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Design, synthesis, and biological activity of novel factor Xa inhibitors: Improving metabolic stability by S1 and S4 ligand modification

Satoshi Komoriya,* Shozo Kobayashi, Ken Osanai, Toshiharu Yoshino, Tsutomu Nagata, Noriyasu Haginoya, Yumi Nakamoto, Akiyoshi Mochizuki, Takayasu Nagahara, Makoto Suzuki, Takashi Shimada, Kengo Watanabe, Yumiko Isobe and Taketoshi Furugoori

Tokyo R&D Center, Daiichi Pharmaceutical Co. Ltd, 16-13, Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134-8630, Japan

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Abstract—Serine protease factor Xa (fXa) inhibitor **1** showed good ex vivo anti-fXa activity upon oral administration in rats. However, it has been revealed that **1** had low metabolic stability against human liver microsomes. To improve the metabolic stability, we attempted to modify the S1 and S4 ligands of **1**. These modifications resulted in compound **34b**, which exhibited selective anti-fXa activity and excellent anti-coagulation activity.

1. Introduction

Serine protease factor Xa (fXa) is a key enzyme in the cascade-like activation of the coagulation system. It holds a central position joining the extrinsic and intrinsic activation pathways[1] and converts prothrombin to thrombin (fIIa). Although both fXa and thrombin play crucial roles in the coagulation cascade, compared with fXa, thrombin acts as multifunctional and its platelet activation is important for stopping hemorrhage. From this viewpoint, fXa inhibitors are expected to display less bleeding risk than do thrombin inhibitors.[2]

In previous reports,[3] we have found that the fused ring fXa inhibitor **1** showed good ex vivo anti-fXa activity at oral administration in rats. In vitro human liver microsomal metabolic stability of compound **1** has been measured by a reported method,[4] and we calculated intrinsic clearance (CL_{int}) of this compound from the values[5] (Table 1). Consequently, it has become apparent that **1** had low metabolic stability against human liver microsomes. Similar to **1**, tetrahydrothiazoropyridine derivative **2**,[3] which had the structure with the carbamoyl moiety removed from **1**, showed low metabolic stability. On the other hand, biaryl derivative **3**,[6] which had the structure of tetrahydrothiazolopyridine transformed to pyridinyl-benzene, showed high stability. This finding suggested a possibility of improving metabolic stability by converting the tetrahydrothiazolopyridinyl part.

fXa has two affinity sites for compound **2**, which are the so-called S1 binding site and aryl binding site (S4 binding site). We have previously reported that the 5-chloroindole moiety was a better structure for the S1 site than the 6-chloronaphthalene moiety.[6] An optimization study was carried out for **4**, whereby the structure of the 6-chloronaphthyl moiety of **2** was transformed to 5-chloroindolyl, as a lead compound (Fig. 1).

Life-threatening consequences often result from thrombosis, such as myocardial infarction, pulmonary embolism, deep vein thrombosis, stroke, and so on. Disseminated intravascular coagulation (DIC), termed by McKay,[7] is one of these disorders, and a DIC animal model is used to confirm the efficacy of anti-coagulants. DIC was originally a clinical designation for an abnormality of clotting and the fibrinolysis system resulting

Keywords: Factor Xa; Anti-coagulant; Orally active compound; Non-basic compound; Metabolic stability.
* Corresponding author. Tel.: +81 3 3680 0151; fax: +81 3 5696 8772; e-mail: komor0ni@daiichipharm.co.jp

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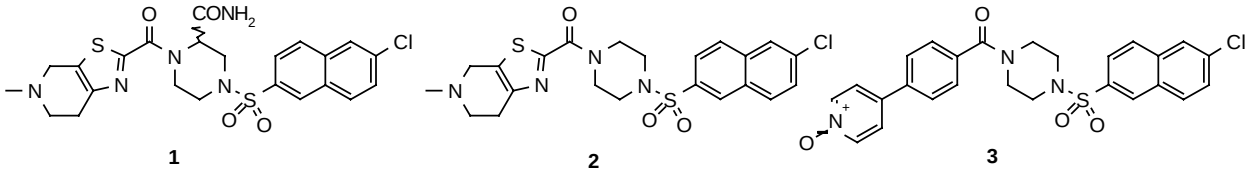
Table 1. In vitro data for compounds **1**–**3**



Compound	fXa (IC_{50}, nM)	fIIa (IC_{50}: μM)	PTCT2[a] in human plasma (μM)	PTCT2 in rat plasma (μM)	CL_{int}[b] ml/min/kg
1	24	1.0	4.4	14	536
2	22	0.56	6	10	750
3	17	0.14	5.3	7.8	19

[a] Clotting time doubling concentration for prothrombin time.
[b] This value calculated from the remaining ratio at 30 min human liver microsomal metabolic stability.[5]

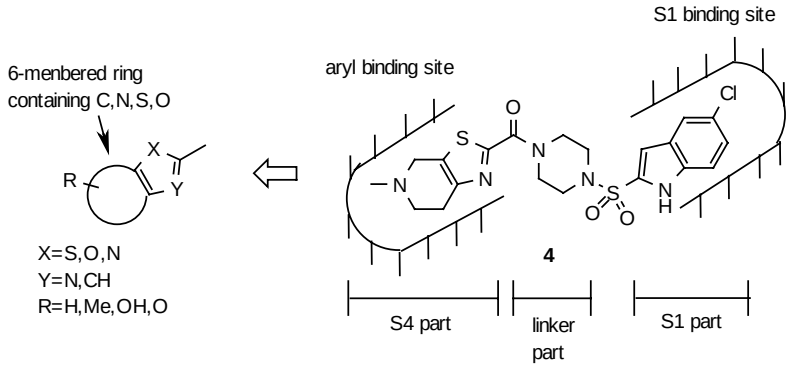


Figure 1. Design of the S4 part.

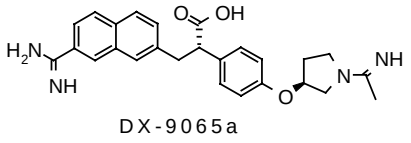


Figure 2. The structure of DX-9065a.

in multiple organ failure (MOF)[8] and histologically only characterized by fibrin microthrombi in the blood vessels of various organs.[9] The principal aims of treating DIC are to improve the causative disease, to prevent the formation and development of fibrin thrombi caused by stimulated coagulation, and to dissolve any thrombi formed.[10] A factor Xa inhibitor, DX-9065a,[11] showed a protective effect in a rat DIC model. We evaluated an optimized compound for its anti-thrombotic activity in a rat DIC model[12] (Fig. 2).

2. Chemistry

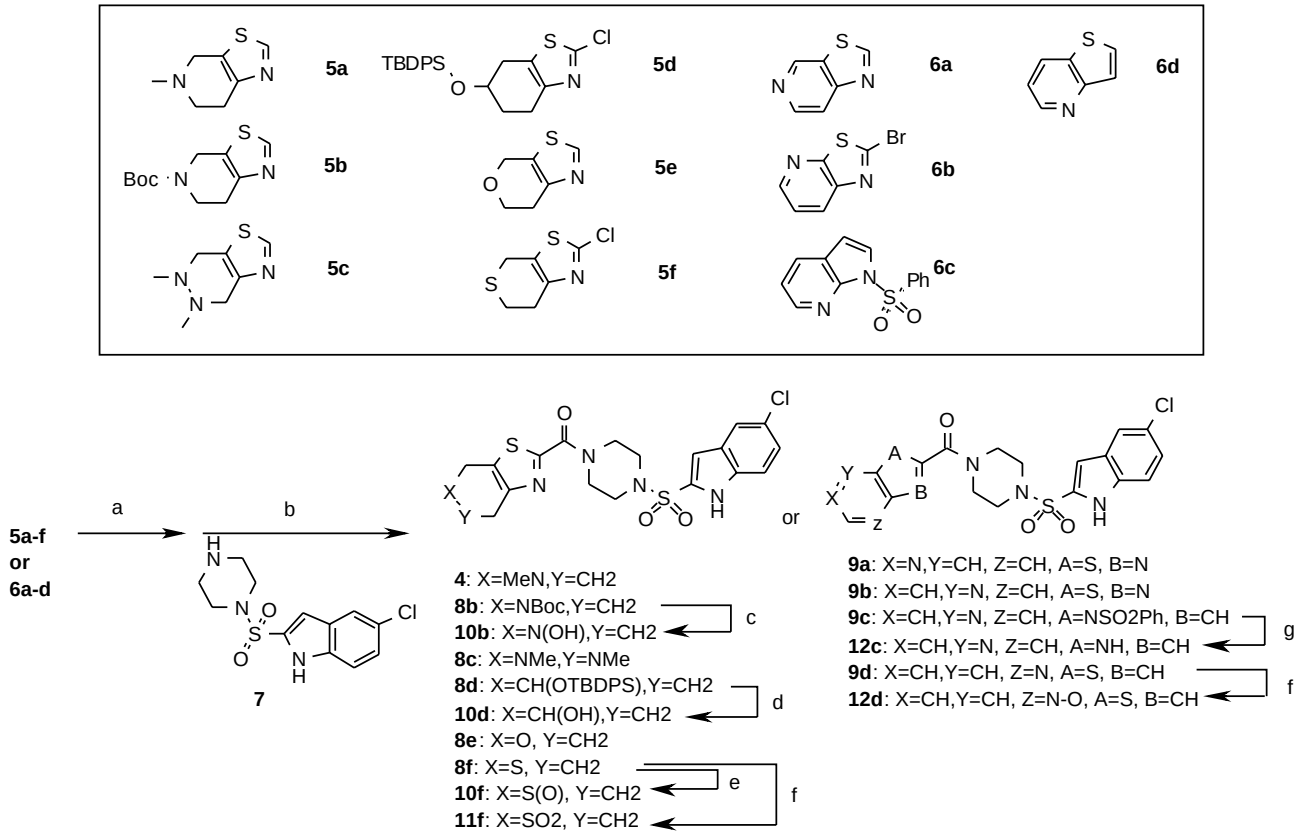
Preparation of compounds containing thiazole, pyrrole, or thiophene in the S4 part is shown in Scheme 1. Carboxylation reaction of compounds **5a**–**f**[3,13,14] and **6a**–**d**[15] using CO_2 gas, followed by condensation with piperazine **7**,[6] gave amides **4**, **8b**–**f**, and **9a**–**d**. Hydroxylamine **10b** was synthesized via successive deprotection of the Boc group, peroxybenzoylation, and debenzoylation of **8b**. Alcohol **10d** was synthesized via desilylation of **8d**. Oxidation of **8f** with sodium periodate or *m*-chloroperbenzoic acid (*m*-CPBA) gave **10f** or **11f**, respectively. Dephenylsulfonylation of **9c** gave **12c**. *N*-Oxide **12d** was synthesized via *m*-CPBA oxidation of **9d.**

Preparation of compounds containing oxazole in the S4 part is shown in Scheme 2. We chose the ester derivatives **5g**,**h** and **6e** as starting materials. Hydrolysis of compounds **5g**,**h** or **6e**,[16] followed by condensation with piperazine **7**, gave **8g**,**h** or **9e**. *N*-Methyl derivative **10g** was synthesized via successive de-*tert*-butoxycarbonylation and N-methylation of **8g**.

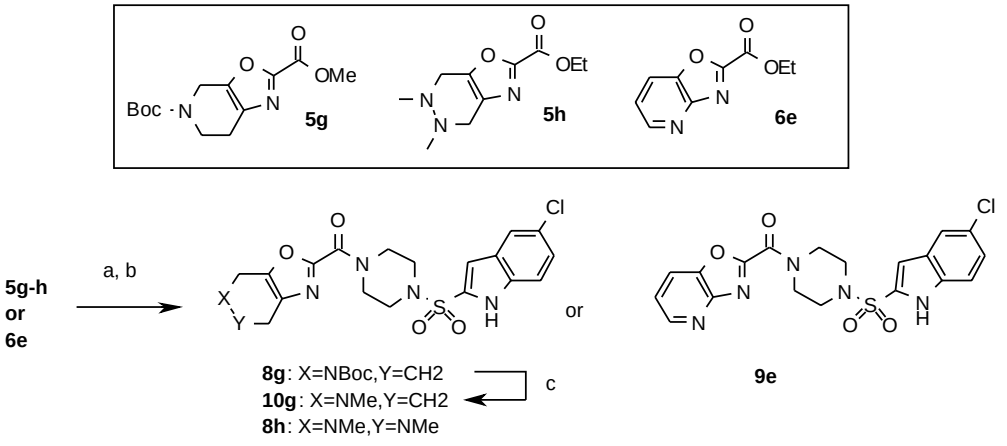
Preparation of compounds containing thiazolo[4,5-*b*]pyridine in the S4 part is shown in Scheme 3. $KMnO_4$ oxidation of **6f**,[17] followed by condensation with piperazine **7**, gave **9f**.

Preparation of **5c**,**d**,**f** and **6b**,**c** is shown in Scheme 4. Dibromination of commercially available **13**, followed by cyclization with dimethylhydrazine, gave **5c**. Reduction and silylation of **15**[18] gave **5d**. Application of the Gewald aminothiazole synthesis[18] to 4-oxothiane (**16**) resulted in the preparation of dihydrothiopyran **17**. The substitutive deamination reaction of **17** gave **5f**. Compound **6b** was also synthesized from **18**[19] by the substitutive deamination reaction. Sulfonylation of azaindole (**19**) with phenylsulfonyl chloride gave **6c**.

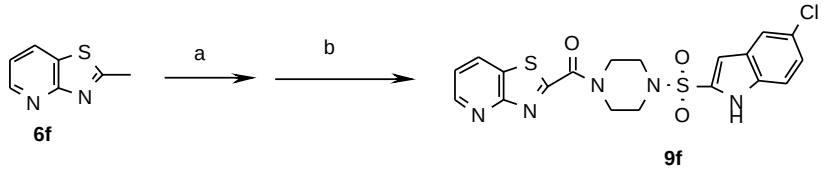
Preparation of **5g**,**h** is shown in Scheme 5. The Wittig reaction of **20**,[20] followed by hydroboration, the



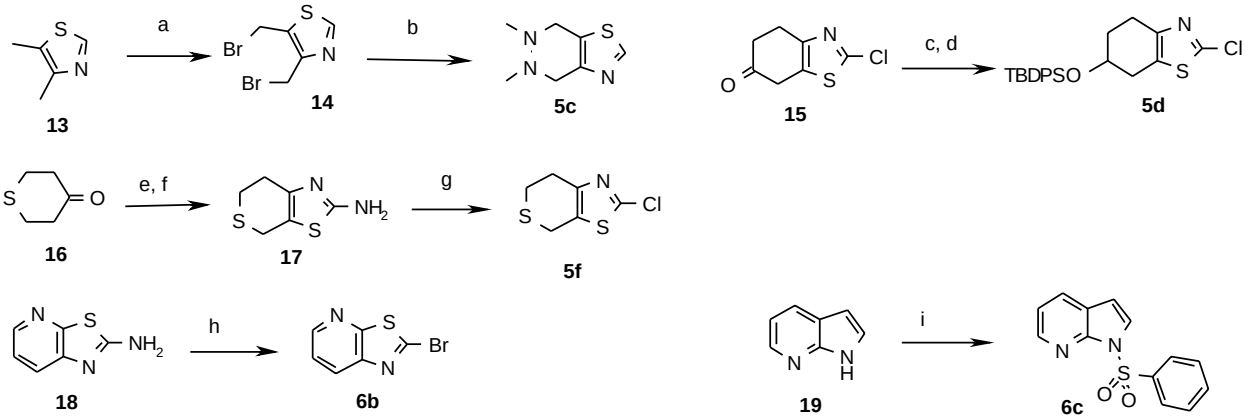
Scheme 1. Reagents and conditions: (a) CO_2 (gas), *n*-BuLi, Et_2O; (b) **7**, WSCI, HOBt, NMM, DMF, 17–69%; (c) i—satd HCl–EtOH, CH_2Cl_2; ii—diphenylperoxyanhydride, CH_2Cl_2, reflux; iii—1 N NaOH aq, MeOH, THF, 22% (three steps); (d) TBAF, THF, 14%; (e) $NaIO_4$, THF, MeOH, 58%; (f) *m*-CPBA, MeOH or EtOH, CH_2Cl_2, 62% (**11f**), 85% (**12d**); (g) 1 N NaOH aq, dioxane, reflux, 52%.



