Synthesis and Structure–Activity Study of Protease Inhibitors. V.[1] Chemical Modification of 6-Amidino-2-naphthyl 4-Guanidinobenzoate

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Research Laboratories, Torii & Co., Ltd.,[a] 3–14–3, Minamiyawata, Ichikawa, Chiba 272, Japan and the Osaka Foundation for Promotion of Fundamental Medical Research,[b] 2nd Nakata-Building #804, 2–1 Toyotsu-cho, Suita, Osaka 564, Japan. Received April 28, 1992

By developing 6-amidino-2-naphthyl 4-guanidinobenzoate (I, FUT-175) as a basic structure, its various derivatives were synthesized and their inhibitory activities on trypsin, plasmin, kallikrein, thrombin, C1r̄ and C1s̄ as well as on complement-mediated hemolysis were examined. The protective effect of these compounds on complement-mediated Forssman shock was also examined in guinea pigs. 6-Amidino-2-naphthyl 4-[(4,5-dihydro-1*H*-imidazol-2-yl)amino]-benzoate (41, FUT-187) was found to be a suitable compound for oral administration with anti-complement activity superior to that of compound I.

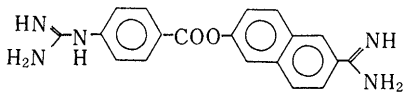
Keywords protease inhibition; C1r̄, C1s̄; anti-complement activity; Forssman shock; amidinonaphthol; structure–activity relationship

Serine proteases, a group of endopeptidases, have a serine molecule in their active center and are classified into trypsin-like serine protease, chymotrypsin-like serine protease and elastase according to substrate specificity.

Representative trypsin-like serine protease includes thrombin and plasmin related to the blood coagulation and fibrinolysis system, kallikrein related to the progress of various inflammatory reactions and C1r̄ and C1s̄, complementary enzymes which participate in the host defense against infection.[2]

These enzymes are interconnected in a complicated manner and are important in the maintenance of homeostasis. They are also known to interact in inflammatory reactions when activated abnormally.[3] Thus, a compound capable of inhibiting these enzymes may be expected to be a therapeutic agent for various inflammatory diseases such as pancreatitis, nephritis and rheumatoid arthritis.

We previously synthesized various ester derivatives containing amidino group, and found compound I (FUT-175) which inhibits protease activities of C1r̄, C1s̄, B, D and C3bBb in the complement system.[1,4]



I (FUT-175)

In this paper, we describe several modified compounds with guanidino and amidino groups which were synthesized to develop suitable substances for oral administration and more potent anti-complement activity than compound I.

Chemistry The principal synthetic routes for the preparation of these derivatives are outlined in Charts 1—5 and described in detail in the Experimental section.

The guanidinobenzoic acid derivatives (**5**—**16**) were synthesized by preparing the thioureidobenzoic acids (**1**, **2**) from 4-aminobenzoic acid with ammonium thiocyanate or methylisothiocyanate, followed by conversion to the isothioureidobenzoic acids (**3**, **4**) with methyl iodide and further conversion to derivatives **5**—**10** with the corresponding amines (Chart 1). Other guanidinobenzoic acids (**17**—**20**) were synthesized by the reaction of 4-aminobenzoic acids with cyanamide, acetylcyanamide or benzoylcyanamide (Chart 2).

4-(2-Thiomethyl-1-imidazolynyl)benzoic acid (**23**) was synthesized by preparing 4-aminoethylaminobenzoic acid (**21**) by the reaction of 4-chlorobenzoic acid with ethylenediamine, followed by conversion to **22** with carbon disulfide and further conversion to **23** with methyl iodide. 4-(2-Amino-1-imidazolynyl)benzoic acid (**24**) was obtained by the reaction of **23** with ammonia in methanol.

2-Amino-5-carboxy benzimidazole (**27**) was synthesized by preparing 2-mercapto-5-carboxybenzimidazole (**25**) by the reaction of 3,4-diaminobenzoic acid with carbon disulfide, followed by conversion to 2-methylthio-5-carboxybenzimidazole (**26**) with methyl iodide and further conversion to **27** with aqueous ammonia (Chart 3).

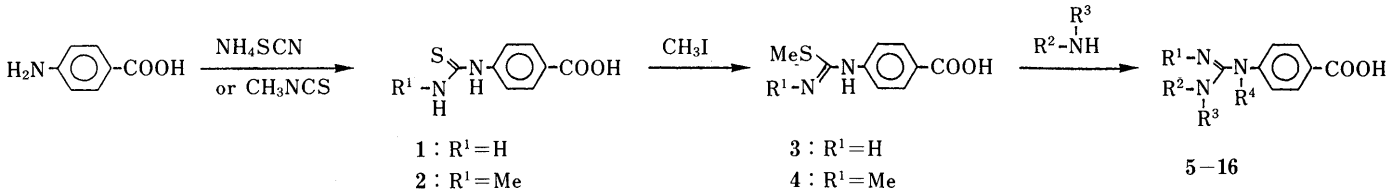
6-(2-Imidazolyl)-2-naphthol (**30**) together with the bis-derivative (**29**) were synthesized by preparing the imidate **28** from 6-cyano-2-naphthol with hydrogen chloride in methanol, followed by reaction with ethylenediamine in methanol. 6-(N^1-Methylamidino)-2-naphthol (**31**) was synthesized from the imidate **28** and methylamine in methanol (Chart 4).

Finally, these guanidinobenzoic acids **5**—**20**, **24** and **27** were treated with 6-amidino-2-naphthol (**32**) and its derivatives (**30**—**32**) in the presence of dicyclohexylcarbodiimide (DCC) to yield the corresponding esters **33**—**49** (Chart 5).

Results and Discussion

The screening system used for these newly synthesized compounds was the same as that described previously.[5–9] Namely, inhibitory activities on representative trypsin-like serine proteases and on complement-mediated hemolysis were used as an index of the *in vitro* system, while guinea pig Forssman shock[10] (Fs) mediated by activation of the complement system, especially its classical pathway, was used as an index of the *in vivo* system.

