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Orally active zwitterionic factor Xa inhibitors with long duration of action

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ABSTRACT

We have optimized 2-aminomethylphenylamine derivative as a factor Xa inhibitor. Several polar functional groups were introduced in the central phenyl ring, and we focused on zwitter ionic compound showing continuous inhibitory activity in oral administration test. In vitro and oral activities were improved by optimization of S1 and S4 ligands. Incorporating the interaction with S1-β pocket enhanced in vitro factor Xa inhibitory activity to less than 1 nM. Many zwitter ionic compounds showed long duration of action and potent inhibitory activity and high AUC values in oral administration tests to monkeys.
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In clinical treatment, safe, easy to use and orally available anticoagulant agents are needed. The extrinsic and intrinsic coagulation systems converge at the activation of factor X to Xa. Activated factor X (fXa) is a trypsin-like serine protease and it has an important role in conversion of prothrombin to thrombin, which produces blood clots.[1] Therefore, many pharmaceutical companies have concentrated on exploring an orally active fXa inhibitor.[2] We also have continuously studied non-amidine compounds,[3,4] and have finally discovered an orally active fXa inhibitor, edoxaban (free form of DU-176b).[5] Nowadays, several compounds such as rivaroxaban,[6] apixaban[7] and edoxaban have been studied in clinic. In order to increase the possibility of launching our fXa inhibitor into the market, we have been studying another novel scaffold for further exploration, and found 2-aminomethylphenylamine derivative **1** as a novel fXa inhibitor (Fig. 1).[4]

Previously, we reported that compound **1** showed potent in vitro and in vivo fXa inhibitory activity, as well as favorable PK profiles after oral administration to monkeys. Herein, we report the optimization of 2-aminomethylphenylamine derivatives and identification of novel zwitter ionic fXa inhibitors with long duration of action. Compound **1** still leaves room for improvement, such as relative low solubility in neutral aqueous solution and moderate metabolic stability.

To increase the solubility, we first planned the introduction of a polar functional group into the phenyl ring. On the other hand, oxidation of phenyl ring was expected in metabolic process owing to high electronic density of phenyl ring having electronic donating groups. We thought that the introduction of polar electron withdrawing groups into the phenyl ring could be a favorable modification to increase solubility and metabolic stability.

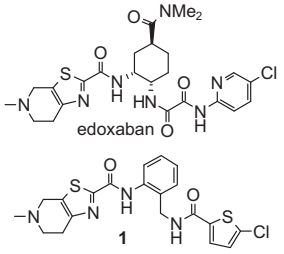

Figure 1. Structures of edoxaban and compound **1**.

Referring to the X-ray structure of the complex with fXa and compound **1**,[4] *ortho*-, *meta*- and *para*-positions in phenyl ring from thiazolopyridine carboxamide unit which are expressed as **a**, **b** and **c** in the structure of Table 1, were placed on the solvent side of fXa. In particular, the **a** and **b** position seemed to be more favorable owing to the existence of large space. Therefore, we executed the introduction of a carboxyl group or a carboxamide group into **a**, **b** and **c**-positions of the phenyl ring (Table 1).

All carboxyl compounds **2** and its amide analogs **3**–**5** showed higher metabolic stability in human microsomes than **1**. From the results of the fXa inhibitory and anticoagulant activities of carboxylic acid derivatives **2A–C**, **a** and **b**-positioned carboxylic acid

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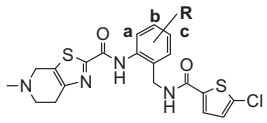
Table 1
In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), solubility (JP1 and JP2) and metabolic stability for substituents of phenyl ring



Compound	R=	Position	IC_{50} (nM)	PTCT2[a] (μM)	Solubility JP1[b] (μg/ml)	Solubility JP2[c] (μg/ml)	MS[d] (%)
1	H	—	1.7	0.5	150	17	53
2A	COOH	a	6.1	2.1	710	>800	n.t.[e]
2B	COOH	b	2	0.85	55	>850	71
2C	COOH	c	20	3.7	72	13	95
3A	$CONH_2$	a	3.5	0.68	740	710	94
3B	$CONH_2$	b	4.8	1.4	30	<2.4	77
4A	CONHMe	a	5.6	0.65	830	130	80
4B	CONHMe	b	8.3	0.89	140	2.7	85
5A	$CONMe_2$	a	2.1	0.61	900	980	81
5B	$CONMe_2$	b	6.3	0.9	>900	830	67
6	COOMe	b	5	1.7	890	52	42
7	$CONHSO_2Me$	b	6	6.1	990	1000	n.t.
8	Tetrazol-5-yl	b	4.7	4.7	320	470	n.t.
9	3*H*-[1,3,4]Oxadiazol-2-one	b	4	2.7	n.t.[e]	n.t.[e]	n.t.

