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PREPARATION OF *meta*-AMIDINO-*N,N*-DISUBSTITUTED ANILINES AS POTENT INHIBITORS OF COAGULATION FACTOR Xa[1]

John M. Fevig,* Joseph Cacciola, Richard S. Alexander, Robert M. Knabb, Gilbert N. Lam,# Pancras C. Wong and Ruth R. Wexler

DuPont Pharmaceuticals Company, P.O. Box 80500, Wilmington, DE 19880-0500, U.S.A.

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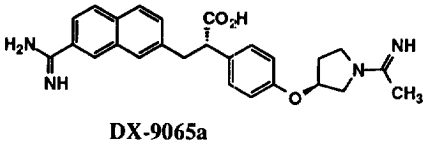
Abstract: The serine protease factor Xa is a critical enzyme in the blood coagulation cascade. Recently, the inhibition of factor Xa has begun to emerge as an attractive strategy for the discovery of novel antithrombotic agents. Here we describe a series of *meta*-amidino-*N,N*-disubstituted anilines as structurally simple and very potent inhibitors of factor Xa.

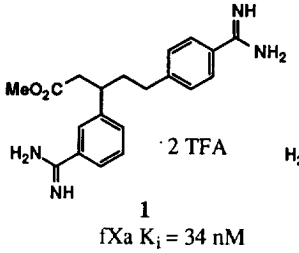
The serine protease factor Xa occupies a central position in the blood coagulation cascade, linking the extrinsic and intrinsic activation mechanisms. Factor Xa (fXa) combines with factor Va, Ca^{2+} and phospholipids to form a prothrombinase complex that generates thrombin by the proteolysis of prothrombin. Thrombin has several important procoagulant functions, including the activation of platelets, the feedback activation of other coagulation factors and the conversion of fibrinogen to insoluble fibrin, which crosslinks aggregating platelets. Direct small molecule thrombin inhibitors have been intensely investigated as potential antithrombotic agents.[2] More recently, the inhibition of thrombin generation by direct inhibition of fXa has emerged as another attractive strategy for the discovery of novel antithrombotic agents.[3]

Several dibasic fXa inhibitors have been reported, the most advanced of which is Daiichi's DX-9065a,[4] which is currently in clinical trials (Figure 1). Recently, we have reported potent bis-benzamidine fXa inhibitors such as **1**,[5] in which the aromatic rings are linked by an acyclic tether, and **2**,[6] which contains an isoxazoline core structure. These compounds are thought to bind in the fXa active site such that the *meta*-substituted benzamidine residue engages in a bidentate interaction with Asp189 in the S_1 specificity pocket while the *para*-substituted benzamidine residue binds in the aryl binding pocket, bordered by residues Phe174, Tyr99 and Trp215.

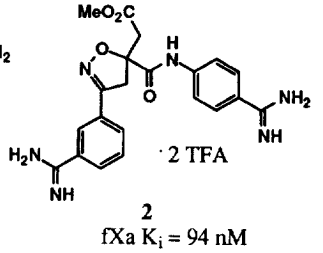
Figure 1.



