Supporting Information

Inhibitors of Human Histone Deacetylase: Synthesis, Enzyme and Cellular Activity of Straight Chain Hydroxamates

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Experimental Section. All chemicals were obtained from commercial suppliers and used without further purification. Flash column chromatography was performed with silica (Merck EM9385, 230-400 mesh). ^{1}H and ^{13}C NMR spectra were recorded at 500 or 300 and at 125 or 75 MHz respectively. Proton and carbon chemical shifts are expressed in ppm relative to internal tetramethylsilane, coupling constants (*J*) are expressed in Hertz. Cells were obtained from American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA.

Method A: Amide Formation Via Mixed Anhydride. To a THF solution of **5** (1 equiv.), the aryl amine (1.3 – 1.5 equiv.) and *i*-BuOCOCl (1.1 equiv.) was added 1-methylmorpholine (1.5 – 2 equiv.) and the mixture stirred overnight. The reaction was diluted with EtOAc, washed with water, brine, dried ($MgSO_4$) and evaporated. The resulting residue was purified as described.

Method B: Hydroxamate Formation Using $HONH_2$/KOH/MeOH. KOH (2 – 4 equiv.) was added to a solution of $HONH_3Cl$ (1.5 – 2 equiv.) in MeOH (to give ca. 1 M solution of $HONH_3Cl$) and the mixture stirred at room temperature 15 – 45 min. Ester (1 equiv.) was added and the mixture stirred until no ester was present (TLC). The reaction was diluted with water (equal to volume of MeOH) and HCl added until pH ≈ 6. The solid was filtered and purified by chromatography or recrystallization.

Method C: One Pot Ester Hydrolysis and *O*-Benzylhydroxamate Formation. $LiOH•H_2O$ (2 equiv.) was added to a THF/H_2O (1:1) solution of ester (ca. 0.2 M) and the mixture was stirred overnight. $PhCH_2ONH_3Cl$ (1.5 equiv.), HOBT (1.5 equiv.) and EDCI (1.5 equiv.) were added to the solution. After 7 h the mixture was extracted with EtOAc, the combined extracts washed with brine, dried ($MgSO_4$) and evaporated. The residue was purified by chromatography or recrystallization.

Method D. Hydrogenation. The *O*-benzylhydroxamate was dissolved in EtOH, with THF used as a cosolvent if necessary, and 10% Pd/C was added (0.1 – 0.15 equiv. Pd). The resulting suspension was stirred under H_2 (balloon) overnight and filtered through Celite®. The filtrate was evaporated and the product purified by chromatography or crystallization.

***N*-Hydroxy–*N'*-(4-methoxyphenyl)-octanediamide, 7a.** A solution of **5** (4.8 g, 25.8 mmol), **6a** (3.5 g, 28.3 mmol), and EDCI (2.8 g, 14.6 mmol) in 60 mL of anhydrous THF was stirred at ambient temperature for 17 h, diluted with brine (150 mL) and extracted with EtOAc. The extract was dried ($MgSO_4$) and filtered through silica. Hexane was added to the filtrate and the resulting precipitate was filtered and dried to give 8-oxo-8-(4-methoxyphenylamino)-octanoic acid methyl ester as a white solid (5.2 g, 68%): ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 1H), 7.48 (d, 2H), 6.85 (d, 2H), 3.70 (s, 3H), 3.57 (s, 3H), 2.27 (m, 4H), 1.53 (m, 4H), 1.17 (m, 4H); ^{13}C NMR (DMSO-d_6) 173.3, 170.6, 154.9, 132.4, 120.4, 113.7, 55.0, 51.1, 36.1, 33.1, 28.3, 28.1, 24.9, 24.2; m/z 294 (MH+); Anal. ($C_{15}H_{22}N_2O_4$), C, H, N; mp 93 - 95. KOH (1.4 g, 25.0 mmol) was added to a solution of $HONH_3Cl$ (0.9 g, 13.7 mmol) in 15 mL of methanol. After 10 minutes ester (2.0 g, 7.1 mmol) was added. The mixture was filtered after 4 d, the filter cake washed with water, suspended in 0.1 M HCl (50 mL) and stirred for 2 h. The insoluble product was filtered, washed with water and dried to yield **7a** as a white powder (1.66g, 80%): ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 1H), 8.67 (s, 1H), 7.48 (d, 2H), 6.85 (d, 2H), 3.70 (s, 3H), 2.24 (t, 2H), 1.94 (t, 2H), 1.56 (t, 2H), 1.48 (t, 2H) 1.27 (d, 4H); ^{13}C NMR (DMSO-d_6) 170.6, 169.0, 154.9, 132.4, 120.5, 113.7, 55.0, 36.1, 32.2, 28.3, 28.3, 25.0, 25.0; m/z 295 (MH+); Anal. ($C_{16}H_{23}N_2O_4$), C, H, N; mp 159-160.

