# Synthesis and Evaluation of 1-Arylsulfonyl-3-piperazinone Derivatives as a Factor Xa Inhibitor[1,2] II. Substituent Effect on Biological Activities

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**Intravascular clot formation is an important event in a number of cardiovascular diseases. The prevention of blood coagulation has become a major target for new therapeutic agents. Factor Xa (FXa) is a trypsin-like serine protease that plays a key role in the blood coagulation cascade and represents an attractive target for anticoagulant drug development. We have investigated substituents in the central part of a lead compound (3: M55113), and discovered that compound M55551 (34: (*R*)-4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic acid) is a potent inhibitor of FXa ($IC_{50}$=0.006 $\mu$M), with high selectivity for FXa over trypsin and thrombin. The activity of this compound is ten times more powerful than the lead compound.**

**Key words** factor Xa inhibitor; 1-arylsulfonyl-3-piperazinone; structure–activity relationship; optical isomer; M55551

Factor Xa (FXa) is a serine protease which plays a critical role in the coagulation cascade, serving as the point of convergence of intrinsic and extrinsic pathways.[3—7] It represents an attractive target for anticoagulant drug development.[8—14]

Most nonpeptide FXa inhibitors reported in the literature are dibasic compounds. Nagahara *et al.*[15,16] have reported the synthesis and evaluation of a series of bis(amidino)-derivatives, and through investigation, DX-9065a (**1**)[17—21] was found to be a selective FXa inhibitor. Further, YM-60828 (**2**),[22—27] which is closely related to **1** in terms of structure, was found to have a more potent inhibitory effect on FXa.

On the other hand, as described in the preceding paper,[1] we have synthesized M55113 (**3**) as a potent inhibitor of FXa ($IC_{50}$=0.06 $\mu$M) with high selectivity for FXa over trypsin and thrombin.

Compared with the structure of compound **3**, the features of compound **1** and compound **2** include a functional acid group in the linker part of the molecule. According to the X-ray structure of the complex of compound **1** in des-Gla-FXa,[28] it is clear that the nitrogen atom of Gln-192 forms a weak hydrogen bond with the acid function of compound **1**.

In order to obtain a compound which has powerful inhibitory activity on FXa, it is necessary to introduce a functional group into the position at which compound **3** and Gln-192 of FXa protein are expected to form a hydrogen bond.

In this paper, we wish to report the continuing search for potent compounds that have a substituent at the piperazine and piperidine rings of compound **3**. Thus, the structure-activity relationships of compound **3** derivatives were examined.

## Chemistry

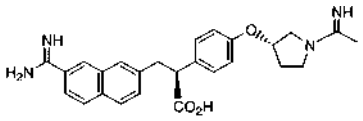
A key intermediate **7** was prepared from glycine ethyl ester hydrochloride, as illustrated in Chart 1. The compound **5**, obtained by the reaction of glycine ethyl ester hydrochloride (**4**) with bromoacetaldehyde diethyl acetal in the presence of cesium carbonate ($Cs_2CO_3$)[29,30] and sodium iodide, was treated with 6-chloro-2-naphthalenesulfonyl chloride under basic conditions, and the resulting sulfonamide **6** was hydrolyzed with aqueous trifluoroacetic acid (TFA) to give the formyl compound **7**.

Synthetic routes of compounds **12** and **17** are shown in Chart 2. Compound **9** was prepared by protection of the primary amino group with di-*tert*-butyl dicarbonate ($Boc_2O$) and subsequent debenzylation of a secondary amino group with $H_2$ over Pd/C. Condensation of **9** with 4-chloropyridine 1-oxide under basic conditions gave the 4-piperidinopyridine 1-oxide derivative **10**. Hydrogenolysis of **10** over Raney Ni[31] in methanol (MeOH), followed by treatment with HCl–MeOH, yielded compound **11**. The final compound **12** was obtained smoothly by reductive condensation of **11** with the intermediate **7**.[32]

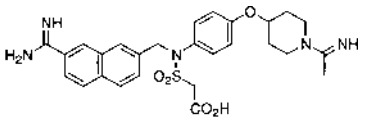
Reaction conditions in the preparation of compound **17** from compound **13**[33] were not much different from the conversion of **8** to **12**.

Synthesis of 4-substituted piperidine derivatives containing another functional group (compound **18**—**20**) in place of the hydroxyl group in **12** was accomplished as shown in Chart 3.

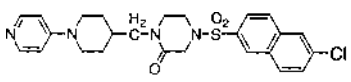
Swern oxidation[34] of **17**, followed by condensation of the resulting aldehyde with hydroxylamine under traditional con-



DX-9065a 1



YM-60828 2



M55113 3

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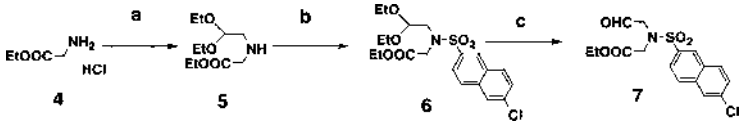
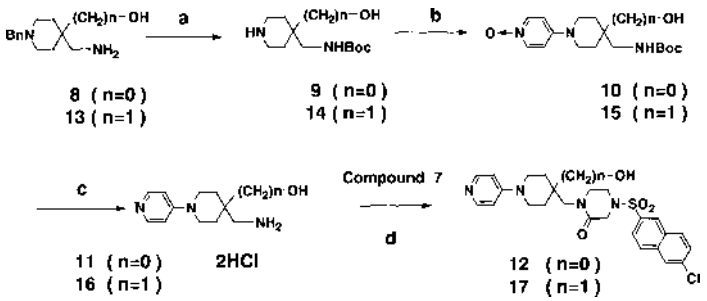


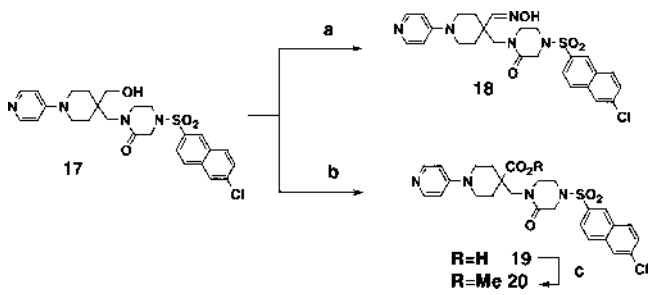
Reagents: a. Bromoacetaldehyde diethyl acetal, $Cs_2CO_3$, NaI, DMF ;
b .6-Chloro-2-naphthalenesulfonyl chloride, $Et_3N$, $CH_2Cl_2$ ;
c. TFA, $H_2O$- $CHCl_3$

Chart 1. Synthesis of Intermediate **7**



Reagents: a. i) $Boc_2O$, NaOH, Dioxane-$H_2O$ ; ii) Pd/C, $HCO_2NH_4$, MeOH ;
b. 4-Chloropyridine 1-oxide, $NaHCO_3$, Isoamylalcohol ;
c. i) $H_2$, Raney-Ni, MeOH ; ii) 10% HCl-MeOH ;
d. AcOH, $NaBH(OAc)_3$, $CH_2Cl_2$

Chart 2. Synthesis of Compounds **12** and **17**



Reagents: a. i) $(COCl)_2$, DMSO, $CH_2Cl_2$ ; ii) $HONH_2$-HCl, AcONa, EtOH ;
b. i) $(COCl)_2$, DMSO, $CH_2Cl_2$ ; ii) $NaClO_2$ ; c. TMS-$CHN_2$

Chart 3. Synthesis of 4-Substituted Piperidine Derivatives

ditions, afforded 4-(hydroxyiminomethy)piperidine (**18**). When the aldehyde was treated with $NaClO_2$ and trimethylsilyldiazomethane, the carboxylic acid (**19**) and the corresponding methyl ester (**20**) were obtained in good yields, respectively.

