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Discovery of a Tetrahydropyrimidin-2(1*H*)-one Derivative (TAK-442) as a Potent, Selective, and Orally Active Factor Xa Inhibitor

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Coagulation enzyme factor Xa (FXa) is a particularly promising target for the development of new anticoagulant agents. We previously reported the imidazo[1,5-*c*]imidazol-3-one derivative **1** as a potent and orally active FXa inhibitor. However, it was found that **1** predominantly undergoes hydrolysis upon incubation with human liver microsomes, and the human specific metabolic pathway made it difficult to predict the human pharmacokinetics. To address this issue, our synthetic efforts were focused on modification of the imidazo[1,5-*c*]imidazol-3-one moiety of the active metabolite **3a**, derived from **1**, which resulted in the discovery of the tetrahydropyrimidin-2(1*H*)-one derivative **5k** as a highly potent and selective FXa inhibitor. Compound **5k** showed no detectable amide bond cleavage in human liver microsomes, exhibited a good pharmacokinetic profile in monkeys, and had a potent antithrombotic efficacy in a rabbit model without prolongation of bleeding time. Compound **5k** is currently under clinical development with the code name TAK-442.

Introduction

Thrombotic disease, such as deep vein thrombosis, pulmonary embolism, acute myocardial infarction, unstable angina, and ischemic stroke is a major cause of morbidity and mortality in developed countries.[1] Anticoagulant therapy with heparins, low molecular weight heparins (LMWH),[2] and warfarin[3] has been widely used for the treatment and prevention of thrombotic diseases. Warfarin is the only oral anticoagulant available for long-term use, but has several drawbacks including a narrow therapeutic window, large inter- and intrapatient variability, drug−drug interactions, food effects, slow onset and offset of action, and the requirement of frequent coagulation monitoring. Therefore, there is a significant unmet medical need for oral anticoagulants with a superior safety for chronic usage.[4] Factor Xa (FXa[a]) is a trypsin-like serine protease that is located at the convergence of the intrinsic and extrinsic pathways of the coagulation cascade and plays a pivotal role in blood coagulation.[5] FXa forms a prothrombinase complex with factor Va and calcium ion on a phospholipid surface, generating thrombin via proteolysis of prothrombin. Thrombin causes fibrin formation, activates platelets, and provides positive feedback to the coagulation cascade, resulting in thrombus formation. Extensive preclinical studies have shown that inactivation of FXa by highly selective inhibitors effectively inhibits a burst of thrombin generation and prevents both venous and arterial thrombosis in animals, without a significant increase in bleeding.[6] Hence, selective FXa inhibitors have been sought as new safe and efficacious anticoagulants,[7] and indeed, a wide variety of novel and selective FXa inhibitors have been reported.[8] Rivaroxaban[9] was approved for the prevention of venous thromboembolism in both the E.U. and Canada in 2008, and several orally active FXa inhibitors such as Apixaban,[10] Eribaxaban,[11] and Edoxaban[12] have been advancing in late clinical stages.

We previously reported on the discovery of the imidazo[1,5-*c*]-imidazol-3-one derivative **1** as a potent and orally active FXa inhibitor.[13] Compound **1** exhibited potent inhibitory activity against human FXa (IC_{50} = 4.8 nM) and potent anticoagulant activity (PT_2 = 1.0 μM, the concentration of compound required to double the clotting time in a human prothrombin time (PT) assay) with a favorable pharmacokinetic profile in monkeys (bioavailability: BA = 46%). However, during the course of our preclinical development program of **1**, it was found that the metabolic hydrolysis of **1** was observed on incubating with human liver microsomes (HLM) (Table 1). In a metabolic study of **1** using HLM in the absence of NADPH, the carboxylic acid **2**, which might be liberated by hydrolytic metabolism of **1**, was detected in 23.3% yield in addition to the parent compound **1** (Table 1). On the other hand, in the case of incubation with monkey liver microsomes, intact **1** remained with no detectable amount of **2** (Table 1). The fact that the stability toward metabolic hydrolysis depended on the species made human pharmacokinetics difficult to be predicted. To minimize the influence of the human specific metabolic pathway on the pharmacokinetics, we focused our research on identifying new

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[a] Abbreviations: FXa, factor Xa; HLM, human liver microsomes; LMWH, low molecular weight heparins; PT, prothrombin time; BA, bioavailability; NADPH, nicotinamide adenine dinucleotide phosphate; CYP, cytochrome P450; WSC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole hydrate; CDI, 1,1′-carbonyldiimidazole; DBU, 1,8-diazabicyclo[5.4.0]-7-undecene; t-PA, tissue plasminogen activator.

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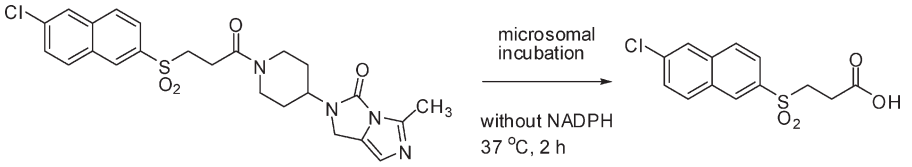
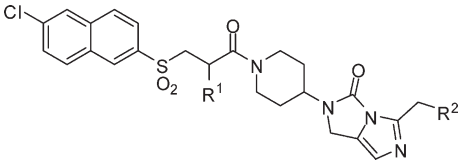
Table 1. Hydrolytic Conversion of **1** to **2** in Human or Monkey Liver Microsomes



1 **2**

liver microsomes	% of **1** remaining	% yield of **2**
human	70.5	23.3
monkey	99.9	0.0

Table 2. In Vitro Activities and Metabolic Stabilities for Compounds **1**, **3a**, **3b** and **4**



					metabolic stability, NADPH (−)
compd	R^1	R^2	human FXa IC_{50} (nM)[a]	PT_2 (μM)[b]	(% yield of acid)[c], human/monkey
1	H	H	4.8 (4.6−5.1)	1.0	23.3/0.0
3a	(*S*)-OH	H	2.1 (1.9−2.2)	0.92	4.4/0.0
3b	(*R*)-OH	H	21 (17−25)	1.9	0.6/0.0
4	H	OH	8.0 (7.5−8.6)	0.83	10.6/0.0

[a] Inhibitory activity against human FXa. Data are from duplicate experiments. IC_{50} values and 95% confidence limits are calculated from the concentration−response curves generated by logistic regression and shown in parentheses. [b] PT_2 is defined as the concentration of compound required to double the time to clot formation in the PT (prothrombin time) assay. PT_2 values shown are the mean of duplicate measurements. [c] Metabolic stability is expressed as the percent yield of the corresponding acid liberated after incubation with human or monkey liver microsomes in the absence of NADPH at 37 °C for 2 h.

FXa inhibitors with improved stability toward hydrolysis in HLM.

In the course of metabolic studies on **1**, the α-hydroxy derivatives **3a** and **3b**, and the hydroxymethyl derivative **4** were identified as active metabolites after oral administration in rats or monkeys.[14] Their FXa inhibitory activities (IC_{50} = 2.1, 21, and 8.0 nM for **3a**, **3b**, and **4**, respectively) and anticoagulant activities (PT_2 = 0.92, 1.9, and 0.83 μM for **3a**, **3b**, and **4**, respectively) are shown in Table 2. A significantly improved stability toward hydrolysis in HLM was observed for **3a** and **3b** (4.4% and 0.6% yield of the corresponding acid, respectively) compared to the parent compound **1** (23.3% yield of **2**). This result indicated that the hydroxy groups at the α-position of the amide carbonyl group in **3a** and **3b** might prevent the close approach of a nucleophile to the amide carbonyl group by serving as a substantial blocking substituent.[15] Thus, we thought that the α-hydroxy groups of **3a** and **3b** might be the key substituent for the improvement of stability toward hydrolysis. Hydroxymethyl derivative **4** was also more stable toward hydrolysis in HLM (10.6% yield of **2**) than the parent compound **1**. Since the introduction of a hydroxymethyl group into the imidazoimidazolone moiety, distant from the amide bond, led to improved stability of **4**, we postulated that the imidazoimidazolone moiety might be involved in substrate recognition of hydrolytic enzymes. Therefore, we thought that modification of the imidazoimidazolone moiety in **3a**, the eutomer of the α-hydroxy derivatives, could also improve the stability toward hydrolysis.

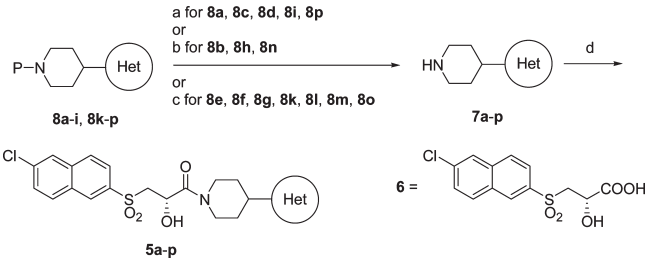
Meanwhile, our binding model for **3a** in FXa revealed that the imidazoimidazolone moiety in **3a** formed no obvious hydrogen bond but the hydrophobic interactions with the aromatic rings of Tyr99, Phe174, and Trp215 in the S4 site of FXa.[14] The imidazoimidazolone moiety in **3a** seemed to be replaceable with other bicyclic or monocyclic heterocycles that can form hydrophobic interactions with the S4 site. Thus, our synthetic efforts were focused on optimization of the imidazoimidazolone moiey in **3a** to improve the stability toward hydrolytic metabolism in HLM without the loss of FXa inhibitory activity. In this report, we describe the discovery of a highly potent and orally active FXa inhibitor **5k** (TAK-442)[16] as a clinical candidate with improved metabolic stability in HLM.

Chemistry

The synthesis of compounds **5a**−**p** is outlined in Scheme 1. Compounds **5a**−**p** were prepared by the condensation of carboxylic acid **6**[14] with piperidine derivatives **7a**−**p** using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC) and 1-hydroxybenzotriazole hydrate (HOBt), without deterioration of enantiomeric purity. Piperidines **7a**−**i** and **7k**−**p** were synthesized via deprotection of protected piperidines **8a**−**i** and **8k**−**p** under the appropriate conditions, while **7j** was commercially available.

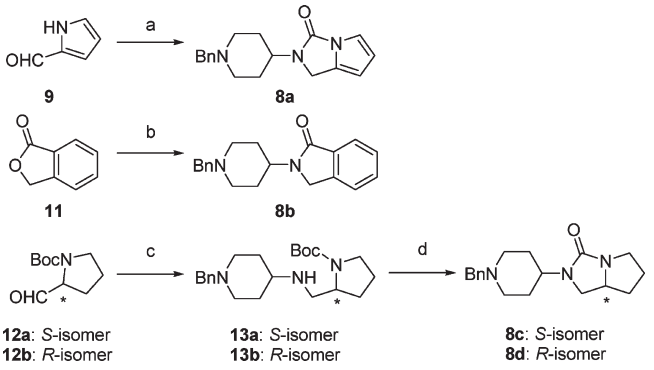
The piperidine derivatives bearing a bicyclic heterocycle **8a**−**d** were synthesized as shown in Scheme 2. The reductive amination of aldehyde **9** with 4-amino-1-benzylpiperidine **10** followed by cyclization using 1,1′-carbonyldiimidazole (CDI) and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) gave **8a**. The isoindolin-1-one derivative **8b** was synthesized by condensation of isobenzofuran-1(3*H*)-one **11** with **10**. Reductive amination of commercially available aldehydes **12a** and **12b** with **10** gave amines **13a** and **13b**, respectively. Trifluoroacetic acid

Scheme 1. Synthesis of Compounds **5a**−**p**[a]



[a] Reagents and conditions: (a) $Pd(OH)_2/C$, H_2, MeOH, room temp., 67%−quant.; (b) Pd/C, HCO_2NH_4, MeOH, reflux, 90%−quant.; (c) Pd/C, H_2, MeOH, room temp., quant.; (d) **6**, WSC, HOBt, DMF, room temp., 9−54%.

