Substituted 3-(Phenylsulfonyl)-1-phenylimidazolidine-2,4-dione Derivatives as Novel Nonpeptide Inhibitors of Human Heart Chymase

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A series of 3-(phenylsulfonyl)-1-phenylimidazolidine-2,4-dione derivatives have been synthesized and evaluated for their ability to selectively inhibit human heart chymase. The structure−activity relationship studies on these compounds gave the following results. The 1-phenyl moiety participates in a hydrophobic interaction where an optimum size is required. At this position, 3,4-dimethylphenyl is the best moiety for inhibiting chymase and showed high selectivity compared with chymotrypsin and cathepsin G. A 3-phenylsulfonyl moiety substituted with hydrogen-bond acceptors such as nitrile and methoxycarbonyl enhances its activity. Molecular-modeling studies on the interaction of 3-[(4-chlorophenyl)sulfonyl]-1-(4-chlorophenyl)-imidazolidine-2,4-dione (**29**) with the active site of human heart chymase suggested that the 1-phenyl moiety interacts with the hydrophobic P_1 pocket, the 3-phenylsulfonyl moiety resides in the $S_{1'}$−$S_{2'}$ subsites, and the 4-carbonyl of the imidazolidine ring and sulfonyl group interact with the oxyanion hole and the His-45 side chain of chymase, respectively. The complex model is consistent with the structure−activity relationships.

Introduction

Angiotensin-I converting enzyme (ACE), a dipeptidyl carboxylase, is a key enzyme in the formation of angiotensin II (Ang II) from angiotensin I (Ang I).[1] However, the conversion of Ang I to Ang II has been demonstrated to occur in the presence of ACE inhibitors in many tissues including the heart.[2−5] The non-ACE enzyme is inhibited by chymostatin, an inhibitor of chymotrypsin-like enzymes.[3,4,6,7] DNA analysis indicates that this enzyme belongs to chymases.[8] Chymase converts Ang I to Ang II with greater efficiency and selectivity than ACE.[4,9] Although physiological and pathological roles of this enzyme have not been fully elucidated yet, increases in chymase activity and mRNA level have been observed in balloon-injury induced hypertrophied vessels in dogs[10] and also in the heart of cardiomyopathic hamsters.[11] Thus, chymase may play an important role in vascular hypertrophy and in cardiovascular diseases such as hypertension, ischaemic heart disease, and congestive heart failure.

Several synthetic inhibitors of chymase are known, such as 2-(Z-$NHCH_2CONH$)$C_6H_4SO_2F$,[12] methoxysuccinyl-Ala-Ala-Pro-(L)boro-Phe-OH,[13] Boc-Val-Pro-Phe-CO_2Me,[14] Z-Ile-Glu-Pro-Phe-CO_2Me, and (F)-Phe-CO-Glu-Asp-ArgOMe.[15] At present, however, no clinically applicable chymase inhibitor has been found. In the course of our study to provide nonpeptide inhibitors of chymotrypsin-type serine proteases, we found that compound **29** showed potent inhibitory activity for chymase. We will describe here the structure−activity relationships of **29** and its related compounds and the fitting and interaction studies with a chymase model.

Results and Discussion

Most of compounds **1**−**15**, **18**−**24**, **27**−**29**, **33**−**38**, **40**−**46**, **48**, and **50** were generally prepared by the reaction of the corresponding arylsulfonyl isocyanates or benzoyl isocyanate and *N*-aryl or heteroarylglycine or their ester derivatives, followed by cyclization with ethyl chloroformate as shown in Scheme 1. The corresponding arylsulfonyl isocyanates were obtained commercially or by the reaction of sulfonamide with chlorosulfonyl isocyanate.[16] Substituted *N*-phenylglycine derivatives were prepared by reductive alkylation of the corresponding anilines and glyoxylic acid with $NaBH_3CN$. *N*-(3,4-Dimethylphenyl)glycine was obtained by *N*-alkylation of 3,4-dimethylacetoanilide with ethyl bromoacetate in the presence of NaH, followed by hydrolysis by hydrochloric acid. *p*-Chlorobenzoyl derivative **47** was prepared by the reaction of 1-phenylimidazolidine-2,4-dione with *p*-chlorobenzoyl chloride in pyridine (Scheme 3-1). Carboxylic acid derivatives **16**, **17**, **30**−**32** were obtained by treating the corresponding allyl esters with Pd complex catalyst[17] or treating the corresponding *tert*-butyl esters with TFA (Scheme 2). Subsequently, the corresponding carboxylic acid amides **25**, **26**, and **39** were prepared by the usual method (Scheme 2). 1-[(*p*-Chlorophenyl)sulfonyl]imidazolidine-2,4-dione derivative **49** was obtained by cyclizing *N*-[(*p*-chlorophenyl)-sulfonyl]glycine with *p*-chlorophenyl isocyanate (Scheme 3-2). 3-[(Benzoyloxy)methyl]imidazolidine-2,4-dione de-

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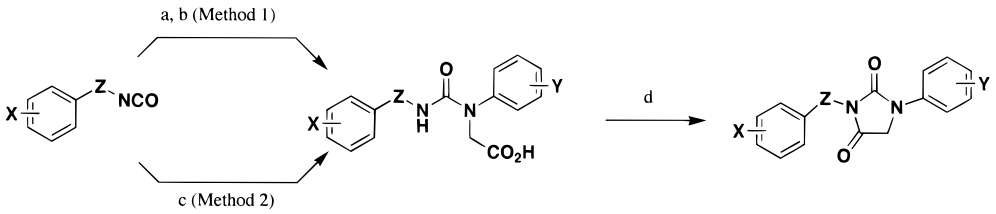
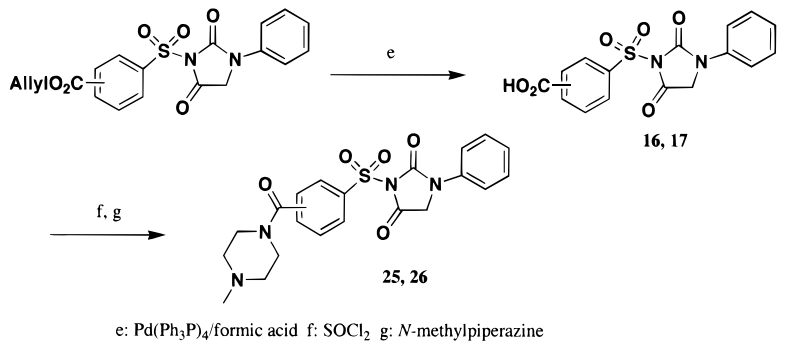
Scheme 1[a]

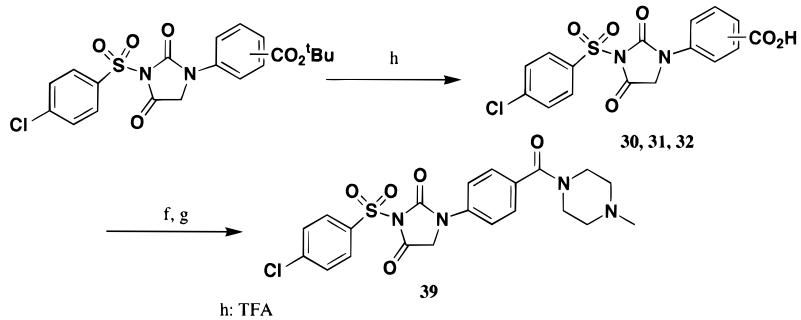


[a] (a) Substituted *N*-phenylacetic acid methyl ester; (b) 1 N NaOH; (c) substituted *N*-phenylacetic acid; (d) ethyl chloroformate/Et_3N.

