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Synthesis and evaluation of myxochelin analogues as antimetastatic agents

Satoshi Miyanaga [a], Hiroaki Sakurai [b], Ikuo Saiki [b], Hiroyasu Onaka [a], Yasuhiro Igarashi [a,*]

[a] Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan
[b] Department of Bioscience, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

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ABSTRACT

Myxochelin A (**1**) is an inhibitor of tumor cell invasion produced by the bacterium belonging to the genus *Nonomuraea*. In order to obtain more potent inhibitors, a series of myxochelin analogues [**2** and (*S*)-**3**–**17**] were synthesized through the coupling of lysine or diaminoalkane derivatives and appropriately protected hydroxybenzoate, followed by modification of functional groups and deprotection. These compounds were evaluated for their inhibitory activity against invasion of murine colon 26-L5 carcinoma cells. Among the synthetic analogues tested, compound (*S*)-**6** which possesses carbamoyl group at C-1 was found to be the most potent antiinvasive agent and is considered to be a promising lead molecule for the antimetastasis. Compound (*S*)-**6** was also shown to inhibit gelatinase activities of MMP-2 and MMP-9 and in vivo lung metastasis in mice.

1. Introduction

Tumor metastasis is the most common cause of death in cancer patients. It is the process by which a tumor cell leaves the primary tumor, disseminates to a distant site via the circulatory system, and establishes a secondary tumor.[1] During the metastatic cascade, translocation of tumor cells across the extracellular matrix barriers, namely invasion, is an essential step to accomplish the metastasis. The process of invasion is understood to mainly consist of tumor cell adhesion, enzymatic degradation of extracellular matrix proteins, and migration. Although the genetic basis of tumorigenesis can vary greatly, the steps required for metastasis are similar for all tumor cells. Therefore, drugs that block metastasis will be useful in treating cancers of various genetic origins. In our previous screening for antiinvasive compounds from natural products, distinct inhibitory effects on tumor cell invasion was found in myxochelin A (**1**), a secondary metabolite produced by the actinomycete of the genus *Nonomuraea*.[2] Compound **1** is composed of three building blocks, a diaminoalkane part derived from L-lysine and two identical 2,3-dihydroxybenzoate units, which are connected by two amide bonds. This combinatorial structural feature prompted us to synthesize analogues and assess the structure–activity relationship of this class of compounds as antiinvasive agents. We herein report the synthesis of myxochelin analogues, structure–activity relationship and in vitro and in vivo antitumor activities.

2. Results and discussion

2.1. Synthesis of myxochelin analogues

The myxochelin analogues were synthesized by the coupling of lysine or diaminoalkane derivatives and the benzyl-protected 2,3-dihydroxybenzoic acid and the following modification of functional groups and deprotection. Ten analogues possessing various types of functionality at C-1 position [(*S*)-**1**–**9** and (*R*)-**1**] were prepared as shown in Scheme 1. Synthesis of both enantiomers of **1** was described in our previous paper.[2] Compound **2** was prepared from 1,5-diaminopentane. Compounds (*S*)-**3**, (*S*)-**4** and (*S*)-**5** were synthesized starting from L-lysine methyl ester. Compound (*S*)-**6** was prepared from L-lysinamide. The nitrile analogue (*S*)-**7** was obtained from the same starting material through the dehydration of the amide. Compounds (*S*)-**8** and (*S*)-**9** were prepared by the reported procedure.[3] In addition, four analogues were synthesized to elucidate the involvement of phenolic hydroxyl groups in bioactivity. Compound (*S*)-**10** in which four phenolic hydroxyl groups of (*S*)-**3** were substituted by methoxy groups was prepared by the coupling of L-lysine methyl ester and 2,3-dimethoxybenzoic acid. Compound (*S*)-**11** that lacked hydroxyl groups in the benzene ring located far from the C-1 functionality was prepared from a protected L-lysine methyl ester (Scheme 2). Similarly, compounds (*S*)-**12** and (*S*)-**13** lacking hydroxyl groups in the benzene ring located near to the C-1 functionality were synthesized from protected L-lysine methyl ester and L-lysinamide, respectively (Schemes 2 and 3). Compound (*S*)-**14** which had an acetyl group instead of the benzoyl group was prepared from a protected L-lysine methyl ester

* Corresponding author. Tel.: +81 766567500; fax: +81 766562498.
E-mail address: yas@pu-toyama.ac.jp (Y. Igarashi).

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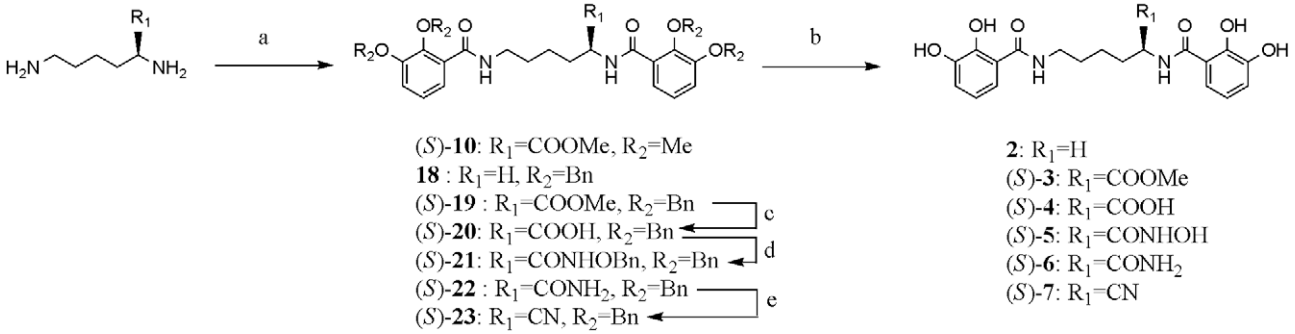
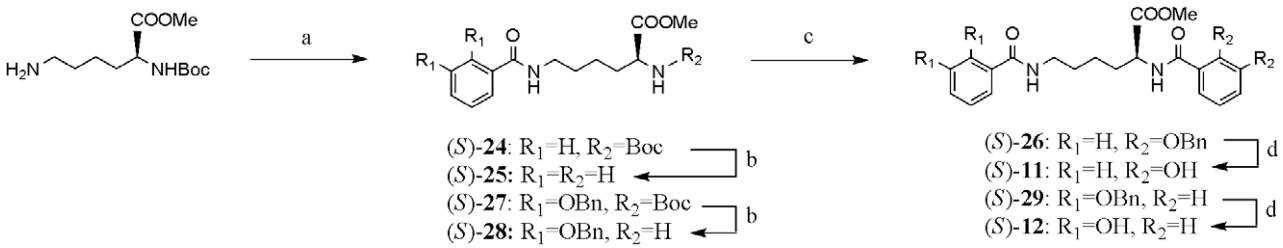
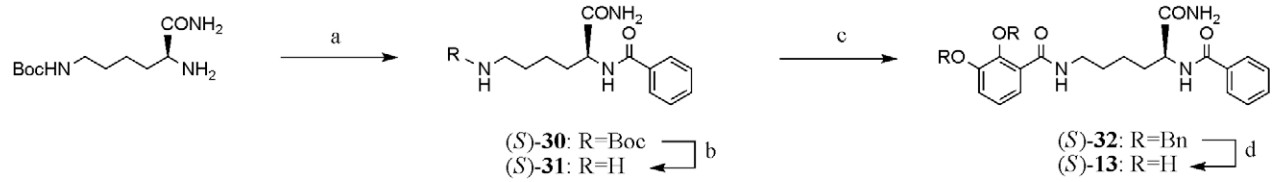


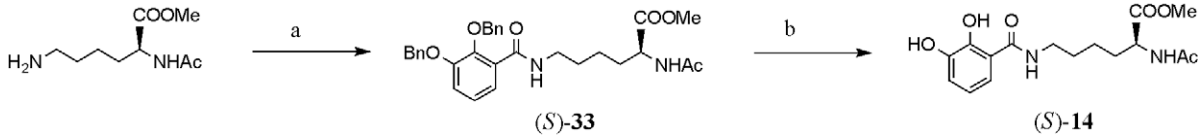
Scheme 1. Reagents: (a) ArCOOH, BOP reagent; (b) H_2, Pd/C, MeOH; (c) NaOH; (d) $BnONH_2$, BOP reagent; (e) triphosgene.



Scheme 2. Reagents: (a) BzCl, pyr or ArCOOH, BOP reagent; (b) HCl/MeOH; (c) ArCOOH, BOP reagent or BzCl, pyr; (d) H_2, Pd/c, MeOH.



Scheme 3. Reagents: (a) BzCl, pyr; (b) HCl/MeOH; (c) ArCOOH, BOP reagent; (d) H_2, Pd/c, MeOH.

