Design and Synthesis of a Potent Histone Deacetylase Inhibitor

Tao Liu, Galina Kapustin, and Felicia A. Etzkorn*

Virginia Tech, Department of Chemistry MC 0212, Blacksburg, Virginia 24061-0212

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Abstract: Histone deacetylase (HDAC) inhibitors have potential for cancer therapy. An HDAC inhibitor based on a cyclic peptide mimic of known structure, linked by an aliphatic chain to a hydroxamic acid, was designed and synthesized. The chimeric compound showed potent competitive inhibition of nuclear HDACs, with an IC_{50} value of 46 nM and a K_i value of 13.7 nM. The designed inhibitor showed 4-fold selectivity for HDAC1 (57 nM) over HDAC8 (231 nM).

DNA is assembled into nucleosomes, which are the fundamental repeating subunits of all eukaryotic chromatin. Nucleosomes are made up of DNA (146 base pairs) wrapped around an octamer of histones.[1] Post-translational modification of the *N*-terminal tails of the histones, including acetylation, phosphorylation, and methylation, alter the chromatin structure and regulate gene transcription.[2] Histones can exist in two forms, acetylated and deacetylated, which are controlled by histone acetyltransferases (HATs[a]) and histone deacetylases (HDACs). HDACs are divided into three classes based on mechanism,[3] of which classes I and II are therapeutic targets for the treatment of leukemia[4] and solid tumors.[5,6] Recent studies have shown that inhibition of HDACs silences the growth of tumor cells by introducing terminal differentiation, growth arrest, and apoptosis.[7,8] Quite a few inhibitors are in phase I or II clinical trials:[9] suberoylanilide hydroxamic acid (SAHA),[10] butyrate,[11] 4-(acetylamino)-*N*-(2-aminophenyl) benzamide (CI-994),[12,13] and cyclo[(2*Z*)-2-amino-2-butenoyl-L-valyl-(3*S*,4*E*)-3-hydroxy-7-mercapto-4-heptenoyl-D-valyl-D-cysteinyl], cyclic (3→5)-disulfide (FK228).[14]

Many HDAC inhibitors have been reported, both naturally occurring, such as trichostatin A (TSA),[15] apicidin,[16] and trapoxin,[17] and synthetic, such as SAHA[18] (Figure 1). These HDAC inhibitors can be divided into categories according to their structural characteristics, such as hydroxamates, carboxylates, benzamides, and cyclic peptides, though most are hydroxamic acid derivatives.[19] We have shown that charged phosphorus-based compounds are poor inhibitors of HDACs,[20] which may distinguish the mechanism of HDAC-catalyzed deacetylation from that of zinc protease amide hydrolysis.[21] Based on SAR studies, efficient HDAC inhibitors should have three features: (1) a hydrophobic region that binds the rim of the active site and blocks the entrance, (2) a coordinating group to chelate to Zn^{2+} at the bottom of the tubular pocket, and (3) a five- to seven-atom linker from the hydrophobic region to the coordinating group.[19] The linker helps insert the hydroxamic acid into the bottom of the tubular active site.

