Synthesis and Evaluation of 1-Arylsulfonyl-3-piperazinone Derivatives as Factor Xa Inhibitors[1—6] VI. A Series of New Derivatives Containing *N*,*S*- and *N*,*SO*$_2$-Spiro Acetal Scaffolds

Fumihiko Saitoh,* Hidemitsu Nishida, Takafumi Mukaihira, Naoto Kosuga, Munetaka Ohkouchi, Tomokazu Matsusue, Ikuya Shiromizu, Yoshitaka Hosaka, Miwa Matsumoto, and Ichiro Yamamoto

Pharmaceutical Research Center, Mochida Pharmaceutical Co., Ltd.; 722 Jimba-aza-uenohara, Gotemba, Shizuoka 412–8524, Japan. Received October 26, 2006; accepted November 20, 2006; published online November 24, 2006

In the course of development of factor Xa (FXa) inhibitors, we have found unique compounds containing an *N*,*O*- and an *N*,*N*-spiro acetal structure. It appeared that the difference in overall conformation due to the *N*,*X*-spiro acetal structure might be important for FXa inhibitory activity. Therefore, other *N*,*X*-spiro acetal structures, an *N*,*S*- and an *N*,*SO*$_2$-spiro acetal, were developed as analogues of the *N*,*X*-spiro acetal structure. Compound 7b (*N*,*S*-spiro acetal structure) was found to have the strongest activity in these series of *N*,*X*-spiro acetal compounds, which had ever been synthesized.[4,5]

Key words factor Xa inhibitor; structure–activity relationship; *N*,*S*-spiro acetal; *N*,*SO*$_2$-spiro acetal; intramolecular cyclization

Factor Xa (FXa), a trypsin-like serine protease, occupies a central position in the blood coagulation cascade in linking the intrinsic and extrinsic mechanisms. FXa is known to activate prothrombin to thrombin. Thrombin has several procoagulant functions, including the activation of platelets, feedback activation of other coagulation factors, and conversion of fibrinogen to insoluble fibrin clots.[7—11] Comparison of hirudin[12—16] (a thrombin inhibitor) and tick anticoagulant peptide[17—22] (a FXa inhibitor) suggests that inhibition of FXa may result in less risk of bleeding, leading to a more favorable safety/efficacy ratio.[23—27]

Direct inhibition of FXa has therefore emerged as an attractive strategy for the discovery of novel antithrombotic agents.[28—34] Fondaparinux (Arixtra®),[35—37] which was approved as the first selective FXa inhibitor in 2002, has been proven to be clinically useful. However, because fondaparinux is a penta-saccharide limited to subcutaneous administration, novel orally administratable FXa inhibitors have been desired. However, no approved orally administratable FXa inhibitor is available in the world.

In previous papers,[4,5] we reported that spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (*N*,*O*-spiro acetal) derivatives and their nitrogen analogues, which were spiro[imidazo[1,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5(1*H*)-one (*N*,*N*-spiro acetal) derivertives, were found to be potent inhibitors of FXa.

With change of the central atom from oxygen to nitrogen, the inhibitory activity of FXa tended to increase. This improvement of activity might be based on the slight difference in the conformation between the *N*,*O*- and the *N*,*N*-spiro acetal structures.

As shown in Table 1, the *N*,*S*- and *N*,*SO*$_2$-spiro acetals are quite different from the *N*,*O*- and *N*,*N*-spiro acetal structures (as calculated by MOPAC PM3).[38]

Therefore, these *N*,*S*- and *N*,*SO*$_2$-spiro acetal structures, which can be prepared by changing the central atom to sulfur, would have the potential for increasing inhibitory activity of FXa.

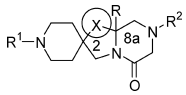
In this paper, we discuss the synthesis and structure–activity relationships of compounds containing the *N*,*S*-spiro acetal, which is spiro[5*H*-thiazolo[3,2-*a*]pyradine-2(3*H*)-piperidin]-5-one scaffold, and containing the *N*,*SO*$_2$-spiro acetal, which is spiro[5*H*-thiazolo[3,2-*a*]pyradine-2(3*H*)-piperidin]-5-one 1,1-dioxide scaffold, as analogues to the central part of the compound.

Chemistry

First, the amino-thiol **4**, an acyclic precursor, was prepared as shown in Chart 1.

The carboxylic acid **1**, which had been reported,[39] was converted to the corresponding amide **2** with ethyl chlorofor-

Table 1. Cyclic *N*,*O*- and *N*,*N*-Spiro Acetal *vs.* Cyclic *N*,*S*- and *N*,*SO*$_2$-Spiro Acetal



X	O	N(R)	S	SO_2
Bond length C2-X (Å)	1.44	1.50	1.85	1.87
Bond length X-C8a (Å)	1.43	1.50	1.87	1.91
Bong angle C2-X-C8 (°)	111.7	110.3	94.7	93.7

Calculated by MOPAC PM3.

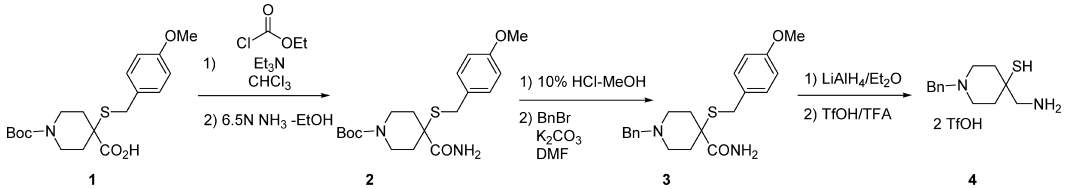


Chart 1. Synthesis of the Amino-Thiol 4 as an Acyclic Precursor

* To whom correspondence should be addressed. e-mail: fsaitoh@mochida.co.jp

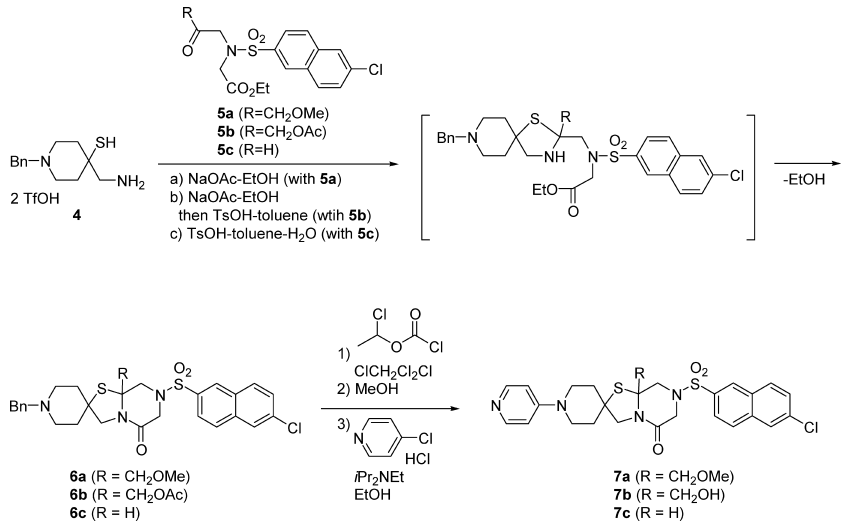


Chart 2. Synthesis of the Key Tricyclic Intermediates **6a**—**c** and the *N*,*S*-Spiro Acetal Compounds **7a**—**c**

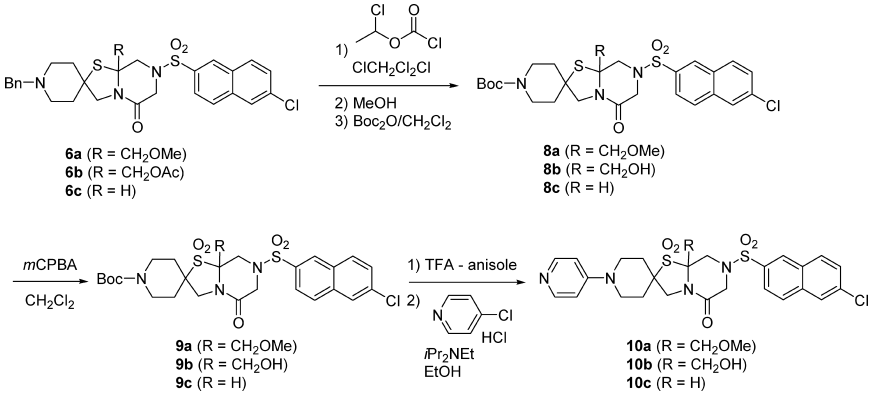


Chart 3. Synthesis of the *N*,SO_2-Spiro Acetal Compounds **10a**—**c**

mate and 6.5 N ammonia–ethanol. The Boc group in amide **2** was deprotected, and a benzyl moiety was introduced to obtain the *N*-benzyl amide **3**. The amide moiety of **3** was reduced to an aminomethyl moiety with lithium aluminum hydride, and the *p*-methoxybenzyl moiety of the thioether in the compound was deprotected with trifluoromethansulfonic acid and trifluoroacetic acid[40] to obtain the amino-thiol **4** in good yield.