The experimental methods were described in Ref. 4.
[a] Anticoagulant activities were evaluated with PTCT2 (concentration required to double prothrombin time) in human plasma.
[b] JP1: Japanese Pharmacopoeia First Fluid (pH 1.2).
[c] JP2: Japanese Pharmacopoeia Second Fluid (pH 6.8).
[d] MS: in vitro metabolic stability in human liver microsome.
[e] Not tested.

derivatives **2A** and **2B** kept strong inhibitory activities and showed higher solubility in neutral aqueous solution (JP2: pH6.8) than **1**. Particularly, **2B** showed strong fXa inhibitory and anticoagulant activities comparable to compound **1**, while **c**-position derivative **2C** apparently showed lower activities. Therefore we investigated only **a**- and **b**-positioned amide derivatives. All amide derivatives showed very strong fXa inhibitory and anticoagulant activities, and there was a tendency that **a**-positioned amide derivatives showed stronger activity than the corresponding **b**-positioned derivatives. Interestingly, most of **a**-positioned amide derivatives showed higher solubility. We selected acid **2B** and carboxamide **3A** for oral administration tests in fasting monkeys at a dose of 1.0 mg/kg, because they showed potent anticoagulant activity and high metabolic stability (Table 2).

Compound **2B** and **3A** showed moderately inhibitory activity and AUC values in plasma, however their transition of activities were apparently different. While **3A** showed inhibitory activity for short term and it's concentration in plasma was not detected at 24 h after p.o. administration, acid **2B** showed more continuously inhibitory activity until 8 h after p.o. administration and it's concentration in plasma had been detected until 24 h after p.o. administration. This striking duration seemed to be big advantage to achieve per once administration a day, therefore we carried out further optimization about carboxylic acid derivative. **2B** was thought to be lower permeability owing to decrease of lipophilicity by introducing carboxylic acid. Then, we investigated the conversion of carboxylic acid into ester **6** or bioisosteres **7**–**9** as shown in Table 1. All these bioisosteres showed high inhibitory activities, but lower anticoagulant activities than **2B**.

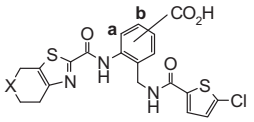
As another approach to increase the lipophilicity, we focused the modification of thiazolopyridine unit as S4 ligand. The *N*-methyl unit was modified into *N*-isopropyl or an oxygen atom referring to our previous optimization[3a,3f] (Table 3). *N*-isopropyl derivatives **10A** and **10B** showed stronger inhibitory and anticoagulant activity than the corresponding *N*-methyl derivatives. Although *N*-isopropyl derivatives showed higher Log*D* values, they showed higher metabolic stability than **2B**. On the other hand, dihydropyranothiazole derivatives **11A** and **11B** definitely declined its biological activity. Based on **10B**, N-substituent was further modified to change the steric volume and basicity. As the lower activity of *N*-Et derivative **12** and *N*-alkylether **13**, isopropyl group seemed to be the most preferred size. To change the basicity keeping similar size of the isopropyl group, the introduction of fluorine into the isopropyl group and conversion into acetyl or sulfonyl group were carried out. Interestingly, the inhibitory and anticoagulant activity became weaker in proportion to the number of fluorine (**14**, **15**). There was decreasing tendency for predicted pK_a values[8] of amine by introduction of fluorine, while Log*D* values of fluorine derivatives (**14**, **15**) were very similar to the value of **10B**. In addition, neutral *N*-acetyl and *N*-sulfonyl derivatives (**16**, **17**) showed very low activity. These results suggested that the basicity of *N*-alkyl unit greatly affected both inhibitory and anticoagulant activities.

Table 2
Ex vivo anti-fXa inhibitory activities in plasma, AUC and concentration with an oral administration to monkeys at 1.0 mg/kg

Compound	Ex vivo (%)						AUC	C_{max}	$C_{24\ h}$
	0.5 h	1 h	2 h	4 h	8 h	24 h	(ngh/ml)	(ng/ml)	
1	79.0	82.0	75.1	64.9	41.4	23.2	346	95.9	0.6
2B	41.3	37.8	25.4	18.2	20.0	1.5	247	25.8	4.0
3A	29.1	39.4	35.3	10.9	1.8	−4.5	285	109	0

Ex vivo and PK tests were examined with an oral administration to fasting two monkeys as HCl salts. The experimental methods were described in Ref. 4.

