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Aminosuberoyl hydroxamic acids (ASHAs): A potent new class of HDAC inhibitors

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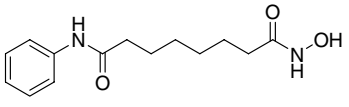
Abstract—Histone deacetylase (HDAC) inhibitors that target Class I and Class II HDACs are currently in advanced clinical trials for the treatment of cancer. Vorinostat (Zolinza™, SAHA) is a hydroxamic acid approved for the treatment of patients with cutaneous T-cell lymphoma who have progressive, persistent or recurrent disease on or following two systemic therapies. As part of an on-going effort to better understand the nature of the HDAC enzyme/inhibitor interaction and design highly effective HDAC inhibitors, we herein report the design, synthesis and HDAC inhibitory activity of a vorinostat-derived series of substrate-based HDAC inhibitors.

Class I/II histone deacetylase (HDAC) enzymes are an emerging therapeutic target for the treatment of cancer and other diseases.[1–5] X-ray crystallographic studies (PDB ID code 1C3S) have demonstrated that the clinical agent vorinostat (vorinostat, **1**) binds directly to the catalytic site on HDAC enzymes and thereby blocks substrate access (Fig. 1).[1,3,6,7] To better understand the structural relationship between vorinostat and HDAC substrates, we have explored a series of branched vorinostat analogues on the basis that vorinostat acts as a substrate mimetic and that closer mimics of the *N*-acetyllysine moieties in the natural substrates will improve binding and therefore inhibitor activity.[8–10] We thereby discovered and disclose herein, a series of aminosuberoyl hydroxamic acids (ASHAs) of remarkable HDAC inhibitory activity (Fig. 2).[11,12]

ASHAs were prepared as summarized in Scheme 1. *N*-Boc-aminosuberic acid ω-methyl ester **2** was coupled with the requisite amine in the presence of EDC and HOBT, affording amide **3**. TFA deprotection of **3** and functionalization with the requisite acid chloride provided methyl ester **4**, which was converted to **5** by treatment with 50% aqueous hydroxylamine in methanol.

The ASHAs were tested for their abilities to inhibit HDAC1 activity and the proliferation of murine erythroleukemia cells (SC-9).[13] IC_{50} values are reported in Table 1. These data demonstrate that the ASHAs comprise a potent new class of HDAC inhibitors that are generally more potent than vorinostat (**1**). This is also reflected in antiproliferative assays, with several compounds displaying IC_{50} value below 100 nM (Table 1).

Anilide and aminoquinolide containing compounds generally show improved enzymatic and proliferation inhibitory activity compared to the corresponding benzylamine derivatives. For example, compounds **5a**, **5e**, and **5i** are 10–30 times more active than the corresponding *N*-benzyl amide **5m**. Furthermore, with



Vorinostat (1)
HDAC1 IC_{50} = 48 nM
SC9 MTS IC_{50} = 606 nM

Figure 1. Vorinostat (**1**).

Keywords: HDAC; Histone deacetylase inhibitor; HDAC inhibitor; Anticancer drug; SAHA; Hydroxamic acids.
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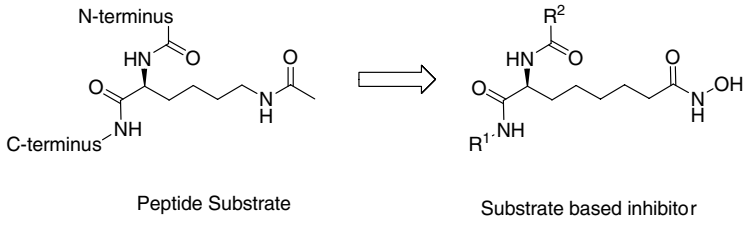
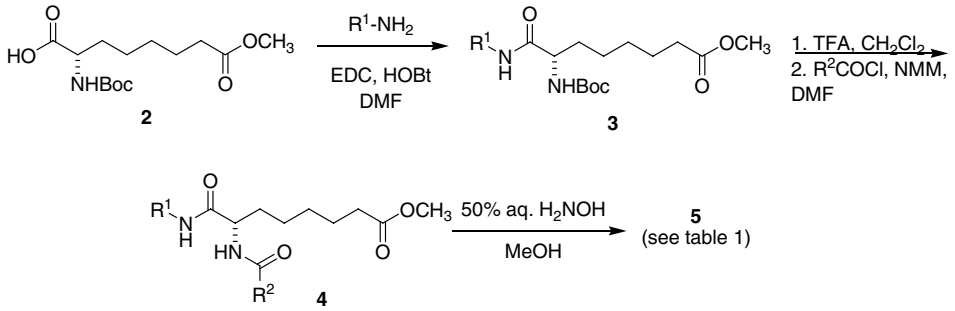
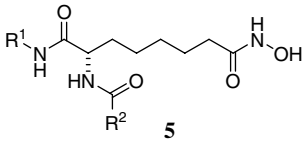


Figure 2. Design rationale for the aminosuberoyl hydroxamic acids (ASHAs).



