Supporting Information

Rational Design and Simple Chemistry Yield a Superior, Neuroprotective HDAC6 Inhibitor, Tubastatin A

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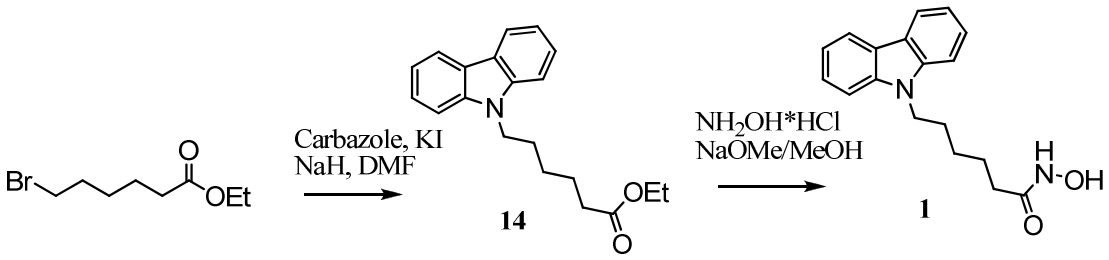
Synthetic Methods and Procedures

General Information for Synthetic Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer with TMS as an internal standard. Standard abbreviation indicating multiplicity was used as follows: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet and br = broad. ^{13}C APT experiments: up - C, CH_2; down - CH, CH_3. MS experiments were performed on a Hewlett Packard Series 1100MSD machine using electrospray ionization. HRMS experiment was performed on Q–TOF–2TM (Micromass). The progress of all reactions was monitored by TLC on precoated *silica gel* plates (Merck *Silica Gel* 60 F254). Column chromatography was performed using Merck *silica gel* (40–60 mesh). Column chromatography was performed using silica gel unless otherwise indicated. Medium pressure automated column chromatography (MPCC) was performed on a Combiflash Rf machine. Solvents and reagents were obtained from commercial sources. Solvents were anhydrous unless otherwise noted. Tubacin was provided by the laboratory of Stuart Schreiber, Harvard University.

HPLC Methods. Solvents: 0.05% TFA in water (solvent A); 0.05% TFA in 1:1 mixture of water and MeOH (solvent B); and 0.05% TFA in MeOH (solvent C). Method A: Column: Synergi 4um (150 × 4.6 mm), flow rate 1.4 mL/min. Machine: Agilent 1100. Gradient: t = 0 min, 100% A; t = 5 min, 100% B; t = 12 min, 100% C; t = 16 min, 100% C; t = 20 min, 40% A, 60% B; t = 25 min, 40% A, 60% B. Method B: Column: Synergi 4μm (150 × 4.6 mm), flow rate 1.4 mL/min. Machine: Agilent 1100. Gradient: t = 0 min, 100% A; t = 8 min, 100% B; t = 18 min, 100% C; t = 21 min, 100% C; t = 24 min, 80% A, 20% B; t = 29 min, 80% A, 20% B.

Procedures

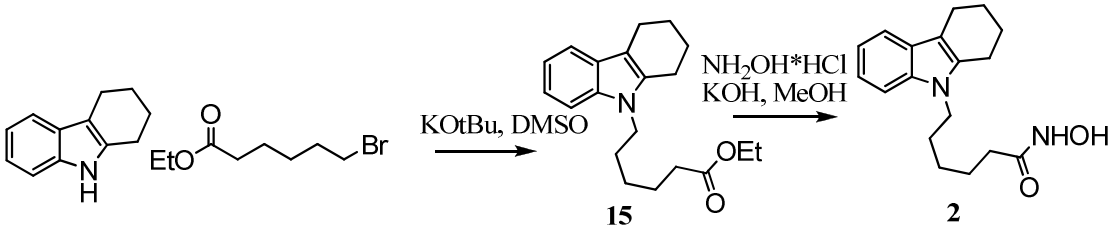
General Procedure A: To KOH (4.8 g) stirring in methanol (20 mL) at 0°C was added hydroxylamine hydrochloride (5.2 g) and allowed to stir at that temperature for 30 minutes. The mixture was filtered and the filtrate transferred to a round bottom flask. A solution of the ester starting material in a minimal amount of methanol was added to the flask and allowed to stir for 1 h. The reaction mixture was neutralized by addition of saturated aqueous NH_4Cl and the volume reduced by rotary evaporation to remove methanol. The reaction mixture was transferred to a separatory funnel with ethyl acetate (50 mL) and water (30 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated.



6-Carbazol-9-yl-hexanoic acid ethyl ester (14): Carbazole (2.0 g, 12.0 mmol) and sodium hydride (60 wt. % in mineral oil, 0.29 g, 12.0 mmol) were placed under argon and dissolved in DMF (5 mL). After stirring for 30 minutes, 6-bromo-hexanoic acid ethyl ester (2.0 mL, 12.0 mmol) and potassium iodide (10 mg) were added to the reaction. The reaction was heated to 80 °C for 2 h. The reaction was then quenched with water (30 mL) followed by addition of ethyl acetate (30 mL). The organic layer was isolated and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with water (2 x 20 mL), brine (15 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (0-80% gradient of ethyl acetate in hexane) afforded the title compound (2.7 g, 73%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (d, 2H, J = 7.7 Hz), 7.56 (d, 2H, J = 8.2 Hz), 7.42 (m, 2H), 7.26 (m, 2H), 4.34 (t, 2H, J = 7.0 Hz), 4.13 (q, 2H, J = 7.1 H), 2.29 (t, 2H, J = 7.3 Hz), 1.93 (m, 2H), 1.70 (m, 2H), 1.45 (m, 2H), 1.25 (t, 3H, J = 7.1 Hz). ^{13}C NMR (100 MHz, DMSO): δ 173.1, 140.4, 126.1, 122.5,

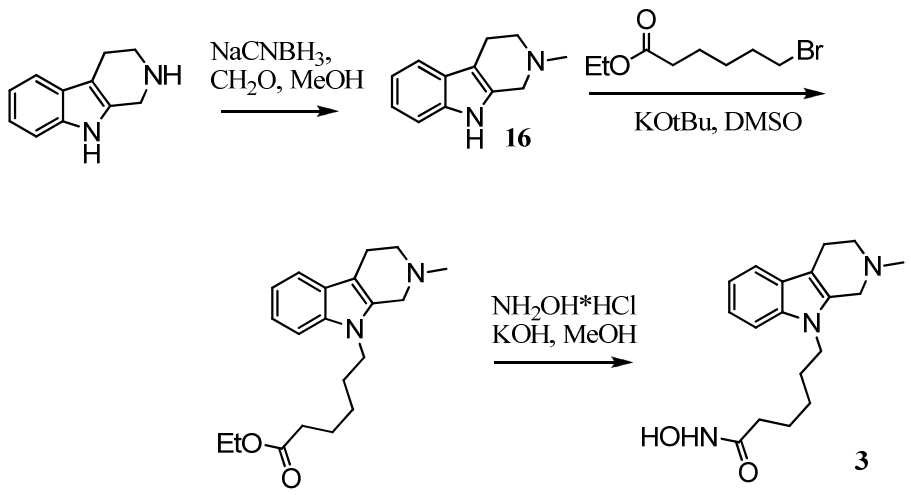
120.7, 119.0, 109.6, 60.0, 42.5, 33.8, 28.7, 26.4, 24.7, 14.5. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{23}NO_2$, 310.1802; found, 310.1792.

6-Carbazol-9-ylhexanoic acid hydroxyamide (1): 6-Carbazol-9-ylhexanoic acid ethyl ester (**14**) (1.0 g, 3.2 mmol) and hydroxylamine hydrochloride (1.4 g, 19.4 mmol) were placed under argon and dissolved in 5 mL of methanol. To it was added a 25 wt. % sodium methoxide solution in methanol (5.6 g, 25.9 mmol) which resulted in the formation of a white precipitate. The reaction was stirred for 24 h at room temperature after which the reaction was diluted with ethyl acetate (20 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (0.41 g, 41%) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ 10.29 (s, 1H), 8.64 (s, 1H), 8.15 (d, 2H, J = 7.7 Hz), 7.59 (d, 2H, J = 8.2 Hz), 7.46 (m, 2H), 7.19 (m, 2H), 4.37 (t, 2H, J = 7.1 Hz), 1.90 (t, 2H, J = 7.3 Hz), 1.76 (m, 2H), 1.51 (m, 2H), 1.30 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 169.4, 140.4, 126.1, 122.4, 120.7, 119.1, 109.6, 42.6, 32.6, 28.7, 26.6, 25.3. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{20}N_2O_2$, 297.1598; found, 297.1591. Analytical HPLC: Purity = 99%, t_R = 10.54 min, Method A.



