

Synthesis and Importance of Bulky Aromatic Cap of Novel SAHA Analogs for HDAC Inhibition and Anticancer Activity

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On the basis of potent HDAC-inhibitory activity and anticancer activity of SAHA, novel SAHA derivatives **3a-d** and **7** with a bulky cap such as *p*-dimethylaminophenyl, 4-phenylaminophenyl, 4-phenyloxyphenyl, 9*H*-fluorenyl or naphthalenyl ring were synthesized starting from the corresponding aryl amines or naphthalenyl acetic acid using an EDC-mediated amide coupling reaction in the presence of HOBt followed by a nucleophilic addition-elimination reaction with hydroxylamine. Compounds **3b**, **3c** and **3d** showed more potent inhibitory activity on total HDACs (14~27-fold), HDAC1 (8~15-fold), HDAC2 (1.3~25-fold) and HDAC7 (1~3-fold) and more potent anticancer activity (2~22-fold) against MCF-7, MDA-MB-231, MCF-7/Dox, MCF-7/Tam, SK-OV-3, LNCaP and PC3 human cancer cell lines than SAHA.

Key Words : HDAC inhibition, Anticancer activity, SAHA, Bulky cap

Introduction

Nucleosomes are made up of DNA wrapped around an octamer of histones. Post-transcriptional modifications of the positively charged *N*-terminal regions of the histones, including acetylation, methylation and phosphorylation, change the chromatin structure and regulate gene transcriptions.^{1,2} Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are involved in epigenetic regulation of genes through modifications of histone tails of H3 and H4.³ HDAC enzymes have been noticed as a very promising target for anticancer agents, due to having ability for an epigenetic modification. Recent studies have shown that inhibition of HDACs silences the proliferation of tumor cells by inducing cell-cycle arrest, terminal differentiation, and/or apoptosis.^{4,5}

The HDACs can be divided into two families: (1) the Zn²⁺ dependent HDAC family composed of class I (HDACs 1, 2, 3 and 8), class II a/b (HDACs 4, 5, 6, 7, 9 and 10), and class IV (HDAC 11) and (2) Zn²⁺ independent NAD⁺-dependent class III SIRT enzymes.⁶ The class I HDACs, the true histone deacetylases are localized in the nucleus of cells. The class II a/b deacetylases have both histone and non-histone proteins as substrates and are primarily localized in the cytoplasm.

Many synthetic and naturally occurring HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza[®]),⁷ apicidin,⁸ trichostatin A,⁹ psammaplin A¹⁰ and trapoxin B¹¹ have been reported (Fig. 1). Hydroxamic acid-type HDAC inhibitors including SAHA reduced the tumor burden of breast, melanoma, prostate, ovarian, colon, lung and cervical cancers.^{12,13} SAHA is a class I/II synthetic

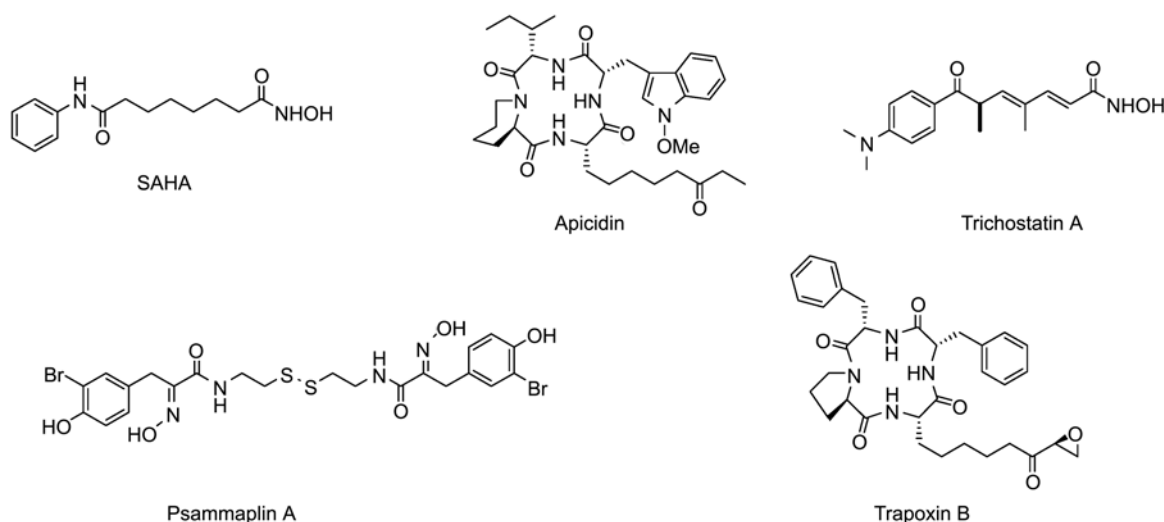


Figure 1. Synthetic and naturally occurring HDAC inhibitors.

inhibitor, and the hydroxamic acid is the crucial moiety to obtain high inhibitory activity, since modifications or substitutions at this site reduce their anti-HDAC effect.¹⁴

SAHA reacts with and blocks the catalytic site of HDAC enzymes. SAHA can cause growth arrest and death of a broad variety of transformed cells both *in vitro* and in tumor-bearing animals at concentrations not toxic to normal cells.^{12,15,16} SAHA has many protein targets whose structure and function are altered by acetylation, including chromatin-associated histones, nonhistone gene transcription factors and proteins involved in regulation of cell proliferation, migration and death. A new drug application was approved by the US Food and Drug Administration for SAHA for treatment of cutaneous T-cell lymphoma.

