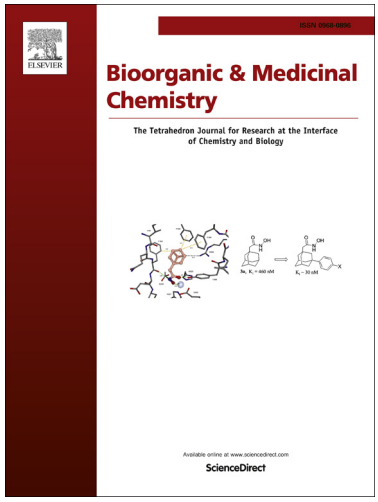

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Design and Synthesis of Novel and Highly-Active Pan-Histone Deacetylase (pan-HDAC) Inhibitors

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ABSTRACT

Histone deacetylase (HDAC) inhibitions are known to elicit anticancer effects. We designed and synthesized several HDAC inhibitors. Among these compounds, compound **40** exhibited a more than 10-fold stronger inhibitory activity compared with that of suberoylanilide hydroxamic acid (SAHA) against each human HDAC isozyme *in vitro* (IC_{50} values of **40**: HDAC1, 0.0038 μM; HDAC2, 0.0082 μM; HDAC3, 0.015 μM; HDAC8, 0.0060 μM; HDAC4, 0.058 μM; HDAC9, 0.0052 μM; HDAC6, 0.058 μM). The dose of the administered HDAC inhibitors that contain hydroxamic acid as the zinc-binding group may be reduced by **40**. Because the carbostyril subunit is a time-tested structural component of drugs and biologically active compounds, **40** most likely exhibits good absorption, distribution, metabolism, excretion, and toxicity (ADMET). Thus, compound **40** is expected to be a promising therapeutic agent or chemical tool for the investigation of life process.

1. Introduction

At the molecular level, histone deacetylases (HDACs)[1, 2] catalyze the deacetylation of ε-N-acetyl lysine residues not only on histone proteins but also on non-histone proteins[3-5], such as tumor suppressor proteins, transcriptional factors, nuclear receptors, and signal mediators, and therefore play an important role in epigenetic transcriptional regulation[6]. From the point of view of cancer, HDACs have much influence on proliferation, differentiation, cell cycle arrest, and/or apoptosis. The deacetylation of lysine residues on histone proteins leads to DNA transcriptional repression because the coilings of chromatin are too tight for transcription factors to access them. In this deacetylated state of chromatin, genes such as $p21^{WAF1/CIP1}$, Gadd 45, FAS, and caspase-3, which are related to cell cycle arrest and apoptosis in tumor cells, are transcriptionally inactivated[7]. Thus, HDAC inhibition on histone proteins is promising strategy to achieve anticancer effects. However, the association of HDACs with their non-histone protein substrates has been revealed. The acetylation of p53 or Runx3, which is a tumor suppressor protein, increases its stability and its binding affinity to DNA, and the acetylation of p53 results in an increase in related transcriptional activation[3-5]. Thus, HDAC inhibition on non-histone proteins may also result in anticancer effects. In addition, HDAC overexpression has been recognized in many types of human cancers[8]. Therefore, HDAC inhibitors are potential anticancer drug candidates.

The 18 human HDACs are subdivided into four classes based on their amino acid sequences. Class I (HDACs 1-3 and 8), class IIa (HDACs 4, 5, 7, and 9), class IIb (HDACs 6 and 10), and class IV (HDAC 11) are zinc-dependent metallohydrolases, whereas class III HDACs (sirtuins 1-7) are NAD^+-dependent. p53[9] and Runx3[10] have been implicated in HDAC1, and Runx3[10] is implicated in HDAC5. It has been suggested that the overexpression of HDAC1-3 is associated with breast cancer[11, 12] and that HDAC8 is associated with T-cell lymphoma[13] and neuroblastoma tumorigenesis[14]. As previously described, HDAC isozymes are intricately interrelated with histone proteins and non-histone proteins.

Vorinostat (suberoylanilide hydroxamic acid, SAHA) and romidepsin have been approved for the treatment of cutaneous T-cell lymphoma (CTCL) by the US Food and Drug Administration (FDA) (Figure 1). Vorinostat was used for the treatment of CTCL in 21 countries in 2011. Recently several HDAC inhibitors have been investigated in clinical trials as potential cancer treatments[6]. Novel HDAC inhibitors are expected to cure patients suffering from a variety of cancers. In the present study, we synthesized novel and highly-active pan-HDAC inhibitors.

2. Chemistry

Compounds **1-43** were designed and synthesized in this study. Their structures and synthetic routes are outlined in Schemes 1-7 and Tables 1-4. The letters X, Y, Z, Y, and B in Schemes 1-5 correspond to those in Tables 1-3. The analysis of the structural

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features shows that the carbostyril subunit, which is often used in biologically active compounds, as the cap group and hydroxamic acid as the zinc-binding group are tethered with an alkyl chain. The synthetic route of compounds **1**-**11** is described in Scheme 1. The nucleophilic substitution of the corresponding carbostyril[15] or coumarin phenoxides **44** to bromides yielded the ethers **45**. The hydroxamic acids **1**-**11** were obtained by the replacement of esters **45** with NH_2OH. Second, the synthetic route of compounds **12**-**18**, **26**, **30**, and **32** is described in Scheme 2. Dicarboxylic acid mono esters and the corresponding carbostyril anilines **46** were condensed with EDCI, and the esters **47** were then replaced with NH_2OH[16] to afford compounds **12**-**18**, **26**, **30**, and **32**. Third, the synthetic route used to produce **19**-**25**, **27**-**29**, and **31** is described in Scheme 3. In this route, amines and the corresponding carbostyril or coumarin carboxylic acids **48** were condensed with EDCI, and the esters **49** were replaced with NH_2OH[16] to afford compounds **19**-**25**, **27**-**29**, and **31**.

Compounds **33**-**35** were then synthesized using the route shown in Scheme 4. *N*-Boc-bromobenzylamine **51** was obtained through Gabriel amine synthesis[17] and subsequent protection. Ethyl acrylate was introduced through a Heck reaction[18] in good yield. Through the coupling of deprotected amines **52** and carbostyril carboxylic acids with EDCI, the esters were converted to compounds **33**-**35** with NH_2OH[16]. Amines[19] were added to the carbostyril sulfonyl chlorides **54**[20], and the esters were converted with NH_2OH[16] to yield compounds **36**-**38** (Scheme 5). In reverse, the addition of 6-amino-3,4-dihydrocarbostyril to sulfonic acid **56** derived from 7-bromoheptanoic acid methyl ester **55** provided **39** (Scheme 6).

The synthetic route used for the production of **40**-**43** is described in Scheme 7. These routes are started from the trans and cis forms of 1,4-cyclohexanedimethanol **58**. After monobenzylation, the other hydroxyl group was mesylated and then replaced with NaN_3. The amines provided by the hydrogenolysis of azides were coupled to 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid with EDCI to form amides **59**. The hydroxyl groups obtained by the high-presure hydrogenolysis of benzyl groups were oxidized with Dess-Martin periodinane[21] and then subject to a Horner-Emmons reaction to generate esters **60**. The replacement of esters with NH_2OH[16] gave compounds **40** and **42**. In contrast, through a reduction of the double bond by hydrogenation, the replacement of esters with NH_2OH[16] produced compounds **41** and **43**.

3. Results and discussion

Two types of enzyme assays, using rat liver HDACs and human HDAC isozymes, were performed in this study. The results of the enzyme assays performed with SAHA[22] and **1**-**43** using rat liver HDACs are shown in Tables 1-4. All of the compounds in which the cap group and the linker were connected by an ether bond showed low activity at 0.1 μM and high activity at 10 μM with the exception of compound **4** (Table 1). Although it is well-known, the linker of HDAC inhibitors has the appropriate length[23]. The weak activity of **4**, which possessed a shorter linker than the other compounds, reflects this fact. The linker lengths of SAHA and **1**-**11**, with the exception of **4**, were similar. It is thus apparent that the ether bonds in this region of the molecules are repulsive to HDAC enzymes. Therefore, the ether bonds were replaced with an amide bond in the next design, and the ether series compound with the longer linker was not synthesized.

Interestingly, some of the compounds with an amide connection elicited stronger activity than SAHA (Table 2). This suggests the existence of a hydrogen bond between this amide connection and HDAC proteins. The structural features of the cap group resulted in different activity results. The rank order of activity strength based on X-Y tended to be CH=CH > $C(CH_3)_2$-CH_2 > CH_2-CH_2. Thus, in addition to the zinc-binding group, the interaction of both the cap group and the connection unit between the linker and the cap group to HDAC proteins is crucial for the activity. The linkers with n = 5 were slightly shorter based on the results of **16** and **23** at 0.1 μM. Compared with **17** and **24**, compounds **13** and **20** showed higher activity at 0.1 μM, respectively, which indicates that a linker length of n = 6 is the best in this amide connection series. The comparisons of the amide and the corresponding reverse amide at 0.1 μM, e.g., **12** (66%) and **19** (68%), **13** (51%) and **20** (45%), **14** (58%) and **21** (44%), **15** (49%) and **22** (57%), and **26** (55%) and **29** (50%), which are shown in Tables 2 and 3 with the percent inhibition in parentheses, did not obviously reveal which type of amide is better in this assay using rat liver HDACs. The case of A = NHCO in Table 2 is preferable in drug design because toxic aniline metabolites cannot be formed.

The assay results with the modified compounds based on these findings are shown in Table 3. The compounds with a hydrophobic cap group, i.e., compounds **26**-**32**, elicited higher activity than SAHA. The comparisons of the results obtained with the corresponding less hydrophobic compounds at 0.1 μM, for example, **26** (55%) and **14** (58%), **27** (63%) and **19** (68%), **28** (51%) and **20** (45%), **29** (50%) and **21** (44%), **30** (52%) and **14** (58%), **31** (70%) and **19** (68%), and **32** (42%) and **14** (58%), which are shown in Tables 2 and 3 with the percent inhibition in parentheses, revealed that both cap forms with a hydrophobic character gave almost the same results in the *in vitro* enzyme assay. The amide bonds and the related substituent groups on positions 1 and 2 of carbostyril did not markedly interact with HDAC proteins. However, the *in vitro* and *in vivo* cell assays show that the both cap forms may give different results due to other factors, such as membrane permeability. The compounds possessing sulfonamide and reverse sulfonamide as the connection unit, i.e., compounds **36**-**39**, had low activity. Of compounds with a linker containing an aromatic ring, the para form **34** elicited stronger activity than SAHA.

The compounds with a linker containing an aromatic ring exhibited low solubility. To improve this low solubility, the benzene ring was saturated to form a cyclohexane ring in the molecular design. The assay results of the resulting compounds are shown in Table 4. The trans forms **40** and **41** elicited stronger activity than SAHA, but the cis forms **42** and **43** elicited weaker activity. The lengths between the carbonyl oxygen atom of the hydroxamic acid group and the carbonyl oxygen atom of the connection amide group on position 6 of carbostyril were calculated by MM2 stabilization in a vacuum state using the Chem3D, software (version 13). Although the approximate rank order of the linker length was **40** (11.33 Å) > **41** (10.56 Å) > **42** (9.33 Å) > **43** (8.87 Å), showing calculated length in parentheses, the rank order of the activity strength was **40** > **41** > **43** > **42**. The cyclohexyl linkers were so rigid that the calculated lengths presumably reflect the actually observed lengths. The linker length of **40** resulted in the best interaction with HDAC proteins.

The results of enzyme assays with SAHA and several of the synthesized compounds using human HDAC isozymes are shown in Table 5. The rank order of the activity strength of the cyclohexyl series was **40** > **41** = **43** > **42**, which is mostly consistent with that obtained in the enzyme assays using rat HDACs. Surprisingly, compound **40** elicited a more than 10-fold increase in pan-spectrum inhibitory activity compared with that of SAHA against human HDAC isozymes, particularly against HDAC8. Structurally, the active site of HDAC proteins forms a cylindrical cave, and the zinc is located at the bottom of this cave[24]. The analysis of the interaction mode between HDAC proteins and

HADC inhibitors shows that the zinc-binding groups chelate the zinc atoms at the bottom of the cave and that the cave mouths are covered with the cap groups. Furthermore, the linkers interact with the wall surface of the cave. Thus, it is hypothesized that cyclohexyl linkers such as that found in compound **40** settle in the cylindrical cave in a thermodynamically more stable state than straight-chain linkers, such as that found in SAHA, due to various factors, such as hydrophobic bonds.

▪ 4. Conclusions

The carbostyril derivatives designed and synthesized in this study elicited HDAC inhibitory activity. Of these studied structures, compound **40** exhibited much stronger pan-spectrum HDAC inhibitory activity than SAHA. Pan-spectrum HDAC inhibitors and selective-spectrum HDAC inhibitors have their own advantages. It is true that selective HDAC inhibitors are very effective for the treatment of chronic diseases due to their few side effects, but their effect in the treatment of acute diseases is different. In these cases, pan-spectrum HDAC inhibitors will exert serious effects even though they might yield some side effects, depending on the conditions. Many types of cancers are considered representative examples of acute diseases and have complicated pathological mechanisms. The analysis of HDACs, which catalyze the deacetylaion of histone proteins and non-histone proteins as their substrates, has shown that these proteins are involved in a variety of processes, including suppression, onset, progression, and apoptosis of cancer cells, through complicated mechanisms. If that is true, it is thus possible that pan-spectrum HDAC inhibitors may be attractive agents for treatment of cancers. This hypothesis is supported by the fact that SAHA and romidepsin have been approved for the treatment of CTCL by FDA. Therefore, the stronger pan-spectrum HDAC inhibitory activity of compound **40** is one of its advantages. The dose of the administered HDAC inhibitors that contain hydroxamic acid as the zinc-binding group may be reduced by compound **40**, which may result in low side effects and toxicities. Moreover, because the carbostyril subunit is a time-tested structural component of drugs and biologically active compounds[25-28], **40** may possess good features of absorption, distribution, metabolism, excretion, and toxicity (ADMET). Thus, compound **40** is expected to be a good therapeutic agent for cancers and can be used as a chemical tool to investigate the process of life phenomena, particularly epigenetics, concerning HDACs.

