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Carbonic Anhydrase Inhibitors: SAR and X-ray Crystallographic Study for the Interaction of Sugar Sulfamates/Sulfamides with Isozymes I, II and IV

Angela Casini,[a] Jochen Antel,[b,*] Francesco Abbate,[a] Andrea Scozzafava,[a] Samuel David,[b] Harald Waldeck,[b] Siegfried Schäfer[b] and Claudiu T. Supuran[a,*]

[a]*Dipartimento di Chimica, Università degli Studi di Firenze, Via della Lastruccia 3, Rm. 188, I-50019 Sesto Fiorentino (Firenze), Italy*

[b]*Solvay Pharmaceuticals GmbH, Hans Böckler-Allee 20, D-30173 Hannover, Germany*

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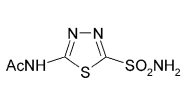
This paper is dedicated to the 60th birthday of Professor George M. Sheldrick[†]

Abstract—A series of sugar sulfamate/sulfamide derivatives were prepared and assayed as inhibitors of three carbonic anhydrase (CA) isozymes, hCA I, hCA II and bCA IV. Best inhibitory properties were observed for the clinically used antiepileptic drug topiramate, which is a low nanomolar CA II inhibitor, and possesses good inhibitory properties against the other two isozymes investigated here, similarly with acetazolamide, methazolamide or dichlorophenamide. The X-ray structure of the complex of topiramate with hCA II has been solved and it revealed a very tight association of the inhibitor, with a network of seven strong hydrogen bonds fixing topiramate within the active site, in addition to the Zn(II) coordination through the ionized sulfamate moiety. Structural changes in this series of sugar derivatives led to compounds with diminished CA inhibitory properties as compared to topiramate.

Introduction

The carbonic anhydrases (CAs) represent a class of ubiquitous zinc enzymes widespread in the bacterial, vegetal and animal kingdoms.[1–3] They catalyze one of the simplest physiological reactions, the interconvertion between CO_2 and bicarbonate, which is also one of the most important chemical processes in all living organisms, being at the basis of different fundamental metabolic pathways (gluconeogenesis, lipogenesis and ureagenesis), and also for the pH and CO_2 homeostasis of such organisms, electrolyte secretion in a variety of tissues/organs, bone resorption, calcification and tumorigenicity (in the case of vertebrates).[1–3]

Due to the important roles of CAs in higher vertebrates, compounds possessing CA inhibitory properties, mainly aromatic/heterocyclic sulfonamides (such as acetazolamide **1**, methazolamide **2**, dichlorophenamide **3** and ethoxozolamide **4**) have been used for more than 45 years as drugs in the therapy of different pathologies such as glaucoma, various neurological/neuromuscolar disorders (essential tremor and Parkinson's disease), epilepsy, acid-base disequilibria, or as diuretics.[1–3] This family of pharmacological agents takes advantage of the sulfonamide moiety as anchoring group to coordinate the zinc ion within the active site of the enzyme, leading to ligands with micro-nanomolar affinity (for example the above mentioned drugs **1**–**4**, possess affinities in the 8–38 nM range for the physiologically most relevant isozyme, hCA II—see Table 1).[4,5]



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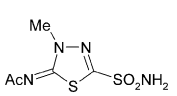

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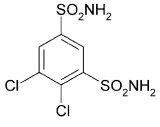

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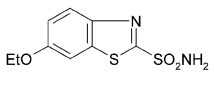

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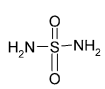

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*Corresponding authors: Tel.: +49-511-857 2154; fax: 49-511-857 2195; e-mail: jochen.antel@solvay.com (J. Antel); tel: +39-055-4573005; fax: +39-055-4573385; e-mail: claudiu.supuran@unifi.it (C.T. Supuran)

[†]Some of his X-ray crystallographic programs were used for the refinement of the structure presented here.

