Selenocarbamates As a Prodrug-Based Approach to Carbonic Anhydrase Inhibition

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1. Synthesis and characterisation of selenocarbamates 3

General procedure for the synthesis of selenocarbamates 3

Selenocarbamates were prepared following our recently developed procedure.[1] To a stirred solution of selenol 1 (1.0 mmol, 1.1 equiv.) in anhydrous acetonitrile (1 mL) at room temperature under a nitrogen atmosphere, isocyanate 2 (0.91 mmol, 1.0 equiv.) was added. After stirring for 10 minutes the solvent was removed under vacuum and the crude material purified by precipitation or subjected to flash column chromatography (petroleum ether/Ethyl acetate) to afford selenocarbamates 3.

Synthesis of Se-phenyl (3,5-dimethylphenyl)carbamoselenoate 3a

Following the General Procedure, benzeneselenol 1a (126 mg, 0.8 mmol) and 1-isocyanato-3,5-dimethylbenzene 2a (107 mg, 0.73 mmol) gave, after precipitation from Et2O/pentane, 3a as a white solid (201 mg, 91%). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 2.25 (6H, s), 6.75 (1H, s), 6.93 (2H, s), 7.01 (1H, bs, NH), 7.42-7.48 (3H, m), 7.73-7.75 (2H, m). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm): 21.9, 117.8 (bs), 127.2 (bs), 130.3, 130.5, 137.3, 137.8, 139.5. $^{77}$Se NMR (CDCl$_3$, 76 MHz) $\delta$ (ppm): 538.3.

Synthesis of Se-phenyl phenylcarbamoselenoate 3b

Following the General Procedure, benzeneselenol 1a (63 mg, 0.4 mmol) and isocyanatobenzene 2b (44 mg, 0.37 mmol) gave, after precipitation from Et$_2$O/pentane, 3b as a white solid (87 mg, 86%). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 7.16 (1H, ap t, $J$ = 6.8 Hz), 7.31-7.38 (5H, m), 7.46-7.53 (3H, m), 7.79-7.81 (2H, m). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm): 119.4 (bs), 124.7, 126.6, 129.0, 129.7, 129.8, 136.6, 137.3, 161.2 (bs).

Synthesis of Se-phenyl p-toly carbamoselenoate 3c

Following the General Procedure, benzeneselenol 1a (63 mg, 0.4 mmol) and 1-isocyanato-4-methylbenzene 2c (49 mg, 0.37 mmol) gave, after precipitation from Et$_2$O/pentane, 3c as a
white solid (96 mg, 91%). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 2.30 (3H, s), 7.08 (2H, ap d, $J = 8.2$ Hz), 7.14 (1H, bs, NH), 7.20 (2H, ap d, $J = 8.2$ Hz), 7.41-7.48 (3H, m), 7.75 (2H, ap d, $J = 6.6$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm): 20.8, 119.2 (bs), 126.7, 129.56, 129.61, 129.8, 134.8, 136.6.

**Synthesis of Se-phenyl (3-methoxyphenyl)carbamoselenoate 3d**

Following the General Procedure A, benzeneselenol 1a (94 mg, 0.6 mmol) and 1-isocyanato-3-methoxybenzene 2d (81 mg, 0.55 mmol) gave, after precipitation from Et$_2$O/pentane, 3d as a pale yellowish solid (120 mg, 71%). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 3.80 (3H, s), 6.71 (1H, dd, $J = 1.8, 8.1$ Hz), 6.81 (1H, d, $J = 8.1$ Hz), 7.12 (1H, t, $J = 1.8$ Hz), 7.21 (1H, t, $J = 8.1$ Hz), 7.32 (1H, bs, NH), 7.45-7.52 (3H, m), 7.77-7.79 (2H, m). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm): 55.3, 105.2 (bs), 110.6, 111.5 (bs), 129.8, 129.9, 136.7, 138.5, 160.2, 161.3 (bs). $^{77}$Se NMR (CDCl$_3$, 76 MHz) $\delta$ (ppm): 541.4.

