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Synthesis and biological activity of novel 1,4-diazepane derivatives as factor Xa inhibitor with potent anticoagulant and antithrombotic activity

Hiroyuki Koshio,* Fukushi Hirayama, Tsukasa Ishihara, Yuta Taniuchi, Kazuo Sato, Yumiko Sakai-Moritani, Seiji Kaku, Tomihisa Kawasaki, Yuzo Matsumoto, Shuichi Sakamoto and Shin-ichi Tsukamoto

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd, 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

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Abstract—Factor Xa (fXa) is a serine protease involved in the coagulation cascade, which has received great interest as a potential target for the development of new antithrombotic drugs. Herein we report a novel series of fXa inhibitors in which the 1,4-diazepane moiety was designed to interact with the S4 aryl-binding domain of the fXa active site. Compound **13** (YM-96765) showed potent fXa inhibitory activity ($IC_{50} = 6.8$ nM) and effective antithrombotic activity without prolonging bleeding time.

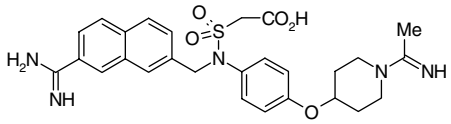
1. Introduction

Warfarin[1] has been widely used for many years in anticoagulant therapy. It exerts its potent anticoagulant effect by inhibiting the biosynthesis of a vitamin K-dependent coagulation factor. Its indirect mechanism results in some disadvantages, such as difficulty in the control of anticoagulant activity and the adverse effect of bleeding. Therefore, novel oral anticoagulant agents are required, which have new mechanisms of action and are much safer and easier to use than warfarin.

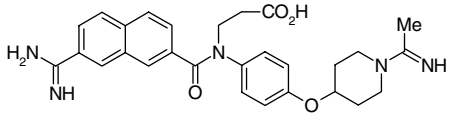
Factor Xa (fXa) is a trypsin-like serine protease that forms a prothrombinase complex with factor Va, Ca^{2+} and phospholipid to produce thrombin. This key enzyme is at the convergent point of the intrinsic and extrinsic coagulation pathway. This process involves signal amplification, with one molecule of fXa activating many molecules of prothrombin to thrombin.[2] Inhibition of fXa may therefore be more effective than the inhibition of thrombin itself. Moreover, the risk of bleeding is expected to decrease because fXa inhibitors specifically affect coagulation but not platelet function.[3]

We have reported in previous publications the discovery of potent and selective fXa inhibitors YM-60828[4] and YM-169964,[5] which contain the naphthamidine moiety for the S1 part and 1-acetoimidoyl-4-piperidyloxy moiety for the S4 part (Fig. 1). In these studies we have presented the following structure–activity relationships (SAR):

(1) The naphthamidine moiety deeply occupies the S1 pocket and the amidine group makes a salt bridge to Asp-189. Replacement of naphthylamidine with benzamidine derivatives results in a minor loss of fXa potency.
(2) Molecular modeling studies indicate that the acetic acid moiety is positioned outside the enzyme pocket



YM-60828



YM-169964

Figure 1. YM-60828 and YM-169964.

Keywords: Antithrombotics; Anticoagulants; Enzyme inhibitors; Factor Xa.
* Corresponding author. Tel.: +81-29-854-1548; fax: +81-29-852-5387; e-mail: koshio@yamanouchi.co.jp

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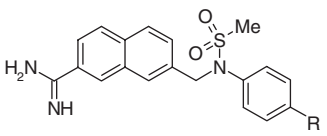
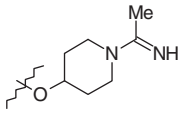
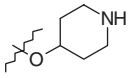
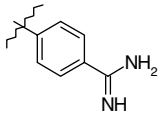
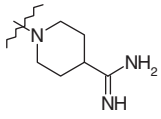
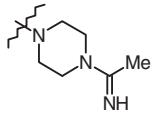
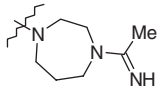
Table 1. SAR from the P4 moiety modification



Compd	R	IC_{50} (nM)[a]
1[b]		3.8
2[b]		127
9a		84
9b		62
10c		121
10d		7.9

[a] Human purified enzyme were used. IC_{50} values represent the average of three determinations with the average standard error of the mean <10%.

[b] Taken from Ref. 4.

and extends into the solvent. Modification of this moiety affords no significant loss of fXa inhibition.

However few SAR around the P4 part have been reported except the observation that removal of the acetimidoyl moiety from the piperidine ring results in a decrease in the activity (Table 1, **1** vs **2**). Therefore, we concentrated our investigations on modification of this section and herein describe a novel series of inhibitors, which contain a 1,4-diazepane moiety for the P4 part.

2. Chemistry

The syntheses of the intermediates of naphthoamidine derivatives **8a**–**g** are illustrated in Scheme 1. Treatment of **5** with various amines provided the 4-substituted nitrobenzenes **6b**–**g**. The cyanonaphthalene intermediates **8b**–**g** were synthesized by reduction of the nitro group, reductive alkylation of 2-cyanonaphthalene-7-carboxyaldehyde[4] and acylation with several sulfonyl chlorides. However, the corresponding reductive alkylation of **3** provided a complex mixture. Thus biphenyl derivative **8a** was obtained by alkylation of **4**, which was prepared by mesylation of 4′-amino-1,1′-biphenyl-4-carbonitrile **3**, with 2-bromomethyl-7-cyanonaphthalene.[6] The syntheses of the amidine derivatives are shown in Scheme 2. Treatment of the intermediates **8a**–**g** under Pinner conditions (HCl/EtOH) afforded the imidates, which were immediately reacted with excess ammonium acetate to provide the corresponding amidine derivatives **9a**–**g**. The amidine derivatives **9c**, **d** and **f** were converted to bis-amidines **10c**, **d** and **f** by reaction with ethyl acetimidate and triethylamine. Hydrolysis of **9f**, **g** and **10f** under acidic conditions provided the carboxyl derivatives **11**, **12** and **13**.

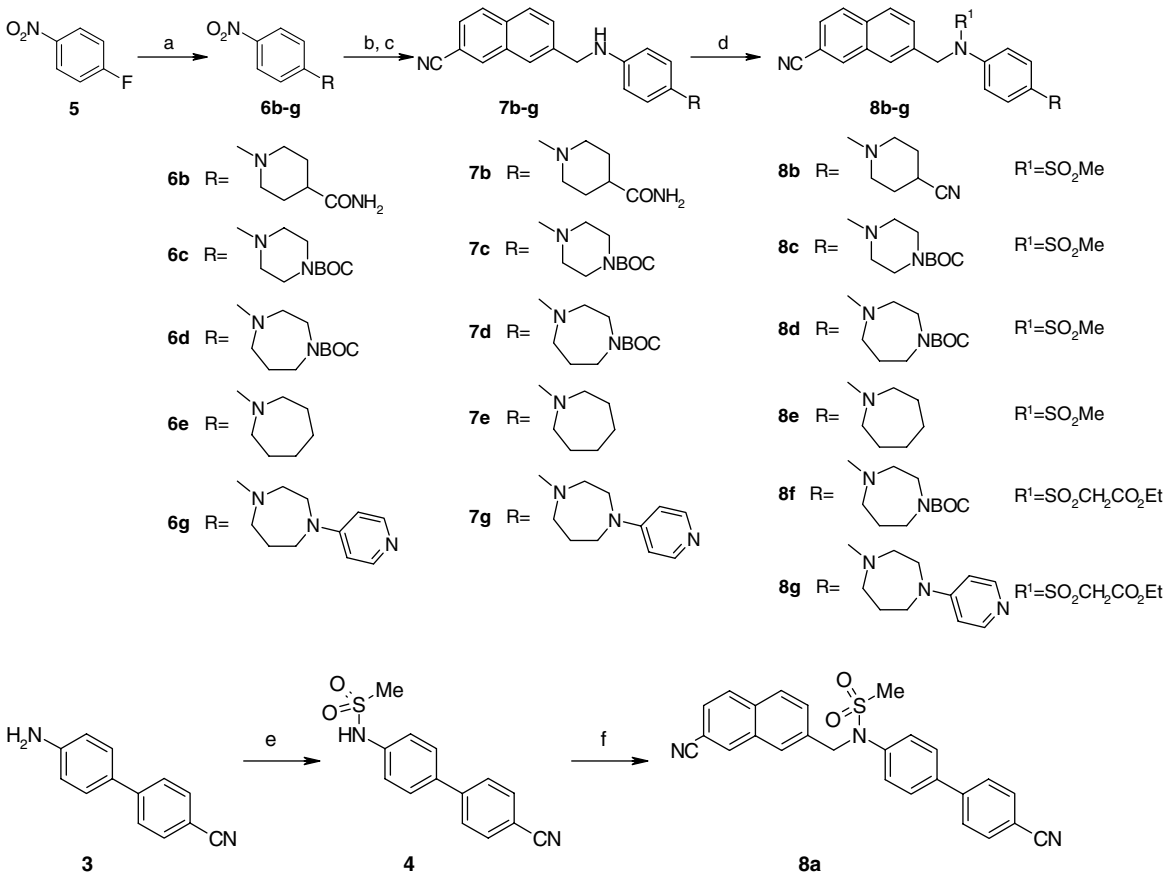
4-Alkylated 1,4-diazepane derivatives **16a**–**c** were synthesized as depicted in Scheme 3. Following the removal of the *tert*-butoxycarbonyl (Boc) protecting group from **8f** under acidic conditions, the resulting 1,4-diazepane derivative was converted to the 4-substituted 1,4-diazepane derivatives **14a**–**c** by reductive alkylation with several alkylaldehydes. Compounds **14a**–**c** were also converted to the amidine derivatives **16a**–**c** in an analogous procedure to that described above.

