From cosubstrate similarity to inhibitor diversity - inhibitors of ADP-ribosyltransferases from kinase inhibitor screening

Benjamin Maurer, Ulf Mathias, Panagiotis Papatheodorou, Suhaib Shekfeh, Joachim Orth, Thomas Jank, Carsten Schwan, Wolfgang Sippl, Klaus Aktories, Manfred Jung*

Supplementary Information

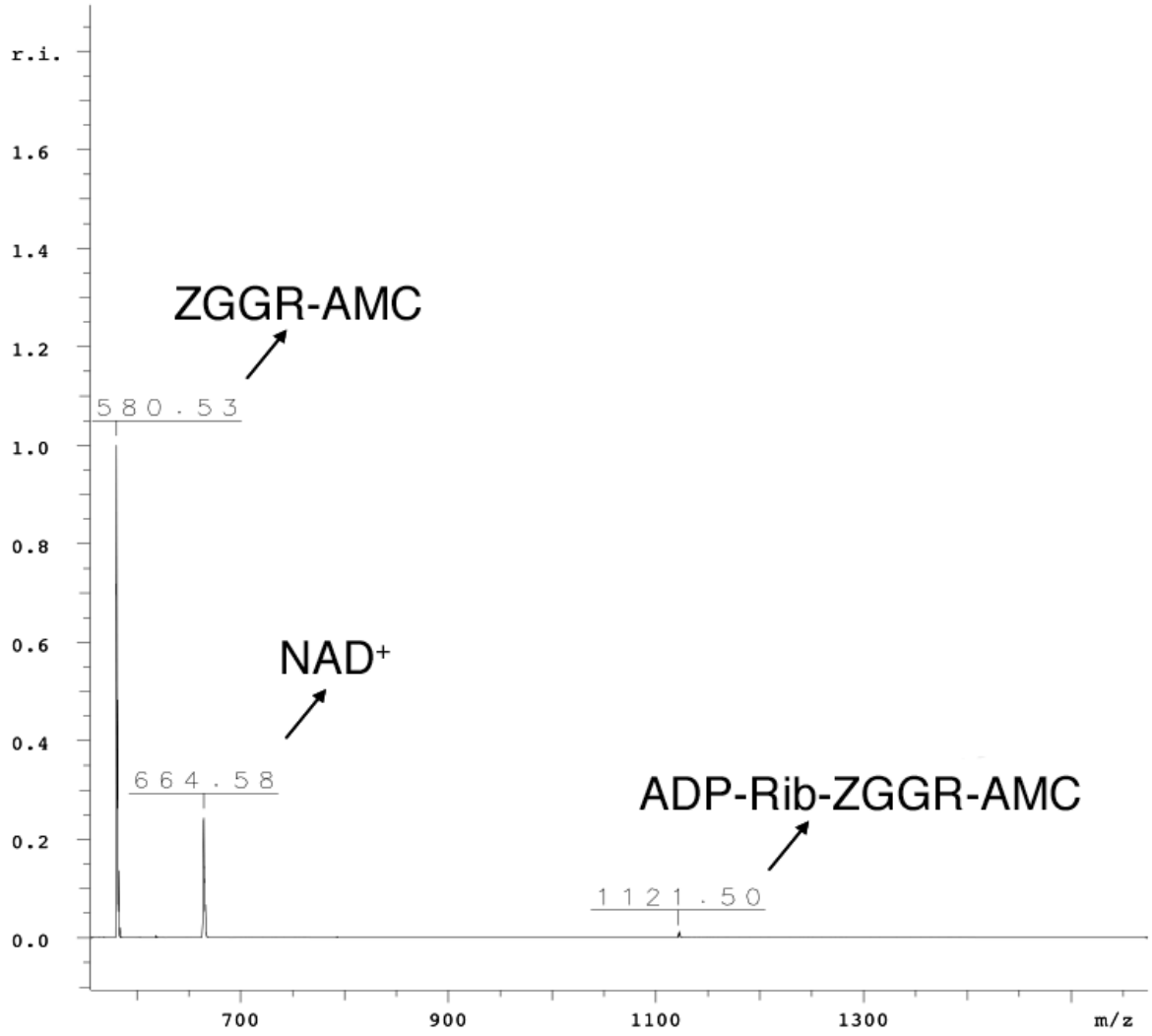


Fig. S1 - MALDI-TOF-detection of ZGGR-AMC, NAD^+ and the ADP-ribosylated substrate (ADP-Rib-ZGGR-AMC).

