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LETTER

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Synthesis and Biological Evaluation of JAHAs: Ferrocene-Based Histone Deacetylase Inhibitors

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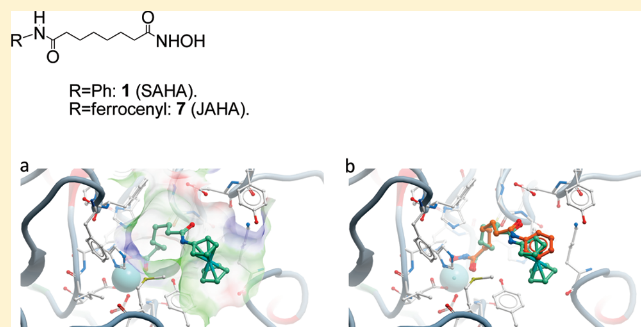
Supporting Information

ABSTRACT: N^1-Hydroxy-N^8-ferrocenyloctanediamide, JAHA (7), an organometallic analogue of SAHA containing a ferrocenyl group as a phenyl bioisostere, displays nanomolar inhibition of class I HDACs, excellent selectivity over class IIa HDACs, and anticancer action in intact cells (IC_{50} = 2.4 μM, MCF7 cell line). Molecular docking studies of 7 in HDAC8 (a,b) suggested that the ferrocenyl moiety in 7 can overlap with the aryl cap of SAHA and should display similar HDAC inhibition, which was borne out in an in vitro assay (IC_{50} values against HDAC8 (μM, SD in parentheses): SAHA, 1.41 (0.15); 7, 1.36 (0.16). Thereafter, a small library of related JAHA analogues has been synthesized, and preliminary SAR studies are presented. IC_{50} values as low as 90 pM toward HDAC6 (class IIb) have been determined, highlighting the excellent potential of JAHAs as bioinorganic probes.



KEYWORDS: Ferrocene, HDAC inhibitor, bioinorganic, anticancer, hydroxamic acid

The dynamic process of lysine acetylation and deacetylation is fundamental to chromatin remodelling and the regulation of gene-specific transcription.[1,2] Histone acetyl transferases promote N-acetylation of ε-lysine tails on histone proteins, whereas histone deacetylases (HDACs) catalyze the remove of acetyl groups, leading to transcriptional repression via a condensed chromatin where DNA is wrapped tightly around the positively charged histone core. The consequence of the latter is transcriptional silencing, which, in cancer, contributes to evasion of apoptosis and cancer progression. HDACis (HDAC inhibitors) are attractive anticancer agents since restoring a relaxed, hyperacetylated, chromatin structure leads to gene transcription and the upregulation of a number of proapoptotic and growth arrest proteins (e.g., p21/cip1). Modulation of acetylation of nonhistone proteins may also contribute to anticancer effects (e.g., p53, hsp90). Vorinostat **1** (SAHA, suberoylanilide hydroxamic acid) is the archetypal HDACi.[3−7] It has been approved for clinical use against cutaneous T-cell lymphoma (CTCL) and shown to synergize with many anticancer agents including kinase inhibitors and proteasome inhibitors as well as radiation therapy.[8−10]

A number of recent publications have explored subtle manipulations of the so-called "cap-tail-linker" pharmacophore of SAHA to probe structure−activity relationships (SARs) and binding modes (Figure 1). HDAC inhibitory activity is improved by the presence of electron-donating groups on the aryl cap, as in the dimethyl analogue **2**, with the exception of ortho substituents, which lead to decreased activity due to steric clashes upon enzyme binding.[11] Other research groups have probed the effects of substituents on the physiochemical properties and selectivities of HDACis as in the branched analogues **3a** and **3b**.[12,13] Alkyl substitutents adjacent to the hydroxamic acid lead to a loss of HDAC inhibitory action as in **3c**, attributed to conformational disruption of binding of the hydroxamic acid to zinc in the active site of the enzyme.[14,15]