***N*-Hydroxy–*N*'-2-pyridinyl-octanediamide, 7b.** Method A was followed to couple **5** (4.66 g, 24.8 mmol) and **6b** (3.11 g, 32.9 mmol) to afford 8-oxo-8-(2-pyridinylamino)-octanoic acid methyl ester (1.75 g, 27%) after flash chromatography (25 – 50% EtOAc/hexanes): ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1H), 8.24 (m, 2H), 7.72 (m, 1H), 7.05 (m, 1H), 3.66 (s, 3H), 2.40 (m, 2H) 2.31 (m, 2H), 1.68 (m, 4H) 1.37 (m, 4H); ^{13}C NMR (DMSO-d_6) 174.17, 171.92, 151.55, 147.20, 138.74, 119.59, 114.22, 51.49, 37.52, 33.97, 28.78, 25.10, 24.84; m/z 265 (MH^+); Anal. ($C_{14}H_{20}N_2O_3 \bullet 0.25\ H_2O$): C, H. Method B was used to convert the ester (1.48 g, 5.60 mmol) to **7b,** which was obtained as a white solid after recrystallization from MeOH/H_2O (270 mg, 18%); ^{1}H NMR (DMSO-d_6) 1.25 (m, 4H), 1.47 (m, 2H), 1.55 (m, 2H), 1.93 (t, 7.2 Hz, 2H), 2.36 (t, 7.4 Hz, 2H), 7.06 (m, 1H), 7.06 (m, 1H), 7.74 (m, 1H), 8.08 (d, 8 Hz, 1H), 8.28 (d, 3.5 Hz) 1H, 8.67 (br. s, 1H), 10.34 (s, 1H), 10.42 (s, 1H); ^{13}C NMR (DMSO-d_6) 25.21, 25.38, 28.75, 32.60, 36.38, 113.75, 119.50, 138.42, 148.24, 152.50, 169.44, 172.56; m/z 295 (MH+); Anal. ($C_{13}H_{19}N_3O_3$): C, H, N; mp 142.3 – 143.6.

***N*-Hydroxy–*N*'-3-pyridinyl-octanediamide, 7c.** Method A was followed to couple **5** (4.77 g, 25.4 mmol) and **6c** (3.10 g, 32.9 mmol) to afford 8-oxo-8-(3-pyridinylamino)-octanoic acid methyl ester (1.99, 30%) after flash chromatography (100% EtOAc): ^{1}H NMR ($CDCl_3$) δ 8.55 (d, 2.6 Hz, 1H), 8.33 (dd, 1.3, 4.7 Hz, 1H), 8.22 (d 8.3 Hz), 8.02 (s, 1H), 7.27 (m, 1H) 3.67 (s, 3H), 2.39 (t, 7.4 Hz, 2H), 2.32 (t, 7.4 Hz, 2H), 1.74 (m, 2H), 1.64 (m, 2H), 1.36 (m 4H); m/z 265 (MH^+); Anal. ($C_{14}H_{20}N_2O_3$) C, H, N. Method B was used to convert the ester (1.91 g, 7.23 mmol) to **7c**; purification by reverse phase HPLC gave a white solid (894 mg, 47%). A sample of **7c** (355 mg) was recrystallized from water (177 mg white solid obtained): ^{1}H NMR (DMSO-d_6) 10.33 (s, 1H), 10.08 (s, 1H), 8.71 (d, 2.3 Hz, 1H), 8.66 (s, 1H), 8.23 (d, 4.6 Hz, 1H), 8.03 (d, 8.34 Hz, 1H), 7.32 (dd, 4.7, 8.3 Hz, 1H), 2.32 (t, 7.4 Hz, 2H), 1.94 (t, 7.3 Hz, 2H), 1.54 (m, 4H), 1.28 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 172.18, 169.45, 144.29, 141.05, 136.28, 126.28, 123.94, 36.56, 32.60, 28.74, 25.38, 25.23; m/z 266 (MH^+); Anal. ($C_{13}H_{19}N_3O_3$): C, H, N; mp 173.7 – 174.7.