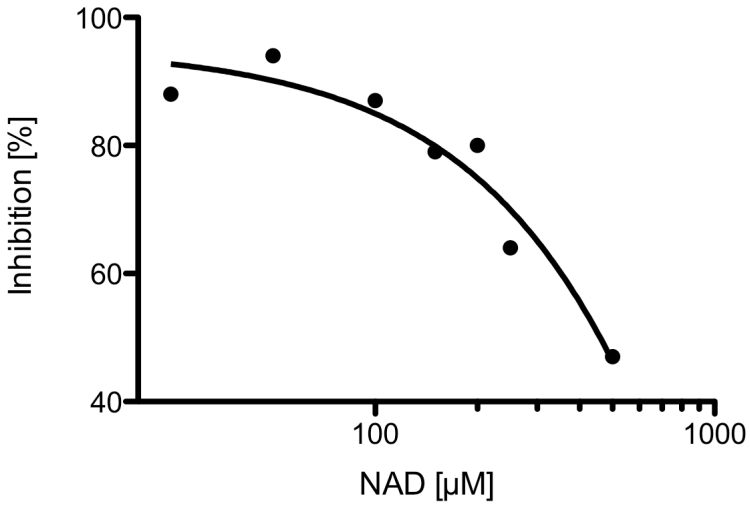
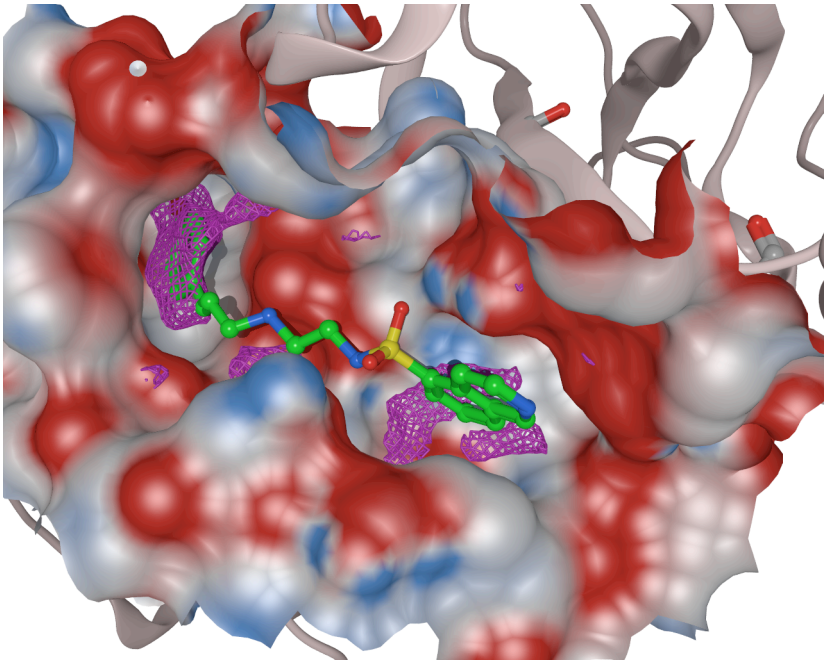


Fig. S2 - Decreasing MTX inhibition by H-89 with increasing concentrations of NAD^+

A



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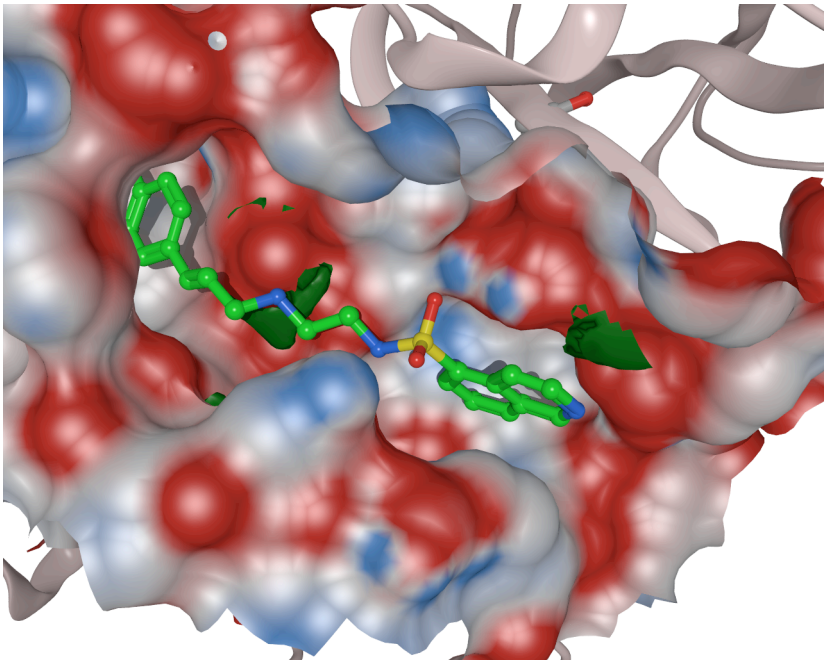

Fig. S3 - Favourable interaction sites at the MTX-NAD^+ binding pocket calculated with the GRID program. The molecular surface is colored according to the electrostatic potential (red color = electronegative region, blue color = electropositive region). A) Favourable hydrophobic interactions obtained with the methyl probe (colored magenta, contour level -3 kcal/mol). B) Favourable interaction sites obtained with an ammonium probe (colored green, contour level -7.0 kcal/mol).

