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Structural analysis of inhibitor binding to human carbonic anhydrase II

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Abstract

X-ray crystal structures of carbonic anhydrase II (CAII) complexed with sulfonamide inhibitors illuminate the structural determinants of high affinity binding in the nanomolar regime. The primary binding interaction is the coordination of a primary sulfonamide group to the active site zinc ion. Secondary interactions fine-tune tight binding in regions of the active site cavity >5 Å away from zinc, and this work highlights three such features: (1) advantageous conformational restraints of a bicyclic thienothiazene-6-sulfonamide-1,1-dioxide inhibitor skeleton in comparison with a monocyclic 2,5-thiophenedisulfonamide skeleton; (2) optimal substituents attached to a secondary sulfonamide group targeted to interact with hydrophobic patches defined by Phe131, Leu198, and Pro202; and (3) optimal stereochemistry and configuration at the C-4 position of bicyclic thienothiazene-6-sulfonamides; the C-4 substituent can interact with His64, the catalytic proton shuttle. Structure-activity relationships rationalize affinity trends observed during the development of brinzolamide (Azopt™), the newest carbonic anhydrase inhibitor approved for the treatment of glaucoma.

Keywords: drug design; protein crystallography; zinc enzyme

Human carbonic anhydrase II (CAII; EC 4.2.1.1) is a zinc metalloenzyme of 260 amino acids that catalyzes the reversible hydration of carbon dioxide to form bicarbonate ion plus a proton, $\text{H}_2\text{O} + \text{CO}_2 \leftrightarrow \text{HCO}_3^- + \text{H}^+$ (Silverman & Lindskog, 1988; Christianson & Fierke, 1996). At the base of the active site cavity, zinc is liganded by His94, His96, His119, and hydroxide ion with tetrahedral coordination geometry (Håkansson et al., 1992). Zinc-bound hydroxide donates a hydrogen bond to the hydroxyl side chain of Thr199, which in turn donates a hydrogen bond to Glu106. With $k_{\text{cat}}/K_M = 10^8 \text{ M}^{-1} \text{ s}^{-1}$, CAII is one of only a handful of enzymes that exhibits reaction kinetics approaching the limit of diffusion control. Catalytic turnover requires transfer of the product proton to bulk solvent via shuttle group His64 (Tu et al., 1989).

Although the carbonic anhydrase isozymes are localized in various organs, tissues, and cells throughout the body, CAII is of particular pharmaceutical interest. The activity of this isozyme is linked

to increased intraocular pressure, a major symptom of glaucoma (Friedenwald, 1949; Kinsey, 1953). Sulfonamide CAII inhibitors are effective in the control of intraocular pressure and are therefore useful in the treatment of glaucoma (Mann & Keilin, 1940; Becker, 1954; Maren, 1987). The high resolution crystal structures of CAII (Håkansson et al., 1992) and various sulfonamide inhibitor complexes show that an ionized sulfonamide nitrogen displaces zinc-bound hydroxide to form a stable enzyme-inhibitor complex with submicromolar to nanomolar affinity (Baldwin et al., 1989; Vidgren et al., 1990; Prugh et al., 1991; Bunn et al., 1994; Håkansson & Liljas, 1994; Jain et al., 1994; Smith et al., 1994; Boriack et al., 1995; Stams et al., 1998). Valuable insight on enzyme-inhibitor affinity is gained by studying these structures, and this insight is critical for understanding the molecular action of brinzolamide (Azopt™; Stams et al., 1998), the newest carbonic anhydrase inhibitor approved for the treatment of glaucoma.

Here, we report the crystal structure determinations of CAII-inhibitor complexes that illuminate specific affinity determinants in the CAII active site explored during the development of brinzolamide. The inhibitors found in Table 1 are novel in comparison with other CAII inhibitors reported to date, in that they contain two sulfonamide groups: a primary sulfonamide targeted for the critical zinc coordination interaction, and a secondary sulfonamide through which various N-substituted functional groups modulate enzyme-inhibitor interactions (Fig. 1). The structures of these CAII-