Scheme 2

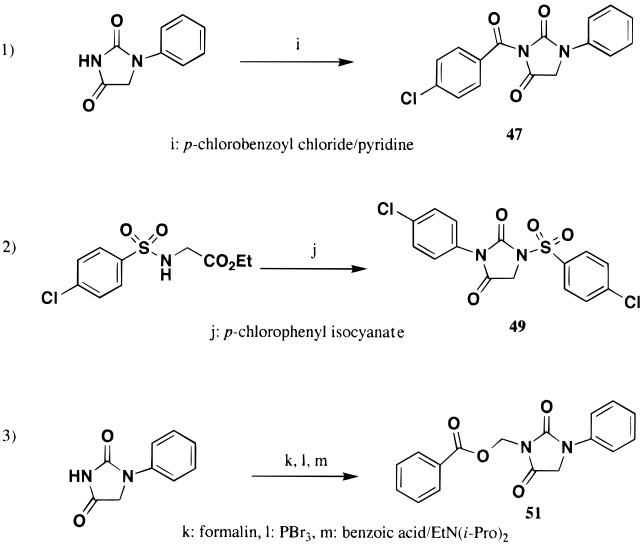


e: $Pd(Ph_3P)_4$/formic acid f: $SOCl_2$ g: *N*-methylpiperazine



h: TFA

Scheme 3



i: *p*-chlorobenzoyl chloride/pyridine

j: *p*-chlorophenyl isocyanate

k: formalin, l: PBr_3, m: benzoic acid/$EtN(i\text{-}Pro)_2$

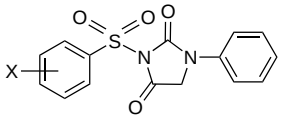
rivative **51** was prepared from 3-(bromomethyl)imidazolidine-2,4-dione derivative and benzoic acid under basic conditions (Scheme 3-3).[18]

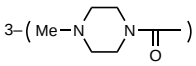
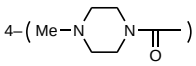
The compounds were tested *in vitro* for inhibition of human heart chymase, bovine pancreas α-chymotrypsin, and human neutrophil cathepsin G. The results are given as IC_{50} values as shown in Tables 1−4.

First, we investigated the substituent effect of the phenylsulfonyl moiety as shown in Table 1. This substitution gave IC_{50} values between 10^{-6} and 10^{-8} M. The unsubstituted compound (**1**) was the least active among them. Substituents such as chlorine (**2**−**4**), fluorine (**5**), bromine (**6**), methoxy (**9** and **10**), nitro (**13**), and phenoxy (**20** and **21**) increased the activity moderately (IC_{50} = ∼10^{-7} M). Methyl substitution did not improve potency as much (IC_{50} = 1.2−2.6 μM). On the other hand, nitrile (**11** and **12**) and methoxycarbonyl substitutions (**14** and **15**) remarkably increased potency (IC_{50} = ∼10^{-8} M). The following observations were made regarding the effect of substitution patterns. Ortho-substituted analogs tended to be less potent than both meta- and para-substituted analogs. Meta- and para-substituted analogs were approximately equipotent except for the carboxylic acid (**16** vs **17**), (methoxycarbonyl)methyl (**18** vs **19**), and 4-methylpiperazinyl amide (**25** vs **26**) analogs. Among these exceptions, meta substitution was more effective than para substitution. Meta,para-disubstituted compounds (**22** and **23**) were some of the most active compounds. It is interesting that 3,4-dimethyl derivative **23** gave excellent activity despite the poor activity of 4-methyl derivative **8**. These results show that the region binding phenylsulfonyl moieties is rather hydrophobic and has enough space to allow a phenoxyphenyl group (**20** and **21**). The potent activity of nitrile (**11** and **12**) and methoxycarbonyl derivatives (**14** and **15**) may be the result of their hydrogen-bond-accepting properties.

Next, we examined the substitution on the 1-phenyl moiety (Table 2). Generally, hydrophilic substituents

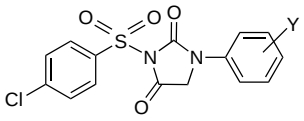
Table 1. Enzyme Inhibitory Activities of Substituted Benzenesulfonyl Derivatives

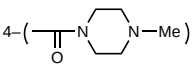


		IC$_{50}$,[a] μM		
compd	X	chymase	chymotrypsin	cathepsin G
1	H	6.2	1.4	4.5
2	2-Cl	1.1	1.2	2.2
3	3-Cl	0.13	>100	2.5
4	4-Cl	0.38	1.1	0.7
5	4-F	0.37	2.6	1.5
6	4-Br	0.17	2.4	0.60
7	2-Me	1.8	1.1	1.8
8	4-Me	2.6	7.0	4.5
9	3-OMe	0.58	3.7	8.0
10	4-OMe	0.55	5.8	4.2
11	3-CN	0.068	0.73	0.78
12	4-CN	0.040	15	0.33
13	4-NO_2	0.70	8.0	6.5
14	3-CO_2Me	0.082	1.7	0.64
15	4-CO_2Me	0.029	1.8	0.34
16	3-CO_2H	0.86	8.5	1.0
17	4-CO_2H	5.9	44	1.9
18	3-CH_2CO_2Me	0.50	4.0	5.4
19	4-CH_2CO_2Me	5.0	11	6.5
20	3-OPh	0.80	1.4	3.3
21	4-OPh	0.70	8.0	3.7
22	3,4-Cl_2	0.018	3.8	0.23
23	3,4-Me_2	0.030	2.0	0.22
24	3,4-$(OMe)_2$	0.11	>100	4.1
25		0.90	1.4	4.8
26		3.3	6.4	16

[a] Concentration inhibiting 50% of the corresponding enzyme. Deviations of data are less than 10% of the mean. Conditions are described in the Experimental Section.

Table 2. Enzyme Inhibitory Activities of Substituted Phenyl Derivatives

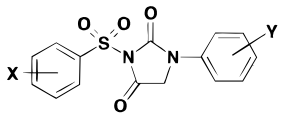


		IC$_{50}$,[a] μM		
compd	Y	chymase	chymotrypsin	cathepsin G
4	H	0.38	1.1	0.7
27	2-Cl	0.22	20	2.0
28	3-Cl	0.066	0.20	3.9
29	4-Cl	0.30	0.50	1.5
30	2-CO_2H	3.3	73	26
31	3-CO_2H	65	>100	>100
32	4-CO_2H	100	42	>100
33	2-OPh	4.6	37	5.9
34	4-OPh	28	39	4.8
35	4-NHAc	>100	>100	>100
36	3,4-Cl_2	0.023	4.0	22
37	3,4-Me_2	0.035	43	3.7
38	3,4-$OMe)_2$	14	>100	>100
39		27	>100	>100

[a] Concentration inhibiting 50% of the corresponding enzyme. Deviations of data are less than 10% of the mean. Conditions are described in the Experimental Section.

were less potent than hydrophobic substituents. Hydrophilic substituents such as carboxylic acid (**30**, **31**, and **32**), *N*-acetylamino (**35**), and 4-methylpiperazinyl amide (**39**) decreased the activity. The rather hindered phenoxy derivative (**34**) also decreased the activity. The highest activity observed for the 3,4-disubstituted derivatives (**36** and **37**) was on the order of 10^{-8} M. These data show that the 1-phenyl moiety is bound by hydrophobic interaction in a rather small space. This space is most likely the P_1 pocket in chymase.