***N*-Hydroxy–*N*'-4-pyridinyl-octanediamide, 7d.** Method A was followed to couple **5** (4.85 g, 25.8 mmol) and **6d** (3.16 g, 33.5 mmol) to afford 8-oxo-8-(4-pyridinylamino)-octanoic acid methyl ester (3.16 g, 46%) after flash chromatography (100% EtOAc): ^{1}H NMR ($CDCl_3$) δ 8.48 (dd, 1.5, 4.9 Hz, 2H), 8.13 (s, 1H), 7.52 (dd, 1.5, 4.9 Hz, 2H), 3.68 (s, 3H), 2.38 (t, 7.3 Hz, 2H), 2.32 (t, 7.3 Hz, 2H), 1.73 (m, 2H), 1.63 (m, 2H), 1.37 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 174.36,172.26, 150.52, 145.36, 113.54, 51.58, 37.50, 33.88, 28.61, 24.99, 24.56; m/z 265 (MH^+); Anal. ($C_{14}H_{20}N_2O_3$) C, H, N. Method B was used to convert the ester (3.03 g, 11.5 mmol) to **7d**, which was obtained as a white solid after crystallization from MeOH/CH_2Cl_2 (1.28 g, 42%): ^{1}H NMR (DMSO-d_6) δ 1.26 4H, 1.48 2H, 1.57 2H, 1.93 (t, 7.3Hz 2H), 2.33 (t, 7.3Hz 2H), 7.55 (dd, 1.5, 4.9 Hz), 8.39 (d, 6 Hz, 2H) 8.68 (s, 1H), 10.25 (s, 1H), 10.34 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.04, 25.37, 28.72, 32.59, 36.82, 113.39, 146.12, 150.68 169.44, 172.86; m/z 266 (MH^+); Anal. ($C_{13}H_{19}N_3O_3$): C, H, N; mp 194.5 – 195.1.

6-[[4-(Dimethylamino)benzoyl]methylamino]-hexanoic acid methyl ester, 9. NaH (164 mg, 7.50 mmol) was added to a solution of **8** (2.00 g, 6.80 mmol) in THF (68 mL) at 0 °C and the reaction was warmed to room temperature. After 30 min. MeI (979 mg, 6.80 mmol) was added, the reaction stirred for 1 h and refluxed for 5 h. The cooled mixture was concentrated under reduced pressure, the residue dissolved in EtOAc (70 mL), washed with brine (20 mL), the organic layer dried over $MgSO_4$, concentrated and the residue purified by flash chromatography (20 – 70% EtOAc/hexanes) to give **9** as a yellow oil (1.83 g, 87% yield): ^{1}H NMR ($CDCl_3$) δ 7.30 (d, 8.6 Hz, 2H), 6.64 (d, 6.6 Hz, 2H), 3.63 (s, 3H), 3.40 (m, 2H), 2.99 (s, 3H), 2.96 (s, 6H), 2.27 (t, 7.2 Hz, 2H), 1.60 (m, 4H), 1.26 (m, 2H); 13C NMR ($CDCl_3$) δ 174.0, 172.1, 151.2, 128.8, 123.7, 111.3, 51.5, 40.3, 34.0, 27.4, 26.2, 24.6; m/z 306 (MH+).

4-(Dimethylamino)-*N*-[6-(hydroxyamino)-6-oxohexyl]-*N*-methyl-benzamide, 10. Method C was used to convert **9** (2.78 g, 9.10 mmol) to 4-(dimethylamino)-*N*-methyl-*N*-[6-oxo-6-[(phenylmethoxy)amino]hexyl]-benzamide, which was obtained as an oil after flash chromatography (2% CH_3OH-EtOAc, 2.28 g, 63% yield). ^{1}H NMR ($CDCl_3$) δ 7.35-7.26 (m, 7H), 6.63 (d, 8.8 Hz, 2H),

