4-Cyanamidobenzenesulfonamide derivatives: a novel class of human and bacterial carbonic anhydrase inhibitors

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ABSTRACT
A one-pot two-step protocol was developed for the synthesis of a series of novel 4-cyanamidobenzenesulfonamides from easily accessible methyl (4-sulfamoylphenyl)-carbamimidothioate. The new sulphonamides were investigated as inhibitors of the enzyme carbonic anhydrase (CA, EC 4.2.1.1), the human (h) cytosolic isoforms hCA I, II, VII, and XIII, as well as three bacterial enzymes belonging to the β-CA class, MscCA from Mammallicoccus (Staphylococcus) sciuri and StCA1 and StCA2, from Salmonella enterica (serovar Typhimurium). The human isoforms were generally effectively inhibited by these compounds, with a clear structure-activity relationship privileging long aliphatic chains (C6, C7 and C18) as substituents at the cyanamide functionality. The bacterial CAs were also inhibited by these compounds, but not as effective as the hCAs. The most sensitive enzyme to these inhibitors was StCA1 (KIs of 50.7–91.1 nM) whereas SscCA was inhibited in the micromolar range (KIs of 0.86–9.59 μM).

GRAPHICAL ABSTRACT

Introduction
Sulphonamides are one of the crucial classes of bioactive compounds that played a pivotal role in the field of drug discovery and development due to their diversified pharmacological activities, with more than seventy FDA-approved medications containing one or more sulphonamide motifs in their structure. The best known and striking biological features of primary sulphonamide-containing compounds is their ability to inhibit the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) by binding to the zinc ion within its catalytic site. CAs are widespread in all types of organisms, in the three domains of life, Archaea, Bacteria and Eukarya. Presently, 15 different CA isoforms (CA I, II, III, IV, VA, VB, VI, VII, VIII, IX, X, XI, XII, XIII, and XIV) were described in humans/primates, 12 of which being able to catalyse the reversible interconversion of CO2 to HCO₃⁻ and a proton, whereas CA VIII, X, and XI are devoid of CO2 hydrase activity. This reaction plays fundamental roles in many physiological and pathological events, including respiration and CO2 transport, secretion of electrolytes, pH and other ions homeostasis, bone reabsorption, calcification, tumorigenesis, etc. Therefore, the involvement of various CA isoform in such processes can be and is exploited for the development of different types of therapeutic agents, among which diuretics, anti-glaucoma, antiepileptic, anti-obesity and anti-tumour medications. CAs are on the other hand widespread in bacteria, with at least four CA genetic families being present in these organisms, the α-, β-, γ-, and δ-class enzymes, of the eight CA classes described so far. Many members of such CAs were discovered, isolated and characterised extensively, mainly in pathogenic bacteria, in the search of inhibitors acting as antibiotics with a diverse mechanism of action compared to the clinically used such agents. Indeed, recent studies demonstrated that bacterial infections difficult to treat due to the drug resistance problems, such as those provoked by vancomycin-resistant Enterococci or Neisseria spp., can be managed by using CA inhibitors of the sulphonamide type. Such promising results opened new directions in the design of CAls selective for the various CA isoforms.
target drugs as well as unravelling the mechanism of various pathological processes. Importantly, compounds containing electrophilic cyanamide warhead displayed significant inhibitory activities against human cathepsins\textsuperscript{17,18}, inhibiting the lysosomal cysteine protease cathepsin S (CatS)\textsuperscript{19,20} (Scheme 1).

In this regard, in the next stage, we may study the inhibitory activity of the same set of compounds reported in this paper against cathepsin(s).

In light of the above-mentioned facts, we hypothesised that compounds possessing sulphonamide and cyanamide moieties might show efficient inhibitory activity against various human and bacterial CAs. Thus, in continuation of our interest in the development of selective CA inhibitors (CAIs)\textsuperscript{21}, herein, we present the synthesis of novel cyanamide-containing sulphonamides (Scheme 2) and evaluate their capability to inhibit various human and bacterial CAs.

