Bioorganic & Medicinal Chemistry 17 (2009) 8221–8233

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

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

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ARTICLE INFO

Article history:
Received 15 August 2009
Revised 10 October 2009
Accepted 13 October 2009
Available online 17 October 2009

Keywords:
Anticoagulant
Factor Xa inhibitor
Solubility
Food effect

ABSTRACT

A series of *cis*-1,2-diaminocyclohexane derivatives possessing a 6–6 fused ring for the S1 moiety were synthesized as novel factor Xa (fXa) inhibitors. The synthesis, structure–activity relationship (SAR), and physicochemical properties are reported herein, together with the discovery of compound **45c**, which has potent anti-fXa activity, good physicochemical properties and pharmacokinetic (PK) profiles, including a reduced negative food effect.

1. Introduction

Thromboembolic disorders including acute myocardial infarction, deep vein thrombosis, pulmonary embolism, and ischemic stroke are the leading cause of morbidity and mortality in developed countries. Anticoagulants currently in clinical use for the prevention and treatment of these diseases include parentally administered unfractionated heparin and low molecular weight heparins (LMWHs), as well as orally administered warfarin (coumarins). Warfarin inhibits the biosynthesis of prothrombin, coagulation factor VII, IX, and X. This mechanism leads to a slow onset of action. Additionally, careful monitoring of the clotting time to achieve efficacy and dose titration to minimize excessive bleeding are necessary.[1] Thus, a novel anticoagulant which can be administered more conveniently and safely is desired.[2,3] Factor Xa (fXa) is a key serine protease located at the convergence of the intrinsic and extrinsic pathways.[4] In the prothrombinase complex consisting of fXa, factor Va, and Ca^{2+}, fXa catalyzes the conversion of prothrombin to thrombin. Thrombin is responsible for the formation of fibrin clots, the activation of platelets, and the feedback activation of other coagulation factors, resulting in the amplification of its own formation. It is anticipated that selective inhibition of fXa will provide an antithrombotic effect by diminishing the amplified formation of thrombin without compromising normal hemostasis and platelet activation because the basal thrombin level is maintained.[5] Therefore, fXa is a particularly attractive target and extensive clinical trials are now ongoing for a number of clinical candidates.[6,7]

In the preceding paper, we reported[8] the exploration of 5–6 fused ring systems as alternative S1 moieties for compound **A**, a potent fXa inhibitor selected as a clinical candidate (Fig. 1),[9] to reduce the negative food effect. The study indicated that the food effect could be reduced by improving solubility. We continued the

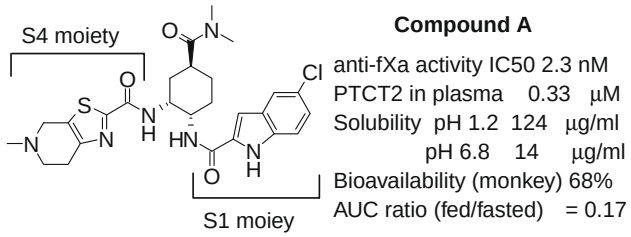

Figure 1. Properties of compound **A**.

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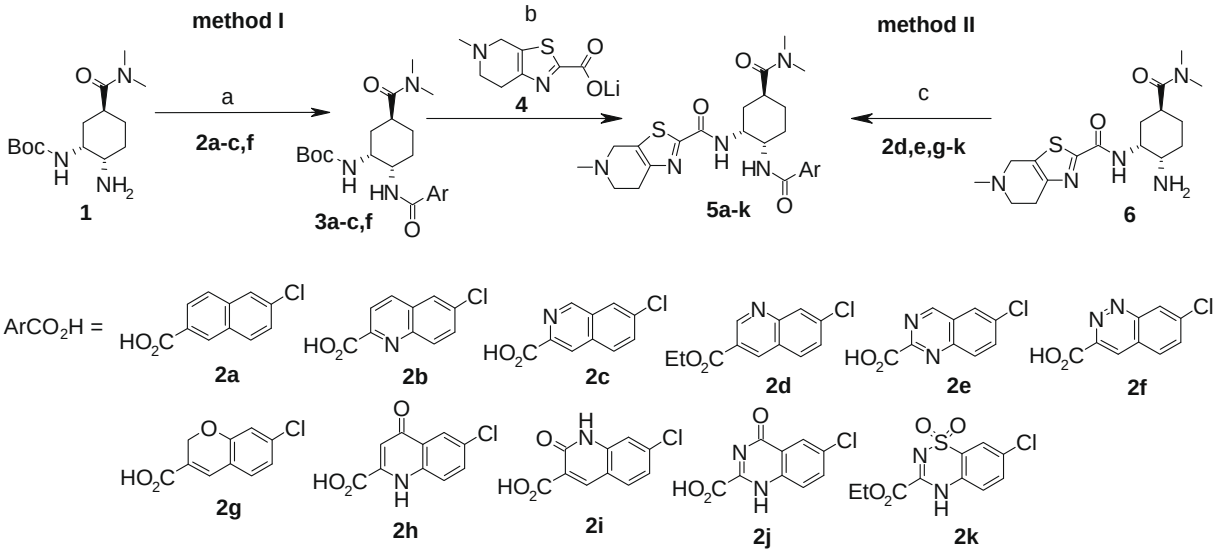
doi:10.1016/j.bmc.2009.10.024



Scheme 1. Reagents and conditions: (a) $ArCO_2H$ (**2a–c**, **f**), EDC·HCl, HOBt, DMF; (b) (i) HCl, EtOH, CH_2Cl_2; (ii) **4**, EDC·HCl, HOBt, DMF; (c) $ArCO_2H$ (**2d, e, g–k**), EDC·HCl, HOBt, DMF. In the case of **2d** and **2k**, corresponding carboxylic acids were prepared by hydrolysis prior to use.

optimization of our clinical candidate, compound **A**, aiming to obtain a more potent derivative possessing better PK profiles. In this paper, we report the results of the exploration of 6–6 fused ring systems as alternative S1 moieties, as well as the finding of a novel S4 moiety, which resulted in the discovery of **45c**.

2. Chemistry

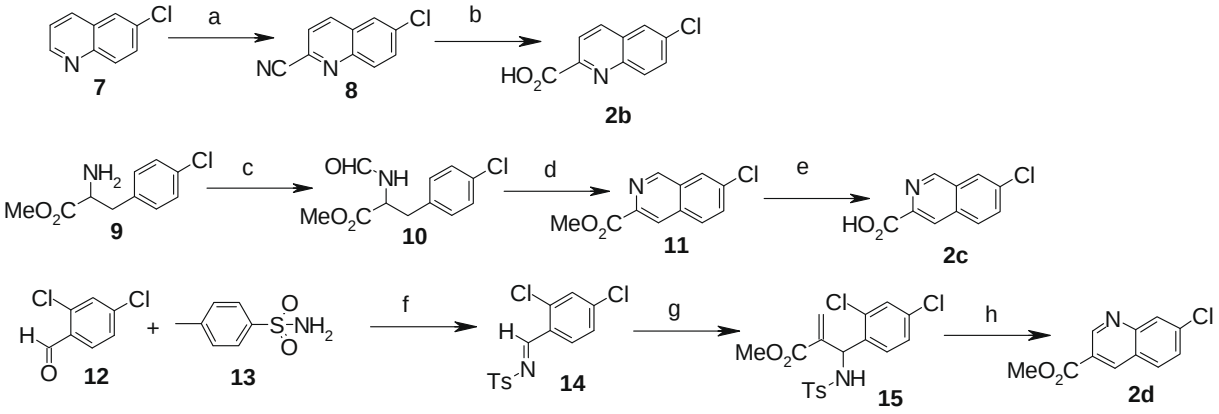
Compounds **5a–k** were synthesized as shown in Scheme 1. In method I, starting material amine **1**[8] was reacted with carboxylic acid **2** to give **3**. Removal of the Boc group on amide **3**, followed by condensation with lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylate (**4**)[10] afforded target compound **5**. In method II, condensation of amine **6**[8] with S1 fragment **2** led to target compound **5**. In the case of **2d** and **2k**, corresponding carboxylic acids were prepared by hydrolysis prior to use.

Reissert reaction[11] of **7** gave nitrile **8**, which was hydrolyzed to afford **2b** (Scheme 2). Phenethylamide **10** was prepared by the treatment of **9** with formic acid. Bischler–Napieralski reaction[12] of **10** directly afforded isoquinoline **11**, which was hydrolyzed to give carboxylic acid **2c**. Imine **14** was synthesized by the reaction of aldehyde **12** and sulfonamide **13**. Baylis–Hillman reaction[13] of **14** with methyl acrylate afforded **15**, which was employed in a cyclization reaction to give **2d**.[14]

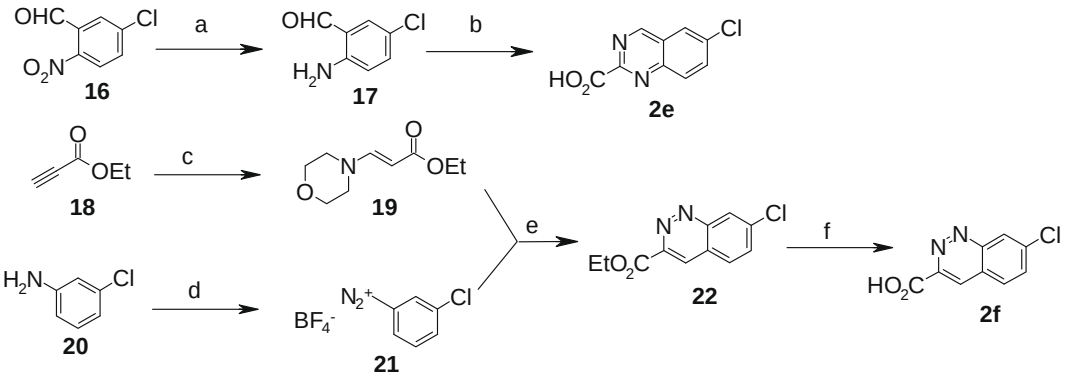
Reduction of nitro group of **16** afforded 2-amino-5-chlorobenzaldehyde (**17**), which was then cyclized to give **2e** (Scheme 3). Michael addition of morpholine to alkyne **18** provided **19**. Separately, diazonium salt **21** was prepared by treating aniline **20** with sodium nitrite. Compounds **19** and **21** were employed in the cyclization affording cinnoline **22**,[15] which was hydrolyzed to give **2f**.

