Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2043–2046

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Substituted Acrylamides as Factor Xa Inhibitors: Improving Bioavailability by P1 Modification†

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Received 21 January 2002; accepted 5 March 2002

Abstract—To overcome the low bioavailability of our substituted acrylamide P1 benzamidine factor Xa inhibitors reported previously, neutral and less basic groups were used to replace the benzamidine. As a result, a series of P1 aminoisoquinoline substituted acrylamide Xa inhibitors was identified to be potent, selective, and orally bioavailable. Modification of P4 moiety of these compounds further improved their pharmacokinetic properties. © 2002 Elsevier Science Ltd. All rights reserved.

Factor Xa is a serine proteinase which cleaves prothrombin to thrombin, leading to blood clot formation within the blood coagulation cascade. It is the sole enzyme responsible for activation of thrombin in the cascade. Because of its important role in blood coagulation, factor Xa has emerged as an attractive target for development of new antithrombotic agents.[2] Our research objective is to discover and develop orally active factor Xa inhibitors for treatment of thrombotic disorders.

In our previous communication, we have described a series of substituted acrylamides as potent and selective factor Xa inhibitors, as exemplified by compound **1** in Figure 1.[3] However, these compounds containing P1 benzamidine functionality suffered from low oral bioavailability. It is generally believed that the highly basic benzamidine group adversely effects bio-absorption of these compounds when they are orally administered. In order to improve their oral bioavailability, we replaced the P1 benzamidine group with neutral and less basic moieties. As a result, a series of potent, selective and bioavailable factor Xa inhibitors have been discovered. In this paper, we will discuss the structure–activity relationship (SAR) of these compounds on P1 modification as well as P4 modification. We will also report the improved pharmacokinetic properties of these compounds.

The SAR study of P1 modification is shown in Table 1. Replacement of P1 benzamidine with 3-aminophenyl and 3-hydrazinophenyl resulted in inactive compounds **2** and **3**. The more basic 3-aminomethylphenyl compound **4** gave improved potency. Compounds with neutral substituted phenyl P1 (**5**–**8**) all had micromolar inhibitory activity. Insertion of an extra carbon between the direct phenyl–vinyl linkage (**9**) increased the potency slightly. The thioamide compound **11** gave better activity as compared to the amide **10**.

As we know, Asp189 in the S1 pocket of factor Xa plays an important role in anchoring benzamidine P1 moieties by forming a salt bridge with the amidine group.[4] To take advantage of a dipole–charge interaction of a P1 group with the Asp189 carboxylate anion, we designed compound **12** containing a P1 group with a significant dipole moment due to the strong electron-withdrawing nitro group at the *para* position (shown in Fig. 2). This compound displayed enhanced activity over the desnitro compound **2** by more than 40-fold, even though its activity ($IC_{50} = 0.25\,\mu M$) needs to be improved further.

Previous literature indicated that 1-aminoisoquinoline could be used as a benzamidine surrogate.[5] In our series, the aminoisoquinoline compound **13** was shown to be quite active with an IC_{50} value of $0.014\,\mu M$, representing a

†For a preliminary account of part of this work, see ref 1.

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0960-894X/02/$ - see front matter
PII: S0960-894X(02)00304-9

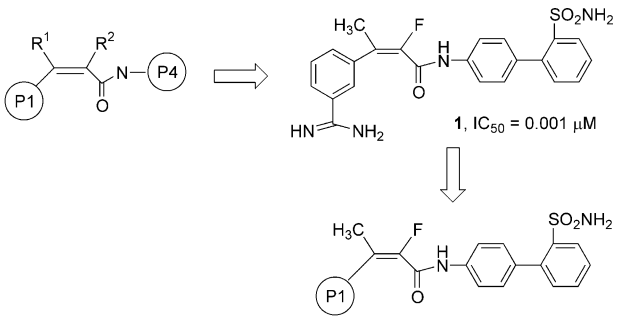


Figure 1. Replacement of P1 benzamidine with new P1 moieties.

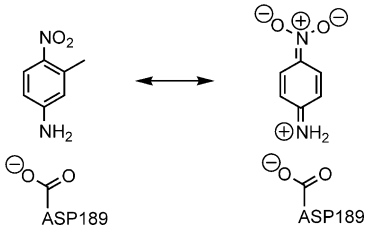


Figure 2. Dipole–charge interaction of P1 (**12**) with Asp189 in S1 pocket.

modest 14-fold reduction in potency as compared to benzamidine analogue **1**. However, the corresponding '*trans*' isomer **14** (shown in Fig. 3) was 60-fold less active than **13**, indicating that the '*cis*' geometry of P1 and P4 moieties is also highly preferred in this series. The corresponding quinazoline compound **15** was 20-fold less active than **13**, while the diaminoquinazoline compound **16** was virtually inactive. The unsubstituted cinnamamide **17** (Fig. 3) had an IC_{50} of 0.078 μM, about 6-fold less active than the methyl-fluoro analogue **13**.

