Supporting Information

Design, synthesis and biological evaluation of 2,4-imidazolinedione derivatives as HDAC6 isoform-selective inhibitors

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1. Approved HDAC inhibitors and the well accepted pharmacophore of HDAC inhibitors

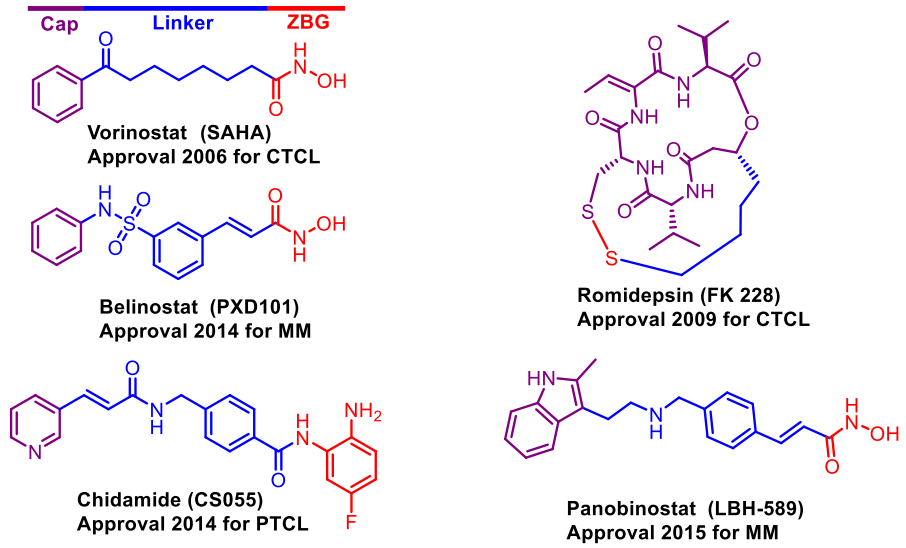
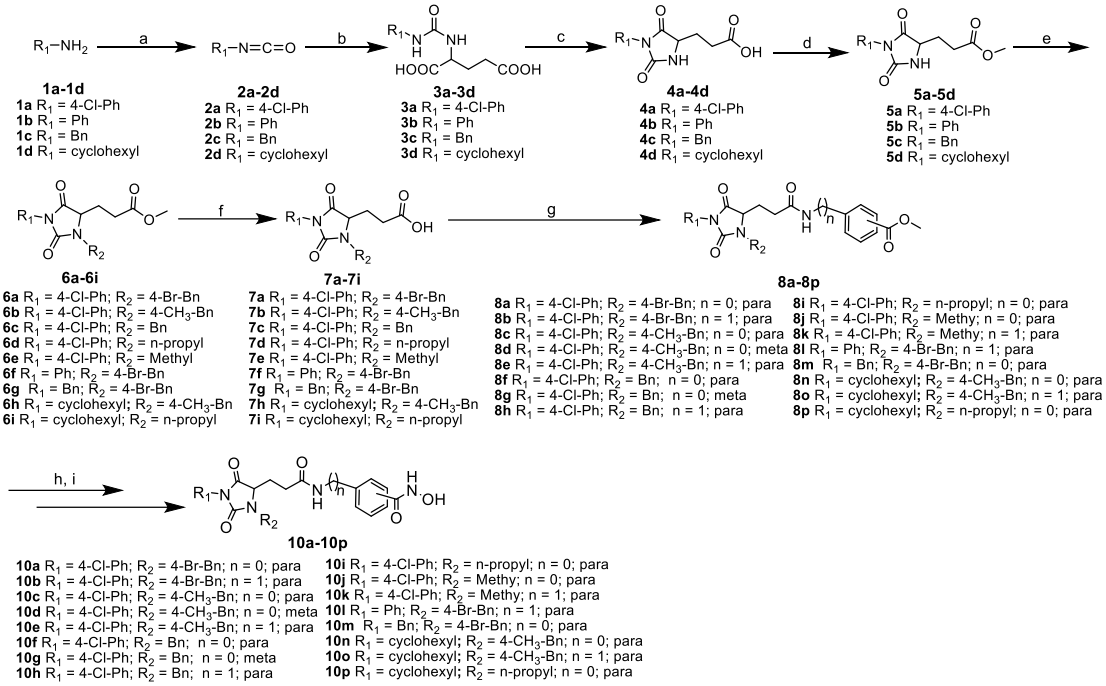


Figure 1. Approved HDAC inhibitors by the FDA/CFDA

2. Experimental

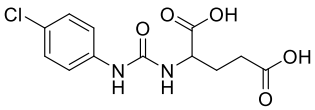
In our work, all start materials, reagents and solvents are analytical grade and used without further purification unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates (60GF-254) and the spots were visualized with UV light, chloride ferric and iodine vapor. Melting points were determined by the RY-1 electrothermal melting point apparatus. ^{1}H NMR were obtained on a Brucker DRX spectrometer at 400 MHz and ^{13}C NMR spectra were obtained on Bruker AVANCE III HD 600 MHz. Chemical shifts are showed in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard and significant. ^{1}H NMR data are reported in the order: multiplicity (m, multiplet; q, quartet; t, triplet; d, doublet; s, singlet;) number of protons. High-resolution mass spectra (HRMS) were conducted on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver with electrospray ionization (ESI).



Scheme 1. Reagents and conditions: (a) triphosgene, $NaHCO_3$, CH_2Cl_2; (b) (i)Toluene, 2 M NaOH, 0 °C, 4 h, (ii)6 M HCl, 44%-72%; (c) Hydrochloric acid, reflux, 3 h, 88%-92%; (d) CH_3COCl, MeOH, reflux, 5h, 95%-97%; (e) K_2CO_3, KI, DMF, overnight, 45%-93%; (f) LiOH•H_2O, THF/H_2O, rt, 6 h, 78%-97%; (g) HATU, DIPEA, CH_2Cl_2, rt, overnight, 40%-95%; (h) LiOH•H_2O, THF/H_2O, rt, 6 h; (i) isobutyl chlorocarbonate, 4-Methylmorpholine, THF, NH_2OH•HCl, KOH, MeOH, rt, 6h, 17%-65%.

3. Synthesis and characterization of target compounds 10a-10p (all target compounds are racemates)

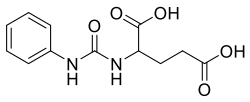
((4-chlorophenyl)carbamoyl)glutamic acid (3a)



The solution of 4-chloroaniline (10.2 g, 80 mmol) with 1 mL triethylamine in 150 mL CH_2Cl_2 was dropwise added to the solution of triphosgene (8.0 g, 27 mmol) at 0 ˚C. When the addition was completed, the reaction was quenched with saturated $NaHCO_3$ solution. Dried the organic phase and filtered, then the solvent was evaporated under vacuum to give compound **2a**.

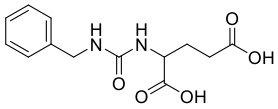
Added the solution of **2a** in 150 mL toluene to the solution of glutamic acid (11.77 g, 80 mmol) in 150 mL 2 M NaOH at 0 ˚C and stirred the mixture for 4 h. The water phase was separated and acidified it to pH 2 with 6 M HCl, then extracted with ethyl acetate three times. Combined organic phases, dried it over with anhydrous $MgSO_4$ and the solvent was evaporated to give crude product. Purified it by silica gel column chromatography to afford compound **3a** as a white solid 14.48 g, Yield: 68%, mp:158-161 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (s, 2H), 8.75 (s, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H), 6.50 (d, J = 8.0 Hz, 1H), 4.20 (td, J = 8.1, 5.3 Hz, 1H), 2.39 – 2.18 (m, 2H), 2.01 (td, J = 13.5, 7.6 Hz, 1H), 1.80 (td, J = 14.4, 8.3 Hz, 1H).

(Phenylcarbamoyl)glutamic acid (3b)



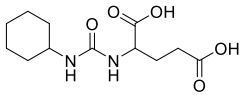
The title compound was synthesized from aniline (**1b**), triphosgene and glutamic acid in a manner similar to that described for the preparation of **3a**. Yield: 72%, mp: 143-145 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.46 (s, 2H), 8.60 (s, 1H), 7.37 (d, J = 7.8 Hz, 2H), 7.22 (t, J = 7.8 Hz, 2H), 6.90 (t, J = 7.3 Hz, 1H), 6.45 (d, J = 8.0 Hz, 1H), 4.20 (dd, J = 13.1, 8.1 Hz, 1H), 2.38 – 2.18 (m, 2H), 2.05 – 1.93 (td, J = 13.6, 7.7 Hz, 1H), 1.79 (td, J = 14.3, 8.4 Hz, 1H).

(Benzylcarbamoyl)glutamic acid (3c)



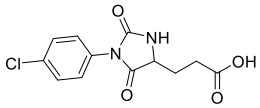
The title compound was synthesized from benzylamine (**1c**), triphosgene and glutamic acid in a manner similar to that described for the preparation of **3a**. Yield: 44%, mp: 141-143 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (s, 2H), 7.31 (t, J = 7.4 Hz, 2H), 7.22 (dd, J = 13.0, 7.0 Hz, 3H), 6.49 (t, J = 4.7 Hz, 1H), 6.27 (d, J = 8.1 Hz, 1H), 4.23 (dd, J = 16.0, 6.1 Hz, 2H), 4.14 (td, J = 8.3, 5.3 Hz, 1H), 2.36 – 2.12 (m, 2H), 1.94 (td, J = 13.9, 7.8 Hz, 1H), 1.73 (td, J = 14.5, 8.6 Hz, 1H).

(Cyclohexylcarbamoyl)glutamic acid (3d)



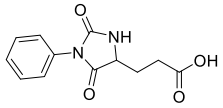
The title compound was synthesized from cyclohexylamine (**1d**), triphosgene and glutamic acid in a manner similar to that described for the preparation of **3a**. Yield: 61%, mp: 170-171 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 2H), 6.01 (d, J = 8.3 Hz, 1H), 5.93 (d, J = 7.9 Hz, 1H), 4.09 (dd, J = 13.5, 8.2 Hz, 1H), 2.35 – 2.13 (m, 2H), 1.91 (td, J = 13.6, 7.1 Hz, 1H), 1.82 – 1.57 (m, 5H), 1.56 – 1.46 (m, 1H), 1.16 (ddt, J = 48.5, 22.5, 11.2 Hz, 5H).

3-(1-(4-chlorophenyl)-2, 5-dioxoimidazolidin-4-yl)propanoic acid (4a)



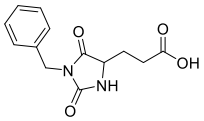
Dissolved compound **3a** (9.32 g, 35 mmol) with hydrochloric acid (70 mL) and refluxed for 3h. Cooled the reaction solution down and there was white crystal precipitated. Compound **4a** was isolated by filtered as a white solid. Yield: 90%, mp: 218-221 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 8.65 (s, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.41 (d, J = 8.7 Hz, 2H), 4.25 (t, 1H), 2.42 (t, J = 7.6 Hz, 2H), 2.04 (td, J = 13.2, 7.7 Hz, 1H), 1.87 (td, J = 14.7, 7.4 Hz, 1H).

3-(2, 5-dioxo-1-phenylimidazolidin-4-yl)propanoic acid (4b)



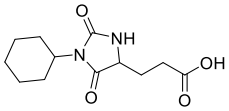
The title compound was synthesized from **3b** in a manner similar to that described for the preparation of **4a**. Yield: 88%, mp: 171-173 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 8.54 (s, 1H), 7.47 (t, J = 7.6 Hz, 2H), 7.41 – 7.29 (m, 3H), 4.26 (t, J = 6.1 Hz, 1H), 2.41 (t, J = 7.6 Hz, 2H), 2.05 (td, J = 13.2, 7.7 Hz, 1H), 1.87 (td, J = 14.7, 7.4 Hz, 1H).

3-(1-benzyl-2, 5-dioxoimidazolidin-4-yl)propanoic acid (4c)



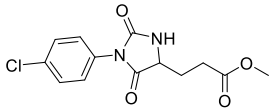
The title compound was synthesized from **3c** in a manner similar to that described for the preparation of **4a.** Yield: 89%, mp: 154-156 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 8.38 (s, 1H), 7.33 (t, J = 7.2 Hz, 2H), 7.26 (dd, J = 13.9, 7.0 Hz, 3H), 4.65 – 4.35 (m, 2H), 4.26 – 4.07 (m, 1H), 2.43 – 2.16 (m, 2H), 1.96 (td, J = 13.7, 7.7 Hz, 1H), 1.73 (td, J = 14.1, 7.8 Hz, 1H).

3-(1-cyclohexyl-2,5-dioxoimidazolidin-4-yl)propanoic acid (4d)



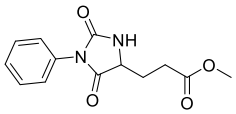
The title compound was synthesized from **3d** in a manner similar to that described for the preparation of **4a.** Yield: 92%, mp: 157-158 ˚C. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.06 (s, 1H), 8.21 (s, 1H), 4.00 (t, J = 6.0 Hz, 1H), 3.70 (ddd, J = 12.3, 8.6, 3.8 Hz, 2H), 2.38 – 2.19 (m, 2H), 2.11 – 1.85 (m, 3H), 1.84 – 1.64 (m, 3H), 1.64 – 1.50 (m, J = 14.7 Hz, 3H), 1.24 (dd, J = 26.2, 13.0 Hz, 2H), 1.09 (dd, J = 25.5, 12.8 Hz, 1H).

