Bioorganic & Medicinal Chemistry Letters 20 (2010) 6808–6811

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Property based optimization of δ-lactam HDAC inhibitors for metabolic stability

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ARTICLE INFO

Article history:
Received 6 June 2010
Revised 3 August 2010
Accepted 24 August 2010
Available online 17 September 2010

Keywords:
Histone deacetylase
Inhibitor
In vitro ADME
Optimization
Lactam

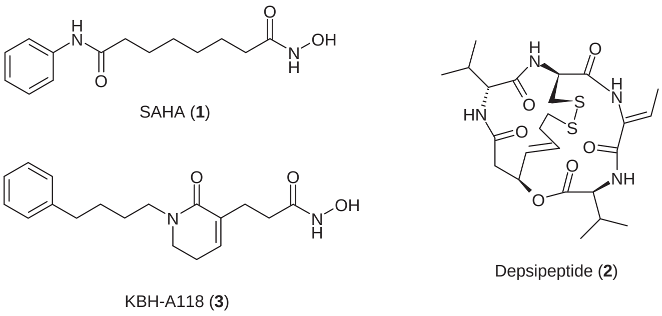
ABSTRACT

The novel δ-lactam based HDAC inhibitor, KBH-A118 (**3**) shows a good HDAC enzyme and cancer cell growth inhibitory activities but has undesirable pharmacokinetics profiles because of instability in mouse liver microsome. To improve metabolic stability, various analogues were prepared with substituents on aromatic ring of cap group and various chain lengths between the cap group and δ-lactam core. The newly prepared analogues showed moderate to potent in vitro activities. Among them six compounds (**8a**, **8e**, **8j**, **8n**, **8t**, and **8v**) were evaluated on mouse liver microsome assay and it turned out that the microsomal stabilities were dependent on lipophilicity and the number of the rotatable bonds. Finally, the animal pharmacokinetic profiles of **8e** displayed improving oral exposure and oral bioavailability.

Histones are the main components of chromatin and play an important role in DNA packing. Tails and globular domain of histones can be occurred post-translational modification, such as acetylation, phosphorylation, methylation, ubiquitination, and sumoylation. These post-translational modification changes DNA–histone interaction and binding-transcription factors.[1] Histone acetylation and deacetylation involved in chromatin remodeling and epigenetic gene expression by histone acetyltransferase (HAT) and histone deacetylase (HDAC). HAT lead to acetylation of histone lysine moieties and loose of DNA–histone interaction, this result recruits additional transcriptional proteins and initiates transcription. The other way, HDAC induces DNA–histone condensation and suppresses gene expression through deacetylation of ε-amino group in lysines residues located the nearby N-terminal of histone proteins. In tumor cells, HDACs cause down-regulation of tumor suppressor genes and they HDAC have been considered as promising targets for anti-cancer chemotherapy.[2–4]

For the structural features, HDAC inhibitors are classified as hydroxamic acid, benzamide, cyclic peptide, and short chain fatty acid.[5] Recently, many HDAC inhibitors are developed clinically for monotherapy or combination with other anti-cancer drugs. Among them, SAHA (Zolinza®, Vorinostat)[6] and depsipeptide (Istodax®, Romidepsin)[7] are approved US FDA for treatment of cutaneous T-cell lymphoma (CTCL) in 2006 and 2009, respectively (Fig. 1).

We have designed and prepared the series of novel δ-lactam based HDAC inhibitors containing zinc binding moiety and cap group. Hydroxamic acid was introduced as a zinc binding group for strong inhibition of HDAC.[8] The cap groups were important for the enzyme inhibition depending on their hydrophobicity. And the carbon chain length connected cap group and δ-lactam core also affect HDAC inhibition while larger the bond length gave higher inhibition of HDAC enzymes. The prepared δ-lactam analogues have evaluated their anti-proliferative activities in vitro and in vivo levels.[9,10] Among these HDAC inhibitors, KBH-A118 (**3**) showed a good HDAC enzyme inhibitory activity and in vitro and in vivo tumor growth inhibitory activities. For the use of oral purpose, however, the pharmacokinetic (PK) profiles of KBH-A118 (**3**) were not desirable. Thus, we here report optimization



SAHA (1)

KBH-A118 (3)

Depsipeptide (2)

Figure 1. Synthetic HDAC inhibitors.

