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Anthranilamide-based *N,N*-dialkylbenzamidines as potent and orally bioavailable factor Xa inhibitors: P4 SAR

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ABSTRACT

Anthranilamide-based benzamidine compound **4** and its N-substituted analogs were designed and examined as factor Xa inhibitors using substituted benzamidines as unconventional S4 binding element. A group of *N,N*-dialkylbenzamidines (**11**, **17** and **24**) have been discovered as potent factor Xa inhibitors with strong anticoagulant activity and promising oral PK profiles.

Activated by both the extrinsic and the intrinsic pathways, the serine protease factor Xa (fXa) plays a pivotal role in the blood coagulation cascade. In the prothrombinase complex comprised 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] FXa is a particularly attractive target for anticoagulant therapy for the treatment of severe cardiovascular diseases.[2] Selective inhibition of fXa provides antithrombotic effects by diminishing the amplified generation of thrombin without affecting existing thrombin levels. 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]

Small molecule, reversible and orally bioavailable fXa inhibitors have been pursued extensively for a decade. We have discovered a series of neutral and mono-basic anthranilamide-based fXa inhibitors as exemplified by compounds **1**, **2** and **3** (Fig. 1), wherein the chloropyridine ring interacts with the S1 specificity pocket.[5,6] Anthranilamides **1**, **2** and **3** are potent and selective fXa inhibitors, with inhibitory IC_{50} values of 6.7 nM, 1.5 nM and 4.7 nM, respectively.[5,7] The biphenyl group of compound **1**, the cyclized guanidine group of compound **2** and the oxazolidin-2-imine group of compound **3** are thought to bind to the highly aromatic fXa S4 pocket either through hydrophobic or non-specific π-cation interactions.[6c,f] Though orally bioavailable (*F* 35% in rat), compound **1** shows very poor in vitro anticoagulant activity in our human plasma-based thrombin generation assay (2 × TG >5 μM).[5a,8] Compounds **2** and **3** with increased P4 hydrophilicity provides improved functional activity (2 × TG 0.58 μM and 0.56 μM, respectively), possibly due to their lower human plasma protein binding. The guanidine moiety's high basicity in compound **2** (calculated pK_a[9] ~14) likely leads to its low oral absorption (*F* <1% in rat)

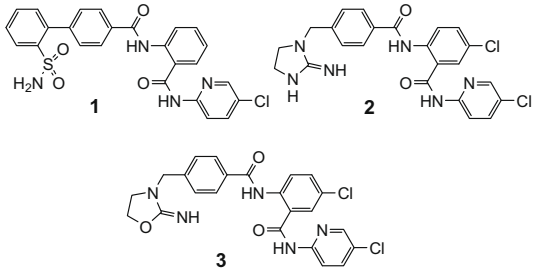

Figure 1. Anthranilamide-based fXa inhibitors.

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and high clearance, while compound **3** displays good oral pharmacokinetic (PK) profile (F 44%, $t_{1/2}$ 8.5 h, CL 18.3 mL/min/kg, V_d 13.6 L/kg in rat).

From our SAR explorations, we learned that a variety of positively charged groups are potent P4 motifs possibly due to π-cation interactions. Additionally, modulation of S4 binding elements provides a promising strategy to improve anticoagulant activity and oral PK properties.