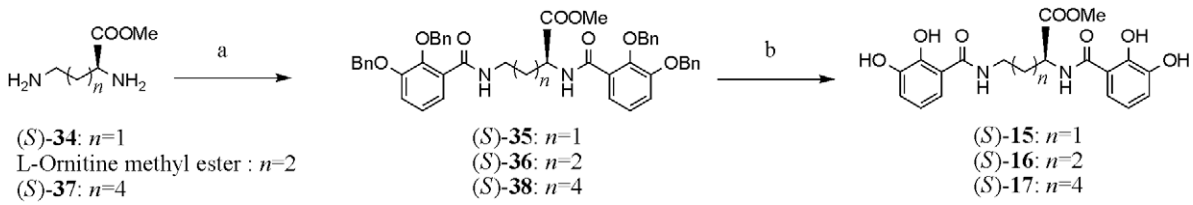


Scheme 4. Reagents: (a) ArCOOH, BOP reagent; (b) H_2, Pd/C, MeOH.

(Scheme 4). Furthermore, three analogues with different methylene chain length were prepared (Scheme 5). Compounds (*S*)-**15** and (*S*)-**16** which had a shorter methylene chain were synthesized from (*S*)-2,4-diaminobutyric acid and L-ornithine, respectively. Compound (*S*)-**17** which had a longer methylene chain was synthesized from (*S*)-2,7-diaminoheptanoate.[4] The structures of synthetic compounds were confirmed by NMR and high resolution ESITOFMS analyses.

2.2. Structure–activity relationship of myxochelin analogues

We have previously shown that the inversion of the absolute configuration at C-2 of **1** from *S* to *R* resulted in the decrease in activity.[2] In the present study, a series of analogues with *S*-configuration were designed and synthesized. The synthetic myxochelin analogues were tested for inhibitory activity against invasion of murine colon 26-L5 cells into the reconstituted basement mem-



Scheme 5. Reagents: (a) ArCOOH, BOP reagent; (b) H_2, Pd/C, MeOH.

brane, Matrigel.[5] Cytotoxicity was also examined in the same cell line. The bioassay results are summarized in Table 1.

2.2.1. Effect of functional group at C-1 position on antiinvasive activity

Among the analogues with different C-1 functionality, the highest activity was shown by compound (*S*)-**6** which has the carbamoyl group at C-1. It is noteworthy that, in spite of its increased potency, the cytotoxicity of (*S*)-**6** was lower than the parent natural product (*S*)-**1** (Table 1 and Fig. 1). Activities of compounds (*S*)-**5**, (*S*)-**8** and (*S*)-**9** were almost equivalent to that of (*S*)-**1**. For the enantiomer (*R*)-**1**, reduction in inhibition of invasion was observed, indicating that the configuration at C-2 is important for activity. Compound (*S*)-**3** showed modest activity. Compounds **2**, (*S*)-**4** and (*S*)-**7** showed either very weak or no activity at the highest concentration (2.5 μM) examined in this study. Interestingly, compound **2** that has no substitution at C-2 was highly toxic and (*S*)-**7** was moderately toxic while other compounds were either weakly toxic or not toxic at the highest concentration (7.5 μM). Although it is not clear what kind of physical property of the functional group (e.g., bulkiness, polarity and acidity) is related, these results indicate that the C-1 functionality affects the inhibitory activity of tumor cell invasion and cytotoxicity as well.

2.2.2. Effect of hydroxyl groups in benzene rings on antiinvasive activity

Based on the preceding structure–activity relationship information, compounds (*S*)-**10**–(*S*)-**13** were designed and synthesized to elucidate the role of hydroxyl groups in benzene rings. At first, compound (*S*)-**10** in which all hydroxyl groups in (*S*)-**3** have been substituted with methoxy groups lost antiinvasive activity. This result indicated that phenolic hydroxyl groups had an essential role in inhibition of invasion. Then, we examined two analogues (*S*)-**11** and (*S*)-**12** which lacked the hydroxyl groups in one of the aromatic rings. Compound (*S*)-**11** completely lost the activity, clearly showing that the hydroxyl groups in the benzene ring far from the C-1

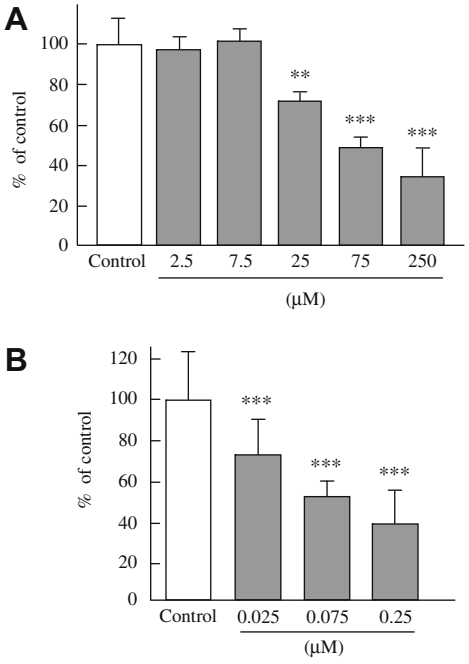
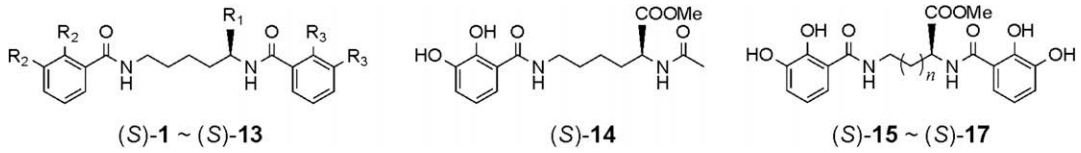
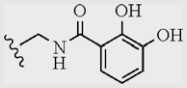


Figure 1. Cytotoxicity (A) and in vitro antiinvasive activity (B) of compound (*S*)-**6** against murine colon 26-L5 cells. The data were expressed as the mean ± SD. $^{**}p < 0.025$, $^{***}p < 0.001$ as compared with the control by student's two-tailed *t*-test.

Table 1
Antiinvasive activity and cytotoxicity of myxochelin analogues



Compound	R_1	R_2	R_3	*n*	IC_{50} (μM)	
					Invasion[a]	Cell growth[b]
(*S*)-**1**	CH_2OH	OH	OH	—	0.59	>7.5 (20%)
(*R*)-**1**	CH_2OH	OH	OH	—	2.2	>7.5 (30%)
2	H	OH	OH	—	>2.5 (45%)	2.7
(*S*)-**3**	COOMe	OH	OH	—	1.5	>7.5 (10%)
(*S*)-**4**	COOH	OH	OH	—	>2.5 (30%)	>7.5 (0%)
(*S*)-**5**	CONHOH	OH	OH	—	0.98	>7.5 (0%)
(*S*)-**6**	$CONH_2$	OH	OH	—	0.091	>7.5 (0%)
(*S*)-**7**	CN	OH	OH	—	>2.5 (45%)	7.5
(*S*)-**8**	CH_2NH_2	OH	OH	—	0.73	>7.5 (40%)
(*S*)-**9**		OH	OH	—	0.52	>5.6 (0%)
(*S*)-**10**	COOMe	OMe	OMe	—	>2.5 (25%)	>7.5 (0%)
(*S*)-**11**	COOMe	H	OH	—	>2.5 (0%)	>7.5 (0%)
(*S*)-**12**	COOMe	OH	H	—	1.0	>7.5 (0%)
(*S*)-**13**	$CONH_2$	OH	H	—	0.26	>7.5 (0%)
(*S*)-**14**	—	—	—	—	2.4	>7.5 (0%)
(*S*)-**15**	—	—	—	1	>2.5 (40%)	>7.5 (13%)
(*S*)-**16**	—	—	—	2	2.5	>7.5 (45%)
(*S*)-**17**	—	—	—	4	1.7	1.6

[a] Inhibition percentage at 2.5 μM is indicated in parentheses.
[b] Inhibition percentage at 5.6 μM for (*S*)-**9** and 7.5 μM for other compounds is indicated in parentheses.

functionality is essential to inhibitory activity. On the other hand, the activity was retained by the analogue (*S*)-**12** that lacks the phenolic hydroxyl groups near to C-1. The C-1 carbamoyl analogue (*S*)-**13** was shown again to exhibit higher activity than the ester analogue (*S*)-**12**. Furthermore, the benzene ring near to the C-1 functionality is not necessary for inhibitory activity as shown by (*S*)-**14**.

2.2.3. Effect of length of methylene chain on antiinvasive activity

The preceding results indicated that both the C-1 functionality and the phenolic hydroxyl groups located far from C-1 were involved in inhibition against tumor cell invasion. Then, we turned our attention to the alkyl linker connecting the two parts. Two methylene shorter analogues, (*S*)-**15** and (*S*)-**16**, and a methylene longer analogue (*S*)-**17** were synthesized and evaluated. Compounds (*S*)-**15** and (*S*)-**16** showed much lower activity than their counterpart (*S*)-**3**. Compound (*S*)-**17** was highly toxic and showed no invasion inhibition at non-cytotoxic concentrations. These results confirmed that the methylene chain length of the natural product is the best for antiinvasive activity.

