Identification of Two Hydrophobic Patches in the Active-Site Cavity of Human Carbonic Anhydrase II by Solution-Phase and Solid-State Studies and Their Use in the Development of Tight-Binding Inhibitors[1]

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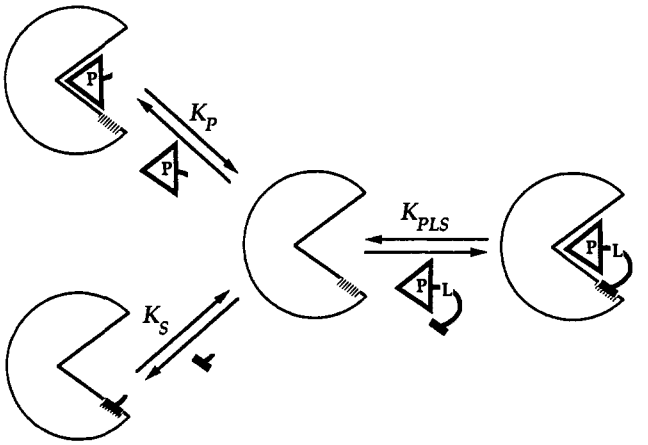
This paper describes inhibitors for human carbonic anhydrase II (HCAII, EC 4.2.1.1) that bind with nanomolar dissociation constants. These inhibitors were developed by exploiting interactions with hydrophobic "patches" in the lip of the active site of this enzyme. These patches are molecular surfaces presented by a phenylalanine on one face of the active-site cleft (Phe-131) and three adjacent hydrophobic residues on the opposite face (Leu-198 and Pro-201/202). Comparison of the affinities of molecules that can occupy either one or both of the two sites indicates that these hydrophobic interactions can contribute factors of 10^2–10^3 to binding constants and that the strength of the interaction is relatively insensitive to the structure of the hydrophobic ligand. One of these inhibitors, the competitive inhibitor *N*-[*N*-[*N*-(4-sulfamoylbenzoyl)phenylglycyl]glycyl]glycine benzyl ester (17), has been studied by X-ray crystallographic methods in its complex with HCAII at 1.9-Å resolution. The geometry of binding of the arylsulfonamide group of 17 is similar to geometries observed in other HCAII·arylsulfonamide complexes. The aromatic side chain of the phenylglycine residue of the inhibitor is inferred to pack against the hydrophobic Phe-131 face, and this interaction "steers" the peptide backbone of the inhibitor toward a region in the HCAII active site different from that occupied in the related triglycyl peptide. Attempts to design inhibitors capable of binding simultaneously to Phe-131 and Leu-198/Pro-201/202 did not lead to molecules that bound more tightly than those binding to these hydrophobic sites individually.

Introduction

We wish to describe the synthesis and assay of a class of tight-binding ($K_d \approx 1$ nM) inhibitors of structure $H_2NO_2SC_6H_4CONH(S)$ for human carbonic anhydrase II (HCAII, EC 4.2.1.1).[2] We have synthesized a number of structures to explore the enthalpic advantage that can be obtained by using inhibitors that can bind by both an active site specific interaction to the primary binding site and by a structure nonspecific hydrophobic interaction to a second,[3–5] and in principle a third, hydrophobic site in the cleft of HCAII (Scheme 1). We chose to use hydrophobic interactions to provide secondary contributions to the primary binding of the arylsulfonamide because hydrophobic interactions have relatively loose geometrical constraints. The principal question that we address in this paper is the magnitude of the increase in the strength of binding that can be achieved by combining a structure-specific interaction (involving the benzenesulfonamide group) and a structure-insensitive interaction (involving a hydrophobic group) in an inhibitor. By virtue of these two separate interactions, the inhibitor becomes bivalent and should (in appropriately designed inhibitors) benefit from an advantage in ΔG at modest cost in $T\Delta S$ for binding as a result. We identified Phe-131 as a candidate for this hydrophobic interaction by examining the X-ray crystal structure of the native enzyme (Phe-131 has 52 Å^2 of solvent-accessible surface). Figure 1 shows a representative section of the active site of HCAII as it is occupied by one of the inhibitors described here.[6] The contribution to binding of hydrophobic interactions directed at Phe-131 was remarkably insensitive to the structure of the group S of the inhibitors. We also identified another hydrophobic surface—a site comprising Leu-198/Pro-201/202—that is also exposed to solvent (113 Å^2). This second patch is mostly shielded from water when certain benzyl esters of oligoglycine-based inhibitors are bound to the enzyme.[7] We infer, from the relative affinities of several inhibitors in which a benzyl group is placed progressively further from the arylsulfonamide group, that only inhibi-

Scheme 1.[a] A Bivalent Tight-Binding Ligand for a Receptor Benefits from Two Enthalpically Favorable Interactions



[a] The affinity of the PLS inhibitor possessing both the primary (P) and secondary (S) recognition element linked by an appropriate linker (L) will be greater than that of either element alone. In the case shown here, the solvent-exposed surface of a hydrophobic region of the receptor is partly covered by the secondary recognition element.