Synthesis of piperazine derivatives containing a functional carbon group on the 6-position of the piperazine ring was accomplished according to the route shown in Chart 4.

The crude carbaldehyde (**22**) which was obtained by Swern oxidation of 1-(4-pyridinyl)-4-piperidinemethanol[35] (**21**) was treated with 3-(*tert*-butoxycarbonylamino)alanine ethyl ester[36] under reductive conditions to give compound **23**. Compound **24** was yielded by the acylation of **23** with chloroacetyl chloride in the presence of triethylborane ($Et_3B$) under basic conditions, followed by deprotection with HCl–EtOH and then ring cyclization with $Et_3N$ in DMF.

The sulfonylation of compound **24** with 6-chloro-2-naphthalenesulfonyl chloride gave compound **25**, and the corresponding carboxylic acid (**26**) was obtained by the hydrolysis of **24** with aqueous NaOH. Compound **27** was prepared by heating **26** with methanolic ammonia in a sealed tube. Compound **28** was yielded by the reduction of compound **25** with lithium borohydride. Compound **29** was obtained by the methylation of compound **28** with dimethyl sulfate. Compound **28** was treated with phthalimide by Mitsunobu reaction, and with hydrazine, successively, to give compound **30**. The oxidation of **28**, followed by condensation of the resulting aldehyde with hydroxylamine under traditional conditions, afforded 6-(hydroxyiminomethyl)-2-piperazinone (**31**).

## Results and Discussion

The FXa inhibitory activity of the compounds synthesized above was measured by a method similar to that described in the preceding paper.[1] The results are listed in Tables 1 and 2.

It is conceivable that the activity of compounds increases when a substituent having hydrogen bond donating ability is introduced into the molecule. In fact, **12** and **17** showed slightly higher activity than lead compound **3**, as listed in Table 1. The carboxylic acid (**19**), however, showed very low activity (1/10), contrary to our anticipation, while the activity of the corresponding ester (**20**) is about same degree as that of the original compound **3**.

As described above, the substituent effect at the 4-position of the piperidine ring on the activity is indistinct at present. Accordingly, no further compounds with a substituent at the 4-position were synthesized.

Next, the activity of the compounds containing a substituent at the 6-position of the piperazine moiety was examined. As summarized in Table 2, all the compounds in this series showed higher inhibitory activity than the lead compound **3**.

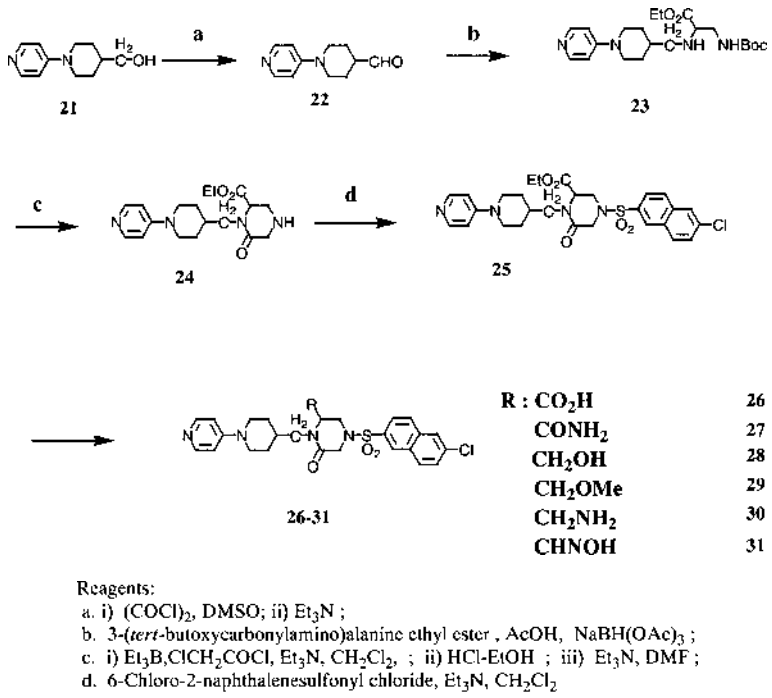
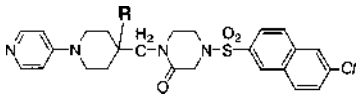


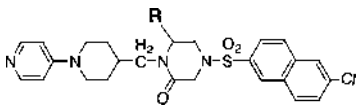

Chart 4. Synthesis of 6-Substituted 2-Piperazinone Derivatives

Table 1. FXa Inhibitory Activity of Compounds **3**, **12**, **17**—**20**



| Compd. | R | $IC_{50}$ ($\mu$M) |
|---|---|---|
| **3** | –H | 0.060 |
| **12** | –OH | 0.031 |
| **17** | $-CH_2OH$ | 0.038 |
| **18** | –CHNOH | 0.048 |
| **19** | $-CO_2H$ | 0.683 |
| **20** | $CO_2Me$ | 0.065 |

Table 2. FXa Inhibitory Activity of Compounds **3**, **25**—**31**

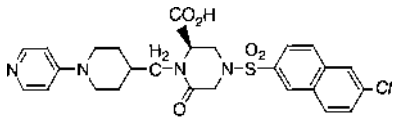


| Compd. | R | $IC_{50}$ ($\mu$M) |
|---|---|---|
| **3** | –H | 0.060 |
| **25** | $-CO_2Et$ | 0.030 |
| **26** | $-CO_2H$ | 0.010 |
| **27** | $-CONH_2$ | 0.017 |
| **28** | $-CH_2OH$ | 0.012 |
| **29** | $-CH_2OMe$ | 0.031 |
| **30** | $-CH_2NH_2$ | 0.012 |
| **31** | –CHNOH | 0.013 |

When any substituent in which especially a hydrogen bond is possible was introduced into the molecule, the FXa inhibitory activity of the compounds increased. Especially, the activity of compound **26** was six times more powerful than the lead compound **3**.

Table 3. Selectivity of M55551 for FXa over Thrombin and Trypsin

| Enzyme | $IC_{50}$ ($\mu$M) | Selectivity (enzyme/FXa) |
|---|---|---|
| FXa | 0.006 | |
| Thrombin | >100 | >16000 |
| Trypsin | >100 | >16000 |



M55551 **34**

At the final stage of the present investigation, the comparison of stereo isomers (*R*,*S*) of 6-substituted 2-piperazinones on the inhibitory activity was carried out.

The activity of stereo isomers (*R* and *S*) of **25** was examined; (*R*)-isomer (**32**, 0.017 $\mu$M) had more powerful activity than (*S*)-isomer (**33**, 0.070 $\mu$M). Then the (*R* and *S*)-isomer of free carboxylic acid (**26**) was obtained by the hydrolysis of **32** and **33** under acidic conditions. The activity of the stereoisomers (*R* and *S*) of **26** was examined; (*R*)-isomer (**34**, 0.006 $\mu$M) had more powerful activity than (*S*)-isomer (**35**, 0.079 $\mu$M).

The activity of this compound (**34**: M55551) is ten times more powerful than the lead compound (**3**: M55113).

It should also be mentioned that M55551 (**34**) showed clear selectivity for FXa over related serine proteases; in particular, the compound is 16000-fold more selective for FXa than for thrombin and trypsin, as shown in Table 3.

On the basis of these results described in the preceding and present papers, M55551 (**34**) is evaluated to be the best

compound among all the derivatives in a series of 1-arylsulfonyl-3-piperazinones, not only in FXa inhibition activity but also in selectivity between FXa and other serine proteases.

Crystallization of a complex of M55551 with FXa was attempted in order to determine whether the carboxylic acid of M55551 and Gln-192 of FXa protein form a hydrogen bond.

