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Discovery of *N*-[(1*R*,2*S*,5*S*)-2-{[(5-chloroindol-2-yl)carbonyl]amino}-5-(dimethylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride: A novel, potent and orally active direct inhibitor of factor Xa

Tsutomu Nagata [a,*], Toshiharu Yoshino [b], Noriyasu Haginoya [b], Kenji Yoshikawa [b], Masatoshi Nagamochi [b], Syozo Kobayashi [b], Satoshi Komoriya [b], Aki Yokomizo [b], Ryo Muto [a], Mitsuhiro Yamaguchi [a], Ken Osanai [a], Makoto Suzuki [b], Hideyuki Kanno [c]

[a] *R&D Division, Daiichi Sankyo Co., Ltd., 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[b] *R&D Division, Daiichi Sankyo Co., Ltd, 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan*
[c] *Daiichi Sankyo RD Associe Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

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ABSTRACT

In the early 1990's, we reported on the low-molecular selective fXa inhibitor **DX-9065a** having two amidino groups. However, it had poor oral bioavailability due to its strong basic amidino groups. To obtain fXa inhibitors with improved oral bioavailability, we investigated various non-amidino fXa inhibitors and finally discovered *cis*-1,2-diaminocyclohexane derivative **4c** to have potent fXa inhibition, promising anticoagulant activity, and good oral bioavailability, compared with amidino compound **DX-9065a**. In addition, we will discuss the influence of the third substituent on the cyclohexane ring on anti-fXa activity, anticoagulant activity, PK profile, and lipophilicity.

1. Introduction

Thrombotic events are major causes of fatal diseases, such as myocardial infarction and cerebral infarction. For the treatment and prevention of such events, several anticoagulants, as well as antiplatelet agents, are clinically prescribed. Among them, warfarin is the only anticoagulant that can be administered orally. However, warfarin has several defects regarding its use. For instance, the anticoagulant effect of warfarin is influenced by other drugs or foods and it needs regular monitoring to adjust anticoagulant effect individually.[1]

Activated blood coagulation factor X (fXa) is a key enzyme that plays an important role in converting prothrombin (fII) to thrombin (fIIa) to begin blood clot formation within the coagulation cascade. This enzyme is located at the start of the common pathway of the extrinsic and intrinsic coagulation systems.[2] Thus, we thought fXa could be an attractive target for the prophylaxis and treatment of thrombosis and related diseases.

We initiated an investigation of fXa inhibitors in 1981 and our efforts to identify a novel fXa inhibitor culminated in the discovery of the first low-molecular selective fXa inhibitor, **DX-9065a**, in the early 1990's (Fig. 1).[3] **DX-9065a** showed not only a potent fXa inhibitory activity both in vitro and ex vivo study in animals, but also exerted antithrombotic effects in both animal models and patients undergoing percutaneous coronary intervention.[4a–c] Therefore, we believed that **DX-9065a** could be a promising new anticoagulant to improve the defects of warfarin for patients.

However, to our great disappointment, the human oral bioavailability of **DX-9065a** was found to be significantly low ($F = 2$–3%) in clinical trials, owing to its strong basic amidino groups.[4d] Therefore, **DX-9065a** could be used only as an injectable formulation in further clinical studies. After **DX-9065a** was disclosed, a number of fXa inhibitors which had amidino groups were developed in clinical trials. Nevertheless, none of these compounds seem to be under development at this time, indicating that the amidino group must be responsible for their low oral bioavailability.

* Corresponding author. Tel.: +81 3 3492 3131.
E-mail address: nagata.tsutomu.ni@daiichisankyo.co.jp (T. Nagata).

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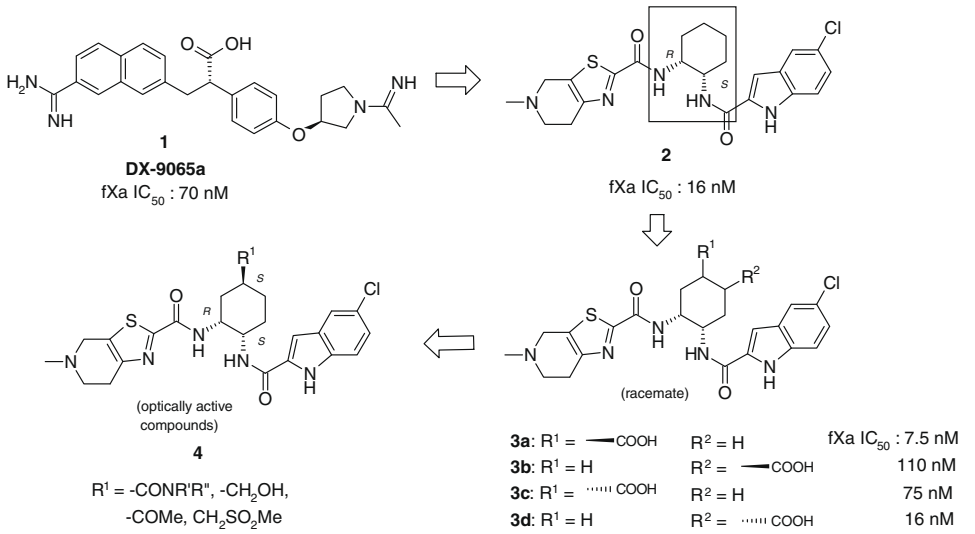


Figure 1. Structure of our factor Xa inhibitors.

To improve its poor oral bioavailability, we have synthesized various non-amidino derivatives that were expected to have anticoagulant activity since the discovery of **DX-9065a**. Recently, we reported that *cis*-1,2-diaminocyclohexane derivative (−)-**2**[5] has potent inhibitory activity for blood coagulation factor Xa (Fig. 1). However, compound (−)-**2** showed poor oral bioavailability ($F = 6.1\%$) in monkeys. The reason for such poor bioavailability of (−)-**2** can be explained by its low metabolic stability, evaluated by measuring its remaining rate (46%) in a human liver microsome test (HLM test). Thus, to improve the metabolic stability, we introduced a carboxylic group onto the cyclohexane ring of racemic **2** with reduced lipophilicity. We eventually designed acids **3a–d**, with a carboxyl group at 4- or 5-position, since there appeared to be sufficient space for substitution on the basis of the analysis of the X-ray structure of (−)-**2** in fXa (Fig. 2). As we anticipated, acids 3a–d were found to be more stable compounds for HLM testing and we identified **3a** as the most potent compound with fXa IC_{50} of 7.5 nM.[6] However, the low lipophilicity of compound **3a** also resulted in low oral activity, possibly due to low permeability. Therefore, to obtain more potent fXa inhibitors with improved oral bioavailability, we still need to balance the lipophilicity of **3a** in terms of both metabolic stability and permeability. In this paper, we focused on the replacement of the carboxylic group of compound **3a** with another polar functional group containing nitrogen and/or oxygen atoms. We will discuss the influence of the third substituent on the cyclohexane ring, as exemplified by compound **4**, for anti-fXa activity, anticoagulant activity, PK profile, and lipophilicity.

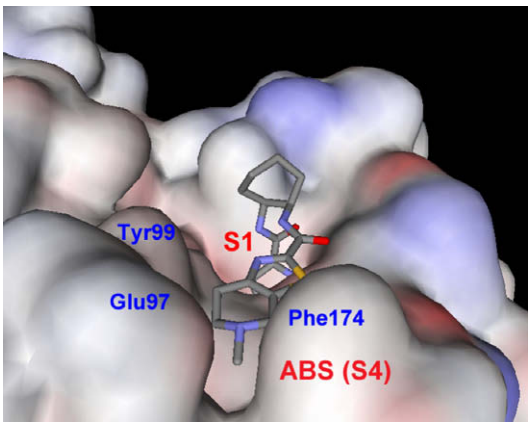


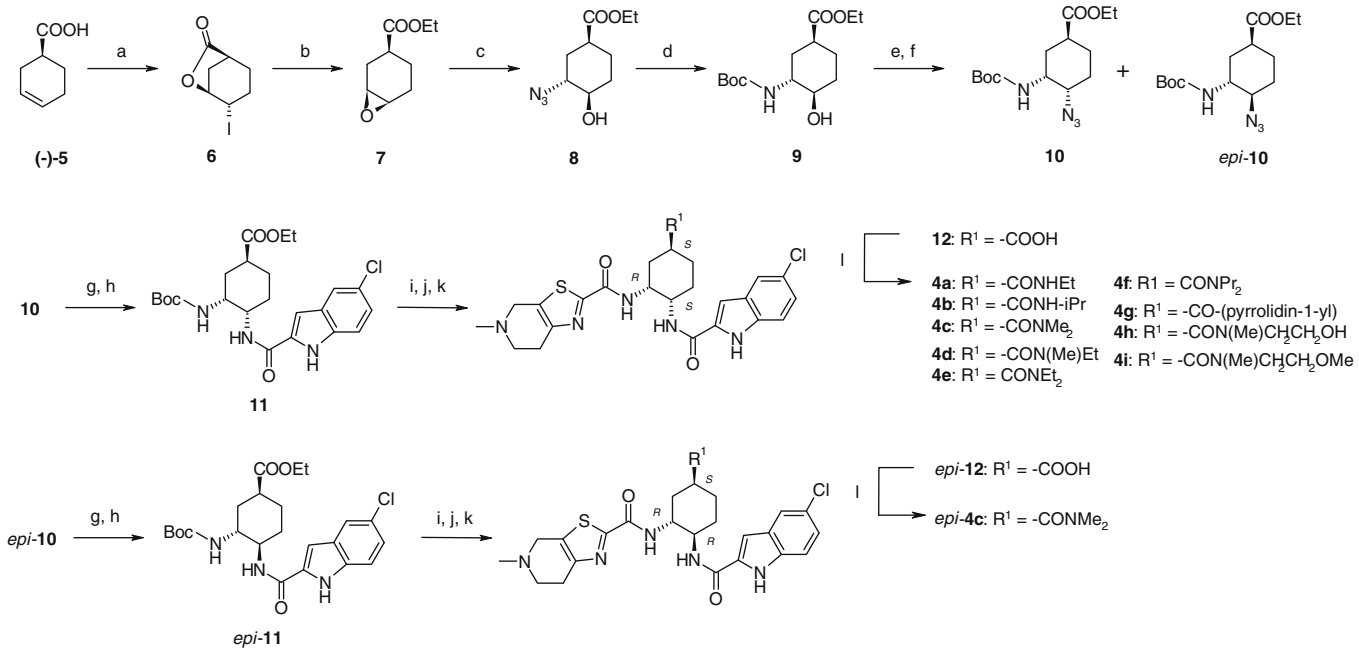
Figure 2. X-ray structure of **2** in fXa.

