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RESEARCH PAPER



Novel thiazolone-benzenesulphonamide inhibitors of human and bacterial carbonic anhydrases

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ABSTRACT

A small library of novel thiazolone-benzenesulphonamides has been prepared and evaluated for their ability to inhibit three human cytosolic carbonic anhydrases (hCA I, hCA II, and hCA VII) and three bacterial carbonic anhydrases (MscCA β , StCA1, and StCA2). All investigated hCAs were inhibited by the prepared compounds **4a–4j** in the low nanomolar range. These compounds were effective hCA I inhibitors (K_s of 31.5–637.3 nM) and excellent hCA II (K_s in the range of 1.3–13.7 nM) and hCA VII inhibitors (K_s in the range of 0.9–14.6 nM). The most active analog in the series, 4-((4-oxo-5-propyl-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide **4d**, strongly inhibited bacterial MscCA β , with K_i of 73.6 nM, considerably better than AAZ (K_i of 625 nM). The tested compounds displayed medium inhibitory potency against StCA1 (K_s of 69.2–163.3 nM) when compared to the standard drug (K_i of 59 nM). However, StCA2 was poorly inhibited by the sulphonamides reported here, with K_s in the micromolar range between 275.2 and 4875.0 nM.

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Carbonic anhydrase inhibitors; sulphonamides; thiazolones; *Mammaliococcus (Staphylococcus) sciuri*; *Salmonella enterica* (serovar *Typhimurium*)

Introduction





The carbonic anhydrases (CAs; EC 4.2.1.1) are a ubiquitous family of metalloenzymes that are found in both prokaryotes and eukaryotes and catalyse the reversible reaction between carbon dioxide and water with formation of bicarbonate and a proton¹. This simple reaction is fundamental to a variety of physiological and pathological processes in which cellular pH buffering plays an essential role². In humans, 15 carbonic anhydrase isoforms (hCAs) have been identified till date, among which 3 have non-catalytic functions (VIII, X, and XI) and 12 are catalytically active (CA I–IV, VA–VB, VI–VII, IX, and XII–XIV)³. Emerging scientific evidence indicates that CAs are involved in an array of disorders, including haemolytic anaemia (CA I), edoema (CA II), altitude sickness (CA II), glaucoma (CA II, IV), obesity (CA VA), neuropathic pain (CA VII), cancer (CA IX, XII), sterility (CA XIII), and many others⁴. They are therefore a target for drug discovery and since so far dozens of CA inhibitors have been FDA-approved for the treatment/management of various diseases (see Figure 1)⁵. On the other hand, a possible new approach for the treatment of infectious diseases which are caused by microorganisms such as bacteria, fungi or parasites is based on the inhibition of carbonic anhydrases families being present in these organisms⁶.

Sulphonamide moiety (R-SO₂NH₂) is a common substructure in marketed small-molecule drugs^{5,7} and a relevant “warhead” to target CAs, mainly because of its strong zinc-binding affinity⁸. The large number of publications on the development of novel sulphonamide-based compounds and investigation of their inhibitory capacity against CAs for expanding the database of CAls implies

that the field is a hot topic in current drug discovery research⁹. However, a major challenge faced when designing hCA inhibitors for therapy is the lack of selectivity for a specific hCA isoform at least for the first and second generation compounds¹⁰.

For about two decades the tail approach was employed for developing isoform selective CA inhibitors. In this strategy, various moieties are attached to the terminal part of scaffolds of simple sulphonamides that may lead to interactions with the external part of the CA isoforms active site, which is the region at the entrance of the cavity that is more diverse than the active site residues between the 12 catalytically active human isoforms¹¹.

Very recently, the research group of Hassan has synthesised a small library of thiazolone-linked benzenesulphonamides (Figure 2(a)) and reported their strong and selective hCA IX inhibitory activity over hCA II, the most active human CA isoform¹². Furthermore, it is reported that some of those compounds showed significant inhibitory effect against triple-negative breast cancer cell lines. In parallel with anticancer properties of thiazolone derivatives¹³, compounds possessing this heterocyclic ring have also been reported to exhibit antibacterial and antifungal activities¹⁴. Based on these literatures and in continuation of our interest in sulphonamide CAls¹⁵, herein, we present a novel, highly efficient and step-economic method for the synthesis of thiazol-2(5H)-one-containing sulphanilamides (Figure 2(b)) and evaluate their capability to inhibit three bacterial and three human cytosolic carbonic anhydrases.

