

Supplemental Information

X-Ray Crystallography-Promoted Drug Design of Carbonic Anhydrase

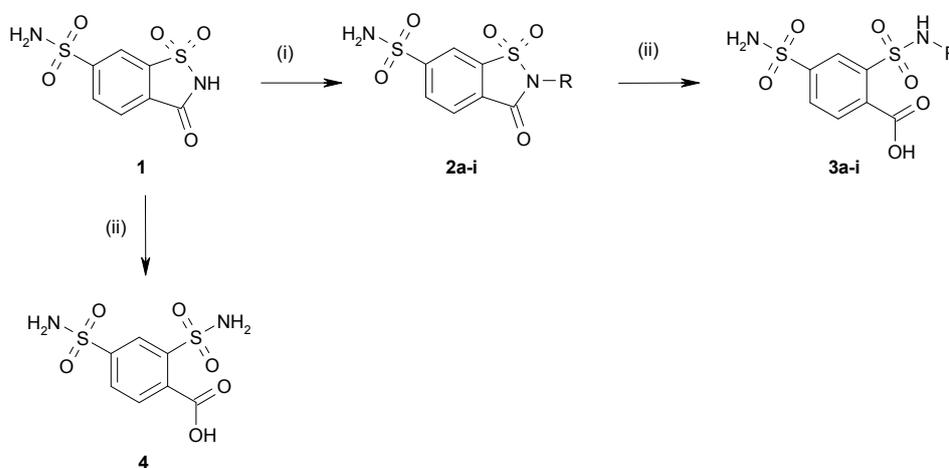
Inhibitors

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Zalubovskis, Claudiu T. Supuran and Kaspars Tars

A. Chemistry

Reagents and starting materials were obtained from commercial sources and used as received. Compounds **1** were synthesized according literature procedures.¹ The solvents were purified and dried by standard procedures prior to use. Melting points were determined on an OptiMelt automated melting point system. NMR spectra were recorded on Bruker (400 MHz) spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-*d*₆ signal (¹H 2.50; ¹³C 39.52)² as an internal standard.



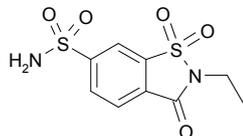
Scheme 1. (i) *R-Br*, K₂CO₃, DMF, H₂O, 90 °C (ii) NaOH, THF/MeOH/H₂O, RT.

General procedure for the synthesis of 2-alkyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxides (2-alkyl-6-sulfamoylsaccharines)

6-Sulfamoylsaccharin (0.50 g, 1.91 mmol) was suspended in H₂O (20 mL). K₂CO₃ (0.13 g, 0.95 mmol) was slowly added with stirring to the reaction mixture. Reaction mixture was evaporated and the residue was suspended in DMF (10 mL). The corresponding bromide (1.91 mmol) was added. The reaction mixture was stirred at 90 °C for 16 h. After cooling to room temperature CH₂Cl₂ (20 mL), water (20 mL), and 2 M HCl (20 mL) were added, and the product was then extracted with CH₂Cl₂

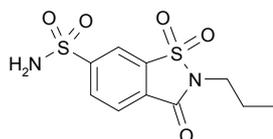
(3×20 mL), washed with brine (30 mL), and dried over Na₂SO₄. CH₂Cl₂ was evaporated leaving DMF solution. Addition of H₂O gave a precipitate which was collected by filtration and dried in vacuum.

2-Ethyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2a)



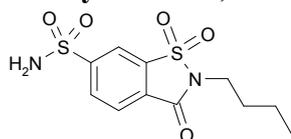
Obtained from 6-sulfamoylsaccharin (0.30 g, 1.14 mmol), K₂CO₃ (0.08 g, 0.57 mmol) and ethyl bromide (0.09 mL, 1.14 mmol) as white solid (0.21 g, 63%). **Mp**: 214-215 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 8.61 (dd, *J* = 0.6, 1.5 Hz, 1H), 8.35 (dd, *J* = 1.5, 8.0 Hz, 1H), 8.30 (dd, *J* = 0.6, 8.0 Hz, 1H), 7.83 (s, 2H), 3.80 (q, *J* = 7.2 Hz, 2H), 1.33 (t, *J* = 7.2 Hz, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 157.2, 150.3, 137.4, 132.2, 129.0, 126.5, 118.8, 34.3, 13.6 ppm. **HRMS** (ESI, *m/z*): calcd for C₉H₉N₂O₅S₂ [M-H]⁻ 288.9953, found 288.9954.

