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Synthesis and Structure–Activity Study of Protease Inhibitors. III.[1,2] Amidinophenols and Their Benzoyl Esters

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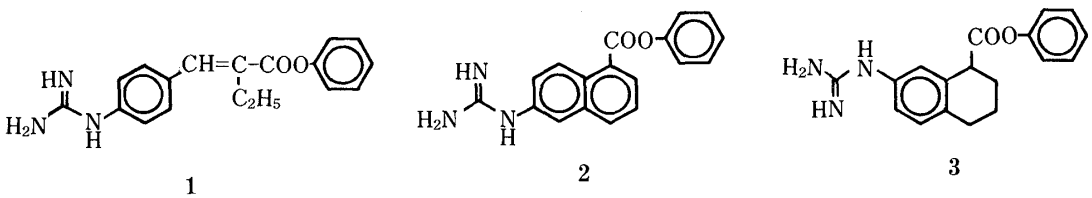
Various amidinophenol derivatives (**27**—**47**) and their benzoates (**4**—**26**) were synthesized and evaluated for inhibitory activities against trypsin, plasmin, kallikrein, thrombin, $C\bar{1}r$ and $C\bar{1}s$ as well as *in vitro* complement-mediated hemolysis. 4-(β-Amidinoethenyl)phenyl 4-guanidinobenzoate (**15**) and 4-amidino-2-benzoylphenyl 4-guanidinobenzoate (**26**) were found to have potent inhibitory activities with IC_{50}s of 9×10^{-8} M (trypsin) and 2×10^{-7} M ($C\bar{1}s$) for the former and 3×10^{-8} M (trypsin) and 2×10^{-7} M ($C\bar{1}s$) for the latter.

Keywords——protease inhibition; trypsin; kallikrein; thrombin; $C\bar{1}r$; $C\bar{1}s$; complement-mediated hemolysis; amidinophenol; amidinophenyl benzoate; structure–activity relationship

The serine-proteases such as trypsin, plasmin, kallikrein, thrombin, $C\bar{1}r$ and $C\bar{1}s$, which are essential in the maintenance of normal homeostasis, may cause various diseases through their anomalous activation. Thus, attempts have been made to develop inhibitors of these proteases;[3a–e] in fact, some, for example, *p*-carbethoxyphenyl ε-guanidinocaproate,[3a] *N*,*N*-dimethylamino *p*-(*p'*-guanidinobenzoyloxy)benzylcarbonyloxy glycolate[3b] and 4'-(2''-carboxy)ethylphenyl *trans*-4-aminomethylcyclohexane carboxylate,[3c] have already been applied in clinical practice with appreciable success.

We have also been interested in synthetic protease inhibitors, and have synthesized various compounds, mainly guanidino- or amidino-containing ester derivatives, particularly aryl ester derivatives, for evaluation of their inhibitory activities against serine-proteases as well as against complement-mediated hemolysis *via* the classical activation pathway, in which the contributions of $C\bar{1}r$ and $C\bar{1}s$ have been well established.[4] We have already reported that some compounds having the guanidino group, **1**, **2** and **3**, show potent inhibition of $C\bar{1}s$, trypsin and thrombin.[1,5]



1 2 3

Chart 1

We have now extended our investigation to amidino-containing ester derivatives. With 4-amidinophenyl benzoate (**5**), already known to be a protease inhibitor,[3e] as a key compound, various amidino-containing ester derivatives, *i.e.* those having a carbon chain introduced

between either the amidino group and benzene ring or the ester linkage and benzene ring (**8**—**15**) and those having substituents on the benzene ring (**16**—**26**), have been synthesized and evaluated.

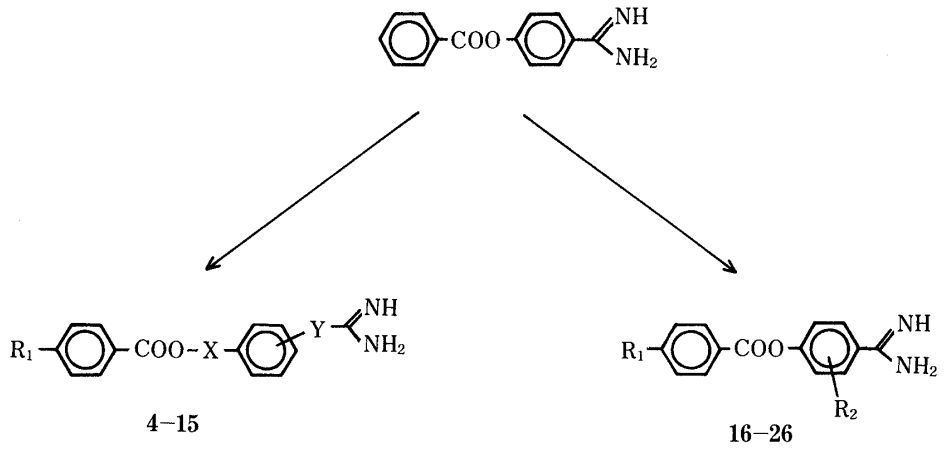


Chart 2

The introduction of an ethenyl group (**13**) between the amidino group and benzene ring and the introduction of a benzoyl group (**25**) on the benzene ring were found to be effective in enhancing protease inhibitory activities, and the 4-guanidinobenzoyl esters (**15**, **26**) of **13** and **25** exhibited potent activities.

This paper describes the synthesis of these compounds. The inhibitory activities on serine-proteases and *in vitro* complement-mediated hemolysis are also reported, and the structure–activity relationship is discussed.

Synthesis

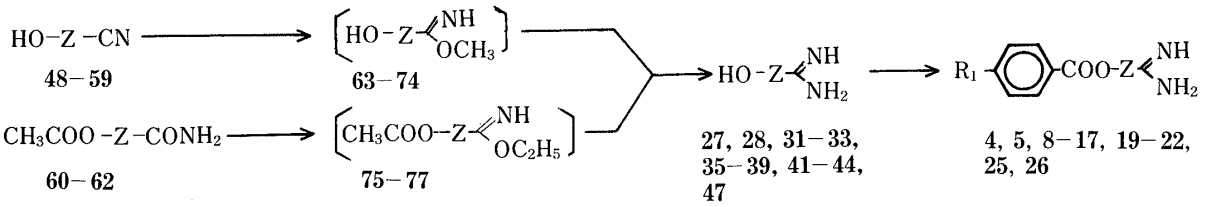
The compounds synthesized were, in principle, prepared from nitriles (**48**—**59**)[6] and amides (**60**—**62**),[7] as shown in Chart 3.

