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Chemistry, Biology, and QSAR Studies of Substituted Biaryl Hydroxamates and Mercaptoacetamides as HDAC Inhibitors—Nanomolar-Potency Inhibitors of Pancreatic Cancer Cell Growth

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The histone deacetylases (HDACs) are able to regulate gene expression, and inhibitors of the HDACs (HDACIs) hold promise in the treatment of cancer as well as a variety of neurodegenerative diseases. To investigate the potential for isoform selectivity in the inhibition of HDACs, we prepared a small series of 2,4′-diaminobiphenyl ligands functionalized at the para*-amino group with an appendage containing either a hydroxamate or a mercaptoacetamide group and coupled to an amino acid residue at the* ortho*-amino group. A smaller series of substituted phenylthiazoles was also explored. Some of these newly synthesized ligands show low-nanomolar potency in HDAC inhibition assays and display micromolar to low-nanomolar IC_{50} values in tests against five pancreatic cancer cell lines. The isoform selectivity of these ligands for class I HDACs (HDAC1–3 and 8) and class IIb HDACs (HDAC6 and 10) together with QSAR studies of their correlation with lipophilicity are presented. Of particular interest is the selectivity of the mercaptoacetamides for HDAC6.*

Introduction

The post-translational acetylation status of chromatin is determined by the competing activities of two enzyme classes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). In general, HATs function to acetylate the ε-amino group of conserved lysine residues within the N-terminal tails of histones, resulting in charge neutralization on the histones and a more open, transcriptionally active chromatin structure, whereas HDACs function to deacetylate and promote transcriptional repression. A shift in the balance of acetylation on chromatin may result in changes in the regulation of patterns of gene expression.[1–4] Because many cancers are associated with aberrant transcriptional activity, and the HDACs can affect transcription factors and gene regulation, these enzymes have been identified as attractive targets for cancer therapy. Indeed, chemical inhibitors of HDACs have been shown to inhibit tumor cell growth and induce differentiation and cell death.[5] Several such inhibitory agents, including suberoylanilide hydroxamic acid (SAHA, vorinostat) and depsipeptide (FR901228) have reached clinical trials,[6–8] and SAHA has been approved by the US Food and Drug Administration for use in cutaneous T-cell lymphoma (CTCL). HDAC inhibitors (HDACIs) also enhance the cytotoxic effects of both radiation and chemotherapeutic drugs.[9,10] Moreover, other studies support the possible use of HDAC inhibitors to treat neurodegenerative disorders such as Parkinson's and Huntington's diseases, wherein they lead to the expression of certain neuroprotective proteins. While a number of different structural classes HDACIs have now been identified, the majority of these have not been tested for their selectivity against the individual HDAC isoforms of which there are now 11 that operate through zinc-dependent mechanisms. These include both the class I HDACs 1, 2, 3, and 8, class II that includes 4, 5, 6, 7, 9, and 10, and class IV that contains HDAC11.[11] To learn more about the role that the individual HDACs play in cell growth and differentiation, neuronal protection, and apoptosis, it is important to develop agents that show selectivity for individual isoforms or a small subset of these isoforms. Although some rather limited degree of isoform selectivity has been shown by a few compounds,[12] the problem of identifying selective inhibitors is far from solved, and is rather complicated by the functional interactions between different HDAC isoforms together with the formation of co-repressor complexes with other proteins that could possibly alter their interaction with various small-molecule inhibitors.

The active site of class I, II, and IV HDACs is found within a highly conserved catalytic domain containing a divalent zinc cation that is coordinated to both histidine and aspartate residues. Deacetylation of the HDAC substrates occurs through attack by a water molecule that is activated through interaction with this zinc cation coupled with deprotonation through

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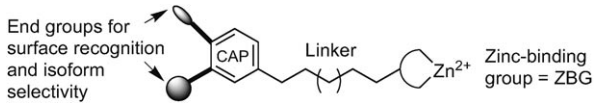
a histidine–aspartate charge-relay system. Based on what is currently known about the structure–activity relationships of various HDAC inhibitors together with co-crystal structural information of the bacterial HDAC homologue HDLP[13] as well as human HDAC7 (PDB: 2PQO and 2PQP) and HDAC8 (PDB: 1VKG, 1T67, 1T64, 1T69, and 2V5X)[14,15] in complex with certain known hydroxamate-based HDAC inhibitors, we have designed and synthesized certain hydroxamate-based HDACIs that present topologically unique end groups or CAPs. As the CAP region of the HDACIs is able to interact with the surface of the protein, in the region immediately outside of the catalytic gorge, it is able to serve as a recognition motif that may differentiate among the different HDAC isoforms. Similar research was exemplified by the identification of the putative HDAC6-selective tubacin from Schreiber's group by employing combinatorial synthesis and cell-based screening of a ~7000-compound library.[16] (However, recent work suggests that tubacin's selectivity for HDAC6 over HDAC1 may only be 4-fold in enzyme assays.[17]) On the other hand, our own work revealed that certain small-molecule HDACIs bearing a mercaptoacetamide group as the zinc-binding group (ZBG) preferably inhibit HDAC6 over other HDACs.[18] Certain other types of HDACIs



containing thiol[19] or benzamide-based ZBGs[20] have also been reported to show some level of isoform or class selectivity. However, strict head-to-head comparisons of the potency and selectivity of the non-hydroxamate-based HDACIs over those containing a hydroxamate group as the ZBG are relatively rare.

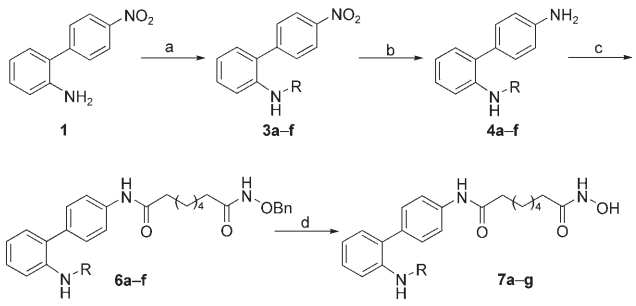
To pursue our plan of generating HDACIs containing topologically differentiated end groups, we designed ligands that are composed of a 2,4′-diaminobiphenyl scaffold in which the *para*-amino group bears an appendage that contains either a hydroxamate or mercaptoacetamide group that interacts with the catalytic zinc atom. The other amino group is used for the introduction of functionality that may discern among the various HDACs through a combination of steric or electronic effects. In particular, as presented in Schemes 1 and 2, we have prepared a series of diaminobiphenyls in which the *ortho*-amino group is coupled with one of the known amino acids. As all protein–protein interactions are governed by complementary amino acid interactions, we reasoned that this design strategy might best lead to reasonably active structures that show some degree of isoform selectivity. Five natural amino acids, namely glycine, phenylalanine, proline, tryptophan, and tyrosine were explored in these studies and were selected based on considerations of structural diversity.

For comparison, we also investigated the effect of replacing the biphenyl group with a phenylthiazole containing a substituent at either the 2- or 3-position of the phenyl ring. This structural modification was explored based on the realization that connectivity through the five-membered thiazole ring will situate the phenyl ring substituent closer to the HDAC protein surface. Moreover, we were inspired to investigate these particular analogues, as previously some related phenylthiazoles were shown to provide very potent antiproliferative agents.[21] As discussed below, in this series of compounds we found that certain bulky alkyl groups led to a substantial increase in inhibitory activity for HDAC6. We detail the procedures used to prepare these compounds, and then provide the biological results including cell-based assays together with the preliminary QSAR study for the synthetic ligands.

Results and Discussion

Chemical synthesis

Synthesis of the biphenyl hydroxamic acid series outlined in Scheme 1 started from 4′-nitro-2-biphenylamine (**1**) prepared



Scheme 1. Synthesis of ligands **7a–g**: a) $POCl_3$, pyridine, −15 °C, 1 h, **2** (**a** = Boc-Gly-OH, **b** = Boc-L-Phe-OH, **c** = Boc-L-Pro-OH, **d** = Boc-L-Trp-OH, **e** = Boc-L-Tyr(Bn)-OH, **f** = Boc_2O, **g** = Boc-D-Phe-OH); for **2f**: toluene 100 °C, overnight; b) H_2, $Pd(OH)_2$/C, room temperature, 4 h; for **3g**: $SnCl_2$, MeOH–dioxane, reflux, overnight; c) **5** (7-benzyloxycarbamoylheptanoic acid), PyBOP, DIPEA, DMF, room temperature, overnight; d) for **7a–f** (**a** R = Gly, **b** R = L-Phe, **c** R = L-Pro, **d** R = L-Trp, **e** R = L-Tyr, **f** R = H): 1) TFA, 2) H_2, $Pd(OH)_2$/C, 4 h; for **7g** (R = Boc): $Pd(OH)_2$/C, 4 h.

from commercially available 2-biphenylamine according to a known procedure.[22] The *ortho*-amino substituent was selected for functionalization with various amino acids, as this position would best allow possible surface interactions. The phenylalanine, tryptophan, and tyrosine-based hydroxamates, respectively **5b**, **5d**, and **5e**, were reported in our previous paper in comparison with certain mercaptoacetamides in neuroprotection assays carried out using cortical neurons from homocysteate (HCA)-induced apoptosis.[18] Thus, compound **1** was coupled with the protected amino acids **2a–e** using $POCl_3$ in dry pyridine[23] to give the corresponding amides **3a–e**. Concerning possible racemization of the amino acid in the peptide coupling step, we compared the optical purity of amide **3b** with its enantiomer **3g** prepared from Boc-D-Phe-OH by using chiral HPLC. In agreement with related work,[23] we found that no racemization had taken place in this amide-forming reaction using $POCl_3$. Reduction of the nitro group was carried out by $Pd(OH)_2$-catalyzed hydrogenation, or in the case of the tyrosine intermediate **3e**, by using tin(II) chloride to avoid undesirable *O*-benzyl group cleavage. The resulting biphenylamines **4a–e** were coupled with 7-benzyloxycarbamoylheptanoic acid

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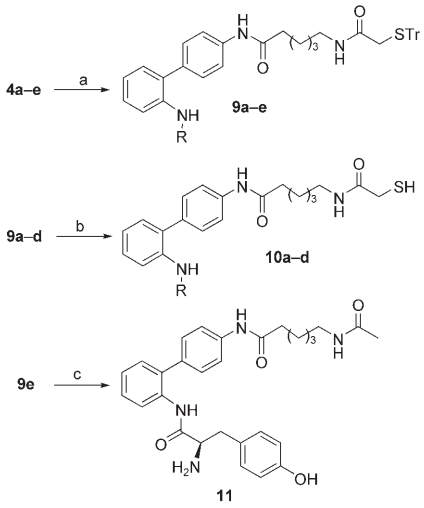
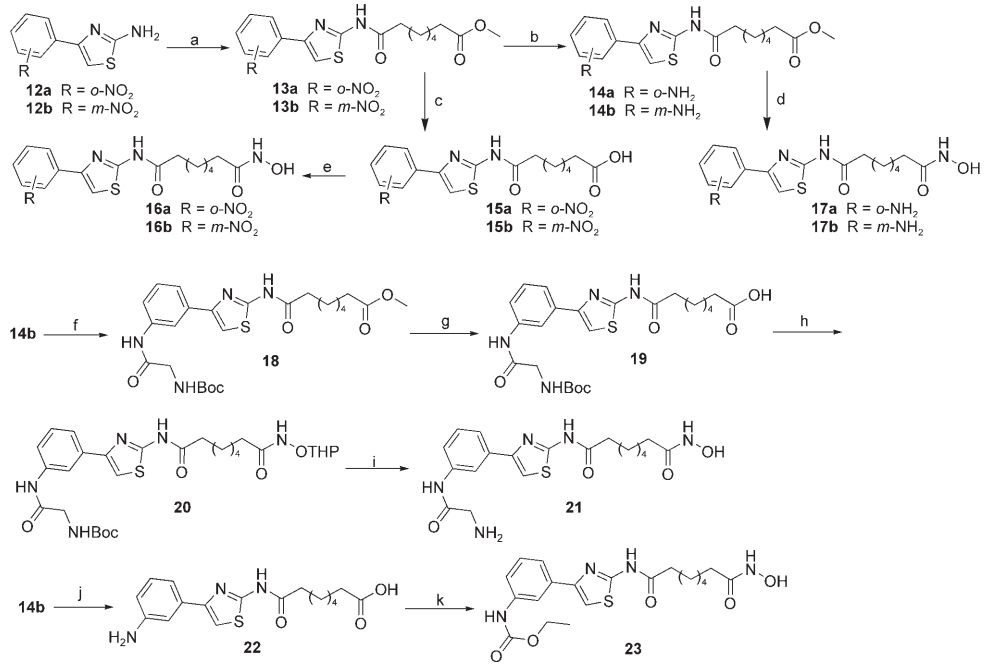
(**5**)[24] by PyBOP to afford the corresponding amides **6a**–**e**. Acid deprotection of the Boc group followed by hydrogenation led to the hydroxamates **7a**–**e**. The compounds containing an unsubstituted NH_2 group **7f** and the Boc-protected amine group **7g** were also prepared to permit appropriate comparisons with their amino acid substituted counterparts **7a**–**e**. Additionally, the analogue without *ortho* substitution **7h** was prepared to better gauge the contribution this substituent makes to HDAC inhibitory activity.[25]

The amino acid based biphenyl-bearing mercaptoacetamides were prepared as shown in Scheme 2. The biphenylamines **4a**–**e** were coupled with 7-(2-tritylsulfanylacetylamino)-heptanoic acid (**8**)[26] by PyBOP, followed by one-pot deprotection of both the trityl and Boc groups of **9a**–**d** with TFA/triethylsilane to afford the mercaptoacetamides **10a**–**d**. Removal of the benzyl group in the tyrosine intermediate **9e** by catalytic hydrogenation was sluggish, and extended reaction times led to cleavage of the thiol group to afford **11**.



