Supporting Information

The structural requirements of histone deacetylase inhibitors: Suberoylanilide hydroxamic acid analogs modified at the C6 position

Sun Ea Choi and Mary Kay Pflum

Department of Chemistry, Wayne State University, Detroit, MI 48202

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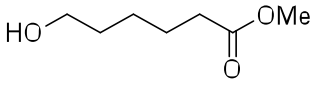
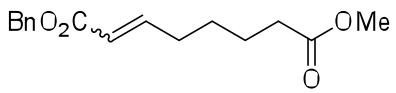
I. Materials and Methods.

Starting materials, reagents, and solvents for reactions obtained from Acros, Sigma-Aldrich, and VWR were used as purchased. Moisture-sensitive reactions were performed under argon with dried glassware and dry solvent. Iron-sensitive reactions were performed with acid-washed glassware and were purified with silica gel that was washed with acid (conc. HCl:distilled water 1:1). Thin-layer chromatography with 60Å, 250μm Partisil® K6F fluorescent indicator plates was used to monitor reactions. Flash chromatography was performed with 60 Å, 230-400 mesh silica gel (Whatman). Solvents were removed by rotary evaporation and a vacuum pump. NMR spectra were recorded in $CDCl_3$ or CD_3OD using a Varian Unity 300 MHz or Varian L900 400 MHz instrument. The peaks around δ 7.24 in the ^{1}H NMR and δ 77 in ^{13}C NMR are due to $CDCl_3$ solvent. The peaks around δ 4.95 and 3.3 in the ^{1}H NMR and δ 58.0 in ^{13}C NMR are due to CD_3OD solvent. Mass spectrometric analysis was performed at Wayne State University's Lumigen Instrument Center using a Waters LCT Premier XE ESI-LC-MS TOF or a Waters GCT EI-TOF. IR spectra were recorded using a Jasco FT/IR – 4100.

II. Experimental Procedures and Compound Characterization



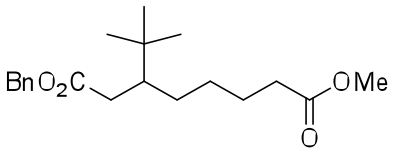
Methyl 6-hydroxyhexanoate (4). Concentrated aqueous sulfuric acid (adjusting to pH 6 using pH paper) was dropwise added to a solution of ε–caprolactone **2** (5.54 mL, 50 mmol) in MeOH (50 mL). The mixture was stirred for 20 min. The mixture was subsequently diluted with anhydrous diethyl ether (25 mL) and washed with distilled water (equal volume to organic layer). The aqueous layer was extracted with diethyl ether (equal volume to organic layer) at least 3 times. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (12% acetone/CH_2Cl_2) on silica gel to give **3** (7.23 g, 99%). ^{1}H-NMR (δ, ppm, CHLOROFORM-D): 1.30 (m, 2H), 1.50 (m, 2H), 1.56 (m, 2H), 2.24 (m, 2H), 2.59 (bs, 1H), 3.52 (m, 2H), 3.58 (s, 3H); ^{13}C-NMR (δ, ppm, CHLOROFORM-D): 24.8, 25.5, 32.4, 34.1, 51, 62.5, 174.5; IR: 3424, 2940, 2866, 1738, 1438, 1205, 857, 744 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+Na] 169.0840, calc. for $C_7H_{14}O_3Na$, 169.0841.



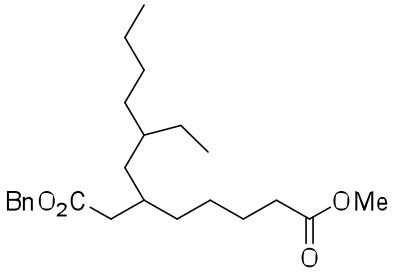
(E/Z)-1-Benzyl 8-methyl oct-2-enedioate (6). To a solution of DMSO (1.02 mL, 14.39 mmol) in CH_2Cl_2 (44 mL) was added 2 M oxalyl chloride in dichloromethane (3.27 mL, 6.54 mmol) dropwise and then methyl 6-hydroxyhexanoate **3** (0.638 g, 4.36 mmol) stepwise at -78ºC. The reaction mixture was stirred for 45 min before triethylamine (TEA, 4.12 mL, 29.66 mmol) was added dropwise at -78 ºC. The mixture was warmed to room temperature and stirred for an additional 1 h. The reaction was quenched by adding distilled water (44 mL) and then washed consecutively with 1.0 M aqueous hydrochloric acid (44 mL), an aqueous solution of saturated $NaHCO_3$ (44 mL), and brine (44 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude organic layer containing methyl 6-oxohexanoate **5** was used immediately without purification.

To a solution of NaH (0.513 g, 12.8 mmol) in THF (85 mL) was added benzyl dimethyl phosphonoacetate (3.31 g, 12.8 mmol) dropwise at 0 ºC and the mixture was stirred for 15 min. To this mixture was added crude methyl 6-oxohexanoate **5** (1.23 g, 8.55 mmol) at -78 ºC and the mixture was stirred for 15 min. The mixture was

allowed to warm to room temperature and stirred for an additional 1 h. The reaction was quenched by addition of an aqueous solution of saturated NH_4Cl until evolution of gas was not observed. The mixture was washed with distilled H_2O (85 mL). The organic layer was collected and the aqueous layer was extracted with diethyl ether (equal volume to aqueous layer) at least 3 times. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (diethyl ether: petroleum ether 1:6) on silica gel to give **6** (2.17 g, 92%). (E+Z)-isomers ^{1}H-NMR (δ, ppm, CHLOROFORM-D): 1.48 (m, 2H), 1.63 (m, 2H), 2.20 (q, 2H), 2.30 (t, 2H), 3.64 (s, 3H), 5.16 (s, 2H), 5.84 (d, 1H, J=180 Hz), 6.98 (m, 1H), 7.35 (m, 5H); ^{13}C-NMR (δ, ppm, CHLOROFORM-D): 24.6, 27.6, 32.1, 33.9, 51.7, 66.2, 121.6, 128.3, 128.4, 128.8, 136.3, 149.5, 166.6, 174.0; IR: 3671, 2974, 1735, 1455, 1258, 1066, 907, 748, 698 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+Na] 299.1269, calc. for $C_{16}H_{20}O_4Na$, 299.1259.

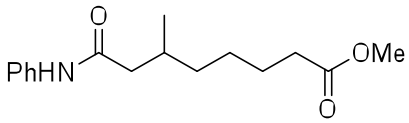


1-Benzyl 8-methyl 3-(*tert*-butyl)octanedioate (7c). To a solution of Cu(I)I (827 mg, 4.34 mmol) in THF (10.9 mL) was added 1.6 M *tert*-butyllithium in pentane (5.43 mL, 8.69 mmol) dropwise at -15 ºC and the mixture was stirred for 20 min. After cooling to -78 ºC, trimethylsilyl chloride (TMSCl, 1.67 mL, 13.03 mmol) was added dropwise to the reaction mixture and then (E/Z)-1-benzyl 8-methyl oct-2-enedioate **6** (300 mg, 1.09 mmol) was added stepwise at -78 ºC. The mixture was stirred for 3 h at -78 ºC and then quenched by addition of an aqueous solution of saturated NH_4Cl: saturated NH_4OH (1:1) until the color of the mixture turned to blue ($(NH_3)_4CuCl_2$(aq)). The mixture was washed with an aqueous solution of saturated NH_4Cl: NH_4OH (1:1) (14.5 mL). The organic layer was collected and the aqueous layer was extracted with diethyl ether (equal volume to the aqueous layer). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was purified by column chromatography (diethyl ether: petroleum ether 1:6) on silica gel to give **7c** (313 mg, 89%). ^{1}H-NMR (δ, ppm, CHLOROFORM-D): 0.84 (s, 9H), 1.05 (m, 1H), 1.17-1.35 (m, 2H), 1.47-1.62 (m, 3H), 1.68 (m, 1H), 2.10 (q, 1H), 2.23 (t, 2H), 2.41 (q, 1H), 3.64 (s, 3H), 5.09 (s, 2H), 7.34 (m, 5H); ^{13}C-NMR (δ, ppm, CHLOROFORM-D): 25.5, 27.6, 28.5, 31.0, 33.8, 34.2, 36.3, 45.2, 51.6, 66.4, 128.4, 128.6, 128.7, 136.3, 174.3, 174.5; IR: 2952, 2869, 1737, 1457, 1367, 1151, 914, 737 cm^{-1}. MS (ESI, *m/z*): found [M^+ +Li] 341.28, calc. for $C_{20}H_{30}O_4Li$, 341.23.



