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Design, synthesis, and SAR of *cis*-1,2-diaminocyclohexane derivatives as potent factor Xa inhibitors. Part I: Exploration of 5–6 fused rings as alternative S1 moieties

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ABSTRACT

A series of *cis*-1,2-diaminocyclohexane derivatives were synthesized with the aim of optimizing previously disclosed factor Xa (fXa) inhibitors. The exploration of 5–6 fused rings as alternative S1 moieties resulted in two compounds which demonstrated improved solubility and reduced food effect compared to the clinical candidate, compound **A**. Herein, we describe the synthesis and structure–activity relationship (SAR), together with the physicochemical properties and pharmacokinetic (PK) profiles of some prospective compounds.

1. Introduction

Venous and arterial thromboembolic disorders have a significant impact on human morbidity and mortality. Unfractionated heparin, low molecular weight heparins and warfarin have been used for the treatment or prevention of thrombosis. However, these drugs have some limitations (parenteral administration, risk of bleeding, strong drug–drug interaction, requirement of frequent monitoring, etc.) and a novel anticoagulant which overcomes these problems is desired.[1–3] Factor Xa (fXa) is an attractive target because it is located at the convergence of intrinsic and extrinsic pathways in the coagulation cascade.[4] It is considered that fXa inhibitors will have less bleeding side effects than thrombin inhibitors.[5]

We have previously reported the discovery of potent and orally active fXa inhibitor compound **A**.[6] It binds to fXa with occupying two sites, that is, the S1 and S4 sites, where the 5-chloroindole moiety and the *N*-methyl-tetrahydrothiazolo[5,4-*c*]pyridine (THTP) moiety fit into, respectively (Fig. 1).

Although compound **A** possesses strong in vitro activity and good bioavailability (BA), its plasma exposure (AUC) was decreased by food intake in an animal model. We anticipated that this feature could lead to variability in the absorption and thereby affect the efficacy. Food can alter BA by various means, thus it is difficult to figure out the details of the cause for the AUC decrease. However, it is considered that the food effects are generally least likely to occur with drugs which belong to Biopharmaceutics Classification System (BCS) Class I,[7,8] in which drugs possess both high permeability[9] and high solubility.[10] Compound **A** which exhibits high permeability[9] and low solubility[10] is considered to belong to Class II. Therefore, we envisioned that the negative food effect should be reduced by improving the solubility. The problem lies in the neutral pH region, while solubility in the acidic pH region is not the case, as displayed in the Figure 1. Thus, we aimed to improve the solubility in the neutral pH region.

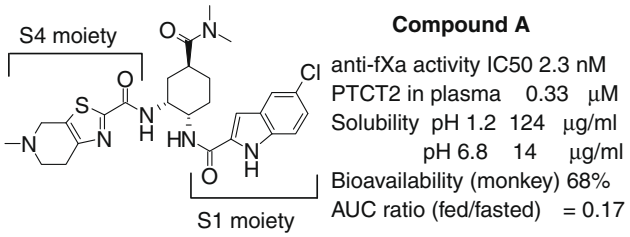

Figure 1. Properties of compound **A**.

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Our research effort was mainly focused on modification of the S1 moiety because the 5-chloroindole fragment seemed to contribute to the poor solubility more than the S4 moiety. Exploration of the other 5–6 fused rings was conducted, together with incorporation of substituents on the indole ring. Herein, we describe the results of SAR, including anti-fXa activity (IC_{50}), anticoagulant activity (PTCT2), and ex vivo anti-fXa activity, along with the physicochemical properties and pharmacokinetics of a number of prospective compounds.

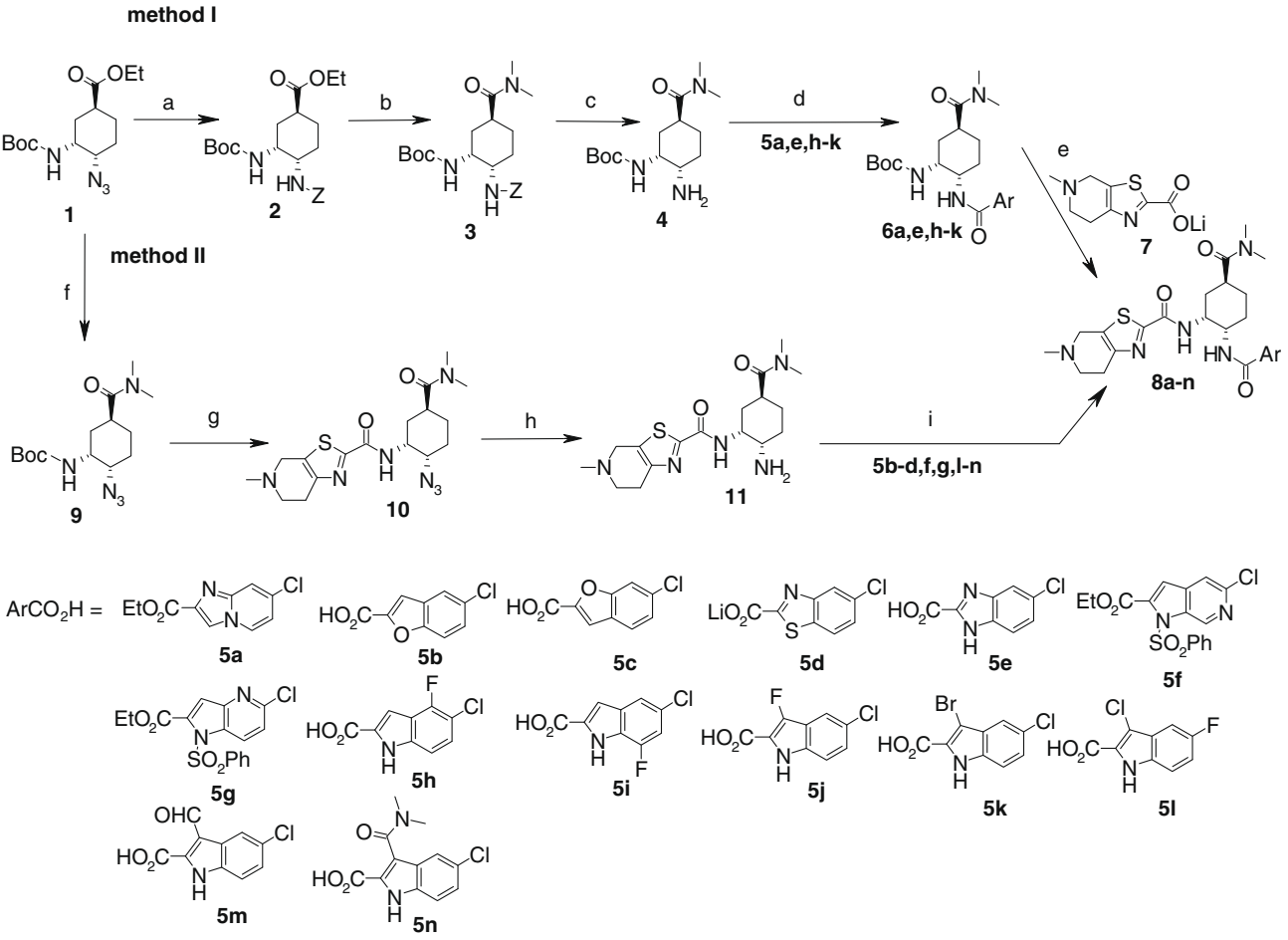
2. Chemistry

Compounds **8a**–**8n** were synthesized in the two methods as shown in Scheme 1. In method I, reduction of the azide group of starting material **1**[6], followed by protection with the Z-group provided compound **2**, which was then hydrolyzed and reacted with dimethylamine to give compound **3**. Removal of the Z-group of **3** and amidation of the resulting amine **4** with carboxylic acid **5** gave compound **6**. Deprotection of **6**, followed by condensation with lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylate (**7**)[11] afforded target compound **8**. In method II, hydrolysis of compound **1**, followed by amidation with dimethylamine gave compound **9**. Removal of the Boc group of **9** and successive condensation with **7** gave azide **10**, which was then reduced to amine **11**. Condensation of **11** with carboxylic acid **5** led to target compound **8**. In the case of **5a**, **5f**, and **5g**, corresponding carboxylic acids were prepared by hydrolysis prior to use. The benzenesulfonyl group of **5f** or **5g** was removed simultaneously during the condensation reaction.

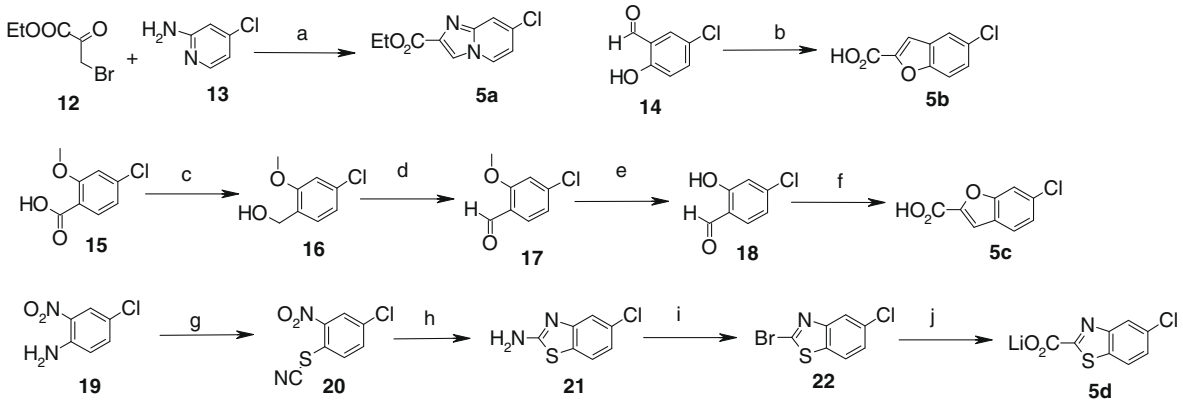
The preparation of **5a**, **5b**, **5c**, and **5d** is shown in Scheme 2. Ethyl bromopyruvate (**12**) was treated with 2-amino-4-chloropyridine (**13**) in DME to give **5a**. Condensation of 5-chlorosalicylaldehyde (**14**) with diethyl bromomalonate, followed by hydrolysis afforded **5b**. Reduction of 4-chloro-2-methoxybenzoic acid (**15**) with $BH_3{\cdot}Me_2S$ gave alcohol **16**, which was then converted to aldehyde **17** by Swern oxidation. After cleavage of the methyl group with LiCl in DMF,[12] the resulting 4-chlorosalicylaldehyde (**18**) was treated with diethyl bromomalonate, and successive hydrolysis gave **5c**. 4-Chloro-2-nitrophenyl thiocyanate (**20**) was synthesized from 4-chloro-2-notroaniline (**19**) by a Sandmeyer reaction. 5-Chloro-2-aminobenzothiazole (**21**) was formed after the reduction of the nitro group of **20** with tin. A Sandmeyer reaction, lithium–halogen exchange, and successive addition of carbon dioxide provided **5d**.

Introduction of a cyanomethyl group to **23** by exploiting (4-chlorophenoxy)acetonitrile[13] gave two isomers, **25a** and **25b** (Scheme 3). Each compound was utilized for the syntheses of **5f** and **5g** via reductive cyclization and subsequent introduction of benzenesulfonyl group, followed finally by anion mediated ethoxycarbonylation.