DX-9065a
fXa IC_{50} = 41 nM



1
fXa K_i = 34 nM



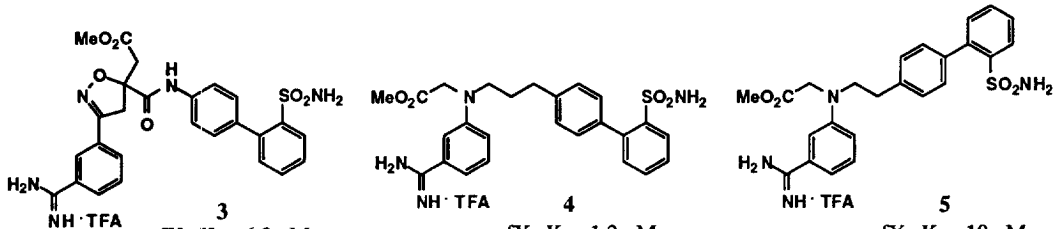
2
fXa K_i = 94 nM

PII: S0960-894X(98)00574-5

We expected that bis-benzamidines such as **1** and **2** would not have adequate oral absorption or pharmacokinetics to be useful as oral antithrombotic agents. Based on some earlier results from our thrombin inhibitor program,[7] and in part to reduce the basicity of these compounds, the *para*-substituted benzamidine in the isoxazoline series was replaced by a biphenylsulfonamide as in **3**[8] (Figure 2), resulting in a monobasic compound with improved affinity for fXa relative to **2**. This result prompted us to replace the *para*-substituted benzamidine of compound **1** with the neutral biphenylsulfonamide residue in a similar manner. This was considered to be a first step in the elaboration of this lead series into an orally active drug, provided that potency toward fXa was maintained. We also wanted to simplify the structure of **1** by removing the stereogenic center. Other work from these laboratories has demonstrated that the stereogenic methine carbon of this series can be replaced by nitrogen, resulting in a series of dibasic *N,N*-disubstituted anilines as fXa inhibitors.[9]

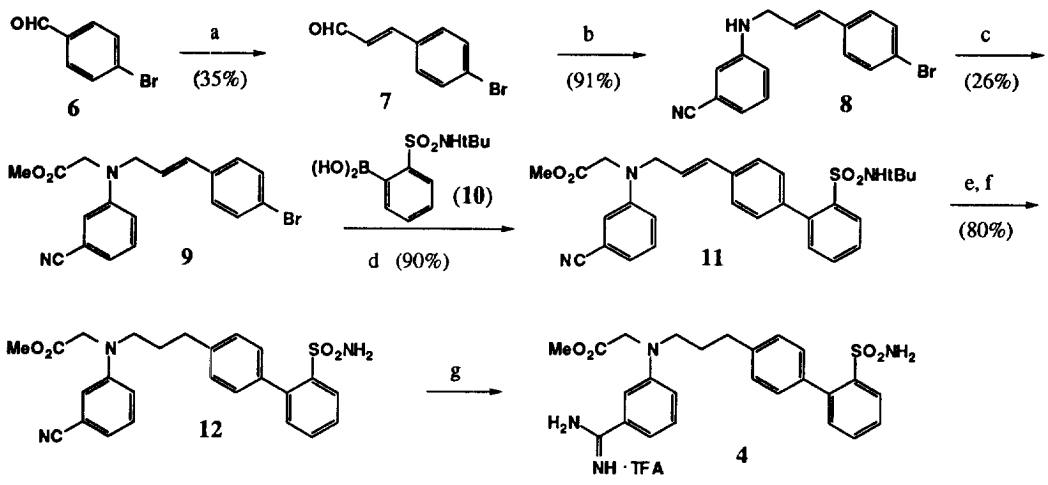
As initial targets, compounds **4** and **5** were prepared (Figure 2). Compound **4**, with a four-atom tether between the benzamidine and biphenyl residues, had a fXa K_i of 1.3 nM. Compound **5**, with the three-atom tether, was less potent than **4** but still was threefold more potent than the lead compound **1**. These results, especially for **4**, were significant in that we were able to replace a pharmacokinetically undesirable benzamidine residue with a neutral biphenylsulfonamide residue while simultaneously increasing the potency more than 25-fold. Compound **4**, an achiral, potent and structurally simple fXa inhibitor, became our lead compound. The remainder of this manuscript will describe the synthesis and biological profile of **4** as well as some of the SAR of side chain and linker analogs of **4**.

Figure 2.



Compound **4** was prepared as described in Scheme 1. Wittig reaction of 4-bromobenzaldehyde **6** with (triphenylphosphoranylidene)acetaldehyde gave the unsaturated aldehyde **7**,[10] the purification of which was complicated by the presence of polymeric products. Reductive amination of **7** with 3-aminobenzonitrile afforded the aniline **8** in high yield. *N*-Alkylation of **8** with methyl bromoacetate was very sluggish and required a sodium carbonate/TDA-1 phase transfer system at 80 °C to prepare the *N,N*-disubstituted aniline **9** in only modest yield. Suzuki coupling of **9** with 2-(*tert*-butylaminosulfonyl)phenyl boronic acid **10** smoothly gave the biphenylsulfonamide **11**. Catalytic hydrogenation of the double bond and TFA-catalyzed removal of the *t*-butyl group gave **12**, which then was subjected to standard Pinner conditions (HCl, methanol, 0 °C; then ammonium carbonate, methanol) to afford compound **4**.