Scheme 2. Synthesis of ligands **10a**–**d** and **11**: a) **8** (6-(2-tritylsulfanylacetylamino)hexanoic acid), PyBOP, DIPEA, DMF, room temperature, overnight; b) TFA, Et_3SiH, 0 °C, 2 h; c) 1) TFA, Et_3SiH, 0 °C, 2 h, 2) H_2, $Pd(OH)_2$/C, room temperature, 10 h.

The preparation of the *ortho*- and *meta*-amino-substituted phenylthiazole-based HDACIs is outlined in Scheme 3 starting from commercially available 4-(2-nitrophenyl)thiazol-2-ylamine (**12a**) and 4-(3-nitrophenyl)thiazol-2-ylamine (**12b**). This chemistry proceeds in a relatively straightforward fashion, as most phenylthiazole-based intermediates are solid and easily purified by washing with appropriate solvents. Compound **16b** was chosen previously as one example of a hydroxamate to compare with the mercaptoacetamides described in our earlier neuroprotection studies.[17] In the present isoform selectivity study, we also prepared the non-substituted phenylthiazole **24** for use as control, employing a similar synthetic protocol. The simple phenylthiazole ligand and some of its substituted counterparts were first reported by researchers at Abbott in 2004, and such compounds appear to act as relatively potent pan-HDAC inhibitors.[12] The synthesis of the glycine-bearing phenylthiazole analogue is also outlined in Scheme 3. Thus, **14b** was coupled with Boc-Gly-OH using EEDQ, and then the ester **18** was hydrolyzed by LiOH to afford acid **19**. In this synthetic route, we chose the THP-protected hydroxylamine as the precursor to the hydroxamate, and in the final step, both the THP and Boc groups were removed by treatment with TFA



Scheme 3. Synthesis of ligands **16a**, **16b**, **17a**, **17b**, **21**, and **23**: a) $POCl_3$, pyridine, suberic acid monomethyl ester, −15 °C, 1 h; b) H_2, Pd/C, EtOH, AcOH, 50 °C, 2 h; c) LiOH; d) NH_2OH, KOH, MeOH, room temperature, 1 h; e) 1) isobutyl chloroformate, Et_3N, 0 °, 2) NH_2OH; f) EEDQ, Boc-Gly-OH; g) LiOH; h) $THPONH_2$, EDCI, HOBt, Et_3N, room temperature; i) TFA; j) LiOH; k) 1) ethyl chloroformate, Et_3N, 0 °C, 2) NH_2OH.

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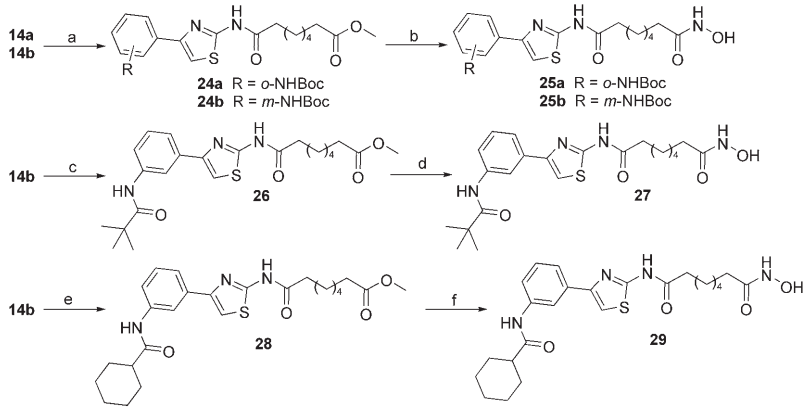
to afford **21**. In another attempt to prepare this hydroxamate using the mixed anhydride, we obtained the ethyl carbamate **23**.

Scheme 4 presents the synthesis of phenylthiazoles **25a**, **25b**, **27**, and **29** imbued with an amide or urethane residue on the benzene ring in linkage with a bulkier alkyl group.



Scheme 4. Synthesis of ligands **25a**, **25b**, **27**, and **29**: a) Boc_2O, THF, reflux, overnight; b) NH_2OH, KOH, MeOH, room temperature, 1 h; c) trimethylacetic anhydride, THF, reflux, overnight; d) NH_2OH, KOH, MeOH, room temperature, 1 h; e) cyclohexanecarbonyl chloride, THF, reflux, overnight; f) NH_2OH, KOH, MeOH, room temperature, 1 h.

HDAC isoform inhibition assays

The putative HDACIs described above were screened in vitro for both enzyme inhibitory activity and then for their ability to block cancer cell growth. The inhibitory effects of compounds on HDAC activity were determined by a fluorescence-based assay with electrophoretic separation of substrate and product carried out using a microfluidic system followed by quantitation of fluorescence intensity in the substrate and product peaks. The assays were performed with isolated HDAC isoforms that had been expressed as His_6-tagged fusion proteins in a baculovirus expression system in Sf9 cells. HDACs 1, 2, 3, 6, and 8 were expressed as full-length fusion proteins. The HDAC10 fusion protein was expressed as a carboxy-terminal deletion of 38 amino acids (residues 632–669). HDAC3 was co-expressed with a fragment of the SMRT gene (residues 395–489) to generate enzymatically active protein. The data are presented as IC_{50} values in Table 1 for the biphenyl HDACIs and in Table 2 for the phenylthiazole HDACIs. Trichostatin A (TSA) was used as a positive control. The recently published inhibitory data for SAHA against a panel of recombinant HDACs are also presented for comparison.[27] As apparent from Table 1, the unsubstituted biphenyl hydroxamate **7h** shows marginal selectivity for inhibition of HDACs 3 and 6 over HDACs 1, 2, and 10, with all of these being in the nanomolar range; for HDAC8, the IC_{50} value was 1.87 μM. Introduction of an *ortho*-amino or substituted *ortho*-amino group (NH_2, BocNH, glycine, or proline) as in **7g**, **7f**, **7a**, and **7c**, respectively, resulted in an approximate 2–4-fold decrease in inhibitory activity for the isoforms tested. The incorporation of the additional functional group thus appears not to play a role in the discrimination of the isoforms. In the case of the respective phenylalanine-, tryptophan-, and tyrosine-derived ligands **7b**, **7d**, and **7e**, their inhibitory activities against HDAC1 are almost same as that of **7h**; the only modest difference between these three ligands relative to **7h** is their decreased inhibitory activity toward HDAC2 (3–5-fold). In general, all five of the amino acid bearing biphenyl hydroxamates are relatively potent HDAC inhibitors, however, they fail to show any real isoform discrimination.

Data for four amino acid bearing biphenyl mercaptoacetamides **10a–d** are shown in Table 1. Compared with their corresponding hydroxamates, these mercaptoacetamides preferentially inhibit HDAC6 over HDACs 1, 2, and 10. The most selective mercaptoacetamide was the proline-containing derivative **10c**, with an IC_{50} value of 1.95 μM against HDAC1 and 0.2 μM against HDAC6. The inhibition pattern and activity range shown by these mercaptoacetamides against the different isoforms agree well with the data previously reported for such mercaptoacetamides from our research group.[18] Taken together, these data suggest that the mercaptoacetamide group represents a structurally unique type of ZBG that embodies some inherent selectivity for HDAC6.

The use of a phenylthiazole as the CAP group for HDAC inhibitors has previously been reported by Glaser et al.[28] using either an α-ketoamide or a hydroxamate as the ZBG. As is readily apparent from Table 2, the phenylthiazole HDACIs are more potent inhibitors than the biphenyl HDACIs listed in Table 1, with IC_{50} values for HDAC1 close to that of TSA. In comparison with the unsubstituted phenylthiazole **24**, the introduction of an amino group as in **17a** and **17b** or a glycineamide residue as in **21** caused little change in either activity or isoform selectivity. The nitro-containing phenylthiazoles **16a** and **16b** are also reasonably potent, although the *ortho*-nitro compound **16a** is almost 10-fold less potent than the corresponding amine analogue **17a**. The *meta*-substituted ethyl carbamate **23** was as potent as its amine analogue **17b** against HDAC1 and HDAC2, but it showed a 3-fold improvement in its HDAC6 inhibitory activity. On further changing the ethyl carbamate in **23** to a *tert*-butyl carbamate as in **25b**, a further increase in HDAC6 inhibitory activity was observed ($IC_{50} <$ 0.2 nM), with no change in inhibitory activity toward either HDAC1 or HDAC2. Also, in comparison with the unprotected *meta*-amino-bearing ligand **17b**, introduction of a Boc protecting group as in **25b** leads to a >15-fold enhancement in the inhibitory activity toward HDAC6 with little change in inhibitory potency toward HDAC1 and HDAC2. Interestingly, replacement of the *tert*-butyloxy group of **25b** by a cyclohexyl group as in **29** leads to subnanomolar potency against both HDAC2 and HDAC3 (IC_{50} values <0.2 nM, >200-fold increase against

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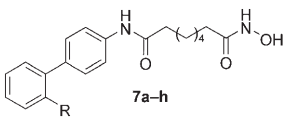
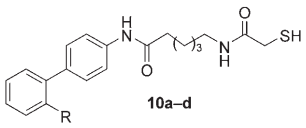
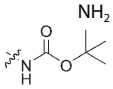
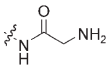
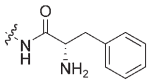
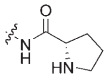
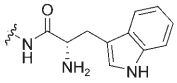
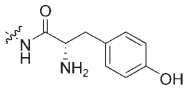
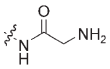
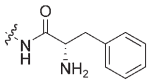
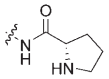
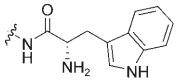
Table 1. HDAC inhibitory activity of the biphenyl-bearing hydroxamates, mercaptoacetamides, SAHA, and TSA.





Compd	R	IC_{50} [nM][a]					
		HDAC1	HDAC2	HDAC3	HDAC8	HDAC10	HDAC6
SAHA[b]	–	68	164	48	1524	NA[d]	90
TSA	–	4	14	2	1380	5	1
7h	H	33	46	7	1870	46	5
7g	NH_2	99	244	ND[c]	2500	139	16
7f		57	74	18	1720	83	11
7a		102	364	ND	3480	146	28
7b		41	156	ND	1600	46	8
7c		52	193	ND	2660	70	16
7d		27	167	ND	1720	28	5
7e		37	205	ND	2060	42	9
10a		3960	15980	ND	6030	7430	387
10b		2760	>30000	ND	5190	7220	452
10c		1950	12490	ND	4230	6070	205
10d		7090	>30000	4330	11250	>30000	656

[a] The isoform inhibition was tested at Amphora Discovery Corporation (http://www.amphoracorp.com/). [b] Taken from reference [27]. [c] Not determined. [d] Not available.

HDAC2 and >20-fold increase against HDAC3), while the IC_{50} value for HDAC6 was still below 0.2 nM. On the other hand, the *ortho*-substituted *tert*-butylcarbamate **25a** showed a 2-fold decrease in activity toward HDAC1 and HDAC2, with similar inhibitory potency against HDAC6 relative to the unprotected *ortho*-NH_2 ligand **17a**. Also, conversion of the Boc-protected ligand **25b** to the closely related pivaloyl derivative **27** results in a >10-fold decrease in HDAC6 inhibition and in only modest changes in the inhibition of the other isoforms, suggesting that the carbamate linkage and its extra oxygen atom can influence isoform(s) differentiation.

Antiproliferative activity

Pancreatic cancer is the fourth leading cause of cancer death in the United States, and remains an incurable disease with a five-year survival of rate of <5%. Increasing evidence indicates that signaling and transcriptional pathways are dysregulated in pancreatic cancer. Recently, SAHA was tested against six pancreatic cancer cell lines and was found to induce pancreatic cancer cell apoptosis, G_2 cell-cycle arrest, and differentiation. Also, the combination of SAHA and the DNA methylation inhibitor 5-aza-2′-deoxycytidine had an enhanced antiproliferative effect on pancreatic cancer cells.[29]

As a further measure of the activity of the present series of compounds, we examined their growth-inhibiting effects

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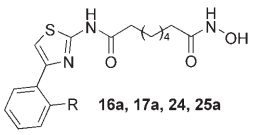
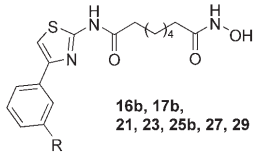
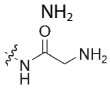
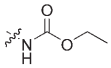
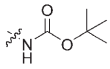
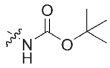
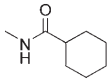
Table 2. HDAC inhibitory activity of the phenylthiazole-bearing hydroxamates.





Compd	R	IC_{50} [nM][a]					
		HDAC1	HDAC2	HDAC3	HDAC8	HDAC10	HDAC6
24	H	3	35	ND[b]	1900	4	3
16a	NO_2	38	222	ND	3930	44	8
16b	NO_2	9	113	ND	4090	11	4
17a	NH_2	3	14	2	1430	3	3
17b	NH_2	4	27	ND	1950	4	3
21		2	19	2	1940	3	1
23		3	25	1	787	2	0.8
25a		12	42	4	1850	14	4
25b		4	21	2	2580	6	< 0.2
27		11	46	6	1990	14	2
29		2	< 0.2	< 0.2	3950	4	< 0.2

[a] The isoform inhibition was tested at Amphora Discovery Corporation (http://www.amphoracorp.com/). [b] Not determined.