3-Oxo-2-propyl-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2b)



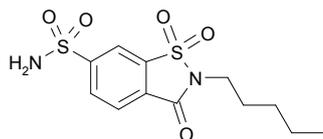
Obtained from 6-sulfamoylsaccharin (0.30 g, 1.14 mmol), K₂CO₃ (0.08 g, 0.57 mmol) and *n*-propyl bromide (0.10 mL, 1.14 mmol) as white solid (0.20 g, 57%). **Mp**: 174-175 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 8.62 (dd, *J* = 0.6, 1.5 Hz, 1H), 8.35 (dd, *J* = 1.5, 8.0 Hz, 1H), 8.30 (dd, *J* = 0.6, 8.0 Hz, 1H), 7.84 (s, 2H), 3.71 (t, *J* = 7.3 Hz, 2H), 1.76 (app sextet, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 157.6, 150.3, 137.2, 132.2, 128.9, 126.5, 118.8, 40.7, 21.2, 11.0 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₀H₁₁N₂O₅S₂ [M-H]⁻ 303.0109, found 303.0116.

2-Butyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2c)



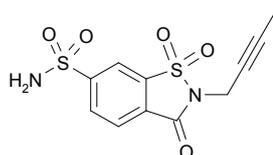
Obtained from 6-sulfamoylsaccharin (0.29 g, 1.11 mmol), K₂CO₃ (0.08 g, 0.55 mmol) and *n*-butyl bromide (0.12 mL, 1.11 mmol) as white solid (0.22 g, 63%). **Mp**: 159-160 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 8.61 (dd, *J* = 0.6, 1.5 Hz, 1H), 8.35 (dd, *J* = 1.5, 8.0 Hz, 1H), 8.30 (dd, *J* = 0.6, 8.0 Hz, 1H), 7.84 (s, 2H), 3.74 (t, *J* = 7.3 Hz, 2H), 1.77-1.68 (m, 2H), 1.43-1.33 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 157.5, 150.3, 137.3, 132.2, 128.9, 126.5, 118.8, 38.8, 29.8, 19.3, 13.4 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₁H₁₃N₂O₅S₂ [M-H]⁻ 317.0266, found 317.0273.

3-Oxo-2-pentyl-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2d)



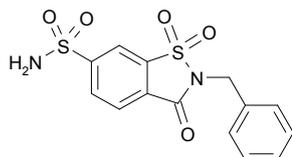
Obtained from 6-sulfamoylsaccharin **1** (0.50 g, 1.91 mmol), K_2CO_3 (0.13 g, 0.95 mmol) and *n*-pentyl bromide (0.24 mL, 1.91 mmol) as white solid (0.06 g, 10%). **Mp**: 126-128°C. **1H NMR** (400 MHz, $DMSO-d_6$): δ 8.61 (dd, $J = 0.8, 1.5$ Hz, 1H), 8.35 (dd, $J = 1.5, 8.1$ Hz, 1H), 8.29 (dd, $J = 0.8, 8.1$ Hz, 1H), 7.84 (s, 2H), 3.73 (t, $J = 7.3$ Hz, 2H), 1.68-1.80 (m, 2H), 1.29-1.36 (m, 4H), 0.87 (t, $J = 7.0$ Hz, 3H) ppm. **^{13}C NMR** (100 MHz, $DMSO-d_6$): δ 157.5, 150.3, 137.3, 132.2, 129.0, 126.6, 118.8, 39.1, 28.2, 27.4, 21.6, 13.8 ppm. Analytical data consistent with those previously reported.¹

2-(But-2-yn-1-yl)-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2e)



