Data S1. Synthesis of CBUD-1001.

Methods

General chemistry. All reagents and solvents used in the present study were commercially available and used without any purification unless otherwise noted. Column chromatography was performed with silica gel 60 (70-230 mesh) using a mixture of DCM and hexane as the eluent. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR) spectrometer in deuterated chloroform ($CDCl_3$) with tetramethylsilane as an internal reference. Data are reported as (ap=apparent, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad; coupling constant(s) in Hz, integration). High-resolution mass spectroscopy was performed using a magnetic sector analyzer. All of the new compounds were identified using ^{1}H-NMR and ^{13}C-NMR data recorded on a 400-MHz spectrometer (Bruker, US) and high resolution mass spectroscopy. The novel HDAC inhibitor (compound 13, CBUD-1001) was prepared in 3 parts (Figs. S1 and S2) with several modifications described in the literature (1,2).

Synthesis of HDAC inhibitors

tert-butyl 4-bromo-2-nitrophenylcarbamate (compound 2). A solution of di-*tert*-butyl dicarbonate (0.31 g, 1.40 mmol) in dichloromethane (10 ml) was added dropwise to a mixture of 4-bromo-2-nitroaniline (0.22 g, 1.00 mmol), trimethylamine (0.15 g, 1.50 mmol), and 4-dimethylaminopyridine (0.02 g, 0.20 mmol) in dichloromethane (20 ml). The reaction mixture was stirred at room temperature for 15 h before quenching with aq. 1M HCl (20 ml). The mixture was extracted with dichloromethane (50 ml) and washed with water (50 ml x2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to a small volume *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: hexane 0:100 to 1:100) on a silica gel to yield compound 2 (0.15 g, 48%) as a light yellow solid. m.p. 103-105°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.52 (d, *J*=8.8 Hz, 1H), 8.34 (d, *J*=2.8 Hz, 1H), 7.70 (dd, *J*=9.2 Hz, 2.4 Hz, 1H), 1.56 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.0, 138.6, 136.2, 135.2, 128.3, 122.3, 113.8, 82.4, 28.2. HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{11}H_{14}BrN_2O_4$=317.0137; found 317.0135.

tert-butyl 2-nitro-4-(thiophen-2-yl)phenylcarbamate (compound 3). Compound 2 (0.32 g, 1.00 mmol), thiophene-2-boronic acid (0.15 g, 1.20 mmol), $Pd(PPh_3)_4$ (0.06 g, 0.05 mmol) and K_2CO_3 (0.55 g, 4.00 mmol) were added to THF. The reaction mixture was stirred at reflux for 15 h before evaporation to dryness. The crude solid was extracted with ethyl acetate (50 ml) and washed with water (50 ml x2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification using flash column chromatography (dichloromethane: hexane 1:4 to 1:3) yielded compound 3 (0.19 g, 60%) as a yellow solid. m.p. 124-126°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.67 (s, 1H), 8.61 (d, *J*=8.8 Hz, 1H), 8.41 (d, *J*=2.4 Hz, 1H), 7.83 (dd, *J*=8.8 Hz, 2.4 Hz, 1H), 7.37-7.33 (m, 2H), 7.12 (dd, *J*=4.8 Hz, 3.6 Hz, 1H), 1.58 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 141.3, 136.0, 134.8, 132.9, 128.7, 128.3, 125.6, 123.9, 122.3, 121.2, 82.0, 28.2. HRMS (ESI) m/z $(M+H)^+$calcd for $C_{15}H_{17}N_2O_4S$=321.0909; found 321.0914.

tert-butyl 2-amino-4-(thiophen-2-yl)phenylcarbamate (compound 4). Compound 3 (0.32 g, 1.00 mmol) was added to methanol, following the portion-wise addition of palladium on carbon (0.032 g). The reaction mixture was stirred at r.t. for 15 h under hydrogen gas. The mixture was filtered through celite, and the solution was evaporated to dryness. The residue was dissolved in dichloromethane and purified by flash column chromatography (ethyl acetate: hexane 1:4 to 1:3) on silica gel to yield compound 4 (0.24 g, 84%) as a white solid. m.p. 169-171°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J*=8.4 Hz, 1H), 7.27-7.21 (m, 2H), 7.10-7.03 (m, 3H), 6.29 (s, 1H), 3.60 (br s, 2H), 1.55 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 144.1, 139.8, 132.3, 127.9, 124.9, 124.4, 122.8, 117.6, 115.1, 80.7, 28.3. HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{15}H_{19}N_2O_2S$=291.1167; found 291.1169.

