Synthesis and Conformational Analysis of a Non-Amidine Factor Xa Inhibitor That Incorporates 5-Methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine as S4 Binding Element

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Our exploratory study was based on the concept that a non-amidine factor Xa (fXa) inhibitor is suitable for an orally available anticoagulant. We synthesized and evaluated a series of *N*-(6-chloronaphthalen-2-yl)sulfonylpiperazine derivatives incorporating various fused-bicyclic rings containing an aliphatic amine expected to be S4 binding element. Among this series, 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine type **61** displayed orally potent anti-fXa activity and evident prolongation of prothrombin time (PT) with the moderate bioavailability in rats. The X-ray crystal analysis afforded an obvious binding mode that 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine and 6-chloronaphthalene respectively bound to S4 and S1 subsites. In this X-ray study, we discovered a novel intramolecular S−O close contact. Ab initio energy calculations of model compounds deduced that conformers with the most close S−O proximity were most stable. The Mulliken population analysis proposed that this energy profile was caused by both of electrostatic S−O affinity and N−O repulsion. The results of these calculations and X-ray analysis suggested a possibility that the restricted conformation effected the affinity to S4 subsite of fXa.

Introduction

Anticoagulants such as heparin and warfarin have been prescribed for the treatment of thromboembolism, which is a major cause of such ischemic diseases as cardiac infarction, cerebral infarction, deep vein thrombosis, and unstable angina. The acceptance of these conventional drugs, however, does not seem to be satisfactory because of the following reasons. As for heparin, it is not suitable for long-term treatment of these diseases because of low compliance of its parenteral administration and side effects such as bleeding tendency.[1] Although warfarin is administered orally and is used for long-term treatment, the drug requires several days of administration to obtain anticoagulant activity and also requires frequent monitoring to maintain that activity at appropriate levels.[2] Thus, needs exist for a novel oral anticoagulant with faster onset of action and lower risk of bleeding.

Among several approaches to address the unmet needs, the inhibition of factor Xa (fXa) is known as one of the most popular.[3] This is mainly because fXa inhibitors seemed to have a lower risk of bleeding than heparin and warfarin. The reason would be attributed to the inhibition position in the coagulation cascade. As is well-known, fXa catalyzes thrombin production and is situated at the confluence of the intrinsic and extrinsic pathways. Thus, fXa inhibitors do not block thrombin directly; rather, they block the confluent position of the coagulation cascade.

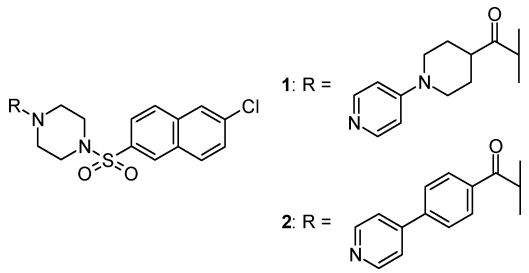

Figure 1. *N*-(6-Chloronaphthalen-2-yl)sulfonylpiperazine derivatives as factor Xa inhibitors.

A number of fXa inhibitors have been synthesized up to the present.[4] Such fXa inhibitors can be classified into amidine derivatives exemplified by DX-9065a[5] and non-amidine derivatives. Most of the amidine-type inhibitors were found to be insufficiently absorbed when administered orally, because of strongly basic amidine groups (pK_a of benzamidine = 11.6)[4b,6a,k] which adopt ionic foams. Therefore, the trend in synthetic studies of fXa inhibitors seems to be shifted to non-amidine type inhibitors containing weakly basic groups such as aminoisoquinoline, imidazole, pyridine, and aniline.[6] Especially, a series of *N*-(6-chloronaphthalen-2-yl)sulfonylpiperazine derivatives **1** and **2**[7] (Figure 1) have interesting structural aspects in terms of their binding mode to fXa, because 6-chloronaphthalene is predicted to replace an amidino benzene or naphthalene as a preferred binding element to the S1 subsite of fXa. Zhu and Scarborough, in their modeling study, proposed that the 6-chloronaphthalene ring of **2** inserted in S1 whereas the pyridine moiety located in S4, and the piperazine ring, func-

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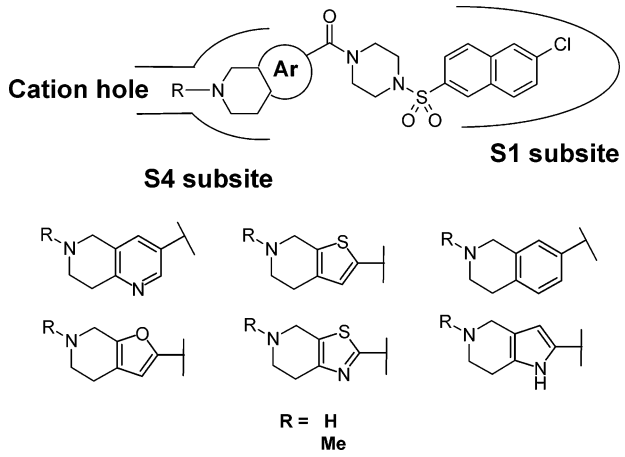


Figure 2. Our design for an orally available factor Xa inhibitor.

tioned as a spacer.[4b] Aventis's group reported that the 6-chlorobenzo[*b*]thiophene ring, regarded as the steric isostere of 6-chloronaphthalene, actually occupied the S1 subsite of fXa in crystal complex.[8] In their report, the chloro group on the benzothiophene ring occupied a small cavity composed of Tyr 228 and Val 213 in the S1 subsite.

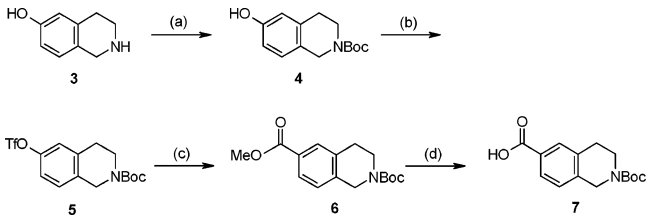
We took note of the utility of 6-chloronaphthalene as the S1 binding element, assumed that the piperazine ring functioned as an appropriate linker between the S1 and S4 binding elements, and furthermore expected a possibility to acquire the novel orally available fXa inhibitor on exploring S4 binding element. We were prompted to introduce the fused-bicyclic ring which contains an aliphatic amine as the S4 binding element to the *N*-(6-chloronaphthalen-2-yl)sulfonylpiperazine unit. We thought the structural aspects of S4 matched the fused-bicyclic ring, because S4, named the "aryl binding site", composed of the residue Tyr 99, Phe 174, and Trp 215 forms a box-shaped site and has a hydrophobic nature. Figure 2 shows the designed compounds containing various fused-bicyclic rings. The calculated pK_a values[9] of secondary (R = H) and tertiary amines (R = Me) in the designed compounds were respectively 6.93−9.23 and 5.53−7.98 whereas that of benzamidine was 12.01. We expected that this weakly basic group also did not cause such poor absorption as the amidine group. In fact, some compounds which contain only one amino group were reported to show good bioavailability in various animals.[10] In addition, the tertiary amine substituted with such as the *N*-alkyl group is further less basic than the secondary in aqueous solution because of the solvent effect of water molecules,[11] so that this amine is possible not to be protonated and exist in a molecular form which is suitable for oral absorption. Furthermore, the *N*-substituent on the tertiary amine is expected to insert into the cavity in the terminus of S4, named the "cation hole", to increase the affinity to S4.

In this paper, we report on our discovery of a non-amidine fXa inhibitor having a fused-bicyclic ring as the novel S4 binding element, and our investigation into the binding mode of this derivative by X-ray crystallography and ab initio calculations.

Chemistry

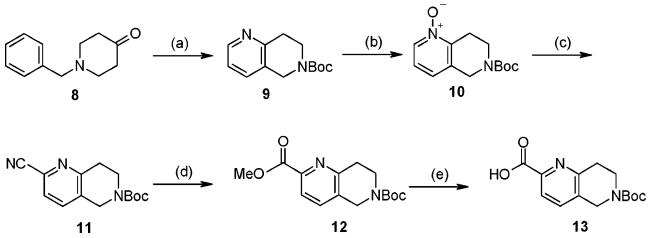
Preparation of 2-*tert*-butoxycarbonyl-1,2,3,4-tetrahydroisoquinolin-6-carboxylic acid (**7**) is shown in Scheme 1. After protection of 6-hydroxy-1,2,3,4-tetrahydroisoquinoline (**3**),[12] the resulting **4** was trifluorosulfonated and then a methoxycarbonyl group was introduced by CO insertion with $Pd(OAc)_2$. Hydrolysis of ester **6** gave carboxylic acid **7**.

Scheme 1[a]



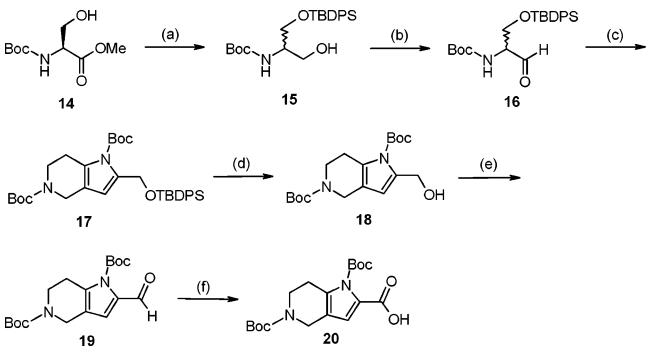
[a] Reagents: (a) $(Boc)_2O$, Et_3N/MeOH; (b) Tf_2O/pyridine; (c) $Pd(OAc)_2$, 1,3-bis(diphenylphosphino)propane, Et_3N, CO/MeOH; (d) aq NaOH/THF−MeOH.

Scheme 2[a]



[a] Reagents: (a) i. Et_3N, 3-acrylaldehyde, pyridinium acetate, ii. 10% Pd−C, H_2/MeOH, iii. $(Boc)_2O$, aq NaOH/toluene; (b) *m*CPBA/CH_2Cl_2; (c) TMSCN, $(CH_3)_2NCOCl/CH_2Cl_2$; (d) i. HCl(c)/MeOH, ii. $(Boc)_2O$/aq $NaHCO_3$−THF; (e) aq NaOH/THF−MeOH.

Scheme 3[a]

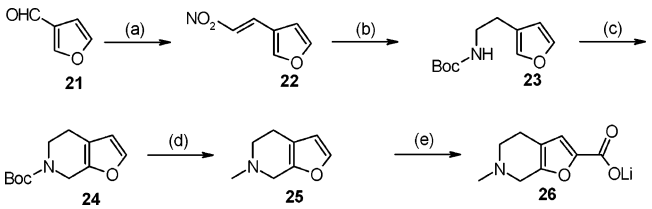


[a] Reagents: (a) i. TBDPSCl, imidazole/DMF, ii. $NaBH_4$/THF-MeOH; (b) Dess−Martin periodinane/CH_2Cl_2; (c) i. LDA/THF then 4-Boc-piperidone, ii. concentrated HCl; (d) HF−Pyr/Pyr; (e) Dess−Martin periodinane/CH_2Cl_2; (f) 2-methyl-2-butene, $NaClO_4$, NaH_2PO_4/*t*-BuOH.

The preparation of 6-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydro-1,6-naphthyridine-2-carboxylic acid (**13**) is shown in Scheme 2. An aldol reaction condensed *N*-benzylpiperidone (**8**) and 3-acrylaldehyde to give 5,6,7,8-tetrahydro-1,6-naphthyridine **9**.[13] After N-oxidation of **9**, reduction and regioselective cyanation[14] of **10** afforded **11** followed by methanolysis to give **12**. The resulting methyl ester **12** was hydrolyzed to carboxylic acid **13**.

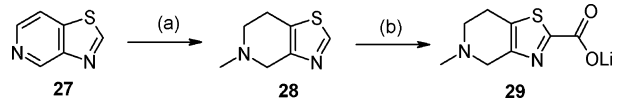
The preparation of 1,5-bis(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (**20**) is shown in Scheme 3. Protection of *N*-Boc-L-serine methyl ester (**14**) with TBDPSCl followed by reduction with $NaBH_4$ gave alcohol **15**. After oxidation of **15**, the resulting aldehyde **16** and *N*-Boc-piperidone were condensed by aldol reaction with LDA and then treated with concentrated HCl to give tetrahydropyrropyridine

Scheme 4[a]



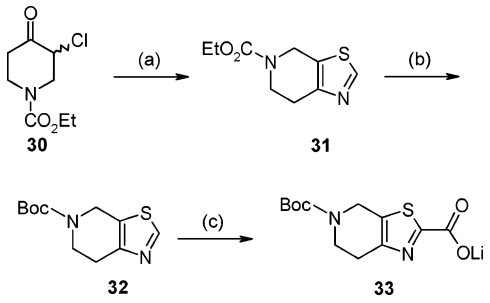
[a] Reagents: (a) CH_3NO_2, NaOH/EtOH−H_2O; (b) i. $LiAlH_4$/THF, ii. $(Boc)_2O/CH_2Cl_2$; (c) paraformaldehyde, *p*-TsOH/toluene; (d) i. HCl/MeOH, ii. $NaBH(OAc)_3$, Et_3N, aq HCHO, $AcOH/CH_2Cl_2$; (e) *n*BuLi/THF, then CO_2.

Scheme 5[a]



[a] Reagents: (a) i. CH_3I/DMF, ii. $NaBH_4$/MeOH; (b). *n*BuLi/THF, then CO_2.

Scheme 6[a]



[a] Reagents: (a) thioformamide, molecular sieves 4 Å/EtOH; (b) 5 N NaOH, then $(Boc)_2O$; (c) *n*-BuLi/ether, then CO_2.

