Synthesis and Evaluation of 1-Arylsulfonyl-3-piperazinone Derivatives as Factor Xa Inhibitors[1—3] IV. A Series of New Derivatives Containing a Spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one Skeleton

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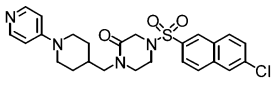
In the course of development of factor Xa (FXa) inhibitor in an investigation involving the synthesis of 1-arylsulfonyl-3-piperazinone derivatives, we found new compounds containing a unique spiro skeleton. Among such compounds, (−)-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (28, M55529) had activity more favorable than those of previously reported compounds. The inhibitory activity of M55529 for FXa is IC_{50}=2 nM, with high selectivity for FXa over thrombin and trypsin.

Key words factor Xa inhibitor; *N*,*O*-spiro acetal; M55529; structure–activity relationship; intramolecular cyclization

Factor Xa (FXa), a trypsin-like serine protease, holds the central position that links the intrinsic and extrinsic mechanisms in the blood coagulation cascade. FXa is known to activate prothrombin to thrombin. Thrombin has several procoagulant functions that include the activation of platelets, feedback activation of other coagulation factors, and conversion of fibrinogen to insoluble fibrin clots.[4—8] Comparison of hirudin[9—13] (a thrombin inhibitor) and tick anticoagulant peptide[14—20] (a FXa inhibitor) suggests that inhibition of FXa may result in less bleeding risk, leading to a more favorable safety/efficacy ratio.[21—24]

Direct inhibition to FXa has therefore emerged as an attractive strategy for the discovery of novel antithrombotic agents.[25—31] In preceding papers,[1,2] we reported the synthesis and evaluation of compounds in a series of 1-arylsulfonyl-3-piperazinone derivatives, of which M55113 (**1**) 4-[(6-chloro-2-naphthalenyl)sulfonyl]-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinone, M55196 (**2**) 4-[(6-chloro-2-naphthalenyl)sulfonyl]-1-[[4-hydroxy-1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinone and M55551 (**3**) (*R*)-4-[(6-chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic acid were found to be potent inhibitors of FXa (IC_{50}=60 nM, 31 nM, 6 nM, respectively) with high selectivity for FXa over trypsin and thrombin.



M55113

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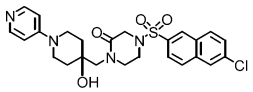

M55196

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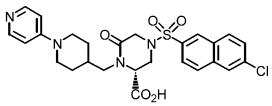

M55551

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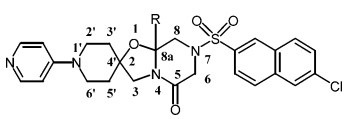
In more recent investigations, fixation of the conformation of testing compounds is believed to affect the strength of interaction between such compounds and the target enzyme. Accordingly, in the next stage of investigation our interest was focused on the synthesis of compounds containing a rigid structure in the central part of the compound (**2**, **3**), and on comparison of the inhibitory activities of the compounds thus synthesized for FXa with those of previously reported compounds. A molecule with a spiro structure in between the piperidine moiety and piperazine moiety was therefore designed as the next candidate for further development of FXa inhibitor. The present paper concerns the synthesis of a series of compounds **4** with a spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one skeleton, together with the FXa inhibitory activities of these new compounds.

Chemistry

First, acyclic precursor **9** was prepared as shown in Chart 1. Sulfonylamidation of glycine ethyl ester hydrochloride (**5**) with 6-chloro-2-naphthalenesulfonyl chloride (**6**) under traditional conditions yielded the corresponding naphthalenesulfonylamide **7**. When **7** was treated with 1-acetoxy-3-chloroacetone (**8**) in DMF in the presence of potassium carbonate, **9** was obtained in good yield as expected.

When 4-(aminomethyl)-1-benzyl-4-piperidinol (**10**) was allowed to react with acyclic precursor **9** under acidic conditions, a product **11** containing a spiro *N*,*O*-acetal structure on the piperazinone ring was obtained, as expected.

The reaction pathway of the formation of the spiro skeleton from **9** and **10** is illustrated in Chart 2. In the first step, a Schiff base was formed by dehydration between a carbonyl group in **9** and a primary amino group in **10**. Subsequent nucleophilic addition of a hydroxyl group to an azomethine



Spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4-piperidin]-5-one derivatives

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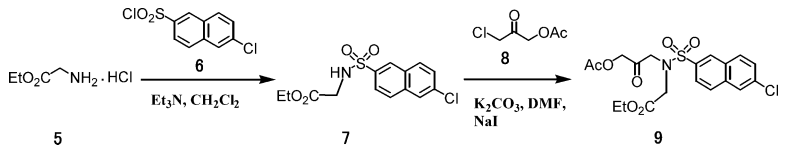


