Thrombin Inhibitors. 3. Carboxyl-Containing Amide Derivatives of N^{α}-Substituted L-Arginine

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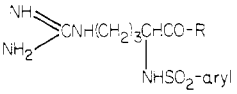
A series of N^{α}-(arylsulfonyl)-L-arginine amide derivatives having carboxamide N-substituents with a carboxyl group was prepared and tested as inhibitors of the clotting activity of thrombin. The most inhibitory compounds were obtained when a carboxyl group was introduced into the carbon next to the amide nitrogen of N^{α}-(arylsulfonyl)-L-arginine amide derivatives, e.g., N^{α}-(arylsulfonyl)-L-arginyl-*N*-butyl-, *N*-(methoxyethyl)- or *N*-(tetrahydrofurfuryl)glycine and 4-alkyl-1-[N^{α}-(arylsulfonyl)-L-arginyl]-2-piperidinecarboxylic acid, with an I_{50} of 1–3 × 10^{-7} M.

We have previously reported that a series of the ester and amide derivatives of N^{α}-substituted arginine showed a potent thrombin inhibitory effect.[1,2] The amide derivatives shown below were highly specific thrombin inhibitors and stable toward enzymatic hydrolysis, but their high toxicity rendered them unsuitable to be used as antithrombotics.[3] We postulated that the high toxicity of this

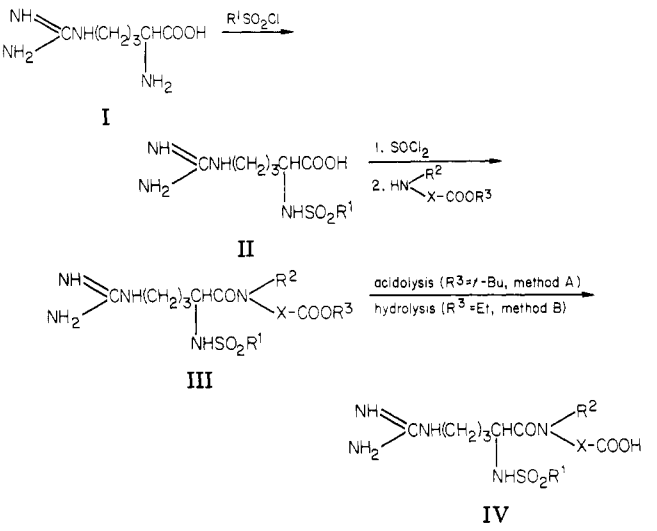


R = alkylimino, dialkylimino, c-NC_5H_9-4-CH_3, and c-NC_5H_9-4-C_2H_5

series of compounds resulted from their strong basicity and, hence, we tried to decrease the basicity by introducing a carboxyl group into these arginine derivatives to obtain thrombin inhibitors of low toxicity. After examination of a variety of compounds, we succeeded in obtaining potent thrombin inhibitors having a carboxyl group at R, which had extremely low toxicity and were stable toward enzymatic hydrolysis. We describe the relationship between the structure of arginine derivatives with a carboxyl group and the thrombin inhibitory effect.

Chemistry. All compounds listed in Tables I–VII were synthesized via the general routes illustrated in Schemes I and II. N^{α}-(Arylsulfonyl)-L-arginine (II), which was easily derived from L-arginine (I) and arylsulfonyl chloride, was reacted with $SOCl_2$ in the presence of a few drops of DMF to give N^{α}-(arylsulfonyl)-L-arginyl chloride. This compound was allowed to react immediately with an appropriate amino acid ester derivative to give N^{α}-(arylsulfonyl)-L-arginyl amino acid ester (III). Acidolysis (R^3 = *t*-Bu; method A) or hydrolysis (R^3 = Et; method B) of III gave N^{α}-(arylsulfonyl)-L-arginyl amino acid derivative (IV) (Scheme I). By an alternative rout (method C, Scheme II), N^{α}-(*tert*-butoxycarbonyl)-N^{ω}-nitro-L-arginine (V) was condensed with an appropriate amino acid ester by the mixed anhydride method using isobutyl chloroformate and triethylamine to give N^{α}-(*tert*-butoxycarbonyl)-N^{ω}-nitro-L-arginyl amino acid ester (VI), which was purified by column chromatography. After removal of the *tert*-butoxycarbonyl group of VI, N^{ω}-nitro-L-arginyl amino acid ester (VII) was sulfonylated with an appropriate arylsulfonyl chloride to afford N^{α}-(arylsulfonyl)-N^{ω}-nitroarginyl amino acid ester (VIII). Compound VIII was also purified by column chromatography. Hydrolysis of VIII, followed by hydrogenolysis of the nitro group, gave N^{α}-(arylsulfonyl)-L-arginyl amino acid (IV) (method C).

Scheme I. Methods A and B



All new amino acid ester derivatives were prepared according to the modified procedure of Speziale et al.,[4] and 4-substituted 2-piperidinecarboxylic acid esters were prepared according to the modified procedure of Bonnett et al.[5] or Böhme et al.[6] The arylsulfonyl chlorides were prepared by the chlorination of the corresponding arylsulfonic acid or its salts. Further details are available under Experimental Section.

