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Mercaptoamide-based non-hydroxamic acid type histone deacetylase inhibitors

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Abstract—Inhibitors of histone deacetylases (HDAC) are emerging as a promising class of anti-cancer agents. A mercaptoamide functionality was designed as a bidentate zinc chelator and incorporated into the hydroxamic acid based SAHA (**1**) scaffold in order to identify non-hydroxamate compounds as potential inhibitors of histone deacetylases. Two sets of mercaptoamides **2** and **3** with varying spacer length were synthesized and their HDAC inhibitory activity was evaluated. Low micromolar inhibition was observed for mercaptoamides **2e**, **3b**, and **3d**.

Inhibitors of histone deacetylases (HDACs) are emerging as a promising class of anti-cancer agents.[1–5] The primary activity of the HDACs is to catalyze the hydrolysis of acetyl groups from amino terminal lysine residues of the nucleosomal core histones.[6] HDAC inhibitors inhibit proliferation of tumor cells by inducing terminal differentiation of tumor cells. Inhibition of HDAC activities in cancer cells also leads to cell cycle arrest and induction of apoptosis. Many of these inhibitors have potent anti-tumor effect in vivo and some of them are currently in phase I/II clinical trials.[7,8]

Class I and II HDACs are metalloenzymes. The co-crystal structure of the histone deacetylase-like protein (HDLP) with SAHA[9] (**1**, Fig. 1), the most advanced hydroxamate compound in clinical trials, reveals an active site comprising a tubular pocket with a zinc ion at the bottom of the pocket. While the hydroxamate head group of SAHA interacts with the active site zinc ion in a bidentate fashion, the linker spans the tube-like portion of the binding pocket and the aromatic cap group makes contact with the pocket entrance. In addition, hydrogen bonding of the hydroxamic acid with the amino acid residues of the enzyme, imidazole groups in the histidines (H131, H132), aspartates (D173, D166) along with active site tyrosine (Y297), provides further rationale for the potent inhibitory activity of these agents. The crystal structure of human HDAC8 complexed with SAHA, reported recently,[10,11] also sheds light on such a catalytic mechanism of the HDACs.

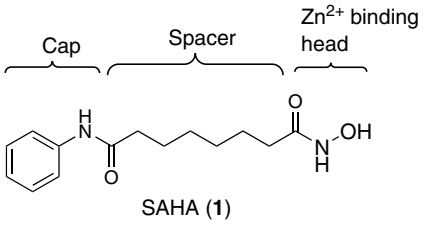

Figure 1. Structural motifs of HDAC inhibitor SAHA.

Hydroxamic acid HDAC inhibitors, such as SAHA or-LAQ824,[12] do not discriminate well among the HDAC subtypes. However, *o*-aminoanilide non-hydroxamate SAHA analogs have shown subtype selectivity.[13–16] By contrast, modifications of the cap group of SAHA has produced selectivity for tubulin acetylation versus histone acetylation.[15] Inhibitors that contain hydroxamic acid as zinc binding group (ZBG) have produced poor results in clinical trials for other diseases because of poor pharmacokinetics that may be associated with the hydroxamic acid functionality.[17–20]

Keywords: Histone deacetylase inhibitor; Non-hydroxamates; Mercaptoamides.
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There are non-hydroxamate HDAC inhibitors such as benzamide, MS-275[21] and a depsipeptide, FRK228[22] currently in phase I/II clinical trials. Other non-hydroxamate functionalities such as trifluoromethyl ketones,[23] ketoamides,[24] phosphonates,[25] and *N*-formylhydroxylamine[26] are also reported. However, these later compounds have either reduced potency or metabolic liability. Therefore, there is a clear need to develop HDAC inhibitors with new non-hydroxamate ZBG to improve the efficacy, pharmacokinetics and selectivity of these promising anti-cancer agents.

The thiol functionality has been shown to be a good monodentate chelator for zinc-dependent enzymes such as angiotensin converting enzyme[27] and matrix metalloproteinases.[28] Thiol-based SAHA analogs are reported to be potent HDAC inhibitors[29] in spite of their monodentate zinc binding ability in comparison to SAHA's bidentate hydroxamic acid moiety. Also it has been shown that the disulfide group in the depsipeptide, FRK228, undergoes reduction within the cells to release the zinc binding thiol moiety leading to potent HDAC inhibition.[22] Here we have designed compounds, structurally as close as possible to SAHA, but having the mercaptoamide replacing the hydroxamate moiety as a bidentate ZBG and tested them for HDAC inhibitory activity.

