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Design and synthesis of thiazole-5-hydroxamic acids as novel histone deacetylase inhibitors

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Abstract—We have designed and synthesized a series of structurally novel hydroxamic acid-based histone deacetylase (HDAC) inhibitors characterized by a zinc chelating head group attached directly to a thiazole ring. The thiazole ring connects to a piperazine spacer, which is capped with a sulfonamide group. These novel molecules potently inhibit an HDAC enzyme mixture derived from HeLa cervical carcinoma cells and show potent antiproliferative activity against the breast cancer cell line MCF7.

Epigenetics refers to the phenomenon in which gene expression can be altered by reversible chemical modifications to DNA or nucleosomal core histones.[1] Epigenetics plays a major role in a number of biological events, including cell differentiation, embryonic development, inflammation, and cancer. One of the major epigenetic activities is the acetylation of lysine side chains of histone tails, which is carried out by a family of histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs).[1] HDAC inhibitors have been shown to have antiproliferative effects on tumor cells in vitro through a number of mechanisms including cell cycle arrest, apoptosis, and induction of terminal differentiation.[1–4] In vivo efficacy in xenograft tumor models has further substantiated HDACs as valid target for developing novel anticancer agents.[5,6] Both hydroxamic acid-based HDAC inhibitors, for example, Vorinostat (SAHA)[7,8] (**1**), LBH589[9–12] (**2**), and PXD101[13,14] (**3**), and non-hydroxamic acid-based HDAC inhibitors, for example, MS275[15,16] (**4**) and FK228[17] (Fig. 1), are currently in the clinic. Clinical trials to date have shown these inhibitors to have activity against a variety of malignancies.[3,4] SAHA is the first HDAC inhibitor to be approved by the FDA for the treatment of cutaneous T-cell lymphoma. The non-hydroxamate inhibitor MS275 has been examined in a phase I trial,[16] LBH589 has been tested in hematologic malignancies,[12] and PXD101 has been tested in an ovarian cancer trial.[14] In each case histone hyperacetylation was observed in peripheral blood mononuclear cells.

The bacterial HDAC homolog HDLP[18] and human HDAC8[19,20] has been co-crystallized with hydroxamic acid-based inhibitor, SAHA. Several HDAC inhibitors have been designed and synthesized based on these crystallographic data.[21–24] In all of these designs the common structural motif for an HDAC inhibitor consists of a metal binding head group (A), which interacts with the Zn^{2+} ion at the bottom of the active site, a linker domain (B), which occupies the narrow tubular pocket, and a cap group (C), which interacts with the residues on the rim of the active site (**5**, Fig. 1).

In our present study, we have designed novel HDAC inhibitors **6** with a hydroxamic acid as the zinc chelating head group attached directly to a thiazole ring with a piperazine spacer and a sulfonamide cap (Fig. 2). A modification to the cap region leading to amides, as in **7**, or *N*-alkyl/arylamines, as in **8,** was also synthesized (Fig. 2). We also prepared a series of sulfonamides **9** with the zinc chelating hydroxamic acid extended from the thiazole ring via a double bond for comparison of

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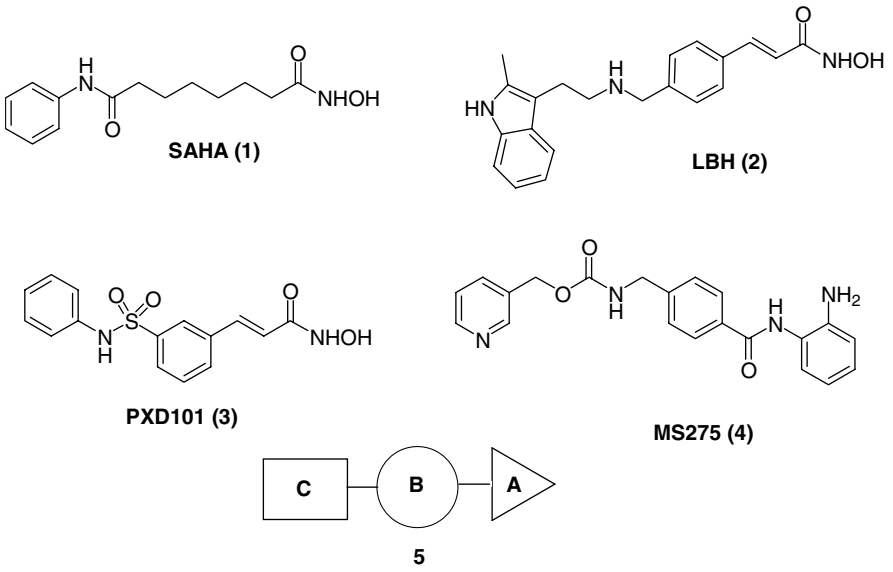


Figure 1. HDAC inhibitors in clinical trial and their structural motif.