The common pharmacophore of the inhibitors for class I/II HDAC enzymes is composed of three parts: (1) a hydrophobic region that binds a cap moiety of inhibitors, (2) an enzyme inhibition region chelating with a zinc cation near the bottom of the catalytic pocket, and (3) a linker region connecting the hydrophobic region with the enzyme inhibition region.¹⁷

According to SAR studies previously reported, HDAC inhibitors with a four- to six-atom linker and the functionality of hydroxamic acid that can bind the enzyme inhibition region showed very good activity.¹⁷ Thus, we investigated the cap effect of HDAC inhibitors on the inhibitory activities against HDACs and several human cancer cell lines, without any structural changes of the hexamethylene linker and the functionality of hydroxamic acid of SAHA. In addition, the effect of transposed amide functionality was examined on the inhibitory activities against HDACs and anticancer activity.

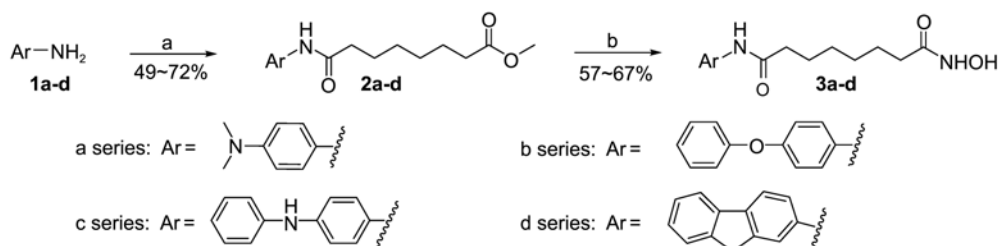
As part of our continuous efforts searching for novel potent HDAC inhibitors, it was interesting to synthesize

SAHA analogues with a bulky cap such as 4-phenoxyphenyl, 4-dimethylaminophenyl, and 4-phenylaminophenyl rings, instead of the phenyl ring of SAHA, and a transposed amide analog with a naphthalenyl ring at the cap moiety. Herein, we wish to report the synthesis of novel SAHA derivatives possessing a bulky cap, and their inhibitory activities against HDAC enzymes and several human cancer cell lines.

Results and Discussion

Chemistry. Common structural feature of all the final compounds **3a-c** (Scheme 1) is that they bear a hexamethylene unit as their linkers and hydroxamic acid to bind to zinc cation existing at the active site of HDAC. A strategy for the synthesis of SAHA derivatives is outlined in Scheme 1. Reaction of suberic acid monomethyl ester with aromatic amines such as *N,N*-dimethylbenzene-1,4-diamine, 4-phenoxyaniline, and *N*-phenyl-*p*-phenylenediamine in the presence of EDC (*N*-ethyl-*N*-(3-dimethylaminopropyl)-carbodiimide) hydrochloride and HOBt (1-hydroxybenzotriazole)^{18,19} gave the corresponding amides, respectively, in 49-62% yields. Although the long reaction time of 5-10 days was needed, treatment of methyl esters **2a-c** with 50% hydroxylamine aqueous solution afforded SAHA analogs **3a-c** in 57-67% yields.

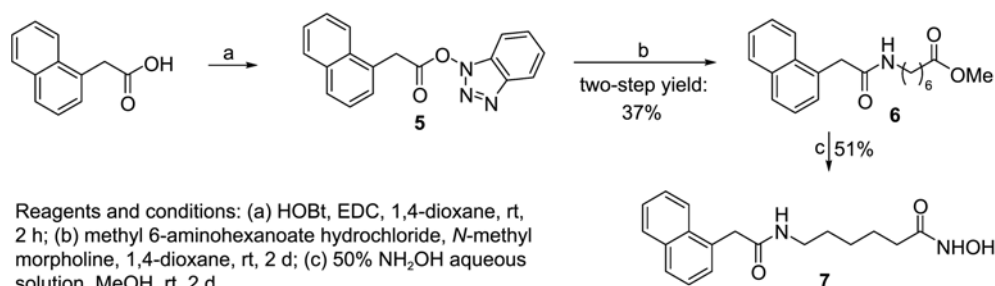
Compound **7** in which the amide functionality of the SAHA structure was transposed and in which the phenyl ring of SAHA was replaced by a naphthalenyl ring was synthesized to evaluate an effect of the reversed amide functionality on HDAC inhibition and anticancer activity (Scheme 2). For the synthesis of compound **7** with the reversed amide functionality, commercially available 1-



Reagents and conditions:

(a) suberic acid monomethyl ester, EDC, HOBt, CH₂Cl₂, rt, 2-4 d; (b) 50% NH₂OH aqueous solution, MeOH, rt, 5-10 d.

