Design and Synthesis of Carboxylate Inhibitors for Matrix Metalloproteinases

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A series of carboxylate compounds were prepared from N^{α}-substituted 2,3-diaminopropionic acid and were tested for efficacy as matrix metalloproteinase (MMP) inhibitors. During modeling of the initial compound 10a, we utilized three-dimensional structure modeling software (InsightII/Discover Ver. 2.98). Some of the prepared carboxylate derivatives, such as carbamate compounds (12c, d, 22) and sulfonamide compounds (14b, c), proved to be effective MMP-1 inhibitors (with IC_{50} values of a 10^{-6} M order), depending on the substituent at the N^{α}-position of 2,3-diaminopropionic acid. Some of them were also evaluated for inhibition of stromelysin-1 (MMP-3), and the sulfonamide compound 14c exceeded the lead compound 5b in its MMP-3 inhibitory potency. For the carbamate compounds, we investigated the minimum molecular size at which the MMP-1 inhibitory potency was maintained, and found that this was P_3–P_1' compound 10b.

Key words human fibroblast collagenase; computerized drug design; inhibitor; carboxylate; stromelysin-1

Matrix metalloproteinases (MMPs) are zinc endometallopeptidases that are involved in the degradation and remodeling of connective tissues. This family of enzymes shows proteolytic activity towards virtually all of the constituents of the extracellular matrix. The members of this family, currently numbering 20, can be classified into four groups, which are the collagenases that cleave triple-helical interstitial collagen; the gelatinases that cleave denatured collagen, elastin, and type IV and V collagen; the stromelysins that mainly cleave proteoglycans; and the membrane-type MMPs that have a C-terminal transmembrane domain for anchoring to the cell membrane. The MMPs are involved in crucial physiological and physiopathological events, such as wound healing, nerve growth, angiogenesis, and pregnancy. In these physiological processes, MMP activity is tightly regulated.[1—5] However, excessive MMP synthesis and release can lead to connective tissue degradation and destruction, which occurs in tumor invasion, metastasis,[6] corneal ulceration,[7] arthritis disease,[8a] periodontal disease,[9] and multiple sclerosis.[10] For example, increased levels of fibroblast collagenase (MMP-1) and stromelysin-1 (MMP-3) have been observed in the cartilage and synovium of patients with rheumatoid arthritis and osteoarthritis, and are correlated with the severity of the disease.[8b] Therefore, MMPs inhibitors may have a potent therapeutic effect on various proteolytic diseases.

Many MMP inhibitors have been synthesized and developed. Several compounds are currently undergoing advanced clinical trials, such as batimastat (**1**),[11] marimastat (**2**),[12] trocade (**3**),[13] and AG3340 (**4**)[14] (Fig. 1). These inhibitors mimic the sequence of the substrate–cleavage site of MMPs and have hydroxamate as a zinc-chelating group (ZCG). This kind of design has been widely applied for other MMPs inhibitors.

Previously, we also developed a series of peptidyl hydroxamates as MMP-1 inhibitors (Fig. 2),[15] our compounds mimicked the P_4–P_1 substrate rather than the P_1–P_1' or P_1–P_2' sites in the above-mentioned MMP inhibitors (**1**)—(**4**). Inhibitors that mimic the P_n' site have been studied extensively but little is known about P_n site-mimicking inhibitors like our compounds because of their only modest inhibitory potency (IC_{50} values in the order of 10^{-6} M for MMP-1). However, we thought that this type of inhibitor could show further improvement of its inhibitory activity, since hydroxamate[15] and phosphorous[16] have been examined as ZCGs.

Design Beside hydroxamate and phosphorous, the ZCGs utilized most frequently are thiol or carboxylate. The free thiols are unstable to oxidation in air at neutral pH during preparation and storage.[17] To avoid the disadvantage, we selected a carboxylate, which was expected to be relatively stable and to show good pharmacokinetics, as the ZCG. Although the carboxylate is a weaker ZCG than the others for MMP-1,[5] we designed the 2,3-diaminopropionic acid derivative (**6**) to compensate for this weakness. The amino acid moiety was expected to chelate the active site zinc ion by

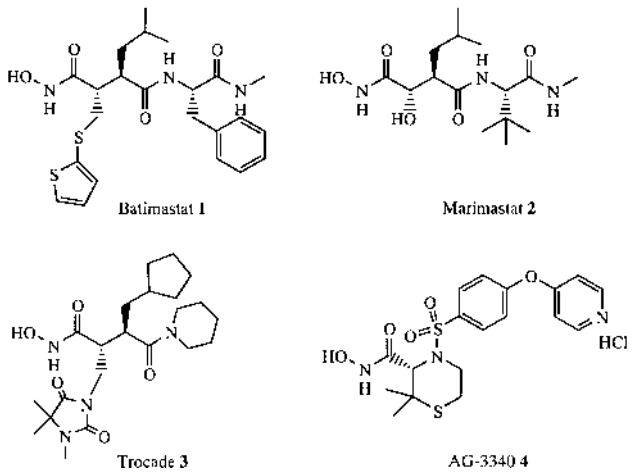
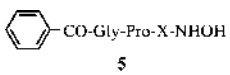


Fig. 1. Selected MMP Inhibitors



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5a; X = Leu-Ala,
5b; X = D-Leu-D-Ala

Fig. 2. Structures of P_4–P_1 Substrate Mimicked Inhibitors That Possess Hydroxamate as ZCG

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both amino and carboxylate groups in a similar manner as the copper complex of amino acid (Fig. 3: in peptide chemistry, the complex is used to selectively protect the lysine residue). However, the 2,3-diaminopropionic acid derivative (**6**) showed no inhibitory activity against MMP-1. This unexpected result led us to perform molecular modeling of compound **6** after it was docked with MMP-1. No report of X-ray crystallographic studies have yet been reported on a complex between the P_n site of mimicking inhibitors and MMP-1. There is only one report about X-ray crystallography between neutrophil collagenase (MMP-8) and Pro–Leu–Gly–NHOH[18] which also mimicked P_n site of collagen cleavage site, but exhibited weaker MMP-1 inhibitory potency than **5a**.[15] However, the sequence homology of the catalytic domain is high between MMP-1 and MMP-8, so both three-dimensional structures are very similar. Therefore, we generated a three-dimensional structured model of a MMP-1 : compound **6** complex based on the crystal structure of MMP-1 (PDB entry: 1CGL) and the structure of a complex between MMP-8 and Pro–Leu–Gly–NHOH (PDB entry: 1JAN) using InsightII/Discover Ver2.98 (Molecular Simulations Inc., San Diego, CA, U.S.A.) (The details of the procedure are described in the experimental section). Certainly, Pro–Leu–Gly–NHOH, well explored P_n site-mimicking inhibitor, was fit into the MMP-1 active site and confirmed that the hydroxamate was bound in a bidentate manner to the active site zinc ion on this software. Before generation of MMP-1 : compound **6** complex, we checked binding feature of MMP-1 : compound **5a** complex. Each amino acid residues were well accommodated to corresponding subsites in a manner similar to the case of Pro–Leu–Gly–NHOH. And N-terminal Bz–Gly–moiety was fit into a shallow hydrophobic pocket consisting of Phe185, Pro189, Ile191, and Gly192. Binding features observed in the model of the compound **6** docked with MMP-1 are represented in Fig. 4 with the interatomic distances for important polar interactions. As a result, the only carboxylate of compound **6** was coordinated with the active site zinc ion in a bidentate manner, while the amino group formed a hydrogen bond with Ala 182 and did not interact with the zinc ion. Comparison with the model of a potent initial inhibitor (**5a**) docked with MMP-1 showed that the hydrogen bond between the carbonyl at the leucine in compound **6** and Ala 184 in MMP-1 was completely absent. In the case of compound **6**, loss of this hydrogen bond might decrease its affinity for MMP-1. This model could indicate that a hydrophobic pocket, consisting of the residues Leu 181, Val 215, and Tyr 240, is located in the vicinity of the N-terminal group of compound **6**. It may be presumed that substitution at the amino group might create a new hydrophobic interaction with this pocket. This hypothesis prompted us to measure the MMP-1 inhibitory activity of intermediate (**10a**) and it is noteworthy that compound **10a** showed potent MMP-1 inhibitory activity (the IC_{50} value was 5.0 μM). Discovery of this novel binding mode led us to explore further N-substitutents with improved inhibitory potency. We prepared compounds with five different bond structures, that is, compounds with carbamate (**12a**—**e**), amide (**13a**—**f**), sulfonamide (**14a**—**c**), alkylamino (**15a**, **b**), and urea (**16**) bonds (Chart 1). In order to determine the smallest molecule exhibiting potent MMP-1 inhibitory activity, the peptide was truncated along the sequence from its N-terminal based on compound **10a**. Furthermore, to assess the interaction between the S_1 pocket of the enzyme and the methyl group, a 2,3-diaminobutanoic acid derivative (**22**) was also designed.