5. Experimental procedures

5.1 Chemistry

The commercial solvents and reagents used in this study were generally used without further purification. Column chromatography was conducted on silica gel 60 (230-400 mesh, Merck). The ^{1}H-NMR spectra were recorded on a Bruker Avance 300 spectrometer. The chemical shifts in the ^{1}H-NMR spectra are given in parts per million (ppm, δ) relative to tetramethylsilane (δ0.00) as the internal standard, and the coupling constants are reported in hertz. The multiplicities are given as s (singlet), br (broad), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), and m (multiplet). The LRMS spectra were recorded on a Shimadzu LCMS-2020 instrument. The HRMS spectra were recorded on a Shimadzu AXIMA Confidence instrument. The melting points were measured using Yanako micro metling point apparatus and were uncorrected.

5.1.1. **6-(3,4-dihydrocarbostyril-6-yloxy)hexanoic acid ethyl ester** (representative of compound **45**) as the general procedure for the synthesis of the intermediate of **1-11**

A solution of 6-hydroxy-3,4-dihydrocarbostyril (corresponding to **44**) (2.00 g, 12.26 mmol) in DMF (10 ml) was added dropwise to a suspension of NaH (60% in mineral oil, 0.59 g, 14.71 mmol) in DMF (5 ml) for 14 min at 0°C with a $CaCl_2$ tube. After stirring for 20 min at 0°C, a solution of 6-bromohexanoic acid ethyl ester (3.06 g, 13.48 mmol) in DMF (7 ml) was added dropwise to the reaction mixture for 7 min at 0°C. The reaction mixture was stirred overnight, during which time the ice was allowed to gradually melt and was subsequently stirred for 3 h at 110°C. After cooling, H_2O (100 ml) was added to the mixture, and the resulting mixture was extracted with AcOEt (100 ml × 3). The combined organic layer was washed with H_2O (100 ml × 1) and brine (100 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 1:2) to afford a colorless solid (2.56 g, 8.38 mmol, y. 68%). Colorless needles (*n*-hexane / AcOEt). Mp. 79-80°C. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.26 (3H, t, *J* = 7.2 Hz, $CH_2C\underline{H}_3$), 1.47-1.52 (2H, m, CH_2), 1.65-1.81 (4H, m, CH_2 × 2), 2.33 (2H, t, *J* = 7.4 Hz, CH_2), 2.61 (2H, t, *J* = 7.5 Hz, CH_2), 2.93 (2H, t, *J* = 7.4Hz, CH_2), 3.92 (2H, t, *J* = 6.5 Hz, CH_2), 4.13 (2H, q, *J* = 7.2 Hz, $C\underline{H}_2CH_3$), 6.65-6.69 (2H, m, ArH), 6.72 (1H, s, ArH), 7.91 (1H, s, NH).

5.1.2. **6-(3,4-dihydrocarbostyril-6-yloxy)hexanoic acid hydroxyamide** (**2**) as the general procedure for the synthesis of **1-43**

A solution of NH_2OH in 50% water (0.40 ml, 6.55 mmol) was added dropwise to a suspension of 6-(3,4-dihydrocarbostyril-6-yloxy)hexanoic acid ethyl ester (corresponding to **45**) (200 mg, 0.66 mmol) in MeOH (1.2 ml) at 0°C. After stirring for 4 min at 0°C, NaOMe in 25% MeOH (0.75 ml, 3.28 mmol) was added dropwise to the reaction mixture. After stirring for 1 h at 0°C, sat.NH_4Cl (10 ml) was added to the mixture. The precipitate was gathered, washed with H_2O and then *n*-hexane, and dried to afford a colorless solid (173 mg, 0.59 mmol, y. 90%). Colorless powder (AcOEt). Mp. 178-179°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.31-1.41 (2H, m, CH_2), 1.49-1.59 (2H, m, CH_2), 1.62-1.71 (2H, m, CH_2), 1.96 (2H, t, *J* = 7.2 Hz, CH_2), 2.39 (2H, t, *J* = 7.5 Hz, CH_2), 2.82 (2H, t, *J* = 7.7 Hz, CH_2), 3.87 (2H, t, *J* = 6.5 Hz, CH_2), 6.69 (1H, dd, *J* = 8.6, 2.6 Hz, ArH), 6.75 (1H, d, *J* = 8.1 Hz, ArH), 6.76 (1H, s, ArH), 8.66 (1H, s, NH), 9.88 (1H, s, NH), 10.33 (1H, bs, NOH). LRMS (ESI+) m/z 293 $[M+H]^+$. LRMS (ESI-) m/z 291 $[M-H]^-$.

5.1.3. **6-(carbostyril-6-yloxy)hexanoic acid hydroxyamide** (**1**)

Colorless powder (AcOEt). Mp. 199-200°C . ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.37-1.45 (2H, m, CH_2), 1.52-1.59 (2H, m, CH_2), 1.70-1.75 (2H, m, CH_2), 1.98 (2H, t, *J* = 7.2 Hz, CH_2), 3.97 (2H, t, *J* = 6.5 Hz, CH_2), 6.48 (1H, d, *J* = 9.3 Hz, ArH), 7.13 (1H, dd, *J* = 9.0 Hz, 2.7 Hz, ArH), 7.19 (1H, d, *J* = 2.4 Hz, ArH), 7.24 (1H, d, *J* = 9.0 Hz, ArH), 7.83 (1H, d, *J* = 9.6 Hz, ArH), 8.68 (1H, s, NH), 10.38 (1H, s, NH), 11.62 (1H, s, NOH). LRMS (ESI+) m/z 291 $[M+H]^+$. LRMS (ESI-) m/z 289 $[M-H]^-$.

5.1.4. **6-(4,4-dimethyl-3,4-dihydrocarbostyril-6-yloxy)hexanoic acid hydroxyamide** (**3**)

Colorless powder (*n*-hexane / AcOEt). Mp. 99-102°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.20 (6H, m, CH_3 × 2), 1.38-1.40 (2H, m, CH_2), 1.52-1.55 (2H, m, CH_2), 1.65-1.70 (2H, m, CH_2), 1.97 (2H, t, *J* = 7.2 Hz, CH_2), 2.29 (2H, s, CH_2), 3.89 (2H, t, *J* =

6.5 Hz, CH_2), 6.71 (1H, dd, *J* = 8.7, 2.4 Hz, ArH), 6.78 (1H, d, *J* = 8.4 Hz, ArH), 6.81 (1H, d, *J* = 2.4 Hz, ArH), 8.66 (1H, s, NH), 9.95 (1H, s, NH), 10.34 (1H, s, NOH). LRMS (ESI-) m/z 319 $[M-H]^-$.

5.1.5. **4-(3,4-dihydrocarbostyril-6-yloxy)butanoic acid hydroxyamide** (**4**)

Colorless powder (AcOEt). Mp. 190-191°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.85-1.94 (2H, m, CH_2), 2.11 (2H, t, *J* = 7.4 Hz, CH_2), 2.39 (2H, t, *J* = 7.7 Hz, CH_2), 2.82 (2H, t, *J* = 7.5 Hz, CH_2), 3.88 (2H, t, *J* = 6.3 Hz, CH_2), 6.70 (1H, dd, *J* = 8.7, 2.7 Hz, ArH), 6.75 (1H, d, *J* = 7.8 Hz, ArH), 6.76 (1H, d, *J* = 2.7 Hz, ArH), 8.69 (1H, s, NH), 9.89 (1H, s, NH), 10.40 (1H, bs, NOH). LRMS (ESI+) m/z 265 $[M+H]^+$. LRMS (ESI-) m/z 263 $[M-H]^-$.

5.1.6. **6-(coumarin-6-yloxy)hexanoic acid hydroxyamide** (**5**)

Colorless powder (*n*-hexane / AcOEt). Mp. 124-126°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.37-1.45 (2H, m, CH_2), 1.51-1.61 (2H, m, CH_2), 1.68-2.00 (2H, m, CH_2), 1.98 (2H, t, *J* = 7.2 Hz, CH_2), 4.00 (2H, t, *J* = 7.2 Hz, CH_2), 6.48 (1H, d, *J* = 9.6 Hz, ArH), 7.19 (1H, dd, *J* = 9.0, 3.0 Hz, ArH), 7.28 (1H, d, *J* = 2.7 Hz, ArH), 7.33 (1H, d, *J* = 9.0 Hz, ArH), 8.00 (1H, d, *J* = 9.6 Hz, ArH), 8.67 (1H, d, *J* = 1.2 Hz, NH), 10.35 (1H, s, NOH). LRMS (ESI+) m/z 292 $[M+H]^+$. LRMS (ESI-) m/z 290 $[M-H]^-$.

5.1.7. **6-(carbostyril-7-yloxy)hexanoic acid hydroxyamide** (**6**)

Colorless powder (AcOEt). Mp. 174-175°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.35-1.45 (2H, m, CH_2), 1.51-1.61 (2H, m, CH_2), 1.69-1.78 (2H, m, CH_2), 1.98 (2H, t, *J* = 7.2 Hz, CH_2), 3.99 (2H, t, *J* = 6.3 Hz, CH_2), 6.29 (1H, d, *J* = 9.6 Hz, ArH), 6.76-6.79 (2H, m, ArH), 7.54 (1H, d, *J* = 9.3 Hz, ArH), 7.79 (1H, d, *J* = 9.3 Hz, ArH), 8.67 (1H, s, NH), 10.35 (1H, s, NH), 11.55 (1H, s, NOH). LRMS (ESI+) m/z 291 $[M+H]^+$. LRMS (ESI-) m/z 289 $[M-H]^-$.

5.1.8. **6-(3,4-dihydrocarbostyril-7-yloxy)hexanoic acid hydroxyamide** (**7**)

Colorless powder (AcOEt). Mp. 130-132°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.33-1.38 (2H, m, CH_2), 1.52-1.57 (2H, m, CH_2), 1.65-1.70 (2H, m, CH_2), 1.97 (2H, t, *J* = 7.5 Hz, CH_2), 2.41 (2H, t, *J* = 7.5 Hz, CH_2), 2.78 (2H, t, *J* = 7.4 Hz, CH_2), 3.87 (2H, t, *J* = 6.5 Hz, CH_2), 6.42 (1H, d, *J* = 2.4 Hz, ArH), 6.47 (1H, dd, *J* = 8.3 Hz, 2.6 Hz, ArH), 7.03 (1H, d, *J* = 8.1 Hz, ArH), 8.66 (1H, s, NH), 9.96 (1H, s, NH), 10.35 (1H, s, NOH). LRMS (ESI+) m/z 293 $[M+H]^+$. LRMS (ESI-) m/z 291 $[M-H]^-$.

5.1.9. **6-(4,4-dimethyl-3,4-dihydrocarbostyril-7-yloxy)-hexanoic acid hydroxyamide** (**8**)

Colorless powder (*n*-hexane / AcOEt). Mp. 62-63°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.18 (6H, s, CH_3 × 2), 1.34-1.41 (2H, m, CH_2), 1.49-1.57 (2H, m, CH_2), 1.63-1.70 (2H, m, CH_2), 1.97 (2H, t, *J* = 7.4 Hz, CH_2), 2.30 (2H, s, CH_2), 3.87 (2H, t, *J* = 6.5 Hz, CH_2), 6.43 (1H, d, *J* = 2.7 Hz, ArH), 6.52 (1H, dd, *J* = 8.4, 2.4Hz, ArH), 7.15 (1H, d, *J* = 8.4 Hz, ArH), 8.66 (1H, s, NH), 10.01 (1H, s, NH), 10.33 (1H, bs, NOH). LRMS (ESI+) m/z 321 $[M+H]^+$. LRMS (ESI-) m/z 319 $[M-H]^-$.

5.1.10. **6-(3,4-dihydrocarbostyril-5-yloxy)hexanoic acid hydroxyamide** (**9**)

Colorless powder (AcOEt). Mp. 159-160°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.37-1.45 (2H, m, CH_2), 1.51-1.60 (2H, m, CH_2), 1.67-1.76 (2H, m, CH_2), 1.97 (2H, t, *J* = 7.2 Hz, CH_2), 2.40 (2H, t, *J* = 7.8 Hz, CH_2), 2.79 (2H, t, *J* = 7.7 Hz, CH_2), 3.94 (2H, t, *J* = 6.3 Hz, CH_2), 6.47 (1H, d, *J* = 7.8 Hz, ArH), 6.58 (1H, d, *J* = 8.1 Hz, ArH), 7.06 (1H, t, *J* = 8.1 Hz, ArH), 8.66 (1H, s, NH), 10.00 (1H, s, NH), 10.33 (1H, s, NOH). LRMS (ESI+) m/z 293 $[M+H]^+$. LRMS (ESI-) m/z 291 $[M-H]^-$.

5.1.11. **6-(carbostyril-8-yloxy)hexanoic acid hydroxyamide** (**10**)

Colorless powder (AcOEt). Mp. 122-124°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.43-1.50 (2H, m, CH_2), 1.53-1.63 (2H, m, CH_2), 1.78-1.87 (2H, m, CH_2), 2.00 (2H, t, *J* = 7.1 Hz, CH_2), 4.08 (2H, t, *J* = 6.3 Hz, CH_2), 6.52 (1H, d, *J* = 9.6 Hz, ArH), 7.07-7.14 (2H, m, ArH), 7.22 (1H, dd, *J* = 6.0, 3.0 Hz, ArH), 7.88 (1H, d, *J* = 9.6 Hz, ArH), 8.68 (1H, s, NH), 10.37 (1H, s, NH), 10.72 (1H, s, NOH). LRMS (ESI+) m/z 291 $[M+H]^+$. LRMS (ESI-) m/z 289 $[M-H]^-$.

