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Cap-Modified Hydroxamate Analogues as Histone Deacetylases Inhibitors and Antitumor Agents

Qing-Wei Zhang, Juan Feng,† and Jian-Qi Li*

*Novel Technology Center of Pharmaceutical Chemistry, Shanghai Institute of Pharmaceutical Industry, Shanghai 200437, P.R. China. *E-mail: lijq@sipi.com.cn*
†College of Chemical & Pharmaceutical Engineering, Hebei University of Science and Technology, Shijiazhuang 050018, P.R. China
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Two series of SAHA-liked hydroxamate analogues were designed, synthesized and evaluated for their biological activities against nuclear HDACs. Compounds of Series I were found to be very effective inhibitors of cancer cell growth in the PC-3, Hut78, K562 and Jurkat E6-1 cancer cell lines with mean IC_{50} values from 0.54 μM (**Ic**, Jurkat E6-1) to 7.73 μM (**Ib**, K562), indicating that they are cell permeable and the benzimidazolyl-based ligands are flexible enough to occupy the binding site of HDAC.

Key Words : Histone deacetylases, SAHA, Molecular docking, Antitumor activity

Introduction

Histone modifications alter the chromatin remodeling, with acetylation giving the inactive chromatin and DNA transcription repression. The reversible acetylation of lysine residues in histone tails is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs).[1,2] Mammalian HDACs consist of four classes, according to their sub-cellular distribution, sequence homology and catalytic activity. Class I (HDAC1, -2, -3, -8), class II (HDAC4, -5, -6, -7, -9, -10), and class IV (HDAC11) enzymes are zinc-dependent HDACs, whereas class III (Sirtuins 1-7) enzymes require NAD^+ for activity. Class II enzymes are subdivided into class II_a (HDAC4, -5, -7, -9) and class II_b (HDAC6, -10).[3-6] Studies using knockout and transgenic mice, as well as RNA interference (RNAi) have revealed that overexpression of HDACs has been observed in certain cancers, and treatment of tumor cells with HDAC inhibitors (HDACi) results in growth arrest, differentiation and apoptosis, promoting these enzymes as promising targets for anticancer therapy.[7-11]

A number of natural products including Trichostatin A (TSA), Romidepsin (FK228), as well as synthetic small molecules such as Vorinostat (SAHA), Entinostat (MS-275) and Scriptaid (Fig. 1) have been identified as potent HDACi and demonstrated antitumor activity. Among these, SAHA and FK228 have recently been approved by the FDA for treating cutaneous T-cell lymphoma (CTCL).[12,13]

The X-ray crystal structures of a bacterial HDAC-like protein (HDLP) bound to TSA and SAHA have revealed structural details of the active site of these zinc-dependent HDACs and the binding interactions with small molecule inhibitors. The active site consists of a deep pocket spanning the length equivalent to a four- to six-carbon linear chain. A zinc ion bound at the bottom of the pocket cooperates with two His-Asp charge-relay systems and facilitates the deacetylation catalysis.[14,15] The chemical structures of HDACi reported so far include a zinc-binding motif, a hydrophobic cavity-binding linker, and a surface recognition cap which can produce specific interactions with external surface of the protein, leading to enhancing HDACi activity.[16-19]

