Thiol-Based Potent and Selective HDAC6 Inhibitors Promote Tubulin Acetylation and T-Regulatory Cell Suppressive Function.

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Supporting Information

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1. CHEMISTRY

General remarks

All starting materials and solvents were purchased from commercial suppliers at reagent purity and, unless otherwise noted, were used as obtained without any further purification. Dry solvents used as media in moisture-sensitive reactions were purchased from Sigma-Aldrich at anhydrous grade and handled under argon. All reactions were carried out in dry conditions, under inert (argon) atmosphere. Microwave reactions were run in a Biotage Initiator microwave reactor. Reactions were monitored by thin layer chromatography on silica gel-coated glass plates (TLC LuxPlate Silica gel 60 F_{254}, Merck), with visualization at 254 nm, and/or using appropriate dyes. Where indicated, synthetic intermediates were purified by 230−400 mesh silica gel flash chromatography on a CombiFlash system, using appropriate solvent mixtures. Final products were purified by preparative HPLC using a Shimadzu preparative liquid chromatograph [ACE 5AQ (150 × 21.2 mm) with 5 μm particle size. Method 1: 25−100% MeOH/H_2O, 30 min; 100% MeOH, 5 min; 100−25% MeOH/H_2O, 4 min. Method 2: 8−100% MeOH/H_2O, 30 min; 100% MeOH, 5 min; 100−8% MeOH/H_2O, 4 min. Method 3: 0% MeOH, 5 min; 0−100% MeOH/H_2O, 25 min; 100% MeOH, 5 min; 100−0% MeOH/H_2O, 4 min. Flow rate = 17 mL/min], with monitoring at 254 and 280 nm. Both solvents were spiked with 0.05% TFA. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100.6 MHz, respectively, on Bruker DPX-400 or AVANCE-400 spectrometers. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to TMS. ^{1}H NMR spectra are reported in this order: multiplicity and number of protons; signals were characterized as: *s* (singlet), *d* (doublet), *dd* (doublet of doublets), *t* (triplet), *m* (multiplet), *bs* (broad signal). HRMS spectra were recorded using ESI with an LCMS-IT-TOF (Shimadzu). Purity of all final compounds was determined by analytical HPLC [ACE 3AQ C18 column (150 × 4.6 mm, particle size 3 μM); 0.05% TFA in H2O/0.05% TFA in MeOH gradient eluting system; flow rate = 1.0 mL/min]. All compounds were tested at >95% purity as determined by HPLC analysis.

Products synthesis and characterization

General procedure A: synthesis of *tert*-Butyl (4-bromobutyl)carbamate (6a).

To a solution of *tert*-butyl (4-hydroxybutyl)carbamate (**5a**) (1.0 g, 5.3 mmol) and PPh_3 (2.09 g, 8 mmol) in 20 mL of THF, a solution of CBr_4 (2.7 g, 8 mmol) in 10 mL of THF was added dropwise, under stirring, at 0 °C. The mixture was allowed to warm to room temperature and stirred for 4 h. The solvent was evaporated *in vacuo*, then the residue was purified by silica gel flash chromatography, eluting with hexanes, then hexanes/ethyl acetate, from 95/5 to 8/2, affording 1.31 g (5.2 mmol, 98% yield) of pure, target product, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.54 (*bs*, 1 H); 3.44 (*t*, 2 H); 3.17-3.14 (m, 2 H); 1.92-1.87 (*m*, 2 H); 1.69-1.63 (*m*, 2 H); 1.46 (*s*, 9 H).

Synthesis of *tert*-Butyl (5-bromopentyl)carbamate (6b).

Compound **6b** (colorless oil, 0.856 g, 3.2 mmol, 95% yield) was prepared from *tert*-butyl (5-hydroxypentyl)carbamate, according to General procedure A.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.53 (*bs*, 1 H); 3.42 (*t*, 2 H); 3.14-3.13 (*m*, 2 H); 1.91-1.87 (*m*, 2 H); 1.50-1.46 (*m*, 13 H).

Synthesis of *tert*-Butyl (6-bromohexyl)carbamate (6c).

Compound **6c** (colorless oil, 3 g, 10.7 mmol, 78% yield) was prepared from *tert*-butyl (6-hydroxyhexyl)carbamate (**5c**), according to General procedure A.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.58 (*bs*, 1 H); 3.38 (*t*, 2 H); 3.09-3.08 (*m*, 2 H); 1.85-1.80 (*m*, 2 H); 1.49-1.40 (*m*, 13 H); 1.33-1.27 (*m*, 2 H).

General procedure B: synthesis of *tert*-Butyl (4-(quinolin-8-ylamino)butyl)carbamate (7a).

