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Identification of arylsulfonamides as Aquaporin 4 inhibitors

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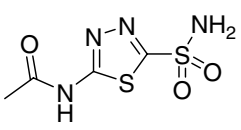
Abstract—Carbonic anhydrase inhibitors AZA, EZA, and 4-acetamidobenzsulfonamide were found to inhibit human AQP4-M23 mediated water transport by 80%, 68%, and 23%, respectively, at 20 μM in an in vitro functional assay. AZA was found to have an IC_{50} against AQP4 of 0.9 μM. Phloretin was inactive under the same conditions.

Aquaporin 4 (AQP4)[1] is a water specific channel protein belonging to the Aquaporin family of transmembrane water and water/glycerol transporters.[2,3] This protein is present in high concentrations in mammalian brain tissues,[4] particularly glial astrocytes, and is widely distributed in kidney, lung, muscle, and gastrointestinal tissues.[5] It is generally considered that AQP4 plays a significant auto-regulatory role in the brain because of its significant presence in glial cells, and hence might be a suitable target for drug discovery.

The precise role played by AQP4 in human physiology and pathology is not known. However, in addition to the auto-regulatory transport of intercellular water, evidence suggests that AQP4 is involved in the pathologies of edema,[6] epilepsy,[7,8] schizophrenia,[9,10] and possibly abnormal cytoskeletal morphology.[11] Modulators of this protein might be useful as therapeutic agents for any of these diseases. However, the study of the physiological roles played by AQP4, and its role in these and other pathologies has undoubtedly been hindered by the lack of any identified ligands or modulators for this protein.

Currently, only the tetraethylammonium cation (TEA) has been shown to inhibit AQP4 mediated water transport.[12] Unfortunately, the lack of SAR that could be developed around TEA suggested that it was not a viable starting point for our study.[13] Instead we turned to known ligands for other AQP isozymes. Acetazolamide (**1**, AZA) is a widely used pan-carbonic anhydrase (CA) inhibitor[14] and was recently shown to be a potent inhibitor of AQP1.[15,16] Phloretin (**2**) is a flavoid known to possess broad inhibitory activity toward a variety of ion channels,[17,18] and has also been shown to modulate several AQP isoforms, in particular the aquaglyceroporins, specifically AQP3, AQP7, and AQP9.[19–21] While AQP1 is closer to AQP4 in terms of sequence homology,[22] the lack of information regarding what types of binding interactions are available to AQP4 made it impossible to exclude either compound *a priori*.



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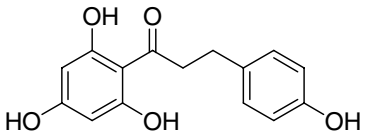

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During the initial phases of this project, the electron diffraction structure of the rat Aquaporin 4-M23 isozyme (rAQP4b) was generously made available to us by professor Fujiyoshi, Kyoto University.[23] Like all members of the Aquaporin family, AQP4 forms a protein homotetramer, where each protein monomer unit has a viable

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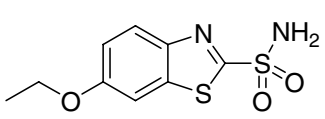
and presumably functional pore (Figure S1). The lack of an obvious gating mechanism or protein bridged binding site capable of blocking the water channel led us to consider modeling only a single protein monomer in lieu of the entire biological unit. The coordinates of one protein monomer were then imported into the BioMedCAChe/ActiveSite software environment for our virtual screening study.[24,25]

The virtual docking studies indicated that the preferred conformation for both compounds was quite similar. Moreover, the docking scores for **1** and **2** with rAQP4b were found to be essentially identical, −63.666 and −62.293 kcal/mol, respectively. In its final docked conformation (Fig. 1a), **1** was stabilized by electrostatic interactions between the sulfonamide group of **1** and guanidyl group of Arg-216 and the backbone carbonyl of Gly-209, as well as the acetamide group of **1** and carboxyl group of Asp-69. Additional hydrophobic interactions between **1** and Trp-59, Thr-56 and Ile-73 were also indicated. These interactions were consistent with that described between **1** and AQP1.[16] Likewise, the final docked structure of **2** (Fig. 1b) also indicated the presence of electrostatic interactions between the phenol and Arg-216. Additional electrostatic interactions were found between the ligand's trihydroxyphenone group and Asp-69 and His-151. Hydrophobic interactions were also identified between **2** and Thr-56, Ile-73 and Ile-205.

