Synthesis and Evaluation of 1-Arylsulfonyl-3-piperazinone Derivatives as Factor Xa Inhibitors\(^1\)–\(^3\) IV. A Series of New Derivatives Containing a Spiro[5H-oxazolo[3,2-a]pyrazine-2(3H),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, \((-\rangle)-7\)-(6-chloro-2-naphthalenyl)sulfonyl|tetrahydro-\(8a\)-(methoxymethyl)-1'-4-pyridinyl|spiro|5H-oxazolo[3,2-a]pyrazine-2(3H),4'-piperidin]-5-one (28, M55529) had activity more favorable than those of previously reported compounds. The inhibitory activity of M55529 for FXa is \(IC_{50}=2 \text{nM}\), with high selectivity for FXa over thrombin and trypsin.

Key words factor Xa inhibitor; N\(_2\)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 coagulant 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) \((-\rangle)-(6\text{-chloro-2-naphthalenyl})\text{sulfonyl}-1\text{-}[1\text{-}-(4\text{-pyridinyl})\text{-}4\text{-piperidinyl}]\text{methyl}\text{-}2\text{-piperazinone}\), M55196 (2) \((-\rangle)-(6\text{-chloro-2-naphthalenyl})\text{sulfonyl}-1\text{-}[4\text{-hydroxy-1\text{-}-(4\text{-pyridinyl})\text{-}4\text{-piperidinyl}]\text{methyl}\text{-}2\text{-piperazinone}\), and M55551 (3) \((R\rangle)-(6\text{-chloro-2-naphthalenyl})\text{sulfonyl}-6\text{-oxo-1\text{-}[(4\text{-pyridinyl})\text{-}4\text{-piperidinyl}]\text{methyl}\text{-}2\text{-piperazinecarboxylic acid were found to be potent inhibitors of FXa (IC}_{50}=60 \text{nM, 31 \text{nM, 6 \text{nM, respectively with high selectivity}} for FXa over trypsin and thrombin. 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[5H-oxazolo[3,2-a]pyrazine-2(3H),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-acetyloxy-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 group in 11 allow the reaction to proceed further.
bond (C=N) followed by intramolecular amide formation gave rise to the spiro skeleton.

\(^1\text{H- and }^{13}\text{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 \( \text{11} \) to various derivatives were carried out. When compound \( \text{12} \) prepared by the deprotection of \( \text{11} \) with 1-chloroethyl chloroformate in 1,2-dichloroethane and with 1 N NaOH in MeOH was treated with 4-chloropyridine, the desired compound \( \text{13} \) was obtained. Then, compound \( \text{13} \) was methylated with dimethyl sulfate in the presence of a phase-transfer catalyst (benzyltriethylammonium chloride) to obtain the methyl ether \( \text{14} \). Compound \( \text{13} \) was treated with phthalimide by Mitsunobu reaction and with hydrazine to obtain the corresponding amino compound \( \text{16} \).

Synthesis of compounds \( \text{21} \) and \( \text{22} \) was achieved as shown in Chart 4. Compound \( \text{17} \), prepared by the reaction of the key intermediate \( \text{11} \) with benzyl chloroformate in the presence of a phase-transfer catalyst (benzyltriethylammonium chloride) to obtain the methyl ether \( \text{14} \). Compound \( \text{17} \) was treated with phthalimide by Mitsunobu reaction and with hydrazine to obtain the corresponding amino compound \( \text{16} \).
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$_2$Cl$_2$ 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 piperazine 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 piperazine type 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
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 it 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 CDCl3, dimethyl sulfoxide-d6 (DMSO-d6) or CD3OD using tetramethylsilane as the internal reference. High-resolution mass spectra (HR-MS) were obtained using JEOL JMS-GCMAET. 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 nm); human thrombin (Sigma Co., 0.09 U/ml) and S-2238 (Chromogenix AB, 0.2 nm); human trypsin (Athens Research and Technology, Inc., 15 ng/ml) and S-2222 (Chromogenix AB, 0.4 nm). 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-naphthyl]sulfonylglucinate (7)** Ethyl glycine hydrochloride (5) (9.88 g, 70.7 mmol) was suspended in CH2Cl2 (500 ml). Et2N (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 M HCl, and the reaction mixture was extracted with CH2Cl2. The organic layer was washed with brine and dried over dry Na2SO4. 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 C18H15ClNO2S, 327.0373. Observed: 327.0374. 

**Ethyl N-(O-Acetoxy-2-oxo-propyl)-N-[6-chloro-2-naphthyl]sulfonylglucinate (9)** To DMF (25 ml) solution of compound 7 (2.50 g, 7.63 mmol) were added K2CO3 (1.58 g, 11.4 mmol) and NaI (1.14 g, 7.63 mmol), and a solution of 1-acectoxy-3-chloroacetone (8) (1.78 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 EtO. The organic layer was washed with brine and dried over dry Na2SO4. The solvent was distilled off under reduced pressure. The resulting residue was crystallized in EtO, 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 C23H21ClNO5S, 441.0690. Observed: 441.0692. 