Scheme 1. Synthesis of the target compounds **3a-3d**.



Reagents and conditions: (a) HOBt, EDC, 1,4-dioxane, rt, 2 h; (b) methyl 6-aminohexanoate hydrochloride, *N*-methylmorpholine, 1,4-dioxane, rt, 2 d; (c) 50% NH₂OH aqueous solution, MeOH, rt, 2 d.

Scheme 2. Synthesis of the target compound **7**.

naphthaleneacetic acid was used. First, reaction of 1-naphthaleneacetic acid with HOBt in the presence of EDC gave 1-naphthaleneacetic acid-HOBt ester adduct **5**, which was coupled with methyl 6-aminohexanoate in the presence of *N*-methyl morpholine as a tertiary amine catalyst to give the reversed amide compound **6** in 37% yield from naphthaleneacetic acid. Treatment of compound **6** with 50% hydroxylamine aqueous solution converted methyl ester into hydroxamic acid to afford compound **7** in a moderate yield. It should be noted that the number of the linker atoms between naphthalenyl ring and hydroxamic acid in compound **7** is same as that between phenyl ring and hydroxamic acid in SAHA.

HDAC Enzymes Inhibition. The effects of the final compounds **3a-c** and **7** on total HDACs, HDAC1, HDAC2, HDAC3, HDAC4, and HDAC7 enzyme activity in nuclear protein isolated from the compound-treated SK-OV-3 cells were examined. As shown in Table 1, compounds **3b** and **3c** inhibited the total HDAC enzymes, HDAC1, HDAC2, and HDAC7 very potently and the IC₅₀ values were lower than those of SAHA used as a positive control. In case of HDAC3 and HDAC4, IC₅₀ values of **3b** and **3c** were similar to those of SAHA. In total HDACs and HDAC1, HDAC enzyme-inhibitory activity of compound **3a** was similar to or a little higher (2~6 fold times) than that of SAHA, but in the other HDACs examined, the inhibitory activities were similar to or lower than that of SAHA. A common characteristic of **3b** and **3c** is to markedly inhibit both HDAC1 and HDAC7 enzymes as well as the total HDAC enzymes, compared with SAHA. Compounds **3b** and **3c** inhibited the total HDACs activity in more than 13-fold and 17-fold, respectively and HDAC1 activity in more than 7-fold and 10-fold, respec-

tively, compared with SAHA. However, compound **7** with reversed amide functionality exhibited lower inhibitory activity than SAHA against HDACs examined. Our findings show that **3b** and **3c** are much more significant HDACs inhibitors than SAHA on both total HDACs enzymes and each HDACs enzyme. The superior inhibition to SAHA might result from the bulkiness of their cap moieties. Therefore, compound **3d** with a fluorenyl group as a bulky cap was synthesized and its HDACs inhibitions were examined. As expected, **3d** exhibited the most potent inhibitory effect on total HDAC enzymes, HDAC1 and HDAC2 (27-, 15- and 25-fold potent, respectively, compared to SAHA) and a similar inhibitory effect on HDAC3 to compounds **3b** and **3c**. These results imply that the cap size is very closely related to HDAC inhibitory activity.

Anticancer Activity. The therapeutic efficacy of HDAC inhibitors was tested in a variety of human cancer cell lines including breast cancer cell lines (MCF-7, MDA-MB-231, MCF-7/Dox, and MCF-7/Tam), ovary cancer cell line (SK-OV-3) and prostate cancer cell lines (LNCaP, DU145, and PC3). The antiproliferative effect of the final compounds **3a-d** and **7** on various cancer cell lines was determined by MTT assay and the results are shown in Table 2. Compound **3d** exhibited the most potent inhibitory activity against all cancer cell lines, except MCF-7/Tam cancer cell lines and its IC₅₀ values indicated sub-micromolar concentrations (0.30-0.76 μM), except MCF-7/Tam (2.11 μM) cancer cell lines. Compound **3c** showed the next potent inhibitory activity against cancer cell lines, and its IC₅₀ values indicated sub-micromolar concentrations (0.35-0.92 μM), except MCF-7/Tam (1.51 μM), and DU145 (2.73 μM) cancer cell lines. Compound **3b** also showed potent anticancer activities on

Table 1. Inhibition of HDAC activity by SAHA and compounds **3a-3d** and **7**

HDAC inhibitors	Compounds, IC ₅₀ (μM)					
	SAHA	3a	3b	3c	3d	7
Total HDAC (HeLa nuclear extract)	5.882	2.903	0.428	0.336	0.214	10.145
HDAC1	1.517	0.272	0.192	0.147	0.099	7.099
HDAC2	2.574	2.457	1.987	2.015	0.105	5.784
HDAC3	1.965	7.093	2.054	2.453	2.547	8.025
HDAC4	10.311	31.570	16.781	12.228	41.086	53.487
HDAC7	24.729	26.350	7.839	9.673	25.501	67.630

