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Bicyclic peptides as potent inhibitors of histone deacetylases: Optimization of alkyl loop length

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ABSTRACT

Bicyclic tetrapeptide hydroxamic acids were prepared as histone deacetylase (HDAC) inhibitors, and the evaluated inhibitory activity shows that they are potent against HDAC1 and HDAC4. The in vivo activity depends on alkyl loop length.

Dynamic acetylation and deacetylation of the ε-amino groups of lysines at *N*-terminal tails of core histones are balanced by histone acetyl transferase (HAT) and histone deacetylase (HDAC) enzymes.[1] Imbalance in histone acetylation and deacetylation can lead to transcriptional deregulation of genes that are involved in the control of cell cycle progression, differentiation, and/or apoptosis. Aberrant histone deacetylation caused by the disrupted HAT activity or abnormal recruitment of HDACs has been related to carcenogenesis.[2] Inhibition of HDAC enzymatic activity is expected to induce re-expression of differentiation-inducing genes.

Several natural and synthetic compounds have been reported so far as HDAC inhibitors. Among them, trichostatin A (TSA),[3] depsipeptide FK228[4] and the cyclic tetrapeptide family including trapoxin (TPX),[5] chlamydocin,[6] HC-toxins[7] and apicidin[8] are naturally occurring HDAC inhibitors. As synthetic inhibitors, suberoylanilide hydroxamic acid (SAHA)[9] and the benzamide MS-275[10] have been designed. Recently cyclic tetrapeptide-based HDAC inhibitors, CHAPs,[11] SCOPs[12] and ketone-based chlamydocin analogues[13] have also been reported. However, current HDAC inhibitors in clinical trials are regarded as broad spectrum HDAC inhibitors with moderate anticancer effect. Therefore, it is desirable clinically to develop specific anticancer drugs that are effective for a particular HDAC that is over expressed in cancer.[14] Of various approaches to achieve this, one is to modify the cap group of the HDAC inhibitors. As the area surrounding the opening to the binding pocket has less homology between HDAC isoforms compared to the active site, the modification of the cap group allows to have a significant impact upon isoform selectivity.[15] So that, for cap group modification several cyclic tetrapeptide HDAC inhibitors have been designed and synthesized.[16] On the other hand, synthesis of constrained peptide by ring closing metathesis (RCM) using ruthenium complexes has been reported.[17] On the basis of these reports, we have previously designed and synthesized a fused bicyclic peptide (Fig. 1, compound **1**) to increase the size and the constraint of ali-

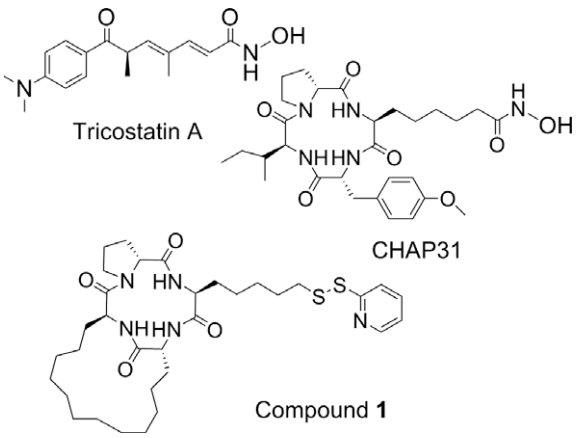

Figure 1. Some selected HDAC inhibitors.

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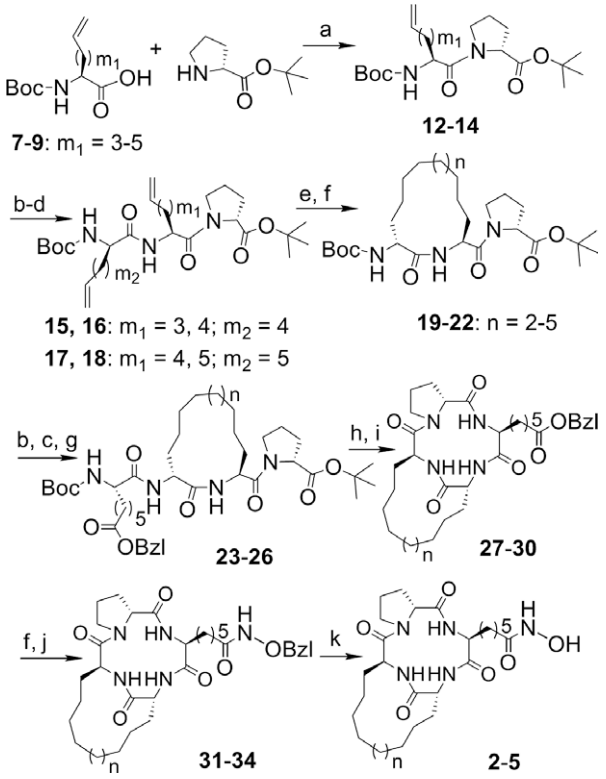
phatic cap group.[18] The bicyclic peptide was found to be active in both cell free and cell based conditions. Moreover, it showed some selectivity among the HDAC isoforms. These results prompted us to make further investigation on bicyclic peptide HDAC inhibitors. In the present study, we design and synthesize a series of bicyclic tetrapeptides by changing the length of the aliphatic loop to explore the effect of the loop length on the activity of the inhibitors. The sequence and configuration of amino acids in CHAP31 are considered as the basis for designing the inhibitors. We herein describe the synthesis of bicyclic tetrapeptides using RCM, and a brief description of the interesting biological results.