tert-butyl 2-(4-(chloromethyl)benzamido)-4-(thiophen-2-yl) phenylcarbamate (compound 5). A solution of 4-(chloromethyl) benzoyl chloride (0.26 g, 1.40 mmol) in dichloromethane (10 ml) was added dropwise to a mixture of compound 4 (0.29 g, 1.00 mmol) and trimethylamine (0.15 g, 1.50 mmol) in dichloromethane (20 ml). The reaction mixture was stirred at room temperature for 3 h before quenching with aq. 1M HCl (20 ml). The mixture was extracted with dichloromethane (50 ml) and washed with water (50 ml x 2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to a small volume *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: hexane 1:5 to 1:3) on silica gel to yield compound 5 (0.41 g, 92%) as a white solid. m.p. 176-178°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 7.97 (d, *J*=8.4 Hz, 3H), 7.46 (d, *J*=8.0 Hz, 2H), 7.35 (dd, *J*=8.4 Hz, 2.0 Hz, 1H), 7.28-7.20 (m, 3H), 7.04 (dd, *J*=5.2 Hz, 3.6 Hz, 1H), 6.99 (s, 1H), 4.64 (s, 2H), 1.52 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 154.6, 143.1, 141.3, 133.9, 132.2, 130.9, 129.3, 128.7, 128.0, 127.9, 124.9, 123.4, 122.9, 81.5, 45.4, 28.3. HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{23}H_{24}ClN_2O_3S$=443.1196; found 443.1198.

tert-butyl 2-(4-(aminomethyl)benzamido)-4-(thiophen-2-yl) phenylcarbamate (compound 6). Compound 5 (0.44 g, 1.00 mmol) and potassium phthalimide (0.37 g, 2.00 mmol) were added to *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80°C for 3 h. The mixture was cooled to room temperature and sat. aq. NH_4Cl (20 ml) was added to the mixture. The precipitate was filtered and then washed with water (50 ml x2) and dried by heating. The solid was then added to ethanol (20 ml), and hydrazine monohydrate (0.1 g, 2.00 mmol) was added. The reaction mixture was stirred at 80°C for 1 h and then quenched with aq. 2M HCl (20 ml). The precipitate was filtered and washed with water (50 ml x2) and then with dichloromethane (50 ml x2). The solid was basified with 2M NaOH (20 ml) and extracted with ethyl acetate (50 ml) and brine (50 ml). The organic layer was washed with water (50 ml x2), then dried over anhydrous Na_2SO_4 and concentrated to dryness to give compound 6 (0.22 g, 52%) as a

white solid. m.p. >300°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 7.96 (s, 1H), 7.92 (d, J=8.0 Hz, 2H), 7.40-7.19 (m, 7H), 7.02 (dd, J=5.2 Hz, 3.6 Hz, 1H), 3.94 (s, 2H), 1.66 (br s, 2H), 1.50 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 154.6, 147.1, 143.3, 132.5, 131.9, 130.9, 129.5, 128.0, 127.7, 127.1, 124.8, 123.3, 122.9, 81.2, 46.0, 28.3. HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{23}H_{26}N_3O_3S$=424.1695; found 424.1697.

