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Design and synthesis of non-hydroxamate histone deacetylase inhibitors: identification of a selective histone acetylating agent

Takayoshi Suzuki,* Azusa Matsuura, Akiyasu Kouketsu, Shinya Hisakawa, Hidehiko Nakagawa and Naoki Miyata*

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan

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Abstract—A series of suberoylanilide hydroxamic acid (SAHA)-based non-hydroxamates was designed, synthesized, and evaluated for their histone deacetylase (HDAC) inhibitory activity. Among these, methyl sulfoxide **15** inhibited HDACs in enzyme assays and caused hyperacetylation of histone H4 while not inducing the accumulation of acetylated α-tubulin in HCT116 cells.

1. Introduction

The dynamic homeostasis of the nuclear acetylation of histones is regulated by the opposing activity of the enzymes, histone acetyl transferases (HATs) and histone deacetylases (HDACs). The reversible acetylation of histone lysine residues by HATs and HDACs is one of the several possible regulatory mechanisms of gene expression.[1] When HDACs are inhibited, histone hyperacetylation occurs. The disruption of the chromatin structure by histone hyperacetylation leads to the transcriptional activation of genes[2] associated with some disease states such as cancer[3] and inflammation.[4] Indeed, HDAC inhibitors such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) (Fig. 1) have potent anti-cancer effects and some of them are currently in phase I/II clinical trials.[5]

Previously reported HDAC inhibitors are mostly hydroxamic acid derivatives,[6] typified by TSA and SAHA, which are thought to chelate the zinc ion in the active site in a bidentate fashion through its CO and OH groups.[7] However, hydroxamic acids occasionally have produced problems such as poor pharmacokinetics and severe toxicity.[8] In addition, although isozyme-selective HDAC inhibitors are considered to be useful not only as tools for probing the biology of the enzyme but also as drugs with low toxicity, many of the known hydroxamate HDAC inhibitors do not distinguish well among the HDAC isozymes.[9] Therefore, it is desirable to find non-hydroxamate HDAC inhibitors to improve the pharmacokinetics, toxicity, and isozyme selectivity of hydroxamates. To date, some types of non-peptide non-hydroxamate HDAC inhibitors have been reported.[10] However, many of these have either reduced potency or metabolic disadvantages. Thus, there remains a need to develop non-hydroxamate HDAC inhibitors. Here, we report the design, synthesis, enzyme inhibition, and in-cell selective histone deacetylase inhibition of SAHA-based non-hydroxamates.

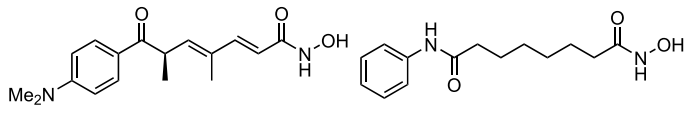

Figure 1. Structures of TSA and SAHA.

Keywords: Histone deacetylase inhibitor; Non-hydroxamate.
* Corresponding authors. Tel./fax: +81 52 836 3407; e-mail addresses: suzuki@phar.nagoya-cu.ac.jp; miyata-n@phar.nagoya-cu.ac.jp

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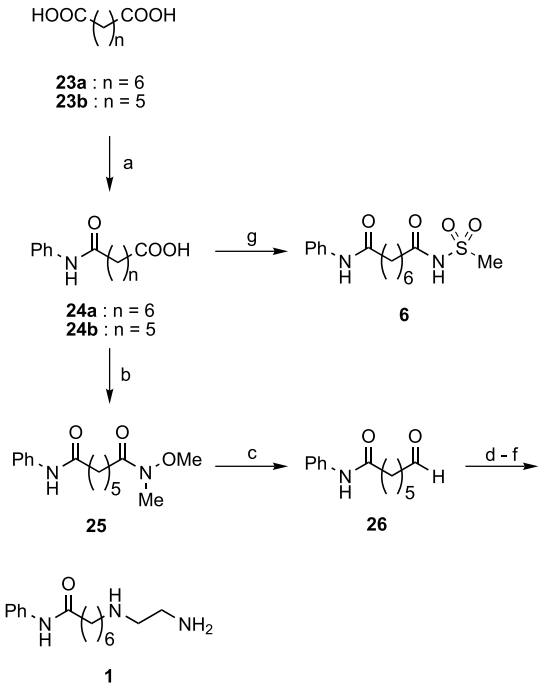
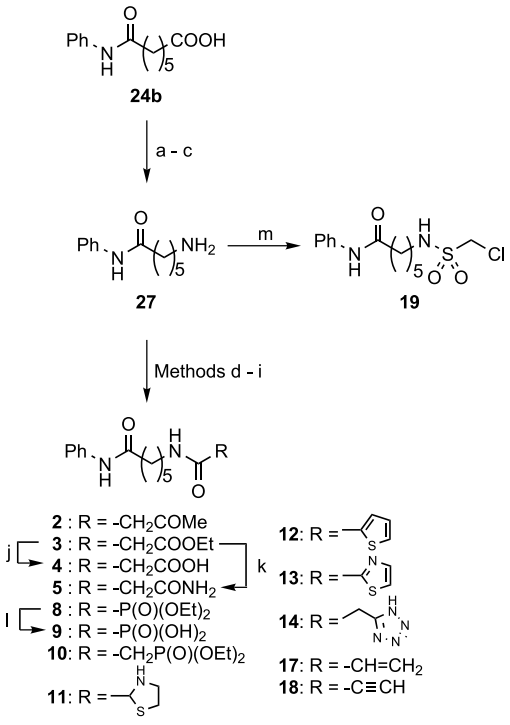
2. Chemistry

The compounds prepared for this study are shown in Table 1. The routes used for synthesis of the compounds are shown in Schemes 1–4. Compounds **1** and **6** were prepared as shown in Scheme 1, starting with dicarboxylic acids **23**. The condensation of dicarboxylic acids **23** with an equivalent amount of aniline gave mono-anilides **24**. The carboxylic acid **24b** was converted to Weinreb amide **25** in the presence of EDCI and HOBt. Compound **25** was allowed to react with lithium aluminum hydride at 0 °C to give aldehyde **26**. Reductive amination of **26** with mono-Boc ethylenediamine, Boc protection of the resultant amine, chromatographic purification, and removal of the two Boc groups afforded ethylenediamine **1**. Carboxylic acid **24a** was converted to acylsulfonamide **6** by acylation of methanesulfonamide in the presence of CDI and DBU in DMF.

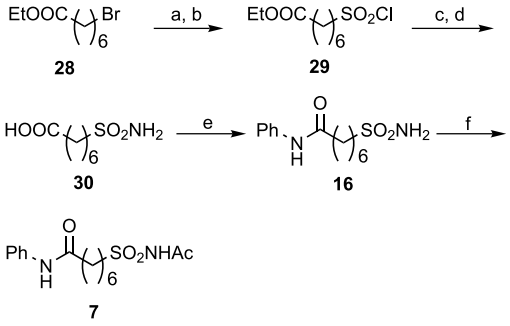
Amine **27** served as a key common intermediate for the synthesis of compounds **2**–**5**, **8**–**14**, **17**, and **18** (Scheme 2). Carboxylic acid **24b** was converted to amine **27** by Curtius rearrangement of carboxylic acid **24b**, treatment of the resulting isocyanates with benzyl alcohol, and removal of the Z group by hydrogenolysis. Amine **27** underwent a reaction with diketene to give acetoacetamide **2**. An appropriate carboxylic acid was reacted with amine **27** in the presence of EDCI and HOBt in DMF to give compounds **3**, **12**, **13**, **17**, and **18**. Hydrolysis of ester **3** provided carboxylic acid **4**, and treatment of **3** with 25% aqueous ammonia produced amide **5**. Amine **27** was converted to diethyl carbamoylphosphonate **8**



Scheme 1. Reagents and conditions: (a) aniline, 180 °C; (b) *N*,*O*-dimethylhydroxylamine hydrochloride, Et_3N, EDCI, HOBt, DMF, rt; (c) $LiAlH_4$, THF, 0 °C; (d) *N*-(2-aminoethyl)carbamic acid *tert*-butyl ester, $NaBH(OAc)_3$, CH_2Cl_2, AcOH, rt; (e) $(Boc)_2O$, Et_3N, rt; (f) TFA, CH_2Cl_2, rt; (g) $MsNH_2$, 1,1′-carbonyldiimidazole, DBU, DMF, rt.