**Synthesis of Se-phenyl (2-fluorophenyl)carbamoselenoate 3e**

Following the General Procedure A, benzeneselenol 1a (63 mg, 0.4 mmol) and 1-fluoro-2-isocyanatobenzene 2e (50 mg, 0.37 mmol) gave, after precipitation from Et$_2$O/pentane, 3e as a pale yellowish solid (76 mg, 71%). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 6.99-7.16 (4H, m), 7.36 (1H, bs, NH), 7.44-7.52 (3H, m), 7.77-7.78 (1H, m), 8.12 (1H, t, $J = 7.8$ Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm): 114.9 (d, $^{13}$C-F = 18.9 Hz), 121.1 (bs), 121.7 (bs), 124.6, 124.7, 124.8, 126.2, 130.0, 161.5 (bs). $^{77}$Se NMR (CDCl$_3$, 76 MHz) $\delta$ (ppm): 544.3.

**Synthesis of Se-phenyl (4-fluorophenyl)carbamoselenoate 3f**

Following the General Procedure A, benzeneselenol 1a (126 mg, 0.8 mmol) and 1-fluoro-4-isocyanatobenzene 2f (100 mg, 0.73 mmol) gave, after precipitation from Et$_2$O/pentane, 3f as a pale yellowish solid (165 mg, 77%). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 7.02 (2H, t, $J = 8.6$ Hz), 7.19 (1H, bs, NH), 7.30-7.34 (2H, m), 7.46-7.54 (3H, m), 7.78-7.80 (2H, m). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ (ppm): 116.4 (d,
\[ J_{C\, F} = 22.7 \text{ Hz}, \ 121.9 \text{ (bs), } 127.1, 130.4, 130.5, 134.0, 137.3, 160.3 \text{ (bd, } J_{C\, F} = 246.2 \text{ Hz).} \]

**77Se NMR (CDCl}_3, 76 MHz) \delta \text{ (ppm): 537.6.}**

**Synthesis of Se-phenyl (4-chlorophenyl)carbamoselenoate 3g**

Following the General Procedure A, benzeneselenol 1a (63 mg, 0.4 mmol) and 1-chloro-4-isocyanatobenzene 2g (56 mg, 0.37 mmol) gave, after precipitation from Et\textsubscript{2}O/pentane, 3g as a colourless solid (94 mg, 83%). \[^1\text{H NMR (CDCl}_3, 400 MHz) \delta \text{ (ppm): 7.21-7.26 (5H, m), 7.41-7.48 (3H, m), 7.72-7.74 (2H, m).} \]

**13C NMR (CDCl}_3, 100 MHz) \delta \text{ (ppm): 120.7 (bs), 126.3, 129.1, 129.8, 129.9, 135.9, 136.7, 161.6 (bs).}**

**Synthesis of Se-phenyl (4-bromophenyl)carbamoselenoate 3h**

Following the General Procedure A, benzeneselenol 1a (94 mg, 0.6 mmol) and 1-bromo-4-isocyanatobenzene 2h (109 mg, 0.55 mmol) gave, after precipitation from Et\textsubscript{2}O/pentane, 3h as a white solid (166 mg, 85%). \[^1\text{H NMR (CDCl}_3, 400 MHz) \delta \text{ (ppm): 7.21 (2H, d, J = 8.7 Hz), 7.37 (2H, d, J = 8.7 Hz), 7.41-7.48 (4H, m overlapped with bs of NH), 7.73 (2H, ap d, Js = 6.5 Hz).} \]

**13C NMR (CDCl}_3, 100 MHz) \delta \text{ (ppm): 118.0 (bs), 121.7 (bs), 127.0, 130.4, 130.5, 132.7, 137.1, 137.3, 162.4 (bs).}**

**Synthesis of Se-phenyl (furan-2-ylmethyl)carbamoselenoate 3i**

Following the General Procedure A, benzeneselenol 1a (86 mg, 0.5 mmol) and 2-(isocyanatomethyl)furan 2i (55 mg, 0.45 mmol) gave, after precipitation from Et\textsubscript{2}O/pentane, 3i as a brownish oil (98 mg, 81%). \[^1\text{H NMR (CDCl}_3, 400 MHz) \delta \text{ (ppm): 4.40 (2H, d, J = 5.5 Hz), 5.83 (1H, bs), 6. 18 (1H, d, J = 2.6 Hz), 6.29-6.30 (1H, m), 7.32 (1H, ap s), 7.33-7.41 (3H, m), 7.66-7.68 (2H, m).} \]