As part of our efforts to discover novel HDACi and further

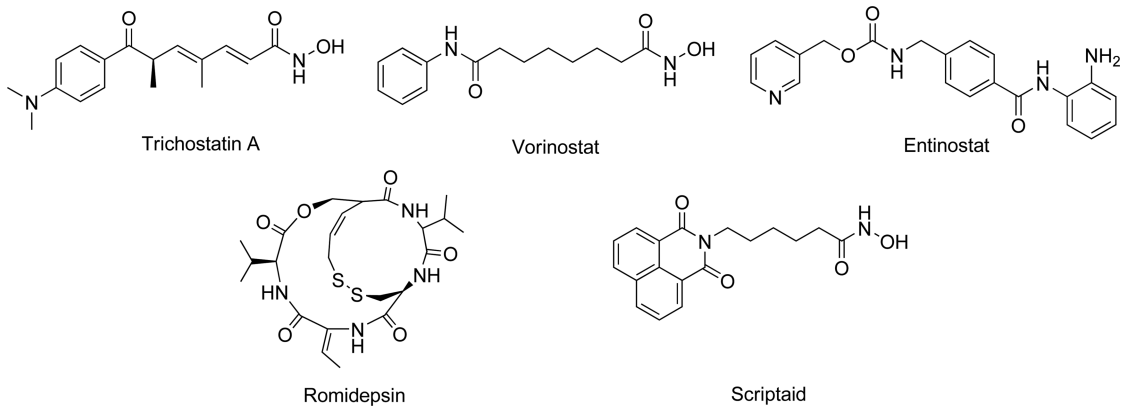


Figure 1. Known HDAC inhibitors.

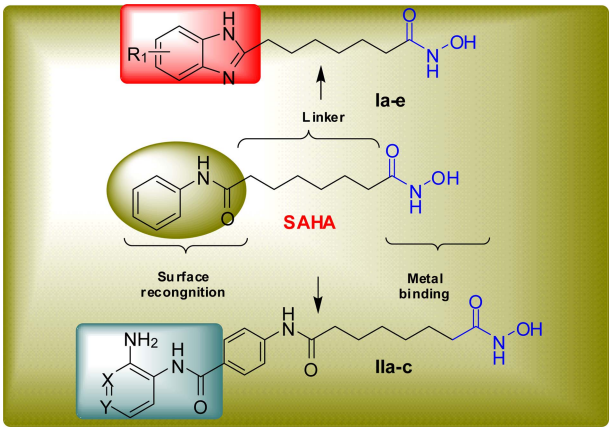
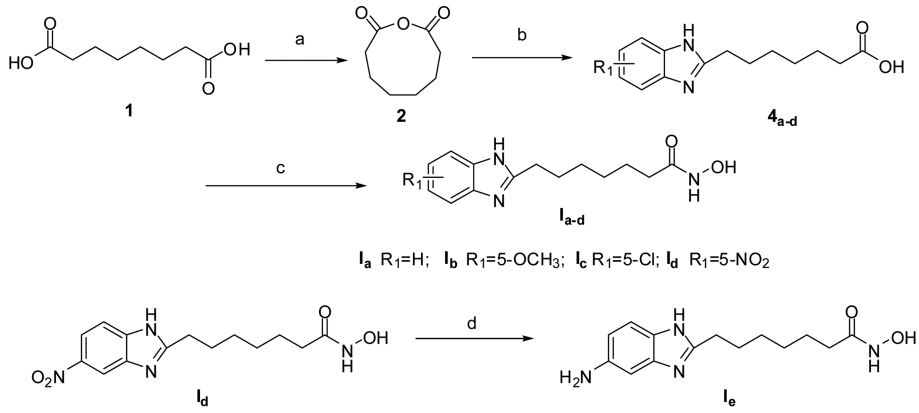


Figure 2. Functional domains of novel HDACi.

enhance our understanding of the roles of specific interactions between the enzyme outer rim and inhibitor cap groups in HDAC inhibitory activity, we designed a series of potent SAHA-liked cap-modified hydroxamate analogues (Fig. 2). Specifically, while maintaining hydroxamic acid metal binding head and the hexamethylene linker domain from SAHA, we replaced the aryl connection unit with benzimidazolyl homologs,[20] 4-((2-aminophenyl)carbamoyl)phenyl, 4-((2-aminopyridin-3-yl)carbamoyl)phenyl, or 4-((3-aminopyridin-4-yl)carbamoyl)phenyl ring, which incorporate the benzamide moiety of MS-275 and also possess HDAC inhibitory activity *in vitro*. Herein we described the synthesis and biological characterization of these novel classes of compounds with HDACs inhibitory activity.

