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RESEARCH ARTICLE

# Synthesis, antioxidant and carbonic anhydrase I and II inhibitory activities of novel sulphonamide-substituted coumarylthiazole derivatives

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**Abstract**

New secondary benzenesulphonamide-substituted coumarylthiazole derivatives were synthesized and their inhibitory effects on purified carbonic anhydrase I and II were evaluated using $CO_2$ as a substrate. The result showed that all the synthesized compounds exhibited inhibitory activity on both hCA I and hCA II with *N*-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)naphthalene-2-sulphonamide (**5f**, $IC_{50}$ value of 5.63 and 8.48 μM, against hCA I and hCA II, respectively) as the strongest inhibitor revealed from this study. Structure–activity relationship revealed that the inhibitory activity of the synthesized compounds is related to the type of the halogen and bulky substituent on the phenyl ring. In addition, the cupric reducing antioxidant capacities (CUPRAC) and ABTS cation radical scavenging abilities of the synthesized compounds were assayed. 4-methoxy-*N*-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5e**) exhibited the strongest ABTS and CUPRAC activity with $IC_{50}$ value of 48.83 μM and $A_{0.50}$ value of 23.29 μM, respectively.

**Keywords**

ABTS, antioxidant, carbonic anhydrase, coumarin, CUPRAC, sulphonamide

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## Introduction

The carbonic anhydrases (CAs; EC 4.2.1.1) are a superfamily of metalloenzymes, which catalyse the interconversion between $CO_2$ and $HCO_3^-$ by using a metal hydroxide nucleophilic mechanism[1–4]. These metalloenzymes are known in nature as five different, genetically distinct families, the α-, β-, γ-, δ- and ζ-CAs[5–7]. Additionally, a new genetic family of CAs, which was called the η-CA class, was discovered by Supuran's group in the last year[8]. The α-, β-, δ-CAs use Zn(II) ions at the active site, the γ-CAs are Fe(II) enzymes [but they are active also with bound Zn(II) or Co(II) ions], while the ζ-class uses Cd(II) or Zn(II) to perform the physiologic reaction catalysis[5–7]. Sixteen different α-CA isoforms were isolated 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[9,10]. The isozyme CA I is found in many tissues, and a presented study from Gao et al.[11] demonstrated that this enzyme is involved in retinal and cerebral edema, and its inhibition may be a valuable tool for fighting these conditions. The CA II is involved in several diseases, such as glaucoma, edema, epilepsy, and probably altitude sickness[12]. Although there are many studies on this enzyme, the CA enzyme family continues to capture the attention of drug discovery scientists and clinicians as the knowledge regarding the therapeutic implications associated with this enzyme class continues to grow[4,13,14].

The CA inhibitor (CAI) targeting enzymes from mammals, which belong to the α-CA has been classified as (i) metal ion binders [inorganic anions; sulphonamides and their isosteres (such as the sulfamates, sulfamides, *N*-hydroxy-sulphonamides)]; (ii) compounds which anchor to the zinc-coordinated water molecule/hydroxide ion (phenols, polyamines, sulfocoumarins, etc.); (iii) compounds occluding the entrance of the active site (coumarins and their isosteres); and (iv) compounds which bind in an unknown manner (secondary/tertiary sulphonamides, imatinib, nilotinib, etc.)[15–22].

Sulphonamides are the best known CAIs and are used for the treatment of glaucoma in medicinal chemistry. Members of this class include aromatic, heterocyclic or aliphatic primary sulphonamides, but most drugs belong to the heterocyclic class[23,24]. The coumarin is a common moiety found in many biologically active natural and therapeutic products and thus represents a very important pharmacophore[25–27]. Besides the numerous activities of coumarin compounds, they were recently shown to constitute a novel class of inhibitors of the metalloenzyme CA[28–30]. In addition, Supuran et al.[31] reported that the hydrolysis of the sulfocoumarin to the vinyl sulfonic acid is mediated by the zinc hydroxide nucleophile from the CA active site, as for the coumarins which are transformed to 2-hydroxycinnamic acids. Interestingly, this sulfonic acid moiety is not coordinated to the