**13C NMR (CDCl}_3, 100 MHz) \delta \text{ (ppm): 38.3, 107.7, 110.4, 126.3, 129.1, 129.4, 129.6, 131.5, 136.5, 142.3, 150.3, 162.8.}**
Synthesis of Se-phenyl carbamoselenoate 3j

Following the General Procedure A, benzeneselenol 1a (63 mg, 0.4 mmol) and isocyanatotrimethylsilane 2j (42 mg, 0.37 mmol) gave, after precipitation from Et₂O/hexane, 3j as a white solid (59 mg, 78%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 5.70 (2H, bs, NH₂), 7.39-7.44 (3H, m), 7.69-7.62 (2H, m). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 126.7 (C), 129.7 (CH), 129.8 (CH), 136.6 (CH), 165.7 (C).

Synthesis of Se-((p-tolyl) (4-bromophenyl)carbamoselenoate 3k

Following the General Procedure A, 4-methylbenzeneselenol 1b (86 mg, 0.5 mmol) and 1-bromo-4-isocyanatobenzene 2h (90 mg, 0.45 mmol) gave, after precipitation from Et₂O/pentane, 3k as a yellowish solid (127 mg, 77%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 2.41 (3H, s), 7.08 (1H, bs, NH), 7.19 (2H, d, J = 8.1 Hz), 7.24-7.26 (2H, m), 7.38 (2H, d, J = 8.1 Hz), 7.62 (2H, d, J = 7.7 Hz). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 21.4, 120.8 (bs), 122.8, 130.9, 132.0, 136.4, 136.7, 140.4.

Synthesis of Se-((o-tolyl) (4-bromophenyl)carbamoselenoate 3l

Following the General Procedure A, 2-methylbenzeneselenol 1c (43 mg, 0.25 mmol) and 1-bromo-4-isocyanatobenzene 2h (45 mg, 0.23 mmol) gave, after precipitation from Et₂O/pentane, 3l as a yellowish solid (59 mg, 72%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 2.55 (3H, s), 7.03 (1H, bs, NH), 7.15-7.19 (2H, m), 7.23-7.27 (1H, m), 7.36-7.39 (2H, m), 7.40-7.41 (2H, m), 7.77 (1H, ap d, J = 7.5 Hz). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 23.4, 120.8, 127.4, 127.6, 130.8, 131.0, 132.1, 136.4, 138.3, 142.9.

Synthesis of Se-dodecyl (4-bromophenyl)carbamoselenoate 3m

Following the General Procedure A, dodecane-1-selenol 1d (63 mg, 0.25 mmol) and 1-bromo-4-isocyanatobenzene 2h (45 mg, 0.23 mmol) gave, after precipitation from Et₂O/cyclohexane, 3m as a white solid (78 mg, 77%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 0.86 (3H, t, J
= 6.7 Hz), 1.23-1.31 (16H, m), 1.69-1.76 (2H, m), 2.99 (2H, t, \( J = 7.3 \) Hz, CH₂Se), 7.24 (1H, bs, NH), 7.29 (2H, ap d, \( J = 8.6 \) Hz), 7.39 (2H, ap d, \( J = 8.6 \) Hz). \(^{13}\)C NMR (CDCl₃, 100 MHz) \( \delta \) (ppm): 14.1, 22.7, 27.7, 29.1, 29.3, 29.5, 29.57, 29.61, 29.63, 29.9, 30.9, 31.9, 120.3, 121.2, 132.1, 136.7, 162.0.

Synthesis of Se-dodecyl carbamoselenoate 3n

Following the General Procedure A, dodecane-1-selenol \( 1d \) (63 mg, 0.25 mmol) and isocyanatotrimethylsilane \( 2j \) (26 mg, 0.23 mmol) gave, after precipitation from Et₂O/hexane, \( 3n \) as a white solid (58 mg, 88%). \(^1\)H NMR (CDCl₃, 400 MHz) \( \delta \) (ppm): 0.88 (3H, t, \( J = 6.8 \) Hz), 1.21-1.33 (18H, m), 1.66-1.75 (2H, m), 2.95 (2H, t, \( J = 7.3 \) Hz, CH₂Se), 5.56 (2H, bs, NH₂). \(^{13}\)C NMR (CDCl₃, 100 MHz) \( \delta \) (ppm): 14.1, 22.7, 27.3, 29.1, 29.4, 29.5, 29.6, 29.7, 29.9, 30.9, 31.9, 165.2.

