Electronic Supplementary Information

An azumamide C analogue without the zinc-binding functionality

Jesper S. Villadsen,[a] Betül Kitir,[ab] Kathrine Wich,[b] Tina Friis,[c] Andreas S. Madsen,[ab] and Christian A. Olsen*[ab]

General Experimental

Materials and methods. All chemicals and solvents were analytical grade and used without further purification. Vacuum liquid chromatography (VLC) was performed on silica gel 60 (particle size 0.015−0.040μm). UPLC−MS analyses were performed on a Waters Acquity ultra high-performance liquid chromatography system. A gradient with eluent I (0.1% HCOOH in water) and eluent II (0.1% HCOOH in acetonitrile) rising linearly from 0% to 95% of II during t = 0.00–2.50 min was applied at a flow rate of 1 mL/min. Analytical HPLC was performed on a [150 mm × 4.6 mm, C_{18} Phenomenex Luna column (3 μm)] using an Agilent 1100 LC system equipped with a UV detector. A gradient, B, with eluent III (95:5:0.1, water−MeCN−TFA) and eluent IV (0.1% TFA in acetonitrile) rising linearly from 0% to 95% of IV during t = 2−20 min was applied at a flow rate of 1 mL/min. Preparative reversed-phase HPLC was performed on a [250 mm × 20 mm, C_{18} Phenomenex Luna column (5 μm, 100 Å)] using an Agilent 1260 LC system equipped with a diode array UV detector and an evaporative light scattering detector (ELSD). A gradient C with eluent III (95:5:0.1, water−MeCN−TFA) and eluent IV (0.1% TFA in acetonitrile) rising linearly from 0% to 95% of IV during t = 5−45 min was applied at a flow rate of 20 mL/min. 1D and 2D NMR spectra were recorded on a Varian INOVA 500 MHz instrument, a Bruker Ascend 400 MHz or a Varian Mercury 300 instrument. All spectra were recorded at 298 K. For the Varian INOVA 500 MHz instrument and the Varian Mercury 300 instrument 1D NMR spectra were recorded at 499.9 MHz and 300 MHz for 1H and 100 MHz and 75 MHz for 13C, respectively. The correlation spectroscopy (COSY) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 6k × 6k, collecting 8 FIDs and 1k × 512 data points. Heteronuclear single quantum coherence (HSQC) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 6k × 25k, collecting 16 FIDs and 1k × 128 data points. Heteronuclear 2-bond correlation (H2BC) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 4k × 35k, collecting 16 FIDs at 295 K and 1k × 256 datapoints. Heteronuclear multiple-bond correlation (HMBC) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 6k × 35 k, collecting 32 FIDs and 1k × 256 datapoints. Finally, on the Bruker Ascend 400 MHz the 1D NMR spectra were recorded at 400 MHz for 1H and 100 MHz for 13C. The correlation spectroscopy (COSY) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 3k × 3k, collecting 4 FIDs and 1k × 128 data points. The heteronuclear single quantum coherence (HSQC) spectra were recorded with a relaxation delay of 1.5 sec before each scan, a spectral width of 4800 × 16600, collecting 4 FIDs and 1k × 256 datapoints. Chemical shifts are reported in ppm relative to deuterated solvent peaks as internal standards (δH, DMSO-d_6 2.50 ppm; δC, DMSO-d_6 39.52 ppm, δH, $CDCl_3$ 7.26 ppm; δC, $CDCl_3$ 77.16 ppm). Coupling constants (J) are given in hertz (Hz). Multiplicities of ^{1}H NMR signals are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

