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Discovery of piperazinylimidazo[1,2-*a*]pyridines as novel S4 binding elements for orally active Factor Xa inhibitors

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Abstract—We have recently reported the discovery of orally active sulfonylalkylamide Factor Xa (FXa) inhibitors, as typified by compound **1** (FXa IC_{50} = 0.061 μM). Since the pyridylpiperidine moiety was not investigated in our previous study, we conducted detailed structure–activity relationship studies on this S4 binding element. This investigation led to the discovery of piperazinylimidazo[1,2-*a*]pyridine **2b** as a novel and potent FXa inhibitor (FXa IC_{50} = 0.021 μM). Further modification resulted in the discovery of 2-hydroxymethylimidazo[1,2-*a*]pyridine **2e** (FXa IC_{50} = 0.0090 μM), which was found to be a selective and orally bioavailable FXa inhibitor with reduced CYP3A4 inhibition.

1. Introduction

Intravascular clot formation is an essential factor in a number of cardiovascular diseases such as myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism, and ischemic stroke. The interruption of the coagulation cascade is one of the most effective strategies for inhibition of clot formation, which is an important approach for the prevention and treatment of these thrombotic disorders. Warfarin prevents the synthesis of vitamin K-dependent coagulation factors in the liver and inhibits the activation of blood coagulation leading to thrombus formation. As the only oral anticoagulant in a clinical setting, warfarin is 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.[1] 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 which are orally active, have long duration of action, and have useful in vivo potency without unpredictable or serious bleeding complications.

The trypsin-like serine protease Factor Xa (FXa), converting prothrombin to thrombin, occupies a unique position at the final convergence point of the intrinsic and the extrinsic coagulation pathways.[2] Since this process involves signal amplification, with one molecule of FXa activating many molecules of prothrombin to thrombin,[3] FXa inhibitors are expected to be more efficacious in interrupting the coagulation cascade than direct thrombin inhibitors. Moreover, it was also shown recently in in vivo studies that FXa inhibitors may have less bleeding risk than thrombin inhibitors.[4] Thus, FXa has been identified as an attractive target enzyme for antithrombotic therapy.[5]

A variety of low-molecular-weight FXa inhibitors have been described[6] and recently many orally active FXa inhibitors have been extensively explored.[5e–h] For example, some non-amidine FXa inhibitors such as apixaban[5f] and rivaroxaban[7] have been reported to be orally active FXa inhibitors and are under clinical study.

Keywords: Factor Xa inhibitor; Anticoagulant; Piperazinylimidazo[1,2-*a*]pyridines; Oral bioavailability.
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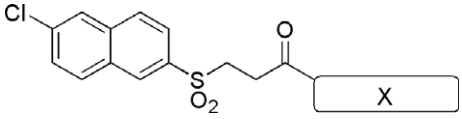
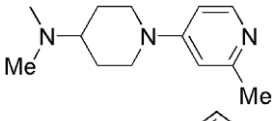
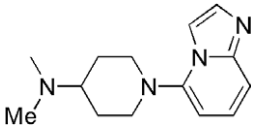
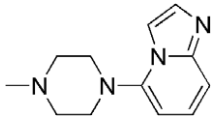
We have previously reported orally active sulfonylalkylamide FXa inhibitors, as typified by compound **1**.[8] The structure–activity relationships (SAR) for this series revealed that the FXa enzyme rigorously recognized the 6-chloronaphthalene moiety in the S1 site of FXa, whereas the recognition of the pyridylpiperidine moiety as the S4 binding element appeared to be loose. Our binding model of compound **1** in FXa[8] indicated that the basic pyridine ring formed no salt bridge with the carboxyl group of any amino acid residue in the S4 site, however, it did form hydrophobic contacts with the aromatic rings of Tyr99, Phe174, and Trp215 in 'the S4 aryl binding site'.[9] These results prompted us to modify the S4 binding element of compound **1**, because it remained uninvestigated in our previous work. Zhu and Scarborough suggested that rather diverse S4 binding elements can be utilized to give highly potent FXa inhibitors and that diverse S4 binding elements would provide opportunities to modulate the physiochemical profiles.[6d] We conducted preliminary chemical modification in an attempt to enhance the hydrophobic contact by extension of the π electron system (e.g., a 5,6-fused heteroaromatic ring, such as an imidazo[1,2-*a*]pyridine, an imidazo[1,2-*b*]pyridazine, and a benzimidazole ring). As a result of these initial efforts, we found piperazinylimidazo[1,2-*a*]pyridine derivative **2a** to be a weak FXa inhibitor and **2b**, which showed more potent FXa inhibitory activity in vitro (FXa IC_{50} = 0.021 μM) than **1**, as shown in Table 1. Therefore, we decided to conduct detailed SAR studies on the S4 binding element using **2b** as a new lead, since there is no report on FXa inhibitors bearing an imidazo[1,2-*a*]pyridine ring and the introduction of the varied substituents on the imidazo[1,2-*a*]pyridine ring appears to be possible by use of robust chemistry. In this paper, we describe the synthesis, SAR, ex vivo anticoagulant activity, selectivity, and pharmacokinetics of the piperazinylimidazo[1,2-*a*]pyridine derivatives.[10] It is also worthwhile to report the synthesis and the properties of imidazo[1,2-*a*]pyridines bearing an alkylamino group at the 5-, 6-, or 7-position, because there are few literature reports on imidazo[1,2-*a*]pyridines.[11]

Table 1. In vitro activities of **1**, **2a**, and **2b**



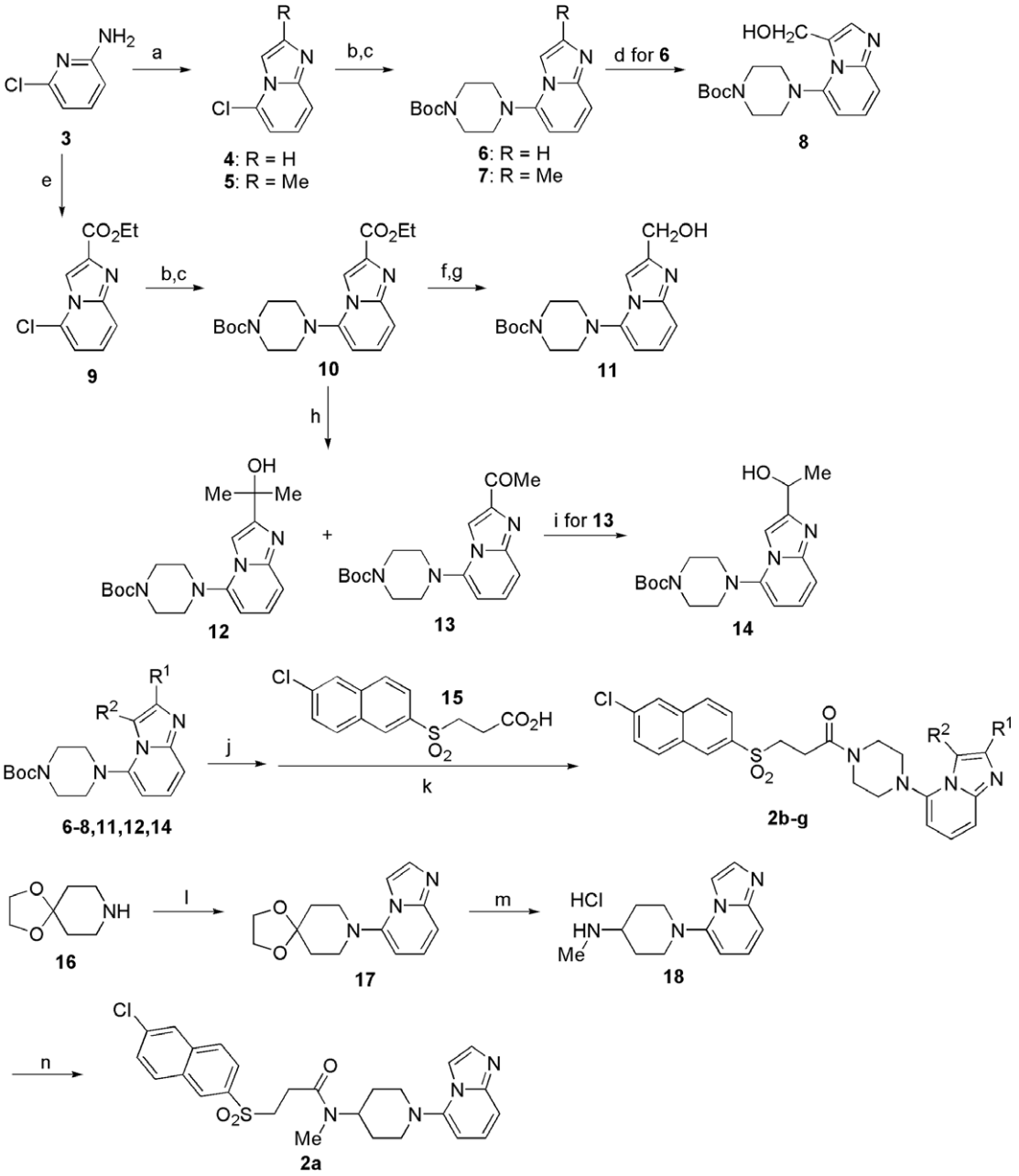
Compound	X	Human FXa IC_{50}[a] (μM)
1		0.061
2a		0.84
2b		0.021

[a] Inhibitory activity against human FXa. IC_{50} values shown are means of duplicate measurement.

2. Chemistry

The synthesis of the 5-(1-piperazinyl)imidazo[1,2-*a*]pyridines **2a–r** is shown in Schemes 1–3. 5-Chloroimidazopyridines **4**[12] and **5**, which were obtained by the condensation of 2-amino-6-chloropyridine **3**[12] and chloroacetaldehyde or bromoacetone, were heated with piperazine without solvent at 100–150 °C followed by *tert*-butoxycarbonylation to give the 5-(1-piperazyl)imidazopyridines **6** and **7** as shown in Scheme 1. Using formaldehyde in ethanol, direct hydroxymethylation at the 3-position of the imidazopyridine ring of **6** gave alcohol **8**.[13] The key intermediate ester **10** was prepared by condensation of **3** and ethyl bromopyruvate followed by displacement of the chlorine atom with piperazine and introduction of a Boc group. Hydrolysis of **10** followed by reduction of the carboxylic group with borane–tetrahydrofuran complex (BH_3–THF) afforded alcohol **11**. Treatment of **10** with 3 equivalent of methylmagnesium bromide gave a mixture of alcohol **12** and ketone **13**. Reduction of the acetyl group of **13** with $NaBH_4$ afforded alcohol **14**. Removal of the Boc group from imidazopyridines **6–8**, **11**, **12**, and **14** followed by coupling with 3-(6-chloronaphthalen-2-yl)sulfonylpropionic acid **15**[8] using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC) and 1-hydroxybenzotriazole hydrate (HOBt) gave the desired amides **2b–g**. 5-Chloroimidazopyridine **4** was heated with piperidine **16** without solvent at 100–150 °C to afford piperidylimidazopyridine **17**. Cleavage of the 1,3-dioxolane ring of **17** with 4 M HCl, followed by reductive amination with methylamine using sodium triacetoxyborohydride, introduction of a Boc group for easy purification by silica gel chromatography, and removal of the Boc group, gave *N*-methyl-*N*-piperidinamine **18**. Amide **2a** was prepared by coupling of amine **18** with carboxylic acid **15**.

