Design, Synthesis, Structure−Selectivity Relationship, and Effect on Human Cancer Cells of a Novel Series of Histone Deacetylase 6-Selective Inhibitors

Yukihiro Itoh,[†] Takayoshi Suzuki,*[,†] Akiyasu Kouketsu,[†] Nobuaki Suzuki,[†] Satoko Maeda,[‡] Minoru Yoshida,[‡,§] Hidehiko Nakagawa,[†] and Naoki Miyata*[,†]

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan, Chemical Genetics Laboratory, RIKEN, Saitama 351-0198, Japan, and CREST Research Project, Japan Science and Technology Agency, Saitama 332-001, Japan

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To uncover novel histone deacetylase 6 (HDAC6)-selective inhibitors and to elucidate the structural requirements for their inhibitory activity, we designed and prepared a series of thiolate analogues based on the structure of an HDAC6-selective substrate and evaluated their properties by Western blotting and enzyme assays. Several thiolate analogues were found to be potent and selective HDAC6 inhibitors. Study of the structure−selectivity relationship revealed that the presence of a bulky alkyl group and *tert*-butylcarbamate group in these compounds is important for HDAC6-selective inhibition. Compounds **16b** and **20b**, the most selective and active compounds in this series, exerted a synergistic inhibition of cancer cell growth in combination with paclitaxel. They also blocked the growth of estrogen receptor α-positive breast cancer MCF-7 cells that had been treated with estrogen. These findings suggested that HDAC6-selective inhibitors have potential as anticancer agents.

Introduction

Reversible protein acetylation is an important post-translational modification that regulates the function of histones and many nonhistone proteins.[1] Acetylation of histone lysine residues is controlled by histone acetyltransferases and histone deacetylases (HDACs[a]) and is closely connected with gene expression and cell cycle progression.[2] The inhibition of HDACs causes histone hyperacetylation and leads to the transcriptional activation of genes such as $p21^{WAF/CIP1}$ and Gadd 45, which are associated with growth arrest and apoptosis in tumor cells.[3] Thus far, 18 HDAC family members have been identified and categorized into two groups, namely, zinc-dependent enzymes (HDAC1−11) and NAD^+-dependent enzymes (SIRT1−7).[2a,4] Among these, HDAC6, one of the zinc-dependent HDAC family members, is unique in that it deacetylates nonhistone proteins, such as α-tubulin, HSP90, and cortactin, and is involved in microtubule stabilization, molecular chaperone activity, and cell motility.[5] Furthermore, recent studies have revealed that HDAC6 is associated with several disease states. It has been reported by Hideshima et al. that the inhibition of HDAC6 causes growth inhibition of multiple myeloma cells without affecting noncancerous cells,[6] and Saji et al. reported that expression of HDAC6 is induced by estrogen stimulation of estrogen receptor α (ERα)-positive breast cancer cells.[7] In addition, HDAC6 inhibition has been reported to be strongly involved in neuroprotection.[8] Therefore, HDAC6-selective inhibitors are of great interest not only as tools for probing the biological functions of the isoform, but also as therapeutic agents having few side effects.

Although a large number of HDAC inhibitors have been found to date,[9] including trichostatin A (TSA, **1**),[10] suberoylanilide hydroxamic acid (SAHA, vorinostat, **2**),[11] trapoxin B (TPX B, **3**),[12] and MS-275 (**4**;[13] Chart 1), most lack HDAC6 selectivity. Most hydroxamate HDAC inhibitors, such as **1** and **2**, exhibit little or no preference for HDAC6, whereas most nonhydroxamate inhibitors, such as **3** and **4**, do not inhibit HDAC6.[5b,c,14] It has been reported that HDAC6 is selectively inhibited by tubacin (**5**; Chart 1), which was discovered by the screening of a 7392 small molecule library.[15] However, there is only limited information on HDAC6-selective inhibitors, and therefore, there is a need to develop novel candidates and to then elucidate their structural requirements for inhibiting HDAC6.

In the course of our study on nonhydroxamate HDAC inhibitors,[14e,16] we found a new class of small molecule thiol-based analogues including NCH-26 (**6**) and NCH-31 (**7**;[16b] Chart 2). Thiols are presumed to inhibit HDACs by coordinating the zinc ion, which is required for deacetylation of the acetylated lysine substrate. Furthermore, the *S*-isobutyryl prodrugs NCH-47 (**8**) and NCH-51 (**9**;[17] Chart 2), which are thought to be hydrolyzed to their free thiol forms within cells, showed potent inhibition of cancer cell growth. Following these findings, we recently performed further investigation of thiolate analogues to uncover those that were HDAC6-selective, and discovered highly potent and selective HDAC inhibitors.[18] In the present report, we describe in full detail the design, synthesis, structure−selectivity relationship, and cellular activity of thiol-based HDAC6-selective inhibitors.

Chemistry

The compounds prepared for this study are shown in Figures 1 and 3 and Table 1. Syntheses were carried out as outlined in Schemes 1−4. The route for synthesis of compounds **11b**−**25b** is described in Scheme 1. (*S*)-2-amino-7-bromoheptanoic acid **42**[19] was treated with $(Boc)_2O$ to yield *N*-Boc compound **43**. Condensation of acid **43** with an appropriate amine afforded amides **11c**−**25c**. Bromides **11c**−**25c** were treated with thio-

* To whom correspondence should be addressed. Tel. and fax: +81-52-836-3407. E-mail: suzuki@phar.nagoya-cu.ac.jp (T.S.); miyata-n@phar.nagoya-cu.ac.jp (N.M.).

† Graduate School of Pharmaceutical Sciences, Nagoya City University.

‡ Chemical Genetics Laboratory, RIKEN.

§ CREST Research Project.

[a] Abbreviations: HDAC, histone deacetylase; SIRT, sirtuin; ERα, estrogen receptor α; TSA, trichostatin A; SAHA, suberoylanilide hydroxamic acid; TPX B, trapoxin B; PTX, paclitaxel; E2, 17β-estradiol.

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Chart 1. Examples of HDAC Inhibitors

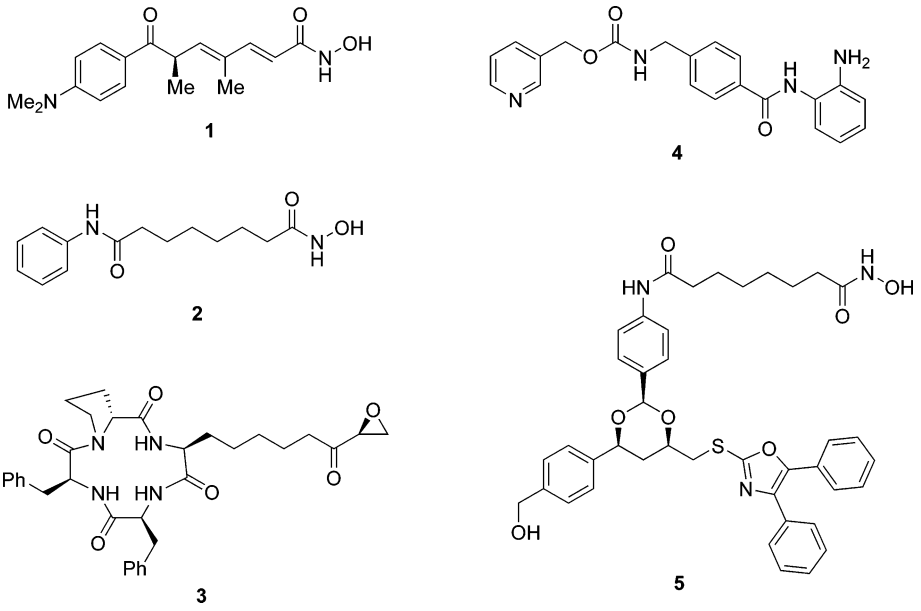
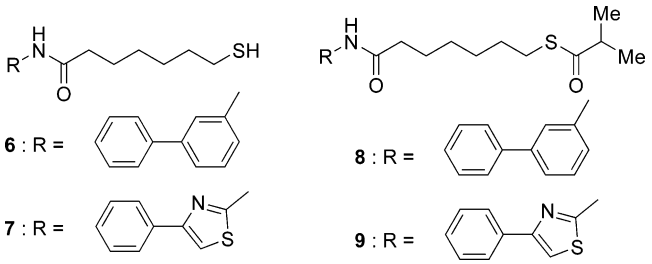


Chart 2. Thiolate HDAC Inhibitors



isobutyric acid under alkaline conditions to yield the desired thioesters **11b**−**25b**.

Compounds **26b**−**41b** were synthesized from thioester **16b** by the route shown in Scheme 2. Removal of the Boc group of **16b** gave amine **26b**. Amine **26b** was allowed to react with the corresponding acid chloride, carboxylic acid, chloroformate, isocyanate or thioisocyanate to give **27b**−**41b**.

Compounds **13a**, **15a**−**20a**, and **26a** were prepared from the corresponding bromides **13c** and **15c**−**20c** (Scheme 3). Treatment of the bromides with potassium thioacetate and subsequent hydrolysis of the thioesters gave thiols **13a** and **15a**−**20a**. Deprotection of the Boc group of **16a** gave amine **26a**.

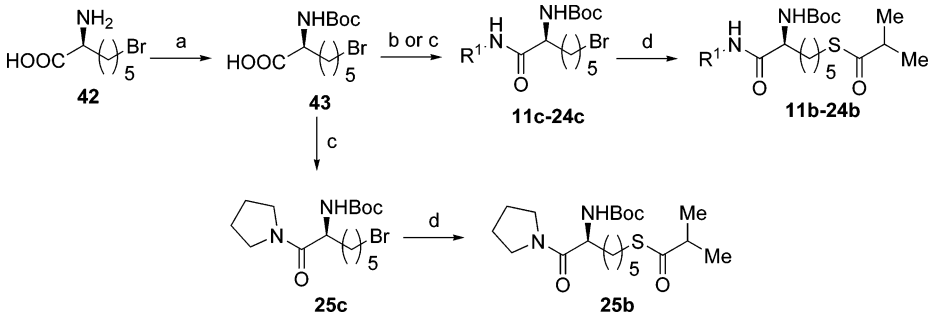
Thiols **27a**, **28a**, **31a**, **33a**, **35a**−**38a**, and **40a** were synthesized from thioester **16d** as outlined in Scheme 4. Thioester **16d** was converted to thiols **27a**, **28a**, **31a**, **33a**, **35a**−**38a**, and **40a** by Boc deprotection of **16d**, treatment of amine **26d** with an appropriate acid chloride, carboxylic acid, chloroformate, isocyanate, or thioisocyanate, and subsequent hydrolysis of thioacetates **27d**, **28d**, **31d**, **33d**, **35d**−**38d**, and **40d**.

Results and Discussion

In designing novel HDAC6-selective inhibitors, we focused initially on small molecule HDAC6-selective substrate **10** (Chart 3), which was discovered by Jung and co-workers.[20] According to their report, compound **10** is selectively deacetylated by HDAC6 in preference to HDAC1 and HDAC3, and the presence of *N*-Boc and trifluoromethyl coumaryl amide in this compound was found to be important for HDAC6 selectivity. This indicated that the structure of *N*-Boc and trifluoromethyl coumaryl amide of compound **10** is selectively recognized by HDAC6, and so, we considered that compound **11a**, in which the acetamide of **10** is replaced by a thiol, might selectively inhibit HDAC6 (Chart 3).

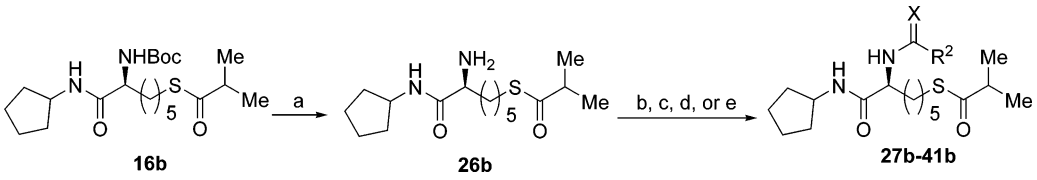
Because HDAC6 has been reported as an α-tubulin deacetylase, inhibition of HDAC6 and that of other HDACs can be assessed according to the accumulation of acetylated α-tubulin and acetylated histones, respectively, using Western blot analysis. We initially evaluated the histone H4/α-tubulin acetylation selectivity of compounds **2**, **8**, **9**, and **11b**, the *S*-isobutyryl prodrug of **11a**, using Western blot analysis (Figure 2). As expected, compound **11b** seemed to induce the accumulation

Scheme 1[a]



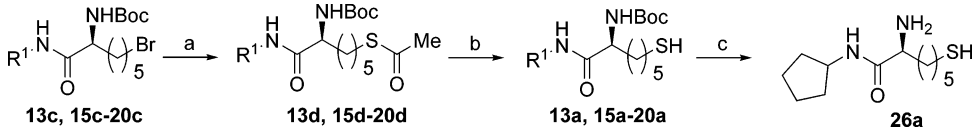
[a] Reagents and conditions: (a) $(Boc)_2O$, Et_3N, THF, H_2O, rt, 95%; (b) R^1NH_2, $POCl_3$, pyridine, −15 °C, 10−48%; (c) R^1NH_2, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 1-hydroxybenzotriazole hydrate (HOBt·H_2O), THF, rt, 69−93%; (d) thioisobutyric acid, Et_3N, EtOH, rt, 19−100%.