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>IC_{50} (nm)</th>
<th>Selectivity (Enzyme/FXa)</th>
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<tbody>
<tr>
<td>Thrombin</td>
<td>&gt;10000</td>
<td></td>
</tr>
<tr>
<td>AP C</td>
<td>&gt;10000</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>&gt;10000</td>
<td></td>
</tr>
<tr>
<td>Plasmin</td>
<td>&gt;10000</td>
<td></td>
</tr>
<tr>
<td>r-PA</td>
<td>1900</td>
<td>950</td>
</tr>
<tr>
<td>Urokinase</td>
<td>&gt;10000</td>
<td></td>
</tr>
</tbody>
</table>

**Thrombin (Sigma Co., 0.09 U/ml) and S-2238 (Chromogenix AB, 0.2 nm); human trypsin (Athens Research and Technology, Inc., 15 ng/ml) and S-2222 (Chromogenix AB, 0.4 nm).** 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}.
401

VoL 52, No. 4

(20 mL) was added and extracted with CH2Cl2. The organic layer was washed with water and dried over dry Na2SO4. The solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel column chromatography (eluents: CH2Cl2 : MeOH = 10 : 1) to give compound 12 (626 mg, 81%) as pale yellow powder, mp 165—166 °C.

1H-NMR (300 MHz, CD2OD) δ: 8.52—7.61 (6H, m, naphthyl), 4.27 (1H, d, J = 11.9 Hz, C2-H), 4.23 (1H, d, J = 16.9 Hz, C3-H), 4.16 (1H, d, J = 11.8 Hz, C4-H), 3.78—3.68 (2H, m, CH2OH), 3.48 (1H, d, J = 11.8 Hz, C5-H), 3.28 (1H, d, J = 11.8 Hz, C6-H), 3.23—2.83 (4H, m, C3-H, C4-H of piperidine), 2.88 (1H, d, J = 11.9 Hz, C6-H), 2.06—1.35 (4H, m, C3-H, C4-H of piperidine). IR (film) cm−1: 1747, 1673, 1417, 1349, 1224, 1168, 698.

8a-(Aminomethyl)-7-[6-chloro-2-naphthalenylsulfonyl][tetrahydro-1-(4-pyridyl)-spiro][5H-oxazolo[3,2-a]pyrazine-2(3H)-4'-piperidine]-5-one (16) To a suspension of compound 15 (2.45 g, 3.66 mmol) in EtOH (50 mL) was added hydroxylamine hydrochloride (10.37 mL, 1.2 mol/L) and the mixture was heated under reflux for 16 h. The reaction mixture was cooled to room temperature and the resulting residue was purified by silica gel column chromatography (eluents: CH2Cl2 : MeOH = 4 : 1) to give the compound 16 (1.43 g, 72%) as colorless oil.

1H-NMR (270 MHz, CDCl3) δ: 8.39—6.58 (10H, m, naphthyl and phenyl), 4.53 (1H, d, J = 11.9 Hz, C4-H), 4.38 (1H, d, J = 16.8 Hz, C5-H), 4.26 (1H, d, J = 11.9 Hz, C6-H), 3.55—3.22 (4H, m, C3-H, C4-H of piperidine), 3.37 (1H, d, J = 16.8 Hz, C6-H), 3.18 (1H, d, J = 13.5 Hz, CH2NH), 3.17 (1H, d, J = 11.9 Hz, C3-H), 2.89 (1H, d, J = 13.5 Hz, CH2NH), 2.24 (1H, d, J = 11.9 Hz, C4-H), 1.94—1.48 (4H, m, C3-H, C4-H of piperidine). 13C-NMR (75 MHz, CDCl3) δ: 162.77 (C1), 154.31 (C2, C4 of pyridine), 149.92 (C2, C4 of pyridine), 135.71—123.40 (10C, naphthyl), 108.53 (C5 of pyridine), 96.51 (C5 of pyridine), 85.61 (C6 of pyridine), 64.76 (CH2OH), 52.09 (C7 of pyridine), 49.07 (C7 of pyridine), 43.56 (C3 of piperidine), 43.14 (C4 of piperidine), 35.72 (C3 of piperidine), 35.04 (C4 of piperidine). IR (film) cm−1: 3395, 2920, 2360, 2270, 2130, 1673, 1417, 1390, 1168, 698.

1'-Benzyloxy carbonyl-7-[6-chloro-2-naphthalenylsulfonyl][tetrahydro-1-(4-pyridyl)-spiro][5H-oxazolo[3,2-a]pyrazine-2(3H)-4'-piperidine]-5-one (17) The compound 11 (67.2 g, 112 mmol) and 1.8-bis(N, N-dimethylamino)naphthalene (4.80 g, 22.5 mmol) were dissolved in CICH2CH3 (670 mL) and benzyl chloroformate (32.1 mL, 224.7 mmol) was added dropwise to the solution under the reaction temperature maintained at 0 °C. The reaction mixture was stirred at room temperature for 2 h, and sat. NaHCO3 was added. The mixture was extracted with CH2Cl2 and the resulting residue was purified by column chromatography (eluents: CH2Cl2 : MeOH = 4 : 1) to give the compound 17 (67.9 g, quant.) as pale yellow powder, mp 96—98 °C.