**Materials and methods**

**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 300 spectrometer with chemical shifts values (\(\delta\)) in ppm relative to TMS using the HRMS) were recorded on a mass spectrometer with a Q-TOF micro mass analyser using the ESI technique.

**Synthesis**

4-Thioureidobenzenesulfonyamide (4)

\[
\text{H}_2\text{NO}_2\text{S}^-\xrightarrow{\text{N}}\text{C}_{\text{N}}\text{N}\xrightarrow{\text{R}}
\]

4-Aminobenzensulfonamide (30.00 g, 174.3 mmol) was dissolved in 180 ml of 3.5 M HCl at 70 °C. After cooling to room temperature, KSCN (16.94 g, 174.3 mmol) was added and the mixture was refluxed for 3 h. After cooling to room temperature, the reaction...
mixture was added to the solution and the mixture was heated at 40°C. The reaction mixture was cooled to room temperature and precipitated K2CO3 (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100°C for 1.5 h. The reaction mixture was cooled to room temperature, MeI (0.164 ml, 2.04 mmol) was added and the mixture was stirred at 100°C for 1.5 h. The reaction mixture was cooled to room temperature, EtI (0.127 ml, 2.04 mmol) was added and the mixture was stirred at 100°C for 1 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 20 ml). The combined organic phases were washed with aq. sat. NaHCO3 (1 x 20 ml) and aq. sat. NH4Cl in water (1 x 20 ml) and dried over Na2SO4. Solvent removal in vacuo afforded 5 (223 mg, 70%) as a white powder.

1H NMR (300 MHz, DMSO-d6) δ = 7.32 (s, 2H), 7.69 (d, 2H, J = 8.6 Hz), 7.77 (d, 2H, J = 8.6 Hz), 10.02 (s, 1H) ppm 13C NMR (75 MHz, DMSO-d6) δ = 122.8, 127.3, 139.8, 143.9, 182.8 ppm MS (ESI) [M + H]+: m/z 232.0.

Methyl (4-sulfamoylphenyl)carbamimidothioate (5)

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K2CO3 (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100°C for 1.5 h. After cooling to room temperature, Mel (0.127 ml, 2.04 mmol) was added and the mixture was stirred at 100°C for 1 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 20 ml). The combined organic phases were washed with aq. sat. NaHCO3 (2 x 20 ml) and aq. sat. NH4Cl (1 x 20 ml) and dried over Na2SO4. Solvent removal in vacuo and the residue was washed with EtOAc (2 x 10 ml) to afford 6 (103 mg, 24%) as a white powder.

1H NMR (300 MHz, DMSO-d6) δ = 3.42 (s, 3H), 7.32 (d, 2H, J = 8.8 Hz), 7.37 (s, 2H), 7.89 (d, 2H, J = 8.8 Hz) ppm 13C NMR (75 MHz, DMSO-d6) δ = 137.7, 114.1, 115.8, 128.5, 139.5, 144.2 ppm HRMS (ESI) [M + H]+: m/z calcd for (C8H9N3O2S) 211.0415. Found 211.0419.

4-(N-Methylcyanamido)benzenesulfonamide (8)

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K2CO3 (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100°C for 1.5 h. After cooling to room temperature, Mel (0.172 ml, 2.04 mmol) was added and the mixture was stirred at 100°C for 1 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 20 ml). The combined organic phases were washed with aq. sat. NaHCO3 (2 x 20 ml) and aq. sat. NH4Cl (1 x 20 ml) and dried over Na2SO4. Solvent removal in vacuo and the residue was washed with EtOAc (2 x 10 ml) to afford 6 (103 mg, 24%) as a white powder.

