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Amido-(Propyl and Allyl)-hydroxybenzamidines: Development of Achiral Inhibitors of Factor Xa

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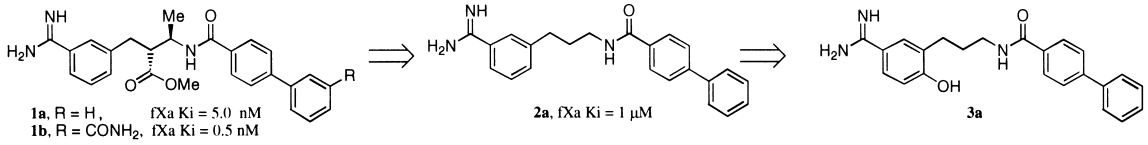
Abstract—The design, synthesis and SAR of amido-(propyl and allyl)-hydroxybenzamidine coagulation factor Xa inhibitors is described. These achiral inhibitors are selective for fXa vis a vis structurally related serine proteases and are readily prepared in 6–7 linear steps. The most potent member **9j** (fXa $K_i = 0.75$ nM) is selective (>1000-fold) and an effective anticoagulant in mammalian plasma.

The serine protease component of the prothrombinase complex, factor Xa (fXa), is the singular enzyme responsible for the conversion of prothrombin to thrombin (fIIa).[1] Inhibitors of thrombin, the terminal enzyme of the coagulation cascade,[2] have been studied for some time as potential antithrombotic agents.[3] More recently, inhibitors of fXa, the penultimate enzyme in the cascade, have been pursued as anticoagulant therapies.[4,5] A variety of factor Xa inhibitors have now been described and several comprehensive reviews have appeared recently.[4–9]

Compounds **1a** and **b** are representative of the β-amidoester series of fXa inhibitors, discovered in our laboratories.[10,11] Previously, the optimization of the biaryl group (P-4) and the structure activity relationships for the branching substituents were described. The ester function, a possible hydrolytic center, was found to be necessary for optimal activity and the *R*,*R* stereoisomer was the most active. A chiral synthesis was described which requires a diazomethane mediated homologation of an amino acid, a costly and potentially hazardous process.

Given the perceived drawbacks of the chiral series and the liabilities of the ester function, we sought to develop simpler analogues in which the branched chain is replaced by an achiral tether (e.g. **2a**). Maintaining potency appeared to be a daunting task since the simple prototype **2a** (fXa $K_i \sim 1$ μM) was much less potent[12,13] than the β-amidoester **1a** (fXa $K_i = 5.0$ nM). Several groups,[14–16] including ourselves,[17,18] have described the potency enhancing effects of incorporating hydroxyl groups *para* to the amidine attachment point of the P-1 benzamidine. This strategy was applied to the amidopropylbenzamidines as a way to increase anti-fXa activity. The development of potent fXa inhibitors based on compound **3a** is described.



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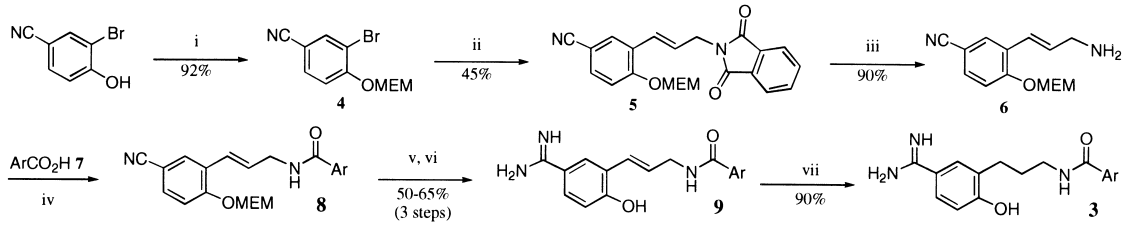
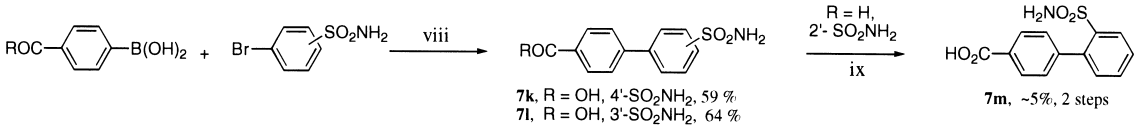


