Supporting Information

Inhibition of Histone Deacetylases 1, 2, And 3 Enhances Clearance of Cholesterol Accumulation in Niemann Pick C1 Fibroblasts

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Synthetic procedures for compounds and components described. Design and synthesis of modified HDAC 1, 2, and 3 inhibitors (computational modeling and design). Synthetic procedures for additional compounds and components tested, but not described in main text. Additional broad spectrum HDACi that clear cholesterol from NPC mutant fibroblasts and images.

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Abbreviations:

NPT, N=number of particles, P=pressure, T=temp

NVT, N=number of particles, V=volume, T=temp

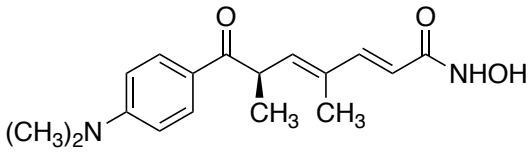
In these supplemental methods we describe the preparation and characterization of compounds and components developed at the University of Notre Dame presented in this study and others tested but found ineffective for cholesterol clearance (not shown) but that may prove informative for other studies.

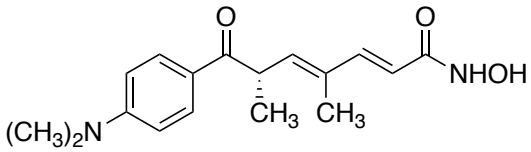
General Information

Reactions were generally conducted under an inert atmosphere of argon with oven-dried glassware. Crimp cap vials were purchased from MicroSolv. Reactions were monitored using thin layer chromatography (TLC) performed on silica gel 60 F254 aluminum-backed plates. Liquid chromatography was performed using a Biotage Isolera Prime, Version 1.5.2 flash purification system with Silicycle, Inc. SiliaSep 12-g cartridges (FLH-R10030B-ISO12). Tetrahydrofuran and toluene were purified by passage through a solvent purification system (Innovative Technology) and stored in a glovebox over 4Å molecular sieves. An assay sample of pyridin-3-ylmethyl (E)-(4-phenyl)phenyl)carbamoyl)benzyl)carbamate was obtained according to the literature procedure [1]. All other reagents were purchased from commercial sources and were used as received.

^{1}H and ^{13}C spectra were obtained using either a Bruker AVANCE III HD 400 instrument operating at 400 MHz and 100 MHz, respectively, or a Bruker AVANCE III HD 500 instrument operating at 500 MHz and 125 MHz, respectively. All ^{1}H and ^{13}C data are reported in ppm (δ) relative to the residual $CDCl_3$ peak at 7.26 ppm and 77.23 ppm or DMSO peak at 2.50 ppm and 39.52 ppm, respectively. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (*J*) (Hz), and integration. High resolution mass spectrometry (HRMS) was performed on a Bruker microTOF-Q II instrument. Infrared spectra were recorded on a Thermo Nicolet IR 200 spectrometer.

I. Synthetic Procedures For Compounds and Components Described in Text

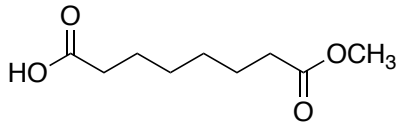




Racemic Trichostatin A (TSA) synthesis and chiral separation of *(R)*-TSA and *(S)*-TSA

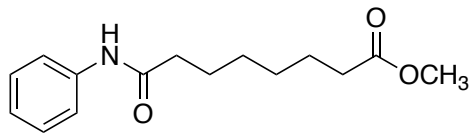
Racemic TSA was obtained via synthetic methods previously described [2]. Racemic TSA was purified using a Waters XBridge Prep C18 5 µm OBD column (19 x 50 mm) and water/acetonitrile gradient (10-90% ACN in water in 10 min, pH = 7; 14 min run, flow rate = 20 ml/min; racemic TSA RT = 7.0 min). With analytically pure racemic TSA in hand, a method was developed for the chiral separation. Racemic TSA was determined to be separable using a Daicel ChiralPAK AD-H 5 µm column (amylose tris(3,5-dimethylphenylcarbamate-coated 5 µm silica gel, 4.6 X 250 mm) and heptane/isopropanol gradient (10-90% IPA in heptane over 15 min, no additives; total 25 min run, flow rate = 1.0 ml/min). ***(S)***-TSA eluted at 14.64 min. ***(R)***-TSA eluted at RT = 15.77 min. These samples were collected and comparison of ***(R)***-TSA to the commercially available ***(R)***-TSA was performed on the same chromatography column. Commercially available ***(R)***-TSA was confirmed >95% enantiomeric purity under the same mobile phase conditions (***(S)***-TSA is not commercially available at the time of this report). Co-injection on chiral HPLC of commercially available ***(R)***-TSA and the isolated ***(R)***-TSA sample derived from the separated racemic mixture confirmed that a single enantiomer was isolated and matched known material. ^{1}H NMR and LCMS data conform to previously reported structural data [2]. Commercially available (*R*)-TSA was purchased from TCI AMERICA (Lot No. CZU7I-GC) for use in HPLC analysis.

SAHA and Precursors Synthesis



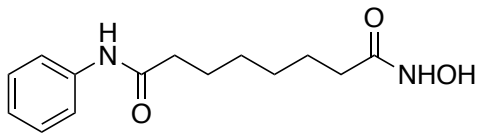
8-Methoxy-8-oxooctanoic acid (suberic acid monomethyl ester) (5.11) [3]

H_2SO_4 (2.5 mL) was added to a stirred solution of octanedioic acid (25 g, 144 mmol) in methanol (120 mL), and the mixture was heated at reflux for 12 h. After the mixture was cooled to 25 °C, the solvent was removed under reduced pressure. Ice water (200 mL) was added to the residue, and the mixture was extracted with diethyl ether (3 x 200 mL). The combined organic phases were washed with satd aq $NaHCO_3$ (150 mL) and brine (100 mL), dried with $MgSO_4$, and concentrated under reduced pressure to afford the diester as a colorless oil. Without further purification, the crude diester was dissolved in methanol (150 mL), and KOH (8.86 g, 158 mmol) was added. The solution was stirred at 25°C for 4 h. The solvent was removed under reduced pressure, and water and diethyl ether were added to the crude residue. The pH of the aq phase was adjusted to 3 with 10% aq HCl and then extracted with diethyl ether (3 x 150 mL). The combined organic phases were washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure. A sticky white solid was obtained, which was vigorously washed with hexanes and filtered. The filtrate was concentrated under reduced pressure to afford the title compound (10.54 g, 39%) as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.65 (s, 3H), 2.33 (t, J = 7.5 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.69 – 1.54 (m, 4H), 1.41 – 1.27 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.89, 174.51, 51.66, 34.09, 28.85, 28.78, 24.83, 24.60. ([3] ^{1}H NMR)



Methyl 8-oxo-8-(phenylamino)octanoate (11) [4].

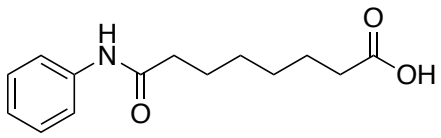
A dry flask containing the preceding suberic acid monomethyl ester (10.8 g, 57.2 mmol) was sealed, evacuated of air and refilled with argon (3 cycles). Aniline (5.8 mL, 63.5 mmol) was added, and the solution was cooled to 0 °C. 4-(*N,N*-Dimethylamino)pyridine (699 mg, 5.7 mmol) and *N*-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (12.1 g, 63.1 mmol) was added, and the system was flushed with argon. The reaction was warmed to 25 °C, and after 3.5 h, another portion of EDC (2.4 g, 12.5 mmol) was added. The mixture was stirred for an additional 1 h and then diluted with CH_2Cl_2 (125 mL). The solution was washed with 1M aq HCl (2 x 50 mL), satd aq $NaHCO_3$ (2 x 50 mL), and brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to provide slightly pink crystals (15.3 g, quantitative). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.8 Hz, 2H), 7.31 (t, *J* = 7.9 Hz, 2H), 7.30 (br s, 1H), 7.09 (t, *J* = 7.4 Hz, 1H), 3.66 (s, 3H), 2.38 – 2.27 (m, 4H), 1.78 – 1.70 (m, 2H), 1.68 – 1.59 (m, 2H), 1.46 – 1.31 (m, 4H). ([4] ^{1}H NMR, ^{13}C NMR)



Suberoylanilide hydroxamic acid (SAHA, Zolinza™, vorinostat) (12) [4]

The preceding methyl ester (15.3 g, 58.1 mmol) was dissolved in THF (41 mL) and MeOH (41 mL). 50 Wt% aq hydroxylamine (41 mL) and KCN (380 mg, 5.8 mmol) were added. The mixture was stirred for 15 h, and then another portion of KCN (80 mg, 1.2 mmol) was added. After an additional 10 h of stirring, the THF and MeOH were evaporated under reduced pressure, and water (400 mL) was added. The suspension was stirred for 30 min and filtered, and the resulting crystalline solid was dried in a vacuum oven for 7 h at 50°C. Acetonitrile (150 mL) and 29% aq NH_4OH (25 mL) were added to the solid at 25 °C. The suspension was heated to 55

°C, stirred at that temperature for 1.5 h, cooled to 25 °C, and stirred for another 1 h. The suspension was filtered and the resulting white crystalline solid was dried in a vacuum oven for 1 h at 50°C to give analytically pure vorinostat (11.0 g, 72%). ^{1}H NMR (400 MHz, DMSO) δ 9.86 (s, 1H), 7.58 (d, *J* = 7.7 Hz, 2H), 7.27 (t, *J* = 7.9 Hz, 2H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.24 (s, 2H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.94 (t, *J* = 7.4 Hz, 2H), 1.62 – 1.53 (m, 2H), 1.53 – 1.44 (m, 2H), 1.34 – 1.20 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 171.2, 169.0, 139.3, 128.6, 122.9, 119.0, 36.3, 32.3, 28.38, 28.37, 25.03, 24.99; HRMS (ES+ TOF) calcd for $C_{14}H_{21}N_2O_3$ [M + H] 265.1547, found 265.1561. ([4] ^{1}H NMR, ^{13}C NMR)



8-Oxo-8-(phenylamino)octanoic acid (inactive vorinostat precursor, SACA)[5]

A solution of octane-1,8-dioic acid (5.00 g, 28.7 mmol) in acetic anhydride (10 mL) was heated at reflux for 1 h. The mixture was cooled to 25 °C and concentrated under vacuum. The remaining yellow solid was recrystallized from acetonitrile to afford the cyclic anhydride (1.75 g; 20%) as a white solid, which was used in the next step. The anhydride was dissolved in anhyd THF (50 mL), and aniline (1.03 g, 1.01 mL; 11.1mmol) was added to the solution at 25 °C. After being stirred for 30 min, the mixture became cloudy white. Water (20 mL) was added, and the resulting white solid was isolated by filtration. Recrystallization from water gave 8-oxo-8-(phenylamino)octanoic acid (0.92 g, 33%) as a white powder. ^{1}H NMR (400 MHz, CH_3OD) δ 7.54 (d, 2H), 7.27 (t, 2H), 7.06 (m, 1H) 2.30 (m, 2H), 2.27 (m, 2H), 1.61 (m, 4H), 1.39 (m, 4H). ^{13}C NMR (126 MHz, CH_3OD) δ 176.43, 173.45, 138.72, 128.58, 120.08, 36.72, 33.67, 28.82,

28.75, 25.59, 24.75. HRMS *m/z* calcd for $C_{14}H_{20}NO_3$ (M+1) 250.1438; found 250.1457. ([5] ^{1}H NMR, ^{13}C NMR).