6-(1,2,3,4-Tetrahydrocarbazol-9-yl)hexanoic acid ethyl ester (15): A RB flask fitted with reflux condenser containing tetrahydrocarbazole (1.71 g, 10.0 mol) was dissolved in DMSO (30 mL), treated with potassium tert-butoxide (1M solution in THF, 12 mL) and stirred at 110 °C for 20 min. Ethyl 6-bromohexanoate (1.67 mL, 10.0 mmol) was added and the mixture stirred at 110 °C for 60 min. The reaction was quenched with a 1:1 brine:water solution (120 mL) and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Purification by column chromatography (25% ethyl acetate in hexane) afforded the title compound (1.01 g, 32%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (m, 1H), 7.27 (m, 1H), 7.19 (m, 1H), 7.12 (m, 1H), 4.18 (q, 2H, J = 7.1 Hz), 4.04 (t, 2H, J = 7.4 Hz), 2.78-2.73 (m, 4H), 2.33 (t, 2H, J = 7.5 Hz), 2.00-1.92 (m, 4H), 1.80-1.75 (m, 2H), 1.72-1.66 (m, 2H), 1.42 (m, 2H), 1.30 (t, 3H, J = 6.9 Hz). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{27}NO_2$, 314.2115; found, 314.2103.

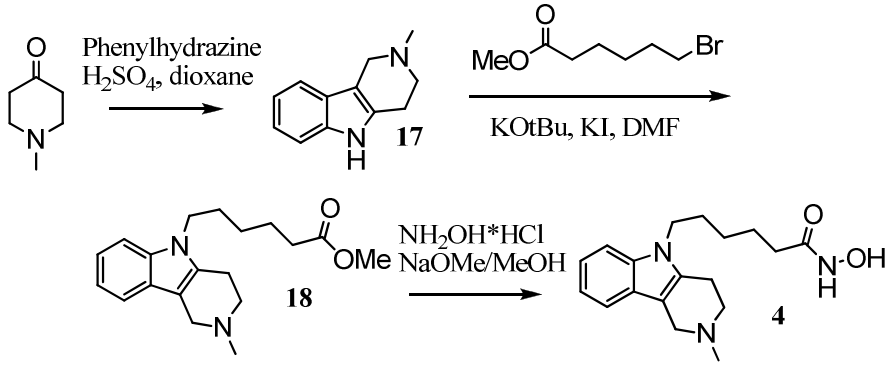
6-(1,2,3,4-Tetrahydrocarbazol-9-yl)hexanoic acid hydroxyamide (2): 6-(1,2,3,4-Tetrahydrocarbazol-9-yl)hexanoic acid ethyl ester (**15**) (200 mg, 0.64 mmol) was converted to hydroxamic acid by procedure A. Purification by HPLC afforded the product (43 mg, 22%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.35 (d, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 8.1 Hz), 7.06 (t, 1H, J = 7.7 Hz), 6.95 (t, 1H, J = 7.1 Hz), 4.02 (t, 2H, J = 7.1 Hz), 2.70 (m, 4H), 2.05 (t, 2H, J = 7.3 Hz), 1.94 (m, 2H), 1.85 (m, 2H), 1.72 (m, 2H), 1.62 (m, 2H), 1.33 (m, 2H). ^{13}C NMR APT (100 MHz, $CDCl_3$): δ 178.1 (up), 171.7 (up), 135.2 (up), 127.3 (up), 120.5 (down), 118.5 (down), 117.8 (down), 109.2 (up), 108.7 (down), 42.6 (up), 33.8 (up), 32.4 (up), 29.9 (up), 26.4 (up), 25.2 (up), 23.3 (up), 22.2 (up), 21.0 (up). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{24}N_2O_2$, 301.1911; found, 301.1898. Analytical HPLC: Purity = 100%, t_R = 8.04 min, Method A.



2-Methyl-2,3,4,9-tetrahydro-1H-β-carboline (16): 2,3,4,9-Tetrahydro-1H-β-carboline (0.50 g, 2.9 mmol) and $NaCNBH_3$ (0.44 g, 7.0 mmol) were added to a round bottomed flask, dissolved in MeOH (35 mL), and treated with 3.23 mL of a 27% solution of formaldehyde in water. This mixture was stirred for 2 h, after which, 2N HCl (50 mL) was added, followed by stirring for 15 min. The mixture was taken to pH = 11 by addition of concentrated, aqueous NaOH and extracted with methylene chloride (3 x 30 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by MPCC (0-10% gradient of MeOH in CH_2Cl_2), giving the title compound (511 mg, 95%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.39 (d, 1H, J = 7.7 Hz), 7.27 (d, 1H, J = 8.0 Hz), 7.05 (t, 1H, J = 7.5 Hz), 6.96 (t, 1H, J = 7.7 Hz), 3.68 (s, 2H), 2.86 (m, 4H), 2.53 (s, 3H). ^{13}C NMR APT (100 MHz, CD_3OD): δ 136.1 (up), 131.7 (up), 127.2 (up), 121.2 (down), 119.2 (down), 117.9 (down), 110.8 (down), 107.8 (up), 53.0 (up), 52.1 (up), 45.5 (down), 21.4 (up). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{12}H_{14}N_2$, 187.1230; found, 187.1233

6-(2-Methyl-1,2,3,4-tetrahydro-β-carbolin-9-yl)hexanoic acid hydroxyamide, trifluoroacetic acid salt (3): A round bottom flask fitted with reflux condenser containing 2-methyl-2,3,4,9-tetrahydro-1H-β-carboline (**16**) (0.20 g, 1.1 mmol), and sodium hydride (60% by wt. in mineral oil, 0.055 g, 1.35 mmol) was vacuum purged and filled with argon, followed by addition of DMF (4 mL). After stirring at 60 ℃ for 20 min, ethyl 6-bromo-hexanoate (0.24 g, 1.1 mmol) was added and the mixture was stirred at 60 ℃ for 6 h. The reaction was quenched by addition of water (30 mL), transferred to a separatory funnel and extracted with ethyl acetate (3 X 20 mL). The combined organic layers were washed with brine (2 X 20 mL), dried (Na_2SO_4) and concentrated. The product was purified by MPCC (0-10% gradient of MeOH in CH_2Cl_2), giving 190 mg of 6-(2-methyl-1,2,3,4-tetrahydro-b-carbolin-9-yl)hexanoic acid ethyl ester.

6-(2-Methyl-1,2,3,4-tetrahydro-β-carbolin-9-yl)hexanoic acid ethyl ester (150 mg) and hydroxylamine hydrochloride (190 mg, 2.74 mmol) were placed under argon and dissolved in 1 mL of methanol. To it was added a 25 wt. % sodium methoxide solution in methanol (0.7 g, 3.2 mmol) which resulted in the formation of a white precipitate. The reaction was stirred for 24 h at room temperature after which the reaction was diluted with ethyl acetate (20 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (49 mg) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.51 (d, 1H, J = 8.0 Hz), 7.42 (d, 1H, J = 8.4 Hz), 7.23 (t, 1H, J = 7.4 Hz), 7.10 (t, 1H, J = 7.1 Hz), 4.64 (br, 2H), 4.12 (m, 2H), 3.70 (br, 2H), 3.18 (m, 5H), 2.07 (t, 2H, J = 7.3 Hz), 1.80 (m, 2H), 1.63 (m, 2H), 1.35 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.7, 125.8, 125.7, 122.5, 119.9, 118.5, 109.6, 104.8, 52.2, 50.2, 43.6, 42.3, 29.1, 25.7, 24.6, 18.2 ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{25}N_3O_2$, 316.2020; found, 316.2015. Analytical HPLC: Purity = 99%, t_R = 1.58 min, Method A.

