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Design and Synthesis of Dual Inhibitors for Matrix Metalloproteinase and Cathepsin

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Abstract—The first example of dual inhibitors for matrix metalloproteinase (MMP) and cathepsin is described. An appropriate alignment of peptide-parts and two different specific functional groups in one molecule led to the discovery of a potent dual inhibitor (**3a**).

Introduction

Proteinases are classified into four types (metalloproteinase, cysteine proteinase, serine proteinase and aspartic proteinase) by differences in hydrolysis mechanisms of peptide bonds.[1] A large number of proteinase inhibitors categorized in peptide-type and non-peptide-type inhibitors have been investigated by many scientists. For the design of peptide-type inhibitors, it is important to align appropriately peptide-parts and a specific functional group for respective classes.[1,2] Peptide-parts represent a frame which is mimicked from amino acid sequence of a substrate cleaved by proteinases. On the other hand, a specific functional group represents a group which interacts with the active site of an enzyme to accelerate hydrolysis of a peptide bond. There are some general functional groups used for the design of proteinase inhibitors. For example, in the case of metalloproteinase inhibitors, the introduction of a functional group chelating metal in an enzyme, such as hydroxamic acid, carboxylic acid or phosphonic acid group, is essential to exhibit potent activity.[3] Concerning cysteine proteinase inhibitors, a functional group interacting with thiol group in an enzyme, such as aldehyde or ketone, is needed.[4,5]

It has been reported that the activation of MMPs and cathepsins is associated with cartilage and bone degradation,[6] such as osteoporosis and rheumatoid arthritis. Therefore a potential dual inhibitor for MMP and cathepsin would be useful for the treatment of these diseases.

Compounds which have inhibitory activity against two proteinases belonging to the same class are known. For instance dual inhibitors against both angiotensine converting enzyme (ACE) and neutral endpeptidase (NEP), which belong to metalloproteinase, have already been reported.[7] However, dual inhibitors for two proteinases belonging to different classes are not known yet because it is very difficult to align appropriately peptide-parts and two specific functional groups for each class.

This paper reports the synthesis and biological activity of dual inhibitors for two proteinases belonging to different classes, which are matrix metalloproteinase (MMP-1) belonging to metalloproteinase and cathepsin L belonging to cysteine proteinase.

Design for Dual Inhibitor

A representative MMP inhibitor[8] and cathepsin inhibitor[5] are shown in Figure 1.

MMP inhibitor **1** has hydroxamic acid as a functional group chelating zinc in an active site of an enzyme. The P1′ and P2′ moieties were optimized from amino acid sequence of the substrate cleaved by MMP, and the optimized ones are Leu and Phe, respectively.[1,2,9] On the other hand, aldehyde group is generally used as a functional group for cathepsin inhibitors because the aldehyde group interacts with thiol group in the active

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site. The modification of peptide parts of this inhibitor was mimicked from the sequence of the left-hand parts at the cleaved site of the substrate.

MMP inhibitor **1** contains benzyl group at P2′ position and methyl group at P3′ position. Then cathepsin inhibitor **2** also has these groups at P2 position and P1 position, respectively. We can see that there is a close similarity between P2′ and P3′ substituents in MMP inhibitor **1** and P2 and P1 substituents in cathepsin inhibitor **2**, even though these proteinases belong to different classes. Therefore we focused on these peptide-parts and designed dual inhibitor **3a** for MMP and cathepsin by introducing respective specific functional groups, hydroxamic acid group for MMP and aldehyde group for cathepsin, into these peptide-parts.

In addition, we investigated the profile of inhibitory activity against each enzyme by the modification of peptide-parts.

