# Discovery of Imidazo[1,5-c]imidazol-3-ones: Weakly Basic, Orally Active Factor Xa Inhibitors

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The coagulation enzyme factor Xa (FXa) has been recognized as a promising target for the development of new antithrombotic agents. We previously found compound **1** to be an orally bioavailable FXa inhibitor in fasted monkeys; however, **1** showed poor bioavailability in rats and fed monkeys. To work out the pharmacokinetic problems, we focused our synthetic efforts on the chemical conversion of the 4-(imidazo[1,2-a]pyridin-5-yl)piperazine moiety of **1** to imidazolylpiperidine derivatives (fused and nonfused), which resulted in the discovery of the weakly basic imidazo[1,5-c]imidazol-3-one **3q** as a potent and selective FXa inhibitor. Compound **3q** showed favorable oral bioavailability in rats and monkeys under both fasted and fed conditions and antithrombotic efficacy in a rat model of venous thrombosis after oral administration, without a significant increase in bleeding time (unlike warfarin). On the basis of these promising properties, compound **3q** was selected for further evaluation.

# Introduction

Abnormal intravascular clot formation is an important factor in a number of cardiovascular diseases such as deep vein thrombosis, pulmonary embolism, myocardial infarction, unstable angina, and ischemic stroke. Agents inhibiting the coagulation cascade, such as heparins, low-molecular-weight heparin, fondaparinux, and vitamin K antagonists (warfarin, acenocoumarol, and fluindione), have been available for the treatment and prevention of these thromboembolic diseases. Vitamin K antagonists prevent the synthesis of vitamin K dependent coagulation factors in the liver and inhibit the activation of blood coagulation leading to thrombus formation. As the only three oral anticoagulants used in a clinical setting, warfarin, acenocoumarol, and fluindione are widely used for the prophylaxis and treatment of venous thromboembolic disease. However, warfarin therapy necessitates a routine blood coagulation test and dose adjustment for each individual because it has substantial drawbacks such as narrow therapeutic dose window, slow onset and offset of action, and interaction with many drugs and food.<sup>1</sup> Despite careful medical treatment, it is difficult to protect patients from the risk of bleeding. Therefore, there is a clear need to develop improved anticoagulants that are orally active, have long duration of action, and have useful in vivo potency without unpredictable or serious bleeding complications.

Factor Xa (FXa<sup>*a*</sup>) is a key serine protease in the coagulation cascade and essential in the formation of a thrombin, a key mediator of both fibrin formation and platelet activation. It plays an important role in the coagulation network at the common pathway that connects both the tissue factor-activated extrinsic pathway and the surface-activated intrinsic pathway.<sup>2</sup> FXa forms

the prothrombinase complex with calcium ion and factor Va, and the complex amplifies the procoagulant action of FXa. FXa acts at an earlier stage in the coagulation cascade than thrombin. Inactivation of FXa by specific inhibitors does not influence preformed thrombin but does effectively prevent the generation of thrombin. Extensive preclinical and clinical proof-of-principal data show that inhibition of FXa is effective in both venous and arterial thrombosis.<sup>3</sup> In animal models, a better therapeutic index (antithrombotic efficacy vs antihemostatic effects, such as bleeding) has been shown for direct FXa inhibitors compared with those for direct thrombin inhibitors and warfarin.<sup>4</sup> On the basis of these data, a cleaner side effect profile would be expected for a FXa inhibitor relative to a thrombin inhibitor in the clinical setting.<sup>3,5</sup>

A variety of direct and low-molecular-weight FXa inhibitors have been described,<sup>6</sup> and orally active FXa inhibitors have been extensively explored.<sup>5d–h</sup> For example, some nonamidine FXa inhibitors such as rivaroxaban<sup>7</sup> and apixaban<sup>8</sup> have been reported to be orally active FXa inhibitors and are under clinical study.

As part of our FXa inhibitor program, we have previously reported that 4-(imidazo[1,2-*a*]pyridin-5-yl)piperazine 1<sup>9</sup> significantly inhibited human FXa with an IC<sub>50</sub> of 0.0097  $\mu$ M, and its in vitro anticoagulant activity PT<sub>2</sub> (the concentration of compound required to double the clotting time in a human prothrombin time (PT) assay) was 1.4  $\mu$ M. Compound 1 demonstrated good selectivity for FXa versus other serine proteinases and favorable oral bioavailability (BA) in fasted monkeys (BA = 30.5%). Unfortunately, compound 1 showed poor oral bioavailability in fed monkeys, fasted rats, and fed rats. We hoped that the pharmacokinetic behavior might be improved by the modification of the 4-(imidazo[1,2-*a*]pyridin-5-yl)piperazine moiety of compound 1 as the S4 binding element.<sup>6c,8,10</sup>

Our binding model of compound **1** in FXa<sup>9</sup> demonstrated that the 2-hydroxymethylimidazo[1,2-*a*]pyridine moiety formed no salt bridge or hydrogen bond with any amino acid residue in the S4 site, but it did form hydrophobic contacts with the aromatic rings of Tyr99, Phe174, and Trp215 in "the S4 aryl binding site".<sup>11</sup> In particular, the imidazo[1,2-*a*]pyridine ring extends across the face of Phe174 phenyl ring with the favorable  $\pi-\pi$  stacking interaction. This result suggests that the 4-(imi-

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<sup>&</sup>lt;sup>*a*</sup> Abbreviations: FXa, factor Xa; PT, prothrombin time; PT<sub>2</sub>, the concentration of compound required to double the clotting time in a human prothrombin time assay; BA, bioavailability; WSC, 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole hydrate; TFA, trifluoroacetic acid; CDI, 1,1'-carbonyldiimidazole; DBU, 1,8-diazabicyclo[5.4.0]-7-undecene; PDB, Protein Data Bank; CYP, cyto-chrome P450; t-PA, tissue plasminogen activator.



 $^a$  Inhibitory activity against human FXa. IC\_{50} values shown are the mean of duplicate measurements. The 95% confidence intervals are shown in parentheses.

dazo[1,2-a]pyridin-5-yl)piperazine moiety might not be essential for inhibitory activity, but the hydrophobic contacts at the S4 aryl binding site might be important, so we hypothesized that the imidazo [1,2-a] pyridine moiety of 1 as the S4 binding element could be replaced with other basic heteroaromatic rings to improve the pharmacokinetic properties without loss of inhibitory activity. Thus, we initially attempted to replace the piperazine ring with a piperidine ring, shift the substitution position of piperidine ring on the imidazo[1,2-*a*]pyridine ring, and remove the 2-hydroxymethyl group to modulate the physiochemical profiles. This initial effort resulted in the discovery of 4-(imidazo[1,2-a]pyridin-3-yl)piperidine 2 as an interesting new lead (Table 1). The quite similar FXa inhibitory activities between 1 and 2 (IC<sub>50</sub> = 0.0097  $\mu$ M for 1 vs 0.012  $\mu$ M for 2) opened up the possibility to explore imidazolylpiperidine derivatives (fused and nonfused). There are no reports on FXa inhibitors bearing an imidazolylpiperidine moiety. Therefore, we were prompted to design and synthesize new imidazole and bicyclic imidazole derivatives 3 (Figure 1) in an attempt to further improve the pharmacokinetic properties.

In this paper, we describe the synthesis and structure–activity relationships for **3** and show that weakly basic imidazo[1,5-c]imidazol-3-one **3q** is a potent and selective direct FXa inhibitor with excellent in vivo activity and favorable oral bioavailability unaffected by food intake, thus making it a promising drug candidate for further evaluation.<sup>12</sup>

## Chemistry

The synthesis of imidazo[1,2-*a*]pyridine **2** and tetrahydroimidazo[1,2-*a*]pyridine **3a** is shown in Scheme 1. The key intermediate piperidine **7a** was prepared by condensation of bromoacetaldehyde **4**<sup>13</sup> and 2-aminopyridine **5** followed by removal of the Boc group. Catalytic hydrogenation of imidazo[1,2-*a*]pyridine **6** using PtO<sub>2</sub> followed by deprotection of the Boc group gave tetrahydroimidazo[1,2-*a*]pyridine **7b**. Piperidines **7a** and **7b** were coupled with 3-(6-chloronaphthalen-2-yl)sulfonylpropionic acid **8**<sup>14</sup> using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC) and 1-hydroxybenzotriazole hydrate (HOBt) and successively treated with concentrated HCl to give the desired amides **2** and **3a**.

4-(Imidazol-5(4)-yl)piperidines 3b-e were synthesized as shown in Scheme 2. Piperidines 9a and  $9b^{15,16}$  were coupled with carboxylic acid 8 to give the desired amides 3b and 3c. Regioselective N-methylation to obtain 1-methylimidazoles 3dand 3e was carried out by N-tritylation on the less hindered nitrogen atom in the imidazole rings of **3b** and **3c**, subsequent formation of the quaternary imidazolium salt using iodomethane, and finally removal of the trityl group under acidic conditions.<sup>16</sup>

4-(Imidazol-1-yl)piperidines 3f-i were prepared by the method outlined in Scheme 3. The displacement reaction of mesylate 11 with imidazoles 12a and 12b gave piperidines 13a and 13b. Removal of the Boc group from piperidines  $13a-d^{17}$  followed by coupling with carboxylic acid 8 afforded amides 3f-i.

The synthesis of 4-(imidazol-2-yl)piperidines 3j and 3k is depicted in Scheme 4. Removal of the Boc group from piperidines  $14^{17}$  and 15, prepared by methylation of 14, followed by coupling with carboxylic acid 8 gave the desired amides 3j and 3k.

Alkylene-linked imidazoles 3l-o were synthesized as described in Scheme 5. Intermediates 17a-d were prepared from 2-methylimidazole by alkylation with 4-(bromoalkyl)piperidine  $16a-d^{18}$  in the presence of sodium hydride. Removal of the Boc group from 17a-c followed by condensation with carboxylic acid 8 and treatment with 4 M HCl in EtOAc gave the desired amides 3l-n. Removal of the Boc group from 17d followed by coupling with carboxylic acid 8 gave amide 30.

The synthesis of alkylene-linked imidazole 3p was shown in Scheme 6. Alkylation of 2-methylimidazole with  $18^{19}$  in the presence of sodium hydride gave amide 19. Treatment with trifluoroacetic acid (TFA) and subsequent reduction of the amide bond with lithium aluminum hydride afforded piperazine 20, which was condensed with carboxylic acid 8 to give the desired amide 3p.

Imidazo[1,5-*c*]imidazol-3-ones **3q** and **3r** were synthesized as follows (Scheme 7). Reductive amination of imidazolecarbaldehydes **22a** and **22b** with 4-aminopiperidine **21** gave aminomethylimidazole **23a** and **23b**. Cyclization of **23a** or **23b** using 1,1'-carbonyldiimidazole (CDI) and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) afforded imidazo[1,5-*c*]imidazol-3-one **24a** or **24b**. Removal of the Boc group from **24a** and **24b** with concentrated HCl and successive condensation with **8** provided the corresponding amides **3q** and **3r**.