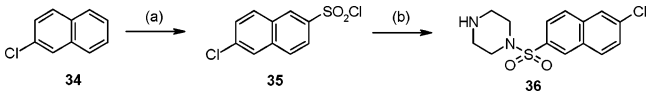
17. After deprotection of **17**, the resulting **18** was oxidized with Dess−Martin reagent and $NaClO_4$ in order to give carboxlic acid **20**. The crude **20** was used unpurified for the following reaction.

Preparation of lithium 6-methyl-4,5,6,7-tetrahydrofuro[2,3-*c*]pyridine-2-carboxylate (**26**) is shown in Scheme 4. Nitro aldol reaction with 3-furaldehyde (**21**) gave nitro olefin **22**. After reduction of the nitro group, the resulting (2-amino)ethylfuran was protected with *tert*-butyl dicarbonate then cyclized with paraformaldehyde and *p*-TsOH. 6-Boc-4,5,6,7-tetrahydrofuro[2,3-*c*]pyridine (**24**) was deprotected under acidic condition then a methyl group introduced by reductive alkylation with sodium triacetoxyborohydride. Introduction of a carboxylate to the furan with *n*-BuLi and CO_2 gave **26**. The crude **26** was used unpurified for the following reaction.

Preparation of lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[4,5-*c*]pyridine-2-carboxylate (**29**) is shown in Scheme 5. Quarternalization and reduction of the pyridine moiety of thiazolo[4,5-*c*]pyridine (**27**)[15] gave 5-methyl-4,5,6,7-tetrahydrothiazolo[4,5-*c*]pyridine (**28**). Introduction of a carboxylate to the thiazole with *n*-BuLi and CO_2 gave **29**.

Preparation of lithium 5-*tert*-butoxycarbonyl-4,5,6,7-tetrahydrothiazolo[4,5-*c*]pyridine-2-carboxylate (**33**) is shown in Scheme 6. Reaction of 3-chloro-1-ethoxycarbonyl-4-piperidone (**30**)[16] with thioformamide gave 5-ethoxycarbonyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine (**31**). After removal of the ethoxycarbonyl group of **31** by alkali hydrolysis, the resulting 4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine was protected with di-*tert*-butyl dicarbonate then treated with *n*-BuLi and CO_2 to give **33**.

Scheme 7[a]



[a] Reagents: (a) i. concd H_2SO_4, ii. $SOCl_2$/DMF; b) i. *tert*-butyl 1-piperazinecarboxylate, Et_3N/CH_2Cl_2, ii. HCl/EtOH.

5-Boc-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine-2-carboxylic acid and 6-Boc-4,5,6,7-tetrahydrothieno- [2,3-*c*]-pyridine-2-carboxylic acid were prepared with the method reported by Katano et al.[17]

Preparation of 1-(6-chloronaphthalen-2-yl)sulfonylpiperazine (**36**) is shown in Scheme 7. (6-Chloronaphthalen-2-yl)sulfonyl chloride (**35**) was synthesized via *β*-sulfonylation and successive chlorination of 2-chloronaphthalene (**34**). Compound **35** was treated with *tert*-butyl 1-piperazinecarboxylate, and the resulting compound was deprotected to give **36**.

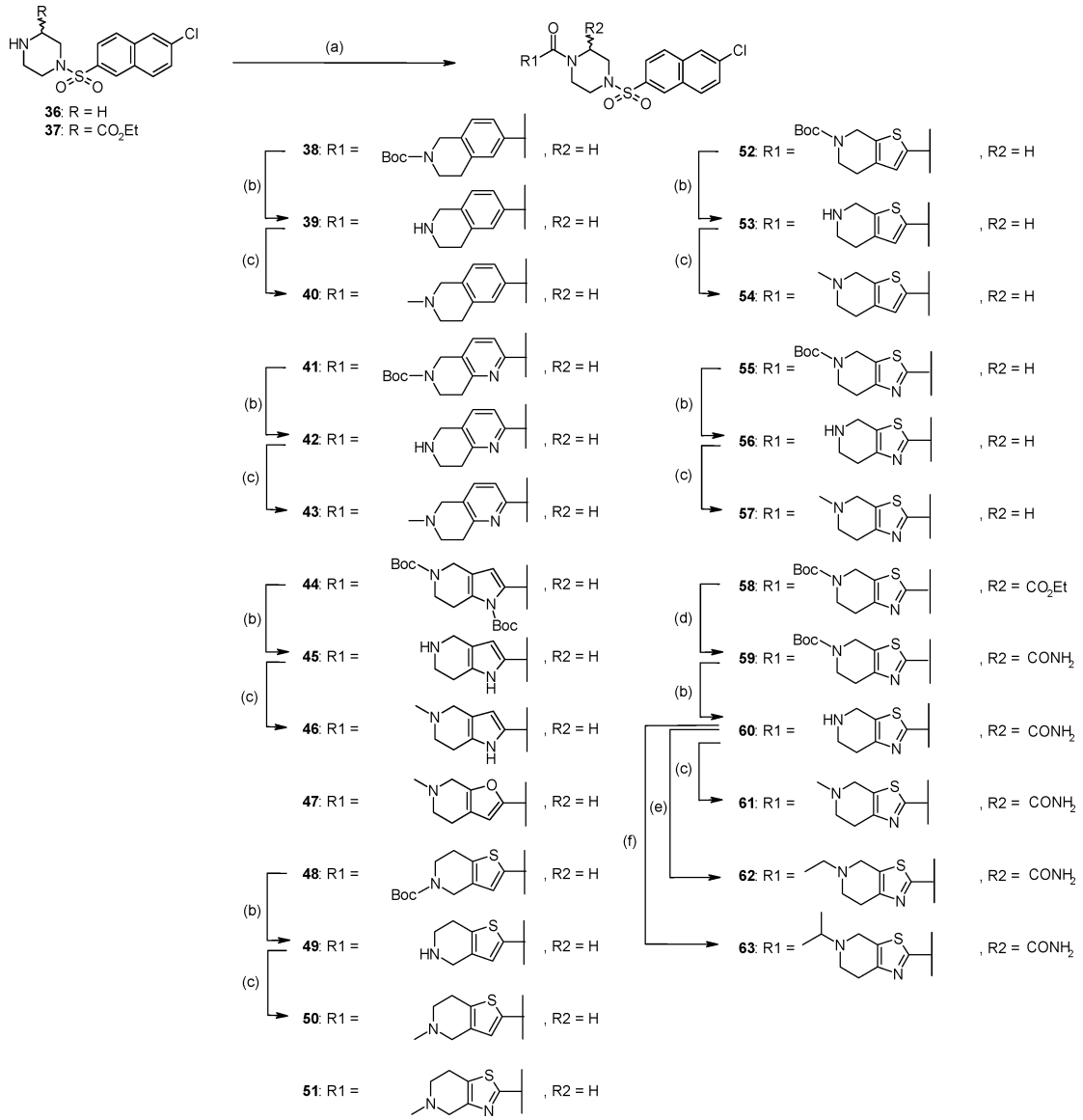
N-(6-Chloronaphthalen-2-yl)sulfonylpiperazine derivatives **36** and **37** were condensed with various carboxylic acids to give the corresponding compounds (Scheme 8). 4-(6-Chloronaphthalen-2-yl)sulfonyl-2-ethoxycarbonylpiperazine (**37**) was prepared with the method reported by Nishida et al.[18] Ethoxycarbonyl derivative **58** was hydrolyzed under alkali conditions, and then the resulting carboxylic acid was condensed with ammonia to give carbamoyl **59**. Deprotection of Boc derivatives **38**, **41**, **44**, **48**, **52**, **55**, and **59** gave the corresponding **39**, **42**, **45**, **49**, **53**, **56**, and **60**, and then alkylation by reductive methylation with formaldehyde gave the corresponding **40**, **43**, **46**, **50**, **54**, **57**, and **61**. Alkylation of **60** with EtI gave **62**. The reductive alkylation with acetone gave isopropyl **63**.

Results and Discussion

At first, we investigated anti-fXa activities of compounds having various hetero aromatic rings involving a secondary or tertiary amine. The in vitro anti-fXa activities are summarized in Table 1. Tetrahydroisoquinoline **39**, **40**, tetrahydronaphthylidine **42**, **43**, tetrahydropyrrolopyridine **45**, **46**, and tetrahydrofuropyridine derivative **47** only exhibited weak activities. On the other hand, tetrahydrothienopyridine **49**, **50**, **53**, **54**, and tetrahydrothiazolopyridine **56** and **57**, which contain the S atom in their heterocycles, had a tendency to show more potent activity than those derivatives without the S atom. Tertiary amino derivatives **50**, **54**, and **57** resulted in 1.5- to 3-fold more activity relative to the corresponding secondary derivatives **49**, **53**, and **56**. However, 5-methyl-4,5,6,7-tetrahydrothiazolo[4,5-*c*]pyridine **51** showed 5-fold less activity than **57**. These results suggested that both of the S and N atoms in the heterocyclic ring, and the appropriate position of the tertiary amino group were required for potent anti-fXa activity for a 5−6 fused-system.

Second, we executed optimizations of tetrahydrothiazolo[5,4-*c*]pyridine **57** as a lead compound. For the purpose of elevating potency, we noted the amino moiety of tetrahydrothiazolo[5,4-*c*]pyridine or the piperazine ring. Because it is thought that the introduction of various alkyl groups on the amino moiety is the ap-

Scheme 8[a]

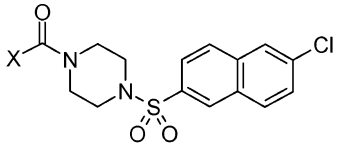


[a] (a) Carboxylic acids, EDC or PyBOP, Et_3N/DMF or CH_2Cl_2; (b) HCl-EtOH or TFA/CH_2Cl_2; (c) aq HCHO, Et_3N, AcOH, $NaBH(OAc)_3$/CH_2Cl_2; (d) i. aq NaOH/EtOH, ii. isobutyl chloroformate, *N*-methylmorpholine/THF, iii. NH_3/CH_2Cl_2; (e) EtI, K_2CO_3/DMF; (f) acetone, Et_3N, AcOH, $NaBH(OAc)_3$/CH_2Cl_2.

proach to acquisition of the most suitable substituent fitting to "cation hole" in S4. The other reason is that the introduction of the polar group on the piperidine ring regarded as a linker is expected to yield the ability to form hydrogen bonds with the amino acids such as Gly 216 or Gly 219. There are some reports that the moiety linking S1 and S4 binding elements has the ability to form hydrogen bonds with Gly 216 or Gly 219 of fXa in modelings or crystal structures.[8b,19] We expected this ability from the carbamoyl group. According to the expectations described above, we designed and synthesized the carbamoyl derivatives **60**−**63** which are substituted, respectively, methyl, ethyl, and isopropyl on the amino moiety. In Table 2, the in vitro anti-fXa, anti-thrombin, and anticoagulant activities are summarized. Although the introductions of carbamoyl groups did not increase anti-fXa activity, *N*-methyl **61** was superior in anticoagulant activity as $PTCT_2$ in human plasma and selectivity over thrombin to the corresponding **57**. All compounds tended to exhibit more potent anticoagulant activities in human plasma than these in rat plasma. *N*-Ethyl **62** and *N*-isopropyl **63** showed 4- to 5-fold less activity than **61**. This result indicated that the *N*-methyl group was a suitable alkyl substituent on the amino moiety.

With respect to compounds **57** and **61**, which showed potent activity in vitro, we executed preliminary ex vivo tests for measuring of anti-fXa activity with oral administration of 30 mg/kg to rats. Whereas compound **57** only displayed 27.6 ± 3.3% inhibition at 1h after oral administration ($n = 3$), compound **61** did 69.0 ± 2.1% inhibition at same time point. As the anticoagulant activity ($PTCT_2$) of **61** in human plasma is more than 3-fold potent relative to that in rat plasma as shown in Table 2, we expected that the orally evident anticoagulant activity in rats would indicate a possibility of the useful antithrombotic agent. In the time course ex vivo test with oral administration of 30 mg/kg to rats ($n = 4$), compound **61** displayed almost equipotent anti-fXa activity of 67.4 ± 1.5% to the preliminary, showed evident anti-fXa activity until 6 h after administration, and also showed our expected anticoagulant activity,

Table 1. In Vitro Anti-fXa Activities for Synthetic Compounds



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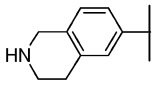
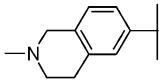
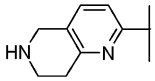
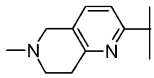
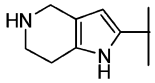
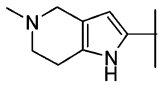
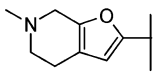
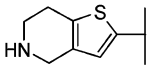
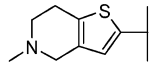
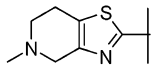
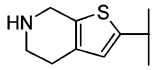
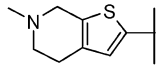
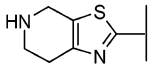
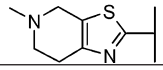
39		640
40		1200
42		920
43		2200
45		6200
46		320
47		550
49		270
50		190
51		210
53		160
54		105
56		60
57		22

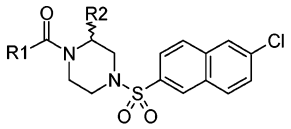
namely, evident prolongation of prothrombin time (PT) of 1.13-fold at 1 h (Table 3). Figure 3 shows the serum concentration of compound **61** administered intravenously (5 mg/kg iv) or orally (5 mg/kg po). The changes of anti-fXa activity ex vivo and the orally serum concentration on time course were correlative. In the oral administration at 5 mg/kg dosing, CL, V_{dss}, C_{max}, and $T_{1/2}$ of **61** were respectively 55.95 mL/min/kg, 10.59 L/kg, 0.35 μg/mL, and 1.6 h. The $AUC_{iv\ or\ po}$ and bioavailability, were respectively 4.20 μg·h/mL (5 mg/kg iv), 1.49 μg·h/mL (5 mg/kg po) and 35%.