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Table 1. *CAII inhibitors*

Name	Label	Structure	K_d (nM)	PDB accession code
<i>N</i> -[(4-methylphenyl)methyl]-2,5-thiophenedisulfonamide	AL5917		0.46 ± 0.06	1BN1
<i>N</i> -[(4-methoxyphenyl)methyl]-2,5-thiophenedisulfonamide	AL5927		0.49 ± 0.11	1BN4
<i>N</i> -(2-thienylmethyl)-2,5-thiophenedisulfonamide	AL5415		0.83 ± 0.35	1BNW
3,4-dihydro-4-hydroxy-2-(2-thienylmethyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide	AL5300		0.20 ± 0.02	1BNU
3,4-dihydro-4-hydroxy-2-(4-methoxyphenyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide	AL5424		0.16 ± 0.01	1BNT
(<i>R</i>)-4-ethylamino-3,4-dihydro-2-(2-methoxyethyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide	AL4623		0.32 ± 0.05	1BNQ
(<i>R</i>)-4-ethylamino-3,4-dihydro-2-(3-methoxypropyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide (brinzolamide)	AL4862		0.13 ± 0.03	1A42
3,4-dihydro-2-(3-methoxyphenyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide	AL7182		0.10 ± 0.01	1BNN
(<i>R</i>)-3,4-dihydro-2-(3-methoxyphenyl)-4-methylamino-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide	AL7089		0.10 ± 0.01	1BNM
(<i>S</i>)-3,4-dihydro-2-(3-methoxyphenyl)-4-methylamino-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide	AL7099		1.70 (single)	1BNV
2-(3-methoxyphenyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide	AL6528		0.13 ± 0.03	1BN3

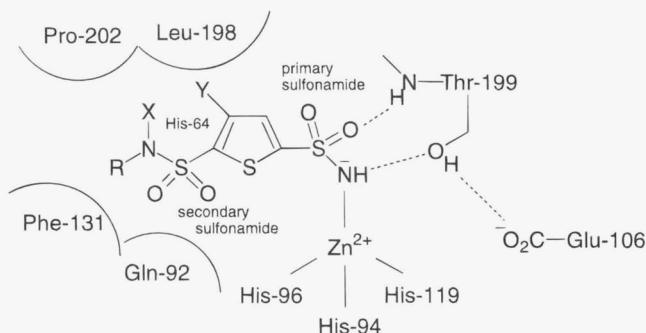


Fig. 1. General scheme of sulfonamide inhibitor binding to CAII. In this paper, the term “primary sulfonamide” refers to the group that ionizes and coordinates to the active site zinc ion and hydrogen bonds with Thr199. The term “secondary sulfonamide” refers to the group with the di- or tri-substituted nitrogen atom that interacts with secondary binding site determinants in the CAII active site, such as Phe131, Pro202, Leu198, Glu92, and/or His64.

inhibitor complexes allow for the rationalization of affinity trends observed early on in the exploration of inhibitor designs.

Results and discussion

For each sulfonamide inhibitor, the primary determinant of enzyme-inhibitor affinity is similar: the ionized nitrogen of the primary sulfonamide group coordinates to zinc and displaces hydroxide ion, thus maintaining tetrahedral coordination geometry. In addition, this nitrogen donates a hydrogen bond to the hydroxyl group of Thr199. One sulfonamide oxygen accepts a hydrogen bond from the backbone NH group of Thr199, and the other sulfonamide oxygen makes no intermolecular interactions. General intermolec-

ular interactions of the primary sulfonamide group are indicated schematically in Figure 1.

The primary sulfonamide group is connected to the C-2 atom of a thiophene ring in all inhibitors studied, so the resulting thiophene-2-sulfonamide comprises a molecular core that is common to all the inhibitors in Table 1. Derivatization of this core by substituting different functional groups on the secondary sulfonamide group at the C-5 atom of the thiophene ring, or by forming a bicyclic thienothiazine-6-sulfonamide-1,1-dioxide by fusing a six-membered ring to thiophene atoms C-4 and C-5, results in CAII inhibitors with varied affinity properties. Stereochemical and configurational variations at the C-4 position of thienothiazine-6-sulfonamide-1,1-dioxides also modulate enzyme-inhibitor affinity. Resultantly, inhibitor affinity is modulated by design variations at secondary sites somewhat distant ($>5\text{ \AA}$) from the primary site. The remainder of this section outlines three different secondary inhibitor design features that influence structure-affinity relationships.