Enzyme Results and Discussion

Thrombin inhibitors which have a carboxyl group at the carboxamide portion of the arginine derivatives were obtained by introducing a carboxylalkyl group at the nitrogen of the amide group of N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginine methoxyethylamide, reported earlier as a potent thrombin inhibitor.[1] The inhibitory effect of these compounds are shown in Table I. The introduction

(1) S. Okamoto, K. Kinjo, A. Hijikata, R. Kikumoto, Y. Tamao, K. Ohkubo, and S. Tonomura, *J. Med. Chem.*, **23**, 827 (1980).
(2) R. Kikumoto, Y. Tamao, K. Ohkubo, T. Tezuka, S. Tonomura, S. Okamoto, Y. Funahara, and A. Hijikata, *J. Med. Chem.*, **23**, 830 (1980).
(3) S. Okamoto, A. Hijikata, R. Kikumoto, Y. Tamao, K. Ikezawa, and E. Mori, International Committee on Thrombosis and Haemostatsis, 22nd Annual Meeting, Kyoto, Japan, 1976.
(4) A. J. Speziale and E. G. Jaworski, *J. Org. Chem.*, **25**, 728 (1960).
(5) R. Bonnett, V. M. Clark, A. Giddey, and A. Alexander Todd, *J. Chem. Soc.*, 2087 (1959).
(6) H. Böhme, H. Ellenberg, Otto-Erich Herboth, and W. Lehners, *Chem. Ber.*, **92**, 1608 (1959).

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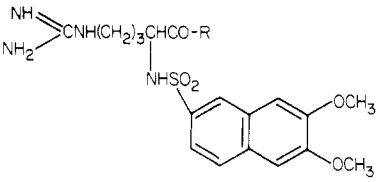
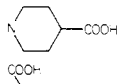
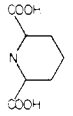
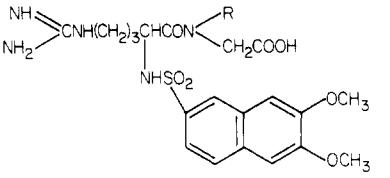
Table I. N^{α}-(6,7-Dimethoxy-2-naphthalenesulfonyl)-L-arginine Amide Derivatives



no.[a]	R	synth method	formula[b]	I_{50},[c] M
1	$N(CH_2CH_2OCH_3)CH_2COOH$	B	$C_{23}H_{33}N_5O_8S$	3.0×10^{-7}
2	$N(CH_2CH_2OCH_3)CH_2CH_2COOH$	B	$C_{24}H_{35}N_5O_8S$	3.7×10^{-6}
3	$N(CH_2CH_2OCH_3)CH_2CH_2CH_2COOH$	A	$C_{25}H_{37}N_5O_8S$	4.0×10^{-5}
4	$N(CH_2CH_2OCH_3)CH(CH_3)COOH$	A	$C_{24}H_{35}N_5O_8S$	5.0×10^{-6}
5		C	$C_{24}H_{33}N_5O_7S$	4.5×10^{-6}
6		C	$C_{24}H_{33}N_5O_7S$	4.0×10^{-5}
7		C	$C_{24}H_{33}N_5O_7S$	1.5×10^{-4}
8		C	$C_{25}H_{33}N_5O_9S$	$>1.0 \times 10^{-4}$

[a] All compounds were amorphous solids. [b] All compounds were analyzed for C, H, N, and S; analytical results were within ±0.4% of the theoretical values. [c] I_{50} was defined as the concentration at which the clotting time was prolonged by twice that of the control.

Table II. N^{α}-(6,7-Dimethoxy-2-naphthalenesulfonyl)-L-arginine Amide Derivatives

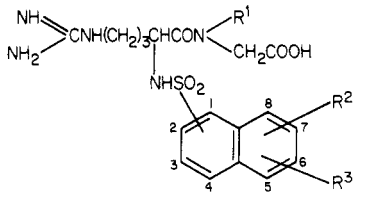


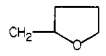
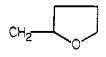
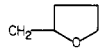
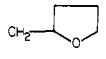
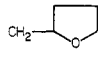
no.[a]	R	synth method	formula[b]	I_{50},[c] M
9	CH_3	C	$C_{21}H_{29}N_5O_7S$	$>1.0 \times 10^{-4}$
10	n-C_3H_7	A	$C_{23}H_{33}N_5O_7S$	8.0×10^{-6}
11	n-C_4H_9	A	$C_{24}H_{35}N_5O_7S$	3.0×10^{-7}
12	n-C_5H_{11}	A	$C_{25}H_{37}N_5O_7S$	1.0×10^{-6}
13	n-C_6H_{13}	A	$C_{26}H_{39}N_5O_7S$	1.5×10^{-7}
14	n-C_8H_{17}	A	$C_{28}H_{43}N_5O_7S$	$>1.0 \times 10^{-4}$
15	$CH_2CH(CH_3)_2$	A	$C_{24}H_{35}N_5O_7S$	2.0×10^{-6}
1	$CH_2CH_2OCH_3$	B	$C_{23}H_{33}N_5O_8S$	3.0×10^{-7}
16	$CH_2CH_2CH_2OCH_3$	B	$C_{24}H_{35}N_5O_8S$	5.0×10^{-6}
17	$CH_2CH_2SCH_2CH_3$	A	$C_{24}H_{35}N_5O_7S_2$	5.0×10^{-6}
18	c-C_6H_{11}	A	$C_{26}H_{37}N_5O_7S$	5.0×10^{-6}
19	c-C_7H_{13}	A	$C_{27}H_{39}N_5O_7S$	1.5×10^{-5}
20	CH_2-c-C_6H_{11}	A	$C_{27}H_{39}N_5O_7S$	1.5×10^{-5}
21	$CH_2C_6H_5$	C	$C_{27}H_{33}N_5O_7S$	2.5×10^{-6}
22	$CH_2CH_2C_6H_5$	C	$C_{28}H_{35}N_5O_7S$	3.0×10^{-6}
23		C	$C_{25}H_{35}N_5O_8S$	2.0×10^{-7}

