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Cycloalkanediamine derivatives as novel blood coagulation factor Xa inhibitors

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Abstract—This paper describes the synthesis of orally available potent fXa inhibitors **2** and **3** by modification of the piperazine part of lead compound **1**. Carbonyl derivative **3** showed potent fXa activity but not sulfonyl derivative **2**. Among the compounds synthesized, cyclohexane derivatives **3g** and **3h** and cycloheptane derivative **3j** had potent anticoagulant activity as well as anti-fXa activity. Synthetic study of the optical isomers of **3g** demonstrated that (−)-**3g** had more potent activity.
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The extrinsic and intrinsic coagulation systems converge at the activation of factor X to Xa. Activated factor X (fXa) has an important role in conversion of prothrombin (fII) to thrombin (fIIa), which produces blood clots.[1] Thus, fXa is a key enzyme in the coagulation cascade and also an attractive target enzyme for the therapy of thrombosis and related diseases.

A decade ago, we reported a low-molecular selective fXa inhibitor **DX-9065a** (Fig. 1) having two amidino groups.[2] **DX-9065a** showed potent fXa inhibitory activity in vitro and ex vivo, while it had poor oral bioavailability (10% in monkeys) probably due to its strong basic amidino groups. As a result, **DX-9065a** has only been used as an injectable formulation in clinical studies.

Many researchers have attempted synthesis of fXa inhibitors with improved oral bioavailability.[3a] Zeneca's researchers have reported epoch-making non-amidino fXa inhibitors, 1-[(6-chloro-2-naphthyl)sulfonyl]-4-[(1-pyridin-4-ylpiperidin-4-yl)carbonyl]piperazine.[3b] We also previously synthesized a variety of non-amidino derivatives, and reported compound **1** having 5-chloroindole and 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine

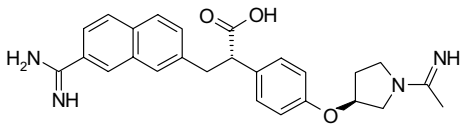

Figure 1. Structure of **DX-9065a**.

groups. The study showed that 5-chloroindole and 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine groups were suitable sub-structures for the S1 and aryl (S4) binding sites of fXa,[4,5] respectively (Fig. 2).[6] However, the oral bioavailability of compound **1** was not satisfactory.

Therefore, we examined the synthesis of orally available potent fXa inhibitors by modification of the piperazine part of compound **1**. Figure 3 shows the drug design

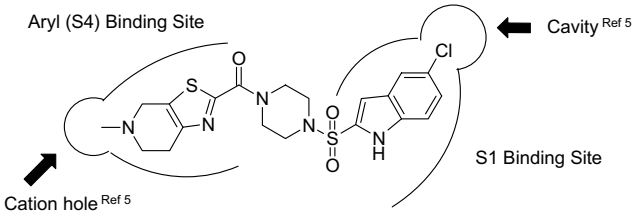

Figure 2. Binding mode of fXa and compound **1**.

Keywords: Factor Xa inhibitors; Cycloalkanediamine; Non-amidine; Anticoagulant.
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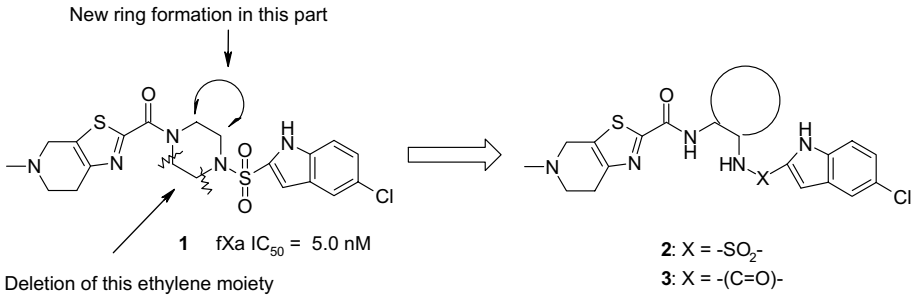


Figure 3. Drug design.

of cycloalkanediamine derivatives. Sulfonyl derivative **2** was first synthesized based on the drug design, though it did not have potent activity. In the next step, we attempted the synthesis of carbonyl derivative **3**, which showed highly potent activity. In this paper, we describe the synthesis and pharmacological properties of cycloalkanediamine derivatives **2** and **3**.