Second, the key tricyclic compound **6a**, which was an *N*,*S*-spiro acetal scaffold, was prepared by an intramolecular cyclization reaction, as shown in Chart 2.

The amino-thiol **4** was coupled with the keto-ester **5a**[41,42] under slightly acidic conditions to obtain the key tricyclic compound **6a** in a fashion similar to the method described in previous papers.[4,5]

The other key tricyclic compounds **6b** and **6c** were obtained with the amino-thiol **4** and the other keto-ester **5b**[4] or the aldehyde-ester **5c**[2] instead of the keto-ester **5a**.

To synthesize compound **6b**, which has the acetoxymethyl moiety as a side chain, reaction for amide formation was carried out by adding *p*-toluene sulfonic acid, and toluene following the first *N*,*S*-sipro acetal formation step. Compound **6c** was obtained in toluene–H_2O in the presence of *p*-toluene sulfonic acid.

These key tricyclic compounds **6a**—**c** were converted to the desired compounds **7a**—**c** by deprotection reaction of the *N*-benzyl moiety with α-chloroethyl chloroformate and by the pyridine coupling reaction, as shown in Chart 2.

Meanwhile, the *N*,SO_2-spiro acetal compounds **10a**—**c** were converted from the key tricyclic compounds **6a**—**c** as shown in Chart 3.

Compounds **8a**—**c**, which were derived from the key tricyclic compounds **6a**—**c** by deprotection reaction of the *N*-benzyl group and by inducing the Boc group on the piperidine ring, were oxidized with *m*-chloroperoxybenzoic acid to obtain the *N*,SO_2-spiro acetal compounds **9a**—**c**, respectively.

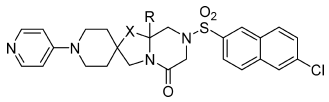
The Boc group in compounds **9a**—**c** was removed in trifluoroacetic acid, and pyridine coupling reaction was carried out to obtain compounds **10a**—**c**, respectively.

Results and Discussion

The FXa inhibitory activities of new compounds with the *N*,*S*- and the *N*,SO_2-spiro acetal structure synthesized in the present investigation were measured using the same method as described in previous papers.[4,5] Results were summarized in Table 2.

As might have been expected, the *N*,*S*- and the *N*,SO_2-spiro acetal compounds **7a** and **10a**, with methoxymethyl moiety as a side chain, had high activity (IC_{50}; **7a**: 1.3 nM, **10a**: 2.3 nM). These activities were almost equivalent compared with

Table 2. Comparison of FXa Inhibitory Activity of Cyclic *N*,*S*- and *N*,SO_2-Spiro Acetal Compounds



Compound	X	R	IC_{50} (nM)
7a	S	CH_2OMe	1.3
10a	SO_2	CH_2OMe	2.3
11	NH	CH_2OMe	1.5[5]
12	NMe	CH_2OMe	1.6[5]
13	O	CH_2OMe	5.0[4]
7b	S	CH_2OH	1.2
10b	SO_2	CH_2OH	1.9
14	NH	CH_2OH	2.4[5]
15	NMe	CH_2OH	1.7[5]
16	O	CH_2OH	5.0[4]
7c	S	H	1.5
10c	SO_2	H	3.0
17	NH	H	5.7[5]
18	NMe	H	18.2[5]
19	O	H	17.2[5]

N,*N*-acetal compounds **11** and **12**,[5] and were superior to *N*,*O*-acetal compound **13**.[4]

Compound **7b** and **10b**, which had hydroxymethyl moiety as a side chain, also inhibited activity of FXa (IC_{50}; **7b**: 1.2 nM, **10b**: 1.9 nM). These compounds also had approximately equivalent activity compared to corresponding *N*,*N*-acetal compounds **14** and **15**,[5] and had slightly higher activity than corresponding *N*,*O*-acetal compound **16**.[4]

In addition, compound **7c** and **10c**, in which a substituent was changed to a proton from the methoxymethyl or the hydroxymethyl moiety as a side chain, had high activities (IC_{50}; **7c**: 1.5 nM, **10c**: 3.0 nM). Although corresponding *N*,*O*- or *N*,*N*-acetal compounds **17**—**19** tended to reduce their activity,[5] compounds **7c** and **10c** could maintain high activity.

Based on these results, we have thought that the features of *N*,*S*- and *N*,SO_2-spiro acetal scaffolds were as below.

First, sulfur atom is larger than nitrogen or oxygen atom and thus both C–S(O_2) bond length must be longer than C–O or C–N bond. In addition, the bond angles of C_2–S(O_2)–C_{8a} must be smaller than C_2–N–C_{8a} or C_2–O–C_{8a} angles as mentioned in Table 1. Therefore, conformations of the *N*,*S*- and the *N*,SO_2-acetal scaffolds could be slightly changed and the overall conformations of these compounds could be more advantageous for inhibiting the FXa active site.

As shown in Fig. 1, it is essential for FXa inhibitory activity that the pyridine ring on the compound is located in the S4 pocket and that the naphthyl ring enters into the S1 pocket. In addition, the halogen-π interaction between the chlorine atom on the naphthyl ring of the compound and phenyl ring on Tyr228, which exists in the bottom of S1 pocket, is important for this activity. The tricyclic scaffold including *N*,*X*-acetal structures (*X*=*O*, *N*, or *S*(O_2)) plays a role of a linker which connects pyridine ring and naphthyl ring being suitable position. In particular, the *N*,*S*- and *N*,SO_2-acetal scaffolds could fit the above pharmacophore.

Second, one of the reasons why compounds **7c** and **10c**, without a side chain, also had high activity might be that not only overall conformations of *N*,*S*- and *N*,SO_2-acetal scaffolds could be more suitable, but also they could widely occupy S2/S3 regions where the tricyclic scaffold located.

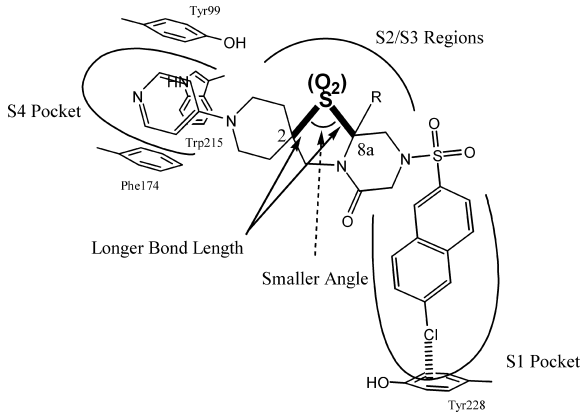


Fig. 1. Overall conformations of *N*,*S*- or *N*,SO_2-acetal scaffolds are better than *N*,*N*- or *N*,*O*-acetal scaffolds for binding in S1 and S4 pockets of FXa.

In contrast, the *N*,*O*- or *N*,*N*- acetal scaffolds, without a side chain, might be slightly smaller for that steric occupation[43] and they might have less suitable overall conformations for binding with FXa active site. Hence the *N*,*O*- or *N*,*N*-acetal scaffolds would generally need a side chain (R: in Fig. 1) like the methoxymethyl or hydroxymethyl moieties to maintain the FXa inhibitory activity.

In conclusion, we have reported novel active FXa inhibitors which have *N*,*S*- and *N*,SO_2-spiro acetal structures as analogues of *N*,*O*- and *N*,*N*-spiro acetals and demonstrated that both compounds with and without side chains have high FXa inhibitory activity.

Further investigations regarding other *N*,*X*-spiro acetal parts are going in our laboratory and results will be published in due course.

Experimental

Melting points (mp) were determined by using METTLER FP82 hotstage melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were taken with JEOL JNM-EX270 FT-NMR or JEOL JNM-LA300 in $CDCl_3$, dimethyl sulfoxide-d_6 (DMSO-d_6) using tetramethylsilane as the internal reference. Mass spectra (MS) were obtained using BRUKER Auto FLEX TOF/TOF or Waters FranctionLynx® MS System (LC/ESI-MS). Infrared absorption spectra (IR) were run using HORIBA FT-720 FT-IR.