[a-c] See corresponding footnotes in Table I.

of a carboxymethyl group (1) showed the most potent effect, the introduction of the 2-carboxyethyl group (2) made its I_{50} about 10 times that of 1, and the introduction of 3-carboxypropyl group (3) made its I_{50} about 100 times that of 1. Thus, it was found that the inhibitory effect was reduced as the methylene chain of the carboxyalkyl group became longer. For the branched methylene, such as 4, the inhibitory effect was also less than that of 1. When a carboxyl group was introduced into the piperidine ring of 1-[N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginyl]piperidine, introduction at the 2 position gave the most potent inhibitor, and the inhibitory potency decreased in the order of the substitution position, 3 (6), 4 (7). Further, it was found that disubstitution at the 2 and 6 (8) positions showed a total loss of the inhibitory potency. Thus, it was shown that the inhibitory effect of arginine derivatives having a COOH group at the amide portion varies largely with the position of the introduced COOH and that the compound (8) with two COOH groups showed no inhibitory effect. The most potent inhibitory effect was shown

Table III. N^{α}-Substituted-naphthalenesulfonyl-L-arginine Amide Derivatives



no.[a]	R^1	substituted position of SO_2	R^2	R^3	synth method	formula[b]	I_{50},[c] M
24	n-C_4H_9	2	H	H	A	$C_{22}H_{31}N_5O_5S$	$>1.0 \times 10^{-4}$
25	n-C_4H_9	2	7-CH_3	H	A	$C_{23}H_{33}N_5O_5S$	3.0×10^{-7}
26	n-C_4H_9	1	5-OCH_3	H	A	$C_{23}H_{33}N_5O_6S$	4.0×10^{-6}
27	n-C_4H_9	2	6-OCH_3	H	A	$C_{23}H_{33}N_5O_6S$	5.0×10^{-7}
28	n-C_4H_9	2	7-OCH_3	H	A	$C_{23}H_{33}N_5O_6S$	5.0×10^{-7}
29	n-C_4H_9	1	5-$N(CH_3)_2$	H	A	$C_{24}H_{36}N_6O_5S$	4.0×10^{-6}
30	n-C_4H_9	2	4-OCH_3	6-OCH_3	A	$C_{24}H_{35}N_5O_7S$	2.0×10^{-6}
31	$CH_2CH_2OCH_3$	1	H	H	C	$C_{21}H_{29}N_5O_6S$	3.0×10^{-5}
32	$CH_2CH_2OCH_3$	2	7-CH_3	H	C	$C_{22}H_{31}N_5O_6S$	3.5×10^{-7}
33	$CH_2CH_2OCH_3$	1	5-OCH_3	H	C	$C_{22}H_{31}N_5O_7S$	2.5×10^{-6}
34	$CH_2CH_2OCH_3$	2	7-OCH_3	H	C	$C_{22}H_{31}N_5O_7S$	5.0×10^{-7}
35	$CH_2CH_2OCH_3$	1	5-$N(CH_3)_2$	H	C	$C_{23}H_{34}N_6O_6S$	5.0×10^{-6}
36	$CH_2CH_2OCH_3$	1	6-CH_3	7-CH_3	C	$C_{23}H_{33}N_5O_6S$	1.0×10^{-5}
37	$CH_2CH_2OCH_3$	2	4-OCH_3	6-OCH_3	C	$C_{23}H_{33}N_5O_8S$	4.0×10^{-6}
38	$CH_2CH_2OCH_3$	2	6-OC_2H_5	7-OC_2H_5	C	$C_{25}H_{37}N_5O_8S$	1.5×10^{-5}
39		1	H	H	C	$C_{23}H_{31}N_5O_6S$	3.0×10^{-5}
40		2	7-CH_3	H	C	$C_{24}H_{33}N_5O_6S$	2.0×10^{-7}
41		2	7-OCH_3	H	C	$C_{24}H_{33}N_5O_7S$	1.5×10^{-7}
42		1	5-$N(CH_3)_2$	H	C	$C_{25}H_{36}N_6O_6S$	2.0×10^{-6}
43		2	6-CH_3	7-CH_3	C	$C_{25}H_{35}N_5O_6S$	3.0×10^{-6}

[a-c] See corresponding footnotes in Table I.

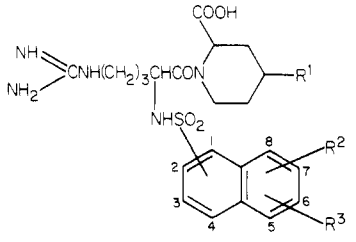
by the introduction of COOH to the carbon next to the amide nitrogen.

Subsequently, several derivatives of N^{α}-substituted arginylglycine were synthesized and their thrombin inhibitory effect was investigated. As shown in Table II, various alkyl groups were introduced into the glycine nitrogen of N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginylglycine. When the introduced alkyl group was as long as four carbon atoms, as in **1** and **11**, the most potent inhibitory effect was exhibited. With alkyl groups whose carbon number is less than 3 or more than 5, their effects were largely reduced, particularly with a CH_3 (**9**) or C_8H_{17} (**14**) group. This effect of the size of the alkyl group observed in this experiment was quite the same as in the amide derivatives in the previous paper, where butylamide derivatives were the most inhibitory.[2] The introduction of other bulky groups (**18–22**) made the compounds less than one-tenth as inhibitory as **1**. The reduced inhibitory potency of these compounds may be due to the bulkiness of the substituents. However, tetrahydrofurfuryl derivative **23**, whose size is similar to cyclohexylmethyl derivative **20**, was as inhibitory as **1**.