Chart 1. Synthesis of Acyclic Precursor **9**

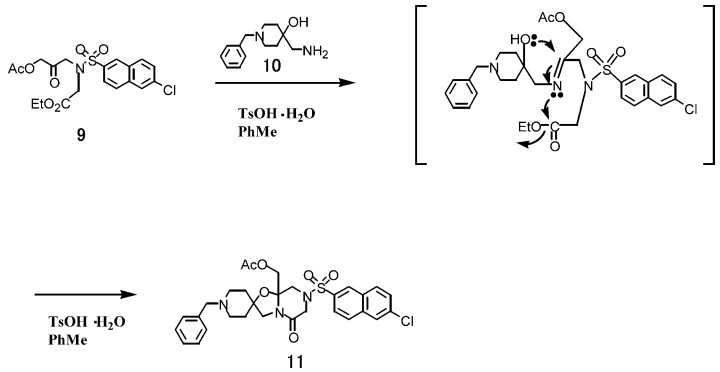


Chart 2. The Reaction Mechanism of the Spiro Skeleton

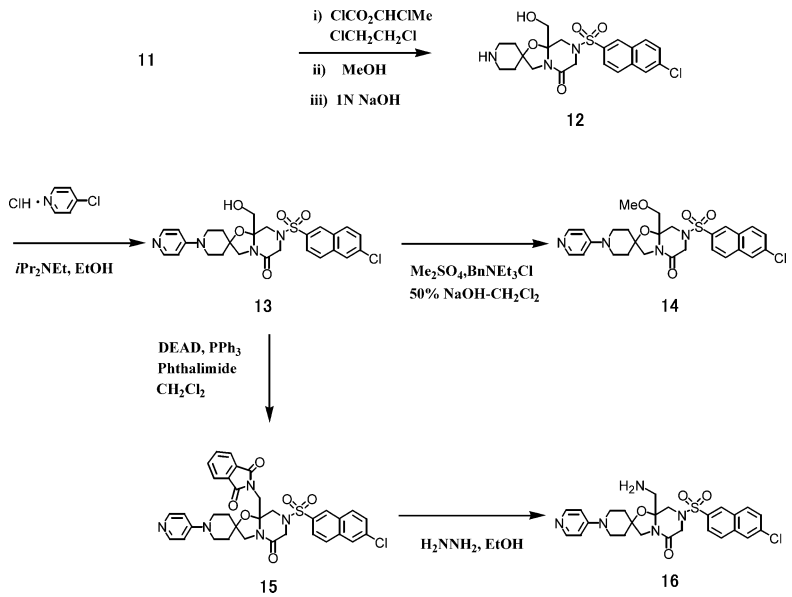


Chart 3. Synthesis of Compounds **13**, **14** and **16**

bond (C=N) followed by intramolecular amide formation gave rise to the spiro skeleton.

^{1}H- and ^{13}C-NMR spectral data for the product are consistent with the proposed spiro structure, as shown in Fig. 1. In addition, the results of high-resolution MS are in good agreement with the structure.

As shown in Chart 3, conversions of the spiro compound **11** to various derivatives were carried out. When compound **12** prepared by the deprotection of **11** with 1-chloroethyl chloroformate in 1,2-dichloroethane and with 1 N NaOH in MeOH was treated with 4-chloropyridine, the desired compound **13** was obtained. Then, compound **13** was methylated with dimethyl sulfate in the presence of a phase-transfer catalyst (benzyltriethylammonium chloride) to obtain the methyl ether **14**. Compound **13** was treated with phthalimide by Mitsunobu reaction[32] and with hydrazine to obtain the corresponding amino compound **16**.

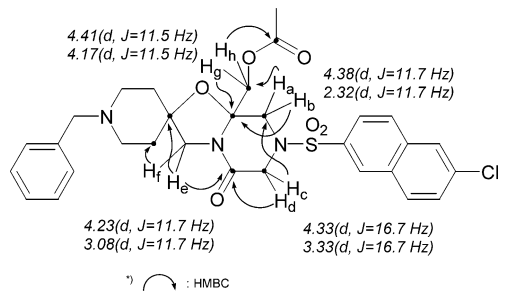


Fig. 1. Assignment of NMR Data

Synthesis of compounds **21** and **22** was achieved as shown in Chart 4. Compound **17**, prepared by the reaction of the key intermediate **11** with benzyl chloroformate in the pres-

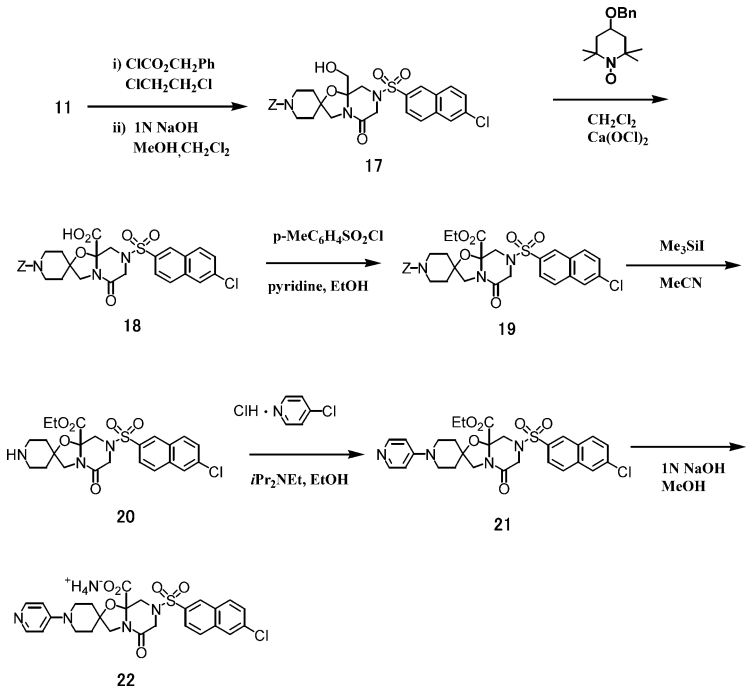
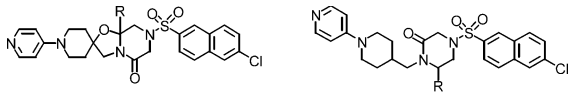


Chart 4. Synthesis of Compounds **21** and **22**

Table 1. Comparison of FXa Inhibitory Activity of Spiro Skeleton and Piperazinone Derivative