3. Result and discussion

The IC_{50} value for the inhibition of fXa enzymatic activity was determined for all the compounds prepared. The CT_2 values for the prothrombin time (PT) were also determined for selected inhibitors, as an indicator of in vitro anticoagulant activity. CT_2 value was defined as the concentration required to double clotting time. Moreover, oral anticoagulant activities were also evaluated by prolongation of PT subsequent to oral administration in mice.

Table 1 illustrates the effect of varying the S4 ligand on in vitro biological activity. Replacing the 1-acetoimidoyl-4-piperidyloxy group of compound **1** for a P4 part with a 4-amidinophenyl group or a 4-amidinopiperidine-1-yl group conferred a significant decrease in the fXa inhibitory activity (**1** vs **9a** and **9b**). An additional two-fold decrease in activity was noted with the 4-acetoimidoylpiperadine-1-yl analogue (**10c**) compared with **9a** and **9b**. However, compound **10d**, that expanded the piperazine ring of **10c**, substantially improved the inhibitory activity and afforded inhibitors with similar potency to **1**.

Potent fXa inhibitor **10d** was further evaluated for both its in vitro anticoagulant activity and oral anticoagulant activity. In vitro anticoagulant activity of **10d** was comparable to that of YM-60828, however it showed poor oral anticoagulant activity (Table 2). Previous studies on YM-60828 have demonstrated that the presence of a carboxyl group, particularly an acetic acid moiety gave potent oral anticoagulant activity in mice.[4] In view of the effect of the carboxyl group, a sulfonyl acetic acid derivative **13** was synthesized, which as pre-

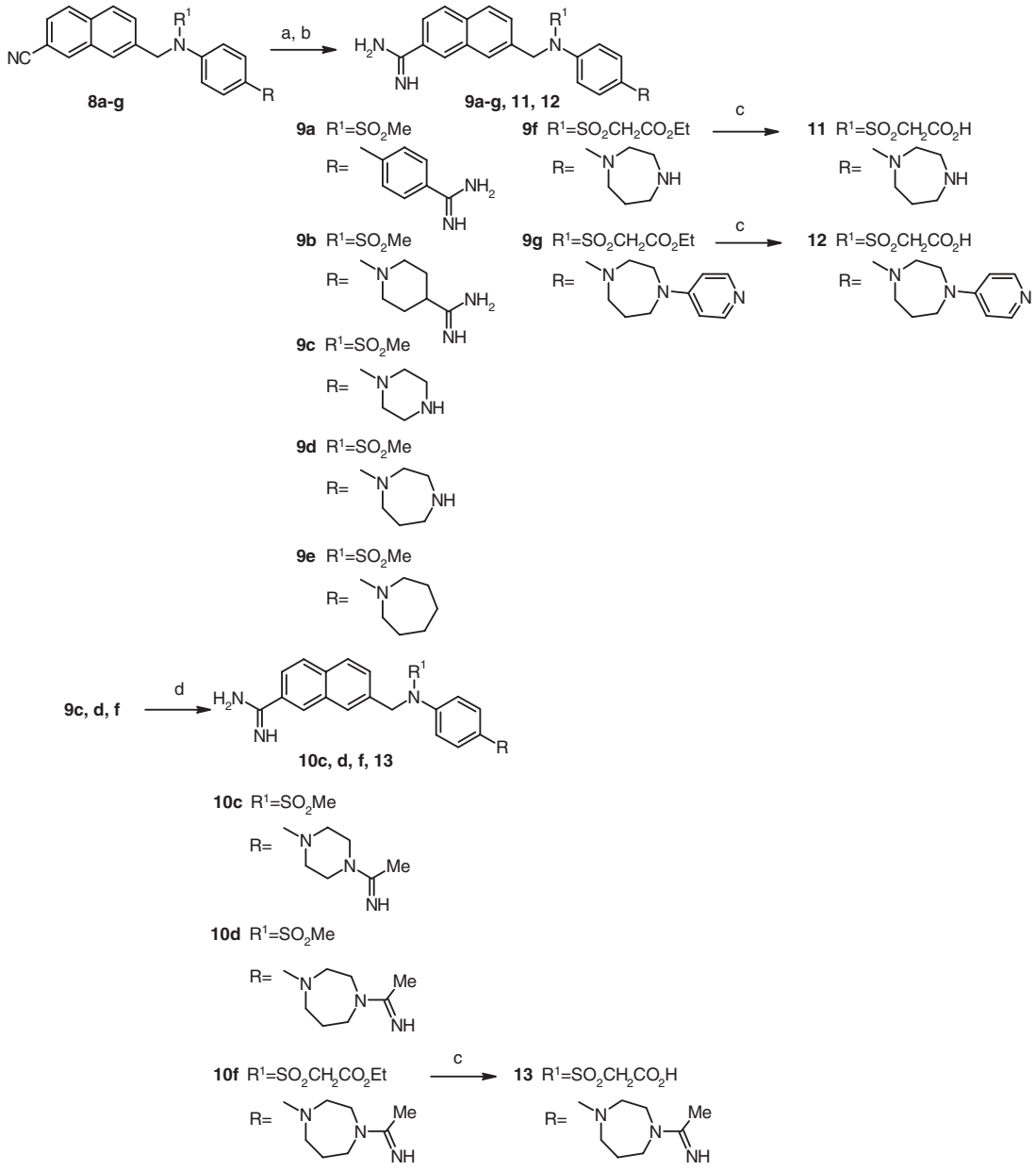


Scheme 1. Reagents and conditions: (a) amine, K_2CO_3, DMF; (b) H_2, 10% Pd–C, EtOH; (c) 7-formyl-2-naphthonitrile, $NaB(OAc)_3H$, 1,2-dichloroethane, AcOH; (d) MsCl for **8b**–**8e**, pyridine, $ClO_2SCH_2CO_2Et$ for **8f** and **8g**, pyridine; (e) MsCl, pyridine; (f) 2-bromomethyl-7-naphthonitrile, K_2CO_3, DMF.

dicted exhibited improved oral anticoagulant activity without affecting its fXa inhibitory activity. As a result, further modification of the acetoimidoyl moiety of compound **13** was undertaken. Surprisingly compound **11**, in which the acetoimidoyl moiety was absent, retained fXa inhibitory activity similar to that of compound **13**. This result suggested that 1,4-diazepane derivatives showed different SAR compared with that of YM-60828 derivatives, which contained a 1-acetoimidoyl-4-piperidyloxy moiety. Furthermore, replacing the acetoimidoyl group with a methyl group provided compound **16a**, which showed comparable activity to compound **11**. Introduction of more bulky substituents such as the butyl group (**16b**) or benzyl group (**16c**), resulted in a minor loss of fXa inhibitory potency. 4-Pyridyl substitution at the same position was also acceptable (**12**). To evaluate the importance of the basicity of the 1,4-diazepane in fXa inhibitory activity, the corresponding azepane derivative **9e** was prepared. Compound **9e** caused a significant reduction in fXa inhibitory activity, which suggested that the basicity of 1,4-diazepane might be important to activity.

The oral anticoagulant activity in mice of the inhibitors (**11**, **12**, **16a**), which were nearly equal to **13** in in vitro anticoagulant activity, were evaluated. Compound **16a** prolonged PT by more than twofold at 0.5 and 1.0 h. In contrast, compound **12** showed poor activity under the same conditions. Compound **13**, which exhibited good oral anticoagulant activity in mice, equivalent to that of compound **16a**, also showed prolongation PT in human plasma (CT_2, PT = 0.12 μM) equally compared with that of in mice plasma. Compound **13** was further evaluated enzymatic selectivity and pharmacokinetic profiles. Compound **13** showed high selectivity for fXa over trypsin (>100-fold). In particular, it showed exceptional selectivity against the related serine protease thrombin (>10,000-fold). Compound **13** was evaluated by a pharmacokinetic study following oral administration in dogs and cynomolgus monkeys, showing the absolute oral bioavailability at 58% and 14.7% (Table 3).

The oral antithrombotic effect in rats thromboplastin-induced venous thrombosis model and the prolongation of bleeding time were examined with warfarin and the hydrogen bromide salt of compound **13** (Fig. 2). Compound **13** showed oral antithrombotic activity in a dose dependent manner. No difference between the antithrombotic effect (ID_{50}) and the prolongation of bleeding time (ED_2, which was the dose that caused a twofold prolongation of bleeding time in the control group) was observed with the typical antithrombotic drug warfarin ($ED_2/ID_{50} = 0.71$). However, the antithrombotic effect of compound **13** was notably separate from its effect on bleeding time ($ED_2/ID_{50} = 27$). The clinical administration of agents such as warfarin are strictly controlled to



Scheme 2. Reagents and conditions: (a) HCl, EtOH; (b) NH_4OAc, EtOH; (c) cHCl; (d) ethyl acetimidate hydrochloride, Et_3N, EtOH.

prevent hemorrhage. These data suggest that compound **13** can exert its antithrombotic effect without significantly prolonging bleeding time and is therefore a much safer agent than warfarin.