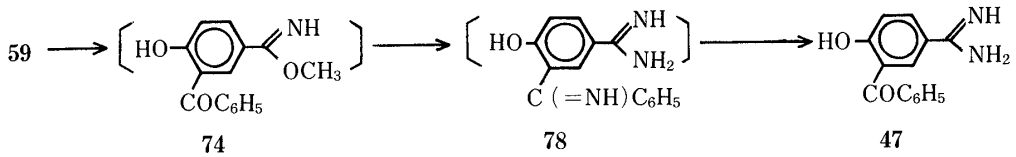
Amidinophenols (**27**,[8a] **28**,[8a] **31**—**33**, **35**—**39**, **41**—**44**, **47**) were prepared either by conversion of nitriles (**48**—**59**) to imidates (**63**—**74**) by treatment with HCl–MeOH, followed by reaction of these imidates with NH_3 in MeOH or by conversion of amides (**60**—**62**) to imidates (**75**—**77**) with $Et_3O^+BF_4^-$, followed by treatment with NH_3 in MeOH, as mentioned above, to obtain amidinophenols with simultaneous deacetylation. The 2-benzoyl derivative (**47**) was prepared by treating the 2-imino derivative (**78**), formed by the reaction of **74** with NH_3, with 3 N HCl under heating.

The 2,6-dibromo derivative (**40**) and 2-nitro derivative (**45**) were synthesized by bromination and nitration of **28**. The 2-amino derivative (**46**) and ethyl derivative (**34**) were synthesized by catalytic reduction, using 10% Pd–C as catalyst, of **45** and **36** as shown in Chart 4. Ester derivatives (**4**,[8a] **5**,[8a] **8**—**10**, **12**—**26**) were synthesized from amidinophenols (**27**, **28**, **31**—**33**, **35**—**47**) either with benzoyl chloride in pyridine or with benzoic acid by dehydrative condensation using dicyclohexyl-carbodiimide (DCC). Benzoylation of **46** with benzoyl chloride gave the dibenzoyl derivative (**24**). 4-(β-Amidinoethyl)phenyl benzoate (**11**) was prepared by catalytic reduction of **13** using 10% Pd–C as a catalyst. Guanidinophenols (**29**, **30**)[8b] were synthesized by reaction of aminophenols (**79**, **80**) with cyanamide, and were converted to esters (**6**, **7**) by reaction with benzoyl chloride.

Assay of Inhibition of Protease- and the Complement-Mediated Hemolysis

The effectiveness of the compounds was determined as the concentration (μM) required to inhibit 50% of the enzyme activity to hydrolyze the substrate (IC_{50}), the substrates used being





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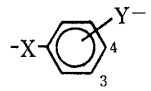
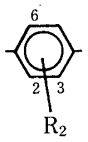
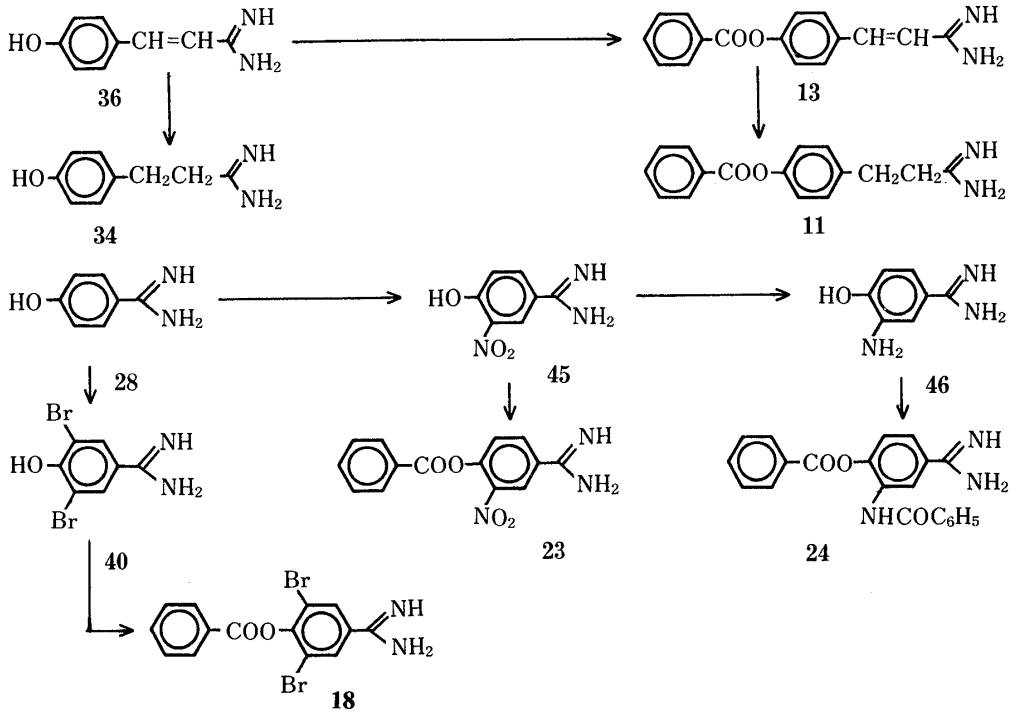




	X	Y	position	R_2
48, 63	—	—	3	**54, 69** 2,6-$(OCH_3)_2$
49, 64	—	—	4	**55, 70** 2-OCH_3
50, 65	$-CH_2-$	—	3	**56, 71** 2-COOH
51, 66	$-CH_2-$	—	4	**57, 72** 2-$COOCH_3$
52, 67	—	$-CH_2-$	4	**58, 73** 2-Cl
53, 68	—	$-CH=CH-$	3	**59, 74** 2-COC_6H_5
60, 75	—	$-CH=CH-$	4	**62, 77** 3-CH_3
61, 76	—	$-CH=C(C_2H_5)-$	4	

Chart 3



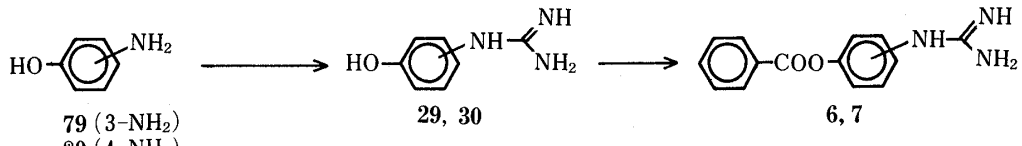


Chart 4

N^{α}-tosylarginine methyl ester (TAME)[9a] for trypsin, plasmin, thrombin and kallikrein, acetyl-arginine methyl ester (AAME)[9b] for $C1\bar{r}$ and acetyltyrosine ethyl ester (ATEE)[9c] for $C1\bar{s}$. The *in vitro* complement-mediated hemolysis system employed was the classical pathway-mediated one, in which $C1\bar{r}$ and $C1\bar{s}$ are involved, using sensitized sheep erythrocytes and guinea-pig sera as the complement source,[9d] and the inhibitory effectiveness was expressed as IC_{50} (μM).

