for typical examples of the synthetic method (Scheme I).

N^{α}-Dansyl-L-arginine (12). To a solution of L-arginine (10.8 g, 62 mmol) and K_2CO_3 (23.7 g, 172 mmol) in H_2O (100 mL) was added dansyl chloride (25 g, 92.7 mmol) in Et_2O (100 mL) with stirring at 0–5 °C. After the solution was stirred for 2 days at room temperature, the precipitate was collected, washed with H_2O (30 mL) and Et_2O (30 mL) successively, and then recrystallized from 50% aqueous MeOH to give **12** (20.4 g, 81.0%): mp 199–200 °C.

N^{α}-Dansyl-L-arginine Methyl Ester Dihydrochloride Monohydrate (10). A suspension of **12** (1.0 g, 2.5 mmol) in MeOH (15 mL) was cooled to 0–5 °C and $SOCl_2$ (2.4 g, 25 mmol) was added dropwise with vigorous stirring. After stirring for 2 h at room temperature, the mixture was refluxed for 2 h. After evaporation of the solvent from the mixture, the residue was triturated in cooled H_2O and Et_2O. The solid obtained was recrystallized from MeOH–Et_2O to yield **10** (1.2 g, 92%): mp 147–150 °C.

N^{α}-Dansyl-L-arginine *n*-Butyl Ester Bis(*p*-toluenesulfonate) (15). A mixture of **12** (1.0 g, 2.5 mmol), *p*-TsOH·H_2O (1.0 g, 5.3 mmol), and *n*-BuOH (10 mL) was heated for 30 min at 100 °C. After the addition of benzene (100 mL), the mixture was refluxed for 3 h with removing water by azeotropic distillation. The solvent was evaporated in vacuo and the residue was triturated in petroleum ether–Et_2O, filtered, and recrystallized from acetone to give **15** (1.9 g, 95%): mp 160–164 °C.

Inhibition Studies of Clotting Activity of Thrombin. To the mixture of 0.8 mL of 0.12% fibrinogen dissolved in borate–saline buffer (pH 7.4) and 0.1 mL of various concentrations of the inhibitor to be tested was added 0.1 mL of 5 units/mL bovine thrombin (Mochida Pharmaceutical). The assay was carried out at 25 °C, and the time from the addition of thrombin to the formation of a clot was recorded.

Hydrolysis Studies by Thrombin and Trypsin. The rates of hydrolysis of test compounds were determined titrimetrically at 37 °C and pH 8.0 with a pH-stat fitted with an automatic burette (Toa Electronics). The reaction mixture contained 1 mM test compounds, 0.15 M KCl, and 50 units of thrombin or 1.8 μg of trypsin in a total volume of 7 mL.

Acknowledgment. The authors thank M. Sugano and A. Sugiyama for their technical assistance and Dr. S. Hattori, General Manager of Biosciences Laboratory, Central Research Laboratories, Mitsubishi Chemical Industries Limited, for his valuable advice and encouragement throughout the work. We are also indebted to members in Systems Engineering Laboratory, Central Research Laboratories, Mitsubishi Chemical Industries Limited, for elemental analyses.

Thrombin Inhibitors. 2. Amide Derivatives of N^{α}-Substituted L-Arginine

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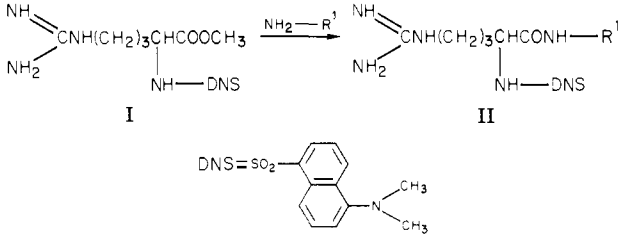
A series of N^{α}-(arylsulfonyl)-L-arginine amide derivatives with substituted or unsubstituted naphthalene and heterocyclic compounds as the N^{α}-substituent was prepared and tested as inhibitors of the clotting activity of thrombin. *N*-*n*-Butyl and *N*-*n*-butyl-*N*-methyl derivatives of N^{α}-dansyl-L-arginine amide were the most inhibitory of *N*-alkyl and *N*,*N*-dialkyl derivatives of N^{α}-dansyl-L-arginine amide. Their inhibitory effect was as potent as that of N^{α}-dansyl-L-arginine *n*-butyl ester, with an I_{50} of 2×10^{-6} M. N^{α}-Substituted naphthalenesulfonyl-L-arginine amide derivatives of 4-methyl- and 4-ethylpiperidine also showed a potent inhibition with an I_{50} of 10^{-7} to 10^{-6} M. The most potent inhibitor in this study was 1-[N^{α}-(4,6-dimethoxynaphthalene-2-sulfonyl)-L-arginyl]-4-methylpiperidine, with an I_{50} of 7.5×10^{-8} M. Arginine amide derivatives of 4-methyl- or 4-ethylpiperidine with tetralin or an oxygen-containing heterocyclic compound as a N^{α}-substituent showed an inhibition with an I_{50} less than 10^{-5} M. N-Monosubstituted derivatives of N^{α}-dansyl-L-arginine amide were not hydrolyzed at all by thrombin and were hydrolyzed very slowly by trypsin, and N,N-disubstituted derivatives were not hydrolyzed at all by both enzymes.

