Borinostats: Solid Phase Synthesis of Carborane-Capped Histone Deacetylase Inhibitors with a Tailor-Made Selectivity Profile

Christoph Selg, Andrea Schöler, Julian Schliehe-Diecks, Maria Hanl, Laura Sinatra, Arndt Borkhardt, Menyhárt B. Sárosi, Sanil Bhatia, Evamarie Hey Hawkins and Finn K. Hansen

a Institute for Drug Discovery, Medical Faculty, Leipzig University, Brüderstraße 34, 04103 Leipzig (Germany).
b Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Heinrich-Heine University Düsseldorf, Düsseldorf (Germany).
c Pharmaceutical Institute, Department of Pharmaceutical and Cell Biological Chemistry, University of Bonn, An der Immenburg 4, 53121 Bonn (Germany).
d Institute of Inorganic Chemistry, Faculty of Chemistry and Mineralogy, Leipzig University, Johannallee 29, 04103 Leipzig (Germany).

E-mail: finn.hansen@uni-bonn.de

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1 Abbreviations

AMC: 7-Amino-4-methylcoumarin  
DIPEA: N-Ethyl-N-(propan-2-yl)propan-2-amine  
DMF: N,N-Dimethylformamide  
COMU: (1-Cyano-2-ethoxy-2-oxoethylhydroxaminoxy)dimethyl-amino-morpholinoo-carbenium-hexafluorophosphate  
HATU: 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate  
HOBT: 1-Hydroxybenzotriazole, benzotriazol-1-ol  
MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide  
ZMAL: Benzyl 5-acetamido-1-(4-methyl-2-oxo-2H-chromen-6-yl)amino-1-oxopentan-2-yl carbamate

2 General Information

Unless otherwise noted, all commercially available compounds were used as provided without further purification. NMR spectra were recorded on a Bruker Avance DRX 400 MHz (1H NMR 400.16 MHz and 13C NMR 100.59 MHz) and a Bruker Avance III 400 MHz (1H NMR 400.20 MHz, 13C NMR 100.64 MHz) spectrometer using the solvent peak as internal reference for 1H and 13C NMR (CDCl3: δH 7.26; δC 77.0). 11B NMR chemical shifts were calculated according to the Z-scale.¹ Multiplicities are indicated, s (singlet), d (doublet), t (triplet), q (quartet), quint (quinquartet), sept (septet), m (multiplet), b (broad singlet); coupling constants (J) are in Hertz (Hz). High-resolution mass spectra were recorded using a Bruker Daltonics Impact II with electrospray-ionization and time-of-flight detection (TOF). TLC analysis was performed on precoated silica gel 60 F254 slides and visualised by UV-light (254 nm) or palladium staining (5% H2O2, Pd(OAc)2 solution in MeOH). Flash chromatography was carried out using silica gel, particle size 0.2–0.063 mm and spherical silica gel, particle size 25 µm using the indicated mobile phase as correlated with TLC analysis. Analytical HPLC analysis were carried out using a Thermo Fisher Scientific UltiMate 3000 system equipped with an UltiMateTM HPG-3400SD pump, an UltiMateTM 3000 diode array detector, an UltiMateTM 3000 autosampler, and a TCC-3000SD standard thermostated column compartment by Dionex. The system was operated using a Macherey-Nagel NUCLEODUR 100-5 C18 ec column (250 mm x 4.6 mm). UV absorption was detected at 210 and 254 nm with a linear gradient of 5% B to 95% B within 45 min. Acidified HPLC-grade water (0.1% TFA; solvent A) and acidified HPLC-grade acetonitrile (0.1% TFA; solvent B) were used for elution at a flow rate of 1 mL/min. Diethyl ether was distilled from sodium/benzophenone under argon (note: for both carborane reactions the solvent has to be free of water and oxygen or yields will drop significantly). Peptide-grade Dimethylformamide was purchased from Iris Biotech GmbH and used as received. All other solvents were distilled prior to use. n-Butyllithium was titrated with N-benzyl benzamide prior to use and stored under argon. All solid phase reactions were carried out in PP-reactors with PE frits (sizes: 5 mL, pore size 23µm, MultiSynTech GmbH) and a 2-chlorotrityl chloride resin (200–400 mesh, 1.60mmol/g, Iris Biotech GmbH) was used.

ortho-Carboranylacetic acid 3² and hydroxamic acids immobilized on resin (HAIRs 7a-d)³ were prepared according to known literature procedures. meta-Carborane carboxylic acid 4 was synthesized after a slightly modified protocol of KASAR et al. where instead of the portion wise addition of dry ice, gaseous CO2 was bubbled through the reaction solution.⁴ All physical characteristics were identical to those reported. All other chemicals were purchased from commercial sources and used as received.

