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BISBENZAMIDINE ISOXAZOLINE DERIVATIVES AS FACTOR Xa INHIBITORS

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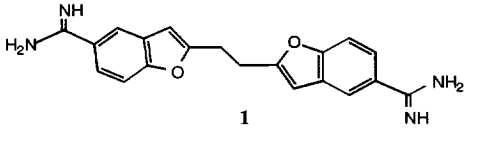
Abstract: Factor Xa is an important serine protease in the blood coagulation cascade. It generates thrombin and holds the central position that links the intrinsic and extrinsic activation mechanism in the final common pathway of coagulation. Therefore, inhibition of factor Xa has potential therapeutic applications in the treatment of both arterial and venous thrombosis. We have designed and synthesized a series of bisbenzamidine isoxazoline derivatives as factor Xa inhibitors. The most potent compound in this series has a K_i of 18 nM against factor Xa. © 1997 The DuPont Merck Pharmaceutical Company. Published by Elsevier Science Ltd.

Factor Xa (FXa) is a serine protease in the blood coagulation cascade that converts prothrombin to thrombin. FXa holds the central position linking the intrinsic and extrinsic activation mechanisms in the final common pathway of coagulation. This process involves signal amplification, with one molecule of the FXa activating many molecules of prothrombin to thrombin. Therefore, inhibition of FXa may be more effective than inhibition of thrombin in interrupting the blood coagulation cascade. Consequently, FXa is a target enzyme for new therapeutic agents with potential for treatment of arterial and venous thrombosis.

Several bisamidinoaryl compounds have been shown to be potent inhibitors of FXa. Tidwell[1] has published a series of bisamidines, represented by 1,2-di(5-amidino-2-benzofuranyl)ethane (DABE, **1**), which is a selective inhibitor of FXa (K_i = 570 nM, bovine FXa). Stürzebecher et al.[2] have reported a series of bisamidines with the most potent compound being 2,7-bis-(4-amidinobenzylidene)-cycloheptan-1-one (BABCH, **2**) having a FXa K_i of 13 nM. More recently, Nagahara et al.[3] have reported on a series of dibasic (amidinoaryl)-propanoic acid derivatives from which DX-9065a (**3**) was selected for development. DX-9065a is a selective FXa inhibitor with an IC_{50} of 41 nM, which does not inhibit thrombin at a concentration of 2000 μM.[3]



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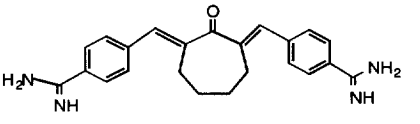

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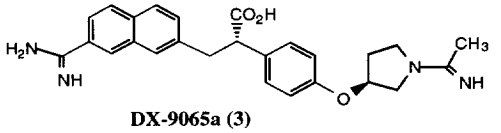
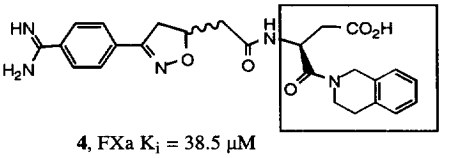


DX-9065a (3)



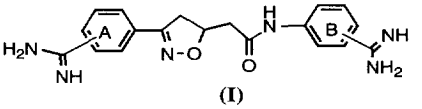
4, FXa K_i = 38.5 μM

Herein we wish to report on a series of isoxazoline compounds as FXa inhibitors. The lead in this series resulted from screening the DuPont Merck library of compounds originally synthesized as GPIIb/IIIa receptor antagonists.[4] Compounds, including **4** , were designed to mimic the sequence of ArgGlyAsp (RGD), which is similar to the GluGlyArg (EGR) substrate sequence of prothrombin for FXa. Therefore, when we embarked on a FXa program, this library of IIb/IIIa compounds was evaluated and we found that a few compounds had weak affinity for FXa.

Since several bisbenzamidine compounds are known to have FXa affinity,[1-3] we decided to maintain the benzamidine isoxazoline as the core structure. Replacement of the right-hand portion of compound **4** with a benzamidine moiety afforded the bisbenzamidines of Table 1. These compounds are inactive against the GPIIb/IIIa receptor. As shown in Table 1, low micromolar FXa affinity was observed. The most potent compound in this series (**6**) had submicromolar affinity with a *para*-amidine at the A-ring and a *meta*-amidine at the B-ring. Although these compounds are not very selective against other trypsin-like serine proteases, compounds with a *meta*-amidine group at the A-ring (**7** and **8**) are more selective over thrombin than compounds with a *para*-amidine at the A-ring (**5** and **6**).