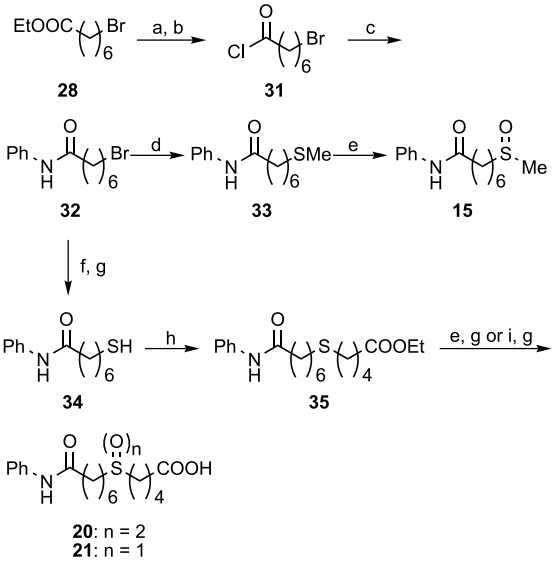


Scheme 2. Reagents and conditions: (a) diphenylphosphoryl azide, Et_3N, benzene, reflux; (b) BnOH, reflux; (c) H_2, 5% Pd–C, MeOH; (d) diketene, rt (for **2**); (e) RCOOH, EDCI, HOBt, DMF, rt (for **3**, **12**, **13**, **17**, and **18**); (f) $EtSCOP(O)(OEt)_2$, rt (for **8**); (g) (i) bromoacetyl chloride, Et_3N, THF, rt; (ii) $P(OEt)_3$, tetrabutyl ammonium iodide, reflux (for **10**); (h) (i) thiazolidine-2,3-dicarboxylic acid 3-*tert*-butyl ester, EDCI, HOBt, DMF, rt; (ii) 4NHCl·AcOEt, AcOEt, rt (for **11**); (i) tetrazole-5-acetic acid, bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 0 °C to rt (for **14**); (j) 2 N aq NaOH, MeOH, rt; (k) 25% aq NH_3, MeOH, rt; (l) TMSBr, MeCN, rt; (m) chloromethylsulfonyl chloride, Et_3N, 0 °C to rt.



Scheme 3. Reagents and conditions: (a) Na_2SO_3, EtOH, H_2O, reflux; (b) $SOCl_2$, DMF, toluene, reflux; (c) 25% aq NH_3, 4-(dimethylamino)pyridine, pyridine, CH_2Cl_2, rt; (d) 2 N aq NaOH, EtOH, rt; (e) aniline, EDCI, HOBt, DMF, rt; (f) AcOH, 1,1′-carbonyldiimidazole, DBU, DMF, rt.

upon reacting with *S*-ethyl diethylphosphonothiolformate, and following ester group removal with trimethylsilyl bromide gave carbamoylphosphonic acid **9**. Acylation of amine **27** with bromoacetyl chloride followed by an Arbazov reaction provided diethyl phosphonate **10**. Coupling between amine **27** and *N*-Boc



Scheme 4. Reagents and conditions: (a) $LiOH{\cdot}H_2O$, EtOH, THF, H_2O, rt; (b) $(COCl)_2$, DMF, CH_2Cl_2, rt; (c) aniline, Et_3N, CH_2Cl_2, rt; (d) 15% aq NaSMe, EtOH, rt; (e) 1 equiv of *m*-chloroperoxybenzoic acid, CH_2Cl_2, rt; (f) AcSK, EtOH, rt; (g) 2 N aq NaOH, EtOH, rt; (h) 5-bromovaleric acid ethyl ester, NaOEt, EtOH, rt; (i) 2 equiv of *m*-chloroperoxybenzoic acid, CH_2Cl_2, rt.

thiazolidine-2-carboxylic acid in the presence of EDCI and HOBt and subsequent deprotection of the Boc group under acidic conditions produced 2-thiazolidinecarboxamide **11**. Amine **27** was reacted with tetrazole-5-acetic acid in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride to give tetrazole-5-acetamide **14**. Chloromethyl sulfonamide **19** was obtained by the coupling of amine **27** with chlorosulfonyl chloride.

The preparation of sulfonamide derivatives **7** and **16** was achieved via sulfonyl chloride **29** (Scheme 3). Bromide **28** was converted to sulfonyl chloride **29** by a reaction with Na_2SO_3 and by subsequent treatment with thionyl chloride. Treatment of the sulfonyl chloride **29** with 25% aqueous ammonia and subsequent hydrolysis yielded sulfonamide **30**. The condensation of carboxylic acid **30** with aniline gave compound **16**. Sulfonamide **16** was acetylated to the reversed acylsulfonamide **7** in the same procedure employed in the preparation of **6**.

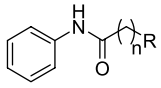
Compounds **15**, **20**, and **21** were synthesized from ester **28** as depicted in Scheme 4. Acid chloride **31** was prepared from ester **28** by hydrolysis of the ethyl ester and subsequent reaction with oxalyl chloride. The amino group of aniline was acylated with the acid chloride **31** to give the amide **32**. Sulfide **33** was obtained by the alkylation of methylmercaptan with bromide **32**. Oxidation of **33** with 1 equiv of MCPBA gave the sulfoxide **15**. Bromide **32** was also treated with potassium thioacetate to give the thioester, after which deacetylation of the thioacetate under alkaline conditions gave thiol **34**. Thiol **34** was alkylated with ethyl 5-bromovalerate, oxidized with 1 or 2 equivalent amounts of MCPBA, and then hydrolyzed to give compounds **20** and **21**.

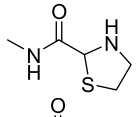
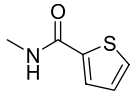
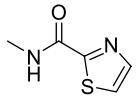
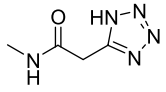
3. Results and discussion

3.1. Enzyme assays

The compounds synthesized in this study were tested with an in vitro assay using a HeLa nuclear extract rich in HDAC activity. The results are summarized in Table 1. SAHA was used as a positive control, which inhibited 100% of the HDAC activity at 100 μM and had an IC_{50} of 0.28 μM.

Table 1. HDAC enzyme inhibition data for compounds **1**–**22**[a]



Compound	R	*n*	% inhibition at 100 μM
1	$-NHCH_2CH_2NH_2$	6	12 ± 1.2
2	$-NHCOCH_2COMe$	5	12 ± 1.3
3	$-NHCOCH_2COOEt$	5	0 ± 1.4
4	$-NHCOCH_2COOH$	5	4.1 ± 0.56
5	$-NHCOCH_2CONH_2$	5	1.1 ± 2.2
6	$-CONHSO_2Me$	6	21 ± 3.0
7	$-SO_2NHAc$	6	2.5 ± 1.9
8	$-NHCOP(O)(OEt)_2$	5	0 ± 1.6
9	$-NHCOP(O)(OH)_2$	5	5.8 ± 1.3
10	$-NHCOCH_2P(O)(OEt)_2$	5	0 ± 0.36
11		5	0 ± 1.5
12		5	21 ± 4.6
13		5	2.0 ± 1.1
14		5	17 ± 1.7
15	–S(O)Me	6	62 ± 2.0
16	$-SO_2NH_2$	6	10 ± 0.97
17	$-NHCOCH{=}CH_2$	5	11 ± 3.5
18	$-NHCOC{\equiv}CH$	5	3.7 ± 2.3
19	$-NHSO_2CH_2Cl$	5	0 ± 0.56
20	$-SO_2(CH_2)_4COOH$	6	6.1 ± 3.3
21	$-S(O)(CH_2)_4COOH$	6	12 ± 6.1
22	$-SO_2Me$	6	33 ± 2.0[b]

[a] Values are means ± standard deviations of a minimum of three separate experiments.

[b] Data taken from the literature (Ref. 10i).

The co-crystal structure of an archaebacterial HDAC homologue (HDAC-like protein, HDLP) with SAHA revealed that the hydroxamic acid group coordinates the zinc ion in the active site through its CO and OH groups.[7] Based on these data, we designed compounds **1**–**14** which were expected to chelate zinc ion in the active site in a bidentate fashion. Notably, an acylsulfonamide moiety has been reported to bind zinc ion in a bidentate fashion through its CO and SO_2 groups,[11] and carbamoylphosphonic acids are known to be matrix

metalloproteinase inhibitors,[12] another form of zinc-dependent metalloenzyme. However, these compounds were found to be totally inactive, although acylsulfonamide **6** and 2-thiophenecarboxamide **12** showed a certain level of inhibitory activity against HDACs. The reason that compounds **1**–**14** were weakly active is unclear, but it is probably because they lack interaction with amino acid residues such as Tyr and His in the active site of HDACs.[7]

Next, compounds with methyl sulfoxide (**15**) and sulfonamide[13] (**16**), which are expected to coordinate zinc ions monodentately, were evaluated for anti-HDAC activity. Interestingly, although the inhibitory ability of monodentate zinc-binding groups (ZBGs) was thought to be less than that of bidentate ZBGs, methyl sulfoxide **15** was more potent than compounds **1**–**14** in inhibiting 68% of the HDAC activity at 100 μM, with an IC_{50} of 48 μM.