**Experimental**

Nuclear magnetic resonance (NMR) spectra were taken with JEOL JNM-EX270 FT-NMR (JEOL, Ltd.) or JEOL JNM-LA300 (JEOL, Ltd.) in $CDCl_3$ or dimethyl sulfoxide-$d_6$ (DMSO-$d_6$) using tetramethylsilane as the internal reference. Data measured by JEOL JNM-LA300 are marked with an asterisk. The following abbreviations were used: s=singlet, d=doublet, dd=double doublet, dt=double triplet, t=triplet, q=quartet, m=multiplet and br=broad. High-resolution mass spectra (HR-MS) were taken with JEOL JMS-GCMATE (JEOL, Ltd.). Optical rotations were determined in the indicated solvents on a JASCO DIP-1000 digital polarimeter (JASCO Corporation). Elemental analysis was performed on a CHNS-O EA1108 elemental analyzer (Carlo Erba Instruments).

**Measurement of Factor Xa, Thrombin and Trypsin Inhibition** The enzyme solution was mixed with a test compound dissolved at various concentrations in dimethyl sulfoxide (DMSO). A 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. The following enzymes and substrates were used: 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 of a control containing no test compound. The inhibitory activity of the test compound was expressed as $IC_{50}$.

***N*-(2,2-Diethoxyethyl)glycine Ethyl Ester (5)** To the suspension of glycine ethyl ester hydrochloride **4** (1.00 g, 7.2 mmol) and bromoacetaldehyde diethyl acetal (1.08 ml, 7.0 mmol) in *N*,*N*-dimethylformamide (DMF) (30 ml), cesium carbonate (4.67 g, 14.4 mmol) and sodium iodide (107 mg, 0.7 mmol) were added. The reaction mixture was stirred for 4 h at 100 °C. After cooling, the reaction mixture was acidified with 1 N aqueous HCl to pH 2 and was washed with AcOEt. Then the aqueous layer was made basic with 1 N aqueous NaOH to pH 11, and was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. Compound **5** was obtained (860 mg, 55%) as a pale yellow oil. $^1$H-NMR ($CDCl_3$) $\delta$: 4.63—4.57 (1H, m), 4.24—4.14 (2H, m), 3.78—3.64 (2H, m), 3.61—3.48 (2H, m), 3.44 (2H, s), 2.76 (2H, d, $J$=6 Hz), 1.32—1.15 (9H, m).

***N*-[(6-Chloro-2-naphthalenyl)sulfonyl]-*N*-(2,2-diethoxyethyl)glycine Ethyl Ester (6)** To the suspension of compound **5** (504 mg, 2.3 mmol) in $CH_2Cl_2$ (20 ml), triethylamine ($Et_3N$) (336 $\mu$l, 2.4 mmol) and 6-chloro-2-naphtalenesulfonyl chloride (600 mg, 2.3 mmol) were added at 0 °C. The reaction mixture was stirred at ambient temperature overnight. Then brine was added to the reaction mixture and the reaction mixture was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; *n*-hexane : AcOEt=20 : 1—4 : 1) to give compound **6** (750 mg, 74%). $^1$H-NMR (*$CDCl_3$) $\delta$: 8.40 (1H, s), 7.93—7.82 (4H, m), 7.55 (1H, dd, $J$=2, 9 Hz), 4.68—4.63 (1H, m), 4.33 (2H, s), 3.96 (2H, q, $J$=7 Hz), 3.77—3.64 (2H, m), 3.60—3.47 (2H, m), 3.37 (2H, d, $J$=6 Hz), 1.23—1.13 (6H, m), 1.12—1.04 (3H, m).

***N*-[(6-Chloro-2-naphthalenyl)sulfonyl]-*N*-(2-oxoethyl)glycine Ethyl Ester (7)** To a mixture of trifluoroacetic acid (5 ml), $CHCl_3$ (1.5 ml) and $H_2O$ (2.5 ml), the solution of compound **6** (560 mg, 1.3 mmol) in $CHCl_3$ (1 ml) was added with vigorous stirring at 0 °C. After 1.5 h, the reaction mixture was made basic with saturated sodium hydrogencarbonate ($NaHCO_3$) aq. to pH 8, and was then extracted with diethyl ether ($Et_2O$). The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; *n*-hexane : AcOEt=5 : 1—2 : 1) to give compound **7** (240 mg, 51%). $^1$H-NMR (*$CDCl_3$) $\delta$: 9.74—9.70 (1H, m), 8.39 (1H, s), 7.98—7.85 (3H, m), 7.85—7.78 (1H, m), 7.62—7.53 (1H, m), 4.24—4.03 (6H, m), 1.21—1.13 (3H, m).

**[(4-Hydroxy-4-piperidinyl)methyl]carbamic Acid 1,1-Dimethylethyl Ester (9)** To a solution of 4-(aminomethyl)-1-(phenylmethyl)-4-piperidinol (**8**) (7.5 g, 34 mmol) in 1,4-dioxane–$H_2O$ (66—34 ml), 1 N aqueous NaOH (34 ml) and $Boc_2O$ (8.16 g, 37.4 mmol) were added, successively. The reaction mixture was stirred at 60 °C for 4 h. After cooling, the reaction mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure to give [[4-hydroxy-1-(phenylmethyl)-4-piperidinyl]methyl] carbamic acid 1,1-dimethylethyl ester (8.19 g, 75%) as a colorless crystal. $^1$H-NMR (*$CDCl_3$) $\delta$: 7.36—7.22 (5H, m), 4.98—4.82 (1H, m), 3.55 (2H, s), 3.15 (2H, d, $J$=6.2 Hz), 2.69—2.56 (2H, m), 2.48—2.32 (2H, m), 1.71—1.51 (4H, m), 1.44 (9H, s).

To the suspension of [[4-hydroxy-1-(phenylmethyl)-4-piperidinyl]methyl] carbamic acid 1,1-dimethylethyl ester (8.19 g, 25.6 mmol) and 10% Pd–C (800 mg) in MeOH (300 ml), ammonium formate (4.84 g, 76.8 mmol) was added, and the reaction mixture was refluxed for 2 h. Then the catalyst was removed by filtration and the filtrate was condensed to afford **9** (6.03 g, 99%) as a colorless crystal. $^1$H-NMR (*DMSO-$d_6$) $\delta$: 6.62—6.52 (1H, m), 2.89 (2H, d, $J$=6.2 Hz), 2.79—2.55 (4H, m), 1.44—1.21 (4H, m), 1.38 (9H, s).

**[[4-Hydroxy-1-(1-oxido-4-pyridinyl)-4-piperidinyl]methyl]carbamic Acid 1,1-Dimethylethyl Ester (10)** To a suspension of compound **9** (2.00 g, 8.7 mmol) and 4-chloropyridine 1-oxide (1.12 g, 8.7 mmol) in isoamylalcohol (35 ml), $NaHCO_3$ (1.75 g, 20.8 mmol) was added, and the reaction mixture was refluxed for 6 h. After cooling, water was added to the reaction mixture and the reaction mixture was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=9 : 1—4 : 1) to give compound **10** (1.03 g, 37%). $^1$H-NMR (*DMSO-$d_6$) $\delta$: 7.90—7.83 (2H, m), 6.92—6.84 (2H, m), 6.78—6.70 (1H, m), 4.58 (1H, br s), 3.64—3.50 (2H, m), 3.17—3.04 (2H, m), 2.92 (2H, d, $J$=6Hz), 1.60—1.30 (4H, m), 1.35 (9H, s).