Table 3
In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), solubility (JP1 and JP2), log*D* value (pH=7.4) and metabolic stability for S4 ligands



Compound	Position	X	IC_{50} (nM)	PTCT2 (μM)	Log*D*[a]	Solubility JP1 (μg/ml)	Solubility JP2 (μg/ml)	MS (%)	pK_a of amine[b]
2A	a	N-Me	6.1	2.1	−0.38	710	>800	n.t.	8.26
2B	b	N-Me	2	0.85	−0.29	55	>850	71	8.26
10A	a	N-Pr	2.9	0.56	0.28	1000	1000	96	9.08
10B	b	N-Pr	0.93	0.62	0.57	49	41	96	9.08
11A	a	O	231	n.t.	−0.12	2	930	57	—
11B	b	O	53	n.t.	0.02	2	950	82	—
12	b	N-Et	4.9	2.1	0.14	230	1000	97	8.79
13	b	N-CH(CH_3)CH_2OMe	4.9	2.7	0.41	610	1000	100	7.59
14	b	N-CH(CH_3)CH_2F	2.9	2.2	0.45	160	580	94	7.00
15	b	N-CH(CH_2F)$_2$	6.9	6.8	0.44	120	870	100	5.55
16	b	N-COMe	49	11	n.t.	n.t.	n.t.	n.t.	—
17	b	N-SO_2Me	9.1	5.8	n.t.	n.t.	n.t.	n.t.	—

The experimental methods were described in Ref. 4.
[a] The distribution coefficients (log*D*) were measured between 1-octanol and phosphate buffered saline (pH = 7.4).
[b] pK_a values of amine were predicted by Pallas program.

To reduce metabolic or toxicological risks,[9] previously we had modified the thiophene ring into the other aromatic rings and reversed the amide bond on compound **1** (left side of Fig. 2).[4] However, all these derivatives showed very low inhibitory activity. Referring to the X-ray structure of the S1 part in fXa with compound **1**, hydrogen bond between amide nitrogen of S1 ligand and carbonyl oxygen of Gly218 and hydrophobic contact with chlorine seemed to be essential to perform strong inhibitory activity. Then, as shown on the right side of Figure 2, we designed the phenylpropionylamide derivatives to reverse amide bond and lengthen alkyl chain at the same time. Table 4 summarized in vitro data of these compounds.

Phenylpropionyl amide derivatives **18**–**21** showed potent inhibitory and anticoagulant activity. Moreover, their metabolic stability and aqueous solubility were preserved. *N*-isopropyl derivatives **20B** and **21** showed stronger inhibitory and anticoagulant activities respectively than the corresponding *N*-methyl derivatives **18** and **19** similar to the thiophene derivative. However, phenylpropionyl amide derivatives **20A, 20B** and **21** showed a little lower anticoagulant activity than the corresponding thiophene derivatives **10A** and **10B**. Then we replaced chlorine with fluorine and bromine. Inhibitory activity of bromine derivative **23** was equivalent to **20B**, but anticoagulant activity was lower. On the other hand, fluorine derivative **22** evidently showed lower inhibitory activity than bromine derivative **23**, but equal anticoagulant activity. Concerning Log*D* values, compound **23** showed higher values than **20B**, while **22** showed lower. From these results, low lipophilicity seemed to be favorable to anticoagulant activity.

An X-ray crystal structure of **20B** in human fXa was examined (Figs. 3 and 4).[10] Chlorophenyl unit and the thiazolopyridine unit were placed in S1 and S4 pockets, respectively. In the S1 pocket, there was a hydrogen bond between anilide nitrogen and carbonyl oxygen of Gly218 (2.99 Å). The chlorine in the phenyl ring makes contact with hydrophobic side chains of Tyr228, Val213, Ala190 and Gly226. In the S4 pocket, the thiazolopyridine unit was placed similarly to compound **1**. Thiazolopyridine ring was placed parallel to the indole ring of Trp215 to make contact with the side chain of Tyr99, Phe174 and Trp215, and the isopropyl group was positioned at the end of S4. The carbonyl carbon of thiazolopyridine–carboxamide group was placed on the carbonyl oxygen of Gly216 to receive electrostatic interaction (3.11 Å). The central phenyl ring was positioned near S1β pocket (esterbinding pocket),[11] but shifted to the solvent side from that of compound **1**. This shift generated the space to improve affinity with fXa.

