Discovery of Novel Selective Inhibitors Targeting Carbonic Anhydrase IX

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INTRODUCTION:

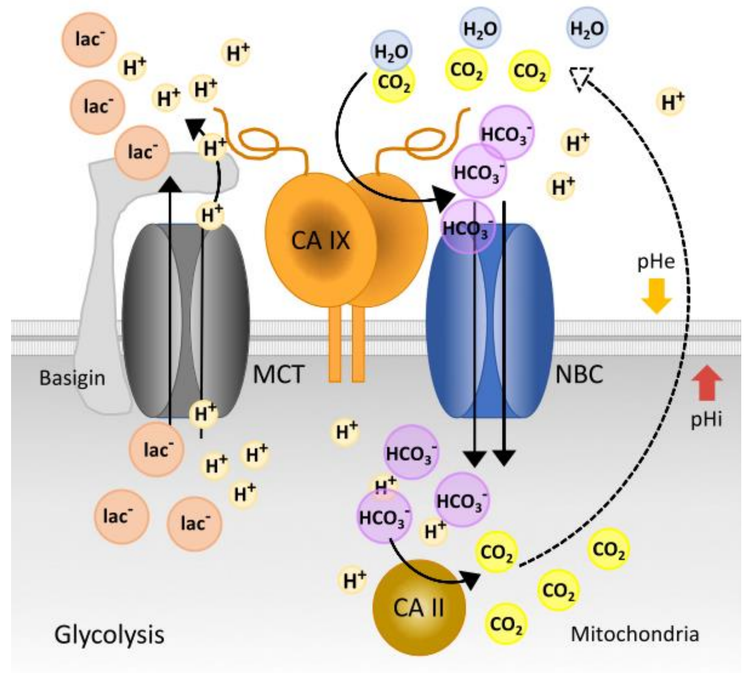
Carbonic anhydrase family catalyzes the reversible hydration of carbon dioxide to bicarbonate and protons:

$$CO_2 + H_2O \xleftrightarrow{\text{CA IX catalysis}} H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

Physiological conditions: CA IX contributes to acid–base balance, facilitates CO_2 transport across membranes, and helps stabilize local pH, particularly under conditions of limited oxygen availability. Its expression is tightly controlled by the hypoxia-inducible factor HIF-1α.

Tumor microenvironment: CA IX is strongly upregulated in response to hypoxia, a hallmark of many solid tumors. By promoting extracellular acidification while maintaining a neutral intracellular pH, CA IX enables cancer cells to survive, proliferate, and invade under metabolic stress. It is also associated with enhanced metastatic potential, modulation of the tumor microenvironment, and resistance to radiotherapy and certain chemotherapies.

