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PREPARATION OF PYRROLIDINE AND ISOXAZOLIDINE BENZAMIDINES AS POTENT INHIBITORS OF COAGULATION FACTOR Xa

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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 pyrrolidine and isoxazolidine benzamidines as novel and 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 which 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 to form a fibrin blood clot. Direct small molecule thrombin inhibitors have been intensely investigated as potential antithrombotic agents.[1] Direct small molecule fXa inhibitors are increasingly being reported, representing another attractive strategy for the discovery of novel antithrombotic agents.[2]

Recent reports from our laboratories have shown that isoxazoline benzamidines such as **1**[3] and *meta*-amidino-*N,N*-disubstituted anilines such as **2**[4] are potent inhibitors of fXa (Figure 1). These inhibitors differ only in the nature of the core template linking the benzamidine and biphenylsulfonamide residues. As part of an effort to introduce further structural diversity into our fXa inhibitor program, inhibitors containing additional core heterocycles were prepared. Here we report fXa inhibitors **3** based on a pyrrolidine (Z = CH_2) or isoxazolidine (Z = O) core template.

Compounds **3** wherein R or R' represent an ester residue can be viewed as ring-constrained analogs of compound **2**. We had conceived of the pyrrolidine series (**3**, Z = CH_2) knowing that these compounds were readily accessible via [3+2] azomethine ylide-olefin cycloaddition. Molecular modeling[5,6] of **3** (R = CO_2Me, R' = H) was performed in a model of the fXa active site. The amidine was expected to engage in a bidentate interaction with Asp189 in the S1 specificity pocket while the biphenylsulfonamide was expected to reside in the S4 aryl binding pocket, as we have observed in related fXa inhibitors.[3,4] The ester group was designed to interact with Arg143, Gln192 or Gly218. Molecular modeling of the pyrrolidine series revealed that one hydrogen of the ring methylene group of the pyrrolidine had a close contact with the backbone NH of Gly216 of fXa. The isoxazolidine series (**3**, Z = O) was designed to remove this steric liability.

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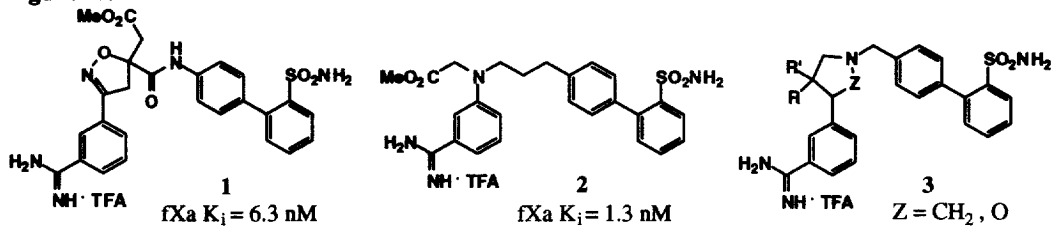
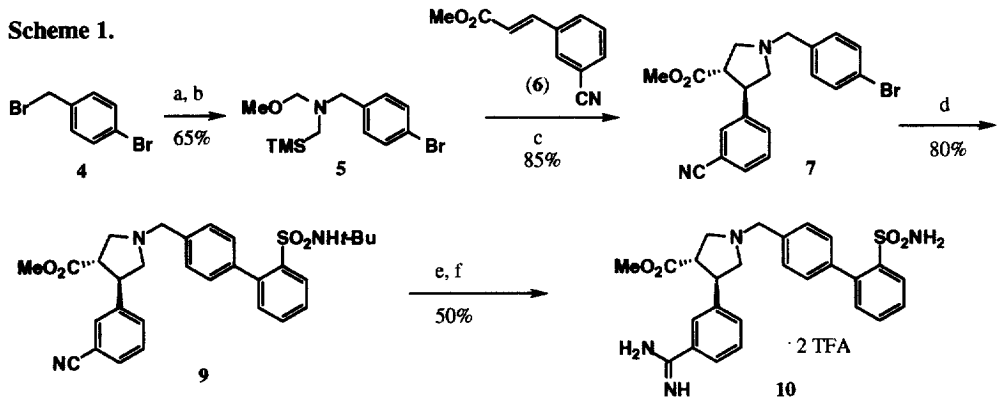
Figure 1.