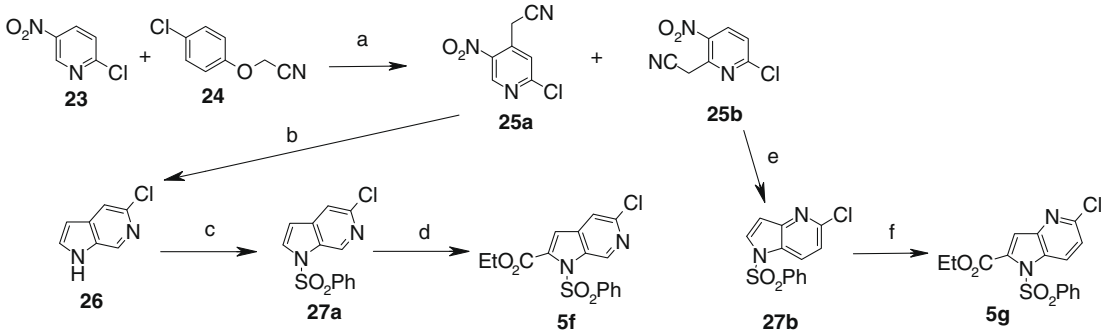
The preparation of indole derivatives **5h**–**5l** is shown in Scheme 4. Benzylbromide **28** was oxidized to aldehyde **29**, which was used



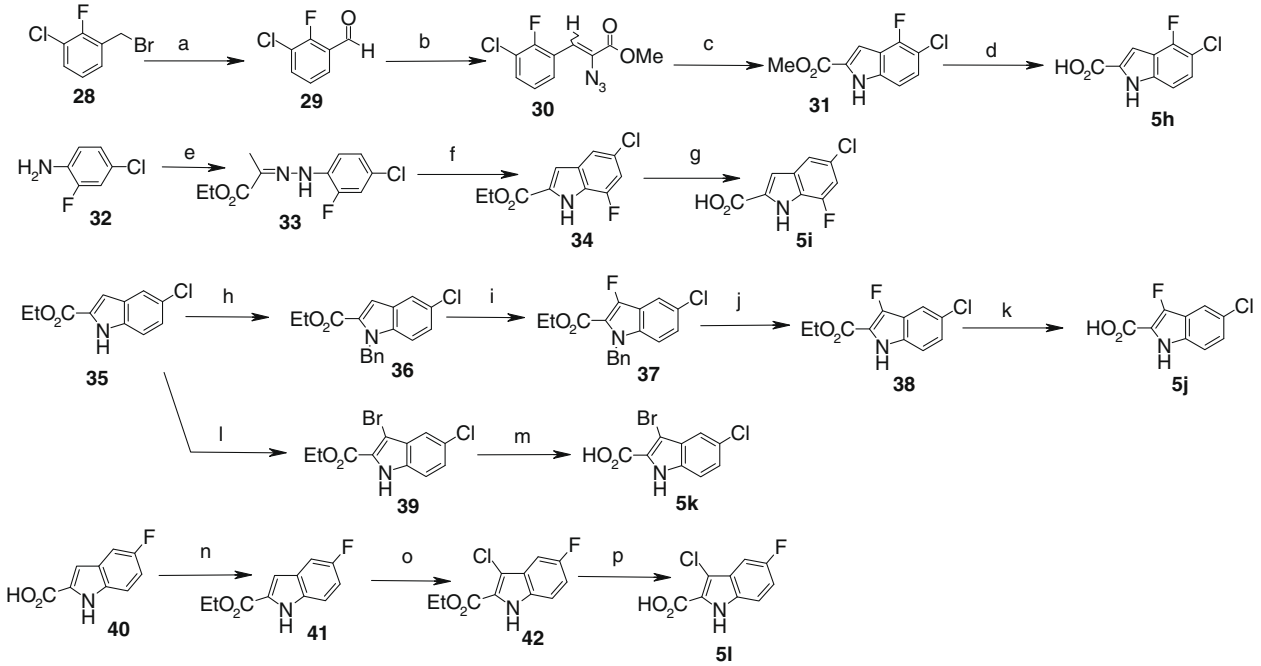
Scheme 1. Reagents and conditions: (a) (i) Pd/C, H_2, EtOH, EtOAc, (ii) Z–Cl, $NaHCO_3$, THF, H_2O; (b) (i) LiOH, THF, H_2O, (ii) $HNMe_2{\cdot}HCl$, EDC·HCl, TEA, HOBt, CH_2Cl_2; (c) Pd/C, H_2, MeOH; (d) $ArCO_2H$ (**5a**, **e**, **h**–**k**), EDC·HCl, HOBt, DMF; (e) (i) HCl, EtOH, (ii) **7**, EDC·HCl, HOBt, DMF; (f) (i) LiOH, THF, H_2O; (ii) $HNMe_2{\cdot}HCl$, EDC·HCl, NMM, HOBt, CH_2Cl_2; (g) (i) HCl, EtOH, CH_2Cl_2; (ii) **7**, EDC·HCl, HOBt, DMF; (h) Pd/C, H_2, EtOH; (i) $ArCO_2H$ (**5b**–**d**, **f**, **g**, **l**–**n**), EDC·HCl, HOBt, DMF. In the case of **5a**, **5f**, and **5g**, corresponding carboxylic acids were prepared by hydrolysis prior to use.



Scheme 2. Reagents and conditions: (a) DME, reflux; (b) (i) diethyl bromomalonate, K_2CO_3, AcOEt; (ii) KOH, EtOH; (c) $BH_3{\cdot}Me_2S$, THF; (d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (e) LiCl, DMF; (f) (i) diethyl bromomalonate, K_2CO_3, 2-butanone; (ii) KOH, EtOH; (g) (i) H_2SO_4, $NaNO_2$, H_2O; (ii) KSCN, CuSCN, H_2O; (h) Sn, concd HClaq; (i) $CuBr_2$, *tert*-BuONO, DMF; (j) (i) *tert*-BuLi, THF; (ii) CO_2.



Scheme 3. Reagents and conditions: (a) NaH, DMF; (b) Raney Ni, H_2, EtOH; (c) $Bu_4N{\cdot}HSO_4$, NaOH, $PhSO_2Cl$, CH_2Cl_2; (d) (i) *tert*-BuLi, Et_2O, THF; (ii) $ClCO_2Et$, Et_2O, THF; (e) (i) Raney Ni, H_2, EtOH; (ii) $Bu_4N{\cdot}HSO_4$, NaOH, $PhSO_2Cl$, CH_2Cl_2; (f) (i) *tert*-BuLi, Et_2O, (ii) $ClCO_2Et$, Et_2O.



Scheme 4. Reagents and conditions: (a) 2-nitropropane, NaH, EtOH; (b) methyl azidoacetate, NaH, MeOH; (c) xylene, 130–140 °C; (d) LiOH, THF, H_2O; (e) (i) $NaNO_2$, HClaq; (ii) ethyl 2-methylacetoacetate, EtOH, H_2O, pH 4–5; (f) polyphosphoric acid; (g) NaOH, THF, H_2O; (h) BnCl, K_2CO_3, DMF; (i) 2,6-dichloro-1-fluoropyridinium triflate, CH_2Cl_2; (j) $AlCl_3$, anisole; (k) LiOH, THF, H_2O; (l) NBS, DMF; (m) LiOH, THF, H_2O; (n) $SOCl_2$, EtOH; (o) NCS, DMF; (p) NaOH, EtOH, H_2O.

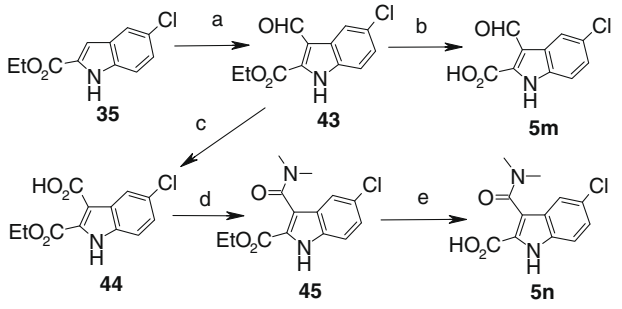
for a Knoevenagel reaction with methyl azidoacetate to give α,β-unsaturated ester **30**. Cyclization of **30** in xylene, followed by hydrolysis of methyl ester afforded carboxylic acid **5h**. Conversion of 4-chloro-2-fluoroaniline (**32**) to diazo compound, and successive treatment with ethyl 2-methylacetoacetate via retro-Claisen condensation[14] gave **33**. Fischer indole cyclization of **33** in polyphosphoric acid, followed by hydrolysis afforded **5i**. Protection of the nitrogen of **35** with a benzyl group, followed by fluorination with 2,6-dichloro-1-fluoropyridinium triflate, followed by debenzylation with $AlCl_3$ and hydrolysis, provided **5j**. Bromination of **35** with NBS, followed by hydrolysis gave **5k**. Esterification of **40** with thionyl chloride in EtOH, followed by chlorination with NCS and hydrolysis furnished **5l**.

A Vilsmeier reaction of **35** gave aldehyde **43**, which was then converted to **5m** by hydrolysis (Scheme 5). Oxidation of aldehyde **43** provided the carboxylic acid **44**. Condensation of **44** with dimethylamine, followed by hydrolysis gave **5n**.

Compounds **8o** and **8p** were prepared as displayed in Scheme 6. Reduction of **1** afforded amine **46**, which was treated with corresponding carboxylic acids to give **47a** or **47b**. Deprotection of the Boc groups of **47a** and **47b** and successive condensation with **7** afforded **48a** and **48b**, respectively. These esters were hydrolyzed and reacted with dimethylamine to afford **8o** and **8p**.

3. Results and discussion

The anti-fXa activity (IC_{50}), anticoagulant activity (PTCT2), solubility, and distribution coefficients (Log *D*) are shown in Table 1. Several potent compounds were orally administered to rats at the dose of 10 mg/kg and the ant-fXa activities in plasma were measured at 0.5, 1, 2, and 4 h after administration. The maximum anti-fXa activity among them is shown in Table 1.



Scheme 5. Reagents and conditions: (a) PhN(Me)CHO, $POCl_3$, $ClCH_2CH_2Cl$; (b) NaOH, EtOH, H_2O; (c) $NaClO_2$, H_2NSO_3H, *tert*-BuOH, H_2O; (d) $Me_2NH{\cdot}HCl$, EDC·HCl, HOBt, DMF; (e) NaOH, EtOH, H_2O.

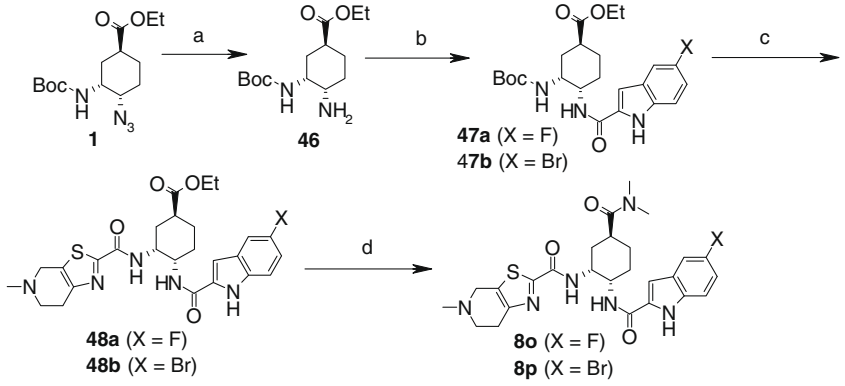
7-Chloroimidazopyridine **8a** showed only weak anti-fXa activity although the solubility was distinctly improved. The anti-fXa activities of benzofuran **8b** and **8c** and benzothiazole **8d** decreased significantly, while benzimidazole **8e** sustained strong anti-fXa activity. Pyrrolopyridine **8f** and **8g** showed moderate anti-fXa activity. These results can be rationalized by the binding mode of compound **A** toward fXa (Fig. 2).[6] The chlorine atom of the indole fits into a cavity composed of Tyr228, Val213, and Ala190 in the bottom of the S1 site. Another characteristic feature lies in hydrogen bonding between the indole NH moiety and the carbonyl oxygen of Gly218 in fXa, which seems to contribute to the tight binding. Thus, the change of position of the chlorine atom and the absence of the hydrogen bond donor presumably caused a drop in the anti-fXa activity in **8b**, **8c**, and **8d**.

Among these compounds, **8e** showed both strong in vitro activity and good solubility, and was subjected to a rat ex vivo assay. Although the metabolic stability was almost comparable to that for compound **A**, it did not exhibit strong anti-fXa activity after oral administration, presumably because of its lower permeability (Table 2).

Then, we moved on to the introduction of a substituent on the indole ring to increase solubility without compromising the permeability and anti-fXa activity. The assay results of these compounds are summarized in Table 3.

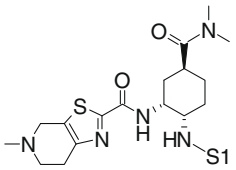
Incorporation of a fluorine atom at the 3-position caused significant increase in the solubility at both pH 1.2 and pH 6.8 without a loss of anti-fXa activity, in contrast to that at the 4- or 7-position. (see **8h**, **8i**, and **8j**). Therefore, we concentrated on the modification of the 3-position of the indole ring, and found that the solubility of all the compounds (**8k–8n**) improved significantly.

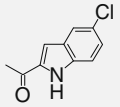
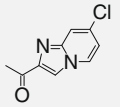
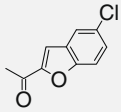
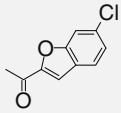
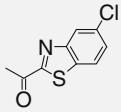
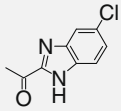
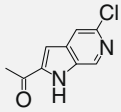
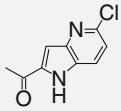
The exchange of the chlorine atom at the 5-position to the other halogen atom gave us further information. Comparison of the anti-fXa activity (IC_{50}) of **8o** and **8p** with compound **A** demonstrated that both chlorine and bromine were suitable for the cavity in the S1 site. We suppose that a chlorine or bromine atom makes contact with the benzene ring of Tyr228 in the S1 site, similar to other inhibitors.[15–17] We assumed that a fluorine atom was not suitable for maintaining this contact and thus caused a drop in anti-fXa activity. On the other hand, the anticoagulant activity (PTCT2) of **8o** was relatively strong, considering its modest anti-fXa activity. This difference between IC_{50} and PTCT2 varied among the compounds (Table 4). There is a propensity for a more polar compound (possessing a lower Log *D* value) to show a smaller PTCT2/IC_{50} ratio. It is assumed that higher lipophilicity causes higher non-specific protein binding and results in a larger decrease in anticoagulant activity in plasma.[6,18] Differences in lipophilicity seemed to affect their solubility as well. Namely, **8o** exhibited in-



Scheme 6. Reagents and conditions: (a) (i) H_2, Pd/C, MeOH; (b) 5-fluoroindole-2-carboxylic acid (**40**) or 5-bromoindole-2-carboxylic acid, EDC·HCl, HOBt, CH_2Cl_2, DMF; (c) (i) HCl, EtOH; (ii) **7**, EDC·HCl, HOBt, TEA, DMF; (d) (i) LiOH, THF, H_2O; (ii) $Me_2NH{\cdot}HCl$, EDC·HCl, HOBt, NMM, DMF.