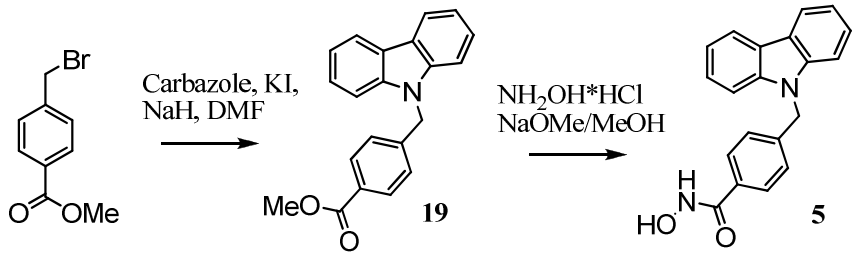


2-Methyl-2,3,4,5-tetrahydro-1H-pyrido[4,3-*b*]indole (17): Phenyl hydrazine (1.0 g, 9.3 mmols) and 1-methyl-piperidin-4-one (1.1 g, 9.3 mmols) were dissolved in 1,4-dioxane (35 mL) and cooled to 0°C. Concentrated sulfuric acid (5 mL) was added dropwise to the reaction at 0°C with stirring upon which a precipitate formed. The reaction was then heated to 60°C for one hour after which the precipitate was fully dissolved. The reaction was stirred for an additional hour at 60°C. The reaction was then cooled to room temperature and the pH was adjusted to approximately 12 by the addition of saturated aqueous sodium bicarbonate solution followed by small portions of solid sodium hydroxide. The organic products were extracted with chloroform (3x20 mL) and the combined organic extracts were washed with brine (15 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (0-80% gradient of ethyl acetate in hexane) afforded the final product (1.6 g, 93% yield) as a beige solid. ^{1}H NMR (400 MHz, DMSO): δ 10.80 (s, 1H), 7.30 (m, 2H), 6.98 (m, 2H), 3.53 (s, 2H), 2.79 (t, J = 5.2 Hz, 2H), 2.71 (t, 2H, J = 5.4 Hz), 2.43 (s, 3H). ^{13}C NMR APT (100 MHz, $CDCl_3$): δ 136.2 (up), 132.0 (up), 126.0 (up), 121.0 (down), 119.1 (down), 117.4 (down), 110.7 (down), 108.3 (up), 52.5 (up), 51.8 (up), 45.8 (down), 23.5 (up). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{12}H_{14}N_2$, 187.1230; found, 187.1228

6-(2-Methyl-1,2,3,4-tetrahydro-pyrido[4,3-*b*]indol-5-yl)hexanoic acid methyl ester (18): 2-Methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**17**) (0.50 g, 2.7 mmol) was placed under argon and dissolved in 5 mL of anhydrous DMF. Potassium *tert*-butoxide (0.32 g, 2.8 mmol) was dissolved in 3 mL of anhydrous DMF and added slowly to the reaction at room temperature. The reaction turned from orange to dark brown. After 15 min, 6-bromohexanoic acid methyl ester (0.56 g, 2.7 mmol) and 5 mg of potassium iodide were added to the reaction at room temperature. The reaction was heated to 80 °C for 2 h upon which a precipitate formed and the reaction turned from dark brown to dark orange. The reaction was then diluted with 30 mL of ethyl acetate and 30 mL of water. The organic layer was isolated and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with water (2 x 20 mL), brine (15 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by MPCC (0-80% gradient of ethyl acetate in hexane) afforded the title compound (0.35 g, 40%) as a yellow oil. ^{1}H NMR (400 MHz, DMSO): δ 7.46 (m, 2H), 7.17 (m, 1H), 7.06 (m, 1H), 4.46 (m, 2H), 4.10 (t, 2H, J = 7.0 Hz), 3.66 (m, 2H), 3.56 (s, 3H), 3.16 (m, 2H), 3.00 (s, 3H), 2.28 (t, 2H, J = 7.4 Hz), 1.66 (m, 2H), 1.55 (m, 2H), 1.29 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 173.7, 136.7, 131.3, 124.7, 121.9, 119.7, 118.0, 110.3, 101.8, 51.6, 51.0, 50.5, 42.9, 42.2, 33.6, 29.8, 26.1, 24.6, 19.8. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{19}H_{26}N_2O_2$, 315.1959; found, 315.1945.

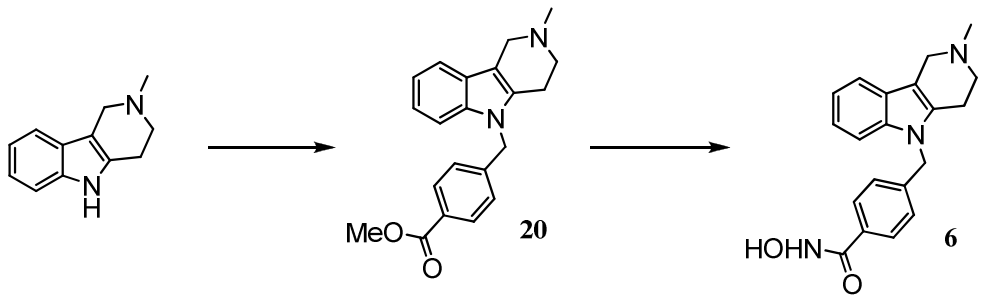
6-(2-Methyl-1,2,3,4-tetrahydro-pyrido[4,3-*b*]indol-5-yl)hexanoic acid hydroxyamide, trifluoroacetic acid salt (4): 6-(2-Methyl-1,2,3,4-tetrahydro-pyrido[4,3-*b*]indol-5-yl)hexanoic acid ethyl ester (**18**) (0.35 g, 1.1 mmol) and hydroxylamine hydrochloride (0.44 g, 6.4 mmol) were placed under argon and dissolved in 5 mL of methanol. To it was added a 25% sodium methoxide solution in methanol (1.84 g, 8.5 mmol) which resulted in the formation of a white precipitate. The reaction was stirred for 24 h at room temperature after which the reaction was diluted with 20 mL ethyl acetate and 20 mL of saturated sodium bicarbonate. The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in*

vacuo. The crude extract was purified by HPLC to yield the title compound (TFA salt, 0.11 g, 23%) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ 10.36 (s, 1H), 10.22 (s, 1H), 7.46 (m, 2H), 7.17 (t, 1H, J = 7.2 Hz), 7.06 (t, 1H, J = 7.4 Hz), 4.46 (m, 2H), 4.10 (t, 2H, J = 5.9 Hz), 3.54 (m, 2H), 3.16 (s, 2H), 3.00 (s, 3H), 1.92 (t, 2H, J = 7.3 Hz), 1.64 (m, 2H), 1.50 (m, 2H), 1.26 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 169.0, 136.3, 130.9, 124.3, 121.6, 119.4, 117.7, 109.9, 101.4, 50.6, 50.2, 42.6, 41.8, 32.2, 29.5, 25.9, 24.9, 19.5. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{18}H_{25}N_3O_2$, 316.2020; found, 316.2007. Analytical HPLC: Purity = 99%, t_R = 5.32 min, Method A.



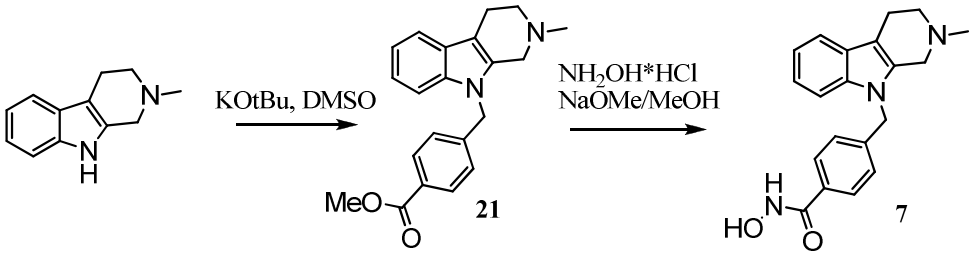
4-Carbazol-9-ylmethylbenzoic acid methyl ester (19): Carbazole (1.0 g, 6.0 mmol) and sodium hydride (60 wt. % in mineral oil, 0.14 g, 6.0 mmol) were placed under argon and dissolved in 5 mL of DMF. The mixture was stirred at room temperature for 30 min, followed by addition of 4-bromomethylbenzoic acid methyl ester (1.4 g, 6.0 mmol) and 5 mg of potassium iodide. The reaction was heated to 80 °C for 2 h upon which a precipitate formed and the reaction turned from dark brown to dark orange. The reaction was then quenched with water (30 mL) and ethyl acetate (30 mL). The organic layer was isolated and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with water (2 x 20 mL), brine (15 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (0-80% gradient of ethyl acetate in hexane) afforded the title compound (0.95 g, 50%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO): δ 8.19 (d, 2H, J = 7.7 Hz), 7.51 (d, 2H, J = 8.3 Hz), 7.41 (m, 4H), 7.23 (m, 4H), 5.74 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 166.4, 143.8, 129.9, 127.3, 126.4, 125.9, 120.8, 119.6, 118.9, 111.4, 109.9, 52.5, 45.8. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{17}NO_2$, 316.1323; found, 316.1314.

