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Synthesis and biological evaluation of the metabolites of 2-(1-{3-[(6-chloro-naphthalen-2-yl)sulfonyl]propanoyl}piperidin-4-yl)-5-methyl-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one

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ABSTRACT

We have recently discovered imidazo[1,5-*c*]imidazol-3-one derivative **1** as a potent, selective, and orally bioavailable factor Xa (FXa) inhibitor. In this study, we have synthesized metabolites of **1** and evaluated their biological activities. As a result, we identified the active metabolites ***S*-5** and **6** with a potent FXa inhibitory activity comparable to **1** and a favorable pharmacokinetic profile in monkeys.

1. Introduction

Thrombotic disorders represent the leading causes of mortality and morbidity worldwide. Because of the limitations associated with the current antithrombotic therapies, using low molecular weight heparins[1] and warfarin,[2] there are still unmet medical needs for novel, efficacious, and safe agents.[3] Recently, factor Xa (FXa), a serine protease involved in the coagulation cascade, has emerged as an important target for the development of new antithrombotic agents.[4] Currently a variety of orally active FXa inhibitors such as rivaroxaban[5] and apixaban[6] have been reported.[7]

We previously described the discovery of an orally bioavailable FXa inhibitor, imidazo[1,5-*c*]imidazol-3-one derivative **1**.[8] Compound **1** selectively inhibits human FXa with an IC_{50} value of 4.8 nM, and its in vitro anticoagulant potency PT_2 (the concentration of compound required to doubles the human prothrombin time) was 1.0 μM. Moreover, compound **1** possessed favorable pharmacokinetics in rats (BA = 24%) and cynomolgus monkeys (BA = 46%) and demonstrated good antithrombotic efficacy in a rat venous thrombosis. During our preclinical development program for **1**, studies on identification of metabolites of **1** were performed. Six metabolites **2**–**7** were observed in the plasma and urine of rats, dogs, and monkeys after oral administration of compound **1**. The structures of the six metabolites were estimated based on their LC/MS/MS data (Fig. 1).

In this report, we describe the synthesis of the metabolites of compound **1** and their biological evaluation, leading to a serendipitous identification of the active metabolite ***S*-5** and **6**, which showed potent FXa inhibitory and anticoagulant activities, high selectivities over other serine proteases and favorable pharmacokinetic profiles in cynomolgus monkeys.

2. Chemistry

Since the synthesis of metabolite **2**, has already been established,[9] we focused our attention on the synthesis of metabolites **3**–**7**. Although 6-chloro-2-naphthalenesulfinic acid, presumed metabolite **3**, was prepared by the reduction of the corresponding sulfonyl chloride **8** by $NaBH_4$, we were unable to obtain an analytically pure sample, presumably because of its liability to disproportionation.[10] We therefore decided to prepare metabolite **3** as the relatively stable sodium salt, as shown in Scheme 1. Reduction of **8** with Na_2SO_3 afforded crude sodium salt **3-Na**, which was contaminated with inseparable impurities. To remove these impurities, the crude **3** was added to *tert*-butyl acrylate, and the resultant Michael adduct **9** was purified by recrystallization. Treatment of **9** with 1 equiv of NaOH effected a retro-Michael reaction to furnish the desired sodium salt **3-Na** in an analytically pure state.

Aminopiperidine derivative **4** was prepared by condensation of commercially available amine **10** with carboxylic acid **2** using

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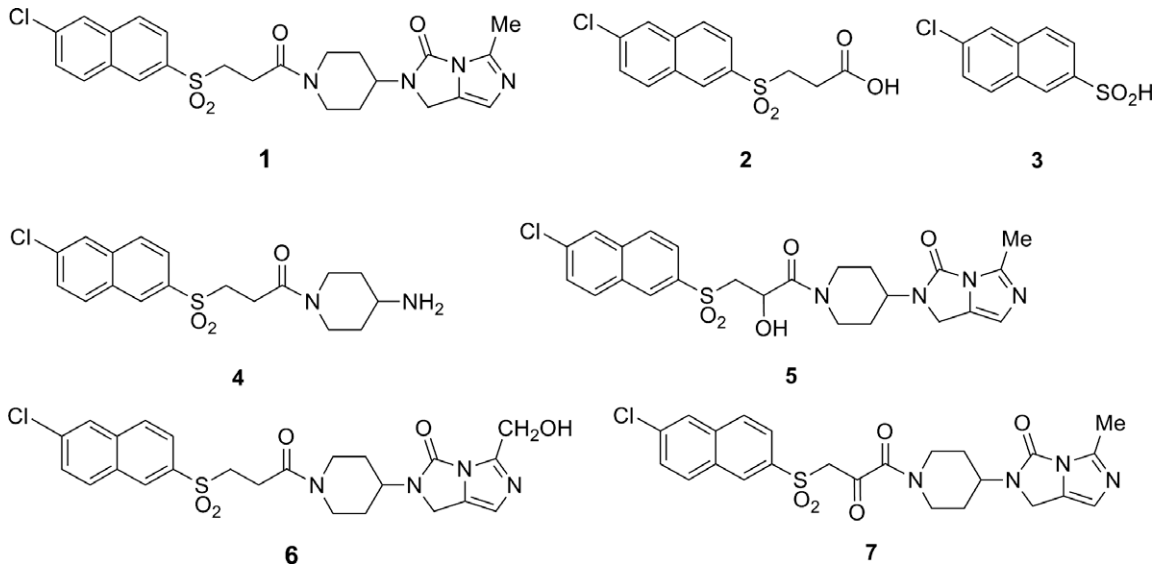
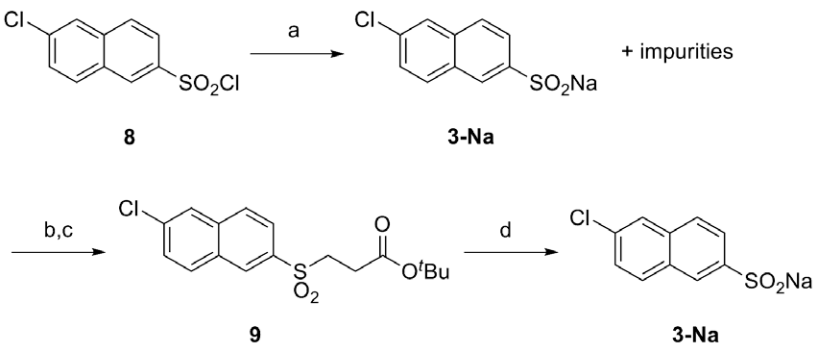


Figure 1. Presumed structures of metabolites of **1**.

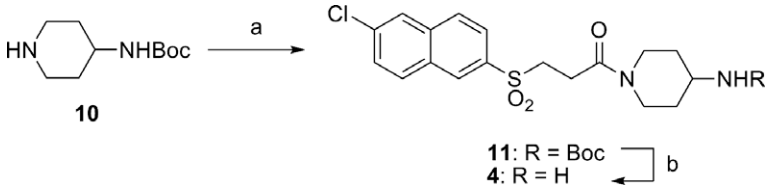


Scheme 1. Synthesis of sodium salt of sulfinic acid **3-Na**. Reagents: (a) Na_2SO_3, $NaHCO_3$; (b) 1 M HCl; (c) *tert*-Butyl acrylate, Et_3N (49% for three steps); (d) 1 M NaOH (quant.).

