Efficacious and Orally Bioavailable Thrombin Inhibitors Based on a 2,5-Thienylamidine at the P1 Position: Discovery of *N*-Carboxymethyl-D-diphenylalanyl-L-prolyl[(5-amidino-2-thienyl)methyl]amide

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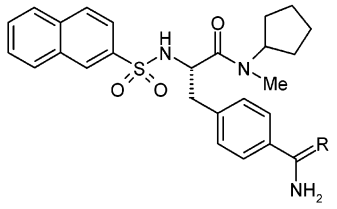
Thrombin, a crucial enzyme in the blood coagulation, has been a target for antithrombotic therapy. Orally active thrombin inhibitors would provide effective and safe prophylaxis for venous and arterial thrombosis. We conducted optimization of a highly efficacious benzamidine-based thrombin inhibitor LB30812 (**3**, K_i = 3 pM) to improve oral bioavailability. Of a variety of arylamidines investigated at the P1 position, 2,5-thienylamidine effectively replaced the benzamidine without compromising the thrombin inhibitory potency and oral absorption. The sulfamide and sulfonamide derivatization at the N-terminal position in general afforded highly potent thrombin inhibitors but with moderate oral absorption, while the well-absorbable N-carbamate derivatives exhibited limited metabolic stability in S9 fractions. The present work culminated in the discovery of the *N*-carboxymethyl- and 2,5-thienylamidine-containing compound **22** that exhibits the most favorable profiles of anticoagulant and antithrombotic activities as well as oral bioavilability (K_i = 15 pM; F = 43%, 42%, and 15% in rats, dogs, and monkeys, respectively). This compound on a gravimetric basis was shown to be more effective than a low molecular weight heparin, enoxaparin, in the venous thrombosis models of rat and rabbit. Compound **22** (LB30870) was therefore selected for further preclinical and clinical development.

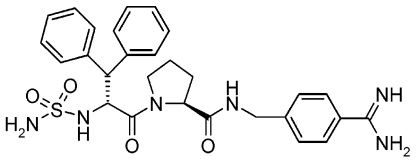
Introduction

Thrombin is a trypsin-like serine protease that plays a central role in thrombosis by cleaving fibrinogen to insoluble fibrin in the blood coagulation cascade. This enzyme also potently activates platelet aggregation by proteolytic activation of the thrombin receptor. Current anticoagulant therapy is limited to indirect thrombin inhibitors including heparin, low molecular heparins (LMWH), and a vitamin K antagonist, warfarin. However, neither heparin nor LMWH can be administered orally, and both heparin and warfarin require careful monitoring for dose adjustments to prevent bleeding complications. Accordingly, direct-acting and orally active thrombin inhibitors would be effective and safe in the treatment as well as prophylaxis of venous and arterial thrombosis.[1]

Small-molecule inhibitors of thrombin have been of considerable interest to medicinal chemists over the past decade.[2] Some years ago, we undertook a research program aimed at the discovery of orally active small-molecule thrombin inhibitors. Our earlier efforts focusing on arylsulfonylphenylalanine amides (so-called argatroban type) led us to a highly potent, selective, and orally active compound, **1** (LB30057, K_i = 0.4 nM), in the benzamidrazone P1 class,[3,4] along with other compounds in the benzylamine series derived from the same template (e.g., **2**) that are also highly orally bioavailable in rats.[5] However, the phase I clinical study of **1** revealed less than desired anticoagulant activity and shorter duration of action, and thus, its further development was limited. These clinical results, after all, led us to seek orally active thrombin inhibitors with sufficient efficacy that would clearly satisfy the criteria for clinically useful anticoagulants.



1, R = NNH_2; **2**, R = H_2



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Our continuing research efforts have been devoted to a class of D-Phe-Pro-agmatine-type[6] tripeptides, given that noncovalent inhibitors lacking an electrophilic serine trap are fast-binding and more efficacious and thus have a better therapeutic index than covalent inhibitors.[7,8] One strategic approach in this work was to judiciously combine the intrinsically potent D-diphenyl-Ala-Pro dipeptide[9,10] with our previously defined P1 elements such as benzamidrazone, benzylamine, and

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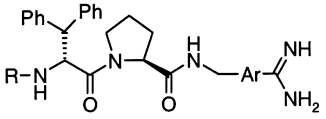
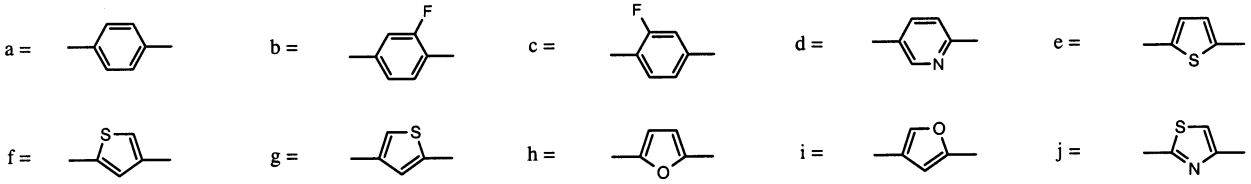
Table 1. Thrombin Inhibitory Activity and Pharmacokinetic Parameters for Compounds **3**−**25**





compd	R	aromatic group	thrombin[a] K_i (nM)	plasma concn[b] ($n = 3$) C_{max} (μg/mL)	plasma concn[b] ($n = 3$) AUC (μg·min/mL)
3	$H_2NS(O)_2$	a	0.003	2.8	272
4	$H_2NS(O)_2$	b	0.008	2.4	206
5	$H_2NS(O)_2$	c	0.010	1.1	89
6	$H_2NS(O)_2$	d	0.015	1.6	246
7	$H_2NS(O)_2$	e	0.004	2.2	282
8	$H_2NS(O)_2$	f	0.02	2.0	254
9	$H_2NS(O)_2$	g	0.16	0.5	
10	$H_2NS(O)_2$	h	0.43	1.0	136
11	$H_2NS(O)_2$	i	0.02	NT[c]	NT[c]
12	$H_2NS(O)_2$	j	0.12	NT[c]	NT[c]
13	$MeS(O)_2$	a	0.003	2.8	263
14	$MeS(O)_2$	e	0.005	1.3	85
15	MeOC(O)	a	0.03	4.0	594
16	MeOC(O)	d	0.017	14	1564
17	MeOC(O)	e	0.015	4.1	426
18	MeOC(O)	f	0.17	4.6	850
19	MeOC(O)	g	0.60	8.6	1297
20	MeOC(O)	i	1.4	7.1	1113
21	HO_2CCH_2	a	0.013	1.3	120
22	HO_2CCH_2	e	0.015	9.5	1390
23	HO_2CCH_2	g	0.10	4.6	212
24	HO_2CCH_2	j	0.64	11.1	1126
25	$HO_2CCH_2CH_2$	e	0.010	4.9	503
argatroban			4.0		

[a] Human thrombin. [b] Concentration after oral dosing at 30 mg/kg in rats as a water solution or as a 20−30% cosolvent (PEG400/EtOH/Tween20 = 85:10:5) solution in water. [c] NT: not tested.

benzamidine as a means to improve oral bioavailability (pK_a of ∼8.9,[5] ∼8.8,[11] and ∼10.2,[12] respectively). These P1 functionalities are substantially less basic than the agmatine but are still capable of strong binding in the S1 specificity pocket of thrombin via ionic interaction with Asp189. To this end, incorporation of the benzamidine P1 led to the identification of a series of extremely potent and orally absorbable thrombin inhibitors with excellent antithrombotic activity as exemplified by the sulfamide compound **3** (LB30812, K_i = 3 pM).[13] However, because of limited bioavailability (e.g., ∼10% in rats for **3**) and a short period of duration after oral administration, these compounds were unsuitable for further development. In this paper, we describe potent thrombin inhibitors with improved oral pharmacokinetic profile that resulted from further optimization studies with the amidine P1-based noncovalent tripeptides.

Results and Discussion

As shown in Table 1, the in vitro activity of compounds was expressed as the inhibition constant K_i. The oral absorption behavior of compounds in this study was determined primarily by the peak plasma concentration (C_{max}) and the area under the curve (AUC) measured after oral administration in rats.

The study focused herein is the investigation of a variety of arylamidines as replacements for the benzamidine at P1, and the structure−activity and structure−absorption relationships study began with the sulfamide lead **3**. On the basis of our previous experience that a fluorine substituent on the P1 phenylene ring of the amidrazone compound **1** has an effect on enhancing oral absorption,[14] fluorophenylene P1 linkers were examined for the amidine class of compounds. While both compounds **4** and **5** maintained thrombin inhibitory potency, there was no improvement in absorption in either case. The pyridine heterocyle as in compound **6** was beneficial to the aqueous solubility but did not improve the oral absorption. For the analogue **7** possessing 2,5-thiophene, both potency and oral absorption were found to be substantially comparable to those of **3**, consistent with the SAR for the sulfonate derivatives **13** and **14**.[15] On the other hand, incorporation of its regioisomeric thiophene nuclei as in the analogues **8** and **9** was detrimental to the potency. A potency decrease also resulted upon employing the furan and thiazole replacements as in compounds **10**−**12**. A relatively dramatic potency drop for the 2,5-furan analogue **10** can be rationalized by molecular modeling, which suggests that the highly bent bond angle of side chains on the furan ring (∼135°) causes unfavorable interaction of the

Table 2. Pharmacokinetic Parameters and Antithrombotic Effect of Selected Compounds in Rats ($n = 3-4$)

compd	Cl [a] (mL/min/kg)	Vdss [a] (mL/kg)	$t_{1/2}$ [a] (min)	AUC [a] (μg·min/mL)	F (%)	DVT [b] (rat, %)	ACT [c] (rat, n-fold)
3	8.6	297	45	1181	8	100	11
7	11.6	310	34	864	11	100	5.0
15	3.0	174	69	3407	6	100	6.8
16	2.1	139	81	4823	11	100	6.0
17	5.4	152	53	1870	8	93	5.2
19	1.7	230	45	6139	7	98	3.7
22	9.6	258	47	1059	43	100	12
24	6.0	189	50	1746	21	NT	NT
argatroban						82	1.9

[a] Parameter obtained after iv application at 10 mg/kg. [b] Inhibition of thrombus formation in thromboplastin-induced deep vein thrombosis (DVT) rat model after iv bolus dosing at 1 mg/kg. [c] Ex vivo activated clotting time (ACT) over the control (ca. 70 s).

amidine group with Asp189 in the S1 specificity pocket of thrombin. On the other hand, the 2,5-thienylamidine P1 (bond angle of ~158°) is well tolerated in the specificity pocket with the amidine group still forming a bidentate salt-bridge interaction with Asp189 (vide supra).