5.1.12. **6-(3,4-dihydrocarbostyril-8-yloxy)hexanoic acid hydroxyamide** (**11**)

Colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.49-1.54 (2H, m, CH_2), 1.70-1.82 (4H, m, CH_2 × 2), 2.23 (2H, t, *J* = 6.9 Hz, CH_2), 2.61 (2H, t, *J* = 7.5 Hz, CH_2), 2.94 (2H, t, *J* = 7.5 Hz, CH_2), 3.99 (2H, t, *J* = 6.0 Hz, CH_2), 6.71-6.75 (2H, m, ArH), 6.91 (1H, t, *J* = 8.0 Hz, ArH), 8.24 (1H, s, NH), 9.84 (1H, bs, NOH). LRMS (ESI+) m/z 293 $[M+H]^+$. LRMS (ESI-) m/z 291 $[M-H]^-$.

5.1.13. **7-(3,4-dihydrocarbostyril-6-ylcarbamoyl)heptanoic acid benzyl ester** (representative of **47**) as the general procedure for the synthesis of the intermediate of **12**-**18**, **26**, **30**, and **32**

The reaction mixture of 6-amino-3,4-dihydrocarbostyril (corresponding to **46**) (78 mg, 0.48 mmol), suberic acid mono-benzyl ester (128 mg, 0.48 mmol), HOBt (65 mg, 0.48 mmol), Et_3N (49 mg, 0.48 mmol), and EDCI·HCl (93 mg, 0.48 mmol) in DMF (2 ml) was stirred overnight. Sat.$NaHCO_3$ (30 ml) was added to the mixture, and the resulting mixture was extracted with AcOEt (30 ml × 3). The combined organic layer was washed with 2 N HCl (30 ml × 1) and then brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (AcOEt) to afford a pale orange solid (101 mg, 0.25 mmol, y. 51%). Colorless powder (*n*-hexane / AcOEt). Mp. 87-88°C. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.36-1.38 (4H, m, CH_2 × 2), 1.63-1.74 (4H, m, CH_2 × 2), 2.29-2.39 (4H, m, CH_2 × 2), 2.61 (2H, t, *J* = 7.5 Hz, CH_2), 2.94 (2H, t, *J* = 7.5 Hz, CH_2), 5.11 (2H, s, CH_2), 6.71 (1H, d, *J* = 8.4 Hz, ArH), 7.17 (1H, dd, *J* = 8.4 Hz, 2.1 Hz, ArH), 7.23 (1H, s, ArH), 7.34-7.37 (5H, m, ArH), 7.50 (1H, s, NH), 8.13 (1H, s, NH). LRMS (ESI+) m/z 409 $[M+H]^+$. LRMS (ESI-) m/z 407 $[M-H]^-$.

5.1.14. **7-(carbostyril-6-ylcarbamoyl)heptanoic acid hydroxyamide** (**12**)

Light green powder ($CHCl_3$ / MeOH). Mp. 210-211°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.27-1.29 (4H, m, CH_2 × 2), 1.44-1.51 (4H, m, CH_2 × 2), 1.94 (2H, t, *J* = 7.2 Hz, CH_2), 2.27-2.31 (2H, m, NCH_2), 6.47 (1H, d, *J* = 9.6 Hz, CH=), 7.23 (1H, d, *J* = 9.0 Hz, ArH), 7.56 (1H, dd, *J* = 8.7, 2.1 Hz, ArH), 7.85 (1H, d, *J* = 9.6 Hz, CH=), 7.99 (1H, d, *J* = 1.8 Hz, ArH), 8.66 (1H, br, NH), 9.95 (1H, br, NH), 10.34 (1H, br, NH), 11.69 (1H, br, NOH). LRMS (ESI+) m/z 332 $[M+H]^+$. LRMS (ESI-) m/z 330 $[M-H]^-$.

5.1.15. **7-(3,4-dihydrocarbostyril-6-ylcarbamoyl)heptanoic acid hydroxyamide** (**13**)

Colorless powder (AcOEt). Mp. 196-197°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26-1.28 (4H, m, CH_2 × 2), 1.46-1.56 (4H, m, CH_2 × 2), 1.94 (2H, t, *J* = 7.4 Hz, CH_2), 2.25 (2H, t, *J* = 7.2 Hz, CH_2), 2.41 (2H, t, *J* = 7.5 Hz, CH_2), 2.82 (2H, t, *J* = 7.5 Hz, ArH), 6.76 (1H, d, *J* = 8.4 Hz, ArH), 7.29 (1H, dd, *J* = 8.6, 2.3 Hz, ArH), 7.43 (1H, d, *J* = 1.5 Hz, ArH), 8.65 (1H, s, NH), 9.73

(1H, s, NH), 9.98 (1H, s, NH), 10.33 (1H, s, NOH). LRMS (ESI+) m/z 334 $[M+H]^+$. LRMS (ESI-) m/z 332 $[M-H]^-$.

5.1.16. **7-(4,4-dimethyl-3,4-dihydrocarbostyril-6-yl-carbamoyl)heptanoic acid hydroxyamide** (**14**)

Colorless powder (AcOEt). Mp. 167-168°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.20 (6H, s, CH_3 × 2), 1.26-1.28 (4H, m, CH_2 × 2), 1.46-1.56 (4H, m, CH_2 × 2), 1.94 (2H, t, J = 7.4 Hz, CH_2), 2.25 (2H, t, J = 7.7 Hz, CH_2), 2.31 (2H, s, CH_2), 6.77 (1H, d, J = 8.7 Hz, ArH), 7.39 (1H, dd, J = 8.4, 2.1 Hz, ArH), 7.51 (1H, d, J = 2.1 Hz, ArH), 8.64 (1H, s, NH), 9.74 (1H, s, NH), 10.04 (1H, s, NH), 10.32 (1H, bs, NOH). LRMS (ESI-) m/z 360 $[M-H]^-$.

5.1.17. **7-(8-fluoro-4,4-dimethyl-3,4-dihydrocarbostyril-6-ylcarbamoyl)heptanoic acid hydroxyamide** (**15**)

Colorless powder ($CHCl_3$ / DMSO). Mp. 173-175°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.21 (6H, s, CH_3 × 2), 1.25-1.27 (4H, m, CH_2 × 2), 1.46-1.58 (4H, m, CH_2 × 2), 1.93 (2H, t, J = 7.2 Hz, CH_2), 2.26 (2H, t, J = 7.4 Hz, CH_2), 2.37 (2H, s, CH_2), 7.21 (1H, s, ArH), 7.54 (1H, dd, J = 13.0, 2.1 Hz, ArH), 8.66 (1H, br, NH), 9.95 (1H, br, NH), 10.06 (1H, br, NH), 10.39 (1H, br, NOH). LRMS (ESI+) m/z 380 $[M+H]^+$. LRMS (ESI-) m/z 378 $[M-H]^-$.

5.1.18. **6-(3,4-dihydrocarbostyril-6-ylcarbamoyl)hexanoic acid hydroxyamide** (**16**)

Colorless powder ($CHCl_3$ / DMSO). Mp. 186-188°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.17-1.30 (2H, m, CH_2), 1.45-1.61 (4H, m, CH_2 × 2), 1.94 (2H, t, J = 7.4 Hz, CH_2), 2.43 (2H, t, J = 7.2 Hz, CH_2), 2.39-2.43 (2H, m, CH_2), 2.79-2.84 (2H, m, CH_2), 6.75 (1H, d, J = 8.4 Hz, ArH), 7.28 (1H, dd, J = 8.4, 2.1 Hz ArH), 7.43 (1H, d, J = 1.5 Hz ArH), 8.67 (1H, br, NH), 10.14 (1H, br, NH), 10.33 (1H, br, NOH). LRMS (ESI+) m/z 320 $[M+H]^+$. LRMS (ESI-) m/z 318 $[M-H]^-$.

5.1.19. **8-(3,4-dihydrocarbostyril-6-ylcarbamoyl)octanoic acid hydroxyamide** (**17**)

Colorless powder (MeOH). Mp. 182-183°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.22-1.32(6H, m, CH_2 × 3), 1.45-1.60 (4H, m, CH_2 × 2), 1.93 (2H, t, J = 7.2 Hz, CH_2), 2.24 (2H, t, J = 7.2 Hz, CH_2), 2.41 (2H, t, J = 7.5 Hz, CH_2), 2.82 (2H, t, J = 7.4 Hz, CH_2), 6.75 (1H, d, J = 8.7 Hz, ArH), 7.28 (1H, d, J = 8.7 Hz, ArH), 7.43 (1H, s, ArH), 8.66 (1H, br, NH), 9.72 (1H, br, NH), 9.99 (1H, br, NH), 10.33 (1H, br, NOH). LRMS (ESI+) m/z 348 $[M+H]^+$. LRMS (ESI-) m/z 346 $[M-H]^-$.

5.1.20. **7-(coumarin-6-ylcarbamoyl)heptanoic acid hydroxyamide** (**18**)

Colorless powder ($CHCl_3$ / DMSO). Mp. 187-188°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26-1.32 (4H, m, CH_2 × 2), 1.44-1.64 (4H, m, CH_2 × 2), 1.94 (2H, t, J = 7.2 Hz, CH_2), 2.31 (2H, t, J = 7.4 Hz, CH_2), 6.48 (1H, d, J = 9.6 Hz, CH=), 7.36 (1H, d, J = 9.0 Hz ArH), 7.65 (1H, dd, J = 9.0, 2.7 Hz ArH), 8.05-8.09 (2H, m, ArH, CH=), 8.66 (1H, br, NH), 10.10 (1H, br, NH), 10.33 (1H, br, NOH). LRMS (ESI+) m/z 333 $[M+H]^+$. LRMS (ESI-) m/z 331 $[M-H]^-$.

5.1.21. **7-(1,4,4-trimethyl-3,4-dihydrocarbostyril-6-yl-carbamoyl)heptanoic acid hydroxyamide** (**26**)

Colorless powder ($CHCl_3$ / AcOEt). Mp. 118-121°C. ^{1}H-NMR (300 MHz / MeOH-d_4) δ1.28 (6H, s, CH_3 × 2), 1.38-1.40 (4H, m, CH_2 × 2), 1.61-1.68 (4H, m, CH_2 × 2), 2.10 (2H, t, J = 7.4 Hz, CH_2), 2.37 (2H, t, J = 7.4 Hz, CH_2), 2.48 (2H, s, CH_2), 3.34 (3H, s, NCH_3), 7.10 (1H, d, J = 8.7 Hz, ArH), 7.50 (1H, d, J = 8.7 Hz, ArH), 7.60 (1H, s, ArH). LRMS (ESI+) m/z 376 $[M+H]^+$. LRMS (ESI-) m/z 374 $[M-H]^-$.

5.1.22. **7-(3,4-dihydro-4,4-dimethyl-2-methoxyquinolin-6-ylcarbamoyl)heptanoic acid hydroxyamide** (**30**)

Colorless powder (MeOH). Mp. 66-71°C. ^{1}H-NMR (300 MHz / MeOH-d_4) δ1.23 (6H, s, CH_3 × 2), 1.38-1.40 (4H, m, CH_2 × 2), 1.61-1.73 (4H, m, CH_2 × 2), 2.09 (2H, t, J = 7.4 Hz, CH_2), 2.31 (2H, s, CH_2), 2.36 (2H, t, J = 7.6 Hz, CH_2), 3.87 (3H, s, OCH_3), 7.08 (1H, d, J = 8.4 Hz, ArH), 7.34 (1H, dd, J = 8.4, 2.4 Hz, ArH), 7.56 (1H, d, J = 2.4 Hz, ArH). LRMS (ESI+) m/z 376 $[M+H]^+$. LRMS (ESI-) m/z 374 $[M-H]^-$.

5.1.23. **7-(3,4-dihydro-4,4-dimethyl-2-ethoxyquinolin-6-yl-carbamoyl)heptanoic acid hydroxyamide** (**32**)

Colorless powder (*n*-hexane / AcOEt). Mp.162-164°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.23 (6H, s, CH_3 × 2), 1.25-1.31 (7H, m, CH_2 × 2 and $OCH_2C\underline{H}_3$), 1.46-1.58 (4H, m, CH_2 × 2), 1.94 (2H, t, J = 7.2 Hz, CH_2), 2.23-2.29 (4H, m, CH_2 and CH_2), 4.27 (2H, q, J = 7.2 Hz, $OC\underline{H}_2CH_3$), 6.95 (1H, d, J = 8.4 Hz, ArH), 7.32 (1H, br, NH), 7.43 (1H, dd, J = 8.4, 2.4 Hz, ArH), 7.52 (1H, d, J = 2.4 Hz, ArH), 9.78 (1H, br, NH), 10.32, (1H, br, OH). LRMS (ESI+) m/z 390 $[M+H]^+$. LRMS (ESI-) m/z 388 $[M-H]^-$.

5.1.24. **7-[(4,4-dimethyl-3,4-dihydrocarbostyril-6-carbonyl)amino]heptanoic acid benzyl ester** (representative of **49**) as the general procedure for the synthesis of the intermediate of **19**-**25**, **27**-**29**, and **31**

The reaction mixture of 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (corresponding to **48**) (251 mg, 1.25 mmol), 7-aminoheptanoic acid benzyl ester (251 mg, 1.31 mmol), HOBt (178 mg, 1.31 mmol), Et_3N (133 mg, 1.31 mmol), and EDCI·HCl (252 mg, 1.31 mmol) in DMF (6 ml) was stirred overnight at room temperature. Then, sat.$NaHCO_3$ (50 ml) was added to the mixture, and the resulting mixture was extracted with AcOEt (50 ml × 3). The combined organic layer was washed with H_2O (50 ml × 1), 1 N HCl (50 ml × 1) and then brine (50 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 1:2) to afford a colorless oil (117 mg, 0.29 mmol, y.22%). ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.26 (6H, s, CH_3 × 2), 1.39-1.43 (4H, m, CH_2 × 2), 1.62-1.69 (4H, m, CH_2 × 2), 2.28-2.39 (2H, m, CH_2), 2.51 (2H, s, CH_2), 3.40-3.46 (2H, m, CH_2), 5.11 (2H, s, CH_2), 6.07 (1H, t, J = 5.4 Hz, NH), 6.79 (1H, d, J = 8.1 Hz, ArH), 7.34-7.36 (5H, m, ArH), 7.52 (1H, dd, J = 8.1, 1.8 Hz, ArH), 7.80 (1H, d, J = 1.5 Hz, ArH), 8.18 (1H, s, NH).