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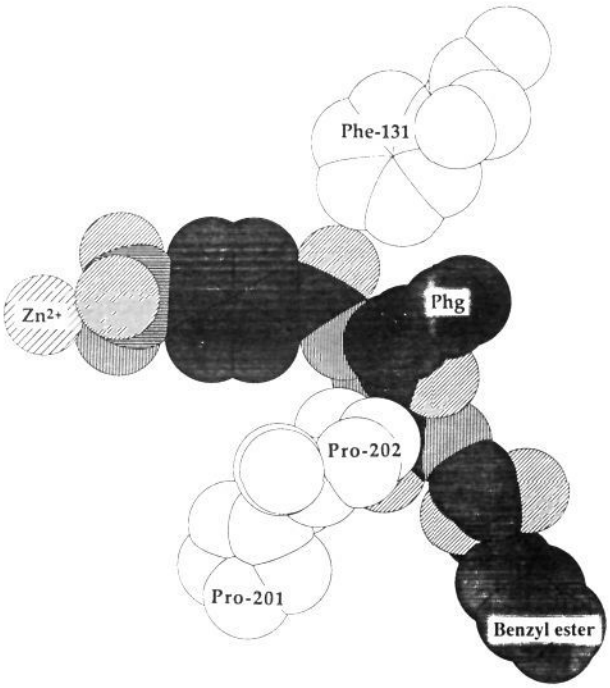
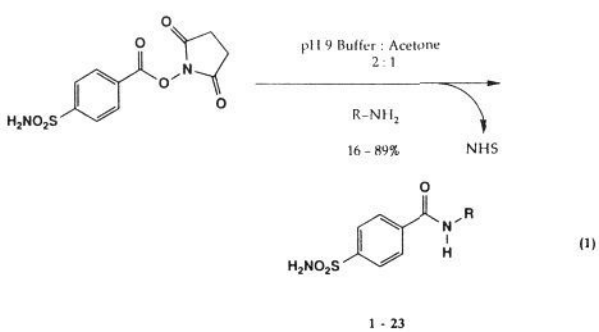


Figure 1. Three binding sites may be invoked by an inhibitor like the phenylglycylglycylglycine derivative of 4-sulfamylbenzoic acid (**17**) shown here. The primary site is the divalent ainc ion at the left. The two proximate hydrophobic sites are Phe-131 and Pro-201/202. The position of the terminal GlyGlyOBn group is not defined by the crystallographic data and is shown in this figure only for scale, without implication of a preferred conformation.

tors that retain their benzyl termini *within* the active-site cleft are able to interact with this second hydrophobic patch (Table 1, entries 17–23).

Results and Discussion

Preparation of Hydrophobic Inhibitors. We prepared inhibitors **1–23** by unexceptional procedures (eq 1).[8]



(1)

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Hydrophobic Amides of 4-Sulfamoylbenzoic Acid Bind to HCAII 20–400 Times More Tightly Than 4-Sulfamoylbenzamide. For a primary recognition element, we chose $H_2NO_2SC_6H_4CO$, because its structure could be readily changed (eq 1) and because a range of inhibitors having this basic structure had been previously studied.[4,5] In several cases, data are available from high-resolution X-ray crystallographic studies of complexes of HCAII with molecules belonging to this class of inhibitors.[4,9,10] Phe-131 is 12 Å from the base of the conical cleft of HCAII, and is therefore accessible to arylsulfonamides possessing an amide linkage between the benzenesulfonamide ring and the hydrophobic moiety.[11] The dissociation constants of 15 inhibitors bearing a hydrophobic group appropriately situated to interact with Phe-131 are shown in Table 1 (entries 1–15). Dissociation constants were measured using a fluorescence assay.[12]

Table 1. Dissociation Constants of Hydrophobic Inhibitors $H_2NO_2SC_6H_4CONH(S)$ from HCAII

