Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1671–1674

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Optimization of the β-Aminoester Class of Factor Xa Inhibitors. Part 2: Identification of FXV673 as a Potent and Selective Inhibitor with Excellent In Vivo Anticoagulant Activity

Kevin R. Guertin,*,† Charles J. Gardner, Scott I. Klein, Allison L. Zulli, Mark Czekaj, Yong Gong, Alfred P. Spada, Daniel L. Cheney, Sebastian Maignan, Jean-Pierre Guilloteau, Karen D. Brown, Dennis J. Colussi, Valeria Chu, Christopher L. Heran, Suzanne R. Morgan, Ross G. Bentley, Christopher T. Dunwiddie, Robert J. Leadley and Henry W. Pauls*

Drug Innovation and Approval, Aventis Pharmaceuticals, Route 202-206, Bridgewater, NJ 08807, USA

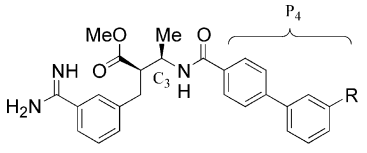
Received 11 January 2002; accepted 28 February 2002

Abstract—Further optimization of the β-aminoester class of factor Xa (fXa) inhibitors is described culminating in the identification of **9c** (**FXV673**), a potent and selective factor Xa inhibitor with excellent in vivo anticoagulant activity. An X-ray structure of **FXV673** bound to human fXa is also presented. Based on its selectivity, potent in vivo activity and favorable pre-clinical safety profile, **FXV673** was selected for further development and is currently undergoing clinical trials.

Factor Xa (fXa) is a pivotal serine protease situated at the juncture of the intrinsic and extrinsic pathways of the blood coagulation cascade. Its singular role in thrombin activation and potentiating effects on clot formation identify it as a target for therapeutic intervention.[1] Thus, the inhibition of fXa (in the physiologically relevant prothrombinase complex) represents an attractive target for the development of novel antithrombotic agents.[2] In response, numerous reports describing small molecule factor Xa inhibitors have recently appeared in the literature.[3] Compounds **1** and **2** are representative of the β-aminoester class of factor Xa inhibitors developed at Aventis.[4]

Parenteral antithrombotic agents include intravenous heparin,[5] low molecular weight heparins (LMWH),[5] IV GPIIbIIIa antagonists[6] and recently, direct thrombin (fIIa) inhibitors such as IV argatroban.[7] Each of the preceding therapies have documented limitations.[8]

A fXa inhibitor may have considerable therapeutic potential for the treatment of acute coronary syndromes. In terms of clot penetration, a small, synthetically derived fXa inhibitor would provide a significant advantage over biologically derived antithrombotics of high molecular weight (e.g., Heparin).[9] Furthermore, there are theoretical arguments to support the potential superiority of fXa inhibitors over direct fIIa inhibitors such as decreased bleeding[10] and elimination of rebound thrombosis.[11]



1, R = H

2, R = CH_2NH_2

Possible indications for an intravenously-administered fXa inhibitor include the treatment of deep vein thrombosis,[12] unstable angina,[13] adjunctive treatment with thrombolytics (acute MI[14]), or in percutaneous transluminal coronary angioplasty.[15] Taken together, these large patient populations present a major medical need for improved antithrombotic therapy.

*Corresponding authors. Fax: +1-973-235-7122; e-mail: kevin.guertin@roche.com (K. R. Guertin); fax: +1-908-231-3577; e-mail: henry.pauls@aventis.com (H.W. Pauls).

†Current address: Roche Research Center, 340 Kingsland St., Nutley, NJ 07110, USA.

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00213-5

In the preceding publication,[4b] methyl was established as the preferred C_3 sidechain of the β-aminoester class of fXa inhibitors. P_4 optimization indicated that aromatic rings substituted with H-bonding and/or positively charged functions were favored, for example **2**. Herein, we report further optimization of the biaryl P_4 moiety culminating in the identification of a potent and selective factor Xa inhibitor for further development.