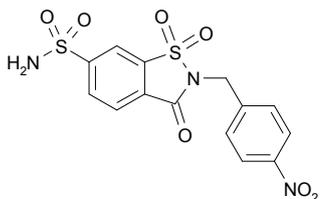
Obtained from 6-sulfamoylsaccharin **1** (0.50 g, 1.91 mmol), K_2CO_3 (0.13 g, 0.95 mmol) and but-2-ynyl bromide (0.17 mL, 1.91 mmol) as white solid (0.16 g, 27%). **Mp**: 229-231°C. **1H NMR** (400 MHz, $DMSO-d_6$): δ 8.63 (d, $J = 1.5$ Hz, 1H), 8.36 (dd, $J = 1.5, 8.1$ Hz, 1H), 8.32 (d, $J = 8.1$ Hz, 1H), 7.86 (s, 2H), 4.55 (q, $J = 2.3$ Hz, 2H), 1.80 (t, $J = 2.3$ Hz, 3H) ppm. **^{13}C NMR** (100 MHz, $DMSO-d_6$): δ 157.0, 150.6, 137.6, 132.4, 128.8, 126.9, 119.1, 80.9, 72.2, 28.5, 3.2 ppm. Analytical data consistent with those previously reported.¹

2-Benzyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2f)



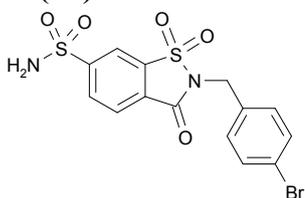
Obtained from 6-sulfamoylsaccharin **1** (0.29 g, 1.11 mmol), K_2CO_3 (0.08 g, 0.55 mmol) and benzyl bromide (0.13 mL, 1.11 mmol) as white solid (0.20 g, 51%). **Mp**: 223-225 °C. **1H NMR** (400 MHz, $DMSO-d_6$): δ 8.65 (d, $J = 1.4$ Hz, 1H), 8.37 (dd, $J = 1.4, 8.0$ Hz, 1H), 8.32 (d, $J = 8.0$ Hz, 1H), 7.85 (s, 2H), 7.29-7.47 (m, 5H), 4.95 (s, 2H) ppm. **^{13}C NMR** (100 MHz, $DMSO-d_6$): δ 157.6, 150.4, 137.3, 134.8, 132.2, 128.9, 128.5, 127.9, 127.8, 126.7, 118.9, 42.0 ppm. Analytical data consistent with those previously reported.¹

2-(4-Nitrobenzyl)-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2g)



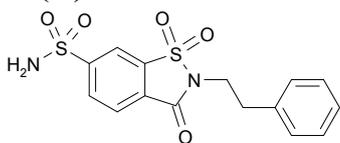
Obtained from 6-sulfamoylsaccharin (0.50 g, 1.91 mmol), K_2CO_3 (0.13 g, 0.95 mmol) and 4-nitrobenzyl bromide (0.41 g, 1.91 mmol) as white solid (0.60 g, 79%). **Mp**: 200-201 °C. **1H NMR** (400 MHz, $DMSO-d_6$): δ 8.67 (dd, $J = 0.5, 1.5$ Hz, 1H), 8.38 (dd, $J = 1.5, 8.0$ Hz, 1H), 8.33 (dd, $J = 0.5, 8.0$ Hz, 1H), 8.25-8.21 (m, 2H), 7.85 (s, 2H), 7.75-7.70 (m, 2H), 5.13 (s, 2H) ppm. **^{13}C NMR** (100 MHz, $DMSO-d_6$): δ 157.7, 150.5, 147.1, 142.6, 137.3, 132.3, 129.0, 128.9, 126.8, 123.6, 119.0, 41.2 ppm. **HRMS** (ESI, m/z): calcd for $C_{14}H_{10}N_3O_7S_2$ $[M-H]^-$ 395.9960, found 395.9967.

2-(4-Bromobenzyl)-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2h)



Obtained from 6-sulfamoylsaccharin (0.29 g, 1.11 mmol), K_2CO_3 (0.08 g, 0.55 mmol) and 4-bromobenzyl bromide (0.28 mg, 1.11 mmol) as white solid (0.41 g, 86%). **Mp**: 254-255 °C. **1H NMR** (400 MHz, $DMSO-d_6$): δ 8.65 (dd, $J = 0.5, 1.5$ Hz, 1H), 8.36 (dd, $J = 1.5, 8.0$ Hz, 1H), 8.31 (d, $J = 8.0$ Hz, 1H), 7.85 (s, 2H), 7.59-7.55 (m, 2H), 7.43-7.38 (m, 2H), 4.94 (s, 2H) ppm. **^{13}C NMR** (100 MHz, $DMSO-d_6$): δ 157.6, 150.4, 137.3, 134.4, 132.2, 131.4, 130.2, 128.9, 126.7, 121.1, 118.9, 41.3 ppm. **HRMS** (ESI, m/z): calcd for $C_{14}H_{10}BrN_2O_5S_2$ $[M-H]^-$ 428.9215, found 428.9219.