2. Chemistry

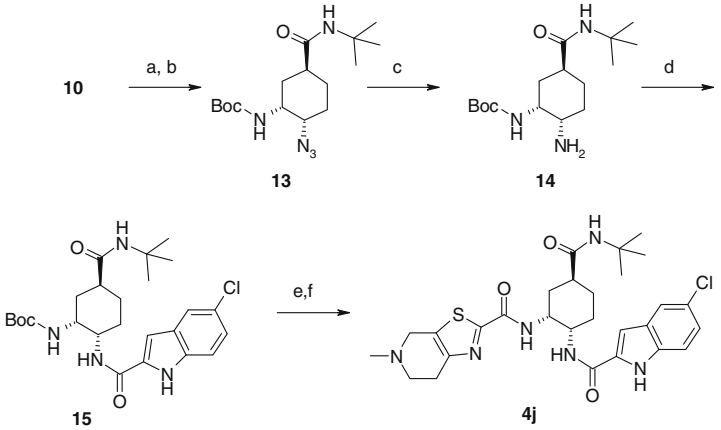
We have already reported a regio- and stereoselective synthesis for preparing racemic 3,4-diamonocyclohexane carboxylic acid derivatives **3** in detail.[6] Following this method, the optically active compound **4** was readily synthesized from (−)-3-cyclohexenecarboxylic acid ((−)-**5**)[7] as described in Scheme 1. Iodolactonization with I_2–KI of acid afforded (−)-4-iodo-6-oxabicyclo[3.2.1]octan-7-one ((−)-**6**).[8] Conversion of lactone **6** to the *cis*-epoxide (−)-**7** was accomplished by ethanolysis and subsequent oxirane ring formation in one pot under an alkali condition. *cis*-Epoxide **7** was treated with sodium azide in the presence of ammonium chloride to afford azide **8** as a single isomer. Catalytic hydrogenation of **8** in the presence of Boc_2O gave alcohol **9** in a quantitative yield. Alcohol **9** was converted into mesylate, followed by treatment of sodium azide to afford the separable mixture of the desired key intermediate **10** and its epimer *epi*-**10**. The azide group of compound **10** was reduced by H_2/Pd–C, followed by acylation with commercially available 5-chloroindole-2-carboxylic acid to give **11**. Removal of the Boc group under acidic condition followed by acylation with thiazolopyridinecarboxylic acid provided ester. Finally, hydrolysis of the ester group followed by condensation with various amimes gave target compounds **4a–i**. Additionally, compound *epi*-**4c** was prepared from by-product *epi*-**10** in six steps by a similar method.

tert-Butyl amide **4j** could not be prepared by condensation with acid **12** and *tert*-butylamine because of less activity of the hindered amine. Thus, alternatively, hydrolysis of intermediate **10** followed by treatment of oxalylchloride/DMF gave acid chloride, which was coupled with *tert*-butylamine to afford amide **13** (Scheme 2). Amide **13** was readily converted to **4j** in three steps by a procedure similar to that described above.

Alcohol **4k**, sulfoxide **4l** and ketone **4m** were prepared according to Scheme 3. Conversion of ester **11** to alcohol **16** was accomplished by reduction employing excess DIBAL-H. Deprotection of **16** followed by acylation gave **4k**. Alcohol **16** was converted into mesylate, followed by treatment of sodium thiomethoxide to afford **17**. Oxidation of **17** followed by deprotection and acylation afforded sulfoxide **4l**. Weinreb amide **19** obtained from acid **12** was treated with methyl lithium to yield ketone **4m**.



Scheme 1. Reagents and conditions: (a) KI, I_2, $NaHCO_3$; (b) 1.2 equiv 2 N-NaOH, EtOH; (c) 1.5 equiv NaN_3, 1.5 equiv NH_4Cl, DMF; (d) H_2, Pd–C, Boc_2O, EtOAc; (e) MsCl, Et_3N CH_2Cl_2; (f) NaN_3, DMF; (g) H_2, Pd–C, EtOH/EtOAc; (h) 5-chloroindole-2-carboxylic acid, WSCI, HOBt, Et_3N, CH_2Cl_2; (i) saturated HCl/EtOH, EtOH; (j) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt, WSCI, HOBt, DMF; (k) 1 N-NaOH, EtOH/THF; (l) amines, WSCI, HOBt, DIPEA or Et_3N, DMF.



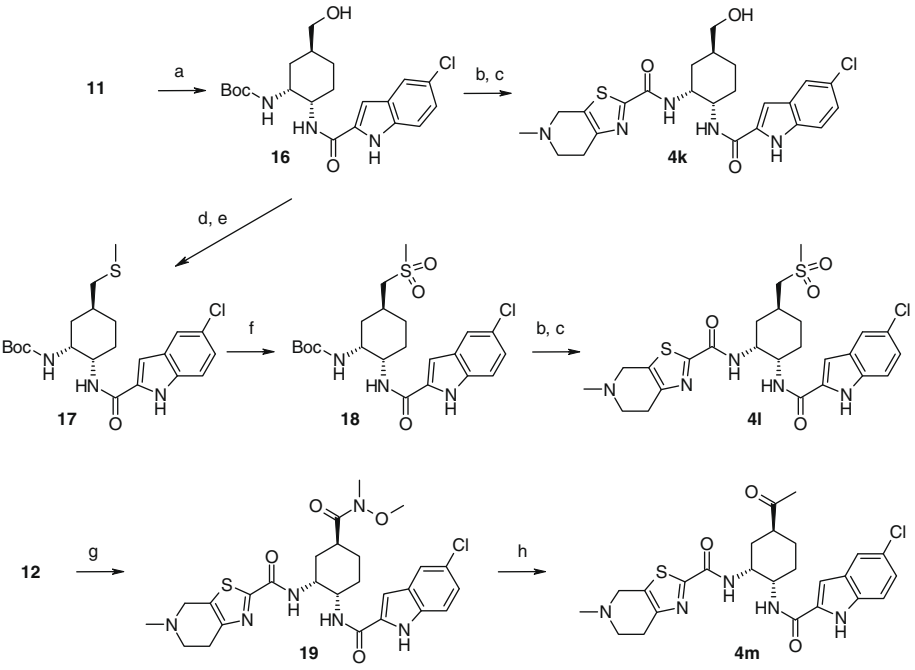
Scheme 2. Reagents and conditions: (a) LiOH, THF, H_2O; (b) *tert*-$BuNH_2$, $(COCl)_2$, CH_2Cl_2, DMF; (c) H_2, Pd–C, MeOH; (d) 5-chloroindole-2-carboxylic acid, WSCI, HOBt, DIPEA, DMF; (e) saturated HCl/MeOH, MeOH; (f) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt, WSCI, HOBt, DIPEA, DMF.

3. Results and discussion

The synthesized compounds were evaluated for in vitro anti-fXa activity (IC_{50}) and anticoagulant activity (PTCT2: the concentration required to double the prothrombin time). The ratio of fXa IC_{50} to PTCT2 as an index was calculated by [PTCT2 (μM)]/[IC_{50} (μM)]. Lipophilicity was measured with octanol to the Japanese Pharmacopoeia Second Fluid distribution coefficient (Log *D* @ pH 6.8). The oral activity of the selected compounds was evaluated by AUC (area under curve) when the compounds were dosed at 1.0 mg/kg po ($N = 3$). These results are summarized in Table 1.

Introduction of a polar functional group on the central cyclohexane ring resulted in an enhancement of anti-Xa activity compared with unsubstituted compound **2**. Almost all the compounds in this series exhibited potent anti-fXa activity with IC_{50} of 2.2–12 nM, suggesting that any polar functional groups were well tolerated as a substituent on the cyclohexane ring. While amides **4c**, **4f**, and **4i** were the most potent compounds, with IC_{50} of 2.2–2.3 nM, the replacement of amide to sulfone decreased the potency (**4l**, IC_{50}: 12 nM).

In general, anticoagulant activity (PTCT2) was correlated with fXa IC_{50}. However, some compounds with more potent anti-fXa activity showed less anticoagulant activity, possibly due to its core structure or functional groups. For instance, acid **3a** (IC_{50}: 7.5 nM, PTCT2: 2.8 μM) was 9-fold more potent for anti-fXa activity but 6-fold less active for anticoagulant activity than **DX-9065a** (IC_{50}: 70 nM, PTCT2: 0.5 μM). Therefore, we adopted PTCT2/IC_{50} ratio as an index to clarify this phenomenon. Comparing both the ratio and the structure of these two compounds, acid **3a** (PTCT2/IC_{50}: 373-fold) and **DX-9065a** (PTCT2/IC_{50}: 7-fold), we considered that **DX-9065a** prolonged the blood clot formation more efficiently than acid **3a** in nature probably due to its amidino groups. Thus, for years we had thought that it would be quite difficult to enhance the anticoagulant activity without amidino groups. However, to our surprise, this series of *cis*-1,2-diaminocyclohexane derivatives without any amidino groups were found to have the PTCT2 values



Scheme 3. Reagents and conditions: (a) DIBAL-H, CH_2Cl_2; (b) saturated HCl/EtOH or MeOH; (c) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt, WSCI, HOBt, DIPEA, DMF; (d) MsCl, Et_3N, CH_2Cl_2; (e) NaSMe, DMF; (f) mCPBA, CH_2Cl_2; (g) MeNHOMe/HCl, WSCI, HOBt, *N*-methylmorpholine, DMF; (h) MeLi, THF.

Table 1
Substitution on cyclohexane ring

Compound	R^1	Anti-fXa IC_{50} (nM)	PTCT2 in human plasma (μM)	PTCT2/fXa IC_{50}	AUC[a] (ng*h/mL)	Log *D*[b] @ pH 6.8
DX-9065a	—	70	0.5	7	NT	NT
2	–H	16	2.9	181	170 (3 mg/kg)	>3.6
3a	—COOH	7.5	2.8	373	NT	NT
4a	–CONHEt	3.5	0.50	143	312	3.2
4b	–CONH-*i*Pr	3.4	0.60	176	315[c]	3.6
4j	–CONH-*tert*-Bu	4.5	1.1	244	7[c]	NT
4c	$-CONMe_2$	2.3	0.33	143	985	2.8
4d	–CON(Me)Et	2.6	0.40	154	356[c]	3.2
4e	$-CONEt_2$	2.9	0.50	172	3	3.4
4f	$-CONPr_2$	2.3	1.3	565	NT	3.6
4g	–CO–(pyrrolidin-1-yl)	3.5	0.50	143	209[c]	3.2
4h	$-CON(Me)CH_2CH_2OH$	2.5	0.30	120	0	2.3
4i	$-CON(Me)CH_2CH_2OMe$	2.2	0.30	136	23	2.8
4k	$-CH_2OH$	4.2	0.49	117	NT	2.8
4m	–COMe	5.4	0.41	76	407[c]	2.4
4l	$-CH_2SO_2Me$	12	0.54	45	535[c]	2.5

NT indicates 'not tested'.
[a] Discrete dosing, monkey, 1 mg/kg, oral.
[b] *n*-Octanol to the Japanese Pharmacopoeia Second Fluid (pH 6.8) distribution coefficient.
[c] Cassette dosing, monkey, 1 mg/kg, oral.

of submicromolar, more potent or equipotent anticoagulant activity compared with **DX-9065a**. In particular, amide **4c**, **4h**, and **4i** with PTCT2 of 0.30–0.33 μM were the most potent anticoagulants, 1.5-fold more active than **DX-9065a**. We speculate that this achievement may be attributed to both the enhanced anti-Xa activity and reduced PTCT2/IC_{50} ratio compared with acid **3a**.

With respect to oral activity, several compounds of this series (administrated at 1.0 mg/kg) exhibited higher AUC values compared with unsubstituted compound **2** (administrated at 3.0 mg/kg), indicating that the introduction of a polar functional group increased the oral activity in monkeys. Among them, amide **4c** showed the highest AUC in monkeys.

It is likely that the PTCT2/IC_{50} ratio is correlated with the Log *D* values in this series of the compounds, suggesting lipophilicity can influence anticoagulant activity. This tendency can be confirmed by the fact that sulfone **4l** with relatively lower lipophilicity (Log *D*: 2.5) had the lowest PTCT2/IC_{50} ratio (45-fold) and amide **4f** with higher lipophilicity (Log *D*: 3.6) had the highest ratio (565-fold).

On the other hand, it is of great interest that stereoisomer *epi*-**4c**, readily prepared from by-product *epi*-**10**, showed the same potency as **4c** in anti-fXa activity, but 3-fold less anticoagulant activity with the PTCT2 of 0.83 μM (Table 2). Namely, *epi*-**4c** has threefold higher PTCT2/IC_{50} ratio than **4c**. To elucidate the different PTCT2/IC_{50} ratios of these two compounds, which are only stereoisomers, we measured their protein binding (%) to human albumin. The result was that they had unexpectedly different protein binding (**4c**: 72%, *epi*-**4c**: 91%), demonstrating that compound **4c** is likely to have favorable stereochemistry for reducing protein binding. Considering these results, we reasoned that the nature of higher protein binding of the compound could prevent its anticoagulant

activity, and hence the PTCT2/IC_{50} ratio was increased. As anticoagulants need to work in the blood where a lot of proteins exist, it is natural that their anticoagulant activity can be influenced by the value of in vitro protein binding.[9] Furthermore, as protein binding is generally correlated with lipophilicity within the same template, PTCT2/IC_{50} ratio would be correlated well with Log *D* values.

