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Highly Selective and Orally Active Inhibitors of Type IV Collagenase (MMP-9 and MMP-2): *N*-Sulfonylamino Acid Derivatives

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Various *N*-sulfonylamino acid derivatives were synthesized and evaluated for their in vitro and in vivo activities to inhibit type IV collagenase (MMP-9 and MMP-2). When the amino acid residue and the sulfonamide moiety were modified, their inhibitory activities were greatly affected by the structure of the sulfonamide moiety. A series of aryl sulfonamide derivatives containing biaryl, tetrazole, amide, and triple bond were found to be potent and highly selective inhibitors of MMP-9 and MMP-2. In addition, these compounds were orally active in animal models of tumor growth and metastasis. These results revealed the potential of the *N*-sulfonylamino acid derivatives as a new type of candidate drug for the treatment of cancer.

Introduction

Matrix metalloproteinase (MMP) is a typical metalloproteinase which requires zinc ion at its active sites. As many as 18 kinds of MMP have been isolated and cloned thus far and are collectively called the MMP family.[1,2] X-ray crystallographic analyses and NMR studies of some MMPs have been published,[3−6] and the molecular mechanisms underlying their enzyme activities are being revealed based on the molecular interaction between the MMP and its specific inhibitor. Such structural information is very helpful for the design and optimization of the structure of inhibitors specific to certain types of MMPs.

MMPs are produced by fibroblasts, chondrocytes, osteoblasts, neutrophils, and macrophages, as well as tumor cells. Although their physiological functions are not fully understood yet, they are postulated to regulate the homeostasis of a variety of tissues under the control of the tissue inhibitor metalloproteinase (TIMP), which associates with and inhibits the activity of MMP. Therefore, it is thought that aberrant production of MMPs and stoichiometric imbalance between MMPs and TIMPs will result in a variety of morbid states, such as tumor metastasis,[7] rheumatoid arthritis,[8] and multiple sclerosis.[9] Moreover, certain MMP inhibitors have recently been shown to exert antitumor activity other than tumor metastasis.[10]

Basement membrane is composed mainly of type IV collagen and is likely to prevent invasion of tumor cells owing to its strong architecture. Thus, the disruption of vascular basement membranes by type IV collagenase (MMP-9 and MMP-2) probably plays an important role in triggering the process of tumor growth. Since the involvement of MMPs in tumor invasion and metastasis was postulated, type IV collagenase inhibitors have been studied extensively in search of a new type of anticancer drug.[11−15] Among them, marimastat[16] and AG-3340 for tumor growth inhibition and Galardin for corneal ulceration[17] are now undergoing clinical trials.

We tried to develop type IV collagenase inhibitors after screening our compound library for a seed compound. Compounds were tested for their inhibitory activities against human gelatinase B (MMP-9) and gelatinase A (MMP-2). We found that 2-(benzenesulfonylamino)-*N*-hydroxy-3-phenylpropionamide ((*RS*)-**1a**) in Figure 1 exhibited inhibitory activity with the IC_{50} value of 1.0 μM against MMP-9. In considering the significant biological effect of chirality, two enantiomers of this compound were prepared to determine their inhibitory activities. The IC_{50} value of (*R*)-**1b** was found to be 0.73 μM while (*S*)-**1c** exhibited no inhibitory activity (IC_{50} > 1000 μM). We also found that another amino acid derivative, (*S*)-**2** with the configuration of (*S*), exhibited inhibitory activity with the IC_{50} value of 0.80 μM. These simple amino acid derivatives were expected to be orally active, while most MMP inhibitors thus far reported are peptidic compounds which generally are poorly absorbed across the gastrointestinal tract.

With these seed compounds in hand, we examined the structure−activity relationship of the amino acid residue and the sulfonamide moiety of (*R*)-*N*-sulfonylamino acid derivatives.

Chemistry

The carboxylic or hydroxamic acid−based inhibitors in Tables 1 and 2 were synthesized as shown in Scheme 1. The protected amino acid ester **3** was coupled with various sulfonyl chlorides in the presence of *N*-methylmorpholine (NMM) to give the corresponding sulfonylamino ester **4**. Removal of ester protective groups by alkaline or trifluoroacetic acid (TFA) hydrolysis or palladium-catalyzed hydrogenation afforded the carboxylic acid **5**. Compound **5** was also synthesized from an amino acid and a sulfonyl chloride by the Shotten−Baumann method. Conversion of **5** to the hydroxamic acid **7** was performed by two methods. One method consists of conversion of **5** to the protected *O*-benzyl hydroxamate **6** in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxybenzotriazole (HOBt), and NMM followed by palladium-catalyzed hydrogenation. The other method includes conversion of **5** to the acid chloride followed

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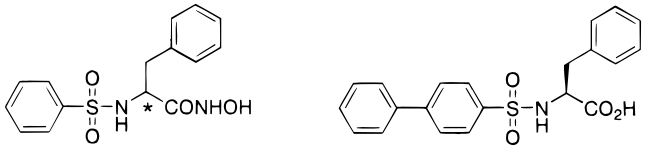


(*RS*)-**1a**: IC_{50} = 1 μM
(*R*)-**1b**: IC_{50} = 0.73 μM
(*S*)-**1c**: IC_{50} > 1000 μM

(*S*)-**2**: IC_{50} = 0.80 μM

Figure 1. IC_{50} values of compounds **1** and **2** vs that of MMP-9.

by coupling with hydoxylamine in the presence of aqueous sodium hydrogencarbonate ($NaHCO_3$). Starting material **3** was commercially available except for **3a** (R^1 = (3-indoyl)CH_2, R^3 = Me, HX = *p*-TsOH) and **3b** (R^1 = (3-indoyl)CH_2, R^3 = Bn, HX = TFA). Compound **3a** was synthesized by a method described in the literature,[18] and **3b** was synthesized using benzyl alcohol in step d of Scheme 1 followed by deprotection with TFA. Various sulfonyl chlorides were also commercially available or synthesized by methods described in the literature[19] or the methods shown in Scheme 2.[20] The sulfonyl chloride used in the synthesis of **5i** was synthesized from 4-(chlorosulfonyl)phenyl isocyanate and aniline.

Syntheses of derivatives containing biaryl, triple bond, tetrazole, and amide moieties are shown in Schemes 3−5. Key intermediates **8**, **9**, **14**, and **18** were synthesized from **3** and 4-iodobenzene-, 5-bromo-2-thiophene-, 4-styrene-, and 4-nitrobenzenesulfonyl chlorides, respectively, in a manner similar to that described above. The aryl halide intermediates **8** and **9** were modified by carbon−carbon bond formation methodologies, Suzuki or Sonogashira reaction, i.e., coupling of **8** and **9** with substituted phenylboronic acids in the presence of palladium(0) and K_2CO_3 produced the biaryl compound **10**,[21,22] and reaction of **8** and **9** with substituted phenylacetylenes in the presence of palladium(II), CuI(I), and triethylamine produced the acetylenic compound **12**.[23,24] Ozonolysis of the styrene intermediate **14** followed by treatment with methyl sulfide afforded the aldehyde. Since an indole ring was unstable under ozonolysis, oxidation with catalytic OsO_4 in the presence of trimethylamine *N*-oxide followed by treatment with $NaIO_4$ was used in the case of compounds containing the tryptophan residue. Without further purification, this crude aldehyde was coupled with benzenesulfonyl hydrazide to give the hydrazone **15**. 1,5-Dipolar cyclization between **15** and diazonium salt prepared from substituted anilines and sodium nitrite afforded the tetrazole ester **16**.[25] Palladium-catalyzed hydrogenation converted the nitro intermediate **18** to the amine **19**, which was coupled with substituted benzoyl chlorides in the presence of NMM to give the amide **20**. Compound **5g** was also synthesized from 4-(chlorosulfonyl)benzoic acid and aniline. Removal of the protective group of esters **10**, **12**, **16**, and **20** in a manner similar to that described above afforded the desired carboxylic acid derivatives **11**, **13**, **17**, and **21**.

Results and Discussion

Enzymatic Activity. All compounds described herein were evaluated for inhibitory activities against human gelatinase B (MMP-9) and gelatinase A (MMP-2). Some of the selected compounds were evaluated for their pharmacokinetic profiles and activities against in vivo animal cancer models.

