**Supporting Information**

# Discovery of Novel Multi-acting Topoisomerase I/II and Histone Deacetylase Inhibitors

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## Table of contents

**Table S1** Relative inhibition rate of the evodiamine derivatives for Top1 and Top2α

| Compounds | Top1 (Relative inhibition rate) | Compounds | Top2α (Relative inhibition rate) |
|---|---|---|---|
| **DNA** | 1.0 | **DNA** | 1.0 |
| **CPT** | 0.49 | **Etoposide** | 0.47 |
| **1** | 0.20 | **1** | 0.15 |
| **7** | 0.47 | **7** | 1.0 |
| **8a** | 1.0 | **8a** | 0.19 |
| **8b** | 0.90 | **8b** | 0.75 |
| **8c** | 1.0 | **8c** | 0.77 |
| **9a** | 0.24 | **9a** | 0 |
| **9b** | 0.29 | **9b** | 0.46 |
| **9c** | 0.35 | **9c** | 0.48 |
| **10a** | 0.30 | **10a** | 0.80 |
| **10b** | 0.10 | **10b** | 0.44 |
| **10c** | 0.07 | **10c** | 0.81 |

**Table S2.** *In Vitro* HDAC Inhibition

| Compd | HDAC1 $K_i$ (nM) | HDAC2 $K_i$ (nM) | HDAC3 $K_i$ (nM) | HDAC6 $K_i$ (nM) | HDAC8 $K_i$ (μM) |
|---|---|---|---|---|---|
| **SAHA** | 12 ± 1.1 | 87 ± 35 | 28 ± 2.3 | 12 ± 1.0 | 4.4 ± 0.35 |
| **7** | 15 ± 2.3 | | | NT[a] | NT[a] |
| **8a** | 161 ± 11 | | | NT[a] | NT[a] |
| **8b** | 95 ± 6.5 | | | NT[a] | NT[a] |
| **8c** | 12 ± 1.6 | 137 ± 26 | 35 ± 3.2 | 7 ± 0.85 | 1.3 ± 0.15 |
| **9a** | >5000 | | | NT[a] | NT[a] |
| **9b** | 47 ± 2.9 | | | NT[a] | NT[a] |
| **9c** | 45 ± 4.0 | | | NT[a] | NT[a] |
| **10a** | 263 ± 22 | | | NT[a] | NT[a] |
| **10b** | 137 ± 12 | | | NT[a] | NT[a] |
| **10c** | >5000 | | | NT[a] | NT[a] |
| **10d** | 32 ± 2.7 | | | NT[a] | NT[a] |

[a]NT = not tested

## Synthetic procedures of the target compounds

### Chemistry

**General methods.** Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE300 or AVANCE500 spectrometer (Bruker Company, Germany), with TMS as an internal standard and $d_6$-DMSO as the solvent. Chemical shifts ($\delta$ values) and coupling constants ($J$ values) are given in ppm and Hz, respectively. ESI mass spectra were performed on an API-3000 LC–MS spectrometer. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60 G (Qindao Haiyang Chemical, China). Commercial solvents were used without any pretreatment.

**Methyl 8-((4-(hydroxymethyl)phenyl)amino)-8-oxooctanoate (4)**. A solution of compound **2** (0.5 g, 4.5 mmol), suberate monomethyl (0.61 g, 4.95 mmol), HBTU (1.6 g, 6.72 mmol) and $Et_3N$ (2.5 mL) in DMF (10 mL) was stirred at room temperature for 2 h. Then, the mixture was diluted with water (80 mL), and extracted with EtOAc (50 mL × 3). Then the combined organic layers were dried over $MgSO_4$, concentrated and purified by silica gel column chromatography ($CH_2Cl_2$-MeOH = 100:2) to give compound **4** (0.81 g, 76%) as a yellow solid. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.20-1.35 (m, 4H), 1.49-1.51 (m, 4H), 3.27 (t, $J$ = 7.2 Hz, 2H), 3.09 (t, $J$ = 6.9 Hz, 2H), 3.56 (s, 3H), 4.40 (d, $J$ = 5.5 Hz, 2H), 5.06 (d, $J$ = 5.6 Hz, 1H), 7.20 (d, $J$ = 8.2 Hz, 2H), 7.51 (d, $J$ = 8.4 Hz, 2H), 9.79 (s, 1H). MS (ESI, positive) m/z calcd for $C_{16}H_{23}NO_4$ (M+H): 294.2; found 294.4.

**Methyl 8-((4-formylphenyl)amino)-8-oxooctanoate (5).** To a stirred solution of PCC (1.25 g, 3.42 mmol) in $CH_2Cl_2$ (100 mL) was added compound **4** (0.4 g, 1.71 mmol). The reaction mixture was stirred at room temperature for 2 h. Then the solution was evaporated under reduced pressure, and the residual was purified by silica gel column chromatography ($CH_2Cl_2$-MeOH = 100:4) to give title compound **5** (0.38 g, 95%) as a yellow solid. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.25-1.32 (m, 4H), 1.46-1.61 (m, 4H), 2.28 (t, $J$ =7.4 Hz, 2H), 2.34 (t, $J$ =7.4 Hz, 2H), 3.56 (s, 3H), 7.82 (dd, $J$ =15.0 Hz, 8.8 Hz, 4H), 9.85 (s, 1H), 10.29 (s, 1H). MS (ESI, positive) m/z

calcd for $C_{16}H_{21}NO_4$ (M + H): 292.2; found 292.2.

**3-((4-(8-Methoxy-8-oxooctanamido)benzyl)amino)-10-hydroxylevodiamine (6).** A solution of aldehyde **5** (0.16 g, 0.55 mmol), amine **1** (0.15 g, 0.46 mmol) and a drop of acetic acid in THF (20 mL) was stirred at room temperature for 30 min. Then $NaBH_3CN$ (0.07 g, 1.1 mmol) was added and the resulting solution was stirred at room temperature for another 3 h. After reaction, the mixture solution was evaporated under reduced pressure, and the residual was partitioned between 2N NaOH (50 mL) and EtOAc (50 mL). The organic phase was evaporated under reduced pressure. The residual yellow oil was purified by silica gel column chromatography ($CH_2Cl_2$-MeOH = 100:2) to give the title compound **6** (0.09 g, 46%) as a yellow solid. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.24-1.32 (m, 4H), 1.46-1.58 (m, 4H), 2.23-2.31 (m, 7H), 2.65-2.78 (m, 2H), 3.03-3.13 (m, 1H), 3.57(s, 3H), 4.21 (d, $J$ =4.9 Hz, 2H), 4.57-4.62 (m, 1H), 5.83 (s, 1H), 6.28 (t, $J$ =5.2 Hz, 1H), 6.62 (dd, $J$ =8.6 Hz, 2.2Hz, 1H), 6.78 (d, $J$ =1.4 Hz, 1H), 6.81 (d, $J$ =2.5 Hz, 1H), 6.96 (d, $J$ =8.5 Hz, 1H), 7.06 (d, $J$ = 2.6 Hz, 1H), 7.14 (d, $J$ = 8.6 Hz, 1H), 7.26 (d, $J$ = 8.4 Hz, 2H), 7.52 (d, $J$ = 8.4 Hz, 2H), 8.68 (s, 1H), 9.81 (s, 1H), 10.89 (s, 1H). MS (ESI, positive) m/z calcd for $C_{35}H_{39}N_5O_5$ (M + H): 610.3; found 610.6.

Compounds **16a-c** and **23a-b** were synthesized according to the same protocol described for **6.**

**3-((4-(8-(Hydroxyamino)-8-oxooctanamido)benzyl)amino)-10-hydroxylevodiamine (7).** To a stirred solution of hydroxylamine hydrochloride (4.67 g, 67 mmol) in MeOH (24 mL) was added a solution of potassium hydroxide (5.61 g, 100 mmol) in MeOH (12 mL) dropwise at 0°C. After addition, the mixture was stirred for 30 min at 0°C. The precipitate was filtered and the filtrate formed a solution of free hydroxylamine in MeOH. Then, compound **6** (0.067 g, 0.11 mmol) was dissolved in above freshly prepared solution of hydroxylamine in MeOH (15 mL). The mixture was stirred at room temperature for 45 min, and then adjusted to pH 7 with acetic acid. The mixture was concentrated and the residue was washed with water to afford compound **7** (0.06 g, 89%) as a yellow solid, mp 214 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.25-1.35 (m, 4H), 1.43-1.58 (m, 4H), 1.93 (t, $J$ =7.2 Hz, 2H), 2.24-2.35 (m,

5H), 2.65-2.78 (m, 2H), 3.05-3.15 (m, 1H), 4.21 (d, *J* =4.8 Hz, 2H), 4.52-4.65 (m, 1H), 5.83 (s, 1H), 6.28 (t, *J* =5.2 Hz, 1H), 6.63 (dd, *J* =8.5 Hz, 1.9 Hz, 1H), 6.78 (d, *J* =1.4 Hz, 1H), 6.82 (d, *J* =2.6 Hz, 1H), 6.96 (d, *J* =8.5 Hz, 1H), 7.06 (d, *J* = 2.3 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H), 7.27 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 8.68 (s, 1H), 8.71 (s, 1H), 9.83 (s, 1H), 10.34 (s, 1H), 10.89 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$: 20.33, 25.47, 28.83, 32.68, 36.77, 37.44, 46.86, 69.48, 102.71, 110.10, 111.06, 112.40, 118.75, 119.59, 123.77, 124.13, 126.81, 127.86, 130.25, 131.72, 135.00, 138.33, 140.74, 145.59, 151.00, 164.45, 169.66, 171.65. HRMS (ESI, positive) m/z calcd for $C_{34}H_{38}N_6O_5$ (M + H): 611.2982; found 611.2986. HPLC purity: 95.8% at 254 nM.