The 50% inhibitory concentration (IC_{50}) of compounds with guanidinobenzoic acid (**5**—**20**, **24**, **27**) or amidino-



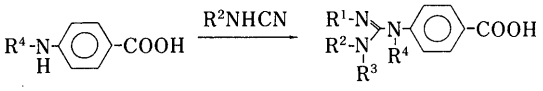
1 : R^1=H
2 : R^1=Me

3 : R^1=H
4 : R^1=Me

5—16

	R^1	R^2	R^3	R^4
5	H	Me	Me	H
6	H	Me	H	H
7	Me	Me	H	H
8	$-CH_2-CH_2-$		H	H
9	$-CH_2-CH_2-CH_2-$		H	H
10	$-CH_2-CH(Me)-$		H	H
11	$-CH_2-CH_2-$		Me	H
12			H	H
13			H	H
14	$-CH=CH-$		H	H
15	H	$-CH_2-CH_2-N(H)-C(=NH)-NH-C_6H_4-COOH$	H	H
16	H	$-CH_2-(CH_2)_2-N(H)-C(=NH)-NH-C_6H_4-COOH$	H	H

Chart 1



17—20

	R^1	R^2	R^3	R^4
17	H	H	H	H
18	H	H	H	Me
19	H	MeCO	H	H
20	H	PhCO	H	H

Chart 2

naphthol (**30**—**32**) in their structure to various enzymes were relatively low, 10^{-3} M order or more, except compounds **20** and **32**. Compound **20** had IC_{50} of 10^{-4} M order on plasmin, kallikrein and $C1\bar{s}$, and compound **32**[3] had IC_{50} of 10^{-4} to 10^{-5} M order on plasmin, $C1\bar{r}$ and $C1\bar{s}$. It was thus confirmed that the inhibitory activities on proteases were markedly augmented by esterification of guanidinobenzoic acids with 6-amidino-2-naphthol (**32**) as shown in Table I.

Amidinonaphthyl ester derivatives listed in Table I are classified into A, B, C and D groups. The inhibitory effect of these guanidino N^1-, N^2- and N^3-methyl derivatives (**33**—**36**; group A) on proteases and on complement-mediated hemolysis using 200-fold diluted serum (hemolysis 200F) were compared with those of compound I. Potent inhibitory effect was observed with compound **33** at 10^{-8} M order against thrombin, and with compounds **34** and **36** at 10^{-7} M order against kallikrein. Furthermore, the inhibitory activity of compounds **33**—**36** maintained an order of 10^{-6} to 10^{-7} M against $C1\bar{s}$, while their inhibitory activities to trypsin and $C1\bar{r}$ were 10^{-6} and 10^{-5} M, respectively, which were lower than those of compound I. In the complement-mediated hemolysis using 2-fold diluted serum (hemolysis 2F), the inhibitory effects of these compounds were similar to that of compound I.

With respect to the effect on Fs(i.v.), compounds **33** and **36** were ineffective at doses of 0.3 and 1 mg/kg. In addition, the protective effect of these compounds could not be evaluated at 1 and 3 mg/kg, because death occurred after intravenous injection of an individual compound alone before Fs was induced. The inhibitory effects of compounds **37** and **38** prepared by acylation of the guanidino group of compound I at N^3 position (group A) were reduced overall, and were especially remarkable in hemolysis 200F and 2F. This evidence together showed that introduction of one or two methyl groups to N^1, N^2 and N^3 of the guanidino group in the basic skeleton of I resulted in a decrease of inhibitory effect on enzymes, but a retention of complement-mediated hemolysis compared to those of compound I. By acylation at N^3 portion of the guanidino group in the basic skeleton of I, inhibitory effects of these compounds were decreased both enzymes and complement-mediated hemolysis. This evidence suggested that the basic guanidino group was essential for