Table 2. Effect of SAHA and compounds **3a-3d** and **7** on cytotoxicity of human cancer cell lines

Cell lines	Origin	Compounds, IC ₅₀ (μM) at 48 hr					
		SAHA	3a	3b	3c	3d	7
MCF-7	Breast	4.21	5.48	1.08	0.92	0.54	12.54
MDA-MB-231	Breast	5.78	6.29	2.43	0.87	0.76	10.37
MCF-7/Dox	Breast	3.14	4.25	1.37	0.56	0.42	> 20
MCF-7/Tam	Breast	5.24	> 20	2.58	1.51	2.11	> 20
SK-OV-3	Ovary	2.25	3.12	1.25	0.58	0.42	6.67
LNCaP	Prostate	2.43	2.42	0.48	0.86	0.30	> 20
DU145	Prostate	0.88	4.79	1.48	2.73	0.48	> 20
PC3	Prostate	5.26	0.48	0.42	0.35	0.24	4.85

prostate cancer cell lines including LNCaP (0.48 μM), and PC3 (0.42 μM) cancer cell lines, and on the other cancer cells, IC_{50} values for anticancer activity were in range of 1.08–2.58 μM . These three compounds, **3b**, **3c** and **3d** exhibited 2–22-fold more potent anticancer activity than SAHA in all cancer cell lines, except DU145 cancer cell line. Compound **3a** with a dimethylaminophenyl group as a cap showed more potent anticancer activity than SAHA only on PC3 cancer cell lines and exhibited similar anticancer activity to SAHA on the other cancer cell lines. Compound **7** which had a naphthalenyl ring as the cap moiety and a transposed amide structure showed similar anticancer activity to or less potent anticancer activity than SAHA, depending on the type of human cancer cell lines. It is notable that all the synthesized compounds **3a–d** and **7** showed more potent anticancer activity than SAHA on PC3 cancer cell lines. These results show that **3b**, **3c** and **3d** may be a promise drug with a broad anticancer spectrum.

In summary, SAHA derivatives **3a–d** and **7** with a bulky cap were synthesized in order to search for more potent anticancer agents with significant HDAC-inhibitory activity. EDC-mediated amide coupling reaction and a nucleophilic addition-elimination reaction with hydroxylamine were employed for the synthesis of the target compounds. Whereas compounds **3a** and **7** showed a similar or a little less inhibitory effect to SAHA on total HDACs and sub-HDACs, compounds **3b**, **3c** and **3d** showed significantly potent inhibitory activities on total HDACs (14–27-fold), HDAC1 (8–15-fold), HDAC2 (1.3–25-fold) and HDAC7 (1–3-fold), compared with SAHA. Although compound **7** showing the lowest HDAC inhibitory activity exhibited less potent anticancer activity than SAHA, compound **3a** with a similar HDAC inhibitory activity to SAHA showed a similar anticancer activity to SAHA. Especially, compounds **3b**, **3c** and **3d** showing the highly potent HDAC inhibitory activity exhibited significantly potent anticancer activity (2–22-fold) against MCF-7, MDA-MB-231, MCF-7/Dox, MCF-7/Tam, SK-OV-3, LNCaP and PC3 human cancer cell lines, compared with SAHA. This study indicates proper bulkiness of the cap moiety of SAHA analogs may be closely correlated to high HDAC inhibition and anticancer activity.

Experimental Section

Melting points are uncorrected. NMR data were recorded on a 400 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard, and the chemical shifts are reported in ppm (δ). Coupling constants are reported in hertz. The abbreviations used are as follows: s (singlet), d (doublet), m (multiplet), dd (doublet of doublet), br s (broad singlet) and quint (quintet). All the reactions described below were performed under argon or nitrogen atmosphere and monitored by thin layer chromatography (TLC). All anhydrous solvents were distilled over CaH_2 or Na/benzophenone prior to use.

Methyl 8-(4-(Dimethylamino)phenylamino)-8-oxooctanoate (2a). To a stirred solution of N^1, N^1 -dimethylbenzene-