Compared to compound **2**, the corresponding benzamidine analog **4** (Table 1) has lower basicity (calculated pK_a ~10), leading us to examine the benzamidine groups as the P4 motifs in our anthranilamide scaffold for fXa inhibition. For proof of concept, we designed compound **4** bearing a primary benzamidine as the P4 group, though this moiety had been well-know for its strong PK liability in the fXa inhibitor discovery arena. Not surprisingly, compound **4** suffers poor oral absorption (F <5% in rat). To our delight, it displays potent fXa inhibitory activity (IC_{50} 4 nM) and excellent selectivity over other serine proteases, with IC_{50} values >10 μM for thrombin, trypsin, tissue plasminogen activator (*t*-PA), activated protein C (aPC), plasmin and IC_{50} value of 4.9 μM for kallikrein. Unlike the early fXa inhibitors bearing the amidine group to ionically interact with Asp189 in the fXa S1 pocket, we believed that the amidine group in compound **4** interacts with the highly aromatic S4 pocket through π-cation interactions. Based on this hypothesis, we rationalized that this benzamidine group could be N-substituted without compromising the fXa binding affinity,[10] whereas the benzamidine group interacting with the fXa S1 pocket is generally not tolerated for any substitution due to negative steric interactions. Further rationale for us to explore N-substituted benzamidines as S4 binding element was that these substitutions could provide significantly improved oral absorption from the primary benzamidine analog, previously reported in the platelet GP IIb–IIIa antagonist literature.[11]

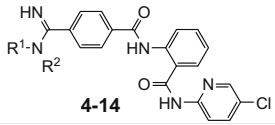
For the P4 SAR exploration around compound **4**, we first examined N-substitution of the benzamidine using a variety of moieties (Table 1). Benzamidines substituted by a hydroxyl, methoxy, amino, dimethylamino, methyl and ethyl group (**5–10**) are less potent than compound **4**. Increasing the bulkiness of the alkyl group reduces fXa potency. Interestingly, the N,N-dialkylated benzamidine compounds **11–14** are more potent than the N-monoalkylated analogs **9–10**. Smaller methyl group is preferred over ethyl, and the *N*,*N*-dimethylbenzamidine **11** (IC_{50} 3 nM) is the most potent fXa inhibitor among all the compounds studied.

To further investigate the P4 N,N-disubstituted benzamidines, we synthesized compounds **15–22** using various cyclic amine staring materials (Table 2). Piperidine compound **17** (IC_{50} 3 nM) is more potent than the azetidine **15**, pyrrolidine **16**, morpholine **18** and thiomorpholine **19** analogs. Oxidation of the thiomorpholine to the corresponding sulfone **20** decreases the fXa potency significantly. The lower activity of compounds **21** and **22** suggests the second basic nitrogen atom in the ring structure detrimental to binding in the fXa S4 pocket.

A group of P4 cyclized amidine moieties have also been studied in this anthranilamide-based fXa inhibitor series (Table 3). Imidazoline **23** and tetrahydro-pyrimidine **26** are approximately equipotent fXa inhibitors. *N*-Methylation profoundly increases the fXa potency, especially in the case of *N*-methylimidazoline **24** (IC_{50} 20 nM). Replacement of the methyl substituent by a bulkier ethyl group decreases the inhibitory potency by threefold in compound **25**.

All the compounds listed in Tables 1–3 have excellent enzyme selectivity profile toward fXa as shown by compound **4**. Based on their fXa inhibitory activity and structural diversity, compounds **11**, **17** and **24** were selected for further in vitro and in vivo studies (Table 4). Their measured human plasma protein binding ranges between 73% and 89%.[12] All three compounds show good in vitro anticoagulant activity (2 × TG 0.36–1.1 μM). The cLog*D* values[9] of these compounds are 0.17, 0.94 and 0.22, respectively at pH 7.4. For comparison, compounds **1–3** (Fig. 1) have cLog*D* values of 3.78, −1.26 and 0.80, respectively at pH 7.4. Compared with compound **11** (IC_{50} 3 nM; 2 × TG 0.54 μM), compound **17** (IC_{50} 3 nM; 2 × TG 0.36 μM) has similar anti-fXa potency and slightly stronger in vitro anticoagulant activity. Dosed at 0.4 mg/kg IV and 6 mg/kg PO in Sprague–Dawley rat, all three compounds demonstrated promising PK profiles. Compound **11** (F 31%; CL 16.8 mL/min/kg; V_d 6.3 L/kg) gives significantly better oral bioavailability (F), lower clearance (CL) and lower volume of distribution (V_d) than compound **17** (F 15%; CL 32.2 mL/min/kg; V_d 16.7 L/kg). Thus compound **11** was chosen over compound **17** as the lead among this class of fXa inhibitors. The PK profiles of compounds **11** and **24**

