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Discovery of a new chemical lead for a matrix metalloproteinase inhibitor

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Abstract—A series of *N*-benzoyl γ-aminobutyric hydroxamic acids were synthesized and evaluated as matrix metalloproteinase inhibitors. First, we focused on chemical modification of the *N*-benzoyl residue. Introduction of electron-rich *para*-substituents was effective to increase the inhibitory activity. Especially, some of the analogs with relatively more planar *N*-acyl residues, such as **10** and **11**, demonstrated more potent activity. Second, chemical modification of the γ-aminobutyric hydroxamic acid moiety was carried out to optimize the three-dimensional arrangement of the two pharmacophores (hydroxamic acid and *N*-acyl residues). Among the tested, the γ-aminobutyric hydroxamic acid moiety was found to be the best spacer for connecting the above-mentioned two pharmacophores. Synthesis and structure–activity relationships are discussed.

1. Introduction

Matrix metalloproteinases (MMPs) are produced by fibroblasts, neutrophils, and macrophages, as well as by tumor cells.[1] These enzymes are postulated to regulate the homeostasis of a variety of tissues under the control of tissue inhibitor of metalloproteinases (TIMPs), which bind to and block the activity of MMPs. As a result, a stoichiometric imbalance between MMPs and TIMPs could theoretically result in a variety of pathological states, ranging from tumor metastasis to rheumatoid arthritis and multiple sclerosis.[1]

At least 20 members of this enzyme family, which share significant sequence homology, have been reported.[1,2] They can be divided into (1) collagenases (MMP-1, -8, -13, and -18), (2) gelatinases (MMP-2 and -9), (3) stromelysins (MMP-3, -10, and -11), and (4) membrane-type MMPs (MT-MMPs) (MMP-14, -15, -16, and -17).

For more than 30 years, MMPs have been heralded as promising targets for the treatment of the above-mentioned types of diseases. A large number of synthetic MMP inhibitors (MMPIs) have been reported. Depending upon their zinc-binding, MMPIs can be assigned to several classes, such as the carboxylic acids, hydroxamic acids, thiols, phosphorus-based ligands, or sulfodiimines, among others.[1a]

In the preceding paper,[3] we reported on the fragment-based generation of a new chemical lead using the in silico technique. Search of a standard LUDI library,[6] followed by conventional molecular design, resulted in lead structure **1** that was targeted for further optimization. Here we report on the discovery of a series of *N*-acyl γ-aminobutyric hydroxamic acids, **10** and **11** (Fig. 1), that are effective MMP inhibitors.

2. Chemistry

Synthesis of the test compounds listed in Tables 1–4 is outlined in Schemes 1–4. Synthesis of compounds **1**–**16** is described in Scheme 1. Condensation of **21b**–**m** and **21p** with *O*-2-methoxypropane hydroxylamine, followed by acidic deprotection, afforded **2**–**13** and **16**, respectively. Condensation of **21a**, **21n**, and **21o** with *O*-benzylhydroxyamine, followed by catalytic hydrogenation, resulted in **1**, **14**, and **15**, respectively.

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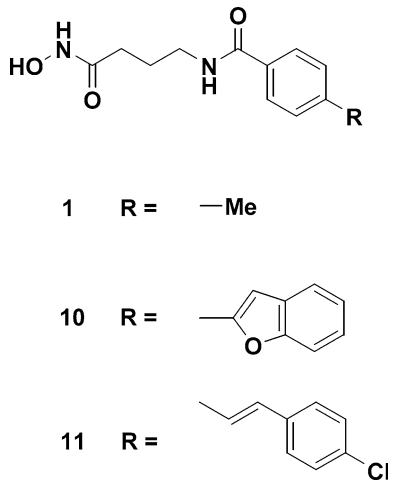


Figure 1. A new class of inhibitors of matrix metalloproteinases.

Synthesis of **17** is described in Scheme 2. N-Methylation of **22**[5] with methyl iodide in the presence of sodium hydride gave **23**. Alkaline hydrolysis of **23**, followed by the condensation with *O*-2-methoxypropane hydroxylamine, provided hydroxamic acid **17**.

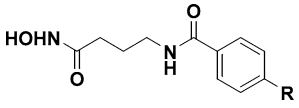
Compound **18** was prepared from **25** as outlined in Scheme 3. O-Alkylation of **25** with ethyl 5-bromopentanoate in the presence of potassium carbonate yielded **26**, after which catalytic hydrogenation resulted in **27**. Palladium-catalyzed coupling of trifluoromethane sulfonate **28**, which was prepared from **27** using trifluoromethanesulfonic anhydride, with the boric acid **29** resulted in **30**. Alkaline hydrolysis of **30** gave **31**, which was converted to the hydroxamic acid **18** by the condensation with *O*-2-methoxypropane hydroxylamine.

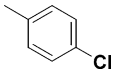
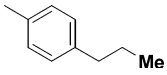
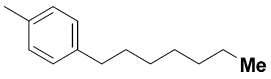
Synthesis of **19** and **20** is described in Schemes 4A and B, respectively. A key intermediate **36** for the synthesis of both compounds **19** and **20** was derived from the malic acid dimethyl ester **32**. Chemoselective reduction of **32** with borane dimethyl sulfide complex afforded the diol **33**.[4] Tosylation of **33**, followed by S_N2 substitution of the formed tosylate **34** with sodium azide, produced the azide **35**. Catalytic hydrogenation of **35**, followed by N-acylation with an acid chloride, afforded **36**. O-Alkylation of **36** with chloromethyl methyl ether in the presence of diisopropylethylamine resulted in **37**. Treatment of **37** with aqueous sodium hydroxide gave an elimination product **38**, which was converted to the hydroxamic acid **19** by the condensation with *O*-2-methoxypropane hydroxylamine. Compound **39** was prepared by O-acylation of **36** with an acetic anhydride in pyridine. Another elimination product **40** was obtained by treatment of the acetate **39** with DBU. Alkaline hydrolysis of **40** led to **41**, which was converted to a hydroxamic acid **20** by condensation with *O*-2-methoxypropane hydroxylamine.

3. Results and discussion

The compounds listed in Tables 1–4 were tested for their inhibitory activity against MMP-1 (human collagenase), MMP-2 (human gelatinase A), MMP-9 (human gelatinase B), and MMP-3 (recombinant human stromelysin). Since the discovery of the *N*-benzoyl γ-aminobutyric hydroxamic acid **1** as a new chemical lead for an MMP-2 and MMP-9 inhibitor, further chemical modification of **1** to identify superior structure has been continued.

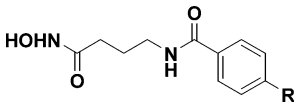
Table 1. Effect of the *para*-substituent of *N*-benzoyl residue on the activity profiles

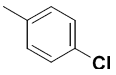
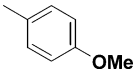
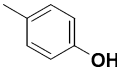
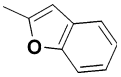
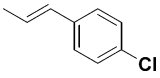


Compound	R	IC_{50} (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
1		59%[a]	18%[a]	40%[a]	NT[b]
2		>100%[a]	65%[a]	NT[b]	>100
3		>100	49%[a]	NT[b]	>100
4		NT[b]	0.038	NT[b]	2.8
5		>100	0.13	NT[b]	16
6		NT[b]	2.2	NT[b]	17

[a] Inhibition percentage at 100 μM.
[b] Not tested.

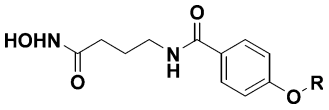
Table 2. Effect of the *para*-substituent of *N*-benzoyl residue on the activity profiles

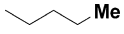
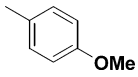
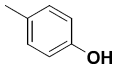
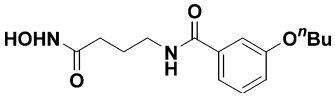


Compound	R	IC_{50} (μM)				
		MMP-1	MMP-2	MMP-9	MMP-3	MMP-3/MMP-2
4		NT[b]	0.038	NT[b]	2.8	73
7		21.1	0.058	NT[b]	3.8	65
8		NT[b]	0.13	NT[b]	16	123
9		59%[a]	0.801	NT[b]	34%[a]	—
10		53%[a]	0.0029	NT[b]	1.8	620
11		>100	0.0071	0.052	0.83	117

[a] Inhibition percentage at 100 μM.
[b] Not tested.