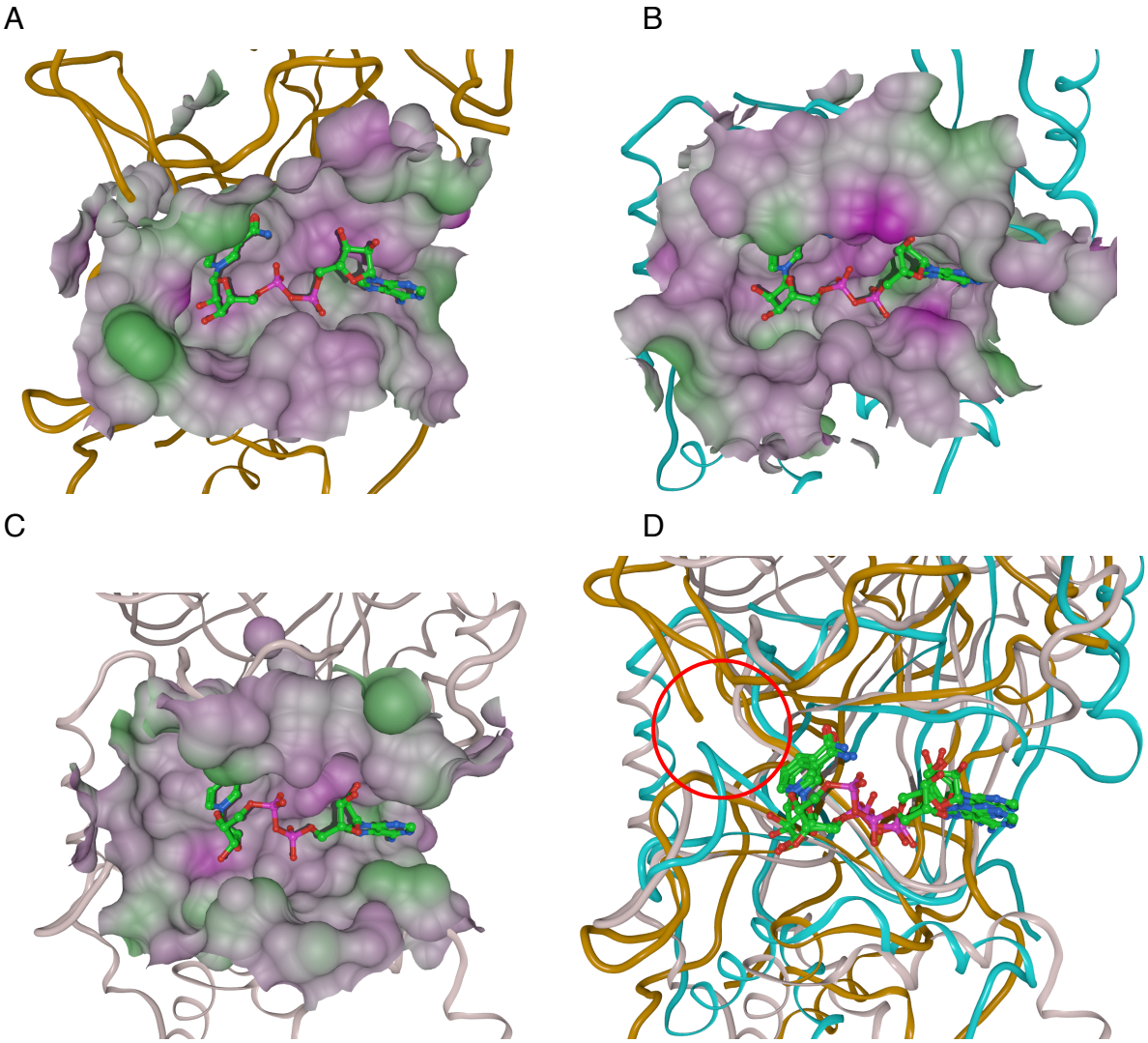

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Fig. S4 - Comparison of the X-ray structures of MTX (brown ribbon) (A), C3bot (green-blue ribbon) (B) and CDTa (mauve backbone) (C). The high similarity of the conformation of the bound NAD^+ molecules (green carbons) can be recognized. The molecular surface of the binding pocket is colored according to the hydrophobicity (green = hydrophobic, magenta = hydrophilic). (D) Superimposition of the three X-ray structures based on their conserved backbone regions. The same coloring scheme for the backbone ribbon is used as in Fig. S4A. Structural variation is mainly detectable in the region neighbouring the nicotinamide group of NAD^+ (marked by the red circle).

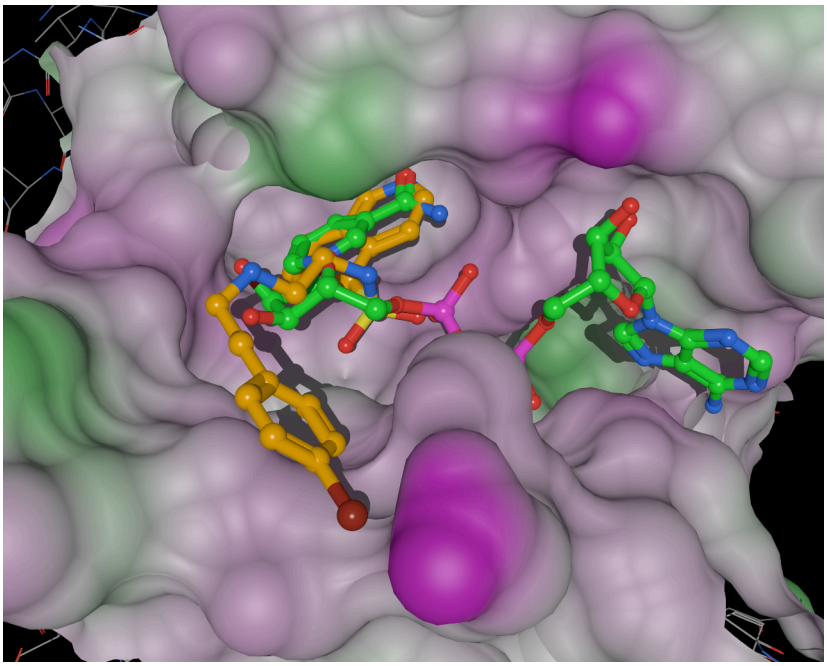


Fig. S5 - Docking solution for H-89 (carbon atoms in orange) at the NAD^+ binding site of C3bot toxin. The cocrystallized NAD^+ molecule is show with green carbon atoms. The molecular surface is colored according the lipophilicity (green = hydrophobic, magenta = polar).

