

RESEARCH ARTICLE

Synthesis and evaluation of new thiadiazole derivatives as potential inhibitors of human carbonic anhydrase isozymes (hCA-I and hCA-II)

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Abstract

2-[[5-(2,4-Difluoro/dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio] acetophenone derivatives (**3a–s**) were designed as human carbonic anhydrase isozymes (hCA-I and hCA-II) inhibitors and synthesized. hCA-I and hCA-II were purified from erythrocyte cells by the affinity chromatography. The inhibitory effects of 18 newly synthesized acetophenones on hydratase activity of these isoenzymes were studied *in vitro*. The average IC₅₀ values of the new compounds for hydratase activity ranged from 0.033 to 0.14 μM for hCA-I and from 0.030 to 0.11 μM for hCA-II. Among the newly synthesized compounds, 2-[[5-(2,4-dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-bromoacetophenone (**3n**) can be considered as a promising hCA-II inhibitor owing to its selective and potent inhibitory effect on hCA-II.

Keywords

Carbonic anhydrase, hydratase activity, thiadiazole

History

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Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are a superfamily of zinc-containing metalloenzymes, present in prokaryotes and eukaryotes, being encoded by five distinct, evolutionarily unrelated gene families: the α -, β -, γ -, δ -, and ϵ -CAs. 16 different α -CA isoforms were isolated and characterized in mammals, where they play crucial physiological roles. Some of them are cytosolic (CA I, CA II, CA III, CA VII, CA XIII), others are membrane-bound (CA IV, CA IX, CA XII, CA XIV and CA XV), CA VA and CA VB are mitochondrial, and CA VI is secreted in saliva and milk. Three acatalytic forms are also known, the CA related proteins (CARP), CARP VIII, CARP X and CARP XI^{1–4}.

CAs, which catalyze the interconversion of carbon dioxide and water to bicarbonate and protons, have attracted a great deal of interest as important targets for drug discovery due to their essential roles in crucial physiological processes connected with respiration and transport of CO₂/bicarbonate between metabolizing tissues and lungs, pH and CO₂ homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity and many other physiologic or pathologic processes⁴.

Inhibition of CA isoenzymes is a promising approach for pharmacologic intervention in a variety of disorders such as glaucoma, epilepsy, obesity, and cancer. In the last decades, CA inhibition has also emerged as a major focus of pharmaceutical research for the design of anti-infective agents with a novel mechanism of action^{1–9}.

Thiadiazole has received considerable attention as a privileged scaffold due to its significant therapeutic potential. The sulfur atom of thiadiazole ring imparts improved liposolubility and the mesoionic nature of 1,3,4-thiadiazoles also allows these compounds to cross cellular membranes and interact with biological targets with distinct affinities^{10,11}. Several derivatives of 5-amino-1,3,4-thiadiazole-2-sulfonamide have been synthesized and used as carbonic anhydrase inhibitors. One of them is acetazolamide (AAZ) (*N*-(5-sulfamoyl-1,3,4-thiadiazole-2-yl)acetamide), which is used in the treatment of glaucoma (Figure 1)^{12–15}. Some researchers have reported the potency of the SH moiety as a zinc-binding function in the design of carbonic anhydrase inhibitors and carried out considerable research for the synthesis and evaluation of 5-amino-1,3,4-thiadiazole-2-thiol derivatives as carbonic anhydrase inhibitors^{16–18}.

On the basis of afore-mentioned findings, herein we report the synthesis and biological evaluation of a series of 2-[[5-(2,4-difluoro/dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio] acetophenone derivatives as hCA-I and hCA-II inhibitors.

Methods

Chemistry

All reagents were purchased from commercial suppliers and used without further purification. Melting points (m.p.) were

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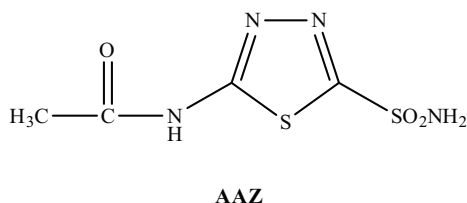


Figure 1. Chemical structure of the clinically used sulfonamide acetazolamide (AAZ).

determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. Proton nuclear magnetic resonance (^1H -NMR) spectra were recorded on a Bruker 500 MHz spectrometer (Bruker, Billerica, MA). Chemical shifts were expressed in parts per million (ppm) and tetramethylsilane was used as an internal standard. Mass spectra were recorded on a Agilent LC-MSD-Trap-SL Mass spectrometer (Agilent, Minnesota, MN). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser (Perkin-Elmer, Norwalk, CT).

