Synthesis and Evaluation of 1-Arylsulfonyl-3-piperazinone Derivatives as Factor Xa Inhibitors\textsuperscript{1,2)} III. Effect of Ring Opening of Piperazinone Moiety on Inhibition

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Compounds containing an ethylenediamine structure in place of the piperazine ring of M55113 (1) and M55551 (2) were synthesized to investigate the effects of a piperazine moiety and evaluated for activity as factor Xa (FXa) inhibitors. Most such compounds, however, exhibited lower activity (1/10—1/100) than that of M55113 and M55551 as FXa inhibitors.

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

Recently, direct inhibition of factor Xa (FXa), which links the intrinsic and extrinsic mechanisms in the blood coagulation cascade,\textsuperscript{3—7)} has emerged as an attractive strategy for the discovery of novel antithrombotic agents.\textsuperscript{8—11)} In previous papers,\textsuperscript{1,2)} we reported the synthesis and biological activity of new compounds in a series of 1-arylsulfonyl-3-piperazinone derivatives, and through this investigation M55113 (1) (IC\textsubscript{50}: 0.06 \textmu M) and M55551 (2) (IC\textsubscript{50}: 0.006 \textmu M) were confirmed to be somewhat favorable as FXa inhibitors.

In order to investigate the role played a piperazine moiety in these compounds, we focused on comparison of the activity of compounds which have an ethylenediamine structure in place of the piperazine ring of M55113 and M55551. The present paper concerns the synthesis of such compounds together with their inhibition of FXa.

Chemistry

Synthesis of ethylenediamine derivative 9 corresponding to M55113 and M55551 was achieved as shown in Chart 1. Compound 6, prepared by the reaction of \textit{N-}\texttl{tert}-butoxycarbonyl-1,2-diaminoethane hydrochloride (4) with 6-chloro-2-naphthalenesulfonyl chloride (5) under basic conditions, was deprotected with trifluoroacetic acid (TFA) to yield compound 7. The condensation of 7 with 1-(4-pyridinyl)-4-piperidinecarboxaldehyde (8) prepared by Swern oxidation\textsuperscript{12)} of the corresponding methanol yielded the desired ethylenediamine derivative 9 in the presence of reducing agent\textsuperscript{13)}.

The route of synthesis of compound 13, which contains an amide group on the ethylenediamine moiety of compound 9, is shown in Chart 2. Compound 11, prepared by the reaction

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of glycine (10) with sulfonyl chloride 5 under basic conditions, was treated with 1-(4-pyridinyl)-4-aminomethylpiperidine (12), which was obtained by Mitsunobu reaction of the corresponding methanol, in the presence of \(N,N'\)-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole hydrate (HOBt) to yield the amide 13.

Synthesis of \((R)\)-3-aminoalanine derivative 19 was accomplished according to the route shown in Chart 3. Sulfonylation of ethyl ester 15 derived from \((R)\)-3-amino-2-(benzyl)oxy carbonylamino)propionic acid (14) with sulfonyl chloride 5 in \(CH_2Cl_2\) in the presence of \(Et_3N\) resulted in smooth formation of the sulfonamide 16. Deprotection of 16 with trimethylsilyl iodide (TMSI) in MeCN followed by reductive condensation with 8 afforded compound 18. Hydrolysis of 18 in hydrochloric acid and acetic acid yielded the desired 3-aminoalanine derivative 19. Starting from \((S)\)-3-amino-2-(benzyl oxy carbonylamino)propionic acid, the method used to synthesize compound 19 was repeated to obtain compound 20. Throughout the synthesis described above, no racemization was observed.

### Results and Discussion

The FXa inhibitory activities of these compounds were measured by a method similar to that described in the preceding paper,\(^1\),\(^2\) and are listed in the Table 1 as IC\(_{50}\) values. The following conclusions are derived from these data, though they are still provisional.

First, compared with the activity of compound 1 (M55113), those of compounds 9 and 13 are quite low. Similarly, the activity of compound 19 was shown to be 1/100 that of compound 2 (M55551). A similar difference in activity was observed between 3 and 20. These data suggest that rigidity of the central part of the molecules tested in this series may be an important factor for significantly FXa inhibitory activity.

