116.5, 103.7; Anal. Calcd.: $C_{15}H_{13}N_3O_2S_2$: C, 54.36; H, 3.95; N, 12.68; O, 9.66; S, 19.35. Found: C, 54.87; H, 3.85; N, 12.98; O, 9.46; S, 19.05.

4–(4–(4-Nitrophenyl)thiazol-2-ylamino) benzenesulfonamide (5b)

Yield 87% (0.327 g), m.p. 284–86 °C; IR (KBr, ν, cm^{-1}): 3326 (NH), 3225 (NH_2), 1738 (C=N), 1333 (NO_2), 1310 and 1156 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.70 (1H, –NH), 8.30 (2H, d), 8.28 (2H, d), 7.90 (2H, d), 7.82 (2H,d), 7.62 (1H, s, thiazole ring), 7.18 (2H, s, NH_2), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 161.1, 150.2, 145.5, 139.3, 138.2, 130.8, 127.6, 127.1, 124.5, 116.8, 109.9; Anal. Calcd.: $C_{15}H_{12}N_4O_4S_2$: C, 47.86; H, 3.21; N, 14.88; O, 17.00; S, 17.04. Found: C, 47.56; H, 3.41; N, 14.55; O, 17.42; S, 17.64.

4–(4–(4-Cyanophenyl)thiazol-2-ylamino) benzenesulfonamide (5c)

Yield 82% (0.291 g), m.p. 297–98 °C; IR (KBr, ν, cm^{-1}): 3300 (NH), 3212 (NH_2), 2226 (C\equiv*N*), 1738 (C=N), 1314 and 1155 (S=O); ^{1}HNMR (300 MHz, DMSO-d_6, ppm): 10.58 (1H, s), 8.10 (2H, d), 7.98 (2H, d), 7.90 (2H, d), 7.79 (2H, =d), 7.40 (1H, s, thiazole ring), 7.10 (2H, s, NH_2), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 162.6, 149.3, 144.3, 138.9, 136.3, 132.8, 127.5, 126.8, 119.3, 118.6, 116.7, 110.6; Anal. Calcd.: $C_{16}H_{12}N_4O_2S_2$: C, 53.92; H, 3.39; N, 15.72; O, 8.98; S, 17.99. Found: C, 53.58; H, 3.62; N, 15.22; O, 8.65; S, 17.75.

4–(4–(4-Fluorophenyl)thiazol-2-ylamino) benzenesulfonamide (5d)

Yield 92% (0.321 g), m.p. 233–35 °C; IR (KBr, ν, cm^{-1}) 3330 (NH), 3298 (NH_2), 1738 (C=N), 1316 and 1148 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.70 (1H, s, NH), 8.0 (2H, m), 7.92 (2H, d) 7.88 (2H, d), 7.4 (1H, s, thiazole ring), 7.22 (2H,t), 7.20 (2H, d, NH_2), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 163.0, 160.5, 149.9, 145.5, 132.6, 129.8, 127.6, 116.9, 116.5, 110.2, 102.3; Anal. Calcd.: $C_{15}H_{12}FN_3O_2S_2$: C, 51.56; H, 3.46; F, 5.44; N, 12.03; O, 9.16; S, 18.35. Found: C, 51.17; H, 3.25; F, 5.64; N, 12.53; O, 9.55; S, 18.62.

4–(4–(4-Chlorophenyl)thiazol-2-ylamino) benzenesulfonamide (5e)

Yield 78% (0.277 g), m.p. 225–26 °C; IR (KBr, ν, cm^{-1}): 3320 (NH), 3310 (NH_2) 1738 (C=N), 1316 and 1147 (S=O);

^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.55 (1H, s, –NH), 7.98 (2H, d), 7.95 (2H, d), 7.8 (2H, d), 7.40 (2H,d), 7.18 (1H, s, thiazole ring), 7.05 (2H, s, NH_2) ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 163.1, 149.8, 144.4, 136.4, 133.6, 133.0, 129.0, 127.7, 127.6, 116.6, 105.2; Anal. Calcd.: $C_{15}H_{12}ClN_3O_2S_2$: C, 49.24; H, 3.31; Cl, 9.69; N, 11.49; O, 8.75; S, 17.53. Found: C, 49.65; H, 3.71; Cl, 9.38; N, 11.88; O, 8.34; S, 17.13.