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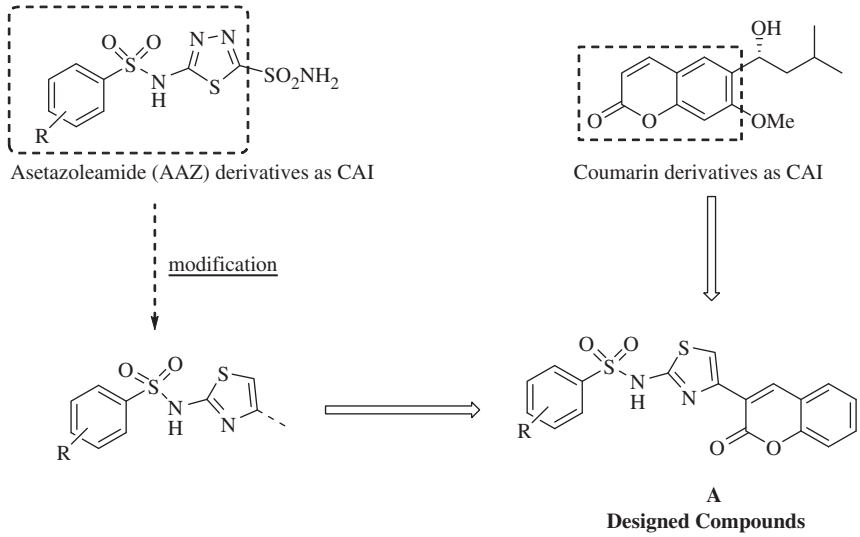



Figure 1. Design strategy of the reported compounds.

Zn(II) ion but it is anchored to the zinc-bound water molecule/hydroxide ion[31].

In this work, novel sulphonamides substituted coumarylthiazole were synthesized and their antioxidant activities and inhibitory effects on the activity of purified human carbonic anhydrase (hCA) I and II were evaluated. In the present study, our main aim is to check whether the new secondary sulphonamide derivatives substituted coumarin modified by the addition of thiazole ring (**A**) (Figure 1) may show higher inhibitory effect on CA enzymes with respect to the compounds previously investigated.

## Methods

### Chemistry

Melting points were taken on a Barnstead Electrothermal 9200 (Staffordshire, UK). IR spectra were registered on a Shimadzu Prestige-21 (200 VCE) spectrometer (Columbia, MD). $^{1}$H and $^{13}$C-NMR spectra were registered on a Varian Infinity Plus spectrometer at 300 and at 75 Hz, respectively. $^{1}$H and $^{13}$C chemical shifts are referenced to the internal deuterated solvent. The elemental analyses were carried out with a Leco CHNS-932 (St. Joseph, MI) instrument. Spectrophotometric analyses were performed by a BioTek Power Wave XS (BioTek, Winooski, VT). Sepharose 4B, L-tyrosine, sulphonamide, synthetic starting material, reagents and solvents were purchased from Merck, Alfa Easer, Sigma-Aldrich and Fluka.

### General procedure for the synthesis of 3-acetylcoumarin (2)

A mixture of benzaldehyde (3 mmol), reactive methylene compound (3 mmol) and L-proline (10 mol%) was heated under neat conditions for 0.5 h. The reaction was monitored by thin-layer chromatography (TLC). After completion of reaction, the reaction mixture was cooled and recrystallized from ethanol to get pure crystalline 3-acetylcoumarin **(2)** in 92% yield (0.52 g). Spectral data of this compound matched with the literature[32].

### General procedure for the synthesis of 3-(bromoacetyl)-coumarin (3)

To a solution of **2** (0.01 mol) in 20 mL chloroform was added 0.01 mol of bromine in 5 mL chloroform, with intermittent shaking and warming to decompose an addition product. The mixture was heated for fifteen minutes on a water-bath to expel most of the hydrogen bromide, then cooled and filtered. The solid was washed with ether and recrystallizated with acetic acid. About 2.60 g product was obtained in 98% yield. Spectral data of this compound matched with the literature[33].

### General procedure for the synthesis of 3-(2-amino-1,3-thiazol-4-yl)coumarin (4)

Thiourea (5 mmol) was added to the solution of **3** (5 mmol) in boiling ethanol (20 mL). The mixture was refluxed for 1 h, then cooled and neutralized with aqueous ammonia. The precipitate was filtered off, washed with ethanol and used directly without crystallization or other purification. About 1.098 g product was obtained in 90% yield. Spectral data of this compound matched with the literature[34].

### General procedure for the synthesis of sulphonamides substituted coumarylthiazole (5a–j)

To a solution of **4** (1 mmol) in dry pyridine was added sulfonylchloride derivatives (1 mmol). The mixture was refluxed for 12 h with stirring, than cooled and added to ice water and 10% HCl. The product was filtered off, washed with water and dried under vacuum. The products were recrystallizated from ethanol over 98% purity. Compounds **5a–j** were obtained with 68–82% yields.

