Bioorganic Chemistry 82 (2019) 123–128

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

# 3-Aminobenzenesulfonamides incorporating acylthiourea moieties selectively inhibit the tumor-associated carbonic anhydrase isoform IX over the off-target isoforms I, II and IV

Tanzeela Abdul Fattah[a], Silvia Bua[b], Aamer Saeed[a,*], Ghulam Shabir[a], Claudiu T. Supuran[b,*]

[a] Departmentof Chemistry, Quaid-i-Azam University-45320, Islamabad, Pakistan
[b] University of Florence, NEUROFARBA Department, Pharmaceutical and Nutraceutical Sciences Section, via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence), Italy

ARTICLE INFO

*Keywords:*
Carbonic anhydrase
Inhibitors
Thioureas
Isothiocyanates
3-Aminobenzenesulfonamide
Acyl-thioureas

ABSTRACT

We describe the synthesis of a series of novel 1-aroyl/acyl-3-(3-aminosulfonylphenyl) thioureas **(4a–k)** acting as human carbonic anhydrase (hCA, EC 4.2.1.1) inhibitors. Reaction of alkyl/aryl isothiocyanates with 3-aminobenzenesulfonamide afforded a series of the title compounds incorporating a variety of short as well as highly lipophilic long tails. The newly synthesized sulfonamides were evaluated against 4 physiologically relevant CA isoforms (hCA I, II, IV, and IX). Several compounds showed interesting inhibitory activity. The tumor-associated hCA IX was the most sensitive isoform to inhibition with these compounds, with $K_I$s in the range of 21.5–44.0 nM and selectivity ratios over the major cytosolic isoform hCA II in the range of 3.35–37.3. The sulfonamides incorporating the phenylacetylthioureido and pentadecanoylthioureido moieties were the most hCA IX-selective inhibitors detected in this work, making them of interest for further investigations.

## 1. Introduction

The zinc metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) catalyzes the rapid hydration of carbon dioxide into a proton and the bicarbonate ion utilizing a metal hydroxide nucleophilic mechanism, this being a simple but vital reaction, involved in many physiological processes including respiration, pH homeostasis, electrolyte secretion, biosynthesis of important molecules (urea, glucose and lipids etc.) and as shown recently, tumorigenicity [1,2]. These processes are crucial for most organisms, and as a result, during the evolution of life on Earth, seven genetically diverse families of this enzyme (classified as α-, the β-, γ-, δ-, ζ-, η- and θ-CAs) evolved [3–5]. There are 15 CA isoforms known in humans (hCAs) and they belong to the α-CA class [1–3]. These enzymes differ in their catalytic/inhibition activity, subcellular localization (mitochondria, cytosol, anchored to the cell membrane, transmembrane or secreted isoforms), tissue distribution and affinity for the different classes of inhibitors [6,7]. CA II is the most investigated and physiologically dominant isoform, being expressed in many tissues and organs in mammals [1–3]. Its presence in the endothelium of neovessels in several cancer tissues, including esophageal, melanoma, renal and lung cancers [8] made it of interest also as an antitumor target. However, CA this cytosolic isoform is crucial for the anti-glaucoma effects of the CA inhibitors (CAIs) of the sulfonamide type, which are clinically used agents for decades [1,3]. hCA I and hCA II inhibitors are utilized for the management of glaucoma, cerebral edema and altitude sickness among others [9–12]. CAIs are in clinical use for more than 70 years as antiglaucoma drugs and diuretics. Glaucoma is a multi-factorial ocular disorder characterized by optic nerve deterioration usually associated with high intraocular pressure (IOP) which may lead to blindness [13]. Sulfonamide CAIs such as brinzolamide (BRZ) are effective in reducing IOP after topical administration. Examples of other clinically employed CAIs are Acetazolamide AAZ, Ethoxzolamide EZA, Brinzolamide BRZ, and Methazolamide MZA, and some of the structurally related sulfonamides found in the literature are shown in Fig. 1. Due to larger number of hCA isoforms, there is a need to improve the selectivity profile and inhibition of such clinically used CAIs, in order to avoid the side effects [14–17].

Dysregulation of CAs expression has been linked to many diseases apart glaucoma, including cancer and epilepsy [10–11]. CA II is one of the most effective catalyst for $CO_2$ hydration [12].