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Table 1. CA inhibition data with standard inhibitors **1–4**, sulfamic acid **5**, sulfamide **6**, and some sugar sulfamides/sulfamates derivatives investigated in this paper (**7–16**), by the nitrophenyl acetate esterase method[15]

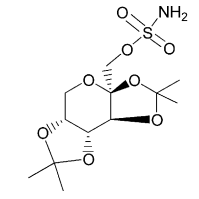
No.	Compd	K_I (nM)		
		hCA I[a]	hCA II[a]	bCA IV[b]
1	Acetazolamide	250	12	70
2	Methazolamide	50	14	36
3	Dichlorophenamide	1200	38	380
4	Ethoxzolamide	25	8	13
5	Sulfamic acid	21,000	97,000	nt
6	Sulfamide	35,000	82,000	nt
7	Topiramate	250	5	54
8		750	68	425
9		420	24	45
10		1200	71	130
11		>100 μM	>100 μM	>100 μM
12		420,000	21,000	33,000
13		>100 μM	25,000	46,000
14		>100 μM	15,000	30,000
15		>100 μM	>100 μM	>100 μM
16		400,000	13,000	27,000

nt, Not tested.
[a]Human cloned isozymes.
[b]Isolated from bovine lung microsomes.

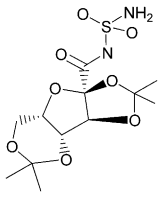
It has been recently reported by our group that the simplest compounds incorporating a sulfonamide moiety, that is sulfamide **5** and sulfamate **6**, bind to the catalytical Zn(II) ion of hCA II giving rise to a different hydrogen-bonding network with respect to the one offered by the classical inhibitors of the aromatic/heterocyclic sulfonamide type.[6] The reason for such different binding modes is due to the presence of an additional heteroatom (N and O, respectively) linked to the SO_2NH_2 group in derivatives **5** and **6**, as compared to the previously investigated CA inhibitors (CAIs).[1–3] These findings are important since they provide additional possibilities of drug design in the field of CAIs. Up to now, most such compounds were built up on the basis of the C–SO_2NH_2 zinc-binding group, as reported mainly by the drug design studies based on the X-ray crystallographic work of Liljas', Lindskog's and Christianson's groups.[4a,7–9] Taking advantage of the recently evidenced interactions between sulfamide/sulfamic acid and hCA II,[6] we developed new types of CAIs bearing these two zinc-binding functions, reporting here a detailed SAR study for sugar sulfamates as CAIs, and new X-ray crystallographic data on the adduct of hCA II with the best inhibitor investigated here, topiramate **7**, a clinically used antiepileptic drug.[10,11] Indeed, topiramate is a sugar sulfamate derivative possessing good anti-epileptic activity.[11] It is particularly interesting from a structural point of view, because it is derived from a monosaccharide and bears a sulfamate functional group that is considered to be responsible for its anticonvulsant properties, even if the mechanism of action of this drug seems to be rather complicated and not entirely understood at this moment.[10,11] The anticonvulsant effects of topiramate or related sulfonamides[10] are probably due to CO_2 retention secondary to inhibition of the red cell and brain enzymes,[1–3] but other mechanisms of action, such as blockade of sodium channels and kainate/AMPA receptors, as well as enhancement of GABA-ergic transmission, were also hypothesized/proved for some of these drugs.[11] In fact topiramate shows a positive modulatory effect on some types of GABA-A receptors, antagonizes kainate/AMPA receptors and inhibits the generation of action potentials in neurons via antagonizing the activation of Na^+ channels.[11] Another mechanism of action of topiramate that has not been viewed as critical up to now, but that we consider here in detail, is the inhibition of different CA isozymes.

Chemistry

Compounds **7–16** investigated for their interaction with isozymes CA I, II and IV were prepared as described in the literature,[12] by reaction of the appropriately protected sugars with sulfamoyl chloride or *N*-(*tert*-butoxycarbonyl)sulfamoyl chloride, followed by removal of the Boc protecting group with TFA,[12,13] or by acylation reactions of sulfamide/aminosulfamide with the corresponding sugar carboxylic acid derivatives.[14]



7 Topiramate



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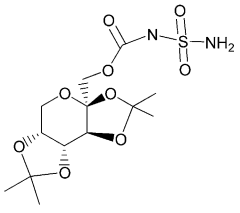