We prepared two sets of compounds possessing an internal amide or reverse amide attached to the zinc binding thiol functionality (Fig. 2). Both the mercaptoethylamide in **2** and the mercaptoacetamide in **3** can potentially function as a bidentate Zn^{2+} chelator. While, the mercaptoacetamides are recently shown to be a ZBG in HDAC inhibitors,[30–32] we have explored the possibility of mercatoethylamide as the ZBG in SAHA based scaffold. We have also varied the distance between the mercaptoamide head group to the cap group to see its effect on the HDAC enzyme inhibition. As in SAHA, a benzamide cap was chosen in compounds **2**, but a quinolinamide cap was incorporated in compounds **3**, since it is shown that incorporation of such a cap have produced improved HDAC potency.[29]

The synthesis of mercaptoamides **2** and **3** is outlined in Schemes 1 and 2 and the compounds prepared for this study are listed in Table 1. Accordingly, the commercially available acid chlorides **4b**–**g** (n = 1–6) were treated with aniline in presence of diisopropylethyl amine (DIEA) to obtain the benzamides **5b**–**g** (n = 1–6). While the benzamide **5a** (n = 0) is available commercially, the benzamide **5h** (n = 7) was obtained by treating the carboxylic acid **4h** (n = 7) with aniline in presence of EDCI and HOBT. The esters of **5a**–**h** were then hydrolyzed using lithium hydroxide to obtain the carboxylic acids,

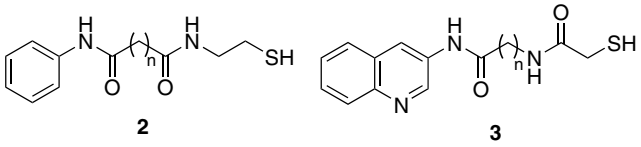
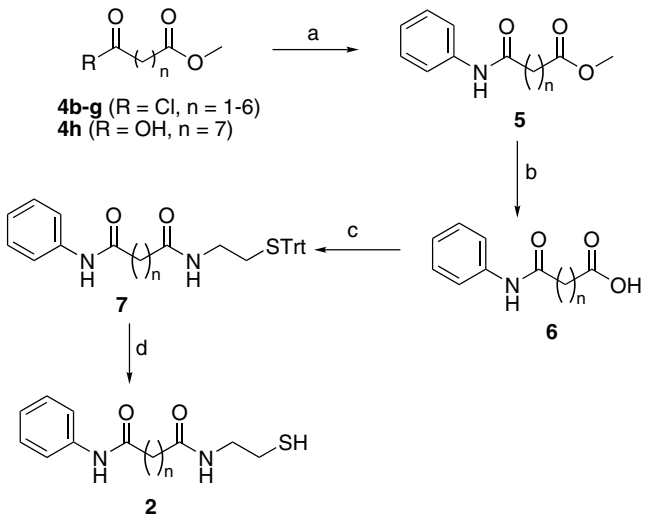


Figure 2. Mercaptoamide based non-hydroxamate HDAC inhibitors.



