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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.

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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 Ca²⁺ 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}

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Anthranilamides **1**, **2** and **3** are potent and selective fXa inhibitors, with inhibitory IC₅₀ 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.^{6,c,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 ($2 \times \text{TG} > 5 \mu\text{M}$).^{5a,8} Compounds **2** and **3** with increased P4 hydrophilicity provides improved functional activity ($2 \times \text{TG}$ 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_a⁹ ~14) likely leads to its low oral absorption (*F* <1% in rat)

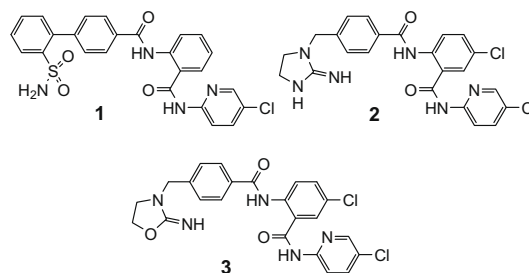


Figure 1. Anthranilamide-based fXa inhibitors.

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.

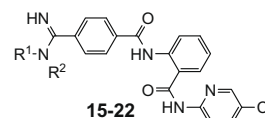
Compared to compound **2**, the corresponding benzamidine analog **4** (Table 1) has lower basicity (calculated $pK_a \sim 10$), leading us to examine the benzamidine groups as the P4 motifs in our anthranilamide scaffold for fXa inhibition. For proof of concept, we designed compound **4** bearing a primary benzamidine as the P4 group, though this moiety had been well-known 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 μ M for thrombin, trypsin, tissue plasminogen activator (t-PA), activated protein C (aPC), plasmin and IC_{50} value of 4.9 μ M 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 starting materials (Table 2). Piperidine compound **17** (IC_{50} 3 nM) is

Table 2

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



Compd	NR ¹ R ²	fXa IC ₅₀ (nM)
15	1-azetidiny	8
16	1-pyrrolidiny	8
17	1-piperidiny	3
18	4-morpholiny	7
19	4-thiomorpholiny	12
20	4-(1,1-dioxo)thiomorpholiny	256
21	1-piperaziny	38
22	4-Me-1-piperaziny	714

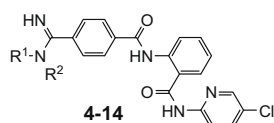
more potent than the azetidine **15**, pyrrolidine **16**, morpholine **18** and thiomorpholine **19** analogs. Oxidation of the thiomorpholine to the corresponding sulfone **20** decreases the fXa potency significantly. The lower activity of compounds **21** and **22** suggests the second basic nitrogen 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 ($2 \times$ TG 0.36–1.1 μ M). The cLogD values⁹ of these compounds are 0.17, 0.94 and 0.22, respectively at pH 7.4. For comparison, compounds **1–3** (Fig. 1) have cLogD values of 3.78, –1.26 and 0.80, respectively at pH 7.4. Compared with compound **11** (IC_{50} 3 nM; $2 \times$ TG 0.54 μ M), compound **17** (IC_{50} 3 nM; $2 \times$ TG 0.36 μ M) 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

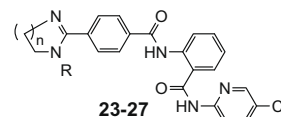
Benzamidine **4** and its N-substituted analogs as fXa inhibitors



Compd	NR ¹ R 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 3

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



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

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