Conformationally-locked thienothiazine-6-sulfonamide-1,1-dioxides bind more tightly than 2,5-thiophenedisulfonamides

The 2,5-thiophenedisulfonamide inhibitors AL5917, AL5927, and AL5415 bind with nearly equal affinities in the 0.5 nM range (Table 1). The lack of significant affinity variation among these inhibitors is notable given that different aromatic substituents are substituted on the secondary 5-sulfonamide group; however, each aromatic substituent interacts with Phe131. To illustrate, an electron density map of the CAII-AL5415 complex is found in Figure 2. Quadrupole-quadrupole interactions between the aromatic substituent of the inhibitor and Phe131 fix the inhibitor in place with optimal aromatic ring centroid-centroid separations of $\sim 5.5\text{ \AA}$ (Burley & Petsko, 1988). His64 adopts the “out” conformation (i.e., pointing away from the active site; see Nair & Christianson, 1991) in these

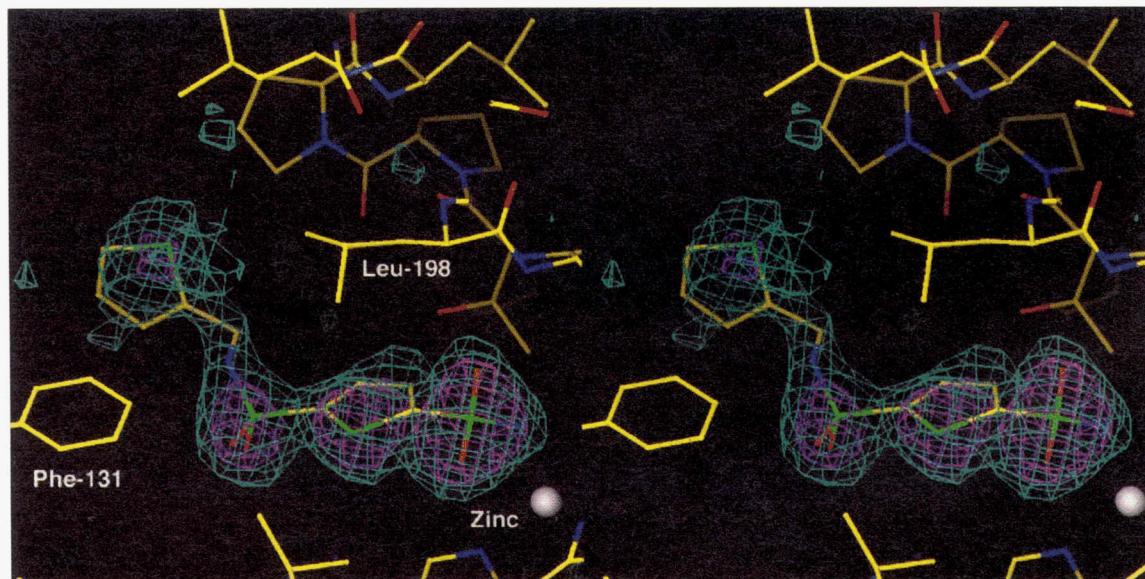


Fig. 2. Difference electron density map of the CAII-AL5415 complex calculated with Fourier coefficients $|F_o| - |F_c|$ and phases derived from the final model less the inhibitor. The cyan map is contoured at 2.5σ and the magenta map is contoured at 6.0σ (the higher contour level highlights the four electron rich sulfur atoms of the inhibitor); refined atomic coordinates are superimposed.

complexes with the 2,5-thiophenedisulfonamide inhibitors even though it is not sterically forced to do so (data not shown).