A microwave vessel, equipped with a magnetic stir bar and a septum, was charged with a mixture of cesium carbonate (3.26 g, 10 mmol), 8-aminoquinoline (0.59 g, 5.2 mmol), and **6a** (1.31 g, 5.2 mmol) in DMF (4 mL). The vessel was sealed and irradiated at 120 °C for 40 min. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate, and the organic layer was washed with 10% aqueous lithium chloride. The organic fraction was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by silica gel flash chromatography, eluting with hexanes/diethyl ether, from 95/5 to 7/3, affording 0.28 g (0.9 mmol, 17% yield) of pure, target product, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.71 (*dd*, 1 H); 8.06 (*dd*, 1 H); 7.41-7.36 (*m*, 2 H); 7.05 (*d*, 1 H); 6.67 (*d*, 1 H); 6.14 (*bs*, 1 H); 4.57 (*bs*, 1 H); 3.38-3.34 (*m*, 2 H); 3.22-3.20 (*m*, 2 H); 1.86-1.79 (*m*, 2 H); 1.73-1.66 (*m*, 2 H); 1.46 (*s*, 9 H).

Synthesis of *tert*-Butyl (5-(quinolin-8-ylamino)pentyl)carbamate (7b).

Compound **7b** (colorless oil, 0.179 g, 0.5 mmol, 17% yield) was prepared from compound **6b**, according to General procedure B.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.72-8.71 (*m*, 1H), 8.06 (*dd*, 1 H); 7.41-7.35 (*m*, 2 H); 7.04 (*dd*, 1 H); 6.67 (*d*, 1 H); 4.55 (*bs*, 1 H); 3.76 (*t*, 2 H); 3.33-3.32 (*m*, 2 H); 3.17-3.16 (*m*, 2 H); 1.88-1.80 (*m*, 2 H); 1.60-1.51 (*m*, 2 H); 1.46 (*s*, 9 H).

Synthesis of *tert*-Butyl (6-(quinolin-8-ylamino)hexyl)carbamate (7c).

Compound **7c** (colorless oil, 0.515 g, 1.5 mmol, 14% yield) was prepared from compound **6c**, according to General procedure B.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.72 (*dd*, 1 H); 8.06 (*dd*, 1 H); 7.41-7.35 (*m*, 2 H); 7.04 (*d*, 1 H); 6.67 (*d*, 1 H); 6.12 (*bs*, 1 H); 4.54 (*bs*, 1 H); 3.34-3.29 (*m*, 2 H); 3.14-3.13 (*m*, 2 H); 1.83-1.74 (*m*, 2 H); 1.54-1.37 (*m*, 15 H).

General procedure C: synthesis of *N1*-(Quinolin-8-yl)butane-1,4-diamine (8a).

Trifluoroacetic acid (0.67 mL, 8.9 mmol) was added to a solution of **7a** (0.28 g, 0.89 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 2 h. After this time, the reaction mixture was cooled to 0 °C, then 1 M aqueous NaOH was added, under stirring (pH was adjusted to 10). The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent *in vacuo* afforded 0.144 g (0.7 mmol, 76% yield) of target product, as a yellow oil. This was used in the following step without any further purification.

Synthesis of *N1*-(Quinolin-8-yl)pentane-1,5-diamine (8b).

Compound **8b** (yellow oil, 0.113 g, 0.5 mmol 91% yield) was prepared from compound **7b**, according to General procedure C.

Synthesis of *N1*-(Quinolin-8-yl)hexane-1,6-diamine (8c).

Compound **8c** (yellow oil, 0.357 g, 1.5 mmol, 98% yield) was prepared from compound **7c**, according to General procedure C.

General procedure D: synthesis of *N*-(4-(Quinolin-8-ylamino)butyl)-2-(tritylthio)acetamide (9a).

2-(Tritylthio)acetic acid (0.074 g, 0.8 mmol), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP, 0.488 g, 0.94 mmol), and triethylamine (0.19 mL, 1.34 mmol) were added to **8a** (0.144 g, 0.67 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated and the crude was purified by silica gel flash chromatography, eluting with hexanes/ethyl acetate, from 9/1 to 6/4, affording 0.242 g (0.5 mmol, 68% yield) of pure, target product, as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.71 (*dd*, 1 H); 8.06 (*dd*, 1 H); 7.50-7.37 (*m*, 17 H); 7.04 (*d*, 1 H); 6.65 (*d*, 1 H); 6.09 (*bs*, 1 H); 3.40 (*t*, 2 H); 3.31 (*t*, 2 H); 3.14 (*s*, 2 H); 1.82-1.78 (*m*, 2 H); 1.72-1.69 (*m*, 2 H).

Synthesis of *N*-(5-(Quinolin-8-ylamino)pentyl)-2-(tritylthio)acetamide (9b).

Compound **9b** (yellow oil, 0.200 g, 0.4 mmol, 74% yield) was prepared from compound **8b**, according to General procedure D.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.70 (*dd*, 1 H); 8.06 (*dd*, 1 H); 7.50-7.42 (*m*, 8 H); 7.35-7.24 (*m*, 9 H); 7.04 (*d*, 1 H); 6.66 (*d*, 1 H); 6.05 (*bs*, 1 H); 3.40 (*t*, 2 H); 3.31 (*t*, 2 H); 3.15 (*s*, 2 H); 3.05 (*t*, 2 H); 1.81-1.75 (*m*, 2 H); 1.43-1.42 (*m*; 2 H).

