Supporting Information

Discovery of a new class of histone deacetylase inhibitors with a novel zinc binding group

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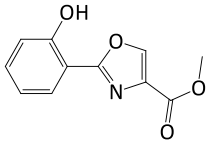
Synthetic Methods and Procedures

General Information

All reagents were purchased from Acros Organics, Alfa Aesar, Fisher Scientific, Matrix Scientific, Oakwood Products, Sigma-Aldrich, TCI America, VWR International and were used without further purification except as noted below. Solvents tetrahydrofuran (THF), dichloromethane (DCM), methanol, dimethylformamide (DMF), acetonitrile and ethyl acetate were purified through SG Water® Glass Contour 6-position solvent purification system. Moisture-sensitive reactions were performed under argon with dried glassware and dry solvent. Preparative scale chromatographic procedures were carried out using Teledyne Isco Combiflash®Rf with normal phase disposable columns RediSep®Rf (Gold/Universal, 4 g, 12 g, 24 g or 40 g). Thin-layer chromatography was conducted on TCL Silica gel 60 F254. Microwave reactions were carried out by using Biotage Initiator 2.5. Solvents were removed by rotary evaporation (Büchi Rotavapor R-114 and Büchi Water Bath B-490) and a vacuum pump (Heidolph Rotavac Valve Control). All ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD, or DMSO-d_6 using a Varian Mercury 400 MHz FT-NMR or a Varian Unity INOVA 400 MHz FT-NMR and all chemical shifts were reported as δ values referenced to TMS. Mass spectrometric analysis was performed at a Waters Mass-Directed Autopurification system (LC/MS) using a 3100 single quadrupole Mass Detector. HPLC analysis was performed through a Waters ACQUITY H-Series UPLC with an Acquity UPLC® BEH C18 1.7 μm 2.1 X 500 mm column using a gradient of 95% to 5% Buffer A over 10 min (Buffer A = water with 0.1% TFA; Buffer B = HPLC grade acetonitrile) at 0.5 mL/min at room temperature. All compounds were found to be of 95% purity or better.

Experimental procedures and compound characterization

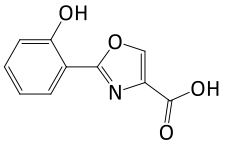
Methyl 2-(2-hydroxyphenyl)oxazole-4-carboxylate (7)



Step A: A 25 ml round bottom single-neck flask was charged with L-serine methyl ester hydrochloride **5** (0.156 g, 1 mmol), magnesium sulfate (0.121 g, 1 mmol) and tetrahydrofuran (5 ml). The mixture was treated with salicylaldehyde **4** (0.122 g, 1 mmol) and triethylamine (0.202 g, 2 mmol). The resulting mixture was stirred at room temperature for 12 hours and filtered through a 0.45 μM PTFE syringe filter. The combined filtrate containing **6** was concentrated to dryness and used for the next step without further purification.

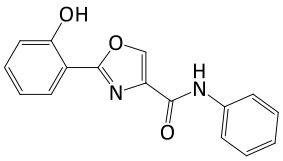
Step B: The crude oxazolidine **6** from step A was dissolved in 5 ml DCM, and the solution was cooled to 0 °C and treated with bromotrichloromethane (0.298 ml, 3 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.453 ml, 3 mmol). The resulting mixture was stirred at 0 °C for 2 hours and then at room temperature for another 10 hours. The reaction mixture was poured directly onto a 40g normal phase disposable RediSep®Rf column. The product was eluted (0-40% ethyl acetate/hexane) to afford the title compound **7** (0.053 g, 23%) as a white, amorphous solid. ^{1}H NMR (δ, ppm, $CDCl_3$): 3.97 (s, 3H), 7.00 (t, 1H), 7.12 (d, 1H), 7.43 (t, 1H), 7.86 (dd, 1H), 8.28 (s, 1H), 10.68 (s, 1H). UPLC: 5.458 min. ESI-MS m/z: calculated: 220.058; found: 220.223 $[M+H]^+$.

2-(2-Hydroxyphenyl)oxazole-4-carboxylic acid (8)



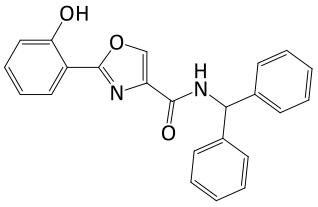
A 5 ml aqueous solution of lithium hydroxide monohydrate (0.04 g, 1 mmol) was added dropwise over a period of 15 min to a 10 ml THF solution of compound **7** (0.03 g, 0.14 mmol) at 0 ˚C. The reaction mixture was slowly warmed up to room temperature and stirred overnight. Then the whole mixture was again cooled to 0 ˚C and acidified by adding 1 M dilute HCl solution to PH = 3. Later the whole mixture was extracted with three 50 ml portions of ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate and filtered. The solvent was then concentrated to yield the product **8** as a white, amorphous solid (0.025 g, 88%). ^{1}H NMR (δ, ppm, CD_3OD): 7.00 (t, 1H), 7.05 (d, 1H), 7.44 (t, 1H), 7.90 (t, 1H), 8.61 (s, 1H). UPLC: 4.572 min. ESI-MS m/z: calculated: 206.048; found: 206.147 $[M+H]^+$.