Measurement of Factor Xa Inhibition Enzyme solution was mixed with a test compound dissolved at various concentrations in dimethyl sulfoxide (DMSO). Synthetic substrate was added and incubated in a 20 mM Tris–HCl buffer (pH 7.5) containing 0.13 M NaCl at 37 °C. The absorbance at 405 nm was measured continuously. Enzyme and substrate were used as follows: human factor Xa (Enzyme Research Laboratories, Inc., 0.019 U/ml) and S-2222 (Chromogenix AB, 0.4 mM). To calculate the inhibitory activity of the test compound, the initial reaction velocity was compared with the value for a control containing no test compound. The inhibitory activity of a test compound was expressed as IC_{50}.

4-Carbamoyl-4-[[(4-methoxyphenyl)methyl]thio]piperidine-1-carboxylic Acid *tert*-butyl Ester (2) To the solution of compound **1**,[39] (2.8 g) in $CHCl_3$ (50 ml) were added Et_3N (1.23 ml) and ethyl chloroformate (0.77 ml) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. Then 6.5 N NH_3–EtOH (5.6 ml) was added into the reaction mixture and the reaction mixture was stirred for 30 min at room temperature. To the reaction mixture was added water at 0 °C and the reaction mixture was extracted with CH_2Cl_2. The organic solvent was washed with 1 N HCl, water, saturated $NaHCO_3$ aqueous solution, and brine, respectively and dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel flash column chromatography (eluant: Hex/AcOEt=1/1) to afford compound **2** (2.12 g, 76% yield) as a colorless amorphous solid.

^{1}H-NMR (270 MHz, DMSO-d_6, 100 °C) δ: 7.23—7.14 (2H, m), 6.95 (2H, br s), 6.88—6.78 (2H, m), 3.73 (3H, s), 3.69 (2H, s), 3.52—3.39 (2H, m),

3.34—3.22 (2H, m), 2.12—1.99 (2H, m), 1.75—1.62 (2H, m), 1.40 (9H, s).

4-[[(4-Methoxyphenyl)methyl]thio]-1-(phenymethyl)piperidine-4-carboxamide (3) [Step 1]: To the compound **2** (2.47 g) was added 10% HCl–MeOH (40 ml) and the reaction mixture was stirred at room temperature overnight. Additionally, 10% HCl–MeOH (20 ml) was added to the reaction mixture and the reaction mixture was stirred at 30 °C for 2 h. The reaction mixture was concentrated *in vacuo*. To the resulting residue was washed with Et_2O and the supernatant was decanted. The remaining Et_2O was removed under reduced pressure to afford the deprotected compound—hydrochloric acid salt (1.87 g, 91% yield) as a colorless amorphous solid.

^{1}H-NMR (300 MHz, DMSO-d_6, 100 °C) δ: 8.91 (2H, m), 7.25—7.15 (2H, m), 7.14 (1H, br s), 6.88—6.82 (2H, m), 3.75 (3H, s), 3.71 (2H, s), 3.22—3.12 (2H, m), 3.03—2.88 (2H, m), 2.39—2.27 (2H, m), 2.07—1.05 (2H, m).

[Step 2]: The deprotected compound (1.8 g) which was afforded in Step 1, was dissolved in *N*,*N*-dimethylformamide (80 ml). To the reaction mixture were added K_2CO_3 (1.96 g) and benzyl bromide (0.74 ml) at 0 °C. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* to approximately 20 ml volume and 1 N HCl was added to the concentrated mixture. Then this mixture was washed with AcOEt for removing excess benzyl bromide and the water layer was adjusted to pH 10 with 1 N NaOH and with saturated $NaHCO_3$ aqueous solution. The water layer was extracted with AcOEt and the organic layer was washed with water, brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel flash column chromatography (eluant: CH_2Cl_2/MeOH=98/2—95/5—90/10) to afford compound **3** (1.74 g, 83% yield) as a colorless amorphous solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.34—7.17 (7H, m), 6.86—6.79 (2H, m), 6.38 (1H, br s), 5.34 (1H, br s), 3.79 (3H, s), 3.66 (2H, s), 3.51 (2H, s), 2.67—2.45 (4H, m), 2.32—2.19 (2H, m), 1.90—1.78 (2H, m).

4-Aminomethyl-1-(phenylmethyl)piperidine-4-thiol bis(trifluoromethanesulfonate) (4) [Step 1]: To a suspension of $LiAlH_4$ (0.88 g) in Et_2O (15 ml) was dropwised the suspension of compound **3** (1.23 g) in Et_2O (18 ml) at 0 °C. The reaction mixture was stirred at room temperature for 15 min and then was refluxed 3.5 h. After cooling the reaction mixture, water (0.9 ml), 1 N NaOH (0.9 ml), and water (2.7 ml) were added to the reaction mixture at 0 °C for quenching. Additionally, Et_2O was added to the mixture and stirred at room temperature for 30 min. Then insoluble matter was removed by Celite® filtration and was washed with THF and Et_2O. The filtrate was concentrated *in vacuo* to afford amino-compound (1.12 g, 95% yield) as a colorless amorphous solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.34—7.20 (7H, m), 6.87—6.80 (2H, m), 3.79 (3H, s), 3.54 (2H, s), 3.54 (2H, s), 2.68—2.48 (4H, m), 2.65 (2H, s), 1.79—1.55 (4H, m).

[Step 2]: The amino-compound (0.69 g) which was afforded in Step 1, was dissolved in trifluoroacetic acid (10 ml). To the reaction mixture were added anisole (0.69 ml) and trifluoromethansulfonic acid (6.9 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 40 min. Then to the reaction mixture was added Et_2O for precipitating compound **4**. The supernatant was decanted and The remaining Et_2O was removed under reduced pressure to afford the compound **4** (0.89 g, 86% yield) as a colorless amorphous solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.35—7.20 (5H, m), 7.17 (2H, br s), 3.55 (2H, s), 2.75—2.65 (2H, m), 2.72 (2H, s), 2.52—2.40 (2H, m), 1.76—1.58 (4H, m).

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(phenylmethyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (6a) The solution of compound **4** (0.79 g) and compound **5a** (0.61 g) in EtOH (12 ml) was refluxed for 1 h. Then sodium acetate (0.12 g) was added into the reaction mixture and the reaction mixture was refluxed for another hour. The reaction mixture was poured into a sealed tube and toluene (11 ml) was additionally added to the reaction mixture. It was stirred at 150—160 °C for 2.5 h. After cooling, the reaction mixture was concentrated *in vacuo* and the resulting residue was purified by silica gel flash column chromatography (eluant: CH_2Cl_2/MeOH=99.5/0.5—99/1—98.5/1.5—98/2) to afford compound **6a** (0.70 g, 72% yield) as a pale yellow amorphous solid.

MALDI-TOF-HR-MS *m/z* (M+H): Calcd for $C_{29}H_{33}{}^{35}ClN_3O_4S_2$: 586.1601. Found: 586.1572. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.17—8.12 (1H, m), 8.00—7.87 (3H, m), 7.78 (1H, dd, *J*=1.8, 8.8 Hz), 7.60 (1H, dd, *J*=2.0, 8.8 Hz), 7.32—7.18 (5H, m), 4.68 (1H, d, *J*=12.1 Hz), 4.39 (1H, d, *J*=12.1 Hz), 4.32 (1H, d, *J*=17.1 Hz), 3.88 (1H, d, *J*=9.0 Hz), 3.54 (1H, d, *J*=9.0 Hz), 3.45 (3H, s), 3.45 (2H, s), 3.34 (1H, d, *J*=17.1 Hz), 3.13 (1H, d, *J*=12.1 Hz), 2.72—2.47 (2H, m), 2.56 (1H, d, *J*=12.1 Hz), 2.30—2.05 (2H, m), 1.98—1.80 (2H, m), 1.70—1.40 (2H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.85, 138.06, 135.69, 135.49, 133.01, 130.84, 130.47, 129.05, 129.02 (3C), 128.98, 128.23 (2C), 127.11, 126.84, 123.55, 74.99, 71.68, 62.86, 59.67, 56.81, 56.67, 52.30, 51.01, 50.72, 47.80, 38.50, 36.28. IR (KBr) cm^{-1}: 1664, 1452, 1410, 1350, 1165, 1119, 1078, 729, 692.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (7a) [Step 1]: To the solution of compound **6a** (0.45 g) in 1,2-dichloroethane (9 ml), were added 1,8-bis(dimethylamino)naphthalene (0.033 g) and α-chloroethyl chloroformate (0.21 ml) at 0 °C. The reaction mixture was stirred at room temperature for 30 min then was refluxed for 1 h. It was concentrated *in vacuo*. To the resulting residue was added MeOH (9 ml) and the mixture was refluxed for 30 min. After cooling, the reaction mixture was concentrated *in vacuo*. To the resulting residue was added Et_2O for precipitation. The supernatant was decanted. Then the above washing was carried out several times, the precipitate was collected by filtration. The precipitate was dissolved in water and the aqueous solution was made basic to pH 9—10 with saturated $NaHCO_3$ aqueous solution. The aqueous solution was extracted with CH_2Cl_2 and the organic layer was washed with brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by amino-silica gel column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: Hex/CH_2Cl_2=1/1—0/100) to afford deprotected compound (0.34 g, 90% yield) as a colorless amorphous solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.38—8.33 (1H, m), 8.00—7.90 (3H, m), 7.79 (1H, dd, *J*=1.8, 8.6 Hz), 7.61 (1H, dd, *J*=2.2, 8.6 Hz), 4.70 (1H, d, *J*=12.3 Hz), 4.41 (1H, d, *J*=12.1 Hz), 4.34 (1H, d, *J*=17.1 Hz), 3.89 (1H, d, *J*=9.0 Hz), 3.46 (3H, s), 3.35 (1H, d, *J*=17.1 Hz), 3.13 (1H, d, *J*=12.3 Hz), 3.00—2.58 (4H, m), 2.58 (1H, d, *J*=12.1 Hz), 1.90—1.74 (2H, m), 1.60—1.40 (2H, m).