*4-Methyl-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5a**)*

Yellow powder, 78% yield (310 mg), m.p.: 235–237 °C; IR: 3366 3287, 3042, 1712, 1634, 1599, 1522, 1365, 1271, 1134, 1081, 765 cm$^{-1}$; $^{1}$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$/ppm: 2.51 (3H, s), 7.37–7.48 (4H, m), 7.57 (1H, s), 7.67 (2H, t, $J = 8.8$ Hz), 7.77 (2H, t, $J = 7.9$ Hz), 8.63 (1H, s); $^{13}$C NMR (DMSO-d$_6$, 75 MHz) $\delta$/ppm: 21.6, 109.5, 116.7, 119.1, 119.4, 125.7, 126.7, 129.5, 130.2, 133.0, 133.3, 139.7, 139.8, 143.3, 153.0, 158.9, 169.5. Anal. Calcd. for $C_{19}H_{14}N_2O_4S_2$: C, 57.27; H, 3.54; N, 7.03; found: C, 57.24; H, 3.50; N, 7.10.

*2,5-Dimethyl-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5b**)*

Orange powder, 82% yield (338 mg), m.p.: 160–162 °C; IR: 3328, 3124, 2965, 1710, 1607, 1528, 1361, 1292, 1131, 1060, 794, 593 cm$^{-1}$; $^{1}$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$/ppm: 2.35 (3H, s),

2.58 (3H, s), 7.24–7.38 (2H, m), 7.43 (2H, t, $J = 7.9$ Hz), 7.64 (1H, s), 7.69 (2H, t, $J = 7.9$ Hz), 7.77 (1H, s), 8.48 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 20.2, 21.1, 111.0, 116.7, 119.0, 125.7, 126.2, 128.8, 129.5, 132.9, 133.3, 133.4, 134.0, 135.8, 139.7, 140.1, 142.9, 153.0, 158.3, 168.1. Anal. Calcd. for $C_{20}H_{16}N_2O_4S_2$: C, 58.24; H, 3.91; N, 6.79; found: C, 58.28; H, 3.95; N, 6.75.

*4-Isopropyl-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5c**)*

Orange powder, 80% yield (340 mg), m.p.: 182–184 °C; IR: 3276, 3142, 2960, 1712, 1606, 1524, 1454, 1360, 1306, 1148, 1090, 938, 755, 569 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 1.21 (6H, d, $J = 5.8$ Hz), 2.93–2.99 (1H, m), 7.37–7.51 (4H, m), 7.63 (1H, s), 7.69 (2H, d, $J = 7.6$ Hz), 7.79 (2H, d, $J = 8.5$ Hz), 8.46 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 24.1, 34.0, 111.2, 116.7, 119.9, 125.7, 126.8, 127.6, 129.5, 132.1, 133.3, 136.8, 139.6, 140.0, 143.8, 153.0, 158.4, 168.1. Anal. Calcd. for $C_{21}H_{18}N_2O_4S_2$: C, 59.14; H, 4.25; N, 6.57; found: C, 59.10; H, 4.28; N, 6.55.

*4-(Tert-butyl)-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5d**)*

Orange powder, 72% yield (316 mg), m.p.: 250–252 °C; IR: 3368, 2961, 1716, 1518, 1442, 1361, 1287, 1145, 1106, 1084, 755, 578 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 1.28 (9H, s), 7.34–7.51 (3H, m), 7.58–7.69 (4H, m), 7.79 (2H, d, $J = 8.7$ Hz ), 8.46 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 31.4, 35.4, 109.4, 111.2, 116.4, 116.7, 119.0, 125.3, 125.7, 126.5, 126.8, 127.7, 129.5, 133.3, 139.6, 153.0, 158.4, 168.1. Anal. Calcd. for $C_{22}H_{20}N_2O_4S_2$: C, 59.98; H, 4.58; N, 6.36; found: C, 59.95; H, 4.56; N, 6.38.

*4-Methoxy-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5e**)*

Yellow powder, 75% yield (310 mg), m.p.: 259–261 °C; IR: 3333, 3117, 2943, 1712, 1521, 1438, 1365, 1304, 1261, 1140, 1088, 927, 749, 556 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 3.82 (3H, s), 7.10 (2H, d, $J = 7.9$ Hz), 7.37–7.46 (3H, m), 7.64–7.69 (2H, m), 7.81 (2H, d, $J = 8.2$ Hz), 8.46 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 56.3, 109.4, 111.3, 114.9, 116.7, 119.1, 125.4, 125.7, 128.8, 129.5, 133.2, 134.1, 139.6, 153.0, 158.4, 162.8, 168.1. Anal. Calcd. for $C_{19}H_{14}N_2O_5S_2$: C, 55.06; H, 3.40; N, 6.76; found: C, 55.09; H, 3.45; N, 6.75.