Design and Synthesis of Modified HDAC 1, 2, and 3 Inhibitors

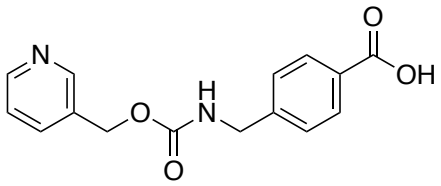
Computational Modeling and Design

Simulations of N-1,2-C and N-1,2-D were performed in both HDAC1 and HDAC3. The HDAC3 complexes were constructed using the crystal structure of HDAC3 (pdb 4A69, 2.06 Å resolution) [6] with each of the ligands docked using the GLIDE software from Schrodinger [7]. The HDAC1 complexes were constructed using the HDAC1 crystal structure (pdb 5ICN. 3.30 Å resolution) [8] and ligands docked in the same manner. Nonbonded parameters for the Zn^{2+} metal ion were determined using the MCPB.py module [9] in Amber18 [10] with the empirical ZAFF parameterization method [11]. The rest of the system was treated using the AMBER14SB force field [12] and GAFF2 [13] for the protein and ligands, respectively. Ligand geometries were initially optimized in Gaussian09 [14] using the M062X/6-31g(d) basis set and functional followed by charge optimization using M062X/6-31++g(d,p) and RESP charge fitting in the *antechamber* module of Amber18. The *pmemd* module of Amber18 was used for the Molecular Dynamics (MD) simulations, where the systems were solvated by a truncated octahedron of TIP3P waters which extended at least 15 Å away from the protein.

All MD simulations used the particle mesh Ewald method for treating long-range electrostatic interactions, a 10 Å cutoff for nonbonded van der Waals interactions, and periodic boundary conditions. Hydrogens were all constrained using the SHAKE algorithm and a timestep of 2 fs was used to integrate the equations of motion. The systems were minimized in 1000 step increments that gradually reduced restraints on the atoms. The system was then heated to 300 K over 30 ps, followed by equilibration for 10 ps, followed by an NPT equilibration for

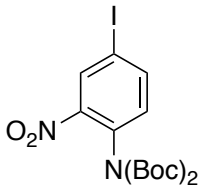
100 ps. The temperature and pressure were maintained using a Langevin thermostat and a Berendsen barostat with isotropic scaling, respectively. Each system was simulated for 25 ns in an NVT ensemble. Structure files in pdb format were generated using the cpptraj module in Amber18 and visualized in PyMOL (PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC). These MD simulations were used to refine the design of selective inhibitors based on the earlier analysis of the selectivity of BRD3227 [15-17]. We envisioned the replacement of the thiophene moiety of BRD3227 with both hydrogen bond acceptors (N-1,2-A) or hydrogen bond donors (N-1,2-C,D) with different linkers that positioned them at different positions in the 14 Å internal pockets [16] of HDACs. This internal pocket is known to have different sizes in HDAC1/2 vs. HDAC3 due to the replacement of a Ser113 with a Tyr. Supplemental Figure 3 shows the calculated models of compounds N-1,2-C (pink) and N-1,2-D (green) bound to HDAC1 (left) and HDAC3 (right). As can be seen, the *trans*-alkene in N-1,2-D positions the hydroxyl function to hydrogen bond with Ser113, while the geometry of the alkyne on N-1,2-C maintains shape complementarity with the internal pocket but does not allow for hydrogen bonding (Suppl. Fig. 3 left). As shown in Suppl. Fig 3 right, neither interaction is possible in the smaller internal pocket of HDAC3, which in fact leads to repulsive interaction. These results are consistent with the experimentally observed larger selectivity of N-1,2-D towards HDAC1/2 and the weak binding of either compound to HDAC3.

Synthesis of Modified Inhibitors



4-((((pyridin-3-ylmethoxy)carbonyl)amino)methyl)benzoic acid (1)[18]

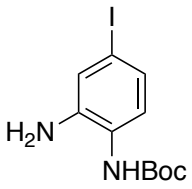
To a dry flask containing 1,1'-carbonyldiimidazole (12.54 g, 77.2 mmol) in dry THF (42 mL) at 0°C was added a solution of 3-pyridinemethanol (7.5 mL, 77.2 mmol) in dry THF (21 mL) under argon. The resulting mixture was warmed to 25 °C, stirred for 1 h, and poured into a slurry of 4-(aminomethyl)benzoic acid (11.68 g, 77.2 mmol) in dry THF (77 mL), dry triethylamine (10.8 mL, 77.2 mmol), and dry 1,8-diazabicyclo[5.4.0]undec-7-ene (11.6 mL, 77.2 mmol). The resulting suspension was flushed with argon and stirred for 4.5 h, and the THF and triethylamine were evaporated under reduced pressure. The resulting suspension was diluted with water (100 mL), and 6M HCl was added until a pH of *ca.* 3 was attained. The resulting precipitate was filtered and washed with water (20 mL) and MeOH (20 mL). KOH (s) was added to the filtrate until a pH of *ca.* 5 was attained, and another portion of precipitate was formed. The solid was filtered off, washed with water (20 mL) and MeOH (20 mL), combined with the previously obtained solid, and dried in a vacuum oven at 50 °C for 3 h. The resulting white crystalline solid (17.14 g, 78 %) corresponded analytically (^{1}H NMR) to a previous literature report [18].



1-[*N*,*N*-Bis(*tert*-butoxycarbonyl)amino]-4-iodo-2-nitrobenzene (HDAC-068A).

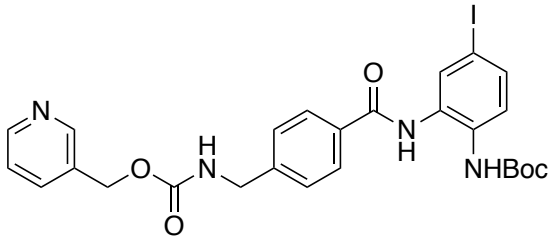
4-Iodo-2-nitroaniline (5.00 g, 18.9 mmol) and 4-dimethylaminopyridine (0.231 g, 1.89 mmol) were dissolved in dry THF (60 mL), and di-*tert*-butyl dicarbonate (4.55 g, 20.8 mmol) in dry THF (20 mL) was added dropwise at 0 °C over 4 h. The mixture was stirred at 0 °C and slowly warmed up to 25 °C for 20 h before being quenched with water (20 mL). The aq layer was extracted with EtOAc (4 x 30 mL). The combined organic layers were dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure. Recrystallization from chloroform

gave the di-Boc protected product (5.96 g). The mother liquid was purified via Biotage (20%-50% EtOAc in hexane; 25 g column) to give an additional 2.07 g of product. In total, 8.03 g (91%) was obtained as a yellow solid. mp: 124-125 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (d, J = 1.9 Hz, 1H), 7.94 (dd, J = 8.3, 2.0 Hz, 1H), 7.04 (d, J = 8.3 Hz, 1H), 1.40 (m, 18H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.13, 146.16, 142.89, 133.96, 133.30, 132.94, 92.41, 84.38, 27.99. HRMS (ESI) calcd for $C_{16}H_{21}N_2NaO_6$ $[M + Na]^+$ 487.0337, found 487.0347; IR (neat): 1763, 1724, 1524, 1108, 871 cm^{-1}



2-Amino-1-[*N*-(*tert*-butoxycarbonyl)amino]-4-iodobenzene (HDAC-078).

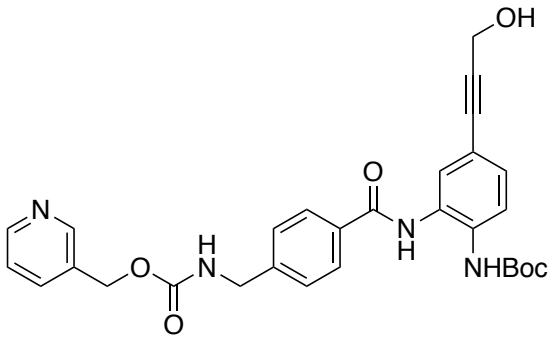
To a stirred mixture of the preceding di-Boc nitro derivative **HDAC-068A** (2.07 g, 4.46 mmol) in ethanol (50 mL) and water (10 mL) was added NH_4Cl (1.91 g, 35.7 mmol) and $FeCl_2$ (1.70 g, 13.4 mmol). Powdered zinc (0.875 g, 13.4 mmol) was then added, and the mixture was heated to 50 °C and stirred for 17 h. The mixture was cooled to 25 °C and filtered through Celite. The filter cake was washed with ethanol (20 mL), and the filtrate was concentrated under reduced pressure to give a residue, which was purified via Biotage (5% MeOH in dichloromethane, 10 g cartridge). The product **HDAC-078** was obtained as a white solid (539 mg, 36%, or 93% based on recovery of 781 mg of starting material **HDAC-068A**). **HDAC-078**: mp: 154-155 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.14 (s, 1H), 7.03 – 6.85 (m, 2H), 1.50 (s, 9H). ^{13}C NMR (126 MHz, MeOD) δ 156.25, 144.38, 127.99, 127.81, 126.11, 125.21, 90.65, 81.15, 28.65. HRMS (ESI) calcd for $C_{11}H_{16}IN_2O_2$ $[M + H]^+$ 335.0251, found 335.0253. IR (neat): 3403, 3339, 2977, 1680, 1587, 1503, 1488, 1411, 1307, 1298, 1246, 1157, 1054 cm^{-1}



Pyridin-3-ylmethyl (4-((2-((tert-butoxycarbonyl)amino)-5-iodophenyl)carbamoyl)benzyl)--carbamate (HDAC 076)

4-((((Pyridin-3-ylmethoxy)carbonyl)amino)methyl)benzoic acid (**1**, 120 mg, 0.418 mmol) and **HDAC-078** (127 mg, 0.380 mmol) were dissolved in dichloromethane (5 mL) under Ar, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (146 mg, 0.760 mmol) and 4-dimethylaminopyridine (4.64 mg, 0.038 mmol) were added. The mixture was stirred at 25 °C for 12 h. The solvent was removed under vacuum, and the residue was purified via Biotage (25 g cartridge, 0 ~ 8% MeOH in dichloromethane). The product **HDAC-076** was obtained as a yellow oil (161.5 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.40 (s, 1H), 8.57 (s, 1H), 8.53 (d, *J* = 4.0 Hz, 1H), 7.98 (d, *J* = 1.7 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.43 – 7.34 (m, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 2.5 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 5.78 (t, *J* = 5.6 Hz, 1H), 5.14 (s, 2H), 4.41 (d, *J* = 5.9 Hz, 2H), 1.48 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.62, 156.33, 154.40, 149.43, 149.40, 142.73, 135.96, 134.84, 134.17, 132.78, 132.09, 131.59, 130.43, 127.87, 127.44, 125.87, 123.48, 88.67, 81.51, 64.35, 44.69, 28.28. HRMS (ESI) calcd for $C_{26}H_{28}IN_4O_5$ $[M + H]^+$ 603.1104, found 603.1099. IR (neat): 3316, 1678, 1522, 1289, 1240, 1045, 872 cm^{-1}

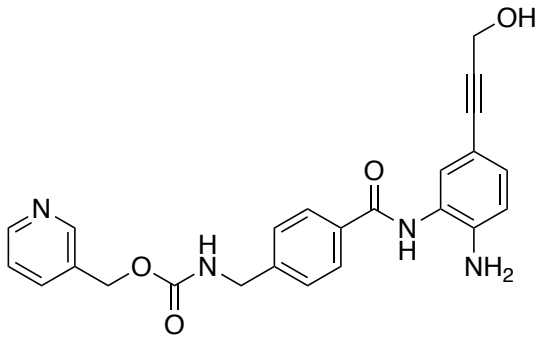
N-NC-A:



Pyridin-3-ylmethyl (4-((2-((tert-butoxycarbonyl)amino)-5-(3-hydroxyprop-1-yn-1-yl)phenyl)carbamoyl)benzyl)carbamate (HDAC-157)

