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Discovery of novel tetrahydroisoquinoline derivatives as potent and selective factor Xa inhibitors

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Abstract—A series of novel 2,7-disubstituted tetrahydroisoquinoline derivatives were designed and synthesized. Among these derivatives, compounds **1** and **2** (JTV-803) exhibited potent inhibitory activity against FXa and good selectivity with respect to other serine proteases (thrombin, plasmin, and trypsin). In addition, compound **2** exhibited potent anti-FXa activity after intravenous and oral administration to cynomolgus monkey, and showed a dose-dependent antithrombotic effect in a rat model of venous thrombosis.

Activation of the intravascular coagulation system is involved in a number of cardiovascular diseases such as deep vein thrombosis (DVT), disseminated intravascular coagulation (DIC), pulmonary embolism, ischemic stroke, and unstable angina. Interruption of the clotting cascade has been investigated to inhibit clot formation and for the prevention or treatment of these thrombotic disorders. Heparin and warfarin are the most widely used anticoagulants for the prophylaxis and treatment of thrombus-based diseases. However, these anticoagulants have clinical limitations due to dependence on antithrombin III and antagonism of vitamin K, respectively. For instance, heparin administration is sometimes associated with the development of an antibody to platelet factor (PF4) that causes thrombocytopenia, while warfarin requires a longer time for the onset of its action, needs continual monitoring, and sometimes interacts with food or other drugs. Factor Xa (FXa) is a trypsin-like serine protease that is an important enzyme in the clotting cascade, since it represents the confluence of the intrinsic and extrinsic pathways. Factor Xa forms the prothrombinase complex, together with nonenzymatic cofactor Va and Ca^{2+} on the surface phospholipids of platelets or endothelial cells, and this complex is responsible for the conversion of prothrombin to thrombin. Then thrombin catalyzes the cleavage of fibrinogen to fibrin, initiating a process that ultimately leads to clot formation. Therefore, we have been interested in achieving inhibition of FXa by the development of a novel FXa inhibitor.

Since the discovery of compound **3** (DX-9065a),[1] a variety of other compounds have been reported.[2] The release of fondaparin sodium in 2002, which is a synthetic pentasaccharide, proved the clinical effectiveness of FXa inhibitors as anticoagulants.[3] This situation prompted us to disclose our experimental results on novel FXa inhibitors (Fig. 1).

From analysis of the binding of FXa to compound **3**,[4] it has been demonstrated that the basic part of both ends of the compound is important for inhibition of FXa. We focused on the two basic parts for S1 and S4 sites, and designed a fundamental skeleton (compound **4**) as follows. At first, we selected an *N*-amidinotetrahydroisoquinoline ring that was expected to completely fill the S1 pocket of FXa and to interact with Asp 189.[5] Then a 4-piperidinylmethyloxy group was selected for position-7 of the tetrahydroisoquinoline ring as a spacer for the introduction of basic substituents because the nitrogen atom of the piperidinyl group could be oriented toward the S4 site.