Our next P1 variation study was conducted with the methyl carbamate and carboxymethyl derivatives, **15** and **21**. Compound **15** in our previous paper was superior to the sulfamide **3** in oral absorption,[13] and the carboxymethyl functionality,[16,17] as exemplified by melagatran,[18] has been widely utilized in the thrombin inhibitor area as a means of improving aqueous solubility as well as oral bioavailability, although this element in the case of the benzamidine **21** was not advantageous for oral absorption. As the P1 aryl was varied, a potency trend in the carboxymethyl series of analogues **21**−**24** was very similar to that observed for the sulfamide series while a slightly different trend was seen for the carbamate series in which **15**−**17** are all similarly potent. When evaluated for oral absorption in rats, the methyl carbamate series displayed a better profile than the sulfamide series. In particular, compounds **16** and **19** in this series displayed 3- to 5-fold higher C_{max} and AUC values compared to **3**. In the series of carboxymethyl derivatives, good oral absorption behavior was displayed by compounds **22** and **24**; this is in contrast to that shown by compounds **21** and **23** despite that they are all very similar in structure.[19] These results suggest that these hydrophilic compounds are presumably absorbed by an active transport mechanism. Most notable in this series is compound **22**, which has an excellent profile of thrombin inhibition and oral absorption as well. Extension of the carboxymethyl chain as in **25** maintained potency compared to **22**. These results are consistent with those of X-ray crystallography of **22**, where the carboxylic acid group is directed toward the solvent, making no interactions with the thrombin active site (vide supra).

A set of key compounds was selected to determine absolute oral bioavailability and antithrombotic activity in rats (Table 2). Upon intravenous dosing, the carbamate derivatives **15**−**19** revealed relatively low clearance and moderate oral bioavailability despite their excellent C_{max} and AUC values observed after oral dosing. The carboxymethyl derivatives **22** and **24** displayed a similar level of clearance but superior oral bioavailability (43% and 21%, respectively), while the sulfamide derivatives **3** and **7** exhibited modest bioavailability. The antithrombotic efficacy for primary in vivo screening was determined using the thromboplastin-induced rat venous thrombosis model at an intravenous dose of 1 mg/kg (Table 2). Like compound **3**, compounds **7**, **15**, **16**, and **22** completely inhibited thrombus formation, reflecting their strong in vitro potency, whereas argatroban used as a reference was 82% effective and our earlier compound **1** (LB30057) was only 50% effective in this model. Activated clotting time (ACT) was also measured ex vivo from the rat plasma of the same animal setting to determine potential anticoagulant activity. Consistent with the results of antithrombotic activity, the ACT for each compound shown in Table 2 was highly increased and at least a 5-fold ACT over the control (ca. 70 s) was required for complete inhibition of thrombosis. For this series of thrombin inhibitors, it appears that the in vitro potency was well translated into ACT anticoagulant activity. Inhibitors with relatively lower K_i potency such as **10** and **20** exhibited a less than 2-fold ACT prolongation over the control (data not shown) despite them being more potent than argatroban.

Table 3. In Vitro Metabolic Stability of Selected Compounds

	recovery[a] (%)			
compd	rat	dog	monkey	human
3	90	93	87	92
7	84	96	81	86
15	80	65	39	86
17	60	91	<10	38
19	49	83	<10	13
21	87	95	80	97
22	89	99	83	95

[a] Recovery of compound after 30 min incubation in liver S9 at the initial concentration of 20 μM.

Compounds were evaluated for their metabolic stability using S9 fractions from rat, dog, monkey, and human livers (Table 3). In this assay, the sulfamoyl and carboxymethyl derivatives were generally quite stable in all species, but the methyl carbamate analogues were relatively unstable, particularly in the monkey. Consequently, such poor metabolic stability, together with low oral bioavailability, precluded further characterization of the carbamate analogues.

The selected compounds were evaluated in vitro for their ability to prolong activated partial thromboplastin time (APTT) in the rat and human plasma. Compared to argatroban, compounds **3**, **7**, and **22** exhibited excellent anticoagulant activity, doubling APTT at 0.14, 0.19, and 0.23 μM, respectively, in human plasma (Table 4). The superiority of these compounds was further demonstrated by their strong ability for ex vivo ACT prolongation in rats. Compound **22** doubled ACT at 0.85 μM. Hence, despite the 5-fold lower K_i potency in inherent thrombin inhibition, the anticoagulant efficacy

Table 4. In Vitro and Ex Vivo Anticoagulant Activity of Selected Compounds[a]

	anticoagulant activity (μM)		
compd	2 × APTT (human)	2 × APTT (rat)	2 × ACT (rat)
3	0.14	1.24	1.23
7	0.19	1.50	2.09
17	0.34	2.85	3.75
19	0.37	5.20	6.86
22	0.23	0.97	0.85
argatroban	0.57	2.50	1.97

[a] At concentration of inhibitor required to double APTT and ACT.

Table 5. Selectivity Profile for Compound **22**

enzyme[a]	K_i (nM)	selectivity (*n*-fold)
thrombin	0.015	1
factor Xa	2250	>10000
trypsin	0.30	20
trypsin[b]	3.0	200
plasmin	7400	>10000
t-PA	433	>10000

[a] Human. [b] Bovine.

of **22** compares favorably to that of **3**, which is possibly attributed to their difference in the plasma protein binding. Compound **22** exhibited 82% and 49% protein binding in the rat and human plasma, respectively, without significant variation in a concentration range of 1−30 μg/mL. On the other hand, compound **3** was 95% protein-bound in both species.

Because of its superior pharmacokinetic and pharmacological properties, compound **22** was selected for further in vitro and in vivo characterization. In addition to its excellent thrombin inhibitory activity, this compound displayed good selectivity toward other serine proteases important to hemostasis and thrombolysis (Table 5). Pharmacokinetic parameters for compound **22** in dogs and monkeys are shown in Table 6. Compound **22** was well absorbed in dogs after oral dosing at 5 mg/kg (at 5 mg/kg, $C_{max} = 3.8$ μg/mL, $F = 42\%$, $t_{1/2} = 68$ min). In cynomolgus monkeys, **22** was also orally bioavailable (at 20 mg/kg, $F = 15\%$, $t_{1/2} = 58$ min). Finally, **22** was compared with the most commonly used therapeutic anticoagulant LMWH in the rat and rabbit venous thrombosis models. Upon intravenous infusion at four different doses in the rats, compound **22** inhibited thrombus formation in a dose-dependent manner, showing complete inhibition at a bolus dose of 0.2 mg/kg with an intravenous infusion dose of 8 μg/(kg·min), which compares favorably to a dose of 2.5 mg/kg + 100 μg/(kg·min) for enoxaparin with an average MW of 5000 (Figure 1). In this study, the APTT of **22** was increased by approximately 1.7-, 2.8-, 3.6-, and 5.6-fold over the control. Consistent with these results, oral administration of this compound in the same rat model caused dose-dependent inhibition of thrombosis with an ID_{50} of 10.3 mg/kg and prolonged APTT by approximately 1.2-, 1.2-, 1.8- and 2.4-fold over the control, respectively (Figure 2). In a rabbit model, thrombosis was induced by insertion of silk threads saturated with thromboplastin into a vena cava. Compound **22** significantly inhibited rabbit venous thrombosis at a dose of 12.5 μg/kg + 0.5 μg/(kg·min), which caused a 1.5-fold increase in APTT (Figure 3). On the other hand, enoxaparin, a positive reference used in comparison, required a much higher dose on a gravimetric basis for a similar effect of thrombosis inhibition in rabbits (300 μg/kg + 5 μg/(kg·min)).

The crystal structure of human thrombin complexed with **22** has been obtained at 1.8 Å (Figure 4).[20] The inhibitor is bound in the active site in a fashion very similar to that of the previously reported compounds.[9,10a] As expected, the diphenyl and proline rings span the distal and proximal hydrophobic pockets, respectively.

