Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 4, pp 765–778, 1997

PII: S0968-0896(97)00028-X
0968-0896/97 $17.00 + 0.00

Synthesis and Biological Evaluation of Orally Active Matrix Metalloproteinase Inhibitors

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Abstract—The synthesis and biological evaluation of orally active inhibitors of matrix metalloproteinase are reported. Modifications of the P2′ position and the α-substituent of hydroxamic acid derivatives were carried out, and revealed that the P2′ substituent influenced the MMP inhibitory activities in vitro and in plasma after oral administration. The hydroxamates with phenylglycine at the P2′ position were absorbed well orally. Compound **15e**, which exhibited the longest duration of inhibitory activity in plasma after oral administration among the phenylglycine derivatives (**5a–5d**, **15a**, **15c**, **15e**), was evaluated in a rat adjuvant arthritis model. A reduction in hind foot pad swelling and improvements of some inflammatory parameters were demonstrated when the compound was administered orally. These results indicate the potential of MMP inhibitors for rheumatoid arthritis. © 1997 Elsevier Science Ltd.

Introduction

The matrix metalloproteinases (MMP), such as collagenase, stromelysin, and gelatinase, are a family of zinc endopeptidases, and have been implicated in a wide variety of biological processes.[1] For example, increased levels of these enzymes have been observed in the cartilage and synovium of patients with rheumatoid and osteoarthritis and correlate to the severity of the disease.[2] MMP are also involved in cancer metastasis and proliferation.[3] Furthermore, it has been reported recently that an MMP-like enzyme can hydrolyze the membrane-bound precursor of the proinflammatory cytokine TNF-α to yield mature, soluble TNF-α.[4] These results broaden the potential use of MMP inhibitors for diseases not generally considered to be associated with matrix degradation. Thus, MMP inhibitors have become the topic of considerable focus in medicinal chemistry, and the rational designs of low molecular weight MMP inhibitors have recently been reported for the purpose of developing anti-arthritis, anti-tumor drugs, and so on.[5] Those designs were based on two structural features: (1) the amino acid sequence around the glycine–isoleucine and glycine–leucine of the cleavage site in the collagen molecules and (2) a ligand to bind to the active site zinc(II) ion, such as hydroxamic acid, thiol, carboxylic acid, and phosphoric acid. Structure–activity studies of MMP inhibitors have shown that (1) inhibitors having hydroxamic acid at the *N*-terminal end are more potent (right-hand side inhibitor), (2) the selectivity of inhibitors to MMPs is mainly dependent on the P1′ substituent, and (3) a variety of substituents can be accommodated at the P2′ and P3′ positions.[5]

There have been many reports on the synthesis and in vitro inhibitory activities of MMP inhibitors, but only a few papers have described the in vivo effects of MMP inhibitors in animal models,[6] presumably due to their poor bioavailability. The discovery of orally active MMP inhibitors is desirable because most of the potential indication of MMP inhibitors would require chronic therapy.

Another important issue in the development of enzyme inhibitors as therapeutic agents is whether broad-spectrum inhibitors or selective inhibitors represent a superior pharmacological effect. Selective MMP inhibitors against gelatinase or stromelysin have been developed through modification of the P1′ substituent of the hydroxamic acid derivatives.[7] However, we believe that broad-spectrum inhibitors have therapeutic advantages in the treatment of various diseases because many studies have revealed that some MMPs are often co-expressed.[8]

The structure–activity relationship of hydroxamates for in vitro MMP inhibitory activities has been studied and is well documented, but with respect to oral absorption, the activities are not clear. Our strategy to discover new orally active inhibitors is as follows: the P1′ substituent is fixed to an isobutyl group, which has been shown to be the best P1′ substituent for broad-spectrum inhibitors,[5] and modifications of the P2′ substituent and the α-substituent of the hydroxamic acid moiety are examined. Thus far, modifications of the P2′ substituent have been made with natural L-amino acids; unnatural amino acids have rarely been used. Hydrophilic groups,

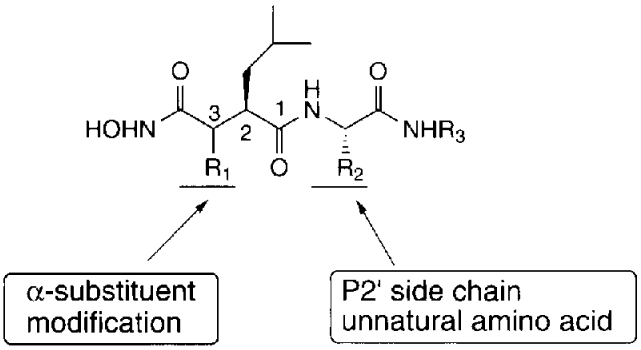


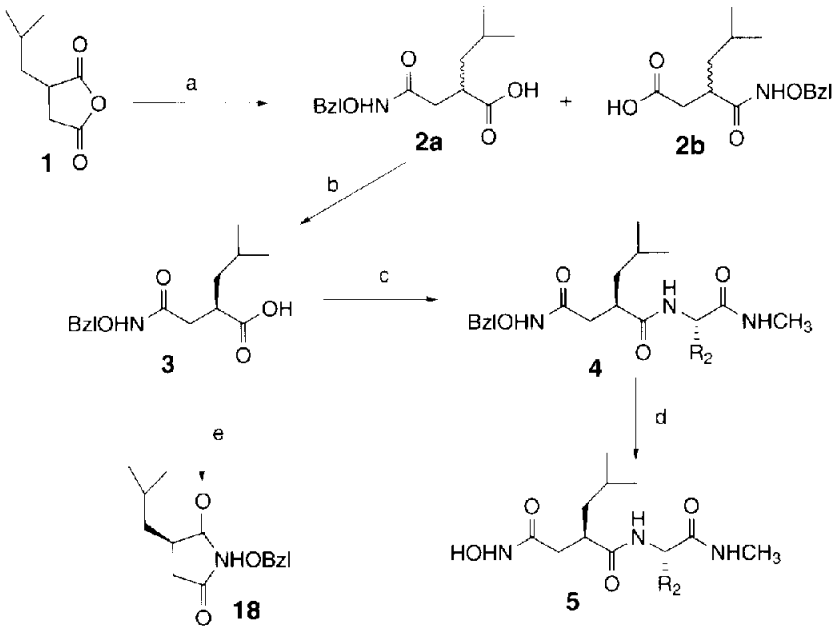
Figure 1. Design of MMP inhibitors.

such as substituted amines are introduced into the α-position in an attempt to increase the water solubility for good oral absorption. This paper reports the preparation of new hydroxamate inhibitors and their biological evaluation, and describes the oral effects against a rat adjuvant arthritis model.

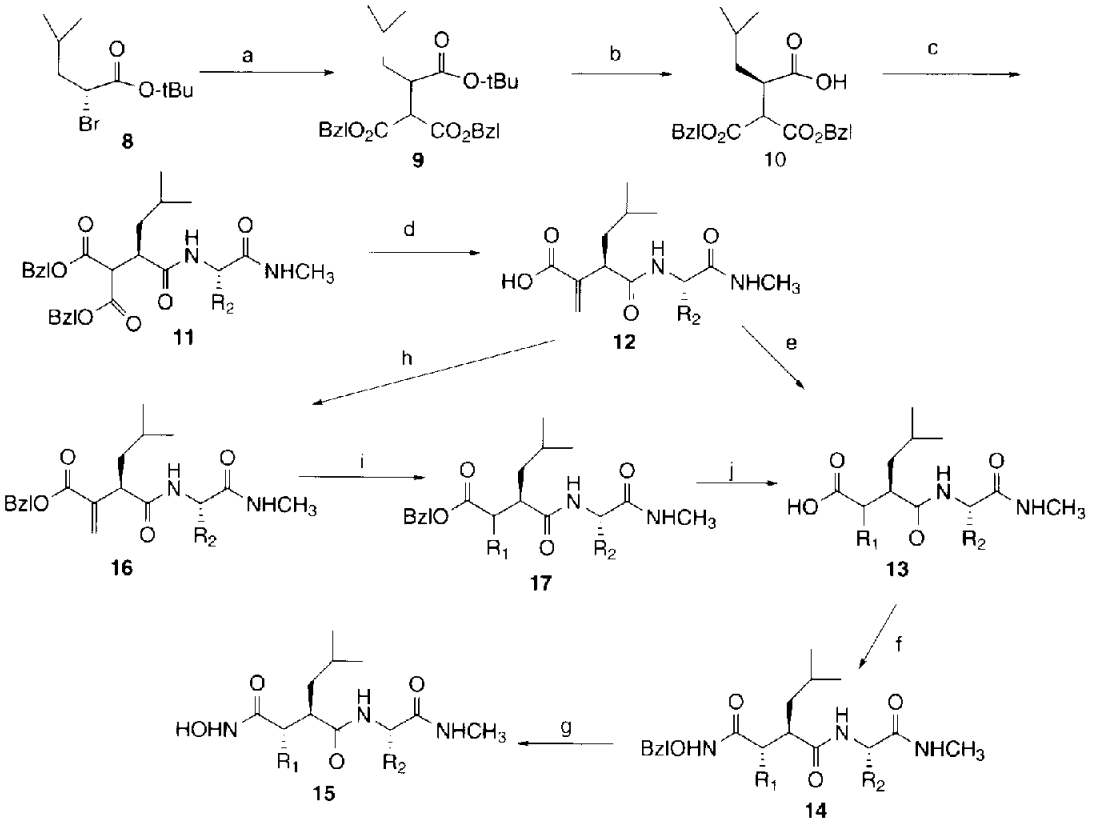
Chemistry

Hydroxamates (**5**) with no substituent at the α-position (the 3-position of the succinic acid) were synthesized by the route shown in Scheme 1. Succinic acid derivative (**3**), the key intermediate, was prepared from 2-isobutylsuccinic anhydride (**1**) with high optical purity.[9] The reaction of anhydride (**1**) with *O*-benzylhydroxylamine gave a separable 2:1 mixture of positionally isomeric *O*-benzylhydroxamates (**2a,b**), with the desired isomer (**2a**) being predominant. The optically active (*R*)-isomer (**3**) was obtained by optical resolution using (+)-phenylethylamine, and then was acylated with various unnatural L-amino acids (**7**) using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (WSC), to give **4** in good yield. When *N*-hydroxybenzotriazol (HOBT) was added in this reaction, no acylated compounds (**4**) were obtained, but instead the succinimide derivative (**18**) was afforded. Deprotection of the *O*-benzylhydroxamate (**4**) with 10% palladium on carbon and hydrogen yielded the hydroxamate derivatives (**5**).

The hydroxamates bearing a substituent at the α-position were prepared as outlined in Scheme 2. *tert*-Butyl (2*S*)-bromo-4-methylpentanoate (**8**), prepared from diazotization of D-leucine followed by esterification, was allowed to react with dibenzyl malonate in the presence of potassium *tert*-butoxide to give tri-ester derivatives (**9**).[10] This reaction did not proceed completely by the S_N2 mechanism to give an isomeric mixture (the *R*:*S* ratio was ca. 3:1, which was determined by HPLC with an optically active column after hydrolysis of the *tert*-butyl ester.). However, after hydrolysis, the racemic acid (**10'**) fortunately precipitated out and the desired (*R*)-acid (**10**) was obtained from the filtrate with high optical purity and good yield. After the condensation of **10** with various amino acid *N*-methylamides (**7**), the obtained di-benzyl ester (**11**) was hydrogenated and treated with formaldehyde and piperidine under Mannich reaction condition to give a methylene derivative (**12**). A conjugated nucleophilic addition of secondary amines or thiols to **12** afforded **13** as a diastereomeric mixture. Fortunately, the major product was the desired diastereomer, because of the selectivity of the nucleophilic addition by steric hindrance of the (*R*)-isobutyl group, as described in the discussion. The condensation of **13** with *O*-benzylhydroxylamine and the following catalytic hydrogenation of **14** gave a mixture of hydroxamates. The major diastereomer **15** was obtained in good purity by recrystallization of the mixture.

