

Supplementary Materials

A Proof-of-Concept Fragment Screening of a Hit-Validated 96-Compound Library Against Human Carbonic Anhydrase II

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Table S1: Crystallographic data for hCAII in complex with fragments. ^a

	hCAII-1 (6RM1)	hCAII-2 (6RMP)	hCAII-3 (6SB7)	hCAII-4 (5M78)	hCAII-5 (6SAS)
Data collection and processing					
Beamline	ELETTRA XRD1	ELETTRA XRD1	BESSY II 14.2	DESY P13	BESSY II 14.1
Wavelength / Å	1.0000	1.0000	0.9184	0.7999	0.9184
Space group	P2 ₁	P2 ₁	P2 ₁	P2 ₁	P2 ₁
<i>a,b,c</i> / Å	42.5, 41.5, 72.0	42.4, 41.6, 72.1	42.5, 41.7, 72.5	42.4, 41.4, 72.2	42.4, 41.5, 72.1
β / °	104.7	104.7	104.3	104.8	104.3
Matthews coefficient / Å ³ Da ⁻¹ ^b	2.1	2.2	2.2	2.2	2.1
Solvent content / % ^b	42.1	44.1	42.9	44.1	42.5
Diffraction data					
Resolution range / Å	41.5 – 1.68 (1.78 – 1.68)	41.5 – 1.22 (1.29 – 1.22)	41.7 – 1.09 (1.16 – 1.09)	41.4 – 1.08 (1.083 – 1.08)	41.5 – 1.10 (1.16 – 1.10)
Unique reflections / Å	27053 (4228)	72050 (11150)	98467 (14806)	105910 (16480)	97293 (15236)
<i>CC</i> _{1/2} / % [1]	99.7 (99.2)	99.8 (85.3)	99.8 (93.0)	99.8 (84.6)	99.8 (78.9)
<i>R</i> _{sym} / % [2]	5.1 (7.3)	6.8 (43.3)	5.2 (23.3)	6.6 (42.6)	6.7 (45.8)
Completeness	96.8 (94.5)	98.6 (94.8)	97.2 (91.1)	98.3 (95.2)	97.8 (95.4)
Wilson <i>B</i> factor / Å ²	8.7	9.9	8.1	8.9	9.0
Multiplicity	3.8 (3.6)	3.7 (3.7)	3.6 (3.5)	3.7 (3.6)	3.6 (3.6)
<i>I</i> / $\sigma(I)$	19.1 (12.1)	10.2 (2.4)	13.5 (4.5)	9.7 (2.2)	9.9 (2.3)
Refinement					
Resolution range / Å	35.6 – 1.68	34.9 – 1.22	35.8 – 1.09	35.6 – 1.08	41.1 – 1.10
Reflections used in refinement (work/free) [3] ^c	27052 (25699/1353)	72050 (68447/3603)	98465 (93541/4924)	103053 (97900/5153)	97283 (92418/4865)
Final <i>R</i> values for all reflections (work/free) [3] ^c	0.153/0.181	0.132/0.162	0.118/0.137	0.125/0.144	0.132/0.149
Protein residues	258	259	258	257	257
Fragment atoms	11	12	10	10	12
Water molecules	166	208	153	152	211
RMSD from ideality					
Bond lengths / Å	0.008	0.007	0.009	0.007	0.006
Bond angles / °	1.00	0.99	1.11	0.97	0.94
Ramachandran plot / % ^d					
Residues in most favored regions	88.0	88.5	88.9	89.9	88.9
Residues in additionally allowed regions	11.5	11.0	11.1	9.6	10.6
Regions in generously allowed regions	0.5	0.5	0	0.5	0.5
Residues in disallowed regions	0	0	0	0	0
Mean <i>B</i> factor / Å ² ^e					
Protein non-hydrogen atoms	10.5	12.9	10.8	11.8	11.4
Fragment	8.3	15.5	14.0	17.0	23.5
Water molecules	18.3	24.5	20.7	24.1	22.8

^a Data in parentheses refer to the highest resolution shell unless stated otherwise. Calculated using the program *Phaser Cell Content Analysis* from the *CCP4* suite [4]. ^c 5 % of all reflections were used for *R*_{free} calculation. ^d Calculated using the program *PROCHECK* [5]. ^e Calculated using the program *MOLEMAN* [6].

