Research Article

Hydrophobic Substituents of the Phenylmethylsulfamide Moiety Can Be Used for the Development of New Selective Carbonic Anhydrase Inhibitors

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A new series of compounds containing a sulfamide moiety as zinc-binding group (ZBG) has been synthesized and tested for determining inhibitory properties against four human carbonic anhydrase (hCA) isoforms, namely, CAs I, II, IX, and XII. The X-ray structure of the cytosolic dominant isoform hCA II in complex with the best inhibitor of the series has also been determined providing further insights into sulfamide binding mechanism and confirming that such zinc-binding group, if opportunely derivatized, can be usefully exploited for obtaining new potent and selective CAIs. The analysis of the structure also suggests that for drug design purposes the but-2-yn-1-olxy moiety tail emerges as a very interesting substituent of the phenylmethylsulfamide moiety due to its capability to establish strong vanderWaals interactions with a hydrophobic cleft on the hCA II surface, delimited by residues Phe131, Val135, Pro202, and Leu204. Indeed, the complementarity of this tail with the cleft suggests that different substituents could be used to discriminate between isoforms having clefts with different sizes.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metalloenzymes found in prokaryotes and eukaryotes, which catalyze the reversible hydration of carbon dioxide to bicarbonate ion and proton \( \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+ \) [1, 2]. In humans 15 different isoforms have been identified so far, among which 12 are catalytically active (CAs I-IV, VA-VB, VI-VII, IX, and XII-XIV), whereas the remaining three (CAs VIII, X, and XI), named as CA-related proteins (CARPs), are devoid of any catalytic activity [2]. All the catalytically active isoforms contain in their active site a zinc ion tetrahedrally coordinated by three conserved histidine residues and a water molecule/hydroxide ion [1, 2].

Over the past few years, the discovery of the involvement of several CA isoforms in human diseases has greatly increased the attention on these enzymes in regard to their consideration as interesting targets for drug design [3]. Indeed, a wealth of derivatives, mainly containing a primary sulfonamide \( \text{R}	ext{SO}_2\text{NH}_2 \) [1, 2, 4–6] and its biosiosteres, such as the sulfamate \( \text{ROSO}_2\text{NH}_2 \) [1, 7, 8] and sulfamide \( \text{RNHSO}_2\text{NH}_2 \) [1, 2, 9–18] as zinc anchoring groups, have been investigated as CA inhibitors (CAIs) with some of them (principally sulfonamides and sulfamates) being explored for
the treatment of a variety of disorders such as glaucoma [19–22], acid-base disequilibria [23], epilepsy [24, 25] neuromuscular diseases [26], edema [27], and obesity [28, 29] and for the management of hypoxic tumors [30]. Acetazolamide (AAZ) 1 [31], methazolamide (MZA) 2 [31], topiramate (TPM) 3 [32], ethoxzolamide (EZA) 4 [33], and dichlorphenamide (DCP) 5 [31] represent some examples of such pharmacologically relevant CAIs (Figure 1). However, it is important to highlight that none of the currently clinically used CAIs shows selectivity for a specific isozyme [1].

The knowledge of the inhibition profile of CAIs against all human isoforms and of their detailed binding to the enzyme (which can be obtained from crystallographic data) can allow for a better understanding of their mechanism of action and can provide an efficient molecular basis for the rational drug design of isozyme-selective compounds [1, 34]. In the last decade a huge number of X-ray structural studies of CA adducts principally with sulfonamides and sulfamates have been reported. On the contrary, sulfamide-containing derivatives have been only poorly investigated as CAIs, because they were initially supposed not to be particularly suitable for obtaining potent CA inhibitors, exhibiting just a moderate-to-weak inhibition potency [35, 36]. However, many recent studies, predominantly by Supuran’s group, have supported the idea that sulfamide derivatives can be considered interesting candidates for obtaining CAIs, showing such several compounds with relatively high CA affinity [12–15]. At present, only 5 sulfamide-containing derivatives have been characterized by means of X-ray crystallography for their interaction with CAs: the simple sulfamide 6 [9, 16], the N-hydroxy-sulfamide 7 [10, 18], the sulfamide derivative of the antiepileptic drug topiramate 8 [11–15, 17], the boron containing derivative 9 [37], and the nitroimidazole-sulfamide 10 [38] (Figure 1). Thus we decided to investigate

\[ \text{Figure 1: Chemical formulas of inhibitors 1–16.} \]
2. Results and Discussion