[Step 2]: To a solution of the deprotected compound (50 mg) which was afforded in Step 1 in EtOH (0.8 ml) were added 4-chloropyridine hydrochloride (23 mg) and *i*-Pr_2NEt (88 μl). The mixture was stirred at 150—160 °C in a sealed tube for 4 h then it was concentrated *in vacuo*. The resulting residue was purified by amino-silica gel column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: Hex/CH_2Cl_2=1/1—CH_2Cl_2–CH_2Cl_2/MeOH=99/1) then was re-purified by silica gel column chromatography (eluant: CH_2Cl_2–CH_2Cl_2/MeOH=99/1—98/2) to afford compound **7a** (14 mg, 24% yield) as a colorless amorphous solid.

MALDI-TOF-HR-MS *m/z* (M+H): Calcd for $C_{27}H_{30}{}^{35}ClN_4O_4S_2$: 573.1397. Found: 573.1426. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.37 (1H, s), 8.24 (2H, d, *J*=6.2 Hz), 7.80 (1H, dd, *J*=1.5, 8.6 Hz), 7.62 (1H, dd, *J*=2.0, 8.6 Hz), 6.59 (2H, d, *J*=6.2 Hz), 4.72 (1H, d, *J*=12.5 Hz), 4.43 (1H, d, *J*=12.3 Hz), 4.36 (1H, d, *J*=17.1 Hz), 3.91 (1H, d, *J*=8.8 Hz), 3.75—3.43 (2H, m), 3,59 (1H, d, *J*=8.8 Hz), 3.47 (3H, s), 3.38 (1H, d, *J*=17.1 Hz), 3.22 (1H, d, *J*=12.5 Hz), 3.15—2.90 (2H, m), 2.61 (1H, d, *J*=12.3 Hz), 2.05—1.90 (2H, m), 1.75—1.50 (2H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.97, 154.35, 150.31 (2C), 135.72, 135.60, 133.01, 130.85, 130.47, 129.13, 129.05, 129.03, 126.84, 123.53, 108.63 (2C), 74.98, 72.20, 59.70, 56.83, 56.74, 52.04, 47.90, 45.63, 43.90, 37.45, 35.24. IR (film) cm^{-1}: 1662, 1597, 1462, 1412, 1348, 1240, 1165, 970, 692.

8*a*-(Acetoxymethyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-1′-(phenylmethyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (6b) To the solution of compound **4** (5.0 g) and compound **5b** (4.1 g) in EtOH (50 ml) was added sodium acetate (1.5 g). The reaction mixture was refluxed for 4 h. Then the reaction mixture was concentrated *in vacuo*. The resulting mixture was dissolved in toluene (500 ml) and TsOH–H_2O (20 mg) was added into the solution. The reaction mixture was refluxed for 1 h. After cooling, to the reaction mixture was added water and the mixture was extracted with AcOEt. The organic layer was washed with brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel flash column chromatography (eluant: Hex/AcOEt=70/30—50/50—30/70) to afford compound **6b** (4.0 g, 70% yield) as a colorless amorphous solid.

MALDI-TOF-HR-MS *m/z* (M+H): Calcd for $C_{30}H_{33}{}^{35}ClN_3O_5S_2$: 614.1550. Found: 614.1580. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.37—8.33 (1H, m), 7.98—7.90 (3H, m), 7.77 (1H, dd, *J*=1.8, 8.8 Hz), 7.61 (1H, dd, *J*=2.2, 8.8 Hz), 7.3—7.18 (5H, m), 4.70 (1H, d, *J*=12.5 Hz), 4.46—4.25 (4H, m), 3.44 (2H, s), 3.36 (1H, d, *J*=17.1 Hz), 3.16 (1H, d, *J*=12.5 Hz), 2.71 (1H, d, *J*=12.5 Hz), 2.70—2.50 (2H, m), 2.10 (3H, s), 2.28—2.03 (2H, m), 1.97—1.80 (2H, m), 1.70—1.58 (1H, m), 1.57—1.45 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 169.98, 162.64, 137.96, 135.73, 135.59, 132.86, 130.83, 130.43, 129.14, 129.11, 129.07, 129.00 (2C), 128.25 (2C), 127.14,

126.85, 123.50, 70.62, 65.96, 62.85, 57.44, 56.36, 52.35, 51.54, 50.76, 47.69, 38.67, 36.40, 20.74. IR (KBr) cm^{-1}: 1747, 1668, 1454, 1408, 1225, 1165, 1078, 694.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(hydroxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (7b) [Step 1]: To the solution of compound **6b** (4.0 g) in CH_2Cl_2 (80 ml), were added 1,8-bis(dimethylamino)naphthalene (0.28 g) and α-chloroethyl chloroformate (1.78 ml) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*. MeOH (80 ml) was added to the resulting residue and the reaction mixture was refluxed for 1 h. After cooling, the reaction mixture was concentrated *in vacuo*. To the resulting residue was added Et_2O for precipitation. The supernatant was decanted. Then the above washing was carried out several times, the precipitate was dissolved in EtOH (40 ml) and 1 N NaOH (40 ml). While stirring the above solution, the precipitate was appeared. The precipitate was collected by filtration to afford deprotected compound (2.7 g, 86% yield) as a pale yellow amorphous solid.

^{1}H-NMR (270 MHz, DMSO-d_6) δ: 8.59 (1H, s), 8.35—8.13 (3H, m), 7.89 (1H, dd, J=2.0, 8.9 Hz), 7.74 (1H, dd, J=2.3, 8.9 Hz), 5.50 (1H, br s), 4.50 (1H, d, J=11.9 Hz), 4.15 (1H, d, J=12.2 Hz), 3.99 (1H, d, J=16.2 Hz), 3.77 (1H, dd, J=4.3, 10.6 Hz), 3.67—3.45 (2H, m), 3.13 (1H, d, J=12.2 Hz), 3.00—2.30 (4H, m), 2.85 (1H, d, J=11.9 Hz), 1.80—1.60 (2H, m), 1.55—1.28 (2H, m).

[Step 2]: To a solution of the deprotected compound (0.43 g) which was afforded in Step 1 in EtOH (20 ml) were added 4-chloropyridine hydrochloride (0.20 g) and *i*-Pr_2NEt (0.78 ml). The mixture was stirred at 140—160 °C in a sealed tube for 9 h then it was concentrated *in vacuo*. The resulting residue was purified by amino-silica gel column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: AcOEt–AcOEt/MeOH=90/10) to afford compound **7b** (58 mg, 12% yield) as a colorless amorphous solid.