Keywords: Potent and selective factor Xa (FXa) inhibitor; Tetrahydroisoquinoline derivatives; JTV-803; Antithrombotic effect on venous thrombosis in rats; Inhibition of human FXa.
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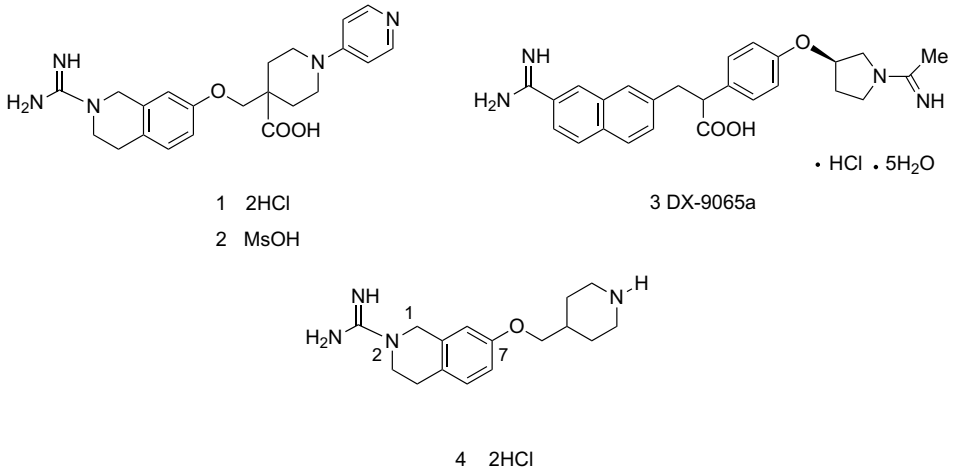
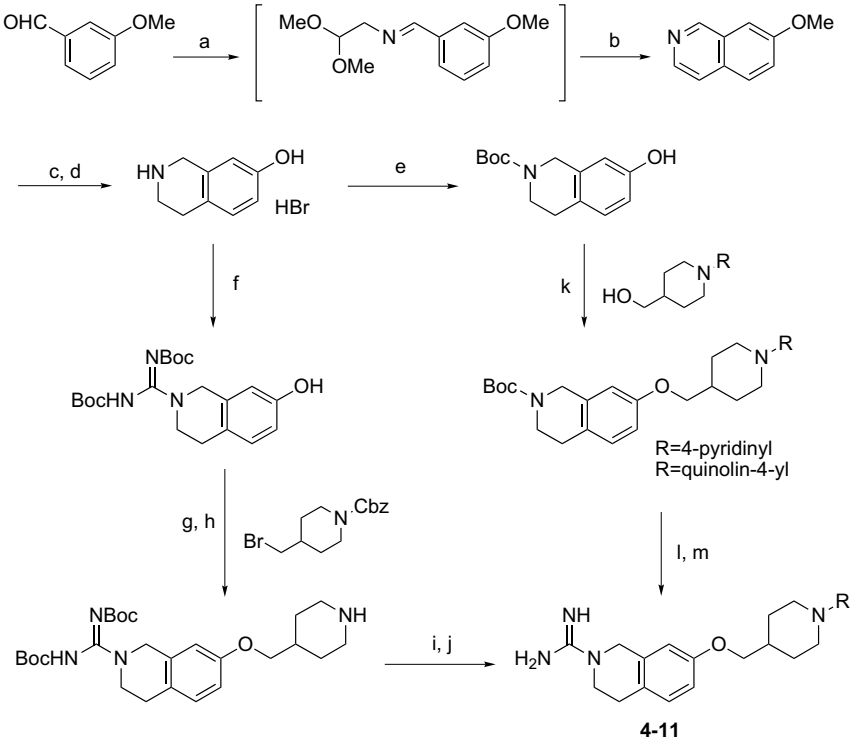


Figure 1.

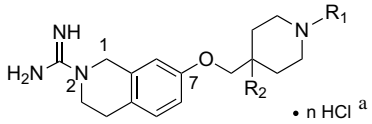
We initially prepared compound **4**, which showed moderate FXa inhibitory activity (IC_{50} = 3.0 μM),[1a] demonstrating that it had sufficient potential for further studies on structure–activity relationships. Introduction of the basic substituents on a nitrogen atom of the piperidine ring was carried out to obtain compounds **6**–**11** (Scheme 1 and Table 1).[6] Among them, compound **10** with a 4-pyridinyl group showed potent FXa inhibitory activity (IC_{50} = 0.06 μM) without inhibition of factor IIa (thrombin) (>10 μM). The potent activity of compound **10** might be explained by the interaction of protonated pyridine ring with S4 site which favors positive charge[7] and by hydrogen bonding of protonated nitrogen atom of pyridine with Glu 97, as expected from the docking simulation of the compounds with FXa.

