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Discovery of betrixaban (PRT054021), *N*-(5-chloropyridin-2-yl)-2-(4-(*N*,*N*-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide, a highly potent, selective, and orally efficacious factor Xa inhibitor

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Dedicated to the memory of Dr. Robert M. Scarborough (1953–2006), co-founder and former Senior VP of Chemistry at Portola Pharmaceuticals, Inc.

ABSTRACT

Systematic SAR studies of in vitro factor Xa inhibitory activity around compound **1** were performed by modifying each of the three phenyl rings. A class of highly potent, selective, efficacious and orally bioavailable direct factor Xa inhibitors was discovered. These compounds were screened in hERG binding assays to examine the effects of substitution groups on the hERG channel affinity. From the leading compounds, betrixaban (compound **11**, PRT054021) has been selected as the clinical candidate for development.

Serine protease factor Xa (fXa), positioned at the juncture of the intrinsic and the extrinsic pathways, plays a pivotal role in the blood coagulation cascade. In the prothrombinase complex consisting of fXa, factor Va and Ca^{2+} assembled on the platelet surface, fXa catalyzes the conversion of prothrombin to thrombin, the final enzyme responsible for fibrin clot formation in this cascade.[1] To safely interrupt blood coagulation without disrupting primary hemostasis, inhibition of fXa should be more effective than direct inhibition of thrombin, which has made fXa a particularly attractive target for the treatment of severe cardiovascular diseases.[2] Selective inhibition of fXa without affecting the existing thrombin levels may cause less impairment of primary hemostasis and thus should be a safer anticoagulant therapy than direct inhibition of thrombin. Clinical findings have confirmed the potential of fXa inhibition for producing excellent antithrombotic efficacy with minimal bleeding risk when compared to direct thrombin inhibitors.[3,4]

In the preceding communication, we reported the discovery of anthranilamide-based compound **1**, *N*-(5-chloropyridin-2-yl)-2-(4-(*N*,*N*-dimethylcarbamimidoyl)-benzamido)benzamide, as a potent fXa inhibitor (IC_{50} 3 nM; K_i 1.4 nM).[5,6] This compound has strong anticoagulant activity in our human plasma based thrombin generation assay (2 × TG 0.54 μM) and good oral bioavailability in rat (*F* 31%). The 4-(*N*,*N*-dimethyl-carbamimidoyl)benzamidine motif, binding in the fXa S4 pocket, delivers the most potent fXa inhibitory activity among all the *N*-substituted benzamidines investigated. To further improve its in vitro fXa potency and anticoagulant activity, we decided to systematically examine aromatic rings A, B and C of compound **1** to expand the SAR scope of this class of fXa inhibitors.

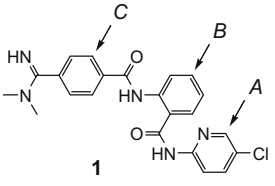

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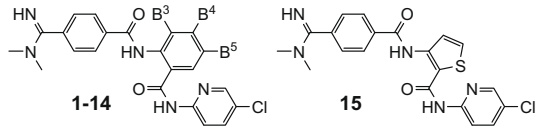
Table 1
B ring substitution SAR



Compound	B^3	B^4	B^5	fXa IC_{50} (nM)
1	H	H	H	4
2	Cl	H	H	21
3	H	Cl	H	2
4	H	H	Cl	0.5
5	H	H	Me	0.7
6	H	H	CF_3	1.3
7	H	H	Et	1.0
8	H	H	C≡CH	<0.5
9	H	H	CN	1.2
10	H	H	F	1.4
11	H	H	OMe	1.5
12	H	H	SMe	<0.5
13	H	H	SO_2Me	1.5
14	H	H	SEt	1.3
15				2

We started our investigation of compound **1** by modifying the central phenyl B ring with a chloro substitution group as B^3, B^4 or B^5 (**2**–**4**, Table 1). Among these three analogs, compound **4** with B_5-chloro is the most potent (IC_{50} 0.5 nM). Its high potency is retained in the human plasma based thrombin generation assay (2 × TG 0.27 μM). In rat, compound **4** has a clearance (CL) of 29.7 mL/min/kg, half-life ($t_{1/2}$) of 12.8 h, and an oral bioavailability (F) of 26.1% when dosed at 0.2 mg/kg IV and 6 mg/kg PO. Replacement of the B^5 chloro with methyl and trifluoromethyl yielded analogs **5** and **6** with IC_{50} values of 0.7 nM and 1.3 nM, respectively, indicating minimal electronic effects.