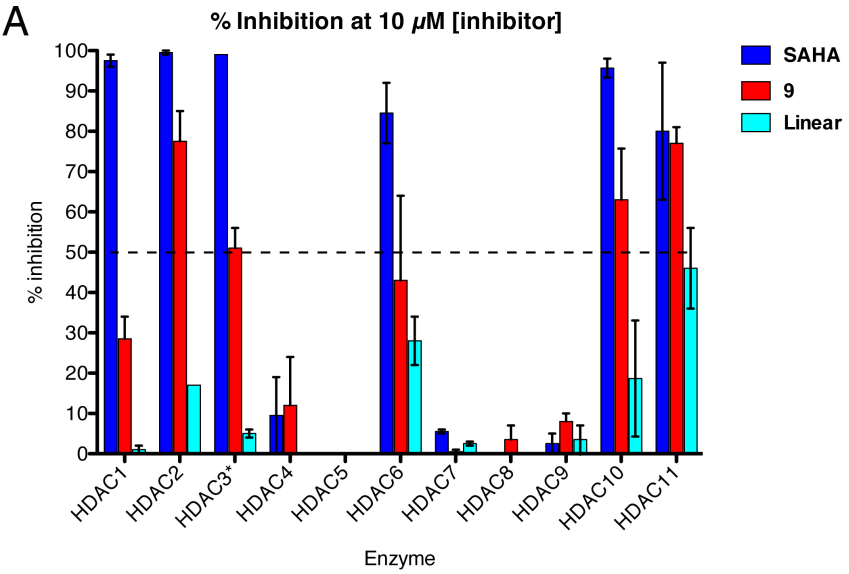
Assay materials. HDAC1 (Purity ≥62% by SDS-PAGE according to the supplier), HDAC2 (Full length, purity ≥94% by SDS-PAGE according to the supplier), HDAC3-NCoR2 complex (Purity ≥80% by SDS-PAGE according to supplier, HDAC4 (Purity ≥60% by SDS-PAGE according to the supplier) and HDAC 5 (Full length, purity ≥90% by SDS-PAGE according to the supplier), HDAC8 (Purity ≥90% by SDS-PAGE according to the supplier), HDAC9 (Full length, purity ≥76% by SDS-PAGE according to the supplier) and HDAC10 (Purity ≥21% by SDS-PAGE according to the supplier) were purchased from BPS Bioscience (San Diego, CA 92121). HDAC 7 (Purity ≥90% by SDS-PAGE according to the supplier) was purchased from Millipore (Temecula, CA 92590). HDAC6 (Purity ≥85% by SDS-PAGE according to the supplier) and HDAC11 (Purity ≥50% by SDS-PAGE according to the supplier) were purchased from Enzo Life Sciences (Postfach, Switzerland). The HDAC assay buffer (50 mM tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$) was added bovine serum albumin (0.5 mg/mL). Trypsin (10,000 units/mg, TPCK treated from bovine pancreas) was from Sigma Aldrich (Steinheim, Germany). All peptides were purified to homogeneity (>95% purity by $HPLC_{230nm}$ using reversed-phase preparative HPLC), and the white fluffy materials obtained by lyophilization were kept at –20 °C.

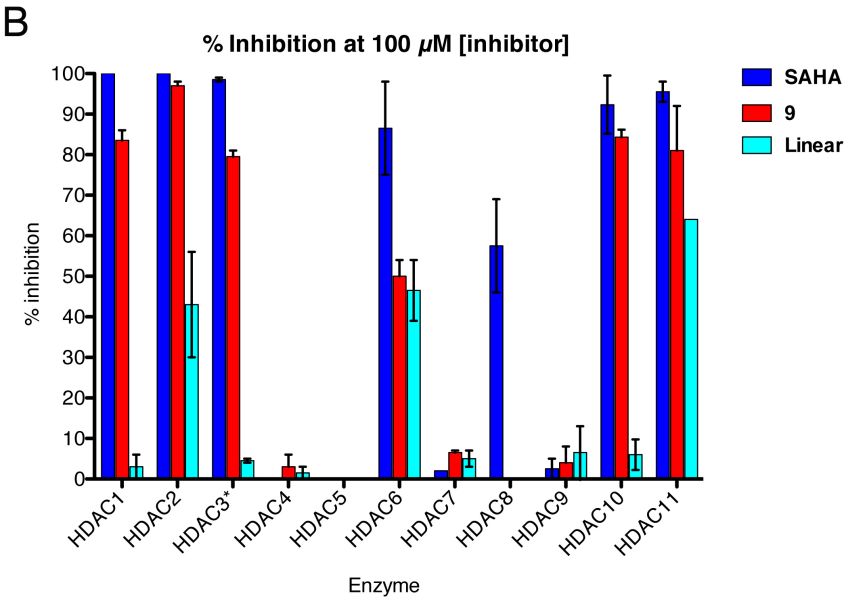
Table S1. Comparison of ^{1}H and ^{13}C NMR data for reference and compound 15

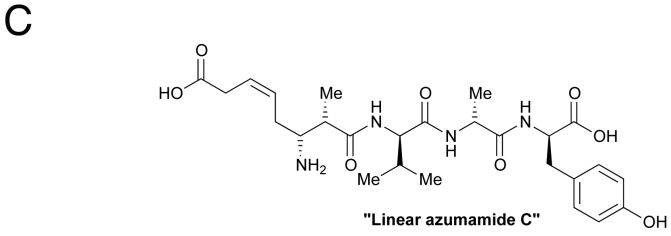
Position	^{1}H MacMillan[a]	^{13}C MacMillan	^{1}H Compound **15**	^{13}C Compd. **15**
1		174.9		175.0
2	2.71–2.62 (m, 1H)	43.9	2.65 (m, 1H)	44.1
3	3.94–3.84 (m, 1H)	53.0	3.93–3.82 (m, 1H)	53.2
4	1.50–1.38 (m, 2H)	34.0	1.43 (m, 2H)	34.2
5	1.38–1.25 (m, 2H)	19.4	1.31 (m, 2H)	19.6
6	0.92 (bt, *J* = 6.6 Hz, 3H)	13.8	1.15 (d, *J* = 7.2 Hz, 3H)	13.9
OMe	3.58 (s, 3H)	51.8	3.67 (s, 3H)	51.9
α-Me	1.17 (d, *J* = 7.2 Hz, 3H)	13.1	1.15 (d, *J* = 7.2 Hz, 3H)	13.2
Ar	7.41–7.30 (m, 5H)	136.5; 128.5; 128.1×2;	7.38 –7.28 (m, 5H)	136.7; 128.6; 128.2×2;
$PhCH_2$	5.10 (s, 2H)	66.7	5.09 (s, 2H)	66.8
NH	4.93 (d, *J* = 9.5 Hz, 1H)		4.95 (d, *J* = 9.7 Hz, 1H)	
Carbamate		156.0		156.2

[a] Ref 32, J. E. Wilson, A. D. Casarez and D. W. MacMillan, *J. Am. Chem. Soc*., 2009, **131**, 11332-11334.