	Inhibitor or S	K_d,[a] nM	
	acetazolamide	6.8	(3)
	MK-927[b]	0.7	
	MK-417(*S*)[b]	0.61	
	MK-417(*R*)[b]	71	
	H	120	(4)
	CH_3	150[c]	(4)
1	benzyl	1.1	(6)
2	4-(dimethylamino)benzyl	1.4	(3)
3	4-nitrobenzyl	2.0	(6)
4	furfuryl	5.6	
5	(phenylthio)methyl	1.7	
6	2-pyridylmethyl	4.3	
7	3-pyridylmethyl	4.0	
8	4-pyridylmethyl	1.4	
9	2-methylbenzyl	2.2	
10	1-naphthylmethyl	0.6	
11	1-octyl	2.5[c]	(2)
12	$CH_2(CF_2)_6CF_3$	0.3	(2)
13	cyclohexylmethyl	1.1	(4)
14	1-adamantylmethyl	2.1	(2)
15	trifluorobenzyl[d]	2.3	(2)
16	L-PheGlyGlyOH	21	(3)
17	L-PhgGlyGlyOBn[e]	0.9	(4)
18	L-PheGlyGlyOBn	5.3	(5)
19	D-PheGlyGlyOBn	36	(5)
20	GlyGlyOBn	71	(2)
21	GlyGlyGlyOBn[f]	75	(4)
22	GlyGlyGlyO-4′-BuBn[g]	53	(3)
23	GlyGlyGlyGlyOBn	210	(3)

[a] These values were all determined at 298 ± 2 and are accurate to ±50%, for values of $K_d < 5$ nM, or to ±20%, for others. The numbers in parentheses are the number of experiments used to determine that value. In cases where there is no number, the value of K_d is from one experiment. [b] Data from Baldwin et al. (ref 4). [c] Compare with data fromKing and Burgen (ref 5), where $K_d = 80$ and 1.2 nM for the methyl and 1-heptyl derivatives, respectively, at an unknown temperature. [d] Mixture of isomeric trifluorobenzylamides. [e] Phg is phenylglycine. [f] For comparison, consider the affinity of the corresponding methyl ester, where $K_d = 300$ nM. [g] This compound is the *p*-butylbenzyl ester of the triglycine derivative.

The affinity of these inhibitors was remarkably insensitive to the precise structure of the hydrophobic group, with the exception of the fluorianted derivative **12**. This compound binds to HCAII with an affinity 8 times that of the structurally analogous perhydro derivative **11**. There are several plausible origins for this increase in affinity—differences in local electrostatic character of C–F and C–H bonds, interfacial free energies involving alkanes and perfluoroalkanes, and relative surface areas of hydrocarbons and fluorocarbons—and it is the subject of a separate study.[13,14]

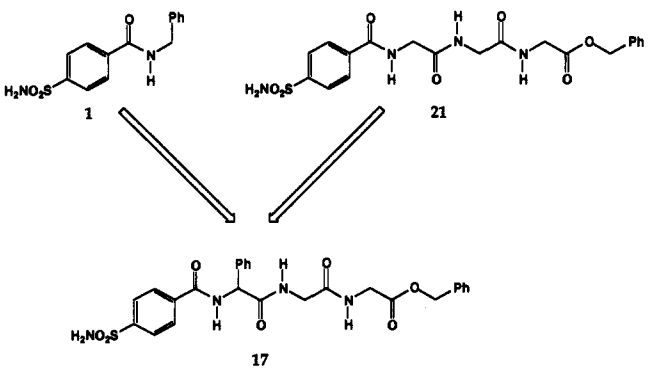
The observation that the structures of these inhibitors generally did not affect their dissociation constants from HCAII suggests that the exact shape and size of the hydrophobic group need not be precisely defined when preparing inhibitors that use secondary, hydrophobic interactions to increase binding constants. Analysis of the complex of HCAII with a typical tight-binding inhibitor, the benzylamide derivative of 4-sulfamoylbenzoic acid (**1**), shows that binding of this molecule to the protein decreases the solvent-exposed surface area of Phe-131 by 23 Å^2 (from 52 to 29 Å^2).[7] This inhibitor *also* reduces the solvent-accessible surface area of Leu-198/Pro-201–202 by 26 Å^2 (from 113 to 87 Å^2) and makes van de Waals contacts with Leu-198 and Pro-202. Although the benzyl

group of this inhibitor is not within van der Waals contact of the side chain of Phe-131, this decrease in solvent-accessible surface area probably contributes to the high affinity of inhibitor 1 for HCAII.