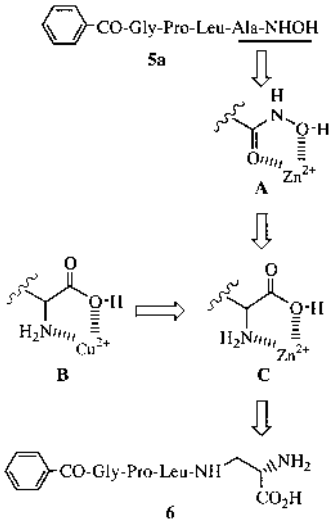


Fig. 3. The Design of α-Amino Acid Type Inhibitor

Synthesis Preparation of the carboxylate compounds is summarized in Charts 1 and 2. The key intermediate 2,3-diaminopropionic acid (**8**) was prepared from Boc–Ser–OMe under Mitsunobu conditions[19] or from Boc–Asn–OMe with iodosobenzene diacetate.[20] Using *N*-ethyl *N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)/1-hydroxy-

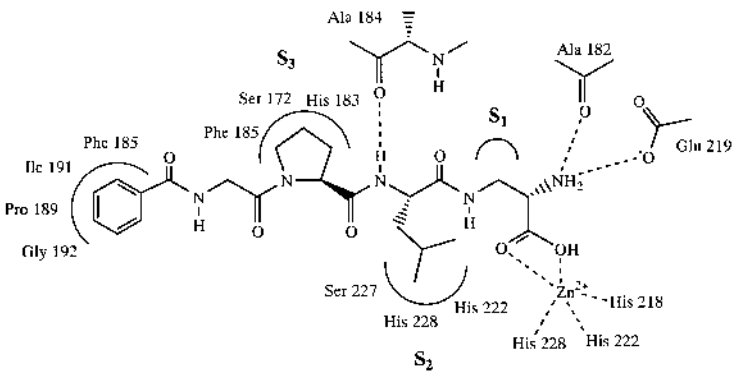
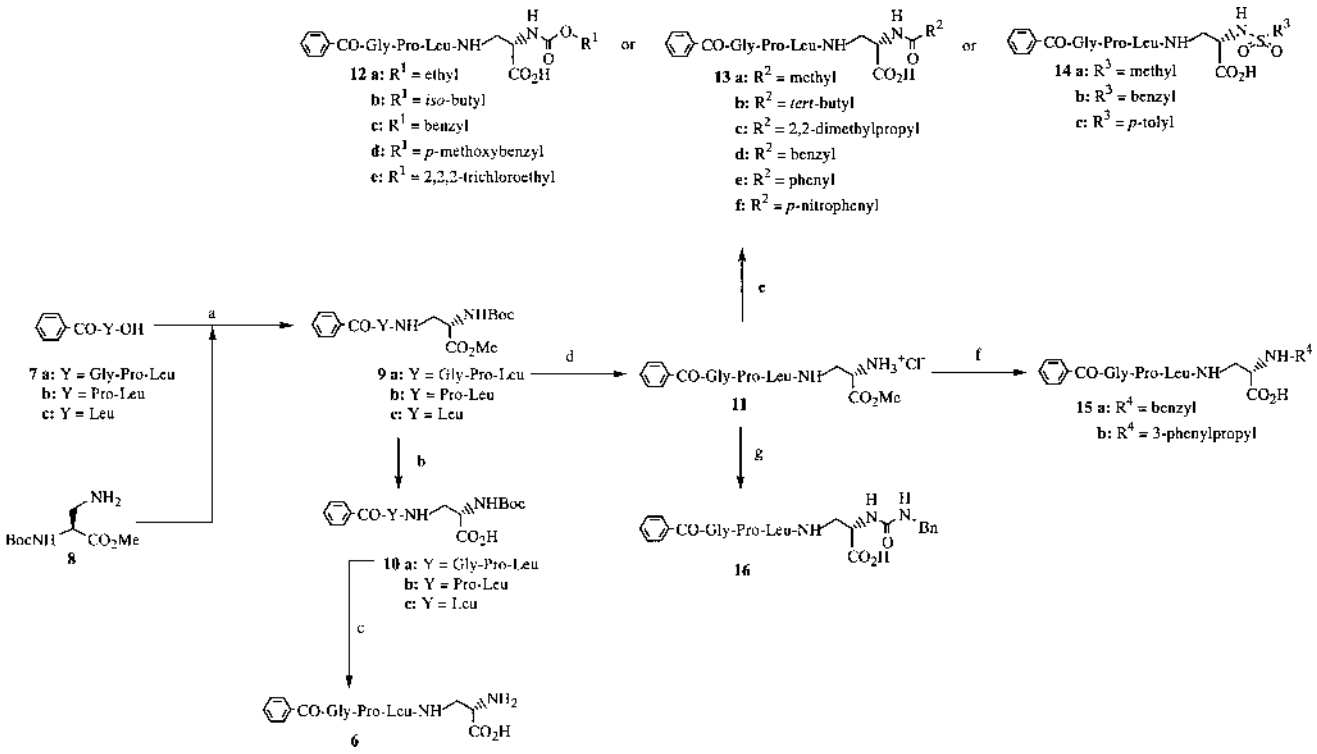


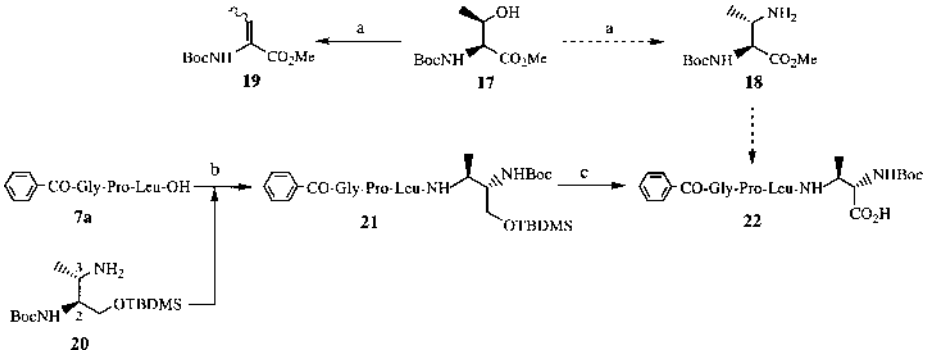
Fig. 4. Schematic Representation of the Docking Simulation of MMP-1 : Compound **6** Complex

Hydrogen bonds are shown with dotted line.



Reagent: (a) EDC/HOBt; (b) aq. NaOH/MeOH; (c) (1) 4 N HCl/EtOAc (2) neutralization; (d) 4 N HCl/EtOAc; (e) (1) R^1O–CO–Cl, R^2–CO–Cl or R^3–SO_2–Cl (2) aq. NaOH/MeOH; (f) (1) R^4–CHO (2) H_2/Pd–C (3) aq. NaOH/MeOH; (g) (1) CDI/Et_3N/Bn–NH_2 (2) aq. NaOH/MeOH.

Chart 1. Synthesis of 2,3-Diaminopropionic Acid Derivatives



Reagent: (a) (1) Ph_3P/diethyl azodicarboxylate/HN_3 (2) H_2/Pd-C; (b) EDC/HOBt; (c) (1) 70% aq. AcOH (2) pyridinium dichromate.

Chart 2. Synthesis of 2,3-Diaminobutanoic Acid Derivative

benzotriazol (HOBt) as the coupling reagent, a fully protected peptide (**9a**) was obtained from compound **7** and **8** after easy work-up. The original amino acid compound **6** was obtained by saponification of the ester in **9a**, followed by treatment with 4 N hydrogen chloride in ethyl acetate (4 N HCl in EtOAc). Truncated compounds, **10b** and **10c**, were also prepared in the same manner as **10a**. The amino group at the α position of the carboxylate provided a functional handle onto which various moieties were introduced, as follows. The Boc group in the fully protected compound **9a** was removed using 4 N HCl in EtOAc. Then the residue was treated with various alkyloxycarbonyl chlorides (for **12a**—**e**), acyl chlorides (for **13a**—**f**), or alkylsulfonyl chlorides (for **14a**—**c**) in the presence of Et_3N, followed by saponification of the methyl ester. *N*-Alkylation of **11** was performed by reductive amination with benzaldehyde or 3-phenylpropionaldehyde, followed by saponification of the methyl ester to give **15a** and **15b**. The substituted ureido compound **16** was prepared as follows. Compound **11** was treated with 1,1′-carbonyldiimidazole (CDI)/Et_3N, followed by reaction with benzylamine and then saponification to the carboxylate was accomplished under the same conditions described above.

