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Optimization of the β-Aminoester Class of Factor Xa Inhibitors. Part 1: P_4 and Side-Chain Modifications for Improved In Vitro Potency

Mark Czekaj,* Scott I. Klein, Kevin R. Guertin,*,† Charles J. Gardner, Allison L. Zulli, Henry W. Pauls, Alfred P. Spada, Daniel L. Cheney, Karen D. Brown, Dennis J. Colussi, Valeria Chu, Robert J. Leadley and Christopher T. Dunwiddie

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

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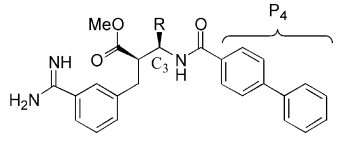
Abstract—A systematic modification of the C_3 side-chain of the β-aminoester class of factor Xa inhibitors and a survey of P_4 variations is described. These changes have resulted in the identification of sub-nanomolar inhibitors with improved selectivity versus related proteases. Coagulation parameters (i.e., APTT doubling concentrations) are also improved.

The inhibition of the serine protease component of the prothrombinase complex, factor Xa (fXa), is generally recognized as a viable strategy for the development of novel antithrombotic agents.[1] The unique position of fXa at the convergence of the intrinsic and extrinsic pathways, its singular role in thrombin activation and in the amplification of the coagulation cascade combine to make it an attractive intervention point.[2] In response, a growing number of publications have appeared describing small molecule fXa inhibitors and several comprehensive reviews have been published.[3]

In a previous publication,[4] we reported the discovery and initial SAR of a novel series of β-aminoester derived fXa inhibitors **1** and **2**. Therein, we established the requirement for the ester moiety, the optimal stereochemistry at the C_2 and C_3 positions (*R*,*R*) and in a preliminary way, examined the SAR of the biaryl P_4 moiety.

A modeling study of **2** in the active site of des1-45 factor Xa predicted that the benzamidine moiety binds in the S_1 pocket while the biphenyl group fills the aromatic pocket (S_4). The model also suggested that the C_1 methoxycarbonyl group is positioned near the catalytic triad, however, our biochemical studies did not support this view.[4] Neither were we able to elucidate the exact role and importance of the C_3 styryl side-chain. Subsequent X-ray analysis indicated that the methoxycarbonyl group occupies a shallow groove remote from the catalytic triad.[5,6]



1, R = CH_2CH_2Ph

2, R = CH=CHPh

In this communication, we describe the systematic variation of the C_3 β-aminoester side-chain using a common synthetic approach. In addition, the SAR of the P_4 group was further elucidated. This work resulted in the identification of nanomolar inhibitors of factor Xa with selectivity against related trypsin-like serine proteases.[7] The subsequent article expands this theme to identify the β-aminoester **FXV673** as a development candidate for acute thrombosis.[6]

Chemistry

The β-aminoesters **4** bearing the requisite *R*-configuration at C_3 are prepared by Arndt–Eistert homologation of the corresponding Boc-D-amino acid derivatives

*Corresponding authors. E-mail: mark.czekaj@aventis.com (M. Czekaj); kevin.guertin@roche.com (K. R. Guertin).
†Current address: Roche Research Center, 340 Kingsland St., Nutley, NJ 07110, USA.

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and proceed via the intermediary diazomethyl ketones **3**.[8] As expected, the homologated esters **4** are obtained with retention of configuration at the asymmetric center (Scheme 1).[9] Subsequent deprotonation of **4** and alkylation with 3-cyanobenzyl bromide affords the adducts **5** as single diastereomers (>95% de as determined by ^{1}H NMR). The Boc protecting group is then removed under standard conditions providing the alkylated β-aminoesters **6**, which are then coupled to appropriate 4-biphenylcarboxylic acids. Finally, conversion of the nitrile moiety of intermediates **7** to their corresponding benzamidines is carried out using Pinner/ammonolysis chemistry delivering the target β-aminoester inhibitors **8**, **9a**, and **9c**–**g** in good yield.