Scheme 2. Synthesis of Piperidine Derivatives **8a**−**d**[a]



[a] Reagents and conditions: (a) (1) 4-amino-1-benzylpiperidine **10**, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 0 °C to room temp., (2) CDI, DBU, CH_3CN, reflux, 29% for two steps; (b) **10**, neat, 200 °C, 49%; (c) **10**, $NaBH_3CN$, AcOH, MeOH, room temp., 86−89%; (d) (1) TFA, CH_2Cl_2, room temp., (2) CDI, DBU, CH_3CN, room temp., 88−89% for two steps.

mediated removal of the Boc group in **13a** and **13b**, followed by cyclization using CDI, afforded **8c** and **8d**, respectively.

The synthesis of piperidines having a varied monocyclic ring is depicted in Scheme 3. Reductive amination of the commercially available piperidone **14** with amines **15a** and **15b** provided compounds **16a** and **16b**, respectively. Cleavage of the Boc group in **16a** and **16b**, using 4 M HCl in EtOAc, followed by cyclization using CDI and DBU, afforded **8e** and **8k**, respectively. Oxazolidinone **8g** and oxazinanone **8l** were prepared by a similar method using **17a** and **17b** as the amines for the reductive amination. Acylation of the secondary amino group in **18a** with chloroacetyl chloride and subsequent intramolecular cyclization using sodium hydride gave the morpholinone **8m**. The piperidine derivatives **8h**, **8i**, **8n**, and **8p** were prepared by acylation or sulfonylation of the primary amino group of piperidine **10** with the corresponding acid chlorides in the presence of pyridine, followed by intramolecular alkylation in the presence of a base.

Scheme 4 illustrates the synthesis of the imidazolone analogue **8f** and the tetrahydropyrazinone analogue **8o**. Nucleophilic addition of 2-aminoacetaldehyde dimethyl acetal to isocyanate **19** gave **20**, which was subsequently treated with aqueous HCl afforded **8f**. Boc-protected hydrazine **21**, which was synthesized from **14** and *tert*-butyl carbazate by reductive amination, was converted into **22** by alkylation and acylation, and successive removal of the Boc group in **22** furnished **8o**. Compound **23** was synthesized by condensation of **2** with piperidine **7k** using WSC and HOBt, as shown in Scheme 5.

Results and Discussion

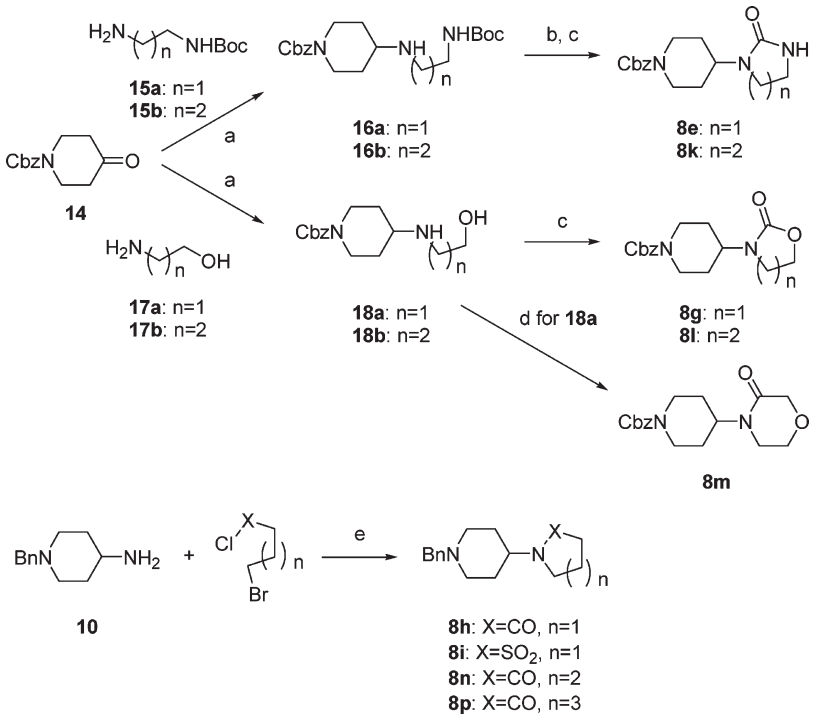
The compounds thus synthesized were evaluated for their in vitro inhibitory activity against human FXa, expressed as IC_{50} values, and for their activity in the prolongation of human PT, expressed as PT_2. To estimate their stability toward metabolic hydrolysis, the yield of acid **6** was determined after incubation with human or monkey liver microsomes in the absence of NADPH at 37 °C for 2 h. The oxidative metabolic stability of compounds was determined after incubation with human or monkey liver microsomes in the presence of NADPH at 37 °C for 20 min, and is expressed as the percent elimination. The log *D* values were determined at pH 7.4 by the reported method.[17]

Replacement of the imidazoimidazolone moiety in **3a** with the other heterocycles resulted in improved hydrolytic stability in HLM, and no detectable amount of the carboxylic acid **6** was observed after incubation with HLM in the absence of NADPH, except **5i** (Tables 3−5). Minor structural changes, such as changing one nitrogen atom to a carbon atom and removing one methyl group from **3a**, made a remarkable change in stability. These observations indicated that the imidazoimidazolone moiety in **3a** might be specifically recognized by the metabolizing enzyme(s). In contrast, log *D* values seemed not to affect the stability toward hydrolysis in HLM, at least for log *D* values in the range 1.56−2.60.

The in vitro activities of compounds **5a**−**d** bearing various bicyclic rings in place of the imidazoimidazolone ring of **3a** were investigated, and the results are shown in Table 3. Pyrrolo[1,2-*c*]imidazol-3-one derivative **5a** was evaluated to examine the effect of the nitrogen atom at the 6-position of the imidazoimidazolone ring in **3a** on potent FXa inhibitory activity. Compound **5a** (IC_{50} = 1.7 nM) was found to exhibit comparable FXa inhibitory activity to that of **3a** (IC_{50} = 2.1 nM). Replacement of the pyrrole ring in **5a** with a phenyl ring resulted in **5b** (IC_{50} = 2.3 nM) that has an equipotent FXa inhibitory activity to **5a**. These results supported our hypothesis that the imidazole ring in **3a** was not essential for potent FXa inhibitory activity and could be replaced. The saturated bicyclic analogues **5c** and **5d** (IC_{50} = 24, 51 nM, respectively) showed more than 10× lower FXa inhibitory activities compared to the aromatized analogue **5a**. These bicyclic imidazolones **5a**−**d** have less potent anticoagulant activity than **3a**.

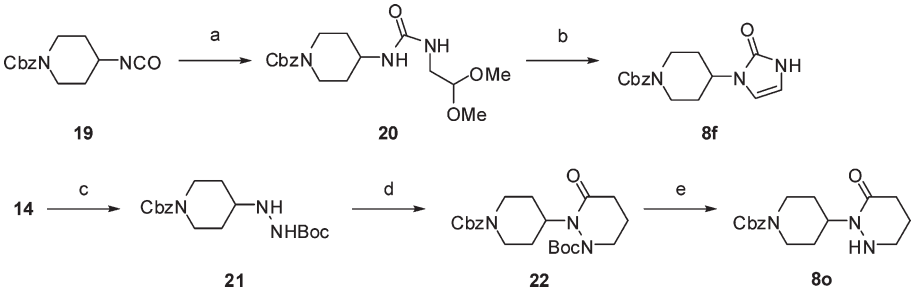
Furthermore, compounds **5a**−**d** showed poor stability toward oxidative metabolism in human or monkey liver microsomes compared to **3a** (Table 3). Since this metabolic instability seemed to be derived from the bicyclic imidazolone moieties, we decided to remove the methylimidazole part in **3a**. As can be seen from Table 3, imidazolidinone analogue **5e** exhibited improved stability toward oxidative metabolism and moderate FXa inhibitory activity with excellent resistance against metabolic hydrolysis. Interestingly, imidazolidinone derivative **5e** has more potent anticoagulant activity (PT_2 = 1.1 μM) than **5a**−**d** (PT_2 = 1.7−2.7 μM) in spite of its moderate FXa inhibitory activity (IC_{50} = 23 nM). These results suggested that improvement in the anticoagulant activity of **5e** might be attributed to its lower lipophilicity (log *D* = 1.73 and 2.16−2.60 for **5e** and **5a**−**d**, respectively). It has been reported that high lipophilicity is likely to result in high plasma protein binding; therefore, the FXa inhibitory activities of the less lipophilic compounds are more effectively translated into in vitro anticoagulant activities.[18] Thus, we chose to investigate the monocyclic analogue of **5e** to enhance the FXa inhibitory

Scheme 3. Synthesis of Piperidines Bearing a Monocyclic Ring **8e**, **8g**−**i**, **8k**−**n** and **8p**[a]



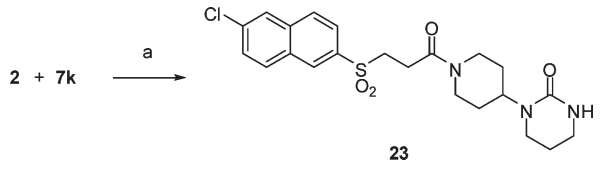
[a] Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane, 0 °C to room temp., quant.; (b) 4 M HCl/EtOAc, EtOAc, room temp., 85−91%; (c) CDI, DBU, CH_3CN, room temp., 83%−quant.; (d) (1) chloroacetyl chloride, Et_3N, THF, 0 °C, (2) NaH, DMF, 0 °C to room temp., and then 80 °C, 44% for two steps; (e) (1) pyridine, CH_2Cl_2, 0 °C to room temp., (2) NaH, THF, room temp., and then reflux, 33−80% for two steps.

Scheme 4. Synthesis of Piperidines Bearing a Monocyclic Ring **8f** and **8o**[a]



[a] Reagents and conditions: (a) $H_2NCH_2CH(OMe)_2$, CH_3CN, room temp., quant.; (b) aq. HCl, MeOH−H_2O, room temp., 90%. (c) $H_2NNHBoc$, $NaBH_3CN$, AcOH, MeOH, 0 °C to room temp., quant.; (d) (1) $Br(CH_2)_3COCl$, Et_3N, THF, 0 °C, (2) NaH, THF, room temp., quant. for two steps; (e) TFA, CH_2Cl_2, room temp., quant.

Scheme 5. Synthesis of Compound **23**[a]



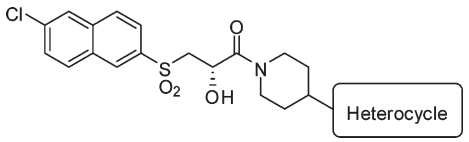
[a] Reagents and conditions: (a) WSC, HOBt, Et_3N, DMF, room temp., 76%.

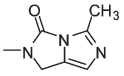
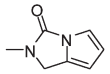
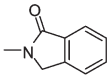
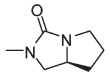
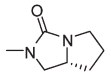
and anticoagulant activities while maintaining good metabolic stability.