The 4-(3,4-dichloro-phenylureido)thioureido-benzene sulfonamide A having thiourea moiety was shown to be an efficient *in vitro* inhibitor of *Plasmodium falciparum* CA (PfaCA) [18]. Various anti-plasmodial 7-chloro-4-aminoquinolyl-derived sulfonamides, as well as sulfonamides of type B (Fig. 1) have been prepared and tested against chloroquine-resistant and chloroquine susceptible *P. falciparum* [19]. Previously,

* Corresponding authors.
*E-mail addresses:* asaeed@qau.edu.pk (A. Saeed), claudiu.supuran@unifi.it (C.T. Supuran).

https://doi.org/10.1016/j.bioorg.2018.10.006
Received 23 August 2018; Received in revised form 26 September 2018; Accepted 4 October 2018
Available online 04 October 2018
0045-2068/ 

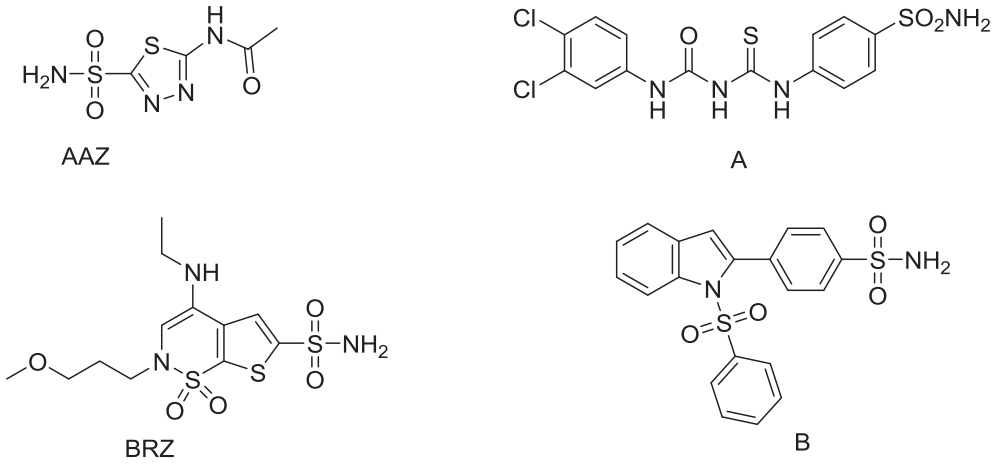



**Fig. 1.** Pharmacologically used CAIs, AAZ and BRZ. Compounds A and B were also reported to possess interesting pharmacological effects [8].

some of us reported sulfanilamide thiourea hybrids acting as antimicrobial agents and urease inhibitors but those compounds have not yet been assayed for their CA inhibitory properties against all physiologically relevant isoforms [20]. In fact, aminobenzenesulfonamide-thiourea conjugates were assayed only as bovine CA II inhibitors [21]. In this paper we report the synthesis of 3-aminobenzenesulfonamide thioureas with aliphatic acid chlorides and determined their inhibition activity against hCA I, II, IV and IX.

## 2. Experimental

### 2.1. Methods and materials

The chemicals, 3-aminobenzenesulfonamide, potassium thiocyanate, thionyl chloride, substituted acid chlorides, all synthetic starting materials, reagents and solvents were of analytical reagent grade or of the highest quality commercially available and were purchased from Sigma Aldrich Merck (Milan, Italy) and were used without further purification. Acetone was freshlydried over $KMnO_4$ and distilled. $R_f$-values were determined using pre-coated silica gel aluminum plates $60F_{254}$ from Merck (Germany). All Melting points were determined in open glass capillaries using Stuart™ SMP3 (UK) melting point apparatus and are uncorrected. Infrared spectra (IR) of the compounds were recorded on a Bio-Rad-Excalibur Series Model No. FTS 300 MX spectrophotometeras pure compounds. $^1$H and $^{13}$C NMR spectra were obtained on a Bruker 300 MHz and 75.5 MHz NMR spectrometer using teteramethylsilane (TMS) as internal reference standard. Elemental analyses were conducted using a LECO-183 CHNS analyser.

### 2.2. Synthesis of 1-aroyl/acyl-3-(3-aminosulfonylphenyl) thioureas (4a–k)

A solution of suitable carboxylic acids **1a–k** (1.0 mmol) and DMF (0.05 ml) in thionyl chloride (1.2 mmol) were refluxed for 3.5 h. After cooling at room temperature the mixture was concentrated to afford the acid chlorides **2a–k.** A solution of acid chlorides **2a–k** (1.0 mmol) in dry distilled acetone (20 ml) was added drop wise to a suspension of potassium thiocyanate (1.0 mmol) in dry acetone and refluxed for 1.5 hr at 50 °C to afford the corresponding isothiocyanates. After cooling on room temperature, a solution of 3-aminobenzenesulfonamide **3** (1.0 mmol) in dry acetone was added in it and the reaction mixture was refluxed for 9 h. After completion checked by TLC (*n*-Hexane: Ethyl acetate 1: 1) the reaction mixture was poured onto crushed ice and the resulting precipitates were collected via simple filtration, washed, dried and recrystallized from ethanol to obtain the 1-aroyl/acyl-3-(3-aminosulfonylphenyl) thioureas **4a–k** in excellent yields (Scheme 1).