2-(2-Hydroxyphenyl)-*N*-phenyloxazole-4-carboxamide (9)



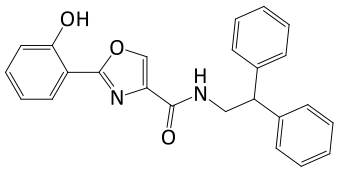
Compound **8** (0.015 g, 0.07 mmol) was dissolved in 3 ml DCM in a 5 ml microwave reaction tube and aniline (0.007 g, 0.08 mmol) was added into above solution followed by HATU (0.038 g, 0.1 mmol) and DIPEA (0.013 g, 0.1 mmol). Microwave reaction was carried out in Biotage Initiator 2.5 for 25 min at 80˚C. The reaction mixture was extracted with water and DCM and organic layer was later washed by 0.1 M dilute HCl solution, saturated sodium bicarbonate solution and brine consecutively. Then the organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated to give crude product. Purification by Combiflash (20%-100% ethyl acetate/hexane) afforded a pure product **9** as a white, amorphous solid (0.025 g, 88%).^{1}H NMR (δ, ppm, $CDCl_3$): 7.04 (td, 1H), 7.14 (d, 1H), 7.21 (t, 1H), 7.42 (t, 2H), 7.47 (td, 1H), 7.71 (d, 2H), 7.91 (dd, 1H), 8.38 (s, 1H), 8.41 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 110.27, 117.42, 120.16, 120.25, 125.04, 126.72, 129.20, 133.55, 136.09, 136.92, 140.86, 156.90, 157.55, 162.32. UPLC: 6.211 min.; m.p. 173-174°C; ESI-MS m/z calculated: 281.0957; found: 281.232 $[M+H]^+$.

***N*-Benzhydryl-2-(2-hydroxyphenyl)oxazole-4-carboxamide (10)**



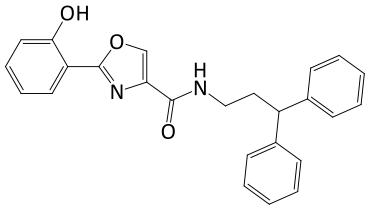
Compound **10** was made from compound **8** (0.04 g, 0.22 mmol), benzhydrylamine (0.046 g, 0.25 mmol), HATU (0.114 g, 0.3 mmol) and DIPEA (0.039 g, 0.3 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 95% yield as an off-white, amorphous solid (0.07 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 6.49 (d, 1H), 7.02 (t, 1H), 7.09 (d, 1H), 7.37-7.47 (m, 9H), 7.45 (t, 1H), 7.89 (dd, 1H), 8.29 (s, 1H), 10.24 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 56.71, 110.33, 117.32, 120.08, 126.63, 127.51, 127.76, 128.85, 133.40, 135.64, 140.59, 140.90, 156.83, 158.90, 161.30. UPLC: 7.032 min.; m.p. 226-227°C; ESI-MS m/z calculated: 371.140; found: 371.274 $[M+H]^+$.

***N*-(2,2-Diphenylethyl)-2-(2-hydroxyphenyl)oxazole-4-carboxamide (11)**



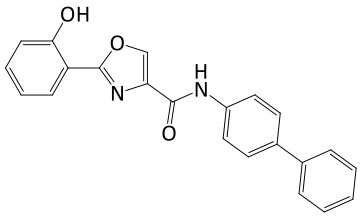
Compound **11** was made from compound **8** (0.05 g, 0.25 mmol), 2,2-diphenylethylamine (0.04 g, 0.2 mmol), HATU (0.114 g, 0.3 mmol) and DIPEA (0.039 g, 0.3 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 86% yield as a white, amorphous solid (0.066 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 4.13 (q, 2H), 4.34 (t, 1H), 6.73 (brs, 1H), 6.99 (t, 1H), 7.08 (d, 1H), 7.26-7.44 (m, 11H), 7.83 (d, 1H), 8.23 (s, 1H), 9.88 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 43.44, 50.45, 110.24, 117.36, 119.95, 126.57, 127.11, 128.05, 128.90, 133.34, 133.36, 135.53, 135.57, 140.03, 140.06, 141.54, 156.92, 159.66, 161.12. UPLC: 7.053 min.; m.p. 206-208°C; ESI-MS m/z calculated: 385.155; found: 385.294 $[M+H]^+$.