Spiro skeleton Piperazinone derivative

R	Spiro skeleton		Piperazinone derivative[2)]	
	Compd. No.	IC_{50} (nM)	Compd. No.	IC_{50} (nM)
$-CH_2OH$	**13**	5	**23**	12
$-CH_2OMe$	**14**	5	**24**	31
$-CH_2NH_2$	**16**	2	**25**	12
$-CO_2Et$	**21**	5	**26**	30
$-CO_2-NH_4^+$	**22**	3	**27**	10

ence of 1,8-bis(*N*,*N*-dimethylamino)naphthalene and with 1 N NaOH in MeOH, was oxidized with $Ca(OCl)_2$ in the presence of 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl benzoate in CH_2Cl_2 to obtain carboxylic acid **18** in good yield. The ethyl ester **19** was followed by esterification of **18** with EtOH in traditional conditions, and compound **20** was afforded by deprotection of the **19**. Desired compound **21** was obtained by coupling reaction of compound **20** with 4-chloropyridine hydrochloride under basic conditions. When compound **21** was treated with 1 N NaOH, the corresponding carboxylic acid **22** was obtained. The spectral data for all of these products are in good agreement with the proposed structures.

Throughout the chemical modifications described above, the spiro structure is fairly stable, although the skeleton has an *N*,*O*-acetal linkage.

Results and Discussion

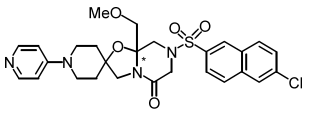
The FXa inhibitory activities of new compounds with a spiro structure synthesized in the present investigation were measured using the same method as described in the preceding paper.[1)]

Compared with the activities of previously tested piperazinone type compounds, those of new compounds were higher for all the derivatives containing the corresponding substitution.

As listed in Table 1, the inhibitory activities (IC_{50}) of new compounds varied from twice to six times those of piperazinone (prototype) compounds. It is conceivable that the rigidity of structures in the linker moiety of the testing compounds is important for exhibition of FXa inhibitory activity. An *N*,*O*-spiro acetal skeleton fixes the conformation of the new compounds, and their shape may be more suitable for inhibition of FXa. On the other hand, the conformation of prototype compounds is relatively loose.

In addition to the above, little effect on activity was observed by introducing a functional group to the new compounds (IC_{50} 2—5 nM), while some effects on activity were

Table 2. Selectivity of M55529 for FXa over Other Serine Protease



28 M55529

	M55529	
	IC_{50} (nM)	Selectivity (Enzyme/FXa)
FXa	2	—
Thrombin	>10000	>5000
APC	>10000	>5000
Trypsin	>10000	>5000
Plasmin	>10000	>5000
t-PA	1900	950
Urokinase	>10000	>5000

observed for the corresponding derivatives of prototype compounds (IC_{50} 10—30 nM).

Based on these data, the steric shape of the molecule is concluded to more strongly affect FXa inhibition than the hydrogen bond-forming ability of the functional group introduced.

At the final stage of the present investigation, comparison was made of stereoisomers including a substitution at the 8-position of the new skeletons for inhibitory activity. It is known that compounds with polar substitution undergo little absorption in the body, and that ester substitution will tend to induce hydrolysis in the body. Compound **14** was therefore considered likely to exhibit the best absorption in the body, as a candidate for development.

The activities of stereoisomers (**28**, **29**) of compound **14** obtained by optical resolution with liquid column chromatography were measured. (−)-Isomer **28** (IC_{50}=2 nM) had stronger activity than (+)-isomer **29** (IC_{50}=129 nM).

Though we are investigating absolute configuration of the compound **28** and **29**, it has not resolved yet.

Compound **28** (M55529) exhibited clear selectivity for FXa over related serine protease, and was 5000-fold more selective for FXa than for thrombin, as shown in Table 2.

The above results suggest that M55529 is promising as FXa inhibitor. Crystallization of a complex of M55529 with FXa has already been successfully performed in our laboratory, and the results of X-ray crystallographic analysis will be published in the near future.

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) or CD_3OD using tetramethylsilane as the internal reference. High-resolution mass spectra (HR-MS) were obtained using JEOL JMS-GCMATE. Infrared absorption spectra (IR) were run using HORIBA FT-720 FT-IR. High performance liquid chromatographies (HPLC) were conducted by using Shimadzu LC-10A. Optical rotations were measured with JASCO DIP-1000 digital polarimeter.

Measurement of Factor Xa, Thrombin and Trypsin 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); human thrombin (Sigma Co., 0.09 U/ml) and S-2238 (Chromogenix AB, 0.2 mM); human trypsin (Athens Research and Technology, Inc., 15 ng/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}.

Ethyl *N*-[(6-Chloro-2-naphthalenyl)sulfonyl]glycinate (7) Ethyl glycinate hydrochloride (**5**) (9.88 g, 70.7 mmol) was suspended in CH_2Cl_2 (500 ml). Et_3N (20.2 ml, 144.9 mmol) and 6-chloro-2-naphthalenesulfonyl chloride (**6**) (17.6 g, 67.4 mmol) were added to the suspension under cooling with ice. After stirring at room temperature for 1 h, the mixture was adjusted to pH 2 by addition of 1 N HCl, and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure. After washing the resulting crystals in hexane, the crystals were collected by filtration and air-dried to give compound **7** (22.4 g, quant.) as pale yellow crystal, mp 93—94 °C.

HR-MS *m/z*: Calcd for $C_{14}H_{14}{}^{35}ClNO_4S$: 327.0332. Found : 327.0325. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.43—7.57 (6H, m, naphthyl), 5.22—5.15 (1H, m, NH), 4.01 (2H, q, J=7.1 Hz, $CO_2CH_2CH_3$), 3.82 (2H, d, J=5.3 Hz, $CH_2CO_2CH_2CH_3$), 1.11 (3H, t, J=7.1 Hz, $CH_2CO_2CH_2CH_3$). IR (film) cm^{-1}: 1745, 1330, 1159, 1120, 1079, 696.

