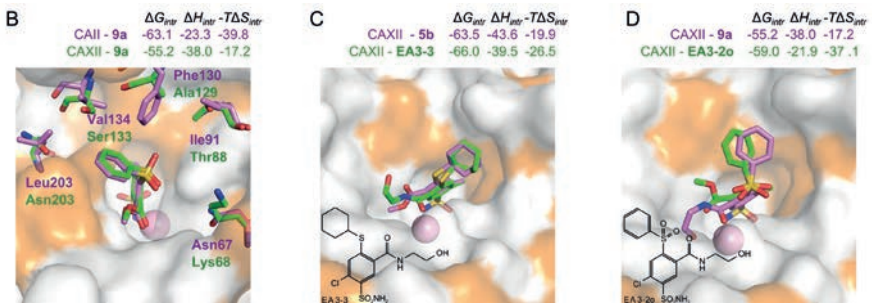

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Structure and Thermodynamics for Drug Design

We measure the energies of protein–ligand binding and apply structure–thermodynamics correlations to design compounds of greater affinity and selectivity for target proteins. We attempt to improve the structure–activity relationships and the principles of rational drug design. We are primarily focused on the human family of twelve catalytically active carbonic anhydrase (CA) isozymes as a disease protein-target. These enzymes have essentially the same fold and a similar shape of the active site suitable for the testing of isozyme selectivity. Sometimes structurally similar compounds exhibit highly different affinities. Deep understanding of the underlying forces that determine the affinity and selectivity is one of the main goals of our laboratory.

In an effort to design CA isozyme-selective compounds, we have assembled a database of 1092 chemical compounds binding to CA isozymes, including

(a) the X-ray crystallographic structures of 180 CA-compound complexes,
(b) the thermodynamics of CA-compound interaction, including the standard observed and intrinsic dissociation constant, Gibbs energy, enthalpy, entropy, volume, and heat capacity changes upon binding,
(c) the kinetics of CA-compound binding, including the on- and off-rates.

Our group of over 30 researchers and students include molecular biologists, biochemists, organic chemists, biophysicists, physicists, computer modellers, biologists and medical doctors. Organic chemists design and synthesize novel compounds, molecular biologists clone, express (both in bacterial and in human cell cultures), and purify various target proteins (Hsp90, HDAC isozymes, functional proteins of COVID-19 coronavirus, etc.), biothermodynamicists determine the energies of binding (by isothermal titration calorimetry, thermal shift assay, and enzyme inhibition assay), crystallographers determine the X-ray crystallographic structures of protein-compound complexes, *in silico* modellers perform compound docking, while cell biologists perform drug-candidate compound studies in biological systems including cancer cell cultures, zebrafish, and mice. Together with medical doctors, we are developing the inhibitors of anticancer target protein CAIX for tumour visualization and treatment.

SELECTED PUBLICATIONS

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3. DeLeeuw, L. W., Monsen, R. C., Petrauskas, V., Gray, R. D., Baranauskiene, L., Matulis, D., Trent, J. O. and Chaires, J. B. POT1 stability and binding measured by fluorescence thermal shift assays. *PLOS ONE*. 2021, 16: e0245675.
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5. Zakšauskas, A., Čapkauskaitė, E., Paketurytė-Latvė, V., Smirnov, A., Leitans, J., Dvinskis, E., Stančaitis, L., Mickevičiūtė, A., Jachno, J., Jezepčikas, L. et al. Methyl 2-Halo-4-Substituted-5-Sulfamoyl-Benzoates as High Affinity and Selective Inhibitors of Carbonic Anhydrase IX. *Int J Mol Sci*. 2021, 27.

Design of CAIX-Selective EA Compounds

Among the twelve catalytically active carbonic anhydrase isozymes present in the human body, the CAIX is highly overexpressed in various solid tumours. The enzyme acidifies the tumour microenvironment enabling invasion and metastatic processes. Therefore, many attempts have been made to design chemical compounds that would exhibit high affinity and selective binding to CAIX over the remaining eleven catalytically active CA isozymes to limit undesired side effects. It has been postulated that such drugs may have anticancer properties and could be used in tumour treatment.

Here we have designed a series of compounds, methyl 5-sulfamoylbenzoates, which bear a primary sulfonamide group, a well-known marker of CA inhibitors, and determined their affinities for all twelve CA isozymes. Variations of substituents on the benzenesulfonamide ring led to compound 4b, which exhibited an extremely high *observed* binding affinity to CAIX; the K_d was 0.12 nM. The *intrinsic* dissociation constant, where the binding-linked protonation reactions have been subtracted, reached 0.08 pM. The compound also exhibited more than 100-fold selectivity over the remaining CA isozymes. The X-ray crystallographic structure of compound 3b bound to CAIX showed the structural position, while several structures of compounds bound to other CA isozymes showed structural reasons for compound selectivity towards CAIX. Since this series of compounds possesses physicochemical properties suitable for drugs, they may be developed for anticancer therapeutic purposes (Zaksauskas et al. *Int J Mol Sci.* 2021, 27).