Results and Discussion

Benzoates (**4**—**26**) as well as amidinophenols (**27**—**47**) were synthesized and evaluated for inhibitory effectiveness against trypsin, plasmin, kallikrein, thrombin, $C1\bar{r}$ and $C1\bar{s}$, as well as against complement-mediated hemolysis.

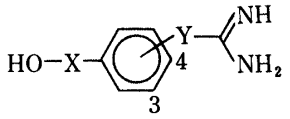
As shown in Table I, the activities of a series of amidinophenols including those derivatives having X- and Y-groups in their molecules were, as a whole, low with IC_{50} values of more than 10^{-3} M. Introduction of substituents (R_2) on the benzene ring of 4-amidinophenol (**28**), on the other hand, was found to be effective in enhancing activities against proteases in the complement system, as demonstrated by the 2,6-dimethoxy (**39**), 2,6-dibromo (**40**), 2-methoxy (**41**) and 2-benzoyl (**47**) derivatives (Table II).

As described above, in a series of amidinophenols, a modification of the mode of binding of either the hydroxyl group or the amidino group to the benzene ring, as represented in Table I, was not effective, while introduction of substituents (R_2) on the benzene ring was effective in enhancing the inhibitory activities, as in **39**, **40**, **41** and **47** (Table II). However, the overall activities were still low, with IC_{50} of 3×10^{-5} M at maximum.

The activities were remarkably enhanced by ester formation with benzoic acid, as shown in Table III.

Considering the position of the amidino-containing group relative to the hydroxyl group in the amidinophenols, 4-substitution was generally effective as compared with 3-substitution,

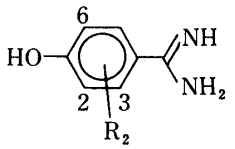
TABLE I. Inhibitory Effects of Amidinophenols on Proteases and Complement-Mediated Hemolysis



Compd. No.	X	Y	Position	Trypsin	Plasmin	Kallikrein	Thrombin	$C1\bar{r}$	$C1\bar{s}$	Hemolysis[b]
27	—	—	3	>1000[a]	>1000	>1000	>1000	>1000	1000	>1000
28	—	—	4	>1000	>1000	>1000	>1000	>1000	>1000	>1000
29	—	–NH–	3	>1000	>1000	>1000	>1000	>1000	>1000	>1000
30	—	–NH–	4	>1000	>1000	>1000	>1000	>1000	>1000	>1000
31	$-CH_2-$	—	3	>1000	>1000	>1000	>1000	>1000	>1000	>1000
32	$-CH_2-$	—	4	>1000	>1000	>1000	>1000	>1000	>1000	>1000
33	—	$-CH_2-$	4	>1000	>1000	>1000	>1000	>1000	>1000	>1000
34	—	$-(CH_2)_2-$	4	600	>1000	>1000	>1000	>1000	>1000	>1000
35	—	–CH=CH–	3	>1000	>1000	>1000	>1000	>1000	>1000	>1000
36	—	–CH=CH–	4	>1000	>1000	>1000	>1000	>1000	800	>1000
37	—	$-CH=\underset{\displaystyle C_2H_5}{\overset{\displaystyle |}{C}}-$	4	>1000	>1000	>1000	>1000	>1000	>1000	>1000

a) Compound concentration for 50% inhibition (μM).
b) Hemolysis: complement-mediated hemolysis.

TABLE II. Inhibitory Effects of Amidinophenols on Proteases and Complement-Mediated Hemolysis



Compd. No.	R_2	Trypsin	Plasmin	Kallikrein	Thrombin	$Cl\bar{r}$	$Cl\bar{s}$	Hemolysis[b]
28	H	>1000[a]	>1000	>1000	>1000	>1000	>1000	>1000
38	3-CH_3	>1000	>1000	>1000	>1000	>1000	>1000	>1000
39	2,6-$(OCH_3)_2$	>1000	>1000	>1000	>1000	>1000	300	>1000
40	2,6-$(Br)_2$	>1000	>1000	>1000	>1000	200	30	>1000
41	2-OCH_3	>1000	>1000	>1000	>1000	500	300	>1000
42	2-COOH	>1000	>1000	>1000	1000	>1000	1000	>1000
43	2-$COOCH_3$	>1000	>1000	>1000	>1000	>1000	>1000	>1000
44	2-Cl	>1000	>1000	>1000	>1000	1000	500	>1000
45	2-NO_2	>1000	>1000	>1000	>1000	700	>1000	>1000
46	2-NH_2	>1000	>1000	>1000	>1000	>1000	>1000	>1000
47	2-COC_6H_5	>1000	>1000	>1000	>1000	800	600	>1000

a) Compound concentration for 50% inhibition (μM).
b) Hemolysis: complement-mediated hemolysis.

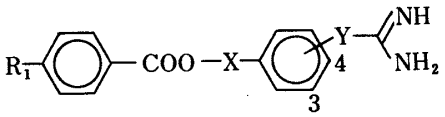
as in (**4** and **5**), (**6** and **7**) and (**12** and **13**). However, this did not apply to the esters (**8**, **9**) having a methylene linkage between the hydroxyl group and the benzene ring, *i.e.* esters with aliphatic alcohols; thus, ester formation with the phenolic hydroxyl group might be essential for enhanced inhibitory activities. As to the effects of Y in 4-substituted phenols, methylene (**10**) and ethylene (**11**) groups as well as the amino group in 4-guanidinophenyl benzoate (**7**) considerably lowered the activities as compared with **5**. An ethenyl group as Y, however, significantly enhanced the activities, as shown by compound **13**, which was the most active among the compounds synthesized in a series of X and Y derivatives. Another finding of interest was that introduction of an ethyl group on the α-carbon of compound **13** gave a compound (**14**) with much lower activities (Table III).