Ethyl *N*-(3-Acetyloxy-2-oxopropyl)-*N*-[(6-chloro-2-naphthalenyl)sulfonyl]glycinate (9) To DMF (25 ml) solution of compound **7** (2.50 g, 7.63 mmol) were added K_2CO_3 (1.58 g, 11.4 mmol) and NaI (1.14 g, 7.63 mmol), and a solution of 1-acetoxy-3-chloroacetone (**8**) (1.72 g, 11.4 mmol) in DMF (7 ml) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 1.5 h, and the mixture was extracted with Et_2O. The organic layer was washed with brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure. The resulting residue was crystallized in Et_2O, and the crystals were collected by filtration and air-dried to obtain the compound **9** (2.72 g, 81%) as pale yellow crystal, mp 75—76 °C.

HR-MS *m/z*: Calcd for $C_{19}H_{20}{}^{35}ClNO_7S$: 441.0649. Found: 441.0600. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.42—7.52 (6H, m, naphthyl), 4.84 (2H, s, $CH_2COCH_2OCOCH_3$), 4.31 (2H, s, $CH_2COCH_2OCOCH_3$), 4.15 (2H, s, $CH_2CO_2CH_2CH_3$), 4.06 (2H, q, J=7.1 Hz, $CH_2CO_2CH_2CH_3$), 2.16 (3H, s, $OCOCH_3$), 1.17 (3H, t, J=7.1 Hz, $CH_2CO_2CH_2CH_3$). IR (film) cm^{-1}: 1743, 1461, 1340, 1234, 1159, 586.

8*a*-[(Acetyloxy)methyl]-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-1′-benzyl-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (11) To a solution of compound **9** (1.6 g, 3.62 mmol) and 4-(aminomethyl)-1-benzyl-4-piperidinol (**10**) (800 mg, 3.62 mmol) in PhMe (200 ml) was added *p*-toluenesulfonic acid monohydrate (34.0 mg, 0.18 mmol), and the mixture was refluxed for 1 h by using a Dean-Stark apparatus. The reaction mixture was allowed to cool, and the solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel column chromatography (eluent: EtOAc) to give the compound **11** (1.08 g, 50%) as ivory crystal, mp 84—86 °C.

HR-MS *m/z*: Calcd for $C_{30}H_{32}{}^{35}ClN_3O_6S$: 597.1700. Found: 597.1650. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.36—7.19 (11H, m, naphthyl and phenyl), 4.41 (1H, d, J=11.5 Hz, CH_2OCOCH_3), 4.38 (1H, d, J=11.7 Hz, C^8-H), 4.33 (1H, d, J=16.7 Hz, C^6-H), 4.23 (1H, d, J=11.7 Hz, C^3-H), 4.17 (1H, d, J=11.5 Hz, CH_2OCOCH_3), 3.46 (2H, s, CH_2Ph), 3.33 (1H, d, J=16.7 Hz, C^6-H), 3.08 (1H, d, J=11.7 Hz, C^3-H), 2.62—2.21 (4H, m, $C^{2',6'}$-H of piperidine), 2.32 (1H, d, J=11.7 Hz, C^8-H), 2.11 (3H, s, $OCOCH_3$), 1.93—1.34 (4H, m, $C^{3',5'}$-H of piperidine). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.05 (CH_2OCOCH_3), 162.74 (C^5), 138.09—123.47 (16C, naphthyl and phenyl), 90.21 (C^{8a}), 81.57 (C^2), 64.65 (CH_2OCOCH_3), 62.73 (CH_2Ph), 51.54 (C^3), 50.37 ($C^{2',6'}$ of piperidine), 50.16 (C^8), 49.89 ($C^{2',6'}$ of piperidine), 47.86 (C^6), 36.69 ($C^{3',5'}$ of piperidine), 36.07 ($C^{3',5'}$ of piperidine), 20.83 (CH_2OCOCH_3). IR (film) cm^{-1}: 1749, 1673, 1224, 1168, 698.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(hydroxymethyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (12) To a solution of compound **11** (1.0 g, 1.67 mmol) in $ClCH_2CH_2Cl$ (10 ml) were added 1-chloroethyl chloroformate (0.46 ml, 4.18 mmol) and 1,8-bis(*N*,*N*-dimethylamino)naphthalene (72 mg, 0.34 mmol), and the mixture was heated under reflux for 30 min. The reaction mixture was allowed to cool, and the solvent was distilled off under reduced pressure. To the residue was added MeOH (10 ml), and the mixture was heated under reflux for 30 min. The reaction mixture was allowed to cool, and the solvent was distilled off under reduced pressure. To a solution of the residue in MeOH (14 ml) was added 1 N NaOH (4 ml) and the mixture was stirred for 1 h at room temperature. The solvent was distilled off under reduced pressure. To the residue, water

(20 ml) was added and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel column chromatography (eluents: CH_2Cl_2 : MeOH = 10 : 1) to give compound **12** (626 mg, 81%) as pale yellow powder, mp 165—166 °C.