The synthesis of imidazo[1,5-*c*]imidazol-3-one **3s**, a piperazine analogue of **3q**, is depicted in Scheme 8. Hydrazone **26**, prepared from hydrazine **25**<sup>18</sup> and **22a**, was reduced with borane-tetrahydrofuran complex and subsequently treated with hydrochloric acid for decomposition of aminopiperazine-borane complex to give hydrazine **27**. Cyclization of **27** with CDI afforded imidazo[1,5-*c*]imidazol-3-one **28**, which was converted into piperazine **29** by palladium-catalyzed debenzylation with ammonium formate. Coupling of carboxylic acid **8** with piperazine **29** gave the desired amide **3s**.

## **Results and Discussion**

The compounds thus synthesized were evaluated in vitro for inhibitory potency against human FXa, expressed as  $IC_{50}$  values, and for their activity in the prolongation of human PT, expressed as  $PT_2$ . To estimate the oral bioavailability of these compounds, the ex vivo PT prolonging activity was also determined 1 h after oral administration to mice at a dose of 30 mg/kg and expressed as ratios of the PT of the compound in treatment mice to that of the untreated group.

The activities of 4-(imidazol-5(4)-yl)piperidine derivatives are shown in Table 2. Tetrahydroimidazo[1,2-*a*]pyridine **3a** exhibited slightly less potent FXa inhibitory activity (IC<sub>50</sub> = 0.023  $\mu$ M for **3a** vs 0.012  $\mu$ M for **2**) compared to **2**, while **3a** showed more potent in vitro anticoagulant activity compared to **2** (PT<sub>2</sub> = 0.48  $\mu$ M for **3a** vs 2.2  $\mu$ M for **2**). This result suggests that



Figure 1

Scheme 1<sup>a</sup>



<sup>*a*</sup> Reagents and conditions: (a) EtOH, reflux, 60%; (b) concd HCl, 91%; (c) (1) H<sub>2</sub>, PtO<sub>2</sub>, (2) concd HCl, 95%; (d) (1) WSC, HOBt, DBU, Et<sub>3</sub>N, (2) concd HCl, 81–82%.

Scheme 2<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) 8, WSC, HOBt, DBU, Et<sub>3</sub>N, 50–91%; (b) TrCl, Et<sub>3</sub>N, 60–100%; (c) (1) MeI, (2) AcOH, 48–76%.

Scheme 3<sup>c</sup>



 $^a$  Reagents and conditions: (a)  $K_2CO_3$  or NaH, 3–11%; (b) (1) concd HCl, (2)  ${\bf 8},$  WSC, HOBt, DBU, Et\_3N, 66–98%.

the improvement in anticoagulant potency of **3a** may be attributed to lower lipophilicity (log D = 2.2 for **3a**, 2.5 for **2** at pH 7.4)<sup>20</sup> and increased basicity (p $K_a = 8.2$  for **3a**, 5.9 for **2**).<sup>21</sup> Thus, we speculated that the lipophilicity and basicity of the S4 binding element influenced the in vitro anticoagulant activities of these compounds.<sup>22</sup> The 1-methylimidazoles **3d** and **3e** are equipotent to tetrahydroimidazo[1,2-*a*]pyridine **3a** in



<sup>*a*</sup> Reagents and conditions: (a) MeI, NaH, 100%; (b) (1) concd HCl, (2) **8**, WSC, HOBt, DBU, Et<sub>3</sub>N, 54–70%.

human FXa inhibitory potency and in vitro anticoagulant activity. On the other hand, compounds **3b** and **3c** ( $\mathbb{R}^1 = \mathbb{H}$ ) are slightly less potent than **3d** and **3e**. The basicity and the lipophilicity of **3c** ( $pK_a = 7.9$ , log D = 2.0 at pH 7.4) are similar to those of **3d** and **3e** (i.e.,  $pK_a = 8.2$ , log D = 2.0 at pH 7.4 for **3e**). This result suggests that the existence of a free hydrogen atom on either nitrogen atom in the imidazole rings of **3b** and **3c** might interrupt the interaction between the S4 site of FXa

Scheme 5<sup>a</sup>



<sup>*a*</sup> Reagents and conditions: (a) NaH, 7–100%; (b) (1) concd HCl, (2) **8**, WSC, HOBt, DBU, Et<sub>3</sub>N, (3) 4 M HCl/EtOAc, 53–74%; (c) (1) concd HCl, (2) **8**, WSC, HOBt, DBU, Et<sub>3</sub>N, 15%.

Scheme 6<sup>a</sup>



 $^a$  Reagents and conditions: (a) 2-methylimidazole, NaH, 68%; (b) (1) TFA, (2) LiAlH<sub>4</sub>, 40%; (c) **8**, WSC, HOBt, Et<sub>3</sub>N, 2%.

and the imidazole ring. As a result, the 1-methylimidazole moiety of **3d** and **3e** was found to be a new effective S4 binding element.

To investigate the influence of the substitution position of the 4-piperidine moiety on the imidazole ring, some 4-(imidazol-1-yl)piperidines (3f-i) and 4-(imidazol-2-yl)piperidines (3j and 3k) were examined and the results are shown in Table 3. Among them, the 2-methyl (3i) and 2-ethyl (3f) derivatives showed the most potent anticoagulant activities. The FXa inhibitory activity of 4-(2-methylimidazol-1-yl)piperidine 3i was slightly weaker than that of the regioisomer 4-(1-methylimidazol-5-yl)piperidine 3d. Compound 3j, lacking an N-substituent, exhibited notably less potent in vitro activity. In comparison to 3i, 4-(1-methylimidazol-2-yl)piperidine 3k inhibited FXa slightly more potently, whereas its anticoagulant activity was slightly less potent. The FXa inhibitory activity, basicity, and lipophilicity of 3k  $(IC_{50} = 0.056 \,\mu\text{M}, \, pK_a = 6.9, \, \log D = 2.1 \text{ at pH } 7.4)$  are similar to those of **3d** (IC<sub>50</sub> = 0.056  $\mu$ M, p $K_a$  = 7.2, log D = 2.0), but 3k was nevertheless less potent with respect to in vitro anticoagulant activity than 3d (PT<sub>2</sub> = 2.1 for 3k vs 0.49  $\mu$ M for 3d). These data suggested that the position of the imidazole ring nitrogen might be more important for in vitro anticoagulant activity than basicity or lipophilicity for these inhibitors. 4-(Imidazol-5-yl)piperidines 3d and 3e were found to be the most potent FXa inhibitors in our 4-(imidazolyl)piperidine series.

In our effort to achieve further enhancement of FXa potency, a molecular modeling study of **2** and **3a** was carried out using the program GOLD<sup>23</sup> and the X-ray structure of FXa reported by Sanofi-Aventis for an inhibitor complex (PDB code 1EZQ)<sup>24</sup> (Figure 2). In this model, the imidazo[1,2-*a*]pyridine ring of **2** (orange) and the tetrahydroimidazo[1,2-*a*]pyridine ring of **3a** (white) are located at the S4 aryl binding site of FXa.<sup>25</sup> In this site, an open space around the imidazole rings (yellow circle)

was found to be available to accommodate structural modifications and the imidazole rings appeared able to shift into the deeper S4 aryl binding site as shown in Figure 2 (yellow arrow). Thus, we designed our next set of compounds to incorporate varied alkylene chains between the imidazole ring and the piperidine ring, hoping to enhance the interaction with this site.

The in vitro anti-FXa and PT prolonging activities obtained for the compounds thus synthesized, of type 3i, are listed in Table 4. Ethylene-linked imidazole **3m** (FXa IC<sub>50</sub> =  $0.037 \,\mu$ M) was found to be more potent than the other alkylene-linked imidazoles 31, 3n, 3o, and original compound 3i. The in vitro anticoagulant activity of 3m was equipotent to that of 3i (PT<sub>2</sub> = 0.91 for **3m** vs 0.99  $\mu$ M for **3i**). The in vitro activity of piperazine **3p**, which has an ethylene linker, was approximately equal to that of piperidine 3m. A molecular modeling study of **3i**, **3m**, and **3p** was carried out using the same model described above (Figure 3). In this model, the imidazole ring of 3i (orange) and the imidazolylethyl groups of **3m** (white) and **3p** (green) are located at the S4 aryl binding site of FXa (yellow circle). In this site, the imidazolylethyl groups of **3m** and **3p** appeared to occupy the deeper S4 aryl binding site more than the imidazole ring of 3i. Because 3m and 3p were found to be more potent FXa inhibitors than 3i, we speculated that the imidazolylethyl group in 3m and 3p might contribute to the enhancement of interaction with the S4 site of FXa compared to the imidazolyl group of 3i.

Compound 3m was considered to have poor oral bioavailability in mice, as discussed in more detail below, which might be ascribed to the flexible ethylene linker. We expected that lowering the flexibility of the ethylene linker by cyclization with the imidazole ring might improve oral bioavailability because of a decrease in the number of rotatable bonds.<sup>26</sup> Moreover, we attempted to lower the basicity of **3m** by introduction of an electron-withdrawing carbonyl group onto the imidazole nitrogen atom as a component for cyclization because the basicity of compound 1 ( $pK_a = 5.6$ ), which showed favorable oral bioavailability in mice and fasted monkeys, was lower than for compounds that had poor oral bioavailability in mice ( $pK_a >$ 7.1 for **3a**, **3d**, **3e**, and **3m**), as discussed in more detail below.<sup>27</sup> Hence, we designed and synthesized the weakly basic bicyclic imidazo[1,5-c]imidazol-3-one moiety as a novel S4 binding element by introduction of an electron-withdrawing carbonyl group on the imidazole nitrogen atom as a component for cyclization. The imidazo[1,5-c]imidazo[-3-one] derivatives have not been reported previously; however, the chemical synthesis is simple and robust. The results of imidazo[1,5-c]imidazol-3ones 3q-s (p $K_a = 5.0-5.4$ ) are shown in Table 5. Interestingly, compounds 3q and 3r showed more potent FXa inhibitory activity than imidazolylethylpiperidine 3m, with IC<sub>50</sub> values in the single-digit nanomolar range (FXa  $IC_{50} = 0.0048 - 0.0053$  $\mu$ M for **3q** and **3r** vs 0.037  $\mu$ M for **3m**). Piperazine **3s** showed potent FXa inhibitory activity (IC<sub>50</sub> =  $0.0069 \,\mu\text{M}$ ) comparable to that of the corresponding piperidine 3q. Surprisingly, in vitro anticoagulant activities of 3q-s were similar to that of 3m (PT<sub>2</sub> = 0.61-1.2  $\mu$ M for 3q-s vs 0.91  $\mu$ M for 3m) despite the unambiguous difference in their FXa inhibitory activities. The reason for these results could not be explained in terms of lipophilicity (log D = 2.1-2.5 for **3q**-s, 2.2 for **3m** at pH 7.4); however, it might be attributed to the difference in basicity ( $pK_a$ ) = 5.0-5.4 for 3q-s, 7.9 for 3m) caused by the existence of a carbonyl group on the imidazo[1,5-c]imidazol-3-one ring.

