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The structural requirements of histone deacetylase inhibitors: Suberoylanilide hydroxamic acid analogs modified at the C6 position

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Abstract

Suberoylanilide hydroxamic acid (SAHA, Vorinostat), the first FDA-approved histone deacetylase (HDAC) inhibitor, was modified at the C6 position to study the structural requirements for high potency and selectivity. Substituents on the C6 position only modestly influenced inhibitor potency, with poorer activity observed as substituent size increased. Interestingly, C6 substituents also modestly influenced selectivity compared to the parent compound, SAHA. This systematic study documenting the influence of substituents on the SAHA linker region will aid development of anti-cancer drugs targeting HDAC proteins.

Keywords

Histone Deacetylase; HDAC inhibitor; Isoform Selectivity; Vorinostat

Histone deacetylase (HDAC) proteins are transcription factors that influence gene expression by altering the acetylation status of lysine residues on nucleosomal histones. The HDAC protein family consists of 18 members and is divided into four classes based on size, cellular localization, number of catalytic active sites, and homology to yeast HDAC proteins.[1] The eleven class I, II, and IV HDAC proteins are metal ion-dependant proteins and sensitive to the inhibitors discussed here.[2]

HDAC proteins are over-expressed in many cancers, making them attractive targets of anti-cancer drugs.[3] In fact, two HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA, Vorinostat, Figure 1) and Romidepsin, were approved for treatment of cutaneous T-cell lymphoma.[4] In addition to lymphoma, HDAC activity has been associated with a variety of other cancers. Over-expression of class I HDAC proteins (HDAC1, 2, and 3) was observed in ovarian cancer.[5] Altered HDAC2 activity in gastric cancers,[6] mutations of HDAC1 and HDAC3 in lung cancers,[7] and abnormal HDAC8 protein activity in acute myeloid leukemia tissues have been reported for the class I proteins.[8] Overproduction of class II HDAC6 was observed in breast cancer tissues.[9] Because individual HDAC isoforms play independent roles in specific cancers, development of isoform-selective inhibitors as anti-cancer drugs

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Supplementary Material
Synthetic procedures, characterization of all compounds, and HDAC assay data are provided as supplementary material.

has been proposed. However, most HDAC inhibitors nonspecifically target all eleven metal ion-dependent HDAC proteins, including SAHA.[10] It has been proposed that the non-selectivity of HDAC inhibitors may cause cancer patients in the clinic to suffer from the side effects, such as fatigue, anorexia, diarrhea, and cardiac arrhythmia.[11] Unfortunately, the clinical toxicity of HDAC inhibitors is poorly characterized because few isoform-selective molecules have been reported. In addition, sequence similarity in the active site of the isoforms has challenged selective inhibitor design.[12]

Most HDAC inhibitors, including SAHA (Figure 1), have a similar construction consisting of a capping group that is solvent-exposed, a carbon linker that is surrounded by a hydrophobic tunnel, and a metal binding moiety that is buried in the protein active site.[13] While the capping group and metal binding moiety have been modified extensively, few structure activity relationship studies focusing on the linker area of SAHA have been reported.[12] However, MS275 (Figure 1) contains an intralinker aryl group and displays nanomolar class I selectivity,[10, 14] while a recently reported nanomolar HDAC6-selective inhibitor (**1**, Figure 1) contains a cyclic substituent at the C7 position of SAHA.[15] These examples highlight the possible role of the linker region in selectivity and the need for a systematic study of linker substituents.

To study the linker region, SAHA analogs containing hydrophobic substituents on the C2 and C3 positions were previously reported (Figure 1).[16] Positioning substituents near the hydroxamic acid reduced inhibitor potency, with IC_{50} values in the μM range. However, the C3-ethyl SAHA analog displayed 12-fold selectivity for HDAC6 over HDAC3. Combining the data from compound **1** and the C2- and C3-SAHA analogs, we theorized that SAHA analogs with substituents positioned nearer to the solvent-exposed capping group might display potent inhibition with HDAC isoform selectivity.