The crystal structure of the HDLP/SAHA complex made it clear that there are nucleophilic amino acids such as His in the active center of HDACs.[7] Compounds bearing acrylamide (**17**), 2-propynamide (**18**), and chloromethanesulfonamide (**19**) could form covalent bonds with nucleophilic amino acids of the enzyme and function as irreversible HDAC inhibitors. However, these compounds were only weakly active against HDACs.

According to the data of the crystal structure of the HDLP/SAHA complex, there is a 14 Å long internal cavity adjacent to the active site.[7,14] We designed the cavity-targeting compounds **20** and **21** modeled after sulfoxide **15** and sulfone **22**. Sulfone **22** has been reported to inhibit HDACs.[10i] The carboxylic acid of **20** and **21** could interact with Arg 27 (HDLP numbering), a component in the construction of the 14 Å internal cavity, and these compounds were anticipated to be more potent HDAC inhibitors. However, contrary to expectation, compounds **20** and **21** did not show strong activity as compared with their parent compounds **15** and **22**. At the time this work was ongoing, the X-ray structure of human HDAC8 was published.[15] According to this work, there is no such large internal cavity adjacent to the active site. This may be the reason why compounds **20** and **21** did not exhibit inhibitory activity against human HDACs.

3.2. Cellular assays

HDACs are also responsible for the deacetylation of non-histone proteins.[1c] Notably, HDAC6, one of the isozymes of HDAC, has recently been reported to be a α-tubulin deacetylase (TDAC).[16] The reversible acetylation of α-tubulin is involved in microtubule stability. Therefore, compounds with high HDAC/TDAC selectivity are desirable for biological studies and medicinal use. However, many hydroxamate HDAC inhibitors such as TSA and SAHA cause the accumulation of acetylated histones as well as acetylated α-tubulin, which indicates that they do not discriminate between HDAC6 and the other isozymes.[9] To investigate the selectivity of sulfoxide **15**, the most active compound in this study,

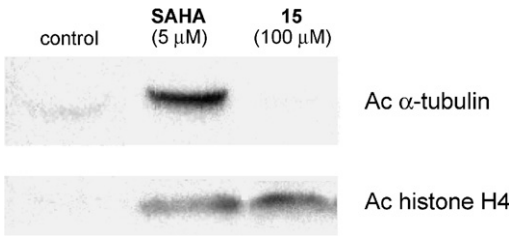


Figure 2. Western blot analysis of histone hyperacetylation and α-tubulin acetylation induction in HCT 116 cells produced by compound **15** and by reference compound SAHA.

Western blot analysis was performed (Fig. 2). HCT116 human colon cancer cells were treated with SAHA or compound **15** for 8 h at 37 °C. As reported previously, 5 μM of SAHA caused the accumulation of both acetylated histone H4 and acetylated α-tubulin. On the other hand, 100 μM of compound **15** did not induce α-tubulin acetylation although the same level of histone H4 hyperacetylation as with SAHA was observed. These results suggested that compound **15** is inactive toward TDAC (HDAC6) in cells.

4. Conclusion

We have designed and prepared a series of SAHA-based non-hydroxamate compounds as (i) analogues bearing functional groups expected to chelate zinc ion bidentately (compounds **1**–**14**) or monodentately (compounds **15** and **16**), (ii) irreversible inhibition-oriented compounds (compounds **17**–**19**), and (iii) 14 Å internal cavity-targeting compounds (compounds **20** and **21**), and evaluated their inhibitory effect on HDACs. Among them, methyl sulfoxide **15** inhibited HDACs in enzyme assays and showed great HDAC/TDAC selectivity in cellular assays.

In conclusion, we have identified a novel lead compound, from which potent HDAC isozyme-selective inhibitors can be developed. The findings of this study should also pave the way for the development of new medicines without side effects caused by interference with microtubule dynamics associated with HDAC6. Currently, detailed structure–activity relationship studies of methyl sulfoxide-based HDAC inhibitors are under way.

5. Experimental section

5.1. Chemistry

Melting points were determined using a Yanagimoto micro melting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a JEOL JNM-LA400 or JEOL JNM-LA500 spectrometer in solvent as indicated. Chemical shifts (δ) were reported in parts per million relative to the internal standard tetramethylsilane. Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the

calculated values. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-SX102A mass spectrometer. GC–MS analyses were performed on a Shimadzu GCMS-QP2010. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku and used without purification. Flash column chromatography was performed using Silica Gel 60 (particle size 0.046–0.063 mm) supplied by Merck.

5.1.1. 6-Phenylcarbamoylhexanoic acid (24b). A mixture of aniline (5.80 g, 62.3 mmol) and pimeric acid (**23b**, 10.0 g, 62.4 mmol) was stirred at 180 °C for 1 h. After cooling, the mixture was diluted with AcOEt–THF and the slurry was filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ and the aqueous layer was acidified with concentrated HCl. The precipitated crystals were collected by filtration to give 7.11 g (49%) of **24b** as a white solid: ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 11.97 (1H, broad s), 9.83 (1H, s), 7.58 (2H, d, J = 7.8 Hz), 7.27 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.4 Hz), 2.67 (2H, t, J = 7.4 Hz), 2.21 (2H, t, J = 7.3 Hz), 1.62–1.49 (4H, m), 1.34–1.27 (2H, m).

5.1.2. Heptanedioic acid methoxymethylamide phenylamide (25). To a solution of **24b** (5.7 g, 24.2 mmol) obtained above and *N*,*O*-dimethylhydroxylamine hydrochloride (4.80 g, 49.2 mmol) in DMF (70 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (6.00 g, 31.3 mmol), 1-hydroxy-1*H*-benzotriazole monohydrate (4.80 g, 31.3 mmol), and triethylamine (7.50 mL, 54.0 mmol) and the mixture was stirred overnight at room temperature. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water, 2 N aqueous HCl, saturated aqueous $NaHCO_3$ and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo and separation by silica gel flash chromatography (*n*-hexane/AcOEt = 8:1) gave 6.34 g (94%) of **25** as a colorless crystal: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.54 (2H, d, J = 7.8 Hz), 7.41 (1H, broad s), 7.31 (2H, t, J = 7.6 Hz), 7.09 (1H, t, J = 7.6 Hz), 3.67 (3H, s), 3.18 (3H, s), 2.45 (2H, t, J = 7.3 Hz), 2.39 (2H, t, J = 7.3 Hz), 1.77 (2H, quintet, J = 7.3 Hz), 1.70 (2H, quintet, J = 7.6 Hz), 1.44 (2H, quintet, J = 7.8 Hz).

5.1.3. 7-Oxoheptanoic acid phenylamide (26). To a suspension of lithium aluminum hydride (300 mg, 7.91 mmol) in THF (30 mL) was added a solution of **25** (2.50 g, 8.98 mmol) obtained above in THF (20 mL) dropwise with cooling by an ice-water bath. The reaction mixture was stirred at 0 °C for 30 min. To the mixture were added water (0.3 mL), 15% aqueous NaOH (0.3 mL), and water (0.9 mL) in a sequential order and the slurry was filtered. After the solid was washed with THF (10 mL), the combined filtrates were concentrated in vacuo. The residue was purified by silica gel flash chromatography (*n*-hexane/AcOEt = 1:1) to give 1.42 g (72%) of **26** as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 9.77 (1H, s), 7.52 (2H, d, J = 8.1 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.25 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.47 (2H, t, J = 7.1 Hz), 2.37 (2H, t, J = 7.3 Hz), 1.76 (2H, quintet, J = 7.3 Hz), 1.68 (2H, quintet, J = 7.4 Hz), 1.42 (2H, quintet, J = 7.3 Hz).