4–(4–(4-Methoxyphenyl)thiazol-2-ylamino) benzenesulfonamide (5f)

Yield 83% (0.299 g), m.p. 213–15 °C; IR (KBr, ν, cm^{-1}): 3337 (NH), 3300 (NH_2), 1738 (C=N), 1319 and 1146 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.65 (1H, s, –NH), 7.95 (2H, d), 7.93 (2H, d), 7.8 (2H, -d), 7.27 (2H, s, thiazole ring), 7.20 (2H, s, NH_2), 7.0 (2H,d), 3.8(3H, s, OCH_3), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 162.8, 159.5, 150.8, 144.5, 136.2, 129.8, 127.8, 127.6, 116.5, 114.3, 101.8, 55.6; Anal. Calcd.: $C_{16}H_{15}N_3O_3S_2$: C, 53.17; H, 4.18; N, 11.63; O, 13.28; S, 17.74. Found: C, 53.65; H, 4.52; N, 11.23; O, 13.62; S, 17.34.

4–(4-*p*-Tolylthiazol-2-ylamino)benzenesulfonamide (5 g)

Yield 89% (0.307 g), m.p. 241–42 °C; IR (KBr, ν, cm^{-1}): 3318 (NH), 3300 (NH_2), 1738 (C=N), 1320 and 1150 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm):10.50 (1H, s, NH), 7.90 (2H, d), 7.86 (2H, d), 7.80 (2H, d), 7.22 (2H, d), 7.12 (1H, s, thiazole ring), 7.10 (2H, s, NH_2), 2.1 (3H, s, $–CH_3$), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 160.5, 149.6, 144.2, 137.5, 129.6, 127.5, 126.2, 118.6, 116.5, 110.8, 103.83, 21.8; Anal. Calcd.: $C_{16}H_{15}N_3O_2S_2$: C, 55.63; H, 4.38; N, 12.16; O, 9.26; S, 18.56. Found: C, 55.94; H, 4.52; N, 12.28; O, 9.53; S, 18.15.

4–(4–(4-Hydroxyphenyl)thiazol-2-ylamino) benzenesulfonamide (5 h)

Yield 84% (0.291 g), m.p. 204–05 °C; IR (KBr, ν, cm^{-1}): 3394 (OH), 3312 (NH), 3225 (NH_2), 1738 (C=N), 1311 and 1150 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.50 (1H, s, NH), 7.95 (2H, d), 7.90 (2H, d), 7.82 (2H, d), 7.22 (1H, s, thiazole ring), 7.12 (2H, s, NH_2), 7.02 (2H, d), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 160.4, 158.5, 148.2, 146.3, 129.7, 129.2, 128.5, 125.5, 116.8, 116.2, 101.5; Anal. Calcd.: $C_{15}H_{13}N_3O_3S_2$: C, 51.86; H, 3.77; N, 12.10; O, 13.82; S, 18.46. Found: C, 51.45; H, 3.56; N, 12.40; O, 13.41; S, 18.66.

4–(4-(2-Nitrophenyl)thiazol-2-ylamino) benzenesulfonamide (5i)

Yield 84% (0.315 g), m.p. 263–64 °C; IR (KBr, ν, cm^{-1}): 3360 (NH), 3333 (NH_2), 1738 (C=N), 1333 (NO_2), 1316 and 1145 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.68 (1H, s, –NH), 8.79 (1H, d), 8.40 (1H, d), 8.20(1H, d), 7.9 (2H, d), 7.8 (2H, d), 7.72 (1H, –t), 7.70 (1H, s, thiazole ring), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 163.3, 148.9, 148.5, 144.3, 136.7, 136.5, 132.4, 130.6, 127.6, 122.6, 1207, 116.7, 107.0; Anal. Calcd.: $C_{15}H_{12}N_4O_4S_2$: C, 47.86; H, 3.21; N, 14.88; O, 17.00; S, 17.04 Found: C, 47.55; H, 3.41; N, 15.23; O, 17.20; S, 17.34.