The assay results for five-membered monocyclic analogues **5f**−**j** are shown in Table 4. Introduction of a double bond into the dihydroimidazolone ring in **5e** resulted in a slight decrease in FXa inhibitory activity (**5f**, IC_{50} = 46 nM). Replacement of a nitrogen atom in **5e** with an oxygen atom also slightly reduced the activity (**5g**, IC_{50} = 60 nM). Pyrrolidinone **5h** (IC_{50} = 14 nM, PT_2 = 0.97 μM) exhibited comparable FXa inhibitory and anticoagulant activities to **5e** (IC_{50} = 23 nM, PT_2 = 1.1 μM), while the FXa inhibitory activities of cyclic sulfonamide analogue **5i** (IC_{50} = 198 nM) and pyrroridine analogue **5j** (IC_{50} = 147 nM) were significantly decreased. These results indicated that the carbonyl group in the terminal ring is important for FXa inhibitory activity by bringing the terminal ring into a perpendicular arrangement to the inner piperidine ring to make a favorable hydrophobic interaction with the S4 site.

To increase the FXa inhibitory and anticoagulant activities, we investigated six- and seven-membered heterocycles bearing a carbonyl group, which were designed to increase hydrophobic interactions with the S4 site by ring expansion (Table 5). Among the compounds synthesized, tetrahydropyrimidinone **5k** and morpholinone **5m** displayed the most potent FXa inhibitory and anticoagulant activities (IC_{50} = 3.5 and 3.6 nM, PT_2 = 0.58 and 0.74 μM, respectively). One atom

Table 3. In Vitro Activities for Compounds **3a** and **5a**−**e**



compd	Heterocycle	human FXa IC_{50} (nM)[a]	human PT PT_2 (μM)[b]	metabolic stability NADPH (−) (% yield of acid)[c] human/monkey	metabolic stability NADPH (+) (% elimination)[d] human/monkey	log D[e]
3a		2.1 (1.9–2.2)	0.92	4.4/0.0	14.4/25.6	2.13
5a		1.7 (1.4–1.9)	1.7	0.0/0.0	44.8/56.8	2.60
5b		2.3 (2.1–2.5)	2.0	0.0/0.0	22.8/38.0	2.58
5c		24 (22–27)	1.7	0.0/0.0	65.2/85.2	2.16
5d		51 (43–59)	2.7	0.0/0.0	26.0/48.8	2.16
5e		23 (21–25)	1.1	0.0/0.0	3.2/8.8	1.73

[a,b,c] Refers to Table 2. [d] Metabolic stability is expressed as the percent elimination of compounds after incubation with human or monkey liver microsomes in the presence of NADPH at 37 °C for 20 min. [e] Measured at pH 7.4.

exchange in the tetrahydropyrimidinone ring in **5k** has an apparent effect on activity. For example, 1,3-oxazinanone **5l**, piperidinone **5n**, and tetrahydropyridazinone **5o** showed decreased FXa inhibitory and anticoagulant activities (IC_{50} = 20, 9.1, and 49 nM, PT_2 = 1.1, 0.81, and 2.5 μM, respectively). Extension of the ring size also resulted in reduced activities (**5p**, IC_{50} = 28 nM, PT_2 = 1.5 μM). These results suggested that among the five- to seven-membered rings a six-membered ring might be the most favorable size for hydrophobic interactions with the aromatic rings in the S4 site.

Regarding the stability toward oxidative metabolism, the monocyclic derivatives **5e**−**g** and **5k**−**m** showed improved stability in HLM and monkey liver microsomes relative to bicyclic derivatives **3a** and **5a**−**d**. Introduction of a heteroatom into the rings seems to increase the stability, and the compounds with lower log D values tended to be more stable.

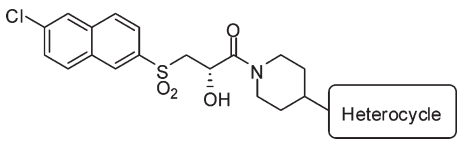
To confirm the impact of the presence of the hydroxy group in **5k** on stability toward hydrolysis in HLM, the stability of **23** without the hydroxy group was examined (Table 6). Compound **23** was less stable compared to **5k**, and this result shows that the hydroxy group at the α-position of the amide carbonyl is also important for suppression of metabolic hydrolysis in HLM. In addition, the hydroxy group of **5k** also had an incremental effect on FXa inhibitory activity, as observed for **3a** vs **1** (IC_{50} = 3.5, 11, 2.1, and 4.8 nM for **5k**, **23**, **3a**, and **1**, respectively), which was explained by the X-ray structural analysis described below.

Considering their excellent anticoagulant activity and good metabolic stability in HLM, **5k** and **5m** were selected for further evaluation. To estimate their oral bioavailability in monkeys, cassette dosing experiments were performed on **5k** and **5m**, and their pharmacokinetic parameters are shown in Table 7. Tetrahydropyrimidinone derivative **5k** showed better oral bioavailability and longer duration at a dose of 1 mg/kg (AUC = 760 ng·h/mL, BA = 52.5%, MRT_{po} = 6.96 h) than **5m** (AUC = 335 ng·h/mL, BA = 33.2%, MRT_{po} = 5.58 h). The better stability of **5k** toward oxidative metabolism in monkey liver microsomes compared to **5m** is reflected in its better pharmacokinetic profile in monkey. Futhermore, **5k** showed better metabolic stability toward oxidative metabolism in HLM than in monkey liver microsomes. Therefore, we chose **5k** as a clinical candidate, which is expected to avoid the potential concern of metabolic hydrolysis in humans and to exhibit a favorable pharmacokinetic profile in humans comparable to that found in monkeys.

Since selectivity over other trypsin-like proteases is an important consideration for the development of FXa inhibitors,[19] the selectivity profile of **5k** was evaluated against other human serine proteases such as thrombin, factor IXa, kallikrein, t-PA, trypsin, activated protein C, and plasmin (Table 8). Compound **5k** displayed good selectivity for FXa over these serine proteases (more than 500-fold).

Finally, the in vivo antithrombotic efficacy of compound **5k** was evaluated in a rabbit venous thrombosis model.[20] It was found that compound **5k** at 50 and 100 μg/kg, i.v. (given as a bolus [1/2 of total dose] followed by a constant infusion for 1 h) significantly inhibited venous thrombus formation by 50% and 81%, respectively, and did not affect the bleeding

Table 4. In Vitro Activities for Compounds **5e**−**j**



compd	Heterocycle	human FXa IC_{50} (nM)[a]	human PT PT_2 (μM)[b]	metabolic stability NADPH (−) (% yield of acid)[c] human/monkey	metabolic stability NADPH (+) (% elimination)[d] human/monkey	log D[e]
5e		23 (21–25)	1.1	0.0/0.0	3.2/8.8	1.73
5f		46 (42–50)	2.3	0.0/0.0	3.6/7.2	1.79
5g		60 (57–63)	1.9	0.0/0.0	7.6/12.4	1.75
5h		14 (13–15)	0.97	0.0/0.0	8.0/33.2	1.96
5i		198 (178–220)	5.0	1.8/0.0	38.0/73.6	1.69
5j		147 (133–163)	1.7	0.0/0.0	2.0/20.8	1.56

[a,b,c,d,e] Refers to Table 2 and Table 3.

time even at a higher dose of 500 μg/kg, i.v. Detailed pharmacological evaluation of **5k** will be reported in due course.

On the basis of its potent inhibitory activity against FXa, potent anticoagulant activity, improved stability toward metabolic hydrolysis in HLM, good selectivity over other human serine proteases, favorable pharmacokinetic profile in monkeys, and in vivo antithrombotic efficacy without prolongation of bleeding time in rabbit model, compound **5k** was selected as a development candidate and is currently undergoing phase II clinical trials.

X-ray Crystallography of Compound 5k

The X-ray structure of compound **5k** in complex with human FXa was obtained at a very high resolution of 1.45 Å (Figure 1). The 6-chloronaphthyl group occupies the S1 substrate binding site and points into the site where it makes a hydrophobic interaction with the aromatic ring of Tyr228. A hydrogen-bonding interaction between the oxygen atom of the amide carbonyl in **5k** and the backbone amide nitrogen of Gly219 is observed. The hydroxy group at the α-position of the amide carbonyl forms a hydrogen bond with the nitrogen atom of the main chain Gly216. This hydrogen-bonding interaction could account for the increase in FXa inhibitory activity of **5k** compared to compound **23**. The tetrahydropyrimidinone moiety is located in the S4 site making hydrophobic interactions with the aromatic rings of Tyr99, Phe174, and Trp215. The carbonyl oxygen of the tetrahydropyrimidinone moiety makes a water-mediated interaction with the backbone carbonyl oxygen of Lys96, and brings it into a perpendicular arrangement to the inner piperidine ring. The sulfone oxygen is involved in a hydrogen bond with the Gln192 main chain nitrogen. Overall, compound **5k** fits into the FXa active site in a highly complementary manner.

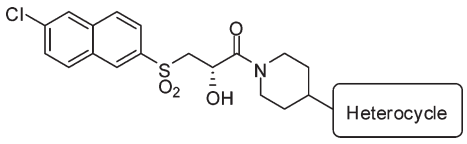
Conclusion

To address the vulnerability of our FXa inhibitor **1** toward metabolic hydrolysis in HLM, chemical modification of the imidazoimidazolone moiety in active metabolite **3a**, which showed improved stability toward hydrolysis, was conducted. Replacement of the imidazoimidazolone ring in **3a** with other bicyclic and monocyclic rings resulted in the discovery of tetrahydropyrimidinone derivative **5k** as a potent and selective FXa inhibitor. Compound **5k** showed no detectable formation of carboxylic acid **6** in HLM and exhibited a favorable pharmacokinetic profile in monkeys and potent antithrombotic efficacy in a rabbit model without prolongation of bleeding time. Compound **5k** was selected and is under clinical trials.

Experimental Section

Melting points were determined with a Yanagimoto melting point apparatus or a Büchi melting point apparatus B-545 and are uncorrected. ^{1}H NMR spectra were obtained at 200 or 300 MHz on a Varian Gemini-200 or a Varian Ultra-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; brs, broad singlet; m, multiplet. Specific rotations were measured with a JASCO DIP-370 or a JASCO P-1030 digital polarimeter. Elemental analyses were carried out by Takeda Analytical Laboratories Ltd. Reactions were followed by TLC on Silica gel 60 F 254 precoated TLC plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.).

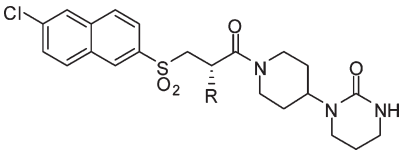
Table 5. In Vitro Activities for Compounds **5k−p**



compd	Heterocycle	human FXa IC_{50} (nM)[a]	human PT PT_2 (μM)[b]	metabolic stability NADPH (−) (% yield of acid)[c] human/monkey	metabolic stability NADPH (+) (% elimination)[d] human/monkey	log D^e
5k (TAK-442)		3.5 (3.3–3.7)	0.58	0.0/0.0	6.4/16.4	1.81
5l		20 (18–21)	1.1	0.0/0.0	2.0/8.8	1.67
5m		3.6 (3.3–3.9)	0.74	0.0/0.0	11.2/24.8	1.85
5n		9.1 (7.9–10)	0.81	0.0/0.0	31.6/77.2	2.19
5o		49 (46–51)	2.5	0.0/0.0	18.8/39.2	1.85
5p		28 (26–30)	1.5	0.0/0.0	91.2/100	2.52

[a,b,c,d,e] Refers to Table 2 and Table 3.

Table 6. Effect of the Hydroxy Group in **5k** on Activity and Hydrolytic Stability in HLM



compd	R	human FXa IC_{50} (nM)[a]	human PT PT_2 (μM)[b]	metabolic stability, NADPH (−) (% yield of acid)[c], human/monkey
5k	OH	3.5 (3.3−3.7)	0.58	0.0/0.0
23	H	11 (10−12)	1.4	4.6/0.0

[a,b,c] Refers to Table 2.

