Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1797–1800

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Development of Potent and Selective Factor Xa Inhibitors

Roopa Rai, Aleksandr Kolesnikov, Yong Li, Wendy B. Young,* Ellen Leahy, Paul A. Sprengeler, Erik Verner, William D. Shrader, Jana Burgess-Henry, Joan C. Sangalang, Darin Allen, Xi Chen, Bradley A. Katz, Christine Luong, Kyle Elrod and Lynne Cregar

Departments of Medicinal Chemistry, Structural Chemistry and Enzymology, Axys Pharmaceuticals, Inc., 385 Oyster Point Blvd., South San Francisco, CA 94080, USA

Received 5 March 2001; accepted 14 March 2001

Abstract—The development of potent and selective small molecule inhibitors of factor Xa is described.

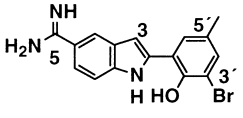
Introduction

The coagulation cascade is a complex scheme of enzymatic reactions that ultimately generates fibrin, the foundation of all blood clots. Disruption or malfunctioning of this homeostatic process leads to thromboembolic disease. Much effort over the past few decades has focused on direct inhibition of key enzymes within this pathway to affect blood coagulation and to potentially generate safer antithrombotic agents. Factor Xa (fXa), a pivotal enzyme in blood coagulation, catalyzes the conversion of prothrombin to thrombin (fIIa). Positioned at the junction of the intrinsic and extrinsic pathways, it is believed that intervention at this key position may enable concomitant control over both pathways which, in turn, may lead to a more effective drug therapy.

There are numerous reports detailing the design and synthesis of peptide, peptidomimetic and small molecule factor Xa inhibitors.[1–5] Although there are no direct factor Xa inhibitors currently being marketed as drugs, there are several compounds in early phase clinical trials.[4] At Axys Pharmaceuticals, we have focused on the generation of selective, small molecule inhibitors of factor Xa that utilize unique binding modes.[6–10] Herein, we describe the structure-based design and development of **1** to a selective subnanomolar inhibitor of factor Xa.

Chemistry: Results and Discussion

2-(3-Bromo-2-hydroxy-5-methyl-phenyl)-1*H*-indole-5-carboxamidine (**1**) was identified in-house as a non-selective serine protease inhibitor.[10] This compound demonstrated good inhibition for factor Xa ($K_i' = 100$ nM), as well as submicromolar inhibition of thrombin (fIIa), urokinase-type plasminogen activator (uPa), trypsin, and low micromolar inhibition of plasmin.[11]



1

	K_i' (μM)
fXa	0.10
fIIa	0.36
uPa	0.11
plasmin	1.2
trypsin	0.64

A crystal structure of **1** complexed with thrombin revealed a surprising array of hydrogen bond interactions between the ligand and the catalytic residues of the protein.[10] The key interactions in this array include three extremely short, low barrier H-bonds between the phenolate of **1**, Ser195 hydroxyl, and a water bound in the oxyanion hole as depicted in Figure 1a. Additional ligand–protein hydrogen bonds occur between the indole NH, 195 OH, the phenol O and the protonated His57 NH. This binding motif appears to be general since it is observed for many structurally related small molecules in a number of different serine proteases.[12] Utilizing this crystal structure, we constructed a model of **1** in factor Xa as depicted in Figure 1b. The model suggested that the core scaffold **1** could be further functionalized to exploit entry into the S1′, S1β, and potentially, the S4 pockets of factor Xa for binding. Our intent was to improve the potency of **1** for factor Xa

*Corresponding author. Fax: +1-650-829-1019; e-mail: wendy_young@axyspharm.com

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00311-0

while also enhancing its selectivity versus key anti-targets such as thrombin, uPa, plasmin, and trypsin.

Initial chemistry efforts focused on preparing C3′ substituted analogues of the original bromo derivative **1** to probe the S1′ pocket. Accordingly, analogues **2**–**5** were generated as depicted in Table 1. Replacement of the bromine of **1** with a hydrogen, chlorine, methyl or phenyl gave analogues that were all less efficient at inhibiting factor Xa, although the chlorine analogue was nearly equipotent. The bromine at C3′ may be optimal for several reasons. First, there are favorable interactions with the residues (including His57) of the relatively small S1′ pocket of fXa and secondly, the bromine shields the network of short hydrogen bonds from water which increases the enthalpic contribution.[12] Thirdly, the electronic properties of the bromine may strengthen the short hydrogen bond network.

