Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3515–3518

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

INHIBITION STEREOCHEMISTRY OF HYDROXAMATE INHIBITORS FOR THERMOLYSIN

Yonghao Jin and Dong H. Kim,*

Center for Biofunctional Molecules and Department of Chemistry
Pohang University of Science and Technology
San 31 Hyojadong, Pohang 790-784, Korea

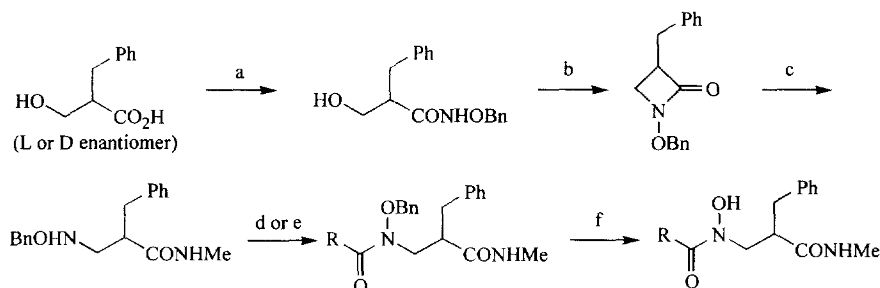
Received 9 September 1998; accepted 21 October 1998

Abstract: *N*-Acyl-*N*-hydroxy-β-amino acid derivatives were prepared and tested as inhibitors for thermolysin to find that these inhibitors show the L-stereospecificity in contrast to the corresponding hydroxamates prepared from α-amino acid, which exhibit the D-stereochemistry. *N*-Formyl-*N*-hydroxy-β-L-Phe-NHMe is the most potent inhibitor having the K_i value of 1.66 μM. © 1998 Elsevier Science Ltd. All rights reserved.

Thermolysin isolated from *Bacillus thermoproteolyticus* is a zinc-containing endopeptidase which catalyzes hydrolysis of the peptide bond on the imino side of amino acid residue having a hydrophobic side chain.[1] The enzyme represents a large number of medicinally important zinc-containing proteases such as angiotensin converting enzyme (ACE), neutral endopeptidases (NEP), and matrix metalloproteases (MMP), and has been used as a model target enzyme for the development of inhibitors of therapeutic potential. Inhibitors of zinc-containing proteases carry, in common, a functional group which acts as a ligand for the zinc ion at the active site of the enzyme: Numerous functional groups such as carboxylate, thiol, aldehyde, hydroxamate, phosphoramidate, and phosphonamidate are incorporated into basic substrate structures of these enzymes.[2] Of these functional groups hydroxamate occupies a prominent position because of its strong chelating property towards zinc ion, and has been extensively utilized in the design of zinc protease inhibitors.[3] Previously, we have reported that the inhibition of thermolysin by *N*-formyl-*N*-hydroxy-Leu-OMe is stereospecific with the inhibitory activity being vested mainly with the D-isomer.[4] We wish to report in this communication that *N*-acyl-*N*-hydroxy-β-amino acid derivatives also exhibit potent inhibitory activity against thermolysin but remarkably the stereochemistry of the inhibition is reversed compared with that for hydroxamates from α-amino acid.

0960-894X/98/$ - see front matter © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(98)00643-X

The inhibitors (**1** – **3**)[5] were prepared starting with optically active α-benzyl-β-hydroxypropionic acid according to the route depicted in Scheme 1, and are listed in Table 1 together with their inhibitory constants determined for thermolysin by the method of Feder[6] using *N*-[3-(2-furyl)acryloyl]-Gly-Leu-NH_2 as substrate.[7]



Scheme 1. Reagents, conditions, and (yields): (a) $BnONH_2$ (2.0 eq), EDCI (1.5 eq), THF-H_2O (98%); (b) DEAD (1.0 eq), PPh_3 (1.0 eq), THF (85%); (c) 40% $MeNH_2$ (10.0 eq), MeOH (100%); (d) Ac_2O (8.0 eq), HCO_2H, 0 °C (100%); (e) acyl chloride (1.0 eq), TEA (1.1 eq), 0 °C, CH_2Cl_2 (98%); (f) H_2, Pd/C, MeOH (98%).