Michael addition of **23** to **24** and successive Horner–Emmons type cyclization provided **25**,[16] which was hydrolyzed to afford carboxylic acid **2g** (Scheme 4).

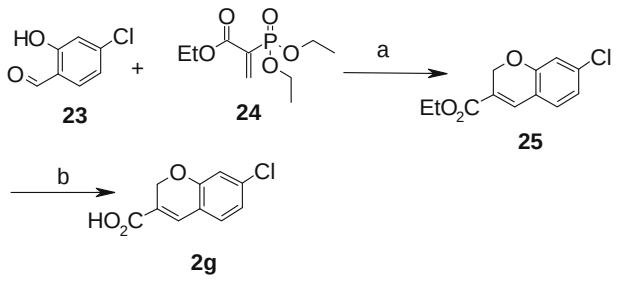
Michael addition of **26** to **27** afforded arylaminoacrylate, which was cyclized to quinolone **28** in a manner similar to a Conrad–Limpach reaction[17] (Scheme 5). Quinolone **28** was hydrolyzed to give **2h**. Knoevenagel condensation of **29** with diethyl malonate, followed by reduction of the nitro group and successive cyclization provided 2-quinolone **31**, which was hydrolyzed to give **2i**. Reaction of amide **32** with ethyl chlorooxoacetate (**33**) gave **34**, which was hydrolyzed to afford **2j**. 4-Chloroaniline (**35**) was treated with chlorosulfonylisocyanate, and subsequent intramolecular cyclization gave benzothiadiazin-3(4*H*)-one 1,1-dioxide **36**. Ring opening of **36** in 50% sulfuric acid, followed by condensation with **33**, affor-



Scheme 2. Reagents and conditions: (a) (i) *m*-CPBA, CH_2Cl_2; (ii) TMSCN, $ClCONMe_2$, CH_2Cl_2; (b) concd HClaq; (c) HCO_2H, EDC·HCl, HOBt, NMM, CH_2Cl_2; (d) (i) $(COCl)_2$, CH_2Cl_2; (ii) $FeCl_3$; (iii) concd H_2SO_4, MeOH; (e) concd HCl aq; (f) toluene, MS 4 Å, reflux; (g) methyl acrylate, DABCO, THF; (h) **13**, K_2CO_3, DMF.



Scheme 3. Reagents and conditions: (a) $NaHSO_3$, Na_2CO_3, H_2O, MeOH; (b) $AcONH_4$, glyoxylic acid, EtOH; (c) morpholine, CH_2Cl_2; (d) (i) $NaNO_2$, HCl, H_2O; (ii) $NaBF_4$; (e) CH_3CN, reflux; (f) NaOH, H_2O, THF.



Scheme 4. Reagents and conditions: (a) NaH, THF; (b) LiOH, THF, EtOH, H_2O.

ded **38**. Compound **2k** was finally obtained by cyclization of **38** with NaOMe.

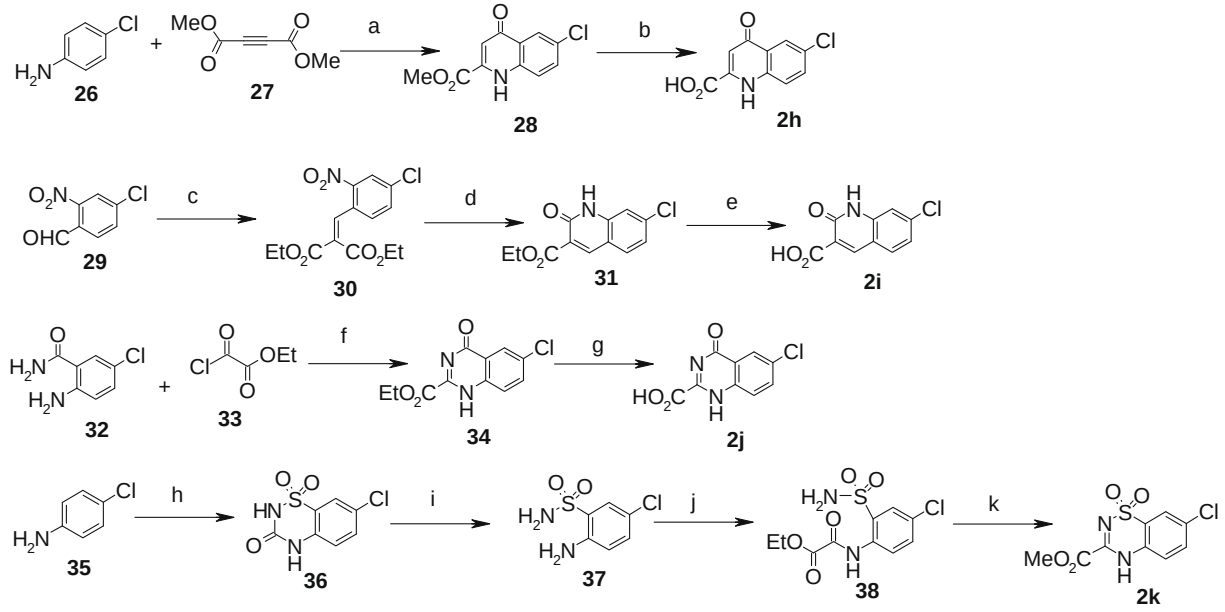
Substitution of dibromide **40**[18] with sulfonamide **39** gave dihydropyrrolothiazole **41** (Scheme 6). Removal of the sulfonyl group, followed by reductive methylation, gave **43**, which was treated with *tert*-BuLi and then successively with carbon dioxide to give **44**. The condensation of **3a** or **3c** with **44** afforded **45a** or **45c**, respectively.

Hydrolysis of **46**[9] and successive amidation with dimethylamine gave **47**, which was then treated with HCl and condensed with dihydropyrrolothiazole **44**, to afford **48** (Scheme 7).

3. Results and discussion

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

Naphthalene variant **5a** exhibited strong anti-fXa activity and improved solubility. The introduction of nitrogen at the 1-position (**5b**) of the naphthalene moiety caused a drastic decrease in activity, while introduction at the 3- or 4-position (**5c** or **5d**) sustained strong activity. In the case of diazanaphthalene derivatives, 3,4-diazanaphthalene (cinnoline) **5f** displayed almost the same



Scheme 5. Reagents and conditions: (a) (i) MeOH, reflux; (ii) Ph_2O, 240 °C; (b) NaOH, dioxane, H_2O; (c) diethyl malonate, $NaHCO_3$, Ac_2O; (d) Fe, AcOH; (e) NaOH, H_2O, THF, EtOH; (f) (i) Py; (ii) Ac_2O, AcOH; (g) LiOH, THF, H_2O; (h) (i) $ClSO_2NCO$, $EtNO_2$; (ii) $AlCl_3$; (i) 50% H_2SO_4aq; (j) **33**, Et_3N, AcOH; (k) MeONa, MeOH.

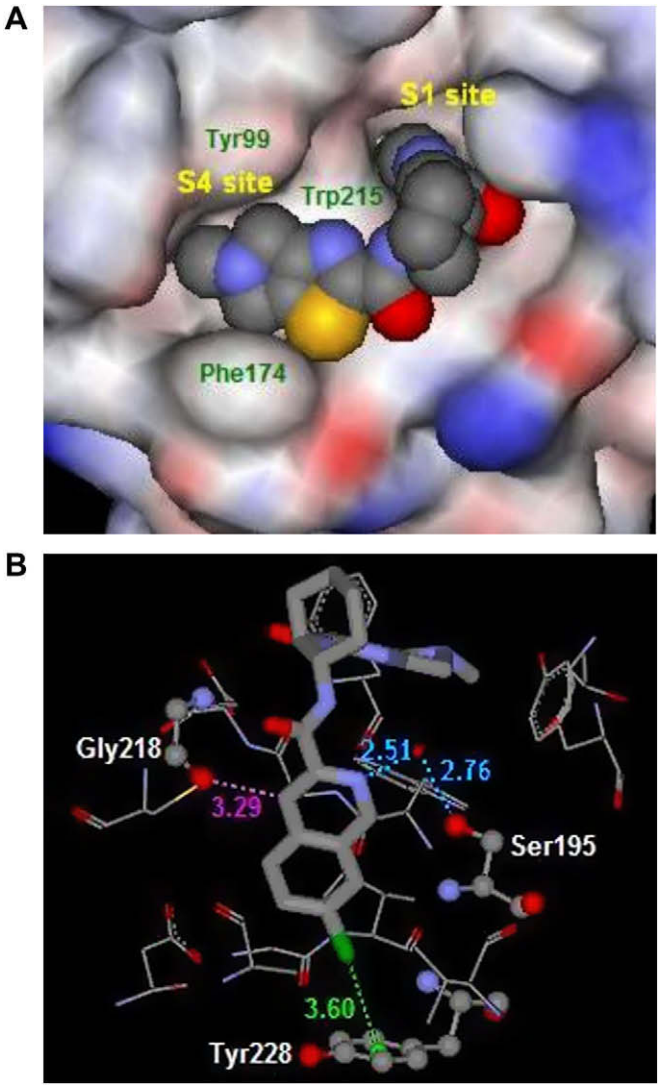


Figure 2. X-ray crystal structure of **45c** in fXa. (A) Surface of fXa S4 site and **45c** (CPK model) are illustrated. The S4 site consisted of Try99, Phe174, and Trp215. (B) The binding mode utilized in the S1 site is shown. There are water-mediated hydrogen bonds (blue line), an aromatic CH···O hydrogen bond (pink line), and Cl-aromatic contact (green line). Ser195, Gly218, and Tyr228 are depicted in the ball and stick model.

magnitude of activity as compound **A**, while 1,3-diazanaphtalene (quinazoline) **5e** was found to be much less potent. Compound **5f** also exhibited significant improvement of solubility in the neutral pH region. The anti-fXa activity of chromene **5g** decreased. 6–6 Fused ring derivatives possessing an NH moiety, which was expected to make a hydrogen bond between Gly218 of fXa like in compound **A**,[9] were then examined. Quinolone **5h**, 1*H*-quinazolin-4-one **5j**, and benzo[1,2,4]thiadiazine **5k** showed very strong anti-fXa activity. However, these compounds did not exhibit strong anti-fXa activity in a rat ex vivo assay except for **5j**. The results of the metabolic stability and permeability measurements are shown in Table 2. Unfortunately, it was revealed that the introduction of an NH moiety to increase the anti-fXa activity also resulted in a decrease in permeability (**5h**, **5i**, and **5j**).

