[Chem. Pharm. Bull. 33(4)1458—1471(1985)]

Synthesis and Structure–Activity Study of Protease Inhibitors. IV.[1] Amidinonaphthols and Related Acyl Derivatives[2]

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(Received June 9, 1984)

Various amidinonaphthols and their acyl derivatives were synthesized and evaluated for inhibitory activities against trypsin, plasmin, kallikrein, thrombin, $C\bar{1}r$ and $C\bar{1}s$, as well as against *in vitro* complement-mediated hemolysis. 6-Amidino-2-naphthyl 4-guanidinobenzoate (**74**, FUT-175) was found to have potent inhibitory activities, particularly against trypsin (IC_{50}: 0.02 μM), $C\bar{1}r$ (IC_{50}: 0.1 μM) and $C\bar{1}s$ (IC_{50}: 0.02 μM), and to be highly effective in inhibiting the complement-mediated hemolysis (IC_{50}: 0.03 μM). FUT-175, furthermore, showed considerable effectiveness in the systemic Forssman shock reaction, in which involvement of the complement system as the pathogenetic factor is well established.

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

It is well known that anomalous activation of serine proteases such as trypsin, plasmin, kallikrein, thrombin, $C\bar{1}r$, $C\bar{1}s$ and others, which are essential in maintaining normal homeostasis, can cause various diseases, and attempts to develop inhibitors of these proteases have been made by many investigators.[3-10] Since these proteases were shown to recognize the arginyl residue in hydrolyzing the amido bond in protein molecules, compounds having guanidino groups have been widely studied.[3-6] Compounds having amidino groups have also been investigated as analogues of guanidino-containing compounds.[7,8] In fact, Walsmann *et al.*[7] reported that *p*-amidinophenylpyruvic acid (APPA) was an effective thrombin inhibitor among various amidino derivatives studied, and Tanizawa *et al.*[9] studied *p*-nitrophenyl *p*-amidinobenzoate as a trypsin substrate.

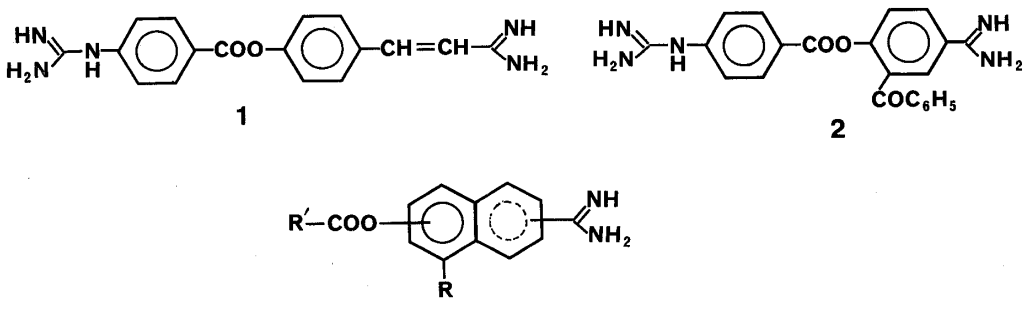


Chart 1

We have also been interested in synthetic protease inhibitors and have reported the

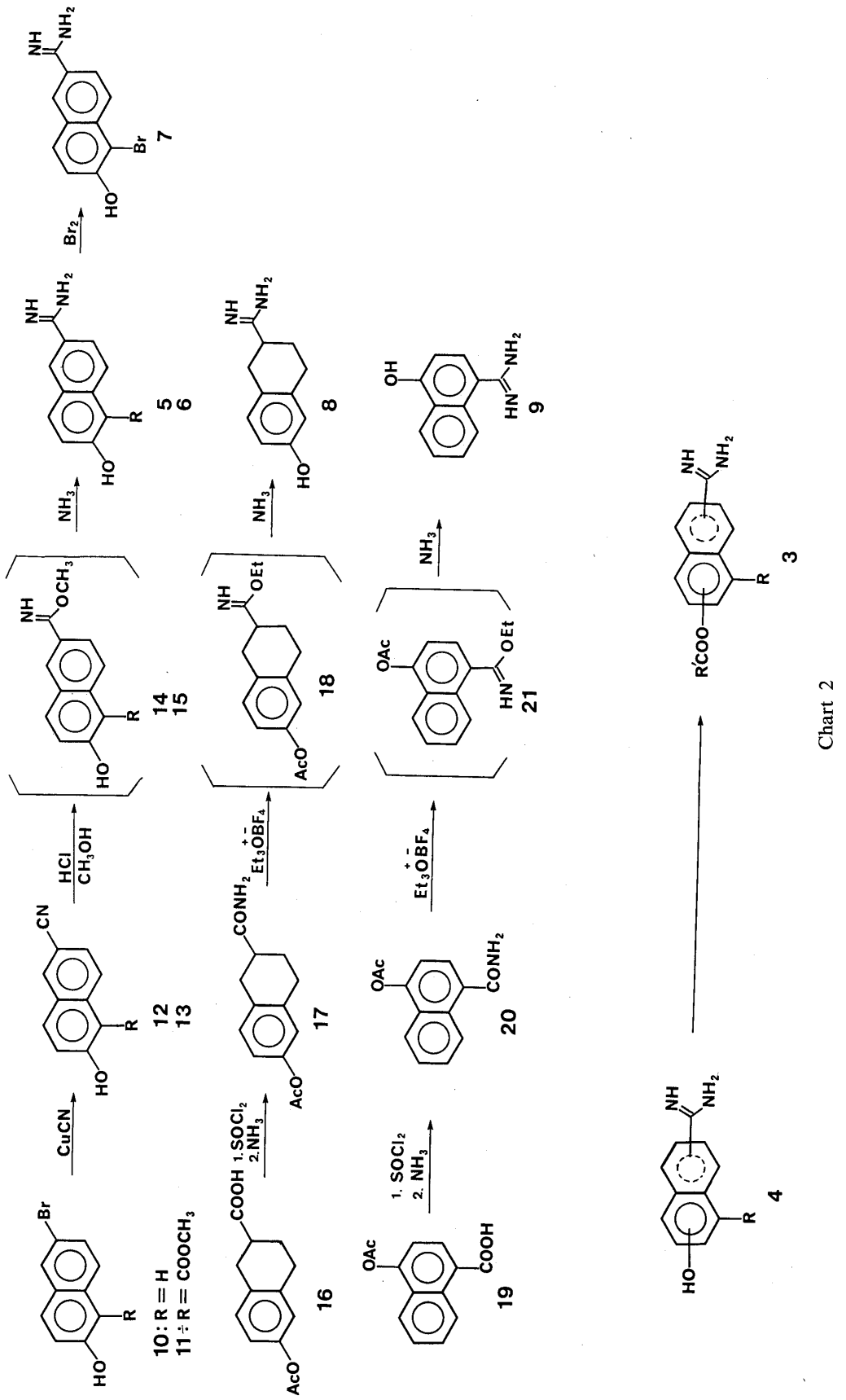


Chart 2

results of our studies on the synthesis of various guanidino- and amidino-containing esters and their inhibitory effects on proteases and complement-mediated hemolysis.[1,11,12]

Among the compounds studied, aryl esters, as a whole, have been found to be active, and in particular, aryl esters of amidino-containing phenols such as compounds **1** and **2** were highly effective as inhibitors of $C1\bar{r}$ and $C1\bar{s}$ and of the complement-mediated hemolysis.[1] Markwardt *et al.*[8] reported that *p*-amidinophenyl benzoate inhibited kallikrein, and Tanizawa *et al.*[10] have also reported that amidinophenol esters bind to the active site of trypsin as "inverse substrates."