MALDI-TOF-HR-MS m/z (M+H): Calcd for $C_{26}H_{28}{}^{35}ClN_4O_4S_2$: 559.1240. Found: 559.1218. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.40—8.39 (1H, m), 8.28—8.22 (2H, m), 8.00—7.93 (3H, m), 7.80 (1H, dd, J=1.8, 8.6 Hz), 7.63 (1H, dd, J=2.0, 9.0 Hz), 6.66—6.58 (2H, m), 4.74 (1H, d, J=12.5 Hz), 4.48—4.33 (2H, m), 4.17 (1H, d, J=11.2 Hz), 3.80 (1H, d, J=11.2 Hz), 3.78—3.66 (1H, m), 3.64—3.54 (1H, m), 3.43 (1H, d, J=16.9 Hz), 3.24 (1H, d, J=12.5 Hz), 3.18—2.97 (2H, m), 2.71 (1H, d, J=12.7 Hz), 2.05—1.94 (2H, m), 1.85—1.45 (2H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.70, 154.33, 149.98 (2C), 135.71, 135.63, 133.11, 130.80, 130.43, 129.16, 129.10, 128.94, 126.84, 123.32, 108.61 (2C), 74.07, 65.82, 57.39, 56.65, 50.67, 47.85, 45.61, 43.94, 37.65, 35.51. IR (film) cm^{-1}: 1657, 1599, 1462, 1346, 1165, 692.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-1′-(phenylmethyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (6c) To the suspension of compound **4** (5.0 g) and compound **5c** (3.45 g) in toluene (500 ml) was added sodium acetate (1.53 g). Water (5 ml) was added into the mixture. The reaction mixture was stirred at room temperature for 1 h then was refluxed for 2 h. Then the reaction mixture was concentrated *in vacuo*. To the resulting mixture was added water and the mixture was extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ aqueous solution, brine, and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and Et_2O was added to the resulting residue for crystallization. The precipitate was collected by filtration to afford compound **6c** (4.7 g, 93% yield) as colorless crystals.

mp 170.5—171.8 °C. MALDI-TOF-HR-MS m/z (M+H): Calcd for $C_{27}H_{29}{}^{35}ClN_3O_3S_2$: 542.1339. Found: 542.1389. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.37—8.32 (1H, m), 8.00—7.90 (3H, m), 7.77 (1H, dd, J=1.8, 8.8 Hz), 7.60 (1H, dd, J=2.0, 9.0 Hz), 7.35—7.20 (5H, m), 5.06 (1H, dd, J=4.2, 10.3 Hz), 4.40 (1H, d, J=12.3 Hz), 4.37—4.20 (2H, m), 3.47 (2H, s), 3.33 (1H, d, J=16.3 Hz), 3.13 (1H, d, J=12.3 Hz), 2.75—2.50 (2H, m), 2.57 (1H, dd, J=10.3, 12.3 Hz), 2.30—2.10 (2H, m), 2.00—1.50 (4H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.23, 138.01, 135.70, 135.55, 132.87, 130.82, 130.43, 129.13, 129.09, 129.03 (3C), 128.27 (2C), 127.15, 126.84, 123.54, 62.89, 59.45, 57.72, 55.87, 51.99, 51.15, 49.70, 47.90, 38.41, 36.54. IR (KBr) cm^{-1}: 1662, 1421, 1350, 1271, 1165, 970, 694.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-1′-(4-pyridinyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (7c) [Step 1]: To the solution of compound **6c** (4.7 g) in CH_2Cl_2 (50 ml), were added 1,8-bis(dimethylamino)naphthalene (0.37 g) and α-chloroethyl chloroformate (2.4 ml) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and MeOH (70 ml) was added to the resulting residue and the mixture was refluxed for 4 h. After cooling, the reaction mixture was concentrated *in vacuo*. To the resulting residue was added Et_2O for precipitation. The precipitate was collected by filtration and the filtrate was dissolved in saturated $NaHCO_3$ aqueous solution. The aqueous solution was extracted with AcOEt and the organic layer was washed with water and brine and then was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by amino-silica gel column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: Hex/AcOEt=1/1—AcOEt–AcOEt/MeOH=90/10) to afford the deprotected compound (3.9 g, quant.) as a pale red amorphous solid.

^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.35 (1H, d, J=2.0 Hz), 8.99—8.91 (3H, m), 7.78 (1H, dd, J=2.0, 8.6 Hz), 7.61 (1H, dd, J=2.0, 8.6 Hz), 5.08 (1H, dd, J=4.3, 10.2 Hz), 4.43 (1H, d, J=12.2 Hz), 4.32 (1H, d, J=16.8 Hz), 4.32—4.22 (1H, m), 3.34 (1H, d, J=16.7 Hz), 3.12 (1H, d, J=12.2 Hz), 3.05—2.85 (2H, m), 2.80—2.65 (2H, m), 2.58 (1H, dd, J=10.2, 12.2 Hz), 1.90—1.35 (4H, m).

[Step 2]: To a solution of the deprotected compound (1.0 g) which was afforded in Step 1 in EtOH (20 ml) were added 4-chloropyridine hydrochloride (0.5 g) and *i*-Pr_2NEt (1.9 ml). The mixture was stirred at 140—160 °C in a sealed tube for 3 h then it was concentrated *in vacuo*. The resulting residue was purified by amino-silica gel column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: Hex/AcOEt=1/2—AcOEt–AcOEt/MeOH=95/5) to afford compound **7c** (163 mg, 14% yield) as a colorless amorphous solid.

MALDI-TOF-HR-MS m/z (M+H): Calcd for $C_{25}H_{26}{}^{35}ClN_4O_3S_2$: 529.1135. Found: 529.1110. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.36 (1H, s), 8.26 (2H, d, J=5.9 Hz), 8.02—7.90 (3H, m), 7.84—7.75 (1H, m), 7.67—7.58 (1H, m), 6.62 (2H, d, J=5.9 Hz), 5.20—5.10 (1H, m), 4.48 (1H, d, J=12.3 Hz), 4.40—4.25 (2H, m), 3.80—3.55 (2H, m), 3.38 (1H, d, J=16.9 Hz), 3.18 (1H, d, J=12.3 Hz), 3.15—2.97 (2H, m), 2.67—2.55 (1H, m), 2.05—1.90 (2H, m), 1.87—1.60 (2H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.33, 154.31, 150.30 (2C), 135.68, 135.58, 132.84, 130.79, 130.39, 129.11 (2C), 129.02, 126.80, 123.46, 108.63 (2C), 59.62, 57.66, 55.90, 49.60, 47.93, 45.27, 44.24, 37.38, 35.46. IR (film) cm^{-1}: 1664, 1597, 1421, 1348, 1163, 694.

1′-(*tert*-Butoxycarbonyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]-tetrahydro-8*a*-(methoxymethyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (8a)The deprotected compound (100 mg), which was obtained in the synthesis of compound **7a**; Step 1, was dissolved in CH_2Cl_2 (2 ml). To this solution was added di-*t*-butyl dicarbonate (44.1 mg) at 0 °C. The reaction mixture was stirred at room temperature overnight. Then it was concentrated *in vacuo* and the resulting mixture was purified by silica gel flash column chromatography (eluant: CH_2Cl_2–CH_2Cl_2/MeOH=98/2) to afford compound **8a** (107 mg, 89% yield) as a colorless amorphous solid.

ESI-MS m/z (M+H): 596 (M^++H), 496 (M^+−Boc+H). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.38—8.33 (1H, m), 8.00—7.91 (3H, m), 7.79 (1H, dd, J=1.7, 8.8 Hz), 7.61 (1H, dd, J=2.0, 8.8 Hz), 4.67 (1H, d, J=12.3 Hz), 4.45—4.30 (2H, m), 3.95—3.68 (3H, m), 3.57 (1H, d, J=8.4 Hz), 3.46 (3H, s), 3.35 (1H, d, J=17.0 Hz), 3.17 (1H, d, J=12.3 Hz), 3.10—2.83 (2H, m), 2.57 (1H, d, J=11.9 Hz), 1.87—1.75 (2H, m), 1.68—1.35 (2H, m), 1.41 (9H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.92, 154.46, 135.72, 135.56, 132.97, 130.84, 130.48, 129.11, 129.04 (2C), 126.84, 123.51, 79.94, 74.98, 72.02, 59.69, 57.06, 56.87, 51.02, 47.85, 42.88, 40.97, 38.13, 36.00, 28.36 (3C). IR (KBr) cm^{-1}: 1689, 1672, 1412, 1350, 1240, 1167, 1078, 692.

1′-(*tert*-Butoxycarbonyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]-tetrahydro-8*a*-(methoxymethyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one 1,1-dioxide (9a) To the solution of compound **8a** (95 mg) in CH_2Cl_2 (9.5 ml), was added *m*-chloroperoxybenzoic acid (77 mg) at 0 °C. The reaction mixture was stirred at 0 °C for 50 min and then at room temperature for 30 min. To the reaction mixture was added saturated $NaHCO_3$ aqueous solution and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by amino-silica gel flash column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: Hex/AcOEt=1/1—1/2—AcOEt) to afford compound **9a** (78 mg, 78% yield) as a pale yellow amorphous solid.