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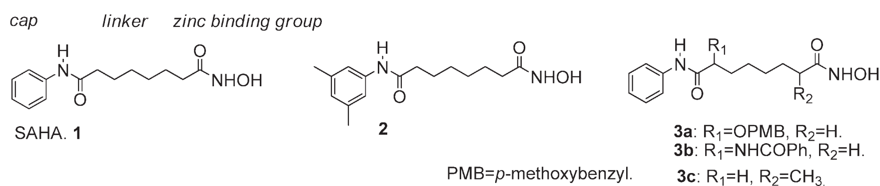
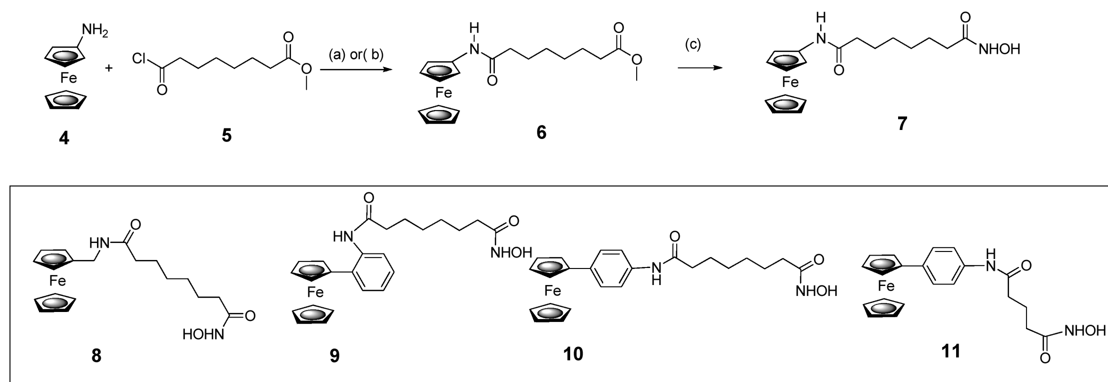


Figure 1. SAHA and analogues.

Scheme 1[a]



[a] Reagents: (a) Base = PS-NMM, and yield = 23%. (b) Base = NEt_3, and yield = 74%; THF, 20 min, μW, 150 °C, 150 W. (c) KOH, $NH_2OH \cdot HCl$, MeOH, 70%.

We describe herein our preliminary findings on designing a metal-based SAHA analogue to probe the effect of a nonplanar phenyl bioisostere, namely, a ferrocene unit, on HDAC inhibitory action. A recent study showed SAHA-*cis*-platin hybrids to have low micromolar activity against HDACs and cancer cell lines,[16] and previous studies from ours and other groups have shown that ferrocene analogues can display impressive anticancer activity and act as effective metallodrugs and bioinorganic probes.[17−19]

The microwave-mediated reaction of ferrocenylamine **4** with methyl-8-chloro-8-oxooctanoate **5** afforded the methyl ester **6** (Scheme 1). High yields were observed when employing triethylamine as a base as opposed to a polystyrene-supported base. Moreover, the former procedure obviated the need for a chromatographic purification step. Next, reaction of **6** with hydroxylamine, generated in situ from its HCl salt, afforded the desired compound Jay Amin hydroxamic acid (JAHA) **7**, in an overall ca. 50% yield. Using related synthetic procedures, a small library of JAHA analogues was synthesized for SAR investigation. Hence, the homologated ferrocene **8** (*homo*-JAHA) was made to probe the effect of introducing a methylene spacer in **7**. A number of aryl-linker JAHA analogues were next synthesized viz. the *ortho*- and *para*-ferrocenyl-substituted **9**−**11**, respectively, to evaluate the effect of the organometallic group linked to a parent SAHA molecule.

The structures of the methyl ester intermediates to **7**, **8**, and **9**, namely, **6**, **12**, and **13** were investigated in the solid state by X-ray crystallography to ascertain the presence of the ferrocene group and to assist our modeling studies on **7** (Figure S2 and Table S1 in the Supporting Information).

The incorporation of organometallic moieties into protein ligands brings the potential benefit of filling space in a way that is not possible with simple planar aromatic groups or alicyclic ring systems, which can enhance affinity.[20,21] In addition, ligands that contain organometallic groups can enable X-ray structure determination of protein−ligand complexes by facilitating phasing. To explore the binding mode of **7** in deacetylases, we performed docking studies, using the structure of an HDAC8-SAHA complex as a starting point.[22] We found that **7** is able to bind in a similar mode to SAHA, exploiting archetypal interactions between the hydroxamate and the catalytic zinc, together with a hydrogen bond between

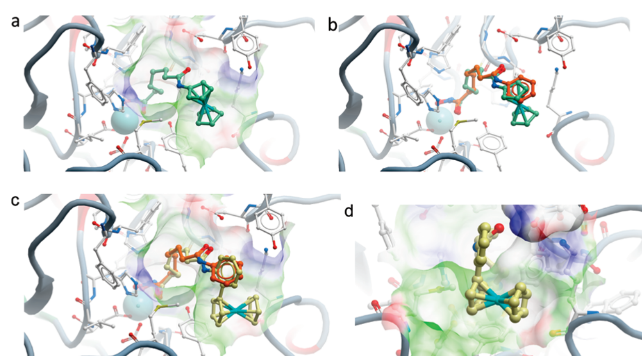


Figure 2. Docked **7**, **9** and comparison to SAHA. (a) Docked **7** in the active site of HDAC8. (b) Comparison of **7** to cocrystallized SAHA (PDB code: 1t69). (c and d) Docked **9** in HDAC8 (superimposed over SAHA in panel c). The zinc ion is depicted in cyan. The residues in the binding pocket and ligands are shown in ball and stick representation. Color coding: nitrogen, blue; oxygen, red; carbon, gray in protein, light green for **7**, and orange for SAHA; and yellow for **9**.