These findings led us to synthesize further aryl esters of amidino-substituted bicyclic alcohols, mainly amidino-substituted naphthols, and to study their activities. Since 6-amidino-2-naphthyl benzoate (**22**) was one of the most active among the compounds synthesized, further investigation was carried out to study the effects of chemical modification of **22** on the protease inhibitory effectiveness. 6-Amidino-2-naphthyl 4-guanidinobenzoate (**74**, FUT-175) was found to be highly active in inhibiting serine proteases and the complement-mediated hemolysis, as well as in protecting guinea pigs from lethal Forssman shock, which is a well-known animal model reaction in which anomalous activation of early components of the complement system is involved as the pathogenetic factor.

This paper describes the synthesis of these compounds, their inhibitory activities against serine proteases and complement-mediated hemolysis and their effectiveness in the systemic Forssman shock reaction. The structure–activity relationship is discussed.

Chemistry

The amidinonaphthols **5** and **6** were synthesized by preparing the nitriles **12** and **13** from the bromo compounds **10** and **11** with cuprous cyanide, followed by conversion to the imidates **14** and **15** with MeOH–HCl and further conversion to **5** and **6** with NH_3 in MeOH. Compound **7** was obtained by bromination of **5** in AcOH. Other amidinonaphthols **8** and **9** were synthesized by subjecting the amides **17** and **20** to reaction with $Et_3O^+BF_4^-$ to obtain the imidates **18** and **21** and subjecting these to reaction with NH_3 in MeOH to obtain **8** and **9**.

The acyl compounds **3** were prepared by esterification of the amidinonaphthols **4** either with acyl chloride or with acid in the presence of dicyclohexylcarbodiimide (DCC) in pyridine.

Assay of Inhibitory Activity towards Proteases 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 N^{α}-tosylarginine methyl ester (TAME)[13] for trypsin, plasmin, thrombin and kallikrein, acetyl arginine methyl ester (AAME)[14] for $C1\bar{r}$ and acetyl tyrosine ethyl ester (ATEE)[15] 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,[16] and the effectiveness of test compounds was expressed as IC_{50} (μM).

Systemic Forssman Reaction

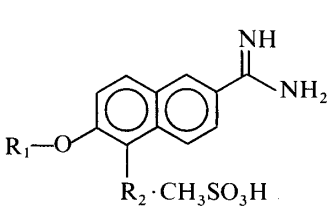
According to the procedure described by Glovsky *et al.*,[17] systemic Forssman reaction was provoked in guinea pigs by intravenous administration of hemolysin, and the effectiveness of test compounds was evaluated in terms of ability to protect animals from death. Compounds were given intravenously 5 min prior to hemolysin administration.

Results and Discussion

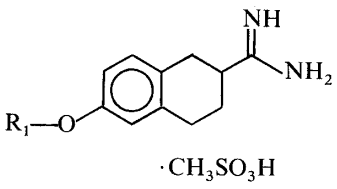
Amidinonaphthols and Their Benzoates

The amidinonaphthols synthesized, as a whole, had only low effectiveness, except for a

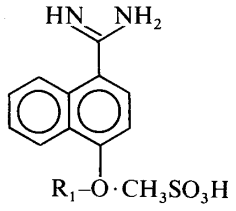
TABLE I. Amidinonaphthol Derivatives



type I



type II



type III

Compd. No.	Type	R_1	R_2	Trypsin	Plasmin	Inhibitory activities IC_{50} (μM)[a]			Cl$\bar{s}$	Hemolysis[b]
						Kallikrein	Thrombin	Cl$\bar{r}$		
5	I	H	H	>1000	600	>1000	>1000	500	80	>1000
6	I	H	$COOCH_3$	>1000	>1000	>1000	>1000	>1000	500	>1000
7	I	H	Br	>1000	>1000	>1000	>1000	>1000	100	>1000
8	II	H		>1000	>1000	>1000	>1000	>1000	>1000	>1000
9	III	H		1000	>1000	>1000	>1000	>1000	>1000	>1000
22	I	C_6H_5CO	H	4	2	>10	4	1	0.3	0.2
23	I	C_6H_5CO	$COOCH_3$	1	0.8	>10	1	>10	0.8	0.4
24	I	C_6H_5CO	Br	0.4	0.7	>10	0.6	8	0.5	2
25	II	C_6H_5CO		>10	>10	>10	>10	>10	>10	>10
26	III	C_6H_5CO		10	10	>10	0.6	>10	10	>10

a) IC_{50} means the concentration (μM) of the compounds required to inhibit 50% of each enzyme activity. *b*) Complement-mediated hemolysis.

few compounds, *i.e.* **5** against plasmin and Cl$\bar{r}$ with IC_{50}s of the order of 10^{-4}—10^{-5} M and **6** and **7** against Cl$\bar{s}$ with IC_{50}s of the order of 10^{-4} M.

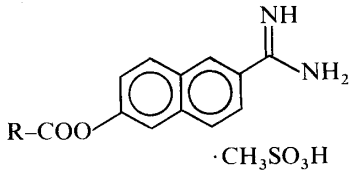
Esterification of amidinonaphthols with benzoic acid, however, was shown to increase the effectiveness markedly, and, in fact, the activities of **22** against Cl$\bar{r}$, Cl$\bar{s}$ and hemolysis and that of **23** against hemolysis were of the order of 10^{-6}—10^{-7} and 10^{-7} M, respectively. The lower effectiveness of **25** as compared with **22** shows that an aromatic amidine group is required for potent protease-inhibitory activity, and the lower activity of **26** (except in one case) against thrombin may reflect a contribution of steric hindrance around the amidino group.