4-Carbazol-9-ylmethyl-*N*-hydroxybenzamide (5): 4-Carbazol-9-ylmethylbenzoic acid methyl ester (**19**) (1.0 g, 3.2 mmol) and hydroxylamine hydrochloride (1.3 g, 19.0 mmol) were placed under argon and dissolved in 5 mL of methanol. To it was added a 25% sodium methoxide solution in methanol (5.48 g, 25.4 mmol) which resulted in the formation of a white precipitate. The reaction was stirred for 24 h at room temperature after which the reaction was diluted with 20 mL ethyl acetate and 20 mL of saturated sodium bicarbonate. The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (0.41 g, 41%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO): δ 11.09 (s, 1H), 8.97 (s, 1H), 8.18 (d, 2H, J = 7.8 Hz), 7.61 (m, 4H), 7.43 (t, 2H, J = 8.0 Hz), 7.19 (m, 4H), 5.71 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 164.0, 140.2, 141.1, 132.0, 127.3, 126.8, 126.0, 122.3, 120.5, 119.2, 109.5, 45.4. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{17}NO_2$, 317.1149; found, 317.1143. Analytical HPLC: Purity = 99%, t_R = 10.69 min, Method A.



4-(2-Methyl-1,2,3,4-tetrahydro-pyrido[4,3-*b*]indol-5-ylmethyl)benzoic acid methyl ester (20): Potassium *tert*-butoxide (0.95 g, 8.5 mmol) was placed under argon and suspended in 1 mL of anhydrous DMF. To it was added 2-methyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole (**17**) (1.5 g, 8.1 mmol) dissolved in 3 mL of DMF upon which the reaction turned a deep orange in color. The reaction was stirred at room temperature for 15 min after which 4-bromomethyl-benzoic acid methyl ester (1.8 g, 8.1 mmol) was added in 1 mL of DMF along with approximately 5 mg of potassium iodide. The reaction then turned a light orange in color. The reaction was stirred at 80°C for two hours after which the reaction was quenched by the addition of 15 mL of water. The pH was adjusted to approximately 12 with 2N NaOH and the organic products were extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with water (15 mL), brine (15 mL), dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Purification by column chromatography (0-80% gradient of ethyl acetate in hexane) afforded the title compound (1.7 g, 61%) as a yellow oil. ^{1}H NMR (400 MHz, CD_3OD): δ 7.83 (d, 2H, J = 8.3 Hz), 7.33 (d, 1H, J = 7.8 Hz), 7.23 (m, 3H), 7.07 (m, 1H), 6.97 (m, 1H), 4.59 (s, 2H), 4.01 (s, 2H), 3.82 (s, 3H), 2.90 (t, 2H, J = 5.5 Hz), 2.84 (t, 2H, J = 5.3 Hz), 2.48 (s, 3H). ^{13}C NMR (100 MHz, MeOD): δ 167.1, 147.5, 134.9, 130.4, 129.2, 128.1, 128.0, 127.4, 120.4, 119.2, 177.7, 108.3, 108.1, 65.4, 51.0, 50.0, 40.2, 29.2, 20.3. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{22}N_2O_2$, 335.1754; found, 335.1769.

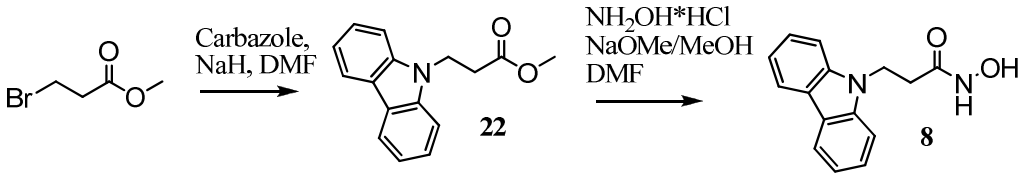
N-Hydroxy-4-(2-methyl-1,2,3,4-tetrahydro-pyrido[4,3-b]indol-5-ylmethyl)benzamide, trifluoroacetic acid salt (6): 4-(2-Methyl-1,2,3,4-tetrahydro-pyrido[4,3-*b*]indol-5-ylmethyl)benzoic acid methyl ester (**20**) (0.50 g, 1.5 mmol) and hydroxylamine hydrochloride (0.62 g, 9.0 mmol) were placed under argon and dissolved in 5 mL of methanol. To it was added a 25% sodium methoxide solution in methanol (2.6 g, 12 mmol) which resulted in the formation of a white precipitate. The reaction was stirred for 24 h after which the reaction was diluted with ethyl acetate (20 mL) and saturated sodium bicarbonate (20 mL). The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (TFA salt, 0.21 g, 31%) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ 11.17 (br, 1H), 10.17 (br, 1H), 9.00 (br, 1H), 7.67 (d, 2H, J = 7.9 Hz), 7.48 (t, 2H, J = 7.9 Hz), 7.17-7.06 (m, 4H), 5.44 (br, 2H), 4.50 (br, 2H), 3.60 (br, 2H), 3.10 (m, 2H), 3.00 (s, 3H). ^{13}C NMR APT (100 MHz, MeOD): δ 141.5 (up), 137.0 (up), 132.3 (up), 131.7 (up), 127.7 (down), 126.9 (down), 124.8 (up), 122.3 (down), 120.2 (down), 118.2 (down), 110.6 (down), 102.7 (up), 51.0 (up), 50.6 (up), 46.0 (up), 42.3 (down), 20.1 (up). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{21}N_3O_2$, 336.1707; found, 336.1708. Analytical HPLC: Purity = 100%, t_R = 5.71 min, Method A.



4-(2-Methyl-1,2,3,4-tetrahydro-β-carbolin-9-ylmethyl)benzoic acid methyl ester (21): A round bottom flask fitted with reflux condenser containing 2-methyl-2,3,4,9-tetrahydro-1H-b-carboline (**16**) (0.30 g, 1.62 mmol) and potassium tert-butoxide (0.22 g, 1.92 mmol) was vacuum purged and filled with argon, followed by addition of DMSO (5 mL). After stirring at 120 °C for 20 min, 4-bromomethyl-benzoic acid methyl ester (0.37 g, 1.62 mmol) was added and the mixture was stirred at 120 °C for 3 h. The reaction was quenched by addition of water (30 mL), transferred to a separatory funnel and extracted with ethyl acetate (3 X 20 mL). The combined organic layers were washed with brine (2 X 20 mL), dried (Na_2SO_4) and concentrated. The product was purified

by MPCC (0 to 5% gradient of MeOH in CH_2Cl_2), which gave 180 mg (33%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$)*:* δ 7.95 (d, 2H, *J* = 8.2 Hz), 7.54 (m, 1H, *J* = 7.6 Hz), 7.18-7.11 (m, 4H), 7.15 (d, 2H, *J* = 8.1 Hz), 5.25 (s, 2H), 3.89 (s, 3H), 3.53 (s, 2H), 2.90 (m, 2H), 2.80 (m, 2H), 2.51 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.5, 141.9, 137.4, 130.4, 126.0, 122.8, 120.2, 118.6, 109.4, 106.7, 52.2, 51.3, 49.7, 46.6, 45.56, 42.1, 18.5. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{22}N_2O_2$, 335.1727; found, 335.1724.