^{1}H-NMR (300 MHz, CD_3OD) δ: 8.52—7.61 (6H, m, naphthyl), 4.27 (1H, d, J=11.9 Hz, C^8-H), 4.23 (1H, d, J=16.9 Hz, C^6-H), 4.16 (1H, d, J=11.8 Hz, C^3-H), 3.78—3.68 (2H, m, CH_2OH), 3.48 (1H, d, J=16.9 Hz, C^6-H), 3.28 (1H, d, J=11.8 Hz, C^3-H), 3.23—2.83 (4H, m, $C^{2',6'}$-H of piperidine), 2.58 (1H, d, J=11.9 Hz, C^8-H), 2.06—1.35 (4H, m, $C^{3',5'}$-H of piperidine). IR (film) cm^{-1}: 1662, 1455, 1348, 1166, 1078, 700.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(hydroxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (13) To a suspension of compound **12** (11.3 g, 24 mmol) and 4-chloropyridine hydrochloride (5.4 g, 36 mmol) in EtOH (210 ml) were added $^i Pr_2NEt$ (21.0 ml, 120 mmol), and the mixture was stirred in a sealed tube at 140—150 °C for 15 h. The reaction mixture was allowed to cool and concentrated. The resulting residue was purified by silica gel column chromatography (ChromatorexNH^{TM}, eluents: CH_2Cl_2 : MeOH = 19 : 1) to give the compound **13** (6.4 g, 49%) as pale yellow powder, mp 141—143 °C.

HR-MS *m/z*: Calcd for $C_{26}H_{27}{}^{35}ClN_4O_5S$: 542.1390. Found: 542.1421. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.38—6.58 (10H, m, naphthyl and pyridinyl), 4.43 (1H, d, J=11.9 Hz, C^8-H), 4.32 (1H, d, J=17.0 Hz, C^6-H), 4.27 (1H, d, J=11.6 Hz, C^3-H), 3.92 (1H, d, J=11.6 Hz, CH_2OH), 3.72 (1H, d, J=11.6 Hz, CH_2OH), 3.54—3.22 (4H, m, $C^{2',6'}$-H of piperidine), 3.40 (1H, d, J=17.0 Hz, C^6-H), 3.19 (1H, d, J=11.6 Hz, C^3-H), 2.42 (1H, d, J=11.9 Hz, C^8-H), 2.22—1.48 (4H, m, $C^{3',5'}$-H of piperidine). ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ: 162.96 (C^5), 154.28 (C^4 of pyridine), 150.02 ($C^{2,6}$ of pyridine), 135.72—123.39 (10C, naphthyl), 108.53 ($C^{3,5}$ of pyridine), 92.51 (C^{8a}), 80.61 (C^2), 64.76 (CH_2OH), 52.09 (C^3), 49.07 (C^8), 48.00 (C^6), 43.56 ($C^{2'}$ or $C^{6'}$ of piperidine), 43.14 ($C^{2'}$ or $C^{6'}$ of piperidine), 35.72 ($C^{3'}$ or $C^{5'}$ of piperidine), 35.04 ($C^{3'}$ or $C^{5'}$ of piperidine). IR (film) cm^{-1}: 3300, 1664, 1599, 1348, 1167, 700.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (14) To a solution of compound **13** (100 mg, 0.18 mmol), benzyltriethylammonium chloride (4.0 mg, 0.018 mmol), and Me_2SO_4 (0.018 ml, 0.198 mmol) in CH_2Cl_2 (2 ml) was gradually added 50% NaOH (0.6 ml) with vigorous stirring under cooling with ice. After stirring the reaction mixture at room temperature for 2 h, water (5 ml) was added under cooling with ice, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel column chromatography (Chromatorex NH^{TM}, eluents: hexane : EtOAc = 1 : 4—1 : 6) to give compound **14** (48.0 mg, 47%) as brown powder, mp 111—113 °C.

HR-MS *m/z*: Calcd for $C_{27}H_{29}{}^{35}ClN_4O_5S$: 556.1547, Found: 556.1540. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.38—6.58 (10H, m, naphthyl and pyridinyl), 4.38 (1H, d, J=11.9 Hz, C^8-H), 4.35 (1H, d, J=16.8 Hz, C^6-H), 4.20 (1H, d, J=11.3 Hz, C^3-H), 3.67 (1H, d, J=10.2 Hz, CH_2OMe), 3.62 (1H, d, J=10.2 Hz, CH_2OMe), 3.53—3.22 (4H, m, $C^{2',6'}$-H of piperidine), 3.43 (3H, s, CH_2OMe), 3.35 (1H, d, J=16.8 Hz, C^6-H), 3.20 (1H, d, J=11.3 Hz, C^3-H), 2.30 (1H, d, J=11.9Hz, C^8-H), 2.03—1.45 (4H, m, $C^{3',5'}$-H of piperidine). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.75 (C^5), 154.23 (C^4 of pyridine), 150.33 ($C^{2,6}$ of pyridine), 135.70—123.46 (10C, naphthyl), 108.54 ($C^{3,5}$ of pyridine), 92.85 (C^{8a}), 80.67 (C^2), 74.88, (CH_2OMe) 59.78 (CH_2OMe), 52.15 (C^3), 49.69 (C^8), 47.98 (C^6), 43.59 ($C^{2'}$ or $C^{6'}$ of piperidine), 43.20 ($C^{2'}$ or $C^{6'}$ of piperidine), 35.82 ($C^{3'}$ or $C^{5'}$ of piperidine), 34.81 ($C^{3'}$ or $C^{5'}$ of piperidine). IR (film) cm^{-1}: 1671, 1594, 1417, 1349, 1168, 1103, 698.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]-8*a*-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl]tetrahydro-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]-pyrazine-2(3*H*),4′-piperidin]-5-one (15) To a solution of phthalimide (4.88 g, 33.1 mmol) and Ph_3P (8.69 g, 33.1 mmol) in CH_2Cl_2 (150 ml) was added dropwise 40% DEAD solution in PhMe (10.0 ml, 33.1 mmol) under cooling with ice. Compound **13** (3.0 g, 5.52 mmol) was added and the mixture was stirred overnight at room temperature. After adding sat. aq. $NaHCO_3$ to the reaction mixture, the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with water and brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure and the resulting residue was purified by silica gel column chromatography (eluents: CH_2Cl_2 : MeOH = 19 : 1) to give compound **15** (2.5 g, 68%) as brown powder, mp 84—87 °C.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.44—6.51 (14H, m, naphthyl, pyridinyl and phthaloyl), 4.54 (1H, d, J=11.7 Hz, C^8-H), 4.35 (1H, d, J=16.7 Hz, C^6-H), 4.28 (1H, d, J=11.9 Hz, C^3-H), 4.19 (1H, d, J=14.5 Hz, CH_2-phthalimide), 4.09 (1H, d, J=14.5 Hz, CH_2-phthalimide), 3.44—3.16 (4H, m, $C^{2',6'}$-H of piperidine), 3.44 (1H, d, J=16.7 Hz, C^6-H), 3.04 (1H, d, J=11.9 Hz, C^3-H), 2.41 (1H, d, J=11.7 Hz, C^8-H), 1.89—1.40 (4H, m, $C^{3',5'}$-H of piperidine). IR (film) cm^{-1}: 1747, 1673, 1417, 1349, 1224, 1168, 698.