Computer-based docking studies using GOLD[7] as well as both published and in-house fXa crystal data were used to analyze the binding mode of compound **4** at the active site of fXa. As shown in Figure 1, the chloropyridine A ring extents into the fXa S1 site with the chloro atom positioned above the phenyl ring of Tyr228 and occupies a hydrophobic pocket formed by Ala190, Val213 and Tyr228. This result is consistent with the disclosed X-ray structure of fXa co-crystallized with an anthranilamide compound similar in structure to compound **4**.[8] The *N*,*N*-dimethylamidine moiety is flanked by the phenyl groups of Tyr99 and Phe174 of the S4 aryl

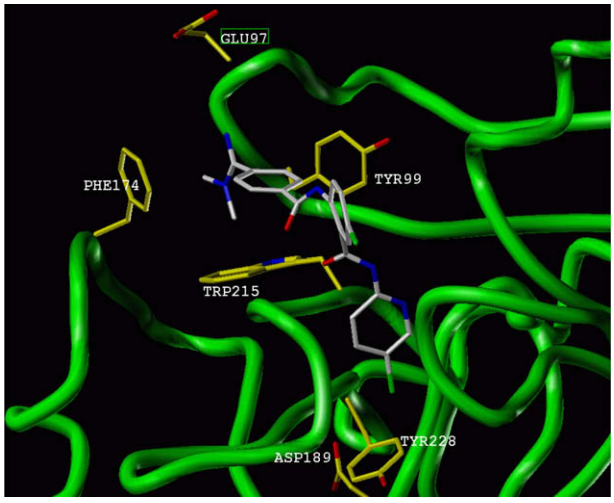


Figure 1. Computer modeling of compound **4** docked in fXa active enzyme pocket.

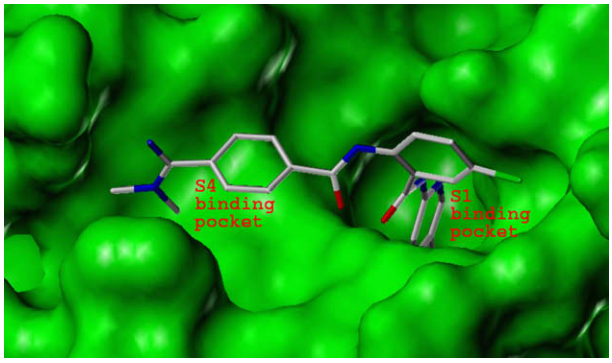


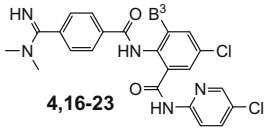
Figure 2. Computer modeling of compound **4** docked in fXa active enzyme pocket.

binding pocket, with the N=C bond resting parallel to the phenyl ring planes and one methyl group inserting into the narrow hydrophobic cleft between the phenyl rings of Phe174 and Trp215. A π-cation interaction presumably exists between the protonated amidino group and the phenyl rings. Furthermore, as shown in Figure 2, B^3 and B^4 are in the open space out of the active binding pocket, thus substitutions at B^3 and B^4 are expected to be tolerated, but to have little impact on potency. The B^5-chloro group points to a small hydrophobic pocket, bordered partially by a disulfide bridge between Cys191 and Cys220; thus a small hydrophobic B^5 group in this pocket could boost the inhibitor's fXa binding affinity. As confirmation of this hypothesis, compounds **5**–**14**, each bearing a small B^5 group capable of hydrophobic interactions, display strong anti-fXa activity with IC_{50} values all in the range of <0.5–1.5 nM. Through analog **15** (IC_{50} 2 nM), we learned that replacement of phenyl B ring with thiophene maintains excellent fXa potency. This docking study also revealed that substitution on the C ring should be tolerated, especially at the position (C^2) next to the carbonyl.

It is worthy noting that highly potent acetylenyl analog **8** (IC_{50} <0.5 nM; 2 × TG 0.21 μM) and methylthio analog **12** (IC_{50} <0.5 nM; 2 × TG 0.24 μM) were found to be stable in rat and human in vitro liver metabolic experiments (S9 incubation $t_{1/2}$ >2 h).[9] In rat PK studies, compound **8** (CL 74.8 mL/min/kg; $t_{1/2}$ 8.4 h; *F* 27.9%) displayed higher clearance and shorter half-life than compound **4**, and compound **12** (CL 77.1 mL/min/kg; $t_{1/2}$ 16.5 h; *F* 26.7%) showed higher clearance. Thus we have concluded that chloro at B^5 is the optimal substitution on B ring based upon the desired PK parameters and superior fXa binding activity of compound **4**.

