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PEPTIDYL ALDEHYDE DERIVATIVES AS POTENT AND SELECTIVE INHIBITORS OF CATHEPSIN L

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Abstract: A series of peptidyl aldehyde derivatives were synthesized and tested for their inhibitory effects on several cysteine proteases and a serine protease. These componds showed no inhibition for α-chymotrypsin at the concentration less than 1000 ng/ml, while they exhibited prominent inhibition for calpain II and cathepsins, especially cathepsin L.

Introduction

Cysteine proteases including calpains and some cathepsins are the enzymes of current interest because of their roles in a number of disease processes such as osteoporosis[1, 2], cancer metastasis[3-5], arthritis[6] and muscular dystrophy[7, 8]. Among them, cathepsin L, a well-characterized cysteine protease, is suggested to play a major role in degradation of extracellular-matrix proteins such as collagen during bone resorption[9]. Therefore selective inhibitors of cathepsin L would be useful to elucidate a role of this enzyme in bone resorption and will be good candidates for new osteoporotic medicines.

Some cystein protease inhibitors classified into chloromethyl ketone[10, 11], epoxide[12, 13] and diazomethane derivatives[14, 15] are known to react irreversibly on the active site of the enzyme. One of the diazomethane derivatives, Z-Phe-Phe-CHN_2 is reported to be a highly selective inhibitor for cathepsin L[16]. However, the instability of diazomethane derivatives are not favourable for clinical use. On the other hand, peptidyl aldehydes form reversible complex with cysteine proteases[17] and serine proteases[18]. Some of di- and tripeptidyl aldehyde derivatives are reported to show almost no inhibition for trypsin, and weak inhibition for α-chymotrypsin, while they exhibited marked inhibition for cathepsins[17].

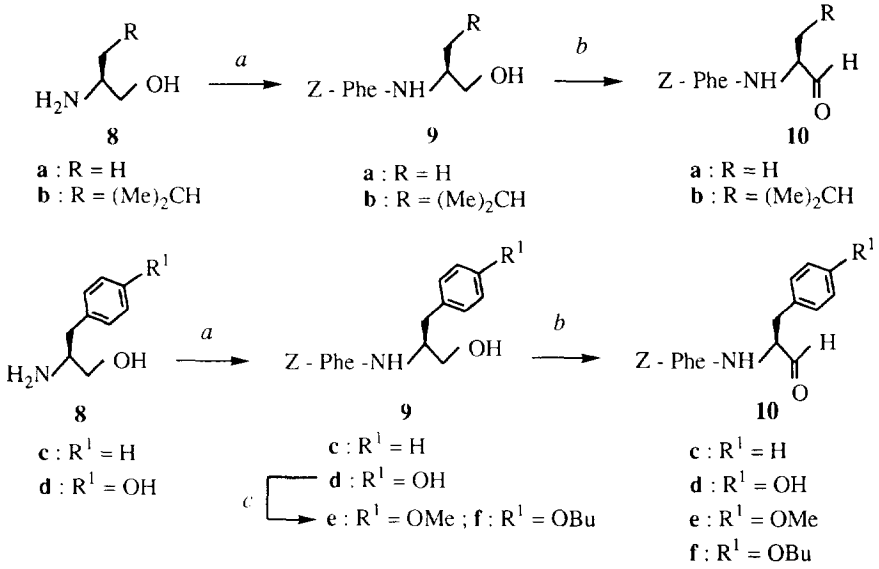
The objective of this study is to synthesize dipeptidyl aldehyde derivatives which show potent and selective inhibition for cathepsin L and to test their effects on bone resorption. Considering from the example of Z-Phe-Phe-CHN_2[18], the amino acid sequence of Phe-Phe

seemed to be preferential for cathepsin L inhibition. Therefore, Z-Phe-Phe-H was selected as the first target compound, and its derivatives with substitution at P1 site were synthesized and evaluated. Synthesis and biological activities of 6 kinds of peptidyl aldehydes were reported in this paper.

