Selective inhibition of Carbonic Anhydrase IX for Cancer Diagnosis and Therapy

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Carbonic anhydrase IX (CA IX) belongs to a group of 15 isoforms of the human carbonic anhydrase enzymes. Typically localized on the cell surface, CA IX is primarily found in specific tissues within the gastrointestinal tract. Its expression is induced in response to local hypoxia, aiding in the regulation of pH levels to accommodate the metabolic production of acidic by-products, thereby promoting cancer cell survival and proliferation. The overexpression of CA IX in solid tumors, coupled with its extracellular presence, suggests its potential utility in cancer diagnosis and therapy.

Primarily, most of CA IX inhibitors feature sulphur-based functional group that coordinates the Zn^{2+} ion in the active site. Although overexpression of CA IX is predominantly associated with tumor tissues, other isoforms are present in normal tissues that contributing critical physiological processes. The high sequence similarity and structural homology among CA isoform family causes off-target inhibition leading to unintended side effects. This underscores the need for developing highly selective inhibitors that minimize off-target effects. The project aims to address these challenges by designing novel functional group to enhance both the affinity and selectivity of CA IX inhibitors.

The active site of CA is situated within the central β-sheet, where the zinc-binding core serves as a key junction for the proposes inhibitors, which are designed with a scaffold capable of attaching enzyme moieties. This scaffold comprises a sulfonimine binding group for metal ion interaction, a functional group for interaction with the hydrophobic regions, and additional heteroaromatic moieties to improve affinity. Structural optimisation of these inhibitors has been conducted by understanding how they are fitting within the enzyme's active site, in order enhancing their affinity for tumor-specific CA IX while restricting interactions with other CA isoforms. Additionally, some potent chelators have been selected for theranostics applications, ensuring they do not compromise the binding capacity of inhibitors.

Recombinant CA IX is produced and expressed in *Escherichia coli* BL21, followed by purification via several chromatographic steps to ensure high protein purity. The purified CA and a series of inhibitors are assessed for affinity using the stopped-flow method to screen a library of inhibitors. To better understand the binding modes between selective inhibitors and the enzyme, X-ray crystallography is employed to achieve high-resolution structures of the compounds. The obtained structural information will guide the modification and optimisation the anchored and sticky groups in design the selective inhibitors. This approach aims to maximize affinity for tumor-specific CA IX while minimizing interactions with other carbonic anhydrase isoforms.