The requisite ester **21** was prepared by condensation of 2-amino-6-fluoropyridine **19**[12] and ethyl 4-chloroacetoacetate followed by displacement of the fluorine atom with piperazine and introduction of a Boc group (Scheme 2). The corresponding displacement reaction using ethyl (5-chloroimidazo[1,2-*a*]pyridin-2-yl) acetate instead of **20** did not proceed. Ester **21** was treated with ammonia solution to give amide **23**. Direct amide formation from **21** with dimethylamine or methylamine using dimethylaluminum chloride afforded dimethylamide **22** and methylamide **24**, respectively. Ester **21** was treated with methylmagnesium bromide and $CeCl_3$[14] to give alcohol **25**. Treatment of dimethylamide **22** with methylmagnesium bromide followed by reduction with $NaBH_4$ provided alcohol **26**. Removal of the Boc group from imidazopyridines **21–26** followed by coupling with **15** gave the corresponding desired amides **2h–m**.



Scheme 1. Synthesis of 5-(1-piperazinyl)imidazo [1, 2-*a*]pyridines. Reagents: (a) $ClCH_2CHO$ or $BrCH_2COMe$; (b) piperazine; (c) $(Boc)_2O$; (d) aq HCHO; (e) $BrCH_2COCO_2Et$; (f) NaOH; (g) BH_3/THF; (h) MeMgBr; (i) $NaBH_4$; (j) concd HCl; (k) WSC, HOBt, DBU, Et_3N; (l) **4**; (m) 1—4 M HCl; 2—$MeNH_2$, $NaBH(OAc)_3$, AcOH; 3—$(Boc)_2O$; 4—concd HCl; (n) **15**, WSC, HOBt, Et_3N, DBU.

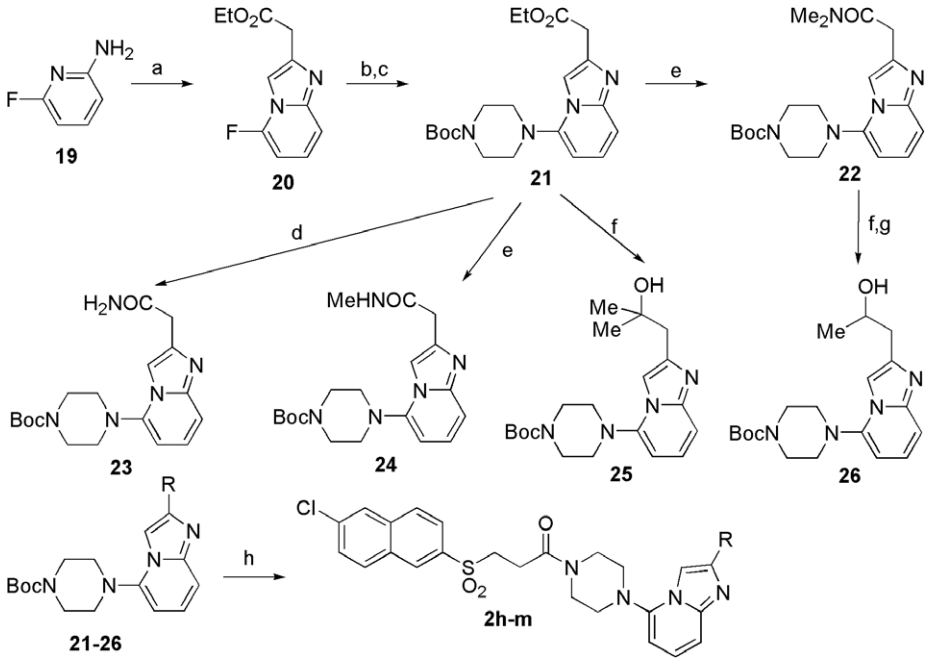
3-Aminoimidazo[1,2-*a*]pyridine **2o** was synthesized by displacement of the chlorine atom in **27**[15] with 1-Boc-piperazine, removal of the Boc group, coupling with **15**, and subsequent reduction of the nitro group with iron powder and $CaCl_2$. 3-Aminoimidazo[1,2-*a*]pyridine **2o** was acetylated to give acetamide **2p** (Scheme 3).

The synthesis of 6-(1-piperazinyl)imidazo[1,2-*a*]pyridine **2q** is depicted in Scheme 4. Displacement of the bromine atom of 5-bromo-2-nitropyridine with 1-Boc-piperazine followed by reduction of nitro group afforded aminopyridine **30**, which was condensed with chloroacetaldehyde to give imidazopyridine **31**. Removal of the Boc group from **31** and subsequent coupling with carboxylic acid **15** afforded the desired amide **2q**.

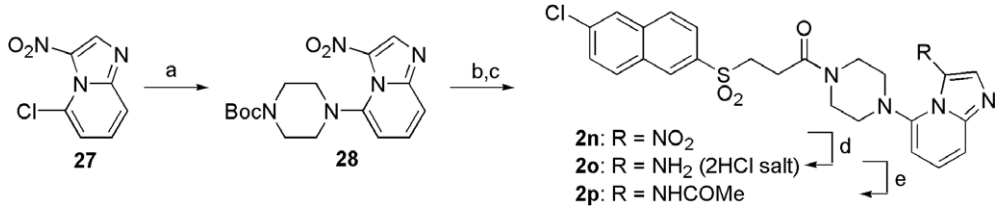
7-(1-Piperazinyl)imidazo[1,2-*a*]pyridine **2r** was synthesized as shown in Scheme 5. 7-(4-Boc-piperazinyl)imidazo[1,2-*a*]pyridine **34** was prepared by displacement of the chlorine atom of 2-amino-4-chloropyridine **32**[16] with 1-Boc-piperazine followed by cyclization with chloroacetaldehyde. Treatment of **34** with concentrated HCl and subsequent coupling with carboxylic acid **15** afforded the desired amide **2r**.

3. Results and discussion

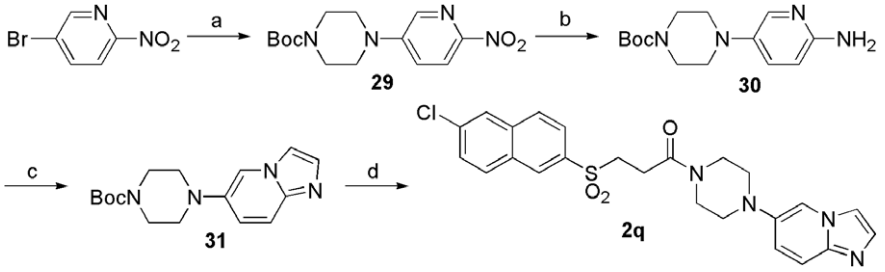
The compounds thus synthesized were evaluated in vitro for inhibitory potency against human FXa, expressed as IC_{50} values, and their activity in the prolongation of hu-



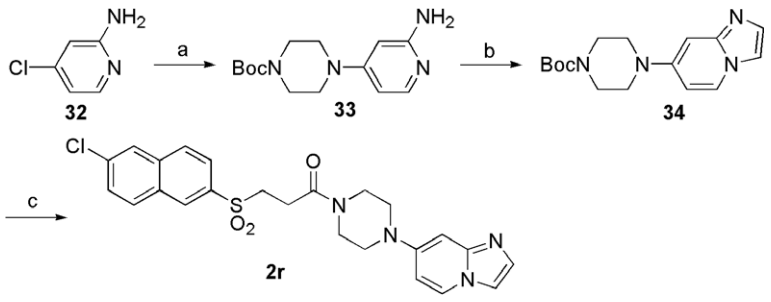
Scheme 2. Synthesis of 5-(1-piperazinyl)imidazo[1,2-*a*]pyridines **2h**–**m**. Reagents: (a) $ClCH_2COCH_2CO_2Et$; (b) piperazine; (c) $(Boc)_2O$; (d) aq NH_3; (e) $MeNH_2$ or Me_2NH, Me_2AlCl; (f) MeMgBr, $CeCl_3$; (g) $NaBH_4$; (h) 1—concd HCl; 2—**15**, WSC, HOBt, DBU, Et_3N.



Scheme 3. Synthesis of 5-(1-piperazinyl)imidazo[1,2-*a*]pyridines **2n**–**p**. Reagents: (a) 1-Boc-piperazine, *i*-Pr_2NEt; (b) concd HCl; (c) 1—**15**, WSC, HOBt, DBU, Et_3N; 2—4 M HCl; (d) Fe, $CaCl_2$; (e) Ac_2O.



Scheme 4. Synthesis of 6-(1-piperazinyl)imidazo[1,2-*a*]pyridine **2q**. Reagents: (a) 1-Boc-piperazine; (b) H_2, Pd/C; (c) $ClCH_2CHO$; (d) 1—concd HCl; 2—**15**, WSC, HOBt, DBU, Et_3N.

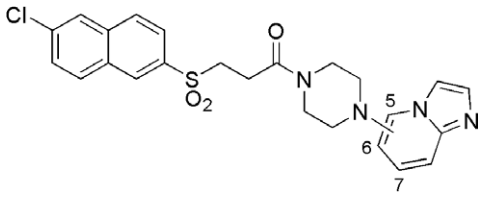


Scheme 5. Synthesis of 7-(1-piperazinyl)imidazo[1,2-*a*]pyridine **2r**. Reagents: (a) 1-Boc-piperazine; (b) $ClCH_2CHO$, $NaHCO_3$; (c) 1—concd HCl; 2—**15**, WSC, HOBt, DBU, Et_3N.

man prothrombin time (PT), expressed as the concentration of compound required to double the clotting time (PT_2) in the PT assay. 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-treatment animals with that of control group.

The results obtained for the imidazo[1,2-*a*]pyridine derivatives, which have a piperazine ring at the 5- (**2b**), 6- (**2q**), or 7-positions (**2r**), in in vitro FXa inhibition and PT assays are listed in Table 2. 5-(1-Piperazinyl)imidazo[1,2-*a*]pyridine **2b** (FXa $IC_{50} = 0.021$ μM) was found to be the most potent FXa inhibitor among these regioisomers. This fact suggests that the configuration of the imidazo[1,2-*a*]pyridine ring in the S4 site in FXa and/or the position of the basic nitrogen atom at the 1-position in the imidazopyridine ring is important for the activity. Additionally, compound **2b** showed the most potent human PT prolonging activity among these regioisomers.

