Supporting Information

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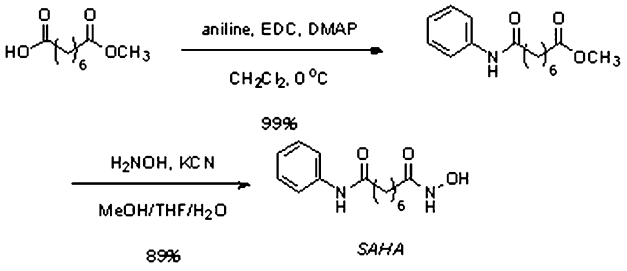
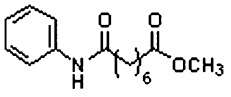
SI Materials and Methods

Synthesis of Histone Deacetylase Inhibitors. Three histone deacetylase inhibitors (HDACi) [CI-994, suberoylanilide hydroxamic acid (SAHA), and racemic trichostatin A (TSA)] and TSA were synthesized in-house using the synthetic processes shown below. Three HDACi [LBH589 (LC Laboratories), thiophene benzamide (Merck), and CI-34051 (Pharmacyclics)] were commercially available.

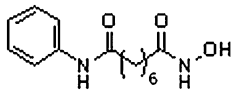
SAHA (Ref. 1 and Scheme S1).



Methyl suberoylanilide (Scheme S2). Monomethyl suberate (200 mg and 1.06 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to 0 °C. The system was placed under argon, and aniline (0.107 mL and 1.17 mmol), 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (244 mg and 1.28 mmol), and 4-(dimethylamino)pyridine (DMAP) (13 mg and 0.106 mmol) were successively added. The mixture was stirred for 4 h and then warmed to 22 °C. The reaction was diluted with CH_2Cl_2 (10 mL) and washed with 1 N HCl (10 mL) and saturated $NaHCO_3$ (10 mL). The organic layer was dried with Na_2SO_4, filtered, and evaporated, yielding 280 mg (99%) of the title compound as a white solid. Melting point (MP) = 65–66 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (d, J = 7.8 Hz, 2H), 7.31 (t, J = 7.8 Hz, 2H), 7.24 (bs, ^{1}H), 7.09 (t, J = 7.2 Hz, ^{1}H), 3.66 (s, ^{3}H), 2.35 (t, J = 7.8 Hz, 2H), 2.31 (t, J = 6.6 Hz, 2H), 1.74 (p, J = 7.2 Hz, 2H), 1.64 (p, J = 7.2 Hz, 2H), 1.41–1.32 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.4, 171.4, 138.1, 129.2, 124.3, 119.9, 51.7, 37.8, 34.1, 28.93, 28.90, 25.5, 24.9, 24.8.

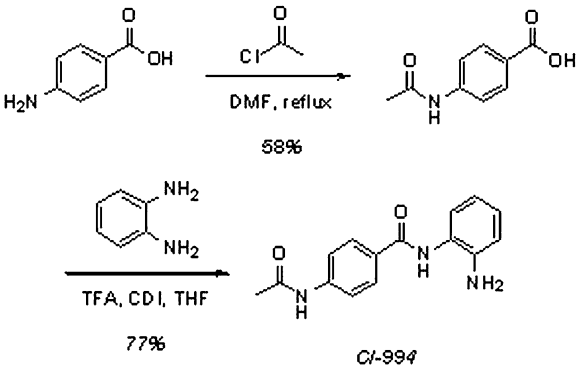


SAHA (Scheme S3). Methyl suberoylanilide (100 mg and 0.38 mmol) was dissolved in a MeOH:THF solution (1:1 and 1 mL) at 22 °C. KCN (2.5 mg and 0.038 mmol) was added followed by 50% aqueous hydroxylamine solution (0.5 mL). The reaction was stirred overnight at 22 °C. The reaction mixture was evaporated, and the remaining solid was triturated in acetonitrile and then washed with water. Drying afforded 89 mg (89%) of the title compound as a white solid. MP = 161–163 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.14 (bs, ^{1}H), 7.58 (d, J = 7.2 Hz, 2H), 7.25 (t, J = 7.8 Hz, 2H), 6.98 (t, J = 7.2 Hz, ^{1}H), 5.43 (bs, ^{1}H), 2.26 (t, J = 7.2 Hz, 2H), 1.83 (t, J = 7.8 Hz, 2H), 1.54 (p, J = 7.2 Hz, 2H), 1.43 (p, J = 7.2 Hz, 2H), 1.28–1.20 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3, 168.0, 139.4, 128.5, 122.7, 119.0, 36.2, 32.7, 28.3, 28.2, 25.5, 25.0.

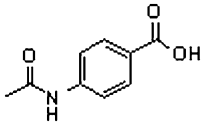


SAHA

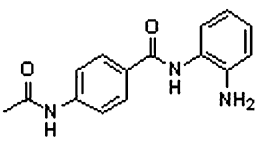
CI-994 (Ref. 2 and Scheme S4).



4-Acetamidobenzoic acid (Scheme S5). p-Aminobenzoic acid (5 g and 36.5 mmol) was added to DMF (10 mL) and stirred at 22 °C. Acetyl chloride (3 mL and 42.3 mmol) was then added by syringe. The solution was heated to reflux and stirred for 3 h. The solution was cooled to 22 °C, and water was added until a precipitate formed. The mixture was filtered, and the solid was recrystallized with EtOH/water. Filtration afforded 3.82 g (58%) of the title compound as a white solid. MP = ^{1}H NMR (300 MHz, DMSO-d6) δ 10.24 (s, ^{1}H), 7.87 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 2.07 (s, ^{3}H).