Table 1
The assay results of **5**–**6** fused ring compounds



Compd	S1	fXa IC_{50} (nM)	PTCT2[a] (μM) in human plasma	Solubility (μg/mL)		Log *D*[b]	Ex vivo anti-fXa activity[c] (%)
				pH 1.2	pH 6.8		
A		2.3	0.33	124	14	2.8	62
8a		120	2.7	830	700	1.3	0.7
8b		1100	20	1000	450	2.4	NT[d]
8c		1900	NT	NT	NT	NT	NT
8d		2300	NT	NT	NT	NT	NT
8e		6.5	0.39	600	390	2.3	2.8
8f		69	2.5	1000	760	1.7	3.9
8g		55	2.0	NT	NT	1.9	4.7

[a] Anticoagulant activities were evaluated with the human plasma clotting time doubling concentration for prothrombin time (PTCT2).
[b] *n*-Octanol to the Japanese Pharmacopoeia Second Fluid (pH 6.8) distribution coefficient.
[c] The maximum anti-fXa activity in plasma after oral administration.
[d] Not tested.

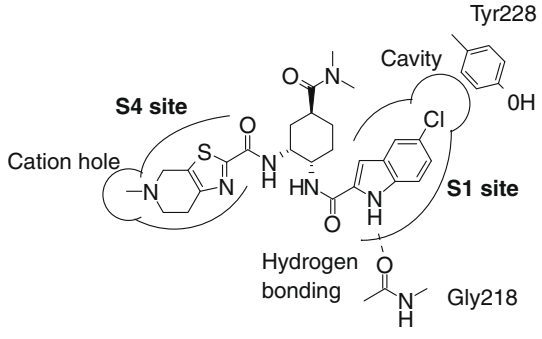


Figure 2. Binding mode of compound **A**.

Table 2
Metabolic stability and permeability

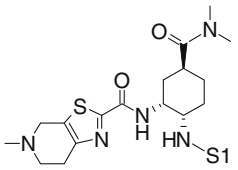
Compd	Metabolic stability[a] (nmol/min/mg)	Permeability AT ratio[b]
A	0.032	>30
8e	0.042	13

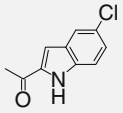
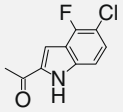
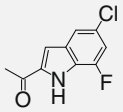
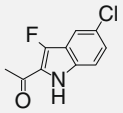
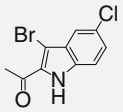
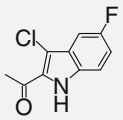
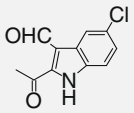
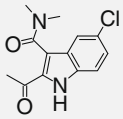
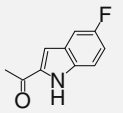
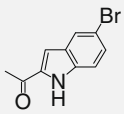
[a] Metabolic stabilities were evaluated by the initial velocity of disappearance (V_{ini}) in rat liver microsomes.
[b] Permeabilities were measured using Caco-2 monolayers. The relative permeability is shown as a ratio compared to atenolol.

creased solubility at both pH 1.2 and pH 6.8, while that of **8p** slightly decreased (Table 3).

Almost all the indole derivatives **8h**–**8p** exhibited moderate to good rat ex vivo activity except for **8n**, which showed relatively

Table 3
The assay results of substituted indole compounds



Compd	S1	fXa IC_{50} (nM)	PTCT2 (μM) in human plasma	Solubility (μg/mL)		Log *D*	Ex vivo anti-fXa activity (%)
				pH 1.2	pH 6.8		
A		2.3	0.33	124	14	2.8	62
8h		1.4	0.44	44	4	3.2	48
8i		49	3.9	110	19	3.1	25
8j		3.5	0.40	880	150	3.1	82
8k		4.8	0.97	740	29	3.6	80
8l		5.7	0.61	1000	280	2.9	66
8m		2.1	0.34	970	86	2.8	54
8n		37	5.7	1000	920	2.1	2.9
8o		15	0.89	550	290	2.1	34
8p		3.2	0.60	100	11	3.0	46

low permeability (Table 5). Among them, **8j**, **8k**, and **8l** demonstrated higher ex vivo activity than compound **A**.

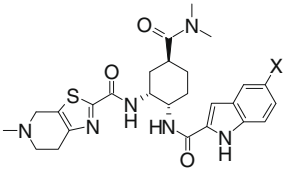
Then oral administration of some prospective compounds to monkeys was conducted in order to examine their PK profiles and food effects (Table 6). The AUC of **8j**, **8o**, and **8p** were comparable to that of compound **A**, while **8k**, **8l**, and **8m** showed substantially smaller AUC. It is noteworthy that **8j** and **8o** exhibited larger AUC than compound **A** in a fed condition. The negative food effect was significantly reduced in **8j** and **8o** which showed improved solubility, as we envisaged.

This finding encouraged us to further explore this series of compounds. The results of the other fused ring compounds will be reported in the following paper.

4. Conclusion

An optimization study of the S1 moiety of *cis*-1,2-diaminocyclohexane derivatives as potent fXa inhibitors was conducted. Exploration of 5–6 fused rings indicated that a hydrogen bond between the carbonyl oxygen of Gly218 and the NH moiety of fused ring is

Table 4
The ratio of PTCT2/IC_{50}



Compd	X	fXa IC_{50} (nM)	PTCT2 (μM) in human plasma	PTCT2/IC_{50} ratio	Log *D*	Protein binding (%)
8o	F	15	0.89	59	2.1	63
A	Cl	2.3	0.33	143	2.8	72
8p	Br	3.2	0.60	189	3.0	NT[a]

[a] Not tested.

Table 5
Metabolic stability and permeability

Compd	Metabolic stability[a] (nmol/min/mg)	Permeability AT ratio[b]
A	0.032	>30
8h	0.038	>30
8i	0.000	>30
8j	0.060	>30
8k	0.042	22
8l	0.067	>30
8m	0.040	ND[c]
8n	0.028	6.8
8o	0.038	>30
8p	0.019	>30

[a] Metabolic stabilities were evaluated by the initial velocity of disappearance (V_{ini}) in rat liver microsomes.
[b] Permeabilities were measured using Caco-2 monolayers. The relative permeability is shown as a ratio compared to atenolol.
[c] Not determined.

important for strong anti-fXa activity. Comparison of the halogen atom at the 5-position of the indole moiety indicated that 5-fluoro derivative, which possessed both a low Log *D* value and low protein binding, was preferable for a small PTCT2/IC_{50} ratio. Introduction of a substituent on the 3-position of the indole moiety effectively improved the solubility. In this series of compounds, the negative food effect was reduced by increasing the solubility in the neutral pH region. We have identified the compounds **8j** and **8o** as potent anti-fXa inhibitors, which are less susceptible to a food effect.

5. Experimental section

5.1. Chemistry

5.1.1. 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. ^{1}H NMR spectra were recorded on a JEOL JNM-EX-400 spectrometer, and chemical shifts are given in ppm (δ) 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. IR spectra were recorded on a HITACHI 270-30 or HORIBA FT-720 spectrometer. Column chromatography was performed with Merck Silica Gel 60 (particle size 0.060–0.200 or 0.040–0.063). Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with Silica Gel 60 F_{254}.

Table 6
PK profiles in monkeys (n = 3, po)

Compd	AUC_{fasted} (ng h/mL)	AUC_{fed} (ng h/mL)	Ratio fed/fasted
A	985	172	0.17
8j	609[a]	260[a]	0.43
8k	82[a]	NT[b]	—
8l	72	NT	—
8	36	NT	—
8o	636	411[a]	0.65
8p	639[a]	NT	—

[a] Cassette dosing: 4 or 5 compounds were administered at the same time. The dose was 1 mg/kg for each compound.
[b] Not tested.

5.1.2. Ethyl (1*S*,3*R*,4*S*)-4-{[(benzyloxy)carbonyl]amino}-3-[(*tert*-butoxycarbonyl)amino] cyclohexanecarboxylate (2)

To a solution of ethyl (1*S*,3*R*,4*S*)-4-azido-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylate (**1**) (303 g, 907 mmol) in EtOAc (8.11 L) were added EtOH (400 mL) and 10% Pd/C (65.0 g). The mixture was stirred for 16 h at room temperature under hydrogen atmosphere (5 atm). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was suspended in THF (3.00 L) and saturated $NaHCO_3$ aqueous solution (3.00 L). To this suspension was added benzyl chloroformate (144 mL, 970 mmol) dropwise over 15 min under ice cooling. After stirring for 15 min under ice cooling, water (3.00 L) and EtOAc (6.00 L) were added. The organic layer was separated and the water layer was extracted with EtOAc (1.00 L). The combined organic layer was washed with brine (2.00 L), dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in EtOAc (1.00 L), and hexane (5.00 L) was added to the solution. The mixture was stirred for 11.5 h at room temperature, and cooled with an ice bath. The resultant precipitate was collected by filtration to give the title compound (255 g, 606 mmol, 67%) as a colorless powder. mp 130–132 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7.1 Hz), 1.26–1.40 (1H, m), 1.44 (9H, s), 1.50–1.65 (1H, m), 1.74–1.87 (1H, m), 1.89–2.08 (3H, m), 2.30–2.41 (1H, m), 3.65–3.73 (1H, m), 4.00–4.15 (1H, br), 4.13 (2H, q, J = 7.1 Hz), 4.50–4.72 (1H, br), 5.03–5.13 (2H, m), 5.18–5.34 (1H, br), 7.29–7.36 (5H, m). HRMS (ESI) *m/z*: Calcd for $C_{22}H_{33}N_2O_6$: 421.23386. Found: 421.23699 $(M+H)^+$.

5.1.3. Benzyl (1*S*,2*R*,4*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-[(dimethylamino)carbonyl] cyclohexylcarbamate (3)

To a solution of **2** (306 g, 727 mmol) in THF (3.00 L) were added water (750 mL) and LiOH (35.2 g, 1.47 mol). The mixture was stirred for 20 h at room temperature, and neutralized with 10% citric acid aqueous solution (1.20 L) under ice cooling, and diluted with EtOAc (6.00 L). The organic layer was separated and the water layer was extracted with EtOAc (1.00 L). The combined organic layer was

washed with brine (1.50 L) and dried over Na_2SO_4. Concentration of the solution in vacuo afforded (1*S*,3*R*,4*S*)-4-{[(benzyloxy)carbonyl]amino}-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylic acid (323 g) as a colorless solid. This compound was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 1.30–1.48 (1H, br), 1.43 (9H, s), 1.50–1.68 (1H, br), 1.70–1.88 (2H, br), 1.90–2.12 (2H, br), 2.35–2.57 (1H, br), 3.64–3.77 (1H, br), 4.00–4.14 (1H, br), 4.60–4.75 (0.5H, br), 5.02–5.14 (2.5H, m), 5.20–5.35 (0.5H, m), 6.30–6.50 (0.5H, br), 7.29–7.37 (5H, m). MS (ESI) *m/z*: 393 $(M+H)^+$.

To a solution of (1*S*,3*R*,4*S*)-4-{[(benzyloxy)carbonyl]amino}-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylic acid (322 g) in CH_2Cl_2 (3.00 L) were added dimethylamine hydrochloride (119 g, 1.45 mol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (209 g, 1.09 mol), 1-hydroxybenzotriazole (HOBt) (148 g, 1.10 mol), and Et_3N (101 mL, 724 mmol). After stirring for four days at room temperature, 10% citric acid aqueous solution (6.00 L) and CH_2Cl_2 (3.00 L) were added. After the precipitate was removed by filtration, the organic layer was separated and washed successively with saturated $NaHCO_3$ aqueous solution (1.50 L), a mixture of 10% citric acid aqueous solution (5.00 L) and brine (6.00 L), saturated $NaHCO_3$ aqueous solution (1.50 L), and then brine (1.50 L). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in EtOAc (1.00 L), and hexane (1.50 L) was added to this solution. The resultant precipitate was collected by filtration to give the title compound (230 g, 548 mmol, 76%) as a colorless solid. mp: 129–132 °C. ^{1}H NMR ($CDCl_3$) δ: 1.23–1.41 (2H, m), 1.44 (9H, s), 1.68–2.05 (4H, m), 2.56–2.66 (1H, m), 2.90 (3H, s), 3.03 (3H, s), 3.67–3.77 (1H, br), 4.09–4.17 (1H, m), 4.60–4.75 (1H, br), 5.03–5.25 (3H, m), 7.29–7.37 (5H, m). MS (ESI) *m/z*: 420 $(M+H)^+$. HRMS (ESI) *m/z*: Calcd for $C_{22}H_{34}N_3O_5$: 420.24985. Found: 420.25018 $(M+H)^+$.