Once chloro had been established as the optimal B_5 substitution, we synthesized compounds **16**–**23** (Table 2) to scan various B^3 groups for different physicochemical properties and improved

Table 2
B ring substitution SAR



Compound	B^3	fXa IC_{50} (nM)
4	H	0.5
16	OMe	0.6
17	OEt	0.6
18	O*i*Pr	0.5
19	NMe_2	0.5
20	NMeEt	0.5
21	$NMe(CH_2CH_2OMe)$	1.2
22	1-piperidinyl	0.9
23	4-morpholinyl	1.3

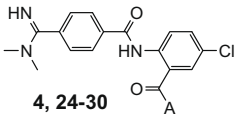
PK profiles. Consistent with the modeling results discussed above, a flat anti-fXa SAR was observed regardless of bulkiness of the B^3 group, with IC_{50} values in the range of 0.5–1.3 nM. However, no improvement was achieved in regard to PK parameters in rat. For example, the bioavailability of compounds **16** and **21** was 11.2% and 8.1%, respectively in rat, compared to that of 26.1% of compound **4**.

With B^5-chloro locked, we focused on the A ring SAR to investigate the S1 binding motif and prepared compounds **24**–**30** (Table 3). Replacement of chloro by fluoro (**24**) and methyl (**25**) slightly compromises fXa potency, probably due to weaker interaction with Tyr228. Replacement of chloro by acetylene (**26**, IC_{50} 0.8 nM; 2 × TG 0.38 μM) retains excellent anti-fXa activity. Though it was stable in rat and human in vitro liver metabolic experiments (S9 incubation $t_{1/2}$ >2 h), compound **26** (CL >100 mL/min/kg; $t_{1/2}$ 6.3 h) displayed higher clearance and shorter half-life than compound **4** in rat PK studies. With the A ring nitrogen moved to the neighboring location, 2-chloro-5-pyridinyl **27** (IC_{50} 3 nM) decreases in fXa potency compared to 5-chloro-2-pyridinyl **4**. With 4-chloroaniline (**28**, IC_{50} 0.7 nM) and 4-acetylenylaniline (**29**, IC_{50} 0.9 nM) as the A ring motifs, in vitro fXa activities comparable to 2-aminopyridines **4** and **26** have been achieved. Due to the concern of aniline's potential toxicity, 2-amino-5-chloropyridine was chosen as the preferred S1 binding motif over the corresponding aniline. Replacement of chloropyridine with chlorothiophene (**30**) reduces fXa potency significantly.

With compound **4** as the starting point, we moved on to the C ring modifications (Table 4). Compounds **31** and **32** with C^3 substitutions are less potent than the corresponding C^2 substituted analogs **33** and **34**. Further modifications at C^2 led to the synthesis of compounds **35**–**44**. Their high fXa potency demonstrates that the C^2 position tolerates a wide range of functional groups, as predicted by the computer-based modeling studies (Figs. 1 and 2). Both pyridine C ring (**45**, IC_{50} 3 nM) and thiophene C ring (**46**, IC_{50} 1.0 nM) slightly reduce fXa potency from lead compound **4**. Among all the analogs in Table 4, the C^2-fluoro compound **33** (IC_{50} <0.5 nM; 2 × TG 0.38 μM) is the most attractive. It has fXa potency very similar to that of lead compound **4**, and displays considerably improved PK profiles in rat with lower clearance (CL 15.3 mL/min/kg) and higher oral bioavailability (*F* 38.0%). The other C^2-substitution groups have resulted in compromises in rat PK parameters. For example, the bioavailability of compounds **40** and **41** was 6.9% and 21.3%, respectively.

After the A, B and C ring SAR had been examined, we went back to modification of the P4 benzamidine N-substitution. Following the previous SAR exploration in this region with compound **1**,[5a] we prepared analogs **47**–**54** (Table 5). All these compounds are highly potent fXa inhibitors, and the SAR overall mirrors that reported in the preceding communication. Judged by the in vitro anti-Xa activity, the *N*,*N*-dimethylbenzamidine as in compound **4** is clearly still the best P4 moiety.

Table 3
A ring SAR



4, 24-30

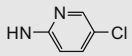
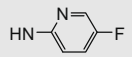
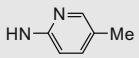
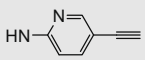
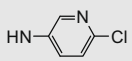
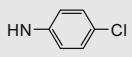
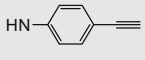
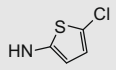
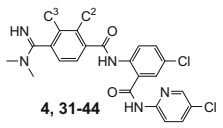
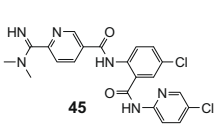
Compound	A	fXa IC_{50} (nM)
4		0.5
24		3
25		1.1
26		0.8
27		3
28		0.7
29		0.9
30		6