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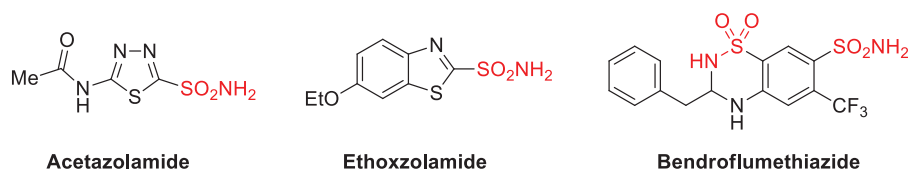


Figure 1. Selected example of FDA-approved sulphonamide carbonic anhydrase inhibitors.

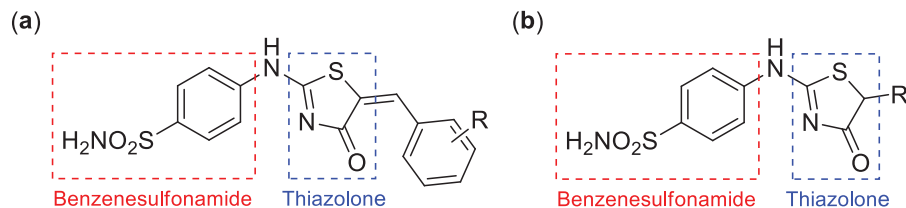


Figure 2. (a) General structure of thiazolone-benzenesulphonamides as selective hCA IX inhibitors developed by Hassan ¹²; (b) General structure of thiazolone-benzenesulphonamides discussed in the paper.

Experimental section

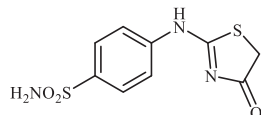
Chemistry

Reagents, starting materials and solvents were obtained from commercial sources and used as received. Thin-layer chromatography was performed on silica gel, spots were visualised with UV light (254 and 365 nm). NMR spectra were recorded on Bruker 500 spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO- d_6 signal (^1H 2.50; ^{13}C 39.52). High-resolution mass spectra (HRMS) were recorded on a mass spectrometer with a Q-TOF micro mass analyser using the ESI technique.

General procedure for the synthesis of compounds 4a-j

To a stirred solution of 1-(4-aminophenyl)thiourea (300 mg, 1.3 mmol) in DMF (4 ml) appropriate α -bromo ester (1.0 equiv., 1.3 mmol) was added at room temperature and the mixture was stirred at 25–40 °C for 15–48 h. Water (20 mL) was added to the reaction mixture and precipitate formed was collected by filtration, washed with water (100 ml) and air dried to afford the corresponding thiazolone-benzenesulphonamides (26–92%) as white solids.

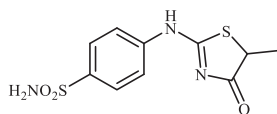
4-((4-Oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4a)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromoacetate (0.143 ml, 1.3 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 15 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4a** (322. mg, 92%) as a white powder.

^1H NMR (500 MHz, DMSO- d_6) δ = 4.06 (2H, s), 7.15 (1H, s), 7.35 (2H, s), 7.86 (3H, s), 11.49 and 11.93 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 35.5, 39.4, 121.0, 122.4, 128.1, 140.6, 141.4, 151.9, 159.1, 175.3, 180.0, 189.3 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for ($\text{C}_9\text{H}_8\text{N}_3\text{O}_3\text{S}_2$) 270.0007. Found 270.0009.

4-((5-Methyl-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4b)

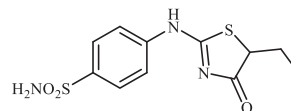


1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromopropanoate (0.169 ml, 1.3 mmol) were dissolved in DMF (4 ml). After

stirring at room temperature for 20 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4b** (311 mg, 84%) as a white powder.

