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Structure and property based design, synthesis and biological evaluation of γ-lactam based HDAC inhibitors: Part II

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ABSTRACT

Histone deacetylases (HDACs) are involved in post-translational modification and *epi*-genetic expression, and have been the intriguing targets for treatment of cancer. In previous study, we reported synthesis and the biological preliminary results of γ-lactam based HDAC inhibitors. Based on the previous results, smaller γ-lactam core HDAC inhibitors are more active than the corresponding series of larger δ-lactam based analogues and the hydrophobic and bulky cap groups are required for better potency which decreased microsomal stability. Thus, γ-lactam analogues with methoxy, trifluoromethyl groups of *ortho*-, *meta*-, *para*-positions of cap group were prepared and evaluated their biological potency. Among them, trifluoromethyl analogues, which have larger lipophilicity, showed better HDAC inhibitory activity than other analogues. In overall, lipophilicity leads to increase hydrophobic interaction between surface of HDAC active site and HDAC inhibitor, improves HDAC inhibitory activity.

Post-translational modifications are inheritable changes in gene expression without changes in DNA sequences.[1] Chromatin comprises DNA–histone complex and undergo structural changes by post-translational modifications which include methylation, acetylation, phosphorylation and ubiquitination.[2] Among the modification above, histone acetylation has been widely studied and known to have various roles for transcriptional regulation and *epi*-genetic gene expression.[3] Histone deacetylase (HDAC) are catalytic enzymes to remove acetyl moiety from ε-amino group in lysine residues of histone.[4] HDACs regulate gene expression by changing of chromatin structure. Deacetylation of histone leads to increase the electrostatic charge of histone and enhance the interaction between DNA and histone. Therefore, the structure of chromatin becomes compact and blocks the access of transcription factors to DNA template, and finally gene expression is suppressed.[5] In addition, HDACs catalyze the deacetylation of sequence-specific DNA binding transcriptional factors.[6] The abnormal transcriptional regulations by HDACs are related with carcinogenesis.[7] Based on these rationales, HDACs have been considered as promising targets for anti-cancer treatment and many HDAC inhibitors have been reported. Among these inhibitors, SAHA (vorinostat, Zolinza®, **1**)[8] and depsipeptide (Romidepsin, Istodax®, **2**)[9] were approved by US FDA for the treatment of cutaneous T-cell lymphoma (CTCL) in 2006 and 2009, respectively (Fig. 1).

We reported novel series of γ-lactam based HDAC inhibitors (**3**) and their anti-cancer evaluation, which are composed with zinc binder, core group and cap group.[10] Hydroxamic acids were introduced as zinc binder for chelation of zinc ion in active site of HDAC enzymes. γ-Lactam is the core linked to diverse substituent cap group by carbon chain. In our previous publication, a smaller

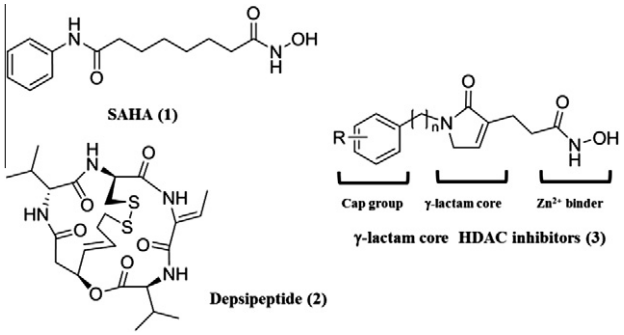

Figure 1. Structure of HDAC inhibitors.