4.84 (s, 2H), 3.39 (br s, 2H), 3.00 (s, 3H), 2.96 (s, 6H), 2.02 (br s, 2H), 1.58 (m, 4H), 1.26 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 172.3, 170.7, 151.2, 135.7, 129.0, 128.9, 128.7, 128.4, 123.3, 111.2, 77.9, 40.2, 32.8, 27.2, 26.1, 25.0; m/z 398 (MH^+). Method D was used to convert the *O*-benzylhydroxamate (1.84g, 4.60 mmol) to **10**. Flash chromatography (5% CH_3OH-CH_2Cl_2) afforded **10** as a tan solid (753 mg, 53% yield): ^{1}H NMR ($CDCl_3$) δ 7.32 (d, 8.6 Hz, 2H), 6.65 (d, 8.8 Hz, 2H), 3.43 (br s, 2H), 3.01 (s, 3H), 2.98 (s, 6H), 2.12 (br s, 2H), 1.60 (br s, 4H), 1.28 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 172.6, 170.9, 151.3, 128.9, 122.9, 111.2, 40.2, 32.5, 26.9, 25.9, 24.9; m/z 308 (MH^+); Anal. ($C_{16}H_{25}N_3O_3$•0.3 H_2O): C, H, N; mp 115.7 – 117.6.

4-(Dimethylamino)-*N*-[6-oxo-6-[methoxyamino]hexyl]-benzamide, 11. 8 (238 mg, 0.813 mmol) was converted to **11** using Method C, replacing $PhCH_2ONH_3Cl$ with $MeONH_3Cl$. After an aqueous work-up, the product was obtained as an oil which crystallized on standing. The crystals were washed with Et_2O to give **11** (150 mg, 60% yield): ^{1}H NMR ($CDCl_3$) δ 7.68 (d, 9 Hz, 2H), 6.68 (d, 9 Hz, 2H), 6.22 (br s, 1H), 3.73 (s, 3H), 3.42 (m, 2H), 3.02 (s, 6H), 1.66 (m, 4H), 1.40 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.02, 165.94, 151.94, 128.39, 121.43, 110.72, 63.06, 32.20, 29.10, 26.04, 24.68; m/z 308 (MH^+); Anal. ($C_{16}H_{25}N_3O_3$), C, H, N; mp 120.7 – 122.1.

6-[(4-Dimethylaminobenzoyl)amino]-hexanoic acid hydrazide, 12. 8 (393.2 mg, 1.345 mmol) was dissolved in 3 mL anhydrous EtOH, hydrazine hydrate (1 mL) added and the mixture was refluxed for 16 h. Evaporation afforded a solid which was recrystallized from EtOAc/*i*-PrOH to give the product as a white solid (316.0 mg, 80%): ^{1}H NMR (DMSO-d_6) δ 8.90 (s, 1H), 8.08 (s, 1H), 7.72 (d, 9 Hz, 2H), 6.68 (d, 9 Hz, 2H), 4.13 (s, 2H), 3.10 (m, 2H), 2.98 (s, 6H), 2.02 (m, 2H), 1.50 (m, 4H), 1.29 (m, 2H); ^{13}C NMR (DMSO-d_6) 171.56, 165.95, 151.94, 128.40, 110.73, 33.40, 29.16, 26.22, 25.04; m/z 293 (MH^+); Anal. ($C_{15}H_{24}N_4O_2$), C, H, N; mp 131.5 - 133.4

6-(Benzoylamino)-heptanoic acid methyl ester, 15a. 14a hydrochloride (1.06 g, 5.42 mmol), **13a** (736 mg, 6.03 mmol), HOBT, (708 mg, 5.24 mmol) and EDCI (1.21 g, 6.31 mmol) were dissolved in DMF and 1-methylmorpholine (2.30 mL, 20.9 mmol) was added dropwise. After 18 h, the mixture was

diluted with water, extracted with EtOAc and the combined organic extract washed with brine, dried ($MgSO_4$), filtered and evaporated to afford an oil. Column chromatography (50% EtOAc/hexanes) afforded **15a** as a clear, colorless oil (1.18 g, 83%): ^{1}H NMR ($CDCl_3$) δ 7.75 (m, 2H), 7.46 (m, 3H), 6.17 (br s, 1H), 3.66 (s, 3H), 3.45 (q, 6.7 Hz, 2H), 2.32 (t, 7.4 Hz, 2H), 1.63 (m, 4H), 1.38 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 174.58, 167.93, 135.22, 131.71, 128.93, 127.25, 51.89, 40.34, 34.34, 29.88, 29.15, 26.99, 25.17; m/z 264 (MH^+).