Scheme 2[a]



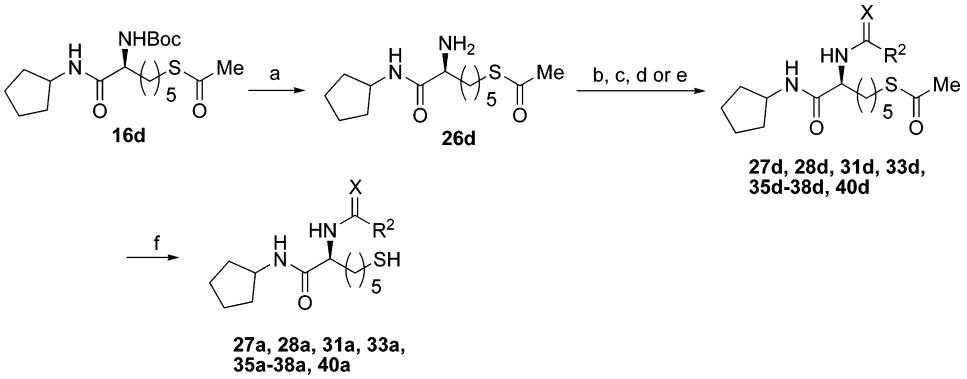
[a] Reagents and conditions: (a) HCl, AcOEt, rt, 100%; (b) R^2COCl, Et_3N, CH_2Cl_2, rt, 11−74%; (c) $(R^2CO)_2O$, 4-dimethylaminopyridine (DMAP), Et_3N, CH_2Cl_2, rt, 52%; (d) R^2COOH, EDCI, $HOBt{\cdot}H_2O$, DMF, 28−62%; (e) R^2NCX, Et_3N, CH_2Cl_2, rt, 24−76%.

Scheme 3[a]



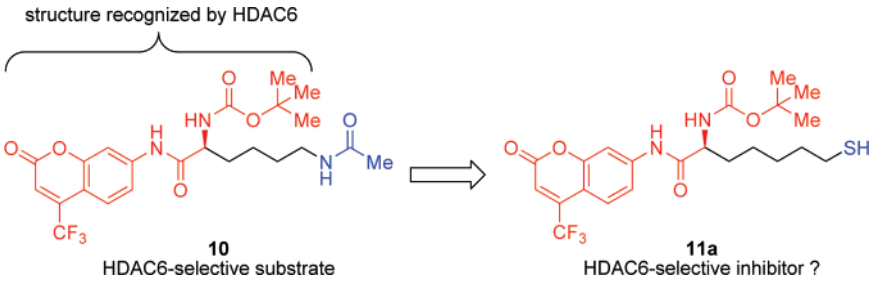
[a] Reagents and conditions: (a) KSAc, EtOH, rt, 57−93%; (b) NaOH, H_2O, EtOH, rt, 53−77%; (c) HCl, AcOEt, rt, 94%.

Scheme 4[a]



[a] Reagents and conditions: (a) HCl, AcOEt, rt, 100%; (b) R^2COCl, Et_3N, CH_2Cl_2, rt, 26−91%; (c) $(R^2CO)_2O$, DMAP, Et_3N, CH_2Cl_2, rt, 52%; (d) R^2COOH, EDCI, $HOBt{\cdot}H_2O$, DMF, 60%; (e) R^2NCX, Et_3N, CH_2Cl_2, rt, 39−95%; (f) NaOH, H_2O, EtOH, rt, 63−100%.

Chart 3. Design of HDAC6-Selective Inhibitors



of acetylated α-tubulin as compared with **2**, **8**, and **9**, which did not have this effect. To obtain highly more selective compounds, the coumarin structure of **11b** was converted to analogs with various functional groups (**12b**−**25b**; Figure 1), and these were examined for their selectivity. Compounds **12b**−**15b** and **21b**, with an aromatic ring, compounds **22b**−**24b**, with a smaller hydrophilic group, and compound **25b**, with a tertiary amide group, did not show high selectivity, whereas compounds **16b**−**20b**, in which R^1 = a bulky alkyl group, induced a dose-dependent increase in α-tubulin acetylation but no major increase in acetylated histone H4 (Figure 2). These results indicated that compounds **16b**−**20b** selectively inhibit HDAC6 in preference to nuclear HDACs in cells.

Having investigated the requirements for the R^1 group (Figures 1 and 2), we next turned our attention to the replacement of the *N*-Boc group (Figures 3 and 4). We employed the cyclopentyl group as the R^1 group and determined the effect of replacement of the *N*-Boc group with other functional groups. We initially evaluated amine **26b** in which the Boc group of **16b** is removed, however, **26b** induced neither histone H4 acetylation nor α-tubulin acetylation. Next, we tested aromatic amides (**27b**−**29b**). Among these, phenyl amide **27b** induced the acetylation of α-tubulin, but its selectivity seemed to decrease when compared with that of the lead compound **16b**. We also examined aliphatic amides (**30b**−**34b**). Although hydroxymethyl compound **30b** was found to be a highly potent α-tubulin acetylating agent, its selectivity seemed to be low as compared with **16b**. Compounds **31b** and **32b** caused the accumulation of both acetylated histone H4 and acetylated α-tubulin, whereas compounds **33b** and **34b** only displayed a weak acetylation activity. It is remarkable that neopentyl amide **34b** did not show any α-tubulin acetylating activity or selectivity at all. In other words, replacement of the oxygen atom of the Boc group with a methylene group significantly decreased both potency and selectivity. This suggests that the carbamate group is important for α-tubulin acetylation selectivity. To confirm the importance of the carbamate structure, we replaced the carbamate group of **16b** with urea (**35b**) and thiourea (**36b**) and

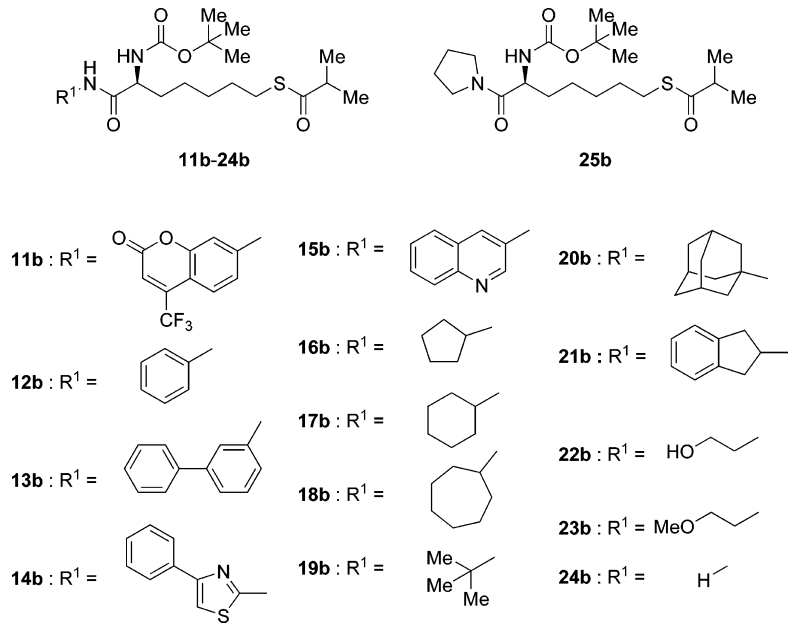


Figure 1. Structures of **11b**−**25b**.

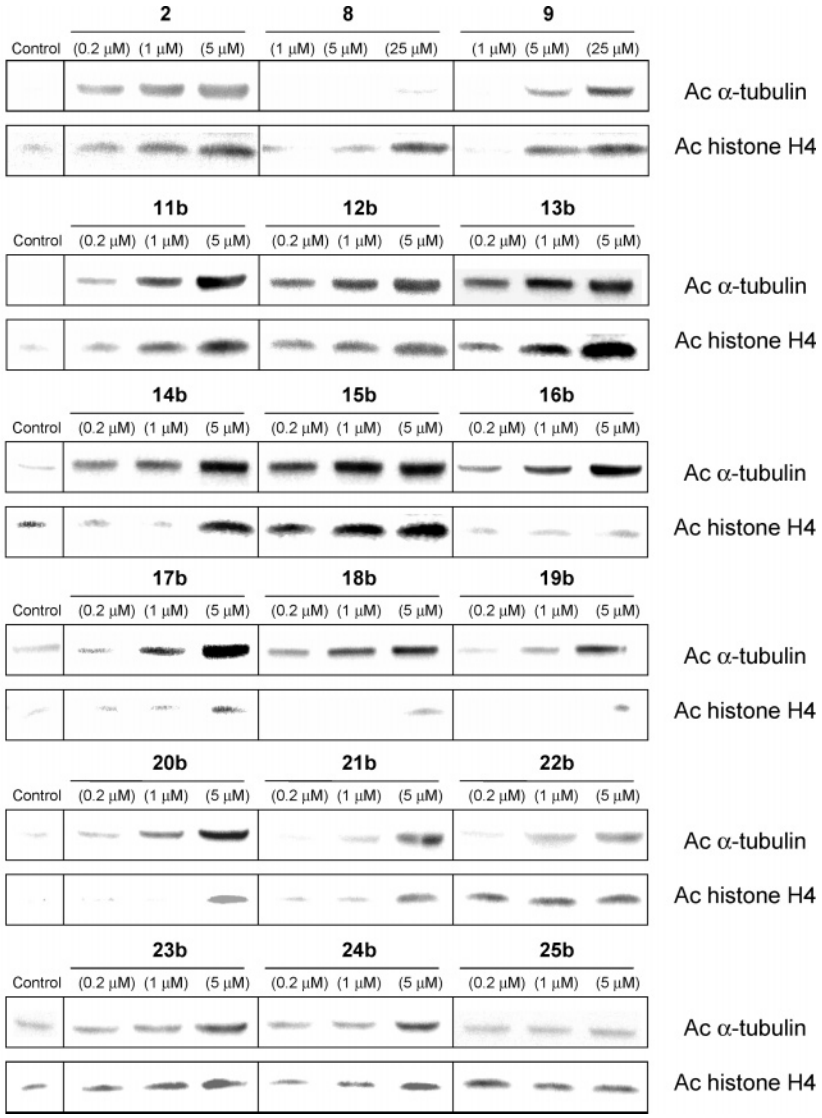


Figure 2. Western blot detection of acetylated α-tubulin and acetylated histone H4 levels in HCT116 cells after 8 h treatment with **2**, **8**, **9**, and **11b**−**25b**.

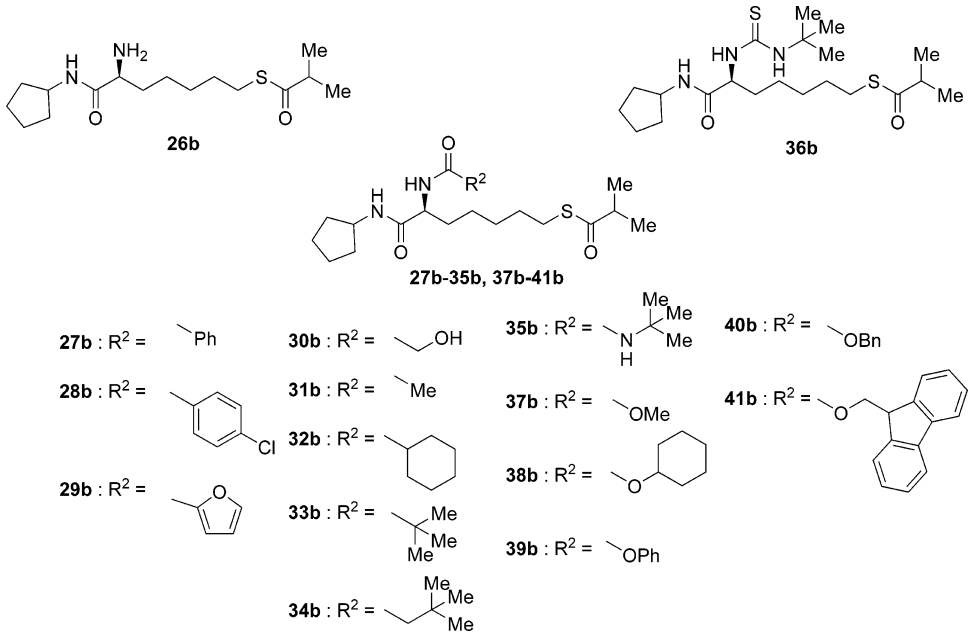


Figure 3. Structures of **26b**−**41b**.

