Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 1067–1085
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Novel amide derivatives as inhibitors of histone deacetylase: Design, synthesis and SAR

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Received 18 March 2008; received in revised form 13 June 2008; accepted 20 June 2008
Available online 27 June 2008

Abstract

Enzymatic inhibition of histone deacetylase (HDAC) activity is emerging as an innovative and effective approach for the treatment of cancer. A series of novel amide derivatives have been synthesized and evaluated for their ability to inhibit human HDACs. Multiple compounds were identified as potent HDAC inhibitors (HDACi), with IC_{50} values in the low nanomolar (nM) range against enzyme activity in HeLa cell extracts and sub-μM for their in vitro anti-proliferative effect on cell lines. The introduction of an unsaturated linking group between the terminal aryl ring and the amide moiety was the key to obtain good potency. This approach yielded compounds such as (*E*)-*N*-[6-(hydroxyamino)-6-oxo-hexyl]-3-(7-quinolinyl)-2-propenamide (**27**) (HDAC IC_{50} 8 nM) which showed potent in vivo activity in the P388 mouse leukemia syngeneic model (an increased lifespan (ILS) of 111% was obtained).

Keywords: HDAC inhibitor; SAR; Hydroxamic acid

1. Introduction

Histones are small basic proteins, which occur in five main classes (H1, H2A, H2B, H3, and H4). A pair of each of H2A, H2B, H3, and H4 together forms a disc-shaped octomeric protein core, around which DNA (about 140 base pairs) is wound to form a nucleosome. In turn, nucleosomes form a structure described as ''beads on a string'' with the DNA, and coil to form the basis of 30 nm chromatin filaments [1]. This packaging of DNA in nucleosomes and higher order chromatin structures blocks accessibility of the transcriptional machinery to their target genes. Transcription is regulated by multiple post-translational modifications of the histone tails within the chromatin, and histone acetylation has been shown to be one of the major regulatory mechanisms for gene expression [2]. Control of expression is dependent on the balance between the competing activities of histone acetyl transferases (HATs) and histone deacetylases (HDACs) [3] on the regulation of chromatin structure by acetylation of lysines on histone tails.

Mammalian HDACs are grouped into four distinct classes. Class I, II and IV enzymes are zinc dependent with classes I and II being homologous to the yeast proteins RPD3 and Hda1, respectively [4], whereas class III HDACs are structurally distinct NAD-dependent enzymes [5,6]. The discovery and study of histone deacetylase inhibitors (HDACi) confirms

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doi:10.1016/j.ejmech.2008.06.020

that these enzymes play an important role in the regulation of a number of cellular processes, including cell proliferation, apoptosis, assembly of the cytoskeleton and regulation of transcription [2,7–9]. In addition, the over-expression of HDACs leads to epigenetic inactivation of apoptotic signaling pathways, cell cycle regulators and tumor suppressor genes [10]. As a consequence of these findings, several HDACi are currently undergoing clinical trials as anticancer drugs [11,12], some of which have been designed as selective inhibitors in addition to the "pan-HDACi" that inhibit all zinc dependent HDACs. The natural product inhibitor Trichostatin A (TSA, (**I**)), and the synthetic inhibitor suberoylanilide hydroxamic acid (SAHA, (**II**)) broadly inhibit the class I, II and IV enzymes and have been reported to inhibit cell growth, induce terminal differentiation, and prevent the formation of tumors in mice [13]. Compound **II** (SAHA, vorinostat, Zolinza®, Merck Inc. [14]) was recently approved for treatment of refractory cutaneous T-cell lymphoma (CTCL [15]). Other synthetic HDACi are currently in clinical development for use in cancer treatment including hydroxymates such as (*E*)-3-(4-(((2-(1*H*-indol-3-yl)ethyl)-(2-hydroxyethyl)amino)methyl)-phenyl)-*N*-hydroxyacrylamide (**III** – panobinostat (LBH589), phase I/II, Novartis [16]) and *N*-hydroxy-3-(3-(*N*-phenylsulfamoyl)phenyl)acrylamide (**IV** – belinostat (PXD101), phase II, TopoTarget [17]), benzamides (4-(2-aminophenylcarbamoyl) benzylamino)methyl nicotinate (**V** – SNDX-275 (MS-275), phase II, Syndax (Bayer-Schering AG) [18]) and *N*-(2-aminophenyl)-4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzamide (**VI** – MGCD0103, phase II, Methylgene [19]) and valproic acid (**VII** – VPA, phase II, TopoTarget [20]) (see Fig. 1 for structures). Furthermore, there is a growing interest in the potential of HDACi in other therapeutic areas, such as inflammation [21], neurodegeneration [22] and malaria [23,24].

HDACi based on an amide-alkyl-hydroxamic acid framework, such as SAHA (**II**) are well known. These conform to a well-described, simple HDAC inhibition pharmacophore A–B–C, where A represents a moiety which confers potency and specificity (usually aryl), B is a linker group, such as amide-alkyl, and C is a zinc liganding group such as hydroxamic acid (Table 1). Most of the SAR exploration studies published to date have investigated the role of the zinc binding group, with considerable effort being used searching for alternatives to hydroxamic acid, and to some extent the linker. The SAR of the aryl moiety has been much less well explored. In the present manuscript we therefore describe the SAR for a novel series of hydroxamic acid containing HDACi with a wide variety of aryl end groups in the side chains which are shown to favorably interact with the residues at the entrance of the binding pocket.

2. Results and discussion

2.1. Chemistry

The routes used for synthesis of the compounds for this study are shown in Schemes 1–3.

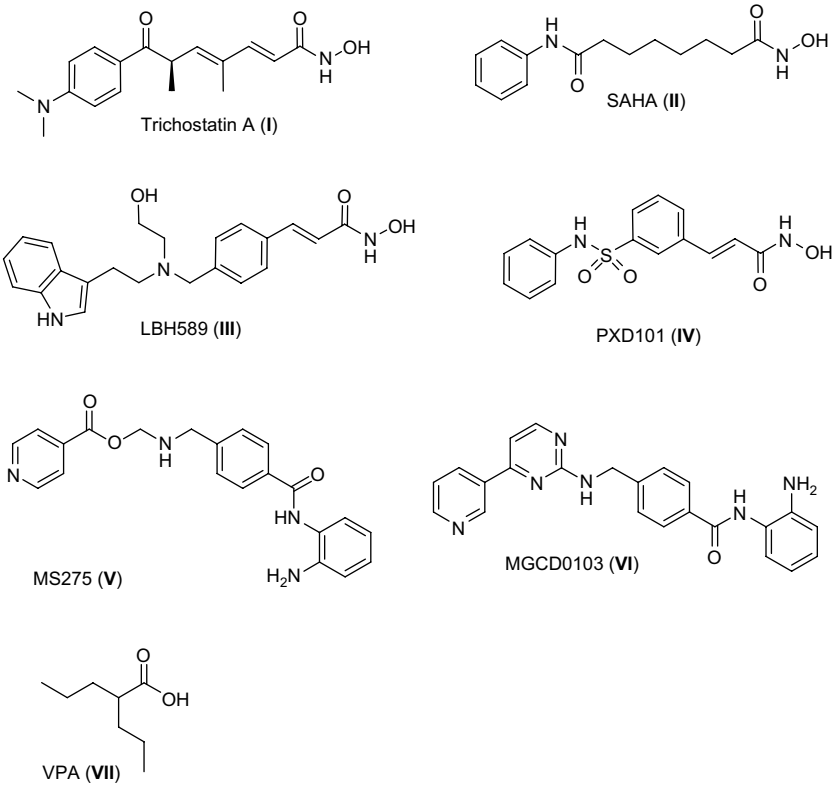
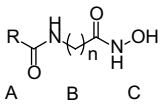
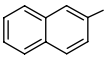
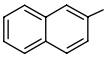
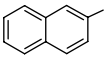
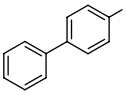
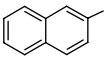
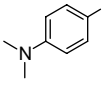
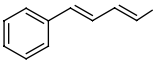
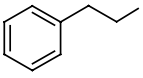
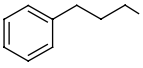
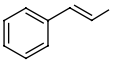
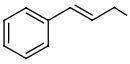
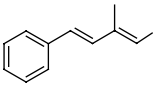
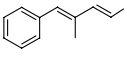
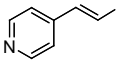
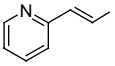


Fig. 1. Natural product and synthetic inhibitors of HDAC.

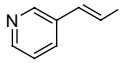
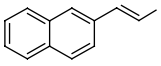
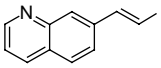
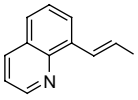
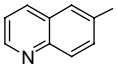
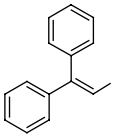
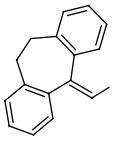
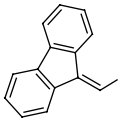
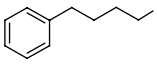
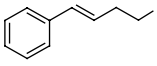
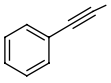
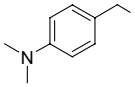
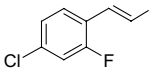
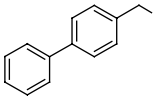
Table 1
Prepared HDAC inhibitors: synthesis sequence and biological activity



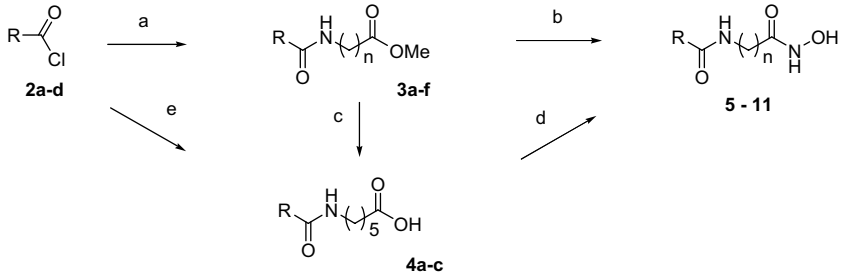
Compound	R	*n*	Synthesis	Biological activity		
			Scheme no.: synthesis sequence	HDAC IC_{50} (nM)	WST-1 IC_{50} (μM)	SAR comment(s)
II (SAHA)				87	3.1	Reverse amide
5		4	1: **2a**, **3a**, **5**	42% at 500 nM	24.7	Chain length; 4 carbons are to short
6		6	1: **2a**, **3b**, **6**	20	0.4	Chain length; 5–6 carbons optimal
7		7	1: **2a**, **3c**, **7**	28	3.6	Chain length
8		5	1: **2b**, **3d**, **8**	38	2.6	
9		5	1: **2a**, **3e**, **4a**, **9**	21	1.1	Chain length; 5–6 carbons optimal
10		5	1: **2c**, **3f**, **4b**, **10**	110	4.1	
11		5	1: **2d**, **4c**, **11**	238	6.3	Simple phenyl; less active as compared to naphthyl
16		5	2: **12a**, **13a**, **16**	29	5.1	Unsaturation in linker; potency retained
17		5	2: **12b**, **13b**, **17**	27% at 500 nM	74.8	Alkyl spacer, potency lost
18		5	2: **12c**, **13c**, **18**	19% at 500 nM	52.7	Alkyl spacer, potency lost
19		5	2: **12d**, **13d**, **19**	32	5.5	Unsaturation, potency retained
20		5	2: **12e**, **13e**, **20**	53% at 500 nM	24.0	Extra methylene lower activity
21		5	2: **12f**, **13f**, **21**	23	4.8	β-substitution allowed
22		3	2: **12g**, **13g**, **22**	22,850	113.5	
23[a]		5	2: **12h**, **13h**, **23**[a]	101	46.4	Pyridyl substitution is allowed
24[a]		5	2: **12i**, **13i**, **24**[a]	46	9.5	Small difference in activity for pyridyl isomers

(*continued on next page*)

Table 1 (*continued*)

Compound	R	*n*	Synthesis	Biological activity		
			Scheme no.: synthesis sequence	HDAC IC_{50} (nM)	WST-1 IC_{50} (μM)	SAR comment(s)
25[a]		5	2: **12j**, **13j**, **25**[a]	141	28.4	
26		5	2: **12k**, **13k**, **26**	7	18.3	Unsaturation and a naphthyl gives potent compounds
27		5	2: **12l**, **13l**, **27**	9	12.3	Quinoline is a preferred substituent
28		5	2: **12m**, **13m**, **28**	25	20.9	Point of substitution affect the activity
29		5	2: **12n**, **13n**, **29**	36	37.9	
30		5	2: **12o**, **13o**, **30**	201	8.5	Diphenyl is less potent
31		5	2: **12p**, **13p**, **31**	338	9.9	To large groups give lower activity
32		5	2: **12r**, **13r**, **32**	5	1.7	Some bulky aromatics are very potent
33		5	2: **12s**, **13s**, **33**	51% at 500 nM	70.8	Alkyl spacer, potency lost
34		5	2: **12t**, **13t**, **34**	625	20.6	
35		5	2: **12u**, **13u**, **35**	62	10.6	Alkyne, potency retained
36		5	2: **12v**, **13v**, **36**	9% at 500 nM	52.7	Methylene spacer, low potency
37		5	2: **12w**, **13w**, **37**	8	6.3	Substituted aryl with unsaturated linker; highly active
38		5	2: **12x**, **13x**, **14**, **15**, **38**	51% at 500 nM	35.9	Methylene spacer; low potency
41			3	39% at 500 nM	44.2	Reverse amide with alkyl spacer; low activity
42			3	46% at 500 nM	13.3	Reverse amide with alkyl spacer; low activity

[a] Isolated as oxalate.