However, most of the compounds listed in Table 1 showed fatal acute toxicity. When these compounds



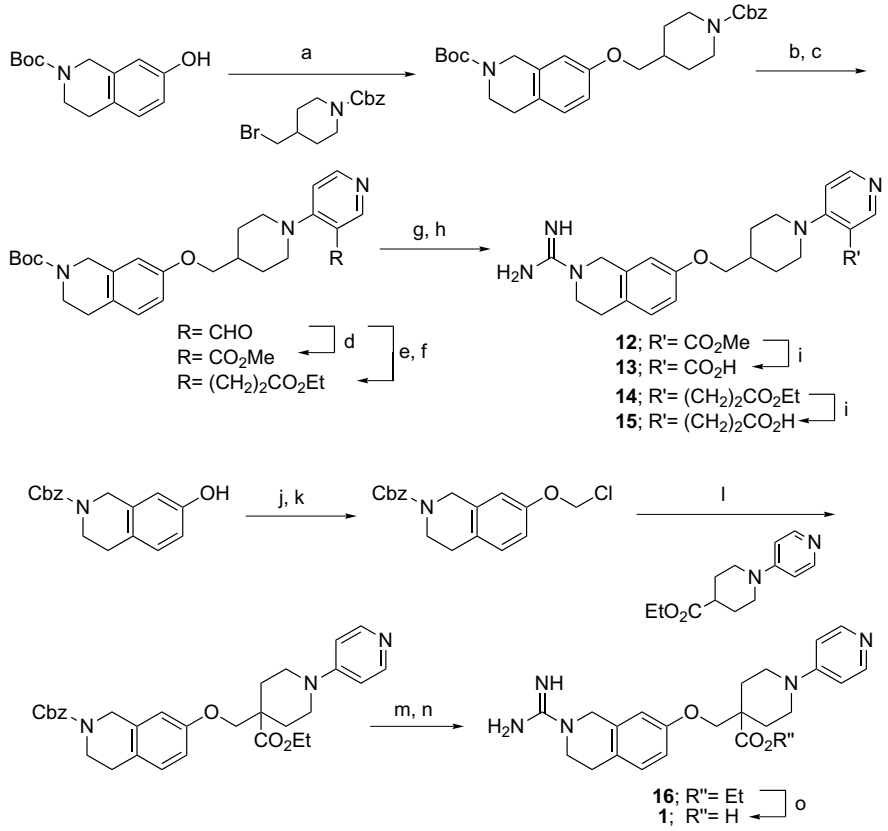
Scheme 1. Synthesis of compounds **4**–**11**. Reagents and conditions:[6] (a) aminoacetal, toluene, reflux; (b) 76% H_2SO_4 aq, 0 °C; (c) H_2 (3 atm), PtO_2 (cat.), AcOH, rt; (d) HBr aq, reflux; (e) Boc_2O, NaOH, dioxane-H_2O, rt; (f) 1*H*-pyrazole-1-(*N*,*N*′-bis-*tert*-butoxycarbonyl) carboxamidine, Et_3N, THE, rt; (g) NaOH, DMSO, rt; (h) H_2 (3 atm), 7.5% Pd/C (cat.), THF–EtOH, rt; (i) ethyl acetoimidate hydrochloride, Et_3N, THF–EtOH, rt for **5**; nitro-benzyl bromide, diisopropylethylamine (DIPEA),THF–DMF, rt, then H_2 (3 atm), 7.5% Pd/C for **6** and **7**; picolyl chloride, DIPEA THF–DMF, rt for **8** and **9**; (j) TFA, $CHCl_3$, rt, then HCl–MeOH; (k) diisopropyl azodicarboxylate (DIPAD), PPh_3, THE, rt; (l) TFA, $CHCl_3$, rt; (m) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, rt, then HCl–MeOH for **10** and **11**.

Table 1. Inhibitory activity for FXa and FIIa (Thrombin),[1a] and acute toxicity in mice (mortality)



