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The structural requirements of histone deacetylase inhibitors: SAHA analogs modified at the C5 position display dual HDAC6/8 selectivity

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ABSTRACT

Histone deacetylase (HDAC) proteins have emerged as important targets for anti-cancer drugs, with four small molecules approved for use in the clinic. Suberoylanilide hydroxamic acid (Vorinostat, SAHA) was the first FDA-approved HDAC inhibitor for cancer treatment. However, SAHA inhibits most of the eleven HDAC isoforms. To understand the structural requirements of HDAC inhibitor selectivity and develop isoform selective HDAC inhibitors, SAHA analogs modified in the linker at the C5 position were synthesized and tested for potency and selectivity. C5-modified SAHA analogs displayed dual selectivity to HDAC6 and HDAC8 over HDAC 1, 2, and 3, with only a modest reduction in potency. These findings are consistent with prior work showing that modification of the linker region of SAHA can alter isoform selectivity. The observed HDAC6/8 selectivity of C5-modified SAHA analogs provide guidance toward development of isoform selective HDAC inhibitors and more effective anti-cancer drugs.

Histone deacetylase (HDAC) proteins have a prominent regulatory role in gene transcription and cell function. HDAC proteins catalyze the removal of the acetyl group from ε-acetyllysine residues on nucleosomal histones. Upon deacetylation of histone proteins, the interaction between histone proteins and DNA increases, which reduces DNA expression and gene transcription.[1,2] In addition, HDAC proteins affect intracellular interactions, protein localization, and protein stability through deacetylation of non-histone substrates.[3–6] Beyond basic cell biology, HDAC proteins are overexpressed in several cancers, as well as other diseases.[7]

HDAC proteins require either metal ions or NAD^+ as cofactors for catalysis.[8] The eleven metal-dependent HDAC isoforms are grouped as classes I, II, or IV, depending on their size, cellular localization, and homology to other HDAC proteins.[9] The seven NAD^+ dependent HDAC proteins are grouped as class III. The metal-dependent HDAC proteins comprise eleven isoforms (HDAC 1–11) and are the focus of this work.

Each metal-dependent HDAC isoform has been associated with various cancers. For example, HDAC1 was overexpressed in lung and colon cancers,[10,11] while HDAC2 displayed aberrant expression in colorectal and gastric cancer.[12] Abnormal activity of HDAC8 was observed in acute myeloid leukemia, T-cell lymphoma, and neuroblastoma.[13] HDAC6 was highly expressed in leukemia, ovarian cancer, and oral squamous cell carcinoma.[14–16] Moreover, HDAC6 plays an important role in cancer cell growth and survival through several non-epigenetic pathways.[17] Importantly, HDAC6 and HDAC8 were expressed at abnormally high levels in various human breast cancer cell lines and were associated with breast cancer invasiveness and metastasis, making them both interesting for anti-cancer drug design.[18]

Due to their fundamental role in cancer, inhibitors targeting HDAC proteins have been developed. HDAC inhibitors showed the ability to reduce proliferation and metastasis, and promote apoptosis in several cancers.[14,18–22] Four HDAC inhibitors have been approved by the FDA for cancer treatment.[20,23–26] For example, SAHA (suberoylamide hydroxamic acid, Vorinostat, Zolinza™) and Belinostat (PXD101, Belodaq™) (Fig. 1) were approved for treatment of T-cell lymphoma,[23–25] while Panobinostat (LBH-589, Farydak™) (Fig. 1) was approved for treatment of multiple myloma.[26] SAHA inhibits most of the eleven metal-dependent HDAC proteins, with only a modest selectivity against HDAC8.[27,28] The non-selectivity of SAHA might explain the side effects observed in clinic, but certainly limits the use of SAHA to study individual HDAC isoforms in cancer biology.[29] In the last four years, several dual HDAC6/HDAC8 selective inhibitors have been developed.[30–33] Dual inhibition of HDAC6 and HDAC8 can possibly have synergistic therapeutic applications in treatment of various cancers, which can improve anti-cancer efficiency compared to current non-selective HDAC inhibitors.[30–34]

SAHA and most of the HDAC inhibitors have a similar pharmacophore that consists of three structural elements (Fig. 1).

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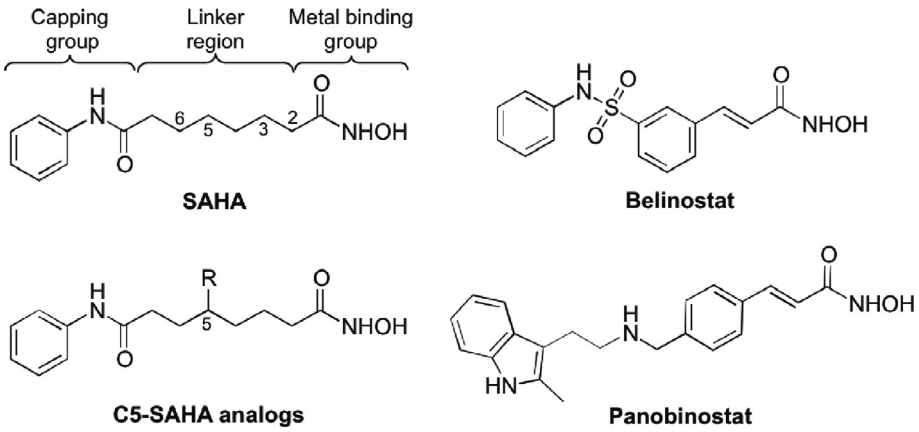


