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Structural study of the location of the phenyl tail of benzene sulfonamides and the effect on human carbonic anhydrase inhibition

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ABSTRACT

The crystal structure of 4-phenylacetamidomethyl-benzenesulfonamide (**4ITP**) bound to human carbonic anhydrase (hCA, EC 4.2.1.1) II is reported. **4ITP** is a medium potency hCA I and II inhibitor (K_Is of 54–75 nM), a strong mitochondrial CA VA/VB inhibitor (K_Is of 8.3–8.6 nM) and a weak transmembrane CA inhibitor (K_Is of 136–212 nM against hCA IX and XII). This elongated compound binds in an extended conformation to hCA II, with its tail lying towards the hydrophobic half of the active site whereas the sulfonamide moiety coordinates the zinc ion. The present structure was compared to that of structurally related aromatic sulfonamides, such as 4-phenylacetamido-benzene-sulfonamide (**3OYS**), 4-(2-mercaptophenylacetamido)-benzene-sulfonamide (**2HD6**) and 4-(3-nitrophenyl)-ureido-benzenesulfonamide (**3N2P**). Homology models of the hCA I, VA, VB, IX and XII structures were build which afforded an understanding of the amino acids involved in the binding of these compounds to these isoforms. The main conclusion of the study is that the orientation of the tail moiety and the presence of flexible linkers as well polar groups in it, strongly influence the potency and the selectivity of the sulfonamides for the inhibition of cytosolic, mitochondrial or transmembrane CA isoforms.

1. Introduction

Sulfonamides are the most important class of carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs).[1–5] Several compounds, among which acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA**, sulthiame **SLT**, dichlorophenamide **DCP**, dorzolamide **DZA**, brinzolamide **BRZ**, sulpiride **SLP** and zonisamide **ZNS**, are in clinical use for years, as diuretics, antiglaucoma agents, as well as antiepileptics[1,2a,6a–f] (Chart 1). Sulfamates such as topiramate **TPM**, estrone-3-O-sulfamate **EMATE**, and irosustat **IRS**, although developed independently of their potential CA inhibitory properties, were also observed to act as potent CAIs and are clinically used as antiepileptics/antiobesity agents (topiramate)[1a,2a,6a–f] or are in advanced clinical trials as dual, steroid sulfatase/CA inhibitors with anticancer effects[6g] (Chart 1). Celecoxib **CLX** and valdecoxib **VLX**, initially discovered as COX-2-selective inhibitors are actually low nanomolar CAIs,[1a,2a] whereas the sweetener saccharin **SAC**, a secondary sulfonamide, also acts as efficient inhibitor on several CA isoforms, of the 15 presently known in humans, CA I–XIV (there are two 'V' type isoforms, CA VA and VB).[1–8] All these compounds directly coordinate to the Zn(II) ion from the enzyme active site in a deprotonated form of their sulfonamide/sulfamate zinc binding groups (ZBG).[1–7]

X-ray crystallography[1b,c,2–5] on adducts of many of these and other such sulfonamide/sulfamate/sulfamide derivatives with several human (h) CA isoforms, was crucial for understanding the factors governing the interaction between the enzyme active site and the inhibitor molecule, which may lead to compounds with selectivity for one (or some) hCAs involved in various pathologies, among which glaucoma,[1,6] cancer,[7] epilepsy,[6] and obesity.[8,9] In fact, the active site architecture of the CAs has a striking feature, not observed in any other enzyme class, as far as we know: half of the active site is lined only with hydrophobic residues, whereas the remaining, opposing half is lined with hydrophilic amino acid residues.[4a] This is a very particular 'bipolar' active site architecture which has been already noticed when the first crystal structure of such an enzyme, hCA II, was reported many years ago.[10a] The most