1,4-diamine (80 mg, 0.58 mmol), HOBt (1-hydroxybenzotriazole, 72 mg, 0.53 mmol), and EDC·HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 153 mg, 0.80 mmol) in anhydrous methylene chloride (2 mL) was dropwise added suberic acid monoethyl ester (100 mg, 0.53 mmol) at 0 °C. After being stirred at room temperature for 48 h, the reaction mixture was concentrated and the residue was diluted with ethyl acetate (2×50 mL) and washed with 0.1 M HCl solution (2×25 mL), and a saturated NaHCO_3 solution (25 mL) subsequently. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by silica gel column chromatography using methylene chloride and MeOH (45:1) as the eluent to give amide **2a** (106 mg, 59%) as a yellowish solid; LRMS (FAB+) m/z 307 $[\text{M}+\text{H}]^+$; ^1H NMR (500 MHz, CDCl_3) δ 7.34 (d, 2 H, $J = 9.0$ Hz, Ph), 7.30 (br s, 1 H, NH), 6.68 (br s, 2 H, Ph), 3.66 (s, 3 H, OCH_3), 2.91 (s, 6 H, $2 \times \text{NCH}_3$), 2.30 (m, 4 H, 2- CH_2 , 7- CH_2), 1.71 (quint, 2 H, $J = 7.5$ Hz, 6- CH_2), 1.62 (quint, 2 H, $J = 7.5$ Hz, 3- CH_2), 1.40–1.33 (m, 4 H, 4- CH_2 , 5- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ 174.51 (1-C=O), 171.36 (8-C=O), 148.14 (Ph), 128.33 (Ph), 122.01 (Ph), 113.40 (Ph), 51.71 (O- CH_3), 41.24 (NCH_3), 37.57, 34.18, 29.04, 29.00, 25.74, 24.95.

Methyl 8-Oxo-8-(4-phenoxyphenylamino)octanoate (2b). 4-Phenoxyphenyl analog **2b** was synthesized by a procedure similar to that described for **2a**: yield 62%; LRMS (FAB+) m/z 356 $[\text{M}+\text{H}]^+$; ^1H NMR (400 MHz, CDCl_3) δ 7.98 (s, 1 H, NH), 7.47 (d, 2 H, $J = 8.8$ Hz, Ph), 7.27 (t, 2 H, $J = 7.6$ Hz, Ph), 7.04 (t, 1 H, $J = 7.2$ Hz, Ph), 6.93 (d, 2 H, $J = 7.2$ Hz, Ph), 6.91 (d, 2 H, $J = 8.8$ Hz, Ph), 3.63 (s, 3 H, OCH_3), 2.30 (t, 2 H, $J = 8.0$ Hz, 2- CH_2), 2.27 (t, 2 H, $J = 7.6$ Hz, 7- CH_2), 1.68 (quint, 2 H, $J = 6.4$ Hz, 6- CH_2), 1.59 (quint, 2 H, $J = 7.2$ Hz, 3- CH_2), 1.32 (m, 4 H, 4- CH_2 , 5- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ 174.58 (1-C=O), 171.92 (8-C=O), 157.77 (Ph), 153.44 (Ph), 133.96 (Ph), 129.94 (Ph), 123.25 (Ph), 121.94 (Ph), 119.74 (Ph), 118.55 (Ph), 51.76 (O- CH_3), 37.52, 34.16, 29.03, 28.97, 25.65, 24.91.

Methyl 8-Oxo-8-(4-phenylamino)phenylamino)octanoate (2c). 4-Phenylaminophenyl analog **2c** was synthesized by a procedure similar to that described for **2a**: yield 49%; LRMS (FAB+) m/z 355 $[\text{M}+\text{H}]^+$; ^1H NMR (400 MHz, CDCl_3) δ 7.56 (s, 1 H, CONH), 7.38 (d, 2 H, $J = 8.8$ Hz, Ph), 7.21 (t, 2 H, $J = 8.4$ Hz, Ph), 7.00 (d, 2 H, $J = 8.8$ Hz, Ph), 6.98 (d, 2 H, $J = 8.8$ Hz, Ph), 6.86 (t, 1H, $J = 7.2$ Hz, Ph), 5.76 (s, 1 H, NH), 3.64 (s, 3H, OCH_3), 2.30 (t, 2 H, $J = 7.2$ Hz, 2- CH_2), 2.27 (t, 2 H, $J = 7.2$ Hz, 7- CH_2), 1.69 (quint, 2H, $J = 6.8$ Hz, 3- CH_2), 1.60 (quint, 2 H, $J = 6.8$ Hz, 6- CH_2), 1.33 (m, 4 H, 4- CH_2 , 5- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ 174.59 (1-C=O), 171.60 (8-C=O), 143.84 (Ph), 139.64 (Ph), 131.94 (Ph), 129.55 (Ph), 121.77 (Ph), 120.71 (Ph), 119.16 (Ph), 117.20 (Ph), 51.76 (OCH_3), 37.59, 34.18, 29.03, 28.98, 25.69, 24.93.