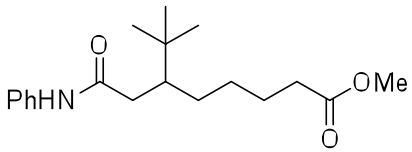
1-Benzyl 8-methyl 3-(2-ethylhexyl)octanedioate (7d). The synthesis was similar to that of **7c** except the following reagents were used: Cu(I)Br-SMe_2 (1.89 g, 9.19 mmol) in THF (15.3 mL), 30-35 wt.% 2-in heptane (10.09 mL, 18.38 mmol), TMSCl (3.52 mL, 27.57 mmol), and (E/Z)-1-benzyl 8-methyl oct-2-enedioate **5** (400 mg, 1.53 mmol). The product was purified by column chromatography (diethyl ether: petroleum ether 1:8) on silica gel to give **7d** (485 mg, 81%). ^{1}H-NMR (δ, ppm, CHLOROFORM-D): 0.79 (m, 3H), 0.88 (t, 3H), 1.23-1.31 (m, 14H), 1.58 (m, 2H), 1.91 (m, 1H), 2.25-2.30 (m, 4H), 3.66 (s, 3H), 5.11 (s, 2H), 7.35 (m, 5H); ^{13}C-

NMR (δ, ppm, CHLOROFORM-D): 10.7, 12.3, 14.40, 23.4, 25.4, 26.1, 28.9, 32.8, 33.1, 34.2, 36.1, 38.5, 44.4, 66.3, 128.4, 128.6, 128.8, 130.4, 173.2, 173.5; IR: 2956, 2858, 1739, 1457, 1167, 912, 741 cm^{-1}. MS (ESI, *m/z*): found $[M^+ +Li]$ 397.36, calc. for $C_{24}H_{38}O_4Li$, 397.29.



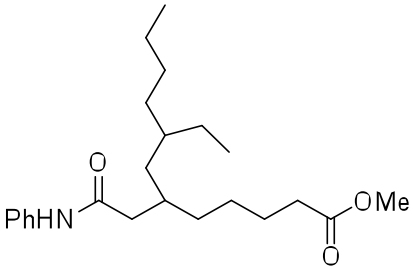
Methyl 6-methyl-8-oxo-8-(phenylamino)octanoate (8a). To a solution of Cu(I)I (1.06 g, 5.57 mmol) in THF (19 mL) was added 1.6M methyllithium in diethyl ether (6.97 mL, 11.15 mmol) dropwise at -15 ºC and the mixture was stirred for 20 min. The reaction mixture was cooled to -78 ºC before addition of trimethylsilyl chloride (TMSCl, 4.25 mL, 33.48 mmol). To the reaction mixture was dropwise added (E/Z)-1-benzyl 8-methyl oct-2-enedioate **6** (513 mg, 1.86 mmol) at -78 ºC. The reaction was stirred for 3 h at -78 ºC to room temperature and then quenched by addition of 1.0 M aqueous hydrochloric acid until a color of the mixture changed to blue ($CuCl_{2(aq)}$). The organic layer was collected and the aqueous layer was extracted with diethyl ether (equal volume to aqueous layer) at least 3 times. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated to obtain compound **7a**, which was used without purification in the next step.

To a solution of crude 1-benzyl 8-methyl 3-methyloctanedioate **7a** (513 mg, 1.86 mmol) in ethyl acetate (19 mL) was added 20% $Pd(OH)_2/C$ (261 mg, 0.372 mmol) and the reaction mixture was purged with H_2 (g) for 30 s. The reaction solution was stirred under H_2 (g) for 3 h and then filtered through a plug of Celite with ethyl acetate (57 mL). The filtrate was concentrated to give 8-methoxy-3-methyl-8-oxooctanoic acid as clear oil. The crude residue, 8-methoxy-3-methyl-8-oxooctanoic acid, was transferred to a flask and dissolved in 19 mL of acetonitrile. TBTU (895 mg, 2.79 mmol), diisopropylethylamine (647 mL, 3.72 mmol), and aniline (254 mL, 2.79 mmol) were added to the flask. The reaction mixture was stirred for 3 h. The mixture was then quenched with 19 mL of saturated $NaHCO_3$ solution, transferred to a separatory funnel and extracted with ethyl acetate (equal volume to aqueous layer) at least 3 times. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated to oil. Flash silica gel chromatography (1:6 diethyl ether: petroleum ether then 1:1 diethyl ether: petroleum ether) afforded 274 mg of the anilide **8a** as a clear oil (53% over 3 steps). ^{1}H-NMR (δ, ppm, CHLOROFORM-D): 0.91 (d, 3H), 1.13-1.34 (m, 4H), 1.56 (m, 2H), 2.09 (m, 2H), 2.27 (m, 3H), 3.61 (s, 3H), 7.02 (t, 1H), 7.24 (t, 2H), 7.52 (d, 2H); ^{13}C-NMR (δ, ppm, CHLOROFORM-D): 19.9, 25.1, 26.6, 30.9, 34.2, 36.5, 45.6, 51.8, 120.0, 124.4, 129.2, 138.2, 171.1, 175.3; IR: 3306, 2952, 2868, 1739, 1601, 1544, 1151, 913, 757 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+H] 278.1764, calc. for $C_{16}H_{24}NO_3$, 278.1756, found [M+Na] 300.1584, calc. for $C_{16}H_{24}NO_3Na$, 300.1576.

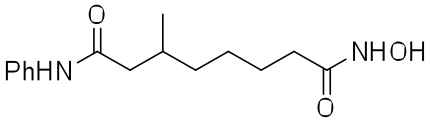


Methyl 6-(*tert*-butyl)-8-oxo-8-(phenylamino)octanoate (8c). A similar procedure to that for **8a** was used, except for the following reagents: 20% $Pd(OH)_2/C$ (210 mg, 0.299 mmol) and 1-benzyl 8-methyl 3-(*tert*-butyl)octanedioate **7c** (414 mg, 0.748 mmol) in ethyl acetate (7.5 mL), TBTU (360 mg, 1.121 mmol) in acetonitrile (7.5 mL), diisopropyethylamine (521 mL, 2.99 mmol), aniline (102 mL, 0.748 mmol) and stirring

for 4 h. The mixture was then quenched with 7.5 mL of saturated $NaHCO_3$ solution, transferred to a separatory funnel and extracted with ethyl acetate (equal volume to aqueous layer) at least 3 times. The organic layer was dried over magnesium sulfate, filtered, and evaporated to oil. In this case, flash silica gel chromatography (1:6 diethyl ether: petroleum ether then 1:1 diethyl ether: petroleum ether) afforded 185 mg of the anilide **8c** as a clear oil (58% over 2 steps). ^{1}H-NMR (δ, ppm, CHLOROFORM-D): 0.91 (s, 9H), 1.13 (m, 1H), 1.33 (m, 1H), 1.42 (m, 1H), 1.55 (m, 3H), 1.82 (m, 1H), 2.09 (dd, 1H), 2.28 (t, 2H), 2.49 (dd, 1H), 3.62 (s, 3H), 7.09 (t, 1H), 7.21 (bs, 1H), 7.31 (t, 2H), 7.50 (d, 2H); ^{13}C-NMR (δ, ppm, CHLOROFORM-D): 25.5, 25.7, 27.8, 28.4, 31.2, 34.1, 45.1, 56.1, 119.2, 124.3, 129.2, 134.7, 168.5, 171.3; IR: 3055, 2952, 2865, 1732, 1600, 1265, 741, 706 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+H] 320.2229, calc. for $C_{19}H_{29}NO_3$, 320.2226, found [M+Na] 342.2048, calc. for $C_{19}H_{29}NO_3Na$, 342.2045.