***N*-(3,3-Diphenylpropyl)-2-(2-hydroxyphenyl)oxazole-4-carboxamide (12)**



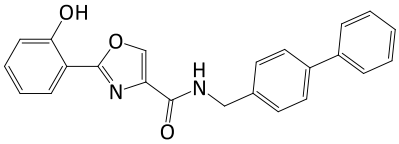
Compound **12** was made from compound **8** (0.026 g, 0.127 mmol), 3,3-diphenylpropylamine (0.032 g, 0.15 mmol), HATU (0.076 g, 0.2 mmol) and DIPEA (0.032 g, 0.25 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 70% yield as a pale tan, amorphous solid (0.035 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 2.45 (q, 2H), 3.48 (q, 2H), 4.05 (t, 1H), 6.72 (t, 1H), 7.03 (t, 1H), 7.12 (d, 1H), 7.18-7.23 (m, 2H), 7.28-7.34 (m, 8H), 7.47 (td, 1H), 7.88 (dd, 1H), 8.24 (s, 1H), 10.18 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 35.13, 38.32, 49.26, 110.37, 117.36, 120.00, 126.53, 126.60, 127.74, 128.70, 133.31, 135.83, 139.99, 144.06, 156.92, 159.67, 161.13. UPLC: 7.321 min. ESI-MS m/z calculated: 399.171; found: 399.353 $[M+H]^+$.

***N*-([1,1'-Biphenyl]-4-yl)-2-(2-hydroxyphenyl)oxazole-4-carboxamide (13)**



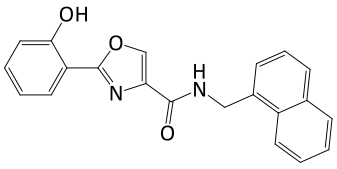
Compound **13** was made from compound **8** (0.028 g, 0.13 mmol), 4-biphenylylamine (0.025 g, 0.15 mmol), HATU (0.068 g, 0.18 mmol) and DIPEA (0.026 g, 0.2 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 84% yield as a white, amorphous solid (0.039 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 7.05 (t, 1H), 7.15 (d, 1H), 7.37 (t, 1H), 7.37-7.40 (m, 3H), 7.45-7.50 (m, 4H), 7.65 (d, 2H), 7.92 (d, 1H), 8.39 (s, 1H), 8.49 (s, 1H), 10.20 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 110.27, 117.44, 120.17, 120.54, 126.74, 126.90, 127.28, 127.78, 128.84, 133.57, 136.07, 136.21, 137.88, 140.35, 140.90, 156.91, 157.55, 161.35. UPLC: 7.408 min.; m.p. 218-219°C; ESI-MS m/z calculated: 357.124; found: 357.220 $[M+H]^+$.

***N*-([1,1'-Biphenyl]-4-ylmethyl)-2-(2-hydroxyphenyl)oxazole-4-carboxamide (14)**



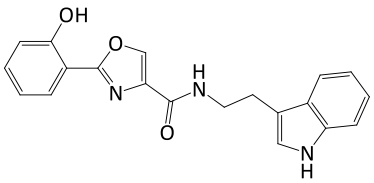
Compound **14** was made from compound **8** (0.022 g, 0.1 mmol), 4-phenylbenzylamine (0.018 g, 0.1 mmol), HATU (0.057 g, 0.15 mmol) and DIPEA (0.02 g, 0.15 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 65% yield as a white, amorphous solid (0.024g). ^{1}H NMR (δ, ppm, $CDCl_3$): 4.73 (d, 2H), 7.02 (td, 1H), 7.08 (d, 1H), 7.12 (t, 1H), 7.36 (t, 1H), 7.39-7.48 (m, 5H), 7.60-7.62 (m, 4H), 7.89 (dd, 1H), 8.32 (s, 1H), 10.21 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 42.98, 110.32, 117.36, 120.03, 126.60, 127.13, 127.40, 127.61, 128.38, 128.81, 133.37, 135.71, 136.65, 140.36, 140.70, 140.82, 156.90, 159.70, 161.30. UPLC: 7.132 min.; m.p. 220-221°C; ESI-MS m/z calculated: 371.1402; found: 371.343 $[M+H]^+$.

2-(2-Hydroxyphenyl)-N-(naphthalen-1-ylmethyl)oxazole-4-carboxamide (15)



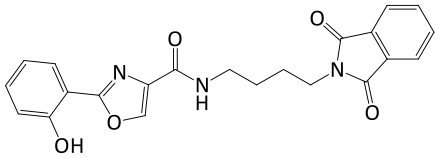
Compound **15** was made from compound **8** (0.022 g, 0.1 mmol), 1-naphthalenemethylamine (0.016 g, 0.1 mmol), HATU (0.057 g, 0.15 mmol) and DIPEA (0.02 g, 0.15 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 81% yield as a white, amorphous solid (0.028g). ^{1}H NMR (δ, ppm, $CDCl_3$): 5.11 (d, 2H), 6.99 (td, 1H), 7.02 (d, 1H), 7.12 (t, 1H), 7.40 (td, 1H), 7.47 (d, 1H), 7.51-7.60 (m, 3H), 7.82-7.87 (m, 2H), 7.90 (d, 1H), 8.10 (d, 1H), 8.30 (s, 1H), 10.19 (s, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 41.33, 110.29, 117.30, 119.96, 123.32, 125.41, 126.07, 126.56, 126.78, 128.87, 131.38, 132.83, 133.28, 133.92, 135.64, 140.37, 156.80, 159.48, 161.17. UPLC: 6.705 min. ESI-MS m/z calculated: 345.124; found: 345.262 $[M+H]^+$.