Table 3. Enzyme Inhibitory Activities of Tetrasubstituted Derivatives



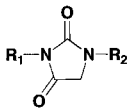
			IC$_{50}$,[a] μM		
compd	X	Y	chymase	chymotrypsin	cathepsin G
40	3,4-Cl_2	3,4-Me_2	0.020	63	20
41	3,4-$(OMe)_2$	3,4-Me_2	0.022	>100	>100
42	3,4-Me_2	3,4-Me_2	0.060	>100	1.7
43	3,4-Cl_2	3,4-Cl_2	0.17	3.7	2.3

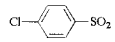
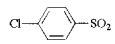
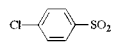
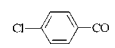
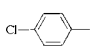
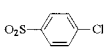
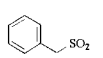
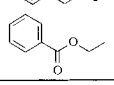
[a] Concentration inhibiting 50% of the corresponding enzyme. Deviations of data are less than 10% of the mean. Conditions are described in the Experimental Section.

From the above results, we examined the hybrid tetrasubstituted derivatives **40**−**43** (Table 3). We found compounds **40**, **41**, and **42** to be equipotent to or have greater potency than the disubstituted analogs **22**, **23**, **24**, and **29**. However, tetrachloro derivative **43** was less potent than **22** and **36**. The 3,4-dimethylphenyl group is presumably the most acceptable residue for the P_1 pocket. Compounds **40** and **41** showed high specificity for chymase although chymase, chymotrypsin, and cathepsin G belong to the same family of serine proteases.

Finally, we prepared other imidazolidindione derivatives (Table 4). Instead of a 1-phenyl moiety, the introduction of cyclohexyl (**44**), 2-pyridyl (**45**), and 2-pyrimidinyl group (**46**), respectively, decreased the activity. Therefore, a benzene ring is optimal for the P_1 pocket. When the (4-chlorophenyl)sulfonyl group was introduced at the 1-position of imidazolidine ring (**49**), the activity disappeared. This might suggest that the sulfonyl group in **49** could not interact with the hydrophobic P_1 pocket. The activity of the amides **47** and **48** was substantially eliminated when compared to the sulfonamide analogs. These compounds were potent selective chymotrypsin inhibitors as illustrated by **48**, which had an IC$_{50}$ of 10^{-8} M for chymotrypsin. This result shows the difference between benzoyl and phenylsulfonyl substitutions and suggests that the sulfonyl group provides a specific interaction and conformational bias for inhibition of chymase whereas amides cannot achieve the same interaction but are selective for chymotrypsin. The incorporation of a 1-benzylsulfonyl group (**50**) in place of the 1-phenylsulfonyl substituent eliminated the activity. The direction of the benzyl group relative to the imidazolidine ring is different from that of the phenyl group and creates steric hindrance in the active site of the enzyme. This may be the reason for the remarkable difference of activity between **50** and **1**. 1-(Benzoyloxy)methyl derivative (**51**), which is used in the case of elastase inhibitors,[18] did not show any activity. In the imidazolidine derivatives, the sulfonyl moiety is essential for activity.

Table 4. Enzyme Inhibitory Activities of Other Derivatives



	R1	R2	IC_{50},[a] μM Chymase	Chymotrypsin	Cathepsin G
44			35	30	75
45			18	>100	8.5
46			33	52	100
47			>100	0.23	3.0
48			20	0.048	7.0
49			>100	>100	>100
50			>100	37	100
51			>100	>100	>100

[a] Concentration inhibiting 50% of the corresponding enzyme. Deviations of data are less than 10% of the mean. Conditions are described in the Experimental Section.

Fitting studies of compound **29** with human chymase were performed based on the hypotheses that the 1-phenyl moiety of inhibitors interacts with the P_1 pocket and the 4-carbonyl group of the imidazolidine ring is located in the oxyanion hole in chymase to interact with the catalytic serine residue. The latter hypothesis was based on the chemical reaction of compound **29** with sodium methoxide[19] in methanol to give *N*-[(4-chlorophenyl)sulfonyl]-*N′*-(4-chlorophenyl)-*N′*-[(methoxycarbonyl)methyl]urea which was the ring-opened product at the N3−C4 bond of the imidazolidine. Thus, a stable complex of compound **29** with the active site of chymase could be formed as shown in Figure 1. In this complex, the position of the 4-carbonyl and 3-*N*-sulfonyl groups of the imidazolidine ring are fixed by hydrogen bonds with the oxyanion hole and the His-45 side chain of chymase, respectively. The 1-(4-chlorophenyl) moiety is located in the P_1 pocket. It seems that directions and positions of the carbonyl and sulfonyl groups relative to the phenyl moiety are fundamental to the inhibition of chymase. The 3-[(4-chlorophenyl)-sulfonyl] moiety was placed near the Arg-130 and Lys-179 side chains in the $S_{1'}-S_{2'}$ subsites, capable of acting as hydrogen-bond donors due to their positive charges.

We could explain the inhibitory data from the complex model. The significant decrease of the inhibitory activity of compounds with bulky or hydrophilic groups at the 1-phenyl moiety in Table 2 may be because these substituents are unable to fit to the P_1 pocket, making the 4-carbonyl and 3-*N*-sulfonyl groups unable to interact with the oxyanion hole and the His-45 side chain of chymase, respectively. In the case of the carboxylic acid substituent on the 1-phenyl moiety (**30**, **31**, and **32**), the activity decreased in the order of ortho, meta, and para. This result may suggest that the *o*-carboxylic acid stays in front of the P_1 pocket, thereby retaining activity. This complex model was consistent with the inhibitory increase by meta and/or para substitution of the 3-phenylsulfonyl moiety with hydrogen-bond acceptors such as methoxycarbonyl and nitrile. In the carboxylic acid (**16** and **17**) and (methoxycarbonyl)methyl (**18** and **19**) analogs, the direction for an electrostatic or hydrogen-bonding interaction may be important for the difference in activity between meta and para positions. It was made certain that the activity and specificity of these inhibitors would be improved by the modification of meta and/or para substitution in the benzenesulfonyl moiety.

In summary, we have reported that 1-phenylimidazolidine-2,4-dione derivatives are novel inhibitors endowed with good potency and selectivity toward human heart chymase.