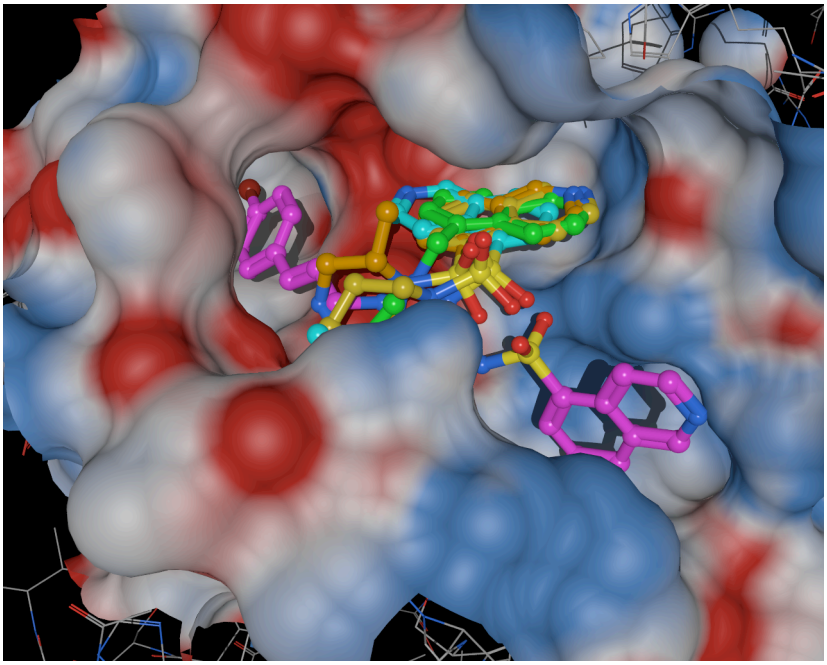


Fig. S6 - Comparison of the docking poses obtained for the active inhibitor H-89 (carbon atoms colored magenta) and four inactive analogs (**4** orange, **5** green, **38** dark yellow, **36** cyan) to MTX. The molecular surface of the protein is shown and is colored according to the electrostatic potential (red – negative, blue – positive).

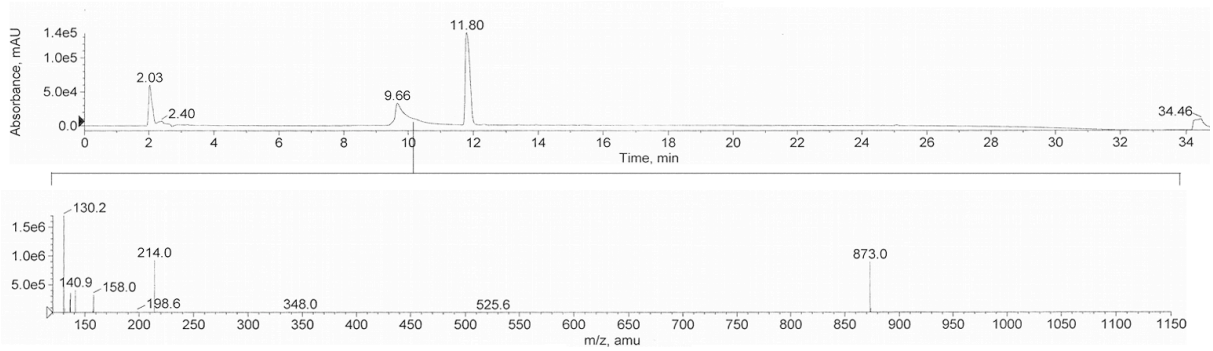


Fig. S7 - ADP-Ribose (10 mM) with Arg-AMC (10 mM) in C3-Gly-Buffer (after 5h) / LC-MS. Bottom row shows m/z peaks at 10.2 min, top row shows the two substrates (ADP-Ribose at 2 min, Arg-AMC at 11.8 min) and the product (9.7 min).