Table 2. In vitro activities of Imidazo[1,2-*a*]pyridines **2b**, **2q**, and **2r**

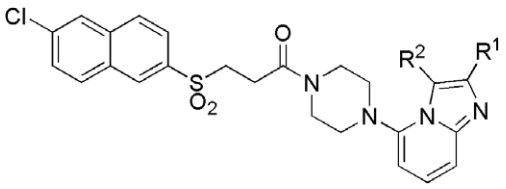


Compound	Position	Human FXa IC_{50}[a] (μM)	Human PT PT_2[b] (μM)
2b	5	0.021	1.4
2q	6	0.087	2.9
2r	7	0.078	2.2

[a] See corresponding footnotes in Table 1.
[b] PT (prothrombin time) is defined as the concentration of compound required to double the time to clot formation in the PT assay. PT_2 values shown are means of duplicate measurement.

The discovery of the 5-(1-piperazinyl)imidazo[1,2-*a*]pyridine **2b** led us to explore the C-2 or C-3 modification on the imidazopyridine ring in an effort to further improve potency as shown in Table 3. All compounds bearing various substituents at the 2-position exhibited FXa inhibitory activities with IC_{50} values of 10^{-8} M order. 2-Methyl derivative **2c** slightly improved the FXa inhibitory activity compared to **2b**. This suggests that the methyl group could fill the S4 hydrophobic site in FXa more effectively through van der Waals interactions leading to an increase in the FXa inhibitory activity. Among the compounds bearing a hydroxyl group, hydroxymethyl derivative **2e**, 1-hydroxy-1-methylethyl derivative **2f**, 1-hydroxyethyl derivative **2g**, 2-hydroxy-2-methylpropyl derivative **2l**, and 2-hydroxypropyl derivative **2m** were equipotent or slightly more potent with respect to anticoagulant activity compared to **2b**. The anticoagulant activities of ester **2h** and amides **2i**–**2k** were equipotent or slightly reduced compared to the corresponding alcohols **2e**–**g**, **2l**, and **2m**. Regarding the 3-substituted imidazo[1,2-*a*]pyridines, as shown in

Table 3. In vitro and ex vivo activities of imidazo[1,2-*a*]pyridines **2b**–**p**



Compound	R^1	R^2	In vitro				Ex vivo
			Human FXa IC_{50}[a] (μM)	Human PT PT_2[b] (μM)	CYP3A4 (% inhibition at 10 μM)	Mouse PT PT_2[b] (μM)	Mouse PT ratio[c]
2b	H	H	0.021	1.4	45	1.7	2.5
2c	Me	H	0.012	0.96	58	1.3	2.9
2e	CH_2OH	H	0.0090	1.4	15	1.4	1.3
2f	$C(Me)_2OH$	H	0.018	2.1	NT[d]	NT[d]	NT[d]
2g	CH(Me)OH	H	0.020	1.2	23	1.8	2.5
2h	CH_2CO_2Et	H	0.093	5.1	NT[d]	NT[d]	NT[d]
2i	CH_2CONMe_2	H	0.057	2.5	NT[d]	NT[d]	NT[d]
2j	CH_2CONH_2	H	0.045	2.1	NT[d]	NT[d]	NT[d]
2k	$CH_2CONHMe$	H	0.041	2.0	NT[d]	NT[d]	NT[d]
2l	$CH_2C(Me)_2OH$	H	0.022	0.86	26	1.7	2.4
2m	$CH_2CH(Me)OH$	H	0.018	1.1	33	1.5	3.2
2d	H	CH_2OH	0.029	1.7	NT[d]	NT[d]	NT[d]
2n	H	NO_2	0.024	3.6	NT[d]	NT[d]	NT[d]
2o	H	NH_2	0.035	0.73	52	1.5	2.4
2p	H	NHAc	0.024	1.8	NT[d]	NT[d]	NT[d]

[a,b] See corresponding footnotes in Tables 1 and 2.
[c] Ex vivo mouse PT prolonging activity was determined 1 h after oral administration of the compound at a dose of 30 mg/kg and expressed as ratios of the PT of the compound-treatment mice with that of control group ($n = 3$).
[d] NT means 'not tested'.

Table 3, amine **2o** showed slightly more potent anticoagulant activity compared to hydroxymethyl derivative **2d**, nitro derivative **2n**, and acetamino derivative **2p**.

The ex vivo PT prolonging activities of a select set of the potent inhibitors in mice were determined and are shown in Table 3. Compounds **2b** and **2c** significantly prolonged mouse PT by 2.5- and 2.9-fold, respectively. These results suggested that **2b** and **2c** resembled the orally active pyridylpiperidine **1** in showing good pharmacokinetics in mice.[8] More polar compounds **2e**, **2g**, **2l**, **2m**, and **2o** significantly prolonged mouse PT by 1.3-, 2.5-, 2.4-, 3.2-, and 2.4-fold, respectively. These results suggested that the polar substituents such as hydroxyl or amino group had not significantly affected the oral bioavailability in mice. The reason why the in vitro mouse PT of **2e** was equipotent to those of other imidazo[1,2-*a*]pyridines **2b**, **2c**, **2g**, **2l**, and **2m** but the ex vivo anticoagulant activity of **2e** was lower than those of them might be attributed to the difference of the plasma concentration of the compounds 1h after oral administration in mice.

In the course of further evaluation of **2b**, it was found to inhibit the human cytochrome P450 enzyme CYP3A4 (45% inhibition at 10 μM), which is known to be a primary factor responsible for the metabolism of most drugs.[17] Drugs that show CYP inhibition influence the pharmacokinetics of other co-administered drugs and can cause undesired effects in patients. It might be reasoned that the inhibitory potency toward CYP3A4 is attributed to coordination of the basic N(1) atom of the imidazopyridine ring ($pK_a = 9.9$ for **2b**)[18] to the heme.[19] We thought that the introduction of a substituent at the 2 or 3 position of the imidazopyridine ring might prevent coordination to the heme and reduce the inhibitory potency. The CYP3A4 inhibition of some hydroxyl-containing derivatives, such as 1-hydroxymethyl derivative **2e** and 1-hydroxyethyl derivative **2g**, was successfully lowered as shown in Table 3. On the basis of the potency, the ex vivo PT prolongation activity, and the weakest CYP3A4 inhibitory potency, **2e** was selected for further evaluation.

Since many trypsin-like proteases have essential physiological functions,[20] an important consideration for the development of FXa inhibitors is the ability of the molecule to bind selectively. In several enzyme inhibition assays, compound **2e** displayed >100-fold selectivity for FXa versus several coagulation (thrombin, kallikrein) and fibrinolytic enzymes (plasmin, tissue plasminogen activator (t-PA)) (Table 4). Furthermore, **2e** showed no significant binding to trypsin.

The pharmacokinetic profile of compound **2e** was evaluated in cynomolgus monkeys. As illustrated in Table 5, **2e** displayed good oral bioavailability (31%) after oral administration at a dose of 3 mg/kg in fasted monkeys.

To predict the binding mode of these inhibitors, a molecular modeling study on **2e** was carried out using the program GOLD and the X-ray structure of FXa complexed with an inhibitor reported by Sanofi-Aventis[21] (Fig. 1). In the model, the imidazo[1,2-*a*]pyridine ring forms no salt bridge with the carboxyl group of Asp189 at the bottom of the S1 pocket, while the 6-chloronaphthyl group is situated in the hydrophobic S1 region and a close contact is observed between the chlorine atom and the benzene ring of Tyr228.[7,9b,22] Compound **2e** represents a parallel example in FXa inhibition wherein a basic group thought to be essential in forming the salt bridge with Asp189[6d,21] can be eliminated and replaced by a neutral group capable of contributing enough favorable interaction to produce good affinity to the enzyme. The imidazo[1,2-*a*]pyridine ring is deeply buried inside the hydrophobic S4 site and makes hydrophobic contacts with the aromatic rings of Tyr99, Phe174, and Trp215 without the salt bridge with any amino acid residue in this site. In particular, the imidazo[1,2-*a*]pyridine ring extends across the face of the Phe174 phenyl ring with a favorable π–π stacking interaction, and thus appears to make the hydrophobic contact with the S4 site in FXa more favorably than the pyridine ring of **1** by extension of the π electron system (Fig. 2). Moreover, a hydrogen bonding interaction between the oxygen atom of the piperazine amide bond and the amide linkage of Gly219 is observed.[21,22c,e,23] On the contrary, a hydrogen bond is not observed be-

Table 4. Selectivity profile for compound **2e**

Serine protease	FXa	Thrombin	Kallikrein	Trypsin	Plasmin	t-PA
K_i[a] (μM)	0.0080	0.96	14	>60	>60	>60

[a] Data are expressed as means of three determinations.

Table 5. In vivo cynomolgus monkey pharmacokinetics of compound **2e** ($n = 3$, fasted)[a]

iv dose	$C_{5\ min}$ (μg/mL)	AUC (μg h/mL)	MRT (h)	$V_{d(ss)}$ (L/kg)	CL_{total} (L/h/kg)
0.3 mg/kg	0.54 ± 0.06	0.73 ± 0.25	1.97 ± 0.63	0.81 ± 0.09	0.44 ± 0.12
po dose	C_{max} (μg/mL)	T_{max} (h)	AUC (μg h/mL)	MRT (h)	B.A. (%)
3 mg/kg	0.50 ± 0.17	1.33 ± 0.58	2.26 ± 0.87	3.60 ± 0.43	30.5 ± 1.5

[a] Values are expressed as means ± SD of three determinations.

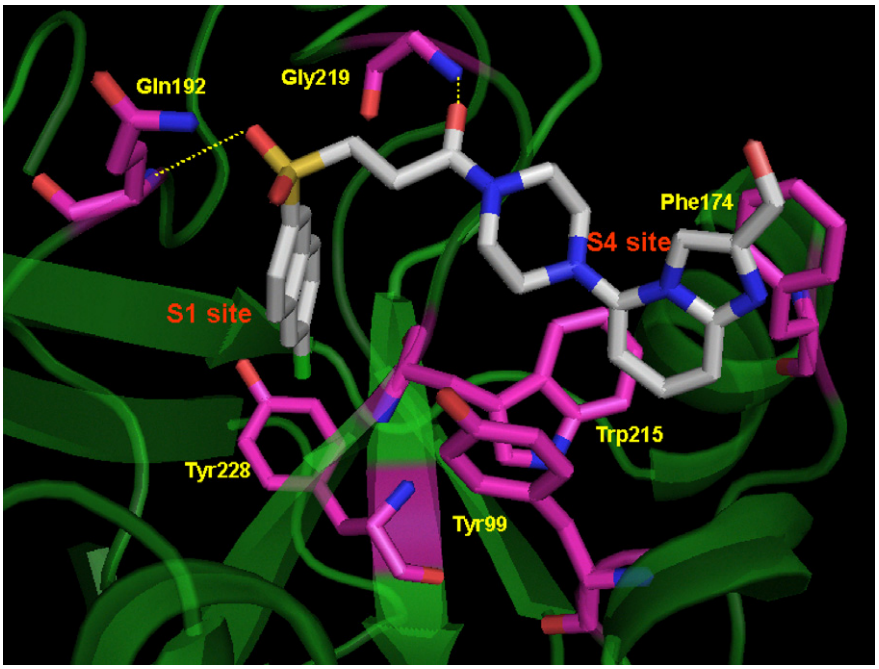


Figure 1. Binding model of compound **2e** in FXa.