3-Oxo-2-(2-phenylethyl)-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (2i)



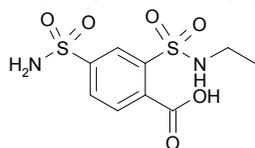
Obtained from 6-sulfamoylsaccharin **1** (0.50 g, 1.91 mmol), K_2CO_3 (0.13 g, 0.95 mmol) and phenylethyl bromide (0.26 mL, 1.91 mmol) as white solid (0.24 g, 34%). **Mp**: 189-191°C. **1H NMR** (400 MHz, $DMSO-d_6$): δ 8.63 (d, $J = 1.5$ Hz, 1H), 8.34 (dd, $J = 1.5, 8.1$ Hz, 1H), 8.27 (d, $J = 8.1$ Hz, 1H), 7.84 (s, 2H), 7.19-7.31 (m, 5H), 3.98 (t, $J = 7.5$ Hz, 2H), 3.06 (t, $J = 7.5$ Hz, 2H) ppm. **^{13}C NMR** (100 MHz, $DMSO-d_6$): δ 157.2, 150.3, 137.6, 137.2, 132.2, 128.8, 128.7, 128.5, 126.7, 126.6, 118.9, 40.2, 33.8 ppm. Analytical data consistent with those previously reported.¹

General procedure for the synthesis of 2-(alkylsulfamoyl)-4-sulfamoylbenzoic acids

The appropriate 2-alkyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide **2** (1 equiv) was dissolved in a mixture of THF/MeOH/ H_2O (2:1:2). NaOH (5 equiv) was added. The reaction mixture was stirred at room temperature for 16 h,

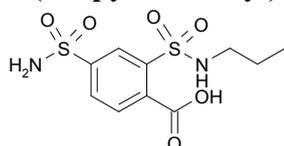
acidified with HCl (conc.) to pH 4, the solvent was evaporated. Re-crystallization from H₂O gave a precipitate which was collected by filtration and dried in vacuum.

2-(Ethylsulfamoyl)-4-sulfamoylbenzoic acid (3a)



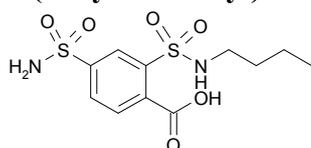
Obtained from 2-ethyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.15 g, 0.52 mmol) and NaOH (0.10 g, 2.59 mmol) in THF/MeOH/H₂O (2:1:2) (12 mL) as white solid (0.09 g, 57%). **Mp**: 198-199 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 13.93 (br s, 1H), 8.31 (d, *J* = 1.8 Hz, 1H), 8.08 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 2H), 7.45 (t, *J* = 5.8 Hz, 1H), 2.97-2.89 (m, 2H), 0.99 (t, *J* = 7.3 Hz, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.1, 145.7, 138.7, 136.2, 130.0, 129.5, 125.5, 37.7, 15.1 ppm. **HRMS** (ESI, *m/z*): calcd for C₉H₁₁N₂O₆S₂ [M-H]⁻ 307.0059, found 307.0063.

2-(Propylsulfamoyl)-4-sulfamoylbenzoic acid (3b)



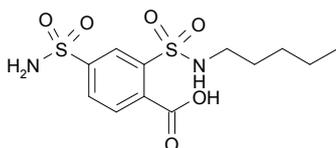
Obtained from 3-oxo-2-propyl-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.15 g, 0.49 mmol) and NaOH (0.10 g, 2.46 mmol) in THF/MeOH/H₂O (2:1:2) (12 mL) as white solid (0.03 g, 19 %). **Mp**: 169-170 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 13.94 (br s, 1H), 8.30 (dd, *J* = 0.4, 1.8 Hz, 1H), 8.08 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.87 (dd, *J* = 0.4, 8.0 Hz, 1H), 7.70 (s, 2H), 7.46 (t, *J* = 5.9 Hz, 1H), 2.89-2.82 (m, 2H), 1.39 (app sextet, *J* = 7.3 Hz, 2H), 0.78 (t, *J* = 7.3 Hz, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.1, 145.6, 138.7, 136.1, 129.9, 129.4, 125.4, 44.4, 22.5, 11.0 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₀H₁₃N₂O₆S₂ [M-H]⁻ 321.0215, found 321.0220.