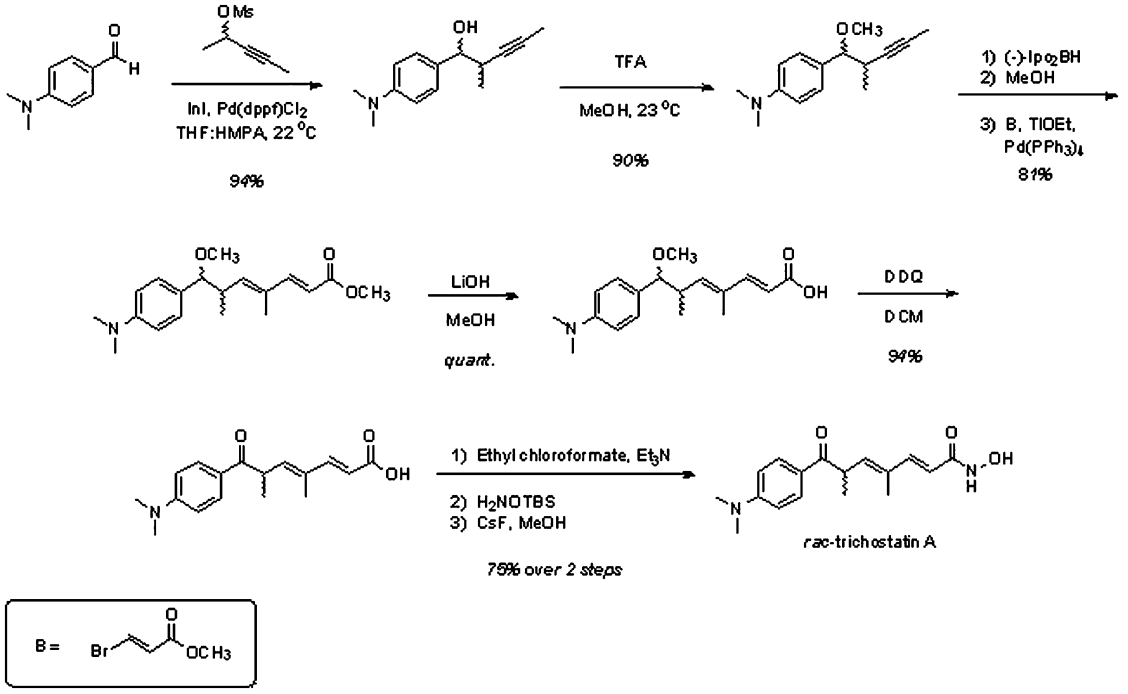


CI-994 (Scheme S6). Benzene-1,2-diamine (9.65 g and 89.4 mmol), 4-acetamidobenzoic acid (2.00 g and 11.2 mmol), and trifluoroacetic acid (TFA) (0.7 mL) were stirred in tetrahydrofuran (THF) (20 mL). When the solution turned white, 1,1'-carbonyldiimidazole (2.03 g and 12.54 mmol) was added to the solution. After 2 h at 22 °C, the solution was filtered and washed with THF to afford 2.31 g (77% yield) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6), δ 10.19 (s, ^{1}H), 9.56 (s, ^{1}H), 7.93 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 7.3 Hz, ^{1}H), 6.96 (t, J = 7.8 Hz, ^{1}H), 6.77 (d, J = 7.8 Hz, ^{1}H), δ 6.59 (t, J = 7.8 Hz, ^{1}H), 4.87 (s, 2H), 2.05 (s, ^{3}H). ^{13}C (75 MHz, DMSO-d_6), δ 168.8, 164.8, 143.2, 142.1, 128.8, 128.7, 126.7, 126.4, 123.5, 118.1, 116.3, 116.2, 24.2. high resolution mass spectrum (HRMS) [fast atom bombardment (FAB)] calculated for $C_{15}H_{15}N_3O_2$ $(M + H)^+$ 269.1164; found: 269.1175.

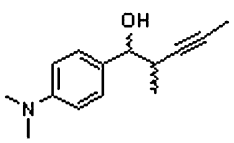


CI-994

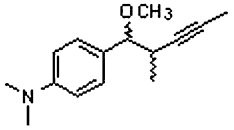
3. TSA (Ref. 3 and Scheme S7).