Dimethylamide **4c** was selected and further evaluated as the most promising compound in in vitro anti-fXa activity, anticoagulant activity, and oral activity in monkeys. The selectivity, anticoagulant activities, and pharmacokinetic data for **4c** are shown in Tables 3–5.

The *Ki* values of **4c** were measured for fXa and other serine proteases (Table 3). Compound **4c** exhibited *Ki* for human fXa of 2.85 nM, 14-fold more active compared with **DX-9065a**. Compound **4c** also exhibited a similar potency for monkeys (*Ki*: 3.04 nM) and rabbits (*Ki*: 1.74 nM), but reduced potency for rats (*Ki*: 8.77 nM). Focusing on selectivity, compound **4c** was found to be a weak inhibitor of thrombin, chymotrypsin, and rt-PA with *Ki* values of 2.54 μM, 67.6 μM and 68.2 μM. respectively, whereas compound **4c** exhibited excellent selectivity against the other serine proteases, trypsin, plasmin, rfVIIa/sTF (>100 μM).

Table 2
Anticoagulant activity and protein binding

Compound	Anti-fXa IC_{50} (nM)	PTCT2 in human plasma (μM)	PTCT2/fXa IC_{50}	Protein binding (%)
4c	2.3	0.33	143	72
epi-**4c**	1.8	0.83	461	91

Table 3
Ki of **4c** for serine proteases

Serin protease	*Ki* (μM)	
	4c	**DX-9065a**
fXa (human)	0.00285	0.041
fXa (rat)	0.00877	ND
fXa (monkey)	0.00304	ND
fXa (rabbit)	0.00174	ND
Thrombim (human)	2.54	>2000
Trypsin (human)	>100	0.62
Chymotrypsin (human)	67.6	>2000
Plasmin (human)	>100	23
rt-PA (human)	68.2	21
rfVIIa/sTF (human)	>100	ND

ND; not determined.

Table 4
Anticoagulant activity of **4c**

Clotting time	Species	CT2 (μM)	
		4c	**DX-9065a**
PT	Human	0.338	0.52
	Rat	0.717	22.2
	Monkey	0.221	ND
	Rabbit	0.300	ND
APTT	Human	0.593	0.97
TT	Human	3.16	ND

ND; not determined.

Table 5
Pharmacokinetic profile (monkey)

Compound	Cl (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	*F* (%)	HLM[c] (%)
2[a]	17.7	2.22	1.4	6.1	46
4c[b]	12.4	1.49	1.5	68	74

[a] 3 mg/kg, oral and iv.
[b] 1 mg/kg, oral and iv.
[c] The remaining rate of the compound after 5 min of incubation with human liver microsomes.

The anticoagulant activity of **4c** was evaluated by measuring the prolongation of prothrombin time (PT, 4 species as in *Ki* measurements), activated partial thromboplastin time (aPTT, human), and thrombin time (TT, human). The concentration of **4c** required to double the clotting time is shown in Table 4. Compound **4c** doubled PT at the concentration of 0.338 μM, exhibiting 1.5-fold more active than **DX-9065a** (PTCT2: 0.52 μM). Prolongation effects of **4c** for PT were similar in humans, monkeys, and rabbits, while reduced activity was observed in rats, reflecting a relatively weak *Ki* value for rat fXa compared with the other species. Compound **4c** also doubled aPTT and TT at the concentrations of 0.593 μM and 3.16 μM, respectively.

The pharmacokinetic profile of **4c** was determined in monkeys by dosing at 1 mg/kg iv and po. The results of a comparison with unsubstituted compound **2** (dosed at 3 mg/kg, iv and po) are depicted in Table 5. While the $t_{1/2}$ (iv) of these two compounds were similar, the lower Cl and V_{dss} of **4c** was observed relative to unsubstituted **2**. Compound **4c** was also found to have a higher remaining ratio in the HLM test, indicating that the metabolic stability of **4c** was improved as a result of the lower lipophilicity. In addition, compound **4c** demonstrated significantly improved oral bioavailability (*F*: 68%) in monkeys compared with **2** (*F*: 6.1%), suggesting that compound **4c** has satisfactory membrane permeability and gut absorption as an orally bioavailable factor Xa inhibitor. The introduction of a dimethylcarbamoyl group, a neutral polar functionality on the cyclohexane ring, enabled the modulation of lipophilicity to achieve the competing goals of good metabolic stability and high permeability.

In our exploratory studies, X-ray analysis of the crystal complex of Gla-less fXa and compound **4c** revealed a binding mode in which the 5-chloroindole and 5-methyltetrahydrothiazolo[5,4-*c*]pyridine moieties served as S1 and S4 binding elements, respectively (Table 6, Figs. 3 and 4). The chlorine substituent of the indole ring occupied a small cavity[10] composed of Tyr228, Val213, and Ala190 in the S1 site of fXa. Intriguingly, this amino acid sequence was not seen in the other related serine proteases, except for fXa and thrombin (Table 7). This might account for the relatively high inhibitory activity of **4c** for thrombin (*Ki*: 2.54 μM) compared to trypsin (>100 μM), chymotrypsin (67.6 μM), plasmin (>100 μM), t-PA (68.2 μM), and factor VIIa (>100 μM). In addition, compound **4c** is expected to have no inhibitory activity for fIXa from this sequence point of view. The NH group of the indole formed a hydrogen bond (2.9 Å) to the carbonyl oxygen of Gly218.[11] The tetrahydrothiazolo[5,4-*c*]pyridine located parallel to the indole ring of Trp215 was sandwiched between the benzene rings of Tyr99 and Phe174 in S4, directing its methyl group into the cation hall.[10] Intramolecular S–O interaction[10] (2.9 Å) was then observed between the sulfur atom in thiazole ring and oxygen atom of the adjacent carbonyl group. A characteristic binding mode for **4c** and fXa was that the cyclohexane core was at an upright position against the surface of fXa in a chair form, directing the dimethylcarbamoyl group outside of fXa. The cyclohexane ring was unlikely to have any interaction with fXa, and served only as a conformationally rigid scaffold. In addition, the indole ring was nearly planar to the neighboring carbonyl group, suggesting that this fixed conformation could contribute to the enhancement of anti-fXa activity. Consequently, the strong binding affinity of **4c** for fXa would be explained by the integration of the rigid conformation of **4c**, mainly the two hydrophobic interactions to the S1 and S4 binding pockets of fXa, and the additional one hydrogen bonding to Gly218.

4. Conclusion

We have been exploring various non-amidino fXa inhibitors with improved oral availability for a decade, employing X-ray analysis to the crystal complex of Gla-less fXa and compounds to enhance anti-fXa activity.[12] In the course of our study, we found that anti-fXa activity was not always well correlated with anticoagulant activity and that the lipophilicity of a compound could influence both anticoagulant activity and oral activity. These included competing profiles for oral fXa inhibitor, high lipophilicity needed for high membrane permeability and low lipophilicity needed for both metabolic stability and anticoagulant activity. In addition, using the PTCT2/IC_{50} ratio we revealed that protein binding was one of the most important factors in nature in determining anticoagulant activity. As a result, we could modulate the lipophilicity of *cis*-1,2-diaminocyclo hexane derivative(−)-**2** with a neutral polar functional group and this finally led to the discovery of non-amidino compound **4c**[13] with well-balanced lipophilicity (Log *D*: 2.8) and favorable stereochemistry for lowering protein binding (71%). Compound **4c** exhibited excellent in vitro anti-fXa activity (IC_{50}: 2.3 nM), potent anticoagulant activity (PTCT2: 0.33 μM) and good oral bioavailability (*F*: 68%), compared with amidino compound **DX-9065a**. We also disclosed the binding mode of **4c** and fXa with high selectivity for fXa against the other related serine proteases. Compound **4c** was anticipated to show significant antithrombotic effects in animal models. The crystalline HCl salt of **4c** was selected as a clinical candidate for further evaluation.

Table 6
Crystal and diffraction data of human factor Xa with **4c**

Crystal parameters	
Space group	$P2_12_12_1$
a (Å)	56.2
b (Å)	72.2
c (Å)	79.3
Resolution (Å)	2.1
R_{sym} (%)	5.4 (30.0)[a]
Completeness (%)	99.6 (99.9)[a]
Number of reflections, redundancy	19353, 3.5
Refinement	
Number of protein atoms (occupancy ≠0)	2239
Average *B* value for protein and ligand atoms (Å^2)	35.0, 40.9
Range of data	25.0–2.1
R value	20.8
R_{free}	25.0
Rmsd from ideality	
Bond length (Å)	0.022
Bond angle (Å)	1.864

[a] Figures in parentheses represent statics in the last shell of data (highest resolution).

5. Experimental

5.1. General

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were determined on a YANACO MP-J3 or a BUCHI B-545 and are uncorrected. Optical rotations were measured with a HORIBA SEPA-300 polarimeter. ^{1}H NMR spectra were recorded on a JEOL JNM-EX-400 spectrometer, and chemical shifts are given in ppm (‰) from tetramethylsilane as the internal standard. FAB mass spectra were recorded on a JEOL JMS-HX110 spectrometer. HR-FAB mass spectra were recorded on a JEOL JMS-700 spectrometer. ESI mass spectra were recorded on a SCIEX API-150EX spectrometer. HR-ESI mass spectra were recorded on a JEOL JMS-T100LP mass spectrometer. Column chromatography was performed with Merck silica gel 60 (particle size 0.060–0.200 or 0.040–0.063). Flash column chromatography was performed with Biotage Si or YAMAZEN Hi-Flash packed columns. Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with silica gel 60 F254.

5.2. (1*S*)-3-Cyclohexene-1-carboxylic acid (5)

(*R*)-(+)-α-Methylbenzylamine salt of (1*S*)-3-cyclohexene-1-carboxylic acid[7] (95.0 g, 384 mmol) was dissolved in ethyl acetate (1.6 L) and 2 N HCl (1.6 L). After the organic layer was separated, the aqueous layer was extracted with ethyl acetate (2 × 500 mL). The organic layers were combined, and the combined organic layer

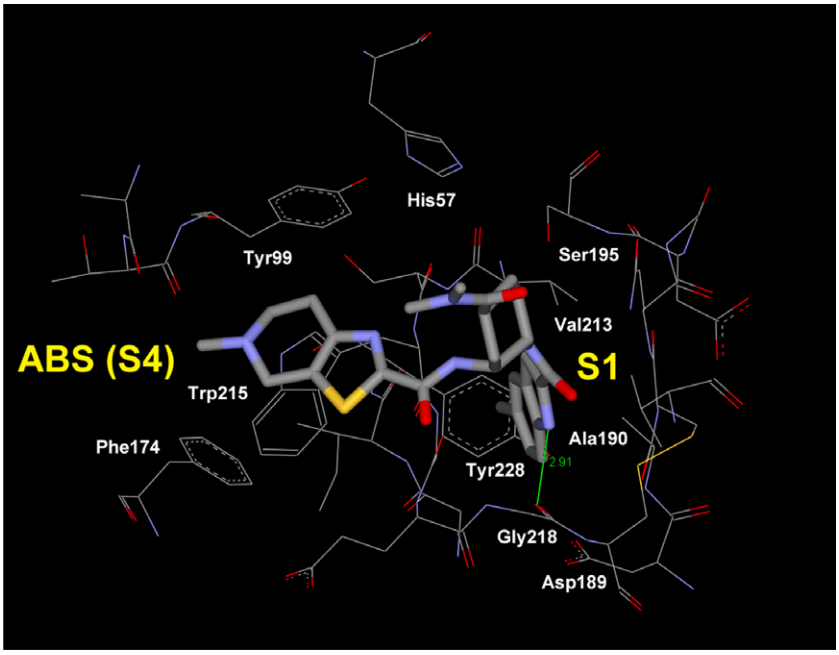


Figure 3. X-ray structure of **4c** in fXa.