As to the effects of substituents (R_2) on the benzene ring (Table IV), 2-substitution was, in general, more favorable than 3-substitution (**16**) or 2,6-disubstitution (**17**, **18**), and the activities of these three compounds, particularly the 2,6-dimethoxy derivative (**17**), were much lower than those of **5**. Among compounds having a substituent at the 2-position, those having a 2-methoxy (**19**) and 2-carboxy (**20**) group were weaker inhibitors while those having a 2-methoxycarbonyl (**21**), 2-chloro (**22**), 2-nitro (**23**), 2-benzamido (**24**) and 2-benzoyl (**25**) group showed stronger inhibitory activities than **5**.

Compounds **13** and **25**, which were the most potent synthesized so far, were substituted at the 4-position of the benzoic acid moiety with a guanidino group to give **15** and **26** respectively, on the basis of our previous finding[5] that guanidino-containing compounds generally exhibited potent trypsin-inhibiting activities. As expected, **15** and **26** showed enhanced trypsin-inhibiting activities, *i.e.* 10 times and 27 times as potent as **13** and **25**, respectively, without any lessening of the inhibitory activities against other proteases studied.

In conclusion, in a series of benzoic acid esters of amidinophenols, favorable structural features for serine-protease inhibiting agents are considered to be as follows; the hydroxyl group participating in ester linkage should be a phenolic one, and the amidino group should be arranged toward the ester linkage within an optimal range of distance in a conjugated system,

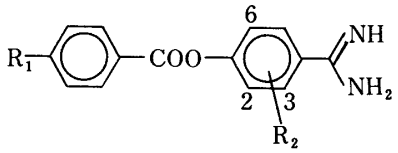
TABLE III. Inhibitory Effects of Amidinophenol Esters on Proteases and Complement-Mediated Hemolysis



Compd. No.	R_1	X	Y	Position	Trypsin	Plasmin	Kallikrein	Thrombin	$Cl\bar{r}$	$Cl\bar{s}$	Hemolysis[b]
4	H	—	—	3	>100[a]	>100	>100	60	>100	>100	>100
5	H	—	—	4	3	2	30	0.3	2	0.5	1
6	H	—	–NH–	3	>100	>100	>100	>100	>100	>100	>100
7	H	—	–NH–	4	20	6	100	>100	5	7	40
8	H	$-CH_2-$	—	3	>100	>100	>100	>100	>100	>100	>100
9	H	$-CH_2-$	—	4	>100	>100	>100	>100	>100	>100	>100
10	H	—	$-CH_2-$	4	>100	>100	>100	>100	90	>100	>100
11	H	—	$-(CH_2)_2-$	4	>100	>100	>100	>100	60	30	>100
12	H	—	–CH=CH–	3	>100	40	>100	100	20	20	>100
13	H	—	–CH=CH–	4	1	2	60	2	0.6	0.3	0.6
14	H	—	$-CH=C(C_2H_5)-$	4	>100	>100	>100	>100	90	30	>100
15	$-NH-C(=NH)-NH_2$	—	–CH=CH–	4	0.09	0.9	10	1	4	0.2	0.3

a) Compound concentration for 50% inhibition (μM). *b*) Hemolysis: complement-mediated hemolysis.

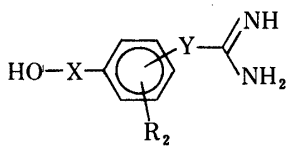
TABLE IV. Inhibitory Effects of Amidinophenol Esters on Proteases and Complement-Mediated Hemolysis



Compd. No.	R_1	R_2	Trypsin	Plasmin	Kallikrein	Thrombin	$Cl\bar{r}$	$Cl\bar{s}$	Hemolysis[b]
5	H	H	3[a]	2	30	0.3	2	0.5	1
16	H	3-CH_3	>100	40	>100	2	6	40	>100
17	H	2,6-$(OCH_3)_2$	>100	>100	>100	>100	>100	>100	>100
18	H	2,6-$(Br)_2$	70	40	>100	6	>100	30	>100
19	H	2-OCH_3	10	20	60	0.2	7	2	30
20	H	2-COOH	3	20	>100	4	30	2	20
21	H	2-$COOCH_3$	0.3	0.6	10	0.1	2	0.3	2
22	H	2-Cl	0.4	0.4	2	0.06	0.2	0.3	0.5
23	H	2-NO_2	0.3	0.4	4	0.1	0.2	0.2	0.1
24	H	2-$NHCOC_6H_5$	0.3	0.4	0.2	0.03	0.9	0.3	0.1
25	H	2-COC_6H_5	0.8	0.4	1	0.03	3	0.3	0.5
26	$-NH-C(=NH)-NH_2$	2-COC_6H_5	0.03	2	0.4	0.2	2	0.2	0.5

a) Compound concentration for 50% inhibition (μM). *b*) Hemolysis: complement-mediated hemolysis.