5.1.25. **7-[(carbostyril-6-carbonyl)amino]heptanoic acid hydroxyamide** (**19**)

Light beige powder (MeOH). Mp. unmeasurable (decomposed at 263°C). ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26-1.33 (4H, m, CH_2 × 2), 1.46-1.55 (4H, m, CH_2 × 2), 1.94 (2H, t, J = 7.5 Hz, CH_2), 3.21-3.29 (2H, m, NCH_2), 6.55 (1H, d, J = 9.6 Hz, CH=), 7.31 (1H, d, J = 8.7 Hz, ArH), 7.93-7.97 (2H, m, ArH, CH=), 8.16 (1H, d, J = 1.8 Hz, ArH), 8.44 (1H, br, NH), 8.66 (1H, br, NH), 10.37 (1H, br, NH) , 11.92 (1H, br, NOH). LRMS (ESI+) m/z 332 $[M+H]^+$. LRMS (ESI-) m/z 330 $[M-H]^-$.

5.1.26. **7-[(3,4-dihydrocarbostyril-6-carbonyl)amino]heptanoic acid hydroxyamide** (**20**)

Colorless powder (AcOEt). Mp. 189-190°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.27-1.28 (4H, m, CH_2 × 2), 1.46-1.51 (4H, m, CH_2 × 2), 1.94 (2H, t, J = 7.4 Hz, CH_2), 2.47 (2H, t, J = 7.2 Hz, CH_2), 2.91 (2H, t, J = 7.5 Hz, CH_2), 3.18-3.24 (2H, m, CH_2), 6.86 (1H, d, J = 8.1 Hz, ArH), 7.63 (1H, d, J = 8.4 Hz, ArH), 7.67 (1H, s, ArH), 8.25 (1H, t, J = 5.4 Hz, NH), 8.64 (1H, s, NH),

10.26 (1H, s, NH), 10.32 (1H, s, NOH). LRMS (ESI+) m/z 334 $[M+H]^+$. LRMS (ESI-) m/z 332 $[M-H]^-$.

5.1.27. **7-[(4,4-dimethyl-3,4-dihydrocarbostyril-6-carbonyl)amino]heptanoic acid hydroxyamide** (**21**)

Colorless powder (AcOEt). Mp. 147-149°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26 (6H, s, CH_3 × 2), 1.29 (4H, m, CH_2 × 2), 1.50 (4H, m, CH_2 × 2), 1.94 (2H, t, *J* = 7.2 Hz, CH_2), 2.38 (2H, s, CH_2), 3.20-3.26 (2H, m, CH_2), 6.89 (1H, d, *J* = 8.4 Hz, ArH), 7.65 (1H, dd, *J* = 8.6, 1.7 Hz, ArH), 7.78 (1H, d, *J* = 1.5 Hz, ArH), 8.32 (1H, t, *J* = 5.9 Hz, NH), 8.66 (1H, s, NH), 10.33 (2H, s, NOH and NH). LRMS (ESI+) m/z 362 $[M+H]^+$. LRMS (ESI-) m/z 360 $[M-H]^-$.

5.1.28. **7-[(8-fluoro-4,4-dimethyl-3,4-dihydrocarbostyril-6-carbonyl)amino]heptanoic acid hydroxyamide** (**22**)

Colorless powder (*n*-hexane / $CHCl_3$). Mp. 168-169°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.27 (6H, s), 1.27-1.32 (4H, m, CH_2 × 2), 1.44-1.54 (4H, m, CH_2 × 2), 1.94 (2H, t, *J* = 7.4 Hz, CH_2), 2.44 (2H, s, CH_2), 3.20-3.26 (2H, m, CH_2), 7.58 (1H, dd, *J* = 11.0, 1.5 Hz, ArH), 7.65 (1H, br, NH), 8.43 (1H, br, NH), 10.33 (1H, br, NH) , 10.36 (1H, br, NOH). LRMS (ESI+) m/z 380 $[M+H]^+$. LRMS (ESI-) m/z 378 $[M-H]^-$.

5.1.29. **6-[(3,4-dihydrocarbostyril-6-carbonyl)amino]hexanoic acid hydroxyamide** (**23**)

Colorless powder (MeOH). Mp. 194-197°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.22-1.32 (2H, m, CH_2), 1.43-1.55 (4H, m, CH_2×2), 1.94 (2H, t, *J* = 7.4 Hz, CH_2), 2.45-2.49 (2H, m, CH_2), 2.91 (2H, t, *J* = 7.5 Hz, CH_2), 3.18-3.24 (2H, m, NCH_2), 6.86 (1H, d, *J* = 8.1 Hz, ArH), 7.11 (1H, br, NH), 7.62-7.68 (2H, m, ArH), 8.26 (1H, br, NH), 8.66 (1H, br, NH), 10.27 (1H, br, NH), 10.33 (1H, br, NOH). LRMS (ESI+) m/z 320 $[M+H]^+$. LRMS (ESI-) m/z 318 $[M-H]^-$.

5.1.30. **8-[(3,4-dihydrocarbostyril-6-carbonyl)amino]octanoic acid hydroxyamide** (**24**)

Colorless powder ($CHCl_3$ / MeOH). Mp. 193-194°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.22-1.31 (6H, m, CH_2 × 3), 1.44-1.53 (4H, m, CH_2 × 2), 1.93 (2H, t, *J* = 7.4 Hz, CH_2), 2.47 (2H, t, *J* = 7.2 Hz, CH_2), 2.91 (2H, t, *J* = 7.7 Hz, CH_2), 3.16-3.24 (2H, m, NCH_2), 6.86 (1H, d, *J* = 8.1 Hz, ArH), 7.61-7.67 (2H, m, ArH), 8.26 (1H, br, NH), 8.65 (1H, br, NH), 10.27 (1H, br, NH), 10.32 (1H, br, NOH). LRMS (ESI+) m/z 348 $[M+H]^+$. LRMS (ESI-) m/z 346 $[M-H]^-$.

5.1.31. 7-**[(4,4-dimethyl-3,4-dihydrocarbostyril-7-carbonyl)amino]heptanoic acid hydroxyamide** (**25**)

Colorless powder (AcOEt / MeOH). Mp. 193-198°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.23 (6H, s, CH_3 × 2), 1.26-1.27 (4H, m, CH_2 × 2), 1.46-1.50 (4H, m, CH_2 × 2), 1.93 (2H, t, *J* = 7.2 Hz, CH_2), 2.36 (2H, s, CH_2), 3.16-3.24 (2H, m, NCH_2), 7.31-7.40 (3H, m, ArH), 8.37 (1H, t, *J* = 5.4 Hz, NH), 8.68 (1H, br, NH), 10.25 (1H, br, NH), 10.36 (1H, br, NOH). LRMS (ESI+) m/z 362 $[M+H]^+$. LRMS (ESI-) m/z 360 $[M-H]^-$.

5.1.32. **7-[(1-methylcarbostyril-6-carbonyl)amino]heptanoic acid hydroxyamide** (**27**)

Light green powder ($CHCl_3$ / MeOH). Mp. 158-161°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26-1.34 (4H, m, CH_2 × 2), 1.44-1.56 (4H, m, CH_2 × 2), 1.94 (2H, t, *J* = 7.2 Hz, CH_2), 3.25 (2H, q, *J* = 6.5 Hz, NCH_2), 3.64 (3H, s, NCH_3), 6.67 (1H, d, *J* = 9.6 Hz, CH=), 7.59 (1H, d, *J* = 9.0 Hz, ArH), 7.97 (1H, d, *J* = 9.6 Hz, CH=), 8.08 (1H, dd, *J* = 8.7, 2.1 Hz, ArH), 8.23 (1H, *J* = 2.1 Hz, ArH), 8.56 (1H, br, NH), 8.67 (1H, br, NH), 10.37 (1H, br, OH). LRMS (ESI+) m/z 346 $[M+H]^+$. LRMS (ESI-) m/z 344 $[M-H]^-$.

5.1.33. **7-[(1-methyl-3,4-dihydrocarbostyril-6-carbonyl)-amino]heptanoic acid hydroxyamide** (**28**)

Colorless powder (H_2O). Mp.168-169°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.24-1.32 (4H, m, CH_2 × 2), 1.45-1.54 (4H, m, CH_2 × 2), 1.94 (2H, t, *J* = 7.2 Hz, CH_2), 2.54-2.59 (2H, m, CH_2), 2.88-2.93 (2H, m, CH_2), 3.23 (2H, q, *J* = 6.6 Hz, NCH_2), 3.27 (3H, s, CH_3), 7.14 (1H, d, *J* = 8.4 Hz, ArH), 7.71-7.77 (2H, m, ArH), 8.34 (1H, br, NH), 8.65 (1H, br, NH), 10.33 (1H, br, OH). LRMS (ESI+) m/z 348 $[M+H]^+$. LRMS (ESI-) m/z 346 $[M-H]^-$.

5.1.34. **7-[(1,4,4-trimethyl-3,4-dihydrocarbostyril-6-carbonyl)amino]heptanoic acid hydroxyamide** (**29**)

Pale cream powder (H_2O). Mp. 173-174°C. ^{1}H-NMR (300 MHz / MeOH-d_4) δ1.32 (6H, s, CH_3 × 2), 1.38-1.42 (4H, m, CH_2 × 2), 1.59-1.69 (4H, m, CH_2 × 2), 2.10 (2H, t, *J* = 7.4 Hz, CH_2), 2.53 (2H, s, CH_2), 3.37 (2H, t, *J* = 7.2 Hz, NCH_2), 3.40 (3H, s, CH_3), 7.22 (1H, d, *J* = 9.6 Hz, ArH), 7.77 (1H, dd, *J* = 8.4 Hz, 2.1 Hz, ArH), 7.85 (1H, d, *J* = 2.1 Hz, ArH). LRMS (ESI+) m/z 376 $[M+H]^+$. LRMS (ESI-) m/z 374 $[M-H]^-$.

5.1.35. **7-[(2-methoxyquinolin-6-carbonyl)amino]heptanoic acid hydroxyamide** (**31**)

Colorless powder (AcOEt). Mp.158-160°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.27-1.32 (4H, m, CH_2 × 2), 1.48-1.56 (4H, m, CH_2 × 2), 1.95 (2H, t, *J* = 7.2 Hz, CH_2), 3.23-3.27 (2H, m, NCH_2), 4.00 (3H, s, OCH_3), 7.08 (1H, d, *J* = 8.7 Hz, CH=), 7.82 (1H, d, *J* = 8.4 Hz, ArH), 8.09 (1H, dd, *J* = 8.7, 2.1 Hz, ArH), 8.32 (1H, d, *J* = 8.7 Hz, CH=), 8.39 (1H, d, *J* = 2.1 Hz, ArH), 8.58 (1H, br, NH), 10.34, (1H, br, NH). LRMS (ESI+) m/z 346 $[M+H]^+$. LRMS (ESI-) m/z 344 $[M-H]^-$.

5.1.36. **(4-bromobenzyl)carbamic acid *tert*-butyl ester** (representative of **51**) as the general procedure for the synthesis of **51**

(a) The reaction mixture of *p*-bromobenzyl bromide (corresponding to **50**) (4.00 g, 16.004 mmol) and phthalimide potassium salt (3.26 g, 17.61 mmol) in DMF (10 ml) was stirred for 6 h at 100°C with a $CaCl_2$ tube. After cooling, H_2O (100 ml) was added, and the resulting mixture was extracted with AcOEt (100 ml × 3). The combined organic layer was washed with H_2O (100 ml × 1) and then brine (100 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure to afford 2-(4-bromobenzyl)isoindole-1,3-dione (5.28 g, quant. y.) as a colorless solid. Colorless cotton-like crystal (*n*-hexane / AcOEt). Mp. 126-129°C. ^{1}H-NMR (300 MHz / $CDCl_3$ / TMS) δ4.80 (2H, s, CH_2), 7.32 (2H, d, *J* = 7.8 Hz, ArH), 7.44 (2H, d, *J* = 8.1 Hz, ArH), 7.71-7.73 (2H, m, ArH), 7.84-7.85 (2H, m, ArH).

(b) The reaction mixture of 2-(4-bromobenzyl)isoindole-1,3-dione (5.01 g, 15.83 mmol) and $H_2NNH_2{\cdot}H_2O$ (100%) (1.59 g, 31.67 mmol) in MeOH (50 ml) was stirred for 1 h at reflux. After cooling, MeOH was removed under reduced pressure. Concentrated HCl (20 ml) was then added and the resulting mixture was stirred for 1.5 h at reflux. After cooling, 2 N NaOH (200 ml) was added to the mixture, and the insoluble matters were removed by filtration. The filtrate was extracted with AcOEt (100 ml × 3). The combined organic layer was washed with H_2O (100 ml × 1) and then brine (100 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure to afford 4-bromobenzylamine (2.73 g, 14.65 mmol, y. 93%) as a yellow oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ3.83 (2H, s, CH_2), 7.20 (2H, d, *J* = 8.4 Hz, ArH), 7.45 (2H, d, *J* = 8.4 Hz, ArH).