Table 3. Antiproliferative activities of SAHA and biaryl HDAC inhibitors against pancreatic cell lines.

Compd	IC_{50} [μM]				
	BxPC-3	HupT3	Mia Paca-2	Panc 04.03	SU 86.86
SAHA	5	0.8	1.1	1.2	1.3
7g	7	1	0.2	3	1
7b	10	10	< 1	10	10
7c	33	23	3	32	23
7e	> 50	25	1	> 50	55
16a	5	2	0.2	3	1
17a	2	< 1	< 1	2	1
17b	1	0.6	0.01	> 10	3
23	1	0.7	0.04	0.6	2

against five pancreatic cancer cell lines using an MTT assay. The preliminary antiproliferative results are outlined in Table 3, with SAHA used as a reference for comparison with our own compounds in this study. As apparent from these data, the unsubstituted biphenyl *ortho*-NH_2 hydroxamate **7g** is as potent as SAHA for inhibiting the growth of the BxPC-3, HupT3, Panc 04.03, and SU 86.86 pancreatic cancer cell lines, whereas the other three phenylalanine-, proline-, and tyrosine-bearing biphenyl hydroxamates **7b**, **7c**, and **7e** are less potent. However, in the case of the Mia Paca-2 cell line, all the biphenyl hydroxamates tested show potencies that are comparable or better than those of SAHA. On the other hand, the substituted phenylthiazole-based inhibitors showed similar or improved potencies relative to SAHA and the biphenyl ligands against all five pancreatic cancer cell lines. Among these ligands, the *meta*-amino-substituted phenylthiazole **17b** gave the best IC_{50} value against the Mia Paca-2 cell line ($IC_{50} = 10$ nM), while its carbamate analogue **23** provided the best overall inhibitory activity against all five pancreatic cancer cell lines.

QSAR studies

To examine the SAR quantitatively, the compounds listed in Tables 1 and 2 were investigated by using classical QSAR (see Table 4). QSARs [Eqs. (1–5), see Table 5 below] were developed from the 23 biphenyls or phenylthiazoles bearing hydroxamates or mercaptoacetamides against HDACs 1, 2, 8, 10, and 6. The pIC_{50} values, the calculated log *P* values (Clog *P*),[30] and the indicator variables I-$NHCOCH_2SH$ and I-Thiazole used in the correlations are listed in Table 4. The indicator variable I-$NHCOCH_2SH$ takes the value of 1.0 for the mercaptoacetamides and 0.0 for all others. The indicator variable I-Thiazole takes the value of 1.0 for the phenylthiazoles and 0.0 for all others.

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Equation (1) in Table 5 shows that the majority of the variance in the inhibitory activity (expressed as pIC_{50}) of the inhibitors against HDAC1 can be explained with the three different compound classes: biphenylhydroxamates, biphenylmercaptoacetamides, and phenylthiazoles. The negative coefficient of I-$NHCOCH_2SH$ indicates that the biphenylmercaptoacetamides are 70-fold (1.844 log units) less potent than the biphenylhydroxamates, and the phenylthiazoles are 9.6-fold (0.983 log units) more potent than the biphenylhydroxamates. The squared correlation coefficient is excellent ($R^2 = 0.920$) and the root mean square error (RMSE) is reasonable (0.322). Figure 1a shows a plot between the observed and calculated pIC_{50} values (for HDAC1) using Equation (1). Similar correlations were obtained against HDAC2 and HDAC10 [Eq. (2) and Eq. (4)], respectively. Two compounds, **10b** and **10d**, have IC_{50} values $>30\,000$ (Table 4). If one includes these with the value of 30 000, an essentially identical correlation is obtained with significantly improved statistics [Eq. (2a)]. The correlation [Eq. (3)] against HDAC8 shows that the biphenylmercaptoacetamides are 2.9-fold (-0.461 log units) less potent than both the phe-

Table 4. HDAC inhibitory activity of biphenyl and phenylthiazole analogues bearing hydroxamates or mercaptoacetamides.[a]

Compd	HDAC1		HDAC2		HDAC8		HDAC10		HDAC6		Clog *P*	I-$NHCOCH_2SH$	I-Thiazole
	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}			
7h	33	7.48	46	7.34	1870	5.73	46	7.34	5	8.30	2.877	0	0
7g	99	7.00	244	6.61	2500	5.60	139	6.86	16	7.80	1.650	0	0
7f	57	7.24	74	7.13	1720	5.76	83	7.08	11	7.96	3.583	0	0
7a	102	6.99	364	6.44	3480	5.46	146	6.84	28	7.55	−0.045	0	0
7b	41	7.39	156	6.81	1600	5.80	46	7.34	8	8.10	1.682	0	0
7c	52	7.28	193	6.71	2660	5.58	70	7.15	16	7.80	0.969	0	0
7d	27	7.57	167	6.78	1720	5.76	28	7.55	5	8.30	1.672	0	0
7e	37	7.43	205	6.69	2060	5.69	42	7.38	9	8.05	1.015	0	0
10a	3960	5.40	15 980	4.80	6030	5.22	7430	5.13	387	6.41	−0.574	1	0
10b	2760	5.56	>30 000	<4.52	5190	5.28	7220	5.14	452	6.34	1.153	1	0
10c	1950	5.71	12 490	4.90	4230	5.37	6070	5.22	205	6.69	0.440	1	0
10d	7090	5.15	>30 000	<4.52	11 250	4.95	>30 000	<4.52	656	6.18	1.143	1	0
24	3	8.52	35	7.46	1900	5.72	4	8.40	3	8.52	2.323	0	1
16a	38	7.42	222	6.65	3930	5.41	44	7.36	8	8.10	2.323	0	1
17a	3	8.52	14	7.85	1430	5.84	3	8.52	3	8.52	1.290	0	1
16b	9	8.05	113	6.95	4090	5.39	11	7.96	4	8.40	2.084	0	1
17b	4	8.40	27	7.57	1950	5.71	4	8.40	3	8.52	1.290	0	1
21	2	8.70	19	7.72	1940	5.71	3	8.52	1	9.00	0.910	0	1
23	3	8.52	25	7.60	787	6.10	2	8.70	0.8	9.10	2.467	0	1
25a	4	8.40	21	7.68	2580	5.59	6	8.22	<0.2	9.70[b]	3.175	0	1
25b	12	7.92	42	7.38	1850	5.73	14	7.85	4	8.40	3.175	0	1
27	11	7.96	46	7.34	1990	5.70	14	7.85	2	8.70	2.758	0	1
29	2	8.70	<0.2	>9.70	3950	5.40	4	8.40	<0.2	9.70[b]	3.552	0	1

[a] IC_{50} values in nM. [b] The IC_{50} value 0.2 was used in the correlations.

Table 5. QSAR equations developed from the 23 biphenyl or phenylthiazoles bearing hydroxamates or mercaptoacetamides listed in Tables 1, 2, and 4 against HDAC1, HDAC2, HDAC8, HDAC10, and HDAC6.

	Equations	*n*	R^2	RMSE	*p*
(1)	pIC_{50} (HDAC1) = −1.844(±0.248) I-$NHCOCH_2SH$ + 0.983(±0.149) I-Thiazole + 7.299(±0.114)	23	0.920	0.322	<0.0001
(2)	pIC_{50} (HDAC2) = −1.963(±0.258) I-$NHCOCH_2SH$ + 0.606(±0.155) I-Thiazole + 6.813(±0.115)	20	0.860	0.326	<0.0001
(2a)	pIC_{50} (HDAC2) = −2.127(±0.195) I-$NHCOCH_2SH$ + 0.606(±0.151) I-Thiazole + 6.813(±0.112)	22	0.918	0.318	<0.0001
(3)	pIC_{50} (HDAC8) = −0.461(±0.097) I-$NHCOCH_2SH$ + 5.668(±0.040)	23	0.518	0.176	<0.0001
(4)	pIC_{50} (HDAC10) = −2.029(±0.222) I-$NHCOCH_2SH$ + 1.007(±0.153) I-Thiazole + 7.192(±0.116)	22	0.916	0.328	<0.0001
(5)	pIC_{50} (HDAC6) = −1.429(±0.246) I-$NHCOCH_2SH$ + 0.711(±0.184) I-Thiazole + 0.046(±0.023) (Clog *P*)^2 + 7.799(±0.163)	23	0.861	0.384	<0.0001
(6)	pIC_{50} (HDAC6) = 0.767(±0.053) pIC_{50} (HDAC1) + 0.046(±0.014) (Clog *P*)^2 + 2.178(±0.374)	23	0.943	0.241	<0.0001
(6a)	pIC_{50} (HDAC6) = 0.844(±0.057) pIC_{50} (HDAC1) + 1.804(±0.432)	23	0.911	0.292	<0.0001
(7)	pIC_{50} (HDAC6) = 0.726(±0.104) pIC_{50} (HDAC2) + 0.275(±0.167) I-Thiazole + 3.032(±0.674)	20	0.863	0.294	<0.0001
(7a)	pIC_{50} (HDAC6) = 0.832(±0.085) pIC_{50} (HDAC2) + 2.435(±0.594)	20	0.849	0.307	<0.0001
(8)	pIC_{50} (HDAC6) = 0.681(±0.060) pIC_{50} (HDAC10) + 0.053(±0.016) (Clog *P*)^2 + 2.892(±0.425)	22	0.914	0.272	<0.0001
(8a)	pIC_{50} (HDAC6) = 0.760(±0.068) pIC_{50} (HDAC10) + 2.539(±0.508)	22	0.863	0.335	<0.0001
(9)	pIC_{50} (HDAC6) = 1.650(±0.447) pIC_{50} (HDAC8) + 0.880(±0.227) I-Thiazole + 0.067(±0.028) (Clog *P*)^2 − 1.840(±2.459)	23	0.775	0.489	<0.0001
(9a)	pIC_{50} (HDAC6) = 1.834(±0.489) pIC_{50} (HDAC8) + 1.059(±0.238) I-Thiazole − 2.633(±2.701)	23	0.709	0.542	<0.0001
(9b)	pIC_{50} (HDAC6) = 2.498(±0.642) pIC_{50} (HDAC8) − 5.863(±3.589)	23	0.419	0.747	<0.0008

[a] QSARs against HDAC1, HDAC2, HDAC8, HDAC10, and HDAC6 are represented respectively in Equations (1)–(5). Equations (6)–(9) are for the selectivity of the inhibitory activity (pIC_{50}) of HDAC6 with respect to those of HDAC1, HDAC2, HDAC10, and HDAC8.

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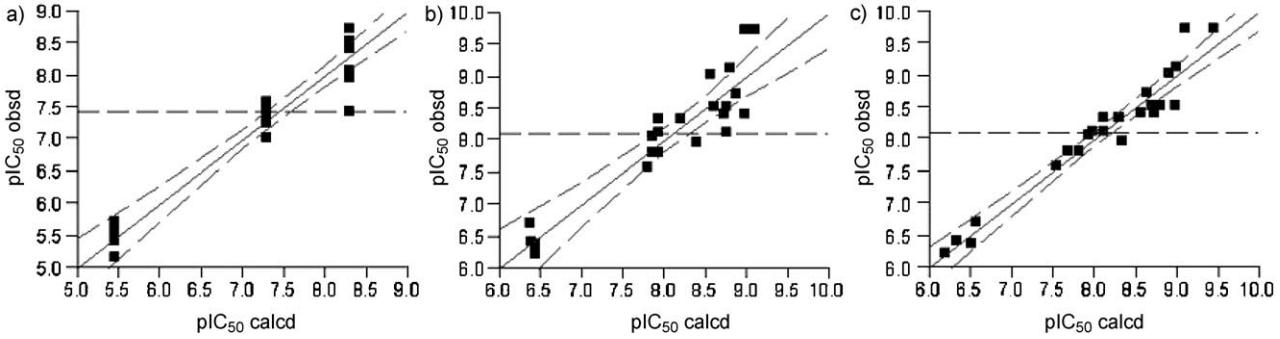

Figure 1. a) Plot between the observed and calculated pIC_{50} values against HDAC1 using Equation (1) in Table 5. Equation (1) shows that the three classes explain 92% of the variance of the inhibitory activity data. The influences of the CAP groups among each of the classes are relatively small and may be involved in the remaining variance of the data. b) Plot between the observed and calculated pIC_{50} values against HDAC6 using Equation (5). c) Plot between the observed and calculated pIC_{50} values against the selectivity of HDAC6 over HDAC1 using Equation (6).

nylthiazoles and the biphenylhydroxamates. The lower R^2 value of Equation (3) is partly due to the narrow range of the pIC_{50} values among the compounds included. However, the small RMSE value (0.176) of Equation (3) shows the goodness of this correlation relative to all the other equations. Equation (5) shows that the inhibitory activity of these compounds against HDAC6 is also influenced by the lipophilicity of the molecules in addition to the similar differences of the biphenylmercaptoacetamides and the phenylthiazoles observed in Equations (1), (2), and (4). Figure 1b shows a plot between the observed and calculated pIC_{50} values (for HDAC6) using Equation (5). Overall, the majority of the variance in pIC_{50} values of the compounds studied is explained by the three classes of compounds. The differences among the CAP groups influence the inhibitory activity only to a minor degree.