We report here the synthesis and evaluation of SAHA analogs with substituents attached at the C6 position (Figure 1). To our knowledge, C6-modified SAHA analogs have not been reported despite the fact that SAHA analogs with substituents at the C7 position, such as **1**, showed potency and selectivity. Due to the symmetrical relationship of the C6 and C3 positions on SAHA, the C6-SAHA analog synthesis had similarities to that of the C3-SAHA analogs, previously reported.[16b] The main difference between the two syntheses was the order of installation of the terminal amide groups. Similar to the C2 and C3-SAHA analogs, we selected hydrophobic substituents since the carbon linker is surrounded by a hydrophobic channel in the HDAC structure.[13]

We initially synthesized the C6-methyl SAHA analog **2a**, as outlined in Scheme 1. Under Fisher conditions, commercially available ε-caprolactone **3** was opened to give alcohol **4**, which was subjected to Swern oxidation to give aldehyde **5**. For the Horner-Wadsworth-Emmons reaction, benzyl dimethyl phosphono-acetate was added to crude compound **5** to give the corresponding α,β-unsturated benzyl ester **6**. A mixture of (E) and (Z)-isomers of ester **6** was treated with a copper (I) iodide and methyl lithium to give the C6-methyl substituted benzyl ester **7a**. Without purification, ester **7a** was deprotected by hydrogenolysis and coupled with aniline to give anilide **8a**, which was directly converted to the methyl hydroxamic acid final product **2a**.

To create the remaining C6-SAHA analogs, purification by column chromatography by was required after 1,4-addition since the mixture of (E) and (Z) isomers **6** incompletely converted to ester **7** (Scheme 2). Using this additional purification step, the C3-*t*-butyl **2c** and C3-2-ethylhexyl 2**d** SAHA analogs were synthesized as described for the methyl variant. Unfortunately, contaminating unsaturated ester **6** was present even after purification of phenyl ester **7b** due to similar polarity. The mixture of unsaturated ester **6** and phenyl

ester **7b** were carried through the reaction series of hydrogenolysis and aniline coupling to give a mixture of anilide **8b** and unsaturated anilide compound **8b´**. Fortunately, after installation of the hydroxamic acid, final compound **2b** was isolated.

HDAC inhibitory activities of the C6-SAHA analogs were measured using the Fluor de Lys® *in vitro* fluorescence activity assay kit (Enzo) using HeLa cell lysates as the source of HDAC activity (Table 1), as previously reported.[16–17] The methyl and phenyl variants **2a** and **2b** were the most potent, displaying nanomolar IC_{50} values, which were only 4-fold reduced compared to SAHA. In addition, the 2-ethylhexyl variant **2d**, which contained the longest substituent of the series, displayed potent inhibitory activity in the nanomolar range. The potent inhibition of these C6 analogs is in contrast to the C3-phenyl SAHA variant (Figure 1, IC_{50} of 73,000 nM), which displayed 811-fold reduced activity versus SAHA.[16b] The results indicate that the active site of HDAC proteins can accommodate a bulky substituent at the C6 position. Interestingly, the *t*-butyl variant **2c**, which contains the bulkiest substituent with methyl groups on the α-carbon, displayed the weakest potency, which was 20-fold reduced compared to SAHA. In summary, the inhibition data show that most C6-SAHA analogs maintain nanomolar potency, but substitution at the α-carbon may decrease inhibitory activity.

The C6-SAHA analogs were next evaluated for potency against individual HDAC isoforms-HDAC1 and HDAC3 representing class I and HDAC6 representing class II. All compounds were tested at a single concentration near their IC_{50} values using the Flour de Lys™ kit (Figure 2). Consistent with previous data,[10, 16a] SAHA exhibited roughly equal inhibition against HDAC1, HDAC3, and HDAC6. The phenyl variant **2b** also similarly inhibited HDAC1, HDAC3, and HDAC6. In contrast, the methyl variant **2a** showed modest dual-preference for HDAC1 and HDAC3 over HDAC6 at 500 nM. The 2-ethylhexyl variant **2d** also showed preference for HDAC3 over HDAC1 and HDAC6. However, the bulkiest analog, the *t*-butyl variant **2c**, displayed preference for HDAC1 and HDAC6 over HDAC3. The data indicate that the methyl, *t*-butyl, and 2-ethylhexyl variants (**2a**, **2c**, and **2d**) display modestly different preferences for each HDAC isoform while still maintaining nanomolar or low micromolar potency.

To more thoroughly assess the selectivity observed in the initial screen, we determined the IC_{50} values of the C6-*t*-butyl variant **2c** against HDAC1, HDAC3, and HDAC6. We selected the *t*-butyl analog because it showed the most potential to create a dual HDAC1/HDAC6-selective inhibitor, which would be useful for the treatment and study of acute myeloid leukemia.[18] As expected based on the initial screen, the C6-*t*-butyl analog **2c** displayed modest preference for HDAC1 and HDAC6 compared to HDAC3 (6-fold and 2-fold, respectively, Table 2). As a control, SAHA showed no selectivity, as expected (Table 2).[10] The analysis shows that substituents on the C6 position modestly influence inhibitor selectivity and may promote creation of dual selective inhibitors.