Methyl 3-(1-(4-chlorophenyl)-2, 5-dioxoimidazolidin-4-yl)propanoate (5a)



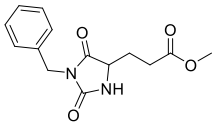
Acetyl chloride (5.4 mL, 75 mmol) was slowly added into the anhydrous methanol (140 mL) in an ice bath and stirred the mixture for 30 min, then added compound **4a** (8.48 g, 30 mmol) to the solution. After refluxing for 5 h, cooled the reaction solution down and removed the solvent under reduced pressure. Added 20 mL n-hexane into the residue and filtered to give compound **5a** as a white solid 8.63 g, Yield: 97%, mp: 133-136 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.7 Hz, 2H), 5.98 (s, 1H), 4.27 (t, J = 5.8 Hz, 1H), 3.72 (s, 3H), 2.57 (t, J = 6.9 Hz, 2H), 2.34 (ddd, J = 18.8, 12.0, 6.7 Hz, 1H), 2.15 (td, J = 13.9, 6.9 Hz, 1H).

Methyl 3-(2, 5-dioxo-1-phenylimidazolidin-4-yl)propanoate (5b)



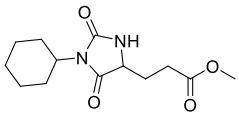
The title compound was synthesized from **4b** in a manner similar to that described for the preparation of **5a.**Yield: 96%, mp: 104-106 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.43 (m, 2H), 7.38 (dd, *J* = 10.4, 4.3 Hz, 3H), 6.32 (s, 1H), 4.29 (t, *J* = 5.7 Hz, 1H), 3.71 (s, 3H), 2.56 (t, *J* = 7.0 Hz, 2H), 2.33 (ddd, *J* = 19.3, 12.3, 6.8 Hz, 1H), 2.16 (td, *J* = 14.0, 6.9 Hz, 1H).

Methyl 3-(1-benzyl-2, 5-dioxoimidazolidin-4-yl)propanoate (5c)



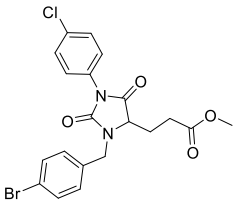
The title compound was synthesized from **4c** in a manner similar to that described for the preparation of **5a.** Yield: 97%, mp: 90-92 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 7.2 Hz, 2H), 7.30 (dt, *J* = 10.0, 4.8 Hz, 3H), 6.11 (s, 1H), 4.73 – 4.56 (m, 2H), 4.10 (t, *J* = 5.9 Hz, 1H), 3.67 (s, 3H), 2.44 (t, *J* = 7.1 Hz, 2H), 2.26 – 2.14 (m, 1H), 2.11 – 1.94 (m, 1H).

Methyl 3-(1-cyclohexyl-2,5-dioxoimidazolidin-4-yl)propanoate (5d)



The title compound was synthesized from **4d** in a manner similar to that described for the preparation of **5a.** Yield: 95%, mp: 109-111 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95 (s, 1H), 4.02 (t, J = 5.5 Hz, 1H), 3.88 (tt, J = 12.6, 3.7 Hz, 1H), 3.69 (s, 3H), 2.45 (t, J = 7.1 Hz, 2H), 2.33 – 1.89 (m, 5H), 1.83 (d, J = 13.0 Hz, 2H), 1.77 – 1.66 (m, J = 8.0 Hz, 3H), 1.47 – 1.05 (m, 4H).

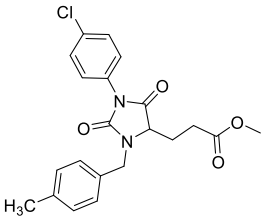
Methyl 3-(3-(4-bromobenzyl)-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanoate (6a)



The solution of **5a** (8.30 g, 28 mmol) in DMF was mixed with K_2CO_3 (11.61 g, 84 mmol) then 4-Bromobenzyl bromide (10.50 g, 42 mmol), KI (0.1 g) was added to the solution. Stirred the mixture at room temperature overnight. Subsequently, the mixture was poured into cool water (3 times) and extracted with ethyl acetate three times. Combined the organic phases and dried it over with $MgSO_4$ for 0.5 h, then it was filtered and concentrated to give the crude product compound **6a**. The crude product was purified by silica gel column chromatography to obtain white solid 11.08 g. Yield: 85%, mp: 71-73 ˚C. (In this reaction, racemization occurs). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.2 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 2H), 7.38 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.2 Hz, 2H), 4.97 (d, *J* = 15.3 Hz, 1H), 4.17 (d, *J* = 15.3 Hz, 1H), 4.00 (dd, *J* = 6.5, 2.7 Hz, 1H),

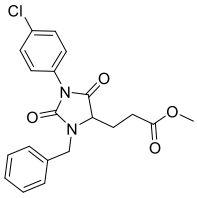
3.68 (s, 3H), 2.51 – 2.26 (m, 3H), 2.20 – 2.08 (m, 1H).

Methyl 3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanoate (6b)



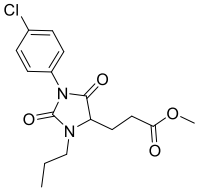
The title compound was synthesized from **5b** and *p*-methylbenzyl bromide in a manner similar to that described for the preparation of **6a.** Yield: 93%, mp: 68-71 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 8.1 Hz, 2H), 5.06 (d, J = 15.0 Hz, 1H), 4.10 (d, J = 15.0 Hz, 1H), 3.99 (dd, J = 6.3, 3.0 Hz, 1H), 3.68 (s, 3H), 2.41 (dd, J = 13.0, 7.0 Hz, 2H), 2.35 (s, 3H), 2.33 – 2.26 (m, 1H), 2.18 (qd, J = 9.0, 5.5 Hz, 1H).

Methyl 3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanoate (6c)



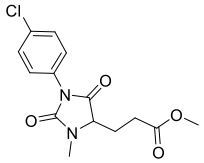
The title compound was synthesized from **5c** and benzyl bromide in a manner similar to that described for the preparation of **6a.** Yield: 82%, mp: 109-112 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.38 (m, 5H), 7.35 (dd, J = 14.1, 5.8 Hz, 4H), 5.08 (d, J = 15.1 Hz, 1H), 4.16 (d, J = 15.1 Hz, 1H), 4.00 (dd, J = 6.4, 2.9 Hz, 1H), 3.68 (s, 3H), 2.51 – 2.37 (m, 2H), 2.37 – 2.27 (m, 1H), 2.18 (qd, J = 9.6, 6.0 Hz, 1H).

Methyl 3-(1-(4-chlorophenyl)-2,5-dioxo-3-propylimidazolidin-4-yl)propanoate (6d)



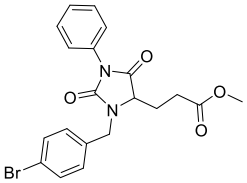
The title compound was synthesized from **5d** and 1-bromopropane in a manner similar to that described for the preparation of **6a.** Yield: 45%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.7 Hz, 2H), 7.37 (d,J = 8.7 Hz, 2H), 4.19 (dd, J = 6.9, 3.1 Hz, 1H), 3.72 (ddd, J = 13.1, 5.7 Hz, 1H), 3.69 (s, 3H), 3.10 (ddd, J = 14.0, 8.7, 5.1 Hz, 1H), 2.61 – 2.43 (m, 2H), 2.44 – 2.31 (m, 1H), 2.24 – 2.09 (m, 1H), 1.81 – 1.58 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H).

Methyl 3-(1-(4-chlorophenyl)-3-methyl-2,5-dioxoimidazolidin-4-yl)propanoate (6e)



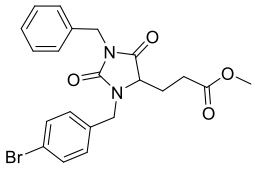
The title compound was synthesized from **5e** and methyl iodide in a manner similar to that described for the preparation of **6a.** Yield: 90%, mp: 114-116 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.7 Hz, 2H), 4.14 – 4.06 (m, 1H), 3.69 (s, 3H), 3.03 (s, 3H), 2.56 – 2.44 (m, 2H), 2.42 – 2.34 (m, 1H), 2.22 (qd, J = 9.5, 5.8 Hz, 1H).

Methyl 3-(3-(4-bromobenzyl)-2,5-dioxo-1-phenylimidazolidin-4-yl)propanoate (6f)



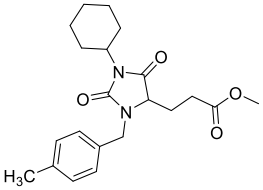
The title compound was synthesized from **5f** and 4-bromobenzyl bromide in a manner similar to that described for the preparation of **6a.** Yield: 90%, mp: 96-98 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (t, *J* = 5.7 Hz, 2H), 7.46 (d, *J* = 7.5 Hz, 2H), 7.39 (dd, *J* = 14.4, 7.3 Hz, 3H), 7.23 (d, *J* = 8.2 Hz, 2H), 4.99 (d, *J* = 15.3 Hz, 1H), 4.18 (d, *J* = 15.3 Hz, 1H), 4.01 (dd, *J* = 6.5, 2.9 Hz, 1H), 3.68 (s, 3H), 2.53 – 2.37 (m, 2H), 2.37 – 2.30 (m, 1H), 2.21 – 2.09 (m, 1H).

Methyl 3-(1-benzyl-3-(4-bromobenzyl)-2,5-dioxoimidazolidin-4-yl)propanoate (6g)



The title compound was synthesized from **5g** and 4-bromobenzyl bromide in a manner similar to that described for the preparation of **6a.** Yield: 87%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 6.6 Hz, 2H), 7.36 – 7.28 (m, 3H), 7.14 (d, *J* = 8.3 Hz, 2H), 4.87 (d, *J* = 15.7 Hz, 1H), 4.67 (q, *J* = 14.5 Hz, 2H), 4.09 (d, *J* = 15.3 Hz, 1H), 3.82 (dt, *J* = 7.4, 3.7 Hz, 1H), 3.62 (s, 3H), 2.42 – 2.13 (m, 3H), 2.11 – 1.88 (m, 1H).

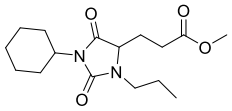
Methyl 3-(1-cyclohexyl-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanoate (6h)



The title compound was synthesized from **5h** and *p*-methylbenzyl bromide in a manner similar to that described for the preparation of **6a.** Yield: 84%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 – 7.09 (m, 4H), 4.94 (d, J = 15.1 Hz, 1H), 3.99 (d, J = 15.1 Hz, 1H), 3.95 – 3.83 (m, 1H), 3.73 (dd, J = 5.8, 2.9 Hz, 1H), 3.66 (s, 3H), 2.43 – 2.31 (m, 4H), 2.30 – 2.23 (m, 2H), 2.22 –

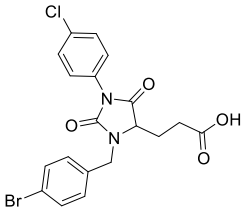
1.98 (m, 5H), 1.81 (t, J = 16.6 Hz, 2H), 1.44 – 1.10 (m, 4H).

Methyl 3-(1-cyclohexyl-2,5-dioxo-3-propylimidazolidin-4-yl)propanoate (6i)



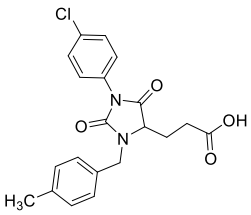
The title compound was synthesized from **5i** and 1-bromopropane in a manner similar to that described for the preparation of **6a.** Yield: 82%, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 – 3.91 (m, 1H), 3.87 (ddd, J = 12.3, 8.1, 4.3 Hz, 1H), 3.68 (s, 3H), 3.63 – 3.54 (m, 1H), 2.99 (ddd, J = 14.0, 8.7, 5.1 Hz, 1H), 2.44 – 2.21 (m, 3H), 2.18 – 1.99 (m, 3H), 1.82 (d, J = 12.8 Hz, 2H), 1.75 – 1.65 (m, 2H), 1.63 – 1.48 (m, 2H), 1.44 – 1.11 (m, 4H), 0.92 (t, J = 7.4 Hz, 3H).

3-(3-(4-bromobenzyl)-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanoic acid (7a)



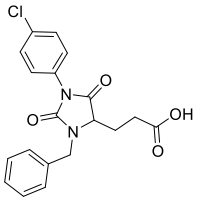
LiOH•H_2O (3.86 g, 92 mmol) was added to the solution of compound **6a** (10.71 g, 23 mmol) in THF/H_2O=3:1(184 mL) and stirred the mixture at room temperature for 6h. Poured 184 mL H_2O into the mixture and extracted the mixture with ethyl acetate two times. Water phase was separated and acidified it to pH 2 with 6 M HCl, then extracted it with ethyl acetate three times. Combined and dried the organic phase, then evaporated the solvent under reduced pressure to obtain compound **7a** as a white solid 9.87 g, Yield: 95%, mp: 89-91 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.57 (dd, *J* = 8.2, 5.4 Hz, 4H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 4.67 (d, *J* = 16.0 Hz, 1H), 4.44 (d, *J* = 15.9 Hz, 1H), 4.22 (t, *J* = 5.2 Hz, 1H), 2.30 – 2.20 (m, 2H), 2.10 – 2.00 (m, 2H).

3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanoic acid (7b)



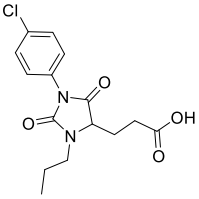
The title compound was synthesized from **6b** in a manner similar to that described for the preparation of **7a.** Yield: 92%, mp: 140-142 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.57 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.17 (d, *J* = 7.9 Hz, 2H), 4.68 (d, *J* = 15.6 Hz, 1H), 4.38 (d, *J* = 15.6 Hz, 1H), 4.20 – 4.14 (m, 1H), 2.29 (s, 3H), 2.21 (dt, *J* = 16.4, 7.0 Hz, 2H), 2.12 – 2.00 (m, 2H).

3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanoic acid (7c)



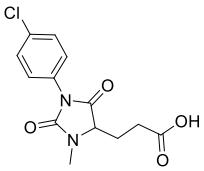
The title compound was synthesized from **6c** in a manner similar to that described for the preparation of **7a.** Yield: 96%, mp: 48-51 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 8.7 Hz, 2H), 7.43 – 7.27 (m, 5H), 4.71 (d, J = 15.8 Hz, 1H), 4.46 (d, J = 15.8 Hz, 1H), 4.29 – 4.14 (m, 1H), 2.33 – 2.15 (m, 2H), 2.13 – 1.99 (m, 2H).