Compounds **8a-c**, **9a-c** and **10a-d** were synthesized according to the same protocol described for **7.**

**4-(1,3-Dioxan-2-yl)benzonitrile (12).** To a stirred solution of compound **11** (2 g, 15.3 mmol) and propane-1,3-diol (4.65 g, 61.2 mmol) in toluene (100 mL) was added 4-methylbenzenesulfonic acid (0.26 g, 1.53 mmol). The resulting mixture was refluxed for 6 h. Then, the mixture solution was washed with saturation sodium bicarbonate (100 mL × 2), brine (100 mL × 2) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated under reduced pressure to give compound **12** (1.68 g, 95%) as white solid. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.41-1.48 (m, 1H), 1.90-2.08 (m, 1H), 3.90-3.99 (m, 2H), 4.12-4.18 (m, 2H), 5.55 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.81 (d, *J* = 8.3 Hz, 2H). MS (ESI, positive) m/z calcd for $C_{11}H_{11}NO_2$ (M+H): 190.1; found 190.3.

Compound **18** was synthesized according to the same protocol described for **12.**

**4-(1,3-Dioxan-2-yl)-*N*'-hydroxybenzimidamide (13).** To a stirred solution of compound **12** (3.45 g, 18.3 mmol) and hydroxylamine hydrochloride (1.89 g, 27.45 mmol) in $CH_3OH$ (50 mL) was added $NaHCO_3$ (3.07 g, 36.6 mmol). The resulting mixture was refluxed for 2 h. After cooling to room temperature, the reaction solution was filtered. The precipitate was crystallized from EtOH to give the title compound **13** (3.19 g, 76%) as white solid. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.42-1.48 (m, 1H), 1.91-2.08 (m, 1H), 3.90-3.99 (m, 2H), 4.12-4.18 (m, 2H), 4.50 (s, 2H), 5.55 (s, 1H),

7.46 (d, $J$ = 8.2 Hz, 2H), 7.81 (d, $J$ = 8.3 Hz, 2H), 9.79 (s, 1H). MS (ESI, positive) m/z calcd for $C_{11}H_{14}N_2O_3$ (M+H): 223.1; found 223.2

**Methyl 4-(3-(4-formylphenyl)-1,2,4-oxadiazol-5-yl)butanoate (15a).** A solution of monomethyl glutarate (0.329 g, 2.25 mmol), compound **13** (0.5 g, 2.25 mmol), HBTU (0.85 g, 2.25 mmol) and DIPEA (0.68g, 5.29 mmol) in DMF (10 mL) were treated by microwave irradiation at 191 °C for 2 min. After reaction, the mixture was diluted with water (100 mL), and extracted with EtOAc (50 mL ×3). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated and purified by silica gel column chromatography (hexane-EtOAc = 4:1) to give compound **14a**. This material was used in the next step without further purification. To a stirred solution of compound **14a** (0.5 g, 1.51 mmol) in aqueous acetone-water (1:1, 10 mL), $Fe(HSO_4)_3$ (0.03 g, 0.08 mmol) was added. The reaction mixture was stirred at 55 °C for 1 h. After completion of the reaction (controlled by TLC), $CH_2Cl_2$ (10 mL) was added. The solution was washed with water (10 mL × 2) and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent gave pure product **15a** (0.35 g, 85%) as a yellow solid. $^1$HNMR (DMSO-$d_6$, 300 MHz) $\delta$: 2.01-2.12 (m, 2H), 2.52 (t, $J$ = 7.4 Hz, 2H), 3.08 (t, $J$ = 7.4 Hz, 2H), 3.60 (s, 3H), 8.09 (d, $J$ = 8.4 Hz, 2H), 8.22 (d, $J$ = 8.1 Hz, 2H), 10.10 (s, 1H). MS (ESI, positive) m/z calcd for $C_{14}H_{14}N_2O_4$ (M + H): 275.1; found 275.3.

Compounds **15b-c** and **22a-c** were synthesized according to the same protocol described for **15a.**

**Methyl 5-(3-(4-formylphenyl)-1,2,4-oxadiazol-5-yl)pentanoate (15b).** Yellow solid: 0.49 g (yield: 86%). 1H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.78-2.32 (m, 4H), 2.51 (t, $J$ = 7.4 Hz, 2H), 3.01 (t, $J$ = 7.4 Hz, 2H), 3.61 (s, 3H), 8.10 (d, $J$ = 8.2 Hz, 2H), 8.22 (d, $J$ = 8.2 Hz, 2H), 10.11 (s, 1H). MS (ESI, positive) m/z calcd for $C_{15}H_{16}N_2O_4$ (M+H): 289.1; found 289.5.

**Methyl 7-(3-(4-formylphenyl)-1,2,4-oxadiazol-5-yl)heptanoate (15c).** Yellow solid: 0.53 g (yield: 85%). $^1$HNMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.29-1.38 (m, 4H), 1.48-1.59 (m, 2H), 1.74-1.84 (m, 2H), 2.30 (t, $J$ = 7.4 Hz, 2H), 3.03 (t, $J$ = 7.4 Hz, 2H), 3.58 (s, 3H), 8.09 (d, $J$ = 8.3 Hz, 2H), 8.22 (d, $J$ = 8.3 Hz, 2H), 10.10 (s, 1H). MS (ESI,

positive) m/z calcd for $C_{17}H_{20}N_2O_4$ (M + H): 317.1; found 317.4.

**3-((4-(5-(7-Methoxy-7-oxoheptyl)-1,2,4-oxadiazol-3-yl)benzyl)amino)-10-hydroxy levodiamine (16c).** Yellow solid: 0.11 g (yield: 45%).$^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.29-1.37 (m, 4H), 1.50-1.55 (m, 2H), 1.74-1.79 (m, 2H), 2.29 (t, $J$ = 7.8 Hz, 2H), 2.28 (s, 3H), 2.26-2.80 (m, 2H), 2.98 (t, $J$ = 7.5 Hz 2H), 3.05-3.10 (m, 1H), 3.57(s, 3H), 4.37 (d, $J$ = 6.8 Hz, 2H), 4.57-4.61 (m, 1H), 5.84 (s, 1H), 6.47 (t, $J$ = 6.0 Hz, 1H), 6.62 (dd, $J$ = 9.1 Hz, 2.2 Hz, 1H), 6.78 (d, $J$ = 2.2 Hz, 1H), 6.82 (dd, $J$ = 9.0 Hz, 3.1 Hz, 1H), 6.97 (d, $J$ = 9.0 Hz, 1H), 7.06 (d, $J$ = 2.2 Hz, 1H), 7.13 (d, $J$ = 8.4 Hz, 1H), 7.54 (d, $J$ = 8.3 Hz, 2H), 7.96 (d, $J$ = 8.3 Hz, 2H), 8.68 (s, 1H), 10.90 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 150 MHz) $\delta$: 20.3, 24.6, 26.1, 26.2, 28.3, 28.4, 33.6, 37.4, 47.1, 51.6, 69.5, 102.7, 110.2, 111.1, 112.4, 112.5, 118.7, 123.8, 124.2, 125.2, 126.8, 127.5, 128.2, 130.2, 131.7, 140.9, 144.5, 145.4, 151.1, 164.4, 167.9, 173.8, 180.7. MS (ESI, positive) m/z calcd for $C_{36}H_{38}N_6O_5$ (M + H): 635.3; found 635.4.

**3-((4-(5-(4-(Hydroxyamino)-4-oxobutyl)-1,2,4-oxadiazol-3-yl)benzyl)amino)-10-h ydroxylevodiamine (8a).** Yellow solid: 0.09 g (yield: 91%), mp 226 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.97-2.01 (m, 2H), 2.10 (t, $J$ = 7.1 Hz, 2H), 2.29 (s, 3H), 2.68-2.76 (m, 2H), 2.99 (t, $J$ = 7.1 Hz, 2H), 3.12-3.15 (m, 1H), 4.37 (d, $J$ = 5.5 Hz, 2H), 4.57-4.60 (m, 1H), 5.83 (s, 1H), 6.44 (t, $J$ = 5.5 Hz, 1H), 6.62 (dd, $J$ = 8.7 Hz, 2.3 Hz, 1H), 6.78 (d, $J$ = 1.7 Hz, 1H), 6.83 (dd, $J$ = 8.7 Hz, 2.3 Hz, 1H), 6.97 (d, $J$ = 8.6 Hz, 1H), 7.06 (d, $J$ = 2.3 Hz, 1H), 7.14 (d, $J$ =8.5 Hz, 1H), 7.37 (d, $J$ = 8.5 Hz, 1H), 7.44 (d, $J$ = 8.5 Hz, 1H), 7.55 (d, $J$ = 8.2 Hz, 2H), 7.96 (d, $J$ = 8.5 Hz, 2H), 8.68 (s, 1H), 10.86 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$: 20.22, 22.43, 25.76, 29.47, 31.70, 37.45, 47.00, 69.48, 102.69, 110.17, 111.04, 112.38, 112.40, 118.73, 122.43, 123.89, 124.18, 126.82, 127.42, 128.38, 130.24, 131.73, 140.99, 144.62, 145.33, 152.05, 163.36, 166.28, 168.87, 169.90. HRMS (ESI, positive) m/z calcd for $C_{32}H_{31}N_7O_5$ (M + H): 594.2465; found 594.2459. HPLC purity: 97.7% at 254 nM.

**3-((4-(5-(5-(Hydroxyamino)-5-oxopentyl)-1,2,4-oxadiazol-3-yl)benzyl)amino)-10-hydroxylevodiamine (8b).** Yellow solid: 0.07 g (yield: 90%), mp 242 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.56-1.52 (m, 2H), 1.71-1.80 (m, 2H), 2.00 (t, $J$ = 7.0 Hz, 2H), 2.28 (s, 3H), 2.73 (m, 2H), 3.00 (t, $J$ = 7.0 Hz, 2H), 3.07-3.13 (m, 1H), 4.37 (s,

2H), 4.59 (d, *J* = 9.8 Hz, 1H), 5.84 (s, 1H), 6.46 (t, *J* = 6.3 Hz, 1H), 6.62 (dd, *J* = 8.5 Hz, 2.0 Hz, 1H), 6.78 (d, *J* = 2.0 Hz, 1H), 6.84 (d, *J* = 2.7 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 1H), 7.07 (dd, *J* = 6.6 Hz, 2.4 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.96 (d, *J* = 8.3 Hz, 2H), 8.23 (s, 1H), 8.75 (s, 1H), 10.38 (s, 1H), 10.89 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 150 MHz) $\delta$: 20.33, 22.50, 25.67, 29.48, 30.44, 31.53, 37.44, 46.14, 47.04, 69.48, 102.69, 110.15, 111.04, 112.37, 118.70, 123.81, 124.21, 125.19, 126.82, 127.57, 128.23, 130.24, 131.73, 140.94, 144.57, 145.41, 151.05, 164.36, 167.88, 168.71. HRMS (ESI, positive) m/z calcd for $C_{33}H_{33}N_7O_5$ (M + H): 608.2621; found 608.2627. HPLC purity: 94.4% at 254 nM.