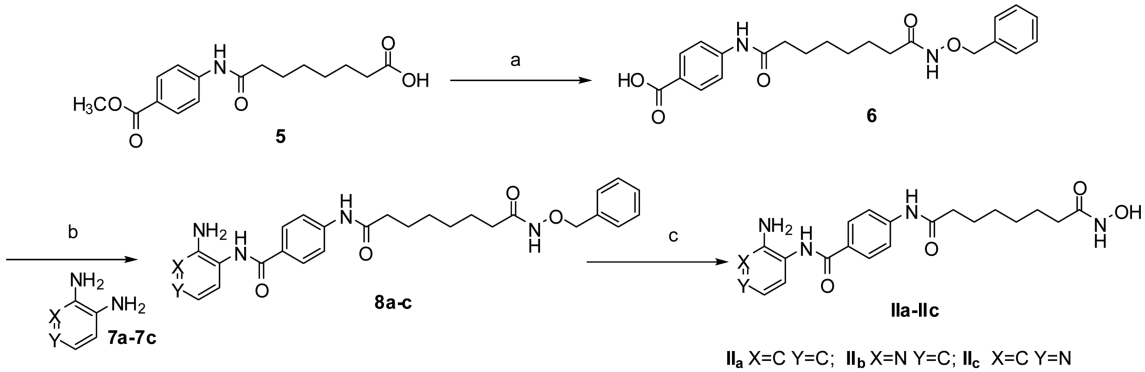
Results and Discussion

Chemistry. An efficient synthesis of a wide range of benzimidazole-based hydroxamic acids (**Ia-Ie**) was developed. Scheme 1 illustrates the general procedure used for preparing hydroxamic acids starting from suberic acid in a 3-step reaction sequence. In general, the suberic acid **1** reacted with acetic anhydride to form suberic α-anhydride **2** at high temperature. Compound **4a-d** were prepared by coupling reaction between compound **2** and appropriate *o*-phenylendiamine substitutions **3**. Treating **4a-d** with ethyl chloro-



Reagents and conditions:
a) acetic anhydride, reflux, 1 h; b) apprppriate *o*-phenylendiamine, 120 °C, 3 h; c) 1. $ClCO_2Et$, Et_3N, THF, rt, 1 h; 2. 2 equiv NH_2OH, KOH, MeOH, rt, 3 h; d) H_2, 10% Pd/C, MeOH, rt, 3.5 h

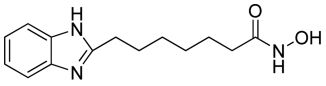
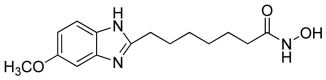
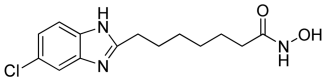
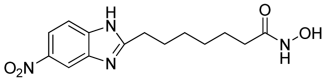
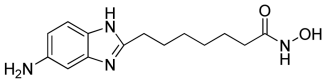
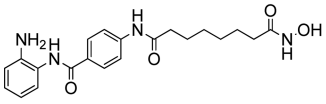
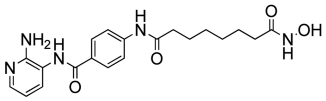
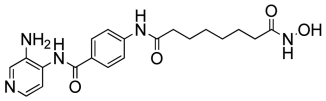
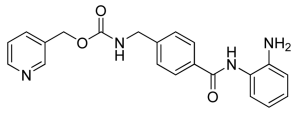
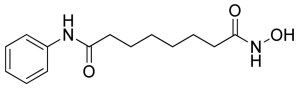
Scheme 1. Synthetic route of **Ia-Ie**.



Reagents and conditions:
a) 1. $BnONH_2$, BOP, Et_3N, rt, 24 h 2. LiOH, MeOH, H_2O, 20 °C, 16 h; b) DMF, Et_3N, BOP, rt, 20 h;
c) H_2, 5% Pd/C, MeOH, rt, 2 h

Scheme 2. Synthetic route of **Ia-Ic**.