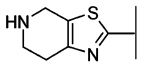
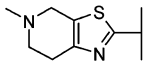
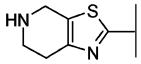
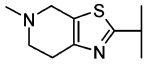
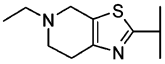
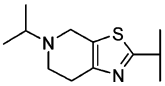
Conformational Analysis with X-ray and ab Initio Calculation Study. To determine the binding mode of **61**, we executed an X-ray study of **61** and Gla-less fXa,[8b,19b,20] which has been used because of the ease of growing crystals. The statistics of data processing and crystallographic refinement for the resulting crystal of **61** and Gla-less fXa are shown in Table 4. The result of the crystal structure confirmed the validity of our prediction. Whereas the 6-Cl group of the naphthalene ring occupied a small cavity composed of Tyr 228, Val 213, and Ala 190 in the S1 subsite in agreement with the result of 6-chlorobenzo[*b*]thiophene,[8b] the 5-methyl-4,5,6,7-tetrahydropyridine located in the S4 subsite lay parallel to the indole ring of Trp215 (Figures 4, 5). This X-ray structure provided further detailed observations for the binding mode of the 5-methy-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine ring to the S4 subsite. One was that the 5-methyl group on tetrahydrothiazolopyridine faced forward toward the "cation hole" in the S4 subsite and occupied the space at the entrance of this hole as shown in Figure 5. We thought that the affinity of the 5-methyl group for this space was caused not by ionic interactions but by van der Waals contacts or a hydrophobic effect, because the tertiary amino moiety containing the 5-methyl group was not protonated and no interactions were confirmed between this amino moiety and the carboxylate of Glu 97. From this observation and the SAR study of Table 2, we assumed that the sizes of the *N*-methyl group on the tetrahydrothiazolopyridine were most suitable for the substituent that fit the space by the "cation hole". The other observation was that an intramolecular S−O contact was recognized in the 2-carbamoylthiazole moiety. The computer-generated drawings for **61** picked out from the structure of the crystal complex were shown in Figures 6 and 7. The distance of S1−O7 was 2.86 Å shorter than the sum of the corresponding van der Waals radii (3.25 Å). The 2-(*N*,*N*-dialkylcarbamoyl)thiazole moiety was found to adopt a plane conformation, since the torsion angle of S1−C1−C2−O7 was 2.15 °.

Several heterocyclic drugs having intramolecular S−O contacts have been reported. Goldstein's group[21] reported extensive studies for this contact in heterocyclic nucleoside analogues containing a thiazole or a thiophene ring. Nagao et al.[22,23] reported the investigations for various types of S−O close contacts in heterocyclic drugs and their properties. Tanaka et al.[24] and Collins et al.[25] respectively, reported similar observations in penem antibiotics and $GABA_A$ receptor agonists. Our result of X-ray analysis is the first report to confirm a novel intramolecular S−O contact in a complexed crystal structure of inhibitor and Gla-less fXa. In all of the reports for the S−O close contacts, the common suggestion was that this close contact restricted the conformation of a compound to effect on its biological activity. This suggestion invoked a prediction that the similar effect increased the affinity of the 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine ring to the S4 subsite.

By using computational studies, Burling et al. proposed that the electrostatic environments consisting of the affinity between the positively charged S atom and the negative O atom, and the repulsion between the negative N atom and the same O atom, restricted the

Table 2. In Vitro Anti-fXa, Anti-thrombin, and Anticoagulant Activities for Synthetic Compounds



entry	R^1 =	R^2 =	anti-fXa IC_{50} (nM)	anti-thrombin IC_{50} (nM)	[a]$PTCT_2$ in human plasma (μM)	$PTCT_2$ in rat plasma (μM)
56		H	60	2900	11.2	16.1
57		H	22	560	6.0	10.0
60		$CONH_2$	83	NT[b]	9.7	28.8
61		$CONH_2$	24	1000	4.4	14.0
62		$CONH_2$	110	NT	7.2	14.6
63		$CONH_2$	80	NT	6.6	17.5

[a] Clotting time doubling concentration for prothronbin time. [b] NT = not tested.

Table 3. Ex Vivo Anti-fXa and Anticoagulant Activities for **61** at 30 mg/kg (po) to Rats

	1 h	3 h	6 h
anti-fXa activity (%)[a]	67.4 ± 1.5	55.8 ± 1.5	24.7 ± 6.0
Test/control PT ratio (fold)[a]	1.13 ± 0.02	1.09 ± 0.02	1.05 ± 0.00

[a] Values expressed as mean ± SE from four rats.

rotation of the glycosyl bond in the thiazole nucleoside.[21a,f] As shown in Figure 8, we also predicted that both this affinity and the repulsion synergistically influenced the restriction of the C−C bond rotation in the 2-carbamoylthiazole moiety. The SAR in Table 1 for thieno and thiazolo types supported this hypothesis. Namely, both the S−O affinity and N−O repulsion restrict the bond rotation to strongly fix the direction of the *N*-methyl group so that there was a big difference of activity between [4,5-*c*]thiazolo **51** and [5,4-*c*]thiazolo **57**, because it is possible that the *N*-methyl group of 4,5-*c* **51** faces and hits the wall of S4 while that of 5,4-*c* **57** faces the "cation hole". On the other hand, in thieno types **50** or **54**, we thought only the S−O affinity restricted the C−C bond rotation so that there was a small difference of activity between 3,2-*c* **50** and 2,3-*c* **54**.

To disclose conformational and electrostatic characteristics lurking in the 2-carbamoylthiazole or thiophene moieties, we performed ab initio MO calculations using the GAMESS program[26] for the model compounds **I** and **II** illustrated in Figure 9.

At first, we examined each relative energy (ΔHF) for the various conformers of **I** and **II** in which torsion angles (χ) of the S−C−C═O moiety changed from 0° to 180°. The value of each ΔHF of **I** and **II** was respectively calculated values on the basis of the energy (0 kcal/mol) of conformer **I-A** (X = N, χ = 0°) and **II-A** (X = CH, χ = 0°) shown in Figure 9. ΔHF of conformers **I-A** and **II-A** with the closest S−O proximity exhibited the minimum value among the respective various conformers. Furthermore, the stability of **I-A** was more remarkable than that of **II-A**. These results suggested that the S−O proximity was related to the stability of conformers and that the N atom on the thiazole ring amplified the stability. It is notable that the torsion angle (2.15°) in the X-ray analysis for compound **61** and Gla-less fXa was almost consistent with that of the conformer **I-A**.

To trace the difference of conformational characters between model compounds **I** and **II**, we examined each electron distribution of heteroatoms by Mulliken population analysis.[27] We thought that this examination could compare electrostatic environments for **I** with **II**. The illustrations of Figure 10 show geometries (χ = 0° or 180°) with respective charges of heteroatoms. Whereas Mulliken charges of S atom (S1) in **I** and **II** were positively charged for both conformers, those of the O atom (O7) in **I** and **II** were negative. In addition, the N atom (N3) on the thiazole ring in **I** was negatively charged. Although respective N atoms (N8) on the carbamoyl group in **I** and **II** were negatively charged,

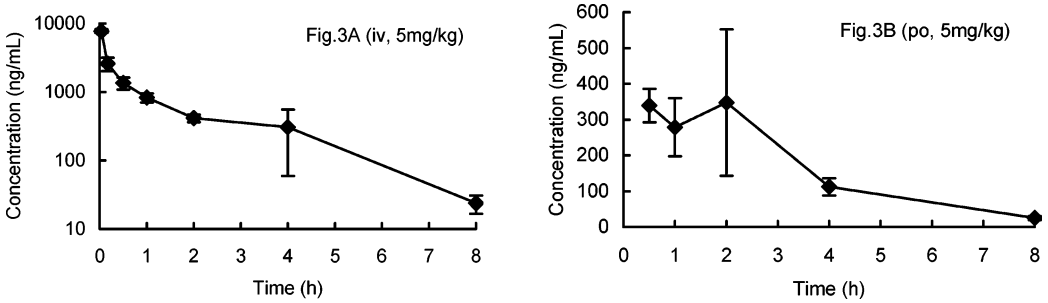


Figure 3. The serum concentration of compound **61** administered intravenously (Figure 3A, 5 mg/kg iv) or orally (Figure 3B, 5 mg/kg po) in rats. Each plot was expressed as ± SD from three rats.

Table 4. Crystal and Diffraction Data of Human Factor Xa with Compound **61**

crystal parameters	
space group	$P2_12_12_1$
a (Å)	56.1
b (Å)	71.9
c (Å)	78.8
resolution (Å)	2.2
R_{sym} (%)	4.4 (25.9)[a]
completeness (%)	90.4 (96.0)[a]
no. of reflections, redundancy	15999, 3.1
refinement	
no. of protein atoms (occupancy ≠ 0)	2145
average *B* value for protein and ligand atoms (Å^2)	36.7, 50.4
range of data	25.0−2.2
R value	19.4
weighted rsmd from ideality	
bond length (Å)	0.019
bond angle (Å)	1.80

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

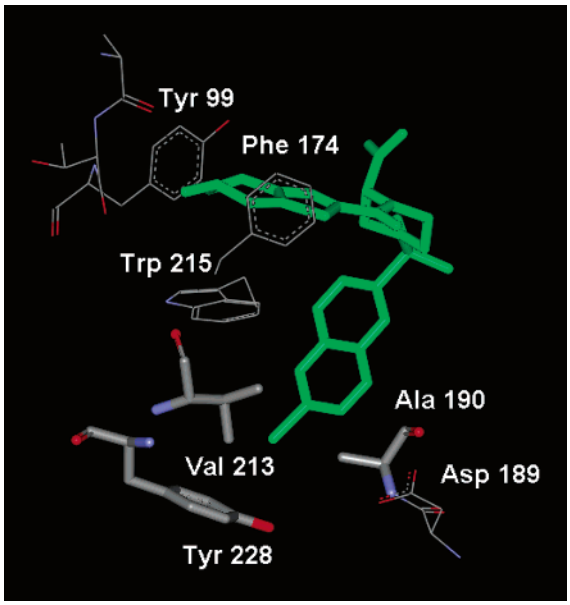


Figure 4. The binding mode of compound **61** shown as the side view. The green sticks drawn are **61**-omitted protons. Ala 190, Val 213, and Tyr 228 comprising the S1 cavity were shown as sticks. Other amino acid residues were shown as lines. Although both of stereo configurations for the carbamoyl group on the piperazine of racemate **61** were confirmed in the crystal, the only *R*-configuration for which more obvious electron density was observed than the *S*-configuration, was shown in the figure.

these charges covered by neutral H atoms were regarded as scarcely affecting the electrostatic affinity and repulsion against the adjacent heterocyclic ring. In conformers **I-A** and **II-A** ($\chi = 0°$), the respective distances of S−O were calculated as 3.00 and 3.01 Å which were shorter than the van der Waals radii. As for other conformers, charges of the respective heteroatoms had almost the same tendency (data not shown). The results of energy and charge calculations led to the suggestion that both the electrostatic S−O affinity and N−O repulsion were synergistically related to the restriction of the C−C bond rotation in **I**.

Conclusion

We have synthesized and evaluated a series of *N*-(6-chloronaphthalen-2-yl)sulfonylpiperazine derivatives to discover a non-amidine fXa inhibitor having a fused-bicyclic ring as the novel S4 binding element. SAR study for anti-fXa activity of this series indicated that both of the S and N atoms in the heterocyclic ring and the appropriate position of the tertiary amino group were required for potent anti-fXa activity for a 5−6 fused-system, so that it was found that 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine was the most preferred S4 binding element. Furthermore, compound **61** showed orally potent anti-fXa activity and evident prolongation of PT with moderate bioavailability. The X-ray crystal study with Gla-less fXa afforded an obvious binding mode of **61** of which thiazolopyridine and 6-chloronaphthalene respectively bound S4 and S1 subsites. In this X-ray study, we discovered a novel intramolecular S−O close contact in the crystal complex. From ab initio MO calculations of model compounds, it was suggested that the restriction of C−C bond rotation was caused by both the electrostatic S−O affinity and N−O repulsion in the 2-carbamoylthiazole moiety to fix the direction of *N*-methyl group required for potent anti-fXa activity.

