A continuous activity assay for HDAC11 enabling re-evaluation of HDAC inhibitors

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Table S1. The activity of selected inhibitors against HDAC6, 8 and 1 reported in the literature

Compound	**Inhibitor class**	**IC_{50} HDAC6**	**IC_{50} HDAC8**	**IC_{50} HDAC1**
Dacinostat (NVP-LAQ824)	hydroxamic acids	5,93[1]	3,84[1]	3,23[1]
Elevenostat (JB3-22)	hydroxamic acids			
Fimepinostat (CUDC-907)	hydroxamic acids	27[2]	191[2]	1,7[2]
FT895		>10000[3]	5600[3]	>10000[3]
Mocetinostat (MGCD0103)	Benzamides	inactive[4]*	inactive[4]	150[5]; 9,26[4]
Pracinostat (SB939)	Hydroxamic acids	1008[6]	140[6]	49[6]
Quisinostat (JNJ-26481585)	Hydroxamic acids	76,8[4]	4,26[4]	0,11[4]
Ricolinostat (ACY1215)	Hydroxamic acids	9[7];4,7[8]	254[7];100[8]	38[7]; 58[8]
Romidepsin (FK228)	Cyclic peptides	>1000[9]; 1220[10]	>1000[9]; >10000[10]	0,8[9]; 1050[10]
SAHA	Hydroxamic acids	165[6]; 5500[11]; 15[7]; 28,6[1]	326[6]; 6,8[11]; 172[7]; 243[1]	110[6]; 13,7[11]; 11[7]; 258[1]
Trapoxin A	Cyclic peptides	524[12]		0,82[12]
Trichostatin A	Hydroxamic acids	0,9[13]; 1,1[14]; 1,5[15]; 0,42[16]	129[13]; 170[14]; 196[15]; 89,5[16]	8,22[17]; 5[13]; 9,2[14]; 10,9[15]; 7,12[16]
Valproate	Aliphatic fatty acids	>2000000[18]; ND[11]	103000[18]; 756000[11]	39000[18];171000[11]

* inactive - not defined

Table S2. Calculated Z' factors and signal to noise ratio of HDAC11 and compound **1**.

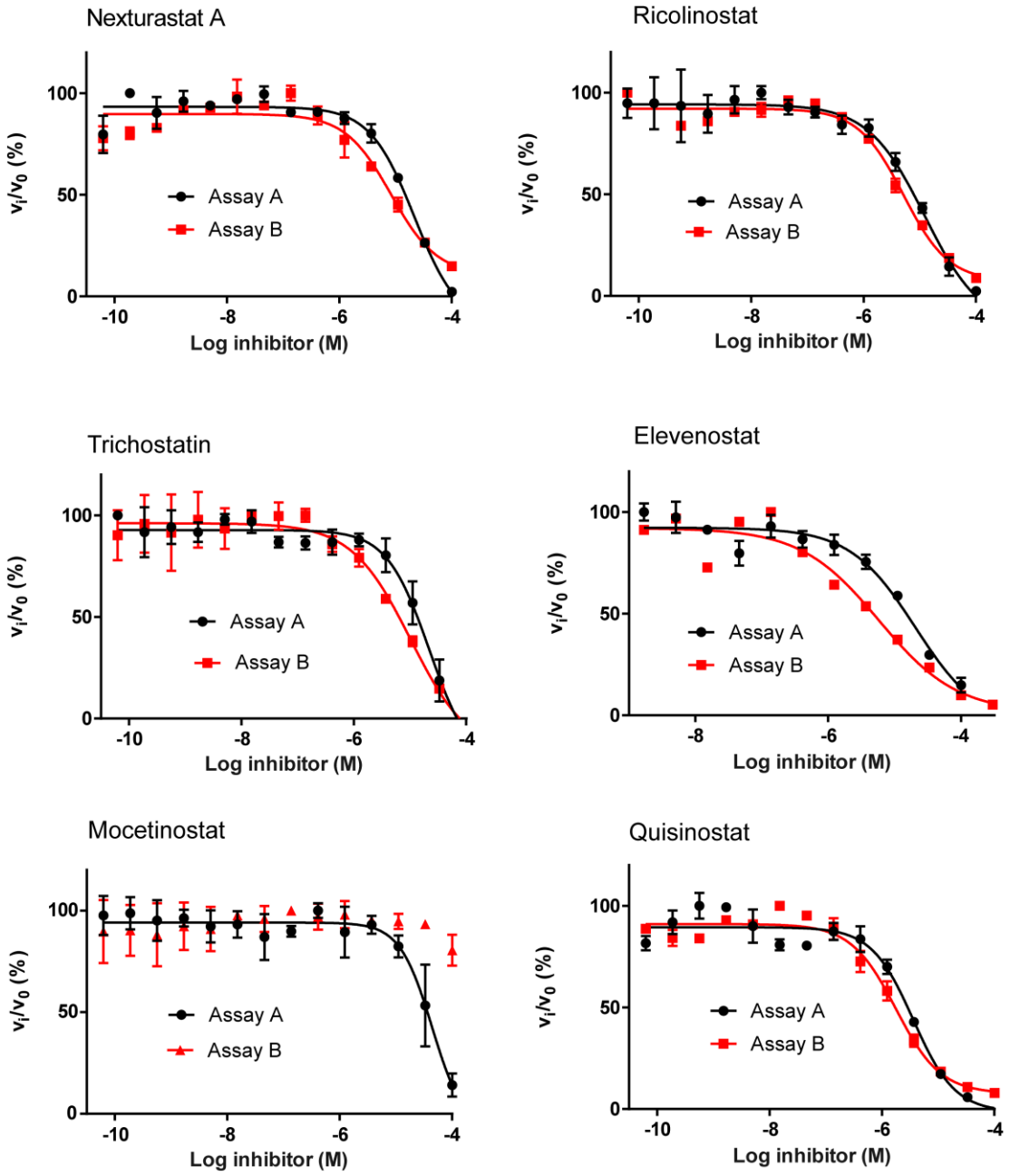
	96 well plate	**384 well plate**	**1536 well plate**
Z'factor	0,95	0,88	0,77
signal/noise ratio	425	258	94