¹ R. H. Harris, E. D. Becker, S. M. Cabral de Menezes, R. Goodfellow and P. Granger, Magn. Reson. Chem. 2002, 40, 489–505.  
² J. Nekvinda, B. Grünner, D. Gabel, W. M. Nau and K. I. Assaf, Chem. Eur. J., 2018, 24, 12970–12975.  
³ L. Sinatra, J. J. Bandolik, M. Roatsch, M. Sönnichsen, C. T. Schoeder, A. Hamacher, A. Schölter, A. Borkhardt, J. Meiler, S. Bhatia, M. U. Kassack and F. K. Hansen, Angew. Chem. Int. Ed., 2020, 59, 22494–22499 and literature cited therein.  
⁴ R. A. Kasar, G. M. Knudsen and S. B. Kahl, Inorg. Chem., 1999, 38, 2936–2940.
3 General Procedure for the Solid-Phase Synthesis of 8a-d, 9a-d, 10 and 11

Following the procedure described by Sinatra et al.\[^3\], prior to synthesis, 5 mg of respective modified resin 7a–d was mixed with 500 μL of 20% piperidine in DMF for 5 min. The solid was filtered off via syringe filtration and the process was repeated once. The filtrates were united and diluted in steps of 10 μL solution per mL of DMF forming a dilution row from 10 to 100 μL/mL. The concentration of the cleaved Fmoc-group was subsequently determined photometrically using a 3500 μL quartz cuvette (100-QS, Hellma Analytics) with a pathlengths of 10 mm and a Shimadzu UV-160A spectrometer at room temperature (ε\(_{300\text{nm}}\) (dibenzofulvene) = 7800 m\(^2\) mol\(^{-1}\)). Resin loadings [mol g\(^{-1}\)] were calculated using Lambert-Beer law:

\[
\text{A} \quad 5 \text{ mL syringe was equipped with a syringe filter and charged with the respective Fmoc-protected resin 7a–d (0.1 mmol, 1.0 equiv.) and 2.0 mL of DMF leaving an air-bubble sufficient for proper mixing. The syringe was closed with plastic cap and gently shaken for 30 min on a laboratory shaker at room temperature. The cap was removed and the excess solvent was pushed out and discarded leaving the now visibly swollen resin. To remove the Fmoc protecting group, 3.0 mL of a freshly prepared cleaving solution (20 vol.-% piperidine in DMF) was sucked into the syringe. The syringe was capped, shaken for 5 min and the excess solution was discarded. The process was repeated once before the resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and again DMF (5 x 3 mL).
\]

For carboxylic acid activation, a mixture consisting of benzoic acid, phenyl acetic acid, 3 or 4 (0.2 mmol, 2.0 equiv.), and the coupling reagent COMU or HATU (0.2 mmol, 2.0 equiv.) in 2 mL of DMF was vortexed for 10 s and upon dissolution DIPEA (388 μL, 0.3 mmol, 3.0 equiv.) was added. The resulting clear solution was agitation for 10 minutes and added to the deprotected resin. The vial was rinsed with 1 mL of DMF which was also added to the reaction mixture. The mixture was agitation for 16 hours at room temperature under exclusion of light. The excess solution was discarded and the residual resin was washed with DMF (5 x 3 mL) and DCM (10 x 3 mL). The plunger was removed carefully and the open syringe was dried inside a round bottom flask connected to high vacuum for 1 h. (Note: The final washing cycle with DCM has to be performed very thoroughly to avoid traces of DMF disturbing the following step). To obtain the free hydroxamate, 4 mL of a freshly prepared acidic cleaving solution (5 vol.-% TFA in DCM) was sucked into the syringe. The syringe was shaken at room temperature for 1 h and the dark red solution was collected. The syringe was extracted once again with 3 mL of the cleaving solution, with MeOH (3 x 3 mL) and DCM (3 x 3 mL). The extracts were united to form a now colourless clear solution. The solvent was removed under reduced pressure and the crude product was subjected to flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%) yielding the respective hydroxamic acids 8a-d, 9a-d, 10 and 11.
4 Experimental Data

7-(1,2-Dicarba-closo-dodecaborane(12)-1-acetamido)-N-hydroxyheptanamide 8a

Synthesized according to the general procedure using resin 7a (127 mg, 0.1 mmol, 1.0 equiv.), HATU (76 mg, 0.2 mmol, 2 equiv.), DIPEA (51 μL, 0.3 mmol, 3 equiv.) and carboranlyacetic acid 3 (41 mg, 0.2 mmol, 2 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, \( R_{f} \) (cyclohexane/EtOAc, 1:1) = 0.18 yielding 8a as colourless oil (31 mg, 0.09 mmol, 90%). \(^1\)H NMR (400 MHz, CD\(_2\)CN) δ 6.97 (s, 1H), 4.75 (s, 1H), 3.14 (t, \( J = 6.4 \) Hz, 2H), 3.11 (s, 2H), 3.50–1.25 (m, 10H, carborane cluster B\( \text{H} \)), 2.09 (t, \( J = 7.4 \) Hz, 2H), 1.58 (t, \( J = 7.1 \) Hz, 2H), 1.48 (t, \( J = 7.0 \) Hz, 2H), 1.33 (h, \( J = 4.8, 4.4 \) Hz, 5H). \(^{11}\)B\(^{1}\)H NMR (128 MHz, CD\(_2\)CN) δ -2.8, -5.8, -9.9, -10.7, -12.9. \(^{13}\)C\(^{1}\)H NMR (101 MHz, CD\(_3\)CN) δ 171.99, 167.08, 71.83, 61.28, 43.67, 40.04, 33.17, 29.48, 29.15, 27.05, 25.99. HRMS-ESI (m/z): [M+Na]\(^+\) calcd for C\(_{33}\)H\(_{45}\)B\(_{15}\)N\(_{3}\)O\(_{4}\): 586.3025, found: 585.2967 with the expected isotopic distribution.