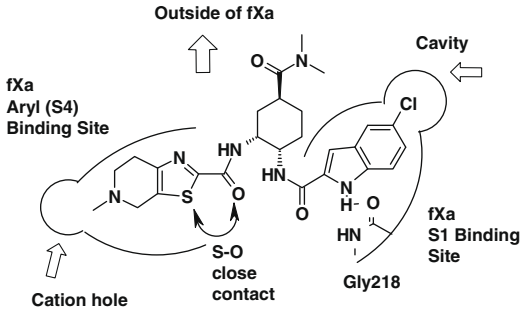


Figure 4. Binding mode of **4c** and fXa.

Table 7
Amino acid sequences in serine proteases

	190	213	228
Factor Xa	***A***	***V***	***Y***
Thrombin	***A***	***V***	***Y***
Trypsin	*S*	***V***	***Y***
Chymotrypsin	*S*	***V***	***Y***
Plasmin	*S*	*T*	***Y***
t-PA	***A***	*I*	***Y***
Factor VIIa	*S*	***V***	***Y***
Factor IXa	*S*	*I*	***Y***

ND; not determined.

was washed with saturated brine (2 × 300 mL). After the organic layer was separated, the aqueous layer was extracted with ethyl acetate (200 mL), and the organic layer was washed with saturated brine (100 mL). All the organic layers were combined, and the combined organic layer was dried over sodium sulfate anhydrate, followed by concentration under reduced pressure, to thereby give **5** (48.3 g, 99%) as an oil.

$[\alpha]_D^{25}$ = −104 (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.66–1.77 (1H, m), 2.00–2.20 (3H, m), 2.20–2.38 (2H, m), 2.57–2.65 (1H, m), 5.65–5.75 (2H, m).

5.3. (1*S*,4*S*,5*S*)-4-Iodo-6-oxabicyclo[3.2.1]octan-7-one (6)

To a mixture of compound **5** (48.0 g, 380 mmol), methylene chloride (580 mL), potassium iodide (82.1 g), sodium hydrogencarbonate (42.0 g), and water (530 mL) was added iodine (125.4 g, 494 mmol) at an internal temperature of 5 °C, followed by stirring at room temperature for 3 h. To the reaction mixture was added 1 N aqueous sodium thiosulfate (800 mL), and the thus-obtained mixture was extracted with methylene chloride (1 L, 500 mL). The organic layer was washed with aqueous sodium hydrogencarbonate (300 mL), water (500 mL), and saturated brine (300 mL), and was dried over anhydrous magnesium sulfate, followed by concentration. The precipitated crystals were collected by filtration, and were washed with hexane, followed by drying, to thereby give **6** (89.5 g, 93%) as a colorless solid.

Mp 130–131 °C; $[\alpha]_D^{25}$ = −41 (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.78–1.96 (2H, m), 2.12 (1H, dd, *J* = 16.5, 5.2 Hz), 2.35–2.50 (2H, m), 2.65–2.70 (1H, m), 2.80 (1H, d, *J* = 12.2 Hz), 4.45–4.55 (1H, m), 4.77–4.87 (1H, m); MS (ESI) *m/z* 274 $(M+Na)^+$; HRMS (ESI) calcd for $C_7H_9NaO_2$: 274.95449 $[(M+Na)^+]$. Found: 274.95643.

5.4. (1*S*,3*S*,6*R*)-7-Oxabicyclo[4.1.0]heptane-3-carboxylic acid ethyl ester (7)

To a suspension of **6** (89.3 g, 354 mmol) in ethanol (810 mL) was added 2 N aqueous sodium hydroxide (213 mL, 425 mmol) at room temperature while being stirred, and the thus-obtained mixture was stirred for 3 h. The reaction mixture was concentrated in a bath at a temperature of 35 °C under reduced pressure. Water (500 mL) was added to the resultant oily matter, and the thus-obtained mixture was extracted with methylene chloride (500 mL and 300 mL). The organic layer was washed with water (300 mL), and was dried over anhydrous magnesium sulfate, followed by concentration under reduced pressure. The resultant oily matter was purified by silica gel column chromatography (hexane:ethyl acetate = 85:15), to thereby give **7** (41.3 g, 69%) as a pale brown oil.

$[\alpha]_D^{25}$ = −58 (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.25 (3H, t, *J* = 7.2 Hz), 1.50–1.70 (2H, m), 1.71–1.82 (1H, m), 2.08–2.28 (4H, m), 3.16 (2H, s), 4.12 (2H, q, *J* = 7.2 Hz); MS (ESI) *m/z* 171 $(M+H)^+$; HRMS (ESI) calcd for $C_9H_{15}O_3$: 171.10212 $[(M+H)^+]$. Found: 171.10164.

5.5. (1*S*,3*R*,4*R*)-3-Azido-4-hydroxycyclohexanecarboxylic acid ethyl ester (8)

A mixture of **7** (41.0 g, 241 mmol), *N*,*N*-dimethylformamide (300 mL), ammonium chloride (19.3 g, 362 mmol), and sodium azide (23.5 g, 362 mmol) was stirred at 76 °C for 13 h. After any insoluble matter was collected by filtration, the filtrate was concentrated under reduced pressure while not allowing the solvent to evaporate to dryness. The residue was combined with the solid matter collected by the previous filtration, and the thus-obtained mixture was dissolved in water (500 mL). The solution was extracted with ethyl acetate (500 mL, 300 mL). The extract was washed with water and saturated brine, and was dried over anhydrous magnesium sulfate, followed by concentration, to thereby give **8** (51.5 g, quant.) as an oil.

$[\alpha]_D^{25}$ = +8 (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.28 (3H, t, *J* = 7.1 Hz), 1.37–1.64 (3H, m), 1.86–1.95 (1H, m), 2.04–2.16 (1H, m), 2.32–2.41 (1H, m), 2.44 (1H, br s), 2.68–2.78 (1H, m), 3.45–3.60 (2H, m), 4.17 (2H, q, *J* = 7.1 Hz).

5.6. (1*S*,3*R*,4*R*)-3-[(*tert*-Butoxycarbonyl)amino]-4-hydroxycyclohexanecarboxylic acid ethyl ester (9)

A mixture of **8** (51.2 g, 240 mmol), di-*tert*-butyl dicarbonate (68.1 g, 312 mmol), 5% palladium on carbon (50%wet, 5.0 g), and ethyl acetate (1000 mL) was stirred at room temperature overnight at a hydrogen pressure of 7 kg/cm^2. After the reaction mixture was filtered, the filtrate was concentrated, and the thus-obtained oily matter was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1–3:1). The thus-obtained compound was crystallized from hexane, to thereby give **9** (46.9 g, 68%) as a colorless powder. Furthermore, the mother liquor was purified by silica gel column chromatography (chloroform:methanol = 100:1), to thereby give **9** (6.74 g, 9.8%).

$[\alpha]_D^{25}$ = +25 (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.28 (3H, t, *J* = 7.1 Hz), 1.38–1.57 (3H, m), 1.45 (9H, s), 1.86–1.95 (1H, m), 2.05–2.17 (1H, m), 2.29–2.39 (1H, m), 2.61–2.68 (1H, m), 3.34 (1H, br s), 3.39–3.48 (1H, m), 3.53–3.64 (1H, m), 4.10–4.24 (2H, m), 4.54 (1H, br s); MS (ESI) *m/z* 310 $(M+Na)^+$; HRMS (ESI) calcd for $C_{14}H_{25}NNaO_5$: 310.16304 $[(M+Na)^+]$. Found: 310.16154.

5.7. (1*S*,3*R*,4*S*)-4-Azido-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylic acid ethyl ester (10) and (1*S*,3*R*,4*R*)-4-azido-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylic acid ethyl ester (*epi*-10)

Compound **9** (53.5 g, 186 mmol) and triethylamine (130 mL, 930 mmol) were dissolved in methylene chloride (500 mL), and methanesulfonyl chloride (42 mL, 558 mmol) was added dropwise thereto at −10 to −15 °C over 20 min, followed by stirring at the

same temperature for 20 min, and the resultant mixture was heated to room temperature over 2 h. The reaction mixture was cooled to 0 °C, and 0.5 N HCl (800 mL) was added dropwise thereto, followed by extraction with methylene chloride. The organic layer was washed with saturated aqueous sodium hydrogencarbonate and saturated brine, and was dried over anhydrous magnesium sulfate. The solvent was distilled away under reduced pressure, to thereby give crude (1*S*,3*R*,4*R*)-3-[(*tert*-butoxycarbonyl)amino]-4-[(methylsulfonyl)oxy]cyclohexanecarboxylic acid ethyl ester. The crude product was dissolved in *N*,*N*-dimethylformamide (335 mL), and sodium azide (60.5 g, 930 mmol) was added thereto, followed by stirring at 67–75 °C for 16 h. The reaction mixture was filtered, and the filtrate was concentrated, to thereby evaporate ca. 250 mL of the solvent. The residue was combined with the solid matter collected by the above-described filtration, and the thus-obtained mixture was dissolved in water, followed by extraction with ethyl acetate. The organic layer was washed with saturated brine, and was dried over anhydrous magnesium sulfate. The solvent was distilled away under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate:hexane = 1:4), to thereby give **10** (18.4 g, 32%) as a colorless powder and *epi*-**10** (3.3 g, 5.7%).

Compound **10**: $[\alpha]_D^{25}$ = +62 (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, *J* = 7.1 Hz), 1.35–2.00 (15H, s), 2.60–2.68 (1H, m), 3.80–3.96 (2H, m), 4.15 (2H, q, *J* = 7.1Hz), 4.61 (1H, br s); MS (ESI) *m/z* 335 $(M+Na)^+$; HRMS (ESI) calcd for $C_{14}H_{24}N_4NaO_4$: 335.16952 $[(M+Na)^+]$. Found: 335.16805.

Compound *epi*-**10**: $[\alpha]_D^{25}$ = −19 (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, t, *J* = 7.1 Hz), 1.46 (9H, s), 1.53–2.30 (6H, m), 2.50–2.65 (1H, m), 3.42–3.72 (2H, m), 4.15 (2H, q, *J* = 7.1 Hz), 4.67 (1H, br s); MS (ESI) *m/z* 335 $(M+Na)^+$; HRMS (ESI) calcd for $C_{14}H_{24}N_4NaO_4$: 335.16952 $[(M+Na)^+]$. Found: 335.16840.

5.8. (1*S*,3*R*,4*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-{[(5-chloroindol-2-yl)carbonyl]amino}cyclohexanecarboxylic acid ethyl ester (11)

Compound **10** (4.0 g, 12.8 mmol) was dissolved in a solvent mixture of ethanol (150 mL) and ethyl acetate (150 mL), and 5% palladium on carbon (0.5 g was added thereto, followed by stirring at room temperature for 17 h under hydrogen atmosphere (5 kg/cm^2). After any insoluble matter was filtered off, the solvent was distilled away under reduced pressure, to thereby give (1*S*,3*R*,4*S*)-4-amino-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylic acid ethyl ester (4.2 g, quant.) as a pale brown oil. The oil was dissolved in methylene chloride (50 mL), and to the solution were added 5-chloroindole-2-carboxylic acid (3.33 g, 17.1 mmol), 1-hydroxybenzotriazole monohydrate (2.52 g, 16.5 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.15 g, 16.5 mmol) at room temperature, followed by stirring for 12 h. To the reaction mixture was added 0.1 N aqueous HCl, and the mixture was extracted with methylene chloride. The organic layer was washed with saturated aqueous sodium hydrogencarbonate and saturated brine, and was dried over anhydrous magnesium sulfate. The solvent was distilled away under reduced pressure, and the residue was purified by silica gel column chromatography (ethyl acetate:hexane = 1:1), to thereby give **11** (4.36 g, 64%) as a colorless powder.

$[\alpha]_D^{25}$ = −27 (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, *J* = 7.1 Hz), 1.43 (9H, s), 1.35–2.46 (7H, m), 3.91–4.02 (1H, m), 4.10–4.22 (2H, m), 4.79 (1H, br s), 6.79 (1H, s), 7.18–7.40 (2H, m), 7.59 (1H, s), 8.00 (1H, br s), 9.13 (1H, br s). MS (ESI) *m/z* 486 $(M+Na)^+$; HRMS (ESI) calcd for $C_{23}H_{30}ClN_3NaO_5$: 486.17717 $[(M+Na)^+]$. Found: 486.17600.