^1H NMR (500 MHz, DMSO- d_6) δ = 1.54 (3H, s), 4.37 (1H, s), 7.14 (1H, s), 7.35 (2H, s), 7.85 (3H, s), 11.48 and 11.93 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 19.6, 45.0, 49.7, 121.1, 122.4, 128.1, 140.6, 142.3, 152.0, 157.2, 178.1, 178.5, 192.0 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for ($\text{C}_{10}\text{H}_{10}\text{N}_3\text{O}_3\text{S}_2$) 284.0164. Found 284.0166.

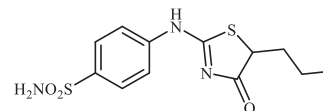
4-((5-Ethyl-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4c)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromobutanoate (0.191 ml, 1.3 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 20 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4c** (298 mg, 77%) as a white powder.

^1H NMR (500 MHz, DMSO- d_6) δ = 0.95 (3H, s), 1.85 (1H, s), 2.00 (1H, s), 4.41 (1H, s), 7.15 (1H, s), 7.35 (2H, s), 7.86 (3H, s), 11.51 and 11.94 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 11.4, 11.9, 26.4, 52.1, 57.1, 121.1, 12.4, 140.6, 142.3, 152.1, 157.3, 177.2, 178.7, 191.0 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for ($\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_3\text{S}_2$) 298.0320. Found 298.0327.

4-((4-Oxo-5-propyl-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4d)

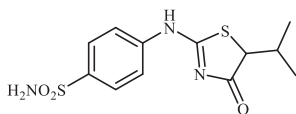


1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromopentanoate (0.426 ml, 2.6 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 36 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4d** (264 mg, 65%) as a white powder.

^1H NMR (500 MHz, DMSO- d_6) δ = 0.91 (3H, s), 1.33 (1H, s), 1.44 (1H, s), 1.77 (1H, s), 1.99 (1H, s), 4.41 (1H, s), 7.15 (1H, s), 7.34 (2H, s), 7.86 (3H, s), 11.49 and 11.95 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 14.4, 20.6, 21.4, 35.4, 35.6, 50.7, 55.7, 121.1, 122.4, 127.9, 128.1, 140.7, 142.4, 152.1, 157.3, 177.4, 178.7,

191.2 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for $(C_{12}H_{14}N_3O_3S_2)$ 312.0477. Found 312.0478.

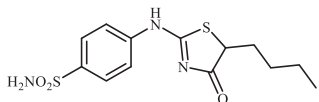
4-((5-Isopropyl-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4e)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromo-3-methylbutanoate (0.424 ml, 2.6 mmol) were dissolved in DMF (4 ml). After stirring at 40 °C for 36 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4e** (106 mg, 26%) as a white powder.

1H NMR (500 MHz, DMSO- d_6) δ = 0.94 (5H, d, J = 9.2 Hz), 1.05 (1H, s), 2.42 (1H, s), 4.50 (1H, s), 7.16 (1H, s), 7.34 (2H, s), 7.86 (3H, s), 11.52 and 11.97 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 16.8, 20.8, 22.2, 30.4, 57.2, 62.6, 120.6, 121.9, 127.4, 127.6, 140.1, 141.8, 151.6, 156.7, 176.2, 178.3, 190.0 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for $(C_{12}H_{14}N_3O_3S_2)$ 312.0477. Found 312.0475.

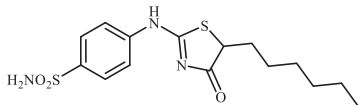
4-((5-Butyl-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4f)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromohexanoate (0.237 ml, 1.3 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 36 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4f** (281 mg, 66%) as a white powder.

1H NMR (500 MHz, DMSO- d_6) δ = 0.60 (3H, s), 1.03 (4H, s), 1.14 (1H, s), 1.50 (1H, s), 1.74 (1H, s), 4.13 (1H, s), 6.87 (1H, s), 7.06 (2H, s), 7.57 (3H, s), 11.21 and 11.67 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 14.7, 22.6, 29.3, 30.1, 32.98, 50.8, 55.9, 121.1, 122.4, 127.9, 128.1, 140.7, 142.3, 152.1, 157.2, 177.3, 178.7, 191.2 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for $(C_{13}H_{16}N_3O_3S_2)$ 326.0633. Found 326.0634.