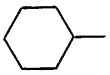
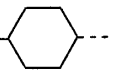
Aliphatic Esters of 6-Amidino-2-naphthols

Increased activities were attained by conversion of **5** into esters (**27**—**34**) with fatty acids. The overall activity (except in one case) against kallikrein depended on the length of the carbon chain of the fatty acid; a length of 3—9 carbons was optimal, and chains of more than 11 carbons resulted in greatly reduced activities. Introduction of either an amino group (**36**) or a guanidino group (**37**) into the fatty acid moiety caused only a slight enhancement of the kallikrein inhibitory activity. The presence of a double bond involving the α-carbon (**38**, **39**) produced a considerable enhancement of the activities, those against trypsin and plasmin, particularly, being more potent than those of **22**. Cyclization (**40**—**42**) was not effective in increasing the activities over those of **22**, except in the case of **40**. Introduction of an aminomethyl group (**44**) into **42** resulted in specifically greater effectiveness of complement-mediated hemolysis inhibition.

Aliphatic esters of 6-amidino-2-naphthol, thus, were not superior to **22**, especially in terms of the complement inhibitory activities.

TABLE II. Aliphatic Esters of 6-Amidino-2-naphthol



Compd. No.	R	Inhibitory activities IC_{50} (μM)[a]						Hemolysis[b]
		Trypsin	Plasmin	Kallikrein	Thrombin	$Cl\bar{r}$	$Cl\bar{s}$	
27	CH_3–	80	>100	>100	>100	10	20	30
28	*n*-C_3H_7–	20	30	>100	20	9	20	20
29	iso-C_4H_9–	30	30	>100	80	2	2	20
30	*n*-C_5H_{11}–	20	20	>100	30	5	4	30
31	*n*-C_9H_{19}–	6	10	>100	>100	6	3	100
32	*n*-$C_{11}H_{23}$–	50	50	>100	>100	>100	>100	>100
33	*n*-$C_{14}H_{29}$–	>100	>100	>100	>100	>100	>100	>100
34	*n*-$C_{17}H_{35}$–	>100	>100	>100	>100	>100	>100	>100
35	Cbz–NH–$(CH_2)_5$–	10	3	>10	>10	4	4	>10
36	H_2N–$(CH_2)_5$– ·CH_3SO_3H	>10	>10	>10	>10	5	>10	>10
37	$HN\!=\!\langle$ H_2N –NH–$(CH_2)_5$– ·CH_3SO_3H	10	>10	10	>10	>10	9	>10
38	C_2H_5–CH=CH–	0.4	0.2	1	6	2	0.4	1
39	CH_3–(CH=CH)$_2$–	0.6	0.3	2	4	10	0.1	2
40		2	4	>10	3	0.2	0.3	3
41		>10	>10	>10	>10	>10	>10	3
42		>10	10	>10	>10	2	3	>10
43	Cbz–NH–CH_2– 	6	0.8	6	>10	0.4	0.4	3
44	H_2N–CH_2–  ·CH_3SO_3H	>10	>10	>10	>10	5	4	0.4
45	$HN\!=\!\langle$ H_2N –NH–CH_2–  ·CH_3SO_3H	>10	>10	8	>10	>10	>10	>10

a, b) See footnotes *a, b*, Table I.

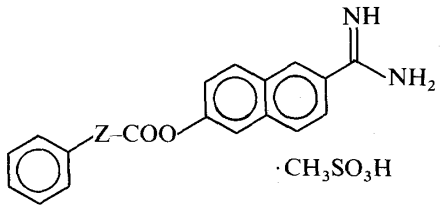
ω-Phenyl Fatty Acid Esters of 6-Amidino-2-naphthol

Introduction of a methylene linkage (**46**) and ethylene linkage (**47**) between the benzene ring and the ester linkage in **22** reduced the activities. The presence of a double bond involving the α-carbon (**48**) was accompanied by an increase in effectiveness, as observed in aliphatic esters, as compared with the corresponding saturated congener, **47**, but the activities were not superior to those of **22**. Introduction of –OAc (**49**) or $=NOCH_3$ (**50**) on the α-carbon also decreased the activities, presumably because of steric hindrance. In this series, compound **48**, though it was less effective than **22**, was the strongest inhibitor, the presence of the conjugated double bond in acyl moiety possibly contributing to this.

Substituted Benzoyl Esters of 6-Amidino-2-naphthol

Since compound **22** was shown to have the greatest effectiveness in terms of protease inhibition among various series of compounds studied so far, we further extended our

TABLE III. ω-Phenyl Fatty Acid Esters of 6-Amidino-2-naphthol



Compd. No.	Z	Inhibitory activities IC_{50} (μM)[a]						Hemolysis[b]
		Trypsin	Plasmin	Kallikrein	Thrombin	$Cl\bar{r}$	$Cl\bar{s}$	
22	—	4	2	>10	4	1	0.3	0.2
46	$-CH_2-$	10	>10	>10	4	>10	>10	>10
47	$-CH_2CH_2-$	10	>10	>10	10	>10	>10	>10
48	$-CH=CH-$	5	3	>10	10	>10	3	3
49	$-CH=C(OAc)-$	10	10	>10	10	2	0.4	>10
50	$-CH_2-C(=NOCH_3)-$	10	>10	10	>10	>10	>10	3
51	$-O-CH_2-$	>10	>10	>10	10	10	10	>10

a, b) See footnotes *a, b*, Table I.

investigation to substituted benzoyl esters as modifications of **22**. First, the effect of position of substitution (**52**—**54**) was studied by introducing a methyl group on the benzene ring. The activity was enhanced in the order of *o*-, *m*- and *p*-substitution, and the *p*-methyl derivative (**54**) was more active against trypsin, plasmin, $Cl\bar{r}$ and $Cl\bar{s}$ than **22**. Thus, other substituents were mainly introduced at the *p*-position.

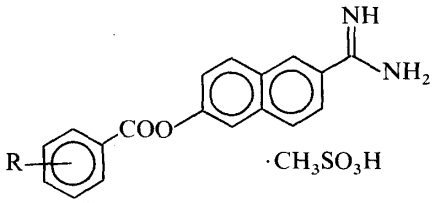
Alkoxy derivatives (**57**—**61**) showed a protease inhibition spectrum similar to that of alkyl derivatives in that the inhibitions of trypsin, $Cl\bar{r}$ and $Cl\bar{s}$ were, as a whole, more potent but that against the complement-mediated hemolysis was 10—100 times less potent than those of **22**.

Introduction of basic substituents (**69**, **70**, **73**, **74**) was also found to improve the effectiveness against trypsin, $Cl\bar{r}$, and $Cl\bar{s}$. As in the case of the cyclic aliphatic ester **44**, compound **73**, having an aminomethyl group, showed enhanced effectiveness in complement-mediated hemolysis inhibition. A guanidino group at the *p*-position on the benzene ring of **22** (compound **74**) resulted in the most potent inhibitory activities against all the proteases studied, and gave inhibitory activity against the *in vitro* complement-mediated hemolysis with IC_{50} of the order of 10^{-8} M. Inhibition of these proteases by **74** was potent, selective and reversible with high affinity, as shown by K_i values of the order of 10^{-7}—10^{-8} M.[18] Electron-withdrawing groups on the benzene ring (**77**—**80**) caused overall reduction of the inhibitory activities.