**4-(Aminomethyl)-1-(4-pyridinyl)-4-piperidinol Hydrochloride (11)** To a solution of compound **10** (300 mg, 0.93 mmol) in MeOH (3 ml), Raney-Ni (catalytic amount) was added, and the reaction mixture was stirred in an atmosphere of hydrogen for 3.5 h at ambient temperature. Then the catalyst was removed by filtration and the filtrate was condensed. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=4 : 1) to give [[4-hydroxy-1-(4-pyridinyl)-4-piperidinyl]methyl]carbamic acid 1,1-dimethylethyl ester (214 mg, 71%). $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.10 (2H, d, $J$=7 Hz), 6.87 (2H, d, $J$=7 Hz), 6.76—6.68 (1H, m), 4.55 (1H, br s), 3.78—3.65 (2H, m), 3.24—3.11 (2H, m), 2.92 (2H, d, $J$=6 Hz), 1.58—1.30 (4H, m), 1.35 (9H, s).

[[4-Hydroxy-1-(4-pyridinyl)-4-piperidinyl]methyl]carbamic acid 1,1-dimethylethyl ester (175 mg, 0.57 mmol) was dissolved in 10% HCl–MeOH (2 ml), and the reaction mixture was stirred for 2 h at ambient temperature. The solvent was removed under reduced pressure. The residue was triturated with $Et_2O$ to give compound **11** (160 mg, 89%). $^1$H-NMR (DMSO-$d_6$) $\delta$: 13.68 (1H, br s), 8.20 (2H, d, $J$=8 Hz),8.13 (2H, br s), 7.22 (2H, d, $J$=8 Hz), 5.43 (1H, s), 4.08—3.96 (2H, m), 3.52—3.32 (2H, m), 2.80 (2H, br s), 1.77—1.46 (4H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-1-[[4-hydroxy-1-(4-pyridinyl)-4-piperidinyl]methyl]piperazinone (12)** Acetic acid (0.02 ml) was added to a suspension of compound **7** (58.4 mg, 0.16 mmol) and compound **11** (50 mg, 0.16 mmol) in $CH_2Cl_2$ (3 ml) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature. Then sodium triacetoxyborohydride ($NaBH(OAc)_3$) (67 mg, 0.32 mmol) was added to the reaction mixture at 0 °C, and the reaction mixture was stirred at ambient temperature overnight. Then the reaction mixture was made basic with 1 N aqueous NaOH to pH 9, and was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=10 : 1), then another silica gel column chromatography (Chromatorex NH$^{TM}$ (Fuji Silysia Chemical, Ltd.) eluents; $CH_2Cl_2$ : MeOH=20 : 1) to give compound **12** (23.3 mg, 29%). HR-MS *m*/*z*: Calcd for $C_{25}H_{27}ClN_4O_4S$: 514.1441. Found: 514.1448. $^1$H-NMR ($CDCl_3$) $\delta$: 8.38—8.32 (1H, m), 8.21—8.13 (2H, m), 7.98—7.90 (3H, m), 7.82—7.75 (1H, m), 7.65—7.58 (1H, m), 6.63—6.56 (2H, m), 3.83 (2H, s), 3.63—3.52 (4H, m), 3.43—3.36 (2H, m), 3.39 (2H, s), 3.29—3.17 (2H, m), 2.02 (1H, br s), 1.59—1.44 (4H, m).

**[[4-(Hydroxymethyl)-4-piperidinyl]methyl]carbamic Acid 1,1-Dimethyl Ethyl Ester (14)** To a solution of 4-(aminomethyl)-1-(phenylmethyl)-4-piperidine-methanol **13** (16.4 g, 70.0 mmol) prepared by a documented method[33] in 1,4-dioxane–$H_2O$ (140–70 ml), 1 N aqueous NaOH (70 ml) and $Boc_2O$ (16.8 g, 77.0 mmol) were added, successively. The reaction mixture was stirred at 60 °C for 1.5 h. After cooling, the reaction mixture

was extracted with EtOAc. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure to give [[4-(hydroxymethyl)-1-(phenylmethyl)-4-piperidinyl]-methyl]carbamic acid 1,1-dimethylethyl ester (21.4 g, 91%) as a colorless crystal. $^1$H-NMR (*DMSO-$d_6$) $\delta$: 7.35—7.20 (5H, m), 6.75—6.60 (1H, m), 4.40—4.30 (1H, m), 3.42 (2H, s), 3.20—3.15 (2H, m), 2.95—2.90 (2H, m), 2.35—2.25 (4H, m), 1.37 (9H, s), 1.40—1.25 (4H, m).

To the suspension of [[4-(hydroxymethyl)-1-(phenylmethyl)-4-piperidinyl]-methyl]carbamic acid 1,1-dimethylethyl ester (0.81 g, 2.4 mmol) in EtOH–$CH_2Cl_2$ (10–5ml), 10% Pd–C (80 mg) was added, and the reaction mixture was stirred in an atmosphere of hydrogen for 2 d at ambient temperature. Then the catalyst was removed by filtration and the filtrate was condensed. The residue was purified by silica gel column chromatography ($CH_2Cl_2$ : MeOH=20 : 1—5 : 1) to give **14** (230 mg, 39%) as a colorless crystal. $^1$H-NMR ($CDCl_3$) $\delta$: 4.92—4.83 (1H, m), 3.39 (2H, s), 3.09 (2H, d, $J$=7 Hz), 2.87—2.74 (4H, m), 1.60—1.20 (4H, m), 1.45 (9H, s).

**[[4-(Hydroxymethyl)-1-(1-oxido-4-pyridinyl)-4-piperidinyl]methyl]-carbamic Acid 1,1-Dimethylethyl Ester (15)** To the suspension of compound **14** (1.88 g, 7.7 mmol) and 4-chloropyridine 1-oxide (1.00 g, 7.7 mmol) in isoamylalcohol (15 ml), $NaHCO_3$ (1.55 g, 18.5 mmol) was added, and reaction mixture was refluxed for 2 h. After cooling, water was added to the reaction mixture, and the reaction mixture was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=20 : 1—13 : 2) to give compound **15** (1.11 g, 43%). $^1$H-NMR ($CDCl_3$) $\delta$: 7.99 (2H, d, $J$=7.9 Hz), 6.62 (2H, d, $J$=7.9 Hz), 5.35—5.20 (1H, m), 4.35—4.10 (1H, br s), 3.42 (2H, s), 3.40—3.22 (4H, m), 3.14 (2H, d, $J$=6.9 Hz), 1.72—1.48 (4H, m), 1.45 (9H, s).

**4-(Aminomethyl)-1-(4-pyridinyl)-4-piperidinemethanol Hydrochloride (16)** To a solution of compound **15** (250 mg, 0.7 mmol) in MeOH (1 ml), Raney-Ni (catalytic amount) was added, and the reaction mixture was stirred in an atmosphere of hydrogen for 1 h at ambient temperature. Then the catalyst was removed by filtration. The filtrate was condensed to afford [[4-(hydroxymethyl)-1-(4-pyridinyl)-4-piperidinyl]methyl]carbamic acid 1,1-dimethylethyl ester (190 mg, 80%) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 8.35—8.07 (2H, m), 6.75—6.50 (2H, m), 5.12—4.95 (1H, m), 3.53—3.06 (9H, m), 1.70—1.33 (4H, m), 1.46 (9H, s).