Table 3. Effect of the *para*-alkoxy substituent at *N*-benzoyl residue on the activity profiles



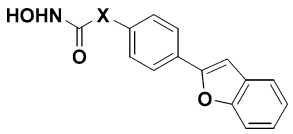
Compound	R	IC_{50} (μM)				
		MMP-1	MMP-2	MHP-9	MMP-3	MMP-3/MMP-2
12		>100	0.75	NT[a]	19	25
13		NT[a]	0.038	NT[a]	2.9	76
14		NT[a]	0.032	0.011	16	500
15		NT[a]	0.028	0.043	13	460
16		>100	>100	NT[a]	>100	—

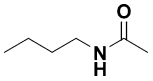
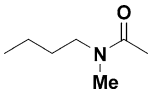
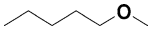
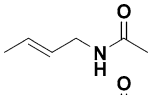
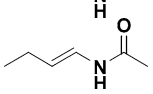
[a] Not tested.

As described in Figure 2, optimization of the *N*-benzoyl residue was expected to be the simplest way to obtain an increase of MMP inhibitory activity. Accordingly, chemical modification was started with optimization of the *para*-substituent of the *N*-benzoyl residue of **1**. Second, optimization of the γ-aminobutyric hydroxamic acid moiety of **1** was performed to find the best spacer for connecting the hydroxamic acid and the *N*-benzoyl residue.

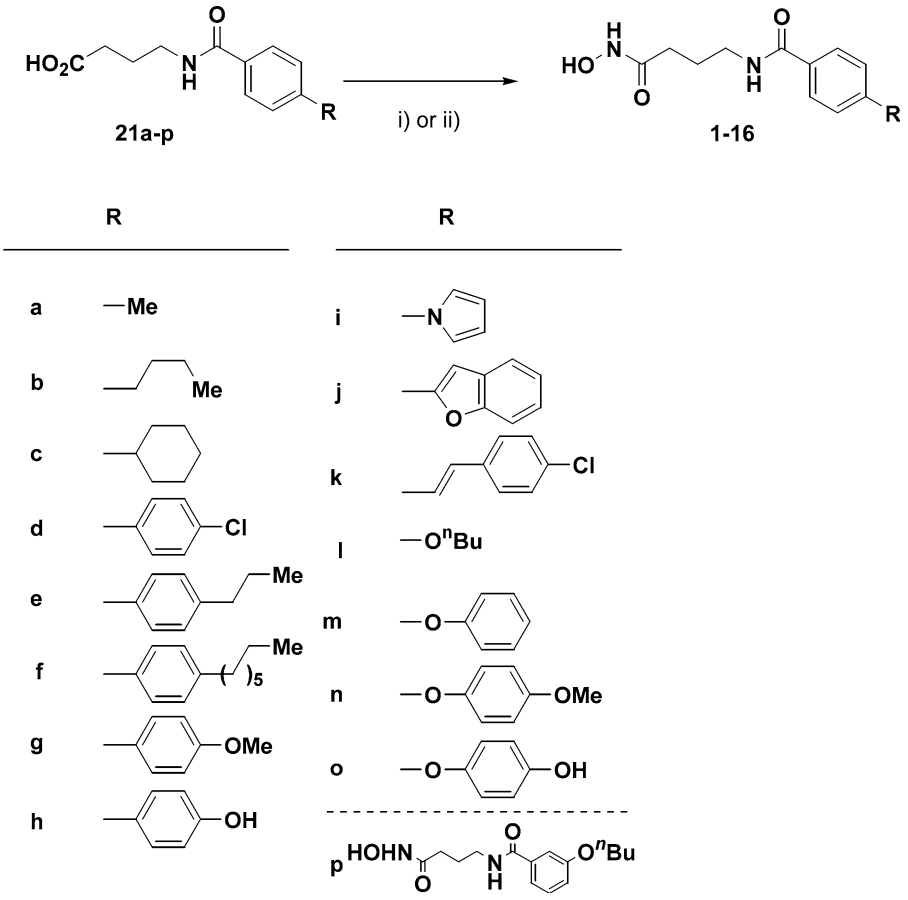
Optimization of the *para*-methyl residue of the *N*-benzoyl moiety of **1** was carried out as shown in Table 1. Chemical modification was focused on identification of a partial structure that was optimal for the *S*1′ pocket

Table 4. Effect of the chemical modification of the 4-aminobutyric acid moiety of 10 on the activity profiles



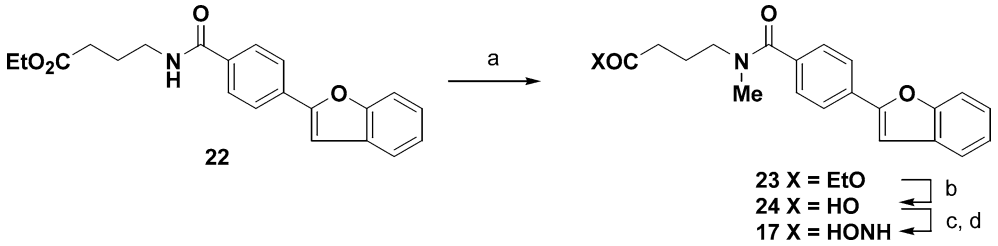
Compound	X	IC_{50} (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
10		53%[a]	0.0029	NT[b]	1.8
17		>100	1.4	NT[b]	9.9
18		>10	>0.1	NT[b]	>100
19		>100	0.81	NT[b]	35
20		>100	0.47	NT[b]	17

[a] Inhibition percentage at 100 μM.
[b] Not tested.

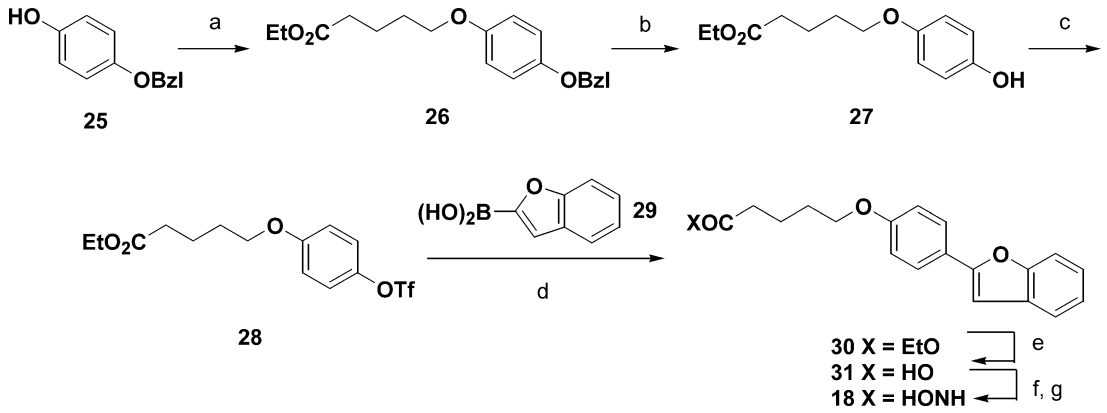


Scheme 1. Synthesis of **1**–**16**. Reagents: (i) $MeOCMe_2ONH_2$, EDC·HCl, $HOBt·H_2O$, Et_3N or $^iPr_2NEt$, DMF then HCl, MeOH; (ii) $BzlONH_2·HCl$, EDC·HCl, $HOBt·H_2O$, Et_3N, DMF then H_2, Pd–C, MeOH, THF.

(Fig. 2) and subtype selectivity. As illustrated by the results of evaluation of **2** and **3**, introduction of a larger *para*-alkyl residue slightly improved the extent of inhibition of MMP-2. Replacement of the *para*-methyl residue of the *N*-benzoyl moiety of **1** with a *para*-chlorophenyl moiety gave **4** which showed a marked increase of both



Scheme 2. Synthesis of **17**. Reagents: (a) MeI, NaH, DMF; (b) NaOH, MeOH; (c) $MeOCMe_2ONH_2$, EDC·HCl, HOBt·H_2O, DMF; (d) HCl, MeOH.