A [3+2] cycloaddition strategy for preparing the pyrrolidine series of inhibitors is shown in Scheme 1. The benzyl bromide **4** was treated with trimethylsilylmethylamine and then with aqueous formaldehyde in methanol to afford the azomethine ylide precursor **5**.[7,8] An equimolar mixture of **5** and readily available cinnamate **6** was treated with a catalytic amount of TFA to effect a smooth [3+2] azomethine ylide-olefin cycloaddition[9] to give racemic pyrrolidine **7**, in which the *E*-olefin geometry has been conserved to produce the *trans* relationship between the ester and aryl residues. The aryl bromide served as a convenient handle for introducing the biaryl residue. Suzuki coupling of **7** with 2-(*tert*-butylaminosulfonyl)phenyl boronic acid **8**[3] afforded the biphenyl **9** in good yield. Pinner reaction to convert the nitrile to an amidine was followed by TFA-catalyzed deprotection of the sulfonamide group to give racemic **10**, which was determined to be a potent inhibitor of fXa (K_i = 4.8 nM).

Scheme 1.

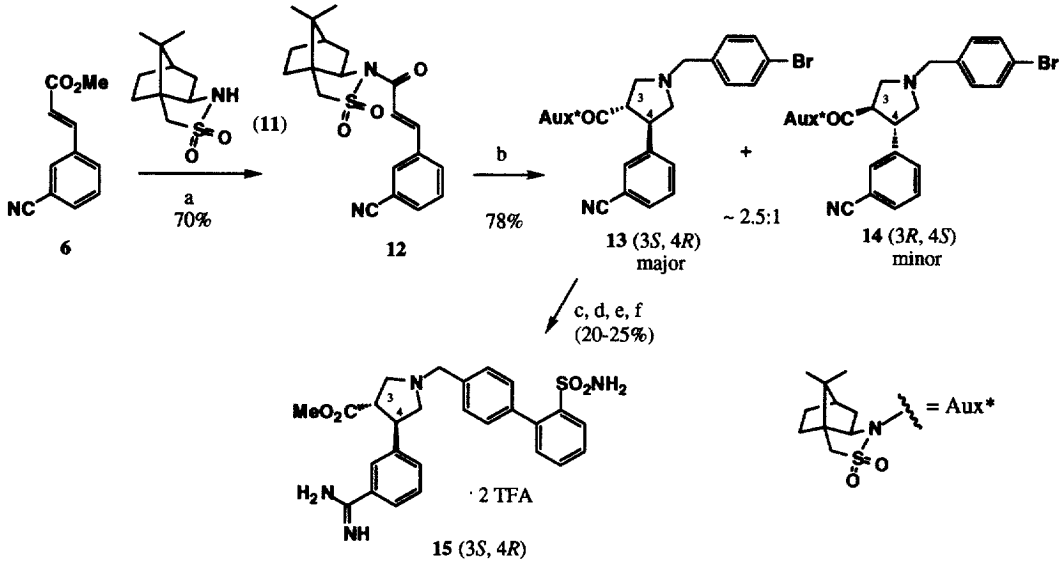


Reagents: (a) $TMSCH_2NH_2$, $NaHCO_3$, THF (b) 37% aq CH_2O, MeOH, 0 °C (c) **6**, TFA (0.1 equiv), CH_2Cl_2 (d) 2-(*t*-BuNHSO$_2$)$C_6H_4B(OH)_2$ (**8**), $(Ph_3P)_4Pd$, Na_2CO_3, H_2O, benzene, 80 °C (e) HCl, MeOH, 0 °C ; then $(NH_4)_2CO_3$, MeOH (f) TFA, reflux