Scheme 1. Synthesis of amido-(propyl and allyl)-benzamidines **9** and **3**. (i) NaH, MEMCl, THF; (ii) AllylNPth, 1% equiv. $Pd(OAc)_2$, 2% equiv. $P(o\text{-}Tol)_3$, Et_3N; (iii) N_2H_4, H_2O, EtOH, reflux, 1 h; (iv) Et_3N, TBTU, DMF; (v) EtOH–HCl; (vi) MeOH–NH_3; HPLC; (vii) 10% Pd/C, H_2.



Scheme 2. Synthesis of biaryl acids. (viii) $Pd(PPh_3)_4$, aq. Na_2CO_3, MeCN, 90 °C; (ix) aq. $NaClO_2/NaH_2PO_4$.

Chemistry[19]

The synthesis begins with the commercially available 3-bromo-4-hydroxybenzonitrile which is protected as the MEM ether **4** (Scheme 1). Heck reaction is followed by removal of the phthaloyl group to yield the free amine **6**. At this stage a variety of carboxylic acids **7** can be coupled to give compounds **8** with a diverse set of P-4 ligands. Pinner reaction followed by ammonolysis gives the amidoallylhydroxybenzamidines **9**. Subsequent catalytic reduction yields the amidopropylhydroxybenzamidines **3**.

The aryl carboxylic acids used in this paper were commercially available (entries **a** and **c**) or prepared by standard methods. The preparation of biaryl acids **7b**, **e**, **f**, **g, h** and **i** has been previously described.[19] The acid required for entry **j** is prepared from the methyl ester of 4-(1-oxypyridin-4-yl)benzoate[19] by application of cyanotrimethylsilane;[20] subsequent acid hydrolysis[21] (85% H_2SO_4) gives the desired **7j**. Alternatively we have found the Suzuki reaction to be a general method for biaryl acid synthesis[22] (Scheme 2). For example, 4-carboxybenzene boronic acid is heated with the appropriate halogenated aryl or heteroaryl in aqueous sodium carbonate and acetonitrile, in the presence of $Pd(PPh_3)_4$. The desired acids **7k** and **l** are obtained by precipitation from the reaction medium and require no further purification. Acids **7n**[23] and **7d**[24] were prepared according to literature methods.

Results and Discussion

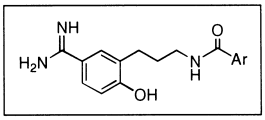
Table 1 summarizes the results for a limited set of amidopropyl hydroxybenzamidines. Note that the *para*-hydroxybenzamidine (**3a**) is 11-fold more active than the comparable unsubstituted benzamidine (**2a**). This substitution, to some degree, compensates for the absent ester function.[26] The entries illustrate the dependence of activity on the nature of the aryl group (Ar), i.e. the P-4 ligand. For example, the most potent analogue **3b** is 35-fold more effective against fXa than the imidazolylphenyl derivative **3f**. Selectivity against thrombin and trypsin varies 10–500-fold depending on the nature of the P-4 ligand.

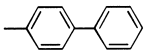
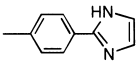
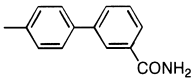
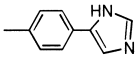
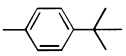
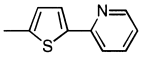
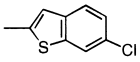
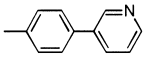
A molecular modeling study of the amidopropyl hydroxybenzamidines bound in the fXa active site was initiated in an effort to enhance the activity of this series.[27] The study suggested that incorporation of unsaturation into the chain would yield conformationally restricted analogues with potentially higher binding affinities. Compound **9a** for example makes many of the same interactions which have been shown to be important for binding of the β-amidoesters to fXa[10,11,28] (Fig. 1). These contacts include a salt bridge (benzamidine/D189), a hydrogen bond (amide carbonyl/G218) and Van der Waals interactions (biaryl/S-4). In addition, the hydroxyl group on the benzamidine is within H-bonding distance of the side chain of S195.