Figure S1. Single-dose HDAC inhibition experiments.[a]

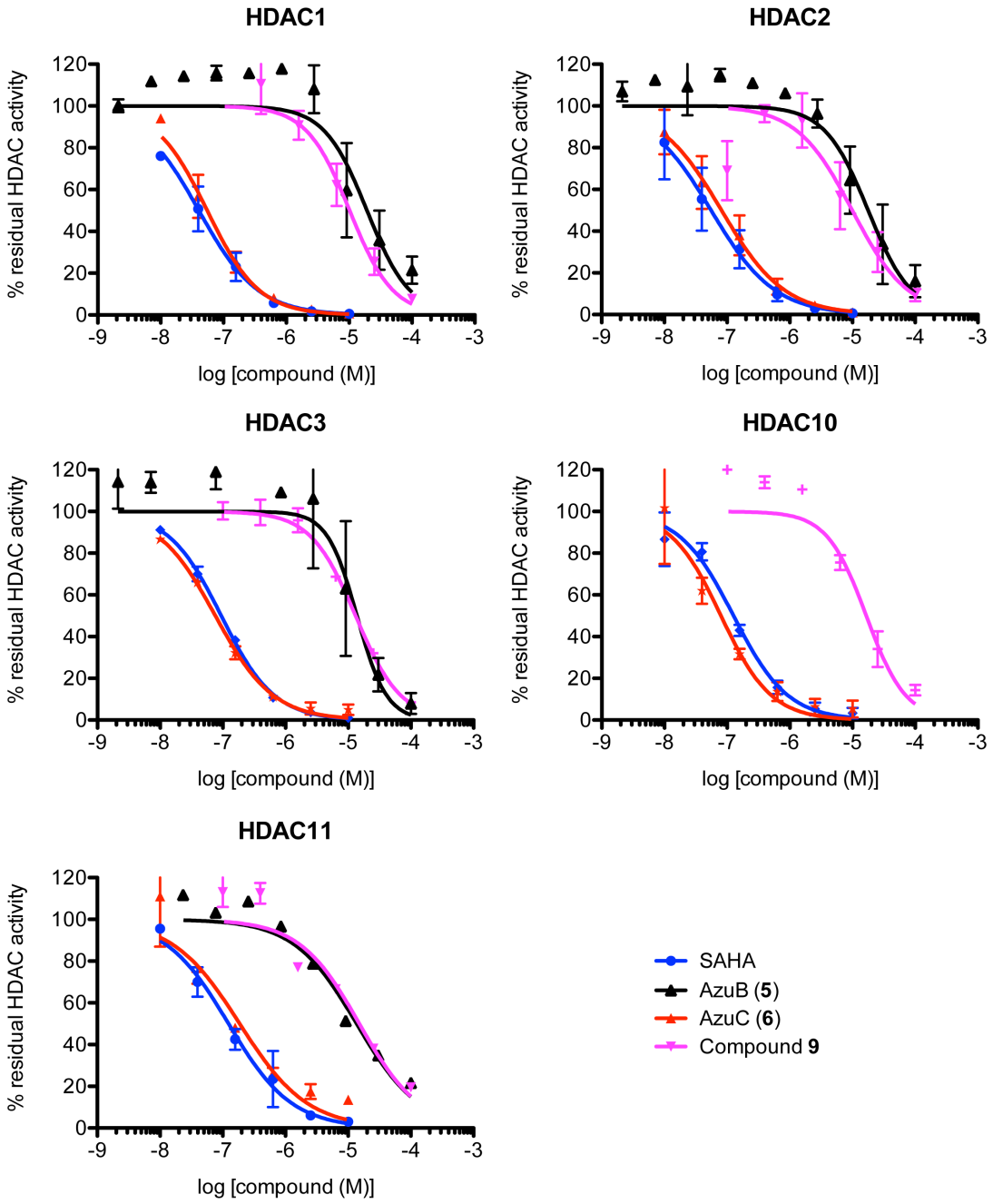




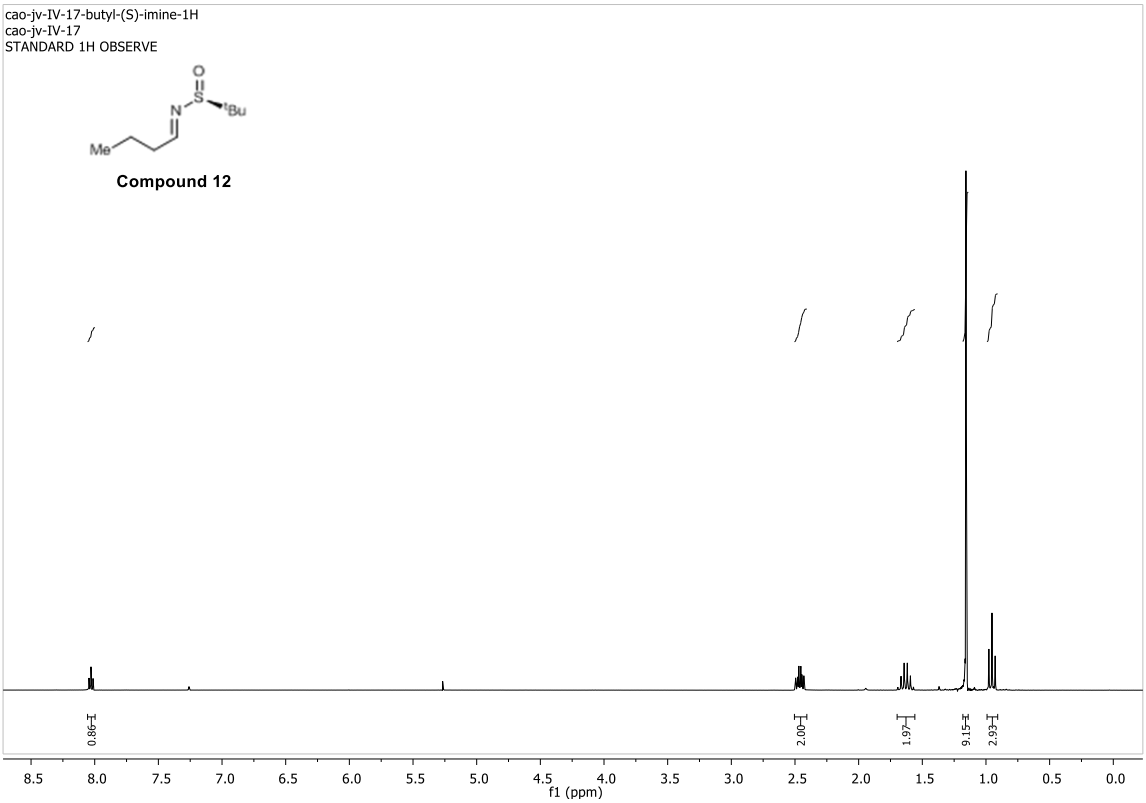


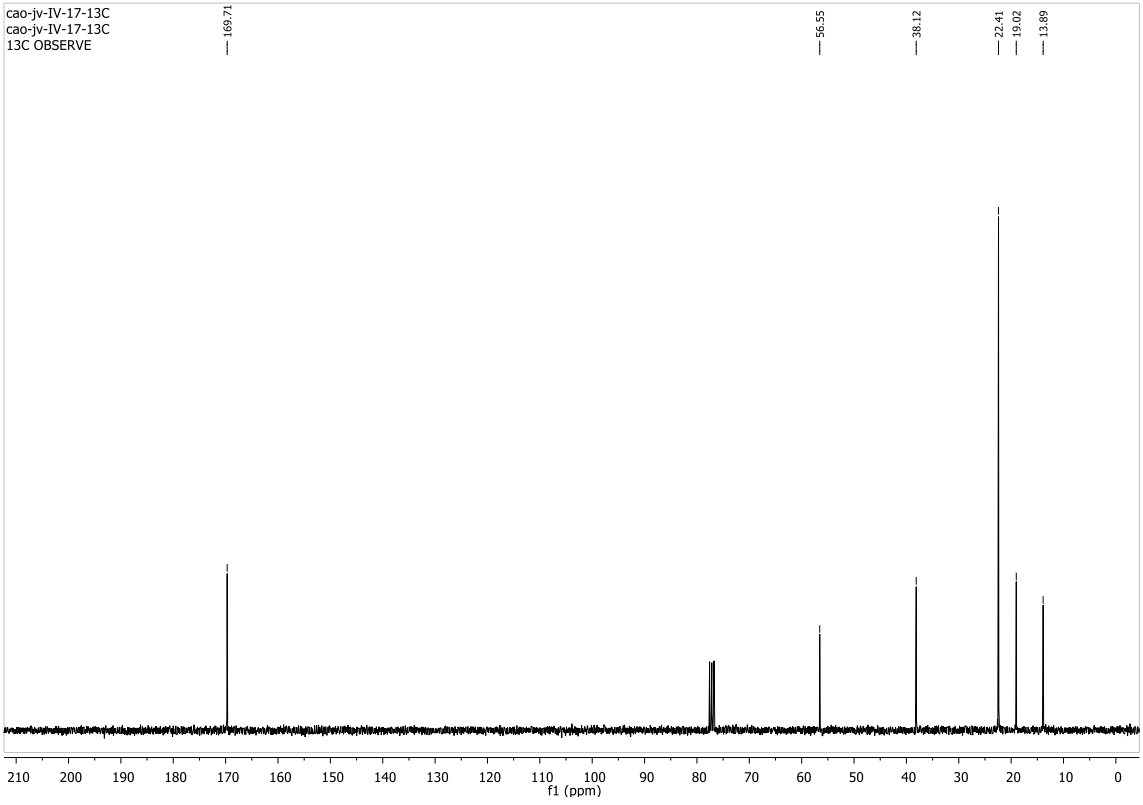
[a]With SAHA (vorinostat) as control compound, we tested the ability of compound **9** as well as the shown linear azumamide C analogue (**C**) to inhibit all the zinc-dependent recombinant human HDAC enzymes at 10 μM (**A**) and 100 μM concentrations (**B**), respectively. *HDAC3 was applied as a complex with NCoR2.