Equations (6)–(9) are correlations that describe the effects of the structural and physicochemical properties on the selectivity of HDAC6 against HDAC1, HDAC2, HDAC10, and HDAC8, respectively. Equation (6a) shows that the inhibitory potency of these molecules between HDAC6 and HDAC1 is highly correlated ($R^2 = 0.911$). Equation (6) indicates that the lipophilicity of the molecules explains an additional 3% of the variance in the selectivity. Figure 1c shows the observed and calculated pIC_{50} values for the selectivity of HDAC6 over HDAC1 using Equation (6). Similar results were obtained for the selectivity between HDAC6 and HDAC10 [Eqs. (8) and (8a)]. The R^2 value for the correlation between HDAC6 and HDAC2 is 0.804 [Eq. (7a)]. The selectivity toward HDAC6 over HDAC2 is accounted for by a 1% improvement in R^2 with an indicator variable I-Thiazole for the phenylthiazoles [compare Eq. (7) with (7a)]. The selectivity between HDAC6 and HDAC8 is explained by a 6% improvement in R^2 with inclusion of the lipophilicity of the molecules [compare Eq. (9) with (9a)] in the correlation. Equation (9b) shows that the phenylthiazoles described with the indicator variable I-Thiazole accounts for 28% of the selectivity of HDAC6 over HDAC8.

Four compounds were not included in some of the QSARs described in Table 5 partially because of the lack of a fixed IC_{50} value. The IC_{50} values of both **10b** and **10d** are $>30\,000$ nM ($pIC_{50} < 4.52$) against HDAC2, and the value of **10d** against HDAC10 was the same. The inhibitory activity of these compounds is the weakest, and these compounds were indeed predicted to have weak inhibitory activity. The calculated pIC_{50} values of these compounds from the corresponding QSAR are 4.85 against HDAC2 and 5.16 against HDAC10, which is the lowest calculated value among the compounds studied in the corresponding case (Table 6). The IC_{50} value of **29** against HDAC2 is <0.2 nM ($pIC_{50} > 9.70$). This compound is predicted to be very potent. The calculated pIC_{50} value of this compound is 7.42, which is the highest calculated value among the compounds studied. Therefore, both the "actives" and "inactives" not included in the QSARs are well predicted. The inhibitory activity of two compounds, **25a** and **29**, against HDAC6 is <0.2 nM ($pIC_{50} > 9.70$). The IC_{50} value of 0.2 nM is used in the QSARs of HDAC6. The calculated pIC_{50} values of these two compounds are 8.98 and 9.09, respectively, the highest potency among the compounds used.

The QSARs obtained for the various HDACIs described in this study suggest that their inhibitory activities toward the different isoforms of HDAC are highly correlated. The QSARs also support the notion that the selectivity between HDAC6 and HDAC1 (as well as HDAC8 and HDAC10) can be increased with a minimum lipophilicity point of zero. Examination of the homology models of these HDACs (data not presented herein) further support this observation, as the pertinent HDACs possess structurally similar binding pockets.

Conclusions

In summary, we have synthesized a series of structurally unique HDAC inhibitors in which the 2,4′-diaminobiphenyl group appropriately decorated with an amino acid residue serves as a potential isoform-differentiating, surface-recognition element. The surface-recognition group is connected through the usual carbon linker to either a hydroxamate or a mercaptoacetamide group that chelates to the catalytic site zinc ion. Different amino acids as well as other structural motifs (e.g., carbohydrates) can be attached to the 2,4′-diaminobiphenyl moiety in order to investigate the possibility of achieving further levels of discrimination among the different HDAC isoforms. While the results obtained from this first generation of amino acid bearing HDACIs reveal some modest

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Table 6. Observed and calculated HDAC inhibitory activity (IC50, nM) of biphenyl and phenylthiazole analogues bearing hydroxamates or mercaptoacetamides.

Compd	pIC_{50} (HDAC1)			pIC_{50} (HDAC2)			pIC_{50} (HDAC8)			pIC_{50} (HDAC10)			pIC_{50} (HDAC6)		
	obsd[a]	calcd[a]	dev[a]	obsd[b]	calcd[b]	dev[b]	obsd[c]	calcd[c]	dev[c]	obsd[d]	calcd[d]	dev[d]	obsd[e]	calcd[e]	dev[e]
7h	7.48	7.30	0.18	7.34	6.81	0.52	5.73	5.67	0.06	7.34	7.19	0.15	8.30	8.18	0.12
7g	7.00	7.30	−0.29	6.61	6.81	−0.20	5.60	5.67	−0.07	6.86	7.19	−0.33	7.80	7.93	−0.13
7f	7.24	7.30	−0.06	7.13	6.81	0.32	5.76	5.67	0.10	7.08	7.19	−0.11	7.96	8.39	−0.43
7a	6.99	7.30	−0.31	6.44	6.81	−0.37	5.46	5.67	−0.21	6.84	7.19	−0.36	7.55	7.80	−0.25
7b	7.39	7.30	0.09	6.81	6.81	−0.01	5.80	5.67	0.13	7.34	7.19	0.15	8.10	7.93	0.17
7c	7.28	7.30	−0.02	6.71	6.81	−0.10	5.58	5.67	−0.09	7.15	7.19	−0.04	7.80	7.84	−0.05
7d	7.57	7.30	0.27	6.78	6.81	−0.04	5.76	5.67	0.10	7.55	7.19	0.36	8.30	7.93	0.37
7e	7.43	7.30	0.13	6.69	6.81	−0.13	5.69	5.67	0.02	7.38	7.19	0.19	8.05	7.85	0.20
10a	5.40	5.46	−0.05	4.80	4.85	−0.05	5.22	5.21	0.01	5.13	5.16	−0.03	6.41	6.39	0.03
10b	5.56	5.46	0.10	<4.52	4.85[f]	>−0.33	5.28	5.21	0.08	5.14	5.16	−0.02	6.34	6.43	−0.09
10c	5.71	5.46	0.25	4.90	4.85	0.05	5.37	5.21	0.17	5.22	5.16	0.05	6.69	6.38	0.31
10d	5.15	5.46	−0.31	<4.52	4.85[f]	>−0.33	4.95	5.21	−0.26	<4.52	5.16[f]	>−0.64	6.18	6.43	−0.25
24	8.52	8.28	0.24	7.46	7.42	0.04	5.72	5.67	0.05	8.40	8.20	0.20	8.52	8.76	−0.24
16a	7.42	8.28	−0.86	6.65	7.42	−0.77	5.41	5.67	−0.26	7.36	8.20	−0.84	8.10	8.76	−0.66
17a	8.52	8.28	0.24	7.85	7.42	0.43	5.84	5.67	0.18	8.52	8.20	0.32	8.52	8.59	−0.06
16b	8.05	8.28	−0.24	6.95	7.42	−0.47	5.39	5.67	−0.28	7.96	8.20	−0.24	8.40	8.71	−0.31
17b	8.40	8.28	0.12	7.57	7.42	0.15	5.71	5.67	0.04	8.40	8.20	0.20	8.52	8.59	−0.06
21	8.70	8.28	0.42	7.72	7.42	0.30	5.71	5.67	0.04	8.52	8.20	0.32	9.00	8.55	0.45
23	8.52	8.28	0.24	7.60	7.42	0.18	6.10	5.67	0.44	8.70	8.20	0.50	9.10	8.79	0.31
25a	8.40	8.28	0.12	7.68	7.42	0.26	5.59	5.67	−0.08	8.22	8.20	0.02	9.70[f][g]	8.98	0.72
25b	7.92	8.28	−0.36	7.38	7.42	−0.04	5.73	5.67	0.07	7.85	8.20	−0.34	8.40	8.98	−0.58
27	7.96	8.28	−0.32	7.34	7.42	−0.08	5.70	5.67	0.03	7.85	8.20	−0.34	8.70	8.86	−0.16
29	8.70	8.28	0.42	>9.70	7.42[f]	>2.28	5.40	5.67	−0.26	8.40	8.20	0.20	9.70[g]	9.09	0.61

[a] Calculated using Equation (1). [b] Calculated using Equation (2). [c] Calculated using Equation (9). [d] Calculated using Equation (3). [e] Calculated using Equation (4). [f] Not used in the correlation. [g] Based on the IC_{50} of 0.2 nM.

degree of isoform selectivity over a panel of six HDAC isoforms, these compounds serve as relatively potent HDAC inhibitors and are able to block the growth of five pancreatic cancer cell lines. The mercaptoacetamide-bearing HDACIs all show some preference for HDAC6 inhibition. Perhaps of greatest interest, the work presented herein has led to the identification of two hydroxamate-bearing *meta*-substituted phenylthiazole CAPs (compounds **25b** and **29**) that exhibit picomolar IC_{50} values in the in vitro HDAC6 inhibition studies; compound **29** also inhibits HDAC2 and HDAC3 with IC_{50} values in the picomolar range. Moreover, several of these phenylthiazoles exhibit submicromolar to low-nanomolar IC_{50} values in the pancreatic cancer cell proliferation studies. We plan to construct a series of second-generation inhibitors for isozyme studies using related scaffolds, but with the putative surface-recognition element located on the aryl ring that is linked to the ZBG. Further modeling studies are underway to better understand the activity of these phenylthiazoles relative to their biphenyl counterparts. Moreover, cell-based experiments will be conducted to obtain some measure of the possible isoform- and tissue-selectivity of these new inhibitors under more biologically relevant conditions, in which the HDACs are able to form complexes with other proteins, including transcription factors and other HDACs. Lastly, we call attention to the fact that in other studies reported recently by us, certain mercaptoacetamides do show useful levels of HDAC6 selectivity.[18]

Experimental Section

Synthesis

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 300/400 MHz and 75/100 MHz, respectively, with TMS as an internal standard. Standard abbreviations indicating multiplicity were used: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintuplet, m = multiplet, and br = broad. HRMS experiments were performed on a Q-TOF-2TM instrument (Micromass). TLC was performed with Merck 250-mm 60 F_{254} silica gel plates. Preparative TLC was performed with Analtech 1000-mm silica gel GF plates. Column chromatography was performed using Merck silica gel (40–60 mesh). HPLC was carried out on ACE AQ columns (100 × 4.6 mm^2 and 250 × 10 mm^2) with detection at $\lambda = 254$ nm on a Shimadzu SPD-10 A VP detector (flow rate = 2.0–3.5 mL min^{-1}; from 10% CH_3CN in H_2O to 100% CH_3CN with 0.05% TFA. Optical rotations were obtained on a Rudolph-Autopol® IV Polarimeter.

Typical procedure for octanedioic acid hydroxyamides 7a, 7c, 7d, 7f, and 7g: The following method represents a typical procedure for the synthesis of the octanedioic acid hydroxyamide-based ligands. The synthesis of **7b**, **7d**, and **7e** were described in our previous work.[18]

(4′-Nitro-2-biphenyl)carbamic acid *tert*-butyl ester (3f): A mixture of 4′-nitro-2-biphenylamine (**1**) (0.857 g, 4.0 mmol) and di-*tert*-butyl dicarbonate (0.870 g, 4.0 mmol) in toluene was heated at 100 °C overnight, and then an additional amount of di-*tert*-butyl dicarbonate (0.175 g, 0.8 mmol) was added. The mixture was kept at 100 °C for another 4 h, and the solvent was then evaporated in vacuo. The solid residue was washed with hexanes/EtOAc 4:1, filtered, and dried to yield (4′-nitro-2-biphenyl)carbamic acid *tert*-butyl ester

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3 f (1.0 g, 79%). ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 1.23 (s, 9H), 7.31–7.41 (m, 4H), 7.65 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 8.7 Hz, 2H).

Octanedioic acid {2′-[2-amino-3-(1*H*-indol-3-yl)propionylamino]-4-biphenyl}amide hydroxyamide (7 d): $POCl_3$ (0.84 g, 5.4 mmol) was added dropwise at −15 °C to a stirred solution of Boc-L-Trp-OH (1.67 g, 5.4 mmol) and 4′-nitro-2-biphenylamine (1.18 g, 5.4 mmol) in dry pyridine (20 mL). The reaction was kept at the same temperature for 1 h, then concentrated to remove part of the pyridine. The residue was dissolved in EtOAc, washed thoroughly with a saturated NH_4Cl solution and brine, the organic phase was separated, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (acetone/hexanes 1:1) to give compound **3 d** (1.6 g, 58%). ^{1}H NMR ($CDCl_3$, 400 Hz): δ = 1.36 (s, 9H), 3.17 (dd, J = 7.6 and 14.4 Hz, 1H), 3.39 (dd, J = 4.0 and 14.4 Hz, 1H,), 4.39–4.46 (m, 1H), 4.95–5.12 (m, 1H), 6.99 (d, J = 8.7 Hz, 1H), 7.00 (s, 1H), 7.18–7.10 (m, 2H), 7.20–7.27 (m, 2H), 7.36–7.45 (m, 2H), 7.53 (s, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 8.4 Hz, 2H), 8.22–8.27 ppm (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 28.5, 56.5, 60.8, 110.4, 111.7, 119.2, 120.5, 122.3, 123.1, 123.8, 124.3, 125.3, 127.5, 130.0, 130.1, 130.2, 130.8, 134.3, 136.6, 144.9, 147.5, 170.4 ppm.