Table 6. Pharmacokinetic Parameters for Compound **22**

species	dose (mg/kg)	*n*	route	Cl (mL/(min·kg))	Vdss (mL/kg)	$t_{1/2}$ (min)	C_{max} (μg/mL)	T_{max} (min)	AUC (μg·min/mL)	*F* (%)
dog	5	3	po			68	3.8	30	363	42
	2.5	3	iv	5.9	223	54			457	
monkey	20	2	po			58	2.1	90	309	15
	2	2	iv	9.8	230	23			205	

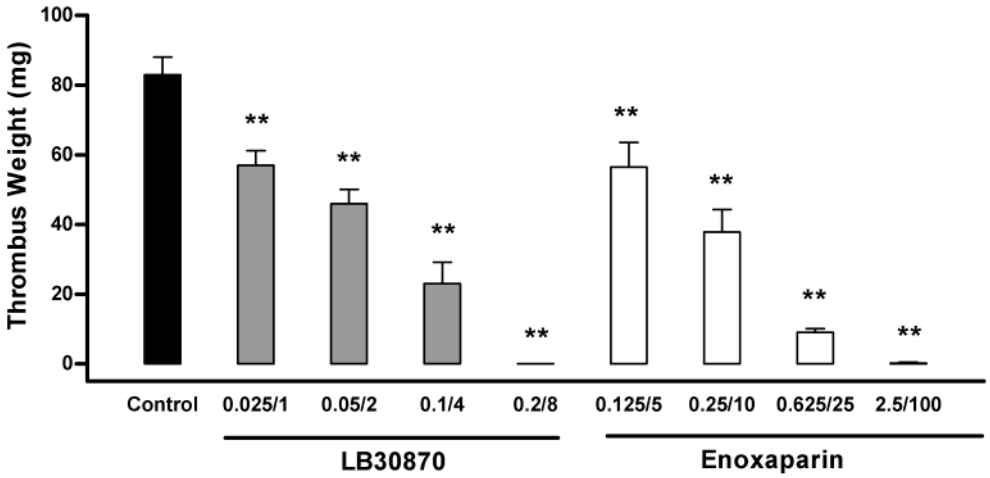


Figure 1. Dose-dependent antithrombotic effect of **22** (LB30870) and LMWH after iv administration in the rat venous thrombosis model: bolus (mg/kg)/infusion (μg/(kg·min)); $n = 10$ for the control group; $n = 6$ for each inhibitor treatment group; (**) $p < 0.01$ vs control; ANOVA, followed by Dunnett's multiple comparison test.

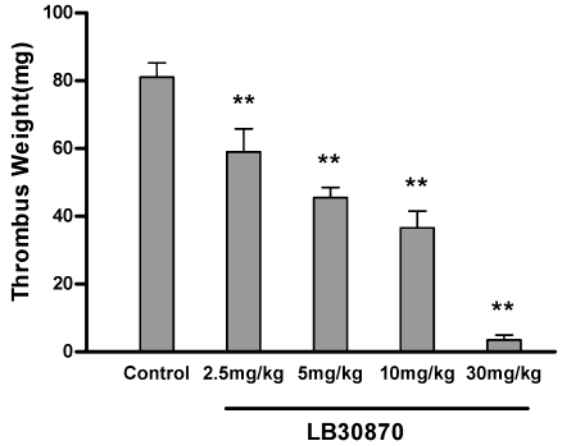


Figure 2. Dose-dependent antithrombotic effect of **22** (LB30870) after oral administration in the rat venous thrombosis model: $n = 6$ for the control and inhibitor treatment groups; (**) $p < 0.01$ vs control; ANOVA, followed by Dunnett's multiple comparison test.

As discussed above, the thienylamidine P1 chain binds in the S1 specificity pocket with the amidine making the bidentate salt bridge interaction with Asp189. The amidine is also hydrogen-bonded to Gly219 and a water molecule. On the other hand, the carboxylic acid moiety is solvent-exposed without interacting with the thrombin active site.

Chemistry

The target compounds were prepared as exemplified in Scheme 1. The N-protected dipeptide **26** was coupled to the P1 precursors **30a**−**i**, and the *N*-Boc group was removed. The free amines **27** were reacted with R−Cl (sulfamoyl chloride, sulfonyl chloride, and chloroformate) in the presence of base. The resulting intermediates were then converted to the amidines **3**−**20** by a modified Pinner reaction that proceeds via a thioimidate intermediate. Compounds **21**−**25** were prepared from **27** by N-alkylation with alkyl bromides, nitrile to amidine conversion, and hydrolysis of the ester groups. In this case, the amidine formation was more efficiently preceded by catalytic hydrogenation of the amidoxime intermediates, **28** and **29**, under acetylation conditions.[21]

The synthesis of the requisite P1 precursors, CN−Ar−CH_2NH_2 **30b**−**j**, is also outlined in Scheme 1. As previously described,[13] compounds **30b**−**e** were typically prepared from the commercially available materials of general structure CH_3−Ar−Br in several steps involving CuCN-mediated cyanation, radical bromination with *N*-bromosuccinimide and a Gabriel-type amination[22] with di-*tert*-butyl iminodicarboxylate. Synthesis of compounds **30f**−**i** began with aldehyde reduction of OHC−Ar−Br (iodoaldehyde[23] for **30g**), and subsequent PPh_3/CBr_4 bromination followed by cyanation afforded the common intermediate $BrCH_2$−Ar−CN. Unlike this pathway, the thiazolecarbonitrile **30j** was prepared from the amide precursor. This thiazole precursor was readily prepared by conversion of *N*-Boc-glycinamide into a thioamide and subsequent cyclocondensation with 3-bromo-2-oxopropionic acid, followed by amidation with aqueous ammonia as previously described by Jung.[24] For the practical preparation of the 2,5-thiophene **30e**, which is a precursor of **22**, the bromide **31** was transformed to an azide followed by iminophosphorylation and subsequent hydrolysis.

Conclusion

We have conducted optimization of a highly efficacious noncovalent tripeptidic inhibitor of thrombin, **3** (LB30812), focusing on improving oral bioavailability. Of a variety of aryl replacements explored at the amidine P1 position, the 2,5-thienylamidine could effectively replace the benzamidine without compromising thrombin inhibitory potency and oral absorption behavior. The sulfamide and sulfonamide derivatization at the N-terminal position, in general, afforded highly potent thrombin inhibitors with good metabolic stability while displaying a moderate level of C_{max} and AUC values upon oral administration to rats. The *N*-carbamate derivatives exhibited superior absorption profile but at the expense of metabolic stability in S9 fractions. The *N*-carboxymethyl derivative **22** demonstrated the best

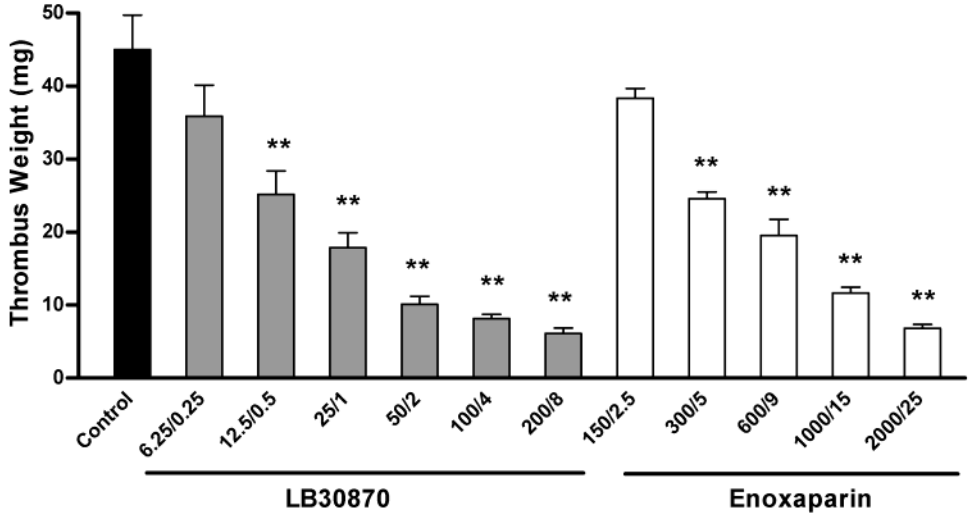


Figure 3. Dose-dependent antithrombotic effect of **22** (LB30870) and LMWH after iv administration in the rabbit venous thrombosis model: bolus (μg/kg)/infusion (μg/(kg·min)); $n = 11$ for the control group; $n = 4$−8 for each inhibitor treatment group; (**) $p < 0.01$ vs control; ANOVA, followed by Dunnett's multiple comparison test.

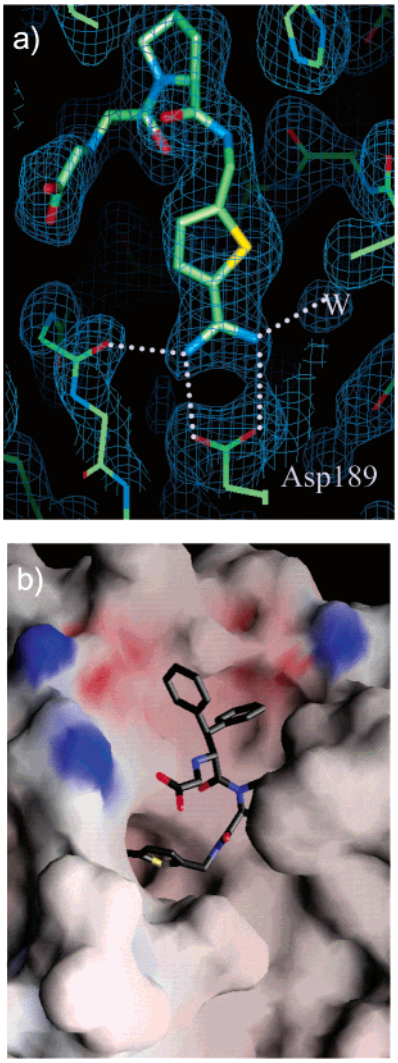


Figure 4. Compound **22** is complexed with human thrombin as determined by X-ray crystallography. (a) The final electron density ($2F_o - F_c$) around the P1 pocket is contoured at the 1.2σ level. Hydrogen bonds are indicated as dashed lines. There is a tightly bound water molecule that serves as a bridge. (b) **22** is bound to thrombin in a surface representation. The diphenyl moiety fits very nicely near the P4 site. Oxygen atoms are shown in red, nitrogen atoms are shown in blue, sulfur is shown in yellow and carbons are shown in either green or dark-gray.

overall profile in this series, which is potent, selective, metabolically stable, and orally bioavailable. This compound also displayed excellent anticoagulant and antithrombotic activity in venous thrombosis models of rat and rabbit. Compound **22** (LB30870) was therefore selected for further preclinical and clinical development.