2.1. Chemistry. Synthesis of aza-benzylidene derivatives of sulfamide, like compound 13, from aryl aldehydes and sulfamide, is reported in the patent literature [39]. Our first efforts to reproduce a published procedure where ethanol was used as a solvent resulted in formation of trace amounts of desired product. After screening of several solvents we found that the use of glacial acetic acid gave reproducible results. With the improved procedure, where equimolar amounts of aryl aldehydes (compounds 20a-b) and sulfamide (compound 6) were used, monosubstituted aza-benzylidene derivatives 13 and 15 were isolated in acceptable yields (Scheme 1). Substituted aryl aldehydes 20a-b were prepared from 4-hydroxybenzaldehyde 17 and corresponding alcohols 18 or 19 under Mitsunobu reaction conditions [40].

For the synthesis of monobenzylic derivatives of sulfamide, we chose one-pot two-step procedure [39], where the first step is the condensation reaction of sulfamide (compound 6) and aryl aldehydes and the second step is the treatment of reaction mixture with NaBH₄, where the reduction of C=N double bond takes place. Under these conditions utilizing aldehydes 20a-b, 17, and 21, monosubstituted sulfamides 11, 12, 14, and 16 were obtained (Scheme 1).

2.2. CA Inhibition and Structure-Activity Relationship (SAR). Sulfamides 11–16 were investigated as inhibitors of four physiologically relevant CA isoforms, the cytosolic hCAs I and II, and the transmembrane, tumor-associated hCAs IX and XII (Table 1). The following SAR can be observed from the data of Table 1.

(i) hCA I was poorly inhibited by sulfamides 11–16, which showed a compact behavior of medium-potency, weak inhibitors, with Kᵢs ranging from 1440 to 4050 nM. Interestingly, the compounds with the bulkier tails, 15 and 16, were more effective as hCA I inhibitors compared to the derivatives with the OH, OMe, or alkynyl-ether moieties 11–14. It may be observed that the standard drug acetazolamide (AAZ, a clinically used drug) was a more effective hCA I inhibitor compared to the sulfamides reported here.

(ii) The new sulfamides inhibited the physiologically dominant cytosolic isoform hCA II with Kᵢs ranging from 9.5 to 188 nM. It is interesting to note that derivatives 13 and 14 were effective hCA II inhibitors (comparable to AAZ),

Scheme 1: Synthesis of compounds 11–16.
Table 1: hCAs I, II, IX, and XII inhibition data with sulfamides 11–16. Acetazolamide (AAZ) has been used as standard drug. Analyses were performed with a CO₂ hydrase, stopped-flow assay [41].

| Compounds | hCA I (nM) | hCA II (nM) | hCA IX (nM) | hCA XII (nM) |
|-----------|------------|-------------|-------------|--------------|
| 11        | 2180       | 74.1        | 40.7        | 5.8          |
| 12        | 4050       | 134         | 60.0        | 6.6          |
| 13        | 1940       | 9.8         | 59.1        | 8.4          |
| 14        | 1810       | 9.5         | 61.7        | 8.1          |
| 15        | 1650       | 188         | 56.3        | 6.5          |
| 16        | 1440       | 43.3        | 62.1        | 6.6          |
| AAZ       | 250        | 12          | 25          | 5.7          |

Mean from 3 different assays, errors in the range of ±10% of the reported values.

with inhibition constants of 9.5–9.8 nM (Table 1). The two compounds incorporate the same but-2-yn-1-yloxy-tail and only differ by the presence of Schiff’s base (imine) moiety in compound 13, which is reduced to the secondary amine in compound 14. It is obvious that this structural modification has a minimal effect on the hCA II inhibitory properties, whereas the nature of the tail present in position 4 of the benzene ring (with respect to the zinc-binding group) has a crucial role in their binding affinity to the enzyme. Indeed, the compounds with such smaller moieties (than the but-2-yn-1-yloxy-one), like 11 and 12, but also those with larger and bulkier such tails (compounds 15 and 16), were less effective CAls compared to compounds 13 and 14 against hCA II. Indeed, compounds 11 and 16 were medium-potency hCA II inhibitors (Kᵢs ranging from 43.3 to 74.1 nM) whereas compounds 12 and 15 were even weaker, with inhibition constants in the range of 134–188 nM (Table 1). The net difference of activity between compounds 11 and 12 which only differ by a CH₃ moiety should be noted. In the case of the imine-amine pair 15, 16, the imine 15 was 4.3 times a weaker hCA II inhibitor compared to the amine 16.