1H NMR (300 MHz, DMSO-d6) δ = 3.42 (s, 3H), 7.32 (d, 2H, J = 8.8 Hz), 7.37 (s, 2H), 7.89 (d, 2H, J = 8.8 Hz) ppm 13C NMR (75 MHz, DMSO-d6) δ = 137.7, 114.1, 115.8, 128.5, 139.5, 144.2 ppm HRMS (ESI) [M + H]+: m/z calcd for (C8H9N3O2S) 211.0415. Found 211.0419.

4-(N-Ethylcyanamido)benzenesulfonamide (9)

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K2CO3 (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100°C for 1.5 h. After cooling to room temperature, EtI (0.164 ml, 2.04 mmol) was added and the mixture was stirred at 100°C for 1 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 20 ml). The combined organic phases were washed with aq. sat. NaHCO3 (2 x 20 ml) and aq. sat. NH4Cl (1 x 20 ml) and dried over Na2SO4. Solvent removal in vacuo and the residue was washed with EtOAc (2 x 10 ml) to afford 6 (103 mg, 24%) as a white powder.
4-(N-Butylcyanamido)benzenesulfonamide (10)

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K₂CO₃ (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100 °C for 1.5 h. After cooling to room temperature, nBuI (0.232 ml, 2.04 mmol) was added and the mixture was stirred at 100 °C for 1 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 × 20 ml). The combined organic phases were washed with aq. sat. NaHCO₃ (2 × 20 ml) and aq. sat. NH₄Cl (1 × 20 ml) and dried over Na₂SO₄. The solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (DCM:MeOH, 95:5) to afford 10 (279 mg, 54%) as a white powder.

1H NMR (300 MHz, DMSO-d₆) δ 0.96 (t, 3H, J = 7.1 Hz), 1.37–1.49 (m, 2H), 1.68–1.77 (m, 2H), 3.77 (t, 2H, J = 7.1 Hz), 7.37–7.39 (m, 4H), 7.88 (d, 2H, J = 8.7 Hz) ppm 13C NMR (75 MHz, DMSO-d₆) δ 14.5, 20.0, 29.8, 49.3, 113.4, 116.4, 128.6, 139.6, 143.6 ppm HRMS (ESI) [M]⁺: m/z calcld for (C₁₁H₁⁵N₃O₂S) 253.0885. Found 253.0888.

4-(N-Octadecylcyanamido)benzenesulfonamide (13)

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K₂CO₃ (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100 °C for 1.5 h. After cooling to room temperature, 1-bromooctadecane (0.335 ml, 2.04 mmol) and KI (680 mg, 4.44 mmol) were added and the mixture was stirred at 100 °C for 1.5 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 × 20 ml). The combined organic phases were washed with aq. sat. NaHCO₃ (2 × 20 ml) and aq. sat. NH₄Cl (1 × 20 ml) and dried over Na₂SO₄. The solvent was removed in vacuo. Residual yellowish solids were washed with cold EtOAc (15 ml) to afford 13 (165 mg, 18%) as a white powder.

1H NMR (300 MHz, DMSO-d₆) δ 0.88 (t, 3H, J = 6.7 Hz), 1.26 (br s, 30H), 1.68–1.77 (m, 2H), 3.75 (t, 2H, J = 6.7 Hz), 7.35–7.38 (m, 4H), 7.88 (d, 2H, J = 8.6 Hz) ppm 13C NMR (75 MHz, DMSO-d₆) δ 14.9, 22.9, 26.9, 27.7, 29.2, 32.1, 49.5, 113.4, 116.4, 128.6, 139.6, 143.5 ppm HRMS (ESI) [M - H]⁻: m/z calcld for (C₁₄H₂₀N₃O₂S) 294.1276. Found 294.1285.

4-(N-Cyclohexylcyanamido)benzenesulfonamide (14)

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K₂CO₃ (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100 °C for 1.5 h. After cooling to room temperature, bromocyclohexane (0.232 ml, 2.04 mmol) and KI (379 mg, 2.24 mmol) were added and the mixture was stirred at 100 °C for 1.5 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 × 20 ml). The combined organic phases were washed with aq. sat. NaHCO₃ (2 × 20 ml) and aq. sat. NH₄Cl (1 × 20 ml) and dried over Na₂SO₄. The solvent was removed in vacuo. Semisolid residue was dissolved in DMSO-d₆ and precipitated by addition of hexane (50 ml). The precipitate formed was filtered, washed with hexane and air dried to afford 14 (290 mg, 48%) as a white powder.

