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Supplementary Information

Benzoxaborole as a New Chemotype for Carbonic Anhydrase Inhibition

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Experimental section

Chemistry

General Methods: The thin layer chromatographies (TLC) were performed on silica plates Merck 60 F354 aluminum. Reactions were monitored by TLC by using alumina plates coated with silica gel and visualized either by using UV light or by charring with ninhydrin solution. Column chromatography was performed on silica gel 60 Å, particle size: 35-70 mesh. ^{1}H and ^{13}C NMR spectra were recorded on Brucker-400 (400 MHz for 1H and 101 MHz for ^{13}C) instrument using the residual solvent signals as an internal reference (d6-DMSO). High-resolution mass spectra (HMRS) were obtained from an ESI-MS spectrometer (SYNAPT G- of Waters).

Synthesis of 6-nitrobenzo[c][1,2]oxaborol-1(3*H*)-ol (2): 1 g (7.4 mmol, 1 equiv.) of commercially available benzoxaborole was added with stirring to 6.4 mL of fuming nitric acid (18.3 equiv.) cooled at -45/-40 °C. The addition was done portionwise and was complete in about 5 min. The mixture was stirred and maintained at -45 to -30 °C, and the progress of the reaction was monitored by TLC (ethyl acetate:petroleum ether = 7:3). After 20 min. the mixture was poured into water and ice and kept at 0-10 °C for 2 hours. The obtained white precipitate was then filtered *in vacuo*, washed with water and lyophilized to afford the compound as a white solid. Yield: 80 %; M.p. 178-180 °C; R*f*: 0.32 (ethyl acetate:petroleum ether = 7:3); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.59 b(s, 1H, OH), 8.58 (ss, 1H, ArH), 8.33 (d, J = 1.7 Hz, 1H, ArH), 8.33 (dd, J^1 = 8.3 Hz, J^2 = 2.2 Hz, 1H, ArH), 7.69 (d, J = 8.5 Hz, 1H, ArH), 5.12 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.6, 147.2, 140.7, 125.6, 123.1, 70.1 (s, CH_2). MS: ESI: *m/z* 178.0 $[M]^+$.

Synthesis 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol (3): AcOH glacial (1.2 mL) was added to a solution of 6-nitrobenzo[c][1,2]oxaborol-1(3H)-ol **2** (2.8 mmol, 1 equiv.) in THF (12 mL). The mixture was vacuum/N_2 purged three times and 10% Pd/C (82 mg) was added. The mixture was again vacuum/N_2 purged three times then vacuum purged again. H_2 was then introduced from a balloon and the reaction was stirred at room temperature for 2 hours. The reaction solution was filtered through a short pad of celite, washed with methanol, and the filtrate was evaporated to afford the product as a foamy orange solid. Yield: 95 %. R*f*: 0.46 (ethyl acetate). ^{1}H NMR 400 MHz (DMSO-d_6): δ 9.05 (bs, 2H, NH_2), 7.03 (d, J = 7.9 Hz, 1H, ArH), 6.89 (s, 1H, ArH), 6.71 (dd, J^1= 7.7 Hz, J^2= 2.0 Hz, 1H, ArH), 4.90 (s, 1H, OH), 4.81 (s, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6): δ 172.1, 147.6, 141.5, 121.5, 117.6, 114.6, 69.7 (s, CH_2). MS: ESI: *m/z* 148.0 $[M]^+$.

General procedure for the synthesis of *N,N'*-disubstituted ureas (4a-f): 6-amino benzoxaborole **3** (6-aminobenzo[c][1,2]oxaborol-1(3H)-ol) (0.67 mmol, 1 equiv.) was dissolved in acetone (1.5 mL) and the solution was stirred at room temperature. Then the opportune isocyanate (0.67 mmol, 1 equiv.) was added dropwise (or in portion) and the solution became pasty. When **3** was consumed,

petroleum ether was added to the mixture, and the precipitate was filtered. The solid was washed with methanol to afford the desired product as a solid.