To incorporate the interaction with S1β pocket and stabilize the conformation, we designed to introduce the substitution into the phenyl ring at ortho position of the alkyl chain. Since S1β pocket is formed by hydrophobic side chain, we selected the substituent not to donate hydrogen as shown in Table 5. Most compounds showed very strong inhibitory activity under 1 nM. Particularly, chloride **25**, trifluoromethyl **26** and methoxy **27** derivatives' inhibitory activities were approximately 0.5 nM. However, their anticoagulant activities were similar to **20B**. High lipophilicity seemed to be unfavorable for anticoagulant activity similar to the observation in anilide unit modification. While neutral aqueous solubilities (JP2) decreased owing to introduction of the hydrophobic substituent, metabolic stabilities maintained relatively high values.

On several zwitter compounds which showed potent anticoagulant activity and high metabolic stability, we examined oral administration tests in fasting monkeys as HCl salts at a dose of

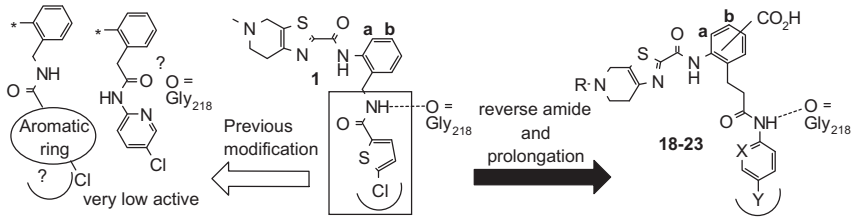
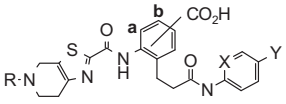


Figure 2. Our tactics of modification for S1 ligand.

Table 4
In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), solubility (JP1 and JP2), log*D* value (pH = 7.4) and metabolic stability for phenylpropionyl amide derivatives



Compound	Position	R	X	Y	IC_{50} (nM)	PTCT2 (μM)	Log*D*	Solubility JP1 (μg/ml)	Solubility JP2 (μg/ml)	MS (%)
18	b	Me	CH	Cl	2	1.0	0.27	430	24	95
19	b	Me	N	Cl	2.4	0.97	−0.28	250	210	92
20A	a	iPr	CH	Cl	1.5	0.77	0.66	1000	830	100
20B	b	iPr	CH	Cl	1.3	0.77	0.82	490	720	100
21	b	iPr	N	Cl	2.3	0.72	0.23	810	86	100
22	b	iPr	CH	F	10	1.1	0.27	980	140	94
23	b	iPr	CH	Br	1.2	1.1	1.08	65	520	88

The experimental methods were described in Ref. 4.

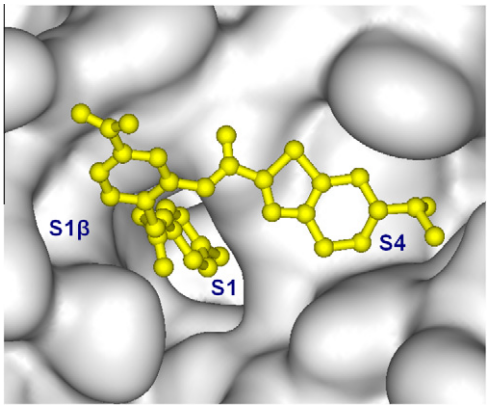


Figure 3. The binding mode of compound **20B**. The surface view is the active site of Gla-less fXa. Compound **20B** is displayed as a yellow ball and stick style without hydrogens.

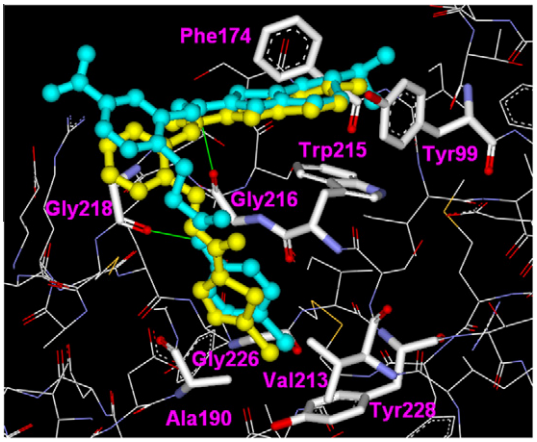


Figure 4. Superposition X-ray structure of **20B** and **1** in fXa. The residues of fXa resulting with **20B** are displayed with carbon, nitrogen, sulfur and oxygen atoms colored by white, blue, yellow and red, respectively. **20B** was displayed as the light blue ball and stick style. **1** is superimposed based on residues of fXa and displayed as a yellow ball and stick style. Hydrogens and water were omitted. Several residues associated with the hydrogen bonds to compound **20B** are displayed by stick style. Meanwhile other residues are displayed by lines. The hydrogen bonds and electrostatic interaction are displayed by green lines.