Chemistry

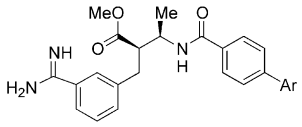
The synthesis of the P_4 modified inhibitors is outlined in Scheme 1.[16] The β-aminoester intermediate **3**[4b] is coupled to the isomeric pyridylphenyl carboxylic acids which are either commercially available, or prepared by Suzuki chemistry as previously described.[17] Conversion of the resulting nitrile intermediates **4a–c** to their corresponding benzamidines is carried out under standard conditions delivering the target β-aminoester fXa inhibitors **5a–c**, in good yield.

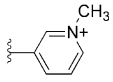
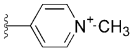
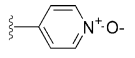
The phenyl-*N*-methylpyridinium and phenylpyridyl-*N*-oxide based inhibitors require *N*-methylation and *N*-oxidation steps, respectively, prior to final conversion to their corresponding benzamidines **7a–c**, **9a–c**. Inhibitors bearing the imidazoylphenyl P_4 group were prepared by coupling of aminoester intermediate **3** with the requisite imidazolyphenyl-carboxylic acids;[17] ammonolysis provides the amidines **10a–c**.

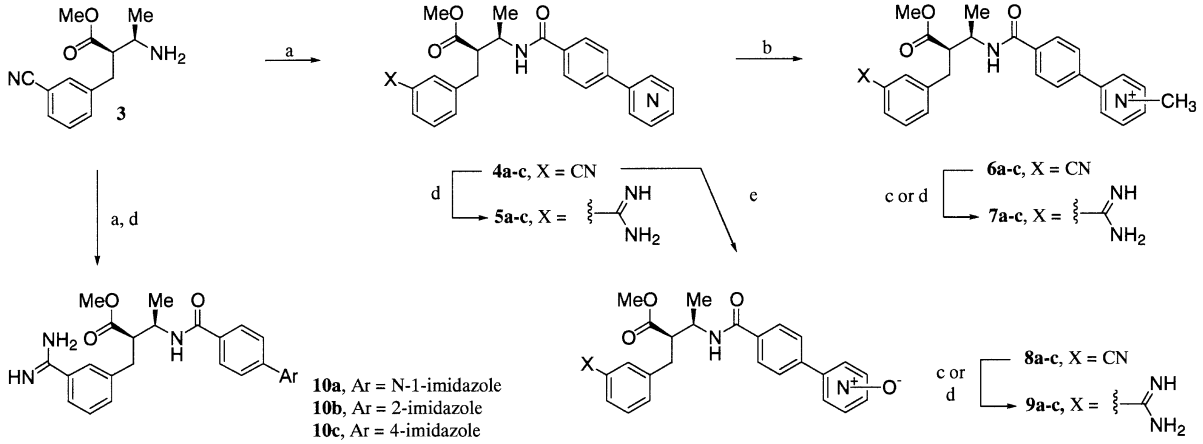
Discussion

Given the desirability of incorporating H-bonding and/or positively charged centers at the distal ring of the P_4 group, compounds were prepared wherein these features were incorporated into the aromatic rings per se. The structure–activity relationships surrounding the inhibitors incorporating heteroaromatic rings into the P_4 ligand are outlined in Table 1. Compounds were assayed against human fXa, thrombin and trypsin as previously described.[18] The improvement in in vitro potency observed for **5a** may be attributed to the hydrogen bonding potential

Table 1. SAR of P-4 modified inhibitors



Compd	Ar	K_i (nM)		
		fXa	flla	Trypsin
1		5.3	3250	69
5a		0.89	2800	51
5b		8.0	4000	368
5c		0.5	>4000	76
7a		1.2	>4000	36
7b		0.8	>4000	52
7c		2.7	>4000	168
9a		0.58	>4000	36
9b		2.0	>4000	130
9c		0.4	>4000	301
10a		6.0	>4000	>2900
10b		46	>4000	490
10c		3.0	>4000	170



Scheme 1. Reagents and conditions: (a) ArCOOH, TBTU, diisopropylamine, DMF, rt; (b) MeI, CH_2Cl_2, reflux; (c) (i) H_2S,pyridine, Et_3N, rt; (ii) MeI, acetone, reflux; (iii) NH_4OAc, MeOH, reflux; (d) (i) HCl, MeOH, rt; (ii) NH_3, MeOH, reflux; (e) *m*-CPBA,CH_2Cl_2, rt.