Scheme 1. Reagents and conditions: (a) $H_2N(CH_2)_nCOOMe\cdots HCl$ (**1**, $n = 4$, 5, 6, or 7), DIPEA, DMF; (b) $H_2NOH\cdots HCl$, aq. NaOH/MeOH; (c) aq. LiOH/THF; (d) CDI/THF, then H_2NOH/DMF; (e) $H_2N(CH_2)_5COOH$, aq. NaOH, dioxane.

Thus, as a starting material for synthesis of hydroxamic acids **5**–**11** (Table 1) served commercially available acid chlorides **2a**–**d** (Scheme 1).

Acid chlorides **2a**–**c** were condensed with appropriate methyl ω-aminoalkanoates **1** ($n = 4$–7) in presence of diisopropylethyl amine in DMF to give the corresponding amidoesters **3a**–**f**. Amidoesters **3a**–**d** were transformed into hydroxamic acids **5**–**8** by treating the former with four-fold excess of hydroxylamine in the presence of NaOH in aqueous methanol medium. Amidoesters **3e**,**f** were hydrolyzed into acids **4a**,**b** by LiOH in aqueous THF. Benzoyl chloride **2d** was directly transformed into carboxylic acid **4c** by the reaction with 6-aminohexanoic acid in the presence of NaOH in water/dioxane (1:1) mixture. The carboxylic acids **4a**–**c** were subsequently converted into the corresponding hydroxamic acids **9**–**11** via imidazolide intermediates followed by hydroxylamine treatment.

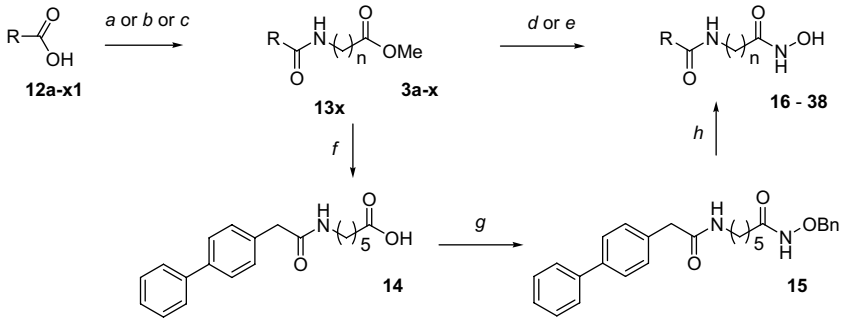
For synthesis of the majority of studied hydroxamic acids, *i.e.* **16**–**38**, (Table 1) appropriate starting carboxylic acids **12a**–**x** were utilized which in turn were provided from commercial suppliers or prepared according to published procedures (see Section 4) (Scheme 2). Syntheses of **16**–**37** were quite straightforward. Thus, amidation of appropriate starting acids **12a**–**x** with methyl 6-aminohexanoate (or methyl 4-aminobutanoate in the case of **12g**) in the presence of suitable condensing agent afforded the expected amidoesters **13a**–**x**. The esters **13a**–**w** were treated with hydroxylamine in the presence of base (NaOH or MeONa) to give the expected hydroxamic acids **16**–**37**. In the case of amidoester **13x** direct treatment with hydroxylamine/base failed. The intermediate ester **13x** was hydrolyzed by LiOH into carboxylic acid **14** and converted into *O*-benzyl hydroxamate **15**. Removal of *O*-benzyl protection of **15** by hydrogenation in the presence of 5% Pd(C) catalyst finished the preparation of hydroxamic acid **38**.

Synthesis of hydroxamic acids **41** and **42** containing a ''reversed amide'' functional group in the central part of the molecule is shown in Scheme 3. Benzylamine **39a** or 2-phenylethylamine **39b** was acylated with imidazolide prepared from 8-methoxy-8-oxooctanoic acid, and the obtained amidoesters **40a**,**b** were treated with hydroxylamine in the presence of NaOH in aqueous methanol affording target hydroxamic acids **41** and **42** (Table 1).

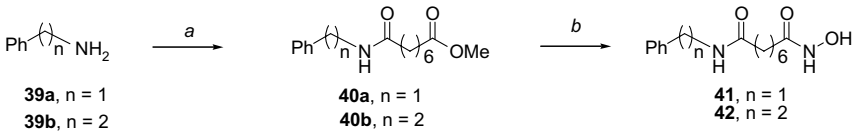
2.2. SAR studies

2.2.1. Chain length

Synthesis of a series of homologues quickly identified that the linking section between the hydroxamic acid and amide must be at least five carbon atoms in length (Table 1), with lengths of five and six being optimal. Predicted binding modes from the results of docking experiments suggested that an extra methyl group in the spacer directs the terminal aryl group into the surface pocket in the correct orientation to pick up favorable interactions (Fig. 2). In addition, the Zn binding distances of compounds with chain lengths of five or six carbon atoms were found to be very similar to reference compounds **I** and **II**. This is consistent with the SAR reported previously by others. Selecting a chain length of five as a basis for



Scheme 2. Reagents and conditions: (a) CDI, **1** ($n = 3$ or 5), TEA, THF; (b) CDI, $H_2N(CH_2)_5COOMe\cdots HCl$, TEA, DMF; (c) ClCOO-*iso*-Bu, TEA/THF, then **1** ($n = 5$); (d) NaOMe, $NH_2OH\cdots HCl$/MeOH; (e) aq. NaOH, $NH_2OH\cdots HCl$/MeOH; (f) aq. LiOH, THF; (g) CDI, $NH_2OBn\cdots HCl$, TEA, DMF; (h) H_2/Pd(C)/MeOH.



39a, n = 1
39b, n = 2

40a, n = 1
40b, n = 2

41, n = 1
42, n = 2

Scheme 3. Reagents and conditions: (a) CDI, $HOOC(CH_2)_6COOMe$/DMF; (b) aq. NaOH, $NH_2OH\cdots HCl$/MeOH.

further work, the effect of introducing a spacer between the amide and the terminal aromatic moiety was examined.

2.2.2. Linker-extension

The simple phenyl analogue **11** had moderate inhibitory activity. Introducing an alkyl spacer length of 2, 3, or 4 reduced both enzyme inhibitory and anti-proliferative potency in **17**, **18**, and **33**. It was also found that a methylene spacer was deleterious to activity, as illustrated by the homologous pairs **8/38** and **10/36** (Table 1). Introduction of 2, 3 or 4 carbon atom spacers significantly altered the orientation of the terminal aryl group of the inhibitors (**17**, **18** and **33**) as opposed to the simple phenyl analogue **11** (Fig. 3). Due to this, compounds with extra carbon atoms demonstrated severe vdW clashes with the residues on the top of the narrow aliphatic channel and the surface aryl pocket, which lead to a significant reduction in the biological activity.

Whenever a saturated carbon was adjacent to the amide e.g., in **20** and **34**, potency remained poor, but when unsaturation was introduced, the compounds showed excellent potency, as in **19** and **16**. Strong potency was also maintained using an alkynyl analogue **35**. The nature of the SP^3 or SP^2 hybridized carbon atom next to the amide linker determined the orientation of the terminal aryl group. Differences in the angles connecting the amide linker and terminal aryl group were governed by the character of SP^3 or SP^2 in the carbon atom next to the amide linker. The entrance of the narrow tunnel leading to the surface pocket is predominantly lipophilic, and small variations in the contact distances governed by the nature of the hybridized carbon atom may change the lipophilic and electrostatic binding contributions. Terminal aryl groups in compounds with unsaturated carbon next to the amide linker (Fig. 4) were oriented in the correct direction to enable favorable interactions with the residues on the entrance of the narrow lipophilic pocket. This guided the identification of compounds with improved biological activity. In general, compounds possessing an alkenyl linker were more potent than their directly linked or methylene linked homologues. Further substitution of the alkyl spacer was allowed in **21**, however, introduction of the alkyl spacer did not compensate for reduction in the length of the linking group between the amide and hydroxamic acid **22**.

2.2.3. SAR of linker-extended reverse amides

In comparison with SAHA (**II**), two compounds (**41** and **42**) were made in which the directionality of the amide was inverted. Interestingly, the SAR appears to mirror that observed above in that the compounds with a methylene (**41**) or ethylene (**42**) spacer were found to be less active than the parent molecule.

2.2.4. Headgroup SAR

Simple aryl systems were generally well tolerated, with the SAR being rather flat (Table 1). Activity of halogen substituted molecules was good. In addition, the activity of pyridyl analogues was less sensitive to the position of substitution in **23–25**, with the 3- and 4-pyridyl compounds being only slightly weaker inhibitors. Interestingly, quinoline substitution yielded potent compounds **27–29**, especially with regard to enzyme inhibitory activity.

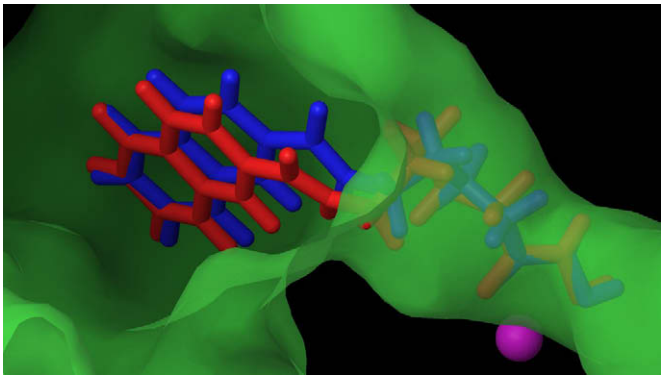


Fig. 2. Superposition of the docked conformations of compound **5** with four carbon chain length (blue), and of compound **6** with six carbon chain length (red) in the HDAC active site. Protein represented in the form of molecular surface, and the active site Zn atom shown in magenta sphere throughout the figures.

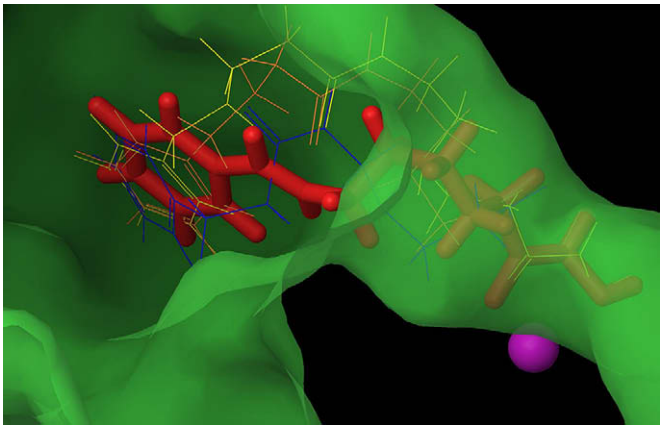


Fig. 3. Superposition of the docked conformations of the compound **11** (red capped sticks), and compounds **17**, **18** and **33** – with two (blue wire frame), three (orange wire frame) and four carbon atom spacer (yellow wire frame) in the HDAC active site.

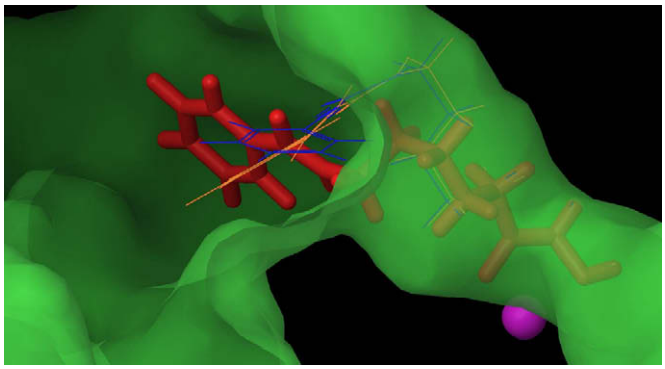


Fig. 4. Superposition of the docked conformations of the compound **11** (red capped sticks), compound **16** (orange wire frame) and compound **19** (blue wire frame) in the HDAC active site.

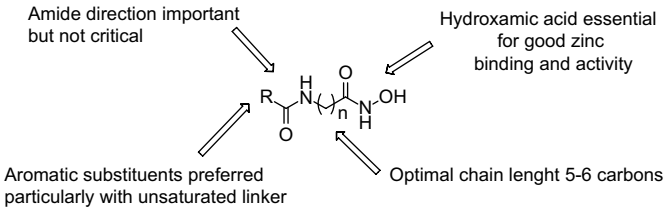


Fig. 6. SAR summary.

However, bulkier head groups revealed that there were some steric constraints to activity. Compared to the simple phenyl analogue **19**, the di-phenyl **30** lost potency. Linking the two aromatic rings to form a tricyclic system did not improve activity **31**. Even so, when the tricyclic system was made smaller and more conformationally constrained, activity returned, with the smallest and most constrained system delivering a highly potent molecule **32**. Superposition of predicted docking orientations of compounds **32** and **19** in the HDAC active site (Fig. 5) revealed the importance of conformationally constrained head groups to achieve tight binding by occupying the surface hydrophobic pocket. A summary of the SAR is given in Fig. 6.