Table 7. Pharmacokinetic Profiles in Monkeys for **5k** and **5m** (Cassette Dosing, $n = 3$)

	0.1 mg/kg, i.v.		1.0 mg/kg, p.o.		
compd	$V_{d(ss)}$ (mL/kg)	CL_{total} (mL/h/kg)	AUC_{po} (ng·h/mL)	MRT_{po} (h)	BA(%)
5k	579 ± 174	708 ± 289	760 ± 220	6.96 ± 1.21	52.5 ± 19.1
5m	687 ± 18	1001 ± 215	335 ± 42.2	5.58 ± 1.12	33.2 ± 6.6

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. Yields are unoptimized. Chemical intermediates were characterized by ^{1}H NMR. The purities of all compounds tested in biological systems were assessed as being >95% using analytical HPLC. The HPLC analyses were performed using a Shimadzu UFLC instrument. Elution was done with a gradient of 5−90% solvent B in solvent A (solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in acetonitrile) through a L-column 2 ODS (3.0 × 50 mm, 2 μm) column at 1.2 mL min^{-1}. Area % purity was measured at 254 nm.

1-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)tetrahydropyrimidin-2(1*H*)-one (5k). To a solution of **6** (0.53 g, 1.70 mmol), **7k** (0.31 g, 1.70 mmol), and HOBt (0.26 g, 1.70 mmol) in DMF (10 mL) was added WSC

Table 8. Inhibitory Activity of Compound **5k** for Human FXa, Thrombin, Factor IXa, Kallikrein, t-PA, Trypsin, Activated Protein C, and Plasmin

enzymes	IC_{50} values (nM)[a]	enzymes	IC_{50} values (nM)[a]
FXa	2.2 (2.0−2.4)	t-PA	44 000 (38 000−52000)
Thrombin	1200 (1100−1300)	Trypsin	> 60 000
Factor IXa	4500 (3700−5400)	Activated protein C	> 60 000
Kallikrein	12000 (11000−13000)	Plasmin	> 60 000

[a] Data were from duplicate experiments. The 95% confidence intervals are shown in parentheses. See Experimental Section.

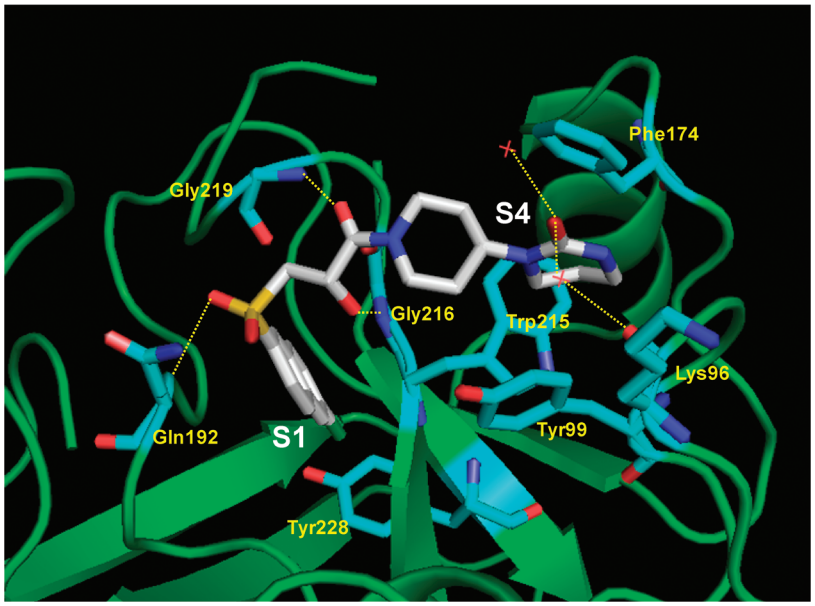


Figure 1. X-ray structure of compound **5k** (white carbons) in complex with human FXa. The amino acids (blue carbons, yellow text) and binding sites (white text) are indicated. The hydrogen bonds are shown as yellow dotted lines, where water molecules are shown as red cross marks.

(0.32 g, 1.70 mmol). The mixture was stirred at room temperature for 15 h and concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and an aqueous solution of $NaHCO_3$. The organic layer was separated, washed with water, 5% aqueous solution of citric acid, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc to EtOAc/MeOH = 10:1). The product was recrystallized from EtOAc to give **5k** (0.12 g, 15%) as colorless crystals, mp 173−174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.57−1.78 (4H, m), 1.90−1.93 (2H, m), 2.70−2.76 (1H, m), 3.11−3.20 (3H, m), 3.27−3.30 (2H, m), 3.40−3.48 (2H, m), 3.78−3.90 (1H, m), 3.94−3.99 (1H, m), 4.56−4.66 (3H, m), 4.97−5.03 (1H, m), 7.59 (1H, dd, J = 1.8, 8.7 Hz), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{22}H_{26}ClN_3O_5S$: C, 55.05; H, 5.46; N, 8.75. Found: C, 54.96; H, 5.57; N, 8.80. $[\alpha]^{25}_D$ +21.6 (c 1.0875, $CHCl_3$).

2-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*c*]imidazol-3-one (5a). Compound **5a** was prepared in a manner similar to that described for **5k** in 48% yield as a colorless powder, mp 195−196 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.60−1.90 (2H, m), 1.90−2.09 (2H, m), 2.66−2.95 (1H, m), 3.11−3.36 (1H, m), 3.38−3.54 (2H, m), 3.62−3.86 (1H, m), 3.98−4.17 (1H, m), 4.20−4.36 (3H, m), 4.61−4.79 (1H, m), 4.93−5.15 (1H, m), 6.06 (1H, d, J = 1.7 Hz), 6.39 (1H, t, J = 3.1 Hz), 7.05−7.16 (1H, m), 7.60 (1H, dd, J = 2.1, 8.7 Hz), 7.87−8.04 (4H, m), 8.52 (1H, s). Anal. Calcd for $C_{24}H_{24}ClN_3O_5S$: C, 57.42; H, 4.82; N, 8.37. Found: C, 57.41; H, 4.79; N, 8.44. $[\alpha]^{25}_D$ +20.8 (c 0.25, $CHCl_3$).

2-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)isoindolin-1-one (5b). Compound **5b** was prepared in a manner similar to that described for **5k** in 25% yield as a colorless powder, mp 200−201 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.68−1.87 (3H, m), 1.88−2.05 (2H, m), 2.77−2.90 (1H, m), 3.22−3.35 (1H, m), 3.44−3.51 (2H, m), 4.05−4.10 (1H, m), 4.33−4.36 (2H, m), 4.52−4.60 (1H, m), 4.68−4.76 (1H, m), 5.00−5.08 (1H, m), 7.46−7.61 (4H, m), 7.86 (1H, d, J = 7.4 Hz), 7.95−7.98 (4H, m), 8.52 (1H, s). Anal. Calcd for $C_{26}H_{25}ClN_2O_5S \cdot H_2O$: C, 58.81; H, 5.12; N, 5.28. Found: C, 58.74; H, 5.17; N, 5.24. $[\alpha]^{25}_D$ +18.6 (c 0.25, $CHCl_3$).

(7a*S*)-2-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)hexahydro-3*H*-pyrrolo[1,2-*c*]imidazol-3-one (5c). Compound **5c** was prepared in a manner similar to that described for **5k** in 39% yield as a colorless powder, mp 154−155 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.18−1.39 (1H, m), 1.49−1.65 (2H, m), 1.78−2.02 (5H, m), 2.63−2.81 (1H, m), 3.00−3.17 (3H, m), 3.39−3.54 (3H, m), 3.59−3.85 (3H, m), 3.88−4.15 (2H, m), 4.55−4.70 (1H, m), 4.90−5.05 (1H, m), 7.59 (1H, dd, J = 2.2, 8.8 Hz), 7.94 (3H, s), 7.96 (1H, d, J = 8.8 Hz), 8.52 (1H, s). Anal. Calcd for $C_{24}H_{28}ClN_3O_5S$: C, 56.97; H, 5.58; N, 8.30. Found: C, 56.91; H, 5.55; N, 8.43. $[\alpha]^{25}_D$ −45.1 (c 0.25, MeOH).

(7a*R*)-2-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)hexahydro-3*H*-pyrrolo[1,2-*c*]imidazol-3-one (5d). Compound **5d** was prepared in a manner similar to that described for **5k** in 52% yield as a colorless powder, mp 166−167 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.22−1.38 (1H, m), 1.48−1.62 (2H, m), 1.71−1.99 (5H, m), 2.66−2.80 (1H, m), 3.03−3.24 (3H, m), 3.36−3.52 (3H, m), 3.61−3.69 (2H, m), 3.72−3.86 (1H, m), 3.96−4.11 (2H, m), 4.61−4.65 (1H, m), 4.95−5.05 (1H, m), 7.59 (1H, dd, J = 2.4, 8.4 Hz), 7.94 (3H, s), 7.96 (1H, d, J = 8.4 Hz), 8.52 (1H, s). Anal. Calcd for $C_{24}H_{28}ClN_3O_5S$: C, 56.97; H, 5.58; N, 8.30. Found: C, 56.94; H, 5.54; N, 8.45. $[\alpha]^{25}_D$ +48.3 (c 0.25, MeOH).

1-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)imidazolidin-2-one (5e). Compound **5e** was prepared in a manner similar to that described for **5k** in 46% yield as a colorless powder, mp 170−171 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.55−1.64 (2H, m), 1.77−1.85 (2H, m), 2.66−2.77 (1H, m), 3.11−3.24 (1H, m), 3.37−3.45 (6H, m), 3.75−3.88 (1H, m), 3.97−4.02 (2H, m), 4.40 (1H, s), 4.61−4.66

(1H, m), 4.98−5.03 (1H, m), 7.59 (1H, dd, J = 2.3, 8.7 Hz), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{21}H_{24}ClN_3O_5S$: C, 54.13; H, 5.19; N, 9.02. Found: C, 54.06; H, 5.16; N, 8.95. $[\alpha]^{25}_D$ +7.3 (c 0.25, MeOH).

1-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)-1,3-dihydro-2*H*-imidazol-2-one (5f). Compound **5f** was prepared in a manner similar to that described for **5k** in 10% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62−1.78 (2H, m), 2.05−2.34 (4H, m), 2.69−2.85 (1H, m), 3.16−3.30 (1H, m), 3.42−3.56 (2H, m), 4.09−4.14 (1H, m), 4.20−4.28 (1H, m), 4.63−4.70 (1H, m), 5.00−5.06 (1H, m), 6.22 (1H, dd, J = 3.0, 12.4 Hz), 6.29−6.30 (1H, m), 7.60 (1H, dd, J = 1.9, 8.7 Hz), 7.95−7.98 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{21}H_{22}ClN_3O_5S\cdot0.5H_2O$: C, 53.33; H, 4.90; N, 8.88. Found: C, 53.56; H, 4.97; N, 8.62. $[\alpha]^{25}_D$ +8.9 (c 0.25, MeOH).

3-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)-1,3-oxazolidin-2-one (5g). Compound **5g** was prepared in a manner similar to that described for **5k** in 41% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.52−1.95 (4H, m), 2.67−2.82 (1H, m), 3.13−3.26 (1H, m), 3.41−3.56 (4H, m), 3.68−3.84 (1H, m), 3.95−4.03 (2H, m), 4.36 (2H, t, J = 8.0 Hz), 4.62−4.67 (1H, m), 4.96−5.06 (1H, m), 7.60 (1H, dd, J = 2.1, 8.9 Hz), 7.91−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{21}H_{23}ClN_2O_6S\cdot0.5H_2O$: C, 52.99; H, 5.08; N, 5.89. Found: C, 52.91; H, 5.06; N, 5.91. $[\alpha]^{25}_D$ +5.5 (c 0.25, MeOH).