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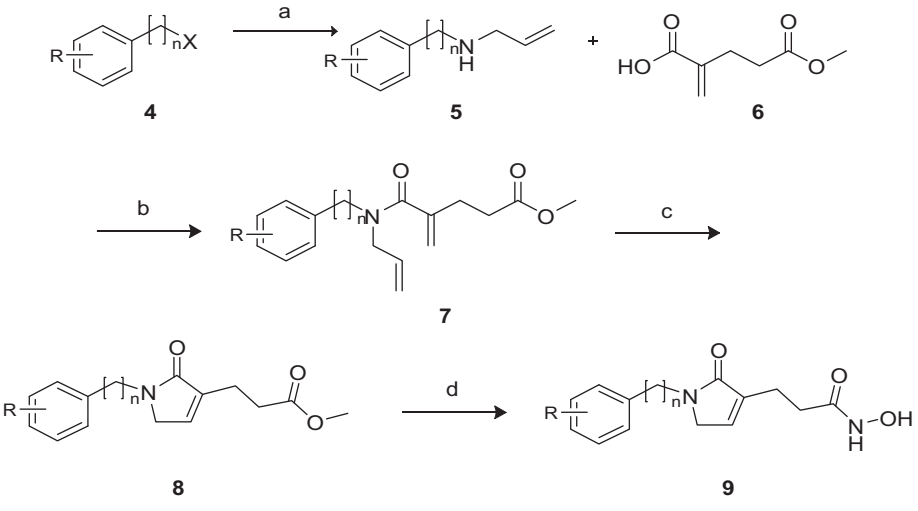
γ-lactam core showed better fit than larger δ-lactam core into narrow hydrophobic pocket of HDAC active site and showed better inhibition profiles of HDACs.[11] In this study, we designed and synthesized γ-lactam HDAC inhibitor analogues with diverse carbon chain linker and various substituents on cap groups to improve HDAC inhibition profiles. We prepared 1–3 carbon chain linker between γ-lactam core and cap group with methoxy, trifluoromethyl substituents of *ortho*-, *meta*-, *para*-positions of cap groups. These modifications of analogues led to better potency, while the metabolic stability slightly decreased.

The Scheme 1 showed preparation of γ-lactam analogues. Commercial benzyl alkyl halide **4** is used as the starting material, secondary amine **5** was synthesized by N-alkylation with allyl amine. Secondary amine **5** was coupled with monoacid **6** by EDC-mediated coupling and to afford amide **7**. γ-Lactam **8** was prepared by ring closing method using 2–3 mol% of Grubb's 2nd catalyst and the methyl ester was converted to hydroxamic acid **9** by $KONH_2$ in MeOH.

We evaluated all prepared γ-lactam analogues on HDAC enzyme inhibitory activity (Table 1). HDAC Flourescent Activity Assay kit was used for evaluation of HDAC inhibitory activity.[12] All of the analogues have 0.07–0.45 μM of IC_{50} values in HDAC inhibitory activity. One exception, **9b**, showed over 10 μM of IC_{50} value and it could be explained through docking study. Among these analogues, the analogues with 2 carbon chain length (**9g**–**l**) showed better HDAC inhibitory activity at 0.07–0.19 μM compare to 1 or 3-carbon analogues. 3-Carbon chain length analogues (**9m**–**o**) showed medium level of HDAC inhibitory activity at 0.13–0.27 μM in the range of IC_{50} whereas 1-carbon chain length ones (**9a**–**f**) showed the poorest HDAC inhibition at 0.12–0.45 μM in the range of IC_{50}. These results can be explained using the Discovery Studio program[13] to perform a docking study with human HDAC-2 (PDB code 3MAX[14]). Figure 2 shows binding modes of the HDAC active site and HDAC inhibitors based on the docking study.[15,16] Compounds **9b** and **9l** have a similar binding mode with SAHA. γ-Lactam HDAC inhibitors bind into the narrow tubular pocket of HDAC active site; hydroxamic acid interacts with Zn^{2+} which is located at the end of the interior active site, and cap groups are located outside of the HDAC active site. Hydroxamic acid of **9l**,[18] the most active compound in these analogues, interacts with His145 and His146 by hydrogen bonding, and γ-lactam core has π–π interaction with two aromatic side chains of Phe155 and Phe210. On the other hand, *ortho*-methoxy benzyl analogue, **9b**, showed the poorest HDAC inhibitory activity, because of short carbon chain linker and o-methoxy substituent in cap group. 1-Carbon chain linker of **9b** is not enough long to bind into narrow tubular pocket of HDAC active site and *ortho*-methoxy substituent hindered to enter deep into HDAC active site. Therefore, **9b** could not enter into HDAC active site enough and it was difficult to chelate with Zn^{2+}. The 2-carbon chain analogues showed proper fitting in active site and they showed better HDAC inhibitory activities than other analogues.

All γ-lactam based HDAC analogues also evaluated their cell growth inhibitory activities using six cancer cell lines; PC-3 (prostate cancer), MDA-MB-231 (breast cancer), ACHN (renal cancer), HCT-15 (colon cancer), NCI-H23 (non-small lung cancer) and NUGC-3 (gastric cancer). In vitro anti-cancer activity showed average 1.9–3.57 μM of GI_{50} value in six cancer cell line and HDAC inhibitory activity and tumor cell growth inhibitory activity are mostly related. Most of these analogues showed good cancer cell growth inhibitory activities. These analogues showed the best activity at 1.9 μM of average GI_{50} in breast cancer cell lines (MDA-MB-231) and at 2.25 μM of average GI_{50} in gastric cell lines (NUGC-3). And they showed poor activity at 3.48 and 3.57 μM of average IC_{50} in prostate cell lines (PC-3) and human colon cancer cell lines (HCT-15), respectively.