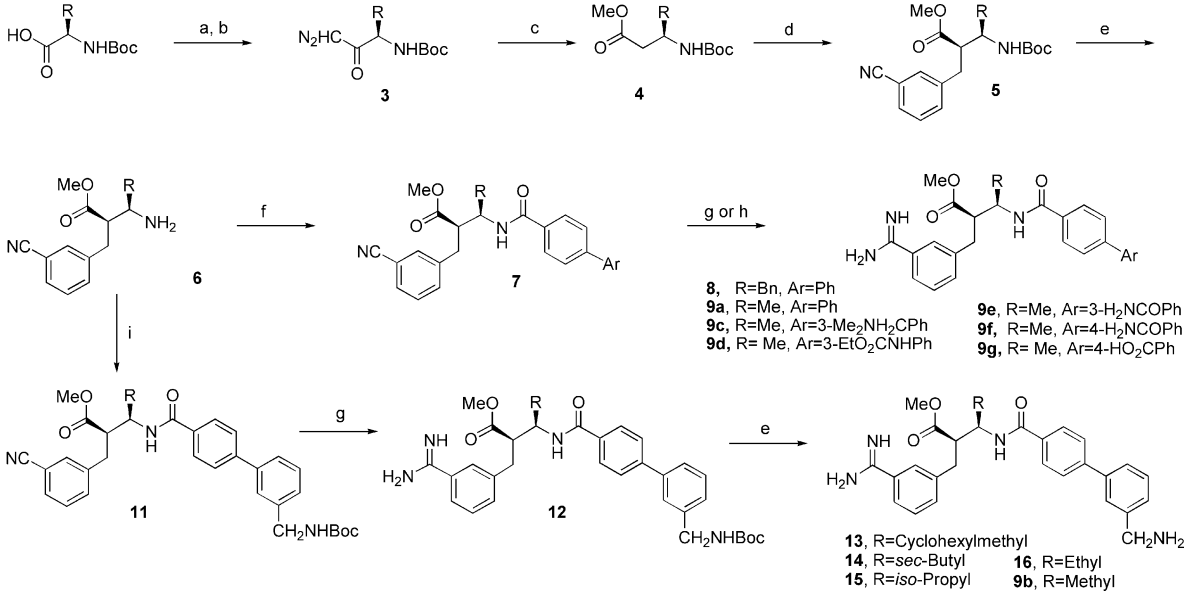
The suitably protected aminomethylbiphenyl carboxylic acid **18** required for compounds **9b** and **13**–**16** is prepared via palladium catalyzed cross coupling of 3-bromobenzonitrile and ethyl-4-iodobenzoate as outlined in Scheme 2. Subsequent reduction affords **17** whose amine function is Boc protected. Base-catalyzed hydrolysis of the ester provides the requisite carboxylic acid **18**, for coupling to amines **6** (Scheme 1). The nitrile moiety of coupled product **11** is converted in a three-step process, to amidines **12**; final Boc deprotection yields the desired inhibitors **9b** and **13**–**16**.

The racemic inhibitor **10** was prepared as outlined in Scheme 3. Acylation of commercially available β-alanine methyl ester hydrochloride with 4-biphenylcarbonyl chloride followed by alkylation with 3-cyanobenzyl bromide afforded intermediate nitrile; which is transformed to amidine **10** using standard conditions.

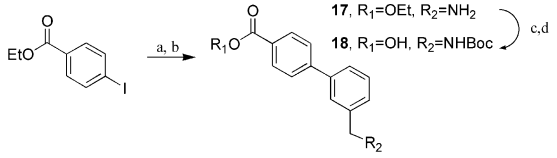
Discussion

Compound **1** is representative of the β-aminoester class of fXa inhibitors originally discovered in our laboratories.[4] Further optimization of this series began with a systematic examination of the C_3 substituent R (Table 1). Initial efforts were focused on compounds bearing a biphenyl moiety in the putative P_4 position. Compounds were assayed against human factor Xa, thrombin and trypsin as previously described.[4] In comparing inhibitors **1**, **2**, **8**, **9a**, and **10**, it is evident that large hydrophobic substituents are not particularly favored at C_3. Complete removal (R = H) resulted in only a modest reduction in fXa potency for the racemate **10**.