1-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)pyrrolidin-2-one (5h). Compound **5h** was prepared in a manner similar to that described for **5k** in 41% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62−1.87 (3H, m), 2.01−2.08 (3H, m), 2.39−2.44 (2H, m), 2.66−2.81 (1H, m), 3.13−3.48 (5H, m), 3.75−4.24 (3H, m), 4.61−4.65 (1H, m), 5.02−5.03 (1H, m), 7.59 (1H, dd, J = 2.1, 8.7 Hz), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{22}H_{25}ClN_2O_5S\cdot0.5H_2O$: C, 55.75; H, 5.53; N, 5.91. Found: C, 55.63; H, 5.74; N, 5.71. $[\alpha]^{25}_D$ +7.1 (c 0.25, MeOH).

(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-1-[4-(1,1-dioxidoisothiazolidin-2-yl)piperidin-1-yl]-1-oxopropan-2-ol (5i). Compound **5i** was prepared in a manner similar to that described for **5k** in 51% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.63−1.84 (2H, m), 1.97−2.08 (2H, m), 2.32−2.42 (2H, m), 2.72−2.94 (1H, m), 3.14−3.30 (5H, m), 3.40−3.46 (2H, m), 3.64−3.81 (2H, m), 3.93−4.02 (1H, m), 4.46−4.58 (1H, m), 4.97−5.02 (1H, m), 7.60 (1H, dd, J = 2.1, 8.9 Hz), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{21}H_{25}ClN_2O_6S_2$: C, 50.34; H, 5.03; N, 5.59. Found: C, 50.02; H, 5.04; N, 5.42. $[\alpha]^{25}_D$ +2.7 (c 0.25, MeOH).

(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-1-oxo-1-[4-(pyrrolidin-1-yl)piperidin-1-yl]propan-2-ol (5j). Compound **5j** was prepared in a manner similar to that described for **5k** in 51% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46−2.05 (8H, m), 2.25−2.31 (1H, m), 2.57(4H, brs), 2.84−3.44 (4H, m), 3.82−3.89 (2H, m), 4.23−4.39 (1H, m), 4.99−5.05 (1H, m), 7.58 (1H, dd, J = 1.9, 8.7 Hz), 7.91−7.97 (4H, m), 8.52 (1H, s). Anal. Calcd for $C_{22}H_{27}ClN_2O_4S\cdot0.5H_2O$: C, 57.44; H, 6.14; N, 6.09. Found: C, 57.35; H, 6.09; N, 5.94. $[\alpha]^{25}_D$ +3.0 (c 0.25, MeOH).

3-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)-1,3-oxazinan-2-one (5l). Compound **5l** was prepared in a manner similar to that described for **5k** in 41% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.65−2.08 (6H, m), 2.66−2.80 (1H, m), 3.17−3.25 (3H, m), 3.40−3.48 (2H, m), 3.68−3.84 (1H, m), 4.00−4.04 (1H, m), 4.25 (2H, t, J = 5.2 Hz), 4.38−4.45 (1H, m), 4.62−4.70 (1H, m), 4.95−5.05 (1H, m), 7.59 (1H, dd, J = 2.1, 8.7 Hz), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{22}H_{25}ClN_2O_6S\cdot0.5H_2O$: C, 53.93; H, 5.35; N, 5.72. Found: C, 54.23; H, 5.36; N, 5.50. $[\alpha]^{25}_D$ +9.6 (c 0.25, MeOH).

4-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)morpholin-3-one (5m). Compound **5m** was prepared in a manner similar to that described for **5k** in 42% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.60−1.82 (4H, m), 2.69−2.83 (1H, m), 3.15−3.29 (3H, m), 3.41−3.48 (2H, m), 3.67−3.90 (3H, m), 4.02−4.05 (1H, m), 4.20 (2H, s), 4.62−4.80 (2H, m), 5.02 (1H, m), 7.58−7.61 (1H, m), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{22}H_{25}ClN_2O_6S\cdot0.5H_2O$: C, 53.93; H, 5.35; N, 5.72. Found: C, 53.82; H, 5.22; N, 5.52. $[\alpha]^{25}_D$ +11.9 (c 0.25, MeOH).

1′-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}-1,4′-bipiperidin-2-one (5n). Compound **5n** was prepared in a manner similar to that described for **5k** in 54% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62−1.79 (9H, m), 2.41−2.45 (2H, m), 2.68−2.82 (1H, m), 3.14−3.26 (3H, m), 3.40−3.48 (2H, m), 3.97−4.01 (1H, m), 4.60−4.68 (1H, m), 4.80−4.84 (1H, m), 4.96−5.04 (1H, m), 7.59 (1H, dd, J = 2.1, 8.7 Hz), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{23}H_{27}ClN_2O_5S\cdot0.2CH_2Cl_2$: C, 54.21; H, 5.77; N, 5.45. Found: C, 54.44; H, 5.56; N, 5.17. $[\alpha]^{25}_D$ +7.7 (c 0.25, MeOH).

2-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)tetrahydropyridazin-3(2*H*)-one (5o). Compound **5o** was prepared in a manner similar to that described for **5k** in 9% yield as a colorless amorphous powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.63−2.07 (6H, m), 2.42−3.24 (6H, m), 3.26−4.04 (5H, m), 4.50−4.76 (2H, m), 5.01 (1H, s), 7.59 (1H, dd, J = 2.0, 8.8 Hz), 7.86−8.05 (4H, m), 8.52 (1H, d, J = 3.0 Hz). Anal. Calcd for $C_{22}H_{26}ClN_3O_5S$: C, 55.05; H, 5.46; N, 8.75. Found: C, 54.91; H, 5.28; N, 8.45. $[\alpha]^{25}_D$ +9.2 (c 0.25, MeOH).

1-(1-{(2*S*)-3-[(6-Chloronaphthalen-2-yl)sulfonyl]-2-hydroxypropanoyl}piperidin-4-yl)azepan-2-one (5p). Compound **5p** was prepared in a manner similar to that described for **5k** in 27% yield as a colorless powder, mp 152−153 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.50−1.71 (10H, m), 2.55−2.57 (2H, m), 2.67−2.81 (1H, m), 3.12−3.24 (3H, m), 3.39−3.46 (2H, m), 3.71−3.87 (1H, m), 3.95−4.00 (1H, m), 4.58−4.66 (1H, m), 4.75−4.83 (1H, m), 5.02 (1H, m), 7.59 (1H, dd, J = 2.3, 8.7 Hz), 7.94−7.97 (4H, m), 8.51 (1H, s). Anal. Calcd for $C_{24}H_{29}ClN_2O_5S$: C, 58.47; H, 5.93; N, 5.68. Found: C, 58.24; H, 5.75; N, 5.56. $[\alpha]^{25}_D$ +6.5 (c 0.25, MeOH).

2-(Piperidin-4-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*c*]imidazol-3-one (7a). A mixture of **8a** (0.90 g, 3.0 mmol) and $Pd(OH)_2$ (50% wet, 0.20 g) and MeOH (50 mL) was stirred at room temperature under H_2 atmosphere (1 atm) for 15 h, followed by filtration through on a pad on Celite, and the filtrate was concentrated in vacuo to afford the desired compound **7a** (0.41 g, 67%) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.59−1.85 (4H, m), 2.67−2.80 (2H, m), 3.14−3.21 (2H, m), 4.04−4.15 (1H, m), 4.30 (2H, m), 6.03−6.05 (1H, m), 6.35−6.38 (1H, m), 7.09−7.11 (1H, m).

(7a*S*)-2-(Piperidin-4-yl)hexahydro-3*H*-pyrrolo[1,2-*c*]imidazol-3-one (7c). Compound **7c** was prepared in a manner similar to that described for **7a** in 74% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27−1.37 (1H, m), 1.53−1.99 (5H, m), 2.67−2.78 (4H, m), 3.01−3.24 (4H, m), 3.49−3.69 (3H, m), 3.80−3.91 (1H, m).

(7a*R*)-2-(Piperidin-4-yl)hexahydro-3*H*-pyrrolo[1,2-*c*]imidazol-3-one (7d). Compound **7d** was prepared in a manner similar to that described for **7a** in 93% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23−1.37 (1H, m), 1.48−1.98 (5H, m), 2.67−2.78 (4H, m), 3.00−3.23 (4H, m), 3.49−3.70 (3H, m), 3.80−3.91 (1H, m).

4-(1,1-Dioxidoisothiazolidin-2-yl)piperidine (7i). Compound **7i** was prepared in a manner similar to that described for **7a** in quantitative yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.59−1.92 (6H, m), 2.28−2.39 (2H, m), 2.70 (2H, dt, J = 2.3, 12.1 Hz), 3.11−3.16 (3H, m), 3.30 (2H, t, J = 6.8 Hz), 3.49−3.60 (1H, m).

1-(Piperidin-4-yl)azepan-2-one (7p). Compound **7p** was prepared in a manner similar to that described for **7a** in quantitative yield as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46−1.69 (11H, m), 2.52−2.56 (2H, m), 2.71 (2H, dt, J = 2.9, 11.9 Hz), 3.09−3.13 (2H, m), 3.28−3.31 (2H, m), 4.53−4.63 (1H, m).

2-(Piperidin-4-yl)isoindolin-1-one (7b). A mixture of **8b** (1.90 g, 6.20 mmol), 10% Pd/C (50% wet, 0.20 g) and ammonium formate (2.50 g, 39.6 mmol), and MeOH (50 mL) was refluxed for 3 h. After cooling to room temperature, the mixture was filtered, and the filtrate was concentrated in vacuo to give **7b** (1.40 g, quant.) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.88−2.15 (4H, m), 2.85−3.01 (2H, m), 3.42 (2H, d, J = 12.6 Hz), 4.41 (2H, s), 4.42−4.56 (1H, m), 7.43−7.60 (3H, m), 7.81−7.90 (1H, m).

1-(Piperidin-4-yl)pyrrolidin-2-one (7h). Compound **7h** was prepared in a manner similar to that described for **7b** in quantitative yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.52−1.68 (4H, m), 1.95−2.05 (2H, m), 2.40 (2H, t, J = 8.1 Hz), 2.67−2.76 (2H, m), 3.12 (2H, d, J = 12.0 Hz), 3.36 (2H, t, J = 6.9 Hz), 4.01−4.12 (1H, m).

1,4′-Bipiperidin-2-one (7n). Compound **7n** was prepared in a manner similar to that described for **7b** in 90% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.57−1.83 (8H, m), 2.41 (2H, t, J = 6.4 Hz), 2.69−2.87 (2H, m), 3.15−3.27 (4H, m), 4.56−4.69 (1H, m).

1-(Piperidin-4-yl)imidazolidin-2-one (7e). A mixture of **8e** (0.50 g, 1.6 mmol) and 10% Pd/C (50% wet, 0.10 g), and MeOH (15 mL) was stirred at room temperature under H_2 atmosphere (1 atm) for 15 h, followed by filtration through a pad of Celite. The filtrate was concentrated in vacuo to afford **7e** (0.32 g, quant.) as a gray powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.50−1.73 (4H, m), 2.66−2.75 (2H, m), 3.13 (2H, d, J = 12.0 Hz), 3.33−3.47 (4H, m), 3.76−3.87 (1H, m).

1-(Piperidin-4-yl)-1,3-dihydro-2*H*-imidazol-2-one (7f). Compound **7f** was prepared in a manner similar to that described for **7e** in quantitative yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.03−2.07 (2H, m), 2.32−2.46 (4H, m), 2.97−3.07 (2H, m), 3.53−3.57 (2H, m), 4.09−4.17 (1H, m), 6.31 (1H, d, J = 3.0 Hz), 6.37 (1H, d, J = 2.6 Hz).