1H NMR (300 MHz, DMSO-d₆) δ 0.89 (t, 3H, J = 6.5 Hz), 1.29 (br s, 5H), 1.37 (br s, 3H), 1.69–1.78 (m, 2H), 3.76 (t, 2H, J = 6.9 Hz), 7.36–7.39 (m, 4H), 7.88 (d, 2H, J = 8.6 Hz) ppm 13C NMR (75 MHz, DMSO-d₆) δ 14.9, 22.9, 26.6, 27.7, 29.2, 32.1, 49.5, 113.4, 116.4, 128.6, 139.6, 143.5 ppm HRMS (ESI) [M - H]⁻: m/z calcld for (C₁₄H₂₀N₃O₂S) 294.1276. Found 294.1285.

4-(N-Heptylcyanamido)benzenesulfonamide (12)

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K₂CO₃ (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100 °C for 1.5 h. After cooling to room temperature, bromocyclohexane (0.252 ml, 2.04 mmol) and KI (372 mg, 2.24 mmol) were added and the mixture was stirred at 100 °C for 1.5 h. The reaction mixture was cooled to room temperature and extracted with EtOAc (3 × 20 ml). The combined organic phases were washed with aq. sat.
NaHCO₃ (2 × 20 ml) and aq. sat. NH₄Cl (1 × 20 ml) and dried over Na₂SO₄. The solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (DCM:MeOH, 95:5) to afford 14 (85 mg, 15%) as a white powder.

\[ ^1H \text{ NMR (300 MHz, DMSO-d}_6) \delta = 1.19–1.27 (m, 1H), 1.41–1.55 (m, 4H), 1.68 (d, 1H, J = 12.1 Hz), 1.83 (s, 2H), 2.02 (s, 2H), 3.99 (s, 1H), 7.38 (s, 2H), 7.43 (d, 2H, J = 8.6 Hz), 7.88 (d, 2H, J = 8.6 Hz) \text{ ppm} \]

\[ ^{13}C \text{ NMR (75 MHz, DMSO-d}_6) \delta = 25.4, 25.6, 31.5, 56.8, 111.9, 117.1, 128.6, 139.8, 143.3 \text{ ppm HRMS (ESI) [M - H]^-:} \] [M - H]: m/z calcd for \((C_{13}H_{16}N_3O_2S) 278.0963. \) Found 278.0969.

\[ 4-(N-Allylcyanamido)benzenesulfonamide (15) \]

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K₂CO₃ (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100 °C for 1.5 h. After cooling to room temperature, allyl bromide (0.176 ml, 2.04 mmol) was added and the mixture was stirred at 100 °C for 1.5 h. The reaction mixture was cooled to room temperature and water was added until precipitate was formed. The precipitate was filtered, washed with hexane and air dried to afford 15 (290 mg, 60%) as a white powder.

\[ ^1H \text{ NMR (300 MHz, DMSO-d}_6) \delta = 4.46 (d, 2H, J = 4.9 Hz), 5.36 (s, 1H), 5.41 (d, 1H, J = 7.8 Hz), 5.94–6.06 (m, 1H), 7.36 (d, 2H, J = 8.6 Hz), 7.38 (s, 2H), 7.88 (d, 2H, J = 8.6 Hz) \text{ ppm} \]

\[ ^{13}C \text{ NMR (75 MHz, DMSO-d}_6) \delta = 52.0, 113.4, 116.5, 120.5, 128.5, 131.6, 139.7, 143.3 \text{ ppm HRMS (ESI) [M - H]^-:} \] [M - H]: m/z calcd for \((C_{10}H_{12}N_3O_2S) 286.0650. \) Found 286.0649.