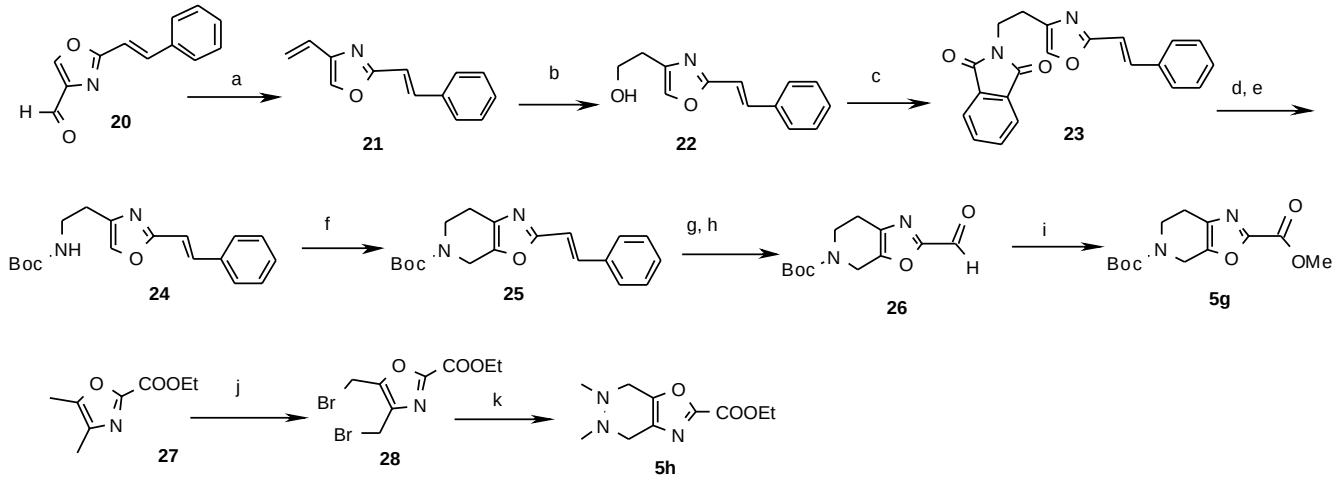
Scheme 2. Reagents: (a) LiOH, H_2O; (b) **7**, WSCI, HOBt, NMM, DMF, 11–62%; (c) i—TFA, CH_2Cl_2; ii—HCHO, $NaBH(OAc)_3$, 98%.



Scheme 3. Reagents: (a) $KMnO_4$, H_2O; (b) **7**, WSCI, HOBt, NMM, DMF, 14% (two steps).



Scheme 4. Reagents: (a) NBS, AIBN, DCE, reflux, 44%; (b) MeNHNHMe, Et_3N, EtOH, 24%; (c) $NaBH_4$, MeOH; (d) TBDPSCl, imidazole, THF, 98% (two steps); (e) pyrrolidine, TsOH, *c*-hexane; (f) S_8, MeOH then H_2N-CN, 44% (two steps); (g) $CuCl_2$, *t*-BuONO, CH_3CN, 15%; (h) $CuBr_2$, *t*-BuONO, CH_3CN, 18%; (i) $PhSO_2Cl$, NaOH, $BnEt_3NCl$, CH_2Cl_2, 53%.



Scheme 5. Reagents and conditions: (a) $CH_3P^+Ph_3Br^-$, *n*-BuLi, THF, 79%; (b) 9-BBN, THF, then H_2O_2, NaOH aq, 99%; (c) phthalimide, PPh_3, DEAD, THF, 95%; (d) H_2NNH_2, EtOH, reflux; (e) Boc_2O, $NaHCO_3$ aq, CH_2Cl_2, 87% (two steps); (f) paraformaldehyde, *p*-TsOH, toluene, reflux, 78%; (g) OsO_4, NMO, THF, acetone, H_2O; (h) $NaIO_4$, THF, MeOH, H_2O, 53% (two steps); (i) MnO_2, NaCN, MeOH, 48%; (j) NBS, AIBN, DCE, reflux, 36%; (k) MeNHNHMe, Et_3N, EtOH, 33%.

Mitsunobu reaction with phthalimide gave **23**. Cleavage of phthalimide with hydrazine, followed by *tert*-butoxycarbonylation and the Pictet–Spengler reaction gave tetrahydrooxazolopyridine **25**. Diol formation from **25** with osmium tetraoxide, followed by $NaIO_4$ oxidation and MnO_2 oxidation, gave **5g**.

Formation of the required oxazolopyridazine skeleton was achieved starting from **27**[21] by the same procedure as for **5c**.

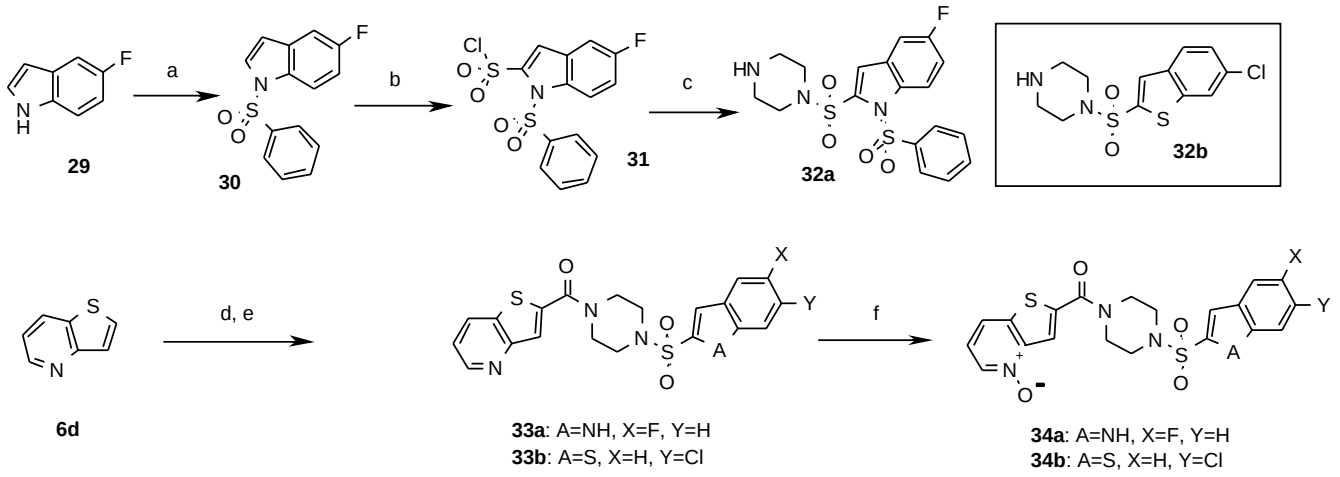
Preparation of **34a**,**b** is shown in Scheme 6. Phenylsulfonylation of 5-fluoroindole (**29**), followed by introducing sulfonyl chloride at the 2-position, condensation of 1-*tert*-butoxycarbonylpiperazine, and cleavage of Boc protection, gave **32a**. Carboxylation of **6d**, followed by condensation with **32a**,**b**,[6] and oxidation using *m*-CPBA, gave **34a**,**b**. In this condensation reaction condition, the phenylsulfonyl moiety was deprotected (**6d** + **32a** → **33a**).

Preparation of **38a**–**d** is shown in Scheme 7. Debenzylation of **35**,[22] followed by monosulfonylation at the 4-position, gave **36**. Carboxylation of **6d**, followed by condensation with **36**, hydrolysis, amide formation with various amines, and oxidation using *m*-CPBA, gave **38a**–**d**.

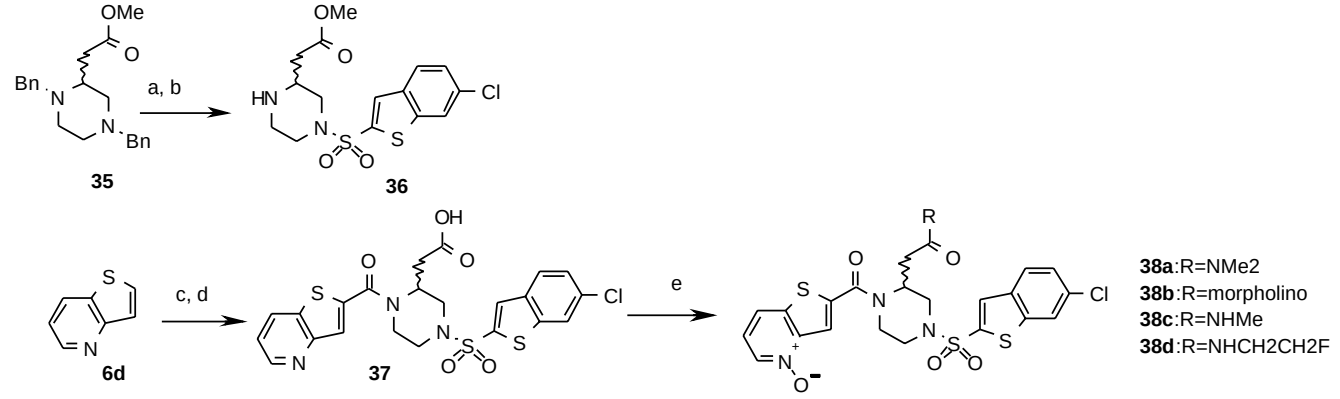
3. Result and discussion

We predicted that the sulfur atom of thiazole and the carbon atom adjacent to nitrogen of tetrahydropyridine are the most likely candidates to be metabolized. Enzyme inhibitory activities, anti-coagulant activities, and metabolic stabilities of synthesized compounds are shown in Tables 2 and 3.

Predictably, the indole derivative **4** improved in vitro anti-fXa activity, but it did not improve human metabolic stability. Transformation of thiazole to oxazole



Scheme 6. Reagents: (a) *n*-BuLi, $PhSO_2Cl$, THF, 99%; (b) i—*t*-BuLi, SO_2 (gas), THF, Et_2O; ii—NCS, CH_2Cl_2, quant.; (c) i—1-*tert*-butoxycarbonylpiperazine, Et_3N, CH_2Cl_2; ii—HCl–EtOH, 39% (two steps); (d) CO_2 (gas), *n*-BuLi, Et_2O; (e) **32a** or **b**, WSCI, HOBt, NMM, DMF, 59% (**33a**), 67% (**33b**); (f) *m*-CPBA, CH_2Cl_2, 64% (**34a**), 65% (**34b**).



Scheme 7. Reagents: (a) H_2, $Pd(OH)_2$, concd HCl, EtOH, 99%; (b) 5-chlorobenzo[*b*]thienyl-2-sulfonyl chloride, Et_3N, CH_2Cl_2, 30%; (c) i—CO_2 (gas), *n*-BuLi, Et_2O; ii—**36**, WSCI, HOBt, DMF, 89%; (d) LiOH, H_2O, THF, 88%; (e) i—amine, WSCI, HOBt, DIPEA, DMF; ii—*m*-CPBA, CH_2Cl_2, 29% (**38a**), 68% (**38b**), 57% (**38c**), 33% (**38d**).

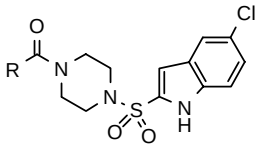
decreased the in vitro anti-fXa activity. On the other hand, various non-aromatized transformations of the tetrahydropyridine part maintained anti-fXa activity, except for **8e**. Thiazolopyridine **9a**, an aromatized analogue of **4**, exhibited 50-fold less anti-fXa activity than that of **4**. However, **9d** and **f**, which include a nitrogen atom at the 4 position, showed only 3-fold less anti-fXa activity. Moreover, **12d**, an *N*-oxide derivative of **9d**, exhibited the best anti-coagulant activity in an array of these compounds (Table 2). We have measured the in vitro human liver microsomal metabolic stability of several indole derivatives. Conversion of a sulfur atom into an oxygen atom improved human metabolic stability (**4** vs **10g**). However, tetrahydrooxazolopyridine **10g** exhibited less satisfactory anti-fXa activity. The other potent compounds also showed a tendency to improve the metabolic stability compared with the parent compound **4**. Especially, thienopyridine *N*-oxide **12d** showed a much higher metabolic stability than **4**.

To determine the binding mode of **12d**, we carried out an X-ray study of **12d** and fXa. The statistics of data processing and crystallographic refinement for the resulting crystal are shown in Table 4.

As a result of the X-ray crystallographic analysis, the chloroindole part located in the S1 subsite with hydrogen bond formation between the nitrogen of chloroindole and the amide carbonyl oxygen of Gly218 on the main chain and a thienopyridine part located in the S4 subsite (Figs. 3 and 4). We have already reported[3] that the tetrahydrothiazolopyridine part of **1** was arranged parallel to the indole ring of Trp215 (panel B). However, the thienopyridine part of **12d** was arranged vertically to the indole ring of Trp215 (panel A).

We have observed an intramolecular S–O close contact (thiazolopyridine-S and carbonyl-O) in the crystal complex of **1** and fXa.[3] In contrast, **12d** has existed without such an intramolecular S–O close contact. The coplanar location of the thienopyridine of **12d** and piperazine amide carbonyl is a disadvantage for stabilizing the conformation, because of repulsion between the thienopyridine 3-H of **12d** and piperazine 3 or 5-H. Ab initio

Table 2. Transformation of tetrahydrothiazolopyridine part



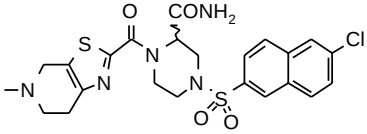
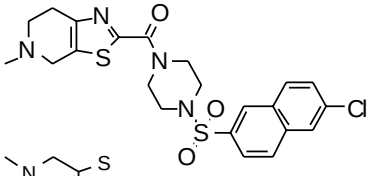
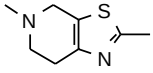
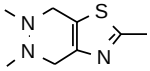
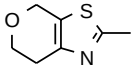
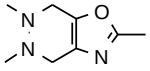
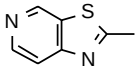
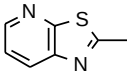
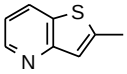
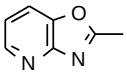
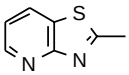
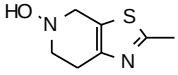
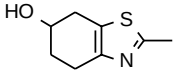
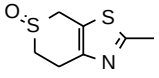
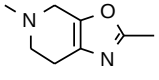
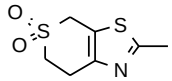
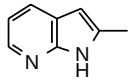
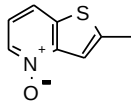
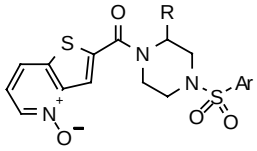
Compound	R=	fXa (IC_{50}, nM)	fIIa (IC_{50}: μM)	PTCT2[a] in human plasma (μM)	PTCT2 in rat plasma (μM)	CL_{int} (ml/min/kg)
1		24	1.0	4.4	14	536
2		22	0.56	6	10	750
4		5	1.05	1.8	2.2	870
8c		8.5	1.3	2.8	3.9	173
8e		24	0.73	11.5	12.6	NT[b]
8h		68	3.5	10	13	NT
9a		248	NT	>20	>20	NT
9b		650	NT	>20	>20	NT
9d		15	3	17	>20	NT
9e		660	NT	>20	>20	NT
9f		16	NT	>20	>20	NT
10b		8.4	1.5	5.4	6.0	333
10d		6.5	0.58	4.8	4.9	93.2
10f		2	NT	1.9	1.8	119
10g		49	4.7	5.1	4.2	75.4

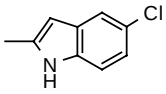
Table 2 (*continued*)

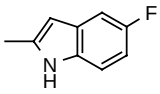
Compound	R=	fXa (IC_{50}, nM)	fIIa (IC_{50}: μM)	PTCT2[a] in human plasma (μM)	PTCT2 in rat plasma (μM)	CL_{int} (ml/min/kg)
11f		6.3	NT	>20	>20	NT
12c		610	NT	>20	>20	NT
12d		2.5	2.5	1.7	3.9	41.7

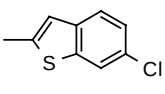
[a] Clotting time doubling concentration for prothrombin time.
[b] NT, not tested.