Both enantiomers of the racemate **10** were prepared to compare their fXa affinities and to obtain additional binding information which might be useful in the further design of new fXa inhibitors. An asymmetric synthesis of the (3*S*,4*R*) enantiomer **15** is shown in Scheme 2. The cinnamate **6** was treated with the aluminum reagent derived from (1*S*)-(-)-2,10-camphorsultam **11** to afford **12** in good yield. The asymmetric [3+2] cycloaddition reaction of **12** with the azomethine ylide derived from amine **5** produced a separable mixture of diastereomeric pyrrolidines, albeit in a disappointing diastereomeric ratio, with the major product **13** having the desired (3*S*,4*R*) stereochemistry.[10] Suzuki coupling of **13** with the boronic

acid **8**, lithium hydroxide removal of the chiral auxiliary, and the Pinner/deprotection sequence as described in Scheme 1 gave the (3*S*,4*R*) enantiomer **15**, which was determined to be of >99% ee, as determined by chiral HPLC analysis. The identical sequence of reactions was performed beginning with (1*R*)-(+)-2,10-camphorsultam to afford the (3*R*,4*S*) enantiomer **16**, also in >99% ee.

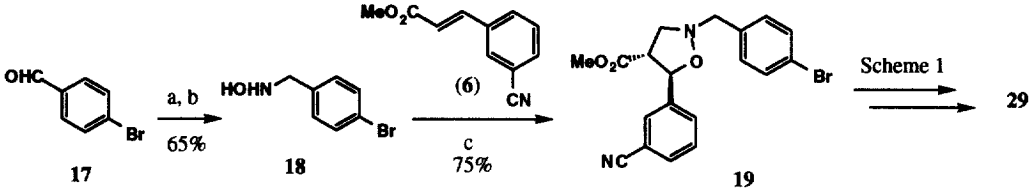
Scheme 2.



Reagents: (a) **11**, $AlMe_3$, toluene (b) **5**, TFA (0.1 equiv), CH_2Cl_2 (c) 2-(*t*-$BuNHSO_2$)$C_6H_4B(OH)_2$ (**8**), $(Ph_3P)_4Pd$, Na_2CO_3, H_2O, benzene, 80 °C (d) LiOH · H_2O, THF/H_2O (e) HCl, MeOH, 0 °C; then $(NH_4)_2CO_3$, MeOH (f) TFA, reflux

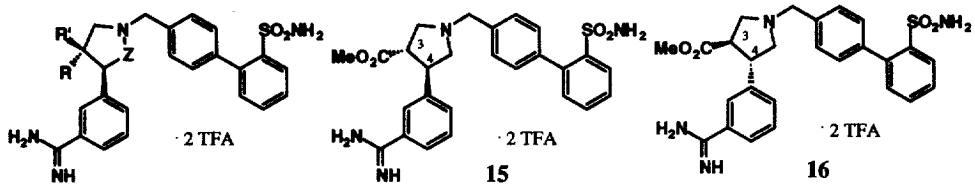
The isoxazolidine core compounds were prepared by a [3+2] nitrone-olefin cycloaddition strategy as shown in Scheme 3. The nitrone precursor, hydroxylamine **18**, was efficiently prepared from aldehyde **17** by reduction of its derived oxime with borane pyridine complex. Heating **18** and cinnamate **6** in the presence of paraformaldehyde and crushed molecular sieves afforded racemic **19** as the only [3+2] cycloaddition product, in accord with literature precedent.[11] Suzuki coupling of **19** and Pinner reaction as described in Scheme 1 led straightforwardly to the isoxazolidine **29**.

Scheme 3.



Reagents: (a) $HONH_2$ · HCl, NaOH, EtOH (b) pyr · BH_3, MeOH, 1N HCl (c) $(CH_2O)_n$, crushed 4 Å mol. sieves, benzene, 80 °C

Table 1.