#### 2.2.1. 1-Butanoyl-3-(3-aminosulfonylphenyl)thiourea (4a)

Off white solid; yield: 74%; m.p: 137 °C; $R_f$: 0.52 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, cm$^{-1}$): 3254(N—H), 1660 (C═O), 1595 (C═C), 1530 (thioamide I), 1331 (C—S), 1240 (thioamide II), 1150, 1092 (thioamide III), 742 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.98 (s, 1H, N—H), 11.90 (s, 1H, N—H), 7.42 ($SO_2NH_2$), 8.04 (1H, brs), 7.50 (1H, t, $J$ = 7.5 Hz), 7.60 (1H, d, $J$ = 7.4 Hz), 7.70 (1H, d, $J$ = 7.6 Hz), 2.17 (t, 2H, $CH_2$), 1.60 (m, 2H, $CH_2$), 0.91 (s, 3H, $CH_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 184.2 (C═S), 178.9 (C═O), 148.1, 140.4, 137.1, 132.2, 128.4, 125.4, 38.9, 20.1, 14.1; Anal. Calcd. for $C_{11}H_{15}N_3O_3S_2$: C, 43.84; H, 5.02; N, 13.94; S, 21.28 found: C, 43.81; H, 5.00; N, 13.91; S, 21.24.

#### 2.2.2. 1-Pentadecanoyl-3-(3-aminosulfonylphenyl)thiourea (4b)

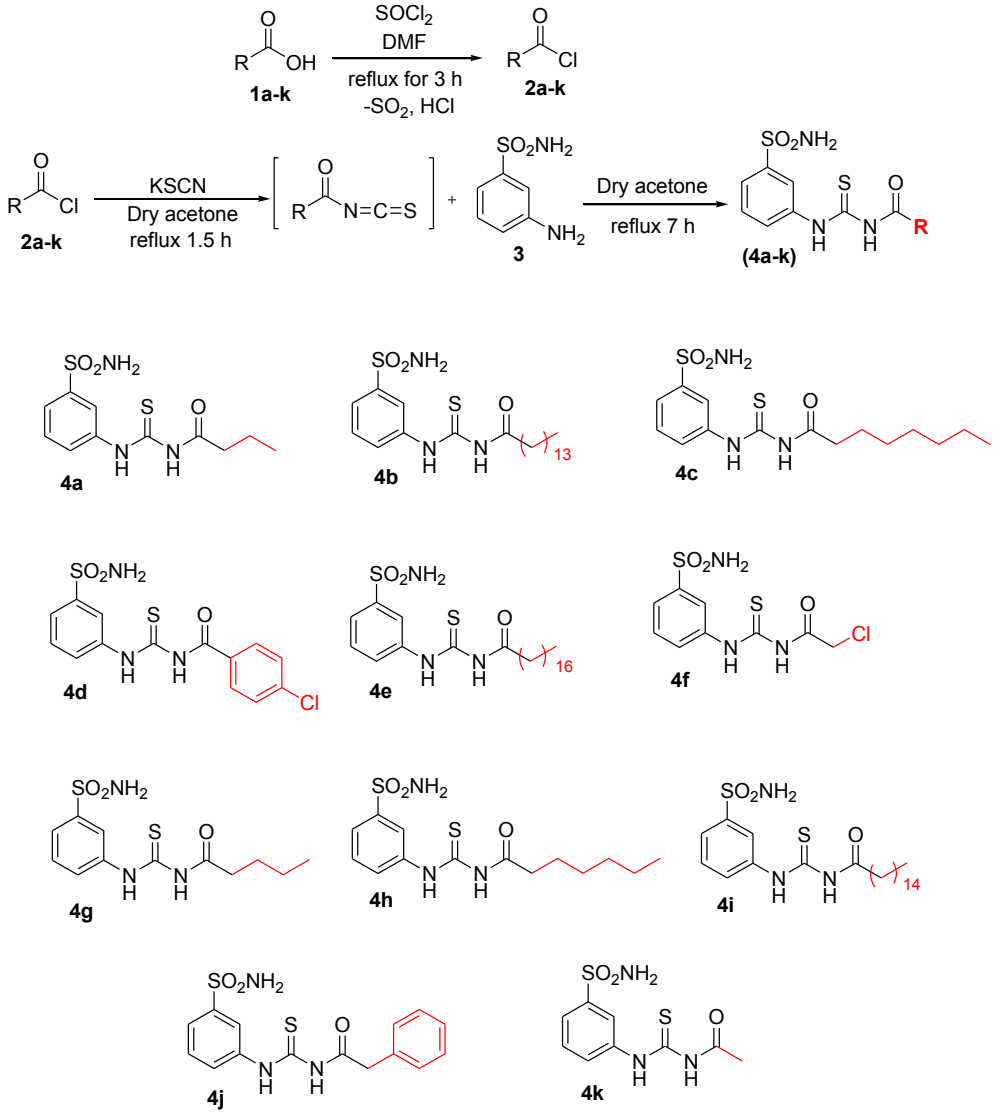
Black solid; yield: 79%; m.p: 129 °C; $R_f$: 0.58 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, cm$^{-1}$): 3250 (N—H), 1669 (C═O), 1589 (C═C), 1529 (thioamide I), 1338 (C—S), 1246 (thioamide II), 1153, 1099 (thioamide III), 743 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.90 (s, 1H, N—H), 11.99 (s, 1H, N—H), 7.44 ($SO_2NH_2$), 8.06 (1H, brs), 7.59 (1H, t, $J$ = 7.4 Hz), 7.62 (1H, d, $J$ = 7.5 Hz), 7.71 (1H, d, $J$ = 7.4 Hz), 2.19 (t, 2H, $J$ = 6.8 Hz), 1.59 (quin, 2H, $CH_2CH_2$), 1.28 (quin, 20H, $CH_2CH_2$), 1.34 (m, 2H, $CH_2CH_3$), 0.92 (t, 3H, $J$ = 7.1 Hz, $CH_3$);$^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 188.2 (C═S), 180.9 (C═O), 147.1, 141.4, 136.2, 132.9, 128.9, 126.4, 38.4, 26.5, 28.9, 29.3, 29.3, 29.1 (6C), 31.8, 22.7, 14.3; Anal. Calcd. for $C_{22}H_{37}N_3O_3S_2$: C, 57.99; H, 8.18; N, 9.22; S, 14.07found: C, 57.96; H, 8.15; N, 9.20; S, 14.05.