Since compound **74** was shown as described above, to be the best compound in terms of serine-protease inhibition in present investigation, a comparative study of the effectiveness in the systemic Forssman shock reaction was carried out on **74**, **22** (the starting compound of the present investigation), **1** and **2**; the latter two were shown in the previous paper[1] to cause potent inhibition of complement-mediated hemolysis. Table V lists the activities of these compounds in protease and hemolysis inhibition.

Figure 1 shows the survival times of guinea pigs observed after intravenous dosing of each compound 5 min prior to intravenous administration of hemolysin; **74** was partially

TABLE IV. Substituted Benzoyl Esters of 6-Amidino-2-naphthol



Compd. No.	R	Trypsin	Plasmin	Inhibitory activities IC_{50} (μM)[a]			Cl$\bar{s}$	Hemolysis[b]
				Kallikrein	Thrombin	Cl$\bar{r}$		
22	H	4	2	>10	4	1	0.3	0.2
52	2-CH_3	10	>10	>10	>10	6	2	>10
53	3-CH_3	2	4	10	4	1	2	6
54	4-CH_3	1	0.5	8	>10	0.4	0.07	2
55	3,4-diCH_3	2	6	10	10	0.9	0.4	>10
56	4-*tert*-C_4H_9	0.3	2	9	3	0.7	0.3	10
57	4-OCH_3	0.5	3	10	3	0.1	0.2	3
58	4-O-*n*-C_4H_9	0.04	10	10	>10	10	0.4	>10
59	3-4-O–CH_2–O–	0.2	5	10	4	0.2	0.2	4
60	4-O–$(CH_2)_2$–$N(C_2H_5)_2$ ·CH_3SO_3H	2	2	2	6	10	5	3
61	4-O–CH_2–C_6H_5	0.04	10	>10	>10	0.4	0.4	>10
62	4-OH	0.3	10	10	7	0.1	0.2	8
63	4-$OCOCH_3$	0.4	2	10	10	0.3	0.3	10
64	4-SCH_3	2	2	10	4	0.5	0.3	2
65	4-F	3	1	10	2	0.2	0.3	3
66	4-Cl	3	0.5	10	10	10	0.4	3
67	4-Br	4	2	10	4	0.7	0.5	4
68	3-CF_3	8	3	>10	10	1	3	>10
69	4-NH_2·CH_3SO_3H	0.06	10	10	>10	0.2	0.2	10
70	4-$N(CH_3)_2$	0.3	>10	>10	>10	0.5	0.3	>10
71	4-$NHCOCH_3$	3	3	>10	>10	0.7	0.4	>10
72	4-CH_2NHCbz	0.6	1	10	>10	0.7	0.5	5
73	4-CH_2NH_2·CH_3SO_3H	4	4	3	>10	0.3	0.2	0.1
74	4-NH-  ·CH_3SO_3H	0.02	0.4	8	0.4	0.1	0.02	0.03
75	4-$COCH_3$	2	3	10	4	2	3	0.4
76	4-CH=N-OCH_3	0.9	0.9	4	3	1	0.4	2
77	4-$COOCH_3$	0.3	0.5	>10	3	7	6	10
78	4-CN	10	3	>10	2	4	8	3
79	4-NO_2	10	3	>10	2	4	4	0.7
80	4-SO_2NH_2	10	2	10	2	3	5	10

a, b) See footnotes *a*, *b*, Table I.

effective even at a dose of 1 mg/kg and was completely effective at a dose of 3 mg/kg (all guinea pigs tested survived). Compound **1** was partially effective at a dose of 3 mg/kg, whereas **2** and **22** were ineffective even at a dose of 3 mg/kg.

The effectiveness of complement-mediated hemolysis inhibition as well as the chemical stability of the ester linkage in the blood might contribute to the result of the *in vivo* animal experiments.

Conclusion

Compound **74**, which has been given the code number FUT-175, has been found to have

TABLE V. Comparative Effectiveness in Serine-Protease Inhibition

Compd. No.	Trypsin	Plasmin	Inhibitory activities IC_{50} (μM)[a]			Cl$\bar{s}$	Hemolysis[b]
			Kallikrein	Thrombin	Cl$\bar{r}$		
1	0.09	0.9	10	1	4	0.2	0.3
2	0.03	2	0.4	0.2	2	0.2	0.5
22	4	2	10	4	1	0.3	0.2
74	0.02	0.4	8	0.4	0.1	0.02	0.03

a, b) See footnotes *a, b*, Table I.

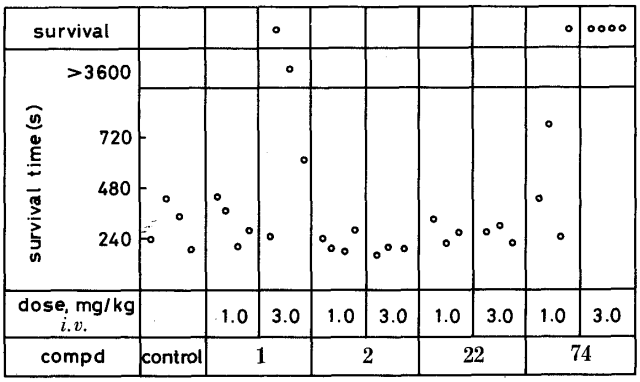


Fig. 1. Effect of Intravenously Administered Compounds on Survival Time in Forssman Shock

Groups of 3—4 guinea pigs were used. Compounds were dosed 5 min before injection of hemolysin (0.5 ml/animal) and deaths were recorded. Each point represents the survival time of an individual guinea pig and "survival" means that the survival time was longer than 24 h.

potent inhibitory activities *in vitro* against serine proteases, particularly trypsin and proteases in the complement system, as well as on the systemic Forssman shock reaction *in vivo*.[18,19] FUT-175, thus, seems promising as a possible therapeutic agent, for instance, in pancreatitis, disseminated intravascular coagulation syndromes, autoimmune diseases and the like *i.e.*, diseases known to be caused by anomalous activation of serine proteases such as trypsin, thrombin and the complement system.