Second, when a carbonyl group or a carboxyl group was introduced into the ethylenediamine skeleton (13, 19), activity clearly increased with activities of 13 and 19 four and five times that of 9, respectively. These findings suggest that carbonyl and carboxyl groups favor interaction with FXa protein.

Finally, regarding the stereochemistry of carboxylic substitution, it is clear that \((R)\)-isomers have higher activity than \((S)\)-isomers in both the piperazine 2 and ethylenediamine 19 skeleton.

These findings may be useful in guiding molecular design in this field.

### 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 CD2OD using tetramethylsilane as the internal reference. High-resolution mass spectra (HR-MS) were obtained using JEOL JMS-GC MAT. 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 X Inhibition** The 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 mM NaCl at 37 °C. The absorbance at 405 nm was measured continuously. 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 IC50.

**2-[6-Chloro-2-naphthalenesulfonyl]glycine-N-[1-(4-pyridyl)-4-piperidinyl]methyl][methylene-2-napthalenesulfonyl amide (9)** A solution of 1-(4-pyridyl)-4-piperidinyl)methyl][methylene-2-napthalenesulfonyl amide (9) was adjusted to pH 9 with 1N NaOH and the mixture was extracted with CH2Cl2: MeOH (3:2). The organic layer was washed with water and brine and dried over dry Na2SO4. The solvent was evaporated under reduced pressure. The residue was crystallized from CH2Cl2 : MeOH (2:1) to give compound 9 (52.5 g, 0.20 mol) in dry CH2Cl2 (1130 ml), compound 9 (200 mg, 0.70 mmol) was added and the mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents: CH3Cl : MeOH : H2O = 95 : 5 : 80 : 20) to give compound 9 (148 mg, 46%) as colorless powder, mp 147—148 °C.

**Ethyl (R)-Amino-2-(benzyloxycarbonylamino)propionate Hydrochloride** (15) To a 20% w/w HCl–EtOH solution (1.5l) under cooling ice bath, (R)-3-amino-2-benzyl-amino-propionic acid (14) (68.0 g, 0.28 mol) was added and the mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the residue was crystallized with EtOAc and EtOH to yield compound 15 (75.0 g, 87%) as white powder, mp 136—137 °C.

**Ethyl (R)-2-(Benzyloxycarbonylamino)propionate** (16) To a solution of 6-chloro-2-naphthalene sulfonyl chloride (5) (52.5 g, 0.20 mol) in dry CH2Cl2 (1130 ml), compound 15 (60.9 g, 0.20 mol) and Et3N (56.4 ml, 0.4 mol) were added and the mixture was stirred at room temperature for three days under Ar. Water (700 ml) was added to the reaction mixture and the mixture was extracted with CH2Cl2. The organic layer was washed with water and brine and dried over anhydrous Na2SO4. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents: CH2Cl2 : MeOH : H2O = 95 : 5 : 80 : 20) to give compound 16 (548 mg, 1.83 mmol) was added dropwise to a solution in THF (27 ml) of compound 11 (548 mg, 1.83 mmol). To the mixture, DCC (415 mg, 2.01 mmol) and HOBt (308 mg, 2.01 mmol) were added and the mixture was stirred at room temperature for 17 h under Ar. The reaction mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents: CH3Cl : MeOH = 85:15 : 80 : 20) to give compound 16 (469 mg, 54%) as colorless powder, mp 215—217 °C.

**Ethyl (R)-3-Amino-2-(benzyloxycarbonyl)amino)propionate** (17) To a solution in MeCN (460 ml) of compound 16 (28.2 g, 0.09 mol) and Et3N (46.0 ml, 0.4 mol) were added and the mixture was stirred at room temperature for 20 min, 2-NH2Cl (220 ml) was added and the reaction mixture was washed with CH2Cl2. The organic layer was washed with water and brine and dried over anhydrous Na2SO4. The solvent was evaporated under reduced pressure under Ar. The residue was crystallized with hexane and EtO to yield compound 16 (97.7 g, 97%) as white powder, mp 96—97 °C.
(4-pyrindinyl)-4-piperidinecarbaldehyde (8) (2.63 g, 14 mmol) and compound 17 (5.0 g, 14 mmol) in dry CH2Cl2 (117 ml) containing AcOH (1.67 ml) were stirred at room temperature for 50 min under Ar. To the reaction mixture, NaBH(OAc)3 (7.42 g, 35 mmol) was added and the mixture was stirred overnight at room temperature. Water (200 ml) was added to the reaction mixture, which was adjusted to pH 7 by sat. aq. NaHCO3 and the mixture was extracted with CH2Cl2. The organic layer was washed with water and brine and dried over dry Na2SO4. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluents: CH2Cl2 : MeOH = 95 : 5 — 80 : 20) to give compound 18 (3.96 g, 53%) as colorless amorphous.