Benzyl Esters of Oligoglycine-Derived Benzenesulfonamides Bind to HCAII Approximately 2 Times More Tightly than 4-Sulfamoylbenzamide. Leu-198 and Pro-m01/202 are located ca. 14 Å from the divalent zinc ion at the base of HCAII, near the lip of the active site cleft. They present a 93 Å^2 solvent-exposed hydrophobic surface and are another potential target for an appropriately tethered hydrophobic group extending from the benzenesulfonamide. The benzyl esters of di- and triglycine-derived benzenesulfonamides (**20–22**) possess a tether of appropriate length and allow the benzyl group to occupy a position that leaves only 13 Å^2 of the hydrophobic surface of the proline residues remaining exposed to solvent in modeling experiments.[7] Although these compounds have the *potential* to bind to the hydrophobic site at Leu-198/Pro-201/202, they exhibit K_d values ranging from 53 to 75 nM: that is, they are only modestly tight-binding. The three-dimensional structure of the HCAII complex with a benzyl ester of a triglycine arylsulfonamide shows that the peptide chain, and not the benzyl group, of the inhibitor interacts with the Pro-202 region.[15]

The longer tetraglycine tether of **23** affords an inhibitor that binds less strongly to the enzyme than the shorter di- and triglycine analogs (K_d = 210 nM, measured using bovine CAII, which has complete active site homology with HCAII). Therefore, the results of solution studies and X-ray crystallographic studies of HCAII·inhibitor indicate that only hydrophobic groups tethered close to the arylsulfonamide portion of the inhibitors will reduce the solvent accessible surface area of the Pro-202 region as well as Phe-131.

Inhibitors That Possess Structures Capable of Placing a Phenyl Ring Near Phe-131 *and* a Benzyl Group Near Leu-198/Pro-201/202 Bind to HCAII 20–130 Times More Tightly Than 4-Sulfamoylbenzamide; There is Apparently No Cooperativity in Binding to These Two Hydrophobic Sites. By superimposing inhibitors 1and **21**, we obtained the structure **17**, the



phenylglycylglycylglycine benzyl ester of 4-sulfamoylbenzoic acid. We hoped that the benzyl ester of this molecule could interact with secondary hydrophobic binding site Leu-198/Pro-201/202, while the phenylglycine side chain interacted with Pro-202 and Phe-131, and that its affinity for HCAII would accordingly be greater than that of derivatives capable of interacting with only one of the two sites. The results in Table 1 indicate that although **17** binds slightly more tightly to HCAII than **1**, it does not bind with the significantly increased binding affinity we would have anticipated if the two sites were able to interact with the inhibitor in a cooperative sense or even independently. The results from crystallography of HCAII·**17** indicate that the benzyl ester of **17** is disordered; these results do, however, support the simultaneous interaction of the phenylglycine side chain with Phe-131 and Pro-202, as reflected in a reduction in the solvent-accessible surface of these residues on binding of **17**.

We also examined the enantiomers **18** and **19**, in order to assess the influence of stereochemistry on binding.[16] The 7-fold difference (5.3 versus 36 nM) in the affinities of these enantiomers is modest, but is consistent with what we expected on the basis of the molecular modeling of HCAII with these inhibitors; when bound in a fashion that would allow the side chain of its phenylalanine residue to approach Phe-131 of HCAII, the benzyl ester terminus of **19** must necessarily extend into solvent. These same modeling studies showed that enantiomer **18** could potentially span both sites.

The results of our studies of carboxylic acid **16** were also consistent with our conception of the active site of HCAII. This molecule lacks the benzyl ester terminus but has the correct stereochemistry to interact with Phe-131; as expected, it binds to HCAII with an affinity about 4-fold less than that of **18**[17] (21 versus 5.3 nM).