[[4-(Hydroxymethyl)-1-(4-pyridinyl)-4-piperidinyl]methyl]carbamic acid 1,1-dimethylethyl ester (60 mg, 0.19 mmol) was dissolved in 10% HCl–MeOH (0.6 ml), and the reaction mixture was stirred for 10 min at 40 °C. The solvent was removed under reduced pressure. Then $Et_2O$ was added to the residue, and it crystallized to afford compound **16** (46 mg, 75%) as a white powder. $^1$H-NMR (DMSO-$d_6$) $\delta$: 13.7—13.4 (1H, br), 8.25—8.16 (2H, m), 8.10—7.90 (2H, br), 7.23—7.12 (2H, m), 5.37—5.21 (1H, br), 3.80—3.30 (6H, m), 2.95—2.84 (2H, m), 1.65—1.51 (4H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-1-[[4-(hydroxymethyl)-1-(4-pyridinyl)-4-piperidinyl]methyl]piperazinone (17)** Acetic acid (28 $\mu$l) was added to a suspension of compound **7** (100 mg, 0.27 mmol) and compound **16** (89 mg, 0.27 mmol) in $CH_2Cl_2$ (3 ml) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature. Then $NaBH(OAc)_3$ (114 mg, 0.54 mmol) was added to the reaction mixture at 0 °C, and the reaction mixture was stirred at ambient temperature overnight. Then the reaction mixture was made basic with 1 N aqueous NaOH to pH 8, and was extracted with $CHCl_3$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=20 : 1—5 : 1) to give compound **17** (43 mg, 30%). HR-MS $m/z$: Calcd for $C_{26}H_{29}ClN_4O_4S$: 528.1598. Found: 528.1594. $^1$H-NMR ($CDCl_3$) $\delta$: 8.39—8.35 (1H, m), 8.29—8.22 (2H, m), 7.99—7.93 (3H, m), 7.83—7.77 (1H, m), 7.65—7.60 (1H, m), 6.65—6.60 (2H, m), 3.86 (2H, s), 3.57—3.38 (8H, m), 3.31 (2H, s), 3.26 (1H, br s), 3.29—3.06 (2H, m), 1.66—1.53 (2H, m), 1.50—1.38 (2H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-1-[[4-(hydroxyiminomethyl)-1-(4-pyridinyl)-4-piperidinyl]methyl]piperazinone (18)** The solution of DMSO (99 $\mu$l) in $CH_2Cl_2$ (4.5 ml) was added to the solution of oxalyl chloride (61 $\mu$l) in $CH_2Cl_2$ (4.5 ml) below −70 °C. The solution of compound **17** (185 mg, 0.35 mmol) in DMSO–$CH_2Cl_2$ (2.2–2.2 ml) was added to the above solution below −70 °C. The reaction mixture was stirred for 1 h between −60 and −65 °C. Then $Et_3N$ (292 $\mu$l, 2.1 mmol) was added to the reaction mixture at −78 °C. After the temperature was raised to room temperature, water was added to the reaction mixture. The reaction mixture was extracted with $CH_2Cl_2$, washed with brine and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. Without purification, the residue was dissolved in EtOH–$CHCl_3$ (6–1.2ml). Then sodium acetate (48 mg, 0.6 mmol) and hydroxylamine hydrochloride (38 mg, 0.6 mmol) were added to the reaction mixture. The reaction mixture was stirred at ambient temperature overnight. Water was added to the reaction mixture, and this mixture was made basic with saturated $NaHCO_3$ aq. to pH 8, then extracted with $CHCl_3$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=20 : 1—5 : 1) to give compound **18** (100 mg, 54%). $^1$H-NMR (DMSO-$d_6$) $\delta$: 10.73 (1H, s), 8.58 (1H, s), 8.32—8.24 (2H, m), 8.17 (1H, d, $J$=9 Hz), 8.12—8.05 (2H, m), 7.90—7.84 (1H, m), 7.78—7.70 (1H, m), 7.15 (1H, s), 6.73—6.65 (2H, m), 3.70 (2H, s), 3.60—3.20 (8H, m), 2.88—2.72 (2H, m), 1.60—1.44 (2H, m), 1.40—1.24 (2H, m).

**4-[4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxopiperazinyl]methyl-1-(4-pyridinyl)-4-piperidinecaboxylic Acid (19)** A solution of DMSO (23 $\mu$l) in $CH_2Cl_2$ (1.0 ml) was added to the solution of oxalyl chloride (14 $\mu$l) in $CH_2Cl_2$ (1.0 ml) below −70 °C. A solution of compound **17** (37 mg, 0.35 mmol) in DMSO–$CH_2Cl_2$ (0.4–0.4 ml) was added to the above solution below −70 °C. The reaction mixture was stirred for 1 h between −60 and −65 °C. Then $Et_3N$ (60 $\mu$l, 0.4 mmol) was added to the reaction mixture at −78 °C. After the temperature was raised to room temperature, water was added to the reaction mixture. This reaction mixture was extracted with $CH_2Cl_2$ and washed with brine, then dried over $Na_2SO_4$. The solvent was removed under reduced pressure. Without purification, 2-methyl-2-butene (56 $\mu$l, 0.5 mmol) was added to the solution of the residue in *tert*-butanol–$CHCl_3$ (0.5–0.1 ml). Then the solution of $NaClO_2$ (11.7 mg, 0.1 mmol) and $NaH_2PO_4$–$2H_2O$ (16.1 mg, 0.1 mmol) in water (0.4 ml) was added to the solution. The reaction mixture was vigorously stirred at ambient temperature overnight. Saturated $NH_4Cl$ aq. was added to reaction mixture. Then $CHCl_3$ and MeOH were added to the reaction mixture, successively. $Na_2SO_4$ was added to the reaction mixture, and the solvent was evaporated under reduced pressure. The residue was triturated with THF to give compound **19** (40 mg, 93%) as a white powder. $^1$H-NMR (DMSO-$d_6$) $\delta$: 8.58 (1H, s), 8.32—8.10 (5H, m), 7.90—7.84 (1H, m), 7.78—7.70 (1H, m), 6.96—6.88 (2H, m), 3.84—3.00 (8H, m), 3.72 (2H, s), 2.98—2.82 (2H, m), 1.88—1.72 (2H, m), 1.42—1.28 (2H, m).

**4-[4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxopiperazinyl]methyl-1-(4-pyridinyl)-4-piperidinecaboxylic Acid Methyl Ester (20)** To the solution of compound **19** (10 mg, 0.02 mmol) in MeOH–$CH_2Cl_2$ (0.5–0.5 ml), trimethylsilyldiazomethane (12 $\mu$l, 0.02 mmol; 2.0 M in *n*-hexane) was added at 0 °C. The reaction mixture was stirred at ambient temperature for 2 h. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=40 : 1—8 : 1) to give compound **20** (4.5 mg, 44%). HR-MS $m/z$: Calcd for $C_{27}H_{29}ClN_4O_5S$: 556.1547. Found: 556.1543. $^1$H-NMR ($CDCl_3$) $\delta$: 8.36—8.33 (1H, m), 8.28—8.16 (2H, m), 7.99—7.92 (3H, m), 7.82—7.77 (1H, m), 7.65—7.59 (1H, m), 6.67—6.58 (2H, m), 3.79 (2H, s), 3.75—3.63 (2H, m), 3.73 (3H, s), 3.54 (2H, s), 3.37 (4H, s), 2.98—2.84 (2H, m), 2.17—2.05 (2H, m), 1.60—1.48 (2H, m).

**1-(4-Pyridinyl)-4-piperidinecarboxaldehyde (22)** A solution of oxalyl chloride (1.77 ml, 20 mmol) in $CH_2Cl_2$ (85 ml) was cooled to −78 °C under Ar. To the cooled solution, a solution of DMSO (3.25 ml, 42 mmol) in dry $CH_2Cl_2$ (85 ml) was added dropwise for 20 min. Then, a solution of compound **21** (3.0 g, 15.6 mmol) prepared by a documented method[35] in dry $CH_2Cl_2$ (48 ml) and dry DMSO (48 ml) was added dropwise for another 20 min. The reaction mixture was stirred at between −65 °C and −60 °C for 1 h, then cooled to −78 °C, and $Et_3N$ (8.31 ml, 60 mmol) was added. The reaction mixture was allowed to stand at room temperature, water (200 ml) was added, and the mixture was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The resulting aldehyde **22** was rather unstable and should be used in the next reaction without purification. MS $m/z$: 190 ($M^+$). $^1$H-NMR (*$CDCl_3$) $\delta$: 9.56 (1H, s), 8.16—7.99 (2H, m), 6.82—6.69 (2H, m), 3.83—3.71 (2H, m), 3.02—2.90 (2H, m), 2.61—2.45 (1H, m), 1.90—1.78 (2H, m), 1.52—1.36 (2H, m).