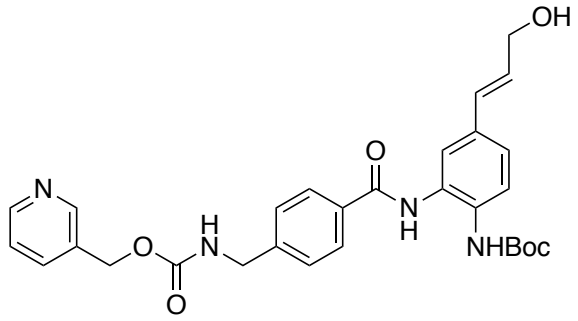
HDAC-076 (103 mg, 0.171 mmol), tetrakis(triphenylphosphine)palladium(0) (19.76 mg, 0.017 mmol), and copper(I) iodide (6.51 mg, 0.034 mmol) were placed in a 10 mL flask under Ar in the glove box and dissolved in DMF (5 mL). Diisopropylamine (0.048 mL, 0.342 mmol) and prop-2-yn-1-ol (0.049 mL, 0.855 mmol) were added in sequence. The mixture was stirred at 25 °C for 4.5 h before the solvent was removed under vacuum. The residue was purified via Biotage (0%-10% MeOH in dichloromethane; 10 g column). The product was obtained as a yellow oil (91 mg, 100%). ^{1}H NMR (500 MHz, MeOD): δ 8.57 (s, 1H), 8.48 (d, *J* = 3.8 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.66 (s, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.45 – 7.35 (m, 3H), 7.27 (dd, *J* = 8.4, 1.9 Hz, 1H), 5.17 (s, 2H), 4.39 (s, 2H), 4.38 (s, 2H), 1.48 (s, 9H). ^{13}C NMR (126 MHz, MeOD): δ 168.20, 158.62, 155.86, 149.62, 149.58, 145.21, 137.86, 134.95, 134.04, 133.48, 131.08, 130.52, 130.19, 128.92, 128.39, 125.20, 125.04, 120.74, 88.96, 84.66, 81.96, 64.93, 51.19, 45.16, 28.58. HRMS (ESI) calcd for $C_{29}H_{31}N_4O_6$ $[M + H]^+$ 531.2244; found 531.2240. IR (neat): 3258, 2977, 1698, 1516, 1235, 1148 cm^{-1}.

N-1,2-C:



Pyridin-3-ylmethyl (4-((2-amino-5-(3-hydroxyprop-1-yn-1-yl)phenyl)carbamoyl)benzyl)-carbamate (HDAC-118)

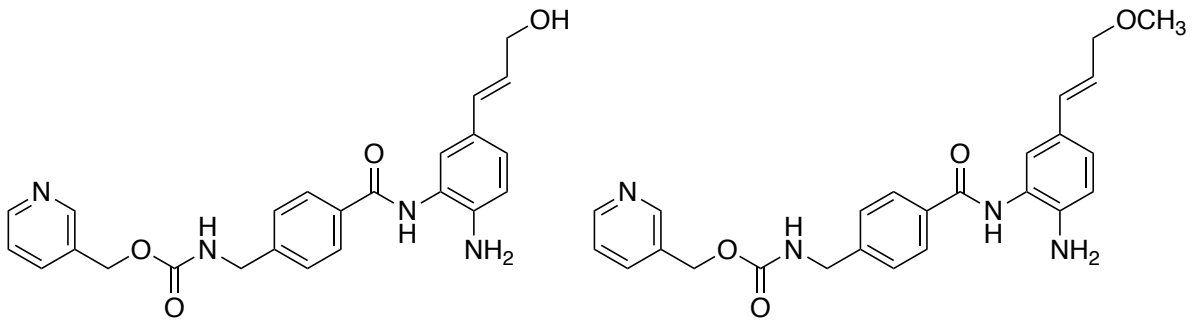
HDAC-157 (41.2 mg, 0.078 mmol) was dissolved with dichloromethane (5 mL) and TFA (0.2 mL). The mixture was stirred at 0 ºC and slowly warmed up to 25 °C for 6 h, before it was carefully quenched with satd aq $NaHCO_3$ (5 mL). The aq layer was extracted with dichloromethane (3 x 5 mL). The combined organic layers were dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified on a preparative silica TLC plate (20 cm x 20 cm) to give **HDAC-118** as a yellow oil (5.2 mg, 16%). ^{1}H NMR (600 MHz, MeOD) δ 8.58 (s, 1H), 8.49 (d, *J* = 4.1 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 2H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.45 (dd, *J* = 7.7, 5.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.27 (s, 1H), 7.13 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 5.18 (s, 2H), 4.38 (s, 2H), 4.36 (s, 2H); ^{13}C NMR (151 MHz, MeOD) δ 168.78, 158.64, 149.62, 149.57, 144.94, 144.87, 137.89, 134.98, 134.22, 132.05, 131.28, 129.09, 128.29, 125.22, 124.29, 117.84, 113.10, 86.41, 85.80, 64.93, 51.32, 45.19.; HRMS (ESI) calcd for $C_{24}H_{23}N_4O_4$ $[M + H]^+$ 431.1719, found 431.1677; IR (MeOH): 3677, 2968, 2866, 2357, 1452 cm^{-1}



Pyridin-3-ylmethyl (*E*)-(4-((2-((tert-butoxycarbonyl)amino)-5-(3-hydroxyprop-1-en-1-yl)phenyl)carbamoyl)benzyl)carbamate (HDAC-244)

HDAC-076 (68 mg, 0.113 mmol), (*E*)-3-(tributylstannyl)prop-2-en-1-ol (196 mg, 0.564 mmol), Pd(dppf)Cl_2 dichloromethane complex (9.22 mg, 0.011 mmol), lithium chloride (14.36 mg, 0.339 mmol), and DMF (4 mL) were placed in a sealed vial in a glove box, and the mixture was stirred at 25 °C for 15 h. The solvent was removed under vacuum, and the residue was purified on Biotage (25 g column, 0~8% MeOH in dichloromethane). Product **HDAC-244** was obtained as a yellow oil (40.2 mg, 67% yield). ^{1}H NMR (500 MHz, MeOD) δ 8.58 (s, 1H), 8.49 (d, *J* = 4.2 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 2H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.66 (s, 1H), 7.54 – 7.34 (m, 4H), 7.30 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.60 (d, *J* = 15.9 Hz, 1H), 6.36 (dt, *J* = 15.9, 5.5 Hz, 1H), 5.18 (s, 2H), 4.39 (s, 2H), 4.23 (dd, *J* = 5.5, 1.3 Hz, 2H), 1.49 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 168.16, 158.63, 156.17, 149.69, 149.65, 149.57, 149.54, 145.15, 137.87, 135.69, 134.97, 134.20, 132.20, 131.55, 130.42, 130.40, 128.88, 128.41, 125.50, 125.33, 125.20, 124.93, 81.76, 64.93, 63.58, 45.17, 28.62. HRMS (ESI) calcd for $C_{29}H_{33}N_4O_6$ $[M + H]^+$ 533.2372, found 533.2395; IR (MeOH): 3357, 2363, 1693, 1672, 1532, 1502, 1417, 1313, 1253, 1160 cm^{-1}

N-1,2-A and N-1,2-D:

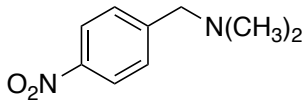


Pyridin-3-ylmethyl (*E*)-(4-((2-amino-5-(3-hydroxyprop-1-en-1-yl)phenyl)carbamoyl)benzyl)-carbamate (HDAC-245) and pyridin-3-ylmethyl (*E*)-(4-((2-amino-5-(3-methoxyprop-1-en-1-yl)phenyl)carbamoyl)-benzyl)carbamate (HDAC-245B)

To a vial of **HDAC-244** (40.2 mg, 0.075 mmol) was added 0.6M hydrogen chloride in MeOH (3 mL). The mixture was stirred at 25 °C for 2 h. The solvent was removed under vacuum, and the HCl salt was obtained. The substrate was dissolved in MeOH again and carefully neutralized to pH=7 with satd aq $NaHCO_3$. The crude material was loaded on 2 20-cm x 20-cm preparative silica TLC plates. The plates were developed with dichloromethane:MeOH = 8:1. The desired free amine/alcohol **HDAC-245** was obtained as a yellow oil (5.9 mg, 18%), and the methoxy derivative **HDAC-245B** was also obtained as a yellow solid (25.1 mg, 75%). **HDAC-245**: ^{1}H NMR (600 MHz, MeOD) δ 8.48 (s, 1H), 8.39 (d, *J* = 4.2 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.40 – 7.24 (m, 3H), 7.16 (s, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 6.39 (d, *J* = 15.8 Hz, 1H), 6.08 (dt, *J* = 15.7, 5.9 Hz, 1H), 5.08 (s, 2H), 4.28 (s, 2H), 4.08 (d, *J* = 5.5 Hz, 2H). ^{13}C NMR (151 MHz, MeOD) δ 168.70, 158.64, 149.62, 149.58, 144.83, 143.54, 137.89, 135.00, 134.33, 131.65, 129.17, 129.08, 128.30, 126.76, 126.58, 125.88, 125.22, 125.06, 118.57, 64.92, 63.95, 45.19. HRMS (ESI) calcd for $C_{24}H_{25}N_4O_4$ [M + H]$^{+}$ 433.1876, found 433.1907; IR (MeOH): 3319, 2939, 2829, 2466, 2068, 1450 cm^{-1}

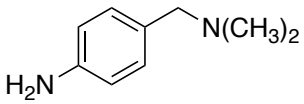
HDAC-245B: mp: 190-191 °C; ^{1}H NMR (500 MHz, MeOD) δ 8.57 (s, 1H), 8.49 (d, *J* = 4.1 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.51 – 7.36 (m, 3H), 7.28 (s, 1H), 7.17 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.51 (d, *J* = 15.8 Hz, 1H), 6.11 (dt, *J* = 15.8, 6.3 Hz, 1H), 5.17 (s, 2H), 4.38 (s, 2H), 4.04 (dd, *J* = 6.3, 0.9 Hz, 2H), 3.34 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 168.70, 158.63, 149.63, 149.56, 144.81, 143.85, 137.86, 134.27, 134.03, 129.08, 128.67, 128.30, 126.86, 126.10, 125.22, 124.94, 123.17, 118.47, 74.39, 64.90, 57.90, 49.85, 49.51, 49.34, 49.17, 49.16, 49.01, 49.01, 49.00, 48.99, 48.99, 48.98, 48.97, 48.85, 48.84, 48.84, 48.83, 48.82, 48.82, 48.81, 48.67, 48.67, 48.66, 48.65, 48.65, 48.49, 45.17. HRMS (ESI) calcd for $C_{25}H_{27}N_4O_4$ $[M + H]^+$ 447.2050, found 447.2027; IR (MeOH): 3319, 2359, 2353, 1684, 1650, 1615, 1530, 1494, 1427, 1256, 1117, 1049, 966 cm^{-1}

II. Synthetic Procedures for Additional Compounds and Components Tested, But Not Described in Text



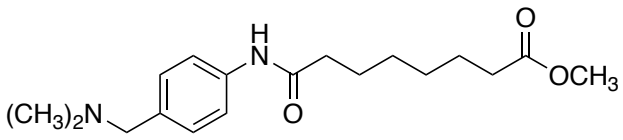
N,*N*-Dimethyl-1-(4-nitrophenyl)methanamine (5.7)[19]

4-Nitrobenzylbromide (8.0 g, 37 mmol), K_2CO_3 (15 g, 55 mmol), and acetonitrile (100 mL) were added to a round-bottom flask. The mixture was stirred at 0 °C and aq 2M dimethyl amine (24 mL, 48 mmol) was added dropwise. The mixture was stirred at 22 °C for 2 h, followed by removal of the solvent under reduced pressure. The crude material was dissolved in water and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford 6.45 g (97%) of the title compound as an orange liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.8 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 2H), 3.49 (s, 2H), 2.23 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.04, 129.58, 123.64, 63.65, 45.59. ([19] ^{1}H NMR)



4-((Dimethylamino)methyl)aniline (5.8)[20]

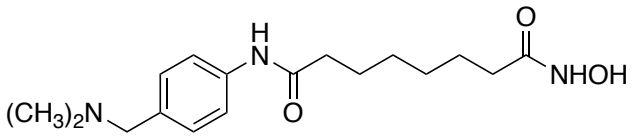
N,*N*-Dimethyl-1-(4-nitrophenyl)methanamine (1.25 g, 6.94 mmol) was dissolved in methanol (60 mL) and 37% aq HCl (1.5 mL, *ca.* 10 mmol) was added. The solution was evacuated and backfilled with Ar three times. Then, 5% Pd on C (125 mg) was added to the reaction solution, and the flask was evacuated and backfilled with Ar. H_2 was bubbled through the black mixture for 5 min, and then the mixture was placed under H_2 (1 atmos) and stirred for 2 h at 22 °C. Ar was then bubbled through the reaction mixture for 10 min. The mixture was filtered through Celite, and the filtrate was evaporated. Et_2O (20 mL) was added to afford a green solid, which was filtered and washed with cold Et_2O. The solid was dissolved in water, and satd aq Na_2CO_3 was added (20 mL). CH_2Cl_2 (50 mL) was added, and the two layers were separated. The aq layer was extracted with CH_2Cl_2 (4 x 50 mL), and the combined organic layers were dried with $MgSO_4$ and evaporated under reduced pressure to afford the title compound as an orange liquid (970 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 8.4 Hz, 2H), 3.62 (s, 2H), 3.32 (s, 2H), 2.22 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.71, 130.52, 128.74, 115.14, 64.05, 45.29. ([20] ^{1}H NMR).