Table 1. The structures and inhibitory activities of compounds **Ia-Ie** and **IIa-c** against HDACs

Compd	Structure	Inhibition ratio towards HDACs *in vitro*/%[a]		
		31.25 μmol·L^{-1}	1.95 μmol·L^{-1}	0.49 μmol·L^{-1}
Ia		100.10	96.41	87.92
Ib		99.94	97.36	89.77
Ic		99.96	98.88	94.39
Id		100.22	97.32	90.72
Ie		97.72	95.07	88.44
IIa		97.53	79.83	54.44
IIb		96.97	80.86	57.75
IIc		96.96	76.44	49.45
MS-275		53.98	25.69	8.51
SAHA		94.86	59.32	28.62

[a]Values are means of three experiments.

formate and subsequently hydroxylamine gave the desired hydroxamates **Ia-d** without isolation of intermediates. The target compound **Ie** was obtained via hydrogenolysis with Pd/C as catalyst. As shown in Scheme 2, compound **5** was converted to the benzamide-containing hydroxamic acids **IIa-c** by multiple steps of benzyl protection, hydrolysis, coupling reaction and deprotection by hydrogenolysis. The final compounds were characterized by ^{1}H-NMR, mass spectroscopy and elemental analysis, which were detailed in the experimental section.

***In Vitro* HDACs Inhibition.** Based on the fact that all zinc-dependent HDACs were highly conserved in their active sites and the crude HeLa cell nuclear extract HDACs is readily available in our group, we used HDACs as the enzyme source to screen our target compounds, with MS-275 and SAHA as positive controls. The results were listed in Table 1.

The results in Table 1 demonstrated that most compounds were more potent than the positive controls MS-275 and SAHA against HeLa cell nuclear extract HDACs at concentrations of 31.25, 1.95 and 0.49 μM. Furthermore, compounds of Series I are more potent than those of Series II. Additional structure-activity relationship (SAR) analysis revealed that the introduction of benzimidazolyl cap groups could greatly increase compounds' inhibitory activities against HDACs.

In order to gain insights into the possible interactions that improved activities, we utilized Discovery Studio 3.1 (DS. 3.1) software and docked compound **Ia** to the binding site of HDLP (histone deacetylase-like protein, the bacterial homologue of human class I HDACs) crystal structures available from Protein Data Bank (PDB entry: 1C3S).

The analysis of predicted binding modes from the docking experiments suggested that in HDLP isoform (Fig. 3), when the zinc ion was coordinated by hydroxamic acid group and the tunnel of the active site was occupied by carbon chain, the malleable protein surface at the rim of the active site could adjust itself to accommodate complex structures, such as benzimidazolyl cap groups. Moreover, we could presume

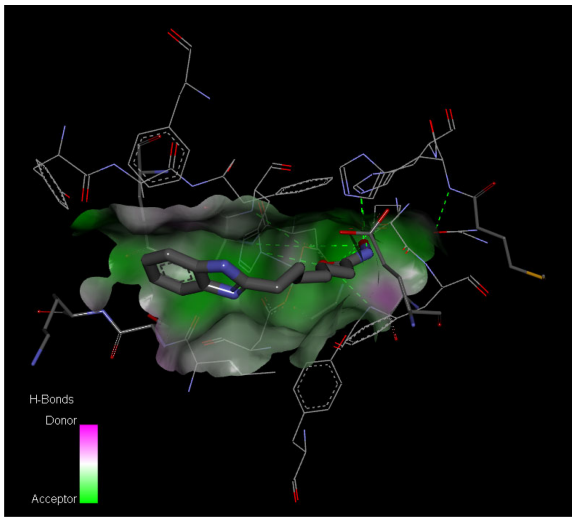


Figure 3. 3D model of the interaction between compound **Ia** and HDLP binding site.

that unlike benzamide-based ligands the benzimidazolyl-based ligands were oriented in the correct direction to enable favor interactions with the residues on the entrance of narrow lipophilic pocket. This guided the recognition of Series **I** compounds contributed to the improved biological activity. However, due to the remarkable malleability and complexity of the HDACs isoform surface, we would not further discuss the docking results here as they may not represent the true binding mode.