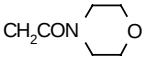
Table 3. Transformation of the S1 site and piperazine side chain



Ar2=Ind1= 

Ar=Ind2= 

Ar2=BT= 

Compound	R=	Ar=	fXa (IC_{50}, nM)	fIIa (IC_{50}: μM)	PTCT2[a] in human plasma (μM)	PTCT2 in rat plasma (μM)	CL_{int} (ml/min/kg)
12d	H	Ind1	2.5	0.14	1.7	3.9	41.7
34a	H	Ind2	44	0.66	3.8	>20	48.2
34b	H	BT	5.6	0.15	3.2	7.4	6.97
38a	CH_2CONMe_2	BT	4.4	0.7	1.5	3.2	525
38b		BT	2.1	0.7	0.9	2.5	334
38c	$CH_2CONHMe$	BT	3.1	NT[b]	0.96	1.4	162
38d	$CH_2CONH(CH_2CH_2F)$	BT	1.5	NT	1.0	2.3	171

[a] Clotting time doubling concentration for prothrombin time.
[b] NT, not tested.

Table 4. Crystal and diffraction data of human fXa with compound **12d**

Crystal parameters	
Space group	$P2_12_12_1$
a (Å)	56.6
b (Å)	72.5
c (Å)	79.0
Resolution (Å)	2.3
R_{sym}[a] (%)	6.4 (33.8)
Completeness[a] (%)	90.6 (84.3)
No. of reflections, redundancy	13,656
Refinement	
No. of protein atoms (occupancy ≠ 0)	2156
Average *B* value for protein and ligand atoms (Å^2)	46.7, 57.5
Range of data	25.0–2.3
R value	19.1
Weighted rsmd from ideality	
Bond length (Å)	0.022
Bond angle (Å)	2.12

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

calculation[23] results of the model compounds **A** and **B**, illustrated in Figure 5, supported this disadvantage. ΔHF of conformers **B-I** and **B-II** exhibited the minimum value and the second minimum value among the respective various conformers. Because of the closest S–O proximity, the ΔHF of **B-I** was lower than that of **B-II**. On the other hand, the ΔHF of conformer **A-I** exhibited the maximum value (Fig. 6). These results suggested that a preferable conformation of **12d** would be a twisted relationship between the thienopyridine face and carbonyl face. The chloroindole part of **12d** fits so tightly into the S1 binding site that its location was not flexible. Considering these structural limitations, the thienopyridine part of **12d** should be arranged vertically to the indole ring of Trp215. In many crystal structural analyses of low molecular weight compounds and the fXa complex, the aromatic moiety of the ligand, which was located in the neighborhood of the phenyl ring (Phe174) and indole ring (Trp215), was arranged vertically to the indole ring of Trp215.[6,24] Consequently, the conformation of **12d** in that crystal structure is the

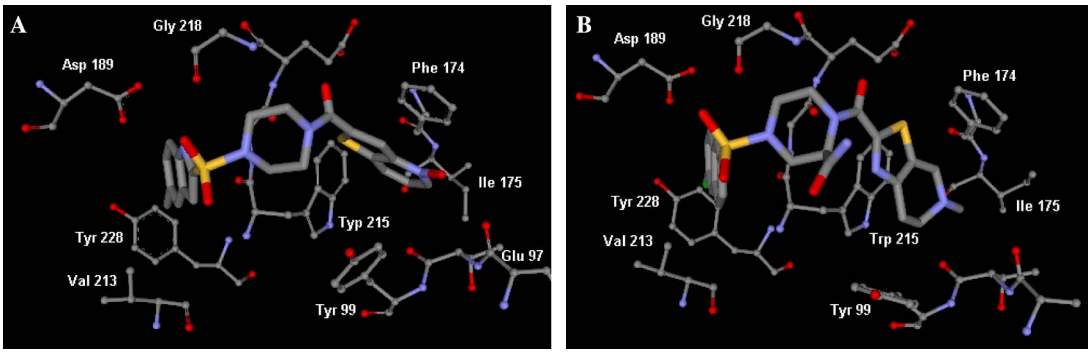


Figure 3. Comparison of the binding modes of **12d** and **1** to fXa. The thick sticks (gray, carbon; blue, nitrogen; red, oxygen, and yellow; sulfur) are **12d** (A) and **1** (B) without protons.

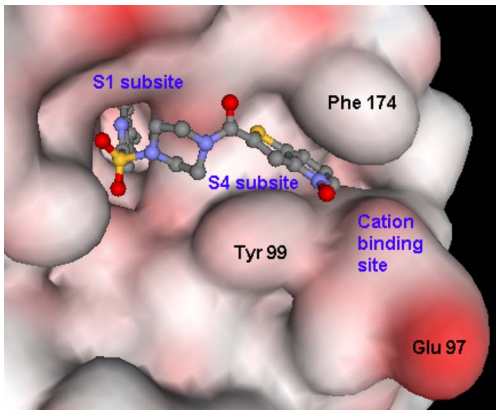


Figure 4. The binding mode of **12d** as viewed from top. The surface view is the active site of Gla-less fXa. The ball and stick drawn (gray, carbon; blue, nitrogen; red, oxygen; and yellow, sulfur) indicates **12d** with protons omitted.

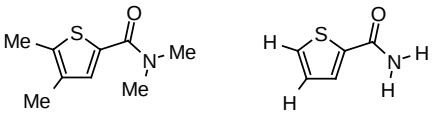


Figure 5. Structures of model compounds **A** and **B**.

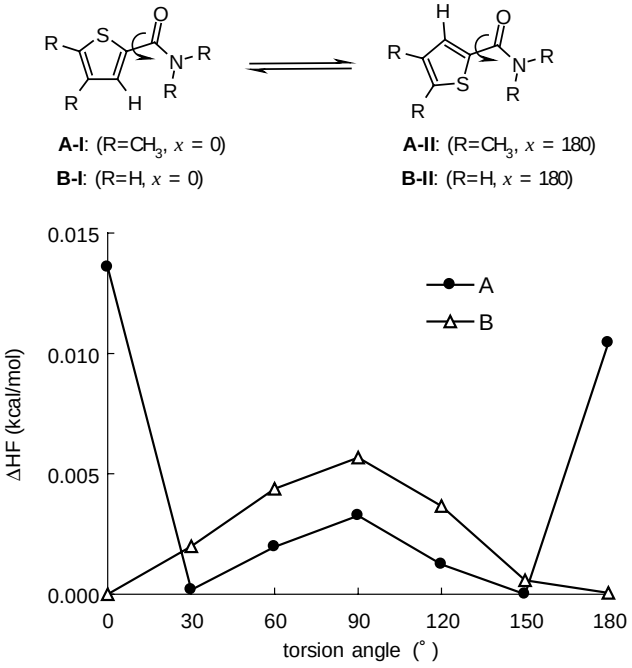


Figure 6. Energy versus torsion angle (χ) of the S–C–C=O moiety. The illustrations on the top are geometries of model compounds A and B at $\chi = 0°$ or 180°. Ab initio calculations were performed at the RHF/STO-3G level.

desirable conformation for stabilization of both the molecule and the binding mode to the S4 binding site. Moreover, the cationic pyridine ring interacted with the cation binding site the same as reported by others.[6,24c,25] This would be the reason why **12d** exhibited a 6-fold more active fXa inhibition than did **9d**.

The optimized study of **12d** is shown in Table 3. In our X-ray study, the S1 part of **12d** tightly bound to fXa and the linker part of **12d** was located on the surface of fXa. We hoped that introducing a hydrophilic moiety in the piperazine ring would improve metabolic stability as a result of changing the physicochemical property without reducing the fXa activity. Therefore, we designed compounds which had carbamoyl moiety on the piperazine ring and halogen substituted 5,6 fused aromatic ring in the place of 5-chloroindole. Metabolic stability of the benzothiophene analogue **34b** was improved 80 times from **1**. The rate of microsomal metabolic turnover for carbamoylmethyl derivatives was higher than that of the parent compound **34b**. This approach maintained or improved the in vitro anti-fXa activity, but could not reduce the microsomal metabolism.

Thienopyridine analogues, **12d** and **34b**, were tested in the Caco-2 assay (gastrointestinal cell assay). Much

Table 5. Caco-2 permeability of **12d** and **34b**

Compound	Caco-2 AT ratio[a]
12d	10.3
34b	23.8

[a] The value of AT ratio[26] means the ratio of Papp (test compound) to Papp (atenolol) in Caco-2 cell permeability assay. Atenolol has been reported to show oral bioavailability of 50% in human.[27]

Table 6. Ex vivo anti-fXa and anti-coagulant activities for **34b** at 30 mg/kg (po) to rats

	0.5 h	1 h	2 h	4 h
Anti-fXa activity (%)[a]	95.3 ± 0.40	96.1 ± 1.30	95.3 ± 1.00	93.9 ± 1.60
Test/control PT ratio (fold)[a]	1.34 ± 0.02	1.39 ± 0.03	1.37 ± 0.04	1.27 ± 0.04

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

higher permeability of **34b** was observed than that of **12d** in the Caco-2 system (Table 5). After oral administration of **34b** to rats, at a dose of 30 mg/kg, potent anti-fXa activities in the plasma and prolongation ratio of the prothrombin time until 4 h were observed. The results are shown in Table 6.

Equi-potent fXa inhibitors **34b** and **1** were evaluated in the rat DIC model;[28] the data are presented in Figures 7 and 8. A dose-dependent anti-thrombotic effect of **34b** had been exhibited at doses of 1–10 mg/kg po. The dose required for 50% inhibition of platelet consumption (ID_{50plt}) was estimated as being 6.5 mg/kg, and the dose required for 50% inhibition of TAT (thrombin–anti-thrombin III complex) formation (ID_{50TAT}) was estimated as being 7.3 mg/kg. In the case of **1**, ID_{50plt} and ID_{50TAT} were calculated as being 19.4 mg/kg and 24.3 mg/kg, respectively.

4. Conclusion

Compound **34b** exhibited excellent anti-coagulation activity and selective anti-fXa activity. Furthermore, its metabolic stability in human microsomes was improved 80 times compared with that of **1**. It has been demonstrated that this compound was highly efficacious in an animal thrombosis model. Good permeability and metabolic stability may contribute to high anti-coagulant activity in an in vivo model after oral administra-

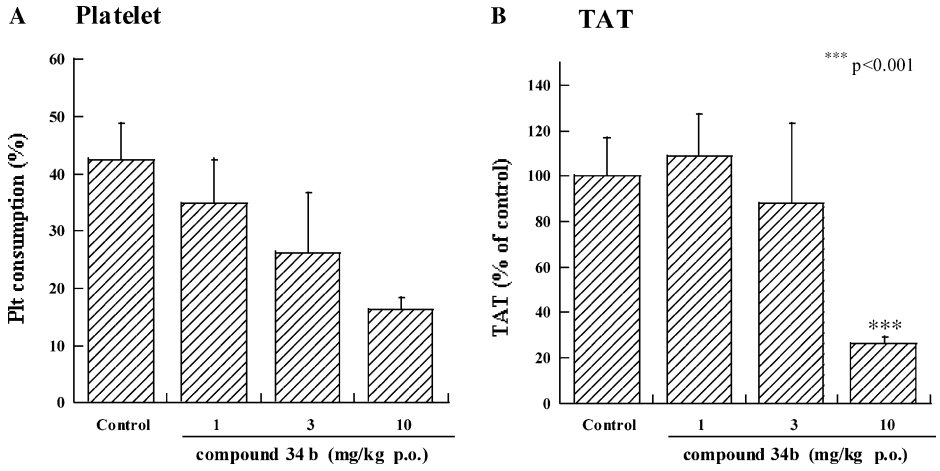


Figure 7. Anti-thrombotic effects of **34b** in the rat DIC model. (A) Platelet consumption. (B) Concentration of thrombin–anti-thrombin III complex (TAT). Data represent means ± SE from six rats. ***$P < 0.001$ significantly different from control.

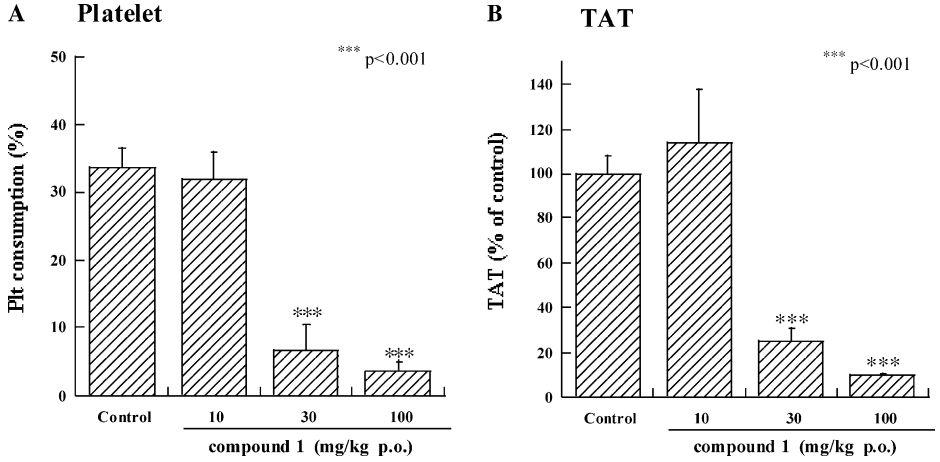


Figure 8. Anti-thrombotic effects of **1** in the rat DIC model. (A) Platelet consumption. (B) Concentration of thrombin–anti-thrombin III complex (TAT). Data represent means ± SE from six rats. ***$P < 0.001$ significantly different from control.

tion. We obtained a non-basic and highly selective fXa inhibitor, and it is expected that this compound will be useful for preventing some thrombotic disorders by oral administration.

5. Experimental

5.1. General

Melting points were determined with a Büchi 520 apparatus in glass capillary tubes and are uncorrected. Column chromatography was performed with Merck silica gel 60 particle size (0.060–0.200 mm or 0.040–0.063 mm) or Sephadex LH-20 (Amersham Biosciences Corp.). Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC aluminum sheets with silica gel 60 F_{254} and detected by UV quenching at 254 nm or by spraying with phosphomolybdic acid or ninhydrin. All analytical samples were found to be homogeneous by TLC.

^{1}H NMR spectra were recorded on a JEOL JNM-EX400 spectrometer and chemical shifts are given in parts per million (δ) from tetramethylsilane as the internal standard. Mass spectra were performed with a JEOL JMS-AX505W (EI) or a JEOL JMS-HX110 (FD, FAB) spectrometer. IR spectra were recorded on a HITACHI 270-30 spectrometer.

5.2. Preparation of the crystals

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., MA). 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. After several days, 2 mM of compound **12d** was added to the drop and then benzamidine/Gla-less fXa crystal[3] was seeded to the drop by the streak-seeding method.[29] The obtained micro-crystal was then grown to a sufficient size for X-ray experiments by the micro-seeding method.[29]

5.3. X-ray data collection and processing

A co-crystal was sealed in a glass capillary together with mother liquor. An all X-ray data set was collected at room temperature on an *R*-Axis IIc imaging plate detector (RIGAKU, Japan) with an RU200 rotating anode generator (RIGAKU, Japan). Data processing was carried out using *d*trek*.[30]

5.4. Structure solution and crystallographic refinement

A previously reported Gla-less fXa structure (PDB code 1HCG[31]) was used as the initial structure. Phase refinement and model rebuilding was carried out by using *refmac5*[32] and *Turbo Frodo*.[33] Low resolution data (<25 Å^2) were included and Babinet bulk solvent scaling[34] was applied. Stereochemistry checks indicate that the refined protein model is in good agreement with expectations within each resolution range. The statistics of the crystallographic refinement are shown in Table 4. Atomic coordinates have been deposited with the Protein Data Bank (PDB code: 2D1J).