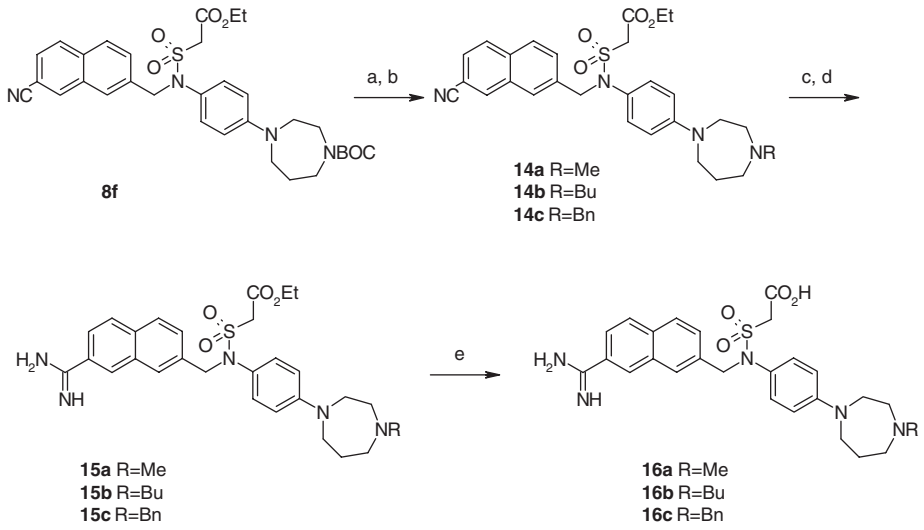
4. Conclusion

We have designed and synthesized a series of fXa inhibitors based on a 1,4-diazepane template as the P4 moiety. Among these compounds, compound **13** (YM-96765) showed nanomolar potency in fXa inhibitory activity and had excellent oral anticoagulant activity in mice and good pharmacokinetic profiles in dogs and cynomolgus monkeys. Furthermore, this compound showed effective oral antithrombotic activity in rats without prolonging bleeding time. Further optimization based on compound YM-96765 will be reported in future publications.

5. Experimental

5.1. Chemistry

^1H NMR spectra were measured with a JEOL EX90, EX400 or GX500 spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (in NMR description, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad peak). Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. ODS column



Scheme 3. Reagents and conditions: (a) TFA, CH_2Cl_2; (b) HCHO for **14a** or EtCHO for **14b** or PhCHO for **14c**, $NaB(OAc)_3H$, 1,2-dichloroethane, AcOH; (c) HCl, EtOH; (d) NH_4OAc, EtOH; (e) cHCl.

chromatography was performed on YMC gel (ODS-A 120-230/70).

5.2. *N*-(4′-Cyanobiphenyl-4-yl)methanesulfonamide (4)

To a stirred solution of 4′-amino-1,1′-biphenyl-4-carbonitrile (230 mg, 1.18 mmol) in pyridine (12 mL) at ambient temperature was added methanesulfonyl chloride (162 mg, 1.42 mmol). After 17 h the reaction mixture was concentrated in vacuo. The resulting residues was chromatographed on silica gel eluting with hexane/ethyl acetate (1/2) to give **4** (320 mg, quant yield) as a colourless amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.11 (3H, s), 6.64 (1H, s), 7.33 (2H, d, $J = 8.4$ Hz), 7.59 (2H, d, $J = 8.8$ Hz), 7.65 (2H, d, $J = 8.8$ Hz), 7.73 (2H, d, $J = 8.4$ Hz); FABMS m/e $(M+H)^+$ 273.

5.3. 1-(4-Nitrophenyl)piperidine-4-carboxamide (6b)

Compound **6b** was synthesized from **5** and pipridine-4-carboxamide according to the same procedure as that for **6d**. Compound **6b** was obtained as a yellow powder (87% yield): ^{1}H NMR ($CDCl_3$) δ 1.82–1.93 (2H, m), 1.96–2.06 (2H, m), 2.39–2.51 (1H, m), 2.99–3.09 (2H, m), 3.94–4.03 (2H, m), 6.82 (2H, d, $J = 9.4$ Hz), 8.11 (2H, d, $J = 9.4$ Hz); FABMS m/e $(M+H)^+$ 250.

5.4. *tert*-Butyl 4-(4-nitrophenyl)piperazine-1-carboxylate (6c)

Compound **6c** was synthesized from **5** and *tert*-butyl piperazine-1-carboxylate according to the same procedure as that for **6d**. Compound **6c** was obtained as a yellow powder (51% yield): ^{1}H NMR ($CDCl_3$) δ 1.19 (9H, s), 3.34–3.46 (4H, m), 3.55–3.68 (4H, m), 6.80 (2H, d, $J = 9.5$ Hz), 8.15 (2H, d, $J = 9.5$ Hz); FABMS m/e $(M)^+$ 307.

5.5. *tert*-Butyl 4-(4-nitrophenyl)-1,4-diazepane-1-carboxylate (6d)

To a stirred solution of *tert*-butyl 1,4-diazepane-1-carboxylate (1.8 g, 8.99 mmol) in DMF (10 mL) was added **5** (1.62 g, 11.5 mmol), potassium carbonate (1.84 g, 13.3 mmol), and stirring continued at 90 °C for 13 h. After the reaction mixture was cooled, the reaction mixture was diluted with ethyl acetate and washed with H_2O and saturated saline. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulted residue was chromatographed on silica gel eluting with chloroform/MeOH (100/1) to give **6d** (2.4 g, 83% yield) as a yellow amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.85–2.10 (2H, m), 3.19–3.35 (2H, m), 3.58–3.72 (6H, m), 6.71 (2H, d, $J = 9.5$ Hz), 8.12 (2H, d, $J = 9.5$ Hz); FABMS m/e $(M+H)^+$ 321.

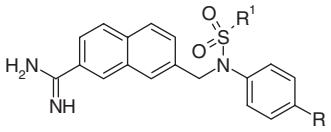
5.6. 1-(4-Nitrophenyl)azepane (6e)

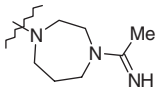
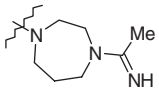
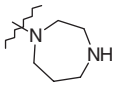
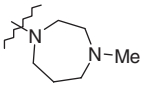
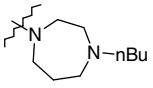
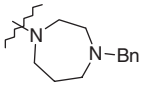
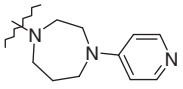
Compound **6e** was synthesized from **5** and hexamethyleneimine according to the same procedure as that for **6d**. Compound **6e** was obtained as a yellow powder (50% yield): ^{1}H NMR ($CDCl_3$) δ 1.49–1.56 (4H, m), 1.72–1.81 (4H, m), 3.35–3.45 (4H, m), 6.61 (2H, d, $J = 9.3$ Hz), 8.10 (2H, d, $J = 9.3$ Hz); FABMS m/e $(M+H)^+$ 221.

5.7. 1-(4-Nitrophenyl)-4-(pyridin-4-yl)-1,4-diazepine (6g)

Compound **6g** was synthesized from **5** and 1-(pyridyl-4-yl)-1,4-diazepine according to the same procedure as that for **6d**. Compound **6g** was obtained as a yellow powder (54% yield): ^{1}H NMR ($CDCl_3$) δ 2.02–2.21 (2H, m), 3.42–3.70 (8H, m), 6.55 (2H, d, $J = 3.2$ Hz), 6.69 (2H, d, $J = 9.5$ Hz), 8.14 (2H, d, $J = 9.5$ Hz), 8.26 (2H, d, $J = 3.2$ Hz); FABMS m/e $(M+H)^+$ 299.

Table 2. SAR from the P4 moiety modification



Compd	R	R^1	IC_{50} (nM)[a]	CT_2 (μM)[b]	PT/control PT[d]		
				PT[c]	0.5 h	1.0 h	2.0 h
10d		Me	7.9	0.37	1.38	1.27	1.22
13		CH_2CO_2H	6.8	0.36	2.25	2.07	1.68
11		CH_2CO_2H	6.2	0.67	1.77	2.07	1.62
16a		CH_2CO_2H	6.5	0.76	2.21	2.04	1.75
16b		CH_2CO_2H	10	1.00	1.84	NT[e]	NT[e]
16c		CH_2CO_2H	18	NT[e]	NT[e]	NT[e]	NT[e]
12		CH_2CO_2H	8.6	0.31	1.21	1.36	1.24
9e		Me	141	NT[e]	NT[e]	NT[e]	NT[e]
	YM-60828		6.0	0.21	2.55	2.25	1.74

[a] Refer to Table 1.
[b] Values represent the concentration required to double clotting time and represent the average of four determination with the average standard error of the mean <10%.
[c] Prothrombin time using mice plasma.
[d] The relative prothrombin time compared with that measured using normal mice plasma at 0.5, 1.0 and 2.0 h after oral administration (100 mg/kg, $n = 3$).
[e] Not tested.

Table 3. Oral pharmacokinetic properties of compound **13**

Spaces	Iv $t_{1/2}$ (min)	CL_{tot} (mL/min/kg)	Oral C_{max} (ng/mL)	F (%)
Dogs[a]	62	11.5	336	58
Cynomolgus monkey[b]	120	4.0	674	14.7

[a] Compound was administered at a dose of 10 mg/kg, po ($n = 3$) and 0.3 mg/kg, iv ($n = 3$).
[b] Compound was administered at a dose of 10 mg/kg, po ($n = 3$) and 1 mg/kg, iv ($n = 3$).

5.8. 1-(4-{[(7-Cyano-2-naphthyl)methyl]amino}phenyl)-piperidine-4-carboxamide (7b)

Compound **7b** was synthesized from **6b** according to the same procedure as that for **7d**. Compound **7b** was obtained as a white amorphous powder (18% yield): ^{1}H NMR (DMSO-d_6) δ 1.63–1.82 (4H, m), 2.32–2.40 (1H, m), 3.12–3.19 (2H, m), 3.33–3.56 (2H, m), 4.44 (2H, s), 6.48–6.68 (2H, m), 6.83–6.96 (2H, m), 7.61–7.78 (2H, m), 7.85–8.05 (3H, m), 8.20 (1H, s); FABMS m/e $(M+H)^+$ 384.