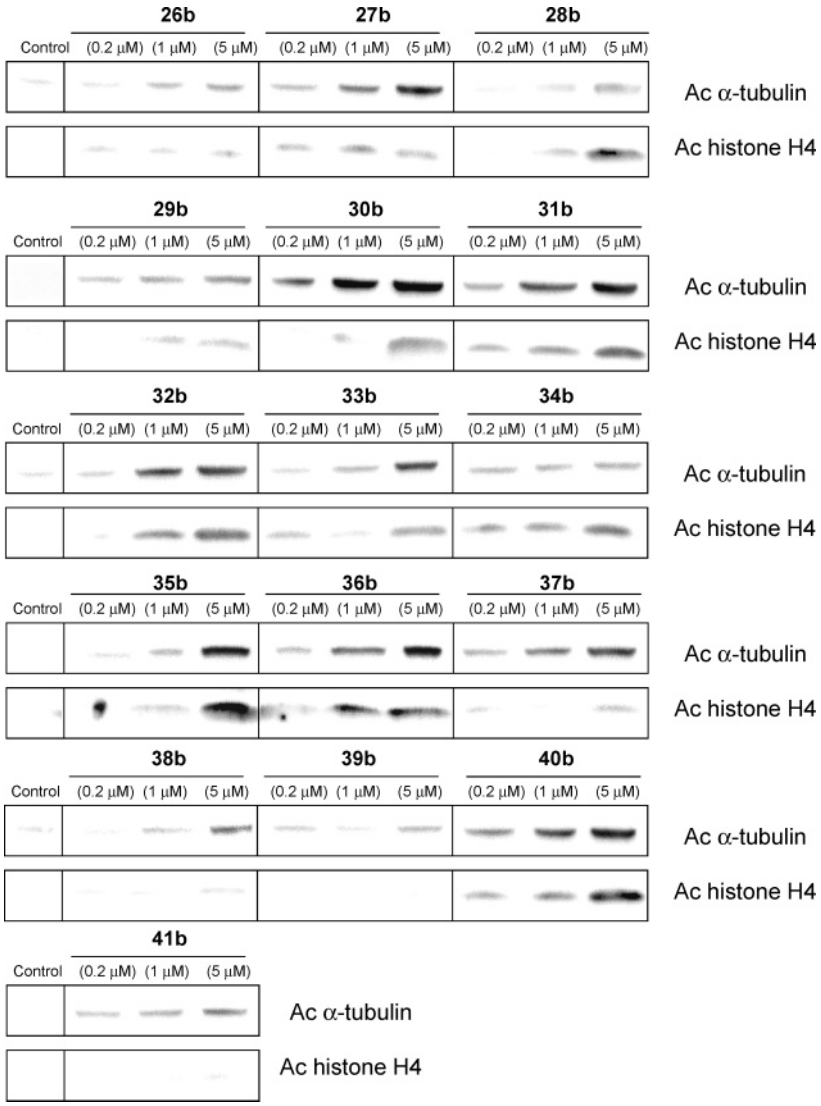
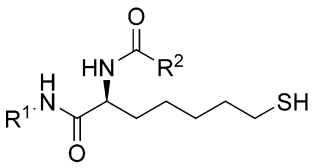


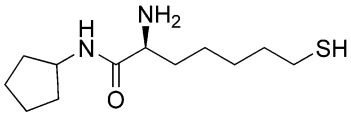
Figure 4. Western blot detection of acetylated α-tubulin and acetylated histone H4 levels in HCT116 cells after 8 h treatment with **26b**−**41b**.

evaluated these two compounds. While compounds **35b** and **36b** sustained α-tubulin acetylation activity, selectivity was significantly reduced. These results highlighted the importance of the carbamate group in α-tubulin/histone acetylation selectivity.

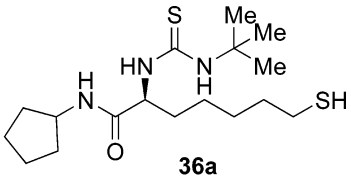
Table 1. In Vitro HDAC1-, HDAC4-, and HDAC6-Inhibitory Activities of **13a**, **15a**−**20a**, **26a**−**28a**, **31a**, **33a**, **35a**−**38a**, and **40a**[a]



13a, 15a-20a, 27a, 28a, 30a, 33a, 35a, 37a, 38a, 40a



26a



36a

			IC$_{50}$ (nM)			selectivity	
cmpd	R^1	R^2	HDAC1	HDAC4	HDAC6	HDAC1/HDAC6	HDAC4/HDAC6
1			21	34	81	0.26	0.42
5			ND[c]	ND	ND	4[b]	4[b]
7			48	32	41	1.2	0.78
13a	3-biphenyl	-O*t*-Bu	62	38	54	1.2	0.70
15a	3-quinolinyl	-O*t*-Bu	51	33	32	1.6	1.0
16a	cyclopentyl	-O*t*-Bu	1210	1030	29	42	36
17a	cyclohexyl	-O*t*-Bu	1270	1140	36	35	32
18a	cycloheptyl	-O*t*-Bu	900	840	23	39	37
19a	-*t*-Bu	-O*t*-Bu	3000	1900	71	42	26
20a	1-adamantyl	-O*t*-Bu	3800	4200	82	46	51
26a			52 300	31 200	3860	14	8.1
27a	cyclopentyl	-Ph	560	280	190	3.0	1.5
28a	cyclopentyl	4-chlorophenyl	1430	650	510	2.8	1.3
30a	cyclopentyl	-CH$_2$OH	1980	970	150	13	6.5
33a	cyclopentyl	-*t*-Bu	1430	730	430	3.3	1.7
35a	cyclopentyl	-NH*t*-Bu	460	310	100	4.6	3.1
36a			400	220	170	2.4	1.3
37a	cyclopentyl	-OMe	2320	1160	120	19	9.7
38a	cyclopentyl	cyclohexyloxy	1030	580	120	8.6	4.8
40a	cyclopentyl	-OBn	130	130	78	1.7	1.7

[a] Values are means of at least three experiments. [b] Taken from the literature (ref 22). [c] ND = No data.

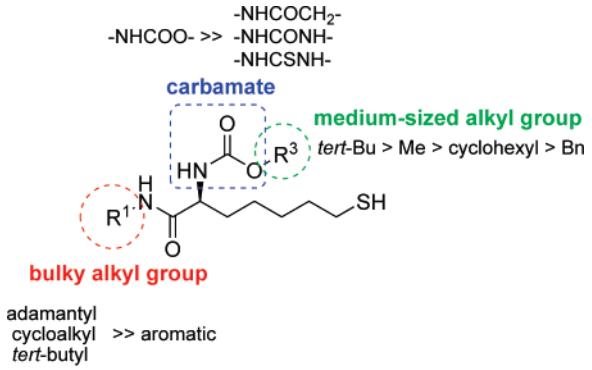


Figure 5. Structure−selectivity relationship.

Next, we converted the *tert*-butyl group of **16b** to other functional groups (**37b**−**41b**). While methyl group (**37b**) retained the α-tubulin acetylation activity and selectivity to some extent, larger alkyl groups (**38b**, **40b**, and **41b**) and a phenyl group (**39b**) caused a decrease in either selectivity or potency. Medium-sized alkyl groups such as the *tert*-butyl group seem to be preferred as the substituents attached to the carbamate. As a result, the *tert*-butoxy group (**16b**) was the best R^2 group choice for the selective accumulation of acetylated α-tubulin in cells.

To confirm HDAC6 selectivity and to examine the structure−selectivity relationship for this series of inhibitors, we performed in vitro enzyme assays using HDAC1, HDAC4, and HDAC6. A selected set of thiols was prepared and evaluated for enzyme inhibition activity. The results of the enzyme assays are shown in Table 1. Compound **1** was chosen as a reference compound. We initially tested compound **7** and compounds with various R^1 groups (**13a**, **15a**, and **16a**−**20a**). Consistent with the results of Western blotting, compound **7** and aromatic compounds **13a** and **15a** did not discriminate among HDAC1, HDAC4, and HDAC6. The strong HDAC1 inhibitory activity of **13a** and **15a** is consistent with the results observed in similar aromatic group-containing HDAC inhibitors.[21] The HDAC6 inhibitory activity of compounds **16a**−**20a**, in which R^1 = a bulky alkyl group, was similar to or greater than that of **1** (IC$_{50}$ of 81 nM, **16a** 29 nM, **17a** 36 nM, **18a** 23 nM, **19a** 71 nM, **20a** 82 nM). Furthermore, while **1** inhibited HDAC1 and HDAC4 rather than HDAC6 (HDAC1 IC$_{50}$/HDAC6 IC$_{50}$ = 0.26; HDAC4 IC$_{50}$/HDAC6 IC$_{50}$ = 0.42), compounds **16a**−**20a** efficiently inhibited HDAC6 in preference to HDAC1 and HDAC4 (HDAC1 IC$_{50}$/HDAC6 IC$_{50}$ = 35−46; HDAC4 IC$_{50}$/HDAC6 IC$_{50}$ = 26−51). The HDAC6 selectivity of compounds **16a**−**20a** was much higher than that of **5**, which showed only about a 4-fold selectivity for HDAC6 over HDAC1 and HDAC 4 in enzyme assays.[22] In addition, in the case of compounds with various R^2 groups (**26a**−**28a**, **30a**, **33a**, **35a**−**38a**, and **40a**), a reasonable correlation between cellular and enzyme assay data was observed. Amine **26a** did not show strong HDAC inhibition, and aromatic amides **27a** and **28a** and aliphatic amides **30a** and **33a** were nonselective inhibitors as compared with **16a**. Conversion of the carbamate of **16a** to urea (**35a**) and thiourea (**36a**) significantly reduced the HDAC6 selectivity. As for carbamate derivatives, methyl carbamate **37a** exerted a moderate level of HDAC6 selectivity (HDAC1 IC$_{50}$/HDAC6 IC$_{50}$ = 19; HDAC4 IC$_{50}$/HDAC6 IC$_{50}$ = 9.7), whereas cyclopentyl **38a** and benzyl **40a** showed a decrease in HDAC6 selectivity as compared with that of **16a** and **37a**. As a result, compounds **16a**−**20a**, in which R^1 = a bulky alkyl group and R^2 = a *tert*-butoxy group, were the most potent and selective HDAC6 inhibitors.

As shown in Figure 5, the results of Western blotting and enzyme assays clarified the structural requirements for HDAC6-selective inhibition. For R^1, bulky alkyl groups such as

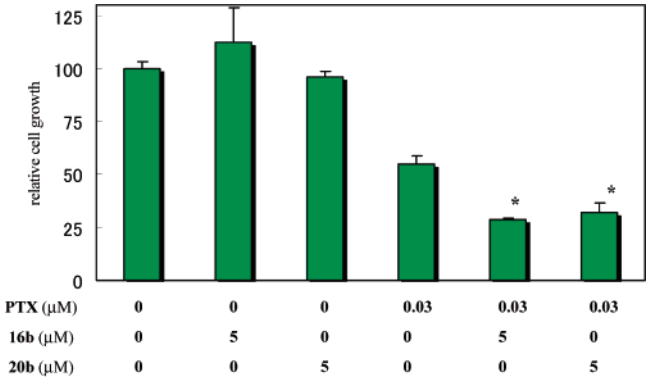


Figure 6. Cell growth inhibition of HCT116 cells using different combinations of HDAC6 inhibitor **16b** or **20b** and paclitaxel (PTX). *$P < 0.05$; ANOVA and Bonferroni-type multiple *t* test results indicated differences between PTX (0.03 μM) and a combination of PTX (0.03 μM) with **16b** (5 μM) and between PTX (0.03 μM) and a combination of PTX (0.03 μM) with **20b** (5 μM).