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 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Varian T-60 or JNM-FX-60Q spectrometer employing Me_4Si as an internal standard. Abbreviations used are as follows: DMF, dimethylformamide; DMSO, dimethylsulfoxide; DCC, dicyclohexylcarbodiimide; MSA, methanesulfonic acid; TAME, N^{α}-tosylarginine methyl ester; ATEE, N^{α}-acetyltyrosine ethyl ester; AAME, N^{α}-acetylarginine methyl ester; ArH, aromatic H; Am^+, $-C(=NH_2^+)-NH_2$

Methyl 6-Bromo-2-hydroxy-1-naphthoate (11)——**11** was prepared by bromination of methyl 2-hydroxy-1-naphthoate with Br_2 in acetic acid at room temperature. **11**: mp 81.5—82 °C (recrystn. solvent, *n*-hexane). IR ν_{max}^{KBr} cm^{-1}: 1650, 1233. ^{1}H-NMR (acetone-d_6) δ: 4.10 (3H, s, CH_3), 7.15 (1H, d, J=8.4 Hz, ArH-3), 7.62 (1H, dd, J=8.4, 3.0 Hz, ArH-7), 7.90 (1H, d, J=8.4 Hz, ArH-4), 7.95 (1H, d, J=3.0 Hz, ArH-5), 8.55 (1H, d, J=8.4 Hz, ArH-8), 12.11 (1H, s, OH). *Anal.* Calcd for $C_{12}H_9BrO_3$: C, 51.27, H, 3.23. Found: C, 51.37; H, 3.09.

6-Cyano-2-naphthol (12)——A mixture of 6-bromo-2-naphthol (22.3 g, 10 mmol) and CuCN (10.8 g, 12 mmol) in DMF (25 ml) was heated at 160—170 °C for 3 h with vigorous stirring under a nitrogen atmosphere. After cooling of the reaction mixture, 10% NaOH was added, and the whole was triturated and filtered. The filtrate was acidified with 10% HCl and the precipitate was collected to give **12** (14.0 g, 83%). Recrystallization from EtOH–H_2O afforded an analytical sample as a pale brown powder, mp 165—166 °C (lit.,[20] mp 164—166 °C).

Methyl 6-Cyano-2-hydroxy-1-naphthoate (13)——**13** was prepared from **11** according to the method for **12**. **13**, mp 185—186 °C (recrystn. solvent, acetone–$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2240, 1650. ^{1}H-NMR (DMSO-d_6) δ: 3.98 (3H, s, CH_3), 7.26—8.59 (5H, m, ArH), 11.15 (1H, s, OH). *Anal.* Calcd for $C_{13}H_9NO_3$: C, 68.72; H, 3.99; N, 6.16. Found: C, 68.68; H, 3.83; N, 6.06.

6-Amidino-2-naphthol (5)——**12** (8.5 g, 50 mmol) was added to a cooled saturated MeOH–HCl solution (50 ml)

TABLE VI. Physicochemical Properties

Compd. No.	Salt	mp (°C)	Recrystn. solvent	Yield[a] (Method[b])	IR ν_{max}^{KBr} cm^{-1} (Ester)	Formula	Analysis (%) Calcd (Found) C	H	N
5	MSA[c]	227—228	EtOH	65		$C_{11}H_{10}N_2O \cdot CH_4O_3S$	51.05 (51.04	5.00 4.96	9.92 9.86)
6	MSA	228—229	MeOH	88		$C_{13}H_{12}N_2O_3 \cdot CH_4O_3S$	49.41 (49.31	4.74 4.74	8.23 8.22)
7	MSA	252.5—254	EtOH	42		$C_{11}H_9BrN_2O \cdot CH_4O_3S$	39.90 (39.87	3.63 3.59	7.76 7.84)
8	MSA	177—180	EtOH	31		$C_{11}H_{14}N_2O \cdot CH_4O_3S$	50.34 (50.00	6.34 6.47	9.78 9.58)
9	MSA	225—226.5	EtOH	37 (A)		$C_{11}H_{10}N_2O \cdot CH_4O_3S$	51.05 (51.06	5.00 5.67	9.92 9.12)
22	MSA	258—258.5	MeOH	62 (A)	1730	$C_{18}H_{14}N_2O_2 \cdot CH_4O_3S$	59.06 (58.90	4.69 4.59	7.25 7.26)
23	MSA	136—139	EtOH	44 (A)	1725 (1708)	$C_{20}H_{16}N_2O_4 \cdot CH_4O_3S$	56.75 (56.26	4.54 4.41	6.30 6.53)
24	MSA	222—224	EtOH	73 (A)	1742	$C_{18}H_{13}N_2O_2 \cdot CH_4O_3S \cdot H_2O$	47.22 (47.52	3.96 3.70	5.80 5.85)
25	MSA	234—235	EtOH	55 (A)	1728	$C_{18}H_{18}N_2O_2 \cdot CH_4O_3S$	58.45 (58.28	5.68 5.70	7.17 7.08)
26	MSA	189—192	EtOH	38 (A)	1735	$C_{18}H_{14}N_2O_2 \cdot CH_4O_3S$	59.06 (58.95	4.69 4.65	7.25 7.00)
27	MSA	221—222	EtOH	30 (A)	1758	$C_{13}H_{12}N_2O_2 \cdot CH_4O_3S$	51.84 (51.77	4.97 4.95	8.64 8.60)
28	MSA	249—251	MeOH	29 (B)	1755	$C_{15}H_{16}N_2O_2 \cdot CH_4O_3S$	54.53 (54.49	5.72 5.71	7.95 7.95)
29	MSA	264—265	MeOH	31 (B)	1753	$C_{16}H_{18}N_2O_2 \cdot CH_4O_3S$	55.72 (55.67	6.05 6.08	7.64 7.63)
30	MSA	178—180	EtOH–Et_2O	31 (B)	1757	$C_{17}H_{20}N_2O_2 \cdot CH_4O_3S$	56.83 (56.86	6.36 6.39	7.36 7.32)
31	MSA	165—168	EtOH–Et_2O	16 (B)	1754	$C_{21}H_{28}N_2O_2 \cdot CH_4O_3S$	60.53 (60.58	7.39 7.45	6.42 6.43)
32	MSA	—203	MeOH	15 (B)	1751	$C_{23}H_{32}N_2O_2 \cdot CH_4O_3S$	62.04 (62.01	7.81 7.92	6.03 6.05)

