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**Supplementary Information**

# Carbonic anhydrase inhibitors: X-Ray crystallographic studies for the binding of N-substituted benzenesulfonamides to human isoform II

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## EXPERIMENTAL SECTION

### Chemistry an CA Inhibition

The sulfonamides **2-4** were prepared as reported in the literature.[1] The CA inhibition has been assayed by the $CO_2$ hydrase method of Khalifah.[2]

### Crystallization, X-ray data collection and refinement of hCA II/inhibitor complexes

Crystals of hCA II/inhibitor adducts have been obtained by the soaking technique. In particular, hCA II native crystals were grown at 20 °C by the vapor diffusion hanging drop method. Drops were prepared by mixing equal volumes of protein (10 mg/ml in 0.1 M TRIS-HCl pH 8.5) and reservoir (2.6 M ammonium sulphate, 0.3 M sodium chloride, 0.1 M Tris-HCl, pH 8.5 and 5 mM 4-(hydroxymercurybenzoate)) solution, and were then equilibrated against 1 ml reservoir. A few hCA II native crystals of 0.3 x 0.2 x 0.1 mm size were then transferred in a 3 μl drop, containing the precipitant solution and the inhibitor at the concentration of 20 mM. These crystals were kept in the soaking solution only for a few minutes since they started to be damaged, and then were washed briefly in a cryoprotectant solution containing 15% glycerol and flash-frozen in gas nitrogen stream. A complete dataset was collected for hCA II/**3** and hCA II/**4** complexes at 1.75 Å and 1.65 Å resolution, respectively, using a copper rotating anode generator developed by Rigaku and equipped with Rigaku Saturn CCD detector. All data were processed using HKL2000 package.[3] Crystal parameters and data collection statistics are reported in Table S1.

The structures of hCA II/inhibitor adducts have been analyzed by difference Fourier techniques, using the PDB file 1CA2 as starting model for refinement,[4] which was carried out with the program CNS 1.0.2.[5] In particular, an initial round of rigid body refinement was followed by simulated annealing and isotropic thermal factor (B-factor) refinement. The inspection of electron density maps in correspondence of the active site region after this single round of refinement revealed the

presence in both hCA II/inhibitor complexes of an inhibitor molecule, which was easily built into the model. Solvent molecules were introduced into the model if they displayed electron density in the Fo-Fc map greater than 3σ and were located at hydrogen bonding distances from appropriate atoms. Several alternating cycles of energy minimization, individual temperature factor refinement and manual model building gave the final models with R-factor/R-free values of 0.171/0.183 and 0.179/0.211 for hCA II/**3** and hCA II/**4**, respectively. The overall quality of the both models was high, with 98.6% of the nonglycine residues located in the allowed regions of the Ramachandran plot. The statistics for refinement are summarized in Table S1. Coordinates and structure factors have been deposited in the Brookhaven Protein Data Bank (Accession code 3T5U and 3T5Z for hCA II/**3** and hCA II/**4**, respectively).

**References**

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**Table S1** Crystal parameters, data collection and refinement statistics for hCA II/**3** and hCA II/**4** complexes

| | **hCA II/3** | **hCA II/4** |
|---|---|---|
| ***Cell parameter*** | | |
| Space group | $P2_1$ | $P2_1$ |
| a (Å) | 42.26 | 42.11 |
| b (Å) | 41.54 | 41.32 |
| c (Å) | 72.09 | 71.84 |
| β (°) | 104.43 | 104.32 |
| ***Data collection statistics*** | | |
| Resolution (Å) | 20.00-1.75 | 20.00-1.65 |
| Temperature (K) | 100 | 100 |
| Total reflections | 109954 | 79525 |
| Unique reflections | 24290 | 28042 |
| Completeness (%) | 98.2 (91.2) | 96.4 (87.6) |
| R-sym* | 0.063 (0.097) | 0.043 (0.061) |
| Mean I/sigma(I) | 21.38 (10.92) | 21.03 (13.45) |
| ***Refinement statistics*** | | |
| Resolution (Å) | 20.00-1.75 | 20.00-1.65 |
| R-factor** (%) | 17.1 | 17.9 |
| R-free** (%) | 18.3 | 21.1 |
| rmsd from ideal geometry: | | |
| Bond lengths (Å) | 0.007 | 0.007 |
| Bond angles (°) | 1.4 | 1.5 |
| Number of protein atoms | 2077 | 2090 |
| Number of inhibitor atoms | 44 | 12 |
| Number of water molecules | 265 | 356 |
| Average B factor ($Å^2$) | 15.41 | 13.67 |

*$R_{sym} = \Sigma|I_i-\langle I\rangle|/\Sigma I_i$; 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 parenthesis are referred to the highest resolution shell (1.81-1.75 Å and 1.71-1.65 Å for hCA II/**3** and hCA II/**4** complexes, respectively).

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