Experimental Section

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 **61**. The benzamidine/Gla-less fXa crystal was dialyzed in a microdialysis button against soak solution 1 (20% PEG6000/15%

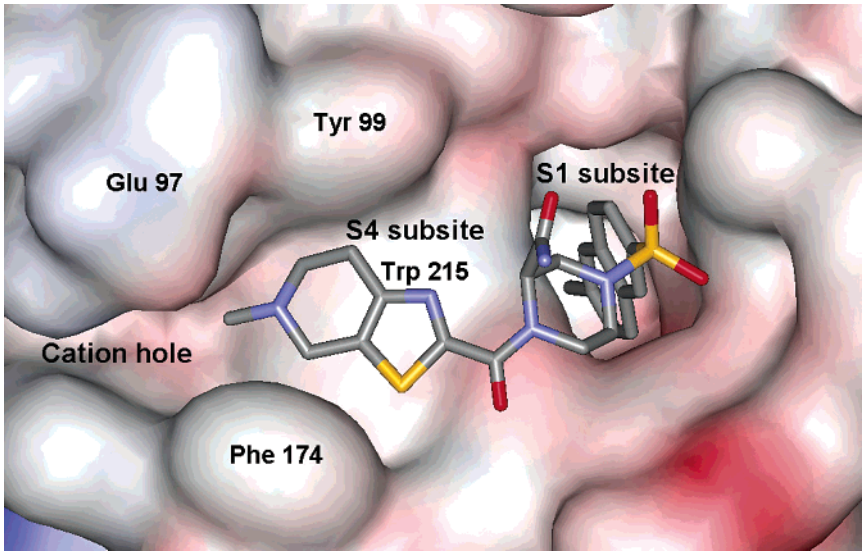


Figure 5. The binding mode of compound **61** shown as the top view. The surface view is the active site of Gla-less fXa. The stick drawn (gray: carbon, blue: nitrogen, red: oxygen, yellow: sulfur) indicate **61**-omitted protons.

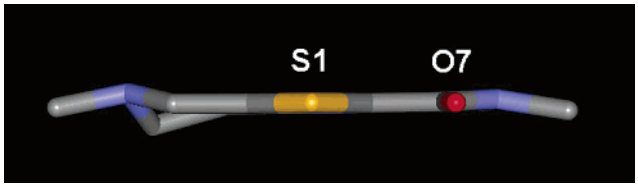


Figure 6. The side view of the tetrahydrothiazolo[5,4-*c*]-pyridine moiety of compound **61** picked up from the X-ray coordinates of the complex crystal structure. The torsion angle of the S1−C1−C2═O7 moiety, which adopted the plane conformation, was 2.15°.

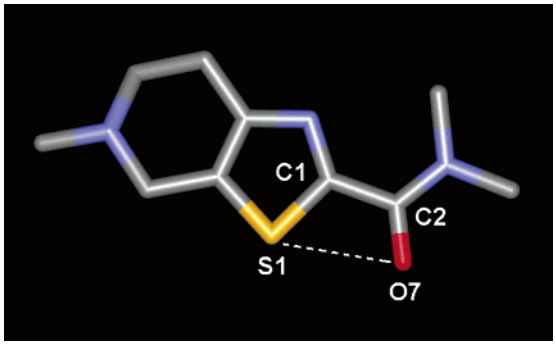


Figure 7. The top view of the tetrahydrothiazolo[5,4-*c*]-pyridine moiety. The distance (a dotted line) S1−O7 was 2.86 Å, which is shorter than the sum of the van der Waals radii (3.25 Å).

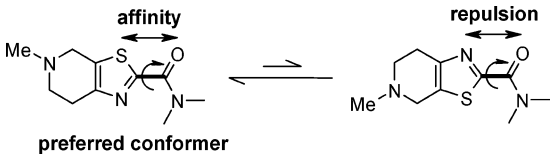


Figure 8. Our hypothesis that both of electrostatic affinity and repulsion restrict the rotation of the C−C bond (bold line) in the 2-carbamoylthiazole moiety.

glycerol/0.3 M AcONa/2.5 mM $CaCl_2$/0.1 M maleate imidazole, pH 5.0) for 5 h and then against soak solution 2 (25% PEG6000/25% glycerol/0.3 M AcONa/2.5 mM $CaCl_2$/0.1 M maleate imidazole, pH 5.0/1 mM of compound **61**. After 1 day, the crystal was picked up and directly exposed to soak solution 2, and soaking was continued for 1 day. All of the soaking process was performed at 20 °C.

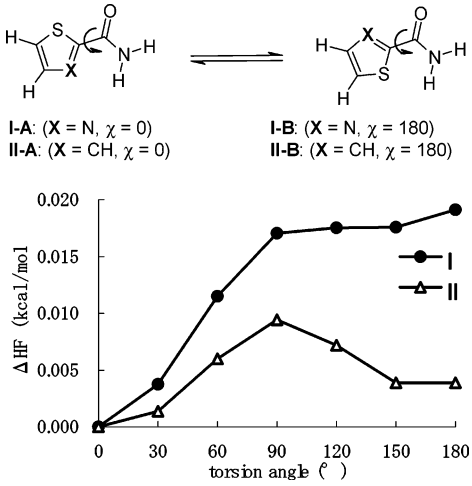


Figure 9. Energy versus torsion angle (χ) of the S−C−C═O moiety. The illustrations on the top are geometries of model compounds **I** and **II** at χ = 0° (A) or 180° (B). Ab initio calculations were perforrmed at RHF/6−31*G level.

X-ray Data Collection and Processing. The soaked crystal was flash-cooled in liquid nitrogen and centered in a gaseous nitrogen stream. The X-ray data set was collected at 100 K on an R-Axis IIc imaging plate detector (Rigaku) using an RU200 rotating anode generator (Rigaku). Data processing was carried out with *Mosflm*[28] and *scala*.[29]

Structure Solution and Crystallographic Refinement. The previously reported Gla-less fXa structure (PDB code: 1HCG[30]) was used as the initial structure. Phase refinement and model improvement was carried out with *refmac*[31] and *Turbo Frodo*.[32] 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 4. Atomic coordinates have been deposited with the Protein Data Bank (PDB code: 1V3X).

Anti-fXa Activity in Vitro. Anti-fXa activity in vitro was measured by using a chromogenic substrate S-2222 (Chromogenix, Inc.) and human fXa (Cosmo Bio-ERL). 5% aqueous DMSO (10 μL) or inhibitors in aqueous DMSO (10 μL) and 0.05 U/mL human fXa (10 μL) were mixed with 0.1 M Tris−0.2 M NaCl−0.2% BSA buffer (pH 7.4; 40 μL). The reaction was started by the addition of 0.75 M S-2222 (40 μL). After the mixture was stirred for 10 s at room temperature, the increase of optical densities (OD/min) were measured at 405

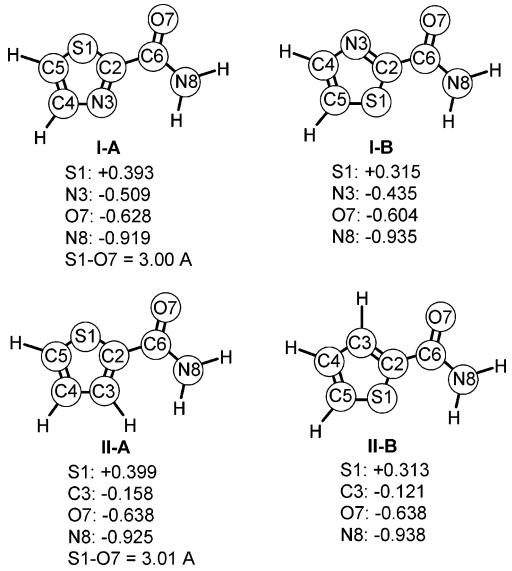


Figure 10. Mulliken charges for respective heteroatoms, S1, N3 (C3), O7, N8 in confomers at $\chi = 0°$ or 180°. Calculations were performed at RHF/6−31*G level.

nm. Both the normal control (no addition of inhibitor) and the positive control (DX-9065a[5] as inhibitor) were used. Anti-fXa activity (inhibition %) was calculated as follows: anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of normal control]. The concentration of an inhibitor required to inhibit enzyme activity by 50% (IC_{50}) was calculated from dose−response curves in which anti-fXa activity was plotted against inhibitor concentration on the statistical probability paper. The average standard errors of positive control ($n = 3$) were the mean <15%.

Anti-thrombin Activity in Vitro. Anti-thrombin activity in vitro was measured by using chromogenic substrate S-2266 (Chromogenix, Inc.) and human thrombin (Sigma Chemical, Inc.). 5% Aqueous DMSO (10 μL) or inhibitors in aqueous DMSO (10 μL) and 4 U/mL human thrombin (10 μL) were mixed with 0.1 M Tris−0.2 M NaCl−0.2% BSA buffer (pH 7.4; 40 μL). The reaction was started by the addition of 0.50 M S-2266 (40 μL). After the mixture was stirred for 10 s at room temperature, the increase of optical densities (OD/min) were measured at 405 nm. Both of the normal control (no addition of inhibitor) and the positive control (DX-9065a[5]) as inhibitor) were used. Anti-thrombin activity (inhibition %) was calculated as follows: anti-thrombin activity = 1 − [(OD/min) of sample/(OD/min) of normal control]. The concentration of an inhibitor required to inhibit enzyme activity by 50% (IC_{50}) was calculated from dose response curves in which anti-thrombin activity was plotted against inhibitor concentration on the statistical probability paper. The average standard errors of positive control ($n = 3$) were the mean <10%.

Anticoagulant Activity in Vitro. Plasma (20 μL) was mixed with inhibitors in saline (20 μL) in the process tube. The coagulation was started by the addition of SIMPLASTIN (Organon Teknica, Inc.) (40μL). Positive control was used the sample containing DX-9065a[5]) as inhibitor. Anticoagulant activity in vitro was evaluated with the plasma clotting time doubling concentration for prothrombin time ($PTCT_2$) calculated from dose−response curves in which anticoagulant activity was plotted against inhibitor concentration. The average standard errors of positive control ($n = 3$) were the mean <10%.

Anti-fXa Activity and Anticoagulant Activity ex Vivo. Male Wistar rats were fasted overnight. Synthetic compounds were dissolved in 0.5% methylcellose solution and administered orally to rats with a stomach tube. For control rats, 0.5% methylcellose solution was administered orally. Rats were anesthetized with halothane at several time points when blood samples were collected in the presence of trisodium citrate. After blood samples were centrifuged, the platelet poor plasma samples were used for measuring their anti-fXa activities or anticoagulant activities. Anti-Xa activity: Plasma (5 μL) was mixed with 0.1 M Tris-0.2 M NaCl−0.2% BSA buffer (pH 7.4; 40 μL) H_2O (5 μL), and 0.1 U/ml human fXa (10 μL). The reaction was started by the addition of 0.75 M S-2222 (40 μL). After the mixture was stirred for 10 s at room temperature, the increase of optical densities (OD/min) were measured at 405 nm. Anti-fXa activity (inhibition %) was calculated as follows; anti-Xa activity = 1 − [(OD/min) of sample/(OD/min) of control]. Anticoagulant activity: Plasma (20 μL) was mixed with inhibitors in saline (20 μL) in the process tube. The coagulation was started by the addition of SIMPLASTIN (40 μL). Anticoagulant activity was evaluated with the prolongation rate of prothrombin time versus control.

Measurement of Serum Concentration. Compound **61** was administered intravenously or orally to Wister rats (5 mg/kg iv, 5 mg/kg po) in aqueous solution. Blood samples were collected into Labospeed tubes (Toyo-kizai Inc.) at 0.03, 0.17, 0.5, 1, 2, 4, and 8 h for intravenous dosing or at 0.5, 1, 2, 4, and 8 h for oral dosing. Respective samples at each time points were collected in $n = 3$. Serum concentrations for compound **61** were determined by LC-MS/MS using Sciex API 365 (Sciex Inc.) coupled with Alliance 2690 HPLC system (Waters Inc.). Compound **61** 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.).

Computational Study. Ab initio calculations obtaining energy profiles and point charges were performed by using the GAMESS program systems[26] at the RHF level of the theory with the 6-31G* basis set. Geometry of model compounds I and II, respectively containing thiazole and thiophen, rings was optimized by fixing the torsion angles of the carbamoyl moiety (O═C−N−H) at 0°.

Relative energies (ΔHF) of each conformer were obtained for values of torsion angle (χ) of the S−C−C═O moiety in the range 0 to 180° and were shown in Figure 9. The χ value was incremented in 30° steps and fixed. The global energy minimum for each model compound I and II was normalized to 0 kcal/mol. The $\chi = 0°$ conformer (**I-A**, **II-A**) refers to the conformation in which the carbonyl oxygen was in closest proximity to the sulfur on the heterocyclic ring, and the relative energy of this conformer was normalized to 0 kcal/mol.

Point charges of respective atoms in all conformers in the range $\chi = 0−180$ ° was obtained by Mulliken population analysis.[27] For only four conformers (**I-A**, **I−B**, **II-A**, **II-B**), Mulliken charges were shown in Figure 10.

Chemistry. Procedures for the preparation of all final products are presented below along with representative procedures for all methods used in the preparation of intermediates. All solvents and reagents were used as acquired from commercial sources without purification. Melting points were determined on a Yanagimoto apparatus and are uncorrected. Column chromatography was performed on Merck silica gel 60 (0.063−0.200 mm). Thin-layer chromatography (TLC) was performed on Merck TLC plates precoated with silica gel 60 F_{254}. ^{1}H NMR specta 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. Mass spectra were performed using a JEOL JMS-AX505W (EI) or a JEOL JMS-HX110 (FD, FAB) spectrometer. IR spectra were recorded on a Hitachi 270-30 spectrometer.

2-*tert*-Butoxycarbonyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (4). To a mixture of **3** (7.87 g, 42.63 mmol as a HCl salt) and Et_3N (4.67 mL, 64.0 mmol) in MeOH (100 mL) was added di-*tert*-butyl dicarbonate (14.0 g, 64.0 mmol). The reaction mixture was stirred for 3 h at room temperature. After evaporation of the solvent, the residue was partitioned between AcOEt and H_2O. The organic layer was washed with 1 N HCl, sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (hexane/AcOEt, 10/1) gave **4** (9.96 g, 94%) a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 2.75 (2H, t, $J = 5.9$ Hz), 3.61 (2H, d, J

= 5.9 Hz), 4.48 (2H, s), 6.25 (1H, br s), 6.64 (1H, d, J = 2.4 Hz), 6.70 (1H, br s), 6.93 (1H, d, J = 7.8 Hz). MS (FAB) m/z 250 $(M + H)^+$.