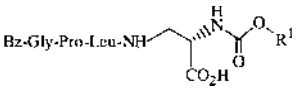
For the preparation of compound **22**, we initially examined the Mitsunobu reaction with Boc–Thr–OMe (**17**) to obtain the protected 2,3-diaminobutanoic acid (**18**), but α,β-unsaturated dehydro amino acid (**19**) was obtained as the sole product (Chart 2). In order to avoid dehydration, N^2,*O*-diprotected 2(*S*),3(*S*)-diaminobutanol (**20**) was prepared from Boc–Thr–OH according to the procedure described by Nakamura *et al.*[21] After condensation of **20** and tripeptide (**7a**)

by the EDC–HOBt condensation method, the *tert*-butyldimethylsilyl group was removed by treatment with 70% aqeous AcOH, followed by oxidation with pyridinium dichromate to give the a desired 2,3-diaminobutylic acid derivative (**22**).

Results and Discussion

The carbamate compounds, **10a** and **12a**—**e**, showed variable of MMP-1 inhibitory activity depending on the size of the substituent (Table 1). The ethyl-substituted compound **12a** exhibited no inhibitory activity, but the compounds with bulkier groups, such as an iso-butyl group (**12b**), showed moderate inhibitory activity (an IC_{50} of 29 μM). The compound with a *tert*-butyl group (**10a**) showed nearly equipotent inhibitory activity compared with the original hydroxamate (**5a**, IC_{50} of 7.7 μM). Likewise, the aromatic ring compounds (**12c**, **d**) exhibited inhibitory activity for MMP-1 in the order of 10^{-6} M. However, the bulkiest substituent of 2,2,2-trichloroethyl group (**12e**), caused a small decrease in activity. According to these results, the S_1' pocket of MMP-1 seemed to be well occupied by *tert*-butyl or *p*-methoxybenzyl groups, while the ethyl group was too small. In order to verify the MMP-1 model, we assessed compound **10a** was docked into MMP-1, and the binding features are shown in Figs. 5 and 6. Coordination of the carboxylate group on the inhibitor with the catalytic zinc atom was observed, and the hydrogen bond between the carbonyl at leucine and Ala 184 was regained. In MMP-1, the lateral wall of the S_1' subsite mainly consists of three amino acids (Leu 181,Val 215, and Tyr 240) and the floor of the pocket is formed by an Arg 214 residue. The *tert*-butyl group was accommodated in this pocket, but did not fully occupy it. Since there was further space between the pocket floorboard and the tip of the *tert*-butyl group, additional elongation of the substituent at the tip of this group might lead to an additional hydrophobic interaction with the S_1' subsite. Replacement of the carbamate group with an amide group led to a dramatic decrease of potency, and all the amide compounds (**13a**—**f**) had no inhibitory activity (0% at 10^{-3} M). Also, inhibitory activity was not observed with alkylamino compounds (**15a**, **b**). Probably, the substituents of the amino groups in amide or alkylamino compounds were not positioned in the S_1' pocket. The difference of potency may be accounted for by the carbamate oxygen atom forming a hydrogen bond with the enzyme and this bond directing the hydrophobic substituent properly the S_1' pocket, but a reasonable explanation of the difference was not obtained from our computerized docking study. The sulfonamide compounds (**14a**—**c**) showed significant inhibition like that observed with the carbamate compounds (Table 2). Although a small substituent methyl group (**14a**) did not generate any inhibitory potency, an aromatic ring substituent (**14b**, **c**) showed inhibitory activity as potent as that of carba-

Table 1. Inhibition of MMP-1 by Carbamate Type Compounds **12**[a)]



Compounds	R^1	Inhibitory activity (IC_{50}, μM)
12a	Ethyl	4% (10^{-4} M)
12b	Iso-butyl	29
10a	*tert*-Butyl	5.0
12c	Benzyl	2.6
12d	*p*-Methoxybenzyl	2.2
12e	2,2,2-Trichloroethyl	28

a) Standard deviations for enzyme assays were typically ±10% of the mean or less.

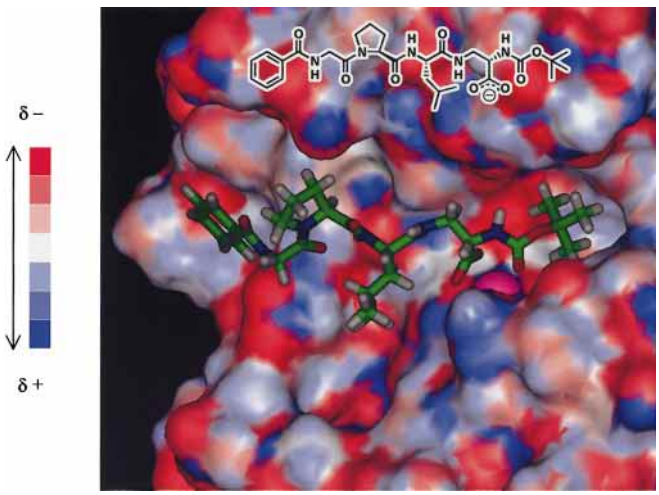


Fig. 5. Expanded View of the MMP-1 : Compound **10a** Complex

Compound **10a** shown as a liquorice model, binds to the MMP-1 active site shown as a Connolly molecular surface of MMP-1. The MMP-1 surface color is ranked according to the electrostatic potential on the MMP-1 surface. As shown with a stacked color bar in the figure, the negative/positive electrostatic potential, the deeper it is colored red/blue. A pink sphere almost buried in the MM-1 surface is a zinc ion coordinated in the MMP-1 active site. The figure was generated by Insight II.

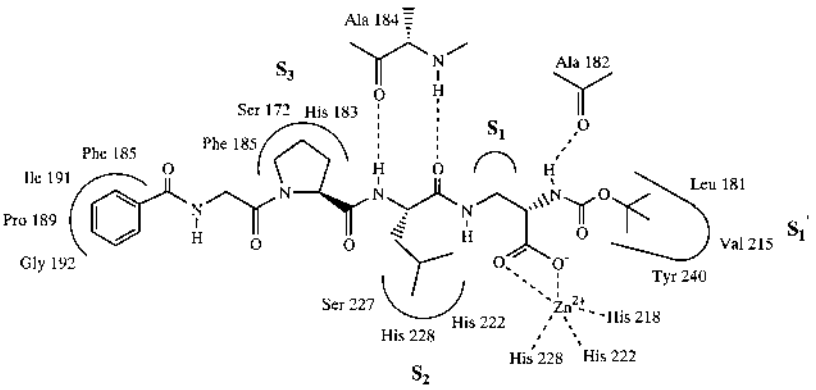


Fig. 6. Schematic Representation of the Docking Simulation of MMP-1 : Compound **10a** Complex

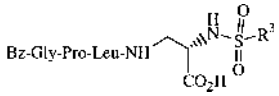
Hydrogen bonds are shown with dotted line.

mate compound **12c**. As described for the carbamate and sulfonamide compounds, a benzyl substituent at the N^{α} position promoted inhibition, so a ureido compound incorporating a benzyl group (**16**) was prepared. However, **16** only exhibited moderate inhibitory activity (IC_{50}=60 μM). Installation of the methyl group at the P_1 position (described as compound **22**) did not cause a dramatic shift of potency compared with that of **10a** (IC_{50}=5.0, 2.4 μM, respectively). This observation as supported by our computerized docking study, in which the installed methyl group was exposed to the solvent and not oriented towards the enzyme.

Comparison between the truncated peptides (**10b**, **c**) and the original peptide (**10a**) showed that loss of the Gly residue (**10b**) represented only caused a 3-fold decrease in inhibition of MMP-1 compared with **10a**, but a shorter peptide with deletion of the Gly–Pro sequence (**10c**) showed complete loss of inhibitory activity (Table 3). Based on these results and the lack of activity of compound **6**, it seems that both the Boc group and the pyrrolidine ring of proline could have a critical role in recognition of the enzyme. The benzoyl group also contributed to increasing the affinity for MMP-1.

For some of the carboxylate compounds that showed micromolar potencies against MMP-1 *i.e.*, **10a**, **12c**, **14c**, and **22**, MMP-3 inhibitory activity was also measured (Table 4). Although the carbamate compounds (**10a**, **12c**, **22**) showed about 25-fold less potency for inhibition of MMP-3 than MMP-1, **14c** exhibited 10 μM potency for MMP-3 (only a 4-fold decrease in potency compared with its inhibition of MMP-1) and was 16-fold more potent than the original hydroxamate compound **5b**. Comparison of these carboxylate compounds and **5b** suggested that the P_1' substituent was important for inhibition of MMP-3, with the sulfonamide structure being especially appropriate.