TABLE V. Physicochemical Properties of Amidinophenols

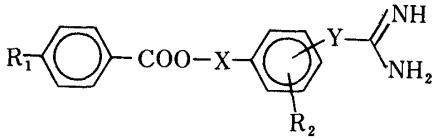


27—47

Compd. No.	Salt	mp (°C)	Recrystn. solvent[g]	Yield (%)	Formula	Analysis (%) Calcd (Found)		
						C	H	N
27	MSA[a]	185—186[c]	A	80	$C_7H_8N_2O \cdot CH_4O_3S$	41.37	5.21	12.06
						(41.38	5.24	12.08)
28	MSA	201—203[d]	A	72	$C_7H_8N_2O \cdot CH_4O_3S$	41.37	5.21	12.06
						(41.43	5.24	12.07)
29	HCl	159—161[e]	A	88	$C_7H_9N_3O \cdot HCl$	44.81	5.37	22.40
						(44.66	5.39	22.32)
30	HCl	194—195[f]	A	39	$C_7H_9N_3O \cdot HCl$	44.81	5.37	22.40
						(44.34	5.37	22.41)
31	MSA	153—154	B	81	$C_8H_{10}N_2O \cdot CH_4O_3S$	43.89	5.73	11.37
						(43.87	5.81	11.32)
32	HCl	219—221	C	83	$C_8H_{10}N_2O \cdot HCl$	51.48	5.94	15.01
						(51.34	6.01	14.93)
33	HCl	242—243	A	91	$C_8H_{10}N_2O \cdot HCl$	51.48	5.94	15.01
						(51.43	5.92	15.05)
34	MSA	100—102	B	79	$C_9H_{12}N_2O \cdot CH_4O_3S$	46.14	6.20	10.76
						(46.04	6.19	10.69)
35	HCl	205—208	B	79	[h]			
36	MSA	182—183.5	A	61	$C_9H_{10}N_2O \cdot CH_4O_3S$	46.50	5.46	10.85
						(46.44	5.48	10.78)
37	MSA	159—160.5	C	15	$C_{11}H_{14}N_2O \cdot CH_4O_3S$	50.34	6.34	9.78
						(50.13	6.35	9.76)
38	TsOH[b]	177.5—179.5	D	64	$C_8H_{10}N_2O \cdot C_7H_8O_3S$	55.89	5.63	8.69
						(55.85	5.92	8.36)
39	MSA	190—191.5	C	93	$C_9H_{12}N_2O_3 \cdot CH_4O_3S$	41.09	5.52	9.58
						(41.05	5.54	9.45)
40	MSA	221.5—223.5	C	56	$C_7H_6Br_2N_2O \cdot C_2H_6O$ CH_4O_3S	27.54	3.70	6.42
						(27.56	3.70	6.42)
41	MSA	152—154	C	75	$C_8H_{10}N_2O_2 \cdot CH_4O_3S$	41.22	5.38	10.68
						(41.33	5.36	10.88)
42	MSA	252 (dec.)	C	56	$C_8H_8N_2O_3 \cdot CH_4O_3S$	39.13	4.38	10.14
						(39.21	4.40	10.01)
43	MSA	181—182	C	16	$C_9H_{10}N_2O_3 \cdot CH_4O_3S$	41.38	4.86	9.65
						(41.37	4.89	9.56)
44	MSA	193—195	D	45	$C_7H_7ClN_2O \cdot CH_4O_3S$	36.03	4.16	10.50
						(35.98	4.15	10.51)
45	MSA	162—163	C	84	$C_7H_7N_3O_3 \cdot CH_4O_3S$	34.66	4.00	15.16
						(34.74	3.99	15.15)
46	2MSA	244—246	C	91	$C_7H_9N_3O \cdot 2CH_4O_3S$	31.48	4.99	12.24
						(31.49	5.00	12.20)
47	MSA	195—196	C	45	$C_{14}H_{12}N_2O_2 \cdot CH_4O_3S$	53.56	4.79	8.33
						(53.56	4.80	8.36)

a) MSA = CH_3SO_3H. *b*) TsOH = CH_3–C_6H_4–SO_3H. *c*) Lit.,[8a] 185—187 °C (HCl).
d) Lit.,[8a] 223—224 °C (HCl). *e*) Lit.,[8b] 155—157 °C. *f*) Lit.,[8b] 198—200 °C.
g) A, MeOH; B, MeOH–Et_2O; C, EtOH; D, EtOH–Et_2O.
h) The structure of **35** was confirmed by the following spectral data: IR ν_{max}^{KBr} cm^{-1}: 3050, 1680. ^{1}H-NMR (DMSO-d_6) δ: 6.47—7.61 (6H, m, arom. H and –HC=CH–), 8.14—10.03 (5H, br,  and –OH). MS *m/z*: 161 (M^+).

TABLE VI. Physicochemical Properties of Amidinophenol Esters



4—26

Compd. No.	Salt	mp (°C)	Recrystn. solvent[d]	Yield (%)	IR ν_{max}^{KBr} cm^{-1} (ester)	Formula	Analysis (%) Calcd (Found) C	H	N
4	MSA[a]	201.5—203[b]	C	52	1722	$C_{14}H_{12}N_2O_2 \cdot CH_4O_3S$	53.56	4.79	8.33
							(53.56	4.79	8.26)
5	MSA	226—228.5[c]	C	61	1744	$C_{14}H_{12}N_2O_2 \cdot CH_4O_3S$	53.56	4.79	8.33
							(53.52	4.82	8.48)
6	MSA	208—209	A	43	1720	$C_{14}H_{13}N_3O_2 \cdot CH_4O_3S$	51.27	4.88	11.96
							(51.17	4.86	11.88)
7	MSA	208.5—210	A	28	1738	$C_{14}H_{13}N_3O_2 \cdot CH_4O_3S \cdot CH_4O$	50.12	5.52	10.96
							(49.78	5.52	10.90)
8	MSA	213—214	A	52	1710	$C_{15}H_{14}N_2O_2 \cdot CH_4O_3S$	54.85	5.18	7.99
							(54.88	5.15	7.86)
9	MSA	180—181	C	19	1720	$C_{15}H_{14}N_2O_2 \cdot CH_4O_3S$	54.85	5.18	7.99
							(54.78	5.17	7.98)
10	HCl	192—194	A	62	1730	[e]			
11	MSA	181—183	B	94	1733	$C_{16}H_{16}N_2O_2 \cdot CH_4O_3S$	56.03	5.53	7.69
							(56.00	5.51	7.54)
12	HCl	82—85	D	77	1730	[f]			
13	MSA	221—222.5	A	81	1723	$C_{16}H_{14}N_2O_2 \cdot CH_4O_3S$	56.34	5.01	7.73
							(56.33	4.98	7.64)
14	MSA	183.5—185	A	80	1722	$C_{18}H_{18}N_2O_2 \cdot CH_4O_3S$	58.45	5.68	7.17
							(58.19	5.69	7.12)
15	2MSA	197—199	B	18	1740	$C_{17}H_{17}N_5O_2 \cdot 2CH_4O_3S$	44.26	4.89	13.58
							(43.89	4.99	13.21)
16	MSA	202—203	C	37	1740	$C_{15}H_{14}N_2O_2 \cdot CH_4O_3S$	54.85	5.18	7.99
							(54.67	5.06	7.92)
17	MSA	264—266	C	62	1746	$C_{16}H_{16}N_2O_4 \cdot CH_4O_3S$	51.51	5.09	7.07
							(51.51	5.06	7.14)
18	MSA	265—267	C	66	1760	$C_{14}H_{10}Br_2N_2O_2 \cdot CH_4O_3S$	36.46	2.86	5.67
							(36.47	2.81	5.67)
19	MSA	205—206	C	78	1740	$C_{15}H_{14}N_2O_3 \cdot CH_4O_3S$	52.45	4.95	7.65
							(52.45	4.96	7.66)
20	MSA	193—194.5	C	34	1725	$C_{15}H_{12}N_2O_4 \cdot CH_4O_3S$	50.52	4.24	7.36
							(50.15	4.46	7.07)
21	MSA	207—208	C	75	1746 [1732]	$C_{16}H_{14}N_2O_4 \cdot CH_4O_3S$	51.77	4.60	7.10
							(51.70	4.59	7.08)
22	MSA	214—216	C	67	1754	$C_{14}H_{11}ClN_2O_2 \cdot CH_4O_3S$	48.59	4.08	7.55
							(48.58	4.01	7.56)
23	MSA	195—197	C	72	1753	$C_{14}H_{11}N_3O_4 \cdot CH_4O_3S$	47.24	3.96	11.02
							(47.28	3.95	11.40)
24	MSA	223.5—224.5	C	66	1744	$C_{21}H_{17}N_3O_3 \cdot CH_4O_3S$	58.01	4.65	9.23
							(57.91	4.55	9.18)
25	MSA	194—195	C	70	1730	$C_{21}H_{16}N_2O_3 \cdot CH_4O_3S$	59.99	4.58	6.36
							(59.87	4.51	6.35)
26	2MSA	218 (dec.)	E	24	1748	$C_{22}H_{19}N_5O_3 \cdot 2CH_4O_3S$	48.56	4.58	11.80
							(48.42	4.59	11.69)