(±)-1-(4-(Dimethylamino)phenyl)-2-methylpent-3-yn-1-ol (Scheme S8). A flame-dried 100-mL round-bottom flask equipped with a stirring bar was charged with rac-pent-3-yn-2-yl methanesulfonate (1.0 g and 6.2 mmol), 4-N,*N*-dimethylamino benzaldehyde (0.76 g and 5.1 mmol), and a degassed solvent mixture of THF (28 mL) and hexamethylphosphoric triamide (HMPA) (7 mL). The reaction flask was kept in an ice bath for 5 min, and then, $PdCl_2$(dppf) (0.19 g, 0.25 mmol, and 5 mole percent) and InI (1.6 g and 6.6 mmol) were added successively. The red reaction mixture turned green almost immediately. The reaction mixture was quenched after 10 min by the addition of water (5 mL) and was diluted with ether (10 mL). The organic layer was separated, and the aqueous (aq) layer was further washed with ether (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and concentrated with a rotary evaporator to obtain a brown pasty mass that was purified using flash chromatography (10% EtOAc in hexane as eluent) to provide the title compound (1.04 g and 94%) as a pale yellow liquid. This compound was isolated as an inseparable mixture of syn and antidiastereomers in a ratio of 52:48 (by ^{1}H NMR). ^{1}H NMR (300 MHz, CDCl3): δH 7.25–7.21 (m, 4H), 6.74–6.70 (m, 4H), 4.62 (dd, J = 5.4, 3.6 Hz, ^{1}H), 4.33 (dd, J = 7.5, 2.7 Hz, ^{1}H), 2.96 (s, 6H), 2.95 (s, 6H), 2.86–2.78 (m, ^{1}H), 2.76–2.68 (m, ^{1}H), 2.50 (d, J = 2.7 Hz, ^{1}H) 2.1 (d, J = 3.6 Hz, ^{1}H), 1.86 (d, J = 2.4 Hz, ^{3}H), 1.80 (d, J = 2.4 Hz, ^{3}H), 1.07 (d, J = 7.2 Hz, ^{3}H), 1.01 (d, J = 6.9 Hz, ^{3}H). ^{13}C NMR (75 MHz, CDCl3): δc 150.6, 150.3, 129.8, 129.7, 127.8, 127.6, 112.5, 112.3, 81.1, 80.9, 78.9, 78.4, 77.9, 76.5, 40.9, 40.8, 35.6, 34.4, 18.0, 16.3, 3.9, 3.8. IR (film): ṽ 3,422, 2,970, 2,917, 2,875, 1,624, 15,241, 1,349 cm^{-1}. HRMS (FAB) calculated for $C_{14}H_{20}NO$ [M+H]+: 218.1545; found: 218.1531.

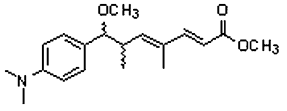


(±)-4-(1-Methoxy-2-methylpent-3-ynyl)-N,N-dimethylaniline (Scheme S9). To a solution of the preceding compound (1.4 g and 6.4 mmol) in MeOH (50 mL), a solution of 0.1% TFA in MeOH (vol/vol; 80 mL) was added, and stirring was continued at 25 °C. During the next 48 h, the light yellow solution turned dark brown, and the starting material disappeared thin layer chromatography (TLC). The reaction mixture was neutralized by careful addition of triethyl amine (TEA), and all of the volatile materials were removed with a rotary evaporator. The dark pasty mass obtained was purified by column chromatography (5% EtOAc in hexane as eluent) to provide a 1:1 mixture of syn and antidiastereomers of the title compound (1.3 g and 90%) as a pale yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δH 7.23 (d, *J* = 9.0 Hz, 2H), 7.18 (d, *J* = 8.7 Hz, 2H), 6.73 (d, *J* = 8.7 Hz, 2H), 6.72 (d, *J* = 9.0 Hz, 2H), 3.94 (d, *J* = 2.4 Hz, ^{1}H), 3.92 (d, *J* = 3.0 Hz, ^{1}H), 3.24 (s, ^{3}H), 3.23 (s, ^{3}H), 2.97 (s, 6H), 2.96 (s, 6H), 2.85–2.66 (m, 2H), 1.84 (d, *J* = 2.4 Hz, ^{3}H), 1.75 (d, *J* = 2.4 Hz, ^{3}H), 1.15 (d, *J* = 6.9 Hz, ^{3}H), 1.01 (d, *J* = 7.2 Hz, ^{3}H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 150.3, 150.2, 128.6, 128.4, 127.4, 127.3, 112.1, 112.0, 87.0, 86.5, 81.6, 81.1, 77.6, 56.9, 56.8, 40.7, 40.6, 33.6, 33.5, 17.9, 17.7, 3.9, 3.7. IR (film): ṽ 2,975, 2,931, 2,917, 2,881, 2,850, 2,814, 1,613, 1,599, 1,521, 1,348, 1,164, 1,107, 1,085 cm^{-1}. HRMS (FAB) calculated for $C_{15}H_{22}NO$ [M+H]+: 232.1701; found: 232.1700.

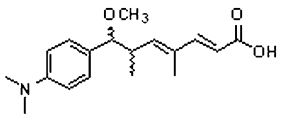


(2E,4E)-Methyl 7-(4-(dimethylamino)phenyl)-7-methoxy-4,6-dimethylhepta-2,4-dienoate (Scheme S10). (−)−Ipc_2BH (0.69 g and 2.4 mmol) was weighed in a 100-mL round-bottom flask under nitrogen. The flask was cooled to 0 °C before adding a solution of (±)-4-(1-methoxy-2-methylpent-3-ynyl)-*N*,*N*-dimethylaniline (0.47 g and 2.0 mmol) slowly dissolving in THF (6 mL). The reaction was stirred for 2 h at that temperature until the solid dissolved, and then, MeOH (0.19 mL and 4.8 mmol) was added. After the homogeneous reaction mixture was stirred at 0 °C for 2 h, a solution of B (0.49 g and 3.0 mmol) in THF (12 mL) was added to

