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Synthesis, enzymatic inhibition, and cancer cell growth inhibition of novel δ-lactam-based histone deacetylase (HDAC) inhibitors

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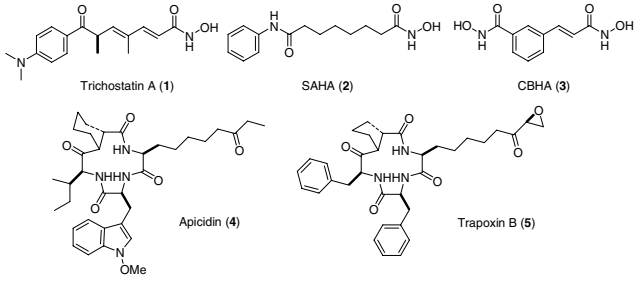
Abstract—δ-Lactam-based hydroxamic acids, inhibitors of histone deacetylase (HDAC), have been synthesized via ring closure metathesis of key diene intermediates followed by conversion to hydroxamic acid analogues. The hydroxamic acids **12a**, **12b**, and **17c** showed potent inhibitory activity in HDAC enzyme assay. The hydroxamic acid **12b** exhibited growth inhibitory activity on five human tumor cell lines, showing good sensitivity on the MDA-MB-231 breast tumor cell.

Histone deacetylase (HDAC) and histone acetyltransferase (HAT) are involved in the co-regulation of chromatin remodeling and the functional regulation of gene transcription.[1] HDAC catalyzes deacetylation of ε-amino group in lysines located near the N-terminal of core histone proteins. Abnormal recruitment of HDAC is related to carcinogenesis.[2] Thus, the identification of potent HDAC inhibitors has been considered as a very intriguing approach for the development of cancer chemotherapy.[3] A number of natural and synthetic HDAC inhibitors have shown an anti-proliferative activity on tumor cells. Among them, trichostatin A (TSA, **1**),[4] apicidin (**4**),[5] trapoxin B (TPX, **5**),[6] and FK-228[7] were classified as natural substances, and suberoylanilide hydroxamic acid (SAHA, **2**)[8] and other TSA or SAHA-like analogues were reported as synthetic HDAC inhibitors.[9]

Intensive efforts have recently been focused on the modifications of the aromatic ring moiety and the aliphatic linker region present in the molecules.



Especially, CBHA (**3**) with the aromatic linker demonstrated toleration of the aromatic ring and the importance of the proper orientation of the linker.[10] Based on the structures of the analogues **1**–**3**, we designed and synthesized novel HDAC inhibitors that incorporate structural features of δ-lactam ring as a linker domain, which is expected to endow the inhibitors with the control of the dihedral angle between surface recognition and metal binding region, and the decreased entropy penalty due to the flexible alkyl chain linker. Herein, we describe our results on the synthesis, enzyme inhibition, and cancer cell growth inhibition of novel δ-lactam-based HDAC inhibitors.

Schemes 1–3 outline the preparation of δ-lactam analogues.[11] Diester **6** was prepared by literature proce-

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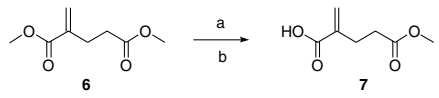
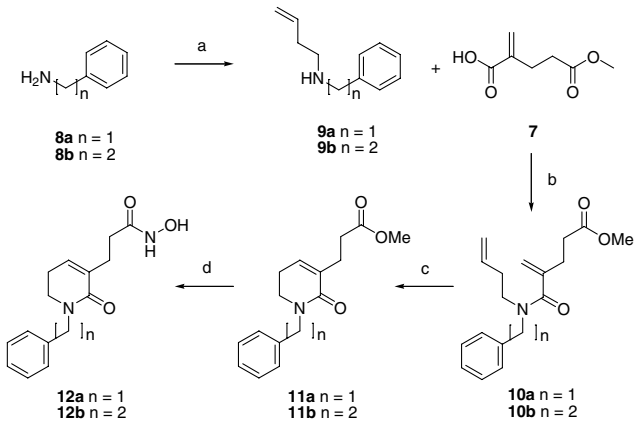
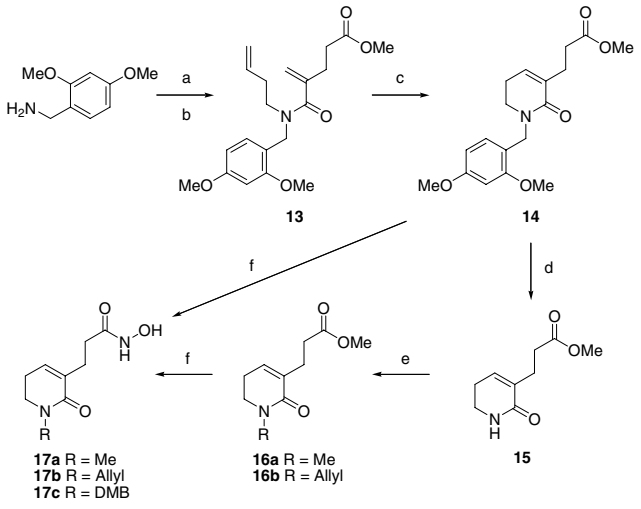


Scheme 1. Reagents: (a) LiOH, THF–H_2O; (b) CSA, MeOH.