Synthesis of *N*-(6-(Quinolin-8-ylamino)hexyl)-2-(tritylthio)acetamide (9c).

Compound **9c** (yellow oil, 0.613 g, 1.1 mmol, 74% yield) was prepared from compound **8c**, according to General procedure D.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.72 (*dd*, 1 H); 8.07 (*dd*, 1 H); 7.45-7.36 (*m*, 8 H); 7.33-7.24 (*m*, 9 H); 7.05 (*d*, 1 H); 6.67 (*d*, 1 H); 6.04 (*bs*, 1 H); 3.31 (*t*, 2 H); 3.16 (*s*, 2 H); 2.99-2.94 (*m*, 2 H); 1.81-1.74 (*m*, 2 H); 1.49-1.44 (*m*, 2 H); 1.37-1.28 (*m*, 4 H).

General procedure E: synthesis of 2-Mercapto-*N*-(4-(quinolin-8-ylamino)butyl)acetamide (2a).

Trifluoroacetic acid (0.35 mL, 4.6 mmol) and triethylsilane (0.15 mL, 0.92 mmol) were added to **9a** (0.242 g, 0.46 mmol) in CH_2Cl_2 (5 mL), at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Volatiles were removed *in vacuo* and 2 mL of MeOH were added. Purification of the crude by preparative HPLC (Method 2) afforded 0.095 g (0.24 mmol, 51% yield) of pure, target product (monomer, TFA salt), as a yellow solid.

^{1}H NMR (400 MHz, MeOD): δ = 8.90 (*d*, 1 H); 8.53 (*d*, 1 H); 7.70-7.69 (*m*, 1 H); 7.61-7.59 (*m*, 1 H); 7.54-7.52 (*m*, 1 H); 7.26 (*d*, 1 H); 3.44 (*t*, 2 H); 3.32-3.29 (*m*, 2 H); 3.14 (*s*, 2 H); 1.87-1.82 (*m*, 2 H); 1.75-1.70 (*m*, 2 H).

^{13}C NMR (100.6 MHz, MeOD): δ = 171.7, 146.2, 138.8, 129.0, 127.7, 121.2, 118.2, 118.4, 111.5, 109.6, 44.8, 38.5, 26.4, 26.1, 24.7.

ESI-HRMS calcd for $[C_{15}H_{19}N_2OS + H]^+$: 290.1322 m/z, found: 290.1322 m/z.

HPLC purity: 99.7%.

Synthesis of 2-Mercapto-*N*-(5-(quinolin-8-ylamino)pentyl)acetamide (2b).

Compound **2b** (yellow solid, monomer, TFA salt, 0.097 g, 0.23 mmol, 87% yield) was prepared from compound **9b**, according to General procedure E.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.69 (*bs*, 2 H); 9.06-9.05 (*dd*, 1 H); 8.62-8.60 (*dd*, 1 H); 7.75-7.71 (*dd*, 1 H); 7.67-7.63 (*t*, 1 H); 7.28-7.26 (*d*, 1 H); 7.03 (*bs*, 1 H); 6.99-6.97 (*d*, 1 H); 3.36-3.33 (*m*, 4 H); 3.25 (*s*, 2 H); 2.02-1.84 (*bs* + *m*, 1 + 2 H); 1.69-1.58 (*m*, 4 H).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 169.3, 143.6, 142.2, 140.8, 130.3, 130.0, 129.8, 120.3, 114.0, 109.8, 43.4, 39.4, 28.5, 27.8, 27.5, 24.1.

ESI-HRMS calcd for $[C_{16}H_{21}N_2OS + H]^+$: 304.1478 m/z, found: 304.1454 m/z.

HPLC purity: 99.7%.

Synthesis of 2-Mercapto-*N*-(6-(quinolin-8-ylamino)hexyl)acetamide (2c).

Compound **2c** (yellow solid, monomer, TFA salt, 0.306 g, 0.7 mmol, 88% yield) was prepared from compound **9c** , according to General procedure E.

^{1}H NMR (400 MHz, MeOD): δ = 8.87 (*dd*, 1 H); 8.43 (*dd*, 1 H); 7.65-7.62 (*m*, 2 H); 7.48 (*d*, 1 H); 7.19 (*d*, 1 H); 3.42 (*t*, 2 H); 3.22 (*t*, 2 H); 3.13 (*s*, 2 H); 3.31 (*t*, 2 H); 1.85-1.77 (*m*, 2 H); 1.59-1.50 (*m*, 2 H); 1.49-1.48 (*m*, 2 H).

^{13}C NMR (100.6 MHz, MeOD) δ 169.1, 142.9, 142.6, 141.2, 130.1, 129.7, 120.3, 113.8, 109.4, 43.6, 39.4, 28.7, 27.9, 27.8, 26.5, 26.1.