**3-((4-(5-(7-(Hydroxyamino)-7-oxoheptyl)-1,2,4-oxadiazol-3-yl)benzyl)amino)-10-hydroxylevodiamine (8c).** Yellow solid: 0.07 g (yield: 92%), mp 201 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.29-1.37 (m, 4H), 1.46-1.56 (m, 2H), 1.70-1.80 (m, 2H), 2.27 (s, 3H), 2.28 (t, *J* = 7.2 Hz, 2H), 2.66-2.76 (m, 2H), 2.96 (t, *J* = 7.4 Hz 2H), 3.01-3.11 (m, 1H), 4.36 (d, *J* = 5.6 Hz, 2H), 4.54-4.62 (m, 1H), 5.83 (s, 1H), 6.47 (t, *J* = 6.3 Hz, 1H), 6.63 (dd, *J* = 8.6 Hz, 2.1 Hz, 1H), 6.78 (d, *J* = 1.9 Hz, 1H), 6.81 (dd, *J* = 8.6 Hz, 2.6 Hz, 1H), 6.96 (d, *J* = 9.0 Hz, 1H), 7.05 (d, *J* = 2.6 Hz, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 2H), 8.22 (s, 1H), 8.68 (d, *J* = 7.8 Hz, 2H), 8.71 (s, 1H), 10.37 (s, 1H), 10.89 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 150 MHz) $\delta$: 20.36, 25.39, 25.88, 26.16, 26.30, 28.49, 28.53, 32.74, 37.39, 47.06, 69.50, 102.70, 110.16, 110.98, 112.28, 112.63, 118.67, 123.72, 124.17, 125.21, 126.83, 127.53, 128.23, 130.08, 131.61, 140.95, 144.53, 145.39, 151.45, 164.35, 167.87, 168.89, 174.20. HRMS (ESI, negative). m/z calcd for $C_{35}H_{37}N_7O_5$ (M - H):634.2778; found 634.2782. HPLC purity: 94.2% at 254 nM.

**Methyl 4-(1,3-dioxan-2-yl)benzoate (18).** White solid: 2.5 g (yield: 93%). $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.41-1.49 (m, 1H), 1.91-2.08 (m, 1H), 3.61 (s, 3H), 3.91-3.99 (m, 2H), 4.10-4.17 (m, 2H), 5.55 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 8.3 Hz, 2H). MS (ESI, positive) m/z calcd for $C_{12}H_{14}O_4$ (M+H): 223.1; found 223.4.

**4-(1,3-Dioxan-2-yl)benzohydrazide (19).** Compound **18** (2 g, 9.01 mmol) was dissolved in a mixture of ethanol and $N_2H_4$•$H_2O$ (1:1) (40 mL). The mixture was

stirred at room temperature for 24 h. After completion of the reaction (controlled by TLC), ethanol was evaporated under reduced pressure and water (50 mL) was added. Then, the aqueous layer was extracted with EtOAc (50 mL × 3). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to give the crude product. The crude product was purified by silica gel column chromatography ($CH_2Cl_2$-MeOH = 100:2) to give compound **19** (1.46 g, 73%) as a white solid. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.41-1.49 (m, 1H), 1.91-2.08 (m, 1H), 3.90-3.99 (m, 2H), 4.12-4.17 (m, 2H), 4.50 (s, 2H), 5.55 (s, 1H), 7.46 (d, $J$ = 8.2 Hz, 2H), 7.80 (d, $J$ = 8.3 Hz, 2H), 9.78 (s, 1H). MS (ESI, positive) m/z calcd for $C_{11}H_{14}N_2O_3$ (M+H): 223.1; found 223.2.

**Methyl 5-(2-(4-(1,3-dioxan-2-yl)benzoyl)hydrazinyl)-5-oxopentanoate (20a).** To a stirred solution of compound **19** (1.0 g, 4.5 mmol), glutarate monomethyl (0.73 g, 4.95 mmol) and HBTU (2.2 g, 5.85 mmol) in DMF (25 mL) was added $Et_3N$ (2.53 mL, 18 mmol). The reaction mixture was stirred at room temperature for 2 h. Then, the mixture was diluted with water (100 mL) and extracted with EtOAc (100mL × 3).The combined organic layers were dried with anhydrous $Na_2SO_4$, filtrated and evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CH_2Cl_2$-MeOH = 100:2) to give compound **20a** (1.13 g, 72%) as a white solid. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.46 (d, $J$ = 12.5 Hz, 1H), 1.75-1.86 (m, 2H), 1.93-2.10 (m, 1H), 2.23 (t, $J$ = 8.0 Hz, 2H), 2.40 (t, $J$ = 8.0 Hz, 2H), 3.61 (s, 3H), 3.91-4.00 (m, 2H), 4.13-4.19 (m, 2H), 5.58 (s, 1H), 7.51 (d, $J$ = 8.2 Hz, 2H), 7.85 (d, $J$ = 8.2 Hz, 2H), 9.87 (s, 1H), 10.31 (s, 1H). MS (ESI, positive) m/z calcd for $C_{17}H_{22}N_2O_6$ (M + H): 351.2; found 351.3.

**Methyl 4-(5-(4-(1,3-dioxan-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)butanoate (21a).** To a stirred solution of **20a** (0.5 g, 1.43 mmol), $Et_3N$ (1.2 mL, 3.43 mmol) in THF (80 mL) was added *p*-toluenesulfonyl chloride (0.82 g, 4.3 mmol). The mixture was heated to reflux for 5 h. Then the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography ($CH_2Cl_2$-MeOH = 100:3) to give title compound **21a** (0.36 g, 78%) as a yellow solid. MS (ESI, positive) m/z calcd for $C_{18}H_{22}N_2O_5$ (M + H): 347.2; found 347.3.

Compound **21b-c** was synthesized according to the same protocol described for **21a**.

**Methyl 4-(5-(4-formylphenyl)-1,3,4-oxadiazol-2-yl)butanoate (22a).** Yellow solid: 0.46 g (yield: 69%). $^1$H NMR ($CDCl_3$-*d*, 300 MHz) $\delta$: 2.17-2.27 (m, 2H), 2.53 (d, $J$ = 7.3 Hz, 2H), 3.05 (d, $J$ = 7.8 Hz, 2H), 3.69 (s, 3H), 8.03 (d, $J$ = 8.2 Hz, 2H), 8.22 (d, $J$ = 8.2 Hz, 2H), 10.10 (s, 1H). MS (ESI, positive) m/z calcd for $C_{14}H_{14}N_2O_4$ (M + H): 275.1; found 275.3.

**Methyl 5-(5-(4-formylphenyl)-1,3,4-oxadiazol-2-yl)pentanoate (22b).** Yellow solid: 0.57 g (yield: 59%). $^1$H NMR ($CDCl_3$-*d*, 300 MHz) $\delta$: 1.75-1.83 (m, 2H), 1.86-1.94 (m, 2H), 2.39 (d, $J$ = 7.1 Hz, 2H), 2.97 (d, $J$ = 7.1 Hz, 2H), 3.66 (s, 3H), 8.00 (d, $J$ = 8.3 Hz, 2H), 8.20 (d, $J$ = 8.3 Hz, 2H), 10.08 (s, 1H). MS (ESI, positive) m/z calcd for $C_{15}H_{16}N_2O_4$ (M + H): 289.1; found 289.3.

**3-((4-(5-(4-(Hydroxyamino)-4-oxobutyl)-1,3,4-oxadiazol-2-yl)benzyl)amino)-10-methoxylevodiamine (9a).** Yellow solid: 0.07 g (yield: 84%), mp 233 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.59-1.65 (m, 2H), 2.14 (t, $J$ = 7.4 Hz, 2H), 2.27 (s, 3H), 2.65-2.73 (m, 2H), 2.85 (t, $J$ = 7.4 Hz, 2H), 3.13-3.27 (m, 1H), 4.37 (d, $J$ = 6.4 Hz, 2H), 4.55-4.59 (m, 1H), 5.81 (s, 1H), 6.52 (t, $J$ = 6.3 Hz, 1H), 6.61 (dd, $J$ = 8.6 Hz, 2.4 Hz, 1H), 6.78 (d, $J$ = 2.2 Hz, 1H), 6.83 (dd, $J$ = 8.6 Hz, 2.75 Hz, 1H), 6.95 (d, $J$ = 8.5 Hz, 1H), 7.03 (d, $J$ = 2.7 Hz, 1H), 7.13 (t, $J$ = 8.5 Hz, 1H), 7.51 (d, $J$ = 8.6 Hz, 2H), 7.87 (d, $J$ = 8.6 Hz, 2H), 8.65 (s, 1H), 8.77 (s,1H), 10.38 (s, 1H), 10.90 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$: 20.32, 22.43, 24.56, 29.47, 31.59, 37.45, 47.00, 69.48, 102.69, 110.17, 111.04, 112.38, 112.46, 118.70, 122.43, 123.79, 124.18, 126.82, 127.02, 128.34, 130.24, 131.73, 140.97, 145.02, 145.33, 151.05, 164.36, 166.68, 168.77. HRMS (ESI, positive) m/z calcd for $C_{32}H_{31}N_7O_5$ (M + H): 594.2465; found 594.2461. HPLC purity: 95.1% at 254 nM.