4–(4-(3-Nitrophenyl)thiazol-2-ylamino) benzenesulfonamide (5j)

Yield 80% (0.300 g), m.p. 259 °C; IR (KBr, ν, cm^{-1}): 3360 (NH), 3334 (NH_2), 1738 (C=N), 1333 (NO_2), 1316 and 1145(S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.78 (1H, s, –NH), 8.78 (1H, s), 8.40 (1H, d), 8.20 (1H, d), 7.98 (2H, d), 7.90 (2H, d), 7.72 (1H, t), 7.70 (1H, s, thiazole ring),7.20 (2H, s, NH_2), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 163.4, 148.9, 148.5, 144.3, 136.8, 136.5, 132.5, 130.6, 127.7, 122.7, 120.7, 116.8, 107.5; Anal. Calcd.: $C_{15}H_{12}N_4O_4S_2$: C, 47.86; H, 3.21; N, 14.88; O, 17.00; S, 17.04. Found: C, 47.55; H, 3.41; N, 14.63; O, 17.48; S, 17.38.

4–(4-(2,4-Dichlorophenyl)thiazol-2-ylamino) benzenesulfonamide (5k)

Yield 93% (0.375 g), m.p. 200 °C; IR (KBr, ν, cm^{-1}): 3388 (NH), 3343 (NH_2),1738 (C=N), 1324 and 1142 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.68 (1H, s, –NH), 8.0 (1H, d), 7.82 (2H, d), 7.80 (2H, d), 7.55 (1H, s), 7.40 (1H, d), 7.38 (1H, s, thiazole ring), 7.10 (2H, d, NH_2), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 162.6, 162.0, 146.4, 144.4, 136.3, 133.3, 132.7, 132.2, 130.3, 127.7, 127.5, 116.6, 109.3; Anal. Calcd.: $C_{15}H_{11}C_{l2}N_3O_2S_2$: C, 45.01; H, 2.77; Cl, 17.71; N, 10.50; O, 7.99; S, 16.02. Found: C, 45.33; H, 2.57; Cl, 17.62; N, 10.33; O, 7.78; S, 16.42.

4–(4-(3,4-Dihydroxyphenyl)thiazol-2-ylamino) benzenesulfonamide (5 l)

Yield 81% (0.294 g), m.p. 214–15 °C; IR (KBr, ν, cm^{-1}): 3400 (OH), 3390 (NH), 3340 (NH_2), 1739 (C=N), 1311 and 1152 (S=O); ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 10.65 (1H, s, –NH), 7.90 (2H, d), 7.78 (2H, d), 7.38 (1H, s, thiazole ring), 7.20 (1H, d), 7.10 (2H, s, NH_2), 6.80 (1H, d), ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 163.0, 150.3, 146.0, 145.7, 144.3, 136.6, 127.6, 126.1, 117.8, 117.0, 116.2, 114.1, 101.0; Anal. Calcd.: $C_{15}H_{13}N_3O_4S_2$: C, 49.57; H, 3.61; N, 11.56; O, 17.61; S, 17.65. Found: C, 49.89; H, 3.42; N, 11.76; O, 17.95; S, 17.98.