ESI-MS m/z (M+H): 528 (M^+−Boc+H). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.35 (1H, d, J=1.8 Hz), 8.00—7.92 (3H, m), 7.78 (1H, dd, J=1.8, 8.8 Hz), 7.62 (1H, dd, J=1.8, 8.8 Hz), 4.83 (1H, d, J=13.0 Hz), 4.39 (1H, d, J=17.1 Hz), 4.26 (1H, d, J=12.7 Hz), 4.04 (1H, d, J=10.5 Hz), 3.94 (1H, d, J=10.5 Hz), 3.82—3.63 (2H, m), 3.42 (3H, s), 3.37 (1H, d, J=17.1 Hz), 3.35—

3.15 (3H, m), 2.89 (1H, d, J=12.7 Hz), 2.13—2.00 (1H, m), 1.93—1.30 (3H, m), 1.42 (9H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 163.32, 154.24, 135.84, 135.80, 132.36, 130.88, 130.46, 129.26, 129.24 (2C), 126.88, 123.40, 80.32, 75.82, 71.74, 60.70, 59.62, 48.90, 48.55, 45.56, 40.79, 40.41, 30.22, 29.21, 28.33 (3C). IR (KBr) cm^{-1}: 1685, 1408, 1352, 1246, 1167, 1136, 1080, 696.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one 1,1-dioxide (10a) [Step 1]: To the compound **9a** (220 mg) were added trifluoroacetic acid (2.2 ml) and anisole (1 drop) at 0 °C. The reaction mixture was stirred at room temperature for 10 min. To the reaction mixture were added 1 N NaOH and saturated $NaHCO_3$ aqueous solution for adjusting to more than pH 10 and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by silica gel flash column chromatography (eluant: CH_2Cl_2–CH_2Cl_2/MeOH=97/3—95/5) to afford the deprotected compound (176 mg, 95% yield) as a colorless amorphous solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.38—8.35 (1H, m), 8.00—7.90 (3H, m), 7.78 (1H, dd, J=1.8, 8.6 Hz), 7.62 (1H, dd, J=2.0, 8.6 Hz), 4.91 (1H, d, J=13.2 Hz), 4.38 (1H, d, J=17.1 Hz), 4.28 (1H, dd, J=1.7, 12.7 Hz), 4.01 (1H, d, J=10.5 Hz), 3.92 (1H, d, J=10.5 Hz), 3.42 (3H, s), 3.37 (1H, d, J=17.1 Hz), 3.26 (1H, d, J=13.2 Hz), 3.13—2.97 (2H, m), 2.88 (1H, d, J=12.7 Hz), 2.81—2.63 (2H, m), 2.08—1.96 (1H, m), 1.88—1.71 (2H, m), 1.40—1.28 (1H, m).

[Step 2]: To the solution of the deprotected compound (72 mg) which was afforded in Step 1 in EtOH (1.4 ml) were added 4-chloropyridine hydrochloride (31 mg) and *i*-Pr_2NEt (120 μl). The mixture was stirred at 150—160 °C in a sealed tube for 5 h then it was concentrated *in vacuo*. The resulting residue was purified by amino-silica gel column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: CH_2Cl_2/MeOH=99/1) and then was re-purified by silica gel flash column chromatography (eluant: CH_2Cl_2/MeOH=50/1—30/1—25/1) to afford compound **10a** (10.4 mg, 13% yield) as a colorless amorphous solid.

MALDI-TOF-HR-MS m/z (M+H): Calcd for $C_{27}H_{30}{}^{35}ClN_4O_6S_2$: 605.1295. Found: 605.1252. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.36 (1H, s), 8.27 (2H, d, J=5.1 Hz), 8.02—7.90 (3H, m), 7.78 (1H, d, J=8.8 Hz), 7.63 (1H, d, J=8.8 Hz), 6.61 (2H, d, J=5.1 Hz), 4.85 (1H, d, J=13.0 Hz), 4.40 (1H, d, J=16.5 Hz), 4.24 (1H, d, J=12.8 Hz), 4.07 (1H, d, J=10.5 Hz), 3.96 (1H, d, J=10.5 Hz), 3.73—3.52 (2H, m), 3.50—3.17 (4H, m), 3.44 (3H, s), 2.94 (1H, d, J=13.0 Hz), 2.30—2.15 (1H, m), 2.08—1.80 (2H, m), 1.75—1.44 (1H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 163.39, 153.84, 150.51 (2C), 135.83 (2C), 132.41, 130.89, 130.46, 129.27, 129.24 (2C), 126.87, 123.38, 108.69 (2C), 75.94, 71.93, 60.41, 59.64, 49.19, 48.59, 45.53, 43.58, 43.45, 29.65, 28.41 IR (film) cm^{-1} : 1678, 1599, 1402, 1350, 1167, 1130, 733, 696, 594.

1′-(*tert*-Butoxycarbonyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]-tetrahydro-8*a*-(hydroxymethyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (8b) The deprotected compound (2.0 g), which was obtained in the synthesis of compound **7b**; Step 1, was dissolved in CH_2Cl_2 (50 ml). To this solution was added di-*t*-butyl dicarbonate (0.91 g) at 0 °C. The reaction mixture was stirred at room temperature overnight. Then it was concentrated *in vacuo* and the resulting mixture was purified by silica gel column chromatography (eluant: Hex/AcOEt=1/1) to afford compound **8b** (2.5 g, quant.) as a pale yellow amorphous solid.

ESI-MS m/z (M+H): 582 (M^++H), 482 (M^+−Boc+H). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.37—8.33 (1H, m), 8.00—7.90 (3H, m), 7.78 (1H, dd, J=1.8, 8.6 Hz), 7.61 (1H, dd, J=2.2, 8.6 Hz), 4.69 (1H, d, J=12.5 Hz), 4.46—4.32 (2H, m), 4.20—4.08 (1H, m), 3.95—3.65 (3H, m), 3.39 (1H, d, J=16.9 Hz), 3.20 (1H, d, J=12.5 Hz), 3.10—2.85 (2H, m), 2.78—2.65 (1H, m), 2.66 (1H, d, J=12.3 Hz), 1.88—1.77 (2H, m), 1.63—1.30 (2H, m), 1.42 (9H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.75, 154.47, 135.74, 135.60, 133.00, 130.86, 130.47, 129.12 (2C), 128.98, 126.86, 123.37, 80.01, 73.88, 65.77, 57.60, 56.79, 50.68, 47.84, 42.93, 41.08, 38.27, 36.17, 28.36 (3C). IR (KBr) cm^{-1}: 3420, 1685, 1666, 1415, 1350, 1242, 1165, 1078, 692.

1′-(*tert*-Butoxycarbonyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]-tetrahydro-8*a*-(hydroxymethyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one 1,1-dioxide (9b) To the solution of compound **8b** (2.1 g) in 1,2-dichloroethane (210 ml), was added *m*-chloroperoxybenzoic acid (1.7 g) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then at 65 °C for another hour. After cooling, silica gel was added into the reaction mixture. This suspension was concentrated *in vacuo* for removing solvent and then silica gel column chromatography was carried out (eluant: Hex/AcOEt=3/1—1/1) to afford compound **9b** (1.8 g, 83% yield) as a colorless amorphous solid.

ESI-MS m/z (M+H): 514 (M^+−Boc+H). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.40—8.36 (1H, m), 8.00—7.92 (3H, m), 7.79 (1H, dd, J=1.7, 8.6 Hz), 7.62 (1H, dd, J=2.0, 8.6 Hz), 4.95 (1H, d, J=13.4 Hz), 4.56 (1H, dd, J=1.7, 12.8 Hz), 4.41 (1H, d, J=17.2 Hz), 4.30—4.05 (2H, m), 3.82—3.65 (2H, m), 3.40 (1H, d, J=17.2 Hz), 3.32—3.15 (2H, m), 3.21 (1H, d, J=13.4 Hz), 2.98—2.88 (1H, m), 2.84 (1H, d, J=12.8 Hz), 2.13—2.01 (1H, m), 1.92—1.65 (2H, m), 1.50—1.30 (1H, m), 1.42 (9H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 163.22, 154.18, 135.84, 135.79, 132.54, 130.92, 130.47, 129.30, 129.25, 129.23, 126.89, 123.33, 80.46, 76.65, 61.92, 61.87, 48.54, 47.74, 44.84, 40.63, 40.40, 29.46, 28.72, 28.32 (3C). IR (KBr) cm^{-1}: 3404, 1672, 1414, 1350, 1304, 1248, 1169, 1134, 696.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(hydroxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one 1,1-dioxide (10b) [Step 1]: To the solution of compound **9b** (1.85 g) in CH_2Cl_2 (50 ml), were added trifluoroacetic acid (3.4 g) and anisole (32.6 mg) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. To the reaction mixture was added Et_2O and the resulting precipitate was collected by filtration to afford deprotected compound (1.9 g, quant.) as a colorless amorphous solid.