In conclusion, SAHA analogs containing substituents on the C6 position in the linker region can display nanomolar IC_{50} values, indicating the subsitutents near the solvent-exposed capping group are accommodated in the HDAC active site. In addition, C6-substituents can also modestly influence selectivity for individual HDAC isoforms. Combined with earlier studies of SAHA analogs substituted on the C2 and C3 positions (Figure 1),[16] the data suggest that the linker region of HDAC inhibitors, particularly near the capping group, is an interesting yet underexplored area of future drug design.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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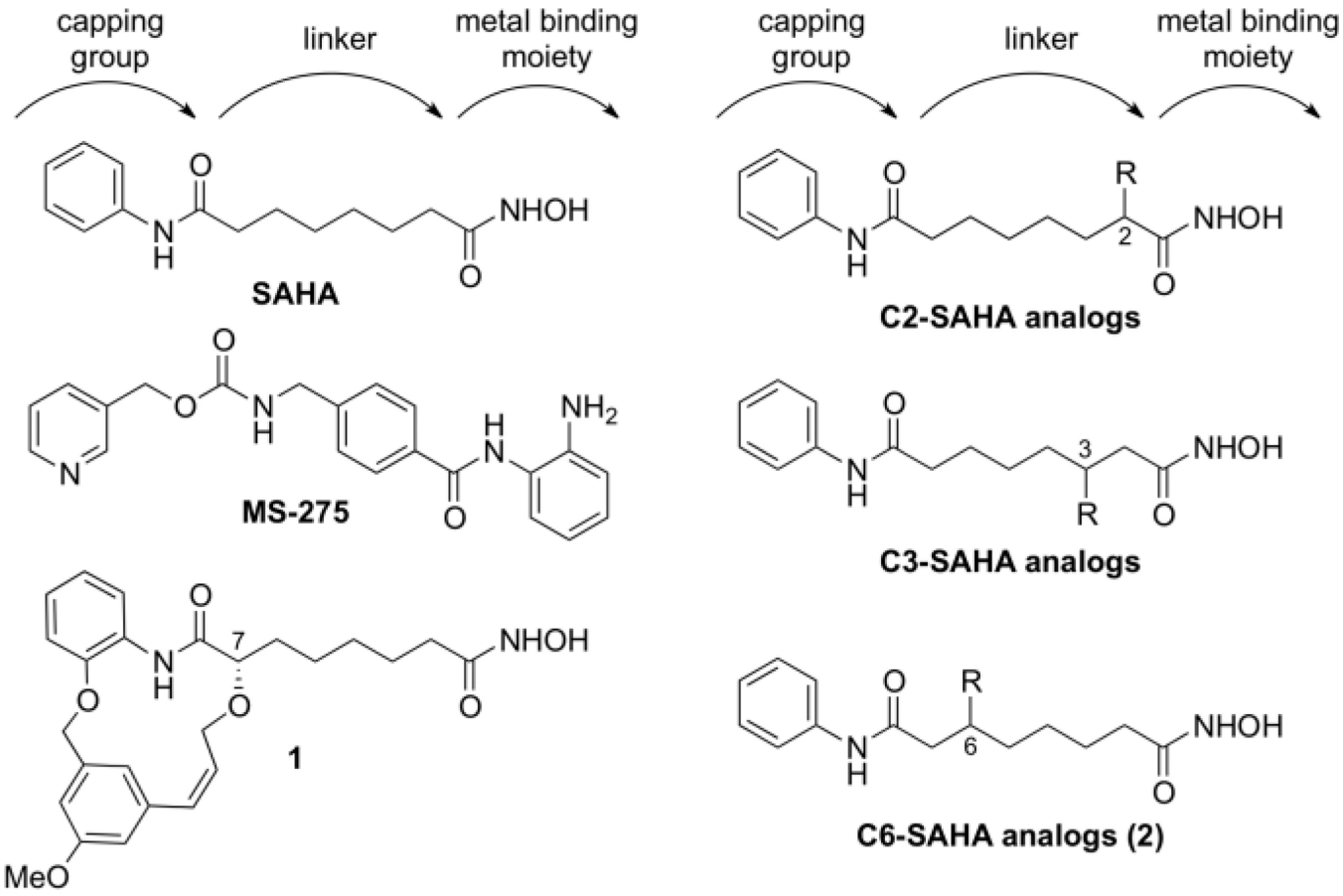