Benzyl 4-(hydroxymethyl)cyclohexanecarboxylate (compound 8). Benzyl bromide (0.26 g, 1.50 mmol) was added dropwise to a stirred mixture of 4-(hydroxymethyl)cyclohexanecarboxylic acid (0.16 g, 1.00 mmol) and K_2CO_3 (0.28 g, 2.00 mmol) in *N*,*N*-dimethylformamide (10 ml). The reaction mixture was stirred at room temperature for 6 h. Water (20 ml) was added to the mixture, and the whole mixture was then extracted with diethyl ether (50 ml). The organic layer was washed again with water (50 ml x 2) and then dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate: hexane 1:5) on silica gel to yield compound 8 (0.17 g, 70%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.30 (m, 5H), 5.15 (s, 2H), 3.50 (d, J=6.4 Hz, 2H), 2.65 (quint, J=4.8 Hz, 1H), 2.11-2.00 (m, 2H), 1.69-1.57 (m, 5H), 1.44 (s, 1H), 1.38-1.25 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 136.3, 128.5, 128.1, 128.0, 67.0, 66.0, 40.4, 38.6, 26.0, 25.8. HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{15}H_{21}O_3$=249.1491; found 249.1494.

Benzyl 4-(tosyloxymethyl)cyclohexanecarboxylate (compound 9). To a mixture of compound 8 (0.25 g, 1.00 mmol) and trimethylamine (0.15 g, 1.50 mmol) in dichloromethane (20 ml), a solution of *p*-toluenesulfonyl chloride (0.29 g, 1.50 mmol) in dichloromethane (10 ml) was added dropwise. The reaction mixture was stirred at room temperature for 15 h before quenching with aq. 1M HCl (20 ml). The solution was extracted with dichloromethane (20 ml) and washed with water (50 ml x2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate: hexane 1:10) on silica gel to yield compound 9 (0.28 g, 75%) as a white solid. m.p. 47–49°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J=8.4 Hz, 2H), 7.41-7.30 (m, 7H), 5.13 (s, 2H), 3.85 (d, J=6.8 Hz, 2H), 2.62 (quint, J=4.8 Hz, 1H), 2.47 (s, 3H), 2.05-1.95 (m, 2H), 1.85-1.72 (m, 1H), 1.65-1.50 (m, 4H), 1.30-1.19 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 144.7, 136.1, 133.1, 129.8, 128.5, 128.1, 128.0, 127.9, 73.9, 66.1, 39.8, 35.6, 25.7, 25.5, 21.6.

Benzyl 4-(fluoromethyl)cyclohexanecarboxylate (compound 10). A mixture of compound 9 (0.40 g, 1.00 mmol) and tetra-*n*-butylammonium fluoride (0.52 g, 2.00 mmol) was added to 2-methyl-2-butanol (10 ml). The reaction mixture was stirred at 80°C for 5 h. The mixture was then cooled to room temperature, extracted with ethyl acetate (20 ml), and washed with water (50 ml x2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate: hexane 1:15) on silica gel to yield compound 10 (0.24 g, 97%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.30 (m, 5H), 5.16 (s, 2H), 4.30 (dd, $^{2}J_{H\text{-}F=}$47.6Hz, $^{3}J_{H\text{-}H=}$6.8 Hz, 2H), 2.67 (quint, J=4.8 Hz, 1H), 2.13-2.05 (m, 2H), 1.90-1.75 (m, 1H), 1.69-1.57 (m, 4H), 1.40-1.29 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 136.2, 128.5, 128.1, 128.0, 88.1&86.5 (d, $^{1}J_{C\text{-}F=}$166.2 Hz, 1C), 66.1, 40.1, 36.8&36.6 (d, $^{2}J_{C\text{-}F=}$17.5 Hz, 1C), 25.9, 24.9 (d, $^{3}J_{C\text{-}F=}$6.6 Hz, 1C). HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{15}H_{20}FO_{2=}$251.1447; found 251.1445.