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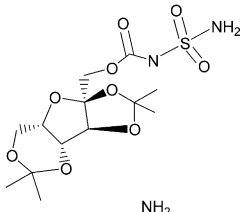

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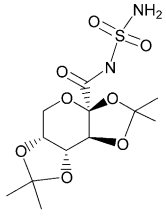

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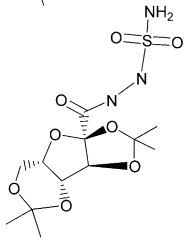

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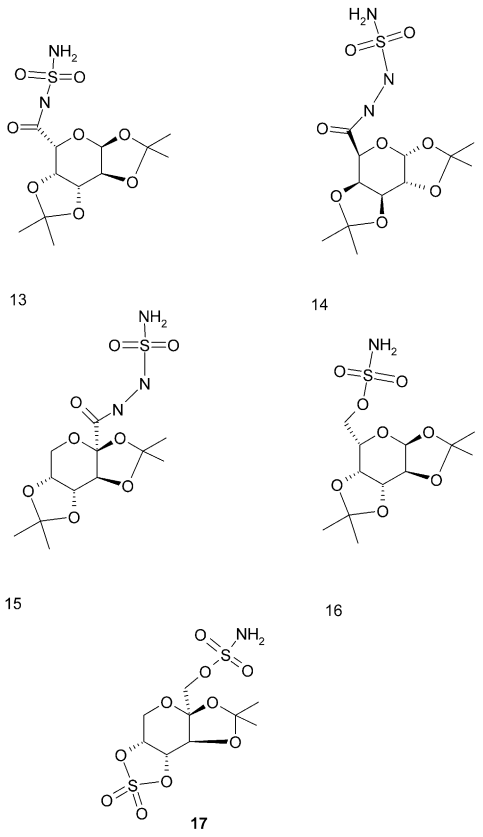
Carbonic anhydrase inhibitory activity

The data of Table 1 show that the compounds incorporating a sugar sulfamate/sulfamide scaffold **7–16** possess a very broad range of activity towards the three investigated isozymes hCA I, hCA II and bCA IV. Thus, two of them, topiramate **7** and the acylated aminosulfamide **9** possessing the same scaffold as topiramate, behave as very potent CAIs, with inhibition constants in the range of 5–24 nM against hCA II, 250–420 nM



against hCA I, and 45–54 nM against bCA IV. Practically these compounds show similar potencies to the clinically used inhibitors **1**–**4** (Table 1). Two other inhibitors in this series, namely **8** and **10**, both incorporating a five-membered ring as compared to the corresponding six-membered ring of topiramate, and acylated aminosulfamide moieties as zinc-anchoring groups, behave as weaker inhibitors as compared to the compounds mentioned earlier (**7** and **9**), with inhibition constants in the range of 68–71 nM against hCA II, 750–1200 nM against hCA I, and 130–425 nM against bCA IV. Two other compounds, **12** and **16**, behave as moderate-weak inhibitors, with inhibition constants in the range of 13–21 μM against hCA II, 40–42 μM against hCA I, and 27–33 μM against bCA IV. All other investigated compounds (such as **11**, and **13**–**15**) behaved as very weak CAIs against the three investigated isozymes, being generally less efficient than the two leads from which they were derived, sulfamic acid **5** and sulfamide **6**, which are moderate, micromolar inhibitors of both hCA I and hCA II (Table 1). Thus, this small library of derivatives offers some very important lessons for the drug design of CAIs: very small structural variations in a series of such derivatives may lead to compounds with completely different biological activity. For example, the nanomolar CA II inhibitor **7** and the totally inactive compound **15** possess the same scaffold, except for the linker to the zinc binding function, which for **15** is slightly longer than for **7**, but the difference in activity between the two compounds is tremendous! It is also clear that the position of the zinc anchoring group is very important for this class of CAIs. For example, compounds **7** and **16** are isomers, but **7** is 2600 times more efficient as a CA II inhibitor as compared to **16**. Practically, all modifications in the structure of **7** that we demonstrate here, led to diminished CA inhibitory properties for the obtained compounds, and this will be better understood why, after examining the X-ray structure of the complex of topiramate **7** with hCA II.