8*a*-(Aminomethyl)-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (16) To a suspension of compound **15** (2.45 g, 3.66 mmol) in EtOH (50 ml) was added hydrazine monohydrate (0.37 ml, 7.47 mmol) and the mixture was heated under reflux for 16 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography (eluents: CH_2Cl_2 : MeOH = 4 : 1) to give the compound **16** (1.43 g, 72%) as colorless oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.39—6.58 (10H, m, naphthyl and pyridinyl), 4.53 (1H, d, J=11.9 Hz, C^8-H), 4.38 (1H, d, J=16.8 Hz, C^6-H), 4.26 (1H, d, J=11.9 Hz, C^3-H), 3.55—3.22 (4H, m, $C^{2',6'}$-H of piperidine), 3.37 (1H, d, J=16.8 Hz, C^6-H), 3.18 (1H, d, J=13.5 Hz, CH_2NH_2), 3.17 (1H, d, J=11.9 Hz, C^3-H), 2.89 (1H, d, J=13.5 Hz, CH_2NH_2), 2.24 (1H, d, J=11.9 Hz, C^8-H), 1.94—1.48 (4H, m, $C^{3',5'}$-H of piperidine). ^{13}C-NMR (75 MHz,, $CDCl_3$) δ: 162.77 (C^5), 154.31 (C^4 of pyridine), 149.92 ($C^{2,6}$ of pyridine), 135.71—123.40 (10C, naphthyl), 108.52 ($C^{3,5}$ of pyridine), 93.27 (C^{8a}), 80.25 (C^2), 51.72 (C^3), 48.91 (C^8), 48.04 (C^6), 46.50 (CH_2NH_2), 43.62 ($C^{2'}$ or $C^{6'}$ of piperidine), 43.21 ($C^{2'}$ or $C^{6'}$ of piperidine), 35.76 ($C^{3'}$ or $C^{5'}$ of piperidine), 35.43 ($C^{3'}$ or $C^{5'}$ of piperidine). IR (KBr) cm^{-1}: 3395, 2920, 2360, 1666, 1597, 1348, 1167.

1′-Benzyloxycarbonyl-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(hydroxymethyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (17) The compound **11** (67.2 g, 112.4 mmol) and 1,8-bis(*N*,*N*-dimethylamino)naphthalene (4.80 g, 22.5 mmol) were dissolved in $ClCH_2CH_2Cl$ (670 ml) and benzyl chloroformate (32.1 ml, 224.7 mmol) was added dropwise to the solution with the reaction temperature maintained at 0 °C. The reaction mixture was stirred at room temperature for 2 h, and sat. aq. $NaHCO_3$ was added under cooling with ice. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure. The resulting residue was dissolved in a mixed solution of MeOH (1.33 l) and CH_2Cl_2 (1.33 l), and 1 N NaOH (140 ml) was added dropwise to this solution under cooling with ice. After stirring at room temperature for 30 min, the solvent was distilled off under reduced pressure. To the residue was added sat. aq. NH_4Cl and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel column chromatography (eluents: CH_2Cl_2 : MeOH = 40 : 1—30 : 1) to give the compound **17** (67.9 g, quant.) as pale yellow powder, mp 96—98 °C.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.37—7.25 (11H, m, naphthyl and phenyl), 5.10 (2H, s, OCH_2Ph), 4.43 (1H, d, J=11.7 Hz, C^8-H), 4.38 (1H, d, J=16.8 Hz, C^6-H), 4.23 (1H, d, J=11.9 Hz, C^3-H), 3.96—3.24 (6H, m, CH_2OH and $C^{2',6'}$-H of piperidine), 3.36 (1H, d, J=16.8 Hz, C^6-H), 3.14 (1H, d, J=11.9 Hz, C^3-H), 2.29 (1H, d, J=11.7 Hz, C^8-H), 1.89—1.34 (4H, m, $C^{3',5'}$-H of piperidine). IR (film) cm^{-1}: 1697, 1670, 1455, 1419, 1238, 1166, 698.

1′-Benzyloxycarbonyl-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-5-oxo-spiro[8*aH*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidine]-8*a*-carboxylic Acid (18) To a solution of compound **17** (68.0 g, 113.4 mmol) in CH_2Cl_2 (680 ml) was added 4-benzyloxy-2,2,6,6-tetramethylpiperidine 1-oxyl (314 mg, 1.14 mmol). 5% aq. $NaHCO_3$ (1.36 l) was added dropwise with stirring under cooling with ice and bleaching powder (54.0 g, 227 mmol) was added. The mixture was vigorously stirred for 1.5 h under cooling with ice, adjusted to pH 1 with 1 N HCl and was extracted with CH_2Cl_2. The organic layer was washed with water, brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure to give the compound **18** (62.7 g, 90%) as pale yellow powder, mp 182—184 °C.