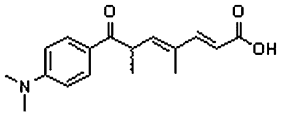
the flask. The ice bath was removed, and $Pd(PPh_3)_4$ (0.23 g, 0.20 mmol, and 10 mol%), water (6 mL), and Tl(OEt) (0.42 mL and 6 mmol) were added successively. The color of the heterogeneous solution changed from yellow to off-white almost immediately. Stirring was continued for 0.5 h at 25 °C before quenching the reaction with 1 N $NaHSO_4$ (4 mL). The reaction mixture was diluted with ether (50 mL). After a few minutes of stirring, the organic layer was separated, and the aq layer was washed with ether (2 × 10 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in a rotary evaporator under reduced pressure to obtain a pasty mass that was purified using flash chromatography (10% EtOAc in hexane as eluent) to provide the title compound as a single diene isomer (pale yellow liquid, 0.52 g and 81%) as an inseparable mixture of syn and antidiastereomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.37 (dd, J = 15.6, 0.6 Hz, ^{1}H), 7.23 (dd, J = 15.6, 0.6 Hz, ^{1}H), 7.11 (d, J = 9.0 Hz, 2H), 7.07 (d, J = 9.0 Hz, 2H), 6.71 (d, J = 9.0 Hz, 2H), 6.67 (d, J = 9.0 Hz, 2H), 5.87 (d, J = 9.3 Hz, ^{1}H), 5.78 (d, J = 15.6 Hz, ^{1}H), 5.71 (d, J = 15.6 Hz, ^{1}H), 5.70 (d, J = 9.0 Hz, ^{1}H), 3.90–3.86 (s, 2H), 3.75 (s, ^{3}H), 3.73 (s, ^{3}H), 3.19 (s, ^{3}H), 3.15 (s, ^{3}H), 2.96 (s, 6H), 2.94 (s, 6H), 2.9–2.78 (m, 2H),). 1.71 (d, J = 1.2 Hz, ^{3}H), 1.60 (d, J = 1.2 Hz, ^{3}H), 1.07 (d, J = 6.6 Hz, ^{3}H), 0.85 (d, J = 6.9 Hz, ^{3}H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_c 168.4, 168.3, 150.5, 150.4, 150.3, 145.8, 144.6, 132.8, 132.5, 128.5, 128.4, 127.9, 127.8, 115.4, 115.3, 112.4, 112.3, 88.1, 87.4, 57.0, 56.9, 51.6, 40.9, 40.8, 40.6, 40.5, 17.1, 16.9, 12.6, 12.5. IR (film): $\tilde{\nu}$ 2,930, 2,821, 1,717, 1,615, 1,520, 1,439, 1,103 cm^{-1}. HRMS (FAB) calculated for $C_{19}H_{28}NO_3$ $[M+H]^+$: 318.2069; found: 318.2072.



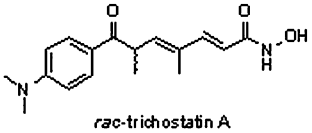
(2E,4E)-7-(4-(Dimethylamino)phenyl)-7-methoxy-4,6-dimethylhepta-2,4-dienoic acid (Scheme S11). (±)-(2*E*,4*E*)-Methyl 7-(4-(dimethylamino)phenyl)-7-methoxy-4,6-dimethylhepta-2,4-dienoate (0.55 g and 1.73 mmol) was dissolved in MeOH (15 mL), and aq LiOH solution (4.2 mL, 0.5 M in water, and 2.1 mmol) was added to it. The resulting solution was stirred at 45 °C for 24 h until the starting disappeared (TLC). The reaction mixture was neutralized by the addition of pH ~ 7 buffer at 25 °C. Most of the volatile materials were removed under reduced pressure, and the remaining yellow residue was suspended in water (10 mL). The pH of this solution was adjusted to pH 4 by the addition of 1 N HCl and was extracted with a mixed solvent (chloroform/methanol = 95/5; 4 × 40 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in a rotary evaporator to obtain the title compound as a yellow viscous liquid (0.51 g, quantitative). This material was found to be sufficiently pure by NMR and was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.44 (d, J = 15.6 Hz, ^{1}H), 7.30 (d, J = 15.6 Hz, ^{1}H), 7.12 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.7 Hz, 2H), 5.93 (d, J = 9.9 Hz, ^{1}H), 5.78 (d, J = 15.6 Hz, ^{1}H), 5.75 (d, J = 9.9 Hz, ^{1}H), 5.71 (d, J = 15.6 Hz, ^{1}H), 3.92–3.87 (m, 2H), 3.20 (s, ^{3}H), 3.16 (s, ^{3}H), 2.97 (s, 6H), 2.95 (s, 6H), 2.93–2.82 (m, 2H), 1.73 (d, J = 1.2 Hz, ^{3}H), 1.63 (d, J = 1.2 Hz, ^{3}H), 1.08 (d, J = 6.9 Hz, ^{3}H), 0.86 (d, J = 6.9 Hz, ^{3}H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_c 173.1, 173.0, 152.4, 152.3, 150.4, 150.2, 146.9, 145.6, 132.9, 132.6, 128.5, 128.4, 115.1, 114.9, 112.5, 112.4, 88.1, 87.3, 56.9, 56.8, 40.8, 40.6, 17.0, 16.8, 12.6, 12.4. IR (film): ν 2,972, 2,923, 2,820, 1,685, 1,620, 1,523, 1,280 cm^{-1}. HRMS (FAB) was calculated for $C_{18}H_{26}NO_3$ $[M+H]^+$: 304.1913; found: 304.1906.