a) MSA = CH_3SO_3H. *b*) Lit.,[8a] 147—148 °C (TsOH). *c*) Lit.,[8a] 233—235 °C ($HClO_4$).
d) A, MeOH; B, MeOH–Et_2O; C, EtOH; D, EtOH–Et_2O; E, H_2O–CH_3COCH_3.
e) The structure of **10** was confirmed by the following spectral data: ^{1}H-NMR (DMSO-d_6) δ: 3.77 (2H, s, $-CH_2-$), 7.14—8.34 (9H, m, arom. H), 8.74—9.63 (4H, br, $-C(=NH_2^+)-NH_2$).
f) MS Calcd for $C_{16}H_{14}N_2O_2$: M, 266.1056. Found *m/z*: M^+, 266.1007.

without steric hindrance. These factors are reflected in the superiority of 1,4-amidinophenol derivatives to 1,3-amidinophenol derivatives and the marked dependence of inhibitory activities upon the structure of Y. As to the ring substituents (R_2), low activities of 3-substituted derivatives and 2,6-di-substituted derivatives might be due to steric hindrance by these substituents of access of the ester linkage to the protease or its active site.

Experimental

Melting points were determined on a Yamato MP-21 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-430 or Jasco IR-A-102 instrument. Nuclear magnetic resonance (NMR) spectra were determined on a Varian T-60 or JEOL JNM FX-60Q spectrometer using tetramethylsilane as an internal standard.

2-Benzoyl-4-cyanophenol (59)——A mixture of 2-benzoyl-4-bromophenol[10a] (37.0 g) and cuprous cyanide (14.6 g) in 37 ml of dimethylformamide (DMF) was refluxed for 4 h with vigorous stirring under an atmosphere of nitrogen. After cooling of the reaction mixture, water was added and the precipitate was collected. A suspension of the precipitate in 10% NaOH was filtered and the filtrate was acidified with conc. HCl. The precipitate was collected and recrystallized from EtOH to give **59** (22.1 g, 74%) as yellow leaflets, mp 120—121 °C. IR ν_{max}^{KBr} cm^{-1}: 3050 (OH), 2220 (CN), 1620 (C=O), 1590. ^{1}H-NMR ($CDCl_3$) δ: 6.84—8.97 (8H, br s, arom. H), 12.48 (1H, br, OH). *Anal.* Calcd for $C_{14}H_9NO_2$: C, 75.33; H, 4.06; N, 6.27. Found: C, 74.94; H, 4.06; N, 6.16.

5-Cyano-2-hydroxybenzoic acid (**56**), methyl 5-cyano-2-hydroxybenzoate (**57**) and 2-chloro-4-cyanophenol (**58**) were prepared from 5-bromo-2-hydroxybenzoic acid, methyl 5-bromo-2-hydroxybenzoate and 4-bromo-2-chlorophenol, respectively, in the same manner. **56**, mp 210—211.5 °C (lit.,[6a] mp 224.0—225.0 °C). **57**, mp 151.5—152.5 °C. IR ν_{max}^{KBr} cm^{-1}: 3070 (OH), 2220 (CN), 1665 (COO). *Anal.* Calcd for $C_9H_7NO_3$: C, 61.02; H, 3.98; N, 7.91. Found: C, 60.63; H, 3.86; N, 7.76. **58**, mp 148.5—149 °C (lit.,[6b] mp 151—152 °C).

3-Cyanobenzylalcohol (50) and 4-Cyanobenzylalcohol (51)——Compounds **50** and **51** were prepared by reduction of 3-cyanobenzaldehyde and 4-cyanobenzaldehyde, respectively, using $NaBH_4$ according to the method of Andrews *et al.*[6c] **50**, oil (lit.,[6d] bp 165 °C/16 mmHg). **51**, mp 132—134 °C (lit.,[6d] mp 133—134 °C).

3-(3-Hydroxyphenyl)propenenitrile (53)——Compound **53** was prepared by reaction of 3-hydroxybenzaldehyde with cyanoacetic acid according to the method of McFarland.[6e] **53**, mp 146—148 °C (lit.,[6f] mp 148 °C).

4-Cyano-2,6-dimethoxyphenol (54) and 4-Cyano-2-methoxyphenol (55)——Compounds **54** and **55** were prepared from 4-hydroxy-3,5-dimethoxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde, respectively, by reaction of the aldehydes with $NH_2OH \cdot HCl$ in formic acid in the presence of sodium formate according to the method of van Es.[6g] **54**, mp 123—124 °C. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2210 (CN), 1600. *Anal.* Calcd for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.30; H, 5.04; N, 7.79. **55**, mp 88—89 °C (lit.,[6h] mp 89—90 °C).