3-(1-(4-chlorophenyl)-2,5-dioxo-3-propylimidazolidin-4-yl)propanoic acid (7d)



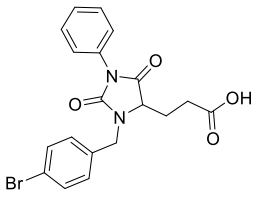
The title compound was synthesized from **6d** in a manner similar to that described for the preparation of **7a.** Yield: 78%, yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 4.34 – 4.08 (m, 1H), 3.71 (dt, J = 15.8, 8.0 Hz, 1H), 3.11 (ddd, J = 14.1, 8.7, 5.2 Hz, 1H), 2.67 – 2.45 (m, 2H), 2.38 (dtd, J = 10.8, 7.6, 3.3 Hz, 1H), 2.24 – 2.06 (m, 1H), 1.79 – 1.52 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H).

3-(1-(4-chlorophenyl)-3-methyl-2,5-dioxoimidazolidin-4-yl)propanoic acid (7e)



The title compound was synthesized from **6e** in a manner similar to that described for the preparation of **7a.** Yield: 97%, mp: 157-158 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.7 Hz, 2H), 4.24 (t, J = 5.0 Hz, 1H), 2.90 (s, 3H), 2.41 – 2.22 (m, 2H), 2.11 (dd, J = 12.7, 7.3 Hz, 2H).

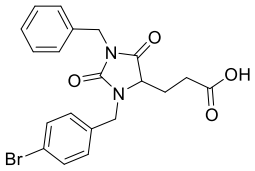
3-(3-(4-bromobenzyl)-2,5-dioxo-1-phenylimidazolidin-4-yl)propanoic acid (7f)



The title compound was synthesized from **6f** in a manner similar to that described for the preparation of **7a.** Yield: 90%, mp: 64-67 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H),

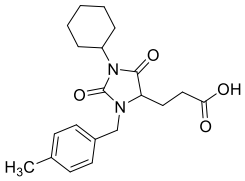
7.57 (d, *J* = 8.3 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 3H), 7.36 (t, *J* = 8.3 Hz, 2H), 4.68 (d, *J* = 15.9 Hz, 1H), 4.44 (d, *J* = 15.9 Hz, 1H), 4.23 (t, *J* = 5.0 Hz, 1H), 2.32 – 2.18 (m, 2H), 2.06 (dd, *J* = 14.6, 7.4 Hz, 2H).

3-(1-benzyl-3-(4-bromobenzyl)-2,5-dioxoimidazolidin-4-yl)propanoic acid (7g)



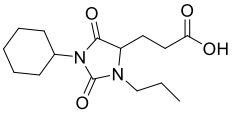
The title compound was synthesized from **6g** in a manner similar to that described for the preparation of **7a.** Yield: 81%, mp: 136-138 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.41 – 7.27 (m, *J* = 16.0, 15.1, 7.2 Hz, 5H), 7.14 (d, *J* = 8.2 Hz, 2H), 4.90 (d, *J* = 15.3 Hz, 1H), 4.67 (q, *J* = 14.5 Hz, 2H), 4.10 (d, *J* = 15.3 Hz, 1H), 3.83 (dd, *J* = 7.1, 3.2 Hz, 1H), 2.49 – 2.12 (m, 3H), 1.96 (td, *J* = 13.8, 7.7 Hz, 1H).

3-(1-cyclohexyl-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanoic acid (7h)



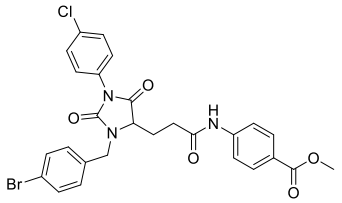
The title compound was synthesized from **6h** in a manner similar to that described for the preparation of **7a.** Yield: 87%, mp: 145-147 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 7.33 – 7.05 (m, 4H), 4.59 (d, J = 15.5 Hz, 1H), 4.25 (d, J = 15.5 Hz, 1H), 3.95 – 3.88 (m, 1H), 3.77 (ddd, J = 12.2, 8.7, 3.6 Hz, 1H), 2.28 (s, 3H), 2.16 – 1.88 (m, 6H), 1.79 (t, J = 16.3 Hz, 2H), 1.60 (d, J = 9.1 Hz, 3H), 1.36 – 1.19 (m, 2H), 1.12 (dt, J = 25.6, 10.0 Hz, 1H).

3-(1-cyclohexyl-2,5-dioxo-3-propylimidazolidin-4-yl)propanoic acid (7i)



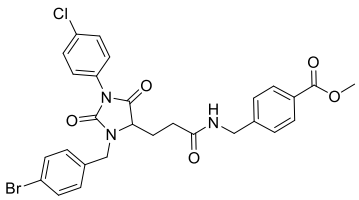
The title compound was synthesized from **6i** in a manner similar to that described for the preparation of **7a.** Yield: 84%, colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.25 (s, 1H), 4.12 (dd, J = 5.5, 3.8 Hz, 1H), 3.84 – 3.66 (m, 1H), 3.39 (dd, J = 14.9, 7.4 Hz, 1H), 3.00 (ddd, J = 13.9, 8.4, 5.2 Hz, 1H), 2.23 – 2.07 (m, 2H), 2.07 – 1.87 (m, 4H), 1.75 (d, J = 12.6 Hz, 2H), 1.66 – 1.38 (m, 5H), 1.34 – 1.17 (m, 2H), 1.09 (dd, J = 25.8, 12.9 Hz, 1H), 0.84 (t, J = 7.4 Hz, 3H).

Methyl 4-(3-(3-(4-bromobenzyl)-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido) benzoate (8a)



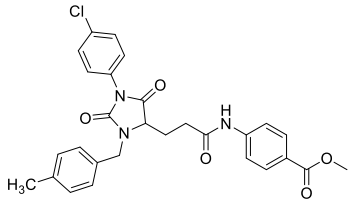
To the solution of compound **7a** (1.81 g, 4.0 mmol), DIPEA (0.73 mL, 4.4 mmol) and HATU (1.83 g, 4.8 mmol) were added, and the mixture was stirred for 30 min at room temperature. After adding methyl 4-aminobenzoate hydrochloride (0.83 g, 4.4 mmol), the mixture was stirred at room temperature overnight. Wash the reaction solution with 1M HCl, saturated $NaHCO_3$ and saturated brine. Dried the organic phase and concentrated it in vacuo to give crude product. Recrystallized with ethyl acetate and compound **8a** was separated as a white solid 1.73 g, yield: 74%, mp: 214 -217 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.56 (dd, J = 11.3, 8.6 Hz, 4H), 7.47 (d, J = 8.7 Hz, 2H), 7.37 (d, J = 8.3 Hz, 2H), 4.73 (d, J = 15.9 Hz, 1H), 4.43 (d, J = 15.9 Hz, 1H), 4.26 (t, J = 4.8 Hz, 1H), 3.82 (s, 3H), 2.41 (dt, J = 15.7, 7.0 Hz, 2H), 2.24 – 2.06 (m, 2H).

Methyl 4-((3-(3-(4-bromobenzyl)-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl) propanamido)methyl) benzoate (8b)



The title compound was synthesized from **7b** and methyl 4-(aminomethyl)benzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethanol to obtain white solid. Yield: 84%, mp: 216-219 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (t, J = 5.9 Hz, 1H), 7.90 (d, J = 8.2 Hz, 2H), 7.56 (dd, J = 8.4, 6.9 Hz, 4H), 7.46 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 8.0 Hz, 4H), 4.70 (d, J = 15.9 Hz, 1H), 4.45 – 4.33 (m, 1H), 4.31 (d, J = 5.8 Hz, 2H), 4.22 (t, J = 4.7 Hz, 1H), 3.84 (s, 3H), 2.31 – 2.16 (m, 2H), 2.16 (s, 2H).

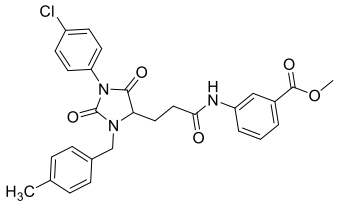
Methyl 4-(3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl) propanamido) benzoate (8c)



The title compound was synthesized from **7c** and 4-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 54%, mp: 196-198 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H), 7.49 (s, 1H), 7.42 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 9.0 Hz,

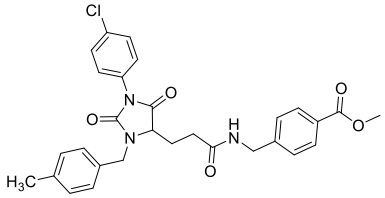
2H),, 7.25 (d, J = 7.2 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 4.96 (d, J = 15.0 Hz, 1H), 4.27 (d, J = 15.0 Hz, 1H), 4.02 (dd, J = 8.1, 2.6 Hz, 1H), 3.90 (s, 3H), 2.62 – 2.44 (m, 3H), 2.33 (s, 3H), 2.14 (dd, J = 16.8, 8.4 Hz, 1H).

Methyl 3-(3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl) propanamido)benzoate (8d)



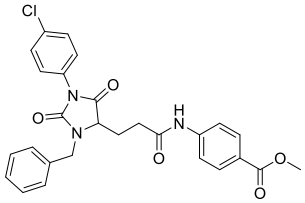
The title compound was synthesized from **7d** and 3-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 70%, mp: 194-196 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.88 – 7.73 (m, 2H), 7.53 – 7.32 (m, 6H), 7.25 (d, J = 5.5 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 4.96 (d, J = 15.0 Hz, 1H), 4.28 (d, J = 15.0 Hz, 1H), 4.04 (dd, J = 19.4, 14.1 Hz, 1H), 3.92 (s, 3H), 2.66 – 2.39 (m, 3H), 2.32 (s, 3H), 2.23 – 2.04 (m, 1H).

Methyl 4-((3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl) propanamido)methyl)benzoate (8e)



The title compound was synthesized from **7e** and methyl 4-(aminomethyl)benzoate hydrochloride in a manner similar to that described for the preparation of **8a.** Evaporated the solvent under reduced pressure to obtain compound **8e.** Yield: 85%, mp: 215-218 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (t, *J* = 5.8 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 7.8 Hz, 2H), 4.72 (d, *J* = 15.6 Hz, 1H), 4.42 – 4.25 (m, 3H), 4.16 (t, *J* = 4.6 Hz, 1H), 3.84 (s, 3H), 2.28 (s, 3H), 2.26 – 2.15 (m, 2H), 2.15 – 2.04 (m, 2H).

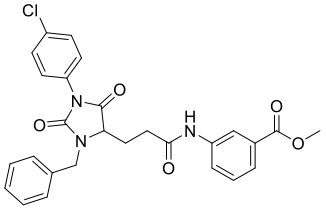
Methyl 4-(3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido) benzoate (8f)



The title compound was synthesized from **7f** and 4-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl

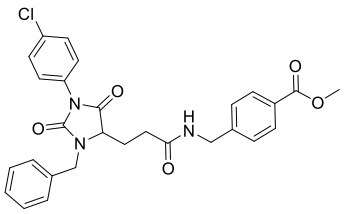
acetate to obtain white solid. Yield: 48%, mp: 182-184 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.6 Hz, 2H), 7.60 (s, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.46 – 7.28 (m, 9H), 5.02 (d, *J* =15.1 Hz, 1H), 4.31 (d, *J* = 15.1 Hz, 1H), 4.02 (dd, *J* = 8.1, 2.5 Hz, 1H), 3.90 (s, 3H), 2.63 – 2.37 (m, 3H), 2.24 – 2.02 (m, 1H).

Methyl 3-(3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido)benzoate (8g)



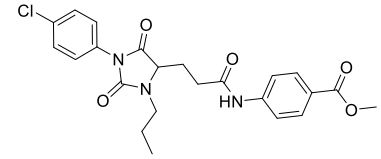
The title compound was synthesized from **7g** and 3-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 50%, mp: 171-172 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.80 (dd, J = 15.9, 7.9 Hz, 2H), 7.54 (s, 1H), 7.49 – 7.29 (m, 10H), 5.02 (d, J = 15.1 Hz, 1H), 4.32 (d, J = 15.1 Hz, 1H), 4.08 – 3.98 (m, 1H), 3.91 (s, 3H), 2.64 – 2.37 (m, 3H), 2.25 – 2.05 (m, 1H).

Methyl 4-((3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido)methyl) benzoate (8h)



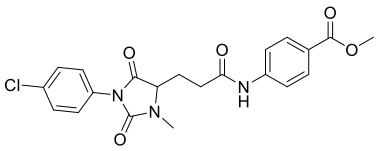
The title compound was synthesized from **7h** and methyl 4-(aminomethyl)benzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 40%, mp: 186-188 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.1 Hz, 2H), 7.43 (d, *J* = 4.9 Hz, 1H), 7.40 (d, *J* = 6.6 Hz, 3H), 7.36 (m, 4H), 7.31 (dd, *J* = 14.8, 6.1 Hz, 3H), 5.78 (s, 1H), 5.03 (dd, *J* = 15.1 Hz, 1H), 4.46 (d, *J* = 5.9 Hz, 2H), 4.30 (d, *J* = 15.2 Hz, 1H), 3.99 (dd, *J* = 7.5, 3.1 Hz, 1H), 3.92 (s, 3H), 2.46 (dd, *J* = 14.2, 6.2 Hz, 1H), 2.32 (ddd, *J* = 19.8, 14.9, 7.9 Hz, 2H), 2.13 (dt, *J* = 11.7, 7.1 Hz, 1H).