General procedure for the synthesis of the compounds

4-(2,4-Difluoro/dichlorophenyl)thiosemicarbazide (**1a–b**)

A mixture of 2,4-difluoro/dichlorophenyl isothiocyanate (0.1 mol) and hydrazine hydrate (0.2 mol) in ethanol (30 ml) was stirred at room temperature for 5 h and then filtered. The residue was crystallized from ethanol^{19,20}.

5-[(2,4-Difluoro/dichlorophenyl)amino]-1,3,4-thiadiazole-2(3H)-thione (**2a–b**)

4-(2,4-Difluoro/dichlorophenyl)thiosemicarbazide (**1a–b**) was dissolved in a solution of sodium hydroxide in ethanol. Carbon disulfide was then added while stirring and the reaction mixture was heated under reflux for 10 h. The solution was cooled and acidified to pH 4–5 with hydrochloric acid solution and crystallized from ethanol²¹.

2-[[5-(2,4-Difluoro/dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]acetophenone derivatives (**3a–s**)

A mixture of 5-(2,4-difluoro/dichlorophenylamino)-1,3,4-thiadiazole-2(3H)-thione (**2a–b**) (0.05 mol) and appropriate phenacyl bromide (0.05 mol) in the presence of potassium carbonate (0.05 mol) in acetone was stirred at room temperature for 8 h. The reaction mixture was filtered and crystallized from ethanol.

2-[[5-(2,4-difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]acetophenone (**3a**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 4.96 (s, 2H), 7.07–7.11 (m, 1H), 7.32–7.37 (m, 1H), 7.56–7.59 (m, 2H), 7.69 (t, J = 7.5 Hz, 1H), 8.04–8.06 (m, 2H), 8.24–8.29 (m, 1H), 10.17 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 364

Anal. Calcd. for $\text{C}_{16}\text{H}_{11}\text{F}_2\text{N}_3\text{O}_2\text{S}_2$: C, 52.88; H, 3.05; N, 11.56. Found: C, 52.90; H, 3.04; N, 11.55.

2-[[5-(2,4-Difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-nitroacetophenone (**3b**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 5.00 (s, 2H), 7.06–7.34 (m, 2H), 8.22–8.27 (m, 3H), 8.37 (d, J = 8.5 Hz, 2H), 10.17 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 409

Anal. Calcd. for $\text{C}_{16}\text{H}_{10}\text{F}_2\text{N}_4\text{O}_3\text{S}_2$: C, 47.05; H, 2.47; N, 13.72. Found: C, 47.07; H, 2.45; N, 13.70.

2-[[5-(2,4-Difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-fluoroacetophenone (**3c**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 4.94 (s, 2H), 7.07–7.11 (m, 1H), 7.32–7.42 (m, 3H), 8.12–8.15 (m, 2H), 8.24–8.29 (m, 1H), 10.16 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 382

Anal. Calcd. for $\text{C}_{16}\text{H}_{10}\text{F}_3\text{N}_3\text{O}_2\text{S}_2$: C, 50.39; H, 2.64; N, 11.02. Found: C, 50.41; H, 2.65; N, 11.00.

2-[[5-(2,4-difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-chloroacetophenone (**3d**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 4.94 (s, 2H), 7.08–7.09 (m, 1H), 7.33–7.35 (m, 1H), 7.65 (d, J = 8.5 Hz, 2H), 8.06 (d, J = 8.5 Hz, 2H), 8.25–8.27 (m, 1H), 10.17 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 398

Anal. Calcd. for $\text{C}_{16}\text{H}_{10}\text{ClF}_2\text{N}_3\text{O}_2\text{S}_2$: C, 48.30; H, 2.53; N, 10.56. Found: C, 48.28; H, 2.54; N, 10.55.

