PICOMOLAR INHIBITORS OF CARBONIC ANHYDRASE: IMPORTANCE OF INHIBITION AND BINDING ASSAYS

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Human carbonic anhydrases (CAs) are targets for drug design due to their role in numerous diseases such as glaucoma, epilepsy, and cancer. Clinically used CA inhibitors–drugs are relatively weak and non-selective for human CA isoforms thus exhibiting toxic side effects. Further drug development should lead to compounds with picomolar affinities and significant selectivities. Currently, the K_i of CA inhibitors is usually determined by the stopped-flow CO_2 hydration assay, the method that directly follows inhibition of CA enzymatic activity. However, the assay has limitations, such as largely unknown concentration of CO_2 and the inability to determine the K_i below several nM. The widely used direct binding assay, isothermal titration calorimetry, also does not determine the K_d below several nM. In contrast, the thermal shift assay can accurately determine picomolar affinities.

The inhibitor dose-response curves were analyzed using Hill and Morrison equations demonstrating that only the Morrison model is applicable for the determination of tight-binding inhibitor K_i. The measurements of interactions between ten inhibitors and seven CA isoforms showed the limitations and advantages of all three techniques. Inhibitor **6**[1] exhibited the K_d of 50 pM and was highly selective towards human CA IX, an isoform which is nearly absent in healthy human, but highly overexpressed in numerous cancers. Combination of inhibition and binding techniques is necessary for precise determination of CA–high-affinity inhibitor (such as **6**) interactions and future drug design[2].

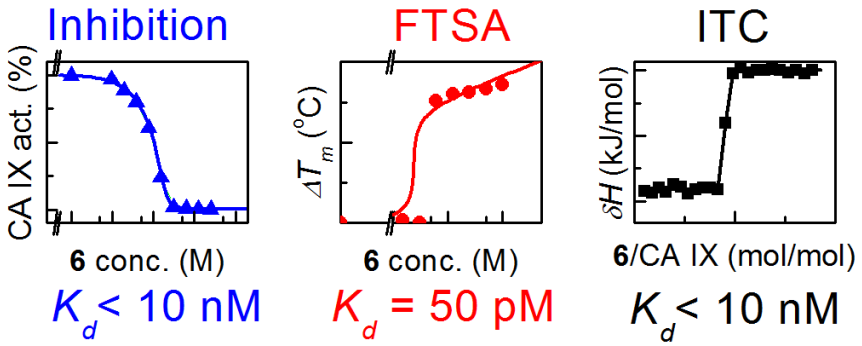

Fig. 1. Comparison of enzymatic activity inhibition assay, fluorescent thermal shift assay and isothermal titration calorimetry.

[1] Dudutienė, V. et al.. Discovery and characterization of novel selective CAIX inhibitors. J. Med. Chem 9435–9446 (2014).
[2] Smirnovienė, J., Smirnovas, V., and Matulis, D. Picomolar inhibitors of carbonic anhydrase: Importance of inhibition and binding assays. Anal. Biochem. **522** 61-72 (2017).