Based on the results described above, *N*-*n*-butyl-, *N*-(2-methoxyethyl)-, and *N*-(tetrahydrofurfuryl)glycine and 4-alkyl-2-piperidinecarboxylic acid were selected as substituents at the carboxamide portion to investigate the effect of N^{α}-substituents on the inhibitory potency. As shown in Tables III and IV, a group of the most potent inhibitors was obtained when a naphthalene ring of N^{α}-substituents was substituted at the 6 or 7 position, such as **25**, **27**, **28**, **32**, **34**, **40**, **41**, **48**, **49**, **53**, and **57–60**. It was also shown in Table II, as well as in Table III, that N^{α}-(6,7-disubstituted-2-naphthalenesulfonyl)-L-arginine derivatives exhibited the potent inhibition of thrombin (**51**, **54**, and **55**). As substituents to a naphthalene ring, the CH_3 or OCH_3 group was found most suitable, and the OC_2H_5 group did not cause potent inhibition as shown by **38** and **52**. The 1-naphthalenesulfonyl group substituted with an OCH_3 or $N(CH_3)_2$ group at the 5 position gave inhibitors with I_{50} values of $2–7 \times 10^{-6}$ M, such as **26**, **29**, **33**, **35**, **42**, and **46**. For compounds with 2-piperidinecarboxylic acid derivatives, introduction of a CH_3 or C_2H_5 group at the 4 position of piperidine increased the inhibitory potency as in dansylarginine amide derivatives. It should be noted that introduction of an *n*-propyl or isopropyl group also increased the inhibitory potency, unlike N^{α}-dansyl-L-arginine amide derivatives.[2] Although introduction of a bulky group, e.g., phenyl group (**61**), showed a reduced inhibitory potency, derivatives with bulky groups, such as **63** and **64**, showed relatively potent inhibition.

When a 1- or 2-tetralinsulfonyl group was introduced as an N^{α}-substituent, *N*-alkylglycine derivatives (**65–69**) were not so potently inhibitory as N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginine derivatives, whereas 1-[N^{α}-(1-tetralinsulfonyl)-L-arginyl]-2-piperidinecarboxylic acid (**70**) was as inhibitory as 1-[N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginyl]-4-methyl-2-piperidinecarboxylic acid (**51**). This suggests that the N^{α}-substituent and carboxamide portion may interact to some extent; hence, the inhibitory potency varies according to the type

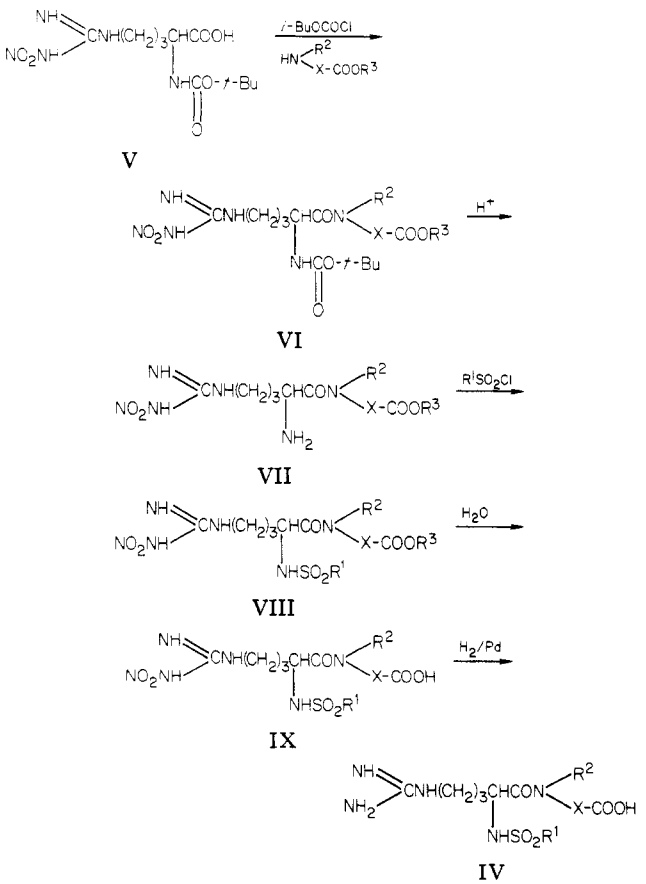
Table IV. N^{α}-Substituted-naphthalenesulfonyl-L-arginine Amide Derivatives