7-(1,2-Dicarba-closo-dodecaborane(12)-1-acetamido)-N-hydroxyhexanamide 8b

Synthesized according to the general procedure using resin 7b (125 mg, 0.1 mmol, 1.0 equiv.), HATU (76 mg, 0.2 mmol, 2 equiv.), DIPEA (51 μL, 0.3 mmol, 3 equiv.) and carboranlyacetic acid 3 (41 mg, 0.2 mmol, 2 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane, gradient elution 20-55%, \( R_{f} \) (cyclohexane/EtOAc, 1:1) = 0.18 yielding 8b as colourless oil (26 mg, 0.08 mmol, 80%). \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) δ 10.33 (s, 1H), 8.21 (t, \( J = 5.6 \) Hz, 1H), 5.13 (s, 1H), 3.18 (s, 3H), 3.10 (s, 2H), 3.02 (q, \( J = 6.4 \) Hz, 2H), 3.50–1.25 (m, 10H, carborane cluster B\( \text{H} \)), 1.94 (t, \( J = 7.4 \) Hz, 2H), 1.50 (q, \( J = 7.5 \) Hz, 2H), 1.39 (t, \( J = 7.4 \) Hz, 2H), 1.25 (q, \( J = 8.2 \), 7.6 Hz, 3H). \(^{11}\)B\(^{1}\)H NMR (128 MHz, DMSO-d\(_6\)) δ -3.1, -5.9, -10.0, -10.7, -12.8. \(^{13}\)C\(^{1}\)H NMR (101 MHz, DMSO-d\(_6\)) δ 169.06, 165.48, 71.51, 61.19, 42.27, 38.61, 32.19, 28.44, 25.99, 24.78. HRMS-ESI (m/z): [M+H]\(^+\) calcd for C\(_{30}\)H\(_{32}\)B\(_{14}\)N\(_{3}\)O\(_{3}\): 331.3036, found: 331.3025.

(\(E\))-3-[[1,2-Dicarba-closo-dodecaborane(12)-1-acetamido]methyl]phenyl]-N-hydroxyacrylamide 8c

Synthesized according to the general procedure using resin 7c (125 mg, 0.1 mmol, 1.0 equiv.), HATU (76 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and carboranlyacetic acid 3 (41 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, \( R_{f} \) (cyclohexane/EtOAc, 1:1) = 0.20) yielding 8c as colourless oil (28 mg, 0.07 mmol, 74%). \(^1\)H NMR (400 MHz, CD\(_3\)OD) δ 7.56 (d, \( J = 7.9 \) Hz, 1H), 7.35 (d, \( J = 8.0 \) Hz, 1H), 6.48 (d, \( J = 15.8 \) Hz, 1H), 4.38 (s, 1H), 3.50–1.25 (m, 10H, carborane cluster B\( \text{H} \)), 3.21 (s, 1H), 2.83 (s, 1H), -C-NH-OH signal could not be detected due to solvent exchange. \(^{11}\)B\(^{1}\)H NMR (128 MHz, CD\(_3\)OD) δ -2.5, -5.5, -9.7, -10.8, -11.7, -12.9. \(^{13}\)C\(^{1}\)H NMR (101 MHz, CD\(_3\)OD) δ 168.42, 166.33, 141.23, 141.17, 135.45, 129.42, 129.02, 118.45, 71.67, 61.59, 44.05, 44.00, 38.89. HRMS-ESI (m/z): [M+H]\(^+\) calcd for C\(_{34}\)H\(_{36}\)B\(_{20}\)N\(_{3}\)O\(_{4}\): 737.2870, found: 737.2874. HPLC t\(_{R}\): 13.39 min, purity: 99.7% rel. area.
4-[(1,2-Dicarba-closo-dodecaborane(12)-1-acetamido)methyl]-N-hydroxybenzamide 8d

Synthesized according to the general procedure using resin 7d (151 mg, 0.1 mmol, 1.0 equiv.), HATU (76 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and carboranylacetic acid 3 (41 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, Rf (cyclohexane/EtOAc, 1:1) = 0.20 yielding 8d as colourless oil (35 mg, 0.1 mmol, 99%). 1H NMR (400 MHz, CD3OD) δ 7.81 – 7.65 (m, 1H), 7.55 – 7.25 (m, 1H), 4.42 (s, 1H), 3.50–1.25 (m, 10H, carborane cluster BH), 3.22 (s, 1H), 2.83 (s, 1H), -C-NH-OH signal could not be detected due to solvent exchange. 13C(1H) NMR (101 MHz, CD3OD) δ -2.5, -5.6, -9.7, 12.9. HRMS (ESI) m/z: [M+H]+ calcd for C12H23B13O3Na: 351.2713, found: 351.2731. HPLC tR: 13.47 min, purity: 95.1% rel. area.

1,2-Dicarba-closo-dodecaborane(12)-N-[6-(hydroxyamino)-6-oxoheptyl]-1-carboxamide 9a

Synthesized according to the general procedure using resin 7a (127 mg, 0.1 mmol, 1.0 equiv.), COMU (86 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and carboranyl carboxylic acid 4 (38 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, Rf (cyclohexane/EtOAc, 1:1) = 0.22) yielding 9a as colourless oil (32 mg, 0.09 mmol, 96%). 1H NMR (400 MHz, CD3OD) δ 3.74 – 3.64 (m, 1H), 3.50–1.25 (m, 10H, carborane cluster BH), 3.14 (t, J = 7.1 Hz, 2H), 2.10 (t, J = 7.4 Hz, 2H), -1.62 (t, J = 7.4 Hz, 2H), 1.55 – 1.43 (m, 2H), 1.43 – 1.21 (m, 5H), -C-NH-OH signal could not be detected due to solvent exchange. 13C(1H) NMR (101 MHz, CD3OD) δ -5.7, -7.7, -11.1, -11.6, -13.3, -13.9, -15.6. HRMS-ESI (m/z): [M+Na]+ calcd for C31H35B13O6N3: 533.2811, found: 533.2850. HPLC tR: 13.47 min, purity: 95.8% rel. area.