Methyl 4-(3-(1-(4-chlorophenyl)-2,5-dioxo-3-propylimidazolidin-4-yl)propanamido) benzoate(8i)



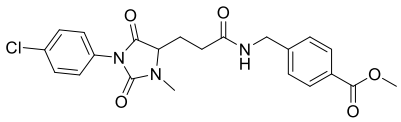
The title compound was synthesized from **7i** and 4-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was purified by silica gel column chromatography to obtain white solid. Yield: 56%, mp: 153-155 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.43 (d, J = 8.7 Hz, 2H), 4.51 – 4.30 (m, 1H), 3.82 (s, 3H), 3.50 (dt, J = 15.4, 7.9 Hz, 1H), 3.14 (ddd, J = 13.8, 8.5, 5.2 Hz, 1H), 2.44 (dd, J = 15.4, 8.5 Hz, 2H), 2.32 – 2.15 (m, 2H), 1.73 – 1.48 (m, 2H), 0.89 (t, J = 7.4 Hz, 3H).

Methyl 4-(3-(1-(4-chlorophenyl)-3-methyl-2,5-dioxoimidazolidin-4-yl)propanamido)benzoate (8j)



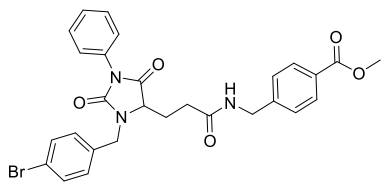
The title compound was synthesized from **7j** and 4-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 66%, mp: 177-179 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6)) δ 10.34 (s, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 8.7 Hz, 2H), 4.31 (t, J = 4.7 Hz, 1H), 3.82 (s, 3H), 2.92 (s, 3H), 2.43 (ddd, J = 23.1, 15.6, 7.9 Hz, 2H), 2.22 (d, J = 6.4 Hz, 2H).

Methyl 4-((3-(1-(4-chlorophenyl)-3-methyl-2,5-dioxoimidazolidin-4-yl)propanamido) methyl)benzoate (8k)



The title compound was synthesized from **7k** and methyl 4-(aminomethyl)benzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 68%, mp: 146-148 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (t, J = 5.8 Hz, 1H), 7.90 (d, J = 8.2 Hz, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.39 (dd, J = 15.1, 8.4 Hz, 4H), 4.35 (dd, J = 16.9, 4.9 Hz, 2H), 4.26 (t, J = 4.2 Hz, 1H), 3.84 (s, 3H), 2.89 (s, 3H), 2.37 – 2.05 (m, 4H).

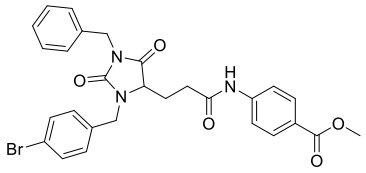
Methyl 4-((3-(3-(4-bromobenzyl)-2,5-dioxo-1-phenylimidazolidin-4-yl)propanamido)methyl) benzoate (8l)



The title compound was synthesized from **7l** and methyl 4-(aminomethyl)benzoate

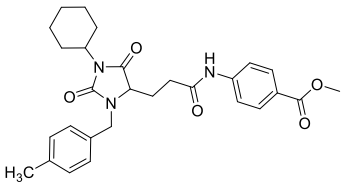
hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 95%, mp: 147-149 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.46 – 7.40 (m, 2H), 7.39 – 7.32 (m, 3H), 7.28 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 8.2 Hz, 2H), 6.04 (t, J = 5.5 Hz, 1H), 4.93 (d, J = 15.2 Hz, 1H), 4.44 (d, J = 5.9 Hz, 1H), 4.24 (d, J = 15.2 Hz, 1H), 3.98 – 3.93 (m, 1H), 3.90 (s, 3H), 2.42 (dt, J = 11.1, 7.2 Hz, 2H), 2.32 (dt, J = 13.0, 6.7 Hz, 1H), 2.18 – 2.05 (m, 1H).

Methyl 4-(3-(1-benzyl-3-(4-bromobenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido)benzoate (8m)



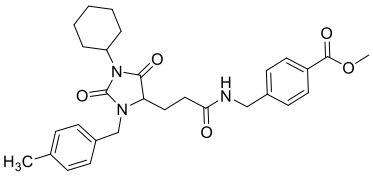
The title compound was synthesized from **7m** and 4-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was purified by silica gel column chromatography to obtain white solid. Yield: 92%, mp: 139-141 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.7 Hz, 2H), 7.46 (dd, J = 8.2, 6.3 Hz, 4H), 7.41 (d, J = 6.4 Hz, 2H), 7.38 – 7.30 (m, 3H), 7.17 (d, J = 8.3 Hz, 2H), 4.85 (d, J = 15.4 Hz, 1H), 4.72 (d, J = 14.4 Hz, 1H), 4.63 (d, J = 14.4 Hz, 1H), 4.14 (d, J = 15.4, 1H), 3.90 (s, 3H), 3.84 (dd, J = 7.8, 3.1 Hz, 1H), 2.46 – 2.29 (m, 2H), 2.27 – 2.17 (m, 1H), 2.08 – 1.97 (m, 1H).

Methyl 4-(3-(1-cyclohexyl-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido) benzoate (8n)



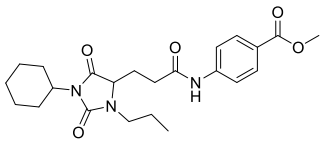
The title compound was synthesized from **7n** and 4-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was recrystallized with ethyl acetate to obtain white solid. Yield: 55%, mp: 185-186 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 7.16 (dd, J = 19.7, 8.0 Hz, 4H), 4.65 (d, J = 15.5 Hz, 1H), 4.26 (d, J = 15.5 Hz, 1H), 3.95 (dd, J = 5.5, 3.4 Hz, 1H), 3.82 (s, 3H), 3.79 – 3.73 (m, 1H), 2.35 – 2.14 (m, 5H), 2.13 – 1.92 (m, 4H), 1.77 (d, J = 12.0 Hz, 2H), 1.63 (s, 3H), 1.37 – 1.20 (m, 2H), 1.18 – 1.01 (m, 1H).

Methyl 4-((3-(1-cyclohexyl-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl) propanamido) methyl) benzoate (8o)



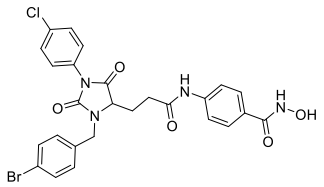
The title compound was synthesized from **7o** and methyl 4-(aminomethyl)benzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was purified by silica gel column chromatography to obtain white solid. Yield: 59%, mp: 168-170 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 7.9 Hz, 2H), 7.15 (q, J = 7.8 Hz, 4H), 5.80 (d, J = 21.5 Hz, 1H), 4.85 (d, J = 15.1 Hz, 1H), 4.43 (t, J = 10.7 Hz, 2H), 4.14 (d, J = 15.1 Hz, 1H), 3.91 (s, 3H), 3.88 (d, J = 12.4 Hz, 1H), 3.79 – 3.68 (m, 1H), 2.30 (s, 3H), 2.26 – 1.94 (m, 6H), 1.81 (d, J = 12.4 Hz, 2H), 1.64 – 1.59 (m, 3H), 1.43 – 1.16 (m, 3H).

Methyl 4-(3-(1-cyclohexyl-2,5-dioxo-3-propylimidazolidin-4-yl)propanamido)benzoate (8p)



The title compound was synthesized from **7p** and 4-aminobenzoate hydrochloride in a manner similar to that described for the preparation of **8a.** The crude product was purified by silica gel column chromatography to obtain white solid. Yield: 62%, mp: 116-117 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 4.18 (dd, J = 5.4, 3.4 Hz, 1H), 3.82 (s, 3H), 3.79 – 3.67 (m, 1H), 3.50 – 3.37 (m, 1H), 3.05 (ddd, J = 13.8, 8.4, 5.1 Hz, 1H), 2.28 (dddd, J = 36.0, 15.5, 9.7, 5.8 Hz, 2H), 2.17 – 1.91 (m, 4H), 1.76 (d, J = 11.6 Hz, 2H), 1.68 – 1.40 (m, 5H), 1.26 (dd, J = 25.5, 12.8 Hz, 2H), 1.17 – 1.01 (m, 1H), 0.84 (t, J = 7.3 Hz, 3H).

4-(3-(3-(4-bromobenzyl)-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10a)[1]

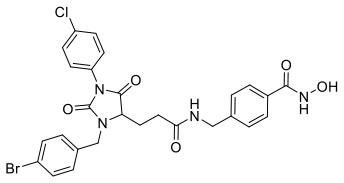


Dissolved compound **8a** (1.17 g, 2.0 mmol) with THF/H_2O=3:1(120 mL) and stirred the mixture for 6h. Then poured 120 mL H_2O into the mixture and extracted with ethyl acetate two times. Separated water phase and 6 M HCl was added to bring the solution to pH 2 and then extracted it with ethyl acetate three times. Dried it over with $MgSO_4$ and the solvent was evaporated under reduced pressure to obtain compound **9a**, yield: 93%.

To the solution of compound **9a** (1.02 g, 1.8 mmol) in THF (30 mL) at 0 ˚C, isobutyl chloroformate (0.27 mL, 2.16 mmol) and *N*-methylmorpholine (0.26 mL, 2.34 mmol) were added and stirred the mixture for 30 min. Filtered and the filtrate was added to freshly prepared

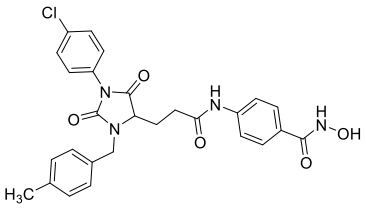
hydroxylamine (0.24 g, 7.2 mmol) in methanol. After stirring the mixture for another 6h at room temperature, removed the solvent and the residue was dissolved in ethyl acetate (50 ml). Washed with 1 M HCl and the crude product was purified by silica gel column chromatography to obtain 0.47 g compound **10a**, Yield: 27%, mp: 210-212 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.16 (s, 1H), 8.95 (s, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.65 – 7.53 (m, 6H), 7.47 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 4.74 (d, J = 15.9 Hz, 1H), 4.43 (d, J = 15.9 Hz, 1H), 4.26 (t, J = 4.9 Hz, 1H), 2.41 (q, J = 7.5 Hz, 2H), 2.16 (q, J = 7.1 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.80, 170.65, 164.40, 155.46, 142.09, 136.35, 132.79, 131.95, 131.40, 130.68, 129.24, 128.86, 128.16, 127.58, 121.20, 118.78, 58.86, 44.19, 30.66, 24.00. HRMS (AP-ESI) m/z, Calcd for $C_{26}H_{22}BrClN_4O_5$, ([M+H]$^+$): 585.0535, found: 585.0539.

4-((3-(3-(4-bromobenzyl)-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido)methyl)-*N*-hydroxybenzamide (10b)



The title compound was synthesized from **8b** in a manner similar to that described for the preparation of **10a.** Yield: 47%, mp: 136-138 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.01 (s, 1H), 8.42 (t, J = 5.7 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H), 7.57 (dd, J = 8.2, 6.2 Hz, 4H), 7.46 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 4.71 (d, J = 15.9 Hz, 1H), 4.39 (d, J = 15.9 Hz, 1H), 4.28 (d, J = 5.7 Hz, 2H), 4.23 (t, J = 4.5 Hz, 1H), 2.31 – 2.15 (m, 2H), 2.10 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.82, 171.33, 164.37, 155.46, 143.12, 136.39, 132.78, 131.95, 131.72, 131.41, 130.65, 129.24, 128.89, 127.53, 127.35, 121.18, 59.00, 44.19, 42.33, 29.49, 24.42. HRMS (AP-ESI) m/z, Calcd for $C_{27}H_{24}BrClN_4O_5$, ([M+H]$^+$): 599.0691, found: 599.0695.

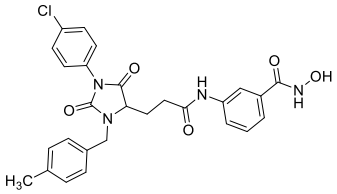
4-(3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10c)



The title compound was synthesized from **8c** in a manner similar to that described for the preparation of **10a.** Yield: 27%, mp: 230-232 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 10.15 (s, 1H), 8.94 (s, 1H), 7.69 (d, J = 8.8 Hz, 2H), 7.66 – 7.52 (dd, J = 8.8, 8.9 Hz, 4H), 7.47 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 7.7 Hz, 2H), 7.16 (d, J = 7.7 Hz, 2H), 4.74 (d, J = 15.6 Hz, 1H), 4.37 (d, J = 15.6 Hz, 1H), 4.21 (t, J = 4.7 Hz,, 1H), 2.46 – 2.31 (m, 2H), 2.26 (s, 3H), 2.22 – 2.08 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.79, 170.66, 164.40, 155.34, 142.11, 137.24, 133.67,

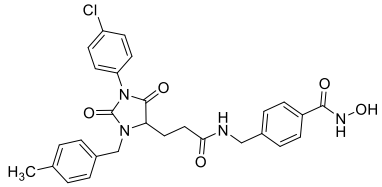
132.76, 131.43, 129.69, 129.24, 128.86, 128.45, 128.15, 127.56, 118.76, 58.64, 44.51, 30.63, 23.96, 21.16. HRMS (AP-ESI) m/z, Calcd for $C_{27}H_{25}ClN_4O_5$, ($[M+H]^+$): 521.1586, found: 521.1581.

