Bioorganic & Medicinal Chemistry 19 (2011) 1623–1642

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

2-Aminomethylphenylamine as a novel scaffold for factor Xa inhibitor

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ARTICLE INFO

Article history:
Received 23 December 2010
Revised 17 January 2011
Accepted 19 January 2011
Available online 25 January 2011

Keywords:
Anticoagulant
Factor Xa
Factor Xa inhibitor
New diamine linker

ABSTRACT

We have been researching orally active factor Xa inhibitor for a long time. We explored the new diamine linker using effective ligands to obtain a new attractive original scaffold 2-aminomethylphenylamine derivative. Compound **1D** showed very strong in vitro and in vivo factor Xa inhibitory activity, as well as favorable PK profiles in po administration to monkeys.

1. Introduction

Thrombosis-related diseases, including myocardial infarction, deep vein thrombosis, and unstable angina often have life-threatening consequences. Current therapies for the treatment and prevention of thrombotic disorders have several problems. They require parenteral administration or careful monitoring of the clotting time to achieve the desired efficacy, and dose titration to minimize excessive bleeding.[1] Therefore, safer and more effective orally administered anticoagulant agents are required.

Factor Xa (fXa) is a trypsin-like serine protease. This key enzyme acts on the site of convergence of the intrinsic and extrinsic coagulation pathways, and forms a prothrombinase complex with factor Va, calcium ion and phospholipids to generate thrombin via the proteolysis of prothrombin.[2] This activation is a highly amplified process.[2b] Therefore, inhibition of fXa may be more effective than the inhibition of thrombin itself. Furthermore, fXa inhibitors are expected to exhibit less bleeding risk than thrombin inhibitors since fXa inhibitors specifically affect the coagulation pathway but not the platelet function.[3]

Many pharmaceutical companies have concentrated on exploring an orally active fXa inhibitor.[4] We have also studied this for many years[5] and finally focused on low-molecule non-amidine compounds to obtain an orally active factor Xa inhibitor, edoxaban (free form of DU-176b[6]). Nowadays, several oral factor Xa inhibitors such as rivaroxaban,[7] apixaban,[8] edoxaban and betrixaban[9] have been studied in clinic (Fig. 1).

In order to increase the possibility of launching our fXa inhibitor into the market, we would like to obtain another novel scaffold for further exploration. Generally, two proper ligands are necessary for an oral fXa inhibitor to occupy S1 and S4 pockets, and several effective ligands have already been determined through our[5] or other's studies.[4] In addition, the overall structure of the inhibitor is supposed to form into an L- or V-like shaped configuration to link S1 ligand and S4 ligand with proper distance and angle to perform strong inhibitory activity. Therefore, we planned to explore new original diamine linkers using the effective ligands, tetrahydrothiazolopyridine unit[5b,c,h–l] (R^1) as S4 ligand, and the chlorothiophene unit[7,10] (R^2) as S1 ligand (Fig. 2).

Referring to the X-ray crystal structure data of fXa and the inhibitor, ethylene diamine, propane-1,3-diamine, or butane-1,4-diamine linker seemed to have the proper length to connect R^1 and R^2 ligands in S4 and S1 pockets respectively. Taking into account the structural diversity and simplicity, at first we focused on 3-carbon length diamine linkers. Then, concerning the strong active scaffold, we designed several derivatives and clarified the basic structural activity relationships.

2. Chemistry

All compounds linked by the diamine structure were synthesized according to the typical synthetic routes (Scheme 1).

Abbreviations: fXa, factor Xa; PTCT2, clotting time doubling concentration for prothrombin time; JP1, Japanese Pharmacopoeia First Fluid; JP2, Japanese Pharmacopoeia Second Fluid.

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doi:10.1016/j.bmc.2011.01.035

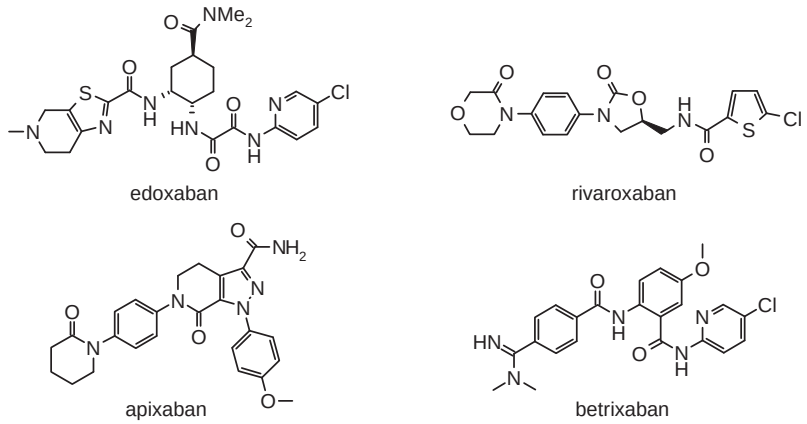


Figure 1. Representative factor Xa inhibitors.

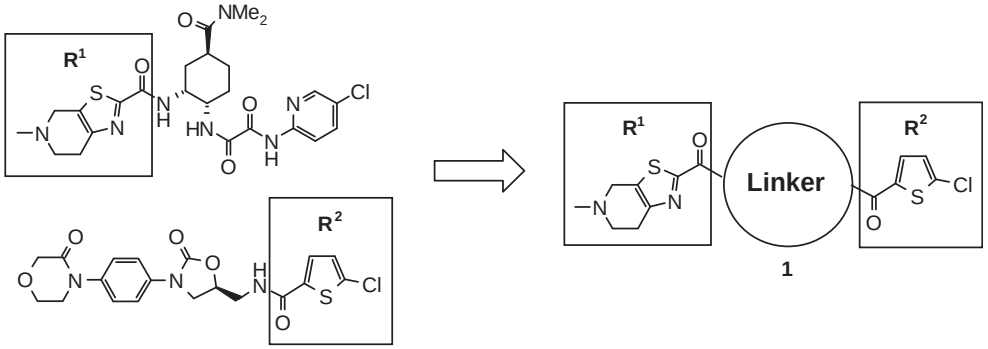
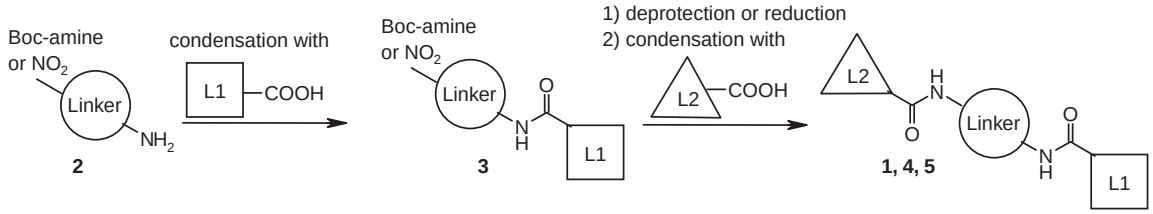


Figure 2. Our tactics to obtain new lead compounds.



Scheme 1. Typical synthetic route of the compound linked by diamine.

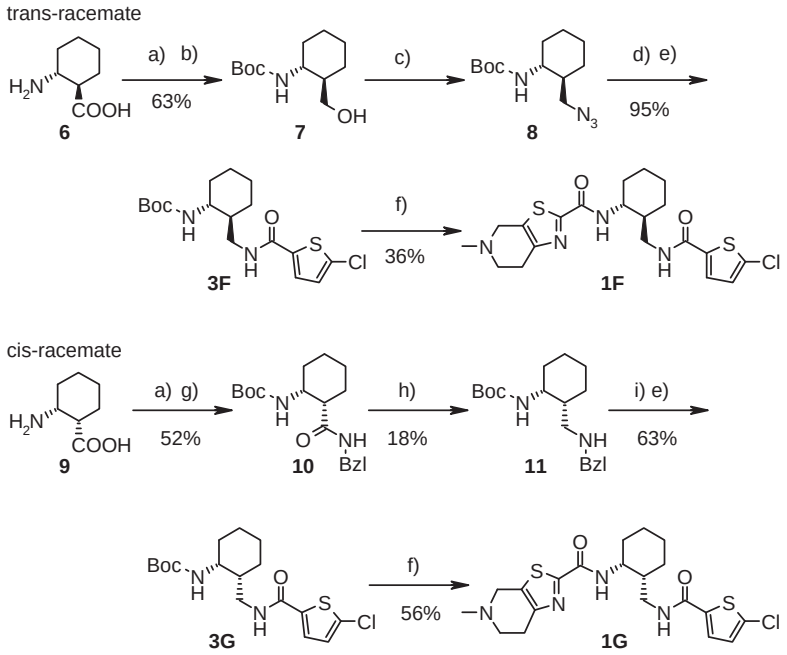
Mono-protected diamine or nitro-amino derivatives **2** were acylated to afford mono-amide derivatives. Mono-amide derivatives **3** were deprotected or reducted, followed by condensation with carboxylic acids to yield the inhibitors linked by the diamine structure.

All starting amines **2** were commercial available except for cyclohexane and pyridine derivatives. Synthetic routes of cyclohexane and pyridine derivatives are shown in Schemes 2 and 3. The amino groups of racemic 2-aminocyclohexane carboxylic acids **6** and **9** were protected by the Boc group. Each carboxyl group was converted into alkylamines by several steps. Obtained amines were condensed with 5-chlorothiophene-2-carboxylic acid to afford mono-amide derivatives (**3F** and **3G**). After deprotection of the Boc group, obtained amines were condensed with 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid[11] to yield the *trans*- and *cis*-cyclohexane derivatives (**1F** and **1G**).

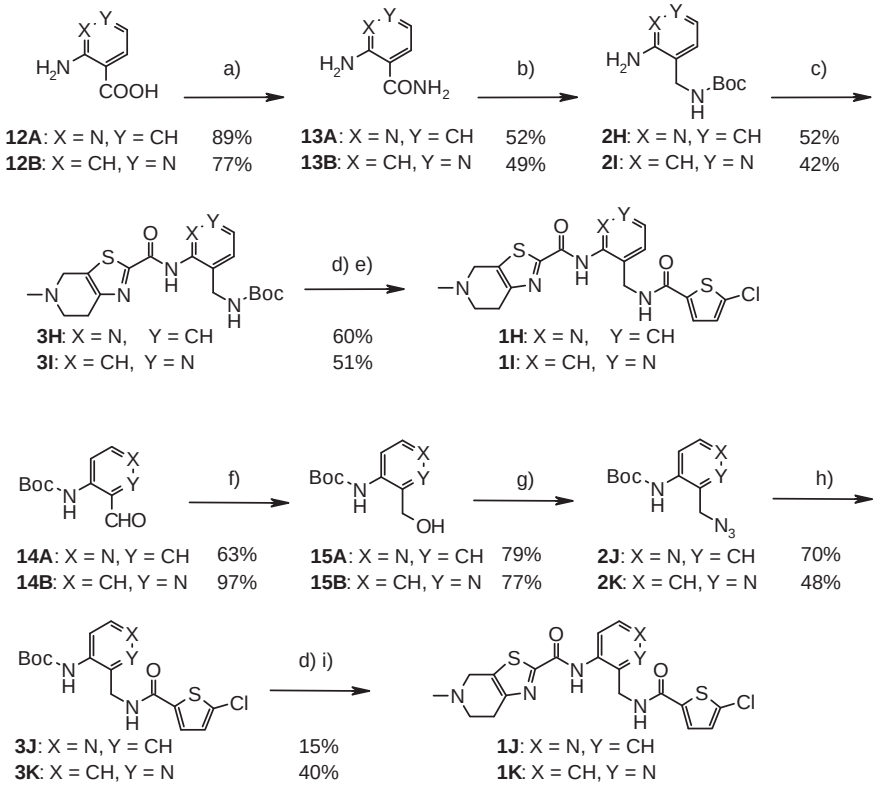
Pyridine derivatives were synthesized by two methods. Compounds **1H** and **1I** were synthesized from **12A** and **12B**, respectively. Carboxyl group was transformed into the Boc-aminomethyl group by condensation, reduction, and protection. Mono-protected diamines (**2H** and **2I**) were condensed with 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid to afford mono-amide derivatives (**3H**, **3I**). After deprotection of the Boc group, obtained amines were condensed with 5-chlorothiophene-2-carboxylic acid to yield the pyridine derivatives (**1H** and **1I**).

On the other hand, compounds **1J** and **1K** were synthesized from aldehydes **14A** and **14B**, respectively. These aldehydes were prepared according to the reported method.[12] Formyl group was transformed into the azidemethyl group by reduction and azidation. Azide derivatives (**2J** and **2K**) were reducted by hydrogenation and successively condensed with 5-chlorothiophene-2-carboxylic acid to obtain mono-amide derivatives (**3J** and **3K**). After deprotection of the Boc group, obtained amines were condensed with 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid to yield the pyridine derivatives (**1J** and **1K**).

Almost all S1 and S4 ligands (acids or esters) were commercial available. Before utilizing these to condense with diamine linker, the esters were hydrolyzed under alkali conditions in general.

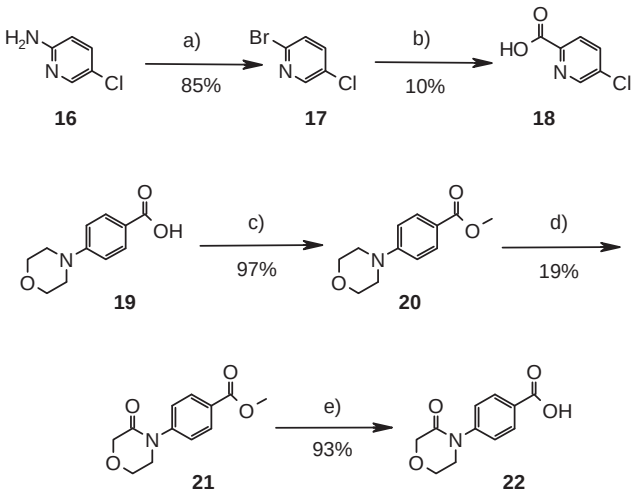


Scheme 2. Synthesis of *trans*- and *cis*-cyclohexane derivatives **1F** and **1G**. Reagents and conditions: (a) Boc_2O, dioxane, H_2O, Et_3N; (b) (1) $ClCO_2{}^iBu$, *N*-methylmorpholine, DME; (2) $NaBH_4$, H_2O; (c) (1) MsCl, Et_3N, CH_2Cl_2; (2) NaN_3, DMF; (d) H_2, Pd/C, MeOH; (e) 5-chlorothiophene-2-carboxylic acid, EDC, HOBt, Et_3N, DMF; (f) (1) HCl–dioxane; (2) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid,[11] EDC, HOBt, Et_3N, DMF; (g) $BzlNH_2$, EDC, HOBt, DMF; (h) BH_3/THF, reflux; (i) H_2, Pd/C, AcOH.



Scheme 3. Synthesis of pyridine derivatives **1H**–**1K**. Reagents and conditions: (a) NH_4Cl, Et_3N, EDC, HOBt, DMF; (b) (1) $LiAlH_4$, THF, reflux; (2) Boc_2O, THF; (c) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carbonylchloride,[11] Py; (d) HCl–dioxane, CH_2Cl_2; (e) 5-chlorothiophen-2-carboxylic acid, EDC, HOBt, Et_3N, DMF; (f) $NaBH_4$, MeOH; (g) DPPA, DBU, THF; (h) (1) H_2, 10% Pd/C, EtOAc; (2) 5-chlorothiophen-2-carboxylic acid, EDC, HOBt, DMF; (i) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid, EDC, HOBt, Et_3N, DMF.

5-Chloropyrrole-2-carboxylate,[13] 4-chloropyrrole-2-carboxylate[14] and 1-isopropylpiperidine-4-carboxylate[15] were prepared according to other reports. Scheme 4 shows the synthetic routes of several S1 and S4 ligands. Amine **16** was subjected to Sandmeyer reaction to obtain bromopyridine **17**. Bromide **17** was lithionated by butyllithium and treated with carbon dioxide gas to give carboxylic acid **18** in a low yield. 4-(3-Oxomorpholino)benzoic acid was prepared from 4-(1-morpholino)benzoic acid **19**. Acid **19** was



Scheme 4. Synthesis of several S1 and S4 ligands. Reagents and conditions: (a) Br_2, $NaNO_2$, 47% HBr, −5 to 15 °C; (b) (1) *n*-BuLi, Et_2O, −78 °C; (2) CO_2gas; (c) $SOCl_2$, MeOH, reflux; (d) $KMnO_4$, benzyltriethylammonium chloride, CH_2Cl_2; (e) $AlCl_3$, Me_2S, CH_2Cl_2.

esterified to afford the corresponding methyl ester **20**. Ester **20** was oxidized with $KMnO_4$ to obtain the oxomorpholine derivative **21**.[16] Since the amide moiety of 4-morpholino-benzoate **21** was easily hydrolyzed under alkali conditions, ester **22** was hydrolyzed by treating with aluminum chloride and dimethylsulfide to give corresponding carboxylic acid **22**.

Synthetic routes of the reverse amide analogs are shown in Scheme 5. Compound **25**, having a reverse amide structure linking to S1 ligand, was synthesized from phenylacetic acid **23**. Compound **23** was condensed with 2-amino-5-chloropyridine **16**, and obtained nitro compound **24** was reduced and acylated to yield compound **25**. Compounds **31**, **33**, and **35**, having a reverse amide structure linking to S4 ligand, were synthesized by condensation of common intermediate **28** and S4 ligands. Intermediate **28** was easily synthesized by condensation and hydrolysis from amine **26**. S4 ligands were prepared according to our or other reports. Protected amine **29**[11] was condensed with intermediate **28** to obtain compound **30**. After deprotection of the Boc group, N-methylation was conducted by reductive amination to yield compound **31**. Aniline **32**[17] and protected amine **34**[18] were utilized as S4 ligand to give reverse amides **33** and **35**, respectively.