1,2-Dicarba-closo-dodecaborane(12)-N-[6-(hydroxyamino)-6-oxohexyl]-1-carboxamide 9b

Synthesized according to the general procedure using resin 7b (125 mg, 0.1 mmol, 1.0 equiv.), COMU (86 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and carboranyl carboxylic acid 4 (38 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, Rf (cyclohexane/EtOAc, 1:1) = 0.18) yielding 9b as colourless oil (26 mg, 0.08 mmol, 82%). 1H NMR (400 MHz, CD3OD) δ 3.74 – 3.60 (m, 1H), 3.33 (p, J = 1.6 Hz, 2H), 3.50–1.25 (m, 10H, carborane cluster BH), 3.14 (t, J = 7.1 Hz, 2H), 2.10 (t, J = 7.5 Hz, 2H), 1.63 (p, J = 7.6 Hz, 2H), 1.56 – 1.39 (m, 2H), 1.39 – 1.21 (m, 2H), -C-NH-OH signal could not be detected due to solvent exchange. 13C(1H) NMR (101 MHz, CD3OD) δ -5.0, -6.3, -7.1, -8.3, -10.5, -10.9, -11.7, -12.6, -13.9, -14.9, -16.3. HRMS-ESI (m/z): [M+Na]+ calcd for C30H34B13O6N2: 539.2687, found: 539.2705. HPLC tR: 13.16 min, purity: 96.5% rel. area.
(E)-3-{4-[(1,2-Dicarba-closododecaborane(12)-1-acetamido)methyl]phenyl}-N-hydroxyacrylamide 9c

Synthesized according to the general procedure using resin 7c (125 mg, 0.1 mmol, 1.0 equiv.), COMU (86 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and carborane carboxylic acid 4 (38 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, DCM/cyclohexane) gradient elution 20-55%, Rf (cyclohexane/EtOAc, 1:1) = 0.20 yielding 9c as colourless oil (33 mg, 0.09 mmol, 92%). 1H NMR (400 MHz, CD3OD) δ 7.57 (d, J = 15.9 Hz, 1H), 7.53 (d, J = 7.8 Hz, 2H), 7.24 (d, J = 7.9 Hz, 2H), 6.47 (d, J = 15.7 Hz, 1H), 4.35 (s, 2H), 3.70 (s, 1H), 3.50–1.25 (m, 10H, carborane cluster BH), 3.19 – 1.36 (m, 2H), -CH2N2OH signal could not be detected due to solvent exchange. 11B(1H) NMR (128 MHz, CD3OD) δ -5.1, -6.4, -8.0, -10.5, -11.6, -12.6, -13.9, -14.8, -16.3. 13C(1H) NMR (101 MHz, CD3OD) δ 166.34, 162.77, 141.38, 141.18, 135.25, 128.95, 128.56, 118.36, 77.21, 56.89, 44.81. HRMS-ESI (m/z): [M+Na]+ calcld for C13H23B10N3O3Na: 385.2533, found: 385.2536. HPLC tR: 13.28 min, purity: 95.0% rel. area.

4-{1,2-Dicarba-closododecaborane(12)-1-acetamido)methyl]-N-hydroxybenzamide 9d

Synthesized according to the general procedure using resin 7d (151 mg, 0.1 mmol, 1.0 equiv.), COMU (86 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and carborane carboxylic acid 4 (38 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, Rf (cyclohexane/EtOAc, 1:1) = 0.20 yielding 9d as colourless oil (27 mg, 0.08 mmol, 81%). 1H NMR (400 MHz, CD3OD) δ 7.89 – 7.62 (m, 1H), 7.30 (d, J = 8.1 Hz, 1H), 4.38 (s, 1H), 3.78 – 3.60 (m, 1H) 3.50–1.25 (m, 10H, carborane cluster BH), -CH2N2OH signal could not be detected due to solvent exchange. 11B(1H) NMR (128 MHz, CD3OD) δ -5.7, -7.5, -11.0, -11.6, -13.3, -15.5. 13C(1H) NMR (101 MHz, CD3OD) δ 167.91, 162.80, 143.45, 132.47, 128.33, 128.16, 77.16, 56.91, 44.75. HRMS-ESI (m/z): [M+H]+ calcld for C13H21B10N3O3: 337.2556, found: 337.2566. HPLC tR: 13.67 min, purity: 95.1% rel. area.

(E)-N-Hydroxy-3-{4-[[2-phenylacetamido)methyl]phenyl]acrylamide 10

Synthesized according to the general procedure using resin 7c (151 mg, 0.1 mmol, 1.0 equiv.), HATU (76 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and phenylacetic acid (38 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, Rf (cyclohexane/EtOAc, 1:1) = 0.20 yielding 10 as colourless oil (24 mg, 0.07 mmol, 77%). 1H NMR (400 MHz, DMSO-d6) δ 9.37 (b, 1H), 8.59 (t, J = 5.9 Hz, 1H), 7.50 (d, J = 7.9 Hz, 2H), 7.42 (d, J = 15.8 Hz, 1H), 7.31 – 7.28 (m, 4H), 7.28 – 7.25 (m, 2H), 6.45 (d, J = 15.8 Hz, 1H), 4.29 (d, J = 5.9 Hz, 3H), 3.49 (s, 3H). 13C(1H) NMR (101 MHz, DMSO-d6) δ 170.17, 162.68, 140.95, 137.88, 136.36, 133.44, 129.00, 128.20, 127.70, 127.43, 126.36, 118.76, 42.35, 41.96. HRMS-ESI (m/z): [M+H]+ calcld for C18H19N3O3: 311.1390, found: 311.1408. HPLC tR: 11.70 min, purity: 95.5% rel. area.
4-(Benzamidomethyl)-N-hydroxybenzamide 11