3-(3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10d)



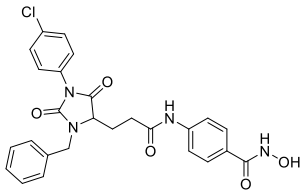
The title compound was synthesized from **8d** in a manner similar to that described for the preparation of **10a.** Yield: 48%, mp: 161-163 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (s, 1H), 10.07 (s, 1H), 9.02 (s, 1H), 7.95 (s, 1H), 7.70 (d, J = 6.9 Hz, 1H), 7.57 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 8.7 Hz, 2H), 7.41 – 7.32 (m, 2H), 7.28 (d, J = 7.9 Hz, 2H), 7.16 (d, J = 7.8 Hz, 2H), 4.74 (d, J = 15.5 Hz, 1H), 4.37 (d, J = 15.6 Hz, 1H), 4.21 (t, J = 4.7 Hz, 1H), 2.44 – 2.31 (m, 2H), 2.26 (s, 3H), 2.21 – 2.07 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.80, 170.46, 164.72, 155.36, 139.75, 137.25, 133.96, 133.69, 132.77, 131.43, 129.69, 129.25, 129.16, 128.88, 128.45, 122.00, 121.51, 118.57, 58.67, 44.54, 30.56, 24.08, 21.16. HRMS (AP-ESI) m/z, Calcd for $C_{27}H_{25}ClN_4O_5$, ($[M+H]^+$): 521.1586, found: 521.1582.

4-((3-(1-(4-chlorophenyl)-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido)methyl)-*N*-hydroxybenzamide (10e)



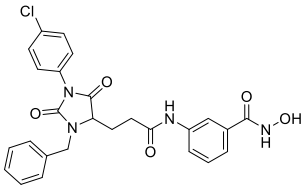
The title compound was synthesized from **8e** in a manner similar to that described for the preparation of **10a.** Yield: 45%, mp: 202-204 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 9.01 (s, 1H), 8.45 (dt, J = 11.5, 5.9 Hz, 1H), 7.79 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.7 Hz, 2H), 7.40 – 7.22 (m, 4H), 7.17 (d, J = 7.8 Hz, 2H), 4.73 (d, J = 15.5 Hz, 1H), 4.42 – 4.20 (m, 3H), 4.16 (t, J = 4.5 Hz, 1H), 2.29 (s, 3H), 2.27 – 2.15 (m, 2H), 2.09 (d, J = 5.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.81, 171.35, 164.50, 155.34, 143.16, 137.23, 133.69, 132.76, 131.72, 131.43, 129.69, 129.24, 128.88, 128.42, 127.52, 127.35, 58.73, 44.50, 42.32, 29.50, 24.38, 21.19. HRMS (AP-ESI) m/z, Calcd for $C_{28}H_{27}ClN_4O_5$, ($[M+H]^+$): 535.1743, found: 535.1744.

4-(3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10f)



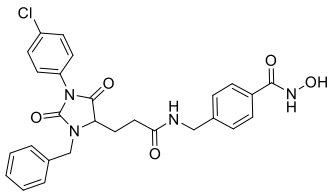
The title compound was synthesized from **8f** in a manner similar to that described for the preparation of **10a.** Yield: 36%, mp: 228-220 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 10.38 (s, 1H), 8.97 (s, 1H), 7.71 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.38 (dt, J = 14.8, 7.3 Hz, 4H), 7.30 (t, J = 7.1 Hz, 1H), 4.79 (d, J = 15.7 Hz, 1H), 4.46 (d, J = 15.7 Hz, 1H), 4.24 (t, J = 4.8 Hz, 1H), 2.49 – 2.35 (m, 2H), 2.24 – 2.13 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.81, 170.76, 164.42, 155.42, 142.24, 136.80, 132.79, 131.43, 129.24, 129.11, 128.97, 128.41, 128.12, 128.03, 127.46, 118.77, 58.77, 44.75, 30.76, 24.12. HRMS (AP-ESI) m/z, Calcd for $C_{26}H_{23}ClN_4O_5$, ([M+H]$^+$): 507.1430, found: 507.1432.

3-(3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10g)



The title compound was synthesized from **8g** in a manner similar to that described for the preparation of **10a.** Yield: 37%, mp: 110-111 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 10.10 (s, 1H), 9.06 (s, 1H), 7.96 (s, 1H), 7.71 (d, J = 7.0 Hz, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 7.45 – 7.34 (m, 6H), 7.30 (t, J = 7.0 Hz, 1H), 4.79 (d, J = 15.8 Hz, 1H), 4.45 (d, J = 15.7 Hz, 1H), 4.25 (t, J = 4.8 Hz, 1H), 2.48 – 2.30 (m, 2H), 2.25 – 2.11 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.63, 171.82, 170.47, 155.43, 139.75, 136.81, 133.97, 132.79, 131.43, 129.25, 129.16, 129.13, 128.90, 128.39, 128.04, 121.99, 121.52, 118.56, 58.81, 44.81, 30.59, 24.13. HRMS (AP-ESI) m/z, Calcd for $C_{26}H_{23}ClN_4O_5$, ([M+H]$^+$): 507.1430, found: 507.1428.

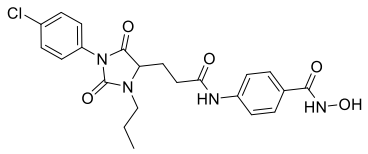
4-((3-(3-benzyl-1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)propanamido)methyl)-*N*-hydroxybenzamide (10h)



The title compound was synthesized from **8h** in a manner similar to that described for the preparation of **10a.** Yield: 40%, mp: 195-197 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.00 (s, 1H), 8.41 (dd, J = 13.5, 7.7 Hz, 1H), 7.69 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 8.7 Hz, 2H), 7.42 – 7.34 (m, 4H), 7.32 (dd, J = 5.5, 3.0 Hz, 1H), 7.28 (d, J = 8.1 Hz,

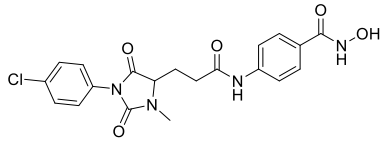
2H), 4.75 (d, *J* = 15.7 Hz, 1H), 4.40 (d, *J* = 15.8 Hz, 1H), 4.27 (d, *J* = 4.2 Hz, 2H), 4.21 (t, *J* = 4.6 Hz, 1H), 2.27 – 2.16 (m, 2H), 2.15 – 2.02 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.83, 171.32, 164.51, 155.42, 143.17, 136.84, 132.77, 131.72, 131.44, 129.25, 129.12, 128.90, 128.36, 128.03, 127.52, 127.35, 58.93, 44.80, 42.30, 29.46, 24.41. HRMS (AP-ESI) m/z, Calcd for $C_{27}H_{25}ClN_4O_5$, ([M+H]$^+$): 521.1586, found: 521.1588.

4-(3-(1-(4-chlorophenyl)-2,5-dioxo-3-propylimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10i)



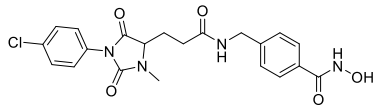
The title compound was synthesized from **8i** in a manner similar to that described for the preparation of **10a.** Yield: 17%, mp: 188-190 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.20 (s, 1H), 8.95 (s, 1H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 2H), 4.41 (t, *J* = 4.7 Hz, 1H), 3.57 – 3.45 (m, 1H), 3.14 (ddd, *J* = 13.8, 8.4, 5.1 Hz, 1H), 2.41 (dt, *J* = 15.3, 6.9 Hz, 2H), 2.31 – 2.10 (m, 2H), 1.75 – 1.47 (m, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.09, 170.77, 164.38, 155.03, 142.11, 132.65, 131.49, 129.22, 128.77, 128.15, 127.59, 118.77, 58.37, 42.63, 30.65, 24.02, 20.96, 11.63. HRMS (AP-ESI) m/z, Calcd for $C_{22}H_{23}ClN_4O_5$, ([M+H]$^+$): 459.1430, found: 459.1429.

4-(3-(1-(4-chlorophenyl)-3-methyl-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10j)



The title compound was synthesized from **8j** in a manner similar to that described for the preparation of **10a.** Yield: 65%, mp: 203-205 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.21 (s, 1H), 8.95 (s, 1H), 7.70 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 8.7 Hz, 2H), 4.31 (t, J = 4.7 Hz, 1H), 2.92 (s, 3H), 2.48 – 2.32 (m, 2H), 2.30 – 2.13 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.03, 170.80, 164.42, 155.33, 142.13, 132.66, 131.55, 129.24, 128.72, 128.16, 127.60, 118.79, 60.54, 30.83, 28.32, 23.92. HRMS (AP-ESI) m/z, Calcd for $C_{20}H_{19}ClN_4O_5$, ([M+H]$^+$): 431.1117, found: 431.1115

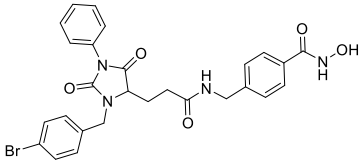
4-((3-(1-(4-chlorophenyl)-3-methyl-2,5-dioxoimidazolidin-4-yl)propanamido)methyl)-*N*-hydroxybenzamide (10k)



The title compound was synthesized from **8k** in a manner similar to that described for the

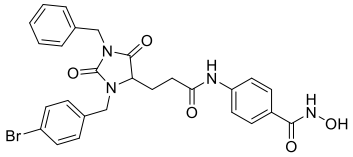
preparation of **10a.** Yield: 29%, mp: 132-134 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.01 (s, 1H), 8.47 (t, J = 5.7 Hz, 1H), 7.69 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.41 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 4.29 (dd, J = 17.6, 11.9 Hz, 3H), 2.89 (s, 3H), 2.32 – 2.04 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.05, 171.47, 164.51, 155.33, 143.15, 132.66, 131.73, 131.57, 129.24, 128.75, 127.53, 127.36, 60.62, 42.35, 29.63, 28.32, 24.31. HRMS (AP-ESI) m/z, Calcd for $C_{21}H_{21}ClN_4O_5$, ([M+H] $^{+}$): 445.1273, found: 445.1272

4-((3-(3-(4-bromobenzyl)-2,5-dioxo-1-phenylimidazolidin-4-yl)propanamido)methyl)-*N*-hydroxybenzamide (10l)



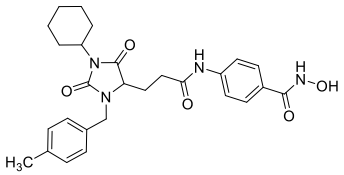
The title compound was synthesized from **8l** in a manner similar to that described for the preparation of **10a.** Yield: 40%, mp: 210-211 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.01 (s, 1H), 8.44 (dd, *J* = 12.8, 7.0 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.53 – 7.46 (m, 2H), 7.41 (t, *J* = 6.1 Hz, 3H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 4.72 (d, *J* = 15.9 Hz, 1H), 4.40 (d, *J* = 15.9 Hz, 1H), 4.30 (d, *J* = 9.4 Hz, 2H), 4.24 (t, *J* = 4.5 Hz, 1H), 2.32 – 2.16 (m, 2H), 2.16 – 2.04 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.99, 171.32, 164.53, 155.78, 143.17, 136.49, 132.52, 131.97, 131.74, 130.65, 129.21, 128.45, 127.53, 127.38, 127.28, 121.17, 58.95, 44.21, 42.34, 29.42, 24.44. HRMS (AP-ESI) m/z, Calcd for $C_{27}H_{25}BrN_4O_5$, ([M+H] $^{+}$): 565.1081, found: 565.1082.

4-(3-(1-benzyl-3-(4-bromobenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10m)



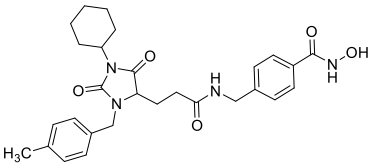
The title compound was synthesized from **8m** in a manner similar to that described for the preparation of **10a.** Yield: 45%, mp: 195-197 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.15 (s, 1H), 8.95 (s, 1H), 7.69 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.2 Hz, 2H), 7.43 – 7.21 (m, 7H), 4.68 (d, J = 15.9 Hz, 1H), 4.60 (q, J = 15.5 Hz, 2H), 4.38 (d, J = 15.9 Hz, 1H), 4.18 (dd, J = 6.4, 3.3 Hz, 1H), 2.37 (ddd, J = 15.1, 9.5, 5.3 Hz, 1H), 2.24 (ddd, J = 15.4, 9.6, 6.0 Hz, 1H), 2.18 – 2.07 (m, 1H), 2.07 – 1.94 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.87, 170.54, 164.40, 156.67, 142.09, 136.97, 136.59, 132.00, 130.54, 129.05, 128.14, 127.92, 127.77, 127.57, 121.17, 118.79, 58.89, 44.11, 42.17, 30.67, 24.14. HRMS (AP-ESI) m/z, Calcd for $C_{27}H_{25}BrN_4O_5$, ([M+H] $^{+}$): 565.1081, found: 565.1089.

4-(3-(1-cyclohexyl-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10n)



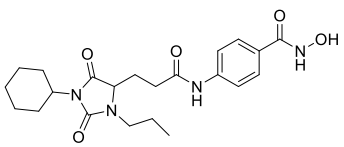
The title compound was synthesized from **8n** in a manner similar to that described for the preparation of **10a.** Yield: 60%, mp: 218-220 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 10.09 (s, 1H), 8.95 (s, 1H), 7.69 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.16 (dd, J = 17.7, 7.4 Hz, 4H), 4.65 (d, J = 15.5 Hz, 1H), 4.25 (d, J = 15.4 Hz, 1H), 3.94 (s, 1H), 3.80 (t, J = 11.5 Hz, 1H), 2.38 – 2.13 (m, 5H), 2.13 – 1.90 (m, 4H), 1.77 (d, J = 11.1 Hz, 2H), 1.63 (s, 3H), 1.38 – 1.20 (m, 2H), 1.18 – 1.02 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.67, 170.54, 164.40, 156.48, 142.11, 137.18, 133.97, 129.71, 128.31, 128.13, 127.54, 118.76, 57.79, 51.24, 44.27, 30.20, 29.57, 29.49, 25.82, 25.31, 23.90, 21.13. HRMS (AP-ESI) m/z, Calcd for $C_{27}H_{32}N_4O_5$, ([M+H] $^{+}$): 493.2446, found: 493.2443.