**3-((4-(5-(5-(Hydroxyamino)-5-oxopentyl)-1,3,4-oxadiazol-2-yl)benzyl)amino)-10-methoxylevodiamine (9b).** Yellow solid: 0.04 g (yield: 90%), mp 280 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.53-1.60 (m, 2H), 1.68-1.75 (m, 2H), 2.01 (t, $J$ = 7.4 Hz, 2H), 2.28 (s, 3H), 2.70-2.72 (m, 2H), 2.91 (t, $J$ = 7.4 Hz, 2H), 3.04-3.09 (m, 1H), 4.36 (d, $J$ = 6.4 Hz, 2H), 4.56 (d, $J$ = 10.7 Hz, 1H), 5.82 (s, 1H), 6.52 (t, $J$ = 6.4 Hz, 1H), 6.62 (dd, $J$ = 8.6 Hz, 2.4 Hz, 1H), 6.78 (d, $J$ = 2.4 Hz, 1H), 6.82 (dd, $J$ = 8.6 Hz, 2.4

Hz, 1H), 6.95 (d, $J$ = 8.6 Hz, 1H), 7.03 (d, $J$ = 2.8 Hz, 1H), 7.12 (t, $J$ = 6.4 Hz, 1H), 7.56 (d, $J$ = 8.6 Hz, 2H), 7.94 (d, $J$ = 8.6 Hz, 2H), 8.70 (s, 1H), 8.76 (s,1H), 10.49 (s, 1H), 10.88 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$: 20.32, 24.75, 24.91, 25.79, 32.13, 37.43, 46.99, 69.48, 102.71, 110.10, 111.03, 112.37, 112.48, 118.77, 122.39, 123.74, 124.11, 126.80, 126.97, 128.36, 130.22, 131.71, 140.95, 145.04, 145.31, 151.07, 164.32, 164.37, 167.00. HRMS (ESI, positive) m/z calcd for $C_{33}H_{33}N_7O_5$ (M + H): 608.2621; found 608.2630. HPLC purity: 97.8% at 254 nM.

**3-((4-(5-(7-(Hydroxyamino)-7-oxopentyl)-1,3,4-oxadiazol-2-yl)benzyl)amino)-10-methoxylevodiamine (9c).** Yellow solid: 0.09 g (yield: 91%), mp 191 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.40-1.58 (m, 4H), 1.61-1.81 (m, 2H), 1.92 (t, $J$ = 7.1 Hz, 2H), 2.18 (t, $J$ = 7.0 Hz, 2H), 2.27 (s, 3H), 2.60-2.78 (m, 2H), 2.89 (t, $J$ = 7.1 Hz 2H), 3.01-3.17 (m, 1H), 4.37 (d, $J$ = 3.8 Hz, 2H), 4.58 (d, $J$ = 11.9 Hz, 1H), 5.82 (s, 1H), 6.47 (t, $J$ = 6.2 Hz, 1H), 6.61 (d, $J$ = 8.3 Hz, 1H), 6.76-6.83 (m, 2H), 6.96 (d, $J$ = 8.0 Hz, 1H), 7.05 (s, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.56 (d, $J$ = 7.8 Hz, 2H), 7.93 (d, $J$ = 7.9 Hz, 2H), 8.67 (s, 2H), 10.32 (s, 1H), 10.88 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 150 MHz) $\delta$: 20.32, 24.98, 25.38, 26.17, 28.48, 32.65, 37.44, 47.00, 69.49, 102.70, 110.18, 111.05, 112.39, 118.69, 122.44, 123.79, 124.18, 126.82, 126.98, 128.39, 130.24, 131.73, 140.97, 144.99, 145.33, 151.05, 164.29, 164.35, 167.12, 169.55. HRMS (ESI, positive) m/z calcd for $C_{35}H_{37}N_7O_5$ (M + H): 636.2934; found 636.2944. HPLC purity: 98.8% at 254 nM.

**3-(5-Methoxy-5-oxopentanamido)-10-methoxylevodiamine (25a).** Reaction of compound **24** (0.2 g, 0.575 mmol) and monomethyl glutarate (0.092 g, 0.633 mmol) as described for the synthesis of **4**, followed by purification using silica gel column chromatography ($CH_2Cl_2$-MeOH = 100:3) gave **25a** (0.19 g, 71%) as a yellow solid. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.82-1.86 (m, 2H), 2.32-2.37 (m, 4H), 2.63 (s, 3H), 2.79-2.83 (m, 2H), 3.14-3.19 (m, 1H), 3.59 (s, 3H), 3.76 (s, 3H), 4.61-4.64 (m, 1H), 6.01 (s, 1H), 6.75 (dd, $J$ = 8.9 Hz, 2.3 Hz, 1H), 6.99 (d, $J$ = 2.3 Hz, 1H), 7.08 (d, $J$ = 8.6 Hz, 1H), 7.25 (d, $J$ = 8.9 Hz, 1H), 7.75 (dd, $J$ = 8.9 Hz, 2.34 Hz, 1H), 8.05 (d, $J$ = 2.3 Hz, 1H), 9.93 (s, 1H), 11.01 (s, 1H). MS (ESI, positive) m/z calcd for $C_{26}H_{28}N_4O_5$ (M + H): 477.2; found 477.4.

Compounds **25b-d** were synthesized according to the same protocol described for **25a.**

**3-(6-Methoxy-6-oxohexanamido)-10-methoxylevodiamine (25b).** Yellow solid: 0.21 g (yield: 75%). $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.55-1.61 (m, 2H), 2.26-2.31 (m, 4H), 2.64 (s, 3H), 2.83 (t, $J$ = 8.9 Hz, 2H), 3.08-3.17 (m, 2H), 3.17-3.20 (m, 1H), 3.58 (s, 3H), 3.77 (s, 3H), 4.62-4.66 (m, 1H), 6.02 (s, 1H), 6.77 (dd, $J$ = 8.9 Hz, 2.3 Hz, 1H), 7.00 (d, $J$ = 2.3 Hz, 1H), 7.08 (d, $J$ = 8.6 Hz, 1H), 7.25 (d, $J$ = 8.9 Hz, 1H), 7.77 (dd, $J$ = 8.9 Hz, 2.3 Hz, 1H), 8.05 (d, $J$ = 2.3 Hz, 1H), 9.88 (s, 1H), 11.00 (s, 1H). MS (ESI, positive) m/z calcd for $C_{27}H_{30}N_4O_5$ (M + H): 491.2; found 491.4.

**3-(7-Methoxy-7-oxoheptanamido)-10-methoxylevodiamine (25c).** Yellow solid: 0.14 g (yield: 68%).$^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.32-1.55 (m, 4H), 1.59-1.63 (m, 2H), 2.21 (t, $J$ = 7.2 Hz, 2H), 2.28 (t, $J$ = 7.2 Hz, 2H), 2.64 (s, 3H), 2.80-2.83 (m, 2H), 3.18 (s, 1H), 3.59 (s, 3H), 3.75 (s, 3H), 4.61-4.66 (m, 1H), 6.00 (s, 1H), 6.75 (dd, $J$ = 8.6 Hz, 2.4 Hz, 1H), 6.98 (d, $J$ = 2.3 Hz, 1H), 7.07 (d, $J$ = 8.7 Hz, 1H), 7.25 (d, $J$ = 8.6 Hz, 1H), , 7.75 (s, 1H), 8.07 (d, $J$ = 2.4 Hz, 1H), 9.96 (s, 1H), 11.01 (s, 1H). MS (ESI, positive) m/z calcd for $C_{28}H_{32}N_4O_5$ (M+H): 505.2; found 505.3.

**3-(8-Methoxy-8-oxooctanamido)-10-methoxylevodiamine (25d).** Yellow solid: 0.12 g (yield: 63%).$^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.31-1.36 (m, 4H), 1.51-1.63 (m, 4H), 2.18 (t, $J$ = 7.1 Hz, 2H), 2.29 (t, $J$ = 7.3 Hz, 2H), 2.63 (s, 3H), 2.79-2.83 (m, 2H), 3.18 (s, 1H), 3.58 (s, 3H), 3.76 (s, 3H), 4.61-4.65 (m, 1H), 6.01 (s, 1H), 6.75 (dd, $J$ = 8.6 Hz, 2.3 Hz, 1H), 6.98 (d, $J$ = 2.4 Hz, 1H), 7.07 (d, $J$ = 8.7 Hz, 1H), 7.24 (d, $J$ = 8.7 Hz, 1H), 7.76 (s, 1H), 8.06 (s, 1H), 9.95 (s, 1H), 11.02 (s, 1H). MS (ESI, positive) m/z calcd for $C_{29}H_{34}N_4O_5$ (M+H): 519.3; found 519.4.

**3-(5-(Hydroxyamino)-5-oxopentanamido)-10-methoxylevodiamine (10a).** Yellow solid: 0.17 g (yield: 84%), mp 252 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.73-1.83 (m, 2H), 1.99 (t, $J$ = 7.3 Hz, 2H), 2.28 (t, $J$ = 7.1 Hz, 2H), 2.62 (s, 3H), 2.81 (d, $J$ = 4.0 Hz, 2H), 3.10-3.21 (m, 1H), 3.74 (s, 3H), 4.62 (d, $J$ = 12.2 Hz, 1H), 6.00 (s, 1H), 6.74 (dd, $J$ = 8.6 Hz, 2.2 Hz, 1H), 6.97 (d, $J$ = 2.0 Hz, 1H), 7.06 (d, $J$ = 8.8 Hz, 1H), 7.23 (d, $J$ = 8.8 Hz, 1H), 7.74 (dd, $J$ = 8.6 Hz, 2.2 Hz, 1H), 8.06 (d, $J$ = 1.3 Hz, 1H), 8.68 (d, $J$ = 1.3 Hz, 1H), 9.96 (s, 1H), 10.39 (s, 1H), 11.00 (s, 1H). $^{13}$C NMR

(DMSO-$d_6$, 75 MHz) $\delta$: 20.20, 22.19, 31.57, 36.50, 41.74, 55.80, 69.78, 71.81, 100.62, 111.80, 113.53, 113.78, 117.83, 124.63, 126.53, 128.75, 130.55, 132.03, 132.23, 134.48, 144.41, 154.20, 164.34, 168.90, 171.26. HRMS (ESI, positive) m/z calcd for $C_{25}H_{27}N_5O_5$ (M + H): 478.2090; found 478.2099. HPLC purity: 95.2% at 254 nM.