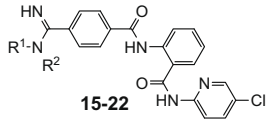
Table 1
Benzamidine **4** and its N-substituted analogs as fXa inhibitors



4-14

Compd	NR^1R^2	fXa IC_{50} (nM)
4	NH_2	4
5	NHOH	35
6	NHOMe	290
7	$NHNH_2$	25
8	$NHNMe_2$	30
9	NHMe	23
10	NHEt	93
11	NMe_2	3
12	NMeEt	6
13	NMePr	9
14	NEt_2	10

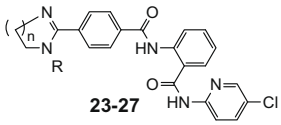
Table 2
N,N-Disubstituted benzamidines **15–22** as fXa inhibitors



15-22

Compd	NR^1R^2	fXa IC_{50} (nM)
15	1-azetidinyl	8
16	1-pyrrolidinyl	8
17	1-piperidinyl	3
18	4-morpholinyl	7
19	4-thiomorpholinyl	12
20	4-(1,1-dioxo)thiomorpholinyl	256
21	1-piperazinyl	38
22	4-Me-1-piperazinyl	714

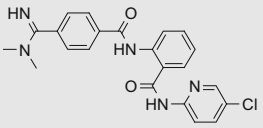
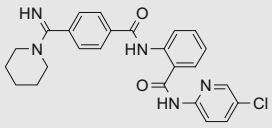
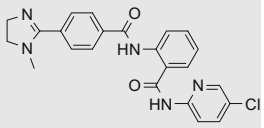
Table 3
Cyclic benzamidines **23–27** as fXa inhibitors



23-27

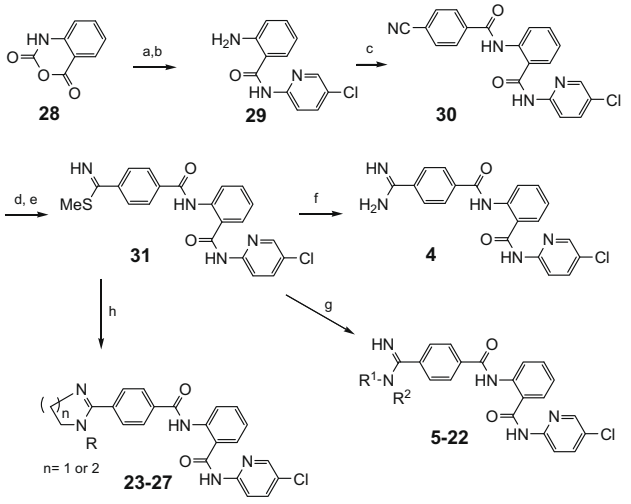
Compd	*n*	R	fXa IC_{50} (nM)
23	1	H	316
24	1	Me	20
25	1	Et	64
26	2	H	226
27	2	Me	78

Table 4
Biological and animal pharmacokinetic data for the leading fXa inhibitors

Compd	11	17	24
			
fXa IC_{50} (nM)	3	3	20
2 × TG (μM)	0.54	0.36	1.1
human plasma protein binding (%)	73	89	82
cLog*D* (pH 7.4)	0.17	0.94	0.22
***Rat* PK**			
F (%)	31	18	22
$t_{1/2}$ (h)	4.3	6.0	>6.0
CL (mL/min/kg)	16.8	32.2	7.1
V_d (L/kg)	6.3	16.7	3.7
***Dog* PK**			
F (%)	69	nd	50
$t_{1/2}$ (h)	3.5	nd	2.2
CL (mL/min/kg)	45.7	nd	49.6
V_d (L/kg)	13.7	nd	9.2

were further assessed in beagle dog (dosed at 0.1 mg/kg IV and 0.5 mg/kg PO). They both were well absorbed with oral bioavailability values of 69% and 50%, respectively.