33	MSA	—225	MeOH	33 (B)	1755	$C_{26}H_{38}N_2O_2 \cdot CH_4O_3S$	64.00 (63.98	8.35 8.39	5.53 5.44)
34	MSA	227—228	MeOH	34 (B)	1750	$C_{29}H_{44}N_2O_2 \cdot CH_4O_3S$	65.66 (65.82	8.82 8.84	5.10 4.99)
35	MSA	145—150	EtOH	66 (B)	1750	$C_{25}H_{27}N_3O_4 \cdot CH_4O_3S$	58.97 (58.74	5.90 5.89	7.93 7.94)
36	2MSA	148—149	EtOH	22 (C)	1750	$C_{17}H_{21}N_3O_2 \cdot 2CH_4O_3S$	46.42 (46.14	5.95 6.04	8.55 8.37)
37	2MSA	170—173	EtOH	11 (B)	1745	$C_{18}H_{23}N_5O_2 \cdot 2CH_4O_3S$	45.02 (44.95	5.86 5.88	13.12 12.81)
38	MSA	222—223	MeOH	47 (B)	1735	$C_{16}H_{16}N_2O_2 \cdot CH_4O_3S$	56.03 (55.64	5.53 5.55	7.69 7.64)
39	MSA	222—225	EtOH–MeOH	36 (B)	1728	$C_{17}H_{16}N_2O_2 \cdot CH_4O_3S$	57.43 (57.42	5.36 5.33	7.44 7.39)
40	MSA	248—250	MeOH	48 (A)	1745	$C_{15}H_{14}N_2O_2 \cdot CH_4O_3S$	54.85 (54.76	5.18 5.13	7.99 7.88)
41	MSA	258—259	MeOH	51 (B)	1750	$C_{16}H_{16}N_2O_2 \cdot CH_4O_3S$	56.03 (55.89	5.53 5.47	7.69 7.68)
42	MSA	270—271	EtOH	29 (A)	1748	$C_{18}H_{20}N_2O_2 \cdot CH_4O_3S$	58.15 (58.13	6.16 6.16	7.14 7.04)
43	MSA	217—218 (dec.)	MeOH	22 (A)	1740	$C_{27}H_{29}N_3O_4 \cdot CH_4O_3S$	60.53 (60.39	5.99 6.00	7.56 7.50)
44	2MSA	198—200	MeOH	67 (C)	1735	$C_{19}H_{23}N_3O_2 \cdot 2CH_4O_3S$	48.73 (48.52	6.04 6.03	8.12 8.09)
45	2MSA	249—250	MeOH	34 (B)	1754	$C_{20}H_{25}N_5O_2 \cdot 2CH_4O_3S$	47.22 (46.98	5.94 6.00	12.51 12.44)
46	MSA	220—221	MeOH	42 (B)	1750	$C_{19}H_{16}N_2O_2 \cdot CH_4O_3S$	59.99 (59.58	5.03 4.93	7.00 6.98)
47	MSA	202—203	EtOH	76 (C)	1756	$C_{20}H_{18}N_2O_2 \cdot CH_4O_3S$	60.86 (60.76	5.35 5.30	6.76 6.80)
48	MSA	238—240	EtOH	49 (A)	1730	$C_{20}H_{16}N_2O_2 \cdot CH_4O_3S$	61.15 (60.99	4.89 4.81	6.79 6.78)
49	MSA	224—225	MeOH	18	1734 (1770)	$C_{22}H_{18}N_2O_4 \cdot CH_4O_3S$	58.72 (58.56	4.71 4.75	5.95 5.99)
50	MSA	204—205	EtOH	25 (B)	1732	$C_{21}H_{19}N_3O_3 \cdot CH_4O_3S$	57.76 (57.38	5.07 4.97	9.18 9.06)
51	MSA	205—206	MeOH	10 (B)	1760	$C_{19}H_{16}N_2O_3 \cdot CH_4O_3S$	57.68 (57.62	4.84 4.82	6.73 6.72)

TABLE VI. (continued)

Compd. No.	Salt	mp (°C)	Recrystn. solvent	Yield[a] (Method[b])	IR ν_{max}^{KBr} cm^{-1} (Ester)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
52	MSA	248—249	MeOH	20 (B)	1735	$C_{19}H_{16}N_2O_2 \cdot CH_4O_3S$	59.99 (59.90	5.03 4.95	7.00 6.99)
53	MSA	221—222	MeOH	31 (B)	1725	$C_{19}H_{16}N_2O_2 \cdot CH_4O_3S$	59.99 (59.84	5.03 5.03	7.00 6.97)
54	MSA	266—267	MeOH	58 (A)	1724	$C_{19}H_{16}N_2O_2 \cdot CH_4O_3S$	59.99 (59.92	5.03 4.99	7.00 6.97)
55	MSA	251—252	MeOH	38 (B)	1730	$C_{20}H_{18}N_2O_2 \cdot CH_4O_3S$	60.86 (60.82	5.35 5.35	6.76 6.76)
56	MSA	291—292	MeOH	31 (A)	1728	$C_{22}H_{22}N_2O_2 \cdot CH_4O_3S$	62.43 (62.47	5.92 5.91	6.33 6.32)
57	MSA	263—264	MeOH	64 (A)	1715	$C_{19}H_{16}N_2O_3 \cdot CH_4O_3S$	57.68 (57.49	4.84 4.83	6.73 6.66)
58	MSA	238—240	MeOH	51 (A)	1720	$C_{22}H_{22}N_2O_3 \cdot CH_4O_3S$	60.25 (60.22	5.72 5.75	6.11 6.07)
59	MSA	—230	MeOH	49 (A)	1716	$C_{19}H_{14}N_2O_4 \cdot CH_4O_3S$	55.81 (55.67	4.21 4.18	6.51 6.45)
60	MSA	217—218	MeOH	60 (B)	1720	$C_{24}H_{27}N_3O_3 \cdot 2CH_4O_3S$	52.25 (52.15	5.90 5.91	7.03 6.89)
61	MSA	259—260	MeOH	55 (A)	1718	$C_{25}H_{20}N_2O_3 \cdot CH_4O_3S$	63.40 (63.14	4.91 4.86	5.69 5.61)
62	MSA	244—246	MeOH	74 (C)	1728	$C_{18}H_{14}N_2O_3 \cdot CH_4O_3S$	56.71 (56.57	4.51 4.50	6.96 6.94)
63	MSA	244—245	MeOH	34 (A)	1731 (1755)	$C_{20}H_{16}N_2O_4 \cdot CH_4O_3S$	56.75 (56.58	4.54 4.49	6.30 6.20)
64	MSA	266—267	MeOH	46 (B)	1725	$C_{19}H_{16}N_2O_2S \cdot CH_4O_3S$	55.54 (55.48	4.66 4.58	6.48 6.44)
65	MSA	251—252	MeOH	30 (A)	1732	$C_{18}H_{13}FN_2O_2 \cdot CH_4O_3S$	56.43 (56.40	4.24 4.10	6.93 6.82)
66	MSA	245—246	MeOH	28 (B)	1725	$C_{18}H_{13}ClN_2O_2 \cdot CH_4O_3S$	54.22 (54.13	4.07 3.81	6.66 6.64)
67	MSA	257—258	MeOH	51 (A)	1725	$C_{18}H_{13}BrN_2O_2 \cdot CH_4O_3S$	49.04 (49.07	3.68 3.69	6.02 6.00)