ESI-HRMS calcd for $[C_{17}H_{23}N_3OS + H]^+$: 318.1635 m/z, found: 318.1611 m/z.

HPLC purity: 97.9%.

Synthesis of *tert*-Butyl (7-(methoxy(methyl)amino)-7-oxoheptyl)carbamate (11).

To a solution of 7-((*tert*-butoxycarbonyl)amino)heptanoic acid (**10**) (3.0 g, 12.2 mmol) in CH_2Cl_2 (50 mL) was added 1-ethyl-(3-(3-dimethylamino)propyl)-carbodiimide hydrochloride (EDC, 3.51 g, 18.3 mmol) and triethylamine (3.4 mL, 24.4 mmol), followed by *N*,*O*-dimethylhydroxylamine hydrochloride (1.31 g, 13.4 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.147 g, 1.2 mmol). The resulting reaction mixture was stirred at room temperature overnight. Water was added, then the layers were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic fractions were washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude by silica gel flash chromatography afforded 3.15 g (10.9 mmol, 90% yield), as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.28 (*bs*, 1 H); 3.65 (*s*, 3 H); 3.14 (*s*, 3 H); 3.07 (*t*, 2 H); 2.38 (*t*, 2 H); 1.62-1.59 (*m*, 2 H); 1.47-1.37 (*m*, 11 H); 1.32-1.23 (*m*, 4 H).

Synthesis of *tert*-Butyl (7-oxoheptyl)carbamate (12).

A 1 M THF solution of $LiAlH_4$ (16.35 mL, 16.4 mmol) was added to a cooled (-78 °C) solution of **11** (3.15 g, 10.9 mmol) in 10 mL of THF. The mixture was allowed to warm to 0 °C and stirred at this temperature for 10 min. Saturated aqueous NH_4Cl was slowly added, under stirring, at 0 °C, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by silica gel flash chromatography, affording 2.34 g (10.2 mmol, 94% yield) of pure, target product, as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.75 (*s*, 1 H); 4.56 (*bs*, 1 H); 3.10-3.09 (*m*, 2 H); 2.44-2.40 (*m*, 2 H); 1.64-1.58 (*m*, 2 H); 1.47-1.43 (*m*, 11 H); 1.34-1.29 (*m*, 4 H).

Synthesis of *tert*-Butyl (7-(3,4-dihydroquinolin-1(2*H*)-yl)heptyl)carbamate (13).

A solution of 1,2,3,4-tetrahydroquinoline (0.665 g, 5.0 mmol) in 5 mL of 1,2-dichloroethane was treated with **12** (2.29 g, 10 mmol), followed by sodium triacetoxyborohydride (2.12 g, 10 mmol) and acetic acid (1.4 mL). The resulting suspension was stirred at room temperature overnight. After this time, the mixture was cooled to 0 °C, quenched with 10 mL of 1 M NaOH and stirred for 20 minutes. The layers were separated, then the aqueous phase was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude by silica gel flash chromatography afforded 0.360 g (1.0 mmol, 21% yield) of pure, target product, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06 (*t*, 1 H); 6.95 (*d*, 1 H); 6.59-6.54 (*m*, 2 H); 4.56 (*bs*, 1 H); 3.29 (*t*, 2 H); 3.24 (*t*, 2 H); 3.13-3.12 (*m*, 2 H); 2.77 (*t*, 2 H); 1.99-1.93 (*m*, 2 H); 1.62-1.59 (*m*, 2 H); 1.47 (*s*, 11 H); 1.39-1.31 (*m*, 6 H).

Synthesis of 7-(3,4-Dihydroquinolin-1(2*H*)-yl)heptan-1-amine (14).

Compound **14** (colorless oil, 0.240 g, 0.97 mmol, 94% yield) was prepared from compound **13**, according to General procedure C.

Synthesis of *N*-(7-(3,4-Dihydroquinolin-1(2*H*)-yl)heptyl)-2-(tritylthio)acetamide (15).

Compound **15** (yellow oil, 0.500 g, 0.9 mmol, 89% yield) was prepared from compound **14**, according to General procedure D.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45-7.43 (*m*, 6 H); 7.34-7.31 (*m*, 6 H); 7.27-7.24 (*m*, 3 H); 7.06 (*t*, 1 H); 6.95 (*d*, 1 H); 6.59-6.54 (*m*, 2 H); 6.04 (*bs*, 1 H); 3.28 (*t*, 2 H); 3.23 (*t*, 2 H); 3.16 (*s*, 2 H); 2.98-2.93 (*m*, 2 H); 1.99-1.93 (*m*, 2 H); 1.59-1.57 (*m*, 2 H); 1.47 (*s*, 11 H); 1.36-1.29 (*m*, 6 H); 1.25-1.23 (*m*, 2 H).