Preparation of hemolysate and purification from blood red cells

Blood samples (25 ml) were taken from healthy human volunteers. They were anticoagulated with acid-citrate-dextrose, centrifuged at 1000*g* for 20 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then hemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 3100*g* for 25 min at 4 °C, and the pH of the hemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 ml hemolysate was applied to an affinity column containing L-tyrosine-sulfonamide-Sepharose-4B[29] equilibrated with 25 mMTris–HCl/0.1 M Na_2SO_4 (pH 8.5). The affinity gel was washed with 50 ml of 25 mM Tris–HCl/22 mM Na_2SO_4 (pH 8.5). The human CA (hCA) isozymes were then eluted with 0.1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), which recovered hCA-I and II, respectively. Fractions (3 ml) were collected and their absorbance was measured at 280 nm.

Hydratase activity assay

Carbonic anhydrase activity was measured by the Wilbur and Anderson method, which is based on determination of the time required for the pH to decrease from 10.0 to 7.4 due to CO_2 hydration[30]. The assay solution was 0.5 M Na_2CO_3/0.1 M $NaHCO_3$ (pH 10.0) and Phenol Red was added as the pH indicator. CO_2-hydratase activity [enzyme units (EU)] was calculated using the equation t_0–tc/tc, where t_0 and tc are the times for pH change of the non-enzymatic and the enzymatic reactions, respectively.

Esterase activity assay

Carbonic anhydrase activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenyl-acetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25 °C using a

Table 1. IC_{50} (μM) values of the sulfonamide-bearing thiazole compounds.

5a–l Inhibitor	Hydratase hCA I IC_{50} (μM)	Hydratase hCA II IC_{50} (μM)	Esterase hCA I *Ki* (μM)	Esterase hCA II *Ki* (μM)	Inhibition type
5a	0.55	0.77	0.40	0.11	Noncompetitive
5b	0.35	0.35	0.33	0.33	Noncompetitive
5c	0.40	0.45	0.51	0.58	Noncompetitive
5d	0.37	0.40	0.33	0.41	Noncompetitive
5e	1.34	1.46	1.23	1.44	Noncompetitive
5f	0.45	0.40	0.59	0.59	Noncompetitive
5g	0.92	0.96	1.01	0.81	Noncompetitive
5h	1.22	1.56	1.21	1.99	Noncompetitive
5i	1.05	1.07	1.04	0.88	Noncompetitive
5j	0.49	0.57	0.42	0.48	Noncompetitive
5k	0.59	0.75	0.48	0.72	Noncompetitive
5l	0.72	0.53	0.78	0.50	Noncompetitive

spectrophotometer (HACH LANGE DV 6000 UV–VIS) according to the method described in the literature[31]. The enzymatic reaction, in a total volume of 3.0 ml, contained 1.4 ml of 0.05 M Tris–SO_4 buffer (pH 7.4), 1 ml of 3 mM 4-nitrophenylacetate, 0.5 ml H_2O and 0.1 ml enzyme solution. A reference measurement was obtained by preparing the same cuvette without enzyme solution. The inhibitory effects of the sulfonamide derivatives were examined. All compounds were tested in triplicate at each concentration level. Different concentrations of the compounds were used.

In vitro inhibition studies

For the inhibition studies of sulfonamide, different concentrations of these compounds were added to the enzyme. Activity percentage values of CA for different concentrations of each sulfonamide were determined by regression analysis using Microsoft Office 2000 Excel (Microsoft, Redmond, WA). CA enzyme activity without a sulfonamide solution was accepted to be 100% activity. Inhibitory effects of compounds **5a–l** on enzyme activities were tested under *in vitro* conditions; K_i values were calculated from the Lineweaver–Burk graphs and are given in Table 1[28].

Results and discussion

For the evaluation of inhibitory effects of sulfonamides on physiologically relevant human CA isozymes hCAI and II, several sulfonamide-bearing thiazole compounds were subjected to CA inhibition assay with CO_2 as substrate.