*N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)naphthalene-2-sulphonamide (**5f**)*

Cream powder, 70% yield (304 mg), m.p.: 251–253 °C; IR: 3292, 3122, 3049, 1709, 1599, 1441, 1367, 1301, 1115, 1078, 922, 752, 669 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 7.39 (2H, t, $J = 8.4$ Hz), 7.62–7.64 (5H, m), 7.84 (1H, d, $J = 8.7$ Hz), 8.00 (1H, d, $J = 7.9$ Hz), 8.09 (1H, d, $J = 8.7$ Hz), 8.17 (1H, d, $J = 6.7$ Hz), 8.42 (1H, s), 8.52 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 111.3, 116.7, 119.0, 122.8, 125.7, 127.1, 128.2, 128.4, 129.3, 129.5, 129.9, 130.0, 132.3, 133.3, 134.8, 139.3, 139.7, 153.0, 158.4, 164.8. Anal. Calcd. for $C_{22}H_{14}N_2O_4S_2$: C, 60.82; H, 3.25; N, 6.45; found: C, 60.84; H, 3.28; N, 6.40.

*4-fluoro-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5g**)*

Reddish powder, 74% yield (297 mg), m.p.: 248–250 °C; IR: 3299, 3123, 3073, 1702, 1608, 1524, 1432, 1366, 1292, 1069, 921, 753, 582 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 7.38–7.55 (3H, m), 7.60–7.74 (6H, m), 8.49 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 111.3, 113.4, 113.7, 116.2, 116.7, 119.0, 120.0, 120.3, 122.8, 125.7, 129.5, 132.2, 132.3, 133.3, 139.7, 144.7, 144.8, 153.0, 158.3, 160.7, 163.9, 168.9. Anal. Calcd. for $C_{18}H_{11}FN_2O_4S_2$: C, 53.72; H, 2.76; N, 6.96; found: C, 53.74; H, 2.75; N, 6.94.

*4-Chloro-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5h**)*

Yellow powder, 82% yield (342 mg), m.p.: 264–266 °C; IR: 3145, 3043, 1720, 1567, 1526, 1440, 1362, 1306, 1267, 1139, 1086, 928, 752 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 7.39–7.51 (3H, m), 7.64–7.70 (4H, m), 7.85 (2H, d, $J = 8.2$ Hz), 8.48 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 111.3, 116.8, 119.0, 125.8, 128.5, 129.9, 133.4, 137.8, 139.8, 140.2, 141.7, 144.7, 153.1, 158.4, 162.7, 168.4. Anal. Calcd. for $C_{18}H_{11}ClN_2O_4S_2$: C, 51.61; H, 2.65; N, 6.69; found: C, 51.63; H, 2.62; N, 6.67.

*4-Bromo-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5i**)*

Reddish powder, 76% yield (351 mg), m.p.: 266–268 °C; IR: 3275, 3043, 1726, 1605, 1529, 1441, 1366, 1307, 1269, 1139, 1083, 936, 759, 553 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 7.35–7.45 (4H, m), 7.50–7.76 (5H, m), 8.45 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 111.2, 116.2, 116.8, 119.0, 122.7, 125.7, 128.6, 129.6, 132.8, 133.4, 139.8, 141.0, 153.1, 158.3, 162.4, 168.6. Anal. Calcd. for $C_{18}H_{11}BrN_2O_4S_2$: C, 46.66; H, 2.39; N, 6.05; found: C, 46.64; H, 2.35; N, 6.08.

*4-Iodo-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)benzenesulphonamide (**5j**)*

Dark yellow powder, 68% yield (347 mg), m.p.: 244–246 °C; IR: 3382, 3154, 3042, 1701, 1605, 1532, 1438, 1361, 1265, 1140, 1086, 941, 759, 554 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 300 MHz) $\delta$/ppm: 7.36–7.45 (2H, m), 7.58–7.67 (5H, m), 7.94 (2H, d, $J = 7.6$ Hz), 8.46 (1H, s); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) $\delta$/ppm: 111.2, 116.8, 119.0, 125.7, 128.3, 129.6, 133.4, 137.5, 138.6, 139.8, 142.2, 147.4, 153.1, 158.4, 161.1, 168.6. Anal. Calcd. for $C_{18}H_{11}IN_2O_4S_2$: C, 42.36; H, 2.17; N, 5.49; found: C, 42.39; H, 2.15; N, 5.47.

## Preparation and purification of haemolysate from blood red cells

Preparation and purification of haemolysate from blood red cells made by the literature[35]. Blood samples (25 mL) were taken from healthy human volunteers. They were anticoagulated with acid–citrate–dextrose, centrifuged at 2000 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 haemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 2000 g for 25 min at 4 °C, and the pH of the haemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 mL haemolysate was applied to an affinity column containing L-tyrosine-sulphonamide-Sepharose-4B equilibrated with 25 mM Tris–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 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 hCA-II, respectively. Fractions of 3 mL were collected and their absorbance measured at 280 nm.

## CA enzyme assay

CA activity was measured by the Maren 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[36]. 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 by using the equation $t_0-t_c/t_c$ where $t_0$ and $t_c$ are the times for pH change of the non-enzymatic and the enzymatic reactions, respectively.