Tethering the secondary 5-sulfonamide group of AL5415 back to the C-4 atom of the thiophene ring to form the thienothiazine-6-sulfonamide-1,1-dioxide skeleton of AL5300 results in greater than fourfold enhancement of enzyme-inhibitor affinity (Table 1). The crystal structure of the CAII-AL5300 complex reveals a binding mode nearly identical to that of AL5415, with the coordination of the primary sulfonamide group to zinc being the principal affinity determinant. Since the affinity measurement was made on a racemic mixture, and since only the *R* enantiomer binds to the enzyme, the affinity enhancement is likely to be underestimated. Despite an $\sim 45^\circ$ difference in the orientation of the secondary sulfonamide group in the CAII-AL5415 and CAII-AL5300 complexes, the location of the thiophene ring and its interaction with Phe131 are identical for the two inhibitors (Fig. 3). Therefore, intermolecular interactions of the secondary sulfonamide group appear to be less critical for enzyme-inhibitor affinity than interactions of the aromatic substituent attached to this group. With primary and secondary binding interactions conserved between these two inhibitors, we conclude that most of the greater than fourfold increase in binding affinity observed for AL5300 results from preorganizing the secondary sulfonamide in an orientation that optimizes the edge-to-face interaction of its aromatic substituent with Phe131.

The six-membered thiazine ring of AL5300 adopts a conformation that we designate ‘‘half-chair₁’’; this places the C-4 hydroxyl group in a pseudo-equatorial conformation that allows it to hydrogen bond with the hydroxyl side chain of Thr200. However, results with AL7089 and AL7182 discussed later suggest that intermolecular interactions of certain C-4 substituents do not significantly impact affinity.

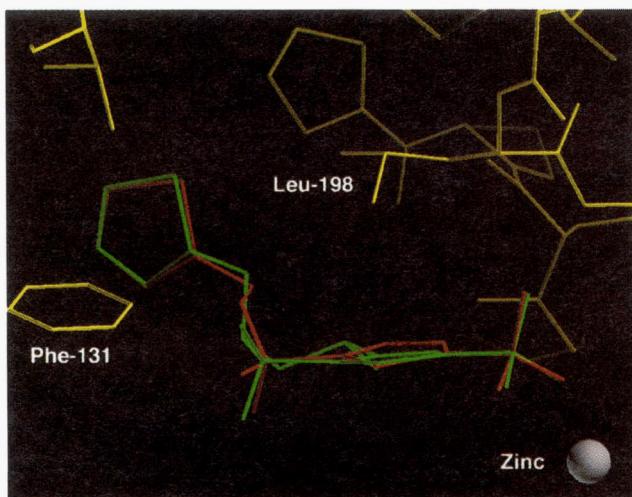


Fig. 3. Superposition of the atomic coordinates of AL5300 (green) and AL5415 (red). For clarity, only the protein atoms of CAII in the CAII-AL5415 complex are shown (yellow). Primary sulfonamide-zinc coordination geometry, and edge-to-face interactions between the thiophene ‘‘tail’’ of the inhibitor and Phe131, are identical in the two complexes. Binding difference are localized to the conformation of the secondary sulfonamide group.

Thienothiazine-6-sulfonamide-1,1-dioxide affinity is modulated by N-substituents on the secondary sulfonamide group

Inhibitors with certain aromatic or aliphatic groups attached to the secondary sulfonamide bind with K_d values in the 0.10–0.32 nM range (Table 1). First, consider the methoxyphenyl-substituted inhibitor AL5424, which binds with affinity roughly equal to that of AL5300. Not unexpectedly, the structure of the CAII-AL5424 complex reveals intermolecular interactions similar to those observed in the CAII-5415 and CAII-AL5300 complexes: the aromatic methoxyphenyl group attached to the secondary sulfonamide group engages in an edge-to-face interaction with Phe131. Furthermore, the thiazine ring adopts the same half-chair₁ conformation as observed for AL5300 (data not shown).

Notably, aliphatic ethers substituted in place of aromatic substituents on the secondary sulfonamide group are accommodated with minimal loss of affinity. First, the structures of the CAII-AL4623 and CAII-AL4862 complexes reveal that aliphatic ether substituents associate with Pro202 and Leu198. To illustrate, an electron density map of the CAII-AL4623 complex is found in Figure 4 (the structure of the CAII-AL4862 complex has been reported previously (Stams et al., 1998)). The loss of the quadrupole-quadrupole interaction between the aromatic ring substituent of the secondary sulfonamide group of the inhibitor and Phe131 has only a minor impact on enzyme-inhibitor affinity, as long as the substituted aliphatic group is sufficiently large to desolvate a correspondingly large hydrophobic patch in the enzyme active site. The slightly higher affinity of AL4862 compared with AL4623 may result from additional enzyme-inhibitor contact surface area provided by the additional methylene group; the binding conformations of these two inhibitors are otherwise identical (data not shown).