2.3. Mode of action of myxochelin analogues

The process of invasion into the basement membrane is a cascade of sequential complex steps, mainly consisting of tumor cell adhesion, enzymatic degradation of extracellular matrix proteins, and migration. To gain insight into the mode of action of myxochelin-type compounds, we assessed their effects on tumor cell adhesion and migration. For this purpose, the natural product (*S*)-**1** and the most potent analogue (*S*)-**6** were selected. The results revealed that neither the migration of murine colon 26-L5 cells nor the cell attachment to extracellular matrix proteins (fibronectin and Matrigel) were inhibited by (*S*)-**1** and (*S*)-**6** (Fig. 2), suggesting that degradation of extracellular matrix must be the target step of these compounds.

The process of tumor invasion definitely requires proteolysis of the extracellular matrix by matrix metalloproteinases (MMPs). In particular, two gelatinases, MMP-2 and MMP-9 are the key enzymes in degradation of extracellular matrix by many types of malignant cancer.[6] Therefore, we next examined the inhibitory effect of (*S*)-**1** and (*S*)-**6** on gelatinase activities. In gelatin-based zymography assay, (*S*)-**1** showed 35% and 39% inhibition against MMP-2 and MMP-9 at 2.5 μM, respectively (Fig. 3A), whereas (*S*)-**6** displayed 44% and 33% inhibition against MMP-2 and MMP-9 at 0.25 μM, respectively (Fig. 3B). A similar trend was also found on comparing the inhibitory activity of (*S*)-**1** and (*S*)-**6** toward tumor cell invasion. We had no access to the inhibition assay for other MMPs, however these results suggest that MMP-2 and MMP-9 are at least the target molecules of (*S*)-**6**.

Some inhibitors of tumor cell invasion inhibit MMPs. Such inhibitors often possess in their structures the chelating-site for a divalent zinc cation in the catalytic domain of MMPs.[7] For example, ONO-4817 inhibits MMPs by binding to the zinc cation with its hydroxamic acid moiety.[8] In case of myxochelin analogues, the hydroxamate analogue (*S*)-**5** was 10-fold less active than the carbamoyl analogue (*S*)-**6**. The hydroxylated benzoyl moiety would be involved in binding to the zinc cation rather than the C-1 functional group.

2.4. Antimetastatic activity of (*S*)-6 in lung metastasis model

Since compound (*S*)-**6** showed the most potent in vitro activity and less cytotoxicity, it was regarded as a candidate for in vivo investigation. To evaluate in vivo antimetastatic effect of (*S*)-**6**, we examined suppression of lung metastasis of colon 26-L5 cells in mouse.[9] Intraperitoneal administration of (*S*)-**6** (50 mg/kg) resulted in a reduction of lung tumor colonies produced by iv injection of colon 26-L5 cells into the tail vein. The inhibitory effect was significant (inhibition rate of 35%, $p < 0.05$), compared to the vehicle control (Fig. 4A). There was no marked body weight loss in the treated mice during the course of the study (Fig. 4B). The oral administration of (*S*)-**6** showed tendency to suppress the lung

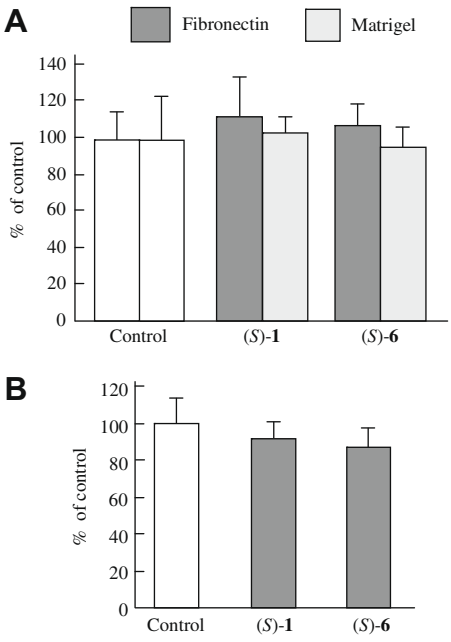


Figure 2. Effects of compounds (*S*)-**1** and (*S*)-**6** at 2.5 μM on tumor cell adhesion (A) and migration (B). The data were expressed as the mean ± SD.

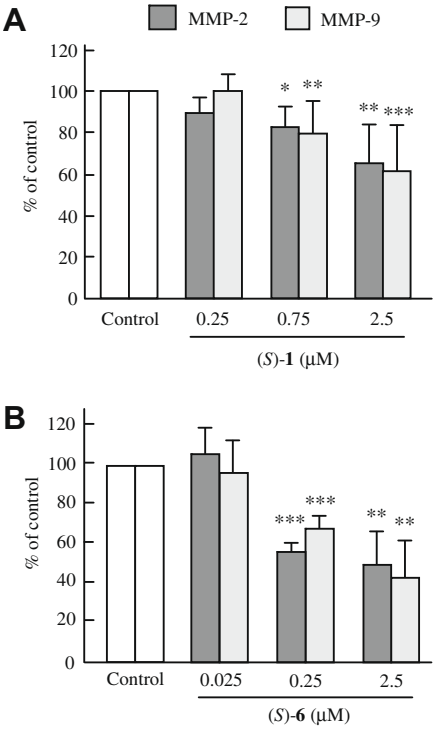


Figure 3. Inhibition of MMP-2 and MMP-9 by compounds (*S*)-**1** (A) and (*S*)-**6** (B). The data were expressed as the mean ± SD. $^{*}p < 0.01$, $^{**}p < 0.025$, $^{***}p < 0.001$ as compared with the control by student's two-tailed *t*-test.

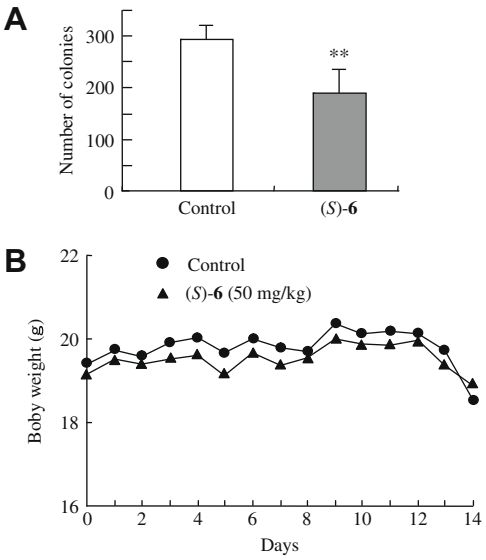


Figure 4. Inhibition of lung metastasis of colon 26-L5 cells by ip administration of compound (*S*)-**6**. (A) Number of tumor colonies formed on lung 14 days after tumor inoculation. (B) Body weight change of mice during 14 days. The data were expressed as the mean ± SD. $^{**}p < 0.025$ as compared with the control by student's two-tailed *t*-test.

metastasis but the effect was not significant ($p = 0.55$, data not shown).

3. Conclusion

In summary, we report the synthesis and biological evaluation of a series of myxochelin analogues. Compound (*S*)-**6** that possesses the carbamoyl group at C-1 exhibited a sevenfold improvement with regard to the parent natural product (*S*)-**1** and showed lower cytotoxicity. Several bioassay results revealed that (*S*)-**6** inhibits the process of extracellular matrix degradation by inhibiting the proteolytic activity of at least two MMPs, MMP-2 and MMP-9. Furthermore, (*S*)-**6** suppressed the lung metastasis of colon 26-L5 cells in mice. Our findings demonstrate that the myxochelin-type compound is a new and interesting scaffold for the development of antimetastatic agents.

4. Experimental

4.1. General

Optical rotations were measured using a JASCO DIP-3000 polarimeter. IR spectra were measured on a Perkin Elmer Spectrum 100. NMR spectra were obtained on a JEOL LA-400 or a Bruker AVANCE 400 spectrometer. HR-ESITOFMS were recorded on a Bruker micro-TOF focus. Silica Gel 60-C18 (Nakalai Tesque 250–350 mesh) was used for ODS column chromatography. HPLC separation was performed using a Cosmosil 5C18-AR-II (Nacalai Tesque Inc., 20 × 250 mm) with a photodiode array detector.

