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RESEARCH ARTICLE

Synthesis and carbonic anhydrase inhibitory activities of new thienyl-substituted pyrazoline benzenesulfonamides

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Abstract

A series of new thienyl-substituted pyrazoline benzenesulfonamides were synthesized and their carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activities were tested on the human (h) isoforms hCA I and hCA II. The inhibition constant (K_i) of these sulfonamides were in the range of 232.16–637.70 nM toward the slow cytosolic isozyme hCA I, and in the range of 342.07–455.80 nM toward hCA II. Many of these compounds showed comparable inhibition with the reference sulfonamide acetazolamide, a clinically used drug. As the sulfonamide CA inhibitors (CAIs) show many therapeutic uses, these derivatives represent interesting examples of a novel class of such derivatives.

Keywords

Benzenesulfonamides, carbonic anhydrase, pyrazoline, synthesis, thiophene

History

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Introduction

Chalcones are open-chain flavonoids, being well-known intermediates for synthesizing various heterocyclic compounds, which are associated with an amazing range of biological activities[1,2]. Chalcones can be used as intermediate compounds for designing and synthesizing pharmacologically active heterocyclic structures such as pyrazolines. Pyrazolines have several biological activities including anticancer, antiviral, antimicrobial and anti-inflammatory activities[3–6].

Carbonic anhydrase (CA) is a superfamily of metalloenzymes that catalyzes the rapid conversion of CO_2 to HCO_3^- and H^+, being involved in many biochemical processes[7]. CA isoforms are found in a variety of tissues where they participate in several important biological processes, such as acid–base balance, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis and electrolyte secretion[7]. Many CA isozymes involved in these processes are important therapeutic targets with the potential to be inhibited/activated for the treatment of a range of disorders, such as edema, glaucoma, obesity, cancer, epilepsy, diuretics, antiepileptics, anticancer and osteoporosis[8–14]. Sulfonamide derivatives, especially aromatic and heterocyclic sulfonamides when the sulfonamido group is unsubstituted[15], are specific and potent carbonic anhydrase inhibitors (CAIs) and still attract much interest[7–9]. *p*-Hydrazinobenzenesulfonamide itself or its condensation derivatives with acetoacetic and levulinic acids had a definite inhibitory activity on carbonic anhydrases when there was no substituent on sulfonamido group[16]. It was reported that sulfonamide compounds having five-membered heterocycle system had better CA activity than six-membered rings[17]. So, the insertion of the thiophene ring into the chemical design can be a useful strategy to obtain compounds with impressive bioactivity.

The aim of this study was to synthesize 4-aryl-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl) benzenesulfonamides, **9**–**16**, which have pyrazoline, sulfonamide and thiophene pharmacophores, to investigate their CA inhibitory activities.

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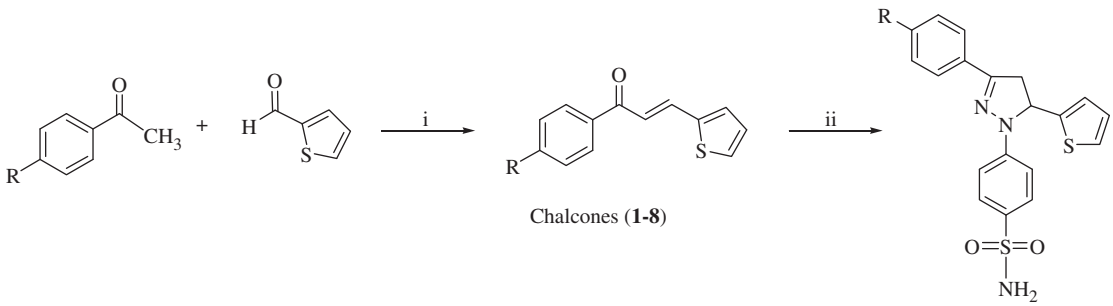
Materials and methods

Melting points were determined on Buchi 530 (Buchi Labortechnik AG, Flawil, Switzerland). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained using a Varian Mercury Plus spectrometer (Varian Inc., Palo Alto, CA). Chemical shifts (δ) were reported in parts per million (ppm). Liquid chromatography ion trap-time of flight tandem mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source, operating in both positive and negative ionization mode. Shimadzu's LCMS Solution software was used for data analysis.