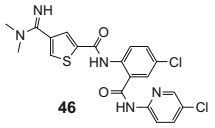
Table 4
C ring substitution SAR



4, 31-44



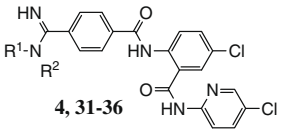
45



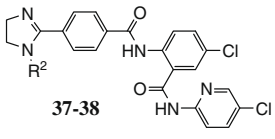
46

Compound	C^3	C^2	fXa IC_{50} (nM)
4	H	H	0.5
31	F	H	1.1
32	OMe	H	7
33	H	F	<0.5
34	H	OMe	0.5
35	H	OCH_2CH_2OMe	1.2
36	H	$OCH_2CH_2NMe_2$	<0.5
37	H	NMe_2	3
38	H	$NMe(CH_2CH_2OMe)$	0.5
39	H	1-azetidinyl	0.4
40	H	1-pyrrolidinyl	2
41	H	1-piperidinyl	<0.5
42	H	4-HO_2C-1-piperidinyl	0.7
43	H	4-morpholinyl	0.7
44	H	1-homopiperidinyl	1.0
45			3
46			1.0

Table 5
P4 benzamidine *N*-substitution SAR

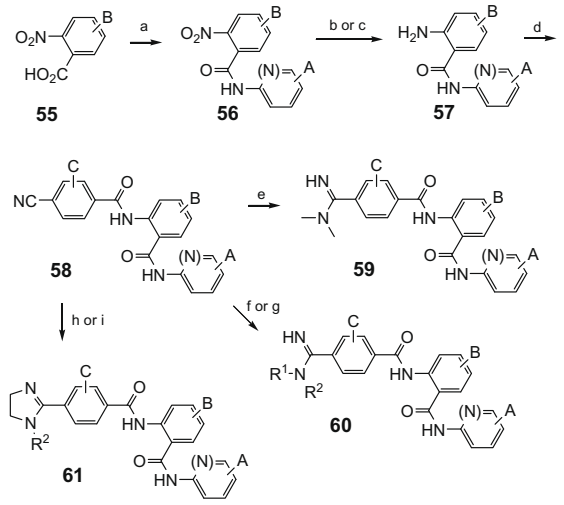


4, 31-36

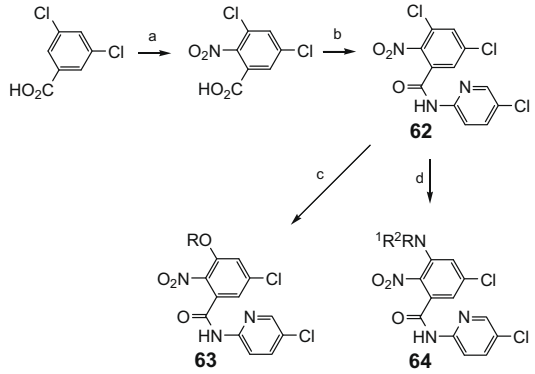


37-38

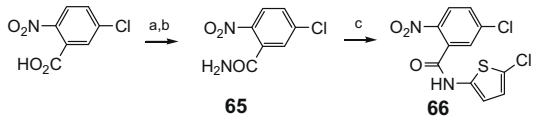
Compound	R^1	R^2	fXa IC_{50} (nM)
4	Me	Me	0.5
47	Et	Me	0.9
48	1-azetidinyl		1.2
49	1-pyrrolidinyl		2
50	1-piperidinyl		1.3
51	4-HO_2C-1-piperidinyl		2
52	4-morpholinyl		2
53		Me	1.5
54		Et	6



Scheme 1. Reagents and conditions: (a) substituted aminopyridine or aniline (1 equiv), $POCl_3$ (1 equiv), pyridine, 0 °C, 60–95%; (b) $SnCl_2{\cdot}2H_2O$ (4 equiv), EtOAc, reflux, 50–70%; (c) H_2 (balloon), 5% Pt(S)/C or 10% Pd/C, EtOAc, 50–90%; (d) substituted 4-cyanobenzoyl chloride (1 equiv), THF, rt, 80–95%; (e) $LiNMe_2$ (4 equiv), THF, 0 °C to rt, 70–90%; (f) (1) H_2S (g), pyridine, Et_3N, rt; (2) MeI (2 equiv), acetone, reflux; (3) NHR^1R^2 (2 equiv), HOAc (3 equiv), MeOH, reflux, 50–90%; (g) (1) HCl (g), dry MeOH, 0 °C; (2) NHR^1R^2 (2 equiv), MeOH, reflux, 50–80%; (h) (1) H_2S (g), pyridine, Et_3N, rt; (2) MeI (2 equiv), acetone, reflux; (3) $H_2NCH_2CH_2NHR^2$ (2 equiv), HOAc (5 equiv), MeOH, reflux, 50–80%; (i) (1) HCl (g), dry MeOH, 0 °C; (2) $H_2NCH_2CH_2NHR^2$ (2 equiv), MeOH, reflux, 50–70%.