2-[[5-(2,4-difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-bromoacetophenone (**3e**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 4.93 (s, 2H), 7.07–7.10 (m, 1H), 7.31–7.36 (m, 1H), 7.79 (d, J = 8.0 Hz, 2H), 7.97 (d, J = 8.5 Hz, 2H), 8.24–8.29 (m, 1H), 10.16 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 443

Anal. Calcd. for $\text{C}_{16}\text{H}_{10}\text{BrF}_2\text{N}_3\text{O}_2\text{S}_2$: C, 43.45; H, 2.28; N, 9.50. Found: C, 43.43; H, 2.30; N, 9.49.

2-[[5-(2,4-difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-cyanoacetophenone (**3f**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 4.97 (s, 2H), 7.08–7.09 (m, 1H), 7.33–7.34 (m, 1H), 8.06 (d, J = 8.5 Hz, 2H), 8.19 (d, J = 8.5 Hz, 2H), 8.24–8.25 (m, 1H), 10.17 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 389

Anal. Calcd. for $\text{C}_{17}\text{H}_{10}\text{F}_2\text{N}_4\text{O}_2\text{S}_2$: C, 52.57; H, 2.60; N, 14.42. Found: C, 52.55; H, 2.59; N, 14.41.

2-[[5-(2,4-Difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-methylacetophenone (**3g**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 2.40 (s, 3H), 4.92 (s, 2H), 7.07–7.11 (m, 1H), 7.33–7.39 (m, 3H), 7.94 (d, J = 8.0 Hz, 2H), 8.24–8.29 (m, 1H), 10.06 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 378

Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{F}_2\text{N}_3\text{O}_2\text{S}_2$: C, 54.10; H, 3.47; N, 11.13. Found: C, 54.11; H, 3.45; N, 11.11.

2-[[5-(2,4-Difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-methoxyacetophenone (**3h**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 3.87 (s, 3H), 4.89 (s, 2H), 7.06–7.12 (m, 3H), 7.33–7.37 (m, 1H), 8.01–8.04 (m, 2H), 8.24–8.29 (m, 1H), 10.15 (s, 1H).

MS (ESI) (m/z): [$M+1$]⁺ 394

Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{F}_2\text{N}_3\text{O}_3\text{S}_2$: C, 51.90; H, 3.33; N, 10.68. Found: C, 51.89; H, 3.31; N, 10.69.

2-[[5-(2,4-Difluorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-methylsulfonylacetophenone (**3i**)

^1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 3.32 (s, 3H), 4.99 (s, 2H), 7.08–7.11 (m, 1H), 7.33–7.37 (m, 1H), 8.12 (d, J = 8.5 Hz, 2H), 8.23–8.28 (m, 3H), 10.18 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 442

Anal. Calcd. for $C_{17}H_{13}F_2N_3O_3S_3$: C, 46.25; H, 2.97; N, 9.52.
Found: C, 46.26; H, 2.95; N, 9.51.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]acetophenone (3j)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 4.97 (s, 2H), 7.42 (dd, $J=9.0$, 2.5 Hz, 1H), 7.58 (t, $J=7.5$ Hz, 2H), 7.64 (d, $J=2.5$ Hz, 1H), 7.70 (t, $J=7.5$ Hz, 1H), 8.05 (d, $J=7.0$ Hz, 2H), 8.33 (d, $J=9.0$ Hz, 1H), 9.95 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 397

Anal. Calcd. for $C_{16}H_{11}Cl_2N_3OS_2$: C, 48.49; H, 2.80; N, 10.60.
Found: C, 48.51; H, 2.79; N, 10.59.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-nitroacetophenone (3k)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 5.02 (s, 2H), 7.42 (dd, $J=9.0$, 2.5 Hz, 1H), 7.64 (d, $J=2.5$ Hz, 1H), 8.27 (d, $J=8.5$ Hz, 2H), 8.32 (d, $J=8.5$ Hz, 1H), 8.39 (d, $J=8.5$ Hz, 2H), 9.96 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 442

Anal. Calcd. for $C_{16}H_{10}Cl_2N_4O_3S_2$: C, 43.55; H, 2.28; N, 12.70. Found: C, 43.53; H, 2.31; N, 12.69.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-fluoroacetophenone (3l)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 4.96 (s, 2H), 7.39–7.43 (m, 3H), 7.63 (d, $J=2.5$ Hz, 1H), 8.12–8.15 (m, 2H), 8.33 (d, $J=8.5$ Hz, 1H), 9.95 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 415