Table S1 continued. ^a

	hCAII-6 (6SAY)	hCAII-7 (6S9Z)	hCAII-8 (6SAC)	hCAII-9 (6SDJ)
Data collection and processing				
Beamline	BESSY II 14.1	DESY P13	BESSY II 14.1	BESSY II 14.2
Wavelength / Å	0.9184	0.7999	0.9184	0.9184
Space group	P2 ₁	P2 ₁	P2 ₁	P2 ₁
<i>a,b,c</i> / Å	42.4, 41.5, 72.2	42.4, 41.3, 72.4	42.4, 41.3, 72.3	42.4, 41.1, 72.2
β / °	104.7	104.7	104.8	104.2
Matthews coefficient / Å ³ Da ⁻¹ ^b	2.1	2.1	2.1	2.1
Solvent content / % ^b	42.2	40.2	40.1	39.9
Diffraction data				
Resolution range / Å	41.5 – 0.95 (1.01 – 0.95)	41.3 – 0.95 (1.01 – 0.95)	41.0 – 1.02 (1.08 – 1.02)	41.1 – 1.02 (1.02 – 1.08)
Unique reflections / Å	146380 (21821)	141261 (19435)	121056 (19033)	120252 (18157)
<i>CC</i> _{1/2} / % [1]	99.9 (79.5)	99.8 (79.0)	99.8 (78.0)	99.8 (96.0)
<i>R</i> _{sym} / % [2]	5.2 (39.9)	6.3 (43.2)	5.3 (39.1)	4.8 (17.5)
Completeness	96.5 (89.1)	92.7 (79.0)	98.3 (96.0)	98.1 (92.1)
Wilson <i>B</i> factor / Å ²	7.9	6.9	8.5	9.6
Multiplicity	3.5 (3.0)	3.8 (3.8)	2.7 (2.5)	3.5 (2.9)
<i>I</i> / $\sigma(I)$	11.4 (2.0)	10.7 (2.3)	10.0 (2.2)	13.5 (3.7)
Refinement				
Resolution range / Å	34.9 – 0.95	35.3 – 0.95	32.0 – 1.02	35.4 – 1.02
Reflections used in refinement (work/free) [3] ^c	146362 (139043/7319)	141257 (134196/7061)	121032 (114983/6049)	120242 (114229/6013)
Final <i>R</i> values for all reflections (work/free) [3] ^c	0.122/0.138	0.119/0.137	0.126/0.142	0.128/0.140
Protein residues	264	265	265	258
Fragment atoms	10	17	9	5
Water molecules	223	241	199	180
RMSD from ideality				
Bond lengths / Å	0.007	0.006	0.008	0.006
Bond angles / °	0.97	0.97	1.07	1.01
Ramachandran plot / % ^d				
Residues in most favored regions	89.6	88.3	87.4	88.5
Residues in additionally allowed regions	9.5	11.2	12.1	11.5
Regions in generously allowed regions	0.9	0.4	0.4	0
Residues in disallowed regions	0	0	0	0
Mean <i>B</i> factor / Å ² ^e				
Protein non-hydrogen atoms	10.7	8.3	10.7	12.7
Fragment	18.5	6.5	5.8	9.0
Water molecules	20.5	20.4	22.5	24.0

^a Data in parentheses refer to the highest resolution shell unless stated otherwise. Calculated using the program *Phaser Cell Content Analysis* from the *CCP4* suite [4]. ^c 5 % of all reflections were used for *R*_{free} calculation. ^d Calculated using the program *PROCHECK* [5]. ^e Calculated using the program *MOLEMAN* [6].