The six-membered thiazine ring of AL4623 adopts the half-chair₁ conformation, with the aliphatic ether and ethylamino groups adopting pseudo-equatorial conformations (Fig. 4). The bulky C-4 ethylamino group sterically displaces His64 to the ‘‘out’’ conformation. This is believed to contribute a factor of 5 to enzyme-inhibitor affinity for certain inhibitors (Smith et al., 1994) and may help compensate for any slight affinity losses that might otherwise result from the loss of inhibitor aromatic edge-to-face interactions with Phe131.

C-4 stereochemistry and configuration can compromise the affinity of thienothiazine-6-sulfonamide-1,1-dioxides

Given the potential importance of a C-4 substituent in triggering the conformational change of His64, we determined the structures of the CAII complexes with inhibitors AL7182, AL7089, and AL7099 to ascertain the precise contribution of this substituent and its stereochemistry on enzyme-inhibitor affinity. Interactions between the primary sulfonamide and zinc, and the secondary sulfonamide methoxyphenyl substituent and Phe131, are maintained among all three inhibitors. Additionally, the side chain of His64 is displaced to the ‘‘out’’ conformation in all three complexes (even though it is not sterically forced to be so in the CAII-AL7182 complex). However, the conformations of the six-membered thiazine rings are quite different (Fig. 5). The thiazene rings of AL7182 and AL7099 adopt similar half-chair₁ conformations, whereas the thiazene ring of AL7089 is flipped to the opposite, half-chair₂ conformation. Slight differences are also observed in the configuration of the thiazene ring nitrogen atom, which tends toward

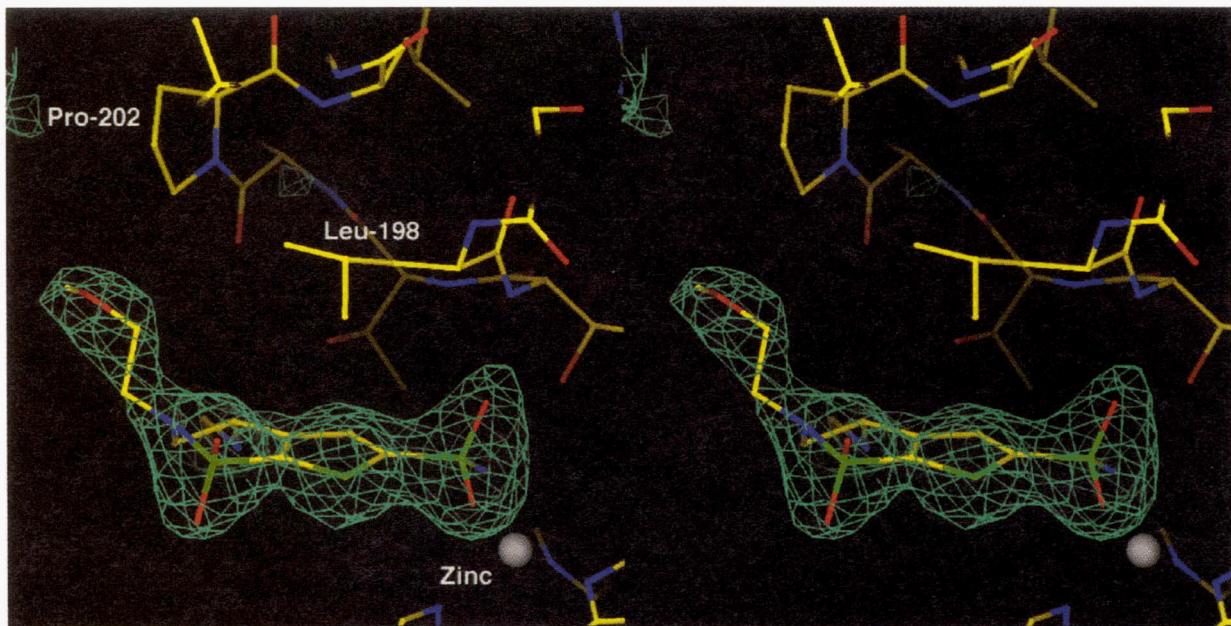


Fig. 4. Difference electron density map of the CAII-AL4623 complex calculated with Fourier coefficients $|F_o| - |F_c|$ and phases derived from the final model less the inhibitor. The map is contoured at 2.5σ and refined atomic coordinates are superimposed.

pyramidal geometry in AL7182 and AL7089 and planar geometry in AL7099 (data not shown). This difference may contribute to the lower affinity of AL7099.

