

Design of Histone Deacetylase Inhibitors with Chlamydocin Framework containing Various Imino Acids instead of Proline Residue

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Introduction

The cyclic tetrapeptide chlamydocin, originally isolated from fungus *Diheterospora chlamydosporia*, is one of the naturally-occurring inhibitors of histone deacetylases [1]. Structure of chlamydocin is *cyclo*(-L-Aoe-Aib-L-Phe-D-Pro-), where Aoe is L-amino-8-oxo-9,10-epoxydecanoic acid. Recently we have synthesized and tested histone deacetylase inhibitory activities of chlamydocin hydroxamic acid analogs [2,3].

In the present study, we designed seven acyclic-imino-acid-containing cyclic tetrapeptide hydroxamic acids such as *cyclo*(-L-Asu(NHOH)-Aib-L-Phe-D-MeAla-), where Asu is aminosuberic acid. The introduction of acyclic imino acids was expected to loosen the chlamydocin framework to afford flexibility in binding to the enzyme. The imino acids, D-MeAla, BzIGly, D-MePhe, and D-MeLeu were employed (Fig. 1).

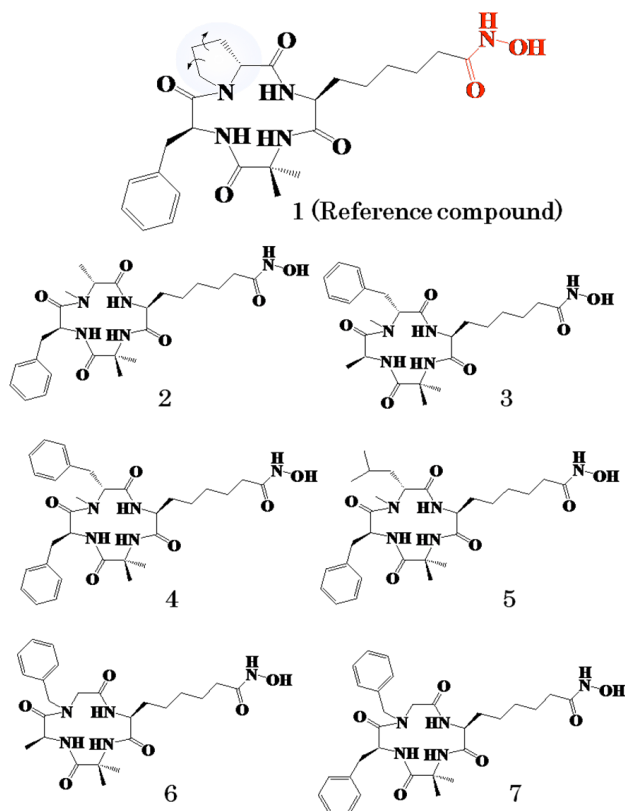


Fig. 1. Structures of acyclic-imino-acid-containing cyclic tetrapeptide hydroxamic acids, *cyclo*(-L-Asu(NHOH)-Aib-L-Phe-D-Pro-) (1), *cyclo*(-L-Asu(NHOH)-Aib-L-Phe-D-MeAla-) (2), *cyclo*(-L-Asu(NHOH)-Aib-L-Ala-D-MePhe-) (3), *cyclo*(-L-Asu(NHOH)-Aib-L-Phe-D-MePhe-) (4), *cyclo*(-L-Asu(NHOH)-Aib-L-Phe-D-MeLeu-) (5), *cyclo*(-L-Asu(NHOH)-Aib-L-Ala-BzIGly-) (6), and *cyclo*(-L-Asu(NHOH)-Aib-L-Phe-BzIGly-) (7).

Results and Discussion

Our aim was to synthesize potent inhibitors of HDACs with the chlamydocin-cyclic tetrapeptide scaffold. Therefore, we intended to synthesize chlamydocin analog containing various acyclic imino acids using solution-phase peptide synthesis strategy as shown in Scheme 1.

	L-Asu	Aib	Xaa	Yaa
Boc	OBzl		OTMSE	
Boc	OBzl	TBAF	OH	H O ^t Bu
Boc	OBzl	DCC, HOBT		O ^t Bu
TFA·H	OBzl	TFA		OH
<i>cyclo</i> (OBzl	HATU, DIEA)

Scheme 1. Synthesis of cyclic tetrapeptides 2 - 7.

After the synthesis of cyclic peptides *cyclo*(-L-Asu(OBzl)-Aib-Xxx-Yyy-), the side chain benzyl ester was converted to the hydroxamic acid. All of the analogs 2 - 7 were characterized by ¹H NMR and high resolution FAB-MS.

Table 1. Characterization of cyclic tetrapeptides 2 - 7.

