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Isoxazolines and Isoxazoles as Factor Xa Inhibitors†

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Abstract—3,4,5-Trisubstituted isoxazolines (**2**) and isoxazoles (**3**) were prepared and evaluated for their in vitro and in vivo antithrombotic efficacy. They were compared to 3,5,5-trisubstituted isoxazolines (1) for Factor Xa selectivity and potency. They were also compared in an arterio-venous (A-V) shunt model of thrombosis.

Factor Xa (fXa) catalyzes the production of thrombin from prothrombin and sits at the junction of the intrinsic and extrinsic pathways of the coagulation cascade.[1] It has recently been suggested that fXa inhibitors may be more effective as antithrombotic agents than direct inhibitors of thrombin and may have less bleeding risk, leading to a better safety-efficacy ratio.[2]

We have previously published on isoxazoline bisamidine inhibitors of fXa.[3] Further optimization of this series resulted in the isoxazoline-5-carboxamides (**1**).[4] These compounds are very potent inhibitors of fXa, selective against other serine proteases and have been shown to inhibit thrombus formation as effectively as thrombin inhibitors. It was our desire to discover what aspects of the isoxazoline core were necessary for activity. A recent report from our laboratories has shown pyrrolidine and isoxazolidine benzamidines to be potent inhibitors of fXa.[5] In this paper we describe our successful efforts to improve activity by changing the point of attachment of the amide moiety and subsequently aromatizing the isoxazoline ring, eliminating all chiral centers.

Table 1 shows a comparison of the fXa inhibition for the 3,4,5-trisubstituted isoxazolines (**2**) relative to the 3,5,5-trisubstituted isoxazolines (**1**).[4] For two pairs of entries, the methyl (**4**) and methoxymethyl (**6**) derivatives show improved fXa inhibition relative to their 3,5,5-trisubstituted isomers. The tetrazoylmethyl derivatives (**8** and **9**) appear to be roughly equivalent in potency. All the compounds are selective inhibitors of fXa relative to thrombin and trypsin. We believe that H-bond acceptors at the 5-position of the regioisomeric isoxazolines (**1** and **2**) enhance potency.

The isoxazole in Table 2 may have different requirements for substituent R because of its flat structure. Charged amino (**10**) and bulky trifluoromethyl (**11**) groups are not well tolerated. Methoxymethyl (**12**) and hydroxymethyl derivatives (**13**) show much greater potency for fXa. Remarkably, the unsubstituted isoxazole ring (SA862) provides the greatest improvement in potency. We believe that with an isoxazoline core, the R group hydrogen bonds to the enzyme and orients the amide bond. With the isoxazole core, this influence on the amide bond may be unnecessary and the R group may be unable to obtain an optimal hydrogen bond to the enzyme.

Molecular models of the isoxazolines and isoxazoles in the active site of the enzyme were compared using Insight II[6] and a model of fXa based on the X-ray crystal structure of fXa determined by Tulinsky.[7] Both molecules have bidentate hydrogen bonds and electrostatic binding to the Asp189 residue. Since these molecules do not interact with Ser 195, this interaction is crucial for activity. Compound **7**, in magenta, and SA862, in white, take different pathways to the S4 region of the active site with SA862 more closely approaching the solvent accessible surface near Tyr99 and Gly216. This closer approach to the enzyme may explain the enhanced potency of SA862. Each molecule is able to position the terminal phenyl ring for a strong edge-to-face interaction with Trp215, a consistent feature