rac-Trichostatic acid (Scheme S12). To a vigorously stirred biphasic mixture of the preceding compound (0.47 g and 1.55 mmol) in dichloromethane (DCM)/H_2O (30 mL; 2/1), dichlorodicyanoquinone (DDQ) (0.42 g and 1.86 mmol) was added in batches at 0 °C. Stirring was continued at that temperature for another 15 min, and then, the layers were separated. The aq layer was washed with DCM (3 × 15 mL). The combined organic layers were washed with water (5 × 20 mL), brined, dried over anhydrous $MgSO_4$, and concentrated with a rotary evaporator to obtain crude product. The remaining brown sticky mass was redissolved in a minimum amount of DCM (15 mL) and filtered through a small pad of celite. The filtrate was evaporated under reduced pressure to obtain *rac*-trichostatic acid (0.42 g and 94%) as a light reddish-brown solid. This material was found to be sufficiently pure by NMR and was used as such for the next step. MP = 75–78 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 7.86 (d, J = 9.0 Hz, 2H), 7.40 (d, J = 15.6 Hz, ^{1}H), 6.67 (d, J = 9.0 Hz, 2H), 6.10 (d, J = 9.6 Hz, ^{1}H), 5.84 (d, J = 15.6 Hz, ^{1}H), 4.41 (dq, J = 9.6, 6.6 Hz, ^{1}H), 3.07 (s, 6H), 1.94 (d, J = 1.2 Hz, ^{3}H), 1.33 (d, J = 6.6 Hz, ^{3}H). ^{13}C NMR (150 MHz, $CDCl_3$): δ_c 198.7, 172.2, 153.7, 151.7, 143.3, 132.9, 130.9, 124.2, 115.9, 111.1, 41.1, 40.3, 17.9, 12.7. IR (film): $\tilde{\nu}$ 3,014, 2,975, 2,928, 2,869, 1,684, 1,615, 1,595, 1,370, 1,237, 1,215, 1,184 cm^{-1}. HRMS [electrospray ionization (ESI)] calculated for $C_{17}H_{22}NO_3$ $[M+H]^+$: 288.1594; found: 288.1596.



rac-TSA (Scheme S13). To a solution of *rac*-TSA (0.39 g and 1.4 mmol) in THF (7 mL), TEA (0.39 mL and 2.8 mmol) and chloroethyl formate (0.15 mL and 1.6 mmol) were added sequentially at 0 °C. Stirring was continued at that temperature for 2 h followed by the addition of NH_2OTBS (0.31 g and 2.1 mmol) in THF (1 mL). After 0.5 h stirring, the cooling bath was removed, and the reaction mixture was allowed to warm to 25 °C, at which time it was further stirred for 1.5 h. During the work-up, the reaction mixture was diluted with ethyl acetate (25 mL), and the organic layer was washed with water (10 mL). The aq layer was further extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in a rotary evaporator to obtain crude protected hydroxamic acid, which was used as such for the next step.



rac-trichostatin A

The crude material obtained from the previous step was dissolved in anhydrous MeOH (4 mL), and CsF (0.3 g and 1.9 mmol; dried by heating for 2 h at 120 °C/0.1 mmHg) was introduced into the reaction flask. After the reaction mixture was stirred for 3 h at 25 °C, it was diluted with EtOAc (20 mL). The organic layer was washed with water (10 mL), and the aq layer was further extracted with EtOAc (3 × 20 mL). The combined organic layers were again washed with water (5 × 20 mL) to remove any water soluble impurity. The organic layer was then washed with brine, dried over anhydrous $MgSO_4$, and concentrated with a rotary evaporator at 25 °C to obtain a pale yellow pasty mass that

solidified after being kept under high vacuum. Trituration with hexane (3 × 10 mL) provided *rac*-TSA as a pale yellow solid (0.32 g and 75% starting from two steps). The spectral data were found to be in close agreement with the reported data. ^{1}H NMR (600 MHz, CD_3OD): δ_H 7.87 (d, J = 9.0 Hz, 2H), 7.18 (d, J = 15.6 Hz, ^{1}H), 6.74 (d, J = 9.0 Hz, 2H), 5.92 (d, J = 9.6 Hz, ^{1}H), 5.87 (d, J = 15.6 Hz, ^{1}H), 4.54 (dq, J = 9.6, 6.6 Hz, ^{1}H), 3.07 (s, 6H), 1.94 (d, J = 1.2 Hz, ^{3}H), 1.27 (d, J = 6.6 Hz, ^{3}H). ^{13}C NMR (150 MHz, CD_3OD): δ_c 201.6, 166.9, 155.6, 146.0, 141.4, 134.5, 132.0, 124.8, 117.2, 112.1, 41.8, 40.2, 18.4, 12.9. IR (film): ν 3,209, 2,984, 296, 1,655, 1,581, 1,539, 1,308, 1,237, 1,186 cm^{-1}. HRMS (ESI) calculated for $C_{17}H_{23}N_2O_3$ $[M+H]^+$: 303.1703; found: 303.1712.

