Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 6204–6208
Bioorganic & Medicinal Chemistry Letters

Carbonic anhydrase inhibitors: X-ray crystallographic studies for the binding of 5-amino-1,3,4-thiadiazole-2-sulfonamide and 5-(4-amino-3-chloro-5-fluorophenylsulfonamido)-1,3,4-thiadiazole-2-sulfonamide to human isoform II

Valeria Menchise,[a] Giuseppina De Simone,[a,*] Anna Di Fiore,[a] Andrea Scozzafava[b] and Claudiu T. Supuran[b,*]

[a]*Istituto di Biostrutture e Bioimmagini-CNR, via Mezzocannone 16, 80134 Naples, Italy*
[b]*Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy*

Received 18 July 2006; revised 7 September 2006; accepted 9 September 2006
Available online 26 September 2006

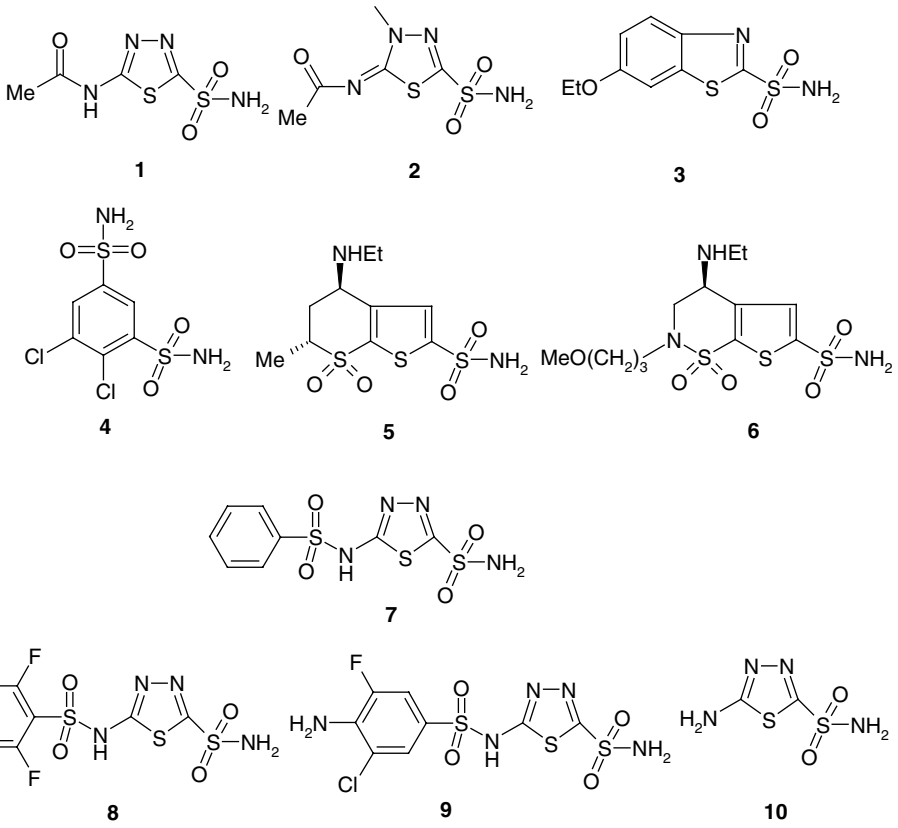
Abstract—The X-ray crystal structures of 5-amino-1,3,4-thiadiazole-2-sulfonamide (the acetazolamide precursor) and 5-(4-amino-3-chloro-5-fluorophenylsulfonamido)-1,3,4-thiadiazole-2-sulfonamide in complex with the human isozyme II of carbonic anhydrase (CA, EC 4.2.1.1) are reported. The thiadiazole-sulfonamide moiety of the two compounds binds in the canonic manner to the zinc ion and interacts with Thr199, Glu106, and Thr200. The substituted phenyl tail of the second inhibitor was positioned in the hydrophobic part of the binding pocket, at van der Waals distance from Phe131, Val 135, Val141, Leu198, Pro202, and Leu204. These structures may help in the design of better inhibitors of these widespread zinc-containing enzymes.

The metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) catalyzes a very simple but critically important physiological reaction: the interconversion between carbon dioxide, generated in huge amounts in all metabolic processes, and the bicarbonate ion.[1–6] Inhibitors of these zinc-containing enzymes show a multitude of applications as diuretic, antiglaucoma, antiobesity or antitumor drugs, being also used as diagnostic tools.[1–6] Various CA isoforms are responsible for specific physiological functions, and drugs with such a diversity of actions target different isozymes of the 15 presently known in humans.[2–6] In all of them, the inhibitor is bound as anion to the catalytically critical Zn^{2+} ion, also participating in extensive hydrogen bond networks and van der Waals interactions with amino acid residues both in the hydrophobic and hydrophilic halves of the enzyme active site, as shown by X-ray crystallographic studies of such enzyme–inhibitor complexes.[7–14] Among the three main classes of potent CA inhibitors (CAIs) described up to now, the sulfonamides, the sulfamates, and the sulfamides, the first one is the most investigated, since classical, clinically used drugs such as acetazolamide **1**, methazolamide **2**, ethoxzolamide **3**, dichlorophenamide **4**, dorzolamide **5**, and brinzolamide **6** all belong to it.[1–6] X-ray crystal stuctures are available for adducts of several isozymes (i.e., CA I, II, IV, V, XII, and XIV)[7–14] mostly with sulfonamides, with several sulfamates and few sulfamides.[10]

Novel sulfonamide derivatives are thus constantly synthesized and investigated as inhibitors of various CA enzymes (from vertebrates or other organisms) in the search of compounds with selectivity for some of the many physiologically relevant isoforms, or for new applications, since the clinically used drugs **1**–**6** unselectively inhibit many CA isozymes and as a consequence, show many undesired side effects.[1–6] For example, a fluorescent sulfonamide recently reported by our group is in clinical development as a diagnostic agent, allowing the imaging of acute hypoxic tumors which are non-responsive to classical chemo- and radio-therapy.[7,15]

Keywords: Carbonic anhydrase; Sulfonamide; 1,3,4-Thiadiazole; X-ray crystallography; Enzyme inhibitor.
* Corresponding authors. Tel.: +39 081 2534579; fax: +39 081 2536642 (G.D.S.), tel.: +39 055 4573005; fax: +39 055 4573385 (C.T.S.); e-mail addresses: gdesimon@unina.it; claudiu.supuran@unifi.it

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.09.022



Benzolamide **7**, an orphan drug[16,17] behaving as a very potent CAI, although quite similar structurally with acetazolamide, **1**, the CAI *par excellence*,[1] possesses different physico-chemical properties, due to the presence of the second, highly acidic (p*K*a of 3.4)[17] sulfonamide moiety in its molecule. In consequence, **7** is much more polar than **1**, does not possess antiglaucoma properties when given systemically (in contrast to acetazolamide or methazolamide), and to a certain extent, crosses biological membranes much more difficultly as compared to other CAIs in clinical use.[1,16,17] However, benzolamide **7** was very much used as a lead molecule for the design of diverse CAIs,[16,18,19] since the X-ray crystal structures of some of its derivatives with CA II[12,20] showed very favorable binding between the enzyme and inhibitor. The modifications of the molecule needed to 'correct' some of its physico-chemical properties were those regarding its too hydrophilic character and lack of water/lipid solubility. Indeed, some benzolamide derivatives reported by this group, which incorporated polyhalogenophenylsulfonamide moieties instead of the phenylsufonamide one of **7**, of types **8** and **9** among others,[19,21] were shown to generally maintain the excellent inhibitory properties of the lead compound **7**, but also to possess much higher solubility both in water as well as organic solvents (due to the presence of the halogen atoms in their molecules) as well as excellent penetrability through biological membranes. Some of these compounds (such as **8**)[19] were shown to act as excellent inhibitors of isoforms CA II and IV (involved in glaucomagenesis) and to possess topical activity as antiglaucoma agents in an animal model of the disease, whereas others, among which **9**, were shown to strongly inhibit the tumor-associated transmembrane isoform CA IX (in addition to CA I and II, the classical, cytosolic isoforms).[21] In order to better understand the interactions between such potent CAIs and the enzyme at molecular level, which might be quite useful for the design of compounds with improved pharmacological properties, we investigated by means of X-ray crystallography the binding of derivatives **8** and **9** to the widespread, physiologically most important isoform of CA, that is, hCA II (h means enzyme of human origin).