4-((3-(1-cyclohexyl-3-(4-methylbenzyl)-2,5-dioxoimidazolidin-4-yl)propanamido)methyl)-*N*-hydroxybenzamide (10o)



The title compound was synthesized from **8o** in a manner similar to that described for the preparation of **10a.** Yield: 52%, mp: 204-206 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.20 (s, 1H), 9.01 (s, 1H), 8.38 (t, J = 5.9 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 7.20 – 7.09 (m, 4H), 4.64 (d, J = 15.5 Hz, 1H), 4.33 – 4.23 (m, 2H), 4.20 (d, J = 15.5 Hz, 1H), 3.90 (s, 1H), 3.77 (t, J = 12.2 Hz, 1H), 2.27 (s, 3H), 2.13 – 1.92 (m, 6H), 1.76 (d, J = 12.5 Hz, 2H), 1.61 (d, J = 11.2 Hz, 3H), 1.26 (q, J = 13.0 Hz, 2H), 1.17 – 1.03 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.71, 171.24, 164.51, 156.47, 143.21, 137.16, 133.98, 131.71, 129.71, 128.28, 127.50, 127.36, 57.88, 51.21, 44.24, 42.30, 29.57, 29.45, 29.07, 25.79, 25.31, 24.32, 21.16. HRMS (AP-ESI) m/z, Calcd for $C_{28}H_{34}N_4O_5$, ([M+H] $^{+}$): 507.2602, found: 507.2603.

4-(3-(1-cyclohexyl-2,5-dioxo-3-propylimidazolidin-4-yl)propanamido)-*N*-hydroxybenzamide (10p)



The title compound was synthesized from **8p** in a manner similar to that described for the preparation of **10a.** Yield: 48%, mp: 203-205 ˚C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 10.15 (s, 1H), 8.94 (s, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 8.7 Hz, 2H), 4.24 – 4.14 (m, 1H), 3.75 (t, J = 12.2 Hz, 1H), 3.41 (dt, J = 21.4, 7.0 Hz, 1H), 3.04 (ddd, J = 13.8, 8.4, 5.1 Hz, 1H),

2.25 (dddd, J = 30.9, 15.4, 9.3, 5.7 Hz, 2H), 2.15 – 1.90 (m, 4H), 1.76 (d, J = 11.9 Hz, 2H), 1.67 – 1.40 (m, 5H), 1.35 – 1.18 (m, 2H), 1.17 – 1.01 (m, 1H), 0.83 (t, J = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.96, 170.67, 164.39, 156.19, 142.12, 128.13, 127.57, 118.77, 57.54, 51.03, 42.30, 30.22, 29.55, 29.47, 25.83, 25.32, 23.99, 21.01, 11.58. HRMS (AP-ESI) m/z, Calcd for $C_{22}H_{30}N_4O_5$, ([M+H]$^+$): 431.2289, found: 431.2283.