Benzenesulfonamide derivatives **5a–l** were prepared as shown in Scheme 1. Sulfanilamide thiourea was prepared from sulfanilamide and ammoniumthiocyanate in HCl. The proposed compounds were prepared from the thiourea compound and α-haloacetophenone derivatives in DMF-Ethanol by heating. The prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. From the ^{1}H NMR spectra, the hydrogen attached to the nitrogen resonances between 10.50 and 11.00 ppm, sulfanilamide NH_2 are seen at around 7.10 ppm and the =CH proton peak on thiazol ring comes around 7.50 ppm. From the ^{13}C NMR spectra, thiazole ring C=N and =C carbon atoms are seen at around 160 and 103 ppm, respectively. In the infrared spectra of compounds **5a–l**, it was possible to observe the absorptions between 3200 and 3400 cm^{-1} relating to NH and NH_2 peaks, As can be seen in the literature[32], there are two peaks assigned to S=O as symmetric and asymmetric stretching. The peaks of asymmetric and symmetric stretch are appeared around 1300 and 1100 cm^{-1}, respectively. All spectra and elemental analyses support the structure of the synthesized compounds.

For evaluating the inhibitory activity of compounds on CA, all synthesized compounds were subjected to CA inhibition assay with CO_2 as substrate. The results showed that all the synthesized compounds (**5a–l**) inhibited the hCA I and II enzyme activity. The inhibition values of the compounds against CAs are summarized in Table 1. We have determined the IC_{50} values of 0.35–11.34 μM (K_i = 0.33–1.21 μM) for hCA I and 0.3–1.56 μM (K_i = 0.11–1.99 μM) for hCA II. Among the compounds, **5b** was found to be the most active for CAs (IC_{50}: 0.35 μM for hCA I and hCA II).

Sulfonamides are coordinated to the zinc (II) ion within the hCAs active site, whereas their organic scaffolds fill the entire enzyme cavity, making an extensive series of van der Waals and polar interactions with amino acid residues both at the bottom, middle and entrance of the active site cavity[33].

There are many classes of compounds for CA inhibitors in the literature: (i) sulfonamides and their isosteres (such as sulfamates, sulfamides and similar derivatives) and metal complexing anions. The primary sulfonamides[34] (RSO_2NH_2) in clinical use for more than 50 years as anti-glaucoma drugs are classical CA inhibitors (CAI). In addition to the established role of these CAIs as anti-glaucoma agents, it has been recently reported that they have potential as anti-convulsant. Sulthiame, topiramate and zonisamide are clinically used antiepileptics, showing potent inhibition of many CA isozymes present in the brain. (ii) Polyamines[35], such as spermine, spermidine and congeners. (iii) Phenols[36], and (iv) the coumarins and thiocoumarins[37], which have an inhibition mechanism not dependent of Zn(II), and bind (in hydrolyzed form) in the same active site region as the activators, occluding the entrance to the active site while the other three CAI groups bind to Zinc(II) ion or Zinc coordinated water molecule/hydroxide ion and the recently reported class of effective CAIs, (v) urea/thiourea compounds[38,39], (vi) β-lactames[40]. Sulfonamides have important inhibitory effect on the CA enzyme due to their ability to gain ionic structure easily and these compounds are very important in medicinal chemistry and they constitute an important class of drugs used extensively as pharmaceutical and agricultural agents[41]. The following conclusions should be noted regarding the CA inhibition data shown in Table 1.

The derivatives **5a–l** investigated here showed inhibitory activity against both two investigated CA isozymes and the affinity in the micromolar range for both cytosolic isozyme hCA I and CA II. The compounds showed IC_{50} in the range of 0.35–1.34 μM and 0.35–1.56 μM against hCA I and CA II, respectively. Nitro group at the *para*-position of the phenyl ring exhibited a greater inhibitory effect than at the *ortho* and *meta*-position. Flouro-substituted compound showed more inhibitory effect than chloro, cyano, methoxy, methyl and unsubstituted compounds. Flourophenylsulphamate adducts were reported that the sulphomates possess a rather variable binding pattern within the hCA active site[42,43]. It should also be mentioned that among the nitro substituted compounds, o-nitro substituted compound (**5i**) seems to be much less effective inhibitors as compared to the meta (**5j**)