The synthesis of compound **4** and its analogs is described in Scheme 1.[13] Isatoic anhydride **28** was reacted with 2-amino-5-chloropyridine and potassium *t*-butoxide to afford compound **29**. Coupling of aniline **29** with 4-cyanobenzoyl chloride furnished benzonitrile **30**. It was converted to methyl thioimidate **31** by treatment with excess hydrogen sulfide gas, followed by *S*-methylation using methyl iodide. Compounds **4**–**27** were prepared from methyl thioimidate **31** using various amines in a parallel synthesis fashion. The yield of this final step was improved by treating the free amines with acetic acid prior to their addition to the methyl thioimidate, to suppress the generation of benzonitrile **30** from **31** promoted by basicity.



Scheme 1. Reagents and conditions: (a) 2-amino-5-chloropyridine (1 equiv), $KOBu^t$ (2 equiv), rt, THF, 10 min; (b) H_2O, 65% for two steps; (c) 4-cyanobenzoyl chloride (1 equiv), THF, rt, 30 min, 95%; (d) H_2S (g), pyridine, Et_3N, rt, overnight; (e) MeI (2 equiv), acetone, reflux, 30 min; (f) NH_4OAc (2 equiv), MeOH, reflux, 10 min, 65% for three steps; (g) NHR^1R^2 (2 equiv), HOAc (3 equiv), MeOH, reflux, 60–85%; (h) $H_2N(CH_2)_nCH_2NHR$ (2 equiv), HOAc (5 equiv), MeOH, reflux, 60–80%.

In summary, we investigated a variety of N-substituted benzamidine moieties as the S4 binding motifs in our anthranilamide-based fXa inhibitor scaffold. Through this exploration, a group of *N*,*N*-dialkylbenzamidine compounds (**11**, **17** and **24**) have been discovered as highly potent and selective fXa inhibitors. These compounds are moderately protein bound in human plasma and demonstrate good anticoagulant activity in our human plasma-based thrombin generation assay. More importantly, these compounds display good oral PK profiles in rat and dog. Compound **11**, *N*-(5-chloropyridin-2-yl)-2-(4-(*N*,*N*-dimethylcarbamimidoyl)-benzamido)benzamide (fXa K_i 1.4 nM),[14] has been chosen as the lead from this class of *N*,*N*-dialkylbenzamidine fXa inhibitors for further modifications.

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12. 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.
13. *Preparation of compound* **11**: (a) To a rapidly stirred solution of KOBu^t (224 mg, 2 mmol) in 10 mL of THF was added 2-amino-5-chloropyridine (128 mg, 1 mmol). After 10 min, isatoic anhydride **28** (163 mg, 1 mmol) was added in three portions. Stirring was allowed to continue for 10 more minutes and the mixture became gelatinous. The reaction was quenched with 10 mL of water, and the resulting slurry was filtered and air-dried to give compound **29** (170 mg) as a pale yellow solid. (b) Compound **29** (123 mg) suspended in 5 mL of THF was treated with 4-cyanobenzoyl chloride (83 mg) at rt for 30 min. The mixture was filtered and washed with small amount of chilled methanol to give compound **30** (145 mg) as a grey solid. (c) Hydrogen sulfide (gas) was bubbled into a suspension of compound **30** (0.1 g) in 5 mL of pyridine and 0.5 mL of triethylamine until saturation. The mixture was stirred at rt for overnight and the volatile was evaporated. The residue was taken up by 3 mL of acetone and to it 0.5 mL of iodomethane was added. The resulting mixture was refluxed for 30 min and evaporated to give a yellow solid, which was treated with a premixed solution of 2 mL of 2 N dimethylamine in THF and 0.2 mL of acetic acid. The reaction mixture was refluxed for 10 min. The desired product **11** was isolated using reverse phase HPLC with water–acetonitrile (0.1% TFA) as its TFA salt (70 mg).
14. The fXa K_i value was determined by the method described in: Betz, A.; Wong, P. W.; Sinha, U. *Biochemistry* **1999**, *38*, 14582.