Methyl 8-(9H-Fluoren-2-ylamino)-8-oxooctanoate (2d). Fluorenyl analog **2d** was synthesized by a procedure similar to that described for **2a**: yield 72%; ^1H NMR (400 MHz, CDCl_3) δ 7.88 (s, 1 H, Ar), 7.66 (m, 2 H, Ar), 7.49 (m, 1 H, Ar), 7.34 (m, 2 H, Ar), 7.24 (t, 1 H, $J = 6.8$ Hz, Ar), 3.82 (s,

2H, benzylic H), 3.65 (s, 3 H, OCH₃), 2.34 (t, 2 H, *J* = 6.8 Hz, 2-CH₂), 2.29 (t, 2 H, *J* = 6.8 Hz, 7-CH₂), 1.73 (quint, 2 H, *J* = 6.0 Hz, 3-CH₂), 1.62 (quint, 2 H, *J* = 6.8 Hz, 6-CH₂), 1.36 (m, 4 H, 4-CH₂, 5-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 174.55 (1-C=O), 171.72 (8-C=O), 144.50 (Ar), 143.38 (Ar), 141.54 (Ar), 138.14 (Ar), 137.11 (Ar), 126.97 (Ar), 126.49 (Ar), 125.19 (Ar), 120.27 (Ar), 119.70 (Ar), 118.83 (Ar), 117.12 (Ar), 51.74 (OCH₃), 37.81, 37.22, 34.16, 28.96, 28.98, 25.61, 24.90.

N¹-(4-(Dimethylamino)phenyl)-N⁸-hydroxyoctanedi- amide (3a). To a stirred solution of **2a** (106 mg, 0.34 mmol) in MeOH (4 mL) was added 50% hydroxylamine aqueous solution (2.2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 d. In succession, to this reaction mixture was added 50% hydroxylamine aqueous solution (0.5 mL) and the reaction mixture was stirred at room temperature for an additional 3 d. The precipitated solid was filtered, washed with water, air-dried, and dried under high vacuum to give hydroxamic acid **3a** as a white solid (61 mg, 57%); mp 155.7-160 °C; LRMS (FAB+) *m/z* 308 [M+H]⁺; ¹H NMR (400 MHz, CD₃OD) δ 7.32 (d, 2 H, *J* = 8.8 Hz, Ph), 6.73 (d, 2 H, *J* = 8.8 Hz, Ph), 2.86 (s, 6 H, 2×NCH₃), 2.30 (t, 2 H, *J* = 7.2 Hz, 2-CH₂), 2.07 (t, 2 H, *J* = 7.2 Hz, 7-CH₂), 1.68-1.59 (m, 4 H, 3-CH₂, 6-CH₂), 1.39-1.33 (m, 4 H, 4-CH₂, 5-CH₂); ¹³C NMR (100 MHz, CD₃OD) δ 173.05 (8-C=O), 171.76 (1-C=O), 148.33 (Ph), 128.78 (Ph), 121.79 (Ph), 113.29 (Ph), 40.20 (NCH₃), 36.48, 32.48, 28.70, 28.62, 25.68, 25.40.

N¹-Hydroxy-N⁸-(4-phenoxyphenyl)octanedi- amide (3b). 4-Phenoxyphenyl analog **3b** was synthesized by a procedure similar to that described for **3a**: yield 67%; mp 162.5-163.1 °C; LRMS (FAB+) *m/z* 357 [M+H]⁺; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.32 (s, 1 H, NHOH), 9.86 (s, 1 H, NH), 8.65 (s, 1 H, OH), 7.57 (d, 2 H, *J* = 8.8 Hz, Ph), 7.32 (t, 2H, *J* = 7.6 Hz, Ph), 7.05 (t, 1 H, *J* = 7.2 Hz, Ph), 6.93 (d, 2 H, *J* = 8.4 Hz, Ph), 6.91 (d, 2 H, *J* = 8.8 Hz, Ph), 2.24 (t, 2 H, *J* = 7.2 Hz, 7-CH₂), 1.90 (t, 2 H, *J* = 7.6 Hz, 2-CH₂), 1.54 (quint, 2H, *J* = 6.4 Hz, 6-CH₂), 1.45 (quint, 2 H, *J* = 6.4 Hz, 3-CH₂), 1.28-1.20 (m, 4 H, 4-CH₂, 5-CH₂); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.70 (8-C=O), 169.74 (1-C=O), 158.13 (Ph), 152.03 (Ph), 135.99 (Ph), 130.60 (Ph), 123.54 (Ph), 121.33 (Ph), 120.15 (Ph), 118.39 (Ph), 36.96, 32.89, 29.08, 25.73, 25.70.

N¹-Hydroxy-N⁸-(4-(phenylamino)phenyl)octanedi- amide (3c). 4-Phenylaminophenyl analog **3c** was synthesized by a procedure similar to that described for **3a**: yield 65%; mp 155.2-155.8 °C; LRMS (FAB+) *m/z* 356 [M+H]⁺; ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.34 (s, 1 H, NHOH), 9.69 (s, 1 H, NH), 8.67 (s, 1H, anilinic NH), 7.99 (s, 1H, OH), 7.45 (d, 2H, *J* = 9.0 Hz, Ph), 7.18 (t, 2H, *J* = 7.5 Hz, Ph), 7.00 (d, 2H, *J* = 9.0 Hz, Ph), 6.98 (d, 2 H, *J* = 8.5 Hz, Ph), 6.74 (t, 1 H, *J* = 7.5 Hz, Ph), 2.24 (t, 2 H, *J* = 7.0 Hz, 7-CH₂), 1.93 (t, 2 H, *J* = 7.5 Hz, 2-CH₂), 1.56 (quint, 2 H, *J* = 6.5 Hz, 6-CH₂), 1.48 (quint, 2 H, *J* = 7.0 Hz, 3-CH₂), 1.33-1.25 (m, 4 H, 4-CH₂, 5-CH₂); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.27 (8-C=O), 169.76 (1-C=O), 144.82 (Ph), 139.15 (Ph), 133.01 (Ph), 129.79 (Ph), 121.01 (Ph), 119.52 (Ph), 118.57 (Ph), 116.28

(Ph), 44.54, 36.92, 32.90, 29.08, 25.83, 25.72.