Table S3. K_i values for listed inhibitors. K_i-values were calculated with the Cheng-Prusoff-relationship[19]. The K_M-value used for the calculation for compound **1** was 12 µM and for compound **3** 200 µM.

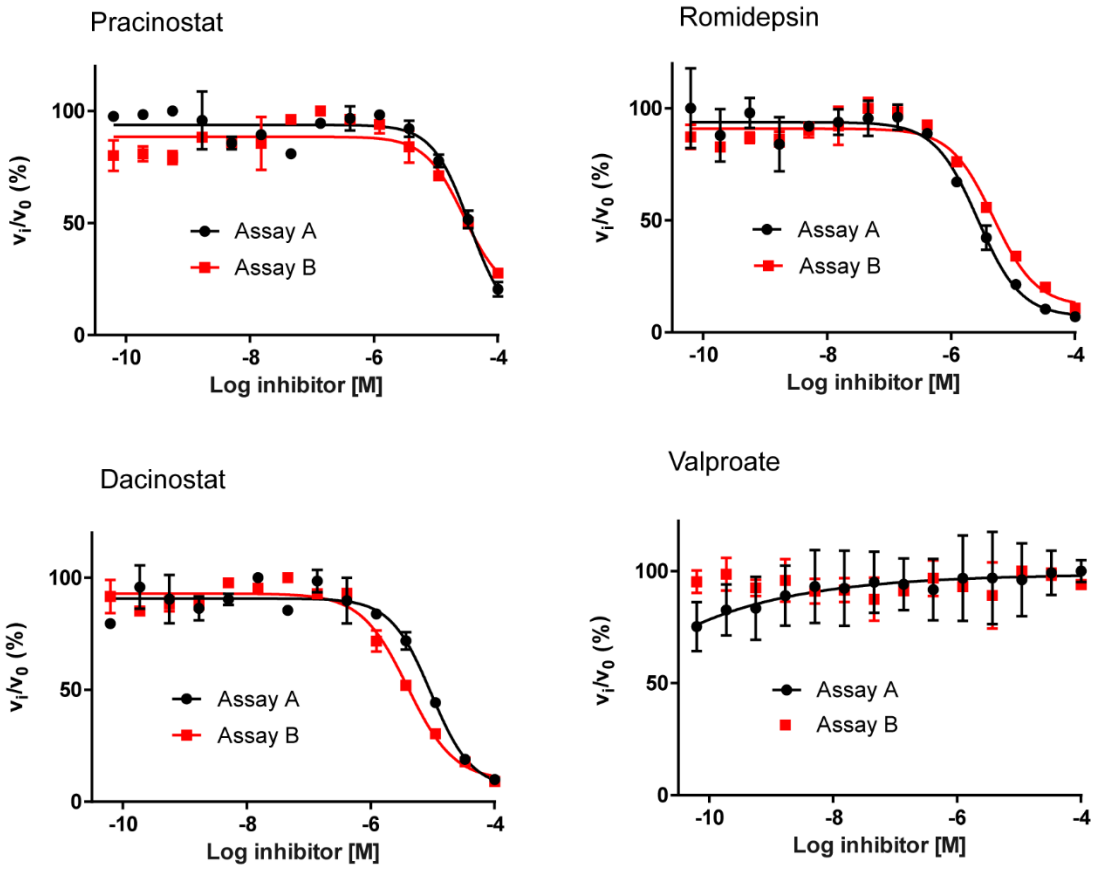
Compound	K_i (nM) lysine derivative 3	K_i (nM) Peptide 1
Dacinostat (NVP-LAQ824)	3750	4188
Elevenostat (JB3-22)	6062	7871
Fimepinostat (CUDC-907)	15	10
Mocetinostat (MGCD0103)	ND	ND
Nexturastat A	7931	ND
Pracinostat (SB939)	26705	15476
Quisinostat (JNJ-26481585)	1681	1454
Ricolinostat (ACY1215)	5125	5444
Romidepsin (FK228)	4577	1198
Trapoxin A	74	4
Trichostatin	9762	9769
Valproate	ND	ND