Compound[a]	R_1	R_2	FXa IC_{50} (μM)[b]	FIIa IC_{50} (μM)[b]	Mortality (10 mg/kg, iv)
4	H	H	3.0	>10[b]	NT[c]
5	C(=NH)Me	H	0.5	>10	3/3[d]
6	2-Aminobenzyl	H	3.0	>10	NT
7	3-Aminobenzyl	H	4.0	>10	NT
8	Pyridin-2-ylmethyl	H	1.0	>10	NT
9	Pyridin-4-ylmethyl	H	0.8	>10	3/3
10	Pyridin-4-yl	H	0.06	>10	3/3
11	Quinolin-4-yl	H	0.4	>10	3/3
12	(3–CO_2Me)pyridin-4-yl	H	0.2	>10	3/3
13	(3–CO_2H)pyridin-4-yl	H	2.0	>10	0/3
14	(3–$(CH_2)_2CO_2$Et)pyridin-4-yl	H	0.2	>10	3/3
15	(3–$(CH_2)_2CO_2$H)pyridin-4-yl	H	0.2	>10	0/3
16	Pyridin-4-yl	CO_2Et	0.08	>10	3/3
1	Pyridin-4-yl	CO_2H	0.03	>10	0/3
17	2-Methylpyridin-4-yl	CO_2H	0.2	>10	0/3
18	1,3-Pyrimidin-4-yl	CO_2H	0.1	>10	0/3
19	4-(1-Methylpyridinium)chloride	CO_2H	1.0	>10	NT

[a] Compounds **1**, **4**, **5**, **10**–**18**: 2HCl salt, compounds **6**–**9**: 3HCl salt, compound **19**: HCl salt.
[b] $n = 2$ (number of animals).
[c] Not tested.
[d] Number of deaths/number of animals tested.



Scheme 2. Synthesis of compounds **1** and **12**–**16**. Reagents and conditions:[6] (a) NaH, DMF, rt; (b) H_2 (3 atm), 7.5% Pd/C (cat.), THF–EtOH, rt; (c) 4-chloro-3-formylpyridine, Et_3N, EtOH, reflux; (d) MnO_2, NaCN (cat.), $CHCl_3$–MeOH, rt; (e) $(EtO)_2POCH_2CO_2Et$, NaH, THF, rt; (f) H_2 (1 atm), 7.5% Pd/C (cat.), THF–EtOH, rt; (g) TFA, $CHCl_3$, rt; (h) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, rt; (i) NaOH, MeOH–H_2O, rt, then HCl aq; (j) $ClCH_2SMe$, NaH, DMF, rt; (k) SO_2Cl_2, CH_2Cl_2, 0 °C; (1) LDA, THF, −70 °C rt; (m) 30% HCl–EtOH, 85 °C; (n) 1*H*-pyrazole-1-carboxamidine, $NaHCO_3$ aq, acetone, rt; (o) c-HCl, reflux.

were administered intravenously at a bolus dose of 10 mg/kg, severe convulsions occurred immediately, and the mice died in a few minutes. Such fatal acute toxicity was also observed in our previous research on other FXa inhibitors, and was avoided by introduction of an acidic functional group.[8] Hence, we tried to introduce a carboxyl group to the most active compound **10**, as shown in Scheme 2 and Table 1 (compounds **13**, **15**, and **1**). As expected, acute toxicity was suppressed when the compounds were given carboxyl groups, while the corresponding esters still showed severe toxicity. Loss of toxicity after introduction of a carboxyl group at different positions suggested that one reason for severe toxicity might be the strong basicity of the compound. Similar to compound **10**, compound **1** exhibited potent inhibitory activity for FXa (IC_{50} = 0.03 μM). This finding was explained by conformational analysis of compounds **10** and **1**. That is, introduction of the carboxyl group at the tertiary carbon of the piperidinyl group did not influence the conformation of compound **10** and the introduced hydrophilic group was oriented towards the aqueous area. (Fig. 2).[9] On the other hand, the introduction of substituents (R_1) at the position-3 of pyridine ring causes the change of the torsional angle between the pyridine ring and the piperidine ring. The loss in activity of compounds **12**–**15** might be due to this fact.