68	MSA	208—209	MeOH	42 (B)	1733	$C_{19}H_{17}F_3N_2O_2 \cdot CH_4O_3S$	52.86 (52.81	3.77 3.71	6.16 6.18)
69	2MSA	158—163	EtOH	95 (C)	1735	$C_{18}H_{15}N_3O_2 \cdot 2CH_4O_3S \cdot H_2O$	46.59 (46.51	4.89 4.54	8.15 8.09)
70	MSA	250—252 (dec.)	MeOH	16 (B)	1705	$C_{20}H_{19}N_3O_2 \cdot CH_4O_3S$	58.73 (58.56	5.40 5.32	9.78 9.70)
71	MSA	277—279	EtOH	29 (B)	1720	$C_{20}H_{17}N_3O_3 \cdot CH_4O_3S$	56.88 (56.54	4.77 4.87	9.48 9.27)
72	MSA	209—210	MeOH	75 (B)	1730	$C_{27}H_{23}N_3O_4 \cdot CH_4O_3S$	61.19 (61.05	4.95 4.96	7.65 7.58)
73	MSA	250—251	EtOH	65 (A)	1730	$C_{19}H_{17}N_3O_2 \cdot 2CH_4O_3S$	49.31 (49.29	4.93 4.95	8.21 8.23)
74	2HCl	275 (dec.)	Acetone–H_2O	50 (B)	1706	$C_{19}H_{17}N_5O_2 \cdot 2HCl$	54.30 (53.83	4.56 4.52	16.66 16.65)
74	2MSA	260 (dec.)	H_2O	40 (B)	1741	$C_{19}H_{17}N_5O_2 \cdot 2CH_4O_3S$	46.75 (46.76	4.67 4.66	12.98 12.99)
75	2MSA	241—242	EtOH	20 (B)	1730	$C_{20}H_{16}N_2O_3 \cdot CH_4O_3S$	58.87 (58.62	4.70 4.77	6.54 6.44)
76	MSA	240—242	MeOH	26 (B)	1728	$C_{20}H_{17}N_3O_3 \cdot CH_4O_3S$	56.88 (56.39	4.77 4.85	9.48 9.35)
77	MSA	257—259	MeOH	44 (A)	1723	$C_{20}H_{16}N_2O_4 \cdot CH_4O_3S$	56.75 (56.72	4.54 4.52	6.30 6.24)
78	MSA	264—265	MeOH	38 (A)	1735	$C_{19}H_{13}N_3O_2 \cdot CH_4O_3S$	58.39 (58.34	4.16 4.00	10.21 10.19)
79	MSA	268—271	MeOH	37 (A)	1743	$C_{18}H_{13}N_3O_4 \cdot CH_4O_3S$	52.90 (52.88	3.97 3.87	9.74 9.64)
80	MSA	271—272	MeOH	59 (B)	1730	$C_{18}H_{15}N_3O_4S \cdot CH_4O_3S$	49.03 (48.65	4.11 4.33	9.03 8.76)

a) Refers to crude yield unless otherwise noted. No attempt was made to maximize the yield. *b*) See Experimental for details. *c*) MSA = CH_3SO_3H.

and the mixture was stirred overnight at room temperature, then concentrated. The residue was dissolved in MeOH (50 ml) and gaseous NH_3 was introduced into the solution at 50 °C for 3 h. The mixture was concentrated *in vacuo* and saturated $NaHCO_3$ solution was added to the residue with stirring. The precipitate was collected, washed with water and then with acetone, and a suspension of the precipitate in MeOH (10 ml) was treated with MSA (5.8 g, 60 mmol). Et_2O was added to the solution and the precipitate was collected to give **5**·MSA (9.2 g, 65%). Recrystallization from EtOH afforded an analytical sample as a pale yellow powder: mp 227—228 °C (lit.,[21] benzenesulfonic acid salt, mp 274—275.5 °C). IR ν_{max}^{KBr} cm^{-1}: 3350, 3150, 1668, 1625. ^{1}H-NMR (DMSO-d_6) δ: 2.51 (3H, s, CH_3SO_3), 7.05—8.58 (6H, m, ArH), 8.87—9.56 (4H, br, Am^+), 10.39 (1H, s, OH).

Methyl 6-Amidino-2-hydroxy-1-naphthoate (6)——**6** was prepared from **13** according to the method for **5**. **6**·MSA, mp 228—229 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3320, 3160, 1690, 1680, 1640, 1620. ^{1}H-NMR (DMSO-d_6) δ: 2.48 (3H, s, CH_3SO_3), 3.98 (3H, s, CH_3O), 7.17—8.71 (5H, m, ArH), 8.87—9.75 (4H, br, Am^+), 10.65—11.45 (1H, br s, OH).

6-Acetoxy-1,2,3,4-tetrahydro-2-naphthoic Acid (16)——**16** was prepared by acetylation of 6-hydroxy-1,2,3,4-tetrahydro-2-naphthoic acid[22] using Ac_2O and AcONa. **16**, mp 127—131 °C (recrystn. solvent, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3150—2500, 1750, 1695, 1210. ^{1}H-NMR ($CDCl_3$) δ: 1.73—3.27 (7H, m), 2.25 (3H, s, CH_3CO), 6.63—7.28 (3H, s, ArH), 7.57—8.07 (1H, br, OH). *Anal.* Calcd for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.94; H, 5.83.

6-Acetoxy-1,2,3,4-tetrahydro-2-naphthamide (17)——**17** was prepared by reaction of **16** with $SOCl_2$ followed by reaction of the acid chloride with NH_3. **17**, mp 144—145.5 °C (recrystn. solvent, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3380, 3180, 1760, 1642, 1210. ^{1}H-NMR (DMSO-d_6) δ: 1.67—3.02 (7H, m), 2.23 (3H, s, CH_3CO), 6.34—8.19 (5H, m, ArH and NH_2). *Anal.* Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.95; H, 6.41; N, 5.97.

4-Acetoxy-1-naphthamide (20)——**20** was prepared from 4-acetoxy-1-naphthoic acid[16] according to the method for **17**. **20**, mp 162—163 °C (recrystn. solvent, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3440, 3210, 1740, 1660, 1210. ^{1}H-NMR (acetone-d_6) δ: 2.45 (3H, s, CH_3CO), 6.73—8.90 (8H, m, ArH and NH_2). *Anal.* Calcd for $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11. Found: C, 68.10; H, 4.88; N, 6.09.

4-Amidino-1-naphthol (9)——A solution of $Et_3O^+BF_4^-$ (4.2 g, 22.1 mmol) in anhydrous CH_2Cl_2 (20 ml) was added dropwise to a suspension of **20** (4.6 g, 20 mmol) in CH_2Cl_2 (50 ml) at room temperature with stirring and the mixture was stirred at room temperature for 3 h. Et_2O was then added, and the precipitate was collected and dissolved in anhydrous MeOH (20 ml). Gaseous NH_3 was introduced into the solution at 50 °C for 3 h, then the precipitate was collected and a suspension of the precipitate in MeOH (10 ml) was treated with MSA (2.9 g, 30 mmol). Et_2O was added to the solution and the resulting precipitate was collected to give **9**·MSA (2.1 g, 37%). Recrystallization from EtOH afforded an analytical sample as pale yellow prisms, mp 225—226.5 °C. IR ν_{max}^{KBr} cm^{-1}: 3400—3000, 1665, 1580. ^{1}H-NMR (DMSO-d_6) δ: 2.47 (3H, s, CH_3SO_3), 7.07 (1H, d, ArH-2), 7.37—8.55 (5H, m, ArH), 8.98—9.67 (4H, br s, Am^+), 10.79—11.57 (1H, br s, OH).