The ex vivo PT prolongation activity after oral administration in mice and cytochrome P450 (CYP) 3A4<sup>28</sup> inhibitory activities of the more potent compounds described above are shown in

### Scheme 7<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) NaBH(OAc)<sub>3</sub>, AcOH, 100%; (b) CDI, DBU, 77–97%; (c) (1) concd HCl; (2) 8, WSC, HOBt, Et<sub>3</sub>N, 50–73%.

### Scheme 8<sup>a</sup>



<sup>*a*</sup> Reagents and conditions: (a) **22a**, 88% (b) (1) BH<sub>3</sub>·THF, (2) 6 M HCl, 100%; (c) CDI, DBU, 65%; (d) HCO<sub>2</sub>NH<sub>4</sub>, 10% Pd/C, 93%; (e) **8**, WSC, HOBt, Et<sub>3</sub>N, 76%.

Table 2. In Vitro Activities of 4-(Imidazol-5(4)-yl)piperidines 3a-e



				••		
compd	$\mathbb{R}^1$	$\mathbb{R}^2$	human FXa, $IC_{50} (\mu M)^a$	human PT, PT <sub>2</sub> ( $\mu$ M) <sup>b</sup>	$\log D^c$	$pK_a^{\ d}$
2			0.012 (0.0099-0.014)	2.2	2.5	5.9
3a	- (0	$(H_2)_4 -$	0.023 (0.022-0.024)	0.48	2.2	8.2
3b	Н	Н	0.17 (0.15-0.20)	3.0	2.0	6.9
3c	Н	Me	0.12 (0.10-0.14)	2.5	2.0	7.9
3d	Me	Н	0.056 (0.052-0.059)	0.49	2.0	7.2
3e	Me	Me	0.031 (0.028-0.034)	0.56	2.0	8.2

<sup>*a*</sup> Inhibitory activity against human FXa. IC<sub>50</sub> values shown are the mean of duplicate measurements. The 95% confidence intervals are shown in parentheses. <sup>*b*</sup> PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT<sub>2</sub> values shown are the mean of duplicate measurements. <sup>*c*</sup> Measured at pH 7.4. <sup>*d*</sup> Measured using a titration method.

Table 6. Tetrahydroimidazo[1,2-a]pyridine **3a**, imidazolylpiperidine derivatives 3d, 3e, and 3i, and imidazolylethyl derivatives 3m and 3p exhibited low ex vivo mouse PT ratios, and so these compounds were judged to have poor oral bioavailability in mice. On the other hand, imidazo[1,5-c]imidazol-3-one derivatives 3q-s showed significant mouse PT prolonging activities and thus appeared to have favorable oral bioavailability in mice. These results support our assumption that lowering the flexibility and basicity of the S4 binding element could dramatically improve oral bioavailability. 5-Methylimidazo[1,5climidazol-3-ones 3q and 3s showed considerably weak CYP3A4 inhibitory activity at 10 µM, whereas 5,7-dimethylimidazo[1,5c]imidazol-3-one 3r inhibited CYP3A4 significantly. On the basis of their ex vivo PT prolongation activities in mice and their weak CYP3A4 inhibitory potencies, compounds 3q and **3s** were selected for evaluation in cynomolgus monkeys.

In order to estimate the oral bioavailability in fasted and fed monkeys, the ex vivo PT prolonging activities in cynomolgus monkeys were determined for **3q** and **3s**. After a 3 mg/kg oral dose, both compounds prolonged PT significantly with long duration of action under fasted conditions (Figure 4A). Under fed conditions, compound **3q** showed as potent a PT prolonging activity as under fasted conditions, whereas compound **3s** showed prolonged PT to a lesser extent following oral dosing (Figure 4B).

Since many trypsin-like proteases have essential physiological functions,<sup>29</sup> an important consideration for the development of FXa inhibitors is the ability of the molecule to bind selectively. Compound **3q** displayed > 3000-fold selectivity for FXa versus several coagulation (thrombin), fibrinolytic (plasmin, tissue plasminogen activator (t-PA)), and digestive enzymes (trypsin) (Table 7).

Table 3. In Vitro Activities of 4-(Imidazol-1-yl)piperidines  $3f\!-\!i$  and 4-(Imidazol-2-yl)piperidines 3j and 3k

CI					R
compd	type	R	human FXa, $IC_{50} (\mu M)^a$	human PT, $PT_2$ ( $\mu$ M)	b
3d			0.056 (0.052-0.059)	0.49	
3f	А	Et	0.16 (0.14-0.17)	1.0	
3g	А	Pr	0.23 (0.19-0.28)	2.0	
3h	Α	Н	1.4 (1.3-1.6)	7.0	
3i	Α	Me	0.17 (0.16-0.18)	0.99	
3j	В	Н	1.6(1.4 - 1.8)	>10	
3k	В	Me	0.056 (0.053-0.060)	2.1	

<sup>*a*</sup> Inhibitory activity against human FXa. IC<sub>50</sub> values shown are the mean of duplicate measurements. The 95% confidence intervals are shown in parentheses. <sup>*b*</sup> PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT<sub>2</sub> values shown are the mean of duplicate measurements.



Figure 2. Superimposed binding model of compounds 2 (orange) and 3a (white) in FXa.

Table 4.	In Vitro	Activities	of	Alkylene-Linked	Imidazoles	3l-p
	CI	$\sim$		0		



<sup>*a*</sup> Inhibitory activity against human FXa. IC<sub>50</sub> values shown are the mean of duplicate measurements. The 95% confidence intervals are shown in parentheses. <sup>*b*</sup> PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT<sub>2</sub> values shown are the mean of duplicate measurements.

The pharmacokinetic profile of compound 3q was evaluated after single intravenous and oral administration to male Sprague–Dawley rats and male cynomolgus monkeys. As illustrated in Table 8, 3q displayed favorable oral bioavailability after oral administration at a dose of 3 mg/kg in fed rats (24%) and monkeys (46%), respectively. As shown in Table 9, the values of  $C_{max}$  in fasted animals were higher than those in the fed state, whereas the values of AUC<sub>0-24h</sub> in the fasted and fed states were almost the same. These results indicated that 3q



Figure 3. Superimposed binding model of compounds 3i (orange), 3m (white), and 3p (green) in FXa.

has favorable oral bioavailability in rats and monkeys without significant influence from food intake. Furthermore, **3q** showed considerably weak inhibition of CYP isoforms at 10  $\mu$ M (CYP2C8, 35% inhibition;<sup>30</sup> CYP2C9, 0% inhibition;<sup>30</sup> CYP2D6, 5% inhibition<sup>31</sup>).

In order to evaluate the efficacy of compound 3q as an oral anticoagulant agent, it was tested in a rat model of venous thrombosis (Figure 5). Compound 3q at doses of 1, 3, and 10 mg/kg po reduced thrombus formation by 32%, 53%, and 86%, respectively. On the other hand, warfarin, an orally active antithrombotic agent, reduced thrombus formation by 82% at 1 mg/kg po; however, its antithrombotic effect was reduced remarkably at 0.3 mg/kg po.

The bleeding times for compound 3q and warfarin were determined in rats and are shown in Table 10. Compound 3q at 30 mg/kg po had little effect on the bleeding time, and a higher dose (100 mg/kg, po) tended to prolong bleeding time to 2.3 times the control value. Meanwhile, warfarin at 1 mg/kg po (minimal dose preventing thrombus formation in rat model) showed significant prolongation of bleeding time to 4.4 times the control value. These results suggest that compound 3q could achieve antithrombotic efficacy without a significant increase in bleeding time in rats, unlike warfarin. On the basis of its potency, selectivity, pharmacokinetic profile, and in vivo efficacy, compound 3q was selected for further evaluation.

To predict the binding mode of compound 3q, a molecular modeling study was carried out using the program GOLD and the X-ray structure of FXa reported by Sanofi-Aventis for an inhibitor complex (PDB code 1EZQ)<sup>24</sup> (Figure 6). In the model, the imidazo[1,5-c]imidazol-3-one moiety forms no salt bridge with the carboxyl group of Asp189 at the bottom of the S1 pocket, while the 6-chloronaphthyl group is deeply buried inside the hydrophobic S1 region with the chlorine atom pointing toward the center of the Tyr228 aromatic ring.<sup>7,11b,22b,32</sup> Compound 3q represents a related example in FXa inhibition, wherein a basic group thought to be essential in forming a crucial salt bridge with Asp189<sup>6d,24</sup> can be eliminated and replaced by a neutral group capable of providing high affinity for the enzyme. A hydrogen bonding interaction between the amide carbonyl group in 3q and the backbone amide nitrogen of Gly219 is observed. Recently, other researchers have reported that the important interaction for high affinity to FXa is not an ionic interaction with Asp189 but hydrogen bonding interactions with the carbonyl groups in the amide linkages of Gly216 and/ or Gly219.<sup>24,32c,33</sup> The imidazo[1,5-c]imidazol-3-one moiety is

Table 5. In Vitro Activities of Imidazo[1,5-c]imidazol-3-ones 3q-s

С



					R		
compd	$\mathbb{R}^1$	$\mathbb{R}^2$	Х	human FXa, IC <sub>50</sub> $(\mu M)^a$	human PT, $PT_2 (\mu M)^b$	$\log D^c$	$pK_a^d$
3m				0.037 (0.036-0.039)	0.91	2.2	7.9
3q	Me	Н	CH	0.0048 (0.0046-0.0051)	1.0	2.3	5.0
3r	Me	Me	CH	0.0053 (0.0048-0.0059)	1.2	2.5	5.4
3s	Me	Н	Ν	0.0069 (0.0061-0.0078)	0.61	2.1	5.0

<sup>*a*</sup> Inhibitory activity against human FXa. IC<sub>50</sub> values shown are the mean of duplicate measurements. The 95% confidence intervals are shown in parentheses. <sup>*b*</sup> PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT<sub>2</sub> values shown are the mean of duplicate measurements. <sup>*c*</sup> Measured at pH 7.4. <sup>*d*</sup> Measured using a titration method.

Table 6. In Vitro and Ex Vivo Activities of Potent FXa Inhibitors

in vitro			ex vivo.	CYP3A4.	
compd	human PT PT <sub>2</sub> $(\mu M)^a$	mouse PT PT <sub>2</sub> ( $\mu$ M) <sup><i>a</i></sup>	mouse PT Ratio <sup>b</sup>	% inhibition at 10 $\mu$ M	
3a	0.48	3.0	1.1	42	
3d	0.49	2.7	1.2	82	
3e	0.56	3.4	1.2	25	
3i	0.99	3.3	1.1	34	
3m	0.91	1.7	1.1	38	
3р	0.99	2.1	1.1	53	
3q	1.0	2.0	1.7	23	
3r	1.2	2.5	2.0	62	
3s	0.61	0.9	2.8	38	

<sup>*a*</sup> PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT<sub>2</sub> values shown are the mean of duplicate measurements. <sup>*b*</sup> Ex vivo mouse PT prolonging activity was determined 1 h after oral administration to mice at a dose of 30 mg/kg and expressed as ratios of the PT of the compound in treatment mice with that of the untreated group (n = 3).