#### 2.2.3. 1-Octanoyl-3-(3-aminosulfonylphenyl)thiourea (4c)

Light brown solid; yield: 85%; m.p: 138 °C; $R_f$: 0.89 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, cm$^{-1}$): 3257 (N—H), 1672 (C═O), 1579 (C═C), 1535 (thioamide I), 1336 (C—S), 1252 (thioamide II), 1188, 1100 (thioamide III), 750 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.99 (s, 1H, N—H), 11.01 (s, 1H, N—H), 7.42 ($SO_2NH_2$), 8.06 (1H, brs), 7.62 (1H, t, $J$ = 7.3 Hz), 7.59 (1H, d, $J$ = 7.6 Hz), 7.68 (1H, d, $J$ = 7.3 Hz), 2.17 (t, 2H, $J$ = 6.9 Hz), 1.58 (quin, 2H, $CH_2CH_2$), 1.28 (quin, 6H, $CH_2CH_2$), 1.38 (m, 2H, $CH_2CH_3$), 0.99 (t, 3H, $J$ = 7.2 Hz, $CH_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 189.1 (C═S), 176.2 (C═O), 149.2, 144.2, 134.4, 133.4, 128.7, 126.6, 37.4, 26.1, 28.8 (2C), 31.9, 22.5, 14.3; Anal. Calcd. for $C_{15}H_{23}N_3O_3S_2$: C, 50.40; H, 6.48; N, 11.75; S, 17.94found: C, 50.37; H, 6.45; N, 11.72; S, 17.91.

#### 2.2.4. 1-(4-Chorobenzoyl)-3-(3-aminosulfonylphenyl)thiourea (4d)

Brown; yield: 78%; m.p: 219 °C; $R_f$: 0.65 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, cm$^{-1}$): 3278 (N—H), 1674 (C═O), 1593 (C═C), 1529 (thioamide I), 1316 (C—S), 1250 (thioamide II), 1150, 1001 (thioamide III), 749 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.51 (s, 1H, N—H), 11.73 (s, 1H, N—H), 7.80 (d, 2H, H-Ar), 7.47 ($SO_2NH_2$),




**Scheme 1.** Synthesis of 1-aroyl/acyl-3-(3-aminosulfonylphenyl) thioureas (**4a–k**).

7.30 (d, 2H, $J$ = 5.7 Hz, H−Ar), 6.39 (d, 1H, $J$ = 4.6 Hz, H-Ar), 7.16 (d, 1H, H-Ar); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 181.7 (C═S), 170.9 (C═O), 139.5 (C−S), 138.8 (C−Cl), 136.1 (C-Ar), 135.1 (C−CO), 129.7 (2C−Ar), 129.1 (C−Ar), 128.6 (2C−Ar), 128.1 (C−Ar), 124.7 (C−Ar), 123.9 (C−Ar); Anal. Calcd. for $C_{14}H_{12}ClN_3O_3S_2$: C, 45.46; H, 3.27; Cl, 9.59; N, 11.36; S, 17.34 found: C, 45.43; H, 3.25; Cl, 9.61; N, 11.30; S, 17.29.