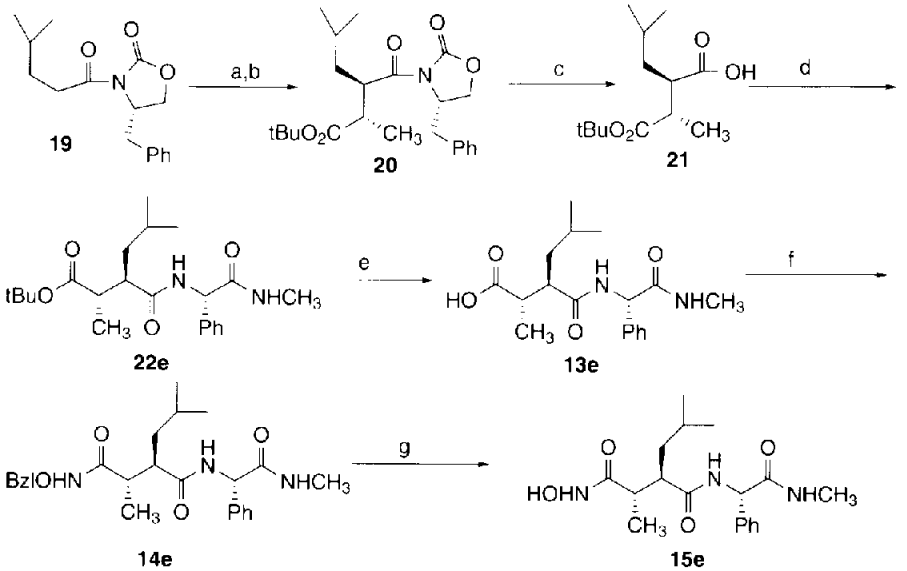


Scheme 1. The synthesis of hydroxamate inhibitors **5**. Reagents and conditions: (a) H_2NOBzl, ether; (b) (i) (*R*)-1-phenylethylamine; (ii) HCl; (c) $H_2NCHR_2CONHCH_3$ (**7**), WSC, DMF; (d) H_2, 10%Pd/C, MeOH; (e) $H_2NCHR_2CONHCH_3$, HOBT, WSC, DMF.



Scheme 2. The synthesis of hydroxamate inhibitors **15**. Reagents and conditions: (a) Dibenzyl malonate, tBuOK, DMF; (b) HCOOH; (c) $H_2NCHR_2CONHCH_3$ (7), WSC, DMF; (d) (i) H_2, 10%Pd/C, or $HCOONH_4$, 10%Pd/C, MeOH; (ii) HCHO, piperidine; (e) nucleophile or H_2, 10%Pd/C; (f) H_2NOBzl, HOBT, WSC, DMF; (g) H_2, 10%Pd/C; (h) benzyl bromide, $NaHCO_3$, DMF; (i) amine; (j) H_2, 10%Pd/C.

After catalytic hydrogenation of **12**, the resultant diastereomeric mixture of **13e** was condensed with *O*-benzylhydroxylamine in a mixture of DMF and dichloromethane to give the major diastereomer **14e** as a precipitate. A minor diastereomer **14f** was isolated by preparative HPLC. Catalytic hydrogenation of **14e** and **14f** gave **15e** and **15f**, respectively. In order to confirm the configuration at the α-position of the main product (**15e**), the hydroxamate **15** with the (*S*)-configuration at the α-position was prepared from (2*R*)-isobutyl-(3*S*)-methylsuccinic acid (**21**), as shown in Scheme **3**.[11] The hydroxamate **15** derived from **21** was identical to the



Scheme 3. The stereoselective synthesis of hydroxamate inhibitors **15e**. Reagents and conditions: (a) (i) diisopropylamine, BuLi, THF; (ii) $CH_3CH(OSO_2CF_3)CO_2Bzl$; (b) (i) H_2, 10%Pd/C; (ii) isobutene, H_2SO_4; (c) 30% H_2O_2, LiOH; (d) $H_2NCHPhCONHCH_3$, WSC, DMF; (e) HCOOH; (f) H_2NOBzl, HOBT, WSC, DMF; (g) H_2, 10%Pd/C.

main product (**15e**) in all respects. This confirmed that the configuration at the α-position of the main product (**15e**) was the (*S*)-configuration.

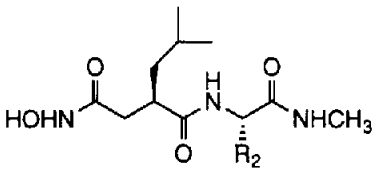
Results and Discussion

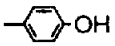
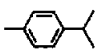
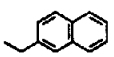
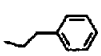
In vitro enzyme inhibitory activities

Hydroxamates (**5a–i**, **15a–i**) were evaluated as inhibitors of MMP-1 (collagenase) and MMP-9 (gelatinase B). The results of their inhibitory activities (IC_{50}) are reported in Tables 1 and 2.

As seen in Tables 1 and 2, all compounds, except for **15f**, show strong inhibitory activities against both enzymes, with IC_{50} values of 10^{-8}–10^{-10} M. All α-substituent groups (R_1) examined here generally increase the activities against MMP-1 and MMP-9, but the morpholinomethyl (**15a**) and (4-methylpiperazino)methyl (**15c**) groups confer no enhancement of activity against MMP-9. The stereochemistry of α-substituent is critical for strong inhibitory activity (**15e** vs **15f**) and (*S*)-configuration is crucial for strong binding to the enzymes. As for the morpholinomethyl (**15a,g**), (1,2,3,4-tetrahydroisoquinolin-2-yl)methyl (**15b,h**) and (4-methylpiperazino)methyl (**15c**) derivatives, the main products were active isomers, and would have the same configuration as the (3*S*)-methyl derivatives (**15e**).

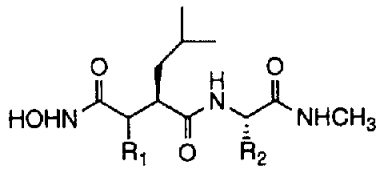
Table 1. In vitro enzyme inhibitory activities

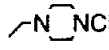
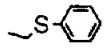


Compound no.	R_2	IC_{50} MMP-1	IC_{50} MMP-9
5a		15 nM	14 nM
5b		8.7 nM	13 nM
5c		22 nM	23 nM
5d		16 nM	13 nM
5e		8.1 nM	4.9 nM
5f		0.35 nM	0.4 nM
5g		2.1 nM	0.47 nM
5h		3.7 nM	3.7 nM
5i		1.1 nM	0.53 nM
BB-94		0.4 nM	0.53 nM

It is interesting that among the non-α-substituted hydroxamates, the 1-naphthylalanine derivative (**5f**) exhibited nearly the same inhibitory activities as BB-94, the strongest broad-spectrum inhibitor. On the other hand, the 2-naphthylalanine derivative (**5g**) exhibited lower potency than the 1-naphthylalanine derivative (**5f**) against MMP-1. It is thought that the P2′ side chain does not play a dominant role in enzyme binding.[5] However, the above result suggests that some interaction between the P2′ side chain and the enzyme exists to assist in binding. In order to investigate this hypothesis, molecular modeling studies of the complex of a truncated MMP-1[12] and our inhibitors were carried out (data not shown). The results showed that the P2′ side chain of **5f** was orientated away from the enzyme and toward the outside, and did not interact with the truncated MMP-1, similar to other inhibitors. Therefore, the 1-naphthyl group of the hydroxamate (**5f**) should interact with the cleaved C-terminal part of the full-length enzyme for binding. Regarding the α-substituent, it was confirmed from modeling studies that the 3-methyl group in the (3*R*)-isomer(**15f**) disturbed the binding to the enzyme. However, the effect of the (3*S*)-methyl group, which enhanced the inhibition did not become clear from the modeling studies using the truncated enzyme data. Therefore,

Table 2. In vitro enzyme inhibitory activities



Compound no.	R_1	R_2	IC_{50} MMP-1	IC_{50} MMP-9
15a	 (*R*)		3.5 nM	45 nM
15b	 (*R*)		0.71 nM	8.3 nM
15c	 (*R*)		4.3 nM	43 nM
15d	 (*S*)		0.9 nM	0.7 nM
15e	CH_3 (*S*)		3.0 nM	3.9 nM
15f	CH_3 (*R*)		2.1 μM	7.8 μM
15g	 (*R*)		0.4 nM	0.76 nM
15h	 (*R*)		0.3 nM	0.46 nM
15i	CH_3 (*S*)		0.15 nM	0.2 nM
15j (BB-94)	 (*S*)		0.4 nM	0.2 nM

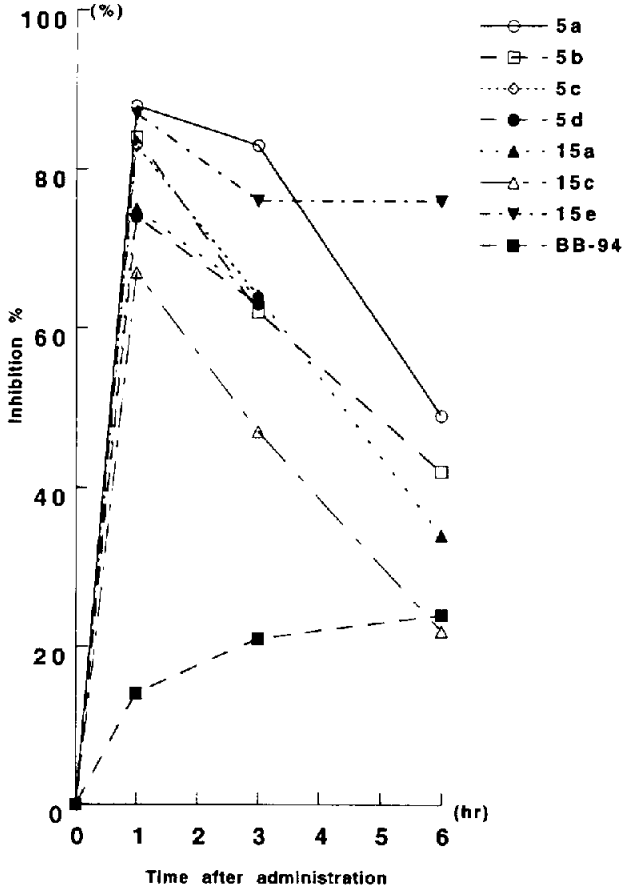


Figure 2. MMP-1 inhibitory activities in plasma after oral administration to mice (30 mg/kg).

three-dimensional data of the full-length MMP-1 will be needed to determine the effects of the 1-naphthylmethyl and α-substituent groups.

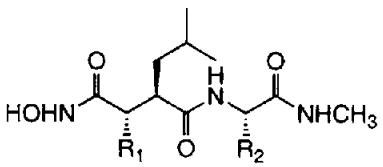
Oral bioavailability

To evaluate their oral activities, compounds **5** and **15** were administered orally (30 mg/kg) to mice, and the percentage inhibition of MMP-1 activity in plasma was measured ex vivo after 1, 3, and 6 h. It was apparent that the phenylglycine derivatives (**5a–5d**, **15a**, **15c**, **15e**) were absorbed well orally and the plasma samples from mice treated with these compounds exhibited a complete inhibition of the enzyme, as shown in Figure 2. On the other hand, plasma samples from mice treated with BB-94 or the other substituted alanine derivatives did not exhibit good inhibition.