2.3. HDAC selectivity

A representative set of compounds were tested for HDAC isoform selectivity. Using recombinant HDAC enzymes (HDAC 1–9) inhibitory activity of the compounds was determined (Table 2). These compounds were found to be more potent than SAHA, but have a similar selectivity profile. Thus, a broad and potent (nM) inhibition of all HDAC enzymes except HDAC8 was found for compounds **21**, **27**, **32** and **37**. HDAC8 was inhibited at approximately 200–900 times higher concentration (IC_{50}). These results correspond well with recently published data where the HDAC selectivity for 3 hydroxamic acid HDACi was studied [25]. In this study, it is concluded that bulkier head groups interacting with the entrance of the binding pocket may give some selectivity between HDAC class I (HDAC1) and class II (HDAC6) enzymes. However, a more pronounced difference is observed between the HDAC8 (class I) enzyme and the others, which is in agreement with what we found. The disfavored binding of long chain amide HDACi to HDAC8 as compared to the potent inhibition of other HDACs is suggested to be due to a possible change of divalent ion in the active site from Zn(II) to Fe(II) in this isoform [26].

2.4. In vivo biology

A small number of compounds were selected for in vivo evaluation in the syngeneic P388 mouse leukemia i.p. model. This model was valuable as a first in vivo screening model determining anti-tumor activity and general animal tolerance. The compounds were administered i.p. thus, as an intra-tumor treatment, which was believed to maximize exposure and limit pharmacokinetic influences. Activity in this model was most commonly observed using compounds with bulkier aryl groups, or suitable substitutions on the aryl ring. Activity was equal to, and in some cases, superior to that shown by compounds currently under clinical investigation as HDACi.

Of the four compounds tested, none produced increased survival when dosed at 20 mg/kg/day for 5 days. However, when dosed at 60 mg/kg/day, two compounds showed anti-tumor effect. Compound **27** (ILS 111%) was the most active followed by compound **32** (ILS 78%). Compound **37** at 60 mg/kg/day had a minor anti-tumor effect in vivo (ILS 22%), while

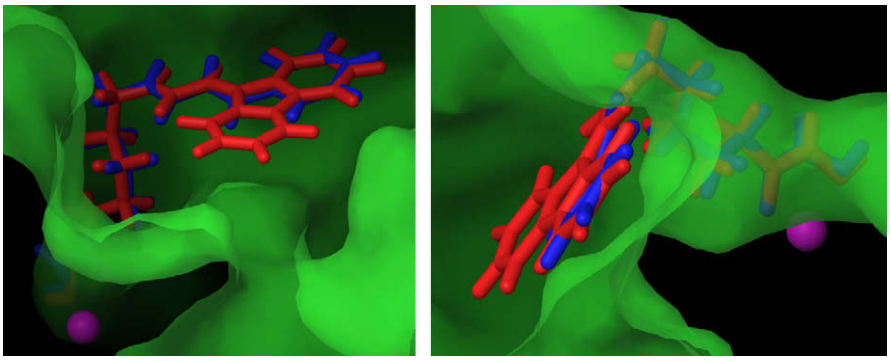


Fig. 5. Superposition of the docked conformations of the compound **32** (red capped sticks), compound **19** (blue capped sticks) in the HDAC active site. For the sake of clarity two orthogonal views are shown in the left and right hand panels.

Table 2
Inhibition of HDAC isoforms (IC_{50}, nM)

Enzyme/compound	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC6	HDAC7	HDAC8	HDAC9
21	3.5	3.1	5.3	3.7	ND[a]	3.5	4.4	2116.3	4.2
27	2.9	2.5	6.7	5.3	7.6	3.4	3.0	2028.3	2.8
32	1.6	0.8	2.8	1.2	ND[a]	2.0	ND[a]	779.0	2.3
37	3.2	3.0	4.9	4.3	ND[a]	2.5	6.3	1619.7	3.9
II (SAHA)	68.1	163.6	67.1	100.9	107.3	110.6	102.4	1374.7	107.2

[a] Not determined.

SAHA (**II**) failed to show any survival benefit at 60 mg/kg/day using the P388 mouse leukemia model (Table 3). At higher concentrations SAHA was found weakly active in this model (ILS 14%, 100 mg/kg/day).

3. Conclusion

A novel series of chain-extended compounds, based on the amide linker template of HDACi, were designed and synthesized using computational and medicinal chemistry. Compounds within this novel series showed potent HDAC enzymatic inhibition, as well as anti-proliferative activity superior to compounds currently undergoing clinical investigation. Two of these novel compounds also demonstrated large survival benefits when administered to an in vivo mouse cancer model, underlining their potential for further development.

Available structural information (from sequence comparison and crystallography [4]) indicates that the HDAC isoforms are well conserved in the vicinity of the catalytic machinery, but more variable at the cavity entrance. Interactions with these surface regions would therefore be expected to differ between HDAC family members and potentially confer HDAC subtype selectivity, a topic of great current interest. However, the inhibitors described in this report did not show a selective interaction with this region of the HDAC isoforms leading to isoform selectivity, with the exception of a decreased activity towards HDAC8. Using data obtained through this and other recent investigations, we are currently exploring further compounds which may exhibit improved isoform selectivity.

Table 3
In vivo survival test using the P388 mouse leukemia i.p. model

Treatment compound ID	Dose (mg/kg/day)	Schedule	Median survival (days)	*N*	ILS (%)	*p*-Value
Vehicle control		×1 i.p. days 3–7	9	18		
32	20	×1 i.p. days 3–7	10	9	11	NS
32	60	×1 i.p. days 3–7	16	9	78	<0.05
37	20	×1 i.p. days 3–7	9	9	0	NS
37	60	×1 i.p. days 3–7	11	9	22	<0.05
II (SAHA)	20	×1 i.p. days 3–7	10	9	11	NS
II (SAHA)	60	×1 i.p. days 3–7	9	9	0	NS
27	20	×1 i.p. days 3–7	9	9	0	NS
27	60	×1 i.p. days 3–7	19	9	111	<0.05

N = number of animal per group. Statistics: LogRank analysis, $p > 0.05$ is considered "not significant" (NS).

4. Experimental

4.1. Chemistry

4.1.1. General

Nuclear magnetic spectra (^{1}H NMR) were recorded on WH-90/DS or Mercury 200 (Varian) spectrometers at ambient temperatures. The purity of synthesized hydroxamic acids (>96%) was assessed by reverse phase analytical HPLC using Symmetry C_{18} column (column size: 3.9 × 150 mm; mobile phase: acetonitrile–0.1 M phosphate buffer with pH 2.5); measurements were performed on a Varian ProStar HPLC system equipped with a spectrophotometer. Elemental analyses were obtained with a Carlo Erba EA 1108 instrument. Melting points were measured on a "Boëtius" or "Fisher" micro melting point apparatus and are uncorrected. Silica gel, 0.035–0.070 mm, (Acros) was employed for column chromatography (CC). All the solvents were purified before use by routine techniques.

2-Naphthoyl chloride (**2a**), benzenecarbonyl chloride (**2d**), [1,1′-biphenyl]-4-carbonyl chloride (**2b**), 2-[1,1′-biphenyl]-4-ylacetic acid (**12x**), 4-(dimethylamino)benzenecarbonyl chloride (**2c**), 2-[4-(dimethylamino)phenyl]acetic acid (**12v**), aniline, benzylamine (**39a**), methyl 3,3-diphenylacrylate, 2-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yliden)acetic acid (**12p**), 7-aminoheptanoic acid, 8-aminooctanoic acid, and 8-methoxy-8-oxooctanoic acid were purchased from Aldrich, 3-phenylpropanoic acid (**12b**), 4-phenylbutanoic acid (**12c**), 5-phenylpentanoic acid (**12s**), (*E*)-3-phenyl-2-propenoic acid (**12d**), (*E*)-4-phenyl-3-butenoic acid (**12e**), 3-phenyl-2-propynoic acid (**12u**), 2-phenyl-1-ethanamine (**39b**), (*E*)-3-(4-chloro-2-fluorophenyl)-2-propenoic acid (**12w**), 6-quinolinecarboxylic acid (**12n**), 5-aminopentanoic acid, and 6-aminohexanoic acid were from *Acros*, (2*E*,4*E*)-5-phenyl-2,4-pentadienoic acid (**12a**), (*E*)-3-(4-pyridinyl)-2-propenoic acid (**12h**), (*E*)-3-(3-pyridinyl)-2-propenoic acid (**12j**), (*E*)-3-(2-naphthyl)-2-propenoic acid (**12k**), and 9-fluorenylideneacetic acid (**12r**) were from *Alfa Aesar*, (*E*)-3-(2-pyridinyl)-2-propenoic acid (**12i**) was from *Apollo Sci.*, and methyl 4-aminobutanoate was from *Fluka*.

3,3-Diphenyl-acrylic acid (**12o**), methyl 5-aminopentanoate, methyl 6-aminohexanoate, methyl 7-aminoheptanoate, and methyl 8-aminooctanoate were prepared by routine protocols from methyl 3,3-diphenylacrylate, 5-aminopentanoic acid, 6-aminohexanoic acid, 7-aminoheptanoic acid, and 8-aminooctanoic acid, accordingly. SAHA was prepared as described in Ref. [27].

*4.1.3. General synthesis of amidomethyl esters **3a–d** from carboxylic acid chlorides **2a**,**b** (method A)*

To a solution of ω-amino acid methyl ester hydrochloride **1** ($n = 4$, 5, 6, or 7) (2.75 mmol) and diisopropyl ethylamine (0.96 ml, 5.5 mmol) in anhydrous *N*,*N*-dimethylformamide (3 ml) appropriate carboxylic acid chlorides **2a**,**b** (2.75 mmol) in dimethylformamide (3 ml) was added. The mixture was stirred for 3 h at room temperature, diluted with brine (30 ml), and extracted with ethyl acetate (3 × 25 ml). The organic phase was washed with brine (2 × 15 ml), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified on silica gel (20 g) with chloroform/ethyl acetate as eluent, affording the corresponding amidoester derivatives **3a–d**.

*4.1.4. General synthesis of hydroxamic acids **5–8** from amidoesters **3a–d** (method B)*

Appropriate amidoesters **3** (1 mmol) and hydroxylamine hydrochloride (278 mg, 4 mmol) were dissolved in methanol (3–5 ml), and a solution of NaOH (320 mg, 8 mmol) in water (1 ml) was added. After stirring for 15–45 min at ambient temperature, the resultant mixture was acidified with 1 N HCl to pH 3 and extracted with ethyl acetate (3 × 30 ml). The organic phase was evaporated under reduced pressure by adding several times of benzene to remove traces of water. The crude product was washed with a small amount of ethyl acetate and crystallized from acetonitrile to give the corresponding hydroxamic acid.

*4.1.5. Preparation of **5***

*4.1.5.1. Methyl 5-[(2-naphthylcarbonyl)amino]pentanoate (**3a**).* Compound **3a** was obtained from 2-naphthoyl chloride (**2a**) and methyl 5-aminopentanoate hydrochloride by the method A, yield 83%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.41–1.89 (m, 4H), 2.23–2.52 (m, 2H), 3.32–3.60 (m, 2H), 3.67 (s, 3H), 6.60 (br s, 1H), 7.36–7.63 (m, 2H), 7.72–8.00 (m, 4H), 8.27 (s, 1H).

*4.1.5.2. N-[5-(Hydroxyamino)-5-oxopentyl]-2-naphthamide (**5**).* Compound **5** was obtained from methyl 5-[(2-naphthylcarbonyl)amino]pentanoate (**3a**) by the method B, yield 92%. M.p. 155–156 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.44–1.62 (m, 4H), 1.94–2.06 (m, 2H), 3.20–3.30 (m, 2H), 7.52–7.64 (m, 2H), 7.86–8.08 (m, 4H), 8.43 (s, 1H), 8.58–8.75 (m, 2H), 10.36 (s, 1H). Anal. Calcd for $C_{16}H_{18}N_2O_3$: C 67.12, H 6.34, N 9.78. Found: C 67.00, H 6.33, N 9.83.

*4.1.6. Preparation of **6***

*4.1.6.1. Methyl 7-[(2-naphthylcarbonyl)amino]heptanoate (**3b**).* Compound **3b** was obtained from 2-naphthoyl chloride (**2a**) and methyl 7-aminoheptanoate hydrochloride by the method A, yield 85%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.16–1.83 (m, 8H), 2.32 (t, $J = 7.0$ Hz, 2H), 3.49 (q, $J = 6.0$ Hz, 2H), 3.63 (s, 3H), 6.32 (br s, 1H), 7.40–7.56 (m, 2H), 7.72–8.05 (m, 4H), 8 27 (s, 1H).

*4.1.6.2. N-[7-(Hydroxyamino)-7-oxoheptyl]-2-naphthamide (**6**).* Compound **6** was obtained from methyl 7-[(2-naphthylcarbonyl)amino]heptanoate (**3b**) by the method B, yield 97%. M.p. 136–137 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.48–1.62 (m, 8H), 1.95 (t, $J = 6.8$ Hz, 2H), 3.20–3.30 (m, 2H), 7.52–7.64 (m, 2H), 7.86–8.08 (m, 4H), 8.43 (s, 1H), 8.52–8.68 (m, 2H), 10.33 (s, 1H). Anal. Calcd for $C_{18}H_{22}N_2O_3$: C 68.77, H 7.05, N 8.91. Found: C 68.50, H 7.08, N 8.96.

*4.1.7. Preparation of **7***

*4.1.7.1. Methyl 8-[(2-naphthylcarbonyl)amino]octanoate (**3c**).* Compound **3c** was obtained from 2-naphthoyl chloride (**2a**) and methyl 8-aminooctanoate hydrochloride by the method A, yield 93%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 0.98–1.89 (m, 10H), 2.34 (t, $J = 7.0$ Hz, 2H), 3.47 (q, $J = 6.0$ Hz, 2H), 3.63 (s, 3H), 6.31 (br s, 1H), 7.40–7.52 (m, 2H), 7.72–8.00 (m, 4H), 8.27 (s, 1H).