Figure 1.
Structures of HDAC inhibitors discussed in the text

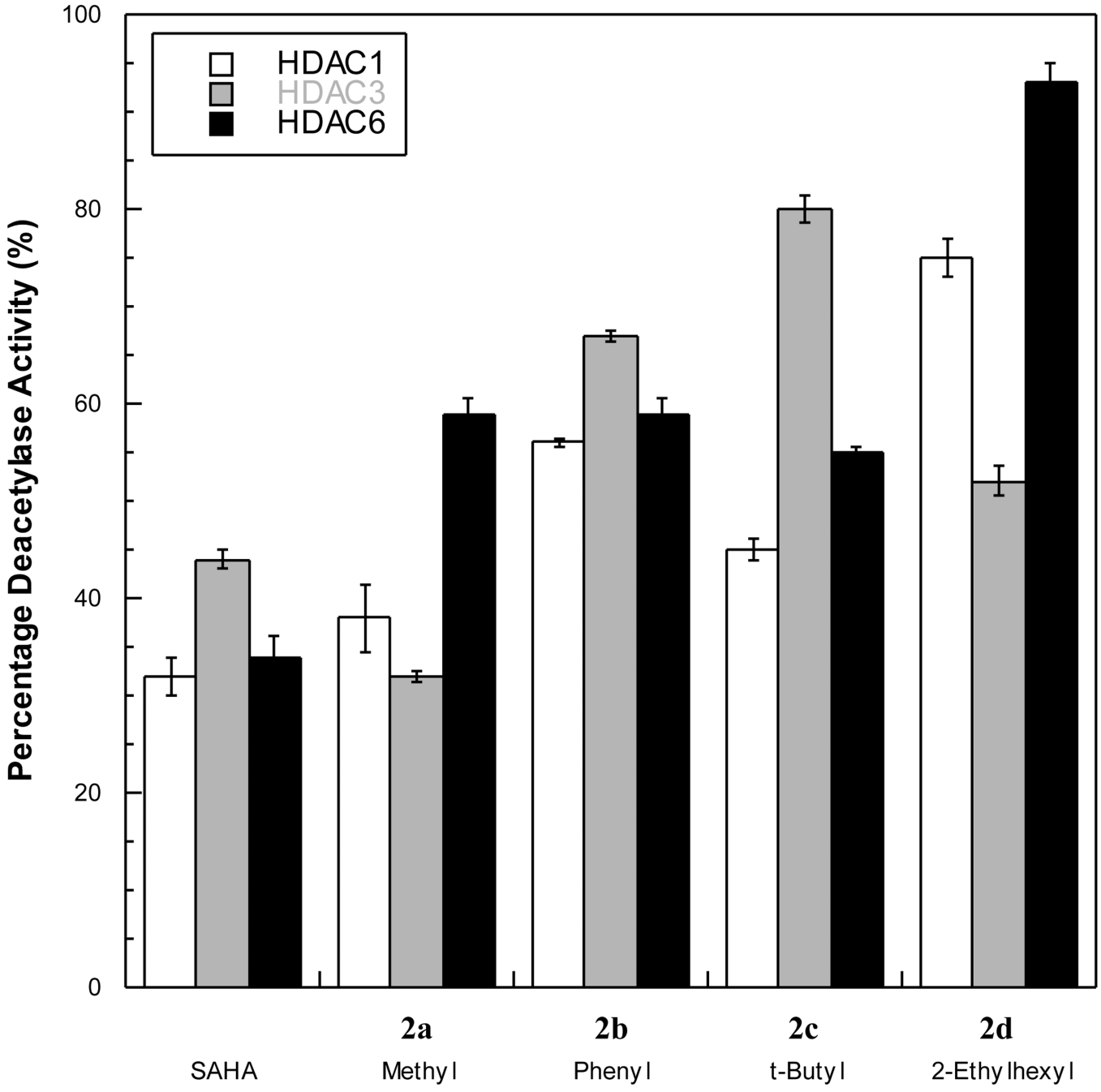