Removal of the methylene unit between the isoxazoline ring and the amide carbonyl of compound **8** increased the FXa affinity by more than fivefold (**9**, Table 2). Molecular modeling studies of **9** indicated that the *meta*-benzamidine fits in the S_1 subsite better than the *para*-benzamidine, and this was shown by comparison of the potential energies of the two groups modeled in the FXa active site. The *meta*-benzamidine interacts with Asp^{189} through ionic and hydrogen bonding interactions. The *para*-benzamidine extends into the aryl binding site where the amidine NH interacts favorably with the π cloud of the phenyl rings of Phe^{174} and Tyr^{99}. In addition, the carbonyl may form a weak hydrogen bond with the NH of Gly^{218} of the enzyme. Morever, in comparison with **9**, the carbonyl of **8** is farther away from Gly^{218} and there appears to be a sterically unfavorable close contact between the proton at the chiral center in **8** and the enzyme backbone. These two factors may account for the lower FXa affinity of **8**.

Table 1. Bisbenzamidine Compounds



(I)

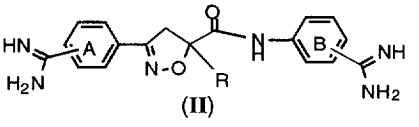
Ex.	A	B	[a]FXa K_i (μM)	[a]Thrombin K_i (μM)	[a]Trypsin K_i (μM)
5	*p*	*p*	1.7	2.2	not tested
6	*p*	*m*	0.87	2.2	0.51
7	*m*	*m*	1.8	11.2	>1.2
8	*m*	*p*	1.4	11.1	not tested

[a]See ref 5 for assay conditions.

To further improve FXa potency, it was postulated that a functionality such as CO_2R might be able to form a hydrogen bond with Tyr^{99} or Gln^{192} of the enzyme. Modeling studies suggested a substituent α to the carbonyl could be favorable. As shown in Table 2, substitution at the chiral center did indeed improve affinity for FXa. Carboxymethyl substitution provided a twofold improvement in FXa activity (**10**) as compared with compound **9**, while carbomethoxymethyl substitution increased the FXa affinity by threefold (**11**). Compound **12**, bearing a methyl glycinamide linkage, improved the potency by 15-fold (K_i = 18 nM). Molecular modeling suggested that the carbonyl oxygen in compounds **10** and **11** forms a weak hydrogen bond with the OH group of Tyr^{99} of FXa and this likely contributes to the increase in binding affinity for these two compounds. The same carbonyl oxygen in **12** does not participate in hydrogen bonding. However, the carbonyl oxygen from the ester group is well positioned to form a strong hydrogen bond with the OH group of Tyr^{99} of FXa. Figure 1 shows the comparison of the two compounds modeled in the FXa active site.

The position of the amidine group was also investigated. It was found to be preferable to have a *meta*-amidine substituted at one phenyl ring and a *para*-substituted at the other. Similar FXa affinity was observed with **11** and **13**. Molecular modeling showed that the two compounds might have different binding modes. In both cases, the *meta*-benzamidine fits into the S_1 pocket better than the *para*-benzamidine, whereas the *para*-benzamidine fits into the aryl binding site better than the *meta*-benzamidine. Figure 2 shows **11** and **13** modeled in the FXa active site with their *meta*-benzamidine moieties docked in the S_1 pocket. In compound **11**, the carboxamide oxygen forms a weak hydrogen bond with the NH of Gly^{219}, and the carbonyl oxygen of the ester side-chain also forms a weak hydrogen bond with the OH group of Tyr^{99} of FXa. In compound **13**, the carboxamide oxygen is capable of forming a stronger hydrogen bond with the NH of Gly^{216}, while the carbonyl oxygen of the ester side-chain does not appear to participate in hydrogen bonding but does fill up the space available in this region.

Table 2. Bisbenzamidine Compounds



(II)

Ex.	A	B	R	[a]FXa K_i (μM)	[a]Thrombin K_i (μM)	[a]Trypsin K_i (μM)
9	*m*	*p*	H	0.27	16	0.70
10	*m*	*p*	CH_2CO_2H	0.143	>21	>1.2
11	***m***	***p***	**$CH_2CO_2CH_3$**	**0.094**	**16**	**0.48**
12	***m***	***p***	**$CH_2CONHCH_2CO_2CH_3$**	**0.018**	**3.1**	**0.42**
13	*p*	*m*	$CH_2CO_2CH_3$	0.117	14.1	>1.2
14	*m*	*m*	$CH_2CO_2CH_3$	0.8	11.8	0.23

[a]See ref 5 for assay conditions.