Cell Proliferation Study. Encouraged by their potent anti-HDAC profiles, all of the compounds were progressed to *in vitro* antiproliferative activity assay against a panel of human cancer cells: prostate carcinoma (PC-3), breast carcinoma (MDA-MB-435S) and leukemia (Hut78, K562 and Jurkat E6-1). As shown in Table 2, the Series **I** compounds **Ia-e** demonstrated good in vitro antiproliferative potency against PC-3 cells, Hut78 cells, K562 cells and Jurkat E6-1 cells with low IC_{50} values, ranging from 0.54 μM (**Ic**, Jurkat E6-1) to 7.73 μM (**Ib**, K562), with significant selectivity over MDA-MB-435S (IC_{50} > 30 μM) cancer cell lines compared with MS-275 and SAHA. Interestingly, even though the HDACs inhibitory activities of Series **II** compounds were all superior to SAHA, they showed poor cancer antiproliferative activities. Based on the results of anti-proliferative capacity, we could presume compounds **II** exhibiting two zink binding groups were unable to permeate plasmalemma and nuclear membrane to inhibit HDACs, which probably mainly enable interactions with other intracellular metalloproteinase.

Table 2. The antiproliferative activities of compounds

Compd	IC_{50} (μM)[a]				
	PC-3	MDA-MB-435S	Hut78	K562	Jurkat E6-1
Ia	2.73	32.94	1.32	4.99	0.80
Ib	3.50	>100	3.77	7.73	1.14
Ic	1.89	>100	1.94	7.47	0.54
Id	6.60	>100	5.99	2.81	5.26
Ie	4.88	>100	4.53	3.49	4.30
IIa	21.42	46.26	74.70	>100	>100
IIb	34.25	>100	>100	>100	>100
IIc	21.06	>100	>100	>100	>100
MS-275	0.50	19.80	5.28	4.66	0.56
SAHA	0.93	26.31	6.52	6.09	0.50

[a]IC_{50} values are reported as the average of at least two separate determinations.

In summary, two novel series of SAHA-liked hydroxamate analogues with modified cap groups were synthesized as HDAC inhibitors. All synthesized compounds showed SAHA-comparable activity towards HDACs and superior anti-HDAC potency. Molecular docking experiment indicates that the surface-recognition domain of the benzamide-based ligands could adjust itself well enough to accommodate complex structures. In contrast to Series **II** compounds, all Series **I** compounds displayed a broad cytotoxicity against PC-3 cells, Hut78 cells, K562 cells and Jurkat E6-1 cells with mean IC_{50} values from 0.54 μM (**Ic**, Jurkat E6-1) to 7.73 μM (**Ib**, K562). Furthermore, detailed experiments, such as SAR studies, *in vitro* and *in vivo* evaluation of these compounds on antitumor activity are underway.

Experimental Section

Melting points were determined on a Yanaco MP-S3 micro-melting point apparatus and were not corrected. Mass spectra were recorded on a Finnign-MAT 212 spectrometer. ^{1}H-NMR spectra were recorded on a Varian INOVA 400 (400 MHz) spectrometers with tetramethylsilane as an internal standard. Elemental analyses were obtained with a Carlo Erba EA 1108 instrument. All the solvents were purified before use by routine techniques. All the reactions described below were monitored by thin layer chromatography (TLC).

General Synthesis of Corresponding Benzimidazole-containing Heptanoic Acid 4a-d from Appropriate *o*-phenylendiamine 3. A solution of suberic acid (5.00 g, 28.70 mmol) in acetic anhydride (10 mL) was heated under reflux for 1 h. After cooling to rt, the solvent was removed *in vacuo*. The crude yellow oil was used without any further purification for the next step. Appropriate *o*-phenylendiamine **3** (28.70 mmol) was added to the produced anhydride. After stirring at 120 °C for 30 minutes, the reaction mixture was diluted with water until a colorless solid precipitated, which was collected by filtration. Recrystallisation from water/acetonitrile gave the pure compound **4a-d** as a colorless solid.