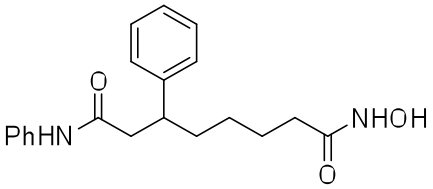


Methyl 8-ethyl-6-(2-oxo-2-(phenylamino)ethyl)dodecanoate (8d). A similar procedure to that for **8a** was used, except for the following reagents: 20% $Pd(OH)_2$/C (345 mg, 0.492 mmol) and 1-benzyl 8-methyl 3-(2-ethylhexyl)octanedioate **7d** (480 mg, 1.229 mmol) in ethyl acetate (12.3 mL), TBTU (592 mg, 1.844 mmol) in acetonitrile (12.3 mL), diisopropyethylamine (856 mL, 4.916 mmol), aniline (168 mL, 1.844 mmol) and stirring for 3 h. In this case, the mixture was quenched with 12.3 mL of saturated $NaHCO_3$ solution, transferred to a separatory funnel and extracted with CH_2Cl_2 (equal volume to aqueous layer) at least 3 times. The organic layer was dried over magnesium sulfate, filtered, and evaporated to oil. The product was purified by column chromatography (diethyl ether: petroleum ether 1:8) on silica gel to give **8d** (349 mg, 76% over 2 steps). ^{1}H-NMR (δ, ppm, CHLOROFORM-D): 0.81-0.87 (m, 6H), 1.10-1.34 (m, 15H), 1.60 (m, 2H), 2.03 (m, 1H), 2.22-2.32 (m, 4H), 3.65 (s, 3H), 7.08 (t, 1H), 7.32 (t, 2H), 7.51 (d, 2H); ^{13}C-NMR (δ, ppm, CHLOROFORM-D): 10.7, 14.4, 23.4, 25.3, 25.8, 26.0, 28.9, 33.1, 33.2, 33.6, 33.8, 36.2, 38.5, 43.2, 51.7, 120.0, 124.3, 129.2, 138.3, 171.4, 174.6; IR: 3322, 3066, 2956, 1740, 1661, 1171, 912, 746, 694 cm^{-1}; MS (ESI, *m/z*): found [M^+ +Li] 382.41, calc. for $C_{23}H_{37}NO_3Li$, 382.29.



***N*^{8}-Hydroxyl-3-methyl-*N*^{1}-phenyloctanediamide (2a).** To a solution of NH_2OH-HCl (677 mg, 9.735 mmol) in methanol (10 mL) was added KOH (1.092 g, 19.469 mmol) at 0 °C in an acid-washed 25mL round-bottom flask. After stirring for 20 min, methyl 6-methyl-8-oxo-8-(phenylamino)octanoate **8a** (270 mg, 0.974 mmol) was added and the mixture was stirred for 8 h at 0°C. The reaction mixture was adjusted to pH 6 by adding concentrated aqueous hydrochloric acid. The mixture was extracted with 30 mL of ethyl acetate, and the organic extraction was washed with 30 mL of distilled water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (9% methanol in CH_2Cl_2) using acid-washed silica gel to give **2a** (159 mg, 58%) as a clear oil. ^{1}H-NMR (δ, ppm, METHANOL-D4): 0.98 (d, 3H), 1.24-1.44 (m, 4H), 1.61 (m, 2H), 2.02 (m, 1H), 2.09 (t, 2H), 2.16 (dd, 1H), 2.34 (dd, 1H), 7.07 (t, 1H), 7.29

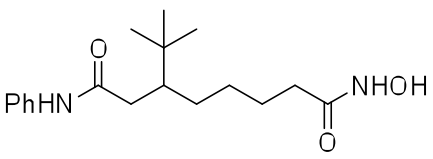
(t, 2H), 7.52 (d, 2H); ^{13}C-NMR (δ, ppm, METHANOL-D4): 18.7, 25.7, 26.3, 30.8, 32.5, 36.3, 44.4, 120.2, 124.0, 128.6, 138.6, 171.8, 172.9; IR: 3270, 2928, 2868, 1643, 1600, 1500, 1418, 1116, 977, 759, 693 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+H] 279.1710, calc. for $C_{15}H_{23}N_2O_3$, 279.1709, found [M+Na] 301.1531, calc. for $C_{15}H_{23}N_2O_3Na$, 301.1528.



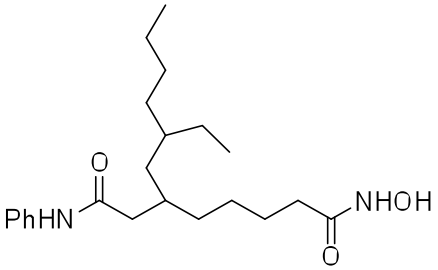
***N*^{8}-Hydroxyl-*N*^{8}, 3-diphenyloctanediamide (2b).** The synthesis of **7b** was similar to that for **7c** except the following reagents were used: Cu(I)I (827 mg, 4.34 mmol) in THF (14.5 mL), 2.0 M phenyl lithium in dibutylether (4.34 mL, 8.69 mmol), TMSCl (1.67 mL, 13.03 mmol), and (E/Z)-1-benzyl-8-methyl oct-2-enedioate **6** (400 mg, 1.45 mmol). The residue was purified by column chromatography (diethyl ether: petroleum ether 1:6) on silica gel to give a mixture of **7b** and **6** (3:1), which was used in the next step.

The synthesis of methyl 6-phenyl-8-oxo-8-(phenylamino)octanoate **8b** was similar to that of **8a** except the following reagents were used: 20% $Pd(OH)_2/C$ (153 mg, 0.218 mmol), the crude reaction mixture containing 1-benzyl 8-methyl 3-phenyloctanedioate **7b** (193 mg, 0.546 mmol) in ethyl acetate (5.5 mL), TBTU (263 mg, 0.818 mmol) in acetonitrile (5.5 mL), diisopropyethylamine (190 mL, 1.09 mmol), aniline (75 mL, 0.818 mmol) and stirring for 4 h. In this case, the reaction mixture was quenched with 5.5 mL of saturated $NaHCO_3$ solution, transferred to a separatory funnel and extracted with CH_2Cl_2 (equal volume to aqueous layer) at least 3 times. The organic layer was dried over magnesium sulfate, filtered, and evaporated to oil. Flash silica gel chromatography (diethyl ether: petroleum ether 1:1) afforded the mixture of phenyl substituted anilide **8b** and α, β unsaturated anilide **8b´** as a clear oil, which was used in the next step.

To a solution of NH_2OH-HCl (696 mg, 10.721 mmol) in methanol (10.7 mL) was added KOH (1.203 g, 21.442 mmol) at 0 ˚C in an acid-washed 25mL round-bottom flask. After stirring for 20 min, the mixture of methyl 6-phenyl-8-oxo-8-(phenylamino)octanoate **7b** and α, β unsaturated anilide **7b´** (380 mg, 1.072 mmol) was added and the reaction mixture was stirred for 8 h at 0°C. The rest of the reaction procedure was similar to that for **2a**. The residue was purified by column chromatography (4% methanol/CH_2Cl_2) on acid-washed silica gel to give **2b** (221 mg, 60% over 4 steps). ^{1}H NMR (δ, ppm, METHANOL-D4): 1.19 (m, 2H), 1.56 (m, 2H), 1.70 (m, 2H), 2.00 (t, 2H), 2.58 (m, 2H), 3.14 (m, 1H), 7.03 (t, 1H), 7.13-7.27 (m, 7H), 7.39 (d, 2H); ^{13}C-NMR (δ, ppm, METHANOL-D4): 25.5, 26.9, 32.5, 35.5, 42.8, 44.5, 120.4, 124.1, 126.4, 127.5, 128.4, 128.5, 138.4, 144.1, 171.7, 171.9; IR: 3235, 3027, 2928, 2859, 1874, 1641, 1599, 1544, 1498, 1467, 1116, 977, 757, 699 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+H] 341.1877, calc. for $C_{20}H_{25}N_2O_3$, 341.1865, found [M+Na] 363.1697, calc. for $C_{20}H_{25}N_2O_3Na$, 363.1685.