Synthesized according to the general procedure using resin 7c (151 mg, 0.1 mmol, 1.0 equiv.), HATU (76 mg, 0.2 mmol, 2.0 equiv.), DIPEA (51 μL, 0.3 mmol, 3.0 equiv.) and phenylacetic acid (38 mg, 0.2 mmol, 2.0 equiv.). The crude oil was purified by flash chromatography (1.5 x 12 cm, EtOAc/cyclohexane) gradient elution 20-55%, Rf (cyclohexane/EtOAc, 1:1) = 0.20) yielding 10 as colourless oil (21 mg, 0.08 mmol, 79%). 1H NMR (300 MHz, CD3OD) δ 7.89 – 7.81 (m, 1H), 7.75 – 7.65 (m, 1H), 7.55 – 7.38 (m, 3H), 4.62 (s, 1H), -C-NH-OH signal could not be detected due to solvent exchange. 13C{1H} NMR (75 MHz, CD3OD) δ 170.26, 168.00, 144.35, 135.44, 132.83, 132.34, 130.96, 129.63, 128.59, 128.35, 128.33, 44.16. HRMS-ESI (m/z): [M+H]+ calcd for C15H15N2O3: 271.1077, found: 271.1082. HPLC tR: 11.27 min, purity: 95.4% rel. area.
5 Biological Evaluation

Reagents

Stock solutions (10 mM) of investigated compounds were prepared in DMSO. All cell culture material was provided by Sarstedt (Newton, USA). All other reagents were supplied by PAN Biotech (Germany) unless otherwise stated.

Cell Line and Cell Culture

The human ovarian carcinoma cell line A2780 (ECACC no. 93112519) was obtained from the European Collection of Cell Cultures (ECACC, UK) and cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum, 2 mM L-Glutamine, 50 U/mL penicillin and 50 µg/mL streptomycin. The cells were grown at 37 °C under humidified air with 5 % CO₂ to 80%–90% confluency before being used in further assays.

MTT Cell Viability Assay

The intrinsic cytotoxicity of test compounds was evaluated by an MTT assay as previously described.\[5\] A2780 cells were seeded in 96 well plates (Starlab GmbH, Hamburg, Germany) at a density of 8000 cells/well and incubated for 12 h. Subsequently cells were exposed to increasing concentrations of the test compounds. After 72 h MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (BioChemica, Applichem GmbH, Germany) solution (5 mg/mL in phosphate-buffered saline) was added to determine cell survival. The formazan dye was dissolved after 1 h in DMSO (Sigma-Aldrich, Germany) and absorbance was measured at 570 nm and 690 nm in a Multiskan microplate photometer (Thermo Fisher Scientific, USA).

All compounds were evaluated in triplicate in at least three independent experiments.

In Vitro Human HDAC1, 2, 3 and 6 Assay

OptiPlate-96 black microplates (Perkin Elmer) were used with an assay volume of 50 µL. 5 µL test compound or control, diluted in assay buffer (50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 0.1 mg/mL BSA), were incubated with 35 µL of the fluorogenic substrate ZMAL (Z-Lys(Ac)-AMC) (21.43 µM in assay buffer) and 10 µL of human recombinant HDAC1 (BPS Bioscience, Catalog# 50051), HDAC2 (BPS Bioscience, Catalog# 50052), HDAC3 (BPS Bioscience, Catalog# 50003) or HDAC6 (BPS Bioscience, Catalog# 50006) at 37 °C. After an incubation time of 90 min, 50 µL of 0.4 mg/mL trypsin in trypsin buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl) were added, followed by further incubation at 37 °C for 30 min. Fluorescence was measured with an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a Fluoroskan Ascent microplate reader (Thermo Scientific). All compounds were evaluated in duplicates in at least two independent experiments.

In Vitro Human HDAC8 Assay

OptiPlate-96 black microplates (Perkin Elmer) were used with an assay volume of 50 µL. 5 µL test compound or control, diluted in assay buffer (50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1.0 mg/mL BSA), were incubated with 35 µL of the fluorogenic substrate Boc-Lys(Tfa)-AMC (Bachem, Catalog# 4060676, 100 µM in assay buffer) and 10 µL of human recombinant HDAC8 (BPS Bioscience, Catalog# 50008) at 37 °C. After an incubation time of 90 min, 50 µL of 1 mg/mL trypsin in trypsin buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl) were added, followed by further incubation at 37 °C for 30 min. Fluorescence was measured with an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a Fluoroskan Ascent microplate reader (Thermo Scientific). All compounds were evaluated in duplicates in at least two independent experiments.