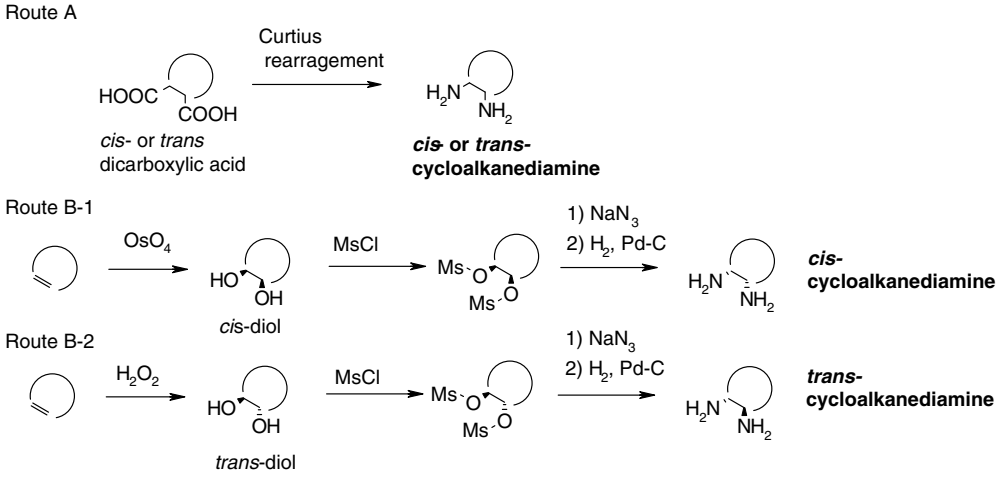
Scheme 1 shows the synthetic pathway of the key intermediate cycloalkanediamines. The cycloalkanediamines were synthesized from commercially available dicarboxylic acids, which were converted to the corresponding cycloalkanediamines via a Curtius rearrangement (Route A). Cycloalkanediamines were also synthesized from commercially available diols or their precursor cycloalkenes (Route B). Oxidation of the cycloalkenes to *cis*- or *trans*-diols was carried out by use of OsO_4/NMO (Route B-1) or H_2O_2 (Route B-2). The diols were mesylated to give di-mesylated compounds, which were successively treated with sodium azide and reduced over Pd–C to yield cycloalkanediamines.

The synthetic pathway of sulfonyl derivative **2** and carbonyl derivative **3** is outlined in Scheme 2. Treatment of cycloalkanediamine **4** with (1-benzenesulfonyl-5-chloroindol-2-yl)sulfonyl chloride[4] (**5**) afforded sulfonamide **6**. Compound **6** was condensed with 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt[7] (**7**) to give the desired sulfonyl derivative **2**. The 1-benzenesulfonyl group was removed in the course of the condensation.

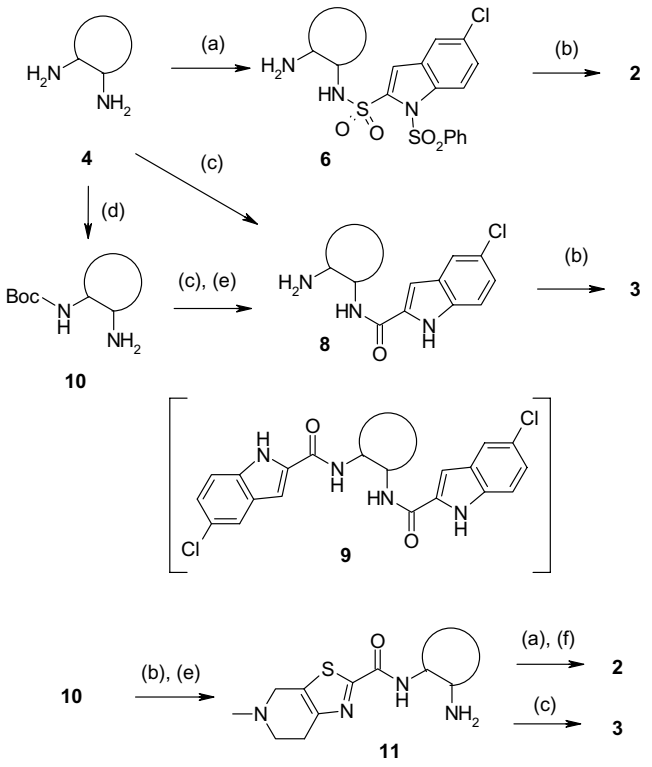
Cycloalkanediamine **4** was acylated with commercially available 5-chloroindole-2-carboxylic acid to give mono-amide **8**, which was successively condensed with thiazolopyridinecarboxylic acid **7** to provide carbonyl derivative **3**. However, sometimes the yield of mono-amide **8** from cycloalkanediamine **4** (especially in the acylation of *trans*-diamine) was significantly low because of the production of diacylated compound **9**. In that case, mono-amide **8** was synthesized via mono-Boc protected derivative **10** in a better yield.