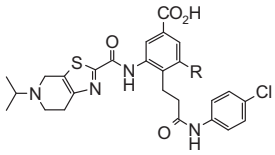
1.0 mg/kg (Table 6). While **a**-carboxylic acids **10A** and **20A** showed lower inhibitory activity and lower AUC values than **2B**, many **b**-carboxylic acids derivatives showed higher inhibitory activity and higher AUC values than **2B**. Compounds **10B**, **20B**, **21** and **25** showed potent and long acting inhibitory activity. Particularly, **25** showed strong inhibitory activity until 24 h after oral administration. Compounds **10B**, **20B**, **21** and **25** showed high AUC values, and their concentrations in plasma were observed until 24 h too. In particular, **20B** showed highest AUC values and $C_{24\ h}$. In comparison with C_{max} and $C_{24\ h}$, the ratios of C_{max} and $C_{24\ h}$ were very small on **20B** ($C_{max}/C_{24\ h}$ = 3.3) and **25** ($C_{max}/C_{24\ h}$ = 3.9). We thought that their relatively small change of blood concentration contribute to the thrombotic effect and the reduction of bleeding risks, and considered **20B** and **25** as favorable compounds for further evaluation to the clinical candidates. We also investigated the bioavailability about **10B**, **20B** and **25** as representative oral active compounds.[12] These compounds **10B**, **20B** and **25B** showed good bioavailability with 51%, 67% and 52%, respectively.

Scheme 1 illustrates a representative synthetic route for the compounds in Tables 1 and 3 using **10B** as an example. Carboxylic acid **30** was esterified into *tert*-butyl ester **31** in anhydrous acid condition. After treatment with ammonia, crude amine **32** was condensed with 5-chlorothiophene-2-carboxylic acid to give **33**. Reduction of nitro group with $FeCl_3$ and zinc, gave a key intermediate **34**. Thiazolopyridine derivatives **35**[13] was deprotected and N-alkylated to obtain isopropyl derivative **36**. The thiazole carbon of **36** was lithionated and subsequently treated with carbon dioxide to provide acid **37** as S4 ligand. After **37** was condensed with **34**, *tert*-butyl ester of amide **38** was deprotected with hydrochloric acid to give **10B**. 5-Methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxylic acid[13] and 6,7-dihydro-4*H*-pyrano[4,3-d]thiazole-2-carboxylic acid[3f] were prepared according to the reported method.

Carboxylic acid bioisosters **7**–**9** were prepared from **2B** or **6** as shown in Scheme 2. Acylsulfoneamide **7** was synthesized by EDCI-assisted condensation of carboxylic acid **2B** with methanesulfoneamide. After EDCI-assisted condensation of **2B** with 3-aminopropionitrile, tetrazole ring was formed by Mitsunobu-type moderate condition.[14] Protected tetrazole derivative **40** was treated in alkali condition to give tetrazole derivative **8**. Amidation of ester **6** with hydrazine gave compound **41**, and followed by treatment with carbonyldiimidazole to form an oxadiazolone ring in a low yield.

Scheme 3 illustrates representative synthetic routes for the compounds in Table 4 using **20B**. Carboxylic acid **42** was esterified with *tert*-butanol in anhydrous condition. After the resultant ester **43** was converted into enamine **44**, oxidation with $NaIO_4$ gave aldehyde **45**.[15] **45** was reacted with Wittig–Horner reagent to get

Table 5
In vitro fXa inhibitory activity (IC_{50}), anticoagulant activity (PTCT2), solubility (JP1 and JP2), log *D* value (pH = 7.4) and metabolic stability for substituents of center phenyl ring