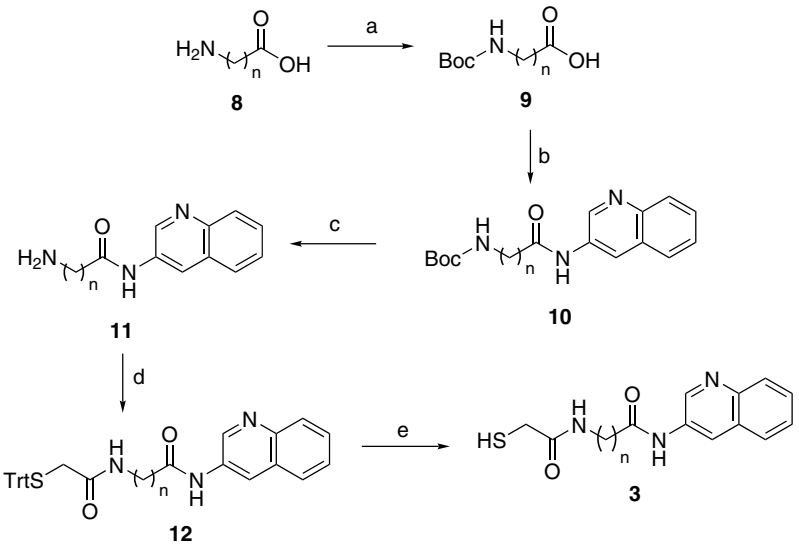
Scheme 1. Reagents and conditions: (a) aniline, DIEA, DCM, 0–10 °C for 10 min, rt for 30 min (83–85%) or EDCI, HOBT, DIEA, rt, 9 h (84–87%); (b) LiOH, EtOH, H_2O, rt, 3 h (90–95%); (c) *S*-trityl cysteamine, EDC, HOBT, DIEA, DCM, rt, 12 h (45–50%); (d) TFA, triisopropylsilane, DCM, 1 h (20–25%).

6a–**h**, which were then coupled with *S*-trityl protected cysteamine in presence of EDCI and HOBT to give trityl protected mercaptoamides **7a**–**h**. The *S*-trityl protected cysteamine was prepared from the corresponding cysteamine by treating with trityl alcohol and trifluroacetic acid. Deprotection of the trityl group from **7** with trifluoroacetic acid in presence of triisopropylsilane resulted in the desired mercaptoethylamides **2a**–**h**.

Mercaptoacetamides, **3a**–**d** (n = 2–5) were prepared according to Scheme 2. The amino acids **8a**–**d** (n = 2–5) were *N*-protected with the Boc group using di-*t*-butyldicarbonate in presence of sodium hydroxide and then coupled with 3-aminoquinoline to obtain amides **10a**–**d**. Amides **10a**–**d** were then treated with hydrochloric acid in diethylether to obtain free amines **11a**–**d**, which were then coupled with *S*-tritylmercaptoacetic acid to obtain trityl protected mercaptoacetamides **12a**–**d** in presence of EDCI and HOBT. Tritylmercaptoacetic acid was prepared from the corresponding mercaptoacetic acid using trityl alcohol and borontrifluoride etherate. Deprotection of the trityl group from **12a**–**d** afforded the desired mercaptoacetamides **3a**–**d** (n = 2–5).

The mercaptoamides, **2a**–**h** and **3a**–**d** were tested with an in vitro assay using a HeLa nuclear extract.[33] Inhibition of HDAC enzyme (IC_{50}) results are summarized in Table 1. SAHA was used as a positive control,[34] which had an IC_{50} value of 0.06 μM in our assays.

The IC_{50} values reported in Table 1 suggest that the replacement of hydroxamic acid with mercaptoamide functionality results in moderately potent HDAC inhibitors. The mercaptoethylamide, **2e** or the mercaptoacetamide, **3d**, whose bidentate Zn^{2+} binding motif (Fig. 3) resembles that of SAHA with a similar overall spacer length, was found to be ~20-fold less potent than SAHA. Interestingly, the mercaptoethylamide **2e** is



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, NaOH, *t*-BuOH, 12 h (82–93%); (b) 3-aminoquinoline, HOBT, EDCI, DIEA, DCM, rt, 12 h (84–88%); (c) HCl/Et_2O, 0 °C, 4 h; (d) *S*-tritylmercaptoacetic acid, HOBT, EDCI, DIEA, DCM, rt, 12 h (80–85%); (e) TFA, DIEA, DCM, 1 h (41–51%).

Table 1. HDAC inhibition data for mercaptoamides **2** and **3**[a]

Entry	Compounds	*n*	IC_{50} (μM)
1	**2a**	0	2.80
2	**2b**	1	70.0
3	**2c**	2	12.5
4	**2d**	3	3.10
5	**2e**	4	1.50
6	**2f**	5	2.50
7	**2g**	6	2.35
8	**2h**	7	10.2
9	**3a**	2	39.5
10	**3b**	3	0.66
11	**3c**	4	2.80
12	**3d**	5	1.10

[a] Average value of two independent experiments.

more potent toward HDAC inhibition compared to the literature reported mercaptoethylamine **13**[29] (IC_{50} > 100 μM) suggesting that the presence of the amide functionality in mercatoethylamide **2e** enhances HDAC inhibition.