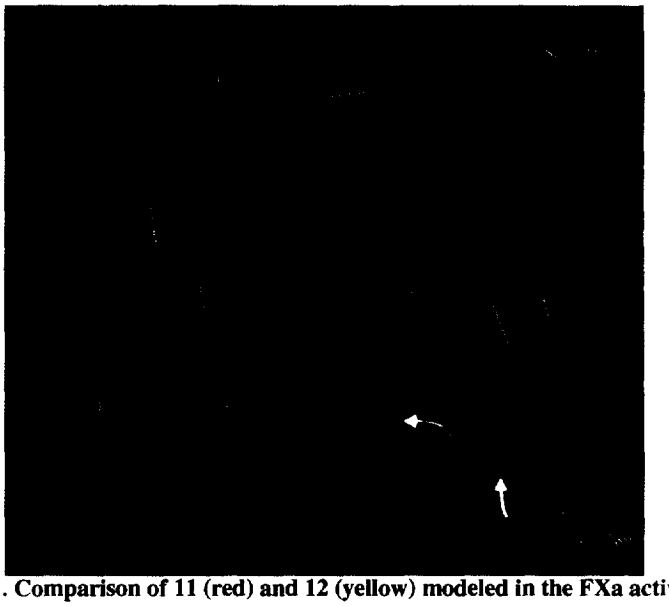


Figure 1. Comparison of 11 (red) and 12 (yellow) modeled in the FXa active site.

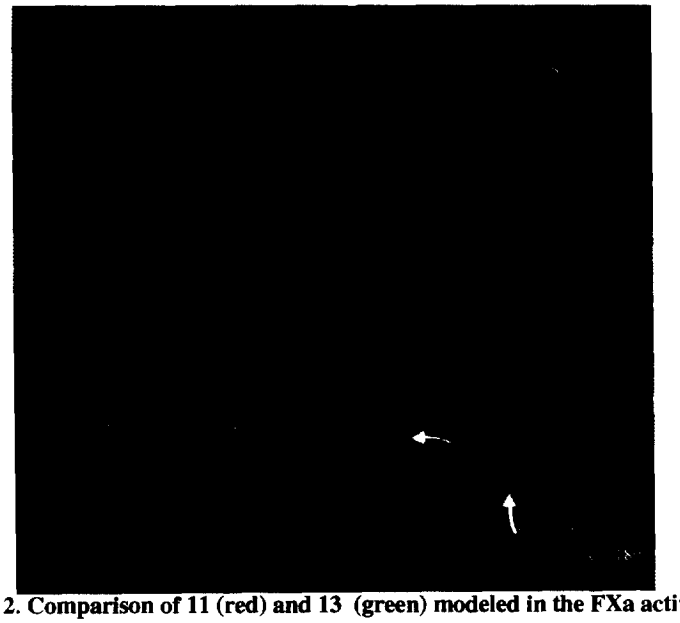
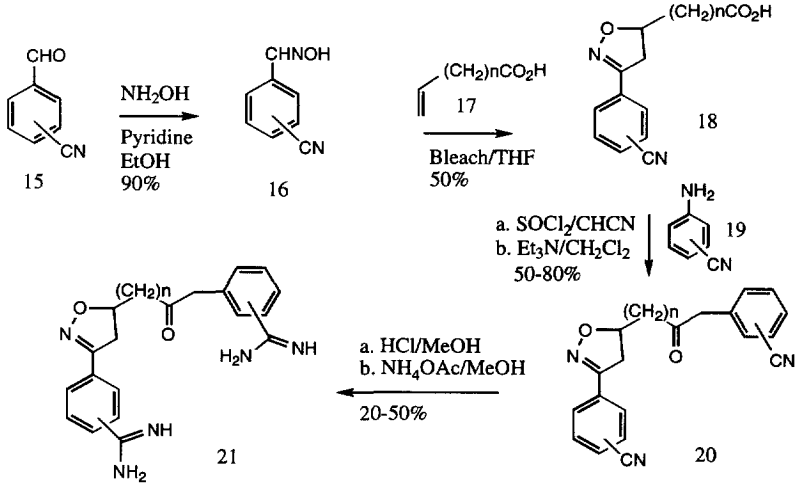


Figure 2. Comparison of 11 (red) and 13 (green) modeled in the FXa active site.