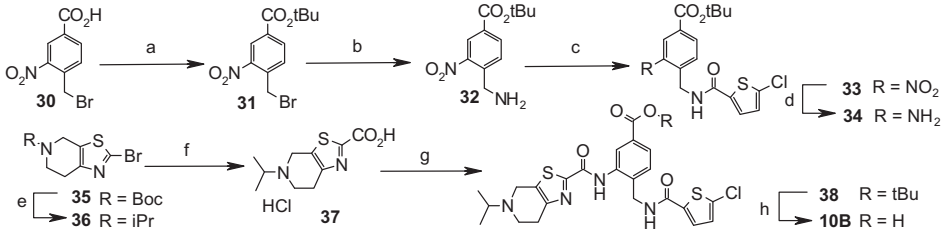
Compound	R	IC_{50} (nM)	PTCT2 (μM)	Log *D*	Solubility JP1 (μg/ml)	Solubility JP2 (μg/ml)	MS (%)
20B	H	1.3	0.77	0.82	490	720	100
24	F	0.8	0.62	1.10	970	11	100
25	Cl	0.38	0.73	1.61	450	160	93
26	CF_3	0.48	0.79	1.64	700	310	77
27	OMe	0.54	0.5	1.15	810	39	87
28	OEt	0.74	0.76	1.62	370	3	93
29	OiPr	1.6	2.6	1.91	320	130	86

The experimental methods were described in Ref. 4.

Table 6
Ex vivo anti-fXa inhibitory activities in plasma, AUC and concentration after an oral administration to monkeys at 1.0 mg/kg

Compound	Ex vivo (%)						AUC	C_{max}	$C_{24\ h}$	$C_{max}/C_{24\ h}$
	0.5 h	1 h	2 h	4 h	8 h	24 h	(ngh/ml)	(ng/ml)		
2B	41.3	37.8	25.4	18.2	20.0	1.5	247	25.8	4.0	6.4
10A	13.6	51.3	47.9	25.1	8.8	4.1	221	50.5	2	25.3
10B	49.6	84.5	79.5	72.5	67.9	32.1	605	67.1	7.1	9.5
20A	−2.9	0.1	0.4	−1.5	3.9	4.6	n.d[a]	n.d.	n.d.	n.d.
20B	44.9	69.6	69.4	67.5	68.8	32.7	1368	87	26	3.3
21	66.5	76	81.3	78.7	64.4	23.9	785	98	9.5	10.3
24	40	64	53.9	55.7	55.2	23.3	448	32	6.1	5.2
25[b]	74.9	86.3	73	73.5	71.2	54.1	724	67	17	3.9
27	1.8	4.4	2.1	2.5	1.5	7.2	n.d.	n.d.	n.d.	n.d.

Ex vivo and PK tests were examined with an oral administration to fasting two monkeys as HCl salts. The experimental methods were described in Ref. 4.
[a] Not detected.
[b] PK tests were examined with four monkeys.



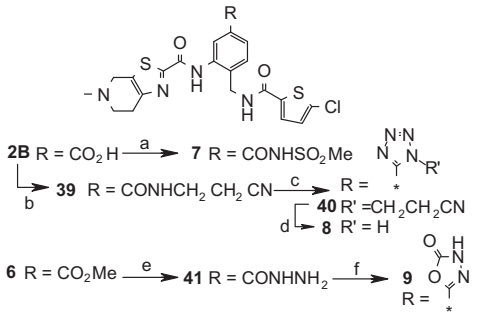
Scheme 1. (a) *t*BuOH, H_2SO_4, $MgSO_4$, CH_2Cl_2, rt, overnight; (b) NH_3-MeOH, rt, 2 h, 54% for 2 steps; (c) 5-chlorothiophene-2-carboxylic acid, EDC/HCl, HOBt, DMF, rt, 2 h, 77%; (d) $FeCl_3$, Zn, DMF, H_2O, 100 °C, 67%; (e) (i) TFA, CH_2Cl_2, 95%; (ii) acetone, $NaBH(OAc)_3$, AcOH, Et_3N, CH_2Cl_2, 94%; (f) (i) *n*-BuLi, CO_2, Et_2O, −78 °C, (ii) HCl–EtOH, 79%; (g) **34**, EDC/HCl, HOBt, DMF, rt, overnight, 92%; (h) 4 N HCl–dioxane, CH_2Cl_2, rt, 87%.

α,β-unsaturated ester **46**. Alkali hydrolysis of ethyl ester and subsequently EDCI-assisted condensation with 4-chloroaniline gave amide **48**. Nitro and alkenyl groups were reduced by hydrogenation to obtain aniline **49**. After acid **37** was treated with oxalylchloride, the resultant acid chloride was condensed with aniline **49**. The resultant amide **50** was treated with hydrochloric acid to give **20B**.