^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.83 (1H, br s), 8.63 (1H, s), 8.53 (1H, br s), 8.35—8.15 (3H, m), 7.92 (1H, dd, J=1.8, 8.8 Hz), 7.76 (1H, dd, J=2.0, 8.8 Hz), 4.94 (1H, d, J=13.9 Hz), 4.09 (1H, d, J=12.3 Hz), 4.01 (1H, d, J=16.5 Hz), 3.96 (1H, d, J=11.9 Hz), 3.85 (1H, d, J=11.9 Hz), 3.74 (1H, d, J=16.5 Hz), 3.40—3.00 (5H, m), 3.22 (1H, d, J=12.3 Hz), 2.10—1.83 (3H, m), 1.77—1.62 (1H, m).

[Step 2]: To the solution of the deprotected compound (50 mg) which was afforded in Step 1 in EtOH (3.0 ml) were added 4-chloropyridine hydrochloride (17.9 mg) and *i*-Pr_2NEt (51.4 mg). The mixture was stirred at 150—160 °C in a sealed tube for 4 h. After cooling, water was added into the reaction mixture and then the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The resulting residue was purified by preparative TLC (CH_2Cl_2/MeOH=4/1) to afford compound **10b** (4.8 mg, 10% yield) as a colorless amorphous solid.

MALDI-TOF-HR-MS m/z (M+H): Calcd for $C_{26}H_{28}{}^{35}ClN_4O_6S_2$: 591.1139. Found: 591.1100. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.39 (1H, s), 8.27 (2H, d, J=5.5 Hz), 8.05—7.92 (3H, m), 7.80 (1H, d, J=8.6 Hz), 7.63 (1H, d, J=8.6 Hz), 6.61 (2H, d, J=5.5 Hz), 4.97 (1H, d, J=13.8 Hz), 4.54 (1H, d, J=13.0 Hz), 4.42 (1H, d, J=17.2 Hz), 4.27 (1H, d, J=12.7 Hz), 4.16 (1H, d, J=12.7 Hz), 3.75—3.55 (2H, m), 3.43 (1H, d, J=17.2 Hz), 3.38—3.23 (2H, m), 3.28 (1H, d, J=13.8 Hz), 2.90 (1H, d, J=13.0 Hz), 2.35—1.67 (4H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 163.32, 153.84, 150.38 (2C), 141.49, 135.85, 132.59, 130.92, 130.47, 129.30, 129.27 (2C), 126.89, 123.34, 108.72 (2C), 76.84, 62.02, 61.53, 48.58, 48.02, 44.88, 43.53, 43.19, 28.91, 27.94. IR (film) cm^{-1}: 1670, 1600, 1456, 1338, 1167, 1128, 696.

1′-(*tert*-Butoxycarbonyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]-tetrahydro-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (8c) The deprotected compound (2.0 g), which was obtained in the synthesis of compound **7c**; Step 1, was dissolved in CH_2Cl_2 (50 ml). To this solution was added di-*t*-butyl dicarbonate (1.0 g) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. To the reaction mixture was added *n*-hexane for precipitation. The precipitate was collected by filtration to afford compound **8c** (2.3 g, 94% yield) as colorless crystals.

mp 212.9—214.2 °C. ESI-MS m/z (M+H): 552 (M^++H), 452 (M^+−Boc+H). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.35 (1H, d, J=2.0 Hz), 8.00—7.90 (3H, m), 7.78 (1H, dd, J=2.0, 8.8 Hz), 7.61 (1H, dd, J=2.2, 8.8 Hz), 5.11 (1H, dd, J=4.0, 10.3 Hz), 4.43 (1H, d, J=12.3 Hz), 4.33 (1H, d, J=16.7 Hz), 4.33—4.25 (1H, m), 4.00—3.70 (2H, m), 3.35 (1H, d, J=16.7 Hz), 3.14 (1H, d, J=12.3 Hz), 3.05—2.92 (2H, m), 2.59 (1H, dd, J=10.1, 12.3 Hz), 1.85—1.75 (2H, m), 1.70—1.35 (2H, m), 1.43 (9H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.34, 154.47, 135.73, 135.60, 132.85, 130.83, 130.44, 129.15, 129.12, 129.07, 126.85, 123.51, 80.00, 59.56, 57.85, 56.14, 49.69, 47.94, 42.58, 41.51, 38.06, 36.16, 28.37 (3C). IR (KBr) cm^{-1}: 1691, 1662, 1419, 1342, 1244, 1165, 962, 694.

1′-(*tert*-Butoxycarbonyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]-tetrahydro-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one 1,1-dioxide (9c) To the solution of compound **8c** (100 mg) in CH_2Cl_2 (10 ml), was added *m*-chloroperoxybenzoic acid (65.6 mg) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 30 min. To the reaction mixture was additionally added *m*-chloroperoxybenzoic acid (65.6 mg) and the reaction mixture was stirred at room temperature

for 3 h. To the reaction mixture was added saturated $NaHCO_3$ aqueous solution and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by amino-silica gel column chromatography (Fuji Silysia Chemical Ltd., Chromatorex NH®, eluant: Hex/AcOEt=1/1) to afford compound **9c** (82.2 mg, 78% yield) as colorless crystals.

mp 247.0—248.1 °C. ESI-MS *m/z* (M+H): 484 (M^+−Boc+H). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.40—8.33 (1H, m), 8.00—7.92 (3H, m), 7.78 (1H, dd, *J*=1.8, 8.8 Hz), 7.63 (1H, dd, *J*=2.0, 8.8 Hz), 4.66—4.56 (1H, m), 4.60 (1H, d, *J*=13.0 Hz), 4.50—4.40 (1H, m), 4.39 (1H, d, *J*=17.1 Hz), 3.83—3.65 (2H, m), 3.37 (1H, d, *J*=17.1 Hz), 3.37—3.23 (2H, m), 3.21 (1H, d, *J*=13.0 Hz), 2.92 (1H, dd, *J*=10.1, 12.7 Hz), 2.17—2.04 (1H, m), 2.00—1.87 (1H, m), 1.78—1.66 (1H, m), 1.63—1.35 (1H, m), 1.44 (9H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.70, 154.19, 135.90, 135.87, 132.45, 130.88, 130.43, 129.38, 129.30 (2C), 126.90, 123.40, 80.48, 69.06, 60.02, 49.97, 48.51, 42.48, 40.56, 40.21, 29.69, 29.08, 28.34 (3C). IR (KBr) cm^{-1}: 1693, 1658, 1439, 1415, 1350, 1246, 1165, 1132, 970, 698.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-1′-(4-pyridinyl)-spiro[5*H*-thiazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one 1,1-dioxide (10c) [Step 1]: To the solution of compound **9c** (73 mg) in CH_2Cl_2 (4 ml), were added trifluoroacetic acid (0.2 ml) and anisole (1 drop) at 0 °C. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and Et_2O was added into the resulting residue for precipitation of deprotected compound. The precipitate was collected by filtration to afford deprotected compound (70.5 mg, 94% yield) as a colorless amorphous solid.

^{1}H-NMR (270 MHz, DMSO-d_6) δ: 8.65 (1H, s), 8.56 (2H, br s), 8.32—8.16 (3H, m), 7.96 (1H, dd, *J*=2.0, 8.9 Hz), 7.76 (1H, dd, *J*=2.3, 8.9 Hz), 4.93 (1H, dd, *J*=4.9, 9.2 Hz), 4.72 (1H, d, *J*=13.2 Hz), 4.40—4.23 (1H, m), 4.03 (1H, d, *J*=16.5 Hz), 3.68 (1H, d, *J*=16.5 Hz), 3.50—3.00 (6H, m), 2.15—1.85 (3H, m), 1.80—1.65 (1H, m).

[Step 2]: The deprotected compound, which was afforded in Step 1, was neutralized with saturated $NaHCO_3$ aqueous solution and the basic suspension was ordinarily worked up. to afford the salt free substrate. To the solution of the salt free substrate (50 mg) in EtOH (3 ml), was added 4-chloropyridine (120 mg). The mixture was stirred at 130 °C for 2 h and at 150 °C for 2 h. After cooling, saturated $NaHCO_3$ aqueous solution was added into the reaction mixture and then the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The resulting residue was purified purified by silica gel column chromatography (CH_2Cl_2/MeOH=90/10) to afford compound **10c** (13.2 mg, 23% yield) as a colorless amorphous solid.