**3-(*tert*-Butoxycarbonylamino)-*N*-[[1-(4-pyridinyl)-4-piperidinyl]-methyl]alanine Ethyl Ester (23)** Crude product **22** (0.56 g, 2.9 mmol), obtained by Swern oxidation, was suspended in dry $CH_2Cl_2$ (16 ml). To the suspension, $\beta$-(*tert*-butoxycarbonylamino)-alanine ethyl ester (0.70 g, 3.0 mmol) obtained by a documented method[36] and AcOH (0.37 ml) were added in that order. After stirring the resulting mixture at room temperature for 30 min under Ar, $NaBH(OAc)_3$ (1.6 g, 7.5 mmol) was added and the reaction mixture was stirred overnight at room temperature. Water (20 ml) was added to the reaction mixture, then it was adjusted to pH 9 with 1 N sodium

hydroxide solution, followed by extraction with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated at reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=99 : 1—80 : 20) to give compound **23** (0.62 g, 52%). $^1$H-NMR ($CDCl_3$) $\delta$: 8.24 (2H, d, $J$=5 Hz), 6.65 (2H, d, $J$=5 Hz), 5.03—4.90 (1H, m), 4.20 (2H, q, $J$=7 Hz), 3.95—3.83 (2H, m), 3.58—3.17 (3H, m), 2.90—2.77 (2H, m), 2.58 (1H, dd, $J$=7, 11 Hz), 2.44—2.34 (1H, m), 1.94—1.59 (3H, m), 1.44 (9H, s), 1.29 (3H, t, $J$=7 Hz), 1.37—1.19 (2H, m).

**6-Oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic Acid Ethyl Ester (24)** To the solution of the compound **23** (2.9 g, 7.1 mmol) in dry $CH_2Cl_2$ (12 ml), triethylborane ($Et_3B$, 8.4 ml, 8.4 mmol; 1.0 M in THF) was added at 0 °C. The mixture was cooled to −78 °C. To the cooled solution, a solution of chloroacetyl chloride (0.68 ml, 8.4 mmol) in dry $CH_2Cl_2$ (2 ml) and $Et_3N$ (1.2 ml, 8.4 mmol) were added in that order, and the mixture was stirred at −78 °C for 1 h. The reaction mixture was allowed to stand at room temperature for 5 h. After cooling the reaction mixture with ice, water was added, and it was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. To the solution of residue (5.1 g) in EtOH (38 ml), 20 wt% HCl–EtOH (38 ml) was added at 0 °C for 5 min and stirred at 0 °C. The mixture was allowed to stand at room temperature with stirring for 2 h. The solvent was evaporated under reduced pressure and the residue was used in the next reaction without purification. A solution of the residue in anhydrous dimethylformamide (DMF) (6.7 ml) was cooled with ice. To the cooled solution, $Et_3N$ (14.8 ml, 0.11 mol) was added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [Chromatorex NH$^{TM}$] (eluents; $CH_2Cl_2$ : MeOH=95 : 5—90 : 10) to give compound **24** (1.04 g, 43%). $^1$H-NMR (*$CDCl_3$) $\delta$: 8.13 (2H, d, $J$=7 Hz), 6.97 (2H, d, $J$=7 Hz), 4.27 (2H, q, $J$=7 Hz), 4.23—4.17 (3H, m), 3.94 (1H, dd, $J$=8.14 Hz), 3.57 (2H, s), 3.43—3.11 (4H, m), 2.66 (1H, dd, $J$=7, 14 Hz), 2.20—2.03 (1H, m), 1.98—1.81 (2H, m), 1.44—1.24 (2H, m), 1.32 (3H, t, $J$=7 Hz).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic Acid Ethyl Ester (25)** Compound **24** (52 mg, 0.15 mmol) was dissolved in $CH_2Cl_2$ (5 ml). To the solution, $Et_3N$ (0.1 ml, 0.7 mmol) and 6-chloro-2-naphthalenesulfonyl chloride (39.1 mg, 0.15 mmol) were added, in that order, and the mixture was stirred overnight at room temperature. Water was added to the reaction mixture, which was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=99 : 1—9 : 1) to give compound **25** (48 mg, 56%). HR-MS *m/z*: Calcd for $C_{28}H_{31}ClN_4O_5S$: 570.1703. Found: 570.1681. $^1$H-NMR ($CDCl_3$) $\delta$: 8.34 (1H, s), 8.25—8.18 (2H, m), 7.98—7.90 (3H, m), 7.77 (1H, dd, $J$=2, 9 Hz), 7.64—7.58 (1H, m), 6.64—6.57 (2H, m), 4.38—4.04 (5H, m), 3.96—3.75 (3H, m), 3.46 (1H, d, $J$=17 Hz), 3.07—2.96 (1H, m), 2.88—2.68 (2H, m), 2.66—2.55 (1H, m), 1.93—1.75 (1H, m), 1.73—1.55 (2H, m), 1.32 (3H, t, $J$=7 Hz), 1.32—1.14 (2H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic Acid (26)** To a solution of the compound **25** (18 mg, 0.03 mmol) in MeOH (0.5 ml), 2 N NaOH solution (63 $\mu$l) was added, and the mixture was stirred at 40 °C for 30 min. The reaction mixture was concentrated under reduced pressure and the resulting residue was dissolved in water and rendered weakly acidic with 0.1 N HCl. The supernatant was removed by decantation, and the residue was dissolved in MeOH, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure to give compound **26** (9 mg, 53%). $^1$H-NMR (*$CD_3OD$) $\delta$: 8.47 (1H, s), 8.16—7.99 (5H, m), 7.90—7.83 (1H, m), 7.64 (1H, dd, $J$=2, 9 Hz), 7.02 (2H, d, $J$=8 Hz), 4.18—3.91 (5H, m), 3.81 (1H, dd, $J$=8, 14 Hz), 3.58 (1H, d, $J$=16 Hz), 3.30—3.20 (1H, m), 3.12—2.93 (2H, m), 2.72 (1H, dd, $J$=7, 14 Hz), 2.08—1.92 (1H, m), 1.82—1.71 (1H, m), 1.62—1.52 (1H, m), 1.31—1.04 (2H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxamide (27)** Ammonia gas was blown into a solution of compound **26** (0.20 g, 0.35 mmol) in 2 N ammonia–MeOH (5 ml), and the solution was stirred in a sealed tube at 80—90 °C for 8 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=9 : 1) to give compound **27** (0.14 g, 74%). HR-MS *m/z*: Calcd for $C_{26}H_{28}ClN_5O_4S$: 541.1550. Found: 541.1509. $^1$H-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 8.48—8.44 (1H, m), 8.14—8.00 (5H, m), 7.85 (1H, dd, $J$=2, 9 Hz), 7.65 (1H, dd, $J$=2, 9 Hz), 6.72 (2H, d, $J$=6 Hz), 4.19 (1H, t, $J$=3 Hz), 4.13—4.04 (2H, m), 3.96—3.80 (3H, m), 3.55 (1H, d, $J$=17 Hz), 3.24 (1H, dd, $J$=4, 12 Hz), 2.86—2.69 (2H, m), 2.62 (1H, dd, $J$=7, 14 Hz), 1.98—1.80 (1H, m), 1.75—1.50 (2H, m), 1.35—1.10 (2H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-(hydroxymethyl)-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]piperazinone (28)** Compound **25** (0.15 g, 0.26 mmol) was dissolved in MeOH (10 ml). To the solution, lithium borohydride (0.60 g, 27.5 mmol) was added in three portions at 30-min intervals. To the reaction mixture, 10% HCl–MeOH was added under cooling with ice to make it acidic, and the mixture was then concentrated to dryness. Water was added to the residue, then saturated $NaHCO_3$ aq. was added to make the residue alkaline, and the mixture was extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography [Chromatorex NH$^{TM}$] (eluents; EtOAc) to give compound **28** (60 mg, 43%). HR-MS *m/z*: Calcd for $C_{26}H_{29}ClN_4O_4S$: 528.1598. Found: 528.1636. $^1$H-NMR ($CDCl_3$) $\delta$: 8.36 (1H, s), 8.21 (2H, d, $J$=7 Hz), 8.00—7.90 (3H, m), 7.84—7.76 (1H, m), 7.62 (1H, dd, $J$=2, 9 Hz), 6.58 (2H, d, $J$=7 Hz), 4.28—4.12 (2H, m), 3.95—3.72 (5H, m), 3.48—3.35 (2H, m), 2.84—2.63 (4H, m), 2.05—1.46 (3H, m), 1.34—1.13 (2H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-(methoxymethyl)-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]piperazinone (29)** Compound **28** (43 mg, 0.08 mmol) was suspended in $CH_2Cl_2$ (1 ml). To the stirred suspension, 50% NaOH solution (0.3 ml) was added under cooling with ice. Then, benzyltriethylammonium chloride (3 mg, 0.013 mmol) and dimethyl sulfate (9 $\mu$l, 0.09 mmol) were added, and the mixture was stirred for 2 h under cooling with ice. Ice water was added to the reaction mixture to quench the reaction, and the mixture was extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=9 : 1) to give compound **29** (16 mg, 36%). HR-MS *m/z*: Calcd for $C_{27}H_{31}ClN_4O_4S$: 542.1754. Found: 542.1739. $^1$H-NMR (*$CDCl_3$) $\delta$: 8.36 (1H, s), 8.26—8.19 (2H, m), 7.98—7.92 (3H, m), 7.80 (1H, dd, $J$=2, 9 Hz), 7.61 (1H, dd, $J$=2, 9 Hz), 6.62—6.55 (2H, m), 4.17 (1H, d, $J$=17 Hz), 4.05 (1H, d, $J$=12 Hz), 3.94—3.70 (3H, m), 3.70—3.43 (3H, m), 3.38 (3H, s), 3.38 (1H, d, $J$=17 Hz), 2.88—2.66 (4H, m), 2.08—1.90 (1H, m), 1.71—1.54 (2H, m), 1.38—1.07 (2H, m).