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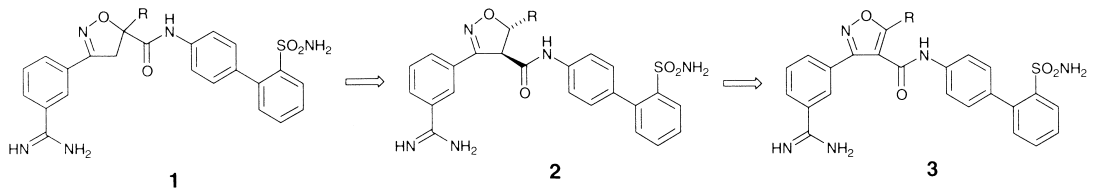
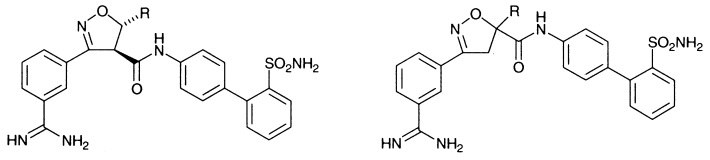


Table 1. Comparison of the fXa activity of isoxazoline 4- and 5-carboxamides



Compound	Structure	R	fXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)
4	A	Me	1.8	4500	110
5[3]	B	Me	11.0	5800	120
6	A	CH_2OMe	0.55	4600	190
7[3]	B	CH_2OMe	3.4	2400	70
8	A	CH_2-tetrazole	2.3	5700	180
9[3]	B	CH_2-tetrazole	1.6	900	91

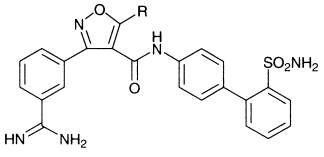
of all our inhibitors. The overall modeled orientation of SA862 is maintained in the X-ray crystal structure determined in bovine trypsin. However, the amide bond is not in conjugation with the isoxazole ring and is oriented towards His57 rather than Gly216 in the model (Figs. 1 and 2).

The 3,5,5-trisubstituted isoxazolines showed enhanced fXa potency when the internal phenyl ring was replaced with a pyridyl ring.[4] Table 3 describes the results of these same changes with the isoxazole core. Unlike the 3,5,5-trisubstituted isoxazolines, the phenylpyridine (**14**) does not improve fXa potency relative to the biphenyl (**12**). However, as with the 3,5,5-trisubstituted isoxazolines,[4] replacing the sulfonamide (SA862) with a methyl sulfone (**15**) resulted in a compound with similar potency.

In a pharmacokinetic comparison of the 3,5,5-trisubstituted isoxazolines with the isoxazoles, compounds were administered to dogs intravenously and their profile was observed (Table 4). The two isoxazoles tested had higher clearance and volumes of distribution than the representatives from the isoxazoline class. SA862 had a significantly longer half-life than the other three compounds tested.

In the rabbit A-V shunt model of thrombosis (Table 5), the isoxazoles were compared to the 3,4,5- and 3,5,5-trisubstituted isoxazolines. In this model,[8] a plastic tubular shunt, containing a thrombogenic silk thread, is placed between the femoral artery and vein. After 40 min, the resulting clots are removed and their weights determined. An ID_{50} can be derived by determining the doses required to diminish the clot size by 50%. These data were compared to the relative fXa K_i in rabbit. The potencies of each of the inhibitors for fXa in the rabbit blood were within a 2-fold window. This explains the

Table 2. SAR of isoxazole 4-carboxamides



Compound	R	fXa K_i (nM)	Thrombin K_I (nM)	Trypsin K_i (nM)
10	NH_2	3.4	4000	117
11	CF_3	9.5	11000	400
12	CH_2OMe	0.63	3800	76
13	CH_2OH	0.50	1900	50
SA862	H	0.15	2000	21

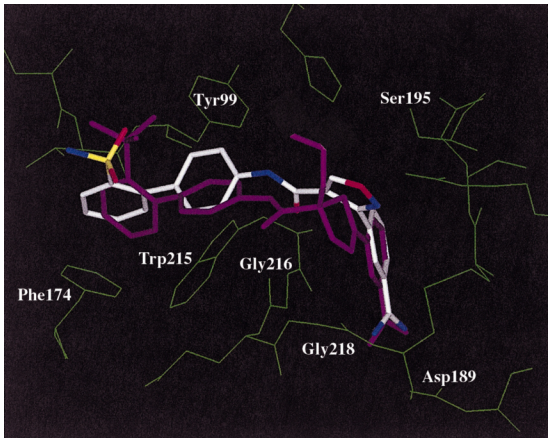


Figure 1. Modeling of **7** vs SA862 in factor Xa.