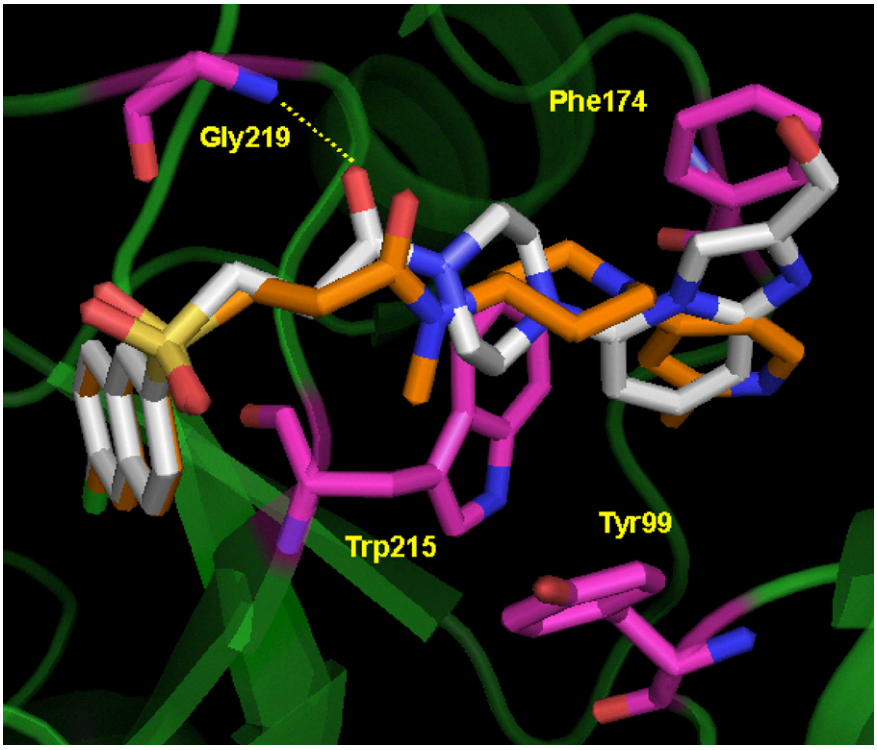


Figure 2. Binding model of compounds **2e** (white) and **1** (orange) in FXa.

tween compound **1** and Gly219 (Fig. 2). Recently, other researchers have reported that the important interaction for high affinity to FXa is not an ionic interaction with Asp189, but a hydrogen bonding interaction with the carbonyl group in the amide linkage of Gly219.[9b,21,22c] The reason why imidazo[1,2-*a*]pyridine **2e** is more potent than pyridylpiperidine **1** could be due to the π–π stacking interaction and the hydrogen bond (Fig. 2). The sulfone oxygen is involved in a hydrogen bond with the Gln192 main chain nitrogen.[6d]

4. Conclusion

In order to clarify SAR of the S4 binding element of our FXa inhibitor **1**, we conducted modification of its S4

binding element. This investigation led to the discovery of piperazinylimidazo[1,2-*a*]pyridine **2b** as a potent FXa inhibitor. Further investigation resulted in discovery of the 2-hydroxymethylimidazo[1,2-*a*]pyridine **2e**, which is a potent, selective, and orally bioavailable FXa inhibitor with reduced CYP3A4 inhibition. Various other optimization strategies using this imidazo[1,2-*a*]pyridine series will be reported in due course.

5. Experimental

5.1. Chemistry

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 300 or 200 MHz on a Varian Ultra-300 or a Varian Gemini-200 spectrometer. Chemical shifts are given in δ 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 (Chromatorex® NH, 100–200 mesh, Fuji Silysia Chemical Ltd) using the indicated eluents. Yields are unoptimized. Chemical intermediates were characterized by ^{1}H NMR.

5.1.1. Ethyl 5-chloroimidazo[1,2-*a*]pyridine-2-carboxylate (9). A mixture of **3**[12] (27.7 g, 216 mmol) and ethyl bromopyruvate (50.5 g, 259 mmol) in EtOH (400 mL) was refluxed for 15 h. The solvent was evaporated in vacuo and the residue was partitioned between $CHCl_3$ (250 mL) and aqueous K_2CO_3 (250 mL) solution. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was washed with diisopropyl ether (IPE) to give **9** as a beige powder (41.5 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (3H, t, J = 7.2 Hz), 4.49 (2H, q, J = 7.2 Hz), 6.99 (1H, dd, J = 0.9 and 7.5 Hz), 7.24–7.29 (1H, m), 7.67 (1H, dt, J = 0.9 and 9.0 Hz), 8.39 (1H, d, J = 0.9 Hz).

5.1.2. 5-Chloro-2-methylimidazo[1,2-*a*]pyridine (5). Compound **5** was prepared in a manner similar to that described for **9**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.49 (3H, d, J = 0.9 Hz), 6.82 (1H, dd, J = 7.5 and 0.9 Hz), 7.11 (1H, dd, J = 7.2 and 9.0 Hz), 7.47 (1H, dt, J = 0.9 and 9.0 Hz), 7.52 (1H, t, J = 0.9 Hz).

5.1.3. *tert*-Butyl 4-(imidazo[1,2-*a*]pyridin-5-yl)piperazine-1-carboxylate (6). A mixture of **4**[12] (4.58 g, 30 mmol) and piperazine (25.8 g, 300 mmol) was heated at 125 °C for 18 h under argon atmosphere. The reaction mixture was partitioned between $CHCl_3$ (200 mL) and water (200 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in EtOH (100 mL) and di-*tert*-butyl dicarbonate ($(Boc)_2O$; 6.55 g, 30.0 mmol) was added to the solution at room temperature. The resulting mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was diluted with water (200 mL) and extracted with EtOAc (200 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 10:1) to give **6** (8.39 g, 93%) as a pale yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.50 (9H, s), 3.06–3.11 (4H, m), 3.54–3.82 (4H, m), 6.30 (1H, d, J = 7.2 Hz), 7.18 (1H, dd, J = 9.2 and 7.2 Hz), 7.42 (1H, d, J = 8.8 Hz), 7.57 (1H, s), 7.66 (1H, s).

5.1.4. *tert*-Butyl 4-(2-methylimidazo[1,2-*a*]pyridin-5-yl)piperazine-1-carboxylate (7). Compound **7** was prepared in a manner similar to that described for **6**. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.50 (9H, s), 2.48 (3H, s), 2.97–3.15 (4H, m), 3.58–3.78 (4H, m), 6.23 (1H, d, J = 8.2 Hz), 7.13 (1H, dd, J = 8.8 and 7.0 Hz), 7.28–7.35 (2H, m).

5.1.5. *tert*-Butyl 4-[3-(hydroxymethyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (8). To a solution of **6** (5.00 g, 16.5 mmol) in EtOH (20 mL) was added aqueous formaldehyde solution (37%; 50.5 mL, 678 mmol) and the resulting mixture was stirred at 85 °C for 16 h. The reaction mixture was concentrated in vacuo, and water (20 mL) was added to the residue. The solution was basified with 8 M NaOH and extracted with $CHCl_3$ (30 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 10:1) to give **8** (3.28 g, 60%) as a colorless solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.50 (9H, s), 2.85–2.97 (2H, m), 3.12–3.38 (4H, m), 3.68 (1H, br s), 4.12–4.34 (2H, m), 4.87 (2H, s), 6.57 (1H, dd, J = 7.2 and 1.2 Hz), 7.20 (1H, dd, J = 8.8 and 1.2 Hz), 7.50 (1H, d, J = 8.8 Hz), 7.56 (1H, s).

5.1.6. *tert*-Butyl 4-(2-ethoxycarbonylimidazo[1,2-*a*]pyridin-5-yl)piperazine-1-carboxylate (10). A mixture of **9** (12.0 g, 53.4 mmol) and piperazine (46.0 g, 534 mmol) in acetonitrile (200 mL) was refluxed for 72 h under argon atmosphere. The reaction mixture was concentrated in vacuo and the residue was partitioned between $CHCl_3$ (250 mL) and water (250 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in EtOH (150 mL), and $(Boc)_2O$ (11.7 g, 53.4 mmol) was added to the solution at room temperature. The resulting mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was diluted with water (200 mL) and extracted with EtOAc (200 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 10:1 EtOAc/EtOH) to give **10** (17.2 g, 86%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.43–1.51 (12H, m), 2.98–3.17 (4H, m), 3.58–3.85 (4H, m), 4.47 (2H, q, J = 5.7 Hz), 6.36 (1H, d, J = 7.2 Hz), 7.22–7.28 (1H, m), 7.46 (1H, d, J = 10.8 Hz), 8.16 (1H, s).

5.1.7. *tert*-Butyl 4-[2-(hydroxymethyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (11). A mixture of **10** (8.26 g, 22.1 mmol) and 8 M NaOH (5.5 mL,

44.1 mmol) in EtOH (80 mL) was stirred at room temperature for 30 min. The reaction mixture was neutralized with concentrated HCl and EtOH was evaporated in vacuo. The residue was diluted with water (50 mL), acidified to pH 3–4 with concentrated HCl, and extracted with $CHCl_3$ (100 mL). The extract was dried over anhydrous $MgSO_4$ and concentrated in vacuo. To a solution of BH_3–THF in THF (1 M, 68.2 mL, 68.2 mmol) was added the residue and the resulting mixture was stirred at room temperature under argon atmosphere for 1 h. The reaction mixture was poured into ice-water (300 mL) and acidified to pH 1–2 with concentrated HCl cautiously. After stirring at room temperature for 1 h, the mixture was basified to pH 10–11 with 8 M NaOH and extracted with EtOAc (150 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 5:1 EtOAc/EtOH) to give **11** (4.77 g, 65%) as a colorless solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.44 (9H, s), 2.94–3.10 (4H, m), 3.51–3.67 (4H, m), 4.61 (2H, d, J = 5.4 Hz), 5.16 (1H, t, J = 5.4 Hz), 6.41–6.45 (1H, m), 7.21–7.24 (2H, m), 7.60 (1H, s).