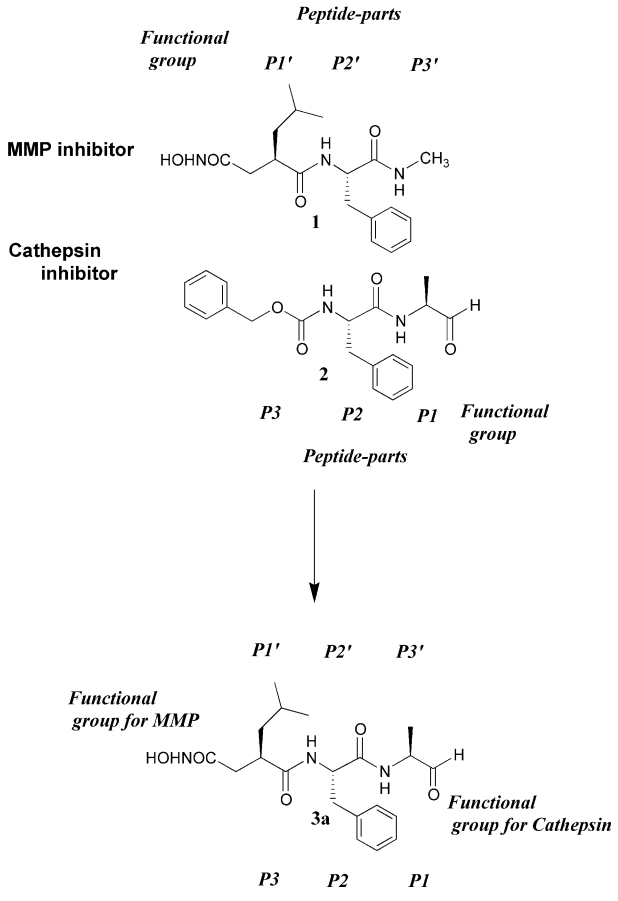


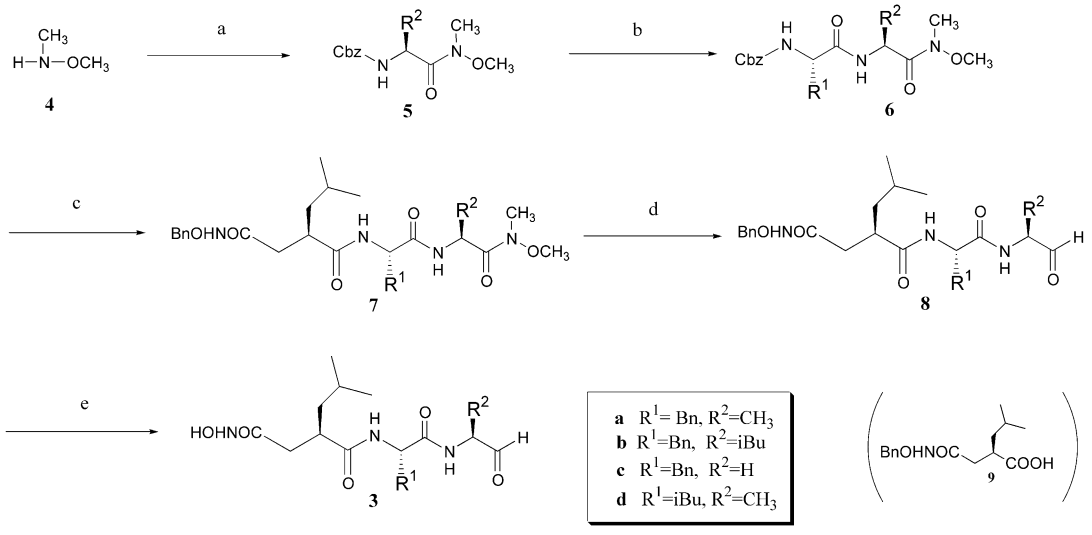
Figure 1.

Chemistry

Compound **3** was synthesized as shown in Scheme 1. *N*,*O*-Dimethylhydroxylamine **4** was condensed with the corresponding amino acid protected by benzyloxy-carbonyl group (Cbz) to give compound **5**. The deprotection of benzyloxycarbonyl group followed by coupling with Cbz-amino acid provided compound **6**. After deprotection of benzyloxycarbonyl group of **6**, the resulting amine was condensed with carboxylic acid **9**[10] in the presence of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC) to give benzyloxy-amide **7**. The reduction of **7** with lithium aluminum hydride provided aldehyde **8**, and then the benzyl group of **8** was deprotected to give compound **3**.