N^{α}-Dansyl-L-arginine *n*-propyl or *n*-butyl ester has been shown to inhibit the clotting activity of thrombin but to be hydrolyzed by thrombin and trypsin.[1] Therefore, these compounds are considered to be hydrolyzed easily in vivo and would be inappropriate as an antithrombotic agent. It is, however, noteworthy that compounds with a high affinity to thrombin were found in L-arginine derivatives. We therefore aimed to obtain thrombin-inhibitory L-arginine amide derivatives which have the advantage of protection from hydrolysis. In this paper, we synthesized various N^{α}-(arylsulfonyl)-L-arginine amide derivatives with substituted or unsubstituted naphthalene and heterocyclic compounds in N^{α}-substituents and examined the inhibitory effect on thrombin in order to investigate the relationship between a structure and an inhibitory effect on thrombin. A part of this work has been reported briefly[2] and presented orally.[3,4]

Scheme I



(1) S. Okamoto, K. Kinjo, A. Hijikata, R. Kikumoto, Y. Tamao, K. Ohkubo and S. Tonomura, *J. Med. Chem.*, preceding paper in this issue.

(2) S. Okamoto, A. Hijikata, K. Kinjo, R. Kikumoto, K. Ohkubo, S. Tonomura, and Y. Tamao, *Kobe J. Med. Sci.*, **21**, 43 (1975).

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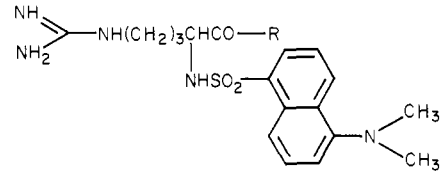
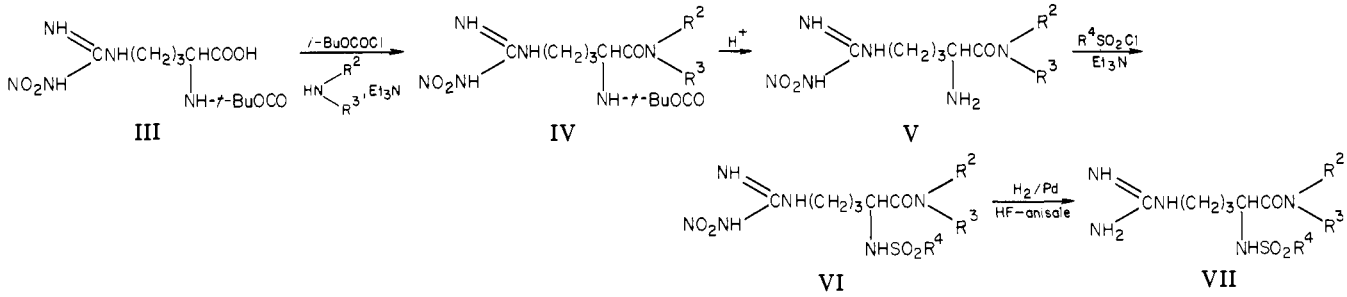
Table I. N^{α}-Dansylarginine Amide Derivatives



no.[a]	R	mp,[b] °C	formula[c]	I_{50},[d] M
1	NHC_2H_5	220-222	$C_{20}H_{30}N_6O_3S\cdot H_2O$	1.0×10^{-4}
2	NH-*n*-C_3H_7	150-153	$C_{21}H_{32}N_6O_3S\cdot H_2O$	1.5×10^{-5}
3	NH-*n*-C_4H_9	145-148	$C_{22}H_{34}N_6O_3S\cdot H_2O$	2.1×10^{-6}
4	NH-*n*-C_5H_{11}	140-143	$C_{23}H_{36}N_6O_3S\cdot H_2O$	1.3×10^{-4}
5	NH-*n*-C_6H_{13}	133-135	$C_{24}H_{38}N_6O_3S\cdot H_2O$	2.0×10^{-5}
6	NH-*n*-C_7H_{15}	240-243	$C_{25}H_{40}N_6O_3S$	1.0×10^{-4}
7	$NHCH(CH_3)_2$	218-220.5	$C_{21}H_{32}N_6O_3S$	1.1×10^{-3}
8	$NHCH_2CH(CH_3)_2$	157-160	$C_{22}H_{34}N_6O_3S$	1.0×10^{-4}
9	$NHCH_2CH{=}CH_2$	140-145	$C_{21}H_{30}N_6O_3S\cdot H_2O$	1.0×10^{-5}
10	NH-c-C_3H_5	165-168	$C_{21}H_{30}N_6O_3S\cdot 2H_2O$	5.0×10^{-4}
11	NH-c-C_6H_{11}	*e*	$C_{24}H_{36}N_6O_3S$[f]	5.0×10^{-4}
12	$NHCH_2$-c-C_6H_{11}	253-256	$C_{25}H_{38}N_6O_3S$	4.0×10^{-5}
13	$NHCH_2$-C_6H_5	244-246	$C_{25}H_{32}N_6O_3S$	5.0×10^{-5}
14	$NHCH_2CH_2$-C_6H_5	143-147	$C_{26}H_{34}N_6O_3S$	1.5×10^{-4}
15	$NHCH_2$-C_6H_4-OCH_3	143-148	$C_{26}H_{34}N_6O_4S\cdot 2H_2O$	5.0×10^{-4}
16	$NH(CH_2)_2OCH_3$	130-135	$C_{21}H_{32}N_6O_4S\cdot 2H_2O$	4.0×10^{-6}
17	$NH(CH_2)_3OCH_3$	*e*	$C_{22}H_{34}N_6O_4S$	6.0×10^{-5}
18	$NH(CH_2)_2OC_2H_5$	230-232	$C_{22}H_{34}N_6O_4S$	5.0×10^{-6}

[a] These samples, except 11, were synthesized via the general route illustrated in Scheme I. See Experimental Section. [b] Melting points are uncorrected. [c] All compounds were analyzed for C, H, N, S, and halogen; analytical results were within ±0.4% of the theoretical values. [d] I_{50} was defined as the concentration at which the clotting time was prolonged by twice the control. [e] Amorphous solid. [f] $2CH_3COOH$ salt. This sample was synthesized via the general route illustrated in Scheme II. See Experimental Section.