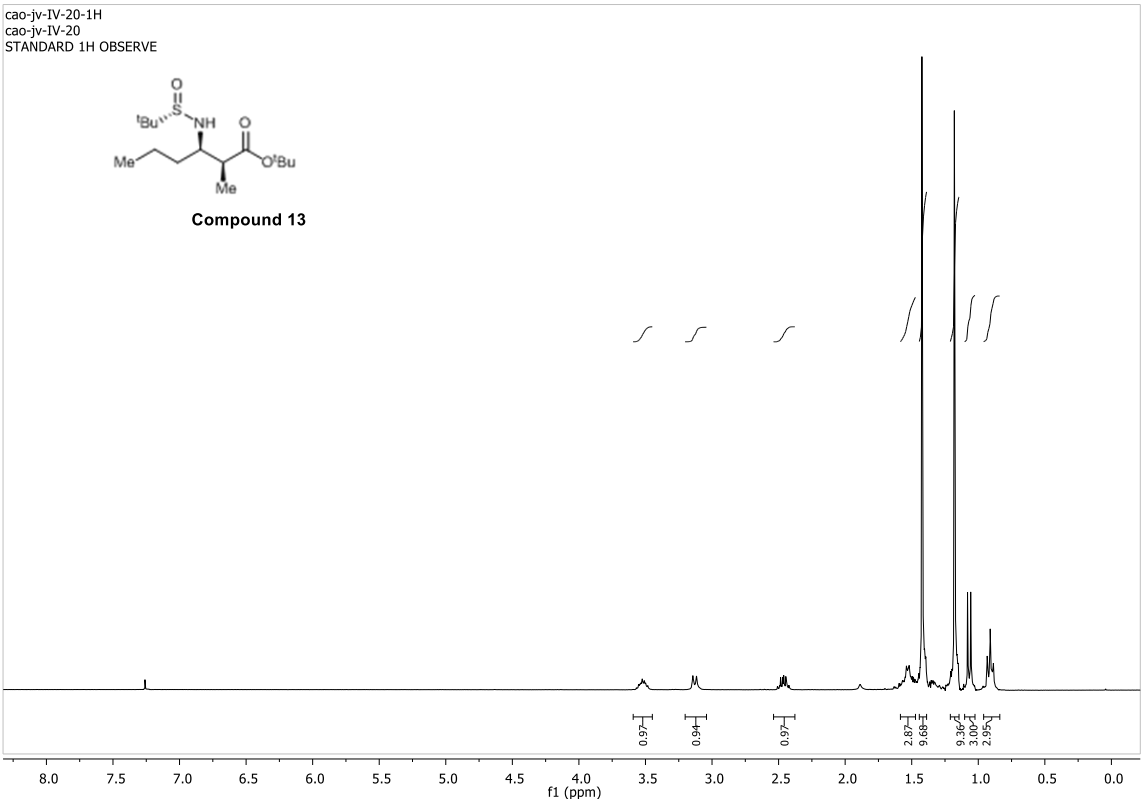
Figure S2. Dose-response HDAC inhibition experiments.[a]

