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Modification of cap group in δ-lactam-based histone deacetylase (HDAC) inhibitors

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Abstract—Novel δ-lactam-based HDAC inhibitors which have various substituted benzyl, bi-aromatic cap groups were prepared using ring closure metathesis reaction, and evaluated their HDAC inhibitory activities and anti-proliferative effects. Among prepared analogues, **11m** and **11o** have very strong HDAC enzymatic inhibition and showed the most potent growth inhibitory activity to five human tumor cell lines including PC-3, ACHN, NUGC-3, HCT-15, and MBA-MB-231 tumor cell lines. Compounds **11m** and **11o** also showed good tumor growth inhibition of MDA-MB-231 cells in in vivo xenograft model. Structure–activity relationship study using docking model explained the significance of hydrophobic aromatic cap groups for their in vitro activities.

Histone deacetylase (HDAC) and histone acetyltransferase (HAT) are involved in chromatin remodeling and epigenetic regulation of genes.[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] and the control of HDAC enzymes has been considered as a very intriguing target for anti-cancer chemotherapy.[3] A number of natural and synthetic HDAC inhibitors have been reported (Fig. 1); trichostatin A (TSA, **1**),[4] apicidin (**4**),[5] trapoxin B (TPX, **5**),[6] and FK-228.[7] And suberoylanilide hydroxamic acid (SAHA, **2**)[8] was approved for the treatment of cutaneous T-cell lymphoma (CTCL).

We reported that design, synthesis, and biological evaluation of novel δ-lactam core HDAC inhibitors (**5**, Fig. 2), which incorporate structural features of δ-lactam ring in a linker domain between surface recognition cap group and zinc binding hydroxamate region.[9] We found that the compounds with two methylene carbon units between zinc binding hydroxamates and δ-lactam core showed potent HDAC inhibitory activity but others showed no activity at all. And the length between δ-lactam core and hydrophobic cap groups is tolerable to the inhibitory activities.

Herein, we report the results of the cap group modification approaches of δ-lactam-based HDAC inhibitors; synthesis, HDAC inhibition, and in vitro and in vivo cancer cell growth inhibition.[10]

Scheme 1 outlines the preparation of δ-lactam analogues.[9] Commercial amines **6** were alkylated with 4-bromo-1-butene in S_N2 manner and resulted in secondary amines **7**. Compound **7** were reacted with monoacid **8** to give amides **9**. Upon treatment of catalytic 2–3 mol % of Grubb's catalyst (I), unsaturated cyclic δ-lactams **10** were obtained. These methyl esters of **10** were converted to hydroxamic acids **11** with $KONH_2$ in MeOH, which have various cap groups on δ-lactam nitrogen.

Keywords: Histone deacetylase; HDAC; Anticancer chemotherapy; Enzyme inhibitor; Growth inhibition; In vivo xenograft model; Docking model.
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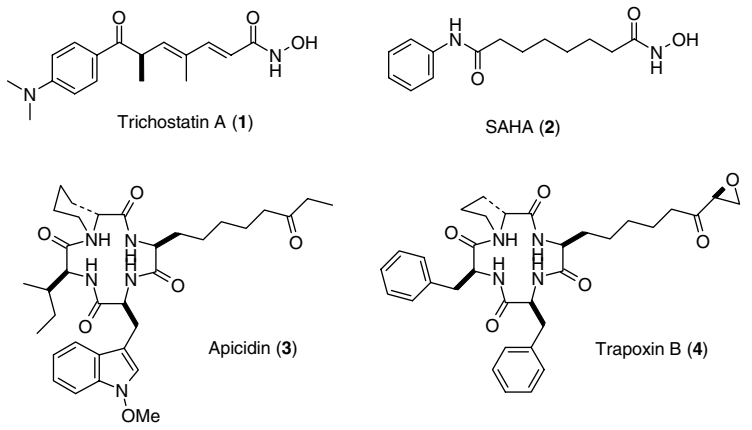


Figure 1. The natural and synthetic HDAC inhibitors.