2-(Butylsulfamoyl)-4-sulfamoylbenzoic acid (3c)



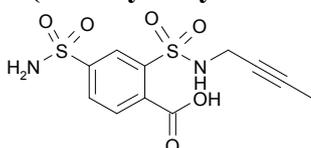
Obtained from 2-butyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.15 g, 0.47 mmol) and NaOH (0.04 g, 2.36 mmol) in THF/MeOH/H₂O (2:1:2) (12 mL) as white solid (0.10 g, 63%). **Mp**: 178-179 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 13.93 (br s, 1H), 8.30 (d, *J* = 1.8 Hz, 1H), 8.08 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 2H), 7.43 (t, *J* = 5.8 Hz, 1H), 2.92-2.85 (m, 2H), 1.41-1.32 (m, 2H), 1.27-1.17 (m, 2H), 0.79 (t, *J* = 7.3 Hz, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.1, 145.7, 138.7, 136.2, 129.9, 129.5, 125.5, 42.3, 31.2, 19.1, 13.4 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₁H₁₅N₂O₆S₂ [M-H]⁻ 335.0372, found 335.0377.

2-(Pentylsulfamoyl)-4-sulfamoylbenzoic acid (3d)



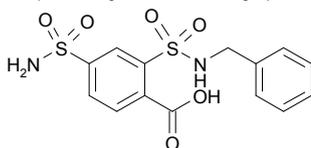
Obtained from 3-oxo-2-pentyl-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.20 g, 0.60 mmol) and NaOH (0.12 g, 3.01 mmol) in THF/MeOH/H₂O (2:1:2) (15 mL) as white solid (0.14 g, 66 %). **Mp**: 182-183 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 13.89 (br s, 1H), 8.31 (d, *J* = 1.8 Hz, 1H), 8.08 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.68 (s, 2H), 7.39 (t, *J* = 5.9 Hz, 1H), 2.93-2.85 (m, 2H), 1.43-1.33 (m, 2H), 1.23-1.12 (m, 4H), 0.82-0.76 (m, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.1, 145.7, 138.7, 136.1, 129.9, 129.4, 125.4, 42.6, 28.8, 28.1, 21.6, 13.8 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₂H₁₇N₂O₆S₂ [M-H]⁻ 349.0528, found 349.0534.

2-(But-2-yn-1-ylsulfamoyl)-4-sulfamoylbenzoic acid (3e)



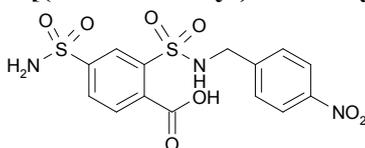
Obtained from 2-(but-2-yn-1-yl)-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.20 g, 0.64 mmol) and NaOH (0.13 g, 3.18 mmol) in THF/MeOH/H₂O (2:1:2) (15 mL) as light brown solid (0.11 g, 52%). **Mp**: 188-189 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 14.02 (br s, 1H), 8.35 (d, *J* = 1.8 Hz, 1H), 8.09 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 2H), 7.66 (t, *J* = 6.3 Hz, 1H), 3.82-3.76 (m, 2H), 1.46 (t, *J* = 2.4 Hz, 3H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.1, 145.7, 139.2, 135.7, 130.1, 129.6, 126.1, 80.6, 74.3, 32.6, 2.7 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₁H₁₁N₂O₆S₂ [M-H]⁻ 331.0059, found 331.0062.

2-(Benzylsulfamoyl)-4-sulfamoylbenzoic acid (3f)



Obtained from 2-benzyl-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.20 g, 0.57 mmol) and NaOH (0.11 g, 2.84 mmol) in THF/MeOH/H₂O (2:1:2) (15 mL) as white solid (0.19 g, 90%). **Mp**: 217-218 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 13.99 (br s, 1H), 8.28 (d, *J* = 1.8 Hz, 1H), 8.05 (dd, *J* = 1.8, 8.0 Hz, 1H), 8.02 (t, *J* = 6.2 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.68 (s, 2H), 7.29-7.17 (m, 5H), 4.14 (d, *J* = 6.2 Hz, 2H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.1, 145.6, 138.9, 137.5, 135.9, 129.9, 129.4, 128.2, 127.6, 127.2, 125.6, 46.1 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₄H₁₃N₂O₆S₂ [M-H]⁻ 369.0215, found 369.0217.