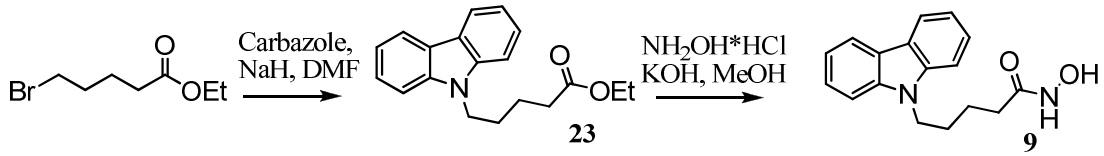
N-Hydroxy-4-(2-methyl-1,2,3,4-tetrahydro-β-carbolin-9-ylmethyl)benzamide, trifluoroacetic acid salt (7): 4-(2-Methyl-1,2,3,4-tetrahydro-b-carbolin-9-ylmethyl)benzoic acid methyl ester (**21**) (0.15 g, 0.45 mmol) and hydroxylamine hydrochloride (0.19 g, 2.7 mmol) were placed under argon and dissolved in 2 mL of methanol. To it was added a 25% sodium methoxide solution in methanol (0.76 g, 3.6 mmol) which resulted in immediate precipitation of a white solid. The reaction was stirred for 24 h at room temperature after which was taken up in 20 mL ethyl acetate and 20 mL of saturated sodium bicarbonate. The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (TFA salt, 28 mg, 14%) as a white solid. 1H NMR (400 MHz, MeOD): δ 7.70 (d, 2H, *J* = 6.63 Hz), 7.58 (d, 1H, *J* = 7.9 Hz), 7.38 (d, 1H, *J* = 8.17 Hz), 7.22 (t, 1H, *J* = 6.97 Hz), 7.13 (m, 3H), 5.46 (s, 2H), 4.49 (m, 2H), 3.49 (m, 2H), 3.20 (t, 2H, *J* = 6.48 Hz), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 206.7, 158.6, 141.2, 137.2, 132.4, 127.8, 127.0, 126.0, 122.7, 120.1, 118.89, 110.6, 106.0, 51.6, 49.5, 46.2, 42.5, 18.7. ESI-HRMS (m/z): $[M-H]^-$ calcd. for $C_{20}H_{21}N_3O_2$, 334.1561; found, 334.1535. Analytical HPLC: Purity = 98%, t_R = 8.07 min, Method B.



3-Carbazol-9-yl-propionic acid methyl ester (22): Carbazole (1.0 g, 5.98 mmol) and sodium hydride (60 wt. % in mineral oil, 0.36 g, 8.97 mmol) were placed under argon, dissolved in DMF (10 mL) and stirred for 20 min at 60 °C. This was followed by addition of 6-bromo-propanoic acid methyl ester (0.65 mL, 5.98 mmol). The reaction was stirred at 60 °C for 4 h. The reaction was then diluted with ethyl acetate (30 mL) and water (30 mL). The organic layer was isolated and the aqueous layer extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with brine (3 x 30 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by MPCC (0-20% gradient of ethyl acetate in hexane) afforded the title compound (735 mg, 49%). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.12 (d, 2H, *J* = 7.8 Hz), 7.49 (m, 4H), 7.27 (t, 2H, *J* = 6.5 Hz), 4.68 (t, 2H, *J* = 7.3 Hz), 3.67 (s, 3H), 2.89 (t, 2H, *J* = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 140.0, 125.8, 123.1, 120.4, 119.2, 108.6, 51.9, 38.7, 33.3. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{16}H_{15}NO_2$, 253.1103; found, 254.1154.

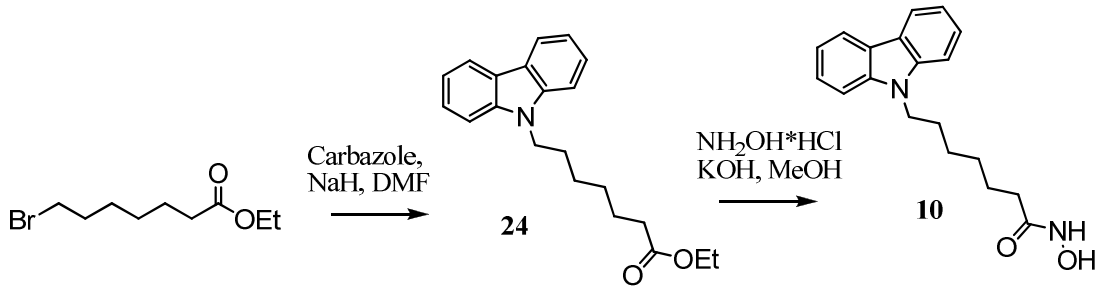
3-Carbazol-9-yl-N-hydroxy-propionamide (8): 3-Carbazol-9-yl-propionic acid methyl ester (**22**) (0.50 g, 1.97 mmol) and hydroxylamine hydrochloride (0.82 g, 12 mmol) were placed under argon and dissolved in DMF (8 mL). To it was added a 25% sodium methoxide solution in methanol (3.4 g, 16 mmol) which resulted in immediate precipitation of a white solid. The reaction was stirred for 24 h at room temperature after which was taken up in ethyl acetate (20 mL), water (10 mL) and of saturated aqueous $NaHCO_3$ (10 mL). The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (234 mg, 47%) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ 10.46 (s, 1H), 8.75 (s, 1H), 8.14 (d, 2H, *J* = 7.7 Hz), 7.60 (d, 2H, *J* = 8.0 Hz), 7.45 (t, 2H, *J* = 7.2 Hz), 7.20 (t, 2H, *J* = 7.6 Hz), 4.61 (t, 2H, *J* = 6.8 Hz), 2.48 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 140.2, 126.2, 122.6, 120.6, 119.3, 109.8, 39.3, 32.3. ESI-HRMS

(m/z): $[M+H]^+$ calcd. for $C_{15}H_{14}N_2O_2$, 255.1128; found, 255.1140. Analytical HPLC: Purity = 97%, t_R = 5.62 min, Method A.



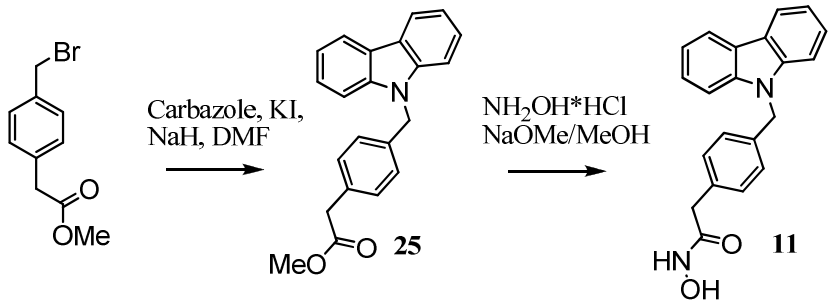
5-Carbazol-9-yl-pentanoic acid methyl ester (23): To a round bottom flask fitted with reflux condenser containing carbazole (1.00 g, 5.98 mmol) and sodium hydride (60 wt. % in mineral oil, 0.29 g, 7.18 mmol), was added DMF (22 mL). After stirring at 50 °C for 20 min, ethyl 5-bromopentanoate (0.95 mL, 5.98 mmol) was added and the mixture stirred at 80°C overnight. The reaction was quenched by addition of 5% aqueous NH_4Cl (100 mL), transferred to a separatory funnel and extracted with ethyl acetate (3 X 40 mL). The combined organic layers were washed with brine (2 X 20 mL), dried (Na_2SO_4) and concentrated. Purification by MPCC (0-40% gradient of ethyl acetate in hexane) afforded the title compound (0.87 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (d, 2H, J = 7.8 Hz), 7.51 (t, 2H, J = 7.2 Hz), 7.43 (d, 2H, J = 8.2 Hz), 7.27 (t, 2H, J = 7.4 Hz), 4.35 (t, 2H, J = 7.1 Hz), 4.13 (q, 2H, J = 7.1 Hz), 2.35 (t, 2H, J = 7.3 Hz), 1.98-1.91 (m, 2H), 1.79-1.72 (m, 2H), 1.25 (t, 3H, J = 7.1 Hz). ^{13}C NMR APT (100 MHz, $CHCl_3$): δ 173.2 (up), 140.3 (up), 125.6 (down), 122.89 (up), 120.4 (down), 118.9 (down), 108.6 (down), 60.4 (up), 42.7 (up), 33.9 (up), 28.4 (up), 22.7 (up), 14.2 (down). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{19}H_{21}NO_2$, 296.1645; found, 296.1650.