Compounds **2** and **3** were also prepared via mono-amide **11**, which was prepared from cycloalkanediamine **10** using a method similar to that of mono-amide **8**.

Since these cycloalkanediamines are racemates, we have synthesized optical isomers to study pharmacological properties. We selected a compound (**3g**) and synthesized its optical isomers. The synthetic pathway of the optical isomers (+)-(1*S*,2*R*)-**3g** and (−)-(1*R*,2*S*)-**3g** is outlined in Scheme 3. Optical resolution[8] of racemic *cis*-cyclohexanediamine **12** afforded optical isomers **13** and **14**. Optical isomers **13** and **14** were condensed with 5-chloroindole-2-carboxylic acid to give (+)-(1*S*,2*R*)-**3g** and (−)-(1*R*,2*S*)-**3g**, respectively. The absolute configuration of (−)-(1*R*,2*S*)-**3g** was determined by X-ray



Scheme 1. Synthesis of cycloalkanediamine spacers.



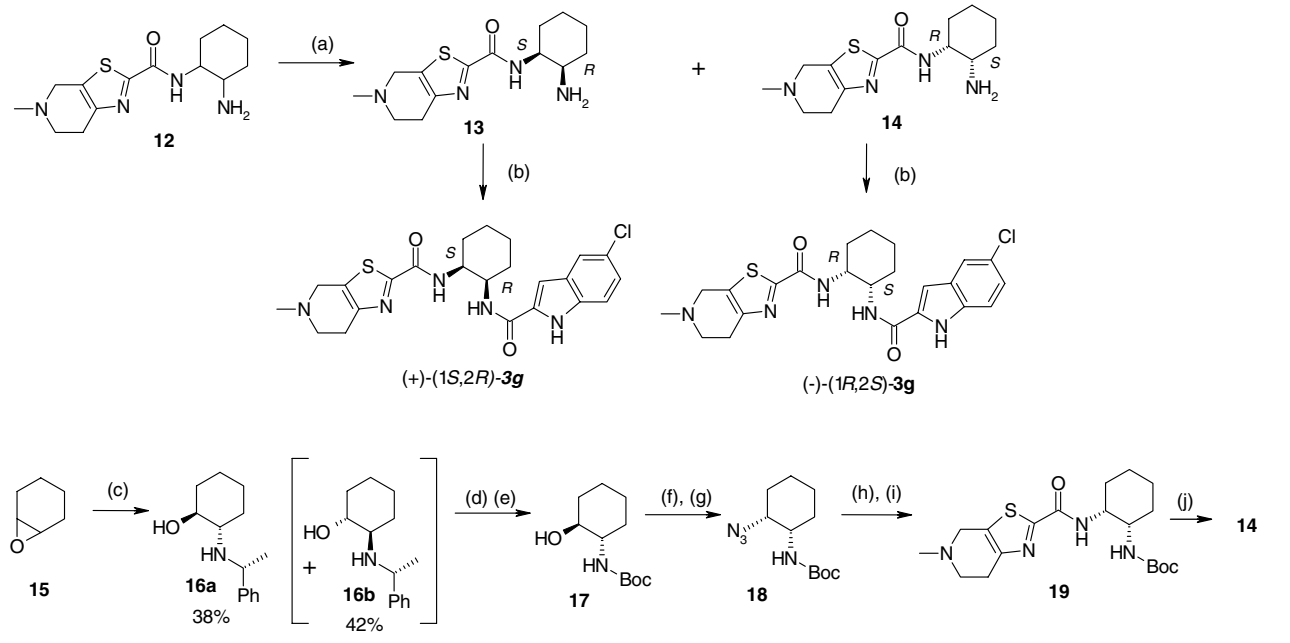
Scheme 2. Reagents: (a) (1-benzenesulfonyl-5-chloroindol-2-yl)sulfonyl chloride (**5**), TEA, DCM; (b) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt (**7**), WSCI, HOBt, DMF; (c) 5-chloroindole-2-carboxylic acid, WSCI, HOBt, DMF; (d) $(Boc)_2O$, DCM or BocON, TEA, DCM; (e) HCl/EtOH or TFA, DCM; (f) 1 N-NaOH, THF, EtOH.