3-(Piperidin-4-yl)-1,3-oxazolidin-2-one (7g). Compound **7g** was prepared in a manner similar to that described for **7e** in quantitative yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.51−1.65 (2H, m), 1.76−1.81 (2H, m), 2.70 (2H, dt, J = 2.4, 12.3 Hz), 3.15 (2H, d, J = 12.7 Hz), 3.54 (2H, t, J = 8.1 Hz), 3.73−3.86 (1H, m), 4.30−4.36 (2H, m).

1-(Piperidin-4-yl)tetrahydropyrimidin-2(1*H*)-one (7k). Compound **7k** was prepared in a manner similar to that described for **7e** in quantitative yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.71−1.95 (6H, m), 2.58−2.88 (4H, m), 3.20−3.40 (6H, m), 4.32−4.40 (1H, m).

3-(Piperidin-4-yl)-1,3-oxazinan-2-one (7l). Compound **7l** was prepared in a manner similar to that described for **7e** in quantitative yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.55−1.78 (4H, m), 1.95−2.10 (2H, m), 2.72 (2H, dt, J = 2.7, 12.1 Hz), 3.15 (2H, dd, J = 2.0, 10.0 Hz), 3.26 (2H, t, J = 6.1 Hz), 4.13−4.33 (3H, m).

4-(Piperidin-4-yl)morpholin-3-one (7m). Compound **7m** was prepared in a manner similar to that described for **7e** in quantitative yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.54−1.69 (5H, m), 2.75 (2H, dt, J = 3.0, 11.3 Hz), 3.13−3.17 (2H, m), 3.31 (2H, t, J = 5.1 Hz), 3.88 (2H, t, J = 5.1 Hz), 4.19 (2H, s), 4.52−4.63 (1H, m).

2-(Piperidin-4-yl)tetrahydropyridazin-3(2*H*)-one (7o). Compound **7o** was prepared in a manner similar to that described for **7e** in quantitative yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.54−1.66 (2H, m), 1.71−1.87 (2H, m), 1.90−2.02 (2H, m), 2.47 (2H, t, J = 7.3 Hz), 2.70 (2H, dt, J = 2.7, 12.7 Hz), 3.00 (2H, t, J = 6.7 Hz), 3.06−3.22 (2H, m), 4.37−4.53 (1H, m).

2-(1-Benzylpiperidin-4-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*c*]imidazol-3-one (8a). $NaBH(OAc)_3$ (12.7 g, 60 mmol) was added portionwise to a solution of 4-amino-1-benzylpiperidine **10** (5.71 g, 30 mmol), pyrrole-2-carbaldehyde **9** (2.85 g, 30 mmol), and AcOH (1.80 g, 30 mmol) in CH_2Cl_2 (250 mL) at 0 °C. After stirring at room temperature for 15 h, the mixture was basified with 1 M NaOH solution and an aqueous solution of K_2CO_3. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in acetonitrile (150 mL), and to the mixture was added CDI (5.84 g, 36 mmol) and DBU (4.57 g, 30 mmol). After refluxing for 15 h, the mixture was cooled to room temperature and concentrated in vacuo. The residue was partitioned between water and CH_2Cl_2, and the organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc) to give **8a** (2.54 g, 29%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.68−1.89 (4H, m), 2.08−2.24 (2H, m), 2.99 (2H, d, J = 11.7 Hz), 3.53 (2H, s), 3.93−4.10 (1H, m), 4.29 (2H, s), 6.03 (1H, d, J = 1.5 Hz), 6.36 (1H, t, J = 3.0 Hz), 7.09 (1H, d, J = 2.3 Hz), 7.17−7.40 (5H, m).

2-(1-Benzylpiperidin-4-yl)isoindolin-1-one (8b). A mixture of isobenzofuran-1(3*H*)-one **11** (7.68 g, 57.2 mmol) and **10** (10.9 g, 57.2 mmol) was stirred at 200 °C for 3 days. After cooling to room temperature, the mixture was partitioned between EtOAc and a saturated aqueous solution of $NaHCO_3$. The separated organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc) to give **8b** (8.60 g, 49%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.81−1.92 (4H, m), 2.12−2.23 (2H, m), 2.99 (2H, d, J = 12.0 Hz), 3.54 (2H, s), 4.25−4.36 (3H, m), 7.23−7.40 (5H, m), 7.43−7.57 (3H, m), 7.85 (1H, d, J = 8.4 Hz).

***tert*-Butyl (2*S*)-2-{[(1-Benzylpiperidin-4-yl)amino]methyl}-pyrrolidine-1-carboxylate (13a).** $NaBH_3CN$ (0.38 g, 6.00 mmol) was added to a solution of *tert*-butyl (2*S*)-2-formylpyrrolidine-1-carboxylate **12a** (1.00 g, 5.00 mmol) and **10** (0.95 g, 5.00 mmol) in MeOH (45 mL) and AcOH (5 mL), and the mixture was stirred at room temperature overnight, followed by basifying with a saturated aqueous solution of K_2CO_3. After removal of the solvents in vacuo, the mixture was extracted with CH_2Cl_2. The 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 (hexane/EtOAc = 4:1 to EtOAc) to give **13a** (1.60 g, 86%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30−1.43 (2H, m), 1.46 (9H, s), 1.78−1.89 (6H, m), 1.96−2.05 (2H, m), 2.38−2.55 (2H, m), 2.78−2.88 (3H, m), 3.25−3.35 (2H, m), 3.48 (2H, s), 3.75−3.90 (1H, m), 7.22−7.31 (5H, m).

***tert*-Butyl (2*R*)-2-{[(1-Benzylpiperidin-4-yl)amino]methyl}-pyrrolidine-1-carboxylate (13b).** Compound **13b** was prepared in a manner similar to that described for **13a** using **12b** in 89% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28−1.43 (2H, m), 1.46 (9H, s), 1.79−1.89 (6H, m), 1.96−2.05 (2H, m), 2.40−2.55 (2H, m), 2.80−2.85 (3H, m), 3.30−3.40 (2H, m), 3.49 (2H, s), 3.75−3.90 (1H, m), 7.21−7.32 (5H, m).

(7a*S*)-2-(1-Benzylpiperidin-4-yl)hexahydro-3*H*-pyrrolo[1,2-*c*]-imidazol-3-one (8c). TFA (10 mL) was added to a solution of **13a** (1.50 g, 4.00 mmol) in CH_2Cl_2 (20 mL) at room temperature. After stirring at room temperature overnight, the mixture was basified with 1 M NaOH solution. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue and DBU (1.22 g, 8.00 mmol) were dissolved in THF (50 mL), and to the solution was added CDI (0.78 g, 4.8 mmol) at room temperature. After stirring at room temperature overnight, the solvent was removed in vacuo. The residue was diluted with EtOAc, washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/EtOAc = 1:4) to give **8c**

(1.06 g, 89%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21−1.35 (1H, m), 1.57−1.96 (7H, m), 2.01−2.11 (2H, m), 2.92 (2H, d, J = 11.1 Hz), 2.99−3.07 (1H, m), 3.18−3.21 (1H, m), 3.46−3.52 (3H, m), 3.56−3.69 (2H, m), 3.72−3.83 (1H, m), 7.20−7.36 (5H, m).

(7a*R*)-2-(1-Benzylpiperidin-4-yl)hexahydro-3*H*-pyrrolo[1,2-*c*]-imidazol-3-one (8d). Compound **8d** was prepared in a manner similar to that described for **8c** using **13b** in place of **13a** in 88% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21−1.35 (1H, m), 1.58−1.98 (7H, m), 2.03−2.11 (2H, m), 2.93 (2H, d, J = 11.1 Hz), 2.99−3.08 (1H, m), 3.18−3.21 (1H, m), 3.43−3.54 (3H, m), 3.59−3.69 (2H, m), 3.75−3.84 (1H, m), 7.22−7.37 (5H, m).

Benzyl 4-({2-[(*tert*-Butoxycarbonyl)amino]ethyl}amino)piperidine-1-carboxylate (16a). To a mixture of benzyl 4-oxopiperidine-1-carboxylate **14** (12.4 g, 53.0 mmol), *tert*-butyl(2-aminoethyl)carbamate **15a** (8.50 g, 53.0 mmol) and AcOH (3.18 g, 53.0 mmol) in 1,2-dichloroethane (350 mL) was added portionwise $NaBH(OAc)_3$ (28.1 g, 133 mmol) at 0 °C. After stirring at room temperature for 15 h, the mixture was basified with 1 M NaOH solution. The separated organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **16a** (20.0 g, quant.) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.09−1.41 (2H, m), 1.44 (9H, s), 1.74−1.94 (2H, m), 2.47−2.69 (1H, m), 2.70−2.81 (2H, m), 2.81−2.98 (2H, m), 3.20 (2H, q, J = 5.8 Hz), 3.97−4.22 (2H, m), 4.89 (1H, brs), 5.12 (2H, s), 7.27−7.41 (5H, m).

Benzyl 4-({3-[(*tert*-Butoxycarbonyl)amino]propyl}amino)piperidine-1-carboxylate (16b). Compound **16b** was prepared in a manner similar to that described for **16a** using **15b** in quantitative yield as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.25−1.33 (3H, m), 1.43 (9H, s), 1.59−1.68 (2H, m), 1.83−1.87 (2H, m), 2.57−2.64 (1H, m), 2.69 (2H, t, J = 6.6 Hz), 2.87−2.95 (2H, m), 3.17−3.23 (2H, m), 4.07−4.10 (2H, m), 5.09 (1H, brs), 5.12 (2H, s), 7.30−7.36 (5H, m).

Benzyl 4-(2-Oxoimidazolidin-1-yl)piperidine-1-carboxylate (8e). To a solution of **16a** (2.00 g, 5.30 mmol) in EtOAc (5 mL) was added 4 M HCl/EtOAc (30 mL). The mixture was stirred at room temperature for 5 h. The resulting precipitate was collected by filtration and washed with EtOAc to give benzyl 4-[(2-aminoethyl)amino]piperidine-1-carboxylate dihydrochloride (1.58 g, 85%) as a beige powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.39−1.63 (2H, m), 2.05 (2H, d, J = 11.1 Hz), 2.66−3.01 (2H, m), 3.20 (4H, s), 3.25−3.42 (1H, m). 4.08 (2H, d, J = 13.6 Hz), 5.09 (2H, s), 7.23−7.47 (5H, m). To a solution of benzyl 4-[(2-aminoethyl)amino]piperidine-1-carboxylate dihydrochloride (1.55 g, 4.40 mmol) and DBU (1.48 g, 9.70 mmol) in acetonitrile (100 mL) was added CDI (0.79 g, 4.90 mmol). The mixture was stirred at room temperature for 15 h and concentrated in vacuo. The residue was partitioned between water and EtOAc. The organic layer was separated, washed with water and a 5% solution of citric acid, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **8e** (1.20 g, 90%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.42−1.64 (2H, m), 1.64−1.82 (2H, m), 2.69−3.01 (2H, m), 3.28−3.49 (4H, m), 3.75−4.01 (1H, m), 4.18−4.41 (3H, m), 5.13 (2H, s), 7.29−7.42 (5H, m).