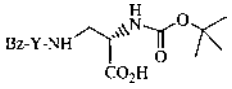
Table 2. Inhibition of MMP-1 by Sulfonamide Type Compounds **14**[a)]



Compounds	R^3	Inhibitory activity (IC_{50}, μM)
14a	Methyl	0% (10^{-5} M)
14b	Benzyl	4.7
14c	*p*-Tolyl	2.5

a) Standard deviations for enzyme assays were typically ±10% of the mean or less.

Table 3. Inhibition of MMP-1 by Truncated Carbamate Type Compounds **10**[a)]



Compounds	Y	Inhibitory activity (IC_{50}, μM)
10a	Gly–Pro–Leu	5.0
10b	Pro–Leu	17
10c	Leu	0% (10^{-3} M)

a) Standard deviations for enzyme assays were typically ±10% of the mean or less.

Conclusion

In summary, we have discovered non-hydroxamate MMP inhibitors with a carboxylate ZCG that show no decrease of inhibitory activity compared with the lead compounds **5**. During construction of the carboxylate compounds, we generated a three-dimensional model of an MMP-1: compound **6** complex based on the crystal structure of MMP-1 and the complex of MMP-8 with an inhibitor (Pro–Leu–Gly–NHOH) using computerized docking. We found structurally new MMP inhibitors, such as carbamate compound (**10a**, **12c**, **12d**, **22**) and sulfonamide compound (**14b**, **c**), which were effective against MMP-1 with IC_{50} values in the order of 10^{-6} M. Compound **10a** showed that important features for the inhibitory potency were both the Boc group and the pyrrolidine ring of proline. With respect to MMP-3 inhibitory potency, compound **14c** showed stronger inhibitory activity compared with the lead compounds **5**.

Because hydroxamates are often unstable and there is a risk of chronic toxicity arising from metabolic degradation to hydroxylamine, our carboxylate compounds may be safe and have better pharmacokinetics. Further chemical and pharmacological evaluation of this series will be reported in due course and it is hoped that these potent MMPs inhibitors will aid in the treatment of various enzymatic diseases.

Experimental

Model Building The atomic coordinates of the crystal structure of MMP-1 and MMP-8 complexed with an inhibitor (Pro–Leu–Gly–NHOH) were retrieved from the RCSB Protein Data Bank[22)] (PDB entries: 1CGL[23)] and 1JAN,[24)] respectively). To form the S_2 pocket of MMP-1, Asp186 residue was moved outside of the pocket in a similar manner to Gln165 of MMP-8. Thus, Pro–Leu–Gly–NHOH could be accommodated in the MMP-1 active site. Then the three-dimensional structure of compound **5a** was con-

Table 4. Inhibition of MMP-3 by Carboxylate Compounds[a)]

Compounds	Inhibitory activity (IC_{50}, μM)
5b	160
10a	128
22	57
12d	65
14c	10

a) Standard deviations for enzyme assays were typically ±10% of the mean or less.

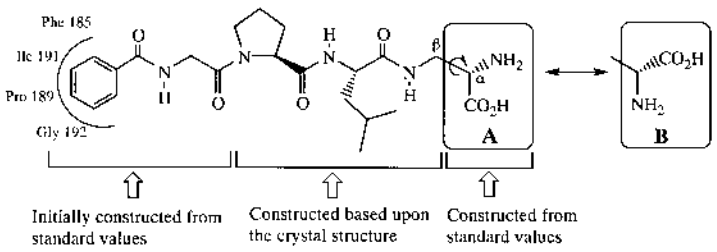


Fig. 7. Construction of Compound **6** Structure

structed based upon Pro–Leu–Gly–NHOH to check the binding feature and decide the three-dimensional structure of Bz–Gly–Pro–Leu–NH–CH moiety, which is common structure of compound **6**. The Bz–Gly– moiety was initially constructed from standard values (bond length, bond angle, and torsion angle) followed by searching for a pocket that could accommodate an aromatic ring by altering all of the torsion angles except for the amide bond. As a result, we found that the aromatic ring of the Bz–Gly– moiety could fit into a hydrophobic pocket consisting of Phe185, Pro189, Ile191, and Gly192. Eventually, based upon the structure of **5a**, three-dimensional structure of compound **6** was constructed. The amino acid moiety was also constructed utilizing standard values (bond length, bond angle, and torsion angle). For modeling of the amino acid moiety, two conformations were assessed based on Cα–Cβ free rotation, which are described as conformers A and B in Fig. 7. After docking of conformer B into MMP-1, strong electrostatic repulsion could be expected between both the carboxylate of the amino acid moiety and the Glu 219 residue, resulting in a failure of docking simulation. For this reason, we chose conformer A as the docking model and then carried out energy minimization using Discover (Ver 2.98, Molecular Simulations Inc., San Diego, CA, U.S.A.). Minimization was preformed by 300 steps of the steepest descent, followed by 3000 steps of the VA09A method to remove steric clashes.

Chemistry Melting points were determined on a Yanagimoto melting point apparatus without correction. IR spectra were taken with a PERKIN ELMER PARAGON 1000 FT-IR spectrometer. Column chromatography was performed on Silica gel BW-200 (Fuji Silysia Chemical Ltd.). TLC was performed on silica gel (Silica gel 60 F_{254}, Merck). *Rf* values refer to the following v/v solvent system: Rf_1, $CHCl_3$–MeOH–AcOH (5 : 2 : 1); Rf_2, $CHCl_3$–MeOH–AcOH (80 : 10 : 5); Rf_3, $CHCl_3$–MeOH (10 : 1); Rf_4, $CHCl_3$–MeOH (20 : 1). ^{1}H-NMR was recorded on a JEOL FX90A or JEOL JMN-AL300 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane (TMS), which was used as the internal standard. Mass spectra were obtained on a JEOL JMS 700 spectrometer by electrospray ionization (ESI) or fast atom bombardment (FAB) ionization techniques. Optical rotations were measured in a JASCO DIP-140 apparatus.

General Procedure for the Preparation of Compounds (9) EDC (1.1 eq of **7**) was added to a mixture of 3-amino-2(*S*)-(*tert*-butyloxycarbonyl-amino)propionic acid methyl ester (**8**) (1.05 eq of **7**), HOBt (1.05 eq of **7**), compound **7**, and *N*,*N*-dimethylformamide (DMF) (4—7% v/w) at −12 °C. The mixture was stirred at −3 °C for 6 h then 0 °C for 15 h. The reaction mixture was evaporated employing a rotary evaporator and a vacuum pump. The residue was dissolved in EtOAc, washed with 1 N aq. HCl, sat. aq. NaCl, 10% aq. Na_2CO_3 and sat. aq. NaCl successively, dried ($MgSO_4$), evaporated *in vacuo* to give a crude product, which was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH = 50 : 1) and precipitated from Et_2O to give **9** as colorless powder.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*tert*-butyloxycarbonylamino)propionic Acid Methyl Ester (**9a**): Yield: 67%. mp; 176—181 °C, $[\alpha]_D^{25}$ −65.8° (*c* = 1.0, MeOH), Rf_4 0.17. IR (neat) cm^{-1}: 3332, 3070, 2954, 2872, 1747, 1708, 1690, 1654. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m), 1.1—1.9 (14H, m), 1.9—2.4 (2H, m), 3.1—3.4 (2H, m), 3.4—3.8 (5H, m), 3.9—4.6 (5H, m), 6.16 (1H, br d, *J* = 8.0 Hz), 6.46 (1H, m), 6.67 (1H, br d, *J* = 8.0 Hz), 6.94 (1H, br d, *J* = 7.9 Hz), 7.4—7.6 (3H, m), 7.7—8.1 (2H, m). *Anal.* Calcd for $C_{29}H_{43}N_5O_8$: C, 59.07; H, 7.35; N, 11.88. Found: C, 59.23; H, 7.31; N, 11.69.