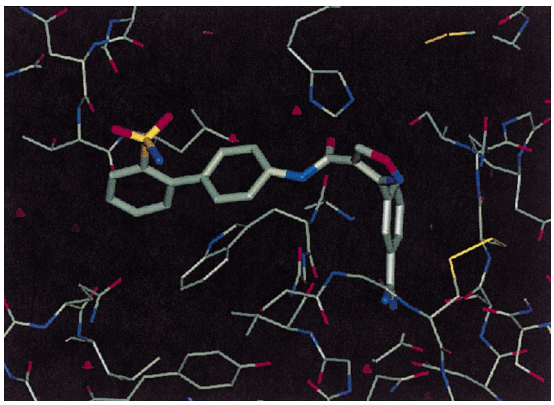


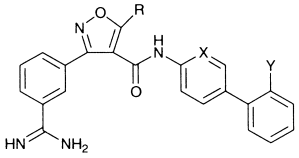
Figure 2. SA862 X-ray structure in bovine trypsin.

tight range of activity seen in the A-V shunt model, implying that each of these compounds is a potent antithrombotic.

Scheme 1 shows the synthetic route used to prepare the 3,4,5-trisubstituted isoxazolines. The oxime of the commercially available 3-cyanobenzaldehyde was chlorinated[9] and the crude product reacted with a 3-substituted acrylate. This [3 + 2] cycloaddition determined the relative stereochemistry.[3] Trimethylaluminum promoted amide bond formation[10] was followed by amidine formation via the methyl imidate.[11]

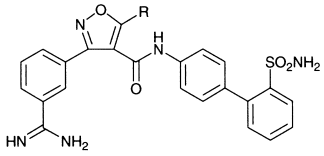
In Scheme 2, the general synthetic route to the isoxazoles is outlined. A β-ketoester was initially reacted

Table 3. Substituent effects on biphenyl system of isoxazole

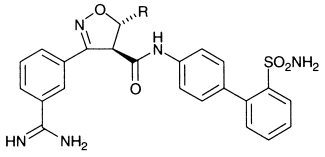


Compound	R	X	Y	fXa K_i (nM)	Thrombin K_i (nM)	Trypsin K_i (nM)
12	CH_2OMe	H	SO_2NH_2	0.63	3800	76
14	CH_2OMe	N	SO_2NH_2	0.75	12000	200
SA862	H	CH	SO_2NH_2	0.15	2000	21
15	H	CH	SO_2Me	0.10	540	25

Table 4. Isoxazole versus isoxazoline[a] dog pharmacokinetic data



A

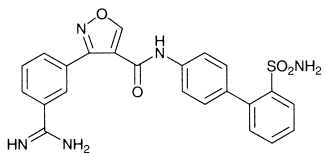


B

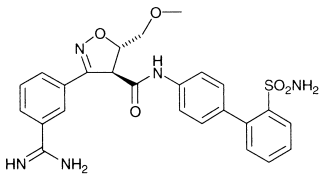
Compound[b]	Structure	R	fXa K_i (nM)	CL (L/h/kg)	$t_{1/2}$ (h)	Vdss (L/g)
12	A	CH_2OMe	0.63	1.7	1.2	0.85
SA862	A	H	0.15	1.5	4.3	1.7
6	B	CH_2OMe	0.55	0.99	1.2	0.38
8	B	CH_2-tetrazole	2.3	0.49	1.0	0.24

[a]All isoxazolines are racemic.
[b]Compounds infused at 1 mg/kg/h iv.