5.9. *tert*-Butyl 4-(4-{[(7-cyano-2-naphthyl)methyl]-amino}phenyl)piperazine-1-carboxylate (7c)

Compound **7c** was synthesized from **6c** according to the same procedure as that for **7d**. Compound **7c** was

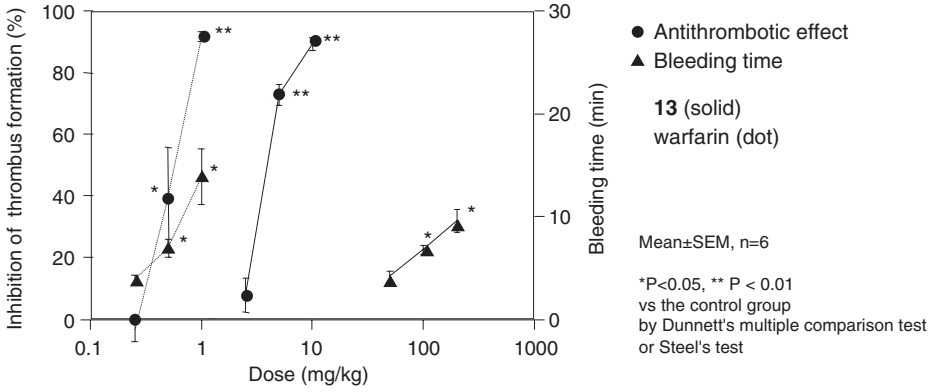


Figure 2. Antithrombotic activity and bleeding time of **13** (HBr salt) and warfarin after oral administration in rats ($n = 6$).

obtained as a white amorphous powder (25% yield): ^{1}H NMR ($CDCl_3$) δ 1.54 (9H, s), 2.82–3.15 (4H, m), 3.59 (4H, t, $J = 4.9$ Hz), 4.89 (2H, s), 6.50–6.71 (2H, m), 6.83–7.01 (2H, m), 7.56–7.80 (2H, m), 7.84–8.03 (3H, m), 8.25 (1H, s); FABMS m/e $(M+H)^+$ 443.

5.10. *tert*-Butyl 4-(4-{[(7-cyano-2-naphthyl)methyl]-amino}phenyl)-1,4-diazepane-1-carboxylate (7d)

To the solution of **6d** (14.12 g, 439 mmol) in EtOH (44 mL) was added 10% Pd–C powder (0.7 g) and stirred in hydrogen atmosphere at ambient temperature for 16 h. The reaction mixture was filtrated through a pad of Celite and concentrated in vacuo to give intermediate aniline compound. To a stirred solution of crude aniline compound and 7-formyl-2-naphthonitrile (9.55 g, 52 mmol) in 1,2-dichloromethane (440 mL) and AcOH (26 mL) at ambient temperature was added sodium triacetoxyborohydride (22.3 g, 105 mmol). After 5 h, the reaction mixture was washed with 10% potassium carbonate solution and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulted residue was chromatographed on silica gel eluting with ethyl acetate/hexane (1/3) to give **7d** (15.8 g, 66%) as a yellow amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s), 1.80–2.09 (2H, m), 3.07–3.55 (8H, m), 4.47 (2H, s), 6.61 (4H, s), 7.50–7.71 (2H, m), 7.82–7.94 (3H, m), 8.17 (1H, s); FABMS m/e $(M+H)^+$ 457.

5.11. 7-({[(4-Azepan-1-yl)phenyl]amino}methyl)-2-naphthonitrile (7e)

Compound **7e** was synthesized from **6e** according to the same procedure as that for **7d**. Compound **7e** was obtained as a white amorphous powder (quant yield): ^{1}H NMR ($CDCl_3$) δ 1.19–1.58 (4H, m), 1.70–1.79 (4H, m), 3.35–3.39 (4H, m), 4.66 (2H, s), 6.59 (2H, d, $J = 9.0$ Hz), 6.79 (2H, d, $J = 9.0$ Hz), 7.56 (1H, dd, $J = 1.5$, 6.4 Hz), 7.67 (1H, dd, $J = 1.5$, 6.4 Hz), 7.82–7.92 (3H, m), 8.17 (1H, s); FABMS m/e $(M+H)^+$ 355.

5.12. 7-[({4-[4-(Pyridin-4-yl)-1,4-diazepan-1-yl]phenyl}-amino)methyl]-2-naphthonitrile (7g)

Compound **7g** was synthesized from **6g** according to the same procedure as that for **7d**. Compound **7g** was obtained as a white amorphous powder (62% yield): ^{1}H NMR ($CDCl_3$) δ 2.05–2.16 (2H, m), 3.35–3.48 (4H, m), 3.15–3.58 (2H, m), 3.63–3.68 (2H, m), 4.48 (2H, s), 6.52–6.58 (2H, m), 6.79 (1H, d, $J = 8.9$ Hz), 7.35 (1H, d, $J = 8.9$ Hz), 7.56–7.60 (1H, m), 7.63–7.69 (2H, m), 7.86–7.90 (2H, m), 8.20–8.27 (2H, m), 8.70 (1H, s); FABMS m/e $(M+H)^+$ 434.

5.13. *N*-(4′-Cyano-1,1′-biphenyl-4-yl)-*N*-[(7-cyano-2-naphthyl)methyl]methanesulfonamide (8a)

To a stirred solution of **4** (310 mg, 1.14 mmol) in DMF (11 mL) at ambient temperature was added 7-bromomethyl-2-naphthonitrile (281 mg, 1.14 mmol) and potassium carbonate (318 mg, 2.3 mmol). After 24 h the reaction mixture was diluted with ethyl acetate and washed with 10% aqueous potassium carbonate. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting residues was chromatographed on silica gel eluting with hexane/ethyl acetate (1/1) to give **8a** (570 mg, quant yield) as a colourless amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.07 (3H, s), 5.01 (2H, s), 7.41 (2H, d, $J = 8.8$ Hz), 7.52 (2H, d, $J = 8.4$ Hz), 7.56–7.61 (3H, m), 7.67–7.73 (4H, m), 7.84–7.89 (4H, m), 8.13 (1H, s); FABMS m/e $(M+H)^+$ 438.

5.14. *N*-[(7-Cyano-2-naphthyl)methyl]-*N*-[4-(4-cyanopiperidin-1-yl)phenyl]methanesulfonamide (8b)

Compound **8b** was synthesized from **7b** and methanesulfonyl chloride according to the same procedure as that for **8d**. Compound **8b** was obtained as a white amorphous powder (90% yield): ^{1}H NMR ($CDCl_3$) δ 1.89–2.06 (4H, m), 2.75–2.81 (1H, m), 2.98 (2H, s), 3.04–3.13 (2H, m), 3.33–3.42 (2H, m), 4.97 (2H, s), 6.79 (2H, d, $J = 9.2$ Hz), 7.12 (2H, d, $J = 9.2$ Hz), 7.58 (1H, dd, $J = 1.5$, 8.4 Hz), 7.66 (1H, s), 7.67 (1H, dd, $J = 1.5$,

8.2 Hz), 7.80–7.88 (2H, m), 8.13 (1H, s); FABMS *m/e* $(M+H)^+$ 444.

5.15. *tert*-Butyl 4-{4-[[(7-cyano-2-naphthyl)methyl](methylsulfonyl)amino]phenyl}piperazine-1-carboxylate (8c)

Compound **8c** was synthesized from **7c** and methanesulfonyl chloride according to the same procedure as that for **8d**. Compound **8c** was obtained as a white amorphous powder (65% yield): ^{1}H NMR ($CDCl_3$) δ 1.59 (9H, s), 2.80–3.02 (4H, m), 3.16 (3H, s), 3.41–3.59 (2H, s), 6.82–7.05 (2H, m), 7.55–7.78 (2H, m), 8.24 (1H, s); FABMS *m/e* $(M+H)^+$ 521.

5.16. *tert*-Butyl 4-{4-[[(7-cyano-2-naphthyl)methyl](methylsulfonyl)amino]phenyl}-1,4-diazepane-1-carboxylate (8d)

To a stirred solution of **7d** (400 mg, 0.88 mmol) in 1,2-dichloroethane (5 mL) was added pyridine (208 mg, 2.63 mmol) and methanesulfonyl chloride (201 mg, 1.76 mmol) and stirred at ambient temperature. After 12 h, the reaction mixture was diluted with chloroform and washed with saturated sodium hydrogencarbonate, water, aqueous 10% citric acid and water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was recrystallized from MeOH to obtain **8d** (342 mg, 73%) as a white powder: ^{1}H NMR ($CDCl_3$) δ 1.30 (4H, s), 1.38 (5H, s), 1.84–1.94 (2H, m), 2.97 (3H, s), 3.15–3.33 (2H, m), 3.44–3.56 (6H, m), 4.94 (2H, s), 6.55 (2H, d, $J = 9.2$ Hz), 7.02–7.10 (2H, m), 7.58 (1H, dd, $J = 1.9$, 8.4 Hz), 7.65–7.73 (2H, m), 7.84 (1H, d, $J = 9.9$ Hz), 7.88 (1H, d, $J = 9.9$ Hz), 8.13 (1H, s); FABMS *m/e* $(M+H)^+$ 534.