Figure 4. Ex vivo PT prolonging activities of compounds 3q and 3s in cynomolgus monkeys (n = 3) at 3 mg/kg, po, under fasted conditions (A) and fed conditions (B).

located deeply in the hydrophobic S4 aryl binding site. The S4 pocket is a narrow hydrophobic channel defined by the aromatic rings of Tyr99, Phe174, and Trp215, and hydrophobic interaction with the three aromatic rings in the S4 site has been reported for many potent FXa inhibitors.<sup>24,32c,33</sup> The imidazo[1,5-*c*]imidazol-3-one is sandwiched between the aromatic rings of Tyr99 and that of Phe174 and forms  $\pi - \pi$  interactions with the aromatic rings of Tyr99 and Phe174 and a CH $-\pi$  interaction with the indole ring of Trp215. The carbonyl group of the

Table 7.	Selectivity	Profile	for	Compound	30
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	serine protease				
	FXa	thrombin	trypsin	plasmin	t-PA
$K_i \ (\mu M)^a$	$0.0020 \pm 0.0010$	$6.2\pm0.4$	>60	>60	>60

<sup>*a*</sup> Data are expressed as the mean  $\pm$  SEM of three determinations.

Table 8. Pharmacokinetic Parameters for 3q in Fed Rats and Monkeys<sup>a</sup>

	-	•
iv (0.3 mg/kg)	rat	monkey
$C_{5\min}$ (ng/mL)	$291 \pm 45$	$612 \pm 98$
$AUC_{0-24h}$ (ng•h/mL)	$245\pm38$	$565 \pm 94$
MRT (h)	$0.7 \pm 0.1$	$1.0 \pm 0.2$
$V_{\rm d(ss)}$ (mL/kg)	$888 \pm 149$	$556\pm38$
CL <sub>total</sub> (mL/h/kg)	$1245\pm189$	$541 \pm 90$
po (3 mg/kg)	rat	monkey
$C_{\rm max}$ (ng/mL)	$145 \pm 26$	$269 \pm 79$
$T_{\rm max}$ (h)	$1.2 \pm 0.8$	$2.3 \pm 1.5$
$AUC_{0-24h}$ (ng · h/mL)	$597 \pm 159$	$2592\pm580$
MRT (h)	$3.6 \pm 0.6$	$7.0 \pm 0.6$
BA (%)	$24 \pm 8$	$46 \pm 6$

<sup>*a*</sup> Data are expressed as the mean  $\pm$  SD of three determinations.

**Table 9.** Pharmacokinetic Parameters for 3q after Oral Administration at a Dose of 3 mg/kg in the Fed and Fasted States<sup>*a*</sup>

fed state	rat	monkey
$C_{\rm max}$ (ng/mL)	$145 \pm 26$	$269\pm79$
$T_{\rm max}$ (h)	$1.2 \pm 0.8$	$2.3 \pm 1.5$
MRT (h)	$3.6 \pm 0.6$	$7.0 \pm 0.6$
$AUC_{0-24h}$ (ng·h/mL)	$597 \pm 159$	$2592\pm580$
fasted state	rat	monkey
$C_{\rm max}$ (ng/mL)	$570 \pm 90$	$1204 \pm 451$
$T_{\rm max}$ (h)	$0.3 \pm 0.0$	$0.8 \pm 0.3$
MRT (h)	$1.5 \pm 0.4$	$1.6 \pm 0.2$
$AUC_{0-24h}$ (ng·h/mL)	$417\pm10$	$2122\pm1030$

<sup>*a*</sup> Data are expressed as the mean  $\pm$  SD of three determinations.

imidazo[1,5-*c*]imidazol-3-one does not appear to interact directly with FXa but rather effects a planarization of the imidazo[1,5-*c*]imidazol-3-one ring and brings it into a perpendicular arrangement with respect to the piperidine ring. The sulfone oxygen is involved in a hydrogen bond with the Gln192 main chain nitrogen.<sup>6d</sup>

#### Conclusion

In order to address the pharmacokinetic liabilities of our FXa inhibitor 1, chemical modification of its 4-(imidazo[1,2-a]pyridin-5-yl)piperazine moiety to other basic substituents led to the discovery of potent FXa inhibitor 4-(imidazo[1,2-a]pyridin-3-yl)piperidine 2 as an interesting new lead. Further modification focused on shifting the imidazole ring into the deeper S4 site and lowering the flexibility and basicity resulting in the discovery of the imidazo[1,5-c]imidazol-3-ones 3q, 3r, and 3s as highly potent FXa inhibitors. As a result of further investigation, 3q showed favorable oral bioavailability in rats and monkeys without significant influence from food intake, good enzyme selectivity, and weak inhibition of CYP3A4. Oral administration of 3q prevented thrombus formation dosedependently in rats without a significant increase in bleeding time, unlike warfarin. On the basis of its excellent in vitro and in vivo efficacy and a favorable pharmacokinetic profile, compound 3q was selected for further evaluation.

#### **Experimental Section**

Melting points were determined with a Yanagimoto melting point apparatus or a Büchi melting point apparatus B-545 and are uncorrected. <sup>1</sup>H NMR spectra were obtained at 300 or 200 MHz on a Varian Ultra-300 or a Varian Gemini-200 spectrometer. Chemical shifts are given in  $\delta$  values (ppm) using tetramethylsilane as the internal standard. 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 (ChromatorexNH, 100–200 mesh, Fuji Silysia Chemical Ltd.) using the indicated eluents. Yields are unoptimized. Chemical intermediates were characterized by <sup>1</sup>H NMR.

*tert*-Butyl 4-(Imidazo[1,2-*a*]pyridin-3-yl)piperidine-1-carboxylate (6). A solution of 4 (13.7 g, 44.7 mmol) and 5 (2.63 g, 27.9 mmol) in EtOH (100 mL) was refluxed for 3 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between EtOAc and water. The aqueous layer was separated, basified with 8 M NaOH to pH 9, and extracted with EtOAc. The extract was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 10/1) to give 6 (8.11 g, 60%) as a pale-yellow solid. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.49 (9H, s), 1.59–1.85 (2H, m), 1.98–2.17 (2H, m), 2.83–3.10 (3H, m), 4.18–4.38 (2H, m), 6.83 (1H, t, J = 6.4 Hz), 7.16 (1H, t, J = 8.0 Hz), 7.41 (1H, s), 7.62 (1H, d, J = 9.2 Hz), 7.97 (1H, d, J = 6.8 Hz).

**3-(Piperidin-4-yl)imidazo[1,2-***a***]pyridine Dihydrochloride (7a). A solution of 6 (9.04 g, 30.0 mmol) in concentrated HCl (49 mL) was stirred at room temperature for 10 min. The reaction mixture was diluted with EtOH and concentrated in vacuo. The precipitated powder was collected by filtration and washed with EtOH and Et<sub>2</sub>O to give <b>7a** (7.92 g, 91%) as a colorless powder. <sup>1</sup>H NMR (200 MHz, D<sub>2</sub>O)  $\delta$ : 1.94–2.22 (2H, m), 2.39–2.59 (2H, m), 3.23–3.48 (2H, m), 3.52–3.78 (3H, m), 7.48–7.61 (1H, m), 7.86 (1H, s), 7.98–8.04 (2H, m), 8.71 (1H, d, *J* = 7.0 Hz).

**3-(Piperidin-4-yl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine Dihydrochloride (7b).** A mixture of **6** (1.51 g, 5.01 mmol) and platinum dioxide (114 mg) in acetic acid (15 mL) was hydrogenated at atmospheric pressure and room temperature for 16 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was diluted with water, basified with 8 M NaOH to pH 12, and extracted with CHCl<sub>3</sub>. The extract was washed with brine, dried over anhydrous MgSO<sub>4</sub>, and concentrated in vacuo to give *tert*-butyl 4-(5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-3-yl)piperidine-1-carboxylate (1.53 g, quantitative) as a pale-yellow amorphous powder.

A mixture of *tert*-butyl 4-(5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-3-yl)piperidine-1-carboxylate (1.53 g, 5.01 mmol) and concentrated HCl (8.20 mL) was stirred at room temperature for 10 min. The reaction mixture was diluted with 2-propanol and concentrated in vacuo. The precipitate was collected by filtration and washed with 2-propanol and Et<sub>2</sub>O to give **7b** (1.32 g, 95%) as a colorless powder. <sup>1</sup>H NMR (200 MHz, D<sub>2</sub>O)  $\delta$ : 1.74–2.19 (6H, m), 2.20–2.37 (2H, m), 2.95–3.31 (5H, m), 3.50–3.66 (2H, m), 4.11 (2H, t, J = 5.4 Hz), 7.20 (1H, s).

3-(1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperidin-4-yl)imidazo[1,2-a]pyridine Hydrochloride (2). To a solution of 8 (0.74 g, 2.48 mmol) and HOBt (0.57 g, 3.72 mmol) in acetonitrile (15 mL) was added WSC (0.72 g, 3.75 mmol), and the mixture was stirred at room temperature for 15 min. A solution of 7a (0.82 g, 2.99 mmol), DBU (0.90 mL, 6.02 mmol), and Et<sub>3</sub>N (1.05 mL, 7.50 mmol) in acetonitrile (5 mL) was added, and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with water and extracted with CHCl<sub>3</sub>. The extract was washed with brine, dried over anhydrous MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/ EtOH = 10/1). To a solution of this product in EtOH (5 mL) was added concentrated HCl (0.38 mL, 4.60 mmol), and the resulting mixture was concentrated in vacuo. The residue was diluted with EtOH and Et<sub>2</sub>O, and the precipitate was collected by filtration to give 2 (1.14 g, 81%) as a colorless powder, mp 224–225 °C. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ: 1.28-1.39 (1H, m), 1.39-1.68 (1H, m), 1.93–2.11 (2H, m), 2.62–2.84 (3H, m), 3.12–3.28 (1H, m), 3.32-3.57 (1H, m), 3.61-3.72 (2H, m), 3.88-4.01 (1H, m), 4.31-4.45 (1H, m), 7.54 (1H, t, J = 6.6 Hz), 7.73 (1H, dd, J =7.4 and 6.0 Hz), 7.93-8.04 (4H, m), 8.19 (1H, d, J = 8.7 Hz), 8.26–8.31 (2H, m), 8.68 (1H, s), 9.02 (1H, d, *J* = 6.9 Hz). Anal.  $(C_{25}H_{24}ClN_3O_3S \cdot HCl \cdot H_2O) C, H, N.$ 

**3-(1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperidin-4-yl)-5,6,7,8-tetrahydroimidazo[1,2-***a***]pyridine Hydrochloride (<b>3a**). Compound **3a** was prepared in a manner similar to that described for **2** in 82% yield as a colorless powder, mp 213–214 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 1.12–1.58 (2H, m), 1.76–2.08 (6H, m), 2.48–2.68 (2H, m), 2.75 (2H, t, *J* = 7.4 Hz), 2.84–3.19 (3H, m), 3.64 (2H, t, *J* = 7.0 Hz), 3.34–3.99 (1H, m), 4.00–4.12 (2H, m), 4.26–4.40 (1H, m), 7.34 (1H, s), 7.74 (1H, dd, *J* = 8.8 and 2.2 Hz), 8.01 (1H, dd, *J* = 8.8 and 1.8 Hz), 8.17–8.32 (3H, m), 8.67 (1H, s). Anal. (C<sub>25</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>3</sub>S•HCl•H<sub>2</sub>O) C, H, N.