crystallographic analysis.[9] Intermediate **14** was alternatively prepared from epoxide **15** as follows. Epoxide **15** was treated with (*R*)-α-methylbenzylamine at 160 °C in a sealed tube for 2 days to afford a mixture of diastereomers **16a** and **16b**, which were easily separated by silica gel chromatography to give pure **16a** and **16b**.[10] Compound **16a** was treated with 10% Pd–C and $HCOONH_4$, and successively protected using Boc_2O to afford compound **17**. Compound **17** was mesylated and then treated with sodium azide to provide azide **18**. Azide **18** was catalytically hydrogenated and then acylated with thiazolopyridinecarboxylic acid **7** to give compound **19**, which was successively treated with acid to yield intermediate **14**.

Table 1 shows in vitro anti-fXa activity of sulfonyl derivatives **2a**–**f**. The activity of all these compounds was less potent than that of compound **1** (IC_{50}: 5.0 nM). However, it is noted that *cis*-isomers were more potent than *trans*-isomers and that *cis*-cyclopropane derivative **2a** showed the most potent activity.

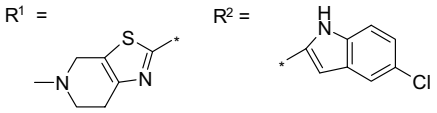
Table 2 shows the in vitro anti-fXa activity of carbonyl derivatives **3a**–**l**. Cyclohexane and cycloheptane derivatives **3g**, **3h**, and **3j** exhibited potent activity, while 3- to 5- and 8-membered ring derivatives **3a**–**f** and **3k**,**l** showed less potent activity. Thus, the ring size significantly impacted on the potency. With respect to configuration, *trans*-5- to 7-membered ring derivatives **3f**, **3h**, and **3j** were more potent than the corresponding *cis*-isomers **3e**, **3g**, and **3i**. However, the order of potency between *cis*- and *trans*-isomers was reversed in 3- and 4-membered ring compounds.

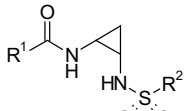
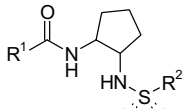
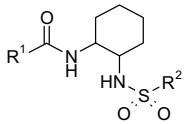
Comparing sulfonyl derivatives **2a**–**f** with carbonyl derivatives **3a**–**l**, there seems to be a distinct structure–activity relationship in the ring size and *cis*-/*trans*-configuration. In the sulfonyl derivatives, *cis*-cyclopropane derivative **2a** was the most potent, while



Scheme 3. Reagents and condition: (a) optical resolution, CHIRAL-PACK AD; (b) 5-chloroindole-2-carboxylic acid, WSCI, HOBt, DMF (76% for (+)-**3g**, 66% for (−)-**3g**); (c) (*R*)-α-methylbenzylamine, sealed tube, 160 °C; (d) 10% Pd–C, $HCOONH_4$, MeOH; (e) Boc_2O, DCM (59%, two steps); (f) MsCl, pyridine (94%); (g) NaN_3, DMF (47%); (h) H_2, 10% Pd–C; (i) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt (**7**), WSCI, HOBt, DMF (44%, two steps); (j) HCl/EtOH (quant.).