MALDI-TOF-HR-MS *m/z* (M+H): Calcd for $C_{25}H_{26}{}^{35}ClN_4O_5S_2$: 561.1033. Found: 561.0994. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.66 (1H, s), 8.35—8.08 (5H, m), 8.00—7.90 (1H, m), 7.80—7.70 (1H, m), 6.84 (2H, d, *J*=5.7 Hz), 4.95—4.83 (1H, m), 4.64 (1H, d, *J*=13.2 Hz), 4.35—4.20 (1H, m), 4.03 (1H, d, *J*=16.5 Hz), 3.71 (1H, d, *J*=16.5 Hz), 3.70—3.10 (6H, m), 2.00—1.70 (3H, m), 1.65—1.50 (1H, m). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 162.59, 153.66, 149.31 (2C), 135.50, 133.95, 132.75, 131.70, 130.40, 129.27, 129.09, 128.31, 126.63, 123.99, 108.48 (2C), 68.86, 59.49, 48.69, 48.19, 42.51, 42.48, 41.43, 27.47, 27.33. IR (KBr) cm^{-1}: 1664, 1601, 1448, 1346, 1163, 1132, 758, 698, 596.

References and Notes

1) Part 1: Nishida H., Miyazaki Y., Kitamura Y., Ohashi M., Matsusue T., Okamoto A., Hosaka Y., Ohnishi S., Mochizuki H., *Chem. Pharm. Bull.*, **49**, 1237—1244 (2001).
2) Part 2: Nishida H., Miyazaki Y., Mukaihira T., Saitoh F., Fukui M., Harada K., Itoh M., Muraoka A., Matsusue T., Okamoto A., Hosaka Y., Matsumoto M., Ohnishi S., Mochizuki H., *Chem. Pharm. Bull.*, **50**, 1187—1194 (2002).
3) Part 3: Nishida H., Miyazaki Y., Mukaihira T., Shimada H., Suzuki K., Saitoh F., Mizuno M., Matsusue T., Okamoto A., Hosaka Y., Matsumoto M., Ohnishi S., Mochizuki H., *Chem. Pharm. Bull.*, **52**, 459—462 (2004).
4) Part 4: Nishida H., Mukaihira T., Saitoh F., Harada K., Fukui M., Matsusue T., Okamoto A., Hosaka Y., Matsumoto M., Shiromizu I., Ohnishi S., Mochizuki H., *Chem. Pharm. Bull.*, **52**, 406—412 (2004).
5) Part 5: Saitoh F., Mukaihira T., Nishida H., Satoh T., Okano A., Yumiya Y., Ohkouchi M., Johka R., Matsusue T., Shiromizu I., Hosaka Y., Matsumoto M., Ohnishi S., *Chem. Pharm. Bull.*, **54**, 1535—1544 (2006).
6) Hosaka Y., Matsumoto M., Shinozaki M., Ohno T., Yatagai Y., Kamiya M., Kurokawa M., Nishida H., Matsusue T., Mizuguchi K., Ishi H., *Eur. J. Pharmacol.*, **529**, 164—171 (2006).
7) Davie E. W., Fujikawa K., Kisiel W., *Biochemistry*, **30**, 10363—10370 (1991).
8) Mann K. G., Nesheim M. E., Church W. R., Haley P., Krishnaswamy S., *Blood*, **76**, 1—16 (1990).
9) Rosenberg J. S., Beeler D. L., Rosenberg R. D., *J. Biol. Chem.*, **250**, 1607—1617 (1995).
10) Harker L. A., Hanson S. R., Kelly A. B., *Thromb. Haemost.*, **78**, 736—741 (1997).
11) Elodi S., Varadi K., *Thromb. Res.*, **15**, 617—629 (1979).
12) Markwardt F., Nowak G., Sturzebecher J., Vogel G., *Thromb. Res.*, **52**, 393—400 (1988).
13) Heras M., Chesbro J. H., Penny W. J., Bailey K. R., Badimon L., Fuster V., *Circulation*, **79**, 657—665 (1989).
14) Markwardt F., *Thromb. Haemost.*, **66**, 141—152 (1991).
15) Markwardt F., *Thromb. Res.*, **74**, 1—23 (1994).
16) Kaplan K. L., Francis C. W., *Semin. Hematol.*, **39**, 187—196 (2002).
17) Waxman L., Smith D. E., Arcuri K. E., Vlasuk G. P., *Science*, **248**, 593—596 (1990).
18) Jordan S. P., Waxman L., Smith D. E., Vlasuk G. P., *Biochemisyry*, **29**, 11095—11100 (1990).
19) Neper M. P., Waxman L., Smith D. E., Schulman C. A., Sardana M., Ellis R. W., Schaffer L. W., Siegel P. K. S., Vlasuk G. P., *J. Biol. Chem.*, **265**, 17746—17752 (1990).
20) Schaffer L. W., Davidson J. T., Vlasuk G. P., Siegl P. K. S., *Circulation*, **84**, 1741—1748 (1991).
21) Dunwiddie C. T., Neeper M. P., Nutt E. M., Waxman L., Smith D. E., Hofmann K. J., Lumma P. K., Garsky V. M., Vlasuk G. P., *Biochemisyry*, **31**, 12126—12131 (1992).
22) Vlasuk G. P., *Thromb. Haemost.*, **70**, 212—216 (1993).
23) Ragosta M., Gimple L. W., Gertz D., Dunwiddie C. T., Vlasuk G. P., Haber H. L., Powers E. R., Roberts W. C., Sarembock I. J., *Circulation*, **89**, 1262—1271 (1994).
24) Fevig J. M., Wexler R. R., *Ann. Rep. Med. Chem.*, **34**, 81—100 (1999).
25) Sanderson P. E. J., *Med. Res. Rev.*, **19**, 179—197 (1999).
26) Hauptmann J., Sturzebecher J., *Thromb. Res.*, **93**, 203—241 (1999).
27) Weitz J. I., Hirsh J., *Chest*, **119**, 95S—107S (2001).
28) Eisenberg P. R., Siegel J. E., Abendschein D. R., Miletich J. P., *J. Clin. Invest.*, **91**, 1877—1883 (1993).
29) Kaiser B., Hauptmann J., *Cardiovasc. Drug Rev.*, **12**, 225—236 (1994).
30) Prager N. A., Abendschein D. R., McKenzie C. R., Eisenberg P. R., *Circulation*, **92**, 962—967 (1995).
31) Wong P. C., Crain E. J., Jr., Nguan O., Watson C. A., Racanelli A., *Thromb. Res.*, **83**, 117—126 (1996).
32) Herault J. P., Bernat A., Pflieger A. M., Lormeau J. C., Herbert J. M., *J. Pharmacol. Exp. Ther.*, **283**, 16—22 (1997).
33) Scarborough R. M., *J. Enzym. Inhib.*, **14**, 15—25 (1998).
34) Kunitada S., *Therap. Res.*, **19**, 7—12 (1998).
35) Petitou M., Duchaussoy P., Jaurand G., Gourvenec F., Lederman I., Strassel J.-M., Bârzu T., Crépon B., Hérault J.-P., Lormeau J.-C., Bernat A., Herbert J.-M., *J. Med. Chem.*, **40**, 1600—1607 (1997).
36) Boneu B., Necciari J., Cariou R., Sie P., Gabaig A. M., Kieffer G., Dickinson J., Lamond G., Moelker H., Mant T., Magnani H., *Thromb. Haemost.*, **74**, 1468—1473 (1995).
37) Vuillemenot A., Schiele F., Meneveau N., Claudel S., Donat F., Fontecave S., Cariou R., Samama M. M., Bassand J. P., *Thromb. Haemost.*, **81**, 214—220 (1999).
38) MOPAC PM3 calculation was used in SYBYL7.1 (Tripos Inc.).
39) Warshawsky A. M., Flynn G. A., WO 9404531 (1994).
40) Fujii N., Yajima H., *Chem. Pharm. Bull.*, **23**, 1596—1603 (1975).
41) Nishida H., Mukaihira T., WO 2002053568 (2002).
42) Nishida H., Saitoh F., Harada K., Shiromizu I., WO 2001002397 (2001).
43) It has been unclear that compound **18** reduced FXa inhibitory activity (IC_{50}: 18.2 nM). The overall conformation of compound **18** might be changed by both *N*-mathylation and without a side chain.