Biological Data

The biological data[11] of compound **3a** are shown in Table 1. As expected, compound **3a** exhibited potent inhibitory activity against both MMP-1 and cathepsin L. Compound **10** having acetal in place of aldehyde group showed potent inhibition against MMP-1 as well as **3a**, but no inhibition for cathepsin L. This result indicates

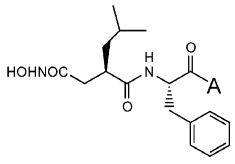


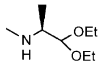
Scheme 1. (a) Cbz-amino acid, EDC, HOBt (**5a**,**d** 69%, **5b** 96%, **5c** 72%); (b) (i) H_2/Pd–C; (ii) Cbz-amino acid, EDC,HOBt (**6a** 71%, **6b** 43%, **6c** 49%, **6d** 84%); (c) (i) H_2/Pd–C; (ii) **9**, EDC (**7a** 56%, **7b** 78%, **7c** 40%, **7d** 38%); (d) $LiAlH_4$; (e) H_2/Pd–C (**3a** 39%, **3b** 14%, **3c** 30%, **3d** 27% from **7**).

that acetal did not interact with thiol group in an active site of cysteine proteinase.

Next, we investigated an inhibitory effect on peptide-parts. As shown in Table 2, the conversion of peptide-parts (at P2′–P3′ position for MMP-1, at P1–P2 position for cathepsin L) gave interesting results. Compound **3b** exhibited potent inhibition against cathepsin L as well as compound **3a**, but less potency against MMP-1. A small substituent seems to be favorable at P3′ position (R^2 substituent) for MMP-1. Actually, it has been reported that the introduction of Leu at this position decreased MMP-1 inhibition.[12] On the other hand, compound **3c** exhibited potent inhibition against MMP-1 as well as compound **3a**, but less potency against cathepsin L. The existence of a substituent at P1 position (R^2 substituent) seems to be essential for the inhibition of cathepsin L. In fact, Yasuma reported that hydrophobic and bulky groups at P1 position were favorable for cathepsin L inhibition.[13] Compound **3d** showed potent inhibition against both MMP-1 and cathepsin L as well as compound **3a**. Leucine at the P2 site is also favorable for cathepsin L inhibition. This result is supported by the S2 pocket of cathepsin L being considered as a hydrophobic and moderately sized cavity.[13] These results indicate that the appropriate combination of peptide-parts would be also important for the design of dual inhibitors of MMP-1 and cathepsin L.

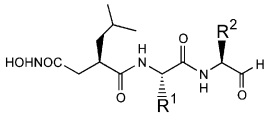
Table 1. Inhibitory activity of compound **3a** against MMP-1 and cathepsin L



	A	IC_{50} (nM)[a]	
		MMP-1	Cathepsin L
3a		25	15
10		29	>1000
1		3	>1000
2		>1000	3

[a]See References and Notes for enzyme assay details.

Table 2. Inhibitory activity of compounds converted at peptide-parts



	R^1	R^2	IC_{50} (nM)[a]	
			MMP-1	Cathepsin L
3a		–CH^3	25	15
3b			1400	7
3c		–H	12	4300
3d		–CH_3	47	23

[a]See References and Notes for enzyme assay details.

The inhibitory activity of these dual inhibitors to other proteinases (e.g., serine proteinase and aspartic proteinase) is not examined, but these inhibitors are probably considered not to show inhibitory activity to serine proteinase and aspartic proteinase because the basic structure of these dual inhibitors is based on the structure of a selective matrix metalloproteinase inhibitor.

In conclusion, we succeeded in the design and synthesis of dual inhibitors for MMP and cathepsin. This paper exhibits the first example of dual inhibitors for two proteinases respectively belonging to different classes. We are in the process of investigating more potent dual inhibitors. This design could be useful for other new dual inhibitors. For example, a dual inhibitor, such as tumor necrosis factor-alpha converting enzyme (TACE)[14] belonging to metalloproteinase and interleukin-1 converting enzyme (ICE)[15] belonging to cysteine proteinase, might be designed by a similar method.