Procedure for Preparation of Amidine Compounds from Nitrile Compounds——Methanesulfonic Acid Salt of 4-Amidino-2-benzoylphenol (**47**): Compound **59** (21.4 g) was added to a cooled saturated solution of dry HCl in anhydrous MeOH (170 ml) and the mixture was stirred overnight at room temperature. Et_2O was added to the mixture and the precipitate was collected to give 2-benzoyl-4-methoxyiminomethylphenol hydrochloride (**74**) (15.6 g). **74** was used for the next reaction without further purification. Gaseous NH_3 was introduced into a cooled suspension of **74** (15.6 g) in anhydrous MeOH (100 ml) with stirring and the mixture was stirred overnight at room temperature, then concentrated. The residue was added to saturated $NaHCO_3$ solution. The precipitate was collected and washed with water and acetone to give carbonic acid salt of 4-amidino-2-benzyliminophenol (**78**) (12.5 g). **78** was used for the next reaction without further purification. A solution of **78** (12.5 g) in 3 N HCl (100 ml) was heated for 1 h on a boiling water bath, then allowed to cool. The precipitate was collected, and washed with an acetone–Et_2O mixture. A suspension of the precipitate in MeOH (20 ml) was treated with methanesulfonic acid (6.0 g). Et_2O was added to the mixture, and the precipitate was collected and recrystallized from EtOH to give **47** (15.1 g, 45%). Recrystallization from EtOH afforded an analytical sample as yellow prisms, mp 195—196 °C. IR ν_{max}^{KBr} cm^{-1}: 3250, 3100, 1680, 1630, 1595. ^{1}H-NMR (DMSO-d_6) δ: 2.46 (3H, s, CH_3SO_3), 7.00—8.21 (8H, m, arom. H), 8.70—9.50 (4H, br, $-C(=NH_2^+)NH_2$), 11.28 (1H, s, OH).

Compounds **27**, **28**, **31**—**33**, **35**, **39** and **41**—**44** were prepared from **48**—**58**, respectively, in the same manner except for the step (**78**→**47**) of hydrolysis of the imino group using 3 N HCl.

Procedure for Amide Compounds——Amide compounds (**60**—**62**) were prepared by acetylation of 4-cumaric acid, α-ethyl-*p*-cumaric acid[10b] and 4-hydroxy-2-methylbenzoic acid,[10c] followed by acid chloride formation with PCl_5, and reaction of the acid chloride with NH_3. **60**, mp 188—190 °C (lit.,[7] mp 189—191 °C). **61**, mp 142—144 °C. IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 3200 (NH), 1760 ($OCOCH_3$), 1645 ($CONH_2$). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (3H, t, J=7.3 Hz, CH_3CH_2–), 2.31 (3H, s, $OCOCH_3$), 2.53 (2H, q, J=7.3 Hz, CH_3CH_2–), 5.97—6.34 (2H, br, $CONH_2$), 6.92—7.46 (5H, m, arom. H and –CH=C<). *Anal.* Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.97; H, 6.47; N, 5.94. **62**, mp 172—173 °C. IR ν_{max}^{KBr} cm^{-1}: 3360 (NH), 3170 (NH), 1750 ($OCOCH_3$), 1650 ($CONH_2$), 1620. ^{1}H-

NMR (DMSO-d_6) δ: 2.25 (3H, s, $OCOCH_3$), 2.38 (3H, s, CH_3), 6.84—7.93 (5H, m, arom. H and $CONH_2$). *Anal.* Calcd for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.17; H, 5.72; N, 7.17.

Procedure for Preparation of Amidine Compounds from Amide Compounds——Methanesulfonic Acid Salt of 4-(β-Amidinoethenyl)phenol (**36**): A solution of $Et_3O^+BF_4^-$ (27.8 g) in anhydrous CH_2Cl_2 (100 ml) was added dropwise to a suspension of **60** (25 g) in CH_2Cl_2 (200 ml) with stirring at room temperature. The mixture was stirred overnight at room temperature and concentrated *in vacuo*. The residue was dissolved in anhydrous MeOH (200 ml) and gaseous NH_3 was introduced into the solution for 3 h at room temperature. The mixture was concentrated, water was added to the residue, the precipitate was collected by filtration, and a suspension of the precipitate in MeOH (30 ml) was treated with methanesulfonic acid (12.2 g). Et_2O was added to the mixture and the precipitate was collected to give **36** (16.5 g, 61%). Recrystallization from MeOH afforded an analytical sample as pale yellow leaflets, mp 182—183.5 °C. IR ν_{max}^{KBr} cm^{-1}: 3350, 3150, 1675. ^{1}H-NMR (DMSO-d_6) δ: 2.53 (3H, s, CH_3SO_3), 6.58 (1H, d, J=16.4 Hz, –CH=C<u>H</u>–), 6.89 (2H, d, J=8.3 Hz, arom. H), 7.52 (2H, d, J=8.3 Hz, arom. H), 7.80 (1H, d, J=16.4 Hz, –C<u>H</u>=CH–), 8.45—9.13 (4H, br, —C(=NH_2^+)–NH_2), 10.23 (1H, s, OH).

Amidine compounds **37** and **38** were prepared in the same manner from **61** and **62**.

Methanesulfonic Acid Salt of 4-Amidino-2-nitrophenol (45)——A solution of **28** (8 g) in conc. H_2SO_4 (20 ml) was treated dropwise with conc. HNO_3 (d=1.38) (3 ml) at 0—10 °C in an ice-salt bath with stirring for 1 h. The mixture was diluted by pouring it into ice water, then the solution was added in small portions to a saturated $NaHCO_3$ solution. The precipitate was collected by filtration and washed with water, then with acetone, and a suspension of the precipitate in MeOH (30 ml) was treated with methanesulfonic acid (6.1 g). Et_2O was added to the mixture and the precipitate was collected to give **45** (9.8 g, 84%). Recrystallization from EtOH afforded an analytical sample as pale yellow needles, mp 162—163 °C. IR ν_{max}^{KBr} cm^{-1}: 3100, 1685, 1625, 1515 (NO_2), 1350, 1327. ^{1}H-NMR (DMSO-d_6) δ: 2.49 (3H, s, CH_3SO_3), 7.34 (1H, d, J=8.8 Hz, H-6), 8.00 (1H, dd, J=2.3, 8.8 Hz, H-5), 8.43 (1H, d, J=2.3 Hz, H-3), 8.65—9.98 (4H, br, —C(=NH_2^+)–NH_2), 10.96—13.12 (1H, br, OH).