Synthesis of *N*-(7-(3,4-Dihydroquinolin-1(2*H*)-yl)heptyl)-2-mercaptoacetamide (3a).

Compound **3a** (brown oil, dimer, di-TFA salt, 0.21 g, 0.2 mmol, 74% yield) was prepared from compound **15**, according to General procedure E.

^{1}H NMR (400 MHz, MeOD): δ = 7.25-7.21 (*m*, 2 H); 7.14-7.12 (*m*, 2 H); 3.55-3.52 (*m*, 2 H); 3.47-3.43 (*m*, 4 H); 3.23 (*t*, 2 H); 2.92 (*t*, 2 H); 2.17-2.10 (*m*, 2 H); 1.77-1.75 (*m*, 2 H); 1.58-1.54 (*m*, 2 H); 1.42-1.39 (*m*, 6 H).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 168.7, 142.8, 129.1, 126.9, 123.8, 118.3, 113.4, 52.7, 48.7, 38.5, 28.9, 28.6, 27.9, 27.0, 26.5, 26.3, 25.3, 20.4.

ESI-HRMS calcd for $[C_{36}H_{54}N_4O_2S_2 + H]^+$: 639.3761 m/z, found: 639.3722 m/z.

HPLC purity: 97.3%.

Synthesis of *tert*-Butyl (6-(3,4-dihydroquinolin-1(2*H*)-yl)-6-oxohexyl)carbamate (17).

N,*N*-Diisopropylethylamine (DIPEA, 3.39 mL, 19.5 mmol) was added to a solution of 6-((*tert*-butoxycarbonyl)amino)hexanoic acid (**16**, 3.0 g, 13.0 mmol) in DMF (13 mL). PyBOP (7.4 g, 14.3 mmol) was then added and the reaction mixture was stirred at room temperature for 10 min. 1,2,3,4-Tetrahydroquinoline (1.8 mL, 14.3 mmol) was then added and the resulting reaction mixture was stirred at room temperature overnight. After this time, the mixture was diluted with ethyl acetate and washed with saturated aqueous NH_4Cl, 10% aqueous LiCl, and brine. The organic phase was dried over anhydrous $Na2SO_4$, filtered and concentrated under reduced pressure. Purification of the crude by silica gel flash chromatography, eluting with hexanes/ethyl acetate, from 9/1 to 4/6, afforded 2.7 g (7.9 mmol, 61% yield) of pure target product, as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18-7.07 (*m*, 4 H); 4.65 (*bs*, 1 H); 3.78-3.75 (*t*, 2 H); 2.71-2.67 (*t*, 2 H); 2.50-2.47 (*t*, 2 H); 2.33-2.30 (*t*, 2 H); 1.97-1.90 (*m*, 2 H); 1.69-1.60 (*m*, 2 H); 1.52-1.33 (*m*, 11 H); 1.22-1.21 (*m*, 2 H).

Synthesis of 6-Amino-1-(3,4-dihydroquinolin-1(2*H*)-yl)hexan-1-one (18).

Compound **18** (yellow oil, 1.0 g, 4.2 mmol, 54% yield) was prepared from compound **17**, according to General procedure C.

Synthesis of *N*-(6-(3,4-Dihydroquinolin-1(2*H*)-yl)-6-oxohexyl)-2-(tritylthio)acetamide (19).

Compound **19** (colorless oil, 0.7 g, 1.4 mmol, 56% yield) was prepared from compound **18**, according to General procedure D.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44-7.42 (*d*, 6 H); 7.29-7.27 (*d*, 6 H); 7.24-7.09 (*m*, 7 H); 6.07-6.05 (*bs*, 1 H); 3.79-3.76 (*t*, 2 H); 3.11 (*s*, 2 H); 2.95-2.92 (*m*, 2 H); 2.72-2.68 (*t*, 2 H); 2.50-2.47 (*t*, 2 H); 1.97-1.90 (*m*, 2 H); 1.68-1.61 (*m*, 2 H); 1.34-1.27 (*m*, 2 H); 1.25-1.20 (*m*, 2 H).

Synthesis of *N*-(6-(3,4-Dihydroquinolin-1(2*H*)-yl)-6-oxohexyl)-2-mercaptoacetamide (3b).

Compound **3b** (colorless oil, monomer, TFA salt, 0.316 g, 0.7 mmol, 55% yield) was prepared from compound **19**, according to General procedure E.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.51 (*bs*, 1 H); 7.28-7.12 (*m*, 4 H); 7.02 (*bs*, 1 H); 3.80-3.77 (*t*, 2 H); 3.29-3.23 (*m*, 4 H); 2.73-2.70 (*t*, 2 H); 2.54-2.50 (*t*, 2 H); 2.00-1.92 (*m*, 3 H); 1.71-1.63 (*m*, 2 H); 1.57-1.48 (*m*, 2 H); 1.34-1.32 (*m*, 2 H).