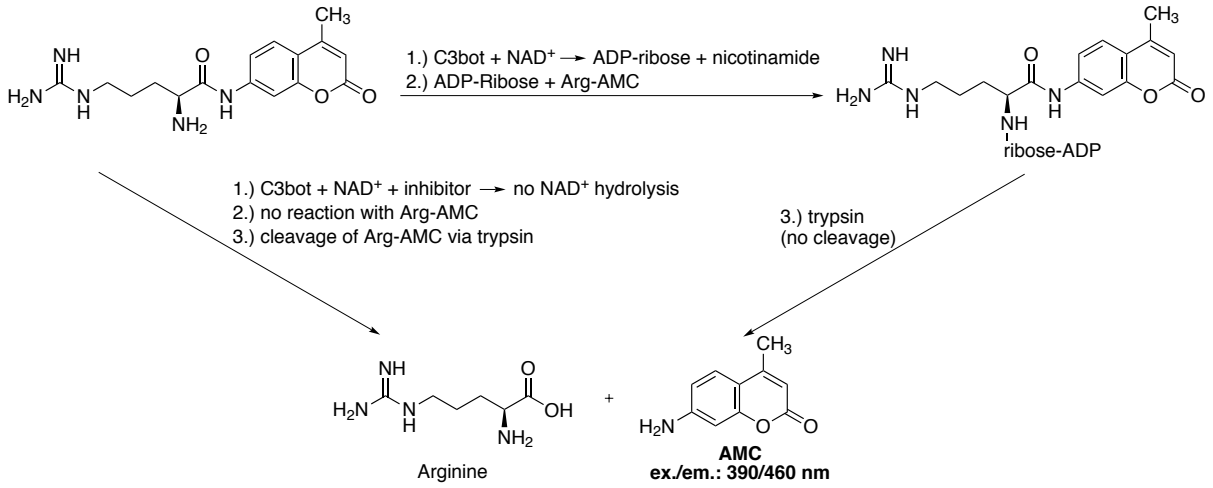


Fig. S8 - Reaction scheme of C3bot-glycohydrolase assay

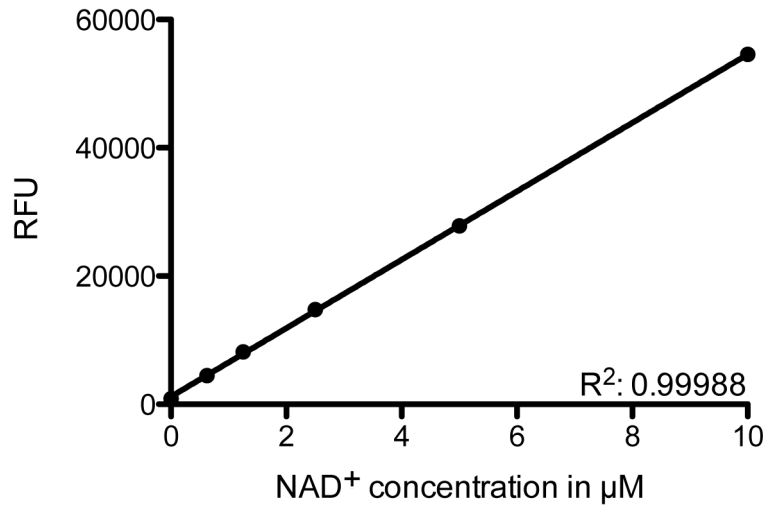


Fig. S9 - NAD^+ calibration curve.

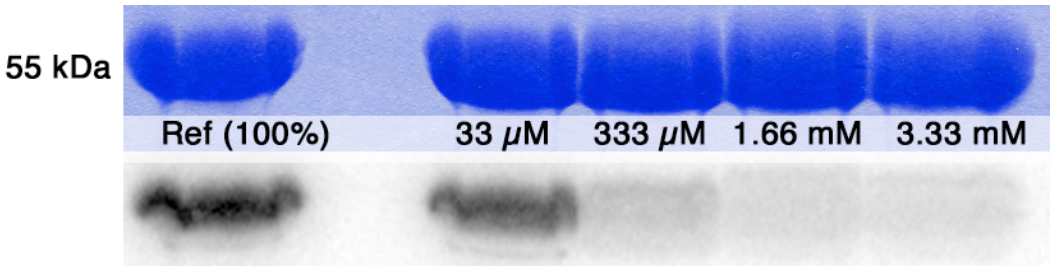


Fig. S10 - Results of the reaction of GST-RhoA, C3bot and ^{32}P-NAD^+ containing diverse concentrations of inhibitor ZM 449829. Top lane shows the coomassie-stained gel as a loading control for GST-RhoA and the bottom lane the radioactive spots of the same area. The concentration of ZM 449829 is given between the lanes (reference contained DMSO).