3-(N^{α}-Benzoyl-prolyl-leucyl-amino)-2(*S*)-(*tert*-butyloxycarbonylamino)-propionic Acid Methyl Ester (**9b**): Yield: 63%. mp; 77—79 °C, $[\alpha]_D^{25}$ −87.5° (*c* = 1.04, MeOH), Rf_3 0.31. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.90 (6H, m), 1.40 (9H, s), 1.5—1.6 (2H, m), 1.78 (1H, m), 1.92 (1H, m), 2.03 (1H, m), 2.21 (1H, m), 2.37 (1H, m), 3.4—3.6 (3H, m), 3.70 (3H, s), 3.8—3.9 (1H, m), 4.30 (2H, m), 4.75 (1H, m), 6.03 (1H, d, *J* = 7.9 Hz), 6.89 (1H, d, *J* = 7.5 Hz), 7.00 (1H, m), 7.4—7.5 (3H, m), 7.56 (2H, m). *Anal.* Calcd for $C_{27}H_{40}N_4O_7$: C, 60.88; H, 7.57; N, 10.52. Found: C, 60.62; H, 7.73; N, 10.51.

3-(N^{α}-Benzoyl-leucyl-amino)-2(*S*)-(*tert*-butyloxycarbonylamino)propionic Acid Methyl Ester (**9c**): Yield: 39%. mp; 69—71 °C, $[\alpha]_D^{25}$ −16.9° (*c* = 1.15, MeOH), Rf_4 0.40. IR (neat) cm^{-1}: 3304, 3072, 2957, 2870, 1751, 1718, 1647, 1604, 1578, 1540. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.96 (6H, m), 1.42 (9H, s), 1.6—1.8 (3H, m), 3.63 (2H, m), 3.70 (3H, s), 4.40 (1H, m), 4.64 (1H, m), 5.59 (1H, d, *J* = 7.7 Hz), 6.74 (1H, d, *J* = 8.1 Hz), 7.4—7.54 (3H, m), 7.80 (2H, m). *Anal.* Calcd for $C_{22}H_{33}N_3O_6 \cdot 3/4H_2O$: C, 58.85; H, 7.74; N, 9.36. Found: C, 58.78; H, 7.80; N, 9.26.

General Procedure for the Preparation of Compounds (10) Two normal (2 N) aq. NaOH (3 eq of **9**) was added to a solution of **9** in MeOH (5—7% v/w) at 0 °C and stirred at room temperature for 1 h. The reaction mixture was neutralized by adding 1 N aq. HCl and MeOH was removed by evaporation. The resultant solution was adjusted to pH 2 by adding 1 N aq. HCl at 0 °C. The organic material was extracted with EtOAc, washed with sat. aq. NaCl, dried ($MgSO_4$) and evaporated *in vacuo*. The resultant residue was precipitated from Et_2O to give the title compound as colorless powder.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*tert*-butyloxycarbonyl-amino)propionic Acid (**10a**): Yield: 95%. mp; 169—172 °C, $[\alpha]_D^{25}$ −109° (*c* = 1.08, MeOH), Rf_1 0.45. Rf_2 0.29. IR (neat) cm^{-1}: 3327, 3066, 2958, 2878, 1719, 1655. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m), 1.0—1.9 (14H, m), 1.9—2.4 (2H, m), 3.1—3.4 (2H, m), 3.4—3.8 (2H, m), 3.9—4.6 (5H, m), 6.16 (1H, br d, *J* = 8.1 Hz), 6.44 (1H, br d, *J* = 8.5 Hz), 6.63 (1H, br d, *J* = 8.6 Hz), 6.73 (1H, br d, *J* = 9.2 Hz), 7.3—7.6 (3H, m), 7.8—8.0 (2H, m). FAB-MS *m*/*z*: 598 $(M+Na)^+$, 576 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{41}N_5O_8 \cdot 1/2H_2O$: C, 57.52; H, 7.24; N, 11.98. Found: C, 57.34; H, 7.28; N, 12.19.

3-(N^{α}-Benzoyl-prolyl-leucyl-amino)-2(*S*)-(*tert*-butyloxycarbonylamino)-propionic Acid (**10b**): Yield: 97%. mp; 110—112 °C, $[\alpha]_D^{25}$ −90° (*c* = 1.24, MeOH), Rf_1 0.56. IR (neat) cm^{-1}: 3280, 3079, 2959, 1727, 1686, 1647, 1560. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.89 (6H, m), 1.40 (9H, s), 1.64 (3H, m), 1.86 (1H, m), 2.03 (1H, m), 2.22 (2H, m), 3.4—3.7 (3H, m), 3.79 (1H, m), 4.2—4.6 (3H, m), 4.72 (1H, t, *J* = 6.8 Hz), 6.07 (1H, d, *J* = 7.2 Hz), 7.2—7.5 (4H, m), 7.55 (2H, m). *Anal.* Calcd for $C_{26}H_{38}N_4O_7 \cdot 1/2H_2O$: C, 58.20; H, 7.33; N, 10.44. Found: C, 58.14; H, 7.48; N, 10.35.

3-(N^{α}-Benzoyl-leucyl-amino)-2(*S*)-(*tert*-butyloxycarbonylamino)propionic Acid (**10c**): Yield: 82%. mp; 127—130 °C, $[\alpha]_D^{25}$ −15.8° (*c* = 1.0, MeOH), Rf_2 0.60. ^{1}H-NMR (CD_3OD, TMS) δ: 0.97 (6H, m), 1.42 (9H, s), 1.6—1.8 (3H, m), 3.41 (1H, dd, *J* = 8.1, 13.8 Hz), 3.71 (1H, dd, *J* = 4.5, 13.8 Hz), 4.26 (1H, m), 4.61 (1H, m), 7.4—7.6 (3H, m), 7.84 (2H, m). *Anal.* Calcd for $C_{21}H_{31}N_3O_6$: C, 59.84; H, 7.41; N, 9.97. Found: C, 59.80; H, 7.45; N, 9.92.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-aminopropionic Acid (6) Compound **10a** (254 mg, 0.441 mmol) was treated with 4 N HCl in EtOAc (10 ml) at 0 °C for 1 h. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in H_2O (10 ml). The solution was adjusted to pH 5 by adding 1 N aq. NaOH then concentrated to *ca.* 2 ml to give crystals, which were collected by filtration, washed with H_2O and dried to afford 140 mg of **6** (96%) as a colorless solid. mp 216—219 °C (dec.), $[\alpha]_D^{25}$ −95.6° (*c* = 1.0, 0.1 N aq. NaOH), Rf_1 0.39. IR (neat) cm^{-1}: 3362, 3096, 2956, 2869, 2360, 2057, 1680, 1594, 1549. ^{1}H-NMR [D_2O+NaOD, 3-(trimethylsilyl)propionic 2,2,3,3-d_4 acid, sodiun salt] δ: 0.88 (3H, d, *J* = 5.1 Hz), 0.93 (3H, d, *J* = 5.3 Hz), 1.6—1.9 (3H, m), 1.9—2.4 (4H, m), 3.2—3.4 (2H, m), 3.6—3.8 (2H, m), 4.2—4.4 (2H, m), 4.45 (1H, m), 4.7—5.0 (m), 7.5—7.9 (5H, m). *Anal.* Calcd for $C_{23}H_{33}N_5O_6 \cdot H_2O$: C, 56.01; H, 7.15; N, 14.20. Found: C, 56.26; H, 7.32; N, 13.93.

General Procedure for the Preparation of Compounds (12)—(14) Compound **9a** was treated with cold 4 N HCl in EtOAc (4% v/w) for 0.5—1.5 h. The reaction mixture was evaporated *in vacuo* and the residue was precipitated from Et_2O, which were collected by filtration, dried under reduced pressure to give hydrochloride salt (**11**) as quantitative yield. To a solution of **11** in DMF (4—7% v/w), Et_3N (2 eq of **11**) and appropriate acyl chloride or sulfonyl chloride (1.05—1.1 eq of **11**) were added successively. The mixture was stirred at room temperature overnight. Then the reaction mixture was diluted with EtOAc, washed with sat. aq. NaCl, water, 10% aq. Na_2CO_3 and sat. aq. NaCl successively, dried ($MgSO_4$) then evaporated *in vacuo*. The residue was dissolved in MeOH (7—10% v/w) and 2 N aq. NaOH (2 eq of acylated compound) was added at 0 °C, then the mixture was stirred for 1—2 h. The reaction mixture was acidified by adding 1 N aq. HCl (pH = 2—3), MeOH was removed by evaporation, extracted with EtOAc, dried ($MgSO_4$) and evaporated *in vacuo*. The residue was precipitated from Et_2O and reprecipitated from appropriate solvent system to give **12**—**14**.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(ethyloxycarbonyl-amino)propionic Acid (**12a**, R^1 = Ethyl): Yield: 62% (reprecipitated from THF–*n*-hexane). mp 105—107 °C, $[\alpha]_D^{25}$ −62.6° (*c* = 1.03, MeOH), Rf_1 0.38. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.75—1.0 (6H, m) 1.13 (3H, t, *J* = 7.1 Hz), 1.5—1.8 (3H, m), 1.9—2.3 (4H, m), 3.30 (2H, m), 3.5—3.8 (2H, m), 3.8—4.3 (5H, m), 4.4—4.6 (2H, m), 6.79 (1H, d, *J* = 7.0 Hz), 7.18 (2H, m), 7.3—7.5 (3H, m), 7.82 (2H, m), 8.23 (1H, m). *Anal.* Calcd for $C_{26}H_{37}N_5O_8 \cdot 1/3H_2O$: C, 56.41; H, 6.86; N, 12.65. Found: C, 56.59; H, 7.00; N, 12.43.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(iso-butyloxycarbonyl-amino)propionic Acid (**12b**, R^1 = Iso-butyl): Yield: 70% (reprecipitated from THF–*n*-hexane). mp 134—137 °C, $[\alpha]_D^{25}$ −72.6° (*c* = 1.01, MeOH), Rf_1 0.42. IR (neat) cm^{-1}: 3312, 3072, 2962, 1746, 1700, 1654, 1558. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (12H, m) 1.4—1.9 (4H, m), 2.0—2.4 (4H, m), 3.25 (2H, m), 3.6—3.9 (4H, m), 3.9—4.3 (3H, m), 4.4—4.6 (2H, m), 6.51