Scheme II



Chemistry. N^{α}-Dansyl-L-arginine amide derivatives of primary amines listed in Table I, except **11**, were synthesized by displacement of the methyl ester portion of the ester N^{α}-dansyl-L-arginine methyl ester described previously[1] with primary amines, as outlined in Scheme I. Other compounds were synthesized via the general route illustrated in Scheme II. N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-nitro-L-arginine (III) was condensed with the appropriate amine by the mixed anhydride method using isobutyl chloroformate and Et_3N in dry THF. After the removal of the Boc group of IV by HCl in AcOEt or CF_3COOH, the amino group of V was sulfonylated with various arylsulfonyl chlorides in the presence of Et_3N in $CHCl_3$. Hydrogenolysis (Pd/H_2) or acidolysis (HF, anisol) of IV afforded N^{α}-(arylsulfonyl)-L-arginine amides.

Enzyme Results and Discussion

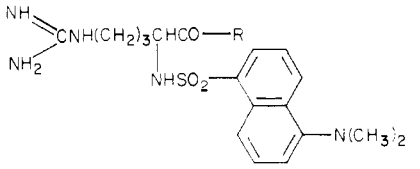
N^{α}-Dansyl-L-arginine *n*-butylamide (**3**) was the most inhibitory to thrombin of the N^{α}-dansyl-L-arginine *n*-alkylamides. The less potent inhibition was observed with *n*-alkylamides whose alkyl carbon number was less than three or more than five, such as **1**, **2**, and **4**–**6**, and other bulky alkyl amides such as **7** and **8**. Although this relationship was very similar to that for N^{α}-dansyl-L-arginine ester derivatives, the effect of the introduction of an *n*-butyl group was more striking in amide derivatives than in ester derivatives. Namely, the effect of the *n*-butylamide was as potent as that of the *n*-butyl ester, with an I_{50} of 10^{-6} M, while ethylamide **1** was 10 times less potent than ethyl ester.[1]

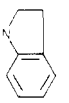
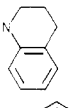
We further investigated the effect of amide derivatives of secondary amines (Table II). *N*-*n*-Butyl-*N*-methylamide **19** retained the high inhibitory potency of *n*-butylamide **3**, but *N*-*n*-butyl-*N*-ethylamide **20** and *N*,*N*-di-*n*-butylamide **21** were less inhibitory than *n*-butylamide **3**. All of the five- to nine-membered unsubstituted cyclic amide derivatives, **24**–**28**, inhibited thrombin potently with an I_{50} of 9×10^{-7} to 3.3×10^{-6} M. Therefore, we selected a six-membered cyclic amide, piperidine derivative, to investigate the effect of methyl group substitution at the 2, 3, and 4 positions of piperidine on affinity to the enzyme

(3) The United States–Japan Cooperative Seminar on Hemorheology and Thrombosis, Kobe, Japan, 1975, Proceedings; see: (a) S. Okamoto, A. Hijikata, K. Ikezawa, K. Kinjo, R. Kikumoto, S. Tonomura, and Y. Tamao, *Thromb. Res., Suppl.* 2, 8, 77 (1976); (b) A. Hijikata, S. Okamoto, E. Mori, K. Kinjo, R. Kikumoto, S. Tonomura, Y. Tamao, and H. Hara, *ibid.*, 8, 83 (1976).

(4) R. Kikumoto, S. Okamoto, Y. Tamao, and A. Hijikata, International Committee on Thrombosis and Haemostasis, 22nd Annual Meeting, Kyoto, Japan, 1976.

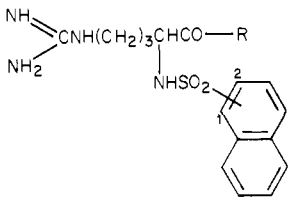
Table II. N^{α}-Dansyl-L-arginine Amide Derivatives of Secondary Amine