5.1.4. 7-(2-Aminoethylamino)heptanoic acid phenylamide ditrifluoroacedic acid salt (1·2TFA). To a solution of **26** (240 mg, 1.09 mmol) obtained above in CH_2Cl_2 (3 mL) and AcOH (1 mL) was added *N*-(2-aminoethyl)carbamic acid *tert*-butyl ester (190 mg, 1.19 mmol) at room temperature. The solution was stirred for 15 min, followed by the addition of sodium triacetoxy borohydride (475 mg, 2.24 mmol). The solution was stirred overnight at room temperature. To the solution were added triethylamine (12 mL) and di-*tert*-butyl dicarbonate (300 mg, 1.37 mmol), and the mixture was stirred at room temperature for 5 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water, saturated aqueous $NaHCO_3$ and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 5:2) gave 120 mg of colorless oil. To a solution of the oil in CH_2Cl_2 (2 mL) was added trifluoroacetic acid (3 mL), and the mixture was stirred at room temperature for 1 h. The solution was concentrated in vacuo to give a solid. The residue was triturated in ether to give 112 mg (21%) of **1·2TFA** as a crude solid. The solid was recrystallized from AcOEt and collected by filtration to give 103 mg of **1·2TFA** as colorless crystals: mp 113–114 °C; ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 9.87 (1H, s), 7.58 (2H, d, J = 7.8 Hz), 7.28 (2H, t, J = 7.6 Hz), 7.02 (1H, t, J = 7.1 Hz), 3.20–3.05 (4H, m), 2.95 (2H, t, J = 8.1 Hz), 2.31 (2H, t, J = 7.1 Hz), 1.66–1.50 (4H, m), 1.42–1.26 (4H, m); Anal. Calcd for $C_{15}H_{25}N_3O$·2TFA: C, 46.44; H, 5.54; N, 8.55. Found: C, 46.25; H, 5.44; N, 8.27.

5.1.5. Phenylcarbamoylheptanoic acid (24a). Compound **24a** was prepared from aniline and suberic acid in 51% yield by using the same procedure described for **24b**: ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 11.98 (1H, broad s), 9.84 (1H, s), 7.58 (2H, d, J = 7.8 Hz), 7.28 (2H, t, J = 7.8 Hz), 7.01 (1H, t, J = 7.3 Hz), 2.29 (2H, t, J = 7.4 Hz), 2.20 (2H, t, J = 7.3 Hz), 1.58 (2H, t, J = 7.2 Hz), 1.50 (2H, t, J = 7.1 Hz), 1.29 (4H, m).

5.1.6. 8-Methanesulfonylamino-8-oxooctanoic acid phenylamide (6). To a solution of **24a** (500 mg, 2.01 mmol) in DMF (5 mL) was added 1,1-carbonyldiimidazole (360 mg, 2.22 mmol). After 1 h, methanesulfonamide (210 mg, 2.21 mmol) and 1,8-diazabicyclo[5,4,0]-7-undecene (330 μL, 2.21 mmol) were added to the reaction mixture. After overnight stirring, the mixture was poured into 2 N aqueous HCl and the precipitated crystals were collected by filtration to give 595 mg (91%) of **6** as a crude solid. The solid was recrystallized from AcOEt and collected by filtration to give 511 mg of **6** as colorless crystals: mp 145–146 °C; ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 11.64 (1H, broad s), 9.84 (1H, broad s), 7.58 (2H, d, J = 8.0 Hz), 7.28 (2H, t, J = 7.8 Hz), 7.01 (1H, t, J = 7.1 Hz), 3.22 (3H, s), 2.29 (2H, t, J = 7.3 Hz), 2.26 (2H, t, J = 7.3 Hz), 1.58 (2H, quintet, J = 7.4 Hz), 1.52 (2H, quintet,

J = 7.3 Hz), 1.36–1.22 (4H, m); MS (EI) m/z: 326 (M^+); Anal. Calcd for $C_{15}H_{22}N_2O_4S$: C, 55.19; H, 6.79; N, 8.58. Found: C, 55.00; H, 6.72; N, 8.73.

5.1.7. 6-Aminohexanoic acid phenylamide (27). To a suspension of **24b** (1.11 g, 4.73 mmol) and triethylamine (699 mg, 6.90 mmol) in benzene (3 mL) was added diphenylphosphoryl azide (1.83 g, 6.64 mmol) and the mixture was heated at reflux temperature for 1 h. Next, benzyl alcohol (1.20 mL, 11.6 mmol) was added and the reaction mixture was stirred at reflux temperature for 24 h. It was then concentrated in vacuo and the residue was dissolved in AcOEt. The AcOEt solution was washed with 0.4 N aqueous HCl, water, saturated aqueous $NaHCO_3$, and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo, and purification by recrystallization from $CHCl_3$–*n*-hexane gave 1.01 g (63%) of (6-phenylcarbamoylpentyl)carbamic acid benzyl ester as a colorless needle: ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 9.81 (1H, s), 7.57 (2H, d, J = 7.8 Hz), 7.37–7.22 (8H, m), 7.00 (1H, t, J = 7.4 Hz), 4.99 (2H, s), 2.99 (2H, q, J = 6.5 Hz), 2.28 (2H, t, J = 7.4 Hz), 1.58 (2H, quintet, J = 7.6 Hz), 1.43 (2H, quintet, J = 7.1 Hz), 1.32 (2H, quintet, J = 7.8 Hz); MS (EI) m/z: 340 (M^+).

A solution of (6-phenylcarbamoylpentyl)carbamic acid benzyl ester(1.00 g, 2.95 mmol) obtained above in MeOH (50 mL) was stirred under H_2 (atmospheric pressure) in the presence of 5% Pd/C (106 mg) at room temperature for 7 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo. The residue was purified by silica gel flash chromatography ($CHCl_3$/MeOH/*i*PrNH$_2$ = 19:1:1) to give 584 mg (96%) of **27** as a white solid: ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 9.83 (1H, s), 7.58 (2H, d, J = 7.6 Hz), 7.27 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.3 Hz), 2.55 (2H, m), 2.29 (2H, t, J = 7.4 Hz), 1.59 (2H, quintet, J = 7.4 Hz), 1.37–1.30 (4H, m).

5.1.8. 6-(3-Oxobutyrylamino)hexanoic acid phenylamide (2). To a solution of **27** (107 mg, 0.52 mmol) obtained above in MeOH (3 mL) was added diketene (0.3 mL, 3.89 mmol) at 0 °C and the resulting mixture was stirred at room temperature for 9 h. The solution was concentrated in vacuo to give a crude solid. The solid was purified by silica gel flash column chromatography (AcOEt only to AcOEt/MeOH = 9:1) to give 108 mg (72%) of **2** as a yellow solid. The solid (108 mg) was recrystallized from *n*-hexane–AcOEt–MeOH and collected by filtration to give 69 mg of **2** as brown crystals: mp 126–128 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.85 (1H, s), 8.02 (1H, s), 7.56 (2H, d, J = 8.2 Hz), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.3 Hz), 3.27 (2H, s), 3.05 (2H, q, J = 6.5 Hz), 2.29 (2H, t, J = 7.5 Hz), 2.12 (3H, s), 1.58 (2H, quintet, J = 7.3 Hz), 1.43 (2H, quintet, J = 7.5 Hz), 1.30 (2H, quintet, J = 7.8 Hz); MS (EI) m/z: 290 (M^+); Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.04; H, 7.40; N, 9.48.

Compounds **3**, **12**, **13**, **17**, and **18** were prepared from amine **27** and an appropriate carboxylic acid using the procedure described for **25**.

5.1.9. *N*-(5-Phenylcarbamoylpentyl)malonamic acid ethyl ester (3). Mp 107–110 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.52 (2H, d, J = 8.2 Hz), 7.32 (2H, t, J = 7.9 Hz), 7.28 (1H, broad s), 7.18 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 4.19 (2H, q, J = 7.1 Hz), 3.31 (2H, q, J = 6.6 Hz), 3.29 (2H, s), 2.37 (2H, t, J = 7.5 Hz), 1.77 (2H, quintet, J = 7.5 Hz), 1.59 (2H, quintet, J = 7.3 Hz), 1.43 (2H, quintet, J = 7.5 Hz), 1.28 (3H, t, J = 7.2 Hz); MS (EI) m/z: 320 (M^+); Anal. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.82; H, 7.65; N, 8.47.