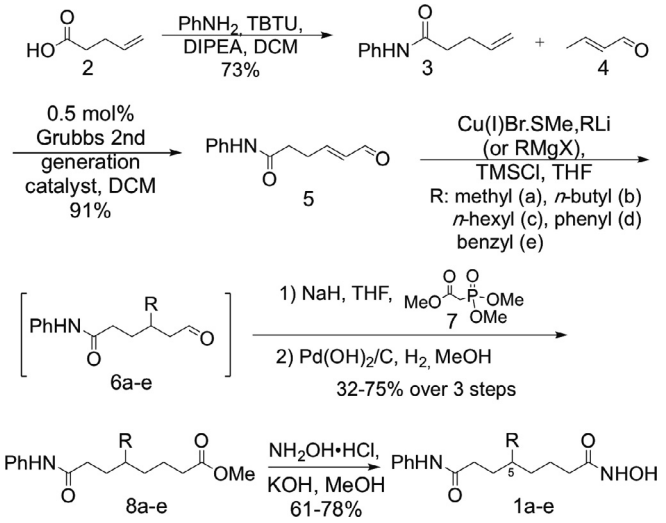
Fig. 1. Chemical structures of the FDA-approved drugs SAHA, Belinostat, and Panobinostat, along with the C5-modified SAHA analogs reported here.

The capping group interacts with solvent-exposed residues of the HDAC active site, while the metal binding group forms key interactions with the catalytic metal deeply buried in the active site (Fig. 1). The linker region that connects the capping and the metal binding groups is positioned in the narrow hydrophobic active site channel. Both the capping and the metal binding groups have been modified extensively in HDAC inhibitor design.[35–40] In contrast, few studies report modification of the linker region.[41–44] To study the effect of substitution on the linker region, SAHA analogs substituted at carbon 2 (C2), 3 (C3), or 6 (C6) of the linker region were synthesized and screened (Fig. 1). C2-hexyl SAHA displayed dual HDAC6/8 selectivity,[44] C3-ethyl SAHA showed HDAC6 selectivity,[42] and C6-butyl SAHA inhibited HDAC1 and 6 over HDAC3.[43] The conclusion of this prior work is that modification of the SAHA linker can alter inhibitor specificity.

Guided by this prior work, here we explored the effect of substituents on the C5 position of SAHA (Fig. 1). SAHA analogs substituted at the C5 were synthesized and tested for potency and selectivity both *in vitro* and *in cellulo*. Several analogs showed dual HDAC6/8 selectivity over HDAC1, 2, and 3, with a modest reduction in HDAC6 inhibition but enhanced HDAC8 inhibition compared to SAHA. This study documents that modifying the linker region of SAHA can alter its selectivity with minimal effect on potency.

Synthesis of C5-modified SAHA analogs

C5-modified SAHA analogs **1a**–**e** were synthesized as shown in Scheme 1. The synthesis started from a coupling reaction of 4-pentenoic acid **2** with aniline using TBTU to obtain amide **3**, which was then reacted with crotonaldehyde **4** via a cross metathesis reaction using second generation Grubbs' catalyst to afford the α,β-unsaturated aldehyde **5**. Aldehyde **5** was substituted with different groups through a 1,4-conjugate addition using organolithium or organomagnesium cuprates to yield intermediates **6a**–**e**. Horner–Wadsworth–Emmons olefination of **6a**–**e** with trimethyl phosphonoacetate followed by reduction gave amide esters **8a**–**e** with a saturated linker. Finally, amide esters **8a**–**e** were reacted with hydroxylamine to afford the C5-substituted SAHA derivatives **1a**–**e** as racemic mixtures.



Scheme 1. Synthesis of C5-modified SAHA analogs (**1a**-**e**).

Table 1
IC_{50} values for SAHA and C5-modified SAHA analogs (**1a**–**1e**) with HeLa cell lysates.[a]

Compounds	R	IC_{50} (μM)
SAHA	H	0.20 ± 0.02
1a	Methyl	0.10 ± 0.01
1b	*n*-Butyl	5.0 ± 0.4
1c	*n*-Hexyl	6.5 ± 0.1
1d	Phenyl	2.2 ± 0.1
1e	Benzyl	6.2 ± 0.2

[a] Mean IC_{50} value and standard error of at least three independent trials are shown (Fig. S52 and Table S1).

In vitro screening of C5-modified SAHA analogs

As a preliminary screen, the new analogs were tested for their global HDAC inhibition with HeLa cell lysates as the source of all HDAC proteins (Table 1). SAHA was also tested as the parent unsubstituted control molecule. The inhibitory activities of the analogs were measured with the HDAC-Glo™ I/II substrate (Promega). C5-methyl SAHA analog **1a** showed greater potency compared to SAHA (100 nM vs 200 nM IC_{50} values, Table 1). However, all other analogs showed weaker potency than SAHA (11- to 33-fold reduction in potency), with IC_{50} values from 2.2 to 6.5 μM (Table 1). The observed lower potencies of compounds **1b**–**1e** may be due to selectivity for specific HDAC isoform(s), which lowered the potency against lysates that contains all HDAC isoforms. The lower potency observed here was similar to what was observed with the C2-modified SAHA analogs.[44]

To test isoform selectivity, the parent molecule, SAHA, and all the C5-modified SAHA analogs were tested at a single concentration using the recently developed ELISA-based HDAC activity assay