5-Carbazol-9-yl-pentanoic acid hydroxyamide (9): 5-Carbazol-9-ylpentanoic acid methyl ester (**23**) (110 mg, 0.37 mmol) was converted to hydroxamic acid by procedure A. Purification by HPLC afforded the title product (26 mg, 25%). ^{1}H NMR (400 MHz, MeOD): δ 8.07 (d, 2H, J = 7.5 Hz), 7.49 (d, 2H, J = 8.2 Hz), 7.43 (t, 2H, J = 7.7 Hz), 7.20 (t, 2H, J = 7.4 Hz), 4.40 (t, 2H, J = 7.0 Hz), 2.09 (t, 2H, J = 7.3 Hz), 1.88 (m, 2H), 1.68 (m, 2H). ^{13}C NMR APT (100 MHz, CD_3OD): δ 140.3 (up), 125.3 (down), 122.7 (up), 119.7 (down), 118.4 (down), 108.5 (down), 41.9 (up), 32.1 (up), 28.1 (up), 23.1 (up). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{17}H_{18}N_2O_2$, 283.1441; found, 283.1448. Analytical HPLC: Purity = 99%, t_R = 5.65 min, Method A.



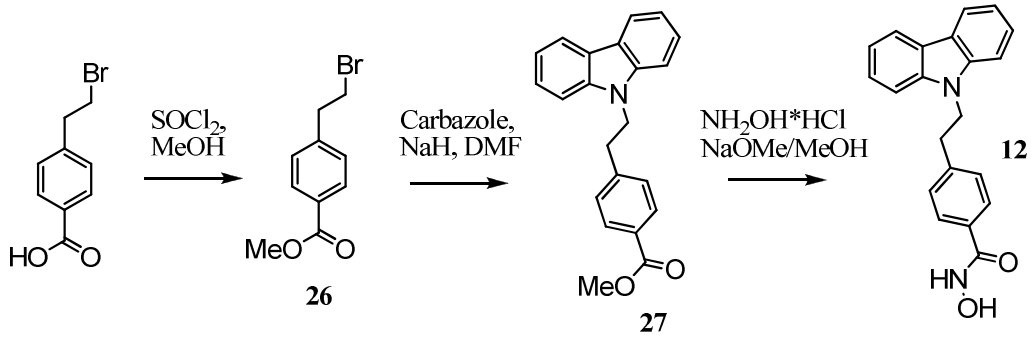
7-Carbazol-9-yl-heptanoic acid ethyl ester (24): A RB flask fitted with reflux condenser containing carbazole (0.60 g, 3.59 mmol) and sodium hydride (60 wt. % in mineral oil, 0.22 g, 5.38 mmol) was vacuum purged and filled with argon, followed by addition of DMF (16 mL). After stirring at 50 °C for 20 min, ethyl 7-bromo-heptanoate (0.85 g, 3.59 mmol) was added and the mixture stirred at 80 °C overnight. The reaction was quenched by addition of 5% aqueous NH_4Cl (75 mL), transferred to a separatory funnel and extracted with ethyl acetate (3 X 30 mL). The combined organic layers were washed with brine (2 X 20 mL), dried (Na_2SO_4) and concentrated. Purification by MPCC (0-50% gradient of ethyl acetate in hexane) afforded the title compound (0.77 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, 2H, J = 7.8 Hz), 7.52 (t, 2H, J = 7.3 Hz), 7.44 (d, 2H, J = 8.1 Hz), 7.28 (t, 2H, J = 7.7 Hz), 4.32 (t, 2H, J = 7.2 Hz), 4.16 (q, 2H, J = 7.1 Hz), 2.30 (t, 2H, J = 7.4 Hz), 1.91 (m, 2H), 1.64 (m, 2H), 1.41 (m, 4H), 1.30 (t, 3H, J = 7.2 Hz). ^{13}C NMR APT (100 MHz, $CDCl_3$): δ 173.7 (up), 140.5 (up), 125.6 (down), 122.9 (up), 120.4 (down), 118.8 (down), 108.7 (down), 60.3 (up), 43.0 (up), 34.3 (up), 28.9 (up), 27.0 (up), 24.8 (up), 14.3 (down). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{25}NO_2$, 324.1958; found, 324.1957.

7-Carbazol-9-yl-heptanoic acid hydroxyamide (10): 7-Carbazol-9-ylheptanoic acid ethyl ester (**24**) (0.25 g, 0.77 mmol) was converted to hydroxamic acid by procedure A. Purification by HPLC gave the title compound (31 mg, 13%) as a white powder. ^{1}H NMR (400 MHz, MeOD): δ 7.97 (d, 2H, J = 7.7 Hz), 7.41-7.32 (m, 4H), 7.09 (t, 2H, J = 7.1 Hz), 4.28 (t, 2H, J = 7.4 Hz), 1.91 (t, 2H, J = 7.3 Hz), 1.78 (m, 2H), 1.47 (m, 2H), 1.34-1.19 (m, 4H). ^{13}C NMR APT (100 MHz, MeOD): δ 171.5 (up), 140.3 (up), 125.2 (down), 122.6 (up), 119.6 (down), 118.3 (down), 108.5 (down), 42.1 (up), 32.2 (up), 28.5 (up), 26.4 (up), 25.2 (up). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{19}H_{22}N_2O_2$, 311.1754; found, 311.1769. Analytical HPLC: Purity = 99%, t_R = 16.17 min, Method B.



(4-Carbazol-9-yl-methyl-phenyl)acetic acid methyl ester (25): Carbazole (1.0 g, 6.0 mmol) and sodium hydride (60 wt. % in mineral oil, 0.14 g, 6.0 mmol) were placed under argon and dissolved in DMF (5 mL). The mixture was stirred at room temperature for 30 min, followed by treatment with (4-bromomethylphenyl)acetic acid methyl ester (1.5 g, 6.0 mmol) and 5 mg of potassium iodide. The reaction was heated to 80°C for 2 h. The reaction was then diluted with ethyl acetate (30 mL) and water (30 mL). The organic layer was isolated and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with water (2 x 20 mL), brine (15 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (0-80% gradient of ethyl acetate in hexane) afforded the title compound (0.71 g, 36%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, 2H, J = 7.7 Hz), 7.49 (m, 2H), 7.40 (d, 2H, J = 8.1 Hz), 7.32 (m, 2H), 7.17 (d, 2H, J = 8.1 Hz), 7.12 (d, 2H, J = 8.1 Hz), 5.52 (s, 2H), 3.71 (s, 3H), 3.61 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 140.3, 135.7, 132.8, 129.3, 126.5, 125.5, 122.8, 120.1, 118.9, 108.5, 51.7, 45.9, 40.4. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{19}NO_2$, 330.1489; found, 330.1494.

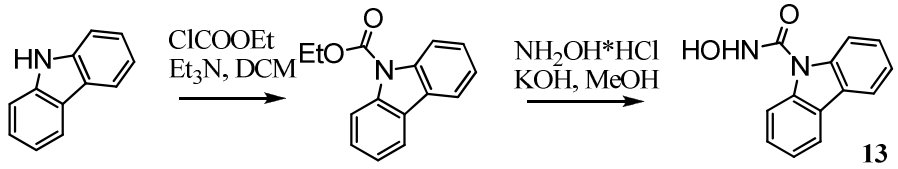
2-(4-Carbazol-9-yl-methylphenyl)-*N*-hydroxyacetamide (11): (4-Carbazol-9-ylmethylphenyl)acetic acid methyl ester (**25**) (0.25 g, 0.8 mmol) and hydroxylamine hydrochloride (0.32 g, 4.6 mmol) were placed under argon and dissolved in 5 mL of methanol. To it was added a 25% sodium methoxide solution in methanol (1.33 g, 6.2 mmol) which resulted in the formation of a white precipitate. The reaction was stirred for 24 h at room temperature after which the reaction was diluted with ethyl acetate (20 mL) and saturated aqueous sodium bicarbonate (20 mL). The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (100 mg, 40%) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ 10.57 (s, 1H), 8.73 (s, 1H), 8.17 (d, 2H, J = 7.7 Hz), 7.61 (d, 2H, J = 8.2 Hz), 7.42 (t, 2H, J = 7.5 Hz), 7.20 (t, 2H, J = 7.4 Hz), 7.11 (m, 4H), 5.62 (s, 2H), 3.18 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 168.9, 140.2, 136.1, 133.8, 128.5, 126.0, 125.0, 122.5, 119.3, 118.4, 108.3, 45.0, 38.4. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{18}N_2O_2$, 331.1441; found, 331.1445. Analytical HPLC: Purity = 99%, t_R = 6.82 min, Method A.