*2.2.5. 1-Octadecanoyl-3-(3-aminosulfonylphenyl)thiourea (**4e**)*

Black solid; yield: 89%; m.p: 161 °C; $R_f$: 0.70 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, cm$^{-1}$): 3251 (N−H), 1688 (C═O), 1560 (C═C), 1555 (thioamide I), 1341 (C−S), 1247 (thioamide II), 1178, 1091 (thioamide III), 743 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 13.01 (s, 1H, N−H), 11.88 (s, 1H, N−H), 7.43 ($SO_2NH_2$), 8.10 (1H, brs), 7.71 (1H, t, $J$ = 7.4 Hz), 7.62 (1H, d, $J$ = 7.4), 7.69 (1H, d, $J$ = 7.4 Hz), 2.17 (t, 2H, $J$ = 6.9 Hz), 1.56 (quin, 2H, $CH_2CH_2$), 1.28 (quin, 26H, $CH_2CH_2$), 1.35 (m, 2H, $CH_2CH_3$), 0.91 (t, 3H, $J$ = 7.1 Hz, $CH_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 185.2 (C═S), 175.3 (C═O), 148.3, 141.2, 136.4, 132.4, 128.4, 126.3, 36.1, 26.2, 28.7, 29.1, 29.6 (9C), 29.3, 31.6, 22.1, 14.9; Anal. Calcd. for $C_{25}H_{43}N_3O_3S_2$: C, 60.32; H, 8.71; N, 8.44; S, 12.88found: C, 60.30; H, 8.69; N, 8.41; S, 12.65.

*2.2.6. 1-(2-chloroacetyl)-3-(3-aminosulfonylphenyl)thiourea (**4f**)*

Dark brown; yield: 67%; m.p: 137 °C; $R_f$: 0.62 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, cm$^{-1}$): 3256 (N−H), 1675 (C═O), 1534 (C═C), 1545 (thioamide I), 1333 (C−S), 1246 (thioamide II), 1159, 1076 (thioamide III), 742 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.81 (s, 1H, N−H), 11.88 (s, 1H, N−H), 7.42 ($SO_2NH_2$), 8.13 (1H, brs), 7.57 (1H, t, $J$ = 7.4 Hz), 7.62 (1H, d, $J$ = 7.3), 7.76 (1H, d, $J$ = 7.3 Hz),4.34 (s, 1H, $CH_2$-Cl); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 188.2 (C═S), 179.2 (C═O), 149.4, 143.1, 136.2, 131.2, 128.1, 126.3, 44.1 ($CH_2$-Cl); Anal. Calcd. for $C_9H_{10}ClN_3O_3S_2$: C, 35.12; H, 3.27; N, 13.65; S, 15.60 found: C, 35.10; H, 3.25; N, 13.61; S, 15.58.

*2.2.7. 1-Pentanoyl-3-(3-aminosulfonylphenyl)thiourea (**4g**)*

White solid; yield: 79%; m.p: 146 °C; $R_f$: 0.51 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, cm$^{-1}$): 3250 (N−H), 1678 (C═O), 1559 (C═C), 1560 (thioamide I), 1330 (C−S), 1242 (thioamide II), 1179, 1099 (thioamide III), 742 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.32 (s, 1H, N−H), 11.60 (s, 1H, N−H), 7.48 ($SO_2NH_2$), 8.00 (1H, brs), 7.76 (1H, t, $J$ = 7.3 Hz), 7.63 (1H, d, $J$ = 7.3 Hz), 7.70 (1H, d, $J$ = 7.3 Hz), 2.19 (t, 2H, $J$ = 6.8 Hz), 1.59 (quin, 2H, $CH_2$), 1.31 (m, 2H, $CH_2$), 0.97 (t, 3H, $J$ = 7.2 Hz, $CH_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 188.1 (C═S), 177.3 (C═O), 148.4, 142.1, 135.4, 132.9, 128.5, 126.6, 36.1, 28.9, 21.6, 13.4; Anal. Calcd. for $C_{12}H_{17}N_3O_3S_2$: C,

45.70; H, 5.43; N, 13.32; S, 15.22 found: C, 45.68; H, 4.47; N, 13.30; S, 15.20.