The allyl analogues were readily obtained (Scheme 1). A modest increase in activity was observed for **9a** versus its saturated analogue **3a** and this trend (up to 3.5-fold) was observed across a range of P-4 moieties (Table 2). Potency was markedly increased by *meta* substitution on the distal phenyl ring with a carboxamide group (**9b**). A similar enhancement was seen in the β-aminoester series.[10,11] This observation was extended to sulfonamide substituents; in this case the *ortho* derivative **9m** was found to be the most effective.[29] Note also that by judicious choice of the P-4 ligand (**9j,** fXa $K_i = 0.75$ nM) it was possible to achieve anti-fXa potency in the amidoallyl hydroxybenzamidine series comparable to the β-aminoesters (**1b,** fXa $K_i = 0.5$ nM).

The most potent inhibitor **9j** was tested against a panel of trysin-like serine proteases and shown to be selective for factor Xa (Table 3). In fact the achiral inhibitors were in general more selective for fXa than the β-amidoesters. This is illustrated by the activities of **3b** and

Table 1. Coagulation factor Xa inhibition data[25] for amidopropylhydroxybenzamidines **3**



Compound	Ar	fXa K_i (nM)	fIIa K_i (nM)	Tryp. K_i (nM)	Compound	Ar	fXa K_i (nM)	fIIa K_i (nM)	Tryp. K_i (nM)
3a		88	>4000	>2900	**3e**		58	>4000	~2900
3b		7.0	>4000	>2900	**3f**		254	>4000	~2900
3c		59	>4000	2200	**3g**		131	>4000	>2900
3d		181	~4000	>2900	**3h**		38	>4000	1900

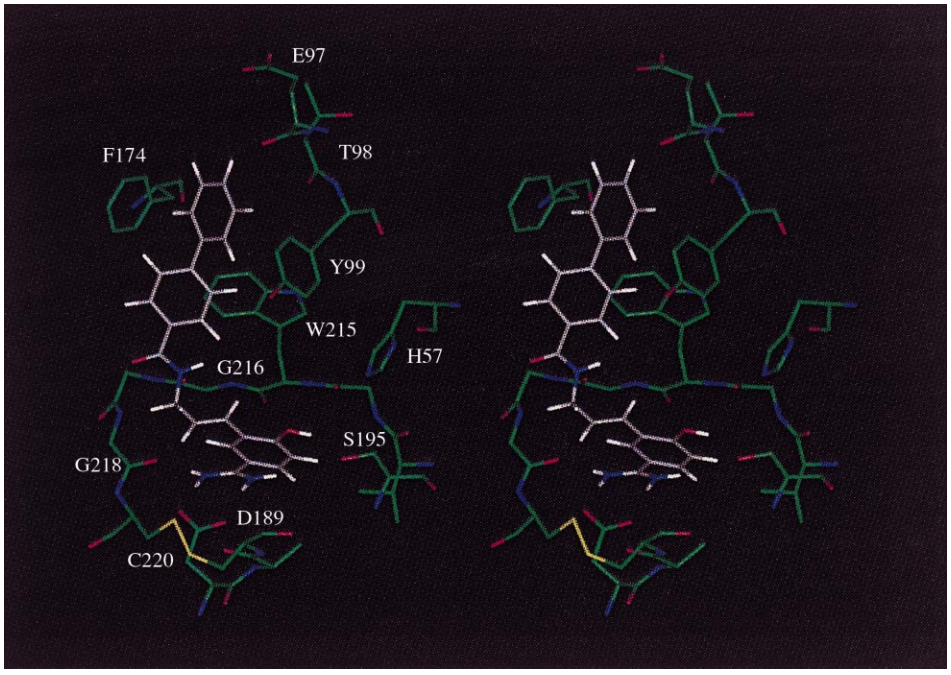


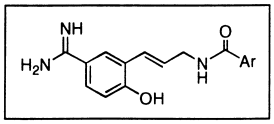
Figure 1. A stereoview of **9a** bound in the active site of factor Xa.[27]

9j as compared to compound **1b** against trypsin and plasmin.