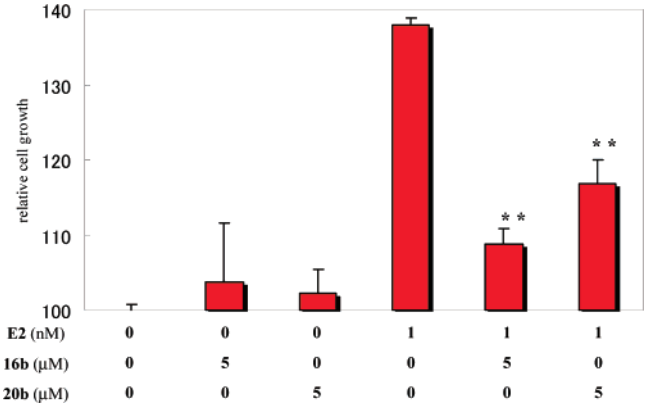


Figure 7. Cell growth inhibition of MCF-7 cells stimulated with estradiol (E2) using HDAC6 inhibitor **16b** or **20b**. **$P < 0.01$; ANOVA and Bonferroni-type multiple *t* test results indicated differences between E2 (1 nM) and a combination of E2 (1 nM) with **16b** (5 μM) and between E2 (1 nM) and a combination of E2 (1 nM) with **20b** (5 μM).

adamantyl and cycloalkyl are preferred. The carbamate structure is also important for HDAC6-selective inhibition. For R^3, medium-sized alkyl groups such as *tert*-butyl are suitable. Because there is no information at present on the three-dimensional structure of HDAC6, the reason for the high HDAC6 selectivity of compounds **16**−**20** is unclear, but it is likely that HDAC6 has at least two hydrophobic pockets where a bulky R^1 group and a medium-sized R^3 group could be placed, as well as an amino acid residue, which could interact with the carbamate group, whereas the other HDAC isoforms lack the pockets and amino acid residue.

To explore the potential of HDAC6-selective inhibitors as anticancer drugs, we first tested compounds **16b** and **20b**, the most selective and active compounds in this series, by means of a cancer cell growth inhibition assay using human colon cancer HCT116 cells. However, they were found to be inactive up to a concentration of 5 μM above which they displayed distinct α-tubulin acetylation on Western blot analysis (Figure 2). We next evaluated whether our HDAC6-selective inhibitors could act synergistically with paclitaxel (PTX) in growth inhibition assays using HCT116 cells. Because PTX exerts its anticancer effect by stabilizing microtubules, where acetylated α-tubulins are most abundant,[5b,c,23] we considered that the combination of our HDAC6-selective inhibitors and PTX might cause a synergistic inhibition of cancer cell growth. As shown in Figure 6, 5 μM of **16b** or **20b** did not show any activity as a single agent, whereas treatment with 0.03 μM of PTX reduced the cell growth by approximately 50%. As expected, however, a combination of 5 μM of **16b** or **20b** and 0.03 μM of PTX reduced cell growth by approximately 70%, suggesting that HDAC6-selective inhibitors have potential as drug candidates when used in combination with PTX.

We also evaluated the effect of HDAC6-selective inhibitors on ERα-positive breast cancer MCF-7 cells. It has been reported that estrogen stimulation of MCF-7 cells increases expression of HDAC6 and enhances cell motility.[7] This report suggested to us that HDAC6 may be associated with the growth of ERα-positive breast cancer cells treated with estrogen. We therefore examined whether HDAC6-selective inhibitors could inhibit growth of MCF-7 cells stimulated with this hormone (Figure 7). As with HCT116 cells, the growth of MCF-7 cells was not influenced by treatment with 5 μM of **16b** or **20b**, whereas treatment with 1 nM of 17β-estradiol (E2)-induced cell growth by approximately 40%. Interestingly, treatment with 5 μM of **16b** or **20b** significantly blocked the growth of MCF-7 cells stimulated with 1 nM of E2. These results suggested that HDAC6 is involved in the growth of ERα-positive breast cancer cells and that HDAC6-selective inhibitors may be useful in the treatment of ERα-positive breast cancer.

Conclusion

We have identified novel HDAC6-selective inhibitors whose designs were based on the structure of HDAC6-selective substrate **10**. Compounds **16**−**20** showed high HDAC6 selectivity in both cellular and enzyme assays and investigation of the structure−selectivity relationship revealed that the presence of a bulky alkyl group, such as adamantyl and cycloalkyl, and a *tert*-butylcarbamate group in these compounds is important for HDAC6-selective inhibition. In biological experiments, the combination of compound **16b** or **20b** and PTX caused a synergistic inhibition of cancer cell growth. The synergistic effect of these HDAC6-selective inhibitors may allow for the reduction of the PTX dosage with consequently fewer side effects. Compounds **16b** and **20b** also showed growth inhibition of MCF-7 cells stimulated by estrogen. Inhibition of ERα-positive breast cancer cell growth by HDAC6-selective inhibitors suggests that these inhibitors may be effective as antibreast cancer drugs. We believe that the findings presented here will provide a basis for constructing new tools for probing the biology of HDAC6 and uncovering new strategies for cancer treatments.

Experimental Section

Chemistry. Melting points were determined using a Yanagimoto micromelting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded JEOL JNM-LA500, JEOL JNM-A500 or BRUKER AVANCE600 spectrometer in solvent as indicated. Chemical shifts (δ) are 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) and fast atom bombardment (FAB) 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.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-[2-oxo-4-(trifluoromethyl)-2*H*-chromen-7-ylamino]heptyl 2-Methylpropanethioate (11b). Step 1: Preparation of (*S*)-7-Bromo-2-*tert*-butoxycarbonylaminoheptanoic Acid (43). To a solution of (*S*)-2-amino-7-bromoheptanoic acid (**42**; 2.01 g, 8.97 mmol) and Et_3N (5 mL, 67.7 mmol) in THF/H_2O (20 mL/40 mL) was added a solution of $(Boc)_2O$ (3.90 g, 17.9 mmol) in THF (20 mL), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into 2 N aqueous NaOH (15 mL), and the whole mixture was washed with $CHCl_3$. To the separated aqueous layer was added 10% aqueous citric acid (15 mL) and was extracted with AcOEt. The organic layer was washed with water and brine and dried over Na_2SO_4. Filtration and concentration in vacuo gave 2.77 g (95%) of **43** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.02 (1H, d, J = 7.0 Hz), 4.31 (1H, s), 3.40 (2H, t, J = 6.7 Hz), 1.87 (1H, m), 1.72−1.65 (1H, m), 1.87 (2H, quintet, J = 7.1 Hz), 1.52−1.43 (13H, m).

Step 2: Preparation of (*S*)-*tert*-Butyl 7-Bromo-1-oxo-1-(2-oxo-4-(trifluoromethyl)-2*H*-chromen-7-ylamino)heptan-2-ylcarbamate (11c). To a solution of 7-amino-4-(trifluoromethyl)coumarin (688 mg, 3.00 mmol) and **43** (973 mg, 3.00 mmol) obtained above in dry pyridine (18 mL) was added phosphoryl chloride (750 μL, 2.97 mmol) at −15 °C, and the solution was stirred at −15 °C for 15 min. The solution was poured into water, and the solution was extracted with AcOEt. The AcOEt layer was separated, washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid, and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3) gave 922 mg (57%) of **11c** as a colorless solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.02 (1H, s), 7.80 (1H, d, J = 1.8 Hz), 7.62 (1H, d, J = 7.9 Hz), 7.46 (1H, d, J = 9.1 Hz), 6.67 (1H, s), 4.97 (1H, s), 4.19 (1H, s), 3.41 (2H, t, J = 6.7 Hz), 2.02−1.96 (1H, m), 1.88 (2H, quintet, J = 7.0 Hz), 1.71−1.60 (1H, m), 1.48 (9H, s), 1.52−1.35 (4H, m).

Step 3: Preparation of (*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-[2-oxo-4-(trifluoromethyl)-2*H*-chromen-7-ylamino]heptyl 2-methylpropanethioate (11b). To a solution of sodium hydrosulfide (4.29 g, 77.9 mmol) in MeOH (50 mL) was added *iso*-butyryl chloride (4 mL, 37.2 mmol) and stirred at 0 °C for 1 h. Then, the solution was stirred at room temperature for 2 h. After that, the reaction mixture was poured into water and extracted with AcOEt. The extract was washed with 1 N aqueous HCl and brine and dried over Na_2SO_4. Filtration and evaporation in vacuo gave 2.29 g (59%) of thioisobutyric acid as a yellow oil. To a solution of **11c** (443 mg, 0.827 mmol) obtained above in EtOH (10 mL) was added a solution of thioisobutyric acid (455 mg, 4.27 mmol) in EtOH (5 mL) and triethylamine (1 mL, 13.6 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into water, and the mixture was extracted with AcOEt. The AcOEt layer was separated, washed with 10% aqueous citric acid and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4), and recrystallization from AcOEt/*n*-hexane gave 250 mg (54%) of **11b** as colorless crystals: mp 163−165 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.07 (1H, s), 7.81 (1H, s), 7.62 (1H, d, J = 7.9 Hz), 7.41 (1H, d, J = 7.3 Hz), 6.68 (1H, s), 5.06 (1H, s), 4.18 (1H, broad s), 2.84 (2H, t, J = 7.8 Hz), 2.73 (1H, quintet, J = 6.9 Hz), 1.99−1.95 (1H, m), 1.70−1.65 (1H, m), 1.60−1.54 (2H, m), 1.47 (9H, s), 1.45−1.40 (4H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.59, 171.00, 159.10, 155.24, 142.34, 128.50, 125.83, 122.40, 116.18, 113.84, 109.36, 107.51, 81.26, 77.24, 43.14, 29.32, 28.31, 28.20, 25.12, 19.42; Anal. ($C_{26}H_{33}F_3N_2O_6S\cdot 1/4H_2O$) C, H, N.

Compounds **12b**−**15b** were prepared from **43** and an appropriate amine using the procedure described for **11b**.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(phenylamino)heptyl 2-Methylpropanethioate (12b). Yield 9%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.22 (1H, s), 7.52 (2H, d, J = 8.0 Hz), 7.31 (2H, t, J = 7.8), 7.10 (1H, t, J = 7.5 Hz), 5.04 (1H, s), 4.17 (1H, s), 2.84 (2H, t, J = 7.3 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 1.96−1.92 (1H, m), 1.65−1.55 (3H, m), 1.50−1.45 (13H, m), 1.19 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.45, 170.26, 129.00, 124.38, 119.87, 77.22, 43.12, 29.36, 28.31, 28.25, 25.13, 19.42; MS (EI) m/z 422 (M^+); HRMS calcd for $C_{22}H_{34}O_4N_2S$, 422.224; found, 422.225.