Scheme 2. Reagents and condition: (a) **8a** → **9a**: 4-bromo-1-butene, hunig base, reflux; (b) EDC, DMAP; (c) Grubb's catalyst (I); (d) $KONH_2$.



Scheme 3. Reagents and condition: (a) 4-bromo-1-butene, hunig base, reflux; (b) **7**, EDC, DMAP; (c) Grubb's catalyst (I); (d) TFA, reflux; (e) KHMDS, MeI or allyl bromide; (f) $KONH_2$.

dures.[12a,12b] Hydrolysis by treatment of LiOH followed by mono-esterification with camphor sulfonic acid (CSA) gave monoacid **7** (Scheme 1).

Commercial amines **8a** and **8b** were alkylated with 4-bromo-1-butene and the resulted **9a** and **9b** were reacted with monoacid **7** to give amides **10a** and **10b**, respectively (Scheme 2). Upon treatment of catalytic 2–3 mol % of Grubb's catalyst (I),[13] unsaturated δ-lactams **11a** and **11b** were obtained and these methyl esters were converted to hydroxamic acids **12a** and **12b**[14] with $KONH_2$ in MeOH, respectively.

For *N*-alkyl analogues, 2,4-dimethoxybenzylamine was alkylated with 4-bromo-1-butene, followed by coupling with **7** to give amide **13** (Scheme 3). Compound **13** was then converted to δ-lactam **14** in the presence of Grubb's catalyst (I). 2,4-Dimethoxybenzyl (DMB) group was removed in acidic condition and the intermediate **15** was methylated with KHMDS/MeI (**16a**) or allylated with KHMDS/allyl bromide (**16b**). Esters **16a**, **16b**, and **14** were converted to hydroxamates **17a**, **17b**, and **17c**,[14] respectively, in the same conditions as Scheme 2.

We have evaluated the HDAC inhibitory activities of the newly prepared δ-lactam analogues on partially purified HDAC enzyme obtained from HeLa cell lysate and their anti-proliferative effects using PC-3 cells as well.[15]

Among propionic hydroxamates, compounds **12a**, **12b**, **17b**, and **17c** were active in the HDAC enzyme assay. The activity of **12b** with two carbon extended phenyl group was two times more than that of **12a** with one carbon extended phenyl group. Compounds **17a–c** with methyl, allyl, and DMB groups for their surface recognition exhibited a molecular size-dependent activity against HDAC: **17a** with a methyl group was not active and **17b** with an allyl group showed a moderate activity, whereas **17c** with an aromatic substituent DMB group showed a potent inhibitory activity against HDAC. These analogues showed some growth inhibitory activities on the PC-3 cell line (Table 1).

Growth inhibitory activities of all the active HDAC inhibitors (**12a**, **12b**, and **17c**) were evaluated in 6 human tumor cell lines. Growth inhibition (GI_{50}) measured by SRB assay[16] of these 3 HDAC inhibitors and the tumor cell line types are listed in Table 2. With a similar pattern to the enzyme inhibition, **12b** exhibited growth inhibitory activity on five human tumor cell lines, while **12a** was active only on the MDA-MB-231 cell line. **17c** with a DMB group exhibited similar inhibitory activity to that of **12b**. In addition, **12b** and **17c** showed more sensitive inhibitory activity on the MDA-MB-231 breast tumor cell line.

We have efficiently prepared novel δ-lactam-based HDAC inhibitors using a ring closure metathesis and evaluated their HDAC inhibitory activities and

Table 1. HDAC enzyme and growth inhibition by δ-lactam analogues and SAHA (**2**)[a]

Compound	IC_{50} (μM) Enzyme[a]	GI_{50} (μM) PC-3
12a	0.67	NA
12b	0.35	8.58
17a	NA	NT
17b	4.27	NA
17c	0.50	4.55
SAHA (**2**)	0.11	0.89

NA, not active.
NT, not tested.
[a] Values are means of a minimum of three experiments.