1H-NMR (300 MHz, CDCl3) δ: 8.37—7.25 (11H, m, naphthyl and phenyl), 5.10 (2H, s, OCH2Ph), 4.43 (1H, d, J = 11.7 Hz, C4-H), 4.38 (1H, d, J = 16.8 Hz, C5-H), 4.23 (1H, d, J = 11.9 Hz, C6-H), 3.96—3.24 (4H, m, C3-H, C4-H of piperidine), 3.21 (4H, m, C3-H, C4-H of piperidine), 3.18 (4H, m, C3-H, C4-H of piperidine). IR (film) cm−1: 3395, 2920, 2360, 2270, 2130, 1673, 1417, 1390, 1168, 698.

1'-Benzyloxy carbonyl-7-[6-chloro-2-naphthalenylsulfonyl][tetrahydro-5-oxo-spiro][5H-oxazolo[3,2-a]pyrazine-2(3H)-4'-piperidine]-5-carboxylic Acid (18) To a solution of compound 17 (68.0 g, 113.4 mmol) in CH2Cl2 (660 mL) was added 4-benzyloxycarbonyl-2,6,6-tetramethylpiperidine 1-oxyl (314 mg, 1.14 mmol). 5% aq. NaHCO3 (1.361 g) 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 CH2Cl2. The organic layer was washed with water, brine and dried over dry Na2SO4. The solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel column chromatography (eluents: CH2Cl2 : MeOH = 4 : 1) to give the compound 18 (62.7 g, 90%) as pale yellow powder, mp 178—180 °C.
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 Na₂SO₄. 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 Compound 19 (40.1 g, 61%) as pale yellow powder, mp 82—84 °C.

**H-NMR** (300 MHz, CDCl₃) δ: 8.36—7.28 (11H, m, naphthyl and phenyl), 5.29 (1H, s, OCH₂Ph), 4.75 (1H, d, J = 11.3 Hz, C₂-H), 3.52 (2H, d, J = 16.6 Hz, C₅-H), 4.12 (2H, q, J = 7.1 Hz, CO₂CH₂CH₃), 4.07 (1H, d, J = 11.5 Hz, C₃-H), 3.81—3.22 (4H, m, C₇-H of piperedine), 3.43 (1H, d, J = 16.6 Hz, C₆-H), 3.29 (1H, d, J = 11.5 Hz, C₃-H), 2.45 (1H, d, J = 11.3 Hz, C₄-H), 1.75—1.44 (4H, m, C₅-S-H of piperidine), 1.34 (3H, t, J = 7.1 Hz, CO₂CH₂CH₃) (IR (film) cm⁻¹: 1745, 1681, 1419, 1238, 1166, 1079, 698.

**1H NMR** (CDCl₃) δ: 3.89 (1H, d, J = 16.5 Hz, C₆-H), 4.58 (1H, d, J = 16.5 Hz, C₆-H), 4.38—4.17 (2H, m, CO₂CH₂CH₃), 4.10 (1H, d, J = 11.4 Hz, C₃-H), 3.16—2.77 (4H, m, C₇-S-H of piperidine), 3.32 (1H, d, J = 11.4 Hz, C₄-H), 2.45 (1H, d, J = 11.4 Hz, C₃-H), 1.90—1.45 (4H, m, C₇-c₇-H of piperidine), 1.44 (3H, t, J = 7.1 Hz, CO₂CH₂CH₃) (IR (film) cm⁻¹: 1749, 1668, 1344, 1102, 1078, 698.

**Ethyl 7-(6-Chloro-2-naphthylamino)sulfonyl]tetrahydro-5-oxo-spiro[8H-oxazolo[3,2-aj pyrazine-2(3H)-j piperidine]-8-carboxylate** (20) To a solution of the compound 19 (40.0 g, 62.2 mmol) in MeCN (400 ml) was added TMSI (222 ml, 155.8 mmol) under cooling with ice. After stirring for 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 the mixture. The mixture was stirred for separation, and the aqueous layer was washed with hexane followed by addition of CH₂Cl₂, 2N NaOH was added with stirring under cooling with ice and the pH of the mixture was adjusted to 11. The mixture was extracted with CH₂Cl₂, and the organic layer was washed with hexane and dried over Na₂SO₄. The solvent was distilled off under reduced pressure to give compound 20 (29.7 g, 94%) as colorless powder, mp 252—254 °C.

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**References and Notes**

1) Part 4 in the series of studies on 1-arylsulfonyl-3-piperazine deriva