Table 5. Comparison of the rabbit A-V-shunt antithrombotic effect



A



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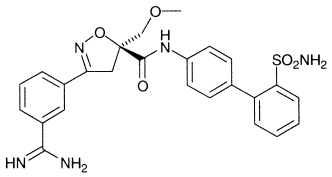
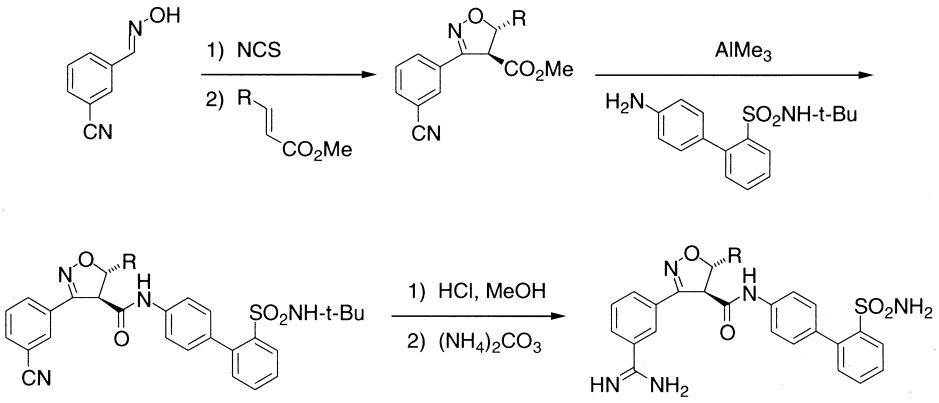


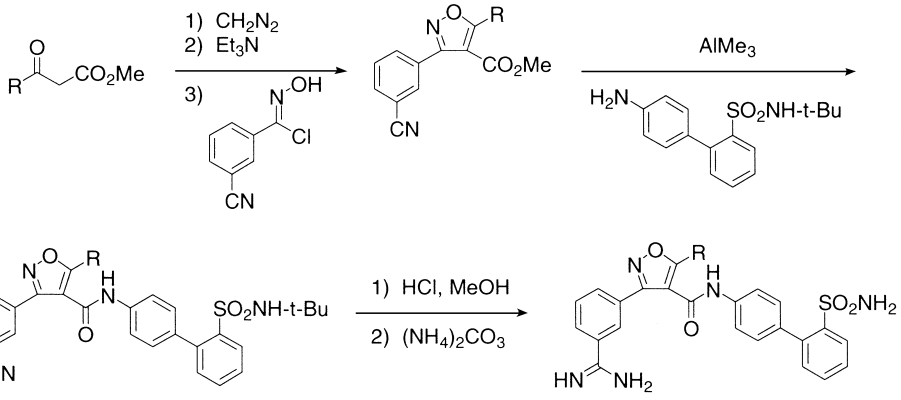
C

Compound	Structure	Human fXa K_i (nM)	Rabbit fXa K_i (nM)	ID_{50}[a] (μmol/kg/h)
SA862	A	0.15	0.40	0.31
6	B	0.55	0.64	0.21
7[3b]	C	3.4	0.80	0.26

[a]ID_{50}s' were determined as described in refs 4a,b.



Scheme 1. General synthesis of isoxazolines.



Scheme 2. General synthesis of isoxazoles.

with diazomethane,[12] followed by triethylamine and the chlorooxime (formed from the NCS reaction with the oxime in Scheme 1) to provide the appropriate regioisomeric isoxazole in moderate yield.[13] Amide bond formation promoted by trimethylaluminum and amidine formation proceeded as in Scheme 1.

We have shown that 4-isoxazolinecarboxamides can result in slightly greater affinities for Factor Xa than their corresponding 5-carboxamide isomers. Changing to a planar isoxazole core further enhances affinity but substitution on the heterocycle is not well tolerated. In dogs, while the clearance was moderate, the half-life for SA862 was attractive. All series were shown to have comparable antithrombotic activity in the A-V-shunt rabbit model. Further elaboration of these findings is ongoing at DuPont Pharmaceuticals.

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