After identification of compound **13**, we carried out SAR study on P4 modification as shown in Table 2. Halogen substitution at 2-position of the biphenylsulfonamide moiety gave compounds **18**–**20** with improved potency (K_i of 0.0011–0.0016 μM) over compound **13**.[6] The pyridylphenyl compound **21** was equally potent as **13**, while the 2-aminocarbonylbiphenyl compound **22** was much less active. Replacement of 2′-aminosulfonyl on the biphenyl moiety with cyano (**23**) and aminocarbonyl (**24**) resulted in reduced activity. The phenylpyridyl compound **25** was also less active as compared to **13**.[7] Although the biphenylsulfoxide **26** was inactive, the biphenylsulfone **27** was 2-fold more active than **13**.

Compounds in this series also showed high selectivity against other serine proteinases. For example, compounds **18**–**20** displayed greater than 1000-fold selectivity for factor Xa versus thrombin and trypsin (Table 2).

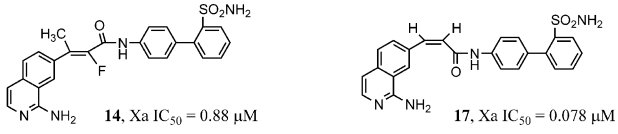
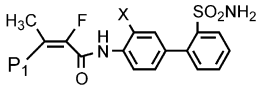


Figure 3. Compounds **14** and **17**.

Table 1. SAR of P1 variation



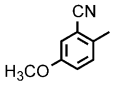
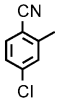
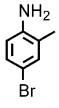
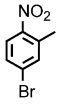
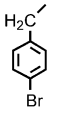
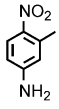
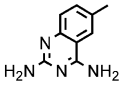
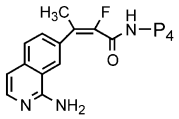
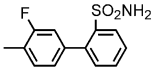
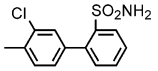
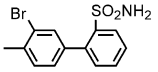
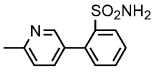
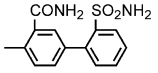
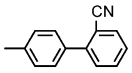
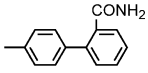
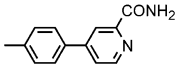
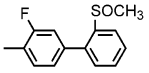
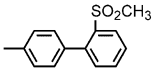
P_1	X	Compd	FXa (IC_{50}, μM)
	H	**2**	>11
	H	**3**	>11
	H	**4**	1.3
	H	**5**	5.7
	H	**6**	9.5
	Br	**7**	>11
	Br	**8**	2.9
	H	**9**	1.8
	H	**10**	0.92
	F	**11**	0.14
	H	**12**	0.25
	H	**13**	0.014
	H	**15**	0.27
	H	**16**	>11

Table 2. SAR of P4 variation



P_4	Compd	Xa	IIa (IC_{50}, μM)	Trypsin	Xa (K_i)
	**18**	0.005	6.3	9.7	0.0016
	**19**	0.011	5.4	>11	0.0011
	**20**	0.007	4.8	2.4	0.0013
	**21**	0.014			
	**22**	1.1			
	**23**	0.19			
	**24**	0.22			
	**25**	0.18			
	**26**	1.5			
	**27**	0.007			

The active aminoisoquinoline compounds **13** and **18**–**21** were found to have greatly improved bioavailabilities and other pharmacokinetic properties compared to the benzamidine analogue **1**, as shown in Table 3. In rats, compound **13** had bioavailability (F) of 6.8%, while the amidine **1** had merely 0.4%. Interestingly, the bioavailability of compounds **13**, **18**, and **19** were well correlated with the halogen substitution on the biphenylsulfonamide moiety. Their bioavailability increased with heavier halogen substitution, from hydrogen (**13**) to fluorine (**18**), to chlorine (**19**). The chloro- and bromo-substituted compounds **19** and **20** had bioavailability of 35 and 30%, respectively. They also showed greatly reduced clearance and volume distribution compared to **1**. It is worth noting that the unsubstituted cinnamamide **17** had oral bioavailability of 1.5% as compared with the methyl-fluoro-substituted counterpart **13** (F, 6.8%), indicating that the methyl-fluoro substitution on the double bond contributes positively to bioavailability. In dogs, compounds **13** and **20** also showed good oral bioavailability of 37 and 33%, respectively.

