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EFFECTS OF YM-51084 AND YM-51085, NEW INHIBITORS PRODUCED BY *STREPTOMYCES SP. Q21705*, ON CATHEPSIN L

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The structures of YM-51084 and YM-51085, new protease inhibitors produced by *Streptomyces sp. Q21705*, were determined by ^{1}H- and ^{13}C-NMR and mass spectrometry. Both were characterized by the basic structures of an acyl tripeptide. YM-51084 was elucidated to be isovaleryl-tyrosyl-valyl-phenylalaninal and YM-51085 was the reduced phenylalaninol form of YM-51084. These compounds proved to strongly inhibit human kidney cathepsin L; the IC_{50} values being 9.6×10^{-9}M and 3.5×10^{-7}M, respectively.

Keywords: Cathepsin L; cysteine protease; protease inhibitor; osteoporosis.

INTRODUCTION

It has been reported that several cathepsins present in lysosomes have collagenolytic activity in acidic conditions.[1] Some cathepsins were termed 'collagenolytic cathepsins', and thereafter named cathepsin L[2] and cathepsin B. In 1986, it was found that a protease resembling cathepsin L was secreted from osteoclasts and that this differed from collagenase, because of the finding that a cysteine protease inhibitor reduced the pits caused by osteoclasts.[3] In an *in vivo* study in rats fed a low calcium diet, calcium levels in blood and hydroxyproline levels in urine, both markers of collagenolysis, were reduced by administration of the cysteine protease inhibitor, E-64.[4] Moreover, recent studies have shown that

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cathepsin L is secreted into osteoclast culture medium in greater proportion to an increase in PTH[5] and also that the concentration of cathepsin L is high at the resorption lacunae of osteoclasts.[6] Together with a report showing that the protease in osteoclasts was cathepsin L or a protease highly homologous to it,[7] these finding suggest that cathepsin L is the enzyme most likely responsible for bone resorption.

We have recently isolated new protease inhibitors, YM-51084 and YM-51085 produced by *Streptomyces sp. Q21705*, and identified their structures. In this study, we also evaluated their inhibitory effects on cathepsin L and other cysteine proteases.

MATERIALS AND METHODS

Materials

YM-51084 and YM-51085 were produced by *Streptomyces sp. Q21705*. Human kidney cathepsin L and human liver cathepsin B were purchased from Protogen AG (Läufelfingen, Switzerland), and human liver cathepsin H and papaya latex papain from Calbiochem-Novabiochem Corp. (CA, USA) and Sigma Corp. (MO, USA), respectively. Benzyloxycarbonyl-Phenyl-Arginyl-methylcoumaryl amide (Z-Phe-Arg-MCA), Z-Arg-Arg-MCA and Z-Arg-MCA, synthetic substrates for cathepsin L, cathepsin B and cathepsin H, respectively, were obtained from Peptide Institute Inc. (Osaka, Japan). Casein, a substrate of papain, was obtained from Wako Pure Chemicals (Tokyo, Japan).

Structural Analyses

Fast atom bombardment spectra (FAB-MS) were measured with a JMS-DX 300 (JEOL, Tokyo) using 10% 3-nitrobenzyl alcohol-DMSO as a matrix. NMR spectra were recorded in CD_3OD and $DMSO_{d6}$ with an ALPHA-500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C, JEOL).

Assay of YM-51084 and YM-51085 for Inhibition of Various Proteases

The inhibitory effects of YM-51084 and YM-51085 on human kidney cathepsin L were determined by modification of a published method.[8] After 10 min incubation at 30°C, fluorescence of MCA was detected. Assay for cathepsin B[9] and H[10] inhibition was determined according to published methods and that for papain by the method of Aoyagi.[11]