***N*-(2-(1*H*-Indol-3-yl)ethyl)-2-(2-hydroxyphenyl)oxazole-4-carboxamide (16)**



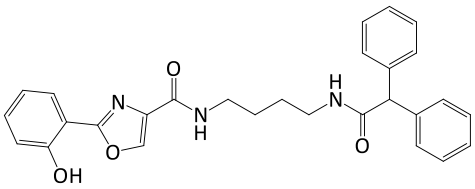
Compound **16** was made from compound **8** (0.022 g, 0.1 mmol), 2-(3-indolyl)ethylamine (0.021 g, 0.12 mmol), HATU (0.057 g, 0.15 mmol) and DIPEA (0.026 g, 0.2 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 72% yield as a pale yellow, amorphous solid (0.025 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 3.14 (t, 2H), 3.81 (q, 2H), 6.89 (brs, 1H), 7.02 (t, 1H), 7.10 (d, 1H), 7.15-7.20 (m, 2H), 7.25 (t, 1H), 7.42-7.46 (m, 2H), 7.69 (d, 1H), 7.87 (d, 1H), 8.23 (brs, 1H), 8.27 (s, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 25.35, 39.13, 110.35, 111.40, 112.63, 117.30, 118.69, 119.66, 120.00, 122.17, 122.41, 126.58, 127.01, 133.29, 135.87, 136.57, 139.94, 156.87, 159.60, 161.10. UPLC: 6.141 min. ESI-MS m/z calculated: 348.135; found: 348.316 $[M+H]^+$.

***N*-(4-(1,3-Dioxoisoindolin-2-yl)butyl)-2-(2-hydroxyphenyl)oxazole-4-carboxamide (17)**



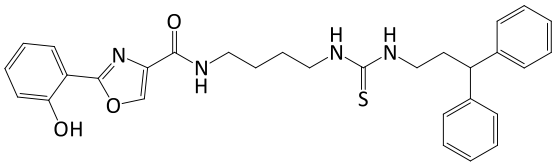
Compound **17** was made from compound **8** (0.04 g, 0.2 mmol), *N*-[4-(amino)butyl]phthalimide hydrochloride (0.056 g, 0.22 mmol), HATU (0.141 g, 0.3 mmol) and DIPEA (0.06 g, 0.5 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9** in 98% yield as a pale yellow, amorphous solid (0.08 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 1.72 (quint, 2H), 1.83 (quint, 2H), 3.56 (q, 2H), 3.78 (t, 2H), 7.00 (td, 1H), 7.09 (d, 1H), 7.14 (t, 1H), 7.42 (td, 1H), 7.71-7.75 (m, 2H), 7.85-7.90 (m, 3H), 8.26 (s, 1H), 10.25 (s, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 26.11, 26.54, 37.24, 38.66, 110.42, 117.35, 119.93, 123.35, 126.59, 132.07, 133.22, 133.99, 135.89, 140.11, 156.90, 159.88, 161.13, 168.57. UPLC: 6.054 min. ESI-MS m/z calculated: 406.141; found: 406.295 $[M+H]^+$.

***N*-(4-(2,2-Diphenylacetamido)butyl)-2-(2-hydroxyphenyl)oxazole-4-carboxamide (18)**



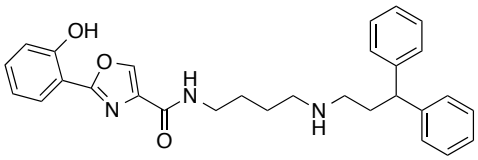
Compound **8** (0.021 g, 0.1 mmol) was dissolved in 10 ml DCM and to above solution was added (4-aminobutyl)-2,2-diphenylacetamide (0.032 g, 0.11 mmol) followed by HATU (0.057 g, 0.15 mmol) and DIPEA (0.02 g, 0.15 mmol). The whole mixture was stirred at 0 ˚C for 10 min and then was allowed warmed up to room temperature and stirred overnight. Resulting solution was washed with 20 ml water and 20 ml brine consecutively. The organic layer was later dried over anhydrous magnesium sulfate and concentrated under vacuum to give the crude compound. The crude compound was purified by column chromatography (0-20% ethyl acetate/hexane) to yield product **18** as an off-white, amorphous solid (0.012 g, 26%). ^{1}H NMR (δ, ppm, $CDCl_3$): 1.63 (m, 4H), 3.37 (t, 2H), 3.50 (t, 2H), 5.00 (s, 1H), 7.02 (t, 2H), 7.12 (t, 2H), 7.34 (m, 6H), 7.88 (dd, 2H), 8.28 (s, 1H), 10.17 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 27.13, 27.20, 38.80, 38.54, 96.26, 109.17, 119.98, 127.29, 128.79, 128.92, 134.59, 135.83, 139.36, 140.09, 152.61. UPLC: 6.492 min. ESI-MS m/z calculated: 470.208; found: 470.339 $[M+H]^+$.