1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-phenylurea (4a): Pale yellow solid. Yield: 42 %. R*f*: 0.70 (ethyl acetate : petroleum ether = 9:1). M.p. 260 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6): δ 9.18 (s, 1H, OH), 8.66 (s, 1H, NH), 8.64 (s, 1H, NH), 7.83 (d, J = 1.8 Hz, 1H, Ar), 7.53 (dd, J^1 =8.2 Hz, J^2 = 2.1 Hz, 1H, Ar), 7.46 (overlapping, 2H, Ar), 7.29 (overlapping, 3H, Ar), 6.97 (t, J = 7.3 Hz,1H, Ar), 4.92 (s, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6):δ 140.1(s, CO), 128.4, 121.4, 121.2, 121.1, 119.5, 117.7, 69.3 (s, CH_2). MS: ESI: *m/z* 269.1 $[M+H]^+$.

1-(4-fluorophenyl)-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)urea (4b): Pale yellow solid. Yield: 42 %. R*f*: 0.48 (ethyl acetate). M.p. 260 °C. ^{1}H NMR 400 MHz (DMSO-d_6):δ 9.17 (bs, 1H, OH), 8.68 (s,1H, NH), 8.67 (s, 1H, NH), 7.82 (d, J = 1.8 Hz, 1H, Ar), 7.53 (dd, J^1 = 8.2 Hz, J^2 = 2.0 Hz, 1H, Ar), 7.48-7.44 (overlapping, 2H, Ar), 7.32 (d, J = 8.2 Hz, 1H, Ar), 7.14-7.09 (overlapping, 2H, Ar), 4.93 (s, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6): δ 140.5 (s, CO), 121.2, 119.6, 119.5, 119.4, 115.0, 114.8, 69.3 (s, CH_2). MS: ESI: *m/z* 287.1 $[M+H]^+$.

1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(4-(trifluoromethyl)phenyl)urea (4c): Pale yellow solid. Yield: 20 %. R*f*: 0.63 (ethyl acetate). M.p. 269-270 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6):δ 9.19 (s, 1H, OH), 9.08 (s, 1H, NH), 8.82 (s, 1H, NH), 7.84 (d, J = 0.9 Hz, 1H, ArH), 7.65-7.64 (overlapping, 3H, Ar), 7.53 (dd, J^1 = 8.4 Hz, J^2 = 1.9 Hz, 1H, Ar), 7.33 (d, J = 8.2 Hz , 1H, Ar), 4.93 (s, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6):δ 140.3 (s, CO), 125.7, 125.7, 121.4, 121.3, 119.8, 117.4, 69.3 (s, CH_2). MS: ESI: *m/z* 337.1 $[M+H]^+$.

1-benzyl-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)urea (4d): White solid. Yield: 41 %. R*f*: 0.48 (ethyl acetate). M.p. 237-239 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6): δ 9.13 (s, 1H, OH), 8.56 (s, 1H, NH), 7.78 (d, J = 1.7 Hz, 1H, NH), 7.49 (dd, J^1 = 2.1 Hz, J^2= 8.2 Hz , 1H, ArH), 7.35-7.30 (overlapping, 4H, ArH), 7.26-7.24 (overlapping, 2H, ArH), 6.60 (t, J = 5.9 Hz, 1H, ArH), 4.90 (s, 2H, CH_2), 4.31 (d, J = 5.9 Hz, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6):δ 140.0 (s, CO), 127.9, 126.8, 126.3, 121.0, 120.7, 119.0, 69.2 (s, CH_2), 42.4 (s, CH_2). MS: ESI: *m/z* 283.1 $[M+H]^+$.