(1H, m), 6.75 (1H, m), 7.04 (1H, m), 7.4—7.55 (3H, m), 7.62 (1H, m), 7.8—7.95 (2H, m). *Anal.* Calcd for $C_{28}H_{41}N_5O_8 \cdot 3/4H_2O$: C, 57.08; H, 7.27; N, 11.89. Found: C, 56.92; H, 7.28; N, 12.10.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(benzyloxycarbonylamino)propionic Acid (**12c**, R^1=Benzyl): Yield: 69% (reprecipitated from MeOH–Et_2O). mp 214—216 °C, $[\alpha]_D^{25}$ −107° (*c*=0.98, MeOH), Rf_1 0.51, Rf_2 0.29. IR (neat) cm^{-1}: 3317, 3066, 2954, 2875, 1735, 1662, 1560. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m) 1.5—1.85 (3H, m), 1.9—2.4 (4H, m), 3.4—3.7 (2H, m), 3.7—4.3 (3H, m), 4.48 (2H, m), 4.97 (2H, m), 6.87 (1H, d, *J*=6.6 Hz), 7.78 (4H, m), 7.1—7.6 (7H, m), 8.15 (1H, m). FAB-MS *m/z* 632 $(M+Na)^+$, 610 $(M+H)^+$. *Anal.* Calcd for $C_{31}H_{39}N_5O_8$: C, 61.07; H, 6.45; N, 11.49. Found: C, 61.14; H, 6.67; N, 11.43.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*p*-methoxybenzyloxycarbonylamino)propionic Acid (**12d**, R^1=*p*-Methoxybenzyl): Yield: 47% (precipitated from EtOAc–Et_2O). mp 194—196 °C, $[\alpha]_D^{25}$ −54.3° (*c*=1.02, MeOH), Rf_1 0.49, Rf_2 0.27. IR (neat) cm^{-1}: 3285, 3068, 2960, 1740, 1696, 1654, 1559, 1254, 1031. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m) 1.45—1.85 (3H, m), 1.9—2.3 (4H, m), 3.29 (2H, m), 3.61 (2H, m), 3.79 (3H, s), 4.0—4.2 (3H, m), 4.50 (2H, m), 4.88 (2H, m), 6.84 (3H, m), 7.1—7.5 (7H, m), 7.79 (2H, m), 8.12 (1H, m). *Anal.* Calcd for $C_{32}H_{41}N_5O_9$: C, 59.25; H, 6.53; N, 10.80. Found: C, 59.38; H, 6.51; N, 10.67.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(2,2,2-trichloroethyloxycarbonyl-amino)propionic Acid (**12e**, R^1=2,2,2-Trichloroethyl): Yield: 61% (reprecipitated from THF–*n*-hexane). mp 119—122 °C, $[\alpha]_D^{25}$ −60.3° (*c*=1.04, MeOH), Rf_1 0.36. IR (neat) cm^{-1}: 3302, 3062, 2957, 2877, 1734, 1654, 1542, 818. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.88 (3H, d, *J*=6.1 Hz) 0.95 (3H, d, *J*=6.1 Hz), 1.5—1.8 (3H, m), 1.9—2.4 (4H, m), 3.37 (2H, m), 3.5—3.8 (2H, m), 4.07 (1H, m), 4.26 (2H, m), 4.48 (2H, m), 4.61 (2H, m), 7.10 (1H, m), 7.23 (m), 7.3—7.6 (4H, m), 7.89 (2H, m), 8.04 (1H, m). *Anal.* Calcd for $C_{26}H_{34}Cl_3N_5O_8 \cdot H_2O$: C, 46.68; H, 5.42; N, 10.47. Found: C, 46.41; H, 5.53; N, 10.54.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(acetylamino)propionic Acid (**13a**, R^2=Methyl): Yield: 67% (reprecipitated from EtOAc–*n*-hexane). mp 89—92 °C, $[\alpha]_D^{25}$ −57.1° (*c*=1.0, MeOH), Rf_1 0.16. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m), 1.1—1.8 (6H, m), 1.9—2.3 (4H, m), 3.30 (2H, m), 3.61 (2H, m), 4.0—4.2 (3H, m), 4.42 (2H, m), 6.80 (1H, d, *J*=7.0 Hz), 7.2—7.5 (5H, m), 7.75 (2H, m), 8.06 (1H, m). *Anal.* Calcd for $C_{25}H_{35}N_5O_7$: C, 58.01; H, 6.82; N, 13.53. Found: C, 58.26; H, 6.79; N, 13.38.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*tert*-butylcarbonylamino)propionic Acid (**13b**, R^2=*tert*-Butyl): Yield: 31% (reprecipitated from THF–Et_2O). mp 142—145 °C, $[\alpha]_D^{25}$ −80.2° (*c*=1.03, MeOH), Rf_1 0.36. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.84 (3H, d, *J*=5.2 Hz), 0.93 (3H, d, *J*=5.4 Hz), 1.23 (9H, s), 1.5—1.8 (3H, m), 2.0—2.3 (4H, m), 3.41 (2H, m), 3.70 (2H, m), 4.10 (1H, m), 4.25 (1H, m), 4.3—4.6 (3H, m), 7.03 (1H, m), 7.20 (1H, m), 7.3—7.6 (4H, m), 7.80 (2H, m), 8.01 (1H, m). *Anal.* Calcd for $C_{28}H_{41}N_5O_7 \cdot 1/4H_2O$: C, 59.61; H, 7.41; N, 12.41. Found: C, 59.64; H, 7.66; N, 12.19.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*tert*-butylacetylamino)propionic Acid (**13c**, R^2=2,2-Dimethylpropyl): Yield: 52% (reprecipitated from THF–Et_2O). mp 119—121 °C, $[\alpha]_D^{25}$ −57.5° (*c*=1.01, MeOH), Rf_1 0.37. IR (neat) cm^{-1}: 3318, 3064, 2956, 2872, 1734, 1654, 1544. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.1 (15H, m), 1.58 (1H, m), 1.78 (2H, m), 1.9—2.4 (6H, m), 3.43 (2H, m), 3.74 (2H, m), 4.23 (1H, m), 4.26 (1H, m), 4.3—4.6 (3H, m), 7.10 (1H, m), 7.20 (1H, m), 7.3—7.5 (4H, m), 7.84 (2H, m), 7.93 (1H, m). FAB-MS *m/z*: 596 $(M+Na)^+$, 574 $(M+H)^+$. *Anal.* Calcd for $C_{29}H_{43}N_5O_7 \cdot 3/4H_2O$: C, 59.32; H, 7.64; N, 11.93. Found: C, 59.53; H, 7.59; N, 11.76.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(benzylcarbonylamino)propionic Acid (**13d**, R^2=Benzyl): Yield: 52% (recrystallized from EtOAc–Et_2O). mp 156—159 °C, $[\alpha]_D^{25}$ −92.8° (*c*=1.05, MeOH), Rf_1 0.55. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m), 1.5—1.8 (3H, m), 2.0—2.3 (4H, m), 3.4—3.7 (6H, m), 3.88 (1H, m), 4.17 (1H, m), 4.3—4.55 (2H, m), 4.54 (1H, m), 7.01 (1H, m), 7.20 (1H, m), 7.3—7.6 (9H, m), 7.86 (2H, m), 8.02 (1H, m). *Anal.* Calcd for $C_{31}H_{39}N_5O_7$: C, 62.72; H, 6.62; N, 11.80. Found: C, 62.84; H, 6.70; N, 11.69.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(benzoylamino)propionic Acid (**13e**, R^2=Phenyl): Yield: 51% (reprecipitated from THF–Et_2O). mp 188—190 °C, $[\alpha]_D^{25}$ −59.0° (*c*=1.15, MeOH), Rf_2 0.59. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m), 1.5—1.8 (3H, m), 1.9—2.3 (4H, m), 3.58 (2H, m), 3.64 (2H, m), 3.90 (1H, m), 4.15 (1H, m), 4.3—4.45 (2H, m), 4.56 (1H, m), 6.10 (1H, m), 6.72 (1H, m), 7.3—7.5 (9H, m), 7.7—7.8 (2H, m), 8.0 (1H, m). *Anal.