no.[a,d]	R^1	substituted position of SO_2	R^2	R^3	formula[b]	I_{50},[c] M
44	H	2	6-OCH_3	H	$C_{23}H_{31}N_5O_6S$	3.0×10^{-6}
45	H	2	7-OCH_3	H	$C_{23}H_{31}N_5O_6S$	5.0×10^{-6}
46	H	1	5-$N(CH_3)_2$	H	$C_{24}H_{34}N_6O_5S$	7.0×10^{-6}
47	CH_3	1	H	H	$C_{23}H_{31}N_5O_5S$	1.0×10^{-6}
48	CH_3	2	7-CH_3	H	$C_{24}H_{33}N_5O_5S$	1.5×10^{-7}
49	CH_3	2	7-OCH_3	H	$C_{24}H_{33}N_5O_6S$	1.5×10^{-7}
50	CH_3	2	4-OCH_3	6-OCH_3	$C_{25}H_{35}N_5O_7S$	3.4×10^{-7}
51	CH_3	2	6-OCH_3	7-OCH_3	$C_{25}H_{35}N_5O_7S$	4.0×10^{-7}
52	CH_3	2	6-OC_2H_5	7-OC_2H_5	$C_{27}H_{39}N_5O_7S$	4.0×10^{-6}
53	C_2H_5	2	7-OCH_3	H	$C_{25}H_{35}N_5O_6S$	1.0×10^{-7}
54	C_2H_5	2	6-OCH_3	7-OCH_3	$C_{26}H_{37}N_5O_7S$	2.5×10^{-7}
55	n-C_3H_7	2	6-OCH_3	7-OCH_3	$C_{27}H_{39}N_5O_7S$	5.0×10^{-7}
56	$CH(CH_3)_2$	2	H	H	$C_{25}H_{35}N_5O_5S$	5.0×10^{-5}
57	$CH(CH_3)_2$	2	6-CH_3	H	$C_{26}H_{37}N_5O_5S$	3.5×10^{-7}
58	$CH(CH_3)_2$	2	7-CH_3	H	$C_{26}H_{37}N_5O_5S$	1.0×10^{-7}
59	$CH(CH_3)_2$	2	7-OCH_3	H	$C_{26}H_{37}N_5O_6S$	1.5×10^{-7}
60	$CH(CH_3)_2$	2	6-Cl	H	$C_{25}H_{34}ClN_5O_5S$	5.0×10^{-7}
61	C_6H_5	2	6-OCH_3	7-OCH_3	$C_{30}H_{37}N_5O_7S$	1.2×10^{-5}

[a-c] See corresponding footnotes in Table I. [d] All compounds were prepared by method C.

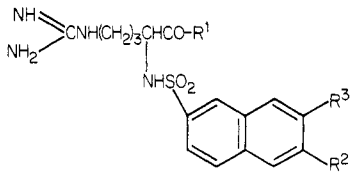
Scheme II. Method C

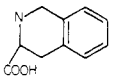


of combination of N^{α}-substituent with the carboxamide portion. In Table VII are shown the derivatives having heterocyclic compounds as N^{α}-substituents. All derivatives, except **73** and **74**, in which the oxygen-containing ring is situated at the terminal end showed a potent thrombin inhibition with an I_{50} at the 10^{-7} M level.

As mentioned above, many potent thrombin inhibitors having the carboxyl group at the carboxamide portion were obtained. Their inhibitory effect varied largely with the substituted position of COOH. This suggests that a part of the carboxamide portion may participate well in the binding through hydrophobic interaction, and it appears that introduction of a hydrophilic COOH around this hydrophobic interaction site interfers with the binding to thrombin. Far less inhibitory potency was observed by the introduction of COOH at the 4 position and to a lesser extent at the 3 position; therefore, the hydrophobic binding site of the carboxamide portion may probably be around the 4 position of the piperidine ring. Furthermore, the much reduced inhibitory potency of the 2,6-piperidinedicarboxylic acid derivative suggests that the 6 position, and probably the 5 position as well as the 4 position, might contribute to hydrophobic interaction in 2-piperidinecarboxylic acid derivatives. Considering the relationship between the size of the 4-position substituents and their inhibitory potency, the most fittable size at the 4 position which will allow the hydrophobic interaction may be as big as CH_3 to C_3H_7. When a straight-chain alkyl substituent is at the carboxamide portion, it would be an alkyl group as large as four carbon atoms that binds at this hydrophobic binding site. Although it is not clear whether COOH participates directly in the binding to thrombin or not, the $NHCH_2COOH$ group alone is not considered to interact with the binding site because of the far less inhibitory potency of N^{α}-substituted arginylglycine. It is essential for the binding that the group capable of hydrophobic binding exists at the carboxamide portion. Thus, inhibitors having a carboxyl group at the carboxamide portion reported in this paper are characterized by the introduction of a hydrophilic carboxyl group near into hydrophobic interaction part without disturbing the hydrophobic interaction. It has been observed that the acute

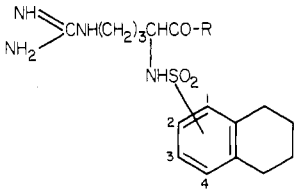
Table V. N^{α}-Substituted-naphthalenesulfonyl-L-arginine Amide Derivatives

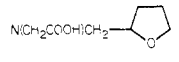
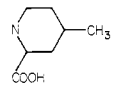


no.[a,d]	R^1	R^2	R^3	formula[b]	I_{50},[c] M
62		H	OCH_3	$C_{24}H_{33}N_5O_6S$	2.5×10^{-6}
63		OCH_3	OCH_3	$C_{27}H_{31}N_5O_7S$	1.5×10^{-6}
64		OCH_3	OCH_3	$C_{28}H_{33}N_5O_7S$	7.0×10^{-7}

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

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



no.[a]	substituted position of SO_2	R	synth method	formula[b]	I_{50},[c] M
65	1	$N(n\text{-}C_4H_9)CH_2COOH$	A	$C_{22}H_{35}N_5O_5S$	2.0×10^{-5}
66	2	$N(n\text{-}C_4H_9)CH_2COOH$	A	$C_{22}H_{35}N_5O_5S$	6.0×10^{-5}
67	1	$N(n\text{-}C_5H_{11})CH_2COOH$	A	$C_{23}H_{37}N_5O_5S$	1.0×10^{-4}
68	1	$N(CH_2CH_2OCH_3)CH_2COOH$	B	$C_{21}H_{33}N_5O_6S$	1.0×10^{-5}
69	1		C	$C_{23}H_{35}N_5O_6S$	6.5×10^{-6}
70	1		C	$C_{23}H_{35}N_5O_5S$	6.0×10^{-7}