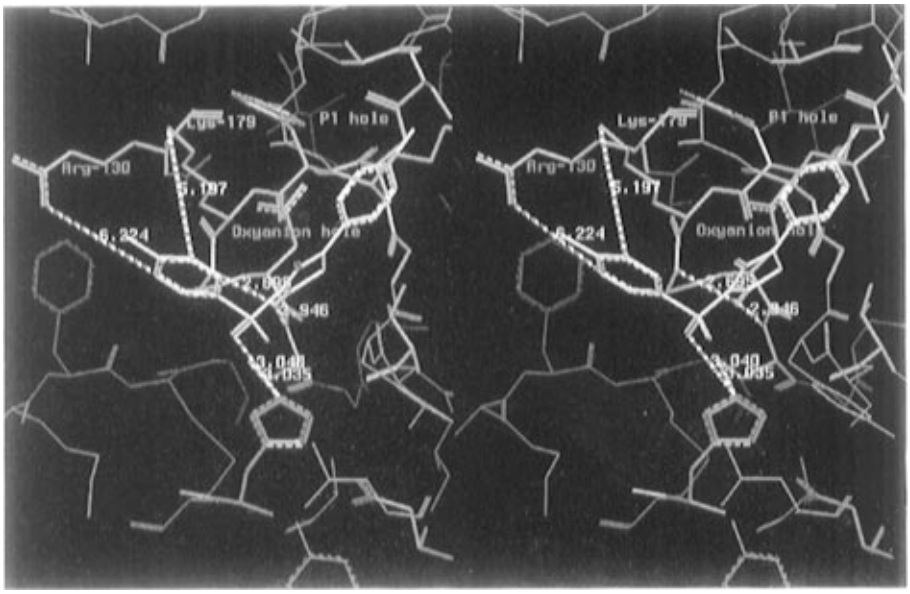


Figure 1. Stereoview of the optimized conformation for human heart chymase and compound **29**. Active triad (Ser-182, His-45, Asp-89), the other residues of chymase and compound **29** are shown by orange, green, and yellow, respectively. 1-Phenyl, imidazolidine, and 3-phenylsulfonyl moieties are placed near the P_1 hole, oxyanion hole and Arg-130/Lys-179 of chymase, respectively, and the 4-carbonyl and 3-N-sulfonyl of the imidazolidine are fixed by hydrogen bonds with the oxyanion hole and His-45 side chain (2.70 and 3.04 Å), respectively.

Experimental Section

Synthesis. Melting points were measured by using a Mettler FP-61 (automatic melting apparatus) and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL FX-100, JEOL EX-400, and Bruker ARX-400 using TMS as an internal reference. Mass spectra were obtained on a JEOL JMX-AX500 by FAB ionization method. Elemental analyses were performed on a Perkin-Elmer 240B elemental analyzer, and all compounds submitted for testing had analytical results 0.4% of the theoretical values.

General Method 1 (Scheme 1). 3-[(4-Chlorophenyl)-sulfonyl]-1-(4-chlorophenyl)imidazolidine-2,4-dione (29). To a solution of methyl [(4-chlorophenyl)amino]acetate (3 g, 15 mmol) in 40 mL of benzene was added (4-chlorophenyl)-sulfonyl isocyanate (2.2 mL, 15 mmol), the mixture was stirred for 1 h. Crystals formed were filtered to obtain *N*-[(4-chlorophenyl)sulfonyl]-*N*′-(4-chlorophenyl)-*N*′-[(methoxycarbonyl)methyl]urea (6.1 g, 100% yield).

The obtained urea derivative (2.6 g, 6.4 mmol) was dissolved in a mixture of MeOH (25 mL) and 1 N NaOH (25 mL) and then stirred for 5 h. The reaction mixture was acidified with 1 N HCl and then extracted with AcOEt. The resulting extract was washed with water, dried over $MgSO_4$, and then evaporated to a residue, which was crystallized from *n*-hexane/AcOEt to give *N*-[(4-chlorophenyl)sulfonyl]-*N*′-(4-chlorophenyl)-*N*′-(carboxymethyl)urea (2.2 g, 88% yield).

A mixture of the above urea (2.2 g, 5.5 mmol) and Et_3N (2 mL, 15.5 mmol) in THF (50 mL) was cooled at 0 °C, and then ethyl chloroformate (0.66 mL, 6.9 mmol) was added. After 1 h, the mixture was stirred for an additional 2 h at room temperature. The resulting solution was extracted with AcOEt, washed with saline, dried over $MgSO_4$, and then evaporated to dryness. The residue was crystallized from *n*-hexane/AcOEt to obtain the title compound (1.3 g) as white crystals: yield 59% (from urea); mp 239−240 °C; ^{1}H-NMR (DMSO-d_6) δ 4.49 (s, 2H), 7.4−8.1 (m, 8H). Anal. ($C_{15}H_{10}Cl_2N_2O_4S$) C, H, N.

By the same manner, compound **1**, **2**, **4**, **7**, **8**, and **48** were prepared. Their physical data, yield, and analytical data were cited as follows.

3-(Phenylsulfonyl)-1-phenylimidazolidine-2,4-dione (1): white crystals; yield 37% (from urea); mp 212.5−214 °C; ^{1}H-NMR (DMSO-d_6) δ 4.53 (s, 2H), 7.1−8.1 (m, 10H). Anal. ($C_{15}H_{12}N_2O_4S$) C, H, N.

3-[(2-Chlorophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (2): white crystals; yield 28% (from (2-chlorophenyl)sulfonyl isocyanate); mp 217−220 °C; ^{1}H-NMR (DMSO-d_6) δ 4.70 (s, 2H), 7.0−8.2 (m, 9H). Anal. ($C_{15}H_{11}ClN_2O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (4): white crystals; yield 81% (from urea); mp 188−192 °C; ^{1}H-NMR (DMSO-d_6) δ 4.27 (s, 2H), 7.2−8.2 (m, 9H). Anal. ($C_{15}H_{11}ClN_2O_4S$) C, H, N.

1-Phenyl-3-(2-tolylsulfonyl)imidazolidine-2,4-dione (7): white crystals; yield 18% (from urea); mp 186−190 °C; ^{1}H-NMR (DMSO-d_6) δ 2.64 (s, 3H), 4.59 (s, 2H), 7.1−8.1 (m, 9H). Anal. ($C_{16}H_{14}N_2O_4S$) C, H, N.

1-Phenyl-3-(4-tolylsulfonyl)imidazolidine-2,4-dione (8): white crystals; yield 32% (from urea); mp 204−206 °C; ^{1}H-NMR (DMSO-d_6) δ 2.42 (s, 3H), 4.25 (s, 2H), 7.2−8.2 (m, 9H). Anal. ($C_{16}H_{14}N_2O_4S$) C, H, N.

3-Benzoyl-1-phenylimidazolidine-2,4-dione (48): white crystals; yield 76% (from urea); mp 188−192 °C; ^{1}H-NMR (DMSO-d_6) δ 4.66 (s, 2H), 7.1−8.1 (m, 10H). Anal. ($C_{16}H_{12}N_2O_3$) C, H, N.