1-(furan-2-ylmethyl)-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)urea (4e): Pale brown solid. Yield: 28 %. R*f*: 0.69 (ethyl acetate). M.p. 270 °C. ^{1}H NMR 400 MHz (DMSO-d_6): δ 9.12 (s, 1H, OH), 8.50 (s, 1H, NH), 7.74 (s, 1H, NH), 7.58 (s, 1H, ArH), 7.48 (d, J = 8.3 Hz, 1H, ArH), 7.26 (d, J = 8.1 Hz, 1H,ArH), 6.51 (m, 1H, ArH), 6.39 (s, 1H, ArH), 6.26 (d, J =2.3 Hz, 1H, ArH), 4.89 (s, 2H, CH_2), 4.29 (d, J = 5.3 Hz, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6): δ 141.6 (s,CO), 121.0, 120.7, 119.0, 110.1, 106.1, 69.2 (s, CH_2), 35.7 (s, CH_2). MS: ESI: *m/z* 273.1 $[M+H]^+$, 295.1 $[M+Na]^+$.

1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(2-methoxy-5-methylphenyl)urea (4f): Pale yellow solid. Yield: 23 %. R*f*: 0.85 (ethyl acetate). M.p. 263 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6): δ 9.31 (s, 1H, OH), 9.16 (s, 1H, NH), 8.16 (s, 1H, NH), 8.00 (s, 1H, Ar), 7.86 (s, 1H, Ar), 7.51 (d, *J* = 8.0 Hz, 1H, ArH), 7.31 (d, *J* = 8.0 Hz, 1H, ArH), 6.88(d, *J* = 8.1 Hz, 1H, ArH), 6.73 (d, *J* = 7.4 Hz, 1H, ArH), 4.92 (s, 2H, CH_2), 3.83 (s, 3H, CH_3), 2.22 (s, 3H, CH_3). ^{13}C NMR 101 MHz (DMSO-d6): δ (s, CO), 121.4, 121.2, 120.8, 119.2, 118.6, 110.2, 69.3(s, CH_2), 55.5(s, CH_3), 20.4 (s, CH_3). MS: ESI: *m/z* 313.1 $[M+H]^+$, 335.1 $[M+Na]^+$

General procedure for the synthesis of *N,N'*-disubstituted thioureas 5a-e: 6-amino benzoxaborole **3** (0.67 mmol, 1 equiv.) was dissolved in acetone (1.5 mL) and the solution was stirred at room temperature. Then the opportune isothiocyanate (0.80 mmol, 1.2 equiv.) was added dropwise (or in portion). The solution was refluxed for about 2-6 hours and the progress of reaction was monitored by TLC (ethyl acetate). Then the solvent was evaporated under reduced pressure, and the crude solid was washed with petroleum ether and n-pentane to afford the desired product as a pale yellow solid.

1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-phenylthiourea (5a): Pale yellow solid. Yield: 45 %. R*f*: 0.33 (dichloromethane : methanol = 94:6). M.p. 164-165 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6):δ 9.83 (s, 1H, OH), 9.72 (s, 1H, NH), 9.23 (s, 1H, NH), 7.74 (d, *J* = 1.6, 1H, ArH), 7.51-7.45 (overlapping, 4H, ArH), 7.38 (d, *J* = 8.2, 1H, ArH),), 7.19-7.14 (overlapping, 2H, ArH), 4.96 (s, 2H, CH_2).^{13}C NMR 101 MHz (DMSO-d_6):δ 184.2, 140.7, 127.0, 125.9, 125.9, 125.7, 121.1, 114.8, 114.6, 99.2, 69.4. MS: ESI: *m/z* 286.1 $[M+H]^+$

1-(4-fluorophenyl)-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl) thiourea (5b): Pale yellow solid. Yield: 25 %. R*f*: 0.44 (dichloromethane : methanol = 94:6). M.p. 172 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6): δ 9.81 (s, 1H, OH), 9.70 (s, 1H, NH), 9.2 (s, 1H, NH), 7.74 (d, *J* = 1.6 Hz, 1H, ArH), 7.47 (overlapping, 3H, ArH), 7.38 (d, *J* = 8.1 Hz, 1H, ArH), 7.16 (t, *J* = 8.8 Hz, 2H, ArH), 4.96 (s, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6):δ 184.0 (s, CS),140.7, 127.0, 125.9, 125.9, 125.7, 121.4, 114.8, 114.6, 99.2, 69.4(s, CH_2). MS: ESI: *m/z* 303.1 $[M+H]^+$.