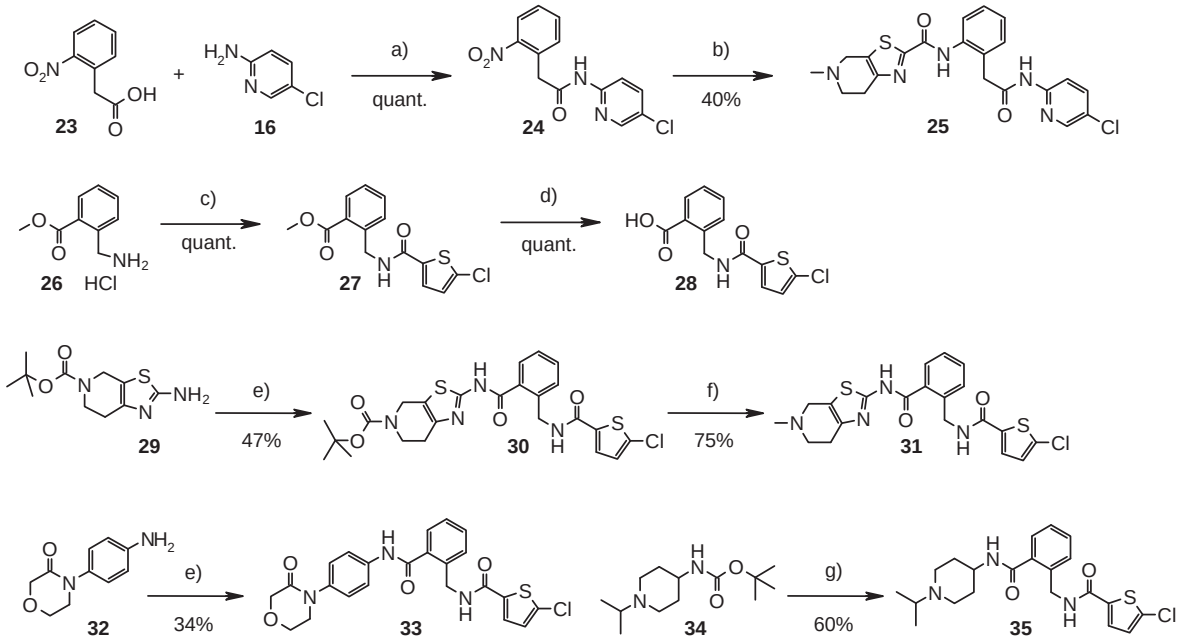
3. Result and discussion

In Table 1, the in vitro anti-fXa and anticoagulant activities (human PTCT2) of our first selection are summarized. Linear compound **1A** showed moderate inhibitory activity despite its high flexibility, but fixed piperidine linkers showed weak activities. Surprisingly, 2-aminomethylphenylamine linker **1D** showed very strong inhibitory and anticoagulant activities comparable to our clinical candidates.[5h] On the other hand, the reverse linking system of **1D** definitely declined its biological activity (for **1E**).

Concerning the scaffold of **1D**, we investigated several **1D** type linkage compounds. In vitro activities, solubilities (JP1 and JP2) and lipophilicities (Log *D*) are shown in Table 2.

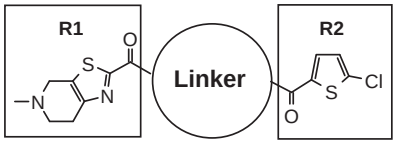
Compound **1D** showed relatively high lipophilicity and low solubility in these compounds. In particular, it showed fairly low solubility in a neutral aqueous solution (JP2: pH 6.8). On the whole, conversions of the benzene ring into the pyridine or cyclohexane ring increased the solubility greatly. Many pyridine derivatives showed lower lipophilicity than **1D**. The *cis*-cyclohexane derivative **1G** indicated moderately strong inhibitory activities, while the activity of *trans*-cyclohexane derivative **1F** was greatly decreased. All four pyridine isomers **1H**–**1K** showed relatively strong inhibitory activity and anticoagulant activity; however, none of these compounds showed stronger activity than **1D**. As a result, the benzene ring seemed to be the most favorable ring on this scaffold.

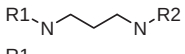
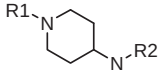
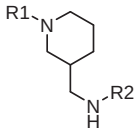
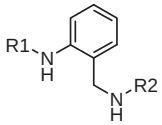
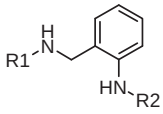
Then, we modified the chlorothiophene moiety as S1 ligand into other aromatic rings substituted with a chloro atom. In Table 3, in vitro inhibitory, anticoagulant activities, solubilities and Log *D* values are summarized. Thiophene ring was changed into the pyridine, benzene or pyrrole ring, taking the substitution position of the chloro moiety into consideration. The aqueous solubility was



Scheme 5. Synthesis of reverse amide type compounds. Reagents and conditions: (a) EDC, HOBt, DMAP, DMF; (b) (1) Raney-Ni, EtOH, 50 °C; (2) 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid, EDC, HOBt, DMF; (c) 5-chlorothiophene-2-carboxylic acid, EDC, HOBt, Et_3N, DMF; (d) $AlCl_3$, Me_2S, CH_2Cl_2; (e) **28**, EDC, HOBt, DMF; (f) 1) 4 N HCl–dioxane; (2) 37% HCHO, $NaBH(OAc)_3$, AcOH, CH_2Cl_2; (g) (1) 4 N HCl–dioxane; (2) **28**, EDC, HOBt, Et_3N, DMF.

Table 1
In vitro fXa inhibitory activity (IC_{50}) and anticoagulant activity (PTCT2), for new scaffolds



Compound	Linker	IC_{50} (nM)	PTCT2[a] (μM)
1A		93	7.6
1B		1025	>20
1C		6830	n.t.[b]
1D		1.7	0.50
1E		5675	n.t.

[a] Anticoagulant activities were evaluated with PTCT2 (clotting time doubling concentration for prothrombin time) in human plasma.
[b] Not tested.

obviously increased by the transformation of the thiophene ring. Heteroaromatic rings showed lower lipophilicity than **1D**. Only the pyrrole derivative **4E** showed moderately strong activity, and the other compounds indicated very low activity. We also investigated the reverse amide analogue of pyridine derivative, but compound **25** showed very low inhibitory activity as well. From these results, only the thiophene ring seemed to be especially suited for the 2-aminomethylphenylamine linker.

To investigate the diversity of S4 ligand on compound **1D**, we modified the tetrahydrothiazolopyridine moiety into some popular S4 ligands and also examined their reverse amide derivative. In Table 4, in vitro inhibitory, anticoagulant activities, solubilities and the Log *D* values are summarized.

Reverse amide analogue **31** showed lower inhibitory and anticoagulant activities than **1D**. 4-(3-Oxomorpholin-4-yl)phenyl unit, which was utilized to the other group's inhibitor[7,10a,b,17,19] such as rivaroxaban was introduced into 2-aminomethylphenylamine linker. Compound **5A** and the reverse amide derivative **33** showed fairly strong inhibitory and anticoagulant activity. Meanwhile, the 1-isopropylpiperidine moiety was also utilized to the other group's research,[20] but there is no comparison between **1D** and 1-isopropylpiperidine derivatives (**5B** and **35**). Concerning the solubility, only **5A** indicated lower solubility than **1D**. Interestingly, the reverse amide analogues (**31**, **33** and **35**) showed higher solubility and lower lipophilicity than their original compounds (**1E**, **5A** and **5B**), respectively.

We continued to further evaluate compounds **1D** and **5A** for their potent inhibitory activities.

Their metabolic stability in human liver microsomes and their oral activities in rats are shown in Table 5.

Metabolic stabilities of these compounds were moderate, and there was a lot of room for improvement in metabolic stability. Compound **5A** showed higher stability than **1D**. However, the oral activities in rats showed inconsistent results. Compound **1D** displayed potent anti-fXa activities in plasma, while **5A** showed very low anti-fXa activities in plasma in spite of its higher metabolic stability compared to **1D**. We thought that a very low solubility of **5A** caused its lower activity in the oral administration test.

As compound **1D** showed the strongest activities in vitro and exhibited potent oral activity in rats, we investigated it in an orally administration test in non-fasting monkeys ($n = 2$) as HCl salts at a dose of 1.0 mg/kg. The anti-fXa activities in plasma are shown in Figure 3. Compound **1D** showed significant and durable inhibitory activity. The pharmacokinetic parameters were measured as shown in Table 6. The AUC and C_{max} values were respectively very large, but slightly inferior to our clinical compound.[6] In short, compound **1D** displayed excellent pharmacodynamic and pharmacokinetic results as the lead compound.

An X-ray crystal structure of **1D** in human fXa is shown in Figures 4 and 5. Chlorothiophene unit and the thiazolopyridine unit were placed in S1 and S4 pockets, respectively. In the S1 pocket, the chlorothiophene unit was inserted similarily to that of other inhibitors.[7] There is a hydrogen bond between amide nitrogen of chlorothiophene carboxamide and carbonyl oxygen of Gly218 (3.12 Å). The chlorine in the thiophene ring makes contact with hydrophobic side chains of Tyr228, Val213, Ala190 and Gly226. Near the amide oxygen of chlorothiophene carboxamide, the water displayed by the red asterisk in Figure 5 was placed to form three hydrogen bonds with amide oxygen of chlorothiophene carboxamide (2.63 Å), oxygen of side chain Ser195 (2.54 Å) and oxygen of backbone Ser 214 (2.68 Å). These hydrogen bonds might cause high inhibitory activity.

In the S4 pocket, the thiazolopyridine unit was placed similarily to our other factor Xa inhibitors.[5b,h] 5-Methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine part makes contact with the side chain of Tyr99, Phe174 and Trp215. Condensed ring was placed parallel to the indole ring of Trp 215, and the methyl group was occupied at the depth of S4. The carbonyl oxygen of thizolopyridine-carboxamide moiety makes a hydrogen bond with NH of Gly218 (3.01 Å). In addition, its carbonyl carbon was placed on the carbonyl oxygen of Gly216. We thought that this close contact could increase conformational stability by electrostatic interaction (2.98 Å).

4. Conclusion

We explored the new diamine linker using the effective ligands to obtain the novel and attractive scaffold 2-aminomethylphenylamine derivative. Then, we designed several derivatives and investigated the basic structural activity relationships. Compound **1D** showed very strong inhibitory and anticoagulant activities on a clinical candidate level. **1D** showed potent oral activities in rats and monkeys, and favorable PK profiles in monkey. Moreover, an X-ray crystal structure of **1D** in human fXa was also determined to clarify the binding mode and several interactions. That is to say, **1D** is a vey promising lead compound for further optimization. However, it leaves room for improvement, such as relative low solubility and moderate metabolic stability.

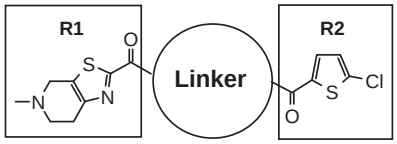
5. Experimental section

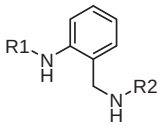
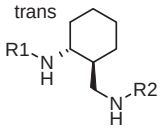
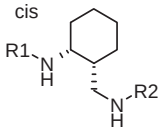
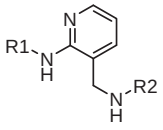
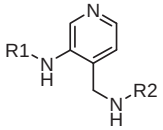
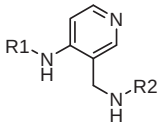
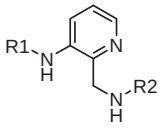
5.1. Chemistry

5.1.1. General

All solvents, reagents and materials were used as acquired from commercial sources without purification. Melting points

Table 2
In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), solubility (JP1 and JP2) and the Log *D* value (pH 7.4) for **1D** type linkage compounds



Compound	Linker	IC_{50} (nM)	PTCT2 (μM)	Solubility JP1[a] (μg/mL)	Solubility JP2[b] (μg/mL)	Log *D* (pH 7.4)
1D		1.7	0.50	150	17	3.3
1F		439	17	>600	440	3.5
1G		27	3.4	800	290	3.7
1H		18	1.5	860	120	2.2
1I		13	1.8	>590	>590	2.6
1J		12	1.4	880	>4.9	3.3
1K		11	1.0	890	67	2.8

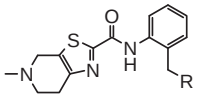
[a] JP1: Japanese Pharmacopoeia First Fluid (pH 1.2).
[b] JP2: Japanese Pharmacopoeia Second Fluid (pH 6.8).

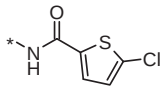
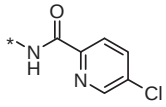
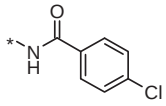
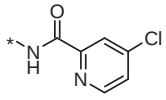
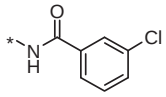
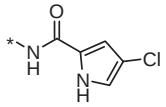
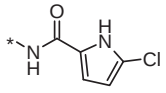
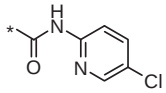
were determined on a Büchi 520, Büchi B-545 or Yanagimoto Micro Melting Point Apparatus and are uncorrected. Column chromatography was performed on Merck Silica Gel 60 (0.063–0.200 mm). Flash chromatography was performed with YAMAZEN HI-FLASH™ column or YAMAZEN ULTRA PACK$_{TM}$ column. Thin-layer chromatography (TLC) was performed on Merck precoated TLC glass sheets with Silica Gel 60 F254 or Merck precoated TLC aluminium sheets with Silica Gel 60 F254. ^{1}H NMR spectra were recorded on a JEOL JNM-EX400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane, which was used as the internal standard. ESI mass spectra were recorded on a SCIEX API-150EX spectrometer, Agilent Technologies Agilent 1100 series LC/MSD or Thermoquest FINNING AQA. FAB mass spectra were recorded on a JEOL JMS-HX110 spectrometer. EI mass spectra were recorded on a JEOL JMS-AX505W spectrometer. HR-ESI mass spectra were recorded on JMS-T100LP AccuTOF LC-plus. IR spectra were recorded on a Hitachi 270–30 spectrometer.

5.1.2. *tert*-Butyl {3-[(5-chlorothiophen-2-yl)carbonylamino]propyl}carbamate (3A)

To the solution of *tert*-butyl *N*-(3-aminopropyl)carbamete (500 μL, 2.87 mmol) in CH_2Cl_2 (10.0 mL) were added 5-chlorothiophene-2-carboxylic acid (533 mg, 3.28 mmol), HOBt (446 mg, 3.30 mmol), EDC·HCl (688 mg, 3.59 mmol) and triethylamine (504 μL, 3.62 mmol). After stirring at room temperature for 2 h, to the reaction mixture were added CH_2Cl_2 and 1 M HCl. After extraction with CH_2Cl_2, combined organics were washed with saturated $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. The mixture was concentrated in vacuo and AcOEt (10 mL) and hexane (10 mL) were added to the residue. The residue was washed for 2 min under ultrasonication and filtrated to obtain the title compound (675 mg) as a white solid. The filtrate was concentrated in vacuo, the residue was washed with AcOEt (10 mL) and hexane (10 mL) and obtain the title compound (123 mg) as a white solid in the same manner. In total, the title compound that obtained was 798 mg (2.50 mmol, 87%). ^{1}H NMR ($CDCl_3$) δ: 1.46

Table 3
In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), solubility (JP1 and JP2) and the Log *D* value (pH 7.4) for S1 ligands



Compound	R	IC_{50} (nM)	PTCT2 (μM)	Solubility JP1 (μg/mL)	Solubility JP2 (μg/mL)	Log *D* (pH 7.4)
1D		1.7	0.50	150	17	3.3
4A		2476	n.t.	350	380	2.8
4B		642	n.t.	560	42	3.4
4C		4145	n.t.	760	150	2.7
4D		1618	n.t.	680	160	3.3
4E		48	2.2	690	170	3.2
4F		>10,000	n.t.	>810	33	3.1
25		623	17	600	100	2.8

(9H, s), 1.58–1.78 (2H, m), 3.17–3.34 (2H, m), 3.38–3.54 (2H, m), 4.74–4.94 (1H, br), 6.90 (1H, d, *J* = 4.2 Hz), 7.28–7.42 (1H, br), 7.35 (1H, d, *J* = 4.2 Hz). ESI-MS *m*/*z*: 219 [(M−Boc)$^+$, ^{35}Cl], 221 [(M−Boc)$^+$, ^{37}Cl], 263 [(M−*t*Bu)$^+$, ^{35}Cl], 265 [(M−tBu)$^+$, ^{37}Cl], 341 [(M+Na)$^+$, ^{35}Cl], 343 [(M+Na)$^+$, ^{37}Cl]. ESI-MS *m*/*z*: 317 [(M−H)$^-$, ^{35}Cl], 319 [(M−H)$^-$, ^{37}Cl].

5.1.3. *N*-{3-[(5-Chlorothiophen-2-yl)carbonylamino]propyl}-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (1A)

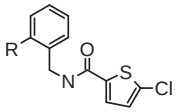
To the mixed solution of **3A** (200 mg, 0.627 mmol) in dioxane (3.0 mL) and MeOH (1.0 mL) was added 4 M HCl solution in dioxane (3.0 mL), and the mixture was stirred at room temperature for 3 h. The mixture was concentrated in vacuo and the obtained residue was dissolved in DMF (4.0 mL). To the solution were added 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (176 mg, 0.750 mmol), HOBt (102 mg, 0.755 mmol), EDC·HCl (153 mg, 0.798 mmol) and triethylamine (322 μL, 2.29 mmol). After stirring at room temperature for 3 d, the solvent was evaporated. To the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 20:1) and the obtained crude product was washed with AcOEt to give the free form of the title compound (185 mg, 0.464 mmol, 74%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.78–1.87 (2H, m), 2.53 (3H, s), 2.84 (2H, t, *J* = 5.7 Hz), 2.93 (2H, t, *J* = 5.4 Hz), 3.42–3.49 (2H, m), 3.52–3.59 (2H, m), 3.74 (2H, s), 6.89 (1H, d, *J* = 4.2 Hz), 7.43 (1H, d, *J* = 4.2 Hz), 7.52–7.63 (2H, m). ESI-MS *m*/*z*: 399 [(M+H)$^+$, ^{35}Cl], 401 [(M+H)$^+$, ^{37}Cl].

To the solution of the free form of the title compound (185 mg) in EtOH (20 mL) was added 1 M HCl solution in EtOH (500 μL) and the solvent was evaporated. Water was added to the residue and evaporated, and then dried in vacuo at room temperature for 14 h to obtain the title compound (202 mg) as a white solid. MP: 84–87 °C (Dec.). IR (ATR) cm^{-1}: 3400, 2931, 1639, 1549, 1520, 1427, 1292, 1140. ^{1}H NMR (DMSO-d_6) δ: 1.68–1.80 (2H, m), 2.91 (3H, s), 3.04–3.34 (6H, m), 3.39–3.54 (1H, m), 3.61–3.82 (1H, m), 4.35–4.49 (1H, m), 4.63–4.77 (1H, m), 7.17 (1H, d, *J* = 4.2 Hz), 7.63 (1H, d, *J* = 4.2 Hz), 8.68 (1H, t, *J* = 5.5 Hz), 8.93 (1H, t, *J* = 6.1 Hz), 11.56 (1H, br s). FAB-MS *m*/*z*: 399 [(M+H)$^+$, ^{35}Cl], 401 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{16}H_{19}ClN_4O_2S_2 \cdot 1.1HCl \cdot H_2O$: C, 42.05; H, 4.87; Cl, 16.29; N, 12.26; S, 14.03. Found: C, 42.25; H, 5.23; Cl, 16.37; N, 12.26; S, 14.10.