General procedure for the synthesis of chalcones (1–8)

An aqueous solution of NaOH (10%, 10 mL) was added into the ethanol (6 mL) solution of 2-thiophene carbaldehyde (20.0 mmol) and a suitable acetophenone (20.0 mmol) (Scheme 1). The mixture was stirred overnight at room temperature and it was then poured on ice-water (100 ml). The mixture was neutralized with a solution of HCl (10%). The colored precipitate formed was filtered and crystallized from methanol–water (**1**–**8**). The yields of the chalcones were in the range of 32–59% [**1** (50%), **2** (43%), **3** (46%), **4** (55%), **5** (59%), **6** (44%), **7** (41%), **8** (32%)][18].



R: H for **1, 9**; CH_3 for **2, 10**; CH_3O for **3, 11**; Cl for **4, 12**; F for **5, 13**; Br for **6, 14**; NO_2 for **7, 15**; OH for **8, 16**.

(i) 10% aq NaOH, EtOH, 0-5 °C, 12 h; (ii) 4-hydrazinobenzenesulfonamide hydrochloride, EtOH/H^+, reflux 12 h.

Scheme 1. Synthesis of 1,3,5-trisubstituted pyrazoline-bearing benzenesulfonamides, **9–16**.

General procedure for the synthesis of pyrazolines (9–16)

The mixture of a suitable chalcone (1.0 mmol) and 4-hydrazino benzenesulfonamide hydrochloride (1.1 mmol) was dissolved in ethanol, and then catalytic amount of glacial acetic acid was added (Scheme 1). The mixture was refluxed for 12 h (**9–16**). Reactions were followed by thin-layer chromotography (TLC). After the reaction was stopped, some of the solvent was removed under vacuum and the mixture was stirred for 12 h at room temperature. The obtained solid was filtered, dried at room temperature and crystallized from methanol–ether (**9–16**)[6].

*4-(3-Phenyl-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**9**)*

M.p. 207–209 °C. Yield: 77%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 7.79$ (d, 2H, $J = 8.1$ Hz), 7.60 (d, 2H, $J = 8.8$ Hz), 7.47–7.40 (m, 3H), 7.36 (d, 1H, $J = 5.0$ Hz), 7.19 (d, 2H, $J = 8.8$ Hz), 7.14 (d, 1H, $J = 3.4$ Hz), 7.03 (s, 2H, NH_2), 6.94 (dd, 1H, $J = 5.0$, 3.4 Hz), 5.99 (dd, 1H, $J = 11.5$, 4.7 Hz), 3.92 (dd, 1H, $J = 17.6$, 11.5 Hz), 3.32 (dd, 1H, $J = 17.6$, 4.7 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 150.7$, 146.7, 145.2, 134.2, 132.4, 130.1, 129.4, 127.7, 127.6, 126.8, 126.3, 126.2, 113.2, 59.2, 43.7; HRMS (ESI-MS): calcd. for $C_{19}H_{18}N_3O_2S_2$ $[M+H]^+$ 384.0835; found 384.0822.