***N*-(4-(3-(3,3-Diphenylpropyl)thioureido)butyl)-2-(2-hydroxyphenyl)oxazole-4- carboxamide (19)**



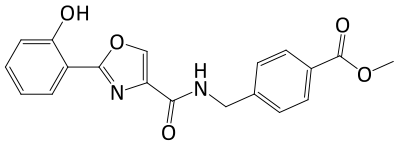
Compound **19** was made from compound **8** (0.006 g, 0.03 mmol), 1-(4-aminobutyl)-3-(3,3-diphenylpropyl)thiourea (0.01 g, 0.03 mmol) followed by HATU (0.019 g, 0.05 mmol) and DIPEA (0.007 g, 0.05 mmol) in 10 ml DCM using the procedure described for the synthesis of compound **18** in 75% yield as a pale yellow, amorphous solid (0.012 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 2.37-2.47 (m, 4H), 3.11-3.20 (m, 4H), 3.47 (q, 2H), 4.01 (dt, 2H), 5.88 (t, 1H), 6.729 (t, 1H), 7.02 (t, 1H), 7.12 (d, 1H), 7.18-7.19 (m, 4H), 7.20-7.32 (m, 6H), 7.44 (td, 1H), 7.88 (dd, 1H), 8.23 (s, 1H), 10.17 (brs, 1H). ^{13}C NMR (δ, ppm, $CDCl_3$): 35.11, 38.62, 39.71, 44.11, 46.88, 48.56, 49.26, 110.36, 117.35, 120.00, 126.52, 126.70, 127.73, 127.78 128.57, 128.79, 133.31, 135.81, 139.97, 143.39, 144.06, 144.35, 156.91, 159.68, 161.13, 161.46, 165.78. UPLC: 7.321 min. ESI-MS m/z calculated 529.228; found: 529.216 $[M+H]^+$.

***N*-(4-((3,3-Diphenylpropyl)amino)butyl)-2-(2-hydroxyphenyl)oxazole-4- carboxamide hydrochloride (20)**



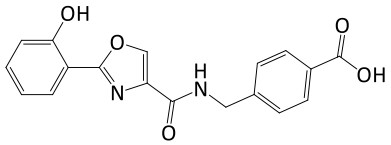
Compound **20** was made from compound **8** (0.037 g, 0.18 mmol), tert-butyl (4-aminobutyl)(3,3-diphenylpropyl)carbamate (0.07 g, 0.18 mmol), HATU (0.11 g, 0.3 mmol) and DIPEA (0.038 g, 0.3 mmol) in 4 ml DCM using the procedure described for the synthesis of compound **9**. The reaction mixture was evaporated to dryness, and the crude N-Boc-protected intermediate compound was dissolved in 10 ml ethyl acetate without further purification. A 5.0 mL portion of HCl in acetic acid was added, and the mixture was stirred at room temperature overnight. The resulting mixture was extracted with water and the aqueous layer was lyophylized to afford final product **20** as a white, amorphous solid (0.057 g, 63%). ^{1}H NMR (δ, ppm, CD_3OD): 1.58 (m, 2H), 2.41 (q, 2H), 2.75 (brs, 2H), 2.89 (brs, 2H), 3.28 (q, 2H), 3.49 (brs, 2H), 4.10 (t, 1H), 7.03 (t, 1H), 7.10 (d, 1H), 7.17-7.20 (m, 2H), 7.21-7.31 (m, 8H), 7.46 (t, 1H), 7.85 (dd, 1H), 8.74 (s, 1H), 8.90 (t, 1H), 9.02 (brs, 1H), 10.41 (s, 1H). ^{13}C NMR (δ, ppm, CD_3OD): 23.59, 26.80, 31.13, 38.86, 46.21, 46.71, 48.06, 111.05, 117.83, 120.29, 126.88, 127.31, 127.94, 129.07, 133.51, 136.50, 141.61, 144.34, 156.65, 159.82, 160.33. UPLC: 5.740 min. ESI-MS m/z calculated 470.245; found: 470.408 $[M+H]^+$.

Methyl 4-((2-(2-hydroxyphenyl)oxazole-4-carboxamido)methyl)benzoate (21)



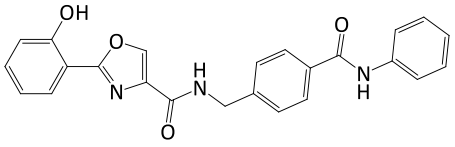
Compound **21** was made from compound **8** (0.025 g, 0.12 mmol), methyl 4-(aminomethyl)benzoate hydrochloride (0.03 g, 0.15 mmol), HATU (0.076 g, 0.2 mmol) and DIPEA (0.033 g, 0.25 mmol) in 10 ml DCM using the procedure described for the synthesis of compound **9** in 40% yield as an off-white, amorphous solid (0.017 g). ^{1}H NMR (δ, ppm, $CDCl_3$): 3.89 (s, 3H), 4.69 (d, 2H), 6.96 (t, 1H), 7.00 (d, 1H), 7.04 (brs, 1H), 7.37-7.42 (m, 3H), 7.84 (dd, 1H), 7.99 (d, 2H), 8.27 (s, 1H), 10.14 (s, 1H). ESI-MS m/z calculated: 353.114; found: 353.298 $[M+H]^+$.