Scheme 1. Synthesis of hydroxamic acids derived from 2-L-aminosuberic acid.

Table 1. Inhibition of histone deacetylase activity and murine erythroleukemia cell proliferation by hydroxamic acids derived from 2-L-aminosuberic acid[a]



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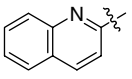
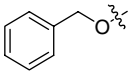
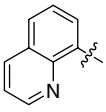
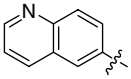
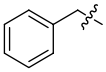
R^1	R^2			
				
	**5a** 12.1/73	**5b** 11.1/250	**5c** 10/33	**5d** 18/102
	**5e** 9.4/43	**5f** 4/367	**5g** 42/165	**5h** 14.5/39
	**5i** 3.5/90	**5j** 1.3/756	**5k** 54/107	**5l** 7.7/110
	**5m** 93/1039	**5n** 120/3423	**5o** 51.3/374	**5p** 48/412

[a] Values shown in the table are for IC_{50} HDAC1/IC_{50} SC9 MTS. Vorinostat (SAHA) IC_{50} HDAC1 = 48 nM and IC_{50} SC9 MTS = 606 nM.

the exception of the R′ = 2-quinolyl series, a general trend in HDAC inhibitory potency of 6-aminoquinolide > 8-aminoquinolide > anilide is observed. Despite being very potent compounds against the enzyme, the 6-aminoquinoline derivatives are generally less active in cell culture than their aniline- and 8-aminoquinoline-derived analogues. The 8-aminoquinoline component, in particular, appears to be optimal in cellular assays.

To further investigate the pharmacologic properties of select ASHAs, **5b** and **5e** were tested in vivo for the ability to attenuate tumor growth in an HCT-116 colon xenograft model in nude mice (Chart 1). Both agents demonstrated marked efficacy (TGI $\geqslant$ 50%) at tolerated doses (<20% body weight loss) when dosed once daily, ip for 21 days. In this tumor model, the ASHAs also demonstrated statistically significant tumor growth inhibition at sub-MTD doses. Similar observations were

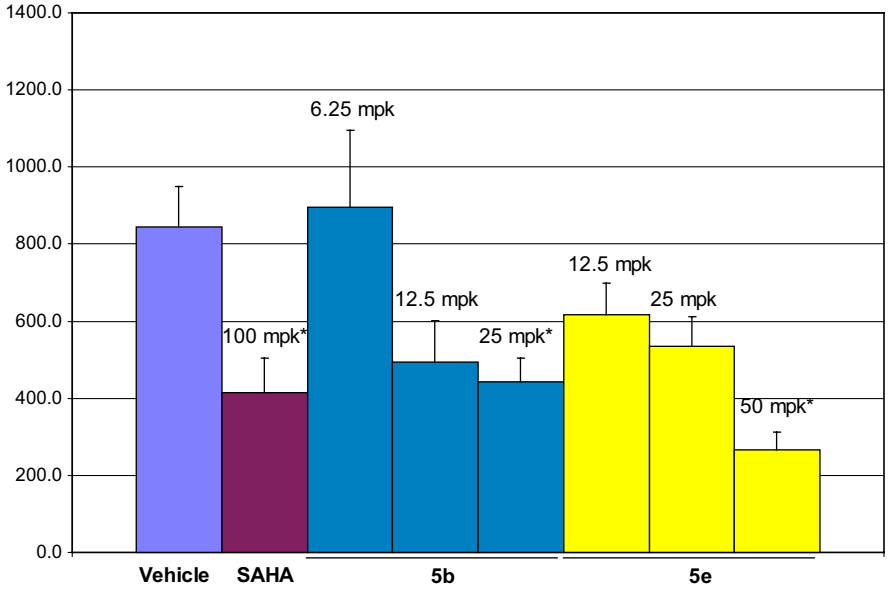


Chart 1. HCT-116 colon xenograft growth inhibition with **5b** and **5e**.[a]

made when these agents were tested in the MDA-231 breast xenograft model (data not shown).

In summary, we have disclosed the design and synthesis of a novel, potent class of HDAC inhibitors, the aminosuberoyl hydroxamic acids (ASHAs). Constituents of the ASHA class of HDAC inhibitors exhibit potent HDAC and cell proliferation inhibitory activities at nanomolar concentrations. Additionally, these agents are active in vivo and have been shown to inhibit tumor growth in both colon and breast xenograft models. Further studies of these agents and related analogues will be reported in due course.

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