5.1.4. *tert*-Butyl (1*R*,2*S*,5*S*)-2-amino-5-[(dimethylamino)carbonyl]-cyclohexylcarbamate (4)

To a solution of **3** (189 g, 454 mmol) in MeOH (8.00 L) was added 10% Pd/C (57.0 g) and the mixture was stirred for 3 h at room temperature under hydrogen atmosphere (7 atm). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. After codistillation of EtOH with toluene, hexane (1.50 L) was added to the residue. The resultant precipitate was collected by filtration to give the title compound (121 g, 424 mmol, 93%) as a colorless powder. ^{1}H NMR ($CDCl_3$) δ: 1.20–1.77 (6H, m), 1.45 (9H, s), 2.20–2.35 (1H, br), 2.63–2.74 (1H, m), 2.92 (3H, s), 3.02 (3H, s), 3.02–3.11 (2H, m), 3.74–3.82 (1H, m), 4.88–5.00 (1H, br). MS (ESI) *m/z*: 286 $(M+H)^+$.

5.1.5. *tert*-Butyl (1*R*,2*S*,5*S*)-2-{[(7-chloroimidazo[1,2-*a*]pyridin-2-yl)carbonyl]amino}-5-[(dimethylamino)carbonyl]cyclohexylcarbamate (6a)

(i) *Hydrolysis step:* To a solution of ethyl 7-chloroimidazo[1,2-*a*]pyridine-2-carboxylate (**5a**) (118 mg, 0.525 mmol) in THF (5 mL) were added LiOH (13.8 mg, 0.578 mmol) and water (2 mL) at room temperature. The mixture was stirred for 3 h and the solvent was evaporated to give lithium 7-chloroimidazo[1,2-*a*]pyridine-2-carboxylate.

(ii) *Condensation step:* The residue was dissolved in DMF (10 mL) and to the mixture were added **4** (136 mg, 0.477 mmol), HOBt (71.0 mg, 0.525 mmol), and EDC·HCl (192 mg, 1.00 mmol). After stirring overnight, the mixture was concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The water layer was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. To the residue was added Et_2O and the resultant precipitate was collected by filtration to give the title compound (203 mg, 0.438 mmol, 92%) as a pale yellow powder. ^{1}H NMR ($CDCl_3$) δ: 1.43 (9H, br s), 1.47–2.15 (6H, m), 2.62–2.75 (1H, m), 2.95 (3H, s), 3.06 (3H, s), 4.12–4.22 (1H, m), 4.23–4.30 (1H, br), 4.81–4.90 (1H, m), 6.83 (1H, dd, J = 7.3, 2.0 Hz), 7.50–7.58 (2H, m), 8.06 (1H, dd, J = 7.3, 0.7 Hz), 8.10 (1H, d, J = 0.7 Hz). MS (ESI) *m/z*: 464 $(M+H)^+$.

5.1.6. *tert*-Butyl (1*R*,2*S*,5*S*)-2-{[(5-chloro-1*H*-benzimidazol-2-yl)-carbonyl]amino}-5-[(dimethylamino)carbonyl]cyclohexylcarbamate (6e)

To a solution of **3** (235 mg, 0.558 mmol) in THF (5 mL) was added 10% Pd/C (50 mg) and the mixture was stirred overnight at room temperature under hydrogen atmosphere (ambient pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in DMF (5 mL) and to this solution were added **5e**[19] (165 mg, 0.844 mmol), HOBt (100 mg, 0.740 mmol), and EDC·HCl (171 mg, 0.892 mmol). After stirring for four days, the mixture was concentrated in vacuo. To the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed ($MeOH/CH_2Cl_2$ = 1:10) to give the title compound (250 mg, 0.539 mmol, 97%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.01–2.00 (6H, m), 1.34 (9H, s), 2.79 (3H, s), 2.80–2.95 (1H, m), 2.98 (3H, s), 3.89–4.06 (2H, m), 7.08 (1H, d, J = 6.6 Hz), 7.31 (1H, d, J = 8.5 Hz), 7.62 (2H, br s), 8.47 (1H, d, J = 8.5 Hz), 13.46 (1H, br s). IR (KBr) cm^{-1}: 3660, 3163, 2971, 1709, 1668, 1622, 1552, 1423, 1242, 1159, 1066, 1057. MS (ESI) *m/z*: 464 $(M+H)^+$.

5.1.7. *N*-{(1*R*,2*S*,5*S*)-2-{[(7-Chloroimidazo[1,2-*a*]pyridin-2-yl)carbonyl]amino}-5-[(dimethylamino)carbonyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (8a)

To a suspension of **6a** (203 mg, 0.438 mmol) in CH_2Cl_2 (5 mL) was added saturated HCl ethanolic solution (5 mL) and the mixture was stirred for 2 h. The solvent was evaporated and to the residue were added MeOH and Et_2O. After concentration, Et_2O was added again and the resultant precipitate was collected by filtration to give *N*-{(1*S*,2*R*,4*S*)-2-amino-4-[(dimethylamino)carbonyl]cyclohexyl}-7-chloroimidazo[1,2-*a*]pyridine-2-carboxamide (193 mg) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.41–1.56 (1H, m), 1.69–1.90 (3H, m), 1.92–2.08 (2H, m), 2.82 (3H, s), 3.07 (3H, s), 3.23–3.33 (1H, m), 3.74 (1H, br s), 4.05–4.15 (1H, m), 7.28 (1H, dd, J = 7.3, 2.0 Hz), 7.86 (1H, d, J = 2.0 Hz), 8.30 (3H, br s), 8.64 (1H, d, J = 6.8 Hz), 8.68 (1H, s), 8.80 (1H, d, J = 7.3 Hz). MS (ESI) *m/z*: 364 $(M+H)^+$.

This solid was dissolved in DMF (5 mL) and lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylate (**7**) (134 mg, 0.657 mmol), HOBt (88.8 mg, 0.657 mmol), and EDC·HCl (168 mg, 0.876 mmol) were added. After stirring for three days, the mixture was concentrated in vacuo. To the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed ($MeOH/CH_2Cl_2$ = 1:9). The obtained compound was dissolved in 1 N HCl ethanolic solution and concentrated in vacuo. To the residue was added Et_2O and the resultant precipitate was collected by filtration to give the title compound (112 mg, 0.175 mmol, 40%) as a colorless powder. Mp 190–195 °C (decomp.). ^{1}H NMR (DMSO-d_6) δ: 1.45–1.60 (1H, m), 1.60–1.90 (3H, m), 1.95–2.25 (2H, m), 2.79 (3H, s), 2.92 (3H, s), 2.93 (3H, s), 3.05–3.40 (2H, m), 3.48 (1H, br s), 3.71 (1H, br s), 4.16 (1H, br s), 4.30–4.90 (4H, br), 7.11–7.22 (1H, m), 7.68–7.82 (1H, m), 8.45 (1H, s), 8.59–8.74 (2H, m), 8.75–8.95 (1H, m), 11.53 (0.5H, br s), 11.65 (0.5H, br s). MS (FAB) *m/z*: 544 $(M+H)^+$. Anal. Calcd for $C_{25}H_{30}ClN_7O_3S\cdot1.6HCl\cdot2H_2O$: C, 47.03; H, 5.62; Cl, 14.44; N, 15.36; S, 5.02. Found: C, 46.83; H, 5.74; Cl, 14.51; N, 15.26; S, 5.29.

5.1.8. *tert*-Butyl (1*R*,2*S*,5*S*)-2-azido-5-[(dimethylamino)carbonyl]cyclohexylcarbamate (9)

Compound **1** (5.00 g, 16.0 mmol) was dissolved in THF (130 mL) and water (25 mL). To this solution was added LiOH (510 mg, 21.3 mmol) under ice cooling. After the mixture was stirred at room temperature for 4 h, LiOH (200 mg, 8.35 mmol) was added. The mixture was stirred at room temperature for an additional 20 h, and then neutralized with 1 N HCl aqueous solution (29 mL). EtOAc and brine were added to the mixture. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo to give (1*S*,3*R*,4*S*)-4-azido-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylic acid (4.77 g) as a colorless solid, which was used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ: 1.45 (9H, s), 1.82–2.10 (6H, m), 2.74–2.76 (1H, m), 3.83–4.05 (2H, br), 5.71 (1H, br s).

To a solution of (1*S*,3*R*,4*S*)-4-azido-3-[(*tert*-butoxycarbonyl)-amino]cyclohexanecarboxylic acid (4.77 g) in CH_2Cl_2 (150 mL) were added dimethylamine hydrochloride (3.26 g, 40.0 mmol), EDC·HCl (4.60 g, 24.0 mmol), HOBt (3.24 g, 24.0 mmol), and *N*-methylmorpholine (8.09 g, 80.0 mmol). The mixture was stirred at room temperature for 18 h. Saturated $NaHCO_3$ aqueous solution was added to the mixture, and the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 1:50) to give the title compound (4.90 g, 15.7 mmol, 98%) as an amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.60–2.09 (6H, m), 2.75–2.81 (1H, m), 2.93 (3H, s), 3.01 (3H, s), 3.73–3.77 (1H, m), 4.10–4.12 (1H, m), 4.59 (1H, br s). MS (FAB) *m/z*: 312 (M+H)$^+$.

5.1.9. *N*-{(1*R*,2*S*,5*S*)-2-Azido-5-[(dimethylamino)carbonyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide (10)

To a solution of **9** (9.13 g, 29.2 mmol) in CH_2Cl_2 (100 mL) was added saturated HCl ethanolic solution (100 mL), and the mixture was stirred at room temperature for 1 min. The mixture was concentrated in vacuo, and the residue was dissolved in DMF (200 mL). To this solution were added **7** (10.0 g, 50.4 mmol), HOBt (4.47 g, 29.2 mmol), and EDC·HCl (16.8 g, 87.7 mmol). The mixture was stirred overnight at room temperature and concentrated in vacuo. To the residue was added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 3:50) to give the title compound (7.38 g, 18.9 mmol, 64%) as a yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.72–1.97 (4H, m), 2.10–2.27 (2H, m), 2.51 (3H, s), 2.80–3.00 (11H, m), 3.68 (1H, d, *J* = 15.4 Hz), 3.74 (1H, d, *J* = 15.4 Hz), 3.88–3.93 (1H, m), 4.54–4.60 (1H, m), 7.24 (1H, d, *J* = 7.6 Hz). MS (FAB) *m/z*: 392 (M+H)$^+$.

5.1.10. *N*-{(1*R*,2*S*,5*S*)-2-Amino-5-[(dimethylamino)carbonyl]-cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide (11)

To a solution of **10** (274 mg, 0.700 mmol) in EtOH (10 mL) was added 10% Pd/C (300 mg) and the mixture was stirred for 20 h at room temperature under a hydrogen atmosphere (ambient pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo to give the title compound (208 mg, 0.569 mmol, 81%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.40–1.90 (6H, m), 2.30–2.40 (1H, m), 2.51 (3H, s), 2.65–3.00 (6H, m), 2.92 (3H, s), 2.99 (3H, s), 3.10–3.20 (1H, m), 3.65–3.78 (2H, m), 4.20–4.30 (1H, m), 7.47–7.54 (1H, m). MS (ESI) *m/z*: 366 (M+H)$^+$.

5.1.11. *N*-{(1*R*,2*S*,5*S*)-2-{[(5-Chloro-1-benzofuran-2-yl)carbonyl]-amino}-5-[(dimethylamino)carbonyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (8b)

Compound **10** (260 mg, 0.665 mmol) was reduced to amine **11** according to the procedure 5.1.10 and the amine was dissolved in DMF (30 mL). To this solution were added **5b** (108 mg, 0.550 mmol), HOBt (84 mg, 0.55 mmol), and EDC (211 mg, 1.10 mmol). After stirring overnight, the mixture was concentrated in vacuo, and the residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 3:97) to give a pale yellow solid. This solid was dissolved in CH_2Cl_2 (2 mL) and 1 N HCl ethanolic solution (0.405 mL, 0.405 mmol) was added. This solution was concentrated in vacuo, and to the residue was added Et_2O. The resultant precipitate was collected by filtration to give the title compound (195 mg, 0.318 mmol, 58%) as a pale yellow solid. Mp 174–178 °C. ^{1}H NMR (DMSO-d_6) δ: 1.46–1.59 (1H, m), 1.62–1.88 (3H, m), 1.90–2.14 (2H, m), 2.80 (3H, s), 2.90 (3H, s), 2.97 (3H, s), 2.98–3.08 (1H, m), 3.11–3.32 (2H,m), 3.56 (2H, br s), 4.05–4.15 (1H, m), 4.32–4.80 (3H, m), 7.46 (1H, dd, *J* = 8.8, 2.2 Hz), 7.51 (1H, s), 7.67 (1H, d, *J* = 8.8 Hz), 7.86 (1H, *J* = 2.0 Hz), 8.50 (1H, d, *J* = 7.8 Hz), 8.69 (1H, d, *J* = 7.8 Hz), 11.76 (1H, br s). MS (FAB) *m/z*: 544 (M+H)$^+$. Anal. Calcd for $C_{26}H_{30}ClN_5O_4S{\cdot}0.9HCl{\cdot}2H_2O$: C, 50.95; H, 5.74; Cl, 10.99; N, 11.43; S, 5.23. Found: C, 51.15; H, 5.81; Cl, 11.10; N, 11.62; S, 5.38.