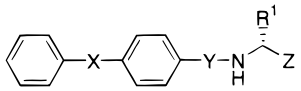
Combination of (*R*)-**1b** and the biphenyl moiety in (*S*)-**2** produced the more potent inhibitor **7a** with the IC_{50} value of 0.03 μM against MMP-9. Using **7a** as a lead compound, the indole derivative **7b** and compound **7c** containing a 4-phenoxyphenyl group were prepared. These compounds were also potent inhibitors. However, we observed that hydroxamic acid derivatives were chemically and pharmacokinetically unstable and broke down in vivo to produce hydroxylamine, a known carcinogenic compound.[26] The hydroxamic acid derivatives are poorly absorbed by oral administration as shown in Table 1. On the other hand, carboxylic acid inhibitors were less potent but showed more excellent pharmacokinetic properties than the corresponding hydroxamic acid inhibitors. Therefore, our basic strategy for SAR studies of the sulfonamide moiety was based on the development of carboxylic acid derivatives which are highly selective and orally active MMP-9 and MMP-2 inhibitors.

From the tryptophan derivative **5b**, which was the most potent inhibitor among **5a**−**c**, the sulfonamide moiety was modified with various groups. Insertion of a suitable spacer such as azo, tetrazole, triple bond, amide, and oxygen atom between the biphenyl ring in **5b** resulted in the inhibitory activities being retained and enhanced (**5k**−**o**), while insertion of methylene, carbonyl, and urea groups led to poor inhibitory activities (**5e**,**h**,**i**). Interestingly, in the case of amide compounds, compound **5n** was a moderately potent inhibitor, but compound **5g** was a poor inhibitor. Linking each phenyl ring of **5o** by carbon−carbon bond greatly decreased the inhibitory activity (**5j**). Since the carbonylamino derivatives **23** and **24** exhibited no significant inhibitory activity, the sulfonamide group seemed to be essential for the activity. These results suggest that modification of the sulfonamide moiety of carboxylic acid derivatives greatly alters the inhibitory activity. Consequently, the four types of sulfonamide compounds containing biaryl, triple bond, tetrazole, and amide were selected for further modification.

The substituent effects of the phenyl ring in the sulfonamide moiety are shown in Tables 3 and 4. First, the derivatives containing the biaryl and the triple bond moieties are shown in Table 3. Compared with the unsubstituted compounds **5b**, **5m**, and **13d**, the para-substituted compounds **11a**, **13a**, **13b**, **13e**, and **13g** exhibited more potent inhibitory activities, but a hydrophilic substituent such as a carboxyl group decreased the inhibitory activity (**13c**). On the other hand, meta (**11b**) or ortho (**13f**) substitution resulted in decreased activity. Generally, valine and phenylalanine derivatives were less potent inhibitors than tryptophan derivatives except for the compound having a para methylmercapto substituent, such as **11f**. However, the derivatives with a thiophene ring directly attached to the sulfonamide group not only enhanced the inhibitory activity (**11g**, **11i**, **13i**, and **13k**) but also improved the pharmacokinetic properties (**11c**, **11d**, **11i**, and **13g**).

The derivatives containing the tetrazole and the amide moieties are shown in Table 4. From the results

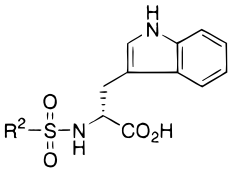
Table 1. Inhibition of MMP-9 and MMP-2 by Hydroxamic or Carboxylic Acid Derivatives and Their Pharmacokinetic Properties

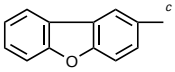
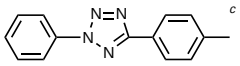


compd	R^1	X	Y	Z	IC_{50} (μM)[a] MMP-9	IC_{50} (μM)[a] MMP-2	C_{max} (μM)[b]
7a	$PhCH_2$		SO_2	CONHOH	0.030	0.017	2.5
5a	$PhCH_2$		SO_2	CO_2H	0.24	0.31	290.8
7b	(3-indoyl)CH_2		SO_2	CONHOH	0.0050	0.0013	ND[c]
5b	(3-indoyl)CH_2		SO_2	CO_2H	0.18	0.053	34.3
7c	$(CH_3)_2CH$	O	SO_2	CONHOH	0.00054	0.0020	5.8
5c	$(CH_3)_2CH$	O	SO_2	CO_2H	1.1	>1.0	334.3
24	$PhCH_2$		CO	CONHOH	>50	>1.0	—[d]
23	$PhCH_2$		CO	CO_2H	>50	>1.0	—[d]

[a] Concentration required for 50% inhibition of enzyme activity. Standard deviations are less than 10% of the mean values. Details of the enzyme assays are described in the Experimental Section. [b] Maximum plasma concentration of the inhibitor after administration of 200 mg/kg po in mice. The conditions are described in the Experimental Section. [c] Not detected. [d] Not done.

Table 2. Inhibition of MMP-9 and MMP-2 by Modification of the Sulfonamide Moiety in Carboxylic Acid Derivatives



compd	R^2	IC_{50} (μM)[e] MMP-9	IC_{50} (μM)[e] MMP-2
5d	Ph[a]	3.4	>1.0
5e	$PhCH_2Ph$[c]	>10	>1.0
5f	2-naphthyl[a]	>10	>1.0
5g	PhNHCOPh[d]	>10	>1.0
5h	PhCOPh[b]	>10	>1.0
5i	PhNHCONHPh[c]	>10	>1.0
5j		>10	>1.0
5b	4-biphenyl[a]	0.18	0.053
5k	Ph-N=N-Ph[a]	0.35	0.050
5l		0.032	0.019
5m	PhC≡CPh[c]	0.24	0.050
5n	PhCONHPh[d]	0.68	<0.04
5o	PhOPh[b]	0.25	0.050

[a] Commercially available sulfonyl chloride. [b] See ref 19. [c] Synthetic methods are shown in Scheme 2. [d] See the Experimental Section. [e] Concentration required for 50% inhibition of enzyme activity. Standard deviations are less than 10% of the mean values. Details of the enzyme assays are described in the Experimental Section.

described above, para substitution generally tended to increase the inhibitory activities, and therefore, para-substituted compounds were only synthesized in the case of tetrazole and amide derivatives. Regardless of the amino acid residue, introduction of both types of substituents, i.e., electron-donating and electron-withdrawing groups, to the para position of the phenyl ring produced more potent inhibitors than the unsubstituted derivatives. But a bulky substituent such as a cyclohexyl group reduced the inhibitory activity (**17h**).

Several compounds were examined for their inhibitory activities against various metalloproteinases (MMP-1, -2, -3, -7, and -9, angiotensin-converting enzyme (ACE), neutral endopeptidase (NEP), and endothelin-converting enzyme (ECE)), and the results are shown in Table 5. Potent compounds **5l**, **11d**, **11f**, **13g**, **17e**, and **21a** containing biaryl, triple bond, tetrazole, and amide moieties were highly selective inhibitors of both MMP-9 and MMP-2 and exhibited no inhibitory activity against other metalloproteinases.

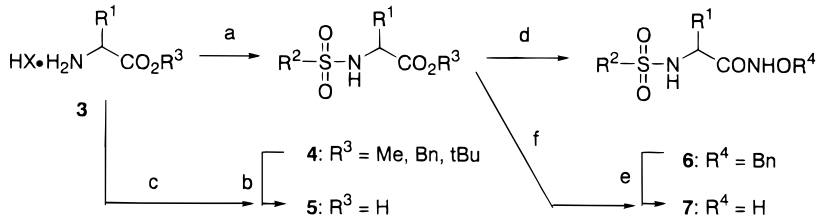
In a recent review, various structural studies on the complexes of MMPs with small molecule inhibitors were reported.[27] As an example of them, it is shown that the sulfonamide moiety of Ciba-Geigy's compound is binding to the S_1' pocket of MMP-1. Though the structures of MMP-9 and MMP-2 have not yet become apparent, it is expected that their structures are similar to those of other MMPs. In addition, since the ability of a carboxylic acid group to coordinate to a metal atom of metalloproteinase is considered to be much weaker than that of a hydroxamic acid group, the sulfonamide moieties of our inhibitors are assumed to occupy the thin and deep S_1' pockets of MMP-9 and MMP-2. Therefore, the selectivity of our inhibitors might be explained by this complementary interaction with the enzymes.

Antitumor Activity. The hydroxamic acid inhibitor **7a** which was a lead compound and the carboxylic acid inhibitors **5l**, **11d**, **11f**, **13g**, **17e**, and **21a** in Table 5 were evaluated for their inhibitory activities in in vivo animal cancer models. As shown in Figure 2, lung colonization of Lewis lung carcinoma cells was significantly suppressed by these inhibitors of type IV collagenase. In addition, antitumor activity was also demonstrated in the human lung cancer model. Ma44 cells grow as a solid tumor on the peritoneum after being implanted ip, and mice bearing Ma44 eventually die within 3 to 4 weeks. Daily oral administration of compound **5l** resulted in prolonged survival of Ma44-bearing mice (26% increased life span against vehicle control, $P < 0.01$, Figure 3). These results indicated that *N*-sulfonylamino acid derivatives are orally active in in vivo animal cancer models.