Table 1. Structures of *N*-acyl-*N*-hydroxy-amino acid derivatives and their inhibitory constants determined for the inhibition of thermolysin

Compd No.	Structure	mp (°C)	$[\alpha]_D^{23}$ ($CHCl_3$)	K_i (μM)
L-**1**	L-HC-NCH$_2$CHCONHMe O OH CH$_2$Ph	69-70	+60.8 (c 1.12)	1.66±0.05
D-**1**	D-HC-NCH$_2$CHCONHMe O OH CH$_2$Ph	69-70	-60.6 (c 1.10)	68.2±5.6
L-**2**	L-CH$_3$C-NCH$_2$CHCONHMe O OH CH$_2$Ph	96-97	+32.1 (c 1.10)	3950±240
D-**2**	D-CH$_3$C-NCH$_2$CHCONHMe O OH CH$_2$Ph	96-97	-32.0 (c1.15)	NI[a]
L-**3**	L-CH$_3$CH$_2$C-NCH$_2$CHCONHMe O OH CH$_2$Ph	102	+27.6 (c 1.50)	7500±250
D-**3**	D-CH$_3$CH$_2$C-NCH$_2$CHCONHMe O OH CH$_2$Ph	101-102	-27.5 (c 1.50)	NI[a]
4	L-HC-NCH$_2$CHCO$_2$Me O OH CH$_2$CHMe$_2$	oil	-18.6 (c 1.28)	22.5±0.7
5	D-HC-N-LeuOMe O OH	oil	-27.1 (c 1.30)	44.0±2.5[b]

[a] No inhibitory activity was observed at the concentration up to 20 mM. [b] Ref. 4

It is apparent from Table 1 that the inhibitory activity is stereospecific with inhibitors belonging to the L-series being much more potent than that having the D-configuration, which is in direct contrast to the inhibitory stereochemistry observed with the corresponding inhibitor prepared from α-amino acid.[4] Thus,

in the case of *N*-formyl-*N*-hydroxy-β-Phe, the L-form is 41-fold more potent than that of the D-form. The K_i value of 1.66 μM exhibited by L-**1** is the lowest for hydroxamate inhibitors evaluated in this study. The other distinctive trend noted from the Table is dependency of the inhibitory activity on the size of the alkyl group in the *N*-acyl portion of the inhibitors: the highest binding affinity shown with L-**1** is sharply curtailed as the formyl group is replaced with an acetyl or propanoyl group, giving the K_i value of 3950 and 7500 μM for L-**2** and L-**3**, respectively. The increase of the K_i value, upon the acetyl group being changed to propanoyl, is only minimal to suggest that there is not much difference between methyl and ethyl groups compared with hydrogen. These results suggest that the crevice leading to the S_3 subsite is shallow and thus a group bulkier than hydrogen cannot be accommodated. Comparison of the thermolysin binding affinity of hydroxamates prepared from α- and β-amino acid, revealed that the K_i value of hydroxamate of L-β-Leu (**4**)[7] is about one half of that of D-α-Leu (**5**), suggesting that hydroxamates of L-β-amino acid possesses higher binding affinity toward thermolysin than hydroxamates prepared from D-α-amino acid. This is noteworthy in light of the fact that most of hydroxamate type inhibitors for zinc proteases are those prepared by incorporating the hydroxamate into basic substrate backbones of α-amino acids. Thus, the replacement of the α-amino acid in the known hydroxamate inhibitors with the corresponding β-amino acid is expected to improve the inhibitory potency.

We have previously proposed a schematic representation for the active site of thermolysin, which assists one to visualize the D-stereospecificity for hydroxamate inhibitors derived from α-amino acid.[4] This active site model may be of value in understanding the reversed inhibitory stereochemistry shown with the hydroxamates of β-amino acid. Figure 1 represents the active site of thermolysin, which is occupied by hydroxamate inhibitors prepared from β-Phe. The active site zinc ion is located at such a locus that it can effectively form the bidendate coordinative bonds only with the hydroxamate of β-Phe having the L-configuration.