*2.2.8. 1-Heptanoyl-3-(3-aminosulfonylphenyl)thiourea (**4h**)*

Brown solid; yield: 81%; m.p: 141 °C; $R_f$: 0.60 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, $cm^{-1}$): 3250 (N—H), 1670 (C═O), 1569 (C═C), 1542 (thioamide I), 1339 (C—S), 1243 (thioamide II), 1178, 1107 (thioamide III), 748 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.43 (s, 1H, N—H), 11.54 (s, 1H, N—H), 7.42 ($SO_2NH_2$), 8.12 (1H, brs), 7.58 (1H, t, $J$ = 7.3 Hz), 7.62 (1H, d, $J$ = 7.6 Hz), 7.69 (1H, d, $J$ = 7.3 Hz), 2.18 (t, 2H, $J$ = 6.9 Hz), 1.59 (quin, 2H, $CH_2CH_2$), 1.27 (quin, 4H, $CH_2CH_2$), 1.37 (m, 2H, $CH_2CH_3$), 0.94 (t, 3H, $J$ = 7.3 Hz, $CH_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 181.1 (C═S), 174.2 (C═O), 149.3, 144.8, 134.3, 133.2, 128.7, 126.2, 36.1, 26.0, 28.3, 31.4, 22.9, 14.5; Anal. Calcd. for $C_{14}H_{21}N_3O_3S_2$: C, 48.96; H, 6.16; N, 12.23; S, 18.67 found: C, 48.91; H, 6.14; N, 12.20; S, 18.65.

*2.2.9. 1-Hexadecanoyl-3-(3-aminosulfonylphenyl)thiourea (**4i**)*

Blackish solid; yield: 88%; m.p: 143 °C; $R_f$: 0.62 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, $cm^{-1}$): 3245 (N—H), 1659 (C═O), 1594 (C═C), 1538 (thioamide I), 1345 (C—S), 1251 (thioamide II), 1165, 1123 (thioamide III), 744 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.78 (s, 1H, N—H), 11.65 (s, 1H, N—H), 7.47 ($SO_2NH_2$), 8.10 (1H, brs), 7.65 (1H, t, $J$ = 7.3 Hz), 7.77 (1H, d, $J$ = 7.4 Hz), 7.72 (1H, d, $J$ = 7.3 Hz), 2.17 (t, 2H, $J$ = 6.9 Hz), 1.58 (quin, 2H, $CH_2CH_2$), 1.29 (quin, 22H, $CH_2CH_2$), 1.31 (m, 2H, $CH_2CH_3$), 0.97 (t, 3H, $J$ = 7.2 Hz, $CH_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 183.2 (C═S), 179.9 (C═O), 147.4, 145.4, 139.2, 1324, 128.1, 126.1, 38.4, 26.1, 28.8, 29.0, 29.7 (7C), 29.5, 31.6, 22.7, 14.4; Anal. Calcd. for $C_{23}H_{39}N_3O_3S_2$: C, 58.81; H, 8.37; N, 8.95; S, 13.65found: C, 58.78; H, 8.35; N, 8.93; S, 13.63.

*2.2.10. 1-(2-phenylacetyl)-3-(3-aminosulfonylphenyl)thiourea (**4j**)*

White solid; yield: 76%; m.p: 154 °C; $R_f$: 0.76 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, $cm^{-1}$): 3267 (N—H), 1674 (C═O), 1539 (C═C), 1540 (thioamide I), 1338 (C—S), 1252 (thioamide II), 1176, 1096 (thioamide III), 744 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.76 (s, 1H, N—H), 11.54 (s, 1H, N—H), 7.43 ($SO_2NH_2$), 8.14 (1H, brs), 7.55 (1H, t, $J$ = 7.3 Hz), 7.63 (1H, d, $J$ = 7.2), 7.78 (1H, d, $J$ = 7.2 Hz), 3.49 (s, 2H, $CH_2$-Ph), 7.09 (d, 2H, Ar-H), 7.16 (dd, 2H, Ar-H), 7.07 (t, 1H, Ar-H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 187.2 (C═S), 177.2 (C═O), 149.8, 143.2, 136.5, 131.4, 128.7, 126.7, 138.5, 129.8 (2C—Ar), 129.2 (2C—Ar), 129.7 (1C—Ar), 40.9 ($CH_2$—Ph); Anal. Calcd. for $C_{15}H_{15}N_3O_3S_2$: C, 51.56; H, 4.33; N, 12.03; S, 18.35found: C, 51.54; H, 4.30; N, 12.00; S, 18.33.