Cell Proliferation by Nuclei Count. HDACi, Cl-995, SAHA, LBH-589, and TSA were added to Niemann-Pick type C1 (NPC1) skin fibroblasts GM03123 cells plated in 384-well plates at a range of concentrations in quadruplicate. The cells were treated with HDACi 1 d after seeding. The final DMSO concentration in each well, including control wells, was 0.2% vol/vol. The cells were incubated for 4, 24, 48, or 72 h. At the end of each time period, cells were washed with PBS, fixed with 2% PFA, and washed with PBS again. Nuclei were stained using 2 nM DRAQ5 (5 μM stock solution in water) in PBS for 45 min at room temperature. Images were acquired using a Nikon 10× Plan Fluor NA objective and Cy5 filter set. Cells were counted by using the count nuclei function of MetaXpress image analysis software. The percent reduction in the number of cells compared with the DMSO control was calculated for each concentration and time.

Lactate Dehydrogenase Cytotoxicity Assay. Cytotoxicity of hit compounds was measured by an lactate dehydrogenase (LDH) release assay kit according to the manufacturer's instructions (Roche Diagnostic). GM03123 cells were plated in 96-well plates (Costar; Corning) at a density of 3,500 cells/well and were incubated for 24 h. Compounds were added to the GM03123 cells at 0 (DMSO solvent control), 5, 10, and 20 μM concentrations in triplicate using methods similar to the dose-dependence assay. After 24-h treatment, 100 μL tissue culture supernatant were removed, and LDH activity was determined by measuring absorbance at 492 nm using a SpectraMax M2 plate reader (Molecular Devices). The experiment was repeated three times, and therefore, an average of nine data points is reported.

Time- and Dose-Dependence Assay. The dose dependence of four HDACi (CI-994, SAHA, LBH589, and TSA) was determined as a function of time after 4-, 24-, 48-, and 72-h treatment of NPC1 fibroblasts (GM03123). Two of the HDACi (CI-994 and SAHA) were tested at concentrations of 0.04, 0.12, 0.37, 1.11, 3.33, and 10 μM. The remaining two HDACi (LBH589 and TSA), which were found to be potent in initial experiments, were treated with concentrations of 0.005, 0.014, 0.040, 0.12, 0.37, and 1.11 μM. Briefly, GM03123 cells were seeded in five different 384-well plates at 450 cells/well in growth medium on day 1. To maintain the same density of cells at the final time point, compounds were added chronologically. After overnight incubation, two-time concentrated compounds diluted in growth medium supplemented with 20 mM Hepes buffer and 1% FBS were added in the first plate (for the 72-h time point) to achieve the desired final concentrations. In the second plate, compounds were added in similar fashion, 48 h after seeding the cells, and allowed to incubate for additional 48 h. In the third plate, compounds were added 72 h after seeding the cells and allowed to incubate for 24 h. Finally, in the fourth plate, compounds were added 92 h after seeding the cells and allowed to incubate for 4 h. DMSO was used as a control in each plate for each concentration. One plate without any treatment was retained as a control to monitor the distribution of cells and growth profile. All five plates were washed with PBS three times, fixed with 1.5% PFA, and stained with 50 μg/mL filipin and nuclear stain DRAQ5. Measurements were made from four wells for each condition and each compound in each experiment, and the experiment was repeated two times. Images were acquired at 10× magnification on ImageXpress^{Micro} automatic fluorescence microscope at four sites per well and analyzed to obtain the lysosomal storage organelle (LSO) compartment ratio (4). The LSO compartment ratio for each concentration was normalized to corresponding DMSO-treated control.

1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-LDL Uptake. Human NPC1 fibroblasts GM03123 cells were plated in poly-D-lysine–coated cover slip dishes in growth medium supplemented with 10% FBS. After 24 h of incubation at 37 °C, the medium was changed to one containing 5% L\lipoprotein deficient serum (LPDS). After 16–18 h of incubation at 37 °C, cells were treated separately with 10 μM CI-994, 10 μM SAHA, 0.37 μM TSA, or 0.04 μM LBH589 in medium supplemented with 5% LPDS and 20 mM Hepes. After 24-h incubation, 5 μg/mL 1,1'-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)-LDL was added to the cells and incubated for additional 24 h in the presence of various HDACi. WT GM05659 fibroblasts were used as a reference, and GM03123 cells treated with DMSO were used as a vehicle control. DiI-LDL containing medium was aspirated, and regular growth medium was added to the cells and incubated at 37 °C for 30 min to remove surface label. Cells were then washed with PBS and fixed with 1.5% PFA for 20 min at room temperature. Finally, cells were stained with filipin (50 μg/mL, 45 min at room temperature). Images were acquired using epifluorescence microscopy and appropriate filter sets for filipin and DiI. All images were corrected for background and shading. Cell area was determined based on the filipin images, and the average DiI intensity per cell area was measured.

1. Stowell JC, Huot RI, van Voast L (1995) The synthesis of *N*-hydroxy-*N*′-phenyloctanidiamide and its inhibitory effect on proliferation of AXC rat prostate cancer cells. *J Med Chem* 38:1411–1413.
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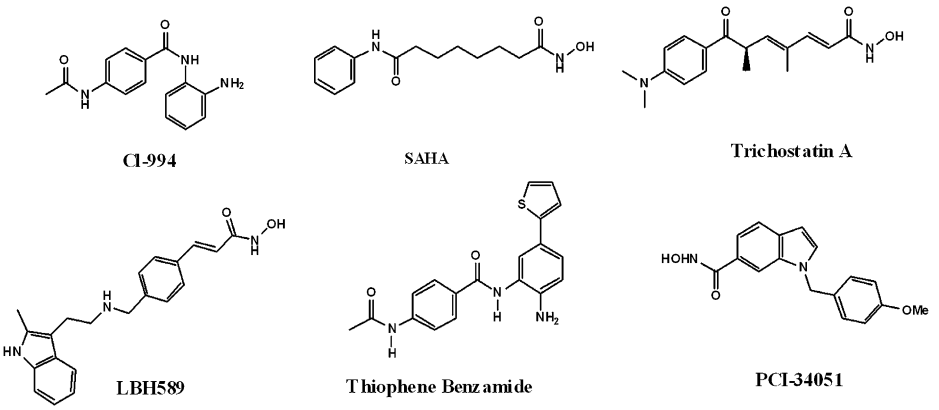

Fig. S1. Structures of HDACi.