Methyl 8-((4-((dimethylamino)methyl)phenyl)amino)-8-oxooctanoate (5.12)

To a flame-dried round-bottomed flask under an atmosphere of Ar was added 8-methoxy-8-oxooctanoic acid (1.36 g, 7.20 mmol) and anhyd DMF (32 mL). The solution was stirred at 25

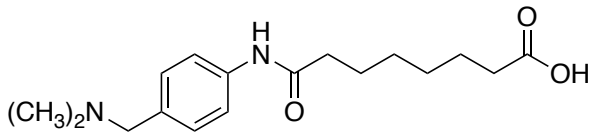
°C, and triethylamine (1.505 mL, 10.8 mmol) and EDCI (1.794 g, 9.36 mmol) were added. The solution was stirred for 5 min, and HOBT (1.26 g, 9.36 mmol) was added. After stirring for an additional 5 min, 4-((dimethylamino)methyl)aniline (1.41 g, 9.36 mmol) was added dropwise, and the solution was stirred for 16 h at 25 °C. The mixture was diluted with water and extracted with EtOAc (3x). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. Following purification via MPLC (5-25% MeOH in dichloromethane), 1.51 g (66%) of the title compound was obtained as yellow solid. mp = 39-41 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 2H), 3.66 (s, 3H), 3.43 (s, 2H), 2.38 – 2.23 (m, 10H), 1.78 – 1.57 (m, 4H), 1.44 – 1.29 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.23, 171.69, 137.48, 133.68, 129.71, 119.86, 63.60, 51.43, 44.99, 37.38, 33.95, 28.79, 28.77, 25.39, 24.71; HRMS (ESI) calcd for $C_{18}H_{28}N_2O_3$ [M + H] 321.2173, found 321.2164.



***N*1-(4-((Dimethylamino)methyl)phenyl)-*N*8-hydroxyoctanediamide (5.3)**

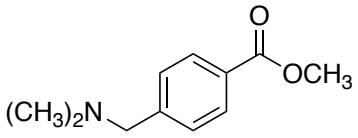
To a stirred solution of methyl 8-((4-((dimethylamino)methyl)phenyl)amino)-8-oxooctanoate (1.39 g, 4.34 mmol) in methanol (20 mL) was added DBU (1.94 ml, 13.01 mmol) at 22 °C. Then, 50% aq hydroxylamine (2.56 mL, 43.4 mmol) was added dropwise, and the mixture stirred for 2 h. The mixture was concentrated under reduced pressure and purified by MPLC (50-90% MeOH in dichloromethane) to afford 982 mg (70%) of the title compound as a white solid. mp = 41-43 °C; ^{1}H NMR (500 MHz, MeOD) δ 7.51 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 8.5 Hz, 2H), 3.40 (s, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 2.20 (s, 6H), 2.06 (t, *J* = 7.4 Hz, 2H), 1.74 –

1.51 (m, 4H), 1.45 – 1.27 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.40, 171.72, 138.16, 133.01, 129.98, 119.86, 63.14, 43.89, 36.68, 32.52, 28.72, 28.64, 25.54, 25.41; HRMS (ESI) calcd for $C_{17}H_{27}N_3O_3$ [M + H] 322.2125, found 322.2138.



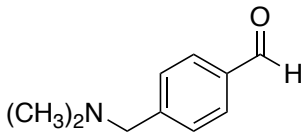
8-((4-((Dimethylamino)methyl)phenyl)amino)-8-oxooctanoic acid (inactive BBBP-SAHA)

To a flame-dried round-bottomed flask under an atmosphere of Ar was added suberic anhydride (182 mg, 1.165 mmol) and anhyd THF (5 mL). The mixture was heated slightly until the mixture became homogenous. The flask was cooled to 0 °C, and a solution 4-((dimethylamino)methyl)aniline (175 mg, 1.16 mmol) in anhyd THF (5 mL) was added dropwise. The solution was warmed to 22 °C and stirred for 12 h. To quench the residual starting amine, benzaldehyde (0.1 mL) was added, and the mixture stirred for 1 h at 25 °C. The mixture was concentrated under reduced pressure and purified by MPLC (35-90% MeOH in dichloromethane) to afford 187 mg (50%) of the title compound as a white solid. mp = 216-218 °C; ^{1}H NMR (400 MHz, CH_3OD) δ 7.56 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 3.52 (s, 2H), 2.36 (t, J = 7.6 Hz, 2H), 2.29 (s, 6H), 2.17 (t, J = 7.6 Hz, 2H), 1.77 – 1.55 (m, 4H), 1.46 – 1.31 (m, 4H); ^{13}C NMR (126 MHz, CH_3OD) δ 182.93, 174.73, 139.66, 133.20, 131.30, 121.08, 63.98, 44.74, 39.05, 37.97, 30.41, 30.12, 27.54, 26.88; HRMS (ESI) calcd for $C_{17}H_{26}N_2O_3$ [M + H] 307.2016, found 307.2040.



Methyl 4-((dimethylamino)methyl)benzoate (5.14)[21]

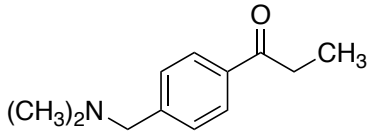
To a solution of methyl 4-(bromomethyl)benzoate 1 (5.00 g, 21.8 mmol) in THF (30 mL) was added dimethylamine (2*M* THF solution, 21.8 mL, 43.6 mmol) at 0 °C. The mixture was stirred for 12 h at 22 °C. The mixture was diluted with satd aq $NaHCO_3$ and EtOAc, and the layers were separated. The aq layer was again extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to afford the title compound as yellow oil (3.41 g, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H), 3.90 (s, 3H), 3.46 (s, 2H), 2.24 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.28, 144.62, 129.82, 129.11, 64.25, 52.25, 45.68. ([21] ^{1}H NMR, ^{13}C NMR)



4-((Dimethylamino)methyl)benzaldehyde (5.15)

To a suspension of $LiAlH_4$ (1.18 mg, 31.0 mmol) and anhyd Et_2O (10 mL) was added methyl 4-((dimethylamino)methyl)benzoate (3.00 g, 15.5 mmol) at 0 °C. The mixture was warmed and stirred at 22 °C for 1.5 h. After cooling the mixture was cooled to 0 °C, a solution of sodium tartrate was added slowly, and the mixture was stirred for 1 h. The mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The resulting residue was dissolved in $CHCl_3$ (10 mL), and MnO_2 (5.00 g, 57.5 mmol) was added. The mixture was heated and stirred at reflux for 4 h. After being cooled to 22 °C, the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure to afford 2.20 g (87%) of title compound as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H), 7.84 (d, J = 8.1 Hz, 2H),

7.49 (d, J = 7.9 Hz, 2H), 3.51 (s, 2H), 2.27 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.17, 146.25, 135.78, 130.02, 129.71, 64.19, 45.62; HRMS (ESI) calcd for $C_{10}H_{13}NO$ [M + H] 164.1070, found 164.1072.

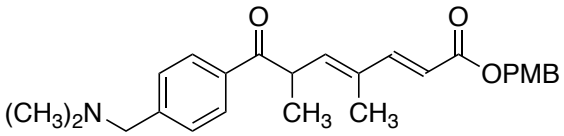


1-(4-((Dimethylamino)methyl)phenyl)propan-1-one (5.16)

Magnesium turnings (328 mg, 13.5 mmol) and a small crystal of iodine were placed into a two-neck, flame dried flask. The flask was flushed with argon, and THF (10 mL) was added. Bromoethane (1.01 mL, 13.5 mmol) was added slowly via syringe, and a vigorous reaction immediately ensued. The mixture was stirred for 1 h, during which time all the magnesium dissolved. 4-Dimethylaminobenzaldehyde (2.00 g, 12.3 mmol), dissolved in anhyd THF (4 mL) was added slowly via syringe at 0 ˚C. After the addition was complete, the mixture was stirred at 22 ˚C for 12 h. The reaction was quenched with satd aq NH_4Cl and extracted with Et_2O. The combined extracts were dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography (3% MeOH in CH_2Cl_2) to furnish 1.49 g (63%) of the corresponding alcohol as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 – 7.20 (m, 4H), 4.56 (t, J = 6.6 Hz, 1H), 3.39 (d, J = 1.4 Hz, 2H), 2.19 (s, 6H), 1.86 – 1.67 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H).

A flame-dried, three-neck flask containing anhyd CH_2Cl_2 (100 mL) was cooled to -78 ˚C followed by the addition of oxalyl chloride (0.61 mL, 7.07 mmol) and DMSO (0.84 mL, 11.8 mmol). The mixture was stirred for 15 min at -78 ˚C. Then a solution of 1-(4-((dimethylamino)methyl)phenyl)propan-1-ol (1.14 g, 5.90 mmol) in anhyd CH_2Cl_2 (10 mL) was added at -78 ˚C, and the mixture was stirred at -78 ˚C for 1 h. Then Et_3N (3.27 mL, 23.6 mmol)

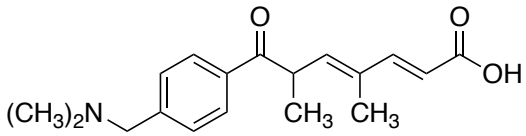
was added at -78°C, and the mixture was warmed to 22 °C and stirred for 3 h. Water was added, and the layers were separated. The aq layer was extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (5 mL) followed by the addition of aniline (0.10 mL) to remove residual aldehyde, and the mixture was stirred for 30 min at 22 °C. Following concentration under reduced pressure, purification by MPLC (2-6% MeOH in CH_2Cl_2) afforded the title compound as a yellow oil (0.85 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 3.48 (s, 2H), 3.00 (q, *J* = 7.3 Hz, 2H), 2.25 (s, 6H), 1.22 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.80, 144.40, 136.16, 129.33, 128.31, 64.19, 45.65, 32.01, 8.52; HRMS (ESI) calcd for $C_{12}H_{17}NO$ [M + H] 192.1383, found 192.1404.