Synthesis of Se-benzyl (3,5-dimethylphenyl)carbamoselenoate 3o

Following the General Procedure A, phenylmethaneselenol \( 1e \) (103 mg, 0.6 mmol) and 1-isocyanato-3,5-dimethylbenzene \( 2a \) (81 mg, 0.55 mmol) gave, after precipitation from Et₂O/cyclohexane, \( 3o \) as a pale yellowish solid (118 mg, 67%). \(^1\)H NMR (CDCl₃, 400 MHz) \( \delta \) (ppm): 2.29 (6H, s), 4.26 (2H, s, CH₂Se), 6.79, (1H, s), 6.97 (1H, s), 7.03 (2H, s), 7.20-7.23 (1H, m), 7.27-7.31 (2H, m), 7.35-7.37 (2H, m). \(^{13}\)C NMR (CDCl₃, 100 MHz) \( \delta \) (ppm): 21.9, 31.2, 127.7, 129.3, 129.6, 139.6, 139.8, 153.0, 160.4 (C=O).

Synthesis of Se-benzyl (4-bromophenyl)carbamoselenoate 3p

Following the General Procedure A, phenylmethaneselenol \( 1e \) (52 mg, 0.3 mmol) and 1-bromo-4-isocyanatobenzene \( 2h \) (55 mg, 0.28 mmol) gave, after precipitation from Et₂O/cyclohexane, \( 3p \) as a pale yellowish solid (65 mg, 75%). \(^1\)H NMR (CDCl₃, 400 MHz) \( \delta \) (ppm): 4.27 (2H, s, CH₂Se), 7.13 (1H, bs, NH), 7.21-7.36 (7H, m), 7.42-7.44 (2H, m). \(^{13}\)C NMR (CDCl₃, 100 MHz) \( \delta \) (ppm): 31.4, 121.9 (bs), 127.8, 129.3, 129.6, 132.8, 137.2 (bs), 139.5, 162.4 (bs). MS (ESI): 391.8 [M+Na]⁺.
Synthesis of Se-(2-(allyloxy)-2-hydroxyethyl) (3,5-dimethylphenyl)carbamoselenoate 3q

According to the General Procedure A, the reaction was carried out with 1-(allyloxy)-3-hydroselenopropan-2-ol 1f (69 mg, 0.35 mmol) and 1-isocyanato-3,5-dimethylbenzene 2a (47 mg, 0.32 mmol). Purification by flash column chromatography (petroleum ether/EtOAc 4:1) gave 3q as a colourless oil (78 mg, 72%). 1H NMR (CDCl3, 400 MHz) δ (ppm): 2.29 (6H, s), 2.91 (1H, bs, OH), 3.11 (1H, dd, J = 6.9, 13.4 Hz, CH₃H₂Se), 3.24 (1H, dd, J = 4.1, 13.4 Hz, CH₃H₂Se), 3.49 (1H, dd, J = 6.7, 9.6 Hz, CH₃H₂O), 3.55 (1H, dd, J = 4.4, 9.6 Hz, CH₃H₂O), 4.03-4.04 (2H, m, OCH₃CH=CH₂), 4.06-4.13 (1H, m, CH=CH₂), 5.19-5.30 (2H, m, CH=CH₂), 5.86-5.95 (1H, m, CH=CH₂), 6.79 (1H, s), 7.03 (2H, s), 7.38 (1H, bs, NH). 13C NMR (CDCl3, 100 MHz) δ (ppm): 21.3, 30.5, 70.3, 72.3, 73.2, 117.4, 126.5, 134.3, 138.9.

Synthesis of Se-(2-hydroxy-3-isopropoxypropyl) (adamantan-1-yl)carbamoselenoate 3r