4-((2-(2-Hydroxyphenyl)oxazole-4-carboxamido)methyl)benzoic acid (22)



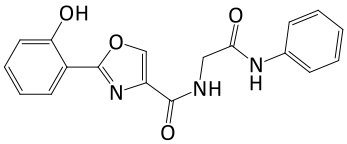
Compound **22** was made from compound **21** (0.017 g, 0.05 mmol) and lithium hydroxide monohydrate (0.021 g, 0.5 mmol) in 10 ml/5 ml THF/H_2O using the procedure described for the synthesis of compound **8** in 83% yield as a pale yellow, amorphous solid (0.014 g). ^{1}H NMR (δ, ppm, CD_3OD): 4.68 (s, 2H), 7.03 (t, 1H), 7.08 (d, 1H), 7.43 (t, 1H), 7.49 (d, 2H), 7.93 (dd, 1H), 8.02 (d, 2H), 8.51 (s, 1H). ; m.p. 248-251°C; ESI-MS m/z: calculated: 339.098; found: 339.294 $[M+H]^+$.

2-(2-Hydroxyphenyl)-N-(4-(phenylcarbamoyl)benzyl)oxazole-4-carboxamide (23)



Compound **23** was made from compound **22** (0.014 g, 0.04 mmol), aniline (0.005 g, 0.05 mmol), HATU (0.016 g, 0.05 mmol) and DIPEA (0.013 g, 0.1 mmol) in 3 ml DCM using the procedure described for the synthesis of compound **9** in 48% yield (0.008 g). ^{1}H NMR (δ, ppm, CH_3OD): 4.67 (s, 2H), 6.99 (t, 1H), 7.05 (d, 1H), 7.13 (t, 1H), 7.34 (m, 1H), 7.40 (s, 2H), 7.47 (d, 2H), 7.65 (d, 2H), 7.88 (m, 3H), 8.34 (s, 1H). ^{13}C NMR (δ, ppm, CH_3OD): 42.73, 110.36, 117.18, 119.89, 120.92, 124.52, 126.62, 127.59, 127.72, 128.76, 133.17, 135.65, 138.16, 140.51, 161.19. UPLC: 6.094 min. ESI-MS m/z calculated: 414.146; found: 414.603 $[M+H]^+$.

2-(2-Hydroxyphenyl)-N-(2-oxo-2-(phenylamino)ethyl)oxazole-4-carboxamide (24)



Compound **24** was made from compound **8** (0.011 g, 0.05 mmol), 2-amino-N-phenylacetamide hydrochloride (0.01 g, 0.05 mmol), HATU (0.038 g, 0.1 mmol) and DIPEA (0.025 g, 0.2 mmol) in 3 ml DCM using the procedure described for the synthesis of compound **9** in 83% yield (0.014 g). ^{1}H NMR (δ, ppm, DMSO-d_6): 4.10 (d, 2H), 7.05 (q, 2H), 7.10 (d, 1H), 7.32 (t, 2H), 7.47 (d, 2H), 7.88 (d, 1H), 8.80 (s, 1H), 9.17 (t, 1H), 10.10 (s, 1H), 10.43 (s, 1H). ^{13}C NMR (δ, ppm, DMSO-d_6): 46.87, 111.04, 117.84, 119.60, 120.34, 123.76, 127.36, 129.25, 133.56, 136.22, 139.37, 141.91, 156.69, 160.30, 160.41, 167.90. UPLC: 5.347 min. ESI-MS m/z calculated: 338.114; found 338.231 $[M+H]^+$.

HDAC Inhibitory Assay Methods

To perform the isoform selectivity study, the procedure was similar except that the HeLa cell nuclear extract was replaced with 11 different HDAC isoforms and appropriate substrate was used for the corresponding isoforms. Recombinant human HDAC1, HDAC2, HDAC3/NcoR2, HDAC5, HDAC6, HDAC8, HDAC9, HDAC10 and HDAC11 were purchased from BPS Biosciences. Recombinant human HDAC4 and HDAC7 were purchased from Millipore. The substrate Boc-Lys(Ac)-AMC and Boc-Lys(Tfa)-AMC were purchased from Bachem. The substrate Ac-RHK(Ac)K(Ac)-AMC was purchased from Enzo Life Sciences. Due to the different specific activities of each isoforms, different amount of enzyme was used in each isoform screening assay. In these assays, TSA, SAHA and DPAHA were employed as positive controls. All determinations were carried out in triplicate, and reported values are the average of these determinations, which in no case varied by more than 3%.