*4-(5-(Thiophen-2-yl)-3-(p-tolyl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**10**)*

M.p. 206–208 °C. Yield: 66%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 7.68$ (d, 2H, $J = 8.6$ Hz), 7.60 (d, 2H, $J = 8.4$ Hz), 7.35 (d, 1H, $J = 5.0$ Hz), 7.25 (d, 2H, $J = 8.4$ Hz), 7.17 (d, 2H, $J = 8.6$ Hz), 7.13 (d, 1H, $J = 3.0$ Hz), 7.03 (s, 2H, NH_2), 6.93 (dd, 1H, $J = 5.0$, 3.0 Hz), 5.96 (dd, 1H, $J = 11.3$, 4.5 Hz), 3.89 (dd, 1H, $J = 17.5$, 11.3 Hz), 3.29 (dd, 1H, $J = 17.5$, 4.5 Hz), 2.33 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 150.8$, 146.8, 145.2, 139.9, 134.0, 130.0, 129.6, 127.7, 127.6, 126.8, 126.26, 126.21, 113.1, 59.1, 43.8, 21.7; HRMS (ESI-MS): calcd. for $C_{20}H_{20}N_3O_2S_2$ $[M+H]^+$ 398.0991; found 398.0984.

*4-(3-(4-Methoxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**11**)*

M.p. 219–221 °C. Yield: 71%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 7.73$ (d, 2H, $J = 8.8$ Hz), 7.58 (d, 2H, $J = 8.8$ Hz), 7.35 (d, 1H, $J = 5.2$ Hz), 7.16–7.12 (m, 3H), 7.01–6.99 (m, 4H), 6.93 (dd, 1H, $J = 5.2$, 3.3 Hz), 5.94 (dd, 1H, $J = 11.6$, 4.0 Hz), 3.88 (dd, 1H, $J = 17.6$, 11.6 Hz), 3.79 (s, 3H, OCH_3), 3.29 (dd, 1H, $J = 17.6$, 4.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 161.0$, 150.7, 146.9, 145.3, 133.8, 128.5, 127.7, 127.6, 126.22, 126.16, 124.9, 114.9, 112.9, 59.0, 56.0, 43.9; HRMS (ESI-MS): calcd. for $C_{20}H_{20}N_3O_3S_2$ $[M+H]^+$ 414.0941; found 414.0925.

*4-(3-(4-Chlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**12**)*

M.p. 184–186 °C. Yield: 41%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 7.80$ (d, 2H, $J = 8.6$ Hz), 7.60 (d, 2H, $J = 8.8$ Hz), 7.51 (d, 2H, $J = 8.8$ Hz), 7.36 (d, 1H, $J = 5.1$ Hz), 7.19 (d, 2H, $J = 8.6$ Hz), 7.14 (d, 1H, $J = 3.5$ Hz), 7.04 (s, 2H, NH_2), 6.93 (dd, 1H, $J = 5.1$, 3.5 Hz), 6.01 (dd, 1H, $J = 11.6$, 4.5 Hz), 3.91 (dd, 1H, $J = 17.6$, 11.6 Hz), 3.32 (dd, 1H, $J = 17.6$, 4.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 149.6$, 146.5, 144.9, 134.6, 134.5, 131.3, 129.5, 128.5, 127.7, 127.6, 126.35, 126.32, 113.3, 59.4, 43.6; HRMS (ESI-MS): calcd. for $C_{19}H_{17}ClN_3O_2S_2$ $[M+H]^+$ 418.0445; found 418.0443.

*4-(3-(4-Fluorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**13**)*

M.p. 200–202 °C. Yield: 85%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 7.84$ (dd, 2H, $J = 8.8$, 5.5 Hz), 7.60 (d, 2H, $J = 8.8$ Hz), 7.36 (d, 1H, $J = 5.1$ Hz), 7.28 (t, 2H, $J = 8.8$ Hz), 7.18 (d, 2H, $J = 8.8$ Hz), 7.14 (d, 1H, $J = 3.2$ Hz), 7.03 (s, 2H, NH_2), 6.93 (dd, 1H, $J = 5.1$, 3.2 Hz), 5.99 (dd, 1H, $J = 11.6$, 4.5 Hz), 3.91 (dd, 1H, $J = 17.6$, 11.6 Hz), 3.31 (dd, 1H, $J = 17.6$, 4.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 163.4$ (d, $^1J = 247$ Hz), 149.9, 146.7, 145.1, 134.3, 129.1, 129.0, 127.7, 127.6, 126.3, 126.2, 116.5 (d, $^2J = 22$ Hz), 113.2, 59.3, 43.8; HRMS (ESI-MS): calcd. for $C_{19}H_{17}FN_3O_2S_2$ $[M+H]^+$ 402.0741; found 402.0734.