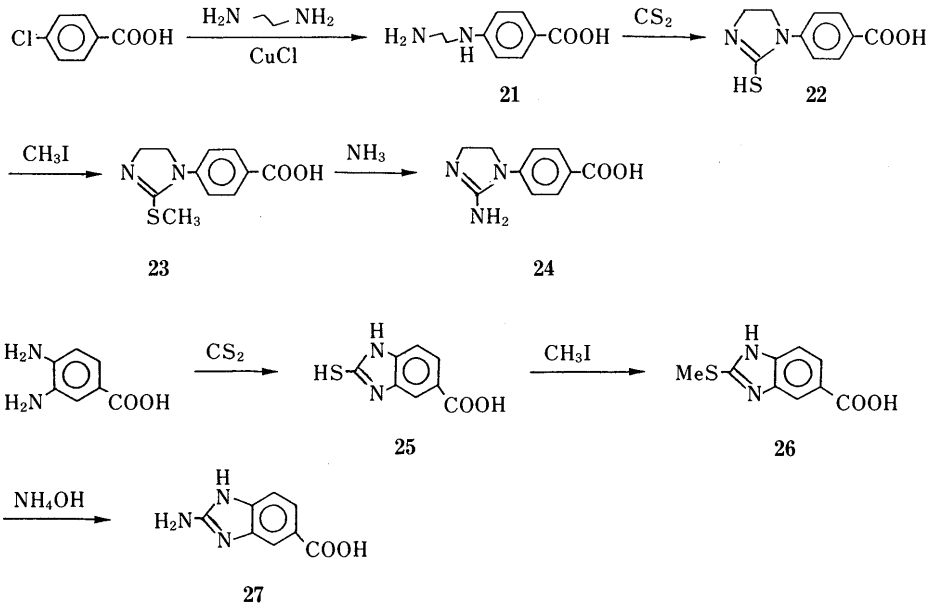


Chart 3

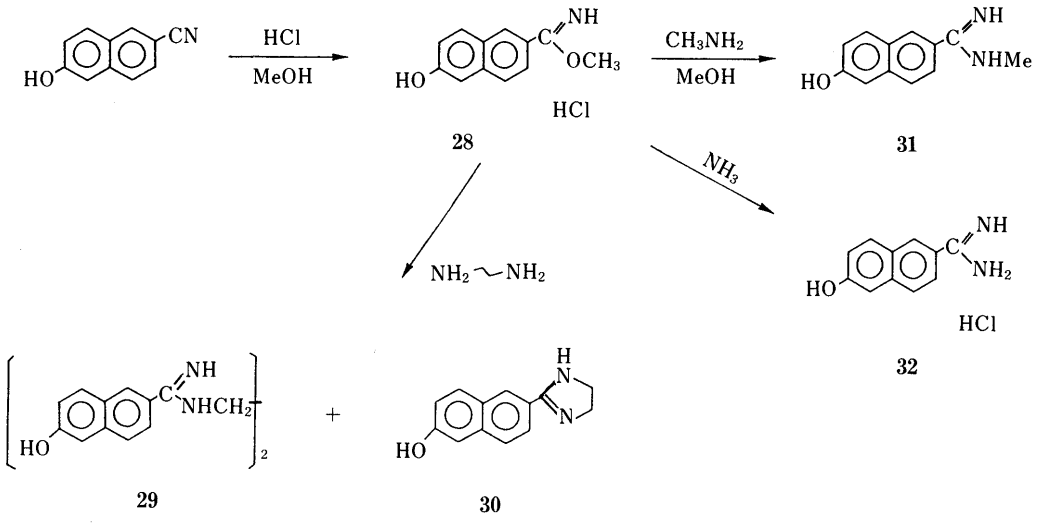


Chart 4

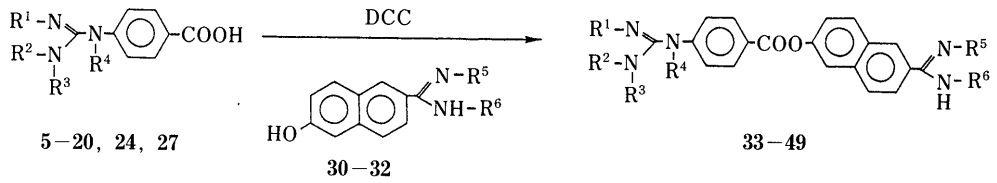


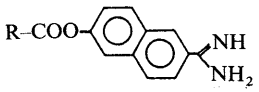
Chart 5

anti-complement activity and contributed to the stability of esters in blood.

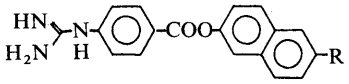
Compounds of group B have the cyclized guanidino groups. The protease inhibitory effects of compounds **39** and **40** were all decreased similarly to the case of N^3 acyl introduction mentioned above. While the effect of compound **39** was markedly decreased in hemolysis 200F activity, the inhibitory effect of compound **40** maintained only 10^{-7} M order in this activity. As for the effect on Fs(i.v.), compound **39** was ineffective at a dose of 3 mg/kg but compound **40** was effective over a prolonged survival period.

Compounds **41**—**47** of group C were prepared by cyclization at N^2 and N^3 of the guanidino group in the basic skeleton of I, and contained a suitable structure to obtain more augmented anti-complement activity. Compared to compound I, the protease inhibitory activity (IC_{50}) was lower overall; each of the effects in most compounds of group C was around the order of 10^{-7} M and was 10^{-7} to 10^{-8} M against kallikrein and $C1\bar{s}$. In addition, most of these compounds in group C were 10^{-6} to 10^{-8} M against hemolysis 200F and half of them were effective at 1×10^{-5} M or above against hemolysis 2F; all, however, were weaker than compound I. Compound **46**

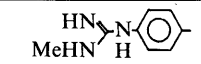
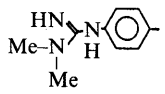
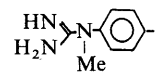
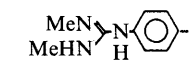
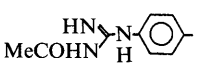
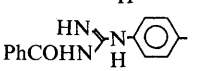
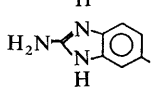
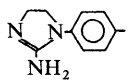
TABLE I. Inhibitory Effects of Amidinonaphtholesters on Proteases and Complement-Mediated Hemolysis

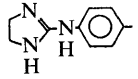
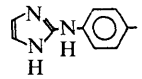
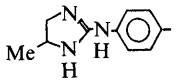
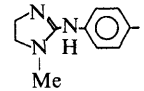
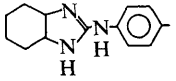
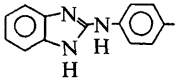
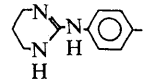


33—47



48, **49**, I

Compd. No.		R	Inhibitory activities IC_{50} (μM)[a]						Hemolysis[b] 200F (μM)	% inhibition on hemolysis 2F[c]								
										1×10^{-4}			1×10^{-5}			1×10^{-6} (M)		
			Trypsin	Plasmin	Kallikrein	Thrombin	Clr	Cls		0 min	5 min	10 min	0 min	5 min	10 min	0 min	5 min	10 min
A	**33**		4	3	3	0.06	>10	2	0.2				82	80	73			
	34		6	2	0.9	0.6	>10	0.4	0.5				54	39	22			
	35		0.6	0.6	>10	>10	3	0.3	0.7				75	57	42			
	36		10	0.8	0.7	9	>10	0.4	0.5				53	53	38			
	37		4	1	>10	>10	>10	0.1	3	12	12	7						
	38		2	3	>10	>10	>10	4	2	8	8	8						
B	**39**		2	>10	4	4	3	0.4	>10	42	34							
	40		4	4	>10	10	>10	3	0.5				80			44	24	12

C	**41**		3	2	4	6	20	0.3	0.2	95	96	96	82	82	77	25	21	21
	42		0.6	5	4	6	3	3	0.3	68	65	57						
	43		3	1	0.4	2	10	0.4	0.1	99	95	97	87	87	83	24	22	21
	44		0.3	1	0.6	0.8	>10	0.3	2				34	27				
	45		2	3	0.9	2	9	0.4	0.03				83		75			
	46		5	0.4	>10	>10	>10	0.05	5	0	0	0						
	47		6	1	0.5	2	>10	0.3	0.4				86	85	83			
D	**48**		0.05	0.3	>10	0.6	5	5	>10				3	0				
	49		0.05	0.3	>10	5	5	7	>10				1	0.3				
	I		0.02	0.4	8	0.4	0.1	0.02	0.08	100	100	100	91	85	78	33	26	23

a) IC_{50} means the concentration (μM) of the compounds required to inhibit 50% of each enzyme activity. *b*) Hemolysis 200F: Complement-mediated hemolysis *via* classical pathway with 200-fold diluted serum. *c*) Hemolysis 2F: Complement-mediated hemolysis *via* classical pathway with 2-fold diluted serum.