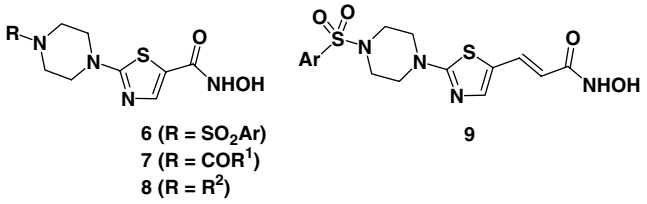
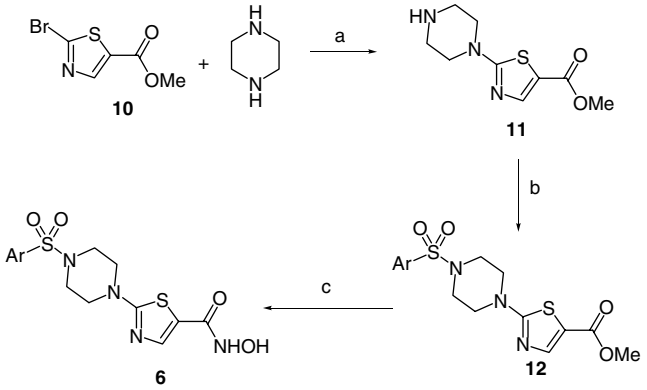


Figure 2. Thiazole based hydroxamic acid HDAC inhibitors.

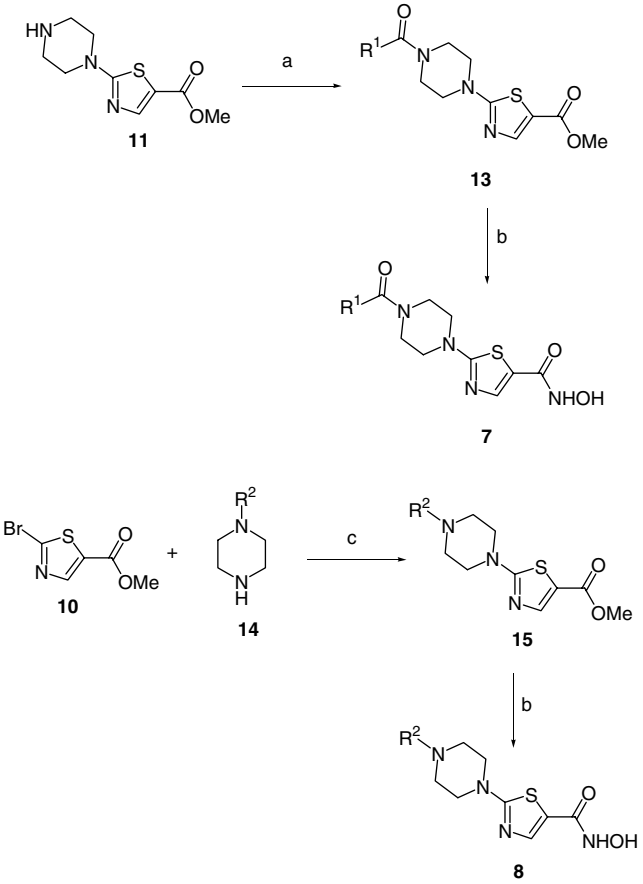
HDAC enzyme activity with that of the **6** series. Here we report the synthesis and biological evaluation of these novel HDAC inhibitors.

Sulfonamide analogs of hydroxamic acids **6** were synthesized from the commercially available 2-bromothiazole ester **10** (Scheme 1). Accordingly, methyl 2-bromothiazole carboxylate **10** was treated with piperazine in the presence of potassium carbonate to obtain thiazole intermediate **11**. The intermediate **11** was treated with arylsulfonyl chlorides to afford sulfonamides **12** which on treatment with 50% aqueous hydroxylamine in the presence of sodium hydroxide resulted in the desired hydroxamic acids **6**.

The preparation of amide analogs of hydroxamic acids **7** was carried out according to Scheme 2. The intermediate



Scheme 1. Reagents and conditions: (a) K_2CO_3, MeCN, 80 °C, 8 h (90%); (b) arylsulfonyl chlorides, Et_3N, DCM, rt, 4–12 h (87%); (c) 50% aq NH_2OH, NaOH, MeOH, DCM, rt, 4–5 h (50%).