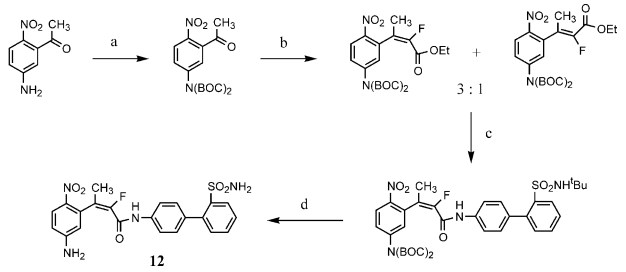
Although compounds **18**–**20** are potent in vitro with K_i of 1 nM, they showed weaker antithrombotic activity in rabbit PT assay (6.3–10.3 μM, Table 3). Not surprisingly, they were also found to be highly protein-bound in plasma (99–99.8%). We are currently modifying these compounds to reduce their plasma protein binding with the goal of enhancing their antithrombotic activity.

Syntheses of compounds of this study are exemplified in Schemes 1 and 2. To synthesize **12**, as shown in Scheme 1, the amino group of 3-amino-6-nitroacetophenone was protected with di-BOC by using $(BOC)_2O$/DMAP. Horner–Emmons reaction of the protected acetophenone with triethyl 2-fluoro-2-phosphonoacetate gave the acrylates as a mixture of *cis*/*trans* isomers in a ratio of 3:1 in favor of the desired '*cis*' isomer. After separation, the acrylate was subject to Weinreb amidation with the biphenylamine to give the acrylamide, which was then treated with trifluoroacetic acid to provide **12**.

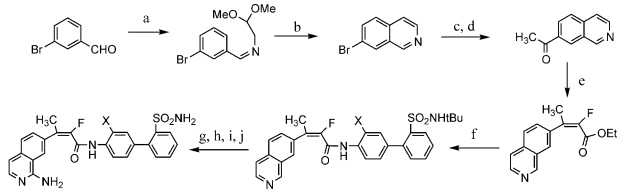
Synthesis of the aminoisoquinoline compounds **13** and **18**–**20** is shown in Scheme 2. Pomeranz–Fritsch isoquinoline synthesis starting with 3-bromobenzaldehyde provided a mixture of 7- and 5-bromoisoquinolines in a ratio of 6 to 4. Stille reaction of the bromide mixture with tributyl(1-ethoxyvinyl)tin, followed by treatment with hydrochloric acid, gave a mixture of the methyl ketone compounds. Upon separation of the desired 7-isomer by chromatography, it was condensed with triethyl 2-fluoro-2-phosphonoacetate ylide to give the acrylates as a mixture of *cis*/*trans* isomers in a ratio of 3 to 1 in favor of the desired '*cis*' isomer, which was then separated by chromatography. Weinreb amidation of the acrylate with the biphenylamine yielded the acrylamide.

Table 3. Pharmacokinetic data

Species	Compd	F (%)	Cl (mL/min/kg)	Vz (L/kg)	$T_{1/2}$ (h)	Rabbit PT (μM)
Rats	**1**	0.4	15.4	4.3	3.23	
	17	1.5	56.4	7.4	1.51	
	13	6.8	48.7	10	2.38	
	18	13.5	25.3	3.7	1.7	6.3
	19	35.1	6.7	0.8	1.43	9.4
	20	30.1	4.4	0.61	1.6	10.3
	21	13	19.1	2.7	1.64	
Dogs	**1**		26.2	13.0	5.7	
	13	36.7	11.4	6.8	7.1	
	20	33.1	10.8	5.5	6.0	



Scheme 1. Synthesis of **12**. Reagents: (a) $(BOC)_2O$, DMAP, TEA, $CHCl_3$; (b) triethyl 2-fluoro-2-phosphonoacetate, $KN(Me_3Si)_2$, THF, $-78\,^{\circ}C$; (c) biphenylamine, $AlMe_3$, CH_2Cl_2; (d) trifluoroacetic acid.



Scheme 2. Synthesis of 1-aminoisoquinoline compounds. Reagents: (a) aminoacetaldehyde dimethyl acetal; (b) concd H_2SO_4, P_2O_5, $160\,^{\circ}C$; (c) tributyl(1-ethoxyvinyl)tin, $Pd(Ph_3P)_4$, toluene, reflux; (d) H_2O/HCl; (e) triethyl 2-fluoro-2-phosphonoacetate, $KN(Me_3Si)_2$, THF, $-100\,^{\circ}C$; (f) biphenylamine, $AlMe_3$, CH_2Cl_2, X = H, F, Cl, Br; (g) mCPBA, acetone; (h) TsCl/pyridine; (i) aminoethanol, CH_2Cl_2; (j) trifluoroacetic acid.

The isoquinoline moiety was transformed to 1-aminoisoquinoline by reaction with *m*CPBA to give the *N*-oxide, which was then treated with tosyl chloride in pyridine, followed by reaction with aminoethanol. Removal of *t*-butyl group of the aminoisoquinoline compound by trifluoroacetic acid gave the final products.

References and Notes

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