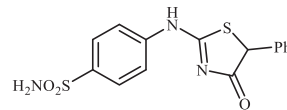
4-((5-Hexyl-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4g)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromooctanoate (0.546 ml, 2.6 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 48 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4g** (221 mg, 48%) as a white powder.

1H NMR (500 MHz, DMSO- d_6) δ = 0.87 (3H, s), 1.27 (6H, s), 1.42 (2H, s), 1.77 (1H, s), 2.02 (1H, s), 7.14 (1H, s), 7.34 (2H, s), 7.85 (3H, s), 11.49 and 11.81 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 14.8, 22.9, 27.1, 27.8, 29.1, 31.9, 33.2, 50.8, 55.8, 121.0, 122.3, 127.9, 128.1, 140.6, 142.3, 152.0, 157.3, 177.3, 178.5, 191.1 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for $(C_{15}H_{20}N_3O_3S_2)$ 354.0946. Found 354.0955.

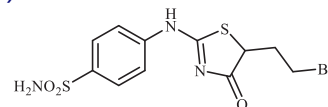
4-((4-Oxo-5-phenyl-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4h)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromo-2-phenylacetate (0.546 ml, 1.3 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 20 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4h** (397 mg, 88%) as a white powder.

1H NMR (500 MHz, DMSO- d_6) δ = 0.60 (3H, s), 5.65 (1H, d, J = 6.2 Hz), 7.25 (1H, s), 7.41 (7H, d, J = 6.2 Hz), 7.88–7.98 (3H, m), 11.71 and 12.23 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 53.9, 58.5, 121.3, 122.5, 127.9, 128.2, 129.1, 129.4, 129.9, 137.2, 137.7, 140.9, 142.3, 151.5, 157.5, 176.2, 178.7, 189.4 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for $(C_{15}H_{12}N_3O_3S_2)$ 346.0320. Found 346.0322.

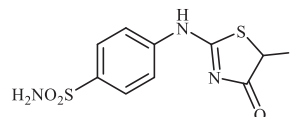
4-((5-(2-Bromoethyl)-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4i)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2,4-dibromobutanoate (0.205 ml, 1.3 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 20 h water (20 ml) was added and the precipitated product was filtered, washed with water (100 ml), and air dried to afford **4i** (396 mg, 81%) as a white powder.

1H NMR (500 MHz, DMSO- d_6) δ = 2.32 (1H, s), 2.62 (1H, s), 3.67 (2H, s), 4.48 (1H, s), 7.16 (1H, s), 7.35 (2H, s), 7.86 (3H, s), 11.58 and 12.00 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 32.6, 33.5, 36.4, 36.9, 49.5, 54.3, 121.2, 122.4, 127.9, 128.1, 140.7, 142.3, 151.8, 157.3, 176.9, 178.7, 190.4 ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for $(C_{11}H_{11}N_3O_3S_2Br)$ 375.9425. Found 377.9416.

4-((5-Fluoro-4-oxo-4,5-dihydrothiazol-2-yl)amino)benzenesulphonamide (4j)



1-(4-Aminophenyl)thiourea (300 mg, 1.3 mmol) and ethyl 2-bromo-2-fluoroacetate (0.153 ml, 1.3 mmol) were dissolved in DMF (4 ml). After stirring at room temperature for 20 h the reaction mixture was transferred to a separating funnel and mixture of water/dichloromethane (15:10 ml) was added and it was shaken vigorously for a few minutes. After 1 h, the precipitated product was filtered, washed with water (100 ml) and CH_2Cl_2 (20 ml), and air dried to afford **4j** (247 mg, 66%) as a white powder.

1H NMR (500 MHz, DMSO- d_6) δ = 6.76 (1H, d, J = 44.6 Hz), 7.18 (1H, d, J = 5.0 Hz), 7.40 (2H, d, J = 14.2 Hz), 7.88 (1H, d, J = 5.4 Hz), 7.92 (2H, s), 11.97 and 12.61 (1H, s and s) ppm. ^{13}C NMR (125 MHz, DMSO- d_6) δ = 94.0 (d, J = 221.8 Hz), 97.6 (d, J = 223.5 Hz), 122.2 (d, J = 42.0 Hz), 128.1 (d, J = 33.8 Hz), 141.4, 141.6 (d, J = 9.8 Hz), 150.9, 153.9, 171.0, 176.1, 184.6 (d, J = 13.6 Hz) ppm. HRMS (ESI) $[M-H]^-$: m/z calcd for $(C_9H_7N_3O_3FS_2)$ 287.9913. Found 287.9910.