Prospective compounds, **5a**, **5c**, **5f**, **5h**, and **5j**, which showed strong rat ex vivo activity and/or in vitro activity were administered to monkeys to obtain the PK profiles (Table 3). Compounds **5a** and **5c** exhibited relatively large AUC, compared to more polar compounds like **5f**, **5h** and **5j**. The negative food effects of **5a**, **5c**, and **5f**, which showed improved solubility, were smaller than that of compound **A**. Since **5a** and **5c** showed better PK profiles than the other compounds, further modification was conducted. Assuming that a smaller S4 moiety is preferable to further increase solubility, a dihydropyrrolo[3,4-*d*]thiazole (DHPT) structure was incorporated to the scaffolds of **5a, 5c**, and compound **A**. The assay results of these compounds are summarized in Table 4.

The introduction of DHPT improved solubility at pH 6.8 in all the compounds. Although the in vitro anti-fXa activity was slightly decreased, the anticoagulant activity of **45c** was comparable to compound **A**. In addition, **45c** showed the highest rat ex vivo activity among those compounds. Considering its anti-fXa activity, compound **45c** exhibited relatively strong anticoagulant activity compared with **45a** and **48**. It is probably due to the lower lipophilicity of compound **45c**. As previously described,[8,9] it is assumed that higher lipophilicity causes higher non-specific protein binding and larger decrease of anticoagulant activity in plasma. The PTCT2/IC_{50} ratio and log *D* value and protein binding were well correlated among these compounds (Table 5).

The food effect of **45c** in monkeys and dogs were then examined (Table 6). Compound **45c** exhibited a substantially smaller food effect than compound **A** in both monkeys and dogs. Moreover, the AUC of **45c** was significantly larger in dogs compared to compound **A**.

The X-ray crystal structure of **45c** in fXa was obtained at a resolution of 1.8 Å. Figure 2A and B show the binding mode of **45c** in

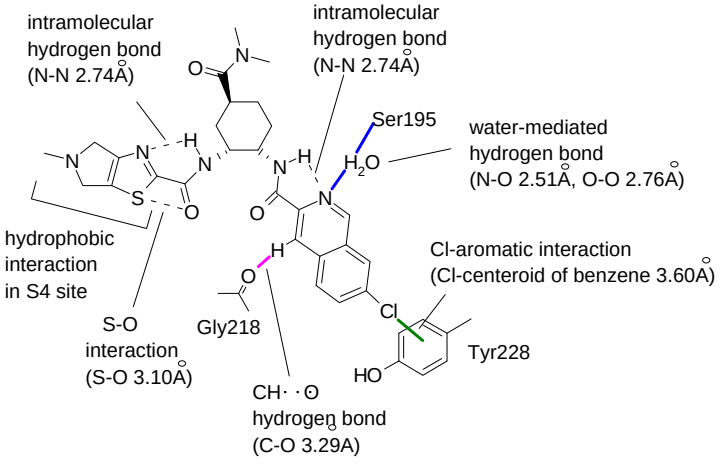
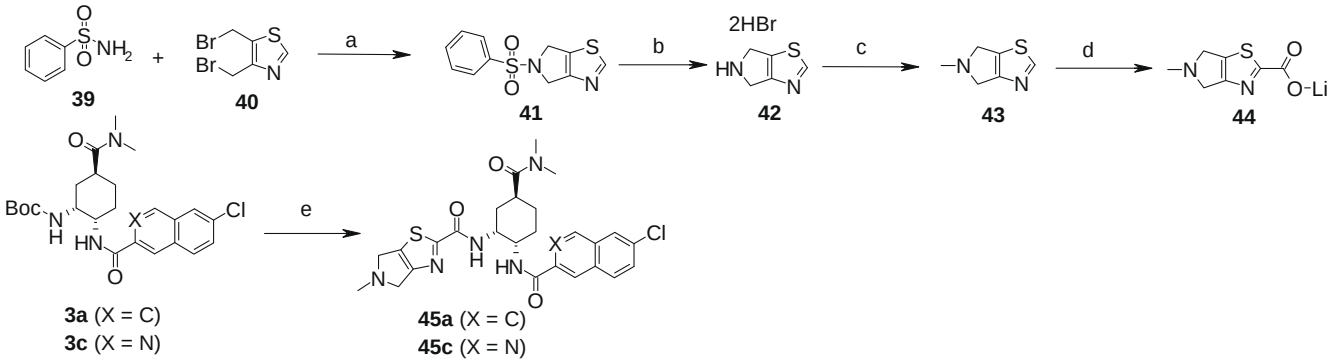
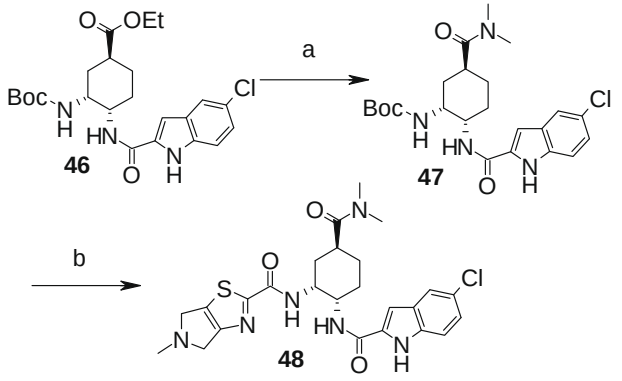


Figure 3. Major interactions between **45c** and fXa and intramolecular interactions.



Scheme 6. Reagents and conditions: (a) NaH, DMF; (b) 47% HBr aq, PhOH; (c) HCHO, Et_3N, AcOH, $NaBH(OAc)_3$, CH_2Cl_2; (d) (i) *tert*-BuLi, THF; (ii) CO_2 gas; (e) (i) HCl, EtOH; (ii) **44**, EDC·HCl, HOBt, DMF.



Scheme 7. Reagents and conditions: (a) (i) NaOH, THF, EtOH, H_2O; (ii) $MeNH_2$·HCl, EDC·HCl, HOBt, Et_3N, DMF; (b) (i) HCl, EtOH; (ii) **44**, EDC·HCl, HOBt, DMF.

the S4 and S1 sites, respectively. The major interactions are summarized in Figure 3.

(i) Interactions of **45c** with fXa (Fig. 2A and B): The DHPT moiety closely fits into the S4 site, the 3-azanaphthalene moiety fits into the S1 site, and cyclohexane moiety acts as a linker to connect these two motifs (Fig. 2A).[19] This binding mode is similar to those observed previously in our laboratory.[9,20] On the other hand, several specific binding modes were observed for compound **45c** with fXa. There is one water-mediated hydrogen bond between the nitrogen of 3-azanaphthalene and the hydroxyl group of Ser195 (Fig. 2B).[21] As well, the aromatic CH moiety in azanaphthalene seems to make a CH···O hydrogen bond[22] with the oxygen of Gly218 (Fig. 2B). It should be noted that the nitrogen of 3-azanaphthalene and the adjacent carbonyl oxygen are located in the opposite direction. This conformation is important to fit the chlorine atom into the cavity of the S1 site. Similar to other inhibitors,[21,23–25] the chlorine makes contact with the benzene ring of Tyr228 which is located at the bottom of the S1 site. (Fig. 2B).

(ii) Intramolecular interactions of **45c** (Fig. 3): The distance between nitrogen of azanaphthalene and adjacent amide nitrogen is 2.74 Å, indicating that there is an intramolecular hydrogen bond.[26] The distance between the sulfur of thiazole and the oxygen of the adjacent carbonyl group is 3.10 Å, which is slightly shorter than the sum of the van der Waals radius (3.25 Å). This result indicates the existence of an S–O interaction.[20,27,28] We assume that this interaction contributes to constrain the rotation of the S4 moiety.[29] As well, an intramolecular hydrogen bond between thiazole nitrogen and the adjacent amide hydrogen seems to work cooperatively.[30]

4. Conclusions

Exploration of 6–6 fused rings as alternative S1 moieties and the modification of S4 moiety were conducted. An S1 moiety having nitrogen at the 3- or 4-position was acceptable, but was unacceptable at the 1-position. On the contrary, an NH moiety at the 1-position contributed to strong anti-fXa activity. However, these compounds did not exhibit good PK profiles, probably due to insufficient permeability. In the course of further optimization, we have identified the novel S4 moiety dihydropyrrolo[3,4-*d*]thiazole, which led to the discovery of compound **45c**. Compound **45c** possesses strong anticoagulant activity, good physicochemical properties, excellent PK profiles, and a reduced food effect in both monkeys and dogs compared with compound **A**. The X-ray crystal structure of **45c** in fXa revealed its binding mode to the enzyme and also indicated the existence of intramolecular interactions. The findings described in this and the previous reports are valuable for further optimization, which will be reported in due course.

5. Experimental section

5.1. Chemistry

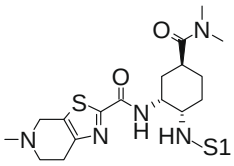
5.1.1. General

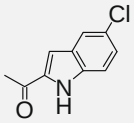
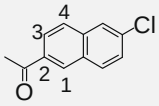
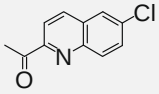
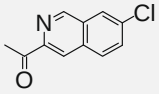
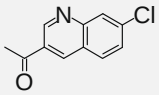
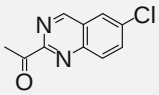
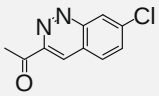
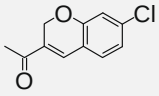
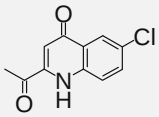
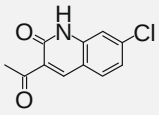
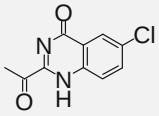
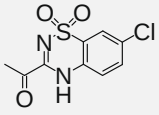
Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were determined on a YANACO MP-J3 or a BUCHI B-545 and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-EX-400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane as the internal standard. FAB mass spectra were recorded on a JEOL JMS-HX110 spectrometer. ESI mass spectra were recorded on a SCIEX API-150EX 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}.