4-Methoxybenzyl (2*E*,4*E*)-7-(4-((dimethylamino)methyl)phenyl)-4,6-dimethyl-7-oxohepta-2,4-dienoate (5.18)

Patterned after the procedure of Costner, *et al.* in a glove box, LiHMDS (107 mg, 0.64 mmol), THF (2.0 mL), and 1-(4-((dimethylamino)methyl)phenyl)propan-1-one (116 mg, 0.61 mmol) were added to a 10-mL crimp cap vial containing a magnetic stir bar and stirred in the glovebox at 22 °C for 15 min. Then, $ZnBr_2$ (205 mg, 0.91 mmol) was added to the Li enolate solution and stirred for 1 h at 22 °C. In a second 10-mL crimp cap vial, bis[2-(NHMe)-2'-Pd(OMs)biphenyl] (7 mg, 0.009 mmol), Q-Phos (13 mg, 0.018 mmol), 4-methoxybenzyl (2*E*,4*E*)-5-bromo-4-methylpenta-2,4-dienoate (208 mg, 0.67 mmol) and THF (0.5 mL) were added, and the vial was crimped. After the catalyst solution had turned dark purple, the Zn enolate solution was added dropwise to the catalyst solution and stirred at 22 °C for 1.5 h. The

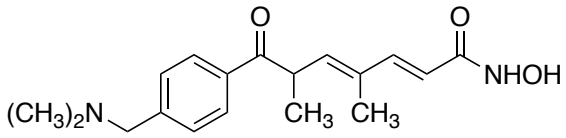
reaction mixture was taken out of the glovebox, diluted with 10% MeOH: 90% CH_2Cl_2 (10 mL), and passed through a silica plug. The plug was washed with 10% MeOH: 90% CH_2Cl_2 (20 mL), and the filtrate was concentrated. Purification of the residue by MPLC (2-15% MeOH in CH_2Cl_2) afforded the title compound (180 mg, 70%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.33 – 7.27 (m, 3H), 6.87 (d, *J* = 8.7 Hz, 2H), 5.98 (d, *J* = 9.7 Hz, 1H), 5.87 (d, *J* = 15.4 Hz, 1H), 5.10 (s, 2H), 4.41 (dq, *J* = 9.6, 6.8 Hz, 1H), 3.79 (s, 3H), 3.45 (s, 2H), 2.23 (s, 6H), 1.89 (d, *J* = 1.2 Hz, 3H), 1.31 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.44, 167.20, 159.85, 149.24, 145.00, 140.64, 135.35, 133.61, 130.30, 129.42, 128.69, 128.47, 117.29, 114.19, 66.21, 64.11, 55.50, 45.67, 41.89, 17.71, 12.76; HRMS (ESI) calcd for $C_{26}H_{31}NO_4$ [M + H] 422.2326, found 422.2349.



(2*E*,4*E*)-7-(4-((Dimethylamino)methyl)phenyl)-4,6-dimethyl-7-oxohepta-2,4-dienoic acid (5.22)

4-Methoxybenzyl (2*E*,4*E*)-7-(4-((dimethylamino)methyl)phenyl)-4,6-dimethyl-7-oxohepta-2,4-dienoate (190 mg, 0.45 mmol) was dissolved in CH_2Cl_2 (2 mL), and Et_3SiH (524 mg, 4.5 mmol) was added via syringe. Trifluoroacetic acid (2 mL) was then added via syringe at 22 °C. The solution was stirred and monitored by TLC (40% MeOH in CH_2Cl_2). Complete consumption of the starting ester was achieved after 15 min. The solvent was evaporated under reduced pressure, and the crude product was purified by MPLC (10-35% MeOH in CH_2Cl_2) to afford **5.22** as yellow oil (130 mg, 96%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.08 (d, *J* = 8.1 Hz, 2H), 7.72 (d, *J* = 8.2 Hz, 2H), 7.25 (d, *J* = 15.7 Hz, 1H), 5.94 (d, *J* = 9.7 Hz, 1H), 5.87 (d, *J* = 15.7 Hz, 1H), 4.64 (dq, *J* = 9.7, 6.8 Hz, 1H), 4.46 (s, 2H), 2.91 (s, 6H), 1.96 (d, *J* = 1.0 Hz, 3H),

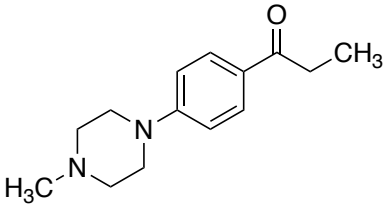
1.32 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 200.93, 169.29, 149.03, 139.75, 137.80, 134.87, 134.39, 131.39, 129.14, 117.42, 60.33, 42.13, 41.96, 16.47, 11.60; HRMS (ESI) calcd for $C_{18}H_{23}NO_3$ [M + H] 302.1754, found 302.1754.



(2*E*,4*E*)-7-(4-((Dimethylamino)methyl)phenyl)-N-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide (5.4)

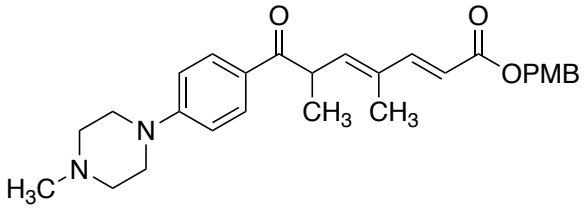
Under an atmosphere of Ar, (2*E*,4*E*)-7-(4-((dimethylamino)methyl)phenyl)-4,6-dimethyl-7-oxohepta-2,4-dienoic acid (27 mg, 0.09 mmol) was dissolved in anhyd THF (0.5 mL) and cooled to 0°C. Et_3N (25 μL, 0.18 mmol) was added via syringe followed by ethyl chloroformate (10 μL, 0.104 mmol). The mixture was stirred at 0 °C for 2 h, and H_2NOTBS (16 mg, 0.108 mmol) was added as a solution in anhyd THF (0.5 mL). The mixture was warmed to 22 °C and stirred for 1 h. The mixture was concentrated under reduced pressure, and the residue was purified by MPLC (2-20% MeOH in CH_2Cl_2) to give the *O*-TBS protected hydroxamate as a white solid (24 mg, 62%). Under an atmosphere of Ar, a portion of the protected hydroxamate (24 mg, 0.051 mmol) was dissolved in anhyd MeOH (1.5 mL), and CsF (15.5 mg, 0.102 mmol) was added. The mixture was stirred at 22 ˚C for 2 h, after which TLC (40% MeOH in CH_2Cl_2) indicated complete consumption of the protected material. The reaction mixture was diluted with CH_2Cl_2 (1 mL) and purified by MPLC (35-100% MeOH in CH_2Cl_2) to afford 13 mg (79% for the second step or 49% over two steps) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 15.5 Hz,

1H), 5.94 – 5.82 (m, 2H), 4.58 (dq, *J* = 9.4, 6.8 Hz, 1H), 3.52 (s, 2H), 2.23 (s, 6H), 1.92 (s, 3H), 1.29 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 201.46, 165.42, 144.11, 143.74, 138.58, 135.73, 134.00, 129.65, 128.45, 116.52, 63.33, 44.22, 41.66, 16.58, 11.57; HRMS (ESI) calcd for $C_{18}H_{24}N_2O_3$ [M + H] 317.1865, found 317.1864.



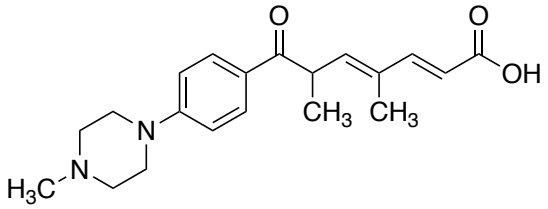
1-(4-(4-Methylpiperazin-1-yl)phenyl)propan-1-one (5.20)

To a flame-dried 35-mL bomb flask was added 1-(4-fluorophenyl)propan-1-one (2.00 g, 13.1 mmol) and 1-methylpiperazine (14.5 mL, 131 mmol). A stream of Ar was bubbled through the solution for 10 min. The flask was then sealed and heated to 120 °C for 20 h at 120 °C. The flask was cooled, and the solution was concentrated under reduced pressure to give a solid. The solid was purified by MPLC (10-25% MeOH in EtOAc) to afford 2.6 g (85%) of the title compound as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 3.47 – 3.28 (m, 4H), 2.93 (q, *J* = 7.3 Hz, 2H), 2.65 – 2.47 (m, 4H), 2.37 (s, 3H), 1.22 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.50, 154.24, 130.22, 127.54, 113.73, 54.98, 47.57, 46.35, 31.37, 8.88; HRMS (ESI) calcd for $C_{14}H_{20}N_2O$ [M + H] 233.1648, found 233.1677.



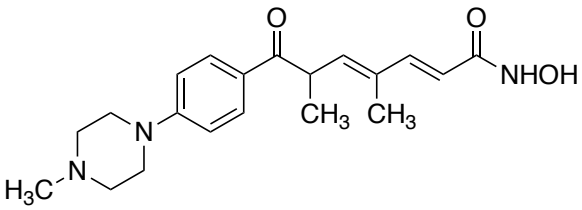
4-Methoxybenzyl (2E,4E)-4,6-dimethyl-7-(4-(4-methylpiperazin-1-yl)phenyl)-7-oxohepta-2,4-dienoate (5.21)

Patterned after the procedure of Cosner, *et al.* in a glove box, LiHMDS (286 mg, 1.71 mmol), 1-(4-(4-methylpiperazin-1-yl)phenyl)propan-1-one (379 mg, 1.63 mmol), and anhyd THF (4.0 mL) were added to a 10-mL crimp cap vial containing a magnetic stir bar. The vial was crimped and stirred in the glovebox at 22 °C for 20 min. In a second 10-mL crimp cap vial, bis[2-(NHMe)-2'-Pd(OMs)biphenyl] (12.5 mg, 0.016 mmol), Q-Phos (23.2 mg, 0.033 mmol), 4-methoxybenzyl (2*E*,4*E*)-5-bromo-4-methylpenta-2,4-dienoate (558 mg, 1.79 mmol) and anhyd THF (2 mL) were added, and the vial was crimped. The lithium enolate solution was added dropwise to the purple catalyst solution and stirred at 22 °C for 10 min. The reaction mixture was taken out of the glovebox, diluted with 10% MeOH: 90% CH_2Cl_2 (10 mL), and passed through a silica plug. The plug was washed with MeOH: 90% CH_2Cl_2 (30 mL), and the filtrate concentrated under reduced pressure. Purification by MPLC (silica gel, CH_2Cl_2/MeOH 1% to 15%) afforded the title compound (708 mg, 94%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 9.1 Hz, 2H), 7.38 – 7.29 (m, 3H), 6.95 – 6.81 (m, 4H), 6.02 (d, *J* = 9.6 Hz, 1H), 5.87 (d, *J* = 15.6 Hz, 1H), 5.13 (s, 2H), 4.37 (dq, *J* = 9.6, 6.8 Hz, 1H), 3.81 (s, 3H), 3.44 – 3.30 (m, 4H), 2.63 – 2.48 (m, 4H), 2.36 (s, 3H), 1.91 (d, *J* = 1.2 Hz, 3H), 1.31 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.76, 167.26, 159.84, 154.38, 149.51, 141.70, 133.06, 130.72, 130.27, 128.55, 126.37, 116.95, 114.19, 113.64, 66.16, 55.52, 54.94, 47.39, 46.34, 41.24, 17.82, 12.71; HRMS (ESI) calcd for $C_{28}H_{34}N_2O_4$ [M + H] 463.2591, found 463.2592.



(2*E*,4*E*)-4,6-Dimethyl-7-(4-(4-methylpiperazin-1-yl)phenyl)-7-oxohepta-2,4-dienoic acid (5.23)

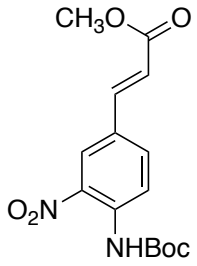
4-Methoxybenzyl (2*E*,4*E*)-4,6-dimethyl-7-(4-(4-methylpiperazin-1-yl)phenyl)-7-oxohepta-2,4-dienoate (600 mg, 1.3 mmol) was dissolved in CH_2Cl_2 (5 mL), and Et_3SiH (2.07 mL, 13.0 mmol) was added via syringe. Trifluoroacetic acid (5 mL) was added via syringe at 22 °C, and the solution was stirred for 10 min. The solvent was evaporated under reduced pressure, and the crude product was purified by MPLC (10-35% MeOH in DMC) to afford **5.23** as yellow solid (427 mg, 96%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (d, *J* = 9.1 Hz, 2H), 7.18 (d, *J* = 15.6 Hz, 1H), 7.02 (d, *J* = 9.1 Hz, 2H), 5.91 – 5.85 (m, 2H), 4.54 (dq, *J* = 9.6, 6.8 Hz, 1H), 3.64 – 3.48 (m, 4H), 3.11 – 2.96 (m, 4H), 2.68 (s, 3H), 1.94 (s, 3H), 1.28 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 200.19, 169.39, 153.25, 149.29, 140.92, 133.67, 130.59, 127.70, 117.09, 114.54, 53.01, 44.89, 42.45, 41.05, 16.86, 11.53; HRMS (ESI) calcd for $C_{20}H_{26}N_2O_3$ [M + H] 343.2016, found 343.2028.



