

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.

CA II	M.....SHHWGYGK.HNGPEHWHKDFPIAKGERQSPVDIDTH	36
CA VI	MRALVLLLSLFLGGQAQHVSDWTYSEGALDEAHWPQHYPACGGQRQSPINLQRT	55
CA II	TAKYDPSLKPLSVSYDQ..ATSLRILNNGHAFNVFDDSQDKAVLKGPLDGTYR	89
CA VI	KVRYNPSLKGLNMTGYETQAGEFPMVNNGHTVQISLPSIMRMTVADG...TVYI	106
CA II	LIQFHFHWGSLD..GQGSEHTVDKKKYAAEELHLVHWNTKYGDFGKAVVQQPDGLAV	142
CA VI	AQQMHFHWGGASSEISGSEHTVDGIRHVIELIHVHYNKYKSYDIAQDAPDGLAV	161
CA II	LGIFLKVGS..AKPGLQKVVDVLDSEIKTKGKSADFTNFDPRGGLPES.LDYWYYP	194
CA VI	LAAFVEVKNYPENTYYSNFIHSLANIKYPGQRTTLTGLDVQDMLPRNLQHYTYH	216
CA II	GSLTTPPLLECVTWIVLKEPISVSSEQVLKFRKLNFNAGEPEELMVDNWRPAQP	249
CA VI	GSLTTPPCTENVHWFVLADFVKLSRTQVWKLNSLLDHR...NKTIHNDYRRTQP	268
CA II	LKNRQIKASFK.....	260
CA VI	LNHRVVESNFPNQEYTLGSEFQFYLHKIEEILDYLRRALN	308

- conserved residues
- similar residues
- conserved residues in the binding site
- unconserved residues in the binding site (mutated in CA VI mimic)

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).

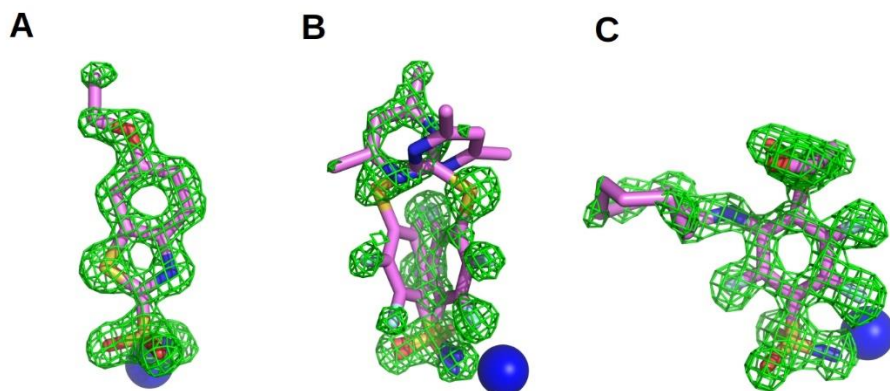


Figure S3. The plot of K_M dependence on pH for CA II (red squares), CA VI-mimic (wine triangles) and CA VI (royal circles).

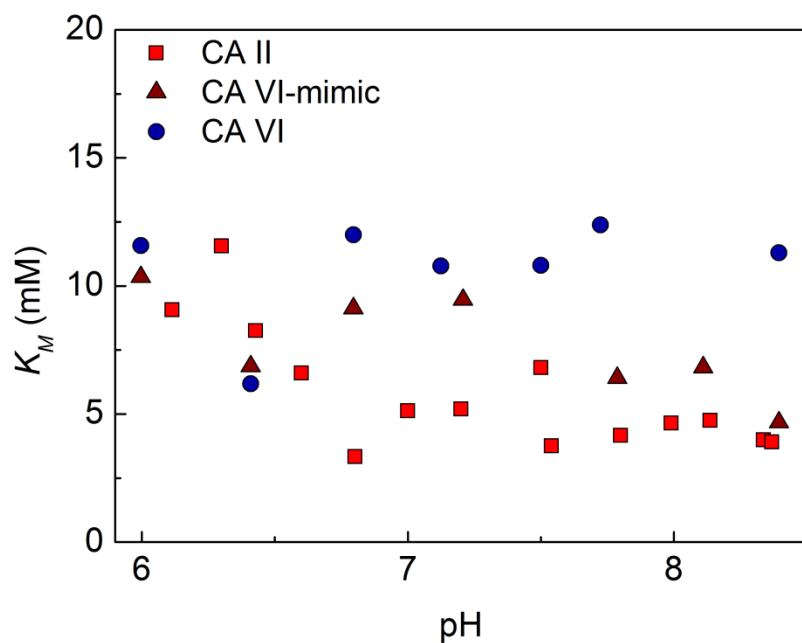


Table S1. Dissociation constants K_d s of selected compounds determined by SFA at pH 7.5, 23 °C.

Inhibitor	Lab. name	p<i>K_a</i>_{SA}	<i>K_d</i>_{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