5.9. (1*S*,3*R*,4*S*)-4-{[(5-Chloroindol-2-yl)carbonyl]amino}-3-{[(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridin-2-yl)carbonyl]amino}cyclohexanecarboxylic acid (12)

Compound **11** (4.2 g, 9.05 mmol) was suspended in ethanol (25 mL), and saturated HCl in ethanol (55 mL) was added at room temperature, followed by stirring for 11 h. The solvent was distilled away under reduced pressure to give a colorless solid (4.15 g). This product (4.15 g) was dissolved in *N*,*N*-dimethylformamide (40 mL), and 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt (2.86 g, 14.0 mmol), 1-hydroxybenzotriazole monohydrate (1.72 g, 11.2 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride (2.15 g, 11.2 mmol) were added to this solution at room temperature, followed by stirring for 39 h. The reaction mixture was concentrated under reduced pressure, and water was added to the residue, followed by extraction with chloroform. The resultant organic layer was washed with saturated brine and dried over magnesium sulfate anhydrate. The solvent was distilled away under reduced pressure, and the resultant residue was purified by silica gel column chromatography (chloroform:methanol = 100:1) to give ethyl (1*S*,3*R*,4*S*)-4-{[(5-chloroindol-2-yl)carbonyl]amino}-3-{[(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]amino}cyclohexanecarboxylate (1.71 g, 35%, two steps) as a colorless amorphous substance.

$[\alpha]_D^{25}$ = −94 (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ: 1.29 (3H, t, *J* = 7.1 Hz), 1.52–1.80 (2H, m), 2.03–2.37 (4H, m), 2.53 (3H, s), 2.57–2.71 (1H, m), 3.73 and 3.78 (each 1H, each d, *J* = 14.4 Hz), 4.08–4.17 (1H, m), 4.18 (2H, q, *J* = 7.2 Hz), 4.55–4.65 (1H, m), 6.85 (1H, br s), 7.21 (1H, dd, *J* = 8.8, 2.0 Hz), 7.33 (1H, d, *J* = 8.8 Hz), 7.48 (1H, d, *J* = 7.6 Hz), 7.63 (1H, d, *J* = 2.0 Hz), 7.98 (1H, d, *J* = 7.6 Hz), 9.30 (1H, s).

The ester (1.6 g, 2.94 mmol) was suspended in a mixture of ethanol (20 mL) and tetrahydrofuran (15 mL), and 1 N aqueous sodium hydroxide (5.9 mL) was added at room temperature, followed by stirring for 12 h at the same temperature. After adding 1 N hydrochloric acid (5.9 mL), the solvent was distilled away under reduced pressure, and the residue was washed with water and diethyl ether to give compound **12** (1.19 g, 78%) as a colorless solid.

Mp 234–236 °C; $[\alpha]_D^{25}$ = −57 (*c* 1.0, methanol); ^{1}H NMR (DMSO-d_6) δ: 1.55–1.85 (3H, m), 1.85–2.05 (2H, m), 2.15–2.25 (1H, m), 2.40 (3H, s), 2.55–2.65 (1H, m), 2.75–2.85 (2H, m), 2.85–2.95 (2H, m), 3.65–3.75 (2H, m), 4.15–4.25 (1H, m), 4.40–4.50 (1H, m), 7.07 (1H, d, *J* = 1.7 Hz), 7.17 (1H, dd, *J* = 8.5, 1.7 Hz), 7.42 (1H, d, *J* = 8.5 Hz), 7.69 (1H, d, *J* = 1.7 Hz), 8.22 (1H, d, *J* = 8.1 Hz), 8.33 (1H, d, *J* = 7.6 Hz), 11.77 (1H, s).

5.10. *N*-[(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(ethylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4a)

Compound **12** (150 mg, 0.291 mmol) was dissolved in *N*,*N*-dimethylformamide (3 mL), and then *N*-ethylamine hydrochloride (119 mg, 1.46 mmol), 1-hydroxybenzotriazole monohydrate (79 mg, 0.516 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (112 mg, 0.582 mmol) and triethylamine (326 μL, 2.35 mmol) were added, and the mixture was stirred at room temperature for four days. The solvent was distilled away under reduced pressure, and saturated aqueous sodium hydrogencarbonate was added to the residue, followed by extraction with methylene chloride. The resultant organic layer was dried over sodium sulfate anhydrate. The solvent was distilled away under reduced pressure, and the resultant residue was purified by silica gel column chromatography (methylene chloride:methanol = 47:3). The thus-obtained solid was dissolved in methylene

chloride, and then 1 N HCl in ethanol (171 μL) was added. The solvent was distilled away under reduced pressure, and methanol and diethyl ether were added to the residue to collect the precipitate formed by filtration to give **4a** (74 mg, 41%) as a colorless powder.

^{1}H NMR (DMSO-d_6) δ: 0.99 (3H, t, J = 7.2 Hz), 1.57–2.02 (6H, m), 2.33–2.38 (1H, m), 2.92 (3H, s), 3.01–3.08 (2H, m), 3.17–3.20 (2H, s), 3.45–3.70 (2H, m), 4.10–4.17 (1H, m), 4.40–4.69 (3H, m), 7.04 (1H, d, J = 2.0 Hz), 7.17 (1H, dd, J = 8.8, 2.0 Hz), 7.41 (1H, d, J = 8.8 Hz), 7.69 (1H, d, J = 2.0 Hz), 7.78–7.81 (1H, m), 8.08–8.12 (1H, m), 8.40 (1H, d, J = 8.1 Hz), 11.23 (1H, br s), 11.79 (1H, br s); MS (FAB) m/z 543 [(M+H)$^+$, ^{35}Cl], 545 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{26}H_{31}ClN_6O_3S{\cdot}HCl{\cdot}2.2H_2O$: C, 50.44; H, 5.93; Cl, 11.45; N, 13.57; S, 5.28. Found: C, 50.29; H, 5.89; Cl, 11.60; N, 13.56; S, 5.29.

5.11. *N*-[(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(isopropylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4b)

Compound **4b** was synthesized from **12** and isopropylamine according the same procedure described for **4a**, colorless powder (125 mg, 77%).

^{1}H NMR (DMSO-d_6) δ: 1.02 (6H, dd, J = 6.5, 2.5 Hz), 1.50–2.10 (6H, m), 2.30 (1H, t, J = 12.0 Hz), 2.91 (3H, s), 3.10–3.75 (4H, m), 3.75–3.90 (1H, m), 4.07–4.20 (1H, m), 4.30–4.57 (2H, br s), 4.57–4.83 (1H, br s), 7.03 (1H, d, J = 1.5 Hz), 7.16 (1H, dd, J = 8.8, 2.1 Hz), 7.41 (1H, d, J = 8.8 Hz), 7.60–7.75 (2H, m), 8.05 (1H, br s), 8.43 (1H, br d, J = 7.8 Hz), 11.63 (1H, br s), 11.79 (1H, s); MS (FAB) m/z 557 [(M+H)$^+$, ^{35}Cl], 559 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{27}H_{33}ClN_6O_3S{\cdot}HCl{\cdot}2.5H_2O$: C, 50.78; H, 6.16; Cl, 11.10; N, 13.16; S, 5.02. Found: C, 50.78; H, 6.03; Cl, 11.24; N, 13.06; S, 5.21.

5.12. *N*-[(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(dimethylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4c)

Compound **12** (900 mg, 1.74 mmol) was dissolved in *N*,*N*-dimethylformamide (50 mL), and then dimethylamine hydrochloride (304 mg, 3.73 mmol), 1-hydroxybenzotriazole monohydrate (262 mg, 1.71 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (369 mg, 1.93 mmol) and diisopropylethylamine (1.83 mL, 10.5 mmol) were added, and the mixture was stirred at room temperature for 12 h. The solvent was distilled away under reduced pressure, and saturated aqueous sodium hydrogencarbonate was added to the residue, followed by extraction with methylene chloride. The resultant organic layer was dried over sodium sulfate anhydrate. The solvent was distilled away under reduced pressure, and the resultant residue was purified by silica gel column chromatography (methylene chloride:methanol = 47:3). The thus-obtained white solids were dissolved in methylene chloride, and then 1 N HCl in ethanol (1.49 mL) was added. The solvent was distilled away under reduced pressure, and methanol and diethyl ether were added to the residue to collect the precipitate formed by filtration to give **4c** (777 mg, 74%) as a white solid.

Mp 248–254 °C (dec); $[\alpha]_D^{18}$ = −53.9 (c 0.505, methanol); enantiomeric excess: >99% (column: CHIRALPAK AD-H, 0.46 × 25 cm, flow: 3.0 mL/min, eluent: hexane/isopropropanol/diethylamine = 65/35/0.5); ^{1}H NMR (DMSO-d_6) δ: 1.45–1.60 (1H, m), 1.70–1.85 (3H, m), 1.90–2.05 (2H, m), 2.80 (3H, s), 2.91 (3H, s), 2.95–3.10 (1H, m), 2.97 (3H, s), 3.10–3.75 (4H, m), 4.05–4.15 (1H, m), 4.35–4.75 (3H, m), 7.05 (1H, s), 7.16 (1H, dd, J = 8.7, 2.1 Hz), 7.41 (1H, d, J = 8.6 Hz), 7.67 (1H, s), 8.30–8.45 (2H, m), 11.63 (1H, br), 11.78 (1H, s); MS (FAB) m/z 543 [(M+H)$^+$, ^{35}Cl], 545 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{26}H_{31}ClN_6O_3S{\cdot}HCl{\cdot}1.5H_2O$: C, 51.48; H, 5.82; Cl, 11.69; N, 13.85; S, 5.29. Found: C, 51.37; H, 5.89; Cl, 12.11; N, 13.77; S, 5.45.

5.13. *N*-{(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-[ethyl(methyl)carbamoyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4d)

Compound **4d** was synthesized from **12** and ethylmethylamine according to the same procedure described for **4a**, as a pale yellow solid (85 mg, 52%).

^{1}H NMR (DMSO-d_6) δ: 0.93–1.13 (3H, m), 1.40–1.64 (1H, m), 1.64–1.88 (3H, m), 1.88–2.10 (2H, m), 2.76 (1/2 of 3H, s), 2.90 (3H, s), 2.93 (1/2 of 3H, s), 3.10–3.80 (7H, m), 4.05–4.17 (1H, m), 4.30–4.85 (3H, m), 7.04 (1H, s), 7.15 (1H, dd, J = 8.8, 1.7 Hz), 7.40 (1H, d, J = 8.8 Hz), 7.67 (1H, s), 8.30–8.50 (2H, m), 11.29 (1H, br s), 11.77 (1H, s); MS (FAB) m/z 557 [(M+H)$^+$, ^{35}Cl], 559 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{27}H_{33}ClN_6O_3S{\cdot}HCl{\cdot}1.5\ H_2O$: C, 52.26; H, 6.01; Cl, 11.43; N, 13.54; S, 5.17. Found: C, 52.27; H, 5.85; Cl, 11.70; N, 13.41; S, 5.33.

5.14. *N*-[(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(diethylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4e)

Compound **4e** was synthesized from **12** and diethylamine according to the same procedure described for **4a**, as a colorless solid (125 mg, 50%).

^{1}H NMR (DMSO-d_6) δ: 0.99, 1.05 (6H, each t, J = 7.1 Hz), 1.53–1.61 (1H, m), 1.74–1.80 (3H, m), 1.96–2.05 (2H, m), 2.88–2.95 (4H, m), 3.17–3.67 (8H, m), 4.11–4.16 (1H, m), 4.45 (1H, br s), 4.55–4.58 (1H, m), 4.66 (1H, br s), 7.06 (1H, d, J = 2.0 Hz), 7.16 (1H, dd, J = 8.9, 1.9 Hz), 7.42 (1H, d, J = 8.9 Hz), 7.69 (1H, d, J = 1.9 Hz), 8.41 (2H, d, J = 7.8 Hz), 11.65 (1H, br s), 11.81 (1H, br s); MS (FAB) m/z 571 [(M+H)$^+$, ^{35}Cl], 573 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{28}H_{35}ClN_6O_3S{\cdot}HCl{\cdot}H_2O$: C, 53.76; H, 6.12; Cl, 11.33; N, 13.43; S, 5.13. Found: C, 53.45; H, 5.99; Cl, 11.59; N, 13.36; S, 5.29.