(2*E*,4*E*)-*N*-Hydroxy-4,6-dimethyl-7-(4-(4-methylpiperazin-1-yl)phenyl)-7-oxohepta-2,4-dienamide (5.5)

(2*E*,4*E*)-4,6-Dimethyl-7-(4-(4-methylpiperazin-1-yl)phenyl)-7-oxohepta-2,4-dienoic acid (260 mg, 0.759 mmol) was dissolved in anhyd DMF (4 mL) and cooled to 0 °C. Et_3N (212 μL, 1.519 mmol) was added via syringe followed by ethyl chloroformate (83 μL, 0.873 mmol). The

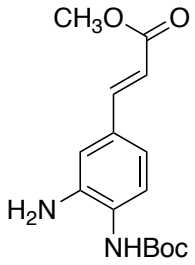
mixture was stirred at 0 °C for 2 h, and H_2NOTBS (134 mg, 0.911 mmol) was added as a solution in anhyd DMF (4 mL). The mixture was warmed to 22 °C and stirred for 1 h. The mixture was purified by MPLC (2-20% MeOH in CH_2Cl_2) to give the *O*-TBS protected hydroxamate as a white solid (164 mg, 46%). The protected hydroxamate (9 mg, 0.019 mmol) was dissolved in anhyd MeOH (0.5 mL), and CsF (5.8 mg, 0.038 mmol) was added. The mixture was stirred at 22 ˚C for 2 h, after which TLC (40% MeOH in CH_2Cl_2) indicated complete consumption of the protected material. The reaction mixture was diluted with CH_2Cl_2 (1 mL) and purified by MPLC (15-100% MeOH in CH_2Cl_2) to afford 5 mg (73% for the second step or 34% over two steps) of the title compound as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 (d, *J* = 9.0 Hz, 2H), 7.15 (d, *J* = 15.5 Hz, 1H), 6.96 (d, *J* = 9.1 Hz, 2H), 5.87 (m, 2H), 4.52 (dq, *J* = 13.6, 6.8 Hz, 1H), 3.45 – 3.35 (m, 4H), 2.64 – 2.52 (m, 4H), 2.33 (s, 3H), 1.91 (s, 3H), 1.26 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 200.41, 165.52, 154.70, 139.41, 133.45, 130.53, 126.19, 116.34, 113.49, 54.50, 46.70, 44.92, 40.83, 16.92, 11.54; HRMS (ESI) calcd for $C_{20}H_{27}N_3O_3$ [M + H] 358.2125, found 358.2123.



(*E*)-Methyl 3-(4-((*tert*-butoxycarbonyl)amino)-3-nitrophenyl)acrylate (3)

To a flask containing K_2CO_3 (108 mg, 0.78 mmol) and *n*-Bu_4NI (116 mg, 0.31 mmol) was added DMF (1.25 mL) and water (0.12 mL). Argon was bubbled through the suspension for 25 min followed by the addition of PPh_3 (16 mg, 0.06 mmol), 4-bromo-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (101 mg, 0.32 mmol), and methyl acrylate (85 mL, 0.94

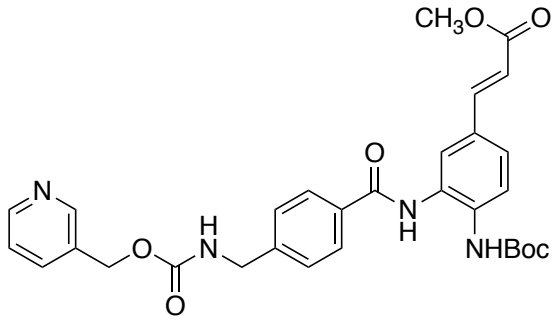
mmol). Argon was bubbled through the suspension for 15 min, and $Pd(OAc)_2$ (9 mg, 0.04 mmol) was added. The mixture was heated to 120 °C for 30 min. After the mixture was cooled to 25 °C, satd aq $NaHCO_3$ (5 mL) was added. The mixture was extracted with EtOAc (3 x 10 mL), and the combined extracts were washed with brine (10 mL), dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure to a brown DMF solution. The crude product was purified on a Biotage KP-Sil 10 g column eluted with hexanes/EtOAc (4:1, 200 mL) followed by hexanes/EtOAc (3:1, 200 mL). After evaporation of the solvents, the product was obtained as a light yellow crystalline solid (51 mg, 51%). Rf = 0.5 (hexanes/EtOAc, 3:1); IR 3372, 3076, 2986, 1736, 1712, 1640, 1619, 1568, 1520, 1435, 1343, 1146 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.77 (s, 1H), 8.63 (d, *J* = 8.9 Hz, 1H), 8.33 (d, *J* = 2.1 Hz, 1H), 7.75 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.63 (d, *J* = 16.0 Hz, 1H), 6.44 (d, *J* = 16.0 Hz, 1H), 3.81 (s, 3H), 1.55 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 152.0, 141.9, 137.3, 135.9, 134.4, 128.5, 125.7, 121.1, 119.2, 82.6, 52.0, 28.3.; HRMS (ES+ TOF) calcd for C15H19N2O6 [M + H] 323.1238, found 323.1257.



(*E*)-methyl 3-(3-amino-4-((*tert*-butoxycarbonyl)amino)phenyl)acrylate (4)

CH_2Cl_2 (1.3 mL) was added to a flask containing the previous nitro derivative **3** (341 mg, 1.06 mmol) and NH_4Cl (68 mg, 1.19 mmol). The resulting suspension was diluted with EtOH (5.3 mL and H_2O (2.7 mL), and argon was bubbled through the slurry for 15 min. Iron powder (295 mg, 5.28 mmol) was added, and argon was bubbled through the slurry for another 20 min.

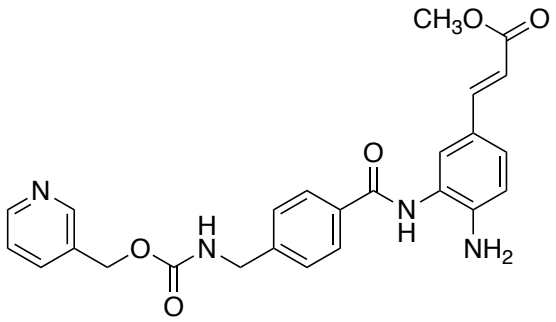
The reaction was heated to reflux and stirred for 35 min, cooled to 25 °C, and diluted with EtOAc (40 mL). The mixture was washed with brine (20 mL), dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure to give a yellow solid. The crude product was purified on a Biotage KP-Sil 25 g column eluted with hexanes/EtOAc 9:1 (200 mL) followed by hexanes/EtOAc 3:1 (200 mL) and hexanes/EtOAc 2:1 (300 mL). The product was collected as a light orange crystalline solid (240 mg, 71%). Rf = 0.4 (hexanes/EtOAc 1:1); IR 3410, 3331, 2980, 1715, 1680, 1640, 1586, 1521, 1499, 1437, 1155 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, *J* = 16.0 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 6.99 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.93 (d, *J* = 1.8 Hz, 1H), 6.40 (br s, 1H), 6.31 (d, *J* = 16.0 Hz, 1H), 3.79 (s, 3H), 3.70 (br s, 2H), 1.51 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 153.5, 144.7, 139.0, 131.7, 127.9, 123.7, 120.5, 117.3, 116.9, 81.1, 51.7, 28.4; HRMS (ES+ TOF) calcd for $C_{15}H_{21}N_2O_4$ [M + H] 293.1496, found 293.1514.



(*E*)-methyl 3-(4-((*tert*-butoxycarbonyl)amino)-3-(4-((((pyridin-3-ylmethoxy)carbonyl)amino)methyl)benzamido)phenyl)acrylate (5).

An oven dried flask, containing acid **1** (215 mg, 0.75 mmol) and amine **4** (200 mg, 0.68 mmol) was sealed, evacuated of air, and refilled with argon (3 cycles). Dry pyridine (6.8 mL) and benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 365 mg, 0.83 mmol) were added at 25 °C. The reaction had not reached completion after 24 h, and another portion of BOP (181 mg, 0.41 mmol) was added. The mixture was stirred for another 22

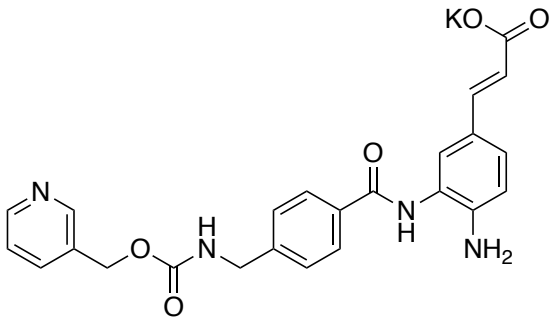
h and then concentrated to a yellow oil. The oil was dissolved in EtOAc (20 mL), washed with satd aq $NaHCO_3$ (15 mL) and brine (5 x 15 mL), dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure to afford a yellow solid. The crude product was purified on a Biotage KP-Sil 25 g column eluted with CH_2Cl_2/MeOH 98:2. The product was collected as an off-white crystalline solid (210 mg, 55%). Rf = 0.5 (CH_2Cl_2/MeOH 9:1); IR 3302, 2979, 2949, 1698, 1608, 1587, 1518, 1237, 1152 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.82 (s, 1H), 8.61 (br s, 1H), 8.55 (br s, 1H), 8.02 – 7.91 (m, 3H), 7.83 (d, *J* = 2.0 Hz, 1H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.63 (d, *J* = 16.0 Hz, 1H), 7.59 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.46 – 7.37 (m, 3H), 6.54 (d, *J* = 16.0 Hz, 1H), 5.12 (s, 2H), 4.31 (d, *J* = 6.2 Hz, 2H), 3.72 (s, 3H), 1.46 (s, 9H); ^{13}C NMR (101 MHz, DMSO) δ 166.6, 165.5, 156.2, 153.0, 149.1, 149.1, 143.8, 143.7, 136.2, 135.7, 134.4, 132.71, 132.66, 129.4, 129.1, 127.8, 126.9, 126.7, 125.8, 123.5, 122.9, 116.9, 80.0, 66.9, 63.3, 51.4, 43.6, 28.0; HRMS (ES+ TOF) calcd for $C_{30}H_{33}N_4O_7$ [M + H] 561.2344, found 561.2340.