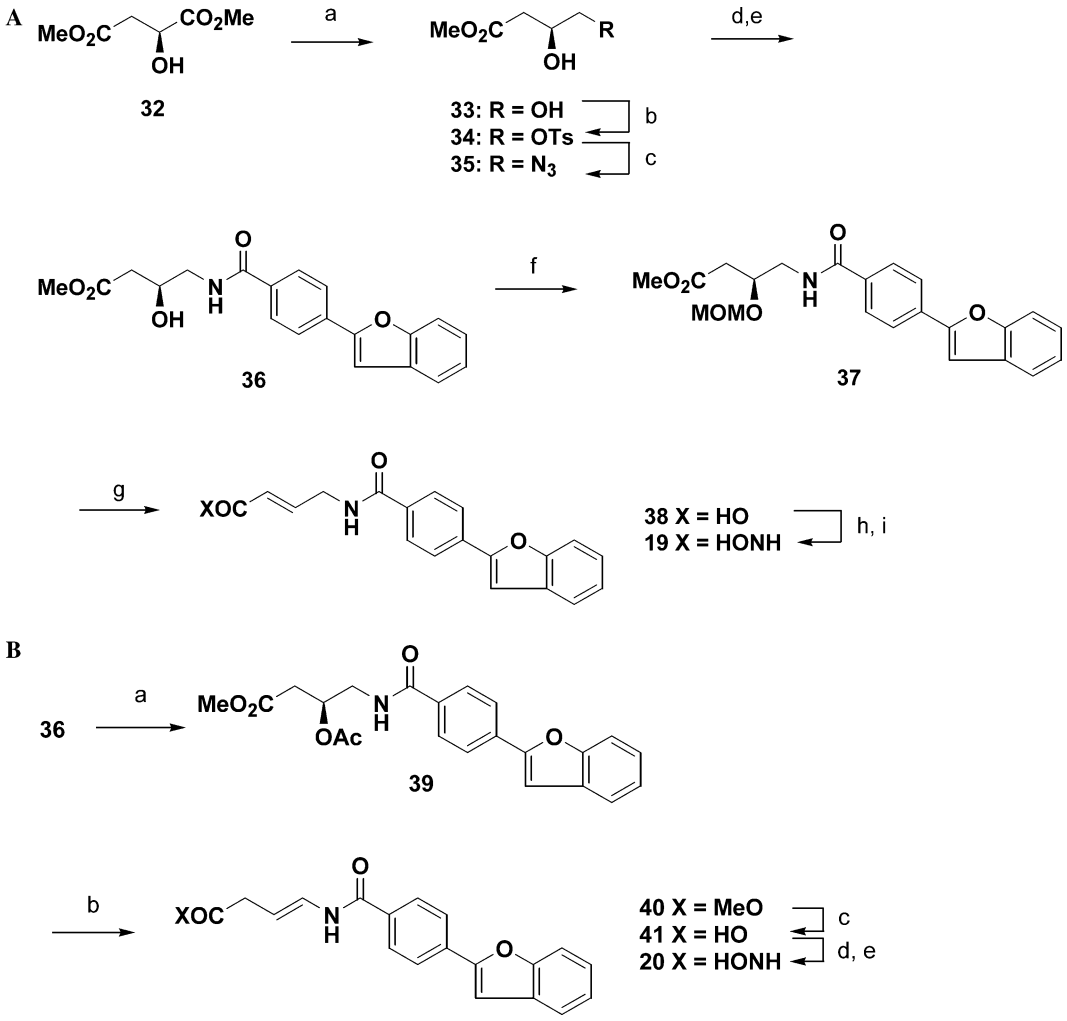


Scheme 3. Synthesis of **18**. Reagents: (a) $EtO_2C(CH_2)_4$ Br, K_2CO_3, DMF; (b) H_2, Pd–C, MeOH; (c) Tl_2O, pyridine; (d) $Pd(PPh^3)_2Cl_2$, Et_3N, OMF; (e) NaOH, THF, MeOH; (f) $MeOCMe_2ONH_2$, EDC·HCl, HOBt H_2O, DMF; (g) HCl, MeOH.

MMP-2 inhibition and MMP-3 inhibition. Replacement of the *para*-chloro residue of **4** with *para*-alkyl residues, such as *n*-propyl and *n*-heptyl residues, led to **5** and **6**, respectively, with a decrease of inhibitory activity for both MMP-2 and MMP-3. These results indicated that the more electron-rich *para*-aryl moieties **4**–**6** were superior to the *para*-alkyl residues **1**–**3** as a *para*-substituent R that was optimum for the S1′ pocket. In particular, a *para*-chlorophenyl residue performed best among the *para*-aryl residues tested.

Further chemical modification of the *para*-substituent R was continued as shown in Table 2. Based on the marked increase of inhibitory activity shown by the *para*-chlorophenyl analog **4**, *para*-methoxyphenyl and *para*-hydroxyphenyl analogs **7** and **8** were also synthesized and evaluated. The *para*-methoxyphenylbenzoyl analog **7** showed stronger inhibitory activity than the *para*-hydroxyphenylbenzoyl analog **8**, while the inhibitory activity of **7** for MMP-2 and MMP-3 was close to that of **4**. Among the heteroaryl analogs, such as **9** and **10**, that were tested, **10** showed significantly stronger inhibitory activity and greater selectivity relative to **4** and **5**, although the pyrrole analog **9** showed significantly weaker activity relative to **4** and **7**. Based on the significant increase of potency and subtype selectivity of **10**, the *para*-chlorocinnamyl analog **11** was also prepared and evaluated. Compound **11** demonstrated slightly less potent inhibition and lower selectivity (MMP-3/MMP-2 ratio) relative to **10**, that it also showed potent inhibitory activity against MMP-9. As a result, conjugated and electron-rich *para*-substituents, such as benzofuran-2-yl and *para*-chlorocinnamyl, were identified as the optimum partial structures. Based on the relatively weaker inhibitory activity of **8** and **9**, their hydrophilic nature seemed to be unsuited to the *S*1′ pocket.

Because of the equipotency of the *para*-methoxyphenyl analog **7** and the *para*-chlorophenyl analog **4**, the *para*-butoxyphenyl analog **12** and three *para*-phenoxyphenyl analogs **13**–**15** were synthesized and tested (Table 3). Replacement of the *para*-methoxyphenyl residue of **7** with a *para*-butoxyphenyl residue led to **12**, with a more than 10-fold decrease of inhibitory activity for MMP-2, while replacement of the methoxyphenyl residue with a phenoxyphenyl residue afforded **13** that retained both potent inhibitory activity and subtype selectivity (MMP-3/MMP-2 ratio). Again, an electron-rich phenoxy residue was found to be more favorable than a larger alkoxy residue. Based on the above findings, another two analogs **14** and **15** were synthesized and evaluated. Introduction of a methoxy residue at the *para*-position of the phenoxy moiety of **13** afforded **14**, which retained inhibitory activity for MMP-2 and showed decreased inhibition of MMP-3. Demethylation of the methoxy residue of **14** gave **15**, which also retained inhibitory activity for MMP-2 and showed decreased inhibition of MMP-3. Compounds **14** and **15** also showed potent inhibitory activity against MMP-9, with **14** being more potent than **15**. It was interesting that the phenol analog **15** showed stronger activity against MMP-2 than compound **8**. This was speculated to be due to the difference of three-dimensional structures between the biphenyl moiety of **8** and the *para*-phenoxybenzoyl moiety of **15**. To confirm the rationale for attempting *para*-substitution of *N*-benzoyl residues, a *meta*-^{n}butoxy isomer **16** was synthesized and evaluated.