A suspension of compound **3 d** (1.80 g, 3.5 mmol) and $Pd(OH)_2$/C (20 wt%, 0.5 g) in a mixture of MeOH (10 mL) and CH_2Cl_2 (10 mL) was stirred under hydrogen atmosphere for 4 h at room temperature. The catalyst was removed by filtration through a pad of Celite, and the solvent was evaporated to give a residue that was purified by flash chromatography (EtOAc/hexanes 1:1 then 2:1) to give compound **4 d** (1.40 g, 83%). ^{1}H NMR ($CDCl_3$, 300 Hz): δ = 1.38 (s, 9H), 3.12–3.28 (m, 1H), 3.30–3.45 (m, 1H), 4.46 (brs, 1H), 5.08 (brs, 1H), 6.35 (brs, 2H), 6.61 (brs, 2H), 6.95 (s, 1H), 7.08–7.19 (m, 4H), 7.22 (t, J = 7.2 Hz, 1H), 7.27–7.40 (m, 3H), 7.62 (d, J = 7.3 Hz, 1H), 7.77 (brs, 1H), 8.35 ppm (brs, 2H); ^{13}C NMR (CD_3OD, 75 MHz): δ = 28.6, 56.7, 80.4, 111.7, 115.9, 119.2, 120.1, 121.0, 122.5, 123.8, 124.7, 125.0, 128.1, 130.2, 130.2, 132.6, 134.7, 136.6, 145.8, 155.6, 170.1 ppm.

DIPEA (0.120 g, 0.92 mmol) was added to a stirred solution of 7-benzyloxycarbamoylheptanoic acid (**5**) (0.13 g, 0.46 mmol) in dry DMF (5 mL), and the mixture was stirred for 10 min at room temperature. Then PyBOP (0.480 g, 0.92 mmol) and biphenyl amine **4 d** (0.220 g, 0.46 mmol) were added sequentially, and stirring was continued overnight. The reaction mixture was diluted with Et_2O, washed with water, a saturated $NaHCO_3$ solution, a saturated NH_4Cl solution, and brine, and dried over Na_2SO_4, filtered, and then concentrated. The crude material was purified by flash chromatography (acetone/hexanes 1:1) to give compound **6 d** (0.233 g, 70%). ^{1}H NMR ($CDCl_3$, 300 Hz): δ = 1.30–1.50 (m, 13H), 1.60–1.85 (m, 4H), 2.06 (brs, 2H), 2.37 (t, J = 7.1 Hz, 2H), 2.94–3.20 (m, 1H), 3.25–3.48 (m, 1H), 4.37 (brs, 1H), 4.92 (s, 2H), 5.21 (brs, 1H), 6.73 (brs, 2H), 6.84 (brs, 1H), 7.18–7.00 (m, 4H), 7.26–7.45 (m, 9H), 7.45–7.60 (m, 2H), 7.68 (brs, 1H), 8.30 (brs, 1H), 8.51 (brs, 1H), 8.83 (brs, 1H).

TFA (2 mL) was added to a solution of compound **6 d** (0.046 mg, 0.063 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 2 h the reaction mixture was diluted with Et_2O, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, then filtered, and concentrated. The crude material was purified by flash chromatography (MeOH/CH_2Cl_2 10:1) to give amine (0.020 g, 51%). ^{1}H NMR ($CDCl_3$, 300 Hz): δ = 1.32–1.78 (m, 8H), 2.04 (brs, 2H), 2.33 (brt, 2H), 3.00 (dd, J = 7.9 and 14.3 Hz, 1H), 3.31 (dd, J = 4.4 and 14.5 Hz, 1H), 3.71 (dd, J = 4.2 and 7.5 Hz, 1H), 4.90 (brs, 2H), 6.84 (s, 1H), 7.04–7.22 (m, 6H), 7.32–7.42 (m, 7H), 7.48 (d, J = 7.9 Hz, 2H), 7.61 (d, J = 7.8 Hz, 1H), 7.80 (brs, 1H), 8.35 (d, J = 8.1 Hz, 1H), 8.48–8.62 (brd, 1H), 9.49 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 24.5, 24.7, 25.9, 26.0, 28.0, 29.3, 29.8, 29.9, 31.4, 32.4, 36.8, 46.6, 50.4, 55.3, 65.4, 77.7, 110.3, 111.0, 118.4, 119.0, 119.6, 120.5, 121.0, 121.6, 123.0, 123.9, 124.1, 127.0, 127.8, 128.2, 128.3, 128.8, 129.4, 129.6, 132.0, 133.5, 134.3, 134.8, 136.0, 137.1, 170.6, 171.5, 172.8 ppm.

A suspension of amine (0.031 g, 0.049 mmol) and $Pd(OH)_2$/C (20 wt%, 0.010 g) in MeOH was stirred under a hydrogen atmosphere at room temperature for 4 h. The catalyst was removed by filtration through a pad of Celite, and the residue was thoroughly washed with MeOH. The solvent was evaporated and the residue was crystallized from MeOH/ether 5:95 to give hydroxamate **7 d** (0.008 g, 30%). $[\alpha]_D^{24}$ = 11.5 (c = 0.27, CH_3OH); ^{1}H NMR (CD_3OD, 300 MHz): δ = 1.30–1.78 (m, 8H), 2.10 (d, J = 7.0 Hz, 2H), 2.37 (d, J = 7.0 Hz, 2H), 3.02–3.15 (m, 1H), 3.25–3.35 (m, 1H), 3.96–4.05 (m, 1H), 6.96–7.08 (m, 1H), 7.09–7.22 (m, 4H), 7.23–7.48 (m, 5H), 7.52–7.67 (m, 3H), 7.74 ppm (d, J = 7.7 Hz, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ = 24.8, 24.9, 26.9, 28.0, 28.1, 31.9, 36.0, 53.5, 99.5, 106.1, 110.9, 114.7, 117.4, 118.6, 119.8, 121.2, 123.8, 125.2, 126.1, 126.4, 127.2, 128.7, 129.8, 132.7, 133.8, 136.0, 136.4, 137.5, 167.5, 171.3, 173.1 ppm; ESI-HRMS calcd for $[C_{31}H_{35}N_5O_4+H]^+$: 542.2761, found: 542.2762; HPLC purity: 95%.

(2*S*)-[1-(4′-Nitro-2-biphenylcarbamoyl)-2-phenylethyl]carbamic acid *tert*-butyl ester (3 b): Compound **3 b** (yield 47%) was prepared from Boc-L-Phe-OH according to the methodology described for the preparation of compound **3 d**. The enantiomeric purity was determined on a Chiralpak AD column 10×250 mm^2, with hexanes/2-propanol 70:30 as the mobile phase at a flow rate of 4 mL min^{-1}. Detection was performed with a UV spectrometer Shimadzu SPD-10 A VP at λ = 254 nm, t_R = 7.4 min. $[\alpha]_D^{24}$ (>99% *ee*) = −3.2 (c = 0.16, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.33 (s, 9H), 3.11 (d, J = 6.8 Hz, 2H), 4.30–4.32 (m, 1H), 4.79 (brs, 1H), 7.18–7.32 (m, 9H), 7.42–7.46 (m, 1H), 7.62 (s, 1H), 8.21 ppm (d, J = 8.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.1, 37.9, 56.7, 80.6, 122.3, 124.0, 125.2, 125.8, 127.2, 128.8, 129.3, 129.6, 129.8, 130.0, 130.8, 133.8, 144.7, 147.2, 169.4 ppm.

(2*R*)-[1-(4′-Nitro-2-biphenylcarbamoyl)-2-phenylethyl]carbamic acid *tert*-butyl ester (3 g): Compound **3 g** (yield 27%) was prepared from Boc-D-Phe-OH according to the methodology described for the preparation of compound **3 d**. Enantiomeric purity was determined on a Chiralpak AD column 10×250 mm^2, with hexanes/2-propanol 70:30 as the mobile phase at a flow rate of 4 mL min^{-1}. Detection was performed with a UV spectrometer Shimadzu SPD-10 A VP at λ = 254 nm, t_R = 9.3 min. $[\alpha]_D^{24}$ (>99% *ee*) = +3.3 (c = 0.31, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.33 (s, 9H), 3.11 (d, J = 6.4 Hz, 2H), 4.30–4.32 (m, 1H), 4.79 (brs, 1H), 7.18–7.32 (m, 9H), 7.44 (t, J = 6.8 Hz, 1H), 7.62 (s, 1H), 8.21 ppm (d, J = 8.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.1, 37.9, 122.3, 124.0, 125.2, 125.8, 127.2, 128.8, 129.3, 129.6, 129.8, 130.0, 130.8, 133.8, 144.7, 147.2, 169.4 ppm.

Octanedioic acid [2′-(2-aminoacetylamino)-4-biphenyl]amide hydroxyamide (7 a): Compound **7 a** was prepared from Boc-Gly-OH according to the methodology described for the preparation of compound **7 d**. ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 1.37 (m, 4H), 1.48 (m, 2H), 1.57 (m, 2H), 1.93 (t, J = 7.2 Hz, 2H), 2.30 (t, J = 7.2 Hz, 2H), 3.43 (m, 2H), 3.62 (brs, 2H), 4.34 (t, J = 4.9 Hz, 1H), 7.32 (m, 5H), 7.50 (d, J = 7.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 8.65 (s, 1H), 9.99 (s, 1H), 10.34 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ = 25.4, 25.5, 28.8, 30.8, 32.6, 34.8, 36.8, 119.4, 125.3, 126.8, 128.0, 128.4, 129.5, 130.8, 133.2, 133.9, 136.2, 139.1, 139.6, 166.0, 169.5, 171.8 ppm; ESI-HRMS calcd for $[C_{22}H_{28}N_4O_4+H]^+$: 413.2189, found: 413.2182; HPLC purity: 96%.

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Octanedioic acid hydroxyamide {2′-[(pyrrolidine-2-carbonyl)amino]-4-biphenyl}amide (7c): Compound **7c** was prepared from Boc-L-Pro-OH according to the methodology described for the preparation of compound **7d**. $[\alpha]_D^{24} = -38.0$ ($c = 1$, CH_3OH); ^1H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 1.09$ (m, 4H), 1.48–1.58 (m, 4H), 1.82–1.94 (m, 5H), 2.19 (m, 1H), 2.30 (m, 3H), 3.15 (m, 2H), 4.17 (m, 1H), 7.17–7.66 (m, 8H), 8.66 (brs, 1H), 9.95 (brs, 1H), 10.00 (brs, 1H), 10.35 ppm (brs, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta = 23.9$, 25.4, 28.8, 29.6, 32.6, 36.8, 46.1, 59.9, 119.3, 125.7, 128.1, 128.6, 129.3, 129.5, 130.7, 133.3, 133.9, 139.2, 169.5, 171.7 ppm; ESI-HRMS calcd for $[C_{25}H_{32}N_4O_4+H]^+$: 453.2502, found: 453.2494; HPLC purity: 98%.

Octanedioic acid (2′-amino-4-biphenyl)amide hydroxyamide (7f): Compound **7f** was prepared from **3f** according to the methodology described for the preparation of compound **7d**. ^1H NMR ([D$_6$]DMSO, 300 MHz): $\delta = 1.28$ (brs, 4H), 1.49–1.59 (m, 4H), 1.94 (t, $J = 6.0$ Hz, 2H), 2.30 (t, $J = 6.0$ Hz, 2H), 4.72 (brs, 2H), 6.61 (t, $J = 6.0$ Hz, 1H), 6.72 (d, $J = 6.0$ Hz, 1H), 6.95 (d, $J = 6.0$ Hz, 1H), 7.01 (t, $J = 6.0$ Hz, 1H), 7.32 (d, $J = 9.0$ Hz, 2H), 7.65 (d, $J = 9.0$ Hz, 2H), 8.66 (s, 1H), 9.93 (s, 1H), 10.33 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 25.4$, 25.5, 28.8, 32.6, 36.8, 115.5, 117.1, 119.7, 125.9, 128.3, 129.2, 130.3, 134.6, 138.4, 145.4, 169.5, 171.6 ppm; ESI-HRMS calcd for $[C_{20}H_{25}N_3O_3+H]^+$: 356.1968, found: 356.1962; HPLC purity: 96%.

[4′-(7-Hydroxycarbamoylheptanoylamino)-2-biphenyl]carbamic acid *tert*-butyl ester (7g): Compound **7g** was prepared from **3f** according to the methodology described for the preparation of compound **7d**, by omitting the use of TFA to remove the Boc protecting group of **6f**, and **7g** was purified by preparative HPLC. ^1H NMR (400 MHz, CD_3OD): $\delta = 1.35$–1.45 (m, 13H), 1.65 (t, $J = 6.8$ Hz, 2H), 1.73 (t, $J = 7.6$ Hz, 2H), 2.11 (t, $J = 7.2$ Hz, 2H), 2.40 (t, $J = 7.6$ Hz, 2H), 7.20–7.24 (m, 1H), 7.27–7.30 (m, 1H), 7.31–7.35 (m, 3H), 7.55 (d, $J = 7.2$ Hz, 1H), 7.64 ppm (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 25.1$, 25.2, 27.1, 28.4, 36.4, 79.5, 119.8, 124.9, 125.8, 127.4, 129.0, 129.9, 134.7, 134.8, 137.8, 154.5, 173.2 ppm; ESI-HRMS calcd for $[C_{25}H_{33}N_3O_5+H]^+$: 456.2493, found: 456.2491; HPLC purity: 96%.

Typical procedure for mercaptoacetamide-based ligands 10a–d: The following method represents a typical procedure for the synthesis of the 6-mercaptoacetylaminohexanoic acid amide-based ligands.