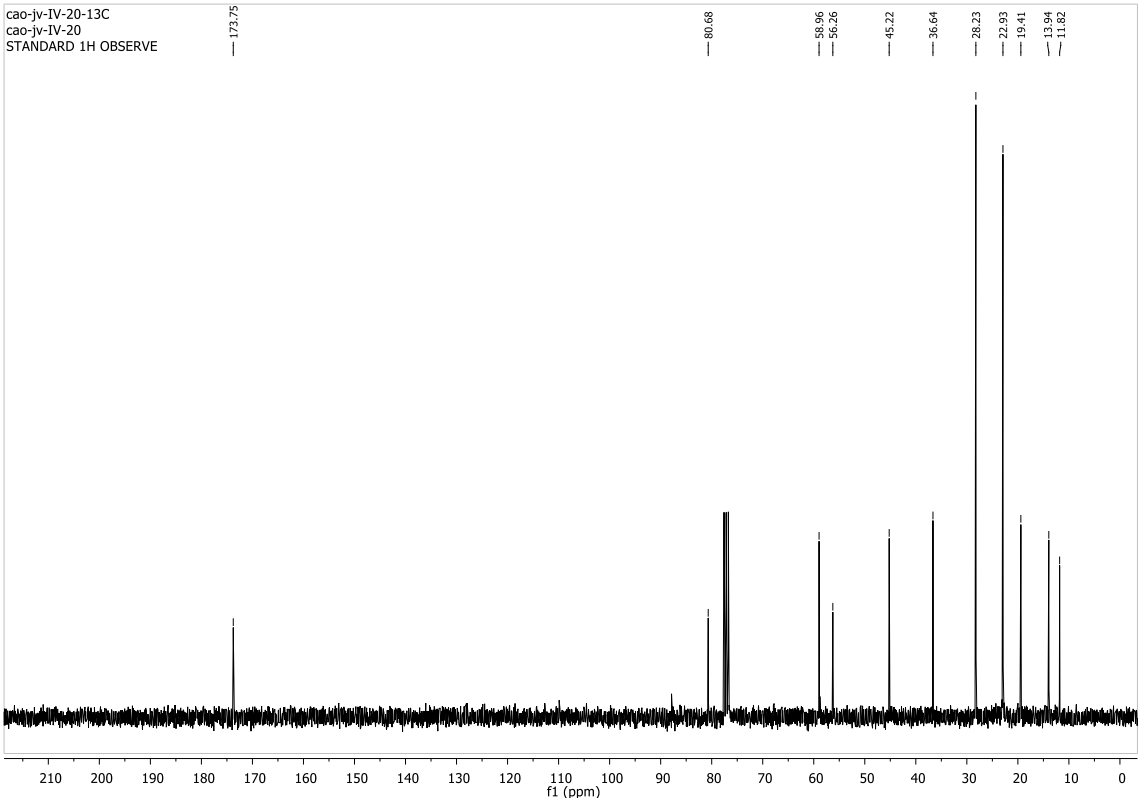


[a]With SAHA (vorinostat) as internal control compound in all assay plates, we performed dose-response experiments in at least two individual assays performed in duplicate. Data for Azumamide B are taken from a previous publication (ref 29, J. S. Villadsen, H. M. Stephansen, A. R. Maolanon, P. Harris, and C. A. Olsen, *J. Med. Chem.*, 2013, **56**, 6512-6520) Data were analyzed and plotted using the GraphPad Prism software. *HDAC3 was applied as a complex with NCoR2.

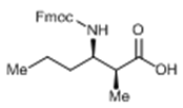

cao-jv-IV-17-butyl-(S)-imine-1H
cao-jv-IV-17
STANDARD 1H OBSERVE
Me
tBu
Compound 12
0.80
2.00
1.97
9.15
2.93
f1 (ppm)


cao-jv-IV-17-13C
cao-jv-IV-17-13C
13C OBSERVE
169.71
56.55
38.12
22.41
19.02
13.89
f1 (ppm)


cao-jv-IV-20-1H
cao-jv-IV-20
STANDARD 1H OBSERVE
Compound 13
0.97
0.94
0.97
2.87
9.68
9.36
3.00
2.95
f1 (ppm)

