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Bioorganic & Medicinal Chemistry Letters 13 (2003) 297–300

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Factor Xa Inhibitors and Their Prodrugs†

Yonghong Song,[a,*] Lane Clizbe,[a] Chhaya Bhakta,[a] Willy Teng,[a] Paul Wong,[b] Brian Huang,[b] Katherine Tran,[b] Uma Sinha,[b] Gary Park,[b] Andrea Reed,[b] Robert M. Scarborough[a] and Bing-Yan Zhu[a]

[a]*Department of Medicinal Chemistry, Millennium Pharmaceuticals, Inc., 256 East Grand Ave., South San Francisco, CA 94080, USA*
[b]*Department of Biology, Millennium Pharmaceuticals, Inc., 256 East Grand Ave., South San Francisco, CA 94080, USA*

Received 6 August 2002; accepted 16 September 2002

Abstract—In addition to our previously reported fluoro acrylamides Xa inhibitors **2** and **3**, a series of potent and novel cyclic diimide amidine compounds has been identified. In efforts to improve their oral bioavailability, replacement of the amidine group with methyl amidrazone gives compounds of moderate potency (**14**, $IC_{50} = 0.028\,\mu M$). In the amidoxime prodrug approach, the amidoxime compounds show good oral bioavailability in rats and dogs. High plasma level of prodrug **26** and significant concentration of active drug **26a** were obtained upon oral administration of prodrug **26** in rats.

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 search for Xa inhibitors based on substituted acrylamides, we serendipitously discovered the cyclic diimide compound **1** as a potent Xa inhibitor with IC_{50} of $0.002\,\mu M$, as shown in Figure 1. Previously, we have also reported identification of the fluoro acrylamides **2** and **3** as potent, selective and efficacious Xa inhibitors.[3,4] However, these amidine compounds generally suffer from low bioavailability. In this communication, we will discuss modifications of the amidine group with the goal of improving the bioavailability of these amidine-containing compounds. Previous literature has shown that amidoximes can be converted to corresponding amidines in vivo (Fig. 1), thus serving as the prodrugs of amidines.[5] In this study, the amidoxime prodrug approach has also been pursued to overcome the low bioavailability of our amidine compounds.

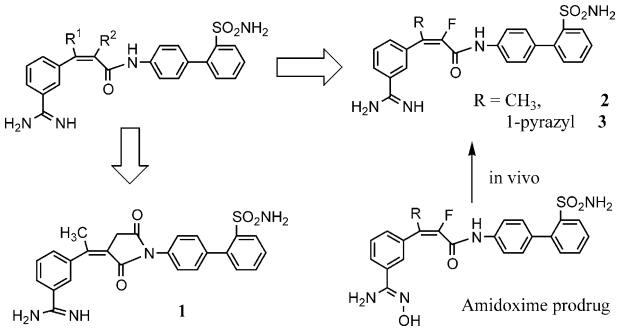

Figure 1. Cyclic diimide **1**, fluoro acrylamides **2** and **3**, and their amidoxime prodrugs.

In an attempted synthesis of a carboxylate substituted acrylamide as factor Xa inhibitor (Scheme 1), a surprising ring closure provided the cyclic diimide compound **1**. This novel compound showed potent anti-Xa activity (IC_{50} of $0.002\,\mu M$) and moderate selectivity against thrombin and trypsin. To determine the importance of the ring structure on potency, the open chain diamide **4** was synthesized. As shown in Table 1, compound **4** is 50-fold less active than **1**, indicating that cyclic structure is optimal for potency. Reduction of the double bond gave the saturated analogue **5** as a mixture of stereoisomers with decreased activity, indicating that