5.1.2. *tert*-Butyl {(1*R*,2*S*,5*S*)-2-[(6-chloro-2-naphthoyl)amino]-5-[(dimethylamino)carbonyl]cyclohexyl}carbamate (3a)

To a solution of *tert*-butyl (1*R*,2*S*,5*S*)-2-amino-5-[(dimethylamino)carbonyl]cyclohexylcarbamate (**1**)[8] (0.17 g, 0.60 mmol) in DMF (5 mL) were added 6-chloronaphtalene-2-carboxylic acid (**2a**) (0.14 g, 0.68 mmol), 1-hydroxybenzotriazole (HOBt) (91 mg, 0.59 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (0.13 g, 0.68 mmol). After stirring for 22 h, the mixture was concentrated in vacuo. The residue was partitioned between AcOEt and water. The organic layer was dried

Table 1
Assay results of 6–6 fused ring derivatives



Compd	S1	fXa IC_{50} (nM)	PTCT2 (μM)[a] in human plasma	Solubility (μg/mL)		log D[b] (pH 6.8)	Ex vivo anti-fXa activity[c] (%)
				pH 1.2	pH 6.8		
A		2.3	0.33	124	14	2.8	62
5a		5.7	0.55	990	61	2.9	81
5b		4000	NT[d]	>1000	43	2.6	NT
5c		3	0.28	>1000	41	2.3	95
5d		4.1	0.44	>1000	110	2	38
5e		870	>20	NT	NT	NT	NT
5f		3.3	0.24	>1000	730	1.7	64
5g		35	1.5	>1000	270	2.7	43
5h		0.43	0.34	>1000	73	1.8	17
5i		12	0.56	>1000	23	2.1	4.2
5j		1.4	0.63	970	>1000	1.6	63
5k		4.9	0.89	>1000	>1000	0.8	1

[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.

Table 2
Metabolic stability and permeability

Compd	Metabolic stability[a] (nmol/min/mg)	Permeability AT ratio[b]
A	0.032	>30
5a	0.029	>30
5c	0.022	>30
5f	0.026	>30
5g	0.059	>30
5h	0.007	4.3
5i	0.017	5.2
5j	0.004	15.4

[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. Relative permeabilities are shown as a ratio compared to atenolol.

Table 3
PK profiles in monkeys (1 mg/kg, po) ($n = 3$)

Compd	AUC_{fasted} (ng h/mL)	AUC_{fed} (ng h/mL)	Ratio fed/fasted
A	985	172	0.17
5a	520	346[a]	0.67
5c	517	333[a]	0.64
5f	182[a]	97[a]	0.53
5h	0	NT[b]	—
5j	271	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.

over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed ($MeOH/CH_2Cl_2 = 1/19$) to give the title compound (0.27 g, 0.57 mmol, 95%) as a pale yellow amorphous solid. ^1H NMR ($CDCl_3$) δ: 1.30–1.59 (10H, m), 1.71–1.94 (2H, m), 2.17 (1H, br s), 2.35 (1H, br s), 2.66 (1H, br s), 2.96 (3H, s), 3.09 (3H, s), 4.00–4.20 (1H, m), 4.20–4.30 (1H, m), 4.75–4.95 (1H, m), 7.44 (1H, d, $J = 9.0$ Hz), 7.70–7.95 (5H, m), 8.31 (1H, s). MS (FAB) m/z: 474 $(M+H)^+$.

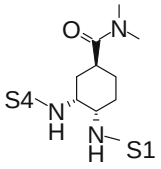
Table 5
The $PTCT2/IC_{50}$ ratio, log D value, and protein binding

Compd	$PTCT2/IC_{50}$ ratio	log D	Protein binding (%)
45a	77	2.7	70
45c	46	2.2	48
48	100	2.6	68

5.1.3. *N*-{(1*R*,2*S*,5*S*)-2-[(6-Chloro-2-naphthoyl)amino]-5-[(dimethylamino)carbonyl]cyclohexyl}-5-methyl-4,5,6,7-tetrahydrothiazolo-[5,4-*c*]pyridine-2-carboxamide hydrochloride (5a)

To a solution of **3a** (0.27 g, 0.57 mmol) in CH_2Cl_2 (10 mL) was added saturated HCl ethanolic solution (10 mL) and the mixture was stirred for 1.5 h. The solvent was evaporated and to the residue was added Et_2O. The resultant precipitate was collected by filtration. This solid was dissolved in DMF (7 mL) and lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylate (**4**)[10] (0.11 g, 0.54 mmol), HOBt (70 mg, 0.46 mmol), and EDC·HCl (0.10 g, 0.52 mmol) were added. After stirring for 23 h, the mixture was concentrated in vacuo. The residue was partitioned between AcOEt and water. The organic layer was 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 MeOH and 1 N HCl ethanolic solution (0.30 mL, 0.30 mmol) was added. The solvent was evaporated and to the residue was added AcOEt. The resultant precipitate was collected by filtration to give the title compound (0.13 g, 0.21 mmol, 37%) as a pale yellow amorphous solid. ^1H NMR (DMSO-d_6) δ: 1.45–1.60 (1H, m), 1.70–1.90 (3H, m), 1.90–2.10 (2H, m), 2.81 (3H, s), 2.91 (3H, s), 3.00 (3H, s), 3.00–3.40 (5H, m), 3.25–3.45 (1H, br), 4.10–4.20 (1H, m), 4.40–4.70 (2H, m), 7.59 (1H, dd, $J = 8.8$, 2.2 Hz), 7.87 (1H, d, $J = 8.5$ Hz), 7.96 (1H, d, $J = 8.5$ Hz), 8.02 (1H, d, $J = 8.8$ Hz), 8.10 (1H, d, $J = 2.2$ Hz), 8.33 (1H, s), 8.43 (1H, d, $J = 8.1$ Hz), 8.52 (1H, d, $J = 7.3$ Hz). MS (FAB) m/z: 554 $(M+H)^+$. Anal. Calcd for $C_{28}H_{32}ClN_5O_3S \cdot HCl \cdot 5/4H_2O$: C, 54.86; H, 5.84; Cl, 11.57; N, 11.42; S, 5.23. Found: C, 54.81; H, 5.90; Cl, 11.28; N, 11.22; S, 5.19.

Table 4
Assay results of **45a**, **45c**, and **48**



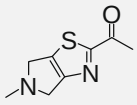
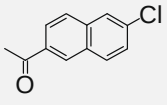
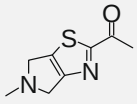
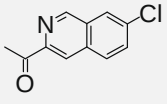
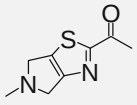
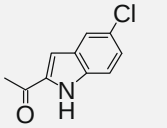
Compd	S4	S1	fXa IC_{50} (nM)	PTCT2 (μM) in human plasma	Solubility (μg/mL)		log D	Ex vivo anti-fXa activity (%)
					pH 1.2	pH 6.8		
45a			12	0.92	>1000	100	2.7	71
45c			9.5	0.44	>1000	120	2.2	90
48			3.4	0.34	270	220	2.6	68

Table 6
Food effect on AUC in monkeys and dogs

Compd	Monkey (po 1 mg/kg)			Dog (po 10 mg/head)		
	AUC_{fasted} (ng h/mL)	AUC_{fed} (ng h/mL)	Ratio fed/fasted	AUC_{fasted} (ng h/mL)	AUC_{fed} (ng h/mL)	Ratio fed/fasted
A	988	172	0.17	301	40	0.13
45c	723	322	0.45	1823	778	0.43

5.1.4. 7-Chloro-*N*-{(1*S*,2*R*,4*S*)-4-[(dimethylamino)carbonyl]-2-{[(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]amino}cyclohexyl}quinoline-3-carboxamide hydrochloride (5d)

(i) Hydrolysis step: Compound **2d** (443 mg, 2.00 mmol) was dissolved in THF (12 mL) and H_2O (1.5 mL), to the solution was added LiOH (72 mg, 3.0 mmol) and the mixture was stirred for 17 h. The solvent was evaporated to give lithium 7-chloro-quinoline-3-carboxylate.

(ii) Condensation step: The residue was dissolved in DMF (15 mL) and to the solution were added *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 (**6**)[8] (320 mg, 0.876 mmol) in DMF (15 mL), HOBt (118 mg, 0.876 mmol), and EDC·HCl (252 mg, 1.31 mmol). After stirring for 2 days, the mixture was concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and water. 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 ($MeOH/CH_2Cl_2$ = 1/19). The obtained solid was dissolved in EtOH (2 mL) and 1 N HCl ethanolic solution (0.8 mL) was added. After stirring for 30 min, the solvent was evaporated and to the residue was added Et_2O. The resultant precipitate was collected by filtration to give the title compound (131 mg, 0.200 mmol, 36%) as a pale orange solid. ^{1}H NMR (DMSO-d_6) δ: 1.45–1.60 (1H, m), 1.65–2.05 (5H, m), 2.81 (3H, s), 2.91, 2.92 (total 3H, each s), 3.02, 3.04 (total 3H, each s), 3.08–3.38 (2H, m), 3.41–3.52 (1H, m), 3.67–3.74 (1H, m), 4.10–4.13 (2H, m), 4.37–4.47 (1H, m), 4.65–4.74 (2H, m), 7.74 (1H, dd, J = 8.8, 2.0 Hz), 8.12–8.16 (2H, m), 8.46–8.51 (1H, m), 8.70–8.79 (2H, m), 9.18 (1H, dd, J = 7.8, 2.0 Hz), 11.52, 11.73 (total 1H, each br s). IR (ATR) cm^{-1}: 3396, 3251, 3058, 2931, 2865, 2700–2300, 1635, 1542, 1517, 1450, 1363, 1309, 1253. MS (ESI) *m/z*: 555 $(M+H)^+$. Anal. Calcd for $C_{27}H_{31}ClN_6O_3S{\cdot}1.8HCl{\cdot}2H_2O$: C, 49.38; H, 5.65; Cl, 15.11; N, 12.80; S, 4.88. Found: C, 49.56; H, 5.71; Cl, 15.08; N, 12.67; S, 5.07.