5.1.8. *tert*-Butyl 4-[2-(1-hydroxy-1-methylethyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (12) and *tert*-butyl 4-(2-acetylimidazo[1,2-*a*]pyridin-5-yl)piperazine-1-carboxylate (13). To a solution of **10** (7.17 g, 19.1 mmol) in THF (60 mL) was added a solution of methylmagnesium bromide in Et_2O (1 M, 60.0 mL, 60.0 mmol) dropwise at 0 °C under argon atmosphere and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into ice-water (100 mL) and extracted with EtOAc (100 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 10:1 EtOAc/EtOH) to give **12** (more polar; 2.96 g, 41%) as a pale yellow oil and **13** (less polar; 2.82 g, 45%) as a pale yellow oil. **12**: ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.50 (9H, s), 1.69 (6H, s), 2.99–3.13 (4H, m), 3.58–3.77 (4H, m), 6.29 (1H, d, J = 7.0 Hz), 7.18 (1H, dd, J = 8.8 and 7.0 Hz), 7.36 (1H, d, J = 8.8 Hz), 7.44 (1H, s). **13**: ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.51 (9H, s), 2.73 (3H, s), 2.98–3.17 (4H, m), 3.58–3.81 (4H, m), 6.37 (1H, d, J = 6.8 Hz), 7.27 (1H, d, J = 9.2 and 7.2 Hz), 7.45 (1H, d, J = 9.2 Hz), 8.13 (1H, s).

5.1.9. *tert*-Butyl 4-[2-(1-hydroxyethyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (14). To a solution of **13** (4.13 g, 12.0 mmol) in EtOH (50 mL) was added $NaBH_4$ (0.55 g, 14.8 mmol) and the mixture was stirred at room temperature for 15 min. The reaction mixture was poured into ice-water (100 mL) and extracted with EtOAc (100 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 10:1) to give **14** (3.95 g, 95%) as a colorless amorphous powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.44–1.63 (12H, m), 2.94–3.12 (4H, m), 3.48–3.70 (4H, m), 4.77–4.95 (1H, m), 5.21 (1H, d, J = 4.8 Hz), 6.41–6.45 (1H, m), 7.17–7.30 (2H, m), 7.54 (1H, s).

5.1.10. 5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridine hydrochloride (2b). A solution of **6** (8.39 g, 27.8 mmol) in concentrated HCl (22.8 mL) was stirred at room temperature for 20 min. The reaction mixture was diluted with EtOH (85 mL) and concentrated in vacuo. The precipitate was collected by filtration and washed with EtOH and Et_2O to give 5-(piperazin-1-yl)imidazo[1,2-*a*]pyridine dihydrochloride (5.24 g, 69%) as a colorless powder.

To a mixture of **15**[8] (0.75 g, 2.5 mmol) and HOBt (0.57 g, 3.72 mmol) in MeCN (15 mL) was added WSC (0.72 g, 3.76 mmol) and the mixture was stirred at room temperature for 20 min. A solution of the 5-(piperazin-1-yl)imidazo[1,2-*a*]pyridine dihydrochloride (0.83 g, 3.02 mmol), Et_3N (1.05 mL, 7.5 mmol), and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU; 0.90 mL, 6.03 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. The solvent was evaporated in vacuo and the residue was partitioned between $CHCl_3$ (50 mL) and water (50 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 5:1) to give 5-(4-{3-[(6-chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridine (1.09 g, 91%) as a pale yellow amorphous powder.

The product obtained (1.09 g, 2.26 mmol) was dissolved in EtOH (15 mL) and concentrated HCl (0.44 mL, 5.40 mmol) was added. The resulting mixture was concentrated in vacuo and the residue was suspended in EtOH and Et_2O. The precipitate was collected by filtration and washed with Et_2O to give **2b** (1.02 g, 73%) as a colorless powder. Mp 137–139 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.83 (2H, t, J = 7.4 Hz), 2.94–3.08 (2H, m), 3.08–3.20 (2H, m), 3.48–3.84 (6H, m), 6.98 (1H, d, J = 7.4 Hz), 7.68–7.76 (2H, m), 7.90–8.04 (2H, m), 8.18–8.24 (2H, m), 8.24–8.32 (3H, m), 8.68 (1H, br s). Anal. Calcd for $C_{24}H_{23}ClN_4O_3S{\cdot}HCl{\cdot}2.2H_2O$: C, 51.56; H, 5.12; N, 10.02. Found: C, 51.87; H, 5.52; N, 9.75.

The following compounds **2c**, **2d**, **2f**, and **2g** were prepared in a manner similar to that described for **2b**.

5.1.11. 5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)-2-methylimidazo[1,2-*a*]pyridine hydrochloride (2c). Yield 66%, as a colorless powder. Mp 125–127 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.52 (3H, s), 2.84 (2H, t, J = 7.4 Hz), 2.94–3.08 (2H, m), 3.08–3.23 (2H, m), 3.23–3.54 (2H, m), 3.54–3.78 (4H, m), 6.93 (1H, d, J = 7.2 Hz), 7.60 (1H, d, J = 8.8 Hz), 7.71–7.76 (1H, m), 7.88 (1H, dd, J = 8.8 and 8.0 Hz), 7.99 (1H, s), 8.03–8.04 (1H, m), 8.18–8.32 (3H, m), 8.68 (1H, br s). Anal. Calcd for $C_{25}H_{25}ClN_4O_3S{\cdot}HCl{\cdot}3H_2O$: C, 51.11; H, 5.49; N, 9.54. Found: C, 51.01; H, 5.46; N, 9.45.

5.1.12. [5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-3-yl]methanol hydrochloride (2d). Yield 47%, colorless powder. Mp 192–194 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.56–3.01 (5H, m), 3.12–3.33 (2H, m), 3.33–3.84 (3H, m),

3.84–4.02 (1H, m), 4.17–4.36 (1H, m), 5.01 (2H, d, J = 6.2 Hz), 7.21–7.28 (1H, m), 7.72–7.86 (2H, m), 7.86–8.11 (2H, m), 8.11–8.37 (4H, m), 8.68 (1H, s). Anal. Calcd for $C_{25}H_{25}ClN_4O_3S{\cdot}HCl{\cdot}H_2O{\cdot}0.5Et_2O$: C, 53.64; H, 5.50; N, 9.27. Found: C, 53.76; H, 5.71; N, 9.49.

5.1.13. 2-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]propan-2-ol hydrochloride (2f). Yield 65%, colorless amorphous powder. Mp > 132 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.63 (6H, s), 2.82 (2H, t, J = 7.2 Hz), 2.92–3.06 (2H, m), 3.06–3.20 (2H, m), 3.44–3.82 (6H, m), 6.97 (1H, d, J = 7.4 Hz), 7.59 (1H, d, J = 8.8 Hz), 7.74 (1H, dd, J = 8.8 and 2.2 Hz), 7.83–8.04 (3H, m), 8.18–8.32 (3H, m), 8.67 (1H, br s). Anal. Calcd for $C_{27}H_{29}ClN_4O_3S{\cdot}HCl{\cdot}H_2O{\cdot}0.4Et_2O$: C, 54.94; H, 5.80; N, 8.96. Found: C, 55.03; H, 6.12; N, 8.73.

5.1.14. 1-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]ethanol hydrochloride (2g). Yield 56%, colorless amorphous powder. Mp 191–193 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.56 (3H, d, J = 6.2 Hz), 2.73–2.90 (2H, t, J = 7.6 Hz), 2.90–3.08 (2H, m), 3.08–3.22 (2H, m), 3.22–3.56 (2H, m), 3.56–3.83 (4H, m), 5.07 (1H, q, J = 6.2 Hz), 6.11 (1H, br s), 6.96 (1H, d, J = 7.8 Hz), 7.60 (1H, d, J = 8.8 Hz), 7.71–7.76 (1H, m), 7.86–8.04 (3H, m), 8.18–8.32 (3H, m), 8.68 (1H, br s). Anal. Calcd for $C_{26}H_{27}ClN_4O_4S{\cdot}HCl{\cdot}H_2O{\cdot}0.4Et_2O$: C, 54.24; H, 5.61; N, 9.17. Found: C, 54.44; H, 5.77; N, 9.04.

5.1.15. [5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]methanol (2e). A solution of **11** (3.99 g, 12.0 mmol) in concentrated HCl (20 mL) was stirred at room temperature for 20 min, and the reaction mixture was diluted with EtOH (50 mL) and 2-PrOH (50 mL). The precipitate was collected by filtration and washed with 2-PrOH and Et_2O to give [5-(piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]methanol dihydrochloride (3.66 g, quant.) as a colorless powder.

To a mixture of **15** (0.75 g, 2.51 mmol) and HOBt (0.57 g, 3.72 mmol) in MeCN (15 mL) was added WSC (0.72 g, 3.76 mmol) and the mixture was stirred at room temperature for 20 min. A solution of [5-(piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]methanol dihydrochloride (0.92 g, 3.01 mmol), Et_3N (1.05 mL, 7.53 mmol), and DBU (0.90 mL, 6.03 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. The solvent was evaporated in vacuo and the residue was partitioned between $CHCl_3$ (50 mL) and water (50 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 5:1) and recrystallized from acetone–EtOH to give **2e** (0.77 g, 60%) as a colorless powder. Mp 194–195 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.88–3.18 (6H, m), 3.56–3.92 (6H, m), 4.88 (2H, s), 6.27 (1H, d, J = 7.4 Hz), 7.19 (1H, dd, J = 9.2 and 7.4 Hz), 7.37 (1H, d, J = 8.8 Hz), 7.52 (1H, s), 7.57–7.63 (1H, m), 7.94–7.98 (4H, m), 8.49 (1H, br s). Anal. Calcd for $C_{25}H_{25}ClN_4O_4S$: C, 58.53; H, 4.91; N, 10.92. Found: C, 58.40; H, 4.84; N, 10.78.

5.1.16. 8-(Imidazo[1,2-*a*]pyridin-5-yl)-1,4-dioxa-8-azaspiro[4.5]decane (17). Compound **17** was prepared in 85% yield by the same method as described above for the synthesis of **10** as a pale yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.96 (4H, t, J = 6.0 Hz), 3.22 (4H, t, J = 4.5 Hz), 4.04 (4H, s), 6.32 (1H, d, J = 7.5 Hz), 7.18 (1H, dd, J = 9.3 and 7.2 Hz), 7.40 (1H, d, J = 8.4 Hz), 7.54 (1H, s), 7.65 (1H, s).