Conclusion

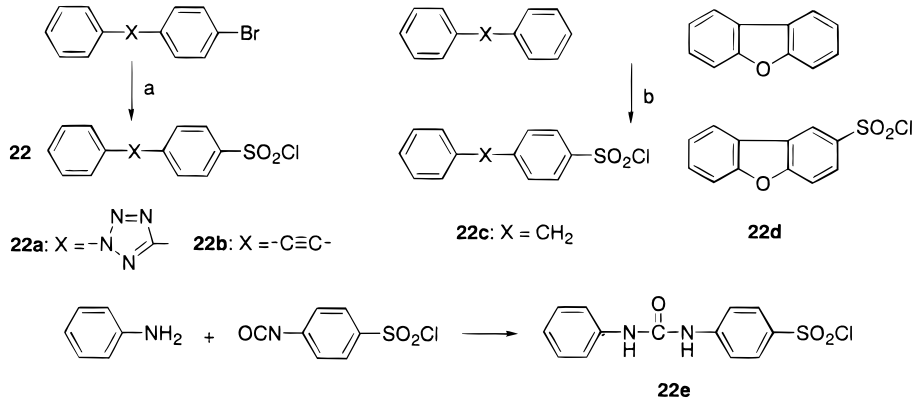
In this paper, we have reported the preparation of simple *N*-sulfonylamino acid derivatives modified in the amino acid residue and the sulfonamide moiety. Evaluation of the enzymatic activity of these compounds showed that the sulfonamide derivatives containing biaryl, triple bond, tetrazole, or amide were selective inhibitors of type IV collagenase (MMP-9 and MMP-2). In addition, selected carboxylic acid inhibitors **5l**, **11d**,

Scheme 1[a]



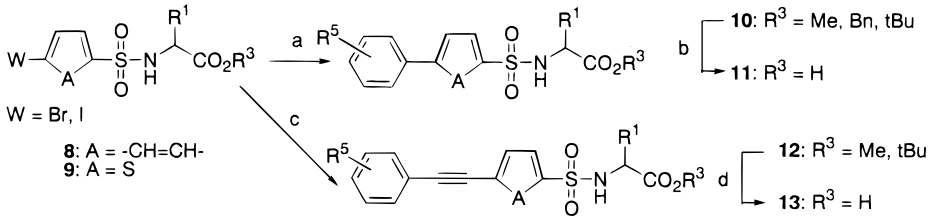
[a] Reagents: (a) R^2SO_2Cl, NMM; (b) NaOH or H_2, Pd−C or TFA; (c) R^2SO_2Cl, aqueous Na_2CO_3; (d) $BnONH_2$·HCl, EDC, HOBt; (e) H_2, Pd−C; (f) (1) $(COCl)_2$, DMF, (2) $HONH_2$·HCl, aqueous $NaHCO_3$.

Scheme 2[a]



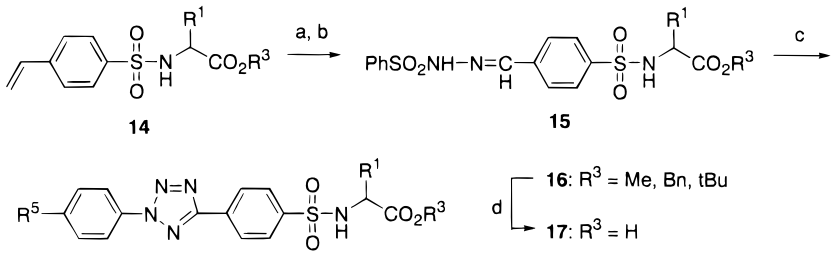
[a] Reagents: (a) (1) *n*-BuLi, then SO_2, (2) SO_2Cl_2; (b) (1) $ClSO_3H$, (2) PCl_5.

Scheme 3[a]



[a] Reagents: (a) substituted phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3; (b) NaOH or H_2, Pd−C or TFA; (c) substituted phenylacetylene, $Pd(PPh_3)_2Cl_2$, CuI(I), Et_3N; (d) NaOH or TFA.

Scheme 4[a]



[a] Reagents: (a) O_3, then Me_2S or (1) OsO_4, trimethylamine *N*-oxide, (2) $NaIO_4$; (b) $PhSO_2NHNH_2$; (c) (4-R^5)$C_6H_4NH_2$, $NaNO_2$, concentrated HCl; (d) NaOH or H_2, Pd−C or TFA.

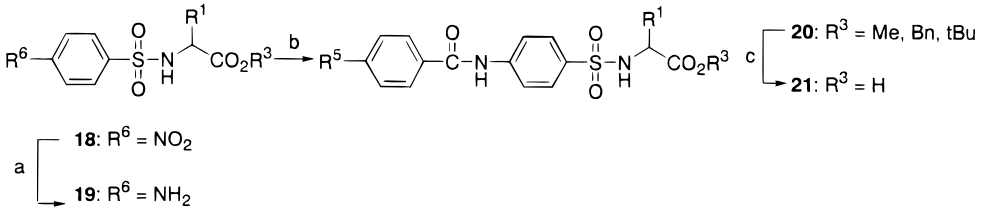
11f, **13g**, **17e**, and **21a** were orally active in animal models of tumor growth and metastasis. These results revealed the potential of *N*-sulfonylamino acid derivatives as a new type of candidate drug for the treatment of cancer.

Experimental Section

Chemistry. Melting points are uncorrected. ^1H NMR spectra were determined at 200 or 300 MHz. Fast atom bombardment mass spectra (FABMS) and high-resolution (HR)-FABMS were determined using *m*-nitrobenzyl alcohol as a matrix. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere with anhydrous solvents that had been dried over type 4A molecular sieves. Drying of an organic phase over anhydrous Na_2SO_4 is simply indicated by the word "dried". Column chromatography using Merck silica gel 60 or a Merck Lobar column is referred to as "chromatography on silica gel".

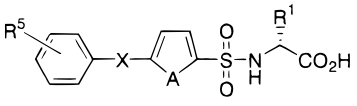
D-Tryptophan Methyl Ester Tosylate (3a). To a solution of 2.04 g (10 mmol) of D-tryptophan and 1.72 g (10 mmol) of anhydrous *p*-toluenesulfonic acid in 20 mL of MeOH was added 2.29 g (12 mmol) of *p*-toluenesulfonyl chloride at room temperature. The mixture was stirred for 4 h at 75 °C. After being cooled to room temperature, the mixture was concentrated in vacuo. The residue was crystallized from MeOH−

Scheme 5[a]



[a] Reagents: (a) H_2, Pd−C; (b) (4-R^5)C_6H_4COCl, NMM; (c) NaOH or H_2, Pd−C or TFA.

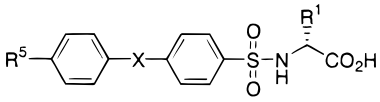
Table 3. Inhibition of MMP-9 and MMP-2 by Derivatives Containing the Biaryl or the Triple Bond Moiety and Their Pharmacokinetic Properties



					IC$_{50}$ (μM)[a]		
compd	R^1	A	X	R^5	MMP-9	MMP-2	C_{max} (μM)[b]
5b	(3-indoyl)CH_2	CH=CH		H	0.18	0.053	34.3
11a	(3-indoyl)CH_2	CH=CH		4-OMe	0.081	0.0065	3.5
11b	(3-indoyl)CH_2	CH=CH		3-OMe	1.1	1.0	−[c]
11d	(3-indoyl)CH_2	S		4-Me	0.061	0.010	251.5
11e	$(CH_3)_2CH$	CH=CH		4-OMe	0.040	<0.1	89.9
11f	$(CH_3)_2CH$	CH=CH		4-SMe	0.040	0.023	189.2
11g	$(CH_3)_2CH$	S		4-OMe	0.072	0.066	97.5
5m	(3-indoyl)CH_2	CH=CH	C≡C	H	0.24	0.050	−[c]
13b	(3-indoyl)CH_2	CH=CH	C≡C	4-Me	0.016	0.034	10.8
13c	(3-indoyl)CH_2	CH=CH	C≡C	4-CO_2H	8.8	>1.0	−[c]
13e	(3-indoyl)CH_2	S	C≡C	4-NO_2	0.023	0.084	−[c]
13f	(3-indoyl)CH_2	S	C≡C	2-NO_2	1.5	>1.0	−[c]
13g	(3-indoyl)CH_2	S	C≡C	4-Me	0.023	0.020	203.5
13h	$(CH_3)_2CH$	CH=CH	C≡C	4-Me	1.2	>1.0	−[c]
13i	$(CH_3)_2CH$	S	C≡C	4-Me	0.014	0.053	284.5
13j	$PhCH_2$	CH=CH	C≡C	4-OMe	7.8	1.0	−[c]
13k	$PhCH_2$	S	C≡C	4-OMe	0.024	0.010	119.1

[a] Concentration required for 50% inhibition of enzyme activity. Standard deviations are less than 10% of the mean values. Details of the enzyme assays are described in the Experimental Section. [b] Maximum plasma concentration of the inhibitor after administration of 200 mg/kg po in mice. The conditions are described in the Experimental Section. [c] Not done.