* Calcd for $C_{30}H_{37}N_5O_7 \cdot 1/2H_2O$: C, 61.25; H, 6.51; N, 11.90. Found: C, 61.14; H, 6.59; N, 12.16.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*p*-nitrobenzoylamino)-propionic Acid (**13f**, R^2=*p*-Nitrophenyl): Yield: 55% (recrystallized from EtOAc–Et_2O). mp 132—135 °C, $[\alpha]_D^{25}$ −52.1° (*c*=1.01, MeOH), Rf_1 0.39. IR (neat) cm^{-1}: 3322, 3075, 2961, 2874, 1734, 1654, 1541. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.92 (3H, d, *J*=5.9 Hz), 1.02 (3H, d, *J*=6.1 Hz), 1.59 (1H, m), 1.76 (2H, m), 1.8—2.5 (4H, m), 3.43 (2H, m), 3.73 (2H, m), 4.23 (1H, m), 4.26 (1H, m), 4.5—4.3 (2H, m), 4.58 (2H, m), 7.01 (1H, m), 7.18 (2H, m), 7.50 (2H, m), 7.87 (4H, m), 8.22 (2H, m), 8.70 (1H, m), 8.89 (1H, d, *J*=4.2 Hz). FAB-MS *m/z*: 647 $(M+Na)^+$, 625 $(M+H)^+$. *Anal.* Calcd for $C_{30}H_{36}N_6O_9$: C, 57.68; H, 5.81; N, 13.45. Found: C, 57.82; H, 5.88; N, 13.21.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(methansulfonylamino)-propionic Acid (**14a**, R^3=Methyl): Yield: 47% (reprecipitated from EtOAc–Et_2O). mp 253—254 °C, Rf_1 0.18. IR (neat) cm^{-1}: 3439, 3368, 3319, 3280, 2959, 1744, 1696, 1654. ^{1}H-NMR (DMSO-d_6, TMS) δ: 0.7—0.9 (6H, m), 1.4—1.7 (3H, m), 1.7—2.1 (4H, m), 2.89 (3H, s), 3.1—3.7 (m), 3.9—4.25 (4H, m), 4.35 (1H, m), 7.4—7.6 (4H, m), 7.78 (1H, m), 7.8—8.0 (2H, m), 8.32 (1H, m), 8.66 (1H, m). *Anal.* Calcd for $C_{24}H_{35}N_5O_8S$: C, 52.07; H, 6.37; N, 12.65. Found: C, 52.24; H, 6.20; N, 12.69.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(benzylsulfonylamino)-propionic Acid (**14b**, R^3=Benzyl): Yield: 51% (reprecipitated from EtOAc–Et_2O). mp 193—194 °C, $[\alpha]_D^{25}$ −52.7° (*c*=1.02, MeOH), Rf_1 0.46, Rf_2 0.25. IR (neat) cm^{-1}: 3318, 3065, 2958, 2875, 1735, 1654, 1542, 1332, 1157. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.7—1.0 (6H, m), 1.4—1.8 (3H, m), 1.9—2.4 (4H, m), 3.1—3.4 (2H, m), 3.63 (2H, m), 4.02 (1H, m), 4.19 (2H, m), 4.31 (2H, s), 4.51 (2H, m), 6.71 (1H, d, *J*=8.8 Hz), 7.02 (1H, m), 7.3—7.6 (9H, m), 7.84 (2H, m), 8.0 (1H, m). FAB-MS *m/z*: 652 $(M+Na)^+$, 630 $(M+H)^+$. *Anal.* Calcd for $C_{30}H_{39}N_5O_8S \cdot 1/2H_2O$: C, 56.41; H, 6.31; N, 10.96. Found: C, 56.36; H, 6.58; N, 10.69.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*p*-toluenesulfonyl-amino)propionic Acid (**14c**, R^3=*p*-Tolyl): Yield: 29% (reprecipitated from EtOAc–Et_2O). mp 120—122 °C, $[\alpha]_D^{25}$ −101° (*c*=0.60, MeOH), Rf_1 0.47, Rf_2 0.26. IR (neat) cm^{-1}: 3310, 3072, 2954, 1735, 1671, 1654, 1542, 1338, 1165. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.8—1.0 (6H, m), 1.5—1.8 (3H, m), 1.9—2.3 (4H, m), 2.44 (3H, s), 3.58 (2H, m), 3.62 (2H, m), 3.90 (1H, m), 4.15 (1H, m), 4.3—4.45 (2H, m), 4.56 (1H, m), 6.04 (1H, m), 6.71 (1H, m), 7.3—7.5 (6H, m), 7.7—7.8 (4H, m), 8.42 (1H, m). FAB-MS *m/z*: 653 $(M+Na)^+$, 631 $(M+H)^+$. *Anal.* Calcd for $C_{30}H_{39}N_5O_8S \cdot 3/4H_2O$: C, 56.02; H, 6.35; N, 10.89. Found: C, 56.17; H, 6.39; N, 10.74.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(benzylamino)propionic Acid (15a, R^4=Benzyl) $NaBH_4$ (31 mg, 0.494 mmol) was added to a mixture of **11** (260 mg, 0.494 mmol), benzaldehyde (50 ml, 0.494 mmol) and MeOH (3 ml) at 0 °C. After stirring for 16 h at room temperature, the reaction mixture was evaporated *in vacuo*, diluted with EtOAc, washed with water and sat. aq. NaCl, dried ($MgSO_4$), then evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH=20 : 1) to give methyl ester of the title compound as a white waxen oil (257 mg, 90%). The methyl ester was saponified by the same method used for preparation of **12** and precipitated from EtOAc–Et_2O to give 130 mg of **15** (55%) as colorless powder. mp 128—131 °C, $[\alpha]_D^{25}$ −64.0° (*c*=1.03, MeOH), Rf_1 0.39. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.87 (3H, d, *J*=6.2 Hz), 0.95 (3H, d, *J*=6.2 Hz), 1.5—1.8 (3H, m), 2.0—2.3 (4H, m), 3.4—3.75 (6H, m), 3.88 (1H, m), 4.17 (1H, m), 4.3—4.45 (2H, m), 4.54 (1H, m), 7.01 (1H, br d, *J*=8.6 Hz), 7.20 (1H, br d, *J*=8.5 Hz), 7.3—7.6 (9H, m), 7.86 (2H, m), 8.02 (1H, d, *J*=8.1 Hz). *Anal.* Calcd for $C_{30}H_{39}N_5O_6 \cdot 1/2H_2O$: C, 62.70; H, 7.02; N, 12.19. Found: C, 62.92; H, 7.09; N, 11.99.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-[(3-phenylpropyl)-amino]propionic Acid (15b, R^4=3-Phenylpropyl) The title compound was prepared from **11** and 3-phenylpropionaldehyde by the same method used for **15** (R^4=benzyl). Yield: 43%. mp 182—187 °C (dec.), $[\alpha]_D^{25}$ −56.9° (*c*=1.0, MeOH), Rf_1 0.44. IR (neat) cm^{-1}: 3348, 2959, 2872, 1684, 1654, 1550. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.88 (3H, d, *J*=6.1 Hz), 0.96 (3H, d, *J*=6.1 Hz), 1.5—1.8 (3H, m), 1.8—2.3 (6H, m), 2.7—3.0 (4H, m), 3.33 (2H, m), 3.59 (2H, m), 4.02 (1H, m), 4.13 (2H, m), 4.38 (2H, m), 7.02 (2H, m), 7.1—7.5 (9H, m), 7.59 (1H, br d, *J*=8.6 Hz), 7.90 (2H, m). FAB-MS *m/z* 616 $(M+Na)^+$, 594 $(M+H)^+$. *Anal.* Calcd for $C_{32}H_{43}N_5O_6 \cdot 3/2H_2O$: C, 61.92; H, 7.47; N, 11.28. Found: C, 62.26; H, 7.59; N, 11.21.