5.1.17. 1-(Imidazo[1,2-*a*]pyridin-5-yl)-*N*-methylpiperidin-4-amine hydrochloride (18). A solution of **17** (6.60 g, 25.4 mmol) in 4 M HCl (14 mL) and acetone (25 mL) was stirred at 50 °C for 6 h, and the reaction mixture was concentrated in vacuo. The concentrated solution was basified with 1 M NaOH to pH 11, saturated with NaCl, and extracted with $CHCl_3$ (100 mL). The extract was washed with brine (10 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in acetic acid (50 mL) and a solution of methylamine (40%, 25 mL) in MeOH was added dropwise over 30 min at 0 °C. After the mixture was stirred at room temperature for 30 min, sodium triacetoxyborohydride (6.30 g, 29.7 mmol) was added and then the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and the concentrated solution was basified with 1 M NaOH to pH 11, saturated with NaCl, and extracted with $CHCl_3$ (100 mL). The extract was washed with brine (10 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in EtOH (100 mL) and di-*tert*-butyl dicarbonate (5.55 g, 25.4 mmol) was added dropwise at room temperature. After the mixture was stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo and the residue was purified by silica gel chromatography (EtOAc/EtOH = 5:1). The obtained product was dissolved in EtOH (10 mL) and concentrated HCl (21 mL) was added. The resulting mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was crystallized from EtOH to give **18** (3.07 g, 38%) as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.91–2.07 (2H, m), 2.34–2.39 (2H, m), 2.83 (3H, s), 2.98–3.11 (2H, m), 3.39–3.51 (1H, m), 3.63–3.70 (2H, m), 7.00 (1H, d, J = 7.6 Hz), 7.58 (1H, d, J = 8.8 Hz), 7.83–7.93 (3H, m).

5.1.18. 3-[(6-Chloronaphthalen-2-yl)sulfonyl]-*N*-[1-(imidazo[1,2-*a*]pyridin-5-yl)piperidin-4-yl]-*N*-methylpropanamide (2a). Compound **2a** was prepared in a manner similar to that described for **2b** in 37% yield as a colorless powder. Mp 190 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.69–1.73 (2H, m), 1.85–1.93 (2H, m), 2.77–3.08 (7H, m), 3.48–3.52 (2H, m), 3.57–3.62 (2H, m), 3.77–3.95 (0.3H, m), 4.53–4.68 (0.7H, m), 6.27–6.34 (1H, m), 7.14–7.24 (1H, m), 7.38–7.45 (1H, m), 7.50–7.52 (1H, m), 7.60 (1H, dd, J = 9.0 and 1.8 Hz), 7.64–7.67 (1H, m), 7.92–7.97 (4H, m), 8.50 (1H, s). Anal. Calcd for $C_{26}H_{27}ClN_4O_3S$: C, 61.11; H, 5.33; N, 10.96. Found: C, 61.03; H, 5.37; N, 11.21.

5.1.19. Ethyl (5-fluoroimidazo[1,2-*a*]pyridin-2-yl)acetate (20). A solution of **19**[12] (5.00 g, 44.6 mmol) and ethyl 4-chloroacetoacetate (7.20 mL, 53.3 mmol) in EtOH (120 mL) was refluxed for 12 h and then concentrated

in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$ (100 mL) and extracted with EtOAc (100 mL ×2). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (2:1 EtOAc/hexane to 20:1 EtOAc/EtOH) to give **20** (7.00 g, 71%) as a brown oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.30 (3H, t, J = 7.0 Hz), 3.89 (2H, s), 4.22 (2H, q, J = 7.0 Hz), 6.42–6.48 (1H, m), 7.14–7.26 (1H, m), 7.40 (1H, dd, J = 9.2 and 0.8 Hz), 7.67 (1H, s).

5.1.20. *tert*-Butyl 4-(2-ethoxycarbonylmethylimidazo[1,2-*a*]pyridin-5-yl)piperazine-1-carboxylate (21). A mixture of **20** (6.40 g, 28.8 mmol) and piperazine (10.0 g, 116 mmol) was stirred at 100 °C for 1 h. After cooling to room temperature, the reaction mixture was diluted with $CHCl_3$ (200 mL) and water (100 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in EtOH (70 mL), and $(Boc)_2O$ (6.33 g, 29.0 mmol) was added dropwise to the solution at room temperature. The resulting mixture was stirred at room temperature for 30 min and concentrated in vacuo. The residue was diluted with water (100 mL) and extracted with EtOAc (100 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 10:1 EtOAc/EtOH) to give **21** (5.50 g, 49%) as a pale yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.30 (3H, t, J = 7.2 Hz), 1.34 (9H, s), 3.08 (4H, t, J = 4.6 Hz), 3.68 (4H, br s), 3.88 (2H, s), 4.22 (2H, q, J = 7.2 Hz), 6.28 (1H, dd, J = 7.4 and 1.0 Hz), 7.17 (1H, dd, J = 9.2 and 7.4 Hz), 7.34 (1H, d, J = 9.2 Hz), 7.55 (1H, s).

5.1.21. *tert*-Butyl 4-[2-(*N*,*N*-dimethylcarbamoylmethyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (22). To a solution of dimethylamine (2 M in THF; 22.5 mL, 129 mmol) and diisopropylethylamine (22.5 mL, 129 mmol) in CH_2Cl_2 (100 mL) was added dimethylaluminum chloride (1 M in hexane; 100 mL, 100 mmol) dropwise at 0 °C. After stirring at 0 °C for 30 min, a solution of **21** (5.00 g, 12.9 mmol) in CH_2Cl_2 (50 mL) was added dropwise. After stirring at room temperature for 12 h, the resulting mixture was poured into saturated aqueous $NaHCO_3$ solution at 0 °C and extracted with $CHCl_3$ (100 mL ×2). The extract was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 5:1 to 2:1) to give **22** (3.45 g, 69%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.50 (9H, s), 2.99 (3H, s), 3.09 (4H, br s), 3.18 (3H, s), 3.66 (4H, br s), 3.93 (2H, s), 6.27 (1H, d, J = 7.0 Hz), 7.16 (1H, dd, J = 9.0 and 7.0 Hz), 7.32 (1H, d, J = 9.0 Hz), 7.57 (1H, s).

5.1.22. *tert*-Butyl 4-(2-carbamoylmethylimidazo[1,2-*a*]pyridin-5-yl)piperazine-1-carboxylate (23). To a solution of **21** (2.50 g, 6.44 mmol) in MeCN (100 mL) was added 25% aqueous ammonia solution (100 mL) and the resulting mixture was stirred at 50 °C for 24 h. MeCN was evaporated in vacuo and extracted with $CHCl_3$ (50 mL ×2). The extract was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 2:1) to give **23** (1.95 g, 84%) as a colorless solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.51 (9H, s), 3.08 (4H, t, J = 4.8 Hz), 3.68 (4H, br s), 3.76 (2H, s), 5.31 (1H, s), 5.48 (1H, br s), 6.33 (1H, dd, J = 7.0 and 1.0 Hz), 7.23 (1H, dd, J = 8.8 and 7.0 Hz), 7.34 (1H, d, J = 8.8 Hz), 7.46 (1H, s).

5.1.23. *tert*-Butyl 4-{2-[(*N*-methylcarbamoyl)methyl]imidazo[1,2-*a*]pyridin-5-yl}piperazine-1-carboxylate (24). Compound **24** was prepared in a manner similar to that described for **22**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.51 (9H, s), 2.82 (3H, s), 3.07 (4H, br s), 3.68 (4H, br s), 3.75 (2H, s), 6.33 (1H, dd, J = 7.4 and 1.2 Hz), 7.19–7.44 (3H, m).

5.1.24. *tert*-Butyl 4-[2-(2-hydroxy-2-methylpropyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (25). A suspension of $CeCl_3$ (5.72 g, 23.2 mmol) in THF (30 mL) was vigorously stirred at room temperature for 12 h. To the suspension was added a solution of methylmagnesium bromide in THF (1 M, 22.0 mL, 22.0 mmol) dropwise at 0 °C and the mixture was stirred at that temperature for 2.5 h. A solution of **21** (1.60 g, 4.11 mmol) in THF (100 mL) was added to the suspension at 0 °C and then the resulting mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into 5% aqueous acetic acid at 0 °C and extracted with EtOAc (50 mL ×3). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 2:1 EtOAc/EtOH). The product obtained was treated with $CeCl_3$ (6.64 g, 26.9 mmol) and methylmagnesium bromide in THF (1 M, 44.0 mL, 44 mmol) by the same method as described above to give **25** (0.22 g, 14%) as a pale yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.26 (6H, s), 1.51 (9H, s), 2.92 (2H, s), 3.08 (4H, t, J = 5.0 Hz), 3.69 (4H, br s), 6.30 (1H, dd, J = 7.4 and 0.9 Hz), 7.15–7.23 (2H, m), 7.31–7.34 (2H, m).

5.1.25. *tert*-Butyl 4-[2-(2-hydroxypropyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (26). A suspension of $CeCl_3$ (5.72 g, 23.2 mmol) in THF (30 mL) was vigorously stirred at room temperature for 12 h. To the suspension was added a solution of **22** (3.00 g, 7.74 mmol) and the mixture was stirred at room temperature for 1 h. A solution of methylmagnesium bromide in THF (1 M, 24.0 mL, 24.0 mmol) was added dropwise to the suspension at 0 °C and the resulting mixture was stirred at that temperature for 1 h. The reaction mixture was poured into 5% aqueous acetic acid at 0 °C and extracted with EtOAc (50 mL ×3). The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc to 2:1 EtOAc/EtOH) to give *tert*-butyl 4-[2-(2-oxopropyl)imidazo[1,2-*a*]pyridin-5-yl]piperazine-1-carboxylate (1.90 g, 69%) as a yellow oil.

To a solution of the product obtained in EtOH (20 mL) was added $NaBH_4$ (0.35 g, 9.40 mmol) at 0 °C and the resulting mixture was stirred at room temperature for 1 h. Water (5 mL) was added to the reaction mixture and EtOH was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and water

(50 mL). The organic layer was separated, washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 5:1) to give **26** (0.88 g, 79%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.30 (3H, d, J = 6.2 Hz), 1.51 (9H, s), 2.79 (1H, dd, J = 14.6 and 8.4 Hz), 2.97 (1H, dd, J = 14.6 and 3.4 Hz), 3.05–3.10 (4H, m), 3.67–3.78 (5H, m), 4.14–4.24 (1H, m), 6.29 (1H, d, J = 7.0 Hz), 7.18 (1H, dd, J = 8.8 and 7.0 Hz), 7.28–7.35 (2H, m).

5.1.26. Ethyl [5-(4-{3-[(6-chloronaphthalen-2-yl)sulfonyl]-propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]acetate hydrochloride (2h). A solution of **21** (1.50 g, 3.86 mmol) in concentrated HCl (10 mL) was stirred at room temperature for 5 min. The reaction mixture was diluted with EtOH (50 mL) and concentrated in vacuo to give ethyl [5-(piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]acetate dihydrochloride (1.40 g, quant.) as a pale yellow amorphous powder.