5.1.5. 6-Chloroquinoline-2-carbonitrile (8)

6-Chloroquinoline (**7**) (2.50 g, 15.3 mmol) was dissolved in CH_2Cl_2 (25 mL), and *m*-chloroperbenzoic acid (*m*-CPBA) (70%, 3.71 g, 15.0 mmol) was added to the solution under ice cooling, followed by stirring at room temperature for 1 h. The mixture was diluted with CH_2Cl_2, washed successively with sodium thiosulfate aqueous solution and NaOH aqueous solution, and dried over Na_2SO_4. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (40 mL). To the solution were added trimethylsilyl cyanide (2.00 mL, 15.9 mmol) and *N,N*-dimethylcarbamoyl chloride (1.50 mL, 16.3 mmol). The mixture was refluxed for 9 h. To the mixture were added additional trimethylsilyl cyanide (1.00 mL, 7.95 mmol) and *N,N*-dimethylcarbamoyl chloride (0.800 mL, 8.69 mmol), and the mixture was refluxed for an additional 16 h. The mixture was diluted with CH_2Cl_2, and 10% K_2CO_3 aqueous solution (40 mL) was added, followed by stirring for 30 min. The organic layer was separated and dried over Na_2SO_4. The solvent was evaporated, and the residue was triturated with CH_2Cl_2 and collected by filtration to give the title compound (1.77 g, 9.38 mmol, 61%) as colorless crystals. The filtrate was concentrated, and chromatographed (CH_2Cl_2) to give another portion of the title compound (0.800 g, 4.24 mmol, 28%) as pale yellow crystals. ^{1}H NMR (DMSO-d_6) δ: 7.94 (1H, dd, J = 9.0, 2.2 Hz), 8.09 (1H, d, J = 8.5 Hz), 8.15 (1H, d, J = 9.0 Hz), 8.29 (1H, d, J = 2.2 Hz), 8.63 (1H, d, J = 8.5 Hz). MS (FAB) *m/z*: 189 $(M+H)^+$.

5.1.6. 6-Chloroquinoline-2-carboxylic acid (2b)

Compound **8** (1.73 g, 9.17 mmol) was dissolved in concd HCl aqueous solution (40 mL), and the solution was refluxed for 19 h. After the mixture was cooled to room temperature, the resultant precipitate was collected by filtration and washed with water to give the title compound (1.81 g, 8.72 mmol, 95%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 7.87 (1H, dd, J = 9.0, 2.4 Hz), 8.10–8.20 (2H, m), 8.24 (1H, d, J = 2.2 Hz), 8.52 (1H, d, J = 8.5 Hz). MS (FAB) *m/z*: 208 $(M+H)^+$.

5.1.7. *rac*-Methyl 4-chloro-*N*-formylphenylalaninate (10)

rac-Methyl 4-chlorophenylalaninate hydrochloride (**9**) (2.00 g, 8.00 mmol) was suspended in CH_2Cl_2 (20 mL), and to the suspension were added EDC·HCl (1.60 g, 8.35 mmol), HOBt (1.23 g, 8.03 mmol), *N*-methylmorpholine (1.90 mL, 17.3 mmol), and formic acid (0.30 mL, 8.0 mmol), followed by stirring for 15 min. Addition of formic acid (0.30 mL) and stirring for 15 min were repeated three times. The mixture was 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 ($MeOH/CH_2Cl_2$ = 1/40) to give the title compound (1.21 g, 5.01 mmol, 63%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 3.10 (1H, dd, J = 13.9, 5.6 Hz), 3.18 (1H, dd, J = 13.9, 5.9 Hz), 3.75 (3H, s), 4.95–5.00 (1H, m), 6.07 (1H, br s), 7.05 (2H, d, J = 8.3 Hz), 7.27 (2H, d, J = 8.3 Hz), 8.18 (1H, s). MS (FAB) *m/z*: 242 $(M+H)^+$.

5.1.8. Methyl 7-chloroisoquinoline-3-carboxylate (11)

Compound **10** (1.45 g, 6.00 mmol) was dissolved in CH_2Cl_2 (40 mL), and oxalyl chloride (0.570 mL, 6.65 mmol) was added dropwise, followed by stirring at room temperature for 30 min. The mixture was cooled to −10 °C and ferric chloride (1.17 g, 7.21 mmol) was added. The mixture was stirred at room temperature for 4 days. To the mixture was added 1 N HCl aqueous solution, and the mixture was diluted with CH_2Cl_2. The organic layer was separated and dried over Na_2SO_4. The solvent was evaporated and the residue was dissolved in MeOH (38 mL). Concd sulfuric acid (2 mL) was added to the solution and the mixture was refluxed for 20 h. Saturated $NaHCO_3$ aqueous solution was added to the mixture and the mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed (EtOAc) to give the title compound (0.250 g, 1.13 mmol, 19%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 4.07 (3H, s), 7.74 (1H, dd, J = 8.8, 2.0 Hz), 7.94 (1H, d, J = 8.8 Hz), 8.06 (1H, d, J = 2.0 Hz), 8.59 (1H, s), 9.28 (1H, s). Anal. Calcd for $C_{11}H_8ClNO_2$: C, 59.61; H, 3.64; Cl, 16.00; N, 6.32. Found; C, 59.40; H, 3.61; Cl, 16.22; N, 6.23.

5.1.9. 7-Chloroisoquinoline-3-carboxylic acid hydrochloride (2c)

Compound **11** (0.230 g, 1.04 mmol) was dissolved in concd HCl aqueous solution (10 mL) and the solution was refluxed for 18 h.

After the mixture was cooled to room temperature, the resultant precipitate was collected by filtration and washed with water to give the title compound (0.21 g, 0.86 mmol, 83%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 7.90–8.00 (1H, m), 8.29 (1H, d, J = 8.5 Hz), 8.44 (1H, s), 8.72 (1H, s), 9.45 (1H, d, J = 6.6 Hz). MS (FAB) m/z: 208 $(M+H)^+$.

5.1.10. *N*-[(*E*)-2,4-Dichlorobenzylidene]-4-methylbenzenesulfonamide (14)

To a solution of 2,4-dichlorobenzaldehyde (**12**) (3.50 g, 20.0 mmol) in toluene (80 mL) were added 4-methylbenzenesulfonamide (**13**) (3.42 g, 20.0 mmol) and molecular sieves (4 Å, powder, 300 mg) and the mixture was refluxed for 2 h. After the solvent was evaporated, Et_2O was added to the residue. The resultant precipitate was collected by filtration and washed with Et_2O to give the title compound (2.56 g, 7.80 mmol, 39%) as a colorless solid. The filtrate was evaporated and the same procedure gave another portion of the title compound (3.60 g, 11.0 mmol, 55%). ^{1}H NMR ($CDCl_3$) δ: 2.45 (3H, s), 7.32 (1H, dd, J = 8.6, 2.0 Hz), 7.36 (2H, d, J = 8.6 Hz), 7.49 (1H, d, J = 2.0 Hz), 7.89 (2H, d, J = 8.6 Hz), 8.09 (1H, d, J = 8.6 Hz), 9.42 (1H, s).

5.1.11. Methyl 2-{(2,4-dichlorophenyl){[(4-methylphenyl)sulfonyl]amino}methyl}acrylate (15)

To a solution of **14** (2.55 g, 7.77 mmol) in THF (50 mL) were added 1,4-diazabicyclo[2.2.2]octane (DABCO) (87.5 mg, 0.780 mmol) and methyl acrylate (2.8 mL, 31 mmol) and the mixture was refluxed for 16 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2. The 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 = 1/2) to give the title compound (2.03 g, 4.90 mmol, 63%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 2.39 (3H, s), 3.63 (3H, s), 5.66 (1H, d, J = 8.0 Hz), 5.85 (1H, d, J = 8.0 Hz), 5.87 (1H, s), 6.28 (1H, s), 7.06 (1H, dd, J = 8.2, 1.9 Hz), 7.17 (2H, d, J = 8.2 Hz), 7.23–7.30 (2H, m), 7.61 (2H, d, J = 8.2 Hz). MS (FAB) m/z: 414 $(M+H)^+$.

5.1.12. Methyl 7-chloroquinoline-3-carboxylate (2d)

To a solution of **15** (1.66 g, 4.00 mmol) in DMF (30 mL) were added 4-methylbenzenesulfonamide (**13**) (0.14 g, 0.80 mmol) and K_2CO_3 (1.11 g, 8.00 mmol) and the mixture was stirred for 16 h at 90 °C. The solvent was evaporated and the residue was partitioned between EtOAc and water. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed (EtOAc/hexane = 1/4) to give the title compound (0.630 g, 2.84 mmol, 71%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 4.02 (3H, s), 7.58 (1H, dd, J = 8.6, 2.2 Hz), 7.88 (1H, d, J = 8.6 Hz), 8.16 (1H, d, J = 1.9 Hz), 8.83 (1H, d, J = 1.9 Hz), 9.45 (1H, d, J = 2.2 Hz). MS (FAB) m/z: 222 $(M+H)^+$.

5.1.13. 6-Chloroquinazoline-2-carboxylic acid (2e)

$NaHSO_3$ (10.4 g, 59.8 mmol) and Na_2CO_3 (5.13 g, 48.4 mmol) were dissolved in water (200 mL) and heated to 60 °C with stirring. To this solution was added dropwise a solution of 5-chloro-2-nitrobenzaldehyde (**16**) (2.04 g, 11.0 mmol) in MeOH (40 mL). The mixture was stirred for 40 min at 60 °C then refluxed for 2.5 h. After cooling, Et_2O was added and the organic layer was separated, washed with water, and dried over Na_2SO_4. The solvent was evaporated to give 2-amino-5-chlorobenzaldehyde (**17**) (0.63 g, 4.0 mmol, 37%) as a yellow oil. This compound was used for the next reaction without further purification.