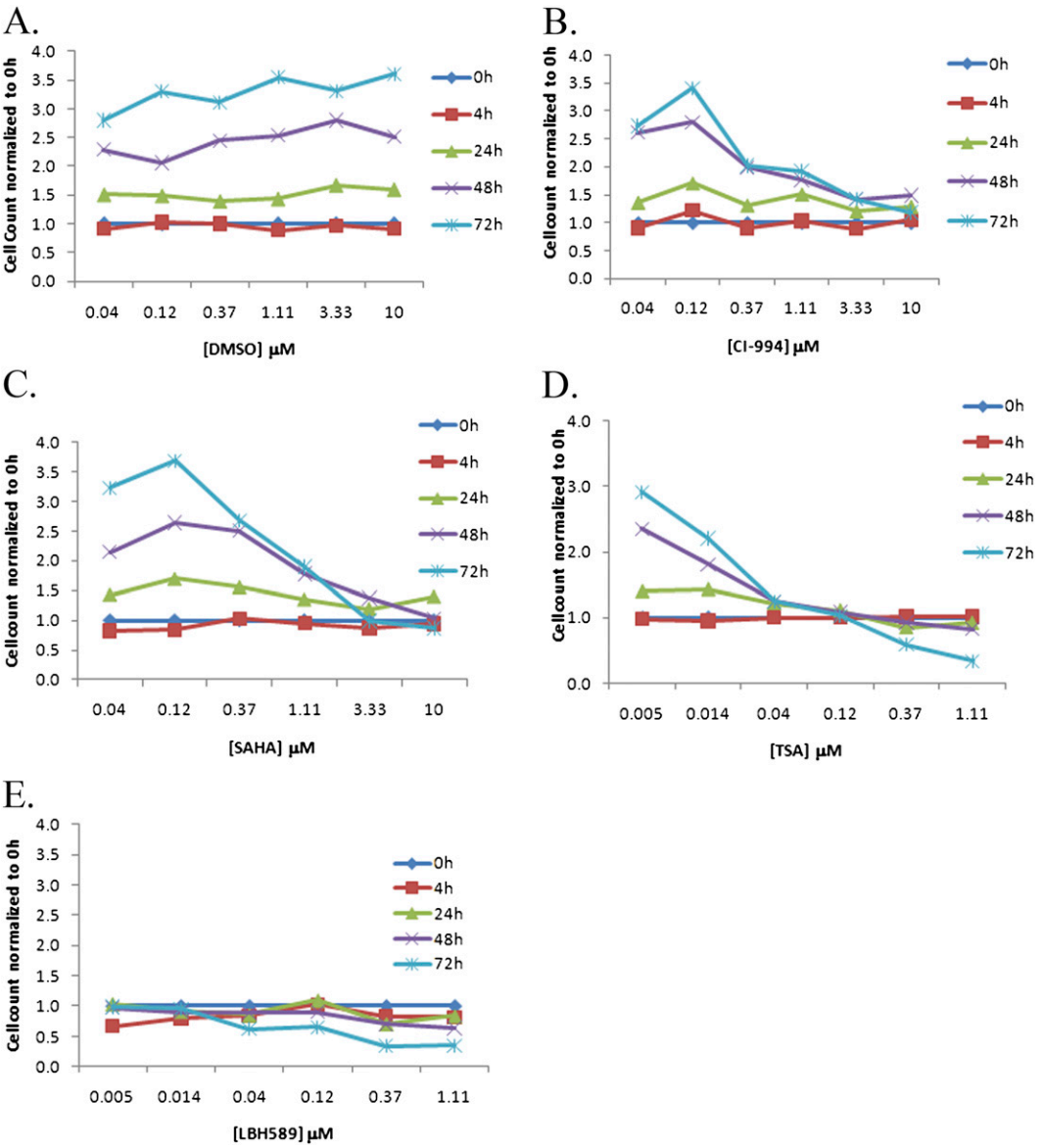

Fig. S2. Cell proliferation assay by cell count. NPC1 human fibroblasts GM03123 were seeded in five 384-well plates in growth medium. (*A* and *B*) HDACi were added to achieve the final concentrations 0.04, 0.12, 0.37, 1.11, 3.33, and 10 μM for two of the above-mentioned HDACi (CI-994, and SAHA). (*C*–*E*) Because of high potency, the remaining two HDACi (LBH-589 and TSA) were treated with a lower concentration range: 0.0037, 0.011, 0.033, 0.11, 0.33, and 1.11 μM. HDACi were added 24 h after plating the cells (*Materials and Methods*). For solvent control, cells were treated with corresponding concentration of DMSO. HDACi were added to five wells for each concentration. Finally, cells were washed with PBS, fixed with PFA, and stained with Draq5 before acquiring images for four sites per well using ImageXpress^{Micro} automated wide-field microscope at 10× magnification and 628/40-nm excitation and 692/40-nm emission filters with a 669-dichroic long pass filter. Images were analyzed to count the number of nuclei, and data were normalized to the number of cells at 0 h of compound addition. Data shown in the plots are the average of three independent experiments totaling 60 images (five wells × four sites × three experiments). Error bars represent SE.