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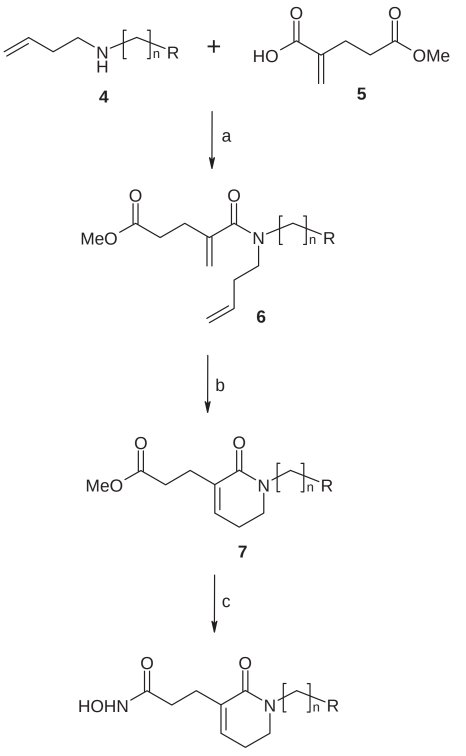
doi:10.1016/j.bmcl.2010.08.117

process to improve the ADME profiles using various in vitro screening tools.

Pharmacokinetic profiles of KBH-A118 (**3**) showed that it has a high systemic clearance (CL = 2.99 l/h/kg), a short half-life ($t_{1/2}$ = 1.29 h) in intravenous administration (Table 4). For the oral administration, it was absorbed rapidly (T_{max} = 15 min) but eliminated very fast ($t_{1/2}$ = 9 min). High oral clearance lowered the oral exposure and resulted in poor oral bioavailability (*F* = 1.0%). The poor oral exposure of KBH-A118 (**3**) may be explained by poor absorption (low C_{max}) or poor metabolic stability (high CL). The ADME profiles of KBH-A118 (**3**) could be examined by caco-2 cell permeability assay and microsomal stability assay (Table 1). KBH-A118 (**3**) showed a good caco-2 cell permeability at 23.9×10^{-6} cm/s, and this result also explained the rapid oral absorption (T_{max} = 15 min). For the liver microsomal stability, KBH-A118 (**3**) was very unstable and remaining only 9% both with and without NADPH in mouse liver microsome assay. These results indicated that KBH-A118 was rapidly degraded in non-oxidative metabolic pathway.[11] Thus, the metabolic instability of KBH-A118 (**3**) accounts for the poor pharmacokinetic profiles and it is the major concern on improvement. Therefore, the new δ-lactam analogues were synthesized and evaluated on their microsomal stability. These analogues contained *meta*- or *para*-substituents on the aromatic cap group for blocking NIH shift, and had various carbon chain numbers between cap group and δ-lactam core for evaluating their lipophilicity.[12]

The Scheme 1 showed preparation of new δ-lactam analogues. Secondary amine **4** was obtained by N-alkylation of synthetic or commercial amines with 4-bromo-1-butene. Amines **4** were coupled with monoacid **5** using EDC and to afford amides **6**. δ-Lactams **7** were prepared by ring closing metastasis using 2–3 mol % of Grubb's catalyst (I) and the methyl ester was altered to hydroxamic acids **8** with $KONH_2$ in MeOH.