To a mixture of **15** (0.96 g, 3.21 mmol) and HOBt (0.74 g, 4.83 mmol) in MeCN (20 mL) was added WSC (0.92 g, 4.8 mmol) and the mixture was stirred at room temperature for 20 min. A solution of the ethyl [5-(piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]acetate dihydrochloride (1.40 g, 3.86 mmol), Et_3N (1.35 mL, 9.69 mmol), and DBU (1.20 mL, 8.04 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. The solvent was evaporated in vacuo and the residue was partitioned between $CHCl_3$ (50 mL) and water (50 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/EtOH = 5:1) to give ethyl [5-(4-{3-[(6-chloronaphthalen-2-yl)sulfonyl]propanoyl}-piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]acetate (1.20 g, 66%) as a colorless oil.

The product (1.20 g, 2.11 mmol) was dissolved in EtOAc (30 mL) and 4 M HCl in EtOAc (1.00 mL, 4.00 mmol) was added and the resulting mixture was stirred at room temperature for 10 min. The precipitate was collected by filtration and washed with EtOAc and Et_2O to give **2h** (0.68 g, 53%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.24 (3H, t, J = 7.2 Hz), 2.83 (2H, t, J = 7.2 Hz), 3.00 (2H, br s), 3.12 (2H, br s), 3.61–3.70 (6H, m), 4.12 (2H, s), 4.17 (2H, q, J = 7.2 Hz), 6.96 (1H, d, J = 7.5 Hz), 7.65 (1H, d, J = 9.0 Hz), 7.73 (1H, dd, J = 8.7 and 2.1 Hz), 7.90 (1H, dd, J = 8.7 and 7.8 Hz), 8.00 (1H, dd, J = 8.4 and 1.8 Hz), 8.11 (1H, s), 8.19 (1H, d, J = 8.4 Hz), 8.26–8.30 (2H, m), 8.67 (1H, d, J = 1.8 Hz). Anal. Calcd for $C_{29}H_{29}ClN_4O_5S{\cdot}HCl{\cdot}1.5H_2O$: C, 54.04; H, 5.16; N, 8.69. Found: C, 54.24; H, 5.35; N, 8.31.

The following compounds **2i**–**m** were prepared in a manner similar to that described for **2h**.

5.1.27. 2-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]-*N*,*N*-dimethylacetamide hydrochloride (2i). Yield 67%, colorless powder. Mp 150–152 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.96 (2H, t, J = 7.5 Hz), 3.03 (3H, s), 3.13 (2H, br s), 3.24–3.26 (5H, m), 3.60 (2H, t, J = 7.5 Hz), 3.80 (4H, br s), 4.26 (2H, s), 6.74 (1H, d, J = 7.4 Hz), 7.59–7.66 (1H, m), 7.73 (1H, d, J = 7.4 Hz), 7.85 (1H, d, J = 8.8 Hz), 7.93–8.00 (5H, m), 8.50 (1H, s). Anal. Calcd for $C_{28}H_{30}ClN_5O_4S{\cdot}HCl{\cdot}0.5H_2O$: C, 54.81; H, 5.26; N, 11.41. Found: C, 54.67; H, 5.18; N, 11.35.

5.1.28. 2-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]acetamide (2j). Yield 62%, colorless powder. Mp 159–161 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.93–3.12 (6H, m), 3.60 (2H, t, J = 7.4 Hz), 3.77–3.81 (6H, m), 5.47 (2H, br s), 6.31 (1H, dd, J = 7.0 and 1.2 Hz), 7.25 (1H, dd, J = 8.8 and 7.0 Hz), 7.37 (1H, d, J = 8.8 Hz), 7.45 (1H, s), 7.61 (1H, dd, J = 8.8 and 2.2 Hz), 7.94–8.00 (4H, m), 8.50 (1H, s). Anal. Calcd for $C_{26}H_{26}ClN_5O_4S{\cdot}H_2O$: C, 55.96; H, 5.06; N, 12.55. Found: C, 56.09; H, 4.90; N, 12.71.

5.1.29. 2-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]-*N*-methylacetamide (2k). Yield 74%, colorless powder. Mp 186–188 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.80–2.82 (3H, m), 2.93–3.15 (8H, m), 3.60 (2H, t, J = 6.4 Hz), 3.75–3.82 (4H, m), 6.42 (1H, dd, J = 7.0 and 1.2 Hz), 7.32–7.64 (5H, m), 7.64–8.00 (4H, m), 8.50 (1H, s). Anal. Calcd for $C_{27}H_{28}ClN_5O_4S{\cdot}H_2O$: C, 56.69; H, 5.29; N, 12.24. Found: C, 56.59; H, 5.18; N, 12.11.

5.1.30. 1-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]-2-methylpropan-2-ol hydrochloride (2l). Yield 50%, colorless amorphous powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.20 (6H, s), 2.82 (2H, t, J = 7.3 Hz), 2.94 (2H, s), 3.01 (2H, br s), 3.12 (2H, br s), 3.64–3.68 (6H, m), 6.95 (1H, d, J = 7.6 Hz), 7.63 (1H, d, J = 8.8 Hz), 7.74 (1H, dd, J = 8.4 and 2.2 Hz), 7.88 (1H, dd, J = 8.8 and 7.6 Hz), 7.96 (1H, s), 8.02 (1H, dd, J = 8.4 and 1.4 Hz), 8.21 (1H, d, J = 8.4 Hz), 8.28–8.32 (2H, m), 8.69 (1H, s). Anal. Calcd for $C_{28}H_{31}ClN_4O_4S{\cdot}HCl{\cdot}2H_2O$: C, 53.59; H, 5.78; N, 8.93. Found: C, 53.72; H, 5.80; N, 8.72.

5.1.31. 1-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-2-yl]propan-2-ol (2m). Yield 73%, colorless powder. Mp 108–109 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (3H, d, J = 6.3 Hz), 2.80 (1H, dd, J = 14.7 and 8.7 Hz), 2.94–2.99 (3H, m), 3.08 (4H, d, J = 26.1 Hz), 3.60 (2H, t, J = 7.2 Hz), 3.73 (4H, br s), 4.17–4.23 (1H, m), 6.26 (1H, d, J = 7.2 Hz), 7.18 (1H, dd, J = 8.7 and 7.2 Hz), 7.33–7.35 (2H, m), 7.59 (1H, dd, J = 8.7 and 2.0 Hz), 7.90–7.96 (4H, m), 8.49 (1H, s). Anal. Calcd for $C_{27}H_{29}ClN_4O_4S{\cdot}0.5H_2O$: C, 58.95; H, 5.50; N, 10.19. Found: C, 59.25; H, 5.78; N, 9.83.

5.1.32. *tert*-Butyl 4-(3-nitroimidazo[1,2-*a*]pyridin-5-yl)piperazine-1-carboxylate (28). A mixture of **27** (9.88 g, 50.0 mmol), 1-Boc-piperazine (14.0 g, 75.2 mmol), and *N*,*N*-diisopropylethylamine (34.8 mL, 200 mmol) in 2-PrOH (300 mL) was refluxed for 4 h. The reaction mixture was concentrated in vacuo. The residue was crystal-

lized from EtOH to give **28** (17.4 g, 67%) as a yellow powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.47 (9H, s), 2.50–3.40 (6H, m), 3.54–4.25 (2H, m), 6.67 (1H, d, J = 7.6 Hz), 7.50 (1H, d, J = 8.3 Hz), 7.59 (1H, dd, J = 8.3 and 7.6 Hz), 8.49 (1H, s).

5.1.33. 5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)-3-nitroimidazo[1,2-*a*]pyridine (2n). To a solution of **28** (17.4 g, 50.0 mmol) in MeOH (300 mL) was added 4 M HCl in EtOAc (80 mL) and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo and the residue was crystallized from EtOH to give 3-nitro-5-(piperazin-1-yl)imidazo[1,2-*a*]pyridine dihydrochloride (16.0 g, quant.) as a yellow powder.

A solution of the product (9.61 g, 30.0 mmol) and DBU (8.95 mL, 60.0 mmol) in MeCN (300 mL) was stirred at room temperature for 10 min. To the solution were added **15** (8.96 g, 95.6 mmol) and HOBt (5.51 g, 36.0 mmol), and the mixture was cooled to 0 °C. Next Et_3N (8.36 mL, 60.0 mmol) and WSC (6.90 g, 36.0 mmol) were added and the resulting mixture was stirred at room temperature for 2 days. The reaction mixture was concentrated in vacuo and the residue was partitioned between CH_2Cl_2 (100 mL) and 10% aqueous Na_2CO_3 solution (100 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc = 1:3) and recrystallized from EtOAc to give **2n** (9.95 g, 63%) as a pale yellow powder. Mp 213–215 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.60–3.10 (5H, m), 3.10–3.95 (6H, m), 4.35–4.65 (1H, br s), 6.66 (1H, dd, J = 7.4 and 1.6 Hz), 7.51–7.66 (3H, m), 7.89–7.99 (4H, m), 8.48 (1H, s), 8.51 (1H, s). Anal. Calcd for $C_{24}H_{22}ClN_5O_5S{\cdot}0.1H_2O$: C, 54.41; H, 4.22; N, 13.22. Found: C, 54.43; H, 4.47; N, 12.94.

5.1.34. 5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-3-amine dihydrochloride (2o). A mixture of **2n** (8.24 g, 15.6 mmol), iron powder (4.36 g, 78.1 mmol), and $CaCl_2$ (3.55 g, 32.0 mmol) in 80% EtOH (600 mL) and DMF (60 mL) was refluxed overnight. After cooling to room temperature, iron powder (4.36 g, 78.1 mmol) was added to the mixture. The resulting mixture was refluxed for 5 h and filtered through a Celite pad. The filtrate was concentrated in vacuo and the residue was partitioned between CH_2Cl_2 (100 mL) and 10% aqueous Na_2CO_3 solution (100 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc/MeOH = 20:1). The product was dissolved in EtOAc (20 mL) and EtOH (10 mL), and then 4 M HCl in EtOAc (5 mL) was added. After stirring at 0 °C for 30 min, the precipitate was collected by filtration and washed with EtOAc to give **2o** (1.02 g, 11%) as a colorless powder. Mp 144–146 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.70–3.10 (4H, m), 3.20–3.36 (2H, m), 3.42–3.72 (3H, m), 3.82–3.94 (1H, m), 4.18–4.30 (1H, m), 5.00–6.20 (4H, br s), 6.81 (1H, d, J = 7.0 Hz), 7.14 (1H, s), 7.48 (1H, d, J = 9.0 Hz), 7.61 (1H, dd, J = 9.0 and 7.0 Hz), 7.75 (1H, dd, J = 8.8 and 2.2 Hz), 8.01 (1H, dd, J = 8.8 and 2.0 Hz), 8.20 (1H, d, J = 8.8 Hz), 8.28–8.34 (2H, m), 8.68 (1H, s), 13.95 (1H, br s). Anal. Calcd for $C_{24}H_{24}ClN_5O_3S{\cdot}2HCl{\cdot}0.3EtOAc$: C, 50.67; H, 4.79; N, 11.72. Found: C, 50.95; H, 4.55; N, 11.75.