2-Amidino-1,2,3,4-tetrahydro-6-naphthol (8)——**8** was prepared from **17** according to the method for **9**. **8**·MSA, mp 177—180 °C (recrystn. solvent, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3550—2900, 1670. ^{1}H-NMR (DMSO-d_6) δ: 1.58—3.31 (7H, m), 2.46 (3H, s, CH_3SO_3), 6.28—7.18 (3H, m, ArH), 8.30—9.27 (4H, br s, Am^+).

6-Amidino-1-bromo-2-naphthol (7)——A solution of Br_2 (3.9 g, 22 mmol) in AcOH (8 ml) was added dropwise to a solution of **5** (5.6 g, 20 mmol) in a mixture of AcOH (40 ml) and H_2O (80 ml) at room temperature, and the mixture was stirred at room temperature for 1 h. The precipitate was collected, washed with acetone and a suspension of the precipitate in EtOH (100 ml) was treated with MSA (5.8 g, 60 mmol). Et_2O was then added and the resulting precipitate was collected to give **7**·MSA (3.0 g, 42%). Recrystallization from EtOH afforded an analytical sample as a colorless powder, mp 252.5—254 °C. IR ν_{max}^{KBr} cm^{-1}: 3450—3000, 1675, 1620. ^{1}H-NMR (DMSO-d_6) δ: 2.47 (3H, s, CH_3SO_3), 7.17—8.73 (5H, m, ArH), 8.91—9.73 (4H, br, Am^+), 11.26 (1H, br s, OH).

Procedure for Acylated Amidinonaphthol Compound——Method A. 6-Amidino-2-naphthyl Benzoate (**22**): Benzoyl chloride (1.4 g, 10 mmol) was added dropwise to a cooled, strirred suspension of **5**·MSA (2.8 g, 10 mmol) in dry pyridine (30 ml). The mixture was stirred at room temperature for 5 h and Et_2O was added. The precipitate was collected and a solution of the precipitate in MeOH (10 ml) was added to saturated $NaHCO_3$ solution (50 ml). The resulting precipitate was collected by filtration, and washed with water then with acetone. A suspension of the precipitate in MeOH (10 ml) was treated with MSA (1.0 g, 12 mmol). Et_2O was added to the solution and the precipitate was collected to give **22**·MSA (2.4 g, 62%). Recrystallization from MeOH afforded an analytical sample as colorless needles, mp 258—258.5 °C. IR ν_{max}^{KBr} cm^{-1}: 3320, 3130, 1730, 1670, 1625. ^{1}H-NMR (DMSO-d_6) δ: 2.50 (3H, s, CH_3SO_3), 7.47—8.83 (11H, m, ArH), 9.13—9.85 (4H, br, Am^+).

Method B. 6-Amidino-2-naphthyl 4-Guanidinobenzoate (**74**): A mixture of 4-guanidinobenzoic acid·HCl (2.2 g, 10 mmol), **5**·MSA (2.8 g, 10 mmol), DCC (2.3 g, 12 mmol) and dry pyridine (40 ml) was stirred overnight at room temperature. The precipitate was collected, and mixed with H_2O (40 ml). The resulting suspension was filtered and $NaHCO_3/H_2O$ (3.8 g/40 ml) solution was added to the filtrate. The precipitate (**74**·$2H_2CO_3$) was collected, washed with acetone, and added to HCl/DMF (0.8 g/20 ml). The mixture was stirred overnight and the precipitate was collected to give **74**·2HCl (2.1 g, 50%). Recrystallization from acetone–H_2O afforded an analytical sample as a colorless powder, mp 275 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3400—3200, 1706, 1680, 1600. ^{1}H-NMR (DMSO-d_6) δ: 7.48—8.22 (14H, m, arom. H and –NH–C(=NH$_2^+$)–NH$_2$), 9.32—9.62 (4H, br, Am^+), 10.77 (1H, s, –NH–C(=NH$_2^+$)–NH$_2$). **74**·2HCl was added

to MSA-Na–H_2O (10 eq, pH 4.4) and the mixture was stirred overnight. The precipitate was collected and recrystallized from H_2O to give **74**·2MSA as a colorless powder, mp 260 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3500—2900, 1741, 1679. ^{1}H-NMR (DMSO-d_6) δ: 2.42 (6H, s, $CH_3SO_3 \times 2$) 7.66—8.23 (14H, m, arom. H and –NH–$\langle^{NH_2^+}_{NH_2}$), 9.19—9.48 (4H, br, Am^+), 10.24 (1H, s, –NH–$\langle^{NH_2^+}_{NH_2}$).

Method C. 6-Amidino-2-naphthyl *trans*-4-Aminomethylcyclohexylcarboxylate (**44**): Hydrogen gas was introduced into a stirred mixture of **43** (1.1 g, 2 mmol), MSA (0.2 g, 2 mmol), 10% Pd–C (0.1 g) and dry DMF (20 ml) at room temperature for 2 h. The mixture was filtered, and Et_2O was added to the filtrate. Concentration afforded an oily residue, which was crystallized from EtOH to give **44**·2MSA (0.7 g, 83%). Recrystallization from MeOH afforded an analytical sample as a colorless powder, mp 198—200 °C. IR ν_{max}^{KBr} cm^{-1}: 3550—2850, 1735, 1665. ^{1}H-NMR (DMSO-d_6) δ: 0.81—3.03 (12H, m), 2.44 (6H, s, $CH_3SO_3 \times 2$), 7.30—8.50 (9H, m, ArH and NH_3^+), 9.11—9.86 (4H, br, Am^+).

Enzyme Inhibition——Bovine trypsin was purchased from Sigma Chemical Co., St. Louis, U.S.A., 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. Human $Cl\bar{r}$ and $Cl\bar{s}$ were prepared by the method of Tamura *et al.*[14] and Okamura *et al.*,[15] respectively. The rates of hydrolysis of TAME by trypsin, plasmin, kallikrein, and thrombin were determined as described by Muramatu *et al.*,[13] that of AAME by $Cl\bar{r}$ as described by Tamura *et al.*,[14] and that of ATEE by $Cl\bar{s}$ as described by Okamura *et al.*,[17] 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.*[16]

References and Notes

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