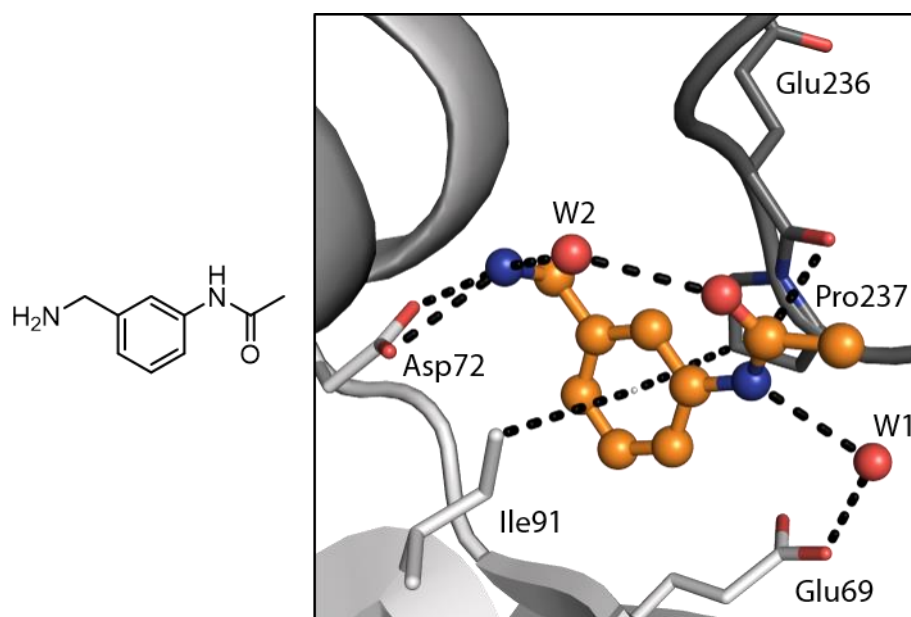


Figure S1: Structural formula and binding mode of fragment **5**. Interactions described in the main article are shown as dashed lines. The phenyl ring centroid is shown as gray dot for clarity. The symmetry-related mate is shown in dark gray.

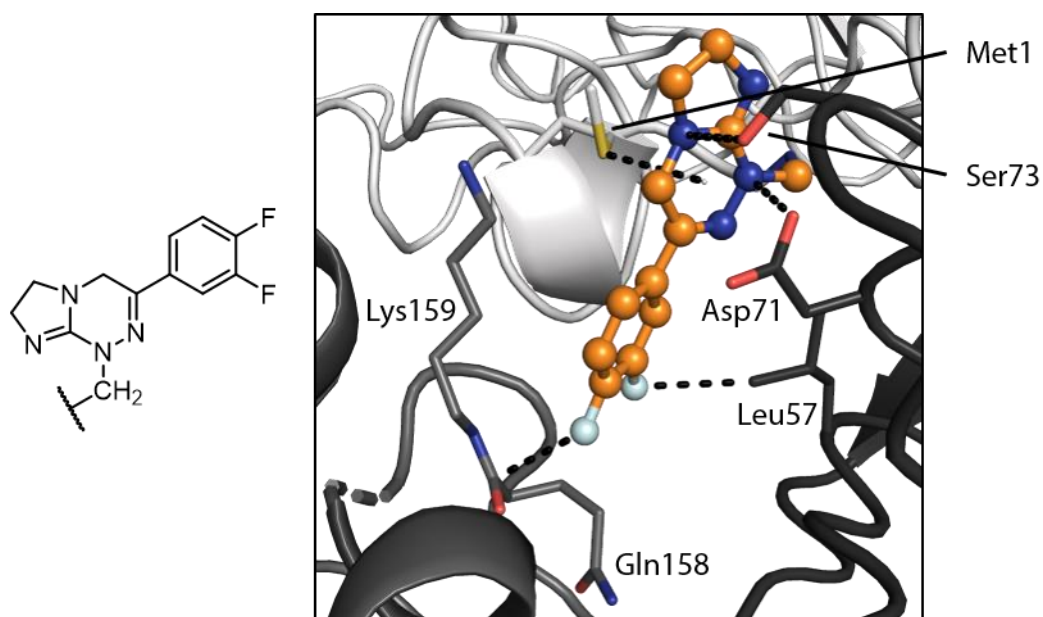


Figure S2: Structural formula and binding mode of fragment **7**. Interactions described in the main article are shown as dashed lines. The centroid of the six-membered heterocycle is shown as gray dot for clarity. Protonation of the heterocyclic scaffold can reasonably be assumed. Symmetry related mates involved in interactions with **7** are shown in darker shades of gray.