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

*Corresponding author. Fax: +1-650-244-9287; e-mail: yonghong.song@mpi.com

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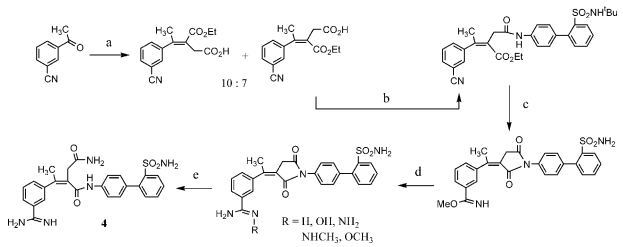
PII: S0960-894X(02)00921-6



Scheme 1. Synthesis of cyclic diimide compounds and **4**. Reagents and conditions: (a) diethyl succinate, KO^{t}Bu, HO^{t}Bu, reflux; (b) biphenylamine, BOP, triethylamine, DMF; (c) MeOH/HCl (g); (d) NH_4OAc (or NH_2OH, or NH_2R), MeOH; (e) NH_3, MeOH, R = H.

the geometry provided by the double bond also contributes to the potency. To explore P4 modification, the sulfonylphenyl was replaced with *t*-butyl (**6**) and pyrrolidylcarbonyl (**7**). However, the replacement reduced the potency by 14- and 90-fold, respectively. Although compound **1** shows potent activity, its bioavailability is low (1.4%) in rats.

Previous literature has shown that replacement of amidine group with amidrazone gave rise to potent and orally bioavailable thrombin inhibitors.[6] In our case, the amidrazone compound **8** shows an IC_{50} of 0.078 μM, a 39-fold decrease from the amidine **1** (Table 2). The methyl amidrazone compound **9** is about 2-fold more active than **8**. Unfortunately, compound **9** does not show improved bioavailability in rats. The amidoxime **10** was also synthesized as prodrug of **1**. However, its bioavailability is also low (1.1%).

We have previously reported a series of fluoro substituted acrylamides as potent and efficacious Xa inhibitors.[3,4] However, these amidine-containing compounds also have low bioavailability (<3%). Replacement of the amidine group with amidrazone and alkylamidrazone was performed in this study. As shown in Table 3, the best compound is again the methyl amidrazone **14** with IC_{50} of 0.028 μM. The unsubstituted amidrazones **11**–**13** are 2–4-fold less active than **14**. Compounds with larger substituting groups (**15** and **16**) and di-substitution (**17**) all give reduced potency. The aminoindazole **18**, which could be viewed as a cyclized amidrazone, is about 100-fold less active than open chain analogues (**11**–**13**).

Modification of the amidine group by alkylation (**19**–**22**) resulted in dramatic decrease in activity, as shown in Table 4. The smaller cyano group (**22**) is the most tolerant.

Previous literature has shown that in many cases, amidoxime compounds can be used as prodrugs of the corresponding amidine compounds, for the amidoxime compounds can be converted to parent amidines in vivo by liver microsomal enzymes. In certain cases, these amidoxime prodrugs have been shown to possess good

Table 1. SAR of cyclic diimides

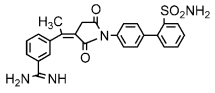
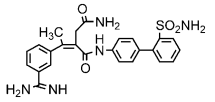
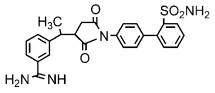
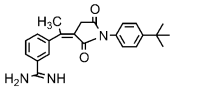
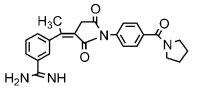
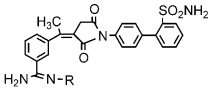
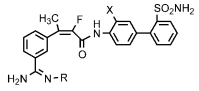
Structure	Compd	FXa	IIa (IC_{50}, μM)	Trypsin	F (%)
	**1**	0.002	0.67	0.18	1.4
	**4**	0.099	1.6	2.5	
	**5**	0.12			
	**6**	0.029	0.076	0.50	1.7
	**7**	0.18	10.7	1.9	

Table 2. Modification of cyclic diimide amidine **1**



R	Compd	FXa (IC_{50}, μM)	F (%)
NH_2	**8**	0.078	
$NHCH_3$	**9**	0.048	1.1
OH	**10**	0.71	1.1