2-*tert*-Butoxycarbonyl-6-trifluoromethanesulfonyloxy-1,2,3,4-tetrahydroisoquinoline (5). Trifluorosulfonic anhydride (8.10 mL, 47.9 mmol) was added to the solution of **4** (9.96 g, 40.0 mmol) in pyridine (100 mL) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and concentrated. Purification of the residue by column chromatography (hexane/AcOEt, 10/1) gave **5** (13.5 g, 88%) as a colorless solid: mp 48−51 °C. ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 2.87 (2H, t, J = 5.9 Hz), 3.66 (2H, d, J = 5.9 Hz), 4.59 (2H, s), 7.06 (1H, br s), 7.08 (1H, d, J = 8.3 Hz), 7.17 (1H, d, J = 8.3 Hz). MS (FAB) m/z 380 $(M - H)^+$.

2-*tert*-Butoxycarbonyl-6-methoxycarbonyl-1,2,3,4-tetrahydroisoquinoline (6). The mixture of **5** (1.34 g, 3.51 mmol), Et_3N (0.73 mL, 5.27 mmol), $Pd(OAc)_2$ (40 mg, 0.18 mmol), and 1,3-bis(diphenylphosphino)propane (145 mg, 0.36 mmol) in dry MeOH (50 mL) was sttred for overnight at 70 °C under an atmosphere of CO. After evaporation of the solvent, purification of the residue by column chromatography (hexane/AcOEt, 15/1) gave **6** (665 mg, 65%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 2.88 (2H, t, J = 5.9 Hz), 3.66 (2H, br s), 3.91 (3H, s), 4.62 (2H, s), 7.17 (1H, d, J = 7.8 Hz), 7.83 (1H, s), 7.84 (1H, d, J = 7.8 Hz). MS (FAB) m/z 290 $(M - H)^+$.

2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinolin-6-carboxylic Acid (7). To a solution of **6** (622 mg, 2.13 mmol) in THF/MeOH (1/1, 10 mL) was added 1 N NaOH (3 mL). The reaction mixture was refluxed for 3 h, cooled to room temperature, and concentrated in vacuo. The residue was separated with 0.05 N HCl (60 mL) and AcOEt (100 mL). The organic layer was dried over Na_2SO_4. Evaporation of the solvent gave **7** (583 mg, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.51 (9H, s), 2.91 (2H, t, J = 5.9 Hz), 3.68 (2H, br s), 4.64 (2H, s), 7.21 (1H, d, J = 7.8 Hz), 7.91 (1H, s), 7.92 (1H, d, J = 7.8 Hz). MS (FAB) m/z 276 $(M - H)^+$.

6-(*tert*-Butoxycarbonyl)-5,6,7,8-tetrahydro-1,6-naphthyridine (9). A mixture of 1-benzyl-4-piperidone (**8**) (3.80 g, 20.1 mmol), 3-acrylaldehyde (2.10 g, 29.5 mmol), Et_3N (1.50 mL, 10.8 mmol), and pyridinium acetate (30.0 mg, 216 mmol) was stirred at 120 °C for 22 h. The reaction mixture was cooled to room temperature and diluted with 3 N HCl. Chloroform was added to the solution, and the separated organic layer was discarded. Saturated Na_2CO_3 was added to the aqueous layer. The aqueous layer was extracted with chloroform. The organic extract was dried over Na_2SO_4 and concentrated. The residue was distilled under reduced pressure (0.90 mmHg, 145−150 °C) to give a colorless oil. The mixture of this oil and 10% Pd−C (50% wet, 500 mg) in AcOH (25 mL) was stirred for 2 h at 50−60 °C under an atmosphere of H_2. The reaction mixture was cooled to room temperature, filtered, and concentrated. To the mixture of the residue and 40% aq NaOH (30 mL) in toluene (20 mL) was added di-*tert*-butyl dicarbonate (3.20 g, 14.7 mmol). The two-phased mixture was stirred for 10 min. The aqueous layer was extracted with toluene. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. Purification of the residue by column chromatography (CH_2Cl_2/AcOEt, 3/1) gave **9** (981 mg, 21%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 3.01 (2H, t, J = 5.9 Hz), 3.76 (2H, t, J = 5.9 Hz), 4.59 (2H, s), 7.13 (1H, dd, J = 7.8, 4.9 Hz), 7.41 (1H, d, J = 7.8 Hz), 8.43 (1H, d, J = 4.9 Hz). MS (FAB) m/z 235 $(M + H)^+$.

6-(*tert*-Butoxycarbonyl)-5,6,7,8-tetrahydro-1,6-naphthyridine-1-oxide (10). To a solution of **9** (1.72 g, 7.34 mmol) in CH_2Cl_2 (40 mL) was added *m*-chloroperbenzoic acid (3.80 g, 22.0 mmol) at 0 °C. After the reaction mixture was stirred for 30 min, dimethyl sulfide (1.62 mL, 22.1 mmol) was added. After the reaction mixture was stirred for 30 min, sat. $NaHCO_3$ was added. The aqueous layer was exracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 10/1) gave **10** (1.80 g, 98%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 3.05 (2H, t, J = 5.9 Hz), 3.75 (2H, t, J = 5.9 Hz), 4.59 (2H, s), 7.04 (1H, d, J = 8.8 Hz), 7.14 (1H, dd, J = 8.8, 5.9 Hz), 8.18 (1H, d, J = 5.9 Hz). MS (FAB) m/z 251 $(M + H)^+$.

6-(*tert*-Butoxycarbonyl)-2-cyano-5,6,7,8-tetrahydro-1,6-naphthyridine (11). To a solution of **10** (760 mg, 3.04 mmol) in CH_2Cl_2 (15 mL) was added trimethylsilyl cyanide (610 μL, 4.57 mmol). After the mixture was stirred for 5 min at room temperature, *N*,*N*-dimethylcarbamyl chloride (420 μL, 4.56 mmol) was added. The reaction was stirred for 41 h at room temperature and then quenched with sat. $NaHCO_3$ (50 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification of the residue by column chromatography (CH_2Cl_2/AcOEt, 2/1) gave a colorless solid. Recrystallization from hexane and CH_2Cl_2 gave **11** (697 mg, 88%) as colorless needles: mp 121−124 °C. IR (KBr) cm^{-1}: 2978, 2933, 2235, 1693, 1685, 1572, 1477, 1458, 1415, 1365, 1267, 1238, 1169, 1161, 1124, 1097, 935. ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 3.05 (2H, t, J = 5.9 Hz), 3.77 (2H, t, J = 5.9 Hz), 4.67 (2H, s), 7.54 (2H, s). MS (FAB) m/z 260 $(M + H)^+$.

6-(*tert*-Butoxycarbonyl)-2-methoxycarbonyl-5,6,7,8-tetrahydro-1,6-naphthyridine (12). To a solution of **11** (1.25 g, 4.82 mmol) in MeOH (40 mL) was added concd HCl (40 mL). The reaction mixture was stirred for 3 h at 100 °C. After being cooled to room temperature, the reaction mixture was poured into aq $NaHCO_3$ (40 g/250 mL) and THF (150 mL). To the two-phased mixture was added di-*tert*-butyl dicarbonate (1.58 g, 7.23 mmol). The reaction was stirred for 30 min, and then the aqueous layer was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and concentrated. Purification of the residue by column chromatography (CH_2Cl_2/AcOEt, 1/1) gave **12** (955 mg, 68%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 3.12 (2H, t, J = 5.9 Hz), 3.77 (2H, t, J = 5.9 Hz), 4.00 (3H, s), 4.67 (2H, s), 7.57 (1H, d, J = 8.1 Hz), 7.98 (1H, d, J = 8.1 Hz). MS (EI) m/z 291 $(M - H)^+$.

6-(*tert*-Butoxycarbonyl)-5,6,7,8-tetrahydro-1,6-naphthyridine-2-carboxylic Acid (13). Starting with **12** and following the procedure for the preparation of **7** gave **13** (quant.) as a colorless solid: mp 181−185 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (9H, s), 2.42−2.48 (4H, m), 3.22−3.28 (2H, m), 7.92 (1H, d, J = 7.8 Hz), 8.25 (1H, d, J = 7.8 Hz). MS (FAB) m/z 277 $(M - H)^+$.

2-(*tert*-Butoxycarbonylamino)-3-(*tert*-butyldiphenylsiloxy)propanol (15). To a solution of *N*-(*tert*-butoxycarbonyl)-L-serine methyl ester (**14**) (13.8 g, 62.9 mmol) in DMF (140 mL) was added imidazole (6.43 g, 94.4 mmol). To the mixture *tert*-butyldiphenylsilyl chloride (19.7 mL, 75.8 mmol) was added dropwise at 0 °C. After stirred for 39 h at room temperature, the reaction mixture was partitioned between AcOEt and H_2O. The aqueous layer was extracted with AcOEt. The combined organic layers were washed with sat. NaCl, dried over Na_2SO_4, and concentrated in vacuo. To the solution of this residue in THF (100 mL) and MeOH (100 mL) was added $NaBH_4$ (7.20 g, 190 mmol) at 0 °C. After being stirred for 2 h at 0 °C, the reaction mixture was diluted with AcOEt and washed with H_2O. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with sat. NaCl, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (hexane/AcOEt, 10/1) gave **15** (24.9 g, 92%) as a colorless solid: mp 74−76 °C. ^{1}H NMR ($CDCl_3$) δ 1.07 (9H, s), 1.44 (9H, s), 2.39 (1H, br s), 3.63−3.85 (5H, m), 5.07 (1H, br s), 7.35−7.48 (6H, m), 7.60−7.67 (4H, m). MS (FAB) m/z 430 $(M + H)^+$.

2-(*tert*-Butoxycarbonylamino)-3-(*tert*-butyldiphenylsiloxy)propanal (16). To a solution of **15** (3.03 g, 7.05 mmol) in CH_2Cl_2 (100 mL) was added Dess−Martin periodinate (3.60 g, 8.49 mmol) at room temperature. After the mixture was stirred for 30 min, sat. $NaHCO_3$ (50 mL) and 10% Na_2SO_3 (50 mL) were added. The separated aqueous layer was extracted with Et_2O. The combined organic layer was dried over Na_2SO_4 and concentrated. Purification of the residue by column chromatography (hexane/AcOEt, 4/1) gave **16** (2.97 g, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.03 (9H, s), 1.46 (9H, s), 3.93 (1H, dd, J = 10.3, 3.9 Hz), 4.18 (1H, d, J = 10.3, 2.9 Hz),

4.27−4.35 (1H, m), 5.33−5.43 (1H, m), 7.32−7.48 (6H, m), 7.55−7.63 (4H, m), 9.66 (1H, s).

1,5-Bis(*tert*-butoxycarbonyl)-2-(*tert*-butyldiphenylsiloxy)methyl-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine (17). To a solution of diisopropylamine (1.35 mL, 16.8 mmol) in dry THF (40 mL) *n*-BuLi (1.66 M in hexanes; 9.20 mL, 15.3 mmol) was added dropwise the at −78 °C under an argon atmosphere. The reaction mixture was stirred for 30 min at −78 °C. To the mixture was added the solution of 4-(*tert*-butoxycarbonyl)piperidone (2.77 g, 13.9 mmol) in THF (10 mL). The reaction mixture was stirred for 90 min at −78 °C. To the mixture the solution of **16** (2.97 g, 6.95 mmol) in THF (10 mL) was added dropwise. After warmed to room temperature for 30 min, the mixture was stirred for 13 h. To the mixture, were added Et_2O and H_2O. The separated aqueous layer was extracted with Et_2O. The combined organic layers were washed with sat. NaCl, dried over Na_2SO_4, and concentrated. To the solution of residue in CH_2Cl_2 (20 mL) concentrated HCl was added dropwise until the pH was 4−5. The mixture was stirred for 2 h. To the mixture were added CH_2Cl_2 and Et_2O. The separated aqueous layer was extracted with Et_2O. The combined organic layers were washed with sat. NaCl, dried over Na_2SO_4, and concentrated. Purification of the residue by column chromatography (hexane/AcOEt, 6/1) gave **17** (2.20 g, 54%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.08 (9H, s), 1.43 (9H, s), 1.49 (9H, s), 2.89 (2H, br s), 3.64 (2H, br s), 4.32 (2H, s), 4.85 (2H, br s), 6.12 (1H, s), 7.30−7.48 (6H, m), 7.60−7.75 (4H, m). MS (FAB) *m*/*z* 613 $(M + Na)^+$.

1,5-Bis(*tert*-butoxycarbonyl)-2-hydroxymethyl-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine (18). To a solution of **17** (2.10 g, 3.55 mmol) in pyridine (20 mL) was added HF-pyridine complex (5.0 mL) at 0 °C. The reaction mixture was stirred for 1 h at room temperature. To the mixture were added ice−water (300 mL) and AcOEt. The separated aqueous layer was extracted with Et_2O. The combined organic layers were washed with sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated. Purification of the residue by column chromatography (hexane/AcOEt, 3/1) gave **18** (882 mg, 70%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.60 (9H, s), 2.85 (2H, br s), 3.45−3.70 (1H, br), 3.64 (2H, br s), 4.29 (2H, s), 4.59 (2H, d, J = 7.3 Hz), 6.01 (1H, s). MS (FAB) *m*/*z* 375 $(M + Na)^+$.