1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(1*H*imidazol-4-yl)piperidine (3b). To a mixture of 8 (0.60 g, 2.00 mmol) and HOBt (0.46 g, 3.00 mmol) in acetonitrile (15 mL) was added WSC (0.58 g, 3.00 mmol), and the mixture was stirred at room temperature for 15 min. A solution of 9a (0.46 g, 2.00 mmol),



Figure 5. Antithrombotic effect of 3q and warfarin in a rat venous thrombosis model. Thrombus formation was stimulated by the combination of endothelial damage and blood stagnation. Data are expressed as the mean  $\pm$  SEM: (\*) p = 0.025 vs vehicle (a one-tailed Williams test), n = 5-6.

Table 10.	Effect of 30	and Warfarir	on Bleeding	Time in Rats <sup>a</sup>
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	-	•
	dose (mg/kg, po)	bleeding time (s)
vehicle		$460 \pm 60$
3q	30	$490 \pm 90$
-	100	$1040\pm296$
vehicle		$360 \pm 35$
warfarin	0.3	$445 \pm 87$
	1	$1590 \pm 158^{b}$

<sup>*a*</sup> Data are expressed as the mean  $\pm$  SEM. <sup>*b*</sup>  $p \leq 0.025$  vs vehicle (a one-tailed Shirley–Williams' test). n = 6.



Figure 6. Binding model of imidazo[1,5-*c*]imidazol-3-one 3q in FXa.

DBU (0.61 g, 4.00 mmol), and Et<sub>3</sub>N (0.61 g, 6.00 mmol) in acetonitrile (15 mL) was added to the mixture, and the resulting mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with aqueous K<sub>2</sub>CO<sub>3</sub> solution and extracted with EtOAc. The extract was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc) to give **3b** (0.43 g, 50%) as a colorless amorphous powder. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub> + CD<sub>3</sub>OD)  $\delta$ : 1.40–1.60 (2H, m), 1.95–2.11 (2H, m), 2.66–2.91 (5H, m), 3.13–3.20 (1H, m), 3.54–3.61 (2H, m), 3.86–3.90 (1H, m), 4.47–4.51 (1H, m), 6.70 (1H, s), 7.53 (1H, d, *J* = 1.2 Hz), 7.59 (1H, dd, *J* = 8.4 and 2.4 Hz), 7.93–7.98 (4H, m), 8.48 (1H, s). Anal. (C<sub>21</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>3</sub>•0.5H<sub>2</sub>O) C, H, N.

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(2-meth-yl-1H-imidazol-4-yl)piperidine (3c).** Compound **3c** was prepared in a manner similar to that described for **3b** in 91% yield as a colorless powder, mp 190–192 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.41–1.60 (2H, m), 1.93–2.10 (2H, m), 2.39 (3H, s), 2.59–2.71 (2H, m), 2.83–2.91 (2H, m), 3.07–3.21 (1H, m), 3.53–3.60 (2H, m), 3.83–3.90 (1H, m), 4.47–4.54 (1H, m), 6.56 (1H, s), 7.58 (1H, dd, *J* = 9.0 and 2.0 Hz), 7.88–7.97 (4H, m), 8.48 (1H, br s). Anal. (C<sub>22</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>3</sub>S•0.2H<sub>2</sub>O) C, H, N.

1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(1-trityl-1H-imidazol-4-yl)piperidine (10a). To a solution of 3b (0.35 g, 0.81 mmol) and  $Et_3N$  (0.10 g, 1.00 mmol) in DMF (10 mL) was added trityl chloride (0.25 g, 0.90 mmol) at 0 °C, and the resulting mixture was stirred at 0 °C for 1 h and then at room temperature for 15 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with saturated aqueous NaHCO3 solution and extracted with EtOAc. The extract was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc) to give **10a** (0.54 g, quantitative) as a colorless amorphous powder. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ: 1.40–1.50 (2H, m), 1.92–2.10 (2H, m), 2.58-2.87 (4H, m), 3.04-3.17 (1H, m), 3.51-3.59 (2H, m), 3.78-3.84 (1H, m), 4.41-4.47 (1H, m), 6.49 (1H, s), 7.09-7.35 (16H, m), 7.57 (1H, dd, J = 8.8 and 1.8 Hz), 7.92–8.02 (4H, m), 8.46 (1H, s).

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(2-methyl-1-trityl-1***H***-imidazol-4-yl)piperidine (10b). Compound 10b was prepared in a manner similar to that described for 10a in 60% yield as a colorless amorphous powder. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) \delta: 1.33–1.59 (2H, m), 1.90–2.06 (2H, m), 2.57–2.74 (2H, m), 2.82–2.89 (2H, m), 3.05–3.13 (1H, m), 3.52–3.57 (2H, m), 3.80–3.84 (1H, m), 4.45–4.49 (1H, m), 6.34 (1H, s), 7.09–7.12 (5H, m), 7.30–7.35 (10H, m), 7.58 (1H, dd,** *J* **= 8.7 and 1.8 Hz), 7.91–7.96 (4H, m), 8.47 (1H, s).** 

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(1-methyl-1***H***-imidazol-5-yl)piperidine (3d). A solution of <b>10a** (0.54 g, 0.80 mmol) and iodomethane (0.10 mL, 1.61 mmol) in DMF (5 mL) was stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in acetic acid (5 mL), water (5 mL), and MeOH (2 mL). After being stirred at 95 °C for 2 h, the reaction mixture was concentrated in vacuo. The residue was basified with saturated aqueous NaHCO<sub>3</sub> solution and extracted with EtOAc. The extract was washed with saturated aqueous NaHCO<sub>3</sub> solution, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc to 10/1 EtOAc/MeOH) to give **3d** (0.27 g, 76%) as a colorless amorphous powder. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.43–1.70 (2H, m), 1.88–2.04 (2H, m), 2.60–2.78 (2H, m), 2.87–2.95 (2H, m), 3.09–3.22 (1H, m), 3.53–3.61 (2H, m), 3.60 (3H, s), 3.91–3.98 (1H, m), 4.55–4.62 (1H, m), 6.76 (1H, s), 7.38 (1H, s), 7.59 (1H, dd, J = 8.8 and 1.8 Hz), 7.93–7.97 (4H, m), 8.48 (1H, s). Anal. (C<sub>22</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>3</sub>S•0.5H<sub>2</sub>O) C, H, N.

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(1,2-dimethyl-1***H***-imidazol-5-yl)piperidine (3e). Compound 3e was prepared in a manner similar to that described for 3d in 48% yield as a colorless amorphous powder. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) \delta: 1.40–1.56 (2H, m), 1.87–2.00 (2H, m), 2.36 (3H, s), 2.60–2.71 (2H, m), 2.87–2.95 (2H, m), 3.09–3.21 (1H, m), 3.45 (3H, s), 3.53–3.61 (2H, m), 3.90–3.96 (1H, m), 4.54–4.60 (1H, m), 6.60 (1H, s), 7.60 (1H, dd, J = 8.8 and 1.8 Hz), 7.93–7.97 (4H, m), 8.49 (1H, s). Anal. (C<sub>23</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>3</sub>S·H<sub>2</sub>O) C, H, N.** 

*tert*-Butyl 4-(2-Ethyl-1*H*-imidazol-1-yl)piperidine-1-carboxylate (13a). A mixture of 11 (10.0 g, 35.8 mmol), 12a (4.13 g, 43.0 mmol), and K<sub>2</sub>CO<sub>3</sub> (4.95 g, 35.8 mmol) in DMF (80 mL) was stirred at 100 °C for 72 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with aqueous NaHCO<sub>3</sub> solution and extracted with EtOAc. The extract was washed with saturated aqueous NaHCO<sub>3</sub> solution, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc to 5/1 EtOAc/MeOH) to give 13a (1.10 g, 11%) as a yellow oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.31–1.39 (3H, m), 1.49 (9H, s), 1.70–1.95 (4H, m), 2.67–2.87 (4H, m), 3.96–4.01 (1H, m), 4.29–4.33 (2H, m), 6.86 (1H, d, *J* = 1.5 Hz), 6.97 (1H, d, *J* = 1.5 Hz).

*tert*-Butyl 4-(2-Propyl-1*H*-imidazol-1-yl)piperidine-1-carboxylate (13b). Compound 13b was prepared in a manner similar to that described for 13a in 3% yield as a colorless amorphous powder. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.98–1.04 (3H, m), 1.49 (9H, s), 1.71–1.94 (6H, m), 2.59–2.69 (2H, m), 2.79–2.87 (2H, m), 3.96–4.04 (1H, m), 4.28–4.33 (2H, m), 6.85 (1H, d, J = 1.2 Hz), 6.97 (1H, d, J = 1.5 Hz).

1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(1Himidazol-1-yl)piperidine (3h). A mixture of 13c (0.28 g, 1.11 mmol) in concentrated HCl (4 mL) was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo, and the residue was azeotroped with EtOH. The residue, DBU (0.33 mL, 2.23 mmol), and Et<sub>3</sub>N (0.47 mL, 3.36 mmol) were dissolved in acetonitrile (15 mL). The solution was added to a mixture of 8 (0.33 g, 1.10 mmol), HOBt (0.26 g, 1.70 mmol), and WSC (0.32 g, 1.70 mmol) in acetonitrile (15 mL), and the resulting mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with aqueous K<sub>2</sub>CO<sub>3</sub> solution and extracted with EtOAc. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc) to give **3h** (0.40 g, 83%) as a yellow gum. <sup>1</sup>H NMR (300 MHz,  $CDCl_3$ ) δ: 1.70-1.92 (2H, m), 2.09-2.22 (2H, m), 2.64-2.72 (1H, m), 2.90-2.97 (2H, m), 3.17-3.25 (1H, m), 3.54-3.61 (2H, m), 4.00-4.09 (1H, m), 4.08-4.21 (1H, m), 4.69-4.73 (1H, m), 6.93 (1H, t, J = 1.2 Hz), 7.08 (1H, d, J = 1.2 Hz), 7.54 (1H, s), 7.60 (1H, dd, J = 8.7 and 2.1 Hz), 7.93-7.97 (4H, m), 8.49 (1H, d, J = 1.2 Hz). Anal.  $(C_{21}H_{22}CIN_3O_3S \cdot 0.5H_2O)$  C, H, N.

The following compounds **3f**, **3g**, and **3i** were prepared in a manner similar to that described for **3h**.