1-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-3-(4-trifluoromethyl) phenyl)thiourea (5c): White solid. Yield: 40 %. R*f*: 0.46 (dichloromethane : methanol = 94:6). M.p. 179 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6): δ 10.09 (s, 1H, OH), 10.08 (s, 1H, NH), 9.24 (s, 1H, NH), 7.76 (d, *J* = 7.4 Hz, 3H, ArH), 7.68 (d, *J* = 8.1 Hz, 2H, ArH), 7.52 (dd, J^1 = 7.8 Hz, J^2 = 1.3 Hz, 1H, ArH), 7.39 (d, *J* = 8.1 Hz, 1H, ArH), 4.97 (s, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6): δ 183.3, 140.7, 126.9, 125.9, 125.8, 125.7, 121.1, 114.7, 114.5, 99.2, 69.4. MS: ESI: *m/z* 353.1 $[M+H]^{+.}$

1-benzyl-l-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)thiourea (5d): White solid. Yield: 40 %. R*f*: 0.47 (dichloromethane : methanol = 94:6). M.p. 172 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6):δ 9.61 (s, 1H, OH), 9.22 (s, 1H, NH), 8.11 (d, *J* = 4.9 Hz, 1H, NH), 7.71 (s, 1H, ArH), 7.48 (d, *J* = 8.0, 1H, ArH), 7.38-7.34 (overlapping, 5H, ArH), 7.25 (m, 1H, ArH), 4.97 (s, 2H, CH_2)., 4.74 (d, *J* = 4.8 Hz, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6):δ 181.2 (s, CS), 150.2, 139.1, 137.9, 128.2, 127.4, 126.8, 121.6, 69.7 (s, CH_2), 47.2. MS: ESI: *m/z* 299.1 $[M+H]^{+.}$

1-(furan-2-ylmethyl)-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl) thiourea (5e): Yellow solid. Yield: 40 %. R*f*: 0.47 (dichloromethane : methanol = 94:6). M.p. 172 °C dec. ^{1}H NMR 400 MHz (DMSO-d_6):δ 9.58 (s, 1H, OH), 9.22 (s, 1H, NH), 8.02 (d, *J* = 4.9 Hz, 1H, NH), 7.69 (d, *J* = 1.7, 1H, ArH), 7.60 (m, *J* = 8.7, 1H, ArH), 7.46 (dd, *J* = 8.1 Hz, *J* = 1.9 Hz, 1 H, ArH), 7.35 (d, J =8.2 Hz, 1H, ArH), 7.42 (m, 1H, ArH), 6.32 (dd, J^1 = 3.1 Hz, J^2 = 0.7 Hz, 1 H, ArH), 4.96 (s, 2H, CH_2)., 4.70 (d, *J* = 5.2 Hz, 2H, CH_2). ^{13}C NMR 101 MHz (DMSO-d_6):δ 183.4 (s, CS), 141.7, 140.15, 121.1, 120.8, 119.1, 110.1, 106.2, 69.3, 35.8 (s, CH_2). MS: ESI: *m/z* 289.1 $[M+H]^{+.}$

Carbonic Anhydrase inhibition assays

An Applied Photophysics stopped-flow instrument was used for assaying the CA catalysed CO_2 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, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. In particular, CO_2 was bubbled in distilled deionized water for 30 min so that the water was saturated (the concentration at a specific temperature is known from literature). In addition, a CO_2 assay kit (from Sigma) was used to measure the concentration in variously diluted solutions obtained from the saturated one (which was kept at the same temperature and a constant bubbling during the experiments). For each inhibitor at least six traces of the initial 5-10% of the reaction was used for determining the initial velocity.[1] 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 distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min - 2 h (or longer, i.e., 4-6 h) at room temperature (at 4°C for the incubation periods longer than 15 min) 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 and the Cheng-Prusoff equation,[2, 3] as reported earlier, and represent the mean from at least three different determinations. hCA I was purchased by Sigma-Aldrich and used without

further purification, whereas all the other hCA isoforms were recombinant ones obtained in-house as reported earlier.[4, 5]