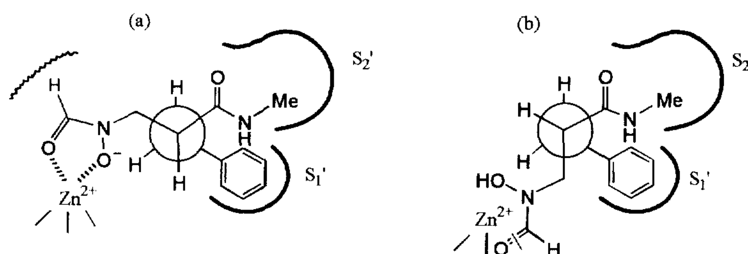


Figure 1. Schematic representation of the active site of thermolysin, which is occupied by hydroxamate inhibitor HCO-N(OH)-CH_2CH(PhCH_2)CONHMe. (a) L-enantiomer; (b) D-enantiomer.

In conclusion, we have demonstrated that in the design of hydroxamate type of thermolysin inhibitors the P_1' amino acid residue may be replaced with α-substituted β-amino acid. The inhibitory potency was somewhat improved by the replacements. It is interest to note that in contrast to the hydroxamate inhibitors prepared from α-amino acid, the present inhibitors which are considered to be homologs of the α-amino acid hydroxamates exhibit the L-stereospecificity in the thermolysin inhibition. In light of the fact that a majority of therapeutic agents display their pharmacological effects through inhibition of enzymes,[8] enzyme inhibitory stereochemistry is of extreme importance in understanding the dissimilar pharmacological effects manifested by stereoisomers of chiral drugs. The result of present study demonstrates that the stereochemistry of enzyme inhibition and thus pharmacological responses can not simply be deduced from the substrate stereospecificity and should be evaluated carefully in a case by case fashion.

Acknowledgement: This work was supported by the Korea Science and Engineering Foundation.

References and Notes

1. (a) Matthews, B. W. *Acc. Chem.Res.* **1988**, *21*, 333 - 340. (b) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed. W.H. Freeman & Co. New York, 1977, pp 418 – 420.
2. Powers, J. C.; Harper, J. W. In *Proteinase Inhibitors*, Barrett, A. J.; Salvesen, G. Eds.; Elsevier; Amsterdam, 1986; pp 219 - 298.
3. (a) Kurzak, B.; Kozolowski, H.; Farkas, E. *Coord. Chem. Rev.* **1992**, *114*, 169 - 200. (b) Browner, M. F.; Smith, W. W.; Castelhano, A. L. *Biochemistry* **1995**, *34*, 6602 – 6610. (c) Rockwell, A.; Melden, M.; Copaland, R. A.; Hardman, K.; Decicco, C. P.; DeGrade, W. F. *J Am. Chem. Soc.* **1996**, *118*, 10337 – 10338. (d) Turbanti, L.; Cerbai, G.; Di Bugno, C.; Giorgi, R.; Garzelli, G.; Criscuoli, M.; Renzetti, A. R.; Subissi, A.; Bramanti, G. *J. Med. Chem.* **1993**, *36*, 699 – 707. (e) Penning, T. D.; Askonas, L. J.; Djuric, S. W.; Haack, R. A.; Yu, S. S.; Mickener, M. L.; Krivi, G. G.; Pylas, E. Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2517 – 2522.
4. Jin, Y.; Kim, D. H. *Tetrahedron: Asymmetry* **1997**, *8*, 3699 – 3702.
5. All new compounds reported in this paper were satisfactorily characterized by the spectral (IR, NMR, MS) and microanalytical data.
6. Feder, J. *Biochem. Biophys. Res. Commun.* **1968**, *32*, 326 – 332.
7. Inhibitor **4** was prepared in a similar fashion starting with (*S*)-2-hydroxymethyl-4-methylpentanoic acid.
8. (a) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*, Academic Press: New York, 1992; chapter 5. (b) Walpole, C. S. J.; Wrigglesworth, R. *Natural Product Report* **1989**, 311 – 346. (c) *Perspective in Medicinal Chemistry*, Testa, B.; Kyburz, E.; Fuhrer, W.; Giger, R. Ed.; Verlag Helvetica Chemical Acta: Basel, 1993; Part B.