Crystal Structure of the Complex between HCAII and 17. The structure of HCAII in the enzyme–inhibitor complex exhibits only minor differences in conformation when compared with that of the native protein; the root mean square (rms) deviation in Cα atoms between the two structures is 0.2 Å. Clear electron density is visible for the arylsulfonamide and the phenylglycine of inhibitor **17** (Figure 2). No hydrogen-bond interactions are observed between the phenylglycine group and the enzyme as judged by distance and stereochemical criteria.[18] Table 2 summarizes selected enzyme–inhibitor contacts. The orientation of the arylsulfonamide group is indistinguishable from that observed in other HCAII·inhibitor complexes.[4,9,10,15] There is, however, a significant difference in the trace of the peptide backbones of the tripeptide inhibitors **17** and **21** (Figure 3). Whereas in the HCAII·**21** complex the peptide backbone of the inhibitor associates with the hydrophobic wall afforded by Leu-198 and Pro-201/202 of the protein,[15] the peptide backbone of **17** in its complex with HCAII seems to be directed out into the hydrophilic cleft. This alternate conformation for the backbone of the inhibitor results from the association of the side chain of phenylglycine with Phe-131 and Pro-202. In other words, the interactions of the side chain "steer" the peptide in an alternate direction.

We note that no electron density is observed for **17** beyond its phenylglycine residue. The electron density about the Cα of phenylglycine is compatible with the hypothesis that the L-isomer is the one bound to the protein. Since this electron density is relatively weak, however, we must leave it as an unresolved ambiguity whether the D- or L-phenylglycine isomer of **17** is that which binds predominantly to HCAII in crystals of the enzyme–inhibitor complex. If the D-isomer is bound in the crystal, however, our projection of the remaining peptide chain must be revised.

Structural studies of **1** and also of an inhibitor derived from the methyl ester of phenylalanine indicate that hydrophobic side chains of the first amino acid residue in peptide inhibitors preferentially associate with the Leu-198/Phe-201/202 hydrophobic wall.[15,19] The side chain of

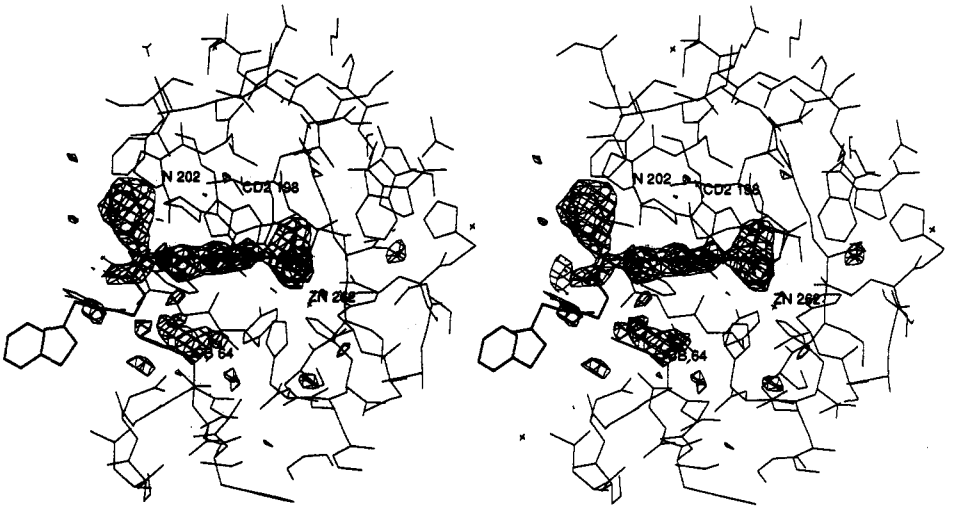


Figure 2. Difference electron density map of the HCAII·17 complex generated with Fourier coefficients $|F_o| - |F_c|$ and phases calculated from the final model less the atoms of the inhibitor contoured at 2.5σ. Enzyme residues His-64, Leu-198, and Pro-202 and the zinc ion are labeled. No density is observed for the inhibitor beyond the first amino acid residue, so this region is simply modeled into the structure in a chemically reasonable fashion (the occupancies for unobserved atoms are set at 0.0 in the coordinate set submitted to the Brookhaven Protein Data Bank). Note the additional density near His-64 that would be consistent with a minor population of the "in" conformer.