5.1.12. Ethyl 7-chloroimidazo[1,2-*a*]pyridine-2-carboxylate (5a)

Ethyl bromopyruvate (**12**) (1.26 mL, 9.05 mmol) and 2-amino-4-chloropyridine (**13**) (1.09 g, 8.46 mmol) were suspended in 1,2-dimethoxyethane (50 mL) and the mixture was refluxed for 2 h. After cooling, the resultant precipitate was collected by filtration. This solid was dissolved in CH_2Cl_2 and the organic layer was washed with saturated $NaHCO_3$ aqueous solution, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 3:97) to give the title compound (880 mg, 3.92 mmol, 46%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.44 (3H, t, *J* = 7.1 Hz), 4.46 (2H, q, *J* = 7.1 Hz), 6.87 (1H, dd, *J* = 7.3, 2.0 Hz), 7.68 (1H, d, *J* = 2.0 Hz), 8.06 (1H, *J* = 7.3 Hz), 8.16 (1H, s). MS (FAB) *m/z*: 225 (M+H)$^+$.

5.1.13. 5-Chloro-1-benzofuran-2-carboxylic acid (5b)

To a solution of 5-chlorosalicylaldehyde (**14**) (7.84 g, 50.1 mmol) in EtOAc (50 mL) were added diethyl bromomalonate (32.0 mL, 190 mmol) and K_2CO_3 (21.0 g, 152 mmol) and the mixture was refluxed for 9 h. The mixture was diluted with EtOAc, washed with 2 N HCl aqueous solution, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in 10% KOH ethanolic solution. After refluxing for 3 h, 2 N HCl aqueous solution was added to the solution to acidify it. The resultant precipitate was collected by filtration and dissolved in ethanol. Water was added to this solution and the resultant precipitate was collected by filtration to give the title compound (6.78 g, 34.5 mmol, 69%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 7.53 (1H, dd, *J* = 8.8, 2.0 Hz), 7.63 (1H, s), 7.76 (1H, d, *J* = 8.8 Hz), 7.87 (1H, d, *J* = 2.0 Hz). IR (KBr) cm^{-1}: 1689, 1574, 1302, 1281, 1061, 945, 930, 893. MS (EI) *m/z*: 196 (M$^+$). Anal. Calcd for $C_9H_5ClO_3$: C, 54.99; H, 2.56; Cl, 18.03. Found: C, 54.94; H, 2.57; Cl, 18.04.

5.1.14. 4-Chloro-2-methoxybenzylalcohol (16)

To a solution of 4-chloro-*o*-anisacid (**15**) (20.0 g, 107 mmol) in THF (100 mL) was added boran–dimethylsulfide complex (11.0 mL, 115 mmol) in portions. Reflux occurred automatically by the heat of the reaction. After the reflux was over, water and EtOAc were added. The organic layer was separated, washed successively with saturated $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. The solvent was evaporated to give the title compound (14.8 g, 86.0 mmol, 80%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 2.23 (1H, t, *J* = 5.9 Hz), 3.85 (3H, s), 4.63 (2H, d, *J* = 5.9 Hz), 6.86 (1H, s), 6.92 (1H, d, *J* = 7.8 Hz), 7.20 (1H, d, *J* = 7.8 Hz). IR (KBr)

cm^{-1}: 1599, 1579, 1493, 1404, 1248, 1036, 1005, 881. MS (EI) *m/z* 172 (M^+).

5.1.15. 4-Chloro-2-methoxybenzaldehyde (17)

To a solution of oxalyl chloride (15.0 mL, 172 mmol) in CH_2Cl_2 (200 mL) was added DMSO (15.0 mL, 211 mmol) in portions at −78 °C and the mixture was stirred for 10 min. To the mixture was added alcohol **16** (14.6 g, 84.7 mmol) in CH_2Cl_2 (40 mL) and the mixture was warmed up to −40 °C over 1 h. Then Et_3N (46.5 mL, 334 mmol) was added and the mixture was stirred for 15 min at room temperature. 2 N HCl aqueous solution was added and the mixture was diluted with CH_2Cl_2. The organic layer was separated, washed successively with saturated $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed (EtOAc/hexane = 1:10). The obtained solid was recrystallized from hexane to give the title compound (9.37 g, 54.9 mmol, 65%) as colorless needles. ^1H NMR ($CDCl_3$) δ: 3.94 (3H, s), 6.99 (1H, s), 7.02 (1H, d, J = 8.8 Hz), 7.77 (1H, d, J = 8.8 Hz), 10.39 (1H, s). IR (KBr) cm^{-1} 3423, 2877, 1678, 1593, 1574, 1483, 1475, 1410, 1396, 1093, 1022, 883, 860, 814. MS (FAB) *m/z*: 171 $(M+H)^+$. Anal. Calcd for $C_8H_7ClO_2$: C, 56.33; H, 4.14; Cl, 20.78. Found: C, 56.33; H, 4.11; Cl, 20.49.

5.1.16. 4-Chlorosalicylaldehyde (18)

To a solution of **17** (8.07 g, 47.3 mmol) in DMF (80 mL) was added LiCl (6.17 g, 146 mmol) and the mixture was refluxed for 40 h. After cooling, 10% NaOH aqueous solution (145 mL) and Et_2O were added. The water layer was separated, acidified with 10% HCl aqueous solution, and extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed (EtOAc/hexane = 1:25) to give the title compound (4.64 g, 29.6 mmol, 63%) as a pale yellow solid. Mp 51 °C. ^1H NMR ($CDCl_3$) δ: 7.00 (1H, d, J = 8.8 Hz), 7.01 (1H, s), 7.49 (1H, d, J = 8.8 Hz), 9.86 (1H, s), 11.07 (1H, s). IR (KBr) cm^{-1}: 1658, 1618, 1566, 1487, 1308, 1225, 1190, 1124, 1076, 926, 860, 806.

5.1.17. 6-Chloro-1-benzofuran-2-carboxylic acid (5c)

To a solution of **18** (4.56 g, 29.1 mmol) in 2-butanone (30 mL) were added diethyl bromomalonate (10.0 mL, 59.4 mmol) and K_2CO_3 (12.4 g, 89.9 mmol). After being refluxed for 9 h, the mixture was diluted with EtOAc and neutralized with 2 N HCl aqueous solution. The organic layer was separated, washed successively with saturated $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was dissolved in 10% KOH ethanolic solution (80 mL) then refluxed for 4 h. The mixture was concentrated to about half volume and acidified with 2 N HCl aqueous solution. The resultant precipitate was collected by filtration and dissolved in EtOH. Water was added to the solution and the resultant precipitate was collected by filtration to give the title compound (5.12 g, 26.0 mmol, 89%) as a colorless solid. ^1H NMR (DMSO-d_6) δ: 7.40 (1H, dd, J = 7.8, 2.0 Hz), 7.68 (1H, s), 7.80 (1H, d, J = 7.8 Hz), 7.90 (1H, br s). IR (KBr) cm^{-1}: 1689, 1614, 1585, 1572, 1435, 1338, 1308, 1292, 1248, 1059, 949, 933, 856, 820. MS (FAB) *m/z* 197 $(M+H)^+$.

5.1.18. 4-Chloro-2-nitrophenyl thiocyanate (20)

4-Chloro-2-nitroaniline (**19**) (17.3 g, 100 mmol) was dissolved in a mixed solvent of concd H_2SO_4 (30 mL) and water (30 mL) and the solution was cooled to 0 °C. To this solution was added 20% sodium nitrite aqueous solution (37.5 mL) and the mixture was stirred for 2 h at 0 °C, then potassium thiocyanate (10.0 g, 104 mmol) was added. The mixture was poured into a solution of cupric thiocyanate (18.0 g, 148 mmol) in water (60 mL) at 0–5 °C. The resultant mixture was stirred for 2 h at 0–5 °C then for 20 min at 70 °C. After cooling, the mixture was filtered and the precipitate was washed with benzene. The organic layer of the filtrate was separated and the water layer was extracted with benzene. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (EtOAc/hexane = 1:8) to give the title compound (11.9 g, 55.3 mmol, 55%) as a pale yellow solid. ^1H NMR (DMSO-d_6) δ: 7.54 (1H, dd, J = 8.8, 2.2 Hz), 8.02 (1H, d, J = 2.2 Hz), 8.35 (1H, d, J = 8.8 Hz). MS (EI) *m/z*: 214 (M^+). Anal. Calcd for $C_7H_3N_2ClO_2S$: C, 39.17; H, 1.41; N, 13.05; Cl, 16.52; S, 14.94. Found: C, 39.08; H, 1.37; N, 12.97; Cl, 16.35; S, 14.92.

5.1.19. 2-Amino-5-chlorobenzothiazole (21)

To a concd HCl aqueous solution (60 mL) were added **20** (5.00 g, 23.3 mmol) and tin (20 g, 0.17 mol), and the mixture was stirred overnight at room temperature then at 100 °C for 3 h. After cooling, the mixture was diluted with water and basified by adding ammonia aqueous solution. After stirring overnight, the tin was filtered and washed successively with benzene, MeOH, THF, CH_2Cl_2 and 1 N NaOH aqueous solution. The water layer was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (EtOAc/hexane = 2:3). The obtained solid was triturated with hexane to give the title compound (1.86 g, 10.0 mmol, 43%) as a colorless solid. ^1H NMR (DMSO-d_6) δ: 7.21 (1H, dd, J = 8.5, 2.2 Hz), 7.30 (1H, d, J = 8.5 Hz), 7.58 (2H, br s) 7.77 (1H, d, J = 2.2 Hz). MS (FAB) *m/z*: 185 (M^+). Anal. Calcd for $C_7H_6N_2ClS$: C, 45.53; H, 2.73; N, 15.17; Cl, 19.20; S, 17.37. Found: C, 45.51; H, 2.78; N, 15.13; Cl, 19.03; S, 17.28.

5.1.20. 2-Bromo-5-chlorobenzothiazole (22)

To 10 mL of DMF were added $CuBr_2$ (2.41 g, 10.8 mmol) and *tert*-butyl nitrite (1.62 mL, 13.5 mmol). The mixture was heated to 50 °C and **21** (1.67 g, 9.00 mmol) was added slowly over 30 min. The mixture was stirred for 45 min at 50 °C and for 30 min at 60 °C. The solvent was evaporated and the residue was chromatographed (EtOAc/hexane = 1:10) to give the title compound (995 mg, 4.00 mmol, 44%) as a pale brown solid. ^1H NMR ($CDCl_3$) δ: 7.44 (1H, dd, J = 8.6, 2.0 Hz), 7.79 (1H, d, J = 2.0 Hz), 7.89 (1H, d, J = 8.6 Hz).

5.1.21. Lithium 5-chlorobenzothiazole-2-carboxylate (5d)

Compound **22** (95 mg, 0.38 mmol) was dissolved in THF (3 mL) and the solution was cooled to −78 °C under argon atmosphere. To the solution was added *tert*-butyl lithium (1.45 M *n*-pentane solution, 0.290 mL, 0.420 mmol) and the mixture was stirred for 2 h at −78 °C, then carbon dioxide was bubbled into the solution for 30 min at the same temperature. After warming the mixture to room temperature, the solvent was evaporated and hexane was added to the residue. The resultant precipitate was collected by filtration to give the title compound (51 mg, 0.23 mmol, 61%) as a pale yellow solid. ^1H NMR (DMSO-d_6) δ: 7.48 (1H, dd, J = 8.8 Hz), 7.95 (1H, d, J = 8.8 Hz), 8.14 (1H, s).