3-(N^{α}-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-*N'*-benzylureidopropionic Acid (16) To a mixture of **11** (260 mg, 0.5 mmol) , Et_3N (70 μl, 0.5 mmol), and THF (10 ml), CDI (97 mg, 0.6 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 2 h. Then benzylamine (55 μl, 0.5 mmol) was added and stirred overnight at the same temperature. The reaction mixture was diluted with EtOAc and washed with 1 N aq. HCl, sat. aq. NaCl, 10% aq. $NaHCO_3$, and sat. aq. NaCl successively, dried ($MgSO_4$), then evaporated *in vacuo*. The residue was purified by silica gel

column chromatography (eluent; $CHCl_3$: MeOH=20 : 1). The methyl ester of the title compound was isolated as a colorless oil (230 mg, 77%). 2 N aq. NaOH (0.5 ml, 1 mmol) was added to a solution of methyl ester (220 mg, 0.37 mmol) in acetone (3 ml) and stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and washed with 1 N aq. HCl, sat. aq. NaCl, 10% aq. $NaHCO_3$, and sat. aq. NaCl successively, dried ($MgSO_4$), then evaporated *in vacuo*. The residue was precipitated from EtOH–Et_2O to give **16** as a white powder (100 mg, 47%). mp 152—154 °C (dec.), $[\alpha]_D^{25}$ −59.2° (c=1.02, MeOH), Rf_1 0.41. IR (neat) cm^{-1}: 3280, 2954, 1735, 1663, 1560. ^{1}H-NMR ($CDCl_3$+MeOH-d_4, TMS) δ: 0.8—1.0 (6H, m) 1.4—1.8 (3H, m), 2.0—2.2 (4H, m), 3.56 (2H, m), 3.6—3.9 (2H, m), 4.0—4.5 (7H, m), 6.07 (1H, m), 6.19 (1H, m), 6.45 (1H, m), 7.1—7.6 (9H, m), 7.79 (2H, m), 8.14 (1H, m). *Anal.* Calcd for $C_{31}H_40N_6O_7\cdot H_2O$: C, 59.41; H, 6.75; N, 13.41. Found: C, 59.40; H, 6.83; N, 13.34.

3(*S*)-(*N*$^{\alpha}$-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-*tert*-(butyloxycarbonylamino)-*O*-*tert*-butyldimethylsilylbutanol (21) Compound **21** was prepared from Bz–Gly–Pro–Leu–OH (**7a**) and 3(*S*)-amino-2(*S*)-(*tert*-butyloxycarbonylamino)-*O*-*tert*-butyldimethylsilylbutanol (**20**)[20] by the same method used for preparation of **9a** to give 950 mg of **21** (69%) as a colorless solid. mp 120—121 °C, $[\alpha]_D^{25}$ −61.7° (c=0.575, MeOH), Rf_4 0.25. IR (neat) cm^{-1}: 3296, 2959, 2873, 1696, 1662, 1550. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.7—0.9 (19H, m), 1.36 (9H, s), 1.4—1.7 (3H, m), 1.9—2.3 (4H, m), 3.2—3.9 (5H, m), 3.9—4.6 (5H, m), 4.86 (1H, br d, J=8.0 Hz), 6.92 (2H, m), 7.2—7.5 (4H, m), 7.6—7.8 (2H, m). *Anal.* Calcd for $C_{35}H_{59}N_5O_7Si\cdot 4/3H_2O$: C, 58.89; H, 8.71; N, 9.81. Found: C, 58.98; H, 8.60; N, 9.74.

3(*S*)-(*N*$^{\alpha}$-Benzoyl-glycyl-prolyl-leucyl-amino)-2(*S*)-(*tert*-butyloxycarbonylamino)butanoic Acid (22) A mixture of **21** (950 mg, 1.37 mmol) and 70% aq. AcOH (20 ml) was stirred at room temperature for 4 h. The reaction mixture was lyophilized and the residue was precipitated from Et_2O to give 750 mg of primary alcohol intermediate (95%) as a colorless powder. To a solution of this intermediate (350 mg, 0.594 mmol) in DMF (4 ml), pyridinium dichromate (1.36 g, 6.31 mmol) was added and stirred at room temperature for 16 h. The reaction mixture was diluted with EtOAc (30 ml) and washed with sat. aq. NaCl, 1 N aq. HCl and sat. aq. NaCl successively, dried ($MgSO_4$), then evaporated *in vacuo*. The residue was precipitated from EtOAc–Et_2O to give 215 mg of **22** (62%) as a pale green powder. mp 201—203 °C, $[\alpha]_D^{25}$ −140° (c=0.96, MeOH), Rf_1 0.41. IR (neat) cm^{-1}: 3400, 3290, 3065, 2976, 1736, 1702, 1656. ^{1}H-NMR ($CDCl_3$+MeOH-d_4, TMS) δ: 0.89 (3H, d, J=6.1 Hz), 0.94 (3H, d, J=6.1 Hz), 1.10 (3H, d, J=6.6 Hz), 1.4—1.7 (12H, m), 1.9—2.3 (4H, m), 3.25 (1H, m), 3.61 (2H, m), 4.0—4.2 (3H, m), 4.3—4.6 (2H, m), 6.21 (1H, br d, J=8.1 Hz), 6.46 (1H, br d, J=8.5 Hz), 6.63 (1H, br d, J=8.6 Hz), 6.94 (1H, br d, J=8.5 Hz), 7.3—7.6 (3H, m), 7.6—7.8 (2H, m). FAB-MS m/z 612 $(M+Na)^+$, 590 $(M+H)^+$. *Anal.* Calcd for $C_{29}H_{43}N_5O_8\cdot 1/2H_2O$: C, 58.18; H, 7.41; N, 11.70. Found: C, 58.00; H, 7.52; N, 11.96.

MMP-1 Inhibition Assay The test was carried out essentially as in Nagai *et al.*[25] In a microtube, 20 μl of proMMP-1 [15 U/ml an assay buffer [50 mM Tris–HCl (pH 7.5), 0.2 M NaCl, 5 mM $CaCl_2$] containing 0.02% BSA] was mixed with 20 μl of 2 mM 4-aminophenylmercuric acetate (APMA) in an assay buffer. And the mixture was incubated at 35 °C for 2 h to activate the proenzyme. A 20 μl aliquot of inhibitor at varying concentrations of inhibitor plus 40 μl of an assay buffer were added to the mixture and incubated further 15 min at 35 °C: In a case of thiol possessing compounds, to ensure that inhibitors remain unoxidized, a 1×10^{-4} M β-mercaptoethanol supplemented with an assay buffer was used. Then 100 μl of 0.05% FITC-labeled collagen in an assay buffer was added, followed by incubation at 35 °C for 2 h. Then the reaction was terminated by adding 10 μl of 80 mM *o*-phenanthroline in 50% ethanol solution. A 200 μl of aliquot of elastase (25 μg/ml assay buffer) was added and the mixture was incubated for 10 min at 35 °C to digest the cleaved FITC-labeled collagen fragment. To the reaction mixture, 400 μl of 70% ethanol in 0.17 M Tris–HCl (pH 9.5)/0.67 M NaCl was added and vortexed for 30 s, followed by centrifugation for 20 min at 3000 rpm to obtain its supernatant fluid. Fluoresence intensity of such a supernatant was measured with (λ_{ex} 495 nm, λ_{em} 520 nm). Inhibitory activity was defined as a percentage of control activity in the absence of inhibitor.

MMP-3 Inhibition Assay In a microtube, 20 μl of proMMP-3 [1 U/ml an assay buffer [50 mM Tris–HCl (pH 7.8), 5 mM $CaCl_2$] containing 0.02% BSA] was mixed with 20 μl of 2 plasmin (20 μg/ml assay buffer). And the mixture was incubated at 37 °C for 2 h to activate the proenzyme, followed by adding 10 μl of 15 mM aq. diisopropyl fluorophosphate to quench the plasmin activity. A 15 μl aliquot of inhibitor at varying concentrations of inhibitor plus 35 μl of an assay buffer were added to the mixture and incubated further 15 min at 35 °C. Then 50 μl of 0.1% FITC-labeled casein in an assay buffer was added, followed by incubation at 37 °C for 2 h. Then the reaction was terminated by adding 120 μl of 5% aq. TCA and vortexed for 30 s, followed by centrifugation for 20 min at 3000 rpm to obtain its supernatant fluid. Fluoresence intensity of such a supernatant was measured with (λ_{ex} 495 nm, λ_{em} 520 nm). Inhibitory activity was defined as a percentage of control activity in the absence of inhibitor.

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