Table 2. Selected Distances between HCAII and 17

inhibitor atom	enzyme residue	separation (Å)
sulfonamide O1	Zn^{2+}	3.1
sulfonamide O2	Thr-199 NH	2.9[a]
sulfonamide N1	Zn^{2+}	2.2
	Thr-199 Oγ	2.8[a]
ester O	HOH	3.3

[a] Possible hydrogen bond as judged from distance and stereochemical criteria.

phenylglycine is, therefore, expected to bind in a fashion similar to that of the side chain of phenylalanine, if the interactions of the rest of the inhibitor (Gly-2 and Gly-3) do not dominate the enzyme–inhibitor association. The binding of 17 shows that the interaction between the phenylglycine residue and Phe-131 of the protein is more favorable than the van der Waals interactions between the peptide backbone of the inhibitor and this wall, as was observed for 21.[15]

Interestingly, the side chain of His-64 is found predominantly in the "out" conformation, even though there appears to be no compelling steric reason for its conformational change away from the native, "in" conformation (Figure 4). This residue is observed to change its conformation when sterically required by inhibitor binding. Since His-64 also undergoes a conformational change to the "out" conformation even when not sterically required by a bound inhibitor,[2] it is unclear what effect its conformation has on enzyme–inhibitor affinity.[10]

Conclusions

We have identified a group of tight-binding inhibitors for HCAII that seem to share a secondary hydrophobic interaction with Phe-131 and Pro-202 in the cleft of the protein. These inhibitors support the value of a strategy for the rational design of tight-binding inhibitors that involves identifying hydrophobic patches in the cleft of this receptor and attaching hydrophobic groups to the primary inhibitory group—the benzenesulfonamide moiety—at distances appropriate to interact with these patches.

An approach to the design of tight-binding inhibitors based on utilizing proximate secondary hydrophobic sites has several advantages relative to approaches that require modification of the structure of the primary inhibitor: (a) because hydrophobic interactions are relatively nondirectional, this strategy is less demanding in terms of design and synthesis than approaches based on inducing a more detailed fit between the molecular shapes of the inhibitor and protein; (b) by choosing an appropriate strategy for synthesis, it is possible to prepare a substantial number of inhibitors easily; and (c) it preserves specificity in recognition.

This routine for the development of tight-binding inhibitors requires, however, that several conditions be met. First, a crystal structure of the enzyme with bound primary inhibitor must be available. Second, there must be a relatively "open" active site, to permit access to the lip of the active site by hydrophobic groups. Third, the linker connecting the primary inhibitor and the secondary hydrophobic group(s) must be designed to introduce as few unfavorable enthalpic and entropic interactions into the bound state as possible.

Experimental Section

Materials and Methods. Pentafluorobenzamide was obtained from PCR Chemicals and was reduced with lithium aluminum hydride to afford a mixture of trifluorobenzylamines. We prepared the amines required for the synthesis of 17–19 by standard methods for peptide synthesis.[20,21] Benzyl esters 20–23 were prepared by treatment of the corresponding acids[12] with the appropriate alcohol in the presence of a catalytic amount of *p*-toluenesulfonic acid, in toluene. The succinimidyl ester of 4-sulfamoylbenzoic acid (ArCONHS) was prepared as described previously. Starting materials for the syntheses of all other inhibitors were of the best grade available, from Aldrich, Fluka, Chemical Dynamics, or Bachem Biochemicals. Syntheses were carried out under nitrogen unless otherwise specified. Yields of inhibitors were not optimized and ranged from 16 to 89%. Silica gel was used for thin-layer and column chromatography; gradients of up to 20% methanol in chloroform were used as eluents for column chromatography. ^{1}H NMR spectra were obtained in Me_2SO-d_6, with a Bruker AM-500 spectrometer operating at 500 MHz for proton, with a dedicated ^{1}H probe. ^{13}C spectra were obtained in Me_2SO-d_6, with a Bruker AM-400 spectrometer operating at 100 MHz for carbon. Elemental analyses were performed by Spang Labs. Mass spectra were obtained at the Harvard University Chemistry Department Mass Spectrometry Facility. One representative synthetic procedure is provided below; data used for the characterization of the other inhibitors is given in the supplementary material.[22]