Table 4. Inhibition of MMP-9 and MMP-2 by Derivatives Containing the Tetrazole or Amide Moiety and Their Pharmacokinetic Properties



				IC$_{50}$ (μM)[a]		
compd	R^1	X	R^5	MMP-9	MMP-2	C_{max} (μM)[b]
5l	(3-indoyl)CH_2	tetrazolyl	H	0.032	0.019	15.8
17a	(3-indoyl)CH_2	tetrazolyl	OMe	0.017	0.013	2.1
17b	(3-indoyl)CH_2	tetrazolyl	Br	0.0041	0.0083	2.5
17c	$(CH_3)_2CH$	tetrazolyl	H	0.11	0.13	−[c]
17e	$(CH_3)_2CH$	tetrazolyl	SMe	0.0059	0.013	47.7
17f	$PhCH_2$	tetrazolyl	H	0.58	0.11	12.1
17g	$PhCH_2$	tetrazolyl	Me	0.086	<0.2	1.2
17h	$PhCH_2$	tetrazolyl	cyclohexyl	1.32	0.28	−[c]
5n	(3-indoyl)CH_2	CONH	H	0.68	<0.04	−[c]
21a	(3-indoyl)CH_2	CONH	Br	0.0027	0.0026	0.2
21c	$(CH_3)_2CH$	CONH	H	2.1	0.050	−[c]
21d	$(CH_3)_2CH$	CONH	NO_2	0.27	<0.04	−[c]
21e	$(CH_3)_2CH$	CONH	SMe	0.016	0.0067	8.3
21f	$PhCH_2$	CONH	H	0.84	<1.0	−[c]
21g	$PhCH_2$	CONH	Me	0.060	<0.04	−[c]

[a] Concentration required for 50% inhibition of enzyme activity. Standard deviations are less than 10% of the mean values. Details of the enzyme assays are described in the Experimental Section. [b] Maximum plasma concentration of the inhibitor after administration of 200 mg/kg po in mice. The conditions are described in the Experimental Section. [c] Not done.

Et_2O to give 3.65 g (94%) of **3a** as colorless crystals: mp 180−181 °C; $[\alpha]^{22}_D$ −10.5° (*c* 1.0, MeOH); IR (KBr) 3375, 1751, 1523, 1173 cm^{-1}; ^1H NMR (CD_3OD) δ 2.36 (s, 3H), 3.27−3.31 (m, 2H), 3.78 (s, 3H), 4.32 (dd, J = 5.4, 7.2 Hz, 1H), 7.00−7.27 (m, 5H), 7.35−7.56 (m, 2H), 7.71 (d, J = 8.2 Hz, 2H). Anal. ($C_{19}H_{22}N_2O_5S\cdot0.3H_2O$) C, H, N, S.

D-Tryptophan Benzyl Ester Trifluoroacetate (3b). A solution of 5.0 g (12.7 mmol) of N^{α}-(*tert*-butyloxycarbonyl)-D-

Table 5. Inhibition of Various Metalloproteinases by Compounds **5l**, **11d**, **11f**, **13g**, **17e**, and **21a**

	IC_{50} (μM)[a]							
compd	MMP-9	MMP-2	MMP-1	MMP-3	MMP-7	ACE	ECE	NEP
5l	0.032	0.019	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
11d	0.061	0.0098	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
11f	0.040	0.023	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
13g	0.023	0.020	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
17e	0.0059	0.013	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
21a	0.0027	0.0026	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0

[a] Concentration required for 50% inhibition of enzyme activity. Standard deviations are less than 10% of the mean values. Details of the enzyme assays are described in the Experimental Section.

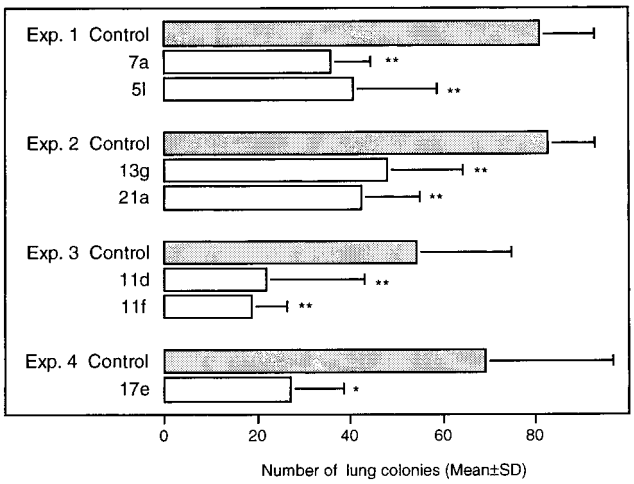


Figure 2. Inhibition of lung colonization by compounds **5l**, **7a**, **11d**, **11f**, **13g**, **17e**, and **21a** in Lewis lung carcinoma model. Control group was treated with vehicle alone. The conditions are described in the Experimental Section. *P < 0.05. **P < 0.01.

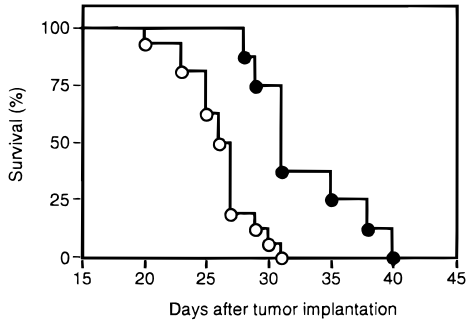


Figure 3. Prolonged survival after compound **5l** (●) administration in Ma44 human lung cancer model. Control group (○) was treated with vehicle alone. The conditions are described in the Experimental Section.

tryptophan benzyl ester[28] in 15 mL of CH_2Cl_2 and 15 mL of TFA was stirred for 1 h at room temperature. The mixture was concentrated in vacuo to give the crude title compound which was used in the next reaction without further purification.

(2*R*)-2-[(4-Biphenylylsulfonyl)amino]-3-phenylpropionic Acid Benzyl Ester (4a). To a solution of 2.50 g (5.85 mmol) of D-phenylalanine benzyl ester tosylate in 60 mL of CH_2Cl_2 were added 1.42 mL (12.87 mmol) of *N*-methylmorpholine and 1.63 g (6.44 mmol) of 4-biphenylsulfonyl chloride at 0 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was poured into water and extracted with CH_2Cl_2. The organic solution was washed with 2 N HCl, 5% $NaHCO_3$ solution, and water, dried, and evaporated. The residue was purified by chromatography on silica gel using 20:1 $CHCl_3$−MeOH to give 2.32 g (84%) of **4a** as colorless crystals: mp 130−131 °C; $[\alpha]^{25}_D$ −16.4° (*c* 0.5, MeOH); IR (Nujol) 3352, 1732, 1341, 1163 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.06 (d, J = 5.8 Hz, 2H), 4.30 (dt, J = 9.0, 5.8 Hz, 1H), 4.89 (s, 2H), 5.12 (d, J = 9.0 Hz, 1H), 6.98−7.81 (m, 19H). Anal. ($C_{28}H_{25}NO_4S$) C, H, N, S.

Compounds **4c**−**f**,**h**−**l**,**o**,**q** were prepared from the corresponding **3** and sulfonyl chloride using a procedure similar to that described for the preparation of **4a**. Compounds **8a**−**d**, **9a**−**c**, **14a**−**c**, and **18a**−**c** were also prepared in a similar manner as above.

(2*R*)-3-(1*H*-Indol-3-yl)-2-[[4-[(phenylamino)carbonyl]-benzenesulfonyl]amino]propionic Acid Benzyl Ester (4g). To a solution of 450 mg (0.94 mmol) of **4q** in 10 mL of THF was added 0.12 mL (1.41 mmol) of oxaryl chloride at 0 °C. The mixture was allowed to warm to room temperature and stirred for 1 h. After being evaporated, the resulting acid chloride was dried in vacuo. To a solution of the crude acid chloride in 10 mL of THF were added 0.34 mL (2.82 mmol) of triethylamine and 0.10 mL (1.13 mmol) of aniline at 0 °C. The mixture was allowed to warm to room temperature and stirred for 24 h. The mixture was poured into water and extracted with EtOAc. The organic solution was washed with 1 N HCl, 5% $NaHCO_3$ solution, and brine, dried, and evaporated. The residue was purified by chromatography on silica gel using 8:1 $CHCl_3$−MeOH to give 292 mg (56%) of **4g** as colorless crystals: mp 207−209 °C; $[\alpha]^{26}_D$ −1.0° (*c* 1.0, DMSO); IR (KBr) 3428, 1731, 1646, 1337, 1168 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.95 (dd, J = 7.5, 14.4 Hz, 1H), 3.11 (dd, J = 7.5, 14.4 Hz, 1H), 4.11 (m, 1H), 4.82 (s, 2H), 6.91−7.14 (m, 6H), 7.23−7.40 (m, 8H), 7.71−7.79 (m, 3H), 7.95 (d, J = 8.1 Hz, 2H), 8.73 (d, J = 7.8 Hz, 1H), 10.34 (s, 1H), 10.83 (br s, 1H); HR-FABMS *m*/*z* 553.1660 M^+ (calcd for $C_{31}H_{27}N_3O_5S$ *m*/*z* 553.1671).