4-(2-Bromo-ethyl)benzoic acid methyl ester (26): 4-(2-Bromo-ethyl)-benzoic acid (1.00 g, 4.37 mmol) was dissolved in MeOH (10 mL) and cooled to 0°C. This was followed by dropwise addition of thionyl chloride (0.48 mL, 6.55 mmol). The mixture was refluxed for 2 h, followed by removal of all volatiles by rotary evaporation. The resulting oil was taken up in EtOAc (50 mL) and washed with water (50 mL). The EtOAc portion was separated, dried (Na_2SO_4) and concentrated to give the product (1.04 g, 98%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, 2H, J = 6.54 Hz), 7.29 (d, 2H, J = 7.1 Hz), 3.92 (s, 3H), 3.59 (t, 2H, J = 7.38 Hz), 3.23 (t, 2H, J = 7.34 Hz). ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{10}H_{11}BrO_2$, 243.0015; found, 243.0007.

4-(2-Carbazol-9-yl-ethyl)benzoic acid methyl ester (27): Carbazole (0.50 g, 2.99 mmol) and sodium hydride (60 wt. % in mineral oil, 0.14 g, 3.59 mmol) were placed under argon and dissolved in 6 mL of anhydrous DMF at room temperature, giving a dark brown solution. Following the evolution of hydrogen gas, 4-(2-bromo-ethyl)benzoic acid methyl ester (**26**) (0.73 g, 2.99 mmol) in DMF (2 mL) was added and the reaction was stirred at 70 °C for 2h. The mixture was taken up in ethyl acetate (30 mL) and water (30 mL), the organic layer was separated and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (2 x 25 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by MPCC (C_{18}, 10-100% gradient of MeOH in H_2O). The product co-eluted with unreacted carbazole. Cold MeOH (8 mL) was added to the product mixture and the suspension was filtered to remove the solid carbazole. The filtrate was concentrated to give the title compound (291 mg, 43%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (m, 2H), 7.92 (d, 2H, J = 8.0 Hz), 7.43 (m, 2H), 7.30 (m, 2H), 7.25 (m, 4H), 4.55 (t, 2H, J = 7.3 Hz), 3.91 (s, 3H), 3.20 (t, 2H, J = 7.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0, 144.1, 140.1, 123.0, 128.9, 125.8, 122.9, 120.4, 119.4, 110.6, 108.4, 52.1, 44.4, 35.2. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{19}NO_2$, 330.1489; found, 330.1460.

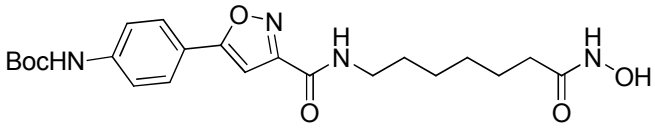
4-(2-Carbazol-9-yl-ethyl)-N-hydroxy-benzamide (12): 4-(2-Carbazol-9-ylethyl)benzoic acid methyl ester (**27**) (0.15 g, 0.46 mmol) and hydroxylamine hydrochloride (0.19 g, 2.7 mmol) were placed under argon and dissolved in DMF (3 mL). To this was added a 25% sodium methoxide solution in methanol (0.79 g, 3.6 mmol) which resulted in immediate precipitation of a white solid. The reaction was stirred for 24 h at room temperature after which it was taken up in 20 mL ethyl acetate and 20 mL of saturated sodium bicarbonate. The organic layer was isolated and the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude extract was purified by HPLC to yield the title compound (10 mg, 7%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO): δ 11.14 (br, 1H), 8.14 (d, 2H, J = 7.8 Hz), 7.62 (m, 4H), 7.40 (m, 4H), 7.18 (t, 2H, J = 7.6 Hz), 4.62 (t, 2H, J = 7.0 Hz), 3.11 (t, 2H, J = 7.6 Hz). ^{13}C NMR (100 MHz, DMSO): δ 140.2, 129.4, 127.3, 126.1, 122.5, 120.7, 119.2, 109.7, 44.1, 34.7. ESI-HRMS (m/z): $[M-H]^-$ calcd. for $C_{21}H_{18}N_2O_2$, 329.1298; found, 329.1273. Analytical HPLC: Purity = 100%, t_R = 9.13 min, Method B.



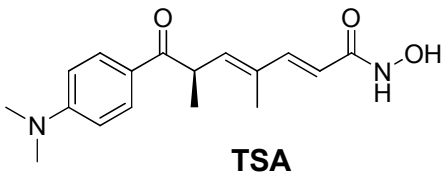
13

Carbazole-9-carboxylic acid hydroxyamide (13): An argon filled RB flask containing carbazole (0.500 g, 2.99 mmol) at 0 ℃ was treated with dichloromethane (12.5 mL) and triethylamine (2.5 mL), followed by slow addition of ethyl chloroformate (0.59 mL, 5.98 mmol). The mixture was stirred at ambient temperature for 16 h, poured into 25 mL of 2N HCl, and extracted with chloroform. The organic portion was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated. The crude product was treated with methanol (4 mL) and filtered. The filtrate was concentrated to give carbazole-9-carboxylic acid ethyl ester (169 mg).
Carbazole-9-carboxylic acid ethyl ester (95 mg, 0.39 mmol) was converted to hydroxamic acid by procedure A. Purification by HPLC gave the title compound (19 mg). ^{1}H NMR (400 MHz, CD_3CN): δ 9.43 (br, 1H), 8.08 (d, 2H, J = 7.7 Hz), 7.50 (d, 2H, J = 8.1 Hz), 7.43 (t, 2H, J = 7.1 Hz), 7.22 (t, 2H, J = 7.1 Hz). ^{13}C NMR APT (100 MHz, CD_3OD): δ 125.8 (down), 120.3 (down), 119.4 (down), 110.6 (down). ESI-MS (m/z): $[M+Na]^+$ 249.6. Analytical HPLC: Purity = 99%, t_R = 8.51 min, Method A.

Additional Chemical Structures



ISOX



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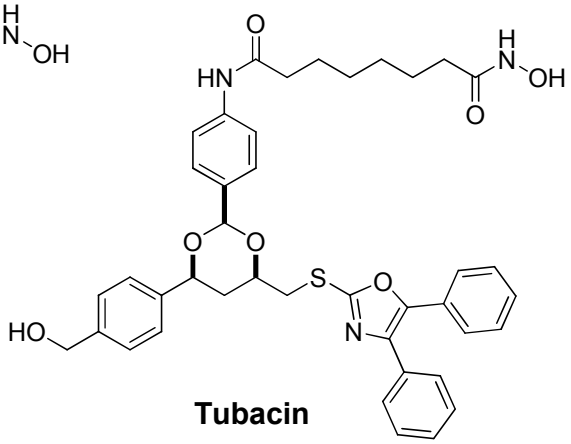
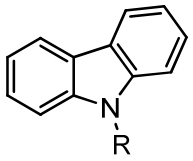
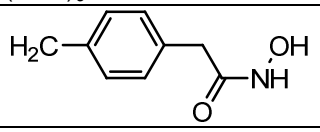
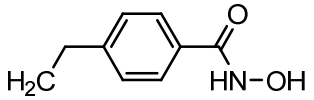


Tubacin

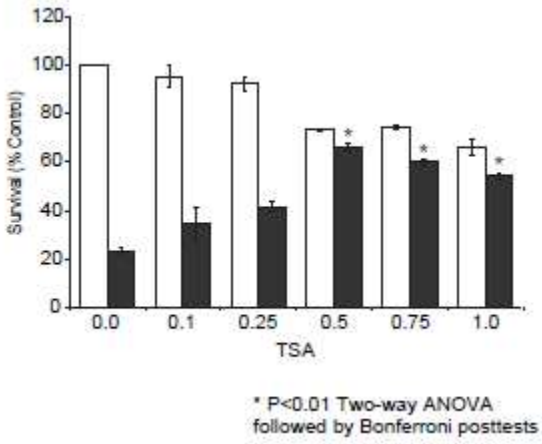
Enzyme Inhibition Assay Methods

Enzyme inhibition assays were performed by the Reaction Biology Corporation, Malvern, PA, using the Reaction Biology HDAC Spectrum platform. (www.reactionbiology.com) The HDAC1, 2, 4, 5, 6, 7, 8, 9, 10, and 11 assays used isolated recombinant human protein; HDAC3/NcoR2 complex was used for the HDAC3 assay. Substrate for HDAC1, 2, 3, 6, 10, and 11 assays is a fluorogenic peptide from p53 residues 379-382 (RHKKAc); substrate for HDAC8 is fluorogenic diacyl peptide based on residues 379-382 of p53 ($RHK_{Ac}K_{Ac}$). Acetyl-Lys(trifluoroacetyl)-AMC substrate was used for HDAC4, 5, 7, and 9 assays. Compounds were dissolved in DMSO and tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 30 µM. Control Compound Trichostatin A (TSA) was tested in a 10-dose IC_{50} with 3-fold serial dilution starting at 5 µM. IC_{50} values were extracted by curve-fitting the dose/response slopes.