There are two different substituents on cap group in this paper. Most of trifluoromethyl analogues showed better HDAC inhibitory activity (0.07–0.26 μM) than methoxy analogues (0.13–0.45 μM) or non-substituted analogues. One of important interactions



Scheme 1. Synthesis of γ-lactam based HDAC inhibitor. Reagents and conditions: (a) allylamine, DIPEA, acetonitrile, 0 °C; (b) EDC, DMAP, methylene chloride; (c) Grubb's 2nd catalyst, methylene chloride; (d) $KONH_2$ (1.7 M in MeOH), MeOH, 0 °C.

Table 1
HDAC enzyme and cancer cell growth Inhibition by γ-lactam analogues

Compound	*n*	R	IC_{50}[a] (μM)	GI_{50}[a] (μM)					
			HDAC	PC-3	MDA-MB-231	ACHN	HCT-15	NCI-H23	NUGC-3
9a	1	H	0.12	4.43	0.99	1.08	5.37	4.29	NT
9b	1	2-Methoxy	>10	3.56	6.03	>10	>10	6.13	>10
9c	1	3-Methoxy	0.38	3.89	1.77	4.43	2.82	1.97	1.75
9d	1	4-Methoxy	0.45	3.12	1.63	2.48	1.72	1.64	1.11
9e	1	3-CF3	0.12	0.88	0.22	0.67	0.54	0.68	0.16
9f	1	4-CF3	0.26	4.36	3.07	4.29	4.54	6.64	3.23
9g	2	H	0.12	4.43	0.99	1.08	5.37	4.29	NT
9h	2	2-Methoxy	0.13	3.51	1.5	2.53	>10	4.49	2.02
9i	2	3-Methoxy	0.16	2.59	1.88	2.48	>10	1.17	3.93
9j	2	4-Methoxy	0.19	3.17	1.08	2.38	>10	4.02	1.42
9k	2	3-CF3	0.12	2.77	2.17	1.17	6.01	1.11	0.92
9l	2	4-CF3	0.07	2.38	1.17	2.48	2.2	1.12	1.08
9m	3	2-Methoxy	0.13	3.51	1.5	2.53	>10	4.49	2.02
9n	3	3-Methoxy	0.16	2.59	1.88	2.48	>10	1.17	3.93
9o	3	4-Methoxy	0.27	7	2.56	4.75	>10	2.51	5.48
Average			0.19	3.48	1.9	2.49	3.57	3.05	2.25

[a] Values are means of a minimum of three independent experiments. NT: not tested.

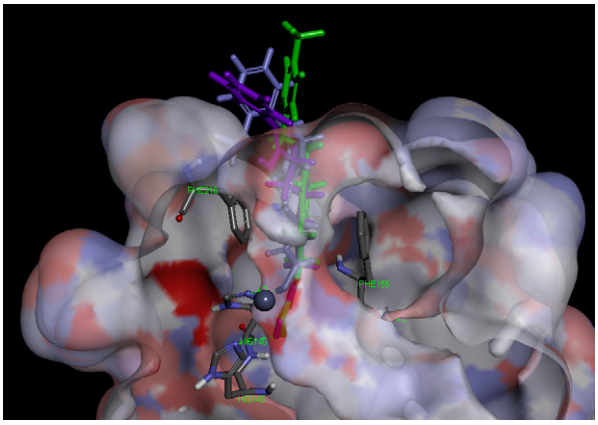


Figure 2. Docking study of γ-lactam core HDAC inhibitors in human HDAC-2 active site: **9b** (blue), **9l** (green) and SAHA (purple).