1,5-Bis(*tert*-butoxycarbonyl)-2-formyl-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine (19). To a solution of **18** (14.0 mg, 39.7 μmol) in CH_2Cl_2 (2.0 mL) was added Dess−Martin periodinate (34.0 mg, 80.2 μmol) at room temperature. After the mixture was stirred for 1 h, AcOEt (10 mL), 10% $Na_2S_2O_3$ (10 mL), and sat. $NaHCO_3$ (10 mL) were added. The separated aqueous layer was extracted with AcOEt. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification of the residue by column chromatography (hexane/AcOEt, 2/1) gave **19** (9.8 mg, 70%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s), 1.63 (9H, s), 2.96 (2H, br t, J = 5.4 Hz), 3.68 (2H, br t, J = 5.4 Hz), 4.37 (2H, s), 6.97 (1H, s), 10.14 (1H, br s). MS (FAB) *m*/*z* 351 $(M + H)^+$.

1-(3-Furyl)-2-nitroethylene (22). To a mixture of 3-furualdehyde (**21**) (10.0 g, 104 mmol) and nitromethane (6.37 g, 104 mmol) in EtOH (200 mL) was added 10 N NaOH (11.0 mL, 110 mmol) at 0 °C. After being stirred for 1 h at 0 °C, the reaction mixture was poured into 15% aq HCl. Collecting the precipitate gave **22** (8.01 g, 55%) as a pale yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 6.57 (1H, d, J = 2.0 Hz), 7.39 (1H, d, J = 13.4 Hz), 7.52 (1H, br s), 7.83 (1H, br s), 7.94 (1H, d, J = 13.4 Hz).

2-(*tert*-Butoxycarbonylamino)-1-(3-furyl)ethane (23). To a suspension of $NaBH_4$ (2.20 g, 58.0 mmol) in THF (170 mL) was added the solution of **22** (8.00 g, 57.5 mmol) for 2 h at room temperature. After being cooled to 0 °C, AcOEt (50 mL) and H_2O (10 mL) were added in order to the mixture. The mixture was stirred for 30 min at room temperature and concentrated in vacuo. To the solution of the residue in CH_2Cl_2 (200 mL) was added di-*tert*-butyl dicarbonate (12.6 g, 57.7 mmol). The reaction mixture was stirred for 1 h and concentrated in vacuo. Purification of the residue by column chromatography (hexane/AcOEt, 8/1) gave **23** (4.30 g, 35%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 2.61 (2H, t, J = 6.8 Hz), 3.25−3.37 (2H, m), 4.57 (1H, br s), 6.29 (1H, s), 7.26 (1H, s), 7.37 (1H, s).

6-(*tert*-Butoxycarbonyl)-4,5,6,7-tetrahydrofuro[2,3-*c*]-pyridine (24). To a solution of **23** (2.20 g, 10.4 mol) in toluene (300 mL) were added paraformaldehyde (635 mg, 20.8 mol) and *p*-toluenesulfonic acid hydrate (50 mg, 0.26 mmol). The reaction mixture was refluxed for 2 h with Dean−Stark trap. After the reaction mixture was cooled to room temperature, to the mixture were added AcOEt and sat $NaHCO_3$, the separated organic layer was washed with sat. NaCl, dried over Na_2SO_4, and concentrated. Purification of the residue by column chromatography (hexane/AcOEt, 10/1) gave **24** (1.04 g, 45%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s), 2.52 (2H, br s), 3.63 (2H, br s), 4.44 (2H, s), 6.25 (1H, s), 7.29 (1H, s). MS (FAB) *m*/*z* 224 $(M + H)^+$.

6-Methyl-4,5,6,7-tetrahydrofuro[2,3-*c*]pyridine (25). To the solution of **24** (1.05 g, 4.70 mmol) in CH_2Cl_2 (2 mL) was added MeOH solution saturated with HCl (30 mL). After stirred for 2 h at room temperature, the mixture was concentrated. To the suspension of the residue in CH_2Cl_2 (20 mL) was added Et_3N (1.31 mL, 9.40 mmol). The mixture was stirred for 15 min at room temperature. To the mixture were added AcOH (810 μL, 14.1 mmol), 37% aq HCHO (610 μL, 7.11 mmol), and sodium triacetoxy borohydride (1.51 g, 7.12 mmol). The reaction mixture was stirred for 1 h at room temperature under an argon atmosphere and poured into sat. $NaHCO_3$. The separated organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 10/1) gave **25** (434 mg, 67%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.48 (3H, s), 2.56 (2H, t, J = 5.6 Hz), 2.67 (2H, t, J = 5.6 Hz), 3.48 (2H, s), 6.23 (1H, d, J = 2.0 Hz), 7.25 (1H, s).

5-Methyl-4,5,6,7-tetrahydrothiazolo[4,5-*c*]pyridine (28). A mixture of thiazolo[4,5-*c*]pyridine (**27**) (700 mg, 5.14 mmol) and CH_3I (648 μL, 10.3 mmol) in DMF (80 mL) was stirred for 4 h at 80 °C. After evaporation of the solvent, H_2O (100 mL) was added to the residue. $NaBH_4$ (583 mg, 15.4 mmol) was added to this solution at 0 °C. The reaction mixture was stirred for 1 h at room temperature. To this mixture were added Et_2O and 1 N NaOH. The separated organic layer was dried over $MgSO_4$ and concentrated. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 25/1) gave **28** (596 mg, 75%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.52 (3H, s), 2.77 (2H, t, J = 5.4 Hz), 2.92−3.00 (2H, m), 3.69 (2H, t, J = 2.0 Hz), 8.61 (1H, s). MS (FAB) *m*/*z* 155 $(M + H)^+$.

Lithium 5-Methyl-4,5,6,7-tetrahydrothiazolo[4,5-*c*]-pyridin-2-carboxylate (29). To a solution of **28** (583 mg, 3.78 mmol) in dry THF (50 mL) was added *n*-BuLi (1.54 M in hexanes; 2.70 mL, 4.16 mmol) at −78 °C under an argon atmosphere. The reaction mixture was stirred for 20 min at −78 °C. After the bubbling of CO_2 gas for 15 min, the reaction mixture was warmed to room temperature. Evaporation of the solvent gave **29** (820 mg, quant.) as a pale brown foam: ^{1}H NMR (DMSO-d_6) δ 2.38 (3H, s), 2.64 (2H, br s), 2.80 (2H, br s), 3.44 (2H, br s). MS (FD) *m*/*z* 199 $(M + H)^+$.

5-Ethoxycarbonyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]-pyridine (31). To a solution of 2-chloro-4-ethoxycarbonylpiperidone (**30**) (150 g, 728 mmol) in *n*-BuOH (350 mL) were added MS 4 Å (200 g) and thioformamide (88.2 g, 1.44 mol), which was prepared with formamide (2000 mL) and P_2S_5 (500 g). After stirred for 2.5 h at 100 °C, the reaction mixture was filtered. The filtrate was diluted with AcOEt and washed with sat $NaHCO_3$. The separated aqueous layer was extracted with AcOEt. The combined organic layers were washed with sat. NaCl, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (hexane/AcOEt, 1/1) gave **31** (79.0 g, 51%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, t, J = 7.3 Hz), 2.96 (2H, br s), 3.82 (2H, br s), 4.19 (2H, t, J = 7.3 Hz), 4.73 (2H, br s), 8.68 (1H, s). MS (FAB) *m*/*z* 213 $(M + H)^+$.

5-*tert*-Butoxycarbonyl −4,5,6,7-tetrahydrothiazolo[5,4-*c*] pyridine (32). To **31** (33.5 g, 158 mmol) was added 3.5 N

NaOH (250 mL). The two-phase mixture was stirred for 2 h at 110 °C. The reactant solution was cooled to room temperature and diluted with MeOH (200 mL). To the mixture was added di-*tert*-butyl dicarbonate (103 g, 470 mmol). The reaction mixture was stirred for 2 h. To the mixture was added 4 N HCl until the pH was 2−3. The mixture was diluted with AcOEt and washed with sat. $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layers were washed with sat. NaCl, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (hexane/AcOEt, 5/1) gave **32** (21.1 g, 55%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 2.94 (2H, br s), 3.76 (2H, br s), 4.68 (2H, s), 8.67 (1H, s). MS (FAB) *m*/*z* 241 $(M + H)^+$.

Lithium 5-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-carboxylate (33). To a stirred solution of **32** (426 mg, 1.77 mmol) in dry ether (100 mL) was added *n*-BuLi (1.57 M in hexanes; 1.16 mL, 1.77 mmol) at −78 °C under an argon atmosphere. The reaction mixture was stirred for 15 min at −78 °C. After the bubbling of CO_2 gas for 5 min, the reaction was warmed to room temperature and concentrated in vacuo. Collecting of the residue and washing with AcOEt gave **33** (421 mg, 82%) as a pale yellow amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.42 (9H, s), 2.71 (2H, br s), 3.60−3.66 (2H, m), 4.55 (2H, s). MS (FAB) *m*/*z* 285 $(M + H)^+$.

6-Chloronaphthalen-2-ylsulfonyl Chloride (35). The suspension of 2-chloronaphthalene (**34**) (50.6 g, 311 mmol) in concd H_2SO_4 (16.6 mL, 311 mmol) was stirred for 6 h at 160 °C. After being cooled to room temperature, the reactant solution was diluted with DMF (200 mL). To the solution was added $SOCl_2$ (34.1 mL, 467 mmol) at 0 °C. The reaction mixture was stirred for 90 min at 0 °C. The collected the precipitate was dissolved in AcOEt. The solution was washed with H_2O, dried over Na_2SO_4, and concentrated in vacuo. Recrystallization of the residue from hexane gave **35** (14.2 g, 17%) as a colorless solid: mp 102−104 °C. ^{1}H NMR ($CDCl_3$) δ 7.64 (1H, dd, J = 8.8, 2.0 Hz), 7.93−8.07 (4H, m), 8.58 (1H, d, J = 1.0 Hz). MS (EI) *m*/*z* 260 M^+.

1-[6-(Chloronaphthalen-2-yl)sulfonyl]piperazine Hydrochloride (36). To a solution of *tert*-butyl 1-piperazinecarboxylate (856 mg, 4.60 mmol) in CH_2Cl_2 (150 mL) were added Et_3N (765μL, 5.52 mmol) and **35** (1.20 g, 4.60 mmol) at room temperature. The reaction mixture was stirred for 15 min and washed with H_2O. The separated organic layer was dried over Na_2SO_4 and concentrated in vacuo. To the residue was added EtOH solution saturated with HCl (30 mL). The reaction mixture was stirred for 3 min and concentrated in vacuo. Recrystallization of the residue from AcOEt gave **36** (1.62 g, quant.) as a colorless solid: mp 248−251 °C. ^{1}H NMR (DMSO-d_6) δ 3.19 (8H, d, J = 7.3 Hz), 7.75 (1H, dd, J = 8.8, 2.0 Hz), 7.86 (1H, dd, J = 8.8, 2.0 Hz), 8.22 (1H, d, J = 8.8 Hz), 8.26−8.32 (2H, m), 8.56 (1H, s), 8.63 (2H, br s). MS (FAB) *m*/*z* 311 $(M + H)^+$.

1-[(2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinolin-6-yl)carbonyl]-4-[(6-chloronaphthalen-2-yl)sulfonyl]-piperazine (38). To a mixture of **7** (460 mg, 1.66 mmol), **36** (637 mg, 1.66 mmol), 1-hydroxybenzotriazole hydrate (381 mg, 2.49 mmol), and Et_3N (230 μL, 1.66 mmol) in CH_2Cl_2 (50 mL) was added 1-(dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (477 mg, 2.49 mmol). The reaction mixture was stirred for overnight at room temperature and then poured into H_2O (50 mL). The separated organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (hexane/AcOEt, 3/1) gave **38** (903 mg, 95%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 1.48 (9H, s), 2.76 (2H, t, J = 5.4 Hz), 3.09 (4H, br s), 3.60 (2H, t, J = 5.4 Hz), 3.77 (4H, br s), 4.52 (2H, s), 7.12−7.25 (3H, m), 7.59 (1H, dd, J = 8.8, 2.0 Hz), 7.75 (1H, dd, J = 8.8, 2.0 Hz), 7.88−7.95 (3H, m), 8.30 (1H, s). MS (FAB) *m*/*z* 570 $(M + H)^+$.

4-[(6-Chloronaphthalen-2-yl)sulfonyl]-1-[(1,2,3,4-tetrahydroisoquinolin-6-yl)carbonyl]piperazine Hydrochloride (39). To the solution of **38** (1.47 g, 0.416 mmol) in CH_2Cl_2 (2 mL) was added EtOH solution saturated with HCl (2 mL). Evaporation of the solvent gave **39** (672 mg, 84%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.89−3.29 (4H, m), 3.20−3.83 (8H, m), 4.25 (2H, s), 7.10−7.25 (3H, m), 7.71 (1H, d, J = 8.3 Hz), 7.81 (1H, d, J = 8.3 Hz), 8.17 (1H, d, J = 8.8 Hz), 8.15−8.25 (2H, m), 8.49 (1H, s), 9.54 (2H, br s). MS (FAB) *m*/*z* 470 $(M + H)^+$. Anal. ($C_{24}H_{24}ClN_3O_3S\cdot HCl\cdot 2H_2O$) C, H, Cl, N, S.