We also examined the effect of spacer length between the cap region and the metal chelating thiol moiety in both series of compounds **2** and **3**. As shown by the data in Table 1, the HDAC inhibition was very much dependent on the chain length with the best activity seen for the compound with a spacer length of $n = 4$ (**2e**), having a similar spacer distance between the head group to cap group as in SAHA. Either shorter ($n = 1$–2) or longer chain lengths ($n = 7$) resulted in reduced HDAC inhibition potency, an observation similar to SAR studies in the hydroxamate series. Similar conclusion can be drawn for the mercaptoacetamide series **3**. Here again, it is clear that the optimal distance is $n = 3$–5. While the inhibitors with chain length of $n = 3$–5, **2d**–**g**, had inhibition in the low micromolar range, it was rather surprising to see low micromolar inhibiton for **2a** with $n = 0$, which could be due to the presence of the 1,2-diketone in **2a** that might provide additional bidentate zinc chelation.

In summary, replacing a hydroxamic acid with a mercaptoamide functionality, as in **2e**, **3b**, and **3d** resulted in HDAC inhibition with IC_{50} in the low micromolar range. The mercaptoamide analog **2e** seemed to be more potent than the structurally similar mercaptoamine analog **13** suggesting that the mercaptoethylamine group

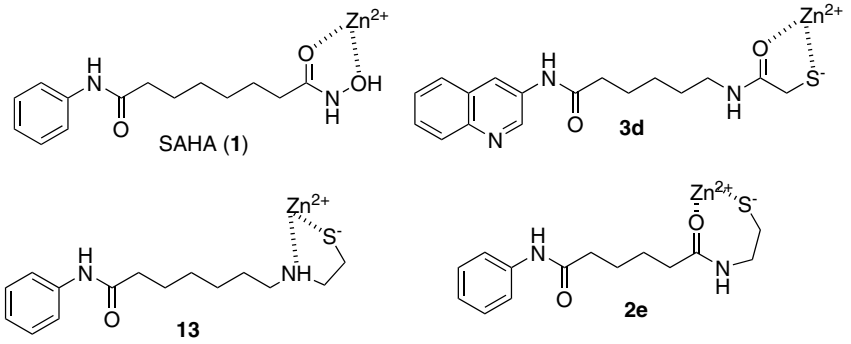


Figure 3. Bidentate chelation of hydroxamic acid in SAHA, mercaptoethylamine **13** and mercaptoamides **2e**, **3d** with Zn^{2+} ion.

adjacent to a carbonyl moiety could have similar bidentate chelating geometry with the zinc ion (Fig. 3) as in SAHA. The reduced potency for **2e** or **3d** compared to SAHA would suggest that the zinc chelation might be weaker with either the mercaptoethylamide or the mercaptoacetamide. Further SAR studies are in progress to improve the HDAC potency, physiological properties and overall pharmacological behavior of this class of inhibitors over the hydroxamate class.

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33. In vitro fluorescent histone deacetylase assay: HDAC inhibition assay was performed using the HDAC fluorescent activity assay kit (Biomol Research Laboratories, Plymouth Meeting, PA). HeLa cell nuclear extract, which contains a number of HDAC isozymes and other nuclear factors, was used as the source of HDAC activity. The final substrate concentration in the assay mixture was 50 μM. The reaction was allowed to proceed for 10 min at room temperature before stopping the reaction. Test compounds were prepared as 20 mM stock solutions in DMSO (Molecular Biology grade, Sigma–Aldrich Co., St. Louis, MO) and stored at −70 °C. DMSO had no significant effect on the activity of this assay at concentrations up to 5%; the final DMSO concentration in the wells will be no more than 2%. Assays were performed in white polystyrene 96-well half-area assay plates (Corning, NY) and measured on a Wallac 1420 fluorescent plate reader (Wallac Oy, Turku, Finland) with an excitation wavelength of 355 nm, an emission wavelength of 460 nm, and a 1 s signal averaging time.
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