*4.1.7.2. N-[8-(Hydroxyamino)-8-oxooctyl]-2-naphthamide (**7**).* Compound **7** was obtained from methyl 8-[(2-naphthylcarbonyl)amino]octanoate by the method B, yield 84%. M.p. 138–139 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.14–1.66 (m, 10H), 1.95 (t, $J = 7.2$ Hz, 2H), 3.22–3.30 (m, 2H), 7.53–7.65 (m, 2H), 7.87–8.08 (m, 4H), 8.43 (s, 1H), 8.55–8.71 (m, 2H), 10.33 (s, 1H). Anal. Calcd for $C_{19}H_{24}N_2O_3$: C 69.49, H 7.37, N 8.53. Found: C 69.20, H 7.40, N 8.52.

*4.1.8. Preparation of **8***

*4.1.8.1. Methyl 6-[([1,1′-biphenyl]-4-ylcarbonyl)amino]hexanoate (**3d**).* Compound **3d** was obtained from [1,1′-biphenyl]-4-carbonyl chloride (**2b**) and methyl 6-aminohexanoate hydrochloride by the method A, yield 47%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.34–1.52 (m, 2H), 1.55–1.78 (m, 4H), 2.34 (t, $J = 7.3$ Hz, 2H), 3.49 (q, $J = 6.6$ Hz, 2H), 3.67 (s, 3H), 6.21 (unresolved t, $J \sim 5.8$ Hz, 1H), 7.33–7.52 (m, 3H), 7.56–7.69 (m, 4H), 7.83 (d, $J = 8.4$ Hz, 2H).

*4.1.8.2. N-[6-(Hydroxyamino)-6-oxohexyl][1,1′-biphenyl]-4-carboxamide (**8**).* Compound **8** was obtained from methyl 6-[([1,1′-biphenyl]-4-ylcarbonyl)amino]hexanoate (**3d**) by the method B, yield 85% (cryst. from acetonitrile, 38%). M.p. 204–206 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.20–1.1.40 (m, 2H), 1.42–1.62 (m, 4H), 1.89–2.01 (m, 2H), 3.17–3.28 (m, 2H), 7.34–7.55 (m, 3H), 7.67–7.79 (m, 4H), 7.93 (d, 2H, $J = 8.2$ Hz), 8.50 (t, 1H, $J = 5.45$ Hz), 8.66 (s, 1H), 10.34 (s, 1H). Anal. Calcd for $C_{19}H_{22}N_2O_3$: C 69.92, H 6.79, N 8.58. Found: C 69.89, H 6.89, N 8.30.

*4.1.9. Preparation of **9***

*4.1.9.1. Methyl 6-[(2-naphthylcarbonyl)amino]hexanoate (**3e**).* To a solution of methyl 6-aminohexanoate hydrochloride

(0.500 g, 2.75 mmol) and diisopropyl ethylamine (0.96 ml, 5.5 mmol) in dry dimethylformamide (3 ml) 2-naphthoyl chloride (**2a**) (0.524 g, 2.75 mmol) in dry dimethylformamide (3 ml) was added. The mixture was stirred for 3 h at room temperature, then diluted with saturated NaCl (30 ml) and extracted with ethyl acetate (3 × 25 ml). The organic phase was washed with saturated NaCl (2 × 15 ml) and dried (Na_2SO_4). The solvent was evaporated to give crude methyl 6-[(2-naphthylcarbonyl)amino]hexanoate (0.825 g) which was used in the next step without further purification.

*4.1.9.2. 6-[(2-Naphthylcarbonyl)amino]hexanoic acid (**4a**).* The crude methyl 6-[(2-naphthylcarbonyl)amino]hexanoate (**3e**) (0.825 g) was dissolved in tetrahydrofuran (5 ml) and 1 N LiOH (5.5 ml, 5 mmol) was added. The reaction mixture was stirred for 3 h at room temperature, washed with ether (2 × 10 ml), and acidified with 2 N HCl up to pH 3. The mixture was extracted with ethyl acetate (3 × 20 ml), the organic layer was washed with brine (3 × 10 ml), and dried (Na_2SO_4). The solvent was evaporated and the residue was chromatographed on silica gel with ethyl acetate as eluent to give pure 6-[(naphthalene-2-carbonyl)amino]hexanoic acid (**4a**) (0.618 g, 79%). ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.11–1.77 (6H, m), 2.22–2.39 (2H, m), 3.47 (2H, dd, $J = 7.0$ Hz), 6.26 (1H, br s), 7.17(1H, s), 7.33–7.57 (2H, m), 7.64–7.93 (4H, m), 8.22 (1H, s).

*4.1.9.3. N-[6-(Hydroxyamino)-6-oxohexyl]-2-naphthamide (**9**).* A solution of 6-[(naphthalene-2-carbonyl)amino]hexanoic acid (**4a**) (0.618 g, 2.17 mmol) in dry tetrahydrofuran (6 ml) was cooled in ice bath under argon atmosphere and 1,1′-carbonyldiimidazole (0.422 g, 2.6 mmol) was added. The mixture was stirred for 30 min and a solution of hydroxylamine (4.35 mmol) in dry dimethylformamide (3 ml) [the solution of hydroxylamine was made from hydroxylamine hydrochloride (0.302 g, 4.35 mmol) and triethylamine (0.61 ml) in dimethylformamide (3 ml) and filtered] was added. After stirring overnight the reaction mixture was diluted with saturated NaH_2PO_4 (30 ml) and extracted with ethyl acetate (3 × 30 ml). The organic phase was evaporated under reduced pressure by adding several times of benzene to remove traces of water. The crude product was crystallized successively from ethyl acetate and acetonitrile to give *N*-[6-(hydroxyamino)-6-oxohexyl]-2-naphthamide (**9**) (0.260 g, 40%). M.p. 96–98 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.21–1.45 (2H, m), 1.46–1.73 (4H, m), 1.89–2.05 (2H, m), 3.25–3.37 (2H, m), 7.65–7.81 (2H, m), 7.86–8.04 (4H, m), 8.42 (1H, s), 8.56–8.72 (2H, m), 10.32 (1H, s). Anal. Calcd: C 67.98, H 6.71, N 9.33. Found: C 68.29, H 6.97, N 8.99.

*4.1.10. Preparation of **10***

*4.1.10.1. Methyl 6-{[4-(dimethylamino)benzoyl]amino}hexanoate (**3f**).* Compound **3f** was obtained from 4-(dimethylamino)benzoyl chloride (**2c**) and methyl 6-aminohexanoate hydrochloride by the method A, yield 67%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.16–1.92 (m, 6H), 2.34 (t, $J = 7.0$ Hz, 2H), 3.03 (s, 6H), 3.43 (q, $J = 6.0$ Hz, 2H), 3.65 (s, 3H), 6.00 (br s, 1H), 6.69 (d, $J = 9.0$ Hz, 2H), 7.79 (d, $J = 9.0$ Hz, 2H).

*4.1.10.2. 6-{[4-(Dimethylamino)benzoyl]amino}hexanoic acid (**4b**).* Compound **4b** was obtained from methyl 6-{[4-(dimethylamino)benzoyl]amino}hexanoate (**3f**) by a similar protocol to **4a**, yield 87%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.16–1.92 (m, 6H), 2.34 (t, $J = 7.0$ Hz, 2H), 3.03 (s, 6H), 3.43 (q, $J = 6.0$ Hz, 2H), 5.97 (br s, 1H), 6.69 (d, $J = 9.0$ Hz, 2H), 7.79 (d, $J = 9.0$ Hz, 2H).

*4.1.10.3. 4-(Dimethylamino)-N-[6-(hydroxyamino)-6-oxohexyl]benzenecarboxamide (**10**).* Compound **10** was obtained from 6-{[4-(dimethylamino)benzoyl]amino}hexanoic acid (**4b**) by a similar protocol to **9**, yield 71%. M.p. 137–138 °C (from acetonitrile). ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.17–1.1.33 (m, 2H), 1.35–1.56 (m, 4H), 1.93 (t, 2H, $J = 7.5$ Hz), 2.95 (s, 6H), 3.11–3.25 (m, 2H), 6.68 (d, 2H, $J = 8.8$ Hz), 7.69 (d, 2H, $J = 8.8$ Hz), 8.01 (t, 1H, $J = 5.45$ Hz), 8.65 (s, 1H), 10.32 (s, 1H). Anal. Calcd for $C_{15}H_{23}N_3$: C 61.41, H 7.90, N 14.32. Found: C 61.87, H 8.08, N 14.01.

*4.1.11. Preparation of **11***

*4.1.11.1. 6-(Benzoylamino)hexanoic acid (**4c**).* To a solution of 6-aminocaproic acid (0.982 g, 7.5 mmol) in water/dioxane mixture (1:1, 30 ml) NaOH (0.8 g, 20 mmol) in H_2O (2 ml) and benzoyl chloride (**2d**) (1.16 ml, 10 mmol) successively were added. The mixture was stirred for 6 h at room temperature and diluted with brine (150 ml). The mixture was washed with diethyl ether (2 × 25 ml), acidified with conc. HCl to pH 4, and extracted with ethyl acetate (4 × 25 ml). The organic solution was washed with brine (3 × 25 ml), dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel with chloroform/ethyl acetate (1:2) as eluent to give 6-(benzoylamino)hexanoic acid (**4c**) (1.589 g, 90%). ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.18–1.85 (m, 6H), 2.34 (t, $J = 7.0$ Hz, 2H), 3.45 (q, $J = 6.0$ Hz, 2H), 6.27 (br s, 1H), 7.29–7.52 (m, 3H), 7.61–7.85 (m, 2H), 10.41 (br s, 1H).

*4.1.11.2. N-[6-(Hydroxyamino)-6-oxohexyl]benzenecarboxamide (**11**).* Compound **11** was obtained from 6-(benzoylamino)hexanoic acid (**4c**) by a similar protocol to **9**, yield 26%. M.p. 102–104 °C (from acetonitrile). ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.15–1.1.30 (m, 2H), 1.42–1.50 (m, 4H), 1.87 (t, $J = 7.0$ Hz, 2H), 3.16 (q, $J = 6.6$ Hz, 2H), 7.34–7.50 (m, 3H), 7.70–7.80 (m, 2H), 8.37 (t, $J = 5.8$ Hz, 1H), 8.60 (s, 1H), 10.27 (s, 1H). Anal. Calcd for $C_{13}H_{18}N_2$: C 62.38, H 7.25, N 11.19. Found: C 62.96, H 7.47, N 10.65.

*4.1.12. Preparation of **16***

*4.1.12.1. Methyl 6-{[(2E,4E)-5-phenyl-2,4-pentadienoyl]amino}hexanoate (**13a**).* 1,1′-Carbonyldiimidazole (0.36 g, 2.2 mmol) was added to a solution of 5-phenyl-penta-2*E*,

4*E*-dienoic acid (**12a**) (0.35 g, 2 mmol) in dry tetrahydrofuran (10 ml) and the obtained mixture was stirred for 1 h at ambient temperature. To the mixture triethylamine (0.30 g 3.0 mmol) and methyl 6-aminohexanoate hydrochloride (0.40 g, 2.2 mmol) were added and the resultant suspension was stirred for 6 h at ambient temperature. The solvent was removed under reduced pressure. To the residue water (15 ml) was added and the precipitate was filtered off, washed with water and dried. Methyl 6-{[(2*E*,4*E*)-5-phenyl-2,4-pentadienoyl]amino}hexanoate (**13a**) (0.36 g, 60%) was obtained as a white solid. M.p. 125–127 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.05–1.72 (m, 6H), 2.29 (t, $J = 7.3$ Hz, 2H), 3.12 (q, $J = 6.0$ Hz, 2H), 3.58 (s, 3H), 6.12 (d, $J = 14.8$ Hz, 1H), 6.91–7.16 (m, 2H), 7.23–7.66 (m, 6H), 8.05 (t, $J = 5.8$ Hz, 1H).

*4.1.12.2. (2E,4E)-N-[6-(Hydroxyamino)-6-oxohexyl]-5-phenyl-2,4-pentadienamide (**16**).* A solution of sodium methylate (6 mmol) in methanol (5 ml) was added to a solution of hydroxylamine hydrochloride (0.28 g, 4 mmol) in methanol (8 ml). The mixture was stirred for 10 min and the precipitate was filtered off. Methyl 6-{[(2*E*,4*E*)-5-phenyl-2,4-pentadienoyl]amino}hexanoate (**13a**) (0.30 g, 1 mmol) was added to the filtrate and the mixture was heated to complete dissolving. The resultant mixture was stirred for 4 h at ambient temperature and the solvent was removed under reduced pressure. The product was dissolved in water (10 ml) and acidified with 3% HCl. The precipitate was filtered off and crystallized from methanol. (2*E*,4*E*)-*N*-[6-(Hydroxyamino)-6-oxohexyl]-5-phenyl-2,4-pentadienamide (**16**) (0.23 g, 66%) was obtained as a white solid. M.p. 160–161 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.05–1.69 (m, 6H), 1.81–2.07 (m, 2H), 3.12 (q, $J = 5.8$ Hz, 2H), 6.14 (d, $J = 14.8$ Hz, 1H), 6.89–7.14 (m, 2H), 7.23–7.65 (m, 6H), 8.05 (t, $J = 6.0$ Hz, 1H), 8.63 (s, 1H), 10.34 (s, 1H). Anal. Calcd for $C_{17}H_{22}N_2O_3$: C 65.68, H 6.61, N 10.21. Found: C 65.63, H 6.60, N 10.17.