The reasons for the good oral bioavailability of compounds **5a–5d**, **15a**, **15c**, and **15e** are that these phenylglycine derivatives have suitable water solubility and lipophilicity (Clog P),[13] as shown in Table 3. The solubility of **15j** (BB94) in water was less than 0.05 mg/mL. The solubility of other arylalanine derivatives was also less than 0.05 mg/mL. The modification by phenylglycine at the P2′ position improved the solubility in water more than 10-fold over that of the corresponding aryalanine derivatives, but the introduction of an α-substituent, such as a methyl or phenylthiomethyl group, decreased their solubility. Thus, it is considered that the hydrophobicities of the (tetrahydroisoquinolin-2-yl)methyl (**15b**) and phenylthiomethyl (**15d**) derivatives are too high to allow absorption from the intestine.

It has been suggested that the energy barrier caused by the desolvation of the peptide backbone upon passage from an aqueous environment to the lipid environment of the membrane is a determining factor in absorption.[14] This energy barrier should be lowered by shielding the amide backbone from hydration. The P2′ phenyl group of compounds **5a–d**, **15a**, and **15e** are located closer to the amide bond as compared with the P2′ benzyl group,

Table 3. Solubility in water and ClogP of MMP inhibitors



Compound no.	R_1	R_2	Solubility (mg/ml)	ClogP
5a	H	Phenyl	3.3	0.75
5b	H	*p*-hydroxyphenyl	5.0	0.09
5c	H	*p*-fluorophenyl	0.9	0.85
5e	H	2-naphthyl	<0.05	1.926
5f	H	1-naphthyl	<0.05	3.17
15a	Morpholinomethyl	Phenyl	1.0[a]	1.04
15b	(Teterahydroisoquinolin-2-yl)methyl	Phenyl	<0.05	3.142
15d	Phenylthiomethyl	Phenyl	<0.05	3.17
15e	Methyl	Phenyl	0.5	1.06
BB-94	Thienylthiomethyl	Benzyl	<0.05	3.09

[a]HCl salt.

and may provide some amide shielding effect to improve the absorption.

The effects of compound 15e in a rat adjuvant arthritis model

The phenylglycine derivatives (**15e**) showed a long duration of inhibitory activity in plasma after oral administration. Next, the in vivo activity of compound **15e** was studied using a rat adjuvant arthritis model. Administration of **15e** to adjuvant arthritic rats at 100 mg/kg, p.o. twice a day for 20 days inhibited increases in hind foot pad swelling on days 17 and 20, as shown in Figure 3. This compound also exhibited suppression of an increase of serum Ca^{2+} ion and improvement in the erythrocyte sedimentation rate, as described in Table 4. These results indicate that MMP inhibitors may offer a new disease-modifying therapy for rheumatoid arthritis. However the effect of compound **15e** against the rat adjuvant model is inferior to that of indomethacin. This may be due to the local concentration of compound **15e** in the inflammation site, as compared with indomethacin.

Conclusion

In summary, we report the synthesis and biological evaluation of MMP inhibitors, which were designed for improved bioavailability by modification of the P2′ position and the α-substituent of the hydroxamate inhibitors. We found that the P2′ substituent of hydroxamate influenced the MMP inhibitory activities in vitro and in plasma after oral administration, and that the hydroxamates with phenylglycine at the P2′ position were absorbed well orally. One of these compounds (**15e**) was evaluated in a rat adjuvant arthritis model and was found to suppress inflammation in this model following oral administration. These results indicate the potential of oral MMP inhibitors for rheumatoid arthritis, but for practical therapy, orally active inhibitors with superior bioavailability will be needed.

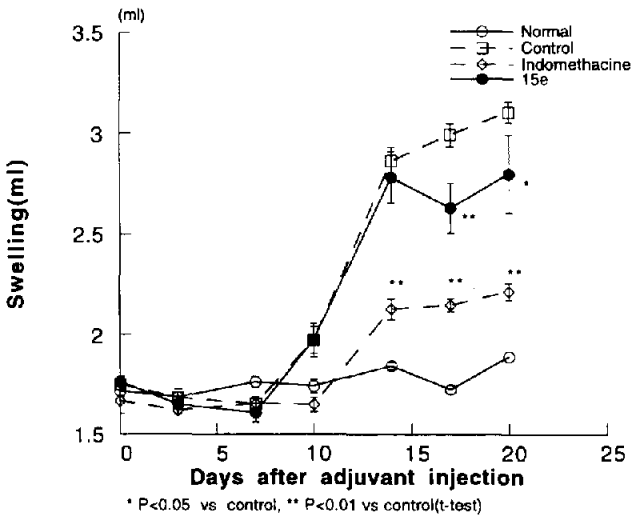


Figure 3. The effect of MMP inhibitor **15e** on left foot pad swelling in a rat adjuvant arthritis model (100 mg/kg, p.o.).

Compound **15e** is an MMP inhibitor bearing good oral activity, and it also has an inhibitory effect on the release of TNF-α from monocytes, as reported recently.[4] Therefore, it is possible that compound **15e** will be applied to other diseases in which excessive production of TNF-α has been implicated, such as septic shock, auto-immune diseases, etc. Those studies are now underway in our laboratories.

Experimental

Melting points were determined with a Buchi capillary melting-point apparatus, Model 535, and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM300 spectrometer using TMS as an internal standard. Elemental analyses were performed on a Yanagimoto CHN-CORDER MT-3. Optical rotations were determined on a JACO digital polarimeter DIP-370 in a 10 cm pathlength cell.

***N*-Benzyloxycarbonyl-L-phenylglycine-*N*-methylamide (6a).** Ethyl chloroformate (16.0 g, 0.147 mol) was added dropwise to a mixture of *N*-benzyloxycarbonyl-L-phenylglycine (35.2 g, 0.123 mol), triethylamine (14.9 g, 0.147 mol) and THF (150 mL) at 5 °C. Methylamine (40% methanol solution, 28.7 g, 0.370 mol) was added to the reaction mixture, which was stirred for 2 h in an ice bath. After filtration, EtOAc was added to the filtrate, and the EtOAc solution was washed with 1N HCl and saturated aqueous $NaHCO_3$ solution, successively. The resultant solution was dried over anhydrous $MgSO_4$, and was concentrated under reduced pressure. The residue was washed with a mixture of *n*-hexane and EtOAc to give **6a** as colorless crystals (33.3 g, 90.5%). ^{1}H NMR (DMSO-d_6): δ 2.55 (3H, d), 4.98 (2H, s), 5.16 (1H, d), 7.2–7.5 (1H, m), 7.6–7.4 (1H, m), 7.95–8.2 (1H, d).

***N*-Benzyloxycarbonyl-L-(*p*-hydroxyphenyl)glycine-*N*-methylamide (6b).** Following the procedure described for **6a**, *N*-benzyloxycarbonyl-L-(*p*-hydroxyphenyl)glycine (5.2 g, 17.3 mmol) led to **6b** (4.6g, 84.8%) as colorless crystals. ^{1}H NMR (DMSO-d_6): δ 2.55 (3H, d), 5.00 (2H, s), 5.02 (1H, d), 6.6–6.7 (2H, m), 7.1–7.2 (2H, m), 7.1–7.4 (5H, m), 7.5–7.6 (1H, m), 7.92 (1H, d), 9.29 (1H, s)

***N*-Benzyloxycarbonyl-L-(*p*-fluorophenyl)glycine-*N*-methylamide (6c).** To a mixture of L-(*p*-fluorophenyl)glycine[15] (220 mg, 1.3 mmol), sodium carbonate (551 mg, 5.2

Table 4. The effect of MMP inhibitor **15e** in a rat adjuvant aithritis model (100 mg/kg, p.o.)

	Erythrocyte sedimentation rate (mm/h)	Serum Ca ion (mg/dl)
Normal	1±0.1	8.6±0.1
Control	33±2	9.5±0.1
Indomehtacine	8±1[a]	9.2±0.1
15e	20.2±2[a]	8.9±0.1[a]

[a] $p < 0.01$ vs control (Dunet test).

mmol), and water (20 mL) was added benzyloxycarbonyl chloride (244 mg, 1.43 mmol) in dioxane (2 mL) with ice cooling. After further stirring with ice cooling for 3 h, the reaction mixture was washed with AcOEt, acidified with 4N HCl, and extracted with chloroform. The organic layer was dried over $MgSO_4$, and concentrated in vacuo to give *N*-benzyloxycarbonyl-L-(*p*-fluorophenyl)glycine (400 mg) as a syrup. This syrup was converted to **6c** (262 mg, 63.7%) following the procedure described for **6a**. ^{1}H NMR ($CDCl_3$): δ 2.8 (3H, d), 5.05 (2H, s), 5.11 (1H, d), 5.6–5.8 (1H, m), 6.0–6.2 (1H, m), 6.8–7.5 (9H, m).

***N*-Benzyloxycarbonyl-L-(*p*-isopropylphenyl)glycine-*N*-methylamide (6d).** Following the procedure described for **6c**, L-(*p*-isopropylphenyl)glycine[15] led to **6d** as colorless crystals. ^{1}H NMR ($CDCl_3$): δ 1.2 (6H, d), 2.75 (3H, d), 5.0 (2H, s), 5.18 (1H, d), 5.4-5.6 (1H, m), 5.9–6.1 (1H, m), 7.1–7.3 (9H, m).

***N*-Benzyloxycarbonyl-L-(2-naphthyl)glycine-*N*-methylamide (6e).** According to the procedure by Inaba et al.,[15] L-(2-naphthyl)glycine was prepared. Following the procedure described for **6c**, L-(2-naphthyl)glycine led to **6e** as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 2.6 (3H, d, J = 4.8 Hz), 5.0 (2H, s), 5.3 (1H, d, J = 8 Hz), 7.22–8.3 (12H, m).

***N*-*tert*-Butoxycarbonyl-L-3-(1-naphthyl)alanine-*N*-methylamide (6f).** Ethyl chloroformate (0.826 g, 7.6 mmol) was added dropwise to a mixture of *N*-*tert*-butoxycarbonyl-L-3-(1-naphthyl)alanine[16] (2 g, 6.3 mmol), triethylamine (0.77 g, 7.6 mmol) and THF (50 mL) at 5 °C. After 5 min, methylamine (40% methanol solution, 2 mL) was added to the reaction mixture, which was stirred for 3 h in an ice bath. EtOAc was added to the mixture, and the EtOAc solution was washed with 1N HCl, saturated aqueous $NaCO_3$ and water, successively. The resultant solution was dried over anhydrous $MgSO_4$, and was concentrated under reduced pressure to give **6f** as colorless crystals (1.87 g, 89.9%). ^{1}H NMR (DMSO-d_6): δ 1.25 (9H, s), 2.58 (3H, d), 3.11 (1H, dd), 3.51 (1H, dd), 4.2–4.3 (1H, m), 6.98 (1H, d), 7.91 (1H, d), 7.4–8.2 (7H, m).

***N*-*tert*-Butoxycarbonyl-L-3-(2-naphthyl)alanine-*N*-methylamide (6g).** Following the procedure described for **6f**, *N*-*tert*-butoxycarbonyl-L-3-(2-naphthyl)alanine[16] led to **6g** as colorless crystals. ^{1}H NMR ($CDCl_3$): δ 1.4 (9H, s), 2.7 (3H, d), 3.2 (2H, d), 4.1–4.6 (1H, m), 5.0–5.3 (1H, m), 5.7–6.1 (1H, m), 7.2–8.0 (7H, m).