The stereochemistry of the C-4 methylamino group affects the binding conformation of the inhibitor as reflected by the orientation of the thiophene rings of AL7089, AL7099, and AL7182 relative to the primary sulfonamide group. The binding mode of AL7182 illustrates the preferred position and conformation of the thienothiazene-6-sulfonamide-1,1-dioxide ring system in the absence of a C-4 substituent. The addition of a methylamino group with *R* stereochemistry (AL7089) changes the orientation of the

thiophene ring by 10° (Fig. 5). The essentially equal affinities of AL7089 and AL7182 (Table 1) suggest that the energetic benefit of any additional contact surface area provided by the C-4 substituent, as well as the possible energetic benefit of triggering the conformational change of His64 to the “out” position, is offset by the slight change in the orientation of the thiophene ring. The addition of a methylamino group with *S* stereochemistry (AL7099) changes the orientation of the thiophene ring by 20° . However, the affinity of AL7099 is 17-fold weaker than that of AL7182. Clearly, this stereochemistry and conformation cannot be easily accommodated in the CAII active site.

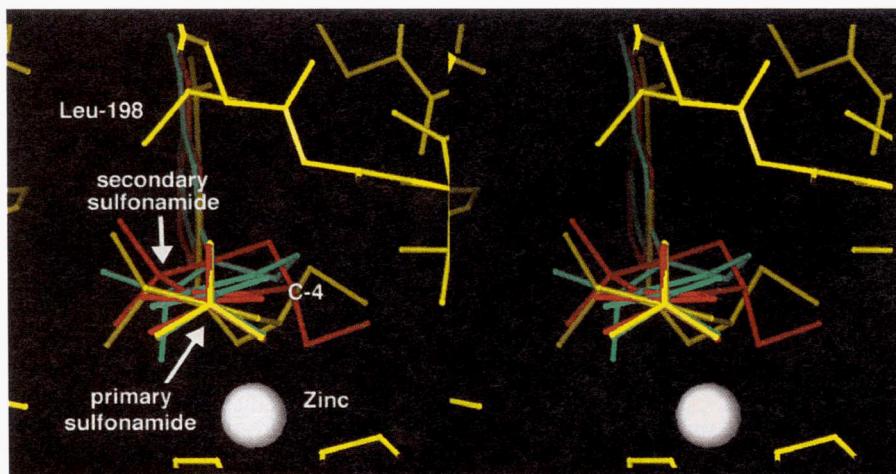


Fig. 5. Superposition of the atomic coordinates of AL7089 (red), AL7099 (yellow), and AL7182 (cyan). For clarity, only the protein atoms of CAII in the CAII-AL7182 complex are shown (yellow). Note the variable conformation of the six-membered thiazine ring, and the primary sulfonamide-thiophene ring dihedral angle, resulting from variations at the inhibitor C-4 atom.