Table 3. Amidrazone analogues of amidine **2**



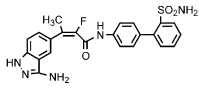
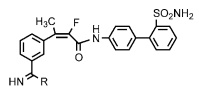
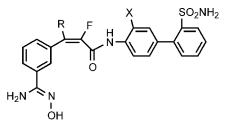
R	X	Compd	FXa (IC_{50}, μM)
H	H	**2**	0.001
NH_2	F	**11**	0.11
NH_2	Cl	**12**	0.041
NH_2	Br	**13**	0.057
$NHCH_3$	H	**14**	0.028
NHPh	H	**15**	0.75
$NHCH_2CF_3$	H	**16**	0.42
$N(CH_3)_2$	H	**17**	0.49
		**18**	5.91

Table 4. Modification of fluoro acrylamide amidine **2**



R	Compd	FXa (IC_{50}, μM)
$NHCH_3$	**19**	0.89
	20	1.61
	21	0.89
NHCN	**22**	0.253

Table 5. Amidoxime prodrugs



R	X	Compd	FXa (IC_{50}, μM)		F (%, rat)[9]
			Amidoxime	Amidine	
CH_3	H	**23**	0.44	0.001 (**2**)	2.5
CH_3	F	**24**	1.59	0.0002 (**24a**)	6
CH_3	Cl	**25**	0.75	0.0005 (**25a**)	16
CH_3	Br	**26**	1.18	0.0005 (**26a**)	52
	H	**27**	3.79	0.001 (**3**)	25 (dog)

oral bioavailability.[5] In this study, we have applied the amidoxime prodrug strategy to our amidine-containing Xa inhibitors. As shown in Table 5, the amidoxime prodrugs (**23**–**27**) are generally inactive, 400–8000-fold less potent than the corresponding active drugs (**2**, **3**, **24a**–**26a**). Although analogue **23** does not have good bioavailability, the bioavailability of additional prodrugs **24**–**26** in rats increases with halogen substitutions within the P4 biphenylsulfonamide moiety, from fluoro (**24**, 6%), to chloro (**25**, 16%), to bromo (**26**, 52%).[7] A similar effect of halogen substitution on bioavailability has also been observed in our previous study in which the P1 amidine was replaced by aminoisoquinoline.[8] Interestingly, the amidoxime prodrug **27** of the pyrazyl acrylamide **3** also shows improved bioavailabilty in dogs (F, 25%) as compared to the parent amidine **3** (F, $<5\%$ in dog). These data demonstrate again that modification of the highly basic amidine group could improve the oral absorption of parent amidine compounds.

Upon oral administration of prodrug **26** in rats, high plasma concentration of the prodrug ($C_{max} = 630$ ng/mL) was obtained; at the same time, significant level of active amidine compound **26a** ($C_{max} = 168$ ng/mL) was also achieved as shown in Figure 2. As compared to the concentration of the prodrug, the level of the active drug is relatively low, indicating that the in vivo conversion of the prodrug to active drug was not optimal (about 20% conversion).

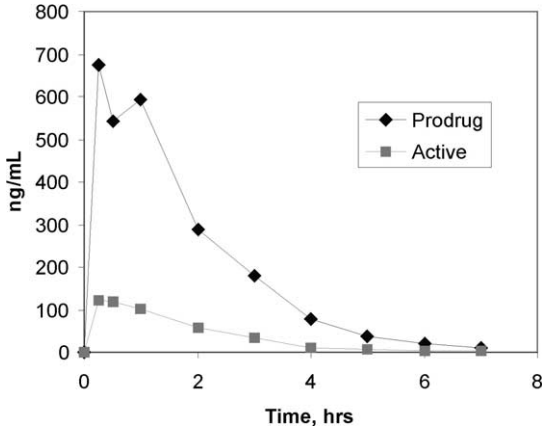
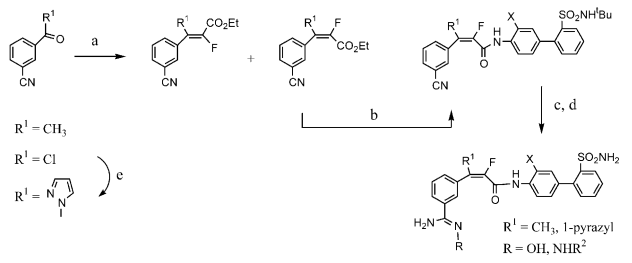