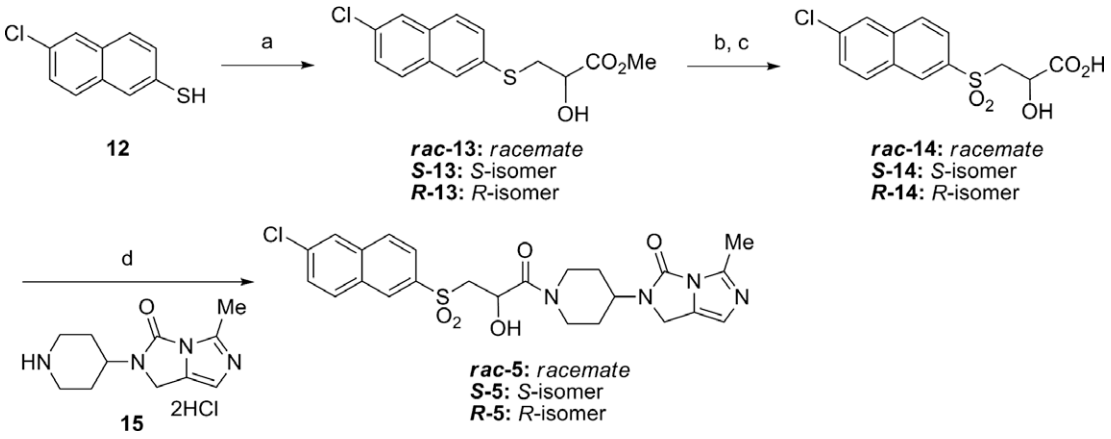
1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC) and 1-hydroxybenzotriazole hydrate (HOBt), followed by acid-promoted deprotection of the Boc group (Scheme 2).

The α-hydroxy derivative **5** was synthesized as illustrated in Scheme 3. The ring opening reaction of methyl glycidate with thiol **12**[9] using EtMgBr as a base led to the formation of α-hydroxy ester **13** in a regioselective manner. When this reaction was employed using triethylamine as a base, a mixture of **13** and its regioisomer were obtained (ca. 1:1). Alkaline hydrolysis of **13** and the subsequent oxidation of the sulfur atom by Oxone® provided the desired α-hydroxyacid **14**, which was then coupled with the piperidine analogue **15**[8] to afford α-hydroxy derivative ***rac*-5**. To evaluate the influence of the stereochemistry of **5** on biological activities, we also prepared both enantiomers in an enantiomerically pure form. By utilizing commercially available (*R*)- and (*S*)-methyl glycidate as chiral synthons, ***S*-5** and ***R*-5** were synthesized through the same route as that for ***rac*-5** without deteriorating the enantiomeric purity.

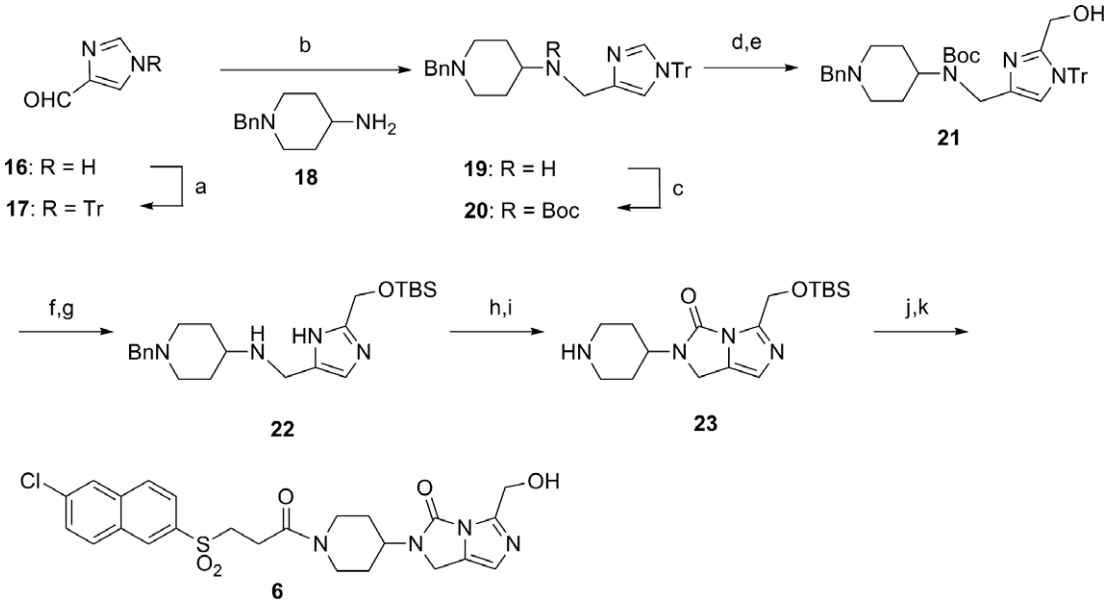
Hydroxymethyl derivative **6** was synthesized as shown in Scheme 4. The reductive amination of 1-trityl-1*H*-imidazole-4-carbaldehyde (**17**) with 4-amino-1-benzylpiperidine (**18**) afforded compound **19**, of which the secondary amino group was subsequently protected with a Boc group to yield fully protected imidazole derivative **20**. The introduction of a hydroxymethyl group at the 2-position of the imidazole ring of **20** was achieved via sequential lithiation, formylation with DMF, and reduction by $NaBH_4$. The trifluoroacetic acid (TFA)-mediated removal of the Trityl and Boc groups in **21**, followed by the protection of the hydroxy functionality with a TBS group furnished cyclization precursor **22**. Construction of an imidazolone ring was accomplished by treatment of **22** with *N*,*N*′-carbonyldiimidazole (CDI) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and subsequent catalytic hydrogenolysis of the *N*-benzyl group afforded the desired piperidine derivative **23**. Condensation of **23** with carboxylic acid **2**, followed by the deprotection of the TBS group with acetic acid provided hydroxymethyl derivative **6**.



Scheme 2. Synthesis of aminopiperidine derivative **4**. Reagents: (a) **2**, WSC, HOBt (60%); (b) concd HCl (91%).



Scheme 3. Synthesis of α-hydroxy derivative **2**. Reagents: (a) EtMgBr then *racemic*, *R*-, or *S*-methyl glycidate (48–77%); (b) 8 M NaOH (82–97%); (c) Oxone® (67–88%); (d) WSC, HOBt (67–73%).



Scheme 4. Synthesis of hydroxymethyl derivative **6**. Reagents and conditions: (a) TrCl, Et_3N (quant.); (b) $NaBH(OAc)_3$ (90%); (c) Boc_2O, Et_3N (98%); (d) BuLi, −40 °C then DMF (89%); (e) $NaBH_4$ (89%); (f) TFA (99%); (g) TBSCl, Et_3N (52%); (h) CDI, DBU (85%); (i) cat. Pd/C, H_2 (quant.); (j) **2**, WSC, HOBt (76%); (k) AcOH (55%).

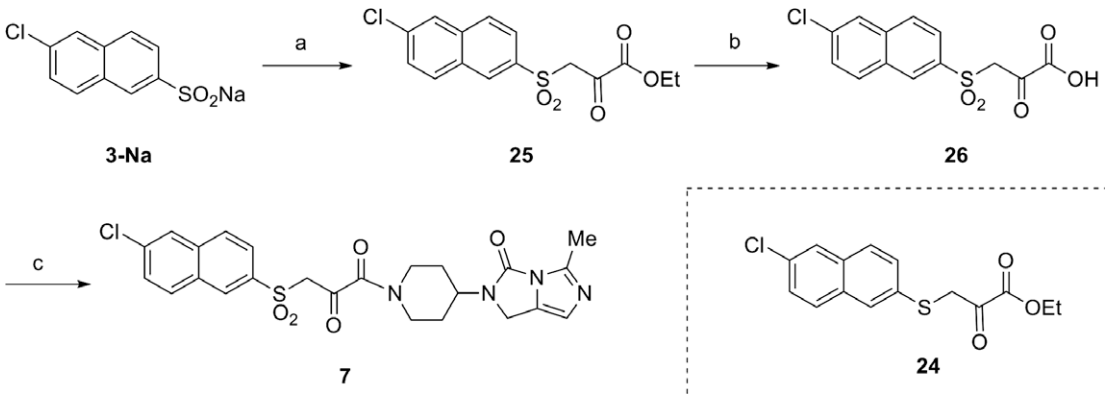
The synthesis of α-ketoamide derivative **7** was depicted in Scheme 5. We first attempted to synthesize compound **7** via the β-thio-α-ketoester **24** as an intermediate, which was readily prepared by the alkylation of thiol **12** with ethyl bromopyruvate. However, oxidation of **24** to the corresponding sulfone gave a complex mixture. We next examined the alkylation of sodium sulfinate **3-Na** as an alternative route to **7** and obtained the product **25**. This material was then subjected to alkaline hydrolysis to yield the desired α-ketoacid **26**. The condensation of **26** with amine **15** did not proceed when WSC or mixed anhydride methods were employed. The use of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)[11] as the dehydrating agent afforded α-ketoamide **7**, although the yield was low.