To a solution of **17** (0.63 g, 4.0 mmol) in EtOH (12 mL) was added ammonium acetate (328 mg, 4.25 mmol) and the mixture was warmed to 35–40 °C. To this mixture was added dropwise a solution of glyoxylic acid monohydrate (0.40 g, 4.4 mmol) in water (4.0 mL) and the mixture was stirred for 15.5 h. After cooling, water was added and the resultant precipitate was collected by filtration. The precipitate was washed with EtOH to give the title compound (74 mg, 0.35 mmol, 8.7%) as a yellow amorphous solid. ^{1}H NMR (DMSO-d_6) δ: 8.00–8.10 (2H, m), 8.32 (1H, s), 9.59 (1H, d, J = 2.4 Hz). MS (EI) m/z: 208 $(M+H)^+$.

5.1.14. Ethyl (*E*)-3-(morpholin-4-yl)-2-acrylate (19)

Morpholine (1.70 mL, 19.5 mmol) was added dropwise to a solution of ethyl propiolate (**18**) (2.00 mL, 19.6 mmol) in CH_2Cl_2 under ice cooling. After stirring at room temperature for 1 h, the mixture was concentrated in vacuo and the residue was chromatographed (MeOH/CH_2Cl_2 = 1/20) to give the title compound (3.72 g) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.1 Hz), 3.21 (4H, t, J = 5.1 Hz), 3.71 (4H, t, J = 5.1 Hz), 4.14 (2H, q, J = 7.1 Hz), 4.70 (1H, d, J = 13.4 Hz), 7.36 (1H, d, J = 13.4 Hz). MS (FAB) m/z: 186 $(M+H)^+$.

5.1.15. Ethyl 7-chlorocinnoline-3-carboxylate (22)

3-Chloroaniline (**20**) (2.00 g, 15.7 mmol) was dissolved in a mixed solvent of water (30 mL) and concd HCl aqueous solution (3.5 mL), and sodium nitrite (1.30 g, 18.8 mmol) was added under ice cooling. After stirring for 10 min, concd HCl aqueous solution (5.30 mL) and sodium tetrafluoroborate (6.90 g, 62.8 mmol) were added. The mixture was stirred for 30 min under ice cooling. The resultant precipitate was collected by filtration and washed successively with water, MeOH, and Et_2O to give 3-chlorobenzenediazonium tetrafluoroborate (**21**) (2.63 g, 11.6 mmol, 74%) as a colorless solid. This compound was used for the next reaction without further purification.

Compound **19** (1.45 g, 7.83 mmol) was dissolved in acetonitrile (100 mL), and to the solution was added compound **21** (1.73 g, 7.64 mmol). The mixture was stirred at room temperature for 1 h then refluxed for 7 days. The solvent was evaporated and the residue was chromatographed twice (EtOAc/CH_2Cl_2 = 1/10, then EtOAc/hexane = 1/1) to give the title compound (0.250 g, 1.06 mmol, 14%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.53 (3H, t, J = 7.1 Hz), 4.62 (2H, q, J = 7.1 Hz), 7.80 (1H, dd, J = 8.8, 2.0 Hz), 7.95 (1H, d, J = 8.8 Hz), 8.64 (1H, s), 8.68 (1H, d, J = 2.0 Hz).

5.1.16. 7-Chlorocinnoline-3-carboxylic acid (2f)

To a solution of compound **22** (0.23 g, 0.97 mmol) in THF (9 mL) was added 1 N NaOH aqueous solution (3.0 mL, 3.0 mmol) and the mixture was stirred for 16 h. After 10% citric acid aqueous solution was added, the solvent was evaporated. The residue was triturated with water and collected by filtration to give the title compound (0.16 g, 0.77 mmol, 79%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ: 8.02 (1H, dd, J = 8.8, 2.0 Hz), 8.34 (1H, d, J = 8.8 Hz), 8.70 (1H, br s), 8.90 (1H, s). MS (FAB) m/z: 209 $(M+H)^+$.

5.1.17. Ethyl 7-chloro-2*H*-chromene-3-carboxylate (25)

4-Chloro-2-hydroxybenzaldehyde (**23**) (510 mg, 3.26 mmol) was dissolved in THF (40 mL), and NaH (60% in oil, 157 mg, 3.91 mmol) was added, followed by stirring at room temperature for 2 h. To the mixture was added a solution of ethyl 2-(diethoxyphosphoryl)acrylate (**24**) (769 mg, 3.26 mmol) in THF (10 mL), and the mixture was stirred at room temperature for 2 h and then refluxed overnight. After the mixture was cooled to room temperature, water and Et_2O were added. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (EtOAc/hexane = 1/10) to give the title compound (247 mg, 1.03 mmol, 32%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.1 Hz), 4.27 (2H, q, J = 7.1 Hz), 4.99 (2H, d, J = 1.2 Hz), 6.85 (1H, d, J = 1.2 Hz), 6.89 (1H, dd, J = 8.1, 2.0 Hz), 7.04 (1H, d, J = 8.1 Hz), 7.38 (1H, br s). MS (EI) m/z: 238 $(M)^+$.

5.1.18. 7-Chloro-2*H*-chromene-3-carboxylic acid (2g)

Compound **2g** was synthesized from **25** according to the procedure used to prepare **2f**. A pale yellow solid. Yield 99%. ^{1}H NMR (DMSO-d_6) δ: 4.92 (1H, d, J = 2.0 Hz), 6.95 (1H, d, J = 2.0 Hz), 7.01 (1H, dd, J = 8.1, 2.2 Hz), 7.35 (1H, d, J = 8.1 Hz), 7.44 (1H, br s). MS (EI) m/z: 210 $(M)^+$.

5.1.19. Methyl 6-chloro-4-oxo-1,4-dihydroquinoline-2-carboxylate (28)

To a solution of 4-chloroaniline (**26**) (12.8 g, 100 mmol) in MeOH (150 mL) was added dimethyl acetylenedicarboxylate (**27**) (13.5 mL, 110 mmol). The mixture was refluxed for 8 h and concentrated in vacuo. The residue was dissolved in diphenyl ether (70 mL) and the solution was stirred at 240 °C for 4 h. After cooling, hexane and Et_2O were added. The resultant precipitate was collected by filtration to give the title compound (11.1 g, 46.7 mmol, 47%) as a yellow powder. ^{1}H NMR (DMSO-d_6) δ: 3.97 (3H, s), 6.66 (1H, br s), 7.76 (1H, dd, J = 9.0, 2.5 Hz), 7.90–8.05 (2H, m), 12.28 (1H, br s). MS (ESI) m/z: 238 $(M+H)^+$.

5.1.20. 6-Chloro-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (2h)

Compound **2h** was synthesized from **28** according to the procedure used to prepare **2f**. A yellow solid. Yield 47%. ^{1}H NMR (DMSO-d_6) δ: 6.90–7.05 (1H, m), 7.90–8.05 (2H, m), 10.10–10.30 (1H, m), 12.13 (1H, br s). MS (ESI) m/z: 224 $(M+H)^+$.

5.1.21. Diethyl (4-chloro-2-nitrobenzylidene)malonate (30)

To a solution of 4-chloro-2-nitrobenzaldehyde (**29**) (10.0 g, 53.9 mmol) in Ac_2O (25 mL) were added diethyl malonate (10.0 mL, 66.2 mmol) and $NaHCO_3$ (8.34 g, 100 mmol), and the mixture was stirred at 100 °C overnight. After cooling, 5% Na_2CO_3 aqueous solution and EtOAc were added to the mixture. The organic layer was separated, washed with brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was recrystallized from EtOH to give the title compound (8.06 g, 24.6 mmol, 46%) as pale brown crystals. ^{1}H NMR ($CDCl_3$) δ: 1.05–1.13 (3H, m), 1.29–1.38 (3H, m), 4.08–4.17 (2H, m), 4.29–4.38 (2H, m), 7.39 (1H, d, J = 8.3 Hz), 7.61 (1H, dd, J = 8.3, 2.1 Hz), 8.09 (1H, s), 8.20 (1H, d, J = 2.1 Hz). MS (FAB) m/z: 328 $(M+H)^+$. Anal. Calcd for $C_{14}H_{14}ClNO_6$: C, 51.31; H, 4.27; Cl, 10.82; N, 4.27. Found: C, 51.43; H, 4.29; Cl, 10.59; N, 4.36.

5.1.22. Ethyl 7-chloro-2-oxo-1,2-dihydroquinoline-3-carboxylate (31)

To a solution of **30** (8.00 g, 24.5 mmol) in AcOH (90 mL) was added iron powder (8.20 g, 147 mmol) and the mixture was stirred at 80 °C for 7 h. After cooling, the mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo. The resultant precipitate was collected by filtration to give the title compound (4.19 g, 16.6 mmol, 68%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.31 (3H, t, J = 6.8 Hz), 4.27 (2H, q, J = 6.8 Hz), 7.27 (1H, d, J = 7.8 Hz), 7.34 (1H, s), 7.86 (1H, d, J = 7.8 Hz), 8.50 (1H, s), 12.10 (1H, s). MS (FAB) m/z: 252 $(M+H)^+$. Anal. Calcd for $C_{12}H_{10}ClNO_3$: C, 57.27; H, 4.01; Cl, 14.09; N, 5.57. Found: C, 57.00; H, 3.97; Cl, 14.09; N, 5.51.

5.1.23. 7-Chloro-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (2i)

Compound **2i** was synthesized from **31** according to the procedure used to prepare **2f**. A colorless solid. Yield 94%. ^{1}H NMR (DMSO-d_6) δ: 7.32–7.55 (2H, m), 8.05 (1H, s), 8.93 (1H, s), 13.80 (1H, br s). MS (FAB) m/z: 224 $(M+H)^+$. Anal. Calcd for $C_{10}H_5ClNO_3{\cdot}0.2H_2O$: C, 53.10; H, 2.41; Cl, 15.67; N, 6.19. Found: C, 52.99; H, 2.69; Cl, 15.57; N, 6.09.