5.5. Computational study

Ab initio calculations obtaining energy profiles and point charges were performed by using the Gaussian 94 program systems[35] at the RHF level of theory with the STO-3G basis set. Geometry of model compounds **A** and **B** was optimized by fixing the torsion angles of the carbamoyl moiety (O=C–N–R) 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 0–180° and are shown in Figure 6. The χ value was incremented in 30° steps and fixed. The global energy minimum for each model compound **A** and **B** was normalized to 0 kcal/mol.

5.6. 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). Aqueous DMSO (5% v/v; 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 increases of optical density (OD/min) were measured at 405 nm. Anti-fXa activity (inhibition percentage) was calculated as follows: anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. The IC_{50} value was obtained by plotting the inhibitor concentration against the anti-fXa activity.

5.7. Anti-fIIa activity in vitro

Anti-thrombin activity in vitro was measured by using chromogenic substrate S-2266 (Chromogenix, Inc.) and human thrombin (Sigma Chemical, Inc.). Aqueous DMSO (5% v/v; 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 increases of optical density (OD/min) were measured at 405 nm. Anti-thrombin activity (inhibition percentage) was calculated as follows: anti-thrombin activity = 1 − [(OD/min) of sample/(OD/min) of control]. The IC_{50} value was obtained from the inhibition percentage on the statistical probability paper.

5.8. Anti-coagulant activity in vitro

Anti-coagulant activity in vitro was evaluated with the plasma clotting time doubling concentration for pro-

thrombin time (PTCT2). Plasma (20 μl) was mixed with inhibitors in saline (20 μl) in the process tube. Coagulation was started by the addition of Simplastin (Organon Teknica, Inc.) (40 μl).

5.9. Anti-fXa activity and anti-coagulant activity ex vivo

Male Wistar rats were fasted overnight. Synthetic compounds were dissolved in 0.5% (w/v) methylcellulose solution and administered orally to rats via a stomach tube. For control rats, 0.5% (w/v) methylcellulose solution was administered orally. Rats were anesthetized with ravonal at several time points when blood samples were collected into tubes containing trisodium citrate. After blood samples were centrifuged, the platelet poor plasma samples were used for measuring their anti-fXa activities or anti-coagulant activities. Anti-fXa activity: plasma (5 μl) was mixed with 0.1 M Tris–0.2 M NaCl–0.2% BSA buffer (pH 7.4; 40 μl), H_2O (5 μl) and 0.1 U/ml human fXa (10 μl). 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 density (OD/min) was measured at 405 nm. Anti-fXa activity (inhibition percentage) was calculated as follows; anti-fXa activity = 1 − [(OD/min) of sample/(OD/min) of control]. Anti-coagulant activity: plasma (20 μl) was mixed with inhibitors in saline (20 μl) in the process tube. Coagulation was started by the addition of Simplastin (40 μl). Anti-coagulant activity was evaluated by comparing the prolongation rate of prothrombin time versus control.

5.10. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]piperazine hydrochloride (4)

To a stirred solution of 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine **5a** (6.43 g, 41.7 mmol) in dry THF (200 ml) was added *n*-BuLi (1.47 M in hexanes; 34.0 ml, 50.0 mmol) at −78 °C under an argon atmosphere. The reaction mixture was stirred for 40 min at −78 °C. After the bubbling of CO_2 gas for 1 h, the reaction mixture was warmed up to room temperature and concentrated in vacuo. Collection of the residue and washing with hexane gave lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-carboxylate (9.42 g) as a pale brown amorphous solid. To a mixture of lithium 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-carboxylate (175 mg), 1-[(5-chloroindol-2-yl)sulfonyl]piperazine (**7**) (400 mg, 1.33 mmol), 1-hydroxybenzotriazole hydrate (10.5 mg, 0.08 mmol), and *N*-methylmorpholine (86.8 mg, 0.86 mmol) in *N*,*N*-dimethylformamide (100 ml) was added 1-(dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (194 mg, 1.01 mmol). The reaction mixture was stirred for 10 h and then concentrated in vacuo. The residue was added to H_2O and AcOEt. The separated organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (AcOEt/MeOH = 10/3), and then the residue was added to 1 N HCl–EtOH and H_2O. The solution was concentrated in vacuo to give **4** (68 mg, 17%) as a pale yellow powder.

Mp 193–206 °C (dec). ^{1}H NMR ($CDCl_3$): δ 2.49 (3H, s), 2.78–2.83 (2H, m), 2.85–2.94 (2H, m), 3.15–3.28 (4H, br), 3.67 (2H, s), 3.82–3.95 (2H, br), 4.50–4.65 (2H, br), 6.96 (1H, d, J = 2.0 Hz), 7.32 (1H, dd, J = 8.8, 2.0 Hz), 7.36 (1H, d, J = 8.8 Hz), 7.67 (1H, s), 8.71 (1H, br). Anal. Calcd for $C_{20}H_{22}ClN_5O_3S_2{\cdot}HCl{\cdot}0.5H_2O$: C, 44.64; H, 4.76; Cl, 13.18; N, 13.02; S, 11.92. Found: C, 44.69; H, 4.72; Cl, 13.36; N, 12.76; S, 11.76. MS (FAB) *m*/*z* 480 [(M+H)$^+$, Cl^{35}], 482 [(M+H)$^+$, Cl^{37}]. IR (KBr) cm^{-1} 3367, 3106, 2924, 2700–2450, 1624, 1473, 1352, 1157, 953.

5.11. 1-[(5-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]-4-[(5-chloroindol-2-yl)sulfonyl]piperazine (8b)

Starting with **5b** (0.80 g, 3.3 mmol) and following the procedure for the preparation of **4** gave **8b** (570 mg, 30%) as a colorless amorphous solid. The title compound was purified only through silica gel column chromatography without transformation into the HCl salt.

^{1}H NMR ($CDCl_3$): δ 1.48 (9H, s), 2.85 (2H, br s), 3.22 (4H, br s), 3.73 (2H, br s), 3.89 (2H, br s), 4.58 (2H, br s), 4.65 (2H, br s), 6.97 (1H, s), 7.32 (1H, dd, J = 8.8 and 2.0 Hz), 7.37 (1H, d, J = 8.8 Hz), 7.66 (1H, d, J = 2.0 Hz), 8.72 (1H, s). Anal. Calcd for $C_{24}H_{28}ClN_5O_5S_2$: C, 50.92; H, 4.99; N, 12.37. Found: C, 50.60; H, 4.97; N, 12.19. MS (FAB) *m*/*z* 566 [(M+H)$^+$, Cl^{35}], 568 [(M+H)$^+$, Cl^{37}]. IR (ATR) cm^{-1} 3276, 3149, 3043, 2979, 1687, 1614, 1477, 1415, 1351, 1272, 1236, 1159, 1106, 995, 954, 800.

5.12. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(5,6-dimethyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridazin-2-yl)carbonyl]piperazine hydrochloride (8c)

Starting with **5c** (62.0 mg, 0.37 mmol) and following the procedure for the preparation of **4** gave **8c** (60.0 mg, 29%) as a colorless powder.

Mp 156–159 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.65 (3H, br s), 2.76 (3H, br s), 3.13 (4H, br s), 3.74 (2H, br s), 4.10–4.50 (6H, br), 7.03 (1H, d, J = 1.5 Hz), 7.31 (1H, dd, J = 8.8, 2.0 Hz), 7.48 (1H, d, J = 8.8 Hz), 7.76 (1H, d, J = 2.0 Hz), 12.42 (1H, s). Anal. Calcd for $C_{20}H_{23}ClN_6O_3S_2{\cdot}HCl{\cdot}2.2H_2O$: C, 42.06; H, 5.01; Cl, 12.42; N, 14.72; S, 11.23. Found: C, 42.31; H, 4.89; Cl, 12.63; N, 14.44; S, 11.19. MS (FAB) *m*/*z* 495 [(M+H)$^+$, Cl^{35}], 497 [(M+H)$^+$, Cl^{37}]. IR (KBr) cm^{-1} 3122, 2921, 2869, 1619, 1448, 1349, 1307, 1278, 1155, 1101, 1056, 993, 950, 929, 916, 809.

5.13. 1-[(5-*tert*-Butyldiphenylsilyloxy-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)carbonyl]-4-[(5-chloroindol-2-yl)sulfonyl]piperazine (8d)

Starting with **5d** (702 mg, 1.6 mmol) and following the procedure for the preparation of **4** gave **8d** (300 mg, 35%) as a colorless amorphous solid. The title compound was purified only through silica gel column chromatography without transformation into the HCl salt.

No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 1.02 (9H, s), 1.81–2.01 (2H, m), 2.62–2.99 (6H, m), 3.19–3.21 (4H, m), 3.87 (1H, br s), 4.19–4.23 (1H, m), 4.57 (1H, br s), 6.95 (1H, d, J = 1.0 Hz), 7.29–7.69 (13H, m). MS (FAB) *m/z* 719 [(M+H)$^+$, Cl^{35}], 721 [(M+H)$^+$, Cl^{37}].

5.14. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(4,5-dihydro-7*H*-pyrano[4,3-*d*]thiazol-2-yl)carbonyl]piperazine (8e)

Starting with **5e** (253 mg, 1.9 mmol) and following the procedure for the preparation of **4** gave **8e** (420 mg, 45%) as a colorless amorphous solid. The title compound was purified only through silica gel column chromatography without transformation into the HCl salt.

^{1}H NMR (DMSO-d_6): δ 2.82 (2H, t, J = 5.6 Hz), 3.12 (4H, t, J = 4.9 Hz) 3.28–3.35 (2H, m), 3.73 (1H, br s), 3.93 (2H, t, J = 5.6 Hz), 4.39 (1H, br s), 4.79 (2H, s), 7.03 (1H, s), 7.30 (1H, dd, J = 8.8, 2.2 Hz), 7.47 (1H, d, J = 8.8 Hz), 7.76 (1H, s), 12.39 (1H, br s). Anal. Calcd for $C_{19}H_{19}ClN_4O_4S_2$: C, 48.87; H, 4.10; N, 12.00; S, 13.73. Found: C, 49.10; H, 4.26; N, 11.90; S, 13.45. MS (FAB) *m/z* 467 [(M+H)$^+$, Cl^{35}], 469 [(M+H)$^+$, Cl^{37}]. IR (KBr) cm^{-1} 3276, 2944, 2881, 2840, 1735, 1616, 1351, 1157, 952.

5.15. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(6,7-dihydro-4*H*-thiopyrano[4,3-*d*]thiazol-2-yl) carbonyl]piperazine (8f)

Starting with **5f** (293 mg, 1.53 mmol) and following the procedure for the preparation of **4** gave **8f** (260 mg, 35%) as a colorless amorphous solid. The title compound was purified only through silica gel column chromatography without transformation into the HCl salt. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 2.96–2.99 (2H, m), 3.06–3.09 (2H, m), 3.17–3.25 (4H, m), 3.40–3.44 (2H, m), 3.86 (2H, s), 5.31 (2H, br s), 6.96 (1H, s), 7.28–7.39 (2H, m), 7.65 (1H, d, J = 2.0 Hz). MS (FAB) *m/z* 483 [(M+H)$^+$, Cl^{35}], 485 [(M+H)$^+$, Cl^{37}].

5.16. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(thiazolo[5,4-*c*]pyridin-2-yl)carbonyl]piperazine (9a)

Starting with **6a** (344 mg, 1.53 mmol) and following the procedure for the preparation of **4** gave **9a** (334 mg, 48%) as a pale yellow form. The title compound was purified only through silica gel column chromatography without transformation into the HCl salt.

^{1}H NMR (DMSO-d_6): δ 3.20 (4H, br s), 3.84 (2H, br s), 4.35 (2H, br s), 7.03 (1H, s), 7.25–7.35 (1H, m), 7.47 (1H, dd, J = 8.8, 2.0 Hz), 7.74 (1H, d, J = 2.0 Hz), 8.05 (1H, d, J = 5.4 Hz), 8.67 (1H, d, J = 5.4 Hz), 9.44 (1H, s), 12.41 (1H, s). MS (FAB) *m/z* 462 [(M+H)$^+$, Cl^{35}], 464 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m/z* 462.0460 (M+H)$^+$ (calcd for $C_{19}H_{17}ClN_5O_3S_2$: 462.0461). IR (KBr) cm^{-1} 3380, 3282, 2923, 2856, 1621, 1500, 1409, 1349, 1305, 1280, 1243, 1151, 1097, 1051, 991, 950, 889, 806.

5.17. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(thiazolo[5,4-*b*]pyridin-2-yl)carbonyl]piperazine hydrochloride (9b)

Starting with **6b** (260 mg, 1.21 mmol) and following the procedure for the preparation of **4** gave **9b** (270 mg, 44%) as a colorless powder.

Mp 244–246 °C. ^{1}H NMR (DMSO-d_6): δ 3.17–3.20 (4H, m), 3.82 (2H, br), 4.38 (2H, br), 7.04 (1H, s), 7.30 (1H, dd, J = 8.8, 1.0 Hz), 7.48 (1H, d, J = 8.8 Hz), 7.64–7.68 (1H, m), 7.76 (1H, s), 8.51 (1H, d, J = 8.5 Hz), 8.73–8.74 (1H, m), 12.43 (1H, s). Anal. Calcd for $C_{19}H_{16}ClN_5O_3S_2 \cdot 1.1HCl \cdot 0.5H_2O$: C, 44.65; H, 3.57; Cl, 14.57; N, 13.70; S, 12.55. Found: C, 44.87; H, 3.54; Cl, 14.67; N, 13.83; S, 12.43. MS (FAB) *m/z* 462 [(M+H)$^+$, Cl^{35}], 464 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m/z* 462.0436 (M+H)$^+$ (calcd for $C_{19}H_{17}ClN_5O_3S_2$: 462.0461). IR (KBr) cm^{-1} 3284, 3145, 3050, 2925, 2867, 2692, 2530, 1627, 1353, 1159, 806, 578.

5.18. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(1-phenylsulfonyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)carbonyl]piperazine (9c)

Starting with **6c** (413 mg, 1.6 mmol) and following the procedure for the preparation of **4** gave **9c** (555 mg, 63%) as a pale yellow form. The title compound was purified only through silica gel column chromatography without transformation into the HCl salt. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 3.18–3.42 (4H, m), 3.45–3.65 (1H, m), 3.65–3.80 (2H, m), 4.10–4.23 (1H, m), 6.52 (1H, s), 6.99 (1H, dd, J = 2.0, 0.74 Hz), 7.20 (1H, dd, J = 8.0, 4.8 Hz), 7.28 (1H, dd, J = 8.8, 2.0 Hz), 7.35 (1H, d, J = 8.8 Hz), 7.40–7.50 (2H, m), 7.50–7.58 (1H, m), 7.67 (1H, d, J = 2.0 Hz), 7.81 (1H, dd, J = 8.0, 1.6 Hz), 8.20–8.27 (2H, m), 8.45 (1H, dd, J = 4.8, 1.6 Hz), 9.00 (1H, br s). MS (FAB) *m/z* 584 [(M+H)$^+$, Cl^{35}], 586 [(M+H)$^+$, Cl^{37}].

5.19. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(thieno[3,2-*b*]pyridin-2-yl)carbonyl]piperazine hydrochloride (9d)

Starting with **6d** (117 mg, 0.86 mmol) and following the procedure for the preparation of **4** gave **9d** (178 mg, 39 %) as a colorless powder.