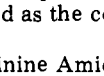


no.[a]	R	formula[b,c]	I_{50},[d] M
19	$N(CH_3)$-*n*-C_4H_9	$C_{25}H_{36}N_6O_3S \cdot 2CH_3COOH$	2.0×10^{-6}
20	$N(C_2H_5)$-*n*-C_4H_9	$C_{26}H_{38}N_6O_3S \cdot 2CH_3COOH$	5.0×10^{-5}
21	$N(n\text{-}C_4H_9)_2$	$C_{28}H_{42}N_6O_3S \cdot 2CH_3COOH$	4.0×10^{-5}
22	$N(C_2H_5)_2$	$C_{22}H_{34}N_6O_3S \cdot 2CH_3COOH$	5.5×10^{-5}
23	$N(CH_3)CH_2\text{-}C_6H_5$	$C_{26}H_{34}N_6O_3S \cdot 2CH_3COOH$	7.0×10^{-6}
24	$N\frown(CH_2)_4$	$C_{22}H_{32}N_6O_3S \cdot 2CH_3COOH$	3.3×10^{-6}
25	$N\frown(CH_2)_5$	$C_{23}H_{34}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-6}
26	$N\frown(CH_2)_6$	$C_{24}H_{36}N_6O_3S \cdot 2CH_3COOH$	9.0×10^{-7}
27	$N\frown(CH_2)_7$	$C_{25}H_{38}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-6}
28	$N\frown(CH_2)_8$	$C_{26}H_{40}N_6O_3S \cdot 2CH_3COOH$	2.0×10^{-6}
29		$C_{24}H_{36}N_6O_3S \cdot 2CH_3COOH$	1.3×10^{-6}
30		$C_{24}H_{36}N_6O_3S \cdot 2CH_3COOH$	6.5×10^{-6}
31		$C_{24}H_{36}N_6O_3S \cdot 2CH_3COOH$	3.0×10^{-7}
32		$C_{25}H_{38}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-7}
33		$C_{25}H_{38}N_6O_3S \cdot 2CH_3COOH$	8.0×10^{-6}
34		$C_{26}H_{40}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-6}
35		$C_{26}H_{40}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-6}
36		$C_{27}H_{42}N_6O_3S \cdot 2CH_3COOH$	2.0×10^{-6}
37		$C_{29}H_{38}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-4}
38		$C_{24}H_{36}N_6O_4S \cdot 2CH_3COOH$	4.0×10^{-6}
39		$C_{26}H_{38}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-5}
40		$C_{22}H_{32}N_6O_4S \cdot 2CH_3COOH$	6.0×10^{-7}
41		$C_{22}H_{32}N_6O_4S \cdot 2CH_3COOH$	2.0×10^{-6}
42		$C_{24}H_{36}N_6O_4S \cdot 2CH_3COOH$	2.0×10^{-5}
43		$C_{26}H_{32}N_6O_3S \cdot 2CH_3COOH$	1.0×10^{-6}
44		$C_{27}H_{34}N_6O_3S \cdot 2CH_3COOH$	1.7×10^{-5}
45		$C_{26}H_{32}N_6O_3S \cdot 2CH_3COOH$	6.7×10^{-7}
46		$C_{23}H_{35}N_7O_3S \cdot 3CH_3COOH$	2.0×10^{-6}
47		$C_{24}H_{35}N_7O_4S \cdot 2CH_3COOH$	3.5×10^{-6}

[a] These samples were synthesized via general route illustrated in Scheme II. See Experimental Section. [b] All compounds were amorphous solids. [c] All compounds were analyzed for C, H, N, S, and halogen; analytical results were within ±0.4% of the theoretical values. [d] I_{50} was defined as the concentration at which the clotting time was prolonged by twice the control.

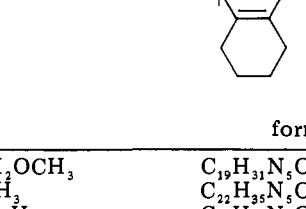
Table III. N^{α}-(Naphthalenesulfonyl)-L-arginine Amide Derivatives



no.[a]	substituted position of SO_2-	R	formula[b,c]	I_{50},[d] M
48	1	NH-*n*-C_4H_9	$C_{20}H_{29}N_5O_3S\cdot CH_3COOH$	5.0×10^{-5}
49	1	N(CH_3)-*n*-C_4H_9	$C_{21}H_{31}N_5O_3S\cdot CH_3COOH$	5.0×10^{-5}
50	1		$C_{22}H_{31}N_5O_3S\cdot CH_3COOH$	4.0×10^{-6}
51	1		$C_{23}H_{33}N_5O_3S\cdot CH_3COOH$	1.0×10^{-6}
52	1		$C_{22}H_{31}N_5O_3S\cdot CH_3COOH$	1.0×10^{-5}
53	1		$C_{20}H_{27}N_5O_4S\cdot CH_3COOH$	2.0×10^{-5}
54	1		$C_{27}H_{33}N_5O_3S\cdot CH_3COOH$	1.0×10^{-4}
55	2	N(CH_3)-*n*-C_4H_9	$C_{21}H_{31}N_5O_3S\cdot CH_3COOH$	5.0×10^{-5}
56	2		$C_{22}H_{31}N_5O_3S\cdot CH_3COOH$	4.5×10^{-6}
57	2		$C_{23}H_{33}N_5O_3S\cdot CH_3COOH$	1.0×10^{-6}
58	2		$C_{22}H_{31}N_5O_3S\cdot CH_3COOH$	2.0×10^{-5}
59	2		$C_{20}H_{27}N_5O_3S\cdot CH_3COOH$	2.0×10^{-5}

[a] These samples were synthesized via the general route illustrated in Scheme II. See Experimental Section. [b] All compounds were amorphous solids. [c] All compounds were analyzed for C, H, N, and S; analytical results were within ±0.4% of the theoretical values. [d] I_{50} was defined as the concentration at which the clotting time was prolonged by twice the control.