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(2-eth-yl-1H-imidazol-1-yl)piperidine (3f).** Yield 98%, colorless amorphous powder. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.36 (3H, t, J = 7.4 Hz), 1.65–1.86 (2H, m), 1.96–2.05 (2H, m), 2.61–2.68 (1H, m), 2.71 (2H, q, J = 7.5 Hz), 2.91–2.98 (2H, m), 3.16–3.24 (1H, m), 3.54–3.62 (2H, m), 4.01–4.13 (2H, m), 4.73–4.77 (1H, m), 6.81 (1H, d, J = 1.8 Hz), 6.98 (1H, d, J = 1.5 Hz), 7.60 (1H, dd, J = 8.7 and 1.8 Hz), 7.94–7.97 (4H, m), 8.49 (1H, d, J = 0.6 Hz). Anal. (C<sub>23</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>3</sub>S·0.5H<sub>2</sub>O) C, H, N.

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(2-propyl-1***H***-imidazol-1-yl)piperidine (3g). Yield 66%, colorless amorphous powder. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) \delta: 1.02 (3H, t,** *J* **= 7.5 Hz), 1.68–2.05 (6H, m), 2.62–2.69 (3H, m), 2.90–2.99 (2H,**  m), 3.14-3.26 (1H, m), 3.54-3.63 (2H, m), 4.01-4.14 (2H, m), 4.71-4.78 (1H, m), 6.80 (1H, d, J = 1.4 Hz), 6.97 (1H, d, J = 1.0 Hz), 7.60 (1H, dd, J = 8.8 and 1.8 Hz), 7.89-7.98 (4H, m), 8.49 (1H, s). Anal. ( $C_{24}H_{28}CIN_3O_3S \cdot 0.5H_2O$ ) C, H, N.

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(2-methyl-1***H***-imidazol-1-yl)piperidine (<b>3**i). Yield 83%, colorless amorphous powder. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.68–1.84 (2H, m), 1.97–2.09 (2H, m), 2.42 (3H, s), 2.61–2.69 (1H, m), 2.91–2.98 (2H, m), 3.15–3.24 (1H, m), 3.54–3.62 (2H, m), 4.02–4.13 (2H, m), 4.73–4.77 (1H, m), 6.81 (1H, d, *J* = 1.5 Hz), 6.94 (1H, d, *J* = 1.5 Hz), 7.60 (1H, dd, *J* = 8.9 and 2.0 Hz), 7.91–7.97 (4H, m), 8.50 (1H, s). Anal. (C<sub>22</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>3</sub>S·H<sub>2</sub>O) C, H, N.

*tert*-Butyl 4-(1-Methyl-1*H*-imidazol-2-yl)piperidine-1-carboxylate (15). To a solution of 14 (0.25 g, 1.00 mmol) in DMF (3 mL) was added NaH (60% in oil, 40 mg, 1.00 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. Iodomethane (0.07 mL, 1.12 mmol) was added to the mixture, and then the resulting mixture was stirred at 0 °C for 2 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with aqueous NaHCO<sub>3</sub> solution and extracted with EtOAc. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was purified by silica gel chromatography (CHCl<sub>3</sub>/MeOH = 10/1) to give 15 (0.27 g, quantitative) as a pale-yellow oil. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.46 (9H, s), 1.80–1.91 (4H, m), 2.70–2.96 (3H, m), 3.61 (3H, s), 4.18–4.25 (2H, m), 6.79 (1H, d, *J* = 1.0 Hz), 6.94 (1H, d, *J* = 1.0 Hz).

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(1***H***-<b>imidazol-2-yl)piperidine (3j).** Compound **3j** was prepared in a manner similar to that described for **3h** in 54% yield as a colorless amorphous powder. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.60–1.85 (2H, m), 1.98–2.14 (2H, m), 2.72–3.04 (4H, m), 3.15–3.24 (1H, m), 3.49–3.61 (2H, m), 3.90–3.95 (1H, m), 4.46–4.50 (1H, m), 6.96 (1H, br s), 7.00 (1H, br s), 7.58 (1H, dd, J = 2.1 and 9.0 Hz), 7.90–7.97 (4H, m), 8.48 (1H, d, J = 0.9 Hz), 8.88 (1H, br s). Anal. (C<sub>21</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>3</sub>S) C, H, N.

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-(1-methyl-1***H***-imidazol-2-yl)piperidine (3k). Compound 3k was prepared in a manner similar to that described for 3h in 70% yield as a colorless amorphous powder. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) \delta: 1.60–2.00 (4H, m), 2.70–2.94 (4H, m), 3.11–3.26 (1H, m), 3.52–3.61 (2H, m), 3.61 (3H, s), 3.93–4.00 (1H, m), 4.45–4.52 (1H, m), 6.79 (1H, d, J = 1.2 Hz), 6.92 (1H, d, J = 1.4 Hz), 7.58 (1H, dd, J = 2.0 and 8.8 Hz), 7.89–7.98 (4H, m), 8.48 (1H, s), 8.88 (1H, br s). Anal. (C<sub>22</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>3</sub>S•0.5H<sub>2</sub>O) C, H, N.** 

*tert*-Butyl 4-[(2-Methyl-1*H*-imidazol-1-yl)methyl]piperidine-1-carboxylate (17a). To a solution of 16a (8.50 g, 30.6 mmol) and 2-methylimidazole (1.80 g, 21.9 mmol) in DMF (100 mL) was added NaH (60% in oil, 0.87 g, 21.8 mmol) at room temperature. After the mixture was stirred at 80 °C for 12 h, the reaction mixture was concentrated in vacuo. The residue was diluted with CHCl<sub>3</sub>, washed with aqueous K<sub>2</sub>CO<sub>3</sub> solution, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 5/1 EtOAc/MeOH) to give 17a (0.43 g, 7%) as a brown oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.13–1.26 (2H, m), 1.46 (9H, s), 1.68 (1H, m), 2.37 (3H, s), 2.67 (2H, t, J = 11.4 Hz), 3.86 (2H, t, J = 7.5 Hz), 4.11 (2H, br s), 6.80 (1H, d, J = 1.5 Hz), 6.91 (1H, d, J = 1.5 Hz).

The following compounds 17b-d were prepared in a manner similar to that described for 17a.

*tert*-Butyl 4-[2-(2-Methyl-1*H*-imidazol-1-yl)ethyl]piperidine-1-carboxylate (17b). Yield 80%, brown oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.13–1.28 (2H, m), 1.46 (9H, s), 1.57–1.63 (4H, m), 1.82 (1H, m), 2.37 (3H, s), 2.65 (2H, t, J = 12.9 Hz), 3.71 (2H, d, J = 7.5 Hz), 4.16 (2H, br s), 6.78 (1H, d, J = 1.5 Hz), 6.91 (1H, d, J = 1.5 Hz).

*tert*-Butyl 4-[3-(2-Methyl-1*H*-imidazol-1-yl)propyl]piperidine-1-carboxylate (17c). Yield quantitative, brown oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.03–1.35 (4H, m), 1.45 (9H, s), 1.59–1.82 (5H, m), 2.37 (3H, s), 2.66 (2H, t, J = 12.4 Hz), 3.81 (2H, t, J = 7.0 Hz), 4.06 (2H, br s), 6.80 (1H, d, J = 1.4 Hz), 6.90 (1H, d, J = 1.4 Hz). *tert*-Butyl 4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]piperidine-1-carboxylate (17d). Yield 47%, brown oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.03–1.35 (4H, m), 1.45 (9H, s), 1.59–1.82 (7H, m), 2.37 (3H, s), 2.66 (2H, t, J = 12.4 Hz), 3.81 (2H, t, J = 7.0 Hz), 4.06 (2H, br s), 6.80 (1H, d, J = 1.4 Hz), 6.90 (1H, d, J = 1.4 Hz).

1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-[(2methyl-1H-imidazol-1-yl)methyl]piperidine Hydrochloride (3l). 17a (0.43 g, 1.54 mmol) was dissolved in concentrated HCl (4 mL) and diluted with EtOH (20 mL). The solution was concentrated in vacuo, and the residue was dissolved in acetonitrile (20 mL) with Et<sub>3</sub>N (0.63 mL, 4.51 mmol) and DBU (0.46 mL, 3.26 mmol). The solution was added to a mixture of 8 (0.45 g, 1.50 mmol), WSC (0.43 g, 2.24 mmol), and HOBt (0.35 g, 2.29 mmol) in acetonitrile (10 mL), and then the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo, diluted with saturated aqueous NaHCO<sub>3</sub> solution, and extracted with CHCl<sub>3</sub>. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc), and the product was dissolved in EtOAc. An amount of 4 M HCl in EtOAc (0.30 mL, 1.20 mmol) was added, and then the mixture was stirred at room temperature for 5 min. The resulting mixture was concentrated in vacuo, and the residue was triturated with  $Et_2O$  to give **31** (0.55 g, 74%) as a beige amorphous powder. <sup>1</sup>H NMR (200 MHz, DMSO- $d_6$ )  $\delta$ : 1.04–1.25 (2H, m), 1.63–1.73 (2H, m), 1.91 (1H, m), 2.37 (3H, s), 2.52 (1H, m), 2.82–3.04 (3H, m), 3.51–3.60 (2H, m), 3.70 (2H, d, *J* = 7.4 Hz), 3.88 (1H, d, J = 13.4 Hz), 4.56 (1H, d, J = 13.4 Hz), 6.76 (1H, d, J = 1.4 Hz), 6.92 (1H, d, J = 1.4 Hz), 7.60 (1H, dd, J = 2.0 and 8.8 Hz), 7.87-7.97 (4H, m), 8.48 (1H, s). Anal.  $(C_{23}H_{26}CIN_3O_3S \cdot HCl \cdot H_2O \cdot 0.25Et_2O) C, H, N.$ 

The following compounds **3m** and **3n** were prepared in a manner similar to that described for **3l**.