N¹-(9H-Fluoren-2-yl)-N⁸-hydroxyoctanedi- amide (3d). Fluorenyl analog **3d** was synthesized by a procedure similar to that described for **3a**: yield 57%; mp 170.5-170.8 °C; ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.36 (s, 1 H, NHOH), 9.95 (s, 1 H, NH), 8.70 (s, 1 H, OH), 7.94 (s, 1 H, Ar), 7.77 (d, 2 H, *J* = 7.5 Hz, Ar), 7.53 (d, 1 H, *J* = 8.0 Hz, Ar), 7.51 (d, 1 H, *J* = 7.5 Hz, Ar), 7.32 (t, 1 H, *J* = 7.5 Hz, Ar), 7.23 (t, 1 H, *J* = 7.0 Hz, Ar), 2.31 (t, 2 H, *J* = 7.0 Hz, 2-CH₂), 1.94 (t, 2 H, *J* = 7.0 Hz, 7-CH₂), 1.59 (quint, 2 H, *J* = 6.0 Hz, 3-CH₂), 1.49 (quint, 2 H, *J* = 6.5 Hz, 6-CH₂), 1.28 (m, 4 H, 4-CH₂, 5-CH₂); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.89 (1-C=O), 169.85 (8-C=O), 144.36 (Ar), 143.40 (Ar), 141.71 (Ar), 139.08 (Ar), 136.76 (Ar), 127.36 (Ar), 126.66 (Ar), 125.64 (Ar), 120.70 (Ar), 120.03 (Ar), 118.53 (Ar), 116.56 (Ar), 37.15, 32.93, 29.10, 25.79, 25.73.

Methyl 6-(2-(Naphthalen-1-yl)acetamido)hexanoate (6). To a stirred solution of 2-(naphthalen-1-yl)acetic acid (200 mg, 1.07 mmol) in 1,4-dioxane (2 mL) were added HOBt (363 mg, 2.68 mmol) and EDC·HCl (309 mg, 1.61 mmol) successively at room temperature with stirring. This reaction mixture was stirred constantly at the same temperature for 2 h and the volatiles were evaporated under reduced pressure. The concentrated residue was diluted with ethyl acetate (50 mL) and washed with NaHCO₃ (25 mL). The organic layer was dried over anhydrous MgSO₄, filtered, and evaporated under reduced pressure to give compound **5** (310 mg, 95%) as a sticky yellowish oil. To a stirred solution of **5** (310 mg, 1.02 mmol) in 1,4-dioxane (2 mL) were added methyl 6-aminohexanoate hydrochloride (186 mg, 1.02 mmol) and *N*-methylmorpholine (0.19 mL, 2.04 mmol) successively at room temperature with stirring. After the reaction mixture was stirred constantly at the same temperature for 2 d, the resulting solution was filtered off through a pad of Celite and washed with methylene chloride and MeOH (70:1). The filtrate was concentrated and was purified by silica gel column chromatography using methylene chloride and MeOH (70:1) as the eluent to give amide **6** (126 mg, 37% from **4**) as a yellow oil; LRMS (FAB+) *m/z* 314 [M+H]⁺; ¹H NMR (400 MHz, CDCl₃) δ 7.93-7.90 (m, 1 H, Ph), 7.85-7.83 (m, 1 H, Ar), 7.78 (d, 1 H, *J* = 7.2 Hz, Ar), 7.52-7.45 (m, 2 H, Ar), 7.41 (t, 1 H, *J* = 7.6 Hz, Ar), 7.35 (d, 1 H, *J* = 7.2 Hz, Ar), 5.42 (br s, 1 H, NH), 3.96 (s, 2 H, CH₂Ar), 3.59 (s, 3 H, OCH₃), 3.08 (q, 2 H, *J* = 6.8 Hz, 6-CH₂), 2.12 (t, 2 H, *J* = 7.6 Hz, 2-CH₂), 1.43 (quint, 2 H, *J* = 8.0 Hz, 3-CH₂), 1.26 (quint, 2 H, *J* = 7.2 Hz, 5-CH₂), 1.07-0.98 (m, 2 H, 4-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 174.15 (1-C=O), 171.05 (NHC=O), 134.14 (Ar), 132.25 (Ar), 131.46 (Ar), 128.98 (Ar), 128.66 (Ar), 128.55 (Ar), 126.93 (Ar), 126.40 (Ar), 125.85 (Ar), 124.04 (Ar), 51.69 (OCH₃), 42.04, 39.49, 33.97, 29.20, 26.31, 24.56.

