Engineered Carbonic Anhydrase VI-Mimic Enzyme Switched the Structure and Affinities of Inhibitors

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Supplementary

Figure S1. The structural alignment of CA II and CA VI sequences (UniProt codes CAH2_HUMAN and CAH6_HUMAN). The residues in the binding site and the mutated amino acids of CA II to design CA VI-mimic protein are indicated underneath the sequences. The binding site residues shown here correspond to the residues shown in Figure 1D.

Figure S2. Crystal structures described in this study. The electron density |F(o)-F(c)| of the ligands is calculated in the absence of ligand and contoured at 3σ. Zinc ion is shown as blue sphere. (A) Compound EZA bound to CA VI-mimic (PDB ID 6QL2). (B) Compound **14** bound to CA VI-mimic (PDB ID 6QL1). (C) Compound **25** bound to CA VI-mimic (PDB ID 6QL3).

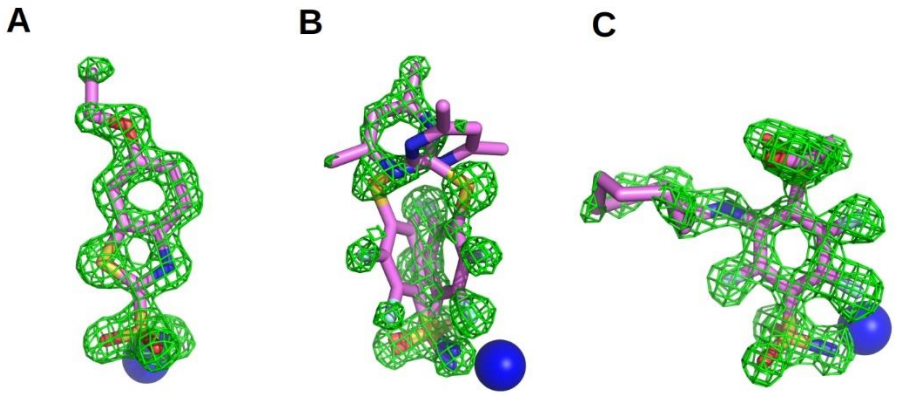

Figure S3. The plot of K_M dependence on pH for CA II (red squares), CA VI-mimic (wine triangles) and CA VI (royal circles).

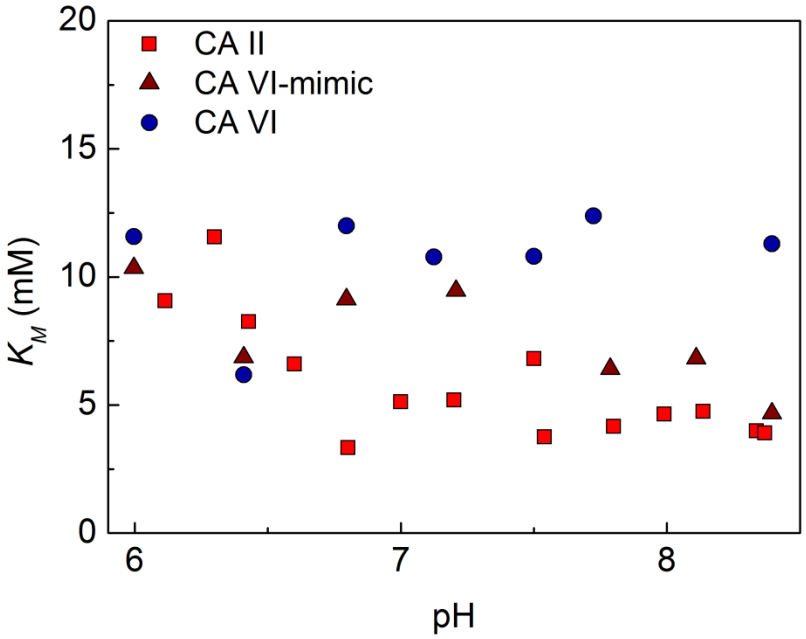

Table S1. Dissociation constants K_ds of selected compounds determined by SFA at pH 7.5, 23 ^{o}C.

Inhibitor	**Lab. name**	**pK_{a_SA}**	***$K_{d\ obs}$ (nM)***		
			CA II	**CA VI-mimic**	**CA VI**
2.	EZA	7.82	<2.5	<25	<25
14.	VD10-50	8.02	<20	67	290
23.	VD12-10	10.2	<17	91	1800
39.	E11-18	8.90	<25	140	750