For further pharmacological evaluation, compound **2** (MsOH salt) was used instead of compound **1** because stable crystals could be obtained. As shown in Table 2, there was selectivity of **2** for FXa relative to other serine proteases (thrombin, plasmin, and trypsin). The anti-FXa activity of compound **2** was evaluated after intravenous and oral administration to cynomolgus monkey based on inhibition of human FXa in plasma (see Fig. 3).[10] After oral administration of compound **2** at a dose of 10 mg/kg, inhibition increased to a maximum of 41 ± 4% at 120 min and then gradually declined. The plasma concentration (C_{max}) was 0.39 μg/mL at 120 min.[11] Thus, compound **2** showed anti-FXa activity after intravenous and oral administration to cynomolgus monkey. Subsequently, the antithrombotic effect of **2** was examined in a rat venous thrombosis model.[12] As shown in Figure 4, compound **2** had a dose-dependent antithrombotic effect at doses of 0.1–1 mg/kg/h, and the effect was statistically significant at 0.3 or 1 mg/kg/h. Low molecular weight heparin (LMWH) also showed a dose-dependent antithrombotic effect in this model at doses of 30–300 U/kg/h.

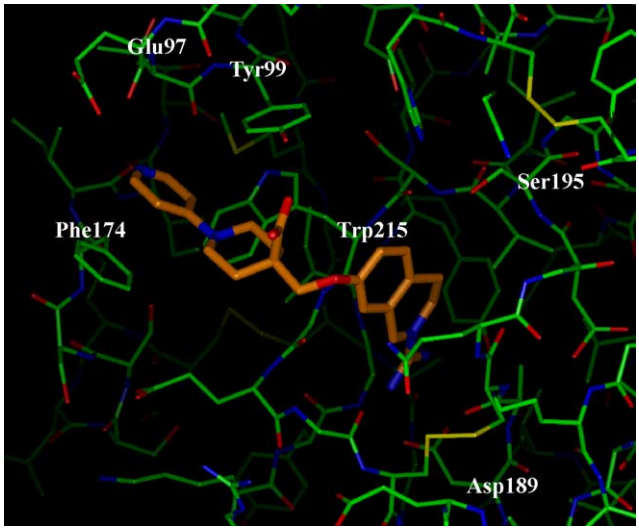


Figure 2. Complex model of factor Xa (green) and compound **1** (orange).

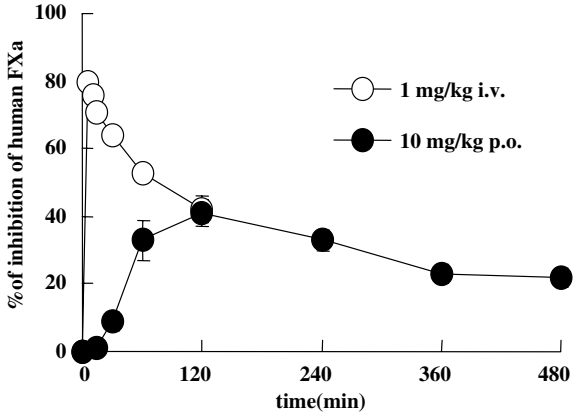


Figure 3. Percent inhibition of human factor Xa after intravenous and oral administration of compound **2** to cynomolgus monkeys.[10] Data represent the mean ± SEM (n = 6).

In conclusion, a series of tetrahydroisoquinoline derivatives were designed and synthesized. Among them, compounds **1** and **2** exhibited the most potent inhibition of FXa. The selectivity of compound **2** (JTV-803) for FXa relative to other serine proteases (thrombin, plasmin, and trypsin) was demonstrated. Moreover, compound **2** showed good efficacy when pharmacological evaluation was done in a rat venous thrombosis model, and it displayed oral activity in cynomolgus monkeys. After further evaluation of toxicology and physical properties, compound **2** was finally selected as a candidate of FXa inhibitor for clinical studies.

Table 2. Selectivity of compound **2** for serine proteases; inhibitory activity (K_i value: μM) for Factor Xa, Thrombin, Plasmin, and Trypsin

Compound	Factor Xa	Thrombin	Plasmin	Trypsin
2	0.019 ± 0.001 μM	>100	78.2 ± 2.8	13.6 ± 1.8
3 (ref.)	0.041 ± 0.002 μM	>100	23.0 ± 0.8	0.62 ± 0.08

The K_i values for each enzyme were determined from Dixon's plot constructed at two substrate concentrations. Data represent the mean ± SEM (n = 3).