General method 2 (Scheme 1). 3-[(4-Phenoxyphenyl)-sulfonyl]-1-phenylimidazolidine-2,4-dione (21). To a solution of (phenylamino)acetic acid (1.25 g, 8.29 mmol) in THF (100 mL) was added (4-phenoxyphenyl)sulfonyl isocyanate (2.0 g, 8.29 mmol) at room temperature, and the mixture was stirred for 16 h. After completion of the reaction, the mixture was cooled to 0 °C and reacted with Et_3N (2.09 g, 20.7 mmol) and ethyl chloroformate (0.9 g, 8.29 mmol) followed by stirring at 0 °C for 1 h. After the reaction, the resulting solution was poured into 1 N HCl (40 mL) and saline (60 mL) and subjected to extraction with AcOEt. The extract was washed with saline, dried over Na_2SO_4, and then concentrated to dryness. The residue was purified by silica gel chromatography (*n*-hexane/AcOEt = 3:1) and crystallized from *n*-hexane/AcOEt to give the title compound (0.31 g) as white crystals: yield 9.2%; mp 157−157.5 °C; ^{1}H-NMR ($CDCl_3$) δ 4.32 (s, 2H), 7.0−8.2 (m, 14H). Anal. ($C_{21}H_{16}N_2O_5S$) C, H, N.

By the same manner, compound **3**, **5**, **6**, **9**−**15**, **18**−**20**, **22**−**24**, **27**, **28**, **33**−**38**, **40**−**46**, and **50** were prepared. Their physical data, yield, and analytical data were cited as follows.

3-[(3-Chlorophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (3): white crystals; yield 21% (from (3-chlorophenyl)sulfonyl isocyanate); mp >250 °C; ^{1}H-NMR (DMSO-d_6) δ 4.52 (s, 2H), 7.1−8.1 (m, 9H). Anal. ($C_{15}H_{11}ClN_2O_4S$) C, H, N.

3-[(4-Fluorophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (5): white crystals; yield 50% (from (4-fluorophenyl)sulfonyl isocyanate); mp 196.5−197 °C; ^{1}H-NMR (DMSO-d_6) δ 4.51 (s, 2H), 7.1−8.2 (m, 9H). Anal. ($C_{15}H_{11}FN_2O_4S$) C, H, N.

3-[(4-Bromophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (6): white crystals; yield 57% (from (4-bromophenyl)sulfonyl isocyanate); mp 190−191 °C; ^{1}H-NMR (DMSO-d_6) δ 4.50 (s, 2H), 7.0−8.0 (m, 9H). Anal. ($C_{15}H_{11}BrN_2O_4S$) C, H, N.

3-[(3-Methoxyphenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (9): white crystals; yield 3.8% (from (3-methoxyphenyl)sulfonyl isocyanate); mp 201−201.5 °C; ^{1}H-NMR ($CDCl_3$) δ 3.89 (s, 3H), 4.31 (s, 2H), 7.1−7.8 (m, 9H). Anal. ($C_{16}H_{14}N_2O_5S$) C, H, N.

3-[(4-Methoxyphenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (10): white crystals; yield 35% (from (4-methoxyphenyl)sulfonyl isocyanate); mp 210.5−211 °C; ^{1}H-NMR (DMSO-d_6) δ 3.86 (s, 3H), 4.51 (s, 2H), 7.1−8.0 (m, 9H). Anal. ($C_{16}H_{14}N_2O_5S$) C, H, N.

3-[(3-Cyanophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (11): white crystals; yield 26% (from (3-cyanophenyl)sulfonyl isocyanate); mp 194−195 °C; ^{1}H NMR (DMSO-d_6) δ 4.52 (s, 2H), 7.1−8.5 (m, 9H). Anal. ($C_{16}H_{11}N_3O_4S$) C, H, N.

3-[(4-Cyanophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (12): white crystals; yield 15% (from (4-cyanophenyl)sulfonyl isocyanate); mp 224−226 °C; ^{1}H NMR (DMSO-d_6) δ 4.53 (s, 2H), 7.1−8.3 (m, 9H). Anal. ($C_{16}H_{11}N_3O_4S$) C, H, N.

3-[(4-Nitrophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (13): white crystals; yield 12% (from (4-nitrophenyl)-sulfonyl isocyanate); mp 203.5−204.5 °C; ^{1}H-NMR (DMSO-d_6) δ 4.52 (s, 2H), 7.1−8.5 (m, 9H). Anal. ($C_{15}H_{11}N_3O_6S$), C, H, N.

3-[[3-(Methoxycarbonyl)phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione (14): white crystals; yield 47% (from [3-(methoxycarbonyl)phenyl]sulfonyl isocyanate); mp 170−171 °C; ^{1}H-NMR (DMSO-d_6) δ 3.92 (s, 3H), 4.51 (s, 2H), 7.1−8.4 (m, 8H), 9.28 (s, 1H). Anal. ($C_{17}H_{14}N_2O_6S$) C, H, N.

3-[[4-(Methoxycarbonyl)phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione (15): white crystals, yield 32% (from [4-(methoxycarbonyl)phenyl]sulfonyl isocyanate); mp 186.5−187.5 °C; ^{1}H-NMR (DMSO-d_6) δ 3.90 (s, 3H), 4.52 (s, 2H), 7.1−7.6 (m, 5H), 8.1−8.3 (m, 4H). Anal. ($C_{17}H_{14}N_2O_6S$) C, H, N.

3-[[3-[(Methoxycarbonyl)methyl]phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione (18): white crystals; yield 27% (from [3-[(methoxycarbonyl)methyl]phenyl]sulfonyl isocyanate); mp 151.5−152 °C; ^{1}H-NMR (DMSO-d_6) δ 3.63 (s, 3H), 3.89 (s, 2H), 4.54 (s, 2H), 7.1−8.0 (m, 9H). Anal. ($C_{18}H_{16}N_2O_6S$) C, H, N.

3-[[4-[(Methoxycarbonyl)methyl]phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione (19): white crystals, yield 33% (from [4-[(methoxycarbonyl)methyl]phenyl]sulfonyl isocyanate); mp 204−206 °C; ^{1}H-NMR (DMSO-d_6) δ 3.62 (s, 3H), 3.86 (s, 2H), 4.53 (s, 2H), 7.1−8.1 (m, 9H). Anal. ($C_{18}H_{16}N_2O_6S$) C, H, N.

3-[(3-Phenoxyphenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (20): white crystals; yield 45% (from (3-phenoxy-

phenyl)sulfonyl isocyanate); mp 150−151 °C; ^{1}H-NMR (DMSO-d_6) δ 4.52 (s, 2H), 7.1−7.8 (m, 14H). Anal. ($C_{21}H_{16}N_2O_5S$) C, H, N.

3-[(3,4-Dichlorophenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (22): white crystals; yield 5.0% (from (3,4-dichlorophenyl)sulfonyl isocyanate); mp 217−218 °C; ^{1}H-NMR (DMSO-d_6) δ 4.42 (s, 2H), 7.3−7.6 (m, 5H), 7.8−8.3 (m, 3H). Anal. ($C_{15}H_{10}Cl_2N_2O_4S$) C, H, N.

3-[(3,4-Dimethylphenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (23): white crystals; yield 50% (from (3,4-dimethylphenyl)sulfonyl isocyanate); mp 227−228 °C; ^{1}H-NMR (DMSO-d_6) δ 2.32 (s, 6H), 4.52 (s, 2H), 7.1−7.8 (m, 8H). Anal. ($C_{17}H_{16}N_2O_4S$) C, H, N.

