

Synthesis of HC-toxin Hydroxamic Acid Analogs and Their Inhibitory Activity toward Histone Deacetylases

Yasuhiko Ohtsuka¹, Izumi Shiraishi¹, Shokichi Ohuchi², Tamaki Kato¹, and Norikazu Nishino^{1*}

¹Graduate School of Life Sciences and Systems Engineering, Kyushu Institute of Technology, Kitakyushu, 808-0196, Japan; ²Faculty of Information Science and Engineering, Kyushu Institute of Technology, Iizuka, 820-8502, Japan

E-mail: nishino@life.kyutech.ac.jp

Introduction

A number of cyclic tetrapeptides have been discovered as biologically active substances. Some of them showed the inhibition of histone deacetylase (HDAC). Previously we have synthesized cyclic tetrapeptide hydroxamic acid analogs by replacing a common amino acid, Aoe (2-amino-8-oxo-9,10-epoxydecanoic acid) with hydroxamic acid-bearing amino acid, Asu(NHOH) [1,2]. The analogs based on trapoxin, Cyl-1, WF3161, and chlamydocin exhibited potent activities in HDAC inhibition and MHC assay. However, the hydroxamic acid analog of HC-toxin (*cyclo*(L-Aoe-D-Pro-L-Ala-D-Ala-)) was not a remarkable inhibitor probably due to the low lipophilicity [3]. In the present study, we attempted to increase the hydrophobicity by incorporating an aromatic ring variously on HC-toxin framework.

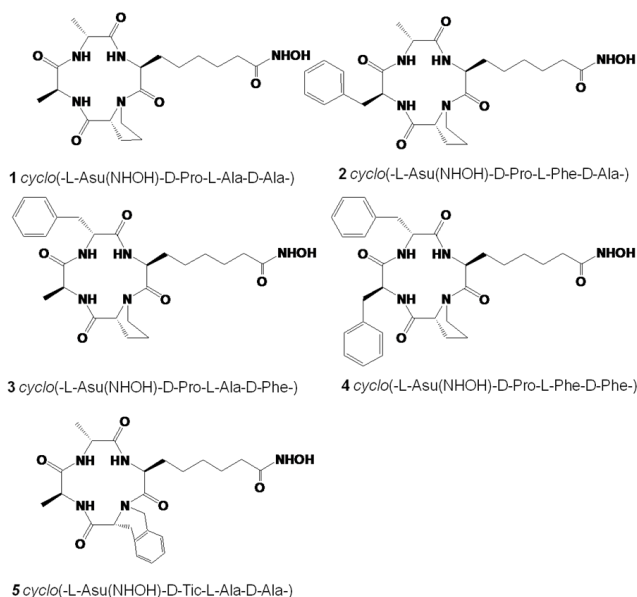


Fig. 1. Structure of *cyclo*(-L-Asu(NHOH)-D-Pro-L-Ala-D-Ala-) (1), *cyclo*(-L-Asu(NHOH)-D-Pro-L-Phe-D-Ala-) (2), *cyclo*(-L-Asu(NHOH)-D-Pro-L-Ala-D-Phe-) (3), *cyclo*(-L-Asu(NHOH)-D-Pro-L-Phe-D-Phe-) (4), and *cyclo*(-L-Asu(NHOH)-D-Tic-L-Ala-D-Ala-) (5),

In the present study, we designed five aromatic ring-containing cyclic tetrapeptide hydroxamic acids such as *cyclo*(-L-Asu(NHOH)-D-Pro-L-Phe-D-Ala-), where Asu is aminosuberic acid. The increase in the hydrophobicity of HC-toxin analog was expected by the introduction of

aromatic ring to the cyclic peptide framework to enhance the hydrophobic interaction with the enzyme (Fig. 1).

Results and Discussion

Our aim was to synthesize potent inhibitors of HDACs with the HC-toxin cyclic scaffold. Therefore, we intended to synthesize HC-toxin analogs containing aromatic ring in various positions in the cyclic tetrapeptide scaffold using a conventional solution-phase peptide synthesis strategy as shown in Scheme 1.

L-Ala or L-Phe	D-Ala or D-Phe	L-Asu	D-Pro or D-Tic
Boc — OH	H —	Boc — OBzl	H — O ^t Bu
DCC, HOBT		DCC, HOBT	
70-80%		70-80%	
Boc —	OBzl	Boc — OBzl	O ^t Bu
H ₂ , Pd-C		4 N HCl/dioxane	
88-95%		87-99%	
Boc —	OH HCl·H	OBzl	O ^t Bu
DCC, HOBT, Et ₃ N		OBzl	
67-84%		OBzl	
TFA·H	TFA	OBzl	OH
82-96%		OBzl	
HATU, DIEA			
36-66%			

Scheme 1. Synthesis of cyclic tetrapeptides 2 - 5.

Table 1. Characterization of cyclic tetrapeptides 2 - 5.