4. ^{1}H-NMR, ^{13}C-NMR, HRMS spectrum of representative compounds

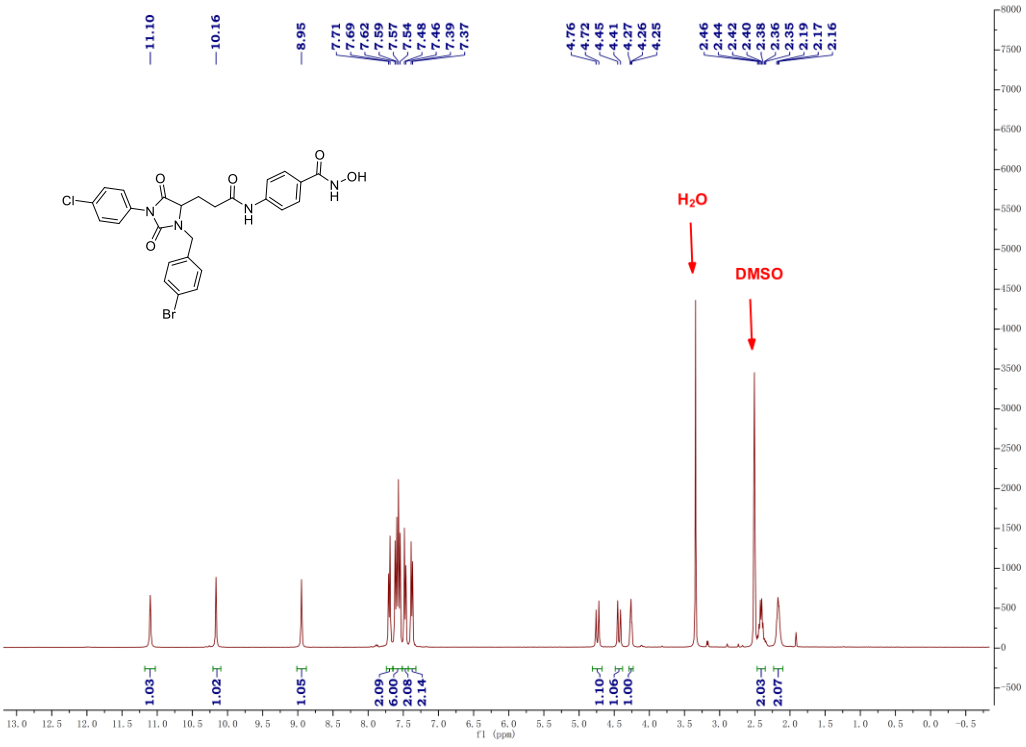


Figure 2. ^{1}H NMR Spectra of compound **10a**

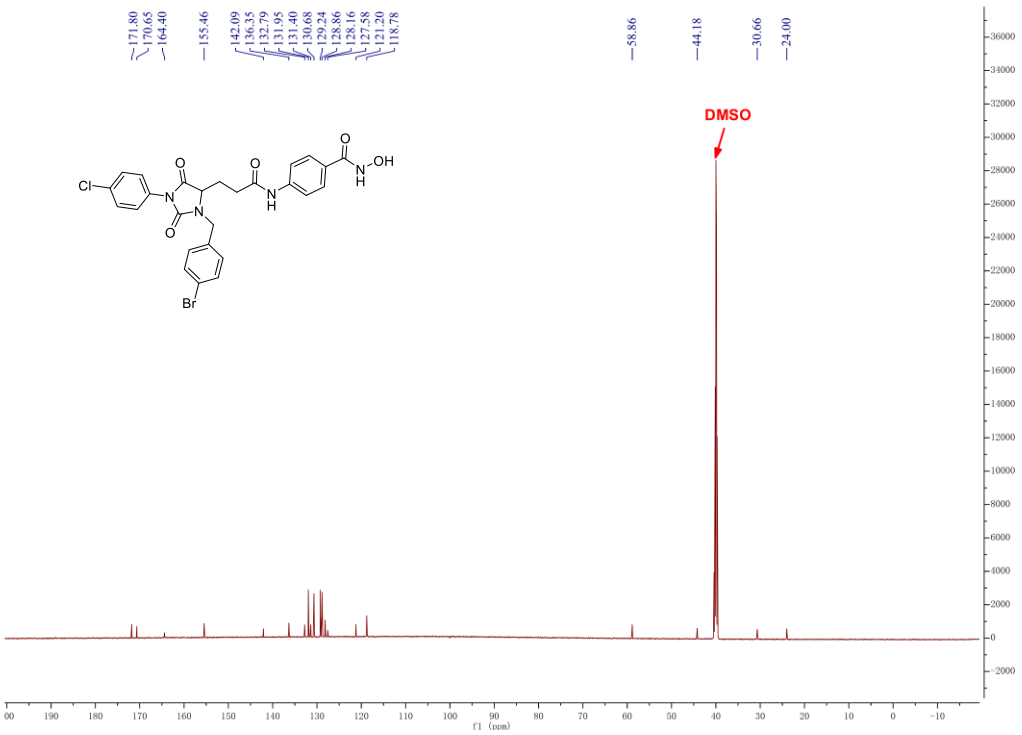


Figure 3. ^{13}C NMR Spectra of compound **10a**

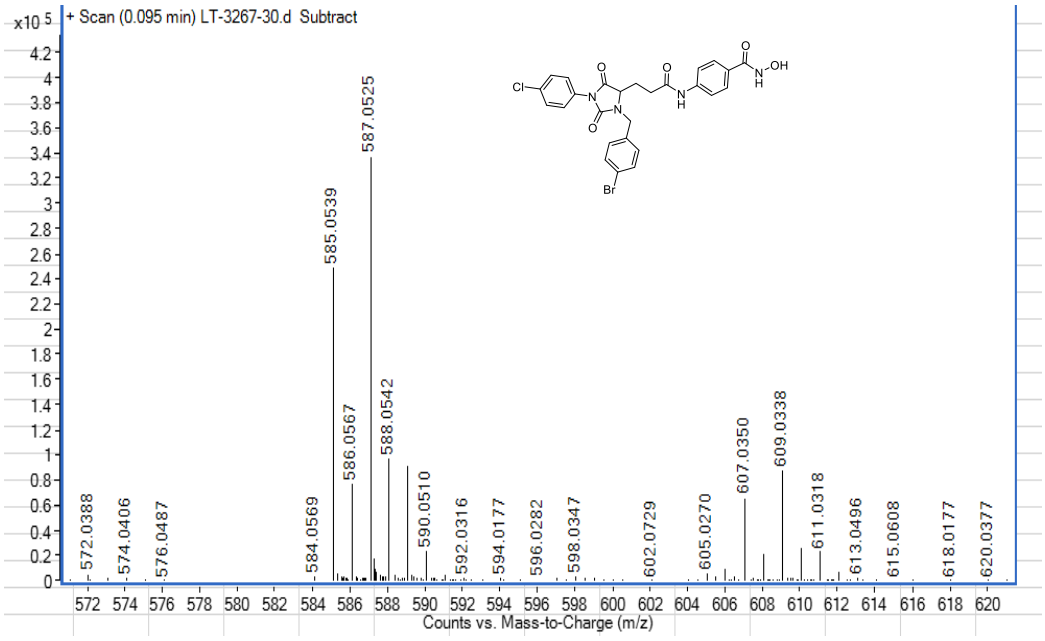


Figure 4. HRMS Spectra of compound **10a**

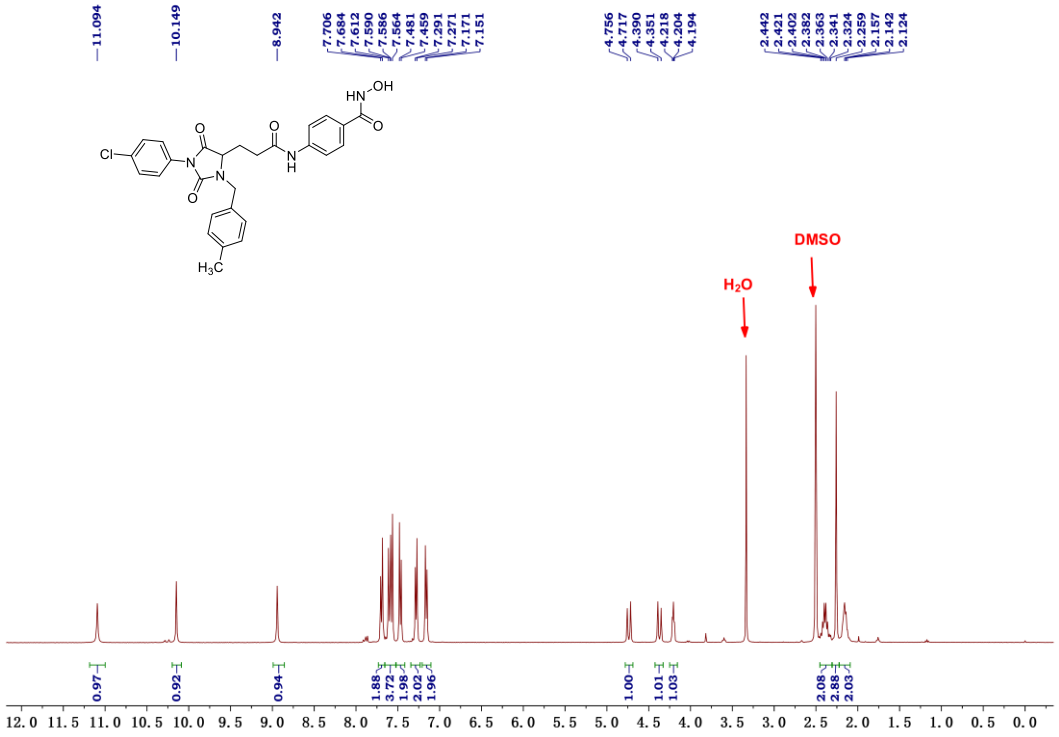


Figure 5. ^{1}H NMR Spectra of compound **10c**

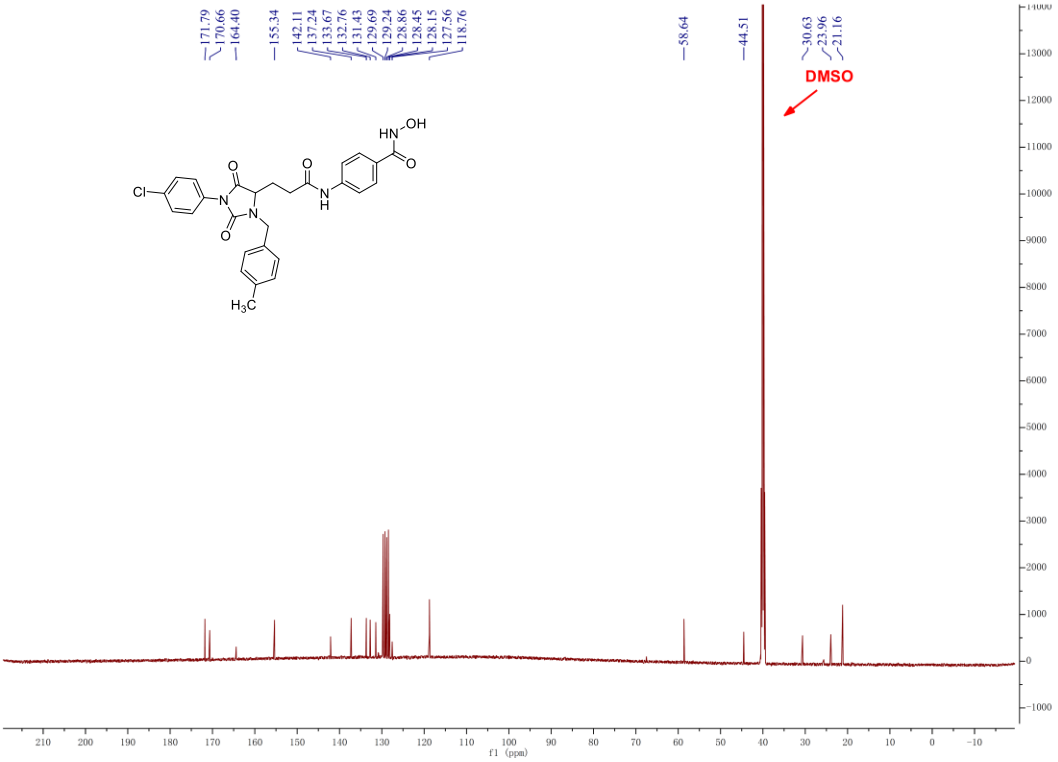


Figure 6. ^{13}C NMR Spectra of compound **10c**

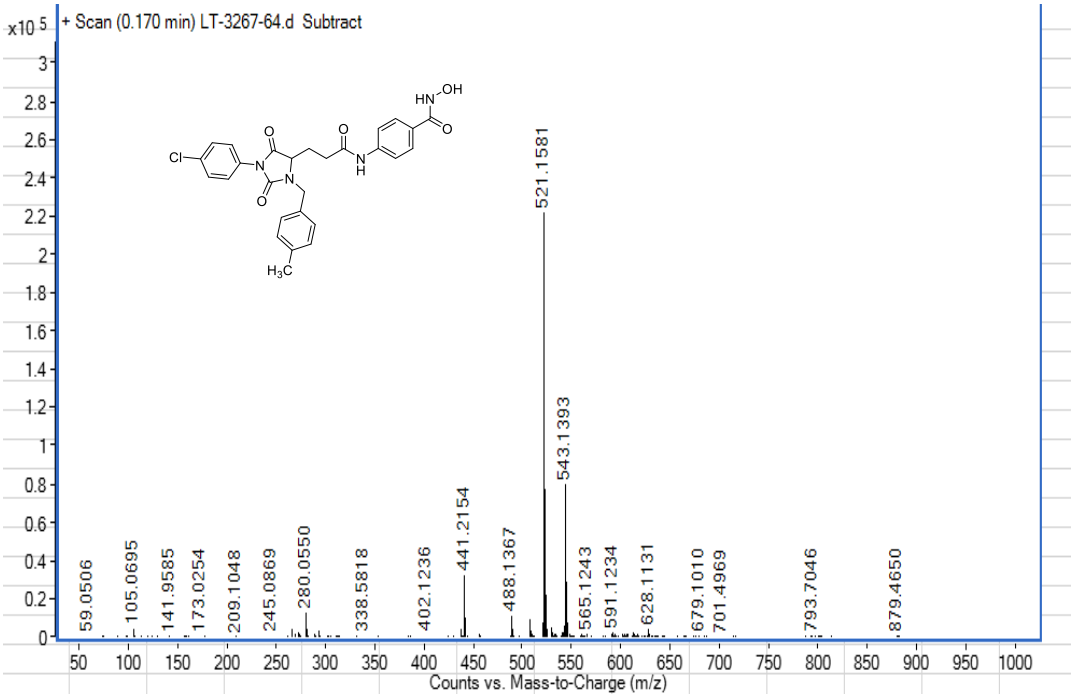


Figure 7. HRMS Spectra of compound **10c**

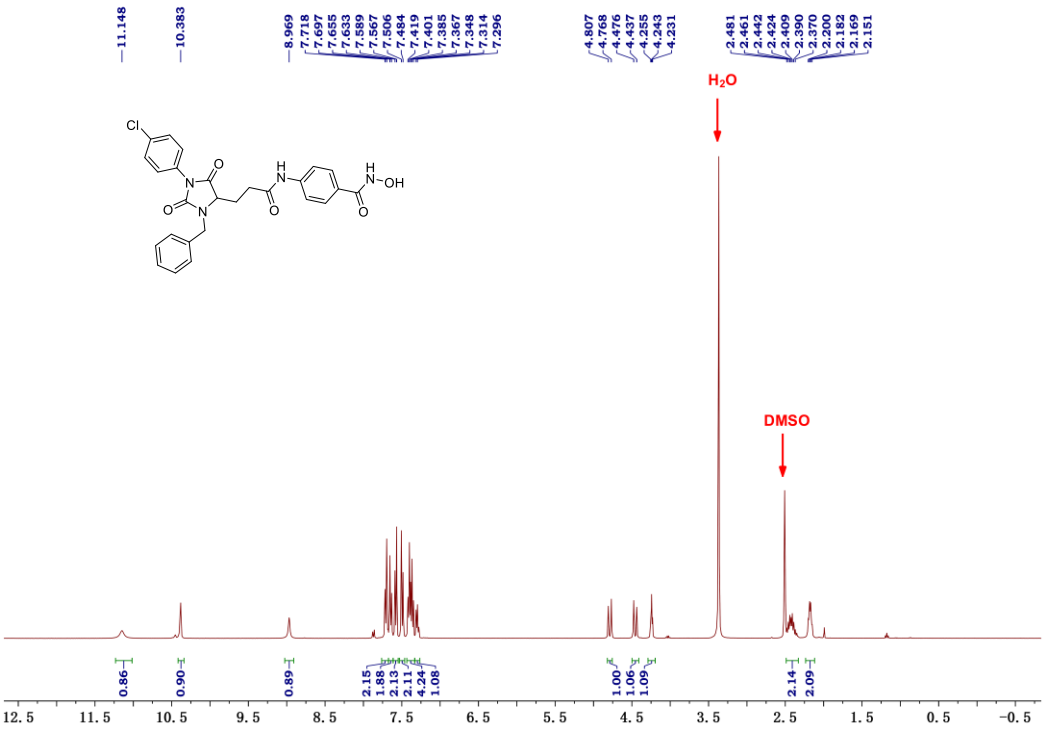


Figure 8. ^{1}H NMR Spectra of compound **10f**

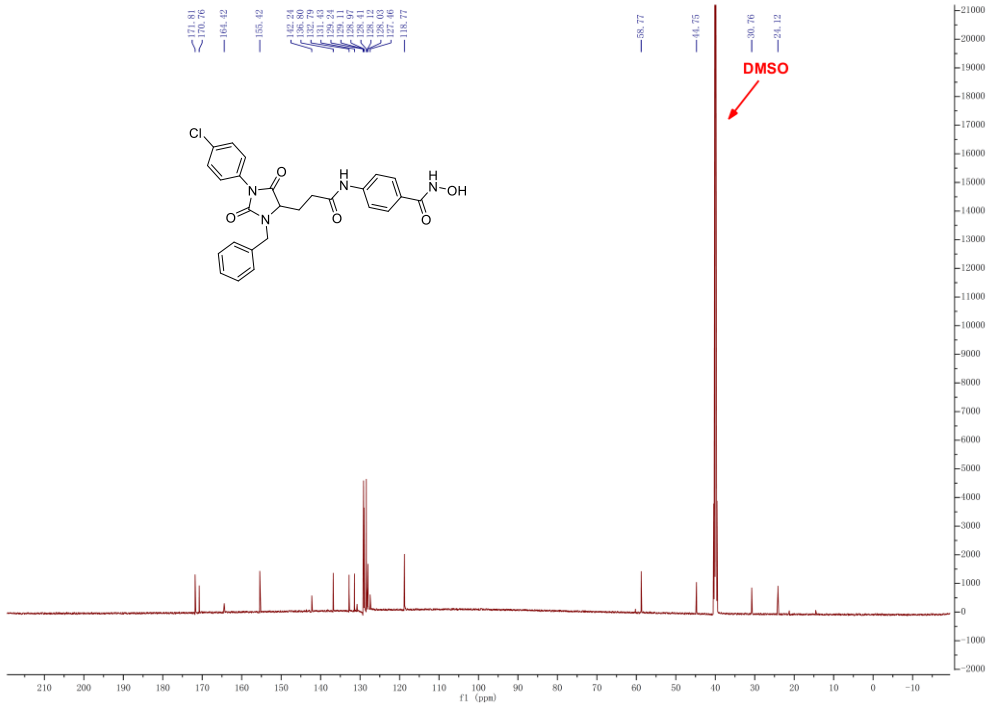


Figure 9. ^{13}C NMR Spectra of compound **10f**

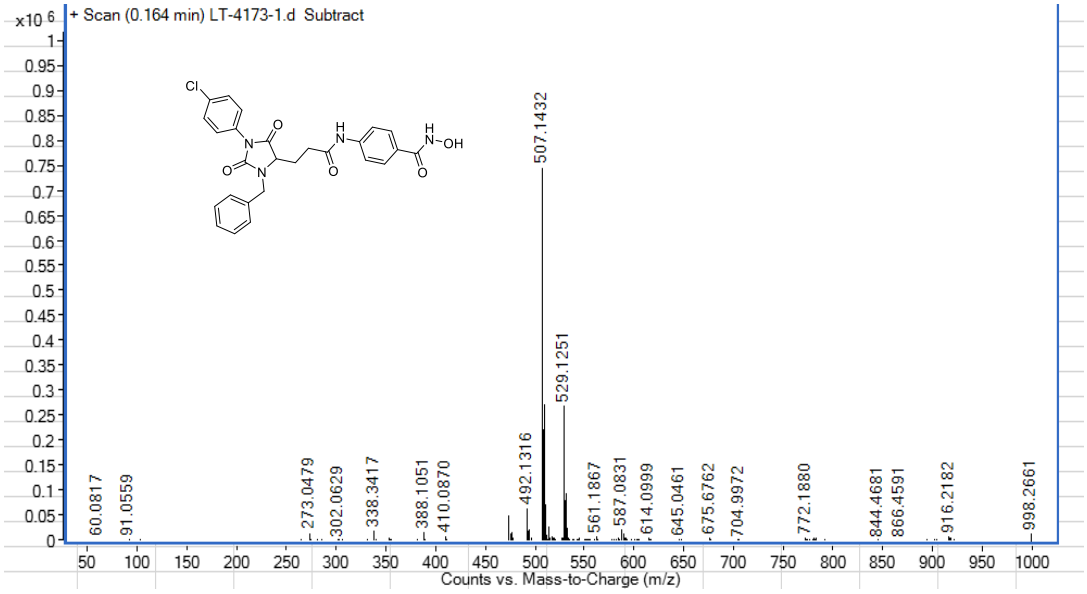


Figure 10. HRMS Spectra of compound **10f**

5. HPLC analysis.[2]

The purity of all targeted compounds was determined to be >90.0% by HPLC. HPLC analysis was performed with a C_{18} column (150 mm × 4.6 mm, 5μm, Diamonsil) at a flow rate of 1mL/min. The mixture of methanol (60%) and H_2O (40%) with 0.1% formic acid were used as

mobile phase.

6. HDAC6 and HDAC isoform inhibitory assay[3]

All enzymatic reactions were conducted at 37 °C. After incubated HDAC with different concentrations of compounds solutions for 5 min, fluorogenic substrate (Ac-LeuGlyLys(Ac)-AMC substrate for HDAC1, 2, 3 and 6; Ac-LeuGlyLys(tfa)-AMC substrate for HDAC4, 5, 7, 8, 9; Ac-ArgThr- Lys(Ac)Lys(Ac)-AMC for HDAC10; tetrapeptide Ac-LeuGlyLys(Ac)-AMC as substrate for HDAC11) was added. After 60 min, the reaction was quenched by developer (50 μL) containing trypsin and TSA. The suspension was incubated at 37 °C for another 60 min, and fluorescence intensity was analyzed with an excitation of 350-360 nm and an emission wavelength of 450-460 nm at SpectraMax M5 microtiter plate reader. The IC_{50} values were calculated using nonlinear regression with normalized dose-response fit using Prism GraphPad software.

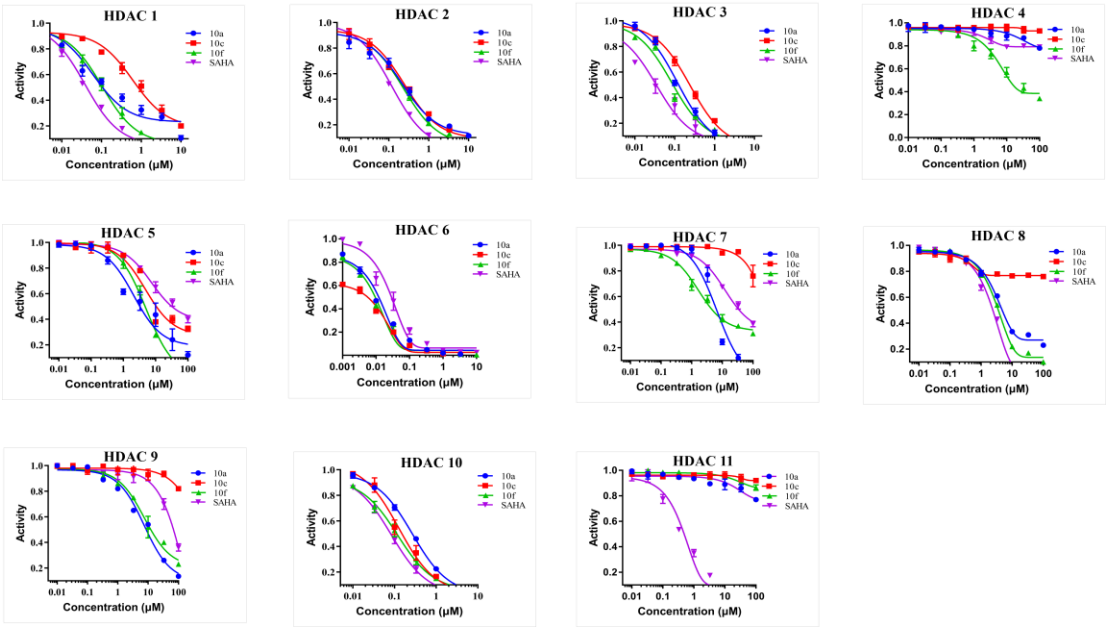


Figure 11. Dose-effect curves of compounds **10a, 10c** and **10f** against HDAC1-11

7. In vitro antiproliferative assay (MTT assay)

All the cell lines were cultured with RPMI1640 medium (10% FBS) at 37 ˚C in a 5% CO_2 humidified incubator as previous research[3]. In brief, the cells were seeded at 3500–4000 cells per well (100 μL/well) in 96-well plates for 8 h and were then treated with various concentrations of compound solutions (100 μL /well) for 48 h. The solution of 0.5% MTT (10 μL /well) was added, and the solution was incubated for 4 h. A total of 150 μL DMSO was added and shaken for dissolution for 15 min at 37 ˚C. Absorbance was determined with a microtiter-plate reader at 570 nm to calculate the inhibition ratios and the IC_{50} values.