The synthetic route of nitrobenzoate intermediates for the compounds in Table 5 was shown in Scheme 4. After esterification of acid **51**, one of the two nitro groups was selectively reduced by treatment with iron powder and hydrochloric acid to give nitroaniline **52**. Amino group of **53** was converted into halogen or hydroxide by the Sandmeyer method to give nitrobenzoate **54**, **55** and **56**. From acid **56**, esterification and O-alkylation by one-pot or stepwise reaction gave nitrobenzoates **57**–**60**. Trifluoromethyl intermediate **63** was prepared from acid **61** by nitration and esterification.

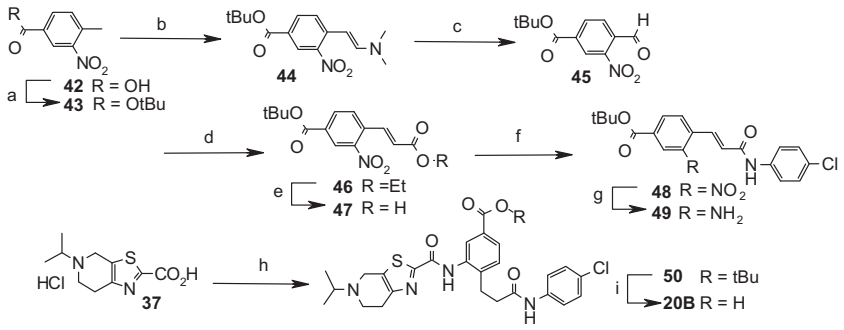
Scheme 5 illustrates a representative synthetic route for the compounds in Table 5 using **25** as an example. Benzylic position of nitrobenzoate **54** was brominated with NBS. The resultant benzylbromide **64** was used in alkylation of *tert*-butyl malonate to give diester **65**. After hydrolysis in acidic condition, copper-catalyzed decarboxylation gave mono carboxylic acid **66**, which was condensed with 4-chloroaniline to give amide **67**. Reduction of the nitro group with $FeCl_3$ and zinc gave aniline **68**. Similar to synthesis of **20B**, S4 ligand was introduced into **68** by treatment with acid chloride derived from **37**. Alkali hydrolysis of the resultant amide **69** gave **25**.

In conclusion, we have explored the fXa inhibitor based on 2-aminomethylphenylamine derivative **1**. From the results of

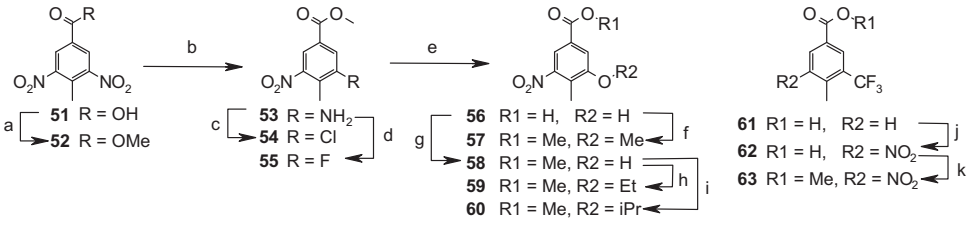


Scheme 2. (a) $MeSO_2NH_2$, EDC/HCl, Et_3N, DMAP, CH_2Cl_2, rt, 2d, 29%; (b) 3-aminopropionitrile, EDC/HCl, HOBt, Et_3N, CH_2Cl_2, rt, 2d, 68%; (c) $TMSN_3$, DEAD, Ph_3P, MeCN, rt, 5d, 61%; (d) NaOHaq, MeOH, rt, 3d, 67%; (e) hydrazine monohydrate, MeOH, reflux, 6d, 73%; (f) CDI, Et_3N, THF, 40–60 °C, 6 d, 18%.