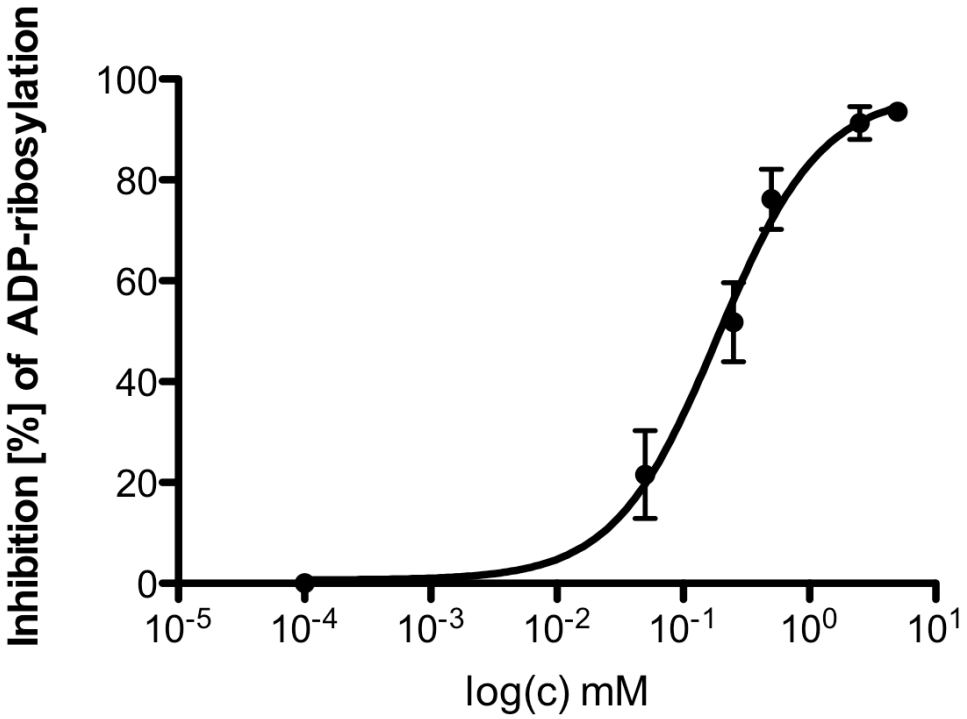
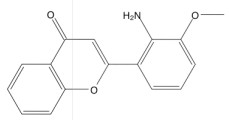
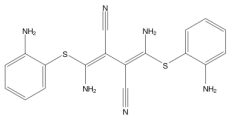
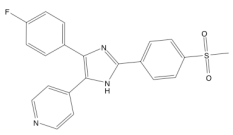
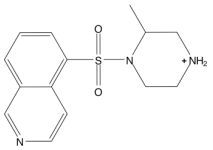
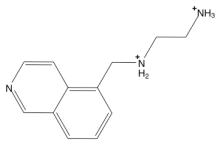
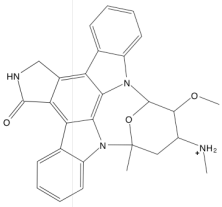
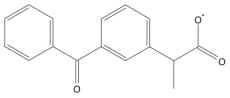
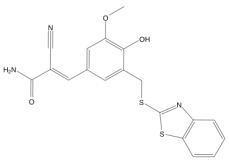
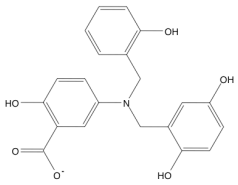
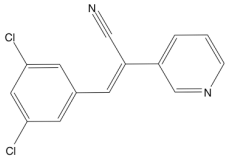
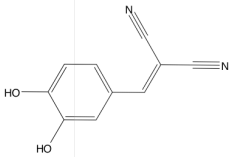
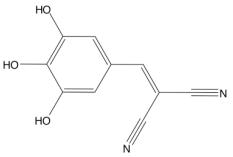
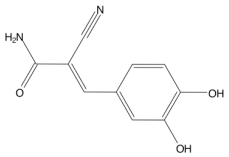
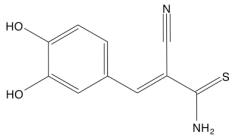
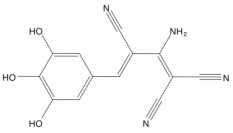
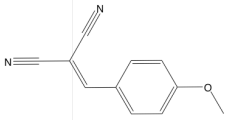
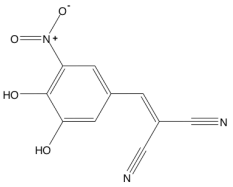
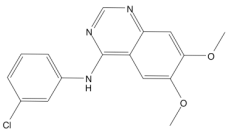
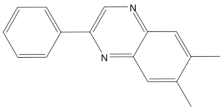
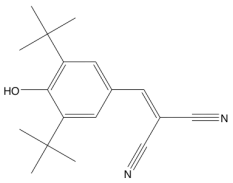
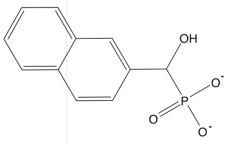
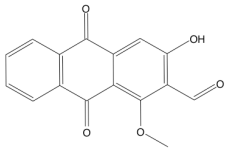
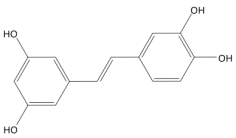
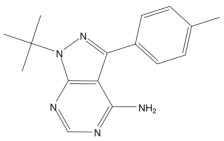
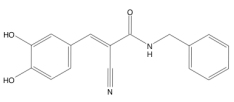
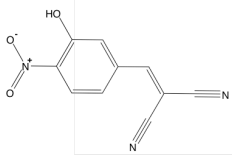
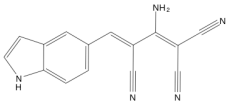
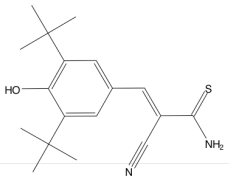
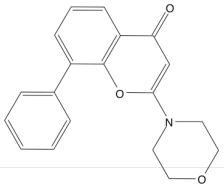
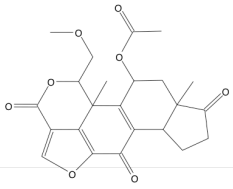
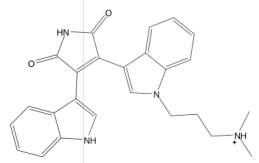
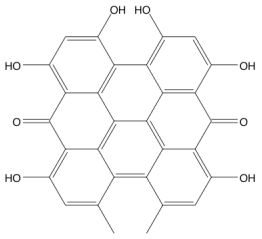
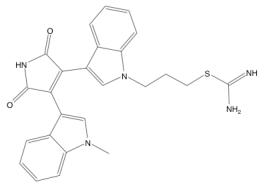
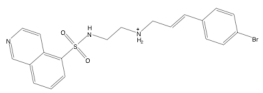
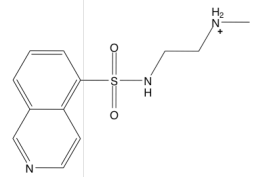
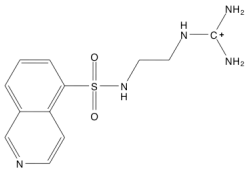
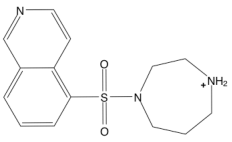
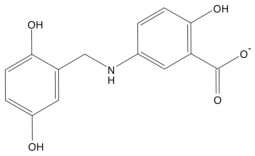
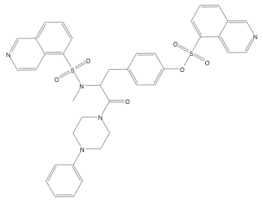
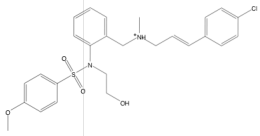
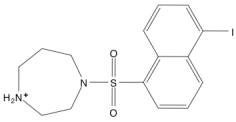
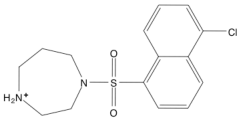
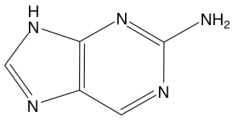
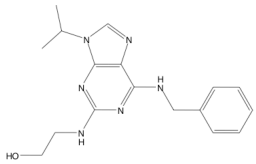
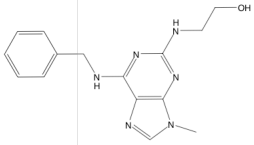
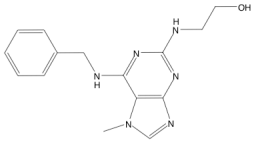
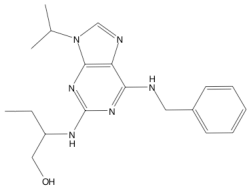
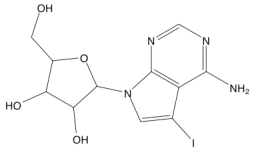
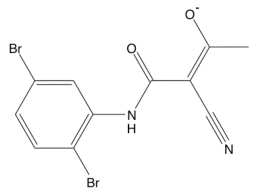
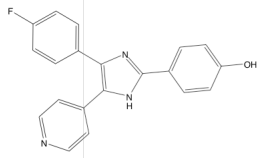
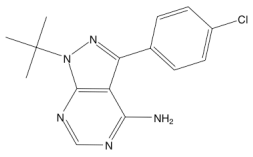
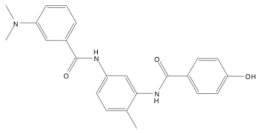
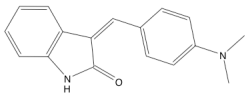
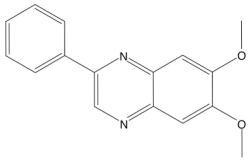
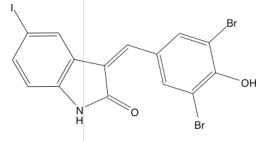
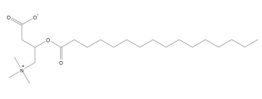
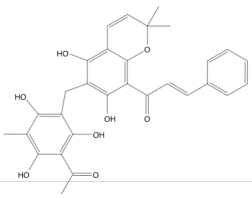
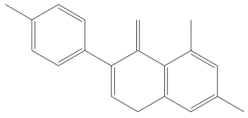
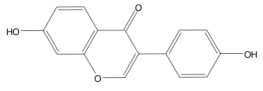