(*S*)-*S*-7-(Biphenyl-3-ylamino)-6-(*tert*-butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (13b). Yield 6%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.24 (1H, broad s), 7.79 (1H, s), 7.58 (2H, d, J = 7.6 Hz), 7.48 (1H, d, J = 7.9 Hz), 7.44−7.34 (5H, m), 4.98 (1H, s), 4.18 (1H, s), 2.84 (2H, t, J = 7.3 Hz), 2.72 (1H, quintet, J = 7.0 Hz), 2.00−1.95 (1H, m), 1.71−1.65 (1H, m), 1.60−1.54 (2H, m), 1.45−1.40 (13H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.43, 170.44, 142.12, 140.66, 129.35, 128.73, 127.47, 127.20, 123.16, 118.68, 81.28, 77.24, 43.13, 29.36, 28.33, 28.26, 25.16, 19.43; MS (EI) m/z 498 (M^+); HRMS calcd for $C_{28}H_{38}O_4N_2S$, 498.255; found, 498.255.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(4-phenylthiazol-2-ylamino)heptyl 2-Methylpropanethioate (14b). Yield 28%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.97 (1H, broad s), 7.82 (2H, d, J = 7.0 Hz), 7.41 (2H, t, J = 7.6 Hz), 7.32 (1H, d, J = 7.3 Hz), 7.14 (1H, s), 5.05 (1H, d, J = 7.5 Hz), 4.37 (1H, s), 2.83 (2H, t, J = 7.3 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 2.00−1.95 (1H, m), 1.71−1.65 (1H, m), 1.60−1.54 (2H, m), 1.48 (9H, s), 1.45−1.40 (4H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.42, 170.36, 157.27, 150.08, 134.34, 128.72, 128.05, 126.12, 107.95, 80.94, 77.24, 54.61, 43.12, 31.75, 29.29, 28.30, 28.20, 25.00, 19.42; MS (EI) m/z 505 (M^+); HRMS calcd for $C_{25}H_{35}O_4N_3S_2$, 505.207; found, 505.214.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(quinolin-3-ylamino)-heptyl 2-Methylpropanethioate (15b). Yield 3%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.76 (1H, s), 8.74 (2H, s), 8.03 (1H, d, J = 8.8 Hz), 7.88 (1H, d, J = 7.9 Hz), 7.62 (1H, d, J = 7.2 Hz), 7.53 (1H, d, J = 7.3 Hz), 5.01 (1H, s), 4.25 (1H, s), 2.85 (2H, t, J = 7.5 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 2.04−1.95 (1H, m), 1.71−1.65 (1H, m), 1.60−1.54 (2H, m), 1.50−1.45 (13H, m), 1.19 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.59, 171.08, 143.97, 131.40, 128.94, 128.29, 128.17, 127.74, 127.25, 123.88, 77.22, 43.14, 29.33, 28.31, 28.18, 25.13, 19.43; MS (EI) m/z 473 (M^+); HRMS calcd for $C_{25}H_{35}O_4N_3S$, 473.235; found, 473.234.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (16b). Step 1: Preparation of (*S*)-*tert*-Butyl 7-Bromo-1-(cyclopentylamino)-1-oxoheptan-2-ylcarbamate (16c). To a solution of **43** (2.49 g, 7.68 mmol) obtained above in THF (12 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI; 2.22 g, 11.6 mmol), 1-hydroxy-1*H*-benzotriazole monohydrate (HOBt·H_2O; 1.77 g, 11.6 mmol), and cyclopentylamine (781 μL, 7.92 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with 10% aqueous citric acid, water, saturated $NaHCO_3$, and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 2/5) gave 2.28 g (76%) of **16c** as a colorless solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.97 (1H, d, J = 7.6 Hz), 4.98 (1H, s), 4.18 (1H, sextet, J = 7.0 Hz), 3.95 (1H, s), 3.40 (2H, t, J = 6.7 Hz), 1.97 (2H, m), 1.90−1.78 (3H, m), 1.71−1.53 (5H, m), 1.49−1.30 (15H, m).

Step 2: Preparation of (*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (16b). To a solution of sodium hydrosulfide (426 mg, 7.60 mmol) in EtOH (12 mL) was added *iso*-butyryl chloride (820 μL, 7.62 mmol) dropwise at 0 °C, and the mixture was stirred at room temperature for 1 h. Then, to the mixture was added a solution of **16c** (296 mg, 0.756 mmol) in EtOH (7 mL) and Et_3N (1 mL, 13.6 mmol), and the resulting mixture was stirred overnight at room temperature. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) gave 314 mg (100%) of **16b** as a yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.97 (1H, d, J = 7.6 Hz), 4.97 (1H, s),

4.18 (1H, sextet, $J = 6.9$ Hz), 3.94 (1H, s), 2.83 (2H, t, $J = 7.2$ Hz), 2.73 (1H, septet, $J = 6.9$ Hz), 1.97−1.94 (2H, m), 1.83−1.78 (1H, m), 1.68−1.53 (5H, m), 1.44 (9H, s), 1.47−1.37 (8H, m), 1.19 (6H, t, $J = 6.7$ Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.29, 170.59, 155.72, 77.23, 50.37, 43.11, 35.04, 34.95, 29.40, 28.43, 28.32, 28.30, 27.99, 25.06, 24.36, 19.42; Anal. ($C_{21}H_{38}N_2O_4S\cdot 1/3H_2O$) C, H, N.

Compounds **17b** and **19b**−**25b** were prepared from **43** and an appropriate amine using the procedure described for **16b**.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclohexylamino)-7-oxoheptyl 2-Methylpropanethioate (17b). Yield 19%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.89 (1H, d, $J = 8.2$ Hz), 4.99 (1H, s), 3.94 (1H, s), 3.75 (1H, s), 2.83 (2H, t, $J = 7.4$ Hz), 2.73 (1H, quintet, $J = 7.0$ Hz), 1.90−1.78 (3H, m), 1.72−1.68 (2H, m), 1.65−1.50 (4H, m), 1.47−1.28 (15H, m), 1.21−1.10 (9H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.35, 170.87, 155.69, 77.23, 48.13, 43.11, 33.04, 29.39, 28.41, 28.32, 25.49, 25.03, 24.72, 19.42; MS (EI) *m*/*z* 428 (M^+); HRMS calcd for $C_{22}H_{40}O_4N_2S$, 428.270; found, 428.276.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(*tert*-butylamino)-7-oxoheptyl 2-Methylpropanethioate (19b). Yield 33%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.80 (1H, s), 4.97 (1H, s), 3.88 (1H, s), 2.83 (2H, t, $J = 7.3$ Hz), 2.73 (1H, septet, $J = 6.9$ Hz), 1.81−1.74 (1H, m), 1.59−1.50 (2H, m), 1.48−1.40 (11H, m), 1.39−1.34 (11H, m), 1.18 (6H, d, $J = 6.7$ Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.29, 171.11, 77.23, 51.32, 43.11, 32.33, 29.41, 28.71, 28.46, 28.31, 25.02, 19.42; Anal. ($C_{20}H_{38}N_2O_4S\cdot 2/3H_2O$) C, H, N.

(*S*)-*S*-7-(Adamant-1-ylamino)-6-(*tert*-Butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (20b). Yield 66%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm), 5.62 (1H, s), 4.99 (1H, m), 3.88 (1H, m), 2.83 (2H, t, $J = 7.3$ Hz), 2.72 (1H, septet, $J = 6.7$ Hz), 2.07 (3H, s), 1.98 (6H, s), 1.77 (1H, sextet, $J = 7.0$ Hz), 1.67 (6H,s), 1.56−1.30 (16H, m), 1.18 (6H, d, $J = 6.7$ Hz); ^{13}C NMR ($CDCl_3$,500 MHz, δ, ppm), 204.27, 170.87, 155.69, 79.90, 55.01, 43.12, 41.58, 36.33, 36.20, 32.51, 29.55, 29.50, 29.43, 28.49, 28.34, 25.00, 19.42; MS (FAB) *m*/*z* 481 (MH^+); Anal. ($C_{26}H_{44}N_2O_4S$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2,3-dihydro-1*H*-inden-2-ylamino)-7-oxoheptyl 2-Methylpropanethioate (21b). Yield 87%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.23−7.14 (4H, m), 6.24 (1H, d, $J = 7.6$ Hz), 4.94 (1H, m), 4.71 (1H, m), 3.94 (1H, m), 3.30 (2H, m), 2.76 (4H, m), 2.69 (2H, septet, $J = 6.7$ Hz), 1.79 (1H, m), 1.58−1.51 (3H, m), 1.48−1.51 (13H, m), 1.18 (6H, d, $J = 6.7$ Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.32, 171.76, 155.68, 140.71, 126.81, 124.79, 80.09, 54.54, 50.57, 43.11, 40.09, 39.99, 32.24, 29.70, 28.37, 28.28, 25.05, 19.41; MS (FAB) *m*/*z* 463 (MH^+); Anal. ($C_{25}H_{38}N_2O_4S\cdot 1/2\ H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2-hydroxyethylamino)-7-oxoheptyl 2-Methylpropanethioate (22b). Yield 46%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.43 (1H, m), 4.98 (1H, m), 3.99 (1H, m), 3.72 (2H, q, $J = 4.8$ Hz), 3.43 (2H, m), 2.61 (1H, broad s) 1.82 (1H, m), 1.57−1.30 (16H, m), 1.18 (6H, d, $J = 6.7$ Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.68, 173.06, 155.57, 80.37, 61.96, 54.89, 43.13, 42.34, 32.12, 29.35, 28.32, 28.26, 28.39, 28.18, 24.95, 19.42; MS (FAB) *m*/*z* 391 (MH^+); Anal. ($C_{18}H_{34}N_2O_5S\cdot 1/2H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2-methoxyethylamino)-7-oxoheptyl 2-Methylpropanethioate (23b). Yield 72%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.30 (1H, s), 4.98 (1H, m), 4.02 (1H, m), 3.45 (4H, m), 3.35 (3H, m), 2.83 (2H, t, $J = 7.3$ Hz), 2.72 (1H, septet, $J = 6.9$ Hz), 1.82 (1H, sextet, $J = 7.0$ Hz), 1.60−1.55 (2H, m), 1.50−1.29 (14H, m), 1.18 (6H, d, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.28, 172.02, 155.61, 80.02, 71.06, 58.77, 54.59, 43.12, 39.20, 32.57, 29.42, 28.43, 28.32, 25.03, 19.41; MS (FAB) *m*/*z* 495 (MH^+); Anal. ($C_{19}H_{36}N_2O_5S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-7-Amino-6-(*tert*-butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (24b). Yield 36%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.04 (1H, broad s), 5.36 (1H, broad s), 4.97 (1H, m), 4.10 (1H, m), 2.83 (2H, t, $J = 7.6$ Hz), 2.72 (1H, septet, $J = 6.7$ Hz), 1.84 (1H, m), 1.60−1.31 (16H, m), 1.18 (6H, d, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.37, 174.46, 155.74, 80.23, 54.03, 43.13, 32.10, 29.38, 28.33, 28.26, 25.94, 19.33; MS (FAB) *m*/*z* 347 (MH^+); Anal. ($C_{16}H_{30}N_2O_4S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(pyrrolidin-1-yl)heptyl 2-Methylpropanethioate (25b). Yield 74%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.32 (1H, d, $J = 8.5$ Hz), 4.40 (1H, m), 3.63 (1H, m), 3.53 (1H, m), 3.41 (2H, m), 2.82 (2H, t, $J = 7.3$ Hz), 2.72 (2H, quintet, $J = 6.7$ Hz), 1.97 (2H, quintet, $J = 6.7$ Hz), 1.87 (2H, m), 1.66 (1H, m), 1.60−1.29 (16H, m), 1.18 (6H, d, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.86, 155.58, 79.48, 51.84, 46.45, 45.94, 33.83, 33.11, 28.40, 28.10, 26.07, 24.48, 24.17; MS (FAB) *m*/*z* 401 (MH^+); MS (EI) *m*/*z* 400 (M^+); HRMS calcd for $C_{20}H_{36}N_2O_4S$, 400.240; found, 400.240.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cycloheptylamino)-7-oxoheptyl 2-Methylpropanethioate (18b). Compound **18b** was prepared from **43** using the procedure described for **11b** (step 2) and **16b** (step 2) in 63% yield: yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.98 (1H, d, $J = 8.2$ Hz), 4.98 (1H, s), 3.95−3.91 (2H, m), 2.83 (2H, t, $J = 7.3$ Hz), 2.73 (1H, septet, $J = 7.0$ Hz), 1.88−1.87 (2H, m), 1.81−1.78 (1H, m), 1.61−1.36 (26H, m), 1.18 (6H, d, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.31, 171.16, 77.23, 60.40, 50.37, 43.11, 32.27, 29.40, 28.32, 28.30, 27.99, 25.06, 24.03, 21.06, 19.42, 14.21; Anal. ($C_{23}H_{42}N_2O_4S\cdot 1/2H_2O$) C, H, N.

(*S*)-*S*-6-Amino-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate Hydrochloride (26b·HCl). To a solution of **16b** (962 mg, 2.32 mmol) in AcOEt (12 mL) was added 4 N HCl/AcOEt (6 mL), and the mixture was stirred at room temperature for 2 h. Then, the solvent was removed in vacuo to give 813 mg (100%) of **26b**·HCl as a colorless oil: ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 8.59 (1H, d, $J = 7.3$ Hz), 8.19 (3H, broad s), 4.03 (1H, sextet, $J = 6.7$ Hz), 3.67 (1H, m), 2.81 (2H, t, $J = 7.3$ Hz), 2.73 (1H, septet, $J = 6.7$ Hz), 1.85−1.76 (2H, m), 1.69−1.58 (4H, m), 1.52−1.20 (10H, m), 1.10 (6H, t, $J = 7.0$ Hz); ^{13}C NMR (DMSO-d_6, 500 MHz, δ, ppm) 203.17, 167.70, 52.14, 50.55, 42.40, 32.32, 31.83, 30.87, 28.82, 27.61, 27.59, 23.60, 23.38, 23.33, 19.09; MS (FAB) *m*/*z* 315 (MH^+ − HCl); Anal. ($C_{16}H_{30}N_2O_2S\cdot HCl\cdot H_2O$) C, H, N.