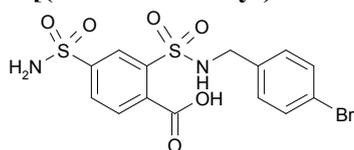
2-[(4-Nitrobenzyl)sulfamoyl]-4-sulfamoylbenzoic acid (3g)



Obtained from 2-(4-nitrobenzyl)-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.30 g, 0.76 mmol) and NaOH (0.15 g, 3.78 mmol) in

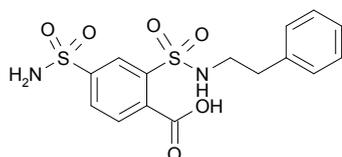
THF/MeOH/H₂O (2:1:2) (24 mL) as light yellow solid (0.19 g, 61%). **Mp**: 140-141 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 14.01 (br s, 1H), 8.27 (t, *J* = 6.3 Hz, 1H), 8.20 (d, *J* = 1.7 Hz, 1H), 8.13-8.08 (m, 2H), 8.03 (dd, *J* = 1.7, 8.0 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.66 (s, 2H), 7.53-7.48 (m, 2H), 4.31 (d, *J* = 6.3 Hz, 2H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.0, 146.6, 145.6, 145.5, 138.7, 135.8, 130.0, 129.5, 128.6, 125.6, 123.3, 45.4 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₄H₁₂N₃O₈S₂ [M-H]⁻ 414.0066, found 414.0070.

2-[(4-Bromobenzyl)sulfamoyl]-4-sulfamoylbenzoic acid (3h)



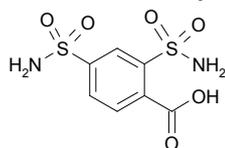
Obtained from 2-(4-bromobenzyl)-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.20 g, 0.46 mmol) and NaOH (0.09 g, 2.32 mmol) in THF/MeOH/H₂O (2:1:2) (15 mL) as white solid (0.14 g, 67%). **Mp**: 185-186 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 13.99 (br s, 1H), 8.29 (d, *J* = 1.8 Hz, 1H), 8.11 (t, *J* = 6.2 Hz, 1H), 8.06 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.69 (s, 2H), 7.48-7.43 (m, 2H), 7.23-7.19 (m, 2H), 4.12 (d, *J* = 6.2 Hz, 2H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.0, 145.7, 138.8, 137.2, 135.9, 131.1, 130.0, 129.8, 129.5, 125.6, 120.3, 45.3 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₄H₁₂BrN₂O₆S₂ [M-H]⁻ 446.9320, found 446.9325.

2-[(2-Phenylethyl)sulfamoyl]-4-sulfamoylbenzoic acid (3i)



Obtained from 3-oxo-2-(2-phenylethyl)-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.20 mg, 0.55 mmol) and NaOH (0.11 g, 2.73 mmol) in THF/MeOH/H₂O (2:1:2) (15 mL) as white solid (0.16 g, 76%). **Mp**: 205-206 °C. **¹H NMR** (400 MHz, DMSO-*d*₆): δ 13.95 (br s, 1H), 8.32 (d, *J* = 1.8 Hz, 1H), 8.08 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 2H), 7.53 (t, *J* = 5.9 Hz, 1H), 7.27-7.21 (m, 2H), 7.20-7.12 (m, 3H), 3.18-3.11 (m, 2H), 2.73-2.66 (m, 2H) ppm. **¹³C NMR** (100 MHz, DMSO-*d*₆): δ 168.1, 145.7, 138.6, 138.4, 136.0, 130.0, 129.5, 128.7, 128.4, 126.3, 125.5, 44.0, 35.5 ppm. **HRMS** (ESI, *m/z*): calcd for C₁₅H₁₅N₂O₆S₂ [M-H]⁻ 383.0372, found 383.0376.