Table IV. N^{α}-(Tetralinsulfonyl)-L-arginine Amide Derivatives



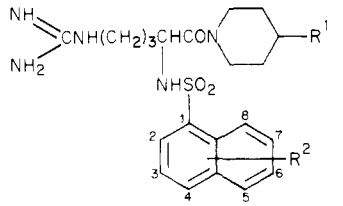
no.[a]	substituted position of SO_2-	R	formula[b,c]	I_{50},[d] M
60	1	$NHCH_2CH_2OCH_3$	$C_{19}H_{31}N_5O_4S\cdot CH_3COOH$	4.0×10^{-5}
61	1	c-NC_5H_9-CH_3	$C_{22}H_{35}N_5O_3S\cdot CH_3COOH$	1.0×10^{-6}
62	1	c-NC_5H_9-C_2H_5	$C_{23}H_{37}N_5O_3S\cdot CH_3COOH$	5.0×10^{-7}
63	1	c-$N(CH_2CH_2)_2O$	$C_{20}H_{31}N_5O_4S\cdot CH_3COOH$	6.5×10^{-6}
64	2	NH-*n*-C_4H_9	$C_{20}H_{33}N_5O_3S\cdot CH_3COOH$	5.0×10^{-6}
65	2	c-NC_5H_9-CH_3	$C_{22}H_{35}N_5O_3S\cdot CH_3COOH$	6.0×10^{-7}
66	2	c-NC_5H_9-C_2H_5	$C_{23}H_{37}N_5O_3S\cdot CH_3COOH$	7.0×10^{-7}
67	2	c-NC_5H_9	$C_{20}H_{31}N_5O_3S\cdot CH_3COOH$	7.0×10^{-6}

[a-d] See corresponding footnotes in Table III.

active site. 1-(N^{α}-Dansyl-L-arginyl)-4-methylpiperidine (**31**) was found to be the most inhibitory of the methyl-substituted piperidine derivatives, and the inhibitory potency decreased in the order of 3-methylpiperidine **30**, 2-methylpiperidine **29**. Since the substitution at the 4 position of piperidine afforded the best inhibitor, a series of 4-substituted piperidine derivatives was examined for the thrombin inhibitory effect. 1-(N^{α}-Dansyl-L-arginyl)-4-ethylpiperidine (**32**) was more inhibitory than the 4-methylpiperidine derivative **31** and was the best inhibitor of substituted piperidine derivatives. The introduction of other bulky groups to the piperidine ring resulted in a slight decrease in the inhibition potency, with the exception of the 4-phenylpiperidine derivative **37** in which much less activity was observed. It is of interest that derivatives of 4,4-dimethylpiperidine **33**, 4-methylpiperazine **46**, and 4-acetylpiperazine **47** were approximately 10 times less inhibitory than the 4-methylpiperidine derivative **31**. N^{α}-Dansyl-L-arginine amide derivatives of morpholine **40** and thiomorpholine **41**, whose molecular size are similar to that of piperidine, were as inhibitory as piperidine derivative **25**. Derivatives of indoline **43** and isoindoline **45** were also as inhibitory as **25**, although indoline or isoindoline are relatively bulky groups.

Subsequently, we examined the thrombin inhibitory effect of N^{α}-(arylsulfonyl)-L-arginine derivatives having the typical structures in the carboxamide portion (Tables III and IV). The variation in the thrombin inhibitory effect

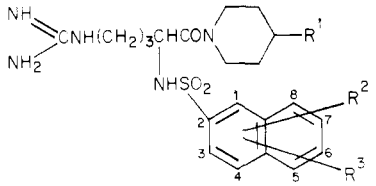
Table V. N^{α}-Substituted (Naphthalene-1-sulfonyl)-L-arginine Amide Derivatives



no.[a]	R^1	R^2	formula[b,c]	I_{50},[d] M
68	C_2H_5	4-OCH_3	$C_{24}H_{35}N_5O_4S\cdot CH_3COOH$	2.0×10^{-6}
69	CH_3	5-OCH_3	$C_{23}H_{33}N_5O_4S\cdot CH_3COOH$	3.0×10^{-7}
70	C_2H_5	5-OCH_3	$C_{24}H_{35}N_5O_4S\cdot CH_3COOH$	6.0×10^{-7}
71	CH_3	8-OCH_3	$C_{23}H_{33}N_5O_4S\cdot CH_3COOH$	4.0×10^{-6}
72	C_2H_5	5-OC_2H_5	$C_{25}H_{37}N_5O_4S\cdot CH_3COOH$	1.0×10^{-7}
73	C_2H_5	5-O-*n*-C_3H_7	$C_{26}H_{39}N_5O_4S\cdot CH_3COOH$	5.0×10^{-7}
74	C_2H_5	5-O-*i*-C_3H_7	$C_{26}H_{39}N_5O_4S\cdot CH_3COOH$	4.0×10^{-7}
75	C_2H_5	7-CH_3	$C_{24}H_{35}N_5O_3S\cdot CH_3COOH$	1.0×10^{-6}
76	C_2H_5	5-$N(C_2H_5)_2$	$C_{27}H_{42}N_6O_3S\cdot 2CH_3COOH$	3.0×10^{-7}
77	C_2H_5	5-OH	$C_{23}H_{33}N_5O_4S\cdot CH_3COOH$	6.0×10^{-6}
78	C_2H_5	5-CN	$C_{24}H_{32}N_6O_4S\cdot CH_3COOH$	6.0×10^{-6}
79	C_2H_5	5-Cl	$C_{23}H_{32}ClN_5O_3S\cdot CH_3COOH$	2.0×10^{-6}
80	C_2H_5	5-NO_2	$C_{23}H_{32}N_6O_5S\cdot CH_3COOH$	1.0×10^{-6}
81	C_2H_5	5-COOH	$C_{24}H_{33}N_5O_5S$	1.0×10^{-3}

[a-d] See corresponding footnotes in Table III.