3-(*tert*-Butyl)-N^8-hydroxyl-N^1-phenyloctanediamide (2c). A similar procedure to that for **2a** was used, except for the following reagents: NH_2OH-HCl (348 mg, 5.008 mmol) in methanol (7.4 mL), KOH (562 mg, 10.018 mmol), methyl 6-(*tert*-butyl)-8-oxo-8-(phenylamino)octanoate **8c** (160 mg, 0.501 mmol) and stirring for 4 h. In this case, the product was purified by column chromatography (4 % methanol in CH_2Cl_2) using acid-washed silica gel to give **2c** (158 mg, 99%). ^{1}H-NMR (δ, ppm, METHANOL-D4): 0.92 (s, 9H), 1.15 (m, 1H), 1.28 (m, 1H), 1.40 (m, 1H), 1.58 (m, 3H), 1.77 (m, 1H), 2.04 (t, 2H), 2.16 (dd, 1H), 2.53 (dd, 1H), 7.07 (t, 1H), 7.29 (t, 2H), 7.50 (d, 2H); ^{13}C-NMR (δ, ppm, METHANOL-D4): 26.1, 26.7, 28.6, 30.8, 32.6, 33.3, 38.5, 45.0, 120.2, 123.9, 128.6, 138.8, 171.8, 173.9; IR: 3350, 2956, 2870, 1938, 1648, 1547, 1500, 1119, 976, 757, 693 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+H] 321.2180, calc. for $C_{18}H_{29}N_2O_3$, 321.2178, found [M+Na] 343.1998, calc. for $C_{18}H_{29}N_2O_3Na$, 343.1988.



3-(2-Ethylhexyl)-N^8-hydroxyl-N^1-phenyloctanediamide (2d). A similar procedure to that for **2a** was used, except for the following reagents: NH_2OH-HCl (629 mg, 9.054 mmol) in methanol (9 mL), KOH (1.016 g, 18.108 mmol), methyl 8-ethyl-6-(2-oxo-2-(phenylamino)ethyl)dodecanoate **8d** (340 mg, 0.905 mmol), and stirring for 6 h. In this case, the product was purified by column chromatography (4 % methanol in CH_2Cl_2) using acid-washed silica gel to give **2d** (199 mg, 58%). ^{1}H-NMR (δ, ppm, METHANOL-D4): 0.85 (m, 6H), 1.19-1.37 (m, 16H), 1.61 (m, 2H), 2.02 (bs, 1H), 2.09 (t, 2H), 2.24 (m, 2H), 7.07 (t, 1H), 7.29 (t, 2H), 7.53 (d, 2H); ^{13}C-NMR (δ, ppm, METHANOL-D4): 9.7, 9.9, 13.4, 23.0, 26.0, 28.5, 28.8, 32.6, 32.8, 33.0, 34.0, 36.1, 38.4, 42.3, 120.1, 124.0, 128.6, 138.7, 171.8, 173.1; IR: 3391, 3256, 3065, 2956, 1643, 1600, 1539, 1500, 1308, 903, 756, 692 cm^{-1}; HRMS (EI-TOF, *m/z*): found [M+H] 377.2798, calc. for $C_{22}H_{37}N_2O_3$, 377.2804.

III. HDAC assay procedure

HDAC activity was measured using the Fluor de Lys® activity assay (Enzo) using the manufacturer's protocol. To measure global HDAC inhibition, HeLa lysates were incubated with or without small molecule inhibitor in HDAC assay buffer (50 mM TrisHCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$) at a final volume of 25 μL for 30 min at 30 ˚C with shaking. Concentrations of small molecule between 1 nM and 1 mM final concentration were used to determine IC_{50} values. Because the small molecules were stored in DMSO, dilution with HDAC buffer ensured that a maximum of 2% DMSO was present in the final reaction mixture. After the initial incubation, Fluor de Lys™ substrate in HDAC assay buffer (100 μM final concentration) was added to make a total reaction volume of 50μL. The reaction mixture was incubated at 30˚C for 30 min with shaking. To quench the reaction and allow color development, Fluor de Lys™ developer (2.5 μL of 20X diluted up to 50 μL in HDAC assay buffer) was added to give a final 100 μL volume and incubated with shaking for 5 min at room temperature. The fluorescence intensity was determined using a Geniosplus Fluorimeter (Tecan) with excitation at 360 nm and emission at 465 nm.

To perform the isoform selectivity studies, the procedure was similar except that the HeLa cell lysates were replaced with 0.2 µL HDAC1 (0.88 mg/mL), 0.3 µL HDAC3 (30 ng/μL) or 2 µL HDAC6 (0.13 mg/mL),

purchased from Enzo Life Science. In addition, the Fluor de Lys™ substrate was used at a final concentration of 50 μM for HDAC1 and HDAC6 or 25μM for HDAC3.

For each trial, a no enzyme control reaction was used to assess the background. The background-corrected fluorescence units of small molecule-treated samples were then compared to that of untreated (DMSO control) samples (set to 100%) to give a percentage deacetylase activity. IC_{50} values were obtained by plotting the percentage deacetylase activity versus the small molecule concentration and fitting the data to a sigmoidal dose-response curve ($y=100/(1+(x/m3)^{m4})$ using KaleidaGraph software where m3 is the IC_{50} value in Molar units. All experiments were performed in triplicate with the mean and standard error reported in the tables and figures.

IV. Supporting Figures

Table S.1. Percentage HDAC activity after incubation of SAHA with Hela Lysate

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
3.125×10^{-8}	75	75	ND*	75	-
6.25×10^{-8}	63	56	51	57	3
1.25×10^{-7}	44	35	40	40	3
2.5×10^{-7}	31	21	26	26	3
5.0×10^{-7}	20	16	15	17	2

*ND = Not determined.

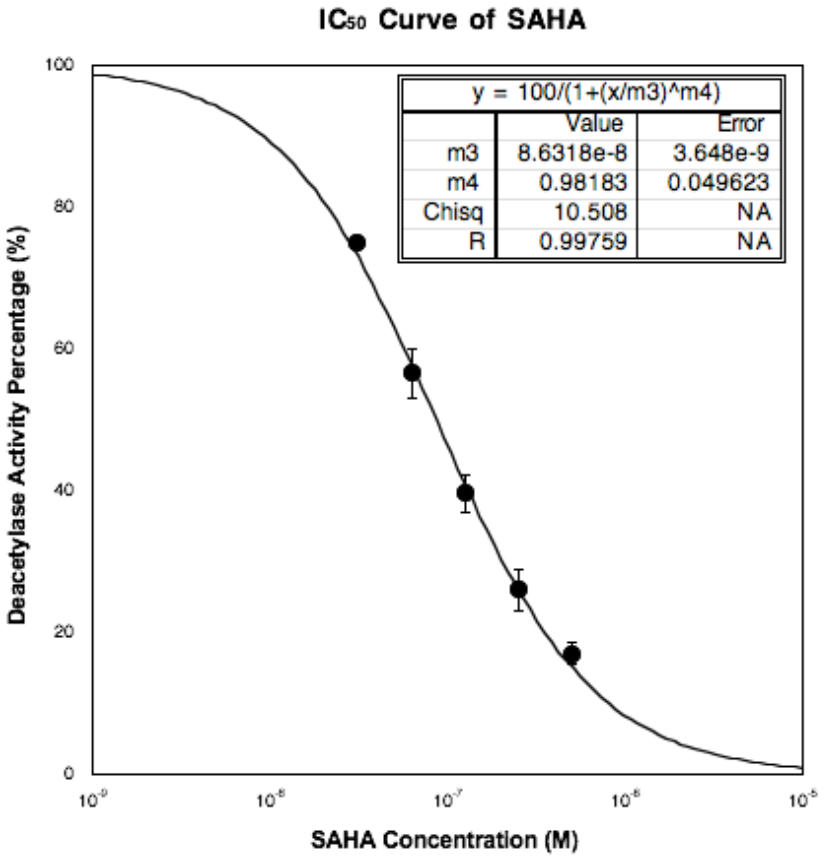


Figure S.1. Dose response curve of SAHA tested using the HDAC activity from HeLa cells lysates from three independent trials with error bars indicating standard error (see Table S.1). In some case, the error bar is smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 1 of the manuscript.