Chemistry

Peptidyl aldehydes were prepared as shown in scheme 1. Peptidyl alcohols were synthesized by using DCC coupling of amino alcohols with Z-Phe-OH in THF solution (yield: **9a**: 45%; **9b**: 53%; **9c**: 34%; **9d**: 34%)[19]. The varying O-alkyl groups in N-acyl tyrosinol were introduced by using alkyl iodides in the presence of potassium carbonate in acetone (yield: **9e**: 37%; **9f**: 54%). In the process of oxidation, sulfur trioxide pyridine complex[20] oxidated the peptidyl alcohols to their corresponding aldehydes much more effectively than the other oxidation reagents such as DMSO-oxalyl chloride[21] or PCC[22] (yield: **10a**: 48%; **10b**: 98%; **10c**: 95%; **10d**: 90%; **10e**: 77%; **10f**: 95%).

Scheme 1



Reagents: a : Z-PheOH, DCC, THF; *b* : Pyridine - SO_3 / Et_3N, DMSO - CH_2Cl_2; *c* : MeI or BuI / K_2CO_3, Acetone.

Table I: Inhibition of cathepsin B and L by dipeptidyl aldehyde derivatives and leupeptin.

	IC_{50} (nM)			B / L
	calpain II	cathepsin L	cathepsin B	
(1)Z-Phe-Phe-H	104	0.74	69.7	94.2
(2)Z-Phe-Tyr-H	184	0.85	85.1	100
(3)Z-Phe-Leu-H	-	0.78	17.7	22.7
(4)Z-Phe-Ala-H	-	15.5	45.1	2.91
(5)Z-Phe-Tyr(Me)-H	104	2.95	97.7	33.1
(6)Z-Phe-Tyr(Bu)-H	79.6	6.96	836	120
Ac-Leu-Leu-Arg-H (Leupeptin)	-	70.3	117	1.66

Results and Discussion

The synthesized dipeptidyl aldehydes, Z-Phe-X-H [X represents Phe, Tyr, Tyr(Me), Tyr(Bu), Ala, Leu] were tested for their inhibitory activity on several cysteine proteases and α–chymotripsin, one of serine proteases (by the method of Barrett and Kirschke[23]). These compounds showed no inhibition for α-chymotrypsin (IC_{50}>1000ng/ml), while they markedly exhibited inhibition for cathepsin B, L, and calpain. As shown in table I, these compounds exhibited stronger inhibition for cathepsin L than leupeptin which is one of the natural occurring peptidyl aldehyde cysteine protease inhibitor. The selectivity for cathepsin L was indicated by the values of B/L which means the ratio of IC_{50} for each cathepsin. Cathepsins are widely distributed in the living body and non-selective inhibitors for cathepsins will bring unfavorable side effects when used clinically. The selectivity for cathepsin L was affected by the amino acid residue at P1 position. The order of selectivity for cathepsin L was Tyr>Phe>Leu>Ala at P1 position. As for inhibitory potency, compounds having Tyr, Phe and Leu at P1 site showed equivalently potent inhibition for cathepsin L, while the compound with Ala at P1 site showed weaker inhibition. Although Z-Phe-Tyr(Bu)-H has the highest B/L value, inhibitory potency of O-Me and O-Bu derivatives for cathepsin L was 5-10 times lower than that of Z-Phe-Tyr-H. From these data, it is suggested that the P1 residue takes part in the selectivity and the potency for cathepsin L inhibition and aromatic amino acids such as Tyr and Phe at P1 site increase the selectivity for cathepsin L. Furthermore, alkylation of hydroxyl group of Tyr resulted in marked loss of inhibitory activity.

The effects of these dipeptidyl aldehydes on bone resorption *in vitro* were confirmed. All these inhibitors suppressed PTH-stimulated ^{45}Ca release in organ culture of chick calvaria (by the modified method of Saito *et al*[24]) by about 50% at 10 μg/ml. Furthermore, they completely suppressed isolated osteoclastic pit formation (by the method of McSheehy and Chambers[25]) at the concentration of 1 μg/ml. Intraperitoneal administration of the Z-Phe-Tyr-H for 4 weeks recovered the bone weight loss of ovariectomyzed mice, a model of osteoporosis, in a dose dependent manner (2.5-10mg/kg). The effects of these inhibitors on bone resorption *in vitro* and *in vivo* will be presented in detail later. Those suppressive activity for bone resorption described above are considered to be resulted from the inhibition of bone collagen degradation by these inhibitors. From these data we can conclude that cathepsin L selective inhibitors, dipeptidyl aldehyde derivatives will be good candidates for new medicines for osteoporosis.

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