(iii) Both transmembrane isoforms, hCA IX and XII, were effectively inhibited by sulfamides 11–16, with little SAR evident from data of Table 1. Thus, for hCA IX the inhibition constants only ranged between 40.7 and 62.1 nM, whereas against hCA XII they were in the range of 5.8–8.4 nM. Thus all these sulfamides were medium-potency hCA IX inhibitors and were highly effective as hCA XII inhibitors (Table 1).

2.3. Crystallography. To better understand at structural level the molecular features determining the inhibition profiles of this new series of compounds against hCAs, we have solved the crystal structure of the cytosolic dominant isoform hCA II in complex with its highest affinity inhibitor (compound 14) in the series.

Crystals of hCA II/14 adduct were isomorphous with those of the native protein [42], allowing for the analysis of the structure by difference Fourier techniques. Data collection and refinement statistics are shown in Table 2. Inhibitor binding did not generate major changes in the structure of hCA II as proved by the low value of the RMSD calculated by superposing the Ca atoms in the adduct and the noninhibited enzyme (0.3 Å). The overall quality of the model was high, with 88.6% of the non-glycine residues located in the allowed regions of the Ramachandran plot (Table 2).

The inspection of the electron density maps at various stages of the crystallographic refinement revealed the binding of two inhibitor molecules: the first one on the protein surface...
and the second in the active site cavity (Figure 2). The binding of the inhibitor on the protein surface will not be discussed here, since it occurs far from the active site; thus it is not correlated with the inhibition properties of the molecule. On the contrary the binding of the molecule in the active site will be analyzed in detail since it is clearly associated with the high inhibitory potency of the investigated sulfamide.

As clearly evidenced in Figure 3(a) the electron density for the molecule bound in the active-site is very well defined for the phenylmethyl sulfamide moiety and slightly less defined for the but-2-yn-1-yloxy tail indicating some flexibility of this region. The compound is anchored to the active site coordinating the catalytic Zn$^{2+}$ ion by means of one nitrogen atom of the sulfamide group (N1) and displacing the zinc bound water molecule/hydroxide ion (Figure 3(a)), similarly to what is observed for other sulfamides (compounds 6–10) and sulfonamides/sulfamates whose crystal structures in adduct with CAs have been reported [16–18, 34, 37]. The same nitrogen atom N1 also interacts with Thr199 forming a hydrogen bond with its side chain, whereas one of sulfamide oxygen atoms forms a second hydrogen bond with the backbone nitrogen atom of the same residue (Figure 3(a)). It is interesting to note that the single bond N2-C1 adopts a trans-conformation (dihedral angle S1-N2-C1-C2 of about 175°), close to the trans-conformation expected for compound 13, which contains in the same position a double bond. Thus it is tempting to speculate that this behavior should be at the basis of the almost identical affinity that the two molecules show for hCA II (see Table 1).

The phenyl ring of the inhibitor resides in the middle of the active site channel, making various van der Waals interactions with the side chains of Phe131, Val135, Pro202, and Thr200 while the but-2-yn-1-yloxy tail lies in a small hydrophobic cleft on the protein surface, defined by residues Phe131, Val135, Pro202, and Leu204 (Figure 3(b)). This cleft has already been identified as an important region in the recognition of CAIs [1, 43]. In agreement with these data, this interaction seems to have important consequences on the inhibitory properties of this series of compounds against hCA II (see Table 1); indeed, inhibitors containing the but-2-yn-1-yloxy tail (compounds 13 and 14) are those with the best inhibitory properties against the enzyme, while compounds with shorter (compounds 11 and 12) or bulkier (compounds 15 and 16) tails have less inhibitory potency. Indeed, compounds with shorter tails probably establish less extensive interactions with this cleft, while those with bulkier tails are unable to interact with it.