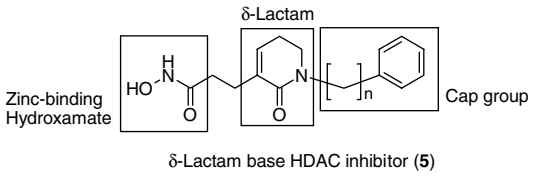
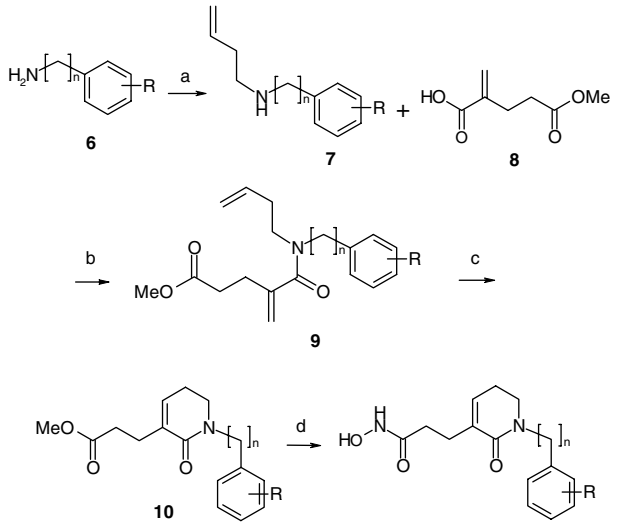


Figure 2. Structural features of δ-lactam-based HDAC inhibitor.

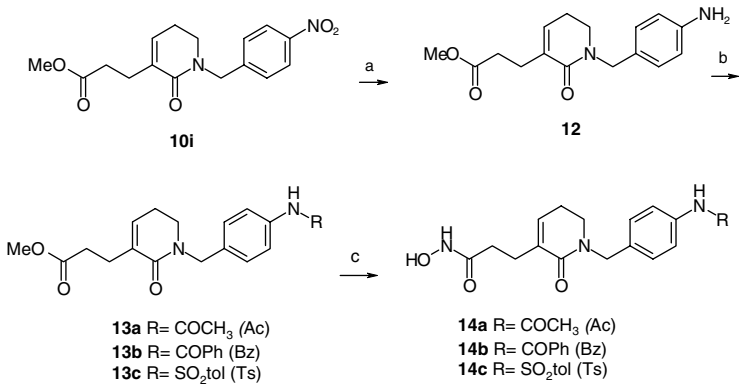


Scheme 1. Reagents: (a) 4-bromo-1-butene, Hunig base, reflux; (b) EDC, DMAP; (c) Grubb's catalyst (I); (d) $KONH_2$.

4-*N*-acyl and sulfonylated aryl analogues were prepared from 4-nitro compound **10i** (Scheme 2). Compound **10i** was reduced to amino compound **12** with zinc and **12** was acylated with acyl chloride or tosyl chloride to give amide **13a** and **13b** or sulfonamide **13c**. The methyl esters of **13** were converted to hydroxamates **14a**, **14b**, and **14c**, respectively, in the same conditions as Scheme 1.

We have evaluated the HDAC inhibitory activities of the newly prepared analogues on partially purified HDAC enzyme obtained from HeLa cell lysate (Table 1).[11] Among prepared compounds, the substituted benzyl analogues **11a–i** ($n = 1$ in Scheme 1), methoxy-substituted analogues (**11d–f**) are more active than methyl analogues (**11a–c**) in the HDAC enzyme assay. For the position of benzyl group, 4-substituted methoxy benzyl analogue (**11f**) showed the best HDAC inhibitory activity at 0.44 μM of IC_{50} value among these analogues. 4-Fluoro analogue (**11g**) and 4-bromo analogue (**11h**) showed the same range of activities to 4-methoxy analogue (**11f**), and 4-nitro analogue (**11i**) is better in inhibitory activities. Surprisingly, 2-naphthyl analogue (**11j**) did not show any activity while its chain elongated analogues ($n = 2$, **11m**; $n = 3$, **11o**)[12] showed very strong inhibitory activity at 37 and 30 nM of IC_{50}, respectively. 1-Naphthyl analogues (**11l**, **11q**) are less active than 2-naphthyl analogues. 4-Biphenyl analogues (**11k**, **11n**, and **11p**) also showed less active inhibitory activities at 0.1–0.2 μM of IC_{50}s. 4-*N*-Acyl and sulfonylated aryl analogues (**14a–c**) showed moderate inhibitory activities ranging from 0.5 to 0.8 μM of IC_{50}. Among these prepared analogues, 2-naphthyl analogues (**11m** and **11o**) are the best compounds in HDAC inhibition assay.