between HDAC active site and HDAC inhibitor is hydrophobic interaction at the surface of HDAC active site. Hydrophobic interaction could be predicted by physicochemical properties. The number of rotational bond, topological polar surface area (tPSA), lipophilicity at pH 7.4 (log*D*) values are calculated using PreADME program[17] (Table 2). In these results, methoxy analogues showed larger number of rotational bond by one, and larger tPSA value than trifluoromethyl or non-substituent analogues. Trifluoromethyl analogues showed larger log*D* value than methoxy and non-substituent analogues. Methoxy and non-substituent analogues have similar log*D* value. Three analogues of 2-carbon chain linker were compared. (**9g**, **9j**, **9l**) These analogues have 0.12, 0.19 and 0.07 μM of IC_{50} for HDAC inhibition, respectively. Trifluoromethyl analogue (**9l**) showed the best HDAC inhibitory activity (0.07 μM) in three analogues, and methoxy analogue (**9j**) showed slightly lower HDAC inhibitory activity than non-substituent (**9g**) (0.19 vs 0.12, IC_{50}). This difference in HDAC inhibitory activity could be explained with physicochemical properties. Three analogues have similar values of tPSA (78.87 and 69.64) and number of rotational bond (6 and 7). However, **9l**, the best active analogue, has significantly larger value of log*D* than other analogues. Log*D* value of **9l** is 1.50, it is about three times larger than log*D* value of **9g** (0.59) or **9j** (0.54). Therefore, **9l** is the most lipophilic compound and it lead to the highest HDAC inhibitory activity at 0.07 μM of IC_{50}. Increasing values of log*D* with lipophilic cap group induced increase hydrophobic interaction at surface of HDAC enzyme, and increase lipophilicity of γ-lactam based HDAC analogues lead to improve HDAC enzyme inhibitory activity.

Compound **9l**, the most lipophilic compound, showed the most potent HDAC inhibition profiles. Increasing lipophilicity leads to an increase in HDAC inhibition activity while it leads to a decrease in microsomal stability. Three analogues with 2-carbon chain linkers were selected to confirm the metabolic stability of γ-lactam HDAC inhibitors (**9g**, **9j**, **9l**). The most lipophilic compound **9l** showed a slight decrease, but remained moderately stable in the mouse liver microsome (Table 3).

In conclusion, we prepared γ-lactam based HDAC inhibitors which have 1–3 carbon chain linker and methoxy and trifluoromethyl substituent in *ortho*-, *meta*-, *para*-position of cap group.

Table 2
Physicochemical properties of γ-lactam based analogues

Compound	Rotatable bond number	tPSA	log*D* (pH 7.4)
9a	5	69.64	0.43
9b	6	78.87	0.37
9c	6	78.87	0.42
9d	6	78.87	0.39
9e	5	69.64	1.39
9f	5	69.64	1.35
9g	6	69.64	0.59
9h	7	78.87	0.53
9i	7	78.87	0.58
9j	7	78.87	0.54
9k	6	69.64	1.54
9l	6	69.64	1.5
9m	8	78.87	0.98
9n	8	78.87	1.03
9o	8	78.87	0.99

Table 3
The metabolic stability of **9g**, **9j**, **9l**

Compound	% Remaining at 30 min[a]		
	−NADPH	+NADPH	Buffer
9g	82.9	85.5	96.7
9j	98.3	92.0	101.6
9l	75.9	73.9	101.6
Positive control[b]	106.2	0.1	107.7

[a] Test compounds (1 μM) were incubated with 0.5 mg/ml of pooled mouse liver microsomes in the presence or absence of NADPH for 30 min at 37 °C.
[b] Buspirone.

These analogues showed good HDAC inhibitory activity and cancer cell growth inhibitory activities. The analogues with 2-carbon chain linker and trifluoromethyl substituents showed the best HDAC inhibitory activity, because 2-carbon chain linker provides proper fitting into active site of HDAC and trifluoromethyl substituents on cap group increases lipophilicity. Larger lipophilicity of the analogues increases hydrophobic interaction between surface of HDAC active site and HDAC inhibitor and leads better HDAC inhibitory activity.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.045. These data include MOL files and InChiKeys of the most important compounds described in this article.

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18. *N*-Hydroxy-3-{2-oxo-1-[2-(4-trifluoromethyl-phenyl)-ethyl]-2,5-dihydro-1*H*-pyrrol-3-yl}-propionamide (**9l**): ^{1}H NMR (DMSO-d_6) δ 7.55–7.47 (m, 4H), 6.85 (s, 1H), 3.88 (s, 2H), 3.73 (t, 2H, J = 7.3 Hz), 3.00 (t, 2H, J = 7.2 Hz), 2.55 (t, 2H, J = 7.0 Hz), 2.33 (t, 2H, J = 7.5 Hz); ^{13}C NMR (DMSO-d_6) δ 170.96, 168.91, 141.24, 138.07, 136.77, 133.55, 130.03, 125.88, 123.69, 51.23, 43.39, 34.35, 30.79, 22.14; ESI (m/z) 343 (MH^+), 365 (MNa^+); HRMS (FAB$^+$) calcd for $C_{16}H_{20}N_2O_4$ (MH^+) 343.1270, found 343.1268; HPLC (t_R: purity = 9.78 min: 99.69%).