Figure 2. Plasma concentrations of prodrug **26** and active drug **26a** versus time upon po administration of prodrug **26** to four conscious rats at a dose of 6 mg/kg, as determined by LC/MS/MS.

In summary, in addition to our previously reported fluoro acrylamides Xa inhibitors, a series of potent and novel cyclic diimide amidine compounds have been identified. In efforts to improve their oral bioavailability, replacement of the amidine group with methyl amidrazone gives compounds of moderate potency (**14**, $IC_{50} = 0.028$ μM). In the amidoxime prodrug approach, the amidoxime compounds show good oral bioavailability in rats and dogs. High plasma level of prodrug **26** and significant concentration of active drug **26a** were obtained upon oral administration of prodrug **26** in rats. Whether improved oral bioavailability of prodrug **26** will translate into in vivo oral activity is to be demonstrated. However, the in vivo conversion of the prodrug to active drug is not complete and could afford significant inter-subject variability.

In an attempted synthesis of a carboxylate substituted acrylamide as shown in Scheme 1, Stobbe condensation of 3-cyano acetophenone with diethyl succinate gave the mono acid as a mixture of *E*/*Z* isomers with a ratio of 10:7. After separation by chromatography, the *Z* isomer was coupled with the biphenylamine by using BOP to give the amide. However, during the transformation of the nitrile to corresponding amidine under Pinner conditions, the amide underwent cyclization in methanol saturated with hydrogen chloride, to yield the cyclic diimide as methyl imidate. Treatment of the imidate with ammonium acetate, hydroxylamine, and hydrazine gave corresponding amidine, amidoxime and amidrazone analogues, respectively. It was also found that the cyclic diimide ring could be opened by nucleophiles such as ammonia at the less conjugated carbonyl site, providing the open chain diamide **4**.

Synthesis of the fluoro acrylamide compounds is shown in Scheme 2. In case of $R^1 = CH_3$, Horner–Emmons reaction of the cyanoacetophenone with triethyl 2-fluoro-2-phosphonoacetate gave the acrylates in favor of the desired isomer (with the cyanophenyl *cis* to the carboxylate). In case of $R^1 = 1$-pyrazole, 3-cyanobenzoyl chloride was treated with pyrazole to give the acylpyrazole. Interestingly the acylpyrazole was found to undergo Horner–Emmons reaction with triethyl



Scheme 2. Synthesis of fluoro acrylamide compounds. Reagents and conditions: (a) triethyl 2-fluoro-2-phosphonoacetate, $KN(Me_3Si)_2$, THF, −78 °C; (b) biphenylamine, X = H, F, Cl, Br, $AlMe_3$, CH_2Cl_2; (c) MeOH/HCl (g); (d) NH_2OH (or NH_2R), MeOH; (e) pyrazole, triethylamine, CH_2Cl_2.

2-fluoro-2-phosphonoacetate, giving the desired acrylate as a minor isomer. Similar Wittig reaction of acylimidazole with phosphine ylide was observed previously in literature, indicating that these acyl azoles behave as ketones rather than acylating agents.[10] Weinreb amidation of these acrylates with the biphenylamine generated the acrylamides. Conversion of the nitrile to corresponding amidine, amidoxime and amidrazone was similarly performed under Pinner conditions.

References and Notes

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7. One of the referees asked whether we made any halogenated analogues of compound **10** for the cyclic diimide series, to see whether the same trend holds for that series. Unfortunately, we do not have such data at this point of time. However, we see the same trend in our aminoisoquinoline series.[8]
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