Mp > 270 °C. ^{1}H NMR (DMSO-d_6): δ 3.11–3.22 (4H, br), 3.70–3.82 (4H, br), 7.04 (1H, s), 7.33 (1H, dd, J = 8.8, 2.0 Hz), 7.51 (1H, d, J = 8.8 Hz), 7.52–7.64 (1H, br), 7.80 (1H, d, J = 2.0 Hz), 7.82–7.88 (1H, m), 8.63–8.87 (2H, br), 12.45–12.53 (1H, br). Anal. Calcd for $C_{20}H_{17}ClN_4O_3S_2 \cdot 0.85HCl \cdot 1.5H_2O \cdot 0.2EtOH$: C, 46.39; H, 4.21; Cl, 12.42; N, 10.61; S, 12.14. Found: C, 46.54; H, 4.19; Cl, 12.33; N, 10.64; S, 12.37. MS (FAB) *m/z* 461 [(M+H)$^+$, Cl^{35}], 463 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m/z* 461.0489 (M+H)$^+$ (calcd for

$C_{20}H_{18}ClN_4O_3S_2$: 461.0509). IR (KBr) cm^{-1} 3114, 3089, 1628, 1431, 1340, 1281, 1149, 960.

5.20. 1-[(5-*tert*-Butoxycarbonyl-4,5,6,7-tetrahydrooxazolo[5,4-*c*]pyridin-2-yl)carbonyl]-4-[(5-chloroindol-2-yl)sulfonyl]piperazine (8g)

To a solution of **5g** (311 mg, 1.1 mmol) in THF (8.0 ml) were added lithium hydroxide (25.0 mg, 1.0 mmol) and H_2O (2.0 ml). The reaction mixture was stirred for 10 min at room temperature and then concentrated in vacuo to give lithium (5-*tert*-butoxycarbonyl-4,5,6,7-tetrahydrooxazolo[5,4-*c*]pyridin-2-yl)carboxylate (280 mg) as a colorless amorphous solid. To a mixture of the lithium salt (105 mg), **7** (119 mg, 0.40 mmol) and 1-hydroxybenzotriazole hydrate (8.6 mg, 0.06 mmol) in *N*,*N*-dimethylformamide (4.0 ml) was added 1-(dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (80.0 mg, 0.42 mmol). The reaction mixture was stirred for 1.5 h and then concentrated in vacuo. The residue was added to H_2O and AcOEt. The separated organic layer was washed with saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1/1), and then the solution was concentrated in vacuo to give **8g** (107 mg, 49%) as a colorless amorphous solid.

^1H NMR ($CDCl_3$): δ 1.46 (9H, s), 2.64 (2H, br s), 3.22 (4H, br s), 3.71 (2H, br s), 3.90 (2H, br s), 4.42 (2H, br s), 4.53 (2H, br s), 6.97 (1H, d, J = 2.0 Hz), 7.33 (1H, dd, J = 8.8, 2.0 Hz), 7.37 (1H, d, J = 8.8 Hz), 7.67 (1H, s), 8.71 (1H, br). MS (FAB) *m*/*z* 550 [$(M+H)^+$, Cl^{35}], 552 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 550.1539 $(M+H)^+$ (calcd for $C_{24}H_{29}ClN_5O_6S$: 550.1527). IR (KBr) cm^{-1} 3276, 2975, 2927, 2863, 1700, 1631, 1525, 1411, 1349, 1268, 1222, 1211, 1155, 1099, 1054, 1022, 954, 916, 873, 808.

5.21. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(5,6-dimethyl-4,5,6,7-tetrahydrooxazolo[4,5-*d*]pyridazin-2-yl)carbonyl]piperazine hydrochloride (8h)

Starting with **5h** (207 mg, 0.92 mmol) and following the procedure for the preparation of **8g** gave 1-[(5-chloroindol-2-yl)sulfonyl]-4-[(5,6-dimethyl-4,5,6,7-tetrahydrooxazolo[4,5-*d*]pyridazin-2-yl)carbonyl]piperazine as a crude compound. The crude was added to 1 N HCl–EtOH solution and concentrated in vacuo. The precipitation of the residue from AcOEt gave **8h** (312 mg, 62%) as a colorless powder.

Mp 153–160 °C (dec). ^1H NMR (DMSO-d_6): δ 2.60–2.85 (6H, br), 3.11 (4H, br s), 3.75 (2H, br s), 4.10–4.45 (6H, br), 7.03 (1H, d, J = 1.5 Hz), 7.31 (1H, dd, J = 8.8, 2.0 Hz), 7.48 (1H, d, J = 8.8 Hz), 7.77 (1H, d, J = 2.0 Hz), 12.42 (1H, s). Anal. Calcd for $C_{20}H_{23}ClN_6O_4S{\cdot}HCl{\cdot}0.7H_2O{\cdot}0.2EtOAc$: C, 45.79; H, 4.99; Cl, 12.99; N, 15.40; S, 5.88. Found: C, 45.71; H, 5.08; Cl, 12.82; N, 15.31; S, 5.91. MS (FAB) *m*/*z* 479 [$(M+H)^+$, Cl^{35}], 481 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 479.1253 $(M+H)^+$ (calcd for $C_{20}H_{24}ClN_6O_4S$: 479.1268). IR (KBr) cm^{-1} 3118, 3014, 2973, 2865, 1639, 1529, 1502, 1438, 1349, 1307, 1278, 1153, 1110, 1097, 1056, 1020, 950, 931, 873, 808.

5.22. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(oxazolo[4,5-*b*]pyridin-2-yl)carbonyl]piperazine (9e)

Starting with **6e** (105 mg, 0.55 mmol) and following the procedure for the preparation of **8g** gave **9e** (29 mg, 11%) as a colorless amorphous solid.

^1H NMR (DMSO-d_6): δ 3.10–3.25 (4H, m), 3.80–3.90 (2H, m), 4.10–4.20 (2H, m), 7.04 (1H, d, J = 1.4 Hz), 7.25–7.35 (1H, m), 7.49 (1H, d, J = 8.8 Hz), 7.55–7.65 (1H, m), 7.77 (1H, d, J = 2.0 Hz), 8.25–8.35 (1H, m), 8.60–8.70 (1H, m), 12.46 (1H, s). Anal. Calcd for $C_{19}H_{16}ClN_5O_4S{\cdot}H_2O$: C, 49.19; H, 3.91; N, 15.10. Found: C, 49.10; H, 3.75; N, 14.84. MS (FAB) *m*/*z* 446 [$(M+H)^+$, Cl^{35}], 448 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 446.0675 $(M+H)^+$ (calcd for $C_{19}H_{17}ClN_5O_4S$: 446.0690). IR (KBr) cm^{-1} 3428, 3120, 3081, 3018, 2925, 2861, 1650, 1544, 1504, 1438, 1407, 1359, 1307, 1268, 1160, 1118, 1056, 1020, 954, 877, 802.

5.23. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(thiazolo[4,5-*b*]pyridin-2-yl)carbonyl]piperazine hydrochloride (9f)

To a solution of 2-methylthiazolo[4,5-*b*]pyridine (500 mg, 3.4 mmol) in H_2O (15 ml) was added $KMnO_4$ (810 mg, 5.1 mmol). The reaction mixture was refluxed for 17 h. The precipitation was filtered out, and the filtrate was added to H_2O and CH_2Cl_2. The separated aqueous layer was acidified by HCl and concentrated in vacuo. The residue was washed with H_2O and Et_2O to give (thiazolo[4,5-*b*]pyridin-2-yl)carboxylic acid (134 mg). To a mixture of the carboxylic acid (60 mg), 1-[(5-chloroindol-2-yl)sulfonyl]piperazine (**7**) (100 mg, 0.33 mmol), 1-hydroxybenzotriazole hydrate (70 mg, 0.50 mmol), and *N*-methylmorpholine (70 mg, 0.67 mmol) in *N*,*N*-dimethylformamide (6.0 ml) was added 1-(dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (96 mg, 0.50 mmol). The reaction mixture was stirred for 5 days and then concentrated in vacuo. The residue was added saturated aqueous $NaHCO_3$ solution and CH_2Cl_2. The separated organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/1), and then the residue was added to 1 N HCl–EtOH and H_2O. The solution was concentrated in vacuo to give **9f** (110 mg, 14%) as a pale yellow amorphous solid.

^1H NMR (CD_3OD): δ 3.90–3.91 (4H, m), 4.47 (4H, br s), 7.00 (1H, s), 7.23 (1H, dd, J = 8.8, 1.7 Hz), 7.42 (1H, d, J = 8.8 Hz), 7.64 (1H, d, J = 2.0 Hz), 7.81–7.84 (1H, m), 8.90–8.94 (2H, m). Anal. Calcd for $C_{19}H_{16}ClN_5O_3S_2{\cdot}0.8HCl{\cdot}1.5H_2O{\cdot}0.1Et_2O$: C, 44.34; H, 3.99; Cl, 12.14; N, 13.33. Found: C, 44.59; H, 4.26; Cl, 12.47; N, 13.69. MS (FAB) *m*/*z* 462 [$(M+H)^+$, Cl^{35}], 464 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 462.0431 $(M+H)^+$ (calcd for $C_{19}H_{17}ClN_5O_3S_2$: 462.0461). IR(KBr) cm^{-1} 3081, 3006, 2921, 2859, 2019, 1943, 1718, 1629, 1159, 950.

5.24. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(5-hydroxy-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]-piperazine (10b)

To a solution of **8b** (534 mg, 0.94 mmol) in CH_2Cl_2 (5 ml) was added to saturated HCl–EtOH solution (20 ml) and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was concentrated in vacuo and the precipitation of the residue from AcOEt gave 1-[(5-chloroindol-2-yl)sulfonyl]-4-[(4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]piperazine (434 mg) as HCl salt. The HCl salt (419 mg) was added to AcOEt and saturated aqueous $NaHCO_3$ solution. The organic layer was concentrated in vacuo and to a suspension of the residue in CH_2Cl_2 (30 ml) was added benzoyl peroxide (70% pure) (302 mg, 0.87 mmol). The reaction mixture was refluxed for 7 h, and was quenched by saturated aqueous Na_2SO_3 solution. The solution was added to H_2O and CH_2Cl_2, and the organic layer was dried over Na_2SO_4, concentrated in vacuo. Purification of the residue was carried out by silica gel column chromatography (CH_2Cl_2/MeOH = 98/2), and then the solution was concentrated in vacuo to give 1-[(5-benzoyloxy-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridin-2-yl)carbonyl]-4-[(5-chloroindol-2-yl)sulfonyl]piperazine (292 mg) as a yellow amorphous solid. To a solution of the *N*-benzoyloxy compound (292 mg) in THF (5.0 ml) and MeOH (5.0 ml) was added 1 N aqueous NaOH solution. The reaction mixture was stirred for 15 min and then concentrated in vacuo. The residue was added to H_2O and $CHCl_3$. The separated organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 95/5), and then the solution was concentrated in vacuo to give **10b** (100 mg, 22%) as a colorless powder.

Mp 163–166 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.70–3.05 (2H, br), 3.05–3.25 (6H, br), 3.65–4.50 (6H, br), 7.03 (1H, s), 7.30 (1H, dd, J = 8.8, 2.0 Hz), 7.47 (1H, d, J = 8.8 Hz), 7.76 (1H, d, J = 2.0 Hz), 8.35 (1H, s), 12.40 (1H, s). Anal. Calcd for $C_{19}H_{20}ClN_5O_4S_2 \cdot 0.5H_2O \cdot 0.25CH_2Cl_2$: C, 45.14; H, 4.23; Cl, 10.38; N, 13.67; S, 12.52. Found: C, 45.30; H, 4.02; Cl, 10.44; N, 13.63; S, 12.52. MS (FAB) *m*/*z* 482 [(M+H)$^+$, Cl^{35}], 484 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 482.0735 (M+H)$^+$ (calcd for $C_{19}H_{21}ClN_5O_4S_2$: 482.0724). IR (KBr) cm^{-1} 3311, 3033, 2918, 2854, 1608, 1537, 1504, 1473, 1450, 1352, 1333, 1306, 1271, 1205, 1151, 1105, 1055, 997, 955, 939, 916, 893, 871, 827, 809.

5.25. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(6-hydroxy-4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)carbonyl]piperazine (10d)

To a solution of **8d** (390 mg, 0.54 mmol) in THF (10 ml) was added tetrabutylammonium fluoride (1 M in THF, 0.81 ml, 0.81 mmol). The reaction mixture was stirred for 16 h at room temperature and then concentrated in vacuo. The residue was added to H_2O and $CHCl_3$. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (CH_2Cl_2/MeOH = 10/1) gave **10d** (110 mg, 14%) as a pale yellow powder.

Mp 228–230 °C. ^{1}H NMR (CD_3OD): δ 1.89–2.03 (2H, m), 2.73–3.11 (6H, m), 3.21–3.31 (4H, m), 3.83 (1H, br s), 4.16–4.20 (1H, m), 4.45 (1H, br s), 6.99 (1H, s), 7.26 (1H, d, J = 8.8 Hz), 7.43 (1H, d, J = 8.8 Hz), 7.66 (1H, s). Anal. Calcd for $C_{20}H_{21}ClN_4O_4S_2 \cdot H_2O \cdot 0.65CH_2Cl_2$: C, 49.32; H, 4.37; Cl, 8.69; N, 11.45; S, 13.10. Found: C, 49.03; H, 4.43; Cl, 8.75; N, 11.19; S, 12.90. MS (FAB) *m*/*z* 481 [(M+H)$^+$, Cl^{35}], 483 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 481.0779 (M+H)$^+$ (calcd for $C_{20}H_{22}ClN_4O_4S_2$: 481.0771). IR (KBr) cm^{-1} 3315, 3029, 2925, 2856, 1606, 1473, 1351, 1157, 954.

5.26. 2-[[4-[(5-Chloroindol-2-yl)sulfonyl]piperazin-1-yl]carbonyl]-6,7-dihydro-4*H*-thiopyrano[4,3-*d*]thiazole 5-oxide (10f)

To a solution of **8f** (150 mg, 0.31 mmol) in THF (6.0 ml) and MeOH (2.0 ml) was added sodium periodate (87 mg, 0.40 mmol). The reaction mixture was stirred for 14 h at room temperature and then concentrated in vacuo. The residue was added to H_2O and CH_2Cl_2. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (CH_2Cl_2/MeOH = 20/1) gave **10f** (90 mg, 58%) as a colorless amorphous solid.

^{1}H NMR ($CDCl_3$): δ 2.85–2.94 (1H, m), 3.15–3.21 (5H, m), 3.46–3.51 (2H, m), 3.81 (1H, br), 3.89 (1H, br), 4.08 (2H, ABq, J = 16.6 Hz), 4.46 (1H, br), 4.55 (1H, br), 6.95 (1H, s), 7.27–7.36 (2H, m), 7.64 (1H, d, J = 1.5 Hz), 9.45 (1H, s). MS (FAB) *m*/*z* 499 [(M+H)$^+$, Cl^{35}], 501 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 499.0305 (M+H)$^+$ (calcd for $C_{19}H_{20}ClN_4O_4S_3$: 499.0335). IR (KBr) cm^{-1} 3396, 3118, 3023, 2971, 2921, 2867, 2690, 1619, 1159, 952.

5.27. 2-[[4-[(5-Chloroindol-2-yl)sulfonyl]piperazin-1-yl]carbonyl]-6,7-dihydro-4*H*-thiopyrano[4,3-*d*]thiazole 5,5-Dioxide (11f)

To a solution of **8f** (150 mg,0.31 mmol) in CH_2Cl_2 (7.0 ml) and MeOH (3.0 ml) was added *m*-CPBA (270 mg) at 0 °C. After the reaction mixture was stirred for 24 h at room temperature, saturated aqueous $Na_2S_2O_4$ solution, H_2O, and CH_2Cl_2 were added. After the reaction mixture was stirred again for 30 min, the precipitate was collected and washed with CH_2Cl_2 to give **11f** (100 mg, 62%) as a colorless powder.