Anal. Calcd. for $C_{16}H_{10}Cl_2FN_3OS_2$: C, 46.38; H, 2.43; N, 10.14. Found: C, 46.40; H, 2.41; N, 10.15.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-chloroacetophenone (3m)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 4.95 (s, 2H), 7.42 (dd, $J=9.0$, 2.5 Hz, 1H), 7.64–7.66 (m, 3H), 8.06 (d, $J=8.5$ Hz, 2H), 8.33 (d, $J=9.0$ Hz, 1H), 9.95 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 431

Anal. Calcd. for $C_{16}H_{10}Cl_3N_3OS_2$: C, 44.61; H, 2.34; N, 9.75. Found: C, 44.60; H, 2.35; N, 9.72.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-bromoacetophenone (3n)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 4.94 (s, 2H), 7.42 (dd, $J=9.0$, 2.5 Hz, 1H), 7.64 (d, $J=2.5$ Hz, 1H), 7.79–7.81 (m, 2H), 7.97–7.99 (m, 2H), 8.32 (d, $J=9.0$ Hz, 1H), 9.95 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 476

Anal. Calcd. for $C_{16}H_{10}BrCl_2N_3OS_2$: C, 40.44; H, 2.12; N, 8.84. Found: C, 40.42; H, 2.13; N, 8.85.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-cyanoacetophenone (3o)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 4.99 (s, 2H), 7.42 (dd, $J=9.0$, 2.5 Hz, 1H), 7.63 (d, $J=2.5$ Hz, 1H), 8.06 (d, $J=8.5$ Hz, 2H), 8.19 (d, $J=8.5$ Hz, 2H), 8.32 (d, $J=9.0$ Hz, 1H), 9.94 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 422

Anal. Calcd. for $C_{17}H_{10}Cl_2N_4OS_2$: C, 48.46; H, 2.39; N, 13.30. Found: C, 48.45; H, 2.41; N, 13.29.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-methylacetophenone (3p)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 2.41 (s, 3H), 4.93 (s, 2H), 7.38 (d, $J=8.0$ Hz, 2H), 7.42 (dd, $J=9.0$, 2.5 Hz, 1H), 7.64 (d, $J=2.0$ Hz, 1H), 7.95 (d, $J=8.5$ Hz, 2H), 8.33 (d, $J=9.0$ Hz, 1H), 9.94 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 411

Anal. Calcd. for $C_{17}H_{13}Cl_2N_3OS_2$: C, 49.76; H, 3.19; N, 10.24. Found: C, 49.75; H, 3.20; N, 10.25.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-methoxyacetophenone (3r)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 3.86 (s, 3H), 4.90 (s, 2H), 7.07–7.12 (m, 2H), 7.40–7.45 (m, 1H), 7.62–7.65 (m, 1H), 8.03 (d, $J=8.5$ Hz, 2H), 8.33 (d, $J=8.5$ Hz, 1H), 9.94 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 427

Anal. Calcd. for $C_{17}H_{13}Cl_2N_3O_2S_2$: C, 47.89; H, 3.07; N, 9.86. Found: C, 47.90; H, 3.05; N, 9.85.

2-[[5-(2,4-Dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-methylsulfonylacetophenone (3s)

¹H-NMR (500 MHz, DMSO- d_6) δ (ppm): 3.32 (s, 3H), 5.01 (s, 2H), 7.42 (dd, $J=9.0$, 2.5 Hz, 1H), 7.65 (d, $J=2.5$ Hz, 1H), 8.12 (d, $J=8.0$ Hz, 2H), 8.27 (d, $J=8.5$ Hz, 2H), 8.32 (d, $J=9.0$ Hz, 1H), 9.96 (s, 1H).

MS (ESI) (m/z): $[M+1]^+$ 475

Anal. Calcd. for $C_{17}H_{13}Cl_2N_3O_3S_3$: C, 43.04; H, 2.76; N, 8.86. Found: C, 43.03; H, 2.74; N, 8.85.