Compounds **1** and **2** were assayed for inhibition of AQP4 mediated hypoosmotic water transport in an in vitro functional assay using *Xenopus* oocytes expressing the human Aquaporin 4-M23 isozyme (hAQP4b).[26,27] Oocytes were generally handled and prepared as previously described,[28] and the assay was conducted using a modification of existing procedures.[29] The untransformed results, Figure 2, clearly indicated that **1** was able to inhibit hAQP4b mediated water transport, while **2** was not. A more detailed analysis of the data indicated that AZA showed 80 ± 4% inhibition, and was statistically relevant; whereas, **2** had no statistically relevant effect on the rate of water transport.[30]

The dose dependency of hAQP4b inhibition by **1** at ligand concentrations between 0.01 and 10 μM was also investigated. Statistically significant inhibition of water transport was found at 10 and 1 μM, in addition to that at 20 μM. No inhibition was observed for oocytes incubated with either 0.1 or 0.01 μM of **1**. Using those data, the apparent IC_{50} for **1** was found to be 0.9 μM with a maximum inhibition of 85%, Figure 3.

Despite the similar results of the in silico study, and the nearly identical sequences between the human and rat isozymes (approximately 97% identical), only **1** was found to inhibit AQP4 mediated hypoosmotic water transport. The drastic differences found for **1** and **2** lead to a persistent question about the validity of our model, and its utility as a predictive tool. Indeed, **1** and **2** were quite different in their overall structures and chemical properties; therefore, we were interested to compare the modeling results and inhibitory activities of similar compounds, in particular those related to **1**.



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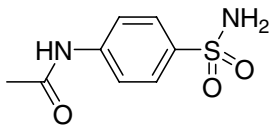

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The potential of additional pan-CA inhibitors to block AQP4 mediated water transport was investigated. Compounds **3** (EZA) and **4** were well-known pan inhibitors of various CA subtypes.[31] Generally, **3** was shown to have inhibitory activity across a range of CA isozymes that was similar to **1**, while **4** was weaker. The docking

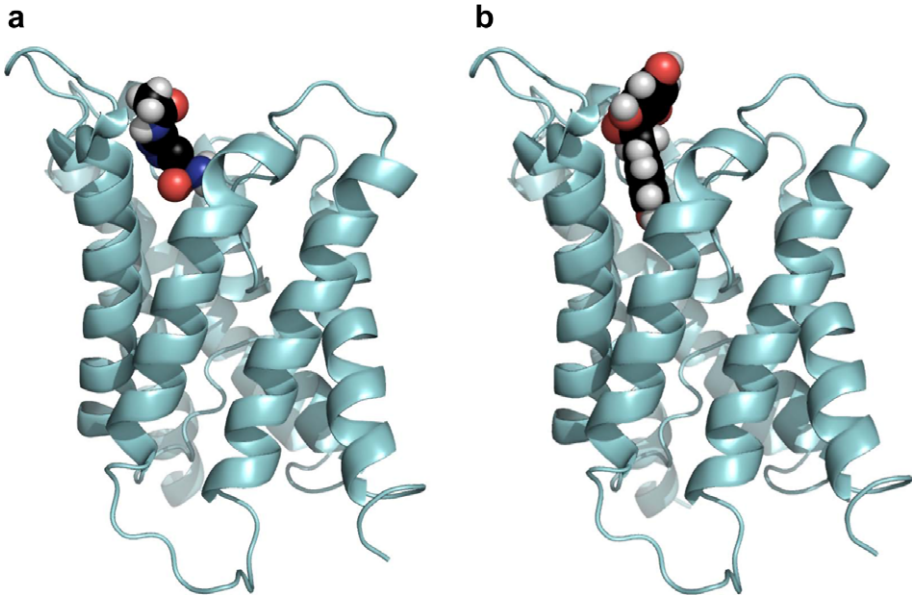

Figure 1. (a) Acetazolamide (**1**) and (b) phloretin (**2**) docked to the rAQP4b protein monomer. The ligands are shown as space-filling models, while the protein is shown as a ribbon schematic structure.