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-[2-(2-methyl-1***H***-imidazol-1-yl)ethyl]piperidine Hydrochloride (3m). Yield 69%, beige amorphous powder. <sup>1</sup>H NMR (200 MHz, DMSO-d\_6) & 1.00–1.24 (2H, m), 1.48 (1H, m), 1.63–1.77 (4H, m), 2.37 (3H, s), 2.49 (1H, t,** *J* **= 13.2 Hz), 2.83–2.90 (2H, m), 2.98 (1H, t,** *J* **= 13.2 Hz), 3.47–3.60 (2H, m), 3.80–3.88 (3H, m), 4.49 (1H, d,** *J* **= 11.4 Hz), 6.79 (1H, s), 6.91 (1H, s), 7.59 (1H, dd,** *J* **= 1.8 and 9.0 Hz), 7.90–7.96 (4H, m), 8.47 (1H, s). Anal. (C<sub>24</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>3</sub>S·HCl·0.25H<sub>2</sub>O) C, H, N.** 

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-[3-(2-methyl-1***H***-imidazol-1-yl)propyl]piperidine Hydrochloride (3n). Yield 53%, beige amorphous powder. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) \delta: 1.00–1.31 (4H, m), 1.52 (1H, m), 1.65–1.88 (4H, m), 2.37 (3H, s), 2.49 (1H, t, J = 11.1 Hz), 2.83–2.90 (2H, m), 3.00 (1H, t, J = 11.1 Hz), 3.47–3.58 (2H, m), 3.84 (1H, d, J = 14.1 Hz), 4.02 (2H, t, J = 6.9 Hz), 4.51 (1H, d, J = 14.1 Hz), 7.05 (1H, s), 7.05 (1H, s), 7.61 (1H, dd, J = 2.1 and 9.0 Hz), 7.89–7.97 (4H, m), 8.47 (1H, s). Anal. (C<sub>25</sub>H<sub>30</sub>ClN<sub>3</sub>O<sub>3</sub>S+HCl+H<sub>2</sub>O) C, H, N.** 

**1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-[4-(2-methyl-1***H***-imidazol-1-yl)butyl]piperidine (30). Compound 30 was prepared in a manner similar to that described for 3h in 15% yield as a beige amorphous powder. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) \delta: 0.91–1.08 (2H, m), 1.22–1.43 (5H, m), 1.61–1.74 (4H, m), 2.37 (3H, s), 2.40–2.54 (1H, m), 2.81–3.02 (3H, m), 3.52–3.60 (2H, m), 3.78–3.85 (3H, m), 4.26 (1H, d, J = 13.2 Hz), 6.79 (1H, d, J = 1.4 Hz), 6.90 (1H, d, J = 1.4 Hz), 7.58 (1H, dd, J = 2.0 and 8.8 Hz), 7.92–7.97 (4H, m), 8.47 (1H, s). Anal. (C<sub>26</sub>H<sub>32</sub>ClN<sub>3</sub>O<sub>3</sub>S·0.5H<sub>2</sub>O) C, H, N.** 

*tert*-Butyl 4-[(2-Methyl-1*H*-imidazol-1-yl)acetyl]piperazine-1-carboxylate (19). To a solution of 2-methylimidazole (1.10 g, 13.3 mmol) in DMF was added NaH (60% in oil, 0.62 g, 15.5 mmol) at 0 °C. After the mixture was stirred at 0 °C for 30 min, 18 (4.09 g, 13.3 mmol) was added to the mixture, and then the resulting mixture was stirred at 50 °C for 1 h. The reaction mixture was concentrated in vacuo, diluted with aqueous K<sub>2</sub>CO<sub>3</sub> solution, and extracted with EtOAc. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to give 19 (2.80 g, 68%) as a colorless oil. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.48 (9H, s), 2.33 (3H, s), 3.44-3.50 (6H, m), 3.60-3.71 (2H, m), 4.67 (2H, s), 6.80 (1H, d, <math>J = 1.6 Hz), 6.94 (1H, d, J = 1.6 Hz).

1-[2-(2-Methyl-1H-imidazole-1-yl)ethyl]piperazine (20). 19 (2.80 g, 9.08 mmol) was dissolved in TFA (15 mL), and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, diluted with saturated aqueous NaHCO<sub>3</sub> solution, and extracted with CHCl<sub>3</sub>. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was dissolved in THF (30 mL), and lithium aluminum hydride (0.46 g, 12.1 mmol) was added to the solution at 0 °C. After being stirred at 60 °C for 1 h, the reaction mixture was cooled to 0 °C. The reaction mixture was quenched with saturated ammonium chloride and extracted with EtOAc. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to give 20 (0.70 g, 40%) as a colorless oil. <sup>1</sup>H NMR (200 MHz, DMSO $d_6$ )  $\delta$ : 2.27 (3H, s), 2.49–2.69 (8H, m), 3.60 (2H, t, J = 9.1 Hz), 3.89 (2H, t, J = 9.1 Hz), 6.69 (1H, d, J = 1.6 Hz), 7.02 (1H, d, J = 1.6 Hz).

1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}-4-[2-(2methyl-1H-imidazol-1-yl)ethyl]piperazine (3p). To a solution of 8 (0.90 g, 3.01 mmol), 20 (0.58 g, 3.01 mmol), Et<sub>3</sub>N (1.26 mL, 9.03 mmol), and HOBt (0.69 g, 4.52 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added WSC (0.87 g, 4.52 mmol) at room temperature. After being stirred at room temperature for 16 h, the reaction mixture was concentrated in vacuo, diluted with saturated aqueous NaHCO3 solution, and extracted with CHCl<sub>3</sub>. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was purified by silica gel chromatography (CHCl<sub>3</sub>/MeOH = 40/1 to 10/1) to give **3p** (0.021 g, 2%) as a brown amorphous powder.  $^{1}$ H NMR (200 MHz, CDCl<sub>3</sub>) δ: 2.39 (3H, s), 2.41–2.46 (4H, m), 2.61-2.67 (2H, m), 2.82-2.89 (2H, m), 3.42-3.60 (6H, m), 3.93 (2H, t, J = 9.3 Hz), 6.85 (1H, d, J = 2.1 Hz), 6.90 (1H, d, J = 2.1 Hz)Hz), 7.59 (1H, dd, J = 2.4 and 13.2 Hz), 7.92–7.94 (4H, m), 8.47 (1H, s). Anal.  $(C_{23}H_{27}ClN_4O_3S \cdot H_2O) C, H, N.$ 

*tert*-Butyl4-{[(2-Methyl-1*H*-imidazol-5-yl)methyl]amino}piperidine-1-carboxylate (23a). A mixture of 21 (2.00 g, 10.0 mmol), 22a (1.10 g, 10.0 mmol), acetic acid (0.69 mL, 12.0 mmol), and sodium triacetoxyborohydride (5.30 g, 25.0 mmol) in MeOH (40 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo, and the residue was basified with aqueous K<sub>2</sub>CO<sub>3</sub> solution. The solution was extracted with EtOAc, and the extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to give 23a (2.94 g, quantitative) as a pale-yellow oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.26 (2H, t, *J* = 7.2 Hz), 1.45 (9H, s), 1.86 (2H, d, *J* = 12.6 Hz), 2.40 (3H, s), 2.61–2.86 (3H, m), 3.74 (2H, s), 3.86–4.18 (2H, m), 6.75 (1H, s).

*tert*-Butyl 4-{[(2,4-Dimethyl-1*H*-imidazol-5-yl)methyl]amino}piperidine-1-carboxylate (23b). Compound 23b was prepared in a manner similar to that described for 23a in quantitative yield as a pale-yellow oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.28–1.40 (2H, m), 1.45 (9H, s), 1.87 (2H, d, J = 12.3 Hz), 2.14 (3H, s), 2.33 (3H, s), 2.67–2.80 (3H, m), 3.71 (2H, s), 4.06 (2H, br s), 6.06 (1H, s).

*tert*-Butyl 4-(5-Methyl-3-oxo-1*H*-imidazo[1,5-*c*]imidazol-2(3*H*)yl)piperidine-1-carboxylate (24a). To a solution of compound 23a (2.94 g, 10.0 mmol) in acetonitrile (20 mL) was added DBU (1.50 mL, 10.0 mmol) and CDI (1.62 g, 10.0 mmol), and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo, diluted with aqueous K<sub>2</sub>CO<sub>3</sub> solution, and extracted with EtOAc. The extract was dried over anhydrous MgSO<sub>4</sub> and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 10/1 EtOAc/MeOH) to give 24a (2.45 g, 77%) as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.47 (9H, s), 1.63–1.73 (2H, m), 1.75–1.98 (2H, m), 2.61 (3H, s), 2.83 (2H, t, *J* = 12.7 Hz), 4.10 (1H, tt, *J* = 12.1 and 4.1 Hz), 4.28 (4H, s), 6.71 (1H, t, *J* = 1.5 Hz).

*tert*-Butyl 4-(5,7-Dimethyl-3-oxo-1*H*-imidazo[1,5-*c*]imidazol-2(3*H*)-yl)piperidine-1-carboxylate (24b). Compound 24b was prepared in a manner similar to that described for 24a in 97% yield as a pale-yellow oil. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.47 (9H, s), 1.57–1.72 (2H, m), 1.79–1.84 (2H, m), 2.15 (3H, s), 2.57 (3H,

s), 2.82 (2H, t, *J* = 12.7 Hz), 4.03–4.15 (1H, m), 4.20 (2H, s), 4.29 (1H, br s).

2-(1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperidin-4-yl)-5-methyl-1,2-dihydro-3H-imidazo[1,5-c]imidazol-3-one (3q). 24a (1.18 g, 3.69 mmol) was dissolved in concentrated HCl (4 mL), and the resulting mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo. The residual solid, 8 (1.00 g, 3.35 mmol), HOBt (0.62 g, 4.02 mmol), and Et<sub>3</sub>N (1.68 mL, 12.1 mmol) were suspended in DMF (7 mL). WSC (0.77 g, 4.02 mmol) was added to the suspension, and then the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was cooled to 0 °C and diluted with water (14 mL). After the mixture was stirred at 0 °C for 2 h, the precipitate was collected by filtration and washed with water. The solid was recrystallized from EtOH-water to give 3q (1.23 g, 73%) as a colorless crystal, mp 195 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ: 1.58-1.72 (2H, m), 1.86-1.99 (2H, m), 2.61 (3H, s), 2.63-2.68 (1H, m), 2.81-3.01 (2H, m), 3.14-3.23 (1H, m), 3.46-3.65 (2H, m), 3.97-4.02 (1H, m), 4.13-4.22 (1H, m), 4.25 (2H, s), 4.69-4.74 (1H, m), 6.70 (1H, d, J = 3.3 Hz), 7.59 (1H, dd, J = 8.7 and 2.1Hz), 7.89-7.96 (4H, m), 8.47 (1H, d, J = 1.5 Hz). Anal.  $(C_{24}H_{25}ClN_4O_4S)$  C, H, N.

**2-(1-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperidin-4-yl)-5,7-dimethyl-1,2-dihydro-3***H***-imidazo[1,5-***c***]imidazol-3one (3***r***). Compound 3***r* **was prepared in a manner similar to that described for 3***q* **in 50% yield as a colorless solid, mp 196–197 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) \delta: 1.62–1.79 (2H, m), 1.83–1.99 (2H, m), 2.15 (3H, s), 2.57 (3H, s), 2.64–2.71 (1H, m), 2.86–2.99 (2H, m), 3.31–3.26 (1H, m), 3.49–3.63 (2H, m), 3.97–4.18 (4H, m), 4.75–4.96 (1H, m), 7.61 (1H, dd, J = 8.8 and 1.8 Hz), 7.89–7.99 (4H, m), 8.49 (1H, s). Anal. (C<sub>25</sub>H<sub>27</sub>ClN<sub>4</sub>O<sub>4</sub>S) C, H, N.** 

**4-Benzyl-N-[(2-methyl-1***H***-imidazol-5-yl)methylene]piperazin-1-amine (26). A suspension of 25 (10.0 g, 52.3 mmol) and 22a (5.80 g, 52.7 mmol) in MeOH (200 mL) was refluxed for 3 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was triturated with Et<sub>2</sub>O to give <b>26** (13.0 g, 88%) as a pale-yellow solid. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 2.40 (3H, s), 2.62 (4H, t, J = 5.2 Hz), 3.09 (4H, t, J = 5.2 Hz), 3.56 (2H, s), 6.97 (1H, s), 7.24–7.34 (5H, m), 7.43 (1H, s), 9.62 (1H, br s).