Scheme 1.



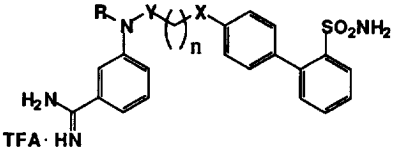
Reagents: (a) Ph_3P=CHCHO, CH_2Cl_2 (b) 3-aminobenzonitrile, $NaCNBH_3$, MeOH (c) $BrCH_2CO_2Me$, Na_2CO_3, TDA-1, DMF, 80 °C (d) **10**, $(Ph_3P)_4Pd$, TBAB, Na_2CO_3, benzene/H_2O, 80 °C (e) H_2 (1 atm), 10% Pd/C, MeOH (f) TFA, 80 °C (g) HCl, MeOH, 0 °C; then $(NH_4)_2CO_3$, MeOH.

Compound **4** was assayed against a range of other serine proteases, including the coagulation enzymes thrombin (K_i = 200 nM) and factor VIIa (K_i = 4200 nM), the fibrinolytic enzymes plasmin (K_i = 300 nM) and tPA (K_i = 33,000 nM), trypsin (K_i = 39 nM) and complement factor I[11] (IC_{50} = 10,000 nM) Compound **4** displayed >100-fold selectivity against all serine proteases examined with the exception of trypsin, for which it has more modest 30-fold selectivity.

As a potent and selective fXa inhibitor, **4** was evaluated in a rabbit arterio-venous (A-V) shunt model[12] to determine if it was a potent antithrombotic agent *in vivo*. Unfortunately, compound **4** was not efficacious in this model, having an ID_{50} > 1.7 μmol/kg/h. The poor efficacy of **4** in the A-V shunt model did not appear to correlate with its potent fXa activity. We suspected that either the methyl ester was metabolically unstable or that **4** suffered from an undesirable pharmacokinetic profile in which it was rapidly cleared from plasma. *In vitro* ester hydrolysis studies on **4** revealed that in rabbit plasma the methyl ester is very rapidly hydrolyzed ($t_{1/2}$ = 10.5 min) to the corresponding carboxylic acid **13**, which was subsequently determined to be a less potent fXa inhibitor (fXa K_i = 12 nM, Table 1). This data might in part explain the poor *in vivo* efficacy of **4**. Ester hydrolysis in human plasma was also rapid ($t_{1/2}$ = 68 min). Clearly, we needed to modify our lead compound such that it has adequate plasma stability to result in good *in vivo* antithrombotic activity.

Given the plasma instability of the acetate side chain of **4**, we attempted to modify this side chain to obtain compounds that were metabolically stable and that retained potent fXa activity (Table 1, compounds **13-20**). We also investigated some analogs of the acyclic tether that we thought might favorably influence metabolic stability (Table 1, compounds **21-26**). The binding data in Table 1 show that a methyl ester is the

Table 1.