***N*-t-Butoxycarbonyl-L-homophenylalanine-*N*-methylamide (6h).** Following the procedure described for **6f**, *N*-*tert*-butoxycarbonyl-L-homophenylalanine[16] led to **6h** as colorless crystals. ^{1}H NMR ($CDCl_3$): δ 1.4 (9H, s), 1.9–2.3 (2H, m), 2.5–2.8 (2H, m), 2.8 (3H, d), 3.9–4.4 (1H, m), 5.2–5.5 (1H, m), 6.3–6.7 (1H, m), 7.2 (5H, s).

***N*-*tert*-Butoxycarbonyl-L-3-(3-benzothienyl)alanine-*N*-methylamide (6i).** Following the procedure described for **6f**, *N*-*tert*-butoxycarbonyl-L-3-(3-benzothienyl)alanine[16] led to **6i** as colorless crystals. ^{1}H NMR (DMSO-d_6): δ 1.29 (9H, s), 2.59 (3H, d, J = 4.5 Hz), 3.01 (1H, dd, J = 9.9 Hz, 14 Hz), 3.19 (1H, dd, J = 4.4 Hz, 14 Hz), 4.24 (1H, dt, J = 4.6 Hz, 9.4 Hz), 6.97 (1H, d, J = 8.6 Hz), 7.3–7.5 (3H, m), 7.8–8.0 (3H, m).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-phenylglycine-*N*-methylamide (4a). Compound **6a** (27 g, 90.6 mmol) was hydrogenated in methanol with 10% Pd/C (0.5 g) under hydrogen atmosphere (4 kg/cm^2) at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The residue was dissolved in DMF (400 mL), and **3** (24.4 g, 87.3 mmol) and (1-(3-dimethylamino)propyl)-3-ethylcarbodiimide (17.8 g, 92.9 mmol) were added. The mixture was stirred at room temperature overnight, and was concentrated under reduced pressure. The resultant residue was dissolved in chloroform (1 L), and washed with 0.1N HCl, saturated aqueous $NaHCO_3$ solution and brine, successively. After drying over anhydrous $MgSO_4$, the organic solution was concentrated in vacuo. The crude crystals were recrystallized from a mixture of EtOAc and methanol to give **4a** (29 g, 73%) as colorless crystals. ^{1}H NMR (DMSO-d_6): δ 0.82 (3H, d, J = 6.3 Hz), 0.87(3H, d, J = 6.3 Hz), 1.0–1.1 (1H, m), 1.35–1.55 (2H, m), 1.97 (1H, dd, J = 7 Hz, 14.4 Hz), 2.13 (1H, dd, J = 7 Hz, 14.4 Hz), 2.57 (3H, d, J = 4.5 Hz), 2.8–2.95 (1H, m), 4.66 (1H, d, J = 11Hz), 4.71 (1H, d, J = 11 Hz), 5.38 (1H, d, J = 7.8 Hz), 7.2–7.4 (10H, m), 8.13 (1H, q, J = 4.5 Hz), 8.45 (1H, d, J = 7.8 Hz), 10.9 (1H, s).

Compounds **4b**, **4c**, **4d** and **4e** were prepared in the same manner as compound **4a**. Their physical data are summarized as follows.

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-(*p*-hydroxyphenyl)glycine-*N*-methylamide (4b). ^{1}H NMR (DMSO-d_6): δ 0.81(3H, d, J = 6.3 Hz), 0.86 (3H, d, J = 6.3 Hz), 1.0–1.1(1H, m), 1.35–1.55 (2H, m), 1.96 (1H, dd, J = 7 Hz, 14.5 Hz), 2.12 (1H, dd, J = 7.3 Hz, 14.5 Hz), 2.56 (3H, d, J = 4.5Hz), 2.6–2.7(1H, m), 4.68 (1H, d, J = 10.9Hz), 4.72 (1H, d, J = 10.9Hz), 5.25 (1H, d, J = 7.7 Hz), 6.67(2H, d, J = 8.5 Hz), 7.16 (2H, d, J = 8.5 Hz), 7.36 (5H, s), 7.97(1H, q, J = 4.5 Hz), 8.28 (1H, d, J = 7.8 Hz), 9.43(1H, s), 10.96 (1H, s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-(*p*-fluorophenyl)glycine-*N*-methylamide (4c). ^{1}H NMR (DMSO-d_6): δ 0.81(3H, d, J = 6.3 Hz), 0.85 (3H, d, J = 6.3 Hz), 1.0–1.1 (1H, m), 1.35–1.52 (2H, m), 1.96 (1H, dd, J = 7.0 Hz, 14.5 Hz), 2.12 (1H, dd, J = 7.4 Hz, 14.5 Hz), 2.57 (3H, d, J = 4.6 Hz), 2.8–2.9 (1H, m), 4.65 (1H, d, J = 12 Hz), 4.69 (1H, d, J = 12 Hz), 5.38 (1H, d, J = 7.8 Hz), 7.11 (2H, dd, J = 8.9 Hz, 8.9 Hz), 7.35 (5H, s), 8.13 (1H, q, J = 7.8 Hz), 8.47 (1H, d, J = 7.8 Hz), 10.95 (1H, s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-(*p*-isopropylphenyl)glycine-*N*-methylamide (4d). ^{1}H NMR (DMSO-d_6): δ 0.82 (3H, d, J = 6.3 Hz), 0.87(3H, d, J = 6.3 Hz), 1.0–1.2 (1H, m), 1.2 (6H, d, J = 6.9 Hz),

1.35–1.5 (2H, m), 1.97(1H, dd, J = 6.8 Hz, 14.5 Hz), 2.12 (1H, dd, J = 7.6 Hz, 14.5 Hz), 2.57 (3H, d, J = 4.6 Hz), 2.75–2.92 (2H, m), 4.65 (1H, d, J = 11 Hz), 4.69 (1H, d, J = 11 Hz), 5.33 (1H, d, J = 7.8 Hz), 7.13 (2H, d, J = 8.1 Hz), 7.29 (2H, d, J = 8.1 Hz), 7.35 (5H, s), 8.06 (1H, q, J = 4.6 Hz), 8.37 (1H, d, J = 7.8 Hz), 10.96 (1H, s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-(2-naphthyl)glycine-*N*-methyl amide (4e). ^{1}H NMR(DMSO-d_6): δ 0.83 (3H, d, J = 6.3 Hz), 0.89 (3H, d, J = 6.3 Hz), 1.03–1.16 (1H, m), 1.4–1.58 (2H, m), 1.99 (1H, dd, J = 6.7 Hz, 14.5 Hz), 2.15 (1H, dd, J = 7.6 Hz, 14.5 Hz), 2.60 (3H, d, J = 4.5 Hz), 2.90–3.01 (1H, m), 4.62–4.70 (2H, m), 5.56 (1H, d, J = 7.8 Hz), 7.2–7.4 (8H, m), 7.8–7.9 (4H, m), 8.18–23 (1H, m), 8.57 (1H, d, J = 7.8 Hz), 10.97 (1H, s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-3-(1-naphthyl)alanine-*N*-methylamide (4f). Compound **6f** (0.8 g, 2.44 mmol) was dissolved in 95% aqueous trifluoroacetic acid (TFA), and the solution was stirred at room temperature for 30 min. Then, the solvents were evaporated in vacuo, and the residue was dissolved into chloroform (20 mL) and washed with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was dissolved in DMF (25 mL) and **3** (670 mg, 2.4 mmol), and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (552 mg, 2.88 mmol) were added. The mixture was stirred at room temperature overnight, and was concentrated in vacuo. The residue was dissolved in chloroform (20 mL), and washed with 0.1N HCl, saturated aqueous $NaHCO_3$ solution, and brine, successively. After drying over $MgSO_4$, the organic solution was concentrated in vacuo to give **4f** (680 mg, 57.9%) as colorless crystals. ^{1}H NMR (DMSO-d_6) δ: 0.72 (3H, d, J = 6.3 Hz), 0.77 (3H, d, J = 6.3 Hz), 0.9–1.02 (1H, m), 1.20–1.38 (2H, m), 1.91(1H, dd, J = 7.1 Hz, 14.4 Hz), 2.07(1H, dd, J = 7.5 Hz, 14.4 Hz), 2.55 (3H, d, J = 4.5 Hz), 2.6–2.7(1H, m), 3.27(1H, dd, J = 8.7 Hz, 13.9 Hz), 3.53 (1H, dd, J = 5.5 Hz, 13.9 Hz), 4.48–4.58 (1H, m), 4.76 (2H, s), 7.32–7.42 (7H, m), 7.48–7.59 (2H, m), 7.76 (1H, d, J = 7.6 Hz), 7.83–7.92 (2H, m), 8.12–8.25 (2H, m), 11.03 (1H, s).

Compounds **4g**, **4h**, and **4i** were prepared in the same manner as compound **4f**. Their physical data are summarized as follows.

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-3-(2-naphthyl)alanine-*N*-methylamide (4g). ^{1}H NMR (DMSO-d_6): δ 0.60 (3H, d, J = 6.3 Hz), 0.71 (3H, d, J = 6.3 Hz), 0.84–0.98 (1H, m), 1.12–1.32 (2H, m), 1.88 (1H, dd, J = 7.4 Hz, 14.4 Hz), 2.04 (1H, dd, J = 7.4 Hz, 14.4 Hz), 2.57 (3H, d, J = 4.5 Hz), 2.54–2.67 (1H, m), 3.02 (1H, dd, J = 6.6 Hz, 13.8 Hz), 3.19 (1H, dd, J = 5.0 Hz, 13.8 Hz), 4.45–4.54 (1H, m), 4.74 (2H, s), 7.36 (5H, s), 7.30–7.49 (3H, m), 7.69 (1H, s), 7.76–7.86 (3H, m), 7.87–7.93 (1H, m), 8.14 (1H, d, J = 8.2 Hz), 10.99 (1H, s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-homophenylalanine-*N*-methyl amide (4h). ^{1}H NMR(DMSO-d_6): δ 0.83 (3H, d, J = 6.5 Hz), 0.88 (3H, d, J = 6.5 Hz), 1.01–1.15 (1H, m), 1.40–1.55 (2H, m), 1.75 (2H, m), 2.04 (1H, dd, J = 6.4 Hz, 14.6 Hz), 2.24 (1H, dd, J = 8.2 Hz, 14.6 Hz), 2.57 (3H, d, J = 4.5 Hz), 2.43–2.68 (2H, m), 2.70–2.83 (1H, m), 4.12–4.22 (1H, m), 4.75 (2H, s), 7.12–7.28 (5H, m), 7.35 (5H, s), 7.70–7.77(1H, m), 8.08 (1H, d, J = 8.1 Hz), 11.06 (1H, s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutylsuccinyl]-L-3-(3-benzothienyl)alanine-*N*-methylamide (4i). ^{1}H NMR (DMSO-d_6): δ 0.72 (3H, d, J = 6.2 Hz), 0.79 (3H, d, J = 6.2 Hz), 0.9–1.0 (1H, m), 1.30–1.40 (2H, m), 1.93 (1H, dd, J = 7.2 Hz, 14.4 Hz), 2.08 (1H, dd, J = 7.3 Hz, 14.4 Hz), 2.57(3H, d, J = 4.5 Hz), 2.6–2.7(1H, m), 3.11 (1H, dd, J = 9.0 Hz, 14.7 Hz), 3.23 (1H, dd, J = 5.1 Hz, 14.7 Hz), 4.5–4.6 (1H, m), 4.75 (2H, s), 7.3–7.5 (8H, s), 7.6–8.0 (3H, m), 8.18 (1H, d, J = 8.1 Hz), 11.02 (1H, s).