Our synthesis was started from the preparation of the building block amino acids. Boc-L-2-amino-6-heptenoic acid (Boc-L-Ae7-OH) (**7**), Boc-L-2-amino-7-octenoic acid (Boc-L-Ae8-OH) (**8**), Boc-L-2-amino-8-nonenoic acid (Boc-L-Ae9-OH) (**9**), Boc-D-2-amino-7-octenoic acid (Boc-D-Ae8-OH) (**10**) and Boc-D-2-amino-8-nonenoic acid (Boc-D-Ae9-OH) (**11**) were synthesized by the reported procedure.[18] Bicyclic tetrapeptides were synthesized according to Scheme 1 by the conventional solution phase method. H-D-Pro-O^{t}Bu was condensed with **7**–**9** using DCC/HOBt, respectively. Boc protection was selectively removed by the reported procedure[19] using 4 M HCl/dioxane, and each amine component was condensed with **10** or **11** by the same DCC/HOBt method to obtain linear tripeptides **15**–**18**. The linear tripeptides **19**–**22** with fused side ring were synthesized by ring closing metathesis between D-Ae8/9 and L-Ae7/8/9 using Grubb's first generation Ru catalyst, in dichloromethane (DCM), followed by catalytic hydrogenation. After selective deprotection of **19**–**22**, Boc protected amino suberic acid benzyl ester [Boc-L-Asu(OBzl)] was incorporated in them to prepare the linear tetrapeptides **23**–**26**. After removal of both side protections by treating with trifluoroacetic acid (TFA), cyclization reaction was carried out by the aid of *N*-[(dimetylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) in dimethylformamide (DMF) under high dilution conditions with minimum amount of diisopropylethyl amine (DIEA) (2.5 equiv) to obtain bicyclic tetrapeptides **27**–**30**. After Bzl deprotection, by catalytic hydrogenation, the carboxyl group was condensed with hydroxylamine benzyl ester, and finally Bzl protection was removed by catalytic hydrogenation to obtain bicyclic tetrapeptide hydroxamic acids **2**–**5**. Reference cyclic tetrapeptide hydroxamic acid (**6**) was also synthesized in a similar manner excluding RCM (Fig. 2).



Scheme 1. Synthesis of bicyclic tetrapeptide hydroxamic acids. Reagents and conditions: (a) DCC, HOBt, DMF, 12 h, 75–80%; (b) 4 M HCl/dioxane, 30 min; (c) Saturated Na_2CO_3, 70–80%; (d) Boc-D-Ae8-OH (**10**) or Boc-D-Ae9-OH (**11**), DCC, HOBt, DMF, 12 h, 75–80%; (e) Grubbs' first generation catalyst, DCM, 48 h, 50–87%; (f) AcOH, Pd–C, H_2, 12 h, 97–100%; (g) Boc-L-Asu(OBzl)-OH, DCC, HOBt, DMF, 12 h, 75–80%; (h) TFA, 3 h, 98–100%; (i) HATU, DIEA, DMF, 4 h, 15–65%; (j) HCl·H_2NOBzl, DCC, HOBt, TEA, DMF, 60–80%; (k) Pd–$BaSO_4$, AcOH, H_2, 70–80%.

The synthesized compounds (**2**–**6**) were assayed for HDAC inhibitory activity using HDAC1, HDAC4 and HDAC6 prepared from 293T cells. Preparation and assay of HDACs, and p21 promoter assay were performed according to the reported methods.[13] The results are summarized in Table 1.