Table S.2. Percentage HDAC activity after incubation of MS-275 with Hela Lysate

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
1.95×10^{-6}	58	59	58	58	0.3
3.91×10^{-6}	44	47	49	47	1
7.81×10^{-6}	32	35	33	33	1
1.56×10^{-5}	27	27	26	27	0.3
6.25×10^{-5}	9	12	11	11	1

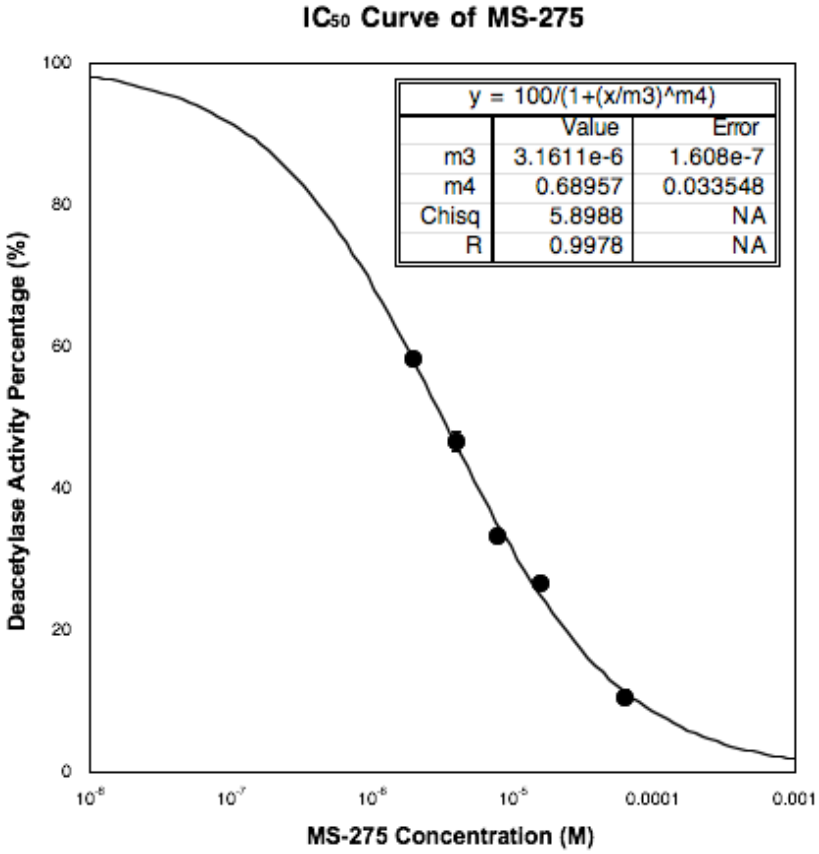


Figure S.2. Dose response curve of MS-275 tested using the HDAC activity from HeLa cells lysates from three independent trials with error bars indicating standard error (see Table S.2). In some cases, the error bars are smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 1 of the manuscript.

Table S.3. Percentage HDAC activity after incubation of C6-SAHA methyl analogue **2a** with Hela Lysate

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
6.25×10^{-8}	104	72	102	92	10
1.25×10^{-7}	94	50	85	76	13
2.5×10^{-7}	66	51	79	65	8
5.0×10^{-7}	30	32	40	34	3
1.0×10^{-6}	24	20	22	22	1

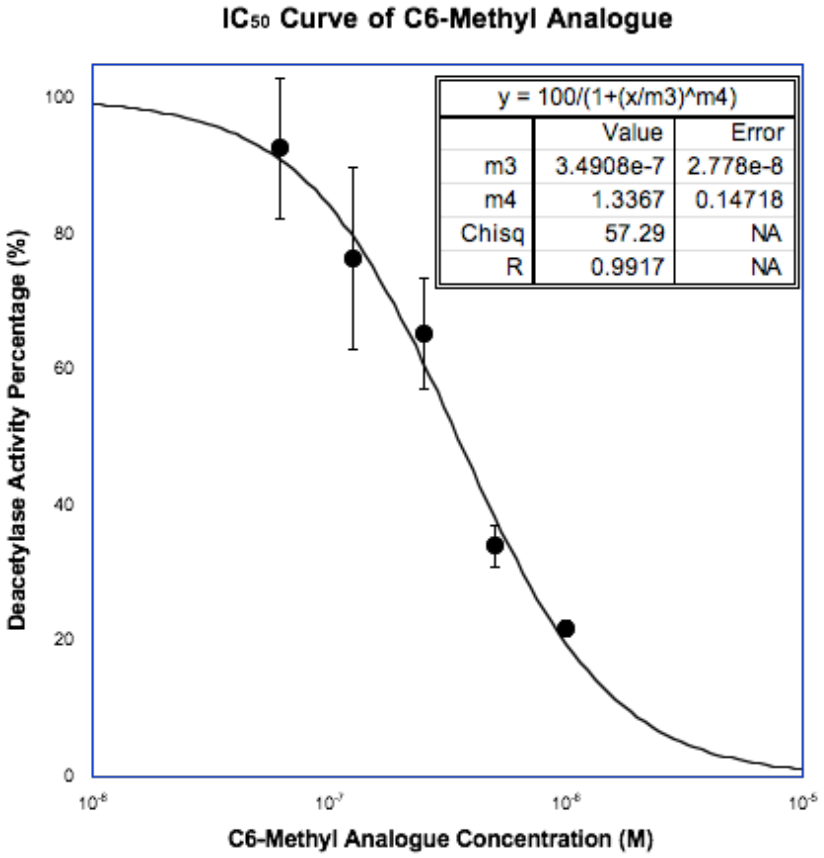


Figure S.3. Dose response curve of C6-SAHA methyl analogue **2a** tested using the HDAC activity from HeLa cells lysates from three independent trials with error bars indicating standard error (see Table S.3). In some cases, the error bar is smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 1 of the manuscript.

Table S.4. Percentage HDAC activity after incubation of C6-SAHA phenyl analogue **2b** with Hela Lysate

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
6.25×10^{-8}	87	87	82	85	1
1.25×10^{-7}	75	73	75	74	0.7
2.5×10^{-7}	59	41	54	51	5
5.0×10^{-7}	45	47	51	47	1
1.0×10^{-6}	27	23	20	23	2

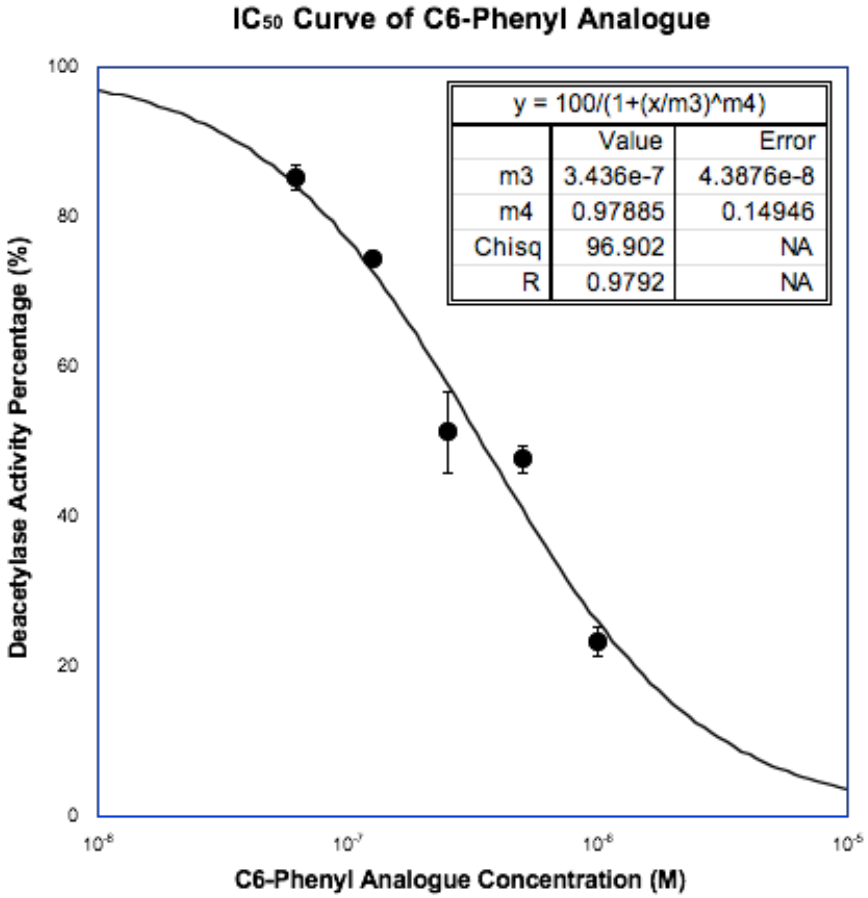


Figure S.4. Dose response curve of C6-SAHA phenyl analogue **1b** tested using the HDAC activity from HeLa cells lysates from three independent trials with error bars indicating standard error (see Table S.4). In some cases, the error bar is smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 1 of the manuscript.