5.1.22. (2-Chloro-5-nitropyridin-4-yl)acetonitrile (25a) and (6-chloro-3-nitropyridin-2-yl)acetonitrile (25b)

NaH (60% in oil, 5.36 g, 134 mmol) was added slowly to DMF (60 mL). The mixture was cooled to −10 °C under argon atmosphere and a solution of 2-chloro-5-nitropyridine (**23**) (9.65 g, 60.9 mmol) and (4-chlorophenoxy)acetonitrile (**24**) (11.2 g, 67.0 mmol) in DMF (120 mL) was added dropwise over 1 h. After stirring for 2 h, 1 N HCl aqueous solution (140 mL) was added. The mixture was extracted with Et_2O (twice). The combined organic layer was dried over Na_2SO_4 and treated with active-charcoal. The solvent was evaporated and the residue was chromatographed (EtOAc/hexane = 3:17) to give **25a** (2.50 g, 12.6 mmol, 21%, eluted

first) as a yellow solid and **25b** (761 mg, 3.85 mmol, 6%, eluted later) as a yellow solid. (Compound **25a**) ^{1}H NMR ($CDCl_3$) δ: 4.30 (2H, s), 7.78 (1H, s), 9.20 (1H, s). MS (EI) *m/z*: 197 $(M)^+$. (Compound **25b**) ^{1}H NMR ($CDCl_3$) δ: 4.40 (2H, s), 7.58 (1H, d, *J* = 8.4 Hz), 8.47 (1H, d, *J* = 8.4 Hz). MS (FAB) *m/z*: 198 $(M+H)^+$.

5.1.23. 5-Chloro-1*H*-pyrrolo[2,3-*c*]pyridine (26)

To a solution of **25a** (104 mg, 0.526 mmol) in EtOH (5 mL) was added Raney Ni (catalytic amount). The mixture was stirred for 5 h at room temperature under hydrogen atmosphere (ambient pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by preparative TLC (EtOAc/hexane = 1:1) to give the title compound (22 mg, 0.14 mmol, 27%) as a pale brown solid. ^{1}H NMR ($CDCl_3$) δ: 6.53–6.55 (1H, m), 7.43–7.44 (1H, m), 7.56 (1H, d, *J* = 0.7 Hz), 8.58 (1H, d, *J* = 0.7 Hz), 8.50–8.65 (1H, br). MS (EI) *m/z*: 153 $(M)^+$.

5.1.24. 5-Chloro-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine (27a)

To a suspension of **26** (90 mg, 0.59 mmol) in CH_2Cl_2 (5 mL) were added tetrabutylammonium hydrogensulfate (20 mg, 0.059 mmol), NaOH (59 mg, 1.5 mmol), and benzenesulfonyl chloride (0.113 mL, 0.885 mmol). After the mixture was stirred for 1 h, saturated NH_4Cl aqueous solution was added and the mixture was extracted with CH_2Cl_2 (twice). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC (EtOAc/hexane = 1:4) to give the title compound (163 mg, 0.557 mmol, 94%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 6.63 (1H, d, *J* = 3.7 Hz), 7.48–7.51 (3H, m), 7.60 (1H, t, *J* = 7.2 Hz), 7.72 (1H, d, *J* = 3.7 Hz), 7.91 (2H, d, *J* = 7.3 Hz), 9.06 (1H, s). MS (EI) *m/z*: 293 $(M)^+$.

5.1.25. Ethyl 5-chloro-1-(phenylsulfonyl)-1*H*-pyrrolo[2,3-*c*]pyridine-2-carboxylate (5f)

To a solution of **27a** (200 mg, 0.683 mmol) in a mixed solvent of Et_2O (7 mL) and THF (3 mL) was added *tert*-butyl lithium (1.6 M *n*-pentane solution, 1.07 mL, 1.71 mmol) at −78 °C under argon atmosphere. After stirring for 2 h at −78 °C, ethyl chloroformate (0.163 mL, 1.71 mmol) was added. The mixture was warmed to 0 °C and stirred for 3 h. Saturated NH_4Cl aqueous solution and Et_2O were added and the organic layer was separated and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by preparative TLC (EtOAc/hexane = 1:3) to give the title compound (143 mg, 0.392 mmol, 57%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.41 (3H, t, *J* = 7.1 Hz), 4.45 (2H, q, *J* = 7.1 Hz), 7.01 (1H, s), 7.52–7.56 (3H, m), 7.63–7.67 (1H, m), 8.08–8.10 (2H, m), 9.23 (1H, s).

5.1.26. 5-Chloro-1-phenylsulfonyl-1*H*-pyrrolo[3,2-*b*]pyridine (27b)

To a solution of **25b** (761 mg, 3.85 mmol) in EtOH (40 mL) was added Raney Ni (catalytic amount) and the mixture was stirred for 3 h at room temperature under hydrogen atmosphere (ambient pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (40 mL) and tetrabutylammonium hydrogensulfate (131 mg, 0.385 mmol), NaOH (385 mg, 9.60 mmol), and benzenesulfonyl chloride (0.739 mL, 5.78 mmol) were added. After stirring for 6.5 h, saturated NH_4Cl aqueous solution was added and the mixture was extracted with CH_2Cl_2 (twice). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (EtOAc/hexane = 1:3) to give the title compound (294 mg, 1.00 mmol, 26%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 6.81 (1H, dd, *J* = 3.8, 0.7 Hz), 7.26 (1H, d, *J* = 8.7 Hz), 7.47–7.52 (2H, m), 7.58–7.63 (1H, m), 7.81 (1H, d, *J* = 3.8 Hz), 7.85–7.88 (2H, m), 8.23 (1H, dd, *J* = 8.7, 0.7 Hz). MS (ESI) *m/z*: 293 $(M+H)^+$.

5.1.27. Ethyl 5-chloro-1-(phenylsulfonyl)-1*H*-pyrolo[3,2-*b*]pyridine-2-carboxylate (5g)

Compound **5g** was synthesized from **27b** according to the procedure used to prepare **5f**. A pale yellow solid. Yield 40%. ^{1}H NMR ($CDCl_3$) δ: 1.40 (3H, t, *J* = 7.1 Hz), 4.42 (2H, q, *J* = 7.1 Hz), 7.21 (1H, d, *J* = 0.7 Hz), 7.36 (1H, d, *J* = 8.9 Hz), 7.52–7.56 (2H, m), 7.65 (1H, tt, *J* = 7.4, 1.4 Hz), 8.03–8.05 (2H, m), 8.41 (1H, dd, *J* = 8.9, 0.7 Hz).

5.1.28. Methyl 5-chloro-4-fluoroindole-2-carboxylate (31)

EtOH (100 mL) was added to NaH (60% in oil, 4.66 g, 0.116 mmol) at 0 °C under argon atmosphere, and the mixture was stirred for 10 min. 2-Nitropropane (11.4 mL, 0.126 mmol) was added to the mixture, and after stirring for 10 min, 1-(bromomethyl)-3-chloro-2-fluorobenzene (**28**) (10.0 g, 44.8 mmol) was added, followed by stirring at room temperature for 3.5 h. The precipitate was removed by filtration, and the filtrate was concentrated in vacuo. To the residue were added Et_2O and water. The organic layer was separated, washed successively with 1 N NaOH aqueous solution, water, and brine, and was dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed (EtOAc/hexane = 3:7) to give a pale yellow oil.

MeOH (20 mL) was added to NaH (60% in oil, 1.6 g) under argon atmosphere at 0 °C, and the mixture was stirred for 10 min. Methyl azidoacetate (4.95 g, 43.0 mmol) and the pale yellow oil, which was obtained above, were dissolved in MeOH (10 mL) and this solution was added to the mixture at −20 °C within 20 min. The mixture was warmed to 0 °C and stirred for 2.5 h. Then water (40 mL) was added. The mixture was concentrated in vacuo, and the residue was extracted with a mixture of CH_2Cl_2 and EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed (toluene/hexane = 3:17) to give a pale yellow oil.

This oil was dissolved in xylene (50 mL), and the solution was stirred at 130–140 °C for 3 h. The mixture was concentrated in vacuo, and the residue was chromatographed (CH_2Cl_2). To the residue were added Et_2O and hexane, and the resultant precipitate was collected by filtration to give the title compound (440 mg, 1.94 mmol, 4%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 4.08 (3H, s), 7.20 (1H, s), 7.31–7.38 (2H, m). MS (FAB) *m/z*: 228 $(M+H)^+$. Anal. Calcd for $C_{10}H_7NO_2ClF$: C, 52.77; H, 3.10; N, 6.15; Cl, 15.58; F, 8.35. Found: C, 52.63; H, 3.08; N, 5.95; Cl, 15.95; F, 8.30.

5.1.29. 5-Chloro-4-fluoroindole-2-carboxylic acid (5h)

Compound **31** (440 mg, 1.94 mmol) was dissolved in THF (10 mL), and an aqueous solution (5 mL) of LiOH (160 mg, 6.68 mmol) was added, followed by stirring at room temperature for 3 h. To the mixture was further added an aqueous solution (5 mL) of LiOH (240 mg, 10.0 mmol), and the mixture was stirred at room temperature for an additional 1 h. The mixture was concentrated in vacuo, and the residue was neutralized with 1 N HCl aqueous solution. The mixture was extracted with EtOAc (three times). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated to give the title compound (392 mg, 1.84 mmol, 95%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 6.79 (1H, s), 7.16–7.26 (2H, m). MS (FAB) *m/z*: 214 $(M+H)^+$. Anal. Calcd for $C_9H_5NO_2ClF$: C, 50.61; H, 2.36; N, 6.56; Cl, 16.60; F, 8.89. Found: C, 50.41; H, 2.30; N, 6.49; Cl, 16.57; F, 8.74.

5.1.30. Ethyl 2-[(4-chloro-2-fluorophenyl)hydrazono]propanoate (33)

4-Chloro-2-fluoroaniline (**32**) (3.20 mL, 29.2 mmol) was dissolved in a mixed solvent of water (60 mL) and concd HCl aqueous solution (12 mL) and the mixture was cooled to 0 °C. To this solution was slowly added sodium nitrite (2.17 g, 31.4 mmol). After

stirring for 30 min, to the mixture were added ethyl 2-methylacetoacetate (4.50 mL, 31.2 mmol) in water (60 mL), EtOH (30 mL), and 50% KOH aqueous solution (10 mL). The pH of the mixture was adjusted to 4–5 by adding 30% sodium acetate aqueous solution. The mixture was stirred for 21 h and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (EtOAc/hexane = 1:10). The obtained compound was dissolved in saturated HCl ethanolic solution. The mixture was stirred for 1 h and diluted with CH_2Cl_2. The organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed (EtOAc/hexane = 1:20) to give the title compound (2.43 g, 9.39 mmol, 32%) as a dark brown solid. ^{1}H NMR (DMSO-d_6) δ: 1.36 (3H, t, J = 7.1 Hz), 2.17 (3H, s), 4.30 (2H, q, J = 7.1 Hz), 7.00–7.30 (2H, m), 7.45–7.70 (1H, m), 12.00–12.30 (1H, br). MS (FAB) m/z: 258 $(M)^+$.

5.1.31. Ethyl 5-chloro-7-fluoroindole-2-carboxylate (34)

Compound **33** (2.43 g, 9.39 mmol) and polyphosphoric acid (10.0 g) were heated at 100 °C for 2 h. Ice-water and CH_2Cl_2 were added to the mixture and the organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (EtOAc/hexane = 1:10) and the obtained compound was triturated with hexane to give the title compound (529 mg, 2.19 mmol, 23%) as a colorless solid. Mp 178–179 °C. ^{1}H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7.1 Hz), 4.43 (2H, q, J = 7.1 Hz), 7.05 (1H, dd, J = 10.5, 1.7 Hz), 7.17 (1H, m), 7.45 (1H, d, J = 1.7 Hz), 9.16 (1H, br). IR (KBr) cm^{-1}: 3290, 1703, 1537, 1379, 1333, 1298, 1248, 1194. MS (FAB) m/z: 241 $(M)^+$.

5.1.32. 5-Chloro-7-fluoroindole-2-carboxylic acid (5i)

Compound **5i** was synthesized from **34** in a manner similar to that described for **5h**. A colorless crystal. Quantitative yield. Mp > 230 °C. ^{1}H NMR (DMSO-d_6) δ: 7.14 (1H, s), 7.21 (1H, d, J = 10.7 Hz), 7.57 (1H, s), 12.49 (1H, s), 13.26 (1H, br s). IR (KBr) cm^{-1}: 3413, 1662, 1543, 1439, 1298, 1265. MS (FAB) m/z: 214 $(M+H)^+$. Anal. Calcd for $C_9H_5ClFNO_2$: C, 50.61; H, 2.36; Cl, 16.60; F, 8.89; N, 6.56. Found: C, 50.60; H, 2.44; Cl, 16.69; F, 8.77; N, 6.22.

5.1.33. Ethyl 1-benzyl-5-chloroindole-2-carboxylate (36)

To a solution of ethyl 5-chloroindole-2-carboxylate (**35**) (1.44 g, 6.44 mmol) in DMF (30 mL) were added K_2CO_3 (2.94 g, 21.3 mmol) and benzyl chloride (2.42 mL, 21.3 mmol). After stirring at 100 °C for 1.5 h, the mixture was concentrated, and the residue was poured into ice-water. The mixture was extracted with EtOAc and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed (EtOAc/hexane = 1:19). To the residue were added Et_2O and hexane, and the resultant precipitate was collected by filtration to give the title compound (1.59 g, 5.07 mmol, 79%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.36 (3H, t, J = 7.1 Hz), 4.33 (2H, q, J = 7.1 Hz), 5.83 (2H, s), 7.00–7.02 (2H, d, J = 7.6 Hz), 7.20–7.38 (6H, m), 7.67 (1H, d, J = 1.7 Hz).