Cmpd*	R	n	X	Y	fXa K_i (nM)**	thrombin K_i (nM)**	trypsin K_i (nM)**
4	$-CH_2CO_2Me$	1	CH_2	CH_2	1.3	200	39
5	$-CH_2CO_2Me$	0	CH_2	CH_2	10	400	56
13	$-CH_2CO_2H$	1	CH_2	CH_2	12	1400	470
14	$-CH_2CONH_2$	1	CH_2	CH_2	19	3500	820
15	$-CH_2CH_2OH$	1	CH_2	CH_2	17	1800	710
16	-H	1	CH_2	CH_2	83	6100	850
17	$-CH_2COCH_3$	1	CH_2	CH_2	16	900	360
18	$-CH_2CH_2OMe$	1	CH_2	CH_2	11	900	330
19	$-CH_2CO_2i$-Pr	1	CH_2	CH_2	7.5	500	74
20	$-CH_2CH_2OPh$	1	CH_2	CH_2	16	300	nt
21	$-CH_2CO_2Me$	1	O	CH_2	0.53	200	19
22	$-CH_2CO_2i$-Pr	1	O	CH_2	2.1	300	52
23	$-CH_2CO_2Me$	2	O	CH_2	6.7	600	77
24	-H	1	CH_2	SO_2	51	17000	1400
25	$-CH_2CO_2Me$	1	CH_2	SO_2	21	9000	180
26	$-CH_2CO_2i$-Pr	1	CH_2	SO_2	37	15000	220

* All compounds were purified by prep HPLC (C18 reverse phase column, elution with a H_2O/CH_3CN gradient with 0.5% TFA), lyophilized and isolated as the trifluoroacetic acid salt.
** Enzyme inhibition assays were performed as described in reference 13. Reported values are averages from at least duplicate experiments.

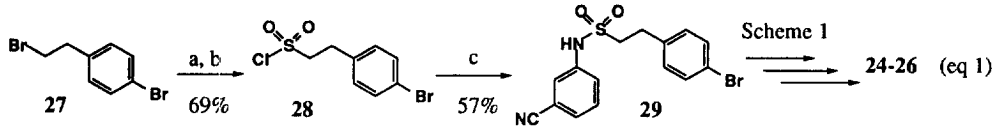
preferred side chain substituent. Most of the side chain modifications led to compounds that are about an order of magnitude less potent than **4**. Complete removal of the side chain (compound **16**) led to a 60-fold drop in potency. The best tolerated substitution is the bulkier *iso*-propyl ester **19**. The bulky phenyl ether **20** is also fairly well tolerated. These substitutions have little effect on trypsin selectivity. The oxygen tethered compounds **21-23** were prepared to block potential metabolism by benzylic oxidation. This substitution resulted in about a threefold increase in fXa potency and led to compound **21**, the most potent compound prepared in this series. The homologous compound **23** (five-atom linker) is an order of magnitude less potent than **21**. This result, taken together with a comparison of **4** and **5**, demonstrates that the four-atom linker is optimal for fXa potency in this series.

The sulfonamide compounds **24-26** were prepared to block potential metabolism occurring through *N*-oxidation of the aniline nitrogen. The sulfonamide **24**, which lacks the side chain, is more potent than the corresponding aniline **16**. Introduction of the acetate side chain in the sulfonamide series, however, did not afford dramatic increases in potency. Thus, **25** and **26** are considerably less potent than **4**, **19**, **21** and **22**.

The side chain analogs **13-15** and **18-20** were prepared by straightforward modifications of the methyl ester side chain of **4**. The methyl ketone **17** was prepared following the general route outlined in Scheme 1 with the following two modifications. First, aniline **8** was *N*-alkylated with α-chloroacetone (KI, Na_2CO_3, TDA-1, DMF, 80 °C; 78% yield) rather than with methyl bromoacetate, and, second, the final Pinner reaction was performed using 5 equiv of methanol in methyl acetate solvent (saturated with HCl). Standard Pinner conditions (methanol solvent saturated with HCl) afforded the decomposition product **16** as the major product, while the methyl acetate modification gave primarily **17** with only about 10% of **16**.

The oxygen analog **21** was prepared following the general route outlined in Scheme 1 with the following modification. *N*-Alkylation of 3-aminobenzonitrile with (4-bromophenoxy)ethyl bromide (K_2CO_3, TDA-1, DMF, 100 °C; 30% yield) produced a phenyl ether intermediate. Methyl bromoacetate *N*-alkylation, Suzuki coupling, TFA deprotection and the Pinner sequence as described in Scheme 1 gave **21**. Transesterification of **21** was cleanly accomplished by refluxing in isopropanol containing a catalytic amount of Ti(O-*i*-Pr)$_4$ to afford **22**.