* ND means: Not determined due to the low activity (IC_{50}>40000)

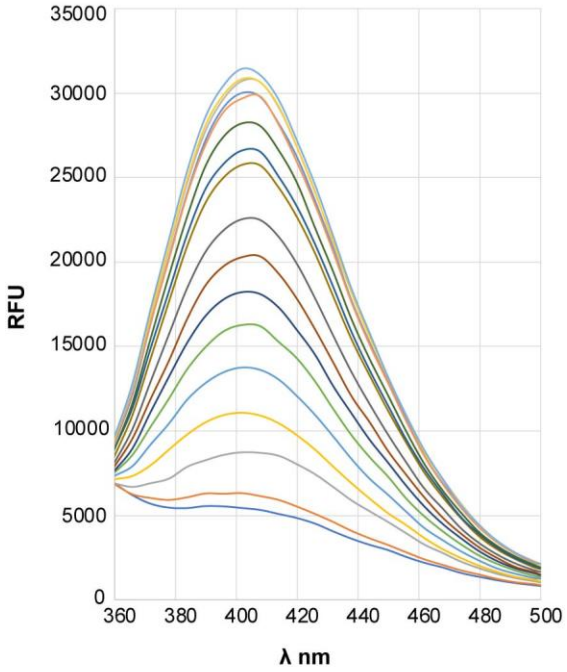
Supplementary figures



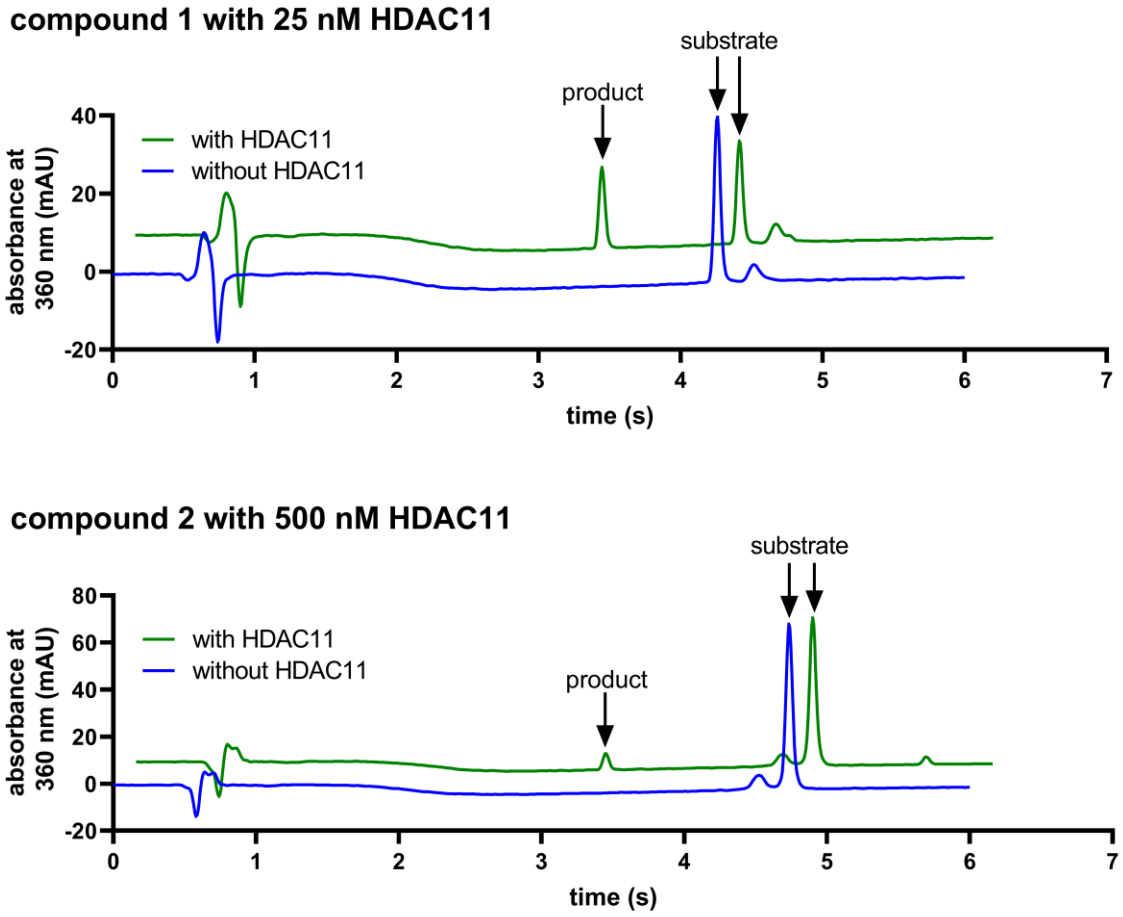
Supplementary figure S1: Comparison of dose response curves of selected inhibitors (Nexturastat A, Ricolinostat, Trichostatin, Elevenostat, Mocetinostat and Quosinostat) measured by Assay A (15 µM substrate **1**, 20 nM HDAC11) and B (10 µM substrate **3**, 60 nM HD11).