Experimental Section

Chemistry. The solvents employed in these experiments were from commercial grade and used without further purification. Proton NMR spectra were recorded on a JEOL 500 spectrometer. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane. Column chromatography was carried out with Merck grade 60 silica gel (230−400 mesh). Purification of the final products was achieved by reverse-phase preparative HPLC on a Delta-Pak C18 column, 100 Å pore size, with trifluoroacetic acid (0.1%)−H_2O−MeOH solvent systems using various linear gradients. The pure fractions were lyophilized to give a white solid. FABMS and HR FABMS were obtained on a JEOL DX300 mass spectrometer. Microanalyses were performed by LG Chem. Analysis Center, Korea, and were within 0.4% of the calculated values.

5-(Aminomethyl)thiophene-2-carbonitrile Hydrochloride (30e). A mixture of 5-methylthiophene-2-carbonitrile (9.9 g, 80.5 mmol), benzoyl peroxide (0.23 g, 0.95 mmol), and *N*-bromosuccinimide (15 g, 84.3 mmol) in carbon tetrachloride (200 mL) was heated at reflux for 6 h. The resulting suspension was filtered, and the filtrate was diluted with dichloromethane (400 mL), washed with a saturated sodium bicarbonate solution, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/*n*-hexane, 1:4) to give 5-(bromomethyl)thiophene-2-carbonitrile[17] as a yellow oil (14.0 g, 86%).

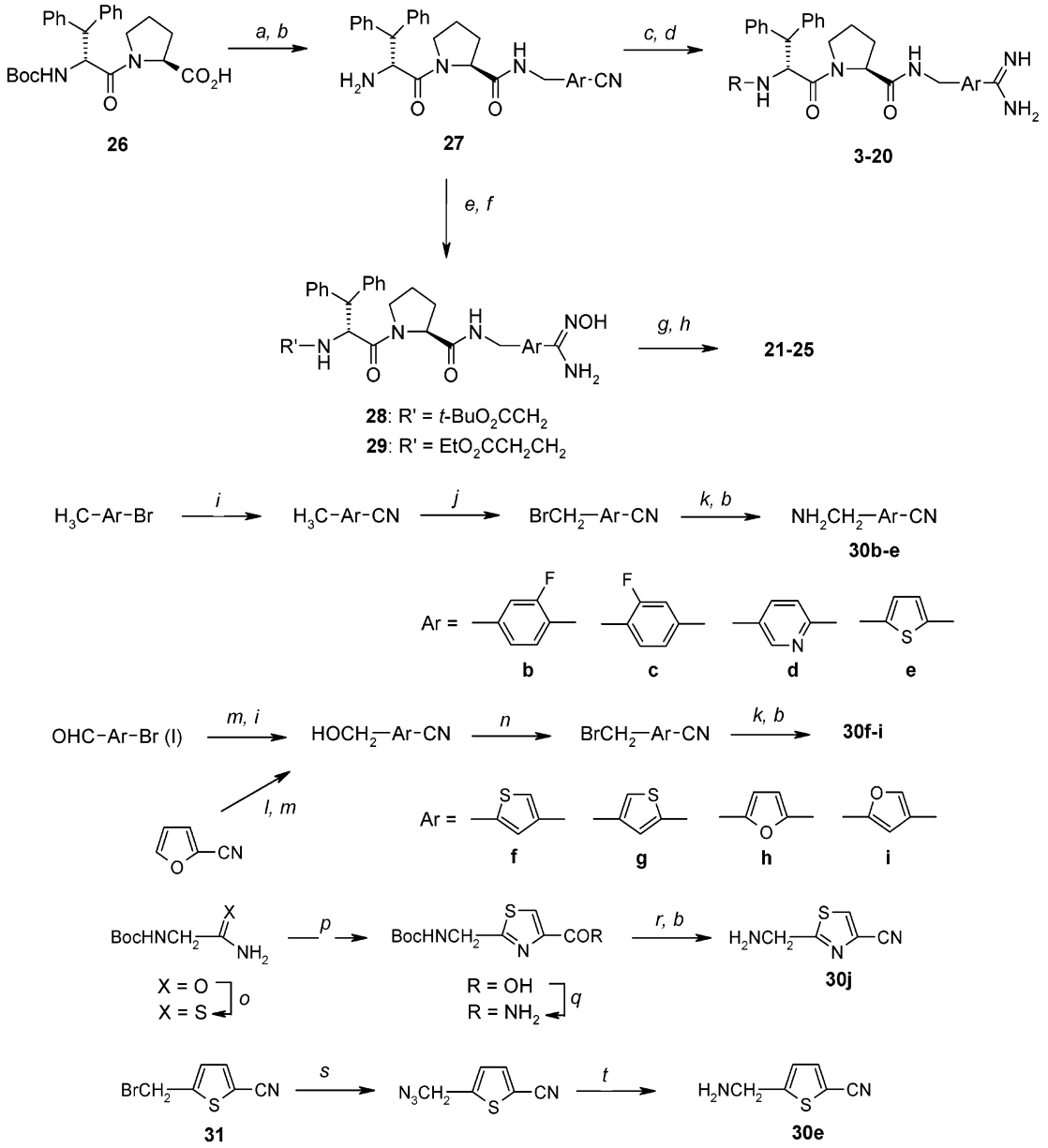
To a cold solution of 5-(bromomethyl)thiophene-2-carbonitrile (4.2 g, 20.8 mmol) in THF (500 mL) was added sodium hydride (60% dispersion in oil, 1.0 g, 25 mmol) in portions. To this suspension was added in portions di-*tert*-butyl iminodicarboxylate (4.9 g, 22.9 mmol, purchased from TCI, Japan). After being stirred for 3 h, the resulting solution was diluted with ethyl acetate (400 mL), washed with water, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/*n*-hexane, 1:4) to give 5-(*N*,*N*-Boc_2-aminomethyl)thiophene-2-carbonitrile as a yellow foam. This solid was dissolved in ethyl acetate (150 mL) and cooled to 0 °C. HCl gas was bubbled through the solution for 10 min, and the mixture was allowed to warm to room temperature. The solvent was removed in vacuo to give the title compound as a pale-yellow solid (2.4 g, 86%). ^{1}H NMR (CD_3OD): δ 7.48 (d, 1H), 7.20 (d, 1H), 4.21 (s, 2H). FAB MS, *m*/*z*: 175 $[M + 1]^+$.

Alternative Preparation of 30e. To a solution of 5-(bromomethyl)thiophene-2-carbonitrile (14.4 g, 71.3 mmol) in benzene (71 mL) was added tetrabutylammonium chloride (0.46 g, 1.43 mmol) and sodium azide (5.57 g, 85.6 mmol), and the mixture was stirred for 1 h at room temperature. The resulting solution was washed with water (20 mL × 2) and cooled to 15 °C. Triphenylphosphine was added portionwise for 1 h, and the mixture was stirred for 1.5 h. After the reaction was completed, 12.8 mL of water was added and the solution was heated at 70 °C for 3 h and extracted with 2 N HCl (30 mL × 2). The aqueous layer was washed with dichloromethane (30 mL), basified with NaOH (4.6 g), and extracted with dichloromethane (30 mL × 2). The combined extracts were concentrated, the residue was dissolved in diethyl ether (70 mL), and 3 N HCl in ethyl acetate (30 mL) was added dropwise keeping the temperature below 25 °C. The white precipitates were collected by filtration and the filter cake was washed with diethyl ether and ethyl acetate to give **30e** as a white solid (10.9 g, 88%).

D-Diphenylalanyl-L-prolyl-[(5-cyano-2-thienyl)methyl]-amide Hydrochloride (27e). To a solution of *N*-Boc-D-diphenylalanyl-L-proline (2 g, 4.8 mmol) in DMF (20 mL) was added 5-(aminomethyl)thiophene-2-carbonitrile hydrochloride (**30e**) (1 g, 5.7 mmol), EDC (1.4 g, 7.2 mmol), HOBT (0.8 g, 6.2 mmol), and triethylamine (2 mL, 14.4 mmol), and the mixture was stirred overnight at room temperature. The solvent was removed in vacuo, and the residue was dissolved in EtOAc and washed sequentially with a saturated sodium bicarbonate solution, 1 N HCl, and brine. After the solvent was dried over magnesium sulfate and concentrated in vacuo, the residue was purified by column chromatography (EtOAc/*n*-hexane, 2:1) to give *N*-Boc-D-diphenylalanyl-L-prolyl[(5-cyano-2-thienyl)methyl]-amide (2.3 g, 86%). ^{1}H NMR ($CDCl_3$): δ 7.83 (s, 1H), 7.55−7.10 (m, 11H), 6.92 (d, 1H), 4.98 (d, 1H), 4.80 (m, 1H), 4.53 (m, 2H), 4.37 (m, 2H), 3.68 (m, 1H), 2.54 (q, 1H), 2.09 (m, 1H), 1.66 (m, 1H), 1.45 (m, 1H), 1.37 (m, 1H), 1.31 (s, 9H). FAB MS, *m*/*z*: 558 $[M + 1]^+$.