*2.2.11. 1-acetyl-3-(3-aminosulfonylphenyl)thiourea (**4k**)*

Light brown; yield: 81%; m.p: 121 °C; $R_f$: 0.72 (*n*-Hexane: Ethyl acetate 1:1); IR (pure, $cm^{-1}$): 3250 (N—H), 1664 (C═O), 1591 (C═C), 1532 (thioamide I), 1329 (C—S), 1241 (thioamide II), 1152, 1082 (thioamide III), 748 (thioamide IV); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm); 12.91 (s, 1H, N—H), 11.92 (s, 1H, N—H), 7.44 ($SO_2NH_2$), 8.12 (1H, brs), 7.54 (1H, t, $J$ = 7.6), 7.64 (1H, d, $J$ = 7.3), 7.79 (1H, d, $J$ = 7.4), 2.05 (s, 3H, $CH_3$); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$); 185.2 (C═S), 181.9 (C═O),149.1, 143.4, 135.1, 133.2, 127.4, 126.4, 23.2; Anal. Calcd. for $C_9H_{11}N_3O_3S_2$: C, 39.55; H, 4.06; N, 15.37; S, 23.46found: C, 39.51; H, 4.04; N, 15.30; S, 23.43.

### 2.3. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed $CO_2$ hydration activity [22]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (pH 7.5) as buffer, and 20 mM $Na_2SO_4$ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed $CO_2$ hydration reaction for a period of 10–100 s. The $CO_2$ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The un catalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were pre-incubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier [23] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier [23,24].

## 3. Results and discussion

### 3.1. Chemistry

A variety of novel 1-aroyl/acyl-3-(3-aminosulfonylphenyl) thioureas **(4a–k)** were synthesized in situ by reaction of corresponding acid chlorides with an equimolar quantity of potassium thiocyanate in dry acetone to afford the isothiocyanates. Treatment of isothiocyanates with 3-aminobenzenesulfonamide in dry acetone in 1:1 M ratio afforded the 1-aroyl/acyl-3-(3-aminosulfonylphenyl) thioureas **(4a–k)** in good yields as shown in Scheme 1.

FT-IR spectrum showed peak at around 3250 $cm^{-1}$ due to N—H stretching vibrations and a strong peak at 1664 $cm^{-1}$ corresponding to C═O (amide). The aromatic stretching frequency bands appear at 1591 $cm^{-1}$. In $^1$H NMR, the characteristic singlets for $N_1$ and $N_3$ protons in the thiourea were found in a comparatively wide range of 11.11–12.99 ppm respectively. The rest of aromatic proton appears in their respective aromatic region. The $-SO_2NH_2$ protons appear at around 7.44 ppm. $^{13}$C NMR showed C═S and C═O signal at 185.2 and 181.9 ppm, while the rest of the aromatic and aliphatic carbons appear at their respective regions in the spectrum.

### 3.2. Carbonic anhydrase inhibitory activity

The inhibitory potential of the synthesized compounds (**4a–k**) on human hCA isoforms (hCAI, hCA II, hCA IV and hCA IX) was evaluated using a stopped-flow $CO_2$ hydrase assay. CA isoforms, the ubiquitous cytosolic hCA I and hCA II, the membrane-anchored hCA IV, and the tumor-associated trans-membrane hCA IX were selected for the biological testing. Results are summarized in Table 1, the standard inhibitor acetazolamide (AAZ) containing sulfonamide moiety was used as a reference compound. All the compounds were able to inhibit the four hCA isoforms. The following structure–activity relationship (SAR) can be drawn from the data of Table 1.

(i) Most thiourea derivatives (**4a–k**) investigated here showed moderate inhibitory properties against the cytosolic isoform hCA I. Compound **4i** exhibited the lowest inhibition of this isoform, with a $K_I$ value of 826.3 nM. It is interesting to note that the slight shortening of the aliphatic chain, as in compound **4b** ($K_I$ = 435.1 nM) respect to **4i,** causes a considerable increase of the inhibitory activity against hCA I.

The introduction of 6 and 7 carbons aliphatic chains determines a further improvement of the inhibitory activity, as shown by **4h** and **4c**, with $K_I$s 486.4 and 438.4 nM, respectively. Also, the introduction of a phenyl group has a positive effect on the inhibitory activity, as in the case of the compounds **4d** and **4j** ($K_I$s = 316.6 and 394.2 nM, respectively), which only differ for a chlorine atom in the aromatic group.

Finally, compound **4f** showed the best activity against hCA I ($K_I$ = 81.6 nM) and is three times more potent than the reference drug

**Table 1**
Inhibition data of human CA isoforms hCA I, II, IV and IX with compounds **4a–k** reported here and the standard sulfonamide inhibitor acetazolamide (AAZ) by a stopped flow $CO_2$ hydrase assay.