5.17. *N*-(4-Azepan-1-ylphenyl)-*N*-[(7-cyano-2-naphthyl)-methyl]methanesulfonamide (8e)

Compound **8e** was synthesized from **7e** and methanesulfonyl chloride according to the same procedure as that for **8d**. Compound **8e** was obtained as a white amorphous powder (62% yield): ^{1}H NMR ($CDCl_3$) δ 1.43–1.54 (4H, m), 1.62–1.77 (4H, m), 2.98 (3H, s), 3.37 (4H, t, $J = 5.7$ Hz), 4.94 (2H, s), 6.53 (2H, d, $J = 9.3$ Hz), 7.02 (2H, d, $J = 9.3$ Hz), 7.57 (1H, dd, $J = 1.5$, 8.4 Hz), 7.67 (1H, s), 7.71 (1H, dd, $J = 1.5$, 8.4 Hz), 7.82–7.89 (2H, m), 8.13 (1H, s); FABMS *m/e* $(M+H)^+$ 432.

5.18. *tert*-Butyl 4-(4-{[(7-cyano-2-naphthyl)methyl][(2-ethoxy-2-oxoethyl)sulfonyl]amino}phenyl)-1,4-diazepane-1-carboxylate (8f)

Compound **8f** was synthesized from **7d** and ethyl (chlorosulfonyl)acetate according to the same procedure as that for **8d**. Compound **8f** was obtained as a white amorphous powder (87% yield): ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, $J = 7.3$ Hz), 1.31 (4H, s), 1.39 (5H, s), 1.75–2.01 (2H, m), 3.13–3.31 (2H, m), 3.45–3.57 (6H, m), 4.04 (2H, s), 4.35 (2H, q, $J = 7.3$ Hz), 5.02 (2H, s), 6.55 (2H, d, $J = 9.0$ Hz), 7.20 (2H, d, $J = 9.0$ Hz), 7.50–7.92 (5H, m), 8.13 (1H, s); FABMS *m/e* $(M+H)^+$ 607.

5.19. Ethyl ({[(7-cyano-2-naphthyl)methyl]{4-[4-(pyridin-4-yl)-1,4-diazepan-1-yl]phenyl}amino}sulfonyl)acetate (8g)

Compound **8g** was synthesized from **7g** and ethyl (chlorosulfonyl)acetate according to the same procedure as that for **8d**. Compound **8g** was obtained as a white amorphous powder (72% yield): ^{1}H NMR ($CDCl_3$) δ 1.92–2.25 (2H, m), 2.98 (3H, s), 3.30–3.58 (8H, m), 4.95 (2H, s), 6.40–6.68 (4H, m), 7.08 (2H, d, $J = 9.0$ Hz), 7.56–7.88 (4H, m), 8.07–8.30 (4H, m); FABMS *m/e* $(M+H)^+$ 512.

5.20. 4′-[({7-[Amino(imino)methyl]-2-naphthyl}methyl)-(methylsulfonyl)amino] biphenyl-4-carboximidamide (9a)

Compound **9a** was synthesized from **8a** according to the same procedure as that for **9d**. Compound **9a** was obtained as a white amorphous powder (67% yield): ^{1}H NMR (DMSO-d_6) δ 3.22 (3H, s), 5.18 (2H, s), 7.61 (2H, d, $J = 8.8$ Hz), 7.72 (1H, dd, $J = 1.5$, 8.3 Hz), 7.76 (2H, d, $J = 8.8$ Hz), 7.82 (1H, dd, $J = 1.5$, 8.3 Hz), 7.86–7.94 (4H, m), 7.98 (1H, s), 8.03 (1H, d, $J = 8.8$ Hz), 8.09 (1H, d, $J = 8.8$ Hz), 8.50 (1H, s), 9.29 (2H, br s), 9.37 (2H, br s), 9.47 (2H, br s), 9.54 (2H, br s); FABMS *m/e* $(M+H)^+$ 472. Anal. Calcd for $C_{26}H_{25}N_5O_2S{\cdot}2.0HCl{\cdot}1.5H_2O$: C, 54.64; H, 5.29; N, 12.25; S, 5.61; Cl, 12.41. Found: C, 54.63; H, 5.40; N, 12.25; S, 5.55; Cl, 12.67.

5.21. 1-{4-[({7-[Amino(imino)methyl]-2-naphthyl}methyl)-(methylsulfonyl)amino]phenyl}piperidine-4-carboximidamide (9b)

Compound **9b** was synthesized from **8b** according to the same procedure as that for **9d**. Compound **9b** was obtained as a white amorphous powder (12% yield): ^{1}H NMR (DMSO-d_6) δ 1.67–1.89 (4H, m), 2.53–2.64 (3H, m), 3.33 (3H, s), 3.74–3.82 (2H, m), 5.00 (2H, s), 6.86 (2H, d, $J = 9.2$ Hz), 7.24 (2H, d, $J = 9.2$ Hz), 7.66 (1H, dd, $J = 1.5$, 8.4 Hz), 7.80 (1H, dd, $J = 1.5$, 8.4 Hz), 7.91 (1H, s), 8.01 (1H, d, $J = 8.4$ Hz), 8.10 (1H, d, $J = 8.4$ Hz), 8.47 (1H, s), 8.92 (4H, br s); FABMS *m/e* $(M+H)^+$ 479. Anal. Calcd for $C_{25}H_{30}N_6O_2S{\cdot}3.0HCl{\cdot}1.3H_2O$: C, 49.11; H, 5.87; N, 13.75; S, 5.24; Cl, 17.40. Found: C, 49.52; H, 6.34; N, 13.87; S, 5.23; Cl, 17.36.

5.22. 7-({(Methylsulfonyl)[4-(piperazin-1-yl)phenyl]amino}methyl)naphthalene-2-carboximidamide (9c)

Compound **9c** was synthesized from **8c** according to the same procedure as that for **9d**. Compound **9c** was obtained as a white amorphous powder (32% yield): ^{1}H NMR (DMSO-d_6) δ 3.11–3.19 (7H, m), 3.35–3.40 (4H, m), 5.00 (2H, s), 6.88 (2H, d, $J = 8.5$ Hz), 7.25 (2H, d,

$J = 8.5$ Hz), 7.68 (1H, d, $J = 8.5$ Hz), 7.74 (1H, d, $J = 8.5$ Hz), 7.99–8.09 (3H, m), 8.53 (1H, s), 9.50 (4H, br s); FABMS m/e $(M+H)^+$ 483.

5.23. 7-{[[4-(1,4-Diazepan-1-yl)phenyl](methylsulfonyl)amino]methyl}naphthalene-2-carboximidamide (9d)

HCl gas bubbled through a solution of **8d** (333 mg, 0.62 mmol) in EtOH (10 mL) under −20 °C for 20 min. The mixture was allowed to stir for 15 h at 5 °C, and then concentrated in vacuo. To the crude imidate dissolved in EtOH (10 mL) at ambient temperature was added ammonium acetate (480 mg, 6.22 mmol). The reaction mixture was stirred at ambient temperature for 24 h and concentrated in vacuo. The resulted residue was chromatographed on ODS-gel eluting with MeOH/H_2O (5/95). MeOH was removed in vacuo, and the aqueous solution was lyophilized after being acidified with 1 N HCl. Compound **9d** (249 mg, 82%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.68–1.75 (2H, m), 2.57–2.65 (2H, m), 2.77–2.84 (2H, m), 3.07 (3H, s), 3.24–3.45 (4H, m), 4.96 (2H, s), 6.57 (2H, d, $J = 9.3$ Hz), 7.14 (2H, d, $J = 9.3$ Hz), 7.90 (1H, s), 8.00 (1H, d, $J = 8.3$ Hz), 8.09 (1H, d, $J = 8.3$ Hz), 8.45 (1H, s); FABMS m/e $(M+H)^+$ 452.

5.24. 7-({4-(Azepan-1-ylphenyl)(methylsulfonyl)amino}methyl)naphthalene-2-carboximidamide (9e)

Compound **9e** was synthesized from **8e** according to the same procedure as that for **9d**. Compound **9e** was obtained as a white amorphous powder (72% yield): ^{1}H NMR (DMSO-d_6) δ 1.39–1.48 (4H, m), 1.61–1.72 (4H, m), 3.09 (3H, s), 3.36 (4H, t, $J = 5.9$ Hz), 4.97 (2H, s), 6.62 (2H, br s), 7.12 (2H, d, $J = 7.8$ Hz), 7.68 (1H, d, $J = 8.8$ Hz), 7.81 (1H, d, $J = 8.8$ Hz), 7.91 (1H, s), 8.14 (1H, d, $J = 8.8$ Hz), 8.09 (1H, d, $J = 8.8$ Hz), 8.47 (1H, s), 9.27 (2H, br s), 9.49 (2H, br s); FABMS m/e $(M+H)^+$ 451. Anal. Calcd for $C_{25}H_{30}N_4O_2S{\cdot}1.9HCl{\cdot}1.0H_2O$: C, 55.82; H, 6.35; N, 10.42; S, 5.96; Cl, 12.52. Found: C, 55.56; H, 6.43; N, 10.41; S, 6.04; Cl, 12.48.

5.25. Ethyl ({({7-[amino(imino)methyl]-2-naphthyl}-methyl)[4-(1,4-diazepan-1-yl)phenyl]amino}sulfonyl)-acetate (9f)

Compound **9f** was synthesized from **8f** according to the same procedure as that for **9d**. Compound **9f** was obtained as a white amorphous powder (99% yield): ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, $J = 7.0$ Hz), 2.01–2.07 (2H, m), 3.03–3.06 (2H, m), 3.11–3.15 (2H, m), 3.41 (2H, t, $J = 6.0$ Hz), 3.62–3.65 (2H, m), 4.24 (2H, q, $J = 7.0$ Hz), 4.36 (2H, s), 5.01 (2H, s), 6.68 (2H, d, $J = 9.2$ Hz), 7.20 (2H, d, $J = 9.2$ Hz), 7.66 (1H, dd, $J = 1.7$, 8.6 Hz), 7.82 (1H, dd, $J = 1.7$, 8.6 Hz), 7.90 (1H, s), 8.02 (1H, d, $J = 8.6$ Hz), 8.10 (1H, d, $J = 8.6$ Hz), 8.49 (1H, s), 9.27 (2H, br), 9.52 (2H, s); FABMS m/e $(M+H)^+$ 524.