The crystallographic structures of adducts of these two compounds with hCA II were solved by difference Fourier techniques, the crystals being isomorphous with those of the native protein.[22] The crystallographic *R*-factor and *R*-free, calculated in the 20.00–1.55 Å resolution range for the structure of the hCA II–**8**, were 0.194 and 0.215, respectively, while the last refinement cycle of the hCA II–**9** structure yielded a *R*-factor of 0.184 and a *R*-free of 0.223, calculated in the 20.00–2.10 Å resolution range. For both structures, 100% of the non-glycine residues were located in the allowed regions of the Ramachandran plot. The statistics for data collection and refinement are summarized in Table 1.

The superimposition of all the Cα atoms of the hCA II proteins in the two adducts with those of the protein in the unbound form (PDB code 1CA2)[22] yielded to r.m.s.d. values of 0.35 and 0.36 Å, respectively. According

Table 1. Crystal parameters, data-collection and refinement statistics for the hCA II–**10** and hCA II–**9** complexes

	hCA II–**10**	hCA II–**9**
Crystal parameters		
Space group	$P2_1$	$P2_1$
Unit-cell parameters (Å,°)	$a = 42.11$	$a = 42.10$
	$b = 41.31$	$b = 41.40$
	$c = 71.89$	$c = 71.73$
	$\beta = 104.35$	$\beta = 104.31$
Data collection statistics		
Resolution range (Å)	20.00–1.55	20.00–2.10
Temperature (K)	100	100
Total reflections	85,927	38,885
Unique reflections	34,175	12,269
Completeness (%)	97.8 (86.0)	87.0 (75.6)
R-sym*	0.048 (=0.164)	0.080 (0.236)
Mean *I*/sigma(*I*)	15.8 (5.52)	13.4 (4.85)
Refinement statistics		
Resolution range (Å)	20.00–1.55	20.00–2.10
R-factor** (%)	0.194	0.184
R-free** (%)	0.215	0.223
r.m.s.d. from ideal geometry		
Bond lengths (Å)	0.007	0.009
Bond angles (°)	1.4	1.3
Number of protein atoms	2049	2059
Number of inhibitor atoms	10	44
Number of water molecules	198	161
Average B factor	13.7	18.9

Values in parentheses refer to the highest resolution shell.
* $R_{sym} = \sum|I_i - \langle I\rangle|/\sum I_i$; over all reflections.
** $R_{factor} = \sum|F_o - F_c|/\sum F_o$; R_{free} calculated with 5% of data withheld from refinement.

to these values, the three-dimensional structure of the hCA II molecule within both complexes was very similar to that observed in the unbound enzyme and in other hCA II–inhibitor complexes reported to date.[3,4]

Inspection of the electron density maps around the catalytic site in the hCA II–**8** structure clearly showed the presence of one inhibitor molecule bound within the active site. However, already from the first stage of the refinement the shape of the electron density around the inhibitor did not appear compatible with its chemical structure. In particular, no electron density was observed for the 2,3,5,6-tetrafluorophenylsulfonyl moiety, suggesting a cleavage of the secondary sulfonamide bond present in **8**. The structure of the species formed after the release of the 2,3,5,6-tetrafluorophenylsulfonyl group, that is, 5-amino-1,3,4-thiadiazole-2-sulfonamide **10**, perfectly fitted the shape of the electron density (Fig. 1). In fact, we obtained the high resolution X-ray crystal structure of the adduct of hCA II with **10**, and not with **8**. In this structure, the tetrahedral geometry of the Zn^{2+} binding site and the key hydrogen bonds between the sulfonamide moiety of the inhibitor **10** and the enzyme active site were all retained with respect to other hCA II–sulfonamide complexes solved so far.[1–3] Thus, the ionized sulfonamide moiety displaced the hydroxyl ion/water molecule present in the native enzyme, coordinating the zinc ion through its nitrogen atom (2.00 Å). This nitrogen atom was also involved in a hydrogen bond with the hydroxyl group of Thr199 (2.72 Å), which