*4.1.13. Preparation of **17***

*4.1.13.1. Methyl 6-[(3-phenylpropanoyl)amino]hexanoate (**13b**).* Compound **13b** from 3-phenylpropionic acid (**12b**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 76%. M.p. 34–35 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 0.99–1.77 (m, 6H), 2.27 (t, $J = 7.2$ Hz, 2H), 2.44 (t, $J = 7.5$ Hz, 2H), 2.95 (t, $J = 7.5$ Hz, 2H), 3.18 (q, $J = 6.0$ Hz, 2H), 3.64 (s, 3H), 5.47 (br s, 1H), 7.22 (s, 5H).

*4.1.13.2. N-Hydroxy-6-[(3-phenylpropanoyl)amino]hexanamide (**17**).* Compound **17** from methyl 6-[(3-phenylpropanoyl)amino]hexanoate (**13b**) by a similar protocol to **16** was prepared, yield 95%. M.p. 107 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.08–1.64 (m, 6H), 1.91 (t, $J = 6.6$ Hz, 2H), 2.33 (m, 2H), 2.66–3.12 (m, 4H), 7.08–7.36 (m, 5H), 7.73 (unresolved t, 2H), 8.61 (br s, 1H), 10.29 (br s, 1H). Anal. Calcd for $C_{15}H_{22}N_2O_3$: C 64.73, H 7.97, N 10.06. Found: C 64.85, H 8.07, N 9.94.

*4.1.14. Preparation of **18***

*4.1.14.1. Methyl 6-[(4-phenylbutanoyl)amino]hexanoate (**13c**).* Compound **13c** from 4-phenylbutyric acid (**12c**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 76%, oil. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.11–2.43 (m, 12H), 2.65 (t, $J = 7.0$ Hz, 2H), 3.23 (q, $J = 5.4$ Hz, 2H), 3.64 (s, 3H), 5.56 (br s, 1H), 7.21 (s, 5H).

*4.1.14.2. N-Hydroxy-6-[(4-phenylbutanoyl)amino]hexanamide (**18**).* Compound **18** from methyl 6-[(4-phenylbutanoyl)amino]hexanoate (**13c**) by a similar protocol to **16** was prepared, yield 73%. M.p. 92–93 °C (from ethyl acetate). ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.11–2.21 (m, 12H), 2.88–3.16 (m, 2H), 7.23 (br s, 5H), 7.74 (unresolved t, 1H), 8.63 (br s, 1H), 10.32 (br s, 1H). Anal. Calcd for $C_{16}H_{24}N_2O_3$: C 65.73, H 8.27, N 9.58. Found: C 65.79, H 8.34, N 9.56.

*4.1.15. Preparation of **19***

*4.1.15.1. Methyl 6-{[(E)-3-phenyl-2-propenoyl]amino}hexanoate (**13d**).* Compound **13d** was obtained from (*E*)-3-phenyl-2-propenoic acid (**12d**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** as a white solid, yield 42%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.11–1.86 (m, 6H), 2.32 (t, $J = 6.5$ Hz, 2H), 3.38 (q, $J = 5.8$ Hz, 2H), 3.65 (s, 3H), 5.68 (br s, 1H), 6.37 (d, $J = 15.6$ Hz, 1H), 7.13–7.61 (m, 5H), 7.59 (d, $J = 15.6$ Hz, 1H).

*4.1.15.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-phenyl-2-propenamide (**19**).* Compound **19** was obtained from methyl 6-{[(*E*)-3-phenyl-2-propenoyl]amino}hexanoate (**13d**) by a similar protocol to **16**, yield 60%. M.p. 154–155 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.05–1.74 (m, 6H), 1.94 (t, $J = 6.4$ Hz, 2H), 3.16 (m, 2H), 6.61 (d, $J = 15.9$ Hz, 1H), 7.16–7.66 (m, 6H), 8.06 (t, $J = 5.3$ Hz, 1H), 8.63 (s, 1H), 10.32 (s, 1H). Anal. Calcd for $C_{15}H_{22}N_2O_3$: C 65.20, H 7.30, N 10.14. Found: C 64.93, H 7.33, N 10.21.

*4.1.16. Preparation of **20***

*4.1.16.1. Methyl 6-{[(E)-4-phenyl-3-butenoyl]amino}hexanoate (**13e**).* Compound **13e** from 4-phenyl-but-3*E*-enoic acid (**12e**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 49%. M.p. 49–51 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.12–1.81 (m, 6H), 2.29 (t, $J = 7.0$ Hz, 2H), 3.14 (d, $J = 6.2$ Hz, 2H), 3.26 (q, $J = 6.5$ Hz, 2H), 3.64 (s, 3H), 5.65 (br s, 1H), 6.27 (dt, $J = 6.2$, 16.0 Hz, 1H), 6.57 (d, $J = 16.0$ Hz, 1H), 7.21–7.52 (m, 5H).

*4.1.16.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-4-phenyl-3-butenamide (**20**).* Compound **20** from methyl 6-{[(*E*)-4-phenyl-3-butenoyl]amino}hexanoate (**13e**) by a similar protocol to **16** was prepared, yield 52%. M.p. 126–128 °C. ^{1}H NMR

(DMSO-d_6, HMDSO) δ: 1.07–1.68 (m, 6H), 1.92 (t, $J = 7.0$ Hz, 2H), 3.03 (d, $J = 5.6$ Hz, 2H), 3.05 (q, $J = 6.2$ Hz, 2H), 6.29 (dt, $J = 5.8$, 16.0 Hz, 1H), 6.48 (d, $J = 16.0$ Hz, 1H), 7.14–7.53 (m, 5H), 7.85 (unresolved t, 1H), 8.63 (br s, 1H), 10.32 (br s, 1H). Anal. Calcd for $C_{16}H_{22}N_2O_3$: C 66.19, H 7.64, N 9.65. Found: C 66.18, H 7.74, N 9.56.

4.1.17. Preparation of ***21***

*4.1.17.1. Methyl 6-{[(2E,4E)-3-methyl-5-phenyl-2,4-pentadienoyl]amino}hexanoate (**13f**).* Compound **13f** was obtained from (2*E*,4*E*)-3-methyl-5-phenyl-2,4-pentadienoic acid [28] (**12f**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a**, yield 90%. M.p. 83–85 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.01–1.78 (m, 6H), 2.28 (t, $J = 7.0$ Hz, 2H), 2.29 (s, 3H), 3.09 (q, $J = 6.0$ Hz, 2H), 3.55 (s, 3H), 5.94 (s, 1H), 6.88 (s, 2H), 7.21–7.67 (m, 5H), 7.96 (unresolved t, 1H).

*4.1.17.2. (2E,4E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-methyl-5-phenyl-2,4-pentadienamide (**21**).* Compound **21** was prepared from methyl 6-{[(2*E*,4*E*)-3-methyl-5-phenyl-2,4-pentadienoyl]amino}hexanoate (**13f**) by a similar protocol to **16**, yield 60%. M.p. 147–149 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.01–1.74 (m, 6H), 1.94 (t, $J = 6.0$ Hz, 2H), 2.29 (s, 3H), 3.09 (q, $J = 6.0$ Hz, 2H), 5.94 (s, 1H), 6.89 (s, 2H), 7.14–7.69 (m, 5H), 7.96 (unresolved t, 1H), 8.67 (s, 1H), 10.29 (s, 1H). Anal. Calcd for $C_{18}H_{24}N_2O_3$: C 68.33, H 7.65, N 8.85. Found: C 68.32, H 7.58, N 8.89.

4.1.18. Preparation of ***22***

*4.1.18.1. Methyl 4-[(2E,4E)-4-methyl-5-phenyl-2,4-pentadienoyl]aminobutanoate (**13g**).* Compound **13g** was obtained from 4-methyl-5-phenylpenta-2,4-dienoic acid [29] (**12g**) and methyl 4-aminobutyrate hydrochloride by a similar protocol to **13a** as white crystals, yield 53%. M.p. 111–113 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.41–1.88 (m, 2H), 2.00 (s, 3H), 2.30 (t, $J = 7.5$ Hz, 2H), 3.12 (q, $J = 6.4$ Hz, 2H), 3.59 (s, 3H), 6.10 (d, $J = 15.0$ Hz, 1H), 6.89 (s, 1H), 7.30 (d, $J = 15.0$ Hz, 1H), 7.48 (s, 5H), 8.10 (unresolved t, 1H).

*4.1.18.2. (2E,4E)-N-[4-(Hydroxyamino)-4-oxobutyl]-4-methyl-5-phenyl-2,4-pentadienamide (**22**).* Compound **22** from methyl 4-[(2*E*,4*E*)-4-methyl-5-phenyl-2,4-pentadienoyl]aminobutanoate (**13g**) by a similar protocol to **16** was prepared, yield 70%. M.p. 127–129 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.42–1.85 (m, 2H), 1.87 (t, 2H), 1.98 (s, 3H), 3.16 (q, $J = 6.2$ Hz, 2H), 6.14 (d, $J = 14.8$ Hz, 1H), 6.87 (s, 1H), 7.25 (d, $J = 14.8$ Hz, 1H), 7.38 (s, 5H), 8.07 (unresolved t, 1H), 9.56 (br s, 2H). Anal. Calcd for $C_{16}H_{20}N_2O_3$: C 66.65, H 6.99, N 9.72. Found: C 66.55, H 6.98, N 9.66.

4.1.19. Preparation of ***23***

*4.1.19.1. Methyl 6-{[(E)-3-(4-pyridinyl)-2-propenoyl]amino}hexanoate (**13h**).* Compound **13h** was obtained from (*E*)-3-(4-pyridinyl)-2-propenoic acid (**12h**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a**, yield 34%. M.p. 92–94 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.09–1.76 (m, 6H), 2.28 (t, $J = 7.5$ Hz, 2H), 3.16 (q, $J = 6.0$ Hz, 2H), 3.57 (s, 3H), 6.81 (d, $J = 16.0$ Hz, 1H), 7.41 (d, $J = 16.0$ Hz, 1H), 7.52 (d, $J = 6.0$ Hz, 2H), 8.23 (unresolved t, 1H), 8.61 (d, $J = 6.0$ Hz, 2H).

*4.1.19.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-(4-pyridinyl)-2-propenamide (**23**).* Compound **23** was obtained from methyl 6-{[(*E*)-3-(4-pyridinyl)-2-propenoyl]amino}hexanoate (**13h**) by a similar protocol to **16** and dissolved in ethanol. To the obtained solution an ethanol solution of oxalic acid (2 equiv) was added. The precipitate was filtered and crystallized from methanol to give (*E*)-*N*-[6-(hydroxyamino)-6-oxohexyl]-3-(4-pyridinyl)-2-propenamide oxalate (**23**), yield 50%. M.p. 168–170 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.09–1.69 (m, 6H), 1.94 (t, $J = 7.5$ Hz, 2H), 3.16 (q, $J = 6.0$ Hz, 2H), 6.81 (d, $J = 16.0$ Hz, 1H), 7.38 (d, $J = 16.0$ Hz, 1H), 7.51 (d, $J = 6.0$ Hz, 2H), 8.19 (unresolved t, 1H), 8.57 (d, $J = 6.0$ Hz, 2H), 10.21 (s, 1H). Anal. Calcd for $C_{14}H_{19}N_3O_3\cdots(COOH)_2\cdots0.25H_2O$: C 51.68, H 5.83, N 11.30. Found: C 51.44, H 5.62, N 11.23.

4.1.20. Preparation of ***24***

*4.1.20.1. Methyl 6-{[(E)-3-(2-pyridinyl)-2-propenoyl]amino}-hexanoate (**13i**).* Compound **13i** was obtained from (*E*)-3-(2-pyridinyl)-2-propenoic acid (**12i**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** as an oil, yield 45%. ^{1}H NMR (DMSO-d_6, HMDSO) δ:1.02–1.72 (m, 6H), 2.28 (t, $J = 7.0$ Hz, 2H), 3.16 (q, $J = 6.0$ Hz, 2H), 3.56 (s, 3H), 7.09 (d, $J = 15.6$ Hz, 1H), 7.23–7.42 (m, 1H), 7.42 (d, $J = 15.6$ Hz, 1H), 7.49 (d, $J = 7.5$ Hz, 1H), 7.76–7.99 (m, 1H), 8.23 (t, $J = 5.6$ Hz, 1H), 8.59 (dd, $J = 2.0$, 4.8 Hz, 1H).

*4.1.20.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-(2-pyridinyl)-2-propenamide oxalate (**24**).* Compound **24** was obtained from methyl 6-{[(*E*)-3-(2-pyridinyl)-2-propenoyl]amino}hexanoate (**13i**) by similar protocols to **16** and **23**, yield 46%. M.p. 126–128 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.15–1.65 (m, 6H), 1.94 (t, $J = 7.2$ Hz, 2H), 3.16 (q, $J = 6.0$ Hz, 2H), 7.08 (d, $J = 15.4$ Hz, 1H), 7.32 (dd, $J = 4.8$, 6.8 Hz, 1H), 7.42 (d, $J = 15.4$ Hz, 1H), 7.56 (d, $J = 7.8$ Hz, 1H), 7.83 (dd, $J = 1.8$, 7.7 Hz, 1H), 8.28 (t, $J = 5.5$ Hz, 1H), 8.60 (d, $J = 4.8$ Hz, 1H), 10.35 (br s, 1H). Anal. Calcd for $C_{14}H_{19}N_3O_3\cdots(COOH)_2\cdots0.5H_2O$: C 51.06, H 5.89, N 11.16. Found: C 50.95, H 5.76, N 11.34.