TABLE II. Protective Effect of Intravenously Administered Compounds in Forssman Shock

Compd. No.		Survival (%)				
		Dose (mg/kg)	0.1	0.3	1	3
A	**33**			0	Death[a)]	Death[a)]
	34			0	0	
	35					0
	36				0	Death[a)]
	37					NT
	38					NT
B	**39**					0
	40					33
C	**41**		0	33	100	100
	42				33	
	43			33	100	
	44					0
	45				0	
	46					0
	47			0	100	100
D	**48**					0
	49				0	
	I				0	100

Each datum obtained from 3 guinea pigs. Guinea pigs were injected intravenously with hemolysin at a dose of 0.5 ml/animal. Compounds were dosed intravenously 5 min prior to hemolysin injection. The effects of compounds were assessed by measuring survival time up to 24 h after hemolysin injection. *a*) Death: animals given compound died before hemolysin injection. NT: Not tested.

was ineffective against hemolysis 2F.

With respect to the effect on Fs(i.v.), in comparison with compound I, compound **41** surpassed always: compounds **42**, **43** and **47** showed a prolonged survival rate, white compound **43** had almost the same survival rate as compound **41**. It was thus suggested that compounds having an imidazoline ring in the group C structure (**41**, **43**, **47**) are effective in increasing the *in vivo* anti-complement activity. On the other hand, the introduction of methyl group at N^1 of the imidazoline ring such as compound **44**, 4,5,6,7-tetrahydro benzimidazole **45** and benzimidazole **46** were ineffective in Fs(i.v.). In addition, the protective effects of compound **42** having imidazoline-converted imidazole ring and compound **47** having imidazoline-converted tetrahydropyrimidine ring in Fs(i.v.) were weaker than the effects of compounds **41** and **43**.

Since anti-complement activity was involved in these compounds with introduced methyl guanidino compounds of group A and cyclized compounds of group C, compound **48** was synthesized by introduction of methyl group to the amidino group of compound I at N^1 position and compound **49** was further synthesized by cyclization of amidino group at N^1 and N^2 positions in group D. The protease inhibitory effects of these compounds were on the order of 10^{-8} M for trypsin, 10^{-7} M for plasmin and 10^{-6} to 10^{-7} M for thrombin, being comparable to that of compound I, but relatively lower against other proteases. Furthermore, the inhibitory activities of hemolysis 200F and 2F were markedly reduced. These compounds were ineffective on Fs(i.v.), correlating with the decreased inhibitory effect on complement-mediated hemolysis. From this evidence non-converted amidino group seemed to be essential to maintain the anti-complement activity of compound I.

TABLE III. Protective Effect of Orally Administered Compounds in Forssman Shock

Compd. No.		Survival (%)		
		Dose (mg/kg) 50	100	200
B	**40**		0	50
C	**41**	50	80	100
	42			0
	43	40	100	80
	47	0	40	71
	I		0	50

Each datum obtained from 3—8 guinea pigs. Guinea pigs were injected intravenously with hemolysin at a dose of 0.5 ml/animal. Compounds were dosed orally 1 h prior to hemolysin injection. The effects of compounds were assessed by measuring survival time up to 24 h after hemolysin injection.

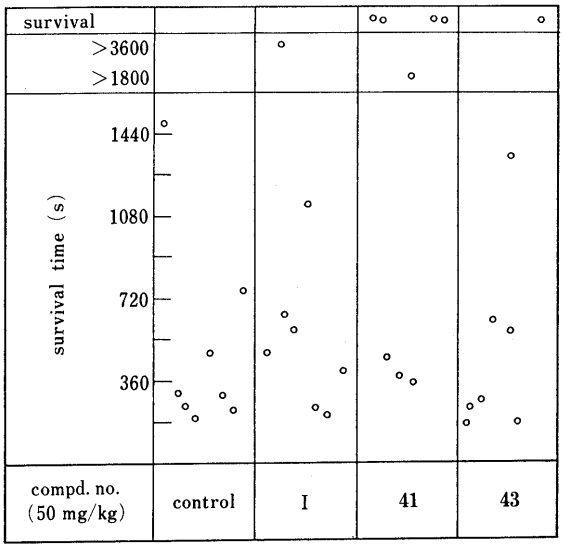


Fig. 1. Comparative Effect of Oral Administered Compound on Survival in Forssman Shock

Each datum obtained from 8 guinea pigs. Guinea pigs were injected intravenously with hemolysin at a dose of 0.5 ml/animal. Compounds were dosed orally at 50 mg/kg 1 h prior to hemolysin injection. The effects of compounds were assessed by measuring survival time up to 24 h after hemolysin injection. Survival means that the survival time was longer than 24 h.

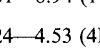
Compounds **40**—**43** and **47** which showed a distinct protective effect on Fs(i.v.) were selected as those having a potent anti-complement activity. The inhibitory effects of these compounds on Fs(*p.o.*) were then compared to that of compound I and arranged in order of effectiveness as follows (Table III): **41**, **43** > **47** > I, **40** ≫ **42**.

Compound **42** was ineffective, however, compounds **41**, **43** and **47** were stronger than and compound **40** was almost equal to compound I in terms of ability to protect animals from death. In addition, compounds **40** and **47** were ineffective on Fs(*p.o.*) at dose of 100 and 50 mg/kg, respectively. To determin their usefulness as an oral preparation, compounds **41** and **43** were selected for further examination.

Compounds **41** and **43** as individual oral compounds with anti-complement activity were compared to compound I in terms of their protective effect on Fs(*p.o.*) (Fig. 1).