4-[(6-Chloronaphthalen-2-yl)sulfonyl]-1-[(2-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)carbonyl]piperazine Hydrochloride (40). To a stirred suspension of **39** (436 mg, 0.861 mmol) in CH_2Cl_2 (50 mL) was added Et_3N (238 μL, 1.72 mmol). After the mixture was stirred for 15 min at room temperature, AcOH (98 μL, 1.72 mmol), 37% aq HCHO (90 μL, 1.05 mmol), and sodium triacetoxy borohydride (292 mg, 1.38 mmol) were added. The mixture was stirred for 2 h at room temperature under an argon atmosphere and poured into H_2O (20 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 50/1) gave a colorless foam. To this material was added 1 N HCl/EtOH (10 mL). The solution was concentrated and dried in vacuo. Codistillation of EtOH with H_2O gave **40** (365 mg, 76%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.88 (3H, s), 2.90−3.80 (13H, m), 4.12−4.56 (1H, m), 7.19 (1H, s), 7.20 (2H, d, J = 6.8 Hz), 7.72 (1H, dd, J = 8.8, 2.0 Hz), 7.81 (1H, d, J = 8.8 Hz), 8.17 (1H, d, J = 8.8 Hz), 8.24−8.28 (2H, m), 8.49 (1H, s), 10.93 (1H, br s). MS (FAB) *m*/*z* 484 $(M + H)^+$. Anal. ($C_{24}H_{24}ClN_3O_3S\cdot HCl\cdot 2.3H_2O$) C, H, Cl, N, S.

1-[(6-*tert*-Butoxycarbonyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)carbonyl]-4-[(6-chloronaphthalen-2-yl)sulfonyl]piperazine (41). Starting with **13** and **36** and following the procedure for the preparation of **38** gave **41** (77%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.50 (9H, s), 2.92 (2H, t, J = 5.7 Hz), 3.11 (2H, br t, J = 4.4 Hz), 3.23 (2H, br t, J = 4.4 Hz), 3.74 (2H, t, J = 5.7 Hz), 3.78 (2H, br t, J = 4.4 Hz), 3.90 (2H, br t, J = 4.4 Hz), 4.59 (2H, s), 7.42 (1H, br d, J = 7.8 Hz), 7.47 (1H, br d, J = 7.8 Hz), 7.58 (1H, dd, J = 8.8, 2.0 Hz), 7.77 (1H, dd, J = 8.5, 2.0 Hz), 7.90 (1H, d, J = 2.0 Hz), 7.92−7.95 (2H, m), 8.30 (1H, br s). MS (FAB) *m*/*z* 571 $(M + H)^+$.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)carbonyl]piperazine Hydrochloride (42). Starting with **41** and following the procedure for the preparation of **39** gave **42** (94%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 3.02 (2H, br t, J = 5.3 Hz), 3.05 (2H, t, J = 6.4 Hz), 3.12 (2H, br s), 3.42−3.49 (2H, m), 3.52 (2H, br t, J = 5.3 Hz), 3.75 (2H, br t, J = 5.3 Hz), 4.33 (2H, br t, J = 5.3 Hz), 7.56 (1H, d, J = 8.3 Hz), 7.89 (1H, d, J = 8.3 Hz), 7.89 (1H, dd, J = 8.8, 1.5 Hz), 7.98 (1H, dd, J = 8.8, 2.0 Hz), 8.34 (1H, d, J = 8.8 Hz), 8.43 (1H, s), 8.44 (1H, d, J = 8.8 Hz), 8.67 (1H, br s), 9.87 (2H, br s). MS (FAB) *m*/*z* 471 $(M + H)^+$. Anal. ($C_{23}H_{23}ClN_4O_3S\cdot 1.9HCl\cdot 0.9H_2O$) C, H, Cl, N, S.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(6-methyl--5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)carbonyl]piperazine Hydrochloride (43). Starting with **42** and following the procedure for the preparation of **40** gave **43** (84%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 3.04 (3H, d, J = 3.9 Hz), 3.17 (2H, br s), 3.26 (2H, br s), 3.38−3.65 (2H, m), 3.68 (2H, br s), 3.39 (2H, br s), 4.40−4.70 (2H. m), 4.57 (2H, br s), 7.57 (1H, d, J = 7.8 Hz), 7.84−7.92 (2H, m), 7.98 (1H, d, J = 8.8 Hz), 8.33 (1H, d, J = 8.3 Hz), 8.42 (1H, s), 8.43 (1H, d, J = 8.8 Hz), 8.67 (1H, s), 11.86 (1H, br s). MS (FAB) *m*/*z* 485 $(M + H)^+$. Anal. ($C_{24}H_{25}ClN_4O_3S\cdot 1.8HCl\cdot 2.2H_2O$) C, H, Cl, N, S.

1-[[1,5-Bis(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl]carbonyl]-4-[(6-chloronaphthalen-2-yl)sulfonyl]piperazine (44). To the solution of **19** (44.0 mg, 126 μmol) in *t*-BuOH (2 mL) were added 2-methyl-2-butene (150 μL), $NaClO_4$ (102 mg, 1.13 mmol), and the solution of NaH_2PO_4 (135 mg, 1.13 mmol) in H_2O (6 mL). The reaction mixture was stirred for 21 h at room temperature. To the mixture were added Et_2O (10 mL) and sat. $(NH_4)_2SO_4$ (40 mL). The separated aqueous layer was extracted with Et_2O. The combined organic layer was dried over Na_2SO_4. Evaporation

of the solvent gave a crude form of 1,5-bis(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (**20**). The crude of **20** was condensed with **36** following the procedure for the preparation of **38**, to give **44** (two steps, 60%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.32 (9H, s), 1.46 (9H, s), 2.83 (2H, br t, J = 5.6 Hz), 3.04 (2H, br), 3.17 (2H, br), 3.55 (2H, br), 3.62 (2H, br t, J = 5.6 Hz), 3.82 (2H, br), 4.25 (2H, s), 5.94 (1H, s), 7.59 (1H, dd, J = 8.8, 2.0 Hz), 7.76 (1H, dd, J = 8.8, 2.0 Hz), 7.87−7.98 (3H, m), 8.30 (1H, br s). MS (FAB) m/z 681 $(M + Na)^+$.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (45). Starting with **44** and following the procedure for the preparation of **39** gave **45** (96%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.77 (2H, br t, J = 5.9 Hz), 3.03 (4H, t, J = 5.3 Hz), 3.30 (2H, br t, J = 5.9 Hz), 3.73 (4H, br t, J = 5.3 Hz), 3.99 (2H, br s), 6.32 (1H, d, J = 2.0 Hz), 7.73 (1H, dd, J = 8.8, 2.0 Hz), 7.83 (1H, dd, J = 8.8, 2.0 Hz), 8.17 (1H, d, J = 8.8 Hz), 8.25 (1H, d, J = 2.0 Hz), 8.28 (1H, d, J = 8.8 Hz), 8.50 (1H, br s), 9.07 (2H, br), 11.38 (1H, br). MS (FAB) m/z 459 $(M + H)^+$. Anal. ($C_{22}H_{23}ClN_4O_3S\cdot 1.1HCl\cdot 0.3H_2O$) C, H, Cl, N, S.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[5-methyl--(4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (46). Starting with **45** and following the procedure for the preparation of **40** gave **46** (64%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.72−2.86 (1H, m), 2.83 (3H, d, J = 4.9 Hz), 2.87−2.99 (1H, m), 3.03 (4H, br t, J = 4.4 Hz), 3.19−3.31 (1H, m), 3.46−3.64 (1H, m), 3.74 (4H, br t, J = 4.4 Hz), 3.97 (1H, dd, J = 14.2, 7.8 Hz), 4.20 (1H, br d, J = 14.2 Hz), 6.32 (1H, d, J = 2.4 Hz), 7.72 (1H, dd, J = 8.8, 2.4 Hz), 7.82 (1H, dd, J = 8.8, 2.0 Hz), 8.16 (1H, d, J = 8.8 Hz), 8.25 (1H, d, J = 2.0 Hz), 8.27 (1H, d, J = 8.8 Hz), 8.51 (1H, br s), 10.84 (1H, br s), 11.42 (1H, br s). MS (FAB) m/z 473 $(M + H)^+$. Anal. ($C_{23}H_{25}ClN_4O_3S\cdot 1.3HCl\cdot 0.7H_2O$) C, H, Cl, N, S.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(6-methyl-4,5,6,7-tetrahydrofuro[2,3-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (47). Starting with **25** and following the procedure for the preparation of **29** gave crude lithium 6-methyl-4,5,6,7-tetrahydrofuro[2,3-*c*]pyridine-2-carboxylate (**26**). The crude **26** was condensed with **36** following the procedure for the preparation of **38**, to give **47** (74.7 mg, two steps 37%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.68 (1H, br d, J = 15.1 Hz), 2.78−2.92 (1H, br), 2.85 (3H, s), 3.04 (4H, br s), 3.26 (1H, br s), 3.52 (1H, br s), 3.72 (4H, br s), 4.20 (1H, br d, J = 15.1 Hz), 4.43 (1H, br d, J = 15.1 Hz), 6.92 (1H, s), 7.71 (1H, dd, J = 8.8, 2.0 Hz), 7.80 (1H, d, J = 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.23 (1H, s), 8.25 (1H, d, J = 8.8 Hz), 8.48 (1H, s), 11.64 (1H, br s). MS (FAB) m/z 474 $(M + H)^+$. Anal. ($C_{23}H_{24}ClN_3O_4S\cdot 1.1HCl\cdot 1.7H_2O$) C, H, Cl, N, S.

1-[[5-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl]carbonyl]-4-[(6-chloronaphthalen-2-yl)sulfonyl]piperazine (48). To a mixture of 5-*tert*-butoxycarbonyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine-2-carboxylic acid (140 mg, 0.494 mmol), **36** (188 mg, 0.494 mmol as a HCl salt), 1-hydroxybenzotriazole hydrate (114 mg, 0.735 mmol), and *N*-methylmorpholine (54 μL, 0.49 mmol) in CH_2Cl_2 (50 mL) was added 1-(dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (142 mg, 0.741 mmol). The reaction mixture was stirred for overnight at room temperature and then poured into H_2O (50 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue using column chromatography (hexane/AcOEt, 3/1) gave **37** (265 mg, 94%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 2.79 (2H, br s), 3.12 (4H, t, J = 4.9 Hz), 3.68 (2H, br s), 3.84 (4H, t, J = 4.9 Hz), 4.42 (2H, s), 6.91 (1H, s), 7.59 (1H, dd, J = 8.8, 2.0 Hz), 7.75 (1H, dd, J = 8.8, 2.0 Hz), 7.90−7.97 (3H, m), 8.30 (1H, s). MS (FD) m/z 575 M^+.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (49). To the solution of **48** (240 mg, 0.416 mL) in CH_2Cl_2 (5 mL) was added EtOH solution saturated with HCl (10 mL). Evaporation of the solvent gave **49** (149 mg, 66%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.99−3.05 (2H, m), 3.08 (4H, t, J = 4.6 Hz), 3.35−3.40 (2H, m), 3.71 (4H, t, J = 4.6 Hz), 4.11 (2H. s), 7.17 (1H, s), 7.71 (1H, dd, J = 8.8, 2.0 Hz), 7.82 (1H, dd, J = 8.8, 2.0 Hz), 8.22−8.28(3H, m), 8.50 (1H, s), 9.38 (2H, br s). MS (FAB) m/z 476 $[(M + H)^+$. Anal. ($C_{22}H_{22}ClN_3O_3S_2\cdot HCl\cdot 1.5H_2O$) C, H, Cl, N, S.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(5-methyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (50). To a stirred suspension of **49** (200 mg, 0.390 mmol) in CH_2Cl_2 (20 mL) was added Et_3N (108 μL, 0.780 mmol). After the mixture was stirred for 15 min at room temperature, AcOH (88 μL, 0.78 mmol), 35% aq HCHO (90 μL, 0.50 mmol), and sodium triacetoxy borohydride (132 mg, 0.624 mmol) were added. The mixture was stirred for 1 h at room temperature under an argon atmosphere and poured into H_2O (20 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue using column chromatography (CH_2Cl_2/MeOH, 50/1) gave a colorless foam. To this material was added 1 N HCl/EtOH (10 mL). The solution was concentrated and dried in vacuo. Codistillation of EtOH with H_2O gave **50** (148 mg, 70%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.87 (3H, s), 3.08 (4H, s), 3.12−3.38 (2H, m), 3.59−3.67 (1H, m), 3.71 (4H, s), 3.90−4.12 (2H, m), 4.33 (1H, d, J = 15.1 Hz), 7.19 (1H, s), 7.73 (1H, d, J = 8.8 Hz), 7.82 (1H, d, J = 8.8 Hz), 8.18 (1H, d, J = 8.8 Hz), 8.24−8.32 (2H, m), 8.51 (1H, s), 11.36 (1H, br s). MS (FAB) m/z 490 $(M + H)^+$. Anal. ($C_{23}H_{24}ClN_3O_3S_2\cdot 1.2HCl\cdot 0.5H_2O$) C, H, Cl, N, S.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(5-methyl-4,5,−6,7-tetrahydrothiazolo[4,5-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (51). Starting with **29** and **36** and following the procedure for the preparation of **48** gave **51** (74%) as a colorless solid: mp 195−200 °C. ^{1}H NMR (DMSO-d_6) δ 2.92 (3H, s), 3.04−3.28 (6H, m), 3.35−3.90 (4H, m), 4.12−4.70 (4H, m), 7.69 (1H, dd, J = 8.8, 2.0 Hz), 7.82 (1H, dd, J = 8.8, 2.0 Hz), 8.14 (1H, d, J = 8.8 Hz), 8.21 (1H, s), 8.25 (1H, dd, J = 8.8, 2.0 Hz), 8.50 (1H, s), 11.27 (1H, br s). MS (FAB) m/z 491 $(M + H)^+$. Anal. ($C_{22}H_{23}ClN_4O_3S_2\cdot HCl\cdot 1.5H_2O$) C, H, Cl, N, S.