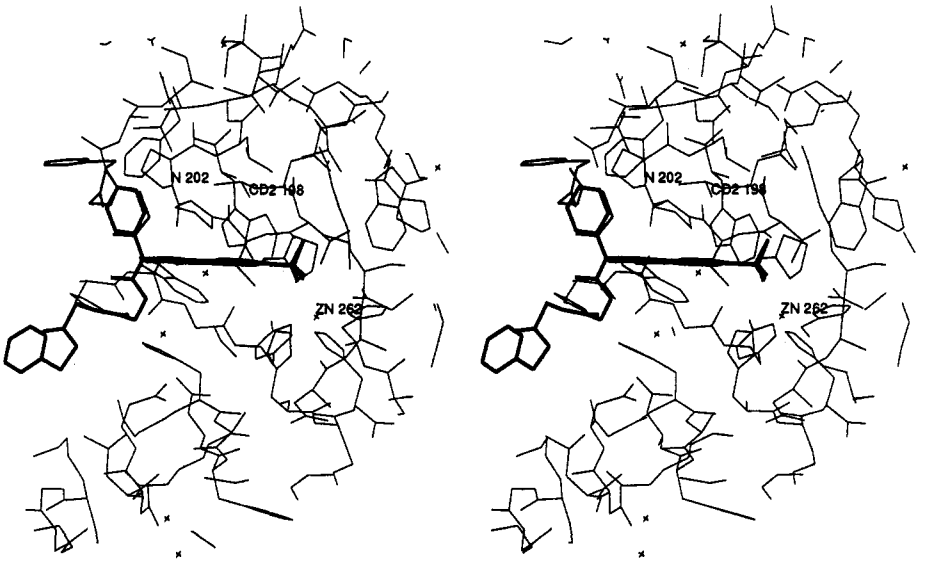


Figure 3. Superposition of the inhibitors **17** (thick bonds) and **21** (medium bonds); for reference, enzyme coordinates appear as thin bonds. Enzyme residues Leu-198 and Pro-202 and the zinc ion are labeled. Note that although the positions of the arylsulfonamide groups are almost identical, the conformation of the peptide tails of each inhibitor are different. As indicated previously, no specific conformation is assigned the terminal groups of **17**.

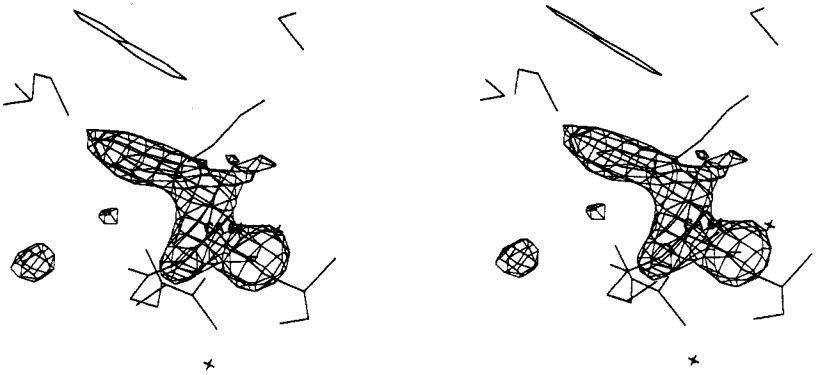


Figure 4. Difference electron density map of His-64 in the HCAII·**17** complex calculated with Fourier coefficients $|F_o| - |F_c|$ and phases derived from the final model less the atoms of His-64. This residue is predominantly in the "out" conformation, but branched electron density is indicative of a minor population of the "in" conformer (which predominates in the native enzyme). The map is contoured at 2.7σ; at lower contours (ca. 2.0σ), two conformers of His-64 will fit into the resulting smear of electron density.

***N*-(4′-Sulfamoylbenzoyl)benzylamine (1).** To ArCONHS (0.3 g, 1.0 mmol) in 3 mL of acetone at room temperature was added benzylamine (110 μL, 1.0 mmol) in 6 mL of 0.1 M KH_2PO_4 (pH 9). The product was isolated by recrystallization of the crude filtrate from hot ethanol, to afford 227 mg (78%). ^{1}H NMR: δ 9.24 (t, 5.4 Hz, 1 H), 8.03 (d, 8.2, 2 H), 7.90 (d, 8.2, 2 H), 7.49 (br s, 2 H), 7.27 (m, 5 H), 4.49 (d, 5.7, 2 H). ^{13}C NMR: δ 165.2, 146.3, 139.4, 137.2, 128.4, 128.0, 128.0, 127.3, 126.9, 125.7, 125.7, 42.8. Anal. Calcd for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65. Found: C, 58.06; H, 4.77; N, 9.40.