5.1.34. Ethyl 1-benzyl-5-chloro-3-fluoroindole-2-carboxylate (37)

To a solution of **36** (2.19 g, 7.00 mmol) in CH_2Cl_2 (30 mL) was added 2,6-dichloro-1-fluoropyridinium triflate (4.35 g, 13.8 mmol), and the mixture was refluxed for three days. The mixture was partitioned between EtOAc and water. The water layer was extracted with EtOAc. The combined organic layer was washed successively with 1 N HCl aqueous solution, water, and brine, and dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed (EtOAc/hexane = 1:24) to give the crude title compound (2.75 g, ca. 8.29 mmol, ca. 60%). A portion of the compound was purified by preparative TLC for the NMR measurement. ^{1}H NMR (DMSO-d_6) δ: 1.25 (3H, t, J = 7.1 Hz), 4.29 (2H, q, J = 7.1 Hz), 5.77 (2H, s), 6.97–6.99 (2H, m), 7.18–7.28 (3H, m), 7.39 (1H, dd, J = 9.0, 2.1 Hz), 7.69 (1H, dd, J = 9.0, 2.1 Hz), 7.78 (1H, d, J = 2.1 Hz).

5.1.35. Ethyl 5-chloro-3-fluoroindole-2-carboxylate (38)

To a solution of crude **37** (1.43 g, ca. 2.28 mmol) in anisole (30 mL) was added $AlCl_3$ (2.88 g, 21.6 mmol) in small portions under ice cooling, followed by stirring at room temperature for 30 min. $AlCl_3$ (2.88 g, 21.6 mmol) was added again, and the mixture was stirred for 18 h. To the mixture was further added $AlCl_3$ (8.00 g, 60.0 mmol), and the mixture was stirred for 5 h. Water was added and the mixture was extracted with EtOAc. The organic layer was washed successively with saturated $NaHCO_3$ aqueous solution and brine and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed (CH_2Cl_2) to give the title compound (470 mg, 1.95 mmol, 84%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7.2 Hz), 4.45 (2H, q, J = 7.2 Hz), 7.25–7.31 (2H, m), 7.66 (1H, d, J = 0.7 Hz), 8.53 (1H, br s). MS(FAB) m/z: 242 $(M+H)^+$. Anal. Calcd for $C_{11}H_9NO_2ClF{\cdot}0.1H_2O$: C, 54.27; H, 3.81; N, 5.75; F, 7.80; Cl, 14.56. Found: C, 54.12; H, 3.88; N, 5.52; F, 7.92; Cl, 14.67.

5.1.36. 5-Chloro-3-fluoroindole-2-carboxylic acid (5j)

Compound **5j** was synthesized from **38** according to the procedure used to prepare **5h**. A colorless powder. Yield 80%. ^{1}H NMR (DMSO-d_6) δ: 7.31 (1H, dd, J = 8.8, 1.9 Hz), 7.42 (1H, dd, J = 8.8, 1.9 Hz), 7.70 (1H, d, J = 1.9 Hz), 11.78 (1H, s). MS (FAB) m/z: 214 $(M+H)^+$. Anal. Calcd for $C_9H_5NO_2ClF$: C, 50.61; H, 2.36; N, 6.56; F, 8.89; Cl, 16.60. Found: C, 50.89; H, 2.80; N, 6.40; F, 8.60; Cl, 16.27.

5.1.37. Ethyl 3-bromo-5-chloroindole-2-carboxylate (39)

N-Bromosuccinimide (440 mg, 2.46 mmol) was added to a solution of ethyl 5-chloroindole-2-carboxylate (**35**) (500 mg, 2.24 mol) in DMF (10 mL) under ice cooling. The mixture was stirred at room temperature for 18 h, and the solvent was evaporated. To the residue were added EtOAc and water, and the organic layer was separated. The water layer was extracted with EtOAc. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed (EtOAc/hexane = 1:9). The obtained colorless powder was triturated with hexane to give the title compound (480 mg, 1.59 mmol, 71%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.42–1.48 (3H, m), 4.43–4.49 (2H, m), 7.30–7.32 (2H, m), 7.65 (1H, d, J = 0.7 Hz), 9.11 (1H, s). MS (FAB) m/z: 303 $(M+H)^+$. Anal. Calcd for $C_{11}H_9NO_2BrCl$: C, 43.67; H, 3.00; N, 4.63; Cl, 11.72. Found: C, 43.36; H, 3.23; N, 4.49; Cl, 11.68.

5.1.38. 3-Bromo-5-chloroindole-2-carboxylic acid (5k)

Compound **5k** was synthesized from **39** according to the procedure used to prepare **5h**. A colorless powder. Yield 93%. ^{1}H NMR (DMSO-d_6) δ: 7.35 (1H, dd, J = 8.8, 2.0 Hz), 7.48–7.53 (2H, m), 12.33 (1H, s). MS (FAB) m/z: 275 [$(M+H)^+$, $^{81}Br^{35}Cl$, $^{79}Br^{37}Cl$]. Anal. Calcd for $C_9H_5NO_2BrCl$: C, 39.38; H, 2.05; N, 5.10; Cl, 12.92. Found: C, 39.28; H, 2.05; N, 4.96; Cl, 12.92.

5.1.39. Ethyl 5-fluoroindole-2-carboxylate (41)

To a suspension of 5-fluoroindole-2-carboxylic acid (**40**) (5.0 g, 28 mmol) in EtOH (100 mL) was added thionyl chloride (6.0 mL) dropwise at 0 °C and then the mixture was refluxed for 3 h. The mixture was concentrated in vacuo, and the residue was diluted with $CHCl_3$. The organic layer was washed successively with saturated $NaHCO_3$ aqueous solution and brine and dried over Na_2SO_4. The solvent was evaporated and hexane was added to the residue. The resultant precipitate was collected by filtration to give the title compound (4.8 g, 23 mmol, 83%) as a pale yellow solid. ^{1}H NMR

($CDCl_3$) δ: 1.42 (3H, t, J = 7.1 Hz), 4.41 (2H, q, J = 7.1 Hz), 7.09 (1H, dt, J = 9.0, 2.4 Hz), 7.18 (1H, dd, J = 2.4, 1.0 Hz), 7.31–7.37 (2H, m), 8.90 (1H, br s).

5.1.40. Ethyl 3-chloro-5-fluoroindole-2-carboxylate (42)

To a solution of **41** (2.0 g, 9.7 mmol) in DMF (20 mL) was added dropwise a solution of *N*-chlorosuccinimide (1.4 g, 11 mmol) in DMF (10 mL) under ice cooling. After stirring at room temperature for 18 h, the mixture was diluted with EtOAc and washed successively with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over Na_2SO_4, and the solvent was evaporated. The residue was chromatographed (EtOAc/hexane = 1:5) to give the title compound (1.9 g, 7.9 mmol, 80%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, t, J = 7.4 Hz), 4.46 (2H, q, J = 7.4 Hz), 7.14 (1H, dt, J = 8.0, 2.7 Hz), 7.32–7.36 (2H, m), 8.91 (1H, br s).

5.1.41. 3-Chloro-5-fluoroindole-2-carboxylic acid (5l)

Compound **5l** was synthesized from **42** in a manner similar to that described for **5h**. A pale brown solid. Quantitative yield. ^{1}H NMR (DMSO-d_6) δ: 7.20 (1H, td, J = 8.8, 2.4 Hz), 7.31 (1H, dd, J = 8.8, 2.4 Hz), 7.46 (1H, dd, J = 8.8, 4.4 Hz), 12.12 (1H, br s).

5.1.42. Ethyl 5-chloro-3-formylindole-2-carboxylate (43)

$POCl_3$ (2.0 mL, 22 mmol) was added to *N*-methylformanilide (2.9 g, 22 mmol), and after stirring for 15 min, 1,2-dichloroethane (50 mL) and **35** (4.0 g, 18 mmol) were added. After being refluxed for 1 h, the mixture was poured into an aqueous solution (28 mL) of sodium acetate (14 g) under ice cooling, and the mixture was stirred for 18 h. The precipitate was collected by filtration, and washed successively with water and Et_2O to give the title compound (3.6 g, 14 mmol, 78%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.38 (3H, t, J = 7.1 Hz), 4.44 (2H, q, J = 7.1 Hz), 7.38 (1H, dd, J = 8.0, 1.4 Hz), 7.56 (1H, d, J = 8.0 Hz), 8.19 (1H, d, J = 1.4 Hz), 10.53 (1H, s).

5.1.43. 5-Chloro-3-formylindole-2-carboxylic acid (5m)

Compound **5m** was synthesized from **43** according to the procedure used to prepare **5h**. A pale brown solid. Yield 96%. ^{1}H NMR (DMSO-d_6) δ: 7.39 (1H, d, J = 8.0 Hz), 7.55 (1H, d, J = 8.0 Hz), 8.20 (1H, s), 10.58 (1H, s), 12.90 (1H, br s).

5.1.44. 5-Chloro-2-(ethoxycarbonyl)indole-3-carboxylic acid (44)

Compound **43** (1.5 g, 6.0 mmol) and sulfamic acid (1.7 g, 18 mmol) were dissolved in a mixed solvent of *tert*-butanol (30 mL) and water (30 mL). To the solution was added sodium chlorite (1.6 g, 18 mmol), and the mixture was stirred for 8 h. The mixture was diluted with water, and the mixture was extracted with EtOAc. The organic layer was washed successively with 1 N HCl aqueous solution and brine, and dried over Na_2SO_4. The solvent was evaporated, and to the residue were added isopropyl ether and hexane. The resultant precipitate was collected by filtration to give the title compound (0.70 g, 2.6 mmol, 43%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.34 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.1 Hz), 7.33 (1H, dd, J = 8.0, 1.4 Hz), 7.52 (1H, d, J = 8.0 Hz), 7.97 (1H, d, J = 1.4 Hz), 12.75 (1H, br s).

5.1.45. Ethyl 5-chloro-3-[(dimethylamino)carbonyl]indole-2-carboxylate (45)

Compound **44** (0.70 g, 2.6 mmol) was dissolved in DMF (10 mL), and to the solution were added dimethylamine hydrochloride (0.26 g, 3.2 mmol), HOBt (0.43 g, 3.2 mmol), and EDC·HCl (1.0 g, 5.2 mmol), followed by stirring at room temperature for two days. The mixture was diluted with EtOAc, and washed successively with 1 N HCl aqueous solution, saturated $NaHCO_3$ aqueous solution, and brine. The organic layer was dried over Na_2SO_4. The solvent was evaporated and to the residue were added isopropyl ether and hexane. The resultant precipitate was collected by filtration to give the title compound (0.60 g, 2.0 mmol, 78%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.29 (3H, t, J = 7.1 Hz), 2.78 (3H, s), 3.04 (3H, s), 4.30 (2H, q, J = 7.1 Hz), 7.31 (1H, dd, J = 8.0, 1.4 Hz), 7.45 (1H, d, J = 1.4 Hz), 7.48 (1H, d, J = 8.0 Hz), 12.29 (1H, s).

5.1.46. 5-Chloro-3-[(dimethylamino)carbonyl]indole-2-carboxylic acid (5n)

Compound **5n** was synthesized from **45** in a manner similar to that described for **5h**. A pale brown solid. Yield 77%. ^{1}H NMR (DMSO-d_6) δ: 2.91 (6H, s), 7.29 (1H, d, J = 8.0 Hz), 7.44 (1H, d, J = 8.0 Hz), 7.47 (1H, s), 12.16 (1H, s).

5.1.47. Ethyl (1*S*,3*R*,4*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-{[(5-fluoroindol-2-yl)carbonyl]amino}cyclohexanecarboxylate (47a)

To a solution of **1** (500 mg, 1.60 mol) in MeOH (10 mL) was added 10% Pd/C (50 mg) and the mixture was stirred for 3 h under hydrogen atmosphere (ambient pressure). The catalyst was removed by filtration and the filtrate was concentrated in vacuo to give ethyl (1*S*,3*R*,4*S*)-4-amino-3-[(*tert*-butoxycarbonyl)amino]-cyclohexanecarboxylate (**46**). This amine was used in the next step without further purification.

Compound **46** was dissolved in CH_2Cl_2 (10 mL) and DMF (10 mL). To this solution were added 5-fluoroindole-2-carboxylic acid (**40**) (345 mg, 1.92 mmol), EDC·HCl (460 mg, 2.40 mmol), HOBt (325 mg, 2.40 mmol), and *N*-methylmorpholine (485 mg, 4.80 mmol). After stirring for 15 h at room temperature, the solvent was evaporated and to the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed ($MeOH/CH_2Cl_2$ = 1:50) to give the title compound (740 mg, quantitative yield) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.1 Hz), 1.52 (9H, s), 1.67–2.41 (7H, m), 3.97 (1H, br s), 4.15 (2H, q, J = 7.1 Hz), 4.08–4.22 (1H, m), 4.85 (1H, br s), 6.83 (1H, s), 7.00–7.05 (1H, m), 7.32–7.36 (1H, m), 7.90–8.00 (1H, m), 8.02 (1H, s), 9.51 (1H, s). MS (FAB) *m/z* 448 $(M+H)^+$.