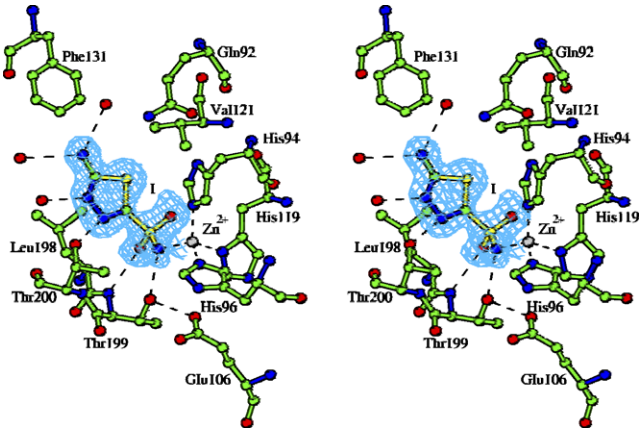


Figure 1. Active site region in the hCA II–**10** complex. The inhibitor is shown associated with simulated annealing omit $|2F_o - F_c|$ electron density map,[25] computed at 1.55 Å and contoured at 1.0σ.

in turn accepted a hydrogen bond from Glu106OE1 atom (2.58 Å). One sulfonamide oxygen formed a hydrogen bond with the backbone NH moiety of Thr199 (2.96 Å), while the other one was semi-coordinated to the Zn^{2+} ion (3.05 Å). The thiadiazole ring was located in the active site channel where it was involved in several van der Waals interactions with the side chains of residues Gln92, Val121, Phe131, Leu198, Thr199, and Thr200. One nitrogen of the heterocyclic ring participated in a hydrogen bond with the hydroxyl group of Thr200 (2.87 Å) and the other one was bound to a water molecule by means of another hydrogen bond (2.87 Å). The exocyclic nitrogen atom was hydrogen bonded to two water molecules (3.01 and 3.28 Å, respectively), while other water molecules and one glycerol molecule filled the active site pocket of the protein. The main protein–inhibitor interactions are schematically shown in Figure 1.

The analysis of the structure of the hCA II–**9** adduct clearly showed the presence of one inhibitor molecule in the active site, which in this case was entirely defined by the electron density map, indicating that the inhibitor was not hydrolyzed. A second molecule of inhibitor **9**, with an occupancy factor of 0.8, was found in the region close to residue Cys206 (outside the active site), stabilized by a face-to-face stacking interaction between its substituted-phenyl ring and the benzene ring of the 4-(hydroxymercury)benzoic acid, a compound usually used in the crystallization solution for enhancing the quality of the crystals, which is covalently bound to Cys206.[7–14] This crystallographic artifact did not affect the binding mode of compound **9** within the enzyme active site, since this second molecule was located on the surface of the protein, far away of the catalytic site (data not shown).