Scheme 2. Reagents and conditions: (a) concd HNO_3 (1.2 equiv), concd H_2SO_4, 0 °C, 70–80%; (b) 2-amino-5-chloropyridine (1 equiv), $POCl_3$ (1 equiv), pyridine, 0 °C, 80–90%; (c) ROH (1.2 equiv), NaH (1.2 equiv), NMP, 110 °C, 50–70%; (d) NHR^1R^2 (2 equiv), Cs_2CO_3 (2 equiv), DMSO, 110 °C, 60–80%.



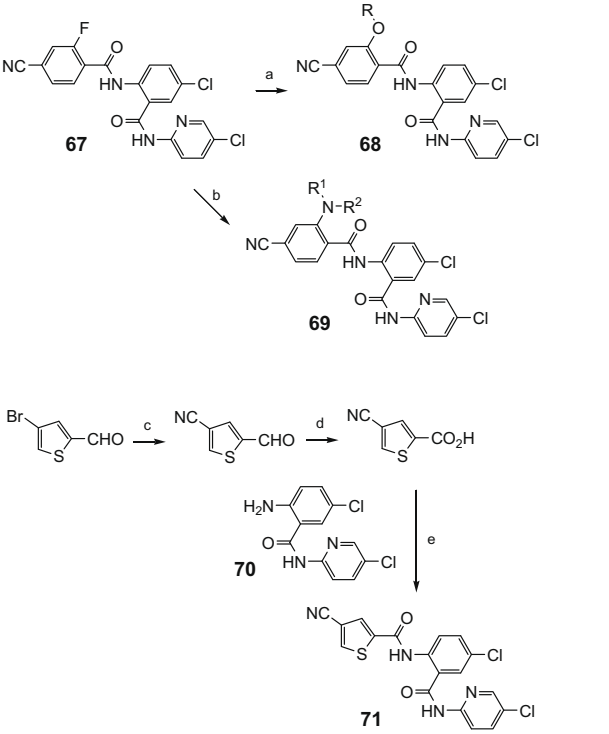
Scheme 3. Reagents and conditions: (a) $(COCl)_2$ (3 equiv), DMF (cat), DCM, rt; (b) conc. NH_4OH (10 equiv), DCM, 0 °C, 90% for 2 steps; (c) 2-chloro-5-iodothiophene (1 equiv), Cs_2CO_3 (2 equiv), CuI (0.1 equiv), *trans*-1,2-diaminocyclohexane (0.1 equiv), dioxane, reflux, 50–60%.

The general synthesis of the compounds shown in Tables 1–5 is described in Scheme 1. Substituted 2-nitrobenzoic acid **55** was coupled with substituted aminopyridine or aniline in pyridine using phosphoryl chloride, or by other standard synthetic methodologies. Reduction of the nitro group of compound **56** was accomplished using tin(II) chloride or hydrogenation with sulfided platinum or palladium on carbon, depending on the compatibility of the substitution groups. The resulting aniline **57** was then treated with substituted 4-cyanobenzoyl chloride, commercially available or freshly produced from the corresponding benzoic acid using oxalyl chloride, to prepare benzonitrile **58**. For target **59** with inert A, B and C substitution groups, it was synthesized by reacting **58** with excessive lithium dimethylamide (commercial 5 wt % suspension in hexane). More generally, target **60** was synthesized via methyl thioimidate or methyl imidate as described in our previous communications.[5a,10] Imidazoline target **61** was prepared from benzonitrile **58** via methyl thioimidate or methyl imidate as well.

The compounds shown in Table 2 were prepared according to Scheme 2. 3,5-Dichlorobenzoic acid was nitrated to produce 3,5-dichloro-2-nitrobenzoic acid, which was next coupled with aminopyridine to obtain **62**. Reaction of **62** with alkoxide, newly produced from the corresponding alcohol and sodium hydride, afforded compound **63**. Treatment of **62** with excessive amount of amine yielded compound **64**. Final targets **16**–**23** were then finished from these two intermediates by the same chemistry illustrated in Scheme 1.

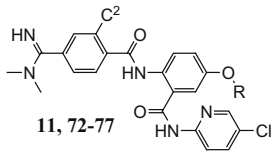
The key steps toward preparation of A ring SAR target **30** (Table 3) are shown in Scheme 3. Chlorothiophene derivative **66** was synthesized by copper-catalyzed coupling reaction of 2-chloro-5-iodothiophene and benzamide **65**, which had been produced from 3-chloro-6-nitrobenzoic acid. Target compound **30** was completed from **66** via the same synthetic route revealed in Scheme 1.