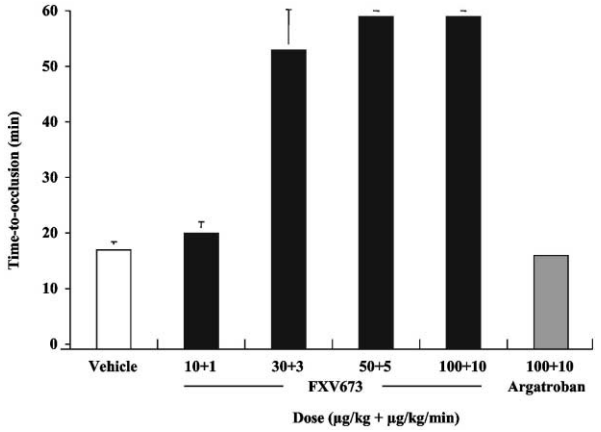


Figure 1. Time-to-occlusion versus dose of **FXV673** in the rat $FeCl_2$-induced carotid artery thrombosis model. Dose is iv bolus + 60 min of infusion.

of the pyridine ring, presumably via a productive interaction with the bound water of the electron rich cation hole. The cation hole, not found in thrombin, trypsin, APC or plasmin, represents an opportunity for enhancing both fXa inhibitory potency and selectivity against these related serine proteases. Structurally related inhibitors, incorporating electron deficient phenyl-*N*-methylpyridinium (e.g., **7a**–**c**) and phenylpyridyl-*N*-oxide (e.g., **9a**–**c**) P_4 groups also showed improved potency relative to the parent biphenyl derivative **1** (Table 1). Compounds based on more basic imidazole rings (**10a**–**c**) offered no advantage over the pyridyl inhibitors.

In particular, the sub-nanomolar *N*-oxide based inhibitor **9c**, designated **FXV673**, showed exquisite selectivity against the related serine proteases fIIa, APC, plasmin and tPA and acceptable selectivity (~750-fold) against trypsin. Clearly, the overall selectivity profile is vastly superior to earlier β-aminoester based prototypes such as inhibitor **1** (Table 2). Related work, published separately,[18] indicated that **FXV673** is a reversible, competitive inhibitor of factor Xa.

Table 2. In vitro comparison of selected β-aminoester fXa inhibitors

Compd	Ratio: K_i enz./K_i fXa					2X APTT (μM)	
	fIIa	Tryp.	APC	Plasmin	tPA	Human	Rat
1	610	13	$>10^3$	87	$>10^3$	3.9	5.1
9c	$>10^4$	750	$>10^4$	$>10^3$	$>10^4$	0.41	0.80

FXV673 inhibited coagulation of human plasma in vitro, doubling activated partial thromboplastin time (APTT) at a concentration of 0.41 μM (Table 2). This illustrates that **FXV673** is an effective inhibitor of fXa in the physiologically relevant human prothrombinase complex; similar results were observed in rat plasma.

The potent fXa inhibitor **FXV673** was evaluated in a rat model of ferrous chloride-induced arterial thrombosis. A dose-dependent increase in time to occlusion (Fig. 1) was observed with a maximal effective dose at about 50 μg/kg bolus and 5 μg/kg/min iv maintenance infusion. This dose corresponded to a 40% reduction in thrombus mass relative to control. At twice the dose, the direct thrombin inhibitor argatroban was not significantly different from vehicle. **FXV673** produced qualitatively