5.1.4. *tert*-Butyl 4-[(5-chlorothiophen-2-yl)carbonylamino]piperidine-1-carboxylate (3B)

tert-Butyl 4-aminopiperidine-1-carboxylate (522 mg, 2.61 mmol), CH_2Cl_2 (10.0 mL), 5-chlorothiophene-2-carboxylic

Table 4
In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), solubility (JP1 and JP2) and the Log *D* value (pH 7.4) for S4 ligands



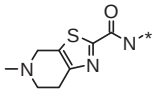
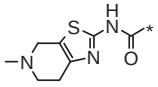
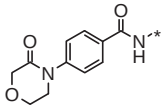
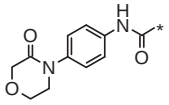
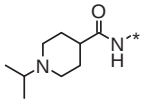
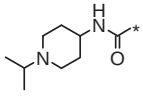
Compound	R	IC_{50} (nM)	PTCT2 (μM)	Solubility JP1 (μg/mL)	Solubility JP2 (μg/mL)	Log *D* (pH 7.4)
1D		1.7	0.50	150	17	3.3
31		59	2.3	>640	100	2.9
5A		4.1	0.91	<2.3	<2.3	2.8
33		9.0	1.0	140	120	2.5
5B		204	5.2	>680	>680	1.5
35		1930	n.t.	810	>820	1.4

Table 5
Human metabolic stability and ex vivo anti-fXa activity on oral administration to rats

Compound	Metabolic stability[a] (%)	Rat ex vivo inhibitory activity[b]				
		Dose (mg/kg)	0.5 h	1 h	2 h	4 h
1D[c]	53	9.2	86±2.3	80±2.8	66±4.2	30±3.9
5A	68	10	17.1±0.7	14.1±3.2	12.7±2.0	12.74±1.8

[a] In vitro metabolic stability was measured in human liver microsome.
[b] Values are expressed as means ± SE from three rats.
[c] Hydrochloride was used.

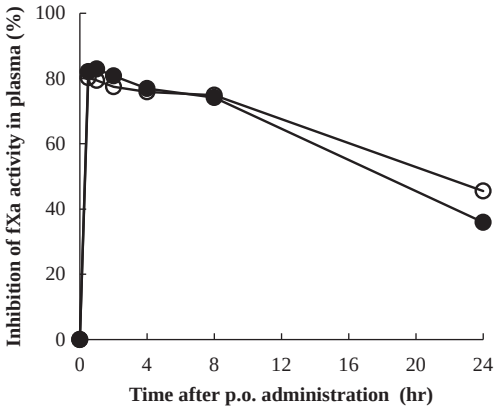


Figure 3. Ex vivo anti-fXa inhibitory activities in plasma with an oral administration of hydrochloride of compound **1D** to two monkeys at 1.0 mg/kg.

Table 6
Monkey pharmacokinetics profile of compound **1D**

Animal	$AUC_{0-24\ h}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)
Monkey 1	697	83.7	0.5	3.8
Monkey 2	705	97.4	1.0	4.3

Hydrochloride was used.

acid (488 mg, 3.00 mmol), HOBt (404 mg, 2.99 mmol), EDC·HCl (632 mg, 3.30 mmol) and triethylamine (462 μL, 3.31 mmol) were treated as described for **3A** to give the title compound (735 mg, 2.13 mmol, 82%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.32–1.56 (2H, m), 1.46 (9H, s), 1.92–2.12 (2H, m), 2.80–3.01 (2H, m), 3.98–4.25 (3H, m), 5.81 (1H, br d, J = 7.6 Hz), 6.89 (1H, d, J = 3.9 Hz), 7.25 (1H, d, J = 3.9 Hz). ESI-MS *m*/*z*: 289 [$(M-{}^tBu)^+$, ^{35}Cl], 291 [$(M-{}^tBu)^+$, ^{37}Cl], 367 [$(M+Na)^+$, ^{35}Cl], 369 [$(M+Na)^+$, ^{37}Cl]. ESI-MS *m*/*z*: 343 [$(M-H)^-$, ^{35}Cl], 345 [$(M-H)^-$, ^{37}Cl].

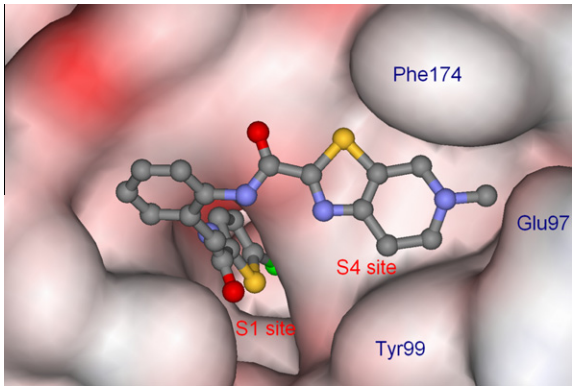


Figure 4. The binding mode of compound **1D** as viewed from the top. The surface view is the active site of Gla-less fXa. Compound **1D** is displayed in a ball and stick style without hydrogens. Carbon, nitrogen, sulfur, oxygen and chlorine atoms are displayed in gray, blue, yellow, red and green, respectively.

5.1.5. *N*-{1-[(5-Methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-yl)carbonyl]piperidin-4-yl}-5-chlorothiophene-2-carboxamide hydrochloride (1B)

Compound **1B** was synthesized in a similar manner to compound **1A**. Compound **3B** (200 mg, 0.580 mmol) was deprotected with 4 M HCl solution in dioxane. Obtained amine, DMF (5.0 mL), 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (164 mg, 0.709 mmol), HOBt (95 mg, 0.703 mmol), EDC·HCl (143 mg, 0.746 mmol) and triethylamine (294 μL, 2.11 mmol) were treated to give the free form of the title compound (223 mg, 53%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.39–1.63 (2H, m), 2.09 (2H, br d, J = 12.0 Hz), 2.53 (3H, s), 2.81–3.02 (5H, m), 3.20–3.35 (1H, m), 3.72 (2H, s), 4.18–4.31 (1H, m), 4.69 (1H, br d, J = 12.0 Hz), 5.42 (1H, br d, J = 12.5 Hz), 6.63 (1H, br d, J = 8.1 Hz), 6.87 (1H, d, J = 4.2 Hz), 7.41 (1H, d, J = 4.2 Hz).

ESI-MS m/z: 425 [$(M+H)^+$, ^{35}Cl], 427 [$(M+H)^+$, ^{37}Cl].

The free form of the title compound (209 mg), EtOH (10 mL) and 1 M HCl solution in EtOH (550 μL) was treated to give the title compound (214 mg) as a white solid. MP: 162–165 °C (Dec.). IR (ATR) cm^{-1}: 3428, 2929, 1620, 1547, 1520, 1427, 1331, 1242, 1976, 1001. ^{1}H NMR (DMSO-d_6) δ: 1.44–1.65 (2H, m), 1.82–2.00 (2H, m), 2.90, 2.91 (total 3H, each s), 2.94–3.28 (3H, m), 3.30–3.53 (2H, m), 3.62–3.77 (1H, m), 3.86–4.15 (1H, m), 4.32–4.51 (2H, m), 4.63–4.77 (1H, m), 5.13 (1H, br d, J = 12.5 Hz), 7.16 (1H, d, J = 3.9 Hz), 7.73 (1H, br s), 8.54 (1H, br s), 11.40–11.66 (1H, m). FAB-MS m/z: 425 [$(M+H)^+$, ^{35}Cl], 427 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{18}H_{21}ClN_4O_2S_2 \cdot 1.1HCl \cdot 0.8H_2O$: C, 45.09; H, 4.98; Cl, 15.53; N, 11.68; S, 13.37. Found: C, 44.94; H, 5.17; Cl, 15.74; N, 11.50; S, 13.18.

5.1.6. *tert*-Butyl {1-[(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-yl)carbonyl]piperidin-3-yl}methylcarbamate (3C)

To the solution of *tert*-butyl (piperidin-3-yl)methylcarbamate (252 mg, 1.18 mmol) in DMF (10 mL) were added 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (330 mg, 1.41 mmol), HOBt (188 mg, 1.39 mmol), EDC·HCl (288 mg, 1.50 mmol) and triethylamine (406 μL, 2.91 mmol). After stirring at room temperature for 18 h, the solvent was evaporated. To the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 30:1→20:1→10:1) and dried in vacuo at room temperature for 2 h to obtain the title compound (436 mg, 1.11 mmol, 94%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.50–1.98 (5H, m), 2.55 (3H, s), 2.80–3.48 (7H, m), 3.69–3.83 (0.25H, m), 3.76 (2H, s), 3.89–4.04 (1H, m), 4.08–4.32 (1.25H, m), 4.62–4.77 (0.25H, m), 4.81–4.99 (0.25H, br), 5.99–6.14 (1H, br). ESI-MS m/z: 395 $(M+H)^+$.

5.1.7. *N*-({1-[(5-Methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-yl)carbonyl]piperidin-3-yl}methyl)-5-chlorothiophene-2-carboxamide hydrochloride (1C)

To the mixed solution of **3C** (365 mg, 0.925 mmol) in MeOH (3.0 mL) was added 4 M HCl solution in dioxane (4.0 mL), and the mixture was stirred at room temperature for 14 h. The mixture was concentrated and dried in vacuo at room temperature for 3.5 h, and the obtained residue was dissolved in DMF (10.0 mL). To the solution were added 5-chlorothiophene-2-carboxylic acid (178 mg, 1.09 mmol), HOBt (147 mg, 1.09 mmol), EDC·HCl (216 mg, 1.13 mmol) and triethylamine (465 μL, 3.34 mmol). The mixture was stirred at room temperature for 19 h. To the solution were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were washed with brine and dried over Na_2SO_4. The solvent was distilled off in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 30:1→20:1→10:1) to give the free form of the title compound (319 mg, 0.727 mmol, 79%) as a light brown solid. ^{1}H NMR ($CDCl_3$) δ: 1.40–2.47 (5H, m), 2.54, 2.57 (total 3H, each s),

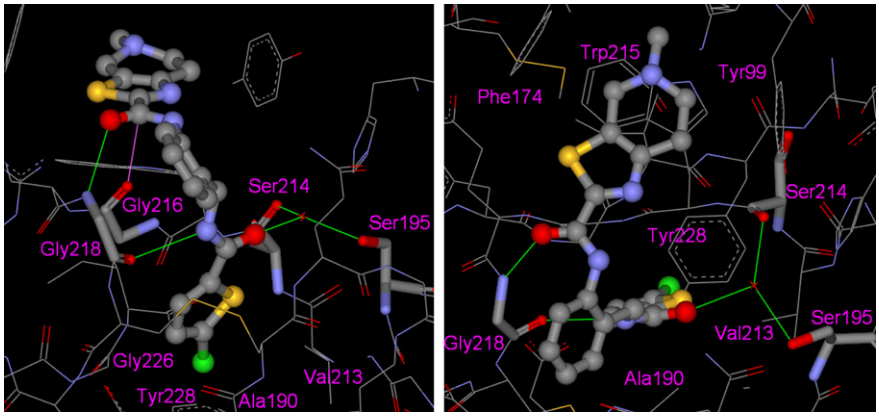


Figure 5. X-ray structure of the complex with fXa and compound **1D**. The residue of fXa and compound **1D** are displayed with carbon, nitrogen, sulfur, oxygen and chlorine atoms displayed in gray, blue, yellow, red, and green, respectively. Hydrogens were omitted. Compound **1D** is displayed in a ball and stick style. Several residues associated with the hydrogen bonds to compound **1D** are displayed by stick style. Meanwhile other residues are displayed by lines. One water oxygen atom associated with the hydrogen bond with **1D** is displayed by a red asterisk, and the other water oxygen atoms are hidden. The hydrogen bonds and electrostatic interaction are displayed by green and pink lines.

2.64–4.17 (11H, m), 4.64 (0.6H, d, J = 13.7 Hz), 4.91 (0.4H, d, J = 13.9 Hz), 6.44–6.54 (0.4H, m), 6.89 (1H, br d, J = 3.7 Hz), 7.10–7.19 (0.6H, m), 7.25–7.32 (0.4H, m), 7.41 (0.6H, br d, J = 3.9 Hz). ESI-MS m/z: 439 [$(M+H)^+$, ^{35}Cl], 441 [$(M+H)^+$, ^{37}Cl].

To the solution of the free form of the title compound (319 mg) in EtOH (10.0 mL) was added 1 M HCl solution in EtOH (750 μL) and the solvent was evaporated. Water was added to the residue and evaporated, and the resultant matter was dried in vacuo at room temperature for 18 h to obtain the title compound (351 mg) as a light brown solid. MP: 64–66 °C. IR (ATR) cm^{-1}: 2929, 1608, 1549, 1464, 1425, 1267, 1074. ^1H NMR (DMSO-d_6) δ: 1.19–1.52 (2H, m), 1.67–1.89 (3H, m), 2.53–2.75 (1H, m), 2.80–3.00 (1H, m), 2.88, 2.90 (total 3H, each s), 3.00–3.77 (6H, m), 4.10–4.51 (2H, m), 4.57–4.77 (1H, m), 4.93–5.30 (1H, m), 7.15–7.22 (1H, m), 7.69 (1H, d, J = 3.7 Hz), 8.71–8.82 (1H, m), 11.39–11.69 (1H, br). ESI-MS m/z: 439 [$(M+H)^+$, ^{35}Cl], 441 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{19}H_{23}ClN_4O_2S_2{\cdot}HCl{\cdot}1.9H_2O$: C, 44.77; H, 5.50; Cl, 13.91; N, 10.99; S, 12.58. Found: C, 44.85; H, 5.74; Cl, 14.13; N, 11.00; S, 12.40.

5.1.8. *N*-(2-Nitrobenzyl)-5-chlorothiophene-2-carboxamide (3D)

To the solution of 2-nitrobenzylamine (377 mg, 2.00 mmol) in DMF (10.0 mL) were added 5-chlorothiophene-2-carboxylic acid (330 mg, 2.03 mmol), HOBt (265 mg, 1.96 mmol), EDC·HCl (384 mg, 2.00 mmol) and triethylamine (280 μL, 2.01 mmol). The reaction mixture was stirred at room temperature for 3 d. Solvent was distilled off in vacuo, and to the residue were added AcOEt and 10% citric acid aqueous solution. After extraction with AcOEt, combined organics were washed with saturated $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. The mixture was concentrated in vacuo, and Et_2O (20 mL) was added to the residue. The residue was washed for 3 min under ultrasonication, filtrated and dried in vacuo at room temperature for 6 h to obtain the title compound (493 mg, 1.66 mmol, 83%) as a pale yellow solid. ^1H NMR ($CDCl_3$) δ: 4.81 (2H, d, J = 6.6 Hz), 6.84–6.94 (1H, br), 6.88 (1H, d, J = 3.9 Hz), 7.26 (1H, d, J = 3.9 Hz), 7.46–7.51 (1H, m), 7.61–7.67 (1H, m), 7.73 (1H, dd, J = 7.7, 1.3 Hz), 8.08 (1H, dd, J = 8.3, 1.2 Hz). ESI-MS m/z: 297 [$(M+H)^+$, ^{35}Cl], 299 [$(M+H)^+$, ^{37}Cl], 319 [$(M+Na)^+$, ^{35}Cl], 321 [$(M+Na)^+$, ^{37}Cl].

5.1.9. *N*-(2-Aminobenzyl)-5-chlorothiophene-2-carboxamide (36)

To the mixed solution of compound **3D** (455 mg, 1.53 mmol) in DMF (10.0 mL) and water (5.0 mL) were added $FeCl_3$ (743 mg, 4.58 mmol) and zinc powder (>90%) (1012 mg, >13.93 mmol). The reaction mixture was refluxed for 30 min. After cooling to room temperature, to the reaction mixture were added AcOEt and saturated $NaHCO_3$ aqueous solution. Insoluble substance was removed by filtration using a celite pad. After extraction with AcOEt, combined organics were washed with brine and water, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Hexane/AcOEt = 4:1→3:1→2:1) to give the title compound (357 mg, 1.34 mmol, 88%) as a white solid. ^1H NMR ($CDCl_3$) δ: 4.53 (2H, d, J = 6.1 Hz), 6.23 (1H, br s), 6.65–6.74 (2H, m), 6.87 (1H, d, J = 3.9 Hz), 7.07–7.17 (2H, m), 7.22 (1H, d, J = 3.9 Hz). ESI-MS m/z: 267 [$(M+H)^+$, ^{35}Cl], 269 [$(M+H)^+$, ^{37}Cl].

5.1.10. *N*-(2-{3-[(5-Chlorothiophen-2-yl)carbonylamino]methyl}phenyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide (1D)

To the solution of compound **43** (253 mg, 0.948 mmol) in DMF (10.0 mL) were added 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (260 mg, 1.11 mmol), HOBt (140 mg, 1.04 mmol), EDC·HCl (234 mg, 1.22 mmol) and triethylamine (320 μL, 2.30 mmol). After stirring at room temperature for 23 h, the solvent was evaporated. To the residue were added 10% MeOH solution in CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. After washing the crude product with AcOEt (3.0 mL), precipitate was collected by filtration and washed with Et_2O to obtain the free form of the title compound (262 mg, 0.586 mmol, 62%) as a pale yellow solid. ^1H NMR ($CDCl_3$) δ: 2.53 (3H, s), 2.85 (2H, t, J = 5.6 Hz), 2.94 (2H, t, J = 5.6 Hz), 3.75 (2H, s), 4.58 (2H, d, J = 5.9 Hz), 6.86 (1H, d, J = 3.9 Hz), 6.97–7.08 (1H, m), 7.21–7.32 (2H, m), 7.33–7.40 (1H, m), 7.46 (1H, d, J = 7.6 Hz), 7.65 (1H, d, J = 7.8 Hz), 9.40 (1H, s). ESI-MS m/z: 447 [$(M+H)^+$, ^{35}Cl], 449 [$(M+H)^+$, ^{37}Cl].