Table VI. N^{α}-Substituted (Naphthalene-2-sulfonyl)-L-arginine Amide Derivatives



no.[a]	R^1	R^2	R^3	formula[b,c]	I_{50},[d] M
82	C_2H_5	H	1-OCH_3	$C_{24}H_{35}N_5O_4S\cdot CH_3COOH$	4.0×10^{-7}
83	C_2H_5	H	4-OCH_3	$C_{24}H_{35}N_5O_4S\cdot CH_3COOH$	1.0×10^{-6}
84	C_2H_5	H	6-OCH_3	$C_{24}H_{35}N_5O_4S\cdot CH_3COOH$	2.0×10^{-7}
85	CH_3	H	7-OCH_3	$C_{23}H_{33}N_5O_4S\cdot CH_3COOH$	1.0×10^{-7}
86	C_2H_5	H	7-OCH_3	$C_{24}H_{35}N_5O_4S\cdot CH_3COOH$	1.0×10^{-7}
87	C_2H_5	H	6-O-*i*-C_3H_7	$C_{26}H_{39}N_5O_4S\cdot CH_3COOH$	8.0×10^{-7}
88	CH_3	4-OCH_3	6-OCH_3	$C_{24}H_{35}N_5O_5S\cdot CH_3COOH$	7.5×10^{-8}
89	C_2H_5	4-OCH_3	6-OCH_3	$C_{25}H_{37}N_5O_5S\cdot CH_3COOH$	1.0×10^{-7}
90	CH_3	6-OCH_3	7-OCH_3	$C_{24}H_{35}N_5O_5S\cdot CH_3COOH$	1.5×10^{-7}
91	C_2H_5	6-OCH_3	7-OCH_3	$C_{25}H_{37}N_5O_5S\cdot CH_3COOH$	1.5×10^{-7}
92	C_2H_5	6-OC_2H_5	7-OC_2H_5	$C_{27}H_{41}N_5O_5S\cdot CH_3COOH$	5.5×10^{-6}
93	C_2H_5	H	6-CH_3	$C_{24}H_{35}N_5O_3S\cdot CH_3COOH$	5.0×10^{-7}
94	C_2H_5	H	6-C_2H_5	$C_{25}H_{37}N_5O_3S\cdot CH_3COOH$	7.0×10^{-7}
95	C_2H_5	H	7-CH_3	$C_{24}H_{35}N_5O_3S\cdot CH_3COOH$	4.0×10^{-7}
96	C_2H_5	H	6-$N(CH_3)_2$	$C_{25}H_{38}N_6O_3S\cdot 2CH_3COOH$	3.0×10^{-7}
97	C_2H_5	H	7-OH	$C_{23}H_{33}N_5O_4S\cdot CH_3COOH$	6.0×10^{-6}

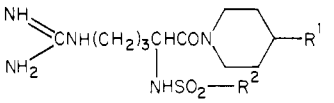
[a-d] See corresponding footnotes in Table III.

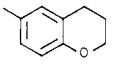
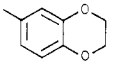
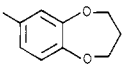
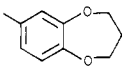
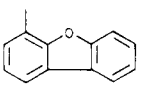
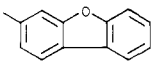
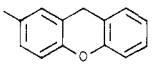
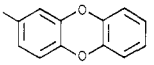
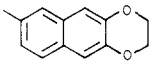
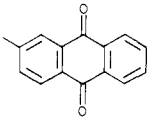
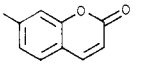
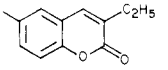
due to the variation in the structure of the carboxamide portion was found comparable to that shown in the study of N^{α}-dansyl-L-arginine amide derivatives. Amide derivatives of N^{α}-(naphthalenesulfonyl)arginine and N^{α}-(tetralinsulfonyl)arginine showed $^1/_{10}$ to $^1/_{30}$ and $^1/_2$ to $^1/_{10}$ as potent inhibition of thrombin, respectively, than the corresponding amide derivatives of N^{α}-dansylarginine, and amide derivatives of 4-methyl- or 4-ethylpiperidine were found the most inhibitory. These results suggest that both the carboxamide portion and the N^{α}-substituent portion of arginine derivatives contribute to the binding to thrombin independently.

Therefore, we investigated the effect of the variation in the structure of the N^{α}-substituent portion on the thrombin inhibition in arginine derivatives with a carboxamide portion fixed to the amide derivative of 4-methyl- or 4-ethylpiperidine. The substitution at the 5 position, as in **69**, **70**, **72–74**, and **76**, but not at the 4 or 8 position, as in **68** and **71**, of the naphthalene ring of a N^{α}-(naphthalene-1-sulfonyl)arginine derivative resulted in an increase in inhibitory potency (Table V). The introduction of OH (**77**), CN (**78**), Cl (**79**), and NO_2 (**80**) into the 5 position scarcely influenced the inhibitory potency. These results suggest that the binding affinity does not depend upon the inductive effect of substituents but upon the bulkiness and hydrophobicity of the substituents. The introduction of a carboxyl group (**81**) resulted in the loss of the inhibitory potency. The negative charge at the 5 position is considered to hinder electrically the binding to thrombin.