\[ 4-(N-Benzylcyanamido)benzenesulfonamide (16) \]

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K₂CO₃ (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100 °C for 1.5 h. After cooling to room temperature, benzyl bromide (0.236 ml, 2.04 mmol) was added and the mixture was stirred at 100 °C for 1.5 h. The reaction mixture was cooled to room temperature and water was added until precipitate was formed. The precipitate was filtered, washed with water (10 ml) and Et₂O (10 ml) and air dried to afford 16 (282 mg, 48%) as a white powder.

\[ ^1H \text{ NMR (300 MHz, DMSO-d}_6) \delta = 5.06 (s, 2H), 7.35–7.44 (m, 9H), 7.86 (d, 2H, J = 8.4 Hz) \text{ ppm} \]

\[ ^{13}C \text{ NMR (75 MHz, DMSO-d}_6) \delta = 52.5, 113.2, 116.2, 128.1, 128.3, 128.9, 135.0, 139.3, 142.8 \text{ ppm HRMS (ESI) [M - H]^-:} \] [M - H]: m/z calcd for \((C_{14}H_{12}N_3O_2S) 380.0655. \) Found 380.0649.

\[ 4-(N-(4-Bromobenzyl)cyanamido)benzenesulfonamide (17) \]

To a solution of methyl (4-sulfamoylphenyl)carbamimidothioate (5) (500 mg, 2.04 mmol) in DMF (8 ml) under stirring K₂CO₃ (564 mg, 4.08 mmol) was added in one portion. The mixture was stirred at 100 °C for 1.5 h. After cooling to room temperature, 4-bromobenzyl bromide (659 mg, 2.04 mmol) was added and the mixture was stirred at 100 °C for 1.5 h. The reaction mixture was cooled to room temperature and water was added until precipitate was formed. The precipitate was filtered, washed with water (10 ml) and Et₂O (10 ml) and air dried to afford 17 (501 mg, 67%) as a white powder.

\[ ^1H \text{ NMR (300 MHz, DMSO-d}_6) \delta = 5.06 (s, 2H), 7.36–7.42 (m, 6H), 7.65 (d, 2H, J = 8.0 Hz), 7.87 (d, 2H, J = 8.0 Hz) \text{ ppm} \]

\[ ^{13}C \text{ NMR (75 MHz, DMSO-d}_6) \delta = 52.3, 113.5, 116.7, 122.6, 128.6, 131.1, 132.8, 134.9, 139.9, 143.2 \text{ ppm HRMS (ESI) [M - H]^-:} \] [M - H]: m/z calcd for \((C_{14}H_{12}N_3O_2SBr) 363.9755. \) Found 363.9758.

CA inhibitory assay

An applied photophysics stopped-flow instrument has been used for assaying the CA catalysed CO₂ hydration activity. 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 and 20 mM Na₂SO₄ (for maintaining constant the ionic strength), followed by 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 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 6 h at room temperature prior to assay in order to allow for the formation of the E – I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng – Prusoff equation, as reported earlier.

Results and discussion

Chemistry

The drug design of the new CAIs reported here considered the benzenesulphonamide scaffold, which has been widely employed earlier for derivatization reactions, mainly by using the tail approach, as it allows a facile chemistry and generally high yields of new products. The general synthetic route of the target compounds is shown in Scheme 3. In order to bypass the need for toxic cyanogen bromide, a multi-step procedure was designed which involves the initial formation of methyl (4-sulfamoylphenyl)
Carboxic anhydrase inhibition