3. Results and discussion

Metabolite identification was conducted by comparing the HPLC retention times and mass spectra of synthetic compounds **2**–**7** with those of the metabolites (Table 1). As a result, compounds **2**–**6** proved to be identical to the respective metabolites, although **3** and **5** were identified as the sodium salt **3-Na** and the racemate ***rac*-5**, respectively.

With all the metabolites of **1** in hand, we turned our attention to their biological activities. The compounds thus synthesized were evaluated in vitro for inhibitory activity against human FXa and for their activity in the prolongation of human PT. The results are summarized in Table 2.

As anticipated, metabolites **2**–**4**, which lacked the imidazoimidazolone moiety, exhibited no inhibitory activity against human FXa at 5 μM. On the other hand, the hydroxylated metabolites **5** and **6** were found to exhibit potent FXa inhibitory activity. Interestingly, compound ***S*-5** had slightly more potent inhibitory activity against FXa than the parent compound **1**, whereas a decrease in activity relative to **1** was observed for its enantiomer ***R*-5**. These results indicated that the configuration of the hydroxy group of **5** had a substantial effect on the activity. Moreover, compound ***S*-5** doubled PT at 0.92 μM, which is a similar potency to that of compound **1**. Introduction of a hydroxymethyl group on the imidazole ring (i.e., **6**) maintained the FXa inhibitory activity and slightly increased the anticoagulant activity. This incremental effect of the



Scheme 5. Synthesis of α-keto derivative **7**. Reagents: (a) ethyl bromopyruvate; (b) 8 M NaOH; (c) **15**, PyBOP, *i*-Pr_2EtN (28% for three steps).

Table 1
HPLC retention times of metabolites **2**–**7**

		2	**3**	**4**	***rac*-5**	**6**	**7**
HPLC retention time[a] (min)	Metabolite	9.8	8.7	14.9[b]	22.3	17.7	12.4
	Synthetic sample	9.9	8.8	14.9[b]	22.2	17.7	12.5

[a] Column: Inertsil ODS-3(4.6 × 250 mm), eluent: MeCN/MeOH/0.01 M aqueous $AcONH_4$ = 25:25:50 v/v, flow rate: 1.0 mL/min, detection: UV-235 nm, temp: 30 °C.
[b] Eluent: MeCN/0.01 M acqueous $AcONH_4$ = a linear gradient of 26:74 to 34:66 v/v, temp: 40 °C.

Table 2
In vitro activities of **1** and its metabolites **2**–**7**

Compd	Human FXa, IC_{50}[a] (nM)	Human PT, PT_2[b] (μM)
1	4.8 (4.6–5.1)	1.0
2	>5000	N.T.[c]
3-Na	>5000	N.T.[c]
4	>5000	N.T.[c]
***S*-5**	2.1 (1.9–2.2)	0.92
***R*-5**	21 (17–25)	1.9
6	8.0 (7.5–8.6)	0.83
7	930 (839–1034)	>10

[a] Inhibitory activity against human FXa.
[b] PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT_2 values shown are the mean of duplicate measurements.
[c] N.T. means 'Not Tested'.

polar group on the anticoagulant potency might be attributed to the reduction of the nonspecific bindings to the plasma proteins.[12] A significant loss in the FXa inhibitory activity was induced by oxo substitution at the α-position of the central amide carbonyl group (i.e., **7**). In the course of the metabolite identification study, compounds ***S*-5** and **6** were serendipitously found as the active metabolites with the potent FXa inhibitory and anticoagulant activities comparable to that of the parent compound **1**.

To investigate contributions of the active metabolites **5** and **6** to the in vivo efficacy of the parent compound **1**, the plasma concentrations of ***S*-5**, ***R*-5** and **6** were measured at 1, 2, 4, and 10 h after oral administration of compound **1** in cynomolgus monkeys (1 mg/kg, po). The results are shown in Table 3. The values of AUC_{0-10h} for ***S*-5**, ***R*-5**, and **6** were ca. 8%, 9% and 18%, respectively, compared to that of the parent compound **1**. Thus, these active metabolites may contribute to the in vivo efficacy of **1**. The metabolites ***S*-5** and ***R*-5** were observed at the similar concentration in plasma, suggesting that metabolic oxidation at the α-position of the central amide carbonyl group of **1** might occur in non-stereoselective manner in cynomolgus monkeys.

After finding the active metabolites ***S*-5** and **6** with potent FXa inhibitory and anticoagulant activities, we next evaluated the selectivity profile against other human serine proteases (Table 4) and their pharmacokinetic profile in monkeys (Table 5). Both of

Table 4
Inhibitory activities and selectivity of **S-5** and **6**

Compd	Serine protease, K_i[a] (nM)				
	FXa	Thrombin	Plasmin	Trypsin	t-PA
S-5	0.50 ± 0.078	1900 ± 120	>60,000	>60,000	>60,000
6	3.8 ± 0.28	7800 ± 560	>60,000	>60,000	>60,000

[a] Data are expressed as mean ± SEM of three determinants.

Table 3
Plasma concentrations of **1**, **S-5**, **R-5** and **6** after oral administration (1 mg/kg po) of **1** in cynomolgus monkey (n = 3)[a]

Compd	Plasma concentration				AUC_{0-10h} (ng h/mL)
	1 h	2 h	4 h	10 h	
1	92.8 ± 10.0	131.6 ± 21.9	65.9 ± 33.9	9.9 ± 5.5	583.2 ± 191.7
***S*-5**	2.7 ± 0.3	6.8 ± 0.5	6.5 ± 1.6	1.8 ± 1.0	44.2 ± 9.6
***R*-5**	0.8 ± 0.7	4.4 ± 0.6	91. ± 1.8	3.6 ± 2.0	54.6 ± 14.3
6	16.6 ± 4.3	25.0 ± 5.3	13.3 ± 1.0	0	107.2 ± 12.1

[a] Data are expressed as the mean ± SD of three determinations.

Table 5
Pharmacokinetic parameters of **S-5** and **6** in monkeys

Compd	iv[a]		po[a]			BA (%)
	V_{dss} (mL/kg)	CL_{total} (mL/h/kg)	C_{max} (ng/mL)	AUC (ng h/mL)	MRT (h)	
S-5	719	852	30.5	307	7.05	25.4
6	588	714	60.1	470	7.56	32.3

[a] Monkeys were administered intravenously at 0.1 mg/kg, and orally at 1.0 mg/kg.