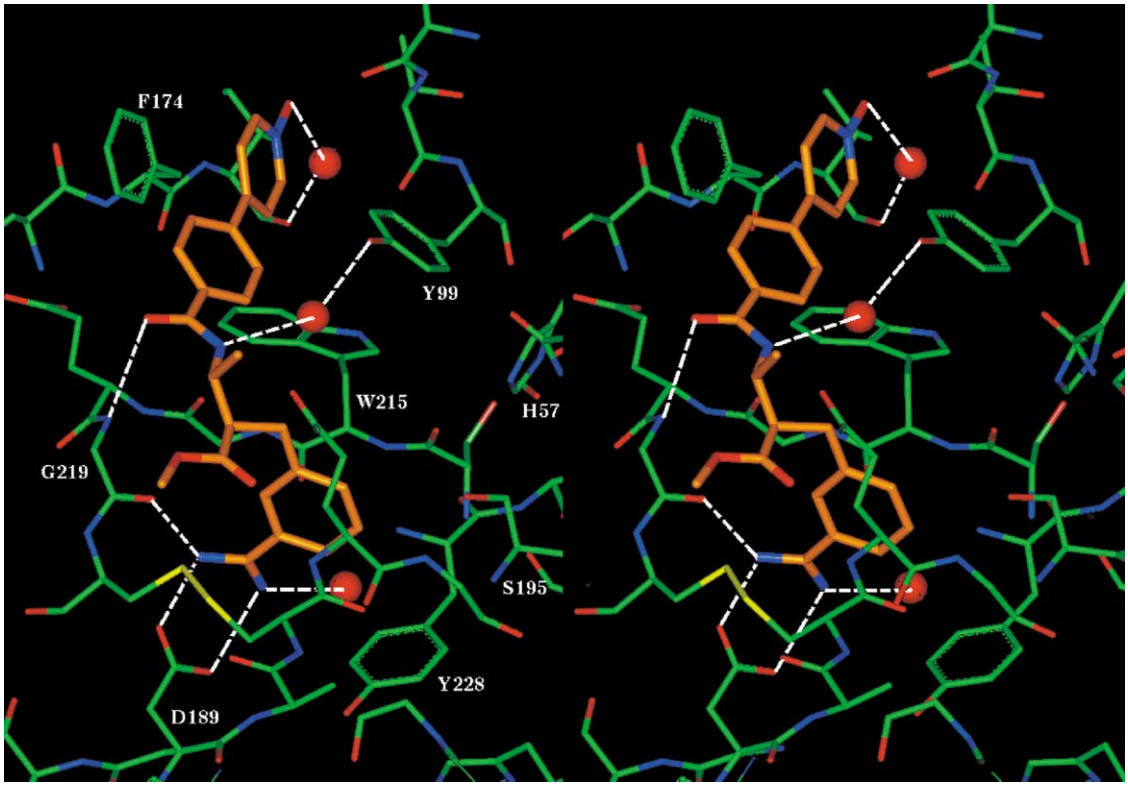


Figure 2. X-ray of **FXV673** in Factor Xa. Red spheres represent bound water.

similar results in an acute canine model of arterial and venous electrolytic injury.

An X-ray structure of **FXV673** bound to human fXa is illustrated in Figure 2. The data reveals an extended inhibitor conformation with the expected bridging interaction between the benzamidine group and D189 as well as a direct H-bond from the amidine to the carbonyl of G219. The phenylpyridyl-*N*-oxide P_4 group is inserted deep into the S_4 site and there are extensive Van der Waals contacts made between three aromatic residues (Y99, F174 and W215) from fXa and the distal pyridine-*N*-oxide ring. The oxide oxygen makes an H-bond to bound water. In addition, there is a hydrogen bond between the inhibitor P_4 amide carbonyl oxygen and the NH of G219. In contrast to our earlier fXa docking studies on inhibitor **1**,[4a] the C_1-carbomethoxy substituent of **FXV673** is remote from the catalytic triad region, fitting snugly into a cleft defined by the disulfide bridge (C191 and C220), G219, Q192 and R147. The C_1 carbomethoxy carbonyl oxygen is involved in a hydrogen bond to the Q192 backbone NH (not shown).

In conclusion, the SAR surrounding the β-aminoester series of fXa inhibitors has been expanded, culminating in the identification of **9c** (**FXV673**) as a potent, competitive and reversible inhibitor ($K_i = 0.4$ nM) with a high level of selectivity against related serine proteases. In vivo experiments have demonstrated that this compound is highly efficacious in animal models of arterial thrombosis. Based on these data and a favorable preclinical safety profile, **FXV673** has been selected for further development and is currently undergoing clinical trials.