(*S*)-*S*-6-Benzamido-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (27b). To a suspension of **26b**·HCl (250 mg, 0.712 mmol) and Et_3N (1 mL, 13.6 mmol) in CH_2Cl_2 (2 mL) was added a solution of benzoyl chloride (248 μL mg, 2.13 mmol) in CH_2Cl_2 (3 mL) dropwise. The reaction mixture was poured into water and was extracted with AOEt. The AcOEt layer was separated, washed with 10% aqueous citric acid and brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by flash column chromatography (AcOEt/*n*-hexane = 1/4 to 1/2) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 208 mg (70%) of **27b** as colorless crystals: mp 116−117 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.78 (2H, m), 7.51 (1H, t, $J = 7.3$ Hz), 7.43 (2H, t, $J = 7.6$ Hz), 6.89 (1H, d, $J = 7.9$ Hz), 6.24 (1H, d, $J = 7.6$ Hz), 4.57 (1H, q, $J = 7.3$ Hz), 4.19 (1H, sextet, $J = 7.0$ Hz), 2.83 (2H, m), 2.72 (1H, septet, $J = 6.7$ Hz), 2.02−1.90 (3H, m), 1.80−1.65 (7H, m), 1.45−1.35 (6H, m), 1.18 (6H, dd, $J = 6.9$, 1.2 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.36, 170.98, 167.24, 133.95, 131.73, 128.57, 127.06, 53.50, 51.34, 43.09, 33.10, 32.94, 32.57, 29.35, 28.35, 28.20, 24.85, 23.75, 23.72, 19.40; MS (EI) *m*/*z* 418 (M^+); HRMS calcd for $C_{23}H_{34}N_2O_3S$, 418.229; found, 418.229; Anal. ($C_{23}H_{34}N_2O_3S$) C, H, N.

Compounds **28b** and **29b** were prepared from **26b**·HCl and an appropriate acid chloride using the procedure described for **27b**.

(*S*)-*S*-6-(4-Chlorobenzamido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (28b). Yield 95%; mp 119−121 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.74 (2H, d, $J = 8.5$ Hz), 7.40 (2H, d, $J = 8.5$ Hz), 6.89 (1H, d, $J = 7.9$ Hz), 6.04 (1H, d, $J = 7.6$ Hz), 4.51 (1H, q, $J = 7.0$ Hz), 4.20 (1H, sextet, $J = 7.0$ Hz), 2.82 (2H, m), 2.72 (1H, septet, $J = 7.0$ Hz), 2.04−1.87 (3H, m), 1.80−1.53 (9H, m), 1.40 (6H, m), 1.18 (6H, dd, $J = 7.0$, 1.5 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.48, 170.88, 166.13,

138.03, 132.32, 128.85, 128.55, 53.37, 51.44, 43.13, 33.13, 32.97, 32.64, 29.64, 28.25, 28.13, 24.76, 23.75, 23.72, 19.43, 19.41; Anal. ($C_{23}H_{33}ClN_2O_3S$) C, H, N.

(*S*)-*S*-7-(Cyclopentylamino)-6-(furan-2-carboxamido)-7-oxoheptyl 2-Methylpropanethioate (29b). Yield 37%; mp 88−89 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.46 (1H, d, J = 0.9 Hz), 7.11 (1H, d, J = 3.6 Hz), 6.88 (1H, d, J = 7.9 Hz), 6.50 (1H, dd, J = 3.5, 1.8 Hz), 6.03 (1H, d, J = 7.0 Hz), 4.46 (1H, q, J = 7.6 Hz), 4.19 (1H, sextet, J = 6.7 Hz), 2.84 (2H, td, J = 7.5, 2.1 Hz), 2.72 (1H, septet, J = 7.0 Hz), 2.02−1.87 (3H, m), 1.74−1.53 (7H, m), 1.45−1.34 (6H, m), 1.18 (6H, dd, J = 7.0, 0.9 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.39, 170.66, 158.20, 147.48, 144.28, 114.66, 112.15, 52.83, 51.39, 43.13, 33.13, 32.98, 32.43, 29.37, 28.35, 28.26, 24.93, 23.76, 23.73, 19.42; MS (EI) *m*/*z* 408 (M^+); HRMS calcd for $C_{21}H_{32}N_2O_4S$, 408.208; found, 408.209; Anal. ($C_{21}H_{32}N_2O_4S$) C, H, N.

Compounds **30b** and **32b**−**34b** were prepared from **26b**·HCl and an appropriate carboxylic acid using the procedure described for **16b** (step 1).

(*S*)-*S*-7-(Cyclopentylamino)-6-(2-hydroxyacetamido)-7-oxoheptyl 2-Methylpropanethioate (30b). Yield 28%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.97 (1H, d, J = 8.2 Hz), 5.99 (1H, d, J = 7.3 Hz), 4.34 (1H, m), 4.21−4.13 (3H, m), 2.91 (1H, broad s), 2.83 (2H, m), 2.74 (1H, septet, J = 7.0 Hz), 1.98 (2H, m), 1.85 (1H, m), 1.71−1.53 (7H, m), 1.44−1.31 (6H, m), 1.18 (6H, dd, J = 7.0, 1.4 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.91, 171.85, 170.86, 62.21, 52.78, 51.38, 43.14, 33.08, 32.92, 32.25, 29.28, 29.24, 29.06, 28.04, 24.73, 23.74, 19.44, 19.42; MS (EI) *m*/*z* 372 (M^+); HRMS calcd for $C_{18}H_{32}N_2O_4S$, 372.208; found, 372.208.

(*S*)-*S*-6-(Cyclohexanecarboxamido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (32b). Yield 62%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.34 (2H, m), 4.29 (1H, q, J = 6.7 Hz), 4.16 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.73 (1H, septet, J = 7.0 Hz), 2.10 (1H, tt, J = 7.0, 3.3 Hz), 1.94 (2H, m), 1.90−1.75 (5H, m), 1.70−1.56 (9H, m), 1.49−1.22 (10H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.39, 176.22, 171.14, 52.75, 51.22, 45.42, 43.13, 33.07, 33.01, 32.03, 29.70, 29.35, 28.33, 28.22, 25.72, 25.68, 25.64, 24.87, 23.74, 19.42; MS (EI) *m*/*z* 424 (M^+); HRMS calcd for $C_{23}H_{40}N_2O_3S$, 424.276; found, 424.275.

(*S*)-*S*-7-(Cyclopentylamino)-7-oxo-6-pivalamidoheptyl 2-Methylpropanethioate (33b). Yield 62%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.21 (1H, d, J = 7.6 Hz), 6.08 (1H, d, J = 7.3 Hz), 4.29 (1H, q, J = 6.7 Hz), 4.17 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.72 (1H, septet, J = 7.0 Hz), 1.96 (2H, m), 1.83 (1H, m), 1.70−1.53 (7H, m), 1.42−1.29 (6H, m), 1.20 (9H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.34, 178.62, 171.17, 52.89, 51.22, 43.13, 38.75, 33.07, 31.98, 29.37, 28.36, 28.23, 27.48, 24.90, 23.74, 23.69, 19.41; MS (EI) *m*/*z* 398 (M^+); HRMS calcd for $C_{21}H_{38}N_2O_3S$, 398.260; found, 398.261.

(*S*)-*S*-7-(Cyclopentylamino)-6-(3,3-dimethylbutanamido)-7-oxoheptyl 2-Methylpropanethioate (34b). Yield 59%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.21 (1H, d, J = 7.3 Hz), 6.05 (1H, d, J = 8.2 Hz), 4.30 (1H, q, J = 6.7 Hz), 4.16 (1H, sextet, J = 6.9 Hz), 2.81 (2H, m), 2.73 (1H, septet, J = 6.7 Hz), 2.07 (2H, m), 1.96 (2H, m), 1.80 (1H, m), 1.70−1.52 (7H, m), 1.42−1.30 (6H, m), 1.18 (6H, d, J = 6.7 Hz), 1.02 (9H, s); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.41, 171.89, 171.10, 53.02, 52.94, 51.23, 50.46, 43.13, 33.09, 32.97, 32.34, 31.90, 30.99, 29.85, 29.33, 28.31, 28.19, 23.75, 23.73, 19.43, 19.42; MS (EI) *m*/*z* 412 (M^+); HRMS calcd for $C_{22}H_{40}N_2O_3S$, 412.276; found, 412.276.

(*S*)-*S*-6-Acetamido-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (31b). To a solution of **26b**·HCl (102 mg, 0.291 mmol) and a catalytic amount of DMAP in CH_2Cl_2 (3 mL) were added acetic acid anhydride (55 μL, 0.582 mmol) and Et_3N (200 μL, 2.72 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 3/1 to AcOEt only) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 54 mg (52%) of **31b** as colorless crystals: mp 130−131 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.10 (1H, d, J = 8.2 Hz), 5.94 (1H, d, J = 7.6 Hz), 4.28 (1H, q, J = 7.3 Hz), 4.17 (1H, sextet, J = 7.3 Hz), 2.82 (2H, m), 2.73 (1H, septet, J = 6.7 Hz), 2.02−1.93 (5H, m), 1.80 (1H, m), 1.74−1.49 (7H, m), 1.46−1.29 (6H, m), 1.18 (6H, dd, J = 7.0, 0.9 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.46, 170.02, 170.02, 53.17, 51.30, 43.14, 33.12, 32.97, 32.34, 29.32, 28.26, 28.16, 24.77, 23.73, 23.71, 23.26, 19.43; MS (EI) *m*/*z* 356 (M^+); HRMS calcd for $C_{18}H_{32}N_2O_3S$, 356.213; found, 356.213; Anal. ($C_{18}H_{32}N_2O_3S$) C, H, N.

(*S*)-*S*-6-(3-*tert*-Butylureido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (35b). To a solution of **26b**·HCl (99 mg, 0.282 mmol) and Et_3N (400 μL, 5.44 mmol) in CH_2Cl_2 (4 mL) was added *tert*-butyl isocyanate (132 μL, 1.14 mmol), and the mixture was stirred at room temperature for 6 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/1) gave 89 mg (76%) of **35b** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.27 (1H, d, J = 7.9 Hz), 4.81 (1H, d, J = 7.9 Hz), 4.48 (1H, s), 4.16 (1H, sextet, J = 6.7 Hz), 4.07 (1H, q, J = 5.8 Hz), 2.92−2.70 (3H, m), 1.94 (2H, m), 1.76 (1H, m), 1.72−1.55 (7H, m), 1.43−1.25 (15H, m), 1.18 (6H, dd, J = 6.7, 0.9 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.82, 172.39, 157.10, 53.85, 51.12, 50.57, 43.14, 33.08, 33.00, 32.07, 29.43, 29.22, 28.21, 28.12, 24.78, 23.73, 23.69, 19.46, 19.44; MS (EI) *m*/*z* 413 (M^+); HRMS calcd for $C_{21}H_{39}N_3O_3S$, 413.271; found, 413.273.

(*S*)-*S*-6-(3-*tert*-Butylthioureido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (36b). Compound **36b** was prepared from **26b**·HCl and *tert*-thioisocyanate using the procedure described for **35b** in 24% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.53 (1H, d, J = 7.0 Hz), 6.09 (1H, m), 5.97 (1H, d, J = 7.0 Hz), 4.85 (1H, m), 4.19 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.73 (1H, septet, J = 7.0 Hz), 1.98 (3H, m), 1.78−1.52 (7H, m), 1.48−1.29 (15H, m), 1.19 (6H, dd, J = 7.0, 1.5 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.47, 179.78, 170.93, 58.73, 52.94, 51.53, 43.12, 33.10, 32.94, 32.64, 29.46, 29.34, 28.33, 28.15, 24.21, 23.69, 23.64, 19.44, 19.42; MS (EI) *m*/*z* 429 (M^+); HRMS calcd for $C_{21}H_{39}N_3O_2S_2$, 429.248; found, 429.248.