The cyanamido-benzensulphonamides 6–17 reported here have been tested as inhibitors of three human (h) CA isoforms, the cytosolic hCA I, II, VII and XIII, as well as the bacterial β-CAs from *Mammallicoccus* (*Staphylococcus*) *sciuri*26 and *Salmonella enterica* (serovar *Typhimurium*), StCA1 and StCA227. It should be mentioned that the first bacterial enzyme, SscCA, was originally reported by us as *Staphylococcus aureus* β-CA, SauCA based on a genomic sequence annotated in the data bases in 201726. A recent reanalysis of that sequence revealed that the original annotation was erroneous, and that the sequence encodes a β-class CA from another *Staphylococcaceae* family member, i.e. *Staphylococcus sciuri*, which is a Gram-positive, oxidase-positive, coagulase-negative member of these infectious bacteria known to provoke disease in humans and animals (it was originally isolated from the squirrel)29. In 2020, Madhaiyan et al. renamed the species as another family member, i.e. *Mammallicoccus sciuri*. In fact, the taxonomy of the *Staphylococcaceae* family is rather complex, and as mentioned earlier, many genome annotations were inexact or overlapping between various genetically similar species20. Thus, we report here that the enzyme previously known as SauCA is in fact MscCA31.

The following should be noted regarding data of Table 1, where the inhibition data of these enzymes are presented:

i. hCA I was effectively inhibited by cyanamido-benzenesulphonamides 6–17 reported here with Ks ranging between 9.3 and 889 nM (acetazolamide, the standard CAI is a medium potency inhibitor with a Ks of 250 nM). The most effective hCA I inhibitors in the series were 10–14, Ks of 9.3–50.6 nM, all of which incorporate rather long aliphatic R moieties at the cyanamide functionality. The optimal substitution seems to be an unsaturated or aromatic one leading to a decrease of the hCA I inhibitor reported here. Smaller aliphatic R moieties, for example the 11-hexyl group, in 11, which is the most effective hCA I inhibitor reported here. Smaller aliphatic R moieties, unsaturated or aromatic ones lead to a decrease of the hCA I inhibitory potency.

ii. hCA II is also effectively inhibited by these sulphamides, with Ks ranging between 5.3 and 148 nM (Table 1). Derivatives 11–13 were the most effective inhibitors, with Ks of 5.3–9.5 nM, even better than AAZ (Ks of 12 nM). Again the

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**Scheme 3.** Reagents and conditions: (i) KSCN, aq. 3.5 M HCl, reflux, 3 h, 31%; (ii) MeI, DMF, 40 °C, 2.5 h, 70%; (iii) K2CO3, DMF, 100 °C, 1.5 h, 89%; (iv) acetic acid (4 equiv.), DMF, RT, 10 min, 59%; (v) (a) K2CO3, DMF, 100 °C, 1.5 h; (b) RX (X = Br or I), DMF, 40 °C, 2.5 h.

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**Table 1.** Inhibition data of human CA isoforms CA I, II, VII, and XIII and bacterial β-CA isoforms SscCA, from *Mammallicoccus* (*Staphylococcus*) *sciuri*, and StCA1 and StCA2, from *Salmonella enterica* (serovar *Typhimurium*), with compounds 6–17 using acetazolamide (AAZ) as standard drug.