References and Notes

1. Davie, E. W.; Fujikawa, K.; Kisiel, W. *Biochemistry* **1991**, *30*, 10363.
2. Kaiser, B.; Hauptmann, J. *J. Cardiovasc. Drug Rev.* **1994**, *12*, 225.
3. For comprehensive reviews, see preceding article.
4. (a) Klein, S. I.; Czekaj, M.; Gardner, C. J.; Guertin, K. R.; Cheney, D. L.; Spada, A. P.; Bolton, S. A.; Brown, K.; Colussi, D.; Heran, C. L.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. *J. Med. Chem.* **1998**, *41*, 437. (b) Czekaj, M.; Klein, S. I.; Guertin, K. R.; Gardner, C.; Zulli, A. L.; Pauls, H. W.; Spada, A. P.; Cheney, D. L.; Brown, K. D.; Colussi, D. J.; Chu, V.; Leadley, R. J.; Dunwiddie, C. T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1667.
5. For a comprehensive review, see: Samama, M. M.; Bara, L.; Gouin-Thibault, I. *Drugs* **1996**, *52*, 8.
6. Jordan, R. E.; Nakada, M. T.; Weisman, H. E. *Biopharm. Ind. Perspect.* **1999**, 35.
7. Hursting, M. J.; Alford, K. L.; Becker, J. C.; Brooks, R. L.; Joffrion, J. L.; Knappenberger, G. D.; Kogan, P. W.; Kogan, T. P.; McKinney, A. A.; Schwartz, R. P. *Semin. Thromb. Hemost.* **1997**, *23*, 503.
8. (a) Leadley, R. J. *Curr. Top. Med. Chem.* **2001**, *1*, 151. (b) Freedman, M. D. *J. Clin. Pharmacol.* **1992**, *32*, 196. (c) Walenga, J. M.; Bick, R. L. *Med. Clin. North Am.* **1998**, *82*, 635. (d) Kleiman, N. S. *Am. Heart J.* **1999**, *138*, S10.
9. Weitz, J. L.; Hudoba, M.; Massel, D.; Maraganore, J.; Hirsh, J. *Biocim. Biophys. Acta* **1994**, *1227*, 113.
10. Schmaier, A. H. *Handb. Exp. Pharmacol.* **2000**, *140*, 277.
11. Gold, H. K.; Torres, F. W.; Garabedian, H. D.; Werner, W.; Jang, I.-K.; Khan, A.; Hagstrom, J. N.; Yasuda, T.; Leinbach, R. C.; Newell, J. B.; Bovill, E. G.; Stump, D. C.; Collen, D. *J. Am. Coll. Cardiol.* **1993**, *21*, 1039.
12. Haas, S. *Hemostasis* **1996**, *26*, 39.
13. Falk, E. *Circulation* **1985**, *71*, 699.
14. Cannon, C. P. *J. Thromb. Thrombolysis* **1995**, *2*, 205.
15. Barry, W. L.; Sarembock, I. J. *Cardiol. Clin.* **1994**, *12*, 517.
16. Satisfactory spectral data were obtained for all compounds. Final products were purified by C18 reverse-phase HPLC (eluant: 0.1% TFA H_2O/CH_3CN gradient).
17. Gong, Y.; Pauls, H. W. *SynLett.* **2000**, *6*, 829. Gong, Y.; Pauls, H. W.; Spada, A.; Czekaj, M.; Liang, G.; Chu, V.; Colussi, D.; Brown, K.; Goa, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 217.
18. Chu, V.; Brown, K.; Colussi, D.; Gao, J.; Bostwick, J.; Kasiewski, C.; Bentley, R.; Morgan, S.; Guertin, K.; Pauls, H. W.; Gong, Y.; Zulli, A.; Perrone, M. H.; Dunwiddie, C. T.; Leadley, R. J. *Thromb. Res.* **2001**, *103*, 309.