4.2. Synthesis of myxochelin analogues

4.2.1. 1,5-Bis[(2,3-dibenzyloxybenzoyl)amino]pentane (18)

To a solution of 1,5-diaminopentane (53 mg, 0.30 mmol), BOP reagent (0.464 g, 1.05 mmol) and anhydrous 1-hydroxybenzotriazole (142 mg, 1.05 mmol) in dry DMF (4 mL) was added 2,3-dibenzyloxybenzoic acid[10] (200 mg, 0.60 mmol) and *N*,*N*-diisopropylethylamine (0.31 mL, 0.60 mmol). After stirring for 14 h at room temperature, the reaction mixture was poured into ice-water and extracted with EtOAc. The organic layer was washed with 0.5 M HCl, saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc = 1:0–1:5) to give **18** (222 mg, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (2H, m), 1.26 (4H, m), 3.19 (4H, m), 5.13 (4H, s), 5.15 (4H, s), 7.13–7.48 (24H), 7.73 (2H, m), 7.89 (2H, t, $J = 5.1$ Hz); HR-ESITOFMS *m*/*z* $[M-H]^-$ 733.3284 (calcd for $C_{47}H_{45}N_2O_6$, 733.3283).

4.2.2. 1,5-Bis[(2,3-dihydroxybenzoyl)amino]pentane (2)

A mixture of compound **18** (134 mg, 0.182 mmol) and 10% Pd/C (91 mg) in MeOH (45 mL) was stirred under H_2 atmosphere at room temperature. After stirring for 3 h, the mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was purified by preparative HPLC (30% CH_3CN in water) and the collected fraction was concentrated to give **2** (23 mg, 34% yield): colorless amorphous solid; ^{1}H NMR (400 MHz, CD_3OD) δ 1.35 (2H, m), 1.56 (4H, m), 3.30 (4H, m), 6.60 (2H, t, $J = 7.6$ Hz), 6.82 (2H, d, $J = 7.3$ Hz), 7.10 (2H, d, $J = 7.6$ Hz); IR ν_{max} 3356, 3189, 1638, 1579, 1554 cm^{-1}; HR-ESITOFMS *m*/*z* $[M-H]^-$ 373.1403 (calcd for $C_{19}H_{21}N_2O_6$, 373.1405).

4.2.3. (*S*)-2,6-Bis[(2,3-dibenzyloxybenzoyl)amino]hexanoic acid methyl ester [(*S*)-19]

In the same manner as described for **18**, L-lysine methyl ester gave (*S*)-**19** in 57% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04–1.28 (4H, m), 1.38 (1H, m), 1.61 (1H, m), 3.10 (2H, m), 3.59 (3H, s), 4.59 (1H, m), 5.05 (2H, s), 5.14 (1H, d, $J = 10.2$ Hz), 5.15 (2H, s), 5.16 (1H, d, $J = 10.2$ Hz), 7.13–7.52 (24H, m), 7.72 (2H, m), 7.85 (1H, t, $J = 5.6$ Hz), 8.48 (1H, d, $J = 7.3$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9, 28.7, 31.5, 52.1, 52.5, 71.22, 71.26, 76.1, 76.3, 116.8, 117.2, 123.25, 123.29, 124.32, 124.37, 126.6, 127.3, 127.6, 127.7, 128.19, 128.23, 128.4, 128.5, 128.63, 128.66, 128.69, 128.74, 136.26, 136.28, 136.3, 136.4, 146.7, 146.9, 151.6, 151.7, 164.9, 164.9, 172.7; HR-ESITOFMS *m*/*z* $[M+Na]^+$ 815.3305 (calcd for $C_{49}H_{48}N_2O_8Na$, 815.3303).

4.2.4. (*S*)-2,6-Bis[(2,3-dihydroxybenzoyl)amino]hexanoic acid methyl ester [(*S*)-3]

In the same manner as described for **2**, (*S*)-**19** gave (*S*)-**3** in 64% yield: colorless amorphous solid; $[\alpha]_D^{24} - 8.0$ (*c* 0.15, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.42 (2H, m), 1.58 (2H, m), 1.79 (1H, m), 1.91 (1H, m), 3.30 (1H, t, $J = 7.1$ Hz), 3.63 (3H, s), 4.53 (1H, dd, $J = 8.6$, 5.1 Hz), 6.59 (1H, t, $J = 7.8$ Hz), 6.62 (1H, t, $J = 7.8$ Hz), 6.82 (1H, d, $J = 7.8$ Hz), 6.84 (1H, d, $J = 7.8$ Hz), 7.08 (2H, d, $J = 7.6$ Hz), 7.22 (1H, d, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 24.4, 29.9, 32.0, 40.1, 52.8, 53.8, 116.78, 116.83, 118.6, 119.47, 119.49, 119.6, 119.7, 147.29, 147.33, 150.0, 150.3, 171.2, 171.5, 174.3; IR ν_{max} 3346, 1729, 1638, 1585, 1535 cm^{-1}; HR-ESITOFMS *m*/*z* $[M-H]^-$ 431.1460 (calcd for $C_{21}H_{23}N_2O_8$, 431.1460).

4.2.5. (*S*)-2,6-Bis[(2,3-dibenzyloxybenzoyl)amino]hexanoic acid [(*S*)-20]

To a solution of (*S*)-**19** (50 mg, 0.063 mmol) in MeOH (5 mL) was added 1 M NaOH (1 mL). After stirring for 2 h at room temperature, the solution was neutralized with 0.5 M HCl and concentrated in vacuo. Then, the residue was acidified with 1 M HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give (*S*)-**20** (47 mg, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15–1.35 (5H), 1.46 (1H, m), 3.11(2H, m), 3.95 (1H, m), 4.99 (2H, s), 5.01 (1H, d, $J = 10.8$ Hz), 5.05 (1H, d, $J = 10.8$ Hz), 5.08 (2H, s), 5.09 (2H, s), 7.04–7.44 (26H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9, 28.8, 30.6, 39.3, 53.0, 71.3, 71.4, 76.4, 76.7, 117.0, 117.6, 123.4, 124.4, 126.0, 127.2, 127.7, 127.8, 128.26, 128.31, 128.6, 126.9, 128.72, 128.8, 128.9, 136.1, 136.27,

136.34, 136.3, 136.4, 147.1, 151.68, 151.74, 165.1, 166.2; HR-ESI-TOFMS *m/z* $[M-H]^-$ 777.3179 (calcd for $C_{48}H_{45}N_2O_8$, 777.3181).

4.2.6. (*S*)-2,6-Bis[(2,3-dihydroxybenzoyl)amino]hexanoic acid [(*S*)-4]

In the same manner as described for **2**, (*S*)-**20** gave (*S*)-**4** in 61% yield: colorless amorphous solid; $[\alpha]_D^{23}$ + 5.4 (*c* 0.13, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.54 (2H, m), 1.67 (2H, m), 1.92 (1H, m), 2.14 (1H, m), 3.38 (2H, t, *J* = 6.4 Hz), 4.61 (1H, br s), 6.67 (1H, t, *J* = 8.1 Hz), 6.71 (1H, t, *J* = 7.8 Hz), 6.89 (1H, d, *J* = 8.1 Hz), 6.92 (1H, d, *J* = 7.6 Hz), 7.16 (1H, d, *J* = 7.8 Hz), 7.31 (1H, d, *J* = 7.8 Hz); ^{13}C NMR (400 MHz, CD_3OD) δ 24.4, 29.9, 32.4, 40.2, 50.4, 116.8, 117.0, 118.6, 119.4, 119.5, 119.7, 147.2, 147.3, 149.8, 150.2, 170.9, 171.5; IR ν_{max} 3353, 1722, 1639, 1583, 1538 cm^{-1}; HR-ESI-TOFMS *m/z* $[M-H]^-$ 417.1296 (calcd for $C_{20}H_{21}N_2O_8$, 417.1303).

4.2.7. (*S*)-2,6-Bis[(2,3-dibenzyloxybenzoyl)amino]-*N*-benzyl-oxyhexanoamide [(*S*)-21]

Compound (*S*)-**20** (80 mg, 0.10 mmol), BOP reagent (88 mg, 0.20 mmol) and anhydrous 1-hydroxybenzotriazole (27 mg, 0.20 mmol) were dissolved in dry DMF (2 mL). To the mixture were then added *O*-benzylhydroxylamine hydrochloride (16 mg, 0.10 mmol) and *N*,*N*-diisopropylethylamine (0.017 mL, 0.10 mmol). After stirring for 14 h at room temperature, the reaction mixture was poured into ice-water and extracted with EtOAc. The organic layer was washed with 0.5 M HCl, saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc = 10:1–1:3) to give (*S*)-**21** (61 mg 67% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08–1.29 (4H, m), 1.60 (1H, m), 2.09 (1H, m), 3.12 (2H, dt, *J* = 6.3, 6.3 Hz), 4.23 (1H, m), 4.94 (2H, s), 5.04 (2H, s), 5.09 (2H, s), 5.11 (2H, s), 5.13 (2H, s), 7.08–7.49 (29H), 7.64 (2H, m), 7.89 (1H, br s), 8.44 (1H, d, *J* = 7.1 Hz), 9.62 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9, 28.7, 30.2, 39.1, 51.0, 71.2, 71.3, 76.1, 76.3, 78.0, 116.8, 117.5, 123.1, 123.2, 124.27, 124.31, 125.9, 127.2, 127.6, 127.7, 128.2, 128.3, 128.4, 128.5, 128.59, 128.61, 128.63, 129.0, 129.1, 135.2, 135.8, 136.2, 136.28, 136.33, 146.7, 146.9, 151.6, 151.7, 164.9, 165.5, 169.1; HR-ESITOFMS *m/z* $[M+Na]^+$ 906.3725 (calcd for $C_{55}H_{53}N_3O_8$-Na, 906.3725).