Table 1. Sulfonyl derivatives



Compound	Structure	Stereochemistry	Anti-fXa IC_{50}[a] (nM)
2a		*cis*	270
2b		*trans*	>10,000
2c		*cis*	3200
2d		*trans*	>10,000
2e		*cis*	4400
2f		*trans*	>10,000

[a] The method for measuring anti-fXa activity was described in Ref. 11.

trans-cycloheptane derivative **3j** was the most potent among the carbonyl derivatives. It is likely that conformational differences, including difference in the distance and bond angle between –NH–SO_2– and –NH–CO–, may affect potency.

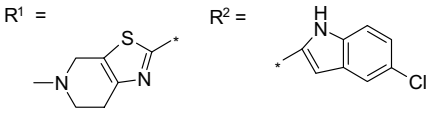
Table 3 shows the anti-fIIa (anti-thrombin) activity and anticoagulant activity (PTCT2) of carbonyl derivatives **3e**–**h**,**j**. All the compounds listed in Table 3 showed high selectivity for fXa versus fIIa. *cis*-Cyclohexane derivative **3g** had the poorest selectivity, though it was still more than 100-fold. Although derivative **3g** was not the most potent fXa inhibitor among the compounds tested, it showed the best anticoagulant activity. In contrast, derivatives **3h** and **3j** had more potent anti-fXa activity and exhibited somewhat less potent anticoagulant activity.

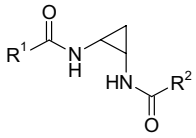
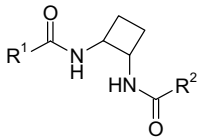
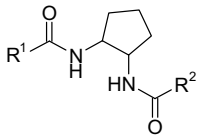
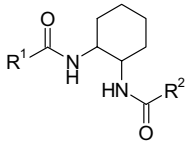
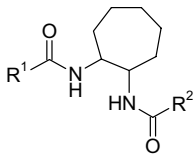
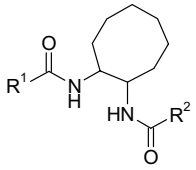
Study of the optical isomers of **3g** demonstrated that (−)-(1*R*,2*S*)-**3g** had potent anti-fXa activity, while (+)-(1*S*,2*R*)-**3g** showed 47-fold less potent activity than the counterpart (−)-isomer. The former compound (−)-(1*R*,2*S*)-**3g** also showed potent anticoagulant activity (2.9 μM).

Table 4 shows ex vivo anti-fXa and anticoagulant activity in rats. Derivative **3g** exhibited over 80% inhibition for fXa from 0.5 to 4 h after oral administration of 30 mg/kg. Under the same conditions, derivative **3g** also showed a potent prolongation effect (1.17- to 1.23-fold) on prothrombin time (PT). These data indicate that derivative **3g** has good oral availability as well as high potency in vivo. On the other hand, derivatives **3f**, **3h**, and **3j** showed less potent activity than **3g**.

Table 5 shows the ex vivo anti-fXa and anticoagulant activity of optically active compound (−)-**3g**. Com-

Table 2. Carbonyl derivatives



Compound	Structure	Stereochemistry	Anti-fXa IC_{50}[a] (nM)
3a		*cis*	187
3b		*trans*	>10,000
3c		*cis*	265
3d		*trans*	2000
3e		*cis*	118
3f		*trans*	86
3g		*cis*	41
3h		*trans*	13
3i		*cis*	175
3j		*trans*	11.3
3k		*cis*	>1400
3l		*trans*	>10,000

[a] The method for measuring anti-fXa activity was described in Ref. 11.

Table 3. Anti-fXa and anti-fIIa activities and anticoagulant activity

Compound	Anti-fXa IC_{50}[a] (nM)	Anti-fIIa IC_{50}[b] (nM)	PTCT2 in human plasma[c] (μM)	PTCT2 in rat plasma[c] (μM)
3e	118	27,000	9.6	17.2
3f	86	36,000	16	30.1
3g	41	4500	4.4	12
(+)-**3g**	753	>100	>20	>20
(−)-**3g**	16	2600	2.9	9.2
3h	13	29,000	6.2	9.5
3j	11.3	>100,000	11.7	14.1

[a] The methods for measuring anti-fXa activitie was described in Ref. 11.