**4-Benzyl-***N***-[(2-methyl-1***H***<b>-imidazol-5-yl)methyl]piperazin-1amine (27).** To a solution of **26** (13.0 g, 45.9 mmol) in THF (100 mL) was added a solution of borane—THF complex (1 M in THF, 140 mL, 140 mmol) at room temperature. After being stirred at room temperature for 15 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in 6 M HCl, and then the mixture was stirred at 100 °C for 2 h. After cooling to 0 °C, the mixture was basified to pH 12 with 6 M NaOH and extracted with CHCl<sub>3</sub>. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to give **27** (14.7 g, quantitative) as a brown oil. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 2.37 (3H, s), 2.53 (4H, br s), 2.75 (4H, br s), 3.52 (2H, s), 3.90 (2H, s), 6.76 (1H, s), 7.29–7.32 (5H, m).

**2-(4-Benzylpiperazin-1-yl)-5-methyl-1,2-dihydro-***3H***-imida-zo[1,5-c]imidazol-3-one (28).** Compound **28** was prepared in a manner similar to that described for **24a** in 65% yield as a colorless solid. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$ : 2.60–2.65 (7H, m), 3.14 (4H, t, *J* = 4.6 Hz), 3.55 (2H, s), 4.43 (2H, s), 6.70 (1H, t, *J* = 2.0 Hz), 7.25–7.34 (5H, m).

5-Methyl-2-(piperazin-1-yl)-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3-one (29). A suspension of 28 (8.00 g, 25.7 mmol), ammonium formate (16.0 g, 254 mmol), and palladium on carbon (1.60 g) in MeOH (100 mL) was refluxed for 6 h. The mixture was cooled to room temperature, and the insoluble material was filtered off through a Celite pad. The filtrate was concentrated in vacuo, and the residue was triturated with EtOAc and Et<sub>2</sub>O to give 29 (5.30 g, 93%) as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 2.61 (3H, s), 2.98–3.03 (4H, m), 3.10–3.15 (4H, m), 4.44 (2H, s), 6.70 (1H, s).

2-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)-5-methyl-1,2-dihydro-3*H*-imidazo[1,5-*c*]imidazol-3one (3s). Compound 3s was prepared in a manner similar to that described for 3p in 76% yield as a colorless solid, mp 230–231 °C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 2.60 (3H, s), 3.13 (2H, t, J = 5.0 Hz), 3.23 (2H, t, J = 5.0 Hz), 3.54–3.59 (4H, m), 3.62 (2H, t, J = 5.0 Hz), 3.69 (2H, t, J = 5.0 Hz), 4.42 (2H, s), 6.70 (1H, t, J = 1.5 Hz), 7.59 (1H, dd, J = 8.6 and 2.3 Hz), 7.89–7.97 (4H, m), 8.47 (1H, s). Anal. (C<sub>23</sub>H<sub>24</sub>ClN<sub>5</sub>O<sub>4</sub>S) C, H, N.

In Vitro Assays for the Inhibition of Human FXa. Human FXa (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<sub>2</sub> at pH 8.3. Enzyme assay was carried out in 96-well microtiter plates. Test compounds were diluted in DMSO. Compound dilutions and 10  $\mu$ L of enzyme solution were added to the well containing buffer and preincubated. The enzymatic reactions were initiated with the addition of 10  $\mu$ L of 3 mM substrate, and the mixture was incubated for 10 min at 37 °C. The reaction was terminated with the addition of 25  $\mu$ L 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 (Multiscan Ascent, Dainippon Sumitomo Pharmaceutical Co.). 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 equation (1 -  $[T]/[C]) \times 100$ . IC<sub>50</sub> values were calculated by nonlinear regression analysis of the concentration-response curve. Data for new compounds were compared to positive control DX-9065a<sup>6a</sup> (FXa IC<sub>50</sub> = 0.13  $\mu$ M). The 95% confidence interval for the IC<sub>50</sub> of DX-9065a was 0.12-0.15 µM (duplicate).

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  $\mu$ L of compound dilutions in DMSO was added to 48.5  $\mu$ L 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  $\mu$ L 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<sub>2</sub>) was calculated from the regression line based on the method of least squares. Data for new compounds were compared to positive control DX-9065a<sup>6a</sup> (PT<sub>2</sub> = 0.81 ± 0.029  $\mu$ M (mean ± SEM, n = 3))

**Measurement of CYP Inhibition Activity.** Inhibition activity of test compounds of CYP3A4 was evaluated by incubating 100  $\mu$ M testosterone with 10 nM CYP3A4 derived from CYP3A4expressing human B-lymphoblastoid cells (BD Biosciences) in the presence of 10  $\mu$ M test compound. The incubation mixture was allowed to stand for 30 min at 37 °C. The concentration of 6 $\beta$ hydroxytestosterone was measured by HPLC system equipped with a UV detector.

Measurement of ex Vivo PT in Mouse after Oral Administration. Male ICR mice (30-35 g, Clea Japan Inc.) fasting for more than 12 h were employed. Test compounds were orally administered to these animals. An hour after administration, 800  $\mu$ L of blood was collected from artery abdominal aorta using 3.8% sodium citrate (whole blood/sodium citrate solution = 9:1, v/v), under anesthesia with pentobarbital (50 mg/kg, ip). The citrated blood was centrifuged at 1000g for 10 min to obtain platelet poor plasma (PPP). PT was measured with an automatic coagulometer. An amount of 50 µL of PPP was preincubated at 37 °C for 4 min. The PPP was mixed with 100  $\mu$ L of thromboplastin solution, and then its coagulation time was measured. Test compounds were suspended in 0.5% methyl cellulose (Metolose cp100, Shin-Etsu Chemical), and as control, 0.5% methylcellulose was administered instead of test compounds. Activity of each test compound was shown as a ratio (%) determined by comparing the clotting time of compound-treatment mice with that of control group. We have

validated the ex vivo PT assay for the evaluation of oral bioavailability in mice by use of the positive control compound DX-9065a.<sup>6a</sup> DX-9065a prolonged mouse PT by 1.1-fold at a dose of 30 mg/kg and by 1.4-fold at a dose of 100 mg/kg.

Measurement of ex Vivo PT in Monkey after Oral Administration. Male cynomolgus monkeys (3.5-4 kg, NAFOVANNY)fasting for more than 12 h were employed. Test compounds were orally administered to these animals. After administration, 1.5 mL of blood was collected from the femoral vein using 3.8% sodium citrate (whole blood/sodium citrate solution = 9:1, v/v) at several time points. The citrated blood was centrifuged at 1000g for 10 min to obtain PPP. Plasma clotting time and anticoagulating activity of drug were determined as described above.

Enzyme Inhibition 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.

Pharmacokinetic Analysis in Rats. Test compound was administered to fasted or fed SD (IGS) rats (male, 8 weeks old, n = 3) intravenously (0.3 mg/kg, DMA/PEG400) and orally (3 mg/kg, 0.5% methylcellulose suspension). At 5, 10, 15, and 30 min and 1, 2, 4, 8, 24 h after intravenous administration or at 15 and 30 min and 1, 2, 4, 8, 24 h after oral administration, blood samples were collected from tail vein. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile. After centrifugation, the supernatant obtained was diluted with 0.01 mol/L HCO<sub>2</sub>NH<sub>4</sub> (adjusted to pH 3.0 with HCO<sub>2</sub>H) and centrifuged again. The compound concentration in the supernatant was measured by LC/ MS/MS with an API3000 triple quadrupole mass spectrometer (Perkin-Elmer 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 L-column ODS (2.1 mm  $\times$  150 mm); mobile phase, 0.01 mol/L HCO<sub>2</sub>NH<sub>4</sub> (adjusted to pH 3.0 with HCO<sub>2</sub>H)/acetonitrile = 6/4; flow rate, 0.2 mL/min; column temperature, 40 °C.

Pharmacokinetic Analysis in Cynomolgus Monkeys. Test compound was administered to fasted or fed cynomolgus monkeys (male, n = 3) intravenously (0.3 mg/kg, DMA/PEG400) and orally (3 mg/kg, 0.5% methylcellulose suspension). At 5, 10, 15, 30 min and 1, 2, 4, 8, 24 h after intravenous administration or at 15 and 30 min and 1, 2, 4, 8, 24 h after oral administration, blood samples were collected from femoral vein. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile. After centrifugation, the supernatant obtained was diluted with 0.01 mol/L HCO<sub>2</sub>NH<sub>4</sub> (adjusted to pH 3.0 with HCO<sub>2</sub>H) and centrifuged again. The compound concentration in the supernatant was measured by LC/MS/MS with an API3000 triple quadrupole mass spectrometer (Perkin-Elmer 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 L-column ODS (2.1 mm  $\times$  150 mm); mobile phase, 0.01 mol/L  $HCO_2NH_4$  (adjusted to pH 3.0 with  $HCO_2H$ )/acetonitrile = 6/4; flow rate, 0.2 mL/min; column temperature, 40 °C.

Rat Venous Thrombosis Model. Male Sprague-Dawley rats (7 weeks old) were anesthetized with sodium pentobarbital. A 10 mL length of inferior vena cava from the distal region staring the left renal vein was isolated, and any side branches were ligated. A balloon catheter was introduced from the left femoral vein to inferior vena cava to injure the endothelium. Denudation of the endothelium was performed by three passes of the inflated balloon catheter. In order to trigger the thrombus formation at the injured region, a silk thread was tied around the vena cava caudal to the left renal vein with a blunt needle (26 gauge), followed by the removal of the needle. Thirty minutes after the starting of partial stasis, the thrombus formed in the vena cava was removed and the wet weight was measured. Blood samples were collected for the measurement of blood coagulation parameters just after the removal of thrombus. Compound 3q and warfarin were orally administered 30 min and 18 h, respectively, before inducing the thrombus formation to examine their antithrombotic potency at the points that give maximal anticoagulating effects. Compound 3q was given after the animals had fasted for more than 12 h.

Measurement of Bleeding Time in Rats after Oral Administration. Male Sprague–Dawley rats (7 weeks old) were anesthetized with sodium pentobarbital. The tail was transected at 2-3mm proximal site from the tip. Blood was blotted every 30 s with filter paper until either bleeding had stopped or 30 min had elapsed. Compound **3q** and warfarin were orally administered 30 min and 18 h before the measurement of bleeding time, respectively.

Docking Studies. The coordinates of FXa were retrieved from the Protein Data Bank (accession code 1EZQ).<sup>24</sup> Compounds 2, 3a, 3i, 3m, 3p, and 3q were docked into the FXa protein crystal structure using GOLD<sup>23</sup> (version 2.0, the Cambridge Crystallographic Data Centre, U.K.) with the standard default settings. The initial structures of the compounds-complexed FXa models were energy-minimized using the MMFF94s force field in MOE (version 2003.02, 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<sup>2</sup> 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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**Supporting Information Available:** Elemental analysis data and the binding model of the protonated **3a** in FXa. This material is available free of charge via the Internet at http://pubs.acs.org.

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