CA inhibition assay

An applied photophysics stopped-flow instrument has been used for assaying the CA catalysed CO₂ hydration activity¹⁶. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer for α -CAs or 20 mM TRIS (pH 8.4) as buffer for β -CAs, and 20 mM Na₂SO₄ (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO₂ hydration reaction for a period of 10 – 100 s. The CO₂ 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 uncatalysed 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 – deionised water, and dilutions up to 0.1 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay in order to allow for the formation of the enzyme – inhibitor complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier^{17–19}, and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier^{20–22} and their concentrations in the assay system ranged between 10 and 14 nM.

Results and discussion

Chemistry

In Scheme 1, the synthesis of the target compounds reported here. The synthesis starts with the selective catalyst- and additive-free S-alkylation of easily accessible 4-thioureidobenzenesulphonamide **1** with commercially available α -bromo esters **2** under mild conditions. Following spontaneous intramolecular cyclisation in *in situ* generated ethyl 2-((N-(4-sulphamoylphenyl)carbamimidoyl)thio)acetate intermediate **3** into the desired products **4** in fair to excellent yields, ranging from 26% to 92%. The analytical and spectroscopic data (NMR and MS spectra) of the prepared compounds are in agreement with the proposed structures. Noteworthy, as observed in NMRs all compounds **4** exist as a mixture of tautomers (Figure 3)²³. All synthesised inhibitors are novel and not known in literature.

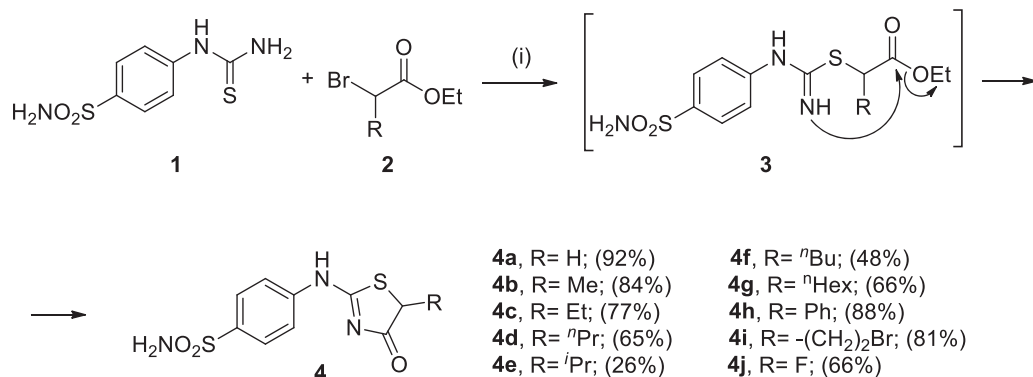
Carbonic anhydrase inhibition

The newly synthesised target compounds **4a–4j** were evaluated for their inhibitory activity towards three human (h) CA isoforms,

the cytosolic hCA I, II, and VII, as well as three bacterial β -CAs from one gram-positive bacteria, *Mammaliicoccus* (*Staphylococcus*) *sciuri*, MscCA β , and one gram-negative bacteria, *Salmonella enterica* (serovar *Typhimurium*), StCA1 and StCA2, by using a stopped-flow CO₂ hydase assay¹⁶.

Some important information from the data of Table 1, where the inhibition data of these enzymes are presented, are listed below:

- i. Ninety percent of the evaluated compounds exhibited superior potency against the slowest cytosolic human isoform hCA I with K_is in the range of 31.5–137.5 nM compared to the standard drug (acetazolamide; AAZ) whose K_i value is 250 nM. The SAR analysis showed that the inhibitory activity was decreased with the increased chain length in alkyl-substituted compounds **4b–4g**. Therefore, the best inhibitor was compound **4b** (K_i of 31.5 nM) with the smallest alkyl group (Me), whereas the poorest inhibitor was compound **4g** (K_i of 637.3 nM) with the longest alkyl group (*n*-Hex). However, the replacement of the aliphatic groups with a hydrogen atom did not increase the inhibitory activity as unsubstituted compound **4a** showed almost equal inhibitory activity with Et-substituted one **4c**. Additionally, no remarkable change was observed when the aliphatic groups were replaced with a benzene ring so that Ph-substituted compound **4h** displayed almost the same inhibitory activity with ⁱPr-substituted one **4e**. However, the inhibitory activity was decreased with the insertion of a fluorine atom at the C5-position. In summary, the relative inhibitory rates of newly designed compounds against hCA I followed the order: Methyl (C1) substituted- > unsubstituted- \approx medium aliphatic chain (C2–C4) substituted- \approx benzene-substituted \geq F-substituted- > long aliphatic chain (C6) substituted derivatives.
- ii. Against the fastest cytosolic human isoform hCA II, similar to hCA I, except long aliphatic chain (C6)-substituted thiazolone-benzenesulphonamide **4g** with K_i values of 13.7 nM, other compounds acted as more effective inhibitors (K_is in the range of 1.3–7.3 nM) compared to the standard drug (K_i of 12.5 nM). Therefore, all substitution patterns present in these compounds lead to highly effective CAs. It should be mentioned that the only aryl-substituted derivative **4h** of the series exhibited the best inhibition for this isozyme which was 10-fold more effective than AAZ. Thus, synthesis and evaluation of novel aryl-substituted thiazolone-benzenesulphonamide may be useful for the development of effective and selective inhibitor of hCA II.
- iii. The brain-associated cytosolic isoform hCA VII which was recently validated as therapeutic target for the relief of



Scheme 1. Reagents and conditions: (i) DMF, 25–40 °C, 15–48 h.

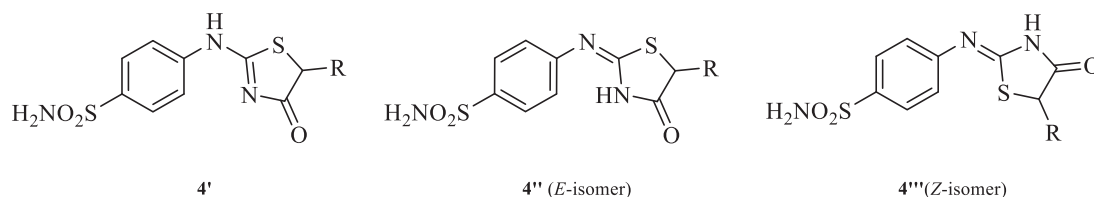


Figure 3. Representative tautomers of compounds **4**.

Table 1. Inhibition data of human CA isoforms hCA I, II, and VII and bacterial β -CA isoforms MscCA, from *Mammaliicoccus (Staphylococcus) sciuri*, and StCA1 and StCA2, from *Salmonella enterica* (serovar *Typhimurium*) with compounds **4a–j** in comparison with the standard sulphonamide inhibitor **AAZ** by a stopped flow CO_2 hydrazide assay.

4a-j

| Compound | R | K_i (nM) ^a | | | | | |
|------------|--|-------------------------|--------|---------|---------------|-------|--------|
| | | hCA I | hCA II | hCA VII | MscCA β | StCA1 | StCA2 |
| 4a | –H | 63.9 | 3.4 | 1.4 | 786.6 | 82.4 | 275.2 |
| 4b | –CH ₃ | 31.5 | 4.3 | 0.9 | 481.4 | 89.9 | 538.1 |
| 4c | –CH ₂ CH ₃ | 66.6 | 3.8 | 1.2 | 411.0 | 86.1 | 480.8 |
| 4d | –(CH ₂) ₂ CH ₃ | 70.7 | 2.3 | 2.0 | 73.6 | 83.3 | 913.1 |
| 4e | –CH(CH ₃) ₂ | 85.4 | 7.3 | 7.5 | 701.3 | 81.1 | 866.9 |
| 4f | –(CH ₂) ₃ CH ₃ | 75.0 | 3.2 | 6.2 | 2425.0 | 72.4 | 904.0 |
| 4g | –(CH ₂) ₅ CH ₃ | 637.3 | 13.7 | 14.6 | 4026.3 | 83.6 | 4875.0 |
| 4h | –C ₆ H ₅ | 90.4 | 1.3 | 7.3 | 1056.7 | 163.3 | 797.4 |
| 4i | –(CH ₂) ₂ Br | 74.6 | 2.2 | 2.1 | 924.5 | 69.2 | 515.1 |
| 4j | –F | 137.5 | 3.7 | 1.9 | 848.7 | 90.9 | 955.9 |
| AAZ | – | 250 | 12.5 | 2.5 | 625 | 59 | 84 |