Scheme 4. (A) Synthesis of **19**. Reagents: (a) i–BH_3·SMe_2, THF then $NaBH_4$; ii—TsOH · H_2O, MeOH; (b) TsCl, pyridine; (c) NaN_3, DMF; (d) H_2, Pd–C, MeOH then HCl; (e) 4-(1-benzofuran-2-yl)benzoyl chloride, Et_3N, CH_2Cl_2; (f) MOMCl, $^iPr_2NEt$, CH_2Cl_2; (g) NaOH, THF, MeOH; (h) $MeOCMe_2ONH_2$, EDC·HCl, HOBt·H_2O, DMF; (i) HCl, MeOH. (B) Synthesis of **20**. Reagents: (a) Ac_2O, pyridine; (b) DBU, THF; (c) NaOH, THF, MeOH; (d) $MeOCMe_2ONH_2$, EDC·HCl, HOBt H_2O, DMF; (e) HCl MeOH.

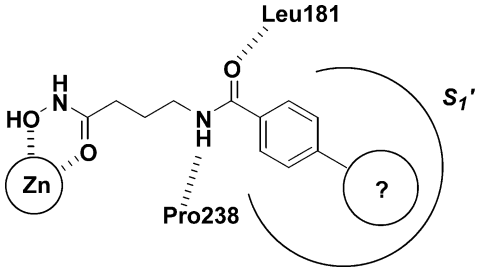


Figure 2. Design of *N*-benzoyl residue.

Compound **16** showed less than 50% inhibition of MMP-1, MMP-2, and MMP-3 at 100 μM. Thus, *para*-substitution was superior to *meta*-substitution for increasing the inhibitory activity, as illustrated by the loss of inhibitory activity with compound **16**.

Optimization of the spacer that connected the hydroxamic acid and the *N*-benzoyl residue was carried out using the best-performing benzofuran-2-yl analog **10**, as shown in Table 4. N-Methylation of the *N*-benzoyl moiety of **10** resulted in **17**, with marked reduction of MMP inhibitory activity. Replacement of the amide moiety of **10** with an ether oxygen afforded **18**, which showed loss of inhibitory activity for MMP-2. As a result, formation of the hydrogen bonds of the amide moiety with Pro238 and Leu181, as described in Figure 2, was strongly suggested to be important for the potent MMP-2 inhibitory activity of **10**. Introduction of a *trans*-double bond into the alkylene of the γ-aminobutyric hydroxamic acid moiety could be suggested to regulate the length and direction of the two pharmacophores, that is the hydroxamic acid and the *N*-benzoyl moiety. Introduction of a conjugated *trans*-2,3-double bond into the trimethylene moiety of **10** afforded **19**, which showed 277-fold weaker inhibitory activity for MMP-2. Introduction of a non-conjugated 3,4-*trans*-double bond into the trimethylene moiety of **10** yielded **20**, which also showed a remarkable decrease of inhibitory activity. Thus, the optimum three-dimensional arrangement of the two pharmacophores (zinc-binding hydroxamic acid and *N*-benzoyl residue) was confirmed to be important for potent inhibitory activity.

The secondary amide moiety of **10** was found to be essential for potent activity because it controlled the conformation and/or the hydrogen bond with Pro238 and Leu181, as described in Figure 2.

In summary, further optimization of a new chemical lead **1**, which was discovered by the in silico fragment-based lead generation technique,[6] was carried out successfully. Chemical modification was focused on the 4-*N*-(4-methyl)benzoyl moiety of **1**, and a series of *N*-benzoyl γ-aminobutyric hydroxamic acids were synthesized and evaluated. 4-(*para*-Substituted phenyl)benzoyl analogs **4** and **7** showed stronger inhibitory activity, while the 4-(*para*-alkylphenyl)benzoyl analogs **5** and **6** showed weaker inhibition. Introduction of electron-rich *para*-substituents, such as benzofuran-2-yl and *para*-chlorocinnamyl moieties, into the *N*-benzoyl moiety was found to be highly effective to enhance the MMP inhibitory activity, as illustrated by compounds **10** and **11**. As shown by the complete loss of inhibitory activity in the case of **16**, *para*-substitution of the *N*-benzoyl moiety was also required for strong activity. Among those tested, γ-aminobutyric hydroxamic acid was found to be the best spacer for connecting the two pharmacophores. A secondary amide was a superior linkage compared to the ether or *N*-methyl amide with respect to the formation of hydrogen bonds with amino acid residues Pro238 and Leu181 in the *S*1′ pocket.

4. Experimental

4.1. General chemical procedures

Analytical samples were homogeneous as confirmed by thin-layer chromatography (TLC) and yielded spectroscopic data consistent with the assigned structures. All ^{1}H NMR spectra were obtained with a Varian Gemini-200 or MERCURY-300 spectrometer. The chemical shift values are reported in ppm (δ) and coupling constants (J) in Hertz (Hz). Fast atom bombardment (FAB) and electron ionization (EI) mass spectra were obtained with a JEOL JMS-DX303HF or JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined by a Hitachi M-1200H spectrometer. Matrix assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectra were obtained on a PerSeptive Voyager Elite spectrometer. IR spectra were measured using a Perkin-Elmer FTIR 1760X or JASCO FTIR-430 spectrometer. Column chromatography was carried out using silica gel [Merck silica gel 60 (0.063–0.200 mm), Wako Gel C200, Fuji Silysia FL60D, or Fuji Silysia BW-235]. TLC was also performed on silica gel (Merck TLC plate, silica gel 60 F_{254}).

4.1.1. 4-[(4-Methylbenzoyl)amino]butanoic acid (21a). To a stirred suspension of 4-aminobutyric acid (1.13 g, 11.0 mmol) in THF (21 mL) was added 1 N NaOH (21.0 mL, 21.0 mmol) at 0 °C, followed by the addition of 4-methylbenzoyl chloride (1.55 g, 10.0 mmol). After being stirred for 3 h at room temperature, the reaction mixture was acidified with 2 N HCl and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Removal of the volatiles in vacuo provided a solid, which was washed with Et_2O to give **21a** (2.00 g, 90%) as a white crystal. TLC $R_f = 0.58$ ($CHCl_3$/MeOH/AcOH, 18/2/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.73 (d, $J = 8.4$ Hz, 2H), 7.40 (m, 1H), 7.21 (d, $J = 8.4$ Hz, 2H), 3.47 (m, 2H), 2.41 (t, $J = 7.3$ Hz, 2H), 2.38 (s, 3H), 1.94 (m, 2H).

4.1.2. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-methylbenzamide (1). To a stirred solution of **21a** (885 mg) in DMF (5 mL) were added *O*-benzyl hydroxylamine hydrochloride (638 mg), EDC·HCl (844 mg), HOBt (673 mg), and diisopropylethylamine (1.74 mL). After being stirred at room temperature for 40 h, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The resulting residue was purified by column chromatography on silica gel to give a protected hydroxamate. A heterogeneous mixture of the above-mentioned residue and 10% palladium on carbon (504 mg) in MeOH (4 mL) was vigorously stirred under H_2 gas for 2 h. Catalyst was removed by filtration and the filtrate was concentrated to give **1** (293 mg, 65%) as a white powder. TLC $R_f = 0.23$ ($CHCl_3$/MeOH, 10/1); MS (APCI, neg. 40 V) m/z 235 $(M-H)^-$; IR (KBr) 1674, 1613, 1608, 1564, 1510, 1458, 1436, 1360, 1332, 1313, 1258, 1233, 1197, 1178, 1101, 1024, 969 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ10.39 (s, 1H), 8.70 (s, 1H), 8.40 (t, $J = 5.2$ Hz, 1H), 7.74 (d, $J = 8.1$ Hz, 2H), 7.25 (d, $J = 8.1$ Hz, 2H), 3.24 (dt, $J = 6.6$, 5.2 Hz, 2H), 2.35 (s, 3H), 2.02 (t, $J = 7.7$ Hz, 2H), 1.74 (m, 2H).

4.1.3. Synthesis of esters 21b–p. Compounds **21b**–**p** were prepared from the corresponding methyl or ethyl esters, respectively, according to the usual alkaline hydrolysis.