Table S.5. Percentage HDAC activity after incubation of C6-SAHA *t*-butyl analogue **2c** with Hela Lysate

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
1.11×10^{-7}	94	99	114	102	6
3.33×10^{-7}	65	87	103	85	11
1.0×10^{-6}	55	58	71	61	4
3.0×10^{-6}	40	37	41	39	1
9.0×10^{-6}	28	20	18	22	3

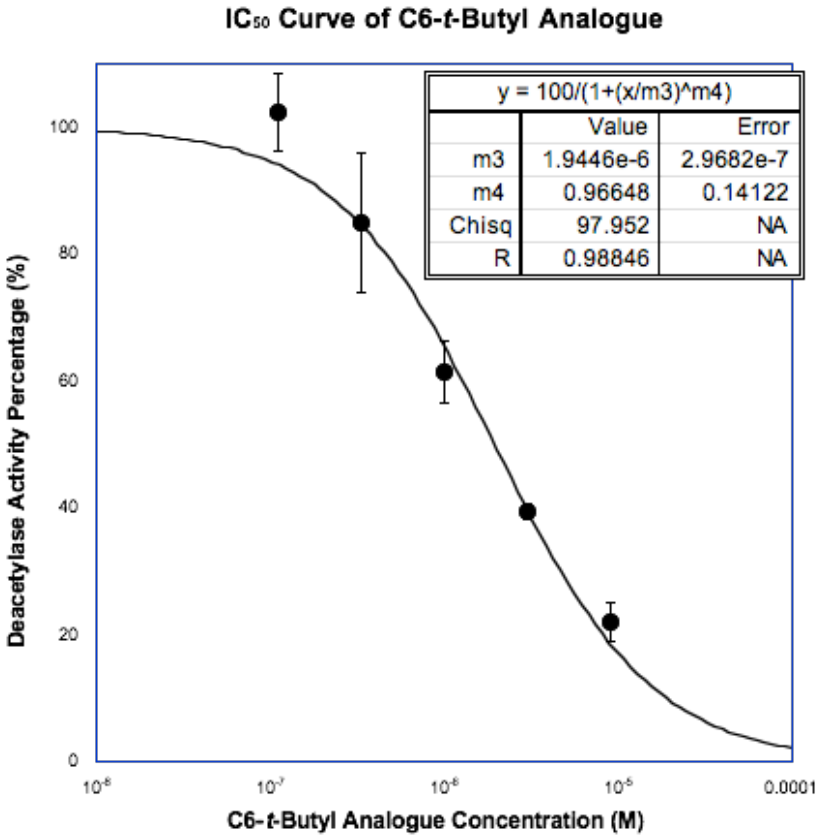


Figure S.5. Dose response curve of C6-SAHA *t*-butyl analogue **2c** tested using the HDAC activity from HeLa cells lysates from three independent trials with error bars indicating standard error (see Table S.5). In some cases, the error bar is smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 1 of the manuscript.

Table S.6. Percentage HDAC activity after incubation of C6-SAHA 2-ethylhexyl analogue **2d** with Hela Lysate

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
1.11×10^{-7}	93	69	78	80	7
3.33×10^{-7}	62	54	55	57	2
1.0×10^{-6}	42	22	38	34	6
3.0×10^{-6}	12	8	13	11	1
9.0×10^{-6}	4	2	2	2.7	0.7

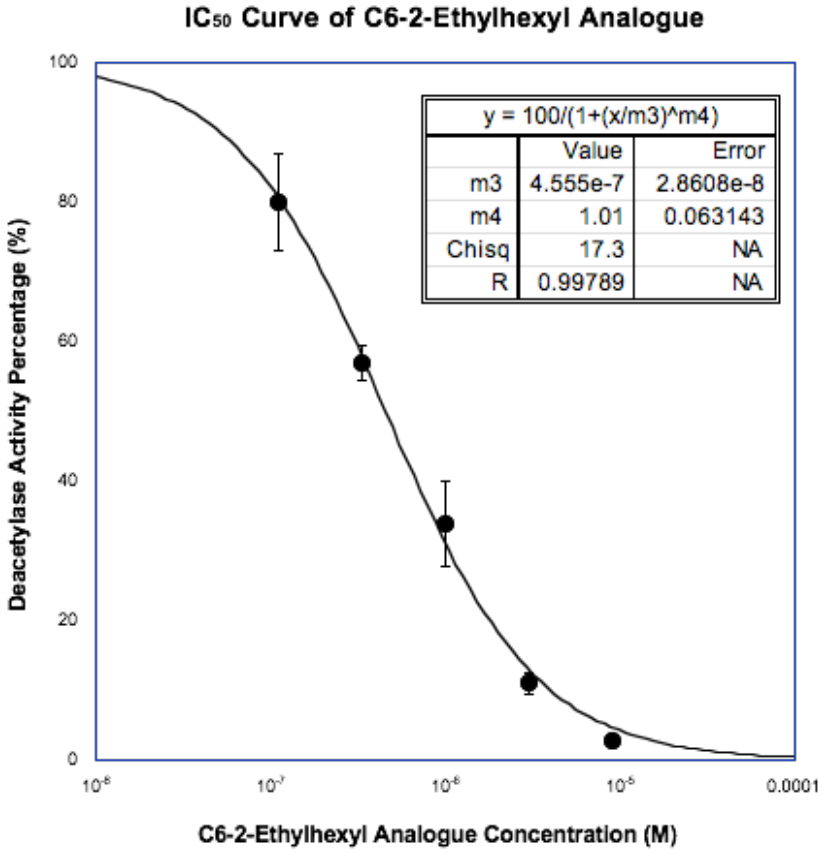


Figure S.6. Dose response curve of C6-SAHA 2-ethylhexyl analogue **2d** tested using the HDAC activity from HeLa cells lysates from three independent trials with error bars indicating standard error (see Table S.6). In some case, the error bar is smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 1 of the manuscript.

Table S.7. Percentage deacetylase activity remaining after incubation of HDAC1, HDAC3, or HDAC6 with SAHA or the C6-SAHA analogues **2a-d***

Compound	HDAC Isoform	Trial 1	Trial 2	Mean	S.E.
SAHA (125 nM)	HDAC1	30	35	32	2
	HDAC3	44	45	44	1
	HDAC6	32	36	34	2
C6-Methyl (500 nM)	HDAC1	35	42	38	3
	HDAC3	32	33	32	0.5
	HDAC6	61	58	59	1
C6-Phenyl (500 nM)	HDAC1	57	56	56	0.5
	HDAC3	68	67	67	0.5
	HDAC6	61	58	59	1
C6-*t*-Butyl (2 μM)	HDAC1	44	46	45	1
	HDAC3	79	82	80	1
	HDAC6	56	55	55	0.5
C6-2-Ethylhexyl (500 nM)	HDAC1	77	73	75	2
	HDAC3	51	54	52	1
	HDAC6	91	95	93	2

*The data are reported in Figure 4 of the manuscript.

Table S.8. Percentage HDAC1 activity after incubation of SAHA.