(2*R*)-2-[(4-Biphenylylsulfonyl)amino]-3-phenylpropionic Acid (5a). A solution of 2.28 g (4.83 mmol) of **4a** in 25 mL of MeOH and 25 mL of EtOAc was hydrogenated using 200 mg of 10% palladium on carbon for 25 min. The mixture was filtered off with Celite, and the filtrate was concentrated in vacuo. The residue was crystallized from 1:1 CH_2Cl_2−hexane to give 1.83 g (99%) of **5a** as colorless crystals: mp 146−147 °C; $[\alpha]^{25}_D$ −4.0° (*c* 1.0, MeOH); IR (Nujol) 3408, 1751, 1325, 1161, 1134 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.77 (dd, J = 8.7, 13.5 Hz, 1H), 2.96 (dd, J = 5.4, 13.5 Hz, 1H), 3.86 (m, 1H), 7.08−7.12 (m, 5H), 7.40−7.75 (m, 9H), 8.13 (br s, 1H). Anal. ($C_{21}H_{19}NO_4S$) C, H, N, S.

Compounds **5d**−**h**,**j**,**l**,**o**, and **5i** were prepared from the corresponding **4** using procedures similar to those described for the preparation of **5a** and **11g**, respectively.

(2*R*)-3-Methyl-2-[(4-phenoxybenzenesulfonyl)amino]-butanoic Acid (5c). A solution of 3.23 g (9.41 mmol) of **4c** in 36 mL of CH_2Cl_2 and 36 mL of TFA was stirred for 3 h at room temperature. The mixture was concentrated in vacuo, and the residue was crystallized from Et_2O−hexane to give 2.62 g (97%) of **5c** as colorless crystals: mp 137−138 °C; $[\alpha]^{24.5}_D$ −3.7° (*c* 1.0, DMSO); IR (KBr) 3154, 1728, 1375, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 2.12 (m, 1H), 3.80 (dd, J = 4.6, 9.6 Hz, 1H), 5.17 (d, J = 9.6 Hz, 1H), 6.95−7.08 (m, 4H), 7.13−7.45 (m, 3H), 7.70−7.85 (m, 2H). Anal. ($C_{17}H_{19}NO_5S\cdot 0.2H_2O$) C, H, N, S.

(2*R*)-3-(1*H*-Indol-3-yl)-2-[[4-(phenylazo)benzenesulfonyl]amino]propionic Acid (5k). To a solution of 250 mg (1 mmol) of D-tryptophan and 288 mg (2 mmol) of sodium carbonate in 10 mL of dioxane and 5 mL of H_2O was added 288 mg (1.03 mmol) of 4-(phenylazo)benzenesulfonyl chloride

at 0 °C. The mixture was allowed to warm to room temperature and stirred for 16 h. The mixture was poured into 10% citric acid solution and extracted with EtOAc. The organic solution was washed with brine, dried, and evaporated. The residue was purified by chromatography on silica gel using 32:1:1 $CHCl_3$−MeOH−H_2O to give 379 mg (84%) of **5k** as orange crystals: mp >235 °C dec; $[\alpha]^{22}_D$ +11.1° (*c* 0.25, DMSO); IR (KBr) 3385, 1747, 1590, 1325, 1159 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.97 (dd, J = 6.9, 15.0 Hz, 1H), 3.13 (dd, J = 3.9, 15.0 Hz, 1H), 3.74 (m, 1H), 6.83−7.04 (m, 2H), 7.08 (d, J = 1.8 Hz, 1H), 7.12 (m, 1H), 7.45 (m, 1H), 7.58−7.74 (m, 3H), 7.78 (d, J = 9.3 Hz, 2H), 7.83 (d, J = 9.3 Hz, 2H), 7.86−8.00 (m, 3H), 10.73 (s, 1H). Anal. ($C_{23}H_{20}N_4O_4S$) C, H, N, S.

Compounds **5b**,**m**,**p** and **23** were prepared from the corresponding amino acid and sulfonyl chloride or carbonylamino chloride using a procedure similar to that described for the preparation of **5k**.

(2*R*)-*N*-(Benzyloxy)-2-[(4-biphenylylsulfonyl)amino]-3-phenylpropionamide (6a). To a solution of 2.0 g (5.24 mmol) of **5a** in 20 mL of DMF were added 1.67 g (10.48 mmol) of *O*-benzylhydroxylamine hydrochloride, 0.70 g (5.24 mmol) of 1-hydroxybenzotriazole monohydrate, 2.90 mL (26.2 mmol) of *N*-methylmorpholine, and 1.53 g (8.0 mmol) of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride at room temperature. After being stirred for 6 h, the mixture was poured into water and extracted with EtOAc. The organic solution was washed with 2 N HCl, 5% $NaHCO_3$ solution, and water, dried, and evaporated. The residue was purified by chromatography on silica gel using 1:1 EtOAc−hexane to give 2.04 g (80%) of **6a** as colorless crystals: mp 171−173 °C; $[\alpha]^{25}_D$ −40.2° (*c* 0.5, DMSO); IR (Nujol) 3248, 1661, 1594, 1333, 1163 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.85−3.60 (m, 2H), 3.86 (m, 1H), 4.77 and 4.82 (AB q, J = 11.4 Hz, 2H), 5.00 (m, 1H), 6.95−7.70 (m, 19H). Anal. ($C_{28}H_{26}N_2O_4S$) C, H, N, S.

Compound **6b** was prepared from **5b** using a procedure similar to that described for the preparation of **6a**.

(2*R*)-[(4-Biphenylylsulfonyl)amino]-*N*-hydroxy-3-phenylpropionamide (7a). A solution of 1.97 g (4.05 mmol) of **6a** in 30 mL of MeOH and 30 mL of EtOAc was hydrogenated using 200 mg of 10% palladium on carbon for 3.5 h. The mixture was filtered off with Celite and the filtrate was concentrated in vacuo. The residue was crystallized from 1:1 CH_2Cl_2−hexane to give 1.35 g (84%) of **7a** as colorless crystals: mp 169−170 °C; $[\alpha]^{25}_D$ +18.5° (*c* 0.5, DMSO); IR (Nujol) 3365, 3295, 1674, 1320, 1159 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.61 (dd, J = 8.6, 13.4 Hz, 1H), 2.80 (dd, J = 6.0, 13.4 Hz, 1H), 3.80 (m, 1H), 7.00−7.20 (m, 5H), 7.40−7.75 (m, 9H), 8.29 (d, J = 9.0 Hz, 1H), 8.89 (s, 1H), 10.66 (s, 1H). Anal. ($C_{21}H_{20}N_2O_4S$) C, H, N, S.

Compound **7b** was prepared from **6b** using a procedure similar to that described for the preparation of **7a**.

(2*R*)-*N*-Hydroxy-3-methyl-2-[(4-phenoxybenzenesulfonyl)amino]butanamide (7c). To a solution of 300 mg (0.854 mmol) of **5c** in 10 mL of CH_2Cl_2 were added 0.37 mL (4.27 mmol) of oxalyl chloride and one drop of *N*,*N*-dimethylformamide at 0 °C. The mixture was allowed to warm room temperature and stirred for 1 h. After being evaporated, the resulting acid chloride was dried in vacuo. To a solution 593 mg (8.54 mmol) of hydroxylamine hydrochloride in 10 mL of THF and 10 mL of H_2O was added 861 mg (10.25 mmol) of sodium hydrogen carbonate at 0 °C. After being stirred for 10 min, a solution of the above acid chloride in 10 mL of THF was added to the mixture. The mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was poured into water and extracted with EtOAc. The organic solution was washed with 1 N HCl solution and brine, dried, and evaporated. The residue was purified by chromatography on silica gel using 20:1 $CHCl_3$−MeOH to give 288 mg (93%) of **7c** as colorless crystals: mp 149−151 °C; $[\alpha]^{25}_D$ −11.2° (*c* 0.7, DMSO); IR (KBr) 3268, 1634, 1584, 1356, 1157 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.76 (d, J = 6.6 Hz, 6H), 1.77 (m, 1H), 3.26 (m, 1H), 7.00−7.18 (m, 4H), 7.23 (m, 1H), 7.37−7.53 (m, 2H), 7.70−7.81 (m, 2H), 8.88 (br s, 1H). Anal. ($C_{17}H_{20}N_2O_5S$) C, H, N, S.