Crystallization and Data Collection

Crystals of native hCA II were grown at room temperature using the hanging drop vapor diffusion method. Two precipitant solutions were used to obtain suitable crystals at different pH values. In particular, at basic pH, we used a precipitant solution, previously reported in literature,[6] containing 1.4 M sodium citrate and 0.1 M TRIS-HCl pH 8.7, whereas at nearly physiological pH, optimal conditions were achieved using a reservoir solution consisting of 2.4 M sodium malonate pH 7.4 and 5 mM 4-(hydroxymercurybenzoate). This precipitant was identified after an initial screening performed using the kits Crystal Screen, Crystal Screen 2 and Index from Hampton Research.[7, 8] In both cases equal volumes of protein (10 mg/mL in 0.02 M TRIS-HCl pH 8.0) and precipitant solution were mixed and equilibrated against 0.5 mL reservoir containing the same precipitant solution. Crystals were grown within two days up to an average size of 0.3 x 0.1 x 0,2 mm. Crystals of hCA II/**1** and hCA II/**4f** complexes were then obtained by the soaking technique. In particular, inhibitor **1** was dissolved at the concentration of 100 mM and 40 mM in the precipitant solutions containing sodium citrate and sodium malonate, respectively, while inhibitor **4f** was dissolved at the concentration of 20 mM in the precipitant solution containing sodium citrate. 15% (v/v) glycerol was added to both solutions to obtain cryoprotectant solutions. A few hCA II native crystals were then transferred in a 2 µl drop of both freshly prepared inhibitor solutions. Crystals were kept in the soaking solution containing inhibitor **1** or inhibitor **4f** for 1 hour or 7 hours, respectively, and then flash-frozen in a gaseous nitrogen stream prior to the diffraction experiment. The complete data sets were collected at 100 K by a copper rotating anode generator developed by Rigaku and equipped with a Rigaku Saturn CCD detector. hCA II/**1** crystals at pH 8.7 and pH 7.4 diffracted up to 1.40 Å and 1.36 Å resolution, respectively, while hCA II/**4f** crystals diffracted up to 1.70 Å. Intensity data were processed and scaled using the program HKL2000. [9] Data collection statistics are shown in Table S1.

Structure Resolution and Refinement

Initial phases of all hCA II/inhibitor complexes were calculated using the PDB entry 1CA2[10] with waters removed. The structures of both hCA II/**1** complexes were refined using the program REFMAC5.8[11] in CCP4i,[12] while the structure of the hCA II/**4f** complex was refined using the program CNS 1.3.[13, 14] For all structures model building and map inspections were performed using the program O.[15] The topologies and parameters for the inhibitors and all linkages were constructed

using JLIGAND.[16] Several rounds of restrained refinement and anisotropic temperature factor refinement alternated with manual rebuilding were necessary to reduce the crystallographic R-factor/R-free values to 0.110/0.148 and 0.116/0.157 for the hCA II/**1** complexes at pH 8.7 and 7.4, respectively, and 0.166/0.201 for the hCA II/**4f** complex. Refinement statistics are summarized in Table S1. The stereochemical quality of the three models was finally assessed using Procheck and Whatcheck programs.[17, 18]

Coordinates and structure factors have been deposited in the Protein Data Bank (accession codes 5JQ0 for hCA II/**1** adduct at pH 8.7, 5JQT for hCA II/**1** adduct at pH 7.4, and 5LMD for hCA II/**4f** adduct).