5.26. Ethyl ({({7-[amino(imino)methyl]-2-naphthyl}-methyl)[4-(4-pyridin-4-yl-1,4-diazepan-1-yl)-phenyl]amino}sulfonyl)acetate (9g)

Compound **9g** was synthesized from **8g** according to the same procedure as that for **9d**. Compound **9g** was obtained as a white amorphous powder (19% yield): ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, $J = 6.8$ Hz), 1.82–1.89 (2H, m), 3.42–3.46 (2H, m), 3.60–3.63 (4H, m), 3.83–3.86 (2H, m), 4.24 (2H, q, $J = 6.8$ Hz), 4.33 (2H, s), 4.98 (2H, s), 6.82 (2H, d, $J = 9.2$ Hz), 7.03–7.10 (2H, m), 7.15 (2H, d, $J = 9.2$ Hz), 7.60 (1H, dd, $J = 1.6$, 8.8 Hz), 7.82 (1H, dd, $J = 1.6$, 8.8 Hz), 7.89 (1H, s), 8.01 (1H, d, $J = 8.8$ Hz), 8.11 (1H, d, $J = 8.8$ Hz), 8.12–8.16 (2H, m), 8.48 (1H, s), 9.31 (2H, s), 9.52 (2H, s), 13.68 (1H, br s); FABMS m/e $(M+H)^+$ 601.

5.27. 7-{[[4-(4-Ethanimidoylpiperazin-1-yl)phenyl](methylsulfonyl)amino]methyl}naphthalene-2-carboximidamide (10c)

Compound **10c** was synthesized from **9c** according to the same procedure as that for **10d**. Compound **10c** was obtained as a white amorphous powder (14% yield): ^{1}H NMR (DMSO-d_6) δ 2.33 (3H, s), 3.12 (3H, s), 3.24–3.38 (4H, m), 3.62–3.70 (2H, m), 3.73–3.80 (2H, m), 5.03 (2H, s), 6.93 (2H, d, $J = 8.8$ Hz), 7.30 (2H, d, $J = 8.8$ Hz), 7.66 (1H, d, $J = 8.8$ Hz), 7.86–7.93 (2H, m), 8.01 (1H, d, $J = 8.8$ Hz), 8.09 (1H, d, $J = 8.8$ Hz), 8.55 (1H, s), 9.50 (2H, br s), 9.72 (2H, br s); FABMS m/e $(M+H)^+$ 479. Anal. Calcd for $C_{25}H_{30}N_6O_2S{\cdot}2.1HCl{\cdot}2.5H_2O$: C, 50.03; H, 6.23; N, 14.00; S, 5.34; Cl, 12.40. Found: C, 50.33; H, 6.20; N, 14.09; S, 5.37; Cl, 12.48.

5.28. 7-{[[4-(4-Ethanimidoyl-1,4-diazepan-1-yl)-phenyl]-(methylsulfonyl)amino]methyl}naphthalene-2-carboximidamide (10d)

To a stirred solution of **9d** (224 mg, 0.5 mmol) in EtOH (10 mL) at ambient temperature was added ethyl acetimidate hydrochloride (650 mg, 3.3 mmol) and Et_3N (683 mg, 6.7 mmol). The mixture was allowed to stir for 24 h at ambient temperature, and then concentrated in vacuo. The resulting residues was chromatographed on ODS-gel eluting with MeOH/H_2O (5/95). MeOH was removed in vacuo, and the aqueous solution was lyophilized after being acidified with 1 N HCl. Compound **10d** (169 mg, 54%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.77–1.87 (2H, m), 2.01 (1.8H, s), 2.24 (1.2H, s), 3.09 (3H, s), 3.44–3.76 (8H, m), 4.98 (2H, s), 6.65–6.73 (2H, m), 7.16–7.23 (2H, m), 7.63–7.68 (1H, m), 7.78–7.84 (1H, m), 7.89–7.93 (1H, m), 7.99–8.04 (1H, m), 8.09 (1H, d, $J = 8.8$ Hz), 8.49 (0.4H, s), 8.51 (0.6H, s), 8.61 (0.6H, s), 8.75 (0.4H, s), 9.24–9.32 (3H, m), 9.51 (2H, s); FABMS m/e $(M+H)^+$ 493. Anal. Calcd for $C_{26}H_{32}N_6O_2S{\cdot}2.2HCl{\cdot}2.7H_2O$: C, 50.25; H, 6.42; N, 13.52; S, 5.16; Cl, 12.55. Found: C, 50.50; H, 6.49; N, 13.59; S, 5.12; Cl, 12.63.

5.29. Ethyl ({({7-[amino(imino)methyl]-2-naphthyl}-methyl)[4-(4-ethanimidoyl-1,4-diazepan-1-yl)phenyl]-amino}sulfonyl)acetate (10f)

Compound **10f** was synthesized from **9f** according to the same procedure as that for **10d**. Compound **10f** was obtained as a white amorphous powder (17% yield): ^{1}H NMR (DMSO-d_6) δ 1.25–1.29 (3H, m), 1.79–1.85 (2H, m), 2.03 (2H, s), 2.25 (1H, s), 3.47–3.75 (8H, m), 4.21–4.27 (2H, m), 4.37 (2H, s), 5.00 (2H, s), 6.68–6.73 (2H, m), 7.17–7.21 (2H, m), 7.62–7.66 (1H, m), 7.82 (1H, d, $J = 8.8$ Hz), 7.90 (1H, s), 8.03 (1H, dd, $J = 4.0$, 8.8 Hz), 8.10 (1H, d, $J = 8.8$ Hz), 8.49 (0.4H, s), 8.51 (0.6H, s), 8.64 (0.6H, s), 8.76 (0.4H, s), 9.29 (2H, s), 9.33 (1H, s), 9.53 (2H, s); FABMS m/e (M+H)$^+$ 565.

5.30. ({({7-[Amino(imino)methyl]-2-naphthyl}methyl)[4-(1,4-diazepan-1-yl)phenyl]amino}sulfonyl)acetic acid (11)

A solution of **9f** (250 mg, 0.42 mmol) in concd HCl (9 mL) was stirred at ambient temperature for 15 h. The reaction mixture was concentrated in vacuo and the residues was chromatographed on ODS-gel eluting with CH_3CN/H_2O (10/90). CH_3CN was removed in vacuo, and the aqueous solution was lyophilized after being acidified with 1 N HCl. Compound **11** (187 mg, 78%) was obtained as a white amorphous powder: ^{1}H NMR (DMSO-d_6) δ 1.99–2.06 (2H, m), 3.03–3.08 (2H, m), 3.12–3.16 (2H, m), 3.41 (2H, t, $J = 6.0$ Hz), 3.61–3.65 (2H, m), 4.23 (2H, s), 5.01 (2H, s), 6.68 (2H, d, $J = 9.1$ Hz), 7.21 (2H, d, $J = 9.1$ Hz), 7.66 (1H, dd, $J = 1.6$, 8.6 Hz), 7.79–7.82 (1H, m), 7.91 (1H, s), 8.02 (1H, d, $J = 8.6$ Hz), 8.10 (1H, d, $J = 8.6$ Hz), 8.47 (1H, s), 9.12 (2H, br s), 9.23 (2H, br s), 9.48 (2H, s); FABMS m/e (M+H)$^+$ 496. Anal. Calcd for $C_{25}H_{29}N_5O_4S{\cdot}3.3HCl{\cdot}3.0H_2O$: C, 48.23; H, 5.57; N, 11.25; S, 4.29; Cl, 15.66. Found: C, 48.26; H, 5.65; N, 11.50; S, 4.45; Cl, 15.60.

5.31. {({7-[Amino(imino)methyl]-2-naphthyl}methyl){4-[4-(pyridin-4-yl)-1,4-diazepan-1-yl]phenyl}amino)sulfonyl}acetic acid (12)

Compound **12** was synthesized from **9g** according to the same procedure as that for **11**. Compound **12** was obtained as a white amorphous powder (23% yield): ^{1}H NMR (DMSO-d_6) δ 1.87–1.89 (2H, m), 3.42–3.45 (2H, m), 3.59–3.63 (4H, m), 3.83–3.86 (2H, m), 4.22 (2H, s), 4.98 (2H, s), 6.69 (2H, d, $J = 8.8$ Hz), 7.03–7.09 (2H, m), 7.15 (2H, d, $J = 8.8$ Hz), 7.61 (1H, dd, $J = 1.6$, 8.4 Hz), 7.83 (1H, dd, $J = 1.6$, 8.3 Hz), 7.89 (1H, s), 8.01 (1H, d, $J = 8.4$ Hz), 8.11 (1H, d, $J = 8.4$ Hz), 8.12–8.15 (2H, m), 8.50 (1H, s), 9.37 (2H, s), 9.55 (2H, s), 13.76 (1H, br s); FABMS m/e (M+H)$^+$ 573. Anal. Calcd for $C_{30}H_{32}N_6O_4S{\cdot}2.4HCl{\cdot}2.5H_2O$: C, 47.80; H, 5.84; N, 11.15; S, 5.10; Cl, 13.55. Found: C, 47.79; H, 5.59; N, 11.15; S, 5.08; Cl, 13.53.