***N*-[7-(Hydroxyamino)-7-oxoheptyl]-benzamide, 16a.** Method C was followed to convert **15a** (963 mg, 3.67 mmol) to *N*-[7-(phenylmethoxyamino)-7-oxoheptyl]-benzamide, which was obtained as a white solid after addition of the reaction mixture to water and filtration of the resulting solid (1.11 g, 85 %, used without further purification): ^{1}H NMR (DMSO-d_6) δ 8.45 (t, 5.1 Hz, 1H), 7.87 (d, 6.6 Hz, 2H), 7.60-7.27 (m, 8H), 4.80 (s, 2H), 3.27 (q, 6.6 Hz, 2H), 1.98 (t, 7.4 Hz, 2H), 1.65-1.39 (m, 4H), 1.39-1.12 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 169.4, 166.1, 136.1, 134.7, 130.9, 128.7, 128.2, 127.1, 76.7, 39.2, 32.2, 29.0, 28.2, 26.2, 24.9; m/z 355 (MH^+). Method D was used to convert the *O*-benzylhydroxamate (1.80 g, 5.08 mmol) to **16a**. The crude material was passed through a plug of silica and the resulting solid was recrystallized from MeOH/EtOAc/hexanes to give **16a** as a white solid (877 mg, 65%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.45 (t, 5.5 Hz, 1H), 7.86 (m, 2H), 7.60-7.35 (m, 3H), 3.26 (q, 6.6 Hz, 2H), 1.96 (t, 7.4, 2H), 1.63-1.40 (m, 4H), 1.40-1.20 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.2, 166.1, 134.7, 130.9, 128.2, 127.1, 38.8, 32.2, 29.0, 28.3, 26.2, 25.1; m/z 287 (M^+ + Na); Anal. ($C_{14}H_{20}N_2O_3$) C, H, N; mp 120.3 – 123.4.

5-[4-(Phenylmethoxy)benzoylamino]-hexanoic acid methyl ester, 15b. Following the procedure described for the preparation of **15a**, **13b** (794.2 mg, 3.380 mmol) was coupled with **14b** (700 mg, 3.86 mmol). The reaction mixture was added dropwise to water, the resulting precipitate was filtered and dried at 50° C at 75 mm Hg to give **15b** as a solid (1.182 g, 96%) which was used without further purification: ^{1}H NMR ($CDCl_3$, 300 MHz) 7.73 (d, 10 Hz, 2H), 7.38 (m, 5H), 6.97 (d, 10 Hz, 2H), 6.11

(s, 1H), 5.11 (s, 2H), 3.66 (s, 3H), 3.43 (q, 5.6 Hz, 2H), 2.33 (t, 6.8 Hz, 2H), 1.65 (m, 4H), 1.40 (m, 2H).

***N*-[6-(Hydroxyamino)-6-oxohexyl]-4-(phenylmethoxy)-benzamide, 16b.** 3.7 M NaOMe in MeOH (0.66 mL) was added to a solution of $HONH_3Cl$ (157 mg, 2.27 mmol) in MeOH (2 mL) resulting in precipitate formation. After 1 h, methyl ester **15b** (383 mg, 1.08 mmol) and 2.0 mL MeOH was added and the mixture stirred 16 h. The suspension was filtered and the solid washed with MeOH. The solid was dissolved in MeOH/HOAc/CH_2Cl_2 (2:1:1), the mixture filtered and the filtrate evaporated to give **16b** as a white solid (251 mg, 65%). ^{1}H NMR (DMSO-d_6) • 8.33 (s, 1H), 7.84, (d, 8 Hz, 2H), 7.39, (m, 5H), 7.05 (d, 8 z, 2H), 5.15, (s, 2H), 3.22 (m, 2H), 1.96 (t, 7 Hz, 2H), 1.53, (br s, 4H), 1.28 (m, 2H); ^{13}C NMR (DMSO-*d*6) 169.03, 165.54, 160.48, 136.80, 128.97, 128.50, 127.96, 127.80, 127.21, 114.29, 69.36, 32.32, 29.02, 26.18, 25.22, 24.98; m/z 357 (MH^+); Anal. ($C_{20}H_{24}N_2O_4$•CH_2Cl_2) C, H, N; mp 210 – 230 dec.