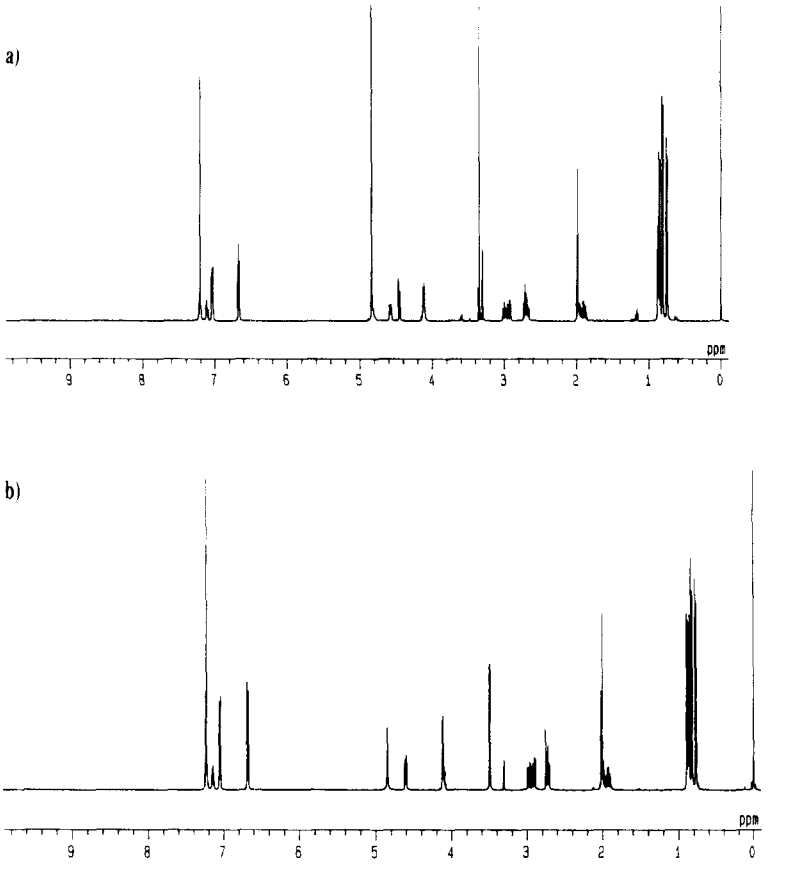

FIGURE 1 ^{1}H-NMR spectra of (a) YM-51084 and (b) YM-51085.

RESULTS

The structures of YM-51084 and YM-51085, produced by *Streptomyces sp. Q21705*, were determined by ^{1}H- and ^{13}C-NMR and mass spectrometry. On the basis of FAB mass spectrometry and ^{13}C-NMR spectroscopy, the molecular formulae of YM-51084 and YM-51085 were determined as $C_{28}H_{37}N_3O_5$ and $C_{28}H_{39}N_3O_5$, respectively. The structures of YM-51084 and YM-51085 were elucidated by ^{1}H NMR, ^{13}C NMR, DEPT, double-quantum-filtered COSY (DQF-COSY), NOESY, ^{1}H-^{13}C COSY and heteronuclear multiple-bond correlation (HMBC). The ^{1}H NMR spectra of YM-51084 and YM-51085 are shown in Figure 1.

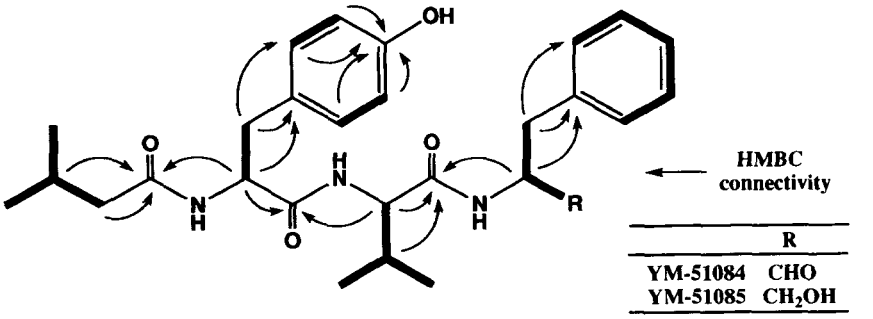

FIGURE 2 Structures of YM-51084 and YM-51085 elucidated by NMR analyses. Bold lines show proton spin networks obtained by the DQF-COSY and arrows indicate ^1H-^{13}C long-range correlations observed in the HMBC spectrum.

The ^{13}C NMR spectrum of YM-51085 revealed the presence of 28 carbon atoms, which were assigned to 4 methyls, 4 methylenes, 14 methines and 6 quaternary carbons by DEPT experiments. The ^1H-^{13}C COSY spectrum established all one-bond ^1H-^{13}C correlations, and the proton spin networks were obtained from DQF-COSY spectrum. The partial structure deduced through DQF-COSY analysis were connected by HMBC spectrum and the final structure of YM-51085 was elucidated as shown in Figure 2. Further confirmation of structure was from its NOESY spectrum.

The ^1H and ^{13}C NMR spectra of YM-51084 were very similar to those of YM-51085. However, NMR analysis in $DMSO_{d6}$ revealed that YM-51084 has an aldehyde group instead of the hydroxymethyl group of the phenylalaninol. The structure of YM-51084 is shown in Figure 2 and the ^1H and ^{13}C chemical shifts for YM-51084 and YM-51085 in CD_3OD are summarized in Table I. Because of the racemization (1:1 ratio) in the assymmetric center of the phenylalaninal residue of YM-51084, several atoms near the assymmetric center of the phenylalaninal showed two set of signals. Therefore, all coupling constants in YM-51084 between protons could not be obtained. Moreover, the ^1H and ^{13}C NMR spectrum in CD_3OD indicated that in CD_3OD, the aldehyde group of YM-51084 is present in the hemiacetal form (δ_H4.46, δ_C98.8).