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-phenylglycine-*N*-methylamide (5a). Compound **4a** (7.3 g, 17.2 mmol) was hydrogenated in methanol (300 mL) with 10% Pd/C (0.2 g) under hydrogen atmosphere (3 kg/cm^2) at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated. The residue was recrystallized from a mixture of THF and methanol to give **5a** (4 g, 69.5%) as colorless crystals: mp 169–170 C; $[\alpha]_D$ +97.4° (c 0.2; MeOH); ^{1}H NMR (DMSO-d_6): δ 0.81 (3H, d, J = 6.3 Hz), 0.87 (3H, d, J = 6.3 Hz), 1.0–1.15 (1H, m), 1.45–1.6 (2H, m), 1.97 (1H, dd, J = 7.4 Hz, 14.4 Hz), 2.13 (1H, dd, J = 6.6 Hz, 14.4 Hz), 2.55 (3H, d, J = 4.5 Hz), 2.8–2.95 (1H, m), 5.38 (1H, d, J = 7.8 Hz), 7.2–7.4 (5H, m), 8.13 (1H, q, J = 4.5 Hz), 8.45 (1H, d, J = 7.8 Hz), 8.72 (1H, s), 10,36 (1H, s). Anal. calcd for $C_{17}H_{25}N_3O_4$: C, 60.78; H,7.51; N, 12.53; found: C, 60.78; H, 7.63; N, 12.36.

Compounds **5b**, **5c**, **5d**, **5e**, **5f**, **5g**, **5h** and **5i** were prepared in the same manner as compound **5a**. Their physical data are summarized as follows.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-(*p*-hydroxyphenyl)glycine-*N*-methylamide (5b). mp 187–188 °C; $[\alpha]_D$ +116.6° (c 0.2; MeOH); ^{1}H NMR (DMSO-d_6): δ 0.80 (3H, d, J = 6.3 Hz), 0.86 (3H, d, J = 6.3 Hz), 1.0-1.15 (1H, m), 1.35–1.6 (2H, m), 1.95 (1H, dd, J = 7.5 Hz, 14.4 Hz), 2.11(1H, dd, J = 6.8 Hz, 14.4 Hz), 2.55 (3H, d, J = 4.5 Hz), 2.8–2.95 (1H, m), 5.22 (1H, d, J = 7.7 Hz), 6.69 (2H, d, J = 8.5 Hz), 7.16 (2H, d, J = 8.5 Hz), 7.98 (1H, q, J = 4.5 Hz), 8.29 (1H, d, J = 7.7 Hz), 8.72 (1H, s), 9.37 (1H, s), 10.34 (1H, s). Anal. calcd for $C_{17}H_{25}N_3O_5$: C, 58.11; H, 7.17; N, 11.96; found: C, 57.88; H, 7.15; N, 11.93.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-(*p*-fluorophenyl)glycine-*N*-methylamide (5c). mp 170–172 °C; $[\alpha]_D$ +78° (c 0.1; MeOH); ^{1}H NMR (DMSO-d_6): δ 0.81 (3H, d, J = 6.3 Hz), 0.85 (3H, d, J = 6.3 Hz), 1.0–1.1 (1H, m), 1.40–1.52 (2H, m), 1.96 (1H, dd, J = 7.3 Hz, 14.4 Hz), 2.12 (1H, dd, J = 7.0 Hz, 14.4 Hz), 2.6 (3H, d, J = 4.6

Hz), 2.8–2.9 (1H, m), 5.36 (1H, d, J = 7.8 Hz), 7.14 (2H, dd, J = 8.9 Hz, 8.9 Hz), 7.4–7.45 (2H, m), 8.13 (1H, d, J = 4.6 Hz), 8.45 (1H, d, J = 7.8 Hz), 10.34 (1H, s). Anal. calcd for $C_{17}H_{24}FN_3O_4$: C, 57.78; H, 6.85; N, 11.89; found: C, 57.83; H, 6.96; N, 11.84.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-(*p*-isopropylphenyl)glycine-*N*-methylamide(5d). mp 219–222 °C; $[\alpha]_D$ +95.3° (c 0.1; MeOH); ^{1}H NMR (DMSO-d_6): δ 0.8 (3H, d, J=6.3 Hz), 0.85 (3H, d, J=6.3 Hz), 1.0–1.08 (1H, m), 1.2 (6H, d, J=6.9 Hz), 1.47–1.52 (2H, m), 1.95 (1H, dd, J=7.5 Hz, 14.4 Hz), 2.15 (1H, dd, J=6.9 Hz, 14.4 Hz), 2.6 (3H, d, J=4.6 Hz), 2.8–2.92 (2H, m), 5.3 (1H, d, J=7.8 Hz), 7.2 (2H, d, J=8.2 Hz), 7.3 (2H, d, J=8.2 Hz), 8.05 (1H, d, J=4.6 Hz), 8.4 (1H, d, J=7.8 Hz), 8.7 (1H, d, J=1.6 Hz). 10.34 (1H, d, J=1.6 Hz). Anal. calcd for $C_{20}H_{31}N_3O_4$: C, 63.64; H, 8.28; N, 11.13; found: C, 63.47; H, 8.30; N, 11.134.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-(2-naphthyl)glycine-*N*-methyl amide (5e). mp 197–199 °C; ^{1}H NMR (DMSO-d_6): δ 0.83 (3H, d, J = 6.3 Hz), 0.88 (3H, d, J = 6.3 Hz), 1.04–1.16 (1H, m), 1.4–1.58 (2H, m), 1.98 (1H, dd, J = 7.4 Hz, 14.5 Hz), 2.15 (1H, dd, J = 7.0 Hz, 14.5 Hz), 2.59 (3H, d, J = 4.5 Hz), 2.87–2.98 (1H, m), 5.54 (1H, d, J = 7.7 Hz), 7.46–7.57 (3H, m), 7.82–7.92 (4H, m), 8.17–8.25 (1H, m), 8.56 (1H, d, J = 7.7 Hz), 8.73 (1H, bs), 10.37(1H, s). Anal. calcd for $C_{21}H_{27}N_3O_4$: C, 65.44; H, 7.06; N, 10.90; found: C, 65.28; H, 7.06; N, 10.73.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-3-(1-naphthyl)alanine-*N*-methyl amide (5f). mp 192–194 °C; ^{1}H NMR (DMSO-d_6): δ 0.71 (3H, d, J = 6.4 Hz), 0.75 (3H, d, J = 6.4 Hz), 0.92–1.03 (1H, m), 1.15–1.38 (2H, m), 1.91 (1H, dd, J = 7.1 Hz, 14.3 Hz), 2.08 (1H, dd, J = 7.6 Hz, 14.3 Hz), 2.5–2.6 (1H, m), 2.57 (3H, d, J = 4.5 Hz), 3.25 (1H, dd, J = 9.2 Hz, 13.9 Hz), 3.58 (1H, dd, J = 5.0 Hz, 13.9 Hz), 4.45–4.56 (1H, m), 7.32–7.42 (2H, m), 7.48–7.60 (2H, m), 7.77 (1H, d, J = 6.8 Hz), 7.91 (1H, d, J = 7.9 Hz), 7.93–8.0 (1H, m), 8.16 (1H, d, J = 8.2 Hz), 8.21 (1H, d, J = 8.2 Hz), 8.80 (1H, bs), 10.44 (1H, s). Anal. calcd for $C_{22}H_{29}N_3O_4$: C, 66.14; H, 7.32; N, 10.52; found: C, 66.05; H, 7.34; N, 10.57.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-3-(2-naphthyl)alanine-*N*-methyl amide (5g). mp 191–192 °C; ^{1}H NMR (DMSO-d_6): δ 0.56 (3H, d, J=6.4 Hz), 0.68 (3H, d, J=6.4 Hz), 0.87–0.97 (1H, m), 1.08–1.30 (1H, m), 1.89 (1H, dd, J=7.4 Hz, 14.3 Hz), 2.05 (1H, dd, J=7.4 Hz, 14.3 Hz), 2.57 (3H, d, J=4.5 Hz), 2.50–2.62 (1H, m), 3.01 (1H, dd, J=9.8 Hz, 13.8 Hz), 3.22 (1H, dd, J=4.9 Hz, 13.8 Hz), 4.45–4.54 (1H, m), 7.36–7.50 (3H, m), 7.69 (1H, s), 7.77–7.88 (3H, m), 7.92–8.00 (1H, m), 8.11 (1H, d, J=8.3 Hz), 8.78 (1H, s), 10.41 (1H, s). Anal. calcd for $C_{22}H_{29}N_3O_4$: C, 66.14; H,7.32; N, 10.52; found: C, 66.10; H, 7.30; N, 10.54.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-homophenylalanine-*N*-methylamide (5h). mp 190–192 °C; ^{1}H NMR (DMSO-d_6): δ 0.82 (3H, d, J = 6.2 Hz), 0.87 (3H, d, J = 6.2 Hz), 1.02–1.16 (1H, m), 1.40–1.57 (2H, m), 1.75–2.00 (2H, m), 2.04 (1H, dd, J = 6.9 Hz, 14.5Hz), 2.24 (1H, dd, J = 7.7 Hz, 14.5 Hz), 2.57 (3H, d, J = 4.5 Hz), 2.42–2.68 (2H, m), 2.68–2.80 (1H, m), 4.07–4.17 (1H, m), 7.13–7.30 (5H, m), 7.73–7.81 (1H, m), 8.06 (1H, d, J = 8 Hz), 8.76(1H, s), 10.44 (1H, s). Anal. calcd for $C_{19}H_{29}N_3O_4$: C, 62.79; H, 8.04; N, 11.56; found: C, 62.64; H, 8.14; N, 11.35.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutylsuccinyl]-L-3-(3-benzothienyl)alanine-*N*-methylamide (5i). mp 195–196 °C; ^{1}H NMR (DMSO-d_6): δ 0.71 (3H, d, J = 6.2 Hz), 0.77 (3H, d, J = 6.2 Hz), 0.9–1.00 (1H, m), 1.2–1.4 (2H, m), 1.92 (1H, dd, J = 7.2 Hz, 14.4 Hz), 2.05 (1H, dd, J = 7.3 Hz, 14.4 Hz), 2.05 (1H, dd, J = 7.3 Hz, 14.4 Hz), 2.54 (3H, d, J = 5.2 Hz), 2.55–2.7 (1H, m), 3.11 (1H, dd, J = 9.3 Hz, 14.7 Hz), 3.26 (1H, dd, J = 4.8 Hz, 14.7 Hz), 4.45–4.6 (1H, m), 7.3–7.5 (3H, m), 7.6–8.0 (3H, m), 8.16 (1H, d, J = 8.1 Hz), 8.77 (1H, d, J = 1.6 Hz), 10.44 (1H, d, J = 1.5 Hz). Anal. calcd for $C_{20}H_{27}N_3O_4S$: C, 59.24; H, 6.71; N, 10.36; found: C, 59.18; H, 6.81; N, 10.34.