No.	Composition	HR FAB-MS [M+H] ⁺		HPLC ¹ (min.)
		Observed	Calcd.	
2	C ₂₅ H ₃₇ N ₅ O ₆	504.2809	504.2824	7.50
3	C ₂₅ H ₃₇ N ₅ O ₆	504.2815	504.2824	7.07
4	C ₃₁ H ₄₁ N ₅ O ₆	580.3163	580.3137	11.58
5	C ₂₈ H ₄₃ N ₅ O ₆	546.3322	546.3293	11.34
6	C ₂₄ H ₃₅ N ₅ O ₆	490.2664	490.2667	7.28
7	C ₃₀ H ₃₉ N ₅ O ₆	566.2972	566.2980	10.81

¹HPLC conditions: Column: Chromolith performance RP-18e (100 × 4.6 mm)
Eluent: 0-50% CH₃CN / 0.1% TFA (Linear gradient over 15 min.)
Detector: 220 nm. Flowrate: 2 mL/min.

We carried out circular dichroism (CD) experiment in two sets in methanol as solvent. In the first set, we compared compounds 2 - 5 containing N-Me amino acids. Here we found that all the compounds showed similar conformation at 250 nm with positive ellipticity. This observation was opposite to compound 1. From 240 nm region all the compounds showed negative ellipticity with similar conformation. In set 2, we compared the conformation of compounds 6 and 7 with N-BzlGly. We found that, from 250 nm they showed similar conformation as compound 1 with negative ellipticity.

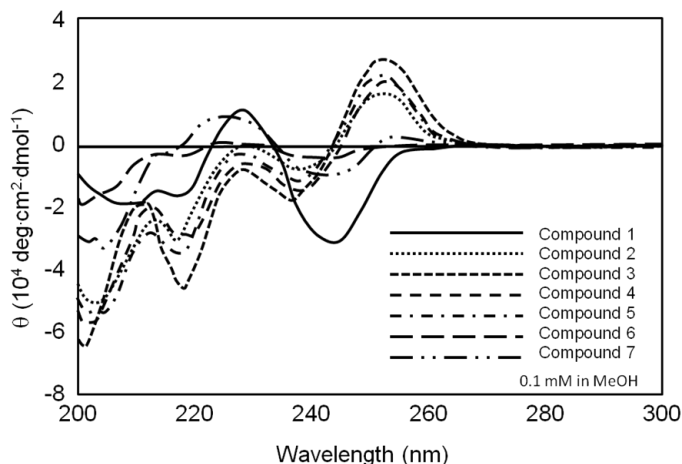


Fig. 2. CD spectra of cyclic tetrapeptides 2 - 7.

Cyclic tetrapeptides 4 and 7 were characterized by ¹H-NMR (1D, 2D-COSY, 2D-HOHAHA, and 2D-NOESY) spectra in CDCl₃. Three dimensional conformations of these compounds were calculated by MOE system using NMR data. Resulted backbone conformations of these two cyclic tetrapeptides resembled compound 1.

Table 1. HDAC Inhibitory activities of cyclic tetrapeptides 2 - 7.

No.	compound	IC ₅₀ (μM)		
		HDAC1	HDAC4	HDAC6
-	Trichostatin A	0.019	0.020	0.028
1	cyclo(-L-Asu(NHOH)-Aib-L-Phe-D-Pro-)	0.0087	0.020	0.16
2	cyclo(-L-Asu(NHOH)-Aib-L-Phe-D-MeAla-)	0.038	0.049	0.96
3	cyclo(-L-Asu(NHOH)-Aib-L-Ala-D-MePhe-)	0.051	0.057	0.57
4	cyclo(-L-Asu(NHOH)-Aib-L-Phe-D-MePhe-)	0.027	0.024	0.49
5	cyclo(-L-Asu(NHOH)-Aib-L-Phe-D-MeLeu-)	NT	NT	NT
6	cyclo(-L-Asu(NHOH)-Aib-L-Ala-BzlGly-)	NT	NT	NT
7	cyclo(-L-Asu(NHOH)-Aib-L-Phe-BzlGly-)	NT	NT	NT

NT = Not tested

The compounds synthesized were subjected to HDAC inhibitory activity using HDAC1, HDAC4 and HDAC6. All of the newly synthesized acyclic-imino- acid-containing cyclic tetrapeptide hydroxamic acids 2 - 4 showed less potent HDAC inhibitory activity than Pro-

containing cyclic tetrapeptide hydroxamic acid 1. The similarity of backbone conformations of compounds 1 and 4 suggested that appropriate backbone conformation is not enough to show potent HDAC inhibitory activity. Although backbone conformations of compounds 1 and 4 are similar, CD spectrum of compound 1 is different from CD spectra of compounds 2 - 4. The introduction of acyclic imino acid affects the aromatic ring orientation arrangements and it may affect CD spectra and HDAC inhibitory activities.

In conclusion, six acyclic-imino-acid-containing cyclic tetrapeptide hydroxamic acids were designed and synthesized. CD spectra of acyclic-imino- acid-containing cyclic tetrapeptide hydroxamic acids 2 - 5 were different from the spectrum of Pro-containing cyclic tetrapeptide hydroxamic acid 1. Backbone conformations of compounds 4 and 7 resemble compound 1. Acyclic-imino-acid-containing cyclic tetrapeptide hydroxamic acids 2 - 4 showed less potent HDAC inhibitory activity than Pro-containing cyclic tetrapeptide hydroxamic acid 1.

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