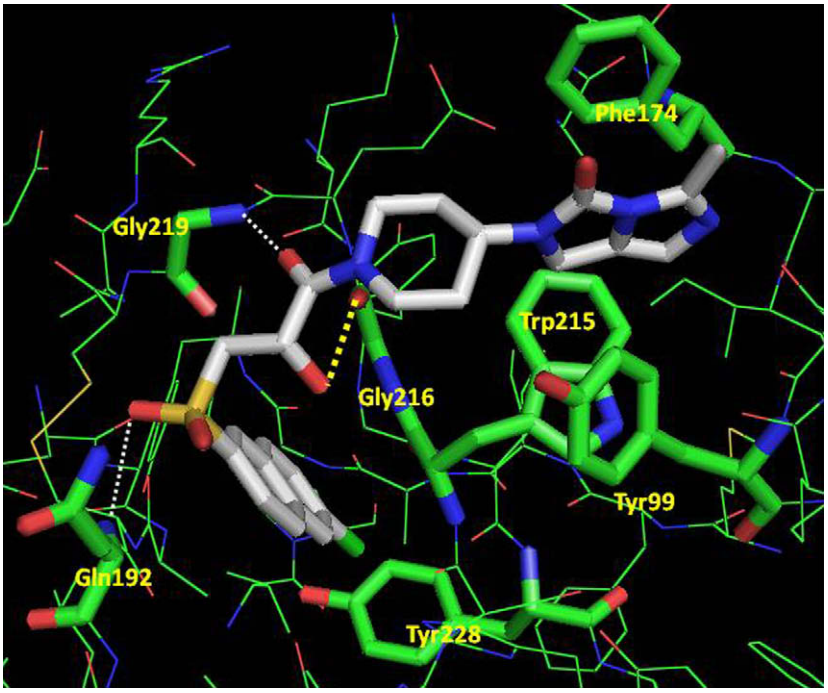


Figure 2. Binding model of ***S*-5** in factor Xa.

the compounds ***S*-5** and **6** displayed the selectivity of more than 2000-fold for FXa over thrombin, plasmin, trypsin and t-PA, and showed favorable pharmacokinetic profiles in monkeys.

To understand the incremental effect on FXa inhibitory activity by introducing the hydroxy group at the α-position of the amide carbonyl group of **1**, molecular modeling study on ***S*-5** was carried out using GOLD programs[13] and the X-ray structure of FXa reported by Sanofi-Aventis for an inhibitor complex (PDB code 1EZQ)[14] (Fig. 2). The overall binding mode of ***S*-5** was predicted to be similar to that of the parent compound **1**.[8] In the model, the 6-chloronaphthylsulfonyl moiety binds to the S1 site with the hydrophobic interaction between the chlorine atom in ***S*-5** and the phenyl ring of Tyr228, and the imidazoimidazolone moiety makes the hydrophobic interactions with the residues Tyr99, Trp215, and Phe174 in the S4 site. The carbonyl oxygen of the central amide and the sulfone oxygen in ***S*-5** are hydrogen-bonded to the backbone nitrogen of Gly219 and Gln192, respectively. Interestingly, the hydroxy group at the α-position of the central amide carbonyl group is oriented to the S2 pocket, forming a hydrogen bond with the carbonyl oxygen of the main chain of Gly216. This newly observed interaction of the hydroxy group might contribute to the slight enhancement in FXa inhibitory activity of ***S*-5** compared to that of the parent compound **1**.

On the other hand, the docking model of ***R*-5** with FXa was also constructed for considering the detrimental effect of the hydroxy group of ***R*-5** on FXa inhibitory activity, and its binding mode was verified to be similar to that of compound **1** as well as ***S*-5**. The energy gaps (ΔG) of the internal energy of the active conformation in FXa from its corresponding local energy minimum for ***R*-5** and the parent compound **1** were calculated. As a result, the ΔG value for ***R*-5** was larger than that of **1** (ΔG = 7.47 kcal/mol for ***R*-5**, 1.65 kcal/mol for **1**), suggesting that the active conformation for ***R*-5** might be less stable than that of **1**.

4. Conclusions

As part of our preclinical development of compound **1**, its metabolites were identified by chemical synthesis, and their biological activities were examined. As a result, we serendipitously identified two active metabolites ***S*-5** and **6** with potent FXa inhibitory and anticoagulant activities, the excellent selectivity over other human serine proteases and favorable pharmacokinetic profile in monkeys. The molecular modeling study reveals that the hydroxy group of ***S*-5** contributes to an increase in FXa inhibitory activity compared to the parent compound **1**. The studies described herein provide not only the data required for the further preclinical development of **1** but also an opportunity to explore for follow-on compounds. Optimization studies based on the active metabolite ***S*-5** and **6** will be reported in due course.

5. Experimental

5.1. Chemistry

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. ^{1}H NMR spectra were ob-

tained at 200 or 300 MHz on a Varian Gemini-200 or a Mercury-300 spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. Peak multiplicities are expressed as follows. Abbreviations are used as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; br s, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz). Infrared absorption spectra (IR) were recorded on a SHIMADZU FTIR-8200PC spectrometer. Optical rotations were determined with a JASCO DIP-370 digital polarimeter. Reactions were followed by TLC on Silica Gel 60 F 254 precoated TLC plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd). Chromatographic separations were carried out on Silica Gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck) or basic silica gel (Chromatorex® NH, 100–200 mesh, Fuji Silysia Chemical Ltd) using the indicated eluents. SCX[15] Bond-Elut prepacked cartridges were purchased from Varian. Elemental analyses were carried out by Takeda Analytical Laboratories Ltd. Yields are not optimized. Compounds **10**, **16**, and **18** were commercially available and used as received. Compounds **2**,[9] **8**,[9] **12**,[9] and **15**[8] were prepared according to the reported methods. Chemical intermediates were characterized by ^{1}H NMR.

5.1.1. *tert*-Butyl 3-[(6-chloronaphthalene-2-yl)sulfonyl]propanoate (9)

An aqueous solution (250 mL) of Na_2CO_3 (25 g, 200 mmol) and $NaHCO_3$ (17 g, 200 mmol) was heated at 70 °C, and **8** (26.1 g, 100 mmol) was added in small portions (CO_2 evolution). After stirring at 70 °C for 2 h, the mixture was allowed to cool to room temperature and left to stand overnight. The precipitate was filtered off, washed with cold acetone and water, and dried to give **3** as a pale yellow powder (25 g), which contained inseparable impurities. An aqueous solution (100 mL) of the crude salt **3** (10.0 g, ca. 40 mmol) was acidified with 1 M HCl solution, and the mixture was extracted with EtOAc (100 mL × 3). The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, and filtered. To the filtrate, *tert*-butyl acrylate (5.9 mL, 40 mmol) and Et_3N (11 mL, 80 mmol) were added, and the mixture was heated at reflux overnight. After cooling to room temperature, the mixture was successively washed with a saturated aqueous solution of $NaHCO_3$ and water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc to give **9** (7.0 g, 49%) as pale yellow needles. ^{1}H NMR ($CDCl_3$) δ: 1.36 (9H, s), 2.69 (2H, t, J = 7.8 Hz), 3.46 (2H, t, J = 7.8 Hz), 7.58 (1H, m), 7.88–7.95 (4H, m), 8.45 (1H, s). Anal. Calcd for $C_{17}H_{19}ClO_4S$: C, 57.54; H, 5.40. Found: C, 57.51; H, 5.62.

5.1.2. Sodium 6-chloronaphthalene-2-sulfinate (3-Na)

One molar NaOH solution (1.4 mL, 1.4 mmol) was added to a solution of **9** (0.50 g, 1.4 mmol) in MeOH (5 mL), and the mixture was stirred at room temperature overnight. After removal of the solvent in vacuo, the residue was suspended in Et_2O, and the precipitate was filtered off to give **3-Na** (0.36 g, quant.) as a pale yellow powder, mp >300 °C. ^{1}H NMR ($CDCl_3$) δ: 7.49 (1H, dd, J = 2.1 and 8.7 Hz), 7.70–7.74 (1H, m), 7.86 (1H, d, J = 9.0 Hz), 7.95–8.00 (3H, m). IR (KBr): 1352, 1325, 1264, 1235, 1179, 1136 cm^{-1}. Anal. Calcd for $C_{10}H_6ClNaO_2S$: C, 48.30; H, 2.43. Found: C, 48.33; H, 2.61.