[a-c] See corresponding footnotes in Table I.

toxicity of these inhibitors was extremely reduced as compared with earlier inhibitors without a carboxyl group.[3] The LD_{50} values of compound 1 were 1150 mg/kg ip and 595 mg/kg iv in mice, whereas that of 1-(N^{α}-dansyl-L-arginyl)-4-ethylpiperidine was 80 mg/kg ip. The investigation concerning acute toxicity will be reported elsewhere in detail. Antithrombotic effects of compound 1 have been reported on the experimental animal thrombosis, such as the arterial thrombosis induced by acetic acid,[7] and the disseminated intravascular coagulation.[8,9]

Experimental Section

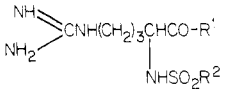
Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The results of elemental analyses were within ±0.4 % of the theoretical values. Compounds were checked by IR on a JASCO IR-A2, and the spectral data were consistent with the assigned structure in all cases. TLC was performed on fluorescent silica gel plates (Merck) to a distance of 20 cm with a solvent system of *n*-BuOH–AcOH–H_2O (3:1:1), and spots were detected under a UV lamp or by exposure to I_2 vapor. The purity of compounds was also checked by high-performance LC (Shimazu LC-3A) on Zorbax ODS column (Du pont) with a solvent system of MeOH–H_2O–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 synthetic methods (Schemes I and II).

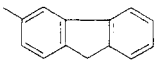
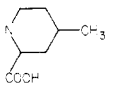
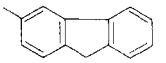
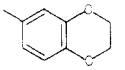
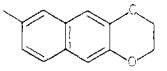
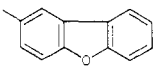
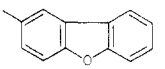
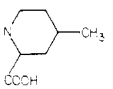
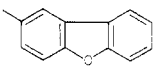
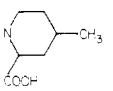
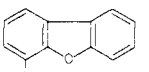
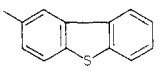
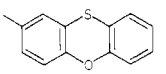
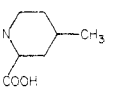
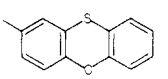
N^{α}-(6,7-Dimethoxy-2-naphthalenesulfonyl)-L-arginyl-*N*-butylglycine (11, Method A). A. Condensation of L-Arginine with 6,7-Dimethoxy-2-naphthalenesulfonyl Chloride. To a solution of L-arginine (80.0 g, 0.46 mol) and K_2CO_3 (72.0 g, 0.521 mol) in H_2O (500 mL) was added a solution of 6,7-dimethoxy-2-naphthalenesulfonyl chloride (109.6 g, 0.382 mol) in benzene (500 mL) under stirring at 60 °C. The mixture was maintained at 60 °C for 3 h and then allowed to cool to room temperature. The precipitate was collected on a filter, washed with EtOH and H_2O, and dried in vacuo to give N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginine (I; 116.1 g, 71.6%), mp 252–253 °C. Anal. ($C_{18}H_{24}N_4O_6S$) C, H, N.

B. Chlorination of I. To a vigorously stirred suspension of I (10.0 g, 23.5 mmol) and $SOCl_2$ (50 mL) was added dropwise 2–3 drops of DMF under N_2 at room temperature for 4 h. Addition of cold dry Et_2O (300 mL) resulted in a precipitate, which was collected by decantation and washed with cold dry Et_2O (3 × 40 mL) to give N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginyl chloride hydrochloride (II) as an amorphous solid, which was used

(7) H. Ikoma, K. Ohtsu, Y. Tamao, R. Kikumoto, E. Mori, Y. Funahara, and S. Okamoto, *Kobe J. Med. Sci.*, **26**, 33 (1980).
(8) H. Hara, Y. Tamao, R. Kikumoto, Y. Funahara, A. Hijikata, and S. Okamoto, *Kobe J. Med. Sci.*, **26**, 47 (1980).
(9) K. Ohtsu, Y. Tamao, R. Kikumoto, K. Ikezawa, A. Hijikata, and S. Okamoto, *Kobe J. Med. Sci.*, **26**, 61 (1980).

Table VII. N^{α}-(Arylsulfonyl)-L-arginine Amide Derivatives



no.[a,d]	R^1	R^2	formula[b]	I_{50},[c] M
71	$N(CH_2CH_2OCH_3)CH_2COOH$		$C_{24}H_{31}N_5O_6S$	4.0×10^{-7}
72			$C_{26}H_{33}N_5O_5S$	1.0×10^{-7}
73	$N(CH_2CH_2OCH_3)CH_2COOH$		$C_{19}H_{29}N_5O_8S$	3.2×10^{-5}
74	$N(CH_2CH_2OCH_3)CH_2COOH$		$C_{23}H_{31}N_5O_8S$	5.7×10^{-5}
75	$N(n\text{-}C_4H_9)CH_2COOH$		$C_{24}H_{31}N_5O_6S$	3.0×10^{-7}
76	$N(CH_2CH_2OCH_3)CH_2COOH$		$C_{23}H_{29}N_5O_7S$	3.5×10^{-7}
77			$C_{25}H_{31}N_5O_6S$	1.0×10^{-7}
78			$C_{25}H_{31}N_5O_6S$	1.5×10^{-7}
79	$N(CH_2CH_2OCH_3)CH_2COOH$		$C_{23}H_{29}N_5O_6S_2$	4.5×10^{-7}
80	$N(CH_2CH_2OCH_3)CH_2COOH$		$C_{23}H_{29}N_5O_7S_2$	3.5×10^{-7}
81			$C_{25}H_{31}N_5O_6S_2$	2.5×10^{-7}

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

in the next step without further purification.