Preparation of 7-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*-hydroxyheptanamide (Ia). To a solution of NH_2OH-HCl (0.14 g, 2.00 mmol) in MeOH (10 mL) was added potassium hydroxide (0.12 g, 2.00 mmol). The reaction mixture was stirred in an ice-bath for 1 h. Filtration to remove the white salt gave a solution of the free NH_2OH in MeOH. A solution of **4a** (0.25 g, 1 mmol) in THF (10 mL) was treated with ethyl chloroformate (0.14 mL, 1.50 mmol) and Et_3N (0.21 mL, 1.50 mmol) and the resulting solution was stirred at rt for 1

h. The prepared free NH_2OH solution was then added to the reaction. Stirring was continued at rt for 3 h. The reaction was concentrated *in vacuo* and the crude was diluted with distd H_2O (50 mL), acidified to pH 2-3 with 1N HCl and extracted with EtOAc (25 mL × 3). The combined organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo*. The residue was recrystallized with acetonitrile to give **Ia** (0.19 g, 72%) as a white solid: mp 173-175 °C. ESI-MS *m/z* 262.1553 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.26-1.28 (m, 4H), 1.45-1.48 (m, 2H), 1.71-1.79 (m, 2H), 1.92-1.96 (m, 2H), 2.95-2.96 (m, 2H), 7.19 (s, 1H), 7.37-7.40 (m, 2H), 7.51 (s, 1H), 7.64-7.68 (m, 2H), 10.30 (s, 1H). Anal. calcd. for $C_{14}H_{19}N_3O_2$: C, 64.35; H, 7.33. found: C, 64.32; H, 7.31.

Preparation of *N*-Hydroxy-7-(5-methoxy-1*H*-benzo[*d*]-imidazol-2-yl)heptanamide (Ib). Compound **Ib** was synthesized by a procedure similar to that described for **Ia**: yield 65%: mp 159-161 °C. ESI-MS *m/z* 292.1680 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.28-1.29 (m, 4H), 1.45-1.49 (m, 2H), 1.76-1.79 (m, 2H), 1.92-1.96 (m, 2H), 2.50-2.72 (m, 2H), 3.76 (s, 3H), 7.10-7.13 (m, 2H), 7.19 (s, 1H), 7.59-7.62 (m, 2H), 10.32 (s, 1H). Anal. calcd. for $C_{15}H_{21}N_3O_3$: C, 61.84; H, 7.27. found: C, 61.82; H, 7.30.

Preparation of 7-(5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-*N*-hydroxyheptanamide (Ic). Compound **Ic** was synthesized by a procedure similar to that described for **Ia**: yield 71%: mp 174-176 °C. ESI-MS *m/z* 296.1166 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.27-1.29 (m, 4H), 1.44-1.47 (m, 2H), 1.69-1.73 (m, 2H), 1.91-1.95 (m, 2H), 2.76-2.80 (m, 2H), 7.12-7.15 (m, 2H), 7.44-7.50 (m, 2H), 8.57 (s, 1H), 10.27 (s, 1H). Anal. calcd. for $C_{14}H_{18}ClN_3O_2$: C, 56.85; H, 6.13. found: C, 56.84; H, 6.10.

Preparation of *N*-Hydroxy-7-(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)heptanamide (Id). Compound **Id** was synthesized by a procedure similar to that described for **Ia**: yield 68%: mp 167-169 °C. ESI-MS *m/z* 307.1405 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.27-1.30 (m, 4H), 1.44-1.48 (m, 2H), 1.72-1.76 (m, 2H), 1.92-1.96 (m, 2H), 2.83-2.87 (m, 2H), 7.64 (s, 1H), 8.06 (d, 1H, *J* = 8.8 Hz), 8.37 (s, 1H), 8.58 (s, 1H), 10.28 (s, 1H), 12.85 (s, 1H). Anal. calcd. for $C_{14}H_{18}N_4O_4$: C, 54.89; H, 5.92. found: C, 54.84; H, 5.91.

Preparation of 7-(5-Amino-1*H*-benzo[*d*]imidazol-2-yl)-*N*-hydroxyheptanamide (Ie). A suspension of compound **Id** (0.052 g, 0.17 mmol) and Pd/C (10 wt %, 20 mg) in MeOH (20 mL) was stirred under hydrogen atmosphere at room temperature for 3.5 h. The catalyst was removed by filtration through a pad of Celite and washed thoroughly with MeOH. The solvent was evaporated. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 2:1) to give compound **Ie** (0.040g, 87%). mp 171-173 °C. ESI-MS *m/z* 275.1885 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.33-1.39 (m, 4H), 1.48-1.55 (m, 2H), 1.83-1.90 (m, 2H), 2.18-2.20 (m, 2H), 2.79 (s, 1H), 3.02-3.18 (m, 4H), 7.33 (dd, 1H, *J* = 2.0, 8.4 Hz), 7.41 (d, 1H, *J* = 4.4 Hz), 7.60 (s, 1H), 7.74 (d, 1H, *J* = 8.4 Hz), 10.31 (s, 1H). Anal. calcd. for $C_{14}H_{20}N_4O_2$: C, 60.85; H, 7.30. found: C, 60.87; H, 7.31.