A different situation is observed in the case of hCA I for which much higher inhibition constants are observed. The structural superposition of hCA II/14 complex with hCA I [46] (Figure 3(f)) can give a reasonable explanation of these data. Indeed, most of the residues involved in the interaction of the inhibitor with hCA II are conserved also in the isoform I. However, the substitution of Thr200 with His200 in hCA I plays an important role in destabilizing the enzyme-inhibitor interaction since this residue is much more bulky and makes the active site narrower (Figures 3(e) and 3(f)). Therefore only an important structural rearrangement of the enzyme active site could allow the binding of the inhibitor, determining the very low affinity toward hCA I, as previously observed for other hCA I/inhibitor complexes [47].

As mentioned above very few papers describing sulfamide-containing derivatives crystallized with hCA II have been reported [16–18, 37, 38]. In these adducts a very weak additional H-bond interaction is observed between the Thr200OG atom and the second nitrogen atom of the sulfamide moiety. This weak interaction is absent in our case. The finding that compound 14 still remains a very good CA inhibitor despite this absence further confirms that such interaction does not have a great effect on the stabilization of the binding.

In conclusion, in this paper we report the X-ray structure of a new sulfamide inhibitor of CAs in complex with hCA II, together with an inhibition study of a family of structurally related compounds for the CA isoforms I, II, IX, and XII. The data reported here provide further insights into sulfamide binding mechanism confirming that this zinc-binding group could be usefully exploited for obtaining new potent and selective CAIs. In particular, the but-2-yn-1-yloxy tail emerges as a very interesting group for this purpose due to its capability to establish strong van der Waals interactions with a hydrophobic cleft on the hCA II surface delimited by residues Phe131, Val135, Pro202, and Leu204. Indeed, the complementarity of the tail with the cleft suggests that
Figure 3: (a) Active site region of the hCA II/14 complex. The inhibitor is shown in association with a σ A-weighted |2Fo – Fc| map (at 1.0 σ). Hydrogen bonds, van der Waals interactions (distance of <4.0 Å), and the active site Zn\(^{2+}\)-ion coordination are also shown. (b, c, d, and e) Solvent accessible surface of hCAs II, IX, XII, and I: the hydrophobic cleft defined by residues 131, 135, 202, and 204 is highlighted in orange (hCA II), blue (hCA IX), green (hCA XII), and magenta (hCA I). For hCA I His200 is also showed in magenta. (f) Structural superposition of the hCA I (magenta) and hCA II (green) active sites. The inhibitor 14 is shown as bound in its complex with hCA II.
different substituents could be used to discriminate between isoforms having cleft with different sizes.

3. Materials and Methods

3.1. Chemistry. Reagents and starting materials were obtained from commercial sources and used as received. Compound 19 was synthesized according to literature procedure [48]. The solvents were purified and dried by standard procedures prior to use; petroleum ether (PE) of boiling range 40–60°C was used. Flash chromatography was carried out using Merck silica gel (230–400 mesh). Thin-layer chromatography was performed on silica gel; spots were visualized with UV light (254 and 365 nm). Melting points were determined on an OptiMelt automated melting point system. NMR spectra were recorded on Varian Mercury (400 MHz) spectrometer using the residual DMSO-6 as an internal standard. Elemental analyses were performed on a Carlo Erba CHNS-O EA-1108 apparatus.

3.1.1. General Procedure for the Synthesis of 4-Alkoxy Substituted Benzaldehydes. To a mixture of 4-hydroxybenzaldehyde (17) (15.58 mmol), PPh3 (16.22 mmol), and dry THF (100 mL) were added. To this mixture at room temperature for 18 h before it was quenched with sat. aq. NH4Cl. EtOH was added and solvent was evaporated in vacuum. H2O was added and mixture was extracted with DCM (3 × 100 mL). Organic layers were combined, dried over Na2SO4, and solvent was evaporated. The crude product was purified by column chromatography on silica gel.