To the solution of the free form of the title compound (262 mg) in EtOH (10.0 mL) was added 1 M HCl solution in EtOH (600 μL) and the solvent was evaporated. Water was added to the residue and evaporated, and the resultant matter was dried in vacuo at room temperature for 18 h to obtain the title compound (282 mg) as a white solid. MP: 259–266 °C (Dec.). IR (ATR) cm^{-1}: 3317, 2366, 1662, 1637, 1591, 1556, 1539, 1504, 1454, 1427, 1298, 1273, 1122, 1078. ^1H NMR (DMSO-d_6) δ: 2.93 (3H, s), 3.08–3.33 (2H, br), 3.42–3.81 (2H, br), 4.31–4.87 (2H, br), 4.42 (2H, d, J = 5.9 Hz), 7.19 (1H, d, J = 4.2 Hz), 7.22–7.39 (3H, m), 7.48 (1H, d, J = 7.6 Hz), 7.71 (1H, d, J = 4.2 Hz), 9.22 (1H, t, J = 5.9 Hz), 10.67 (1H, s), 11.38–11.64 (1H, br). ESI-MS m/z: 447 [$(M+H)^+$, ^{35}Cl], 449 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{20}H_{19}ClN_4O_2S_2{\cdot}HCl{\cdot}0.2H_2O$: C, 49.32; H, 4.22; Cl, 14.56; N, 11.50; S, 13.17. Found: C, 49.40; H, 4.01; Cl, 14.33; N, 11.51; S, 13.21.

5.1.11. *N*-(2-Nitrobenzyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide (3E)

Compound **3E** was synthesized in a similar manner to compound **3C**. 2-nitrobenzylamine (500 mg, 3.29 mmol), DMF (20 mL), 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (848 mg, 3.61 mmol), HOBt (444 mg, 3.29 mmol), EDC·HCl (945 mg, 4.93 mmol) and triethylamine (916 μL, 6.57 mmol) were treated to give the title compound (835 mg, 2.51 mmol, 76%) as a pale yellow powder. ^1H NMR ($CDCl_3$) δ: 2.55 (3H, s), 2.88 (2H, t, J = 5.6 Hz), 2.96 (2H, t, J = 5.6 Hz), 3.77 (2H, s), 4.89 (2H, d, J = 6.8 Hz), 7.47 (1H, td, J = 7.6, 1.2 Hz), 7.63 (1H, td, J = 7.6, 1.2 Hz), 7.72 (1H, dd, J = 7.6, 1.2 Hz), 7.92 (1H, t, J = 6.8 Hz), 8.10 (1H, dd, J = 7.6, 1.2 Hz). ESI-MS m/z: 333 $(M+H)^+$.

5.1.12. *N*-(2-{[(5-Chlorothiophen-2-yl)carbonyl]amino}benzyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (1E)

To the mixed solution of compound **3E** (332 mg, 1.00 mmol) in DMF (10.0 mL) and water (5.0 mL) were added $FeCl_3$ (487 mg, 3.00 mmol) and zinc powder (>90%) (654 mg, >10.0 mmol). The reaction mixture was refluxed for 1 h. After cooling to room temperature, the reaction mixture was filtrated with a celite pad and the filtrate was concentrated in vacuo. The residue was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). After extraction with CH_2Cl_2 (50 mL), the obtained organic phase was washed with brine, dried over Na_2SO_4 and concentrated in vacuo to obtain the crude *N*-(2-aminobenzyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide as a yellow oil. This oil was dissolved in DMF (5.0 mL), and to the solution were added 5-chlorothiophene-2-carboxylic acid (163 mg, 1.00 mmol), HOBt (135 mg, 1.00 mmol) and EDC·HCl (288 mg, 1.50 mmol). The reaction mixture was stirred at room temperature overnight. Solvent was distilled off in vacuo, and the residue was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). After extraction with CH_2Cl_2 (50 mL), the organic phase

was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (8% MeOH–CH_2Cl_2) to obtain the free form of the title compound. After adding 1 M HCl solution in EtOH to the free form of the title compound, the solvent was evaporated. The resultant solid was washed with AcOEt and collected to obtain the title compound (275 mg, 0.545 mmol, 55%) as a white powder. MP: 185–189 °C (Dec.). IR (ATR) cm^{-1}: 3388, 3190, 3055, 2549, 2376, 1676, 1626, 1593, 1568, 1522, 1491, 1442, 1421, 1362, 1331, 1317, 1228, 1119, 1076, 1009, 993, 904, 822, 762, 714, 671, 621, 521, 488, 465, 438. ^1H NMR (DMSO-d_6) δ: 2.92 (3H, s), 3.17 (2H, br s), 3.21–3.68 (2H, m), 4.47 (2H, d, J = 6.3 Hz), 4.49–4.73 (2H, m), 7.24 (1H, td, J = 7.6, 1.2 Hz), 7.28–7.34 (2H, m), 7.38 (1H, dd, J = 7.6, 1.2 Hz), 7.46 (1H, dd, J = 7.6, 1.2 Hz), 7.95 (1H, d, J = 4.2 Hz), 9.53 (1H, t, J = 6.3 Hz), 10.42 (1H, s), 11.35 (1H, br s). ESI-MS m/z: 447 [$(M+H)^+$, ^{35}Cl], 449 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{20}H_{19}ClN_4O_2S_2 \cdot HCl \cdot 0.2H_2O \cdot 0.2AcOEt$: C, 49.50; H, 4.39; Cl, 14.05; N, 11.10; S, 12.71. Found: C, 49.73; H, 4.28; Cl, 13.81; N, 11.00; S, 12.65.

5.1.13. *tert*-Butyl [(1*R**,2*R**)-2-(hydroxymethyl)cyclohexyl]carbamate (7)

To the mixed solution of (1*R**,2*R**)-2-aminocyclohexane carboxylic acid (**6**) (1.00 g, 6.98 mmol) in dioxane (20 mL) and water (20 mL) were added di-*tert*-butyl dicarbonate (1.68 g, 7.68 mmol) and triethylamine (1.46 mL, 10.5 mmol) under ice cooling. After stirring for 3 d at room temperature, solvent was removed in vacuo. The residue was partitioned between AcOEt (150 mL) and 10% citric acid aqueous solution. Organic phase was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was washed with hexane and collected to obtain crude (1*R**,2*R**)-2-(*tert*-butoxycarbonylamino)cyclohexane carboxylic acid (1.61 g, 6.62 mmol, 95%) as a white powder.

This crude (1*R**,2*R**)-2-(*tert*-butoxycarbonylamino)cyclohexane carboxylic acid (1.38 g, 5.67 mmol) were dissolved in 1,2-dimethoxyethane (10 mL), and to the solution was added *N*-methylmorpholine (0.748 mL, 6.81 mmol). After cooling to −20 °C, *iso*-butyl chloroformate (0.894 mL, 6.81 mmol) was added and the mixture was stirred for 15 min. After removing the precipitate by filtration, to the filtrate was added water (1 mL) containing sodium borohydride (321 mg, 8.51 mmol). After stirring for 2 h at room temperature, the solvent was removed in vacuo. To the residue were added CH_2Cl_2 and water. Organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo. The obtained solid was washed with diethyl ether–hexane and collected to obtain the title compound (855 mg, 3.73 mmol, 66%) as a white powder. ^1H NMR ($CDCl_3$) δ: 1.02–1.40 (5H, m), 1.45 (9H, s), 1.48–1.62 (1H, m), 1.62–1.70 (1H, m), 1.71–1.81 (2H, m), 1.91–2.00 (1H, m), 3.31–3.38 (1H, m), 3.39–3.44 (1H, m), 3.75 (1H, dd, J = 12.0, 2.9 Hz). ESI-MS m/z: 252 $(M+Na)^+$, 174 $(M-{}^tBu)^+$.

5.1.14. *tert*-Butyl [(1*R**,2*S**)-2-(azidomethyl)cyclohexyl]carbamate (8)

To the solution of compound **7** (229 mg, 1.00 mmol) in CH_2Cl_2 (3 mL) were added triethylamine (278 μL, 2.00 mmol) and methanesulfonyl chloride (116 μL, 1.50 mmol) at 0 °C, and the mixture was stirred for 1 h. To the mixture was added saturated $NaHCO_3$ aqueous solution (50 mL), and the organic phase was separated. After extraction with CH_2Cl_2 (50 mL) from the aqueous phase, the organic phase was combined, dried over with Na_2SO_4 and concentrated in vacuo. To the obtained yellow solid were added DMF (5 mL) and sodium azide (195 mg, 3.00 mmol), and this mixture was stirred at 70 °C for 3 d. After cooling to room temperature, to the mixture were added water (50 mL) and Et_2O (50 mL). Organic phase was separated, washed with brine (2 × 50 mL) and dried over with Na_2SO_4. The solvent was distilled off in vacuo to obtain the title compound (230 mg, 0.904 mmol, 90%) as a white powder. This compound was used directly in the next steps without further purification. ^1H NMR ($CDCl_3$) δ: 1.03–1.40 (5H, m), 1.45 (9H, s), 1.58–1.79 (2H, m), 1.87–2.03 (2H, m), 3.11–3.22 (1H, m), 3.22–3.33 (1H, m), 3.50 (1H, dd, J = 12.3, 3.8 Hz), 4.34 (1H, br s). ESI-MS m/z: 277 $(M+Na)^+$, 199 $(M-{}^tBu)^+$, 155 $(M-Boc)^+$.

5.1.15. *tert*-Butyl [(1*R**,2*S**)-2-({[(5-chlorothiophen-2-yl)carbonyl]amino}methyl)cyclohexyl]carbamate (3F)

Compound **8** (216 mg, 0.850 mmol) was dissolved in MeOH (10 mL), and to the solution was added wet 10% palladium on carbon (50% water containing, 70 mg). The reaction mixture was stirred under a hydrogen atmosphere for 3 h. After elimination of the catalyst, the filtrate was concentrated in vacuo. The residue was dissolved in DMF (5 mL), and to this solution were added 5-chlorothiophene-2-carboxylic acid (138 mg, 0.850 mmol), HOBt (115 mg, 0.850 mmol), EDC·HCl (244 mg, 1.27 mmol) and triethylamine (118 μL, 0.850 mmol). The reaction mixture was stirred at room temperature overnight. Solvent was distilled off in vacuo and the residue was partitioned between AcOEt (50 mL) and 10% citric acid aqueous solution (50 mL). Organic phase was washed with saturated $NaHCO_3$ aqueous solution and brine (each 50 mL), and dried over Na_2SO_4. Solvent was distilled off in vacuo to obtain the title compound (300 mg, 0.804 mmol, 95%) as a yellow solid. ^1H NMR ($CDCl_3$) δ: 1.10–1.35 (5H, m), 1.47 (9H, s), 1.65–1.83 (3H, m), 1.90–1.97 (1H, m), 2.98–3.06 (1H, m), 3.28–3.42 (1H, m), 3.86–3.96 (1H, m), 4.48 (1H, d, J = 9.3 Hz), 6.90 (1H, d, J = 4.1 Hz), 7.38 (1H, d, J = 4.1 Hz), 7.75–7.86 (1H, m). ESI-MS m/z: 395 $(M+Na)^+$, 317 $(M-{}^tBu)^+$, 273 $(M-Boc)^+$.

5.1.16. *N*-[(1*R**,2*S**)-2-({[(5-Chlorothiophen-2-yl)carbonyl]amino}methyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (1F)

Compound **1F** was synthesized in a similar manner to compound **1A**. Compound **3F** (300 mg, 0.804 mmol) was deprotected with 4 M HCl solution in dioxane. Obtained amine, DMF (5.0 mL), 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (227 mg, 0.967 mmol), HOBt (109 mg, 0.807 mmol), EDC·HCl (231 mg, 1.21 mmol) and triethylamine (224 μL, 1.61 mmol) were treated to obtain the title compound (145 mg, 0.286 mmol, 36%) as a white solid. MP: 150–155 °C (Dec.). IR (ATR) cm^{-1}: 3381, 2929, 2852, 1635, 1545, 1518, 1462, 1425, 1363, 1327, 1290, 1242, 1142, 1097, 1045, 995, 958, 876, 818, 741, 669, 577, 546, 509, 457. ^1H NMR (DMSO-d_6) δ: 0.95–1.35 (3H, m), 1.47–1.92 (6H, m), 2.87–3.01 (5H, m), 3.07–3.22 (2H, m), 3.44–3.70 (2H, m), 4.31–4.81 (3H, m), 7.17 (1H, d, J = 4.2 Hz), 7.61 (1H, d, J = 4.2 Hz), 8.39 (1H, t, J = 6.1 Hz), 8.78 (1H, d, J = 9.0 Hz), 11.22 (1H, br s). ESI-MS m/z: 453 [$(M+H)^+$, ^{35}Cl], 455 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{20}H_{25}ClN_4O_2S_2 \cdot HCl \cdot H_2O$: C, 47.33; H, 5.56; Cl, 13.97; N, 11.04; S, 12.64. Found: C, 47.03; H, 5.54; Cl, 13.87; N, 10.79; S, 12.37.

5.1.17. *tert*-Butyl [(1*R**,2*S**)-2-(benzylcarbamoyl)cyclohexyl]carbamate (10)

(1*R**,2S*)-2-Aminocyclohexane carboxylic acid (**9**) (1.00 g, 6.98 mmol) was treated in a similar manner to the intermediate of compound **7** to obtain (1*R**,2S*)-2-(*tert*-butoxycarbonylamino)cyclohexane carboxylic acid (0.951 g, 3.91 mmol, 56%) as a white powder.

(1*R**,2*S**)-2-(*tert*-Butoxycarbonylamino)cyclohexane carboxylic acid (487 mg, 2.00 mmol) was dissolved in DMF (5 mL), and to this solution were added benzylamine (262 μL, 2.40 mmol), HOBt (109 mg, 0.807 mmol) and EDC·HCl (231 mg, 1.21 mmol). The mixture was stirred at room temperature for 3 d. After the solvent was removed in vacuo, the residue was partitioned between AcOEt

(50 mL) and 10% citric acid aqueous solution (50 mL). Organic phase was washed with saturated $NaHCO_3$ aqueous solution and brine (each 50 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was washed with hexane to obtain the title compound (611 mg, 1.84 mmol, 92%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.41 (9H, s), 1.41–1.46 (1H, m), 1.53–1.68 (4H, m), 1.72–1.80 (2H, m), 1.93–2.06 (1H, m), 2.60–2.66 (1H, m), 3.84 (1H, br s), 4.36–4.50 (2H, m), 5.34 (1H, br s), 6.04 (1H, br s), 7.23–7.29 (3H, m), 7.30–7.37 (2H, m). ESI-MS *m*/*z*: 333 $(M+H)^+$.

5.1.18. *tert*-Butyl [(1*R**,2*R**)-2-(benzylaminomethyl)cyclohexyl]carbamate (11)

To the solution of compound **10** (611 mg, 1.84 mmol) in THF (20 mL) was added 1 M borane–tetrahydrofurane complex THF solution (12.9 mL, 12.9 mmol), and the mixture was refluxed for 1 h. After cooling to the room temperature, solvent was distilled off in vacuo. To the residue were added EtOH (5 mL), water (5 mL) and triethylamine (5 mL), and the mixture was refluxed for 1.5 h. After removing the solvent in vacuo, the residue was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). After extraction with CH_2Cl_2 (50 mL), combined organics were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (5→8% MeOH–CH_2Cl_2) to obtain the title compound (108 mg, 0.340 mmol, 18%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.13–1.39 (3H, m), 1.45 (9H, s), 1.47–1.58 (3H, m), 1.60–1.73 (3H, m), 1.93–2.06 (1H, m), 2.49–2.57 (1H, m), 2.59–2.70 (1H, m), 3.62–3.68 (1H, m), 3.75 (1H, d, *J* = 12.9 Hz), 3.83–3.90 (2H, m), 7.23–7.41 (5H, m). ESI-MS *m*/*z*: 319 $(M+H)^+$.

5.1.19. *tert*-Butyl [(1*R**,2*R**)-2-({[(5-chlorothiophen-2-yl)carbonyl]amino}methyl)cyclohexyl]carbamate (3G)

Compound **10** (95.0 mg, 0.286 mmol) was dissolved in AcOH (5 mL), and to the solution was added wet 10% palladium on carbon (50%water containing, 50 mg). The reaction mixture was stirred under a hydrogen atmosphere for 6 d. After elimination of the catalyst, the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (20 mL), and Na_2CO_3 powder was added to this solution. After stirring for 30 min, insoluble substance was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in DMF (3 mL), and 5-chlorothiophene-2-carboxylic acid (47.2 mg, 0.286 mmol), HOBt (39.2 mg, 0.286 mmol), EDC·HCl (83.4 mg, 0.429 mmol) and triethylamine (40 μL, 0.29 mmol) were added to this solution. The reaction mixture was stirred at room temperature overnight. Solvent was distilled off in vacuo and the residue was partitioned between AcOEt (50 mL) and 10% citric acid aqueous solution (50 mL). Organic phase was washed with saturated $NaHCO_3$ aqueous solution and brine (each 50 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Hexane/AcOEt = 4:1) to obtain the title compound (68.1 mg, 0.183 mmol, 63%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 0.93–1.07 (1H, m), 1.13–1.37 (3H, m), 1.48 (9H, s), 1.50–1.83 (5H, m), 2.38–2.49 (1H, m), 3.75–3.86 (1H, m), 3.92–4.01 (1H, m), 4.79–4.86 (1H, m), 6.89 (1H, d, *J* = 3.9 Hz), 7.38 (1H, d, *J* = 3.9 Hz), 8.00–8.10 (1H, m). ESI-MS *m*/*z*: 395 $(M+Na)^+$, 317 $(M-{}^t\mathrm{Bu})^+$, 273 $(M-\mathrm{Boc})^+$.

5.1.20. *N*-[(1*R**,2*R**)-2-({[(5-Chlorothiophen-2-yl)carbonyl]amino}methyl)cyclohexyl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (1G)

Compound **1G** was synthesized in a similar manner to compound **1A**. Compound **3G** (68.1 mg, 0.183 mmol) was deprotected with 4 M HCl solution in dioxane. Obtained amine, DMF (5.0 mL), 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (51.4 mg, 0.220 mmol), HOBt (24.7 mg, 0.183 mmol), EDC·HCl (52.5 mg, 0.275 mmol) and triethylamine (76 μL, 0.55 mmol) were treated to obtain the title compound (52.2 mg, 0.101 mmol, 56%) as a white solid. MP: 161–168 °C (Dec.). IR (ATR) cm^{-1}: 3400, 2929, 2858, 1732, 1635, 1547, 1520, 1462, 1427, 1371, 1327, 1281, 1252, 1142, 1092, 1043, 997, 960, 916, 877, 810, 746, 714, 606, 553, 511, 451, 417.