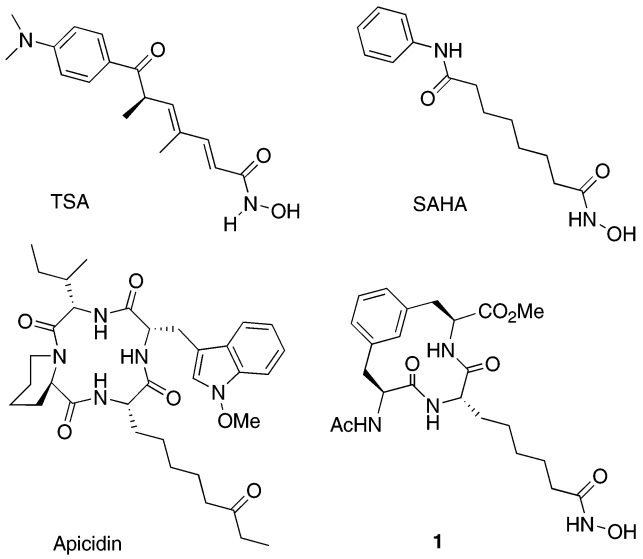

Figure 1. Naturally occurring and synthetic HDAC inhibitors, of which the hydroxamic acid derivatives (TSA[15] and SAHA[18]) are the most common. Cyclic peptides, such as apicidin,[16] and cyclic peptide mimic **1** are another class of potent HDAC inhibitors.

Most inhibitors exhibit IC_{50} values against HDACs in the micromolar range.[19] However, some inhibitors containing a large active site rim recognition element, such as trapoxin, apicidin,[16] and cyclic tetrapeptides,[22−24] give nanomolar IC_{50} values against HDACs. The high potency of these tetrapeptides suggested that introduction of a macrocycle into an HDAC inhibitor would be promising.

HDAC inhibitor (HDI) **1** was designed based on a cyclic peptide mimic previously synthesized in our lab.[25] We have investigated alternative phosphorus-containing head groups for HDAC inhibitors and found them deficient, so we chose the hydroxamic acid head group.[20] Combining the cyclic peptide mimic with the hydroxamic acid functionality not only retained the hydrophobic property of the cyclic peptide mimic, but also introduced the linker and zinc-binding functional group into the targeted molecule **1**. The three-dimensional structure of the cyclic peptide mimic was determined by NMR in previous work.[26] In the molecular modeling, the known 3-D structure of the cyclic peptide mimic[26] was attached to the linker and hydroxamic acid of SAHA from the cocrystal structure with the bacterial histone deacetylase-like protein (HDLP),[27] which is similar to class I HDACs.[28] Compound **1** was docked manually by superposition onto SAHA in the HDLP active site. Several conformers around the Cα−Cβ torsion were minimized in the active site using Sybyl 7.1 (Tripos Associates, see Supporting Information).

Molecular visualization shows that there are several pockets on the HDLP surface (Figure 2). The surface around the entrance to the active site is mostly nonpolar. The aromatic group and the 12-membered ring of compound **1** were predicted to make good contacts with the enzyme surface recognition site, and the hydroxamic acid group is known to bind tightly to Zn^{2+}. The results of the computational study indicated that the designed peptide mimic would be an efficient HDAC inhibitor. Because

* To whom correspondence should be addressed. Phone: 540-231-2235. Fax: 540-231-3255. E-mail: fetzkorn@vt.edu.

[a] Abbreviations: DPPA, diphenylphosphoryl azide; FDDP, pentafluorophenyl diphenyl phosphate; HAT, histone acetyl transferase; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HDAC, histone deacetylase; HDI, histone deacetylase inhibitor; HDLP, histone deacetylase like protein; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, hydroxybenzotriazole; PyAOP, (7-azabenzotriazol-1-yloxy) tripyrrolidino-phosphonium hexafluorophosphate; SAHA, suberoylanilide hydroxamic acid; TBAF, 1-tetra-*n*-butyl ammonium fluoride; TBS, *t*-butyldimethylsilyl; TES, triethylsilane; TFFH, *N*,*N*,*N*′,*N*′-tetramethylfluoroformamidinium hexafluorophosphate; TMSE, trimethylsilylethyl; TSA, trichostatin A.