5.32. ({({7-[Amino(imino)methyl]-2-naphthyl}methyl)[4-(4-ethanimidoyl-1,4-diazepan-1-yl)phenyl]amino}sulfonyl)acetic acid (13)

Compound **13**was synthesized from **10f** according to the same procedure as that for **11**. Compound **13** was obtained as a white amorphous powder (62% yield): ^{1}H NMR (DMSO-d_6) δ 1.49–1.86 (2H, m), 2.03 (2H, s), 2.26 (1H, s), 3.49–3.79 (8H, m), 4.26 (2H, s), 5.00 (1.4H, s), 5.01 (0.6H, s), 6.70–6.74 (2H, m), 7.18–7.22 (2H, m), 7.63–7.67 (2H, m), 7.84–7.90 (1H, m), 8.02 (0.3H, d, $J = 8.4$ Hz), 8.03 (0.7H, d, $J = 8.4$ Hz), 8.10 (1H, d, $J = 8.4$ Hz), 8.54 (0.3H, s), 8.57 (0.7H, s), 8.75 (0.7H, s), 8.92 (0.3H, s), 9.45 (2H, s), 9.48 (1H, s), 9.62 (0.6H, s), 9.63 (1.4H, s); FABMS m/e (M+H)$^+$ 537. Anal. Calcd for $C_{27}H_{32}N_6O_4S{\cdot}3.1HCl{\cdot}1.5H_2O$: C, 47.92; H, 5.67; N, 12.42; S, 4.73; Cl, 16.24. Found: C, 47.91; H, 5.93; N, 12.31; S, 4.73; Cl, 16.46.

5.33. Ethyl ({[(7-cyano-2-naphthyl)methyl][4-(4-methyl-1,4-diazepan-1-yl)phenyl]amino}sulfonyl)acetate (14a)

To a solution of **8f** (610 mg, 1.01 mmol) in 1,2-dichloromethane (11 mL) at ambient temperature was added trifluoroacetic acid (1.1 mL). After stirring at ambient temperature for 3 h, the reaction mixture was concentrated in vacuo. The resulting residues was dissolved in chloroform and washed with 10% aqueous potassium carbonate. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. To a stirred solution of the deprotected intermediate in 1,2-dichloromethane (10 mL) at ambient temperature was added acetic acid (0.6 mL, 10 mmol), 35% aqueous formaldehyde (850 mg, 9.9 mmol) and sodium triacetoxyborohydride (420 mg, 1.97 mmol). After 2 h, the reaction mixture was washed with 10% aqueous potassium carbonate. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulted residue was chromatographed on silica gel eluting with chloroform/MeOH/aqueous NH_3 (100/5/0.5) to give **14a** (480 mg, 92%) as a white amorphous powder: ^{1}H NMR ($CDCl_3$) δ 1.39 (3H, t, $J = 6.9$ Hz), 1.86–1.97 (2H, m), 2.32 (3H, s), 2.49 (2H, t, $J = 4.3$ Hz), 2.61 (2H, t, $J = 4.3$ Hz), 3.37 (2H, t, $J = 4.3$ Hz), 3.46 (2H, t, $J = 4.3$ Hz), 4.07 (2H, s), 4.34 (2H, q, $J = 6.9$ Hz), 5.02 (2H, s), 6.53 (2H, d, $J = 8.3$ Hz), 7.19 (2H, d, $J = 8.3$ Hz), 7.48–7.54 (1H, m), 7.62 (1H, s), 7.65–7.71 (1H, m), 7.77–7.85 (2H, m), 8.03 (1H, s); FABMS m/e (M+H)$^+$ 521.

5.34. Ethyl ({[4-(4-butyl-1,4-diazepan-1-yl)phenyl][(7-cyano-2-naphthyl)methyl]amino}sulfonyl)acetate (14b)

Compound **14b** was synthesized from **8f** and butyraldehyde according to the same procedure as that for **14a**. Compound **14b** was obtained as a white amorphous powder (87% yield): ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, $J = 7.5$ Hz), 1.17–1.31 (2H, m), 1.34–1.45 (5H, m), 1.82–1.93 (2H, m), 2.40 (2H, t, $J = 7.5$ Hz), 2.51 (2H, t, $J = 5.1$ Hz), 2.66 (2H, t, $J = 5.1$ Hz), 3.37 (2H, t, $J = 5.1$ Hz), 3.43 (2H, t, $J = 5.1$ Hz), 4.08 (2H, s), 4.34 (2H, q, $J = 6.9$ Hz), 5.03 (2H, s), 6.53 (2H, d, $J = 9.3$ Hz), 7.19 (2H, d, $J = 9.3$ Hz), 7.42–7.53 (1H, m), 7.60 (1H, s), 7.65–7.69 (1H, m), 7.70–7.82 (2H, m), 7.99 (1H, s); FABMS m/e (M+H)$^+$ 563.

5.35. Ethyl ({[4-(4-benzyl-1,4-diazepan-1-yl)phenyl][(7-cyano-2-naphthyl)methyl]amino}sulfonyl)acetate (14c)

Compound **14c** was synthesized from **8f** and benzaldehyde according to the same procedure as that for **14a**. Compound **14c** was obtained as a white amorphous powder (88% yield): ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, $J = 7.2$ Hz), 1.84–1.92 (2H, m), 2.51–2.57 (2H, m), 2.61 (2H, t, $J = 5.1$ Hz), 3.36–3.45 (4H, m), 3.56 (2H, s), 4.06 (2H, s), 4.34 (2H, q, $J = 7.2$ Hz), 5.02 (2H, s), 6.52 (2H, d, $J = 9.0$ Hz), 7.16–7.35 (10H, m), 7.75–7.84 (2H, m), 8.02 (1H, s); FABMS m/e $(M+H)^+$ 597.

5.36. Ethyl ({({7-[amino(imino)methyl]-2-naphthyl}-methyl)[4-(4-methyl-1,4-diazepan-1-yl)phenyl]amino}-sulfonyl)acetate (15a)

Compound **15a** was synthesized from **14a** according to the same procedure as that for **9d**. Compound **15a** was obtained as a white amorphous powder (40% yield): ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, $J = 7.2$ Hz), 2.04–2.16 (1H, m), 2.28–2.40 (1H, m), 2.71 (1.5H, s), 2.72 (1.5H, s), 2.99–3.09 (2H, m), 3.26–3.42 (4H, m), 3.62–3.76 (2H, m), 4.25 (2H, q, $J = 7.2$ Hz), 4.36 (2H, s), 5.02 (2H, s), 6.67 (2H, d, $J = 8.4$ Hz), 7.22 (2H, d, $J = 8.4$ Hz), 7.67 (1H, d, $J = 8.4$ Hz), 7.83 (1H, d, $J = 8.4$ Hz), 7.90 (1H, s), 8.03 (1H, d, $J = 8.4$ Hz), 8.10 (1H, d, $J = 8.4$ Hz), 8.51 (1H, s), 9.37 (2H, s), 9.56 (2H, s), 11.21 (1H, s); FABMS m/e $(M+H)^+$ 538.

5.37. Ethyl ({({7-[amino(imino)methyl]-2-naphthyl}-methyl)[4-(4-butyl-1,4-diazepan-1-yl)phenyl]amino}-sulfonyl)acetate (15b)

Compound **15b** was synthesized from **14b** according to the same procedure as that for **9d**. Compound **15b** was obtained as a white amorphous powder (26% yield): ^{1}H NMR (DMSO-d_6) δ 0.85–0.89 (3H, m), 1.23–1.30 (5H, m), 1.59–1.72 (2H, m), 2.06–2.13 (1H, m), 2.31–2.41 (1H, m), 2.95–3.05 (4H, m), 3.25–3.45 (4H, m), 3.68–3.73 (2H, m), 4.25 (2H, t, $J = 7.2$ Hz), 4.69 (2H, s), 5.02 (2H, s), 6.66 (2H, d, $J = 8.8$ Hz), 7.22 (2H, d, $J = 8.8$ Hz), 7.66 (1H, dd, $J = 1.6$, 8.8 Hz), 7.82 (1H, dd, $J = 1.6$, 8.8 Hz), 7.91 (1H, s), 8.02 (1H, d, $J = 8.8$ Hz), 8.10 (1H, d, $J = 8.8$ Hz), 8.48 (1H, s), 9.28 (2H, s), 9.51 (2H, s), 10.88 (1H, s); FABMS m/e $(M+H)^+$ 580.

5.38. Ethyl ({({7-[amino(imino)methyl]-2-naphthyl}-methyl)[4-(4-benzyl-1,4-diazepan-1-yl)phenyl]amino}-sulfonyl)acetate (15c)

Compound **15c** was synthesized from **14c** according to the same procedure as that for **9d**. Compound **15c** was obtained as a white amorphous powder (26% yield): ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, $J = 7.6$ Hz), 2.07–2.16 (1H, m), 2.39–2.49 (1H, m), 2.91–3.02 (2H, m), 3.24–3.42 (4H, m), 3.72–3.76 (2H, m), 4.22–4.34 (4H, m), 4.35 (2H, s), 5.02 (2H, s), 6.66 (2H, d, $J = 8.8$ Hz), 7.20 (2H, d, $J = 8.8$ Hz), 7.41–7.44 (3H, m), 7.83 (1H, dd, $J = 1.2$, 8.8 Hz), 7.90 (1H, s), 8.02 (1H, d, $J = 8.8$ Hz), 8.10 (1H, d, $J = 8.8$ Hz), 8.50 (1H, s), 9.34 (2H, s), 9.54 (2H, s), 11.41 (1H, s); FABMS m/e $(M+H)^+$ 634.