5.1.3. *tert*-Butyl (1-{3-[(6-chloronaphthalene-2-yl)sulfonyl]-propanoyl}piperidin-4-yl)carbamate (11)

WSC (2.3 g, 12 mmol) was added to a solution (50 mL) of **2** (3.3 g, 11 mmol), **10** (2.0 g, 10 mmol), HOBt (1.8 g, 12 mmol), and Et_3N (1.7 mL, 12 mmol) in MeCN (50 mL), and the mixture was stirred at room temperature for 5 h. After removal of MeCN in vacuo, the residue was diluted with EtOAc–THF–H_2O (1:1:1, 100 mL). The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from EtOAc to give **11** (3.2 g, 60%) as an off-white powder. ^{1}H NMR ($CDCl_3$) δ: 1.13–1.33 (2H, m), 1.35 (9H, s), 1.88–1.92 (1H, m), 2.00–2.04 (1H, m), 2.65–2.72 (1H, m), 2.82–2.89 (2H, m), 3.05–3.15 (1H, m), 3.49–3.63 (3H, m), 3.75–3.79 (1H, m), 4.34–4.39 (2H, m), 7.58 (1H, dd, J = 2.0 and 8.9 Hz), 7.88–7.95 (4H, m), 8.46 (1H, s). Anal. Calcd for $C_{23}H_{29}ClO_5S$: C, 57.43; H, 6.08; N, 5.82. Found: C, 57.43; H, 6.12; N, 5.86.

5.1.4. 1-{3-[(6-Chloronaphthalene-2-yl)sulfonyl]propanoyl}-piperidin-4-amine hydrochloride (4)

Compound **11** was dissolved in concentrated HCl (5 mL), and the mixture was stirred at room temperature for 10 min. EtOH (200 mL) was added, and the precipitate was collected by filtration and dried to give **4** (2.5 g, 91%) as a pale yellow powder, mp 272–273 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 1.22–1.33 (1H, m), 1.38–1.50 (1H, m), 1.83–1.95 (2H, m), 2.55 (1H, t, J = 11.9 Hz), 2.67–2.81 (2H, m), 3.01 (1H, t, J = 11.9 Hz), 3.16–3.23 (1H, m), 3.61 (2H, t, J = 7.5 Hz), 3.84 (1H, d, J = 13.4 Hz), 4.20 (1H, d, J = 13.4 Hz), 7.73 (1H, dd, J = 2.3 and 8.9 Hz), 7.98 (1H, dd, J = 1.8 and 8.7 Hz), 8.17–8.29 (5H, m), 8.63 (1H, s). IR ν_{max}(KBr), cm^{-1}: 740, 1173, 1456, 1651, 2934, 3321. Anal. Calcd for $C_{18}H_{21}ClN_3O_3S{\cdot}HCl$: C, 51.80; H, 5.31; N, 6.71. Found: C, 51.63; H, 5.32; N, 6.63.

5.1.5. Methyl (2*S*)-3-[(6-Chloronaphthalene-2-yl)thio]-2-hydroxypropanoate (*S*-13)

Under Argon atmosphere, a solution of EtMgBr in Et_2O (3.0 M, 8.6 mL, 18 mmol) was diluted with THF (25 mL) at 0 °C. A solution of **12** (5.0 g, 26 mmol) in THF (50 mL) was then added dropwise at 0 °C, and the mixture was stirred at room temperature for 30 min, giving a white suspension. A solution of (*R*)-methyl glycidate (2.3 mL, 26 mmol) in THF (15 mL) was added dropwise at 0 °C. After stirring at room temperature for 3 h, a saturated aqueous solution of NH_4Cl (50 mL) was added, and the mixture was extracted with EtOAc (100 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc = 3:1 to afford ***S*-13** (5.88 g, 77%) as fluffy needle-like crystals. ^{1}H NMR ($CDCl_3$) δ: 3.12 (1H, d, J = 6.0 Hz), 3.35 (1H, dd, J = 5.7 and 14.1 Hz), 3.48 (1H, dd, J = 4.2 and 14.1 Hz), 3.58 (3H, s), 4.43–4.48 (1H, m), 7.39–7.43 (1H, m), 7.49–7.52 (1H, m), 7.66–7.69 (2H, m), 7.76–7.77 (1H, m), 7.83–7.84 (1H, m). $[\alpha]_D^{25}$ −42.7 (c 0.25, $CHCl_3$).

5.1.6. Methyl (2*R*)-3-[(6-chloronaphthalene-2-yl)thio]-2-hydroxypropanoate (*R*-13)

The title compound was prepared by the procedure similar to that described for ***S*-13**, using **12** and (*S*)-methyl glycidate. Yield 70%, fluffy needle-like crystals. ^{1}H NMR ($CDCl_3$) δ: 3.11 (1H, d, J = 6.0 Hz), 3.34 (1H, dd, J = 5.8 and 14.1 Hz), 3.49 (1H, dd, J = 4.2 and 14.1 Hz), 3.59 (3H, s), 4.44–4.48 (1H, m), 7.39–7.44 (1H, m), 7.49–7.52 (1H, m), 7.66–7.70 (2H, m), 7.76–7.79 (1H, m), 7.82–7.84 (1H, m). $[\alpha]_D^{25}$ +37.2 (c 0.25, $CHCl_3$).

5.1.7. (2*S*)-3-[(6-Chloronaphthalene-2-yl)sulfonyl]-2-hydroxypropanoic acid (*S*-14)

NaOH (8 M) solution (6.8 mL, 54 mmol) was added to a solution of ***S*-13** (5.4 g, 18 mmol) in EtOH (150 mL), and the mixture was stirred at room temperature for 3 h. After removal of EtOH in vacuo, the residue was diluted with H_2O (50 mL) and acidified to pH 3 with 1 M HCl solution. The precipitate was collected by filtration, washed with water, and dried to give (2*S*)-3-[(6-chloronaphthalene-2-yl)thio]-2-hydroxypropanoic acid (5.0 g, 97%) as a white powder. ^{1}H NMR (CD_3OD) δ: 3.27 (1H, dd, J = 6.9 and 14.1 Hz), 3.51 (1H, dd, J = 4.2 and 14.1 Hz), 4.33 (1H, dd, J = 4.2 and 6.9 Hz), 7.40–7.43 (1H, m), 7.51–7.54 (1H, m), 7.71–7.77 (2H, m), 7.82 (1H, s), 7.86 (1H, s). An aqueous solution (150 mL) of Oxone® (6.6 g, 11 mmol) was added dropwise to a suspension of (2*S*)-3-[(6-chloronaphthalene-2-

yl)thio]-2-hydroxypropanoic acid (1.5 g, 5.4 mmol) in acetone (150 mL), and the mixture was vigorously stirred at room temperature for 5 h. The precipitate was collected by filtration, washed with water, and dried to give ***S*-14** as a white powder (1.4 g, 82%). ^{1}H NMR (CD_3OD) δ: 3.65 (1H, dd, J = 8.1 and 14.7 Hz), 3.76 (1H, dd, J = 3.3 and 14.7 Hz), 4.59 (1H, dd, J = 3.3 and 8.1 Hz), 7.62 (1H, dd, J = 2.3 and 8.9 Hz), 7.94–8.09 (4H, m), 8.55 (1H, s). $[\alpha]_D^{25}$ +12.4 (c 0.25, MeOH).

5.1.8. (2*R*)-3-[(6-Chloronaphthalene-2-yl)sulfonyl]-2-hydroxypropanoic acid (*R*-14)

The title compound was prepared by the procedure similar to that described for ***S*-14**, using ***R*-13**. Yield 82%, a white powder. ^{1}H NMR (CD_3OD) δ: 3.55 (1H, dd, J = 8.0 and 14.8 Hz), 3.74 (1H, dd, J = 3.3 and 14.8 Hz), 4.56 (1H, dd, J = 3.3 and 8.0 Hz), 7.61 (1H, dd, J = 2.3 and 8.9 Hz), 7.94–8.09 (4H, m), 8.54 (1H, s). $[\alpha]_D^{25}$ −12.2 (c 0.25, MeOH).