(c) A solution of Boc_2O (1.29 g, 5.91 mmol) in CH_2Cl_2 (10 ml) was added to the suspension of 4-bromobenzylamine (1.00 g, 5.38 mmol) and Et_3N (0.60 g, 5.91 mmol) in CH_2Cl_2 (10 ml) for 5 min at 0°C with a $CaCl_2$ tube. The reaction mixture was stirred overnight at room temperature. Then, H_2O (50 ml) was added to the mixture, and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$ (50 ml × 3). The combined organic layer was washed with H_2O (50 ml × 1) and then brine (50 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure to afford (4-bromobenzyl)carbamic acid *tert*-butyl ester (1.63 g, quant. y.) as a colorless solid. Colorless powder (*n*-hexane). Mp. 73-74°C. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.46 (9H, s, *t*-Bu), 4.26 (2H, d, *J* = 6.0 Hz, CH_2), 4.84 (1H, bs, NH), 7.16 (2H, d, *J* = 8.4 Hz, ArH), 7.45 (2H, d, *J* = 8.4 Hz, ArH).

5.1.37. **(*E*)-3-(4-aminomethylphenyl)acrylic acid ethyl ester** (corresponding to **52**) as the general procedure for the synthesis of **52**

(d) The reaction mixture of (4-bromobenzyl)carbamic acid *tert*-butyl ester (corresponding to **51**) (1.63 g, 5.69 mmol), ethyl acrylate (0.63 g, 6.26 mmol), P(*o*-tolyl)$_3$ (0.35 g, 1.14 mmol), DIEA (2.12 ml, 12.17 mmol), and $Pd(OAc)_2$ (128 mg, 0.57 mmol) in DMF (6 ml) and EtCN (25 ml) was stirred for 10 min at room temperature under N_2 and then stirred for 6.5 h at 90°C under N_2. After cooling, H_2O (100 ml) was added, and the mixture was extracted with AcOEt (100 ml × 3). The combined organic layer was washed with H_2O (100 ml × 1) and then brine (100 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 3:1) to afford (*E*)-3-[4-(*tert*-butoxycarbonyl-aminomethyl)phenyl]acrylic acid ethyl ester (1.33 g, 4.36 mmol, y. 77%) as a yellow solid. Colorless powder (*n*-hexane). Mp .64-65°C. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.34 (3H, t, *J* = 7.1 Hz, $CH_2C\underline{H}_3$), 1.46 (9H, s, *t*-Bu), 4.26 (2H, q, *J* = 7.1 Hz, $C\underline{H}_2CH_3$), 4.33 (2H, d, *J* = 5.1 Hz, CH_2), 4.89 (1H, bs, NH), 6.42 (1H, d, *J* = 15.9 Hz, CH=), 7.30 (2H, d, *J* = 8.1 Hz, ArH), 7.49 (2H, d, *J* = 8.1 Hz, ArH), 7.67 (1H, d, *J* = 15.9 Hz, CH=).

(e) The reaction mixture of (*E*)-3-[4-(*tert*-butoxycarbonyl-aminomethyl)phenyl]acrylic acid ethyl ester (1.27 g, 4.17 mmol) in TFA (3 ml) and CH_2Cl_2 (9 ml) was stirred for 1 h at room temperature. After removing TFA and CH_2Cl_2 under reduced pressure, sat.$NaHCO_3$ (50 ml) was added to the mixture, and the mixture was extracted with AcOEt (50 ml × 3). The combined organic layer was washed with brine (50 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure to afford (*E*)-3-(4-aminomethylphenyl)acrylic acid ethyl ester (0.82 g, 3.98 mmol, y. 95%) as a yellow oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.34 (3H, t, *J* = 7.2 Hz, $CH_2C\underline{H}_3$), 2.52 (2H, bs, NH_2), 3.90 (2H, s, CH_2), 4.26 (2H, q, *J* = 7.1 Hz, $C\underline{H}_2CH_3$), 6.42 (1H, d, *J* = 15.9 Hz, CH=), 7.35 (2H, d, *J* = 8.1 Hz, ArH), 7.50 (2H, d, *J* = 8.1 Hz, ArH), 7.67 (1H, d, *J* = 16.2 Hz, CH=).

5.1.38. **(4,4-dimethyl-3,4-dihydrocarbostyril)-6-carboxylic acid (*E*)-4-[2-(hydroxycarbamoyl)vinyl]benzylamide** (**34**) as the general procedure for the synthesis of **33**-**35**

(f) The reaction mixture of (*E*)-3-(4-aminomethylphenyl)-acrylic acid ethyl ester (corresponding to **52**) (200 mg, 0.97 mmol), 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (214 mg, 0.97 mmol), HOBt (132 mg, 0.97 mmol), Et_3N (99 mg, 0.97 mmol), and EDCI·HCl (187 mg, 0.97 mmol) in DMF (4 ml) was stirred overnight. After sat.$NaHCO_3$ (30 ml) was added, the resulting mixture was extracted with AcOEt (30 ml × 3). The combined organic layer was washed with 1 N HCl (30 ml × 1) and then brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was washed with AcOEt (2 ml), and the insoluble matters were gathered, washed with the solvent (*n*-hexane:AcOEt = 1:1) and then *n*-hexane, and dried to afford (*E*)-3-[4-(4,4-dimethyl-3,4-dihydrocarbostyril-6-carbonyl)aminomethylphenyl]acrylic acid ethyl ester (298 mg, 0.73 mmol, y. 75%) as a colorless solid. Colorless powder (*n*-hexane / AcOEt). Mp. 215-217°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26 (3H, t, *J* = 7.1 Hz, $CH_2C\underline{H}_3$), 1.26 (6H, s, CH_3 × 2), 2.39 (2H, s, CH_2), 4.18 (2H, q, *J* = 7.1 Hz, $C\underline{H}_2CH_3$), 4.49 (2H, d, *J* = 5.7 Hz, CH_2), 6.59 (1H, d, *J* = 16.2 Hz, CH=), 6.91 (1H, d, *J* = 8.1 Hz, ArH), 7.35 (2H, d, *J* = 8.1 Hz, ArH), 7.63 (1H, d, *J* = 16.2 Hz, CH=), 7.68 (2H, d, *J* = 8.1 Hz, ArH), 7.73 (1H, dd, *J* = 8.3, 2.0 Hz, ArH), 7.85 (1H, d, *J* = 1.8 Hz, ArH), 8.97 (1H, t, *J* = 5.9 Hz, NH), 10.38 (1H, s, NH).

(g) This step was performed following the same procedure described for compound **2**. Compound **34**: Colorless powder (AcOEt / MeOH). Mp. 161-165°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26 (6H, s, CH_3 × 2), 2.39 (2H, s, CH_2), 4.61 (2H, d, *J* = 5.7 Hz, CH_2), 6.42 (1H, d, *J* = 15.9 Hz, CH=), 6.91 (1H, d, *J* = 8.4 Hz, ArH), 7.34 (2H, d, *J* = 8.1 Hz, ArH), 7.43 (1H, d, *J* = 15.9 Hz, CH=), 7.52 (2H, d, *J* = 8.1 Hz, ArH), 7.73 (1H, dd, *J* = 8.4, 1.8 Hz, ArH), 7.85 (1H, d, *J* = 1.5 Hz, ArH), 8.96 (1H, t, *J* = 5.7 Hz, NH), 9.02 (1H, s, NH), 10.38 (1H, s, NH), 10.74 (1H, s, NOH). LRMS (ESI+) m/z 394 $[M+H]^+$. LRMS (ESI-) m/z 392 $[M-H]^-$.

5.1.39. **(4,4-dimethyl-3,4-dihydrocarbostyril)-6-carboxylic acid (*E*)-3-[2-(hydroxycarbamoyl)vinyl]benzylamide** (**33**)

Colorless powder (AcOEt / MeOH). Mp. 159-163°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26 (6H, s, CH_3 × 2), 2.39 (2H, s, CH_2), 4.49 (2H, d, *J* = 5.1 Hz, CH_2), 6.46 (1H, d, *J* = 15.9 Hz, CH=), 6.92 (1H, d, *J* = 9.0 Hz, ArH), 7.33-7.60 (5H, m, ArH and CH=), 7.74 (1H, d, *J* = 9.0 Hz, ArH), 7.86 (1H, s, ArH), 9.01 (1H, s, NH), 10.39 (1H, s, NH). LRMS (ESI+) m/z 394 $[M+H]^+$. LRMS (ESI-) m/z 392 $[M-H]^-$.

5.1.40. **(4,4-dimethyl-3,4-dihydrocarbostyril)-7-carboxylic acid (*E*)-3-[2-(hydroxycarbamoyl)vinyl]benzylamide** (**35**)

Colorless powder (AcOEt / MeOH). Mp. 196-200°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.24 (6H, s, CH_3 × 2), 2.37 (2H, s, CH_2), 4.48 (2H, d, *J* = 5.7 Hz, CH_2), 6.44 (1H, d, *J* = 15.6 Hz, CH=), 7.29-7.55 (8H, m, ArH and CH=), 9.00 (1H, t, *J* = 5.9 Hz, NH), 10.27 (1H, s, NH), 10.78 (1H, s, NOH). LRMS (ESI+) m/z 394 $[M+H]^+$. LRMS (ESI-) m/z 392 $[M-H]^-$.

5.1.41. **carbostyril-6-sulfonyl chloride** (representative of **54**) as the general procedure for the synthesis of **54**

The reaction mixture of 2-quinolinol (corresponding to **53**) (1.0 g, 6.8 mmol) and chlorosulfonic acid (3.0 ml, 45.0 mmol) was stirred for 3 h at 65°C under N_2. The mixture was poured into iced water. The precipitate was gathered, washed with H_2O, and dried to afford a colorless solid (1.1 g, 4.6 mmol, y. 68%). ^{1}H-NMR (300 MHz / DMSO-d_6) δ6.51 (1H, dd, *J* = 9.6, 2.1 Hz, CH=), 7.25 (1H, dd, *J* = 8.4 Hz, 2.7 Hz ArH), 7.21 (1H, d, *J* = 8.4 Hz ArH), 7.91 (1H, s, ArH), 7.98 (1H, d, *J* = 9.6 Hz, CH=), 12.14 (1H, br, NH).

5.1.42. **7-(carbostyril-6-sulfonylamino)heptanoic acid hydroxyamide** (**36**) as the general procedure for the synthesis of **36**-**38**

(b) Carbostyril-6-sulfonyl chloride (corresponding to **54**) (138 mg, 0.57 mmol) and then subsequently Et_3N (114 mg, 3.24 mmol) in CH_2Cl_2 (0.5 ml) were added to a solution of 7-aminoheptanoic acid methyl ester hydrochloride (142 mg, 0.73 mmol) in CH_2Cl_2 (2 ml) at room temperature. The reaction

mixture was stirred for 16 h at room temperature. H_2O (10 ml), AcOEt (10 ml), and *n*-hexane (10 ml) were then sequentially added to the mixture. The precipitate was gathered, washed with H_2O, and dried to afford a pink solid (127 mg, 0.35 mmol, y. 61%). ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.15-1.20 (4H, m, CH_2 × 2), 1.29-1.44 (4H, m, CH_2 × 2), 2.22 (2H, t, *J* = 7.2 Hz, CH_2), 2.72 (2H, q, *J* = 6.0 Hz, NCH_2), 3.56 (3H, s, OCH_3) , 6.61 (1H, d, *J* = 9.6 Hz, CH=), 7.43 (1H, d, *J* = 8.7 Hz ArH), 7.53 (1H, t, *J* = 5.9 Hz, NH), 7.84 (1H, dd, *J* = 8.7, 2.1 Hz ArH), 8.07 (1H, d, *J* = 9.6 Hz, CH=), 8.13 (1H, d, *J* = 2.1 Hz, ArH), 12.01 (1H, br, NH).

(c) This step was performed following the same procedure described for compound **2**. Compound **36**: Colorless powder (MeOH). Mp. unmeasurable (decomposed at 242°C). ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.08-1.24 (4H, m, CH_2 × 2), 1.32-1.47 (4H, m, CH_2 × 2), 1.89 (2H, t, *J* = 7.4 Hz, CH_2), 2.72-2.74 (2H, m, NCH_2), 6.61 (1H, d, *J* = 9.6 Hz, CH=), 7.43 (1H, d, *J* = 8.7 Hz, ArH), 7.52 (1H, br, NH), 7.84 (1H, dd, *J* = 8.7, 1.8 Hz ArH), 8.07 (1H, d, *J* = 9.6 Hz, CH=), 8.62 (1H, d, *J* = 1.8 Hz, ArH), 8.96 (1H, br, NH), 10.31 (1H, br, NOH). LRMS (ESI+) m/z 368 $[M+H]^+$. LRMS (ESI-) m/z 366 $[M-H]^-$.

5.1.43. **7-(3,4-dihydrocarbostyril-6-sulfonylamino)heptanoic acid hydroxyamide** (**37**)

Colorless powder ($CHCl_3$ / MeOH). Mp. 181-182°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.14-1.24 (4H, m, CH_2 × 2), 1.30-1.47 (4H, m, CH_2 × 2), 1.90 (2H, t, *J* = 7.4 Hz, CH_2), 2.47 (2H, t, *J* = 7.2 Hz, CH_2), 2.65-2.72 (2H, m, NCH_2), 2.95 (2H, t, *J* = 7.5 Hz, CH_2), 6.99 (1H, d, *J* = 8.1 Hz, ArH), 7.40 (1H, br, NH), 7.53-7.59 (2H, m, ArH), 8.66 (1H, br, NH), 10.37 (1H, br, NH) , 10.45 (1H, br, NOH). LRMS (ESI+) m/z 370 $[M+H]^+$. LRMS (ESI-) m/z 368 $[M-H]^-$.

