Discovery of *N*-[2-[5-[Amino(imino)methyl]-2-hydroxyphenoxy]-3,5-difluoro-6-[3-(4,5-dihydro-1-methyl-1*H*-imidazol-2-yl)phenoxy]pyridin-4-yl]-*N*-methylglycine (ZK-807834): A Potent, Selective, and Orally Active Inhibitor of the Blood Coagulation Enzyme Factor Xa[1]

Gary B. Phillips, Brad O. Buckman, David D. Davey, Keith A. Eagen, William J. Guilford, Josephine Hinchman, Elena Ho, Sunil Koovakkat, Amy Liang, David R. Light, Raju Mohan, Howard P. Ng, Joseph M. Post, Kenneth J. Shaw, Dave Smith, Babu Subramanyam, Mark E. Sullivan, Lan Trinh, Ron Vergona, Janette Walters, Kathy White, Marc Whitlow, Shung Wu, Wei Xu, and Michael M. Morrissey*

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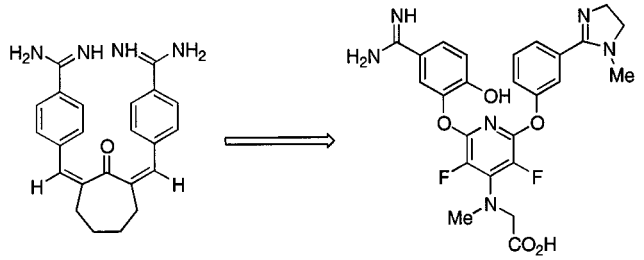
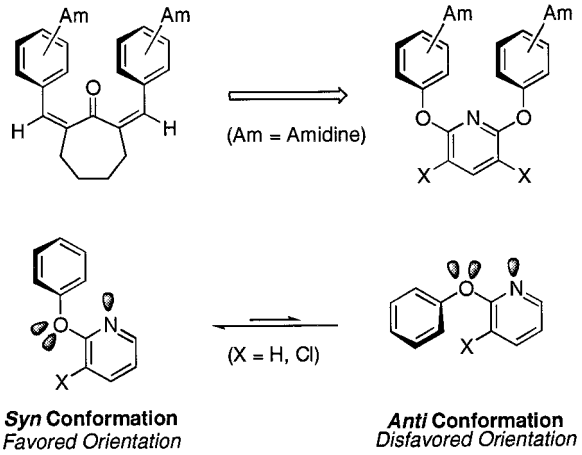
In the preceding communication,[2] we described the discovery and characterization of (*Z*,*Z*)-2,7-bis(4-amidinobenzylidene)cycloheptan-1-one ((*Z*,*Z*)-BABCH,[3] **1**) as a potent, selective and conformationally constrained inhibitor of factor Xa (FXa).[4] The structural and biochemical characteristics of this potent FXa inhibitor presented an excellent opportunity for further inhibitor design. However, the inherent liabilities of this template, specifically the photochemically induced olefin isomerization and the bisamidine functionality, would require significant structural modification before the full potential of this series as viable therapeutics could be realized. In this communication, we outline the transformation of (*Z*,*Z*)-BABCH (**1**) into ZK-807834 (**2**), a highly potent, selective and orally active FXa inhibitor.



Several potential isosteric scaffolds were designed to render the symmetrical cycloheptadienone core of **1** into a photochemically inert and synthetically feasible pharmacophore.[5] Conformational analysis of these scaffolds identified amidine substituted 2,6-diphenoxypyridines as a promising template on which to build potent and selective FXa inhibitors. Computational and experimental data support a model in which the phenyl groups of the 2,6-diphenoxypyridine system share a high degree of overlap with the phenyl groups of the parent (*Z*,*Z*)-BABCH template. AM1 semiempirical molecular orbital calculations (MOPAC) for 2-phenoxypyridine (X = H) suggested that the syn conformation is favored by 4.5 kcal/mol over the anti conformation due to repulsion between the lone pair electrons of the pyridine nitrogen and the phenoxy oxygen atoms.[6] In addition, the crystal structure of 3-chloro-2-phenoxypyridine showed (X = Cl) that the syn conformation predominated in the solid state.[7]



A series of bisamidine substituted 2,6-diphenoxypyridines were prepared[8] and evaluated (Table 1).[9] These analogues demonstrated moderate inhibitory potency for human FXa as well as selectivity against human thrombin (FIIa) and bovine trypsin. Several important trends emerged from this initial survey. The symmetrical meta-substituted bisamidines (**4**, **6**) were more potent and selective than the symmetrical para regioisomers (**3**, **5**) and the asymmetrical meta/para-bisamidine analogue (**8**). This result was in contrast to the SAR observed in the parent (*Z*,*Z*)-BABCH system in which the para-bisamidine regioisomer is nearly 10-fold more potent than the corresponding meta-isomer.[2] It was also observed that substitution on the pyridine ring provided a modest enhancement of the FXa inhibitory potency. Introduction of fluorine groups into the 3- and 5-positions of the pyridine ring (**9**) resulted in a 2−5-fold enhancement of FXa inhibitory potency when compared to the chloro-substituted (**6**) or unsubstituted analogues (**4**). This initial series of compounds also provided the first evidence that the 4-position of the pyridine ring can accommodate a variety of functional groups without loss of FXa inhibitory activity (**7**, **10**). Finally, the inactivity of monoamidine **11** underscored the requirement for substitution on both phenoxy groups.