Against Fs(*p.o.*) *in vivo*, compound I was weak but compounds **41** and **43** maintained a survival rate of 50% and 13%, respectively, at a dose of 50 mg/kg, demonstrat-

TABLE IV. Analytical and Spectral Data for Guanidinocarboxylic Acids and Amidinonaphthols

Compd. No.	Salt[a]	Yield[b] (%)	IR ν_{max}^{KBr} cm^{-1} (carboxyl)	^{1}H-NMR δ (DMSO-d_6) (J, Hz)
5	HCl	93	1697	3.11 (6H, s, $CH_3\times2$), 7.12—8.38 (6H, m, ArH, –NH– ), 9.58 (1H, br s, –NH– )
6	HCl	35	1705	2.87 (3H, d, J=4.1 Hz, CH_3), 7.54—8.46 (7H, m, ArH, –NH– ), 10.10 (1H, br s, –NH– )
7	HCl	42	1704	2.33 (3H, s, CH_3SO_3), 2.71 (6H, d, J=4.2 Hz, $CH_3\times2$), 6.86—8.56 (6H, m, ArH, –NH– ), 9.80 (1H, br s, –NH– )
8	MSA	36	1707	2.48 (3H, s, CH_3SO_3), 3.73 (4H, s, $-CH_2-CH_2-$), 7.36 (2H, d, J=8.5 Hz, ArH), 8.00 (2H, d, J=8.5 Hz, ArH), 8.70 (2H, s, –NH– ), 10.91 (1H, br s, –NH– ), 12.88 (1H, br s, COOH)
9	HCl	24	1655	1.47—2.30 (2H, m, $-CH_2-CH_2-CH_2-$), 2.90—3.66 (4H, m, $-CH_2-CH_2-CH_2-$), 7.32 (2H, d, J=8.5 Hz, ArH), 7.95 (2H, d, J=8.5 Hz, ArH), 8.61 (2H, br s, –NH– ), 10.56 (1H, br s, –NH– )
10	HCl	42	1700	1.28 (3H, d, J=4.5 Hz, CH_3), 2.68—4.58 (3H, m, $-CH_2-CH-$), 7.39 (2H, d, J=8.2 Hz, ArH), 7.98 (2H, d, J=8.2 Hz, ArH), 8.27—9.30 (2H, br, –NH– ), 11.46 (1H, br s, –NH– )
11	HCl	33	1690	3.10 (3H, s, CH_3), 3.70 (4H, m, $-CH_2-CH_2-$), 7.42 (2H, d, J=8.5 Hz, ArH), 8.02 (2H, d, J=8.5 Hz, ArH), 8.44 (1H, br s, –NH– ), 10.28 (1H, br s, –NH– )
12	HCl	19	1700	7.03—8.42 (8H, m, ArH), 12.12 (3H, br s, –NH– )
13	HCl	23	1700	0.80—2.38 (8H, m, $-(CH_2)_4-$), 2.87—4.24 (2H, m, $-CH-\times2$), 7.39 (2H, d, J=8.5 Hz, ArH), 7.99 (2H, d, J=8.5 Hz, ArH), 8.69, 8.80 (2H, each br s, –NH– ), 11.43, 11.75 (1H, each br s, –NH– )
14	HCl	11	1685	6.30—9.20 (8H, m, ArH, –NH–  and $-CH=CH-$), 11.10 (1H, br s, –NH– )
17	MSA	55	1720	2.49 (3H, s, CH_3SO_3), 7.10—8.31 (8H, m, ArH, –NH– ), 10.11 (1H, br s, –NH– ), 12.97 (1H, br s, COOH)
18	HCl	51	1690	3.33 (3H, s, CH_3), 6.63—8.65 (8H, m, ArH, –N– )
19	—	6	1663	2.09 (3H, s, CH_3), 7.50—8.14 (5H, m, ArH, –NH–), 10.22 (1H, br s, –NH– ), 12.64 (1H, br s, NHCO)
20	HCl	34	1665	7.01—8.94 (11H, m, ArH, –NH–), 11.25 (2H, br s, –NH– )
24	HCl	72	1682	3.24—4.53 (4H, m, $-CH_2-CH_2-$), 7.49 (2H, d, J=8.5 Hz, ArH), 8.03 (2H, d, J=8.5 Hz, ArH), 8.39 (2H, br s, $=NH_2^+$), 8.82 (1H, br s, NH)
27	MSA	52	1705	2.49 (3H, s, CH_3SO_3), 7.43 (1H, d, J=8.5 Hz, ArH), 7.67—8.21 (3H, m, ArH), 8.68 (2H, s, $-NH_2$), 12.69 (2H, br s, H_2N– )
30	MSA	26	—	2.45 (3H, s, CH_3SO_3), 4.04 (4H, s, $-CH_2-CH_2-$), 7.11—8.95 (6H, m, ArH), 10.50 (3H, br s, NH_2, OH)
31	HCl	39	—	3.06 (3H, d, J=4.7 Hz, CH_3), 7.04—8.62 (6H, m, ArH), 8.73—11.43 (4H, m, –  , OH)
32[c]	MSA	65	—	2.55 (3H, s, CH_3SO_3), 6.96—8.67 (6H, m, ArH), 8.79—9.74 (4H, br, Am^+), 10.43 (1H, s, OH)

a) MSA: methanesulfonic acid. *b*) Refers to crude yield unless otherwise noted. No attempt was made to maximize the yield. *c*) This compound was previously prepared.[1]

ing that compound **41** had the strongest protective effect.

These results also indicated that it is important for a compound to exhibit an anti-complement activity by the oral route to achieve an inhibitory activity of at least 10^{-7} M on C1$\bar{s}$ and hemolysis 200F with considerably high stability on hemolysis 2F, even if its inhibitory activity on C1$\bar{r}$ was on the order of 10^{-5} M.

Conclusion

We synthesized various compounds by modifying guanidino and amidino groups in compound I and found that compound **41** has a potent inhibitory effect not only on complement-mediated hemolysis *in vitro* but also on Fs(*p.o.*) *in vivo*.

Compound **41** is therefore anticipated to be a useful oral therapeutic agent for autoimmune diseases caused by abnormal activation of a complement system. Compound **41** was designated FUT-187.