Data Analysis

Concentration effect curves were generated using the four-parameter logistic equation (GraphPad Prism 9.0, San Diego, CA) and mean IC₅₀ values were calculated based on at least three distinct experiments.

\[5\] a) H. Mueller, M. U. Kassack and M. Wiese, *J. Biomol. Screen.* 2004, 9, 506–515; b) F. Marighetti, K. Steggemann, M. Hanl and M. Wiese, *ChemMedChem*, 2013, 8, 125–135; c) S. Kraege, K. Stefan, K. Juvale, T. Ross, T. Willmes, M. Wiese, *Eur. J. Med. Chem.*, 2016, 117, 212–229.
Cell culture

The leukemic cell lines HL60 (acute myeloid leukemia or AML), K562 (chronic myeloid leukemia or CML) and HPBALL (T-cell acute lymphoblastic leukemia or T-ALL) were cultured in RPMI 1640 GlutaMax (Life Technology, California, USA, Catalog#61870036) supplemented with 10% FCS. All cells were cultured in a 37 °C humidified incubator with 5% CO₂ according to the suggested culture conditions from DSMZ (https://www.dsmz.de), with the addition of 1% penicillin-streptomycin (Catalog#15070063, Life Technologies).

Cell viability assay

The cell viability assay was performed to determine the IC₅₀ values for the leukemic cell lines. The experimental compounds were first dissolved in DMSO with an initial stock concentration of 10mM and printed on white 384-well plates (Catalog#3570, Thermo Fisher Scientific) with 26 increasing concentrations (0.1 – 25 μM) by using a digital dispenser (D300e, Tecan). Afterwards, 30 μL of cell suspension/well were seeded with a concentration of 0.04 x 10⁶ cells /mL and incubated under standard culture conditions. After 72 h the cell viability was measured utilizing the ATP based CellTiter-Glo luminescent assay (Catalog#G7573, Promega) with a microplate reader (Spark, Tecan). The obtained raw data were normalized to DMSO treated controls (DMSO < 0.5%) and the IC₅₀ values calculated using the sigmoid dose curve (Hill slope) and nonlinear regression (GraphPad Prism Inc.) (n=3). The IC₅₀ data were plotted as a clustered heat map. Each box of the heat map represents the mean of three independent experiments (n=3).

Combinatorial drug screening

Experimental compounds, vorinostat and bortezomib were printed on white 384-well plates with increasing concentrations in a dose-response 8x8 matrices using a digital dispenser. The viability was monitored after 72 h using CellTiter-Glo luminescent assay, using a microplate reader. The ZIP synergy scores calculations were based on the ZIP model using the Synergy Finder tool (https://synergyfinder.fimm.fi/).

Immunoblotting

HL60 cells (0.5 x 10⁶ cells/ml) were treated with the indicated concentration of the compound or vehicle (DMSO) for 24 h under standard culture conditions. Cell pellets were lysed with 300 µL RIPA buffer (50 mM Tris-HCl pH 8.0, 1 % Triton X-100, 0.5 % sodium deoxycholate, 0.1% sodium deoxycholate, 0.1% SDS, 150 mM sodium chloride, 2 mM EDTA, supplemented with protease inhibitors (Catalog#11697498001, Roche) and phosphatase inhibitor (Catalog#4906845001, Roche) cocktail tablets, following manufacturers’ guidelines. After centrifugation, the protein concentration of the whole cell extracts was determined using the Pierce BCA protein assay kit (Catalog#23225, ThermoFisher Scientific), according to manufacture guidelines. 20 μg of total protein extracts were resolved by a n8% or 12% SDS-PAGE at 60 mA for 60 min and transferred at 100 V for 60 min~120 min to nitrocellulose blotting membrane (Catalog#10600002, GE Healthcare, Germany) utilizing the wet mini trans-blot electrophoretic transfer cell system (Catalog#1703930. Bio-Rad). PageRuler Prestained Protein Ladder, 10 to 180 kDa (Catalog#26616, ThermoFisher Scientific) was used as protein molecular weight marker. First, Blots were incubated in 5% BSA/ TBS-T blocking solution for 30 min under slight agitation at room temperature and washed three times for 5 min in TBS-T. Afterwards, the blots were incubated overnight at 4 °C with anti-α-tubulin (Catalog#2144), anti-acetyl-α-tubulin (Catalog#5335), anti-acetyl-histone H3 (Catalog#9677) and anti-GAPDH (Catalog#5174) antibodies from Cell Signaling Technology and anti-acetyl SMC3 (Catalog# MABE1925, Merck Millipore). All primary antibodies were diluted 1:1000 in 5% BSA/ TBS-T as suggested by the manufacturer's guidelines. Afterwards, blots were washed three times for 5 min in TBS-T. Next, blots were incubated with 1:2000 dilution of secondary horseradish peroxidase-conjugated antibodies (Catalog#7074 or #7076, Cell Signalling Technology) for 2 h at room temperature. Blots were washed three times with TBS-T and developed with the ECL system (Catalog#GERPN2109, GE Healthcare), according to the manufacturer’s guidelines. Blots were detected and analyzed with the Jess western blot system (Protein simple).