Crystallography

The hCA II–**7** adduct was subjected to detailed X ray crystallography. The programs SHELX97[16] and O[17] were used to build the model and to compute the Fourier maps. The last refinement cycle yielded a final R factor of 0.18 ($R_{free} = 0.24$) with a final temperature factor of the inhibitor atoms ranging between 8.5 and 32.3 Å^2. The final number of water molecules was 172 and the final rmsd's from ideal geometry for bond lengths and angles were 0.010 Å and 0.021°, respectively. After the structure refinement, the spatial arrangement in the neighborhood of the catalytic Zn(II) ion has been revealed (Fig. 1), with a refinement resolution of 1.8 Å.

Looking at the residual charge density, the assignment of the ligand binding mode to hCA II was unique, with the nitrogen, of the amide group, close to the zinc at the tetrahedral vertex and a distance of 1.97 Å to the catalytic metal ion (Fig. 2). Furthermore, this nitrogen donates a hydrogen bond (of 2.66 Å) to Oγ of Thr 199 via its remaining hydrogen, whereas the backbone NH of Thr 199 donates a hydrogen bond (of 2.85 Å) to one oxygen of the SO_2 group as usually happens for sulfonamide—CA complexes,[4,6] and as was recently reported for the adduct of hCA II with sulfamic acid or sulfamide.[6] Then, an extended network of hydrogen bonds between the inhibitor and some amino acid residues within the cavity strongly stabilize the complex (Fig. 2). Such important hydrogen bond are: Asn 62-Nε_1 to O_4

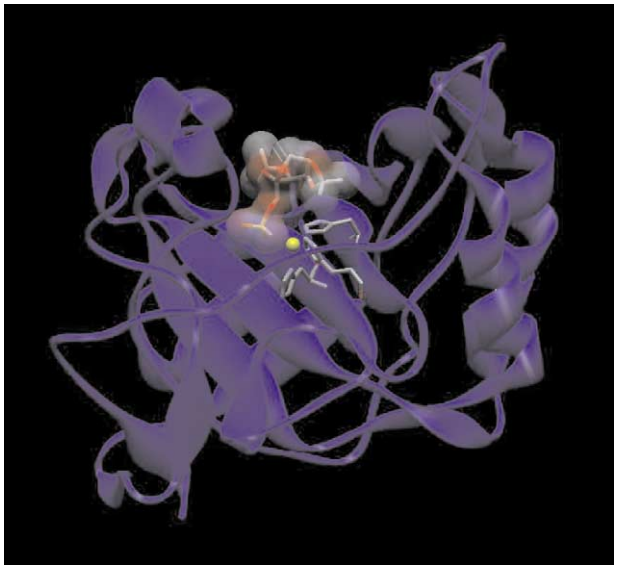


Figure 1. The topiramate-hCA II adduct. The zinc ion (yellow sphere), its three histidine ligands (His 94, His 96 and His 119) as well as the inhibitor molecule are evidenced. Topiramate practically occupies the entire space available within the active site, participating in a multitude of hydrogen bonds and hydrophobic interactions.

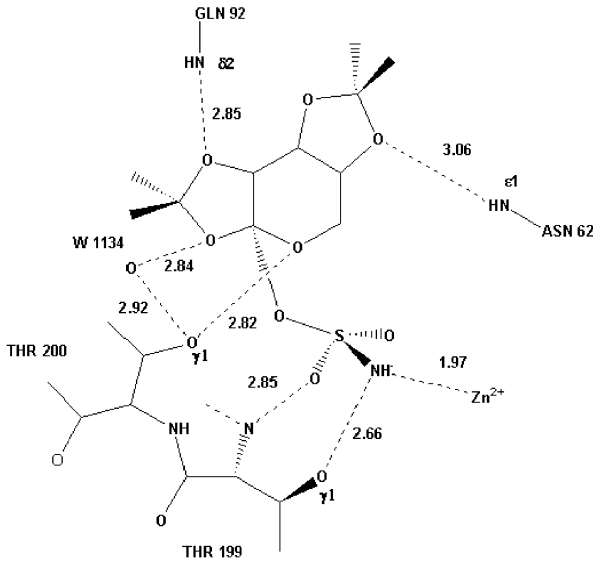


Figure 2. Binding of topiramate **7** to the hCA II active site: the seven hydrogen bonds with critical amino acid residues of the active site and the zinc coordination are emphasized, with the corresponding distances (in Å).