The sulfonamide analogs **24-26** were prepared according to eq 1. Treatment of 4-bromophenethyl bromide **27** with potassium ethyl xanthate followed by chlorine gave the sulfonyl chloride **28**. Sulfonylation of 3-aminobenzonitrile with **28** produced **29**. The methods described in Scheme 1 and the transesterification method described above were used to convert **29** to sulfonamides **24-26**.



Reagents: (a) potassium ethyl xanthate, TDA-1, DMF (b) Cl_2, HOAc/H_2O (4:1), 0 °C (c) 3-aminobenzonitrile, DMAP, pyr.

In an attempt to understand the preference for a side chain ester residue indicated by the results in Table 1, we solved the X-ray crystal structure of compound **21** bound to human thrombin. As expected, the benzamidine is engaged in a bidentate interaction with Asp189 in the S_1 specificity pocket and the biphenyl sulfonamide residue binds in the aryl binding pocket bordered by residues Phe174, Tyr99 and Trp215. The crystal structure also indicates a hydrogen bond (distance = 3.1 Å) between the carbonyl oxygen of the ester side chain and the backbone NH of Glu192. The OMe residue stacks against the hydrophobic side chain methylene groups of Glu192. We would expect similar interactions between the ester side chain and fXa, in which residue 192 is Gln. These two interactions of the ester group of **21** might in part explain some of the results from Table 1. All of the less potent analogs lack either a carbonyl oxygen (**15**, **18**, **20**) or a hydrophobic group (**13-15**), or the hydrophobic group is of a different size than methoxy (**17**, **19**, **20**, **22**, **26**). Methyl ester appears optimal for interaction with Glu192 in thrombin and with Gln192 in fXa.

Finally, even though most of the side chain analogs lost potency relative to the methyl ester **4**, several compounds were selected for pharmacokinetic evaluation in a rabbit PK model.[14] The acid **13**, the methyl ketone **17** and the methyl ether **20** were selected as nonhydrolyzable side chain analogs. In addition,

the esters **19** and **22** were studied to determine if bulkier esters are stable to *in vivo* hydrolysis and to determine the effect of the oxygen in the linking group. Unfortunately, from these studies all compounds were determined to have high clearance rates (all > 1.5 L/kg/h) that were many fold higher than the glomerular filtration rate (GFR) of the rabbit (about 0.2 L/kg/h). Furthermore, all compounds had estimates of plasma half life ($t_{1/2}$) of less than one hour. The overall profile of these compounds is consistent with metabolism as being a major contributor to their high clearance.[15]

In summary, we have prepared a series of fXa inhibitors that are potent, monobasic, achiral and structurally simple. Unfortunately, this series has a narrow SAR in which a small ester group appears to be required in the side chain for optimal activity. Also, this series suffers from uniformly high clearance rates in a rabbit pharmacokinetic model, thought to be in part a consequence of rapid *in vivo* metabolism. Modifications that maintain or improve fXa potency while dramatically lowering clearance are needed for the success of this series. Research along these lines has been continuing.

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References and Notes

* Present address: CoCensys, Inc., 201 Technology Dr., Irvine, CA 92618.
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12. In this model, rabbits are equipped with an AV shunt device containing a thrombogenic silk thread. Test compound is infused iv for 100 min, with blood being circulated through the A-V shunt for only the last 40 min of this infusion. The silk thread is then removed and the clot weight is determined. Antithrombotic potency is expressed as an ID_{50}, which is the dose needed to reduce clot weight by 50% relative to control.
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14. In this model, rabbits were infused with 5-10 mg/kg/h of compound and blood samples were taken prior to, during and up to four hours after the infusion. Blood samples were immediately worked up by solid phase extraction and analyzed for test compound by an *ex vivo* anti-Xa assay or by LC/MS/MS.
15. Preliminary studies on several analogs have demonstrated plasma metabolism of the side chain and in some cases (compounds **13** and **17**) complete loss of the side chain. Christ, D. D.; Mutlib, A., unpublished results.