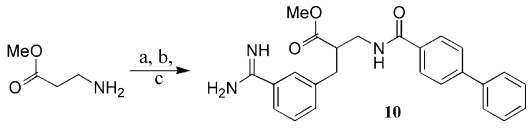
Concurrent work had established the desirability of appending substituents to the distal ring of the biphenyl group;[5] Table 2 summarizes the data. The marked



Scheme 1. Reagents and conditions: (a) *i*-BuCOCl, THF, −30 °C; (b) CH_2N_2, ether, 0 °C; (c) $PhCO_2Ag$, NEt_3, MeOH; (d) (i) LHMDS, THF, −78 to −25 °C; (ii) 3-cyanobenzyl bromide, THF, −78°C to rt; (e) TFA, CH_2Cl_2, 0 °C; (f) Ar–Ph-4–CO_2H, TBTU, *i*Pr_2NEt, DMF, rt; (g) (i) H_2S, pyridine, NEt_3; (ii) MeI, acetone; (iii) NH_4OAc; (h) HCl, MeOH, rt; (ii) NH_3, MeOH, reflux; (i) **18**, TBTU, *i*Pr_2NE_t, DMF, rt.



Scheme 2. Reagents and conditions: (a) *n*BuLi, THF, −78 °C; $ZnCl_2$; 3-bromo-benzonitrile, $Pd(Ph_3P)_2Cl_2$; (b) H_2, Rh on alumina, NH_3, MeOH; (c) Boc_2O, DMAP, DMF, rt; (d) aqueous NaOH.



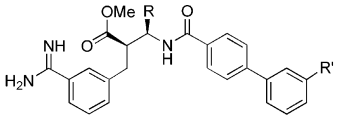
Scheme 3. Reagents and conditions: (a) 4-biphenylcarbonyl chloride, Et_3N, CH_2Cl_2, rt; (b) LHMDS, THF, −78 °C then 3-cyanobenzyl bromide, −78 °C to rt; (c) (i) HCl, MeOH, rt; (ii) NH_3, MeOH, reflux.

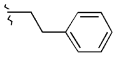
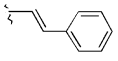
increase in potency observed for **9b** was attributed to the charged nature of the benzyl amine, since a number of dibasic inhibitors make use of the S_4 cation hole of factor Xa for productive interaction.[3b] Although this interpretation was supported by entries **9c** and **9d**, the result for **9e** clearly indicates that a positive charge is not a prerequisite for good activity and H-bonding interactions of the carboxamide with the S_4 pocket may be involved. It appears that a number of different factors are important for binding and a variety of strategies can be employed to optimize interaction with the S_4 pocket.

Variations at C_3, which employ the aminomethylbiphenyl P_4 group, allowed us to explore the SAR around this position in more detail (**13**–**16** and **9b**). The isobutyl substituent (**14**) was the most effective while steric crowding closer to the C_3 branching point (**15**) resulted in decreased fXa inhibitory activity. Although the C_3 substituent is required for stereocontrol at C_2 during synthesis of β-aminoesters (Scheme 1), the potency advantage for larger alkyls over methyl is marginal. Given the simplicity and ready availability of the β-aminobutyric acid precursor **4** (i.e., R = Me), compounds with a methyl group at the C_3 position (e.g., **9a**–**g**) became the focus of further efforts.

The relative activity of several inhibitors verses related trysin-like serine proteases is shown in Table 3. Although very good selectivities have been achieved against thrombin (fIIa), APC and tPA, selectivity against trypsin and plasmin is modest. In comparing compounds **9a**, **9b**, and **2**, the C_3 methyl side-chain derivatives are no less selective than the styryl. The incorporation of the basic aminomethyl substitutent in P_4 appears to impart better potency as well as across the board selectivity (cf., **9a** and **9b**).

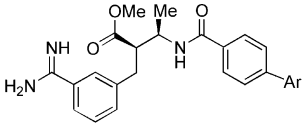
Table 1. SAR of C_3 side-chain



Compd	R	R′	K_i[10] (nM)		
			fXa	fIIa	Tryp.
1[a]			21	810	67
2[a]			9.4	954	221
8			11	709	75
9a			5.3	3250	69
10[b]			20	>3950	230
13			4.0	650	36
14			0.40	990	38
15			8.0	>3950	300
16			0.70	2100	63
9b			0.9	~2900	69

[a]Synthesis described previously.[4]
[b]Racemic.