3-[(3,4-Dimethoxyphenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (24): white crystals; yield 39% (from (3,4-dimethoxyphenyl)sulfonyl isocyanate); mp 209−210 °C; ^{1}H-NMR (DMSO-d_6) δ 3.83 (s, 3H), 3.87 (s, 3H), 4.51 (s, 2H), 7.1−7.7 (m, 8H). Anal. ($C_{17}H_{16}N_2O_6S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(2-chlorophenyl)imidazolidine-2,4-dione (27): white crystals; yield 25% (from (4-chlorophenyl)sulfonyl isocyanate); mp 175−178 °C; ^{1}H-NMR (DMSO-d_6) δ 4.32 (s, 2H), 7.2−8.2 (m, 8H). Anal. ($C_{15}H_{10}Cl_2N_2O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(3-chlorophenyl)imidazolidine-2,4-dione (28): white crystals; yield 2.1% (from (4-chlorophenyl)sulfonyl isocyanate); mp >200 °C; ^{1}H-NMR (DMSO-d_6) δ 4.32 (s, 2H), 7.1−8.2 (m, 8H). Anal. ($C_{15}H_{10}Cl_2N_2O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(2-phenoxyphenyl)-imidazolidine-2,4-dione (33): white crystals; yield 88% (from (4-chlorophenyl)sulfonyl isocyanate); mp 180−181 °C; ^{1}H-NMR (DMSO-d_6) δ 4.32 (s, 2H), 6.8−8.0 (m, 13H). Anal. ($C_{21}H_{15}ClN_2O_5S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(4-phenoxyphenyl)-imidazolidine-2,4-dione (34): white crystals; yield 51% (from (4-chlorophenyl)sulfonyl isocyanate); mp 179.5−181 °C; ^{1}H-NMR (DMSO-d_6) δ 4.50 (s, 2H), 6.9−8.1 (m, 13H). Anal. ($C_{21}H_{15}ClN_2O_5S$) C, H, N.

1-[4-(Acetylamino)phenyl]-3-[(4-chlorophenyl)sulfonyl]-imidazolidine-2,4-dione (35): white crystals; yield 23% (from (4-chlorophenyl)sulfonyl isocyanate); mp 250−251 °C; ^{1}H-NMR (DMSO-d_6) δ 2.06 (s, 3H), 4.54 (s, 2H), 7.5−7.7 (m, 4H), 7.8−8.3 (m, 4H). Anal. ($C_{17}H_{14}ClN_3O_5S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(3,4-dichlorophenyl)-imidazolidine-2,4-dione (36): white crystals; yield 2.1% (from (4-chlorophenyl)sulfonyl isocyanate); mp >200 °C; ^{1}H-NMR (DMSO-d_6) δ 4.31 (s, 2H), 7.3−8.2 (m, 7H). Anal. ($C_{15}H_9Cl_3N_2O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(3,4-dimethylphenyl)-imidazolidine-2,4-dione (37): white crystals; yield 11% (from (4-chlorophenyl)sulfonyl isocyanate); mp 211−213 °C; ^{1}H-NMR (DMSO-d_6) δ 2.23 (s, 3H), 2.25 (s, 3H), 4.23 (s, 2H), 7.11−7.27 (m, 3H), 7.56 (d, 2H), 8.15 (d, 2H). Anal. ($C_{17}H_{15}ClN_2O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(3,4-dimethoxyphenyl)-imidazolidine-2,4-dione (38): white crystals; yield 45% (from (4-chlorophenyl)sulfonyl isocyanate); mp 186−187 °C; ^{1}H-NMR (DMSO-d_6) δ 3.72 (s, 3H), 3.73 (s, 3H), 4.48 (s, 2H), 7.0−8.1 (m, 7H). Anal. ($C_{17}H_{15}ClN_2O_6S$) C, H, N.

3-[(3,4-Dichlorophenyl)sulfonyl]-1-(3,4-dimethylphenyl)-imidazolidine-2,4-dione (40): white crystals; yield 46% (from (3,4-dichlorophenyl)sulfonyl isocyanate); mp 247−249 °C; ^{1}H-NMR (DMSO-d_6) δ 2.23 (s, 3H), 2.26 (s, 3H), 4.31 (s, 2H), 7.1−8.3 (m, 6H). Anal. ($C_{17}H_{14}Cl_2N_2O_4S$) C, H, N.

3-[(3,4-Dimethoxyphenyl)sulfonyl]-1-(3,4-dimethylphenyl)imidazolidine-2,4-dione (41): white crystals; yield 33% (from (3,4-dimethoxyphenyl)sulfonyl isocyanate); mp 195−197 °C; ^{1}H-NMR ($CDCl_3$) δ 2.23 (s, 3H), 2.26 (s, 3H), 4.31 (s, 2H), 7.1−8.3 (m, 6H). Anal. ($C_{19}H_{20}N_2O_6S$) C, H, N.

3-[(3,4-Dimethylphenyl)sulfonyl)-1-(3,4-dimethylphenyl)imidazolidine-2,4-dione (42): white crystals; yield 18% (from (3,4-dimethylphenyl)sulfonyl isocyanate); mp 165−166 °C; ^{1}H-NMR (CD_3OD) δ 2.22 (s, 3H), 2.25 (s, 3H), 4.25 (s, 2H), 7.1−8.0 (m, 6H). Anal. ($C_{19}H_{20}N_2O_4S$) C, H, N.

3[(3,4-Dichlorophenyl)sulfonyl]-1-(3,4-dichlorophenyl)-imidazolidine-2,4-dione (43): white crystals; yield 11% (from (3,4-dichlorophenyl)sulfonyl isocyanate); mp 249−251 °C; ^{1}H-NMR (DMSO-d_6) δ 4.52 (s, 2H), 7.5−8.2 (m, 6H). Anal. ($C_{15}H_8Cl_4N_2O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-cyclohexylimidazolidine-2,4-dione (44): white crystals; yield 16% (from (4-chlorophenyl)sulfonyl isocyanate); mp 175−176 °C; ^{1}H-NMR (DMSO-d_6) δ 1.0−2.0 (m, 10H), 3.72 (m, 1H), 4.20 (s, 2H), 7.89 (d, J = 4.5 Hz, 2H), 8.14 (d, J = 4.5 Hz, 2H). Anal. ($C_{15}H_{17}ClN_2O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(2-pyridyl)imidazolidine-2,4-dione (45): white crystals; yield 4.5% (from (4-chlorophenyl)sulfonyl isocyanate); mp 211−212 °C; ^{1}H-NMR (DMSO-d_6) δ 4.53 (s, 2H), 7.25 (m, 1H), 7.8−8.2 (m, 6H), 8.43 (m, 1H). Anal. ($C_{14}H_{10}ClN_3O_4S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-(2-pyrimidinyl)imidazolidine-2,4-dione (46): white crystals; yield 66% (from (4-chlorophenyl)sulfonyl isocyanate); mp >250 °C dec; ^{1}H-NMR (DMSO-d_6) δ 4.52 (s, 2H), 7.2−8.8 (m, 7H). Anal. ($C_{13}H_9ClN_4O_4S$) C, H, N.