Most reported factor Xa inhibitors exploit the S4 pocket to gain binding affinity. In an attempt to improve the potency of **1** and enhance its overall selectivity profile, we sought to probe the S4 pocket as well. Modeling suggested that substituents at either the indole C3 or the C5′-position of the phenolic ring might bind in this site. A stepwise extension of a benzene moiety from the indole C3 position was accomplished to give **6**–**8**. Of the three carbon homologues generated, compound **6** offered the best binding, demonstrating a 5-fold enhancement in potency for factor Xa as compared to **1**. Additionally, the selectivity versus uPa was improved 15-fold. The model suggested that the one methylene tether of compound **6** is not of sufficient length to allow the phenyl ring to access the S4 pocket. It is more likely that the aromatic ring is bound at the S1β site above the 191–220 disulfide and the selectivity observed is due to differences between the enzymes at this site. Our modeling indicates that the three carbon analogue **8** may bind outside of the S4 pocket, potentially explaining its poor inhibition. Clearly, a range of longer and more complex tethers could be generated at this site to access the factor Xa S4 'cationic binding pocket',[5] although, at this juncture, we avoided this strategy opting for lower molecular weight solutions.

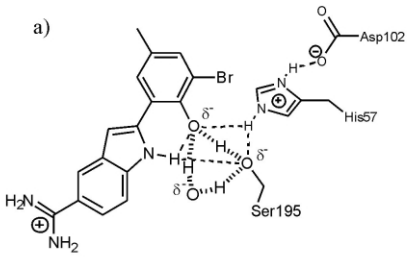
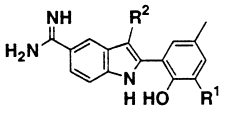


Figure 1. (a) H-bonding array displayed by **1** with the catalytic residues of thrombin in the crystal strucutre; (b) model of **1** in factor Xa built from the thrombin crystal structure showing the S1, S1′ and S4 pockets.

In order to improve the selectivity versus thrombin, we next exploited sequence differences between thrombin and factor Xa at residue 192. Thrombin has a glutamic acid at this position and factor Xa has a glutamine. An anionic moiety in the vicinity should repel the thrombin Glu192 while potentially H-bonding to the factor Xa Gln192. Other factor Xa inhibitors have taken advantage of this difference as well.[5] Our model suggests that substitution at C5′, the position *para* to the phenol, might achieve the desired interaction with amino acid 192 in factor Xa and thrombin.

A series of derivatives that systematically extend a carboxylic acid group from the aryl ring (**9**–**13**) and incorporate varying charged moieties (**14**–**16**) at the C5′ position of **6** is listed in Table 2. Almost all these analogues demonstrated increased inhibition for factor Xa (as compared to **6**) although a range in selectivity versus thrombin was clearly noted. As demonstrated by analogues **10** and **11**, a one carbon spacer between the aryl ring and acid moiety caused the largest decrease in thrombin inhibition. Such a decrease in thrombin inhibition combined with an increase in fXa inhibition provided improved overall selectivity (i.e., 333-fold in the case of **10**). The incorporation of a phosphoric acid group, as in **14**, also demonstrated increased inhibition of factor Xa with 660-fold selectivity versus thrombin. As tetrazoles are known isosteres of carboxylic acids,

Table 1. Modifications at C3′ and C3



Compd	R^1	R^2	K_i (μM)				
			fXa	fIIa	uPa	Plasmin	Trypsin
1	Br	H	0.10	0.36	0.11	1.2	0.64
2	H	H	4.3	23.0	12.4	21.0	8.2
3	Cl	H	0.13	0.46	0.19	1.0	0.7
4	CH_3	H	1.2	6.9	2.3	10.0	5.0
5		H	0.64	4.0	0.11	1.5	1.8
6	Br		0.02	0.26	1.6	4.6	2.4
7	Br		0.35	0.71	2.3	8.8	3.4
8	Br		7.6	6.6	8.6	32	42

compounds **15** and **16** were generated to study this alteration. These analogues showed little to no improvement in potency or selectivity as compared to the nonionic analogue **6**. Notably, the incorporation of anionic moieties had little effect on the inhibition of plasmin and trypsin, although it did enhance uPa activity.

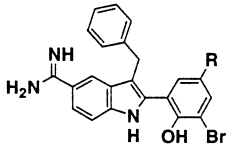
The in vitro anticoagulant effect (activated partial thromboplastin times, aPTTs)[11] of **6** and **9**–**16** are listed in Table 2. For compounds with similar inhibition for factor Xa, the range in efficacies can be attributed to the ability of the compound to bind to plasma proteins and/or the anti-target selectivity profile.

A crystal structure of **10** was obtained in thrombin. The structure reveals that the amidine moiety makes a hydrogen-bonded salt bridge with Asp189 and the array of short hydrogen bonds with the catalytic residues is intact. Interestingly, the benzyl group displays discrete disorder; the major conformer is bound at the S1β disulfide bridge site rather than at S4 which is consistent with our modeling prediction. Also, there is no density for the C5′ carboxylic acid consistent with it being disordered to avoid repulsive interactions with Glu192 (Fig. 2).