| Cmpd | R       | hCA I (x-CA) | hCA II (x-CA) | hCA VII (x-CA) | hCA XIII (x-CA) | MscCA (β-CA) | StCA1 (β-CA) | StCA2 (β-CA) |
|------|---------|-------------|---------------|----------------|----------------|--------------|--------------|--------------|
| 6    | K⁺      | 867         | 130           | 30.6           | 62.6           | 860          | 87.7         | 92.4         |
| 7    | –H      | 889         | 148           | 22.7           | 50.5           | 870          | 91.1         | 94.7         |
| 8    | –CH₃    | 557         | 29.2          | 13.8           | 117            | 1585         | 52.7         | 200          |
| 9    | –CH₂CH₃ | 88.6        | 45.3          | 17.9           | 476            | 3151         | 61.5         | 367          |
| 10   | –(CH₂)₂CH₃ | 16.4       | 32.0          | 1.3            | 70.7           | 4476         | 62.7         | 426          |
| 11   | –(CH₂)₃CH₃ | 9.3         | 5.3           | 1.5            | 83.0           | 6885         | 54.6         | 718          |
| 12   | –(CH₂)₄CH₃ | 38.2       | 7.1           | 1.7            | 31.5           | 7188         | 58.8         | 676          |
| 13   | –(CH₂)₅CH₃ | 17.3       | 9.5           | 2.2            | 155            | 9173         | 66.1         | 791          |
| 14   | –Cy     | 50.6        | 82.3          | 1.9            | 83.8           | 5359         | 69.5         | 579          |
| 15   | –CH₂CH = CH₂ | 74.7       | 54.1          | 2.3            | 97.7           | 5096         | 51.2         | 645          |
| 16   | –CH₂C₆H₅ | 67.3        | 61.4          | 2.0            | 67.8           | 9361         | 50.7         | 692          |
| 17   | –CH₂(4-Br-C₆H₄) | 389      | 93.3          | 2.4            | 53.1           | 9592         | 73.1         | 750          |
| AAZ  | –       | 250         | 12.5          | 2.5            | 16.0           | 625          | 59.0         | 84          |

*Mean from 3 different assays, by a stopped flow technique (errors were in the range of ±10% of the reported values).*
presence of long aliphatic chains (C6, C7 and C18) induced these effective inhibitory effects, whereas shorter, unsaturated or aromatic/benzylic R groups reduce (in some cases only slightly) the potency.

iii. hCA VII, the mainly brain expressed cytosolic isofrom was the most susceptible to inhibition with the newly prepared sulphonamides, which showed Ks ranging between 1.3 and 30.6 nM (Table 1). Thus, all substitution patterns present in these compounds lead to highly effective CAls, with the optimal substitutions being those present in 10–17. Thus, for this isofrom, apart the long aliphatic groups, the unsaturated, benzylic and substituted benzylic moieties afforded highly effective inhibitors.

iv. MscCA was on the other hand poorly inhibited by the cyanamido-benzenesulphonamides 6–17 reported here, with Ks in the micromolar range, more precisely 860–9592 nM (Table 1). The best inhibitors were the unsubstituted cyanamides 6 and 7, which like acetazolamide, are medium potency bacterial CA inhibitors.

v. StCA1 was the best inhibited bacterial CA among the three such enzymes investigated here with cyanamido-benzenesulphonamides 6–17. Indeed, these compounds showed Ks ranging between 50.7 and 91.1 nM. Thus, all of them show a quite flat structure-activity relationship, acting as effective (but not highly potent) CAls.

vi. The second S. enterica (serovar Typhimurium) CA isofrom StCA2 was on the other hand less sensitive to inhibition with these new sulphonamides compared to StCA1, and the Ks were in the range of 92.4–791 nM. In this case, the best inhibitors were the ones with small R groups (H or potassium), compounds 6 and 7, whereas the increase of the R moiety led to a decrease of the inhibitory power (Table 1).

Conclusions

A one-pot two-step protocol was developed for the synthesis of novel 4-cyanamidobenzenesulphonamide derivatives from easily accessible methyl (4-sulfamoylphenyl)carbamimidothioate under catalyst- and additive-free conditions. The small series of prepared compounds was investigated for the inhibition of human and bacterial CAls belonging to the α- and β-CA classes. Several highly effective hCA I, II, and VII inhibitors were detected, whereas hCA XIII was less inhibited by most such compounds. The structure-activity relationship for the inhibition of the human isofroms is rather straightforward, with long aliphatic chains (C6, C7 and C18) at the cyanamide functionality inducing the most effective inhibitory effects. Among the three bacterial CAls investigated here for their inhibition with cyanamidobenzenesulphonamides, StCA1 was the most sensitive to these inhibitors, followed by StCA2, whereas MscCA was inhibited only in the micromolar range. Although these compounds do not show selectivity for the inhibition of bacterial versus human CAls, they may be considered as interesting starting points for the development of novel pharmacological agents belonging to this class of enzyme inhibitors.

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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