The inhibitor **9** (situated within the active site) presented all the interactions generally observed in the complexes of hCA II with sulfonamides and sulfamates.[1–3] The main protein–inhibitor interactions are schematically depicted in Figure 2. In particular, the deprotonated primary sulfonamide nitrogen atom coordinated the Zn^{2+} ion (1.98 Å) and was involved in a hydrogen bond

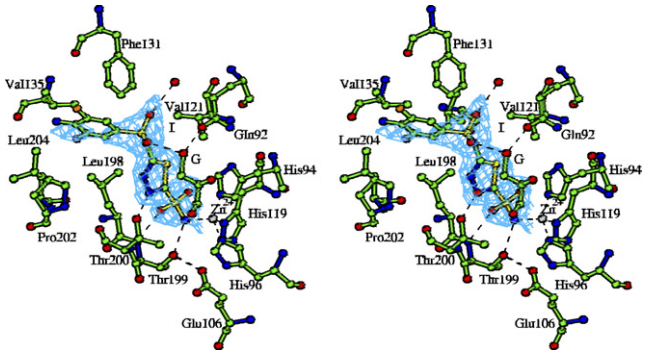


Figure 2. Active site region in the hCA II–**9** complex. The inhibitor is shown associated with simulated annealing omit $|2F_o - F_c|$ electron density map,[25] computed at 2.10 Å and contoured at 1.0σ.

with the side-chain oxygen of Thr199 (2.64 Å), which was also hydrogen bonded to the Glu106OE1 atom (2.40 Å). One oxygen of the primary sulfamoyl moiety also accepted a hydrogen bond from the backbone NH of Thr199 (2.88 Å), while the other one was 2.98 Å away from the catalytic Zn^{2+} ion. The tetrahedral coordination site of the Zn^{2+} ion was completed by the imidazolic nitrogens of His94, His96, and His119. Several polar and hydrophobic interactions stabilized the organic scaffold of the inhibitor **9** within the enzyme active site. In particular, the thiadiazole ring was located in the active site channel where it established several van der Waals interactions with side chains of residues Gln92, Val121, Phe131, Leu198, Thr199, and-Thr200, and it was hydrogen bonded with one of its nitrogen atoms to the hydroxyl group of Thr200 (3.19 Å), similar to the derivative **10** discussed above. One oxygen of the secondary sulfonamide moiety made a hydrogen bond with a water molecule (3.25 Å), while the second one was hydrogen bonded to an oxygen of one glycerol molecule (2.70 Å), which in turn was stabilized by hydrogen bond interactions with the side chain of Gln92 and Asn67, two residues located toward the entrance of the enzyme active site. Finally the substituted phenyl tail of **9** was positioned in the hydrophobic part of the binding pocket, at van der Waals distance from residues Phe131, Val135, Val141, Leu198, Pro202, and Leu204 (all of them known to be important for their interaction with other thiadiazolesulfonamide derivatives complexed with hCA II).[1,3,12,20]

Although **8** and **9** have quite similar structures (being halogeno-substituted benzolamides) and the arrangement of their thiadiazolyl-sulfonamide moieties within the active site of the enzyme is very similar, the chemical behavior of the two molecules as evidenced by these studies is substantially different. Compound **8** was found in the active site in the hydrolyzed form, the electron density maps being defined for its precursor, **10**, that is, 5-amino-1,3,4-thiadiazole-2-sulfonamide. It is interesting to note that although **10** is the precursor of the most investigated and used CAI, acetazolamide **1**, its complexes with CA isozymes have never been investigated up to now by means of X-ray crystallography. Indeed, compound **10** is a rather weak CA II inhibitor (K_I of 60 nM) as compared to acetazolamide **1** (K_I of 12 nM) or the derivative **9** (K_I of 0.3 nM).[1,21] On the other hand, **9** is entirely defined by the electron density maps. This can be ascribed to the different substitution pattern of the phenylsulfonamide moiety in compounds **8** and **9**, which affects the polarization of the sulfur–nitrogen bond of the secondary sulfonamide moiety. In particular, the inductive effect due to the four highly electronegative fluorine atoms on the phenyl ring (compound **8**) makes the sulfur atom much more susceptible to nucleophilic attack and ultimately to hydrolysis. The inductive effect due to the two halogen atoms in compound **9** is probably less intense, and it is counteracted by the electron-donating resonance effect of the amino group in the *para* position. This makes **9** unsusceptible to hydrolysis. Indeed, although **8** and its congeners were very effective and pharmacologically appropriate CAIs for a putative use as topically acting antiglaucoma drugs, they were not developed clinically due to their lack of stability, and hydrolysis after several days/weeks in solution.[19] On the contrary, compounds possessing the substitution pattern present in **9** (i.e., a 4-amino group and only one or two halogen atoms) are not sensitive to hydrolysis, making them much more appropriate for various pharmacological applications.[21,23]