Methanesulfonic Acid Salt of 4-(2-Amidinoethyl)phenol (34)——A mixture of **36** (1.3 g) and 10% Pd–C (0.4 g) in 10 ml of MeOH was hydrogenated with stirring at room temperature under atmospheric pressure. After removal of the catalyst by filtration, the filtrate was poured into Et_2O (50 ml) and the precipitate was collected to give **34** (1.0 g, 79%). Recrystallization from MeOH–Et_2O afforded an analytical sample as colorless leaflets, mp 100—102 °C. IR ν_{max}^{KBr} cm^{-1}: 3300, 3100, 1690. ^{1}H-NMR (DMSO-d_6) δ: 2.30—2.92 (4H, br, –C<u>H</u>$_2$C<u>H</u>$_2$–), 2.47 (3H, s, CH_3SO_3), 6.71 (2H, d, J=8.3 Hz, arom. H), 7.07 (2H, d, J=8.3 Hz, arom. H), 8.49—9.16 (4H, br, —C(=NH_2^+)–NH_2), 9.28 (1H, s, OH).

Compound **11** was prepared from **13** in the same manner. Compound **46** was prepared from **45** in the same manner in the presence of methanesulfonic acid (1 eq).

Methanesulfonic Acid Salt of 4-Amidino-2,6-dibromophenol (40)——Bromine (40 g) was added dropwise to a stirred solution of **28** (17.3 g) in water (200 ml) at room temperature. The mixture was stirred for 1 h at room temperature, then aq. $Na_2S_2O_3$ solution was added to the mixture, followed by saturated $NaHCO_3$ solution. The precipitate was collected by filtration, and washed with water then with acetone. A suspension of the precipitate in EtOH (20 ml) was treated with methanesulfonic acid (7.0 g). Et_2O was added to the mixture, and the precipitate was collected by filtration to give **40** (22.0 g, 56%). Recrystallization from EtOH afforded an analytical sample as colorless prisms, mp 221.5—223.5 °C. IR ν_{max}^{KBr} cm^{-1}: 3300, 3120, 1690, 1460. ^{1}H-NMR (DMSO-d_6) δ: 2.46 (3H, s, CH_3SO_3), 8.08 (2H, s, arom. H), 8.55—9.91 (4H, br, —C(=NH_2^+)–NH_2).

3-Guanidinophenol (29) and 4-Guanidinophenol (30)——Compounds **29** and **30** were prepared by reaction of 3-aminophenol hydrochloride and 4-aminophenol hydrochloride with cyanamide, respectively, according to the method of Hughes *et al.*[8b]

Methanesulfonic Acid Salt of 4-Amidino-2-benzoylphenyl Benzoate (25)——Benzoyl chloride (1.5 g) was added dropwise to a cooled mixture of **47** (3.4 g) in dry pyridine (35 ml) and the mixture was stirred at room temperature for 3 h. After removal of the precipitate by filtration, Et_2O was added to the filtrate to give an oily residue. A solution of the oily residue in water was added to a saturated $NaHCO_3$ solution. The precipitate was collected by filtration, and washed with water, then with acetone. A suspension of the precipitate in MeOH (8 ml) was treated with methanesulfonic acid (0.9 g). Et_2O was added to the mixture and the precipitate was collected by filtration to give **25** (3.1 g, 70%). Recrystallization from EtOH afforded an analytical sample as colorless needles, mp 194—195 °C. IR ν_{max}^{KBr} cm^{-1}: 3280, 3110, 1730 (COO), 1697, 1655 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 2.46 (3H, s, CH_3SO_3), 7.24—8.40 (13H, m, arom. H), 9.01—9.83 (4H, br, —C(=NH_2^+)–NH_2).

Compounds **4**—**10** and **16**—**25** were prepared in the same manner (**24**: 2 mol eq of benzoyl chloride was used).

Dimethanesulfonic Acid Salt of 4-(β-Amidinoethenyl)phenyl 4-Guanidinobenzoate (15)——A mixture of 4-guanidinobenzoic acid hydrochloride (10.6 g), methanesulfonic acid salt of 4-(β-amidinoethenyl)phenol (12.7 g) and dicyclohexylcarbodiimide (12.2 g) in dry pyridine (130 ml) was stirred overnight at room temperature. The precipitate

was collected by filtration, washed with dry pyridine and suspended in DMF (200 ml). After filtration of the suspension, Et_2O was added to the filtrate. The precipitate was collected by filtration and added to a saturated $NaHCO_3$ solution. The precipitate was collected by filtration, and washed with water then with acetone. A suspension of the precipitate in MeOH (20 ml) was treated with methanesulfonic acid (10 g). Et_2O was added to the mixture and the precipitate was collected to give **15** (4.5 g, 18%). Recrystallization from MeOH–Et_2O afforded an analytical sample as colorless leaflets, mp 197—199 °C. IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 3150 (NH), 1740 (COO). ^{1}H-NMR (DMSO-d_6) δ: 2.50 (6H, s, $CH_3SO_3 \times 2$), 6.81 (1H, d, J=16.7 Hz, –CH=CH–), 7.13—8.37 (13H, m), 8.57—9.31 (4H, br, $-C(NH_2^+)NH_2$), 10.28 (1H, br s, NH).

Compounds **12**, **13**, **14** and **26** were prepared in the same manner. **26**, mp 218 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3150 (NH), 1748 (COO), 1667 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 2.47 (6H, s, $CH_3SO_3 \times 2$), 6.98—8.47 (16H, m), 8.83—10.07 (4H, br, $-C(NH_2^+)NH_2$), 10.26 (1H, br s, NH).

Enzyme Inhibition——Bovine trypsin was purchased from Sigma Chemical Co., St. Louis, USA, and dissolved in 0.1 M borate buffer containing 0.01 M $CaCl_2$, pH 8.5. Human plasmin was purchased from Green Cross Co., Osaka, Japan, and porcine kallikrein from Bayer, and they were each dissolved in 0.1 M borate buffer, pH 8.5. Bovine thrombin was purchased from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan, and dissolved in 0.02 M phosphate buffer, pH 7.4. The rates of hydrolysis of TAME by trypsin, plasmin, kallikrein, and thrombin were determined as described by Muramatu *et al.*,[9a] that of AAME by Cl^- as described by Tamura *et al.*,[9b] and that of ATEE by Cl^- as described by Okamura *et al.*,[9c] at a substrate concentration of 10 mM.

Inhibition of Complement-Mediated Hemolysis——Sheep erythrocytes were purchased from Tokyo Faruma Co., Tokyo, Japan, and hemolysin from Denka Seiken Co., Ltd., Tokyo, Japan. Complement-mediated hemolytic activities were determined as described by Baker *et al.*[9d]

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