To a cooled solution of this compound (2 g, 3.7 mmol) in EtOAc (20 mL) was added 4 N HCl in EtOAc (5 mL). After being stirred for 3 h at room temperature, the solution was removed to dryness to give the title compound as a white solid (2.71 g, 100%). ^{1}H NMR ($CDCl_3$): δ 8.52 (m, 1H), 7.55−7.12 (m, 11H), 6.84 (d, 1H), 4.77 (d, 1H), 4.57 (d, 1H), 4.50 (dd, 1H), 4.23 (d, 1H), 4.18 (dd, 1H), 3.77 (t, 1H), 2.43 (q, 1H), 1.94 (m,

Scheme 1[a]



[a] (a) CN−Ar−CH_2NH_2 (**30a**−**i**), NMM, EDC, HOBT, DMF, quantitaive; (b) TFA, CH_2Cl_2 or 4 N HCl/EtOAc, EtOAc; (c) R−Cl (methanesulfonyl chloride, sulfamoyl chloride, methyl chloroformate), Et_3N, CH_2Cl_2; (d) (i) H_2S, Py, Et_3N, (ii) MeI, CH_3CN, reflux, (iii) NH_4OAc, MeOH, reflux; (e) t-BuO_2CCH_2Br or $EtO_2CCH_2CH_2Br$, DIPEA, NaI (cat.), CH_3CN, room temp, 36 h; (f) NH_2OH·HCl, Na_2CO_3, EtOH/H_2O; (g) H_2, 10% Pd−C, Ac_2O, MeOH; (h) 6 N HCl, MeOH for **21**−**24**; LiOH, H_2O for **25**; (i) CuCN, DMF, reflux; (j) NBS, benzoyl peroxide, CCl_4, reflux; (k) $(Boc)_2NH$, NaH, THF; (l) n-BuLi, DMF, THF, −78 °C; (m) $NaBH_4$, MeOH, room temp; (n) CBr_4, PPh_3, CH_2Cl_2; (o) Lawesson's Reagent, DME, room temp; (p) 3-bromo-2-oxopropionic acid, $CaCO_3$, EtOH, room temp; (q) isobutyl chloroformate, NH_4OH, NMM, THF; (r) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (s) NaN_3, Bu_4NCl, benzene, room temp; (t) PPh_3, H_2O, benzene, reflux.

1H), 1.62 (m, 1H), 1.48 (m, 1H), 1.30 (m, 1H). FAB MS, *m*/*z*: 459 $[M + 1]^+$. Anal. Calcd for $C_{26}H_{26}N_4O_2S$·1.0$C_4H_8O_2$·1.0HCl: C, 61.79; H, 6.05; N, 9.61. Found: C, 61.6; H, 5.9; N, 9.8.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(5-amidino-2-thienyl)methyl]amide Trifluoroacetic Acid (7).** To a stirring solution of chlorosulfonyl isocyanate (6.3 g, 45 mmol) in dichloromethane (25 mL) was added dropwise formic acid (2.13 g, 45 mmol). The mixture was heated at reflux for 5 h and cooled to obtain a 1.8 N solution of sulfamoyl chloride in dichloromethane. To a cooled (0 °C) solution of **27e** (3.2 g, 6.47 mmol) in dichloromethane (100 mL) was added the 1.8 N sulfamoyl chloride solution (6 mL) and triethylamine (2.7 mL). After the reaction was completed, the resulting solution was diluted with dichloromethane (40 mL), washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/*n*-hexane, 3:1) to give *N*-aminosulfonyl-D-diphenylalanyl-L-prolyl-[(5-cyano-2-thienyl)methyl]amide (3.1 g, 88%). ^1H NMR ($CDCl_3$): δ 7.77 (d, 1H), 7.51−7.17 (m, 10H), 7.15 (d, 1H), 4.98 (d, 1H), 4.62 (m, 1H), 4.59 (m, 1H), 4.31 (m, 1H), 4.05 (m, 1H), 3.76 (m, 1H), 2.90 (m, 1H), 1.85 (m, 1H), 1.73 (m, 1H), 1.62 (m, 1H), 1.46 (m, 1H). MS, *m*/*z*: 538 $[M + 1]^+$.

A solution of the coupling compound (0.2 g, 0.37 mmol) in pyridine (2 mL) and Et_3N (0.2 mL) was saturated with gaseous H_2S. After the mixture stood for 1 day, the solvent was removed in vacuo to obtain the thioamide as a yellow solid. To this material was added acetonitrile (2 mL) and iodomethane (0.07 mL, 1.12 mmol), and the mixture was heated at reflux for 1 h. After the solvent was evaporated in vacuo, the resulting methyl thioimidate was dissolved in acetonitrile (2 mL). To this solution was added ammonium acetate (0.09 g, 1.12 mmol) over 10 min, and the mixture was heated at reflux for 1 h. The solution was cooled and concentrated and

the residue was purified by column chromatography using 10% methanol in chloroform to give the title compound, which was further purified by preparative HPLC (TFA (0.1%)−H_2O−MeOH gradient). ^{1}H NMR (CD_3OD): δ 8.21 (m, 1H), 7.77 (d, 1H), 7.51−7.25 (m, 11H), 4.98 (d, 1H), 4.62 (dd, 1H), 4.59 (dd, 1H), 4.31 (d, 1H), 4.05 (dd, 1H), 3.76 (m, 1H), 2.90 (q, 1H), 1.85 (m, 1H), 1.73 (m, 1H), 1.62 (m, 1H), 1.46 (m, 1H). FAB MS, *m*/*z*: 555 $[M + 1]^+$. Anal. Calcd for $C_{26}H_{30}N_6O_4S_2 \cdot 1.3C_2HF_3O_2 \cdot 0.3H_2O$: C, 48.3; H, 4.5; N, 11.8. Found: C, 48.3; H, 4.6; N, 11.6.

***N*-(*tert*-Butoxycarbonyl)methyl-D-diphenylalanyl-L-prolyl[(5-hydroxyamidino-2-thienyl)methyl]amide (28e).** To a cooled (0 °C) solution of **27e** (3.03 g, 6.06 mmol) in acetonitrile (60 mL) was added diisopropylethylamine (4.22 mL, 24.24 mmol) and *tert*-butyl bromoacetate (4.22 mL, 9.09 mmol), and the mixture was stirred for 2 days at room temperature. After the reaction mixture was concentrated in vacuo, the residue was purified by column chromatography (EtOAc/*n*-hexane, 7:3) to give *N*-(*tert*-butoxycarbonyl)methyl-D-diphenylalanyl-L-prolyl[(5-cyano-2-thienyl)methyl]amide (2.51 g, 72%). ^{1}H NMR ($CDCl_3$): δ 8.12 (t, 1H), 7.37 (m, 5H), 7.19 (m, 6H), 6.93 (d, 1H), 4.61 (dd, 1H), 4.49 (dd, 1H), 4.25 (m, 2H), 4.12 (dd, 1H), 3.24 (m, 3H), 2.67 (m, 1H), 2.07 (m, 1H), 1.67 (m, 1H), 1.43 (m, 1H), 1.37 (s, 9H), 1.25 (m, 1H). FAB MS, *m*/*z*: 573 $[M + 1]^+$.

To a solution of the above product (2.31 g, 4.03 mmol) in a 4:1 mixture of ethanol and water (60 mL) was added hydroxylamine hydrochloride (1.04 g, 14.91 mmol) and sodium carbonate (726 mg, 6.85 mmol), and the mixture was heated at reflux for 1 h. After the reaction mixture was concentrated in vacuo, the residue was diluted with EtOAc, washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give the title compound (2.36 g, 96%). ^{1}H NMR (CD_3OD): δ 8.13 (t, 1H), 7.42−7.35 (m, 4H), 7.27−7.11 (m, 7H), 7.01 (d, 1H), 6.83 (d, 1H), 4.87 (s, 2H), 4.56 (m, 1H), 4.44 (m, 1H), 4.27 (m, 3H), 3.32 (m, 1H), 3.25 (dd, 2H), 2.73 (m, 1H), 1.80−1.42 (m, 4H), 1.41 (s, 9H). FAB MS, *m*/*z*: 606 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_4S \cdot 1.5H_2O$: C, 60.0; H, 6.1; N, 12.5. Found: C, 60.2; H, 6.3; N, 12.6.

***N*-Carboxymethyl-D-diphenylalanyl-L-prolyl[(5-amidino-2-thienyl)methyl]amide Ditrifluoroacetic Acid (22).** To a solution of compound **28e** (2.36 g, 3.89 mmol) in methanol (45 mL) was added 10% palladium-on-carbon (240 mg) and acetic anhydride (0.74 mL, 7.78 mmol), and the mixture was stirred for 8 h under H_2 (1 atm). The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was purified by preparative HPLC to give the *N*-(*tert*-butoxycarbonyl)methyl-D-diphenylalanyl-L-prolyl-[(5-amidino-2-thienyl)methyl]amide, acetic acid salt (1.6 g, 70%). ^{1}H NMR (CD_3OD): δ 7.77 (d, 1H), 7.59 (m, 2H), 7.44 (m, 2H), 7.31−7.18 (m, 6H), 7.16 (d, 1H), 4.61 (dd, 2H), 4.49 (d, 1H), 4.20 (d, 1H), 4.03 (m, 1H), 3.53 (m, 1H), 2.99 (m, 1H), 1.89 (s, 3H), 1.85−1.68 (m, 3H), 1.42 (s, 9H), 1.35 (m, 1H). FAB MS, *m*/*z*: 590 $[M + 1]^+$.