[b] The methods for measuring anti-fIIa activitie was described in Ref. 12.

[c] Anticoagulant activities in human and rat plasma were evaluated by the plasma clotting time doubling concentration (PTCT2).[13]

Table 4. Ex vivo anti-fXa and anticoagulant activities

Compound	At 30 mg/kg (po) to rats							
	Anti-fXa activity[a] (%)				Prolongation effect of PT[a] (fold)			
	0.5 h	1 h	2 h	4 h	0.5 h	1 h	2 h	4 h
3f	78.6 ± 2.3	80.7 ± 1.0	78.4 ± 3.0	80.6 ± 3.0	1.12 ± 0.01	1.14 ± 0.02	1.26 ± 0.03	1.16 ± 0.02
3g	80.3 ± 1.1	81.5 ± 1.8	81.7 ± 1.8	85.6 ± 0.8	1.20 ± 0.02	1.17 ± 0.02	1.21 ± 0.02	1.23 ± 0.03
3h	84.9 ± 2.1	78.3 ± 3.0	65.7 ± 5.3	57.0 ± 8.7	1.13 ± 0.02	1.07 ± 0.00	1.07 ± 0.01	1.04 ± 0.00
3j	79.0 ± 0.6	66.0 ± 0.90	59.0 ± 2.50	49.0 ± 3.0	1.05 ± 0.01	1.02 ± 0.00	1.00 ± 0.01	1.00 ± 0.01

[a] The methods for measuring ex vivo anti-fXa and anticoagulant activities were described in Ref. 14. Values are expressed as means ± SE from four rats.

Table 5. Ex vivo anti-fXa and anticoagulant activity for compound (−)-**3g**

Compound	At 30 mg/kg (po) to rats							
	Anti-fXa activity[a] (%)				Prolongation effect of PT[a] (fold)			
	1 h	3 h	6 h	24 h	1 h	3 h	6 h	24 h
(−)-**3g**	97.0 ± 0.6	99.5 ± 1.0	90.7 ± 1.9	1.7 ± 1.7	1.56 ± 0.01	1.55 ± 0.01	1.36 ± 0.01	1.02 ± 0.01

[a] The methods for measuring the ex vivo anti-fXa and anticoagulant activities were described in Ref. 14. Values are expressed as means ± SE from four rats.

pound (−)-**3g** showed excellent oral anti-fXa activity and anticoagulant activity in rats over 6 h after oral administration.

Table 6 shows the pharmacokinetic properties of compound (−)-**3g** evaluated using monkeys (po and iv, 3 mg/kg) and human microsomes. For comparison, data of compound **3f** are also shown in Table 6. Compound (−)-**3g** showed poor oral bioavailability ($F = 6.1\%$) when compared with compound **3f** ($F = 37\%$). The reason for such poor bioavailability of (−)-**3g** can be explained by its low remaining rate (46%), which was significantly lower than that of **3f** (89%), in the human liver microsome test.

In summary, we synthesized a series of sulfonyl derivatives **2** and carbonyl derivatives **3**, tested their pharmacological properties, and found that among them carbonyl derivatives **3g**, (−)-**3g**, **3h**, and **3j** had potent anti-fXa activity. In addition, *cis*-/*trans*- and absolute configurations affect anticoagulant activity as well as anti-fXa activity. In the series of compounds having a cycloalkanediamine skeleton, 5-chloroindole-2-carbonyl group was a superior component for aryl (S4) binding site. These results would be useful for drug design of new fXa inhibitors.

Table 6. Pharmacokinetic profiles in monkeys and human microsomes

Compound	Cl (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	F (%)	Remaining rate[b] (%)
3f[a]	14.2	2.54	1.4	37	89
(−)-**3g**[a]	17.7	2.22	1.4	6.1	46

[a] Hydrogen chloride salts were used. Both compounds were administered at a dose of 3 mg/kg po and iv ($n = 3$).

[b] The remaining rate of the two compounds after 5 min of incubation with human liver microsomes.