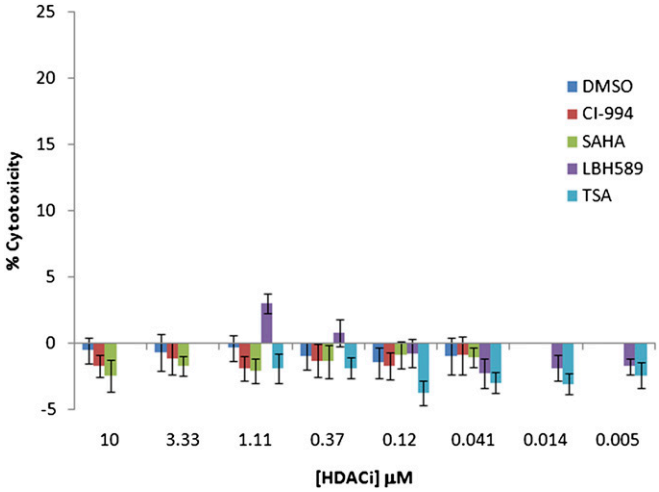

Fig. S3. Cytotoxicity assay by LDH. Cytotoxicity induced by HDACi was measured by LDH release. The percentage of cellular LDH released into the medium was measured in the presence of HDACi at various concentrations. The data were compared with reference to low (no compound treatment) and high (lysed cells) controls.

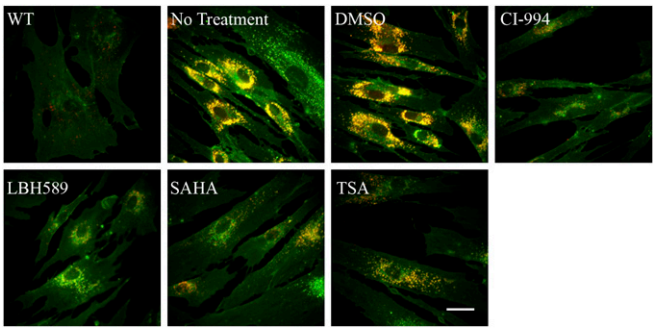

Fig. S4. Representative images showing DiI intensities after various HDACi treatments. Human NPC1 fibroblasts GM03123 were treated with various HDACi at their optimum effective concentrations and incubated for 48 h with 5 μg/mL DiI-LDL added for the final 24 h. Cells were fixed and labeled with filipin. Images were acquired to measure DiI intensities (shown in red) to evaluate LDL uptake (Fig. 5*A*). Filipin staining was used to mark the total cellular area (shown in green). Images were acquired using the ImageXpress^{Micro} imaging system as described in the text. Representative images of WT fibroblast GM05659 (control), untreated human NPC1 mutant fibroblasts GM03123, and GM03123 fibroblasts treated with DMSO (vehicle control), 10 μM CI-994, 40 nM LBH589, 10 μM SAHA, or 370 nM TSA are shown. (Scale bar, 30 μM.)

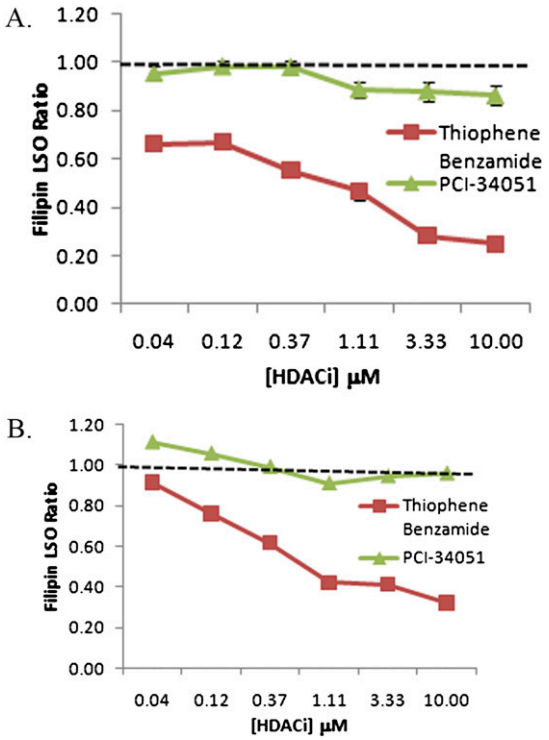

Fig. S5. Dose-dependence plots for NPC1 cells in presence of isoform-specific HDACi (set 2). NPC1 human fibroblasts GM03123 (*A*) and GM18453 (*B*) were treated with isoform-specific HDACi thiophene benzamide and PCI-34051 at various concentrations for 48 h followed by fixing, staining with filipin, and imaging. DMSO at corresponding concentrations was used as a solvent control. Images were analyzed to obtain filipin LSO ratio, and data were normalized to corresponding DMSO-treated cells. Data shown in the plots are averages of three independent experiments totaling 60 images (five wells × four sites × three experiments). The dotted horizontal lines indicated mean values for the solvent control; error bars represent SE.