^{1}H NMR (DMSO-d_6) δ: 1.20–1.67 (8H, m), 1.70–1.83 (1H, m), 2.01–2.12 (1H, m), 2.92 (3H, s), 3.01–3.41 (3H, m), 3.45–3.70 (2H, m), 4.10–4.20 (1H, m), 4.36–4.74 (2H, m), 7.16 (1H, d, *J* = 4.1 Hz), 7.58 (1H, d, *J* = 4.1 Hz), 8.33 (1H, d, *J* = 8.5 Hz), 8.49 (1H, t, *J* = 5.6 Hz), 11.47 (1H, br s). ESI-MS *m*/*z*: 453 $[(M+H)^+, {}^{35}\mathrm{Cl}]$, 455 $[(M+H)^+, {}^{37}\mathrm{Cl}]$. Anal. Calcd for $C_{20}H_{25}ClN_4O_2S_2{\cdot}HCl{\cdot}1.4H_2O$: C, 46.67; H, 5.64; Cl, 13.78; N, 10.89; S, 12.46. Found: C, 46.96; H, 5.45; Cl, 13.61; N, 10.53; S, 12.07.

5.1.21. 2-Aminonicotinamide (13A)

To the suspension of 2-aminonicotinic acid (1.38 g, 10.0 mmol) in DMF (50 mL) were added HOBt (1.35 g, 10.0 mmol), NH_4Cl (1.07 g, 20.0 mmol), EDC·HCl (2.88 g, 15.0 mmol) and triethylamine (2.78 mL, 20.0 mmol). The mixture was stirred overnight at room temperature. Solvent was distilled off in vacuo, and CH_2Cl_2 (150 mL) and saturated $NaHCO_3$ aqueous solution (150 mL) were added to the residue. Organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (3→5%MeOH–CH_2Cl_2) to obtain the title compound (1.21 g, 8.85 mmol, 89%) as a white powder. ^{1}H NMR (DMSO-d_6) δ: 6.55 (1H, dd, *J* = 7.7, 4.8 Hz), 7.17 (2H, s), 7.30 (1H, s), 7.92 (2H, dd, *J* = 7.7, 1.8 Hz), 8.06 (1H, dd, *J* = 4.8, 1.8 Hz). ESI-MS *m*/*z*: 138 $(M+H)^+$.

5.1.22. *tert*-Butyl [(2-aminopyridin-3-yl)methyl]carbamate (2H)

To the suspension of $LiAlH_4$ (330 mg, 8.00 mmol) in THF (50 mL) was added compound **13A** (274 mg, 2.00 mmol) under ice cooling. The mixture was stirred at room temperature for 30 min, and the mixture was refluxed overnight. After cooling to room temperature, to the mixture were sequentially added 5 M NaOH aqueous solution (0.33 mL), water (0.33 mL) and 5 M NaOH aqueous solutio (1.0 mL). The mixture was stirred at room temperature for 2 h. $MgSO_4$ was added to the mixture, and the unsoluble substance was filtered off by a celite pad. The filtrate was concentrated in vacuo to obtain a colorless oil. This oil was dissolved in THF (5 mL) and di-*tert*-butyl dicarbonate (437 mg, 2.00 mmol) was added. After stirring at room temperature for 2 h, the solvent was distilled off in vacuo. The residue was purified by flash chromatography on silica gel (8%MeOH–CH_2Cl_2) to obtain the title compound (230 mg, 1.03 mmol, 52%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 4.20 (2H, d, *J* = 6.3 Hz), 4.92 (1H, br s), 5.25 (2H, br s), 6.57 (1H, dd, *J* = 7.3, 5.6 Hz), 7.26 (1H, dd, *J* = 7.3, 1.7 Hz), 7.99 (1H, dd, *J* = 5.6, 1.7 Hz). ESI-MS *m*/*z*: 224 $(M+H)^+$.

5.1.23. *tert*-Butyl [(2-{[(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-yl)carbonyl]amino}pyridin-3-yl)methyl]carbamate (3H)

5-Methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (469 mg, 2.00 mmol) was suspended in thionylchloride (10 mL). The mixture was refluxed for 1 h and concentrated in vacuo to obtain the corresponding acylchloride as a solid. To the obtained solid were added pyridine (10 mL) and CH_2Cl_2 (10 mL) containing compound **2H** (223 mg, 1.00 mmol). The mixture was stirred at room temperature overnight and stirred at 50 °C overnight. To the mixture was added further prepared acylchloride that is obtained by treating 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (469 mg, 2.00 mmol) and thionylchloride (10 mL) as described before. The mixture was stirred at room temperature for another 3 d. Solvent was distilled off in vacuo and the residue was partitioned

between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). Organic phase was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (8% MeOH–CH_2Cl_2) to obtain the title compound (211 mg, 0.523 mmol, 52%) as a white powder. ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.60 (3H, s), 2.96 (2H, br s), 3.05 (2H, br s), 3.86 (2H, br s), 4.34 (2H, d, J = 6.1 Hz), 5.59 (1H, br s), 7.24 (1H, dd, J = 7.6, 4.9 Hz), 7.91 (1H, d, J = 7.6 Hz), 8.39 (1H, d, J = 4.9 Hz), 9.40 (1H, br s). ESI-MS m/z: 404 $(M+H)^+$.

5.1.24. *N*-[3-({[(5-Chlorothiophen-2-yl)carbonyl]amino}methyl)pyridin-2-yl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide (1H)

Compound **1H** was synthesized in a similar manner to compound **1C**. Compound **3H** (205 mg, 0.508 mmol) was deprotected with 4 M HCl solution in dioxane. Obtained amine, DMF (10 mL), 5-chlorothiophene-2-carboxylic acid (51.4 mg, 0.220 mmol), HOBt (68.7 mg, 0.508 mmol), EDC·HCl (146 mg, 0.762 mmol) and triethylamine (212 μL, 1.52 mmol) were treated to obtain the title compound (167 mg, 0.307 mmol, 60%) as a pale yellow solid. MP: 265–269 °C (Dec.). IR (ATR) cm^{-1}: 3388, 3190, 3055, 2549, 2376, 1676, 1626, 1593, 1568, 1522, 1491, 1442, 1421, 1362, 1331, 1317, 1228, 1119, 1076, 1009, 993, 904, 822, 762, 714., 671, 621, 521, 488, 465, 438. ^{1}H NMR (DMSO-d_6) δ: 2.95 (3H, s), 3.12–3.35 (2H, m), 3.46–3.87 (2H, m), 4.40–4.54 (3H, m), 4.72–4.82 (1H, m), 7.21 (1H, d, J = 3.9 Hz), 7.41 (1H, dd, J = 7.6, 4.9 Hz), 7.72 (1H, d, J = 3.9 Hz), 7.81 (1H, d, J = 7.6 Hz), 8.42 (1H, d, J = 4.9 Hz), 9.23 (1H, br s), 11.02 (1H, br s), 11.33–11.60 (1H, m). ESI-MS m/z: 448 [$(M+H)^+$, ^{35}Cl], 450 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{19}H_{18}ClN_5O_2S_2 \cdot 1.9HCl \cdot 1.5H_2O$: C, 41.93; H, 4.24; Cl, 18.89; N, 12.87; S, 11.78. Found: C, 41.72; H, 4.20; Cl, 19.17; N, 12.70; S, 11.65.

5.1.25. 3-Aminoisonicotinamide (13B)

To the suspension of isonicotinic acid (1.00 g, 7.24 mmol) in DMF (10 mL) were added HOBt (978 mg, 7.24 mmol), 7 M ammonia solution in methanol (2.06 mL, 14.5 mmol) and EDC·HCl (2.08 g, 10.9 mmol). The mixture was stirred overnight at room temperature. Solvent was distilled off in vacuo, and CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution were added to the residue. Organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (5→8% MeOH–CH_2Cl_2) to obtain the title compound (767 mg, 5.60 mmol, 77%) as a pale yellow powder. ^{1}H NMR (DMSO-d_6) δ: 6.59 (2H, br s), 7.39 (1H, d, J = 5.3 Hz), 7.43 (1H, br s), 7.72 (1H, d, J = 5.3 Hz), 8.00 (1H, br s), 8.12 (1H, s). ESI-MS m/z: 138 $(M+H)^+$.

5.1.26. *tert*-Butyl [(3-aminopyridin-4-yl)methyl]carbamate (2I)

Compound **2I** was synthesized in a similar manner to compound **2H**. Compound **13B** (274 mg, 2.00 mmol) was reducted by $LiAlH_4$ (330 mg, 8.00 mmol). Obtained amine and di-*tert*-butyl dicarbonate (349 mg, 1.60 mmol) were treated to obtain the title compound (217 mg, 0.972 mmol, 49%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 4.22 (2H, d, J = 6.6 Hz), 5.00 (1H, br s), 6.93 (1H, d, J = 4.5 Hz), 7.92 (1H, d, J = 4.5 Hz), 8.05 (1H, s). ESI-MS m/z: 224 $(M+H)^+$.

5.1.27. *tert*-Butyl [(3-{[(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-yl)carbonyl]amino}pyridin-4-yl)methyl]carbamate (3I)

Compound **3I** was synthesized in a similar manner to compound **3H**. 5-Methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (352 mg, 1.50 mmol) was treated with thionylchloride (5 mL) to obtain acylchloride. Obtained acylchloride, pyridine (5 mL) and compound **2I** (216 mg, 0.969 mmol) were treated to obtain the title compound (163 mg, 0.404 mmol, 42%) as a light yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.56 (3H, s), 2.90 (2H, t, J = 5.8 Hz), 3.01 (2H, t, J = 5.8 Hz), 3.80 (2H, s), 4.34 (2H, d, J = 6.1 Hz), 5.22 (1H, br s), 7.31 (1H, d, J = 5.0 Hz), 8.46 (1H, d, J = 5.0 Hz), 8.98 (1H, s), 9.47 (1H, br s). ESI-MS m/z: 404 $(M+H)^+$.

5.1.28. *N*-[4-({[(5-Chlorothiophen-2-yl)carbonyl]amino}methyl)pyridin-3-yl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (1I)

Compound **1I** was synthesized in a similar manner to compound **1C**. Compound **3I** (163 mg, 0.404 mmol) was deprotected with 4 M HCl solution in dioxane. Obtained amine, DMF (10 mL), 5-chlorothiophene-2-carboxylic acid (65.7 mg, 0.404 mmol), HOBt (54.6 mg, 0.404 mmol), EDC·HCl (116 mg, 0.606 mmol) and triethylamine (169 μL, 1.21 mmol) were treated to obtain the title compound (106 mg, 0.204 mmol, 51%) as a pale yellow solid. MP: 228–230 °C (Dec.). IR (ATR) cm^{-1}: 3199, 2947, 2667, 2540, 2387, 1685, 1655, 1610, 1541, 1516, 1471, 1423, 1363, 1329, 1294, 1230, 1200, 1134, 1082, 1003, 949, 895, 854, 806, 735, 627, 600, 548, 515, 469, 422. ^{1}H NMR (DMSO-d_6) δ: 2.95 (3H, s), 3.13–3.36 (2H, m), 3.44–3.93 (2H, m), 4.42–4.59 (3H, m), 4.70–4.85 (1H, m), 7.23 (1H, d, J = 4.1 Hz), 7.57 (1H, d, J = 5.4 Hz), 7.79 (1H, d, J = 4.1 Hz), 8.56 (1H, d, J = 5.4 Hz), 8.75 (1H, s), 9.43 (1H, t, J = 5.7 Hz), 11.03 (1H, s), 11.65 (1H, br s). ESI-MS m/z: 448 [$(M+H)^+$, ^{35}Cl], 450 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{19}H_{18}ClN_5O_2S_2 \cdot 1.5HCl \cdot H_2O$: C, 43.83; H, 4.16; Cl, 17.02; N, 13.45; S, 12.32. Found: C, 43.50; H, 4.31; Cl, 17.34; N, 13.34; S, 12.15.

5.1.29. *tert*-Butyl [3-(hydroxymethyl)pyridin-4-yl]carbamate (15A)

tert-Butyl (3-formylpyridin-4-yl)carbamate[12] was dissolved in MeOH (10 mL), and $NaBH_4$ (205 mg, 5.43 mmol) was added. After the mixture was stirred at room temperature for 1 h, the solvent was distilled off in vacuo. The residue was partitioned between CH_2Cl_2 (50 mL) and brine (50 mL). Organic phase was dried over Na_2SO_4 and concentrated in vacuo. Obtained solid was washed with hexane to obtain the title compound (382 mg, 1.70 mmol, 63%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 4.66 (2H, s), 7.93 (1H, s), 8.08 (1H, d, J = 5.9 Hz), 8.28 (1H, d, J = 5.9 Hz), 8.32 (1H, s). ESI-MS m/z: 225 $(M+H)^+$.

5.1.30. *tert*-Butyl [3-(azidomethyl)pyridin-4-yl]carbamate (2J)

Compound **15A** (382 mg, 1.70 mmol) was dissolved in THF (5 mL). To this solution were added diphenylphospholyl azide (732 μL, 3.41 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (509 μL, 3.41 mmol) under ice cooling. After stirring at room temperature overnight, the solvent was distilled off in vacuo. The residue was partitioned between AcOEt (50 mL) and water (50 mL). Organic phase was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (3% MeOH–CH_2Cl_2) to obtain the title compound (333 mg, 1.33 mmol, 79%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.55 (9H, s), 4.36 (2H, s), 8.11 (1H, d, J = 5.6 Hz), 8.36 (1H, s), 8.50 (1H, d, J = 5.6 Hz). ESI-MS m/z: 250 $(M+H)^+$.

5.1.31. *tert*-Butyl (3-{[(5-chlorothiophen-2-yl)carbonylamino]methyl}pyridin-4-yl)carbamate (3J)

Compound **2J** (330 mg, 1.32 mmol) was dissolved in AcOEt (10 mL), and to the solution was added wet 10% palladium on carbon (50%water containing, 100 mg). The reaction mixture was stirred under a hydrogen atmosphere for 2 h. After elimination of the catalyst, the filtrate was concentrated in vacuo. The residue was dissolved in DMF (5 mL), and to this solution were added 5-chlorothiophene-2-carboxylic acid (215 mg, 1.32 mmol), HOBt (179 mg, 1.32 mmol) and EDC·HCl (380 mg, 1.99 mmol). The reaction mix-

ture was stirred at room temperature for 1 h. Solvent was distilled off in vacuo, and the residue was partitioned between AcOEt (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). Organic phase was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (3→5% MeOH–CH_2Cl_2) to obtain the title compound (338 mg, 0.919 mmol, 70%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.57 (9H, s), 4.51 (2H, d, J = 6.3 Hz), 6.90 (1H, d, J = 4.1 Hz), 7.57 (1H, d, J = 4.1 Hz), 8.19–8.28 (3H, m), 8.38 (1H, d, J = 6.1 Hz), 9.52 (1H, s). ESI-MS m/z: 368 [(M+H)$^+$, ^{35}Cl], 370 [(M+H)$^+$, ^{37}Cl].

5.1.32. *N*-[3-({[(5-Chlorothiophen-2-yl)carbonyl]amino}methyl)pyridin-4-yl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (1J)

Compound **1J** was synthesized in a similar manner to compound **1A**. Compound **3J** (238 mg, 0.647 mmol) was deprotected with 4 M HCl solution in dioxane. Obtained amine, DMF (5.0 mL), 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (182 mg, 0.775 mmol), HOBt (87.4 mg, 0.647 mmol), EDC·HCl (186 mg, 0.971 mmol) and triethylamine (271 μL, 1.94 mmol) were treated to obtain the title compound (52.9 mg, 0.0972 mmol, 15%) as a white solid. MP: 255–258 °C (Dec.). IR (ATR) cm^{-1}: 3396, 3062, 3016, 2956, 2538, 1701, 1639, 1595, 1560, 1493, 1425, 1358, 1329, 1302, 1240, 1186, 1149, 1065, 1005, 945, 893, 825, 781, 741, 714, 646, 544, 515, 469, 418. ^{1}H NMR (DMSO-d_6) δ: 2.93 (3H, s), 3.08–3.94 (4H, m), 4.43–4.58 (1H, m), 4.63 (2H, d, J = 5.2 Hz), 4.69–4.86 (1H, m), 7.21 (1H, d, J = 4.1 Hz), 7.75 (1H, d, J = 4.1 Hz), 8.35 (1H, d, J = 6.2 Hz), 8.74 (1H, d, J = 6.2 Hz), 8.80 (1H, s), 9.57 (1H, t, J = 5.2 Hz), 11.14 (1H, s), 11.53–11.85 (1H, m). ESI-MS m/z: 448 [(M+H)$^+$, ^{35}Cl], 450 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{19}H_{18}ClN_5O_2S_2 \cdot 1.9HCl \cdot 1.5H_2O$: C, 41.93; H, 4.24; Cl, 18.89; N, 12.87; S, 11.78. Found: C, 42.00; H, 4.11; Cl, 18.94; N, 12.66; S, 11.66.

5.1.33. *tert*-Butyl [2-(hydroxymethyl)pyridin-3-yl]carbamate (15B)

Compound **15B** was synthesized in a similar manner to compound **15A**. *tert*-Butyl (2-formylpyridin-3-yl)carbamate[12] (1.10 g, 5.05 mmol) was treated with $NaBH_4$ (381 mg, 10.1 mmol) to obtain the title compound (1.10 g, 4.89 mmol, 97%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 3.06–3.86 (1H, m), 4.80 (2H, s), 7.22 (1H, dd, J = 8.5, 4.6 Hz), 7.35 (1H, br s), 8.16 (1H, d, J = 4.6 Hz), 8.31 (1H, d, J = 8.5 Hz). ESI-MS m/z: 225 (M+H)$^+$.