5.15. *N*-[(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(dipropylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4f)

Compound **4f** was synthesized from **12** and dipropylamine according to the same procedure described for **4a**, as a colorless solid (78 mg, 30%).

^{1}H NMR (DMSO-d_6) δ: 0.69 (3H, t, J = 7.3 Hz), 0.79 (3H, t, J = 7.3 Hz), 1.38–1.47 (4H, m), 1.57–1.78 (4H, m), 1.98–2.01 (2H, m), 2.80 (1H, t, J = 11.5 Hz), 2.91 (3H, s), 3.01–3.39 (6H, m), 3.48 (1H, br s), 3.68 (1H, br s), 4.13–4.16 (1H, m), 4.43 (1H, br s), 4.48–4.50 (1H, m), 4.68 (1H, br s), 7.04 (1H, d, J = 2.0 Hz), 7.16 (1H, dd, J = 8.8, 2.2 Hz), 7.41 (1H, d, J = 8.8 Hz), 7.70 (1H, d, J = 2.2 Hz), 8.33 (1H, d, J = 7.6 Hz), 8.41 (1H, d, J = 8.0 Hz), 11.27–11.40 (1H, m), 11.80 (1H, br s); MS (FAB) m/z 599 [(M+H)$^+$, ^{35}Cl], 601 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{39}H_{39}ClN_6O_3S{\cdot}HCl{\cdot}1.5H_2O$: C, 54.37; H, 6.54; Cl, 10.70; N, 12.68; S, 4.84. Found: C, 54.21; H, 6.44; Cl, 10.88; N, 12.59; S, 4.97.

5.16. *N*-[(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(pyrrolidin-1-ylcarbonyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4g)

Compound **4g** was synthesized from **12** and pyrrolidine according to the procedure described for **4a**, as a pale yellow solid (67 mg, 40%).

^{1}H NMR (DMSO-d_6) δ: 1.45–2.10 (10H, m), 2.75–2.90 (2H, m), 2.90 (3H, s), 3.10–3.70 (7H, m), 4.05–4.20 (1H, m), 4.25–4.80 (3H, m), 7.05 (1H, s), 7.17 (1H, d, J = 8.7 Hz), 7.41 (1H, d, J = 8.7 Hz), 7.69 (1H, s), 8.32 (1H, br d, J = 7.6 Hz), 8.38 (1H, br d, J = 7.1 Hz), 11.22 (1H, br s), 11.78 (1H, s); MS (FAB) m/z 569 [(M+H)$^+$, ^{35}Cl], 571 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{28}H_{33}ClN_6O_3S{\cdot}HCl{\cdot}2.5H_2O$: C, 51.69; H, 6.04; Cl, 10.90; N, 12.90; S, 4.93. Found: C, 51.65; H, 6.01; Cl, 11.09; N, 12.85; S, 5.03.

5.17. *N*-{(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-[(2-hydroxyethyl)(methyl)carbamoyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4h)

Compound **4h** was synthesized from **12** and *N*-methylethanolamine according to the procedure describe for **4a**, as a colorless solid (42 mg, 23%).

^{1}H NMR (DMSO-d_6) δ: 1.50–1.55 (1H, m), 1.74–1.84 (3H, m), 1.94–1.97 (2H, m), 2.67(1.5H, s), 2.91 (3H, s), 3.02 (1.5H, s), 3.10–3.68 (9H, m), 4.11–4.13 (1H, m), 4.43–4.66 (4H, m), 7.05 (1H, s), 7.16 (1H, dd, J = 8.7, 2.0 Hz), 7.41 (1H, d, J = 8.7 Hz), 7.68 (1H, s), 8.34–8.40 (2H, m), 11.47 (1H, br s), 11.79 (1H, s); MS (FAB) m/z 573 [(M+H)$^+$, ^{35}Cl], 575 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{27}H_{33}ClN_6O_4S{\cdot}HCl{\cdot}H_2O$: C, 51.67; H, 5.78; Cl, 11.30; N, 13.39; S, 5.11. Found: C, 51.62; H, 5.69; Cl, 11.36; N, 13.30; S, 5.23.

5.18. *N*-{(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-[(2-methoxyethyl)(methyl)carbamoyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4i)

Compound **4i** was synthesized from **12** and *N*-methylethanolamine according to the procedure describe for **4a**, as a colorless solid (83 mg, 45%).

^{1}H NMR (DMSO-d_6) δ: 1.50–1.99 (6H, m), 2.80 (1.5H, s), 2.91 (3H, s), 3.01 (1.5H, s), 3.03 (1H, br s), 3.16 (2H, s), 3.23 (3H, s), 3.35–3.67 (6H, m), 4.09–4.16 (1H, m), 4.43–4.67 (3H, m), 7.04–7.06 (1H, m), 7.16 (1H, dd, J = 8.8, 2.0 Hz), 7.42 (1H, d, J = 8.8 Hz), 7.69 (1H, br s), 8.29–8.41 (2H, m), 11.59 (1H, br s), 11.80 (1H, br s); MS (FAB) m/z 587 [(M+H)$^+$, ^{35}Cl], 589 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{28}H_{35}ClN_6O_4S{\cdot}HCl{\cdot}0.7H_2O$: C, 52.86; H, 5.93; Cl, 11.14; N, 13.21; S, 5.04. Found: C, 52.73; H, 5.78; Cl, 11.35; N, 13.20; S, 5.15.

5.19. (1*S*,3*R*,4*R*)-3-[(*tert*-Butoxycarbonyl)amino]-4-{[(5-chloroindol-2-yl)carbonyl]amino}cyclohexanecarboxylic acid ethyl ester (*epi*-11)

Compound *epi*-**11** was synthesized from *epi*-**10** according to the procedure described for **11**, as a colorless solid (4.4 g 53%).

^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.1 Hz), 1.59 (9H, s), 1.40–2.48 (7H, m), 3.91–4.03 (1H, m), 4.08–4.22 (1H, m), 4.14 (2H, q, J = 7.1 Hz), 4.82 (1H, br s), 6.80 (1H, br s), 7.21 (1H, dd, J = 8.8, 2.0 Hz), 7.33 (1H, d, J = 8.8 Hz), 8.02 (1H, br s), 9.32 (1H, br s); MS (ESI) m/z 464 [(M+H)$^+$, ^{35}Cl], 466 [(M+H)$^+$, ^{37}Cl].

5.20. (1*S*,3*R*,4*R*)-4-{[(5-Chloroindol-2-yl)carbonyl]amino}-3-{[(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridin-2yl)carbonyl]amino}cyclohexanecarboxylic acid (*epi*-12)

Compound *epi*-**12** was synthesized from *epi*-**11** according to the procedure described for **12**, as a colorless solid (800 mg 76%, three steps).

Mp >230 °C; ^{1}H NMR (DMSO-d_6) δ: 1.44–2.23 (6H, m), 2.34 (3H, s), 2.59–2.93 (5H, m), 3.53 and 3.62 (each 1H, each d, J = 5.7 Hz), 3.95–2.28 (2H, m), 7.02 (1H, s), 7.12 (1H, d, J = 8.8 Hz), 7.36 (1H, d, J = 8.8 Hz), 8.29 (1H, d, J = 8.8 Hz), 8.40 (1H, d, J = 8.8 Hz), 11.65 (1H, s); HRMS (FAB) m/z calcd for $C_{24}H_{27}{}^{35}ClN_5O_4S$: 516.1472 [(M+H)$^+$, ^{35}Cl]. Found: 516.1457. Calcd for $C_{24}H_{27}{}^{37}ClN_5O_4S$: 518.1452 [(M+H)$^+$, ^{37}Cl]. Found: 518.1454.

5.21. *N*-[(1*R*,2*R*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(dimethylcarbamoyl)cyclohexyl]-5-methyl-4,5,6,7tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (*epi*-4c)

Compound *epi*-**4c** was synthesized from *epi*-**12** according to the procedure described for **4c**, as a colorless powder (310 mg, 80%).

Mp 223–226 °C; ^{1}H NMR (DMSO-d_6) δ: 1.00–2.05 (7H, m), 2.50 (3H, s), 2.81 (3H, s), 2.92–3.65 (9H, m), 3.95–4.10 (1H, m), 4.50–4.68 (1H, m), 7.08 (1H, s), 7.13 (1H, dd, J = 8.8, 2.0 Hz), 7.37 (1H, d, J = 8.8 Hz), 7.66 (1H, br s), 8.31(1H, d, J = 8.4 Hz), 8.50 (1H, d, J = 9.2 Hz), 11.67 (1H, s); MS (ESI) m/z 543 [(M+H)$^+$, ^{35}Cl], 545 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{26}H_{31}ClN_6O_3S{\cdot}HCl{\cdot}1.5H_2O$: C, 51.48; H, 5.82; Cl, 11.69; N, 13.85; S, 5.29. Found: C, 51.17; H, 5.68; Cl, 11.64; N, 13.60; S, 5.41.

5.22. *tert*-Butyl [(1*R*,2*S*,5*S*)-2-azido-5-(tert-butylcarbamoyl) cyclohexyl]carbamate (13)

Compound **10** (509 mg, 1.63 mmol) was dissolved in tetrahydrofuran (40.0 mL), lithium hydroxide (111 mg, 4.64 mmol) and water (5.0 mL) were successively added under ice cooling, and the mixture was stirred at room temperature for 36.5 h. The solvent was distilled off under reduced pressure, water and 1N hydrochloric acid (4.64 mL) were added to the residue, and the solvent was distilled off again under reduced pressure. Dichloromethane (25 mL) and *N*,*N*-dimethylformamide (260 μL) were added to the residue, and the mixture was stirred under ice cooling. Further, oxalyl chloride (216 μL, 2.48 mmol) was added to continuously stir the mixture at room temperature for 1 h. *tert*-Butylamine (1.13 mL, 10.8 mmol) was added to the reaction mixture under ice cooling to stir the mixture at room temperature for 14 h. After water and dichloromethane were added to the reaction mixture to conduct liquid separation, the resultant organic layer was dried over anhydrous sodium sulfate, and the solvent was distilled off under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane:ethyl acetate = 2:1) to give **13** (197 mg, 36%) as a pale yellow amorphous substance.

^{1}H NMR ($CDCl_3$) δ: 1.25–1.35 (9H, m), 1.35–1.45 (9H, m), 1.55–2.00 (6H, m), 2.20–2.30 (1H, m), 3.70–4.80 (3H, m), 5.30–5.45 (1H, m); MS (FAB) m/z 340 (M+H)$^+$.

5.23. *tert*-Butyl [(1*R*,2*S*,5*S*)-2-amino-5-(*tert*-butylcarbamoyl)cyclohexyl]carbamate (14)

Compound **13** (175 mg, 0.52 mmol) was dissolved in methanol (10 mL), and to the solution 10% palladium on carbon (20 mg) was added to conduct catalytic hydrogenation under atmospheric pressure at room temperature for 14 h. The catalyst was separated by filtration, and the filtrate was concentrated to give **14** (148 mg, 91%) as a pale yellow amorphous substance. This compound was used in the next reaction without further purification.

^{1}H NMR ($CDCl_3$) δ: 1.20–1.35 (9H, m), 1.44 (9H, s), 1.50–2.20 (9H, m), 2.90–3.00 (1H, m), 3.84 (1H, br), 4.94 (1H, br), 5.34 (1H, br).