^{1}H-NMR (300 MHz, CD_3OD) δ: 8.52—7.25 (11H, m, naphthyl and phenyl), 5.08 (2H, s, OCH_2Ph), 4.64 (1H, d, J=11.3 Hz, C^8-H), 4.17 (1H, d, J=16.6 Hz, C^6-H), 4.02 (1H, d, J=11.5 Hz, C^3-H), 3.82—3.17 (4H, m, $C^{2',6'}$-H of piperidine), 3.43 (1H, d, J=16.6 Hz, C^6-H), 3.29 (1H, d, J=11.5 Hz, C^3-H), 2.72 (1H, d, J=11.3 Hz, C^8-H), 1.87—1.39 (4H, m, $C^{3',5'}$-H of piperidine). IR (film) cm^{-1}: 1656, 1423, 1349, 1240, 1164, 698.

Ethyl 1′-Benzyloxycarbonyl-7-[(6-chloro-2-naphthalenyl)sulfonyl]-tetrahydro-5-oxo-spiro[8*aH*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidine]-8*a*-carboxylate (19) To a solution of compound **18** (62.7 g, 0.10 mol) in pyridine (640 ml) was added EtOH (58.4 ml, 1.0 mol). After gradually adding *p*-toluene-sulfonyl chloride (97.3 g, 0.51 mol) with stirring under cooling with ice, the mixture was stirred at room temperature for 4 h.

After addition of ice water, the reaction mixture was extracted with EtOAc. The organic layer was washed with water, 1 N HCl and brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure and the resulting residue was purified by silica gel column chromatography (eluents: hexane : EtOAc = 3 : 1—2 : 1) to obtain the compound **19** (40.1 g, 61%) as pale yellow powder, mp 82—84 °C.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.36—7.28 (11H, m, naphthyl and phenyl), 5.10 (2H, s, OCH_2Ph), 4.75 (1H, d, J=11.3 Hz, C^8-H), 4.32 (1H, d, J=16.6 Hz, C^6-H), 4.12 (2H, q, J=7.1 Hz, $CO_2CH_2CH_3$), 4.07 (1H, d, J=11.5 Hz, C^3-H), 3.81—3.22 (4H, m, $C^{2',6'}$-H of piperidine), 3.43 (1H, d, J=16.6 Hz, C^6-H), 3.29 (1H, d, J=11.5 Hz, C^3-H), 2.45 (1H, d, J=11.3 Hz, C^8-H), 1.75—1.42 (4H, m, $C^{3',5'}$-H of piperidine), 1.34 (3H, t, J=7.1 Hz, $CO_2CH_2CH_3$). IR (film) cm^{-1}: 1745, 1681, 1419, 1238, 1166, 1079, 698.

Ethyl 7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-5-oxo-spiro-[8*aH*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidine]-8*a*-carboxylate (20) To a solution of the compound **19** (40.0 g, 62.2 mmol) in MeCN (400 ml) was added TMSI (22.2 ml, 155.8 mmol) under cooling with ice. After stirring the mixture for 45 min under cooling with ice, the reaction mixture was poured into 1 N HCl under cooling with ice and hexane was added to this mixture. The mixture was stirred for separation, and the aqueous layer was washed with hexane followed by addition of CH_2Cl_2. 2 N NaOH was added with stirring under cooling with ice and the pH of the mixture was adjusted to 11. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine and dried over dry Na_2SO_4. The solvent was distilled off under reduced pressure to give compound **20** (29.7 g, 94%) as colorless powder, mp 252—254 °C.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.38—7.58 (6H, m, naphthyl), 4.74 (1H, d, J=11.4 Hz, C^8-H), 4.31 (1H, d, J=16.5 Hz, C^6-H), 4.38—4.17 (2H, m, $CO_2CH_2CH_3$), 4.10 (1H, d, J=11.4 Hz, C^3-H), 3.16—2.77 (4H, m, $C^{2',6'}$-H of piperidine), 3.33 (1H, d, J=16.5 Hz, C^6-H), 3.32 (1H, d, J=11.4 Hz, C^3-H), 2.45 (1H, d, J=11.4 Hz, C^8-H), 1.90—1.45 (4H, m, $C^{3',5'}$-H of piperidine), 1.34 (3H, t, J=7.1 Hz, $CO_2CH_2CH_3$). IR (film) cm^{-1}: 1749, 1668, 1344, 1162, 1078, 698.

Ethyl 7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-5-oxo-1′-(4-pyridinyl)-spiro[8*aH*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidine]-8*a*-carboxylate (21) To a solution in EtOH (600 ml) of compound **20** (30 g, 59.0 mmol) and 4-chloropyridine hydrochloride (13.4 g, 88.5 mmol) was added ^{i}Pr$_2$NEt (51.2 ml, 295 mmol) and the mixture was stirred in a sealed tube at 150 °C for 15 h. The reaction mixture was allowed to cool and concentrated. The resulting residue was purified by silica gel column chromatography (eluents: CH_2Cl_2 : MeOH = 20 : 1—10 : 1) to give the compound **21** (14.2 g, 41%) as pale yellow powder, mp 106—109 °C.