4-(fluoromethyl)cyclohexanecarboxylic acid (compound 11). Compound 10 (0.25 g, 1.00 mmol) was added to methanol (20 ml), following the portion-wise addition of palladium on carbon (0.03 g). The reaction mixture was stirred at r.t. for 15 h under hydrogen gas. The mixture was filtered through celite and then evaporated to dryness to yield compound 11 (0.16 g, 99%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.2 (br s, 1H), 4.30 (dd, $^{2}J_{H\text{-}F=}$47.2Hz, $^{3}J_{H\text{-}H=}$6.4 Hz, 2H), 2.67 (quint, J=4.8 Hz, 1H), 2.13-2.00 (m, 2H), 1.90-1.73 (m, 1H), 1.73-1.50 (m, 4H), 1.45-1.30 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 88.2&86.5 (d, $^{1}J_{C\text{-}F=}$166.2 Hz, 1C), 39.8, 36.9&36.7 (d, $^{2}J_{C\text{-}F=}$18.2 Hz, 1C), 25.7, 24.9 (d, $^{3}J_{C\text{-}F=}$6.6 Hz, 1C). HRMS (ESI) m/z $(M+H)^+$ calcd for $C_8H_{14}FO_2$=161.0978; found 161.0974.

tert-butyl 2-(4-((4-(fluoromethyl)cyclohexanecarboxamido) methyl)benzamido)-4-(thiophen-2-yl)phenylcarbamate (compound 12). A mixture of compound 6 (0.21 g, 0.50 mmol), compound 11 (0.16 g, 1.00 mmol), EDC (0.19 g, 1.00 mmol), and 4-DMAP (0.01 g, 0.1 mmol) in dichloromethane (20 ml) was stirred at room temperature for 15 h before quenching with sat. aq. $NaHCO_3$ (20 ml). The solution was then extracted with dichloromethane (50 ml) and washed with water (50 ml x 2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: hexane 1:1 to 2:1) on silica gel to yield compound 12 (0.17 g, 61%) as a white solid. m.p. 117-119°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 1H), 7.95 (d, J=1.6 Hz, 1H), 7.83 (d, J=8.0 Hz, 2H), 7.37-7.17 (m, 7H), 7.05 (dd, J=4.8 Hz, 3.6 Hz, 1H), 6.31 (t, J=6.0 Hz, 1H), 4.43 (d, J=6.0 Hz, 2H), 4.35 (dd, $^{2}J_{H\text{-}F=}$47.6 Hz, $^{3}J_{H\text{-}H=}$6.8 Hz, 2H), 2.40-2.35 (m, 1H), 2.00-1.80 (m, 3H), 1.70-1.56 (m, 6H), 1.50 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 165.7, 154.5, 143.2, 142.9, 132.9, 131.9, 130.7, 129.5, 128.0, 127.8, 127.4, 124.9, 124.8, 123.4, 123.3, 122.8, 87.3&85.6 (d, $^{1}J_{C\text{-}F=}$166.9 Hz, 1C), 81.3, 42.9, 42.1, 35.6&35.4 (d, $^{2}J_{C\text{-}F=}$18.3 Hz, 1C), 28.3, 25.9, 24.9 (d, $^{3}J_{C\text{-}F=}$6.5 Hz, 1C). HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{31}H_{37}FN_3O_4S$=566.2489; found 566.2492.

N-(2-amino-5-(thiophen-2-yl)phenyl)-4-((4-(fluoromethyl)cyclohexanecarboxamido) ethyl) benzamide (compound 13). To a stirred solution of compound 12 (0.11 g, 0.20 mmol) in dichloromethane (10 ml), trifluoroacetic acid (5 ml) was added dropwise for 2 min. The reaction was stirred at r.t. for 3 h and then evaporated to dryness. The residue was dissolved in ethyl acetate (20 ml) and basified with 1M NaOH (20 ml) at 0°C. The organic layer was washed with water (50 ml x2) and dried over anhydrous Na_2SO_4. The solution was evaporated to dryness to yield compound 13 (0.09 g, 92%) as a light yellow solid. m.p. 201-203°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.33 (t, J=6.4 Hz, 1H), 7.96 (d, J=8.4 Hz, 2H), 7.48 (d, J=2.0 Hz, 1H), 7.40-7.34 (m, 3H), 7.31 (dd, J=8.4 Hz, 2.4 Hz, 1H), 7.25 (dd, J=3.6 Hz, 1.2 Hz, 1H), 7.06 (dd, J=4.2 Hz, 3.6 Hz, 1H), 6.82 (d, J=8.4 Hz, 1H), 5.15 (s, 2H), 4.43-4.17 (m, 4H), 2.43-2.35 (m, 1H), 1.93-1.70 (m, 3H), 1.60-1.45 (m, 6H). ^{13}C NMR(100 MHz, DMSO-d_6) δ 175.0, 165.7,