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Table S1: Data collection and refinement statistics for the hCA II/inhibitor adducts

	hCA II/**1** pH 8.7	hCA II/**1** pH 7.4	hCA II/**4f**
	Cell parameter		
Space group	$P2_1$	$P2_1$	$P2_1$
Unit cell parameters (Å, °)	a= 42.3 b= 41.2 c= 72.0 β= 104.4	a= 42.2 b= 41.1 c= 71.8 β= 103.9	a= 42.4 b= 41.5 c= 72.1 β= 104.4
	Data collection statistics		
Resolution limits (Å)	50.0-1.40	50.0-1.36	50.0-1.70
Temperature (K)	100	100	100
Total reflections	192053	183415	184684
Unique reflections	42313	50126	25797
Completeness (%)	89.1 (74.2)	97.4 (79.1)	95.4 (70.9)
R-merge*	0.037 (0.210)	0.050 (0.292)	0.081 (0.333)
Mean I/sigma(I)	31.8 (5.7)	20.3 (3.0)	21.4 (3.1)
Redundancy	4.5 (3.5)	3.7 (2.3)	7.2 (3.0)
	Refinement statistics		
Resolution limits (Å)	50.0-1.40	50.0-1.36	50.0-1.70
R-factor** (%)	10.5	12.1	16.6
R-free** (%)	14.6	16.5	20.1
r.m.s.d. from ideal geometry: Bond lengths (Å) Bond angles (°)	 0.013 1.8	 0.017 1.8	 0.013 1.6
Number of protein atoms	2049	2073	2049
Number of inhibitor atoms	22	72	24
Number of water molecules	238	235	231
Average B factor (Å^2) All atoms Protein atoms Inhibitor atoms Water molecules	 14.3 13.1 11.8 25.1	 14.5 13.4 17.0 23.8	 14.0 12.9 20.3 23.4

*R-merge = $\Sigma_{hkl}\Sigma_i|I_i(hkl)-\langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an observation and $\langle I(hkl)\rangle$ is the mean value for its unique reflection; summations are over all reflections; **$R_{factor} = \Sigma|F_o-F_c|/\Sigma F_o$; R_{free} calculated with 5% of data withheld from refinement. Values in parentheses are referred to the highest resolution shell (1.42-1.40 Å for hCA II/**1** at pH 8.7, 1.38-1.36 Å for hCA II/**1** at pH 7.4 and 1.73-1.70 for hCA II/**4f**).

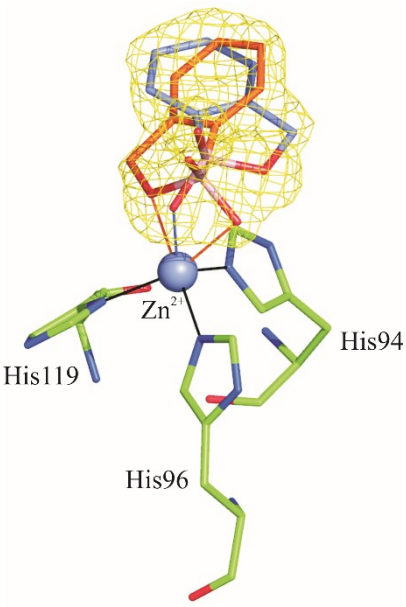

Fig. S1 σ_A-weighted |Fo-Fc| electron density map (contoured at 3.0 σ) calculated removing inhibitor molecule from final coordinates of the hCA II/**1** adduct, crystallized at pH 8.7.

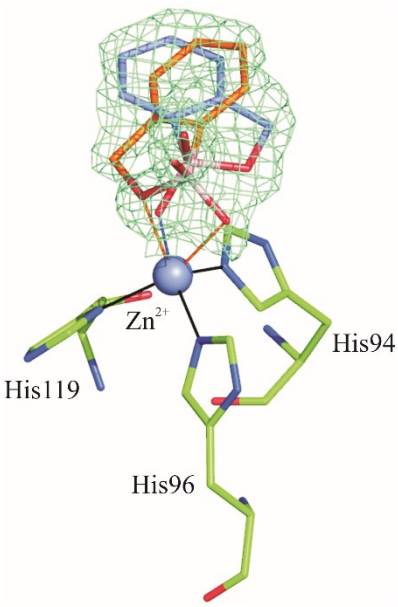

Fig. S2 σ_A-weighted |2Fo-Fc| electron density map (contoured at 1.0 σ) relative to the inhibitor molecule in the hCA II/**1** adduct, crystallized at pH 7.4.