HR-MS *m/z*: Calcd for $C_{28}H_{29}{}^{35}ClN_4O_6S$: 584.1496, Found: 584.1532. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.36—6.57 (10H, m, naphthyl and pyridinyl), 4.76 (1H, d, J=11.6 Hz, C^8-H), 4.33 (1H, d, J=16.7 Hz, C^6-H), 4.30 (2H, dq, J=7.2, 2.2 Hz, $CO_2CH_2CH_3$), 4.11 (1H, d, J=11.6 Hz, C^3-H), 3.54—3.25 (4H, m, $C^{2',6'}$-H of piperidine), 3.38 (1H, d, J=11.6 Hz, C^3-H), 3.36 (1H, d, J=16.7 Hz, C^6-H), 2.49 (1H, d, J=11.6 Hz, C^8-H), 1.89—1.52 (4H, m, $C^{3',5'}$-H of piperidine), 1.36 (3H, t, J=7.2 Hz, $CO_2CH_2CH_3$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 168.02 ($CO_2CH_2CH_3$), 162.04 (C^5), 154.08 (C^4 of pyridine), 150.37 ($C^{2,6}$ of pyridine), 135.68—123.48 (10C, naphthyl), 108.53 ($C^{3,5}$ of pyridine), 90.08 (C^{8a}), 81.75 (C^2), 62.96, ($CO_2CH_2CH_3$), 52.31 (C3), 50.37 (C^8), 47.97 (C^6), 43.27 ($C^{2'}$ or $C^{6'}$ of piperidine), 43.20 ($C^{2'}$ or $C^{6'}$ of piperidine), 35.69 ($C^{3'}$ or $C^{5'}$ of piperidine), 34.46 ($C^{3'}$ or $C^{5'}$ of piperidine), 14.01 ($CO_2CH_2CH_3$). IR (film) cm^{-1}: 1743, 1678, 1595, 1350, 1167, 1080, 870, 696.

7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-5-oxo-1′-(4-pyridinyl)-spiro-[8*aH*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidine]-8*a*-carboxylic Acid (22) To a solution of compound **21** (100 mg, 0.17 mmol) in MeOH (2.4 ml) was added 1 N NaOH (0.684 ml, 0.68 mmol) under cooling with ice and the mixture was stirred at room temperature for 1 h. After adjusting the pH of the mixture to 2—3 with 1 N HCl, the solvent was distilled off under reduced pressure. The resulting residue was dissolved in methanol (5 ml) and ion-exchange resin MSC-1 (100—200 mesh, H-form, manufactured by Muromachi Chemicals Inc., 2.0 g) was added and the mixture was stirred for 30 min. The resin was collected by filtration, washed with methanol and added to 2 N ammonia–methanol solution (5 ml). The mixture was stirred for 30 min. The solution obtained by filtering off the resin was concentrated under reduced pressure to give the compound **22** (54.8 mg, 58%) as pale yellow amorphous.

^{1}H-NMR (300 MHz, CD_3OD) δ: 8.48—6.75 (10H, m, naphthyl and pyridinyl), 4.58 (1H, d, J=11.2 Hz, C^8-H), 4.12 (1H, d, J=16.9 Hz, C^6-H), 3.89 (1H, d, J=11.4 Hz, C^3-H), 3.69—3.22 (4H, m, $C^{2',6'}$-H of piperidine), 3.45 (1H, d, J=16.9 Hz, C^6-H), 3.30 (1H, d, J=11.4 Hz, C^3-H), 2.70 (1H, d, J=11.2 Hz, C^8-H), 1.97—1.52 (4H, m, $C^{3',5'}$-H of piperidine). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 173.44 (CO_2^-), 163.27 (C^5), 155.00 (C^4 of pyridine), 147.60 ($C^{2,6}$ of pyridine), 135.68—123.70 (10C, naphthyl), 107.90 ($C^{3,5}$ of pyridine), 91.59 (C^{8a}), 79.82 (C^2), 52.11 (C^3), 50.53 (C^8), 48.46 (C^6), 42.73 ($C^{2'}$ or C6′ of piperidine), 42.64 ($C^{2'}$ or $C^{6'}$ of piperidine), 35.31 ($C^{3'}$ or $C^{5'}$ of piperidine), 33.93 ($C^{3'}$ or $C^{5'}$ of piperidine). IR (KBr) cm^{-1}: 1657, 1628, 1601, 1396, 1348, 1169.

(−)-7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (28) and (+)-7-[(6-Chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (29) Compound **14** was optically resolved on HPLC [Waters DeltaPrep 4000 manufactured by Waters Inc.: Column used, Daicel Chiralcel™ OD manufactured by Daicel Chemical Industries, Ltd., 2 cm×25 cm: eluent: hexane : EtOH : Et_2NH = 60 : 40 : 1: flow rate, 10 ml/min, detection wavelength, 254 nm] to obtain (+)-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (**29**) as brown powder [retention time: 43.5 min, mp 112—113 °C, $[\alpha]_D^{25}$ +48.8° (c=1.247, $CHCl_3$), $[\alpha]_D^{33}$ +91.3° (c=1.000, MeOH), >99% ee], and (−)-7-[(6-chloro-2-naphthalenyl)sulfonyl]tetrahydro-8*a*-(methoxymethyl)-1′-(4-pyridinyl)-spiro[5*H*-oxazolo[3,2-*a*]pyrazine-2(3*H*),4′-piperidin]-5-one (**28**) as pale yellow powder [retention time: 63.0 min, mp 111—112 °C, $[\alpha]_D^{25}$ −48.4° (c=1.175, $CHCl_3$), $[\alpha]_D^{33}$ −90.7° (c=1.000, MeOH), >99%ee], respectively.

Acknowledgments The authors thank Emeritus Professor Hiroshi Yamanaka of Tohoku University for his encouragement and helpful discussions throughout this study.

References and Notes

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