Biochemistry

Sephacrose-4B, sulfanilamide, L-tyrosine, Tris, Na_2SO_4 , protein assay reagents, and chemicals for electrophoresis were purchased from Sigma-Aldrich Co. (St. Louis, MO). All other chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany).

Purification of carbonic anhydrase isozymes (hCA-I and hCA-II) from human erythrocytes by affinity chromatography

Fresh human blood was obtained from the blood center, Ataturk University, it was stored at 4 °C used within 2–3 d at most. The blood samples were centrifuged to separate erythrocytes at 2500 rpm for 15 min plasma and white blood cells which is the upper part the layers carefully removed and discarded. Then, underlying erythrocytes were washed with 0.9% NaCl solution twice and upper portions were also discarded. The erythrocytes were hemolyzed with distilled water at 0 °C, it was stirred for half an hour at 4 °C. The hemolysate was centrifuged at 20 000 rpm for 30 min and cell membranes were separated. pH was adjusted to 8.7 with solid Tris. So, the hemolysate was recovered to be applied to the column^{22,23}.

The affinity gel was prepared on Sepharose-4B matrix. After Sepharose-4B was activated with CNBr, L-tyrosine was covalently fitted. Then sulfanilamide was coupled to tyrosine with diazotization reaction as a ligand. The hemolysate was applied to the prepared Sepharose-4B-L-tyrosine-sulfanilamide affinity column equilibrated with 25 mM Tris-HCl/0.1 M 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 I and hCA II) isozymes were eluted with 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), respectively. All procedures were performed at 4 °C^{22,24}.

Hydratase activity assay

Carbonic anhydrase activity was determined using the Wilbur-Anderson Method which was modified by Rickli and Sly^{25,26}. This method, as a result hydration of CO₂ is released H⁺ ions and the pH changes were determined by means of bromine thymol blue indicator, based on measuring the elapsed time. Enzyme Unit (EU) were calculated using the equation (to-tc/tc) to and tc are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively.

Inhibition assays

It was studied with compounds **3a-s** to calculate values of IC₅₀ of hCA-I and hCA-II enzymes on the hydratase activity at different concentrations while maintaining constant the substrate concentration. Activities of enzymes in the medium without inhibitors were used as 100% activity. The activity % values of enzymes were calculated by measuring hydratase activity in the presence of different concentrations of inhibitors. The IC₅₀ value was calculated by utilizing graphs of % activity-[I] for each inhibitor^{27,28}.

Protein determination

Protein assay is performed according to the Bradford method for all purification steps. This method bases on the principle which Coomassie brilliant blue G-250 was binding with proteins in medium of phosphoric acid. Formed complex shows a maximum absorbance at 595 nm. The sensitivity of this method is between 1 and 100 µg²⁹.

SDS polyacrylamide gel electrophoresis

Then purifying hCA-I and-II isoenzymes from erythrocytes, discontinuous 3-10% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed in accordance Laemmli and the purity of enzymes were checked³⁰.

Results and discussion

The synthesis of compounds **3a-s** followed the general pathway outlined in Scheme 1. Initially, 4-(2,4-difluoro/dichlorophenyl)thiosemicarbazides (**1a-b**) were obtained by the reaction of