5.24. *tert*-Butyl [(1*R*,2*S*,5*S*)-5-(*tert*-butylcarbamoyl)-2-{[(5-chloroindol-2-yl)carbonyl]amino}cyclohexyl]carbamate (15)

Compound **14** (147 mg, 0.469 mmol) was dissolved in *N*,*N*-dimethylformamide (10 mL), and to the solution were added 5-chloroindole-2-carboxylic acid (217 mg, 1.11 mmol), 1-hydroxy-

benzotriazole (158 mg, 1.17 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (211 mg, 1.10 mmol) at room temperature. Diisopropylethylamine (200 μL, 1.15 mmol) was added to the reaction mixture under ice cooling to stir the mixture at room temperature for 59 h. The reaction mixture was evaporated under reduced pressure, and saturated aqueous sodium hydrogencarbonate was added thereto, followed by extraction with methylene chloride. The resultant organic layer was dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure, and the residue was purified by column chromatography on silica gel (methylene chloride:methanol = 98:2) to give **15** (141 mg, 62%) as a pale yellow solid.

^{1}H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.35–2.30 (16H, m), 3.90–4.05 (1H, m), 4.15–4.25 (1H, m), 5.04 (1H, br), 5.42 (1H, br), 6.65–6.90 (1H, m), 7.19 (1H, dd, J = 8.8, 1.7 Hz), 7.37 (1H, d, J = 8.8 Hz), 7.59 (1H, br), 8.13 (1H, br), 10.51 (1H, s); MS (ESI) m/z 491 [$(M+H)^+$, ^{35}Cl], 493 [$(M+H)^+$, ^{37}Cl].

5.25. *N*-[(1*R*,2*S*,5*S*)-5-(*tert*-Butylcarbamoyl)-2-{[(5-chloroindol-2-yl)carbonyl]amino}cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4j)

Compound **15** (140 mg, 0.29 mmol) was dissolved in methanol (10 mL), and saturated HCl in methanol (10 mL) was added under ice cooling, followed by stirring at room temperature for 4 h. The solvent was distilled away under reduced pressure, and the residue was dissolved in *N*,*N*-dimethylformamide (10 mL). To the solution were added 5-methyl-4,5,6, 7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid lithium salt (83.5 mg, 0.41 mmol), 1-hydroxybenzotriazole (68.6 mg, 0.51 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (80.7 mg, 0.42 mmol) at room temperature. Diisopropylethylamine (176 μL, 1.01 mmol) was added to the reaction mixture under ice cooling to stir the mixture at room temperature for 11 h. The reaction mixture was evaporated under reduced pressure, and saturated aqueous sodium hydrogencarbonate was added thereto, followed by extraction with methylene chloride. The resultant organic layer was washed with saturated brine and dried over magnesium sulfate anhydrate. The solvent was distilled away under reduced pressure, and the resultant residue was purified by silica gel column chromatography (methylene chloride:methanol = 95:5). The thus-obtained solid was dissolved in methylene chloride, and then 1 N HCl in ethanol (171 μL) was added. The solvent was distilled away under reduced pressure, and methanol and diethyl ether were added to the residue to collect the precipitate formed by filtration to give **4j** (89.2 mg, 47%) as a colorless powder.

Mp 225–230 °C (dec); ^{1}H NMR (DMSO-d_6) δ: 1.23 (9H, s), 1.50–2.00 (6H, m), 2.30–2.50 (1H, m), 2.93 (3H, s), 3.10–3.80 (4H, m), 4.05–4.80 (4H, m), 7.03 (1H, d, J = 1.5 Hz), 7.16 (1H, dd, J = 8.8, 2.0 Hz), 7.35–7.45 (2H, m), 7.68 (1H, d, J = 2.0 Hz), 7.90–8.10 (1H, m), 8.42 (1H, d, J = 8.1 Hz), 11.30–11.45 (1H, m), 11.79 (1H, s); MS (FAB) m/z 571 [$(M+H)^+$, ^{35}Cl], 573 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd $C_{28}H_{35}ClN_6O_3S{\cdot}HCl{\cdot}3H_2O$: C, 50.83; H, 6.40; Cl, 10.72; N, 12.70; S, 4.85. Found: C, 50.81; H, 6.26; Cl, 10.94; N, 12.71; S, 5.11.

5.26. *tert*-Butyl [(1*R*,2*S*,5*S*)-2-{[(5-chloroindol-2-yl)carbonyl]amino}-5-(hydroxymethyl)cyclohexyl]carbamate (16)

Compound **11** (900 mg, 1.94 mmol) was dissolved in dichloromethane (15 mL), a 1 N hexane solution (9.0 mL, 9.0 mmol) of isobutyllithium hydride was added at −78 °C, and the mixture was stirred for 2 h. A saturated aqueous solution of ammonium chloride was added, followed by extraction with ethyl acetate, and the resultant organic layer was washed with saturated saline and then dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure, and the residue was purified by **c**olumn chromatography on silica gel (ethyl acetate:hexane = 5:1) to give **16** (340 mg 42%) as a colorless solid.

^{1}H NMR ($CDCl_3$) δ: 1.14–1.73 (13H, m), 1.81–1.90 (1H, m), 1.92–2.01 (1H, m), 2.19–2.34 (1H, m), 3.43–3.59 (2H, m), 3.87–3.99 (1H, m), 4.13–4.21 (1H, m), 4.84–4.96 (1H, m), 6.82 (1H, br s), 7.21 (1H, dd, J = 8.5, 2.1 Hz), 7.34 (1H, d, J = 8.5 Hz), 7.60 (1H, br s), 8.05 (1H, br s), 9.46 (1H, br s).

5.27. *N*-[(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-(hydroxymethyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4k)

Compound **4k** was synthesized from **16** according the procedure described for **4j**. Saturated HCl in ethanol was used instead of saturated HCl in methanol. Colorless powder (224 mg, 65%).

Mp 228–231 °C; $^{1H\ NMR}$ (DMSO-d_6) δ: 1.01–2.08 (7H, m), 2.91 (3H, s), 3.04–3.83 (9H, m), 4.02–4.18 (1H, m), 4.33–4.51 (1H, m), 7.00 (1H, d, J = 2.0 Hz), 7.15 (1H, dd, J = 8.8, 2.0 Hz), 7.40 (1H, d, J = 8.8 Hz), 7.65 (1H, d, J = 2.0 Hz), 7.99 (1H, d, J = 8.0 Hz), 8.38 (1H, d, J = 8.4 Hz), 11.74 (1H, s); MS (ESI) m/z 502 [$(M+H)^+$, ^{35}Cl], 504 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{24}H_{28}ClN_5O_3S{\cdot}HCl{\cdot}2.5H_2O$: C, 49.40; H, 5.87; Cl, 12.15; N, 12.00; S, 5.79. Found: C, 49.44; H, 5.50; Cl, 11.98; N, 11.98; S, 5.79.

5.28. *tert*-Butyl {(1*R*,2*S*,5*S*)-2-{[(5-chloroindol-2-yl)carbonyl]amino}-5-[(methylthio)methyl]cyclohexyl}carbamate (17)

Compound **16** (500 mg, 1.19 mmol) and triethylamine (329 μL, 2.38 mmol) were suspended in a mixture of tetrahydrofuran (8 mL)–methylene chloride (8 mL). Methanesulfonyl chloride (138 μL, 1.79 mmol) was added dropwise thereto at −78 °C, followed by stirring at −5 °C for 15 h. The reaction mixture was evaporated under reduced pressure, and water was added thereto, followed by extraction with methylene chloride. The organic layer was washed with saturated brine, and was dried over sodium sulfate anhydrate. The solvent was distilled away under reduced pressure, to thereby give a pale yellow solid (654 mg). The solid was dissolved in *N*,*N*-dimethylformamide (8 mL), and 15% sodium thiomethoxide solution (1.8 mL) was added. The mixture was stirred at room temperature for 4 h, and the resulting mixture was diluted with water, followed by extraction with ethyl acetate. The resultant organic layer was dried over sodium sulfate anhydrate. The solvent was distilled away under reduced pressure, and the resultant residue was purified by silica gel column chromatography (methylene chloride:methanol = 96:4) to give **17** (492 mg, 91%) as a pale brown solid.

^{1}H NMR ($CDCl_3$) δ: 1.52 (9H, s), 1.87–3.04 (13H, m), 3.91–3.94 (1H, m), 4.12–4.15 (1H, m), 4.95 (1H, br s), 6.81 (1H, s), 7.19 (1H, dd, J = 1.2, 8.8 Hz), 7.35 (1H, d, J = 8.8 Hz), 7.57 (1H, s), 9.82 (1H, br s); MS (ESI) m/z: 452 $(M+H)^+$.

5.29. *tert*-Butyl {(1*R*,2*S*,5*S*)-2-{[(5-chloroindol-2-yl)carbonyl]amino}-5-[(methylsulfonyl)methyl]cyclohexyl}carbamate (18)

Compound **17** (300 mg, 0.664 mmol) was dissolved in methylene chloride (10 mL), and then mCPBA (70%, 400 mg) was added at 0 °C. After stirring for 1 h, the reaction mixture was diluted with water, followed by extraction with methylene chloride. The resultant organic layer was washed with saturated brine, and was dried over sodium sulfate anhydrate. The solvent was distilled away under reduced pressure, and the resultant residue was purified by silica gel column chromatography (methylene chloride:methanol = 96:4) to give crude **18**. Saturated aqueous sodium hydrogencarbonate was added to the crude **18**, followed by extraction with ethyl acetate. The resultant organic layer was dried over sodium

sulfate anhydrate, and then the solvent was distilled away under reduced pressure to give **18** (254 mg, 79%) as a pale yellow solid.

^{1}H NMR ($CDCl_3$) δ: 1.44–2.19 (13H, m), 2.22–2.30 (2H, m), 2.89–3.25 (7H, m), 3.93–4.15 (2H, m), 4.98 (1H, br s), 6.82 (1H, s), 7.21 (1H, dd, J = 2.0, 8.8 Hz), 7.34 (1H, d, J = 8.8 Hz), 7.60 (1H, br s), 9.54 (1H, br s).

5.30. *N*-{(1*R*,2*S*,5*S*)-2-{[(5-Chloroindol-2-yl)carbonyl]amino}-5-[(methylsulfonyl)methyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide hydrochloride (4l)

Compound **4l** was synthesized from **18** according the procedure described for **4j**. Brown solid (55 mg, 19%).

^{1}H NMR (DMSO-d_6) δ: 1.35–1.40 (1H, m), 1.55–1.62 (1H, m), 1.70–1.76 (1H, m), 1.88–1.94 (1H, m), 2.03–2.07 (1H, m), 2.13–2.17 (1H, m), 2.30–2.33 (1H, m), 2.43–3.48 (10H, m), 3.60–3.73 (2H, m), 4.11–4.16 (1H, m), 4.40–4.42 (2H, m), 4.68–4.73 (1H, m), 7.05 (1H, s), 7.16 (1H, dd, J = 2.0, 8.8 Hz), 7.41 (1H, d, J = 8.8 Hz), 7.68 (1H, s), 8.26 (1H, d, J = 7.8 Hz), 8.39 (1H, d, J = 7.8 Hz), 11.78 (1H, br s); MS (ESI) m/z 564 $(M+H)^+$. Anal. Calcd for $C_{25}H_{30}ClN_5O_4S_2{\cdot}HCl{\cdot}1.75H_2O$: C, 47.50; H, 5.50; N, 11.08; S, 10.15; Cl, 11.22. Found: C, 47.88; H, 5.52; N, 10.65; S, 9.95; Cl, 11.43.

5.31. *N*-{(1*R*,2*S*,5*S*)-2-{[(5-chloroindol-2-yl)carbonyl]amino}-5-[methoxy(methyl)carbamoyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide (19)

Compound **19** was synthesized from **12** and *N,O*-dimethylhydroxylamine hydrochloride according the procedure describe for **4a**. Colorless solid (yield: 66%).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.57–1.91 (4H, m), 1.96–2.00 (1H, m), 2.10–2.21 (1H, m), 2.92 (3H, s), 2.93–3.01 (2H, m), 3.08 (3H, s), 3.10–3.28 (2H, m), 3.57 (3H, s), 3.69–3.76 (2H, m), 4.16–4.19 (1H, m), 4.50–4.52 (1H, m), 4.69 (1H, br s), 7.06 (1H, s), 7.17 (1H, dd, J = 1.5, 8.8 Hz), 7.42 (1H, d, J = 8.8 Hz), 7.70 (1H, s), 8.33 (1H, br s), 8.41 (1H, d, J = 7.8 Hz), 11.04 (1H, br s), 11.81 (1H, br s); MS (ESI) m/z 559 $(M+H)^+$.