5.1.34. *tert*-Butyl [2-(azidomethyl)pyridin-3-yl]carbamate (2K)

Compound **2K** was synthesized in a similar manner to compound **2J**. Compound **15B** (1.09 g, 4.86 mmol) was treated with diphenylphospholyl azide (2.09 mL, 9.72 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.45 mL, 9.72 mmol) to obtain the title compound (930 mg, 3.73 mmol, 77%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.54 (9H, s), 4.54 (2H, s), 6.97 (1H, br s), 7.28 (1H, dd, J = 8.3, 4.8 Hz), 8.26 (1H, dd, J = 4.8, 1.6 Hz), 8.30 (1H, dd, J = 8.3, 1.6 Hz). ESI-MS m/z: 250 (M+H)$^+$.

5.1.35. *tert*-Butyl (2-{[(5-chlorothiophen-2-yl)carbonylamino]methyl}pyridin-3-yl)carbamate (3K)

Compound **3K** was synthesized in a similar manner to compound **3J**. Compound **2K** (900 mg, 3.61 mmol) was hydrogenated with wet 10% palladium on carbon (50%water containing, 100 mg). Obtained amine, DMF (15 mL), 5-chlorothiophene-2-carboxylic acid (587 mg, 3.61 mmol), HOBt (488 mg, 3.61 mmol) and EDC·HCl (1.04 g, 5.42 mmol) were treated to obtain the title compound (639 mg, 1.74 mmol, 48%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.55 (9H, s), 4.65 (2H, d, J = 5.9 Hz), 6.87 (1H, d, J = 3.9 Hz), 7.26 (1H, dd, J = 8.3, 4.9 Hz), 7.34 (1H, d, J = 3.9 Hz), 8.02 (1H, t, J = 5.9 Hz), 8.20 (1H, dd, J = 4.9, 2.4 Hz), 8.29 (1H, dd, J = 8.3, 1.5 Hz), 8.46 (1H, s). ESI-MS m/z: 368 [(M+H)$^+$, ^{35}Cl], 370 [(M+H)$^+$, ^{37}Cl].

5.1.36. *N*-[2-({[(5-Chlorothiophen-2-yl)carbonyl]amino}methyl)pyridin-3-yl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (1K)

Compound **1K** was synthesized in a similar manner to compound **1A**. Compound **3K** (304 mg, 0.826 mmol) was deprotected with 4 M HCl solution in dioxane. Obtained amine, DMF (5 mL), 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (233 mg, 0.992 mmol), HOBt (112 mg, 0.826 mmol), EDC·HCl (238 mg, 1.24 mmol) and triethylamine (346 μL, 2.48 mmol) were treated to obtain the title compound (186 mg, 0.334 mmol, 40%) as a white solid. MP: 285–290 °C (Dec.). IR (ATR) cm^{-1}: 3068, 3016, 2954, 2538, 2382, 1685, 1639, 1552, 1520, 1456, 1421, 1371, 1331, 1294, 1221, 1182, 1130, 1074, 997, 947, 893, 870, 818, 762, 723, 644, 611, 586, 517, 478. ^{1}H NMR (DMSO-d_6) δ: 2.94 (3H, s), 3.09–3.33 (2H, m), 3.56–3.79 (2H, m), 4.41–4.52 (1H, m), 4.59 (2H, d, J = 5.6 Hz), 4.70–4.83 (1H, m), 7.18 (1H, d, J = 3.9 Hz), 7.51 (1H, dd, J = 8.1, 4.9 Hz), 7.72 (1H, d, J = 3.9 Hz), 8.05 (1H, dd, J = 8.1, 1.5 Hz), 8.50 (1H, dd, J = 4.9, 1.5 Hz), 9.28 (1H, t, J = 5.6 Hz), 10.94 (1H, s), 11.58 (1H, br s). ESI-MS m/z: 448 [(M+H)$^+$, ^{35}Cl], 450 [(M+H)$^+$, ^{37}Cl]. Anal. Calcd for $C_{19}H_{18}ClN_5O_2S_2 \cdot 2HCl \cdot 2H_2O$: C, 40.98; H, 4.34; Cl, 19.10; N, 12.58; S, 11.52. Found: C, 41.17; H, 4.12; Cl, 18.90; N, 12.69; S, 11.58.

5.1.37. 2-Bromo-5-chloropyridin (17)

2-Amino-5-chloropyridine (3.86 g, 30.0 mmol) was dissolved in 48% HBr (50 mL). To this solution was added bromine (4.61 mL), while the inside temperature was kept from −5 °C to 0 °C. After water (25 mL) containing sodium nitrite (5.18 g, 75.0 mmol) was slowly added to the mixture, inside temperature was slowly raised to 15 °C while stirring the mixture for 3 h. To the solution were added 5 M NaOH aqueous solution (150 mL), sodium thiosulfate aqueous solution (10 mL) and Et_2O (150 mL). Organic layer was separated, washed with brine and dried over Na_2SO_4. Solvent was distilled off in vacuo to obtain the title compound (4.90 g, 25.5 mmol, 85%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 7.44 (1H, dd, J = 8.5, 0.7 Hz), 7.53 (1H, dd, J = 8.3, 2.7 Hz), 8.35 (1H, dd, J = 2.7, 0.7 Hz).

5.1.38. 5-Chloropyridine-2-carboxylic acid (18)

Compound **17** (4.54 g, 23.6 mmol) was suspended in dried Et_2O (100 mL) under argon atmosphere. To the suspension was added 1.54 M *n*-butyllithium solution in hexane (16.9 mL, 26.0 mmol) at −78 °C, and the mixture was stirred for 2 h to obtain a brown solution. Carbon dioxide gas (ca. 2 L) was blown into the solution, and the mixture was stirred for 2 h. After warming to room temperature, the solvent was distilled off in vacuo. Obtained brown solid was washed with hexane and suspended in water (50 mL). After 1 M HCl (30 mL) was added to this suspension, the mixture was concentrated in vacuo. Obtained yellow solid was washed with Et_2O to obtain the title compound (358 mg, 2.27 mmol, 9.6%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ: 8.05 (2H, d, J = 8.4 Hz), 8.12 (1H, dd, J = 8.4, 2.3 Hz), 8.76 (1H, d, J = 2.3 Hz). ESI-MS m/z: 158 [(M+H)$^+$, ^{35}Cl], 160 [(M+H)$^+$, ^{37}Cl].

5.1.39. *tert*-Butyl (2-nitrobenzyl)carbamate (37)

To the suspension of 2-nitrobenzylamine hydrochloride (1.89 g, 10.0 mmol) in CH_2Cl_2 (20 mL) were added di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol) and triethylamine (2.09 mL, 15.0 mmol). After stirring at room temperature for 3 h, solvent was distilled off in vacuo. The residue was partitioned between AcOEt (50 mL) and 10% citric acid aqueous solution (50 mL). Organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo.

Obtained orange solid was washed with hexane to give the title compound (2.23 g, 8.84 mmol, 88%) as an orange solid. ^{1}H NMR ($CDCl_3$) δ: 1.43 (9H, s), 4.57 (2H, d, J = 6.3 Hz), 5.34 (1H, br s), 7.42–7.49 (1H, m), 7.59–7.66 (2H, m), 8.06 (1H, d, J = 8.1 Hz). ESI-MS m/z: 275 $(M+Na)^+$, 197 $(M-{}^tBu)^+$, 153 $(M-Boc)^+$.

5.1.40. *tert*-Butyl {2-[(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-carbonyl)amino]benzyl}carbamate (38)

Compound **37** (2.23 g, 8.84 mmol) was dissolved in AcOEt (50 mL), and wet 10% palladium on carbon (50% water containing, 70 mg) was added to this solution. After stirring for 1 h under hydrogen atmosphere, catalyst was removed by filtration. Filtrate was concentrated in vacuo to obtain a white solid. This solid was dissolved in DMF (30 mL), and to this solution were added 5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxylic acid hydrochloride (2.49 g, 10.6 mmol), HOBt (1.19 g, 8.84 mmol), EDC·HCl (2.54 g, 13.3 mmol) and triethylamine (2.46 mL, 17.7 mmol). The mixture was stirred overnight at room temperature. After removing the solvent in vacuo, the residue was partioned between AcOEt (200 mL) and saturated $NaHCO_3$ aqueous solution (200 mL). Organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Obtained pale yellow powder was washed with Et_2O to obtain the title compound (2.93 g, 7.28 mmol, 82%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.52 (3H, s), 2.86 (2H, t, J = 5.8 Hz), 2.99 (2H, t, J = 5.8 Hz), 3.72 (2H, s), 4.34 (2H, d, J = 5.9 Hz), 5.13 (1H, br s), 7.19 (1H, td, J = 7.8, 1.2 Hz), 7.31–7.38 (2H, m), 7.92 (1H, dd, J = 7.8, 1.2 Hz), 9.52 (1H, br s). ESI-MS m/z: 403 $(M+H)^+$.

5.1.41. *N*-[2-({[(5-Chloropyridin-2-yl)carbonyl]amino}methyl)phenyl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (4A)

Compound **38** (302 mg, 0.751 mmol) was dissolved in CH_2Cl_2 (3 mL). To this solution was added 4 M HCl solution in dioxane (5 mL). After the mixture was stirred at room temperature for 1 h, solvent was distilled off in vacuo to obtain *N*-(2-aminomethylphenyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide dihydrochloride (compound **39**) (255 mg). This compound was used in the next step without purification.

Compound **39** (255 mg) was dissolved in DMF (10.0 mL). To the solution were added 5-chlorothiophene-2-carboxylic acid (118 mg, 0.751 mmol), HOBt (101 mg, 0.751 mmol), EDC·HCl (216 mg, 1.13 mmol) and triethylamine (209 μL, 1.50 mmol). After the mixture was stirred at room temperature for 2 h, solvent was distilled off in vacuo. The residue was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). Organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (5% MeOH–CH_2Cl_2) to give the free form of the title compound. To the free form of the title compound was added 1 M HCl aqueous solution and the mixture was concentrated in vacuo. The obtained solid was washed with AcOEt to obtain the title compound (297 mg, 0.609 mmol, 81%) as a white powder. MP: 265–269 °C (Dec.). IR (ATR) cm^{-1}: 3323, 1662, 1591, 1554, 1500, 1454, 1429, 1367, 1303, 1240, 1196, 1105, 1074, 1014, 962, 868, 831, 773, 721, 688, 631, 577, 525, 440. ^{1}H NMR (DMSO-d_6) δ: 2.94 (3H, s), 3.28 (2H, br s), 3.37–3.70 (2H, m), 4.49 (2H, d, J = 6.3 Hz), 4.53–4.71 (2H, m), 7.25 (1H, td, J = 7.3, 1.5 Hz), 7.31 (1H, td, J = 7.3, 1.5 Hz), 7.43 (1H, dd, J = 7.3, 1.5 Hz), 7.48 (1H, dd, J = 7.3, 1.5 Hz), 8.04 (1H, d, J = 8.5 Hz), 8.14 (1H, dd, J = 8.5, 2.3 Hz), 8.71 (1H, d, J = 2.3 Hz), 9.44 (1H, t, J = 6.3 Hz), 10.87 (1H, s), 11.22 (1H, br s). ESI-MS m/z: 442 $[(M+H)^+, {}^{35}Cl]$, 444 $[(M+H)^+, {}^{37}Cl]$. Anal. Calcd for $C_{21}H_{20}ClN_5O_2S{\cdot}HCl{\cdot}0.5H_2O$: C, 51.75; H, 4.55; Cl, 14.55; N, 14.37; S, 6.58. Found: C, 51.61; H, 4.51; Cl, 14.51; N, 14.33; S, 6.67.

5.1.42. *N*-(2-{[(4-Chlorobenzoyl)amino]methyl}phenyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (4B)

Compound **4B** was synthesized in a similar manner to compound **4A**. Compound **39** (intermediate of compound **4A**) (155 mg, 0.413 mmol), DMF (4.0 mL), 4-chlorobenzoic acid (64.7 mg, 0.413 mmol), HOBt (55.8 mg, 0.413 mmol), EDC·HCl (119 mg, 0.620 mmol) and triethylamine (115 μL, 0.826 mmol) were treated to obtain the title compound (112 mg, 0.231 mmol, 56%) as a white powder. MP: 262–264 °C (Dec.). IR (ATR) cm^{-1}: 3334, 3095, 2918, 2441, 2345, 1655, 1591, 1552, 1516, 1479, 1454, 1435, 1363, 1296, 1265, 1194, 1128, 1092, 1078, 1045, 1012, 962, 893, 847, 764, 721, 683, 652, 588, 573, 525, 465, 434. ^{1}H NMR (DMSO-d_6) δ: 2.93 (3H, s), 3.18 (2H, br s), 3.35–3.69 (2H, m), 4.45 (2H, d, J = 5.6 Hz), 4.46–4.73 (2H, m), 7.25 (1H, td, J = 7.5, 1.6 Hz), 7.31 (1H, td, J = 7.5, 1.6 Hz), 7.38 (1H, dd, J = 7.5, 1.6 Hz), 7.49–7.57 (3H, m), 7.88 (2H, d, J = 8.6 Hz), 9.17 (1H, t, J = 5.6 Hz), 10.74 (1H, s), 11.18–11.64 (1H, m). ESI-MS m/z: 441 $[(M+H)^+, {}^{35}Cl]$, 443 $[(M+H)^+, {}^{37}Cl]$. Anal. Calcd for $C_{22}H_{21}ClN_4O_2S{\cdot}HCl{\cdot}0.25H_2O$: C, 54.83; H, 4.71; Cl, 14.71; N, 11.63; S, 6.65. Found: C, 54.69; H, 4.41; Cl, 14.39; N, 11.55; S, 6.74.

5.1.43. *N*-(2-{[(4-Chloropyridine-2-yl)carbonylamino]methyl}phenyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (4C)

Compound **4C** was synthesized in a similar manner to compound **4A**. Compound **39** (intermediate of compound **4A**) (155 mg, 0.413 mmol), DMF (4.0 mL), 4-chloropyridine-2-carboxylic acid (65.1 mg, 0.413 mmol), HOBt (55.8 mg, 0.413 mmol), EDC·HCl (119 mg, 0.620 mmol) and triethylamine (115 μL, 0.826 mmol) were treated to obtain the title compound (105 mg, 0.142 mmol, 34%) as a white powder. MP: 262–264 °C (Dec.). IR (ATR) cm^{-1}: 3267, 2989, 2945, 2667, 2580, 2538, 1695, 1653, 1587, 1520, 1454, 1360, 1300, 1267, 1236, 1188, 1119, 1070, 1047, 1020, 997, 960, 899, 835, 768, 739, 690, 634, 609, 580, 517, 463, 430. ^{1}H NMR (DMSO-d_6) δ: 2.92 (3H, s), 3.22 (2H, br s), 3.58 (2H, br s), 4.51 (2H, d, J = 6.2 Hz), 4.54–4.63 (2H, m), 7.25 (1H, t, J = 7.6 Hz), 7.31 (1H, t, J = 7.6 Hz), 7.43 (1H, d, J = 7.6 Hz), 7.48 (1H, d, J = 7.6 Hz), 7.78 (1H, dd, J = 5.4, 2.1 Hz), 8.04 (1H, d, J = 2.1 Hz), 8.64 (1H, d, J = 5.4 Hz), 9.49 (1H, t, J = 6.2 Hz), 10.85 (1H, s). ESI-MS m/z: 442 $[(M+H)^+, {}^{35}Cl]$, 444 $[(M+H)^+, {}^{37}Cl]$. Anal. Calcd for $C_{21}H_{20}ClN_5O_2S{\cdot}HCl{\cdot}H_2O$: C, 50.81; H, 4.67; Cl, 14.28; N, 14.11; S, 6.46. Found: C, 50.44; H, 4.70; Cl, 14.18; N, 14.19; S, 6.54.

5.1.44. *N*-(2-{[(3-Chlorobenzoyl)amino]methyl}phenyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (4D)

Compound **4D** was synthesized in a similar manner to compound **4A**. Compound **39** (intermediate of compound **4A**) (155 mg, 0.413 mmol), DMF (4.0 mL), 3-chlorobenzoic acid (64.7 mg, 0.413 mmol), HOBt (55.8 mg, 0.413 mmol), EDC·HCl (119 mg, 0.620 mmol) and triethylamine (115 μL, 0.826 mmol) were treated to obtain the title compound (68.0 mg, 0.136 mmol, 33%) as a white powder. MP: 144–146 °C (Dec.). IR (ATR) cm^{-1}: 3317, 3068, 2947, 2667, 2576, 1695, 1655, 1630, 1587, 1568, 1520, 1454, 1358, 1317, 1298, 1248, 1186, 1161, 1119, 1070, 1018, 995, 957, 899, 837, 758, 717, 681, 613, 580, 507, 471, 428. ^{1}H NMR (DMSO-d_6) δ: 2.92 (3H, s), 3.18 (2H, br s), 3.58 (2H, br s), 4.47 (2H, d, J = 5.6 Hz), 4.58 (2H, br s), 7.27 (1H, t, J = 7.4 Hz), 7.33 (1H, t, J = 7.4 Hz), 7.40 (1H, d, J = 7.4 Hz), 7.48–7.55 (2H, m), 7.61 (1H, d, J = 7.4 Hz), 7.83 (1H, d, J = 7.4 Hz), 7.91 (1H, s), 9.22 (1H, t, J = 5.6 Hz), 10.72 (1H, s), 11.48 (1H, br s). ESI-MS m/z: 441 $[(M+H)^+, {}^{35}Cl]$, 443 $[(M+H)^+, {}^{37}Cl]$. Anal. Calcd for $C_{22}H_{21}ClN_4O_2S{\cdot}HCl{\cdot}1.25H_2O$: C, 52.86; H, 4.94; Cl, 14.18; N, 11.21; S, 6.41. Found: C, 52.82; H, 4.68; Cl, 14.44; N, 11.21; S, 6.63.