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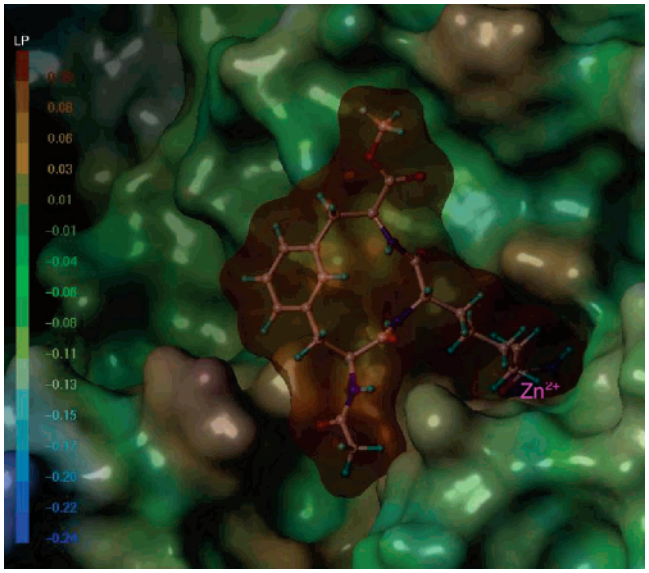
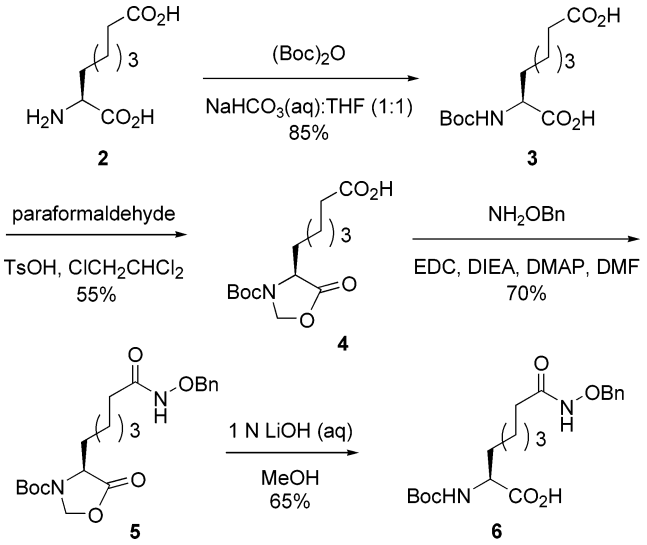


Figure 2. Molecular model of the rim and the active site of the complex of compound **1** (ball and stick with translucent red-orange surface) and HDLP (lipophilic potential surface with scale at left). Created with Sybyl 7.1.

Scheme 1



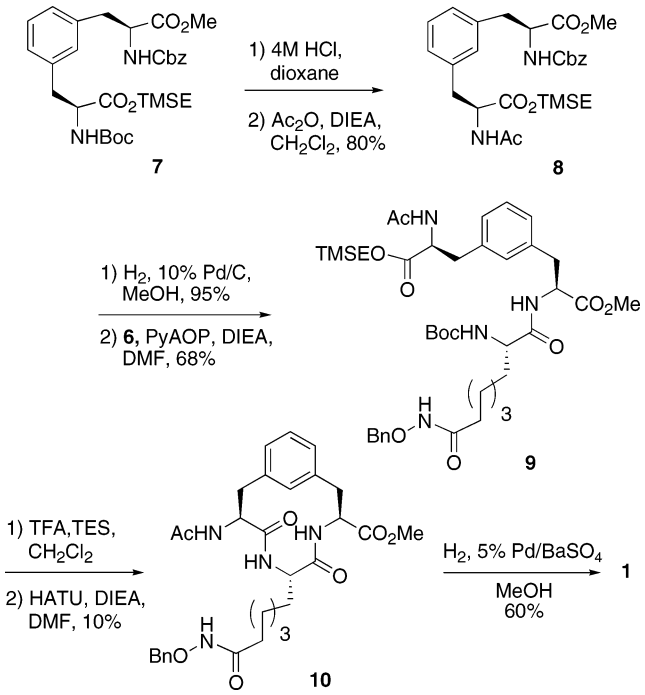
compound **1** does not contain natural α-amino acids, it may be less susceptible to biodegradation than the cyclic peptide inhibitors. We now present the synthesis of compound **1** and describe its inhibitory activity against HDACs.