Benzyl 4-(2-Oxotetrahydropyrimidin-1(2*H*)-yl)piperidine-1-carboxylate (8k). To a solution of **16b** (9.50 g, 24.3 mmol) in EtOAc (10 mL) was added 4 M HCl/EtOAc (20 mL) at 0 °C. After stirring at room temperature for 5 h, the resulting precipitate was collected by filtration to give benzyl 4-[(3-aminopropyl)amino]piperidine-1-carboxylate dihydrochloride (8.00 g, 91%) as a colorless powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.46−1.57 (2H, m), 1.96−2.07 (4H, m), 2.91−3.21 (8H, m), 4.08−4.10 (2H, m), 5.09 (2H, s), 7.32−7.41 (5H, m), 8.09 (2H, brs), 9.33 (2H, brs). To a solution of benzyl 4-[(3-aminopropyl)amino]piperidine-1-carboxylate dihydrochloride (7.85 g, 21.5 mmol) and DBU (7.00 g, 47.4 mmol) in acetonitrile (250 mL) was added CDI (3.84 g, 23.7 mmol) at room temperature. The mixture was stirred at room temperature for 15 h and concentrated in vacuo. The residue was partitioned between EtOAc and water. The organic layer was separated, washed with 5% aqueous solution of citric acid and brine, and concentrated in vacuo. The residue was recrystallized from EtOAc−MeOH to afford **8k** (5.95 g, 87%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.54−1.71 (4H, m), 1.86−1.93 (2H, m), 2.83−2.92 (2H, m), 3.13 (2H, t, J = 5.6 Hz), 3.24−3.29 (2H, m), 4.28 (2H, brs), 4.45−4.56 (1H, m), 4.71 (1H, brs), 5.12 (2H, s), 7.31−7.37 (5H, m).

Benzyl 4-[(2-Hydroxyethyl)amino]piperidine-1-carboxylate (18a). A mixture of benzyl 4-oxopiperidine-1-carboxylate **14** (7.00 g, 30.0 mmol), 2-aminoethanol **17a** (2.80 g, 46.0 mmol), and AcOH (2.70 g, 46.0 mmol) in 1,2-dichloroethane (150 mL) and MeOH (10 mL) was stirred at room temperature for 3 h, and to the mixture was added portionwise $NaBH(OAc)_3$ (12.7 g, 46.0 mmol). After stirring at room temperature for 15 h, the mixture was basified to pH 12 with 1 M NaOH solution. The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **18a** (9.00 g, quant.) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21−1.33 (2H, m), 1.86−1.97 (4H, m), 2.58−2.67 (1H, m), 2.78−2.92 (4H, m), 3.63 (2H, t, J = 5.2 Hz), 4.10 (2H, brs), 5.12 (2H, s), 7.29−7.36 (5H, m).

Benzyl 4-[(3-Hydroxypropyl)amino]piperidine-1-carboxylate (18b). A mixture of benzyl 4-oxopiperidine-1-carboxylate **14** (12.7 g, 54.0 mmol), 3-aminopropanol **17b** (4.50 g, 60.0 mmol), and AcOH (3.60 g, 60.0 mmol) in 1,2-dichloroethane (350 mL) was stirred at room temperature for 2 h. To the mixture was added portionwise $NaBH(OAc)_3$ (23.1 g, 109 mmol), and the mixture was stirred at room temperature for 15 h. The mixture was basified with 2 M NaOH solution and an aqueous solution of K_2CO_3. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **18b** (16.8 g, quant.) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.13−1.36 (2H, m), 1.63−1.75 (2H, m), 1.89 (2H, d, J = 11.7 Hz), 2.56−2.70 (1H, m), 2.87−2.99 (4H, m), 3.76−3.86 (2H, m), 3.93−4.24 (2H, m), 5.12 (2H, s), 7.27−7.44 (5H, m).

Benzyl 4-(2-Oxo-1,3-oxazinan-3-yl)piperidine-1-carboxylate (8l). To a solution of **18b** (15.9 g, 54.4 mmol) and DBU (0.83 g, 5.50 mmol) in THF (400 mL) was added CDI (8.82 g, 54.4 mmol) at room temperature. The mixture was stirred at room temperature for 15 h and refluxed for 15 h. After removal of the solvent in vacuo, the residue was partitioned between EtOAc and water. The organic layer was separated, washed with water, 5% aqueous solution of citric acid, and brine, and concentrated in vacuo to give **8l** (17.5 g, quant.) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48−1.81 (4H, m), 1.94−2.10 (2H, m), 2.75−2.99 (2H, m), 3.19 (2H, t, J = 6.2 Hz), 4.18−4.46 (5H, m), 5.12 (2H, s), 7.28−7.41 (5H, m).

Benzyl 4-(2-Oxo-1,3-oxazolidin-3-yl)piperidine-1-carboxylate (8g). Compound **8g** was prepared in a manner similar to that described for **8l** using **18a** in 83% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.49−1.68 (2H, m), 1.66−1.81 (2H, m), 2.86 (2H, d, J = 9.0 Hz), 3.49 (2H, t, J = 7.8 Hz), 3.83−3.94 (1H, m), 4.20−4.36 (4H, m), 5.13 (2H, s), 7.29−7.40 (5H, m).

Benzyl 4-(3-Oxomorpholin-4-yl)piperidine-1-carboxylate (8m). To a solution of **18a** (7.50 g, 27.0 mmol) and Et_3N (4.10 mL, 29.0 mmol) in THF (70 mL) was added dropwise chloroacetyl chloride (2.2 mL, 28.0 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h. After the solvent was removed in vacuo, the residue was diluted with water, and the mixture was extracted with EtOAc. The extract was washed with a 5% aqueous solution of citric acid and brine, dried over anhydrous Na_2SO_4, and the solvent removed in vacuo. The residue was dissolved in DMF (60 mL) and cooled to 0 °C, and then NaH (60% in mineral oil; 1.20 g, 30.0 mmol) was added to the mixture. The mixture was stirred at 0 °C for 1 h, at room temperature for 1 h, and at 80 °C for 15 h. After removal of the solvent in vacuo, the residue was diluted with water, acidified with 1 N HCl solution, and extracted with EtOAc. The extract was washed with water

and brine and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1:2 to EtOAc) to give **8m** (3.80 g, 44%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.56−1.69 (4H, m), 2.85−2.93 (2H, m), 3.24 (2H, t, J = 5.1 Hz), 3.87 (2H, t, J = 5.1 Hz), 4.19 (2H, s), 4.31 (2H, brs), 4.60−4.71 (1H, m), 5.13 (2H, s), 7.29−7.37 (5H, m).

1-(1-Benzylpiperidin-4-yl)pyrrolidin-2-one (8h). To a solution of **10** (3.81 g, 20.0 mmol) and pyridine (2.37 g, 30.0 mmol) in CH_2Cl_2 (50 mL) was added dropwise 4-bromobutanoyl chloride (2.42 mL, 21.0 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h and at room temperature for 15 h. An aqueous solution of K_2CO_3 was added, and the organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in THF (20 mL), and this solution was added dropwise to a suspension of NaH (60% in mineral oil, 1.60 g, 42.0 mmol) in THF (30 mL). After stirring at room temperature for 1 h and at reflux for 15 h, the mixture was allowed to cool to room temperature. An aqueous solution of K_2CO_3 was added and extracted with EtOAc. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc/MeOH = 10:1) to give **8h** (1.72 g, 33%) as an orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.61−1.79 (4H, m), 1.94−2.13 (4H, m), 2.38 (2H, t, J = 8.1 Hz), 2.94 (2H, d, J = 9.9 Hz), 3.35 (2H, t, J = 6.9 Hz), 3.50 (2H, s), 3.93−4.13 (1H, m), 7.20−7.35 (5H, m).

1-Benzyl-4-(1,1-dioxidoisothiazolidin-2-yl)piperidine (8i). Compound **8i** was prepared in a manner similar to that described for **8h** using 3-chloropropane-1-sulfonyl chloride in 63% yield as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.75−1.88 (4H, m), 2.03−2.12 (2H, m), 2.28−2.38 (2H, m), 2.91−2.95 (2H, m), 3.13 (2H, t, J = 7.6 Hz), 3.28 (2H, t, J = 6.6 Hz), 3.42−3.49 (1H, m), 3.49 (2H, s), 7.25−7.34 (5H, m).

1′-Benzyl-1,4′-bipiperidin-2-one (8n). Compound **8n** was prepared in a manner similar to that described for **8h** using 5-bromopentanoyl chloride in 80% yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.51−1.65 (2H, m), 1.64−1.83 (6H, m), 2.05−2.20 (2H, m), 2.39 (2H, t, J = 6.2 Hz), 2.93 (2H, dd, J = 2.2 and 9.5 Hz), 3.19 (2H, t, J = 5.6 Hz), 3.50 (2H, s), 4.45−4.64 (1H, m), 7.15−7.42 (5H, m).

1-(1-Benzylpiperidin-4-yl)azepan-2-one (8p). A solution of 6-bromohexanoyl chloride (5.61 g, 26.2 mmol) in THF (20 mL) was added dropwise to a solution of 4-amino-1-benzylpiperidine **10** (5.00 g, 26.2 mmol) and Et_3N (3.65 mL, 26.2 mmol) in THF (200 mL) at 0 °C. The mixture was stirred at room temperature for 5 h, and the mixture was partitioned between with EtOAc and water. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo, giving *N*-(1-benzylpiperidin-4-yl)-6-bromohexanamide (8.75 g, 91%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36−1.52 (4H, m), 1.59−1.70 (2H, m), 1.82−1.93 (4H, m), 2.06−2.20 (4 H, m), 2.81 (2H, d, J = 11.7 Hz), 3.38−3.44 (2H, m), 3.49 (2H, s), 3.70−3.88 (1H, m), 5.30 (1H, d, J = 7.8 Hz), 7.14−7.38 (5H, m). To a solution of *N*-(1-benzylpiperidin-4-yl)-6-bromohexanamide (3.00 g, 8.17 mmol) in DMF (150 mL) was added NaH (60% in mineral oil, 359 mg, 8.98 mmol) at 0 °C, and the mixture was stirred at 80 °C for 15 h. After cooling to room temperature, the solvent was removed in vacuo. The residue was diluted with water and extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane/EtOAc = 10:1 to 1:2) to give **8p** (1.43 g, 61%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.51−1.76 (10H, m), 2.06−2.14 (2H, m), 2.48−2.57 (2H, m), 2.86−2.99 (2H, m), 3.25−3.35 (2H, m), 3.49 (2H, s), 4.39−4.63 (1H, m), 7.17−7.39 (5H, m).

Benzyl 4-({[(2,2-Dimethoxyethyl)amino]carbonyl}amino)piperidine-1-carboxylate (20). 2-Aminoacetaldehydedimethylacetal (0.36 g, 3.40 mmol) was added to a solution of commercially available benzyl 4-isocyanatotetrahydro-1(2*H*)-pyridinecarboxylate **19** (0.88 g, 2.40 mmol) in acetonitrile (15 mL), and the mixture was stirred at room temperature for 2 h. After removal of the solvent in vacuo, the residue was diluted with 0.1 M HCl solution and extracted with EtOAc. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **20** (1.24 g, quant.) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (2H, m), 1.91−1.96 (2H, m), 2.90−2.99 (2H, m), 3.30 (2H, t, J = 5.7 Hz), 3.40 (6H, s), 3.72−3.79 (1H, m), 4.09 (2H, m), 4.35 (1H, t, J = 5.1 Hz), 4.57−4.61 (2H, m), 5.12 (2H, s), 7.31−7.36 (5H, m).

Benzyl 4-(2-Oxo-2,3-dihydro-1*H*-imidazol-1-yl)piperidine-1-carboxylate (8f). One M HCl solution (5 mL) was added to a solution of **20** (0.37 g, 1.23 mmol) in water (5 mL) and MeOH (10 mL), and the mixture was stirred at room temperature for 3 days. After removal of the solvents in vacuo, the residue was dissolved in water and extracted with EtOAc. The extract was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **8f** (0.27 g, 90%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.63 (2H, m), 1.92−1.96 (2H, m), 2.88−2.96 (2H, m), 4.09−4.22 (1H, m), 4.34 (2H, brs), 5.14 (2H, s), 6.19 (1H, t, J = 2.4 Hz), 6.30 (1H, t, J = 2.6 Hz), 7.26−7.38 (5H, m), 9.66 (1H, brs).