***N*-[6-(Hydroxyamino)-6-oxohexyl]-4-hydroxybenzamide, 17.** Method D was used to convert **15b** (610 mg, 1.72 mmol) to 5-[4-(hydroxy)benzoylamino]-hexanoic acid methyl ester. Flash chromatography (85% EtOAc/Hex) provided the ester as a syrup (433 mg, 95%): ^{1}H NMR ($CDCl_3$) δ 7.66 (d, 8.6 Hz, 2H), 6.88 (d, 8.6 Hz, 2H), 6.44 (br s, 1H), 3.68 (s, 3H), 3.44 (m, 2H), 2.33 (t, 7 Hz, 2H), 1.64 (m, 4H), 1.40 (m, 2H); ^{13}C NMR ($CDCl_3$) 174.49, 168.37, 160.26, 128.87, 125.35, 115.57, 51.62, 39.93, 33.85, 29.18, 26.36, 24.41; m/z 266 (MH^+), Anal. ($C_{14}H_{19}NO_4$), C, H, N. This ester (352 mg, 1.33 mmol) was treated with $HONH_3Cl$ (194 mg, 2.79 mmol) dissolved in 2 mL MeOH and 2.7 M NaOMe in MeOH (1.10 mL). At 14 h and 38 h additional portions of $HONH_3Cl$ (1 equivalent each) and NaOMe/MeOH (1.5 equivalents each) were added. After 62 h, 5% HCl/MeOH (anhydrous, 5 mL) was added, the mixture stirred for 1 h, filtered and the filtrate evaporated to give an oil. Purification by reverse phase flash chromatography (Aldrich C-18 silica, 5% - 10% MeCN/H_2O) afforded an oil. Trituration of the oil with CH_2Cl_2/acetone afforded **17** as a yellow solid (244.6 mg, 60%): ^{1}H NMR (DMSO-d_6) • 8.66 (s, 1H), 8.17 (t, 5.5 Hz, 1H), 7.70 (d, 8.6 Hz, 2H), 6.8 (d, 8.6 Hz, 2H), 3.35 (s, 1H), 3.19 (m, 2H), 1.94 (t, 7.3 Hz, 2H), 1.50 (m, 4H), 1.27 (m, 2H); ^{13}C NMR (DMSO-d_6) 169.05, 165.74,

159.87, 128.96, 125.39, 114.66, 32.22, 29.00, 26.10, 24.90; m/z 289 (M+Na); Anal. ($C_{13}H_{18}N_2O_4$): C, H, N; mp 119.0 - 121.2.

1,3-Dihydro-*N*-hydroxy-1,3-dioxo-2*H*-isoindole-2-heptanamide, 19a. 1-Methylmorpholine was added dropwise to a mixture of **18a** (316 mg, 1.21 mmol) and *i*-BuOCOCl (0.16 mL, 1.28 mmol) in THF (10 mL), the resulting suspension stirred 6 h, then filtered. In a separate vessel, a solution of $HONH_3Cl$ (89 mg, 1.28 mmol) in pyridine (0.20 mL, 2.47 mmol) was diluted with THF (20 mL) and the above filtrate was added to the mixture. After 18 h, the suspension was diluted with EtOAc, the mixture washed with H_2O, then brine. The organic solution was dried ($MgSO_4$), filtered and evaporated. The resulting solid was recrystallized 3 times from CH_2Cl_2/hexanes to provide **19a** as a white solid (52 mg, 15%): ^{1}H NMR (DMSO-d_6) δ 10.32 (s, 1H), 8.66 (br s, 1H) 7.86 (m 4H),3.55 (t, 7.0 Hz, 2 H), 1.93 (t, 7.3 Hz, 2 H), 1.55 (m, 4H), 1.25 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 168.87, 167.84, 134.26, 131.50, 122.91, 38.59, 31.97, 27.64, 25.77, 24.60; m/z 277 (MH^+); Anal. ($C_{14}H_{16}N_2O_4$), C, H, N, mp 124.7 – 125.3.

1,3-Dihydro-*N*-hydroxy-1,3-dioxo-2*H*-isoindole-2-heptanamide, 19b. Following the procedure described for the preparation of **19a**, **18b** (304 mg, 1.10 mmol) was converted to **19b**. Recrystallization from CH_2Cl_2/hexanes afforded **19b** as a white solid (151 mg, 47 %): ^{1}H NMR (DMSO-d_6) δ 10.35 (br. s, 1H), 7.85 (m, 4H), 3.55 (t, 7.2 Hz, 2H), 1.93 (t, 7.3 Hz, 2H), 1.57 (m, 2H), 1.47 (m, 2H), 1.26 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 169.38, 168.28, 134.71, 131.95, 126.97, 123.33, 37.71, 32.51, 28.48, 28.19, 26.32, 25.32; m/z 291 (MH^+); Anal. ($C_{15}H_{18}N_2O_4$ •0.5 H_2O), C, H, N, mp 96 – 99.