3.1.2. 4-(But-2-yn-1-yl-oxy)benzaldehyde (20a). Compound 20a was obtained from 4-hydroxybenzaldehyde (17) (1.90 g, 15.58 mmol), PPh3 (4.27 g, 16.27 mmol), but-2-yn-1-ol (18) (0.87 mL, 11.54 mmol), and DIAD (3.13 mL, 15.82 mmol). The crude product was purified by column chromatography (toluene) and crystallized from EtOH to yield 20a (1.55 g, 77%) as white solid. Mp 66–68°C. 1H NMR (400 MHz, DMSO-d6) δ: 1.84 (t, 3H, J = 2.3 Hz), 4.88 (q, 2H, J = 2.3 Hz), 7.13–7.17 (m, 2H), 7.86–7.90 (m, 2H), 9.88 (s, 1H).

3.1.3. 4-[(2-Methylquinolin-4-yl)methoxy]benzaldehyde (20b). Compound 20b was obtained from 4-hydroxybenzaldehyde (17) (1.90 g, 15.58 mmol), PPh3 (4.27 g, 16.27 mmol), (2-methylquinolin-4-yl)methanol (19) [48] (2.00 g, 11.54 mmol), and DIAD (3.13 mL, 15.82 mmol). The crude product was purified by column chromatography (PE/EtOAc 1:1) to yield 20b (2.70 g, 85%) as yellow solid. Mp 99–101°C.

3.1.4. General Procedure for the Synthesis Benzylidene Sulfamides. To a mixture of sulfamide (6) (3.12 mmol) glacial acetic acid (5 mL) followed by the corresponding benzaldehyde (3.12 mmol) was added. Reaction mixture was stirred at 60°C for 23 h. EtOH was added and solvent was evaporated in vacuum. The crude product was purified by column chromatography on silica gel.

3.1.5. N-[(4-But-2-yn-1-yloxy)phenyl]methylidene)sulfuric Diamide (13). Compound 13 was obtained from sulfamide (6) (0.30 g, 3.12 mmol) and 4-(but-2-yn-1-yloxy)benzaldehyde (20a) (0.54 g, 3.12 mmol). The crude product was purified by column chromatography (PE/EtOAc 1:2) and crystallized from MeCN/H2O to yield 13 (0.17 g, 41%) as white solid. Mp 192–194°C.

3.1.6. N-[(4-[(2-Methylquinolin-4-yl) methoxy][phenyl]methylidene)sulfuric Diamide (15). Compound 15 was obtained from sulfamide (6) (0.30 g, 3.12 mmol) and 4-[(2-methylquinolin-4-yl) methoxy]benzaldehyde (20b) (0.87 g, 3.12 mmol). The crude product was purified by column chromatography (PE/EtOAc 2:1) then neat EtOAc and crystallized from EtOH/H2O to yield 15 (0.41 g, 37%) as white solid. Mp 99–101°C.

3.1.7. General One-Pot Procedure for the Synthesis of Mono-substituted Sulfamide. To sulfamide (6) (1 eq) glacial acetic acid followed by the corresponding benzaldehyde (1 eq) was added. Reaction mixture was stirred at 60°C for 23 h. NaBH4 (10 eq) portionwise was added followed by extra glacial acetic acid. Reaction mixture was stirred at room temperature for 18 h before it was quenched with sat. aq. NH4Cl. EtOH was added and solvent was evaporated in vacuum. H2O was added and mixture was extracted with
EtOAc. Combined organic layers were dried over Na₂SO₄ and purified by column chromatography on silica gel.

3.1.8. N-(4-Hydroxybenzyl)sulfuric Diamide (11). Compound 11 was obtained from sulfamide (6) (0.30 g, 3.12 mmol), 4-hydroxybenzaldehyde (17) (0.38 g, 3.12 mmol) in AcOH (5 mL), and NaBH₄ (1.18 g, 3.12 mmol) with extra AcOH (15 mL). Reaction mixture was quenched with sat. aq. NH₄Cl (10 mL), diluted with H₂O (40 mL), and extracted with EtOAc (3 × 30 mL). The crude product was purified by column chromatography (PE/EtOAc 1:2) to yield 12 (0.14 g, 21%) as yellow solid. Mp 180–181°C.