Table 1. IC_{50} Values for HDACis versus SAHA (1)[a]

analogue	HDAC1	HDAC2	HDAC3	HDAC6	HDAC8
1	0.001 (0.0002)	0.002 (0.0007)	0.003 (0.0004)	0.00049 (0.00010)	1.41 (0.15)
7	0.011 (0.002)	0.011 (0.003)	0.018 (0.004)	0.0008 (0.0001)	1.36 (0.16)
8	0.033 (0.005)	0.039 (0.0086)	0.072 (0.011)	0.0067 (0.0009)	0.29 (0.02)
9	0.0107 (0.002)	0.010 (0.0026)	0.008 (0.001)	0.0003 (0.00006)	0.002 (0.0002)
10	0.0005 (0.0004)	0.0006 (0,0001)	0.009 (0.0018)	0.00009 (0.00005)	0.54 (0.07)
11	2.56 (0.34)	3.04 (0.16)	3.17 (0.22)	1.40 (0.13)	6.07 (1.18)

[a] IC_{50} values for compounds (μM) with SD in parentheses. Assays were performed in duplicate.

the amide moiety of the ligand and Asp101. The ferrocenyl group of **7** is comfortably accommodated in the shallow pocket formed by Tyr100, Phe152, and Tyr306; this region of the enzyme is reported to be malleable and able to adapt to structures of different ligands. This binding mode suggested that 7 and SAHA should display similar potencies as HDACis (Figure 2).

Compound **9**, with the ferrocene group substituted at the ortho-position on the phenyl group of SAHA, is docked similarly (Figure 2c). The phenyl ring is twisted when compared with cocrystallized SAHA. With the insertion of the phenyl group, when compared to compound **7**, the ferrocene group of compound **9** is pushed further down to the subpocket of the enzyme, leading to a better fit in the hydrophobic groove formed by Phe 152 and Tyr306 as well as Lys33 and Met274 (Figure 2d). The close van der Waals interactions between the ferrocene group and the residues in the subpocket could potentially increase the affinity of compound **9**.

Compound **7** was tested for inhibitory action against class I, IIa, and IIb HDACs (**1**−**9**) and compared with the related SAHA **1**. Both have similar broad HDAC inhibitory profiles, exhibiting similar IC_{50} values, including for HDAC8, which gratifyingly consolidates our modeling studies (Table 1). Compound **8** has a similar profile to that of **7** for HDACs **1**−**3**, although it is ca. 10-fold less potent toward HDAC6 and four times more potent toward HDAC8. Compound **10** considerably outperforms the other ferrocenes as well as SAHA toward HDACs **1**, **2**, and notably, against the class IIb, HDAC6 (IC_{50} = 90 pm), whereas **9** shows the lowest IC_{50} value toward HDAC8 with a IC_{50} = 2 nM. Compound **11**, a shorter length analogue of **10**, displayed poor HDAC inhibition, underpinning the importance of chain length on HDAC inhibition. None of the compounds **7**−**11** showed any significant inhibition of class IIa HDACs (**4**, **5**, **7**, and **9**),[23] and data are not shown.

Critical to the development of chemical probes targeting HDACs is the demonstration of activity in a cellular environment. The compounds were tested for cytotoxicity against a breast cancer cell line, and the results are given in Table 2.[24] SAHA **1** is the most cytotoxic of the series with all of the ferrocenes displaying single digit micromolar activities. Analogues **9** and **10** are less cytotoxic than **1**, which may reflect reduced cellular penetration due to the presence of the ferrocene group.

Additionally, propidium iodide-staining experiments demonstrated that **7** and **10** also induced cell cycle arrest and cell death in MCF7 cells (Figure S1 in the Supporting Information). Consistent with effects of other HDACis, the compounds decreased the proportion of cells in the G1 and S phases of the cell cycle, associated with an accumulation of cells in G2M.[25] The compounds also increased the proportion of cells with sub-G1 DNA content, a marker of cell death.

Table 2. Cytotoxicity of JAHAs and SAHA in MCF7 Breast Cancer Cell Lines

analogue	IC_{50} (μM) with SD[a]
1	0.73 ± 0.38
7	2.44 ± 0.54
8	3.60 ± 0.48
9	1.90 ± 0
10	3.13 ± 1.08
11	5.08 ± 0.55

[a] MCF7 cell line. Mean value of a minimum of two assays.