4.1.21. Preparation of ***25***

*4.1.21.1. Methyl 6-{[(E)-3-(3-pyridinyl)-2-propenoyl]amino}-hexanoate (**13j**).* Compound **13j** from (*E*)-3-(3-pyridinyl)-2-propenoic acid (**12j**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 52%. M.p. 75–77 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ:

1.01–1.78 (m, 6H), 2.25 (t, $J = 7.2$ Hz, 2H), 3.16 (q, $J = 6.0$ Hz, 2H), 3.57 (s, 3H), 6.75 (d, $J = 16.0$ Hz, 1H), 7.46 (d, $J = 16.0$ Hz, 1H), 7.46 (dd, $J = 4.8$, 8.0 Hz, 1H), 7.98 (dt, $J = 1.8$, 8.0 Hz, 1H), 8.14 (t, $J = 5.3$ Hz, 1H), 8.56 (dd, $J = 1.8$, 4.8 Hz, 1H), 8.76 (d, $J = 1.8$ Hz, 1H).

*4.1.21.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-(3-pyridinyl)-2-propenamide oxalate (**25**).* A solution of sodium methylate (6 mmol) in methanol (5 ml) was added to a solution of hydroxylamine hydrochloride (0.28 g, 4 mmol) in methanol (8 ml). The mixture was stirred for 10 min and NaCl was filtered off. Methyl 6-{[(*E*)-3-(3-pyridinyl)-2-propenoyl]amino}hexanoate (**13j**) (0.28 g, 1 mmol) was added to the filtrate and the resultant mixture was stirred for 4 h at ambient temperature. The solvent was removed under reduced pressure, the product was dissolved in ethanol (10 ml) and then oxalic acid (0.36 g, 4 mmol) was added to the solution. The precipitate was filtered and crystallized from water. (*E*)-*N*-[6-(hydroxyamino)-6-oxohexyl]-3-(3-pyridinyl)-2-propenamide oxalate (**25**) (0.22 g, 68%) was obtained as a white solid. M.p. 157–159 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.03–1.72 (m, 6H), 1.96 (t, $J = 7.2$ Hz, 2H), 3.18 (q, $J = 6.0$ Hz, 2H), 6.74 (d, $J = 15.8$ Hz, 1H), 7.44 (d, $J = 15.8$ Hz, 1H), 7.44 (dd, $J = 5.0$, 8.0 Hz, 1H), 7.99 (dt, $J = 1.8$, 8.0 Hz, 1H), 8.18 (t, $J = 5.2$ Hz, 1H), 8.56 (dd, $J = 1.6$, 5.0 Hz, 1H), 8.74 (d, $J = 2.0$ Hz, 1H), 10.34 (br s, 1H). Anal. Calcd for $C_{14}H_{19}N_3O_3\cdots0.5(COOH)_2\cdots2H_2O$: C 50.27, H 6.75, N 11.73. Found: C 50.28, H 6.71, N 11.60.

*4.1.22. Preparation of **26***

*4.1.22.1. Methyl 6-{[(E)-3-(2-naphthyl)-2-propenoyl]amino}-hexanoate (**13k**).* Compound **13k** from (*E*)-3-(2-naphthyl)-2-propenoic acid (**12k**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 94%. M.p. 74–76 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.10–1.78 (m, 6H), 2.25 (t, $J = 6.5$ Hz, 2H), 3.21 (q, $J = 5.6$ Hz, 2H), 3.58 (s, 3H), 6.78 (d, $J = 15.5$ Hz, 1H), 7.46–8.23 (m, 9H).

*4.1.22.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-(2-naphthyl)-2-propenamide (**26**).* Compound **26** from methyl 6-{[(*E*)-3-(2-naphthyl)-2-propenoyl]amino}hexanoate (**13k**) by a similar protocol to **16** was prepared, yield 74%. M.p. 161–163 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.07–1.74 (6H, m, CH_2), 1.81–2.14 (unresolved t, 2H), 3.03–3.41 (m, 2H), 6.74 (d, $J = 16.0$ Hz, 1H), 7.43–8.21 (m, 9H), 8.63 (br s, 1H), 10.32 (br s, 1H). Anal. Calcd for $C_{19}H_{22}N_2O_5\cdots H_2O$: C 66.26, H 7.02, N 8.13. Found: C 66.51, H 7.11, N 8.01.

*4.1.23. Preparation of **27***

*4.1.23.1. Methyl 6-{[(E)-3-(7-quinolinyl)-2-propenoyl]amino}-hexanoate (**13l**).* Compound **13l** from (*E*)-3-(7-quinolinyl)-2-propenoic acid [30] (**12l**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 61%. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.12–1.67 (m, 6H), 2.31 (t, $J = 7.3$ Hz, 2H), 3.19 (q, $J = 6.4$ Hz, 2H), 3.58 (s, 3H), 6.83 (d, $J = 15.8$ Hz, 1H), 7.54 (dd, $J = 4.2$, 8.3 Hz, 1H), 7.62 (d, $J = 15.8$ Hz, 1H), 7.81 (dd, $J = 1.4$, 8.6 Hz, 1H), 8.00 (d, $J = 8.6$ Hz, 1H), 8.15 (s, 1H), 8.19 (t, $J = 5.8$ Hz, 1H), 8.36 (dd, $J = 1.5$, 8.3 Hz, 1H), 8.92 (dd, $J = 1.5$, 4.2 Hz, 1H).

*4.1.23.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-(7-quinolinyl)-2-propenamide (**27**).* Compound **27** from methyl 6-{[(*E*)-3-(7-quinolinyl)-2-propenoyl]amino}hexanoate (**13l**) by a similar protocol to **16** was prepared, yield 58%. M.p. 163–165 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.19–1.38 (m, 2H), 1.38–1.61 (m, 4H), 1.95 (t, $J = 7.2$ Hz, 2H), 3.18 (q, $J = 6.6$ Hz, 2H), 6.83 (d, $J = 15.8$ Hz, 1H), 7.54 (dd, $J = 4.3$, 8.3 Hz, 1H), 7.62 (d, $J = 15.8$ Hz, 1H), 7.81 (dd, $J = 1.4$, 8.5 Hz, 1H), 8.01 (d, $J = 8.5$ Hz, 1H), 8.14 (s, 1H), 8.18 (t, $J = 5.8$ Hz, 1H), 8.36 (dd, $J = 1.6$, 8.3 Hz, 1H), 8.68 (br s, 1H), 8.92 (dd, $J = 1.6$, 4.3 Hz, 1H), 10.35 (br s, 1H). Anal. Calcd for $C_{18}H_{21}N_3O_3\cdot H_2O$: C 62.59, H 6.71, N 12.17. Found: C 62.44, H 6.69, N 11.83.

*4.1.24. Preparation of **28***

*4.1.24.1. Methyl 6-{[(E)-3-(8-quinolinyl)-2-propenoyl]amino}-hexanoate (**13m**).* Compound **13m** from (*E*)-3-(8-quinolinyl)-2-propenoic acid [31] (**12m**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 46%. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.10–1.68 (m, 6H), 2.24 (t, $J = 7.1$ Hz, 2H), 2.97 (q, $J = 6.0$ Hz, 2H), 3.61 (s, 3H), 6.97 (d, $J = 15.8$ Hz, 1H), 7.53–7.78 (m, 2H), 7.95–8.13 (m, 2H), 8.26 (t, $J = 5.2$ Hz, 1H), 8.44 (dd, $J = 1.6$, 8.0 Hz, 1H), 8.60 (d, $J = 15.8$ Hz, 1H), 9.00–9.17 (m, 1H).

*4.1.24.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-3-(8-quinolinyl)-2-propenamide (**28**).* Compound **28** from methyl 6-{[(*E*)-3-(8-quinolinyl)-2-propenoyl]amino}hexanoate (**12m**) by a similar protocol to **16** was prepared, yield 52%. M.p. 120–122 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.19–1.39 (m, 2H), 1.39–1.66 (m, 4H), 1.97 (t, $J = 7.2$ Hz, 2H), 3.20 (q, $J = 6.6$ Hz, 2H), 6.93 (d, $J = 16.0$ Hz, 1H), 7.62 (dd, $J = 4.2$, 8.3 Hz, 1H), 7.67 (t, $J = 8.0$ Hz, 1H), 8.02 (d, $J \sim 8.0$ Hz, 1H), 8.05 (d, $J \sim 8.0$ Hz, 1H), 8.21 (t, $J = 5.6$ Hz, 1H), 8.42 (dd, $J = 1.6$, 8.3 Hz, 1H), 8.63 (d, $J = 16.0$ Hz, 1H), 8.70 (br s, 1H), 9.00 (dd, $J = 1.6$, 4.2 Hz, 1H), 10.37 (br s, 1H). Anal. Calcd for $C_{18}H_{21}N_3O_3\cdots H_2O$: C 62.59, H 6.71, N 12.17. Found: C 62.36, H 6.52, N 11.97.

*4.1.25. Preparation of **29***

*4.1.25.1. Methyl 6-[(6-quinolinylcarbonyl)amino]hexanoate (**13n**).* Compound **13n** from quinoline-6-carboxylic acid (**12n**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 48%. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.15–1.70 (m, 6H), 2.21 (t, $J = 7.1$ Hz, 2H), 2.88

(q, $J = 6.0$ Hz, 2H), 3.64 (s, 2H), 7.65 (dd, $J = 4.0$, 8.0 Hz, C_9HN), 8.10 (d, $J = 8.7$ Hz, 1H), 8.10–8.21 (m, 1H), 8.42–8.61 (m, 2H), 8.51 (t, $J = 5.1$ Hz, 1H), 9.11 (dd, $J = 1.6$, 4.0 Hz, 1H).

*4.1.25.2. N-[6-(Hydroxyamino)-6-oxohexyl]-6-quinolinecarboxamide (**29**).* Compound **29** from methyl 6-[(6-quinolinylcarbonyl)amino]hexanoate (**13n**) by a similar protocol to **16** was prepared, yield 52%. M.p. 180–182 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.21–1.44 (m, 2H), 1.44–1.71 (m, 4H), 1.97 (t, $J = 7.3$ Hz, 2H), 3.31 (q, $J = 6.4$ Hz, 2H), 7.61 (dd, $J = 4.2$, 8.3 Hz, 1H), 8.08 (d, $J = 8.8$ Hz, 1H), 8.17 (dd, $J = 1.8$, 8.8 Hz, 1H), 8.47 (dd, $J = 1.6$, 8.3 Hz, 1H), 8.49 (d, $J = 1.8$ Hz, 1H), 8.70 (br s, 1H), 8.71 (t, $J = 5.6$ Hz, 1H), 8.98 (dd, $J = 1.6$, 4.2 Hz, 1H), 10.36 (br s, 1H). Anal. Calcd for $C_{16}H_{19}N_3O_3\cdots H_2O$: C 60.18, H 6.63, N 13.16. Found: C 59.84, H 6.50, N 13.01.

*4.1.26. Preparation of **30***

*4.1.26.1. Methyl 6-[(3,3-diphenylacryloyl)amino]hexanoate (**13o**).* Compound **13o** from 3,3-diphenyl-acrylic acid (**12o**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 82%, as an oil. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 0.91–1.70 (m, 6H), 2.30 (t, $J = 6.9$ Hz, 2H), 3.01 (q, $J = 5.6$ Hz, 2H), 3.59 (s, 3H), 6.45 (s, 1H), 7.03–7.50 (m, 10H), 7.80 (t, $J = 5.0$ Hz, 1H).

*4.1.26.2. N-[6-(Hydroxyamino)-6-oxohexyl]-3,3-diphenylacrylamide (**30**).* Compound **30** from methyl 6-[(3,3-diphenylacryloyl)amino]hexanoate (**13o**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **16** was obtained, yield 63%. M.p. 123–125 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 0.90–1.63 (m, 6H), 2.01 (t, $J = 7.0$ Hz, 2H), 2.97 (q, $J = 5.5$ Hz, 2H), 6.43 (s, 1H), 7.01–7.47 (m, 10H), 7.78 (t, $J = 5.0$ Hz, 1H), 8.63 (br s, 1H), 10.32 (br s, 1H). Anal. Calcd for $C_{21}H_{24}N_2O_3$: C 71.57, H 6.86, N 7.95. Found: C 71.56, H 6.87, N 7.98.

*4.1.27. Preparation of **31***

*4.1.27.1. Methyl 6-{[2-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yliden)acetyl]amino}hexanoate (**13p**).* Compound **13p** from 2-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yliden)acetic acid (**12p**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 75%. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 0.89–1.65 (m, 6H), 2.21 (t, $J = 7.1$ Hz, 2H), 2.99 (q, $J = 6.0$ Hz, CH_2), 3.05 (s, 4H), 3.59 (s, 3H), 6.18 (s, 1H), 6.89–7.34 (m, 8H), 7.34 (t, $J = 5.2$ Hz, 1H).

*4.1.27.2. 6-{[2-(10,11-Dihydro-5H-dibenzo[a,d]cyclohepten-5-yliden)acetyl]amino}-N-hydroxyhexanamide (**31**).* Compound **31** from methyl 6-{[2-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yliden)acetyl]amino}hexanoate (**13p**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **16** was obtained, yield 64%. M.p. 141–143 °C. (DMSO-d_6, HMDSO) δ: 1.00–1.19 (m, 2H), 1.19–1.33 (m, 2H), 1.42 (qui, $J = 7.0$ Hz, 2H), 1.90 (t, $J = 7.4$ Hz, 2H), 2.96 (q, $J = 6.0$ Hz, 2H), 3.32–3.37 (m, 4H), 6.21 (s, 1H), 7.05–7.15 (m, 3H), 7.15–7.25 (m, 4H), 7.15–7.33 (m, 1H), 7.80 (t, $J = 5.6$ Hz, 1H), 8.66 (br s, 1H), 10.33 (br s, 1H). Anal. Calcd for $C_{23}H_{26}N_2O_3$: C 72.99, H 6.92, N 7.40. Found: C 72.71, H 6.93, N 7.46.