5.1.10. Thiophene-2-carboxylic acid (5-phenylcarbamoylpentyl)amide (12). Mp 139–141 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.53 (2H, d, J = 7.6 Hz), 7.52 (1H, d, J = 4.9 Hz), 7.45 (1H, d, J = 4.9 Hz), 7.35 (1H, broad s), 7.31 (2H, t, J = 7.9 Hz), 7.10 (1H, t, J = 7.3 Hz), 7.04 (1H, t, J = 4.9 Hz), 6.27 (1H, broad s), 3.47 (2H, q, J = 6.6 Hz), 2.40 (2H, t, J = 7.2 Hz), 1.80 (2H, quintet, J = 7.4 Hz), 1.67 (2H, quintet, J = 7.0 Hz), 1.48 (2H, quintet, J = 7.7 Hz); MS (EI) m/z: 316 (M^+); HRMS Calcd for $C_{17}H_{20}N_2O_2S$ 316.125. Found 316.125.

5.1.11. Thiazole-2-carboxylic acid (5-phenylcarbamoylpentyl)amide (13). Mp 130–133 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.84 (1H, broad s), 8.85 (1H, broad s), 8.02 (1H, d, J = 3.4 Hz), 7.99 (1H, d, J = 3.1 Hz), 7.57 (2H, d, J = 7.9 Hz), 7.27 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.0 Hz), 3.26 (2H, q, J = 6.7 Hz), 2.30 (2H, t, J = 7.5 Hz), 1.59 (2H, quintet, J = 7.6 Hz), 1.56 (2H, quintet, J = 7.9 Hz), 1.32 (2H, quintet, J = 7.5 Hz); MS (EI) m/z: 317 (M^+); HRMS Calcd for $C_{16}H_{19}N_3O_2$ S 317.120. Found 317.119.

5.1.12. *N*-(5-Phenylcarbamoylpentyl)acrylamide (17). Mp 166–168 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.84 (1H, s), 8.07 (1H, s), 7.58 (2H, d, J = 7.9 Hz), 7.27 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.5 Hz), 6.20 (1H, dd, J = 10.1, 17.1 Hz), 6.05 (1H, dd, J = 2.2, 17.1 Hz), 5.55 (1H, dd, J = 2.2, 10.1 Hz), 3.12 (2H, q, J = 5.8 Hz), 2.29 (2H, t, J = 7.5 Hz), 1.59 (2H, quintet, J = 7.5 Hz), 1.45 (2H, quintet, J = 7.9 Hz), 1.30 (2H, quintet, J = 7.3 Hz); MS (EI) m/z: 260 (M^+); Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76. Found: C, 68.97; H, 7.76; N, 10.64.

5.1.13. Propynoic acid (5-phenylcarbamoylpentyl)amide (18). Mp 143–145 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.84 (1H, s), 8.70 (1H, s), 7.58 (2H, d, J = 7.9 Hz), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.3 Hz), 4.09 (1H, s), 3.07 (2H, q, J = 6.7 Hz), 2.29 (2H, t, J = 7.3 Hz), 1.58 (2H, quintet, J = 7.3 Hz), 1.43 (2H, quintet, J = 7.6 Hz), 1.28 (2H, quintet, J = 7.3 Hz); MS (EI) m/z: 258 (M^+); HRMS Calcd for $C_{15}H_{18}N_2O_2$: 258.137. Found: 258.137.

5.1.14. *N*-(5-Phenylcarbamoylpentyl)malonamic acid (4). To a solution of **3** (134 mg, 0.42 mmol) in MeOH (3 mL) was added 2 N aqueous NaOH (0.5 mL, 1.00 mmol), and the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated and dissolved in AcOEt. The solution was washed with 2 N aqueous HCl and brine, and was dried over Na_2SO_4. Filtration and

concentration in vacuo gave a crude solid. The solid was recrystallized from *n*-hexane–AcOEt and collected by filtration to give 54 mg (43%) as colorless crystals: mp 128–130 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.84 (1H, s), 8.32 (1H, s), 8.02 (1H, s), 7.58 (2H, d, J = 7.6 Hz), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.5 Hz), 3.10 (2H, s), 3.05 (2H, q, J = 6.4 Hz), 2.29 (2H, t, J = 7.5 Hz), 1.59 (2H, quintet, J = 7.5 Hz), 1.43 (2H, quintet, J = 7.3 Hz), 1.30 (2H, quintet, J = 7.6 Hz); Anal. Calcd for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.67; H, 6.95; N, 9.47.

5.1.15. *N*-(5-Phenylcarbamoylpentyl)acrylamide (5). To a solution of **3** (61 mg, 0.19 mmol) in MeOH (1.5 mL) was added 25% aqueous NH_3 (1.5 mL), and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the residue was triturated in AcOEt to give 16 mg (28%) of **5** as a crude solid. The solid was recrystallized from *n*-hexane–AcOEt–MeOH and collected by filtration to give 13 mg of **5** as colorless crystals: mp 166–168 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.84 (1H, s), 7.97 (1H, t, J = 5.3 Hz), 7.58 (2H, d, J = 7.9 Hz), 7.41 (1H, broad s), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.31 Hz), 6.99 (1H, broad s), 3.05 (2H, q, J = 6.5 Hz), 2.97 (2H, s), 2.28 (2H, t, J = 7.6 Hz), 1.58 (2H, quintet, J = 7.6 Hz), 1.43 (2H, quintet, J = 7.2 Hz), 1.30 (2H, quintet, J = 7.5 Hz); MS (EI) m/z: 291 (M^+); Anal. Calcd for $C_{15}H_{21}N_3O_3$: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.68; H, 7.25; N, 14.17.

5.1.16. (5-Phenylcarbamoylpentylcarbamoyl)phosphonic acid diethyl ester (8). Under Ar atmosphere, to a solution of ethyl chloroformate (1.14 g, 9.12 mmol) in dry toluene (50 mL) was slowly added triethylphosphite (2.82 g, 17.0 mmol) at 0 °C and the resulting mixture was stirred at room temperature for 4 h. The solution was concentrated and purified by silica gel flash column chromatography (AcOEt/*n*-hexane = 1:2) to give 1.25 g (61%) of *S*-ethyl diethylphosphonothiolformate as a crude oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 4.25 (4H, m), 3.01 (2H, q, J = 8.5 Hz), 1.37 (6H, t, J = 5.5 Hz), 1.28 (3H, t, J = 9.3 Hz); MS (EI) m/z 226 (M^+).

To a solution of *S*-ethyl diethylphosphonothiolformate (679 mg, 3.00 mmol) obtained above in MeCN (5 mL) was added **27** (215 mg, 1.04 mmol), and the solution was stirred overnight at room temperature. The solution was concentrated and purified by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/2 to AcOEt only) to give 208 mg (54%) of **8** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.65 (1H, broad s), 7.55 (2H, d, J = 7.9 Hz), 7.30 (2H, t, J = 7.9 Hz), 7.22 (1H, broad s), 7.09 (1H, t, J = 7.5 Hz), 4.22 (4H, m), 3.34 (2H, q, J = 6.6 Hz), 2.36 (2H, t, J = 7.3 Hz), 1.75 (2H, quintet, J = 7.6 Hz), 1.59 (2H, quintet, J = 7.5 Hz), 1.41 (2H, quintet, J = 7.6 Hz), 1.36 (6H, t, J = 7.2 Hz); MS (EI) m/z: 370 (M^+); HRMS Calcd for $C_{17}H_{27}N_2O_5P$ 370.166. Found 370.165.

5.1.17. (5-Phenylcarbamoylpentylcarbamoyl)phosphonic acid (9). A solution of **8** (156 mg, 0.42 mmol) and bromotrimethylsilane (614 mg, 4.01 mmol) in MeCN (5 mL) was stirred overnight at room temperature. After the addition of MeOH (1 mL), the solution was concentrated in vacuo and the residue was triturated in AcOEt–MeOH–*n*-hexane to give 57 mg (43%) of **9** as a crude solid. The solid was recrystallized from AcOEt–MeOH and collected by filtration to give 53 mg of **9** as colorless crystals: mp 154–156 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.85 (1H, s), 8.35 (1H, broad s), 7.58 (2H, d, J = 8.2 Hz), 7.28 (2H, t, J = 8.1 Hz), 7.01 (1H, t, J = 7.3 Hz), 3.10 (2H, q, J = 6.7 Hz), 2.29 (2H, t, J = 7.6 Hz), 1.59 (2H, quintet, J = 7.6 Hz), 1.46 (2H, quintet, J = 7.6 Hz), 1.27 (2H, quintet, J = 7.6 Hz); Anal. Calcd for $C_{13}H_{19}N_2O_5P$: C, 49.68; H, 6.09; N, 8.91. Found: C, 49.51; H, 6.06; N, 8.82.