For evaluating the inhibition of class I HDACs (HDAC1, HDAC2 and HDAC3) and class IIb HDACs (HDAC6 and HDAC10), the 10 μl of test samples was first incubated with 20 ng HDAC1 (specific activity = 500 pmol/min/μg), 20 ng HDAC2 (specific activity = 675 pmol/min/μg), 5 ng HDAC3 (specific activity = 2500 pmol/min/μg), 25 ng HDAC6 (specific activity = 215 pmol/min/μg) and 250 ng HDAC10 (specific activity = 2.3 pmol/min/μg) respectively. Then 25 μl of the Boc-Lys(Ac)-AMC substrate (50 μM final concentration) was added and incubated at 37 °C for 30-60 min, and followed by adding 50 μl of developer (1mg/ml trypsin and 1 uM TSA). The last step is to read the samples in a SpectraMax M5 plate reader (Molecular Devices) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

For evaluating the inhibition of class IIa HDACs (HDAC4, HDAC5, HDAC7 and HDAC9) and class IV HDAC (HDAC11), the 10 μl of test samples was first incubated with 1 ng HDAC4 (specific activity = 15400 pmol/min/μg), 5 ng HDAC5 (specific activity = 2521 pmol/min/μg), 1 ng HDAC7 (specific activity = 26340 pmol/min/μg), 5 ng HDAC9

(specific activity = 3000 pmol/min/μg), and 500 ng HDAC11 (specific activity = 1.8 pmol/min/μg) respectively. Then 25 μl of the Boc-Lys(Tfa)-AMC substrate (50 μM final concentration) was added and incubated at 37 ˚C for 30-60 min, and followed by adding 50 μl of developer. The last step is to read the samples in a SpectraMax M5 plate reader (Molecular Devices) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

For evaluating the inhibition of HDAC8, the 10 μl of test samples was first incubated with 25 ng HDAC8 (specific activity = 298 pmol/min/μg). Then 25 μl of the fluorogenic peptide from p53 residues 379 - 382 (Ac-RHK(Ac)K(Ac)-AMC, 50 μM final concentration) was added and incubated at 37 ˚C for 30-60 min, and followed by adding 50 μl of developer (1mg/ml trypsin and 1 uM TSA). The last step is to read the samples in a SpectraMax M5 plate reader (Molecular Devices) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Attention: Because 2-(oxazol-2-yl)phenol-based HDAC inhibitors usually suffer from solubility issues. For the inhibitory assay, maximum 1% of DMSO which is also a HDAC inhibitor is allowed. Under this restriction, 20 μM is the highest concentration that can be used for this class of analogues.

Additional Inhibition Data

Table S1. Inhibitory activity of TSA (1 μM), SAHA (1 μM), DPAHA (5 μM) and 2-(oxazol-2-yl)phenol analogues 7-24 (20 μM) against 11 human recombinant HDAC isoforms.

No.	HDAC1	HDAC2	HDAC3	HDAC 4	HDAC 5	HDAC6	HDAC7	HDAC8	HDAC9	HDAC 10	HDAC 11
TSA	100%	99.2%	99.8%	2.4%	10.8%	100%	6.3%	17.9%	14.4%	100%	32.8%
SAHA	92.7%	83.2%	97.0%	0	0	97.7%	2.9%	10.7%	14.0%	100%	50.4%
DPAHA	2.1%	14.8%	76.4%	0	12.2%	62.4%	14.2%	4.5%	25.9%	39.2%	38.1%
7	4.6%	30.1%	39.1%	17.6%	13.2%	59.5%	7.1%	22.8%	19.0%	46.7%	41.3%
8	32.6%	54.8%	38.7%	25.6%	20.0%	59.8%	0	19.0%	15.5%	50.0%	48.4%
9	62.5%	29.5%	22.5%	17.1%	9.3%	50.3%	1.3%	0	20.5%	48.8%	21.8%
10	68.0%	54.1%	37.8%	20.7%	14.9%	61.6%	9.2%	23.3%	33.3%	47.1%	23.1%
11	43.9%	34.8%	23.7%	8.5%	0	49.1%	4.1%	16.7%	21.6%	35.7%	39.5%
12	35.9%	28.7%	37.5%	25.8%	11.3%	48.1%	0.7%	7.2%	28.2%	69.8%	45.2%
13	62.2%	31.9%	30.2%	25.5%	0	45.6%	19.4%	7.2%	32.4%	58.6%	28.4%
14	61.4%	52.2%	40.1%	24.4%	9.2%	47.8%	1.4%	17.9%	20.8%	48.7%	47.8%
15	47.8%	45.2%	32.3%	21.3%	3.2%	40.4%	3.8%	10.6%	15.5%	44.5%	49.9%
16	41.7%	36.0%	18.9%	7.5%	0	30.8%	3.8%	7.7%	18.4%	44.8%	26.0%
17	35.2%	25.4%	11.0%	17.5%	10.3%	46.0%	1.1%	9.6%	16.4%	32.1%	33.8%
18	27.2%	0	4.0%	4.8%	1.4%	26.7%	2.2%	0	9.0%	56.5%	30.1%
19	43.3%	1.3%	3.7%	55.8%	43.5%	50.2%	21.9%	0	70.2%	91.2%	69.9%
20	32.2%	19.7%	18.1%	6.5%	6.9%	28.9%	0	14.0%	16.4%	26.2%	39.1%
22	70.1%	56.5%	39.7%	24.2%	12.6%	60.3%	9.3%	20.0%	26.3%	47.2%	49.2%
23	47%	41.3%	15.8%	18.3%	0	30.8%	9.9%	0	24.3%	56.3%	20.1%
24	25.5%	32.0%	36.9%	28.1%	12.5%	59.9%	0	15.0%	14.2%	54.7%	46.6%