(*E*)-methyl 3-(4-amino-3-(4-((((pyridin-3-ylmethoxy)carbonyl)amino)methyl)benzamido)phenyl)acrylate (6)

Boc-protected amine **5** (59 mg, 0.10 mmol) was dissolved in CH_2Cl_2 (0.7 mL) and cooled to 0 °C, and TFA (0.3 mL) was added dropwise. The solution was warmed to 25 °C, stirred for 30 min, and diluted with CH_2Cl_2 (5 mL). 1M aq KOH (7 mL) was added, and the phases were

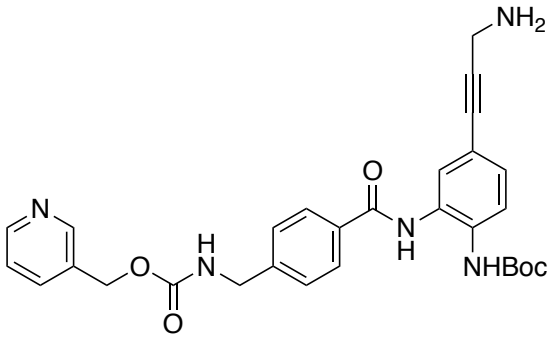
separated. The aq phase was extracted with EtOAc (2 x 5 mL), and the combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to a give a yellow crystalline solid. The crude product was purified on a Biotage KP-Sil 10 g column eluted with CH_2Cl_2/MeOH 9:1. The product was collected as an off white crystalline solid (38 mg, 79%), which turned yellow upon storage. Rf = 0.4 (CH_2Cl_2/MeOH 9:1); IR 3426, 3320, 3033, 2946, 1688, 1644, 1626, 1593, 1493, 1258, 1154 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 9.64 (s, 1H), 8.60 (s, 1H), 8.54 (d, *J* = 4.6 Hz, 1H), 7.98 (t, *J* = 6.2 Hz, 1H), 7.96 – 7.92 (m, 2H), 7.79 (dt, *J* = 7.9, 2.0 Hz, 1H), 7.51 (d, *J* = 15.9 Hz, 1H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.42 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.40 – 7.34 (m, 3H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.27 (d, *J* = 15.9 Hz, 1H), 5.60 (br s, 2H), 5.10 (s, 2H), 4.28 (d, *J* = 6.2 Hz, 2H), 3.67 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 167.2, 165.5, 156.2, 149.6, 149.5, 149.1, 149.1, 146.4, 145.1, 143.2, 135.7, 133.1, 132.7, 127.9, 127.6, 126.7, 123.5, 122.7, 121.8, 115.5, 111.8, 66.9, 63.2, 51.0, 43.6; HRMS (ES+ TOF) calcd for $C_{25}H_{25}N_4O_5$ [M + H] 461.1819, found 461.1799.



Potassium (*E*)-3-(4-amino-3-(4-((((pyridin-3-ylmethoxy)carbonyl)amino)methyl) benzamido)phenyl)acrylate (7).

An oven-dried flask containing the methyl acrylate derivative **6** (24 mg, mmol) was sealed, evacuated of air, and refilled with argon (3 cycles). Anhyd THF (1 mL) and potassium trimethylsilanoate (16 mg, mmol) were added. The mixture was stirred for 22 h, at which point a

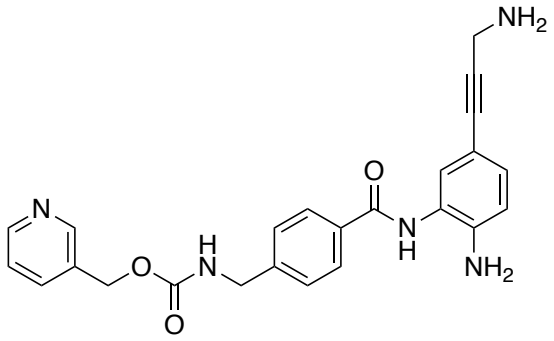
yellow precipitate could be observed. The solvent was removed with a pipette, and residual THF was evaporated under reduced pressure. The resulting solid was dissolved in methanol (0.5 mL) and precipitated by slow addition of THF. The mother liquor was removed by pipette, and the off-white precipitate was dried under reduced pressure to give the desired potassium carboxylate (18 mg, 74%). ^{1}H NMR (500 MHz, methanol-d_4) δ 8.58 (s, 1H), 8.50 (d, J = 4.9 Hz, 1H), 7.99 – 7.91 (m, 2H), 7.89 (d, J = 7.9 Hz, 1H), 7.53 – 7.25 (m, 7H), 6.87 (d, J = 8.3 Hz, 1H), 6.33 (d, J = 15.8 Hz, 1H), 5.19 (s, 2H), 4.39 (s, 2H); HRMS (ES+ TOF) calcd for $C_{24}H_{23}N_4O_5$ [M - K + 2H] 447.1663, found 447.1662.



Pyridin-3-ylmethyl (4-((5-(3-aminoprop-1-yn-1-yl)-2-((tert-butoxycarbonyl)amino)phenyl)-carbamoyl)benzyl)carbamate (HDAC-093)

HDAC-076 (100 mg, 0.166 mmol), $Pd(dppf)_2Cl_2 \cdot$ dichloromethane complex (13.56 mg, 0.017 mmol), and copper(I) iodide (6.32 mg, 0.033 mmol) were placed in a 10 mL flask and degassed with Ar. Prop-2-yn-1-amine (0.054 mL, 0.830 mmol) was added. The system was stirred at 25 °C for 24 h. The solvent was removed under vacuum and the residue purified by Biotage (10 g cartidge, 0~30% MeOH in dichloromethane). The product **HDAC-093** was obtained as a yellow oil (65.9 mg, 75%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.57 (s, 1H), 8.48 (s, 1H), 7.96 – 7.80 (m, 3H), 7.76 (s, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.48 – 7.37 (m, 3H), 7.34 (dd, J = 8.4, 1.8 Hz, 1H), 5.17 (s, 2H), 4.38 (s, 2H), 4.03 (s, 2H), 1.48 (s, 10H), ^{13}C NMR (151 MHz, CD_3OD) δ 166.83, 157.21, 154.38, 148.20, 148.14, 143.90, 136.50, 133.54, 132.96, 132.54,

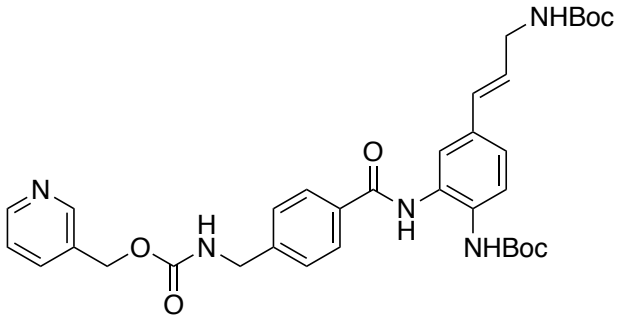
129.65, 129.30, 129.20, 127.51, 127.01, 123.85, 123.58, 117.55, 85.61, 80.72, 80.22, 63.53, 48.04, 43.74, 29.50, 27.18. HRMS (ESI) calcd for $C_{29}H_{32}N_5O_5$ $[M + H]^+$ 530.2398, found 530.2382; IR (MeOH): 3713, 3300, 2956, 2360, 1702, 1156 cm^{-1}



Pyridin-3-ylmethyl (4-((2-amino-5-(3-aminoprop-1-yn-1-yl)phenyl)carbamoyl)benzyl)-carbamate (HDAC-212)

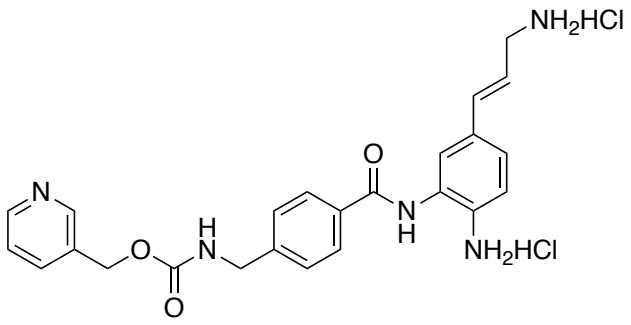
HDAC-093 (88 mg, 0.166 mmol) was dissolved in dichloromethane (6 mL) and TFA (2 mL). The mixture was stirred at 25 °C for 10 h and then carefully quenched with satd aq $NaHCO_3$ (3 mL). A precipitate formed, the liquid phase was removed, and the precipitate was dissolved in MeOH (3 mL). The aq layer was separated and extracted with dichloromethane (5 x 5 mL) and EtOAc (5 x 5 mL). The combined organic phases were dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified on a 20-cm x 20-cm preparative silica TLC plate using dichloromethane:MeOH = 5:1. The desired product **HDAC-212** was obtained as a yellow oil (30 mg, 42%). ^{1}H NMR (400 MHz, MeOD) δ 8.56 (s, 1H), 8.48 (d, *J* = 3.8 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 2H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.50 – 7.27 (m, 4H), 7.14 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 5.17 (s, 2H), 4.37 (s, 2H), 3.84 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.91, 163.29, 162.95, 158.67, 149.63, 149.56, 145.52, 144.89, 137.83, 134.93, 134.19, 132.21, 131.65, 129.08, 128.28, 125.20, 124.15, 122.60, 119.69, 117.69, 116.78, 113.87, 111.84, 86.96, 81.67, 64.92, 45.18, 31.28. HRMS (ESI) calcd for

$C_{24}H_{24}N_5O_3$ $[M + H]^+$ 430.1874, found 430.1870; IR (MeOH): 3065, 2862, 2491, 2069, 1120 cm^{-1}



Pyridin-3-ylmethyl (*E*)-(4-((2-((tert-butoxycarbonyl)amino)-5-(3-((tert-butoxycarbonyl)amino)-prop-1-en-1-yl)phenyl)carbamoyl)benzyl)carbamate (HDAC-191)

HDAC-076 (103 mg, 0.171 mmol), *tert*-butyl (*E*)-(3-(tributylstannyl)allyl)carbamate (336.2 mg, 0.753 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II)-(dichloromethane) (13.96 mg, 0.017 mmol), lithium chloride (8.70 mg, 0.205 mmol), and THF (4 mL) were placed in a septum-sealed vial and heated at 80 °C for 10 h. The solvent was removed under vacuum, and the residue was loaded on Biotage (10 g cartridge, 0~10% MeOH in dichloromethane). **HDAC-191** (93.5 mg, 87 %) was obtained as a yellow oil. ^{1}H NMR (600 MHz, MeOD) δ 8.57 (s, 1H), 8.49 (d, *J* = 4.2 Hz, 1H), 7.91 (t, *J* = 7.9 Hz, 2H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.62 (s, 1H), 7.48 – 7.34 (m, 4H), 7.26 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.49 (d, *J* = 15.9 Hz, 1H), 6.20 (dt, *J* = 15.8, 5.8 Hz, 1H), 5.18 (s, 2H), 4.38 (s, 2H), 3.81 (d, *J* = 5.1 Hz, 2H), 1.48 (s, 9H), 1.46 (s, 9H).^{13}C NMR (151 MHz, MeOD) δ 168.11, 158.61, 158.37, 156.14, 149.61, 149.57, 145.13, 137.87, 135.57, 134.94, 134.15, 132.13, 131.48, 130.81, 128.87, 128.39, 128.02, 125.26, 125.20, 124.83, 81.75, 80.15, 64.92, 45.16, 43.24, 28.79, 28.63. HRMS (ESI) calcd for $C_{34}H_{42}N_5O_7$ $[M + H]^+$ 632.3084, found 632.3078; IR (MeOH): 3304, 2939, 2826, 2360, 2341, 1453, 1417 cm^{-1}



Pyridin-3-ylmethyl (E)-(4-((2-amino-5-(3-aminoprop-1-en-1-yl)phenyl)carbamoyl)benzyl)-carbamate dihydrochloride (HDAC-219)