4.2.8. (*S*)-2,6-Bis[(2,3-dihydroxybenzoyl)amino]- *N*-hydroxy-hexanoamide [(*S*)-5]

In the same manner as described for **2**, (*S*)-**21** gave (*S*)-**5** in 81% yield: colorless amorphous solid; $[\alpha]_D^{23}$ − 6.0 (*c* 0.13, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.50 (2H, m), 1.67 (2H, m), 1.89 (2H, m), 3.38 (2H, t, *J* = 7.3 Hz), 4.50 (1H, t, *J* = 7.1 Hz), 6.67 (1H, t, *J* = 8.1 Hz), 6.69 (1H, t, *J* = 8.1 Hz), 6.89 (1H, d, *J* = 7.6 Hz), 6.91 (1H, d, *J* = 7.6 Hz), 7.17 (1H, d, *J* = 8.0 Hz), 7.31 (1H, d, *J* = 7.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 24.3, 30.0, 32.9, 40.2, 52.5, 116.8, 118.7, 119.5, 119.59, 119.62, 119.7, 147.3, 147.4, 149.8, 150.1, 170.7, 171.2, 171.5; IR ν_{max} 3202, 1635, 1582, 1530 cm^{-1}; HR-ESI-TOFMS *m/z* $[M-H]^-$ 432.1402 (calcd for $C_{20}H_{22}N_3O_8$, 432.1412).

4.2.9. (*S*)-2,6-Bis[(2,3-dihydroxybenzoyl)amino]hexanoamide [(*S*)-6]

In the same manner as described for **2**, (*S*)-2,6-bis[(2,3-dibenzyloxybenzoyl)amino]hexanoamide[3] [(*S*)-**22**] gave (*S*)-**6** in 57% yield: colorless amorphous solid; $[\alpha]_D^{23}$ − 7.0 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.44 (2H, m), 1.51 (2H, m), 1.90 (1H, m), 1.95 (1H, m), 3.38 (2H, *J* = 6.6 Hz), 4.58 (1H, dd, *J* = 8.6, 5.1 Hz), 6.65 (1H, d, *J* = 7.8 Hz), 6.68 (1H, d, *J* = 7.8 Hz), 6.90 (2H, m), 7.17 (1H, d, *J* = 7.8 Hz), 7.30 (1H, d, *J* = 7.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 24.4, 30.0, 32.9, 40.1, 50.6, 116.9, 117.3, 118.7, 119.32, 119.36, 119.4, 119.7, 147.38, 147.43, 150.3, 150.4, 170.9, 171.5, 171.2; IR ν_{max} 3321, 1671, 1637, 1583, 1534 cm^{-1}; HR-ESITOFMS *m/z* $[M-H]^-$ 416.1461 (calcd for $C_{20}H_{22}N_3O_7$, 416.1463).

4.2.10. (*S*)-2,6-Bis[(2,3-dihydroxybenzoyl)amino]hexanenitrile [(*S*)-7]

In the same manner as described for **2**, (*S*)-2,6-bis[(2,3-dibenzyloxybenzoyl) amino]hexanenitrile[3] [(*S*)-**23**] gave (*S*)-**7** in 38% yield: colorless amorphous solid; $[\alpha]_D^{23}$ − 18.1 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.67 (2H, m), 1.92 (2H, m), 2.01 (2H, m), 3.40 (2H, dd, *J* = 13.7, 6.8 Hz), 5.03 (1H, t, *J* = 7.3 Hz), 6.68 (1H, t, *J* = 8.0 Hz), 6.69 (1H, t, *J* = 8.0 Hz), 6.89 (1H, d, *J* = 7.8 Hz), 6.93 (1H, d, *J* = 7.8 Hz), 7.17 (1H, d, *J* = 8.1 Hz), 7.24 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 24.1, 29.7, 33.2, 40.0, 41.5, 116.2, 116.8, 118.7, 119.2, 119.3, 119.4, 119.5, 119.8, 119.9, 147.4, 147.7, 150.4, 151.1, 171.1, 171.6; IR ν_{max} 3337, 2244, 1638, 1585, 1534 cm^{-1}; HR-ESITOFMS *m/z* $[M+Na]^+$ 422.1327 (calcd for $C_{20}H_{21}N_3O_6Na$, 422.1323).

4.2.11. (*S*)-2,6-Bis[(2,3-dimethoxybenzoyl)amino]hexanoic acid methyl ester [(*S*)-10]

In the same manner as described for **18**, condensation of L-lysine methyl ester and 2,3-dimethoxybenzoic acid gave (*S*)-**10** in 92% yield: colorless amorphous solid; $[\alpha]_D^{22}$ − 3.9 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (2H, m), 1.60 (2H, m), 1.79 (1H, m), 1.94 (1H, m), 3.37 (2H, dd, *J* = 12.7, 6.4 Hz), 3.68 (3H, s), 3.79 (3H, s), 3.83 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 4.80 (2H, m), 6.94 (1H, d, *J* = 8.3 Hz), 6.98 (1H, d, *J* = 8.3 Hz), 7.04 (1H, t, *J* = 7.8 Hz), 7.06 (1H, t, *J* = 8.0 Hz), 7.57 (1H, d, *J* = 8.0 Hz), 7.59 (1H, d, *J* = 8.1 Hz); IR ν_{max} 3369, 1740, 1652, 1521 cm^{-1}; HR-ESITOFMS *m/z* $[M+Na]^+$ 511.2057 (calcd for $C_{25}H_{32}N_2O_8Na$, 511.2051).

4.2.12. N^{ε}-Benzoyl-N^{α}-*tert*-butoxycarbonyl-L-lysine methyl ester [(*S*)-24]

To a stirred solution of N^{α}-*tert*-butoxycarbonyl-L-lysine methyl ester (300 mg, 1.0 mmol) in dry pyridine (3.5 mL) was added benzoyl chloride (211 mg, 1.5 mmol) at 0–5 °C. After stirring at the same temperature for 15 h, the mixture was poured into ice-water and extracted with EtOAc. The organic layer was washed with saturated $CuSO_4$ solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc = 1:0–1:1) to give (*S*)-**24** (380 mg, 99% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35–1.59 (4H, m), 1.49 (9H, s), 1.83 (1H, m), 1.98 (1H, m), 3.12 (2H, m), 3.79 (3H, s), 4.60 (1H, br s), 4.82 (1H, m), 6.75 (1H, br s), 7.45 (2H, t, *J* = 7.8 Hz), 7.53 (2H, t, *J* = 6.8 Hz), 7.83 (1H, d, *J* = 7.6 Hz); HR-ESI-TOFMS *m/z* $[M+Na]^+$ 387.1891 (calcd for $C_{19}H_{28}N_2O_5Na$, 387.1890).

4.2.13. N^{ε}-Benzoyl-L-lysine methyl ester [(*S*)-25]

A solution of compound (*S*)-**24** (350 mg, 0.96 mmol) in 1.5 M HCl–MeOH (17.5 mL) was stirred for 3 h at room temperature. The pH of the reaction mixture was adjusted to 8.0 by the addition of aqueous NH_4OH solution. The mixture was then concentrated in vacuo and the remaining aqueous residue was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give (*S*)-**25** (168 mg, 66% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 1.50 (2H, m), 1.72 (2H, m), 1.90 (1H, m), 2.02 (1H, m), 2.93 (2H, t, *J* = 6.3 Hz), 3.34 (3H, s), 4.63 (1H, m), 7.47 (2H, t, *J* = 7.3 Hz), 7.55 (1H, t, *J* = 7.1 Hz), 7.85 (2H, d, *J* = 7.3 Hz); HR-ESITOFMS *m/z* $[M+H]^+$ 265.1555 (calcd for $C_{14}H_{21}N_2O_3$ 265.1547).