and para (**5b**) substituted corresponding compounds. According to the literature[44], this may be due to the sterical impairment of the *ortho*-substituent for the binding of the compounds to the Zn(II) ion within the enzyme active site. K_i values of the compounds for hCA I ($K_i = 0.33–1.21$) are close to the clinically used sulfonamide AZA (acetazolamide, $K_i = 0.250\ \mu M$) whereas the values of the compounds for hCA II ($K_i = 0.11–1.99\ \mu M$) are higher than AZA ($K_i = 0.012\ \mu M$)[26].

In conclusion, we have reported the synthesis, characterization and evaluation of biological activity of a series of sulfonamide-bearing thiazole compounds for the inhibition of the physiologically relevant CA isozymes. They inhibit the CAs with the inhibition constants of 0.35–1.22 μM for hCA I and 0.35–1.56 μM for hCA II. Compound **5b** behaved as a good inhibitor on hCA I and hCA II with an activation constant of 0.35 μM. Enzyme inhibition is a very important issue for drug design and biochemical applications[45–48]. The results put forward that new sulfonamide derivatives inhibited the hCA I and II enzyme activity. Therefore, our results suggested that the sulfonamide derivatives may be used for some medical applications and they may be taken for further evaluation *in vivo* studies. The inhibition of the various enzymes in tissues may cause several disorders, but may also be the opposite. Sometimes, enzyme inhibition may need for the treatment of some diseases. In this regard, enzyme inhibition studies are very critical in the new drug development.