According to the General Procedure A, the reaction was carried out with 1-hydroseleno-3-isopropoxypropan-2-ol 1g (50 mg, 0.25 mmol) and 1-adamantyl isocyanate 2k (40 mg, 0.23 mmol). Purification by flash column chromatography (petroleum ether/EtOAc 3:1) gave 3r as a colourless oil (51 mg, 61%). 1H NMR (CDCl3, 400 MHz) δ (ppm): 1.14 (3H, d, J = 6.1 Hz), 1.15 (3H, d, J = 6.1 Hz), 1.65-1.66 (6H, m), 1.96-1.97 (6H, m), 2.05-2.10 (3H, m), 2.60 (1H, bs, OH), 2.99 (1H, dd, J = 6.9, 13.3 Hz, CH₃H₂Se), 3.11 (1H, dd, J = 4.2, 13.3 Hz, CH₃H₂Se), 3.40 (1H, dd, J = 6.4, 9.4 Hz, CH₃H₂O), 3.47 (1H, dd, J = 4.8, 9.4, CH₃H₂O), 3.54-3.64 (1H, m, CH(CH₃)₂), 3.92-3.98 (1H, m, CHOH), 5.34 (1H, bs, NH). 13C NMR (CDCl3, 100 MHz) δ (ppm): 22.1, 29.4, 30.5, 36.1, 41.8, 54.9, 70.4, 71.2, 72.2, 160.7. MS (ESI): 376.3 [M+H]+.

Synthesis of Se-(2-hydroxycyclohexyl) (3,5-dimethylphenyl)carbamoselenoate 3s

According to the General Procedure A, the reaction was carried out with 2-hydroselenocyclohexan-1-ol 1h (180 mg, 1 mmol) and 1-isocyanato-3,5-dimethylbenzene 2a (134 mg, 0.91 mmol). Purification by flash column chromatography (petroleum ether/EtOAc 4:1) gave 3s as a colourless oil (232 mg, 78%). 1H NMR (CDCl3, 400 MHz) δ (ppm): 1.24-1.41 (4H, m), 1.54-1.64 (1H, m), 1.65-1.79 (2H, m), 2.11-2.22 (1H, m), 2.27 (6H, s), 3.00 (1H, bs, OH), 3.38 (1H, ddd, J = 4.0, 10.1, 12.4 Hz, CHSe), 3.60 (1H,
td, \( J = 4.1, 10.1 \) Hz), 6.76 (1H, s), 7.03 (2H, s), 7.86 (1H, bs, NH). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \) (ppm): 21.3, 24.3, 26.8, 32.8, 35.7, 51.9, 74.7, 117.4, 126.5, 138.9.

**Synthesis of Se-2-hydroxycyclohexyl (furan-2-ylmethyl)carbamoselenoate 3t**

![Formula 3t]

According to the General Procedure A, the reaction was carried out with 2-hydroxyselenocyclohexan-1-ol \( \text{1h} \) (180 mg, 1 mmol) and 2-(isocyanatomethyl)furan \( \text{2i} \) (112 mg, 0.91 mmol). Purification by flash column chromatography (petroleum ether/EtOAc 3:1) gave \( \text{3t} \) as a brownish oil (203 mg, 74%). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 1.31-1.39 (3H, m), 1.51-1.60 (1H, m), 1.65-1.66 (1H, m), 1.76-1.77 (1H, m), 2.11-2.14 (1H, m), 2.19-2.26 (1H, m), 2.85 (1H, bs), 3.30-3.41 (1H, m), 3.50-3.55 (1H, m), 4.40 (1H, dd, \( J = 5.3, 15.4 \) Hz), 4.46 (1H, dd, \( J = 5.4, 15.4 \) Hz), 6.23-6.24 (1H, m), 6.30-6.31 (2H, m overlapped with bs), 7.34 (1H, ap s). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \( \delta \) (ppm): 24.3, 26.8, 32.8, 35.6, 38.4, 51.6, 74.6, 108.0, 110.5, 142.5, 150.4, 164.1.

**Synthesis of (S)-Se-(2-((4-methylphenyl)sulfonamido)propyl) (3,5-dimethylphenyl)carbamoselenoate 3u**

![Formula 3u]