### *In vitro* inhibition studies

For the inhibition studies of sulphonamides, five different concentrations of these compounds were added to the medium in 4.2 mL of total reaction volume including the enzyme solution. Duration (in seconds) of the colour change from red to yellow in solution was measured in a 10-ml glass tube with 1 cm diameter. CA enzyme activity without a compound solution was accepted as 100% activity. All compounds were tested in triplicate at each concentration used. Activity % values of CA for different concentrations of each compound were determined by regression analysis using Microsoft Office 2000 Excel. For the compounds having an inhibition affect, the inhibitor concentration causing up to 50% inhibition ($IC_{50}$ values) was determined from the graphs.

### Antioxidant activity assays

In CUPRAC assay, the absorbance values were used to calculate for $A_{0.50}$, but in ABTS assay, inhibition (%) values were used to calculate for $IC_{50}$.

#### *ABTS cation radical decolourization assay*

$ABTS^{\bullet+}$ scavenging activities of the synthesized compounds were determined as previously reported[37]. The solution of $ABTS^{\bullet+}$ radical was generated by dissolving 19.2 mg of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (7 mM ABTS) and 3.3 mg $K_2S_2O_3$ in distilled water (5 mL). This solution was kept in dark for 24 h at room temperature, and the absorbance of the solution was fixed to ~0.70 at 734 nm by dilution. The solutions of the samples were prepared in *n*-propanol at a concentration of 1000 µg/mL. The absorbance was measured at room temperature at 734 nm, after 6 min from $ABTS^{\bullet+}$ addition. The decrease in the absorption was used to calculate the activities. The results were expressed as $IC_{50}$. Propyl alcohol was used as a control solvent.

#### *Cupric reducing antioxidant capacity assay (CUPRAC)*

Cupric reducing antioxidant capacities of the synthesized compounds were determined in accordance with the literature method[38]. The solutions of compounds and standards were prepared in *n*-propanol at a concentration of 1000 µg/mL. Different volumes (1000 mg/L and 54.5 mL) of the sample were added to a solution prepared by adding 61.0 µL of 10 mM $CuCl_2$, 61.0 µL 7.5 mM neocuproine and 61.0 µL of 1.0 mM $NH_4CH_3COO$ buffer (pH 7), respectively. The absorbance was measured at room temperature at 450 nm, after an hour. The results were calculated as $A_{0.50}$. Propyl alcohol was used as a solvent to controls.

## Results and discussion

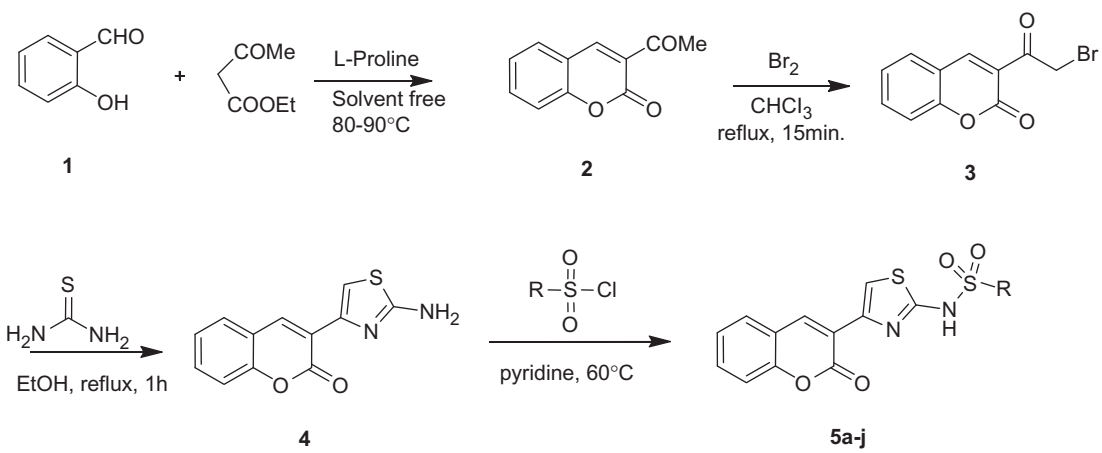
Sulphonamide derivatives are known as the strongest CAIs[23]. The asetazolamide (AAZ) derivatives have also been reported as potent CAI[4]. Our design strategy is that the 1,3,4-thidiazole ring of AAZ was modified to thiazole ring and was added to sulphonamides in order to increase inhibitory activity by forming hydrogen bond. On the other hand, we think that the presence of coumarin moiety contributes to inhibitor activity by hydrolysing to 2-hydroxycinnamic acids or interacting with the enzyme active sites (Figure 1).