of topiramate (3.06 Å), Gln 92- $N\varepsilon_2$ to O_6 of topiramate (2.85 Å) and the water molecule 1134 that makes a bridge between the inhibitor and Thr 200 through two hydrogen bonds, one with O_3 (2.84 Å) of the inhibitor and the other with Thr 200- $O\gamma_1$ (2.92 Å); $O\gamma$ of Thr 200 to the pyranose oxygen of topiramate (2.82 Å). It is interesting to note that the X-ray structure of hCA II with a structurally related inhibitor, **17** (RWJ-37947), has recently been reported.[18] The difference between topiramate and RWJ-37947 consists in the fact that one of the diisopropylidene moieties of topiramate was replaced by a cyclic sulfate group, and this compound has been reported to act as a better CAI as compared to topiramate.[18] Still, CA inhibition data of topiramate reported by its discoverers are rather controversial. Thus, Shank's group initially reported that topiramate is a very weak (millimolar) CA inhibitor.[11a] The same group then recently reported different results, showing that topiramate is a much stronger CA inhibitor (in the micromolar range), but anyhow, with an efficacy 10 times lower than that of acetazolamide, against a large number of CA isozymes.[19] Obviously, the assay methods of this and the above mentioned study[19] are different, as different is the source of enzymes. Our data clearly show that topiramate is a very potent CA II inhibitor, and this statement is also very much supported by the side effects seen in many patients treated with this antiepileptic drug,[14d] which are typical for the strong sulfonamide CA inhibitors used as systemic antiglaucoma agents (such as acetazolamide, methazolamide) and include paresthesias, nephrolithiasis, and weight loss, among others.[1] Furthermore, the X-ray data for the topiramate-hCA II adduct presented here, explain at molecular level why this compound is a nanomolar CA II inhibitor, and may shed some light for the design of even more potent CAIs, with other therapeutic applications. Returning to the published X-ray structure of hCA II–RWJ-37947, we must note that this inhibitor was shown to bind in a completely different manner as compared to topiramate (we should also emphasize that the resolution of the structure reported by Recacha et al.[18] was of 2.1 Å as compared to the resolution of our structure which is 1.8 Å). Thus, RWJ-37947 has a totally different conformation when bound to the active site as compared to topiramate. More precisely, its cyclic sulfate moiety points towards the hydrophobic pocket of the enzyme, interacting with residues Phe 131, Pro 202 and Leu 198, whereas the isopropylidene moiety is stated to make hydrogen bonds (by one of its methyl group) with the side chain amide groups of Asn 67, Asn 62 and His 94. It should be noted that the authors of this study agree that it was rather difficult to accommodate this inhibitor within the active site,[18] difficulties which were not encountered at all with topiramate, whose structure is reported here. Furthermore, not even an oxygen of **17** (except for one such atom belonging to the zinc anchoring sulfamate group) participates in hydrogen bonds with amino acid residues within the active site, whereas topiramate participates in seven such interactions, as shown in Figure 2 and as discussed above. Observing how topiramate fits to the hCA II active site, it is easy to understand why all variations in its structure which we proved here in compounds **8–16**, led to compounds with diminished CA inhibitory properties.

Conclusions

In a series of sugar sulfamate/sulfamide derivatives best CA inhibitory properties were observed for the clinically used antiepileptic drug topiramate, which is a low nanomolar CA II inhibitor. Its X-ray structure in the complex with this isozyme revealed a very tight association, with a network of seven strong hydrogen bonds fixing the inhibitor within the active site, in addition to the Zn(II) coordination through the ionized sulfamate moiety.

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