Acknowledgments

We thank Sincrotrone Trieste C.N.R./Elettra, for giving us the opportunity to collect data at the Crystallographic Beamline. This research was financed in part by a grant of the 6th Framework Programme of the European Union (EUROXY project) and by an Italian FIRB project (MIUR/FIRB RBNE03PX83_001).

References and notes

1. Supuran, C. T.; Scozzafava, A.; Conway, J. *Carbonic Anhydrase—Its Inhibitors and Activators*; CRC Press: Boca Raton, New York, London, 2004, p 1.
2. (a) Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 199; (b) Supuran, C. T.; Scozzafava, A.; Casini, A. Development of Sulfonamide Carbonic Anhydrase Inhibitors. In *Carbonic Anhydrase—Its Inhibitors and Activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC Press: Boca Raton, 2004; p 67.
3. (a) Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146; (b) Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2004**, *14*, 667.
4. Winum, J. Y.; Scozzafava, A.; Montero, J. L.; Supuran, C. T. *Med. Res. Rev.* **2005**, *25*, 186.
5. Winum, J.-Y.; Scozzafava, A.; Montero, J.-L.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2006**, *16*, 27.
6. Winum, J.-Y.; Scozzafava, A.; Montero, J.-L.; Supuran, C.T. *Med. Res. Rev.* **2006**, *26*, in press.
7. Alterio, V.; Vitale, R. M.; Monti, S. M.; Pedone, C.; Scozzafava, A.; Cecchi, A.; De Simone, G.; Supuran, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 8329.
8. Menchise, V.; De Simone, G.; Alterio, V.; Di Fiore, A.; Pedone, C.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 5721.
9. (a) De Simone, G.; Di Fiore, A.; Menchise, V.; Pedone, C.; Antel, J.; Casini, A.; Scozzafava, A.; Wurl, M.; Supuran,

C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2315; (b) Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schafer, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 841; (c) Abbate, F.; Casini, A.; Owa, T.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 217.