To a cooled (0 °C) solution of the above compound (1.4 g, 2.37 mmol) in dichloromethane (15 mL) was added TFA (15 mL), and the mixture was stirred for 3.5 h. After the reaction mixture was concentrated in vacuo, the residue was purified by reverse-phase preparative HPLC to give the title compound (1.2 g, 80%). The analytical sample was obtained as a crystalline solid by neutralizing the TFA salt with 2 N NaOH. ^{1}H NMR (CD_3OD): δ 7.80 (d, 1H), 7.65 (m, 2H), 7.49 (m, 2H), 7.39−7.22 (m, 6H), 7.19 (d, 1H), 5.33 (d, 1H), 4.60 (dd, 2H), 4.58 (d, 1H), 4.06 (m, 1H), 3.84 (dd, 2H), 3.49 (m, 1H), 2.85 (m, 1H), 1.89−1.73 (m, 3H), 1.32 (m, 1H). FAB MS, *m*/*z*: 534 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_4S \cdot 1.5H_2O$: C, 60.0; H, 6.1; N, 12.5. Found: C, 60.2; H, 6.3; N, 12.6.

***N*-(2-Carboxyethyl)-D-diphenylalanyl-L-prolyl-[(5-amidino-2-thienyl)methyl]amide Ditrifluoroacetic Acid (25).** A mixture of **27e** (400 mg, 0.81 mmol), sodium carbonate (690 mg, 6.5 mmol), sodium iodide (609 mg, 4.1 mmol), tetrabutylammonium bromide (79 mg, 0.244 mmol), and methyl 3-bromopropionate (0.2 mL, 1.626 mmol) in toluene (8 mL) was heated at reflux for 5 h. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography (EtOAc/*n*-hexane, 7:3) to give *N*-[2-(methoxycarbonyl)ethyl]-D-diphenylalanyl-L-prolyl[(5-cyano-2-thienyl)methyl]amide (180 mg, 41%). This compound (160 mg) was hydrogenated in the presence of palladium-on-carbon and acetic anhydride using the procedure as described in the preparation of **22** to give *N*-[2-(methoxycarbonyl)ethyl]-D-diphenylalanyl-L-prolyl[(5-amidino-2-thienyl)methyl]amide diacetic acid **29** (91 mg, 54%). ^{1}H NMR (CD_3OD): δ 7.74 (d, 1H), 7.35−7.11 (m, 10H), 6.98 (d, 1H), 4.72 (dd, 1H), 4.30 (m, 1H), 4.17 (s, 2H), 4.16 (m, 1H), 3.53 (s, 3H), 3.52 (m, 1H), 2.89 (m, 2H), 2.75 (m, 1H), 2.38 (m, 2H), 1.89 (m, 2H), 1.62 (m, 1H), 1.40 (m, 1H). FAB MS, *m*/*z*: 562 $[M + 1]^+$

A mixture of the above product (91 mg, 0.16 mmol), 0.5 N LiOH (10 mL), and water (3 mL) was stirred for 3 h at room temperature. The reaction mixture was neutralized with 1 N HCl and concentrated in vacuo. The residue was purified by preparative HPLC (TFA (0.1%)−H_2O−MeOH gradient) to give the title compound (55 mg, 44%). ^{1}H NMR (CD_3OD): δ 7.81 (d, 1H), 7.62 (m, 2H), 7.50 (m, 2H), 7.42−7.32 (m, 6H), 7.21 (d, 1H), 5.12 (d, 1H), 4.61 (dd, 2H), 4.50 (d, 1H), 4.11 (m, 1H), 3.60 (m, 1H), 3.31 (m, 2H), 2.78 (m, 1H), 2.68 (m, 2H) 1.83 (m, 3H), 1.30 (m, 1H). FAB MS, *m*/*z*: 548 $[M + 1]^+$. Anal. Calcd for $C_{29}H_{33}N_5O_4S \cdot 2.0C_2HF_3O_2 \cdot 0.3H_2O$: C, 50.7; H, 4.6; N, 8.9. Found: C, 50.5; H, 4.5; N, 8.9.

In a procedure similar to the synthesis of **7** and **22**, compounds **3**−**6**, **8**−**21**, **23**, and **24** were prepared from the corresponding amines, CN−Ar−CH_2NH_2.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl-[(4-amidinophenyl)methyl]amide Trifluoroacetic Acid (3).** ^{1}H NMR (CD_3OD): δ 7.73 (d, 2H), 7.55−7.18 (m, 12H), 4.91 (d, 1H), 4.45 (d, 1H), 4.39−4.31 (t, 2H), 4.09 (m, 1H), 3.78 (m, 1H), 2.91 (q, 1H), 1.85 (m, 1H), 1.71 (m, 1H), 1.61 (m, 1H), 1.46 (m, 1H). FAB MS, *m*/*z*: 549 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{32}N_6O_4S \cdot 1.3C_2HF_3O_2 \cdot 0.3H_2O$: C, 52.1; H, 4.9; N, 11.9. Found: C, 52.0; H, 4.9; N, 12.1.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(4-amidino-3-fluorophenyl)methyl]amide Trifluoroacetic Acid (4).** ^{1}H NMR (CD_3OD): δ 7.60 (m, 1H), 7.45−7.21 (m, 12H), 5.05 (d, 1H), 4.50 (dd, 1H), 4.35 (m, 2H), 4.07 (m, 1H), 3.78 (m, 1H), 2.88 (m, 1H), 1.85 (m, 1H), 1.72 (m, 1H), 1.61 (m, 1H), 1.47 (m, 1H). FAB MS, *m*/*z*: 567 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}FN_6O_4S \cdot 1.2C_2HF_3O_2 \cdot 0.5H_2O$: C, 51.0; H, 4.7; N, 11.7. Found: C, 50.8; H, 4.8; N, 11.4.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(4-amidino-2-fluorophenyl)methyl]amide Trifluoroacetic Acid (5).** ^{1}H NMR (CD_3OD): δ 7.71−7.21 (m, 13H), 4.98 (d, 1H), 4.56 (m, 1H), 4.35 (m, 2H), 4.08 (m, 1H), 3.78 (m, 1H), 2.90 (m, 1H), 1.84 (m, 1H), 1.75 (m, 1H), 1.61 (m, 1H), 1.47 (m, 1H). FAB MS, *m*/*z*: 567 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}FN_6O_4S \cdot 1.2C_2HF_3O_2 \cdot 0.7H_2O$: C, 50.7; H, 4.7; N, 11.6. Found: C, 50.7; H, 4.8; N, 11.5.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl(6-amidino-3-picolyl)amide Trifluoroacetic Acid (6).** ^{1}H NMR (CD_3OD): δ 8.72 (s, 1H), 8.08 (m, 1H), 7.99 (m, 1H), 7.44−7.35 (m, 4H), 7.26−7.24 (m, 6H), 4.98 (d, 1H), 4.59 (d, 1H), 4.33−4.30 (m, 2H), 4.06 (m, 1H), 3.76 (m, 1H), 2.90 (m, 1H), 1.84−1.45 (m, 4H). FAB MS, *m*/*z*: 550 $[M + 1]^+$. Anal. Calcd for $C_{27}H_{31}N_7O_4S \cdot 2.7C_2HF_3O_2 \cdot 0.7H_2O$: C, 44.9; H, 4.1; N, 11.3. Found: C, 44.7; H, 4.2; N, 11.5.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(4-amidino-2-thienyl)methyl]amide Trifluoroacetic Acid (8).** ^{1}H NMR (CD_3OD): δ 8.10 (m, 1H), 7.51−7.25 (m, 11H), 4.99 (d, 1H), 4.45 (dd, 2H), 4.31 (d, 1H), 4.06 (m, 1H), 3.76 (m, 1H), 2.87 (m, 1H), 1.87 (m, 1H), 1.71 (m, 1H), 1.60 (m, 1H), 1.47 (m, 1H). FAB MS, *m*/*z*: 555 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_4S \cdot 1.3C_2HF_3O_2 \cdot 0.5H_2O$: C, 48.1; H, 4.6; N, 11.7. Found: C, 48.3; H, 4.7; N, 11.5.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(5-amidino-3-thienyl)methyl]amide Trifluoroacetic Acid (9).** ^{1}H NMR (CD_3OD): δ 7.83 (m, 1H), 7.45−7.35 (m, 4H), 7.30−7.15 (m, 7H), 4.95 (d, 1H), 4.45 (m, 1H), 4.32 (d, 1H), 4.21 (d, 1H), 4.04 (m, 1H), 3.76 (m, 1H), 2.89 (m, 1H), 1.85 (m, 1H), 1.77 (m, 1H), 1.61 (m, 1H), 1.46 (m, 1H). FAB MS, *m*/*z*: 555 $[M + 1]^+$.