4.1.4. 4-Butyl-*N*-[4-(hydroxyamino)-4-oxobutyl]benzamide (2). To a stirred solution of **21b** (400 mg, 1.52 mmol) in DMF (3 mL) were added HOBt (470 mg, 2.0 equiv), EDC·HCl (590 mg, 2.0 equiv), and *O*-2-methoxypropane hydroxylamine (320 mg, 2.0 equiv). After being stirred at room temperature for 150 min, the reaction mixture was quenched with satd NH_4Cl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give a protected hydroxamate. The pH value of a solution of the protected hydroxamate in MeOH was adjusted to 3 with 1 N HCl. After being stirred at room temperature for 20 min, the solution was diluted with AcOEt and treated with satd $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The resulting crystalline solid was washed with Et_2O to give **2** (211 mg, 50%) as a white powder. TLC $R_f = 0.37$ ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (MALDI, Pos.) m/z 301 $(M+Na)^+$, 279 $(M+H)^+$; IR (KBr) 3214, 2935, 2849, 1672, 1610, 1564, 1435, 1314, 1176, 1100, 1023, 967, 907, 851, 764, 688, 529, 412 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ10.40 (br s, 1H), 10.20 (s, 1H), 8.41 (t, $J = 5.4$ Hz, 1H), 7.76 (d, $J = 8.0$ Hz, 2H), 7.25 (d, $J = 8.0$ Hz, 2H), 3.23 (dt, $J = 5.4$ Hz, 7.0 Hz, 2H), 2.62 (t, $J = 8.2$ Hz, 2H), 2.02 (t, $J = 7.0$ Hz, 2H),

1.83-1.66 (m, 2H), 1.64 ~ 1.48 (m, 2H), 1.30 (sextet, J = 7.2 Hz, 2H), 0.90 (t, J = 7.2 Hz, 3H).

Compounds **3**–**16** were prepared from **21c**–**p**, respectively, according to the same procedures as described above.

4.1.5. 4-Cyclohexyl-*N*-[4-(hydroxyamino)-4-oxobutyl]benzamide (3). Yield 56%; TLC R_f = 0.40 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (FAB, Pos.) m/z 305 $(M+H)^+$; IR (KBr) 3320, 2923, 2849, 1631, 1546, 1503, 1447, 1306, 1185, 1099, 1029, 996, 843, 766, 662, 632 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ10.39 (s, 1H), 8.43-8.36 (m, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 3.30–3.13 (m, 2H), 2.63–2.54 (m, 1H), 2.01 (t, J = 7.6 Hz, 2H), 1.86–1.65 (m, 6H), 1.48–1.24 (m, 6H).

4.1.6. 4′-Chloro-*N*-[4-(hydroxyamino)-4-oxobutyl]-1,1′-biphenyl-4-carboxamide (4). Yield 87%; TLC R_f = 0.34 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (MALDI, Pos.) m/z 371 $(M+K)^+$, 355 $(M+Na)^+$, 333 $(M+H)^+$; IR (KBr) 3308, 3180, 3020, 1632, 1542, 1484, 1454, 1390, 1302, 1103, 1003, 972, 942, 825, 769, 685, 623 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ10.41 (s, 1H), 8.62–8.52 (m, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.82–7.72 (m, 4H), 7.55 (d, J = 8.4 Hz, 2H), 3.35–3.20 (m, 2H), 2.04 (t, J = 7.5 Hz, 2H), 1.83–1.69 (m, 2H).

4.1.7. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4′-propyl-1,1′-biphenyl-4- carboxamide (5). Yield 60%; TLC R_f = 0.40 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (FAB, Pos.) m/z 341 $(M+H)^+$; IR (KBr) 3302, 3180, 2954, 1630, 1544, 1475, 1371, 1305, 1222, 1096, 1044, 1029, 1003, 972, 943, 834, 812, 769, 687, 658, 628, 536 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.70 (br s, 1H), 8.57–8.49 (m, 1H), 7.93 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H), 3.31–3.20 (m, 2H), 2.61 (t, J = 7.4 Hz, 2H), 2.04 (t, J = 7.2 Hz, 2H), 1.76 (quint, J = 7.2 Hz, 2H), 1.62 (sextet, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H).

4.1.8. 4′-Heptyl-*N*-[4-(hydroxyamino)-4-oxobutyl]-1,1′-biphenyl-4-carboxamide (6). Yield 84%; TLC R_f = 0.34 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (FAB, Pos.) m/z 397 $(M+H)^+$, 279; IR (KBr) 3305, 3185, 2921, 1631, 1543, 1494, 1454, 1307, 1096, 835, 815, 770, 627, 610 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.40 (br s, 1H), 8.57–8.50 (m, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 3.32–3.22 (m, 2H), 2.62 (t, J = 7.7 Hz, 2H), 2.04 (t, J = 7.3 Hz, 2H), 1.76 (quint, J = 7.3 Hz, 2H), 1.69–1.52 (m, 2H), 1.38–1.17 (m, 8H), 0.86 (t, J = 6.6 Hz, 3H).

4.1.9. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4′-methoxy-1,1′-biphenyl-4-carboxamide (7). Yield 70%; TLC R_f = 0.26 ($CHCl_3$/MeOH, 9/1); MS (MALDI, Pos.) m/z 351 $(M+Na)^+$, 329 $(M+H)^+$; IR (KBr) 3324, 3186, 3010, 2952, 1629, 1542, 1495, 1289, 1255, 1206, 1182, 1095, 1036, 1012, 824, 769, 715, 637, 625, 601 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.57–8.47 (m, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 3.81 (s, 3H), 3.26 (q, J = 6.5 Hz, 2H), 2.03 (t, J = 7.5 Hz, 2H), 1.83–1.69 (m, 2H).

4.1.10. 4′-Hydroxy-*N*-[4-(hydroxyamino)-4-oxobutyl]-1,1′-biphenyl-4-carboxamide (8). Yield 36%; TLC R_f = 0.23 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (FAB, Pos.) m/z 315 $(M+H)^+$; IR (KBr) 3310, 1632, 1543, 1495, 1381, 1300, 1265, 1206, 1186, 1110, 829, 769, 673, 626, 529 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ10.40 (s,1H), 9.80–9.45 (br s, 1H), 8.53–8.44 (m, 1H), 7.89 (d, J = 8.5 Hz, 2H), 7.67 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 3.31–3.20 (m, 2H), 2.03 (t, J = 7.4 Hz, 2H), 1.83–1.68 (m, 2H).

4.1.11. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-(1*H*-pyrrol-1-yl)benzamide (9). Yield 74%; TLC R_f = 0.31 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (MALDI, Pos.) m/z 310 $(M+Na)^+$, 288 $(M+H)^+$; IR (KBr) 3322, 3175, 1632, 1545, 1519, 1474, 1331, 845, 723 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.40 (s, 1H), 9.00–8.24 (br s, 1H), 8.52 (t, J = 5.6 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.50–7.44 (m, 1H), 6.34–6.29 (m, 1H), 3.38–3.31 (m, 2H), 2.04 (t, J = 7.5 Hz, 2H), 1.76 (quint, J = 7.5 Hz, 2H).

4.1.12. 4-(1-Benzofuran-2-yl)-*N*-[4-(hydroxyamino)-4-oxobutyl]benzamide (10). Yield 38%; TLC R_f = 0.22 ($CHCl_3$/MeOH/AcOH, 100/10/1); MS (FAB, Pos.) m/z 339 $(M+H)^+$; IR (KBr) 3320, 1633, 1541, 1451, 1306, 1030, 854, 807, 748 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ10.39 (br s, 1H), 8.59 (t, J = 5.8 Hz, 1H), 8.01 (d, J = 9.0 Hz, 2H), 7.96 (d, J = 9.0 Hz, 2H), 7.67 (m, 2H), 7.57 (d, J = 0.5 Hz, 1H), 7.39–7.23 (m, 2H), 3.27 (q, J = 5.8 Hz, 2H), 2.03 (t, J = 7.6 Hz, 2H), 1.76 (m, 2H).

4.1.13. 4-[(*E*)-2-(4-Chlorophenyl)vinyl]-*N*-[4-(hydroxyamino)-4-oxobutyl]benzamide (11). Yield 78%; TLC R_f = 0.17 ($CHCl_3$/MeOH/AcOH, 100/10/1); MS (FAB, Pos.) m/z 359 $(M+H)^+$; IR (KBr) 3266, 1636, 1544, 1503, 1489, 1437, 1323, 1097, 970, 843, 814, 710, 563 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.39 (br s, 1H), 8.50 (t, J = 5.8 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 16.2 Hz, 1H), 7.30 (d, J = 16.2 Hz, 1H), 3.25 (q, J = 5.6 Hz, 2H), 2.02 (t, J = 7.6 Hz, 2H), 1.74 (m, 2H).