Fig. S11 - Inhibition of CDTa by Tyrphostin 47 in the radioactive assay (inhibitor concentration on x-axis). The IC_{50} is 185 μM. (n = 3)

Table S1 - Compounds from the Biomol library

 **Biomol:** 1	 **Biomol:** 2	 **Biomol:** 3	 **Biomol:** 4	 **Biomol:** 5
 **Biomol:** 6	 **Biomol:** 7	 **Biomol:** 8	 **Biomol:** 9	 **Biomol:** 10
 **Biomol:** 11	 **Biomol:** 12	 **Biomol:** 13	 **Biomol:** 14	 **Biomol:** 15
 **Biomol:** 16	 **Biomol:** 17	 **Biomol:** 18	 **Biomol:** 19	 **Biomol:** 20
 **Biomol:** 21	 **Biomol:** 22	 **Biomol:** 23	 **Biomol:** 24	 **Biomol:** 25
 **Biomol:** 26	 **Biomol:** 27	 **Biomol:** 28	 **Biomol:** 29	 **Biomol:** 30

 **Biomol:** 31	 **Biomol:** 32	 **Biomol:** 33	 **Biomol:** 34	 **Biomol:** 35
 **Biomol:** 36	 **Biomol:** 37	 **Biomol:** 38	 **Biomol:** 39	 **Biomol:** 40
 **Biomol:** 41	 **Biomol:** 42	 **Biomol:** 43	 **Biomol:** 44	 **Biomol:** 45
 **Biomol:** 46	 **Biomol:** 47	 **Biomol:** 48	 **Biomol:** 49	 **Biomol:** 50
 **Biomol:** 51	 **Biomol:** 52	 **Biomol:** 53	 **Biomol:** 54	 **Biomol:** 55
 **Biomol:** 56	 **Biomol:** 57	 **Biomol:** 58	 **Biomol:** 59	 **Biomol:** 60

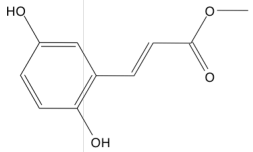
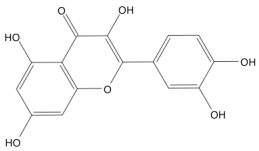
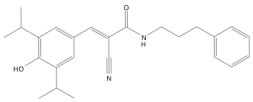
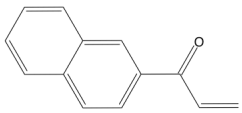
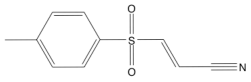
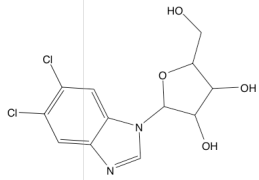
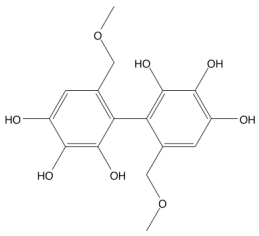
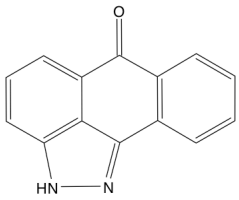
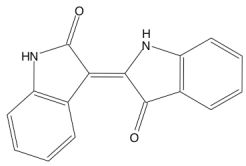
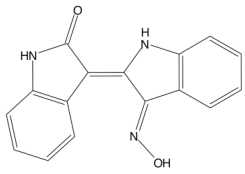
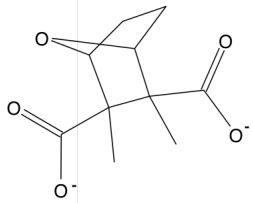
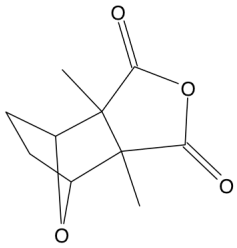
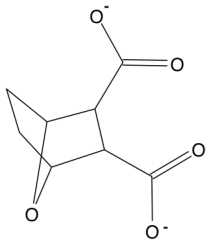
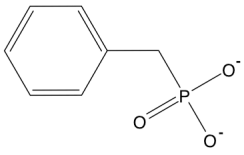
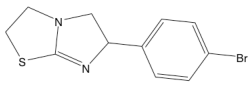
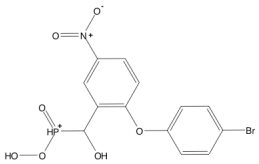
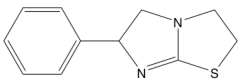
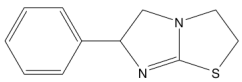
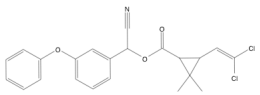
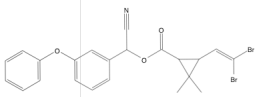
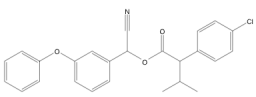
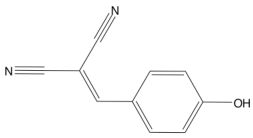
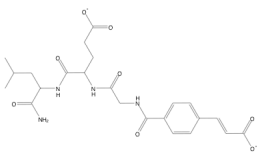
 **Biomol:** 61	 **Biomol:** 62	 **Biomol:** 63	 **Biomol:** 64	 **Biomol:** 65
 **Biomol:** 66	 **Biomol:** 67	 **Biomol:** 68	 **Biomol:** 69	 **Biomol:** 70
 **Biomol:** 71	 **Biomol:** 72	 **Biomol:** 73	 **Biomol:** 74	 **Biomol:** 75
 **Biomol:** 76	 **Biomol:** 77	 **Biomol:** 78	 **Biomol:** 79	 **Biomol:** 80
 **Biomol:** 81	 **Biomol:** 82	 **Biomol:** 83	 **Biomol:** 84	