C. Condensation of II with *N*-Butylglycine *tert*-Butyl Ester. To a stirred solution of *N*-butylglycine *tert*-butyl ester (2.90 g, 15.5 mmol) and Et_3N (4.5 mL) in $CHCl_3$ (50 mL) was added in portions II (6.76 g, 14.1 mmol) at 0–5 °C. The reaction mixture was allowed to stand at room temperature for 3 h. At the end this period, the reaction mixture was washed with aqueous NaCl solution, dried (Na_2SO_4), and evaporated in vacuo. The residue was triturated with a small amount of H_2O to give a crystalline material. This was collected by filtration and recrystallized from EtOH–Et_2O to give N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginyl-*N*-butylglycine *tert*-butyl ester (III; 6.96 g, 82%), mp 164–166 °C. Anal. ($C_{28}H_{43}N_5O_7S \cdot 0.5H_2SO_3$) C, H, N.

D. Acidolysis of III. To a solution of III (2.00 g, 3.32 mmol) in $CHCl_3$ was added AcOEt (50 mL) containing 15% dry HCl with stirring for 5 h at room temperature. After evaporation, the residue was washed with Et_2O and chromatographed on cationic ion-exchange resin (Diaion SK 102, 200–300 mesh, manufactured by Mitsubishi Chemical Industries Ltd.) packed in H_2O, washed with H_2O, and eluted with 3% aqueous NH_3. The main fraction was evaporated to dryness and washed with Et_2O–EtOH to give N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginyl-*N*-butylglycine (11) as an amorphous solid (1.41 g, 79%): IR (KBr) 3360, 3140, 1622 cm^{-1}. Anal. ($C_{24}H_{35}N_5O_7S$) C, H, N.

***N*$^{\alpha}$-(6,7-Dimethoxy-2-naphthalenesulfonyl)-L-arginyl-*N*-(2-methoxyethyl)glycine (1; Method B). A. *N*$^{\alpha}$-(6,7-Dimethoxy-2-naphthalenesulfonyl)-L-arginyl-*N*-(2-methoxyethyl)glycine ethyl ester (IV)** was prepared in manner similar to that for method A, from II and *N*-(2-methoxyethyl)glycine ethyl ester in 91% yield as an amorphous solid. For analysis of the product, a portion of the product was converted to the flavianate, mp 185 °C. Anal. ($C_{25}H_{37}N_5O_8S \cdot C_{10}H_6N_2O_8S$) C, H, N.

B. Hydrolysis of IV. A solution of IV (2.50 g) in EtOH (5 mL) and 1 N NaOH (7 mL) was stirred for 30 h at room temperature. After the solution was concentrated in vacuo, the residue was chromatographed on cationic ion-exchange resin packed in H_2O, washed with H_2O, and eluted 3% aqueous NH_3. The eluted solution was evaporated to dryness, and the residue was purified by reprecipitation with EtOH–Et_2O to give N^{α}-(6,7-dimethoxy-2-naphthalenesulfonyl)-L-arginyl-*N*-(2-methoxyethyl)glycine (1; 1.32 g, 72%) as an amorphous solid: IR (KBr) 3380, 3180, 1630 cm^{-1}. Anal. ($C_{23}H_{33}N_5O_8S$) C, H, N.

1-[*N*$^{\alpha}$-(7-Methoxy-2-naphthalenesulfonyl)-L-arginyl]-4-methyl-2-piperidinecarboxylic Acid (49; Method C). A. Condensation of *N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-*N*$^{\omega}$-nitro-L-arginine (V) with Ethyl 4-Methyl-2-piperidinecarboxylate. A solution of V (3.19 g, 10 mmol) in anhydrous THF (50 mL) was cooled to −20 °C. While the solution was stirred, isobutyl chloroformate (1.36 g, 10 mmol) was added and the temperature was maintained at −20 °C for 15 min. A cold solution ethyl 4-methyl-2-piperidinecarboxylate (1.71 g, 10 mmol) was added with stirring to the mixed anhydride solution at −20 °C. The reaction mixture was stirred at −20 °C for 10 min and at room temperature for additional 1 h. THF was evaporated in vacuo below 40 °C, and 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. The residue was purified by chromatography on silica gel using $CHCl_3$–MeOH (97:3) as eluent. Evaporation of the eluate yielded ethyl 1-[*N*$^{\alpha}$-(*tert*-butoxycarbonyl)-*N*$^{\omega}$-nitro-L-arginyl]-4-methyl-2-piperidinecarboxylate (VI; 3.07 g, 65%) in the form of a syrup.