The synthetic procedures employed to obtain the target compounds **5a–j** are depicted in Scheme 1. Compound **4** was synthesized from salicylaldehydes in three steps according to the literature[39], and then it was reacted with various benzenesulfonyl choloride derivatives in pyridine to get product sulphonamides substituted coumarylthiazole (**5a–j**).

All the new compounds were characterized by $^1$H-NMR, $^{13}$C-NMR, IR and elemental analysis. In the infrared spectra of the synthesized compounds, it was possible to observe the absorptions ~3300 $cm^{-1}$ relating to NH stretch of sulphonamide groups, ~1520 $cm^{-1}$ relating to C=N stretch for thiazole, absorptions in ~1710 $cm^{-1}$ from coumarin carbonyl moiety stretch and absorptions ~1360 $cm^{-1}$ relating to $SO_2$ antisym stretch in sulphonamides. From the $^1$H-NMR spectra, the resonance due to the hydrogen attached to the sulphonamide nitrogen was not detected. The signals for aromatic hydrogens were observed between 7.10 and 8.17 ppm, the signal of proton at thiazole ring was detected at ~8.48 ppm. From the $^{13}$C-NMR spectra, the signals can be seen ~158 and 168 ppm relating to coumarin carbonyl and thiazole ring, respectively.

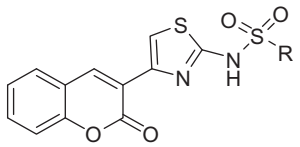
For evaluation, the physiologically relevant human CA isozyme hCA I and II inhibitory activity, all the synthesized compounds were subjected to CA inhibition assay with $CO_2$ as a substrate.

The result showed that all synthesized compounds (**5a–j**) inhibited the hCA I and II enzyme activity. The $IC_{50}$ values for hCA I and II inhibitions are summarized in Table 1. The $IC_{50}$ values were between 5.63 and 22.63 µM for hCA I and between 8.48 and 23.87 µM for hCA II inhibitory activity. Among

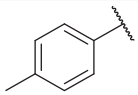
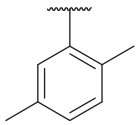
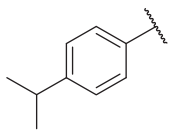
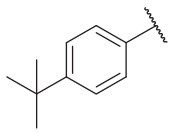
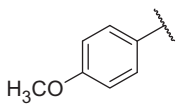
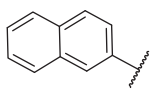
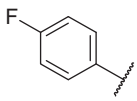
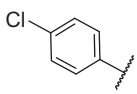
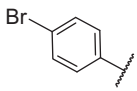
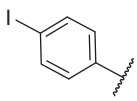



Scheme 1. Synthesis of new sulphonamides substituted coumarylthiazole (**5a–j**) derivatives.

Table 1. *In vitro* inhibition $IC_{50}$ and $A_{0.50}$ values (μM) of sulphonamides substituted coumarylthiazole (**5a–j**) for hCA I and hCA II and antioxidant activities.



5a-j

| Compound | R | hCA I $IC_{50}$ (μM) | hCA II $IC_{50}$ (μM) | $ABTS^{\bullet+}$ $IC_{50}$ (μM)* | CUPRAC $A_{0.50}$ (μM)† |
|---|---|---|---|---|---|
| **5a** |  | 22.63 | 23.87 | 70.95 ± 2.51 | 61.36 ± 0.31 |
| **5b** |  | 8.64 | 11.68 | 54.77 ± 2.87 | 40.26 ± 0.40 |
| **5c** |  | 6.46 | 11.12 | 116.62 ± 1.56 | 43.70 ± 0.32 |
| **5d** |  | 6.21 | 10.91 | 104.77 ± 2.08 | 49.71 ± 0.30 |
| **5e** |  | 10.90 | 18.00 | 48.83 ± 1.38 | 23.29 ± 0.02 |
| **5f** |  | 5.63 | 8.48 | 64.42 ± 3.06 | 80.62 ± 0.33 |
| **5g** |  | 16.21 | 18.30 | 50.45 ± 2.98 | 81.19 ± 0.40 |
| **5h** |  | 9.55 | 15.72 | 60.40 ± 1.11 | 71.06 ± 0.34 |
| **5i** |  | 7.67 | 11.35 | 81.29 ± 1.38 | 49.58 ± 0.30 |
| **5j** |  | 5.88 | 8.69 | 87.65 ± 1.12 | 41.96 ± 0.39 |
| Quercetin‡ | | | | 15.49 ± 2.33 | 18.47 ± 0.04 |

*$IC_{50}$ values represent the mean ± standard error of mean of three parallel measurements ($p < 0.05$).
†$A_{0.50}$ values represent the mean ± standard error of mean of three parallel measurements ($p < 0.05$).
‡Standard.