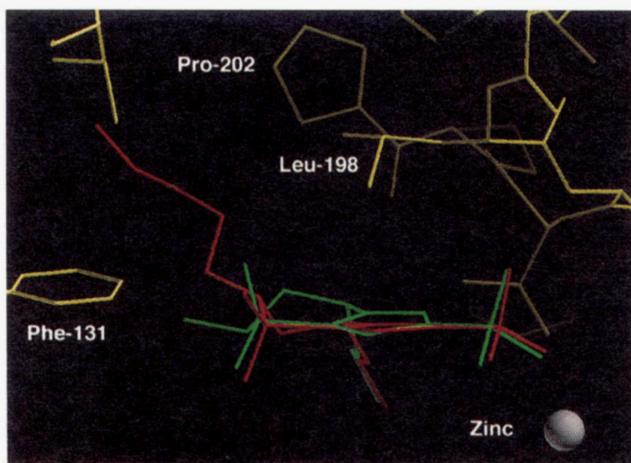


Fig. 6. Superposition of the atomic coordinates of brinzolamide (AzoptTM; AL4862, $K_d = 0.13$ nM) and dorzolamide (TrusoptTM, $K_i = 0.37$ nM; Greer et al., 1994; Smith et al., 1994), the two newest CAII inhibitors approved for the treatment of glaucoma. Brinzolamide is red; dorzolamide is green. For clarity, only the protein atoms of CAII in the CAII-AL4862 (brinzolamide) complex are shown (yellow). Note that the six-membered thiazene ring of brinzolamide adopts a half-chair₁ conformation, whereas the six-membered thieno ring of dorzolamide adopts a half-chair₂ conformation.

Interestingly, in the absence of a C-4 substituent the thiazine ring of AL7182 can be locked by introducing an additional degree of unsaturation between C-3 and C-4 to yield AL6528, which is essentially equipotent (Table 1). This conformationally-rigid inhibitor binds tightly in the enzyme active site in a manner similar to that of AL7182, without benefit of a flexible thiazine ring or a substituent at the C-4 position. Here, too, His64 is displaced to the “out” conformation even though it is not sterically forced to be so (data not shown). Therefore, neither a C-4 substituent nor conformational flexibility of the thiazine ring is required to achieve enzyme-inhibitor affinity in the 0.1 nM regime.

Conclusions

Structure-affinity relationships discerned in the current study are approximations, since other factors such as desolvation and conformational differences between free and complexed enzyme and inhibitor are not taken into account. Nevertheless, it is clear that the bicyclic thienothiazene-6-sulfonamide-1,1-dioxide ring system comprises a superior molecular scaffolding that can easily be derivatized and optimized for high-affinity binding to CAII. Secondary enzyme-inhibitor interactions >5 Å away from the active site zinc ion can be targeted by derivatization of the secondary sulfonamide group with aromatic or aliphatic substituents that interact with hydrophobic patches largely defined by Phe131, Leu198, and Pro202. The conformation and interactions of the secondary sulfonamide group itself appear to be much less important than the interactions of the functional groups attached to it. In particular, the aromatic-aromatic edge-to-face interaction between an inhibitor substituent and Phe131 appears to be critical. It is intriguing that AL7089 is the only thienothiazene-6-sulfonamide-1,1-dioxide inhibitor to adopt the half-chair₂ conformation, and this conformation is accommodated with sub-nanomolar affinity. This is consistent with the binding of dorzolamide (TrusoptTM; Greer et al., 1994; Smith et al., 1994), in which the six-membered thieno ring adopts a similar half-chair₂ conformation (Fig. 6).

Locking the conformation of the six-membered thiazine ring by introducing an additional degree of unsaturation (compare AL7182 and AL6528) maintains enzyme-inhibitor affinity in the 0.1 nM regime. The hybridization of the thiazine nitrogen appears to play some role in governing affinity. Comparison of AL7089, AL7182, and AL7099 binding modes suggests that this nitrogen can undergo a change from pyramidal to planar configurations to accommodate inhibitor binding. The configurational flexibility of the thiazine nitrogen is an unexpected benefit of the thienothiazene-6-sulfonamide-1,1-dioxide ring system and may be exploited in the structure-based design of high affinity ligands to other protein receptors.

Table 2. Data collection and refinement statistics for CAII-inhibitor complexes

Inhibitor	AL5917	AL5927	AL5415	AL5300	AL5424	AL4623	AL7182	AL7089A	AL7099A	AL6528
No. crystal	1	1	1	1	1	1	1	1	1	1
No. measured reflections	51,323	51,206	22,136	31,207	31,321	15,724	39,386	22,271	23,968	31,886
No. unique reflections ($>2\sigma$)	13,725	13,265	10,279	10,603	10,031	7,686	10,818	7,539	8,174	12,088
Completeness of data (%)	93.1	90.0	80.2	77.1	73.0	77.5	96.2	96.4	82.5	94.2
Maximum resolution (Å)	2.1	2.1	2.25	2.15	2.15	2.4	2.3	2.6	2.4	2.2
$R_{\text{merge}}^{\text{a}}$	0.063	0.036	0.088	0.078	0.099	0.102	0.088	0.096	0.085	0.077
No. reflections used in refinement (6.5-max. resolution)	13,151	12,725	9,623	10,206	9,643	7,301	10,237	7,043	7,788	11,539
R_{cryst}	0.166	0.163	0.145	0.156	0.165	0.143	0.160	0.137	0.139	0.170
No. waters in final cycle of refinement	128	128	66	59	62	57	99	67	71	117
RMS derivations										
Bonds (Å)	0.008	0.007	0.012	0.012	0.011	0.014	0.008	0.008	0.009	0.009
Angles (°)	1.7	1.7	3.0	3.0	2.9	3.1	1.8	1.9	1.9	1.7
Improper angles (°)	25.3	25.3	26.1	26.0	26.0	25.9	25.2	25.4	25.0	25.5
Dihedral angles (°)	1.3	1.3	1.2	1.2	1.1	1.2	1.3	1.3	1.4	1.4