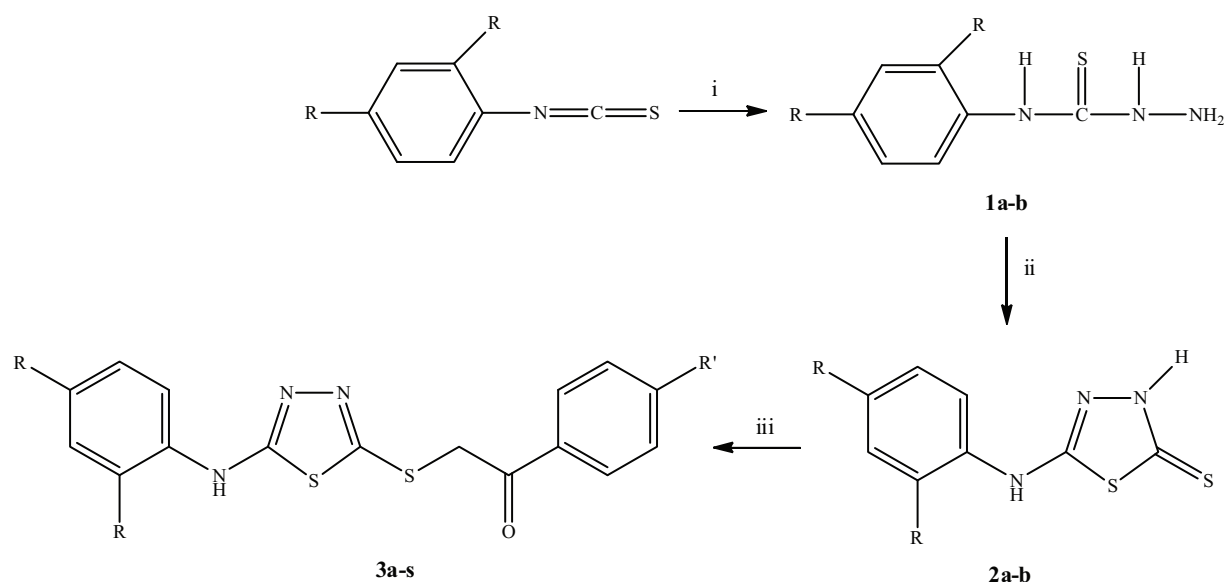
2,4-difluoro/dichlorophenylisothiocyanates with hydrazine hydrate. 5-[(2,4-Difluoro/dichlorophenyl)amino]-1,3,4-thiadiazole-2(3H)-thiones (**2a-b**) were synthesized via the ring closure reaction of 4-(2,4-difluoro/dichlorophenyl)thiosemicarbazides (**1a-b**) with carbon disulfide in the presence of sodium hydroxide. Finally, the nucleophilic substitution reaction of 5-[(2,4-difluoro/dichlorophenyl)amino]-1,3,4-thiadiazole-2(3H)-thiones (**2a-b**) with appropriate phenacyl bromides afforded 2-[[5-(2,4-difluoro/dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]acetophenone derivatives (**3a-s**). Some physicochemical properties of the compounds were given in Table 1.

The structures of compounds **3a-s** were confirmed by ¹H-NMR, mass spectral data, and elemental analysis. In the ¹H-NMR spectra of compounds **3a-s**, the signal due to the S-CH₂ protons was observed in the region 4.89–5.02 ppm as a singlet peak. The signal due to the N-H proton attached to the thiadiazole ring occurred in the region 9.94–10.18 ppm as a singlet. The other aromatic and aliphatic protons were observed at expected regions.

Table 1. Physicochemical properties of the compounds (**3a-s**).

Compound	R	R'	Yield (%)	M.p. (°C)	Log P*	MR*
3a	F	H	78	125	5.63	94.26
3b	F	NO ₂	90	167	–	–
3c	F	F	79	161	5.79	94.67
3d	F	Cl	81	158	6.19	98.87
3e	F	Br	83	155	6.46	101.95
3f	F	CN	85	169	5.66	100.36
3g	F	CH ₃	73	159	6.12	100.16
3h	F	OCH ₃	71	140	5.5	101.51
3i	F	SO ₂ CH ₃	74	179	4.34	109.04
3j	Cl	H	80	168	6.43	102.66
3k	Cl	NO ₂	92	183	–	–
3l	Cl	F	81	203	6.59	103.07
3m	Cl	Cl	83	195	6.99	107.27
3n	Cl	Br	85	184	7.26	110.35
3o	Cl	CN	87	217	6.46	108.76
3p	Cl	CH ₃	75	162	6.92	108.56
3r	Cl	OCH ₃	73	148	6.3	109.91
3s	Cl	SO ₂ CH ₃	76	186	5.14	117.44

*Log P and MR values were calculated using ChemDraw Ultra 7.0 (Cambridge Soft Corporation, Cambridge, MA). Log P and MR values of compounds **3b** and **3k** were not calculated.



Scheme 1. The synthetic route for the preparation of the thiadiazole derivatives (**3a-s**). Reagents and conditions: (i) NH₂NH₂ · H₂O, ethanol, rt, 5 h; (ii) (1) CS₂/NaOH, ethanol, reflux, 10 h; (2) HCl, pH 4–5; (iii) PhCOCH₂Br, K₂CO₃, acetone, rt, 8 h.