1-[(6-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)carbonyl]-4-[(6-chloronaphthalen-2-yl)sulfonyl]piperazine (52). Starting with 6-*tert*-butoxycarbonyl-4,5,6,7- tetrahydrothieno[2,3-*c*]pyridine-2-carboxylic acid and **36** and following the procedure for the preparation of **48** gave **52** (12%) as a colorless amorphous foam: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 2.64 (2H, br s), 3.12 (4H, t, J = 4.9 Hz), 3.64 (2H, br s), 3.84 (4H, t, J = 4.9 Hz), 4.57 (2H, br s), 6.92 (1H, s), 7.59 (1H, dd, J = 8.8, 2.0 Hz), 7.75 (1H, dd, J = 8.8, 2.0 Hz), 7.90−7.96 (3H, m), 8.30 (1H, s). MS (FAB) m/z 576 $(M + H)^+$.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (53). Starting with **52** and following the procedure for the preparation of **49** gave **53** (99%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.83 (2H, t, J = 5.4 Hz), 3.08 (4H, br s), 3.36 (2H, br s), 3.71 (4H, br s), 4.32 (2H, br s), 7.18 (1H, s), 7.71 (1H, dd, J = 8.8, 2.0 Hz), 7.81 (1H, d, J = 8.8 Hz), 8.16 (1H, d, J = 8.8 Hz), 8.21−8.28 (2H, m), 8.49 (1H, s), 9.42 (2H, br s). MS (FAB) m/z 476 $(M + H)^+$. Anal. ($C_{22}H_{22}ClN_3O_3S_2\cdot HCl$) C, H, Cl, N, S.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[(6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)carbonyl]piperazine Hydrochloride (54). Starting with **53** and following the procedure for the preparation of **50** gave **54** (71%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.88 (3H, s), 2.90−3.05 (1H, m), 3.08 (4H, s), 3.22−3.35 (1H, m), 3.55−3.66 (1H, m), 3.72 (4H, s), 3.75−3.95 (1H, m), 4.22−4.35 (1H, m), 4.50−4.65 (1H, m), 7.20 (1H, s), 7.71 (1H, d, J = 8.8 Hz), 7.81 (1H, d, J = 8.8 Hz), 8.17 (1H, d, J = 8.8 Hz), 8.22−8.30 (2H, m), 8.50 (1H, s), 11.04 (1H, br s). MS (FAB) m/z 490 $(M + H)^+$. Anal. ($C_{23}H_{24}ClN_3O_3S_2\cdot HCl\cdot H_2O$) C, H, Cl, N, S.

1-[[5-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrothiazolo-[5,4-*c*]pyridin-2-yl]carbonyl]-4-[(6-chloronaphthalen-2-yl)sulfonyl]piperazine (55). Starting with **33** and **36**, fol-

lowing the procedure for the preparation of **48** gave **55** (55%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 2.84 (2H, br s), 3.19 (4H, br s) 3.72 (2H, t, J = 5.4 Hz), 3.87 (2H, br s), 4.54 (2H, s), 4.63 (2H, br s), 7.57 (1H, dd, J = 8.8, 2.0 Hz), 7.76 (1H, dd, J = 8.8 Hz, 2.0 Hz), 7.87−7.94 (3H, m), 8.30 (1H, s). MS (FAB) m/z 577 $(M + H)^+$

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[[4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl]carbonyl]piperazine Hydrochloride (56). Starting with **55** and following the procedure for the preparation of **49** gave **56** (90%) as a colorless solid: mp 267−270 °C. ^{1}H NMR (DMSO-d_6) δ 3.01 (2H, t, J = 5.9 Hz), 3.11 (4H, br s) 3.44 (2H, br s), 3.74 (2H, br s), 4.32−4.46 (4H, m), 7.71 (1H, dd, J = 8.8, 2.0 Hz), 7.83 (1H, dd, J = 8.8 Hz, 2.0 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.23 (1H, s), 8.26 (1H, d, J = 8.8 Hz), 8.30 (1H, s). MS (FAB) m/z 477 $(M + H)^+$. Anal. ($C_{21}H_{21}ClN_4O_3S_2$·HCl·0.2H_2O) C, H, Cl, N, S.

1-[(6-Chloronaphthalen-2-yl)sulfonyl]-4-[[5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl]carbonyl]-piperazine Hydrochloride (57). Starting with **56** and following the procedure for the preparation of **50** gave **57** (90%) as a colorless solid: mp 157−159 °C. ^{1}H NMR (DMSO-d_6) δ 2.89 (3H, s), 3.10 (6H, br s), 3.32−3.81 (4H, m), 4.30−4.81 (4H, m), 7.71 (1H, dd, J = 8.8, 2.0 Hz), 7.82 (1H, dd, J = 8.8, 2.0 Hz), 8.15(1H, d, J = 8.8 Hz), 8.20−8.28 (2H, m), 8.50 (1H, s), 11.28 (1H, br s). MS (FAB) m/z 491 $(M + H)^+$ Anal. ($C_{22}H_{23}ClN_4O_3S_2$·HCl·0.6H_2O) C, H, Cl, N, S.

1-[[5-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrothiazolo-[5,4-*c*]pyridin-2-yl]carbonyl]-4-[(6-chloronaphthalen-2-yl)sulfonyl]-2-ethoxycarbonylpiperazine (58). To a solution of 4-[(6-chloronaphthalen-2-yl)sulfonyl]-2-ethoxycarbonylpiperazine (**37**) (1.00 g, 2.61 mmol) in DMF (30 mL) were added **33** (756 mg, 2.61 mmol), 1-benzotriazolyloxy-tris-(pyrrolidino)-phosphonium hexafluorophosphite (1.50 g, 2.87 mmol), and Et_3N (398 μL, 2.87 mmol) at room temperature. The reaction mixture was stirred for overnight and concentrated. The residue was dissolved in AcOEt and washed with H_2O. The separated organic layer was washed with sat. NaCl, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (hexane/AcOEt, 4/1) gave **58** (505 mg, 30%) as a pale yellow amorphous foam: ^{1}H NMR ($CDCl_3$) δ 1.24−1.37 (3H, m), 1.47 (9H, s), 2.45−2.60 (1H, m), 2.62−2.71 (1H, m), 2.75−2.90 (2H, m), 3.65−3.94 (3H, m), 4.19−4.31 (2H, m), 4.45−4.72 (4H, m), 5.35 (0.5H, br s), 5.71−5.77 (0.5H, m), 6.72 (1H, br s), 7.58 (1H, dd, J = 8.8 Hz, 2.0 Hz), 7.77 (1H, dd, J = 8.8 Hz, 2.0 Hz), 7.88−7.92 (3H, m), 8.33 (1H, s). MS (FAB) m/z 649 $(M + H)^+$.

1-[[5-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrothiazolo-[5,4-*c*]pyridin-2-yl]carbonyl]-2-carbamoyl-4-[(6-chloronaphthalen-2-yl)sulfonyl]piperazine (59). To a solution of **58** (487 mg, 0.750 mmol) in THF (5 mL) were added MeOH (5 mL) and 1 N NaOH (3 mL). The reaction mixture was stirred for 4 h at room temperature. To the mixture was added 1 N HCl until the pH was 1−2. The mixture was diluted with in AcOEt and washed with H_2O. The separated aqueous layer was extracted with AcOEt. The combined organic layers were washed with sat. NaCl, dried over Na_2SO_4, and concentrated in vacuo. To the solution of the resultant residue in THF (5 mL) were added dropwise *N*-methylmorpholine (91.0 μL, 0.825 mmol) and isobutyl chloroformate (108 μL, 0.825 mmol) at −20 °C in order. The reaction mixture was stirred for 10 min at −20 °C. To the mixture was added the solution of NH_3 in CH_2Cl_2 (500 μL) which was prepared by partitioning between aq NH_3 and CH_2Cl_2. The reaction mixture was stirred for 10 min at −20 °C. To the mixture was added the solution of 1 N HCl/EtOH (10 mL). The reaction was warmed to room temperature and concentrated in vacuo. The solution of the residue in CH_2Cl_2 was washed with 1 N HCl, sat. $NaHCO_3$ and sat. NaCl in order. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 100/1) gave **59** (317 mg, 68%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.41 (9H, s), 2.39−2.86 (4H, m), 3.60−3.80 (4H, m), 4.25−4.34 (1H, m), 4.36−4.34 (0.5H, m), 4.62 (2H, br s), 4.97 (0.5H, br s), 5.44−5.52 (0.5H, m), 6.19 (0.5H, br s), 7.30−7.39 (1H, m), 7.63−7.85(3H, m), 8.15 (1H, d, J = 8.8 Hz), 8.20−8.29 (2H, m), 8.48 (1H, s). MS (FAB) m/z 620 $(M + H)^+$.

2-Carbamoyl-4-[(6-chloronaphthalen-2-yl)sulfonyl]-1-[[4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl]carbonyl]-piperazine Trifluoroacetate (60). To a solution of **59** (303 mg, 0.489 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (1 mL). The reaction mixture was stirred for 3 min. Evaporation of the mixture gave **60** (263 mg, 83%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.39−2.70 (2H, m), 2.92−3.06 (2H, m), 3.42−3.77 (4H, m), 4.25−4.50 (3.5H, m), 4.97 (0.5H, br s), 5.35−5.44 (0.5H, m), 6.14 (0.5H, br s), 7.30−7.39 (1H, m), 7.66−7.73 (2H, m), 7.77−7.82 (1H, m), 8.16 (1H, d, J = 8.8 Hz), 8.21−8.28 (2H, m), 8.49 (1H, s), 9.26 (2H, br s). MS (FAB) m/z 520 $(M + H)^+$. Anal. ($C_{22}H_{22}ClN_5O_4S_2$·TFA·0.6H_2O): C, H, Cl, F, N, S.

2-Carbamoyl-4-[(6-chloronaphthalen-2-yl)sulfonyl]-1-[[5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl]-carbonyl]piperazine Hydrochloride (61). Starting with **60** and following the procedure for the preparation of **50** gave **61** (52%) as a colorless amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.37−2.70 (2H, m), 2.91 (3H, s), 3.00−3.78 (6H, m), 4.28−4.77 (3.5H, m), 4.97 (0.5H, br s), 5.40−5.50 (0.5H, m), 6.14 (0.5H, br s), 7.32−7.40 (1H, m), 7.68−7.75 (2H, m), 7.77−7.83 (1H, m), 8.15 (1H, d, J = 8.8 Hz), 8.21−8.28 (2H, m), 8.49 (1H, s). MS (FAB) m/z 534 $(M + H)^+$. Anal. ($C_{23}H_{24}ClN_5O_4S_2$·HCl·2.5H_2O) C, H, Cl, N, S.

2-Carbamoyl-4-[(6-chloronaphthalen-2-yl)sulfonyl]-1-[[5-ethyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl]-carbonyl]piperazine Hydrochloride (62). To a stirred solution of **60** (570 mg, 0.900 mmol) and Et_3N (249 μL, 1.80 mmol) in DMF (10 mL) was added EtI (142 μL, 1.80 mmol). The mixture was stirred overnight at room temperature and concentrated in vacuo. To the residue were added CH_2Cl_2 and H_2O. The organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 10/1) gave a pale yellow amorphous solid. To this material was added 1 N HCl (2 mL). The solution was concentrated and dried in vacuo to give **62** (231 mg, 41%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 1.00−1.13 (3H, m), 2.35−2.95 (4H, m), 2.95−3.90 (6H, m), 4.25−4.43 (2.5H, m), 4.65−4.78 (1H, m), 4.97 (0.5H, m), 5.39−5.47 (0.5H, m), 6.08−6.17 (0.5H, m), 7.29−7.36 (1H, m), 7.76−7.73 (2H, m), 7.89 (1H, d, J = 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.23 (1H, s), 8.25 (1H, d, J = 8.8 Hz), 8.48 (1H, s). MS (FAB): m/z 548 $(M + H)^+$. Anal. ($C_{24}H_{26}ClN_5O_4S_2$·1.1HCl·2.0H_2O) C, H, Cl, N, S.

2-Carbamoyl-4-[(6-chloronaphthalen-2-yl)sulfonyl]-1-[[5-isopropyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]-pyridin-2-yl]carbonyl]piperazine Hydrochloride (63). To a stirred suspension of **60** (230 mg, 0.363 mmol) in CH_2Cl_2 (20 mL) was added Et_3N (79 μL, 0.57 mmol). After the mixture was stirred for 15 min at room temperature, AcOH (41 μL, 0.72 mmol), acetone (320 μL, 4.32 mmol), and sodium triacetoxy borohydride (918 mg, 4.33 mmol) were added. The mixture was stirred for 1 h at room temperature and poured into H_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (CH_2Cl_2/MeOH, 25/1) gave a pale yellow foam. 1 N HCl (2 mL) was added to this material. The solution was concentrated and dried in vacuo to give **63** (148 mg, 65%) as a pale brown foam: ^{1}H NMR (DMSO-d_6) δ 1.26−1.41 (6H, m), 2.38−2.78 (2H, m), 2.95−3.80 (7H, m), 4.24−4.52 (2.5H, m), 4.55−4.70 (1H, m), 4.97 (0.5H, br s), 5.43 (0.5H, d, J = 14.2 Hz), 6.10 (0.25H, br s), 6.18 (0.25H, br s), 7.28−7.38 (1H, m), 7.65−7.73 (2H, m), 7.58 (1H, d, J = 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz), 8.23 (1H, d, J = 2.0 Hz), 8.26 (1H, d, J = 8.8 Hz), 8.48 (1H, s), 10.95−11.35 (1H, m). MS (FAB) m/z 562 $(M + H)^+$. Anal. ($C_{25}H_{28}ClN_5O_4S_2$·1.4HCl·0.5H_2O) C, H, Cl, N, S.

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