5.1.24. Ethyl 6-chloro-4-oxo-1,4-dihydroquinazoline-2-carboxylate (34)

To a solution of 2-amino-5-chlorobenzamide (**32**) (2.50 g, 14.7 mmol) in pyridine (15 mL) was added ethyl 2-chloro-2-oxoacetate (**33**) (2.00 mL, 17.9 mmol) and the mixture was stirred at room temperature for 18 h. The mixture was concentrated in vacuo and the residue was dissolved in AcOH (50 mL). To the solution was added Ac_2O (5.0 mL) and the mixture was refluxed for 16 h. The solvent was evaporated and EtOH was added to the residue. The resultant precipitate was collected by filtration to give the title compound (2.71 g, 10.7 mmol, 73%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.35 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.1 Hz), 7.85 (1H, d, J = 8.6 Hz), 7.91 (1H, dd, J = 8.6, 2.3 Hz), 8.10 (1H, d, J = 2.3 Hz), 12.85 (1H, br s). MS (ESI) m/z: 253 $(M+H)^+$.

5.1.25. 6-Chloro-4-oxo-1,4-dihydroquinazoline-2-carboxylic acid (2j)

Compound **2j** was synthesized from **34** according to the procedure used to prepare **2f**. A colorless solid. Yield 85%. ^{1}H NMR (DMSO-d_6) δ: 7.50–8.20 (3H, m), 12.44 (1H, br s). MS (ESI) m/z: 265 $(M+H+CH_3CN)^+$.

5.1.26. 7-Chloro-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (36)

To a solution of chlorosulfonylisocyanate (15.0 g, 106 mmol) in nitroethane (120 mL) was added dropwise 4-chloroaniline (**35**) (11.3 g, 90.0 mmol) in nitroethane (20 mL) at −78 °C over 10 min. The mixture was allowed to warm to 0 °C and $AlCl_3$ (15.0 g, 112 mmol) was added to the mixture in one portion. After the mixture became a clear solution, it was allowed to warm to room temperature. The mixture was heated at 115 °C for 25 min and poured into ice-water. The resultant precipitate was collected by filtration and washed successively with water and Et_2O to give the title compound (14.7 g, 63.2 mmol, 60%) as a pale brown powder. ^{1}H NMR (DMSO-d_6) δ: 7.26 (1H, d, J = 8.8 Hz), 7.69 (1H, dd, J = 8.8, 2.0 Hz), 7.82 (1H, d, J = 2.0 Hz), 11.39 (1H, s).

5.1.27. 2-Amino-5-chlorobenzenesulfonamide (37)

To compound **36** (7.83 g, 33.7 mmol) was added 50% sulfuric acid (270 mL, 2.53 mol) and the mixture was heated at 130–140 °C for 3 h. After cooling, the mixture was poured into ice-water (600 mL) and 40% NaOH aqueous solution (500 mL) was added. The pH of the mixture was adjusted to 3 with 1 N HCl aqueous solution. The water layer was extracted with EtOAc (twice). The combined organic layer was washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 1/10) to give the title compound (6.31 g, 30.5 mmol, 97%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 5.99 (2H, br s), 6.82 (1H, d, J = 8.8 Hz), 7.27 (1H, dd, J = 8.8, 2.7 Hz), 7.38 (2H, br s), 7.50 (1H, d, J = 2.7 Hz). IR (ATR) cm^{-1}: 3444, 3392, 3361, 3282, 1626, 1601, 1533, 1477, 1408, 1304, 1254, 1140, 1111, 881, 818. MS (FAB) m/z: 207 $(M+H)^+$.

5.1.28. Ethyl 2-[2-(aminosulfonyl)-4-chloroanilino]-2-oxoacetate (38)

To a solution of **37** (7.83 g, 33.7 mmol) in glacial acetic acid (3.0 mL) was added Et_3N (2.80 mL, 20.1 mmol). To the solution was carefully added ethyl 2-chloro-2-oxoacetate (**33**) (1.12 mL, 10.0 mmol). After stirring for 1 h, water (10 mL) and concd HCl aqueous solution were added. The resultant precipitate was collected by filtration and washed with water (twice). Recrystallization of this solid (2.17 g) from EtOH (12 mL) gave the title compound (710 mg, 2.31 mmol, 23%) as colorless crystals. ^{1}H NMR (DMSO-d_6) δ: 1.33 (3H, t, J = 7.1 Hz), 4.33 (2H, q, J = 7.1 Hz),

7.77 (1H, dd, J = 8.8, 2.4 Hz), 7.87 (1H, d, J = 2.4 Hz), 7.87 (2H, br s), 8.30 (1H, d, J = 8.8 Hz), 10.76 (1H, br s). IR (ATR) cm^{-1}: 3298, 3236, 1745, 1709, 1583, 1523, 1390, 1346, 1259, 1194, 1159, 760, 710, 602, 499. MS (FAB) m/z: 307 $(M+H)^+$.

5.1.29. Methyl 7-chloro-4*H*-1,2,4-benzothiadiazine-3-carboxylate 1,1-dioxide (2k)

To a solution of **38** (700 mg, 2.28 mmol) in MeOH (3.0 mL) were added NaOMe (156 mg, 2.89 mmol) in MeOH (3.0 mL) and phenolphthalein (small amount). After stirring for 29 h, NaOMe (160 mg, 2.96 mmol) was added and the mixture was stirred for an additional 18.5 h. Water (6.0 mL) and 1 N HCl aqueous solution (5.85 mL) were added to the mixture. The resultant precipitate was collected by filtration to give the title compound (491 mg, 1.79 mmol, 78%) as a colorless solid. ^1H NMR (DMSO-d_6) δ: 3.94 (3H, s), 7.80 (1H, d, J = 9.0 Hz), 7.83 (1H, dd, J = 9.0, 2.2 Hz), 7.96 (1H, d, J = 2.2 Hz), 12.91 (1H, br s). IR (ATR) cm^{-1}: 3248, 1734, 1595, 1520, 1475, 1441, 1336, 1306, 1263, 1167, 1146, 1109, 939, 825, 806, 715, 565. MS (FAB) m/z: 275 $(M+H)^+$.

5.1.30. 5-(Phenylsulfonyl)-5,6-dihydro-4*H*-pyrrolo[3,4-*d*]thiazole (41)

Benzenesulfonamide (**39**) (638 mg, 4.06 mmol) and 4,5-bis(bromomethyl)thiazole (**40**) (1.10 g, 4.06 mmol) were dissolved in DMF (10 mL) and cooled to 0 °C. To this solution was added NaH (60% in oil, 357 mg, 8.93 mmol), and the mixture was stirred for 3 h at room temperature. To the mixture were added CH_2Cl_2 and H_2O, and the organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (EtOAc/CH_2Cl_2 = 1/9) to give the title compound (137 mg, 0.515 mmol, 13%) as a colorless solid. ^1H NMR ($CDCl_3$) δ: 4.60–4.63 (2H, m), 4.70–4.73 (2H, m), 7.52–7.64 (3H, m), 7.88–7.92 (2H, m), 8.71 (1H, s). MS (FAB) m/z: 267 $(M+H)^+$. Anal. Calcd for $C_{11}H_{10}N_2O_2S_2$: C, 49.60; H, 3.78; N, 10.52. Found: C, 49.28; H, 3.79; N, 10.41.

5.1.31. 5,6-Dihydro-4*H*-pyrrolo[3,4-*d*]thiazole dihydrobromide (42)

A mixture of **41** (800 mg, 3.00 mmol), phenol (0.800 mL) and 47% HBr aqueous solution (5.00 mL) was refluxed for 2 h. After cooling, EtOAc and H_2O were added. The water layer was separated and concentrated in vacuo. To the residue was added EtOAc and the resultant precipitate was collected by filtration to give the title compound (521 mg, 2.52 mmol, 60%) as a colorless solid. ^1H NMR (DMSO-d_6) δ: 4.42 (2H, br s), 4.56 (2H, br s), 9.14 (1H, s). MS (FAB) m/z: 127 $(M+H)^+$. Anal. Calcd for $C_5H_6N_2S{\cdot}2HBr$: C, 20.85; H, 2.80; Br, 55.49; N, 9.73. Found: C, 20.67; H, 2.78; Br, 55.74; N, 9.39.

5.1.32. 5-Methyl-5,6-dihydro-4*H*-pyrrolo[3,4-*d*]thiazole (43)

To a suspension of **42** (357 mg, 1.24 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (0.407 mL, 2.92 mmol), AcOH (0.167 mL, 2.92 mmol), formalin (0.188 mL, 2.19 mmol), and $NaBH(OAc)_3$ (464 mg, 2.19 mol). After stirring for 30 min, CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution were added. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (MeOH/CH_2Cl_2 = 1/19) to give the title compound (150 mg, 1.07 mmol, 86%) as a brown oil. ^1H NMR ($CDCl_3$) δ: 2.67 (3H, s), 3.95–3.99 (2H, m), 4.01–4.05 (2H, m), 8.69 (1H, s). MS (ESI) m/z: 141 $(M+H)^+$.

5.1.33. Lithium 5-methyl-5,6-dihydro-4*H*-pyrrolo[3,4-*d*]thiazole-2-carboxylate (44)

To a solution of **43** (771 mg, 5.50 mmol) in THF (10 mL) was added *tert*-butyl lithium (1.54 M pentane solution, 3.93 mL, 6.05 mmol) at −78 °C under argon atmosphere. After stirring for 1 h at 0 °C, the mixture was cooled to −78 °C again and CO_2 gas was bubbled into it over 20 min. The mixture was allowed to warm to room temperature and the solvent was evaporated to give the title compound (1.08 g, quant.) as a brown solid. This solid was used for the next reaction without further purification. ^1H NMR (DMSO-d_6) δ: 2.52 (3H, s), 3.73 (2H, t, J = 3.2 Hz), 3.87 (2H, t, J = 3.2 Hz).