Cell Proliferation Assay Methods

Cytotoxicity of test compounds was determined by MTS assay, following the protocol of CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega). The cells used in this assay were MV4-11 (human, macrophage, biphenotypic B myelomonocytic leukemia) and MCF-7 (human, epithelial, adenocarcinoma). 10000 cells /well in 99 µl medium were seeded in a 96-well plate and were treated with 1 µl test compounds in DMSO with gradually increased concentrations (final concentration from 1 µM to 100 µM). The MV-4-11 cells were incubated with the compounds for 24 hours at 37 ˚C in 5% CO2 while the MCF-7 cells were first seeded on the 96-well plate and were allowed to attach for 1 day and then were incubated with different concentrations of the test compound for 72 hours at 37 ˚C in 5% CO2. After 24 or 72 hours 20 µl of MTS reagent solution was added into each well and the cells were incubated for another 4 hours at 37 ˚C under 5% CO2 environment. The absorbance was measured at 490 nm in a SpectraMax M5 plate reader (Molecular Devices) to determine the cell viability. The absorbance was directly proportional to the amount of viable cells. All determinations were carried out in triplicate, and reported values are the average of these determinations, which in no case varied by more than 3%.

Western Blot Methods

MV4-11 cells were first harvested with six different concentrations of test compound (1 µM, 2.5 µM, 5 µM, 7.5 µM, and 10 µM) and two different concentrations of SAHA (0.1 µM and 0.5 µM) under 10 ml IMDM (Iscove's Modified Dulbecco's Media) with 10% FBS (fetal bovine serum) for 4 hours. DMSO was used as the control. Later the cell pellets were collected by centrifuge at 1000 rpm for 5 minutes and then washed by ice-cold PBS (phosphate-buffered saline) twice. The PBS solution was drained and the cell pellets were re-suspended with ice-cold RIPA (radioimmunoprecipitation assay) buffer (150 mM sodium chloride, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0 and cocktail protease inhibitors) and were sonicated three times for 10 seconds each over a period of 30 minutes. The samples were later centrifuged at 20,000g for 15 minutes at 4 ˚C and the supernatants in each tube were collected and placed in a fresh tube kept on ice. BCA (bicinchoninic acid) assay was used to determine the total protein amount. Laemmli buffer (4% SDS, 10% 2-mercaptoehtanol, 20% glycerol, 0.004% bromophenol blue and 0.125 M Tris HCl) was then added into each sample and the mixture was boiled at 95 - 100°C for 5 minutes for denaturation. Around 20 µg protein was loaded into each well and the gels will be submerged in migration buffer which normally contains 25 mM Tris base, 190 mM glycine and 0.1% SDS. Run the gel for 90 - 120 minutes under 125 V. The proteins were then immobilized on a nitrocellulose membrane following electrophoretic transfer from the gel at 20 V at 4 ˚C overnight via wet transfer process. Non-protein binding areas on the membrane were blocked to prevent non-specific binding of antibodies by 5% non-fat milk in TBST (50 mM Tris, ph 7.6, 150 mM NaCl and 0.05% Tween 20) at room temperature for 1 hour and the membranes were incubated with primary antibodies respectively (anti-acetyl-H3K9, and anti-p21) that specifically bound to the protein of interest. Unbound antibodies were removed by washing and a secondary antibody conjugated to an enzyme, a fluorophore was used for detection. The detected signal from the protein:antibody:antibody complex was proportional to the amount of protein on the membrane. Later the membranes were striped and re-probed with loading control primary antibodies respectively (anti-H3) and repeated the previous steps to detect the signal.

Molecular Modeling Procedure

Molecular modeling. All molecular docking studies were performed using the Molecular Operations Environment (MOE) software, version 2010.12. The X-ray coordinates of human HDAC2 bound to 4-(acetylamino)-N-[2-amino-5-(thiophen-2-yl)phenyl]benzamide (PDB code 4LY1) were downloaded from the Protein Data Bank,[34] refined to add hydrogens and partial charges to the system with the Protonate3D application, and the active site was defined as a sphere enclosing residues within 9 Å around the substrate-like peptide inhibitor. The 3D structure of **10** was built and energy minimized using the MM94x field and a convergence value of 0.001 kcal/ mol/Å as the termination criterion.[35] The energy minimized compound **10** was docked in the binding site of HDAC2, tethered energy minimization was conducted to relieve bad

crystallographic contacts or other bad geometries, and poses were scored using ChemPLP. All poses generated by the program were visualized; however, the pose with the highest fitness score was used for elucidating the binding characteristics of **10** in the HDAC2 active site. An interaction diagram of compound **10** in the HDAC2 binding pocket was generated using the MOE software, version 2010.12. The numbering sequence of amino acid residues in 3ZMT is preserved throughout this paper.