4.2.14. (*S*)-6-Benzoylamino-2-[(2,3-dibenzyloxybenzoyl)-amino]hexanoic acid methyl ester [(*S*)-26]

In the same manner as described for **18**, (*S*)-**25** gave (*S*)-**26** in 36% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (4H, m), 1.90 (2H, m), 3.19 (1H, m), 3.33 (1H, m), 3.75 (3H, s), 4.70 (1H, m), 5.04 (1H, d, *J* = 10.5 Hz), 5.06 (1H, d, *J* = 10.5 Hz), 5.15 (2H, s), 6.91 (1H, d, *J* = 7.3 Hz), 7.10–7.50 (16H, m), 7.83 (2H, d, *J* = 8.0 Hz), 7.98 (1H, t, *J* = 5.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 29.1,

31.4, 38.5, 52.4, 52.6, 71.3, 76.4, 116.9, 123.3, 124.4, 127.20, 127.23, 127.7, 128.3, 128.5, 128.68, 128.72, 128.77, 131.6, 136.3, 136.4, 146.7, 151.7, 165.5, 167.3, 173.0; HR-ESITOFMS *m/z* $[M-H]^-$ 579.2513, calcd 579.2501 (for $C_{35}H_{35}N_2O_6$).

4.2.15. (*S*)-6-Benzoylamino-2-[(2,3-dihydroxybenzoyl)amino]-hexanoic acid methyl ester [(*S*)-11]

In the same manner as described for **2**, (*S*)-**26** gave (*S*)-**11** in 43% yield: colorless amorphous solid; $[\alpha]_D^{23}$ −4.4 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.41 (2H, m), 1.56 (2H, m), 1.77 (1H, m), 1.90 (1H, m), 3.27 (2H, t, *J* = 9.3 Hz), 4.50 (1H, dd, 9.5, 5.1 Hz), 6.57 (1H, t, *J* = 7.6 Hz), 6.80 (1H, d, *J* = 7.6 Hz), 7.08 (1H, d, *J* = 7.6 Hz), 7.42 (2H, t, *J* = 7.6 Hz), 7.50 (1H, t, *J* = 7. 4 Hz), 7.71 (2H, d, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 24.5, 29.9, 31.9, 40.8, 52.7, 54.3, 116.7, 118.6, 119.5, 128.5, 129.5, 132.9, 135.1, 147.4, 150.4, 170.6, 171.5, 174.4; IR ν_{max} 3348, 1736, 1637, 1535 cm^{-1}; HR-ESITOFMS *m/z* $[M+Na]^+$ 423.1521 (calcd for $C_{21}H_{24}N_2O_6Na$ 423.1527).

4.2.16. N^{α}-*tert*-Butoxycarbonyl-N^{ε}-(2,3-dibenzyloxybenzoyl)-L-lysine methyl ester [(*S*)-27]

In the same manner as described for **18**, N^{α}-*tert*-butoxycarbonyl-L-lysine methyl ester gave (*S*)-**27** in 98% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (2H, m), 1.38 (2H, m), 1.45 (9H, s), 1.63–1.75 (2H, m), 2.98 (2H, m), 3.67 (3H, s), 4.47 (1H, br s), 4.67 (1H, q, *J* = 7.6 Hz), 5.12 (1H, d, *J* = 10.5 Hz), 5.16 (2H, s), 5.19 (1H, d, *J* = 10.5 Hz), 7.13–7.47 (13H, m), 7.74 (1H, m), 8.51 (1H, d, *J* = 7.6 Hz); HR-ESITOFMS *m/z* $[M+Na]^+$ 599.2719 (calcd for $C_{33}H_{40}N_2O_7Na$ 599.2728).

4.2.17. (*S*)-2-Amino-6-[(2,3-dibenzyloxybenzoyl)amino]-hexanoic acid methyl ester [(*S*)-28]

In the same manner as described for (*S*)-**25**, (*S*)-**27** gave (*S*)-**28** in 84% yield: ^{1}H NMR (400 MHz, CD_3OD) δ 1.25 (3H, m), 1.47 (2H, m), 1.68 (1H, m), 2.67 (2H, m), 3.61 (3H, s), 4.46 (1H, dd, *J* = 8.3, 5.4 Hz), 5.04 (1H, d, *J* = 10.5 Hz), 5.05 (1H, d, *J* = 10.5 Hz), 5.09 (2H, s), 7.05–7.40 (13H, m); HR-ESITOFMS *m/z* $[M+H]^+$ 477.2384 (calcd for $C_{28}H_{33}N_2O_5$ 477.2384).

4.2.18. (*S*)-2-Benzoylamino-6-[(2,3-dibenzyloxybenzoyl)-amino]hexanoic acid methyl ester [(*S*)-29]

In the same manner as described for **18**, (*S*)-**28** gave (*S*)-**29** in 46% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (2H, m), 1.32 (2H, m), 1.47 (1H, m), 1.64 (1H, m), 2.42 (2H, t, *J* = 7.1 Hz), 3.64 (3H, s), 5.02 (4H, s), 7.07–7.44 (18H, m); HR-ESITOFMS *m/z* $[M-H]^-$ 579.2506, calcd 579.2501 (for $C_{35}H_{35}N_2O_6$).

4.2.19. (*S*)-2-Benzoylamino-6-[(2,3-dihydroxybenzoyl)amino]-hexanoic acid methyl ester [(*S*)-12]

In the same manner as described for **2**, (*S*)-**29** gave (*S*)-**12** in 46% yield: colorless amorphous solid; $[\alpha]_D^{23}$ −9.1 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.43 (2H, m), 1.57 (2H, m), 1.81 (1H, m), 1.89 (1H, m), 3.27 (2H, t, *J* = 6.6 Hz), 3.62 (3H, s), 4.54 (1H, dd, *J* = 9.0, 5.1 Hz), 6.59 (1H, t, *J* = 7.6), 6.83 (1H, d, *J* = 7.3 Hz), 7.22 (1H, d, *J* = 7.8 Hz), 7.37 (2H, t, *J* = 7.3), 7.39 (1H, t, *J* = 7.3 Hz), 7.66 (2H, d, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 24.3, 30.0, 32.0, 40.5, 52.8, 53.8, 116.8, 119.4, 119.5, 119.6, 128.2, 129.5, 132.5, 135.8, 147.4, 150.4, 170.4, 171.2, 174.2; IR ν_{max} 3314, 1730, 1637, 1534 cm^{-1}; HR-ESITOFMS *m/z* $[M+Na]^+$ 423.1527 (calcd for $C_{21}H_{24}N_2O_6Na$ 423.1527).

4.2.20. N^{α}-Benzoyl-N^{ε}-*tert*-butoxycarbonyl-L-lysinamide [(*S*)-30]

In the same manner as described for (*S*)-**24**, N^{ε}-*tert*-butoxycarbonyl-L-lysinamide gave (*S*)-**30** in 34% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (9H, s), 1.47 (2H, m), 1.56 (2H, m), 1.84 (1H, m), 2.03 (1H, m), 3.13 (2H, m), 4.70 (1H, m), 5.64 (1H, br s), 6.41 (1H, br s), 6.99 (1H, br s), 7.45 (2H, t, *J* = 7.6 Hz), 7.53 (1H, t, *J* = 7.6 Hz), 7.82 (2H, d, *J* = 7.4 Hz); HR-ESITOFMS *m/z* $[M-H]^-$ 348.1929 (calcd for $C_{18}H_{26}N_3O_4$ 348.1929).

4.2.21. N^{α}-Benzoyl-L-lysinamide [(*S*)-31]

In the same manner as described for (*S*)-**25**, (*S*)-**30** gave (*S*)-**31** in 71% yield: ^{1}H NMR (400 MHz, CD_3OD) δ 1.47–1.60 (2H, m), 1.71 (2H, m), 1.86 (1H, m), 1.99 (1H, m), 2.94 (2H, m), 4.60 (1H, dd, *J* = 9.0, 5.5 Hz), 7.49 (2H, t, *J* = 7.5 Hz), 7.57 (1H, t, *J* = 7.5 Hz), 7.89 (2H, d, *J* = 8.0 Hz); HR-ESITOFMS *m/z* $[M+H]^+$ 250.1558 (calcd for $C_{13}H_{20}N_3O_2$ 250.1550).

4.2.22. (*S*)-2-Benzoylamino-6-[(2,3-dibenzyloxybenzoyl)-amino]hexanoamide [(*S*)-32]

In the same manner as described for **18**, (*S*)-**31** gave (*S*)-**32** in 49% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (4H, m), 1.86 (2H, m), 3.15 (1H, m), 3.29 (1H, m), 4.61 (1H, m), 5.01 (1H, d, *J* = 10.4 Hz), 5.02 (1H, d, *J* = 10.4 Hz), 5.12 (2H, s), 6.12 (1H, br s), 6.98 (1H, br s), 7.06–7.64 (16H, m), 7.81 (2H, m), 7.97 (1H, t, *J* = 5.7 Hz); HR-ESITOFMS *m/z* $[M+Na]^+$ 588.2470 (calcd for $C_{34}H_{35}N_3O_5Na$ 588.2469).