Table S2 - HPLC conditions for the separation shown in Fig. S7.

time [min]	gradient	Solvent A	Solvent B
0 - 4	no	10%	90%
4 - 25	yes	100%	0%
25 - 27	no	100%	0%
27 - 27.5	yes	10%	90%
27.5 - 36	no	10%	90%

Table S3 - Calculated docking scores (Chemscore, Goldscore) and GBSA binding free energies for the 84 tested Biomol inhibitors (compound numbering chosen by us, see supplementary Table S1 for reference).

Cpd.	GBSA score	Chemscore	Goldscore
35	-69.71	32.05	62.52
2	-54.10	32.02	62.55
5	-53.97	33.40	53.68
40	-51.04	27.67	64.11
76	-50.34	29.10	61.44
41	-47.89	29.72	8.57
6	-47.16	26.54	52.17
38	-44.46	32.73	62.86
57	-44.43	28.53	53.93
31	-43.25	37.67	62.66
45	-42.64	23.69	60.29
18	-42.07	26.56	59.80
56	-41.37	26.79	9.26
3	-41.21	32.62	57.08
42	-40.19	29.70	55.48
51	-39.43	31.86	61.58
84	-39.29	19.91	69.64
53	-38.99	28.65	50.41
36	-38.79	32.74	58.38
63	-38.43	35.48	56.35
46	-38.24	23.12	61.81
25	-37.53	30.23	54.50
67	-36.98	20.07	44.85
9	-36.92	27.41	59.92
33	-36.38	34.04	65.24
58	-35.30	26.56	56.39
4	-35.16	31.77	55.65
47	-34.54	22.34	64.27
82	-33.95	28.97	63.72
52	-33.83	21.38	47.34

8	-33.66	33.32	66.23
34	-32.89	31.22	60.22
22	-32.84	24.63	48.52
71	-31.89	17.74	29.92
54	-31.74	28.08	50.13
43	-31.72	30.11	54.60
77	-31.69	22.20	65.47
30	-30.88	23.63	52.06
60	-30.70	24.69	48.77
66	-30.16	23.36	53.10
23	-29.50	25.79	47.49
80	-29.38	32.26	63.48
48	-29.35	23.84	60.38
49	-29.23	18.45	56.25
55	-29.20	22.95	54.26
1	-28.86	24.66	47.57
81	-28.78	26.94	67.13
26	-28.61	21.23	36.11
29	-28.22	28.56	52.80
27	-28.13	28.55	51.15
44	-28.02	17.14	38.25
19	-27.74	25.31	54.09
70	-27.74	28.18	57.76
20	-27.66	26.29	36.18
75	-27.47	22.57	51.06
28	-27.40	27.68	36.08
14	-27.10	26.99	40.37
59	-27.00	30.44	43.43
68	-26.98	28.75	47.96
10	-26.41	29.57	51.37
65	-26.14	25.54	43.14
78	-25.81	21.91	52.25
69	-25.81	27.70	53.64
17	-25.61	21.80	38.01
39	-25.50	29.98	52.38
50	-23.06	27.04	50.03
61	-22.79	22.17	41.29
64	-22.77	27.74	44.23
15	-22.74	23.84	41.13
21	-22.64	29.21	60.19
73	-21.66	18.88	41.37
24	-21.16	21.48	48.74
16	-21.14	24.73	38.51
79	-20.97	21.81	47.76
62	-19.72	21.71	47.29
83	-18.80	23.17	38.07
74	-18.46	23.26	51.64
11	-17.45	25.10	38.79
72	-17.39	20.48	28.83
13	-17.33	26.19	39.34
12	-16.52	23.51	38.04
32	-12.09	29.46	48.70
37	-11.10	28.18	66.44
7	-1.11	30.80	52.82