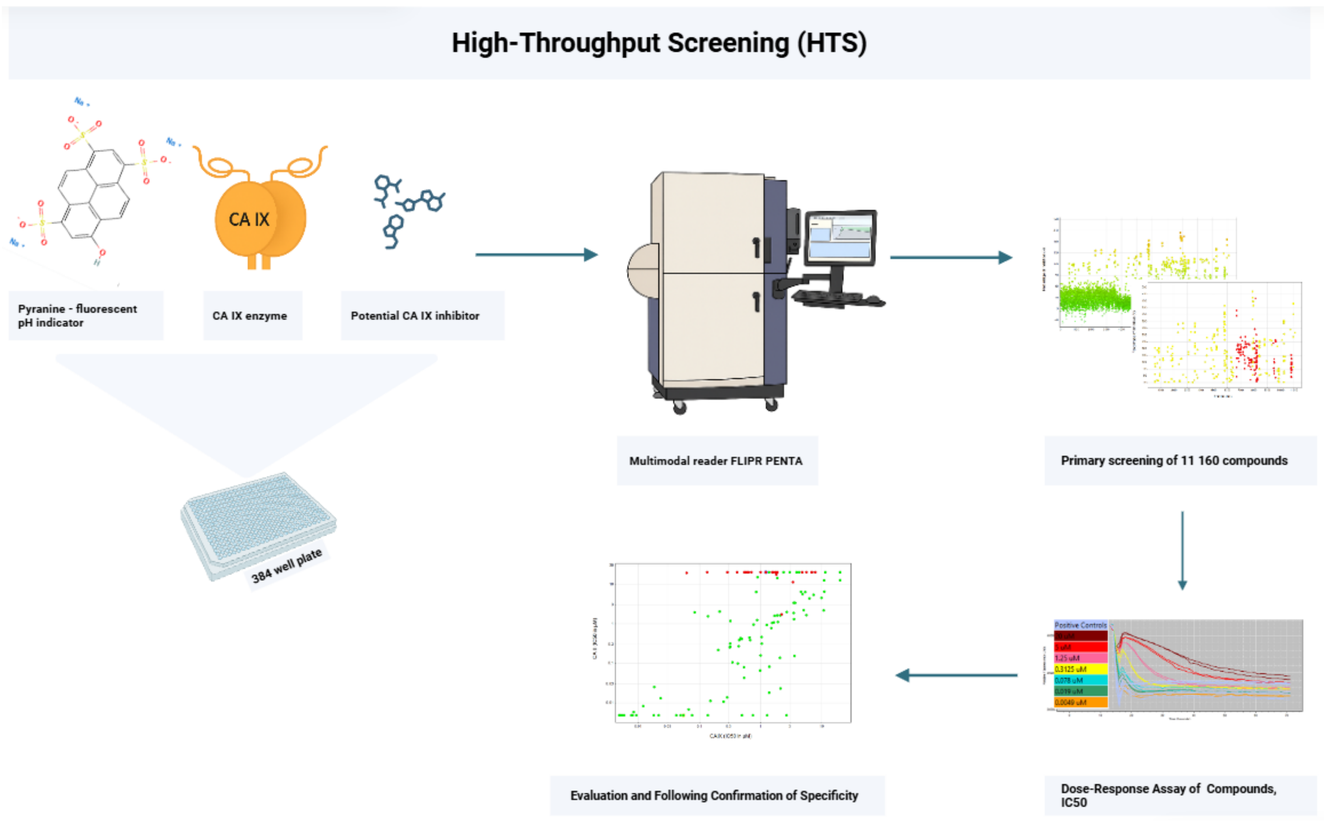
These features make CA IX an attractive therapeutic target.

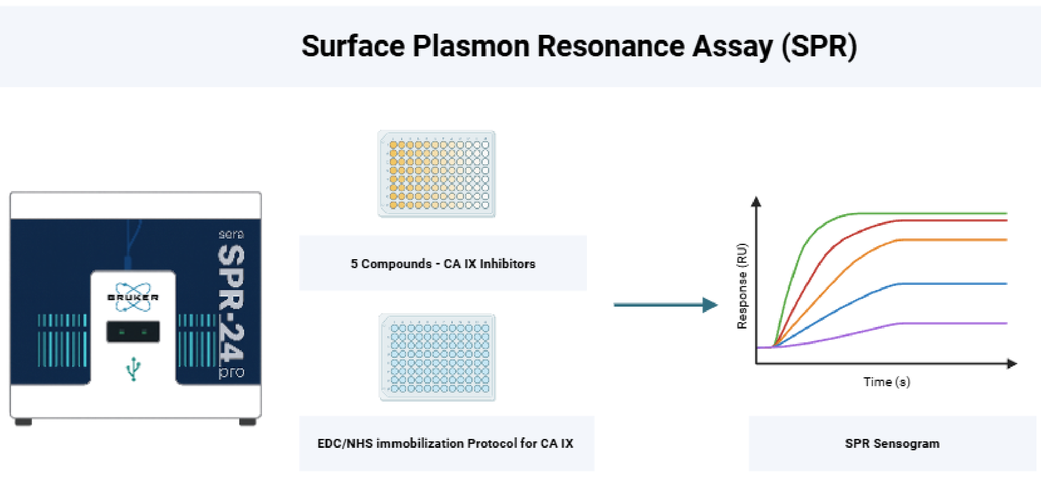


OBJECTIVE:

1. Identification of novel, highly specific inhibitors of carbonic anhydrase IX
2. Selection of best candidates for radiolabeling to improved localization of hypoxic tumors

METHODS:





RESULTS:

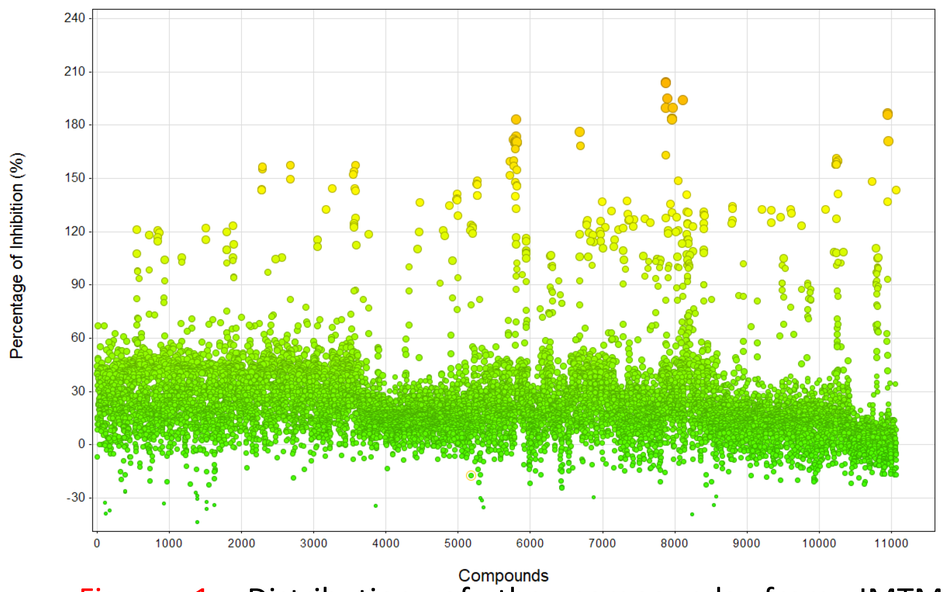


Figure 1: Distribution of the compounds from IMTM Proprietary Library according to their inhibitory effect on CA IX, Z factor = 0,52 – 0,84.

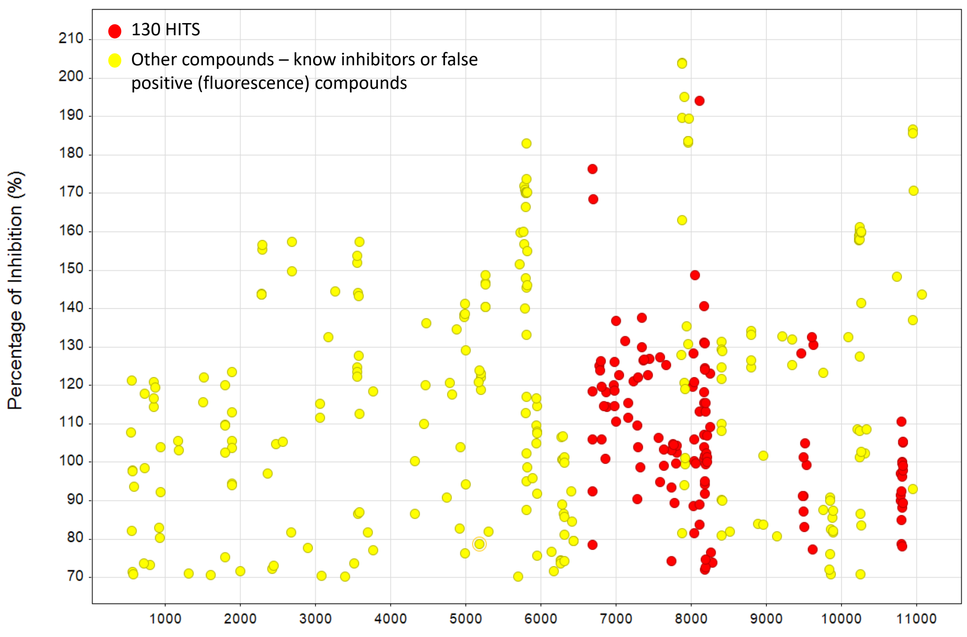


Figure 2: Selected compounds from Figure 1 with PI value > 70%.