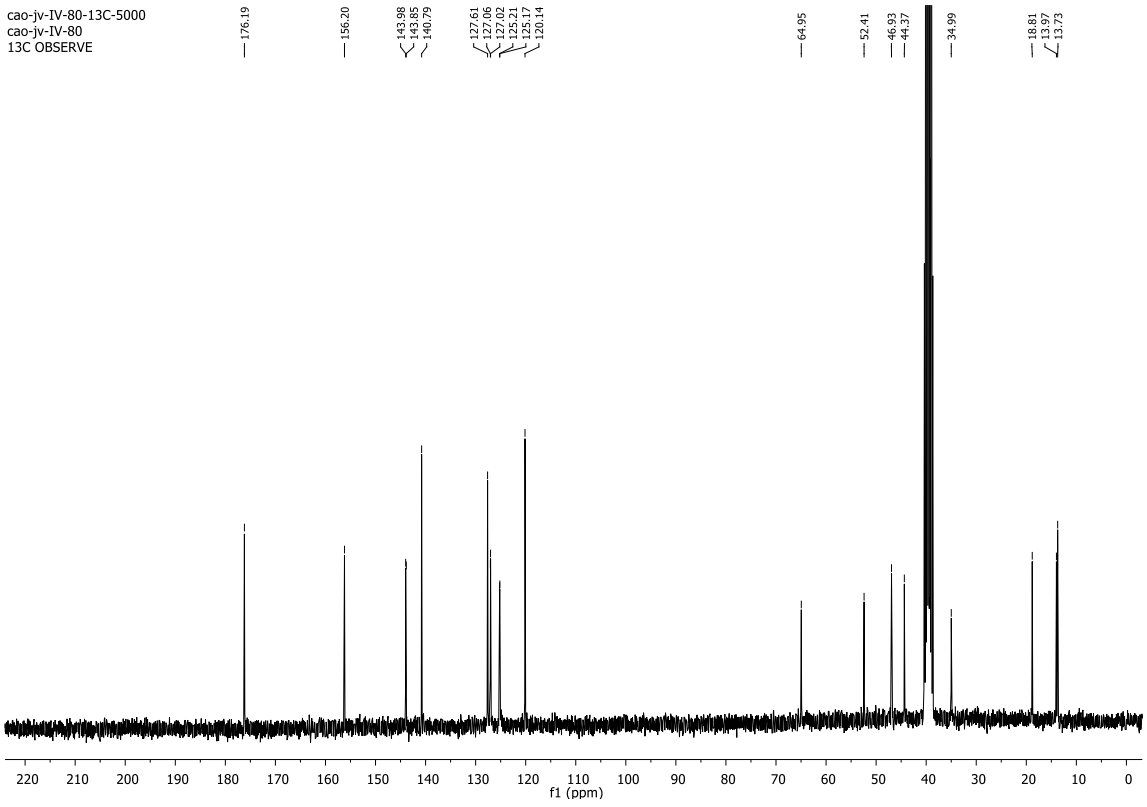

cao-jv-IV-20-13C
cao-jv-IV-20
STANDARD 1H OBSERVE
173.75
80.68
58.96
56.26
45.22
36.64
28.23
22.93
19.41
13.94
11.82
f1 (ppm)

cao-jv-IV-80-1H_1
cao-jv-IV-80
STANDARD 1H OBSERVE

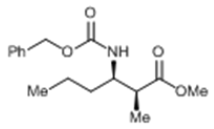


Compound 14

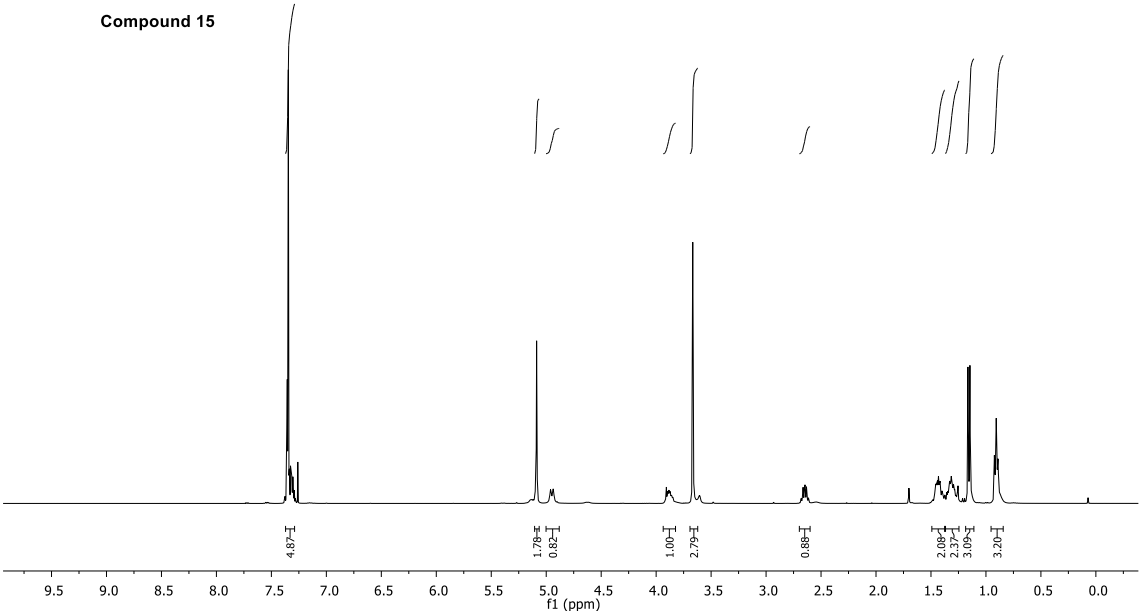
cao-jv-IV-80-13C-5000
cao-jv-IV-80
13C OBSERVE



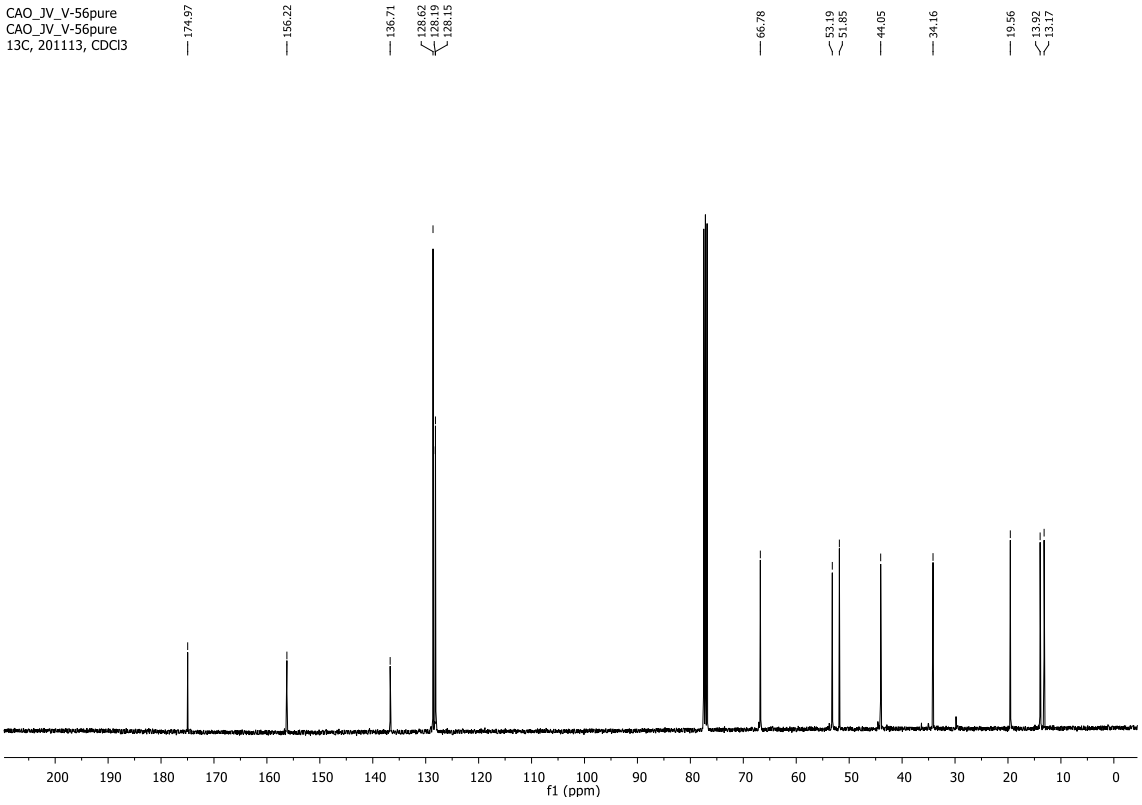
CAO_JV_V-56pure
CAO_JV_V-56pure
1H, 201113, CDCl3