Table 2. GI_{50} and tissue type for cells treated with **12a**, **12b**, and **17c**[a]

Cell line	Tissue	Growth inhibition[b] (μM)			
		12a	**12b**	**17c**	**SAHA**
HCT-15	Colon	>10	8.16	7.98	0.82
LOX-IMVI	Melanoma	>10	5.84	6.63	1.39
PC-3	Prostate	>10	8.58	4.55	0.89
ACHN	Kidney	>10	7.76	9.58	0.73
MDA-MB-231	Breast	4.26	1.62	2.67	0.66
NCI-H23	Lung	>10	>10	>10	0.92

[a] Values are means of a minimum of three experiments.
[b] Growth inhibition was measured by SRB (sulforhodamine B) assay.

anti-proliferative effects. The propionic hydroxamic acids (**12b** and **17c**) have good enzyme inhibitory and cell growth inhibitory activities and showed the most potent growth inhibitory activity to MDA-MB-231 among 6 human tumor cell lines. Further structure–activity relationships of zinc binder and chain unit size from hydroxamic acid or hydrophobic aromatic group to the core δ-lactam will be reported in due course.

Acknowledgments

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11. *General procedures:* **7** (1.2 equiv) was dissolved in methylene chloride and then **9a** in methylene chloride was added via cannular at room temperature under Ar atmosphere. EDC (1.3 equiv) and DMAP (0.3 equiv) were added and the mixture was stirred for 8 h at room temperature. After usual work-ups, **10a**, obtained by flash chromatography, was dissolved in methylene chloride (0.01 M) and the mixture was gassed by Ar bubbling for 30 min. Grubb's catalyst (Type I, 0.02 equiv) was added to the reaction mixture and stirred for 12 h in dark. The mixture was concentrated in vacuo and **11a** was obtained by flash chromatography. Compound **11a** was dissolved in methanol (0.5 M solution) and then NH_2OK (1.7 M suspension in methanol, 5.0 equiv) was added at 0°C. The mixture was stirred for 20 h at room temperature. 10% HCl was added to pH 2–3 and the mixture was concentrated in vacuo. White solid formed was removed by filtration, eluting with methanol/chloroform (1:9). Compound **12a** was obtained by flash chromatography. Compounds **12b** and **17c** were obtained following similar methods, respectively.
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14. *N-Hydroxy-3-(2-oxo-1-phenethyl-1,2,5,6-tetrahydro-pyridin-3-yl)- propionamide* (**12b**): ^{1}H NMR ($CDCl_3$) δ 7.29–7.18 (m, 5H), 6.40 (br t, 1H), 3.62 (t, J = 7.2 Hz, 2H), 3.19 (t, J = 7.1 Hz, 2H), 2.85 (t, J = 7.1 Hz, 2H), 2.54–2.44 (m, 2H), 2.18–2.15 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 169.72, 165.29, 138.95, 136.14, 133.66, 128.89, 128.51, 126.45, 49.29, 46.34, 34.17, 23.70; ESI (*m*/*z*) 289.1 (MH^+); HRMS (*m*/*z*) (MH^+) calcd for $C_{16}H_{20}N_2O_3$ 289.1539; found 289.1547. *3-[1-(2,4-Dimethoxy-benzyl)-2-oxo-1,2,5,6-tetrahydro-pyridin-3-yl]-N- hydroxy-propionamide* (**17c**): ^{1}H NMR ($CDCl_3$) δ 7.12 (d, J = 9.0 Hz, 1H), 6.43–6.33 (m, 3H), 4.51 (s, 2H), 3.75 (s, 3H), 3.74 (s, 3H), 3.27 (t, J = 6.9 Hz, 2H), 2.55 (m, 2H), 2.38 (m, 2H), 2.22 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.1, 165.4, 160.2, 158.5, 135.8, 133.5, 130.4, 117.5, 104.2, 98.3, 55.3, 44.9, 44.6, 32.8, 27.1, 23.8; ESI (*m*/*z*) 357.5 (MNa^+); HRMS (*m*/*z*) (MH^+) calcd for $C_{17}H_{22}N_2O_5$ 335.1602; found 335.1609.
15. *HDAC assay*: HDAC fluorescent activity assays using a Fluror de Lys™ Substrate (Biomol, Plymouth Meeting, PA), which contains an acetylated lysine side chain, were performed according to manufacturer's instructions. In brief, HeLa nuclear extracts, which were used as an HDAC enzyme source, were incubated at 25 °C with 250 mM of Fluror de Lys™ Substrate and various concentrations of each sample. Reactions were stopped after 20 min with Fluror de Lys™ Developer and fluorescence was measured using a microplate spectrofluorometer (LS 50B, Perkin-Elmer) with excitation at 360 nm and emission at 460 nm.
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