***tert*-Butyl 2(*R*)-bromo-4-methylpentanoate (8).** Into a solution of 2(*R*)-bromo-4-methylpentanoic acid (66 g, 0.338 mol)[10] in dichloromethane (200 mL) at −40 °C was bubbled 2-methylpropene (49 g, 0.875 mol), and then the mixture was stirred at room temperature for 24 h after the addition of sulfuric acid (1.6 mL). The mixture was evaporated in vacuo to a half volume, and washed with 10% aqueous $NaCO_3$ solution and dried over MgSO4. Evaporation of the solvent gave *tert*-butyl ester **8** (74.8 g, 75.7%) as an oil: $[\alpha]_D$ +29 ° (c 2; MeOH); ^{1}H NMR ($CDCl_3$): δ 0.92 (3H, d), 0.95 (3H, d), 1.45 (9H, s), 1.7–2.0 (3H, m), 4.18 (1H, t).

Benzyl 2-benzyloxycarbonyl-3(*R*)-hydroxycarbonyl-5-methylhexanoate (10).

To a solution of dibenzyl malonate (68 g, 0.23 mol) in DMF (500 mL) was added potassium *tert*-butoxide (26.5 g, 0.237 mol) in five portions under stirring at 0–5 °C and then a solution of *tert*-butyl ester **8** (54.5 g, 0.217 mol) in DMF (100 mL) was added dropwise at 0 °C for 1 h, and the mixture was stirred at 0–5 °C for 4 days. To the mixture were added EtOAc (500 mL) and saturated aqueous NH_4Cl solution, and the organic layer was separated and the water layer was extracted with EtOAc (500 mL). The organic layers were combined, washed with 10% aqueous NaCl solution and dried over $MgSO_4$. The solvent was removed in vacuo, and the oily residue was purified by silica gel column chromatography (eluent:*n*-hexane/EtOAc = 30/1) to afford tri-ester **9** (48.9 g, 49.6%) as an oil. Tri-ester **9** (23.2 g, 51.1 mmol) was dissolved in 95% aqueous TFA (75 mL) and stirred at room temperature overnight. The solvents were evaporated in vacuo, and the oily residue was dissolved in dichloromethane (100 mL), and washed with brine and dried over $MgSO_4$. After removal of the solvents, the residue was dissolved in diethyl ether (70 mL), and *n*-hexane (200 mL) was added. The precipitated crystals were filtered off, and the filtrate was

evaporated in vacuo to give di-ester **10** (12.5 g, 61.5% as syrup: $[\alpha]_D$ +30 ° (*c* 2; MeOH); ^{1}H NMR (DMSO-d_6): 0.77 (6H, d, *J* = 6.4 Hz), 1.0–1.1 (1H, m), 1.4–1.6 (2H, m), 2.8–3.0 (1H, m), 3.71 (1H, d, *J* = 10 Hz), 5.09–5.25 (4H, m), 7.2–7.4 (10H, m).

(4-Benzyloxy-3-benzyloxycarbonyl-2(*R*)-isobutylsuccinyl)-L-phenylglycine-*N*-methylamide (11a). To a solution of **7a** (10.3 g, 62.7 mmol) in DMF (100 mL) were added **10** (23 g, 57.8 mmol), HOBT (10.6 g, 59 mmol), 4-aminopyridine (3.6 g) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (13.3 g, 69.3 mmol) with ice cooling, and the mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc (800 mL) and washed with water, 0.5 N HCl, saturated aqueous $NaHCO_3$ solution, and brine, successively. After drying over anhydrous $MgSO_4$, the organic solution was evaporated in vacuo to give **11a** (26.6 g, 95%) as white crystals: ^{1}H NMR (DMSO-d_6): δ 0.75 (3H, d, *J* = 6.5 Hz), 0.82 (3H, d, *J* = 6.4Hz), 1.0–1.1 (1H, m), 1.4–1.6 (2H, m), 2.56 (3H, d, *J* = 4.6 Hz), 3.2–3.35 (1H, m), 3.67 (1H, d, *J* = 10 Hz), 4.95 (2H, s), 5.07 (1H, d, *J* = 12.4 Hz), 5.16 (1H, d, *J* = 12.4 Hz), 5.39 (1H, d, *J* = 7.7 Hz), 7.15–7.45 (15H, m), 8.11 (1H, q, *J* = 4.6 Hz), 8.74 (1H, d, *J* = 7.7 Hz).

(4-Hydroxy-2(*R*)-isobutyl-3-methylenesuccinyl)-L-phenylglycine-*N*-methylamide (12a). A mixture of **11a** (26 g, 47.7 mmol), ammonium formate (20 g, 317.2 mmol), 10% Pd/C (6 g) and EtOH (600 mL) was stirred at room temperature for 1.5 h. After removal of the catalyst by filtration, piperidine (5.2 g, 61.7 mmol) was added, and the mixture was stirred at room temperature for 30 min. After addition of 37% aq formaldehyde solution (32.7 mL, 435.6 mmol), the mixture was stirred at room temperature for 18 h, and then refluxed for 1.5 h. The solvents were removed under reduced pressure and the residue was partitioned between 10% aqueous citric acid solution (500 mL) and EtOAc (500 mL). The acid layer was extracted with EtOAc (500 mL × 4). The organic layer was combined and extracted with 10% aqueous potassium carbonate solution (300 mL × 3). These aqueous extracts were acidified to pH 4 with 2 N HCl and extracted with dichloromethane (400 mL × 5). The dichloromethane extracts were dried over $MgSO_4$, and evaporated in vacuo to give **12a** (12.3 g, 69%) as a white solid: ^{1}H NMR (DMSO-d_6): δ 0.83 (3H, d, *J* = 6.4 Hz), 0.88 (3H, d, *J* = 6.4 Hz), 1.3–1.7 (1H, m), 1.4–1.6 (2H, m), 2.57 (3H, d, *J* = 4.6 Hz), 3.6–3.7 (1H, m), 5.37 (1H, d, *J* = 7.8 Hz), 5.6 (1H, s), 6.1 (1H, s), 7.2–7.4 (5H, m), 8.16 (1H, q, *J* = 4.6 Hz), 8.33 (1H, d, *J* = 7.8 Hz), 12.6 (1H, br s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutyl-3(*S*)-methylsuccinyl]-L-phenylglycine-*N*-methylamide (14e). A mixture of **12a** (11.1 g 33.4 mmol), 10% Pd/C (1.0 g), and MeOH (700 mL) was stirred under hydrogen atmosphere for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated to give **13e** as a diastereomer mixture. To a stirred solution of **13e** (11 g, 32.9 mmol) in DMF (150 mL) and dichloromethane (200 mL) were added *O*-benzylhydroxylamine hydrochloride (8.0 g, 50.1 mmol), triethylamine (5.1 g, 50.4 mmol), HOBT (5.5 g, 40.7 mmol) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (7.8 g, 40.7 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature. The resultant precipitate was filtered and washed with aqueous HCl solution and small amount of methanol successively to give **14e** (7.7 g, 53.2%) as a colorless solid: mp 256–257 °C; ^{1}H NMR (DMSO-d_6): δ 0.76 (3H, d, *J* = 2.1 Hz), 0.78 (3H, d, *J* = 2.5 Hz), 0.84 (3H, d, *J* = 6.4 Hz), 0.9 (1H, m), 1.3 (2H, m), 2.1 (1H, m), 2.56 (3H, d, *J* = 4.5 Hz), 2.66 (1H, m), 4.77 (2H, s), 5.46 (1H, d, *J* = 8 Hz), 7.23–7.42 (10H, m), 8.07 (1H, q, *J* = 4.6 Hz), 8.74 (1H, d, *J* = 8.0 Hz), 11.04 (1H, s).

From **20**: To a solution of compound **20**[11] (0.98 g, 2.4 mmol) in THF (30 mL) were added 30% H_2O_2 (1.1 mL) and lithium hydroxide monohydrate (0.15 g,) under stirring at 0–5 °C, and the mixture was stirred at 0–5 °C for 13 h and further at room temperature for 4 h. After addition of sodium nitrite (1.1 g), the mixture was stirred for 30 min, and evaporated in vacuo. Water (10 mL) was added and the mixture was washed with chloroform. The water layer was acidified to pH 4 with 1N HCl, and extracted with EtOAc (10 mL × 3). The organic layers were combined, and washed with brine, dried over $MgSO_4$ and evaporated in vacuo to give **21** (0.45 g,76%) as a colorless oil. (**21**; ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, d, *J* = 6.5 Hz), 0.91 (3H, d, *J* = 6.5 Hz), 1.16 (3H, d, *J* = 6.9 Hz), 1.2 (1H, m), 1.4 (9H, s), 2.57 (1H, m). To a solution of compound **21** (0.45 g, 1.8 mmol) and L-phenylglycine-*N*-methylamide (**7a**; 0.38 g, 2.3 mmol) in DMF (10 mL) and dichloromethane (20 mL) were added HOBT (0.26 g, 1.9 mmol) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (0.42 g, 2.2 mmol) with ice cooling, and the mixture was stirred at room temperature overnight. The precipitates were collected by filtration and washed with diethyl ether to give **22e** (0.3 g, 42%) as colorless crystals. (**22e**; ^{1}H NMR ($CDCl_3$): δ 0.87 (3H, d, *J* = 6.6 Hz), 0.88 (3H, d, *J* = 6.9 Hz), 0.91 (3H, d, *J* = 6.5 Hz), 1.1 (1H, m), 1.42 (9H, s), 1.5 (1H, m), 1.7 (1H, m), 2.4 (1H, m), 2.5 (1H, m), 2.8 (1H, d, *J* = 4.9 Hz), 5.4 (1H, d, *J* = 6.5 Hz), 5.71 (1H, d, *J* = 5 Hz), 7.18 (1H, d, *J* = 6.5 Hz), 7.3 (5H, m)). A solution of compound **22e** (0.3 g, 0.77 mmol) in HCOOH (5 mL) was stirred at room temperature for 3 h. After the solvent was evaporated in vacuo, the residue was dissolved in dicholoromethane (10 mL) and DMF (10 mL). *O*-Benzylhydroxylamine hydrochloride (0.23 g, 1.4 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (0.27 g, 1.4 mmol) and triethylamine 0.15 g, 1.5 mmol) were added with ice cooling, and the mixture was stirred at room temperature for 17 h. The precipitates were collected by filtration and washed with water and diethyl ether successively to give **14e** (0.18 g, 57%) as colorless crystals. The physical properties of this compound were in accord with those of the compound from **12a**.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*S*)-methylsuccinyl]-L-phenylglycine-*N*-methylamide (15e). Compound **14e** (7.7 g, 17.5 mmol) was hydrogenated in MeOH

(500 mL) with 10% Pd/C (0.7 g) under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The crude crystals were recrystallized from methanol to give **15e** (5.2 g, 85.0%) as colorless crystals: mp 217–219 °C; $[\alpha]_D$ +136° (*c* 0.1; MeOH); ^{1}H NMR (DMSO-d_6): δ 0.78 (3H, d, *J* = 6.8 Hz), 0.79 (3H, d, *J* = 6.3 Hz), 0.85 (3H, d, *J* = 6.4 Hz), 0.9–0.95 (1H, m), 1.35–1.5 (2H, m), 2.10 (1H, m), 2.57 (3H, d, *J* = 4.6 Hz), 2.6–2.7 (1H, m), 5.47 (1H, d, *J* = 8 Hz), 7.2–7.4 (5H, m), 8.07 (1H, d, *J* = 4.6 Hz), 8.72 (1H, d, *J* = 8 Hz), 10.42 (1H, s). Anal. calcd for $C_{18}H_{27}N_3O_4$: C, 61.87; H, 7.79; N, 12.03; found: C, 61.70; H, .79; N, 11.97.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*R*)-methylsuccinyl]-L-phenylglycine-*N*-methylamide (15f). A diastereomer mixture of **14e** and **14f**, which was obtained from the filtrate of **14e**, was hydrogenated in MeOH with 10% Pd/C under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The crude crystals were purified by preparative HPLC (GL SCIENCE Inertsil PREP-ODS 20 × 250 mm, eluent: 0.1% aqueous TFA/CH_3CN = 2/1) to give **15f** as colorless crystals: mp 176–173 °C; ^{1}H NMR (DMSO-d_6): δ 0.80 (3H, d, *J* = 6.5 Hz), 0.83 (3H, d, *J* = 6.5 Hz), 0.93 (3H, d, *J* = 7.0 Hz), 1.0–1.2 (1H, m), 1.35–1.55 (2H, m), 2.3–2.4 (1H, m), 2.57 (3H, d, *J* = 4.6 Hz), 2.5–2.65 (1H, m), 5.35 (1H, d, *J* = 7.8 Hz), 7.2–7.4 (5H, m), 8.17 (1H, d, *J* = 4.6 Hz), 8.75 (1H, s) 10.44 (1H, s). Anal. calcd for $C_{18}H_{27}N_3O_4$: C, 61.87; H, 7.79; N, 12.03; found: C, 61.48; H, 7.70; N, 11.89.