The key steps toward C ring SAR targets (Table 4) are described in Scheme 4. The C^2 side-chains were installed by treatment of fluoro precursor **67** with cesium carbonate and the corresponding alcohols or amines. 4-Cyano-2-thiophenecarboxylic acid was made through cyanation and oxidation of commercial 4-bromo-2-thiophenecarboxaldehyde. It was coupled with aniline **70** to produce **71**. All the final targets were then prepared from nitriles **68**, **69** and **71** by the general route shown in Scheme 1.



Scheme 4. Reagents and conditions: (a) ROH (10 equiv), Cs_2CO_3 (5 equiv), NMP, 110 °C, 60–70%; (b) NHR^1R^2 (5 equiv), Cs_2CO_3 (3 equiv), NMP, 110 °C, 70–85%; (c) CuCN (3 equiv), CuI (0.3 equiv), DMF, reflux, 70%; (d) $NaClO_2$ (9 equiv), NaH_2PO_4 (6 equiv), 2-methyl-2-butene, *t*BuOH, acetone, water, 0 °C, 90%; (e) $POCl_3$ (1 equiv), pyridine, 0 °C, 80%.

Table 6
C ring and B ring substitution SAR for compound **11**



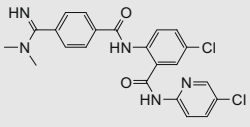
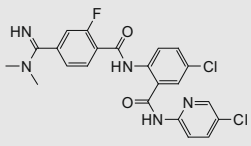
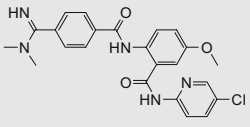
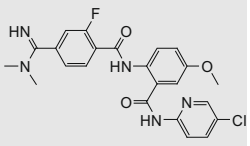
11, 72-77

Compound	R	C^2	fXa IC_{50} (nM)	2 × TG (μM)	hERG K_i (μM)
11	Me	H	1.5	0.33	1.8
72	CF_3	H	17	nd	nd
73	CH_2CF_3	H	10	nd	nd
74	CH_2CO_2H	H	6	1.5	>10
75	Me	F	0.7	0.34	2.1
76	Me	1-piperidinyl	2		0.20
77	Me	4-HO_2C-1-piperidinyl	0.9	0.32	>10

Compounds **4** (fXa K_i 44 pM) and **33** (fXa K_i 60 pM) have displayed good PK profiles in beagle dog and cynomolgus monkey. Dosed at 0.1 mg/kg IV and 1 mg/kg PO, compounds **4** and **33** had bioavailability of 64.9% and 68.8% respectively in dog; dosed at 0.1 mg/kg IV and 1 mg/kg PO, they had bioavailability of 73.0% and 86.1%, respectively in monkey. The outstanding fXa potency, excellent in vitro anticoagulant activity and good PK characteristics of compounds **4** and **33** led to the evaluation of their safety profiles. Unfortunately, both compounds were found to exhibit potent affinity toward the hERG (human ether-a-go-go related gene) ion channel, with K_i values of 0.11 μM and 0.42 μM, respectively in a radioligand (dofetilide or astemizole) hERG transfected membrane binding assay.[11,12] In recent years, the interaction of pharmaceutical agents with the hERG-encoded cardiac potassium channel has represented a significant safety concern and regulatory hurdle for new molecular entities in development.[13,14] Inhibition of the hERG channel can cause delayed ventricular cell repolarization, seen as QT interval prolongation on the electrocardiogram and associated with a potentially fatal cardiac arrhythmia. Thus, the potential risk of this class of excellent fXa inhibitors to cause undesired cardiac repolarization forced us to reexamine our SAR exploration directions.

An extensive screening of the compounds in Tables 1–5 to understand the hERG binding SAR revealed that modifications to the A, B, C rings and the amidine P4 moieties overall have little effect on the hERG channel affinity with the exception of two carboxylic acid-containing compounds **42** and **51** (both have hERG K_i values >10 μM). Their reduced hERG affinity is accredited to the

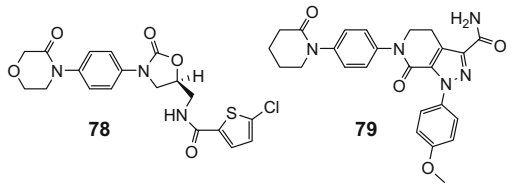
Table 7
Biological and animal pharmacokinetic data for the leading fXa inhibitors

