SELECTIVE INHIBITION OF TUMOR-ASSOCIATED CARBONIC ANHYDRASE IX BY FLUORINATED BENZENESULFONAMIDES

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Carbonic anhydrases (CAs) are ubiquitous metalloenzymes present in living organisms; in humans, they belong to the α-CA family. These enzymes catalyze the reversible hydration of carbon dioxide to bicarbonate and a proton, thereby playing a key role in cellular pH regulation. Among the twelve catalytically active human isoforms, carbonic anhydrase IX (CA IX) is of particular interest due to its overexpression in hypoxic tumor cells, where it contributes to tumor progression and metastasis. Consequently, CA IX represents an attractive target for anticancer therapy. Primary benzenesulfonamides bearing various substituents are well-established CA inhibitors owing to their high affinity and relatively simple synthesis. However, the development of compounds that selectively inhibit CA IX while preserving the physiological activity of other CA isoforms remains a significant challenge [1].

The objective of this study was to identify selective CA IX inhibitors by evaluating newly synthesized fluorinated benzenesulfonamide derivatives bearing cyclic and aliphatic side chains High-throughput fluorescent thermal shift assay (FTSA) was employed to simultaneously screen multiple ligands across a range of concentrations. FTSA determines ligand-induced protein stabilization by monitoring changes in the protein unfolding temperature (ΔT_m), which is subsequently converted into binding constants K_b using advanced thermodynamic models [2]. Isoform selectivity was assessed by performing experiments with CA I, CA II, chimeric CA VI, CA VII, CA IX, CA XII, and CA XIII. In addition, stopped-flow CO_2 hydration assay was used to confirm inhibition of CA catalytic activity. All experimentally obtained binding and inhibition data were deposited into the Protein–Ligand Binding Database (PLBD, plbd.org), enabling their direct use in AI-driven drug discovery and predictive modeling of CA IX inhibitor affinity and selectivity.

Screening of the compound series enabled the identification of the lead compound MZ24-11, which exhibited nanomolar affinity (K_d = 1.4 nM) and pronounced selectivity toward CA IX (**Fig. 1**) over CA I, CA II, chimeric CA VI, CA VII, CA IX, CA XII, and CA XIII. Although the lead inhibitor demonstrated high potency, further structural optimization is required for its development as a viable anticancer drug candidate.

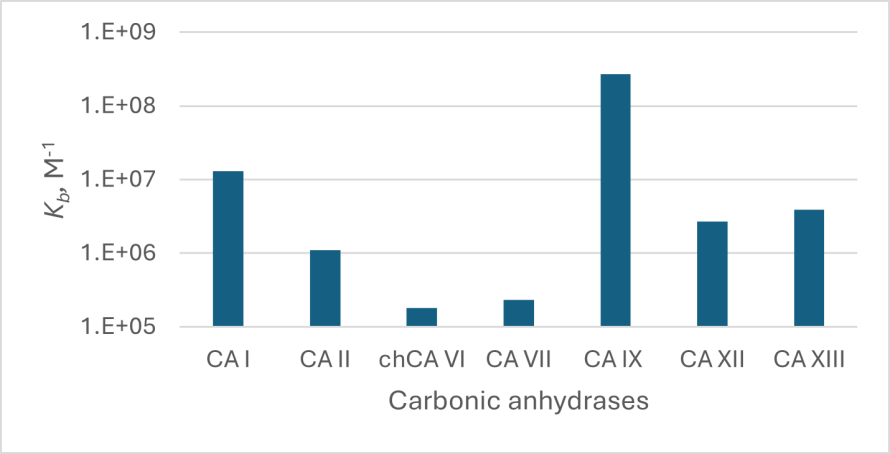

Fig. 1. A diagram comparing binding constants (Kb) of lead compound MZ24-11 binding to CA I, II, chCA VI, CA VII, CA IX, CA XII and CAXIII. CA IX is clearly inhibited by several orders of magnitude stronger than CA I, II, chCA VI, CA VII, CA XII, CA XIII, indicating inhibitor's selectivity towards CA IX.

[1] V. Linkuvienė et al., "Thermodynamic, kinetic, and structural parameterization of human carbonic anhydrase interactions toward enhanced inhibitor design," Quarterly Reviews of Biophysics, vol. 51, p. e10, Jan. 2018, doi: 10.1017/s0033583518000082.

[2] V. Petrauskas, E. Kazlauskas, M. Gedgaudas, L. Baranauskienė, A. Zubrienė, and D. Matulis, "Thermal shift assay for protein–ligand dissociation constant determination," TrAC Trends in Analytical Chemistry, vol. 170, p. 117417, Nov. 2023, doi: 10.1016/j.trac.2023.117417.