10. Abbate, F.; Supuran, C. T.; Scozzafava, A.; Orioli, P.; Stubbs, M.; Klebe, G. *J. Med. Chem.* **2002**, *45*, 3583.
11. (a) Boriack, P. A.; Christianson, D. W.; Kingery-Wood, J.; Whitesides, G. M. *J. Med. Chem.* **1995**, *38*, 2286; (b) Stams, T.; Chen, Y.; Boriack-Sjodin, P. A.; Hurt, J. D.; Liao, J.; May, J. A.; Dean, T.; Laipis, P.; Silverman, D. N.; Christianson, D. W. *Protein Sci.* **1998**, *7*, 556; (c) Kim, C. Y.; Chang, J. S.; Doyon, J. B.; Baird, T. T.; Fierke, C. A.; Jain, A.; Christianson, D. W. *J. Am. Chem. Soc.* **2000**, *122*, 12125; (d) Whittington, D. A.; Waheed, A.; Ulmasov, B.; Shah, G. N.; Grubb, J. H.; Sly, W. S.; Christianson, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9545.
12. Vidgren, J.; Svensson, A.; Liljas, A. *Int. J. Biol. Macromol.* **1993**, *15*, 97.
13. (a) Abbate, F.; Winum, J. Y.; Potter, B. V.; Casini, A.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 231; (b) Lloyd, M. D.; Pederick, R. L.; Natesh, R.; Woo, L. W.; Purohit, A.; Reed, M. J.; Acharya, K. R.; Potter, B. V. *Biochem. J.* **2005**, *385*, 715; (c) Lloyd, M. D.; Thiyagarajan, N.; Ho, Y. T.; Woo, L. W.; Sutcliffe, O. B.; Purohit, A.; Reed, M. J.; Acharya, K. R.; Potter, B. V. *Biochemistry* **2005**, *44*, 6858.
14. (a) Gruneberg, S.; Stubbs, M. T.; Klebe, G. *J. Med. Chem.* **2002**, *45*, 3588; (b) Antel, J.; Weber, A.; Sotriffer, C. A.; Klebe, G. Multiple Binding Modes Observed in X-ray Structures of Carbonic Anhydrase Inhibitor Complexes and Other Systems: Consequences for Structure-Based Drug Design. In *Carbonic Anhydrase—Its Inhibitors and Activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC Press: Boca Raton, 2004; p 45.
15. (a) Svastova, E.; Hulikova, A.; Rafajova, M.; Zat'ovicova, M.; Gibadulinova, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C. T.; Pastorek, J.; Pastorekova, S. *FEBS Lett.* **2004**, *577*, 439; (b) Cecchi, A.; Hulikova, A.; Pastorek, J.; Pastorekova, S.; Scozzafava, A.; Winum, J.-Y.; Montero, J.-L.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 4834.
16. Supuran, C. T.; Ilies, M. A.; Scozzafava, A. *Eur. J. Med. Chem.* **1998**, *33*, 739.
17. Maren, T. H. Benzolamide—A Renal Carbonic Anhydrase Inhibitor. In *Orphan Drugs*; Karch, F. E., Ed.; Dekker: New York, 1982; p 89.
18. Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Starnotti, M.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2867.
19. (a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4542; (b) de Leval, X.; Ilies, M.; Casini, A.; Dogné, J. M.; Scozzafava, A.; Masini, E.; Mincione, F.; Starnotti, M.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 2796.
20. Abbate, F.; Casini, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2357.
21. Ilies, M. A.; Vullo, D.; Pastorek, J.; Scozzafava, A.; Ilies, M.; Caproiu, M. T.; Pastorekova, S.; Supuran, C. T. *J. Med. Chem.* **2003**, *46*, 2187.
22. Eriksson, A. E.; Jones, T. A.; Liljas, A. *Proteins Struct. Funct.* **1988**, *4*, 274.
23. Crystals of hCA II–**10** and hCA II–**9** complexes were obtained by cocrystallization experiments, adding a 5-molar excess of inhibitor[19,21] to a 10 mg/mL protein solution in 100 mM Tris–HCl buffer, pH 8.5. The crystallization solution contained for both adducts 2.5 M $(NH_4)_2SO_4$, 0.3 M NaCl, 100 mM Tris–HCl (pH 8.2), and 5 mM 4-(hydroxymercurybenzoic) acid, to improve the crystal quality. Complete data sets were collected at 100 K, at the Synchrotron source Elettra in Trieste, Italy, using a Mar CCD detector. The resolutions of the data collections were 1.55 and 2.10 Å for the hCA II–**10** and for hCA II–**9** complexes, respectively. Prior to cryogenic freezing, the crystals were transferred to the precipitant solution with the addition of 15% (v/v) glycerol. Diffracted intensities were processed using the HKL crystallographic data reduction package (Denzo/Scalepack).[24] The structures of the complexes were analyzed by difference Fourier techniques using hCA II crystallized in the $P2_1$ space group as the starting model.[22] The refinements were carried out with the program CNS,[25] whereas the model building and map inspections were performed using the O program.[26] The correctness of stereochemistry was finally checked using PROCHECK.[27] Data collection parameters and statistics for refinement of both structures are summarized in Table 1. Coordinates and structure factors have been deposited with the Protein Data Bank (accession code 2HNC and 2HOC, respectively).
24. Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307.
25. Brünger, A. T.; Adams, P. D.; Clore, G. M.; De Lano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr. Sect. D* **1998**, *54*, 905.
26. Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. *Acta Crystallogr. Sect. A* **1991**, *47*, 110.
27. Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton, J. M. *J. Appl. Crystallogr.* **1993**, *26*, 283.