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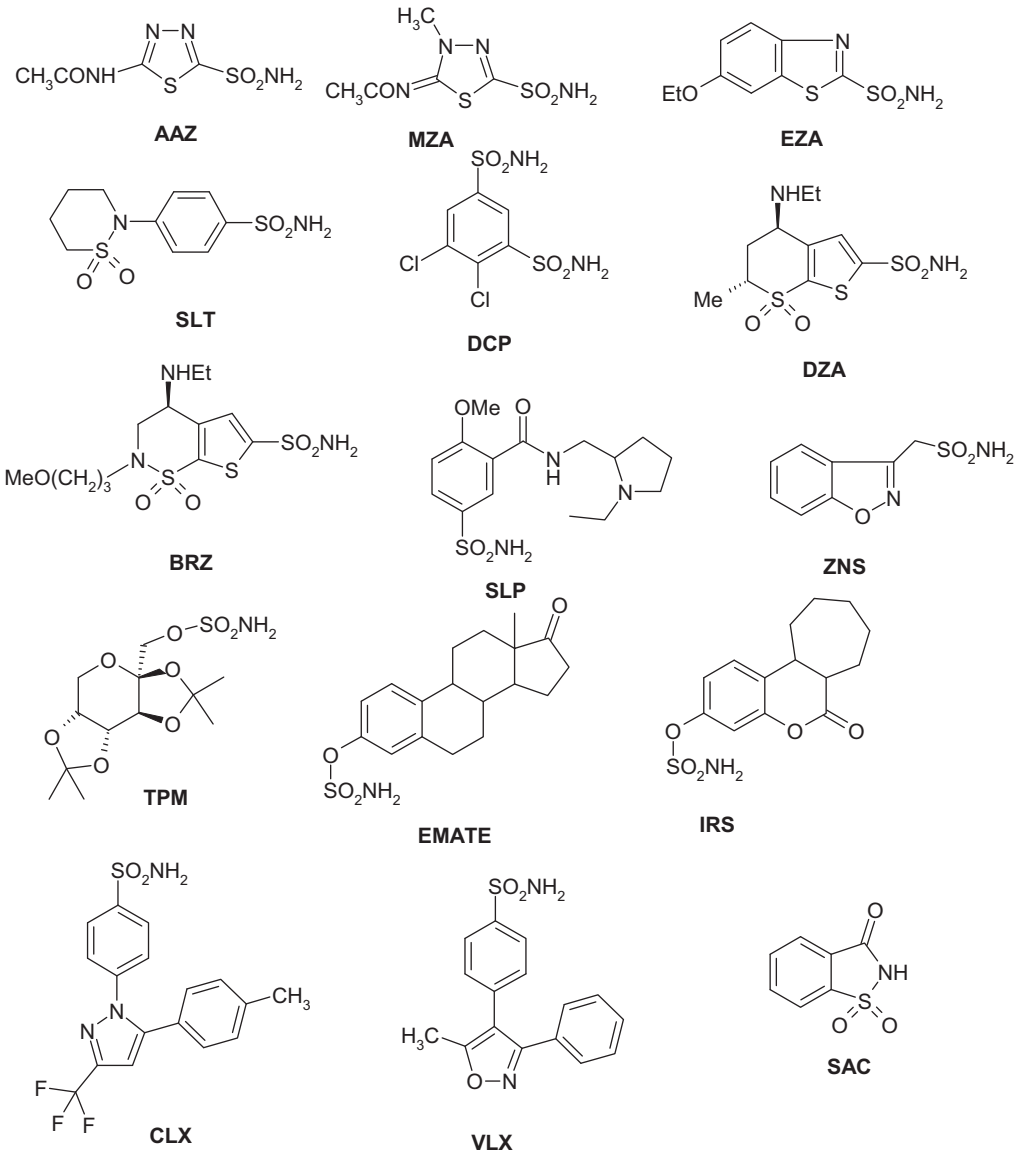

Chart 1. Clinically used sulfonamide/sulfamate CAIs **AAZ-SAC**.

probable explanation for this highly particular active site architecture (which is in fact present also in the other CA families, the β-, γ- and ζ-CA classes)[6] is that the hydrophobic part is used to entrap the CO_2 molecule (a quite hydrophobic gas). Indeed, its binding site is at the bottom of this part of the cavity, as shown in an interesting study by McKenna's group for hCA II.[10b] On the contrary, the hydrophilic half may be the part of the active site through which the polar components generated from the CO_2 hydration reaction (bicarbonate and protons) are released from the cavity towards the environment. At least for the protons, it is in fact well demonstrated that a relay of water molecules and several histidines (proton shuttling residues) are involved in such processes.[10c,d]

Although the details for the interaction between the ZBG and the metal ion are well known now for decades (for the sulfonamide/sulfamate/sulfamide CAIs), only recently a deeper understanding regarding the binding of the scaffolds and especially tails incorporated in them started to be achieved, based on the accumulation of structures for a large variety of such compounds bound to hCA II (as well as few other isoforms).[1b,2–9] Important such observations based on comparisons of a large number of such adducts allowed for some rationalization regarding the binding of the tails to the hydrophobic, or hydrophilic parts of the enzyme active site, which in fact strongly influences the selectivity of the CAIs for various isoforms. However, this process is still in its infancy and analyzing more such derivatives as well as their interactions with different CA isoforms is extremely useful in the drug design of selective, tighter-binding inhibitors. Continuing our interests in this field we report here the X-ray crystal structure for the adduct of a phenylacetamido benzenesulfonamide derivative bound to hCA II and an analysis of its interaction with several other pharmacologically relevant isoforms, such as hCA I, VA, VB, IX and XII.

2. Results and discussion

2.1. Chemistry and CA inhibition

Among the many classes of sulfonamide CAIs reported by our groups, a series of phenacetyl-containing aromatic and heterocyclic sulfonamides[3b,c] attracted attention due to the fact that for some members we evidenced excellent activity against physiologically