Growth inhibitory activities of all the active HDAC inhibitors (**11a–q** and **14a–c**) were evaluated on five human tumor cell lines using PC-3 (prostate cancer), ACHN (renal cancer), NUGC-3 (gastric cancer), HCT-15 (colon cancer), and MDA-MB-231 (breast cancer) cell lines. Cell growth inhibition of the tested analogues was measured by SRB assay[13] and the obtained GI_{50} values of the types of tumor cell lines compared with SAHA are summarized in Table 1. Overall growth inhibitory activities were related to their HDAC inhibitory activities. The most potent analogues **11m** and **11o** exhibited very strong growth inhibitory activities to five human tumor cell lines at 0.28–1.18 μM. For the analogues with comparable HDAC inhibitory activity, **11i**, **11n**, and **11q** showed very comparable growth inhibitory



Scheme 2. Reagents: (a) Zn, AcOH–MeOH, reflux; (b) RCOCl, TEA, DMAP; (c) $KONH_2$.

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

Compound	IC_{50} (μM)[a] HDAC	GI_{50} (μM)[a]				
		PC-3	MDA-MB-231	NUGC-3	HCT-15	ACHN
11a	2.97	NA	4.92	NT	NA	NA
11b	1.90	NA	8.12	NT	NA	4.38
11c	2.10	8.10	1.43	NT	8.51	5.00
11d	0.86	NA	NA	NT	NA	NA
11e	1.94	NA	3.92	NT	9.03	7.60
11f	0.44	7.08	3.86	NT	6.89	1.67
11g	0.56	NT	NT	NT	9.96	NT
1h	0.45	3.15	1.63	NT	1.72	2.48
11i	0.23	3.01	0.47	1.11	NA	5.21
11j	NA	NT	NT	NT	NT	NT
11k	0.23	0.53	0.28	1.65	1.00	1.18
11l	0.32	2.58	2.53	3.26	5.43	6.62
11m	0.037	0.61	0.28	0.37	1.16	1.18
11n	0.27	3.23	1.04	3.70	3.19	2.49
11o	0.030	0.30	0.56	0.79	0.53	0.46
11p	0.20	2.95	1.28	4.20	4.56	2.67
11q	0.11	1.69	1.47	2.01	1.67	1.82
14a	0.82	NA	NT	NT	NT	NT
14b	0.51	NA	6.03	NA	NA	NA
14c	0.59	1.80	0.56	NT	NA	0.37
2	0.11	2.69	2.00	2.79	2.49	2.22

NA, $GI_{50} > 10$ μM; NT, not tested.
[a] Values are means of a minimum of three independent experiments.

activities to SAHA but **11k** showed very strong anti-proliferative activity comparable to the most potent analogues **11m** and **11o**. The analogues which have moderate HDAC inhibitory activity (over 0.5 μM of IC_{50}) showed poor growth inhibitory activity to five human tumor cell lines, while some compounds were active on the PC-3 and MDA-MB-231 cell lines. Thus, these new analogues showed better sensitivity on the MDA-MB-231 breast tumor cell line and PC-3 prostate tumor cell line.

The most promising inhibitor **11m** was selected to further evaluate in vivo tumor growth inhibitory activity and the results are summarized in Table 2.[14] In this model, compound **11m** and SAHA were administered daily (ip, 30 mg/kg) to nude mice after the sizes of MDA-MB-231 human breast xenografts reached 50–60 mm^3. Compound **11m** and SAHA inhibited 51% and 39% in tumor growth, respectively, compared to the group without treatment. Thus, both compound **11m** and SAHA displayed anti-tumor growth activities and **11m** appeared to be better than SAHA in inhibition of the growth of tumor.

In order to understand the binding mode of these inhibitors, we examined a docking model of human HDAC-1 catalytic core based on the crystal structure of a bacterial HDAC homologue (HDLP, PDB code 1C3R) and docked compound **11m** within the site using the program Discover (Fig. 3).[15] The hydroxamic acid moiety of **11m** chelates to the catalytic Zn^{2+} ion bound in the active site and the δ-lactam ring was bounded in the tubular hydrophobic pocket. Furthermore, longer 2-naphthyl hydrophobic cap group gives the extra stabilization energy by hydrophobic interaction. Although HDLP has a large deletion at entrance to the active site, the docking data suggest that the chain length between hydrophobic cap group and δ-lactam ring and the size

Table 2. Inhibition of the growth of the MDA-MB-231 human breast tumor xenograft by compound **11m** and SAHA (**2**)[a]

Ip[b]	Compound	
	11m	**2**
Dose (mg/kg)	30	30
% Inhibition	51*	39**
% Body weight to vehicle	94	100

[a] Nude mice (six per group) were treated.
[b] **11m** and SAHA (**2**) were administered (ip) after the size of tumors reached to 50–60 mm^3.
* $P < 0.05$.
** $P < 0.01$.