4.2.23. (*S*)-2-Benzoylamino-6-[(2,3-dihydroxybenzoyl)amino]-hexanoamide [(*S*)-13]

In the same manner as described for **2**, (*S*)-**29** gave (*S*)-**13** in 59% yield: colorless amorphous solid; $[\alpha]_D^{23}$ −4.8 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.46 (2H, m), 1.59 (2H, m), 1.76 (1H, m), 1.86 (1H, m), 3.30 (2H, t, *J* = 6.5 Hz), 4.47 (1H, d, *J* = 9.2, 5.2 Hz), 6.57 (1H, t, *J* = 7.8 Hz), 6.81 (1H, d, *J* = 7.6 Hz), 7.09 (1H, d, *J* = 7.9 Hz), 7.33 (2H, t, *J* = 7.4 Hz), 7.42 (1H, t, *J* = 7.4 Hz), 7.73 (2H, d, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 24.5, 30.1, 32.9, 40.1, 55.0, 116.9, 118.8, 119.2, 119.3, 128.5, 129.5, 132.8, 135.3, 147.5, 150.7, 170.4, 171.5, 177.3; IR ν_{max} 3310, 1671, 1636, 1578, 1532 cm^{-1}; HR-ESITOFMS *m/z* $[M+Na]^+$ 408.1528 (calcd for $C_{20}H_{23}N_3O_5Na$ 408.1530).

4.2.24. (*S*)-2-Acetyl-6-[(2,3-dibenzyloxybenzoyl)amino]-hexanoic acid methyl ester [(*S*)-33]

In the same manner as described for **18**, N^{α}-acetyl-L-lysine methyl ester gave (*S*)-**33** in 99% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21–1.36 (4H), 1.68 (1H, m), 1.78 (1H, m), 2.06 (3H, s), 3.21 (1H, m), 3.30 (1H, m), 3.71 (3H, s), 4.48 (1H, ddd, *J* = 7.6, 7.6, 4.4 Hz), 5.07 (1H, d, *J* = 10.4 Hz), 5.08 (1H, d, *J* = 10.4 Hz), 5.16 (2H, s), 6.29 (1H, d, *J* = 7.6 Hz), 7.15–7.17 (2H, m), 7.32–7.49 (10H, m), 7.73 (1H, m), 8.00 (1H, d, *J* = 5.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.2, 23.1, 28.9, 31.3, 38.5, 52.1, 52.3, 71.3, 76.4, 117.0, 123.2, 124.4, 127.7, 128.3, 128.71, 128.72, 128.77, 128.80, 136.3, 146.8, 151.7, 165.4, 170.1, 173.0; HR-ESITOFMS *m/z* $[M+Na]^+$ 541.2309 (calcd for $C_{30}H_{34}N_2O_6Na$ 541.2309).

4.2.25. (*S*)-2-Acetyl-6-[(2,3-dihydroxybenzoyl)amino]hexanoic acid methyl ester [(*S*)-14]

In the same manner as described for **2**, (*S*)-**33** gave (*S*)-**14** in 51% yield: colorless amorphous solid; $[\alpha]_D^{24}$ −2.7 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.36 (2H, m), 1.52 (2H, m), 1.60 (1H, m), 1.75 (1H, m), 3.25 (3H, s), 3.28 (2H, t, *J* = 6.8 Hz), 3.58 (3H, s), 4.46 (1H, dd, *J* = 8.8, 5.2 Hz), 6.60 (1H, t, *J* = 7.8 Hz), 6.81 (1H, d, *J* = 7.7 Hz), 7.81 (1H, d, *J* = 7.9 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 22.3, 24.2, 29.9, 32.1, 40.1, 52.6, 53.8, 116.8, 118.7, 119.37, 119.43, 147.5, 150.6, 171.5, 173.4, 174.3; IR ν_{max} 3314, 1733, 1635, 1587, 1537 cm^{-1}; HR-ESITOFMS *m/z* $[M-H]^-$ 337.1405 (calcd for $C_{16}H_{22}N_2O_6$ 337.1405).

4.2.26. (*S*)-2,4-Bis[(2,3-dibenzyloxybenzoyl)amino]butyric acid methyl ester [(*S*)-35]

In the same manner as described for **18**, (*S*)-**34**[11] gave (*S*)-**35** in 46% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (1H, m), 1.90 (1H, m),

3.05 (1H, m), 3.32 (1H, m), 3.61 (3H, s), 4.64 (1H, dt, J = 5.6, 8.4 Hz), 5.08 (1H, d, J = 10.4 Hz), 5.10 (1H, s), 5.11 (1H, s), 5.14 (2H, s), 5.15 (2H, s), 5.21 (1H, J = 10.4 Hz), 7.11–7.47 (24H, m), 7.65–7.70 (2H, m), 8.03 (1H, t, J = 5.6 Hz), 8.50 (1H, d, J = 7.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 32.1, 36.1, 50.6, 52.3, 71.3, 71.4, 76.2, 76.3, 117.0, 117.4, 123.2, 123.4, 124.2, 126.6, 127.6, 127.8, 128.2, 128.3, 128.47, 128.52, 128.65, 128.68, 128.8, 128.9, 136.4, 136.5, 146.8, 147.1, 151.73, 151.75, 165.1, 165.3, 172.3; HR-ESITOFMS m/z $[M+Na]^+$ 787.2988 (calcd for $C_{47}H_{44}N_2O_8Na$ 787.2990).

4.2.27. (*S*)-2,4-Bis[(2,3-dihydroxybenzoyl)amino]butyric acid methyl ester [(*S*)-15]

In the same manner as described for **2**, (*S*)-**35** gave (*S*)-**15** in 31% yield: colorless amorphous solid; $[\alpha]_D^{22}$ − 25.9 (c 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 2.16 (1H, m), 2.27 (1H, m), 3.45 (1H, m), 3.61 (1H, m), 3.70 (3H, s), 4.78 (1H, dd, J = 8.5, 5.0 Hz), 6.71 (1H, t, J = 7.5 Hz), 6.75 (1H, t, J = 8.0 Hz), 6.92 (1H, dd, J = 8.0, 1.0 Hz), 6.96 (1H, dd, J = 7.5, 1.0 Hz), 7.19 (1H, dd, J = 8.0, 1.0 Hz), 7.35 (1H, dd, J = 7.5, 1.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 32.1, 37.1, 51.8, 53.0, 116.68, 116.70, 118.7, 119.3, 119.6, 119.7, 119.9, 147.4, 150.2, 150.3, 171.4, 171.7, 173.8; IR ν_{max} 3342, 1728, 1638, 1585, 1535 cm^{-1}; HR-ESITOFMS m/z $[M+Na]^+$ 427.1112 (calcd for $C_{19}H_{20}N_2O_8Na$ 427.1112).

4.2.28. (*S*)-2,5-Bis[(2,3-dihydroxybenzoyl)amino]pentanoic acid methyl ester [(*S*)-16]

In the same manner as described for **2**, (*S*)-2,5-bis[(2,3-dibenzyloxybenzoyl)amino]pentanoic acid methyl ester[3] [(*S*)-**36**] gave (*S*)-**14** in 24% yield: colorless amorphous solid; $[\alpha]_D^{22}$ − 2.9 (c 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.66 (2H, m), 1.82 (1H, m), 1.95 (1H, m), 3.34 (2H, t, J = 6.8 Hz), 3.64 (3H, s), 4.60 (1H, d, J =8.8, 5.2 Hz), 6.60 (1H, t, J = 8.0 Hz), 6.62 (1H, t, J = 8.0 Hz), 6.81 (1H, d, J = 8.0 Hz), 6.83 (1H, d, J = 8.0 Hz), 7.10 (1H, d, J = 8.0 Hz), 7.23 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 27.0, 29.9, 39.9, 52.8, 53.7, 116.7, 116.9, 118.7, 119.46, 119.52, 119.7, 147.4, 150.3, 150.5, 171.2, 171.7, 174.1; IR ν_{max} 3376, 1728, 1639, 1587, 1537 cm^{-1}; HR-ESITOFMS m/z $[M+Na]^+$ 441.1266 (calcd for $C_{20}H_{22}N_2O_8Na$ 441.1268).

4.2.29. (*S*)-2,7-Bis[(2,3-dibenzyloxybenzoyl)amino]heptanoic acid methyl ester [(*S*)-38]

In the same manner as described for **18**, (*S*)-2,7-diaminoheptanoic acid methyl ester [(*S*)-**37**], which was prepared from (*S*)-2-amino-7-(*tert*-butoxycarboxamido)heptanoic acid methyl ester[4], gave (*S*)-**38** in 28% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03–1.20 (6H, m), 1.37 (1H, m), 1.62 (1H, m), 3.17 (2H, dt, J = 5.6, 6.0 Hz), 3.70 (3H, s), 4.61 (1H, ddd, J = 7.6, 7.2, 5.6 Hz), 5.07 (2H, s), 5.11 (1H, d, J = 10 Hz), 5.150 (2H, s), 5.153 (2H, s), 5.16 (1H, d, J = 10 Hz), 7.12–7.52 (24H, m), 7.72 (2H, m), 7.90 (1H, t, J = 5.6 Hz), 8.48 (1H, d, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 26.6, 28.9, 31.8, 39.6, 52.2, 52.7, 71.30, 71.34, 76.1, 76.4, 116.9, 117.3, 123.3, 123.4, 124.43, 124.45, 127.7, 127.8, 128.27, 128.29, 128.48, 128.52, 128.67, 128.68, 128.71, 128.8, 136.32, 136.35, 136.42, 146.8, 148.0, 151.7, 151.8, 165.0, 165.1, 172.8; HR-ESITOFMS m/z $[M+Na]^+$ 829.3458 (calcd for $C_{50}H_{50}N_2O_8Na$ 829.3459).