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. MS were taken on a VG analytical ZAB-HF mass spectrometer. Nuclear magnetic resonance (NMR) spectra were determined on a JEOL JNM-FX-60Q spectrometer using tetramethylsilane as an internal standard. Abbreviations used are as follows: s, singlet; d, doublet; m, multiplet; br, broad; br s, broad singlet; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DCC, dicyclohexylcarbodiimide; MSA, methanesulfonic acid; ArH, aromatic H; Am^+, –.

4-Thioureidobenzoic Acid (1) and 4-(*S*-Methylisothioureido)benzoic Acid

TABLE V. Physicochemical Properties of Ester Derivatives

Compd. No.	Salt[a]	mp (°C)	Recrystn. solvent	Yield[b] (%)	IR ν_{max}^{KBr} cm^{-1} (Ester)	Formula	Analysis (%) Calcd (Found) C	H	N
33	2HCl	265—267	H_2O–MeOH	32	1720	$C_{20}H_{19}N_5O_2 \cdot 2HCl \cdot H_2O$	53.32 (53.11)	5.39 5.12	15.20 15.48)
34	2MSA	265—267	H_2O–acetone	28	1720	$C_{21}H_{21}N_5O_2 \cdot 2CH_4O_3S$	48.38 (48.67)	5.15 5.15	12.33 12.34)
35	2MSA	252—254	H_2O–acetone	36	1730	$C_{20}H_{15}N_5O_2 \cdot 2CH_4O_3S$	47.72 (47.73)	4.90 4.92	12.70 12.65)
36	2MSA	271—272	H_2O–acetone	57	1730	$C_{21}H_{21}N_5O_2 \cdot 2CH_4O_3S$	48.28 (48.67)	5.18 5.15	12.35 12.34)
37	MSA	259—261	Acetone	51	1720	$C_{21}H_{19}N_5O_3 \cdot CH_4O_3S$	348.1461 (348.1422)		[c,d]
38	2MSA	266—268	H_2O–acetone	33	1710	$C_{26}H_{21}N_5O_2 \cdot 2CH_4O_3S$	52.09 (52.25)	4.56 4.54	10.88 10.88)
39	2MSA	268—270	MeOH–acetone	60	1720	$C_{19}H_{15}N_5O_2 \cdot 2CH_4O_3S$	46.58 (46.92)	4.45 4.31	12.86 13.03)
40	2HCl	280—283	H_2O–acetone	46	1720	$C_{21}H_{19}N_5O_2 \cdot 2HCl$	374.1618 (374.1631)		[c]
41	2MSA	248—250	H_2O–EtOH	42	1700	$C_{21}H_{19}N_5O_2 \cdot 2CH_4O_3S$	48.63 (48.84)	4.83 4.81	12.33 12.38)
42	2HCl	285—288	MeOH–acetone	28	1725	$C_{21}H_{17}N_5O_2 \cdot 2HCl$	56.77 (56.23)	4.31 4.38	15.76 [d] 15.68)
43	2MSA	214—215	DMF–EtOH	41	1722	$C_{22}H_{21}N_5O_2 \cdot 2CH_4O_3S$	49.57 (49.73)	5.03 5.04	12.08 12.08)
44	2MSA	245—247	H_2O–acetone	41	1725	$C_{22}H_{21}N_5O_2 \cdot 2CH_4O_3S \cdot H_2O$	48.39 (48.23)	5.23 5.23	11.80 11.72)
45	2MSA	206—208	MeOH–acetone	22	1725	$C_{25}H_{25}N_5O_2 \cdot 2CH_4O_3S \cdot H_2O$	50.85 (50.46)	5.53 5.47	10.98 10.86)
46	2HCl	284—287	H_2O–MeOH	23	1721	$C_{25}H_{19}N_5O_2 \cdot 2HCl \cdot H_2O$	58.71 (58.60)	4.54 4.52	13.74 13.67)
47	2MSA	240—243	H_2O–EtOH	53	1721	$C_{22}H_{21}N_5O_2 \cdot 2CH_4O_3S$	49.70 (49.73)	4.96 5.04	12.14 12.08)
48	2MSA	240—242	H_2O–acetone	37	1720	$C_{20}H_{19}N_5O_2 \cdot 2CH_4O_3S$	47.73 (47.15)	4.92 5.16	12.65 [d] 12.48)
49	2MSA	258—260	H_2O–acetone	60	1720	$C_{21}H_{18}N_5O_2 \cdot 2CH_4O_3S$	48.64 (48.93)	4.97 4.64	12.28 12.40)

a, b) See footnotes *a, b*), Table IV. *c*) Determined by high-resolution mass spectrometry. Upper figure, calculated for $(M+H)^+$ (**37**; $(M+H-CH_2=C=O)^+$) and lower figure, found (*m/z*). *d*) The structures of **37**, **42** and **48** were confirmed by the following spectral data. ^{1}H-NMR (d_6-DMSO) δ: (**37**): 2.14 (3H, s, CH_3CO), 2.44 (3H, s, CH_3SO_3), 7.32—8.82 (12H, m, ArH, –NH– ), 8.89—9.93 (4H, br, Am^+), 10.44 (1H, br s, –CONH–), (**42**): 7.07—8.81 (14H, m, ArH, –NH–  and –CH=CH–), 8.47—9.82 (4H, br, Am^+), 11.17 (1H, s, –NH–), (**48**): 2.46 (6H, s, $CH_3SO_3 \times 2$), 3.11 (3H, d, J=4.7 Hz, CH_3), 7.15—10.83 (18H, m, ArH, –NH–  , ).

(3) Compound **1**[11] was prepared from 4-aminobenzoic acid and ammoniumthiocyanate in 5% HCl, followed by conversion to **3**·HI[11] by refluxing **1** in anhydrous EtOH with CH_3I.

4-(3-Methyl-2-thioureido)benzoic Acid (2) and 4-(3-Methyl-2-methylisothioureido)benzoic Acid (4) Compound **2**[12] was prepared from 4-aminobenzoic acid and methylisothiocyanate followed by conversion to **4**·HI[12] by refluxing **2** in anhydrous MeOH with CH_3I.