4.1.14. 4-Butoxy-*N*-[4-(hydroxyamino)-4-oxobutyl]benzamide (12). Yield 71%; TLC R_f = 0.29 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (MALDI, Pos.) m/z 333 $(M+K)^+$, 317 $(M+Na)^+$, 295 $(M+H)^+$; IR (KBr) 3321, 2956, 1629, 1543, 1507, 1252, 1178, 1067, 1027, 836, 768, 644 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.70 (br s, 1H), 8.32 (t, J = 5.8 Hz, 1H), 7.80 (d, J = 9.0 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 4.01 (t, J = 6.4 Hz, 2H), 3.35–3.15 (m, 2H), 2.01 (t, J = 7.3 Hz, 2H), 1.81–1.64 (m, 4H), 1.44 (sextet, J = 7.4 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H).

4.1.15. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-phenoxybenzamide (13). Yield 59%; TLC R_f = 0.25 ($CHCl_3$/MeOH/AcOH/H_2O, 100/10/1/1); MS (MALDI, Pos.) m/z 315

$(M+H)^+$; IR (KBr) 3302, 1633, 1544, 1489, 1370, 1303, 1258, 1165, 1100, 1028, 972, 845, 747, 692, 630 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.36 (br s, 1H), 8.69 (br s, 1H), 8.41 (t, J = 5.6 Hz, 1H), 7.85 (dt, J = 8.8 Hz, 2.8 Hz, 2H), 7.37–7.46 (m, 2H), 7.19 (tt, J = 7.2 Hz, 1.2 Hz, 1H), 6.97–7.09 (m, 4H), 3.22 (dt, J = 6.6 Hz, 5.6 Hz, 2H), 1.99 (t, J = 7.6 Hz, 2H), 1.71 (tt, J = 7.6 Hz, 6.6 Hz, 2H).

4.1.16. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-(4-methoxyphenoxy)benzamide (14). Yield 71%; TLC R_f = 0.40 ($CHCl_3$/MeOH/AcOH/H_2O, 50/10/1/1); MS (APCI, Neg, 40 V) *m/z* 343 (M−H)-; IR (KBr) 3300, 1634, 1543, 1508, 1240, 1101, 1035, 837 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.37 and 9.79 (both br s, 1H), 9.02 and 8.70 (both br s, 1H), 8.40 (t, J = 5.5 Hz, 1H), 7.82 (d, J = 9.1 Hz, 2H), 7.06–6.91 (m, 6H), 3.79 (s, 3H), 3.21 (m, 2H), 1.96 (m, 2H), 1.72 (m, 2H).

4.1.17. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-(4-hydroxyphenoxy)benzamide (15). Yield 66%; TLC R_f = 0.25 ($CHCl_3$/MeOH/AcOH/H_2O, 50/10/1/1); MS (APCI, Neg, 40 V) *m/z* 329 (M−H)-; IR (KBr) 3297, 1635, 1543, 1497, 1374, 1326, 1236, 1166, 1096, 971, 855, 838, 777, 633 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 8.37 (t, J = 5.5 Hz, 1H), 7.81 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 9.1 Hz, 2H), 6.90 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 9.1 Hz, 2H), 3.21 (m, 2H), 1.99 (m, 2H), 1.71 (m, 2H).

4.1.18. 3-Butoxy-*N*-[4-(hydroxyamino)-4-oxobutyl]benzamide (16). Yield 78%; TLC R_f = 0.31 ($CHCl_3$/MeOH/AcOH, 9/1/0.5); MS (MALDI, Pos.) *m/z* 317 $(M+Na)^+$, 295 $(M+H)^+$; IR (KBr) 3377, 3292, 2871, 1633, 1542, 1469, 1318, 1239, 1105, 1040, 975, 800, 742 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ10.39 (s, 1H), 8.45 (t, J = 5.2 Hz, 1H), 7.43–7.29 (m, 3H), 7.19–7.01 (m, 1H), 4.01 (t, J = 6.3 Hz, 2H), 3.30–3.18 (m, 2H), 2.02 (t, J = 7.5 Hz, 2H), 1.83–1.64 (m, 4H), 1.49 (sextet, J = 7.3 Hz, 2H), 0.95 (t, J = 7.3 Hz, 3H).

4.1.19. Methyl (3*S*)-3,4-dihydroxybutanoate (33). To a stirred solution of dimethyl (2*S*)-2-hydroxysuccinate (**32**) (92.5 mmol) in THF (170 mL) were added dropwise borane dimethyl sulfide complex (95.3 mmol) at room temperature and then sodium borohydride (4.63 mmol) under cooling with an ice-water bath. After being stirred for 10 min, the reaction mixture was warmed up to room temperature and stirred for additional 30 min. To the stirred reaction mixture were added MeOH (40 mL) and *p*-toluenesulfonic acid monohydrate (4.63 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was evaporated. The resulting residue was diluted with MeOH–benzene and evaporated. This work-up was repeated several times to give **33** (100%) as a yellow oil. TLC R_f = 0.25 (AcOEt).

4.1.20. Methyl (3*S*)-3-hydroxy-4-{[(4-methylphenyl)sulfonyl]oxy}butanoate (34). To a stirred solution of **33** (37.3 mmol) in pyridine (50 mL) was added *p*-toluenesulfonyl chloride (37.3 mmol) under cooling with an ice-water bath. After being stirred for 5 h, the reaction mixture was quenched with 1 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give crude product, which was crystallized from *n*-hexane–Et_2O to give **34** (5.18 g, 48%). TLC R_f = 0.60 (AcOEt/*n*-hexane, 1/1); ^1H NMR (200 MHz, $CDCl_3$) δ7.80 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 4.32–4.18 (m, 1H), 4.05 (d, J = 5.2 Hz, 2H), 3.70 (s, 3H), 3.03 (br s, 1H), 2.57–2.53 (m, 2H), 2.46 (s, 3H).

4.1.21. Methyl (3*S*)-4-azido-3-hydroxybutanoate (35). To a stirred solution of **34** (4.66 g, 16.2 mmol) in DMF (40 mL) was added sodium azide (1.16 g, 17.8 mmol). After being stirred at 80 °C for 45 min, the reaction mixture was evaporated to remove DMF and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give **35** (1.96 g, 77%). TLC R_f = 0.68 (AcOEt/*n*-hexane, 1/1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.28–4.14 (m, 1H), 3.74 (s, 3H), 3.40 (dd, J = 3.1 Hz, 9.6 Hz, 1H), 3.33 (dd, J = 4.1 Hz, 9.6 Hz, 1H), 3.14 (d, J = 4.0 Hz, 1H), 2.58–2.55 (m, 2H).

4.1.22. Methyl (3*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-3-(hydroxy)butanoate (36). To a stirred solution of **35** (1.00 g, 6.36 mmol) in MeOH (50 mL) was added 10% palladium on carbon under argon atmosphere. The resulting heterogeneous mixture was subjected to the catalytic hydrogenation for an hour. Catalyst was removed by filtration and the filtrate was concentrated. The resulting amine was treated with 4 N HCl/AcOEt to obtain the hydrochloride salt. To a stirred solution of the hydrochloride salt in CH_2Cl_2 (20 mL) were added Et_3N (2.7 mL, 19.08 mmol) and 4-(1-benzofuran-2-yl)benzoyl chloride (1.63 g, 6.36 g) under cooling with an ice-water bath. After being stirred at room temperature for 30 min, the reaction mixture was quenched with 1 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give **36** (1.89 g, 84%). TLC R_f = 0.31 (AcOEt/*n*-hexane, 1/1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.57 (t, J = 6.0 Hz, 1H), 8.00 (s, 4H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.40–7.24 (m, 2H), 5.13 (d, J = 5.6 Hz, 1H), 4.18–4.00 (m, 1H), 3.57 (s, 3H), 3.30 (t, J = 6.0 Hz, 2H), 2.55 (dd, J = 4.0 Hz, 15.0 Hz, 1H), 2.31 (dd, J = 4.0 Hz, 15.0 Hz, 1H).