**6-(Aminomethyl)-4-[(6-chloro-2-naphthalenyl)sulfonyl]-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]piperazinone (30)** Azodicarboxylic acid diethyl ester (as 40% toluene solution) (1.71 ml, 3.78 mmol) was added to a solution of triphenylphosphine (0.99 g, 3.78 mmol) and phthalimide (0.56 g, 3.78 mmol) in $CH_2Cl_2$ (30 ml) under cooling with ice. After stirring the mixture at the same temperature for 10 min, compound **28** (0.50 g, 0.95 mmol) was added, and the mixture was stirred at room temperature for 30 min. Saturated $NaHCO_3$ aq. was added to the reaction mixture under cooling with ice, and the mixture was extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=9 : 1) to give 4-[(6-chloro-2-naphthalenyl)-sulfonyl]-6-phthaliminomethyl-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-piperazinone (0.61 g, 98%). $^1$H-NMR (*$CDCl_3$) $\delta$: 8.42 (1H, s), 8.24—8.19 (2H, m), 7.97—7.87 (6H, m), 7.83—7.75 (2H, m), 7.63—7.51 (1H, m). 6.62—6.56 (2H, m), 4.33—4.20 (1H, m), 4.11 (1H, d, $J$=17 Hz), 4.04—3.93 (2H, m), 3.87—3.68 (4H, m), 3.43 (1H, d, $J$=17 Hz), 2.96—2.68 (4H, m), 2.08—1.87 (1H, m), 1.73—1.57 (2H, m), 1.42—1.17 (2H, m).

A portion (0.61 g, 0.93 mmol) of 4-[(6-chloro-2-naphthalenyl)sulfonyl]-6-phthaliminomethyl-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]piperazinone was suspended in EtOH (15 ml). Hydrazine monohydrate (54 $\mu$l, 1.11 mmol) was added to the suspension at room temperature, followed by stirring at room temperature for 20 h. The suspension was then allowed to reflux for 4 h. After leaving the suspension to cool, the insolubles were filtered off and the filtrate was concentrated. The residue was purified by silica gel column chromatography (eluents; $CH_2Cl_2$ : MeOH=9 : 1) to give compound **30** (0.36 g, 77%). HR-MS *m/z*: Calcd for $C_{26}H_{30}ClN_5O_3S$: 527.1758. Found: 527.1780. $^1$H-NMR (*$CDCl_3$) $\delta$: 8.36 (1H, d, $J$=1 Hz), 8.22—8.19 (2H, m), 7.97—7.94 (3H, m), 7.82—7.78 (1H, m), 7.61 (1H, dd, $J$=2, 9 Hz), 6.59—6.57 (2H, m), 4.25—4.16 (2H, m), 3.89—3.76 (3H, m), 3.36 (1H, d, $J$=17 Hz), 3.30—3.22 (1H, m), 3.07 (1H, dd, $J$=10, 13 Hz), 2.96 (1H, dd, $J$=4, 13 Hz), 2.79—2.68 (3H, m), 2.63 (1H, dd, $J$=8, 14 Hz), 2.05—1.87 (1H, m), 1.73—1.57 (2H, m), 1.35—1.14 (2H, m).

**4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxaldehyde 2-Oxime (31)** A solution of oxalyl chloride (33 $\mu$l, 0.38 mmol) in $CH_2Cl_2$ (1 ml) was cooled to −78 °C under Ar. To the cooled solution, a solution of DMSO (60 $\mu$l,

0.76 mmol) in $CH_2Cl_2$ (1 ml) was added dropwise over 20 min. Then, a solution of compound **28** (10 mg, 0.019 mmol) in $CH_2Cl_2$ (1 ml) was added dropwise over another 20 min. The reaction mixture was stirred at between −65 °C and −60 °C for 1 h, the mixture was cooled to −78 °C, and $Et_3N$ (0.16 ml, 1.14 mmol) was added. The reaction mixture was allowed to stand at room temperature, water (4 ml) was added, and the mixture was extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The resulting crude aldehyde was dissolved in EtOH (1 ml). Hydroxylamine hydrochloride (2.5 mg, 0.038 mmol) and sodium acetate (3 mg, 0.038 mmol) were added to the solution. AcOH was added to the reaction mixture to adjust its pH to about 4, then the mixture was stirred overnight at room temperature. The reaction mixture was rendered alkaline by the addition of saturated $NaHCO_3$ aq., then extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried over $Na_2SO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography [Chromatorex NH$^{TM}$] (eluents; $CH_2Cl_2$ : MeOH=97 : 3) to give compound **31** (1.9 mg, 19%). $^1$H-NMR (*$CDCl_3$) $\delta$: 8.37—8.35 (1H, m), 8.17 (2H, d, $J$=6 Hz), 7.98—7.92 (3H, m), 7.83—7.77 (1H, m), 7.65—7.58 (1H, m), 7.46 (0.7H, d, $J$=8 Hz), 6.84 (0.3H, d, $J$=7 Hz), 6.59 (2H, d, $J$=6 Hz), 4.96—4.87 (0.3H, m), 4.18—4.00 (1.7H, m), 3.95—3.48 (5H, m), 3.19—3.05 (1H, m), 2.90—2.64 (3H, m), 2.15—1.95 (1H, m), 1.70—1.45 (2H, m), 1.35—1.10 (2H, m).