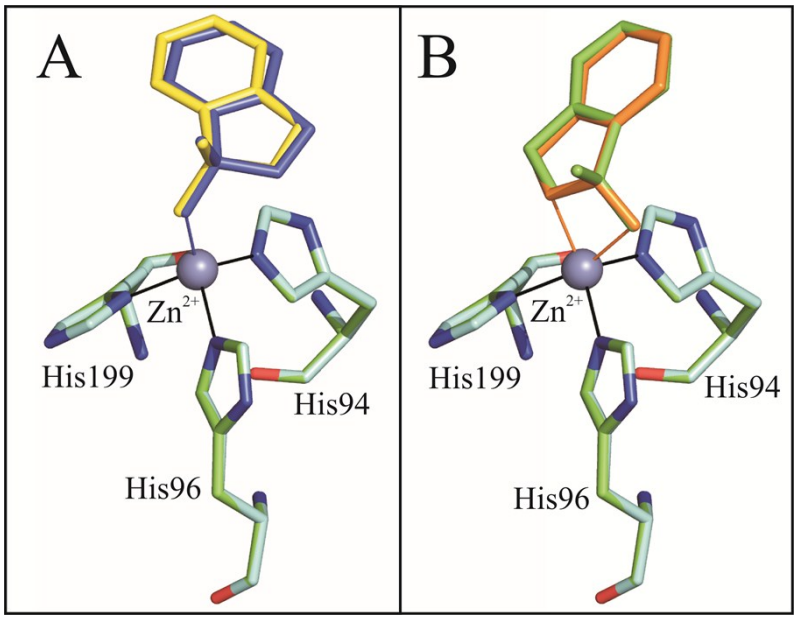

Fig. S3 Structural superposition of the hCA II/**1** adduct obtained at pH 8.7 with that obtained at pH 7.4 in **binding mode A** (A) and **binding mode B** (B). The inhibitor in **binding mode A** is colored in blue and yellow at pH 8.7 and 7.4, respectively, and in orange and green for **binding mode B** at pH 8.7 and 7.4, respectively.

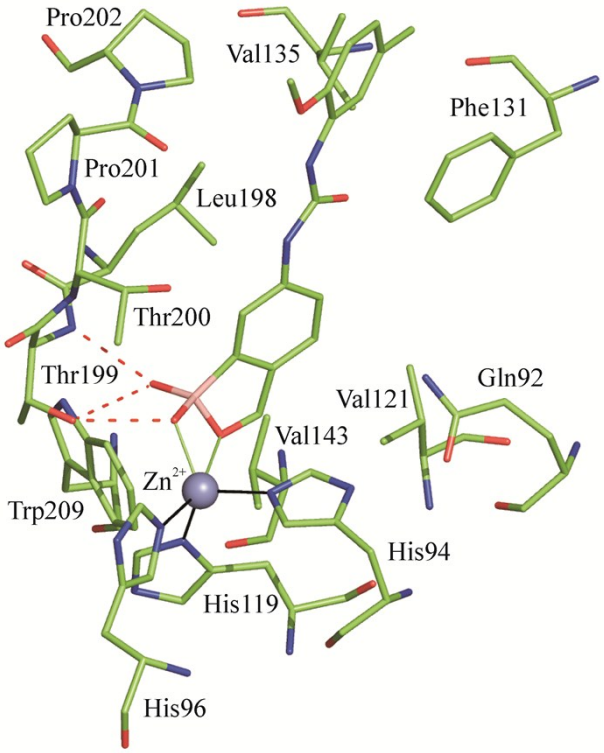

Fig. S4 Active-site region of the hCA II/**4f** adduct. Hydrogen bonds, residues involved in van der Waals interactions (distance <4.0 Å) and the active-site Zn^{2+} ion coordination are shown.