**3-(6-(Hydroxyamino)-6-oxohexanamido)-10-methoxylevodiamine (10b).** Yellow solid: 0.08 g (yield: 90%), mp 285 °C.$^1$HNMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.51-1.57 (m, 4H), 1.98 (t, $J$ = 7.1 Hz, 2H), 2.28 (t, $J$ = 6.7 Hz, 2H), 2.64 (s, 3H), 2.82 (d, $J$ = 8.2 Hz, 2H), 3.14-3.19 (m, 1H), 3.75 (s, 3H), 4.61-4.64 (m, 1H), 6.01 (s, 1H), 6.75 (dd, $J$ = 8.7 Hz, 2.4 Hz, 1H), 6.98 (d, $J$ = 2.2 Hz, 1H), 7.06 (d, $J$ = 8.7 Hz, 1H), 7.25 (d, $J$ = 8.7 Hz, 1H), 7.67 (dd, $J$ = 5.8 Hz, 3.2 Hz, 1H), 7.73 (d, $J$ = 5.8 Hz, 3.2 Hz, 1H), 7.75 (d, $J$ =2.2 Hz, 1H), 8.07 (s, 1H), 9.99 (s, 1H), 11.01 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$: 19.33, 20.19, 25.31, 27.67, 36.53, 36.97, 55.82, 69.78, 71.61, 100.61, 111.80, 112.43, 112.77, 118.83, 125.13, 126.58, 129.15, 130.95, 132.03, 132.15, 134.08, 145.51, 153.80, 164.34, 167.40, 171.36. HRMS (ESI, negative) m/z calcd for $C_{26}H_{29}N_5O_5$ (M - H): 490.2090; found 490.2111. HPLC purity: 95.8% at 254 nM.

**3-(7-(Hydroxyamino)-7-oxoheptanamido)-10-methoxylevodiamine (10c).** Yellow solid: 0.12 g (yield: 83%), mp 245 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.26-1.30 (m, 2H), 1.47-1.50 (m, 2H), 1.55-1.58 (m, 2H), 2.11 (t, $J$ = 7.3 Hz, 2H), 2.27 (t, $J$ = 7.4 Hz, 2H), 2.63 (s, 3H), 2.80-2.83 (m, 2H), 3.13-3.18 (m, 1H), 3.75 (s, 3H), 4.60-4.64 (m, 1H), 6.01 (s, 1H), 6.75 (dd, $J$ = 8.6 Hz, 2.4 Hz, 1H), 6.98 (d, $J$ = 2.4 Hz, 1H), 7.06 (d, $J$ = 8.7 Hz, 1H), 7.25 (d, $J$ = 8.7 Hz, 1H), 7.54 (d, $J$ = 6.9 Hz, 1H), 7.64 (d, $J$ = 2.4 Hz, 8.6 Hz, 1H), 7.75 (dd, $J$ = 8.6 Hz, 2.4 Hz, 1H), 8.07 (d, $J$ = 2.4 Hz, 1H), 9.98 (s, 1H), 11.03 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 150 MHz) $\delta$: 20.20, 24.98, 25.42, 28.78, 36.67, 36.98, 55.85, 69.77, 100.64, 111.83, 112.45, 112.77, 118.82, 120.36, 121.46, 125.11, 126.59, 130.93, 132.17, 134.15, 145.53, 153.82, 164.33, 171.43. HRMS (ESI, positive) m/z calcd for $C_{27}H_{31}N_5O_5$ (M + H): 506.2403; found 506.2411. HPLC purity: 95.1% at 254 nM.

**3-(8-(Hydroxyamino)-8-oxooctanamido)-10-methoxylevodiamine (10d).** Yellow solid: 0.10 g (yield: 88%), mp 272 °C. $^1$H NMR (DMSO-$d_6$, 600 MHz) $\delta$: 1.23-1.34 (m, 4H), 1.42-1.53 (m, 2H), 1.53 (m, 2H), 2.12 (t, $J$ = 7.1 Hz, 2H), 2.27 (t, $J$ = 7.1 Hz,

2H), 2.63 (s, 3H), 2.80-2.82 (m, 2H), 3.17 (s, 1H), 3.76 (s, 3H), 4.63 (d, $J$ = 12.5 Hz, 1H), 6.01 (s, 1H), 6.75 (dd, $J$ = 8.7 Hz, 2.3 Hz, 1H), 6.99 (d, $J$ = 2.4 Hz, 1H), 7.07 (d, $J$ = 8.7 Hz, 1H), 7.24 (d, $J$ = 8.7 Hz, 1H), 7.52 (s, 1H), 7.60 (s, 1H), 7.76 (dd, $J$ = 8.7 Hz, 2.5 Hz, 1H), 8.06 (s, 1H), 9.93 (s, 1H), 11.03 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 150 MHz) $\delta$: 20.19, 25.49, 25.52, 28.85, 32.71, 36.73, 55.84, 69.75, 100.64, 111.81, 112.44 112.75, 118.82, 120.36, 121.47, 125.10, 126.58, 130.91, 132.17, 134.16, 145.52, 153.81, 164.31, 171.45. MS (ESI, positive) m/z calcd for $C_{28}H_{33}N_5O_5$ (M + H): 520.2574; found 520.2560. HPLC purity: 96.3% at 254 nM.

## Experimental protocols of biological assays

**In Vitro HDAC Inhibition.** The HDAC1 enzyme was purchase from Abcam (#AB101661). HDAC2 (#BPS50002), HDAC3 (#BPS50003), HDAC6 (#BPS50006) and HDAC8 (#BPS50008) enzymes were purchase from BPS Bioscience. All of the enzymatic reactions were conducted at 37 °C for 30 minutes. The reaction mixture contained 25 mM Tris (pH 8.0), 1 mM $MgCl_2$, 0.1 mg/ml BSA, 137 mM NaCl, 2.7 mM KCl, HDAC and the enzyme substrate in a final volume of 50 μL. The HDAC protein was incubated with a commercially available fluorogenic HDAC substrate (substrate 3 for HDAC1, HDAC2, HDAC3 and HDAC6, #BPS50037, calss 2a substrate for HDAC8, #BPS50040) at a concentration equivalent to the substrate $K_m$ (8 μM for HDAC1, 4 μM for HDAC2, 8 μM for HDAC3, 8 μM for HDAC6, 200 μM for HDAC8). The compounds were diluted in 10% DMSO and 5 μL of the dilution was added to a 50 μL reaction so that the final concentration of DMSO was 1% in all of reactions. The assay was performed by quantitating the fluorescent product amount of in solution following an enzyme reaction. Fluorescence was then analyzed with an excitation of 350-360 nm and an emission wavelength of 450-460 nm at Spectra Max M5 microtiter plate reader. The $IC_{50}$ values were calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad sofeware. $K_i$ values were calculated based on the Cheng-Prusoff equation,[1] $K_i = IC_{50}/(1 + ([S]/K_m)$. [S],

substrate concentration; $K_m$, Michaelis constant.

**Top1-Mediated Supercoiled pBR322 Relaxation Assay.** DNA relaxation assays were employed according to the procedure described previously.[2] The reaction mixture contained 35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 5 mM spermidine, 0.1% bovine serum albumin (BSA), pBR322 plasmid DNA (0.25 μg), the indicated drug concentrations (1% DMSO), and 1 unit of Top1 (TaKaRa Biotechnology Co., Ltd., Dalian) in a final volume of 20 μL. Reaction mixtures were incubated for 15 min at 37 °C and stopped by addition of 2 μL of 10 × loading buffer (0.9% sodium dodecyl sulfate (SDS), 0.05% bromophenol blue, and 50% glycerol).Electrophoresis was carried out in a 0.8% agarose gel in TAE (Tris-acetate-EDTA) at 8 V/cm for 1 h. Gels were stained with ethidium bromide (0.5 μg/mL) for 60 min. The DNA band was visualized over UV light and photographed with Gel Doc Ez imager (Bio-Rad Laboratories Ltd.).

**Top2-Mediated Supercoiled pBR322 Relaxation Assay.** DNA Top2α inhibitory activity of the compounds was measured using Topoisomerase II Drug Screening Kit (TopoGEN, Inc.).[3] The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 30 μg/mL bovine serum albumin (BSA), 2 mM ATP, pBR322 plasmid DNA (0.25μg), the indicated drug concentrations (1% DMSO), and 0.75 unit of Top2α (TopoGEN, Inc.) in a final volume of 20 μL. Reaction mixtures were incubated for 30 min at 37 °C and stopped by addition of 2 μL 10% SDS. After that, 2 μL 10 × gel loading buffer (0.25% bromophenol blue, 50% glycerol) was added. The reaction products were analyzed on 1% agarose gel at 8 V/cm for 1 h with TAE (Tris-acetate-EDTA) as the running buffer. Gels were stained with ethidium bromide (0.5 μg/mL) for 60 min. The DNA band was visualized over UV light and photographed with Gel Doc Ez imager (Bio-Rad Laboratories Ltd.).

**In Vitro Cytotoxicity Assay.** Cells were plated in 96-well microtiter plates at a density of $5 \times 10^3$/well and incubated in a humidified atmosphere with 5% $CO_2$ at

37 °C for 24 h. Test compounds were added onto triplicate wells with different concentrations and 0.1% DMSO for control. After they had been incubated for 72 h, 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) solution (5 mg/mL) was added to each well and the plate was incubated for an additional 4 h. The formazan was dissolved in 100 μL of DMSO. The absorbance (OD) was read on a WellscanMK-2 microplate reader (Labsystems) at 570 nm. The concentration causing 50% inhibition of cell growth ($IC_{50}$) was determined by the Logit method.[4, 5] All experiments were performed three times.

**Apoptosis Detection Assay.** HCT116 cells ($5 \times 10^5$ cells/mL) were seeded in six-well plates and treated with compounds at concentration of 5 μM for 48 h. the cells were then harvested by trypsinization and washed twice with cold PBS. After centrifugation and removal of the supernatants, cells were resuspended in 400 μL of 1 × binding buffer which was then added to 5 μL of annexin V-FITC and incubated at room temperature for 15 min. After adding 10 μL of PI the cells were incubated at room temperature for another 15 min in dark. The stained cells were analyzed by a Flow Cytometer (BD Accuri C6). All experiments were performed three times.

## Reference

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(2) Dong, G.; Sheng, C.; Wang, S.; Miao, Z.; Yao, J.; Zhang, W. Selection of evodiamine as a novel topoisomerase I inhibitor by structure-based virtual screening and hit optimization of evodiamine derivatives as antitumor agents. *J. Med. Chem.* **2010,** 53, 7521-7531.