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References and Notes

1. Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
2. Zask, A.; Levin, J. I.; Killar, L. M.; Skotnicki, J. S. *Curr. Pharm. Des.* **1996**, *2*, 624.
3. Whittaker, M.; Floyd, C. D.; Brown, P.; Andrew, J. H.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735.
4. Smith, R. A.; Coles, P. J.; Spencer, R. W.; Copp, L. J.; Jones, C. S.; Krantz, A. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 1201.
5. Woo, J.-H.; Sigeizumi, S.; Yamaguchi, K.; Sugimoto, K.; Kobori, T.; Tsuji, T.; Kondo, K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1501.
6. (a) Buttle, D. J.; Handley, C. J.; Ilic, M. Z.; Saklatvala, J.; Murata, M.; Barrett, A. J. *Arthritis Rheum.* **1993**, *36*, 1709. (b) Inui, T.; Ishibashi, O.; Origane, Y.; Fujimori, K.; Kokubo, T.; Nakajima, M. *Biochem. Biophys. Res. Commun.* **1999**, *258*, 173.
7. (a) Robl, J. A.; Cimarusti, M. P.; Simpkins, L. M.; Brown, B.; Ryono, D. E.; Bird, J. E.; Asaad, M. M.; Schaeffer, T. R.; Trippodo, N. C. *J. Med. Chem.* **1996**, *39*, 494. (b) Flynn, G. A.; Beight, D. W.; Mehdi, S.; Koehl, J. R.; Giroux, E. L.; French, J. F.; Hake, P. W.; Dage, R. C. *J. Med. Chem.* **1993**, *36*, 2420.
8. Grams, F.; Crimmin, M.; Hinnes, L.; Huxley, P.; Pieper, M.; Tschesche, H.; Bode, W. *Biochemistry* **1995**, *34*, 14012.
9. Morphy, J. R.; Millican, T. A.; Porter, J. R. *Curr. Med. Chem.* **1995**, *2*, 743.

10. Hirayama, R.; Yamamoto, M.; Tsukida, T.; Matsuo, K.; Obata, Y.; Sakamoto, F.; Ikeda, S. *Bioorg. Med. Chem.* **1997**, *5*, 765.
11. The assay for inhibitory activity of MMP-1 is described in ref 10.

The assay for inhibitory activity of cathepsin L: Inhibitory activities of cathepsin L, purchased from Cosmo Bio Co. Ltd., were measured using Cbz-Phe-Arg-AMC (Cosmo Bio Co. Ltd.) as a substrate. Cathepsin L (human kidney) and a compound were incubated in 400 mM CH_3COOH/NaOH (pH 5.5) containing 4 mM EDTA 2Na at 30 °C for 1 min. A substrate was added, then incubated at 30 °C for 10 min. The incubation was terminated by the addition of 100 mM CH_3COOH/NaOH (pH 4.3) containing 100 mM monochloroacetic acid. The fluorescence intensity (Ex 375 nm, Em 445 nm) was measured. The IC_{50} values were the average of at least two determinations with a standard deviation of less than $\pm 30\%$.

12. Campbell, D. A.; Xiao, X.-Y.; Harris, D.; Ida, S.; Mortezaei, R.; Ngu, K.; Shi, L.; Tien, D.; Wang, Y.; Navre, M.; Patel, D. V.; Sharr, M. A.; DiJoseph, J. F.; Killar, L. M.; Leone, C. L.; Levin, J. I.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1157.
13. Yasuma, T.; Oi, S.; Choh, N.; Nomura, T.; Furuyama, N.; Nishimura, A.; Fujisawa, Y.; Sohda, T. *J. Med. Chem.* **1998**, *41*, 4301.
14. (a) Barlaam, B.; Bird, T. G.; Lambert-van der Brempt, C.; Campbell, D.; Foster, S. J.; Maciewicz, R. *J. Med. Chem.* **1999**, *42*, 4890. (b) Yamamoto, M.; Hirayama, R.; Naruse, K.; Yoshino, K.; Shimada, A.; Inoue, S.; Kayagaki, N.; Yagita, H.; Okumura, K.; Ikeda, S. *Drug Des. Discov.* **1999**, *16*, 119.
15. (a) Revesz, L.; Briswalter, C.; Heng, R.; Leutwiler, A.; Mueller, R.; Wuethrich, H.-J. *Tetrahedron Lett.* **1994**, *35*, 9693. (b) Dolle, R. E.; Singh, J.; Rinker, J.; Hoyer, D.; Prasad, C. V. C.; Graybill, T. L.; Salvino, J. M.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. *J. Med. Chem.* **1994**, *37*, 3863.