2,4-Disulfamoylbenzoic acid (4)



3-Oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide (0.15 g, 0.57 mmol) was dissolved in a mixture of THF/MeOH/H₂O (2:1:2) (12 mL). NaOH (0.11 g, 2.86 mmol) was added. The reaction mixture was stirred at room temperature for 16 h, acidified with HCl (conc.) to pH 4, the solvent was evaporated. Recrystallization from H₂O gave white solid (0.05 g, 28 %). **Mp**: 281-282 °C. **¹H NMR**

(400 MHz, DMSO-*d*₆): δ 14.03 (br s, 1H), 8.38 (d, $J = 1.8$ Hz, 1H), 8.08 (dd, $J = 1.8, 8.0$ Hz, 1H), 7.92 (d, $J = 8.0$ Hz, 1H), 7.72 (s, 2H), 7.38 (s, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.1, 145.9, 141.9, 134.8, 130.4, 129.1, 124.7.

HRMS (ESI, *m/z*): calcd for C₇H₇N₂O₆S₂ [M-H]⁻ 278.9746, found 278.9752.

B. Protein x-ray crystallography

Materials and methods

Protein production and purification. hCA II was produced and purified essentially as described earlier by our groups.³

Crystallization and data collection.

Protein was concentrated to 10 mg/ml in 20 mM tris-HCl pH 8.0 using 10 kDa cutoff Amicon concentrator. Crystallization was done by hanging drop technique in EasyXtal 15-well Tool plates (Qiagen). 5 μ l of protein was mixed with 5 μ l of bottom solution and 0.2 μ l of 100 mM inhibitor in 100% DMSO. Bottom solution contained 1.54M Na citrate, 60 mM tris-HCl pH 9.0. Protein crystals were flash-frozen in liquid nitrogen without addition of extra cryoprotectant.

Data were collected at beamline I911-3, MAX-lab synchrotron, Lund, Sweden.

Structure determination.

Images were processed by MOSFLM⁴ and scaled by SCALA⁵. The structures were refined by REFMAC⁶ using hCA II structure in complex with sulfonamide class inhibitor (PDB code 4BF1)⁷ as an initial model. The parameter files for inhibitors **2i**, **2e** and **2d** were generated by LIBCHECK⁸. The ligand was fitted in electron density in COOT⁹, followed by further REFMAC runs. Data scaling, refinement, and validation statistics are listed in Table 1. Atomic coordinates and structure factors were deposited in PDB with accession code 5AMD, 5AML and 5AMG for complexes hCAII-**2i/3i**, hCAII-**2e/3e** and hCAII-**2d/3d**, respectively.

Table 1. Data processing, refinement and validation statistics for the hCA II-**2i/3i**, hCA II-**2e/3e** and hCAII-**2d/3d** complexes.

Structure	hCAII- 2i/3i	hCAII- 2e/3e	hCAII- 2d/3d
Space group	P2 ₁	P2 ₁	P2 ₁
Cell dimensions			
a (Å)	42.2	42.3	42.3
b (Å)	41.5	41.6	41.7
c (Å)	72.0	72.0	72.0
β (°)	104.5	104.5	104.6
Resolution (Å)	20-1.50	20-1.36	20-1.55
Highest resolution shell (Å)	1.50-1.58	1.36-1.43	1.55-1.63
Number of reflections	36798	52730	34263
Number of reflections in test set	1838	2685	1718
Completeness (%)	95.0 (93.3*)	99.8 (99.7*)	96.7 (95.0*)

R _{merge}	0.06 (0.43*)	0.05 (0.39*)	0.09 (0.53*)
<I/σI>	9.0 (2.1*)	10.0 (2.4*)	7.3 (2.0*)
Average multiplicity	2.1 (2.1*)	2.6 (2.3*)	2.1 (2.1*)
R-factor	0.14 (0.20*)	0.14 (0.19*)	0.14 (0.21*)
R _{free}	0.19 (0.26*)	0.18 (0.24*)	0.19 (0.25*)
Average B factor (Å ²)	11.1	12.7	11.2
Average B factor for inhibitor (Å ²)	15.7	9.7	12.9
 from Wilson plot (Å ²)	8.3	8.9	7.9
Number of protein atoms	2095	2088	2095
Number of inhibitor atoms	25	21	22
Number of solvent molecules	299	304	298
r.m.s. deviations from ideal values			
Bond lengths (Å)	0.010	0.011	0.012
Bond angles (°)	1.50	1.49	1.47
Outliers in Ramachandran plot (%)	0.41	0.41	0.41

*Values in parenthesis are for the high resolution bin

C. CA Inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO₂ hydration activity.¹⁰ Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na₂SO₄ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed 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 uncatalyzed 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 preincubated 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, as reported earlier,¹¹ and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.^{3,11,12}

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