4-(3,3-Dimethylguanidino)benzoic Acid (5) To a solution of **3**·HI (10.1 g, 30 mmol) in anhydrous MeOH (30 ml) was added dropwise a slution of dimethylamine (40% in MeOH) (17 g, 150 mmol) at room temperature with stirring. The mixture was stirred overnight at 90 °C, then concentrated *in vacuo*. The residue in 2-PrOH (10 ml) was treated with conc. HCl (15 ml). The resulting precipitate was collected by filtration, and washed with 2-PrOH. The precipitate was collected to give **5**·HCl (6.7 g, 93%). Compounds **6**·HCl,[12] **7**·HCl[12] were prepared from **3**·HI and **4**·HI, respectively, in the same manner.

4-[(4,5-Dihydro-1*H*-imidazol-2-yl)amino]benzoic Acid (8) To a solution of **3**·HI (10.1 g, 30 mmol) in DMF (15 ml) was added ethylene diamine (1.82 g, 30 mmol) at room temperature. The mixture was stirred overnight at 140 °C, then concentrated *in vacuo*, and the residue was washed with AcOEt, then with Et_2O. **8**·HI was treated with conc. HCl (10 ml) at 0 °C, then added to Et_2O with stirring. The resulting precipitate was collected by filtration and washed with 2-PrOH. The precipitate was collected to give **8**·HCl (2.54 g, 35%). **8**·HCl was added to 1 N NaOH at room temperature with stirring, and the solution was neutralized with 1 N HCl with stirring. The resulting precipitate was collected by filtration and washed with water, then treated with MSA in DMF. The solution was added to acetone, and the resulting precipitate was collected to give **8**·MSA. When MeOH was used instead of DMF, the compounds **8**·HCl (45%) and **15**·2MSA (27.6%) were obtained from **3**·HI. **15**·2MSA: IR ν_{max}^{KBr} cm^{-1}: 3450—2900, 1710, 1655, 1622, 1590, 1562. ^{1}H-NMR (DMSO-d_6) δ: 2.42 (6H, s, $CH_3SO_3 \times 2$), 3.49 (4H, br, $-CH_2-CH_2-$), 7.34 (4H, d, J=8.5 Hz, ArH), 7.64—8.69 (10H, m, ArH, –NH–  $\times 2$), 10.66 (2H, s, –NH–  $\times 2$).

Compounds **9**—**14** and **16** were prepared from **3**·HI in the same manner except for compound **14**. **16**·2HCl: IR ν_{max}^{KBr} cm^{-1}: 3450—2900, 1680—1660, 1615, 1590, 1570. ^{1}H-NMR (DMSO-d_6) δ: 1.42—2.29, 2.63—4.08 (6H, each as m, $-(CH_2)_3-$), 7.35 (4H, d, J=8.5 Hz, ArH), 7.75—8.93 (10H, m, ArH, –NH–  $\times 2$), 10.43 (2H, s, –NH–  $\times 2$).

The synthesis of compound **14**·HCl was achieved using aminoacetaldehyde diethylacetal as reaction reagent. Triethylamine was added to the solution of **3**·HI, and the mixture was stirred for 25 h at 90 °C, then concentrated *in vacuo* and the residue in conc. HCl was stirred for 3.5 h at 50 °C. The resulting precipitate was collected to give **14**·HCl.

4-(1-Methylguanidino)benzoic Acid (18) To a suspension of 4-methylaminobenzoic acid HCl (18.7 g, 0.12 mol) in MeOH (150 ml) was added

cyanamide (8.4 g, 0.2 mol) at room temperature. The mixture was stirred overnight at 50 °C, then concentrated *in vacuo* and the residue was washed with AcOEt. The resulting precipitate was collected by filtration, and washed with AcOEt. The precipitate was collected to give **18**·HCl (14.0 g, 50.8%).

Compounds **17**, **19** and **20** were prepared from 4-aminobenzoic acid in the same manner.

4-(2-Aminoethylamino)benzoic Acid (21) A mixture of 4-chlorobenzoic acid (10 g, 63.5 mmol), cuprous chloride (0.5 g, 5 mmol) and ethylenediamine (42 ml, 635 mmol) was refluxed for 36 h. The reaction mixture was concentrated *in vacuo* and 10% HCl (100 ml) was added to the residue. The resulting precipitate was collected to give **21**·2HCl (10.2 g, 63.0%). IR ν_{max}^{KBr} cm^{-1}: 3300, 3150—2700, 1660, 1600, 1525. ^{1}H-NMR (DMSO-d_6) δ: 2.40—3.99 (4H, m, $-CH_2-CH_2-$), 4.66—9.39 (10H, m, ArH, COOH, NH_2, NH_3^+).

4-(2-Imidazoline-2-mercapto-1-yl)benzoic Acid (22) A mixture of **21**·2HCl (10 g, 39.5 mmol), KOH (4.8 g, 86.7 mmol) and CS_2 (2.6 ml, 43.3 mmol) in H_2O (120 ml)–EtOH (30 ml) was refluxed for 18 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was treated with 1 N HCl, and the resulting precipitate was collected to give **22** (8.5 g, 96.9%). IR ν_{max}^{KBr} cm^{-1}: 3200, 2950, 1670, 1600, 1510. ^{1}H-NMR (DMSO-d_6) δ: 3.14—4.64 (4H, m, $-CH_2-CH_2-$), 7.91 (4H, s, ArH), 8.94 (1H, br s, SH).

Compound **25**[13a,b] was prepared from the 3,4-diaminobenzoic acid in the same manner.

4-(2-Thiomethyl-1-imidazolynyl)benzoic Acid (23) To a suspension of **22** (7 g, 31.5 mmol) and $NaHCO_3$ (5.3 g, 63.1 mmol) in DMF (20 ml) was added CH_3I (2.4 ml, 37.8 mmol) at room temperature. The mixture was stirred for 5 h at room temperature, then added to Et_2O. The resulting precipitate was collected by filtration, washed with 10% HCl and then with 2-PrOH. The precipitate was collected to give **23** (6.3 g, 84.4%). IR ν_{max}^{KBr} cm^{-1}: 3150—2700, 1700, 1600, 1545. ^{1}H-NMR (DMSO-d_6) δ: 2.79 (3H, s, CH_3), 3.68—4.74 (4H, m, $-CH_2-CH_2-$), 7.63 (2H, d, J=8.8 Hz, ArH), 8.08 (2H, d, J=8.8 Hz), 11.44 (1H, br s, COOH).