Compound	**4**	**33**	**11** (betrixaban)	**75**
				
fXa IC_{50} (nM)	0.5	<0.5	1.5	0.7
fXa K_i (nM)	0.044	0.060	0.117	0.105
2 × TG (μM)	0.27	0.29	0.33	0.34
rabbit 2 × PT (μM)	0.83	1.1	1.7	1.5
human plasma protein binding (%)	88.1	87.6	80.0	63.4
hERG K_i (μM)	0.11	0.42	1.8	2.1
patch clamp hERG IC_{50} (μM)	0.33	nd	8.9	4.2
thrombin IC_{50} (μM)	>10	>10	>10	>10
trypsin IC_{50} (μM)	>10	>10	>10	>10
t-PA IC_{50} (μM)	>10	>10	>10	>10
aPC IC_{50} (μM)	>10	>10	>10	>10
plasmin IC_{50} (μM)	>10	>10	>10	>10
kallikrein IC_{50} (μM)	0.51	0.54	6.3	7.2
Rat PK				
F (%)	26.1	38.0	23.8	48.6
$t_{1/2}$ (h)	12.8	5.2	8.8	5.5
CL (mL/min/kg)	29.7	15.3	43.6	57.5
V_d (L/kg)	26.1	6.9	32.9	26.5
AUC_{PO} (ng h/mL)	885	2425	456	770
dose IV; PO (mg/kg)	0.2; 6	0.2; 6	1; 5	1; 5
Dog PK				
F (%)	64.9	68.8	51.6	88.9
$t_{1/2}$ (h)	15.3	13.1	21.2	7.9
CL (mL/min/kg)	34.0	30.5	26.5	24.6
V_d (L/kg)	45.7	35.1	48.8	16.8
AUC_{PO} (ng h/mL)	320	381	825	1500
dose IV; PO (mg/kg)	0.1; 1	0.1; 1	0.5; 2.5	0.5; 2.5
Monkey PK				
F (%)	73.0	86.1	58.7	77.6
$t_{1/2}$ (hr)	19.4	22.8	9.6	11.4
CL (mL/min/kg)	26.7	11.2	18.7	16.2
V_d (L/kg)	36.5	20.5	13.4	16.3
AUC_{PO} (ng hr/mL)	512	1301	4180	6170
dose IV; PO (mg/kg)	0.1; 1	0.1; 1	0.75; 7.5	0.75; 7.5

carboxylic acid residue, which couples with the P4 basic benzamidine moiety to cause compounds **42** and **51** to stay in a zwitterionic form.[11,14] However, this zwitterionic property is strongly detrimental to oral absorption. The oral bioavailability of compound **42**, for example, in rat is <5%. Fortunately, among the remaining compounds shown in Tables 1–5, compound **11** (hERG K_i 1.8 μM) exhibits significantly lower hERG activity than all the others (hERG $K_i \leqslant 0.5$ μM).

To follow up on compound **11** (fXa K_i 117 pM), compounds **72**–**77** (Table 6) were prepared to fine-tune fXa potency and hERG channel affinity. Replacement of methoxy with trifluoromethoxy (**72**) or 2,2,2-trifluoroethoxy (**73**) reduces anti-fXa activity considerably. While the C^2-fluoro analog **75** (IC_{50} 0.7 nM; fXa K_i 105 pM) displays similarly mild hERG activity (hERG K_i 2.1 μM) as compound **11**, the corresponding 1-piperidinyl analog **76** has strong hERG binding potency. Zwitterionic compounds **74** and **77** exhibit greatly decreased hERG affinity, but their oral bioavailability in rat is very low (<5%).

From these systematic SAR explorations, compounds **11** and **75** have been selected for further evaluations (Table 7). They both show excellent in vitro anticoagulant potency, judged by their 2 × TG values of 0.33 μM and 0.34 μM. Compounds **4**, **33**, **11** and **75** all are dose-dependently efficacious in our rabbit deep vein thrombosis model.[15] The concentration required to double the rabbit prothrombin time (2 × PT)[16] is below 2 μM for each fXa inhibitor. Owing to the *N*,*N*-dimethylbenzamidine functionality which is charged in the biological pH range, these four compounds have good solubility in water and exhibit low human plasma protein binding (63–88%).[17] Detailed in vitro biological study and in vivo anticoagulant efficacy study of these compounds will be disclosed in a separate biology-focused publication. To benchmark our lead compounds in anticoagulant activity, we prepared and studied rivaroxaban (BAY59-7939, reported fXa K_i 0.4 nM)[4a] (**78**) and apixaban (BMS-562247, reported fXa K_i 0.08 nM)[4f] (**79**), two oral fXa inhibitors in advanced clinical trials. We found the in-house 2 × TG values for them to be 0.41 μM and 1.8 μM, respectively.