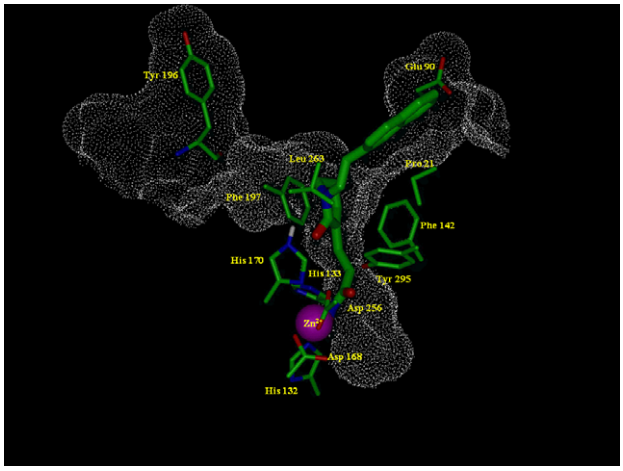


Figure 3. The docked orientations for compounds **11m** bound to the HDAC1 catalytic core of HDLP.

of cap group are quite important to form the flexible conformation in binding pocket.

Collectively, we have prepared novel δ-lactam-based HDAC inhibitors which have various substituted benzyl, bi-aromatic cap groups with 1–4 carbon length between δ-lactam and cap group, and evaluated their HDAC inhibitory activities and anti-proliferative effects. The 2-naphthyl analogues (**11m** and **11o**) have very strong enzymatic and showed the most potent growth inhibitory activity to five human tumor cell lines. Further structure–activity relationship study using docking model explained the significance of hydrophobic aromatic cap groups for their in vitro activities.

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12. *N-hydroxy-3-(1-(2-(naphthalen-2-yl)ethyl)-2-oxo-1,2,5,6-tetrahydropyridin-3-yl)propanamide (**11m**).* ^{1}H NMR ($CDCl_3$) δ 7.72 (br, 3H), 7.59 (s, 1H, 7.26–7.38 (br, 3H), 6.27 (br, 1H), 3.60 (br, 2H), 2.41–3.04 (m, 8H), 1.98 (br, 2H); ^{13}C NMR ($CDCl_3$) δ 170.14, 164.92, 136.35, 135.57, 133.19, 133.13, 131.82, 127.77, 127.28, 127.16, 127.06, 126.84, 125.71, 125.08, 77.42, 77.19, 77.00, 76.57, 49.06, 46.04, 33.95, 32.25, 26.94, 23.30; ESI (*m/z*) 338 (M^+) 361 ($M+Na^+$).
*3-(1-(3-(1-Bromonaphthalen-2-yl)propyl)-2-oxo-1,2,5,6-tetrahydropyridin-3-yl)-N-hydroxypropanamide (**11o**).* ^{1}H NMR ($CDCl_3$)δ 7.73 (br, 3H), 7.57–7.59(d, 1H, $J = 6.0$ Hz), 7.38 (br, 2H), 7.24–7.31 (m, 1H), 6.27 (br, 1H), 3.38–3.43 (br, 2H), 3.20–3.25 (br, 2H), 2.14–2.74 (m, 8H), 1.88 (br, 2H); ^{13}C NMR ($CDCl_3$) δ 170.29, 165.31, 139.034, 135.83, 133.51, 133.43, 131.94, 127.89, 127.51, 127.36, 127.10, 126.27, 125.87 125.12, 46.902, 45.45, 33.28, 32.76, 29.07, 27.23, 23.69; ESI (*m/z*) 353 (M^+) 375 ($M+Na^+$).
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14. *In vivo tumor growth inhibition experiment*. An equal volume of the MDA-MB-231 human breast cancer cells was injected sc in the right flank of each BALB/c nude mouse. When the size of the tumors reached 50–60 mm^3, compounds were intraperitoneally administered daily to the nude mice for 15 days. Tumor length and width were periodically measured until the end of the experiment and tumor volume was calculated using the following formula: Tumor volume = Length $\times$ $(width)^2 \times \pi/6$.
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