Scheme 2. Reagents and conditions: (a) alkyl/arylacid chlorides, Et_3N, DCM, rt, 4–12 h (86%); (b) 50% aq NH_2OH, NaOH, MeOH, DCM, rt, 4–5 h (50%); (c) K_2CO_3, MeCN, 80 °C, 8 h (92%).

ester **11** was treated with either acetyl chloride, phenylacetyl chloride or benzoyl chloride in the presence of triethylamine to obtain the corresponding amide intermediates **13**. Amide intermediates **13** were then converted to hydroxamic acids **7** by reaction with 50% aqueous hydroxylamine in the presence of sodium hydroxide. Synthesis of the corresponding *N*-alkyl/aryl analogs **8** is also shown in Scheme 2. Accordingly, bromothiazole **10** was reacted with the commercially available *N*-alkyl or aryl piperazines **14** in the presence of potassium carbonate to obtain esters **15**, which were then converted to hydroxamic acids **8** by treatment with aqueous hydroxylamine and sodium hydroxide.

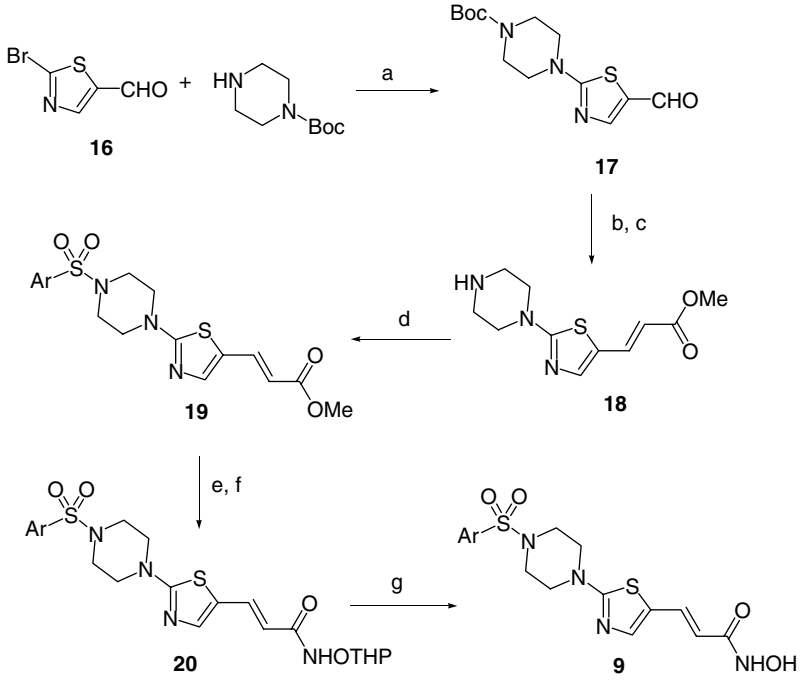
The α, β-unsaturated hydroxamic acids **9** were synthesized according to Scheme 3. Commercially available 2-bromo-5-formylthiazole **16** was treated with *N*-Boc-piperazine and potassium carbonate to obtain formyl intermediate **17**. Intermediate **17** was converted to the α, β-unsaturated ester **18** via a Wittig–Horner reaction with trimethyl 2-phosphonoacetate in the presence of *n*-butyl lithium followed by Boc deprotection. The α, β-unsaturated ester intermediate **18** was then treated with arylsulfonyl chlorides in the presence of triethylamine to obtain the sulfonamide esters **19**. The acids resulting from ester hydrolysis of **19** were coupled with tetrahydropyranyl (THP) protected hydroxylamine in presence of hydroxybenzotriazole and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide to give the THP protected α, β-unsaturated hydroxamic acid intermediates **20**. Removal of the THP group in **20** using hydrochloric acid in ether afforded the desired hydroxamic acids **9**.

The in vitro HDAC inhibitory activity in a HeLa cell nuclear extract[25] and the antiproliferative activity towards the breast cancer cell line MCF7 were determined for hydroxamic acids **6**, **7**, **8**, and **9**. SAHA[26] was used as positive control, affording an IC_{50} value of 0.06 μM and a GI_{50} value of 0.4 μM. The data for hydroxamic acids **6** (Table 1) clearly indicate that these novel hydroxamates potently inhibit HDAC enzymes with the best activity seen for compounds **6c** and **6f** with IC_{50} values of <100 nM. The SAR data suggest that electron-donating groups on the aryl ring afford more potent inhibitors (**6c** and **6f**) compared to electron-withdrawing groups (**6b**

Table 1. HDAC inhibition and cell proliferation data for hydroxamic acids **6**, **7**, and **8**[a]