^a R_{merge} for replicate reflections, $R = \sum |I_{hi} - \langle I_h \rangle| / \sum \langle I_h \rangle$; I_{hi} = intensity measured for reflection h in data set i , $\langle I_h \rangle$ = average intensity for reflection h calculated from replicate data.

^b Crystallographic R -factor, $R_{\text{cryst}} = \sum ||F_o|| - ||F_c|| / \sum ||F_o||$; $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

Materials and methods

Crystals of recombinant CAII were grown using the sitting drop method, requiring the addition of a 5 μL drop containing 6.7–7.4 mg/mL enzyme and 50 mM Tris-HCl (pH 8.0 at room temperature) to a 5 μL drop containing 50 mM Tris-HCl with 1.75–2.50 M ammonium sulfate (pH 8.0 at room temperature) in the crystallization well. For each crystallization trial, the enzyme drops were saturated with methyl mercuric acetate to promote the growth of diffraction quality parallelepipeds within two weeks.

Inhibitors were synthesized using standard techniques; full details of the synthesis, molecular characterization, enzymology, and pharmacology will be reported at a later date (T. Dean, unpubl. results). Inhibition of CAII was determined using published techniques (Chen & Kernohan, 1967; Ponticello et al., 1987). No unusual or slow binding kinetics were observed for any of the inhibitors studied.

CAII-inhibitor complexes were prepared by soaking crystals in buffer solutions containing inhibitors. Prior to crystal soaking experiments, crystals were transferred to a buffer solution of 4.0 M K_2HPO_4 (pH = 10.0) and cross-linked with 0.1% (vol/vol) gluteraldehyde for 6–8 h. Subsequently, crystals were transferred over a period of 2–7 days into a buffer solution containing 4–8 mM inhibitor dissolved in DMSO such that the final concentration of DMSO in the crystal soaking solution was less than 10% (vol/vol). After soaking in inhibitor solutions for 1–2 weeks, crystals were harvested, mounted, and sealed in 0.5 or 0.7 mm glass capillaries with a small portion of mother liquor.

X-ray diffraction data were collected using either a Siemens X-100A multiwire area detector or an R-AXIS IIC image plate detector (Molecular Structure Corporation, The Woodlands, Texas). A Rigaku RU-200HB rotating anode X-ray generator equipped with double focusing mirrors supplied Cu-K α radiation. All X-ray diffraction data were collected at room temperature by the oscillation method. Intensity data were processed with MOSFLM and CCP4 programs (Leslie, 1992; CCP4, 1994). All crystals belonged to space group P2₁ with typical unit cell parameters $a = 42.7 \text{ \AA}$, $b = 41.7 \text{ \AA}$, $c = 73.0 \text{ \AA}$, and $\beta = 104.6^\circ$.

The structure of refined human CAII (Håkansson et al., 1992) was the starting point for refinement with X-PLOR (Brünger et al., 1987). Inhibitor atoms and active site solvent molecules were added and refined when the crystallographic *R*-factor dropped below 0.200. The refinement of each enzyme-inhibitor complex converged smoothly to low final crystallographic *R*-factors and each final model exhibited good stereochemistry. Pertinent refinement statistics for all CAII-inhibitor structures are recorded in Table 2, and the coordinates of all CAII-inhibitor complexes have been deposited in the Brookhaven Protein Data Bank (accession codes are listed in Table 1).

Acknowledgments

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