^{13}C NMR (100.6 MHz, CDCl3): δ = 23.7, 24.8, 25.9, 26.3, 27.7, 28.5 (2 C), 33.8, 39.3, 124.3, 125.1, 125.7, 128.2 (2 C), 138.4, 169.7, 172.8.

ESI-HRMS calcd for $[C_{17}H_{24}N_2O_2S + H]^+$: 321.1631 m/z, found: 321.1494 m/z.

HPLC purity: 97.3%.

Synthesis of *tert*-Butyl (6-(methoxy(methyl)amino)-6-oxohexyl)carbamate (20).

EDC (4.1 g, 21.4 mmol), triethylamine (3.98 mL, 28.5 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (1.53 g, 15.7 mmol) and DMAP (0.174 g, 1.4 mmol) were added in this order to a solution of compound **16** (3.3 g, 14.2 mmol) in dry CH_2Cl_2 (57 mL). The resulting reaction mixture was stirred at room temperature overnight, after which time a white precipitate formed. Saturated aqueous NH_4Cl was added to the reaction mixture and the phases were separated. The organic phase was washed with saturated aqueous NH_4Cl and brine, dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent under reduced pressure afforded 3.9 g (14.2 mmol, quantitative yield) of target product, as a colorless oil. This was used in the following step without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.62 (*bs*, 1 H); 3.64 (*s*, 3 H); 3.14 (*s*, 3 H); 3.09-3.08 (*m*, 2 H); 2.40-2.35 (*m*, 2 H); 1.65-1.58 (*m*, 2 H); 1.51-1.43 (*m*, 2 H); 1.40 (*s*, 9 H); 1.37-1.31 (*m*, 2 H).

Synthesis of *tert*-Butyl (6-oxohexyl)carbamate (21).

Asolution of compound **20** (1.9 g, 6.9 mmol) in dry THF (69 mL) was cooled to -78 °C, then $LiAlH_4$ (0.394 g, 10.4 mmol) was added at this temperature, in one portion. The resulting reaction mixture was stirred at -78 °C for 1.5 h, then allowed to warm to 0 °C. 0.1 N aqueous HCl was carefully added under stirring and the resulting reaction mixture was extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent under reduced pressure gave 1.7 g of a 1/1 mixture of starting material and target product (3.4 mmol, 50% yield), as a colorless oil. This was used in the following step without any further purification.

Synthesis of *tert*-Butyl (6-(6-chloro-3,4-dihydroquinolin-1(*2H*)-yl)hexyl)carbamate (22).

Sodium triacetoxyborohydride (1.67 g, 7.9 mmol) was added in one portion to a stirred solution of 6-chloro-1,2,3,4-tetrahydroquinoline (0.662 g, 3.95 mmol) and **21** (1.7 g, 7.9 mmol) in 1,2-dichloroethane (20 mL). The resulting reaction mixture was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ was added to the reaction mixture and it was extracted with chloroform. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification by silica gel flash chromatography, eluting with hexanes, then hexanes/ethyl acetate, from 95/5 to 85/15, afforded 0.95 g (2.6 mmol, 66% yield) of pure, target product, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.98-6.96 (*dd*, 1 H); 6.90-6.89 (*d*, 1 H); 6.46-6.44 (*d*, 1 H); 4.55 (*bs*, 1 H); 3.27-3.24 (*t*, 2 H); 3.22-3.19 (*t*, 2 H); 3.15-3.10 (*m*, 2 H); 2.73-2.70 (*t*, 2 H); 1.96-1.90 (*m*, 2 H); 1.61-1.55 (*m*, 2 H); 1.53-1.46 (*m* + *s*, 2 + 9 H); 1.41-1.35 (*m*, 4 H).

Synthesis of 6-(6-Chloro-3,4-dihydroquinolin-1(*2H*)-yl)hexan-1-amine (23).

Compound **23** (yellow oil, 0.314 g, 1.2 mmol 86% yield) was prepared from compound **22**, according to General procedure C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98-6.96 (*dd*, 1 H); 6.90 (*s*, 1 H); 6.47-6.44 (*d*, 1 H); 3.27-3.24 (*t*, 2 H); 3.23-3.19 (*t*, 2 H); 2.73-2.70 (*m*, 4 H); 1.96-1.90 (*m*, 2 H); 1.62-1.55 (*m*, 2 H); 1.48-1.45 (*m*, 2 H); 1.37-1.36 (*m*, 4 H); 1.12 (*bs*, 2 H).

Synthesis of *N*-(6-(6-Chloro-3,4-dihydroquinolin-1(*2H*)-yl)hexyl)-2-(tritylthio)acetamide (24).