5.1.9. 2-(1-{(2*S*)-3-[(6-Chloronaphthalene-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)-5-methyl-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (*S*-5)

The title compound was prepared by the procedure similar to that described for **11**, using ***S*-14** and **15**. Yield 71%, a white powder, mp 203–204 °C. ^{1}H NMR (DMSO-d_6) δ: 1.34–1.86 (2H, m), 2.45 (3H, s), 2.49–2.75 (2H, m), 3.01–3.23 (1H, m), 3.57–3.72 (1H, m), 3.76–3.89 (1H, m), 3.91–4.10 (2H, m), 4.21–4.40 (3H, m), 4.75–4.88 (1H, m), 5.73–5.82 (1H, m), 6.67 (1H, d, J = 9.2 Hz), 7.72 (1H, dd, J = 2.2 and 8.5 Hz), 7.98 (1H, dd, J = 1.6 and 8.6 Hz), 8.13–8.18 (2H, m), 8.24–8.30 (2H, m), 8.63 (1H, s). IR ν_{max}(KBr), cm^{-1}: 1140, 1150, 1319, 1634, 1728, 3436. Anal. Calcd for $C_{24}H_{25}ClN_4O_5S$: C, 55.76; H, 4.87; N, 10.84. Found: C, 55.60; H, 4.98; N, 10.85. $[\alpha]_D^{25}$ +22.5 (c 0.25, $CHCl_3$).

5.1.10. 2-(1-{(2*R*)-3-[(6-Chloronaphthalene-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)-5-methyl-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (*R*-5)

The title compound was prepared by the same procedure as descried for ***S*-5** except that (*S*)-methyl glycidate was used in place of its *R*-isomer. Yield 23%, a white solid, mp 203–204 °C. ^{1}H NMR ($CDCl_3$) δ: 1.66–1.99 (4H, m), 2.61 (3H, s), 2.69–2.88 (1H, m), 3.14–3.33 (1H, m), 3.45–3.54 (2H, m), 3.89–4.00 (1H, m), 4.07–4.22 (1H, m), 4.26–4.30 (2H, m), 4.65–4.88 (1H, m), 4.98–5.09 (1H, m), 6.71 (1H, s), 7.59 (1H, dd, J = 1.0 and 8.8 Hz), 7.95 (3H, s), 7.96 (1H, d, J = 8.8 Hz), 8.51 (1H, s). IR ν_{max}(KBr), cm^{-1}: 1143, 1155, 1319, 1635, 1730, 3420. Anal. Calcd for $C_{24}H_{25}ClN_4O_5S$: C, 55.76; H, 4.87; N, 10.84. Found: C, 55.79; H, 4.92; N, 10.94. $[\alpha]_D^{25}$ −21.8 (c 0.25, $CHCl_3$).

5.1.11. 2-(1-{3-[(6-Chloronaphthalene-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)-5-methyl-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (*rac*-5)

The title compound was prepared by the same procedure as descried for ***S*-5** except that racemic methyl glycidate was used in place of its *R*-isomer. Yield 32%, a white amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.62–1.99 (4H, m), 2.61 (3H, s), 2.69–2.85 (1H, m), 3.14–3.33 (1H, m), 3.44–3.52 (2H, m), 3.81–3.93 (1H, m), 4.10–4.22 (1H, m), 4.27–4.30 (2H, m), 4.68–4.80 (1H, m), 4.98–5.09 (1H, m), 6.72 (1H, s), 7.59 (1H, dd, J = 1.0 and 8.8 Hz), 7.95 (3H, s), 7.96 (1H, d, J = 8.8 Hz), 8.51 (1H, s). IR ν_{max}(KBr), cm^{-1}: 1140, 1153, 1335, 1650, 1733, 3431. Anal. Calcd for $C_{24}H_{25}ClN_4O_5S$: C, 55.76; H, 4.87; N, 10.84. Found: C, 55.79; H, 4.92; N, 10.94.

5.1.12. 1-Trityl-1*H*-imidazole-4-carbaldehyde (17)

Trityl chloride (42 g, 160 mmol) was added to a suspension (300 mL) of **16** (15 g, 160 mmol) and Et_3N (23 mL, 160 mmol) in CH_2Cl_2 (300 mL), and the mixture was stirred at room temperature overnight. The precipitate was collected by filtration, washed successively with hexane and water, and dried to give **17** (51 g, quant.) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 7.07–7.13 (6H, m), 7.25–7.41 (9H, m), 7.52 (1H, d, J = 1.5 Hz), 7.60 (1H, d, J = 1.5 Hz), 9.86 (1H, s).

5.1.13. 1-Benzyl-*N*-[(1-trityl-1*H*-imidazol-4-yl)methyl]piperidin-4-amine (19)

$NaBH(OAc)_3$ (3.8 g, 18 mmol) was added to a solution of **17** (5.0 g, 15 mmol), **18** (2.8 g, 15 mmol), and AcOH (1 mL) in CH_2Cl_2 (100 mL), and the mixture was stirred at room temperature overnight. After adding 1 M NaOH solution (50 mL), the mixture was extracted with CH_2Cl_2 (50 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **19** (4.6 g, 90%) as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.51–1.62 (2H, m), 1.83–1.96 (2H, m), 2.04–2.12 (2H, m), 2.58–2.65 (1H, m), 2.88–2.92 (2H, m), 3.57 (2H, s), 3.78 (2H, s), 6.75 (1H, s), 7.05–7.14 (6H, m), 7.23–7.32 (14H, m), 7.39 (1H, s).

5.1.14. *tert*-Butyl (1-benzylpiperidin-4-yl)[(1-trityl-1*H*-imidazol-4-yl)methyl]carbamate (20)

Boc_2O (3.8 g, 18 mmol) was added to a solution of **19** (9.0 g, 18 mmol) and Et_3N (1.8 g, 18 mmol) in EtOH (200 mL), and the mixture was stirred at room temperature overnight. After removal of EtOH in vacuo, the residue was partitioned between EtOAc and water. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **20** (11 g, 98%) as a white amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.28 (9H, s), 1.47–1.52 (2H, m), 1.73–1.81 (2H, m), 1.94–2.04 (2H, m), 2.87 (2H, d, J = 11.4 Hz), 3.45 (2H, s), 3.90–4.05 (1H, m), 4.29 (2H, s), 6.55 (1H, s), 7.09–7.14 (5H, m), 7.20–7.33 (16H, m).

5.1.15. *tert*-Butyl (1-benzylpiperidin-4-yl){[2-(hydroxymethyl)-1-trityl-1*H*-imidazol-4-yl]methyl}carbamate (21)

Under Argon atmosphere, a solution of *n*-BuLi in hexane (1.5 M, 1 mL, 1.5 mmol) was added dropwise at 0 °C to a solution of **20** (0.61 g, 1.0 mmol) in THF (30 mL), and the mixture was stirred at 0 °C for 30 min. Anhydrous DMF (1 mL) was then added at 0 °C, and the mixture was stirred at room temperature overnight. After adding a saturated aqueous solution of NH_4Cl (20 mL), the mixture was extracted with EtOAc (30 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc = 10:1 to give *tert*-butyl (1-benzylpiperidin-4-yl)[(2-formyl-1-trityl-1*H*-imidazol-4-yl)methyl]carbamate (0.57 g, 89%) as pale yellow crystals. ^{1}H NMR ($CDCl_3$) δ: 1.26 (9H, s), 1.42–1.54 (2H, m), 1.74–1.79 (2H, m), 1.95–1.99 (2H, m), 2.88 (2H, d, J = 11.1 Hz), 3.45 (2H, s), 3.98–4.03 (4H, m), 4.34 (2H, s), 6.85 (1H, s), 7.05–7.09 (6H, m), 7.20–7.35 (14H, m), 9.10 (1H, s). $NaBH_4$ (0.53 g, 14 mmol) was added in small portions at 0 °C to a solution of *tert*-butyl (1-benzylpiperidin-4-yl)[(2-formyl-1-trityl-1*H*-imidazol-4-yl)methyl]carbamate (9.0 g, 14 mmol) in EtOH–CH_2Cl_2 (5:1, 120 mL), and the mixture was stirred at room temperature overnight. After removal of the solvents in vacuo, the residue was partitioned between EtOAc and water. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc = 1:1 to give **21** (8.0 g, 89%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 1.28 (9H, s), 1.47–1.61 (2H, m), 1.77–1.89 (2H, m), 1.95–2.00 (2H, m), 2.87–2.93 (2H, m), 2.91 (1H, t, J = 5.4 Hz), 3.46 (2H, s), 3.57 (2H, d, J = 5.4 Hz), 3.96–4.05 (1H, m), 4.24 (2H, s), 6.59 (1H, s), 7.05–7.11 (6H, m), 7.19–7.36 (14H, m).