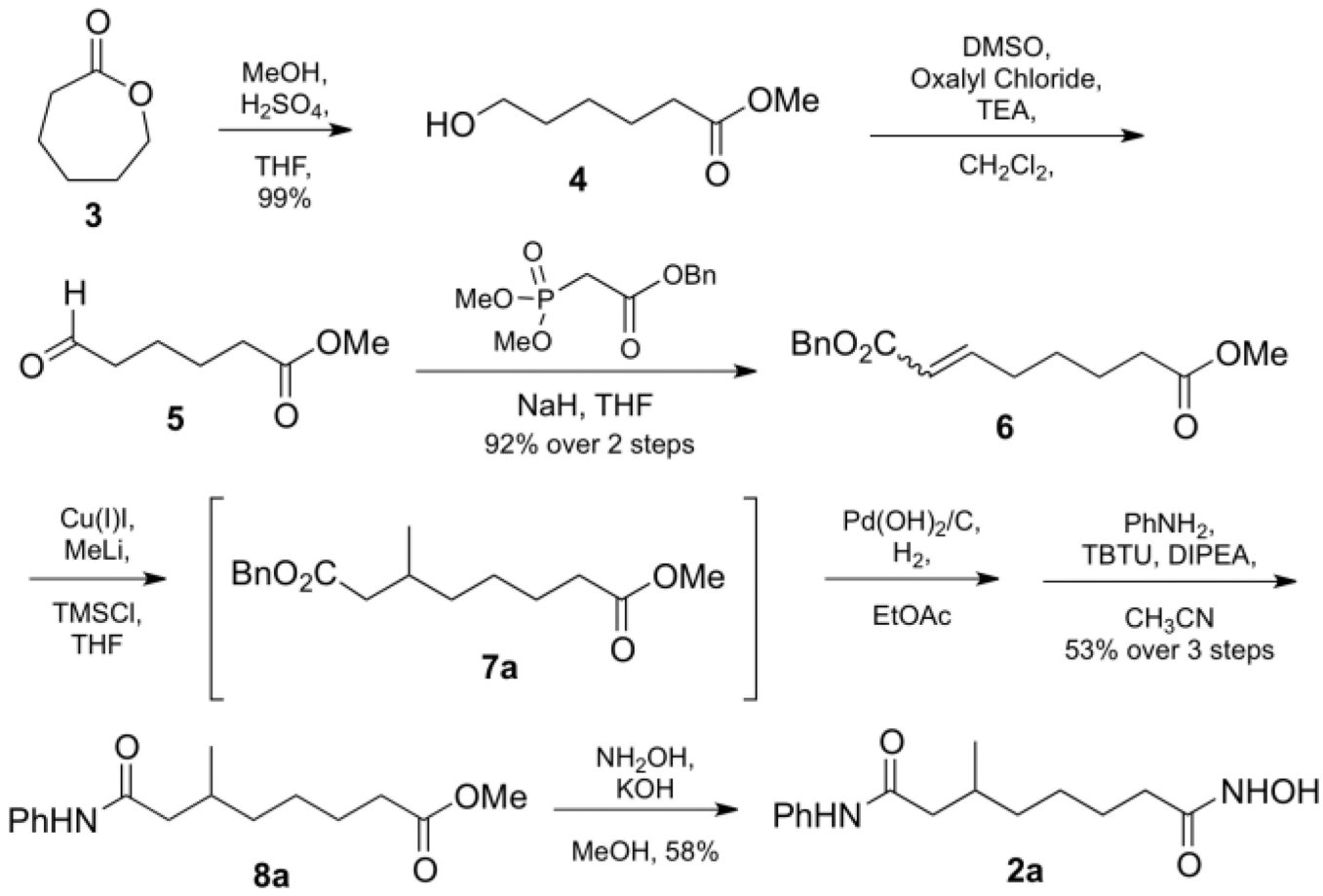
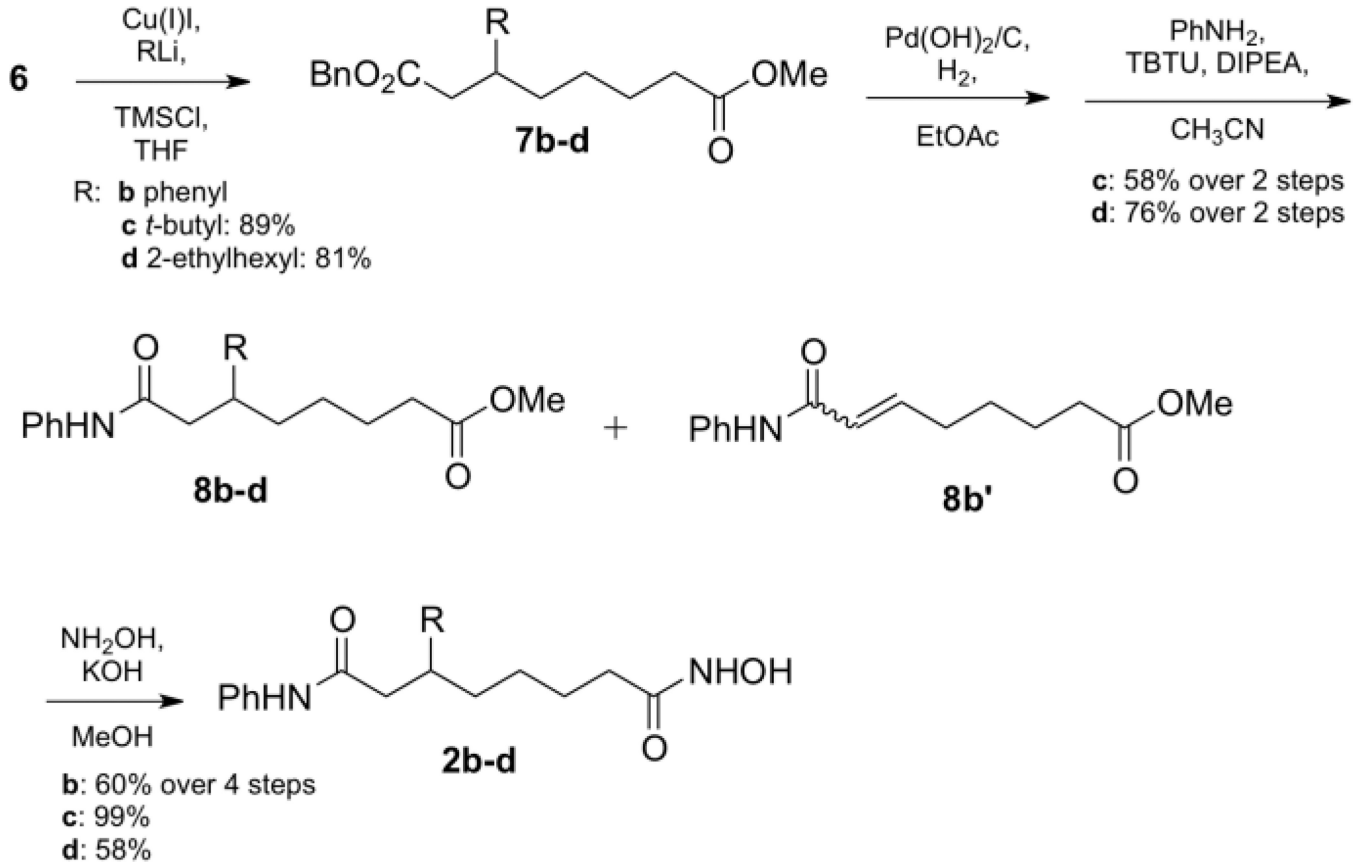