Compound **24** (colorless oil, 0.166 g, 0.3 mmol, 25% yield) was prepared from compound **23**, according to General procedure D.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47-7.45 (*d*, 6 H); 7.35-7.31 (*t*, 6 H); 7.28-7.26 (*d*, 3 H); 7.01-6.98 (*dd*, 1 H); 6.93-6.92 (*d*, 1 H); 6.48-6.46 (*d*, 1 H); 6.05 (*bs*, 1 H); 3.28-3.25 (*t*, 2 H); 3.23-3.20 (*t*, 2 H); 3.16 (*s*, 2 H); 3.01-2.96 (*dd*, 2 H); 2.74-2.71 (*t*, 2 H); 1.97-1.91 (*m*, 2 H); 1.61-1.54 (*m*, 2 H); 1.41-1.28 (*m*, 6 H).

Synthesis of *N*-(6-(6-Chloro-3,4-dihydroquinolin-1(*2H*)-yl)hexyl)-2-mercaptoacetamide (3c).

Compound **3c** (pinkish oil, 0.068 g, 0.2 mmol, 73%) was prepared from compound **24**, according to General procedure E. The compound was isolated as dimer and free base, after neutralization of the collected fractions from preparative HPLC with saturated aqueous $NaHCO_3$, extraction with chloroform, and lyophilization.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98-6.95 (*dd*, 1 H); 6.90-6-89 (*d*, 1 H); 6.76 (*bs*, 1 H); 6.45-6.43 (*d*, 1 H); 3.42 (*s*, 2 H); 3.33-3.28 (*dd*, 2 H); 3.26-3.24 (*t*, 2 H); 3.22-3.19 (*t*, 2 H); 2.73-2.70 (*t*, 2 H); 1.95-1.89 (*m*, 2 H); 1.61-1.54 (*m*, 4 H); 1.43-1.31 (*m*, 4 H).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 168.3, 143.8, 128.6, 126.6, 123.8, 119.6, 111.4, 51.4, 49.3, 42.6, 40.0, 29.4, 28.0, 26.8 (2 C), 22.0.

ESI-HRMS calcd for $[C_{34}H_{48}Cl_2N_4O_2S_2 + H]^+$: 679.2669 m/z, found: 679.2664 m/z.

HPLC purity: 95.2%.

Synthesis of *tert*-Butyl 4-(quinolin-8-ylcarbamoyl)benzylcarbamate (26).

To a solution of 4-(((tert-butoxycarbonyl)amino)methyl)benzoic acid (**25**, 1.0 g, 4.0 mmol) in DMF (5 mL) was added triethylamine (1.12 mL, 8.0 mmol), EDC (1.15 g, 6.0 mmol), followed by 8-aminoquinoline (0.576 g, 4.0 mmol) and DMAP 0.049 g, 0.4 mmol). The resulting mixture was stirred at room temperature overnight. Water was added to the reaction mixture, then the layers were separated and the aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude by silica gel flash chromatography afforded 1.32 g (3.5 mmol, 88% yield) of pure, target product, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.67 (*bs*, 1 H); 8.87 (*dd*, 1 H); 8.80-8.79 (*m*, 1 H); 8.12 (*dd*, 1 H); 7.99 (*d*, 2 H); 7.54-7.48 (*m*, 2 H); 7.43-7.40 (*m*, 3 H); 5.28 (*bs*, 1 H); 4.38-4.37 (*m*, 2 H); 1.47 (*s*, 9 H).

Synthesis of 4-(Aminomethyl)-*N*-(quinolin-8-yl)benzamide (27).

Compound **27** (off-white solid, 0.900 g, 3.2 mmol, 93% yield) was prepared from compound **26**, according to General procedure C.

Synthesis of *N*-(Quinolin-8-yl)-4-((2-(tritylthio)acetamido)methyl)benzamide (28).

Compound **28** (off-white solid, 1.12 g, 1.9 mmol, 59% yield) was prepared from compound **27**, according to General procedure D.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.93 (*dd*, 1 H); 8.86 (*dd*, 1 H); 8.19 (*dd*, 1 H); 8.02 (*d*, 2 H); 7.62-7.56 (*m*, 2 H); 7.54-7.42 (*m*, 8 H); 7.32-7.22 (*m*, 10 H); 8.40 (*bs*, 1 H); 4.24 (*d*, 2 H); 3.23 (*s*, 2 H).

Synthesis of 4-((2-Mercaptoacetamido)methyl)-*N*-(quinolin-8-yl)benzamide (4).

Compound **4** (off-white solid, monomer, TFA salt, 0.340 g, 0.7 mmol, 51%) was prepared from compound **28**, according to General procedure E.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.74 (*bs*, 1 H); 8.93 (*d*, 1 H); 8.89 (*dd*, 1 H); 8.24 (*d*, 1 H); 8.08 (*d*, 2 H); 7.63-7.60 (*m*, 2 H); 7.54-7.48 (*m*, 3 H); 7.15 (*bs*, 1 H); 4.60 (*d*, 2 H); 3.36 (*d*, 2 H); 1.94 (*t*, 1 H).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 168.0, 164.7, 147.8, 141.4, 138.2, 136.4, 134.1, 133.9, 127.7, 127.6, 127.5, 127.2, 121.6, 121.3, 116.7, 43.2, 27.9.