5.1.35. *N*-[5-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridin-3-yl]acetamide (2p). A mixture of **2o** (0.29 g, 0.50 mmol) and acetic anhydride (90 mg, 0.88 mmol) in pyridine (5 mL) was stirred at room temperature for 5 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between CH_2Cl_2 (20 mL) and 10% aqueous Na_2CO_3 solution (20 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/25% NH_4OH = 200:10:1). The product was crystallized from EtOAc and Et_2O to give **2p** (0.20 g, 76%) as a colorless powder. Mp 146–148 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.26 (3H, s), 2.38–2.88 (10H, m), 3.82–4.08 (1H, m), 4.54–4.76 (1H, m), 6.40–6.49 (1H, m), 7.08–7.22 (1H, m), 7.43–7.62 (2H, m), 7.91–8.03 (5H, m), 8.50 (1H, s), 10.19 (1H, s). Anal. Calcd for $C_{26}H_{26}ClN_5O_4S{\cdot}0.6H_2O$: C, 56.69; H, 4.98; N, 12.71. Found: C, 56.60; H, 4.89; N, 12.45.

5.1.36. *tert*-Butyl 4-(6-nitropyridin-3-yl)piperazine-1-carboxylate (29). A solution of 1-Boc-piperazine (2.79 g, 15.0 mmol) and 5-bromo-2-nitropyridine (1.02 g, 5.00 mmol) in *N*-methylpyrrolidone (15 mL) was stirred at 120 °C for 3 h. The reaction mixture was diluted with water and the precipitate was collected by filtration to give **29** (1.27 g, 82%) as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.49 (9H, s), 3.43–3.52 (4H, m), 3.62–3.67 (4H, m), 7.21 (1H, dd, J = 3.0 and 9.0 Hz), 8.14 (1H, d, J = 3.0 Hz), 8.19 (1H, d, J = 9.2 Hz).

5.1.37. *tert*-Butyl 4-(6-aminopyridin-3-yl)piperazine-1-carboxylate (30). A suspension of **29** (0.31 g, 1.01 mmol) and 10% palladium on carbon (50% water, 80 mg) in EtOH (20 mL) was hydrogenated for 2 h. The reaction mixture was filtered and concentrated in vacuo to give **30** (0.28 g, quant.) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48 (9H, s), 2.95 (4H, t, J = 4.5 Hz), 3.55–3.58 (4H, m), 6.51 (1H, dd, J = 0.9 and 9.0 Hz), 7.19 (1H, dd, J = 3.0 and 8.7 Hz), 7.73 (1H, d, J = 2.1 Hz).

5.1.38. *tert*-Butyl 4-(imidazo[1,2-*a*]pyridin-6-yl)piperazine-1-carboxylate (31). A solution of **30** (0.24 g, 0.86 mmol) and aqueous chloroacetaldehyde (40%, 0.34 g, 1.70 mmol) in EtOH (20 mL) was refluxed for 15 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between aqueous $NaHCO_3$ solution (10 mL) and EtOAc (10 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **31** (0.28 g, quant.) as a brown oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.49 (9H, s), 3.00 (4H, t, J = 5.2 Hz), 3.58–3.68 (4H, m), 7.05 (1H, dd, J = 2.2 and 9.8 Hz), 7.51–7.57 (4H, m).

5.1.39. 6-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridine (2q). A solution of **31** (0.26 g, 0.86 mmol) in concentrated HCl (2 mL) and EtOH (2 mL) was stirred at room temperature for 1 h and the reaction mixture was concentrated in vacuo. The water was azeotroped with EtOH to give 6-(piperazin-1-yl)imidazo[1,2-*a*]pyridine dihydrochloride (0.25 g, quant.) as a brown amorphous powder.

To a mixture of **15** (0.26 g, 0.86 mmol) and HOBt (0.20 g, 1.31 mmol) in MeCN (25 mL) was added WSC (0.25 g, 1.30 mmol) and the mixture was stirred at room temperature for 20 min. To the mixture was added a solution of 6-(piperazin-1-yl)imidazo[1,2-*a*]pyridine dihydrochloride (0.25 g, 0.86 mmol), Et_3N (0.26 g, 2.6 mmol), and DBU (0.25 mL, 1.71 mmol), 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 $NaHCO_3$ solution (20 mL), THF (20 mL), and EtOAc (20 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc) to give **2q** (0.13 g, 31%) as a green amorphous powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.91–2.97 (4H, m), 3.06 (2H, t, J = 5.1 Hz), 3.56–3.61 (2H, m), 3.66 (2H, t, J = 5.1 Hz), 3.72 (2H, t, J = 5.1 Hz), 7.01 (1H, dd, J = 2.1 and 9.9 Hz), 7.51–7.60 (5H, m), 7.89–7.97 (4H, m), 8.48 (1H, s). Anal. Calcd for $C_{24}H_{23}ClN_4O_3S{\cdot}0.5H_2O{\cdot}0.4EtOAc$: C, 58.32; H, 5.20; N, 10.63. Found: C, 58.05; H, 5.41; N, 10.76.

5.1.40. *tert*-Butyl 4-(2-aminopyridin-4-yl)piperazine-1-carboxylate (33). A solution of 1-Boc-piperazine (13.0 g, 69.8 mmol) and **32**[16] (6.00 g, 46.7 mmol) in EtOH (50 mL) was refluxed for 7 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between $CHCl_3$ (100 mL) and aqueous K_2CO_3 solution (100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was washed with IPE to give **33** (10.8 g, 83%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48 (9H, s), 3.23–3.28 (4H, m), 3.52–3.57 (4H, m), 4.25 (2H, br s), 5.85 (1H, d, J = 3.3 Hz), 6.18 (1H, dd, J = 3.9 and 9.3 Hz), 7.83 (1H, d, J = 9.2 Hz).

5.1.41. *tert*-Butyl 4-(imidazo[1,2-*a*]pyridin-7-yl)piperazine-1-carboxylate (34). A solution of **33** (1.39 g, 4.99 mol), $NaHCO_3$ (0.42 g, 5.00 mmol), and aqueous chloroacetaldehyde (40%, 1.18 g, 6.00 mmol) in EtOH (20 mL) was refluxed for 3 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between aqueous K_2CO_3 solution (30 mL) and $CHCl_3$ (30 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by basic silica gel chromatography (EtOAc) to give **34** (1.26 g, 83%) as a brown powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.49 (9H, s), 3.17 (4H, t, J = 5.2 Hz), 3.60 (4H, t, J = 5.2 Hz), 6.59 (1H, dd, J = 2.4 and 7.6 Hz), 6.81 (1H, d, J = 2.6 Hz), 7.38 (1H, t, J = 0.6 Hz), 7.47 (1H, d, J = 1.0 Hz), 7.94 (1H, d, J = 7.8 Hz).

5.1.42. 7-(4-{3-[(6-Chloronaphthalen-2-yl)sulfonyl]propanoyl}piperazin-1-yl)imidazo[1,2-*a*]pyridine (2r). Compound **2r** was prepared in a manner similar to that described for **2q** in 24% yield as a colorless powder. Mp 200–202 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.91–2.96 (2H, m), 3.13 (2H, t, J = 5.1 Hz), 3.21 (2H, t, J = 5.1 Hz), 3.56–3.61 (2H, m), 3.65 (2H, t, J = 5.1 Hz), 3.71 (2H, t, J = 5.1 Hz), 6.55 (1H, dd, J = 2.4 and 7.5 Hz), 6.80 (1H, d, J = 2.1 Hz), 7.38 (1H, t, J = 0.6 Hz), 7.48 (1H, d, J = 1.5 Hz), 7.57 (1H, dd, J = 1.8 and 8.7 Hz), 7.89–7.96 (5H, m), 8.48 (1H, d, J = 1.5 Hz). Anal. Calcd for $C_{24}H_{23}ClN_4O_3S{\cdot}0.2H_2O$: C, 59.24; H, 4.85; N, 11.51. Found: C, 59.00; H, 4.69; N, 11.32.

5.2. Biology

5.2.1. In vitro assays for the inhibition of human FXa. Human Factor Xa (0.3 U/mL) was obtained from Roche Diagnostics. Chromogenic 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. Enzyme assay was carried out in 96-well microtiter plates. Test compounds were diluted in DMSO. Compound dilutions and 10 μL of enzyme solution were added to the well containing 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 (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_{50} values were calculated from the regression line based on the method of least squares between inhibitory effect and concentration. Data for new compounds were compared to positive control DX-9065a[6c] (FXa IC_{50} = 0.13 μM). The 95% confidence interval for the IC_{50} of DX-9065a was 0.12–0.15 μM (duplicate).

5.2.2. In vitro PT assays. The assay of plasma clotting time was performed using an automatic coagulometer (STA Compact, Diagnostica Stago). PT was measured with STA PT reagents (Roche Diagnostics). 1.5 μL of compound dilutions in DMSO was added to 48.5 μL of human normal plasma (fresh human plasma: FFP, Sekisui Chemical Co.), and the mixture was preincubated at 37 °C for 4 min. Coagulation was initiated with the addition of 100 μL of thromboplastin, and coagulation time was measured. Coagulation time prolonging ratio (%) was calculated 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. Data for new compounds were

compared to positive control DX-9065a[6c] (PT_2 = 0.81 ± 0.029 μM (mean ± SEM, n = 3)).

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

5.2.4. 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 μL 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 1000*g* for 10 min to obtain platelet poor plasma (PPP). PT was measured with an automatic coagulometer. Fifty microliters of PPP was preincubated at 37 °C for 4 min. The PPP was mixed with 100 μL 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 in 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.[6c] 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.

5.2.5. Enzyme inhibition assays. Enzyme assays using chromogenic substrates were performed as follows. Human Factor Xa was obtained from Roche diagnostics. Human thrombin was obtained from Sigma Chemical Co. Human trypsin was obtained from Atheus Research and Technology, Inc. Human kallikrein and plasmin were 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, kallikrein, 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, kallikrein, and t-PA, respectively. Compound dilutions were added to the wells containing buffer and enzyme, and incubated for 30 min. The enzyme reactions were initiated by the addition of substrate and the color developed from the release of *p*-nitroanilide from each chromogenic substrate was monitored continuously for 5 min at 405 nm on a microtiter plate reader. The K_i values were determined from a Lineweaver–Burk plot, when the optical densities were measured with different concentrations of substrates.

5.2.6. Pharmacokinetic analysis in cynomolgus monkeys. Test compound was administered to fasted cynomolgus monkeys (male, n = 3) intravenously (1 mg/kg, DMA/PEG400) and orally (1 mg/kg, 0.5% methylcellulose suspension). Before and 5, 10, 15, 30 min, 1, 2, 4, 8, 24 h after intravenous administration, or before and 15, 30 min, 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 the same volume of 0.01 mol/L HCO_2NH_4 (adjusted to pH 3.0 with HCO_2H) 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 × 50 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.

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