(*S*)-*S*-7-(Cyclopentylamino)-6-(methoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (37b). To a solution of **26b**·HCl (177 mg, 0.504 mmol) in CH_2Cl_2 (3 mL) were added methyl chloroformate (39 μL, 0.505 mmol) and Et_3N (500 μL, 6.80 mmol), and the mixture was stirred at room temperature for 15 min. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/2 to AcOEt only) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 138 mg (74%) of **37b** as colorless crystals: mp 88−89 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.90 (1H, d, J = 6.7 Hz), 5.24 (1H, broad s), 4.18 (1H, sextet, J = 7.0 Hz), 4.02 (1H, m), 3.68 (3H, s), 2.82 (2H, td, J = 7.6, 2.4 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.89 (2H, m), 1.81 (1H, m), 1.73−1.53 (7H, m), 1.42−1.31 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 171.08, 156.86, 54.99, 52.38, 51.29, 43.13, 33.12, 33.01, 32.61, 29.71, 29.36, 28.30, 28.23, 24.82, 23.74, 19.42; MS (EI) *m*/*z* 372 (M^+); HRMS calcd for $C_{18}H_{32}N_2O_4S$, 372.208; found, 372.208; Anal. ($C_{18}H_{32}N_2O_4S$) C, H, N.

Compounds **39b**−**41b** were prepared from **26b**·HCl and an appropriate chloroformate using the procedure described for **37b**.

(*S*)-*S*-7-(Cyclopentylamino)-7-oxo-6-(phenoxycarbonyl)heptyl 2-Methylpropanethioate (39b). Yield 17%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.36 (2H, t, J = 7.9 Hz), 7.20 (1H, t, J = 7.6 Hz), 7.12 (2H, d, J = 7.9 Hz), 5.85 (1H, d, J = 7.0 Hz), 5.66 (1H, d, J = 8.2 Hz), 4.22 (1H, sextet, J = 7.3 Hz), 4.09

(1H, q, J = 7.3 Hz), 2.83 (2H, td, J = 7.0, 2.4 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.99 (2H, m), 1.86 (1H, m), 1.72−1.53 (7H, m), 1.46−1.36 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.42, 170.69, 154.82, 150.92, 129.33, 125.47, 121.54, 55.06, 51.42, 43.14, 33.15, 33.02, 32.71, 29.37, 28.26, 28.21, 24.76, 23.74, 19.42; MS (FAB) m/z 435 (MH^+); Anal. ($C_{23}H_{34}N_2O_4S$) C, H, N.

(*S*)-*S*-6-(Benzyloxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (40b). Yield 24%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.35 (5H, m), 5.89 (1H, m), 5.31 (1H, m), 5.10 (2H, s), 4.17 (1H, sextet, J = 6.7 Hz), 4.04 (1H, m), 2.82 (2H, td, J = 7.2, 2.1 Hz), 2.72 (1H, septet, J = 7.0 Hz), 1.94 (2H, s), 1.81 (1H, m), 1.70−1.51 (7H, m), 1.42−1.23 (6H, m), 1.17 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.39, 170.98, 156.19, 136.27, 128.56, 128.22, 128.06, 67.04, 55.00, 51.28, 43.12, 33.09, 32.99, 32.52, 29.36, 28.30, 28.23, 24.84, 23.71, 19.42; MS (EI) m/z 448 (M^+); HRMS calcd for $C_{24}H_{36}N_2O_4S$, 448.240; found, 448.239; Anal. ($C_{24}H_{36}N_2O_4S$) C, H, N.

(*S*)-*S*-6-[{(9*H*-Fluoren-9-yl)methoxy}carbonyl]-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (41b). Yield 11%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.76 (2H, d, J = 7.6 Hz), 7.58 (2H, d, J = 7.6 Hz), 7.40 (2H, t, J = 7.6 Hz), 7.31 (2H, td, J = 7.7, 0.9 Hz), 5.82 (1H, m), 5.34 (1H, d, J = 7.7 Hz), 4.40 (2H, m), 4.23−4.15 (1H, m), 4.02 (1H, m), 2.84 (2H, m), 2.73 (1H, septet, J = 6.7 Hz), 1.97 (2H, m), 1.82 (1H, m), 1.68−1.52 (7H, m), 1.45−1.25 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 170.95, 156.19, 143.80, 141.35, 127.75, 127.10, 125.04, 120.02, 120.00, 67.01, 54.99, 51.32, 47.21, 43.14, 33.12, 33.02, 32.59, 29.37, 28.28, 28.23, 24.80, 23.74, 23.72, 19.42; MS (FAB) m/z 537 (MH^+); Anal. ($C_{31}H_{40}N_2O_4S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-6-(Cyclohexyloxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (38b). To a solution of triphosgene (654 mg, 2.20 mmol) in dry Et_2O (4 mL) was added dropwise a mixture of cyclohexanol (682 μL, 6.46 mmol) and pyridine (626 μL, 7.74 mmol) at −78 °C. The mixture was stirred for 1 h at −78 °C and then 1.5 h at room temperature. The reaction mixture was poured into 1 N aqueuos HCl and was extracted with AcOEt, washed with brine, and dried over Na_2SO_4. Filtration and evaporation in vacuo gave a colorless oil. To a solution of **26b**·HCl (94 mg, 0.268 mmol) and Et_3N (300 μL, 4.08 mmol) in CH_2Cl_2 (4 mL) was added the colorless oil, and the mixture was stirred at room temperature for 13 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3) gave 30 mg (26%) of **38b** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.91 (1H, d, J = 7.3 Hz), 5.08 (1H, m), 4.61 (1H, m), 4.18 (1H, sextet, J = 7.6 Hz), 3.98 (1H, m), 2.82 (2H, td, J = 7.0, 1.8 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.98 (2H, m), 1.84 (3H, m), 1.75−1.50 (11H, m), 1.37 (10H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 171.20, 63.46, 54.80, 51.20, 43.11, 33.11, 33.03, 32.41, 31.92, 29.37, 28.34, 28.25, 25.36, 24.92, 23.72, 23.70, 23.69, 19.42; MS (FAB) m/z 441 (MH^+); MS (EI) m/z 440 (M^+); HRMS calcd for $C_{23}H_{40}N_2O_4S$, 440.271; found, 440.270.

(*S*)-*tert*-Butyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (16a). Step 1: Preparation of (*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl Ethanethioate (16d). A solution of **16c** (410 mg, 1.05 mmol) obtained above and KSAc (183 mg, 1.60 mmol) in EtOH (3 mL) was stirred overnight at room temperature. The reaction mixture was evaporated in vacuo. The residue was subjected to silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) to give 303 mg (75%) of **16d** as a yellow solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.97 (1H, d, J = 7.3 Hz), 4.97 (1H, s), 4.18 (1H, m), 3.94 (1H, m), 2.85 (2H, t, J = 7.3 Hz), 2.32 (3H, s), 1.97−1.96 (2H, m), 1.81−1.80 (1H, m), 1.67−1.55 (8H, m), 1.44 (9H, s), 1.39−1.36 (5H, m).

Step 2: Preparation of (*S*)-*tert*-Butyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (16a). To a solution of **16d** (300 mg, 0.778 mmol) obtained above in EtOH (5 mL) was added 2 N aqueous NaOH (2 mL, 4.00 mmol), and the solution was stirred at room temperature for 10 min. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) gave 200 mg (74%) of **16a** as a colorless solid. The solid (106 mg) was recrystallized from AcOEt/*n*-hexane to give 60 mg of **16a** as colorless crystals: mp 108−110 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.93 (1H, d, J = 7.0 Hz), 4.95 (1H, s), 4.19−4.16 (1H, m), 3.94 (1H, s), 2.52 (2H, q, J = 7.3 Hz), 1.98−1.96 (2H, m), 1.85−1.80 (1H, m), 1.67−1.52 (7H, m), 1.44 (9H, s), 1.42−1.31 (7H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.89, 77.23, 48.04, 36.96, 33.79, 33.31, 28.65, 28.04, 25.68, 25.56, 24.88, 24.56; Anal. ($C_{17}H_{32}N_2O_3S$) C, H, N.

Compounds **13a**, **15a**, and **17a**−**20a** were prepared from the corresponding bromide using the procedure described for **16a**.

(*S*)-*tert*-Butyl 1-(Biphenyl-3-ylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (13a). Yield 34%; colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.23 (1H, broad s), 7.79 (1H, s), 7.58 (2H, d, J = 7.3 Hz), 7.48 (1H, d, J = 7.9 Hz), 7.44−7.33 (5H, m), 4.97 (1H, s), 4.18 (1H, s), 2.53 (2H, q, J = 7.3 Hz), 2.00−1.96 (1H, m), 1.71−1.59 (3H, m), 1.47−1.43 (13H, m), 1.33 (1H, t, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.39, 156.27, 142.14, 140.64, 138.12, 129.35, 128.73, 127.48, 127.18, 123.18, 118.65, 118.61, 80.71, 55.27, 33.70, 30.93, 28.33, 27.99, 25.25, 24.46; MS (EI) m/z 428 (M^+); HRMS calcd for $C_{24}H_{34}N_2O_3S$, 428.213; found, 428.213.

(*S*)-*tert*-Butyl 7-Mercapto-1-oxo-1-(quinolin-3-ylamino)heptan-2-ylcarbamate (15a). Yield 39%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.79 (1H, broad s), 8.74 (2H, s), 8.03 (1H, d, J = 8.5 Hz), 7.78 (1H, d, J = 7.9 Hz), 7.62 (1H, t, J = 7.0 Hz), 7.52 (1H, t, J = 7.8 Hz), 5.00 (1H, d, J = 6.7 Hz), 4.26 (1H, m), 2.53 (2H, q, J = 7.6 Hz), 2.00 (1H, m), 1.73−1.63 (3H, m), 1.50−1.36 (13H, m), 1.34 (1H, t, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.17, 145.18, 143.89, 131.37, 128.97, 128.20, 128.07, 127.68, 127.14, 123.66, 81.13, 77.23, 33.65, 30.94, 28.35, 27.97, 25.35, 24.43; MS (EI) m/z 403 (M^+); HRMS calcd for $C_{21}H_{29}N_3O_3S$, 403.193; found, 403.194.

(*S*)-*tert*-Butyl 1-(cyclohexylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (17a). Yield 72%; mp 125−127 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.85 (1H, d, J = 7.9 Hz), 4.96 (1H, s), 3.95 (1H, s), 3.75 (1H, s), 2.51 (2H, q, J = 7.4 Hz), 1.90−1.78 (3H, m), 1.72−1.68 (2H, m), 1.64−1.56 (4H, m), 1.44 (9H, s), 1.43−1.31 (7H, m), 1.19−1.13 (3H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.84, 77.23, 48.13, 33.74, 33.08, 32.93, 30.94, 28.33, 27.99, 25.49, 25.07, 24.71, 24.46; MS (EI) m/z 358 (M^+); HRMS calcd for $C_{18}H_{34}O_3N_2S$, 358.229; found, 358.229; Anal. ($C_{18}H_{34}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(Cycloheptylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (18a). Yield 62%; mp 76−78 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.92 (1H, d, J = 7.6 Hz), 4.96 (1H, s), 3.95−3.91 (2H, m), 2.52 (2H, q, J = 7.3 Hz), 1.88−1.79 (3H, m), 1.62−1.58 (7H, m), 1.54−1.33 (20H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.55, 77.21, 54.60, 50.38, 35.06, 34.96, 33.74, 32.34, 28.33, 27.99, 25.08, 24.47, 24.02; Anal. ($C_{18}H_{34}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(*tert*-Butylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (19a). Yield 58%; mp 90−91 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.78 (1H, s), 4.97 (1H, m), 3.89 (1H, m), 2.52 (2H, q, J = 7.6 Hz), 1.79 (1H, sextet, J = 8.2 Hz), 1.66−1.50 (3H, m), 1.49−1.38 (11H, m), 1.36−1.30 (12H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 171.05, 155.69, 79.90, 54.91, 51.26, 33.68, 32.36, 28.66, 28.26, 27.94, 24.96, 24.39; MS (FAB) m/z 333 (MH^+); Anal. ($C_{16}H_{32}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(Adamant-1-ylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (20a). Yield 41%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.60 (1H, s), 4.97 (1H, m), 3.88 (1H, m), 2.81 (2H, q, J = 7.3 Hz), 2.07 (3H, s), 1.98 (6H, d, J = 2.7 Hz), 1.78 (1H, sextet, J = 7.8 Hz), 1.67 (3H, s), 1.65−1.30 (17H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.87, 155.71, 79.93,

54.98, 41.60, 36.32, 33.76, 32.60, 30.92, 29.55, 29.43, 29.22, 28.35, 28.02, 25.00, 24.47; MS (FAB) *m/z* 411 (MH^+); Anal. ($C_{22}H_{38}N_2O_3S\cdot 1/4H_2O$) C, H, N.