Declaration of interest

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References

1. Ranga R, Sharma V, Kumar V. New thiazolidinly analogs containing pyridine ring: synthesis, biological evaluation and QSAR studies. Med Chem Res 2013;22:1538–48.
2. Marson CM. New and unusual scaffolds in medicinal chemistry. Chem Soc Rev 2011;40:5514–33.
3. Patt WC, Hamilton HW, Taylor MD, et al. Structure activity relationships of a series of 2-amino-4-thiazole containing renin inhibitors. J Med Chem 1992;35:2562–72.
4. Jung KY, Kim SK, Gao ZG, et al. Structure-activity relationships of thiazole and thiadiazole derivatives as potent and selective human adenosine A3 receptor antagonists. Bioorg Med Chem 2004;12: 613–23.
5. Heng S, Gryncel KR, Kantrowitz ER. A library of novel allosteric inhibitors against fructose 1,6-bisphosphatase. Bioorg Med Chem 2009;17:3916–22.
6. Ram S, Celik G, Khloya P, et al. Benenesulfonamide bearing 1,2,4-triazole scaffolds as potent inhibitors of tumor associated carbonic anhydrase isoform hCA IX and hCA XII. Bioorg Med Chem 2014; 22:1873–82.
7. Vogt D, Veber J, Ihlefeld K, et al. Desing, synthesis and evaluation of 2-aminothiazole derivatives as sphingosine kinase inhibitors. Bioorg Med Chem 2014;22:5354–67.
8. Wei MX, Reng L, Li XQ, et al. Synthesis of new chiral 2,5-disubstituted 1,3,4-thiadiazoles possessing γ-butenolide moiety and preliminary evaluation of in vitro anticancer activity. Eur J Med Chem 2009;44:3340–4.
9. Alterio V, Di Fiore A, D'Ambrosio K, et al. Multiple binding modes of inhibitors to carbonic anhydrases: how to desing specific drugs targeting 15 different isoforms? Chem Rev 2012;112:4421–68.
10. Supuran CT, Scozzafava A, Casini A. Carbonic anhydrase inhibitors. Med Res Rev 2003;23:146–89.
11. Supuran CT, Scozzafava A. Applications of carbonic anhydrase inhibitors and activators in therapy. Exp Opin Ther Patents 2002;12: 217–42.
12. Hewett-Emmett D. Evolution and distribution of the carbonic anhydrase gene families. In: Chegwidden WR, Edwards Y, Carter N, eds. The carbonic anhydrase – New horizons. Basel: Birkhauser Verlag; 2000:29–78.
13. Del Prete S, Vullo D, Fisher GM, et al. Discovery of a new family of carbonic anhysrases in tha malaria pathogen plasmodium falciparum-the η-carbonic anhydrases. Bioorg Med Chem Lett 2014;24: 4389–96.
14. Demirdağ R, Çomakli V, Şentürk M, et al. Purification and characterization of carbonic anhydrase from sheep kidney and effects of sulfonamides on enzyme activity. Bioorg Med Chem 2013; 21:1522–5.
15. Innocenti A, Durdagi S, Doostdar N, et al. Nanoscale enzyme inhibitors: fullerenes inhibit carbonic anhydrase by occluding the active site entrance. Bioorg Med Chem 2010;18:2822–8.
16. SitaRam Ceruso M, Khloya P, et al. Functionalized 1,3-diarylpyrazoles bearing 6-aminosulfonylbenzothiazole moiety as potent inhibitors of carbonic anhydrase isoforms hCA I, II, IX and XII. Bioorg Med Chem 2014;22:6945–52.
17. Ozensoy O, Arslan O, Kockar F. Differential in vitro inhibition effects of some antibiotics on tumor associated carbonic anhydrase isozymes of hCA-IX and hCA-XII. J Enzyme Inh Med Chem 2008; 23:579–85.
18. Briganti F, Mangani S, Scozzafava A, et al. Carbonic anhydrase catalyzes cyanamide hydration to urea: is it mimicking the physiological reaction? J Biol Inorg Chem 1999;4:528–36.
19. Guerri A, Briganti S, Scozzafava A, et al. Mechanism of cyanamide hydration catalyzed by carbonic anhydrase II suggested by cryogenic X-ray diffraction. Biochemistry 2000;39:12391–7.
20. Mustafa G, Khan IU, Ashraf M, et al. Synthesis of new sulfonamides as lipoxygenase inhibitors. Bioorg Med Chem 2012;20:2535–9.
21. Gadad AK, Mahajanshetti CS, Nimbalkar S, Raichurkar A. Synthesis and antibacterial activity of some 5-guanylhydrazone/thiocyanato-6-arylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfonamide derivatives. Eur J Med Chem 2000;35:853–67.
22. Li JJ, Anderson D, Burton EG, et al. 12-Diarylcyclopentenes as selective cyclooxygenase-2 inhibitors and orally active antiinflammatory agents. J Med Chem 1995;38:4570–8.
23. Yoshino H, Ueda N, Niijma J, et al. Novel sulfonamides as potential, systemically active antitumor agents. J Med Chem 1992;35:2496–7.
24. Pacchiano F, Carta F, MCDonald PC, et al. Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis. J Med Chem 2011;54:1896–902.
25. Renzi G, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors: topical sulfonamide antiglaucoma agents incorporating secondary amine moieties. Bioorg Med Chem Lett 2000;10:673–6.
26. Alp C, Maresca A, Alp NA, et al. Secondary/tertiary benzenesulfonamides with inhibitory action against the cytosolic human carbonic anhydrase isoforms I and II. J Enzyme Inhib Med Chem 2013;28: 294–8.
27. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–81.
28. Fabrizi F, Mincione F, Somma T, et al. A new approach to antiglaucoma drugs: carbonic anhydrase inhibitors with or without NO donating moieties. Mechanism of action and preliminary pharmacology. J Enzyme Inhib Med Chem 2012;27: 138–47.
29. Arslan O, Nalbantoglu B, Demir N, et al. A new method for the purification of carbonic anhydrase isozymes by affinity chromatography. Turk J Med Sci 1996;26:163–6.
30. Wilbur KM, Anderson NG. Electrometric and colorimetric determination of carbonic anhydrase. J Biol Chem 1948;176:147–54.
31. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. J Biol Chem 1967;242:4221–9.
32. Sen E, Alim Z, Duran H, et al. Inhibitory effect of novel pyrazole carboxamide derivatives on human carbonic anhydrase enzyme. J Enzyme Inhib Med Chem 2013;28:328–36.
33. Maresca A, Temperini C, Pochet L, et al. Deciphering the mechanism of carbonic anhydrase inhibition with coumarins and thiocoumarins. J Med Chem 2010;53:335–44.
34. Supuran CT. Carbonic anhydrase inhibitors. Bioorg Med Chem Lett 2010;20:3467–74.
35. Carta F, Temperini C, Innocenti A, et al. Polyamines inhibit carbonic anhydrases by anchoring to the zinc-coordinated water molecule. J Med Chem 2010;53:5511–17.
36. Sarikaya SBO, Topal F, Sentürk M, et al. In vitro inhibition of α-carbonic anhydrase isozymes by some phenolic compounds. Bioorg Med Chem Lett 2011;21:4259–68.