5.39. ({({7-[Amino(imino)methyl]-2-naphthyl}methyl)-[4-(4-methyl-1,4-diazepan-1-yl)phenyl]amino}sulfonyl)-acetate (16a)

Compound **16a** was synthesized from **15a** according to the same procedure as that for **11**. Compound **16a** was obtained as a white amorphous powder (40% yield): ^{1}H NMR (DMSO-d_6) δ 2.73 (3H, s), 3.01–3.11 (2H, m), 3.31–3.33 (6H, m), 3.52–3.55 (2H, m), 4.23 (2H, s), 5.02 (2H, s), 6.66 (2H, d, $J = 8.8$ Hz), 7.22 (2H, d, $J = 8.8$ Hz), 7.67 (1H, d, $J = 8.8$ Hz), 7.80 (1H, dd, $J = 2.0$, 8.8 Hz), 7.92 (1H, s), 8.02 (1H, d, $J = 8.8$ Hz), 8.10 (1H, d, $J = 8.8$ Hz), 8.47 (1H, s), 9.20 (2H, s), 9.47 (2H, s), 10.73 (1H, s); FABMS m/e $(M+H)^+$ 510. Anal. Calcd for $C_{26}H_{31}N_5O_4S{\cdot}2.9HCl{\cdot}1.5H_2O$: C, 50.16; H, 6.15; N, 10.45; S, 4.78; Cl, 15.34. Found: C, 50.45; H, 6.04; N, 10.50; S, 4.78; Cl, 15.15.

5.40. ({({7-[Amino(imino)methyl]-2-naphthyl}methyl)-[4-(4-butyl-1,4-diazepan-1-yl)phenyl]amino}sulfonyl)acetic acid (16b)

Compound **16b** was synthesized from **15b** according to the same procedure as that for **11**. Compound **16b** was obtained as a white amorphous powder (20% yield): ^{1}H NMR (DMSO-d_6) δ 0.87 (3H, t, $J = 7.4$ Hz), 1.22–1.31 (2H, m), 1.59–1.71 (2H, m), 2.06–2.14 (1H, m), 2.32–2.43 (1H, m), 2.95–3.05 (4H, m), 3.25–3.47 (4H, m), 3.69–3.74 (2H, m), 4.24 (2H, s), 5.02 (2H, s), 6.66 (2H, d, $J = 9.2$ Hz), 7.22 (2H, d, $J = 9.2$ Hz), 7.67 (1H, dd, $J = 1.2$, 8.8 Hz), 7.82 (1H, dd, $J = 1.2$, 8.8 Hz), 7.91 (1H, s), 8.02 (1H, d, $J = 8.8$ Hz), 8.10 (1H, d, $J = 8.8$ Hz), 8.49 (1H, s), 9.32 (2H, s), 9.53 (2H, s), 10.96 (1H, s); FABMS m/e $(M+H)^+$ 552. Anal. Calcd for $C_{28}H_{35}N_5O_4S{\cdot}2.3HCl{\cdot}2.5H_2O$: C, 52.54; H, 6.87; N, 9.88; S, 4.52; Cl, 11.51. Found: C, 53.04; H, 6.90; N, 9.43; S, 4.28; Cl, 11.26.

5.41. ({({7-[Amino(imino)methyl]-2-naphthyl}methyl)[4-(4-benzyl-1,4-diazepan-1-yl)phenyl]amino}sulfonyl)acetic acid (16c)

Compound **16c** was synthesized from **15c** according to the same procedure as that for **11**. Compound **16c** was obtained as a white amorphous powder (18% yield): ^{1}H NMR (DMSO-d_6) δ 2.08–2.16 (1H, m), 2.38–2.49 (1H, m), 2.92–3.02 (2H, m), 3.24–3.46 (4H, m), 3.72–3.77 (2H, m), 4.23 (2H, s), 4.25–4.35 (2H, m), 5.02 (2H, s), 6.65 (2H, d, $J = 8.8$ Hz), 7.21 (2H, d, $J = 8.8$ Hz), 7.41–7.44 (3H, m), 7.60–7.64 (2H, m), 7.67 (1H, dd, $J = 1.5$, 8.8 Hz), 7.82 (1H, dd, $J = 1.2$, 8.8 Hz), 7.90 (1H, s), 8.02 (1H, d, $J = 8.8$ Hz), 8.10 (1H, d, $J = 8.8$ Hz), 8.49 (1H, s), 9.32 (2H, s), 9.53 (2H, s), 11.35 (1H, s); FABMS m/e $(M+H)^+$ 536. Anal. Calcd for $C_{32}H_{35}N_5O_4S{\cdot}2.5HCl{\cdot}3.2H_2O$: C, 53.55; H, 6.33; N, 9.18; S, 4.20; Cl, 11.62. Found: C, 53.46; H, 6.08; N, 9.19; S, 4.22; Cl, 11.24.

6. Biology

6.1. Chromogenic assay

The hydrolysis rates of synthetic substrates were assayed by continuously measuring absorbance at 405 nm at 37 °C with a microplate reader (Model 3550, Bio-Rad, USA). Reaction mixtures (125 μL) were prepared in 96-well plates containing chromogenic substrates and an inhibitor in either 0.05 M Tris–HCl, pH 8.4, 0.15 M NaCl. Reactions were initiated with a 25 μL portion of the enzyme solution. Enzymes and substrates were used as follows: factor Xa and S-2222; thrombin and S-2238; trypsin and S-2222. The concentration of an inhibitor required to inhibit enzyme activity by 50% (IC_{50}) was calculated from dose–response curves in which the logit transformation of residual activity was plotted against the logarithm of inhibitor concentration.

6.2. Plasma clotting time assays

Citrated blood samples from mice and dogs were collected. Platelet-poor plasma was prepared by centrifugation at 3000 rpm for 10 min and stored at −40 °C until use. Plasma clotting times were performed using a KC10A coagulometer (Amelung Co., Lehbrinsweg, Germany) at 37 °C. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using Orthobrain thromboplastin and thrombofax (Ortho Diagnostic Systems Co., Tokyo, Japan), respectively. Coagulation times for each test sample were compared with coagulation times measured using a distilled water control. The concentration required to double the clotting time (CT_2) was estimated from each individual concentration–response curve. Each measurement was performed three times, and represented as the mean value.

6.3. Ex vivo studies

Male mice weighing 30–37 g and male beagle dogs weighing 8.6–12.6 kg were used in these studies. In all animal species used, non-fasted animals for intravenous studies and fasted animals for overnight for the oral studies were used. In mice, the test drug was dissolved in saline and administered to animals intravenously at 1 mg/kg via tail vein or orally at 100 mg/kg using a gastric tube. Citrated blood was collected from the vena cava 1 min after intravenous injection or 30 min after oral administration. In dogs, the test drug was dissolved in saline or 0.5% methylcellulose and administered to animals intravenously at 0.3 mg/kg via cephalic vein or orally at 10 mg/kg using a gastric tube. At a predetermined time before and after the drug administration, citrated blood was collected from the cephalic vein. At a predetermined time before and after the drug administration, citrated blood was collected from the femoral vein. Platelet-poor plasma was prepared by centrifugation for measurement of PT or APTT. All data were expressed as relative fold values, compared with the baseline value (dogs and monkeys) or the vehicle group (mice).

6.4. Thromboplastin-induced venous thrombosis model in rats

Male SD rats weighing 280–320 g were used. In the intravenous studies, the test drug (HBr salt of **13**, heparin or vehicle) was administered to non-fasted animals under anesthesia with urethane (0.96 g/kg ip) by continuous iv infusion using an infusion pump (Harvard apparatus) 2 h before the thrombus formation. In the oral studies, the test drug (HBr salt of **13**, warfarin or vehicle) was orally administered to fasted animals using a gastric tube 1 h (HBr salt of **13** and vehicle) or 18 h (warfarin or vehicle) before the thrombus formation. Thrombus formation was induced by the method of Reyers et al. Under anesthesia with urethane, the abdomen was surgically opened and the inferior vena cava was carefully isolated. Venous thrombosis was induced by injection of 25 μg/kg thromboplastin (Ortho Diagnostic Systems Co., Tokyo, Japan) into the right femoral vein. One minute after the injection of thromboplastin, two tight ligations 1 cm apart were made with cotton thread on the inferior vena cava just below the left renal venous branch. Ten minutes after the ligation, the thrombosed segment was longitudinally opened. The thrombus was gently removed, and dissolved in 2 mL of 0.5 N NaOH. The thrombus protein content was measured by photometry using a due binding assay kit (Bio-Rad, Hercules, CA) and bovine serum albumin (BSA) as a protein standard. The experiments were performed on groups of six animals each. Data represent percentages of inhibition compared with the thrombus wet weight of the saline group. Data represent the means ± SEM.

6.5. Template bleeding time in rats

Male SD rats weighing 300–330 g were used. In the oral studies, the test drug (HBr salt of **13**, warfarin or vehicle) was orally administered to fasted animals using a gastric tube 1 h (HBr salt of **13** and vehicle) or 18 h (warfarin or vehicle) before the measurement of bleeding time. A template bleeding device (Simplate®, Organon Teknika, Tokyo) was placed on the right planta and triggered. Blood flowing from the incision was gently wiped away with filter paper every 30 s. Bleeding time was measured as time elapsed until bleeding stopped. When template bleeding time was prolonged beyond 30 min, measurement was stopped and the bleeding time was recorded as 30 min. The experiments were performed on groups of six animals each. Data represent the means ± SEM.

6.6. Statistical analyses

Statistical analysis was performed by Dunnett multiple comparison test for thrombus formation or Steel's test for bleeding time, compared with the vehicle group. A p value of less than 0.05 was considered significant.

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