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Carbonic anhydrase inhibitors: Binding of an antiglaucoma glycosyl-sulfanilamide derivative to human isoform II and its consequences for the drug design of enzyme inhibitors incorporating sugar moieties☆

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Abstract—*N*-(4-Sulfamoylphenyl)-α-D-glucopyranosylamine, a promising topical antiglaucoma agent, is a potent inhibitor of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1). The high resolution X-ray crystal structure of its adduct with the target isoform involved in glaucoma, CA II, is reported here. The sugar sulfanilamide derivative binds to the enzyme in a totally new manner as compared to other CA–inhibitor adducts investigated earlier. The sulfonamide anchor was coordinated to the active site metal ion, and the phenylene ring of the inhibitor filled the channel leading to the active site cavity. The glycosyl moiety responsible for the high water solubility of the compound was oriented towards a hydrophilic region of the active site, where no other inhibitors were observed to be bound up to now. A network of seven hydrogen bonds with four water molecules and the amino acid residues Pro201, Pro202 and Gln92 further stabilize the enzyme–inhibitor adduct. Topiramate, another sugar-based CA inhibitor, binds in a completely different manner to CA II as compared to the sulfonamide investigated here. These findings are useful for the design of potent, sugar-derived enzyme inhibitors.

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 enzymes show a multitude of applications as diuretic, antiglaucoma, antiobesity or antitumour drugs, and are 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 an anion to the catalytically critical Zn^{2+} ion, also participating in extensive hydrogen bond networks and van der Waals interactions with amino acid residues in both the hydrophobic and hydrophilic halves of the enzyme active site.[7–15] 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 one, 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] mainly with sulfonamides, with some sulfamates and few sulfamides (including the simplest such derivatives,

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☆ Coordinates and structure factors have been deposited with the Protein Data Bank (Accession code 2HL4).
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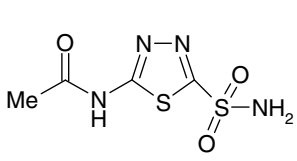
sulfamic acid and $H_2NSO_2NH_2$),[10] providing clear information on zinc coordination pattern in these enzymes as well as in the corresponding enzyme-inhibitor complexes. X-ray crystallography of CA–inhibitor adducts is in fact quite useful for drug design not only of this class of biologically active compounds, but also for investigating a much wider range of metalloenzyme inhibitors.[15] Thus, constantly, novel sulfonamide derivatives are synthesized and investigated as inhibitors of various enzymes in the search of compounds with selectivity for some of the many physiologically relevant isoforms, since the clinically used drugs **1**–**6** unselectively inhibit many isozymes and as a consequence show many undesired side effects.[1–6] For example, the fluorescent sulfonamide **7**, recently reported by our group, is in clinical development as an imaging agent, allowing the precise imaging of acute hypoxic tumours that are non-responsive to classical chemo- and radio-therapy.[7,16] On a totally different research area, we have also reported a series of sugar-sulfanilamide derivatives with potent topical antiglaucoma activity in an animal model of this disease.[17] The most promising compound of that series, the glucose derivative **8**, was shown to be a strong inhibitor of the key isozyme involved in glaucoma formation, that is, hCA II (h means human isoform), with an inhibition constant of 23 nM.[17] The compound was also shown to be highly water soluble and could be formulated as eye drops, which is not a straightforward property for sulfonamides, that are usually very poorly water soluble compounds. In order to understand at the molecular level the strong inhibitory activity of this compound against the target isoform involved in glaucomagenesis, and also for learning more regarding the possibility of using such data to obtain even more effective enzyme inhibitors with the desired physico-chemical properties, we here report the high resolution X-ray crystal structure (at 1.55 Å) of the adduct of hCA II with the sugar sulfonamide derivative **8**. A structural comparison with topiramate **9**, another interesting sugar derivative possessing good CA inhibitory activity and very interesting biological activity, being a clinically used antiepileptic,[15] is also reported. Topiramate possesses a



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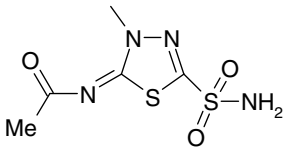

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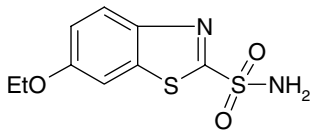

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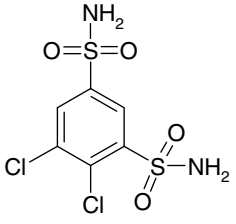

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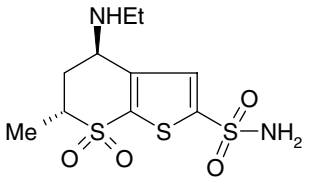

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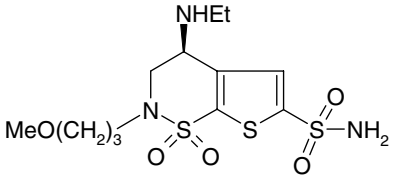

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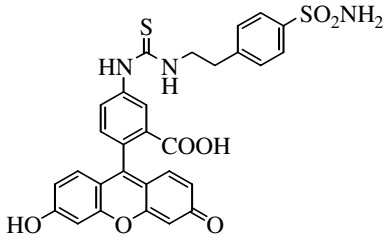

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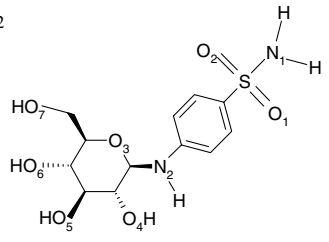

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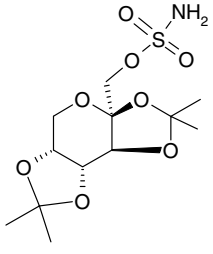

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