The amine group of α-(*S*)-aminosuberic acid **2** was protected by treatment with Boc_2O in dioxane−water (Scheme 1). The formation of oxazolidinone **4** was successfully carried out by treatment with paraformaldehyde and TsOH.[29,30] The Boc group was stable in the presence of TsOH with heating.

The best solvent for the reaction was 1,1,3-trichloroethane, in which **3** was soluble, and with which water forms an azeotrope.[29] Oxazolidinone **4** was coupled with benzyloxyhydroxylamine to give protected hydroxamate **5**. The oxazolidinone of **5** was saponified with LiOH.[29]

The stereoselective synthesis of intermediate **7** was reported previously by Travins and Etzkorn.[25] From our modeling, the size of the Boc group might have hindered efficient binding of the cyclic peptide mimic into the surface site of the enzyme. The Boc group of **7** was deprotected with 4 M HCl[31] because of its compatibility with the trimethylsilylethyl (TMSE) group,

Scheme 2



which could be cleaved if TFA was used to remove Boc (Scheme 2).[32] The amine was blocked with acetyl, as in the modeled inhibitor, to give **8**. Liberation of the amine by hydrogenolysis of the Cbz group of **8** furnished the free amine.

Condensation with **6** to give **9** was accomplished using (7-azabenzotriazol-1-yloxy) 1-tripyrrolidino-phosphonium hexafluorophosphate (PyAOP) as the coupling reagent. PyAOP, which suppresses racemization, is suitable for the coupling of hindered amino acids, difficult short sequences, and cyclic systems.[33,34] We encountered unexpected difficulty in the deprotection of the TMSE ester. Various fluoride ion sources were used to attempt cleavage of the TMSE group, but none of these reactions afforded the desired intermediate. TMSE esters are known to be labile to strong acid, so the TMSE of **9** was cleaved by TFA with triethylsilane (TES) as a scavenger, which simultaneously deprotected Boc.[35−37] Exchange of the TFA salt using HCl in ether did not improve cyclization results in the next step.

Many attempts were made to produce cyclic intermediate **10**, employing different classes of coupling reagents, including the phosphates diphenylphosphoryl azide (DPPA) and pentafluorophenyl diphenyl phosphate (FDDP), the phosphonium salts PyAOP, the uronium salts *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and *O*-(7-azabenzo-triazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexa-fluorophosphate (HATU), and the acid fluorides formed using cyanuric fluoride or *N*,*N*,*N*′,*N*′-tetramethyl-fluoroformamidinium hexafluorophosphate (TFFH).[38] However, only DPPA or HATU coupling produced macrocycle **10**. DPPA is a widely used coupling reagent in peptide cyclizations,[39] however, in our case, the highest yield of cyclized **10** using DPPA was only 10%. Comparable yields were obtained with HATU; the best result was achieved with a 1 h HATU coupling. Long coupling times using HATU or PyAOP produced no product. Steric hindrance was not a problem in previous cyclizations with Ala in the position of the α-aminosuberic acid.[26] The instability of the hydroxamic acid functionality may have contributed to the low yield of **10**. Hydrogenolysis of **10** to expose the key hydroxamic

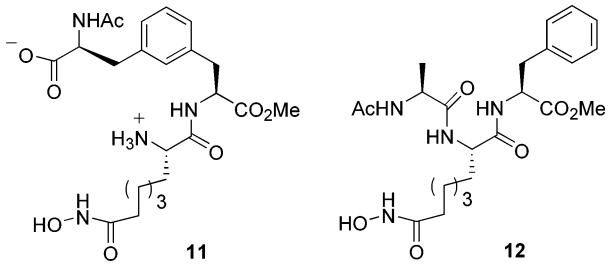
Table 1. In Vitro Inhibition (IC_{50}) of HeLa Nuclear Extract HDACs, HDAC1, and HDAC8 Activity