8. Apoptosis and cell cycle analysis

HL-60 cells (4×10^5) were treated with different concentrations (0.25 μM and 0.45 μM) of **10a, 10c** and SAHA for 48 h. The cells were harvested by centrifuging, and the supernatant was

removed. After washing with cold PBS buffer, the cells were resuspended in 195 μL Annexin V-FITC binding buffer. Then, 2.5 μL Annexin V-FITC and 5 μL propidium iodide (PI) were added. The cells were incubated for 15 min at room temperature, and the samples were kept on ice and analyzed apoptotic cells by flow cytometry.

Following the same method, harvested and resuspended HL-60 cells. Then, the cells were fixed gently in 70% ethanol (in PBS) at 4 ˚C overnight and were washed with PBS. Resuspend the cells with 0.5 mL staining buffer, 25 μL PI (20X), 10 μL RNase A (50X) and incubate for 30 min at 37 ˚C in the dark. Keep the samples on ice and analyze the cell distribution by flow cytometry.

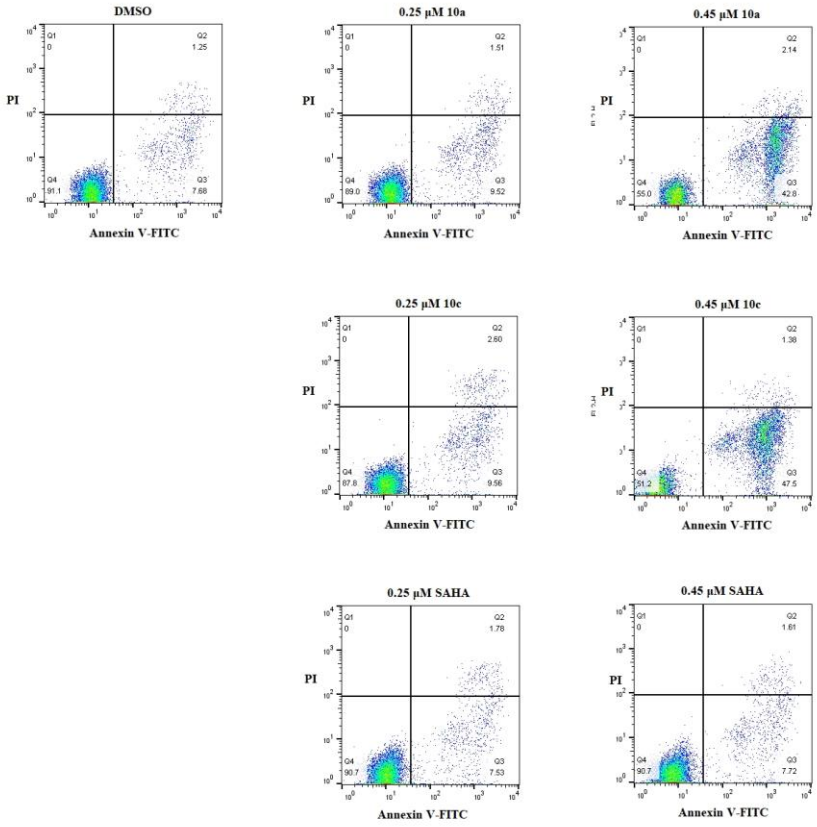


Figure 12. Inducing apoptosis of HL-60 by compound **10a, 10c** and SAHA

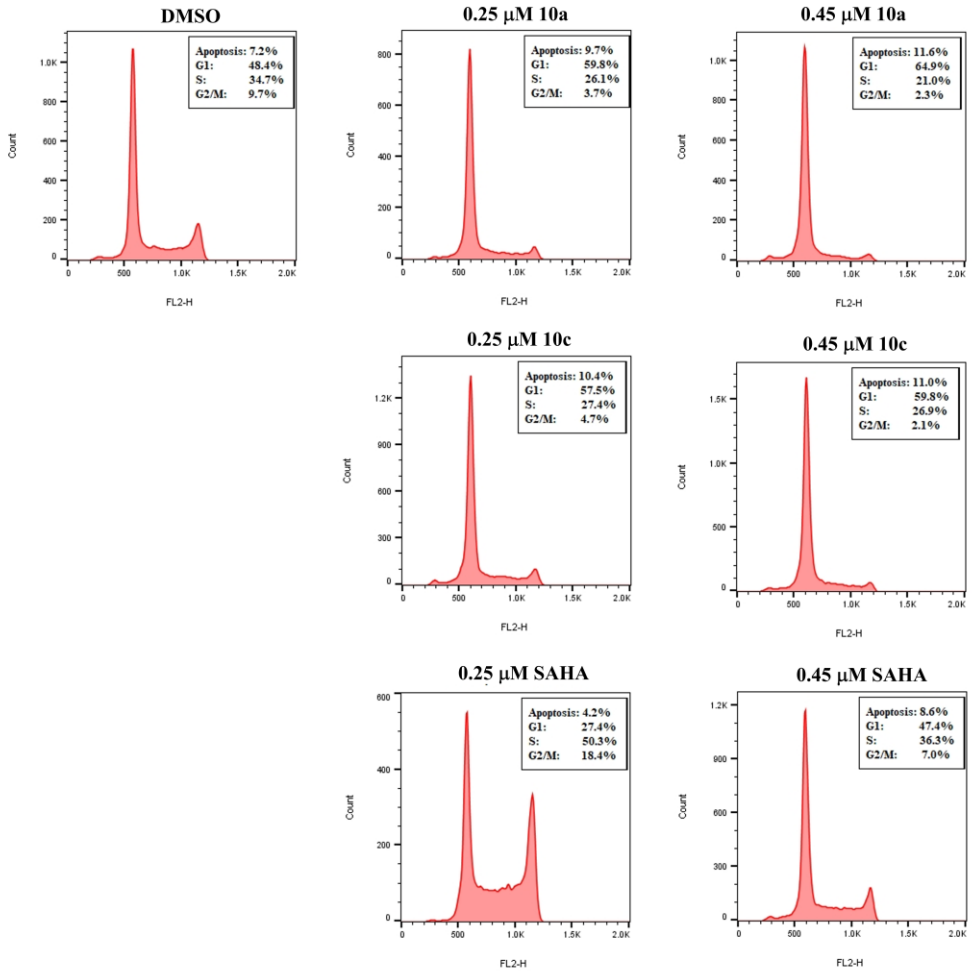


Figure 13. Cell cycle analysis for compound **10a, 10c** and SAHA against HL-60

9. Caspase 3 assay

Incubate HL-60 cells with various concentrations (0.25 μM, 0.45 μM and 0.75 μM) of potent compound **10c** for different time (24h and 48 h), then 1×10^6 cells were collected and washed with PBS. Resuspend cells with 50 μL chilled cell lysis buffer and incubated on ice for 10 min. After transferring the mixture to 96-well plate, added 50 μL of 2X reaction buffer (containing 10 mM DTT) and 5 μL caspase 3 substrates (1mM DEVE-AFC) to each sample. Incubate the mixture at 37 ˚C for 1.5 h and read samples in a microplate reader at the excitation and emission wavelengths of 400 nm and 505 nm. The results are expressed as fold increase in caspase activity of apoptotic cells over that of non-induced cells.

10. Western blot assay

Plate HL-60 cells in 6-well plate and treat it with different concentrations of compound **10c**, ACY-1215 and SAHA. After incubating 24h under humidified conditions (37 °C, 5% CO_2), cells were harvested by centrifuging (900 rpm, 5 min), and the supernatant was removed. Wash twice with ice-cold PBS and extract with RIPA containing 1mM PMSF and 1% phosphatase inhibitor cocktail solution on ice for 30 min. The protein was collected by centrifuging (13000 rpm, 15 min,

4°C), and the concentrations were quantified by the BCA Protein Assay Kit. Add 5X blue loading buffer pack and boil the cell lysates for 5 min. Resolved on 12.5% gradient gels and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% non-fat milk and incubated with specific primary antibodies overnight at 4 ˚C. After washing and incubating with HRP-conjugated secondary antibodies, the bands were detected by scanning blots with an LI-COR Odyssey imaging system.

11. Anti-proliferation activity of intermediate of compound 10c

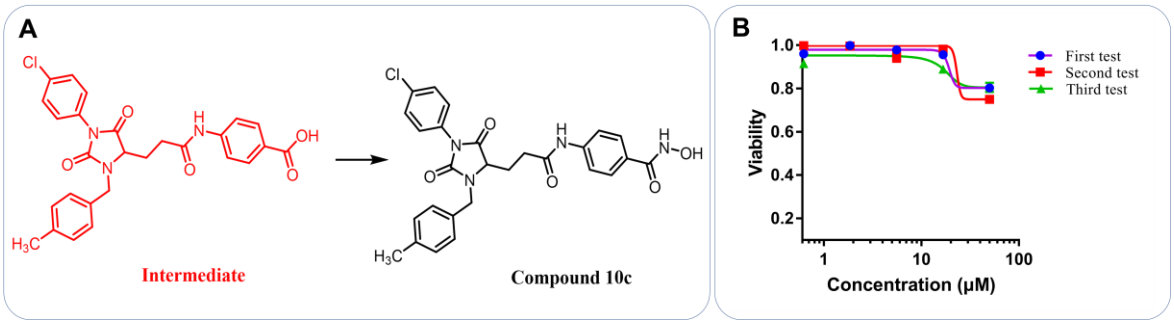


Figure 14. (A) Structure of compound 10c and its intermediate; (B) anti-proliferation activity of intermediate against HL-60 cell.

12. Molecular docking

The crystal structure of HDAC6 (PDB ID: 5EDU) and Tripos/Sybyl 2.1 were used in current study. Molecular docking of compound **10c** and HDAC6 (5EDU) were performed with Surflex-dock according to our previous research.[2] Generally, receptor was dehydrated and added hydrogen. Ligand of HDAC6 (5EDU) was chosen to create the binding pocket and all other settings were default. Optimized the structure and top scored conformation was chosen to plot figures.

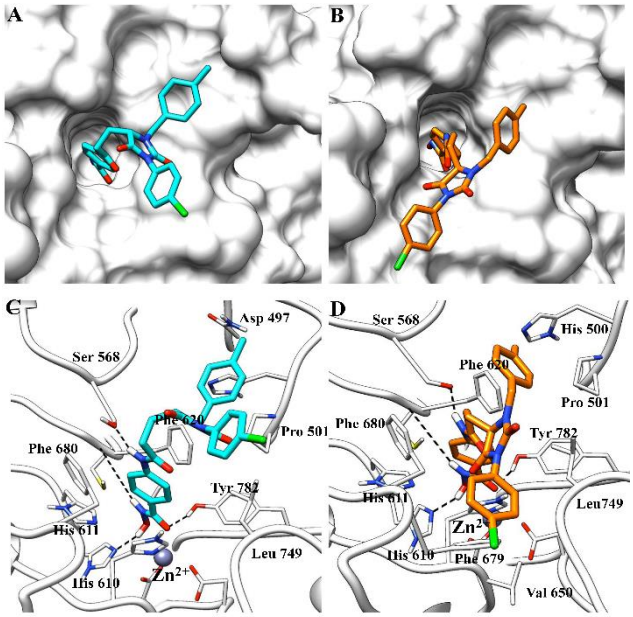


Figure 15. (A) Proposed binding mode of (*S*)-10c (depicted in cyan) in the active site of HDAC6. (B) Proposed binding mode of (*R*)-10c (depicted in orange) in the active site of HDAC6. (C) The interactions between (*S*)-10c and HDAC6 residues (gray). (D) The interactions between (*R*)-10c and HDAC6 residues (gray). Docking study was derived by Surflex-dock in Tripos/Sybyl 2.1. Hydrogen bonds were shown as black dotted line and the zinc ion was shown as a gray sphere

13. References

1 Reddy, A. S.; Kumar, M. S.; Reddy, G. R. A convenient method for the preparation of hydroxamic acids. *Tetrahedron Lett.* **2000**, 41, 6285-6288.

2 Chen, C.; Hou, X.; Wang, G.; Pan, W.; Yang, X.; Zhang, Y.; Fang, H. Design, synthesis and biological evaluation of quino-line derivatives as HDAC class I inhibitors. *Eur J Med Chem*. **2017**, 133, 11-23

3 Jesper, S.; Villadsen, H. M.; Stephansen, A. R.; Maolanon, P. H.; and Christian A. O.; Total Synthesis and Full Histone Deacetylase Inhibitory Profiling of Azumamides A−E as Well asβ 2 -epi-Azumamide E and β3 -epi-Azumamide E. *J. Med. Chem*. **2013**, 56, 6512−6520.