*4-(3-(4-Bromophenyl)-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**14**)*

M.p. 197–199 °C. Yield: 58%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 7.73$ (d, 2H, $J = 8.6$ Hz), 7.64 (d, 2H, $J = 8.6$ Hz), 7.60 (d, 2H, $J = 8.6$ Hz), 7.36 (d, 1H, $J = 5.0$ Hz), 7.19 (d, 2H, $J = 8.6$ Hz), 7.14 (d, 1H, $J = 3.4$ Hz), 7.04 (s, 2H, NH_2), 6.93 (dd, 1H, $J = 5.0$, 3.4 Hz), 6.01 (dd, 1H, $J = 11.7$, 4.4 Hz), 3.91 (dd, 1H, $J = 17.6$, 11.7 Hz), 3.29 (dd, 1H, $J = 17.6$, 4.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 149.7$, 146.5, 144.9, 134.5, 132.4, 131.6, 128.7, 127.7, 127.6, 126.4, 126.3, 123.3, 113.3, 59.3, 43.5; HRMS (ESI-MS): calcd. for $C_{19}H_{17}BrN_3O_2S_2$ $[M+H]^+$ 461.9940; found 461.9929.

*4-(3-(4-Nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**15**)*

M.p. 209–211 °C. Yield: 78%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 8.27$ (d, 2H, $J = 8.8$ Hz), 8.02 (d, 2H, $J = 8.8$ Hz), 7.64 (d, 2H, $J = 8.8$ Hz), 7.38 (d, 1H, $J = 5.1$ Hz), 7.26 (d, 2H, $J = 8.8$ Hz), 7.16 (d, 1H, $J = 3.1$ Hz), 7.07 (s, 2H, NH_2), 6.94 (dd, 1H, $J = 5.1$, 3.1 Hz), 6.13 (dd, 1H, $J = 11.8$, 4.6 Hz), 3.98 (dd, 1H, $J = 17.7$, 11.8 Hz), 3.40 (dd, 1H, $J = 17.7$, 4.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 148.6$, 147.8, 145.9, 144.6, 138.6, 135.2, 127.8, 127.7, 127.6, 126.6, 126.5, 124.7, 113.8, 59.7, 43.2; HRMS (ESI-MS): calcd. for $C_{19}H_{17}N_4O_4S_2$ $[M+H]^+$ 429.0686; found 429.0690.

*4-(3-(4-Hydroxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-*1H*-pyrazol-1-yl)benzenesulfonamide (**16**)*

M.p. 249–251 °C. Yield: 83%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) $\delta = 9.88$ (s, 1H, OH), 7.63 (d, 2H, $J = 8.8$ Hz), 7.57 (d, 2H, $J = 8.8$ Hz), 7.35 (d, 1H, $J = 5.0$ Hz), 7.13–7.11 (m, 3H), 7.01 (s, 2H, NH_2), 6.92 (dd, 1H, $J = 5.0$, 3.6 Hz), 6.82 (d, 2H, $J = 8.8$ Hz), 5.91 (dd, 1H, $J = 11.4$, 4.3 Hz), 3.85 (dd, 1H, $J = 17.6$, 11.4 Hz), 3.24 (dd, 1H, $J = 17.6$, 4.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm) $\delta = 159.6$, 151.0, 146.9, 145.4, 133.6, 128.6, 127.7, 127.5, 126.2, 126.1, 123.3, 116.3, 112.8, 58.9, 43.9; HRMS (ESI-MS): calcd. for $C_{19}H_{18}N_3O_3S_2$ $[M+H]^+$ 400.0784; found 400.0767.