4.1.23. Methyl (3*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-3-(methoxymethoxy)butanoate (37). To a stirred solution of **36** (201 mg, 0.57 mmol) in CH_2Cl_2 (1.0 mL) were added diisopropylethylamine (1.0 mL) and chloromethyl methyl ether (2.83 mmol). After being stirred at room temperature overnight, the reaction mixture was quenched with 0.5 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The resulting residue was purified by column chromatography on silica gel (AcOEt/*n*-hexane, 1/1) to give **37** (176 mg, 78%). TLC R_f = 0.38 (AcOEt/*n*-hexane, 1/1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.65 (t, J = 5.4 Hz, 1H), 8.02 (d, J = 9.2 Hz, 2H), 7.97 (d, J = 9.2 Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.40–7.24 (m, 2H), 4.65 (d, J = 7.0 Hz, 1H), 4.59 (d, J = 7.0 Hz, 1H), 4.20–4.08

(m, 1H), 3.58 (s, 3H), 3.52–3.34 (m, 2H), 2.65 (dd, J = 15.8 Hz, 4.4 Hz, 1H), 2.54–2.43 (m, 1H).

4.1.24. (2*E*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}but-2-enoic acid (38). To a stirred solution of **37** (0.35 mmol) in THF (2.0 mL) was added 1 N NaOH (1.0 mL). After being stirred at room temperature overnight, the reaction mixture was quenched with 1 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give **38** (100%). TLC R_f = 0.21 ($CHCl_3$/MeOH, 10/1).

4.1.25. 4-(1-Benzofuran-2-yl)-*N*-[(2*E*)-4-(hydroxyamino)-4-oxobut-2-enyl]benzamide (19). To a stirred solution of **38** (110 mg, 0.287 mmol) in DMF (2 mL) were added HOBt·H_2O (66 mg, 0.431 mmol), EDC·HCl (83 mg, 0.431 mmol), and *O*-2-methoxypropane hydroxylamine (107 μL, 1.15 mmol). After being stirred at room temperature for 2 h, the reaction mixture was quenched with satd NH_4Cl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The resulting residue was purified by column chromatography on a silica gel to give a protected hydroxamate (TLC R_f = 0.40, $CHCl_3$/MeOH, 10/1). The pH value of the solution of the protected hydroxamate in MeOH was adjusted to 3 with 1 N HCl. After being stirred at room temperature for 30 min, the solution was evaporated. The resulting crystalline solid was washed with Et_2O to give **19** (15 mg, 23% from **38**) as a white powder. TLC R_f = 0.20 ($CHCl_3$/MeOH/AcOH, 100/10/1); MS (MALDI, Pos.) m/z 337 $(M+H)^+$, 359 $(M+Na)^+$; IR (KBr) 3313, 1635, 1537, 1497, 1450, 1306, 854, 808, 749 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.60 (br s, 1H), 8.89 (t, J = 5.8 Hz, 1H), 8.02 (s, 4H), 7.71–7.63 (m, 2H), 7.59 (br s, 1H), 7.40–7.24 (m, 2H), 6.69 (dt, J = 15.4, 4.8 Hz, 1H), 5.86 (d, J = 15.4 Hz, 1H), 4.08–4.02 (m, 2H).

4.1.26. (3*S*)-3-(acetyloxy)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}butanoate (39). To a stirred solution of **36** (0.85 mmol) in pyridine (5.0 mL) was added acetic anhydride (0.6 mL). After being stirred at room temperature for 2 h, the reaction mixture was quenched with 1 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give **39** (325 mg, 97%). TLC R_f = 0.52 (AcOEt/*n*-hexane, 2/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.93 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.63–7.52 (m, 2H), 7.37–7.21 (m, 2H), 7.13 (d, J = 0.6 Hz, 1H), 5.50–5.38 (m, 1H), 3.78–3.69 (m, 2H), 3.71 (s, 3H), 2.74 (d, J = 4.2 Hz, 2H), 2.10 (s, 3H).

4.1.27. Methyl (3*E*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}but-3-enoate (40). To a stirred solution of **39** (0.38 mmol) in THF (1.0 mL) was added DBU (0.872 mmol). After being stirred at 50 °C for 3 h, the reaction mixture was quenched with 1 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give **40** (96 mg, 76%). TLC R_f = 0.70 (AcOEt/*n*-hexane, 2/1); ^1H NMR (200 MHz, DMSO-d_6) δ 10.41 (d, J = 9.6 Hz, 1H), 8.05 (s, 4H), 7.71–7.63 (m, 2H), 7.61 (br s, 1H), 7.41–7.24 (m, 2H), 6.98 (dd, J = 14.4 Hz, 9.6 Hz, 1H), 5.54 (dt, J = 14.4 Hz, 7.1 Hz, 1H), 3.61 (s, 3H), 3.16 (d, J = 7.1 Hz, 2H).

4.1.28. (3*E*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}but-3-enoic acid (41). Compound **41** (52 mg, 69%) was obtained from **40** according to the same procedures as described for the preparation of **38** from **37**. TLC R_f = 0.22 ($CHCl_3$/MeOH/AcOH, 100/10/1); ^1H NMR (200 MHz, DMSO-d_6) δ 10.39 (d, J = 9.8 Hz, 1H), 8.05 (s, 2H), 7.72–7.64 (m, 2H), 7.61 (d, J = 0.6 Hz, 1H), 7.41–7.24 (m, 2H), 6.95 (dd, J = 14.4 Hz, 9.8 Hz, 1H), 5.54 (dt, J = 14.4 Hz, 7.2 Hz, 1H), 3.05 (d, J = 7.2 Hz, 2H).

4.1.29. 4-(1-Benzofuran-2-yl)-*N*-[(1*E*)-4-(hydroxyamino)-4-oxobut-1-enyl]benzamide (20). Compound **20** (32 mg, 62%) was prepared from **41** as a pale yellow powder according to the same procedures as described for the preparation of **19** from **38**. TLC R_f = 0.21 ($CHCl_3$/MeOH/AcOH, 100/10/1); ^1H NMR (200 MHz, DMSO-d_6) δ 10.45 (br s, 1H), 10.37 (d, J = 9.6 Hz, 1H), 8.75 (s, 1H), 8.04 (s, 4H), 7.72–7.63 (m, 2H), 7.61 (s, 1H), 7.41–7.24 (m, 2H), 6.94 (dd, J = 14.2 Hz, 9.6 Hz, 1H), 5.53 (dt, J = 14.2 Hz, 7.8 Hz, 1H), 2.76 (d, J = 7.8 Hz, 2H).

4.1.30. Ethyl 4-[[4-(1-Benzofuran-2-yl)benzoyl](methyl)amino]butanoate (23). To a stirred solution of compound **22**[6] (100 mg, 0.29 mmol) and iodomethane (355 μL, 5.7 mmol) in DMF (3 mL) was added 60% NaH (13 mg, 0.32 mmol) at 0 °C. After being stirred for an hour at room temperature, the reaction mixture was quenched with satd NH_4Cl and extracted with AcOEt. The organic layer was washed with H_2O, brine, dried over $MgSO_4$, and evaporated to give **23** as a crude product.

4.1.31. 4-[[4-(1-Benzofuran-2-yl)benzoyl](methyl)amino]butanoic acid (24). To a stirred solution of **23** (113 mg, 0.31 mmol) in MeOH (10 mL) was added 2 N NaOH (3.1 mL). After being stirred for 30 min at room temperature, the reaction mixture was neutralized with 2 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting crystalline solid was washed with Et_2O to give **24** (83 mg, 87% from **22**) as a white powder. TLC R_f = 0.51 ($CHCl_3$/MeOH, 9/1); ^1H NMR (200 MHz, CD_3OD) δ 7.98 (d, J = 8.4 Hz, 2H), 7.66–7.59 (m, 1H), 7.57–7.46 (m, 3H), 7.37–7.19 (m, 3H), 3.62 and 3.40 (t and t, J = 7.5 Hz and J = 7.5 Hz, 2H), 3.10 and 3.03 (s and s, 3H), 2.45 and 2.20 (t and t, J = 7.5 Hz and J = 7.5 Hz, 2H), 2.10–1.75 (m, 2H).