| Compd | $K_I$ (nM)[a] | | | | Selectivity ratio |
|---|---|---|---|---|---|
| | hCA I | hCA II | hCA IV | hCAIX[b] | hCA II/IX |
| **4a** | 293.1 | 140.1 | 6343.7 | 44.0 | 3.18 |
| **4b** | 435.1 | 968.1 | 8594.8 | 238.1 | 4.06 |
| **4c** | 438.4 | 123.0 | 7315.0 | 46.2 | 2.66 |
| **4d** | 316.6 | 283.2 | 5087.5 | 83.9 | 3.37 |
| **4e** | 582.5 | 919.6 | 8934.5 | 274.2 | 3.35 |
| **4f** | 81.6 | 50.9 | 7406.2 | 23.3 | 2.18 |
| **4g** | 334.7 | 163.1 | 7019.0 | 30.9 | 5.27 |
| **4h** | 486.4 | 127.5 | 7981.4 | 21.5 | 5.93 |
| **4i** | 826.3 | 986.2 | 8030.7 | 26.4 | 37.3 |
| **4j** | 394.2 | 263.3 | 6986.5 | 36.3 | 7.25 |
| **4k** | 712.5 | 148.1 | 5650.0 | 40.2 | 3.68 |
| **AAZ** | 250 | 12 | 74 | 25 | 0.48 |

[a] Mean values from three different assays. Errors were within ± 5–10% of the reported values (data not shown).
[b] Catalytic domain.

AAZ $K_I$ 250 nM, and this result is presumably associated to the presence of halogens, the absence of a long aliphatic chain and likelythe absence of bulky groups.

(ii) The physiologically dominant hCA II was moderately inhibited by most thiourea derivatives reported with $K_I$s values spanning between 123.0 and 986.2 nM. The general tendencies described above are also applicable for this isoform, in fact compound **4f** stood out again as the best hCA II inhibitor with $K_I$ value of 50.9 nM. The presence of aliphatic chains of 13, 14 and 16 carbon atoms determined a reduction of the inhibitory activity of compounds **4b**, **4i** and **4e** ($K_I$s = 968.1, 986.2, 919.6, respectively). Unlike the trend showed against hCA IX discussed below, the compounds **4d** and **4j** showed very similar $K_I$s (283.2 and 263.3 nM, respectively), confirming that the addition of a halogen atom on the phenyl ring has not substantial impact on the inhibitory activity against hCA II. Small variations in the length of the aliphatic chain in this particular type of scaffold correspond to minimal variations of the inhibitory activity as in the cases of the compounds **4a** and **4k** ($K_I$s = 140.1 and 148.1 nM, respectively) and of compounds **4c** and **4h** ($K_I$s = 123.0 and 127.5 nM, respectively). These evidences are nevertheless important to confirm that the compounds with a medium-length aliphatic chain are the best inhibitors against hCA II.

(iii) All derivatives herein studied show low inhibitory activity against the membrane-bound isoform hCA IV (with $K_I$s in the range between 5087.5 and 8934.5 nM) and exhibit rather flat SAR.

(iv) The tumor associated hCA IX was the most inhibited isoform among those considered in this study, with $K_I$s in the range between 21.5 and 274.2 nM. Compounds bearing a medium-length alkyl chain showed the best $K_I$s as in the case of compounds **4a**, **4g**, **4h**, and **4k** ($K_I$s = 44.0, 30.9, 21.5 and 40.2 nM, respectively). Conversely, **4b** and **4e** that possess long alkyl chains, displayed a medium nanomolar ($K_I$s = 238.1 and 274.2 nM, respectively) inhibitory action against hCA IX. It is important to emphasize that compound **4j,** holding an un-substituted phenyl, is an effective inhibitor, with a $K_I$ value of 36.3, whereas the presence of chlorine atom on the phenyl ring determines a considerable reduction of inhibitory potency of compound **4d** with a $K_I$ value of 83.9 nM.

(v) We can conclude that all derivatives bearing medium-length aliphatic chain tails have reported the best CA inhibitory activities particularly against the tumor associated hCA IX. In fact, as seen from the selectivity ratios (Table 1), some of the derivatives possess good such indices for the inhibition of hCA IX over hCA II, in the range of 3.35–37.3. The most hCA IX – selective CAIs designed here were the sulfonamides incorporating the phenylacetylthioureido (**4j**) and pentadecanoylthioureido (**4i**) moieties, that shown selectivity ratios of 7.25–37.3. Following these evidences, compounds **4j** and **4i** have been selected as a guide for the synthesis of new derivatives.

## 4. Conclusions

We have reported the synthesis of a novel series of 1-aroyl/acyl-3-(3-aminosulfonylphenyl) thioureas **(4a–k)** in good yields. The new compounds were evaluated for their human carbonic anhydrase inhibition potential. Several compounds displayed interesting activity, with $K_I$s in the range of 21.5–44.0 nM and selectivity ratios over the major cytosolic isoform hCA II in the range of 3.35–37.3. The sulfonamides incorporating the phenylacetylthioureido and pentadecanoylthioureido moieties were the most hCA IX-selective inhibitors detected in this work, making them of interest for further investigations.

## Acknowledgments

We are thankful to Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan for providing encouraging environment and facilities for research work.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.006.

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