^aMean from three different assays, by a stopped flow technique (errors were in the range of ± 5 –10% of the reported values).

neuropathic pain²⁴ showed a rather similar inhibition profile with hCA I, with sulphonamides investigated here. Therefore, methyl-substituted compound **4b** was the strongest inhibitor with a K_i of 0.9 nM and ⁿHex-substituted compound **4g**, showed poorest inhibitory capacities with a K_i of 14.6 nM. It is worth mentioning that 60% of the compounds investigated here (**4a–d**, **4i**, **4j**), showed even better inhibitory activities against hCA VII in comparison with the reference drug.

- iv. The bacterial β -CA from *Mammaliicoccus (Staphylococcus) sciuri*, MscCA β , was effectively inhibited by some of the obtained sulphonamides, with the K_i values in the range of 73.6 and 4026.3 nM. Indeed, Among the 10 investigated compounds, three exhibited superior inhibitory activity compared to AAZ which all are alkyl-substituted derivatives. Compound **4d** showed the best inhibition against MscCA with a K_i of 73.6 nM, which was 8-fold higher than that of AAZ (K_i of 625 nM). Although this compound **4d** displayed negligible selectivity for MscCA over StCA1 (MscCA/StCA1:1.4), its high selectivity index greater than 10 for MscCA versus StCA2 is remarkable.
- v. One of the *S. enterica* (serovar *Typhimurium*) CA isoform, StCA1, was effectively inhibited by the sulphonamides reported here, with K_i s in the range between 69.2 and 163.3 nM. The most promising compound, **4g**, showed the most significant selectivity for this isozyme versus MscCA β and StCA2, 48- and 58-fold higher selectivity, respectively. Therefore, this compound is an excellent candidate for future *in vivo* preclinical studies.

- vi. The second *S. enterica* (serovar *Typhimurium*) CA isoform, StCA2, was on the other hand poorly inhibited with targeted sulphonamides compared to MscCA β and StCA1, and the K_i s were in the range of 275.2–4875.0 nM. Among the tested compounds, unsubstituted derivative **4a** demonstrated the most potent StCA2 inhibitory activity with a K_i of 275.2 nM, although the activity was three-fold lower than the reference compound (K_i of 84 nM).

Conclusion

A small library of C5-functionalized thiazol-2(5H)-one-containing sulphonamide derivatives **4a–4j** has been prepared through a high atom- and step-economical reaction between easily accessible 4-thioureidobenzenesulphonamide and commercially available α -bromo esters under mild and additive-free conditions. These compounds were tested for their inhibition of three cytosolic human carbonic anhydrase isoforms (hCA I, hCA II, and hCA VII) as well as three bacterial enzymes belonging to the β -CA class (MscCA β , StCA1, and StCA2). The results showed that majority of the examined compounds effectively inhibited hCAs, even better than the standard drug (AAZ). Noteworthy, in all cases these compounds showed significant selectivity for hCA II and hCA VII over hCA I. However, they did not show selectivity for the inhibition of hCA II versus hCA VII. The bacterial CAs were also inhibited by these compounds, but not as effectively as the hCAs. Importantly, compound **4g** which showed the poorest capacity to inhibit human CAs, inhibited StCA1 almost in the same range of AAZ, while it has a 48- and 58-fold high selectivity for this isozyme versus MscCA and StCA2, respectively. The activity of this analog against StCA1 is especially noteworthy, and suggests that it is an excellent candidate for future *in vivo* preclinical studies.

Disclosure statement

No potential conflict of interest was reported by all author(s) except CTS. CT Supuran is Editor-in-Chief of the Journal of Enzyme Inhibition and Medicinal Chemistry. He was not involved in the assessment, peer review, or decision-making process of this paper. The authors have no relevant affiliations of financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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