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutyl-3-(morpholinomethyl)succinyl]-L-phenylglycine-*N*-methylamide (14a). A mixture of compound **12a** (12 g, 36.1 mmol) and morpholine (100 mL) was stirred at 40–45 °C for 2 days. After evaporation of morpholine under reduced pressure, the residue was dissolved in 5% aqueous $NaHCO_3$ solution (300 mL), washed with ether. The water layer was acidified to pH 1 with 2N HCl and concentrated to one-fifth of the volume under reduced pressure, and then the acidic layer was extracted with chloroform (300 mL × 5). The extracts were dried over $MgSO_4$ and evaporated to afford **13a** (11.6 g, 70%) as an diastereomer mixture (the ratio is 5:1.). To a solution of **13a** (2.65 g, 5.81 mmol) in DMF (25 mL) and dichloromethane (50 mL), were added HOBT (1.0 g, 7.40 mmol), *O*-benzylhydroxylamine hydrochloride (1.08 g, 6.8 mmol), 1-(3-(dimethylamino)propyl)-3-ethycarbodiimide (1.29 g, 6.73 mmol) and *N*-methylmorpholine (1.2 mL, 8.7 mmol) at 0–5 °C. The mixture was stirred at room temperature overnight. After the solvents were evaporated under reduced pressure, the resultant residue was dissolved in chloroform, washed with water and aqueous saturated $NaHCO_3$ solution successively, dried over $MgSO_4$, and then evaporated in vacuo. The crude crystals were recrystallized from methanol–ether to give a single diastereomer **14a** (1.56 g, 51%) as colorless crystals: ^{1}H NMR (DMSO-d_6): δ 0.77 (3H, d, *J* = 6.4 Hz), 0.82 (3H, d, *J* = 6.4 Hz), 0.85–0.9 (1H, m), 1.35–1.45 (2H, m), 1.73 (1H, dd, *J* = 3.1 Hz, 11.8 Hz), 1.9–2.0 (2H, m), 2.55–2.7 (2H, m), 2.57 (3H, d, *J* = 4.6 Hz), 3.3–3.5 (4H, m), 4.76 (1H, d, *J* = 11.3 Hz), 4.81 (1H, d, *J* = 11.3 Hz), 5.45 (1H, d, *J* = 8 Hz), 7.2–7.45 (10H, m), 8.1 (1H, q, *J* = 4.6 Hz), 8.71 (1H, d, *J* = 8 Hz), 11.0 (1H, s).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutyl-3(*R*)-(4-methylpiperazino)methyl-succinyl]-L-phenylglycine-*N*-methylamide (14c). To a solution of compound **12a** (500 mg, 1.5 mmol) in DMF (5 mL) were added $NaHCO_3$ (262 mg, 3 mmol) and benzyl bromide (300 mg, 1.18 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with chloroform, washed with water and dried over $MgSO_4$. The chloroform solution was evaporated in vacuo to give benzyl ester **16a** (573 mg, 90%) as a colorless solid. This ester (500 mg, 1.18 mmol) was heated with 1-methylpiperazine (355 mg, 3.54 mmol) at 60 °C for 1 h and the residue was purified by preparative TLC to afford **17c** (341 mg, 55%) as colorless crystals. After the debenzylation of **17c** (340 mg, 0.65 mmol) in MeOH by hydrogenation, the resulting **13c** was allowed to react with *O*-benzylhydroxylamine in a same manner as described above to give **14c** (250 mg, 71.5%) as colorless crystals: ^{1}H NMR(DMSO-d_6): δ 0.77 (3H, d, *J* = 6.4 Hz), 0.83 (3H, d, *J* = 6.4 Hz), 1.05 (1H, m), 1.38 (2H, m), 1.72 (1H, dd, *J* = 3.1 Hz, 12.1 Hz), 1.9 (2H, m), 2.05 (3H, s), 2–2.4 (8H, m), 2.53 (3H, d, *J* = 12.8 Hz), 2.6 (1H, m), 4.76 (2H, q, *J* = 11 Hz), 5.44 (1H, d, *J* = 8.0 Hz), 7.2–7.5 (10H, m), 8.04 (1H, d, *J* = 4.6 Hz), 8.63 (1H, d, *J* = 8.0 Hz), 10.98 (1H, s).

Compounds **14g** and **14h** were prepared in the same manner as compound **14c**. Their physical data are summarized as follows.

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutyl-3(*R*)-morpholinomethylsuccinyl]-L-3-(1-naphthyl)alanine-*N*-methylamide (14g). ^{1}H NMR (DMSO-d_6): δ 0.72 (3H, d, *J* = 6.4 Hz), 0.81 (3H, d, *J* = 6.4 Hz), 0.8–0.9 (1H, m), 1.2–1.35 (3H, m), 1.78 (2H, br), 2.1–2.35 (6H, m), 2.57 (3H, d, *J* = 4.5 Hz), 3.3–3.45 (8H, m), 4.6–4.8 (3H, m), 7.3–7.6 (9H, m), 7.78 (2H, d, *J* = 7.5 Hz), 7.9 (1H, dd, *J* = 9.2 Hz, 1.4 Hz), 8.22 (1H, d, *J* = 8.1 Hz), 8.35 (1H, d, *J* = 8.2 Hz), 10.93 (1H, br).

[4-(*N*-Benzyloxyamino)-2(*R*)-isobutyl-3(*R*)-(1,2,3,4-tetrahydroisoquinolin-2-yl)-methylsuccinyl]-L-3-(1-naphthyl)alanine-*N*-methylamide(14h). ^{1}H NMR (DMSO-d_6): δ 0.74 (3H, d, *J* = 6.4 Hz), 0.83 (3H, d, *J* = 6.4 Hz), 1.3–1.5 (3H, m), 2.0–2.1 (1H, m), 2.2–2.3 (1H, m), 2.35–2.45 (2H, m), 2.54 (3H, d, *J* = 4.5 Hz), 2.6–2.65 (2H, m), 2.8–3.1 (3H, m), 3.2–3.5 (3H, m), 4.6–4.7 (3H, m), 6.9–7.1 (3H, m), 7.15–7.3 (5H, m), 7.7–7.9 (3H, m), 7.95 (1H, s), 8.23 (1H, d, *J* = 8.3 Hz), 8.37 (1H, d, *J* = 9 Hz), 10.93 (1H, s).

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*R*)-(morpholinomethyl)succinyl]-L-phenylglycine-*N*-methylamide (15a). Compound **14a** (1.5g, 2.86 mmol) was hydrogenated in MeOH (40 mL) with 10% Pd/C (0.1 g) at room temperature. The catalyst was removed by filtration,

and the filtrate was evaporated in vacuo. The residue was recrystallized from MeOH and ethyl ether to give **15a** (1.06 g, 85.8%) as colorless crystals: mp 216–217 °C (dec.); $[\alpha]_D$ +85.1° (*c* 0.2; MeOH); ^{1}H NMR (DMSO-d_6): δ 0.78 (3H, d, *J* = 6.4 Hz), 0.83 (3H, d, *J* = 6.4 Hz), 0.85–0.97 (1H, m), 1.35–1.46 (2H, m), 1.72 (1H, dd, *J* = 3 Hz, 11.8 Hz), 1.90–1.92 (2H, m), 2.15–2.35 (3H, m), 2.56 (3H, d, *J* = 4.6 Hz), 2.5–2.57 (2H, m), 3.4–3.45 (4H, m), 5.47 (1H, d, *J* = 8 Hz), 7.2–7.45 (5H, m), 8.10 (1H, d, *J* = 4.6 Hz), 8.66 (1H, d, *J* = 8 Hz), 8.77 (1H, d, *J* = 1.7 Hz), 10.36 (1H, d, *J* = 1.7 Hz). Anal. calcd for $C_{19}H_{29}N_3O_4$ H_2O: C, 58.39; H, 8.02; N, 12.38; found: C, 58.43; H, 7.98; N, 12.39.

15a HCl salt. mp 150–160 °C (dec.); $[\alpha]_D$ +88.4° (*c* 0.2; MeOH); ^{1}H NMR (DMSO-d_6): δ 0.81 (3H, d, *J* = 6.4 Hz), 0.85 (3H, d, *J* = 6.4 Hz), 0.95–1.1 (1H, m), 1.4–1.55 (2H, m), 2.57 (3H, d, *J* = 4.6 Hz), 2.6–3.2 (7H, m), 3.5–3.9 (5H, m), 5.43 (1H, *J* = 7.7 Hz), 7.2–7. 5 (5H, m), 8.20 (1H, q, *J* = 4.6 Hz), 8.92 (1H, d, *J* = 7.7 Hz), 10.37 (1H, s), 10.84 (1H, s). Anal. calcd for $C_{19}H_{29}N_3O_4$ H_2O HCl: C, 54.04; H, 7.63; N, 11.46; found: C, 53.76; H, 0.44; N, 11.34.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*R*)-(1,2,3,4-tetrahydroisoquinolin-2-yl)-methylsuccinyl]-L-phenylglycine-*N*-methylamide (15b). To a solution of compound **12a** (500 mg, 1.5 mmol) in DMF (5 mL) were added $NaHCO_3$ (262 mg, 3 mmol) and benzyl bromide (300 mg, 1.18 mmol) and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with chloroform, washed with water and dried over $MgSO_4$. The chloroform solution was evaporated in vacuo to give benzyl ester **16a** (573 mg, 90%) as a colorless solid. This ester (500 mg, 1.18 mmol) was heated with 1,2,3,4-tetrahydroisoquinoline (470 mg, 3.54 mmol) at 60 °C for 1 h and the residue was purified by preparative TLC to afford **17b** (435 mg, 66.3%), as colorless crystals. After the de-benzylation of **17b** (435 mg, 0.78 mmol) in MeOH by hydrogenation, the resulting **13b** was reacted with *O*-benzylhydroxylamine in the same manner as described above to give **14b** (313 mg, 70%) as colorless crystals. Compound **14b** was hydrogenated in a similar manner to afford **15b** (200 mg, 76.6%) as colorless crystals: mp 199–201 °C (dec.); ^{1}H NMR (DMSO-d_6): δ 0.80 (3H, d, *J* = 6.4 Hz), 0.85 (3H, d, *J* = 6.4 Hz), 0.94 (1H, m), 1.48 (2H, m), 1.89 (1H, m), 2.21 1H, m), 2.57 (3H, d, *J* = 4.5 Hz), 2.5–2.8 (4H, m), 3.13 (1H, d, *J* = 14.4 Hz), 3.3–3.5 (2H, m), 545 (1H, d, *J* = 7.9 Hz), 6.9–7.2 (4H, m), 7.2–7.5 (5H, m), 8.1 (1H, m), 8.7 (1H, m), 10.39 (1H, br s). Anal. calcd for $C_{27}H_{36}N_4O_4$: C, 67.48; H, 7.55; N, 11.66; found: C, 67.04; H, 7.44; N, 11.34.