General Synthesis of Protected N^8-(Benzyloxy)octanediamide 8a-c From 8-((4-(Methoxycarbonyl)phenyl)amino)-8-oxooctanoic Acid 5. To a mixture of 8-((4-(methoxycarbonyl)phenyl)amino)-8-oxooctanoic acid **5** (0.31 g, 1.00 mmol), BOP (benzotriazolyloxy-tris-(dimethylamino)phosphoniumhexafluorophosphate) (0.49 g, 1.10 mmol) and Et_3N (0.28 mL, 2.00 mmol) in DMF (10 mL) was added reagent $BnONH_2$ (*O*-Benzylhydroxylamine) (0.15 g, 1.20 mmol) at rt. After stirring at rt for 24 h, the mixture was poured into water under stirring, and the precipitating intermidate (0.30 g, 72%) was removed by filtration, dried *in vacuo* and used in the next step without further purification. The crude benzyl benzoate analog (0.21 g, 0.50 mmol) was dissolved in MeOH (10 mL), and LiOH (0.05 g, 2.00 mmol) in H_2O (2 mL) was added. After stirring at rt overnight, MeOH was removed under reduced pressure, and the aqueous layer was extracted with ethyl acetate (3 × 10 mL), cooled to 0 °C, and acidified with diluted acetic acid to pH = 5-6. The precipitating product **6** (0.18 g, 88%)was removed by filtration as white solid and dried *in vacuo* without further purification. The benzyl acid analog **6** (0.16 g, 0.40 mmol) was dissolved in dry DMF (5 mL), and BOP (0.20 g, 0.44 mmol), Et_3N (0.12 mL,0.80 mmol), and the respective *o*-phenylendiamine (0.44 mmol) were added. After stirring at room temperature for 20 h, the mixture was poured into water (20 mL) under stirring, and the precipitating product **8a-c** were removed by filtration, dried *in vacuo*, and purified by column chromatography (DCM/MeOH = 50/1-10/1).

Preparation of N^1-(4-((2-Aminophenyl)carbamoyl)-phenyl)-N^8-hydroxyoctanediamide (IIa). A suspension of compound **8a** (0.083 g, 0.17 mmol) and Pd/C (5 wt %, 0.020 g) in MeOH (20 mL) was stirred under hydrogen atmosphere at room temperature for 2 h. The catalyst was removed by filtration through a pad of Celite and washed thoroughly with MeOH. The solvent was evaporated. The residue was purified by column chromatography on silica gel (EtOAc/MeOH = 5:1) to give compound **IIa** (0.054 g, 79%): mp > 250 °C. ESI-MS *m/z* 399.3090 $[M+H]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.22-1.29 (m, 4H), 1.47-1.50 (m, 2H), 1.56-1.60 (m, 2H), 1.93-1.96 (m, 2H), 2.30-2.34 (m, 3H), 4.81 (s, 2H), 6.58-6.65 (m, 1H), 6.77 (d, 1H, *J* = 8.0 Hz), 6.94-6.98 (m, 1H), 3.87 (s, 1H), 7.16 (d, 1H, *J* = 8.0 Hz), 7.70 (d, 2H, *J* = 8.4 Hz), 7.92 (d, 2H, *J* = 8.4 Hz), 9.45 (s, 1H), 10.13 (s, 1H). Anal. calcd. for $C_{21}H_{26}N_4O_4$: C, 63.30; H, 6.58. found: C, 63.27; H, 6.57.