37. Maresca A, Temperini C, Vu H, et al. Non-zinc mediated inhibition of carbonic anhydrases: coumarins are a new class of suicide inhibitors. J Am Chem Soc 2009;131:3057–62.
38. Celik F, Arslan M, Yavuz E, et al. Synthesis and carbonic anhydrase inhibitory properties of novel 1,4-dihydropyrimidinone substituted diarylureas. J Enzyme Inhib Med Chem 2014;29:18–22.
39. Demirhan H, Arslan M, Kaya MO, et al. In vitro inhibition of purified human carbonic anhydrase I and II by novel fluorene derivatives. Maced J Chem Chem Eng 2014;33:199–207.
40. Berber N, Arslan M, Nurcan Bilen Ç, et al. Synthesis and evaluation of new phthalazine substituted β-lactam derivatives as carbonic anhydrase inhibitors. Russ J Bioorg Chem 2015;41:414–20.
41. Jain P, Saravanan C, Singh SK. Sulphonamides: deserving class as MMP inhibitors? Eur J Med Chem 2013;60:89–100.
42. Kim CY, Chang JS, Doyon JB, et al. Contribution of fluorine to protein-ligand affinity in the binding of fluoroaromatic inhibitors to carbonic anhydrase II. J Am Chem Soc 2000;122:12125–34.
43. Winum JY, Innocenti A, Vullo D, et al. Carbonic anhydrase inhibitors; fluorinated phenyl sulfamates show strong inhibitory activity and selectivity for the inhibition of the tumor-associated isozymes IX and XII over the cytosolic ones I and II. Bioorg Med Chem Lett 2009;19:5082–5.
44. Winum JY, Dogne JM, Casini A, et al. Carbonic anhydrase inhibitors: synthesis and inhibition of cytosolic membrane-associated carbonic anhydrase isozymes I, II, and IX with sulfonamides incorporating hydrazino moieties. J Med Chem 2005;48:2121–5.
45. Alim Z, Kilinç N, İşgör MM, et al. Some anti-inflammatory agents inhibit esterase activities of human carbonic anhydrase isoforms I and II: an in vitro study. Chem Biol Drug Design 2015;86:857–63.
46. Karatas MO, Alici B, Cakir U, et al. Synthesis and carbonic anhydrase inhibitory properties of novel coumarin derivatives. J Enzyme Inhib Med Chem 2013;28:299–304.
47. Arslan M, Gencer N, Arslan O, et al. In vitro efficacy of some cattle drugs on bovine serum paraoxonase 1 (PON1) activity. J Enzyme Inhib Med Chem 2012;27:722–9.
48. Sayin D, Cakir DT, Gencer N, et al. Effects of some metals on paraoxonase activity from shark *Scyliorhinus canicu*. J Enzyme Inhib Med Chem 2012;27:595–8.