6-(2-Mercaptoacetylamino)hexanoic acid {2′-[2-amino-3-(1*H*-indol-3-yl)propionylamino]-4-biphenyl}amide (10d): DIPEA (0.126 g, 0.97 mmol) was added to a stirred solution of 6-(2-tritylsulfanylacetylamino)hexanoic acid (**8**) (0.218 g, 0.48 mmol) in dry DMF, and the mixture was stirred for 10 min at room temperature. Then PyBOP (0.508 g, 0.97 mmol) and biphenyl amine **4d** (0.230 g, 0.48 mmol) were added, and stirring was continued overnight. The reaction mixture was diluted with Et_2O, washed consecutively with cold water, a saturated $NaHCO_3$ solution, a saturated NH_4Cl solution, and brine, and dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (acetone/hexanes 1:1) to give compound **9d** (0.224 g, 51%). ^1H NMR (CD_3OD, 300 MHz): $\delta = 1.30$–1.50 (m, 13H), 1.69 (t, $J = 6.7$ Hz, 2H), 2.28–2.40 (m, 2H), 2.90–3.02 (m, 2H), 3.05–3.20 (m, 3H), 3.24–3.45 (m, 1H), 4.38 (brs, 1H), 5.21 (brs, 1H), 6.11 (t, $J = 5.4$ Hz, 1H), 6.68–6.82 (m, 3H), 6.94–7.18 (m, 4H), 7.20–7.48 (m, 22H), 7.53 (d, $J = 7.7$ Hz, 1H), 7.69 (brs, 1H), 8.15 (s, 1H), 8.27 (d, $J = 6.9$ Hz, 1H), 9.08 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 14.6$, 19.3, 21.4, 25.4, 26.7, 28.6, 29.3, 36.4, 37.5, 39.9, 60.8, 68.2, 80.4, 109.9, 111.9, 119.0, 119.8, 121.6, 121.6, 122.3, 123.7, 124.9, 127.5, 127.7, 128.5, 129.8, 130.3, 132.6, 133.6, 134.6, 136.7, 138.0, 144.3, 155.6, 168.6, 170.6, 171.6, 172.2 ppm.

To a solution of compound **9d** (0.070 g, 0.077 mmol) in CH_2Cl_2 at 0 °C, TFA (1 mL) was added. The resulting yellow solution was treated dropwise with triethylsilane until the color disappeared. The mixture was then stirred for 2 h, and the solvent was evaporated. The residue was dissolved in EtOAc and washed consecutively with a saturated $NaHCO_3$ solution and brine, and the organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (MeOH/CH_2Cl_2 1:10) to give compound **10d** (0.026 mg, 60%). $[\alpha]_D^{24} = 11.7$ ($c = 0.16$, CH_3OH); ^1H NMR (CD_3OD, 400 Hz): $\delta = 1.50$–1.48 (m, 2H), 1.53–1.65 (m, 2H), 1.68–1.78 (m, 2H), 2.40 (t, $J = 7.4$ Hz, 2H), 3.03 (dd, $J = 7.1$ and 14.3 Hz, 1H), 3.13 (s, 2H), 3.15–3.28 (m, 3H), 3.69 (dd, $J = 6.9$ and 12.4 Hz, 1H), 6.97 (t, $J = 7.1$ Hz, 1H), 7.02–7.13 (m, 4H), 7.18–7.28 (m, 2H), 7.30–7.40 (m, 2H), 7.49 (d, $J = 8.4$ Hz, 2H), 7.58 (d, $J = 7.9$ Hz, 1H), 7.97 ppm (d, $J = 8.0$ Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 25.4$, 26.4, 26.5, 29.0, 29.0, 30.4, 36.7, 39.5, 39.7, 42.0, 55.9, 109.9, 111.3, 118.5, 118.9, 120.3, 121.5, 123.8, 123.3, 125.3, 127.7, 127.9, 129.6, 130.2, 134.5, 137.2, 138.3, 170.1, 173.4 ppm; ESI-HRMS calcd for $[C_{31}H_{36}N_5O_3S+H]^+$: 558.2539, found: 558.2533; HPLC purity: 96%.

6-(2-Mercaptoacetylamino)hexanoic acid [2′-(2-aminoacetylamino)-4-biphenyl]amide (10a): Compound **10a** was prepared according to the methodology described for the preparation of compound **10d**. ^1H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 1.32$ (m, 2H), 1.45 (m, 2H), 1.61 (m, 2H), 2.32 (t, $J = 7.3$ Hz, 2H), 3.10 (m, 2H), 3.35 (m, 4H), 3.47 (s, 2H), 7.23–7.37 (m, 7H), 7.70 (d, $J = 8.5$ Hz, 2H), 7.91 (d, $J = 7.9$ Hz, 1H), 8.11 (t, $J = 5.3$ Hz, 1H), 9.77 (s, 1H), 10.00 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta = 24.9$, 26.1, 28.8, 36.4, 38.2, 42.0, 42.9, 119.1, 123.5, 125.1, 127.7, 129.3, 130.3, 132.6, 133.8, 134.2, 138.8, 167.6, 168.7, 171.3 ppm; ESI-HRMS calcd for $[C_{22}H_{28}N_4O_3S+H]^+$: 429.1960, found: 429.1953; HPLC purity: 97%.

6-(2-Mercaptoacetylamino)hexanoic acid [2′-(2-amino-3-phenylpropionylamino)-4-biphenyl]amide (10b): Compound **10b** was prepared according to the methodology described for the preparation of compound **10d**. $[\alpha]_D^{24} = -22.0$ ($c = 0.5$, CH_3OH); ^1H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 1.07$ (t, $J = 7.0$ Hz, 1H), 1.17 (t, $J = 7.0$ Hz, 1H), 1.31 (m, 2H), 1.44 (m, 2H), 1.59 (m, 2H), 2.31 (m, 2H), 2.85 (m, 1H), 3.07 (m, 4H), 3.48 (brs, 2H), 3.90 (m, 1H), 7.14–7.37 (m, 10H), 7.64 (d, $J = 8.3$ Hz, 2H), 7.77 (d, $J = 7.7$ Hz, 1H), 8.11 (brs, 1H), 9.81 (brs, 1H), 9.98 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta = 25.2$, 26.5, 29.2, 36.8, 38.2, 42.4, 46.1, 55.0, 119.5, 124.9, 125.9, 127.2, 127.8, 128.8, 129.5, 129.8, 130.6, 132.9, 134.2, 135.0, 136.2, 139.1, 168.0, 169.3, 171.7 ppm; ESI-HRMS calcd for $[C_{29}H_{34}N_4O_3S]^+$: 518.2351, found: 518.2339; HPLC purity: 96%.

Pyrrolidine-2-carboxylic acid {4′-[6-(2-mercaptoacetylamino)hexanoylamino]-2-biphenyl}amide (10c): Compound **10c** was prepared according to the methodology described for the preparation of compound **10d**. $[\alpha]_D^{24} = 7.1$ ($c = 0.07$, CH_3OH); ^1H NMR ([D$_6$]DMSO, 400 MHz): $\delta = 1.25$–1.70 (m, 8H), 1.78 (m, 1H), 1.96 (m, 1H), 2.23–2.35 (m, 4H), 2.55 (m, 1H), 3.08 (m, 3H), 3.59 (m, 1H), 7.13–7.34 (m, 7H), 7.70 (d, $J = 11.2$ Hz, 2H), 8.11 (m, 1H), 8.28 (d, $J = 10.8$ Hz, 1H), 10.00 (brs, 1H), 10.13 ppm (brs, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): $\delta = 25.2$, 26.2, 26.5, 29.2, 30.7, 36.8, 42.4, 46.8, 60.9, 119.5, 120.4, 124.1, 128.6, 129.3, 129.8, 130.4, 132.0, 132.6, 135.4, 139.2, 168.0, 171.7, 173.5 ppm; ESI-HRMS calcd for $[C_{25}H_{32}N_4O_3S]^+$: 468.2195, found: 468.2186; HPLC purity: 98%.

(2-(4-Benzyloxyphenyl)-1-{4′-[6-(2-tritylsulfanylacetylamino)hexanoylamino]-2-biphenylcarbamoyl}ethyl)carbamic acid *tert*-butyl ester (9e): Compound **9e** (yield 76%) was prepared according to

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the methodology described for the preparation of compound **9d**. ^{1}H NMR (CD_3OD, 300 MHz,): δ = 1.20–1.45 (m, 13H), 1.71 (t, J = 6.6 Hz, 2H,), 2.32 (d, J = 7.2 Hz, 2H), 2.90–3.10 (m, 4H), 3.12 (s, 2H), 4.26 (br s, 1H), 4.90–5.10 (m, 3H), 6.07 (br s, 1H), 6.90 (d, J = 8.2 Hz, 2H), 6.95–7.11 (m, 4H), 7.12–7.45 (m, 24H), 7.58 (d, J = 7.9 Hz, 2H), 7.76 (br s, 1H), 7.93 (br s, 1H), 8.35 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz,): δ = 14.6, 21.4, 25.3, 26.6, 28.5, 29.3, 36.3, 37.7, 37.9, 39.8, 60.8, 68.3, 70.3, 80.5, 115.5, 120.4, 121.3, 124.9, 127.5, 127.8, 128.3, 128.6, 128.9, 129.4, 129.8, 129.9, 130.5, 130.7, 132.2, 133.3, 134.5, 137.3, 144.3, 138.4, 158.2, 168.4, 169.8, 171.5, 171.6 ppm.

6-(2-Mercaptoacetylamino)hexanoic acid {2′-[2-amino-3-(4-benzyloxyphenyl)propionylamino]-4-biphenyl}amide (10e): Compound **10e** (yield 45%) was prepared according to the methodology described for the preparation of compound **10d**. ^{1}H NMR (CD_3OD, 300 MHz): δ = 1.65–1.35 (m, 4H), 1.77 (t, J = 7.1 Hz, 2H), 2.38 (t, J = 8.1 Hz, 2H), 2.74 (dd, J = 13.9 and 8.6 Hz, 1H), 3.20 (s, 2H), 3.29 (dd, J = 12.7 and 6.3 Hz, 2H), 3.60 (dd, J = 8.3 and 3.8 Hz, 1H), 5.10–4.95 (m, 1H), 6.98–6.82 (m, 3H), 7.05–7.45 (m, 14H), 7.59 (d, J = 8.0 Hz, 2H), 7.92 (s, 1H), 8.38 (d, J = 8.0 Hz, 1H), 9.57 (s, 1H).

6-Acetylaminohexanoic acid {2′-[2-amino-3-(4-hydroxyphenyl)-propionylamino]-4-biphenyl}amide (11): A suspension of compound **10e** (0.035 g, 0.056 mmol) and $Pd(OH)_2$/C (20 wt%, 0.010 g) in MeOH (5 mL) was stirred under a hydrogen atmosphere at room temperature for 10 h. The catalyst was removed by filtration through a pad of Celite, and the residue was thoroughly washed with MeOH. The solvent was evaporated, and the crude material was dissolved in EtOAc, and the solvent was removed by rotary evaporation. The crude solid was purified by flash chromatography ($MeOH/CH_2Cl_2$ 1:10) to give compound **11** (0.007 g, 24%). ^{1}H NMR (CD_3OD, 300 MHz): δ = 1.50–1.45 (m, 2H), 1.65–1.50 (m, 2H), 1.85–1.70 (m, 2H), 1.93 (s, 3H), 2.41 (t, J = 7.1 Hz, 2H), 2.75 (dd, J = 13.2 and 7.2 Hz, 1H), 2.93 (dd, J = 13.3 and 6.1 Hz, 1H), 3.19 (t, J = 6.9 Hz, 2H), 3.57 (t, J = 6.0 Hz, 1H), 6.74 (d, J = 8.2 Hz, 2H), 7.02 (d, J = 8.2 Hz, 2H), 7.40–7.13 (m, 5H), 7.60 (d, J = 8.3 Hz, 2H), 7.96 ppm (d, J = 7.9 Hz, 1H); ^{13}C NMR (CD_3OD, 75 MHz): δ = 21.5, 25.5, 26.5, 29.1, 36.8, 39.2, 39.7, 56.8, 105.7, 115.4, 120.3, 123.4, 125.4, 127.9, 128.1, 129.6, 130.3, 130.5, 134.4, 138.4, 156.4, 172.2, 173.5, 174.3 ppm.

Typical procedure for 4-phenylthiazolylamides of octanedioic acid hydroxyamide 16a, 16b, 17a, and 17b: The synthesis of ligand **16b** was described in our previous work.[18]

Octanedioic acid [4-(3-nitrophenyl)thiazol-2-yl]amide methyl ester (13b): A stirred solution of 4-(3-nitrophenyl)thiazol-2-ylamine **12b** (2.21 g, 10 mmol) and suberic acid monomethyl ester (1.88 g, 10 mmol) in dry pyridine (20 mL) was cooled to −15 °C, and $POCl_3$ (1.2 mL, 13 mmol) was added dropwise over 30 min. After stirring for another 1 h at the same temperature, the reaction mixture was diluted with EtOAc and washed thoroughly with saturated aqueous $KHSO_4$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude material was washed with EtOAc to give compound **13b** (2.40 g, 62%). ^{1}H NMR ([D_6]DMSO, 400 MHz): δ = 1.30 (br s, 4H), 1.53 (t, J = 6.8 Hz, 2H), 1.61 (t, J = 6.5 Hz, 2H), 2.30 (t, J = 7.3 Hz, 2H), 2.46 (t, J = 7.5 Hz, 2H), 7.74 (t, J = 7.9 Hz, 1H), 3.58 (3H, s), 7.93 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 8.35 (d, J = 7.1 Hz, 1H), 8.74 (d, J = 1.4 Hz, 1H), 12.3 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 24.6, 24.8, 28.5, 28.6, 33.6, 35.2, 51.6, 110.8, 120.5, 122.7, 130.8, 132.1, 136.2, 146.7, 148.7, 158.8, 172.1 ppm.