5.1.44. **7-(4,4-dimethyl-3,4-dihydrocarbostyril-6-sulfonylamino)heptanoic acid hydroxyamide** (**38**)

Colorless powder (AcOEt). Mp. 125-126°C. ^{1}H-NMR (300 MHz / MeOH-d_4) δ1.25-1.30 (4H, m, CH_2 × 2), 1.34 (6H, s, CH_3 × 2), 1.38-1.60 (4H, m, CH_2 × 2), 2.05 (2H, t, *J* = 7.4 Hz, CH_2), 2.50 (2H, s, CH_2), 2.83 (2H, t, *J* = 6.9 Hz, CH_2), 7.02 (1H, d, *J* = 8.4 Hz, ArH), 7.65 (1H, dd, *J* = 8.3, 2.0 Hz, ArH), 7.78 (1H, d, *J* = 2.0 Hz, ArH). LRMS (ESI+) m/z 398 $[M+H]^+$. LRMS (ESI-) m/z 396 $[M-H]^-$.

5.1.45. **7-(chlorosulfonyl)heptanoic acid methyl ester** (**56**)

The reaction mixture of 7-bromoheptanoic acid methyl ester (**55**) (800 mg, 3.6 mmol) and Na_2SO_3 (580 mg, 4.6 mmol) in H_2O (3.6 ml) was stirred for 17 h at reflux. After cooling, the mixture was washed with Et_2O (10 ml × 3) and concentrated under reduced pressure to afford a colorless solid (1.4 g, quant. y.). ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.21-1.34 (4H, m, CH_2 × 2), 1.45-1.59 (4H, m, CH_2 × 2), 2.28 (2H, t, *J* = 7.5 Hz, CH_2), 2.38 (2H, t, *J* = 7.8 Hz, SCH_2), 3.58 (3H, s, OCH_3).

5.1.46. **7-(3,4-dihydrocarbostyril-6-ylsulfamoyl)heptanoic acid methyl ester** (**57**)

A solution of Et_3N (530 mg, 5.2 mmol) in acetone (2 ml) was added to a suspension of 7-(chlorosulfonyl)heptanoic acid methyl ester (**56**) (1.0 g, 4.6 mmol) and trichlorotriazine (926 mg, 5.0 mmol) in acetone (8 ml) for 5 min at room temperature. The mixture was stirred for 20 min at 80°C with a microwave apparatus (Initiator™ Eight, Biotage). After cooling, the mixture was filtered with Celite®. Then, 2 N NaOH (3.0 ml), THF (2.5 ml), and 6-amino-3,4-dihydrocarbostyril (800 mg, 4.9 mmol) were added to the filtrate, and the mixture was stirred for 20 min at 80°C with a microwave apparatus. After cooling, the mixture was filtered with Celite®. The filtrate was diluted with H_2O (15 ml) and then extracted with AcOEt (15 ml × 1, and then 10 ml × 3). The combined organic layer was washed with brine (15 ml × 2), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (AcOEt:MeOH = 7:1) to afford a pink solid (223 mg, 0.62 mmol, y. 13%). ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.30-1.48 (4H, m, CH_2 × 2), 1.61-1.67 (2H, m, CH_2), 1.78-1.89 (2H, m, CH_2), 2.30 (2H, t, *J* = 7.4 Hz, CH_2), 2.61-2.66 (2H, m, CH_2), 2.95-3.0 (2H, m, CH_2), 3.03-3.08 (2H, m, NCH_2), 3.67 (3H, s, OCH_3), 6.31 (1H, br, NH), 6.70 (1H, d, *J* = 8.4 Hz, ArH), 7.02 (1H, dd, *J* = 8.6, 2.7 Hz, ArH), 7.11 (1H, d, *J* = 2.7 Hz, ArH), 7.50 (1H, br, NH).

5.1.47. **7-(3,4-dihydrocarbostyril-6-ylsulfamoyl)heptanoic acid hydroxyamide** (**39**)

Colorless powder (H_2O). Mp. 133-135°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.16-1.36 (4H, m, CH_2 × 2), 1.39-1.49 (2H, m, CH_2), 1.58-1.68 (2H, m, CH_2), 1.91 (2H, t, *J* = 7.4 Hz, CH_2), 2.39-2.44 (2H, m, CH_2), 2.83 (2H, t, *J* = 7.5 Hz, CH_2), 2.95-3.00 (2H, m, NCH_2), 6.80 (1H, d, *J* = 8.4 Hz, ArH), 6.96-7.00 (2H, m, ArH), 7.24 (1H, br, NH), 8.67 (1H, br, NH), 10.04 (1H, br, OH), 10.35 (1H, br, NH). LRMS (ESI+) m/z 370 $[M+H]^+$. LRMS (ESI-) m/z 368 $[M-H]^-$.

5.1.48. **4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*trans*-4-benzyloxymethylcyclohexylmethyl)amide** (representative of **59**) as the general procedure for the synthesis of **59**

(a) A solution of *trans*-1,4-cyclohexanedimethanol (corresponding to **58**) (300 mg, 2.08 mmol) in DMF (2 ml) was added to a suspension of NaH (60% in mineral oil, 42 mg, 1.04 mmol) in DMF (1 ml) for 3 min at 0°C. After stirring for 30 min at 0°C, a solution of BnBr (178 mg, 1.04 mmol) in DMF (2 ml) was added to the mixture for 1 min at 0°C. The mixture was stirred overnight at room temperature, and then for 2 h at 60°C. After cooling, H_2O (30 ml) was added, and the resulting mixture was extracted with AcOEt (50 ml × 3). The combined organic layer was washed with H_2O (30 ml × 1) and then brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 2:1) to afford (*trans*-4-benzyloxymethylcyclohexyl)methanol (177 mg, 0.75 mmol, y. 36%) as a colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ0.94-1.01 (4H, m, CH_2 × 2), 1.26 (1H, m, CH), 1.45 (1H, m, CH), 1.82-1.89 (4H, m, CH_2 × 2), 3.29 (2H, d, *J* = 6.6 Hz, CH_2), 3.46 (2H, d, *J* = 6.3 Hz, CH_2), 4.50 (2H, s, CH_2), 7.33-7.35 (5H, m, ArH).

(b) A solution of MsCl 104 mg (0.904 mmol) in CH_2Cl_2 (2 ml) was added to a solution of (*trans*-4-benzyloxymethylcyclohexyl)methanol (177 mg, 0.75 mmol) and Et_3N 229 mg (2.26 mmol) in CH_2Cl_2 (2 ml) for 1 min at 0°C. The reaction mixture was stirred overnight at room temperature. Then, H_2O (30 ml) was added, and the resulting mixture was extracted with $CHCl_3$ (30 ml × 3). The combined organic layer was washed with H_2O (30 ml × 1) and then brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 4:1) to afford *trans*-methanesulfonic acid 4-benzyloxymethylcyclohexylmethyl ester (199 mg, 0.64 mmol, y. 85%) as a colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ0.97-1.07 (4H, m, CH_2 × 2), 1.61-1.64 (1H, m, CH), 1.67-1.73 (1H, m, CH), 1.83-1.91 (4H, m, CH_2 × 2), 3.00 (3H, s, CH_3), 3.29 (2H, d, *J* = 6.3 Hz, CH_2), 4.03 (2H, d, *J* = 6.3 Hz, CH_2), 4.50 (2H, s, CH_2), 7.30-7.35 (5H, m, ArH).

(c) The reaction mixture of *trans*-methanesulfonic acid 4-benzyloxymethylcyclohexylmethyl ester (200 mg, 0.64 mmol)

and NaN_3 (42 mg, 0.64 mmol) in DMF (2 ml) was stirred overnight at room temperature, and then for 1.5 h at 80°C. After cooling, H_2O (30 ml) was added, and the resulting mixture was extracted with AcOEt (30 ml × 3). The combined organic layer was washed with H_2O (30 ml × 1) and then brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 5:1) to afford (*trans*-4-azidomethylcyclohexylmethoxymethyl)benzene (184 mg, quant. y.) as a colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ0.96-1.03 (4H, m, CH_2 × 2), 1.45-1.58 (2H, bs, CH × 2), 1.81-1.89 (4H, m, CH_2 × 2), 3.13 (2H, d, *J* = 6.9 Hz, CH_2), 3.29 (2H, d, *J* = 6.3 Hz, CH_2), 4.50 (2H, s, CH_2), 7.33-7.34 (5H, m, ArH).

(d) The suspension of (*trans*-4-azidomethylcyclohexylmethoxymethyl)benzene (1.57 g, 6.65 mmol) and 10% Pd-C (0.16 g) in EtOH (15 ml) in a glass autoclave reactor was stirred for 7 h at room temperature under H_2 atmosphere at 0.18 MPa. After the pressure was leaked, the reaction mixture was filtered, washed with MeOH, and concentrated under reduced pressure to afford (*trans*-4-benzyloxymethylcyclohexyl)methylamine (1.36 g, 5.807 mmol, y. 96%) as a colorless solid. Colorless powder (AcOEt / MeOH). Mp. 149-153°C. ^{1}H-NMR (300 MHz / $CDCl_3$) δ0.97-1.03 (4H, m, CH_2 × 2), 1.60-1.63 (2H, m, CH × 2), 1.87-1.90 (4H, m, CH_2 × 2), 2.76 (2H, d, *J* = 6.6 Hz, CH=), 3.26 (2H, d, *J* = 6.6 Hz, CH=), 4.47 (2H, s, CH_2), 4.82 (2H, bs, NH_2), 7.32-7.34 (5H, m, ArH).

(e) The reaction mixture of (*trans*-4-benzyloxymethylcyclohexyl)methylamine (106 mg, 0.46 mmol), 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (100 mg, 0.46 mmol), HOBt (62 mg, 0.46 mmol), Et_3N (46 mg, 0.46 mmol), and EDCI·HCl (87 mg, 0.46 mmol) in DMF (2 ml) was stirred overnight at room temperature. Then, sat.$NaHCO_3$ (30 ml) was added, and the resulting mixture was extracted with AcOEt (30 ml × 3). The combined organic layer was washed with 1 N HCl (30 ml × 1) and then brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 1:4) to afford 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*trans*-4-benzyloxymethylcyclohexylmethyl)amide (representative of **59**) (94 mg, 0.22 mmol, y. 47%) as a colorless solid. Colorless powder (*n*-hexane/AcOEt). Mp. 87-88°C. ^{1}H-NMR (300 MHz / $CDCl_3$) δ0.96-1.07 (4H, m, CH_2 × 2), 1.36 (6H, s, CH_3 × 2), 1.69 (2H, bs, CH × 2), 1.83-1.90 (4H, m, CH_2 × 2), 2.51 (2H, s, CH_2), 3.29 (2H, d, *J* = 6.6 Hz, CH_2), 3.31 (2H, t, *J* = 6.3 Hz, CH_2), 4.49 (2H, s, CH_2), 6.12 (1H, t, *J* = 5.4 Hz, NH), 6.80 (1H, d, *J* = 8.1 Hz, ArH), 7.33-7.34 (5H, m, ArH), 7.51 (1H, dd, *J* = 8.1, 1.8 Hz, ArH), 7.81 (1H, d, *J* = 1.8 Hz, ArH), 8.29 (1H, s, NH). LRMS (ESI+) m/z 435 $[M+H]^+$. LRMS (ESI-) m/z 433 $[M-H]^-$.

5.1.49. **(*E*)-3-[*trans*-4-[[(4,4-dimethyl-3,4-dihydro-carbostyril-6-carbonyl)amino]methyl]cyclohexyl]acrylic acid ethyl ester** (representative of **60**) as the general procedure for the synthesis of **60**

(f) The suspension of 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*trans*-4-benzyloxymethylcyclohexylmethyl)-amide (corresponding to **59**) (81 mg, 0.19 mmol) and 10% Pd-C (8 mg) in EtOH (3 ml) in a glass autoclave reactor was stirred for 7 h at room temperature under H_2 atmosphere at 0.30 MPa. After the pressure was leaked, the reaction mixture was filtered, washed with MeOH, and concentrated under reduced pressure to afford 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*trans*-4-hydroxymethylcyclohexylmethyl)amide (51 mg, 0.15 mmol, y. 80%) as a colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ0.95-1.08 (4H, m, CH_2 × 2), 1.37 (6H, s, CH_3 × 2), 1.55 (2H, bs, CH × 2), 1.85-1.88 (4H, m, CH_2 × 2), 2.51 (2H, s, CH_2), 3.33 (2H, t, *J* = 6.5 Hz, CH_2), 3.47 (2H, d, *J* = 6.3 Hz, CH_2), 6.13 (1H, m, NH), 6.79 (1H, d, *J* = 8.1 Hz, ArH), 7.52 (1H, dd, *J* = 8.3, 2.0 Hz, ArH), 7.81 (1H, d, *J* = 1.8 Hz, ArH), 8.11 (1H, s, NH).

(g) A suspension of Dess-Martin periodinane (68 mg, 0.16 mmol) in CH_2Cl_2 (3 ml) was added to a solution of 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid *trans*-(4-hydroxymethylcyclohexylmethyl)amide (50 mg, 0.15 mmol) in CH_2Cl_2 (1 ml) for 3 min at room temperature. The reaction mixture was stirred overnight at room temperature. A solution of slight amount of $Na_2S_2O_3$ in sat.$NaHCO_3$ (30 ml) was added to the mixture, and the resulting mixture was extracted with $CHCl_3$ (30 ml × 3). The combined organic layer was washed with brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure to afford 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*trans*-4-formylcyclohexylmethyl)-amide (41 mg, 0.12 mmol, y. 83%) as a colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.05-1.18 (4H, m, CH_2 × 2), 1.37 (6H, s, CH_3 × 2), 1.60 (2H, m, CH × 2), 1.93-2.05 (4H, m, CH_2 × 2), 2.51 (2H, s, CH_2), 3.35 (2H, t, *J* = 6.5 Hz, CH_2), 6.15 (1H, t, *J* = 5.7 Hz, NH), 6.81 (1H, d, *J* = 8.1 Hz, ArH), 7.52 (1H, dd, *J* = 8.1, 1.8 Hz, ArH), 7.81 (1H, d, *J* = 1.5 Hz, ArH), 8.39 (1H, s, NH), 9.63 (1H, d, *J* = 1.2 Hz, CHO).