**(*R*)-4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic Acid Ethyl Ester (32)** Using (*R*)-$\beta$-(*tert*-butoxycarbonylamino)alanine ethyl ester, the method used to synthesize compound **25** was repeated to give compound **32**. The optical purity of this compound was measured by HPLC [CHIRALPAK$^{TM}$ AS (Daicel Chemical Industries, Ltd.); methanol : diethylamine=100 : 0.1], and it was found to be 98.3% e.e. HR-MS *m/z*: Calcd for $C_{28}H_{31}ClN_4O_5S$: 570.1703. Found: 570.1664. $[\alpha]_D^{29}$ −49.9° ($c$=1.00, EtOH). $^1$H-NMR ($CDCl_3$) $\delta$: 8.34 (1H, s), 8.25—8.18 (2H, m), 7.98—7.90 (3H, m), 7.77 (1H, dd, $J$=2, 9 Hz), 7.64—7.58 (1H, m), 6.64—6.57 (2H, m), 4.38—4.04 (5H, m), 3.96—3.75 (3H, m), 3.46 (1H, d, $J$=17 Hz), 3.07—2.96 (1H, m), 2.88—2.68 (2H, m), 2.66—2.55 (1H, m), 1.93—1.75 (1H, m), 1.73—1.55 (2H, m), 1.32 (3H, t, $J$=7 Hz), 1.32—1.14 (2H, m).

**(*S*)-4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic Acid Ethyl Ester (33)** Using (*S*)-$\beta$-(*tert*-butoxycarbonylamino)alanine ethyl ester, the method used to synthesize compound **25** was repeated to give compound **33**. The optical purity of this compound was measured by HPLC [CHIRALPAK$^{TM}$ AS (Daicel Chemical Industries, Ltd.); methanol : diethylamine=100 : 0.1], and it was found to be 96.7% e.e. HR-MS *m/z*: Calcd for $C_{28}H_{31}ClN_4O_5S$: 570.1703. Found: 570.1689. $[\alpha]_D^{30}$ +42.1° ($c$=1.00, EtOH). $^1$H-NMR ($CDCl_3$) $\delta$: 8.34 (1H, s), 8.25—8.18 (2H, m), 7.98—7.90 (3H, m), 7.77 (1H, dd, $J$=2, 9 Hz), 7.64—7.58 (1H, m), 6.64—6.57 (2H, m), 4.38—4.04 (5H, m), 3.96—3.75 (3H, m), 3.46 (1H, d, $J$=17 Hz), 3.07—2.96 (1H, m), 2.88—2.68 (2H, m), 2.66—2.55 (1H, m), 1.93—1.75 (1H, m), 1.73—1.55 (2H, m), 1.32 (3H, t, $J$=7 Hz), 1.32—1.14 (2H, m).

**(*R*)-4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic Acid Monohydrate (34)** A solution of compound **32** (3.00 g, 5.3 mmol) in 1 N HCl (300 ml) was heated under reflux for 11 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH (60 ml), and 1 N aqueous NaOH was added to make it alkaline. To the solution, Muromac$^{TM}$ MSC-1 ($H^+$ form, 60 g, Muromachi Kagaku Kogyo Kaisha, Ltd.) was added, and the resulting suspension was stirred at room temperature for 30 min. The ion exchange resin was collected with suction, then washed with water and MeOH. To the resin, 10% (w/w) $NH_3$–MeOH (150 ml) was added. After stirring for 30 min, the resin was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Chromatorex NH$^{TM}$ (Fuji Silysia Chemical, Ltd.) eluents; AcOEt : MeOH=3 : 1—1 : 2) to give (*R*)-4-[(6-chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic acid ammonium salt (2.22 g, 75%). To a solution of the ammonium salt (300 mg, 0.54 mmol), 0.1 M $H_2SO_4$ aq. (2.7 ml) was added, and the resulting precipitate was collected to give compound **34** (258 mg, 86%). The optical purity of this compound was measured by HPLC [CHIRALPAK$^{TM}$ WH (Daicel Chemical Industries, Ltd.); 0.5 mM $CuSO_4$ aq. : acetonitrile=1 : 1], and it was found to be 95.1% e.e. $[\alpha]_D^{22}$ −69.4° ($c$=0.50, 1 M LiOH aq.). $^1$H-NMR (*DMSO-$d_6$) $\delta$: 8.58 (1H, s), 8.29 (1H, d, $J$=9 Hz), 8.26 (1H, d, $J$=2 Hz), 8.17 (1H, d, $J$=9 Hz), 8.10 (2H, d, $J$=7 Hz), 7.87 (1H, dd, $J$=2, 9 Hz), 7.72 (1H, dd, $J$=2, 9 Hz), 6.82 (2H, d, $J$=7 Hz), 4.17—4.04 (2H, m), 3.96—3.62 (3H, m), 3.81 (1H, d, $J$=16 Hz), 3.39 (1H, d, $J$=16 Hz), 3.10—3.01 (1H, m), 2.81—2.67 (2H, m), 2.60—2.47 (1H, m), 1.89—1.73 (1H, m), 1.65—1.54 (1H, m), 1.49—1.38 (1H, m), 1.17—0.85 (2H, m). *Anal.* Calcd for $C_{26}H_{27}ClN_4O_5S$–$H_2O$: C, 55.66; H, 5.21; N, 9.99. Found: C, 55.54; H, 5.04; N, 10.05.

**(*S*)-4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic Acid Monohydrate (35)** A solution of compound **33** (50 mg, 0.088 mmol) in 1 N HCl (5 ml) was heated under reflux for 12 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH (5 ml), and 1 N aqueous NaOH was added to make it alkaline. To the solution, Muromac$^{TM}$ MSC-1 ($H^+$ form, 1 g, Muromachi Kagaku Kogyo Kaisha, Ltd.) was added, and the resulting suspension was stirred at room temperature for 30 min. The ion exchange resin was collected with suction, then washed with water and MeOH. To the resin, 10% (w/w) $NH_3$–MeOH (5 ml) was added. After stirring for 30 min, the resin was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Chromatorex NH$^{TM}$ (Fuji Silysia Chemical, Ltd.) eluents; AcOEt : MeOH=3 : 1—1 : 2) to give (*S*)-4-[(6-Chloro-2-naphthalenyl)sulfonyl]-6-oxo-1-[[1-(4-pyridinyl)-4-piperidinyl]methyl]-2-piperazinecarboxylic acid ammonium salt (33 mg, 65%). To a solution of the ammonium salt (33 mg, 0.058 mmol), 0.1 M $H_2SO_4$ aq. (0.27 ml) was added, and the resulting precipitate was collected to give compound **35** (30 mg, 91%). The optical purity of this compound was measured by HPLC [CHIRALPAK$^{TM}$ WH (Daicel Chemical Industries, Ltd.); 0.5 mM $CuSO_4$ aq. : acetonitrile=1 : 1], and it was found to be 91.3% e.e. $^1$H-NMR (*DMSO-$d_6$) $\delta$: 8.58 (1H, s), 8.29 (1H, d, $J$=9 Hz), 8.26 (1H, d, $J$=2 Hz), 8.17 (1H, d, $J$=9 Hz), 8.10 (2H, d, $J$=7 Hz), 7.87 (1H, dd, $J$=2, 9 Hz), 7.72 (1H, dd, $J$=2, 9 Hz), 6.82 (2H, d, $J$=7 Hz), 4.17—4.04 (2H, m), 3.96—3.62 (3H, m), 3.81 (1H, d, $J$=16 Hz), 3.39 (1H, d, $J$=16 Hz), 3.10—3.01 (1H, m), 2.81—2.67 (2H, m), 2.60—2.47 (1H, m), 1.89—1.73 (1H, m), 1.65—1.54 (1H, m), 1.49—1.38 (1H, m), 1.17—0.85 (2H, m).

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