synthesized compounds, **5f** ($IC_{50} = 5.63$ and 8.48 μM, for hCA I and hCA II, respectively) showed the highest inhibitory activity against hCA I and II. Most of the primary sulphonamide compounds are mentioned very potent inhibitor of the cytosolic isoform hCAs[6]. All the synthesized sulphonamides in this study are moderate inhibitory activity for the hCAs, because they are secondary sulphonamides and contain big bulky groups. Up to now, many sulphonamide and coumarin derivatives have been synthesized as strong CAIs by Supuran's group[28–30,40–45]. The inhibition values of them ranged from low micromolar to nanomolar. These compounds have stronger inhibitory effect than the sulphonamides substituted coumarylthiazole in this study. But, **5f** ($IC_{50} = 5.63$ and 8.48 μM, for hCA I and hCA II, respectively) showed higher inhibitory properties against hCA I and II compared to some sulphonamide and coumarin derivatives, reported in our previously study[46–51], with an $IC_{50}$ value of between 6.79 and 620 μM, for hCA I; between 6.54 and 51.45 μM, for hCA II.

The following structure–activity relationship (SAR) observations can be drawn from data of Table 1: (i) the best inhibitor among the newly synthesized and investigated compounds was naphthalene substituted derivative (**5f**) for hCA I and II; (ii) the alkyl series at the phenyl ring showed a qualitative relationship between increasing inhibitory activity and bulky group for hCA I and II [**5d** (R = 4-*tert*-butyl-benzene, $IC_{50} = 6.21$ and 10.91 μM for hCA I and II, respectively) > **5c** (R = 4-isopropyl-benzene, $IC_{50} = 6.46$ and 11.12 μM for hCA I and II, respectively) > **5b** (R = 2,5-dimethyl-benzene, $IC_{50} = 8.64$ and 11.68 μM for hCA I and II, respectively) > **5a** (R = 4-methyl-benzene, $IC_{50} = 22.63$ and 23.87 μM for hCA I and II, respectively)]; and (iii) the inhibitory activity on both hCA I and II seems to be strongly dependent on the size and polarizability of the halogen substituent at the *para*-position of the phenyl ring [for size and polarizability, I > Br > Cl > F, for inhibitory activity, **5j** (R = 4-iodobenzene, $IC_{50} = 5.88$ and 8.69 μM for hCA I and II, respectively) > **5i** (R = 4-bromobenzene, $IC_{50} = 7.67$ and 11.35 μM for hCA I and II, respectively) > **5h** (R = 4-cholorobenzene, $IC_{50} = 9.55$ and 15.72 μM for hCA I and II, respectively) > **5g** (R = 4-fluorobenzene, $IC_{50} = 16.21$ and 18.30 μM for hCA I and II, respectively)].

According to SAR study, it is clear that the bulky substituents (such as *tert*-butyl, naphthalene and iodine) increase inhibitory activity of the compound due to steric effect. The sulphonamide drug binds in deprotonated form to the catalytically critical Zn(II) ion, also participating in extensive hydrogen bond and van der Waals interactions with amino acid residues both in the hydrophobic and hydrophilic halves of the enzyme active site, as shown by X-ray crystallographic studies of enzyme–inhibitor complexes[6]. The inhibition mechanism of coumarins is different compared to that of the classical CAIs of the sulphonamide type[40]. Several kinetic and X-ray crystallographic studies have revealed that coumarins are mechanism-based inhibitors, which undergo hydrolysis under the influence of the zinc hydroxide, nucleophilically active species of the enzyme, with generation of substituted-2-hydroxycinnamic acids (Figure 2)[28,41,42]. Supuran's group reported that inhibitor (as for coumarin/sulfocoumarin) and enzyme solutions were pre-incubated together for ~6 h prior to assay in order to allow for the formation of the E−I complex or for the eventual active site mediated hydrolysis of the inhibitor[31].

On the other hand, the two endocyclic nitrogen of the 1,3,4-thidiazole ring of AAZ derivatives participates in two hydrogen bonds with the OH of Thr200[4].

Based on the above consideration, we consider that the coumarin ring should not undergo ring opening without pre-incubation on enzyme and inhibitor. Also it is difficult that the sulphonamide moiety of the synthesized compounds could bind to zinc cation in traditional manner due to contain big bulky groups.