Octanedioic acid [4-(2-nitrophenyl)thiazol-2-yl]amide methyl ester (13a): Compound **13a** was prepared according to the methodology described for the preparation of compound **13b**; the crude solid was washed with MeOH to get pure **13a** (yield 48%). ^{1}H NMR ([D_6]DMSO, 400 MHz): δ = 1.28 (br s, 4H), 1.52 (t, J = 6.1 Hz, 2H), 1.57 (t, J = 6.6 Hz, 2H), 2.29 (t, J = 7.4 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 3.58 (s, 3H), 7.52 (s, 1H), 7.61 (t, J = 7.9 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 8.6 Hz, 1H), 12.1 ppm (s, 1H); ^{13}C NMR ([D_6]DMSO, 100 MHz): δ = 24.6, 24.8, 28.5, 28.6, 33.6, 35.1, 51.6, 112.2, 124.4, 128.8, 129.6, 131.2, 132.9, 145.3, 149.0, 158.3, 172.1, 173.7 ppm.

Octanedioic acid [4-(3-aminophenyl)thiazol-2-yl]amide methyl ester (14b): A suspension of compound **13b** (0.391 g, 1 mmol) and Pd/C (10 wt%, 50 mg) in EtOH and AcOH (20 mL + 1 mL) was reacted under hydrogen atmosphere at 50 °C for 2 h. The catalyst was removed by filtration through a pad of Celite. The solvent was evaporated, and the crude material was dissolved in EtOAc, washed consecutively with $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The crude solid was purified by flash chromatography (EtOAc/hexanes 2:1) to give compound **14b** (0.261 g, 72%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.08–1.22 (m, 4H), 1.61–1.45 (m, 4H), 2.06 (dd, J = 7.3 and 4.7 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 3.69 (s, 3H), 6.70–6.66 (m, 1H), 7.10 (s, 1H), 7.16 (br s, 1H), 7.20 (s, 1H), 7.22 (s, 1H), 11.2 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.4, 25.00, 25.09, 29.01, 29.07, 34.3, 36.1, 51.9, 108.1, 113.0, 115.3, 116.9, 130.2, 135.7, 147.3, 150.1, 159.6, 171.8, 174.6 ppm.

Octanedioic acid [4-(2-aminophenyl)thiazol-2-yl]amide methyl ester (14a): Compound **14a** (yield 81%) was prepared according to the methodology described for the preparation of compound **14b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.19–1.30 (m, 4H), 1.55–1.62 (m, 4H), 2.12 (t, J = 7.5 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 3.61 (s, 3H), 4.73 (br s, 1H), 6.76 (m, 2H), 7.07 (s, 1H), 7.17 (t, J = 7.0 Hz, 1H), 7.47 (d, J = 7.0 Hz, 1H), 10.38 ppm (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.5, 24.6, 28.5, 28.6, 33.9, 35.6, 51.5, 108.9, 116.8, 118.4, 119.0, 129.1, 129.4, 158.3, 171.0, 174.1 ppm.

Octanedioic acid hydroxyamide [4-(3-aminophenyl)thiazol-2-yl]amide (17b): KOH (0.772 g, 13.8 mmol) was added at 40 °C for 10 min to a solution of hydroxylamine hydrochloride (0.958 g, 13.8 mmol) in MeOH. The reaction mixture was cooled to 0 °C and filtered. Compound **14b** (0.250 g, 0.69 mmol) was added to the filtrate followed by KOH (50 mg, 0.89 mmol) at room temperature for 30 min. The reaction mixture was extracted with EtOAc, and the organic layer was washed with a saturated NH_4Cl aqueous solution and brine, dried over Na_2SO_4, filtered, and concentrated. The crude solid was purified by preparative HPLC to give compound **17b** (0.110 g, 44%). ^{1}H NMR (CD_3OD, 300 MHz): δ = 1.35–1.50 (m, 4H), 1.65 (t, J = 7.0 Hz, 2H), 1.74 (t, J = 7.0 Hz, 2H), 2.11 (t, J = 7.2 Hz, 2H), 2.51 (t, J = 7.2 Hz, 2H), 7.29 (dd, J = 7.8 and 1.2 Hz, 1H), 7.51 (s, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.90 (s, 1H), 7.98 ppm (d, J = 7.8 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 24.3, 24.7, 27.9, 28.0, 34.6, 108.5, 119.2, 120.8, 125.1, 129.7, 136.3, 147.4, 158.0, 172.1 ppm; ESI-HRMS calcd for $[C_{17}H_{22}N_4O_3S+H]^+$: 363.1485, found: 363.1482; HPLC purity: 97%

Octanedioic acid hydroxyamide [4-(2-aminophenyl)thiazol-2-yl]amide (17a): Compound **17a** (yield 50%) was prepared according to the methodology described for the preparation of compound **17b**. ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.35–1.50 (m, 4H), 1.66 (m, 2H), 1.77 (m, 2H), 2.12 (t, J = 7.3 Hz, 2H), 2.55 (t, J = 7.3 Hz, 2H), 7.35 (d, J = 7.7 Hz, 1H), 7.43 (m, 2H), 7.63 (s, 1H), 7.94 ppm (d, J = 7.6 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 24.3, 24.7, 27.9, 28.0, 31.8, 34.7, 110.3, 122.7, 125.3, 126.7, 128.0, 128.7, 145.8, 171.13, 172.0 ppm; ESI-HRMS calcd for $[C_{17}H_{22}N_4O_3S+H]^+$: 363.1485, found: 363.1485; HPLC purity: 97%.

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Octanedioic acid [4-(3-nitrophenyl)thiazol-2-yl]amide (15b): $LiOH{\cdot}H_2O$ (0.839 g, 20.0 mmol) was added to a solution of compound **13b** (0.391 g, 1.0 mmol) in a mixture of MeOH (10 mL) and water (10 mL), and the mixture was stirred at room temperature for 3 h. The reaction mixture was then acidified to pH 5 with 1 N HCl dropwise and extracted with EtOAc. The organic layer was washed consecutively with water and brine, dried over Na_2SO_4, filtered, and then concentrated. The solvent was evaporated to give compound **15b** (0.322 g, 86%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 1.18–1.30 (m, 4H), 1.49 (t, J = 6.3 Hz, 2H), 1.60 (brs, 2H), 2.19 (t, J = 5.7 Hz, 2H), 2.45 (t, J = 6.5 Hz, 2H), 7.72 (t, J = 8.0 Hz, 1H), 7.91 (s, 1H), 8.16 (dd, J = 7.9 and 2.8 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H), 8.72 (s, 1H), 12.3 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ = 25.1, 25.3, 29.1, 31.2, 34.4, 35.7, 111.2, 120.9, 123.0, 131.2, 132.5, 136.6, 147.1, 149.1, 159.2, 172.5, 175.3 ppm.

Octanedioic acid hydroxyamide [4-(3-nitrophenyl)thiazol-2-yl]amide (16b): Et_3N (0.18 mL, 1.3 mmol) under nitrogen was added to a solution of compound **15b** (0.100 g, 0.26 mmol) in dry THF, and the solution was stirred for 5 min. The solution was cooled to −15 °C and stirred for another 5 min. Isobutyl chloroformate (67 μL, 0.52 mmol) was then added dropwise, and the mixture was stirred for 15 min. The solid was filtered; the filtrate was cooled to 0 °C, and a solution of NH_2OH (aq, 50%, 1 mL) was added over 10 min. The reaction mixture was diluted with EtOAc, washed with saturated aqueous NH_4Cl and brine, dried over Na_2SO_4, filtered, and concentrated. The solvent was removed by rotary evaporation. The crude solid was purified by preparative HPLC to give compound **16b** (0.027 g, 26%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 1.35–1.50 (m, 4H), 1.50 (t, J = 6.1 Hz, 2H), 1.60 (brs, 2H), 1.94 (t, J = 7.2 Hz, 2H), 2.45 (t, J = 7.2 Hz, 2H), 7.73 (t, J = 7.9 Hz, 1H), 7.91 (s, 1H), 8.17 (dd, J = 8.1 and 1.5 Hz, 1H), 8.34 (d, J = 7.7 Hz, 1H), 8.67 (brs, 1H), 8.72 (s, 1H), 10.3 (s, 1H), 12.3 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 24.9, 25.4, 28.7, 32.6, 35.3, 110.8, 120.4, 122.7, 130.8, 132.1, 136.2, 146.7, 148.7, 158.8, 169.5, 172.1 ppm; ESI-HRMS calcd for $[C_{17}H_{20}N_4O_5S+H]^+$: 393.1227, found: 393.1227; HPLC purity: 96%.

Octanedioic acid hydroxyamide [4-(2-nitrophenyl)thiazol-2-yl]amide (16a): Compound **16a** was prepared from **13a** according to the methodology described for the preparation of compound **16b**. ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 1.26 (m, 4H), 1.48 (m, 2H), 1.58 (m, 2H), 1.94 (t, J = 7.04 Hz, 2H), 2.44 (t, J = 7.04 Hz, 2H), 7.52 (s, 1H), 7.61 (t, J = 7.4 Hz, 1H), 7.71–7.79 (m, 2H), 7.89 (d, J = 7.8 Hz, 1H), 10.34 (s, 1H), 12.15 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 25.0, 25.4, 28.7, 32.7, 35.2, 112.2, 124.4, 128.8, 129.7, 131.3, 133.0, 145.3, 149.0, 158.4, 169.5, 172.2 ppm; ESI-HRMS calcd for $[C_{17}H_{20}N_4O_5S+H]^+$: 393.1227, found: 393.1227; HPLC purity: 97%.

Octanedioic acid {4-[3-(2-aminoacetylamino)phenyl]thiazol-2-yl}amide hydroxyamide (21): EEDQ (0.333 g, 1.35 mmol) was added to a solution of compound **14b** (0.100 g, 0.27 mmol) and Boc-Gly-OH (0.242 mg, 1.35 mmol) in dry DMF (10 mL) at room temperature, and the mixture was stirred at 40 °C overnight. The reaction mixture was diluted with EtOAc, washed with water, a saturated $NaHCO_3$ solution, a saturated NH_4Cl solution, and brine, and dried over Na_2SO_4. The solvent was evaporated, and the crude product was purified by flash chromatography (EtOAc/hexanes 1:2 then 1:1) to give compound **18** (0.087 g, 61%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.33 (brs, 4H), 1.45 (s, 9H), 1.62 (t, J = 6.8 Hz, 2H), 1.71 (brs, 2H), 2.31 (t, J = 7.4 Hz, 2H), 2.43 (brs, 2H), 3.68 (s, 3H), 4.04 (brs, 2H), 7.08 (s, 1H), 7.33 (t, J = 7.7 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.54 (d, J = 7.2 Hz, 1H), 7.91 (brs, 1H), 8.60 ppm (brs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.6, 24.7, 28.2, 28.7, 33.9, 35.9, 51.5, 108.1, 117.7, 122.1, 129.4, 138.0, 148.6, 159.4, 171.7, 174.2 ppm.

$LiOH{\cdot}H_2O$ (0.097 g, 2.2 mmol) was added to a solution of compound **18** (0.06 g, 0.11 mmol) in a mixture of MeOH (10 mL) and water (10 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was acidified to pH 5 with 1 N HCl dropwise and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and then filtered. The solvent was evaporated to give compound **19** (0.047 g, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.39 (brs, 4H), 1.46 (s, 9H), 1.65 (t, J = 6.7 Hz, 2H), 1.75 (brs, 2H), 2.35 (t, J = 7.3 Hz, 2H), 2.50 (t, J = 7.4 Hz, 2H), 4.01 (brs, 2H), 7.02 (s, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.38 (brs, 1H), 7.53 (brs, 1H), 7.84 (brs, 1H), 8.78 ppm (brs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.4, 24.7, 28.2, 28.5, 33.9, 35.8, 108.3, 117.9, 119.8, 122.4, 129.4, 134.3, 137.9, 148.5, 160.3, 171.9, 177.0 ppm.

HOBt (0.025 g, 0.178 mmol) and EDCI (0.034 g, 0.178 mmol) were added sequentially to a stirred solution of compound **19** (0.045 g, 0.089 mmol) and $THPONH_2$ (0.021 mg, 0.178 mmol) in dry CH_2CL_2 (10 mL) at room temperature, and stirring was continued overnight. The reaction mixture was diluted with EtOAc, washed with water, a saturated $NaHCO_3$ solution, a saturated NH_4Cl solution, and brine, and dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes 1:1, then EtOAc) to give compound **20** (0.047 g, 89%). ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ = 1.27 (brs, 4H), 1.41 (s, 9H), 1.45–1.70 (m, 10H), 1.98 (t, J = 6.5 Hz, 2H), 2.45 (t, J = 7.1 Hz, 2H), 3.45–3.52 (m, 1H), 3.73 (s, 2H), 3.88–3.95 (s, 1H), 4.80 (s, 1H), 7.30–7.40 (m, 2H), 7.52 (s, 1H), 7.57 (d, J = 7.1 Hz, 1H), 8.29 (s, 1H), 9.98 (s, 1H), 10.90 (s, 1H), 12.28 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 18.7, 25.0, 25.1, 25.2, 28.2, 28.6, 32.5, 35.2, 44.1, 61.7, 78.4, 101.2, 108.4, 117.2, 118.9, 121.0, 129.4, 135.2, 139.7, 149.1, 156.4, 158.3, 168.6, 169.4, 172.0 ppm.