Benzyl 4-[2-(*tert*-Butoxycarbonyl)hydrazino]piperidine-1-carboxylate (21). To a solution of **14** (9.87 g, 42.3 mmol), *tert*-butyl carbazate (14.7 g, 42.3 mmol), and AcOH (2.42 mL, 42.3 mmol) in MeOH (150 mL) was added $NaBH_3CN$ (5.33 g, 84.8 mmol) portionwise at 0 °C. The mixture was stirred at room temperature for 15 h and concentrated in vacuo. The residue was basified with 1 M NaOH solution and extracted with CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **21** (15.0 g, quant.) as colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21−1.41 (2H, m), 1.46 (9H, s), 1.73−1.86 (2H, m), 2.84−3.10 (3H, m), 3.96 (1H, brs), 3.99−4.17 (2H, m), 5.12 (2H, s), 6.04 (1H, brs), 7.28−7.41 (5H, m).

***tert*-Butyl 2-{1-[(Benzyloxy)carbonyl]piperidin-4-yl}-3-oxotetrahydropyridazine-1(2*H*)-carboxylate (22).** To a solution of **21** (1.05 g, 3.00 mmol) and Et_3N (0.46 mL, 3.30 mmol) in THF (30 mL) was added dropwise 4-bromobutyryl chloride (0.35 mL, 3.00 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and concentrated in vacuo. The residue was diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in THF (30 mL), and NaH (60% in mineral oil, 0.18 g, 4.50 mmol) was added. The mixture was stirred at room temperature for 15 h and concentrated in vacuo. The residue was diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **22** (1.28 g, quant.) as a yellow amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (9H, s), 1.54−2.51 (8H, m), 2.75−3.04 (3H, m), 4.09−4.54 (4H, m), 5.06−5.18 (2H, m), 7.28−7.44 (5H, m).

Benzyl 4-(6-Oxotetrahydropyridazin-1(2*H*)-yl)piperidine-1-carboxylate (8o). A solution of **22** (0.84 g, 2.60 mmol) and TFA (1.0 mL) in CH_2Cl_2 (1.5 mL) was stirred at room temperature and concentrated in vacuo. The residue was basified with an aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **8o** (1.28 g, quant.) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.50−1.65 (2H, m), 1.68−1.86 (2H, m), 1.90−2.01 (2H, m), 2.47 (2H, t, J = 7.3 Hz), 2.74−2.94 (2H, m), 2.95−3.03 (2H, m), 3.62 (1H, t, J = 7.7 Hz), 4.17−4.36 (2H, m), 4.47−4.58 (1H, m), 5.12 (2H, d, J = 4.6 Hz), 7.28−7.42 (5H, m).

1-(1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperidin-4-yl)tetrahydropyrimidin-2(1*H*)-one (23). To a solution of **2** (299 mg, 1.00 mmol), **7k** (183 mg, 1.50 mmol), and HOBt (230 mg, 1.50 mmol) in DMF (10 mL) were added WSC (288 mg, 1.50 mmol) and Et_3N (417 μL, 3.00 mmol). The mixture was stirred at room temperature for 15 h and concentrated in vacuo. The residue was diluted with an aqueous solution of $NaHCO_3$ and

extracted with CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc to EtOAc/MeOH = 10:1) to give **23** (353 mg, 76%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.41−1.80 (4H, m), 1.83−2.00 (2H, m), 2.49−2.67 (1H, m), 2.76−2.97 (2H, m), 3.04−3.19 (3H, m), 3.22−3.35 (2H, m), 3.42−3.66 (2H, m), 3.80−3.96 (1H, m), 4.45−4.71 (3H, m), 7.60 (1H, dd, J = 1.9, 8.9 Hz), 7.84−8.02 (4H, m), 8.48 (1H, s). Anal. Calcd for $C_{22}H_{26}ClN_3O_4S$: C, 56.95; H, 5.65; N, 9.06. Found: C, 56.65; H, 5.61; N, 9.00.

In Vitro Assays for the Inhibition of Human FXa. Human factor Xa (0.3 U/mL) was obtained from Roche Diagnostics. Chromogenic substrate, 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. Enzymatic assays were carried out in 96-well microtiter plates. An initial stock solution of test compound at 10 mM was prepared in DMSO, and subsequently diluted with DMSO. Five microliters of compound dilutions (final concentration: 0.2 nM−0.6 μM, 2 nM−6 μM, or 2 nM−60 μM) and 10 μL of enzyme solution were added to the well containing 225 μL of buffer, and 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 with the addition of 25 μL of 50% acetic acid. The color development from the release of *p*-nitroanilide from each chromogenic substrate was measured at 405 nm on a microtiter plate reader (Multiskan Ascent, Dainippon Pharmaceuticals, Japan). Each absorbance [T] was calculated by subtracting the absorbance measured without the substrate. The control [C] was performed using DMSO solution in place of test compound. Inhibitory effect (%) was calculated according to the eq $(1 - [T]/[C]) \times 100$. IC_{50} values (and 95% confidence limits) were calculated from the concentration−response curves generated by logistic regression using the SAS system.

In Vitro PT Assays. The assay of plasma clotting time was performed using an automatic coagulometer (STA Compact, Diagnostica Stago). PT was measured with PT reagent (Roche Diagnostics). An initial stock solution of test compound at 10 mM was prepared in DMSO, and subsequently diluted with DMSO. Compound dilutions of 1.5 μL were 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 (DMSO derived from test compound solution did not exceed a plasma concentration of 3% v/v). Coagulation was initiated with the addition of 100 μL of PT reagent, and coagulation time was measured. Coagulation time was determined on plasma containing three or four different concentrations of test compound. Coagulation time prolonging ratio (%) was calculated based on 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.

Enzyme Affinity Assays. Following enzymes and chromogenic substrates were used in this study: human FXa (Roche Diagnostics), human thrombin and human activated protein C (Sigma Chemical), human factor IXa (MP Biomedicals), human kallikrein (Calbiochem), human recombinant tissue plasminogen activator (t-PA) (Kyowa Hakko Kogyo), human trypsin (Athens Research and Technology), human plasmin (Enzyme Research Laboratories), S-2222, S-2288, S-2302 and S-2366 (Chromogenix-Instrumentation Laboratory), and Spectrozyme FIXa (American Diagnostica). All other reagents were of the highest grade commercially available. Tris-HCl buffer solution (Tris-HCl, 50 mmol/L; NaCl, 145 mmol/L; $CaCl_2$, 2 mmol/L; pH 8.3) (215 μL) and drugs (5 μL) dissolved in DMSO were preincubated at 37 °C for 10 min, and were mixed with enzyme solution (10 μL). A reaction was started with the addition of chromogenic substrate (20 μL). Optical density (O. D.) at 405 nm was then continuously measured by microplate reader for 5 min (plasmin, t-PA, activated protein C) or 10 min (FXa, thrombin, FIXa, kallikrein, trypsin). The following chromogenic substrate for each serine protease was used: S-2222 (0.40 mmol/L) for FXa (0.012 unit/mL), S-2366 (0.24 mmol/L) for thrombin (0.050 unit/mL), Spectrozyme FIXa (0.40 mmol/L) for factor IXa (2.0 μg/mL), S-2302 (0.24 mmol/L) for kallikrein (0.20 μg/mL), S-2288 (0.48 mmol/L) for t-PA (4000 unit/mL), S-2222 (0.18 mmol/L) for trypsin (0.020 μg/mL), S-2366 (0.48 mmol/L) for activated protein C (0.12 μg/mL) and S-2366 (0.48 mmol/L) for plasmin (0.4 μg/mL). Data were expressed as mean ± SEM. IC_{50} values (and 95% confidence limits) were calculated as described above.

Metabolic Stability Assay in the Absence of NADPH. Human and monkey hepatic microsomes were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L KH_2PO_4−K_2HPO_4 phosphate buffer (pH 7.4) and 10 μmol/L test compound in DMSO. The concentration of hepatic microsomal protein was 4 mg/mL. The reaction mixture was incubated at 37 °C for 120 min. The reaction was terminated by the addition of 0.1 mL of acetonitrile to reaction mixture. All incubations were made in duplicate. The hydrolytic metabolite produced in the reaction mixture was measured by HPLC system equipped with a UV detector.

Metabolic Stability Assay in the Presence of NADPH. Human hepatic microsomes were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L KH_2PO_4−K_2HPO_4 phosphate buffer (pH 7.4) and 1 μmol/L test compound in DMSO. The concentration of hepatic microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 50 mmol/L $MgCl_2$, 50 mmol/L glucose-6-phosphate, 5 mmol/L β-$NADP^+$ and 15 unit/mL glucose-6-phosphate dehydrogenase was prepared and added to the incubation mixture with a 10% volume of the reaction mixture. After the addition of the NADPH-generating system, the mixture was incubated at 37 °C for 0 and 20 min. The reaction was terminated by the addition of acetonitrile equivalent to the volume of the reaction mixture. All incubations were made in duplicate. Test compound in the reaction mixture was measured by HPLC system equipped with a UV detector. For metabolic stability determinations, chromatograms were analyzed for parent compound disappearance from the reaction mixtures.

Cassette Dosing in Cynomolgus Monkeys. All experiments were conducted in accordance with the regulations of Animal Care and Use Committee of the Takeda Chemical Industries Ltd. Test compounds were administered as a cassette dosing to cynomolgus 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.

Factor Xa + Compound 5k Crystal Structure Determination. The cDNA encoding human factor Xa was obtained by PCR from a human liver cDNA library and cloned into a modified pBastbacHTb vector (Invitrogen). The final construct contains a baculovirus gp67 signal peptide followed by a His10 tag, a TEV cleavage site and the coding sequence corresponding to residues 127−475 of factor Xa. The expressed glycosylated enzyme was isolated from the cell culture medium by diafiltration using crossflow ultrafiltration followed by passage over a nickel chelate resin (binding buffer: 25 mM Tris [pH 7.9], 200 mM NaCl). After removal of the His10-tag, the protein was activated using snake venom (Enzyme Research, Russels Viper Venom, RVV-X). The activated protein was deglycosylated (BioLabs,

PNGase F, #P0704L) and then further purified over a MonoS column (25 mM MES pH 6.5) followed by a S-200 column (25 mM Tris pH 7.6, 200 mM NaCl) to remove inactive and glycosylated species of the protease. The protein was incubated with 1 mM of compound **5k** for 30 min and concentrated to a final concentration of 15 mg/mL. Crystals were obtained at 20 °C using Syrrx's automated Nanovolume Crystallization technology.[21] The reservoir solution was 25% PEG 3350, 0.1 M Tris pH 8.5. Crystals appeared within 3 days and grew to a maximum size within 7−10 days. X-ray diffraction data were collected at Advanced Light Source (ALS) beamline 5.0.3, and processed using the program HKL2000.[22] The crystals diffracted to 1.45 Å resolution and belong to the *P*212121 space group with one molecule in the asymmetric unit and unit cell parameters of $a = 49.0$ Å; $b = 70.3$ Å; and $C = 78.5$ Å. The structure was determined by molecular replacement using MOLREP, utilizing the published coordinates of factor Xa with accession code 1FJS.[23,24] Subsequent structure refinement and model building were performed utilizing REFMAC and XtalView.[23,25] The final refined crystallographic statistics for the cocrystal structure with compound **5k** are $R = 19.4\%$ (R_{free}, 21.2%), with root-mean-square deviations (rmsd) for bond lengths of 0.007 Å, and for bond angles of 1.15°. Compound **5k** was bound into the active site and clearly visible in the electron density maps.

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