Mp > 260 °C. ^{1}H NMR (DMSO-d_6): δ 3.14 (4H, br), 3.51–3.54 (4H, m), 3.73 (2H, br s), 4.34 (2H, br s), 4.68 (2H, s), 7.02 (1H, s), 7.30 (1H, dd, J = 8.6, 2.0 Hz), 7.47 (1H, d, J = 9.1 Hz), 7.75 (1H, d, J = 2.0 Hz), 12.37 (1H, s). Anal. Calcd for $C_{19}H_{19}ClN_4O_5S_3 \cdot 0.05CH_2Cl_2$: C, 44.06; H, 3.71; Cl, 7.51; N, 10.79; S, 18.53. Found: C, 43.82; H, 3.73; Cl, 7.55; N, 10.77; S, 18.37. MS (FAB) *m*/*z* 515 [(M+H)$^+$, Cl^{35}], 517 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 515.0261 (M+H)$^+$ (calcd for $C_{19}H_{20}ClN_4O_5S_3$: 515.0284). IR

(KBr) cm^{-1} 3334, 3056, 2973, 2937, 2869, 1623, 1317, 1157, 931.

5.28. 1-[(5-Chloroindol-2-yl)sulfonyl]-4-[(1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)carbonyl]piperazine (12c)

To a solution of **9c** (509 mg, 0.87 mmol) in 1,4-dioxane (20 ml) was added 1 N aqueous NaOH solution (2.62 ml). The reaction mixture was refluxed for 4 h, neutralized with 1 N aqueous HCl, and concentrated in vacuo. The residue was added to H_2O and CH_2Cl_2. The separated organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue was carried out by silica gel column chromatography (CH_2Cl_2/MeOH = 20/1 → 10/1) and the solution was concentrated in vacuo to give **12c** (206 mg, 52%) as a pale yellow amorphous solid.

^{1}H NMR (DMSO-d_6): δ 3.12 (4H, t, J = 4.6 Hz), 3.76 (4H, t, J = 4.6 Hz), 6.71 (1H, s), 7.04 (1H, s), 7.10 (1H, dd, J = 7.8, 4.6 Hz), 7.33 (1H, dd, J = 8.8, 2.0 Hz), 7.50 (1H, d, J = 8.8 Hz), 7.79 (1H, d, J = 2.0 Hz), 7.99 (1H, dd, J = 7.8, 1.2 Hz), 8.29 (1H, dd, J = 4.6, 1.2 Hz), 12.02 (1H, br s), 12.47 (1H, br s). Anal. Calcd for $C_{20}H_{18}ClN_5O_3S{\cdot}0.1CH_2Cl_2$: C, 53.36; H, 4.05; Cl, 9.40; N, 15.48; S, 7.09. Found: C, 53.21; H, 4.13; Cl, 9.30; N, 15.52; S, 7.39. MS (FAB) m/z 444 [$(M+H)^+$, Cl^{35}], 446 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) m/z 444.0867 $(M+H)^+$ (calcd for $C_{20}H_{19}ClN_5O_3S$: 444.0897). IR (KBr) cm^{-1} 3307, 3136, 3068, 2989, 2904, 2846, 2690, 1610, 1523, 1448, 1360, 1306, 1267, 1209, 1157, 1090.

5.29. 2-[[4-[(5-Chloroindol-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thieno[3,2-*b*]pyridine *N*-oxide (12d)

To a solution of **9d** (94 mg, 0.20 mmol) in CH_2Cl_2 (40 ml) and EtOH (2.0 ml) was added *m*-CPBA (157 mg) at 0 °C. After the reaction mixture was stirred for 26 h, saturated aqueous $Na_2S_2O_4$ solution was added. After the reaction mixture was stirred again for 30 min, saturated aqueous $NaHCO_3$ was added. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue was carried out by silica gel column chromatography (CH_2Cl_2/MeOH = 35/1) and the solution was concentrated in vacuo to give **12d** (90 mg, 85%) as a colorless powder.

Mp > 155 °C (dec). ^{1}H NMR ($CDCl_3$): δ 3.15–3.35 (4H, br), 3.70–4.00 (4H, br), 7.01 (1H, s), 7.20–7.45 (3H, br), 7.67 (1H, s), 7.80 (1H, d, J = 7.3 Hz), 7.93 (1H, s), 8.40–8.60 (1H, br), 10.80–11.00 (1H, br). Anal. Calcd for $C_{20}H_{17}ClN_4O_4S_2{\cdot}0.65H_2O{\cdot}0.35CH_2Cl_2$: C, 47.15; H, 3.69; Cl, 11.63; N, 10.81; S, 12.37. Found: C, 47.32; H, 3.76; Cl, 11.45; N, 10.63; S, 12.23. MS (FAB) m/z 477 [$(M+H)^+$, Cl^{35}], 479 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) m/z 477.0439 $(M+H)^+$ (calcd for $C_{20}H_{18}ClN_4O_4S_2$: 477.0458). IR (KBr) cm^{-1} 1627, 1417, 1354, 1240, 1159, 1099, 997, 953.

5.30. 1-[(6-Chloroindol-2-yl)sulfonyl]-4-[(5-methyl-4,5,6,7-tetrahydrooxazolo[5,4-*c*]pyridin-2-yl)carbonyl]piperazine hydrochloride (10g)

To a mixture of **8g** (100 mg, 0.18 mmol) in CH_2Cl_2 (3.0 ml) was added trifluoroacetic acid (3.0 ml). The reaction mixture was stirred for 15 min at room temperature and then concentrated in vacuo. To a solution of the residue, Et_3N (50.0 μl, 0.36 mmol), and AcOH (21.0 μl, 0.37 mmol) in CH_2Cl_2 (4.0 ml) were added formalin (23.5 μl, 0.27 mmol) and sodium triacetoxyborohydride (58.0 mg, 0.27 mmol). After the reaction mixture was stirred for 1 h at room temperature, saturated aqueous $NaHCO_3$ solution and CH_2Cl_2 were added. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10/1), and then the residue was added to 1 N HCl–EtOH and H_2O. The solution was concentrated in vacuo to give **10g** (91 mg, 98%) as a colorless amorphous solid.

^{1}H NMR (DMSO-d_6): δ 2.90 (4H, s), 3.11 (3H, br s), 3.25–3.75 (2H, br), 3.35 (2H, s), 3.75 (2H, br s), 4.16 (2H, br s), 4.20–4.75 (2H, br), 7.04 (1H, s), 7.32 (1H, dd, J = 8.8, 1.0 Hz), 7.50 (1H, d, J = 8.8 Hz), 7.78 (1H, d, J = 1.0 Hz), 11.51 (1H, br s), 12.46 (1H, s). Anal. Calcd for $C_{20}H_{22}ClN_5O_4S{\cdot}HCl{\cdot}0.7H_2O$: C, 46.83; H, 4.79; Cl, 13.82; N, 13.65; S, 6.25. Found: C, 46.79; H, 4.81; Cl, 13.82; N, 13.52; S, 6.33. MS (FAB) m/z 464 [$(M+H)^+$, Cl^{35}], 466 [$(M+H)^+$, Cl^{37}]. IR (KBr) cm^{-1} 3325, 1641, 1529, 1437, 1354, 1277, 1157, 1115, 955.

5.31. 4,5-Bis(bromomethyl)thiazole (14)

A solution of 4,5-dimethylthiazole (**13**) (5.00 g, 44.2 mmol), *N*-bromosuccinimide (15.7 g, 88.4 mmol), and 2,2′-azobisisobutyronitrile (362 mg, 2.21 mmol) in 1,2-dichloroethane (500 ml) was refluxed for 1 h. The reaction mixture was concentrated in vacuo. Purification of the residue was carried out by silica gel column chromatography (Et_2O/hexane = 4/1) gave **14** (5.24 g, 44%) as a pale yellow oil. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 4.64 (2H, s), 4.74 (2H, s), 8.75 (1H, s).

5.32. 5,6-Dimethyl-4,5,6,7-tetrahydrothiazolo[4,5-*d*]pyridazine (5c)

To a suspension of **14** (600 mg, 2.21 mmol) and 1,2-dimethylhydrazine dihydrochloride (294 mg, 2.21 mmol) in EtOH (20 ml) was added triethylamine (1.23 ml, 8.84 mmol). The reaction mixture was stirred for 1 h at 50 °C and then concentrated in vacuo. Purification of the residue was carried out by silica gel column chromatography (CH_2Cl_2/MeOH = 95/5) gave **5c** (90 mg, 24%) as a colorless oil. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 2.43 (3H, s), 2.56 (3H, s), 3.92 (2H, s), 4.06 (2H, br s), 8.68 (1H, s). MS (FAB) *m*/*z* 170 $(M+H)^+$.

5.33. 6-*tert*-Butyldiphenylsilyloxy-2-chloro-4,5,6,7-tetrahydrobenzo[*d*]thiazole (5d)

To a solution of 2-chloro-6-oxo-4,5,6,7-tetrahydrobenzo[*d*]thiazole (**15**) (2.7 g, 14 mmol) in MeOH (30 ml) was added sodium borohydride (820 mg, 22 mmol). The reaction mixture was stirred for 1 h at room temperature and then added to $CHCl_3$ and H_2O. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 2/1) gave 2-chloro-6-hydroxy-4,5,6,7-tetrahydrobenzo[*d*]thiazole (2.89 g, 99%) as a colorless oil. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 1.91–2.08 (2H, m), 2.72–3.05 (4H, m), 4.23–4.28 (1H, m). MS (FAB) *m*/*z* 190 [$(M+H)^+$, Cl^{35}], 192 [$(M+H)^+$, Cl^{37}].

To a solution of 2-chloro-6-hydroxy-4,5,6,7-tetrahydrobenzo[*d*]thiazole (2.8 g, 14 mmol) in THF (50 ml) were added imidazole (1.5 g, 22 mmol) and *tert*-butyldiphenylsilyl chloride (4.4 g, 16 mmol). The reaction mixture was stirred for 2 days at room temperature. The precipitate was filtered out and the filtrate was concentrated in vacuo. The residue was added to $CHCl_3$ and H_2O. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 8/1) gave **5d** (6.17 g, 99%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 1.04 (9H, s), 1.82–2.00 (2H, m), 2.58–2.76 (3H, m), 2.88–2.95 (1H, m), 4.17–4.23 (1H, m), 7.34–7.46 (6H, m), 7.60–7.68 (4H, m). MS (FAB) *m*/*z* 428 [$(M+H)^+$, Cl^{35}], 430 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 428.1290 $(M+H)^+$ (calcd for $C_{23}H_{27}ClNOSSi$: 428.1271). IR (ATR) cm^{-1} 3070, 2929, 2890, 2856, 1427, 1108, 1060, 998, 975, 850, 821.

5.34. 2-Amino-6,7-dihydro-4*H*-thiopyrano[4,3-*d*]thiazole (17)

To a solution of 4-oxothiane (5.00 g, 43 mmol) in cyclohexane (50 ml) were added pyrrolidine (3.06 g, 43 mmol) and $TsOH{\cdot}H_2O$ (50 mg, 0.22 mmol). The reaction mixture was refluxed for 1.5 h with a Dean–Stark trap. After cooling, the mixture was filtered and the filtrate was evaporated. To the solution of the residue in dry MeOH (50 ml) was added S_8 (1.38 g, 43 mmol) under water bath cooling. To the stirred mixture was added dropwise a solution of cyanamide (2.01 g, 43 mmol) in dry MeOH (10 ml). The reaction mixture was stirred for 20 h at room temperature. The reaction mixture was concentrated in vacuo. Purification of the residue by silica gel column chromatography (CH_2Cl_2/MeOH = 40/1 → 20/1) gave **17** (3.97 g, 44%) as a brown amorphous solid. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 2.83–2.86 (2H, m), 2.91–2.94 (2H, m), 3.65 (2H, s), 4.84 (2H, br s). MS (FAB) *m*/*z* 173 $(M+H)^+$.

5.35. 2-Chloro-6,7-dihydro-4*H*-thiopyrano[4,3-*d*]thiazole (5f)

To a stirred suspension of copper(II) chloride (3.07 g, 22.8 mmol) in CH_3CN (100 ml) was added *tert*-butyl nitrite (2.94 g, 28.5 mmol). To the stirred mixture was added **17** (3.28 g, 19 mmol) in portions for 1 h. The reaction mixture was stirred for 1 h at room temperature and then stirred for 6 h at 60 °C. The reaction mixture was concentrated in vacuo. Purification of the residue using column chromatography (hexane/AcOEt = 2/1) gave **5f** (0.55 g, 15%) as a yellow oil. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 2.95–2.98 (2H, m), 3.03–3.06 (2H, m), 3.76 (2H, s).

5.36. 2-Bromothiazolo[5,4-*b*]pyridine (6b)

Starting with **18** (1.00 g, 6.61 mmol) and copper(II) bromide (1.77 g, 7.94 mmol), and following the procedure for the preparation of **5f** gave **6b** (260 mg, 18%) as a colorless amorphous solid. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 7.42 (1H, dd, J = 8.1, 4.7 Hz), 8.21–8.23 (1H, m), 8.58 (1H, dd, J = 4.7, 1.5 Hz).

5.37. 1-(Phenylsulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridine (6c)

To a solution of 7-azaindole (0.59 g, 5.0 mmol) in CH_2Cl_2 (10.0 ml) were added NaOH (0.65 g, 16 mmol) and benzyltriethylammonium chloride (0.030 g, 0.13 mmol). Phenylsulfonyl chloride (0.80 ml, 6.3 mmol) was dropped into the reaction mixture under ice bath. The reaction mixture was stirred for 2 h at room temperature, filtered using Celite, and washed with CH_2Cl_2. The filtrate was concentrated in vacuo. Purification of the residue by silica gel column chromatography (CH_2Cl_2) and precipitation from MeOH gave **6c** (0.68 g, 53%) as a colorless amorphous solid.

^{1}H NMR ($CDCl_3$): δ 6.58 (1H, d, J = 4.2 Hz), 7.16 (1H, dd, J = 7.8, 4.7 Hz), 7.43–7.65 (3H, m), 7.71 (1H, d, J = 4.2 Hz), 7.83 (1H, d, J = 7.8 Hz), 8.18 (2H, d, J = 7.8 Hz), 8.41 (1H, d, J = 4.7 Hz). Anal. Calcd for $C_{13}H_{10}ClN_2O_2S$: C, 60.45; H, 3.90; N, 10.85; S, 12.41. Found: C, 60.39; H, 3.95; N, 10.83; S, 12.46. MS (FAB) *m*/*z* 259 $(M+H)^+$. IR (KBr) cm^{-1} 3157, 3093, 3047, 3012, 1579, 1522, 1481, 1468, 1448, 1400, 1369, 1275, 1255, 1174, 1153.

5.38. 2-(*trans*-β-Styryl)-4-vinyloxazole (21)

To a solution of methyltriphenylphosphonium bromide (8.16 g, 22.8 mmol) in THF (80 ml) was added *n*-butyl

lithium (1.54 M in hexane, 14.2 ml, 21.9 mmol) at 0 °C. After the reaction mixture was stirred for 30 min at room temperature, a solution of **20** (3.64 g, 18.3 mmol) in THF (20 ml) was added to the reaction mixture at 0 °C. The reaction mixture was stirred for 2 h at room temperature and then added to H_2O and AcOEt. The separated aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 4/1 → 3/1) gave **21** (2.84 g, 79%) as a pale yellow oil.

^{1}H NMR ($CDCl_3$): δ 5.33 (1H, dd, J = 10.7, 1.5 Hz), 5.98 (1H, dd, J = 17.6, 1.5 Hz), 6.56 (1H, dd, J = 17.6, 10.7 Hz), 6.95 (1H, d, J = 16.6 Hz), 7.31–7.42 (3H, m), 7.49–7.56 (4H, m). MS (FAB) m/z 198 $(M+H)^+$. HRMS (FAB) m/z 198.0919 $(M+H)^+$ (calcd for $C_{13}H_{12}NO$: 198.0919). IR (KBr) cm^{-1} 3149, 3059, 3028, 1643, 1545, 1448, 1402, 1338, 1107, 982, 964.