***N*-Hydroxy-η-oxo-benzeneoctanamide, 21.** *O*-tritylhydroxylamine resin[1] (2.68 g, nominal loading 1.3 mmol/g) was added to a solution of **20** (2.82 g, 12.0 mmol), HOBT (1.63 g, 12.06 mmol) and EDCI (2.31 g, 12.1 mmol) in 20 mL DMF and the mixture agitated gently. After 2 d the suspension was filtered, the resin washed successively with DMF, water, THF and MeOH then air dried 4 h. The resin was suspended in 25 mL HCO_2H/THF (1:3 v/v) and agitated. After 3 h the mixture was filtered,

the resin washed with THF and MeOH and the filtrate evaporated to give **21** as a solid (646 mg): ^{1}H NMR (DMSO-d_6) δ 1.48 (s, 4H), 1.53 (m, 4H), 1.96 (t, 6 Hz, 2H), 3.33 (t, 6 Hz, 2H) 7.54 (t, 6 Hz, 2H), 7.63 (t, 6 Hz, 1H), 7.94 (d, 8 Hz, 2H), 8.65 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 23.66, 24.99, 28.32, 28.70, 32.21, 37.81, 127.82, 128.66, 132.98, 136.69, 169.08, 200.03; m/z 250 (MH^+); Anal. ($C_{14}H_{19}NO_3$): C, H, N; mp 91.0 – 92.2.

p21 Promoter Activation Assay. The assay was carried out as previously described.[2] Figure S1 shows the data from two independent experiments for the reference compound, psammaplin A. This compound reproducibly results in 18- to 22-fold activation over background of the p21 promoter as measured by quantifiying the photon output from the luciferin-luciferase reaction. Results are reported as AC_{50}, the compound concentration that results in 50% of the maximal promoter activation induced by psammaplin A, or 9- to 11-fold induction above background.

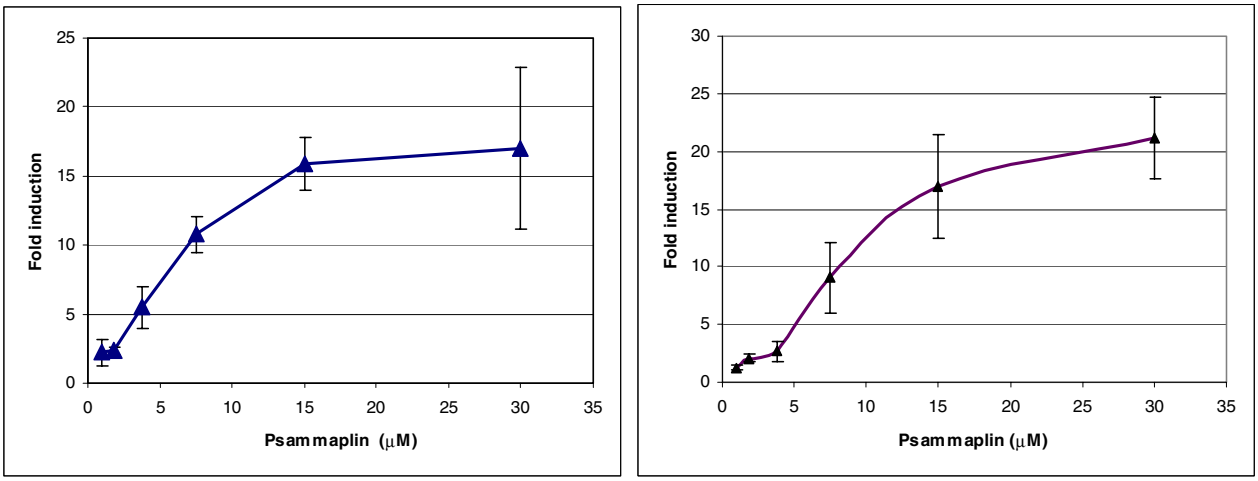

Figure S1. Representative p21 promoter induction for psammaplin A. Values are

HDAC Enzyme Assay. HDAC is partially purified from H1299, human non-small cell lung carcinoma cells. Cells were grown to 70-80% confluence in RPMI media in the presence of 10% fetal calf serum, harvested and lysed using sonication. The lysate was centrifuged at 23,420 x g for 10-15 min, the supernatant applied to a Hiload 26/10 High Performance Q-sepharose column (Amersham Pharmacia Biotech), previously equilibrated with a Buffer A (20mM Tris pH8, 0.1 mM EDTA, 10 mM NH_4Cl_2, 1 mM β-mercaptoethanol, 5% glycerol, 2 μg/mL aprotinin, 1 μg/mL leupeptin, and 400 mM