Compd[a]	R	R'	Z	fXa K_i (nM)[b]	trypsin K_i (nM)[b]
10	-CO_2Me	H	CH_2	4.8	63
15 (3*S*,4*R*)	-CO_2Me	H	CH_2	3.4	52
16 (3*R*,4*S*)	-CO_2Me	H	CH_2	13	200
20	-CO_2H	H	CH_2	600	-
21	H	-CO_2Me	CH_2	170	>1600
22	H	-CO_2H	CH_2	460	-
23	-$CONH_2$	H	CH_2	150	500
24	-$CONMe_2$	H	CH_2	400	-
25	-CO_2*i*-Pr	H	CH_2	5.8	98
26	-$COCH_3$	H	CH_2	36	200
27	-CO_2*n*-Bu	H	CH_2	12	110
28	-CO_2Et	CH_3	CH_2	26	750
29	-CO_2Me	H	O	3.1	69
30	-CO_2*i*-Pr	H	O	1.8	93

a. All compounds are racemic unless otherwise noted.
b. Enzyme inhibition assays were performed as described in reference 12. All values are averages from at least duplicate experiments.

Binding data for **10**, the enantiomers **15** and **16** and several additional pyrrolidine and isoxazolidine fXa inhibitors is presented in Table 1. Most of the analogs are replacements for the methyl ester substituent of **10**. This approach was predicated by the observation that **10** was ineffective in a rabbit arterio-venous (A-V) shunt thrombosis model[13] (ID_{50} > 1.4 μmol/kg/h), a result which did not appear to correlate with its potent fXa affinity. A possible explanation for this result is that in vivo ester hydrolysis was occurring to produce the acid **20**, which we had subsequently determined to be two orders of magnitude less potent than **10**. Our goal was to replace the methyl ester with a nonhydrolyzable or less hydrolyzable substituent while maintaining potent fXa activity.

The results in Table 1 show that **15** is the most potent enantiomer of **10**, although there is not a dramatic difference in affinity between the two enantiomers. Stereochemistry is important in this series, with the cis-pyrrolidine ester **21** being 35-fold less potent than **10**. All replacements for the methyl ester of **10** led to a loss in potency, especially for the carboxylic acid **20** and the amides **23** and **24.** The bulkier esters **25** and **27** were the best tolerated substitutions, while the nonhydrolyzable methyl ketone **26** and the α-substituted ester **28** were about an order of magnitude less potent than **10**. In general, these results parallel those we had observed while concurrently exploring ester modifications of the acyclic series represented by **2**.[4] The isoxazolidine methyl ester **29** was about equipotent to **10** while, in contrast to the pyrrolidine series, the *iso*-propyl ester afforded a modest increase in potency and resulted in **30**, the most

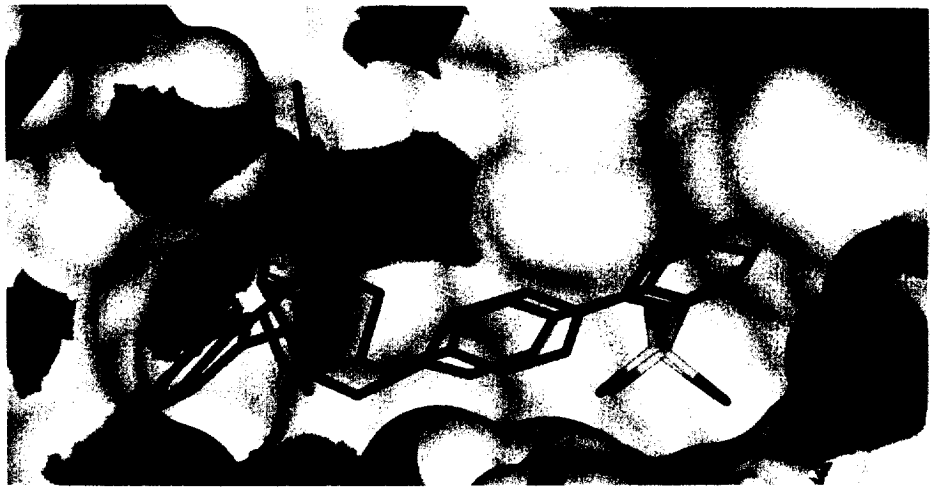


Figure 2. Molecular modeling conformations of the enantiomers **15** (purple) and **16** (green) shown in a Connolly surface representation of the fXa active site (red = oxygen, blue = nitrogen, white = all other atoms).