5.1.48. Ethyl (1*S*,3*R*,4*S*)-4-{[(5-fluoroindol-2-yl)carbonyl]-amino}-3-{[(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]amino}cyclohexanecarboxylate (48a)

To a solution of **47a** (740 mg) in EtOH (15 mL) was added 4 N HCl in dioxane (2 mL) and after stirring for 20 h at room temperature, the solvent was evaporated. The residue was dissolved in DMF (20 mL) and to the solution were added **7** (390 mg, 1.90 mmol), HOBt (325 mg, 2.40 mmol), EDC·HCl (460 mg, 2.40 mmol), and Et_3N (810 mg, 8.00 mmol). After stirring for three days, the mixture was concentrated in vacuo and the residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed ($MeOH/CH_2Cl_2$ = 1:25) to give the title compound (380 mg, 0.720 mmol, two steps, 45%) as a pale yellow amorphous. ^{1}H NMR (CD_3OD) δ: 1.29 (3H, t, J = 7.1 Hz), 1.60–2.34 (6H, m), 2.53 (3H, s), 2.61–2.68 (1H, m), 2.80–2.88 (2H, m), 2.96–2.99 (2H, m), 3.75 (2H, s), 4.12–4.14 (1H, m), 4.18 (2H, q, J = 7.1 Hz), 4.59–4.60 (1H, m), 6.86 (1H, s), 6.99–7.04 (1H, m), 7.27–7.34 (2H, m), 7.47 (1H, d, J = 7.1 Hz), 7.92 (1H, d, J = 5.6 Hz), 9.13 (1H, s). MS (FAB) *m/z*: 528 $(M+H)^+$.

5.1.49. *N*-{(1*R*,2*S*,5*S*)-5-[(Dimethylamino)carbonyl]-2-{[(5-fluoroindol-2-yl)carbonyl]amino}cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (8o)

To a solution of **48a** (380 mg, 0.720 mmol) in THF (10 mL) and H_2O (1.2 mL) was added LiOH (23 mg, 0.94 mmol) and the mixture

was stirred for 17 h. The solvent was evaporated and the residue was dissolved in DMF (5 mL) and CH_2Cl_2 (5 mL). To this solution were added dimethylamine hydrochloride (74 mg, 0.91 mmol), EDC·HCl (130 mg, 0.680 mmol) HOBt (93 mg, 0.68 mmol), and *N*-methylmorpholine (230 mg, 2.23 mmol). After stirring for 19 h, the solvent was evaporated and the residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 1:10) to give the title compound as a free form (147 mg). This solid was dissolved in MeOH and 1 N HCl ethanolic solution (280 μL) was added. The solvent was evaporated and to the residue was added Et_2O. The resultant precipitate was collected to give the title compound (140 mg, 0.240 mmol, 33%) as a pale yellow solid. Mp >270 °C. ^{1}H NMR (DMSO-d_6) δ: 1.48–2.00 (6H, m), 2.60–3.30 (5H, m), 2.80 (3H, s), 2.91 (3H, s), 2.98 (3H, s), 3.70–4.68 (4H, m), 7.00–7.06 (2H, m), 7.37–7.42 (2H, m), 8.36–8.41 (2H, m), 11.12 (1H, br s), 11.69 (1H, s). IR (KBr) cm^{-1}: 3276, 2940, 2539, 2364, 1625, 1531, 1259, 954, 767, 588, 543, 439. MS (FAB) *m/z*: 527 (M+H)$^+$. Anal. Calcd for $C_{26}H_{31}FN_6O_3S$·HCl·1.5H_2O: C, 52.92; H, 5.98; Cl, 6.01; F, 3.22; N, 14.24; S, 5.43. Found: C, 52.78; H, 6.13; Cl, 6.04; F, 3.12; N, 14.00; S, 5.61.

5.1.50. *N*-{(1*S*,2*R*,5*S*)-2-{[(5-Bromoindol-2-yl)carbonyl]amino}-5-(ethoxycarbonyl)cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide (48b)

To a solution of **46** (3.10 g, 10.8 mmol) in CH_2Cl_2 (100 mL) were added 5-bromoindole-2-carboxylic acid (2.38 g, 9.93 mmol), HOBt (1.52 g, 9.93 mmol), and EDC·HCl (2.28 g, 11.9 mmol). After stirring overnight, the mixture was concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 1:50) to give ethyl (1*S*,3*R*,4*S*)-4-{[(5-bromoindol-2-yl)carbonyl]amino}-3-[(*tert*-butoxycarbonyl)amino]cyclohexanecarboxylate (**47b**) as a colorless solid.

This solid was dissolved in CH_2Cl_2 (50 mL) and to the solution was added saturated HCl ethanolic solution (100 mL). After the completion of reaction, the mixture was concentrated in vacuo to give a pale yellow solid. This solid was dissolved in DMF (300 mL) and **7** (2.14 g, 11.5 mmol), HOBt (234 mg, 1.53 mmol), EDC·HCl (1.91 g, 9.95 mmol), and Et_3N (1.06 mL, 7.65 mmol) were added. After stirring for three days, the mixture was concentrated in vacuo. To the residue were added 300 mL of a mixed solvent of CH_2Cl_2 and MeOH (10:1) and water (300 mL). The organic layer was separated and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed (MeOH/EtOAc = 1:25) to give the title compound (2.00 g, 3.40 mmol, 34%) as a pale yellow amorphous. ^{1}H NMR (DMSO-d_6) δ: 1.29 (3H, t, J = 6.8 Hz), 1.57–1.80 (2H, m), 2.03–2.37 (4H, m), 2.52 (3H, m), 2.59–2.61 (1H, m), 2.78–2.88 (2H, m), 2.91–3.02 (2H, m), 3.69–3.80 (2H, m), 4.11–4.25 (3H, m), 4.58–4.69 (1H, m), 6.84 (1H, s), 7.27–7.38 (2H, m), 7.50 (1H, d, J = 7.6 Hz), 7.76 (1H, s), 8.01 (1H, d, J = 5.9 Hz), 10.01 (1H, s). MS (FAB) *m/z*: 588 (M+H)$^+$.

5.1.51. *N*-{(1*S*,2*R*,5*S*)-2-{[(5-Bromoindol-2-yl)carbonyl]amino}-5-[(dimethylamino)carbonyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (8p)

Compound **8p** was synthesized from **48b** according to the procedure used to prepare **8o**. A colorless solid. Yield 67%. ^{1}H NMR (DMSO-d_6) δ: 1.48–1.56 (1H, m), 1.71–1.83 (3H, m), 1.97–1.99 (2H, m), 2.80 (3H, s), 2.92 (3H, s), 2.98 (3H, s), 3.01–3.08 (1H, m), 3.20 (2H, br s), 3.48 (1H, br s), 3.68 (1H, br s), 4.09–4.14 (1H, m), 4.43 (1H, br s), 4.58–4.61 (1H, m), 4.67 (1H, br s), 7.05 (1H, d, J = 2.0 Hz), 7.28 (1H, dd, J = 8.6, 1.8 Hz), 7.37 (1H, d, J = 8.6 Hz), 7.83 (1H, d, J = 1.8 Hz), 8.40 (2H, d, J = 8.1 Hz), 11.41 (1H, br s), 11.80 (1H, br s). MS (FAB) *m/z*: 587 (M+H)$^+$. Anal. Calcd for $C_{26}H_{31}BrN_6O_3S$· HCl· 2.4H_2O: C, 46.80; H, 5.56; N, 12.60; S, 4.81. Found: C, 46.80; H, 5.30; N, 12.56; S, 4.84.

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

The 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 test compounds 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). The absorbance (OD) at 405 nm was monitored every 10 s with a SPECTRAmax 340 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) at room temperature and the reaction velocity (OD/min) was obtained. Anti-fXa activity (inhibition%) was calculated as follows: Anti-fXa activity = {1 − [(OD/min) of sample/(OD/min) of control]} × 100. The IC_{50} value was obtained by plotting the compound concentration against the anti-fXa activity.

5.3. 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 test compounds 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 compound required to double the clotting time (CT2) was estimated from the concentration-response curve by a regression analysis.

5.4. Anti fXa activity ex vivo

Male wister rats were fasted overnight. Test 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. Anti-fXa 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). The absorbance (OD) at 405 nm was monitored every 10 s with a SPECTRAmax 340 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) at room temperature and the reaction velocity (OD/min) was obtained. Anti-fXa activity (inhibition%) was calculated as follows: Anti-fXa activity = {1 − [(OD/min) of sample/(OD/min) of control]} × 100.

5.5. Metabolic stability and permeability

IGS-SD rat liver microsomes (male, pooled) were purchased from XenoTech LLC (Kansas City, KS, USA). The reaction mixtures consisted of 1.0 mg liver microsomal protein/mL in 100 mM sodium–phosphate buffer (pH 7.4), with a final concentration of 25 mM glucose-6-phosphate (G-6-P), 10 mM $MgCl_2$, 1 mM β-$NADP^+$, 2 units/mL G-6-P dehydrogenase, and 10 μM of test compounds (final CH_3OH concentration: 1%, v/v). The total volume of the reaction mixtures was 1.0 mL. Reactions were started by the addition of β-$NADP^+$ after a 3-min pre-incubation period at 37 °C. A 200-μL aliquot was removed and transferred to 800-μL of ice-cold MeOH containing I.S. after 5 min of incubation. After centrifu-

gation at 1500*g* for 10 min, the supernatants were evaporated to dryness and dissolved in 400 μL of a 40:60 (v/v) mixture of 10 mM Ammonium acetate and MeOH. A 20-μL aliquot was injected into the LC–MS system. All reactions were performed in duplicate. The disappearance rate (V_{ini}) was calculated using Eq. 1.

$$V_{ini}\ (\text{nmol/min/mg of protein}) = 10\ (\text{nmol/mL}) \times \left(1 - \frac{\text{PAR}_{t=5\ \text{min}}}{\text{PAR}_{t=0\ \text{min}}}\right) \times \frac{1}{5\ (\text{min})} \times \frac{1}{1\ (\text{mg of protein/mL})} \quad (1)$$

$$\text{Peak area ratio(PAR)} = \frac{\text{Peak area(test compound)}}{\text{Peak area(IS)}}$$

Permeability was measured according to the literature,[20] with slight modifications. Evaluation is based on the ratio toward permeability of atenolol (AT ratio).

5.6. Distribution coefficient and protein binding

The distribution coefficient and protein binding were measured as previously described.[6]

5.7. Pharmacokinetic analysis in cynomolgus monkeys

Female cynomolgus monkeys (3–4 years old) were fasted overnight before the administration. The test compounds were administered orally (1 mg/kg, 0.5% (w/v) methylcellulose suspension, 2 mL/kg, via a stomach tube) in a fasted or non-fasted condition. For the non-fasted condition, 5 pieces of solid food (CPD#5048, LabDiet) were given to each monkey 30 min before the oral administration. Before and 30 min, 1, 2, 4, 8, 24 h after oral administration, blood samples (1.35 mL) were collected from the femoral vein into syringes containing 150 μL of 3.13% trisodium citrate dehydrate. The blood samples were centrifuged at 1500*g* for 10 min to obtain the plasma fraction. The plasma samples were stored at −40 °C until the measurement of the compound concentration. The plasma concentrations of the compounds were measured by HPLC–MS–MS using an Alliance 2690 HPLC system (Waters) and Quattro Ultima (Micromass). The HPLC conditions were as follows: column, symmetry C_{18} (2.1 mm × 150 mm); eluent, 10 mM $AcONH_4$/MeOH = 30:70; flow rate, 0.2 mL/min; column temperature, 40 °C. The chemical composition of CPD#5048 is as follows: protein 26%; fat 5%; carbohydrate, 49% (For more information, see Supplementary data).

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Supplementary data

Supplementary data (Characterizations of further intermediates as well as the final compounds are supplied) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.023.

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9. The permeability assay was conducted using Caco-2 monolayers. The permeability of compound A was more than 30 times higher than that of atenolol. And it is considered that compound A possesses enough permeability.
10. The solubility was assayed using Japanese Pharmacopoeia First Fluid (JP1, pH 1.2) and Japanese Pharmacopoeia Second Fluid (JP2, pH 6.8). JP1 and JP2 simulate gastric pH and intestinal pH respectively. The minimum solubility of compound **A** was 14 μg/mL in pH 6.8. Assuming the effective dose is 60 mg/man, its dose number (Do) is calculated to be ca. 17 according to Eq. 2.

$$\text{Dose Number(Do)} = \frac{M/V_0}{C_s} \quad (2)$$

where C_s (mg/mL) is the solubility, M is the dose (mg/man), and V_0 is the volume of water taken with the dose, which is generally set to be 250 mL.
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