B. Removal of the *tert*-Butoxycarbonyl Group and N$^{\alpha}$-Sulfonylation with 7-Methoxy-2-naphthalenesulfonyl

Chloride. Compound VI (3.0 g, 6.35 mmol) was dissolved in AcOEt (50 mL) containing 10% dry HCl and stirred for 3 h. Cold Et_2O (70 mL) was added, and the precipitated material, ethyl 4-methyl-1-(N^{α}-nitro-L-arginyl)piperidinecarboxylate hydrochloride (VII), was centrifuged and washed with Et_2O (2 × 20 mL) by successive centrifugation and decantation, the precipitation being well suspended in each wash by vortex mixing. The product was dried in vacuo. To a mixture of VII (2.50 g, 6.1 mmol) and Et_3N (2.1 g, 20 mmol) in CH_2Cl_2 (30 mL) was added 7-methoxy-2-naphthalenesulfonyl chloride (1.94 g, 7.5 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). Evaporation of the eluate gave ethyl 1-[N^{α}-(7-methoxy-2-naphthalenesulfonyl)-N^{ω}-nitroarginyl]-4-methyl-2-piperidinecarboxylate (VIII; 3.2 g, 89%) as an amorphous solid. Anal. ($C_{26}H_{36}N_6O_8S$) C, H, N.

C. Removal of the NO_2 Group. Compound VIII (3.20 g, 5.38 mmol) was dissolved in EtOH (20 mL) and AcOH (2 mL), and Pd black (0.50 g) was added. H_2 was bubbled into the mixture for 30 h at room temperature. After we filtered off the catalyst, the filtrate was evaporated to give a viscous oily product. Reprecipitation from EtOH–Et_2O gave ethyl 1-[N^{α}-(7-methoxy-2-naphthalenesulfonyl)-L-arginyl]-4-methyl-2-piperidinecarboxylate (IX; 2.44 g, 83%) as an amorphous solid. For analysis of the product, a portion of the product was converted to the flavianate, mp 188–189 °C. Anal. ($C_{26}H_{37}N_5O_6S \cdot C_{10}H_6N_2O_8S$) C, H, N.

D. Hydrolysis of IX. The title compound (49) as an amorphous solid was prepared in manner similar to that for method B: IR (KBr) 3250 (br), 1625 cm^{-1}. Anal. ($C_{24}H_{33}N_5O_6S$) C, H, N.

Inhibition Studies of the Clotting Activity of Thrombin. The method has been described in the preceding paper.[1] The clotting time was measured at 25 °C in the reaction mixture, at a final volume of 1.0 mL containing 0.096% fibrinogen.

Acknowledgment. The authors thank A. Maruyama and Mrs. K. Sugano 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 the elemental analyses.

Analogues of Chloramphenicol: Circular Dichroism Spectra, Inhibition of Ribosomal Peptidyltransferase, and Possible Mechanism of Action

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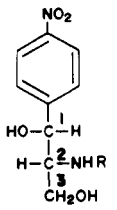
Circular dichroism spectra of a series of chloramphenicol derivatives **1a–r** were measured in water at pH 7. Compounds **1a–o** exhibit two positive Cotton effects at 310–340 and 240–260 nm, respectively, and a weaker negative Cotton effect at 280–300 nm. In analogues **1c**, **11**, and **1m** there is only a minimum between the two positive Cotton effects. Derivatives **1p–r** possess a strong negative Cotton effect at ca. 280 nm. Compounds **1a–r** were examined as inhibitors of the puromycin reaction with *Escherichia coli* 70S ribosome–poly(U)–*N*-AcPhe-tRNA complex. Analogues **11**, **1n**, **1o**, and **1q** are potent competitive inhibitors of puromycin comparable to or better than chloramphenicol (**1b**). Compounds **1k** and **1m** are less active, whereas **1d–g** and **1j** are only moderately effective. The rest of the analogues have marginal or no activity. The results are compared with previous biological data and discussed in terms of a retro-inverso relationship of chloramphenicol (**1b**) to the aminoacyl moiety of puromycin (aminoacyl-tRNA) and to a hypothetical transition state of peptide bond formation.

The antibiotic chloramphenicol (**1b**) is a powerful inhibitor of protein synthesis in bacteria.[1] Extensive studies have established that **1b** inhibits peptide bond synthesis catalyzed by peptidyltransferase at, or close to, the ribosomal A site.[2] Although it has been inferred from these investigations that **1b** is an analogue of aminoacyl-tRNA or puromycin, attempts to relate its structure to various portions of the 3′ terminus of aminoacyl-tRNA (puromycin) have not yet met with success. Thus, X-ray diffraction[3] and NMR[4] studies of **1b** led to postulation of a structure designated "curled" conformation (**2**) stabilized by hydrogen bonding which is probably maintained during binding of **1b** to ribosomes.[5] Another study[6] has shown that conformation **2** is stable in solution even in the absence of hydrogen bonding. According to one proposal,[4] conformer **2** can resemble uridine 5′-phosphate (UMP). There is, however, little to suggest that UMP plays a role in protein synthesis. This concept was later extended[7] to include pyrimidine 5′-nucleotides, in general, because of a similarity of chloramphenicol action to that of the cytosine-containing antibiotics gougerotin and blasticidin.



1a: R = H
1b: R = Cl_2CHCO
1c: R = CHO
1d: R = CH_3CO
1e: R = C_2H_5CO
1f: R = n-C_3H_7CO
1g: R =  CO
1h: R =  CO
1i: R = $HOCH_2CO$
1j: R = FCH_2CO
1k: R = $ClCH_2CO$
1l: R = $CNCH_2CO$
1m: R = N_3CH_2CO
1n: R = BR_2CHCO
1o: R = Cl_3CCO
1p: R = CO
1q: R = CH_3SO_2
1r: R = $ClCH_2PO(ONa)$

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