The effect of the substitution to a naphthalene ring was further examined with N^{α}-(naphthalene-2-sulfonyl)-L-arginine derivatives. The substitution with a methoxy group at the 1, 6, or 7 position (**82** and **84–86**) increased the inhibitory effect significantly (Table VI), but a substitution at the 4 position, as in **83**, did not affect the inhibitory potency of unsubstituted naphthalene derivative **57**.

Table VII. Derivatives with Heterocyclic Compounds Containing Oxygen



no.[a]	R^1	R^2	formula[b,c]	I_{50},[d] M
98	C_2H_5		$C_{22}H_{35}N_5O_4S\cdot CH_3COOH$	1.5×10^{-5}
99	C_2H_5		$C_{21}H_{33}N_5O_5S\cdot CH_3COOH$	2.5×10^{-5}
100	CH_3		$C_{21}H_{33}N_5O_5S\cdot CH_3COOH$	8.0×10^{-6}
101	C_2H_5		$C_{22}H_{35}N_5O_5S\cdot CH_3COOH$	5.0×10^{-6}
102	C_2H_5		$C_{25}H_{33}N_5O_4S\cdot CH_3COOH$	2.0×10^{-7}
103	C_2H_5		$C_{25}H_{33}N_5O_4S\cdot CH_3COOH$	3.0×10^{-7}
104	C_2H_5		$C_{26}H_{35}N_5O_4S\cdot CH_3COOH$	3.0×10^{-7}
105	C_2H_5		$C_{25}H_{33}N_5O_5S\cdot CH_3COOH$	1.5×10^{-7}
106	C_2H_5		$C_{25}H_{35}N_5O_5S\cdot CH_3COOH$	1.0×10^{-5}
107	C_2H_5		$C_{27}H_{33}N_5O_5S\cdot HF$	1.5×10^{-6}
108	C_2H_5		$C_{22}H_{31}N_5O_5S\cdot HF$	2.0×10^{-5}
109	C_2H_5		$C_{24}H_{35}N_5O_5S\cdot HF$	1.0×10^{-6}

[a-d] See corresponding footnotes in Table III.

However, the remarkable increase in the inhibitory potency was observed with dimethoxy substitution at the 4 and 6 position (**88** and **89**), whereas in 6,7-dimethoxy substitution (**90** and **91**) no increase was observed in the inhibitory potency by disubstitution. The derivatives with a methyl or ethyl group at the 6 or 7 position (**93–95**) also showed the potent inhibitory effect with an I_{50} at the 10^{-7} M level.

Since (tetralinsulfonyl)arginine derivatives were more inhibitory than (naphthalenesulfonyl)arginine derivatives as described above, derivatives with other heterocyclic compounds as the N^{α}-substituent were synthesized and examined for their thrombin inhibitory effect. The compounds containing one or two oxygen atoms in a saturated ring decreased the inhibitory potency over 10 times more than the (tetralin-2-sulfonyl)arginine derivative (**61** or **62**), if the oxygen-containing ring is situated at a terminal end of the compound, like in **98–101** and **106** (Table VII). However, the derivatives with a heterocyclic compound containing oxygen in the ring system in which the oxygen-containing ring is not situated at a terminal end, like in **102–105**, showed the potent inhibitory effect with I_{50} at the 10^{-7} M level.

Several compounds with a high inhibitory potency were examined for the susceptibility to hydrolysis by thrombin and trypsin (Table VIII). When incubated with a large amount of thrombin, no compounds tested were hydrolyzed when incubated for 4 h. As for hydrolysis by trypsin,

Table VIII. Hydrolysis of N^{α}-Dansyl-L-arginine Amide Derivatives

	rate of hydrolysis, %	
no.	thrombin[a]	trypsin[b]
3	0	~50
16	0	~50
19	0	0
25	0	0
32	0	0

[a] Values in 4-h incubation. [b] Values in 6-h incubation.

it was observed that *N,N*-disubstituted amide derivatives were not hydrolyzed at all, but N-monosubstituted amide derivatives were hydrolyzed only slowly by large amounts of trypsin. Thus, amide derivatives of N^{α}-dansyl-L-arginine were found to be strikingly stable toward hydrolysis by thrombin or trypsin in comparison with ester derivatives.

1-(N^{α}-Dansyl-L-arginyl)-4-ethylpiperidine has been shown to inhibit thrombin in a competitive manner by the authors[2,5] and recently by others.[6] It has been also shown that its inhibition is highly specific to thrombin and is

(5) A. Hijikata, S. Okamoto, R. Kikumoto, and Y. Tamao, *Thromb. Haemostasis*, **42**, 1039 (1979).
(6) M. E. Nesheim, F. G. Prendergast, and K. G. Mann, *Biochemistry*, **18**, 996 (1979).

more than 100 times weaker for trypsin, plasmin, reptilase, and factor X_a.[2,5,6]

Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The results of elemental analyses were within ±0.4% of the theoretical values. Compounds were checked by IR on a JASCO IR-A2, and the spectral data were consistent with the assigned structure in all cases. TLC was performed on fluorescent silica gel plates (Merck) to a distance of 20 cm with a solvent system of *n*-BuOH–acetic acid–water (3:1:1), and spots were detected under a UV lamp or by exposure to I_2 vapor. The purity of compounds were also checked by high-performance LC (Shimadzu LC-3A) on a Zorbax ODS column (Du pont) with a solvent system of MeOH–water–Pic A (Waters) (65:42:1.5), and peaks were detected by UV (254 nm). In the following, experimental details are presented for typical examples of the synthetic method.