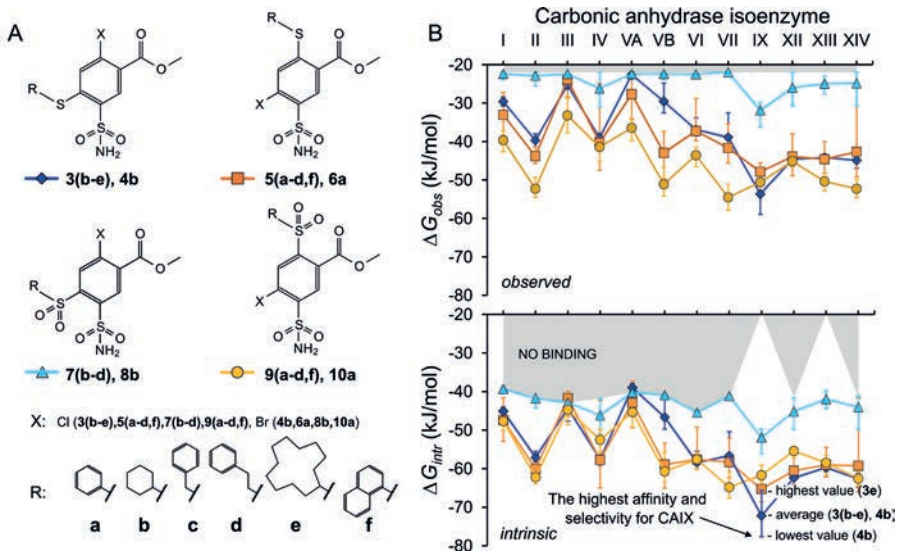

Fig. 1. (**A**) Chemical structures of the four groups of compounds—methyl halo 2- or 4-substituted-5-sulfamoylbenzoates containing chlorine or bromine and substituents listed at the bottom of the panel. (**B**) Averaged change in the standard observed (upper panel) or *intrinsic* (lower panel) Gibbs energy for compounds belonging to one of the four groups: **3(b–e)**, **4b**—blue rhombs, **5(a–d,f), 6a**—orange squares, **7(b–d), 8b**—cyan triangles, and **9(a–d,f), 10a**—yellow circles. The bars show the margin between the strongest and weakest affinity of compounds in that group for the indicated CA isozyme. The *intrinsic* binding affinity of compounds **3(b–e), 4b** to CAIX is highlighted: the blue rhombus is the average of all five interactions, the bar above shows the weakest CAIX interaction with **3e**, and below is the strongest with **4b**. Compounds in this group have the highest selectivity and affinity for CAIX.

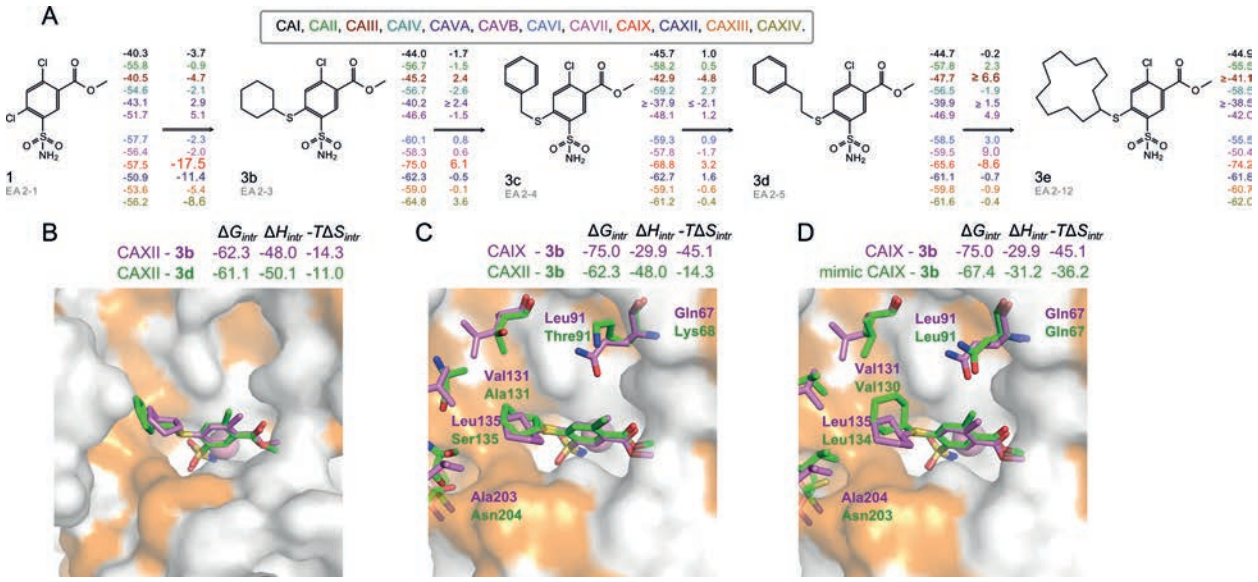

Fig. 2. Correlation of compound chemical structures with the changes in standard intrinsic Gibbs energy upon binding and the comparison of the crystal structures between CA isozymes. (**A**) Differences of ΔG_{intr} between neighbouring compounds are listed on the connecting arrows. All values have units of kJ/mol. Colours represent CA isozymes. (**B**) Compounds **3b** (magenta, PDB ID: 7PP9) and **3d** (green, PDB ID: 7PUW) bound to CAXII. (**C**) Compound **3b** bound to CAXII (green, PDB ID: 7PP9) and CAIX (magenta, PDB ID: 7POM). (**D**) Compound 3b bound to CAIX (magenta, PDB ID: 7POM) and mimic-CAIX (green, PDB ID: 7Q0C). Zinc ion is shown as a pink sphere. The notable nonconservative residues between active sites are shown in the "stick" mode. The protein surface of the CA active site is coloured orange for hydrophobic residues (V, I, L, F, M, A, G, and P) and grey for the residues with charged and polar side chains (R, D, N, E, Q, H, K, S, T, Y, W, and C).