To assess the activity of lead compounds emerging from this research, we cultured K562 erythroleukemia cells with compounds **9** and **10** and performed flow cytometry to quantify changes in bulk chromatin acetylation (the endogenous substrate of class I deacetylases) and acetylation of α-tubulin (a substrate of HDAC6). As shown in Figure 3, cells treated with **9** and **10** (10 μM) produced comparable histone and tubulin acetylation as the FDA-approved HDACi **1** (Figure 3).

JAHA analogues have been synthesized in good yields using a crucial expedited microwave-mediated amide coupling reaction prior to the attachment of the hydroxamic acid zinc binding moiety. Many, apart from **11**, display excellent broad class I HDAC inhibition although, for HDAC8, IC_{50} values are mainly in the micromolar range, with the exception of **9** (IC_{50} = 2 nM). Nanomolar or subnanomolar inhibition of the class IIb HDAC6 was also observed. These results establish that the three-dimensional manipulation of the aryl “cap” of SAHA **1** is a viable strategy for the synthesis of HDACis. Because HDAC inhibition is often improved, these results suggest that the use of nonplanar cap groups may have broader potential in HDACi design. Current studies are aiming to synthesize JAHA analogues by changing the cap (metal/coligands), linker, and binding groups for SAR studies and employing these analogues as structural probes. Further studies have confirmed the cell permeability of compounds **9** and **10** and identify attractive potency against class I and class II HDACs in a cellular context.[25] Compounds **9** and **10** hold promise as bioinorganic probes for HDACs, and we are currently actively investigating their potential for obtaining cocrystal structures in HDACs as well as studies in vivo including *Xenopus*.[26,27]

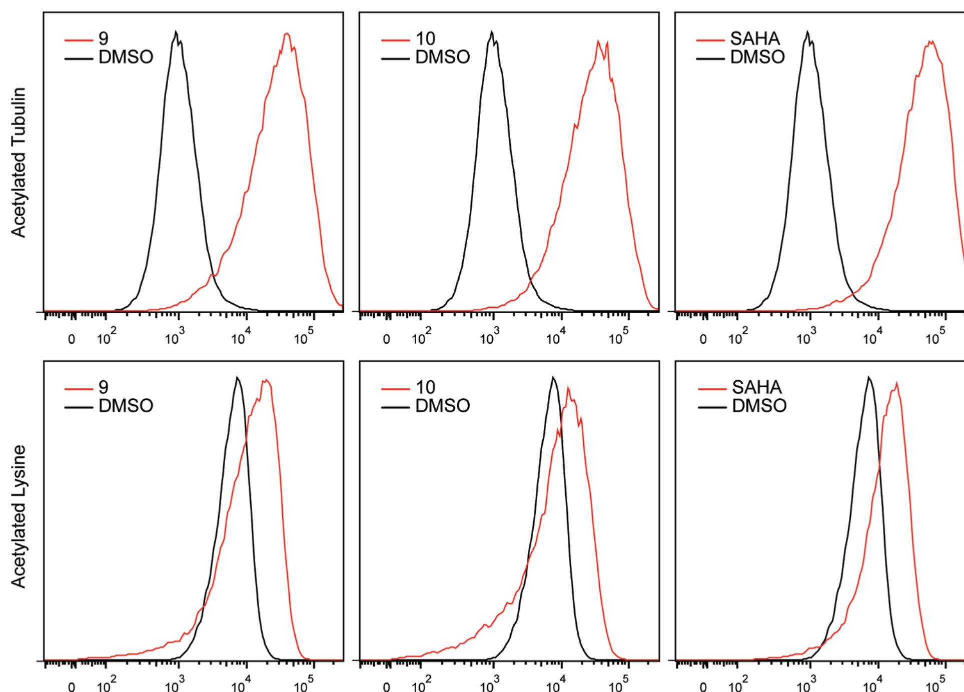


Figure 3. Comparative flow cytometry cell-based data for **9**, **10** and SAHA.

■ ASSOCIATED CONTENT

S Supporting Information. Biological procedures, synthesis of JAHAs, JAHA modeling details, ^{1}H and ^{13}C spectra, and X-ray crystallography.[28] This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DISCLOSURE

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■ DEDICATION

This paper is dedicated to the memory of Mick Pether, James Black Foundation, a brilliant medicinal chemist, sadly and prematurely deceased in 2010.

■ ABBREVIATIONS

JAHA, Jay Amin hydroxamic acid; SAHA, suberoyl anilide hydroxamic acid; HDAC, histone deacetylase; HDACi, HDAC inhibitor; SAR, structure–activity relationship

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