[6] A. Ianevski, L. He, T. Aittokallio and J. Tang, Bioinformatics, 2020, 36, 2645.
6 Technical Details of the Molecular Docking Simulations

The crystal structure of human histone deacetylase 6 catalytic domain 2 (hHDAC6-CD2) as a chimera with maltose-binding protein (MBP) and the HDAC1:MTA1 complex were downloaded from www.rcsb.org (PDB ID: 5EDU and 5ICN).[7,8,9] MBP, all ligands, non-standard residues except for the Zn²⁺ ion, and all water molecules were removed with UCSF Chimera.[10] Protein hydrogen atoms were added using the reduce program without performing automated flips on ASN, GLN, and HIS sidechain residues (NQH), creating N H hydrogens on histidine rings and allowing existing O H and S H groups to rotate.[11]

Ligand structures were constructed in their deprotonated forms with UCSF Chimera.[10] Ligand geometries were optimized with the PM6-D3H4X/COSMO(water) method, as implemented in MOPAC2016 (Version: 19.179L).[12] The linear scaling algorithm MOZYME was used to speed up the calculations. The boron atom radius in MOZYME was set to 0.92 Å. The CHELPG atomic partial charges for each ligand were derived at the HF/6-31G* level, as implemented in ORCA.[13]

Ligand molecules and one hHDAC6-CD2 were prepared for docking with AutoDock Tools 1.5.6.[14] All hydrogen atoms and ligand CHELPG partial atomic charges were kept and all torsion angles within the carborane clusters were set to non-rotatable, but otherwise default protocols were followed for preparing receptor and ligand structures. Gasteiger charges were assigned to each atom of the macromolecule, and boron AutoDock atom types were adapted from literature.[15,16]

Docking was performed with AutoDock 4.2.5.1.[14,17] A 56×52×44 points three-dimensional affinity grid with 0.375 Å grid point spacing was defined around the hHDAC6-CD2 active site cavity. A 60×56×50 points three-dimensional affinity grid with the same grid point spacing was defined around the HDAC1 active site cavity. The number of docking runs was increased to 100, but otherwise default protocols were followed: Lamarckian genetic algorithm (LGA) for ligand conformational search; population size of 150 individuals; maximum of 2.5 × 10⁶ energy evaluations; maximum of 27 000 generations; one top individual to survive to the next generation automatically; mutation rate of 0.02; crossover rate of 0.8; random initial positions and conformations; the probability of performing a local search on an individual in the population was set to 0.06; the maximum number of iterations per local search was set to 300.

UCSF Chimera was used to inspect the docking results and to render images thereof.[10]

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**Figure S1.** 9d docked into human HDAC1 (PDB ID: 5ICN). The docked pose of 8c is shown as magenta wireframe for comparison. Residue numbering according to PDB ID: 5ICN. B: orange, C: grey/green, N: blue, O: red, Zn: purple. Hydrogen atoms are omitted. HDAC1 is shown in grey. HDAC6 backbone trace is shown in light blue for comparison.
7 NMR Data for Compounds 8a-d, 9a-d, 10 and 11

\(^1\)H NMR (CD\(_3\)CN) of 8a.

\(^{13}\)B\(^{1}H\) NMR (CD\(_3\)CN) of 8a.
$^{13}$C{[H]} NMR (CD$_3$CN) of 8a.

$^1$H NMR (DMSO-d$_6$) of 8b.
$^{13}$B$^1$H NMR (DMSO-$d_6$) of 8b.

$^{13}$C$^1$H NMR (DMSO-$d_6$) of 8b.
$^1$H NMR (CD$_3$OD) of $8c$.

$^{13}$B($^1$H) NMR (CD$_3$OD) of $8c$. 
$^{13}$C NMR (CD$_3$OD) of 8c.

$^1$H NMR (CD$_3$OD) of 8d.
$^{13}$B{$_1^1$}H) NMR (CD$_3$OD) of 8d.

$^{13}$C{$_1^1$}H) NMR (CD$_3$OD) of 8d.
$^{1}H$ NMR (CD$_3$OD) of 9a.

$^{1}$B($^1$H) NMR (CD$_3$OD) of 9a.
$^{13}$C(\textsuperscript{1}H) NMR (CD$_3$OD) of 9a.

$^1$H NMR (CD$_3$OD) of 9b.
$^{13}$B($^1$H) NMR (CD$_3$OD) of 9b.

$^{13}$C($^1$H) NMR (CD$_3$OD) of 9b.
$^1$H NMR (CD$_3$OD) of 9c.

$^{11}$B($^1$H) NMR (CD$_3$OD) of 9c.
$^{13}$C\textsuperscript{[\textit{H}]} NMR (CD$_3$OD) of 9c.

$^1$H NMR (CD$_3$OD) of 9d.
$^1$H NMR (CD$_3$OD) of 9d.

$^{13}$C NMR (CD$_3$OD) of 9d.
\[^1\text{H} \text{NMR (DMSO-d}_6\text{)}\] of 10.

\[^{13}\text{C}\{^1\text{H}\} \text{NMR (DMSO-d}_6\text{)}\] of 10.
$^1$H NMR (CD$_3$OD) of 11.

$^{13}$C($^1$H) NMR (CD$_3$OD) of 11.
8 HRMS ESI Data for Compounds 8a-d, 9a-d, 10 and 11

Simulated (top) and measured (bottom) HRMS-ESI mass spectrum (positive mode) of 8a.

Simulated (top) and measured (bottom) HRMS-ESI mass spectrum (positive mode) of 8b.
Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 8c.

Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 8d.
Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 9a.

Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 9b.
Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 9c.

Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 9d.
Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 10.

Simulated (top) and measured (bottom) HRMS-ESI spectrum (positive mode) of 11.
9 HPLC Chromatograms of Key Target Compounds

HPLC chromatogram of 8c. (Thermo Fisher Scientific UltiMate TM 3000).

HPLC chromatogram of 8d. (Thermo Fisher Scientific UltiMate TM 3000).
HPLC chromatogram of 9a. *(Thermo Fisher Scientific UlitMateTM 3000)*.