HR-MS m/z: Calcd for C28H35ClN4O4S: 530.1754. Found: 530.1762. 1H-NMR (75 MHz, CD3OD) δ: 8.43—6.61 (10H, m, naphthyl and pyridinyl), 5.40—5.25 (1H, m, NH), 4.20—4.03 (2H, m, CO2CH2CH3), 3.90—3.79 (2H, m, C15-H of piperidine), 3.32 (1H, dd, J=4.6, 11.9 Hz, CHCH2NHSO2), 3.25 (1H, dd, J=4.6, 7.1 Hz, CH(CH2)NH2), 2.99 (1H, dd, J=7.1, 11.9 Hz, CHCH2NHSO2), 2.85—2.72 (2H, m, C3,5-H of piperidine), 2.47 (1H, d, J=6.6, 11.3 Hz, piperidine-CH2NH), 1.82—1.08 (5H, m, C3,4,5-H of piperidine), 1.22 (3H, t, J=7.2 Hz, CO2CH2CH3). 13C-NMR (75 MHz, CDCl3): δ: 172.43 (C3), 154.66 (C4) of pyridine, 150.06 (C6,8 of pyridine), 137.20—123.51 (10C, naphthyl), 108.32 (C6,8 of pyridine), 61.50 (CO2CH2CH3), 50.49 (CHCH2NHSO2), 53.54 (piperidine-CH2NH), 46.17 (C2 or C6 of piperidine), 44.43 (CH(CH2)NH2), 36.45 (C5 of piperidine), 29.45 (C6 of piperidine), 29.34 (C7 of piperidine), 14.16 (CO2CH2CH3). IR (KBr) cm−1: 2929, 1732, 1599, 1329, 1157. [α]D20 +21.2° (c=1.00, MeOH).

(R)-3-[(6-Chloro-2-naphthyl)sulfonylamino]-2-[1-(4-pyridinyl)-4-piperidinylmethylamino]propionic Acid Dihydrochloride (19) To a solution in AcOH (5.0 ml) of compound 18 (500 mg, 0.94 mmol), c. HCl (5.0 ml) was added and the mixture was heated under reflux for 3.5 h. The reaction mixture was concentrated under reduced pressure to give compound 19 (540 mg, quant.) as brown amorphous.

The optical purity of this compound was measured by HPLC [CHIRAL-PK AD-H (Daicel Chemical Industries, Ltd.); hexane : EtOH : TFA : Et3N = 70: 0.1: 0.1: 0], and it was found to be 94.1% ee.

1H-NMR (300 MHz, CD3OD) δ: 8.53—7.16 (10H, m, naphthyl and pyridinyl), 4.40—4.28 (2H, m, C4,6-H of piperidine), 4.21—4.14 (1H, m, CH(CH2)NH2), 3.65—3.01 (6H, dd, CH(=NH)SO2, C6,8-H of piperidine and piperidine-CH2NH), 2.41—1.34 (5H, m, C15,16-H of piperidine). 13C-NMR (75 MHz, CD3OD) δ: 168.71 (C3), 154.66 (C4) of pyridine, 151.00 (C6,8 of pyridine), 137.20—123.51 (10C, naphthyl), 108.32 (C6,8 of pyridine), 61.50 (CO2CH2CH3), 50.49 (CHCH2NHSO2), 53.54 (piperidine-CH2NH), 46.17 (C2 or C6 of piperidine), 44.43 (CH(CH2)NH2), 36.45 (C5 of piperidine), 29.45 (C6 of piperidine), 29.34 (C7 of piperidine), 14.16 (CO2CH2CH3). IR (KBr) cm−1: 2925, 1741, 1645, 1547, 1331, 1157. [α]D20 +15.3° (c=1.00, MeOH).

References and Notes