5.1.45. *N*-[2-({[(4-Chloro-1*H*-pyrrol-2-yl)carbonyl]amino}methyl)phenyl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (4E)

Methyl 4-chloropyrrole-2-carboxylate[14] (79.8 mg, 0.500 mmol) was dissolved in mixed solvent of THF (1 mL), MeOH (1 mL) and water (1 mL). To this solution was added lithium hydroxide (24.4 mg, 1.00 mmol), and the mixture was stirred at room temperature for 15 min and at 50 °C for 1.5 h. The solvent was distilled off in vacuo. To the obtained residue were added compound **39** (intermediate of compound **4A**) (188 mg, 0.500 mmol) and DMF (5 mL). To this mixture were added HOBt (67.6 mg, 0.500 mmol) and EDC·HCl (144 mg, 0.750 mmol). After the mixture was stirred at room temperature for 1.5 h, the solvent was distilled off in vacuo. The residue was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). Organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (5% MeOH–CH_2Cl_2) to give the free form of the title compound. To the free form of the title compound was added 1 M HCl aqueous solution and the mixture was concentrated in vacuo. The obtained solid was washed with AcOEt to obtain the title compound (118 mg, 0.241 mmol, 48%) as a pale yellow powder. MP: 216–221 °C (Dec.). IR (ATR) cm^{-1}: 3211, 2528, 2372, 1726, 1666, 1633, 1587, 1564, 1522, 1454, 1389, 1360, 1323, 1308, 1240, 1120, 1072, 1043, 993, 935, 897, 837, 766, 717, 648, 607, 580, 546, 509, 457, 442, 422. ^{1}H NMR (DMSO-d_6) δ: 2.94 (3H, s), 3.19 (2H, br s), 3.44–3.83 (2H, m), 4.41 (2H, d, J = 5.9 Hz), 4.44–4.81 (2H, m), 6.83–6.86 (1H, m), 6.96–6.99 (1H, m), 7.23–7.37 (3H, m), 7.52 (1H, d, J = 7.6 Hz), 8.76 (1H, t, J = 5.9 Hz), 10.76 (1H, s), 11.43 (1H, br s), 11.84 (1H, br s). ESI-MS m/z: 430 [$(M+H)^+$, ^{35}Cl], 432 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{20}H_{20}ClN_5O_2S{\cdot}0.9HCl{\cdot}1.5H_2O$: C, 49.05; H, 4.92; Cl, 13.75; N, 14.30; S, 6.55. Found: C, 49.43; H, 4.92; Cl, 13.99; N, 13.95; S, 6.54.

5.1.46. *N*-[2-({[(5-Chloro-1*H*-pyrrol-2-yl)carbonyl]amino}methyl)phenyl]-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (4F)

Compound **4F** was synthesized in a similar manner to compound **4E**. Methyl 5-chloro-1*H*-pyrrole-2-carboxylate[13] (160 mg, 1.00 mmol) was hydrolyzed with lithium hydroxide (48.9 mg, 2.00 mmol). Obtained carboxylic acid, compound **39** (intermediate of compound **4A**) (375 mg, 1.00 mmol), DMF (5 mL), HOBt (135 mg, 1.00 mmol) and EDC·HCl (288 mg, 1.50 mmol) were treated to obtain the title compound (188 mg, 0.381 mmol, 38%) as a pale yellow powder. MP: 231–234 °C (Dec.). IR (ATR) cm^{-1}: 3180, 2947, 2459, 1666, 1633, 1587, 1560, 1523, 1454, 1419, 1402, 1361, 1306, 1240, 1213, 1122, 1072, 1045, 993, 897, 862, 820, 800, 756, 717, 648, 611, 602, 580, 534, 505, 455. ^{1}H NMR (DMSO-d_6) δ: 2.95 (3H, s), 3.05–3.32 (2H, m), 3.38–3.80 (2H, m), 4.40 (2H, d, J = 5.9 Hz), 4.44–4.59 (1H, m), 4.61–4.85 (1H, m), 6.07 (1H, dd, J = 3.7, 2.4 Hz), 6.82 (1H, dd, J = 3.7, 2.8 Hz), 7.22–7.39 (3H, m), 7.53 (1H, dd, J = 7.8, 1.2 Hz), 8.70 (1H, t, J = 5.9 Hz), 10.78 (1H, s), 11.41 (1H, br s), 12.33 (1H, s). ESI-MS m/z: 430 [$(M+H)^+$, ^{35}Cl], 432 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{20}H_{20}ClN_5O_2S{\cdot}0.9HCl{\cdot}1.5H_2O$: C, 49.05; H, 4.92; Cl, 13.75; N, 14.30; S, 6.55. Found: C, 49.43; H, 4.92; Cl, 13.99; N, 13.95; S, 6.54.

5.1.47. *N*-(5-Chloropyridin-2-yl)-2-(2-nitrophenyl)acetamide (24)

(2-Nitrophenyl)acetic acid (1.81 g, 10.0 mmol) and 5-chloropyridin-2-yl-amine (1.29 g, 10.0 mmol) were dissolved in DMF (20 mL). To this solution were added HOBt (135 g, 10.0 mmol), EDC·HCl (2.88 g, 15.0 mmol) and 4-dimethylaminopyridine (123 mg, 1.00 mmol), and the mixture was stirred overnight at room temperature. After distilling off the solvent in vacuo, the obtained residue was partitioned between AcOEt (150 mL) and 10% citric acid aqueous solution (150 mL). The organic layer was washed with brine, saturated $NaHCO_3$ aqueous solution and brine (each 150 mL), and then dried over $MgSO_4$ and concentrated in vacuo. The residue was washed with hexane to obtain the title compound (2.92 g, 10.0 mmol, 100%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 4.08 (2H, s), 7.48–7.54 (2H, m), 7.62–7.68 (2H, m), 8.10–8.16 (2H, m), 8.21 (1H, d, J = 2.7 Hz), 8.35 (1H, br s). ESI-MS m/z: 292 [$(M+H)^+$, ^{35}Cl], 294 [$(M+H)^+$, ^{37}Cl].

5.1.48. *N*-(2-{2-[(5-Chloropyridin-2-yl)amino]-2-oxoethyl}phenyl)-5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamide hydrochloride (25)

To the solution of compound **24** (291 mg, 1.00 mmol) in EtOH (5 mL) was added Raney nickel (1 g). After stirring at 50 °C for 5 h under hydrogen atmosphere, catalyst was filtered off by a celite pad. Filtrate was concentrated in vacuo to obtain a yellow solid. This yellow solid was dissolved in DMF. To this mixture were added 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine-2-carboxylic acid dihydrochloride (282 mg, 1.20 mmol), HOBt (135 mg, 1.00 mmol) and EDC·HCl (288 mg, 1.50 mmol). After the mixture was stirred at room temperature for 2 h, the solvent was distilled off in vacuo. The residue was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ aqueous solution (50 mL). Organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (3% MeOH–CH_2Cl_2) to give the free form of the title compound. To the free form of the title compound was added 1 M HCl aqueous solution, and the mixture was concentrated in vacuo. The obtained solid was washed with AcOEt to obtain the title compound (200 mg, 0.396 mmol, 40%) as a white powder. MP: 180–185 °C (Dec.). IR (ATR) cm^{-1}: 3334, 3251, 2989, 2534, 1680, 1587, 1522, 1454, 1373, 1340, 1296, 1244, 1134, 1111, 1068, 1009, 995, 960, 897, 835, 806, 768, 715, 690, 627, 586, 540, 509, 445, 418. ^{1}H NMR (DMSO-d_6) δ: 2.95 (3H, s), 3.07–3.30 (2H, m), 3.68–3.78 (1H, m), 3.79–3.92 (3H, m), 4.41–4.53 (1H, m), 4.71–4.82 (1H, m), 7.24 (1H, td, J = 7.8, 1.5 Hz), 7.33 (1H, td, J = 7.8, 1.5 Hz), 7.44 (1H, dd, J = 7.8, 1.5 Hz), 7.67 (1H, dd, J = 7.8, 1.5 Hz), 7.90 (1H, dd, J = 9.0, 2.7 Hz), 8.07 (1H, d, J = 9.0 Hz), 8.39 (1H, d, J = 2.7 Hz), 10.72 (1H, s), 11.02 (1H, s), 11.25 (1H, br s). ESI-MS m/z: 442 [$(M+H)^+$, ^{35}Cl], 444 [$(M+H)^+$, ^{37}Cl]. Anal. Calcd for $C_{21}H_{20}ClN_5O_2S{\cdot}1.1HCl{\cdot}1.5H_2O$: C, 49.55; H, 4.77; Cl, 14.62; N, 13.76; S, 6.30. Found: C, 49.72; H, 4.58; Cl, 14.83; N, 13.55; S, 6.38.

5.1.49. Methyl 4-(4-morpholinyl)benzoate (20)

Thionyl chloride (436 μL, 6.00 mmol) was added dropwise to methanol (10 mL) under ice cooling. To this solution was added 4-(4-morpholinyl)benzoic acid (207 mg, 1.00 mmol), and then the mixture was refluxed for 1.5 h. After concentration in vacuo, dichloromethane and water were added to the residue. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo to give the title compound (215 mg, 97%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.28 (4H, t, J = 4.9 Hz), 3.84–3.87 (7H, m), 6.84–6.89 (2H, m), 7.91–7.97 (2H, m). MS (ESI) m/z: 222 $(M+H)^+$.

5.1.50. Methyl 4-(3-oxo-4-morpholinyl)benzoate (21)

To the solution of compound **20** (207 mg, 936 μmol) in dichloromethane (10 mL) were added benzyltriethylammonium chloride (639 mg, 2.81 mmol) and $KMnO_4$ (222 mg, 1.40 mmol). After stirring at room temperature for 2 h, saturated aqueous $NaHSO_3$ solution was added to the reaction mixture. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (Hexane/AcOEt = 1:2) to give the title compound (41 mg, 19%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.80–3.83 (2H, m), 3.92 (3H, s), 4.03–4.07 (2H, m), 4.36 (2H, s), 7.45–7.49 (2H, m), 8.06–8.10 (2H, m). MS (ESI) m/z: 236 $(M+H)^+$.

5.1.51. 4-(3-Oxomorpholin-4-yl)benzoic acid (22)

To the solution of compound **21** (1.21 g, 5.14 mmol) in CH_2Cl_2 (40 mL) were added dimethylsulfide (5.0 mL) and anhydrous aluminium chloride (2.06 g, 15.4 mmol). The mixture was stirred at room temperature for 8 h. After the solvent was distilled off in vacuo, to the obtained residue were added ice and dilute hydrochloric acid. The precipitate was collected by filtration to obtain the title compound (1.06 g, 4.79 mmol, 93%) as a white powder. ^{1}H NMR ($CDCl_3$) δ: 3.83 (2H, t, J = 5.1 Hz), 4.06 (2H, t, J = 5.1 Hz), 4.33 (2H, s), 7.46 (2H, d, J = 8.5 Hz), 8.08 (2H, d, J = 8.5 Hz), 12.35 (1H, br s). ESI-MS m/z: 222 $(M+H)^+$.

5.1.52. *N*-{2-[4-(3-Oxomorpholin-4-yl)benzoylamino]benzyl}-5-chlorothiophene-2-carboxamide (5A)

Compounds **22** (113 mg, 0.510 mmol) and **36** (13550 mg, 0.506 mmol) were dissolved in DMF (5 mL). To the solution were added HOBt (68.4 mg, 0.506 mmol) and EDC·HCl (194 mg, 1.01 mmol). After stirring at room temperature for 3 d, the solvent was distilled off in vacuo. To the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were dried over Na_2SO_4 and concentrated in vacuo. EtOH was added to the residue, and the mixture was stirred at 50 °C for 30 min. After cooling to room temperature, precipitate was collected by filtration. Obtained white powder was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 24:1) to obtain the title compound (92 mg, 0.195 mmol, 39%) as a white powder. MP: 243–24 °C. IR (ATR) cm^{-1}: 3286, 1651, 1622, 1603, 1556, 1520, 1504. ^{1}H NMR (DMSO-d_6) δ: 3.81 (2H, t, J = 5.0 Hz), 4.00 (2H, t, J = 4.9 Hz), 4.24 (2H, s), 4.45 (2H, d, J = 5.9 Hz), 7.20 (1H, dd, J = 4.2, 1.5 Hz), 7.24 (1H, t, J = 7.4 Hz), 7.28–7.36 (2H, m), 7.51 (1H, d, J = 7.8 Hz), 7.59 (2H, d, J = 7.6 Hz), 7.68 (1H, dd, J = 4.2, 1.2 Hz), 8.04 (2H, d, J = 7.6 Hz), 9.20 (1H, t, J = 5.6 Hz), 10.30 (1H, s). ESI-MS m/z: 470 $(M+H)^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_4S$: C, 58.78; H, 4.29; N, 8.94; Cl, 7.54; S, 6.82. Found: C, 58.60; H, 4.33; N, 8.86; Cl, 7.36; S, 6.85.

5.1.53. Lithium 1-isopropylpiperidine-4-carboxylate (40)

To the solution of ethyl 1-isopropylpiperidine-4-carboxylate[15] (3.43 g, 17.2 mmol) in tetrahydrofurane (60 mL) were added water (15 mL) and lithium hydroxide (421 mg, 17.2 mmol). After stirring at room temperature overnight, the mixture was concentrated in vacuo to give the title compound (3.05 g, quant.) as a white solid. IR (KBr) cm^{-1}: 3325, 2966, 2925, 2821, 1689, 1583, 1560, 1427, 1385, 1174, 1132, 748, 694, 634. ^{1}H NMR (CD_3OD) δ: 1.05 (6H, d, J = 6.6 Hz), 1.65–1.78 (2H, m), 1.83–1.94 (2H, m), 2.07 (1H, tt, J = 11.4, 3.9 Hz), 2.20 (2H, dt, J = 2.7, 11.6 Hz), 2.60–2.72 (1H, m), 2.84–2.95 (2H, m). ESI-MS m/z: 172 $(M-Li+2H)^+$.

5.1.54. *N*-(2-{[(5-Chlorothiophen-2-yl)carbonylamino]methyl}phenyl)-1-isopropylpiperidine-4-carboxamide hydrochloride (5B)

Compound **5B** was synthesized in a similar manner to compound **5A**. Compound **40** (116 mg, 0.657 mmol), compound **36** (135 mg, 0.506 mmol), DMF (15 mL), HOBt (67.6 mg, 0.500 mmol) and EDC·HCl (194 mg, 1.01 mmol) were treated to obtain the free form of the title compound (81 mg, 0.19 mmol, 35%) as a light brown amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.11 (6H, s), 1.23–1.34 (1H, m), 1.51–2.03 (5H, m), 2.22–2.51 (2H, m), 2.75–3.03 (2H, m), 4.51 (2H, d, J = 6.3 Hz), 6.90 (1H, d, J = 3.9 Hz), 7.10 (1H, td, J = 7.6, 1.0 Hz), 7.25–7.36 (4H, m), 7.95 (1H, d, J = 7.3 Hz), 9.03 (1H, s). ESI-MS m/z: 420 $(M+H)^+$.

To the free form of the title compound (81 mg, 0.192 mmol) was added 1 M HCl solution in EtOH (210 μL). Solvent was distilled off in vacuo, and to the residue were added EtOH and water. Then it was concentrated in vacuo to obtain the title compound (75 mg) as a pale yellow amorphous solid.

Mp: 225–229 °C. IR (ATR) cm^{-1}: 3216, 2935, 2661, 2513, 1676, 1624, 1589, 1547, 1518. ^{1}H NMR (DMSO-d_6) δ: 1.27 (6H, d, J = 6.6 Hz), 1.91–2.17 (4H, m), 2.69–2.78 (1H, m), 2.92–3.04 (2H, m), 3.42–3.49 (3H, m), 4.39 (2H, d, J = 6.1 Hz), 7.14–7.21 (2H, m), 7.23–7.32 (2H, m), 7.48 (1H, d, J = 7.8 Hz), 7.74 (1H, d, J = 4.2 Hz), 9.30 (1H, t, J = 6.0 Hz), 9.43 (1H, s), 9.85 (1H, s). ESI-MS m/z: 420 $(M+H)^+$. HRMS: Theoretical m/z: for $C_{21}H_{27}ClN_3O_2S$: 420.1513. Observed m/z: 420.1487.

5.1.55. Methyl 2-{[(5-chlorothiophen-2-yl)carbonylamino]methyl}benzoate (27)

To the solution of methyl 2-aminomethylbenzoate hydrochloride (605 mg, 3.00 mmol) and 5-chlorothiophene-2-carboxylic acid (488 mg, 3.00 mmol) in DMF (10.0 mL) were added HOBt (405 mg, 3.00 mmol) and EDC·HCl (1.19 g, 6.00 mmol). The reaction mixture was stirred at room temperature for 28 h. Solvent was distilled off in vacuo, and to the residue were added saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organic layer was dried over Na_2SO_4. The residue was purified by column chromatography on silica gel ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH = 199:1) to obtain the title compound (949 mg, 3.06 mmol, quant.) as a colorless viscosity liquid. ^{1}H NMR ($CDCl_3$) δ: 3.95 (3H, s), 4.73 (2H, d, J = 6.6 Hz), 6.85 (1H, d, J = 3.9 Hz), 7.22 (1H, d, J = 4.2 Hz), 7.37 (2H, td, J = 7.6, 1.2 Hz), 7.52 (1H, td, J = 7.5, 1.2 Hz), 7.61 (1H, d, J = 7.3 Hz), 7.99 (1H, dd, J = 7.8, 1.2 Hz). ESI-MS m/z: 310 $(M+H)^+$.

5.1.56. 2-{[(5-Chlorothiophen-2-yl)carbonyl]amino]methyl}benzoic acid (28)

To the solution of compound **27** (949 mg, 3.00 mmol) in CH_2Cl_2 (20 mL) were added dimethylsulfide (2.20 mL, 30 mmol) and anhydrous aluminium chloride (1.20 g, 9.00 mmol). The mixture was stirred at room temperature for 2 h. After the solvent was distilled off in vacuo, to the obtained residue were added ice and dilute hydrochloric acid. The precipitate was collected by filtration to obtain the title compound (945 mg, 3.15 mmol, quant.) as a white powder. ^{1}H NMR ($CDCl_3$) δ: 4.76 (2H, d, J = 6.8 Hz), 7.23 (1H, d, J = 3.9 Hz), 7.25 (1H, d, J = 3.9 Hz), 7.30–7.40 (1H, m), 7.49 (1H, t, J = 7.3 Hz), 7.56 (1H, d, J = 7.3 Hz), 7.70 (1H, br s), 8.03 (1H, d, J = 7.3 Hz), 8.43–8.56 (1H, br).