Anal. Calcd for $C_{26}H_{30}N_6O_4S_2 \cdot 1.4C_2HF_3O_2 \cdot 0.4H_2O$: C, 47.9; H, 4.5; N, 11.6. Found: C, 48.1; H, 4.6; N, 11.6.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(5-amidino-2-furanyl)methyl]amide Trifluoroacetic Acid (10).** ^{1}H NMR (CD_3OD): δ 7.78 (m, 3H), 7.52 (m, 2H), 7.34 (m, 6H), 6.60 (d, 1H), 4.99 (d, 1H), 4.49 (m, 2H), 4.24 (d, 2H), 4.11 (m, 1H), 3.76 (m, 1H), 2.95 (m, 1H), 1.81−1.37 (m, 4H). FAB MS, *m/z*: 538 $[M + 1]^+$. Anal. Calcd for $C_{26}H_{30}N_6O_5S \cdot 1.2C_2HF_3O_2 \cdot 0.2H_2O$: C, 49.9; H, 4.7; N, 12.3. Found: C, 49.6; H, 4.6; N, 12.5.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(5-amidino-3-furanyl)methyl]amide Trifluoroacetic Acid (11).** ^{1}H NMR (CD_3OD): δ 7.83 (s, 1H), 7.47 (s, 1H), 7.43 (m, 2H), 7.35 (m, 2H), 7.25 (m, 6H), 4.93 (d, 1H), 4.36 (m, 1H), 4.30 (d, 1H), 4.11 (m, 1H), 4.02 (m, 1H), 3.75 (m, 1H), 2.88 (m, 1H), 1.84 (m, 1H), 1.75−1.55 (m, 2H), 1.43 (m, 1H). FAB MS, *m/z*: 539 $[M + 1]^+$. Anal. Calcd for $C_{26}H_{30}N_6O_5S \cdot 1.3C_2HF_3O_2 \cdot 0.3H_2O$: C, 49.4; H, 4.6; N, 12.1. Found: C, 49.6; H, 4.5; N, 11.9.

***N*-Aminosulfonyl-D-diphenylalanyl-L-prolyl[(4-amidino-2-thiazolyl)methyl]amide TFA (12).** ^{1}H NMR (CD_3OD): δ 8.56 (s, 1H), 7.45 (m, 2H), 7.35 (m, 2H), 7.25 (m, 6H), 4.94 (d, 1H), 4.67 (m, 2H), 4.33 (d, 1H), 4.11 (m, 1H), 3.77 (m, 1H), 2.89 (m, 1H), 1.88 (m, 1H), 1.80−1.61 (m, 2H), 1.47 (m, 1H). FAB MS, *m/z*: 556 $[M + 1]^+$. Anal. Calcd for $C_{25}H_{29}N_7O_4S_2 \cdot 1.5C_2HF_3O_2 \cdot 0.2H_2O$: C, 46.1; H, 4.3; N, 13.4. Found: C, 46.2; H, 4.5; N, 13.2.

***N*-Methylsulfonyl-D-diphenylalanyl-L-prolyl[(4-amidinophenyl)methyl]amide Trifluoroacetic Acid (13).** ^{1}H NMR (CD_3OD): δ 7.76 (m, 2H), 7.52 (m, 4H), 7.48−7.18 (m, 8H), 5.02 (d, 1H), 4.44 (m, 2H), 4.34 (d, 1H), 4.09 (m, 1H), 3.72 (m, 1H), 2.98 (m, 1H), 2.84 (s, 3H), 1.80 (m, 2H), 1.67 (m, 1H), 1.40 (m, 1H). FAB MS, *m/z*: 548 $[M + 1]^+$. Anal. Calcd for $C_{29}H_{33}N_5O_4S \cdot 1.5C_2HF_3O_2 \cdot 1.2H_2O$: C, 51.9; H, 5.0; N, 9.5. Found: C, 51.8; H, 5.1; N, 9.6.

***N*-Methylsulfonyl-D-diphenylalanyl-L-prolyl[(5-amidino-2-thienyl)methyl]amide Trifluoroacetic Acid (14).** ^{1}H NMR (CD_3OD): δ 7.78 (d, 1H), 7.50 (m, 2H), 7.42−7.18 (m, 9H), 5.02 (d, 1H), 4.58 (m, 2H), 4.31 (d, 1H), 4.04 (m, 1H), 3.72 (m, 1H), 2.95 (m, 1H), 2.87 (s, 3H), 1.81 (m, 2H), 1.64 (m, 1H), 1.42 (m, 1H). FAB MS, *m/z*: 554 $[M + 1]^+$. Anal. Calcd for $C_{27}H_{31}N_5O_4S_2 \cdot 0.5C_2HF_3O_2 \cdot 2H_2O$: C, 52.0; H, 5.5; N, 10.8. Found: C, 52.0; H, 5.7; N, 10.7.

***N*-Methoxycarbonyl-D-diphenylalanyl-L-prolyl[(4-amidinophenyl)methyl]amide Trifluoroacetic Acid (15).** ^{1}H NMR (CD_3OD): δ 7.82 (m, 2H), 7.18−7.52 (m, 12H), 5.10 (d, 1H), 4.53 (m, 1H), 4.36 (m, 2H), 4.15 (m, 1H), 3.83 (m, 1H), 3.23 (s, 3H), 2.86 (m, 1H), 1.85 (m, 2H), 1.60 (m, 1H), 1.47 (m, 1H). FAB MS, *m/z*: 527 $[M + 1]^+$. Anal. Calcd for $C_{30}H_{33}N_5O_4 \cdot 1.2C_2HF_3O_2 \cdot 0.2H_2O$: C, 58.5; H, 5.2; N, 10.6. Found: C, 58.3; H, 5.3; N, 10.8.

***N*-Methoxycarbonyl-D-diphenylalanyl-L-prolyl(5-amidino-2-picolyl)amide Trifluoroacetic Acid (16).** ^{1}H NMR (CD_3OD): δ 8.67 (s, 1H), 8.09 (d, 1H), 7.96 (d, 1H), 7.41 (m, 2H), 7.35 (m, 2H), 7.21 (m, 6H), 5.15 (d, 1H), 4.59 (m, 1H), 4.42 (m, 1H), 4.38 (d, 1H), 4.09 (m, 1H), 3.82 (m, 1H), 3.33 (s, 3H), 2.90 (m, 1H), 1.91−1.75 (m, 2H), 1.69 (m, 1H), 1.47 (m, 1H). FAB MS, *m/z*: 529 $[M + 1]^+$. Anal. Calcd for $C_{29}H_{32}N_6O_4 \cdot 2.2C_2HF_3O_2 \cdot 0.2H_2O$: C, 50.9; H, 4.4; N, 10.6. Found: C, 50.7; H, 4.5; N, 10.8.

***N*-Methoxycarbonyl-D-diphenylalanyl-L-prolyl[(5-amidino-2-thienyl)methyl]amide Trifluoroacetic Acid (17).** ^{1}H NMR (CD_3OD): δ 7.81 (m, 1H), 7.52−7.18 (m, 11H), 5.14 (d, 1H), 4.66 (m, 1H), 4.52 (m, 1H), 4.36 (dd, 1H), 4.07 (m, 1H), 3.84 (m, 1H), 3.30 (s, 3H), 2.91 (m, 1H), 1.85 (m, 2H), 1.65 (m, 1H), 1.49 (m, 1H). FAB MS, *m/z*: 533 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_4S \cdot 1.0C_2HF_3O_2 \cdot 2.2H_2O$: C, 52.4; H, 5.3; N, 10.2. Found: C, 52.7; H, 5.7; N, 10.3.

***N*-Methoxycarbonyl-D-diphenylalanyl-L-prolyl[(4-amidino-2-thienyl)methyl]amide Trifluoroacetic Acid (18).** ^{1}H NMR (CD_3OD): δ 8.26 (m, 1H), 7.48−7.18 (m, 11H), 5.14 (d, 1H), 4.59 (q, 1H), 4.36 (d, 2H), 4.07 (m, 1H), 3.83 (m, 1H), 3.45 (s, 3H), 2.92 (m, 1H), 1.82 (m, 2H), 1.65 (m, 1H), 1.49 (m, 1H). FAB MS, *m/z*: 516 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_4S \cdot 1.0C_2HF_3O_2 \cdot 3.0H_2O$: C, 51.3; H, 5.5; N, 10.0. Found: C, 51.3; H, 5.7; N, 10.2.

***N*-Methoxycarbonyl-D-diphenylalanyl-L-prolyl[(5-amidino-3-thienyl)methyl]amide Trifluoroacetic Acid (19).** ^{1}H NMR (CD_3OD): δ 7.49 (d, 1H), 7.39 (m, 2H), 7.34 (m, 2H), 7.25 (m, 6H), 6.57 (d, 1H), 5.15 (d, 1H), 4.59 (m, 1H), 4.37 (m, 2H), 4.05 (m, 1H), 3.85 (m, 1H), 3.39 (s, 3H), 2.89 (m, 1H), 1.82−1.46 (m, 4H). FAB MS, *m/z*: 516 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_4S \cdot 1.2C_2HF_3O_2 \cdot 0.2H_2O$: C, 54.2; H, 4.9; N, 10.4. Found: C, 54.5; H, 4.7; N, 10.4.

***N*-Methoxycabonyl-D-diphenylalanyl-L-prolyl[(5-amidino-2-furanyl)methyl]amide Trifluoroacetic Acid (20).** ^{1}H NMR (CD_3OD): δ 7.49 (d, 1H), 7.39 (m, 2H), 7.34 (m, 2H), 7.25 (m, 6H), 6.57 (d, 1H), 5.15 (d, 1H), 4.59 (m, 1H), 4.37 (m, 2H), 4.05 (m, 1H), 3.85 (m, 1H), 3.39 (s, 3H), 2.89 (m, 1H), 1.82−1.46 (m, 4H). FAB MS, *m/z*: 518 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_5 \cdot 1.2C_2HF_3O_2 \cdot 0.3H_2O$: C, 55.5; H, 5.0; N, 10.7. Found: C, 55.4; H, 5.1; N, 10.9.