(h) A solution of triethyl phosphonoacetate (30 mg, 0.13 mmol) in THF (2 ml) was added to a suspension of NaH (60% in mineral oil, 5 mg, 0.13 mmol) in THF (5 ml) for 2 min at 0°C under N_2 using a syringe attached to a needle. After the reaction mixture was stirred for 15 min at 0°C, a solution of 4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*trans*-4-formylcyclohexylmethyl)amide (41 mg, 0.120 mmol) in THF (2 ml) was added to the mixture for 4 min at 0°C under N_2 with a syringe. After stirring for 6 h at 0°C under N_2, THF was removed under reduced pressure. Then, H_2O (30 ml) was added to the residue, and the mixture was extracted with AcOEt (30 ml × 3). The combined organic layer was washed with brine (30 ml × 1), dried over Na_2SO_4 (anhyd.), filtered, and concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 1:4) to afford (*E*)-3-[*trans*-4-[[(4,4-dimethyl-3,4-dihydrocarbostyril-6-carbonyl)-amino]methyl]cyclohexyl]acrylic acid ethyl ester (representative of **60**) (33 mg, 0.08 mmol, y. 66%) as a colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ1.01-1.16 (4H, m, CH_2 × 2), 1.29 (3H, t, *J* = 7.2 Hz, $CH_2C\underline{H}_3$), 1.37 (6H, s, CH_3 × 2), 1.72 (2H, bs, CH × 2), 1.84-1.91 (4H, m, CH_2 × 2), 2.51 (2H, s, CH_2), 3.33 (2H, t, *J* = 6.5 Hz, CH_2), 4.18 (2H, q, *J* = 7.1 Hz, $C\underline{H}_2CH_3$), 5.77 (1H, dd, *J* = 15.8, 1.4 Hz, CH=), 6.14 (1H, t, *J* = 5.7 Hz, NH), 6.80 (1H, d, *J* = 8.1 Hz, ArH), 6.90 (1H, dd, *J* = 15.8 Hz, 6.8 Hz, CH=), 7.52 (1H, dd, *J* = 8.1, 1.8 Hz, ArH), 7.81 (1H, d, *J* = 1.5 Hz, ArH), 8.23 (1H, s, NH).

5.1.50. **4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*E*)-[*trans*-4-(2-hydroxycarbamoylvinyl)cyclohexyl-methyl]amide** (**40**)

Colorless cotton-like crystal (*n*-hexane / AcOEt / MeOH). Mp. 184-187°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.01-1.06 (4H, m, CH_2 × 2), 1.26 (6H, s, CH_3 × 2), 1.52 (2H, bs, CH × 2), 1.73-1.80 (4H, m, CH_2 × 2), 2.38 (2H, s, CH_2), 3.11 (2H, t, *J* = 5.9 Hz, CH_2), 5.70 (1H, d, *J* = 15.6 Hz, CH=), 6.59 (1H, dd, *J* = 15.5, 6.8 Hz, CH=), 6.89 (1H, d, *J* = 8.4 Hz, ArH), 7.67 (1H, d, *J* = 8.1 Hz, ArH), 7.79 (1H, s, ArH), 8.35 (1H, t, *J* = 5.6 Hz, NH), 10.34 (1H, s, NH). LRMS (ESI+) m/z 400 $[M+H]^+$. LRMS (ESI-) m/z 398 $[M-H]^-$. HRMS (MALDI+) m/z calcd for $C_{22}H_{30}N_3O_4^+$ $[M+H]^+$ 400.2231, found 400.2222.

5.1.51. **4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid (*E*)-[*cis*-4-(2-hydroxycarbamoylvinyl)cyclohexyl-methyl]amide** (**42**)

Colorless powder (AcOEt / MeOH). Mp. 210-213°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26 (6H, s, CH_3 × 2), 1.53 (8H, m, CH_2 × 4), 1.73-1.77 (2H, m, CH × 2), 2.38 (2H, s, CH_2), 3.20 (2H, t, *J* = 6.5 Hz, CH_2), 5.74 (1H, d, *J* = 15.6 Hz, CH=), 6.70 (1H, dd, *J* = 15.6, 6.0 Hz, CH=), 6.89 (1H, d, *J* = 8.4 Hz, ArH), 7.66 (1H, dd, *J* = 8.4, 1.8 Hz, ArH), 7.78 (1H, d, *J* = 1.5 Hz, ArH), 8.33 (1H, t, *J* = 5.4 Hz, NH), 8.85 (1H, s, NH), 10.34 (1H, s, NH), 10.55 (1H, s, NOH). LRMS (ESI+) m/z 400 $[M+H]^+$. LRMS (ESI-) m/z 398 $[M-H]^-$. HRMS (MALDI+) m/z calcd for $C_{22}H_{30}N_3O_4^+$ $[M+H]^+$ 400.2231, found 400.2253.

5.1.52. **4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid [*trans*-4-(2-hydroxycarbamoylethyl)cyclohexylmethyl]-amide** (**41**) as the general procedure for the synthesis of **41** and **43**

(j) The suspension of (*E*)-3-[*trans*-4-[[(4,4-dimethyl-3,4-dihydrocarbostyril-6-carbonyl)amino]methyl]cyclohexyl]acrylic acid ethyl ester (representative of **60**) (107 mg, 0.26 mmol) and 10% Pd-C (11 mg) in EtOH (4 ml) in a glass autoclave reactor was stirred for three days at room temperature under H_2 atmosphere at 0.16 MPa. After the pressure was leaked, the reaction mixture was filtered and washed with MeOH. The filtrate was concentrated under reduced pressure. The crude product was purified through open silica gel column chromatography (*n*-hexane:AcOEt = 1:4) to afford 3-[*trans*-4-[[(4,4-dimethyl-3,4-dihydrocarbostyril-6-carbonyl)amino]-methyl]cyclohexyl]propionic acid ethyl ester (78 mg, 0.19 mmol, y. 72%) as a colorless oil. ^{1}H-NMR (300 MHz / $CDCl_3$) δ0.95-1.04 (4H, m, CH_2 × 2), 1.25 (3H, t, *J* = 7.1 Hz, $CH_2\underline{CH_3}$), 1.37 (6H, s, CH_3 × 2), 1.50-1.58 (2H, m, CH_2), 1.66 (2H, bs, CH × 2), 1.77-1.85 (4H, m, CH_2 × 2), 2.31 (2H, t, *J* = 7.8 Hz, CH_2), 2.51 (2H, s, CH_2), 3.31 (2H, t, *J* = 6.3 Hz, NCH_2), 4.12 (2H, q, *J* = 7.2 Hz, $C\underline{H_2}CH_3$), 6.18 (1H, t, *J* = 5.7 Hz, NH), 6.80 (1H, d, *J* = 8.1 Hz, ArH), 7.51 (1H, dd, *J* = 8.3 Hz, 1.8 Hz, ArH), 7.81 (1H, d, *J* = 1.8 Hz, ArH), 8.29 (1H, s, NH).

(k) This step was performed following the same procedure described for compound **2**. **41**: Colorless powder (AcOEt / MeOH). Mp. 195-197°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ0.81-0.88 (4H, m, CH_2 × 2), 1.26 (6H, s, CH_3 × 2), 1.37-1.42 (2H, m, CH_2), 1.47 (2H, bs, CH × 2), 1.71-1.73 (4H, m, CH_2 × 2), 1.95 (2H, t, *J* = 7.7 Hz, CH_2), 2.38 (2H, s, CH_2), 3.08 (2H, t, *J* = 6.3 Hz, NCH_2), 6.88 (1H, d, *J* = 8.4 Hz, ArH), 7.66 (1H, dd, *J* = 8.4, 1.8 Hz, ArH), 7.68 (1H, d, *J* = 1.8 Hz, ArH), 8.32 (1H, t, *J* = 5.7 Hz, ArH), 8.65 (1H, s, NH), 10.34 (2H, s, NOH and NH). LRMS (ESI+) m/z 402 $[M+H]^+$. LRMS (ESI-) m/z 400 $[M-H]^-$. HRMS (MALDI+) m/z calcd for $C_{22}H_{31}N_3O_4Na^+$ $[M+Na]^+$ 424.2207, found 424.2238.

5.1.53. **4,4-dimethyl-3,4-dihydrocarbostyril-6-carboxylic acid [*cis*-4-(2-hydroxycarbamoylethyl)cyclohexylmethyl]-amide** (**43**)

Colorless powder (AcOEt). Mp. 129-132°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.25 (6H, s, CH_3 × 2), 1.30-1.52 (10H, m, CH_2 × 5), 1.70-1.76 (2H, m, CH × 2), 1.95 (2H, t, *J* = 7.5 Hz, $C(O)CH_2$), 2.38 (2H, s, CH_2), 3.20 (1.7H, t, *J* = 6.6 Hz, NCH_2), 6.88 (1H, d, *J* = 8.1 Hz, ArH), 7.66 (1H, dd, *J* = 8.1, 1.5 Hz, ArH), 7.80 (1H, d, *J* = 1.5 Hz, ArH), 8.31 (1H, br, NH), 8.65 (1H, br, NH), 10.33 (2H, br, NOH and NH). LRMS (ESI+) m/z 402 $[M+H]^+$. LRMS (ESI-) m/z 400 $[M-H]^-$. HRMS (MALDI+) m/z calcd for $C_{22}H_{31}N_3O_4Na^+$ $[M+Na]^+$ 424.2207, found 424.2212.

5.2 Biology

The enzyme assays using rat liver HDACs were conducted according to the literature[29, 30]. Rat liver HDACs were purchased from Merck KGaA (Germany). The enzyme assays using human HDAC isozymes were performed based on the protocol[31] generated by Promega. The assay was performed using HDAC-Glo™ I/II assay kit and the G6420 screening system (Promega, USA). The human HDAC isozymes were purchased from Singal Chem (Canada).

5.2.1. Enzyme assay using rat liver HDACs

The incubation buffer composed of 25 mM Tris-HCl, 137 mM NaCl, 2.7 mM KCl, and 1 mM $MgCl_2$ (pH 8.0) was used in this assay. The reagents included 1 μM of the test compound (1% DMSO), 50 μM of the rat liver HDACs (32 μg/ml), and 50 μl of Boc-Lys(Ac)-AMC (250 μM); the final assay concentrations are shown in parentheses. Because the activity of an enzyme depends on the lot number, the concentration used was adjusted when necessary. Immediately after the test compound and the enzyme were mixed, the mixture was read on a fluorometer to determine the background. After preincubation for 15 min at 37°C, pre-warmed Boc-Lys(Ac)-AMC was added to initiate the reaction. The reaction mixture was sealed and incubated for 30 min at 37°C, and then 10 μl of diluted Developer Concentrate was added to quench the reaction. After incubation for 1 h at 37°C, the plate was read on a fluorometer (excitation: 360 nm, emission: 465 nm).

5.2.2. Enzyme assay using human HDAC isozymes

The fluorometric activity assay kit used to determine HDAC activity was the HDAC-Glo™ I/II assay and screening system (G6420, Promega). Arbitrary human HDAC isozymes were incubated with the test compounds for 30 min at room temperature, and the reaction was initiated by the addition of HDAC-Glo™ I/II substrate and the Developer Reagent. The reaction mixtures were incubated for 30 min at room temperature. The plate was read on a fluorometer (excitation: 360 nm, emission: 460 nm). The activity was calculated as a percentage of the activity of the control. The 50% inhibition concentration (IC_{50}) values for the test compounds were calculated using the SigmaPlot software.

Abbreviations

DNA, deoxyribonucleic acid; Gadd 45, growth arrest and DNA damage 45; DMF, *N*,*N*-dimethylformaldehyde; Et, ethyl; rt, room temperature; quant., quantitative; y., yield; Me, methyl; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, hydroxybenzotriazole; Bn, benzyl; Boc, *tert*-butoxycarbonyl; Ac, acetyl; DIEA, *N*,*N*-diisopropylethylamine; TFA, trifluoroacetic acid; MW, microwave; Ms, mesyl; Pa, pascal; compnd, compound; NMR, nuclear magnetic resonance; Mp, melting point; LRMS, low-resolution mass spectrometry; HRMS, high-resolution mass spectrometry; anhyd., anhydrous; Hz, hertz; DMSO, dimethylsulfoxide; Bu, butyl; ESI, electrospray ionization; MALDI, matrix assisted laser desorption / ionization; calcd, calculated; Lys, lysine; AMC, 7-amido-4-methylcoumarin

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22. The SAHA used for the assays performed in this study was synthesized using the same method that was used to synthesize HDAC inhibitors **12**-**14**, with the exception that the carbostyril skeleton was replaced with benzene ring. SAHA: Colorless powder (*n*-hexane / AcOEt / MeOH). Mp. 158-159°C. ^{1}H-NMR (300 MHz / DMSO-d_6) δ1.26-1.29 (4H, m, CH_2 × 2), 1.44-1.51 (2H, m, CH_2), 1.55-1.60 (2H, m, CH_2), 1.94 (2H, t, J = 7.4 Hz, CH_2), 2.29 (2H, t, J = 7.4 Hz, CH_2), 7.01 (1H, t, J = 7.4 Hz, ArH), 7.27 (2H, t, J = 7.8 Hz, ArH), 7.59 (2H, d, J = 7.5 Hz, ArH), 8.66 (1H, s, NH), 9.88 (1H, s, NH), 10.35 (1H, s, NOH). LRMS (ESI+) m/z 265 ([M+H]$^+$). LRMS (ESI-) m/z 263 ([M-H]$^-$).
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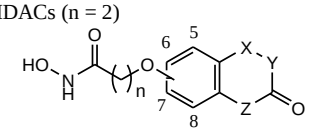
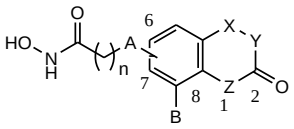
31. Promega Corporation, www.promega.com