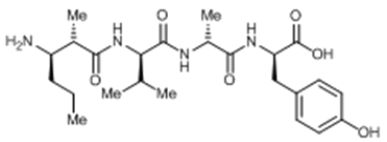
Compound 15



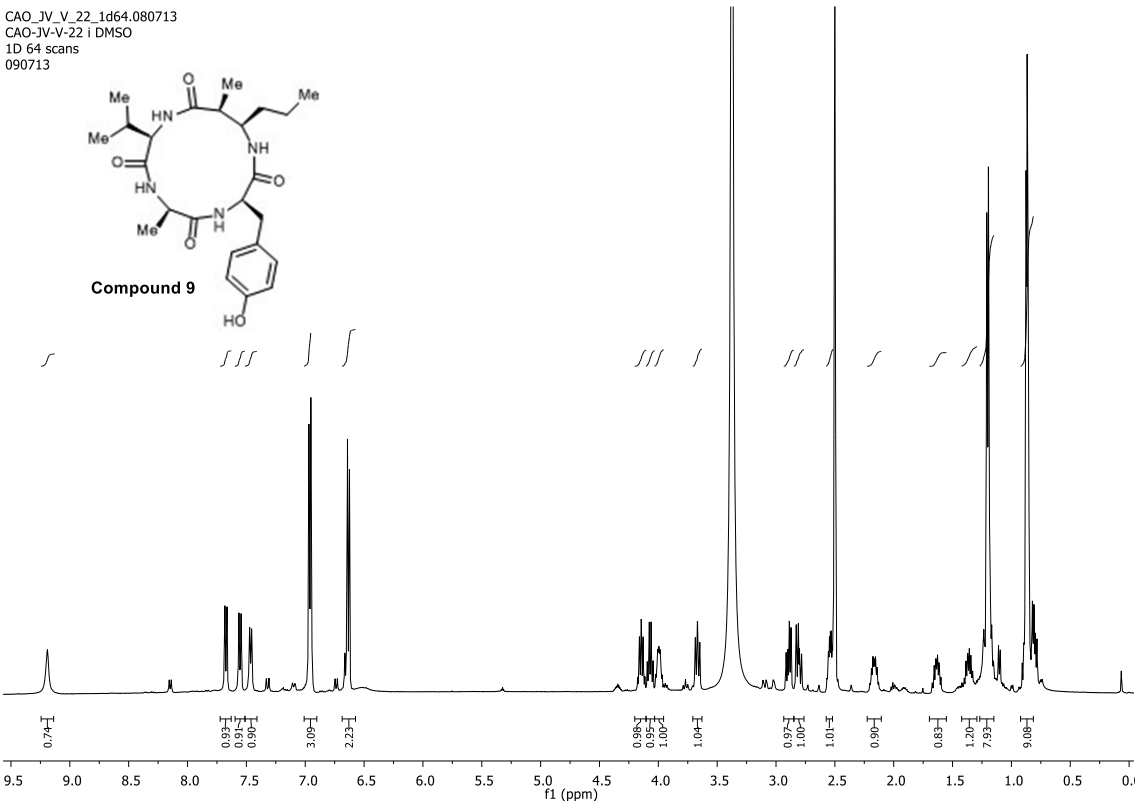
CAO_JV_V-56pure
CAO_JV_V-56pure
13C, 201113, CDCl3



CAO_JV_V-20solid
cao-jv-V-20-solid, 1H, 128 scans, 130613, MeOH



Compound 16


CAO_JV_V_22_1d64.080713
CAO-JV-V-22 i DMSO
1D 64 scans
090713
Compound 9
f1 (ppm)