HDI	HDACs (nM)	HDAC1 (nM)	HDAC8 (nM)
1	46 ± 8	57 ± 10	231 ± 30
12	167 ± 20	174 ± 28	ND[a]
11	8100 ± 2100	$40\,000 \pm 7400$	ND[a]
TSA	41 ± 5	60 ± 2[23]	40[42]
SAHA	110[20]	112[43]	270[20]

[a] Not determined.

acid functionality using the mild catalysts, 5% $Pd(OH)_2/C$[40] or 5% $Pd/BaSO_4$,[41] produced the target HDAC inhibitor **1**.

Inhibition of HDAC activity in HeLa nuclear extracts by compound **1** was measured using a fluorescence-based assay. The IC_{50} value of **1** was 46 ± 8 nM (Table 1). The IC_{50} value for TSA measured in this assay system was 20-fold less potent than that reported in radioactively-labeled histones HDAC assays.[24] This may indicate that our inhibitor may be more potent than we report. The K_i value was measured in the same system to be 13.7 ± 4.1 nM, and the data fit best to a tight-binding competitive inhibition model (see Supporting Information). The K_i value is more robust and may be compared across assay systems because both the inhibitor and the substrate concentrations are varied. The high efficiency of the designed synthetic HDAC inhibitor **1** indicates that the hydrophobicity and the shape of the surface recognition element play an important role in the formation of the enzyme−inhibitor complex.



We also sought to determine the effect of the rigid macrocycle on the inhibition of HDACs. Does the design element that is different from all other HDAC inhibitors contribute to the affinity of the inhibitor **1**? Two compounds, **11** and **12**, were designed as controls to ascertain the effects of the macrocycle. Although it was facile to synthesize from existing intermediates, the uncyclized control **11** had two charged groups, ammonium and carboxylate, that could decrease the affinity for HDACs hydrophobic surface. The linear peptide **12**, with both termini capped as in the inhibitor **1**, eliminated the effect of charge to serve as a direct cyclization control. In addition, the peptide **12** had only two additional atoms, both hydrogens, compared with **1**. Compound **11** inhibited nuclear HDACs with an IC_{50} value of 8.1 μM, considerably less potent than **1**, while compound **12** had an IC_{50} value of 167 nM, a 3.6-fold loss of activity (Table 1). The structure of the surface recognition group, in particular, the conformational restriction and shape of the 12-membered ring, certainly provides favorable interactions of inhibitor **1** with at least one HDAC found in nuclear extracts.

To demonstrate inhibition of specific HDAC enzymes by compound **1**, human HDAC1 and HDAC8 (both class I) were assayed. Generally, synthetic inhibitors have not shown significant selectivity among HDACs. Cyclic peptide inhibitors have shown the greatest distinctions between HDACs. For example, cyclic peptides have been shown to have selectivity for HDAC1 over HDAC6.[44] The IC_{50} values obtained for HDI **1** were 57 nM for HDAC 1 and 231 nM for HDAC8 (Table 1). Thus, the designed inhibitor showed 4-fold selectivity for HDAC1 over HDAC8. All of the IC_{50} values for the designed inhibitor **1** and controls **11** and **12** reported in Table 1 were measured under the same conditions.

In conclusion, we have designed and synthesized a potent HDAC inhibitor based on our own cyclic peptide mimic.[25] The key steps of the synthesis included 12-membered ring cyclization and hydroxamic acid deprotection. The new inhibitor exhibited 4-fold selectivity for HDAC1 over HDAC8. The potency of the HDAC inhibitor demonstrated that the surface recognition region plays an important role in the design of new HDAC inhibitors.

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Supporting Information Available: Computational and experimental procedures, ^1H and ^{13}C NMR spectra of compounds **1**−**12**, HPLC chromatograms of **1**, **11**, and **12**, and COSY, HMQC for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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