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8. Optical resolution: compound **12** (900 mg) was dissolved in isopropanol (6 ml). The solution was purified in 11 portions by preparative HPLC (CHIRALPAK AD. Daicel Chemical Industries, Ltd; 2.0 in diameter × 25 cm) with hexane/isopropanol/diethylamine = 68:32:0.5 as the mobile phase at a flow rate of 6 ml/min. Compound **13**: 320 mg, retention time = 24.8 min. Compound **14**: 390 mg, retention time = 33.4 min.
9. The coordinates of the complex have been deposited in the Protein Data Bank as 2EI6.pdb.
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11. In vitro anti-fXa activity was measured by using a chromogenic substrate S-2222 (Chromogenix, Inc.) and human fXa (Enzyme Research Laboratories). Aqueous

DMSO (5% v/v; 10 μL) or inhibitors in aqueous DMSO (10 μL) and 0.0625 U/mL human fXa (10 μL) were mixed with 0.1 M Tris–0.2 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL). A reaction was started by the addition of 0.75 M S-2222 (40 μL). After the mixture was stirred for 10 s at rt, the increase of optical densities (OD/min) was measured at 405 nm. Anti-fXa activity (inhibition %) was calculated as follows: anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. The IC_{50} value was obtained by plotting the inhibitor concentration against the anti-fXa activity.

12. In vitro anti-thrombin activity was measured by using chromogenic substrate S-2266 (Chromogenix, Inc.) and human thrombin (Sigma Chemical, Inc.). Aqueous DMSO (5% v/v; 10 μL) or inhibitors in aqueous DMSO (10 μL) and 4 U/ml human thrombin (10 μL) were mixed with 0.1 M Tris–0.2 M–NaCl 0.2% BSA buffer (pH 7.4; 40 μL). A reaction was started by the addition of 0.50 M S-2266 (40 μL). After the mixture was stirred for 10 s at room temperature, the increase of optical density (OD/min) was measured at 405 nm. Anti-thrombin activity (inhibition percentage) was calculated as follows: anti-thrombin activity = 1 − [(OD/min) of sample/(OD/min) of control]. The IC_{50} value was obtained by plotting the inhibitor concentration against the anti-fIIa activity.
13. Prothrombin time (PT) was measured with an Amelung KC-10A microcoagulometer (MC Medical, Tokyo, Japan) as follows; First, 50 μL of plasma was mixed with 50 μL of inhibitor or 4% DMSO/saline and incubated for 1 min at 37 °C. Coagulation was started by the addition of 100 μL of thromboplastin C Plus (0.5 U/mL) to the mixture, and the clotting time was measured. he concentration of inhibitor required to double the clotting time (CT2) was estimated from the concentration–response curve by a regression analysis.
14. Anti-fXa activity and anticoagulant activity ex vivo. Male Wister rats were fasted overnight. Synthetic compounds were dissolved in 0.5% (w/v) methylcellulose solution and administered orally to rats via a stomach tube. For control rats, 0.5% (w/v) methylcellulose solution was administered orally. The rats were anesthetized with ravonal at several time points when blood samples were collected in the presence of trisodiumcitrate. After the blood samples were centrifuged, the platelet-poor plasma samples were used for the measurement of their anti-fXa activities or anticoagulant activities. Anti-Xa activity: plasma (5 μL) was mixed with 0.1 M Tris–0.2 M–NaCl–0.2% BSA buffer (pH 7.4; 40 μL), H_2O (5 μL), and 0.1 U/mL human fXa (10 μL). A reaction was started by the addition of 0.75 M S-2222 (40 μL). After the mixture was stirred for 10 s at rt, the increase of optical density (OD/min) was measured at 405 nm. Anti-fXa activity (inhibition %) was calculated as follows: anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. Anticoagulant activity was measured with an Amelung KC-10A microcoagulometer. Plasma (50 μL) was incubated for 1 min at 37 °C in a cup. The coagulation was started by the addition of 100 μL of thromboplastin C Plus (0.5 U/mL) to the mixture and the clotting time was measured. The anticoagulant activity was evaluated by the prolongation rate of prothrombin time versus the control.