Compound **26** (yield 81.8%) was prepared from **25**[13a,b] in the same manner except for the reaction condition (2 h at 70 °C).

4-(2-Amino-1-imidazolynyl)benzoic Acid (24) Compound **23** (10 g, 42.4 mmol) was aminated with liq. NH_3 solution (250 ml, 20% in MeOH) at 60 °C in a sealed tube for 18 h. The resulting precipitate was collected by filtration and washed with EtOH. The precipitate in 2-PrOH (10 ml) was treated with conc. HCl (14.1 ml, 0.17 mol). The resulting precipitate was collected by filtration, and washed with Et_2O. The precipitate was collected to give **24**·HCl (7.4 g, 72.3%).

Compound **27**[14] was prepared from **26** in the same manner except that amination was done with aq. NH_4OH.

6-(2-Imidazolyl)-2-naphthol (30) 2-Cyano-6-naphthol (4.0 g, 23.7 mmol) was added to a cooled HCl saturated MeOH solution (40 ml), the mixture was stirred overnight at room temperature and Et_2O was added. The resulting precipitate was collected to give **28**·HCl.[1] To a suspension of **28**·HCl in anhydrous MeOH (15 ml) was added ethylenediamine (1.5 g, 23.7 mmol) at 0 °C. The mixture was stirred overnight at room temperature, then concentrated *in vacuo* and to the residue was added conc. HCl (10 ml) in 2-PrOH (10 ml) with stirring. The mixture was separated to obtain the precipitate and the filtrate by filtration. The precipitate was added to the saturated $NaHCO_3$ (100 ml) with stirring, then the resulting precipitate was collected, washed with water and then acetone. A suspension of the precipitate in MeOH (5 ml) was treated with MSA (3 g, 31.2 mmol). Et_2O was added to the solution and the precipitate was collected to give **29**·2MSA (1.6 g, 25.8%). IR ν_{max}^{KBr} cm^{-1}: 3420—2950, 1675, 1620, 1570. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (6H, s, $CH_3SO_3 \times 2$), 3.88 (4H, br, $-CH_2-CH_2-$), 6.85—8.83 (12H, m, ArH), 8.88—11.50 (6H, m, $Am^+ \times 2$). In contrast, the filtrate described above was concentrated *in vacuo*. Compound **30**·MSA (3.7 g, 57.1%) was prepared from the residue *via* a carbonate in the same manner.

6-*N*-Methylamidino-2-naphthol (31) To a suspension of **28**·HCl (4.2 g, 17.8 mmol) in anhydrous MeOH (20 ml) was added methylamine (40% in MeOH (5.5 ml, 71.2 mmol) at 0 °C. The mixture was stirred for 3 h at 0 °C, then concentrated *in vacuo* and the residue was added to the water. The resulting precipitate was collected by filtration, washed with water and then with acetone. The precipitate was added to a cooled HCl saturated MeOH solution (20 ml) and the mixture was stirred for 1 h at 0 °C. Et_2O was then added, and the resulting precipitate was collected to give **31**·HCl (1.64 g, 39.0%).

6-Amidino-2-naphthyl 4-[(4,5-Dihydro-1*H*-imidazol-2-yl)amino]benzoate (41) To a mixture of 4-(2-imidazolynyl)aminobenzoic acid (**8**)·MSA (1.03 g, 3.4 mmol), 6-amidino-2-naphthol (**32**)·MSA[1] (0.96 g, 3.4 mmol), 4-dimethylaminopyridine (42 mg, 0.34 mmol), and DCC (1.05 g, 5.1 mmol) was added dry pyridine (5 ml). The mixture was stirred overnight at room temperature. To this was added acetone (50 ml) and the resulting precipitate was collected by filtration. DMF (30 ml) was added to the precipitate with stirring, the resulting precipitate was removed by filtration and the filtrate was added to acetone (400 ml). The resulting precipitate was collected by filtration to give **41**·2MSA (0.8 g, 41.7%). Alternatively, **8**·HCl (4 g, 16.5 mmol), 6-amidino-2-naphthol (**32**)·MSA (4.67 g, 16.5 mmol) and 4-dimethylaminopyridine (0.2 g, 1.65 mmol) were dissolved in dry pyridine (60 ml) and DCC (5.13 g, 24.9 mmol) was added to the solution. The mixture was stirred overnight at room temperature. The reaction mixture was treated as described above, *via* a carbonate salt, to yield **41**·2HCl (2.35 g, 31.9%). IR ν_{max}^{KBr} cm^{-1}: 3500—2800, 1700, 1670, 1600. ^{1}H-NMR (DMSO-d_6) δ: 2.49 (6H, s, $CH_3SO_3 \times 2$), 3.77 (4H, s, $-CH_2-CH_2-$), 7.10—9.06 (12H, m, ArH, $-NH-C(=NH-^+)-NH-$), 9.09—9.90 (4H, br, Am^+), 11.08 (1H, br s, $-NH-C(=NH-^+)-NH-$). Ester compounds **33**—**47** were prepared from **32** and ester compounds **48** and **49** were prepared from **17** in the same manner.

Biological Method Assay of inhibitory activities toward proteases and the complement-mediated hemolysis. The effectiveness of test 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)[5] for trypsin, plasmin, thrombin, and kallikrein, N^{α}-acetylarginine methyl ester (AAME)[6] for $C1\bar{r}$ and N^{α}-acetyltyrosine ethyl ester (ATEE)[7] 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 diluted 200-fold[8] or 2-fold[9] as the complement source. The effectiveness of test compounds was expressed as IC_{50} (μM) in the case of 200-fold diluted guinea pig serum and the inhibitory % of test compounds at the various concentrations and incubation time in the case of 2-fold diluted serum.

Systemic Forssman Reaction According to the procedure described by Glovsky *et al.*,[10] 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. The compounds were given intravenously 5 min and orally 1 h prior to hemolysin administration.

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