3-[(Phenylmethyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (50): white crystals; yield 47% (from (phenylmethyl)-sulfonyl isocyanate); mp 205−206 °C; ^{1}H-NMR (DMSO-d_6) δ 4.56 (s, 2H), 4.97 (s, 2H), 7.1−7.7 (m, 10H). Anal. ($C_{16}H_{14}N_2O_4S$) C, H, N.

Carboxylic Acid Derivatives and the Corresponding Amide Derivatives (Scheme 2). 3-[(3-Carboxyphenyl)-sulfonyl]-1-phenylimidazolidine-2,4-dione (16). By the general method 2, 3-[[3-[(allyloxy)carbonyl]phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione was prepared from [3-[(allyloxy)carbonyl]phenyl]sulfonyl isocyanate (56% yield).

In a 10% solution of formic acid in THF (10 mL) was dissolved the above imidazolidine derivative (0.4 g, 1.0 mmol). The resulting solution was deaerated under reduced pressure. To the solution were added tetrakis(triphenylphosphine)-palladium (50 mg, 0.04 mmol) and triphenylphosphine (50 mg, 0.2 mmol), followed by stirring at room temperature for 2 h under shielding from the light. After completion of the reaction, the mixture was concentrated to a residue, which was crystallized from AcOEt to obtain the title compound (0.35 g) as white crystals: yield 97%; mp 231−232 °C dec; ^{1}H-NMR (DMSO-d_6) δ 4.50 (s, 2H), 7.1−8.4 (m, 8H), 8.54 (s, 1H), 13.68 (s, 1H). Anal. ($C_{16}H_{12}N_2O_6S$) C, H, N.

3-[(4-Carboxyphenyl)sulfonyl]-1-phenylimidazolidine-2,4-dione (17). By the general method 2, 3-[[4-[(allyloxy)-carbonyl]phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione was prepared from [4-[(allyloxy)carbonyl]phenyl]sulfonyl isocyanate (76% yield). By the same manner of the preparation of **16**, compound **17** (1.10 g) was obtained from the above imidazolidine derivative (2.4 g, 5.62 mmol) as white crystals: yield 54%; mp 230−233 °C dec; ^{1}H-NMR (DMSO-d_6) δ 4.52 (s, 2H), 7.1−8.2 (m, 9H), 13.61 (s, 1H). Anal. ($C_{16}H_{12}N_2O_6S$) C, H, N.

3-[[3-[(4-Methylpiperazinyl)carbonyl]phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione (25). A suspension of **16** (200 mg, 0.56 mmol) in $SOCl_2$ (1.0 mL) was warmed at 80 °C for 2 h to form the corresponding acid chloride. The resulting solution was evaporated under reduced pressure. The residue was dissolved in THF (25 mL), and *N*-methylpiperazine (0.065 mL, 0.59 mmol) was added to the resulting solution under ice-cooling, followed by stirring for 30 min. White crystals were precipitated from the reaction solution and collected by filtration to give the title compound as the hydrochloride (0.14 g): yield 53%; mp 151−153 °C; ^{1}H-NMR (DMSO-d_6) δ 2.76 (s, 3H), 2.8−3.8 (m, 8H), 4.52 (s, 2H), 7.1−8.2 (m, 9H), 11.1 (s, 1H). Anal. ($C_{21}H_{22}ClN_4O_5S\cdot HCl$) C, H, N.

3-[[4-[(4-Methylpiperazinyl)carbonyl]phenyl]sulfonyl]-1-phenylimidazolidine-2,4-dione (26). In a similar manner of obtaining **25**, the title compound (0.23 g) was prepared as the hydrochloride from **17** (0.20 g, 0.56 mmol): white crystals; yield 87%; mp 212−214 °C; ^{1}H-NMR (DMSO-d_6) δ 2.75 (s, 3H), 2.9−3.8 (m, 8H), 4.53 (s, 2H), 7.1−8.2 (m, 9H), 11.14 (s, 1H). Anal. ($C_{21}H_{22}ClN_4O_5S\cdot HCl$) C, H, N.

1-(4-Carboxyphenyl)-3-[(4-chlorophenyl)sulfonyl]imidazolidine-2,4-dione (32). By general method 2, 1-[4-(*tert*-butyloxycarbonyl)phenyl]-3-[(4-chlorophenyl)sulfonyl]imidazolidine-2,4-dione was prepared from (4-chlorophenyl)sulfonyl

isocyanate (38% yield). This compound (0.12 g, 0.27 mmol) was dissolved in TFA (1.5 mL) and stirred for 10 min. The resulting solution was evaporated under reduced pressure. The residue was crystallized from Et_2O to give the title compound (90 mg) as white crystals: yield 85%; mp 271−272 °C; ^{1}H-NMR (DMSO-d_6) δ 4.51 (s, 2H), 7.6−8.1 (m, 8H). Anal. ($C_{16}H_{11}ClN_2O_6S$) C, H, N.

1-(2-Carboxyphenyl)-3-[(4-chlorophenyl)sulfonyl]imidazolidine-2,4-dione (30). By general method 2, 1-[2-(*tert*-butyloxycarbonyl)phenyl]-3-[(4-chlorophenyl)sulfonyl]imidazolidine-2,4-dione was prepared from (4-chlorophenyl)sulfonyl isocyanate (6.8% yield). By the same manner of the preparation of **32**, compound **30** (50 mg) was obtained from the above imidazolidine derivative (60 mg, 0.13 mmol) as white crystals: yield 98%; mp 208−209 °C; ^{1}H-NMR (DMSO-d_6) δ 4.46 (s, 2H), 7.4−8.3 (m, 8H). Anal. ($C_{16}H_{11}ClN_2O_6S$) C, H, N.

1-(3-Carboxyphenyl)-3-[(4-chlorophenyl)sulfonyl]imidazolidine-2,4-dione (31). By general method 2, 1-[3-(*tert*-butyloxycarbonyl)phenyl]-3-[(4-chlorophenyl)sulfonyl]imidazolidine-2,4-dione was prepared from (4-chlorophenyl)sulfonyl isocyanate (9.7% yield). By the same manner of the preparation of **32**, compound **31** (160 mg) was obtained from the above imidazolidine derivative (250 mg, 0.55 mmol) as white crystals: yield 75%; mp 254−255 °C; ^{1}H-NMR (DMSO-d_6) δ 4.54 (s, 2H), 7.5−8.3 (m, 8H). Anal. ($C_{16}H_{11}ClN_2O_6S$) C, H, N.

3-[(4-Chlorophenyl)sulfonyl]-1-[4-[(4-methylpiperazinyl)carbonyl]phenyl]imidazolidine-2,4-dione (39). In a manner similar to that of obtaining **25**, the title compound (0.18 g) was prepared as the hydrochloride from **32** (0.20 g, 0.51 mmol): white crystals; yield 38%; mp 172−175 °C; ^{1}H-NMR (DMSO-d_6) δ 2.76 (s, 3H), 3.4−3.6 (m, 8H), 4.55 (s, 2H), 7.6−8.1 (m, 8H). Anal. ($C_{21}H_{21}ClN_4O_5S\cdot HCl$) C, H, N.