Figure 2.
Screen of C6-SAHA analogs against HDAC1, HDAC3, and HDAC6 with 125 nM SAHA, 500 nM **2a**, **2b**, and **2d**, and 2 μM **2c**.



Scheme 1.
Initial synthesis of C6-SAHA methyl analog (**2a**).



Scheme 2.
Modified synthesis of C6-SAHA analog (**1b–d**).

Table 1

HDAC inhibition by SAHA, MS-275, and the C6-SAHA analogs **2a–d** using HeLa cell lysates

Compounds	R	IC_{50}, nM[a]
SAHA		86 ± 4
MS-275		3160 ± 160
2a	Methyl	349 ± 28
2b	Phenyl	344 ± 44
2c	*t*-Butyl	1940 ± 300
2d	2-Ethylhexyl	456 ± 28

[a]Values are the mean of at least three experiments with standard error given.

Table 2

IC_{50} values of SAHA and the C6-SAHA *t*-butyl variant **2c** for HDAC1, HDAC3, and HDAC6

Compound	**IC_{50}/μM**		
	HDAC1	**HDAC3**	**HDAC6**
SAHA	0.096 ± 0.02	0.136 ± 0.01	0.074 ± 0.009
2c	0.99 ± 0.06	5.4 ± 0.7	2.4 ± 0.5