5.32. *N*-[(1*R*,2*S*,5*S*)-5-Acetyl-2-{[(5-chloroindol-2-yl)carbonyl]amino}cyclohexyl]-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide (4m)

Methyl lithium (1.14 N solution, 0.43 mL) was added to a solution of **19** (216 mg, 0.386 mmol) in tetrahydrofuran (3 mL) under ice cooling, and the mixture was stirred for 30 min. Methyl lithium (1.14 N solution, 1.27 mL) was then added to the reaction mixture, and the resultant mixture was stirred for an additional 1 h. An ethanol solution of HCl was added to the reaction mixture, followed by extraction with ethyl acetate. The aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated brine, and was dried over anhydrous magnesium sulfate. The solvent was distilled away under reduced pressure, and the resultant residue was purified by silica gel column chromatography (methylene chloride:methanol = 96:4) to give **4m** (120 mg, 60%) as a yellow solid. The thus-obtained solid was dissolved in tetrahydrofuran, and then 1 N HCl in ethanol was added. The solvent was distilled away under reduced pressure to give HCl salt of **4m**.

Compound **4m**: ^{1}H NMR ($CDCl_3$) δ: 1.62–1.65 (2H, m), 1.96–2.04 (1H, m), 2.13–2.17 (2H, m), 2.22 (3H, s), 2.32–2.35 (1H, m), 2.53 (3H, s), 2.63–2.67 (1H, m), 2.81–2.89 (2H, m), 2.94–2.96 (2H, m), 3.75 (2H, s), 4.09–4.11 (1H, m), 4.59–4.61 (1H, m), 6.85 (1H, d, J = 2.0 Hz), 7.20 (1H, dd, J = 2.0, 8.8 Hz), 7.33 (1H, d, J = 8.8 Hz), 7.47 (1H, d, J = 7.3 Hz), 7.62 (1H, d, J = 1.5 Hz), 8.00 (1H, d, J = 5.6 Hz), 9.60 (1H, s); MS (ESI) m/z: 514 $(M+H)^+$.

HCl salt of **4m**: Anal. Calcd for $C_{25}H_{28}ClN_5O_3S{\cdot}HCl{\cdot}1.5H_2O$: C, 51.99; H, 5.58; N, 12.13; S, 5.55; Cl, 12.28. Found: C, 51.63; H, 5.10; N, 12.12; S, 5.59; Cl, 12.23.

5.33. In vitro anti-fXa activity (IC_{50})

In vitro Anti-fXa activity (IC_{50}) 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.

5.34. Prothrombin time (PTCT2)

Prothrombin time (PT) was measured with an Amelung KC-10A micro coagulometer (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. The concentration of inhibitor required to double the clotting time (CT2) was estimated from the concentration-response curve by a regression analysis.

5.35. Determination of *Ki* values to FXa of various species

All the assays were performed in triplicate using 96-well plates. Forty microliters of the substrates in H_2O was mixed with 10 μL of various concentrations of **4c** in 5% DMSO solution. As a control 5% DMSO solution was added instead of **4c**. The reaction was started by the addition of 50 μL of FXa in 40 mM Tris–HCl, pH 7.4, 300 mM NaCl, 0.2% BSA to 50 μL of the mixed solution (final volume was 100 μL). The reaction velocities (ΔO.D./min) at 405 nm were monitored with SPECTRAmax 340 (Molecular Devices Co., USA) at 30 °C for 10 min. The *Ki* values of **4c** to each enzyme were calculated graphically with a Lineweaver–Burk plot.

5.36. Determination of *Ki* values for serine proteases

All the assays were performed in triplicate using 96-well plates. Inhibitory effects of **4c** on thrombin, trypsin, plasmin, chymotrypsin and rt-PA activities: Forty microliters of the substrates in H_2O was mixed with 10 μL of various concentrations of **4c** in 5% DMSO solution. As a control 5% DMSO solution was added instead of **4c**. The reaction was started by the addition of 50 μL of enzyme in the reaction buffer (40 mM Tris–HCl, pH 7.4, 300 mM NaCl, 0.2% BSA) to the mixed solution (final volume was 100 μL). Inhibitory effects of **4c** on rhFVIIa activity: Forty microliters of Spectrozyme fVIIa in H_2O was mixed with 10 μL of various concentrations of **4c** in 5% DMSO solution. Then 25 μL of sTF was added. As a control 5% DMSO solution was added instead of **4c**. The reaction was started by the addition of 25 μL of rhFVIIa in the reaction buffer (40 mM Tris–HCl, pH 7.4, 300 mM NaCl, 4 mM $CaCl_2$, 0.2% BSA) to the mixed solution (final volume was 100 μL).

The reaction velocities (ΔO.D./min) at 405 nm were monitored with SPECTRAmax 340 (Molecular Devices Co.) at 30 °C for

10 min. The *Ki* values of **4c** to each enzyme were calculated graphically with the Lineweaver–Burk plot. The *Ki* values over 100 μM were expressed as '>100 μM'. The concentrations of enzymes and substrates used in this study were shown as follows. Thrombin (0.03 U/mL, S-2238, 20, 25, 33, 50, 100 μM). Trypsin (0.3 U/mL, S-2222, 40, 50, 67, 100, 200 μM). Chymotrypsin (0.005 U/mL, S-2586, 20, 25, 33, 50, 100 μM). Plasmin (0.004 U/mL, S-2251, 200, 250, 334, 500, 1000 μM). GRTPA inj. (rt-PA, 750 U/mL, S-2288, 200, 250, 334, 500, 1000 μM) rhFVIIa/sTF (2 nM/20 nM, Spectrozyme fVIIa, 200, 250, 334, 500, 1000 μM).

5.37. Preparation of human plasma and rat plasma

Normal human plasma was obtained from healthy volunteers. Blood was collected from the antecubital vein into a syringe containing 3.13% trisodium citrate dihydrate (9:1 v/v). Rat plasma was prepared as follows. Blood was collected from the abdominal aorta of anesthetized normal rats with Thiopental Sodium (100 mg/kg, i.p.) into a syringe containing 3.13% trisodium citrate dihydrate (9:1 v/v). The blood samples were centrifuged at 1500g for 10 min at 4 °C, and then human plasma and rat plasma were stored at −40 °C until the measurement of the coagulation time.

5.38. Anticoagulation activity of 4c

The anticoagulation activity of **4c** was evaluated in three coagulation assays, prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT). These activities were measured by using human plasma with Amelung KC-10A micro coagulometer (MC Medical., Tokyo, Japan). To evaluate the species specificity of **4c** for anticoagulant activity, PT was measured using human plasma, rat plasma, cynomolgus monkey plasma and rabbit plasma. The concentration required to double the clotting time (CT2) was estimated from a linear regression analysis. All the assays were performed in triplicate. PT was measured as follows: Fifty microliters of plasma was mixed with 50 μL of various concentrations of **4c** in 4% DMSO saline and incubated for 1 min at 37 °C. As a control 4% DMSO saline was added instead of **4c**. The reaction was started by the addition of 100 μL of Thromboplastin C Plus (0.5 U/vial) to the mixture and then the clotting time was measured (final volume was 200 μL). APTT was measured as follows: fifty microliters of plasma and 50 μL of Platelin LS were mixed with 25 μL of various concentrations of **4c** in 4% DMSO saline and incubated for 5 min at 37 °C. As a control 4% DMSO saline was added instead of **4c**. The reaction was started by the addition of 25 μL of 50 mM CaCl2 to the mixture and then the clotting time was measured (final volume was 150 μL). TT was measured as follows: Fifty microliters of plasma was mixed with 50 μL of various concentrations of **4c** in 4% DMSO saline and incubated for 1 min at 37 °C. As a control 4% DMSO saline was added instead of **4c**. The reaction was started by the addition of 100 μL of human thrombin (final concentration was 4 U/mL) to the mixture and then the clotting time was measured (final volume was 200 μL).

5.39. Distribution coefficient

The distribution coefficients (log *D*) were determined by the well-known shake-flask method. Four hundred micromolars of compound solution in *n*-octanol (2 mL)/the Japanese Pharmacopoeia Second fluid (2 mL, pH 6.8) was placed on a shaker for 30 min. After centrifuging at 3000 rpm for 10 min, each layer was assayed using LC–MS methodologies (LC–Mass spectrometer: 1100 Series LC/MSD, Agilent; Analytical Column: X Terra® MSC18 3.5 μm, 3.0 × 30 mm, waters; mobile phase: 10 mM ammonium acetate buffer (pH 4.5)/0.05% acetic acid in acetonitrile = 95:5–10:90 v/v). The values of log *D* were analyzed using Analyst software program (version 1.4, Applied Biosystems).

5.40. Protein binding

Protein binding was determined by the ultra filtration method. One hundred micromolars of compound dissolved in DMSO and 1458 μL of human serum solution (CONSERA, lyophilized Human serum, Nissui Pharmaceutical) were distributed into a deep well plate (Waters) and mixed by pipetting. Three hundred microliters of the mixed solution was distributed into each well of a 96-well filter plate (MultiScreen® Filter Plate with Ultracel, Millipore) and was centrifuged at 3000 rpm for 60 min at 37 °C. The filtrate (50 μL) was collected and subjected to LC/MS analysis. The LC/MS system consisted of an Alliance 2795 (Waters), ZQ (Waters), and an X Terra® MS C18 3.5 μm, 3.0 × 30 mm column (Waters). The mobile phase was a 10 mM ammonium format buffer–MeOH; the gradient condition, 80/20–10/90 (v/v). Protein binding was analyzed using QuantLynx (Waters).

5.41. Preparation of the crystal

Purified human Gla-less fXa was purchased from Hematologic Technologies Inc. Without further purification, the purchased protein sample was dialyzed against 5 mM maleate imidazole, pH 5.0/4 mM $CaCl_2$/10 mM benzamidine, and concentrated to 7.5 mg/mL with microcon-10 (Millipore Co.). Concentrated Gla-less fXa was mixed with an equal volume of reservoir solution (15% PEG6000/1 mM $CaCl_2$/0.3 M AcONa/0.1 M maleate imidazole, pH 5.0) and vapor-equilibrated against the same solution at 20 °C. Under this condition, the crystal did not form spontaneously, so micro- and macroseeding methods were needed to obtain crystals of appropriate size. The resultant benzamidine/Gla-less fXa crystal was exposed to a two-step soaking method described below to obtain complex crystals with compound **4c**. The benzamidine/Gla-less fXa crystal was dialyzed in a microdialysis button against soak solution 1 (20% PEG6000/15% glycerol/0.3 M AcONa/2.5 mM $CaCl_2$/0.1 M maleate imidazole, pH 5.0) for 5 h and then against soak solution 2 (25% PEG6000/25% glycerol/0.3 M AcONa/2.5 mM $CaCl_2$/0.1 M maleate imidazole, pH 5.0/1 mM) of compound **4c**. After one day, the crystal was picked up and directly exposed to soak solution 2, and the soaking was continued. All of the soaking process was performed at 20 °C.

5.42. X-ray data collection and processing

The soaked crystal was flash-cooled in liquid nitrogen and centered in a gaseous nitrogen stream. The X-ray data set was collected at 100 K on an R-Axis VII imaging plate detector (Rigaku) using an MicroMax 007 rotating anode generator (Rigaku). Data processing was carried out with *d*trek*.[14]

5.43. Structure solution and crystallographic refinement

The previously reported Gla-less fXa structure (PDB code: 1HCG[15]) was used as the initial structure. Phase refinement and model improvement was carried out with *refmac*[16] and *coot*.[17] Stereochemistry checks indicate that the refined protein model is in good agreement with expectations within each resolution range. The statistics of data processing and crystallographic refinement are shown in Table 6. The atomic coordinates have been deposited with the Protein Data Bank (PDB code: 2EI8).

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