*4.1.28. Preparation of **32***

*4.1.28.1. Methyl 6-{[2-(9H-fluoren-9-yliden)acetyl]amino}hexanoate (**13r**).* Compound **13r** from fluoren-9-ylidene-acetic acid (**12r**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **13a** was obtained, yield 73%. M.p. 54–56 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.05–1.76 (m, 6H), 2.28 (t, $J = 7.3$ Hz, 2H), 3.27 (q, $J = 6.1$ Hz, 2H), 3.59 (s, 3H), 7.11 (s, 1H), 7.22–7.59 (m, 4H), 7.64–7.95 (m, 3H), 8.51 (t, $J = 5.2$ Hz, 1H), 8.62–8.84 (m, 1H).

*4.1.28.2. 6-{[2-(9H-Fluoren-9-yliden)acetyl]amino}-N-hydroxyhexanamide (**32**).* Compound **32** from methyl 6-{[2-(9*H*-fluoren-9-yliden)acetyl]amino}hexanoate (**13r**) and methyl 6-aminohexanoate hydrochloride by a similar protocol to **16** was obtained, yield 84%. M.p. 174–176 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.20–1.41 (m, 2H), 1.41–1.63 (m, 4H), 1.96 (t, $J = 7.2$ Hz, 2H), 3.24 (q, $J = 6.1$ Hz, 2H), 7.09 (s, 1H), 7.25–7.49 (m, 4H), 7.73–7.88 (m, 3H), 8.53 (t, $J = 5.5$ Hz, 1H), 8.65–8.74 (m, 1H), 8.68 (s, 1H), 10.36 (s, 1H). Anal. Calcd for $C_{21}H_{22}N_2O_3$: C 71.98, H 6.33, N 7.99. Found: C 71.91, H 6.37, N 8.03.

*4.1.29. General synthesis of amidomethyl esters **13s–u** from carboxylic acids **12s–u** (method C)*

A solution of carboxylic acids **12s–u** (2.75 mmol) in dry dimethylformamide (3 ml) under argon atmosphere was cooled in ice bath and 1,1′-carbonyldiimidazole (490 mg, 3.01 mmol) was added. The mixture was stirred for 30 min and then triethylamine (1.0 ml, 7.2 mmol) followed by a solution of methyl 6-aminohexanoate hydrochloride (2.75 mmol) in dry dimethylformamide (3 ml) were added. The reaction mixture was stirred at ice bath temperature for 1 h and 20 h at room temperature, diluted with 50 ml of brine and extracted with ethyl acetate (3 × 25 ml). The organic phase was washed with brine, 5% $NaHCO_3$, brine, saturated KH_2PO_4, and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified on silica gel (20 g) with chloroform/ethyl acetate as eluent affording the corresponding amidoester derivatives **13s–u**.

*4.1.30. Preparation of **33***

*4.1.30.1. Methyl 6-[(5-phenylpentanoyl)amino]hexanoate (**13s**).* Compound **13s** from 5-phenylpentanoic acid (**12s**) and methyl 6-aminohexanoate hydrochloride by the method C was obtained, yield 35%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.22–1.81 (m, 10H), 2.02–2.41 (m, 4H), 2.49–2.75

(m, 2H), 3.22 (q, $J = 6.0$ Hz, 2H), 3.65 (s, 3H), 5.52 (br s, 1H), 7.05–7.38 (m, 5H).

*4.1.30.2. N-Hydroxy-6-[(5-phenylpentanoyl)amino]hexanamide (**33**).* Compound **33** was obtained from methyl 6-[(5-phenylpentanoyl)amino]hexanoate (**13s**) by the method B, yield 52%. M.p. 97–98 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.09–1.61 (m, 10H), 1.91 (t, $J = 7.3$ Hz, 2H), 2.06 (t, $J = 6.7$ Hz, 2H), 2.56 (t, $J = 7.2$ Hz, 2H, overlapped with a signal of DMSO), 2.99 (q, $J = 6.3$ Hz, 2H), 7.11–7.34 (m, 5H), 7.75 (t, $J = 5.4$ Hz, 1H), 8.67 (s, 1H), 10.33 (s, 1H). Anal. Calcd for $C_{17}H_{26}N$: C 66.64, H 8.55, N 9.14. Found: C 66.63, H 8.65, N 9.14.

*4.1.31. Preparation of **34***

*4.1.31.1. Methyl 6-[(E)-5-phenyl-4-pentenoyl]aminohexanoate (**13t**).* Compound **13t** from 5-phenyl-pent-4*E*-enoic acid [32] (**12t**) and methyl 6-aminohexanoate hydrochloride by the method C was obtained, yield 82%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.10–1.78 (m, 6H), 2.07–2.69 (m, 6H), 3.25 (q, $J = 6.0$ Hz, 2H), 3.65 (s, 3H), 5.53 (br s, 1H), 6.20 (dt, $J = 6.0$, 16.0 Hz, 1H), 6.49 (d, $J = 16.0$ Hz, 1H), 7.07–7.45 (m, 5H).

*4.1.31.2. (E)-N-[6-(Hydroxyamino)-6-oxohexyl]-5-phenyl-4-pentenamide (**34**).* Compound **34** was obtained from methyl 6-[(*E*)-5-phenyl-4-pentenoyl]aminohexanoate (**13t**) by the method B, yield 10%. M.p. 131–133 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.12–1.54 (m, 8H), 1.90 (t, $J = 6.8$ Hz, 2H), 2.10–2.40 (m, 2H), 3.00 (q, $J = 6.0$ Hz, 2H), 6.25–6.50 (m, 2H), 7.18–7.42 (m, 5H), 7.81 (t, $J = 5.2$ Hz, 1H), 8.65 (s, 1H), 10.32 (s, 1H). Anal. Calcd for $C_{17}H_{24}N$: C 67.08, H 7.95, N 9.20. Found: C 66.67, H 7.94, N 9.17.

*4.1.32. Preparation of **35***

*4.1.32.1. Methyl 6-[(3-phenyl-2-propynoyl)amino]hexanoate (**13u**).* Compound **13u** was obtained from phenylpropynoic acid (**12u**) and methyl 6-aminohexanoate hydrochloride by the method C, yield 89%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.25–1.92 (m, 6H), 2.34 (t, $J = 7.0$ Hz, 2H), 3.34 (q, $J = 6.0$ Hz, 2H), 3.65 (s, 3H), 7.27–7.63 (m, 5H).

*4.1.32.2. N-[6-(Hydroxyamino)-6-oxohexyl]-3-phenyl-2-propynamide (**35**).* Compound **35** was obtained from methyl 6-[(3-phenyl-2-propynoyl)amino]hexanoate (**13u**) by the method B, yield 70%. M.p. 112–113 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.15–1.55 (m, 6H), 1.94 (t, $J = 7.2$ Hz, 2H), 3.10 (q, $J = 6.2$ Hz, 2H), 7.39–7.61 (m, 5H), 8.66 (s, 1H), 8.76 (t, $J = 5.4$ Hz, 1H), 10.33 (s, 1H). Anal. Calcd for $C_{15}H_{18}N_2O_3$: C 65.68, H 6.61, N 10.21. Found: C 65.49, H 6.61, N 10.24.

*4.1.33. General synthesis of amidomethyl esters **13v**,**w** from carboxylic acids **12v**,**w** (method D)*

To a solution of appropriate carboxylic acid **12v** or **12w** (2.0 mmol) in anhydrous tetrahydrofuran (5 ml) under argon atmosphere triethylamine (0.36 ml, 2.6 mmol) was added and the mixture was cooled in an ice bath. Then to the reaction mixture *iso*-butylchloroformate (0.3 ml, 2.3 mmol) was added and the resulting mixture was stirred for 20 min at ice bath temperature. At the same time, a suspension of methyl 6-aminohexanoate hydrochloride (364 mg, 2 mmol), anhydrous tetrahydrofuran (3 ml) and triethylamine (0.31 ml, 2.2 mmol) was prepared and stirred for 20 min at room temperature. This suspension to the above prepared reaction mixture of activated ester was added. The resulting mixture was stirred at ice bath temperature for 15 min and 1 h at room temperature, then diluted with brine (50 ml) and extracted with ethyl acetate (3×25 ml). The organic phase was washed with brine, 5% $NaHCO_3$, brine, saturated KH_2PO_4 and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified on silica gel (20 g) with chloroform/ethyl acetate as eluent affording title product **13v** or **13w**, accordingly.

*4.1.34. Preparation of **36***

*4.1.34.1. Methyl 6-({2-[4-(dimethylamino)phenyl]acetyl}amino)hexanoate (**13v**).* Compound **13v** from (4-dimethylaminophenyl)-acetic acid (**12v**) and methyl 6-aminohexanoate hydrochloride by the method D was prepared, yield 73%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.16–1.92 (m, 6H), 2.34 (t, $J = 7.0$ Hz, 2H), 3.03 (s, 6H), 3.22 (s, 2H), 3.43 (q, $J = 6.0$ Hz, 2H), 3.65 (s, 3H), 6.00 (br s, 1H), 6.69 (d, $J = 9.0$ Hz, 2H), 7.79 (d, $J = 9.0$ Hz, 2H).

*4.1.34.2. 6-({2-[4-(Dimethylamino)phenyl]acetyl}amino)-N-hydroxyhexanamide (**36**).* Compound **36** was obtained from methyl 6-({2-[4-(dimethylamino)phenyl]acetyl}amino)hexanoate (**13v**) by the method B, yield 39%. M.p. 124–126 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.11–1.56 (m, 6H), 1.91 (t, $J = 7.4$ Hz, 2H), 2.84 (s, 6H), 2.98 (q, $J = 6.4$ Hz, 2H), 3.22 (s, 2H), 6.64 (d, $J = 8.6$ Hz, 2H), 7.04 (d, $J = 8.6$ Hz, 2H), 7.85 (t, $J = 5.4$ Hz, 1H), 8.66 (s, 1H), 10.33 (s, 1H). Anal. Calcd for $C_{16}H_{25}N_3O_3$: C 62.52, H 8.20, N 13.67. Found: C 62.32, H 8.21, N 13.68.

*4.1.35. Preparation of **37***

*4.1.35.1. Methyl 6-{[(E)-3-(4-chloro-2-fluorophenyl)-2-propenoyl]amino}hexanoate (**13w**).* Compound **13w** from 3-(4-chloro-2-fluorophenyl)acrylic acid (**12w**) and methyl 6-aminohexanoate hydrochloride by the method D was prepared, yield 76%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.16–1.85 (m, 6H), 2.32 (t, $J = 7.0$ Hz, 2H), 3.40 (q, $J = 6.0$ Hz, 2H), 3.65 (s, 3H), 5.80 (br s, 1H), 6.52 (d, $J = 16.0$ Hz, 1H), 7.00–7.54 (m, 3H), 7.56 (d, $J = 7.0$ Hz, 1H).

*4.1.35.2. (E)-3-(4-Chloro-2-fluorophenyl)-N-[6-(hydroxyamino)-6-oxohexyl]-2-propenamide (**37**).* Compound **37** was obtained from methyl 6-{[(*E*)-3-(4-chloro-2-fluorophenyl)-2-propenoyl]amino}hexanoate (**13w**) by the method B, yield 27%. M.p. 155–157 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ:

1.16–1.60 (m, 6H), 1.94 (t, $J = 7.3$ Hz, 2H), 3.15 (q, $J = 6.2$ Hz, 2H), 6.72 (d, $J = 16.0$ Hz, 1H), 7.35 (dd, $J = 2.2$, 8.4 Hz, 1H), 7.42 (d, $J = 16.0$ Hz, 1H), 7.53 (dd, $J = 2.2$, 11.0 Hz, 1H), 7.68 (t, $J = 8.4$ Hz, 1H), 8.23 (t, $J = 5.4$ Hz, 1H), 8.67 (d, $J = 1.8$ Hz, 1H), 10.34 (s, 1H). Anal. Calcd for $C_{15}H_{18}ClFN_2O_3$: C 54.80, H 5.52, N 8.52. Found: C 54.60, H 5.55, N 8.60.

4.1.36. Preparation of **38**

*4.1.36.1. Methyl 6-{[(E)-3-(4-chloro-2-fluorophenyl)-2-propenoyl]amino}hexanoate (**13x**).* Compound **13x** from 2-[1,1′-biphenyl]-4-ylacetic acid (**12x**) and methyl 6-aminohexanoate hydrochloride by the method C was prepared, yield 79% (white solid). ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.09–1.89 (m, 6H), 2.27(t, $J = 7.0$ Hz, 2H), 3.20 (q, $J = 6.0$ Hz, 2H), 3.58 (s, 2H), 3.65 (s, 3H), 5.43 (br s, 1H), 7.18–7.74 (m, 9H).

*4.1.36.2. 6-[(2-[1,1′-Biphenyl]-4-ylacetyl)amino]hexanoic acid (**14**).* Compound **14** was obtained from methyl 6-{[(*E*)-3-(4-chloro-2-fluorophenyl)-2-propenoyl]amino}hexanoate (**13x**) by a similar protocol to **4a**, yield 87%. M.p. 148–150 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.09–1.69 (m, 6H), 2.32 (t, $J = 7.0$ Hz, 2H), 3.20 (q, $J = 6.0$ Hz, 2H), 3.61 (s, 2H), 5.43 (br s, 1H), 7.29–7.69 (m, 9H).