(3) Dong, G.; Wang, S.; Miao, Z.; Yao, J.; Zhang, Y.; Guo, Z.; Zhang, W.; Sheng, C. New tricks for an old natural product: discovery of highly potent evodiamine derivatives as novel antitumor agents by systemic structure-activity relationship analysis and biological evaluations. *J. Med. Chem.* **2012,** 55, 7593-7613.

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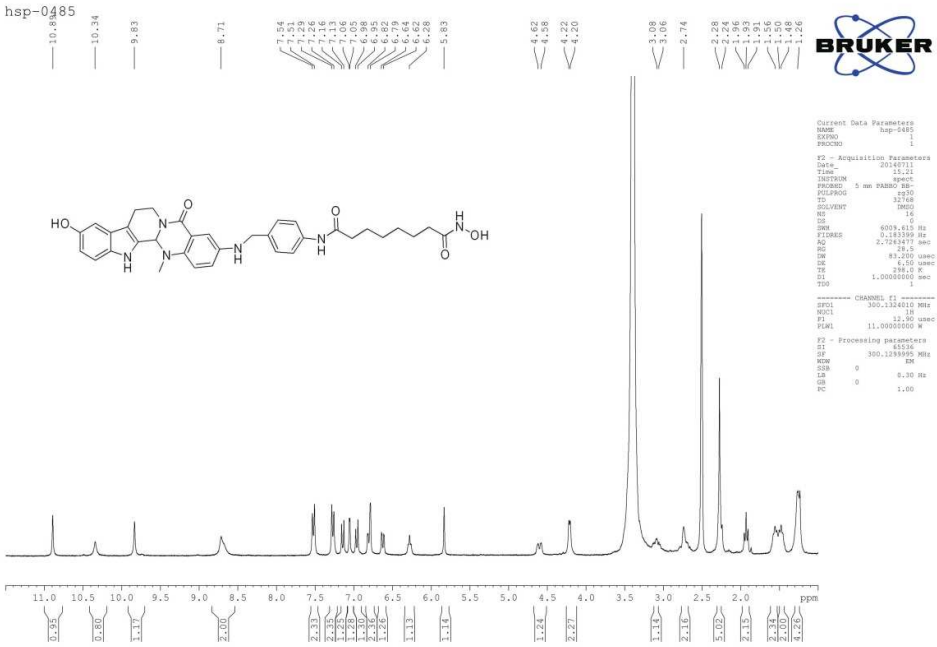
(5) Du, L.; Liu, H. C.; Fu, W.; Li, D. H.; Pan, Q. M.; Zhu, T. J.; Geng, M. Y.; Gu, Q. Q. Unprecedented citrinin trimer tricitinol B functions as a novel topoisomerase IIalpha inhibitor. *J Med Chem* **2011,** 54,

5796-5810.

# Spectral Data

Compund **7** $^{1}$H NMR:

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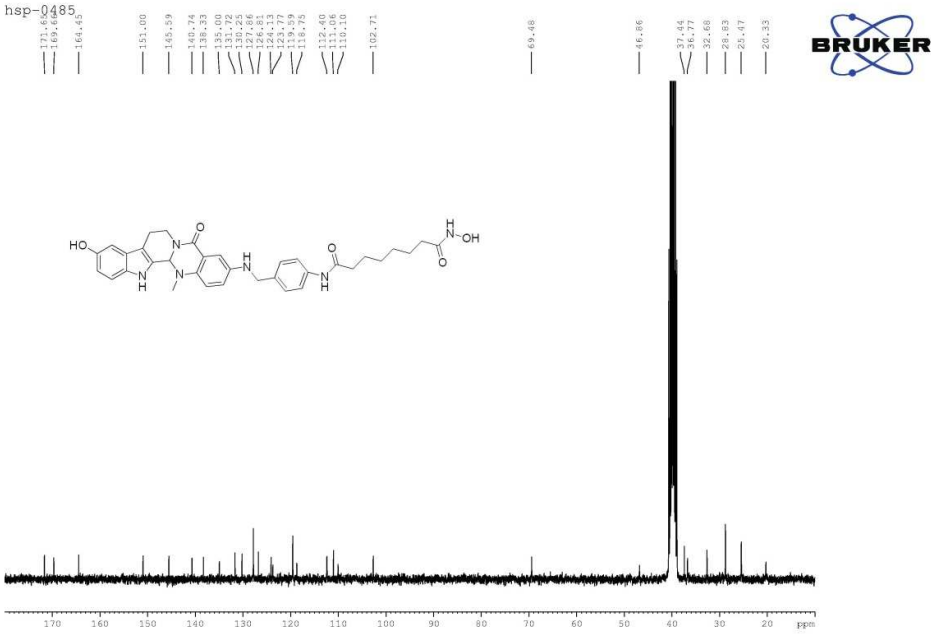

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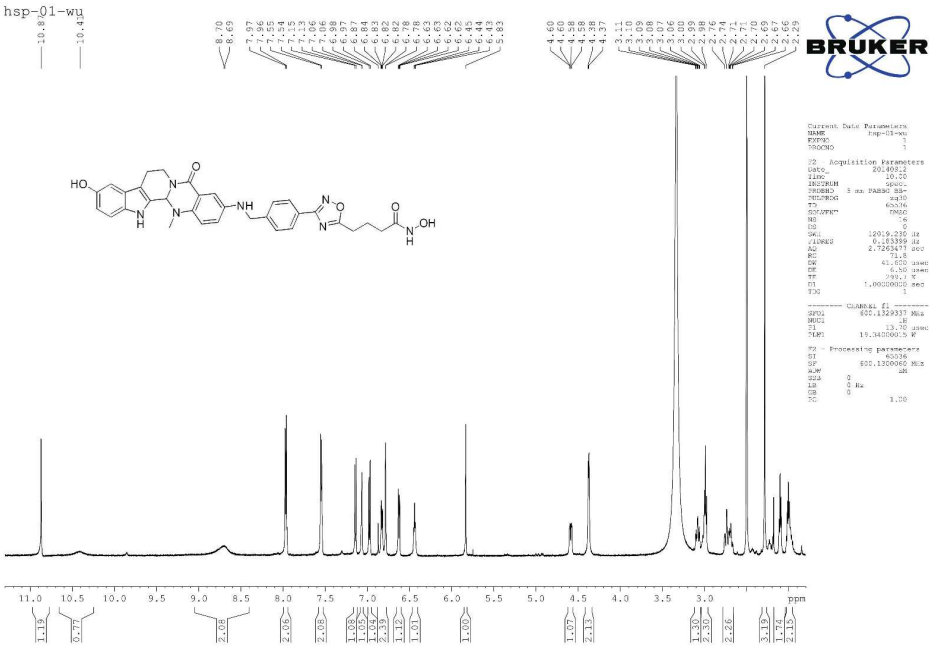


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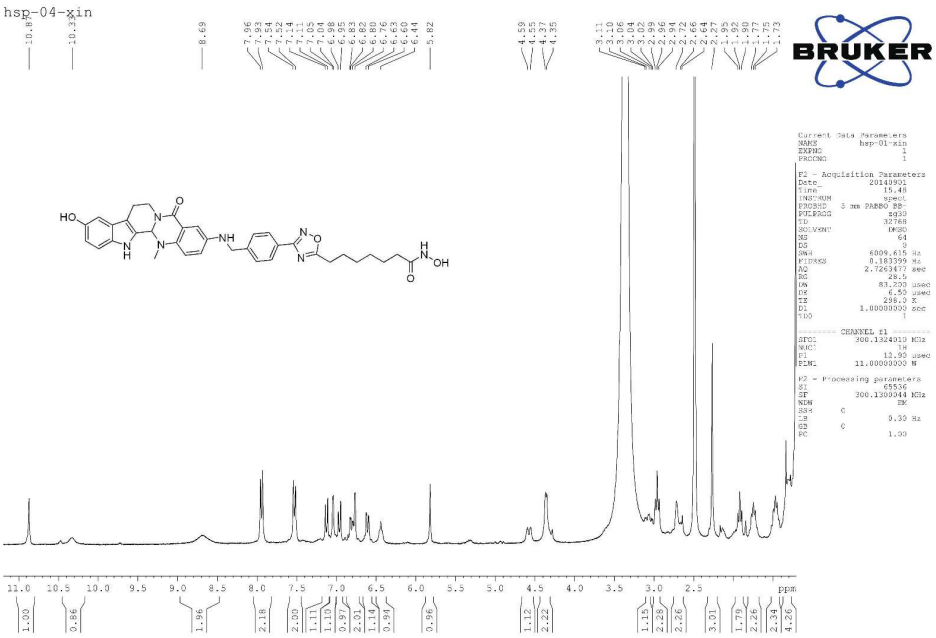

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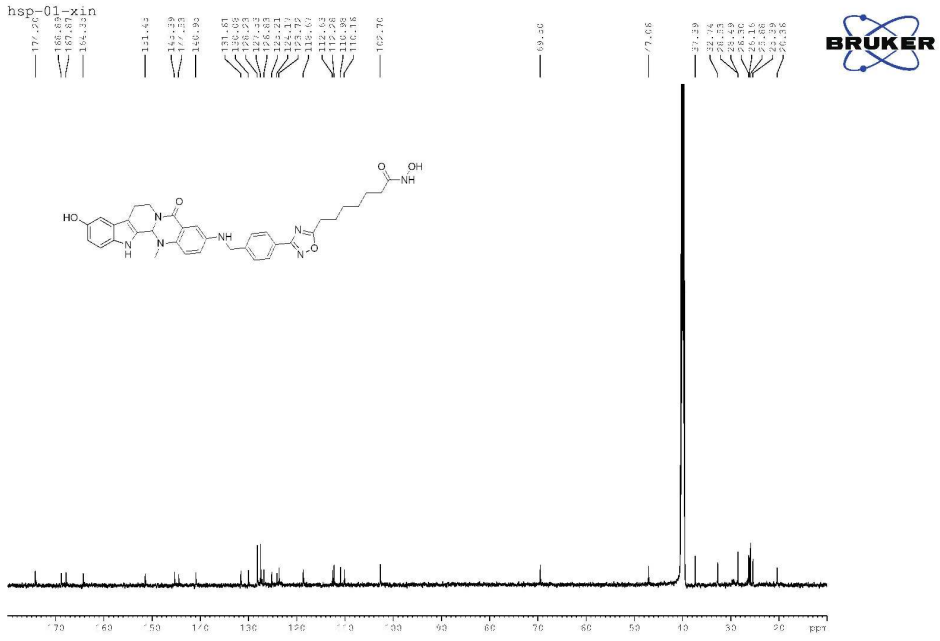