4.2.30. (*S*)-2,7-Bis[(2,3-dihydroxybenzoyl)amino]heptanoic acid methyl ester [(*S*)-17]

In the same manner as described for **2**, (*S*)-**38** gave (*S*)-**17** in 54% yield: colorless amorphous solid; $[\alpha]_D^{22}$ − 9.1 (c 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.33 (4H, m), 1.53 (2H, m), 1.75 (1H, m), 1.82 (1H, m), 3.27 (2H, t, J = 7.2 Hz), 3.64 (3H, s), 4.53 (1H, dd, J = 8.8, 5.2 Hz), 6.60 (1H, t, J = 8.0 Hz), 6.63 (1H, t, J = 8.0 Hz), 6.81 (1H, dd, J = 7.6, 1.2 Hz), 6.84 (1H, dd, J = 8.0, 1.2 Hz), 7.09 (1H, dd, J = 8.0, 1.2 Hz), 7.23 (1H, dd, J = 7.5, 1.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 26.7, 27.5, 30.2, 32.3, 40.3, 52.8, 53.9, 116.8, 116.9, 118.7, 119.50, 119.57, 119.65, 119.74, 147.3, 147.4, 150.0, 150.3, 171.1, 171.5, 174.4; IR ν_{max} 3316, 1746, 1635, 1586, 1541 cm^{-1}; HR-ESITOFMS m/z $[M-H]^-$ 445.1614 (calcd for $C_{22}H_{25}N_2O_8$ 445.1616).

4.3. Biological activity

4.3.1. Cytotoxicity assay

Cytotoxicity was assayed according to the procedure previously described,[2] with some modifications. Briefly, murine colon 26-L5 carcinoma cells were suspended (1×10^5 cells/mL) in RPMI medium containing 10% FCS, L-glutamine and the test compound at various concentrations, and seeded into the wells of a 96-well culture plate (1×10^4 cells/100 μL/well). After incubation for 24 h in a humidified 5% CO_2 incubator at 37 °C, the well was washed with PBS and the cells were fixed with 20% formalin. Then, the wells were washed with PBS and cells were stained with crystal violet for 30 min. After washing with water, the crystal violet dye was extracted with 30% acetic acid and the absorbance at 590 nm was measured. Solutions of test compounds were prepared in DMSO.

4.3.2. Invasion and migration assays

Invasion assay was carried out according to the procedure previously described.[2] Murine colon 26-L5 carcinoma cells were used. Solutions of test compounds were prepared in DMSO.

4.3.3. Cell adhesion assay

Cell adhesion assay was performed in 96-well microculture plates coated with matrix proteins at a concentration of 50 μg/mL overnight and then treated with 100 μL of RPMI medium containing 10% FCS for 1 h to block the remaining protein binding sites. The matrix proteins used were fibronectin and Matrigel. Murine colon 26-L5 cells were pre-treated with compounds (*S*)-**1** or (*S*)-**6** in a humidified 5% CO_2 incubator at 37 °C for 24 h. Then, the cells were harvested and resuspended in RPMI medium containing 10% FCS and L-glutamine, and seeded into a 96-well culture plate at a density of 4×10^4 cells/well. The cells were allowed to attach to the matrix proteins at 37 °C for 1 h. Non-adherent cells were removed by vacuum aspiration. Adherent cells were washed with PBS and fixed with 20% formalin at 4 °C for 1 h. Then, the wells were washed with PBS and cells were stained with crystal violet for 30 min. After washing with water, the crystal violet dye was extracted with 30% acetic acid and the absorbance at 590 nm was measured.

4.3.4. Gelatin-based zymography assay

Gelatinase inhibition assay was carried out according to the procedure previously described,[12] with some modifications. Conditioned media of murine colon 26-L5 carcinoma cells were electrophoresed under non-reducing conditions on gelatin zymogram containing 10% SDS–PAGE co-polymerized with 0.1% gelatin (Sigma Chemical Co.). After electrophoresis, the gels were washed twice with rinsing buffer (50 mM Tris–HCl, 2.5% Triton X-100, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.05% NaN_3) at room temperature for 1 h to remove SDS. Strips of the gel were incubated with the reaction buffer for gelatinolytic activity (50 mM Tris–HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.05% NaN_3) supplemented with various concentrations of test compound at 37 °C. After incubation for 24 h, the gel strips were stained with a staining solution (0.1% coomassie brilliant blue, 10% acetic acid, 10% 2-propanol). The locations of the enzymes were visualized as clear bands on the blue background. Gelatinolytic activities were quantified using Chemi Dos XRS System (Bio-rad).

4.3.5. In vivo lung metastasis

Specific pathogen-free 7-week-old female BALB/c mice were purchased from Japan SLC, Hamamatsu, Japan. The mice were

maintained in the Laboratory for Animal Experiments, Institute of Natural Medicine, University of Toyama under laminar air-flow conditions. Colon 26-L5 cells were harvested with trypsin–EDTA, washed with RPMI medium and resuspended in cold PBS. BALB/c mice were given an intravenous injection of colon 26-L5 cells (3×10^4/200 μL) via the tail vein. Compound (*S*)-**6** (1 mg/mouse/200 μL in PBS) were administered orally or intraperitoneally once a day for 14 consecutive days. In contrast, the control mice were given 200 μL of PBS. The mice were sacrificed after 14 days after tumor inoculation. The lungs were fixed in Bouin's solution and the tumor colonies were counted under a dissecting microscope.

4.3.6. Statistical analysis

The statistical significance of differences between groups was determined by applying the Student's two-tailed *t*-test.

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References and notes

1. (a) Woodhouse, E. C.; Chuaqui, R. F.; Liotta, L. A. *Cancer* **1997**, *80*, 1529; (b) Geho, D. H.; Bandle, R. W.; Clair, T.; Liotta, L. A. *Physiology* **2005**, *20*, 194.
2. Miyanaga, S.; Obata, T.; Onaka, H.; Fujita, T.; Saito, N.; Sakurai, H.; Saiki, I.; Furumai, T.; Igarashi, Y. *J. Antibiot.* **2006**, *59*, 698.
3. Ambrosi, H. D.; Hartmann, V.; Pistorius, D.; Reissbrodt, R.; Trowitzsch-Kienast, W. *Eur. J. Org. Chem.* **1998**, 541.
4. Boyle, T. P.; Bremner, J. B.; Coates, J. A.; Keller, P. A.; Pyne, S. G. *Tetrahedron* **2005**, *61*, 7271.
5. Saito, K. I.; Oku, T.; Ata, N.; Miyashiro, H.; Hattori, M.; Saiki, I. *Biol. Pharm. Bull.*. **1997**, *20*, 345.
6. Brikedal-Hansen, H. *Curr. Opin. Cell Biol.* **1995**, *7*, 728.
7. (a) Maskos, K. *Biochimie* **2005**, *87*, 249; (b) Beckett, R. P.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Whittaker, M. *Drug Discov. Today* **1996**, *1*, 16.
8. Muraishi, Y.; Mitani, N.; Fuse, H.; Saiki, I. *Anticancer Res.* **2001**, *21*, 3845.
9. Yoshioka, I.; Tsuchiya, Y.; Aozuka, Y.; Onishi, Y.; Sakurai, H.; Koizumi, K.; Tsukada, K.; Saiki, I. *Anticancer Res.* **2005**, *25*, 815.
10. Kanai, F.; Isshiki, K.; Umezawa, Y.; Morishima, H.; Naganawa, H.; Takita, T.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1985**, *38*, 31.
11. Macdonald, S. J. F.; Clarke, G. D. E.; Dowle, M. D.; Harrison, L. A.; Hodgson, S. T.; Inglis, G. G. A.; Johnson, M. R.; Shah, P.; Upton, R. J.; Walls, S. B. *J. Org. Chem.* **1999**, *64*, 5166.
12. Lirdprapamongkol, K.; Sakurai, H.; Kawasaki, N.; Choo, M. K.; Saito, Y.; Aozuka, Y.; Singhirunnusorn, P.; Ruchirawat, S.; Svasti, J.; Saiki, I. *Eur. J. Pharm. Sci.* **2005**, *25*, 57.