All the compounds **2**–**6** are active in nanomolar range. For comparison, the inhibitory activity of tricostatin A is also shown. The activity toward HDAC1 slightly changes with the difference of the size of the aliphatic loop. However, the changes in activity toward HDAC4 and HDAC6 are not so remarkable. All the compounds are specific toward HDAC4 compared with HDAC1 and HDAC6. They are about two times more active toward HDAC4 than HDAC1, and about 50 times more active than HDAC6. Most of the currently available HDAC inhibitors have no or very little specificity toward HDAC isoforms. Bicyclic tetrapeptides seem to be promising target for the development of isoform selective inhibitors. Compound **3** showed better selectivity (the ratio of IC_{50} values: HDAC6/HDAC4 = 75).

All of the bicyclic tetrapeptides except for **2** are excellent in p21 promoter-inducing activity. The variation of HPLC retention time, which can be used as a parameter for hydrophobicity, could be correlated with p21 promoter-inducing activity. A linear increase in hydrophobicity was observed when loop length was increased from nine to eleven CH_2 groups. However, increase in hydrophobicity was not remarkable upon further increase in loop length (Fig. 3). Similar increasing trend in p21 promoter-inducing activity was observed (Fig. 3). It seems that the aliphatic loop helped in penetration through the cell membrane, and resulted in increased activity up to certain loop length. In our finding, eleven CH_2 loop length is the optimum for p21 promoter-inducing activity, as no remarkable increase in activity has been observed for further elongation in the loop. Compound **6** was synthesized as a reference compound to compare the activity between cyclic tetrapeptide

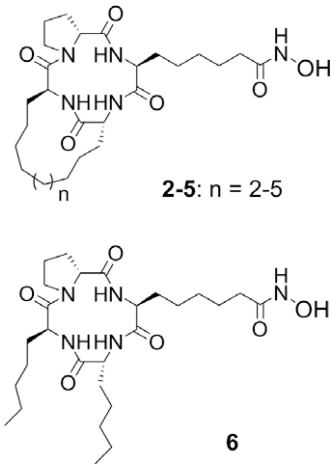


Figure 2. Bicyclic tetrapeptides and a reference compound synthesized as HDAC inhibitors.

Table 1
HDAC inhibitory activity and p21 promoter activity data for bicyclic tetrapeptide hydroxamic acids and reference compounds

Compounds	Loop size	HPLC[20] t_R (min)	IC_{50} (nM)			p21 promoter assay, EC_{1000} (nM)
			HDAC1	HDAC4	HDAC6	
Tricostatin A	—	—	23	44	65	20
2	$-(CH_2)_9-$	4.74	9.1	5.4	330	92
3	$-(CH_2)_{10}-$	6.03	9.1	5.5	410	7.2
4	$-(CH_2)_{11}-$	7.39	11	4.5	280	2.6
5	$-(CH_2)_{12}-$	7.52	13	5.0	240	2.0
6	—	7.51	25	12	340	13

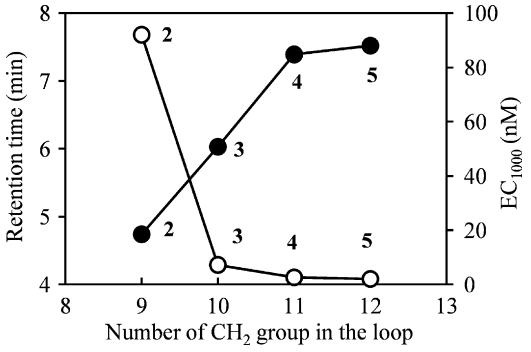


Figure 3. Change in (●) hydrophobicity, and (○) activity in cell based test with loop length of bicyclic tetrapeptides (**2**–**5**).

and bicyclic tetrapeptide. Compound **3** corresponds to the fused ring one of compound **6**. The improvement in activity and selectivity in both cell free and cell based conditions of compound **3** over compound **6** reflects the importance of closed ring.

In summary, in order to find novel and potent non-aromatic HDAC inhibitors, we designed and synthesized CHAP31-based bicyclic tetrapeptide hydroxamic acids by changing the aliphatic loop length. These inhibitors show potent HDAC inhibitory activity in vivo and in vitro. They also show some selectivity among the HDAC isoforms. The aliphatic loop length is important, and eleven CH_2 loop is the optimum for in vivo activity. These results further confirm the hypothesis that modification of the cap group of HDAC inhibitors can lead to potent HDAC inhibitors, which may have potential as anticancer agents. We are also carrying out conformational analysis of these inhibitors by NMR calculation methods. These results will be published elsewhere.

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