In order to clearly discern the role of the carboxylic acid in insulting thrombin, a series of acid analogues of **10** was generated (Table 3). The acid was masked as an ester (**17**), secondary amide (**18**) and a primary amide (**19**). Not surprisingly, in each case, the inhibition for factor Xa was left intact while the inhibition for thrombin increased, thus decreasing the effective selectivity. This result reinforces the notion that Glu192 of thrombin is responsible for the selectivity since the acids, esters, and amides can interact with Gln192 of factor Xa but only the esters and amides are well tolerated by Glu192 of thrombin. Masking the anionic charge removes the repulsive interaction with Glu192 of thrombin and reduces the selectivity for the target enzyme.

As phenols are candidates for in vivo metabolism (glucuronidation and sulfation) and amidines are expected to limit absorption, we investigated the contribution of these moieties on our elaborated lead molecule **12**. We hypothesized that there may be enough other positive interactions between the inhibitor and protein to allow removal of the hydroxyl or amidine. However, both masking the hydroxy as a methyl ether (**20**) and removing it completely (**21**) reduced inhibition for factor Xa 90-fold and 2000-fold, respectively. Although the methoxy analogue **20** still maintained appreciable factor Xa potency, the selectivity versus almost all other anti-targets

Table 2. Modifications at C5′



Compd	R	K_i (μM)					2xaPTT (μM)
		fXa	fIIa	uPa	Plasmin	Trypsin	
6	$-CH_3$	0.02	0.26	1.6	4.6	2.4	3.9
9	$-CO_2H$	0.004	0.33	0.32	1.5	0.44	0.55
10	CO_2H	0.003	1.0	0.43	1.8	1.2	0.38
11	CO_2H	0.01	0.75	0.35	3.0	1.3	0.93
12	CO_2H (2)	0.0005	0.12	0.28	1.7	0.6	0.52
13	CO_2H (3)	0.0007	0.04	0.27	1.5	0.61	0.75
14	OPO_3H_2 (2)	0.0005	0.33	0.40	1.2	3.8	0.42
15	N=N, N-NH	0.01	0.35	0.55	4.2	1.3	0.70
16	N=N, N-NH	0.02	0.44	0.35	2.2	0.84	0.59

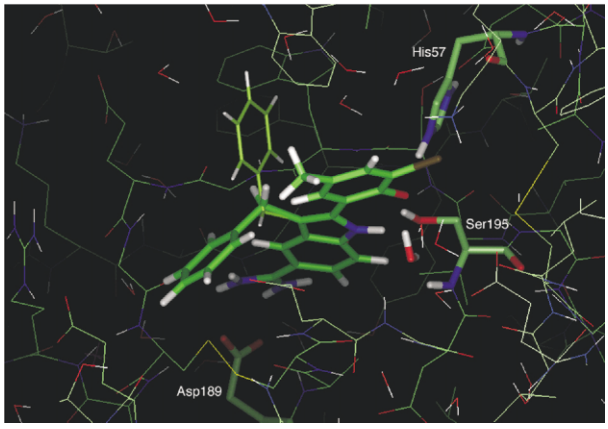
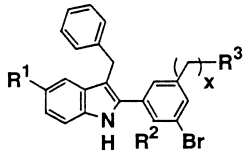
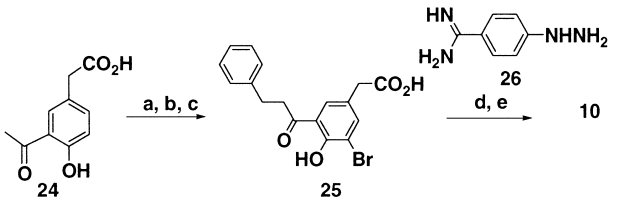


Figure 2. Crystal structure of **10** in thrombin (2.0 Å resolution). Note: the C3 benzyl group is discretely disordered (thick green bonds represent 77% occupancy site, thin light green bonds display 1% occupancy) and the C5′ substituent is modeled as a methyl group due to lack of density.