144.6, 144.2, 143.2, 133.3, 128.7, 128.3, 127.2, 124.3, 124.0, 123.7, 122.9, 121.5, 117.0, 87.5&85.9 (d, $^{1}J_{C\text{-}F=}$164.0 Hz, 1C), 42.1, 41.3, 35.6&35.4 (d, $^{2}J_{C\text{-}F=}$17.5 Hz, 1C), 26.0, 25.0 (d, $^{3}J_{C\text{-}F=}$6.6 Hz, 1C). HRMS (ESI) m/z $(M+H)^{+}$ calcd for $C_{26}H_{29}FN_3O_2S$=466.1965; found 466.1964.

References

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Figure S1. Synthesis routes for the preparation of the benzamide scaffold.

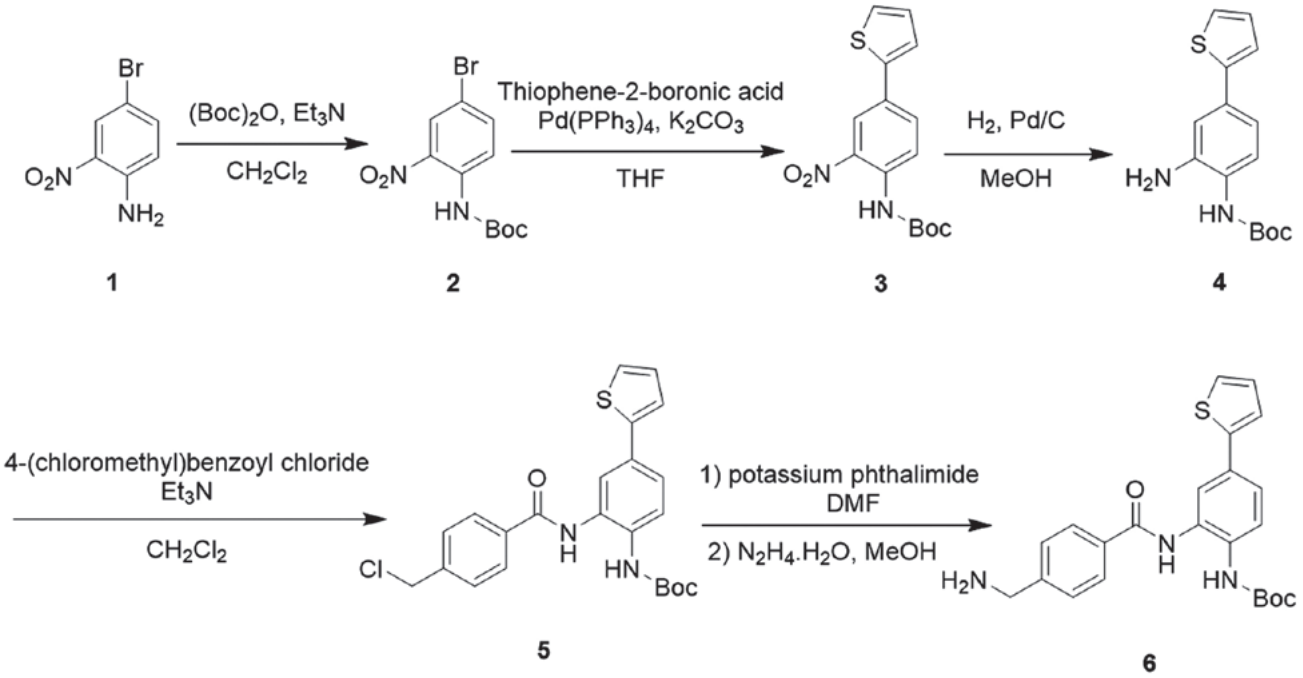

Figure S2. Synthesis routes for the preparation of a novel fluorinated HDAC inhibitor. HDAC, histone deacetylase.