First, in vitro activities of the prepared δ-lactam analogues were evaluated on their HDAC and cancer cell growth inhibitory activities using PC-3 (prostate cancer), MDA-MB-231 (breast cancer), ACHN (renal cancer) NUGC-3 (gastric cancer), HCT-15 (colon cancer), NCI-H23 (lung cancer) cell lines. In general, most of compounds showed HDAC enzyme inhibitory activities and cancer cell growth inhibitory activities. For HDAC inhibition, the activity of the analogues ranges from 0.05 to 0.55 μM for IC_{50}. Most of *meta*- or *para*-substituted analogues are active and relatively bulky groups (**8r**) are more active than small substituent (**8h**). The cyano groups (**8b** and **8c**) and nitro (**8q**) on the aromatic cap group demonstrated relatively weak activities. Cancer cell growth inhibition was influenced by HDAC enzyme inhibitory activities; compounds which have showed lower 0.10 μM of IC_{50} show good GI_{50} (0.3–10 μM) and the rest analogues have moderate activity (Table 2). Mechanism of cancer cell growth arrest by HDAC inhibitors is quite complicated but it is known that up-regulating of $p21^{WAF1/CIP1}$ expression is the most common in many HDAC inhibitors.[10] The correlation between HDAC inhibition and cancer cell growth inhibition of these prepared analogues is relatively high for NCI-H23 and is fairly low for MDA-MB-231. It can be explained that HCI-H23[13] showed the more sensitive to up-relegating $p21^{WAF1/CIP1}$ by HDAC inhibitors, compared to MDA-MB-231.[14]



8a n=1 R=3-Cl-Ph
8b n=1 R=3-CN-Ph
8c n=1 R=4-CN-Ph
8d n=1 R=4-*t*-Bu-Ph
8e n=1 R=2-F, 4-Br-Ph
8f n=1 R=6-Bromonaphtyl
8g n=2 R=3-Me-Ph
8h n=2 R=4-Me-Ph
8i n=2 R=3-OMe-Ph
8j n=2 R=2-F-Ph
8k n=2 R=2-Cl-Ph
8l n=2 R=4-Cl-Ph
8m n=2 R=3-Br-Ph
8n n=2 R=4-Br-Ph
8o n=2 R=3-CF_3-Ph
8p n=2 R=4-CF_3-Ph
8q n=2 R=4-NO_2-Ph
8r n=2 R=4-t-Bu-Ph
8s n=2 R=2-Naphtyl
8t n=3 R=4-*t*-Bu-Ph
8u n=3 R=1-Bromonaphtyl
8v n=4 R=4-Me-Ph
8w n=4 R=4-F-Ph
8x n=4 R=4-Cl-Ph
8y n=4 R=4-Br-Ph

Scheme 1. Reagents and conditions: (a) EDC, DMAP, CH2Cl_2; (b) Grubb's catalyst (I), CH_2Cl_2; (c) $KONH_2$ (1.7 M in MeOH), MeOH, 0 °C.

Table 1
In vitro ADME profiles of KBH-A118 and other reference compounds

Compound	Caco-2 cell	Remaining at 60 min[a] (%)		
	P_{app} × 106 (cm/s)	−NADPH	+NADPH	Buffer
KBH-A118	23.9	8.5	8.7	97.3
Negative ctrl	1.5 ± 0.1[b]	96.2[d]	100.8[d]	
Positive ctrl	29.6 ± 0.8[c]	86.8[e]	1.2[e]	

[a] Microsomal stability was determined by incubating 1 μM of test compounds with female ICR mouse liver microsomes (0.5 mg protein/mL) for 60 min at 37 °C.
[b] Ranitidine.
[c] Metoprolol.
[d] Atenolol.
[e] Chlorpromazine.