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
3.125×10^{-8}	68	91	82	80	7
6.25×10^{-8}	55	62	57	58	2
1.25×10^{-7}	48	27	37	37	6
2.50×10^{-7}	37	40	25	34	5

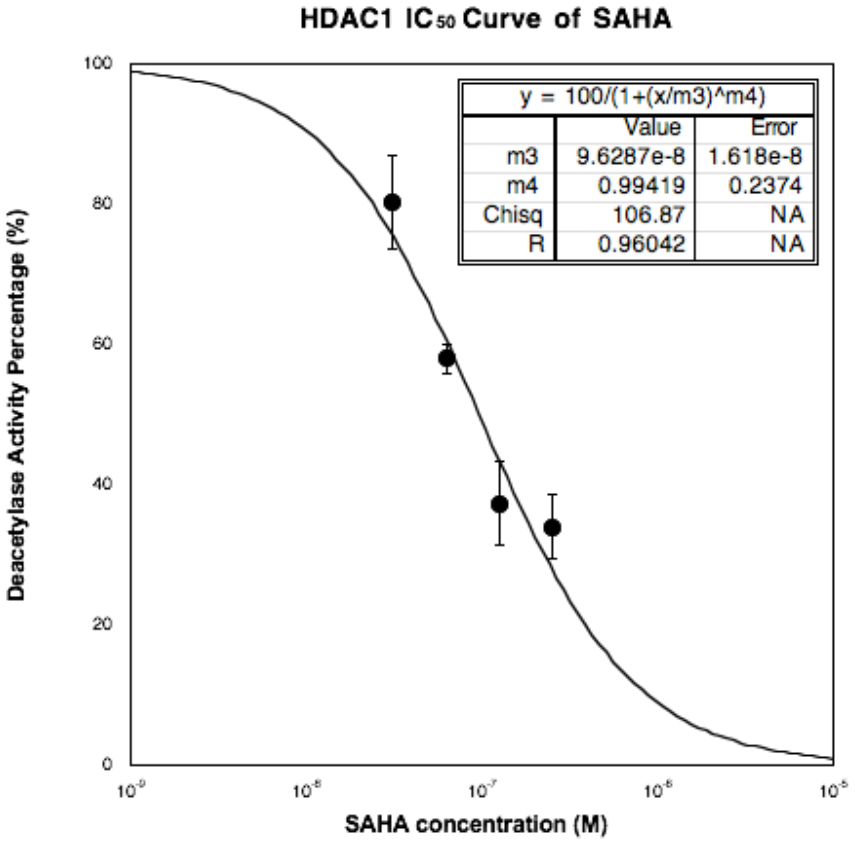


Figure S.7. Dose response curve of SAHA tested with HDAC1 from three independent trials with error bars indicating standard error (see Table S.8). Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 2 of the manuscript.

Table S.9. Percentage HDAC3 activity after incubation of SAHA.

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
3.125×10^{-8}	88	78	74	80	4
6.25×10^{-8}	76	74	70	73	2
1.25×10^{-7}	63	45	44	56	5
2.50×10^{-7}	27	39	37	34	4

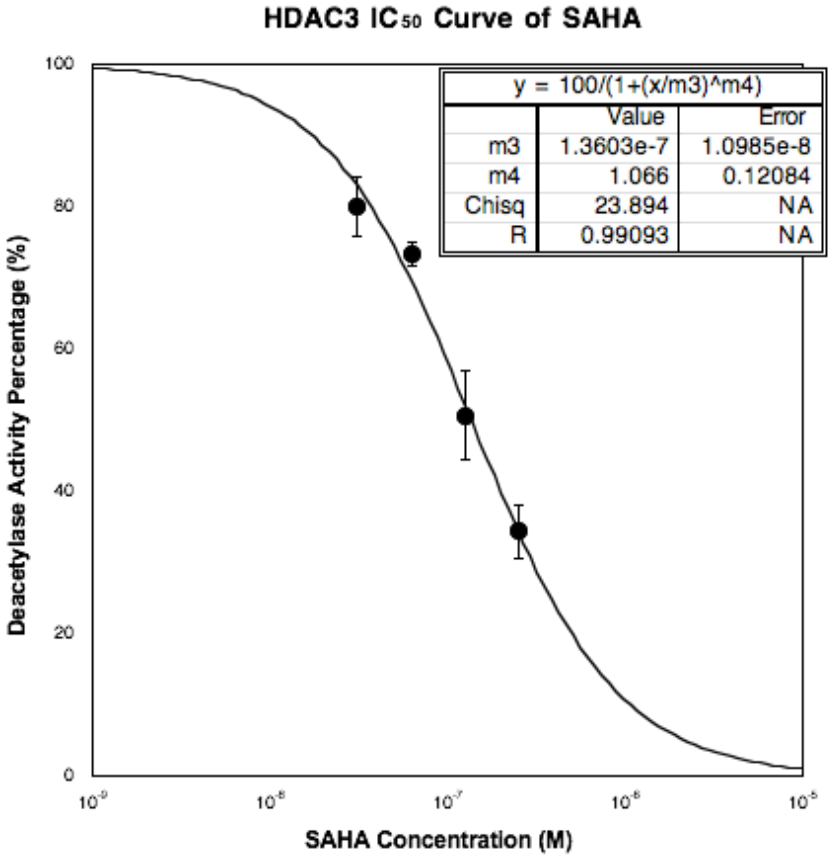


Figure S.8. Dose response curve of SAHA with HDAC3 from three independent trials with error bars indicating standard error (see Table S.9). Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 2 of the manuscript.

Table S.10. Percentage HDAC6 activity after incubation of SAHA.

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
3.125×10^{-8}	66	76	73	72	3
6.25×10^{-8}	64	62	60	62	1
1.25×10^{-7}	37	28	32	32	2
2.50×10^{-7}	13	12	9	11	1

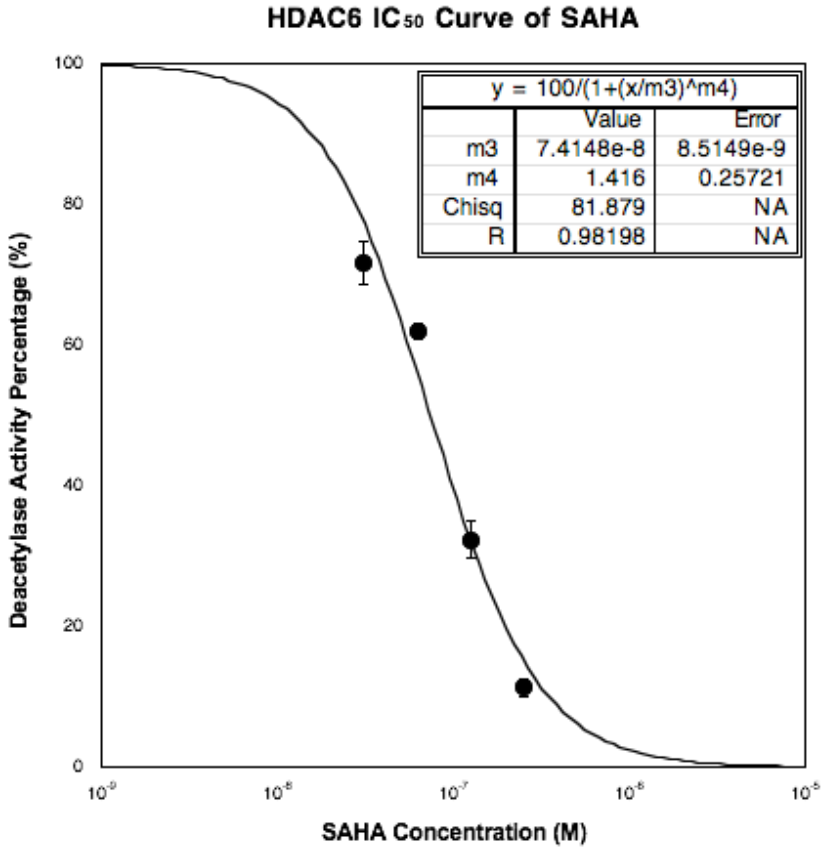


Figure S.9. Dose response curve of SAHA with HDAC6 from three independent trials with error bars indicating standard error (see Table S.10). In some cases, the error bars are smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 2 of the manuscript.

Table S.11. Percentage HDAC1 activity after incubation of C6-SAHA *t*-butyl analogue **2c**.