5.1.16. 1-Benzyl-*N*-{[2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1*H*-imidazol-4-yl]methyl}piperidin-4-amine (22)

An aqueous solution of TFA (30%, 30 mL) was added to a solution of **22** (8.6 g, 13 mmol) in CH_2Cl_2 (60 mL), and the mixture was stirred at room temperature for 3 h. The separated aqueous layer was

washed with hexane (50 mL), basified to pH 12 with K_2CO_3, and extracted with CH_2Cl_2 (50 mL). The extract was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give (4-{[(1-benzylpiperidin-4-yl)amino]methyl}-1*H*-imidazol-2-yl)methanol (4.0 g, 99%) as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$) δ: 1.48–1.55 (2H, m), 1.63–1.75 (2H, m), 1.87–2.02 (2H, m), 2.64–2.65 (1H, m), 2.84–2.88 (2H, m), 3.47 (2H, s), 3.74 (2H, s), 4.46 (2H, s), 5.42 (1H, br s), 6.76 (1H, s), 7.19–7.34 (5H, m). TBDMSCl (0.17 g, 1.0 mmol) was added to a solution of (4-{[(1-benzylpiperidin-4-yl)amino]-methyl}-1*H*-imidazol-2-yl)methanol (0.28 g, 0.90 mmol) and Et_3N (0.26 mL, 2.0 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between CH_2Cl_2 and water, and the separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/EtOH = 10:1) to give **22** (0.20 g, 52%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.10 (6H, s), 0.92 (9H, s), 1.41–1.44 (2H, m), 1.84–1.88 (2H, m), 1.97–2.04 (2H, m), 2.50–2.51 (1H, m), 2.82–2.86 (2H, m), 3.49 (2H, s), 3.75 (2H, s), 4.77 (2H, s), 6.81 (1H, s), 7.18–7.36 (5H, m).

5.1.17. 5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-2-(piperidin-4-yl)-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (23)

CDI (0.094 g, 0.60 mmol) was added to a solution of **21** (0.20 g, 0.50 mmol) and DBU (0.14 mL, 1.0 mmol) in 1,2-dichloroethane (10 mL), and the mixture was stirred at 60 °C for 30 min. The mixture was partitioned between CH_2Cl_2 and water, and the separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/EtOH = 5:1) to give 2-(1-benzylpiperidin-4-yl)-5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (0.18 g, 85%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.13 (6H, s), 0.91 (9H, s), 1.72–1.83 (4H, m), 2.08–2.17 (2H, m), 2.99 (2H, d, J = 11.4 Hz), 3.52 (2H, s), 3.95–4.02 (1H, m), 4.30–4.31 (2H, m), 4.92 (2H, s), 6.79–6.80 (1H, m), 7.24–7.37 (5H, m). A mixture of 2-(1-benzylpiperidin-4-yl)-5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (0.18 g, 0.40 mmol) and 10% Pd/C (50% wet, 36 mg) in MeOH (3 mL) was stirred at room temperature under H_2 atmosphere overnight. The mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo to give **23** (0.16 g, quant.) as a colorless powder. ^{1}H NMR ($CDCl_3$) δ: 0.14 (6H, s), 0.92 (9H, s), 1.61–1.74 (2H, m), 1.85–1.88 (2H, m), 2.71–2.79 (2H, m), 3.20 (2H, d, J = 11.7 Hz), 4.02–4.10 (1H, m), 4.32–4.33 (2H, m), 4.93 (2H, s), 6.80–6.81 (1H, m).

5.1.18. 2-(1-{3-[(6-Chloronaphthalene-2-yl)sulfonyl]propanoyl}-piperidin-4-yl)-5-(hydroxymethyl)-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (6)

5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-2-(1-{3-[(6-chloronaphthalene-2-yl)sulfonyl]propanoyl}piperidin-4-yl)-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one was prepared (76%) by the procedure similar to that described for **11**, using **2** and **23**. ^{1}H NMR ($CDCl_3$) δ: 0.14 (6H, s), 0.92 (9H, s), 1.57–1.74 (2H, m), 1.87–1.99 (2H, m), 2.59–2.66 (1H, m), 2.83–3.01 (2H, m), 3.14–3.22 (1H, m), 3.48–3.65 (2H, m), 4.00 (1H, d, J = 14.4 Hz), 4.08–4.27 (1H, m), 4.27 (2H, s), 4.72 (1H, d, J = 14.1 Hz), 4.91 (2H, s), 6.80–6.81 (1H, m), 7.54–7.61 (2H, m), 7.89–7.96 (3H, m), 8.47 (1H, s). 5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-2-(1-{3-[(6-chloronaphthalene-2-yl)sulfonyl]propanoyl}piperidin-4-yl)-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (0.22 g, 0.40 mmol) was dissolved in AcOH–THF–H_2O (2:1:1, 8 mL), and the mixture was stirred at 60 °C for 3 h. After removal of the solvents in vacuo, the residue was partitioned between $CHCl_3$ and a saturated aqueous solution of $NaHCO_3$. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/EtOH = 10:1) to give **6** (0.099 g, 55%) as a colorless powder, mp 199–200 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 1.56–1.80 (2H, m), 1.89–2.02 (2H, m), 2.80–2.91 (1H, m), 2.95–3.06 (1H, m), 3.17–3.25 (1H, m), 3.46–3.56 (1H, m), 3.59–3.69 (1H, m), 3.86 (1H, t, J = 6.6 Hz), 4.03 (1H, d, J = 15.3 Hz), 4.15–4.23 (1H, m), 4.36 (2H, s), 4.75 (1H, d, J = 11.7 Hz), 4.87 (2H, d, J = 6.6 Hz), 6.78 (1H, s), 7.61 (1H, dd, J = 2.1 and 9.0 Hz), 7.90–7.98 (4H, m), 8.49 (1H, s). IR ν_{max}(KBr), cm^{-1}: 1144, 1302, 1620, 1750, 3638. Anal. Calcd for $C_{24}H_{25}ClN_4O_5S$: C, 55.76; H, 4.87; N, 10.84. Found: C, 55.52; H, 4.94; N, 10.74.

5.1.19. Ethyl 3-[(6-chloronaphthalene-2-yl)sulfonyl]-2-oxopropanoate (25)

A suspension of **3** (6.4 g, 26 mmol) and ethyl bromopyruvate (3.2 mL, 26 mmol) in MeOH (100 mL) was refluxed overnight. After removal of MeOH in vacuo, the residue was partitioned between EtOAc and water. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (hexane/EtOAc = 2:1 to 1:1) to give **25** (4.5 g, 50%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7.2 Hz), 4.24 (2H, q, J = 7.2 Hz), 4.73 (2H, s), 7.59 (1H, dd, J = 2.4 and 8.7 Hz), 7.85–7.93 (4H, m), 8.43–8.44 (1H, m).

5.1.20. 3-[(6-Chloronaphthalene-2-yl)sulfonyl]-2-oxopropanoic acid (26)

NaOH (8 M) solution (2.6 mL, 20 mmol) was added to a solution of **25** (3.5 g, 10 mmol) in MeOH (50 mL), and the mixture was stirred at room temperature for 3 h. After removal of MeOH in vacuo, the residue was suspended in CH_2Cl_2, and the precipitate was collected by filtration to give **26** (1.6 g) as a colorless powder. ^{1}H NMR (DMSO-d_6) δ: 5.09 (2H, s), 7.71–7.79 (1H, m), 7.97–8.05 (1H, m), 8.16–8.35 (3H, m), 8.63 (1H, s).