4.1.32. 4-(1-Benzofuran-2-yl)-*N*-[4-(hydroxyamino)-4-oxobutyl]-*N*-methylbenzamide (17). Compound **17** (65 mg, 85%) was prepared from **24** as a white powder according to the same procedures as described for the preparation of **19** from **38**. TLC R_f = 0.31 ($CHCl_3$/MeOH, 9/1); MS (APCI, Neg, 40 V) m/z 351 $(M-H)^-$; IR (KBr) 3198, 1659, 1607, 1510, 1489, 1450, 1411, 1324, 1260, 1170, 1075, 1032, 919, 850, 808, 748 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.99 (d, J = 8.4 Hz, 2H), 7.66–7.59 (m, 1H), 7.58–7.45 (m, 3H), 7.37–7.20 (m, 3H), 3.70–3.54, and 3.42–3.30 (m, 2H), 3.16–2.95 (m, 3H), 2.30–1.80 (m, 4H).

4.1.33. Ethyl 5-[4-(benzyloxy)phenoxy]pentanoate (26). To a stirred solution of **25** (2.0 g, 10.0 mmol) in DMF (20 mL) were added ethyl 5-bromopentanoate (2.38 g, 15.0 mmol) and K_2CO_3 (3.45 g, 25.0 mmol). After being stirred at 80 °C for 3 h, the reaction mixture was neutralized with 1 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was triturated with *n*-hexane to give **26** (2.88 g, 88%) as a beige powder. TLC $R_f = 0.50$ (*n*-hexane/AcOEt, 4/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.48–7.26 (m, 5H), 6.90 (d, $J = 9.4$ Hz, 2H), 6.81 (d, $J = 9.4$ Hz, 2H), 5.01 (s, 2H), 5.02 (s, 2H), 4.13 (q, $J = 7.2$ Hz, 2H), 3.97–3.86 (m, 2H), 2.45–2.30 (m, 2H), 1.90–1.70 (m, 4H), 1.26 (t, $J = 7.2$ Hz, 3H).

4.1.34. Ethyl 5-(4-hydroxyphenoxy)pentanoate (27). To a stirred solution of **26** (1.50 g, 4.53 mmol) in MeOH (20 mL) was added 10% palladium on carbon under argon atmosphere. The resulting heterogeneous mixture was vigorously stirred under H_2 gas for 3 h. Catalyst was removed by filtration and the filtrate was concentrated to give **27** (1.11 g, 100%) as a purple oil. TLC $R_f = 0.43$ (*n*-hexane/AcOEt, 2/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.85–6.70 (m, 4H), 4.77 (br s, 1H), 4.14 (q, $J = 7.2$ Hz, 2H), 3.96–3.86 (m, 2H), 2.44–2.32 (m, 2H), 1.86–1.74 (m, 4H), 1.26 (t, $J = 7.2$ Hz, 3H).

4.1.35. Ethyl 5-(4-{[(trifluoromethyl)sulfonyl]oxy}phenoxy)pentanoate (28). To a stirred solution of **27** (4.53 mmol) in pyridine (10 mL) was added trifluoromethanesulfonyl anhydride (0.76 ml, 4.85 mmol) under cooling with an ice bath. After being stirred at room temperature for 20 min, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give **28** (1.68 g, 98%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ7.18 (d, $J = 9.0$ Hz, 2H), 6.90 (d, $J = 9.0$ Hz, 2H), 4.13 (q, $J = 7.2$ Hz, 2H), 4.00–3.93 (m, 2H), 2.44–2.34 (m, 2H), 1.90–1.76 (m, 4H), 1.26 (t, $J = 7.2$ Hz, 3H).

4.1.36. Ethyl 5-[4-(1-benzofuran-2-yl)phenoxy]pentanoate (30). To a stirred solution of **28** (1.67 g, 4.05 mmol) in DMF (10 ml) were added benzo[*b*]furan-2-boronic acid (**29**, 1.09 g, 6.75 mmol), Et_3N (5 mL), and $Pd(PPh_3)_2Cl_2$ (316 mg, 0.45 mmol). After being stirred at 50 °C for 4 h, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The resulting residue was purified by chromatography on silica gel and triturated with *n*-hexane to give **30** (388 mg, 26%) as a beige powder. TLC $R_f = 0.42$ (*n*-hexane/AcOEt, 4/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.79 (d, $J = 8.8$ Hz, 2H), 7.60–7.44 (m, 2H), 7.30–7.14 (m, 2H), 6.96 (d, $J = 8.8$ Hz, 2H), 6.88 (d, $J = 0.8$ Hz, 1H), 4.14 (q, $J = 7.2$ Hz, 2H), 4.07–3.97 (m, 2H), 2.50–2.30 (m, 2H), 1.90–1.75 (m, 4H), 1.26 (t, $J = 7.2$ Hz, 3H).

4.1.37. 5-[4-(1-Benzofuran-2-yl)phenoxy]pentanoic acid (31). Compound **31** (94%) was prepared from **30** as a pale yellow powder according to the same procedures as described for the preparation of **38** from **37**. TLC $R_f = 0.50$ ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (200 MHz, $CDCl_3 + CD_3OD$) δ 7.79 (d, $J = 8.8$ Hz, 2H), 7.60–7.46 (m, 2H), 7.43–7.14 (m, 2H), 6.97 (d, $J = 8.8$ Hz, 2H), 6.90 (d, $J = 1.2$ Hz, 1H), 4.10–4.00 (m, 2H), 2.48–2.34 (m, 2H), 1.96–1.76 (m, 4H).

4.1.38. 5-[4-(1-Benzofuran-2-yl)phenoxy]-*N*-hydroxypentanamide (18). Compound **18** (84%) was prepared from **31** as a white powder according to the same procedures as described for the preparation of **2** from **21b**. TLC $R_f = 0.43$ ($CHCl_3$/MeOH, 9/1); MS (APCI, neg. 40 V) *m*/*z* 324 (M−H)-; IR (KBr) 3212, 2939, 1638, 1505, 1453, 1296, 1254, 1178, 1113, 1034, 975, 920, 885, 834, 799, 751, 526, 442 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.90–8.50 (br s, 1H), 7.85 (d, $J = 8.8$ Hz, 2H), 7.66–7.54 (m, 2H), 7.34–7.18 (m, 3H), 7.06 (d, $J = 8.8$ Hz, 2H), 4.04 (t, $J = 6.0$ Hz, 2H), 2.04 (t, $J = 6.6$ Hz, 2H), 1.84–1.56 (m, 4H).

5. Enzyme assays: MMP-1, MMP-2, MMP-9, and MMP-3 assays using synthetic substrate

5.1. MMP-1 assay

Commercially available assay kits (Yagai, Yamagata City, Japan) were used. The solutions provided in the kits were used unless otherwise stated. A 98 μL portion of enzyme solution (0.5 U/mL) and 2 μL of inhibitor solution (DMSO) were incubated with 100 μL of 0.5 mg/mL FITC-labeled type I collagen solution at 37 °C for 3 h. After incubation with 300 L of quenching solution on ice for 30 min, the reaction mixture was centrifuged at 2000*g* for 15 min. Supernatant was used for measurement of fluorescence by RF5300-PC. Excitation and emission wavelengths were 495 and 520 nm, respectively.

5.2. MMP-2 and MMP-9 assays

A mixture of 130 μL MOCAc-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2 (final concentration: 15 μM) and 20 μL of inhibitor solution was incubated at 37 °C for 5 min. Fifty microliters of enzyme solution was added, and the reaction was performed at 37 °C for 10 min. Its fluorescence was measured by f_{max}. Excitation and emission wavelengths were 320 and 390 nm, respectively.

5.3. MMP-3 assay

A mixture of 190 μL MOCAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp- Arg-Lys(Dnp)-NH_2 (final concentration: 15 μM) and 10 μL of inhibitor solution was incubated at 37 °C for 5 min. Fifty microliters of enzyme solution was added and the reaction was performed at 37 °C for 10 min. Its fluorescence was measured by f_{max}. Excitation and emission wavelengths were 320 and 390 nm, respectively.

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