N-Hydroxy-6-(2-(naphthalen-1-yl)acetamido)hexanamide (7). Naphthalenyl analog **7** was synthesized by a procedure similar to that described for **3a**: yield 51%; mp 135.2-135.9 °C; LRMS (FAB+) *m/z* 315 [M+H]⁺; ¹H NMR (500 MHz, CD₃OD) δ 8.03 (d, 1 H, *J* = 8.5 Hz, Ar), 7.87 (d, 1 H, *J* = 8.0 Hz, Ar), 7.79 (d, 1 H, *J* = 7.5 Hz, Ar), 7.53 (t, 1 H, *J* = 7.5

Hz, Ar), 7.49 (t, 1 H, $J = 7.5$ Hz, Ar), 7.44 (t, 1 H, $J = 7.0$ Hz, Ar), 7.41 (d, 1 H, $J = 7.5$ Hz, Ar), 3.97 (s, 2 H, CH₂Ar), 3.16 (t, 2 H, $J = 7.0$ Hz, NCH₂), 2.03 (t, 2 H, $J = 7.5$ Hz, 2-CH₂), 1.56 (quint, 2 H, $J = 7.5$ Hz, 3-CH₂), 1.47 (quint, 2 H, $J = 7.5$ Hz, 5-CH₂), 1.29-1.24 (m, 2 H, 4-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 172.75 (NHC=O), 171.64 (1-C=O), 134.19 (Ar), 132.39 (Ar), 131.84 (Ar), 128.53 (Ar), 127.88 (Ar), 127.70 (Ar), 126.07 (Ar), 125.63 (Ar), 125.43 (Ar), 123.69 (Ar), 40.32, 39.20, 32.41, 28.78, 26.15, 25.15.

General for Cell Culture, Cell Viability Test and Assay of HDAC Enzyme Activity. SAHA and all chemicals used for bioassay were purchased from Sigma-Aldrich (St. Louis, MO, USA). SAHA and test compounds **3a-3d** and **7** were dissolved in sterile dimethyl sulfoxide (DMSO) to generate a 10 mM stock solution. The solution was stored at -80 °C. Subsequent dilutions were made in RPMI-1640 (Gibco, Rockville, MD, USA).

Cell Lines and Culture. The cell lines, MCF-7, MDA-MB-231, PC3, DU-145, LNCaP, and SK-OV-3 cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). All cells were maintained as monolayers at 37 °C in an atmosphere containing 5% CO₂/air in RPMI-1640 (Gibco, Rockville, MD) containing 2 mM L-glutamate, 10% heat-inactivated fetal bovine serum (FBS, Gibco), 1.25 mM HEPES (Gibco) and 100 U/mL penicillin/streptomycin (Gibco).

Cell Viability Test. Cytotoxicity was assessed by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. For the compound treatment, the cells were plated for 48 h in RPMI-1640 containing 10% FBS. The medium was then changed to RPMI-1640 containing 5% charcoal-dextran treated FBS (CD-FBS) with various concentrations of test compounds, SAHA, **3a-3d** and **7**. The cultures were initiated in 96-well microtiter plates at a density of 1×10^4 cells per well. Cells were allowed to attach for 48 h before treatment with SAHA, **3a-3d** and **7**. At the end of the treatment period, 15 μ L of the MTT reagent (5 mg/mL) was added to each well. After 4 h incubation at 37 °C, the supernatant was aspirated, and formazan crystals were dissolved in 100 μ L DMSO at 37 °C for 10 min with gentle agitation. The absorbance per well was measured at 540 nm with a VERS Amax Microplate Reader (Molecular Devices Corp.). Assay was done in triplicate. The IC₅₀ values were then determined for each compounds from a plot of log (compound concentration) versus percentage of cell killed.

Assay of HDAC Enzyme Activity. The HDAC enzymes were purchased from BPS Bioscience (San Diego, CA, USA) and the HDAC enzyme assay was performed using a Fluorogenic HDAC Assay Kit (BPS Bioscience) according to the manufacturer's instructions. Briefly, HDAC enzymes were incubated with vehicle or various concentrations of SAHA, **3a-3d** and **7** for 30 min at 37 °C in the presence of an HDAC fluorimetric substrate. The HDAC assay developer (which produces a fluorophore in the reaction mixture) was

added, and the fluorescence was measured using VICTOR 3 (PerkinElmer, Waltham, MA, USA) with excitation at 360 nm and emission at 460 nm. The measured activities were subtracted by calculated using GraphPad Prism (Graphpad Software, San Diego, CA, USA).

Statistical Analysis. All the data is represented as the average of the values obtained \pm SD. A paired Student's *t*-test was used to determine the statistical significance. A *p*-value < 0.05 , or *p*-value < 0.01 was considered statistically significant.

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