Compound	Composition	HPLC Retention time (min)	HRFAB-MS [M+H] ⁺	
			Observed	Calcd.
1	C ₁₉ H ₃₂ N ₅ O ₆	< 2.50	426.2381	426.2274
2	C ₂₅ H ₃₆ N ₅ O ₆	3.18	502.2692	502.2587
3	C ₂₅ H ₃₆ N ₅ O ₆	4.22	502.2636	502.2587
4	C ₃₁ H ₄₀ N ₅ O ₆	5.70	578.2961	578.2900
5	C ₂₄ H ₃₄ N ₅ O ₆	3.64	488.2514	488.2431

Column: Chromolith Performance RP-18e (100 x 4.6 mm)
Eluent: 10-100% CH₃CN / 0.1% TFA (linear gradient over 15 min)
Detect: 220 nm Flow rate: 2.0 mL / min

After the synthesis of cyclic peptides, the side chain benzyl ester was converted to the hydroxamic acid. All of the analogs 2 - 5 were characterized by ¹H NMR and high resolution FAB-MS.

We carried out the circular dichroism (CD) experiment of newly designed compounds 2 - 5 (Fig. 2). All the compounds showed large negative ellipticity at 210 nm, negative ellipticity at 240 nm, and positive ellipticity at 250 nm. These results showed that all of these compounds take similar conformation.

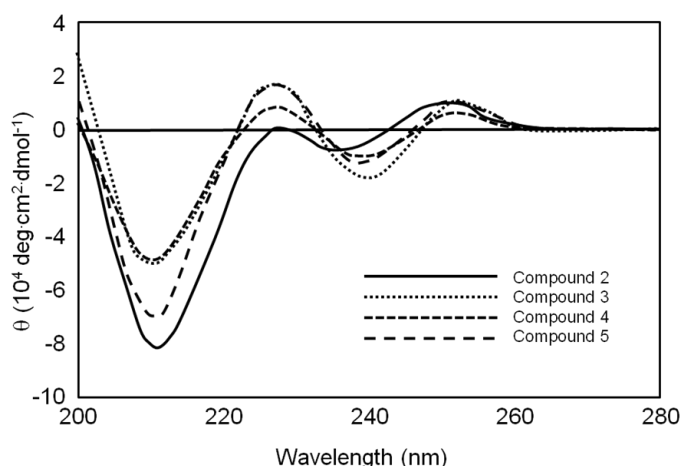


Fig. 2. CD spectra of cyclic tetrapeptides 2 - 5.

Cyclic tetrapeptide **5** containing D-Tic was characterized by $^1\text{H-NMR}$ (1D, 2D-COSY, 2D-HOHAHA, and 2D-NOESY) spectra in CDCl_3 . Three dimensional conformation of the compound was calculated by MOE system using NMR data. Resulted backbone conformation of the cyclic tetrapeptide resembled to the conformation of HC-toxin.

The analogs were subjected to the inhibition of HDAC1, HDAC4, and HDAC6 for the structure-activity-relationship. All of the newly synthesized Phe-containing cyclic tetrapeptide hydroxamic acids **2 - 4** showed similar HDAC inhibitory activities to the activity of reference compound (HC-toxin tetrapeptide hydroxamic acid analog **1**). HPLC retention time of these compounds **1 - 4** indicated that introduction of one or two aromatic rings to the reference compound **1** increased hydrophobicity of the compounds **2 - 4**. The increase in hydrophobicity of these analogs did not affect the inhibitory activity toward HDACs. On the other hand, Tic-containing cyclic tetrapeptide hydroxamic acid **5** showed extremely high HDAC inhibitory activity. Restricted conformational flexibility of Tic may force the aromatic ring to place proper position on the cyclic tetrapeptide backbone to inhibit HDACs.

Table 1. HDAC inhibitory activity of cyclic tetrapeptides 2 - 7.

Compound	IC_{50} (μM)		
	HDAC1	HDAC4	HDAC6
1	0.028	0.041	0.52
2	0.047	0.065	1.8
3	0.032	0.040	0.77
4	0.037	0.052	1.3
5	0.0047	0.0079	0.10

In conclusion, five HC-toxin analogs as cyclic tetrapeptide hydroxamic acids were designed and synthesized. Addition of Phe residue to HC-toxin sequence showed no significant enhancement in HDAC inhibition. Compound **5** containing D-Tic residue at D-Pro position was most potent against HDACs. CD measurements showed that all of these HC-toxin analogs have similar conformation.

Acknowledgments

We would like to thank Ms. S. Maeda, Dr. T. G. Nishino, and Dr. M. Yoshida (RIKEN) for HDAC inhibition assay. This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

References

1. Nishino, N., Jose, B., Shinta, R., Kato, T., Komatsu, Y., and Yoshida, M. (2004) *Bioorg. Med. Chem.*, **12**, 5777-5784.
2. Nishino, N., Yoshikawa, D., Watanabe, L. A., Kato, T., Jose, B., Komatsu, Y., Sumida, Y., and Yoshida, M. (2004) *Bioorg. Med. Chem. Lett.*, **14**, 2427-2431.
3. Furumai, R., Komatsu, Y., Nishino, N., Khochbin, S., Yoshida, M., and Horinouchi, S., (2001) *PNAS*, **98**, 87-92.