Inhibitor **9j** was tested for its ability to prolong the activated partial thromboplastin time (APTT) in plasma from four species.[30] This assay provides a measure of the compound's ability to inhibit the prothrombinase bound factor Xa i.e. the physiologically relevant form of the enzyme. Some species differences were observed; the concentration required to double the APTT for human, dog, rabbit and rat was found to be 0.87, 0.99, 0.51 and 1.7 μM, respectively. This phenomenon has been observed with other factor Xa inhibitors and may reflect subtle structural differences in the factor Xa from different species.[31]

In summary, achiral factor Xa inhibitors have been described whose synthesis is readily achieved in 6–7 linear steps. The amidoallyl hydroxybenzamidines are nanomolar inhibitors of fXa with better than a 1000-fold selectivity against related serine proteases. The effectiveness of inhibitor **9j** against the prothrombinase complex is demonstrated by the APTT prolongation in mammalian plasma.[32]

Table 2. Coagulation factor Xa inhibition data[25] for amidoallyhydroxybenzamidines **9**



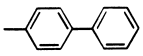
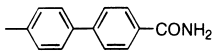
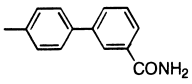
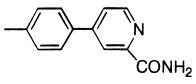
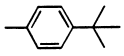
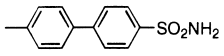
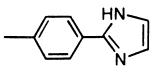
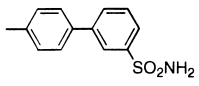
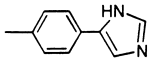
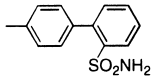
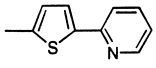
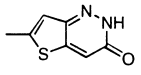
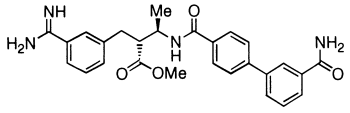
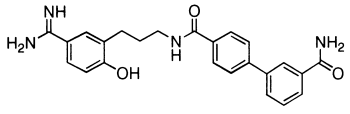
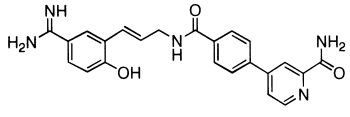
Compound	Ar	fXa K_i (nM)	fIIa K_i (nM)	Tryp. K_i (nM)	Compound	Ar	fXa K_i (nM)	fIIa K_i (nM)	Tryp. K_i (nM)
9a		51	>4000	>2900	**9i**		20	2900	1400
9b		5.0	>4000	>2900	**9j**		0.75	>4000	2200
9c		61	3200	2300	**9k**		12	>4000	1600
9e		128	>4000	1400	**9l**		9.0	>4000	240
9f		19	>4000	1800	**9m**		2.0	>4000	1500
9g		37	>4000	1400	**9n**		184	>4000	1400

Table 3. Selectivity of coagulation factor Xa inhibitors against related serine proteases[25]

	Compound	fXa K_i (nM)	fIIa K_i (nM)	Tryp. K_i (nM)	APC K_i (nM)	Plasm. K_i (nM)	tPA K_I (nM)
1b		0.50	~4000	90	>18,500	140	>8700
3b		7.0	>4000	>2900	15,000	3500	>8700
9j		0.75	>4000	2200	~18,500	>7300	7700

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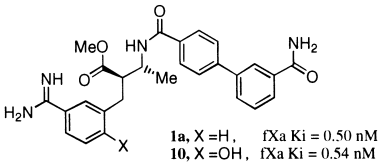

1a, X =H, fXa Ki = 0.50 nM
10, X =OH, fXa Ki = 0.54 nM

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