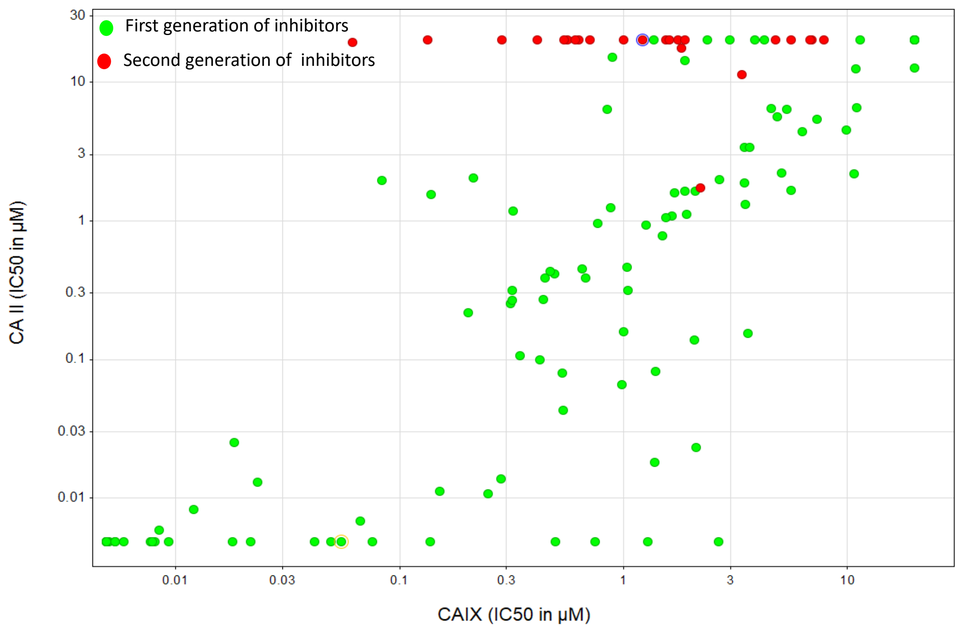


Figure 3: Evaluation of the specificity of HITS compounds, R= IC50(CAII)/IC50(CAIX)

	COMPOUND:	IC50 CA II [μM]	IC50 CA IX [μM]	SELECTIVITY INDEX R R=IC50(CAII)/IC50(CAIX)	K_D
1ST GENERATION OF INHIBITORS	LEM 17808	2.02	0.21	9	2.932×10^{-8}
	LEM 17802	1.94	0.084	23	1.28×10^{-2}
	LEM 18606	1.53	0.14	11	-
2ND GENERATION OF INHIBITORS	LEM 241350	>20	0.13	149	-
	LEM 241354	>20	0.41	49	-
	LEM 241377	19.38	0.06	314	-
	LEM 241378	>20	0.29	70	-

Table 1: Based on the primary HTS results, seven compounds exhibiting the most promising inhibitory profiles toward CA IX were selected for subsequent characterization. These hits are currently being subjected to follow-up biophysical analyses, including determination of the binding affinity (KD) by surface plasmon resonance (SPR), to further validate their potential as selective CA IX inhibitors

CONCLUSION:

HTS screening identified several compounds with notable inhibitory activity and selectivity toward CA IX. A subset of the most promising hits was advanced to SPR analysis; however, the current SPR setup does not yet provide reliable kinetic parameters due to insufficient CA IX immobilization. Further optimization of the immobilization protocol is required to obtain robust K_D values and fully validate the selected inhibitors

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