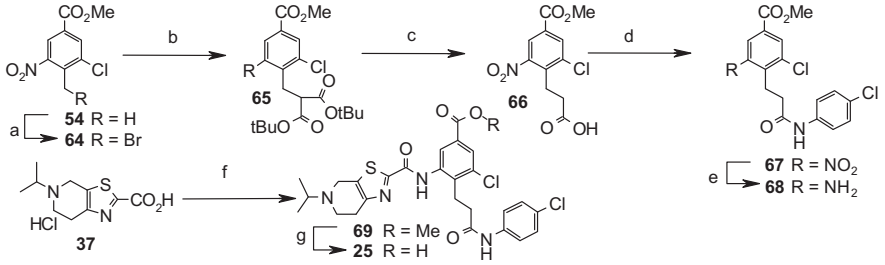
introduction of polar functional groups into the central phenyl ring to improve aqueous solubility and metabolic stability, we focused on zwitter ionic compound **2B** showing continuous inhibitory activity after oral administration in monkeys. By optimization of S4 ligands, we obtained *N*-isopropyl derivatives **10B** which showed more potent fXa inhibitory and anticoagulant activities and higher metabolic stability than **2B**. To reduce metabolic or toxicological risk of thiophene, we designed phenylpropionylamide derivatives and obtained many potent compounds such as **20A**, **20B** and **21**. Based on the X-ray structure of **20B** and fXa, the substituents not to donate hydrogen were introduced into the phenyl ring to interact with S1-β pocket. Owing to this challenge, we obtained many zwitter compounds **24**–**28** showing very strong inhibitory activity under 1 nM. Many promising compounds such as **10B**, **20B**, **21** and **25** showed long duration of action and potent inhibitory activity and high AUC values in oral administration tests with fasting monkeys. While many basic or neutral compounds were reported as an orally active fXa inhibitor, we obtained the orally active zwitter ionic fXa inhibitor. Our fXa inhibitors showed the small change of blood concentration, and we thought that this property could reduce the bleeding risk as anticoagulant. And there are several reports that the introduction of carboxylic acid weakened off-target profiles such as hERG channel binding[16] and CYP3A4 time



Scheme 3. (a) *t*BuOH, H_2SO_4, $MgSO_4$, CH_2Cl_2, rt, 3d, 55%; (b) $(MeO)_2CHNMe_2$, DMF, 80 °C, 10.5 h; (c) $NaIO_4$, THF, H_2O, rt, 3 h, 55% for 2 steps; (d) $(EtO)_2POCH_2CO_2Et$, NaH, THF, 0 °C, 0.5 h, 67%; (e) NaOH, THF, H_2O, 0 °C-rt, 24 h, 86%; (f) 4-chloroaniline, EDC/HCl, HOBt, Et_3N, CH_2Cl_2, rt, 3 d, 87%; (g) H_2, 10%Pd on carbon, THF, rt, 8 h, 64%; (h) (i) $(COCl)_2$, Et_3N, CH_2Cl_2, DMF, rt, 3 h, (ii) **49**, Et_3N, CH_2Cl_2, rt, 82%; (i) HCl–dioxane, rt, 18 h, 98%.



Scheme 4. (a) $SOCl_2$, MeOH, reflux, 3 h, 93%; (b) Fe, HCl, MeOH, dioxane, reflux, overnight, 51%; (c) *t*BuONO, $CuCl_2$, MeCN, 60 °C, 1 h, 82%; (d) HF/Py, $NaNO_2$, 0–110 °C, 29%; (e) $NaNO_2$, H_2SO_4, H_2O, 0 °C-reflux, 63%; (f) MeI, NaH, THF–DMF, rt, 18 h, 83%; (g) MeOH, HNO_3, 1d, reflux, 85%; (h) EtI, K_2CO_3, acetone, reflux, 98%; (i) 2-bromopropane, K_2CO_3, acetone, reflux, 2 d, 67%, (j) HNO_3, H_2SO_4, 100 °C, 1.5 h, 96%; (k) MeOH, H_2SO_4, reflux, 6 h, 92%.



Scheme 5. (a) NBS, $(PhCOO)_2O$, CCl_4, reflux, overnight, 70%; (b) di-*tert*-butyl malonate, NaH, THF, rt, 15 h, 67%; (c) (i) HCl–dioxane, rt, 3d, (ii) TFA, CH_2Cl_2, rt, 2 h, (iii) Cu_2O, MeCN, 80 °C, 16 h, 84%; (d) 4-chloroaniline, EDC/HCl, HOBt, Et_3N, CH_2Cl_2, rt, 24 h, 75%; (e) $FeCl_3$, Zn, DMF, H_2O, 80–90 °C, 10 min, 98%; (f) (i) $(COCl)_2$, Et_3N, CH_2Cl_2, DMF, rt, 3 h, (ii) **68**, Et_3N, CH_2Cl_2, rt, 15 h, 71%; (g) LiOH, THF, H_2O, rt, 21 h, 78%.

dependent inhibition.[17] We expect that our zwitter ionic fXa inhibitors would have superior profiles that other basic and neutral fXa inhibitors didn't have.

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