Table 1. *In vitro* inhibitory activity (%) of SAHA and compounds **1-11** against rat liver HDACs (n = 2)



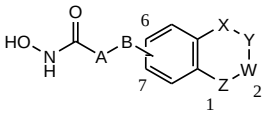
					% inhibition	
compnd	X-Y	Z	position	n	10 μM	0.1 μM
SAHA	-	-	-	-	96	34
1	CH=CH	NH	6	5	94	19
2	CH_2-CH_2	NH	6	5	91	13
3	$C(CH_3)_2$-CH_2	NH	6	5	88	11
4	CH_2-CH_2	NH	6	3	65	9
5	CH=CH	O	6	5	91	12
6	CH=CH	NH	7	5	93	21
7	CH_2-CH_2	NH	7	5	91	13
8	$C(CH_3)_2$-CH_2	NH	7	5	91	11
9	CH_2-CH_2	NH	5	5	86	7
10	CH=CH	NH	8	5	86	7
11	CH_2-CH_2	NH	8	5	80	4

Table 2. *In vitro* inhibitory activity (%) of SAHA and compounds **12-25** against rat liver HDACs (n = 2)



							% inhibition	
compnd	A	B	X-Y	Z	position	n	10 μM	0.1 μM
SAHA	-	-	-	-	-	-	96	34
12	CONH	H	CH=CH	NH	6	6	99	66
13	CONH	H	CH_2-CH_2	NH	6	6	98	51
14	CONH	H	$C(CH_3)_2$-CH_2	NH	6	6	98	58
15	CONH	F	$C(CH_3)_2$-CH_2	NH	6	6	99	49
16	CONH	H	CH_2-CH_2	NH	6	5	95	24
17	CONH	H	CH_2-CH_2	NH	6	7	97	30
18	CONH	H	CH=CH	O	6	6	98	46
19	NHCO	H	CH=CH	NH	6	6	100	68
20	NHCO	H	CH_2-CH_2	NH	6	6	98	45
21	NHCO	H	$C(CH_3)_2$-CH_2	NH	6	6	98	44
22	NHCO	F	$C(CH_3)_2$-CH_2	NH	6	6	99	57
23	NHCO	H	CH_2-CH_2	NH	6	5	94	21
24	NHCO	H	CH_2-CH_2	NH	6	7	98	40
25	NHCO	H	$C(CH_3)_2$-CH_2	NH	7	6	99	63

Table 3. *In vitro* inhibitory activity (%) of SAHA and compounds **26**-**39** against rat liver HDACs (n = 2)



						% inhibition	
compnd	A	B	X-Y	Z-W	position	10 μM	0.1 μM
SAHA	-	-	-	-	-	96	34
26	$(CH_2)_6$	CONH	$C(CH_3)_2$-CH_2	NMe-C=O	6	98	55
27	$(CH_2)_6$	NHCO	CH=CH	NMe-C=O	6	99	63
28	$(CH_2)_6$	NHCO	CH_2-CH_2	NMe-C=O	6	99	51
29	$(CH_2)_6$	NHCO	$C(CH_3)_2$-CH_2	NMe-C=O	6	99	50
30	$(CH_2)_6$	CONH	$C(CH_3)_2$-CH_2	N=C-OMe	6	99	52
31	$(CH_2)_6$	NHCO	CH=CH	N=C-OMe	6	100	70
32	$(CH_2)_6$	CONH	$C(CH_3)_2$-CH_2	N=C-OEt	6	97	42
33	(E) 	NHCO	$C(CH_3)_2$-CH_2	NH-CO	6	95	22
34	(E) 	NHCO	$C(CH_3)_2$-CH_2	NH-CO	6	98	46
35	(E) 	NHCO	$C(CH_3)_2$-CH_2	NH-CO	7	96	27
36	$(CH_2)_6$	$NHSO_2$	CH=CH	NH-CO	6	94	18
37	$(CH_2)_6$	$NHSO_2$	CH_2-CH_2	NH-CO	6	94	15
38	$(CH_2)_6$	$NHSO_2$	$C(CH_3)_2$-CH_2	NH-CO	6	94	17
39	$(CH_2)_6$	SO_2NH	CH_2-CH_2	NH-CO	6	91	11

Table 4. *In vitro* inhibitory activity (%) of SAHA and compounds **40**-**43** against rat liver HDACs (n = 2)

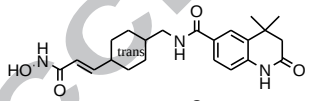
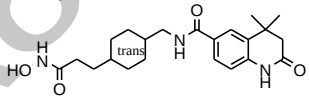
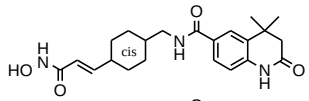
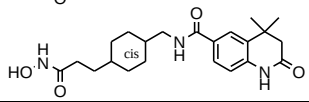
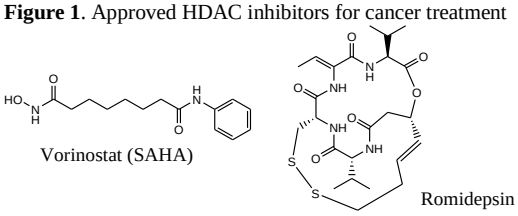
	% inhibition	
compnd	10 μM	0.1 μM
SAHA	96	34
40 	98	55
41 	99	43
42 	92	22
43 	97	27

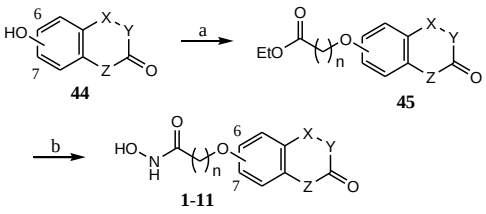
Table 5. *In vitro* inhibitory activity (IC_{50}) of SAHA and various synthesized compounds against human HDAC isozymes (n > 2)

	inhibition (IC_{50}; μM)						
	Class I				Class IIa		Class IIb
compnds	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC9	HDAC6
SAHA	0.030	0.028	0.11	0.11	0.15	0.038	0.40
12	0.035	0.045	0.054	0.048	0.063	0.058	0.066
13	0.014	0.018	0.091	0.094	0.12	0.025	0.63
14	0.0091	0.010	0.089	0.033	0.047	0.045	0.55
19	0.024	0.025	0.18	0.073	0.28	0.046	0.28
20	0.024	0.020	0.085	0.053	0.12	0.035	0.43
21	0.030	0.093	0.069	0.055	0.063	0.039	0.092
27	0.089	0.090	0.098	0.072	0.086	0.041	0.086
29	0.043	0.10	0.028	0.059	0.28	0.12	0.13
31	0.052	0.17	0.028	0.080	0.078	0.034	0.040
33	0.67	0.10	0.21	0.20	2.0	0.57	0.29
34	0.066	0.022	0.065	0.049	0.38	0.037	0.11
40	0.0038	0.0082	0.015	0.0060	0.058	0.0052	0.058
41	0.035	0.035	0.089	0.028	0.22	0.057	0.44
42	0.19	0.31	0.37	0.021	0.75	0.091	0.72
43	0.041	0.025	0.087	0.024	0.33	0.050	0.53

Figure 1. Approved HDAC inhibitors for cancer treatment

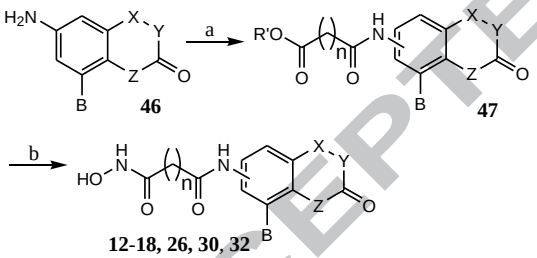


Scheme 1.



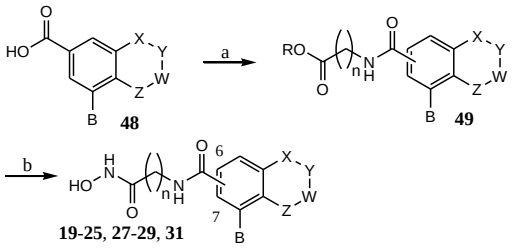
Reagents and conditions: (a) NaH, DMF, 0°C, then $EtO_2C(CH_2)nBr$ (n=3, 5), rt, 25%-quant.y.; (b) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 40%-quant.y.

Scheme 2.



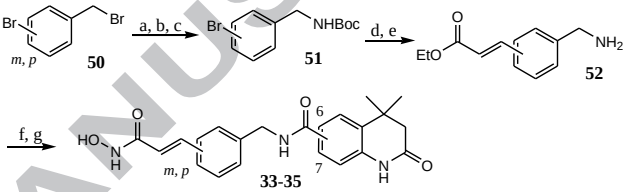
Reagents and conditions: (a) EDCI·HCl, HOBt, Et_3N, DMF, $RO_2C(CH_2)nCO_2H$ (R=Bn, Me; n=5-7), rt, 51-91%; (b) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 31%-quant. y.

Scheme 3.



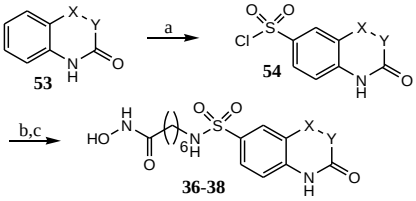
Reagents and conditions: (a) EDCI·HCl, HOBt, Et_3N, DMF, $RO_2C(CH_2)nNH_2$ (R=Bn, Me; n=5-7), rt, 18-86%; (b) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 22-82%.

Scheme 4.



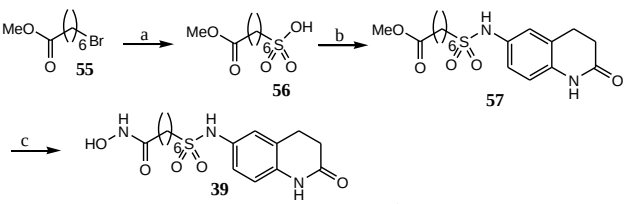
Reagents and conditions: (a) phthalimide potassium salt, DMF, 100°C, quant. y.; (b) $H_2NNH_2·H_2O$, MeOH, reflux, 93-95%; (c) Boc_2O, Et_3N, CH_2Cl_2, 0°C to rt, 95%-quant. y.; (d) ethyl acrylate, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, EtCN, DMF, DIEA, 90°C, N_2, 77-96%; (e) TFA, CH_2Cl_2, rt, 95%-quant. y.; (f) EDCI·HCl, HOBt, Et_3N, DMF, 4,4-dimethyl-3,4-dihydrocarbostyril-6 (or 7)-carboxylic acid, rt, 70-75%; (g) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 11-75%.

Scheme 5.



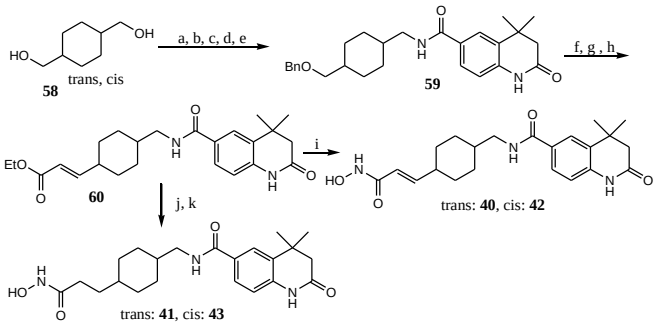
Reagents and conditions: (a) chlorosulfonic acid, 65°C, N_2, 50-89%; (b) $MeO_2C(CH_2)_6NH_2·HCl$, Et_3N, CH_2Cl_2, rt, 58-72%; (c) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 14-67%.

Scheme 6.



Reagents and conditions: (a) Na_2SO_3, H_2O, reflux; (b) trichlorotriazine, Et_3N, acetone, 80°C, MW, then 2 N NaOH, 6-amino-3,4-dihydro-carbostyril, 80°C, MW, 13% for two steps; (c) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 39%.

Scheme 7.



Reagents and conditions: (a) NaH (0.5eq.), DMF, 0°C then BnBr (0.5eq.), 0°C, 22-36%; (b) MsCl, Et_3N, CH_2Cl_2, 0°C to rt, 85-100%; (c) NaN_3, DMF, 80°C, 86%-quant. y.; (d) H_2, 10%Pd-C, EtOH, rt, 0.18 MPa, 95-96%; (e) EDCI·HCl, HOBt, Et_3N, DMF, 4,4-dimethy-3,4-dihydro-carbostyril-6-carboxylic acid, rt, 47-94%; (f) H_2, 10%Pd-C, EtOH, rt, 0.30 MPa, 80-99%; (g) Dess-Martin periodinane, CH_2Cl_2, 0°C to rt, 41-83%; (h) NaH, $(EtO)_2P(=O)CH_2CO_2Et$, THF, 0°C, 60-66%; (i) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 20-67%; (j) H_2, 10%Pd-C, EtOH, rt, 0.16MPa, 55-80%; (k) NH_2OH, H_2O, MeOH, NaOMe, 0°C, 47-80%.

Graphical Abstract

Design and Synthesis of Novel and Highly-Active Pan-Histone Deacetylase (pan-HDAC) Inhibitors

Toshihiko Tashima, Hiroaki Murata, Hidehiko Kodama

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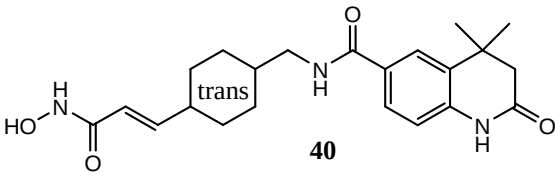

40

IC_{50} values of **40**: HDAC1, 0.0038 μM; HDAC2, 0.0082 μM; HDAC3, 0.015 μM; HDAC8, 0.0060 μM; HDAC4, 0.058 μM; HDAC9, 0.0052 μM; HDAC6, 0.058 μM