phenyl methyl sulfonyl fluoride (PMSF)) and eluted with a linear gradient of 0-500 mM NaCl in Buffer A at a flow rate of 2.5 mL/min. 4 mL fractions were collected and each was titrated for HDAC activity using a modification of the published procedure[3] to determine the optimal amount needed to obtain a signal to noise ratio of at least 5:1. The substrate used is a peptide of amino acid sequence SGRGKGGKGLGKGGAKRHRKVLRD, corresponding to the twenty-four *N*-terminal amino acids of human histone H4, biotinylated at the N-terminus and peracetylated with ^{3}H-acetate at each lysine residue. The substrate is diluted in 10 μL of Buffer B (100 mM Tris pH 8.0, 2 mM EDTA), added to the enzyme mixture and incubated at 37 °C for 1.5 h. The reaction is stopped by the addition of 20 μL of 0.5N HCl/0.08M HOAc, extracted with TBME and an aliquot of the organic layer is added to Opti-Phase Supermix liquid scintillation cocktail (Wallac). The mixture was read on a 1450 MicroBeta Trilux liquid scintillation and luminescence counter (Wallac) with a color/chemical quench and dpm correction and the data corrected for background luminescence.

Monolayer Growth Inhibition Assay. All incubations were carried out at 37 °C. The effect of the HDAIs on monolayer cell proliferation was measured using an adaptation of published procedures.[4] Cells were plated in 96-well plates at initial densities of between 1000 and 3000 cells/well and incubated. After 24 h, test compounds were added to test wells, vehicle added to vehicle control (VC) wells and initial growth control wells (IGC) received 10 μL 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) mixture (prepared on day of use at a ratio of 10 μL of a 0.92 mg/mL solution of phenazine methosulfate (PMS) to a 190 μL of a 2 mg/mL solution of MTS), the plate was incubated for 4 h and the OD_{490} of IGC wells was measured on a Molecular Devices Thermomax at 490 nm using the Softmax program to determine initial cell density values. The plates were incubated for 72 h, 10 μL/well of MTS mixture was added to the test and VC wells and the OD_{490} was measured. OD_{490} values for wells containing cells were corrected for media absorbance. The following formulas were used to calculate percent growth: If $X > T_0$, % Growth

= 100 x ((X-T_0)/(GC -T_0)), if X < T_0, % Growth = 100 x (X-T_0)/T_0); where T_0 = IGC well OD_{490} – background, GC = VC well OD_{490} – background, X = compound treated well OD_{490} – background.

Molecular Modeling. The program SYBYL[5] was used to create a homology model for HDAC-1 based on the crystal structure of HDLP. Both proteins are Class 1 HDACs and share 32% identity. Key residues which are conserved include Asp-168, His-170, and Asp-258, which chelate the Zn^{2+}, and His-131, His-132, and Tyr-297, which interact with the hydroxamic acid (HDLP numbering). His-131 and -132 form charge relays with Asp-166 and Asp-173, respectively, and these residues are also conserved. The alkyl chains of the inhibitors pass through a narrow channel in the binding pocket which is formed by Phe-141 and Phe-198, residues which are also conserved in HDAC-1. At the top of the binding pocket, Glu-92 in HDLP is mutated to Asp in HDAC-1 and Tyr-91 is mutated to Glu. Docking calculations for the analogs were carried out with the program QXP.[6] The hydroxamic acid moiety was constrained to the conformation seen in the crystal structure, while the rest of the inhibitor was free to move. Monte Carlo docking calculations were carried out for 5,000 steps. Mutated residues were allowed to relax during the minimization steps. The surface shown in Figure 2 was created using the MOLCAD option in SYBYL.

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(5) SYBYL, version 6.6.1 (Jan. 19, 2000), is a product of Tripos, Inc., 1699 South Hanley Road, Suite 303, St. Louis, Missouri 63144-2913.

(6) QXP is developed and distributed by Colin McMartin of Thistlesoft in Colebrook, Connecticut 06021; version 98.2S (1998) was used. McMartin C.; Bohacek R. S. QXP: powerful, rapid computer algorithms for structure-based drug design *J. Comput. Aided Mol. Des.* **1997**, *11*, 333 - 344.