Biological activity

Carbonic anhydrase inhibition assay

Both the CA isoenzymes (hCA I and II) were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography in a single purification step as decscribed previously[19]. Thus, pH of the solution was adjusted to 8.7 using solid Tris. Then, supernatant was transferred to the previously prepared Sepharose-4B-L-tyrosine-sulphanilamide affinity column[20]. Subsequently, the proteins from the column were spectrophotometrically determined at 280 nm[21]. For determination of the purity of the hCA isoenzymes, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), having 10% and 3% acrylamide as an eluent and packing gel, respectively, with 0.1% SDS[22], was performed, through which a single band was observed for each isoenzyme.

CA isoenzyme activities were determined following the methods described by Verpoorte et al[23] and the methods reported previously[24]. Absorbance change at 348 nm from *p*-nitrophenylacetate (NPA) to *p*-nitrophenolate (NP) was recorded in 3 min intervals at the room temperature (25 °C) using a spectrophotometer (Shimadzu, UV-VIS Spectrophotometer, UVmini-1240, Kyoto, Japan)[25,26]. Quantity of the protein was measured spectrophotometrically at 595 nm during the purification steps according to the Bradford method[27]. As reported previously, bovine serum albumin was used as a standard protein. An activity (%)–[benzenesulfonamides] graph was depicted to determine the inhibitory effect of each benzenesulfonamides derivative. For K_i values, three different concentrations of **9–16** were tested. NPA was used as a substrate at five different concentrations, and Lineweaver–Burk curves[28] were drawn as described in previous studies[29,30].

Results and discussion

Compounds **9–16** were successfully synthesized by starting from suitable chalcone and their chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. The detailed interpretation of the spectra are presented in the ''Materials and methods'' section. CA inhibitory activities of the compounds were tested on hCA I and II isoenzymes and the results are shown in Table 1.

When IC_{50} values of the compounds were considered, all compounds [compounds(times): **9** (1.9), **10** (2.9), **11** (2.4), **12** (2.0), **13** (1.9), **14** (3.3), **15** (2.9), **16** (2.5)] had 1.9–3.3 times more potent inhibitory potential than acetazolamide (AZA) toward hCA I. The most effective compound toward hCA I in terms of IC_{50} value was **14,** which has bromine substituent, while the least effective ones were fluorine-substituted compound **13** and nonsubstituted compound **9**.

The inhibitory activity of the halogen-bearing compounds toward hCA I was inversly correlated with electronegativity of the halogen [**14** with bromine ($IC_{50} = 299.48$ nM) > **13** with fluorine ($IC_{50} = 521.84$ nM) > **12** with chlorine ($IC_{50} = 502.90$ nM)] by considering IC_{50} values. Any type of substituent on the phenyl ring (except fluorine substituent) was useful modification to increase the inhibitory potential of the compounds by decreasing IC_{50} value toward hCA I.

When the methyl (**10**)- and methoxy (**11**)-substituted compound's IC_{50} values were compared, introduction of oxygen atom in **11** decreased the inhibitory potential by increasing IC_{50} of **11**. When oxygen-bearing methoxylated compound **11** was compared with compound **16** that is hydroxy-substituted one, **16** was more potent inhibitor than **11**. This may suggest that decrease in steric hindrance on oxygen may be helpful to increase the inhibitory potential toward hCA I. On the other hand, when the substituent was nitro (**15**) in which two oxygen atoms are available on nitrogen, inhibitory potential of **15** was more potent than **16**. The order of potency of the compounds toward hCA I in terms of IC_{50} was **15** (nitro) > **16** (hydroxy) > **11** (methoxy).

It was interesting that compound **10** having methyl group on phenyl ring, which is an electron-donating substituent, had similar inhibitory effect (similar IC_{50} values) with **15**, which has an

Table 1. Human CA isoenzymes (hCA I and II) inhibition value of the compounds (**9–16**) by the esterase method with 4-nitrophenyl acetate as substrate.