5.39. 4-(2-Hydroxyethyl)-2-(*trans*-β-styryl)oxazole (22)

To a solution of **21** (13.0 g, 65.9 mmol) in THF (500 ml) was added 9-borabicyclo[3.3.1]nonane (0.5 M in THF, 158 ml, 78.0 mmol) at 0 °C. The reaction mixture was stirred for 15 h at room temperature and then added to H_2O, 3 N aqueous NaOH solution, and 30% aqueous H_2O_2 solution at 0 °C. The reaction mixture was stirred for 6 h at room temperature, and then added to H_2O and AcOEt. The separated aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 2/1 → 0/1) gave **22** (14.1 g, 99%) as a colorless amorphous solid.

^{1}H NMR ($CDCl_3$): δ 2.69 (1H, br s), 2.80 (2H, t, J = 5.6 Hz), 3.90–3.97 (2H, m), 6.91 (1H, d, J = 16.6 Hz), 7.30–7.42 (4H, m), 7.43–7.56 (3H, m). MS (FAB) m/z 216 $(M+H)^+$. HRMS (FAB) m/z 216.1033 $(M+H)^+$ (calcd for $C_{13}H_{14}NO_2$: 216.1025). IR (KBr) cm^{-1} 3298, 3140, 2924, 2871, 1637, 1601, 1525, 1446, 1354, 1053, 1005, 976, 958.

5.40. *N*-[2-[2-(*trans*-β-Styryl)oxazol-4-yl]ethyl]phthalimide (23)

To a solution of **22** (292 mg, 1.36 mmol) in THF (15 ml) were added phthalimide (200 mg, 1.36 mmol), triphenylphosphine (357 mg, 1.36 mmol), and diethyl azodicarboxylate (214 μl, 1.36 mmol). The reaction mixture was stirred for 4 h at room temperature and then concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 3/1) gave **23** (447 mg, 95%) as a colorless amorphous solid.

^{1}H NMR ($CDCl_3$): δ 2.98 (2H, t, J = 7.2 Hz), 4.03 (2H, t, J = 7.2 Hz), 6.88 (1H, d, J = 16.6 Hz), 7.28–7.45 (5H, m), 7.48 (2H, d, J = 7.3 Hz), 7.71 (2H, dd, J = 2.9, 5.4 Hz), 7.84 (2H, dd, J = 2.9, 5.4 Hz). MS (FAB) m/z 345 $(M+H)^+$. HRMS (FAB) m/z 345.1243 $(M+H)^+$ (calcd for $C_{21}H_{17}N_2O_3$: 345.1239). IR (KBr) cm^{-1} 3136, 2947, 2918, 1774, 1713, 1429, 1400, 1365, 1093, 976.

5.41. 4-[2-(*tert*-Butoxycarbonylamino)ethyl]-2-(*trans*-β-styryl)oxazole (24)

To a solution of **23** (6.40 g, 18.6 mmol) in EtOH (150 ml) was added hydrazine hydrate (2.00 ml, 41 mmol). The reaction mixture was refluxed for 3 h and then added to CH_2Cl_2 (150 ml), saturated aqueous $NaHCO_3$ solution (150 ml), and di-*tert*-butyl dicarbonate (13.4 g, 61.4 mmol). The reaction mixture was stirred for 0.5 h at room temperature. The separated aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 2/1 → 1/1) gave **24** (5.06 g, 87%) as a colorless amorphous solid.

^{1}H NMR ($CDCl_3$): δ 1.45 (9H, s), 2.75 (2H, t, J = 6.6 Hz), 3.46 (2H, dt, J = 5.9, 6.6 Hz), 4.92 (1H, br s), 6.91 (1H, d, J = 16.6 Hz), 7.29–7.45 (4H, m), 7.48 (1H, d, J = 16.6 Hz), 7.52 (2H, d, J = 7.3 Hz). MS (FAB) m/z 315 $(M+H)^+$. HRMS (FAB) m/z 315.1724 $(M+H)^+$ (calcd for $C_{18}H_{23}N_2O_3$: 315.1709). IR (KBr) cm^{-1} 3394, 3136, 2978, 2933, 2881, 1687, 1645, 1599, 1518, 1446, 1365, 1246, 1165, 960.

5.42. 5-(*tert*-Butoxycarbonyl)-2-(*trans*-β-styryl)-4,5,6,7-tetrahydrooxazolo[5,4-*c*]pyridine (25)

To a solution of **24** (190 mg, 0.60 mmol) in toluene (15 ml) were added paraformaldehyde (54.5 mg, 1.60 mmol) and *p*-toluenesulfonic acid (7.2 mg, 0.038 mmol). The reaction mixture was refluxed for 1 h and then added to saturated aqueous $NaHCO_3$ solution and AcOEt. The separated aqueous layer was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 3/1 → 2/1) gave **25** (153 mg, 78%) as a colorless viscous syrup.

^{1}H NMR ($CDCl_3$): δ 1.50 (9H, s), 2.67 (2H, br s), 3.73 (2H, br s), 4.55 (2H, s), 6.90 (1H, d, J = 16.1 Hz), 7.29–7.42 (3H, m), 7.46 (1H, d, J = 16.1 Hz), 7.52 (2H, d, J = 7.3 Hz). MS (FAB) m/z 327 $(M+H)^+$, 271 $(M-\text{isobutene}+H)^+$, 227 $(M-\text{Boc}+H)^+$. HRMS (FAB) m/z 327.1690 $(M+H)^+$ (calcd for $C_{19}H_{23}N_2O_3$: 327.1709). IR (KBr) cm^{-1} 2976, 2918, 2846, 1697, 1523, 1477, 1448, 1415, 1394, 1365, 1284, 1248, 1165, 1138, 1093, 964, 916.

5.43. 5-(*tert*-Butoxycarbonyl)-2-formyl-4,5,6,7-tetrahydrooxazolo[5,4-*c*]pyridine (26)

To a solution of **25** (803 mg, 2.46 mmol) in THF (16 ml) were added acetone (8.0 ml), H_2O (4.0 ml), *N*-methylmorpholine *N*-oxide (577 mg, 4.93 mmol), and aqueous osmium tetraoxide solution (0.039 M, 3.20 ml, 0.12 mmol). The reaction mixture was stirred for 14 h at room temperature and then added to 10% aqueous $Na_2S_2O_3$ solution and AcOEt. The separated aqueous

layer was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. To a solution of the residue in THF (16 ml) were added MeOH (8.0 ml), H_2O (8.0 ml), and sodium periodate (790 mg, 3.69 mmol). The reaction mixture was stirred for 3 h at room temperature and then added to H_2O and AcOEt. The separated aqueous layer was extracted with AcOEt. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 4/1 → 2/1) gave **26** (234 mg, 53%) as a colorless amorphous solid. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 1.49 (9H, s), 2.77 (2H, br s), 3.77 (2H, br s), 4.62 (2H, s), 9.70 (1H, s).

5.44. 5-(*tert*-Butoxycarbonyl)-2-methoxycarbonyl-4,5,6,7-tetrahydrooxazolo[5,4-*c*]pyridine (5g)

To a solution of **26** (225 mg, 0.89 mmol) in MeOH (9.0 ml) were added sodium cyanide (220 mg, 4.49 mmol) and manganese (IV) dioxide (780 mg, 8.97 mmol). The reaction mixture was stirred for 0.5 h at room temperature and filtered with Celite. The filtrate was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 3/2 → 1/1) gave **5g** (120 mg, 48%) as a colorless amorphous solid.

^{1}H NMR ($CDCl_3$): δ 1.49 (9H, s), 2.73 (2H, br s), 3.74 (2H, br s), 4.01 (3H, s), 4.59 (2H, s). MS (FAB) *m*/*z* 283 $(M+H)^+$. HRMS (FAB) *m*/*z* 283.1292 $(M+H)^+$ (calcd for $C_{13}H_{19}N_2O_5$: 283.1294). IR (KBr) cm^{-1} 2976, 2929, 2852, 1741, 1697, 1631, 1527, 1406, 1367, 1304, 1201, 1157, 1095, 916.

5.45. Ethyl [4,5-bis(bromomethyl)oxazol-2-yl]carboxylate (28)

Starting with **27** (2.65 g, 15.7 mmol) and following the procedure for the preparation of **14** gave **28** (1.84 g, 36%) as a colorless oil. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 1.44 (3H, t, J = 7.1 Hz), 4.43 (2H, s), 4.49 (2H, q, J = 7.1 Hz), 4.55 (2H, s).

MS (FAB) *m*/*z* 326 (M+H, Br^{79} and $Br^{79})^+$, 328 (M+H, Br^{79} and $Br^{81})^+$, 330 (M+H, Br^{81} and $Br^{81})^+$.

5.46. Ethyl (5,6-dimethyl-4,5,6,7-tetrahydrooxazolo[4,5-*d*]pyridazin-2-yl)carboxylate (5h)

Starting with **28** (920 mg, 2.81 mmol) and following the procedure for the preparation of **5c** gave **5h** (207 mg, 33%) as a pale yellow oil.

^{1}H NMR ($CDCl_3$): δ 1.44 (3H, t, J = 7.1 Hz), 2.44 (3H, s), 2.52 (3H, s), 3.75 (2H, br s), 3.92 (2H, br s), 4.47 (2H, q, J = 7.1 Hz). MS (FAB) *m*/*z* 226 $(M+H)^+$. HRMS (FAB) *m*/*z* 226.1179 $(M+H)^+$ (calcd for $C_{10}H_{16}N_3O_3$: 226.1192). IR (KBr) cm^{-1} 2987, 2948, 2823, 2780, 1729, 1525, 1309, 1187, 1141, 1103, 1024, 960, 850.

5.47. 5-Fluoro-1-phenylsulfonylindole (30)

To a solution of 5-fluoroindole (**29**) (1.0 g, 7.4 mmol) in THF (30 ml) at −78 °C under argon atmosphere was added *n*-butyl lithium solution (1.53 M in hexane, 5.3 ml, 8.1 mmol). After the reaction mixture was stirred for 1 h at −78 °C, a solution of phenylsulfonyl chloride (1.0 ml, 8.1 mmol) was added. After it was warmed up from −78 °C to room temperature for 3 h, the reaction mixture was added to brine and AcOEt. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 9/1) gave **30** (2.0 g, 99%) as a colorless powder. No further purification was attempted on this compound, which was used directly in the next step.

Mp 119–121 °C. ^{1}H NMR ($CDCl_3$): δ 6.62–6.63 (1H, m), 7.02–7.07 (1H, m) 7.18 (1H, dd, J = 8.5, 2.5 Hz), 7.43–7.47 (2H, m), 7.53–7.57 (1H, m), 7.60 (1H, d, J = 3.7 Hz), 7.84–7.87 (2H, m), 7.94 (1H, dd, J = 9.0, 4.4 Hz).

5.48. (5-Fluoro-1-phenylsulfonylindol-2-yl)sulfonyl chloride (31)

To a solution of **30** (2.02 g, 7.3 mmol) in Et_2O (10 ml) and THF (20 ml) was added *tert*-butyl lithium (1.54 M in pentane, 5.3 ml, 8.2 mmol) at −78 °C under argon atmosphere. After the reaction mixture was warmed up to 0 °C, SO_2 gas was introduced to the mixture at −78 °C. After the reaction mixture was warmed up to room temperature, it was stirred for 4 h at room temperature and concentrated in vacuo. To the residue were added hexane and Et_2O, and collecting the precipitate gave a colorless powder. To a solution of the powder in CH_2Cl_2 (70 ml) was added *N*-chlorosuccinimide (1.44 g, 8.1 mmol). After stirring the reaction mixture for 20 h at room temperature, CH_2Cl_2 and H_2O were added. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (hexane/AcOEt = 4/1) gave **31** (2.88 g, quant.) as a colorless oil. No further purification was attempted on this compound, which was used directly in the next step.

^{1}H NMR ($CDCl_3$): δ 7.33–7.51 (4H, m), 7.60–7.64 (2H, m), 8.04–8.07 (2H, m), 8.30 (1H, dd, J = 9.3, 4.2 Hz). MS (FAB) *m*/*z* 374 [$(M+H)^+$, Cl^{35}], 376 [$(M+H)^+$, Cl^{37}].

5.49. 1-[(5-Fluoro-1-phenylsulfonylindol-2-yl)sulfonyl]piperazine (32a)

To a solution of **31** (1.05 g, 2.8 mmol) in CH_2Cl_2 (30 ml) were added 1-(*tert*-butoxycarbonyl)piperazine (520 mg, 2.8 mmol) and triethylamine (570 mg, 5.6 mmol) in an

ice bath. The mixture was stirred for 3 h at room temperature and added to CH_2Cl_2 and 1 N aqueous HCl solution. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the resulting residue by silica gel column chromatography (CH_2Cl_2/MeOH = 5/1) gave 1-(*tert*-butoxycarbonyl)-4-[(5-fluoro-1-phenylsulfonylindol-2-yl)sulfonyl]piperazine as a brown amorphous mass. To a solution of 1-(*tert*-butoxycarbonyl)-4-[(5-fluoro-1-phenylsulfonylindol-2-yl)sulfonyl]piperazine was added 1 N HCl–EtOH solution (15 ml). The reaction mixture was stirred for 20 h at room temperature and concentrated in vacuo. The residue was added to 1 N aqueous NaOH solution and CH_2Cl_2. The separated organic layer was dried over $MgSO_4$ and concentrated in vacuo to give **32a** (465 mg, 39%) as a brown powder. No further purification was attempted on this compound, which was used directly in the next step.

Mp 181–183 °C. ^{1}H NMR ($CDCl_3$): δ 2.95 (4H, t, J = 4.9 Hz), 3.39 (4H, t, J = 4.9 Hz) 7.40–7.56 (6H, m), 8.00–8.02 (2H, m), 8.26 (1H, dd, J = 9.0, 4.4 Hz). MS (FAB) m/z 424 $(M+H)^+$.

5.50. 1-[(5-Fluoroindol-2-yl)sulfonyl]-4-[(thieno[3,2-*b*]pyridin-2-yl)carbonyl]piperazine (33a)

Starting with **6d** (48 mg, 0.35 mmol) and **32a** (150 mg, 0.35 mmol), and following the procedure for the preparation of **4** gave **33a** (100 mg, 59%) as a pale yellow amorphous solid. The title compound was purified only through silica gel column chromatography without transformation into the HCl salt.

^{1}H NMR (CD_3OD): δ 3.30 (4H, br s), 3.84 (4H, br s), 7.02 (1H, s), 7.09–7.15 (1H, m), 7.36 (1H, dd, J = 9.3, 2.4 Hz), 7.46 (1H, dd, J = 9.0, 4.4 Hz), 7.79–7.83 (2H, m), 8.88 (1H, dd, J = 5.4, 1.5 Hz), 8.93 (1H, d, J = 7.8 Hz). MS (FAB) m/z 445 $(M+H)^+$. HRMS (FAB) m/z 445.0833 $(M+H)^+$ (calcd for $C_{20}H_{18}FN_4O_3S_2$ 445.0804). IR (KBr) cm^{-1} 3399, 3089, 3027, 2927, 2877, 2624, 2024, 1629, 1160, 962.

5.51. 1-[(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl]-4-[(thieno[3,2-*b*]pyridin-2-yl)carbonyl]piperazine hydrochloride (33b)

Starting with **6d** (111 mg, 0.82 mmol) and **32b** (349 mg, 0.90 mmol) and following the procedure for the preparation of **4** gave **33b** (282 mg, 67%) as a colorless powder.