Next, we performed microsomal stability assay for evaluating the substituent effect of cap group. The highly lipophilic compounds showed high metabolic clearance. Through in silico profiles using PreADME program,[15] we selected **8a**, **8e**, **8j**, **8n**, **8t**, and **8v**, which have similar *c* log $P^{16,17}$ to KBH-A118 (2.18 ± 0.5) and a diverse substituent and various number of rotatable bond[18] for metabolic stability evaluation. This assay determined by incubation 1 μM of analogues with mouse liver microsome (1 mg protein/ml) for 30 min at 37 °C. The metabolic stabilities of **8a**, **8e**, **8j**, **8n**, **8t**, and **8v** using mouse liver microsome were displayed in Table 3. *c* Log *P* values of the compounds correlated well with their microsomal stabilities. Higher *c* log *P* than 2.67 resulted in poor metabolic stability (**8t** and **8v**). And the higher number of rotatable bond may also result in the negative impact on microsomal stability by increasing lipophilicity. Among the six analogues, **8e** and **8n** are equally stable in microsome and showed a good in vitro activity, but **8e** displayed better in silico parameters, such as the number of rotatable bonds and *c* log P^{12}, and these can be more useful for future design for the further PK optimization.

Finally, **8e**[19] was evaluated in mouse pharmacokinetic model and it showed an improved PK profiling compared to KBH-A118. Half-life had a fivefold increased at 6.2 h compared to 1.29 h ($t_{1/2}$,

Table 2
HDAC enzyme and growth inhibition by δ-lactam analogues, KBH-A118 (3) and SAHA (**1**)

Compound	IC_{50} (μM)[a]			GI_{50} (μM)[a]			
	HDAC	PC-3	MDA-MB-231	ACHN	NUGC-3	HCT-1	NCI-H23
1	0.11	2.69	2.00	4.22	2.94	2.49	2.34
3	0.21	3.25	2.03	1.89	3.19	5.40	5.99
8a	0.36	4.65	3.58	4.48	3.92	6.22	2.96
8b	0.31	8.48	4.18	NT	NT	>10	NT
8c	0.23	>10	8.17	NT	NT	>10	NT
8d	0.05	5.19	2.84	3.00	NT	2.94	NT
8e	0.31	5.81	3.12	NT	NT	5.37	NT
8f	0.13	2.61	2.31	NT	NT	1.10	NT
8g	0.23	>10	4.04	NT	NT	4.41	NT
8h	0.55	6.34	2.84	4.55	3.57	4.00	5.56
8i	0.46	4.84	3.03	7.60	4.26	3.96	7.06
8j	0.52	7.07	3.02	4.47	3.78	3.60	6.09
8k	0.61	7.25	3.91	8.71	5.93	4.21	6.50
8l	0.31	1.51	0.92	1.28	1.06	1.66	2.12
8m	0.30	0.81	0.87	0.30	0.82	1.25	1.83
8n	0.13	3.41	1.87	NT	NT	0.87	NT
8o	0.03	2.47	1.90	2.64	1.70	2.35	3.97
8p	0.12	4.82	1.81	NT	NT	1.70	NT
8q	0.67	>10	>10	>10	>10	>10	>10
8r	0.05	0.50	1.13	2.27	1.10	0.52	0.99
8s	0.10	0.39	0.27	0.38	0.36	0.31	0.43
8t	0.12	1.13	>10	1.72	2.81	1.66	2.00
8u	0.03	0.30	0.56	0.46	0.79	0.53	0.49
8v	0.24	2.53	3.05	2.49	2.13	2.94	2.79
8w	0.50	>10	>10	9.53	9.02	>10	>10
8x	0.38	4.15	2.56	2.46	1.79	3.30	2.88
8y	0.26	4.81	3.54	2.39	2.01	3.19	2.73

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

Table 3
The microsomal stability of δ-lactam analogues

Compound	Remaining at 30 min[a] (%)			In silico parameters[b]	
	−NADPH	+NADPH	Buffer	Rotatable bond #	*c* log *P*
8a	40.3	31.3	102.7	5	1.61
8e	55.0	31.7	102.3	5	1.90
8j	52.7	45.3	103.8	6	1.48
8n	56.6	29.6	103.9	6	2.02
8t	8.0	3.3	103.2	7	3.13
8v	5.7	4.0	99.3	8	2.67
Buspirone[c]	96.6	0.3	105.8		

[a] 1 μM test compounds with female ICR mouse liver microsomes (1 mg protein/ml) for 30 min at 37 °C.
[b] PreADME program, BMDRC.
[c] Positive control.