In a vial **HDAC-191** (55.3 mg, 0.104 mmol) was dissolved in MeOH (2 mL), and 3M hydrogen chloride in EtOAc (1 mL) was added. The mixture was stirred at 25 °C for 3 h. The solvent was removed under vacuum, and the desired HCl salt was obtained, which was neutralized with satd aq $NaHCO_3$. The crude material was loaded on a 20-cm x 20-cm preparative silica TLC plate. The plate was developed using dichloromethane:MeOH = 5:1. The desired salt was formed by addition of 0.6M HCl in MeOH (1 drop), and the solvent was removed under vacuum to give **HDAC-219** as a yellow oil (16.8 mg, 32%). ^{1}H NMR (500 MHz, MeOD) δ 8.93 (s, 1H), 8.85 (d, *J* = 5.2 Hz, 1H), 8.69 (d, *J* = 8.1 Hz, 1H), 8.22 – 8.13 (m, 1H), 8.07 (d, *J* = 8.2 Hz, 2H), 7.61 (d, *J* = 1.6 Hz, 1H), 7.55 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.53 – 7.42 (m, 3H), 6.86 (d, *J* = 15.8 Hz, 1H), 6.49 – 6.33 (dt, *J*= 15.8, 6.7 Hz, 1H), 5.36 (s, 2H), 4.41 (s, 2H), 3.75 (d, *J* = 6.7 Hz, 2H). ^{13}C NMR (126 MHz, MeOD) δ 168.90, 158.05, 146.97, 145.47, 142.26, 141.84, 139.76, 135.48, 133.14, 129.54, 128.58, 128.46, 126.74, 125.88, 125.29, 123.90, 63.49, 45.26, 42.31. HRMS (ESI) calcd for $C_{24}H_{26}N_5O_3$ $[M -2HCl + H]^+$ 432.2036, found 432.2101; IR (MeOH): 3317, 2939, 2829, 1447, 1409 cm^{-1}

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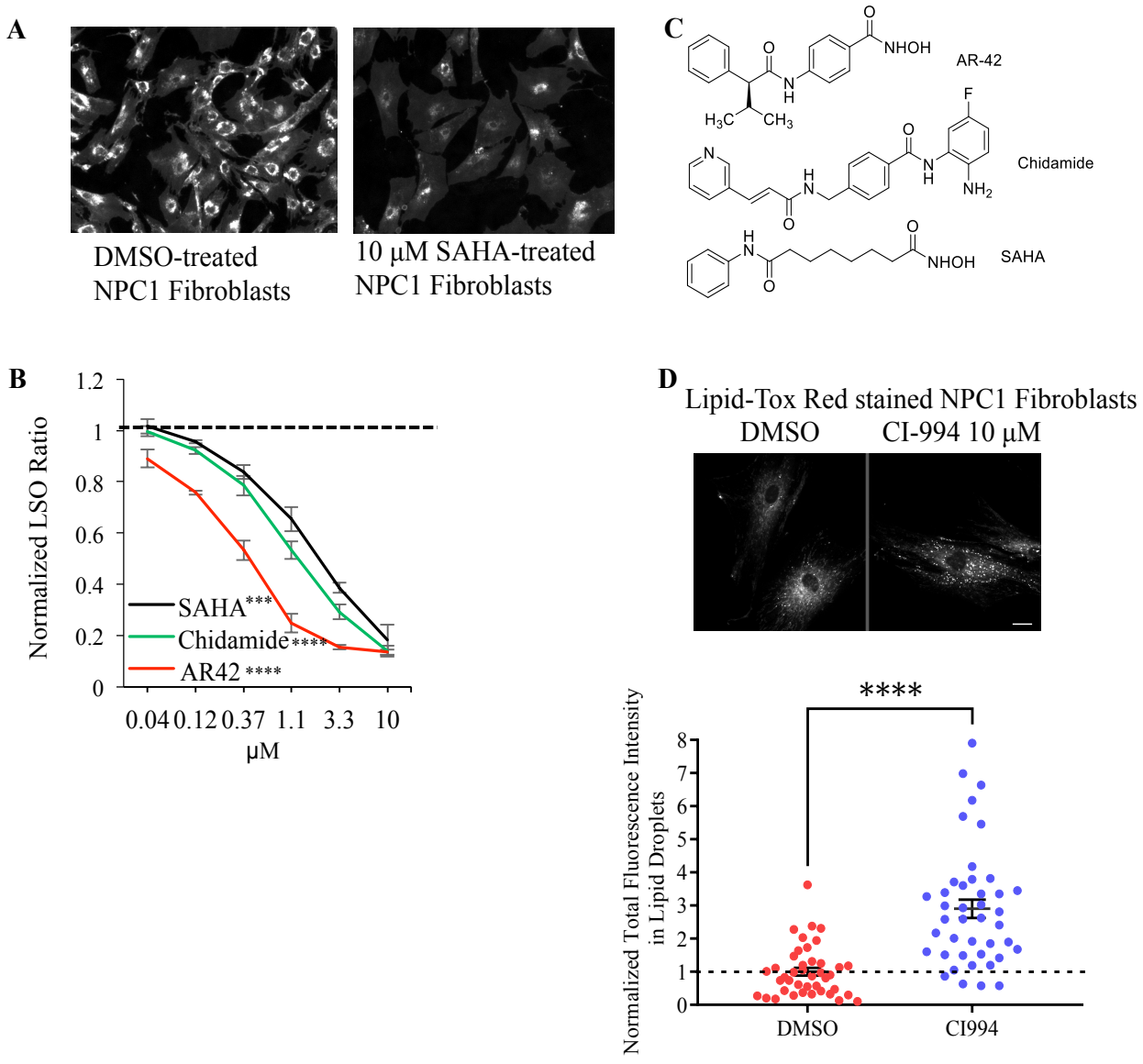
Supplemental Figures and Table:

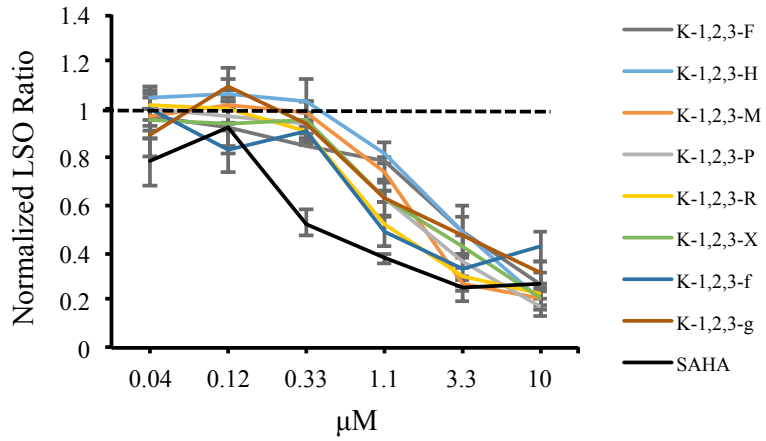


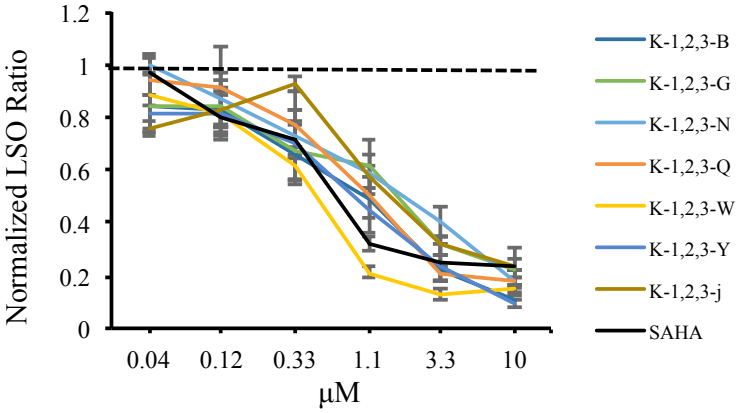
Supplemental Figure 1. Broad spectrum HDAC inhibitors clear cholesterol from NPC1^{I1061T} fibroblasts. A. Filipin-stained NPC1^{I1061T} fibroblasts treated for 72 hours with DMSO (controls) or 10 µM SAHA. Clusters of strongly filipin-stained LSOs are far more abundant in DMSO (left panel) vs. SAHA treated cells (right panel). **B.** Dose-dependent cholesterol clearance response of NPC1^{I1061T} fibroblasts to treatment with broad spectrum HDAC inhibitors. N=3 independent cell culture experiments. Bars are S.E.M. ****, P <0.0001; ***, P <0.001 **C.** Structures of broad spectrum HDAC inhibitors from B. **D.** LipidTox Red stained NPC1^{I1061T} fibroblasts treated with 10 µM CI-994 for 48 hours show increased lipid droplet formation. Representative images are average projections. Scale bar = 25 µm. Plot of total fluorescence intensity in lipid droplets normalized to the DMSO treated cells from 3 independent experiments. Bars are +/- S.E.M., Mann-Whitney P <0.0001.

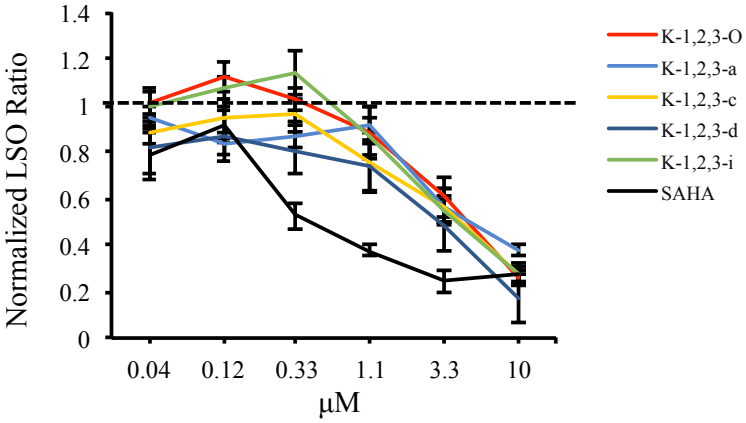
Supplemental Table 1. IC50s and ED50s of broad spectrum HDAC inhibitors.

Broad spectrum HDAC inhibitors have varying specificity for individual HDACs. IC50 values (μM) for each HDAC indicated are shown in color-coded cells. Green bordered boxes indicate low IC50 values and high efficacy for the indicated HDAC. Red bordered boxes indicate no inhibitory activity. IC50 values were obtained from the cited references. Mean ED50 values in μM (and ranges) for reduction of cholesterol accumulation for each compound.

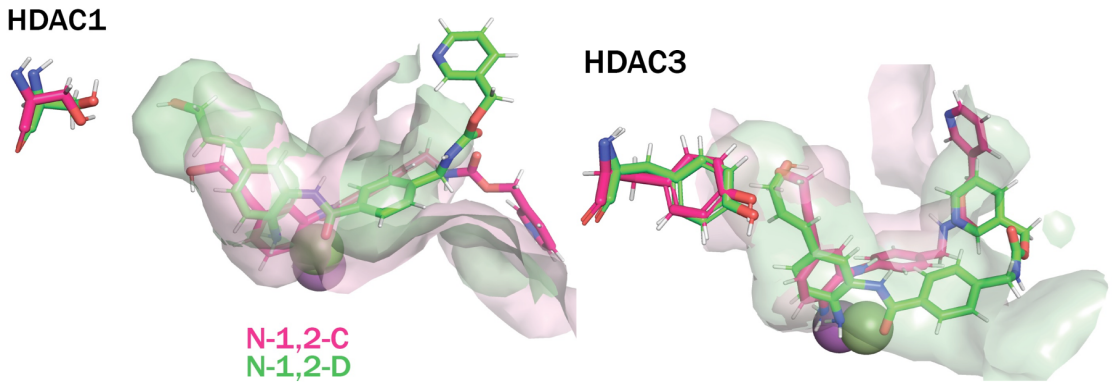
Compound	HDAC1	HDAC2	HDAC3	HDAC6	HDAC8	ED50
Chidamide [22]	0.10	0.16	0.07	>50	0.73	1.11 (0.99-1.29)
AR-42 [23]	0.02	0.02	0.02	0.02	0.02	0.39 (0.37-0.44)







Supplemental Figure 2. Additional inhibitors specific to HDACs 1, 2 and 3 correct the cholesterol accumulation phenotype in NPC1^{I1061T} fibroblast cells. Compounds designed by KDAc therapeutics to inhibit HDACs 1, 2 and 3, increase cholesterol clearance in NPC1^{I1061T} fibroblast cells with 72 hour treatment. Bars are S.E.M.



Supplemental Figure 3. Modeled interactions of selected HDACi and HDACs 1 and 3. The calculated models of N-1,2-C (pink) and N-1,2-D (green) bound to HDAC1 (left) and HDAC3 (right) used in the design of these isoform specific HDAC inhibitors.