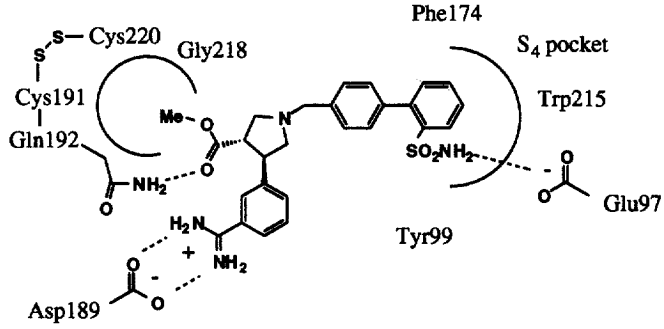


Figure 3. Schematic representation of molecular modeling interactions of **15** in the fXa active site.

potent fXa inhibitor from this series. The analogs **20**, **22**, **23-25**, **27**, and **30** were prepared by straightforward modifications of the methyl ester residue. The cis-pyrrolidine **21**, the methyl ketone **26**, and the α-substituted ester **28** were prepared by the [3+2] cycloaddition strategy of Scheme 1 using the appropriate olefins (corresponding to **6**), which were prepared by standard methods.

The two enantiomers **15** and **16** were modeled into the active site of fXa (Figure 2) in an attempt to rationalize some of the results from Table 1. The two enantiomers model similarly, except for the orientation of the pyrrolidine core, which does not appear to be involved in any specific interactions with fXa. In both compounds, the benzamidine projects into the S_1 pocket to interact in a bidentate fashion with Asp189 and the biphenylsulfonamide resides in the S_4 aryl binding pocket, bordered by Phe174, Trp215, Glu97 and Tyr99. The sulfonamide NH_2 can form a hydrogen bond with the carboxylate of Glu97. The methoxy of the ester residue of both compounds resides in a hydrophobic cleft formed by Gln192, Cys191-S-S-Cys220 and Gly218. The ester carbonyl can form a hydrogen bond with the Gln192 NH_2 while the methoxy group can stack against the side chain methylene groups of Gln192. These interactions are represented for **15** in

schematic form in Figure 3. The methoxy residue of **15** is more deeply buried in the cleft than that of **16**, which might in part explain the higher binding affinity of this enantiomer. This positioning of the methoxy residue might also help to rationalize other results from Table 1. The hydrophobic cleft would tolerate other nonpolar analogs **25-28** and **30** fairly well, while the lower affinity observed for the acid **20** and the amides **23** and **24** might result from less favorable binding of these polar groups in the hydrophobic cleft.

In conclusion, we have designed and synthesized pyrrolidines and isoxazolidines as potent inhibitors of coagulation fXa. These compounds have a narrow SAR, in which an ester group is required for potent activity. The pyrrolidine methyl ester **10** had poor antithrombotic efficacy, probably due to in vivo ester hydrolysis to the acid **20**, and these compounds appear to suffer from high iv clearance rates as well.[14] Subsequent to this work, modifications of the heterocyclic core have led to compounds with greatly improved fXa affinity and antithrombotic efficacy, a broader SAR and improved pharmacokinetics. These results will be the subject of future publications.

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References and Notes

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13. 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.
14. Compounds **25** and **30** were evaluated in a rabbit pharmacokinetic model and both were found to have clearance rates of greater than 2.0 L/h/kg and half-lives ($t_{1/2}$) of less than 1h. In this model, rabbits were infused with 10 mg/kg/h of compound and blood samples were taken prior to, during and up to 4h 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. The high clearance observed for **25** and **30** precluded any further in vivo testing on these compounds.