3-(4-Chlorobenzoyl)-1-phenylimidazolidine-2,4-dione (47) (Scheme 3-1). To a solution of 1-phenylimidazolidine-2,4-dione (0.5 g, 2.84 mmol) in pyridine (20 mL) was added *p*-chlorobenzoyl chloride (0.5 g, 2.86 mmol) at 0−5 °C, and then the mixture was stirred at the same temperature for 2 h. After the reaction, 1 N HCl was added to the solution, and then the precipitate formed was filtered and recrystallized from *n*-hexane/AcOEt to obtain the title compound (0.22 g) as white crystals: yield 25%; mp 182−183.5 °C; ^{1}H-NMR (DMSO-d_6) δ 4.63 (s, 2H), 7.1−8.1 (m, 9H). Anal. ($C_{16}H_{11}ClN_2O_3$) C, H, N.

1-[(4-Chlorophenyl)sulfonyl]-3-(4-chlorophenyl)imidazolidine-2,4-dione (49) (Scheme 3-2). To a mixture of *N*-[(*p*-chlorophenyl)sulfonyl]glycine ethyl ester (2.78 g, 10 mmol), triethylenediamine (60 mg, 0.5 mmol), and chlorobenzene (20 mL) was added *p*-chlorophenyl isocyanate (3.08g, 20 mmol), and the mixture was refluxed for 2 h. After the reaction, AcOEt was added, and the mixture was washed with water and then dried over Na_2SO_4, followed by evaporation to a residue, which was chromatographed on silica gel ($CHCl_3$). The fraction of the product was concentrated and crystallized from *n*-hexane/AcOEt to obtain the title compound (2.98 g) as white crystals: yield 77%; mp 193−194 °C; ^{1}H-NMR ($CDCl_3$) δ 4.56 (s, 2H), 7.3−8.3 (m, 8H). Anal. ($C_{15}H_{10}Cl_2N_2O_4S$) C, H, N.

3-[(Benzoyloxy)methyl]-1-phenylimidazolidine-2,4-dione (51) (Scheme 3-3). A mixture of 1-phenylimidazolidine-2,4-dione (556 mg, 3.16 mmol), 37% formalin (5.0 mL, 66.7 mmol), and MeOH (20 mL) was stirred at 70 °C for 2 h. After the reaction, water (20 mL) was added and the precipitate was filtered and washed with water to give 3-(hydroxymethyl)-1-phenylimidazolidine-2,4-dione (585 mg, 90% yield). This compound (103 mg, 0.5 mmol) was suspended in DMF (2 mL) and then cooled with ice. PBr_3 (19 μL, 0.2 mmol) was added to the resulting suspension, and the mixture was stirred for 1 h. More PBr_3 (10 μL) was added, and the mixture was stirred for 15 min. To the resulting solution was added water (20 mL), and the formed precipitate was collected and washed with water to obtain 3-(bromomethyl)-1-phenylimidazolidine-2,4-dione (116 mg, 86% yield).

To a solution of benzoic acid (54 mg, 0.44 mmol) and diisopropylethylamine (70 μL, 0.40 mmol) in DMF (1 mL) was added the bromo derivative (100 mg, 0.37 mmol), the mixture was stirred at room temperature for 20 min, and then cold water was added. The resulting precipitate was filtered and washed with water and Et_2O successively to give the title compound (91 mg) as white crystals: yield 87%; mp 171−173 °C; ^{1}H-NMR (DMSO-d_6) δ 4.41 (s, 2H), 5.89 (s, 2H), 7.1−8.1 (m, 10H). Anal. ($C_{17}H_{14}N_2O_4$) C, H, N.

Protease Inhibition Assay. Human heart chymase was purified according to the method of Urata et al.[6] Human leukocyte cathepsin G and bovine pancreas α-chymotrypsin were purchased from Calbiochem Co., La Jolla, CA, and Sigma Chemical Co., St. Louis, MO, respectively.

The chymase activity regarding the ability to form Ang II was assessed by using Ang I as a substrate according to the procedure of Urata et al.[20] with a minor modification. To measure an inhibitory activity of the synthetic compounds on human heart chymase, 0.1 unit of the purified enzyme was preincubated with test compounds dissolved in DMSO at 37 °C for 10 min in 20 mM Tris HCl buffer (pH 7.5). The chymase reaction was started by the addition of Ang I, and the reaction was stopped with 30% acetic acid (final 15%) after 30 min incubation. To determine the amount of Ang II formed, 10 μL of the aliquot was applied to a C18 reverse-phase HPLC column (Develosil ODS 5, Nomura Chemical Co. Seto, Japan) that was pre-equilibrated with water containing 0.05% TFA. The column was developed using a 10-min linear acetonitrile gradient (0−60%) at 2 mL/min. The IC_{50} value was calculated from the inhibition of Ang II formation at different doses for each compound. The inhibition experiments were repeated at least three times with deviations of 10% from the mean. IC_{50} against human leukocyte cathepsin G was also determined with the same procedure for chymase. In the case of bovine pancreas chymotrypsin each compound was assayed at 37 °C for 10 min in 20 mM Tris-HCl buffer (pH 7.5) containing 20 mM $CaCl_2$. IC_{50} was determined with the same method as chymase.

Modeling of Human Heart Chymase. The homology alignment of amino acid sequences of human heart chymase and rat mast cell protease II (RMCP II)[21] indicated that the two sequences were 62% identical (conservation of 139 out of 226 residues), and neither insertion nor deletion was found. According to the homology alignment, the preliminary three-dimensional structure model of human heart chymase was constructed by substitution of 85 amino acid residues onto the atomic coordinates of RMCP II structure[22] deposited in the Protein Data Bank (accession number is 3RP2). The initial structure was applied to conjugate gradient minimization and 10 ps molecular dynamics simulation in a Monte Carlo water generated in 10.0 Å around the enzyme molecules. The alignment and graphical operations were performed with a QUANTA/CHARMM system,[23] and the structural optimization was calculated using the implemented parameters for protein molecules.[23−25]

Modeling of Putative Complex of Human Heart Chymase and Compound 29 (Figure 1). The structure of compound **29** was generated and optimized by MM2 force field implemented in a QUANTA/CHARMM system. In order to estimate the position of the phenyl ring of the inhibition in P_1 pocket of human heart chymase, the γ-chymotrypsin structure (stored in the PDB entry 1GCT), which contains the tetrapeptide Pro-Gly-Ala-Tyr[26] bound to active site of the protease, was used. The tetrapeptide was transformed onto the human heart chymase active site by the transformation that superimposes 16 α-carbons of chymotrypsin onto human heart chymase with an RMS error of 0.33 Å, because the backbones of chymases and chymotrypsin are nearly identical in the vicinity of the primary specificity pocket (P_1 hole) and the active site catalytic triad. In the first step of the structural optimization, the inhibitor **29** was docked into the active site of human heart chymase using the CHARMM program to match the 1-phenyl and 4-carbonyl of the imidazolidine ring with the phenyl and carbonyl of the tetrapeptide tyrosine, respectively. The initial structure was applied to conjugate gradient minimization and 10 ps molecular dynamics simulation in a Monte Carlo water generated in 10.0 Å around the enzyme molecules using the parameters[23−25] implemented in a QUANTA/CHARMM system.[23]

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