5.1.18. [(5-Phenylcarbamoylpentylcarbamoyl)methyl]-phosphonic acid diethyl ester (10). A mixture of **27** (618 mg, 3.00 mmol), Et_3N (1.0 mL, 7.20 mmol), and bromoacetyl chloride (1.0 mL, 6.5 mmol) in THF (20 mL) was stirred at room temperature for 1 h. The reaction mixture was diluted with $CHCl_3$. The solution was washed with saturated aqueous $NaHCO_3$, water, and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo, and purification by silica gel flash column chromatography gave 693 mg (71%) of a 1:1 mixture of bromoacetyl 6-anilino-6-oxohexanamide and chloroacetyl 6-anilino-6-oxohexanamide as a light yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.52 (2H, d, J = 8.1 Hz), 7.32 (2H, t, J = 7.8 Hz), 7.10 (1H, t, J = 7.3 Hz), 6.64, 6.57 (1H, broad s), 4.03, 3.86 (2H, s), 3.33 (2H, m), 2.38 (2H, t, J = 7.4 Hz), 1.78 (2H, m), 1.61 (2H, m), 1.43 (2H, m).

To a solution of the solid (164 mg, 0.54 mmol) obtained above in $P(OEt)_3$ (852 mg, 5.13 mmol) was added tetrabutylammonium iodide (81.9 mg, 0.22 mmol). The mixture was stirred overnight at reflux temperature. After cooling, the reaction mixture was evaporated in vacuo to remove triethylphosphite. Purification by silica gel flash column chromatography (AcOEt only to AcOEt/MeOH = 9:1) gave 147 mg of **10** as a colorless oil (74%): ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.94 (1H, broad s), 7.57 (2H, d, J = 7.9 Hz), 7.30 (2H, t, J = 7.9 Hz), 7.08 (1H, t, J = 7.3 Hz), 6.89 (1H, broad s), 4.13 (4H, m), 3.27 (2H, q, J = 6.5 Hz), 2.84 (2H, d, J = 20.7 Hz), 2.35 (2H, t, J = 7.3 Hz), 1.75 (2H, quintet, J = 7.5 Hz), 1.56 (2H, quintet, J = 7.3 Hz), 1.42 (2H, quintet, J = 7.5 Hz), 1.33 (6H, t, J = 7.0 Hz); MS (EI) m/z: 384 (M^+); HRMS Calcd for $C_{18}H_{29}N_2O_5P$ 384.181, Found 384.182.

5.1.19. Thiazolidine-2-carboxylic acid (5-phenylcarbamoylpentyl)amide hydrochloric acid salt (11·HCl). To a solution of thiazolidine-2-carboxylic acid (704 mg, 5.29 mmol) in MeOH (10 mL) was added Boc_2O (1.97 g, 9.04 mmol) and Et_3N (0.5 mL), and the mixture was stirred overnight at room temperature. The solution was concentrated, dissolved with AcOEt and the AcOEt solution was extracted with saturated aqueous $NaHCO_3$ and the aqueous layer was neutralized with 2 N HCl. The aqueous layer was extracted with ether and the organic layer was washed with brine, and dried over Na_2SO_4. Filtration and concentration in vacuo, and

purification by silica gel flash column chromatography (*n*-hexane/AcOEt = 2:1) gave 966 mg of thiazolidine-2,3-dicarboxylic acid 3-*tert*-butyl ester (78%) as a crude solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 11.09 (1H, broad s), 5.33, 5.15 (1H, s), 3.99, 3.83 (2H, m), 3.24, 3.01 (2H, m), 1.48, 1.45 (9H, s).

2-(5-Phenylcarbamoylpentylcarbamoyl)thiazolidine-3-carboxylic acid *tert*-butyl ester was prepared from amine **27** and thiazolidine-2,3-dicarboxylic acid 3-*tert*-butyl ester obtained above in 79% yield using the procedure described for **25**. ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.85 (1H, s), 7.98 (1H, broad s), 7.58 (2H, d, J = 7.9 Hz), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.5 Hz), 5.14, 5.02 (1H, s), 3.75, 3.67 (2H, m), 3.06 (2H, m), 2.98 (2H, m), 2.29 (2H, t, J = 7.7 Hz), 1.58 (2H, quintet, J = 7.5 Hz), 1.40 (2H, m), 1.34 (9H, s), 1.32 (2H, quintet, J = 7.5 Hz); MS (EI) *m*/*z*: 421 (M^+).

To a solution of 2-(5-phenylcarbamoylpentylcarbamoyl)-thiazolidine-3-carboxylic acid *tert*-butyl ester (180 mg, 0.43 mmol) in AcOEt (5 mL) was added 4 N HCl·AcOEt (1.0 mL, 4.00 mmol), and the mixture was stirred overnight at room temperature. The solution was concentrated in vacuo and triturated in AcOEt–MeOH–*n*-hexane to give 112 mg (81%) of **11·HCl** as a crude solid. The solid was recrystallized from AcOEt–MeOH and collected by filtration to give 102 mg as colorless crystals: mp 144–147 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.90 (1H, s), 8.56 (1H, broad s), 7.59 (2H, d, J = 7.6 Hz), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.5 Hz), 5.17 (1H, s), 3.60 (1H, m), 3.45 (1H, m), 3.11 (4H, m), 2.30 (2H, t, J = 7.1 Hz), 1.59 (2H, quintet, J = 7.5 Hz), 1.47 (2H, quintet, J = 7.0 Hz), 1.31 (2H, quintet, J = 7.5 Hz); MS (EI) *m*/*z*: 321 (M^+); HRMS Calcd for $C_{16}H_{23}N_3O_2S$ 321.151. Found 321.151.

5.1.20. 6-(2-1*H*-Tetrazol-5-ylacetylamino)hexanoic acid phenylamide (14). A solution of 1*H*-tetrazole-5-acetic acid (76.8 mg, 0.60 mmol) and **27** (105 mg, 0.51 mmol) in CH_2Cl_2 (1 mL) was cooled to 0 °C and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (206 mg, 0.79 mmol) was added. The mixture was stirred overnight at room temperature and concentrated in vacuo. To the residue was added saturated aqueous NaCl and the precipitate was collected to give 160 mg (99%) of **14** as a pink solid. The solid was recrystallized from MeOH and collected by filtration to give 14 mg of **14** as pink crystals: mp 174–176 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.88 (1H, s), 8.34 (1H, broad s), 7.58 (2H, d, J = 7.6 Hz), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.5 Hz), 3.86 (2H, s), 3.09 (2H, q, J = 6.5 Hz), 2.30 (2H, t, J = 7.5 Hz), 1.59 (2H, quintet, J = 7.1 Hz), 1.45 (2H, quintet, J = 7.5 Hz), 1.31 (2H, quintet, J = 7.8 Hz); Anal. Calcd for $C_{15}H_{20}N_6O_2 \cdot 1/4H_2O$: C, 56.15; H, 6.44; N, 26.19. Found: C, 56.07; H, 6.25; N, 26.30.

5.1.21. 6-Chloromethanesulfonylaminohexanoic acid phenylamide (19). To a solution of **27** (153 mg, 0.74 mmol) and triethylamine (0.40 mL, 2.87 mmol) in $CHCl_3$ (3 mL) was added chloromethylsulfonyl chloride (311 mg, 2.09 mmol) dropwise with cooling by an ice-water bath. The solution was stirred at room temperature for 30 min. The reaction mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was separated, washed with water, 1 N aqueous HCl and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo, and purification by silica gel flash column chromatography (*n*-hexane/AcOEt = 2:1 to 1:1) gave 54 mg (23%) of **19** as a yellow solid. The solid was recrystallized from *n*-hexane–$CHCl_3$ and collected by filtration to give 46 mg of **19** as colorless crystals: mp 122–124 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.51 (2H, d, J = 7.9 Hz), 7.32 (2H, t, J = 7.9 Hz), 7.15 (1H, broad s), 7.11 (1H, t, J = 7.3 Hz), 4.76 (1H, broad s), 4.49 (2H, s), 3.23 (2H, q, J = 6.7 Hz), 2.39 (2H, t, J = 7.2 Hz), 1.77 (2H, quintet, J = 7.5 Hz), 1.65 (2H, quintet, J = 7.2 Hz), 1.48 (2H, quintet, J = 7.7 Hz); MS (EI) *m*/*z*: 318 (M^+); Anal. Calcd for $C_{13}H_{19}ClN_2O_3S$: C, 48.97; H, 6.01; N, 8.79. Found: C, 48.88; H, 5.85; N, 8.71.