ESI-HRMS calcd for $[C_{19}H_{17}N_3O_2S + H]^+$: 352.1114 m/z, found: 352.1080 m/z.

HPLC purity: 99.5%.

2. BIOLOGICAL ASSAYS

Stock solution preparation

To the appropriate compound (neat, 1.0 equiv) was added solid TCEP (1.5 equiv). Dilution with the adequate amount of a 9:1 DMSO/distilled water solution and agitation for 20-30 min (magnetic stirrer) at rt afforded the required stock solution of mercapto-acetamide. All compounds were used immediately after reduction.

HDAC inhibition assays

HDAC inhibition assays were performed by the Reaction Biology Corporation (Malvern, PA) using human full-length recombinant HDAC1 and 6, isolated from a baculovirus expression system in Sf9 cells. An acetylated, fluorogenic peptide derived from residues 379-382 of p53 ($RHKK_{Ac}$) was used as the substrate in the assays. The reaction buffer contained: 50 mM Tris·HCl pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/mL BSA, and a final concentration of 1% DMSO. The enzyme was added into wells of the reaction plate and stock solutions of compounds **2-4** were distributed into the enzyme mixture by Acoustic technology (Echo550 instrument; nanoliter range). The plates were spun down and pre-incubated for 5-10 min. The substrate was then delivered to all reaction wells to initiate the reaction, which was incubated for 2 h at 30 °C. After incubation, developer and TSA were added to quench the reaction and generate fluorescence. Kinetic measurements were then taken for 1.5 h at 15 min intervals to ensure that development was complete. Endpoint readings were taken for analysis after the development reached a plateau. Dose-response curves were generated, and the IC_{50} value for each compound was extrapolated from the generated plots (ten-dose IC_{50} curves were generated using a threefold serial dilution pattern starting at 30 μM).

Tubulin and Histone Acetylation Western Blot Assay.

E17 cells were plated at… A 50 mM stock of compound was then added by serial dilutions in complete medium to the indicated concentrations. Cells were incubated for 24 h under humidified conditions (37 °C, 5% CO_2). Wells were then washed with cold PBS, and cells were lysed in a buffer containing 10 mM Tris-HCl pH 8.0, 10% SDS, 4 mM urea, 100 mM DTT, and 1x protease inhibitor (Roche). Cells were lysed for 30 min on ice and then sonicated for 8 min (8 cycles of 30 s on/30 s rest). Cells were then boiled for 10 min with 6x gel loading buffer and resolved on 4-15% gradient gels and subsequently transferred onto nitrocellulose membranes. Membranes were blocked with 5% milk in PBS-T, and specific antigens were detected using antibodies against acetyl-H3 and H3 (Cell Signaling) and acetyl-α-tubulin and α-tubulin (Sigma). Bands were detected by scanning blots with an LI-COR Odyssey imaging system using both 700 and 800 channels.

Primary cortical cultures were prepared from rat embryos (embryonic day 17; E17). Twenty-four hours later, cultures were treated with serial dilutions of a 5o mM stock of each compound (the final DMSO concentration in growth media was 0.1%). Cells were incubated for 6 h under humidified conditions (37 °C, 5% CO_2). Total proteins were extracted on ice in RIPA buffer (Boston Bioproducts) supplemented with and 1x protease inhibitors (Roche), and sonicated for 2 min. After quantification by Bradford assay, protein lysates were boiled for 3 min with 4x gel loading buffer and resolved on 4-15% gradient gels and subsequently transferred onto nitrocellulose membranes. Membranes were blocked with LI-COR blocking buffer, and specific antigens were detected using antibodies against acetyl-H3 and H3 (Cell Signaling) and acetyl-α-tubulin and α-tubulin (Sigma). Bands were detected by scanning blots with an LI-COR Odyssey imaging system using both 700 and 800 channels, and quantified using Image Studio Lite software.

Treg suppression assays

For *in vitro* studies of the effects of compounds on Treg suppressive function, 5 x 10^4 cell-sorted CD4+YFP+Foxp3+ Treg cells, or bead-isolated CD4+ CD25+ Tregs, were added to 96-well plates and serially diluted in medium (RPMI-1640 and 10% FBS, plus pen/strep), followed by the addition of equal numbers of CFSE-labeled CD4+CD25- T-effector (Teff) cells and γ-irradiated, CD3 mAb (1 μg/ml), and compounds at final concentrations of 0.1, 0.3, 1.0 and 3.0 μM. Cells were cultured for 3 days, and cells were then stained with CD4 mAb (Pacific blue) and CFSE and CD4 positive T cell proliferation was acquired and data analyzed with FlowJo software. Assays were performed in triplicate and repeated at least once, and data are shown as mean ± SD of CFSE+ T cell proliferation at each ratio of Treg:Teff cells cultured in the presence of 1.0 μM of compound. Proliferation at a given ratio of each drug vs. DMSO-treated cells was evaluated using student's t-test.