(2*R*)-[(4-Biphenylylcarbonyl)amino]-*N*-hydroxy-3-(1*H*-indol 3-yl)propionamide (24). Compound **24** was prepared from **23** and hydroxylamine using a procedure similar to that described for the preparation of **6a**: colorless crystals; 22% yield; mp 164−167 °C; $[\alpha]^{24.5}_D$ −3.5° (*c* 0.5, DMSO); IR (KBr) 3278, 1631, 1533 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.04 (d, J = 6.4 Hz, 2H), 4.62 (m, 1H), 7.12−7.55 (m, 7H), 7.65−7.97 (m, 7H); HR-FABMS *m*/*z* 361.1548 (M + H)$^+$ (calcd for $C_{22}H_{21}N_2O_3$ *m*/*z* 361.1552).

(2*R*)-2-[[5-(4-Methoxyphenyl)thiophene-2-sulfonyl]-amino]-3-methylbutanoic Acid Methyl Ester (10g). To a solution of 500 mg (1.40 mmol) of **9b** in 12 mL of THF were added 319 mg (2.10 mmol) of (4-methoxyphenyl)boronic acid, 387 mg (2.80 mmol) of potassium carbonate, and 81 mg (0.07 mmol) of tetrakis(triphenylphosphine)palladium(0) at room temperature. The mixture was degassed and stirred under an argon atmosphere at 75 °C for 48 h. After being cooled to room temperature, the mixture was poured into water and extracted with EtOAc. The organic solution was washed with 1 N HCl, 5% $NaHCO_3$ solution, and brine, dried, and evaporated. The residue was purified by chromatography on silica gel using 1:3 EtOAc−hexane to give 447 mg (83%) of **10g** as colorless crystals: mp 122−123 °C; $[\alpha]^{25}_D$ −21.7° (*c* 1.0, DMSO); IR (KBr) 3261, 1735, 1350, 1167, 1136 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7.0 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 2.10 (m, 1H), 3.54 (s, 3H), 3.85 (s, 3H), 3.87 (dd, J = 5.0, 10.2 Hz, 1H), 5.20 (d, J = 10.2 Hz, 1H), 6.94 (d, J = 9.0 Hz, 2H), 7.11 (d, J = 4.0 Hz, 1H), 7.49 (d, J = 4.0 Hz, 1H), 7.52 (d, J = 9.0 Hz, 2H). Anal. ($C_{17}H_{21}NO_5S_2$) C, H, N, S.

Compounds **10a**−**f**,**h**,**i** were prepared from the corresponding **8** or **9** and substituted phenylboronic acid using a procedure similar to that described for the preparation of **10g**.

(2*R*)-2-[[5-(4-Methoxyphenyl)thiophene-2-sulfonyl]amino]-3-methylbutanoic Acid (11g). To a solution of 390 mg (1.01 mmol) of **10g** in 8 mL of MeOH and 8 mL of THF was added 5.1 mL of 1 N NaOH solution at room temperature. The mixture was stirred for 6 h at 60 °C. After being cooled to room temperature, the mixture was acidified with 1 N HCl solution and extracted with EtOAc. The organic solution was washed with brine, dried, and evaporated. The residue was crystallized from EtOAc−hexane to give 373 mg (100%) of **11g** as colorless crystals: mp 174−176 °C; $[\alpha]^{25}_D$ −12.8° (*c* 1.0, DMSO); IR (KBr) 3261, 1735, 1343, 1163 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.82 (d, J = 6.9 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 1.98 (m, 1H), 3.60 (dd, J = 6.0, 9.3 Hz, 1H), 3.80 (s, 3H), 7.02 (d, J = 8.7 Hz, 2H), 7.41 (d, J = 3.9 Hz, 1H), 7.49 (d, J = 3.9 Hz, 1H), 7.65 (d, J = 8.7 Hz, 2H), 8.28 (d, J = 9.3 Hz, 1H), 12.63 (br s, 1H). Anal. ($C_{16}H_{19}NO_5S_2$) C, H, N, S.

Compounds **11a**,**b**, **11c**,**d**,**i**, and **11e**,**f**,**h** were prepared using procedures similar to those described for the preparation of **5a**, **11g**, and **5c**, respectively.

(2*R*)-3-Methyl-2-[[5-[(4-methylphenyl)ethynyl]thiophene-2-sulfonyl]amino]butanoic Acid Methyl Ester (12i). To a solution of 3.0 g (8.42 mmol) of **9b** in 30 mL of DMF were added 1.28 mL (10.1 mmol) of (4-methylphenyl)-acetylene, 80 mg (0.421 mmol) of copper(I) iodide, 148 mg (0.211 mmol) of dichlorobis(triphenylphosphine)palladium(II), and 2.35 mL (16.8 mmol) of triethylamine at room temperature. The mixture was degassed and stirred under an argon atmosphere at 50 °C for 24 h. After being cooled to room temperature, the mixture was poured into water and extracted with EtOAc. The organic solution was washed with 1 N HCl, 5% $NaHCO_3$ solution, and brine, dried, and evaporated. The residue was purified by chromatography on silica gel using 1:2 EtOAc−hexane to give 2.9 g (88%) of **12i** as colorless crystals: mp 124−125 °C; $[\alpha]^{25}_D$ −5.0° (*c* 1.0, DMSO); IR (KBr) 2204, 1735, 1349, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 6.9 Hz, 3H), 1.00 (d, J = 6.9 Hz, 3H), 2.10 (m, 1H), 2.39 (s, 3H), 3.60 (s, 3H), 3.86 (dd, J = 4.8, 9.9 Hz, 1H), 5.21 (d, J = 9.9 Hz, 1H), 7.14 (d, J = 3.6 Hz, 1H), 7.18 (d, J = 8.1 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 3.6 Hz, 1H). Anal. ($C_{19}H_{21}NO_4S_2$) C, H, N, S.

Compounds **12a**−**c**,**e**,**g**,**h**,**j**,***k*** were prepared from the corresponding **8** or **9** and substituted phenylacetylene using a procedure similar to that described for the preparation of **12i**.

Compounds **13a**−**c**,**e**,**g**,**i**,**k** and **13h**,**j** were prepared from the corresponding **12** using procedures similar to those described for the preparation of **11g** and **5c**, respectively. Compounds **13d**,**f** were also prepared from **5p** and the corresponding substituted phenylacethylene using a procedure similar to that described for the preparation of **12i**.

(2*R*)-3-Methy-2-[[5-[(4-methylphenyl)ethynyl]thiophene-2-sulfonyl]amino]butanoic acid (13i): colorless crystals; mp 157−158 °C; $[\alpha]^{25}_D$ −7.6° (*c* 0.5, DMSO); IR (KBr) 3267, 2208, 1712, 1350, 1163 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.82 (d, J = 7.2 Hz, 3H), 0.87 (d, J = 7.2 Hz, 3H), 2.01 (m, 1H), 2.35 (s, 3H), 3.61 (dd, J = 5.8, 8.8 Hz, 1H), 7.27 (d, J = 7.8 Hz, 2H), 7.39 (d, J = 3.6 Hz, 1H), 7.49 (d, J = 7.8 Hz, 2H), 7.50 (d, J = 3.6 Hz, 1H), 8.47 (d, J = 8.8 Hz, 1H), 12.63 (br s, 1H). Anal. ($C_{18}H_{19}NO_4S_2\cdot 0.2H_2O$) C, H, N, S.

(2*R*)-2-[[4-[(Benzenesulfonylhydrazono)methyl]benzenesulfonyl]amino]-3-methylbutanoic Acid *tert*-Butyl Ester (15b). Ozone gas was bubbled through a solution of 5.09 g (15 mmol) of **14b** in 300 mL of CH_2Cl_2 at −78 °C until the solution had turned pale blue. To the mixture was added 22.0 mL (300 mmol) of methyl sulfide, and the resulting mixture was allowed to warm to room temperature. The mixture was concentrated in vacuo to give 6.03 g of crude aldehyde, which was used for the next reaction without further purification. To a solution of the above product in 60 mL of EtOH and 15 mL of THF was added 2.72 g (15.8 mmol) of benzenesulfonyl hydrazide at room temperature. After being stirred for 2 h, the mixture was concentrated in vacuo. The residue was purified by chromatography on silica gel using 1:4 $CHCl_3$−EtOAc to give 4.44 g (60% for two steps) of **15b** as colorless crystals: mp 163−164 °C; $[\alpha]^{23.5}_D$ −11.6° (*c* 0.5, DMSO); IR (KBr) 3430, 3274, 1711, 1364, 1343, 1172 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.84 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 1.19 (s, 9H), 2.00 (m, 1H), 3.63 (dd, J = 4.5, 9.9 Hz, 1H), 5.16 (d, J = 9.9 Hz, 1H), 7.50−7.68 (m, 5H), 7.73 (s, 1H), 7.78−7.84 (m, 2H), 7.96−8.02 (m, 2H), 8.16 (br s, 1H). Anal. ($C_{22}H_{29}N_3O_6S_2$) C, H, N, S.