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
2.50×10^{-7}	102	92	82	92	5
5.00×10^{-7}	86	85	80	84	1
1.00×10^{-6}	68	44	35	49	9
3.125×10^{-5}	7	1	1	3	2

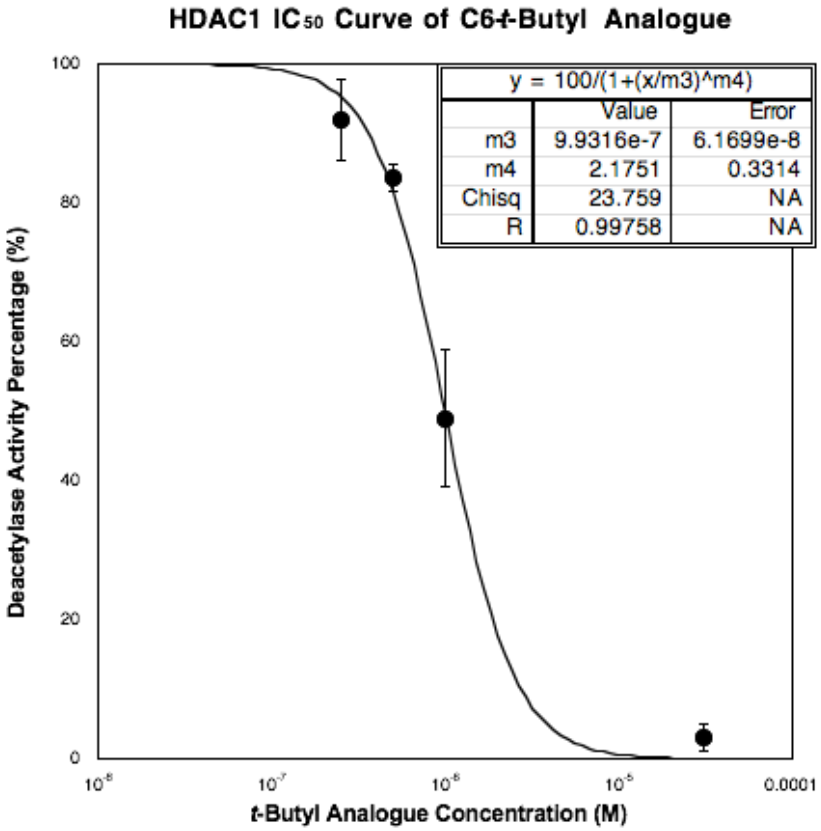


Figure S.10. Dose response curve of C6-SAHA *t*-butyl analogue **2c** tested with HDAC1 from three independent trials with error bars indicating standard error (see Table S.11). Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 2 of the manuscript.

Table S.12. HDAC3 activity percentage after incubation of C6-SAHA *t*-butyl analogue **2c**.

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
1.00×10^{-6}	87	85	81	84	1
4.00×10^{-6}	76	63	52	64	6
1.5625×10^{-5}	21	20	13	18	2
3.125×10^{-5}	7	6	6	6	0.3

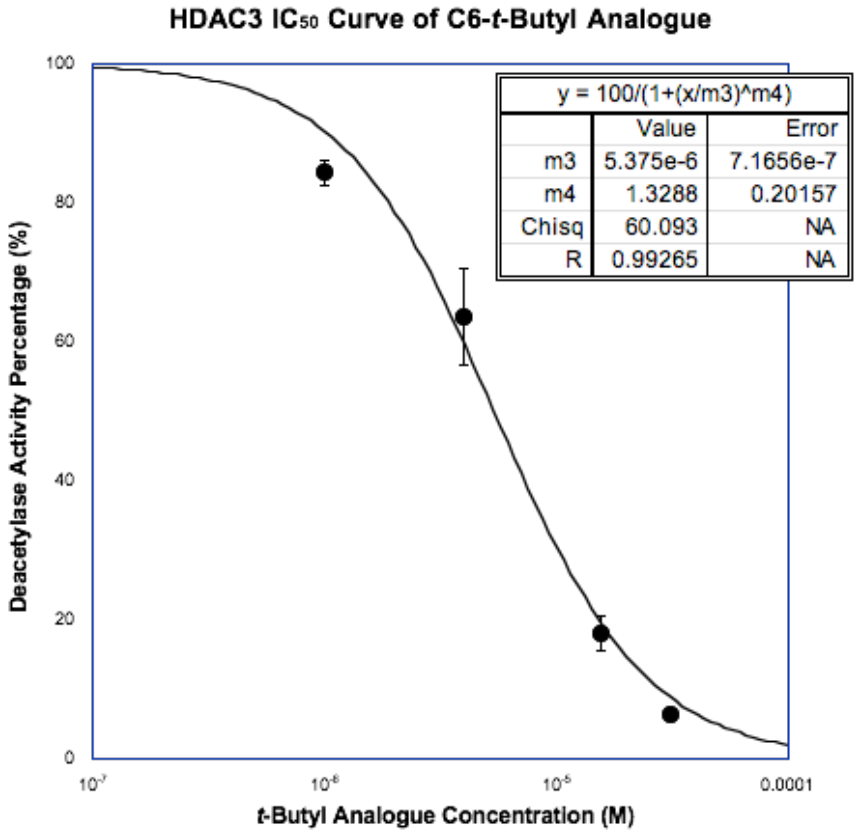


Figure S.11. Dose response curve of C6-SAHA *t*-butyl analogue **2c** tested using the HDAC3 activity from three independent trials with error bars indicating standard error (see Table S.12). In some cases, the error bar is smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 2 of the manuscript.

Table S.13. HDAC6 activity percentage after incubation of C6-SAHA *t*-butyl analogue **2c**.

Concentration (M)	Trial 1	Trial 2	Trial 3	Mean	Standard Error (S.E.)
5.00×10^{-7}	98	90	88	92	3
1.00×10^{-6}	89	82	86	85	2
2.00×10^{-6}	48	35	48	44	4
4.00×10^{-6}	45	29	45	40	5

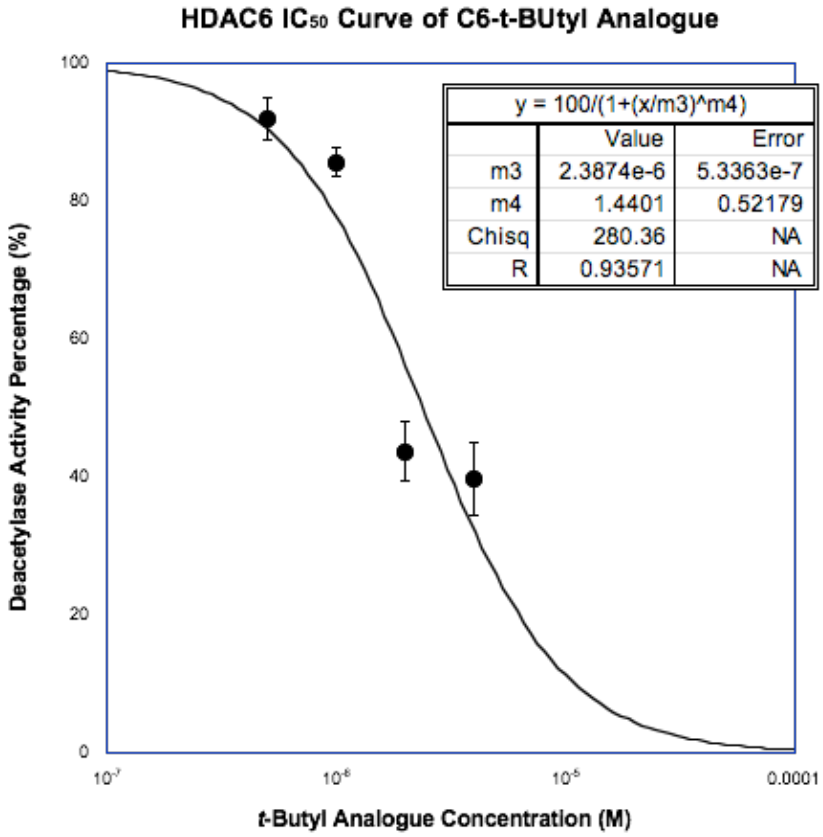


Figure S.12. Dose response curve of C6-SAHA *t*-butyl analogue **2c** tested using the HDAC6 activity from three independent trials with error bars indicating standard error. In some case, the error bar is smaller than the marker size. Data were fit to the sigmoidal curve using Kaleidograph 4.0 (Synergy Software) to determine the IC_{50}. The inset displays the results from the data analysis. The data are reported in Table 2 of the manuscript.