TFA (2 mL) was added to a solution of compound **20** (0.040 mg, 0.066 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 10 min, the reaction mixture was concentrated in vacuo. The crude material was purified by preparative HPLC to give compound **21** (0.016 g, 67%). ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.42 (brs, 4H), 1.65 (t, J = 6.8 Hz, 2H), 1.75 (t, J = 7.0 Hz, 2H), 2.11 (t, J = 7.4 Hz, 2H), 2.50 (t, J = 7.3 Hz, 2H), 3.89 (s, 2H), 7.36 (s, 1H), 7.39 (d, J = 7.9 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 7.7 Hz, 1H), 8.18 ppm (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 24.3, 24.7, 27.9, 28.0, 31.8, 34.6, 40.3, 107.2, 116.6, 118.4, 121.5, 128.4, 135.1, 137.7, 148.8, 157.6, 163.6, 172.0 ppm; ESI-HRMS calcd for $[C_{19}H_{25}N_5O_4S_1+H]^+$: 420.1700, found: 420.1697; HPLC purity: 95%.

Octanedioic acid [4-(3-aminophenyl)thiazol-2-yl]amide (22): Compound **22** (yield 80%) was prepared according to the methodology described for the preparation of compound **15b**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.35–1.50 (m, 4H), 1.69 (t, J = 6.7 Hz, 2H), 1.78 (brs, 2H), 2.40 (t, J = 7.1 Hz, 2H), 2.49 (t, J = 7.3 Hz, 2H), 6.69 (d, J = 7.8 Hz, 1H), 6.99 (s, 2H), 7.07 (d, J = 8.2 Hz, 1H), 7.21 ppm (t, J = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 24.9, 25.5, 28.9, 29.1, 34.4, 130.2, 36.7, 108.1, 113.5, 115.6, 117.3, 135.0, 147.1, 149.7, 160.8, 172.2, 179.6 ppm.

Octanedioic acid hydroxyamide [4-(3-urethanylphenyl)thiazol-2-yl]amide (23): Compound **23** was prepared according to the methodology described for the preparation of compound **16b** by substituting compound **15b** with compound **22** and using ethyl chloroformate. The crude material was purified by preparative HPLC to give the desired product (0.130 g, 12%). ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ = 1.23–1.27 (m, 5H), 1.48 (m, 2H), 1.59 (m, 2H), 1.93 (t, J = 6.8 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 4.13 (q, J = 7.1 Hz, 2H), 7.31

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(brs, 2H), 7.47 (brs, 2H), 8.11 (s, 1H), 9.68 (s, 1H), 10.3 (s, 1H), 12.2 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ = 14.9, 25.0, 25.4, 28.7, 32.6, 35.2, 60.6, 108.3, 116.0, 118.2, 120.3, 129.4, 135.3, 140.0, 149.2, 154.0, 158.3, 169.5, 172.0 ppm; ESI-HRMS calcd for $[C_{20}H_{26}N_4O_5S+H]^+$: 435.1696, found: 435.1694; HPLC purity: 96%.

7-[4-(2-*tert*-Butoxycarbonylaminophenyl)thiazol-2-ylcarbamoyl]-heptanoic acid methyl ester (24a): A mixture of **14a** (0.1 g, 0.27 mmol) and di-*tert*-butyl dicarbonate (0.6 g, 2.7 mmol) in THF was held at reflux overnight. The solvent was then evaporated in vacuo and the residue was dissolved in EtOAc, washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes 1:3) to give compound **24a** (0.087 g, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz,): δ = 1.05–1.11 (m, 2H), 1.16–1.24 (m, 2H), 1.34 (s, 9H), 1.50–1.60 (m, 4H), 1.98 (t, J = 7.6 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 3.67 (s, 3H), 7.07 (t, J = 8.0 Hz, 1H), 7.12 (s, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 8.32 (brs, 1H), 8.98 (brs, 1H), 11.03 ppm (brs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.3, 24.6, 28.4, 28,6, 28.7, 33.9, 35.5, 51.4, 110.5, 121.6, 122.6, 129.2, 129.5, 136.3, 148.0, 152.7, 159.6, 171.3, 174.1 ppm.

{2-[2-(7-Hydroxycarbamoylheptanoylamino)thiazol-4-yl]phenyl}-carbamic acid *tert*-butyl ester (25a): Compound **25a** (yield 57%) was prepared according to the methodology described for the preparation of compound **17b**. ^{1}H NMR (CD_3OD, 400 MHz,): δ = 1.41 (brs, 4H), 1.52 (s, 9H), 1.65 (t, J = 6.8 Hz, 2H), 1.74 (t, J = 6.8 Hz, 2H), 2.10 (t, J = 7.2 Hz, 2H), 2.51 (t, J = 7.2 Hz, 2H), 7.08 (t, J = 7.6 Hz, 1H), 7.28–7.32 (m, 2H), 7.62 (d, J = 8.0 Hz, 1H), 8.00 ppm (d, J = 8.0 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 24.3, 24.7, 26.8, 27.9, 28.0, 31.8, 34.6, 79.6, 109.4, 120.1, 122.3, 123.0, 127.8, 128.0, 135.4, 147.9, 153.2, 157.8, 171.1, 172.0 ppm; ESI-HRMS calcd for $[C_{22}H_{30}N_4O_5S_1+H]^+$: 463.2009, found: 463.2003; HPLC purity: 96%.

{3-[2-(7-Hydroxycarbamoylheptanoylamino)thiazol-4-yl]phenyl}-carbamic acid *tert*-butyl ester (25b): Compound **25b** (yield 64%) was prepared according to the methodology described for the preparation of compound **17b**. ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.40 (brs, 4H), 1.62 (s, 9H), 1.64 (t, J = 6.8 Hz, 2H), 1.73 (t, J = 6.4 Hz, 2H), 2.10 (t, J = 7.6 Hz, 2H), 2.48 (t, J = 7.6 Hz, 2H), 7.28–7.29 (m, 2H), 7.33 (s, 1H), 7.54 (t, J = 2.8 Hz, 1H), 8.03 ppm (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz,): δ = 24.7, 25.1, 27.2, 28.3, 32.2, 35.0, 79.0, 107.2, 116.1, 117.8, 120.3, 125.8, 128.5, 135.1, 139.4, 149.6, 153.9, 171.5, 172.4 ppm; ESI-HRMS calcd for $[C_{22}H_{30}N_4O_5S_1+H]^+$: 463.2009, found: 463.2003; HPLC purity: 96%.

7-{4-[3-(2,2-Dimethylpropionylamino)phenyl]thiazol-2-yl-carbamoyl}heptanoic acid methyl ester (26): A mixture of **14b** (0.120 g, 0.33 mmol) and trimethylacetic anhydride (0.618 g, 3.3 mmol) in dry THF was held at reflux overnight. The solvent was then evaporated, and the residue was dissolved in EtOAc, then washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes 1:3) to give compound **26** (0.117 g, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz,): δ = 1.06–1.14 (m, 2H), 1.17–1.23 (m, 2H), 1.34 (s, 9H), 1.53–1.55 (m, 4H), 2.16 (t, J = 8.0 Hz, 2H), 2.28 (t, J = 8.0 Hz, 2H), 3.68 (s, 3H), 7.16 (s, 1H), 7.34 (t, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.74 (s, 1H), 8.17 (s, 1H), 10.95 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz,): δ = 24.6, 24.7, 27.5, 28.6, 33.8, 35.8, 39.6, 51.5, 108.2, 117.9, 119.6, 121.8, 129.2, 149.1, 158.9, 171.5, 174.3, 176.9 ppm.

Octanedioic acid {4-[3-(2,2-dimethylpropionylamino)phenyl]thiazol-2-yl}amide hydroxyamide (27): Compound **27** (yield 42%) was prepared according to the methodology described for the preparation of compound **17b**. ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.32 (s, 9H), 1.41 (brs, 4H), 1.64 (t, J = 6.8 Hz, 2H), 1.74 (t, J = 6.4 Hz, 2H), 2.11 (t, J = 7.2 Hz, 2H), 2.50 (t, J = 7.2 Hz, 2H), 7.32–7.36 (m, 2H), 7.44 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 8.11 ppm (s, 1H); ^{13}C NMR (CD_3OD, 100 MHz,): δ = 24.7, 25.1, 26.3, 28.3, 32.2, 35.0, 39.0, 107.3, 119.0, 120.7, 121.7, 125.8, 128.4, 135.0, 138.6, 149.4, 157.9, 172.4, 178.5 ppm; ESI-HRMS calcd for $[C_{22}H_{30}N_4O_4S_1+H]^+$: 447.2060, found: 447.2052; HPLC purity: 96%.

7-{4-[3-(Cyclohexanecarbonylamino)phenyl]thiazol-2-yl-carbamoyl}heptanoic acid methyl ester (28): A mixture of **14b** (0.100 g, 0.27 mmol) and cyclohexanecarbonyl chloride (0.45 g, 2.7 mmol) in dry THF was held at reflux overnight. The solvent was then evaporated, and the residue was dissolved in EtOAc, washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes 1:3) to give compound **28** (0.082 g, 61%). ^{1}H NMR ($CDCl_3$, 400 MHz,): δ = 1.05–1.40 (m, 7H), 1.50–1.60 (m, 6H), 1.65–1.70 (m, 1H), 1.78–1.82 (m, 2H), 1.93–1.96 (m, 2H), 2.13 (t, J = 7.6 Hz, 2H), 2.27 (t, J = 7.6 Hz, 2H), 3.68 (s, 3H), 3.97 (t, J = 8.0 Hz, 1H), 5.63–5.65 (m, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.08 (s, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 8.10 (s, 1H), 8.16 (s, 1H), 11.06 ppm (brs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.8, 24.6, 25.6, 28.6, 28.7, 29.1, 29.5, 33.9, 35.7, 46.3, 51.5, 67.6, 107.8, 108.1, 117.8, 119.4, 121.6, 129.2, 134.9, 138.8, 159.0, 171.6, 174.4, 175.2 ppm.

Octanedioic acid {4-[3-(cyclohexanecarbonylamino)phenyl]thiazol-2-yl}amide hydroxyamide (29): Compound **29** (yield 45%) was prepared according to the methodology described for the preparation of compound **17b**. ^{1}H NMR (400 MHz, CD_3OD): δ = 1.27–1.40 (m, 8H), 1.51–1.63 (m, 4H), 1.73–1.75 (m, 2H), 1.83–1.91 (m, 4H), 2.17 (brs, 2H), 2.37–2.42 (m, 1H), 2.49 (t, J = 7.2 Hz, 2H), 7.31–7.34 (m, 2H), 7.45 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 8.14 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 24.7, 25.1, 25.3, 25.4, 28.3, 29.2, 35.0, 45.7, 117.6, 119.3, 121.3, 128.5, 135.1, 138.9, 172.4, 176.3 ppm; MS ESI-HRMS calcd for $[C_{24}H_{32}N_4O_4S_1+H]^+$: 473.2217, found: 473.2207; HPLC purity: 96%.

HDAC inhibition assays

Purified HDACs were incubated with 1 μM carboxyfluorescein (FAM)-labeled acetylated peptide substrate and test compound for 17 h at 25 °C in HDAC assay buffer containing 100 mM HEPES (pH 7.5), 25 mM KCl, 0.1% BSA, and 0.01% Triton X-100. Reactions were terminated by the addition of buffer containing 0.078% SDS for a final SDS concentration of 0.05%. Substrate and product were separated electrophoretically using a Caliper LabChip 3000 system with blue laser excitation and green fluorescence detection (CCD2). The fluorescence intensity in the substrate and product peaks was determined using the Well Analyzer software on the Caliper system. The reactions were performed in duplicate for each sample. IC_{50} values were automatically calculated using the IDBS XLFit version 4.2.1 plug-in for Microsoft Excel and the XLFit 4-Parameter Logistic Model (sigmoidal dose–response model): $((A + ((B-A)/1 + ((C/x)^D))))$, in which x is compound concentration, A and B are respectively the estimated minimum and maximum of percent inhibition, C is the inflection point, and D is the Hill slope of the sigmoidal curve. The standard errors of the IC_{50} values were automatically calculated using the IDBS XLFit version 4.2.1 plug-in for Microsoft Excel and the formula xf4_FitResultStdError().

Cytotoxicity assays

The pancreatic cancer cell lines BxPc-3, HupT3, Mia Paca-2, Panc 04.03, and SU 86.86 were obtained from ATCC (Rockville, MD,

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USA) and were grown in medium (DMEM or RPMI) containing 10% fetal calf serum and L-glutamine. Pancreatic cancer cells were plated out in duplicate into 6 wells of a 96-well microtiter plate at $2.5–4\times10^3$ cells per well. Four hours post plating, individual wells were treated with diluent (DMSO) or varying concentrations of SAHA or the indicated HDACIs from a concentration of 1 nm to 50 μM. Cytotoxicity was measured at time "0", and 72 h post treatment using the colorimetric MTT assay according to the manufacturer's suggestions (Promega, Madison, WI, USA). The IC_{50} values were calculated using XLfit (IDBS Limited, Guildford, UK).

Reagent abbreviations

Boc	*tert*-butyloxycarbonyl
DIPEA	*N*,*N*-diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
EDCI	3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
HOBt	1-hydroxy-1*H*-benzotriazole
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
PyBOP	1-benzotriazolyloxytris(pyrrolidino)phosphonium hexafluorophosphate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TMS	tetramethylsilane

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