Determination of Dissociation Constants. Our method for determining the affinities of inhibitors for HCAII is a modification of the assay originally described by Chen and Kernohan[23] and is based on the fact that the fluorescence of 5-(dimethylamino)-1-naphthalenesulfonamide (DNSA) can be used to determine the concentration of HCAII·DNSA in solutions containing protein and fluorescent and nonfluorescent inhibitors.[12,23]

Fluorescence spectrophotometry was carried out with a Perkin-Elmer MPF-4 spectrofluorimeter, with temperature regulated at 298 ± 2 K using a circulating bath. HCAII was dissolved in 20 mM KH_2PO_4 (pH 7.5) and solutions were stored at 4 °C. Concentrations of stock solutions of HCAII were determined spectrophotometrically ($\epsilon_{280} = 5.7 \times 10^4$ M^{-1} cm^{-1})[24] and were normally 75 μM. The fluorescence probe 5-(dimethylamino)-1-naphthalenesulfonamide (DNSA) was routinely recrystallized from ethanol within 2 days of use and stored under vacuum in a desiccator. Concentrations of solutions of DNSA were determined spectrophotometrically ($\epsilon_{326} = 4640$ M^{-1} cm^{-1}),[24] with typical stock solutions being ~25 μM. Titrant solutions of inhibitors were prepared gravimetrically in Me_2SO-d_6 to a concentration of ~20 mM. The precise concentrations were determined by dilution of this stock solution into Me_2SO-d_6 containing 1.84 mM *N*,*N*-dimethylformamide (DMF) (3.00 μL dissolved in 25.0 g of Me_2SO-d_6), and integration of the resonances of the inhibitors relative to DMF in 40- to 400-μL dilutions.

Molecular Modeling of HCAII·Sulfonamide Complexes. Molecular modeling was done using MacroModel Version 3.0 (Columbia University), on an Evans & Sutherland PS 390 microcomputer. Solvent-accessible surface areas were determined by selecting the residue(s) of interest and using a probe of 1.4-Å radius, in the "Surfaces" submode of "Analyze" mode.

Crystallography. Data were obtained and analyzed precisely as described.[15] Relevant data collection and reduction statistics are recorded in Table 3.I

Neither the inhibitor atoms nor active site water molecules were included in the initial stages of refinement for the enzyme–inhibitor structure. Residue conformations throughout the protein were examined during the course of refinement by using maps calculated with Fourier coefficients $|F_o| - |F_c|$ or $2|F_o| - |F_c|$ and phases derived from the in-progress atomic model. Only minimal ajustments of atomic positions were necessary, and the inhibitor, as well as active-site water molecules, were added when the crystallographic *R* factor dropped below 0.200. During inspection of the difference electron density map, no clear density was observed for the second or third amino acid in the inhibitor; therefore, the occupancy of those atoms was set to zero. Refinement converged smoothly to a final crystallographic *R* factor of 0.181 for the enzyme–inhibitor complex; the final model

Table 3. Data Collection and Refinement Statistics for HCAII·17 Complex

number of crystals	1
number of measured reflections	24 387
number of unique reflections	15 041
maximum resolution (Å)	1.9
R_{merge}[a]	0.073
number of reflections used in refinement (6.5–1.9 Å shell)	13 685
completeness of data (%)	73
R factor[b]	0.181
number of ordered water molecules in final refinement cycle	58
rms deviation from ideal bond lengths (Å)	0.011
rms deviation from ideal bond angles (deg)	2.8
rms deviation from ideal dihedral angles	25.8
rms deviation from ideal improper dihedral angles	1.2

[a] R_{merge} for replicate reflections, $R = \sum\|I_{hi}\| - \langle|I_h|\rangle|/\sum\langle|I|\rangle$; $|I_{hi}|$ = intensity measured for reflection h in data set i; $\langle|I_h|\rangle$ = average intensity of reflection h calculated from replicate data. [b] Crystallographic R factor, $R = \sum\|F_o| - |F_c\|/\sum|F_o|$; $|F_o|$ and $|F_c|$ are the observed and calculated structure factors, respectively.

had excellent stereochemistry with rms deviations from ideal bond lengths and angles of 0.011 Å and 2.8°, respectively.

For the refined HCAII·17 structure, a difference electron density map calculated with Fourier coefficients $|F_o| - |F_c|$ and phases derived from the coordinates of the final model revealed that the highest peaks in the vicinity of the active site were 3.5σ. Additionally, the rms error in atomic positions for each structure was estimated to be ca. 0.2 Å based on relationships derived by Luzzati.[26]

Acknowledgment. Dr. Scott Pollack participated in the initial experiments involving disulfide interchange as an approach to tight-binding inhibitors for HCAII. We thank Satish Nair for helpful scientific discussions. Dr. J. J. Baldwin (Merck) emphasized the importance of hydrophobic interactions involving Phe-131 to us early in this project.

Supplementary Material Available: Experimental data for pertinent compounds (5 pages). Ordering information is given on any current masthead page.

References

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