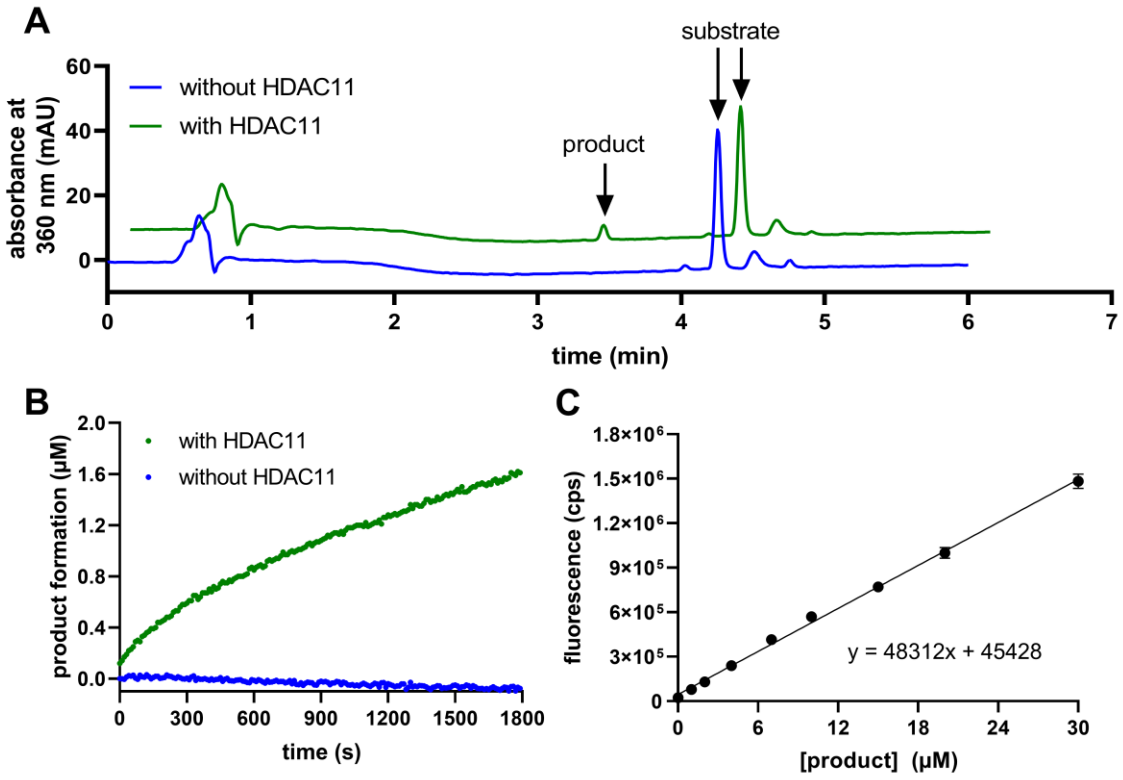
Supplementary figure S2: Comparison of dose response curves of selected inhibitors (Pracinostat, Romidepsin, Dacinostat, Valproate) measured by Assay A (15 µM substrate **1**, 20 nM HDAC11) and B (10 µM substrate **3**, 60 nM HD11).