Table 3. Modifications at C5, C2′, and C5′



Compd	x	R^1	R^2	R^3	K_i (μM)	
					fXa	fIIa
10	1	Am	OH	CO_2H	0.003	1.0
17	1	Am	OH	CO_2Me	0.003	0.08
18	1	Am	OH	$CONMe_2$	0.002	0.043
19	1	Am	OH	$CONH_2$	0.002	0.025
12	2	Am	OH	CO_2H	0.0005	0.12
20	2	Am	OMe	CO_2H	0.045	2.5
21	2	Am	H	CO_2H	1.0	12
22	2	H	OH	CO_2H	300	700
23	2	$CONH_2$	OH	CO_2H	8.4	78



Scheme 1. Synthesis of **10**. Reagents and conditions: (a) PhCHO, NaOH, THF; (b) H_2 Pd/C; (c) NBS, DMF; (d) **26**, triethylamine, MeOH, reflux; (e) PPA, 130 °C.

was significantly diminished. Additionally, removing the amidine completely (**22**) caused a 600,000-fold decrease in potency, while replacement with an amide (**23**) was somewhat less detrimental. Recently, we have generated a series of analogues of **10** in which the 5-amidinoindole moiety has been replaced with a less-basic heterocycle. Such analogues show only a 50-fold loss in factor Xa potency and improved anti-target selectivity. This work will be reported shortly.

The synthesis of the lead inhibitor **10** is illustrated in Scheme 1. All other analogues depicted were synthesized in an analogous manner, although the requisite acetophenones were utilized.[6] All compounds described were generated by utilizing a Fischer–Indole cyclization as the terminal step. The synthesis of **10** begins with 3-acetyl-4-hydroxy-phenylacetic acid (**24**) as previously outlined.[6] Condensation of **24** with benzaldehyde, followed by catalytic hydrogenation, and subsequent treatment with *N*-bromosuccinimide gave **25**. Ketone **25** was then treated with hydrazine **26** and the resultant hydrazone was heated in polyphosphoric acid at 125–130 °C for less than 1 h to give **10**.

Conclusion

Utilizing structure-based design, a nonselective screening lead was optimized to produce a series of selective, subnanomolar inhibitors of factor Xa. Interestingly, this series demonstrates a S1–S1′ binding mode that is unique from most other reported factor Xa inhibitors which generally bind from S1 to S4. Pharmacokinetics, in vivo animal efficacy, and non-amidino P1 analogue development will be the subject of a future publication.

Acknowledgements

The authors wish to thank Michael Venuti and Jochen Knolle for helpful scientific discussions, Liling Fang for analytical support and Barbara Nielsen for aid in preparing this manuscript.

References and Notes

1. Zhu, B.-Y.; Scarborough, R. M. *Curr. Opin. Cardiol. Pulm. Renal Invest. Drugs* **1999**, *1*, 63.
2. Fevig, J. M.; Wexler, R. R. *Annu. Rep. Med. Chem.* **1999**, *34*, 81.
3. Ewing, W. R.; Pauls, H. W.; Spada, A. P. *Drugs Future* **1999**, *24*, 771.
4. Zhu, B.-Y.; Scarborough, R. M. *Annu. Rep. Med. Chem.* **2000**, *35*, 83.
5. Rai, R.; Sprengeler, P. A.; Elrod, K. C.; Young, W. B. *Curr. Med. Chem.* **2001**, *8*, 101.
6. Allen, D. A.; Hataye, J. M.; Hruzewicz, W. N.; Kolesnikov, A.; Mackman, R. L.; Rai, R.; Spencer, J. R.; Verner, E. J.; Young, W. B. PCT International Application WO 00/35886, 2000.
7. Day, R. F.; Young, W. B. PCT International Application WO 99/26933, 1999.
8. Fatheree, P. R.; Jenkins, T. E.; Li, Y.; Linsell, M. S.; Rai, R.; Shrader, W. D.; Trapp, S. G.; Young, W. B. PCT International Application WO 99/26932, 1999.
9. Fatheree, P. R.; Jenkins, T. E.; Linsell, M. S.; Trapp, S. G.; Verner, E. J.; Young, W. B. US6150379, 2000.
10. Verner, E. J.; Katz, B. A.; Spencer, J.; Allen, D.; Hataye, J.; Hruzewicz, W.; Hui, H. C.; Kolesnikov, A.; Li, Y.; Luong, C.; Martelli, A.; Kesavan, R.; Rai, R.; She, M.; Shrader, W.; Sprengeler, P. A.; Trapp, S.; Wang, J.; Young, W. B.; Mackman, R. L. *J. Med. Chem.* **2001**, in press.
11. Inhibition assays for fXa, fIIa, plasmin and trypsin were performed as described (Cregar, L.; Elrod, K. C.; Putnam, D.; Moore, W. R. *Arch. Biochem. Biophys.* **1999**, *366*, 125) with the pH adjusted to 7.4. Coagulation experiments were carried out with human plasma and a coagulation meter (Electra 800) in accordance with the manufacturer's instructions.
12. Katz, B. A.; Elrod, K.; Luong, C.; Rice, M.; Mackman, R. L.; Sprengeler, P. A.; Spencer, J.; Hataye, J.; Janc, J.; Link, J.; Litvak, J.; Rai, R.; Rice, K.; Sideris, S.; Verner, E.; Young, W. *J. Mol. Biol.* **2001**, *307*, 1451.