5.1.21. 2-(1-{3-[(6-Chloronaphthalene-2-yl)sulfonyl]-2-oxopropanoyl}piperidin-4-yl)-5-methyl-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (7)

PyBOP (3.9 g, 7.5 mmol) was added to a suspension of **26** (1.6 g, 5.0 mmol), **15** (1.8 g, 6.0 mmol), and *i*-Pr_2EtN (2.8 mL, 16 mmol) in DMF (30 mL), and the mixture was stirred at room temperature overnight. After removal of DMF in vacuo, the residue was passed through a short column (EtOAc/EtOH = 5:1) and then purified by SCX[15] column (wash protocol: DMF, sample charge, DMF × 10, DCM × 3, MeOH × 3, sample elution with 1 M NH_3 in MeOH) to remove excess **15** and *i*-Pr_2EtN. The sample was further purified by column chromatography ($CHCl_3$/MeOH = 10:1) and preparative HPLC to give **7** as a colorless amorphous powder. The amorphous powder was then suspended in Et_2O–EtOAc (10:1, 50 mL) and stirred overnight to give a white powder (0.72 g, 28%), mp 160–161 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 1.75–1.84 (2H, m), 1.92–2.02 (2H, m), 2.61 (3H, s), 2.80–2.90 (1H, m), 3.22–3.32 (1H, m), 4.11–4.30 (4H, m), 4.56–4.69 (2H, m), 4.89 (1H, d, J = 12.8 Hz), 6.71 (1H, s), 7.63 (1H, dd, J = 2.0 and 8.9 Hz), 7.91–8.00 (4H, m), 8.49 (1H, s). IR ν_{max}(KBr), cm^{-1}: 1151, 1361, 1643, 1743. Anal. Calcd for $C_{24}H_{23}ClN_4O_5S$: C, 55.97; H, 4.50; N, 10.88. Found: C, 55.85; H, 4.70; N, 10.86.

5.2. Biology

5.2.1. In vitro assays for the inhibition of human FXa

Human factor Xa (0.3 U/mL) was obtained from Roche Diagnostics. Chromogenic substrates, S-2765 (Chromogenix-Instrumentation Laboratory) was used for the measurement of the inhibition of FXa. Anti-FXa activity was assayed in a buffer containing 50 mM Tris–HCl, 145 mM NaCl and 2 mM $CaCl_2$ at pH 8.3. Enzyme assay was carried out in 96-well microtiter plates. Test compounds

were dissolved in DMSO. The solution and 10 μL of enzyme solution were added to the well containing buffer, and the mixture was preincubated. The enzymatic reactions were initiated with the addition of 10 μL of 3 mM substrate, and the mixture was incubated for 10 min at 37 °C. The reaction was terminated by the addition of 25 μL of 50% acetic acid. The color development by the release of *p*-nitroanilide from each chromogenic substrate was measured at 405 nm on a microtiter plate reader (MTP32, Corona Electric Co.). Each absorbance [T] was calculated by subtracting the absorbance measured in the absence of the substrate. The control [C] was performed using DMSO solution in the absence of test compounds. Inhibitory effect (%) was calculated according to the equation $(1 - [T][C]) \times 100$. IC_{50} values were calculated from the regression line based on the method of least squares between inhibitory effect and concentration.

5.2.2. In vitro PT assays

The assay of plasma clotting time was performed using an automatic coagulometer (STA Compact, Diagnostica Stago). PT was measured with STA PT reagents (Roche Diagnostics). An amount of 1.5 μL of compound dilutions in DMSO was added to 48.5 μL of human normal plasma (fresh human plasma, FFP, Sekisui Chemical Co.), and the mixture was preincubated at 37 °C for 4 min. Coagulation was initiated with the addition of 100 μL of thromboplastin, and coagulation time was measured. Coagulation time prolonging ratio (%) was calculated on the basis of coagulation time when DMSO was added instead of test compound. The plasma clotting time doubling concentration (PT_2) was calculated from the regression line based on the method of least squares. Data for new compounds were compared to positive control DX-9065a[16] (PT_2 = 0.81 ± 0.029 μM (mean ± SEM, n = 3)).

5.2.3. Enzyme affinity assays

Enzyme assays using chromogenic substrates were performed as follows. Human FXa was obtained from Roche diagnostics. Human thrombin was obtained from Sigma Chemical Co. Human trypsin was obtained from Atheus Research and Technology, Inc. Human plasmin was purchased from BioPur AG. Human t-PA was obtained from Kyowa Hakko Kogyo Co. The chromogenic substrates used were S-2222, S-2366, S-2222, S-2366, S-2302, and S-2288 for FXa, thrombin, trypsin, plasmin, and t-PA, respectively, and obtained from Chromogenix-Instrumentation Laboratory. All enzyme assays were performed at 37 °C in 96-well microtiter plates. The final enzyme concentrations were 0.024 U/mL, 0.080 U/mL, 0.040 μg/mL, 0.040 U/mL, 0.020 U/mL, and 4000 U/mL for FXa, thrombin, trypsin, plasmin, and t-PA, respectively. Compound dilutions were added to the wells containing buffer and enzyme and incubated for 30 min. The enzyme reactions were initiated by the addition of substrate, and the color developed from the release of *p*-nitroanilide from each chromogenic substrate was monitored continuously for 5 min at 405 nm on a microtiter plate reader. The K_i values were determined from a Lineweaver–Burk plot, when the optical densities were measured with different concentrations of substrates.

5.2.4. Measurement of plasma concentrations in cynomolgus monkey after oral administration

All experiments were conducted in accordance with the regulation of Animal Care and Use Committee of the Takeda Chemical Industries Ltd. Male Cynomolgus monkeys (3–4 years old, Shin Nippon Biomedical Laboratories Ltd, Japan) were used. Test compound (1 mg/kg) was orally administered under feeding condition. Blood samplings were performed 1, 2, 4, and 10 h after the administration. Plasma samples were prepared by centrifugation for 15 min at 4 °C. The plasma samples were deproteinized with acetonitrile. After centrifugation, the supernatant obtained was diluted with 0.01 mol/L $CH_3CO_2NH_4$. The compound concentration in the supernatant was measured by LC/MS/MS with an API4000 mass spectrometer (AB/MDS SCIEX). The mass spectrometer was equipped with a turbo ionspray source and operated in positive ion mode. The HPLC conditions were as follows: column, an Chiralcel OD-RH (2.0 mm i.d. × 150 mm); mobile phase, 0.01 mol/L $CH_3CO_2NH_4$/acetonitrile = 65/35; flow rate, 0.2 mL/min; column temperature, 40 °C.

5.2.5. Pharmacokinetic analysis in monkey

All experiments were conducted in accordance with the regulation of Animal Care and Use Committee of the Takeda Chemical Industries Ltd. Test compounds were administered as a cassette dosing to monkeys. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with 0.01 mol/L ammonium acetate and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

5.3. Computer chemistry

5.3.1. Docking studies

The coordinates of FXa were retrieved from the Protein Data Bank (accession code 1EZQ).[14] Compound ***S*-5** was docked into the FXa protein crystal structure using GOLD[13] (version 2.2, the Cambridge Crystallographic Data Centre, UK) with the standard default settings. The initial structure of the compound-complexed FXa model was energy-minimized using the MMFF94s force field in MOE (version 2006.0804, Chemical Computing Group, Montreal, Canada) to obtain the final docking models. During the minimization procedure, the following conditions were adopted. The dielectric constant was set to 4r, where r is the distance between two interacting atoms. The residues, which are 8 Å away from each compound, were fixed. A harmonic force constraint against the initial atomic positions of the backbone was added, using 10 kcal/Å^2 as a force constant. And atomic charges for the protein and the compounds were set according to the AMBER99 force field and the AM1-BCC method, respectively.

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