N^{α}-Dansyl-L-arginine *n*-Butylamide Monohydrate (3). N^{α}-Dansyl-L-arginine methyl ester dihydrochloride hydrate (1.0 g, 2.0 mmol) was dissolved in *n*-butylamine (2 mL) with vigorous agitation. After 2 days at room temperature, *n*-butylamine was evaporated in vacuo. The residual syrup was triturated in H_2O–Et_2O, filtered, and recrystallized from 50% aqueous MeOH to yield **3** (0.84 g, 90%), mp 150–152 °C.

1-[N^{α}-Dansyl-L-arginyl]-4-methylpiperidine Diacetate (31). A. Condensation of N^{α}-(*tert*-Butyloxycarbonyl)-N^{ω}-nitro-L-arginine (III) with 4-Methylpiperidine. A solution of III (3.2 g, 10 mmol) and Et_3N (1.0 g, 10 mmol) in anhydrous THF (40 mL) was cooled to −25 °C and isobutyl chloroformate (1.36 g, 10 mmol) was added. After 15 min, a solution of 4-methylpiperidine (0.99 g, 10 mmol) in anhydrous THF (5 mL) was added and the reaction mixture was maintained at −25 °C for 10 min and then allowed to warm to room temperature for 40 min. The reaction mixture was concentrated under reduced pressure at below 40 °C. The residue was extracted with AcOEt (100 mL), washed (10% aqueous citric acid, saturated $NaHCO_3$, and saturated NaCl), dried (Na_2SO_4), and evaporated to give 1-[N^{α}-(*tert*-butyloxycarbonyl)-N^{ω}-nitro-L-arginyl]-4-methylpiperidine (IV; 2.8 g, 70%).

B. Removal of the *tert*-Butyloxycarbonyl Group and N^{α}-Sulfonylation with Dansyl Chloride. Compound IV (2.8 g), without further purification, was dissolved in AcOEt (50 mL) containing 10% dry HCl and stirred for 2 h. Cold Et_2O (50 mL) was added, and the precipitated material, 4-methyl-1-(N^{ω}-nitro-L-arginyl)piperidine hydrochloride (V), was centrifuged and washed with Et_2O (2 × 20 mL) by successive centrifugation and decantation, the precipitate being well suspended in each wash by vortex mixing. The product was dried in vacuo. To a mixture of V (2.1 g, 6.2 mmol) and Et_3N (1.9 g, 19 mmol) in CH_2Cl_2 (30 mL) was added dansyl chloride (2.0 g, 7.4 mmol) with stirring at 0–5 °C. After 4 h, the mixture was washed with H_2O, dried (Na_2SO_4), and evaporated. The oily residue was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (97:3). The eluate gave 1-(N^{α}-dansyl-N^{ω}-nitro-L-arginyl)-4-methylpiperidine (VI; 3.1 g, 95%) as amorphous solids.

C. Removal of the NO_2 Group. Compound VI (2.0 g, 3.7 mmol) was dissolved in EtOH (20 mL) and AcOH (5 mL), and Palladium black (0.5 g) was added. H_2 was bubbled into the mixture for 2 days at room temperature. After filtering off the catalyst, the filtrate was evaporated to give a viscous oily product. Reprecipitation from MeOH–Et_2O gave **31** (1.8 g, 80%) as amorphous solids.

Free 31. Compound **31** (1.0 g, 1.6 mmol) was dissolved in H_2O (10 mL) and the pH of the solution was adjusted to pH 11.5 with NaOH. After the solution was stirred for 2 h in an ice bath, the precipitate was collected. Recystallization from 50% aqueous MeOH gave free **31** (0.75 g, 95%), mp 212 °C (decomposed). Anal. ($C_{24}H_{36}N_6O_3S$) C, H, N, S.

1-[N^{α}-(Anthraquinone-2-sulfonyl)-L-arginyl]-4-ethylpiperidine Hydrogen Fluoride (107). V (3.5 g, 10 mmol), mentioned above, was reacted with anthraquinone-2-sulfonyl chloride in the same manner to give 1-[N^{α}-(anthraquinone-2-sulfonyl)-N^{ω}-nitro-L-arginyl]-4-ethylpiperidine (4.7 g, 81%) as amorphous solids. Then this was dissolved in anhydrous HF (2 mL) and anisole (0.5 mL) at 0 °C. After 30 min, HF was evaporated in vacuo below 5 °C. The residue was washed with dry Et_2O (5 × 10 mL) by successive centrifugation and decantation. The product was dried in vacuo. Reprecipitation from EtOH–Et_2O gave pure **107** (2.9 g, 65%) as amorphous solids.

Inhibition Studies of the Clotting Activity of Thrombin. The method has been described in the preceding paper.[1]

Hydrolysis Study by Thrombin and Trypsin. The reaction mixture containing 1 mM test compounds, 0.1 M Tris-HCl buffer (pH 8.0), and 100 units of thrombin or 0.15 mg of trypsin in a total volume of 1.0 mL was incubated at 37 °C. An aliquot of sample was taken out periodically and spotted on a silica gel thin-layer sheet (Kodak) and developed with ethanol–chloroform (1:1). Fluorescence intensity of the spots of N^{α}-dansyl-L-arginine and amides of N^{α}-dansyl-L-arginine on the chromatogram was estimated visually under UV light.

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