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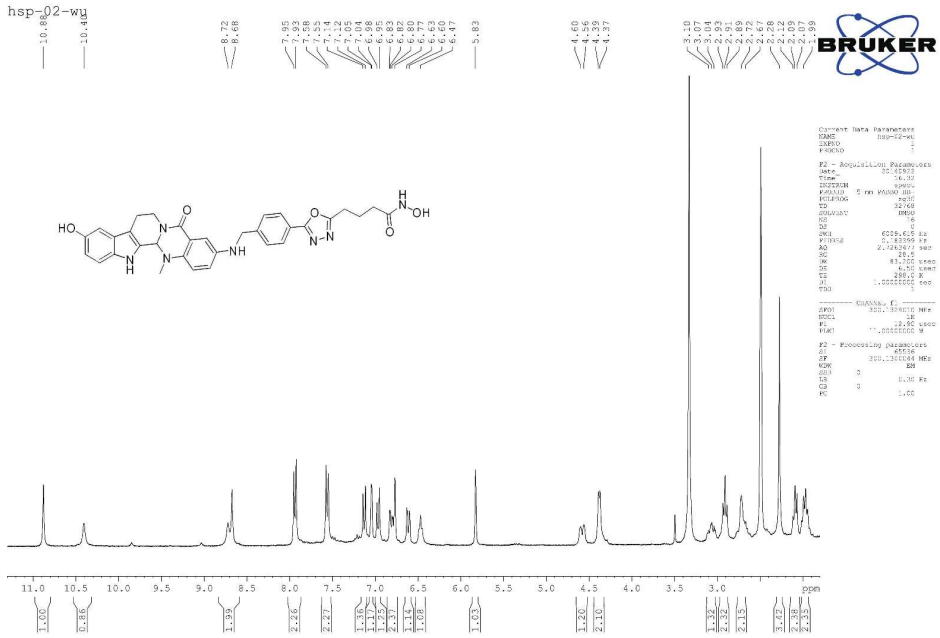

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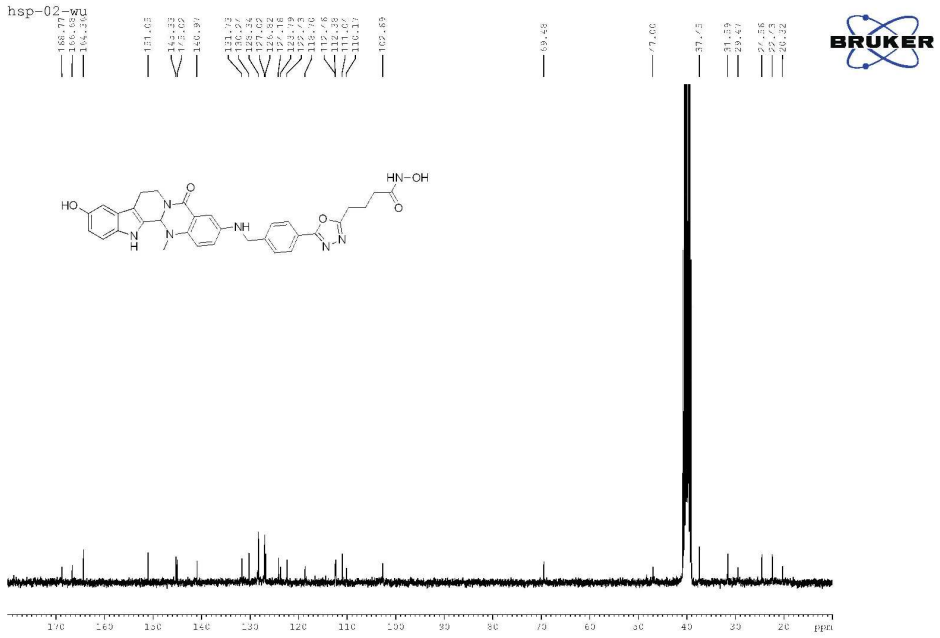


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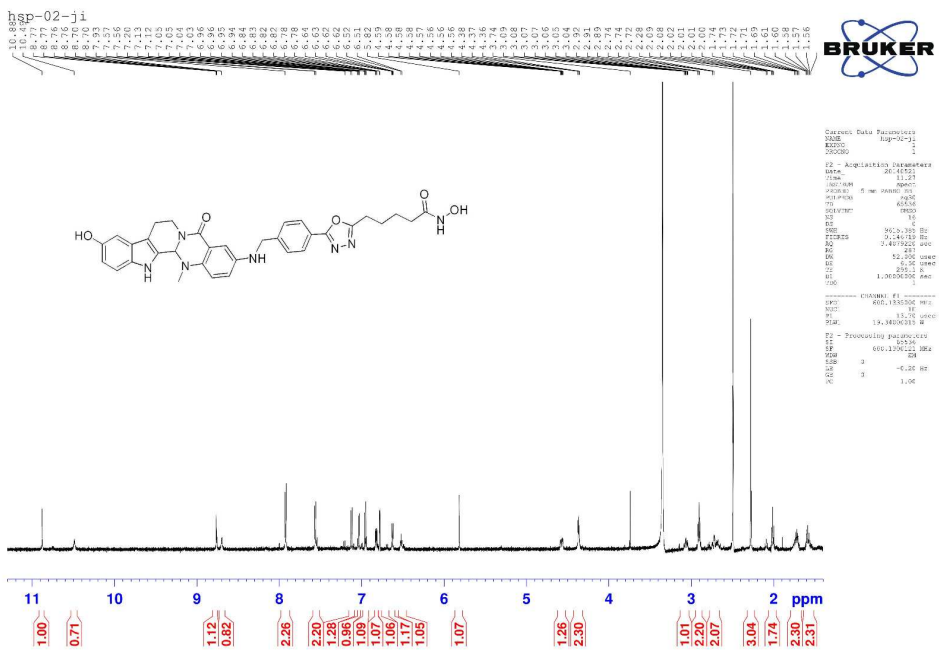

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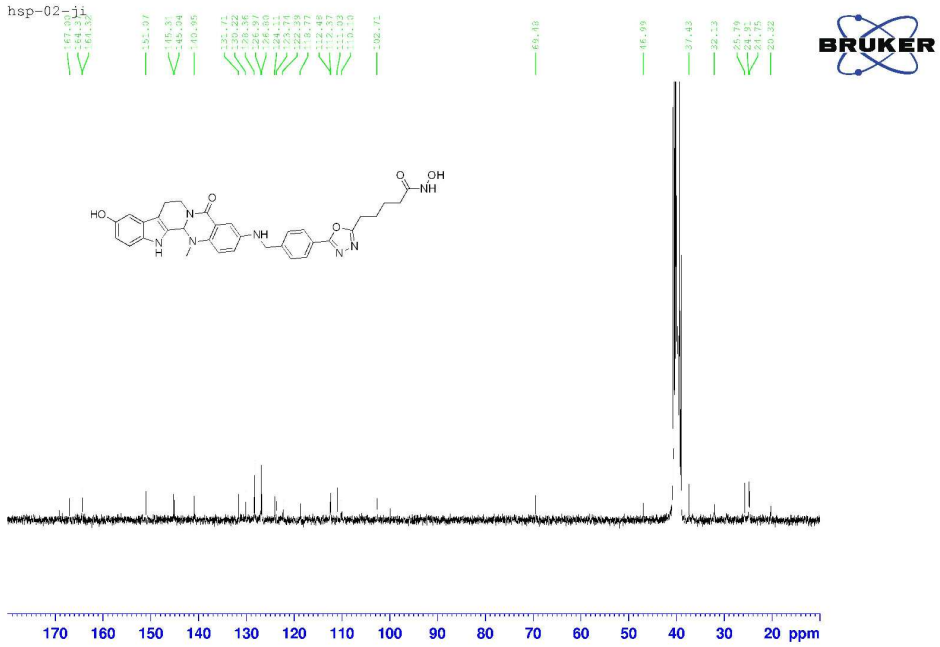


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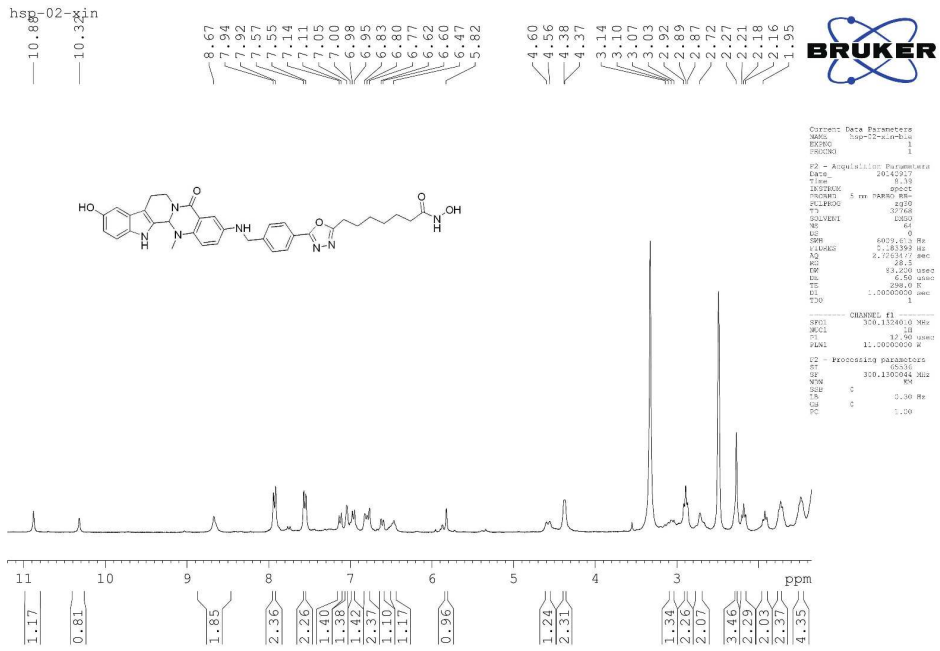