	IC_{50} (nM)				K_i (nM)	
Compounds	hCA I	r^2	hCA II	r^2	hCA I	hCA II
9	520.66	0.9511	427.25	0.9726	441.99 ± 88.26	372.48 ± 67.37
10	338.05	0.9964	491.14	0.9837	232.16 ± 18.17	403.33 ± 71.60
11	412.25	0.9721	508.81	0.9611	432.85 ± 95.90	441.02 ± 110.8
12	502.90	0.9667	487.34	0.9527	637.70 ± 310.3	396.91 ± 92.80
13	521.84	0.9806	472.07	0.9704	630.58 ± 301.1	455.80 ± 128.4
14	299.48	0.9903	436.67	0.9704	276.32 ± 32.91	368.52 ± 79.11
15	337.88	0.9712	482.25	0.9489	291.74 ± 22.74	342.07 ± 94.07
16	401.74	0.9819	523.02	0.9728	270.17 ± 78.36	437.60 ± 88.43
AZA	985.77	0.9811	489.40	0.9972	278.76 ± 44.28	293.43 ± 46.41

Acetazolamide (AZA) was used as a standard inhibitor for all hCA isoenzymes. The results were expressed as nanomolar (nM).

electron-attracting substituent. This may suggest that the activity is not dependent on the electonegativity of the substituents on the phenyl ring toward hCA I isoenzyme.

On the other hand, when IC_{50} value of the compounds toward hCA II were considered, the compounds had similar IC_{50} to AZA. When IC_{50} values of the compounds were considered, the best inhibitor was nonsubstituted compound **9** toward hCA II. Other substituents than hydrogen on phenyl ring decreased the inhibitory activity by increasing IC_{50} values of the compounds. The least effective compound was hydroxy-substituted compound **16**. Increasing steric hindrance on oxygen atom by the replacement of hydrogen with methyl in methoxy-substituted compound **11** increased the activity toward hCA II isoenzyme, while the introduction of nitro substituent instead of hydroxy group was a useful modification to increase the acitivity. IC_{50} values of the halogen-bearing compounds were not dependent on the electronegativities of the substituents.

When K_i value of the compounds toward hCA isoenzymes were considered: K_i values were in the range of 232.16 ± 18.17–637.70 ± 310.30 nM toward hCA I and 342.07 ± 94.07–455.80 ± 128.40 nM toward hCA II, while K_i values of AZA were 278.76 ± 44.28 nM and 293.43 ± 46.41 nM toward hCA I and hCA II, respectively. According to K_i values of the compounds toward hCA I, the most effective compound, which has the lowest K_i value, was methyl-substituted compound **10** and the least effective one was chlorine-substituted **12**. Compound **10** and hydroxy-substituted compound **16** had more potent K_i values than AZA, while bromine-substituted **14** has K_i value similar to AZA toward hCA I.

When the K_i values of the compounds toward hCA II were considered, the most effective one was fluorine-substituted compound **13** and the least effective one was nitro-susbtituted compound **15**. All compounds were less effective than AZA toward hCA II isoenzyme.

Compounds **10**, **12**, **13**, **14** and **16** are reported here for the first time, by detailed spectral analysis and bioactivities. When the IC_{50} values were considered, the most effective compounds were bromine-substituted **14**, which are 3.3 times more potent than AZA, and nonsubstituted compound **9** toward hCA I and II, respectively. On the other hand, according to K_i values, methyl-substituted compound **10** toward hCA I and nitro-substituted compound **15** toward hCA II can be considered as leader compounds for further studies.

In conclusion, we report the synthesis and CA inhibitory activity of a new class of sulfonamides, which showed medium potency against the cytosolic isoforms hCA I and II, presumably due to the very bulky scaffolds present in their molecules. However, such compounds may show interest for the inhibition of other CA isoforms that possess a wider active site, such as hCA IX, XII and XIV[31].

Declaration of interest

The authors report no conflicts of interest. The authors are responsible for the content and writing of this article. This research work was supported by Ataturk University Research Found, Turkey (Project No. BAP: 2013/289).

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