According to the General Procedure A, the reaction was carried out with (S)-\( \text{N}-(1\)-hydroselenopropan-2-yl)-4-methylbenzenesulfonamide \( \text{1i} \) (59 mg, 0.2 mmol) and 1-isocyanato-3,5-dimethylbenzene \( \text{2a} \) (27 mg, 0.18 mmol). Purification by flash column chromatography (petroleum ether/EtOAc 3:1) gave \( \text{3u} \) as a white powder (54 mg, 68%). \(^1\)H NMR (Acetone-\( d_6\), 400 MHz) \( \delta \) (ppm): 1.16 (3H, d, \( J = 6.5 \) Hz), 2.26 (6H, s), 2.37 (3H, s), 3.00-3.05 (2H, m, CH\(_2\)Se), 3.49-3.58 (1H, m, CHN), 6.45 (1H, d, \( J = 6.9 \) Hz, NHTs), 6.76 (1H, s), 7.18 (2H, s), 7.35 (2H, ap d, \( J = 8.5 \) Hz), 7.76 (2H, ap d, \( J = 8.5 \) Hz), 9.22 (1H, s, NHC(O)). \(^{13}\)C NMR (Acetone-\( d_6\), 100 MHz) \( \delta \) (ppm): 20.4, 20.5, 20.7, 32.5, 50.4, 116.8, 125.4, 126.9, 129.4, 138.4, 138.7, 139.0, 142.8, 160.4 (C=O).
2. **$^1$H-NMR spectra of control experiments for compounds 3a and 3o**

$^1$H NMR Spectrum of compound 3a (CDCl$_3$, 400 MHz)

$^1$H NMR spectrum of the raw material recovered after stirring the selenocarbamate 3a in the buffer solution and under conditions used for the kinetic assays.

$^1$H NMR spectrum of the raw material recovered after stirring the selenocarbamate 3a in the buffer solution and under conditions used for the kinetic assays.
\(^1\)H NMR Spectrum of compound 3o (CDCl\(_3\), 400 MHz)

\(^1\)H NMR spectrum of the raw material recovered after stirring the selenocarbamate 3o in the buffer solution and under conditions used for the kinetic assays.
3. Structure determination

The crystal structure of hCA II (PDB accession code: 4FIK) without solvent molecules and other heteroatoms was used to obtain initial phases using Refmac5.\(^2\) 5% of the unique reflections were selected randomly and excluded from the refinement data set for the purpose of \(R_{free}\) calculations. The initial \(|Fo - Fc|\) difference electron density maps unambiguously showed the inhibitor molecules. The inhibitor was introduced in the model with 1.0 occupancy. Refinements proceeded using normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT.\(^3\) The quality of the final models were assessed with COOT and RAMPAGE.\(^4\) Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 7QBH). Graphical representations were generated with Chimera.\(^5\)
4. **Summary of Data Collection and Atomic Model Refinement Statistics for hCA II**

|                              | hCAII + 3o |
|------------------------------|------------|
| PDB ID                       | 7QBH       |
| Wavelength (Å)               | 0.971700   |
| Space Group                  | P21        |
| Unit cell (a, b, c, α, β, γ) (Å,°) | 42.38, 41.48, 72.33, 90.00, 104.53, 90.00 |
| Limiting resolution (Å)      | 41.47-1.22 (1.25-1.22) |
| Unique reflections           | 58985 (1034) |
| Rmerge (%)                   | 6.3 (28.1)  |
| Rmeas (%)                    | 6.9 (38.3)  |
| Redundancy                   | 5.47 (2.05) |
| Completeness overall (%)     | 81.9 (19.6) |
| <I/σ(I)>                     | 15.65 (2.36) |
| CC (1/2)                     | 99.8 (82.6) |

**Refinement statistics**

|                              |          |
|------------------------------|----------|
| Resolution range (Å)         | 41.47-1.22 |
| Rfactor (%)                  | 15.23    |
| Rfree(%)                     | 17.70    |
| r.m.s.d. bonds(Å)            | 0.0145   |
| r.m.s.d. angles (°)          | 1.9515   |

**Ramachandran statistics (%)**

|                              |          |
|------------------------------|----------|
| Most favored                 | 96.9     |
| additionally allowed         | 3.1      |
| outlier regions              | 0.0      |

**Average B factor (Å²)**

|                              |          |
|------------------------------|----------|
| All atoms                    | 13.754   |
| inhibitors                   | 22.519   |
| solvent                      | 22.646   |
5. Crystallographic figures

**Figure S1.** Electron density of inhibitors 3o bound to zinc (grey) in hCA II active site. 2F$_o$-F$_c$ maps and contoured to the 1.0 $\sigma$ level.

**Figure S2.** Compound 3o inside the active site of hCA II. Hydrophobic (red) and hydrophilic (blue) residues are labeled.

**Figure S3.** Overlay of compounds 3o and 5 with hCA II
6. References

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