Table 4
Pharmacokinetics profiles of **KBH-A118** and **8e** in Balb/c mouse

Parameter[*]	Unit	KBH-A118		**8e**	
		IV	PO	IV	PO
Dose	(mg/kg)	5	20	10	20
T_{max}	(h)	–	0.25	–	0.25
C_{max}	(ng/ml)	–	196.8	–	1387.3
$AUC_{0\text{-inf}}$[**]	(h ng/ml)	1671.7	64.4	2587.7	732.3
CL	(l/h/kg)	2.99	–	3.9	–
V_{ss}	(l/kg)	0.27	–	0.8	–
$t_{1/2}$	(h)	1.29	0.15	6.2	–
F	(%)		1	–	14.1

[*] PK parameters were based on mean plasma concentration-time profiles of three animals per time points. PK parameters were calculated by noncompartmental analysis using PK Solutions 2.0 (Summit Research Services, Montrose, CO, USA).
[**] AUC after iv administration was calculated from 0 to infinity, whereas the oral AUC was calculated from 0 to 5 h.

KBH-A118) and plasma exposure rise two times at intravenous (2587.7 h ng/ml vs 1671.7 h ng/ml) and ten times at oral administration (732.3 h ng/ml vs 64.4 h ng/ml). The oral bioavailability of **8e** had been remarkably increased to 14.1% compared to 1% of KBH-A118 (Table 4). The systemic clearance of **8e**, however, was slightly increased to 3.9 l/h/kg compared to 2.99 of KBH-A118. This result indicated that non-oxidative phase I mechanism such as phase II conjugation may be involved in metabolic degradation of these compounds.[10] It turns out that improving microsomal stability is an appropriate to improve in vivo pharmacokinetic profiles in δ-lactam based HDAC inhibitors. The metabolic stability of the compounds depends on their physical chemical properties such as, *c* log *P* and the number of rotatable bonds.

In conclusion, KBH-A118 showed a good HDAC enzyme and cancer cell growth inhibitory activities, but its pharmacokinetics profiles was very poor because of instability in mouse liver microsome. The microsomal instability caused a low oral exposure, high clearance and resulted in a poor oral bioavailability. For improving metabolic stability, *meta*- or *para*-substituents were introduced on aromatic ring of cap group, for blocking NIH shift and reduced carbon chain length, to decrease lipophilicity of the compounds. The newly prepared analogues also showed a good HDAC enzyme inhibitory activities and cancer cell growth inhibitory activities. Based on the microsomal stability screening, **8e** was selected for animal pharmacokinetic experiment, which showed the similar in vitro activity profiles to KBH-A118. As a result, the pharmacokinetic profiles of **8e** displayed a long half-life (6.2 h), a good oral exposure and a moderate oral bioavailability (*F* = 14.1%).

Acknowledgments

This research was supported by National Research Foundation (2009-0092966), Korea Research WCU grant (R31-2008-000-10086-0) and Brain Korea 21 project, Republic of Korea.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.117.

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19. *3-(1-(4-Bromo-2-fluorobenzyl)-2-oxo-1,2,5,6-tetrahydropyridin-3-yl)-N-hydroxypropanamide* (**8e**): ^{1}H NMR (500 MHz, DMSO-*d*6) δ 10.36 (s, 1H), 8.69 (s, 1H), 7.54 (dd, 1H, JA = 9.5 Hz, JB = 2.0 Hz), 7.40 (dd, 1H, JA = 8.0 Hz, JB = 2.0 Hz), 7.24 (t, 1H, *J* = 8.0 Hz), 6.38 (br t, 1H, *J* = 4.0 Hz), 4.53 (s, 2H), 3.31 (d, 2H, *J* = 7.0 Hz), 2.39 (t, 2H, *J* = 7.0 Hz), 2.08 (br q, 2H, JA = 7.5 Hz, JB = 6.5 Hz), 2.08 (t, 2H, *J* = 8.0 Hz).