3.1.9. N-(4-Methoxybenzyl)sulfuric Diamide (12). Compound 12 was obtained from sulfamide (6) (0.30 g, 3.12 mmol), 4-methoxybenzaldehyde (21) (0.38 mL, 3.12 mmol) in AcOH (5 mL), and NaBH₄ (1.18 g, 3.12 mmol) with extra AcOH (15 mL). Reaction mixture was quenched with sat. aq. NH₄Cl (3 mL), with H₂O (50 mL), and extracted with EtOAc (3 × 30 mL). The crude product was purified by column chromatography (PE/EtOAc 2:1) and crystallized from EtOH/H₂O to yield 12 (0.27 g, 40%) as white solid. Mp 118–120°C.

3.1.10. N-[4-(But-2-yn-1-yl-oxy)benzyl]sulfuric Diamide (14). Compound 14 was obtained from sulfamide (6) (0.26 g, 2.67 mmol), 4-(but-2-yn-1-yl-oxy)benzaldehyde (20a) (0.47 g, 2.67 mmol) in AcOH (5 mL), and NaBH₄ (1.01 g, 26.7 mmol) with extra AcOH (15 mL). Reaction mixture was quenched with sat. aq. NH₄Cl (15 mL), diluted with H₂O (40 mL), and extracted with EtOAc (3 × 30 mL). The crude product was purified by column chromatography (PE/EtOAc 2:1 then 1:1) and crystallized from DCM to yield 14 (0.04 g, 9%) as white solid. Mp 93–95°C.

3.2. CA Inhibition Assays. A stopped-flow CO₂ hydration assay with an Applied Photophysics instrument was used for measuring the inhibition of hCAs I, II, IX, and XII by the new compounds reported here. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557nm, with 20mM Heps (pH 7.4) or 20mM Tris (pH 8.3) as buffers, and 20mM NaClO₄ for maintaining the ionic strength constant. The initial rates of the CA-catalyzed CO₂ hydration reaction were followed for a period of 10–100 s [41]. The concentrations of substrate (CO₂) ranged from 1.7 to 17mM for the determination of the inhibition constants, with at least six traces of the initial 5–10% of the reaction being used for determining the initial velocity, for each inhibitor. The uncatalyzed rates were determined and subtracted from the total observed rates. Stock solutions of inhibitors (10mM) were prepared in distilled-deionized water and dilutions up to 0.01 mM were done with the assay buffer. Enzyme and inhibitor solutions were preincubated prior to assay for 15 min (at room temperature), in order to allow for the formation of the E-I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng-Prusoff equation as reported earlier by our groups. The kinetic parameters for the uninhibited enzymes were derived from Lineweaver-Burk plots, as reported earlier [49–51], and represent the mean from at least three different determinations.

3.3. X-Ray Studies. hCA II/14 complex was obtained by adding a 5-molar excess of inhibitor to a 10 mg/mL protein solution in 20 mM Tris-HCl pH 8, 0.1% DMSO. Crystals of the complex were obtained using the hanging drop vapor diffusion technique. In particular, 2 μL of complex solution and 2 μL of precipitant solution (1.4 M Na-Citrate, 100 mM Tris-HCl pH 8.0) were mixed and suspended over a reservoir containing 1 mL of precipitant solution at 20°C. X-ray diffraction data were collected at 100 K, using a copper rotating anode generator developed by Rigaku and equipped with a Rigaku Saturn CCD detector. Prior to cryogenic freezing, the crystals
were transferred to the precipitant solution with the addition of 15% (v/v) glycerol. Data were processed using the HKL2000 package [52]. Diffraction data were indexed in the P2₁ space group with one molecule in the asymmetric unit. Unit cell parameters and data reduction statistics are reported in Table 2. The atomic coordinates of hCA II (PDB entry 1CA2) [42] were used as a starting model for crystallographic refinement after deletion of non-protein atoms. Structure refinement (in the 20.0–1.85 Å resolution range) was carried out using CNS [53] and model building was performed with O [54]. Inhibitor molecules were identified from peaks in |Fₒ| – |Fᶜ| maps and gradually built into the model over several rounds of refinement. Restraints on inhibitor bond angles and distances were taken from similar structures in the Cambridge Structural Database [55] whereas standard restraints were used on protein bond angles and distances throughout refinement. The correctness of stereochemistry was finally checked using PROCHECK [56]. Final refinement statistics are presented in Table 2. The atomic coordinates of hCA II/14 complex were deposited in the Protein Data Bank, accession code 4PQ7.

Conflicts of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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