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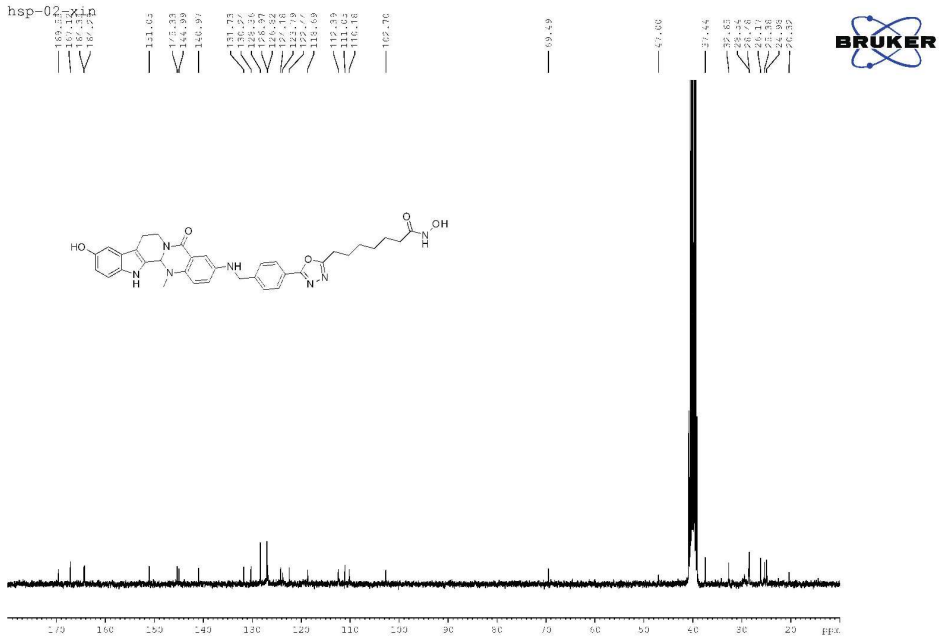


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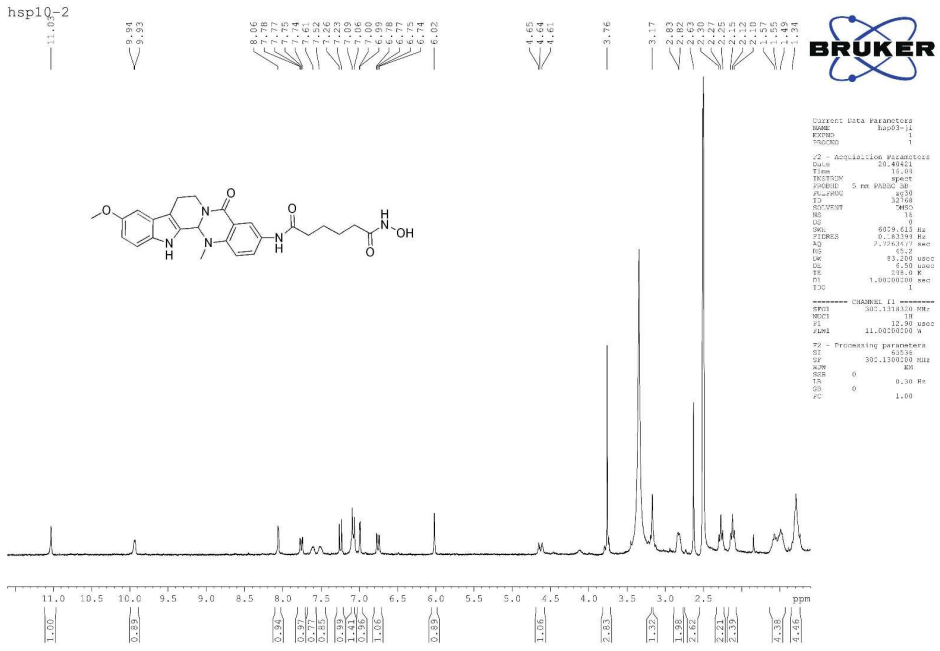

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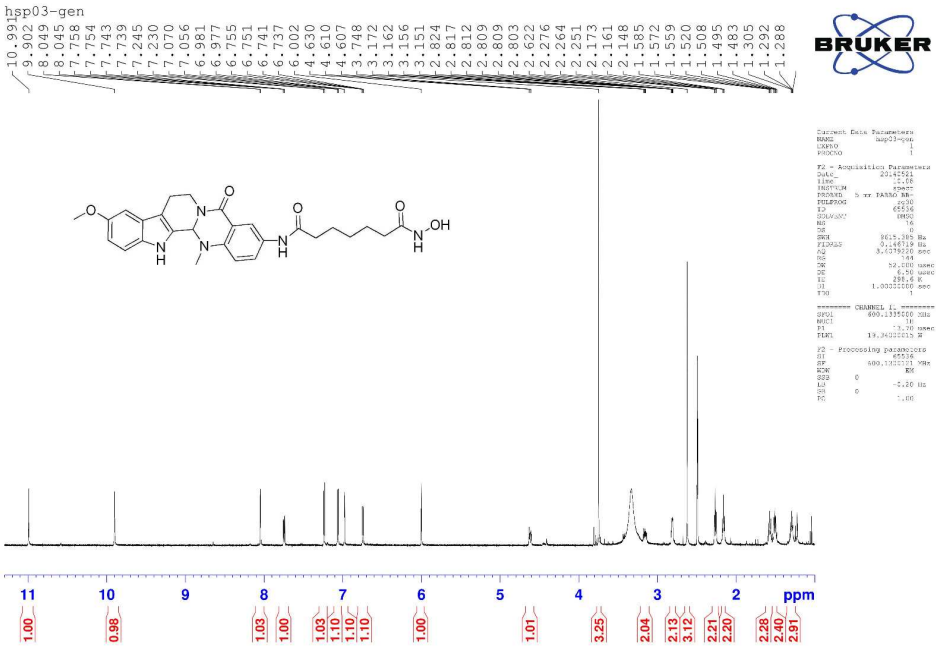


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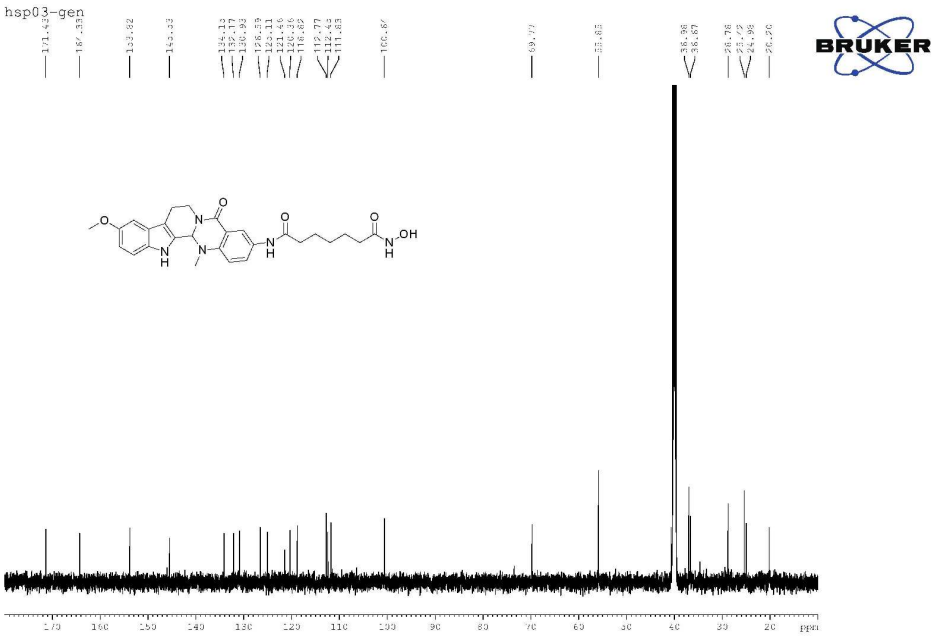

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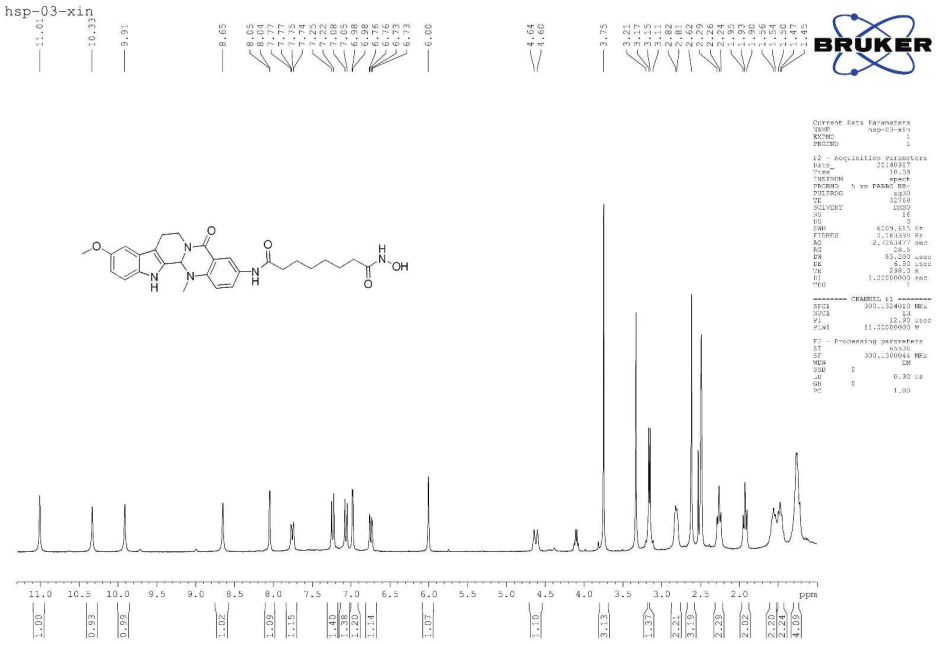


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