Table 2. SAR of biaryl P_4 ligands



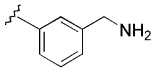
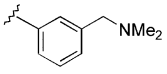
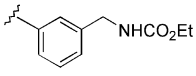
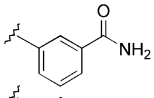
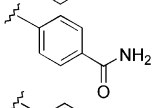
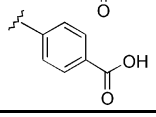
Compd	Ar	K_i[10] (nM)		
		fXa	fIIa	Tryp.
9a		5.3	~3250	69
9b		0.9	2920	69
9c		1.0	2660	95
9d		>1200	>4000	>2900
9e		0.5	>4000	90
9f		2.0	>4000	42
9g		69	>4000	ND

Table 3. In vitro[10] comparisons of selected β-aminoester fXa inhibitors

Compd	fXa K_i (nM)	Ratio: K_i enz/K_i fXa					2 X APTT (μM)	
		fIIa	Tryp.	APC	Plasmin	tPA	Human plasma	Rat plasma
2	9.4	100	24	>2050	80	>965	ND	ND
9a	5.3	610	13	>3500	87	>1600	3.9	5.05
9b	0.9	3250	77	650	230	>9650	0.33	1.7

Anticoagulant activity in plasma from human and rat was measured for select compounds. Inhibitors **9a** and **9b** were effective anticoagulants in vitro and reflected the trend established against free factor Xa (Table 3). Subtle species differences were observed perhaps due to structural variations in enzyme.

Conclusion

A systematic study on the effects of the C_3 branching substituent on the potency and selectivity of the β-aminoester series of factor Xa inhibitors has been carried out. The results reveal that small alkyl substituents are preferred at this position and the C_3 substituent could be completely removed with only a modest reduction in fXa inhibitory potency. Significant increases in potency could also be achieved by substitutions on the distal P_4 phenyl ring.

References and Notes

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7. Parts of this work were communicated in poster form: Czekaj, M.; Klein, S. I.; Gardner, C. J.; Guertin, K. R.; Zulli, A. L.; Pauls, H.; Spada, A. P.; Chu, V.; Brown, K.; Colussi, D.; Leadley, R. J.; Dunwiddie, C. T.; Morgan, S. R.; Heran, C. L.; Perrone, M. H.; Maignan, S.; Guilloteau, J. P.; Liang, G. *Abstracts of Papers*, 218th National Meeting of the American Chemical Society, New Orleans, LA; American Chemical Society: Washington, DC, 1999; MEDI-032.
8. Alternatively, derivative **4** (R = Me) may be readily prepared on a large scale via conjugate addition of Li (R)-*N*-(α-methylbenzyl)benzylamide to methyl crotonate followed by debenzylation using Pearlman's catalyst. See: Davies, S. G.; Ichihara, O. *Tetrahedron: Asymm.* **1991**, *2*, 183. Final Boc protection (BOC_2O, Et_3N, CH_2Cl_2) then provides β-aminoester 4 (R = Me) in good yield.
9. Satisfactory spectral data were obtained for all compounds. Final products were purified by C18 reverse-phase HPLC, eluting with a 0.1% TFA H_2O/CH_3CN gradient, lyophilized and isolated as powders.
10. In vitro enzyme assays and activated partial thromboplastin times (APTT) were performed as described in: Choi-Sledeski, Y. M.; McGarry, D. G.; Green, D. M.; Mason, H. J.; Becker, M. R.; Davis, R. S.; Ewing, W. R.; Dankulich, W. P.; Manetta, V. E.; Morris, R. L.; Spada, A. P.; Cheney, D. L.; Brown, K. D.; Colussi, D. J.; Chu, V.; Heran, C. L.; Morgan, S. R.; Bentley, R. G.; Leadley, R. J.; Maignan, S.; Guilloteau, J. P.; Dunwiddie, C. T.; Pauls, H. W. *J. Med. Chem.* **1999**, *42*, 3572.