5.1.22. 7-Chlorosulfonylheptanoic acid ethyl ester (29). To an aqueous solution (7 mL) of anhydrous sodium sulfite (2.03 g, 16.1 mmol) was added a solution of 7-bromoheptanoic acid ethyl ester (**28**, 2.0 g, 8.43 mmol) in EtOH (5 mL) and the solution was boiled under reflux with stirring for 2 h. The solution was evaporated to dryness and the solid was dried in vacuo at 60 °C. This white solid was placed in a flask, toluene (30 mL) was added followed by a catalytic amount of DMF, and then thionyl chloride (6.2 mL, 85.0 mmol) was added dropwise. The mixture was boiled under reflux with stirring for 5 h, diluted with AcOEt, washed with aqueous saturated cold water, and brine, and dried over $MgSO_4$. Filtration and concentration in vacuo, and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 4:1) gave 2.02 g (93%) of **29**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 4.13 (2H, q, J = 7.1 Hz), 3.66 (2H, t, J = 7.8 Hz), 2.31 (2H, t, J = 7.3 Hz), 2.06 (2H, quintet, J = 7.8 Hz), 1.66 (2H, quintet, J = 7.3 Hz), 1.53 (2H, quintet, J = 7.8 Hz), 1.41 (2H, quintet, J = 7.1 Hz), 1.26 (2H, quintet, J = 7.1 Hz).

5.1.23. 7-Sulfamoylheptanoic acid phenylamide (16). To a mixture of 25% aqueous NH_3 (10 mL), a catalytic amount of 4-(dimethylamino)pyridine, and THF (20 mL) was added a solution of **29** (1.33 g, 5.18 mmol) obtained above in THF (10 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water, saturated aqueous $NaHCO_3$ and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 1:1) gave 1.11 g (91%) of 7-sulfamoylheptanoic acid ethyl ester as a crude oil.

Compound **16** was prepared from 7-sulfamoylheptanoic acid ethyl ester obtained above in 64% yield using the procedure described for **4** and **25**: mp 163–164 °C; ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 9.85 (1H, broad s), 7.58 (2H, d, J = 8.1 Hz), 7.28 (2H, t, J = 7.6 Hz), 7.01 (1H, t, J = 7.1 Hz), 6.72 (2H, broad s), 2.95 (2H, t, J = 7.8 Hz), 2.30 (2H, t, J = 7.6 Hz), 1.69 (2H, quintet, J = 8.0 Hz), 1.59 (2H, quintet, J = 7.6 Hz), 1.40 (2H,

quintet, $J = 7.8$ Hz), 1.32 (2H, quintet, $J = 7.1$ Hz); MS (EI) *m/z*: 284 (M^+); Anal. Calcd for $C_{13}H_{20}N_2O_3S$: C, 54.91; H, 7.09; N, 9.85. C, 54.83; H, 7.18; N, 9.66.

5.1.24. 7-Acetylsulfamoylheptanoic acid phenylamide (7). Compound **7** was prepared from **16** obtained above and acetic acid in 61% yield using the procedure described for **6**: mp 174–176 °C; ^{1}H NMR (DMSO-d_6 400 MHz, δ; ppm) 11.60 (1H, broad s), 9.85 (1H, broad s), 7.58 (2H, d, $J = 7.6$ Hz), 7.28 (2H, t, $J = 7.6$ Hz), 7.01 (1H, t, $J = 7.3$ Hz), 3.34 (2H, t, $J = 7.8$ Hz), 2.29 (2H, t, $J = 7.3$ Hz), 1.65 (2H, quintet, $J = 7.8$ Hz), 1.57 (2H, quintet, $J = 7.3$ Hz), 1.40 (2H, quintet, $J = 7.6$ Hz), 1.30 (2H, quintet, $J = 7.6$ Hz); Anal. Calcd for $C_{15}H_{22}N_2O_4S \cdot 1/10H_2O$: C, 54.89; H, 6.82; N, 8.54. Found: C, 55.03; H, 6.83; N, 8.16.

5.1.25. 7-Bromoheptanoic acid phenylamide (32). 7-Bromoheptanoic acid was prepared from **28** in 99% yield using the procedure described for **4**. In this case, LiOH was used instead of NaOH: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 3.41 (2H, t, $J = 6.8$ Hz), 2.37 (2H, t, $J = 7.3$ Hz), 1.87 (2H, quintet, $J = 6.8$ Hz), 1.66 (2H, quintet, $J = 7.6$ Hz), 1.54–1.32 (4H, m).

To a suspension of 7-bromoheptanoic acid (2.64 g, 12.6 mmol) obtained above in CH_2Cl_2 (30 mL) were added oxalyl chloride (1.65 mL, 18.9 mmol) and a catalytic amount of DMF. The mixture was stirred at room temperature for 2 h. The solvent was removed by evaporation in vacuo to give acid chloride **31**. To a solution of aniline (3.50 g, 37.6 mmol) and triethylamine (5.30 mL, 38.1 mmol) in CH_2Cl_2 (40 mL) was added a solution of **31** obtained above in CH_2Cl_2 (10 mL) dropwise cooling in an ice-water bath. The mixture was stirred at room temperature for 1 h. It was diluted with AcOEt and washed with aqueous saturated $NaHCO_3$, water and brine, before being dried over $MgSO_4$. Filtration and concentration in vacuo, and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 3:1) gave 3.13 g (87%) of **32**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, $J = 8.1$ Hz), 7.32 (2H, t, $J = 7.6$ Hz), 7.15 (1H, broad s), 7.10 (1H, t, $J = 7.6$ Hz), 3.41 (2H, t, $J = 6.8$ Hz), 2.36 (2H, t, $J = 7.3$ Hz), 1.87 (2H, quintet, $J = 7.1$ Hz), 1.75 (2H, quintet, $J = 7.3$ Hz), 1.49 (2H, quintet, $J = 7.6$ Hz), 1.41 (2H, quintet, $J = 6.8$ Hz).

5.1.26. 7-Methylsulfanylheptanoic acid phenylamide (33). To a solution of **32** (300 mg, 1.06 mmol) in EtOH (10 mL) was added methylmercaptan sodium salt (15% in water, 1.50 g, 3.21 mmol) and the solution was stirred at room temperature for 5 h. The reaction mixture was diluted with AcOEt and washed with water and brine, and was dried over $MgSO_4$. Filtration and concentration in vacuo, and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 2:1) gave 262 mg (99%) of **33** as a crude solid. The solid was recrystallized from *n*-hexane–AcOEt and collected by filtration to give 217 mg of **33** as a colorless crystal: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, $J = 8.0$ Hz), 7.32 (2H, t, $J = 7.8$ Hz), 7.16 (1H, broad s), 7.10 (1H, t, $J = 7.6$ Hz), 2.49 (2H, t, $J = 7.1$ Hz), 2.36 (2H, t, $J = 7.3$ Hz), 2.09 (3H, s), 1.74 (2H, quintet, $J = 7.3$ Hz), 1.61 (2H, quintet, $J = 7.3$ Hz), 1.42 (4H, m); MS (EI) *m/z*: 251 (M^+).

5.1.27. 7-Methanesulfinylheptanoic acid phenylamide (15). To a solution of **33** (80 mg, 0.32 mmol) obtained above in CH_2Cl_2 (3 mL) was added 3-chloroperoxybenzoic acid (65%, 85 mg, 0.32 mmol). The mixture was stirred at room temperature for 30 min. To the reaction mixture was added saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was separated, washed with water and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (AcOEt/MeOH = 10:1) gave 66 mg (77%) of **15** as a crude solid. The solid was recrystallized from *n*-hexane–AcOEt and collected by filtration to give 50 mg of **15** as colorless crystals: mp 121–122 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.65 (1H, broad s), 7.54 (2H, d, $J = 7.8$ Hz), 7.31 (2H, t, $J = 7.6$ Hz), 7.09 (1H, t, $J = 7.3$ Hz), 2.80–2.60 (2H, m), 2.56 (3H, s), 2.36 (2H, t, $J = 7.4$ Hz), 1.90–1.65 (4H, m), 1.65–1.35 (4H, m); MS (EI) *m/z*: 267 (M^+); Anal. Calcd for $C_{14}H_{21}NO_2S$: C, 62.89; H, 7.92; N, 5.35. Found: C, 62.64; H, 7.89; N, 5.35.