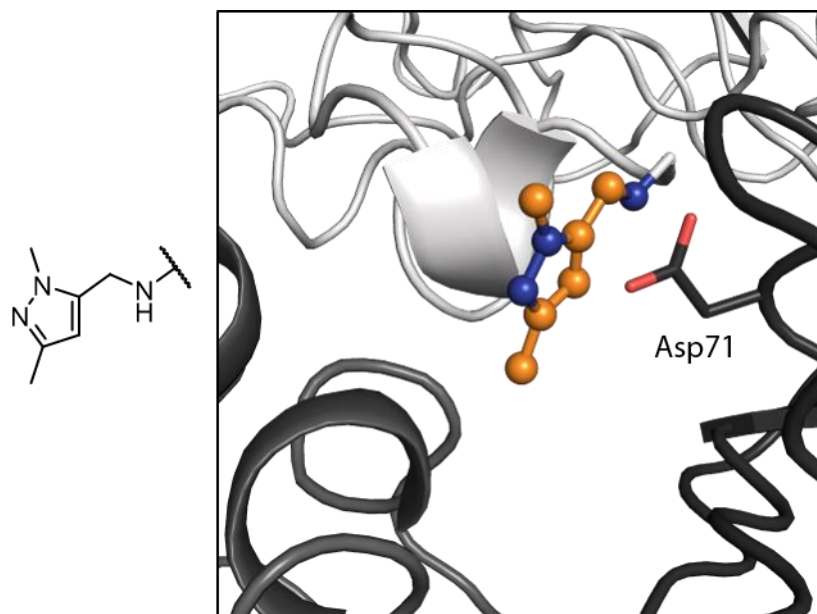
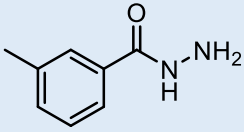
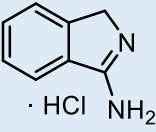
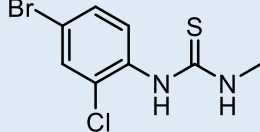
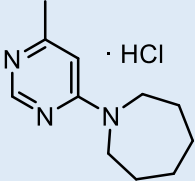
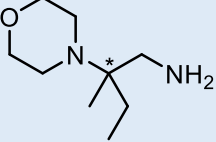
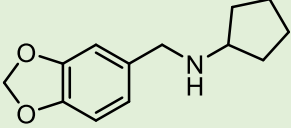
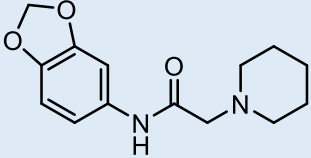
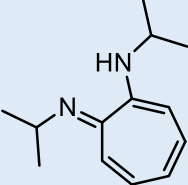
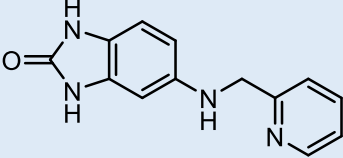
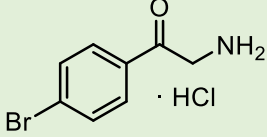
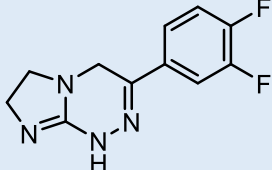
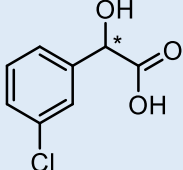
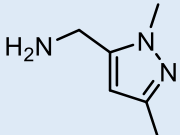
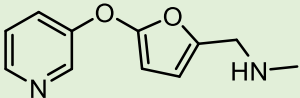
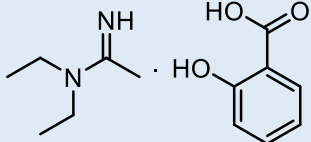
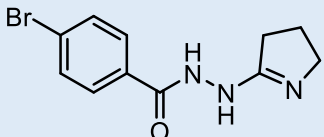
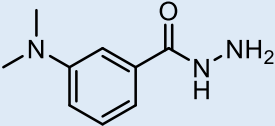
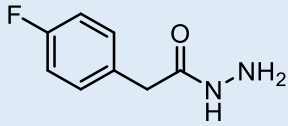
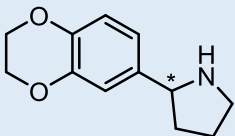
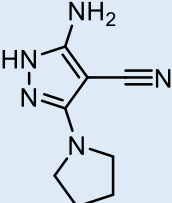
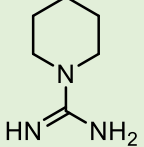
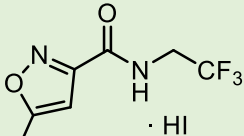
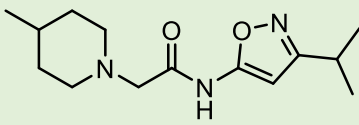
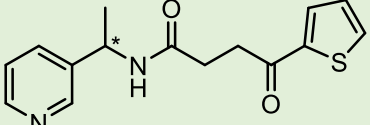