Compound **15c** was prepared from **14c** using a procedure similar to that described for the preparation of **15b**.

(2*R*)-2-[[4-[(Benzenesulfonylhydrazono)methyl]benzenesulfonyl]amino]-3-(1*H*-indol-3-yl)propionic Acid Methyl Ester (15a). To a solution of 9.0 g (23.4 mmol) of **14a** in 100 mL of THF and 10 mL of H_2O were added 2.92 g (25.8 mmol) of trimethylamine *N*-oxide and 7.45 mL of 4% osmium tetroxide solution in H_2O at 0 °C. The mixture was allowed to warm to room temperature and stirred for 4 h. The mixture was poured into 100 mL of 1 M $Na_2S_2O_3$ solution and extracted with EtOAc. The organic solution was washed with 1 N HCl, 5% $NaHCO_3$ solution, and brine, dried, and evaporated. The residual crude diol was used in the next reaction without further purification. To a solution of the above product in 150 mL of THF was added a solution of 6.01 g (28.1 mmol) of sodium periodate in 20 mL of H_2O at 0 °C. After being stirred for 1 h, the mixture was poured into water and extracted with EtOAc. The organic solution was washed with 1 N HCl, 5% $NaHCO_3$ solution, and brine, dried, and evaporated. Without further purification, the residual crude aldehyde was introduced to **15a** using a procedure similar to that described for the preparation of **15b**: colorless crystals; 46% yield for three steps; mp 218−219 °C; $[\alpha]^{25}_D$ +18.3° (*c* 1.0, DMSO); IR (KBr) 3465, 3292, 1726, 1355, 1166 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.84 (dd, J = 7.8, 14.1 Hz, 1H), 3.04 (dd, J = 6.6, 14.1 Hz, 1H), 3.94 (m, 1H), 6.80−6.95 (m, 2H), 7.06 (d, J = 2.4 Hz, 1H), 7.13−7.24 (m, 2H), 7.48−7.70 (m, 7H), 7.88−7.94 (m, 3H), 8.51 (d, J = 8.4 Hz, 1H), 10.76 (s, 1H), 11.79 (br s, 1H); HR-FABMS *m*/*z* 540.1143 M^+ (calcd for $C_{25}H_{24}N_4O_6S_2$ *m*/*z* 540.1138).

(2*R*)-3-Methyl-2-[[4-[2-[4-(methylmercapto)phenyl]-2*H*-tetrazol-5-yl]benzenesulfonyl]amino]butanoic Acid *tert*-Butyl Ester (16e). To a solution of 0.14 mL (1.22 mmol) of 4-(methylmercapto)aniline in 5 mL of EtOH and 5 mL of H_2O was added a solution of 78.4 mg (1.14 mmol) of sodium nitrite in 1 mL of H_2O at 0−5 °C of the internal temperature and stirred for an additional 15 min. To a solution of 496 mg (1 mmol) of **15b** in 5 mL of pyridine was added the above solution at −15 °C. The mixture was allowed to warm to room temperature. The mixture was poured into water and extracted with EtOAc. The organic solution was washed with 2 N HCl, 5% $NaHCO_3$ solution, brine, dried, and evaporated. The residue was purified by chromatography on silica gel using 1:9 $CHCl_3$−EtOAc to give 374 mg (74%) of **16e** as colorless crystals: mp 202−203 °C; $[\alpha]^{25}_D$ −8.8° (*c* 0.25, acetone); IR (KBr) 3310, 1705, 1345, 1171 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.83 (d, J = 6.9 Hz, 3H), 0.86 (d, J = 7.2 Hz, 3H), 1.19 (s, 9H), 2.00 (m, 1H), 2.59 (s, 3H), 3.54 (dd, J = 6.3, 9.6 Hz, 1H), 7.56 (d, J = 8.7 Hz, 2H), 8.00 (d, J = 8.7 Hz, 2H), 8.10 (d, J = 8.7 Hz, 2H), 8.33 (d, J = 9.6 Hz, 1H), 8.34 (d, J = 8.7 Hz, 2H). Anal. ($C_{23}H_{29}N_5O_4S_2\cdot 0.3H_2O$) C, H, N, S.

Compounds **16c**,**f** were prepared from the corresponding **3** and sulfonyl chloride using a procedure similar to that described for the preparation of **4a**. Compounds **16a**,**b**,**d**,**g**,**h** were prepared from the corresponding **15** and substituted aniline using a procedure similar to that described for the preparation of **16e**.

Compounds **17a**,**b** and **17c**−**h** were prepared from the corresponding **16** using procedures similar to those described for the preparation of **11g** and **5c**, respectively.

(2*R*)-3-Methyl-2-[[4-[2-[4-(methylmercapto)phenyl]-2*H*-tetrazol-5-yl]benzenesulfonyl]amino]butanoic acid (17e): colorless crystals; 98% yield; mp 194−195 °C; $[\alpha]^{23.5}_D$ −10.1 (*c* 0.5, DMSO); IR (KBr) 3432, 1720, 1343, 1166 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.81 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.9 Hz, 3H), 1.98 (m, 1H), 2.59 (s, 3H), 3.59 (m, 1H), 7.56 (d, J = 9.0 Hz, 2H), 8.00 (d, J = 8.7 Hz, 2H), 8.11 (d, J = 9.0 Hz, 2H), 8.24 (br s, 1H), 8.33 (d, J = 8.7 Hz, 2H). Anal. ($C_{19}H_{21}N_5O_4S_2$) C, H, N, S.

Componuds **19a**−**c** were prepared from the corresponding **18** using a similar to that procedure for the preparation of **5a**.

(2*R*)-3-Methyl-2-[[4-[(4-nitrobenzoyl)amino]benzenesulfonyl]amino]butanoic Acid *tert*-Butyl Ester (20d). To a solution of 500 mg (1.52 mmol) of **19b** in 10 mL of CH_2Cl_2 were added 423 mg (2.28 mmol) of 4-nitrobenzoyl chloride and 0.33 mL (3.04 mmol) of *N*-methylmorpholine at 0 °C. The mixture was allowed to warm to room temperature and stirred for 24 h. The mixture was poured into water and extracted with EtOAc. The organic solution was washed with 1 N HCl, 5% $NaHCO_3$ solution, and brine, dried, and evaporated. The residue was purified by chromatography on silica gel using 30:1 $CHCl_3$−MeOH to give 680 mg (94%) of **20d** as pale yellow crystals: mp 172−173 °C; $[\alpha]^{22}_D$ +5.8° (*c* 1.0, DMSO); IR (KBr) 3365, 1717, 1528, 1369, 1160, 1138 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.82 (d, J = 6.2 Hz, 3H), 0.85 (d, J = 6.2 Hz, 3H), 1.23 (s, 9H), 1.93 (m, 1H), 3.45 (m, 1H), 7.77 (d, J = 8.6 Hz, 2H), 7.96 (d, J = 8.6 Hz, 2H), 8.01 (br s, 1H), 8.20 (d, J = 8.6 Hz, 2H), 8.39 (d, J = 8.6 Hz, 2H), 10.87 (s, 1H). Anal. ($C_{22}H_{27}N_3O_7S$) C, H, N, S.

Compounds **4n**, **20a**−**c**, and **20e**−**h** were prepared from the corresponding **19** and substituted benzoyl chloride using a procedure similar to that for the preparation of **20d**.

Compounds **5n**, **21a**−**b**, and **21c**−**h** were prepared from **4n** or the corresponding **20** using procedures similar to those for the preparation of **11g** and **5c**, respectively.

(2*R*)-3-Methyl-2-[[4-[(4-nitrobenzoyl)amino]benzenesulfonyl]amino]butanoic acid (21d): pale yellow crystals; 99% yield; mp 240−242 °C; $[\alpha]^{22}_D$ −7.0° (*c* 1.0, DMSO); IR (KBr) 3402, 1726, 1688, 1591, 1347, 1166 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.80 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.6 Hz, 3H), 1.95 (m, 1H), 3.52 (dd, J = 5.7, 9.6 Hz, 1H), 7.78 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 9.6 Hz, 1H), 8.20 (d, J = 8.4 Hz, 2H), 8.39 (d, J = 8.4 Hz, 2H), 10.87 (s, 1H), 12.51 (br s, 1H). Anal. ($C_{18}H_{19}N_3O_7S\cdot 0.4H_2O$) C, H, N, S.