5.1.34. *N*-{(1*R*,2*S*,5*S*)-2-[(6-Chloro-2-naphthoyl)amino]-5-[(dimethylamino)carbonyl]cyclohexyl}-5-methyl-5,6-dihydro-4*H*-pyrrolo[3,4-*d*]thiazole-2-carboxamide hydrochloride (45a)

Compound **45a** was synthesized from **3a** and **44** according to the procedure used to prepare **5a**. A pale yellow solid. Yield 18%. A pale yellow solid. ^1H NMR (DMSO-d_6) δ: 1.48–1.56 (1H, m), 1.71–1.84 (3H, m), 1.95–2.04 (2H, m), 2.81 (3H, s), 3.00 (3H, s), 3.02 (3H, s), 3.06–3.15 (2H, m), 4.13–4.14 (1H, m), 4.52–4.63 (4H, m), 7.60 (1H, d, J = 8.5 Hz), 7.87 (1H, d, J = 8.8 Hz), 7.96 (1H, d, J = 8.5 Hz), 8.01 (1H, d, J = 8.8 Hz), 8.10 (1H, s), 8.32 (1H, s), 8.45 (1H, d, J = 8.1 Hz), 8.51 (1H, d, J = 7.3 Hz). MS (FAB) m/z: 540 $(M+H)^+$. Anal. Calcd for $C_{27}H_{30}ClN_5O_3S{\cdot}0.9HCl{\cdot}2.7H_2O{\cdot}0.1Et_2O$: C, 52.34; H, 5.95; Cl, 10.71; N, 11.14; S, 5.10. Found: C, 52.08; H, 5.55; Cl, 10.72; N, 11.19; S, 5.48.

5.1.35. 7-Chloro-*N*-{(1*S*,2*R*,4*S*)-4-[(dimethylamino)carbonyl]-2-{[(5-methyl-5,6-dihydro-4*H*-pyrrolo[3,4-*d*]thiazol-2-yl)carbonyl]amino}cyclohexyl}isoquinoline-3-carboxamide hydrochloride (45c)

Compound **45c** was synthesized from **3c** and **44** according to the procedure used to prepare **5c**. A colorless solid. Yield 40%. ^1H NMR (DMSO-d_6) δ: 1.47–1.56 (1H, m), 1.71–1.75 (3H, m), 1.95–1.99 (1H, m), 2.12–2.15 (1H, m), 2.78 (3H, s), 2.95 (3H, s), 2.98 (1H, br s), 3.05 (3H, s), 4.19–4.22 (1H, m), 4.44–4.52 (3H, m), 4.74–4.88 (2H, m), 7.87 (1H, dd, J = 8.8, 1.7 Hz), 8.24 (1H, d, J = 8.8 Hz), 8.36 (1H, d, J = 1.7 Hz), 8.58 (1H, s), 8.90–8.92 (2H, m), 9.30 (1H, s), 12.65–12.75 (1H, m). MS (FAB) m/z: 541 $(M+H)^+$. Anal. Calcd for $C_{26}H_{29}ClN_6O_3S{\cdot}1.3HCl{\cdot}1.3H_2O$: C, 51.04; H, 5.42; Cl, 13.33; N, 13.78; S, 5.24. Found: C, 51.29; H, 5.54; Cl, 13.28; N, 13.40; S, 5.56.

5.1.36. *tert*-Butyl {(1*R*,2*S*,5*S*)-2-{[(5-chloroindol-2-yl)carbonyl]amino}-5-[(dimethylamino)carbonyl]cyclohexyl}carbamate (47)

To a solution of ethyl (1*S*,3*R*,4*S*)-3-[(*tert*-butoxycarbonyl)amino]4-{[(5-chloroindol-2-yl)carbonyl]amino}cyclohexanecarboxylate (**46**)[9] (4.00 g, 8.62 mmol) in THF (50 mL) and EtOH (50 mL) was added 1 N NaOH aqueous solution (37 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 5 h. The mixture was then neutralized with 1 N HCl aqueous solution (37 mL) at 0 °C and concentrated in vacuo. The resultant precipitate was collected by filtration to give (1*S*,3*R*,4*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-{[(5-chloroindol-2-yl)carbonyl]amino}cyclohexanecarboxylic acid (7.99 g, 18.3 mmol). 7.89 g (18.1 mmol) of this solid was dissolved in DMF (200 mL) and to the solution were added dimethylamine hydrochloride (3.69 g, 45.2 mmol), EDC·HCl (6.94 g, 36.2 mmol), HOBt (2.44 g, 18.1 mmol), and Et_3N (6.30 mL, 45.2 mmol). After stirring for 14.5 h, the solvent was evaporated in vacuo. The residue was partitioned between EtOAc and water, and the water layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (EtOAc/hexane = 1/19) to give the title compound (8.18 g, 17.7 mmol, 96%) as a gray amorphous solid. ^1H NMR ($CDCl_3$) δ: 1.52 (9H, s), 1.62–1.79 (1H, m), 1.80–1.97 (2H, m), 2.05–2.38 (2H, m), 2.58–2.72 (1H, m), 2.95 (3H, s), 3.07 (3H, s), 3.91–4.10 (1H, m), 4.18–4.27 (1H, m), 4.68–4.88 (1H, m), 6.80 (1H, s), 7.22 (1H, dd, J = 8.8, 1.5 Hz), 7.27 (1H, d, J = 1.5 Hz), 7.35 (1H, d, J = 8.8 Hz), 7.59 (1H, s), 7.98 (1H, s), 9.50 (1H, s).

5.1.37. 5-Chloro-*N*-{(1*S*,2*R*,4*S*)-4-[(dimethylamino)carbonyl]-2-{[(5-methyl-5,6-dihydro-4*H*-pyrrolo[3,4-*d*]thiazol-2-yl)carbonyl]-amino}cyclohexyl}indole-2-carboxamide hydrochloride (48)

Compound **48** was synthesized from **47** and **44** according to the procedure used to prepare **5a**. A colorless solid. Yield 34%. ^{1}H NMR (DMSO-d_6) δ: 1.45–1.55 (1H, m), 1.67–1.83 (3H, m), 1.91–1.99 (2H, m), 2.79 (3H, s), 2.97 (3H, s), 3.01–3.08 (1H, m), 3.05 (3H, s), 4.07–4.13 (1H, m), 4.35–4.91 (5H, m), 7.03 (1H, d, J = 1.5 Hz), 7.15 (1H, dd, J = 8.7, 2.1 Hz), 7.40 (1H, d, J = 8.7 Hz), 7.66 (1H, d, J = 2.1 Hz), 8.36 (1H, d, J = 7.8 Hz), 8.40 (1H, d, J = 8.3 Hz), 11.77 (1H, s), 12.25 (1H, br s). MS (ESI) m/z: 529 $(M+H)^+$. Anal. Calcd for $C_{25}H_{29}ClN_6O_3S{\cdot}HCl{\cdot}1.4H_2O$: C, 50.83; H, 5.60; Cl, 12.00; N, 14.23; S, 5.43. Found: C, 50.85; H, 5.33; Cl, 12.00; N, 14.37; S, 5.74.

5.2. Biological assays

Assays of the in vitro anti-fXa activity, in vitro anticoagulant activity and rat ex vivo anti-fXa activity assay were performed as previously described.[8]

5.3. Metabolic stability and permeability

Metabolic stability and permeability were measured as previously described.[8]

5.4. Distribution coefficient and protein binding

Distribution coefficient and protein binding were measured as previously described.[9]

5.5. Pharmacokinetic analysis in cynomolgus monkey

Pharmacokinetic analysis was performed as previously described.[8]

5.6. Pharmacokinetic analysis in beagle dogs

Male beagle dogs (8–11 months old, n = 5) were fasted overnight. The test compounds were administered orally (10 mg/head, ca. 1 mg/kg, in water suspension, 4 mL/kg, via a stomach tube) in a fasted or non-fasted condition. For the non-fasted condition, ca. 270 g of solid food (TC-2, Aixia) was given to each dog 1–2 h before the oral administration. The chemical composition of TC-2 is as follows: protein 23%; fat 9%; carbohydrate, 51%. Blood sample collection and measurement of the compound concentration in plasma were performed in a similar manner to that was employed in the pharmacokinetic analysis in cynomolgus monkeys.[8]

5.7. 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 these conditions, the crystal did not form spontaneously, so micro- and macroseeding methods were needed to obtain crystals of the 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 **45c**. 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 **45c**. After 1 day, the crystal was picked up and directly exposed to soak solution 2, and the soaking was continued. The entire soaking process was performed at 20 °C.

Table 7
Crystal and diffraction data of human fXa with **45c**

Crystal parameters	
Space group	*P*212121
a (Å)	56.1
b (Å)	72.2
c (Å)	79.2
Resolution (Å)	1.8
R_{sym} (%)	4.8 (26.6)[a]
Completeness (%)	94.5 (79.5)[a]
No. of reflections, redundancy	28,855, 2.66
Refinement	
No. of protein atoms (occupancy ≠0)	2239
Average *B* value for protein and ligand atoms (Å^2)	30.9, 39.2
Range of data	25.0–1.8
R value	19.6
R_{free}	23.0
(Not weighted) rmsd from ideality	
Bond length (Å)	0.025
Bond angle (Å)	2.290

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

5.8. 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 IIc imaging plate detector (Rigaku) using an RU200 rotating anode generator (Rigaku). Data processing was carried out with *Mosflm*[31] and *scala*.[32]

5.9. Structure solution and crystallographic refinement

The previously reported Gla-less fXa structure (PDB code: 1HCG[33]) was used as the initial structure. Phase refinement and model improvement were carried out with *refmac*[34] and *Turbo Frodo*.[35] Stereochemistry checks indicated that the refined protein model was in good agreement with the expectations within each resolution range. The statistics of the data processing and crystallographic refinement are shown in Table 7. The atomic coordinates have been deposited with the Protein Data Bank (PDB code: 3IIT).

Acknowledgements

We are grateful to anticoagulant group in Biological Research Laboratories II. We thank Dr. Kiyoshi Nakayama for helpful discussions and editorial assistance in preparing the manuscript.

Supplementary data

Supplementary data (characterization of further intermediates as well as final compounds are supplied) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.024.

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