Mp 137–140 °C. ^{1}H NMR (DMSO-d_6): δ 3.11–3.28 (4H, br), 3.70–3.87 (4H, br), 7.53–7.63 (2H, br), 7.80–7.90 (1H, br s), 8.05–8.16 (2H, br), 8.30–8.40 (1H, br), 8.65–8.74 (1H, br), 8.75–8.85 (1H, br s). Anal. Calcd for $C_{20}H_{16}ClN_3O_3S_3 \cdot 1.15HCl \cdot 0.95H_2O \cdot 0.1Et_2O$: C, 45.23; H, 3.70; Cl, 13.74; N, 7.76; S, 17.76. Found: C, 45.20; H, 3.65; Cl, 13.81; N, 7.53; S, 17.64. MS (FAB) m/z 478 [$(M+H)^+$, Cl^{35}], 480 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) m/z 478.0104 $(M+H)^+$ (calcd for $C_{20}H_{17}ClN_3O_3S_3$: 478.0121). IR (KBr) cm^{-1} 1631, 1589, 1460, 1431, 1348, 1279, 1252, 1157, 989, 935.

5.52. 2-[[4-[(5-Fluoroindol-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thieno[3,2-*b*]pyridine *N*-oxide (34a)

Starting with **33a** (120 mg, 0.27 mmol) and following the procedure for the preparation of **12d** gave **34a** (80 mg, 64%) as a colorless amorphous solid.

^{1}H NMR (DMSO-d_6): δ 3.25–3.27 (4H, m), 3.87–3.90 (4H, m), 7.03 (1H, d, J = 1.5 Hz), 7.09–7.14 (1H, m), 7.31–7.39 (3H, m), 7.77 (1H, d, J = 8.3 Hz), 7.91 (1H, s), 8.42 (1H, d, J = 6.1 Hz), 9.97 (1H, s). MS (FAB) m/z 461 $(M+H)^+$. HRMS (FAB) m/z 461.0768 $(M+H)^+$ (calcd for $C_{20}H_{18}FN_4O_4S_2$: 461.0754). IR (KBr) cm^{-1} 3099, 2971, 2927, 2856, 2705, 2659, 2181, 1627, 1351, 1162.

5.53. 2-[[4-[(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl]piperazin-1-yl]carbonyl]thieno[3,2-*b*]pyridine *N*-oxide (34b)

Starting with **33b** (145 mg, 0.30 mmol) and following the procedure for the preparation of **12d** gave **34b** (98 mg, 65%) as a colorless powder.

^{1}H NMR ($CDCl_3$): δ 3.22–3.32 (4H, br), 3.88–3.97 (4H, br), 7.29 (1H, dd, J = 8.3, 6.4 Hz), 7.46 (1H, dd, J = 8.6, 1.7 Hz), 7.71 (1H, d, J = 8.3 Hz), 7.79 (1H, s), 7.83–7.88 (3H, m), 8.30 (1H, 1H, d, J = 6.4 Hz). Anal. Calcd for $C_{20}H_{16}ClN_3O_4S_3 \cdot 0.15H_2O$: C, 48.36; H, 3.31; Cl, 7.14; N, 8.46; S, 19.37. Found: C, 48.65; H, 3.46; Cl, 6.94; N, 8.09; S, 19.06. MS (FAB) m/z 494 [$(M+H)^+$, Cl^{35}], 496 [$(M+H)^+$, Cl^{37}]. IR (KBr) cm^{-1} 1631, 1435, 1415, 1350, 1244, 1157, 993, 937.

5.54. 1-[(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl]-3-(methoxycarbonylmethyl)piperazine (36)

To a solution of 1,4-dibenzyl-2-methoxycarbonylmethylpiperazine (**35**) (10.0 g, 29.8 mmol) in EtOH (300 ml) were added palladium hydroxide (418 mg) and concd HCl (30 ml). The reaction mixture was stirred for 5 h at room temperature under hydrogen atmosphere. After filtration of the catalyst, followed by concentration of the filtrate gave 2-methoxycarbonylmethylpiperazine dihydrochloride (6.88 g, 99%) as a brown amorphous solid.

To a solution of 2-methoxycarbonylmethylpiperazine dihydrochloride (6.87 g, 29.7 mmol) in CH_2Cl_2 (300 ml) were added (6-chlorobenzo[*b*]thien-2-yl)sulfonyl chloride (9.54 g, 35.7 mmol) and triethylamine (16.6 ml, 119 mmol). The reaction mixture was stirred for 3.5 h at room temperature and then added to H_2O. The separated aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by silica gel column chromatography (CH_2Cl_2/MeOH = 50/1) gave **36** (3.49 g, 30%) as a pale yellow amorphous solid.

^{1}H NMR ($CDCl_3$): δ 2.28–2.48 (3H, m), 2.55–2.67 (2H, m), 2.85–3.08 (2H, m), 3.14–3.27 (1H, m), 3.59–3.68 (2H, m), 3.69 (3H, s), 7.43 (1H, dd, J = 8.6, 2.0 Hz), 7.74 (1H, s), 7.80 (1H, d, J = 8.6 Hz), 7.85 (1H, d, J = 2.0 Hz). MS (FAB) m/z 389 [$(M+H)^+$, Cl^{35}], 391

[(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m/z* 389.0386 (M+H)$^+$ (calcd for $C_{15}H_{18}ClN_2O_4S_2$: 389.0397). IR (KBr) cm^{-1} 3338, 2852, 1732, 1589, 1452, 1354, 1159, 1007.

5.55. [4-(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl-1-[(thieno[3,2-*b*]pyridin-2-yl)carbonyl]piperazin-2-yl]acetic acid (37)

Starting with **6d** (2.12 g, 16 mmol) and **36** (5.00 g, 13 mmol), and following the procedure for the preparation of **4** gave 4-(6-chlorobenzo[*b*]thien-2-yl)sulfonyl-2-methoxycarbonylmethyl-1-[(thieno[3,2-*b*]pyridin-2-yl)carbonyl]piperazine (6.2 g, 89%) as a pale yellow amorphous solid.

^{1}H NMR (DMSO-d_6): δ 2.55–2.65 (1H, m), 2.70–2.80 (1H, m), 2.80–2.90 (1H, m), 2.95–3.05 (1H, m), 3.40–3.75 (1H, m), 3.70 (3H, s), 3.80–4.00 (2H, m), 4.15–4.60, 5.00–5.50 (total 2H, each br), 7.25–7.35 (1H, m), 7.40–7.50 (1H, m), 7.61 (1H, s), 7.77 (1H, s), 7.83 (1H, d, J = 8.8 Hz), 7.88 (1H, d, J = 1.7 Hz), 8.10–8.20 (1H, m), 8.70 (1H, dd, J = 4.4, 1.5 Hz). MS (FAB) *m/z* 550 [(M+H)$^+$, Cl^{35}], 552 [(M+H)$^+$, Cl^{37}].

To a solution of the methyl ester (4.83 g, 8.78 mmol) in THF (120 ml) were added lithium hydroxide (235 mg, 9.81 mmol) and H_2O (15 ml). The reaction mixture was stirred for 2.5 h at room temperature and then added to 1 N aqueous HCl and concentrated in vacuo. Precipitation of the residue from MeOH–H_2O gave **37** (4.18 g, 88%) as a colorless amorphous solid.

^{1}H NMR (DMSO-d_6): δ 2.60–2.95 (4H, m), 3.10–3.80 (3H, m), 3.90–5.20 (2H, m), 7.35–7.45 (1H, m), 7.56 (1H, dd, J = 8.8, 2.0 Hz), 7.79 (1H, s), 8.00–8.10 (1H, m), 8.33 (1H, d, J = 1.9 Hz), 8.48 (1H, d, J = 8.3 Hz), 8.65–8.70 (1H, m), 12.30–12.70 (1H, br). Anal. Calcd for $C_{22}H_{18}ClN_3O_5S_3{\cdot}0.2H_2O{\cdot}0.2MeOH$: C, 48.83; H, 3.54; Cl, 6.49; N, 7.70; S, 17.62. Found: C, 49.21; H, 3.63; Cl, 6.54; N, 7.32; S, 17.97. MS (FAB) *m/z* 536 [(M+H)$^+$, Cl^{35}], 538 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m/z* 536.0175 (M+H)$^+$ (calcd for $C_{22}H_{19}ClN_3O_5S_3$: 536.0175). IR (KBr) cm^{-1} 3077, 2927, 2871, 1708, 1631, 1558, 1490, 1455, 1396, 1353, 1319, 1270, 1160, 1103, 993, 958.

5.56. 2-[[4-[(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl]-2-[(*N*,*N*-dimethylcarbamoyl)methyl]piperazin-1-yl]carbonyl]thieno[3,2-*b*]pyridine *N*-oxide (38a)

To a solution of **37** (385 mg, 0.71 mmol), 1-hydroxybenzotriazole hydrate (100 mg, 0.74 mmol), dimethylamine (2.0 M in THF, 1.54 ml, 3.08 mmol), and diisopropylethylamine (0.32 ml, 1.83 mmol) in *N*,*N*-dimethylformamide (20 ml) was added 1-(dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (154 mg, 0.80 mmol). The reaction mixture was stirred for 40 h and then concentrated in vacuo. The residue was added to 10% aqueous citric acid solution and CH_2Cl_2. The separated organic layer was washed with saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 98/2) to give 4-(6-chlorobenzo[*b*]thien-2-yl)sulfonyl-2-(*N*,*N*-dimethylcarbamoyl)methyl-1-[(thieno[3,2-*b*]pyridin-2-yl)carbonyl]piperazine (162 mg) as a pale yellow solid. Starting with 4-(6-chlorobenzo[*b*]thien-2-yl)sulfonyl-2-(*N*,*N*-dimethylcarbamoyl)methyl-1-[(thieno[3,2-*b*]pyridin-2-yl)carbonyl]piperazine (162 mg) and following the procedure for the preparation of **12d** gave **38a** (121 mg, 29%) as a pale yellow powder.

Mp 138–141 °C. ^{1}H NMR (CD_3OD): δ 2.68–2.95 (4H, br), 2.89 (3H, br s), 3.00–3.30 (6H, br), 3.75–3.95 (2H, br), 7.48–7.53 (2H, m), 7.91 (1H, s), 7.94 (1H, s), 7.97 (1H, d, J = 8.6 Hz), 8.07 (1H, d, J = 1.7 Hz), 8.16 (1H, d, J = 8.3 Hz), 8.44 (1H, d, J = 6.1 Hz). Anal. Calcd for $C_{24}H_{23}ClN_4O_5S_3{\cdot}0.5H_2O{\cdot}0.3EtOH$: C, 49.09; H, 4.32; Cl, 5.79; N, 9.31; S, 15.98. Found: C, 49.03; H, 4.17; Cl, 5.79; N, 9.04; S, 15.84. MS (FAB) *m/z* 579 [(M+H)$^+$, Cl^{35}], 581 [(M+H)$^+$, Cl^{37}]. IR (KBr) cm^{-1} 1639, 1413, 1356, 1255, 1159, 995, 943.

5.57. 2-[[4-[(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl]-2-[(morpholinocarbonyl)methyl]piperazin-1-yl]carbonyl]thieno[3,2-*b*]pyridine *N*-oxide (38b)

Starting with **37** (246 mg, 0.46 mmol) and morpholine (0.17 ml, 1.95 mmol), and following the procedure for the preparation of **38a** gave **38b** (194 mg, 68%) as a pale yellow powder.

Mp 148–151 °C. ^{1}H NMR ($CDCl_3$): δ 2.55–2.82 (3H, m), 3.05–3.20 (1H, m), 3.25–3.80 (9H, m), 3.80–4.00 (2H, br), 4.15–4.70 (1H, br), 5.00–5.40 (1H, br), 7.25–7.31 (1H, m), 7.46 (1H, d, J = 8.6 Hz), 7.70 (1H, d,J = 8.3 Hz), 7.78 (1H, s), 7.84 (1H, d, J = 8.6 Hz), 7.88 (2H, s), 8.28 (1H, d, J = 6.1 Hz). Anal. Calcd for $C_{26}H_{25}ClN_4O_6S_3H_2O{\cdot}0.1EtOH$: C, 48.88; H, 4.32; Cl, 5.51; N, 8.70; S, 14.94. Found: C, 48.98; H, 4.37; Cl, 5.46; N, 8.56; S, 15.08. MS (FAB) *m/z* 621 [(M+H)$^+$, Cl^{35}], 623 [(M+H)$^+$, Cl^{37}]. HRMS (FAB) *m/z* 621.0697 (M+H)$^+$ (calcd for $C_{26}H_{26}ClN_4O_6S_3$: 621.0703). IR (KBr) cm^{-1} 2854, 1635, 1414, 1358, 1255, 1230, 1159, 1115, 993, 943.

5.58. 2-[[4-[(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl]-2-[(*N*-methylcarbamoyl)methyl]piperazin-1-yl]carbonyl]thieno[3,2-*b*]pyridine *N*-oxide (38c)

Starting with **37** (231 mg, 0.43 mmol) and methylamine hydrochloride (40.6 mg, 0.60 mmol), and following the procedure for the preparation of **38a** gave **38c** (147 mg, 57%) as a pale yellow amorphous solid.

^{1}H NMR (DMSO-d_6): δ 2.50–3.35 (5H, m), 2.57 (3H, d, J = 3.9 Hz), 3.50–3.80 (2H, m), 3.85–5.20 (2H, m), 7.42 (1H, dd, J = 8.1, 6.3 Hz), 7.58 (1H, d, J = 8.5 Hz), 7.80–8.10 (5H, m), 8.33 (1H, s), 8.37 (1H, d, J = 6.1 Hz). Anal. Calcd for $C_{23}H_{21}ClN_4O_5S_3{\cdot}1.1H_2O$: C, 47.23; H, 4.00; Cl, 6.06; N, 9.58; S, 16.45. Found: C, 47.07; H, 3.98; Cl, 6.11; N, 9.40; S, 16.65. MS (ESI) *m/z* 565 [(M+H)$^+$, Cl^{35}], 567 [(M+H)$^+$, Cl^{37}]. IR (KBr) cm^{-1} 3322, 3070, 2850, 1650, 1616, 1548, 1452, 1411, 1361, 1315, 1249, 1159, 1101, 1008, 954.

5.59. 2-[4-[(6-Chlorobenzo[*b*]thien-2-yl)sulfonyl]-2-[[*N*-(2-fluoroethyl)carbamoyl]methyl]piperazin-1-yl]carbonyl]thieno[3,2-*b*]pyridine *N*-oxide (38d)

Starting with **37** (289 mg, 0.43 mmol) and 2-fluoroethylamine hydrochloride (71.6 mg, 0.72 mmol), and following the procedure for the preparation of **38a** gave **38d** (88.0 mg, 33%) as a pale yellow amorphous solid.

^{1}H NMR ($CDCl_3$): δ 2.50–3.00 (4H, m), 3.40–3.70 (3H, m), 3.70–4.00 (2H, m), 4.20–4.60 (3H, m), 4.90–5.30 (1H, m), 6.70–6.90 (1H, br), 7.25–7.35 (1H, m), 7.42 (1H, d, J = 8.1 Hz), 7.70–7.80 (2H, m), 7.80–7.90 (2H, m), 7.93 (1H, s), 8.29 (1H, d, J = 6.1 Hz). Anal. Calcd for $C_{24}H_{22}ClFN_4O_5S_3{\cdot}H_2O{\cdot}0.05CH_2Cl_2$: C, 46.56; H, 4.08; Cl, 6.29; F, 3.06; N, 9.03; S, 15.51. Found: C, 46.85; H, 3.73; Cl, 6.60; F, 3.06; N, 9.05; S, 15.90. MS (FAB) *m*/*z* 597 [$(M+H)^+$, Cl^{35}], 599 [$(M+H)^+$, Cl^{37}]. HRMS (FAB) *m*/*z* 597.0513 $(M+H)^+$ (calcd for $C_{24}H_{23}ClFN_4O_5S_3$: 597.0503). IR (KBr) cm^{-1} 3434, 3316, 3072, 1664, 1629, 1550, 1519, 1450, 1415, 1357, 1253, 1160, 1099, 1051, 1004, 941.

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