78 **79**

In patch clamp hERG assays,[18] compounds **11** and **75** have IC_{50} values of 8.9 μM and 4.2 μM respectively, while that is 0.33 μM for compound **4**. All four compounds are uniformly selective for fXa with very poor activity toward thrombin, trypsin, t-PA, aPC or plasmin inhibition (IC_{50} >10 μM) and only weak activity toward plasma kallikrein inhibition. The plasma kallikrein IC_{50} and K_i values for compound **11** are 6.3 μM and 3.5 μM respectively, and those for compound **75** are 7.2 μM and 3.5 μM, respectively. These compounds are stable in rat, dog, monkey and human liver S9 incubation, and have displayed a profile of good oral bioavailability and oral exposure, long half-life, moderate to high clearance, and high volume of distribution (V_d). Dosed at 0.5 mg/kg IV and 2.5 mg/kg PO, compounds **11** and **75** had bioavailability of 51.6% and 88.9% respectively in dog; dosed at 0.75 mg/kg IV and 7.5 mg/kg PO, they had bioavailability of 58.7% and 77.6% respectively in monkey. The C^2-fluoro group improves oral absorption and lowers CL and V_d parameters in rat, dog and monkey, as shown for compounds **33** and **75** compared to **4** and **11**.

Comparison of compounds **11** and **75** head-to-head in various animal model studies revealed little difference in efficacy. Compound **75** has higher exposure than compound **11** following oral dosing, but there is no difference in PK when administered intravenously. Compound **75** is relatively more potent in the patch clamp hERG assay and commands much higher manufacturing cost than compound **11**. Based upon the overall biological, toxicological and PK/PD profiles, hERG liability concern and manufacturing cost, compound **11** (betrixaban) was chosen as the clinical candidate for this class of anthranilamide-based fXa inhibitors. In addition, a safety window of greater than 30-fold was maintained for the ratio of patch clamp hERG IC_{50} to the expected compound C_{max} adjusted for its unbound fraction.[13c] The Phase II study of betrixaban (PRT054021) as an oral fXa inhibitor for prevention of venous thromboembolic (VTE) events after total knee replacement has been successfully completed in 2008 and has validated its potential to be a safe and effective oral anticoagulant.[19] The ongoing Phase II EXPLORE Xa study will assess the safety, tolerability and efficacy of betrixaban compared with dose-adjusted warfarin in patients with non-valvular atrial fibrillation (AF).

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16. The rabbit 2×PT values were determined by the method described in Ref. 5(b).
17. Human plasma protein binding, expressed as unbound fraction, was determined by this protocol: The compound stock solutions were diluted in 1.0 M HEPES (pH 7.4) to yield a 1.0 mM working solution. The working solution was added to plasma samples (EDTA was used as anticoagulant) in a ratio of 1/100 yielding a final concentration of 10 μM. The mixture was gently mixed and incubated at 37 °C for 30 min. At the conclusion of the incubation 3 aliquots (450 μL) each were added to a centrifugal filter device fitted to a 96 well plate. Standards were prepared in protein free human plasma and transferred to the centrifugal filter device fitted to the same plate. The plates were centrifuged for 25 min at 32 °C in a centrifuge. 15 μL each of the filtrate was transferred to a round bottom 96 well plate and 15 μL of acetonitrile including KN1022 (1 μg/mL) as internal standard was added followed by 60 μL of DI water. Plates were placed on a Multi-Tube Vortexer for 30 s and vortexed. Concentrations in the filtrate were determined by LC/MS/MS and using standard curves prepared in ultra filtrated plasma.
18. The patch clamp assay utilizes cultured, whole Human Embryonic Kidney (HEK) 293 cells stably transfected with the hERG gene to evaluate the potential of a compound to interfere with the potassium-selective I_{Kr} current generated under normoxic conditions. Interference of the potassium channel suggests that the compound could modify (shorten or prolong) the electrocardiographic (ECG) QT interval. (a) Zhou, Z.; Gong, Q.; Ye, B.; Fan, Z.; Makielski, J. C.; Robertson, G. A.; January, C. T. *Biophys. J.* **1998**, *74*, 230; (b) Zhou, Z.; Gong, Q.; Ye, B.; Fan, F.; Makielski, J. C.; Robertson, G. A.; January, C. T. *Biophys. J.* **1997**, *72*, A225; (c) Hancox, J. C.; Levi, A. J.; Witchel, H. J. *Pflugers Arch.* **1998**, *436*, 843; (d) Jurkat-Rott, K.; Lehmann-Horn, F. *Curr. Pharm. Biotechnol.* **2004**, *5*, 387.
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