HPLC chromatogram of 9b. *(Thermo Fisher Scientific UliMateTM 3000)*.
HPLC chromatogram of 9c. (Thermo Fisher Scientific UltiMateTM 3000).

| No. | Ret. Time [min] | Peak Name | Height [n A.U.] | Area [n A.U.*min] | Rel. Area [%] | Amount [n M] | Type |
|-----|----------------|-----------|----------------|------------------|--------------|-------------|------|
| 1   | 10.21          | n.a.      | 2.453          | 0.251            | 0.31         | n.a.        | BM B |
| 2   | 10.09          | n.a.      | 6.069          | 0.195            | 0.05         | n.a.        | BM B |
| 3   | 11.34          | n.a.      | 0.067          | 0.051            | 0.02         | n.a.        | BM B |
| 4   | 11.53          | 95.4      | 3.025          | 0.118            | 0.14         | n.a.        | BM B |
| 5   | 11.81          | n.a.      | 2.005          | 0.187            | 0.08         | n.a.        | BM B |
| 6   | 12.31          | n.a.      | 1.929          | 0.193            | 0.09         | n.a.        | BM B |
| 7   | 13.67          | 211,416   | 53.687         | 2.978            | 2.74         | n.a.        | M B |
| 8   | 14.18          | 24,865    | 3.006          | 1.33             | 1.13         | n.a.        | M B |
| 9   | 14.28          | n.a.      | 4.460          | 0.361            | 0.33         | n.a.        | Rd  |
| 10  | 14.95          | n.a.      | 1.569          | 0.149            | 0.07         | n.a.        | BM B |
| 11  | 14.95          | n.a.      | 0.563          | 0.060            | 0.02         | n.a.        | Rd  |
| 12  | 15.38          | n.a.      | 1.050          | 0.083            | 0.04         | n.a.        | M B |
| 13  | 15.61          | n.a.      | 1.063          | 0.143            | 0.10         | n.a.        | BM B |

Total: 220,503 100.00

HPLC chromatogram of 9d. (Thermo Fisher Scientific UltiMateTM 3000).

| No. | Ret. Time [min] | Peak Name | Height [n A.U.] | Area [n A.U.*min] | Rel. Area [%] | Amount [n M] | Type |
|-----|----------------|-----------|----------------|------------------|--------------|-------------|------|
| 1   | 10.45          | n.a.      | 3.059          | 0.393            | 0.13         | n.a.        | BM B |
| 2   | 10.88          | n.a.      | 1.107          | 0.149            | 0.12         | n.a.        | Ru  |
| 3   | 11.42          | n.a.      | 25.278         | 2.409            | 2.08         | n.a.        | M   |
| 4   | 11.75          | 95.4      | 4.240          | 0.554            | 0.48         | n.a.        | Rd  |
| 5   | 12.75          | n.a.      | 4.029          | 0.542            | 0.46         | n.a.        | M B |
| 6   | 12.56          | n.a.      | 4.025          | 0.582            | 0.49         | n.a.        | BM B |
| 7   | 13.28          | 881,495   | 95.83          | 8.660            | 7.88         | n.a.        | BM B |
| 8   | 13.74          | n.a.      | 0.607          | 0.083            | 0.07         | n.a.        | M   |
| 9   | 14.23          | 881,495   | 95.83          | 8.660            | 7.88         | n.a.        | M   |
| 10  | 14.43          | n.a.      | 6.038          | 0.091            | 0.08         | n.a.        | Rd  |
| 11  | 14.71          | n.a.      | 20.708         | 2.163            | 1.89         | n.a.        | M   |
| 12  | 14.87          | n.a.      | 3.628          | 0.251            | 0.20         | n.a.        | Rd  |
| 13  | 15.02          | n.a.      | 11.124         | 1.188            | 1.01         | n.a.        | M B |
| 14  | 15.27          | n.a.      | 1.038          | 0.153            | 0.13         | n.a.        | BM B |
| 15  | 15.42          | n.a.      | 2.044          | 0.164            | 0.14         | n.a.        | BM B |
| 16  | 15.58          | n.a.      | 3.443          | 0.208            | 0.17         | n.a.        | BM B |
| 17  | 15.75          | 95.4      | 10.447         | 1.197            | 1.08         | n.a.        | BM B |
| 18  | 16.17          | n.a.      | 0.074          | 0.011            | 0.01         | n.a.        | BM B |
| 19  | 16.95          | n.a.      | 0.008          | 0.006            | 0.00         | n.a.        | BM B |
| 20  | 17.41          | n.a.      | 1.532          | 0.125            | 0.11         | n.a.        | BM B |

Total: 881,495 100.00
HPLC chromatogram of 10. *(Thermo Fisher Scientific UltiMate 3000).*

| No. | Ret. Time | Peak Rate | Height | Area | Rel. Area | Amount Type |
|-----|-----------|-----------|--------|------|-----------|-------------|
| 1   | 11.70     | -         | 95,45  | n.a. | n.a.      | n.a.        |
| 2   | 12.52     | -         | 139,752| 3,95 | 0,55      | n.a.        |
|     |           |           | 200,00 |      |           |             |

HPLC chromatogram of 11. *(Thermo Fisher Scientific UltiMate 3000).*

| No. | Ret. Time | Peak Rate | Height | Area | Rel. Area | Amount Type |
|-----|-----------|-----------|--------|------|-----------|-------------|
| 1   | 11.17     | -         | 95,48  | n.a. | n.a.      | n.a.        |
| 2   | 12.51     | -         | 60,268 | 4,85 | 4,60      | n.a.        |
|     |           |           | 100,00 |      |           |             |

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