Compound	Ar/R^1/R^2	HDAC IC_{50} (μM)	MCF7 GI_{50} (μM)
Sulfonamides			
6a	2-Naphthyl	0.22	2
6b	4-Trifluoromethoxyphenyl	1.2	4
6c	4-Methylphenyl	0.09	0.35
6d	4-Biphenyl	0.2	1.8
6e	4-Trifluoromethylphenyl	2.6	4
6f	3,4-Dimethoxyphenyl	0.07	2
6g	Phenyl	0.57	10
6h	4-Nitrophenyl	0.4	10
6i	4-Fluorophenyl	0.15	3.5
Amides			
7a	Methyl	2.8	>100
7b	Phenyl	3.5	40
7c	Benzyl	32	20
Aryl/alkyl amines			
8a	Methyl	>100	>100
8b	Benzyl	>100	4
8c	2-(Phenyl)ethyl	>100	>100

[a] Average value of at least two independent experiments.



Scheme 3. Reagents and conditions: (a) K_2CO_3, MeCN, 80 °C, 8 h (92%); (b) trimethylphosphonoacetate, *n*-BuLi, THF, −50 °C, 2 h (74%); (c) 4 M HCl/dioxane, 2 h (97%); (d) arylsulfonyl chlorides, Et_3N, DCM, rt, 4–12 h (86%); (e) NaOH, MeOH, rt, 5 h (95%); (f) HN_2OTHP, EDCI, HOBT, Et_3N, DCM, rt, 12 h (83%); (g) HCl, MeOH, Et_2O, rt, 5 h (91%).

and **6e**). The sulfonamide analogs **6** show good potency against HDAC enzymes, while the corresponding amide analogs **7** and alkyl/aryl analogs **8** were less potent against the HDAC enzyme and showed less antiproliferative activity against the breast tumor cell line (Table 1). The antiproliferative activity (GI_{50}) toward MCF7 for sulfonamides **6** was in the range of 0.35–10 μM with the best cellular activity seen for **6c** which exhibited a GI_{50} of 350 nM.

Having identified the sulfonamide functionality as the optimal cap group, we explored the possibility of improving both HDAC enzyme activity and cell potency by preparing the α, β-unsaturated hydroxamic acids **9**. Such α, β-unsaturated hydroxamic acids, for example, LAQ824,[7] LBH589,[7] (**2**), and PXD101[9] (**3**), have been shown to be potent HDAC inhibitors. The results presented in Table 2 suggest that such a modification does not, in general, result in improved potency in this series, except for **9a**. Seven compounds out of the nine in the **6** series displayed greater potency against HDAC enzyme than the corresponding α, β-unsaturated analog in the **9** series, with differences of approximately 2- to 50-fold (e.g., **6d**, HDAC IC_{50} of 0.2 μM vs **9d**, HDAC IC_{50} of 11 μM). There was not a direct correlation between HDAC inhibitory activity and cellular potency for all pairwise comparisons within the **6** and **9** series, however **6** analogs demonstrated higher enzyme inhibitory activity and increased antiproliferative activity, for example, **6c** (IC_{50} 0.09 μM, GI_{50} 0.35 μM) versus **9c** (IC_{50} 0.55 μM, GI_{50} 2 μM). The lack of correlation in cellular potency of the **9** series versus the **6** series compounds may be due to improved cellular permeability as a result of the α, β-unsaturated hydroxamic acid function.

In summary, we have designed and synthesized a series of novel HDAC inhibitors having both enzymatic and antiproliferative activity. The sulfonamide analog **6c** was found to have both enzyme and cell potency similar to SAHA. Future efforts will involve the identification of readily synthesized analogs of series **6**, which are potent and chemically stable HDAC inhibitors that avoid any liability resulting from the α, β-unsaturated double bond present in series **9**.

Table 2. HDAC inhibition and cell proliferation data for hydroxamic acids **9**[a]

Compound	Ar	HDAC IC_{50} (μM)	MCF7 GI_{50} (μM)
9a	2-Napthyl	0.05	0.7
9b	4-Trifluoromethoxyphenyl	3.2	6
9c	4-Methylphenyl	0.55	2
9d	4-Biphenyl	11	1.5
9e	4-Trifluoromethylphenyl	15	3
9f	3,4-Dimethoxyphenyl	0.16	4
9g	Phenyl	0.44	3
9h	4-Nitrophenyl	1.6	5
9i	4-Fluorophenyl	4.7	2.5

[a] Average value of at least two independent experiments.

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25. In vitro fluorescent histone deacetylase assay: HDAC inhibition assays were 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 a stop solution was added. 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% with the final DMSO concentration in the assays of no more than 2%. Assays were performed in white polystyrene 96-well half-area assay plates (Corning, 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 one sec signal averaging time.
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