Compound **11** when dosed via intravenous infusion at 1 mg/kg/h and 5 mg/kg/h in a rat vena cava thrombosis model[6] produced 40% and 80% inhibition of thrombus formation, respectively. The ID_{50}, the dose which produced 50% inhibition of thrombus formation, was determined to be 1.6 mg/kg/h in this model. The duration of action of **11** was studied in rat by an ex vivo anti-FXa activity assay. A 2 mg/kg sample was injected by iv bolus. Blood samples were withdrawn before injection and at 30 minute intervals after injection up to 4 h. Compound **11** was extracted by precipitation, evaporation, and reconstituted anti-FXa activity assayed. Activity was compared to spiked plasma sample or by direct assay of the compound concentration by ELISA. The half life of **11** in the rat was found to be 69 minutes.

All the compounds reported herein are racemic. Compounds listed in Table 1 were prepared as shown in Scheme I. Cyanobenzaldehyde **15** was converted to the corresponding oxime **16**. The oxime was oxidatively chlorinated and then dehydrochlorinated to generate the nitrile oxide in situ, which underwent 1,3-dipolar cycloaddition with an alkene to form the isoxazoline **18**. The acid **18** was then converted to the acyl chloride, which reacted with cyanoaniline **19** to give the amide **20**. The biscyano-compound **20** was transformed to the bisbenzamidine **21** under standard Pinner conditions.[7]

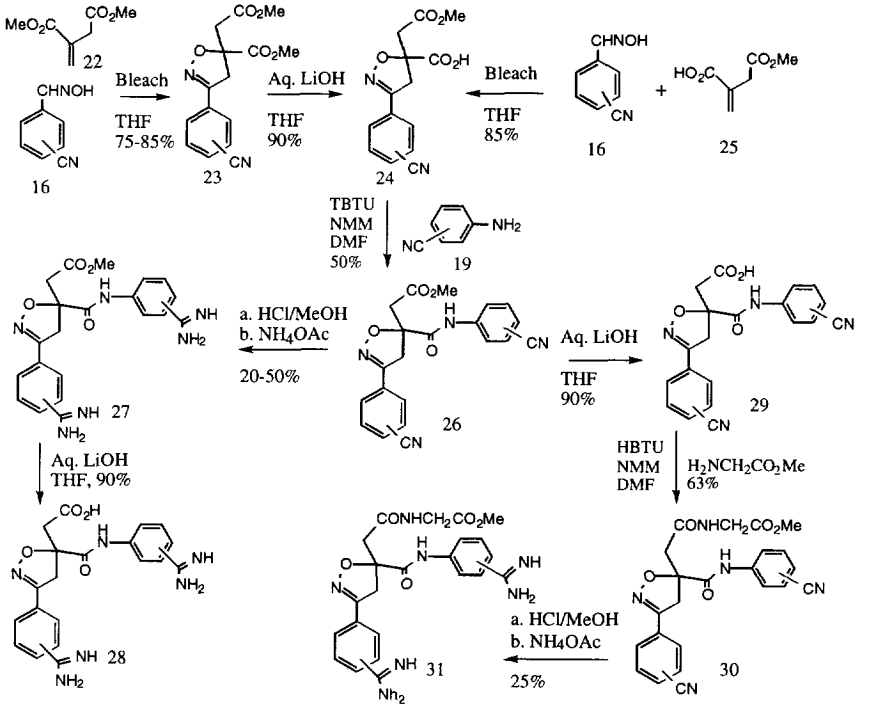
Scheme I



Compounds listed in Table 2 were prepared as shown in Scheme II. Isoxazoline **24** could be prepared either from dimethyl itaconate **22** or monomethyl itaconate **25** as shown. Compound **24** was then coupled with cyanoaniline **19** to give the bisnitrile **26**. A Pinner reaction gave bisbenzamidine **27**. Hydrolysis of **27** afforded the bisbenzamidine-acid **28**. Hydrolysis of compound **26** yielded acid **29**, which was then coupled with glycine methyl ester to afford compound **30**. The bisbenzamidine **15** was obtained using standard Pinner conditions.

In conclusion, we have designed and synthesized a series of bisbenzamidine isoxazoline compounds. These compounds are potent FXa inhibitors with the most potent compound exhibiting a K_i value of 18 nM for human factor Xa. These compounds also showed efficacy in a rat vena cava thrombosis model.

Scheme III



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