Table II shows the inhibitory effects of YM-51084 and YM-51085 against several proteases, including cathepsin L. Their inhibitory effects against human kidney cathepsin L activity were the strongest with IC_{50} values of 9.6×10^{-9} M and 3.5×10^{-7} M, respectively. IC_{50} values were respectively 1.2×10^{-8} M and 7.3×10^{-7} M against human liver cathepsin B, 2.2×10^{-6} M and 8.6×10^{-5} M against

TABLE I ^{1}H and ^{13}C NMR chemical shifts of YM-51084 and YM-51085 in CD_3OD. Because of the racemization, several atoms of YM-51084 show two set of signals (see text).

	YM-51084			*YM-51085*	
	δ_H	δ_C		δ_H	δ_C
iVal			iVal		
1		175.5	1		175.6
2	1.99 (d, 7.9)	46.1	2	2.01 (d, 7.9)	46.1
3	1.90 (m)	27.4	3	1.92 (m)	27.4
4	0.76 (d, 6.1)	22.7	4	0.78 (d, 6.1)	22.8
	0.82 (d, 6.7)			0.83 (d, 6.7)	
Tyr			Tyr		
α	4.58	56.0	α	4.60 (dd, 9.8, 4.9)	56.1
β	2.68	37.9	β	2.73 (dd, 14.0, 9.8)	37.9
	2.93			2.97 (dd, 14.0, 4.9)	
γ		129.3	γ		129.2
δ	7.03	131.2	δ	7.05 (d, 8.5)	131.3
ε	6.68	116.2	ε	6.69 (d, 8.5)	116.3
ξ		157.2	ξ		157.3
CO		173.8, 173.9	CO		173.9
Val			Val		
α	4.11	60.2	α	4.11 (d, 6.7)	60.3
β	1.97	32.2, 32.3	β	2.00 (m)	32.3
γ	0.86	18.6	γ	0.87 (d, 6.7)	18.6
	0.87	19.7		0.89 (d, 6.7)	19.7
CO		173.0, 173.1	CO		173.0
Phenylalaninal			Phenylalaninol		
α	4.13	56.3, 56.4	α	4.11 (m)	54.2
β	2.71	35.6, 36.1	β	2.73 (d, 14.0)	37.9
	3.00			2.90 (dd, 6.7)	
γ		139.7, 139.9	γ		139.7
δ	7.20–7.22	130.3, 130.4	δ	7.22–7.26 (m)	130.4
ε	7.20-7.22	129.3	ε	7.22–7.26 (m)	129.4
ξ	7.11	127.2	ξ	7.15 (m)	127.4
CHO (hemiacetal)	4.46	98.8	CH_2OH	3.49 (d, 5.5)	64.0

TABLE II IC_{50} values of YM-51084 and YM-51085 on various cysteine proteases.

	YM-51084	*YM-51085*
Cathepsin L (Human kidney)	9.6×10^{-9} M	3.5×10^{-7} M
Cathepsin B (Human liver)	1.2×10^{-8} M	7.3×10^{-7} M
Papain (Papaya latex)	2.2×10^{-6} M	8.6×10^{-5} M
Cathepsin H (Human liver)	$>10^{-7}$ M	$>10^{-5}$ M

papain. Although their accurate IC_{50} values were not obtained for cathepsin H which is highly homologous to cathepsin L and B, the values were much greater than those for cathepsin L and B. The IC_{50} values against papain were more than 100-fold greater than those against cathepsin L.

DISCUSSION

YM-51084 and YM-51085 produced by *Streptomyces sp. Q21705* showed potent inhibitory activities against cysteine proteases such as cathepsin L and cathepsin B. However, their inhibitory effects were much weaker against cathepsin H or papain among other cysteine proteases examined. In addition, a recent study by us has revealed that both YM-51084 and YM-51085 inhibited a protease derived from purified rabbit osteoclasts (unpublished data). Although the properties of the protease in the rabbit osteoclasts have not been adequately characterized, there is a high possibility that osteoclast-specific protease may be directly implicated in bone diseases. It is widely considered that bone diseases like osteoporosis are closely related to the action of osteoclasts during the process of bone remodeling or resorption, and to the action of protease found in osteoclasts. Tezuka *et al.*[7] showed that the osteoclast-specific protease is highly homologous to cathepsin L. Since YM-51084 and YM-51085 inhibited osteoclast protease they should be useful in elucidating the relationship of osteoclast protease to the pathogenesis of bone diseases such as osteoporosis, and may be possible candidate drugs for the treatment of osteoporosis.

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