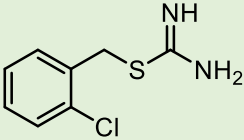
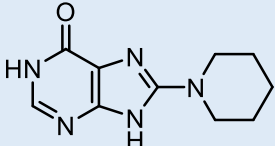
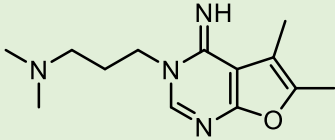
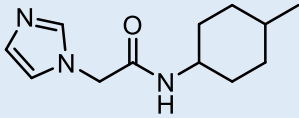
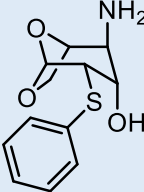
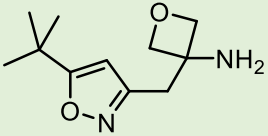
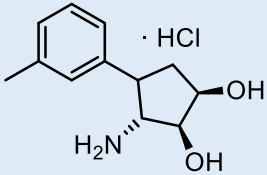
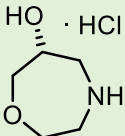
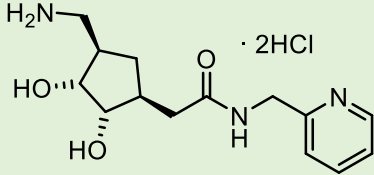
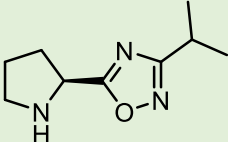
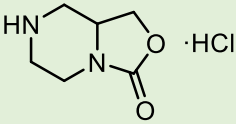
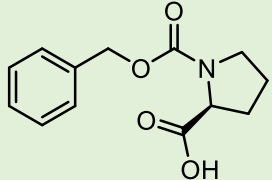
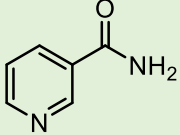
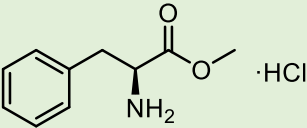
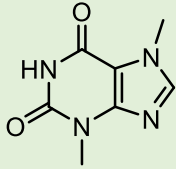
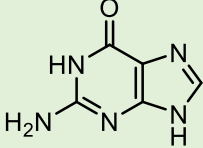
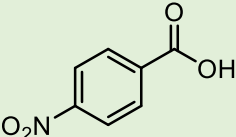
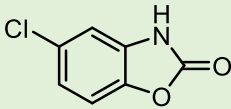
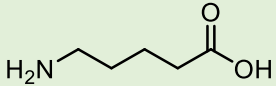
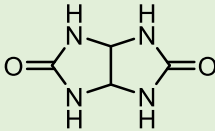
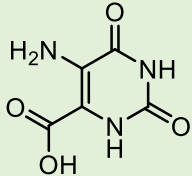
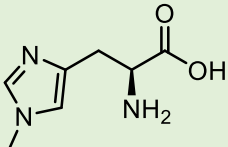
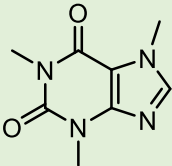
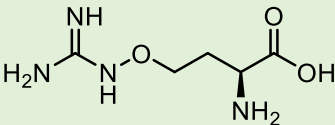
Figure S3: Structural formula and binding mode of fragment **8**. The side chain of Met1 could not be modeled. Protonation of the heterocycle and consequently a charged interaction with Asp71 is unlikely. Symmetry-related mates are shown in darker shades of gray.