5.1.28. 5-(6-Phenylcarbamoylhexylsulfanyl)pentanoic acid ethyl ester (35). To a solution of a mixture of **32** (2.80 g, 9.85 mmol) in EtOH (30 mL) was added potassium thioacetate (1.70 g, 14.9 mmol). The mixture was stirred at reflux temperature for 7 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water, saturated aqueous $NaHCO_3$ and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 5:2) gave 2.70 g (98%) of thioacetic acid *S*-(6-phenylcarbamoylhexyl) ester as a crude solid: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, $J = 8.0$ Hz), 7.32 (2H, t, $J = 7.3$ Hz), 7.22 (1H, broad s), 7.10 (1H, t, $J = 7.3$ Hz), 2.86 (2H, t, $J = 7.1$ Hz), 2.35 (2H, t, $J = 7.3$ Hz), 2.32 (3H, s), 1.73 (2H, quintet, $J = 7.1$ Hz), 1.59 (2H, quintet, $J = 7.1$ Hz), 1.40 (4H, m); MS (EI) *m/z*: 279 (M^+).

Compound **34** was prepared from **32** obtained above in 87% yield using the procedure described for **4**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, $J = 8.0$ Hz), 7.32 (2H, t, $J = 7.6$ Hz), 7.12 (1H, broad s), 7.10 (1H, t, $J = 7.1$ Hz), 2.53 (2H, q, $J = 7.3$ Hz), 2.36 (2H, t, $J = 7.6$ Hz), 1.74 (2H, quintet, $J = 7.1$ Hz), 1.63 (2H, quintet, $J = 7.1$ Hz), 1.42 (4H, m), 1.33 (1H, t, $J = 7.8$ Hz); MS (EI) *m/z*: 237 (M^+).

To a solution of **34** (1.0 g, 4.21 mmol) obtained above and 5-bromovaleric acid ethyl ester (1.0 mL, 6.32 mmol) in EtOH (15 mL) was added sodium ethoxide in EtOH (20%, 1.60 g, 4.70 mmol) with cooling by an ice-water bath. The solution was stirred overnight at room temperature. The reaction mixture was poured into ice water and extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and was

dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 2:1) gave 1.39 g (91%) of **35** as a white solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.52 (2H, d, J = 7.9 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.20 (1H, broad s), 7.10 (1H, t, J = 7.4 Hz), 4.13 (2H, q, J = 7.2 Hz), 2.53–2.48 (4H, m), 2.36 (2H, t, J = 7.4 Hz), 2.32 (2H, t, J = 7.3 Hz), 1.76–1.70 (4H, m), 1.63–1.57 (4H, m), 1.50–1.35 (4H, m), 1.25 (3H, t, J = 7.1 Hz); MS (EI) *m*/*z*: 365 (M^+).

5.1.29. 5-(6-Phenylcarbamoylhexane-1-sulfinyl)pentanoic acid (20). 5-(6-Phenylcarbamoylhexane-1-sulfinyl)pentanoic acid ethyl ester was prepared from **35** obtained above in 81% yield using the procedure described for **15**: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.53 (2H, d, J = 7.9 Hz), 7.48 (1H, broad s), 7.31 (2H, t, J = 7.7 Hz), 7.09 (1H, t, J = 7.3 Hz), 4.13 (2H, q, J = 7.0 Hz), 2.69 (2H, quintet, J = 7.1 Hz), 2.64 (2H, quintet, J = 6.7 Hz), 2.38–2.34 (4H, m), 1.83–1.75 (8H, m), 1.60–1.40 (4H, m), 1.26 (3H, t, J = 7.0 Hz); MS (EI) *m*/*z*: 381 (M^+).

Compound **20** was prepared from 5-(6-phenylcarbamoylhexane-1-sulfinyl)pentanoic acid ethyl ester obtained above in 98% yield using the procedure described for **4**: mp 122–123 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.85 (1H, broad s), 7.54 (2H, d, J = 7.9 Hz), 7.30 (2H, t, J = 7.7 Hz), 7.09 (1H, t, J = 7.4 Hz), 2.78 (2H, m), 2.67 (2H, m), 2.39 (2H, t, J = 6.5 Hz), 2.36 (2H, t, J = 7.6 Hz), 1.88–1.66 (8H, m), 1.58–1.35 (4H, m); MS (EI) *m*/*z*: 353 (M^+); Anal. Calcd for $C_{18}H_{27}NO_4S$·1/5MeOH: C, 60.74; H, 7.79; N, 3.89. Found: C, 60.96; H, 7.73; N, 3.52.

5.1.30. 5-(6-Phenylcarbamoylhexane-1-sulfonyl)pentanoic acid (21). To a solution of **35** (600 mg, 1.64 mmol) in CH_2Cl_2 (10 mL) was added 3-chloroperoxybenzoic acid (65%, 960 mg, 3.62 mmol). The mixture was stirred overnight at room temperature. To the reaction mixture was added saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was separated, washed with water and brine, and was dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 1:3) gave 650 mg (99%) of 5-(6-phenylcarbamoylhexane-1-sulfonyl)pentanoic acid ethyl ester as a crude solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.52 (2H, d, J = 8.0 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.28 (1H, broad s), 7.10 (1H, t, J = 7.6 Hz), 4.14 (2H, q, J = 7.0 Hz), 2.98–2.23 (4H, m), 2.38–2.34 (4H, m),1.95–1.83 (4H, m), 1.81–1.73 (4H, m), 1.51 (2H, quintet, J = 7.7 Hz), 1.45 (2H, quintet, J = 7 Hz), 1.26 (3H, t, J = 7.3 Hz); MS (EI) *m*/*z*: 397 (M^+).

Compound **21** was prepared from 5-(6-phenylcarbamoylhexane-1-sulfonyl)pentanoic acid ethyl ester obtained above in 94% yield using the procedure described for **4**: mp 153–154 °C; ^{1}H NMR (DMSO-d_6 500 MHz, δ; ppm) 12.1 (1H, broad s), 9.85 (1H, s), 7.58 (2H, d, J = 8.6 Hz), 7.28 (2H, t, J = 7.7 Hz), 7.01 (1H, t, J = 7.3 Hz), 3.08 (2H, t, J = 7.3 Hz), 3.05 (2H, t, J = 7.3 Hz), 2.30 (2H, t, J = 7.3 Hz), 2.26 (2H, t, J = 7.3 Hz), 1.73–1.52 (8H, m), 1.41 (2H, quintet, J = 7.4 Hz), 1.33 (2H, quintet, J = 7.3 Hz); MS (EI) *m*/*z*: 369 (M^+); Anal. Calcd for $C_{18}H_{27}NO_5S$: C, 58.51; H, 7.37; N, 3.79. Found: C, 58.43; H, 7.42; N, 3.52.

5.2. Biology

5.2.1. HDAC inhibitory activity assay. The assay of HDAC activity was performed using an HDAC fluorescent activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories). HeLa nuclear extracts (0.5 μL/well) were incubated at 37 °C with 25 μM of Fluor de Lys™ substrate and various concentrations of samples. Reactions were stopped after 30 min by adding Fluor de Lys™ Developer with trichostatin A, which stops further deacetylation. Then, 15 min after addition of this developer, the fluorescence of the wells was measured on a fluorometric reader with excitation set at 360 nm and emission detection set at 460 nm, and the % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells. The concentration of compound which results in 50% inhibition was determined by plotting the log[Inh] versus the logit function of the % inhibition. IC_{50} values of SAHA and compound **15** are determined using a regression analysis of the concentration/inhibition data.

5.2.2. Western blot analysis. HCT116 human colon cancer cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, U.S.A.) and cultured in McCoy5A culture medium containing penicillin and streptomycin, supplemented with fetal bovine serum as described in the ATCC instructions. HCT-116 cells (5×10^5) treated for 8 h with samples at the indicated concentrations in 10% FBS-supplemented McCoy's 5A medium were collected and sonicated. Protein concentrations of the lysates were determined by using a Bradford protein assay kit (Bio-Rad Laboratories); equivalent amounts of proteins from each lysate were resolved in 15% SDS-polyacrylamide gel and then transferred onto nitrocellulose membranes (Bio-Rad Laboratories). After blocking with Tris-buffered saline (TBS) containing 3% skimmed milk for 30 min, the transblotted membrane was incubated with hyperacetylated histone H4 antibody (Upstate Biotechnology) (1:2000 dilution) or acetylated α-tubulin antibody (SIGMA) (1:2000 dilution) in TBS containing 3% skimmed milk at 4 °C overnight. After probed with the primary antibody, the membrane was washed twice with water, followed by incubation with goat anti-rabbit or anti-mouse IgG-horseradish peroxidase conjugates (diluted 1:10000 or 1:5000) for 1.5 h at room temperature and washed twice with water. The immunoblots were visualized by enhanced chemiluminescence.

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