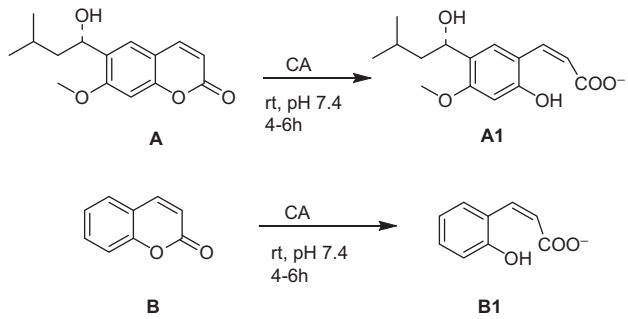



Figure 2. Formation of 2-hydroxy-cinnamic acids A1 and B1 by the CA-mediated hydrolysis of coumarin A and B[28,41,42].

So we suppose that the bulky substituents (such as *tert*-butyl, naphthalene and iodine), increasing inhibitory activity, could locate at the entrance of the enzyme active site. We also think that the nitrogen of the thiazole ring of the synthesized compounds contribute to inhibition by forming hydrogen bonds.

## Antioxidant activity assay

It is known that many natural and synthetic coumarin, sulphonamide and thiazole derivatives have various pharmacological properties. Among these properties, their antioxidant effects were examined[52–54]. Also, Gocer et al.[53] have recently reported that some sulphonamides demonstrated effective antioxidant properties (for $Cu^{2+}$ reducing capabilities and $ABTS^{\bullet+}$). In addition, Supuran's group investigated the the interaction of CA isozymes with some antioxidant compounds, such as resveratrol, dobutamine, curcumin, catechin, silymarin, salicyclates and quercetin. It was reported that all these antioxidant compounds showed effective hCA I and II inhibitory activity[55–58]. In view of these findings, it was considered that the synthesized compounds might possess certain antioxidant activity due to include sulphonamide, coumarin and thiazole moiety containing sulfonyl, carbonyl, imine and methoxy groups.

The ABTS method is based on the ability of hydrogen or electron-donating antioxidants to decolourize the performed radical monocation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) generated due to oxidation of ABTS with potassium persulfate[37]. The results in Table 1 indicated that all synthesized compounds exhibited moderate radical scavenging ability. **5e** and **5g** exhibited the strongest ABTS activity with $IC_{50}$ values of 48.83 and 50.45 μM, respectively. These potencies of **5e** and **5g** was 3.3-fold less than that of quercetin ($IC_{50} = 15.49$ μM) used as the reference compound.

The halogen substituent at the *para*-position of the phenyl ring showed an inverse relationship for increasing ABTS activity with growing size and polarizability [for size and polarizability, I > Br > Cl > F; for ABTS activity, **5j** (R = 4-iodobenzene, $IC_{50} = 87.65$ μM) < **5i** (R = 4-bromobenzene, $IC_{50} = 81.29$ μM) < **5h** (R = 4-cholorobenzene, $IC_{50} = 60.40$ μM) < **5g** (R = 4-fluorobenzene, $IC_{50} = 50.45$ μM)].

CUPRAC assays have a distinct advantage over other electron-transfer based assays (e.g. Folin, FRAP, ABTS, DPPH). This advantage is its realistic pH close to that physiological, favourable redox potential, accessibility and stability of reagents and applicability to lipophilic antioxidants as well as hydrophilic ones[38]. The cupric reducing antioxidant capacities of the synthesized compounds (**5a–j**) were determined according to the literature method[38] using quercetin as the reference compound. Among the synthesized compounds, only **5e** ($A_{0.50} = 23.29$ μM) showed close cupric reducing antioxidant

activity to quercetin ($A_{0.50} = 18.47\,\mu M$). The others have less the cupric reducing antioxidant capacity than quercetin.

Interestingly, in contrast ABTS activity, the CUPRAC activity seems to be strongly dependent on the increasing size and polarizability of the halogen substituent at the *para*-position of the phenyl ring [for size and polarizability, I > Br > Cl > F; for CUPRAC activity, **5j** (R = 4-iodobenzene, $A_{0.50} = 41.96\,\mu M$) > **5i** (R = 4-bromobenzene, $A_{0.50} = 49.58\,\mu M$) > **5h** (R = 4-cholorobenzene, $A_{0.50} = 71.06\,\mu M$) > **5g** (R = 4-fluorobenzene, $A_{0.50} = 81.19\,\mu M$)].

## Conclusions

A series of new secondary benzenesulphonamide substituted coumarylthiazole derivatives (**5a–j**) was synthesized and their activities as hCA I and hCA II inhibitors and structure–activity relationship were examined. All synthesized compounds inhibited hCA I and II. **5f** exhibited the strongest inhibition against hCA I and II with $IC_{50}$ value of 5.63 and 848 μM, respectively. The SARs revealed that the inhibitory activity of the synthesized compounds could also be affected by the type of the halogen substituent on the phenyl ring. Also the bulky substituents (such as *tert*-butyl, naphthalene and iodine) increase inhibitory activity of the compounds due to steric effect. Additionaly, the synthesized compounds showed moderate antioxidant activity.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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