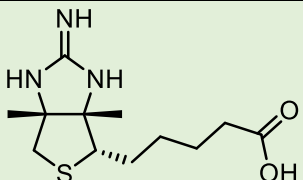
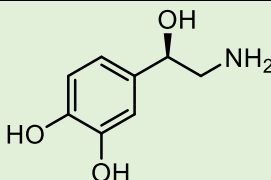
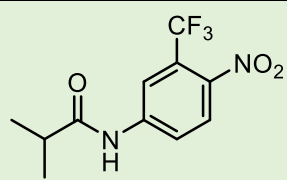
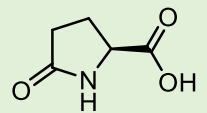
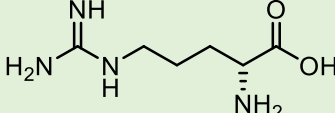
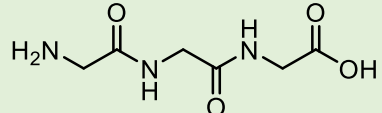
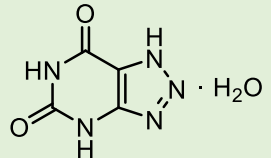
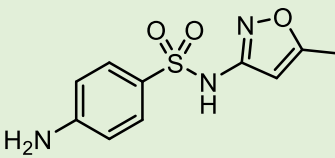
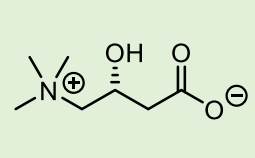
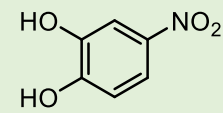
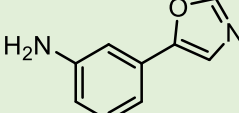
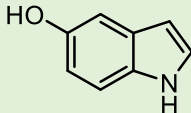
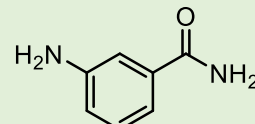
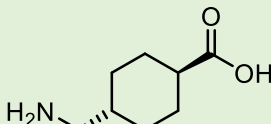
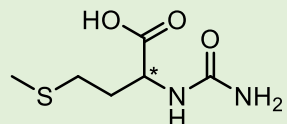
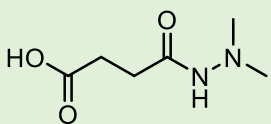
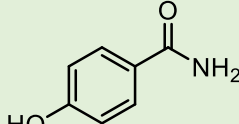
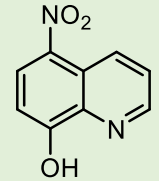
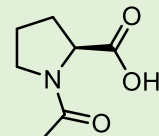
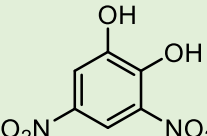
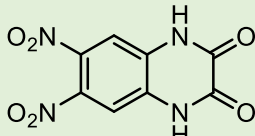
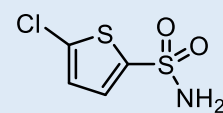
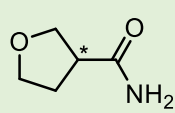
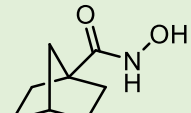
Table S2: Structural formulas of screened fragments. Identified hits are colored in orange and the numbers from the main article are given in parentheses. Blue coloring indicates a soaking time of 15 h and green coloring a soaking time of 3 min.

		
1 (1)	2	3
		
4	5	6
		
7	8	9^a
		
10	11 (7)	12
		
13 (8)	14	15 (4)
		
16	17 (2)	18
		
19	20	21
		
22	23	24

^a Is not fragment 9 from the main article.

25	26	27
28	29	30
31	32	33
34	35	36
37	38	39
40	41	42
43	44	45
46 (5)	47	48

		
49	50	51
		
52	53	54
		
55	56	57
		
58	59 (6)	60
		
61	62	63
		
64	65	66
		
67	68	69
		
70	71	72

		
73	74	75
		
76	77	78
		
79	80	81
		
82	83	84
		
85	86	87
		
88	89	90
		
91	92	93
		
94 (3)	95	96

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