4-(2-Phenyl-2*H*-tetrazol-5-yl)benzenesulfonyl Chloride (22a). To a solution of 38.65 g (0.128 mol) of 5-(4-bromophenyl)-2-phenyl-1,2,3,4-tetrazole in 450 mL of THF was added 88

mL (0.141 mol) of 1.6 M *n*-butyllithium in hexane dropwise over a 30-min period at −78 °C. To the mixture was added a solution of 273 mL (6.4 mol) of sulfur dioxide in 200 mL of THF at −78 °C. A yellow precipitate separated immediately. The mixture was allowed to warm to room temperature and stirred for 24 h. After removal of the solvent in vacuo, the residual crude lithium sulfinate was washed with Et_2O and used in the next reaction without further purification. To a suspension of the above product in 500 mL of CH_2Cl_2 was added 12.9 mL (0.161 mol) of sulfuryl chloride at 0 °C. The mixture was allowed to warm to room temperature and stirred for 24 h. The mixture was poured into ice−water and extracted with EtOAc. The organic solution was washed with brine, and then dried and evaporated. The residue was crystallized from EtOAc−hexane to give 21.28 g (52% for two steps) of **22a** as colorless crystals: mp 157−158 °C; IR (KBr) 1376, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50−7.70 (m, 3H), 8.15−8.30 (m, 4H), 8.54 (d, J = 8.2 Hz, 2H). Anal. ($C_{13}H_9ClN_4O_2S\cdot 0.2H_2O$) C, H, Cl, N, S.

Compound **22b** was prepared from the corresponding bromide using a procedure similar to that described for the preparation of **22a**.

Dibenzofuran-2-sulfonyl Chloride (22d). To a solution of 16.8 g (0.1 mol) of dibenzofuran in 100 mL of $CHCl_3$ was added 6.98 mL (0.105 mol) of chlorosulfonic acid at 0 °C. The mixture was allowed to warm to room temperature and stirred for 24 h. After removal of the solvent in vacuo, the residual crude sulfonic acid was washed with $CHCl_3$−hexane and used in the next reaction without further purification. The mixture of the above product and 20.8 g (0.1 mol) of phosphorus pentachloride was stirred at 80 °C for 6 h. After being cooled to room temperature, the mixture was poured into ice−water and extracted with $CHCl_3$. The organic solution was washed with 5% $NaHCO_3$ solution and brine, dried and evaporated. The residue was washed with hexane to give 9.83 g (74% for two steps) of **22d** as colorless crystals: ^1H NMR ($CDCl_3$) δ 7.40−7.80 (m, 4H), 8.00−8.20 (m, 2H), 8.67 (d, J = 1.8 Hz, 2H).

Compound **22c** was prepared from diphenylmethane using a procedure similar to that described for the preparation of **22d**.

4-(3-Phenylureido)benzenesulfonyl Chloride (22e). To a solution of 0.84 mL (9.19 mmol) of aniline in 20 mL of THF was added 2.0 g (9.19 mmol) of 4-(chlorosulfonyl)phenyl isocyanate at 0 °C. After being stirred for 1.5 h, the mixture was poured into water and extracted with EtOAc. The organic solution was washed with brine, dried, and evaporated. The residue was crystallized from hexane to give 2.70 g (95%) of **22e** as colorless crystals: IR ($CHCl_3$) 3306, 1662, 1375, 1167 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.93−7.50 (m, 7H), 7.53 (d, J = 8.7 Hz, 2H), 9.07 (s, 1H), 9.16 (s, 1H).

Biology. Enzyme assays: MMP-9, MMP-2, MMP-1, and MMP-7 Assays (Using Synthetic Substrate).[29] A mixture of 24 μL of enzyme solution (about 0.2 unit/mL) and 1 μL of inhibitor solution were incubated with 25 μL of 2 mM ABD-F[30] in buffer A (25 mM Hepes-NaOH/5 mM $CaCl_2$, pH 7.0) for 30 min at room temperature and then mixed with 10 μL of 1.25 mM Ac-Pro-Leu-Gly-SCH($CH_2CH(CH_3)_2$)CO-Leu-Gly-OCH_2CH_3[31] in buffer A. Enzyme reaction was performed at 37 °C for 3 h and quenched with 350 μL of 3% aqueous acetic acid. Fluorescence was measured using Shimazu spectroflurophotometer RF-5000. Excitation and emission wavelengths were 360 and 530 nm, respectively.

MMP-9, MMP-2, and MMP-7 Assays (Using Natural Substrate).[29] Commercially available assay kits (Yagai, Yamagata City, Japan) were used. The solutions provided in the kits were used unless otherwise stated. A 30 μL portion of enzyme solution (0.5 unit/mL) was incubated with 1 μL of inhibitor solution (DMSO) and 19 μL of buffer B (50 mM Tris·Cl/0.5 M D-glucose/0.2 M NaCl/5 mM $CaCl_2$/0.05% (w/v) Triton X-100/0.01% NaN_3, pH 7.5) at room temperature for 30 min and then further incubated with 50 μL of 1 mg/mL FITC-labeled type IV collagen solution at 42 °C for 3 h. After being incubated with 300 μL of quenching solution on ice for 30 min, the reaction mixture was centrifuged at 8000*g* for 10 min. Supernatant (340 μL) was used for measurement of fluorescence. Excitation and emission wavelengths were 495 and 520 nm, respectively.

MMP-3 Assay.[29] A mixture of 4 μL of enzyme solution and 2 μL of inhibitor solution were incubated with 74 μL of buffer C (50 mM Tris·Cl/10 mM $CaCl_2$/0.2 M NaCl/0.02% (w/v) NaN_3/0.05% (v/v) Brij-35, pH 7.5) at room temperature for 30 min. A solution of 25 μL of 25 μM MOAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)NH_2[32] (buffer C) was added, and the reaction was performed for 90 min. A 50 μL aliquot of the reaction mixture was mixed with 400 μL of 3% aqueous acetic acid, and its fluorescence was measured. Excitation and emission wavelengths were 325 and 393 nm, respectively.

Phamacokinetics. Ex vivo pharmacokinetic studies were usually carried out in mice. Compounds were dosed in 0.5% methyl cellulose (1500 cP) at 200 mg/kg po, after which the mice were bled into tubes at various time points. The inhibitors in plasma were extracted with CH_3CN. The inhibitor level in plasma was then determined by HPLC on a column of Cosmosil 5C18−300 (4.6 × 150 mm, Nacalai Co., Osaka, Japan) with a linear gradient of CH_3CN in the presence of 0.1% TFA.

Antitumor Assay. All experiments consisted of 6−16 mice per group.[33] Single-cell suspension of Lewis lung carcinoma[34] (3×10^4) was injected into BDF1 mice through the tail vein. All compounds were suspended in vehicle (saline including 0.4% Tween 80, 0.5% carboxymethylcellulose, and 0.9% benzyl alcohol)[35] and were orally administrated 4 h before and 1, 24, 48, and 72 h after tumor injection. On day 14, mice were sacrificed, and the number of the lung colonies was counted. In the case of Ma44 lung cancer, tumor cells (1×10^4) were inoculated ip in nude mice (day 0). Compounds were orally administrated once a day from day 1 to day 20, and the survival of the mice was scored. The statistical significance in the present experiments was evaluated by the Dunnet's test.

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Supporting Information Available: Characteristic data for part of synthetic intermediates and final products and biological data for part of compounds (35 pages). Ordering information is given on any current masthead page.

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(30) 4-Fluoro-7-sulfamoylbenzofurazan (ABD-F) was purchased from Dojindo Laboratories.
(31) Ac-Pro-Leu-Gly-SCH($CH_2CH(CH_3)_2$)CO-Leu-Gly-OCH_2CH_3 was purchased from Bachem.
(32) MOAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2 and Bz-Ala-Ala-Leu-pNA were purchased from Peptide Institute.
(33) Female BDF1 (7−9 weeks old) and female athymic BALB/c-nu/nu mice (8−10 weeks old) used in this study were purchased from Nihon SLC (Shizuoka, Japan) and Clea Japan Inc. (Tokyo, Japan), respectively.
(34) Lewis murine lung carcinoma were obtained from the National Cancer Institute (NCI, Rockville, MD) and maintained by serial sc transplantation as a tumor fragment. Ma44 human lung squamous cell carcinoma was kindly supplied by Dr. Komiya (Habikino Hospital, Osaka, Japan) and maintained by in vitro passage using Eagle's MEM (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal calf serum (Gibco BRM, Rockville, MD). Production of type IV collagenases in these cell lines was confirmed by gelatin zymography.
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