(*S*)-2-Amino-*N*-cyclopentyl-7-mercaptoheptanamide Hydrochloride (26a·HCl). Compound **26a**·HCl was prepared from **16a** using the procedure described for **26b**·HCl in 94% yield: colorless oil; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 8.43 (1H, d, J = 7.3 Hz), 8.13 (3H, broad s), 4.03 (1H, sextet, J = 6.7 Hz), 3.65 (1H, t, J = 6.7 Hz), 2.46 (2H, q, J = 7.3 Hz), 2.28 (1H, t, J = 7.6 Hz), 1.82 (2H, m), 1.66 (4H, m), 1.58−1.23 (10H, m); ^{13}C NMR (DMSO-d_6, 500 MHz, δ, ppm) 167.65, 52.08, 50.46, 32.89, 32.24, 31.75, 30.85, 27.08, 23.50, 23.41, 23.30, 23.24; MS (FAB) *m/z* 245 (MH^+ − HCl); Anal. ($C_{12}H_{24}N_2OS\cdot HCl\cdot 3/4H_2O$) C, H, N.

Compounds **27a** and **28a** were prepared from **16d** using the procedure described for **26b**·HCl, **27b**, and **16a** (step 2).

(*S*)-*N*-(1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl)-benzamide (27a). Yield 69%; mp 171−172 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.79 (2H, m), 7.52 (1H, t, J = 7.3 Hz), 7.44 (2H, t, J = 7.6 Hz), 6.79 (1H, d, J = 8.2 Hz), 6.01 (1H, d, J = 7.0 Hz), 4.54 (1H, q, J = 7.0 Hz), 4.19 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q, J = 7.6 Hz), 1.98 (3H, m), 1.77−1.56 (7H, m), 1.48−1.35 (6H, m), 1.32 (1H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.94, 167.23, 133.94, 131.81, 128.65, 127.04, 53.50, 51.41, 33.71, 33.17, 33.00, 32.76, 28.04, 25.01, 24.46, 23.75, 23.72; MS (EI) *m/z* 348 (M^+); HRMS calcd for $C_{19}H_{28}N_2O_2S$, 348.187; found, 348.187; Anal. ($C_{19}H_{28}N_2O_2S$) C, H, N.

(*S*)-4-Chloro-*N*-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl]benzamide (28a). Yield 54%; mp 178−180 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.73 (2H, d, J = 8.5 Hz), 7.41 (2H, d, J = 8.5 Hz), 6.84 (1H, d, J = 7.6 Hz), 5.95 (1H, d, J = 7.3 Hz), 4.53 (1H, q, J = 7.0 Hz), 4.20 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q J = 7.0 Hz), 1.96 (3H, m), 1.74−1.54 (7H, m), 1.41 (6H, m), 1.32 (1H, t, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.84, 166.10, 138.08, 132.29, 128.88, 128.51, 53.55, 51.44, 33.66, 33.17, 32.96, 32.91, 28.01, 24.94, 24.45, 23.74, 23.71; Anal. ($C_{19}H_{27}ClN_2O_2S$) C, H, N.

Compounds **30a** and **33a** were prepared from **16d** using the procedure described for **26b**·HCl, **16b** (step 1), and **16a** (step 2).

(*S*)-*N*-Cyclopentyl-2-(2-hydroxyacetamido)-7-mercaptoheptanamide (30a). Yield 55%; mp 85−86 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.93 (1H, d, J = 8.5 Hz), 5.90 (1H, d, J = 6.7 Hz), 4.34 (1H, q, J = 7.9 Hz), 4.21−4.10 (3H, m), 2.69 (1H, t, J = 5.7 Hz), 2.51 (2H, q, J = 7.3 Hz), 1.97 (2H, m), 1.83 (1H, m), 1.72−1.53 (7H, m), 1.46−1.30 (7H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 171.82, 170.99, 62.16, 52.97, 51.40, 33.65, 33.10, 32.89, 32.48, 27.93, 25.02, 24.45, 23.73; MS (EI) *m/z* 302 (M^+); HRMS calcd for $C_{14}H_{26}N_2O_3S$, 302.166; found, 302.167; Anal. ($C_{14}H_{26}N_2O_3S$) C, H, N.

(*S*)-*N*-Cyclopentyl-7-mercapto-2-pivalamidoheptanamide (33a). Yield 60%; mp 134−139 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.20 (1H, d, J = 7.9 Hz), 6.08 (1H, d, J = 7.3 Hz), 4.30 (1H, q, J = 7.3 Hz), 4.17 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q, J = 7.3 Hz), 1.96 (2H, m), 1.83 (1H, m), 1.71−1.55 (8H, m), 1.45−1.25 (6H, m), 1.20 (9H, s); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 178.60, 171.13, 52.83, 51.22, 38.75, 33.70, 33.08, 33.03, 32.13, 28.01, 27.47, 24.97, 24.45, 23.73, 23.68; MS (EI) *m/z* 328 (M^+); HRMS calcd for $C_{17}H_{32}N_2O_2S$, 328.218; found, 328.219; Anal. ($C_{17}H_{32}N_2O_2S$) C, H, N.

Compounds **35a** and **36a** were prepared from **16d** using the procedure described for **26b**·HCl, **35b**, and **16a** (step 2).

(*S*)-1-*tert*-Butyl-3-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl]urea (35a). Yield 71%; mp 188−191 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.19 (1H, d, J = 6.7 Hz), 4.92 (1H, d, J = 8.5 Hz), 4.49 (1H, s), 4.16 (1H, sextet, J = 7.3 Hz), 4.09 (1H, q, J = 7.6 Hz), 2.52 (2H, q, J = 7.6 Hz), 1.94 (2H, m), 1.76 (1H, m), 1.72−1.50 (7H, m), 1.46−1.22 (16H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 172.45, 157.02, 53.83, 51.16, 50.57, 33.75, 33.10, 32.98, 32.64, 29.44, 28.07, 25.20, 24.47, 23.72, 23.67; Anal. ($C_{17}H_{33}N_3O_2S\cdot 1/6H_2O$) C, H, N.

(*S*)-1-*tert*-Butyl-3-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl)thiourea (36a). Yield 25%; mp 125−127 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.48 (1H, d, J = 7.6 Hz), 6.06 (1H, s), 5.85 (1H, d, J = 7.6 Hz), 4.87 (1H, m), 4.19 (1H, sextet, J = 7.0 Hz), 2.50 (2H, q, J = 7.0 Hz), 2.02 (3H, m), 1.76−1.58 (7H, m), 1.48−1.36 (16H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 179.92, 171.32, 58.04, 53.03, 51.49, 33.71, 33.10, 32.87, 32.74, 29.42, 28.08, 24.50, 24.45, 23.70, 23.66; MS (EI) *m/z* 359 (M^+); HRMS calcd for $C_{17}H_{33}N_3O_2S$, 359.207; found, 359.205; Anal. ($C_{17}H_{33}N_3O_2S$) C, H, N.

Compounds **37a** and **40a** were prepared from **16d** using the procedure described for **26b**·HCl, **37b**, and **16a** (step 2).

(*S*)-Methyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (37a). Yield 89%; mp 81−82 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.79 (1H, d, J = 7.0 Hz), 5.18 (1H, m), 4.18 (1H, sextet, J = 6.7 Hz), 4.02 (1H, m), 3.68 (3H, s), 2.51 (2H, q, J = 7.6 Hz), 1.98 (2H, m), 1.81 (1H, m), 1.71−1.53 (7H, m), 1.46−1.30 (7H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 171.02, 54.97, 52.41, 51.30, 33.68, 33.14, 33.03, 32.73, 27.93, 24.91, 24.45, 23.71; MS (EI) *m/z* 302 (M^+); HRMS calcd for $C_{14}H_{26}N_2O_3S$, 302.166; found, 302.169; Anal. ($C_{14}H_{26}N_2O_3S$) C, H, N.

(*S*)-Benzyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (40a). Yield 77%; mp 119−122 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.35 (5H, m), 5.80 (1H, m), 5.27 (1H, m), 5.11 (2H, s), 4.17 (1H, q, J = 6.7 Hz), 4.03 (1H, m), 2.50 (2H, q, J = 7.3 Hz), 1.96 (2H, s), 1.84 (1H, m), 1.70−1.53 (7H, m), 1.45−1.26 (7H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.98, 156.18, 136.22, 128.57, 128.24, 128.06, 67.04, 54.17, 51.26, 33.68, 33.11, 32.98, 32.66, 27.93, 24.92, 24.44, 23.71; MS (EI) *m/z* 378 (M^+); HRMS calcd for $C_{20}H_{30}N_2O_3S$, 378.198; found, 378.197; Anal. ($C_{20}H_{30}N_2O_3S\cdot 1/3H_2O$) C, H, N.

(*S*)-Cyclohexyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (38a). Compound **38a** was prepared from **16d** using the procedure described for **26b**·HCl, **38b**, and **16a** (step 2) in 78% yield: mp 110−111 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.85 (1H, m), 5.06 (1H, m), 4.62 (1H, m), 4.18 (1H, sextet, J = 7.0 Hz), 3.99 (1H, m), 2.51 (2H, q, J = 7.0 Hz), 1.97 (2H, m), 1.85 (3H, m), 1.74−1.46 (11H, m), 1.45−1.30 (11H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.21, 156.06, 73.63, 54.75, 51.20, 33.70, 33.12, 33.03, 32.53, 31.91, 29.70, 27.96, 25.35, 24.99, 24.46, 23.76, 23.71, 23.39; MS (FAB) *m/z* 371 (MH^+); Anal. ($C_{19}H_{34}N_2O_3S\cdot 1/4H_2O$) C, H, N.

Biology. Western Blot Analysis. Human colon cancer HCT116 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in McCoy5A culture medium containing penicillin and streptomycin, which was supplemented with fetal bovine serum as described in the ATCC instructions. HCT-116 cells (5×10^5) were treated for 8 h with samples at the indicated concentrations in 10% FBS supplemented with McCoy's 5A medium and were then collected and extracted with SDS buffer. Protein concentrations of the lysates were determined using a Bradford protein assay kit (Bio-Rad Laboratories) with which equivalent amounts of protein from each lysate were resolved in 15% SDS-polyacrylamide gels and then transferred onto nitrocellulose membranes (Bio-Rad Laboratories). After blocking for 30 min with Tris-buffered saline (TBS) containing 3% skimmed milk, the transblotted membranes were incubated overnight at 4 °C with hyperacetylated histone H4 antibody (Upstate Biotechnology; 1:4000 dilution), acetylated α-tubulin antibody (SIGMA; 1:4000 dilution), or β-actin antibody (Abcam; 1:500 dilution) in TBS containing 3% skimmed milk. After probing with the primary antibody, the membrane was washed twice with water and then incubated with goat, antirabbit, or antimouse IgG-horseradish peroxidase conjugates (diluted 1:5000) for 2 h at room temperature and washed twice more with water. The immunoblots were visualized by enhanced chemiluminescence.

Enzyme Assays. The inhibitory activities of the test compounds against partially purified HDAC1, HDAC4, and HDAC6 were assayed according to a method reported in ref 14c.

Cell Growth Inhibition Assay. Human colon cancer HCT116 cells and ERα-positive breast cancer MCF-7 cells, which were purchased from American Type Culture Collection (ATCC, Manassas, VA), were cultured in McCoy5A and Dulbecco's Modified

Eagle's Medium (DMEM) containing penicillin and streptomycin, which was supplemented with fetal bovine serum as described in the ATCC instructions, respectively. HCT116 and MCF-7 cells were plated in 96-well plates at initial densities of 5000 (HCT116) or 1500 (MCF-7) cells/well (50 μL/well) and incubated at 37 °C. After 24 h, cells were exposed to test compounds by adding solutions (50 μL/well) of compounds at various concentrations in McCoy5A (HCT116) and DMEM (MCF-7) medium at 37 °C at 5% CO_2 for 72 h. The mixtures were then treated with 10 μL of alamarBlue, and cells were further incubated at 37 °C for 3 h. The fluorescence in each well was measured on a fluorometric plate reader, with excitation set at 530 nm and emission detection set at 590 nm, and the percentage of cell growth was calculated from the fluorescence readings.

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Supporting Information Available: Results of the elemental analysis of **11b**, **16b**, **18b**−**24b**, **26b**−**29b**, **31b**, **37b**, **39b**−**41b**, **16a**−**20a**, **26a**−**28a**, **30a**, **33a**, **35a**−**38a**, and **40a** are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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