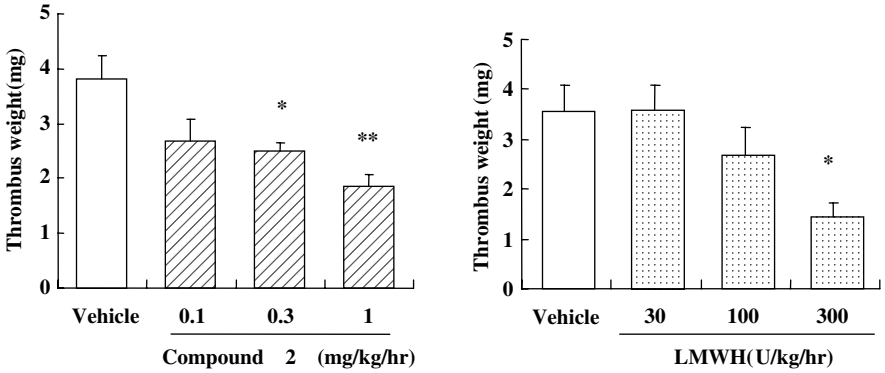


Figure 4. Effect of compound **2** and LMWH on venous thrombosis in rats. (Data represent the mean ± SEM, $^{**}P < 0.01$, $^{*}P < 0.05$, Dunnett's test, $n = 5$–7).[12]

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9. FXa-compound **1** complex model was built by docking compound **1** into the S1 and the aryl binding site on FXa crystal structure (PDB code 1fax). Discover and Insight II program from Accerlys were used for energy calculation and graphical displays, respectively.
10. Ex vivo assessment of inhibition of human factor Xa in cynomolgus monkey plasma—Compound **2** was dissolved in physiological saline and was administrated intravenously to male cynomolgus monkeys at a dose of 1 mg/kg. Before administration and at 5, 10, 15, 30, 60, and 120 min after administration, 1500 μL blood samples were collected from the saphenous vein of each monkey into a syringe containing 300 μL of 3.8% citric acid. Plasma was prepared from each sample by centrifugation at $2000 \times g$ for 10 min at 4 °C. Compound **2** was dissolved in deionized distilled water and administered orally to fasting male cynomolgus monkeys at a dose of 10 mg/kg. Before administration and at 15, 30, 60, 120, 240, 360, and 480 min after oral administration, 1500 μL blood samples were collected and plasma was obtained as described above. Forty microliters of human factor Xa (0.5 U/mL) and 40 μL of a 4-fold diluted plasma sample were incubated in 40 μL of 0.1 M Tris–0.2 M NaCl buffer (pH 8.4) at 37 °C for 10 min. Then, 40 μL of a synthetic substrate (S-2222, adjusted to 0.8 mM) was added and the mixture was incubated at 37 °C for 3 min. The reaction was stopped by addition of 60% acetic acid and the absorbance at 405 nm was measured with a spectrometer (Model 3550, BIO-RAD, Hercules, USA). As the control, plasma obtained prior to administration of compound **2** was measured. Human factor Xa inhibitory activity was calculated as the percent inhibition relative to the control.
11. Pharmacokinetic parameters in cynomolgus monkeys: C_{max} 0.39 μg/mL, $T_{1/2}$ 3.6 h, Bioavailability (B.A.) 10.7% (10 mg/kg po), Clearance 0.25 L/h/kg, Volume of Distribution 0.32 L/kg (1 mg/kg iv).
12. Assessment of the effect on venous thrombosis in rats—Male SD rats were anesthetized with urethane (1.3 g/kg ip). About 1 cm of an abdominal vein was carefully dissected at a site below the left renal vein, and Parafilm (Parafilm M, ANC) was placed on the dorsal aspect of the vein. A 2×3 mm piece of filter paper (No. 1, Whattman) containing 25% $FeCl_3$ was applied to the detached vein and removed after 20 min. Immediately after removing the filter paper, a 5 mm length of the abdominal vein was resected and weighed. The thrombus weight was calculated by subtracting the weight of the vessel walls from the total measured weight. Compound **2** (0.1, 0.3, 1 mg/kg/h) was administered by continuous intravenous infusion from 1 h prior to placement of the filter paper.