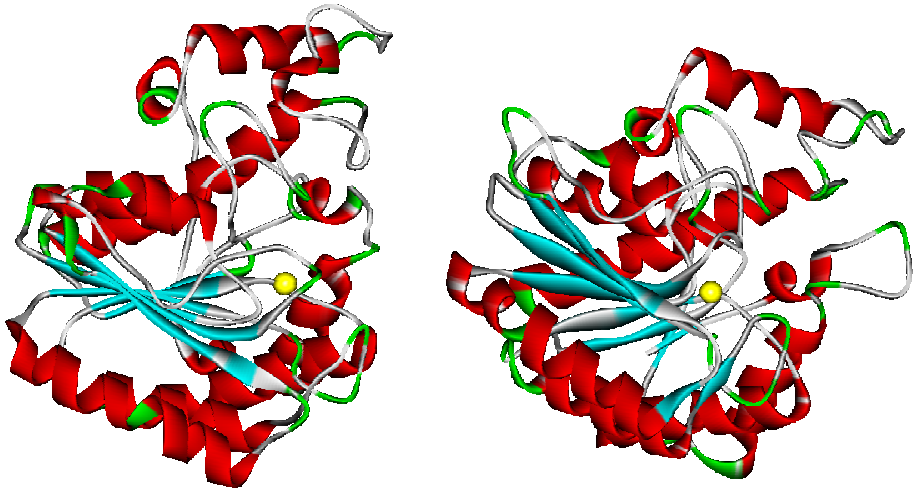
Additional Data

	 R=	HDAC1 IC_{50}(μM) ± SD	HDAC6 IC_{50}(μM) ± SD
8	$(CH2)_2CONHOH$	>30	1.59 ± 0.08
9	$(CH2)_4CONHOH$	12.8 ± 0.7	2.63 ± 0.04
10	$(CH2)_6CONHOH$	0.204 ± 0.087	0.006 ± 0.002
11		>30	0.301 ± 0.009
12		>30	0.180 ± 0.018
13	CONHOH	>30	>30

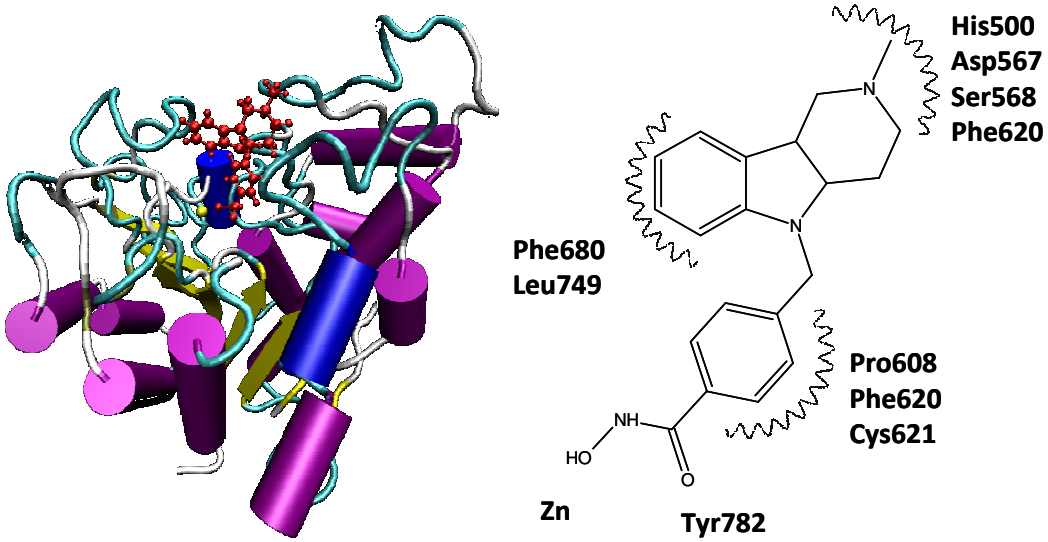
Supplementary Table 1. Enzyme inhibition data for carbazoles with various linker groups. Values are the means of two experiments. Data is shown as IC_{50} values in μM ± standard deviation. Compounds were tested in duplicate in 10-dose IC50 mode with 3-fold serial dilution starting from 30 μM solutions. IC50 values were extracted by curve-fitting the dose/response slopes.



Supplementary Figure 1. TSA Neuroprotection graph. X-axis is TSA concentration in μM. White bars are TSA alone, black bars are TSA + homocysteic acid. *, significant increase in survival relative to HCA-treated control, P < 0.01, by two-way ANOVA followed by Bonferroni post-tests.



Supplementary Figure 2 Homology models for HDAC1 (left) and HDAC6 (right) colored according to secondary motifs. The structures of the HDAC1 and HDAC6 models, similar to the structure of resolved HDACs, form a single compact α/β domain composed of a central parallel β-sheet domain surrounded by 11 α-helices. The main differences between the two models are the loops emerging from the protein core. They define the rims of the catalytic cavity, thus determining the different ligand selectivity. The catalytic site is constituted by a narrow channel, ≈ 10 Å deep, at bottom of which the zinc ion (depicted by yellow sphere) is bound. The region around the metal ion is highly conserved even if a detailed comparison of the two modeled cavities suggests that the HDAC6 channel is wider and less deep than that of HDAC1.



Supplementary Figure 3 Ribbon model for HDAC6 in complex with Tubastatin A (left) and description of the main interactions stabilizing this complex (right). The obtained docking results are in line with bioactivity values. All generated complexes for the HDAC6 isozyme are characterized by significant closeness between the Zn ion and the hydroxamic acid function, indicating tight binding. The catalytic channel is predominately lined by apolar residues thus explaining its ability to accommodate both n-pentyl and tolyl linkers. The carbazole moiety can stabilize a rich set of π–π stacking interactions with the aromatic residues surrounding the rim cavity (i.e., His500, His611, Phe620 and Phe680). Similarly, the carboline moieties preserve the aromatic contacts with His611 and Phe680 and elicit additional polar interactions between the ammonium head and Asp567 and Ser568. Similar docking analyses were carried out on the

HDAC1 cavity, where the hydroxamic acid and linker fit in the catalytic channel, but the tricyclic CAP clashes against Pro101 and Phe150 on one side, as well as P he205 and Leu271 on the other side, thus explaining the marked selectivity of these inhibitors for HDAC6.

Histone Precipitation and Western Blot Analysis

For total histone precipitation, approximately 1×10^7 treated neurons were incubated in 1 mL hypotonic lysis buffer containing 10 mM Tris-Hcl pH8, 1 mM KCl, 1.5 mM $MgCl_2$, 1 mM DTT, 1 mM Aprotinin, 1 mM Pepstatin, and 0.4 mM PMSF for 30 min. rotating at 4 ℃. Nuclei were pelleted by centrifugation for 10 min at 10,000 rpm, resuspended in 200 μL 0.4N H_2SO_4 and rotated for 12 h at 4 ℃. Following centrifugation at 13,000 rpm for 10 min the supernatant was transferred to new tube and histone proteins precipitated by adding 66 μL of 100% TCA drop-wise followed by a 30 min incubation on ice. Histone proteins were pelleted by centrifugation at 13,000 rpm for 10 min, washed twice with ice-cold acetone, dried at room temperature for 20-40 min and resuspend in 50 μL H_2O. Fifteen micrograms of total histone proteins were used in SDS-PAGE and Western blot analysis. For α-tubulin acetylation studies, total cell lysates were obtained by rinsing 5×10^6 treated cortical neurons with cold PBS followed by lysis in NP40 lysis buffer (Boston Bioproducts). Protein concentrations in lysates were quantified by Bradford assay (Bio-Rad). Fifteen micrograms of total lysate were used in SDS-PAGE and Western blot analysis. Immunodetection was performed using a Li-Cor (Li-Cor Biosciences) Odyssey system. The following primary antibodies: Acetylated α-tubulin (Sigma-Aldrich), total α-tubulin (Sigma-Aldrich), histone H4 (Millipore), acetyl histone H4 (Millipore), and MAG (Millipore).