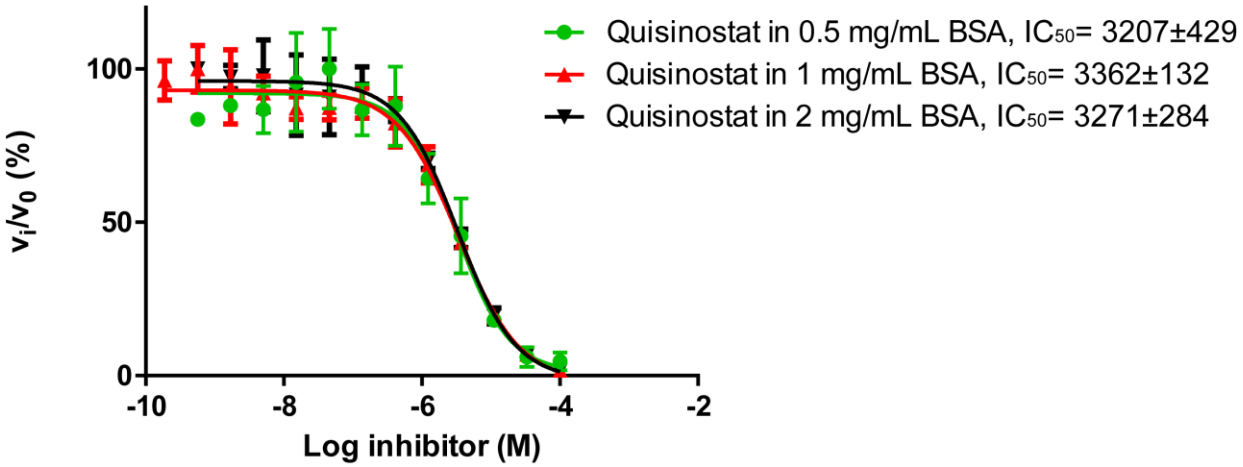
Supplementary figure S3. Fluorescence measurements using peptide 1. Emission fluorescence spectra (λ 360-500; band width 30) of 15 µM substrate **1** excited at 320 nm (band width 20 nm) recorded every 2 minutes after the HDAC11 treatment (enzyme concentration 20 nM).



Supplementary figure S4. HPLC runs of compound 1 and 2 without HDAC11 (blue) and with HDAC11 (green). The reaction mixture (20 µM peptide dissolved in the assay buffer) was incubated at 37 °C for 1h with 25 nM HDAC11 (compound 1) and with 500 nM HDAC11 (compound 2). The product formation was analyzed by HPLC at an absorbance of 360 nm. The product formation was calculated from the product peak area divided by the total peak area. The negative control was treated like the sample but there was buffer used to start the reaction. The product formation of compound 1 was 8.2 µM (41 %) and 1.3 µM (6 %) of compound 2.



Supplementary figure S5. Comparison of product formation of compound 1 between Multilabel plate reader and HPLC. The reaction mixture (peptide 1 dissolved in buffer at 20 µM) was incubated in 96 well plate for 5 min at 25 °C. HDAC11 was added to the mixture (final concentration 30 nM) and the fluorescence increase was monitored with the plate reader (λ_{ex} = 330 ± 75 nm and λ_{em} = 430 ± 8 nm) for 30 min. After that, the reaction was quenched by adding diluted TFA (final concentration 0.5 %) and the mixture was analyzed with HPLC (**A**). The product formation was calculated from the product peak area divided by the total peak area. A calibration line (**C**) was used to calculate the product formation from the change of the fluorescence intensity in µM (**B**). The product formation calculated from HPLC runs was 1.66 µM (8.3 %) and from fluorescence measurements 1.68 µM (8.4 %).



Supplementary figure S6. The influence of BSA concentration in the assay buffer on inhibitor potency. The Quisinostat was selected as a representative of moderately active inhibitors and tested in the assay buffer supplemented with various BSA concentrations (0.5, 1 and 2 mg/mL). No effect of BSA on the IC50 values was observed at BSA concentrations tested.

Supplementary references

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