*4.1.36.3. N-(Benzyloxy)-6-[(2-[1,1′-biphenyl]-4-ylacetyl)amino]hexanamide (**15**).* A solution of 6-[(2-[1,1′-biphenyl]-4-ylacetyl)amino]hexanoic acid (**14**) (0.619 g, 1.9 mmol) in anhydrous dimethylformamide (7.5 ml) under argon atmosphere was cooled in an ice bath and carbonyldiimidazole (0.339 g, 2.09 mmol) was added. The resulting mixture was stirred for 30 min at ice bath temperature and then triethylamine (1.2 ml, 8.6 mmol) followed by a solution of *O*-benzylhydroxylamine hydrochloride (0.456 g, 2.86 mmol) in anhydrous dimethylformamide (7.5 ml) were added. The mixture was stirred at ice bath temperature for 1 h and for 20 h at room temperature. The reaction mixture was diluted with brine, the precipitate was filtered and chromatographed on silica gel (20 g) with chloroform/methanol (9:1) as eluent to give the title compound **15** (0.550 g, 67%). M.p. 150–151 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.07–1.69 (m, 6H), 1.85–2.23 (m, 2H), 3.18 (q, $J = 6.0$ Hz, 2H), 3.58 (s, 2H), 4.87 (s, 2H), 5.56 (br s, 1H), 7.29–7.65 (m, 14H).

*4.1.36.4. 6-[(2-[1,1′-Biphenyl]-4-ylacetyl)amino]-N-hydroxyhexanamide (**38**).* *N*-(Benzyloxy)-6-[(2-[1,1′-biphenyl]-4-ylacetyl)amino]hexanamide (**15**) (0.550 g, 1.28 mmol) was dissolved in chloroform/methanol mixture (1:1, 20 ml) and 250 mg of 5% Pd/C catalyst was added. The suspension was vigorously stirred under hydrogen atmosphere until the starting material disappeared (*ca.* 5 h). The resultant reaction mixture was filtered through a small amount of silica gel, the sorbent was washed with chloroform, and the combined filtrates were evaporated in vacuum to give the title compound **38** as a white solid (0.320 g, 73%). The purity of the material was enhanced by crystallization from acetonitrile. M.p. 226–227 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 11.25–1.48 (m, 6H), 1.92 (t, $J = 7.2$ Hz, 2H), 3.35 (q, $J = 6.0$ Hz, 2H), 3.42 (s, 2H), 7.26–7.54 (m, 5H), 7.56–7.69 (m, 4H), 8.04 (t, $J = 6.0$ Hz, 1H), 8.65 (s, 1H), 10.33 (s, 1H). Anal. Calcd for $C_{20}H_{24}N_2O_3\cdots0.35H_2O$: C 69.28, H 7.18, N 8.08. Found: C 69.33, H 7.24, N 8.15.

4.1.37. General synthesis of amidoesters **40a** and **40b** (method E)

A solution of 8-methoxy-8-oxooctanoic acid (2.75 mmol) in anhydrous dimethylformamide (3 ml) under argon atmosphere was cooled in an ice bath and to the solution carbonyldiimidazole (490 mg, 3.01 mmol) was added. The mixture was stirred for 30 min at ice bath temperature and then a solution of appropriate amine **39a** or **39b** (2.75 mmol) in dimethylformamide (3 ml) was added (1.0 ml triethylamine additionally was added if amine **39a** or **39b** in a salt form was utilized). The reaction mixture was stirred for 1 h at ice bath temperature and 20 h at room temperature. Then the mixture was supplemented with brine (50 ml) and extracted with ethyl acetate (3×25 ml). The organic phase was washed with brine, 5% $NaHCO_3$, brine, saturated KH_2PO_4, and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified on silica gel (20 g) with chloroform/ethyl acetate as eluent affording the corresponding reaction product **40a** or **40b**, accordingly.

4.1.38. Preparation of **41**

*4.1.38.1. Methyl 8-(benzylamino)-8-oxooctanoate (**40a**).* Compound **40a** was obtained from 8-methoxy-8-oxooctanoic acid and benzylamine by the method E, yield 80%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.14–1.83 (m, 8H), 2.21 (t, $J = 7.0$ Hz, 2H), 2.27 (t, $J = 7.0$ Hz, 2H), 3.63 (s, 3H), 4.43 (d, $J = 6.0$ Hz, 2H), 5.69 (br s, 1H), 7.29 (s, 5H).

*4.1.38.2. N^1-Benzyl-N^8-hydroxyoctanediamide (**41**).* Compound **41** was obtained from methyl 8-(benzylamino)-8-oxooctanoate (**40a**) by the method B, yield 60%. M.p. 126–126.5 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.16–1.32 (m, 4H), 1.36–1.60 (m, 4H), 1.92 (t, $J = 7.2$ Hz, 2H), 2.12 (t, $J = 7.4$ Hz, 2H), 4.24 (d, $J = 5.6$ Hz, 2H), 7.16–7.36 (m, 5H), 8.30 (t, $J = 5.6$ Hz, 1H), 8.66 (s, 1H), 10.33 (s, 1H). Anal. Calcd for $C_{15}H_{22}N_2O_3\cdots0.5H_2O$: C 62.70, H 8.07, N 9.75. Found: C 62.84, H 7.83, N 9.73.

4.1.39. Preparation of **42**

*4.1.39.1. Methyl 8-oxo-8-(phenethylamino)octanoate (**40b**).* Compound **40b** was obtained from 8-methoxy-8-oxooctanoic acid and phenethylamine (**39b**) by the method E, yield 63%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.05–1.81 (m, 8H), 2.09 (t, $J = 7.0$ Hz, 2H), 2.27 (t, $J = 7.0$ Hz, 2H), 2.78 (t, $J = 7.0$ Hz,

2H), 3.52 (q, $J = 6.0$ Hz, 2H), 3.65 (s, 3H), 5.56 (br s, 1H), 7.00–7.43 (m, 5H).

*4.1.39.2. N^1-Hydroxy-N^8-phenethyloctanediamide (**42**).* Compound **42** was obtained from methyl 8-oxo-8-(phenethylamino)octanoate (**40b**) by the method B, yield 30%. M.p. 113–114 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ: 1.10–1.30 (m, 4H), 1.34–1.56 (m, 4H), 1.92 (t, $J = 7.2$ Hz, 2H), 2.01 (t, $J = 7.4$ Hz, 2H), 2.68 (t, $J = 7.6$ Hz, 2H), 3.25 (q, $J = 6.8$ Hz, 2H), 7.12–7.34 (m, 5H), 7.85 (t, $J = 5.6$ Hz, 1H), 8.66 (d, $J = 1.6$ Hz, 1H), 10.33 (s, 1H). Anal. Calcd for $C_{16}H_{24}N_2O_3\cdots0.17H_2O$: C 65.05, H 8.30, N 9.48. Found: C 65.04, H 8.25, N 9.44.

4.2. Computational chemistry

All molecular modeling operations were performed using the Schrödinger suite of software tools (Maestro, version70110) running on a HP8200 Linux work station.

4.2.1. Preparation of ligands for docking

The compounds dataset was imported into Maestro and prepared for docking runs using Ligprep module. This process consists of a series of steps for generating 3D structure from 2D (SD file) representation, searching for tautomers and steric isomers, and performing a geometry minimization of ligands.

4.2.2. Protein preparation and refinement

The X-ray crystal structure of bacterial HDAC–TSA complex (PDB_ID:1C3R) was obtained from the Protein Data Bank [33]. Bacterial HDAC–TSA complex is a dimer with duplicate binding sites. Thus, after manual inspection and cleaning of structure, we have retained the 'A' sub unit for modeling calculations. The protein was prepared for docking runs as follows: amino acid residues within a 20 Å radius of the co-crystallized ligand (TSA) were used to define the binding site. The zinc metal atom was assigned with correct atom type (87) and charge (+2) from metal ion. Maestro atom types and ligand (TSA) checked for correct atom types, formal charges, bond orders. Using the *pprep* command, amino acid residues that form salt bridges in the binding site of the protein (except the residues within 3.5 Å radius of ligand) were neutralized and hydrogen bonding conflicts corrected. After adding hydrogen atoms, the prepared ligand and protein entries were subjected to a series of restrained, partial minimizations using *imprep* command. The maximum rms deviation allowed during the minimization process was 0.3. Finally, the combined minimized structure was exported in .mae files for receptor grid calculations.

4.2.3. Docking and scoring

All molecular docking calculations were performed using Glide [34] with the prepared and refined protein structure. Generation of a receptor grid is the first step of the Glide docking process. The shape and properties of protein are represented by different sets of fields on a grid with complimentary steric and electrostatic interactions between putative small molecule inhibitor. A 15 Å grid was computed using the centroid of co-crystallized ligand to perform docking calculations. The following hydrogen bond and metal constraints selected from the protein structure were included in the grid calculation: A. 1036A: His132: NE2:NE2: Hydrogen bond acceptor constraint. B. 1026A: His131: NE2:NE2: Hydrogen bond acceptor constraint. C. 5200A: Tyr 297: HH:HH: Hydrogen bond donor constraint. D. 2790A: Zinc 501: ZN:ZN: Metal constraint. The receptor grid was then used to rank order the docked orientations. Compounds were docked using Glide extra-precision mode with hydrogen bond and metal constraints with up to five poses saved per molecule. The docked poses for each ligand were then analyzed for steric and electrostatic complimentarity with the receptor in order to select the best pose for further analysis.

4.3. In vitro assays

4.3.1. Histone deacetylase activity

Human cervix epithelial cancer HeLa cells (ATCC Ref. No. CCL-2) were cultured in DMEM containing 10% foetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Subconfluent cells were harvested by trypsinisation, and washed twice in ice cold PBS. Cells were then resuspended in two volumes of buffer (60 mM Tris–HCl, pH 7.4, 30% glycerol, 450 mM NaCl), and lysed by three freeze and thaw cycles (dry ice and 30 °C). Cell debris was removed by centrifugation at ~20,000g and supernatant aliquoted and stored at −80 °C.

The activity of the compounds as HDACi was determined using a commercially available fluorescent assay kit (Fluor de Lys™, BioMol Research Labs, Inc., Plymouth Meeting, USA). HeLa extract was incubated for 1 h at 37 °C in assay buffer (25 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, pH 8.0) with 15 μM acetylated substrate in the presence of test compound.

4.3.2. Cell proliferation

HeLa cells were seeded into 96-well plates at a density of 3000 cells/well in 100 μL of culture medium, allowed to settle overnight, and compounds added using increasing concentrations the following day. Plates were then incubated at 37 °C in 5% CO_2 for 48 h, 10 μL/well of WST-1 reagent added, followed by a further 1 h incubation at 37 °C. Absorbance was then measured using a wavelength of 450 nm (reference wavelength 690 nm). Percent activity (% activity) in reducing the number of viable cells was calculated for each test compound as % activity $= \{(SC - B)/(S^0 - B)\} \times 100$. SC denotes signal measured in the presence of the compound being tested, S^0 signal measured in the absence of the compound being tested and B the background signal measured in blank wells containing medium only. IC_{50} values were calculated using the non-linear regression (variable slope) curve fitting option within Prism 3.0 (GraphPad Software Inc., San Diego, CA).

4.3.3. Recombinant human histone deacetylase (rhHDAC) activity

Recombinant HDACs 1–9 were produced and assays performed as described in Ref. [35].

In brief, the deacetylase activity of rhHDACs 1–9 was assayed with appropriate HDAC substrates (Biomol International: HDACs 1–7 and 9 – KI-104; HDAC8 – KI-178) in Hepes buffer (25 mM Hepes, 137 mM NaCI, 2.7 mM KCI and 4.9 mM MgCI2, pH 8.0).

Plates were incubated at 37 °C for 3 h (HDAC8 assays were performed at room temperature) and the reaction quenched with HDAC-FDL Developer (Biomol International: HDACs 1–7 and 9 – KI-105; HDAC8 – KI-176) 20 × stock diluted to 1:400 in Hepes buffer and containing 2 μM TSA. Further incubation for 25 min at room temperature allowed a fluorescence signal to develop. Generated fluorescence was measured at wavelengths 355 nm (excitation) and 460 nm (emission).

4.4. In vivo P388 i.p./i.p. survival studies

The in vivo anti-tumor activity of compounds, **32**, **37**, **II** (SAHA) and **27** was investigated [36]. B6D2F1 female mice were obtained from Taconic M&B (Ry, Denmark) and kept in a light- and temperature-controlled environment with ad libitum access to water and standard laboratory diet. On day 0, mice weighing 19–21 g were inoculated with 10^6 P388 mouse leukemia cells intraperitoneally (i.p.). On days 3–7 mice were treated once daily i.p. with the different HDACi formulated as 8 and 24 mg/ml in 100% DMSO and dosed at 50 μl per mouse per dose i.p. corresponding to 20 and 60 mg/kg/day. For each treatment group, the percent increase in lifespan (%ILS) was calculated as $(T_{MS} - C_{MS})/(C_{MS}) \times 100$. T_{MS} is the median survival in the test group and C_{MS} is the median survival in the vehicle control group. The effect of treatment was compared with vehicle control treated mice using LogRank analyses with p-values of less than 0.05 considered as statistically significant.

Experiments were conducted according to the institutional and national guidelines for the care and use of laboratory animals, and approved by the Danish Experimental Animal Inspectorate, Department of Justice.

Acknowledgements

We kindly thank CuraGen Inc. for providing us with the recombinant HDAC isoforms used for selectivity determinations. We also thank Angela Finn and Ruth Hollinshead for excellent technical assistance.

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