***N*-Carboxymethyl-D-diphenylalanyl-L-prolyl[(4-amidinophenyl)methyl]amide Ditrifluoroacetic Acid (21).** ^{1}H NMR (CD_3OD): δ 7.76 (d, 2H), 7.67 (m, 2H), 7.57 (d, 2H), 7.51 (m, 2H), 7.41−7.26 (m, 6H), 5.33 (d, 1H), 4.59 (d, 1H), 4.49 (dd, 2H), 4.10 (m, 1H), 3.82 (dd, 2H), 3.49 (m, 1H), 2.87 (m, 1H), 1.91−1.72 (m, 3H), 1.30 (m, 1H). FAB MS, *m/z*: 528 $[M + 1]^+$. Anal. Calcd for $C_{30}H_{33}N_5O_4 \cdot 2.3C_2HF_3O_2 \cdot 0.7H_2O$: C, 51.7; H, 4.6; N, 8.7. Found: C, 51.9; H, 4.3; N, 8.4.

***N*-Carboxymethyl-D-diphenylalanyl-L-prolyl[(5-amidino-3-thienyl)methyl]amide Ditrifluoroacetic Acid (23).** ^{1}H NMR (CD_3OD): δ 7.91 (s, 1H), 7.87 (s, 1H), 7.67 (m, 2H), 7.50 (m, 2H), 7.38−7.25 (m, 6H), 5.34 (d, 1H), 4.58 (d, 1H), 4.49 (d, 1H), 4.36 (d, 1H), 4.07 (m, 1H), 3.78 (dd, 2H), 3.53 (m, 1H), 2.89 (m, 1H), 1.83−1.72 (m, 3H), 1.31 (m, 1H). FAB MS, *m/z*: 534 $[M + 1]^+$. Anal. Calcd for $C_{28}H_{31}N_5O_4S \cdot 2.2C_2HF_3O_2 \cdot 0.5H_2O$: C, 49.2; H, 4.4; N, 8.9. Found: C, 49.0; H, 4.4; N, 9.0.

***N*-Carboxymethyl-D-diphenylalanyl-L-prolyl[(4-amidino-2-thiazolyl)methyl]amide Ditrifluoroacetic Acid (24).** ^{1}H NMR (CD_3OD): δ 8.63 (s, 1H), 7.65 (m, 2H), 7.52 (m, 2H), 7.45−7.23 (m, 6H), 5.31 (d, 1H), 4.74 (s, 2H), 4.55 (d, 1H), 4.13 (m, 1H), 3.78 (dd, 2H), 3.52 (m, 1H), 2.86 (m, 1H), 1.81 (m, 3H), 1.37 (m, 1H). FAB MS, *m/z*: 535 $[M + 1]^+$. Anal. Calcd for $C_{27}H_{30}N_6O_4S \cdot 2.2C_2HF_3O_2 \cdot 0.8H_2O$: C, 47.0; H, 4.2; N, 10.4. Found: C, 46.8; H, 4.5; N, 10.5.

Determination of Enzyme Inhibition Constants. Thrombin assay was performed according to the same procedure as described previously[25] except that the concentration of Chromozym TH and thrombin was set to 80 μM and 1.5 mU/mL, respectively, and the hydrolysis reaction was monitored for 1.5 h. Assays for trypsin, factor Xa, plasmin and t-PA were performed according to published procedures.

Determination of Pharmacokinetics. Male Sprague−Dawley rats (250−300 g) were restrained individually on a surgical plate in a supine position. The femoral artery and the femoral vein (iv only) of rats were cannulated with polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ) under light ether anesthesia. After complete recovery from anesthesia, rats were orally given 30 mg/kg of the test compound dissolved in distilled water via gavage or given 10 mg/kg via the femoral vein for intravenous (iv) study. Blood samples (0.25 mL) were collected from the femoral artery at 0 (for control), 1 (iv only), 5, 15, 30, 60, 90 (iv only), 120, 180, and 240 min after dosing.

Male beagle dogs (7−10 kg, Hazleton Research Product Inc., Calamazoo, MI) were housed individually in a metabolic cage for the plasma disposition study. Dogs were orally given 10 mg/kg of the test compound dissolved in distilled water via gavage or injected with 2 mg/(0.2 mL·kg) via the cephalic vein using INTROCAN. Blood samples were withdrawn via the cephalic vein at 0 (for control), 1, 5 (iv only), 15, 30, 60, 90, 120, 180, 240, 360 (po only), and 480 (po only) min after dosing.

Blood samples were placed into heparinized tubes (25 U/mL), deproteinized with 2 volumes of methanol, and centrifuged. The resulting supernatant (60 μL) was analyzed by HPLC, eluting with a mixture of 0.1% trifluoroacetic acid aqueous solution and acetonitrile with a ratio of 81% to 19%. Plasma concentration of the test compound was recorded and

used to calculate the pharmacokinetic parameters: maximum plasma concentration of test compound (C_{max}), time of maximum plasma concentration (T_{max}), area under the curve (AUC), and fraction of test compound absorbed (F).

Determination of Metabolic Stability. A portion of liver of rats ($n = 2$, pooled), dogs ($n = 2$, pooled), monkeys ($n = 2$, pooled), and humans ($n = 2$) was excised, chilled, and blotted dry on the paper tissue. After homogenization by a Tempest tissue homogenizer (The Virtis Company, NY) with 2 volumes of 0.1 M Tris-acetate buffer (pH 7.4) containing 0.1 M KCl and 1 mM EDTA, the homogenate was centrifuged twice at 9000*g* for 20 min using Beckman model J2-M1 (CA). A portion of the resulting supernatant was used as the S9 fraction. The metabolic reaction was initiated by adding the test compound (final concentration of 20 μM) to a mixture of the NADPH generating system (1 mM NADP, 3 mM G6P, 0.2 units/mL G6PD, and 5 mM $MgCl_2$) and liver S9 (6.72 mg/mL) in 100 mM potassium phosphate buffer (pH 7.4) incubated at 37 °C and 150 opm. The volume of the total reaction mixture was 0.5 mL. Aliquots of 100 μL were withdrawn at 0, 10, 30, and 60 min, and 2 volumes of methanol were added to deproteinize the samples for HPLC analysis. The metabolic activity was also measured in the absence of the NADPH generating system to serve as a control.

Determination of Antithrombotic Activity. Rat model.[26] Male Sprague−Dawley rats (300−350 g, six per group) were anesthetized by intraperitoneal injection with urethane solution (1.25 g/kg). The abdomen was surgically opened by a midline incision, and the inferior venae cava was carefully dissected free from surrounding connective tissue. The venae iliolumbar and spermatica were ligated with a silk thread. Thrombus formation was initiated by infusion of a thromboplastin preparation (Simplastin) using an infusion pump (model 100, IITC Life Science) via the left femoral vein at 0.5 mL/(kg·min). Simplastin (Organon Teknika) was reconstituted with 4 mL of distilled water and then given diluted at 1:2.5 in distilled water. At the start of infusion for 30 s, the vena cava was ligatured below the left renal vein. After the end of infusion, the vena cava was also ligatured above the iliac veins 16 mm apart from upper ligature. After 15 min of stasis, the thrombus formed inside the vessel was carefully removed and weighed. Before the sample was weighed, the excess blood was removed by blotting the wet clot on the wet Whitman filter paper.

Saline (control) or the test compound (1 mg/kg) was given by either bolus iv injection only or bolus injection plus infusion via the femoral vein 5 min before the thromboplastin infusion. The bolus injection volume was 0.5 mL/kg. For oral study, the test compound was given 120 min prior to thrombus formation. Antithrombotic activity (%) was expressed as $100 \times (A - B)/A$, where A is the mean clot weight of control group and B is the mean clot weight of test compound group.

Rabbit Model. New Zealand White rabbits (2.5−3.5 kg) were anesthetized by intravenous administration of a mixture of ketamine and xylazine. Maintenance of anesthesia was achieved by periodic intravenous administration of pentobarbital sodium. The animals were administered with either saline or compound **22** via intravenous bolus injection, followed by infusion for 60 min. During the infusion, the vena cava and left femoral vein were isolated for insertion of silk threads. Eight strands of silk thread (USP 4-0, Ethicon), which served as a stimulus for clot formation, were tied at the end of a 2F arterial embolectomy catheter (Baxter) and trimmed to 3 cm in length. The threads were wetted for about 20 min in a diluted thromboplastin solution (1:4 dilution of PT reagents). Fifteen minutes after the onset of infusion, the threads were inserted into the abdominal vena cava via the left femoral vein and were positioned approximately 2 cm below the branch of the left renal vein. Distal and proximal ligatures with PE-230 catheters occluded the vena cava segment (4 cm in length) containing silk threads. A ligature stenosis was formed by removal of the catheter. Infusion continued for a further 45 min to allow the growth of thrombus. The resulting thrombus was excised by surgical dissection, and wet weights of thrombus were measured.

Determination of Anticoagulant Activity. The anticoagulant activity for activated partial thromboplastin time (aPTT) was determined using an automatic coagulometer (Coag-A-Mate RA4, Organon Teknika). For the aPTT, 100 μL of plasma and the inhibitor were mixed with the same volume of the aPTT reagent (Platelin LS, Organon Teknika), and the mixture was incubated at 37 °C for 5 min, followed by the addition of 100 μL of 25 mM $CaCl_2$. For determination of ex vivo coagulation parameters of activated clotting time (ACT), blood samples were immediately collected from the carotid artery, and the clotting time was measured in a Hemotech automate (ACT II, Medtronic. Inc., Minneapolis, MN).

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