Preparation of N^1-(4-((2-Aminopyridin-3-yl)carbamoyl)phenyl)-N^8-hydroxyoctanediamide (IIb). Compound **IIb** was synthesized by a procedure similar to that described for **IIa**: yield 70%. mp 219-220 °C. ESI-MS *m/z* 400.1983 $[M+H]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.22-1.28 (m, 4H), 1.46-1.57 (m, 4H), 1.93-1.98 (m, 1H), 2.30-2.37 (m, 3H), 5.01 (s, 2H), 7.42 (d, 2H, *J* = 5.2 Hz), 7.72 (d, 2H, *J* = 8.4 Hz), 7.80 (d, 1H, *J* = 4.8 Hz), 7.92 (d, 2H, *J* = 8.4 Hz), 8.09 (m, 2H), 9.58 (s, 1H), 10.13 (s, 1H). Anal. calcd. for $C_{20}H_{25}N_5O_4$: C, 60.14; H, 6.31. found: C, 60.17; H, 6.32.

Preparation of N^1-(4-((3-Aminopyridin-4-yl)carbamoyl)phenyl)-N^8-hydroxyoctanediamide (IIc). Compound **IIc** was synthesized by a procedure similar to that described

for **IIa**: yield 70%. mp 214-216 °C. ESI-MS *m/z* 400.1987 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.23-1.30 (m, 4H), 1.49-1.62 (m, 4H), 1.93-1.97 (m, 2H), 2.32-2.36 (m, 2H), 5.68 (s, 2H), 6.62 (dd, 1H, *J* = 4.8, 7.6 Hz), 7.55 (d, 2H, *J* = 7.6 Hz), 7.70 (d, 2H, *J* = 8.8 Hz), 7.85 (d, 1H, *J* = 4.8 Hz), 7.93 (d, 2H, *J* = 8.8 Hz), 9.48 (s, 1H), 10.07 (s, 1H), 10.27 (s, 1H). Anal. calcd. for $C_{20}H_{25}N_5O_4$: C, 60.14; H, 6.31. found: C, 60.15; H, 6.31.

***In Vitro* HDAC Inhibition.** *In vitro* HDAC inhibition was assayed using the HDAC Fluorimetric Assay/Drug Discovery Kit as previously described.[21] Briefly, 15 μL of HeLa nuclear extract was mixed with three concentrations of compound sample and 5 μL of assay buffer. Fluorogenic substrate (25 μL) was added, and reaction was allowed to proceed for 15 min at room temperature and then stopped by addition of a developer containing TSA. Fluorescence was monitored after 15 min at excitation and emission wavelengths of 360 and 460 nm, respectively. The % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells.

***In Vitro* Antiproliferative Assay.** *In vitro* antiproliferative assays were determined by the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) method as described.[22] Briefly, prostate carcinoma (PC-3), breast carcinoma (MDA-MB-435S) and leukemia (Hut78, K562 and Jurkat E6-1) cells were respectively maintained in RPMI1640 medium containing 10% FBS at 37 °C in 5% CO_2 humidified incubator. Before dosing into a 96-well cell plate, cells were allowed to grow for a minimum of 4 h prior to addition of compounds. After compounds addition, the plates were incubated for an additional 48 h, and then 0.5% MTT solution was added to each well. After further incubation for 4 h, formazan formed from MTT was extracted by adding 200 mL of DMSO for 15 min. Absorbance was then determined using an ELISA reader at 570 nm and the IC_{50} values were calculated according to the inhibition ratios.

Molecular Docking. The HDLP-SAHA protein-ligand complex crystal structure (PDB ID: 1C3S) was chosen as the template to compare the docking mode between compound **Ia** bound to HDLP. The molecular docking procedure was performed by using LigandFit protocol within Discovery Studio 3.1. For ligand preparation, the 3D structures of compound **Ia** were generated and minimized using Discovery Studio 3.1. For enzyme preparation, the hydrogen atoms were added, and the water and impurities were removed. The whole HDLP enzyme was defined as a receptor and the site sphere was selected based on the ligand binding location of SAHA, then the SAHA molecule was removed and compound **Ia** was placed during the molecular docking procedure. Types of interactions of the docked enzyme with ligand were analyzed after end of molecular docking. Ten docking poses were saved for each ligand and the final docked conformation was scored.

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