Compounds **15c**, **15d**, **15g**, **15h**, and **15i** were prepared in the same manner as compound **15a**. Their physical data are summarized as follows.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*R*)-(4-methylpiperazino)methyl-succinyl]-L-phenylglycine-*N*-methylamide (15c). mp 175 °C (dec.); ^{1}H NMR (DMSO-d_6): δ 0.8 (3H, d, *J* = 6.4 Hz), 0.82 (3H, d, *J* = 6.4 Hz), 0.98 (1H, m), 1.48 (2H, m), 2.2 (1H, m), 2.57 (3H, d, *J* = 4.5 Hz), 2.72 (3H, s), 2.7 (2H, m), 2.8–3.2 (2H, m), 3.3–3.5 (2H, m), 5.44 (1H, d, *J* = 7.8 Hz), 7.2–7.5 (10H, m), 8.18 (1H, d, *J* = 4.6 Hz), 8.75 (1H, d, *J* = 7.8 Hz), 10.63 (1H, s), 10.9 (1H, bs). Anal. calcd for $C_{23}H_{37}N_5O_4$ $3H_2O$ 2HCl: C, 48.08; H, 7.89; N, 12.19; found: C, 47.77; H, 7.47; N, 12.08.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*R*)-morpholinomethylsuccinyl]-L-3-(1-naphthyl)alanine-*N*-methylamide hydrochloride (15g). mp 185–187 °C (dec.); ^{1}H NMR (DMSO-d_6): δ 0.74 (3H, d, *J* = 6.4 Hz), 0.78 (3H, d, *J* = 6.4 Hz), 0.9–1.0 (1H, m), 1.2–1.5 (2H, m), 2.5–2.8 (4H, m), 2.55 (3H, d, *J* = 4.4 Hz), 3.0–3.4 (4H, m), 3.6–3.9 (4H, m), 4.6–4.7 (1H, m), 7.4–7.6 (4H, m), 7.9–8.0 (1H, m), 7.93 (1H, d, *J* = 8 Hz), 7.97 (1H, d, *J* = 4.6 Hz), 8.28 (1H, d, *J* = 8.1 Hz), 8.56 (1H, d, *J* = 8.2 Hz), 9.0 (1H, br), 10.40 (1H, Br), 10.85 (1H, s). Anal. calcd for $C_{27}H_{38}N_4O_5$ HCl: C, 60.61; H, 7.35; N, 10.47; found: C, 60.24; H, 7.41; N, 10.32.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*R*)-(1,2,3,4-tetrahydroisoquinolin-2-yl)-methylsuccinyl]-L-3-(1-naphthyl)-alanine-*N*-methylamide hydrochloride (15h). mp 147–153 ° C (dec); ^{1}H NMR (DMSO-d_6): δ 0.76 (3H, d, *J* = 6.4 Hz), 0.79 (3H, d, *J* = 6.4 Hz), 0.9–1.05 (1H, m), 1.2–1.8 (4H, m), 2.50 (3H, d, *J* = 4.7 Hz), 2.5–3.3 (8H, m), 3.8–4.1 (1H, m), 4.1–4.3 (1H, m), 4.65 (1H, dd, *J* = 8.0 Hz, 8.1 Hz), 6.8–8.0 (10H, m), 8.28 (1H, d, *J* = 8.3 Hz), 8.62 (1H, br), 9.09 (1H, br), 10.46 (1H, br), 10.89 (1H, br). Anal. calcd for $C_{32}H_{40}N_4O_4$ HCl: C, 66.14; H, 7.11; N, 9.64; found: C, 65.76; H, 7.21; N, 9.56.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*S*)-methylsuccinyl]-L-3-(1-naphthyl)alanine-*N*-methylamide (15i). [4-(*N*-Benzyloxyamino)-2(*R*)-isobutyl-3(*S*)-methylsuccinyl]-L-3-(1-naphthyl)alanine-*N*-methylamide (**14i**) was prepared in the same manner as compound **14e.** The resulting **14i** was hydrogenated in a similar manner to give **15i** as colorless crystals; mp 212–213 °C (dec.); ^{1}H NMR (DMSO-d_6): δ 0.39 (3H, d, *J* = 6.7 Hz), 0.73 (3H, d, *J* = 6.4 Hz), 0.81 (3H, d, *J* = 6.4 Hz), 0.8–0.9 (1H, m), 1.2–1.45 (2H, m), 1.85–2.0 (1H, m), 2.3–2.5 (1H, m), 2.57 (3H, d, *J* = 4.5 Hz), 3.2–3.6 (2H, m), 4.6–4.75 (1H, m), 7.3–7.65 (4H, m), 7.7–7.8 (2H, m), 7.9 (1H, d, *J* = 7.9 Hz), 8.22 (1H, d, *J* = 8.8 Hz), 8.34 (1H, d, *J* = 8.4 Hz), 8.7 (1H, br), 10.35 (1H, s). Anal. calcd for $C_{23}H_{31}N_3O_4$: C, 68.81; H, 7.56; N, 10.16; found: C, 68.65; H, 7.62; N, 10.11.

[4-(*N*-Hydroxyamino)-2(*R*)-isobutyl-3(*S*)-phenylthiomethylsuccinyl]-L-phenylglycine-*N*-methylamide (15d). The suspension of compound **12a** (700 mg, 2.1 mmol) and thiophenol (5 mL) was stirred under argon at 60 °C for 3 days. After addition of ethyl ether:*n*-hexane (60 mL:20 mL), the precipitated solid was collected by filtration and washed with ethyl ether/EtOAc to give **13d** (380 mg, 40.7%) as a colorless solid. To a solution of **13d** (380 mg, 0.86 mmol) in DMF were added HOBT (145 mg, 1.07 mmol) and 1-(3-(dimethylamino)propyl)-1-ethylcarbodiimide (180 mg, 0.94 mmol) at 0–5 °C and the mixture was stirred at same temperature for 2 h. The solution of

hydroxylamine hydrochloride (82 mg, 1.18 mmol) and *N*-methylmorpholine (120 mg, 1.19 mmol) in DMF (3 mL) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc, and washed with 1N HCl, dried over $MgSO_4$ and evaporated in vacuo. The crude solid was recrystallized from isopropanol to afford **15d** (84 mg, 21.4%) as colorless crystals: mp 218–211 °C (dec); ^{1}H NMR (DMSO-d_6): δ 0.80 (3H, d, J = 6.4 Hz), 0.85 (3H, d, J = 6.4 Hz), 0.88–1.0 (1H, m), 1.32–1.53 (2H, m), 2.26–2.37 (1H, m), 2.53–2.61 (1H, m), 2.57 (3H, d, J = 4.5 Hz), 2.72–2.83 (1H, m), 2.93–3.93 (1H, m), 5.53 (1H, d, J = 8.2 Hz), 6.92–6.97 (2H, m), 7.09–7.32 (6H, m), 7.43–7.49 (2H, m), 8.12–8.18 (1H, m), 8.91(1H, s), 8.95 (1H, d, J = 8.2 Hz), 10.58 (1H, s) . Anal. calcd for $C_{24}H_{31}N_3O_4S$: C, 63.00; H, 6.83; N, 9.18; found: C, 62.95; H, 7.86; N, 9.15.

MMP inhibition assay

MMP-1 and -9 were isolated from the human cancer cell line, HT1080, and purified to homogeneity at Kanebo Biochemical Research Center as described before.[17] Enzymatic activities were measured by following the cleavage of the substrate, the fluorescence isothiocyanate-collagens (FITC-collagen) for MMP-1 and the fluorescence isothiocyanate-gelatin (FITC-gelatin) for MMP-9, described by Nagai et al.[18] MMP-1, activated by trypsin, and FITC-collagen were incubated in 50 mM tris-HCl buffer (pH 7.5) containing 0.2 M NaCl, 5 mM $CaCl_2$, 0.05% Briji and 0.02% NaN_3 at 35.5 °C for 16–18 h. The incubation was terminated by the addition of 3 volumes of stop solution (0.1 M Tris-HCl buffer pH 8.8 containing 0.4 M NaCl, 3 mM $CaCl_2$, 0.01% NaN_3/ethanol=8/7). The digested fragments were separated by centrifugation and the fluorescence intensity (Ex 495 nm, Em 520 nm) was measured. Percentage inhibition of collagen degradation was calculated from the difference of fluorescence intensity between the presence and absence of test compounds. MMP-9 activity was measured by the method of Nagai et al.[18] Activated MMP-9 and FITC-labeled gelatin were incubated in 50 mM tris-HCl buffer (pH 7.5) containing 0.2 M NaCl, 5 mM $CaCl_2$, 0.05% Briji and 0.02% NaN_3 at 35.5 °C for 16–18 h. The reaction was terminated by the addition of equal volume of 22% TCA and the supernatant was isolated. Fluorescence intensity of the supernatant was measured after adjusting the pH to neutral condition and percentage inhibition was determined as described in MMP-1 assay. The IC_{50} was calculated from a least-squares fit of the inhibition % and inhibitor concentration.

MMP-1 inhibitory activities in mice plasma after oral administration

Male ddy mice (4-week-old) were purchased from SLC (Hamamatsu, Japan). Test compounds were suspended in 0.5% carboxymethyl cellulose (CMC) and orally administered to three mice per group (30 mg/kg). After 1, 3, and 6 h, the blood was collected from abdominal vein in the presence of heparin (final concn 10 U/ml) under ether anesthesia. Plasma was separated by centrifugation and was added to 0.5 M tris-HCl buffer (pH 7.5), and then heated at 100 °C for 10 min. Denatured protein was removed by centrifugation and the supernatant was filtered through an ultrafiltration unit (Molecut II). Then, the filtrate was added to the MMP-1 assay system and inhibitory activity in plasma was determined as described above.

Rat adjuvant arthritis model

Male Lewis rats (seven-week-old) were purchased from Charlesriver Inc. (Tokyo, Japan). Arthritis was induced in male Lewis rats by intradermal injection of 600 μg of heat killed *Mycobacterium butyricum* suspended in 0.1 ml of mineral oil into the subplantar region of the right hind paw. Immediately after the adjuvant injection, test compounds, suspended in 0.5% CMC, were administered at 100 mg/kg orally. Thereafter, test compounds were administered at every 12 h for 20 days. The effects of test compounds were evaluated by left hind foot pad volume and erythrocyte sedimentation ratio. Left hind foot pad volume was determined by plethysmometer just before and on days 3, 7, 10, 14, 17 and 20 after the adjuvant injection. On the 21st day, blood was drawn from abdominal aorta into a syringe containing 1/5 volume of 3.28% sodium citrate under ether anesthesia. Erythrocyte sedimentaion was determined using Matsuyoshi's erythrocyte sedimentation rack and Ca^{2+} concentrations in serum were measured using Calcium C test Wako (Wako Pure Chemical Industries, Ltd.).

Acknowledgments

We thank Dr Shintaro Inoue for the preparation of MMP-1, -9, and their substrates, and Dr Hideki Tsujishita for the molecular modeling studies.

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(*Received in Japan* 30 *October* 1996; *accepted* 21 *January* 1997)