5.1.57. *tert*-Butyl 2-(2-{[(5-chlorothiophen-2-yl)carbonylamino]methyl}benzoylamino)-6,7-dihydro-4*H*-thiazolo[5,4-*c*]pyridine-5-carboxylate (30)

tert-Butyl 2-amino-6,7-dihydro-4*H*-thiazolo[5,4-*c*]pyridine-5-carboxylate[11] (179 mg, 0.700 mmol) and compound **28** (207 mg, 0.700 mmol) were dissolved in DMF (6 mL). To the solution were added HOBt (94.6 mg, 0.700 mmol) and EDC·HCl (268 mg, 1.40 mmol). After stirring at room temperature for 24 h, the solvent was distilled off in vacuo. To the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 24:1) to obtain the title compound (176 mg, 0.330 mmol, 47%) as a pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.63–2.70 (2H, m), 3.69–3.77 (2H, m), 4.61 (2H, s), 4.67 (2H, d, J = 6.4 Hz), 6.86 (1 H, d, J = 3.9 Hz), 7.29 (1H, d, J = 3.9 Hz), 7.41–7.48 (3H, m), 7.56 (1H, td, J = 7.6, 1.2 Hz), 7.67 (1H, d, J = 7.3 Hz), 7.72 (1H, d, J = 7.3 Hz). ESI-MS m/z: 533 $(M+H)^+$.

5.1.58. *N*-[2-(4,5,6,7-Tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-ylcarbamoyl)benzyl]-5-chlorothiophene-2-carboxamide (41)

4 M HCl solution in dioxane (1.50 mL, 6.00 mmol) was added to compound **30** (176 mg, 0.330 mmol). The mixture was stirred at room temperature for 40 min and at 40–50 °C for 2 h. After adding Et_2O to the reaction mixture, the precipitate was collected by filtra-

tion to obtain the hydrochloride of the title compound (134 mg, 0.309 mmol, 94%) as a white powder. ^{1}H NMR (DMSO-d_6) δ: 2.90 (2H, t, J = 5.1 Hz), 3.40–3.48 (2H, m), 4.32 (2H, s), 4.60 (2H, d, J = 5.9 Hz), 7.19 (1H, d, J = 4.2 Hz), 7.39 (1H, t, J = 7.3 Hz), 7.44 (1H, d, J = 7.8 Hz), 7.53 (1H, t, J = 7.6 Hz), 7.60 (1H, d, J = 7.3 Hz), 7.70 (1H, d, J = 4.2 Hz), 9.22 (1H, t, J = 6.0 Hz), 9.48 (2H, s), 12.70 (1H, s). ESI-MS m/z: 433 $(M+H)^+$.

To the hydrochloride of the title compound (119 mg, 0.244 mmol) was added saturated $NaHCO_3$ aqueous solution. Organics were extracted with mixed solvent (CH_2Cl_2/MeOH = 5:1) five times. Combined organics were dried over Na_2SO_4 and concentrated in vacuo to obtain the title compound (87 mg, 0.20 mmol) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.55–2.60 (2H, m), 2.67–2.72 (1H, br), 3.18 (2H, t, J = 5.7 Hz), 4.06 (2H, s), 4.66 (2H, d, J = 6.4 Hz), 6.86 (1H, d, J = 3.9 Hz), 7.24–7.30 (2H, m), 7.41 (1H, t, J = 7.6 Hz), 7.48 (1H, t, J = 5.9 Hz), 7.54 (1H, t, J = 7.1 Hz), 7.61–7.67 (2H, m). ESI-MS m/z: 433 $(M+H)^+$.

5.1.59. *N*-[2-(5-Methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridin-2-ylcarbamoyl)benzyl]-5-chlorothiophene-2-carboxamide hydrochloride (31)

To the suspension of the free form of compound **41** (87 mg, 0.20 mmol) in CH_2Cl_2 (10 mL) were added acetic acid (23 μL, 0.402 mmol), 37% formalin aqueous solution (32 μL, 0.40 mmol) and triacetoxy sodium borohydride (67.5 mg, 0.320 mmol). After stirring for 5 min, additional triacetoxy sodium borohydride (67.5 mg, 0.320 mmol) was added and the mixture was stirred for 6 h. The mixture was alkalified by the addition of 1 M NaOH aqueous solution (1 mL) and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by preparative thin layer chromatography (CH_2Cl_2/MeOH = 9:1) to obtain the free form of the title compound (72 mg, 0.16 mmol, 80%) as a pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 2.20 (2H, s), 2.48 (3H, s), 2.60 (2H, s), 3.61 (2H, s), 4.65 (2H, d, J = 6.4 Hz), 6.85 (1H, d, J = 4.2 Hz), 7.27 (1H, d, J = 4.2 Hz), 7.35 (1H, t, J = 7.6 Hz), 7.52 (1H, t, J = 7.6 Hz), 7.57–7.66 (3H, m), 11.69 (1H, s).

The free form of the title compound (72 mg, 0.16 mmol) was dissolved in EtOH, and 1 M HCl solution in EtOH was added to this solution. The solvent was distilled off in vacuo to obtain the title compound (75 mg) as a white powder. MP: 232–235 °C. IR (ATR) cm^{-1}: 3356, 3143, 3103, 3049, 2968, 1635, 1624, 1577, 1539, 1520. ^{1}H NMR (DMSO-d_6) δ: 2.93 (3H, s), 2.97 (2H, br s), 3.38–3.51 (1H, m), 3.60–3.75 (1H, m), 4.25–4.40 (1H, m), 4.50–4.65 (1H, m), 4.60 (2H, d, J = 6.1 Hz), 7.19 (1H, d, J = 4.2 Hz), 7.39 (1H, t, J = 7.5 Hz), 7.44 (1H, d, J = 7.6 Hz), 7.54 (1H, t, J = 7.6 Hz), 7.60 (1H, d, J = 7.8 Hz), 7.68 (1H, d, J = 4.2 Hz), 9.18 (1H, t, J = 5.9 Hz), 10.59 (1H, s), 12.74 (1H, s). Anal. Calcd for $C_{20}H_{19}ClN_4O_2S_2$·HCl·0.5H_2O: C, 48.78; H, 4.30; N, 11.34; Cl, 14.40; S, 13.02. Found: C, 48.98; H, 4.18; N, 11.47; Cl, 14.01; S, 13.07.

5.1.60. *N*-{2-[4-(3-Oxomorpholin-4-yl)phenylcarbamoyl]benzyl}-5-chlorothiophene-2-carboxamide (33)

Compound **33** was synthesized in a similar manner to compound **30**. Compound **28** (118 mg, 0.400 mmol), 4-(4-aminophenyl)morpholine-3-one[17] (76.9 mg, 0.400 mmol), DMF (5 mL), HOBt (54.1 mg, 0.400 mmol) and EDC·HCl (153 mg, 0.800 mmol) were treated to obtain the title compound (64 mg, 0.14 mmol, 34%) as a white solid. MP: 174–176 °C. IR (ATR) cm^{-1}: 3394, 3307, 1666, 1637, 1603, 1533. ^{1}H NMR ($CDCl_3$) δ: 3.78 (2H, t, J = 5.1 Hz), 4.05 (2H, t, J = 5.0 Hz), 4.34 (2H, s), 4.60 (2H, d, J = 6.1 Hz), 6.86 (1H, d, J = 4.2 Hz), 7.25–7.28 (1H, m), 7.35 (2H, d, J = 8.8 Hz), 7.41 (1H, t, J = 7.5 Hz), 7.46–7.54 (2H, m), 7.56–7.63 (2H, m), 7.71 (2H, d, J = 8.8 Hz), 8.47 (1H, s). ESI-MS m/z: 470 $(M+H)^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_4S$·0.2H_2O: C, 58.34; H, 4.34; N, 8.87; Cl, 7.49; S, 6.77. Found: C, 58.15; H, 4.21; N, 8.79; Cl, 7.38; S, 6.80.

5.1.61. *N*-[2-(1-Isopropylpioeridin-4-ylcarbamoyl)benzyl]-5-chlorothiophene-2-carboxamide hydrochloride (35)

To *tert*-butyl (1-isopropylpiperidin-4-yl)carbamate[18] (96.9 mg, 0.400 mmol) was added 4 M HCl solution in dioxane (1.00 mL, 4.00 mmol). The mixture was stirred at 50 °C for 80 min. After distillation of the solvent in vacuo, to the solution of the residue in DMF (5 mL) were added compound **28** (118 mg, 0.400 mmol), HOBt (54.1 mg, 0.400 mmol), EDC·HCl (153 mg, 0.800 mmol) and triethylamine (112 μL, 0.800 mmol). After stirring at room temperature for 15 h, the solvent was distilled off in vacuo. To the residue were added CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2, combined organics were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 97:3→19:1→47:3→93:7→23:2) to obtain the free form of the title compound (100 mg, 0.238 mmol, 60%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.11 (6H, d, J = 6.6 Hz), 1.62–1.74 (2H, m), 2.06–2.14 (2H, m), 2.36–2.46 (2H, m), 2.80–2.88 (1H, m), 2.91–2.98 (2H, m), 3.95–4.06 (1H, m), 4.56 (2H, d, J = 6.4 Hz), 6.10 (1H, br s), 6.83 (1H, d, J = 3.9 Hz), 7.34 (1H, t, J = 7.3 Hz), 7.41–7.46 (2H, m), 7.55 (1H, d, J = 7.3 Hz), 7.80 (1H, t, J = 5.6 Hz).

The free form of the title compound (100 mg, 0.238 mmol) was dissolved in EtOH, and 1 M HCl solution in EtOH was added to this solution. The solvent was distilled off in vacuo to obtain the title compound (104 mg) as a white powder. MP: 132–136 °C. IR (ATR) cm^{-1}:.3222, 3062, 3030, 2978, 2943, 2661, 2522, 1631, 1541, 1520. ^{1}H NMR (DMSO-d_6) δ: 1.23 (1/5 × 6H, d, J = 7.3 Hz), 1.25 (4/5 × 6H, d, J = 7.3 Hz), 1.73–2.01 (2H, m), 2.04–2.13 (2H, m), 3.02–3.23 (2H, m), 3.25–3.50 (4H, m), 4.52 (1/5 × 2H, d, J = 5.9 Hz), 4.55 (4/5 × 2H, d, J = 5.9 Hz), 7.20 (1H, d, J = 4.2 Hz), 7.29–7.45 (4H, m), 7.70 (1H, d, J = 3.9 Hz), 8.59 (4/5 × 1H, d, J = 7.3 Hz), 8.64 (1/5 × 1H, d, J = 6.6 Hz), 9.06–9.15 (1H, m), 9.30 (1H, br s). ESI-MS m/z: 420 $(M+H)^+$. Anal. Calcd for $C_{21}H_{26}ClN_3O_2S$·0.95HCl·H_2O: C, 53.37; H, 6.17; N, 8.89; Cl, 14.63; S, 6.78. Found: C, 53.25; H, 6.25; N, 8.96; Cl, 14.42; S, 6.62.

5.2. Anti-fXa activity in vitro

Anti-fXa activity in vitro was measured by using a chromogenic substrate S-2222 (Chromogenix, Inc.) and human fXa (Enzyme Research Laboratories). Aqueous DMSO (5%, 10 μL) or inhibitors in aqueous DMSO (10 μL) and 0.0625 U/mL human fXa (10 μL) were mixed with 0.1 M Tris–0.3 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL). The reaction was started by the addition of 0.75 M S-2222 (40 μL). The absorbance (O.D.) at 405 nm was monitored every 10 seconds with a microplate spectrophotometer SPECTRAmax 340 (Molecular Devices, Sunnyvale, CA, USA) at room temperature and the reaction velocity (mO.D./min) was obtained. Anti-fXa activity (inhibition%) was calculated as follows: Anti-fXa activity = (1 − (reaction velocity of sample) ÷ (reaction velocity of control)) × 100. The IC_{50} value was obtained by plotting the anti-fXa activity against the inhibitor concentration.

5.3. Anticoagulant activity in vitro

Prothrombin time (PT) was measured with an Amelung KC-10A micro coagulometer (MC Medical, Tokyo, Japan) as follows: 50 μL of plasma was mixed with 50 μL of inhibitors or 4% DMSO/saline and incubated for 1 min at 37 °C. The coagulation was started by the addition of 100 μL of Thromboplastin C Plus (0.5 U/mL) to the mixture, and the clotting time was measured. The concentration of inhibitors required to double the clotting time (CT2) was estimated from the concentration-response curve by a regression analysis.

5.4. Solubilities

The solubilities were determined by HPLC analysis. Ten millimolars of compound solution in DMSO (50 μL) was freeze-dried. To the residue the Japanese Pharmacopoeia First Fluid (250 μL, pH 1.2) or the Japanese Pharmacopoeia Second Fluid (250 μL, pH 6.8) was added, and the mixture was stirred by pipette operation. The mixture was saved under shading over 12 h. After filtration of the mixture, the resulting filtrate was diluted 20 times by adding aqueous DMSO solution (1:1 (v/v)) to obtain the measurement sample solution. Five micromolars of compound solution in aqueous DMSO solution (1:1 (v/v)) and one hundred micromolar of compound solution in aqueous DMSO solution (1:1 (v/v)) were prepared to make a calibration curve. The measurement sample solution, five micromolar solution and one hundred micromolar solution were assayed using HPLC methodologies (Analytical Column: X Terra® MSC18 3.5 μm, 3.0 × 30 mm, Waters; Mobile Phase: 10 mM ammonium acetate buffer (pH 4.5)/0.05% acetic acid in acetonitrile = 95:5 to 10:90 v/v; Wave length: PDA 220–420 nm). The solubilities were analyzed using Millenium software (Waters).

5.5. Distribution coefficient

The distribution coefficients (log *D*) between 1-octanol and phosphate buffered saline (PBS) were assayed by a shaking flask method.[21] Equal amounts of PBS and 1-octanol were shaken and left for over 12 h. The upper layer (1-octanol) and lower layer (PBS) were collected individually. Each compound was dissolved in 1-octanol or PBS (200 μM). The same amount of either PBS or 1-octanol was added and the mixture was shaken vigorously for 30 min at room temperature.

Then, both phases were separated and assayed using LC–MS methodologies (LC–Mass spectrometer: 1100 Series LC/MSD, Agilent; Analytical Column: X Terra® MSC18 3.5 μm, 3.0 × 30 mm, Waters; Mobile Phase: 10 mM ammonium acetate buffer (pH 4.5)/0.05% acetic acid in acetonitrile = 95:5 to 10:90 v/v). The values of log *D* were analyzed using Analyst software program (version 1.4, Applied Bio. Systems).

5.6. Metabolic stability

Compounds (final 1 μM) were incubated with human liver microsome in sodium phosphate buffer (pH 7.4) for 20 min at 37 °C. The microsomal protein concentration in the assay was 0.1 mg/mL. Reaction was started by the addition of NADPH generating system at 37 °C and stopped by addition of MeOH after 30 min. After centrifuging each solution separately at 3500 rpm for 10 min at 4 °C, the corresponding loss of parent compound was determined by LC/MS/MS.

5.7. Anti-fXa activity in rat ex vivo

Male Wistar rats were fasted overnight. Synthetic compounds were dissolved in 0.5% methylcellose solution and administered orally to rats by gavage. For control rats, 0.5% methylcellose solution was administered orally. The rats were anesthetized with thiopental sodium (100 mg/kg, iv) 15 min after the oral administration. Blood samples (450 μL) were collected from the jugular vein into syringes containing 50 μL of 3.13% trisodium citrate dihydrate 0.5, 1, 2 and 4 h after dosing. Plasma was prepared by centrifugation. Anti-fXa activity in plasma was measured as follows. Plasma sample (5 μL) was mixed with 0.1 M Tris–0.3 M NaCl–0.2% BSA buffer (pH 7.4; 40 μL), H_2O (5 μL) and 0.1 U/mL human fXa (10 μL). The reaction was started by the addition of 0.75 M S-2222 (40 μL). The reaction velocity and anti-fXa activity (inhibition%) were obtained as mentioned above.

5.8. Anti-fXa activity in cynomolgus monkey ex vivo

Synthetic compound was administered orally to cynomolgus monkey (1 mg/kg) in aqueous solution. Blood samples (900 μL) were collected from the femoral vein into syringes containing 100 μL of 3.13% trisodium citrate dihydrate using Labospeed tubes (Toyo-kizai Inc.) at 0.5, 1, 2, 4, 8 and 24 h after oral dosing. The reaction velocity and anti-fXa activity (inhibition%) was obtained as mentioned above.

5.9. Measurement of serum concentration

Synthetic compound was administered orally to monkeys (1 mg/kg) in aqueous solution. Blood samples were collected using Labospeed tubes (Toyo-kizai Inc.) at 0.5, 1, 2, 4, 8 and 24 h after oral dosing. Serum concentrations for synthetic compound were determined by LC–MS/MS using Sciex API 365 (Sciex Inc.) coupled with Alliance 2690 HPLC system (Waters Inc.). Synthetic compound was separated on a Symmetry C18 column (Waters Inc.). The quantitation limit was 7 ng/mL. Respective pharmacokinetic parameters were carried out using Top Fit ver. 2.0 (Gustav Fischer Inc.).

5.10. Preparation of the crystal

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

Table 7
Crystal and diffraction data of human fXa with compound **1D**

Crystal parameters	
Space group	*P*212121
a (Å)	56.5
b (Å)	72.4
c (Å)	79.1
Resolution (Å)	1.8
R_{sym} (%)	4.2 (33.5)[a]
Completeness (%)	99.4 (99.0)[a]
No. of reflections, redundancy	30,533, 3.1
Refinement	
No. of protein atoms (occupancy ≠0)	2152
Average *B* value for protein and ligand atoms (Å^2)	30.13, 32.02
Range of data	25.0–1.8
R value	19.9
R_{free}	22.5
(Not weighted) rmsd from ideality	
Bond length (Å)	0.015
Bond angle (Å)	1.57

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

$CaCl_2$/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 CaCl2/0.1 M maleate imidazole, pH 5.0/1 mM of compound **1D**). After 1 day, the crystal was picked up and directly exposed to soak solution 2, and soaking was continued. All of the soaking process was performed at 20 °C.

5.11. X-ray data collection and processing

The soaked crystal was flash-cooled in liquid nitrogen and centered in a gaseous nitrogen stream. The X-ray data set was collected at 100 K on an R-Axis VII imaging plate detector (Rigaku) using a MicroMax 007 rotating anode generator (Rigaku). Data processing was carried out with *d*∗*trek*.[22]

5.12. Structure solution and crystallographic refinement

The previously reported Gla-less fXa structure (PDB code: 1HCG[23]) was used as the initial structure. Phase refinement and model improvement were carried out with *refmac*[24] and *Turbo Frodo*.[25] Stereochemistry checks indicate that the refined protein model is in good agreement with expectations within each resolution range. The statistics of data processing and crystallographic refinement are shown in Table 7.

Acknowledgment

We are grateful to the anticoagulant group in Biological Research Laboratory for performing the biological assays.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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