Table 2. Summary of purification for hCA-I and hCA-II from human erythrocyte.

Purification steps	Volume mL	Activity (EU/mL)	Total activity		Protein (mg/mL)	Specific activity (EU/mg)	Purification fold
			EU	%			
Hemolysate	100	42.75	4275	100	155	0.28	–
hCA-I	40	56.5	2260	52.9	0.082	689.0	2460.7
hCA-II	30	62.6	1875	43.9	0.045	1391.1	4968.2

Table 3. IC₅₀ values for the *in vitro* inhibition of hCA-I and hCA-II with compounds **3a–s**.

Compound	IC ₅₀ (μM)		
	hCA-I	hCA-II	hCA-I/hCA-II
3a	0.1	0.055	1.82
3b	0.11	0.074	1.49
3c	0.058	0.055	1.05
3d	0.068	0.056	1.21
3e	0.089	0.080	1.11
3f	0.078	0.078	1.0
3g	0.11	0.084	1.31
3h	0.14	0.10	1.4
3i	0.111	0.098	1.13
3j	0.128	0.096	1.33
3k	0.05	0.11	1.45
3l	0.071	0.044	1.61
3m	0.049	0.062	1.79
3n	0.068	0.072	1.94
3o	0.033	0.054	1.61
3p	0.036	0.056	1.56
3r	0.043	0.030	1.43
3s	*	*	*

*Since compound **3s** activated hCA-I and hCA-II isozymes, IC₅₀ values can not be calculated.

Mass spectral data and elemental analysis results were in full accordance with their depicted structures. hCA-I and hCA-II isoenzymes were purified from human blood using Sepharose-4B-L-tyrosine-sulphanylamide affinity chromatography. hCA-I was purified with a specific activity of 689.0 EU/mg and a yield of 52.9% and hCA-II was purified with a specific activity of 1391.1 EU/mg and a yield of 43.9%. The overall purification was approximately 2460.7-fold for hCA-I and 4968.2-fold for hCA-II (Table 2). The newly synthesized compounds (**3a–s**) were investigated for their *in vitro* inhibitory effects on hCA-I and hCA-II and IC₅₀ values were calculated for all derivatives (Table 3).

Generally, compounds bearing 2,4-dichlorophenyl group except compounds **3j** and **3k** were more effective than compounds bearing 2,4-difluorophenyl group. The inhibitory effects of eight derivatives (**3a**, **3b**, **3g**, **3h**, **3i**, **3j**, **3l**, **3r**) were more significant on hCA-II than hCA-I, whereas the inhibitory effects of four derivatives (**3k**, **3m**, **3o**, **3p**) were more significant on hCA-I than hCA-II.

The inhibitory effects of compounds **3c**, **3d**, **3e** and **3n** on hCA-I and hCA-II were fairly close. Compound **3f** stands out as the only derivative exhibiting the same inhibitory effect on hCA-I and hCA-II. log P and MR values of the compounds were calculated and compared with the inhibitory effects on hCA-I and hCA-II, but no correlation was found. Eleven compounds (**3o** ≈ **3p** > **3r** ≈ **3m** ≈ **3k** > **3c** > **3d** = **3n** > **3l** > **3f** > **3e**) were more effective on hCA-I than other compounds. According to IC₅₀ values, seven derivatives (**3r** > **3l** > **3o** > **3a** = **3c** ≈ **3d** = **3p**) exhibited more inhibitory effect on hCA-II.

Conclusion

In this article, we synthesized a series of 2-[[5-(2,4-difluoro/dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]acetophenone derivatives (**3a–s**) and evaluated their ability to inhibit carbonic anhydrase isozymes (hCA-I and hCA-II). Although compounds **3a–s** do not carry a sulfonamide group, an important pharmacophore for hCA inhibitory activity, it is a remarkable finding that compounds **3a–r** exhibited significant inhibitory effects on hCA-I and hCA-II. Among the newly synthesized compounds, 2-[[5-(2,4-dichlorophenylamino)-1,3,4-thiadiazol-2-yl]thio]-4'-bromoacetophenone (**3n**) can be evaluated as a potential hCA-II inhibitor due to its selective and potent inhibitory effect on hCA-II.

Declaration of interest

The authors report no conflicts of interest.

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