With the encouraging structure−activity data from the initial set of analogues, synthetic efforts were focused on reducing the overall basicity and enhancing the FXa inhibitory potency of the 2,6-diphenoxypyridine template. A series of monoamidines were prepared with isosteric functional groups in place of the second amidine moiety to attenuate the basicity of the compounds. Five examples are outlined in Table 2 (entries **12**−**16**). While these modifications led to a general decrease in

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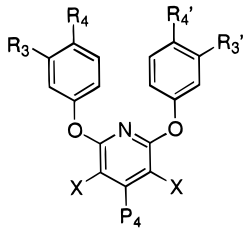
Table 1. Initial SAR for Bisamidino-2,6-diphenoxypyridine FXa Inhibitors



							enzyme inhibition[a] (K_i, nM)		
entry	R_3	R_4	R_3'	R_4'	X	P_4	FXa	FIIa	trypsin
1							0.66	530	33
3	H	Am	H	Am	H	H	400	550	2800
4	Am	H	Am	H	H	H	53	14000	1100
5	H	Am	H	Am	Cl	H	620	1400	2700
6	Am	H	Am	H	Cl	H	130	>5000	2100
7	Am	H	Am	H	H	CO_2H	59	27000	570
8	Am	H	H	Am	H	H	130	1200	1900
9	Am	H	Am	H	F	H	24	37000	1200
10	Am	H	Am	H	F	Me	13	22000	810
11	Am	H	H	H	F	Me	>5000	>5000	>5000

[a] K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean.[9]

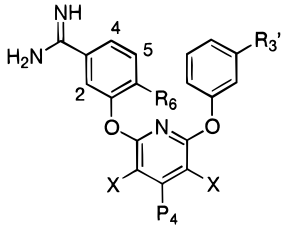
FXa inhibitory potency for the 2,6-diphenoxypyridines, *N*,*N*-dimethylcarboxamide **16** retained the best overall potency and selectivity as a monoamidine FXa inhibitor ($K_i = 80$ nM).

With the benzamidine group as the invariant, FXa inhibitor **16** was then employed as the starting point to systematically introduce substituents into both the amidine-containing (proximal) and carboxamide-containing (distal) phenyl rings to optimize the binding interaction with the FXa active site. Three examples of proximal phenyl ring substitutions are highlighted in Table 2 (entries **17**−**19**). While the majority of substitutions to either phenyl ring resulted in a complete loss of FXa inhibitory activity, incorporation of an amine (**17**) or hydroxyl moiety (**18**) into the 6-position of the proximal phenyl ring led to a maximal 70-fold increase in FXa inhibitory activity.[10] These data suggested that a hydrogen bond donating group at the 6-position of the proximal phenyl ring was critical for enhanced FXa inhibitory activity. This hypothesis was supported by the 6-methoxy analogue (**19**) which was nearly 500-fold less active than its 6-hydroxy counterpart (**18**).

Finally, a number of heterocyclic groups were introduced at the 3-position of the distal phenyl ring (R_3') to further refine the SAR at this site. As outlined in entries **20**−**24** of Table 2, this position can accommodate a variety of heterocyclic moieties with different steric, electronic, and conformational properties. Of this group, the functionalities which lead to the most potent inhibitors share a conformational bias toward a nonplanar orientation with the distal phenyl ring. Nonplanarity of these two groups appears to be more important to FXa inhibitory activity than the electronics or basicity of the 3-position substituent (**16** vs **15**, **21** vs **22**, and **24** vs **23**).

X-ray crystallography studies of 2,6-diphenoxypyridine inhibitors in trypsin demonstrated that the bound conformation of these inhibitors is similar to that determined by Bode and co-workers for DX-9065a[4a] in both trypsin[11] and FXa.[12] The 1.8 Å resolution crystal structure of bovine cationic trypsin[13] was determined in complex with inhibitors **23** and **25**[14] and refined to *R*-factors of 15.5% and 18.7%, respectively (Figure 1).[15] Both **23** and **25** bind to the active site of trypsin in an extended L-shaped conformation (Figure 2),[16] resembling the conformation exemplified by DX-9065a and (*E*,*Z*)-BABCH[2] and different from the U-shaped conformation postulated for (*Z*,*Z*)-BABCH **(1)** in FXa.[2] In both

Table 2. In Vitro Optimization of 2,6-Diphenoxypyridine FXa Inhibitors



					enzyme inhibition[a] (K_i, nM)		
entry	X	P_4	R_6	R_3'	FXa	FIIa	trypsin
12	F	Me	H	NMe_2	160	2600	1900
13	F	Me	H	CH_2NMe_2	640	22000	
14	F	Me	H	$CONH_2$	280	>5000	>5000
15	F	Me	H	CONHMe	1300		
16	F	Me	H	$CONMe_2$	80	15000	2700
17	F	Me	NH_2	$CONMe_2$	14	2300	550
18	F	Me	OH	$CONMe_2$	1.2	1100	1100
19	F	Me	OMe	$CONMe_2$	740	>5000	4300
20	H	CO_2H	OH	$CONMe_2$	3.9	1100	300
21	H	CO_2H	OH	1-Me-2-imidazole	9.0	180	660
22	H	CO_2H	OH	1-imidazole	26	1400	1200
23	H	CO_2H	OH	2(1*H*)-imidazoline	85	>5000	1400
24	H	CO_2H	OH	1-Me-2(1*H*)-imidazoline	2.4	810	220

[a] K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$), and the standard deviations are <30% of the mean.[9]

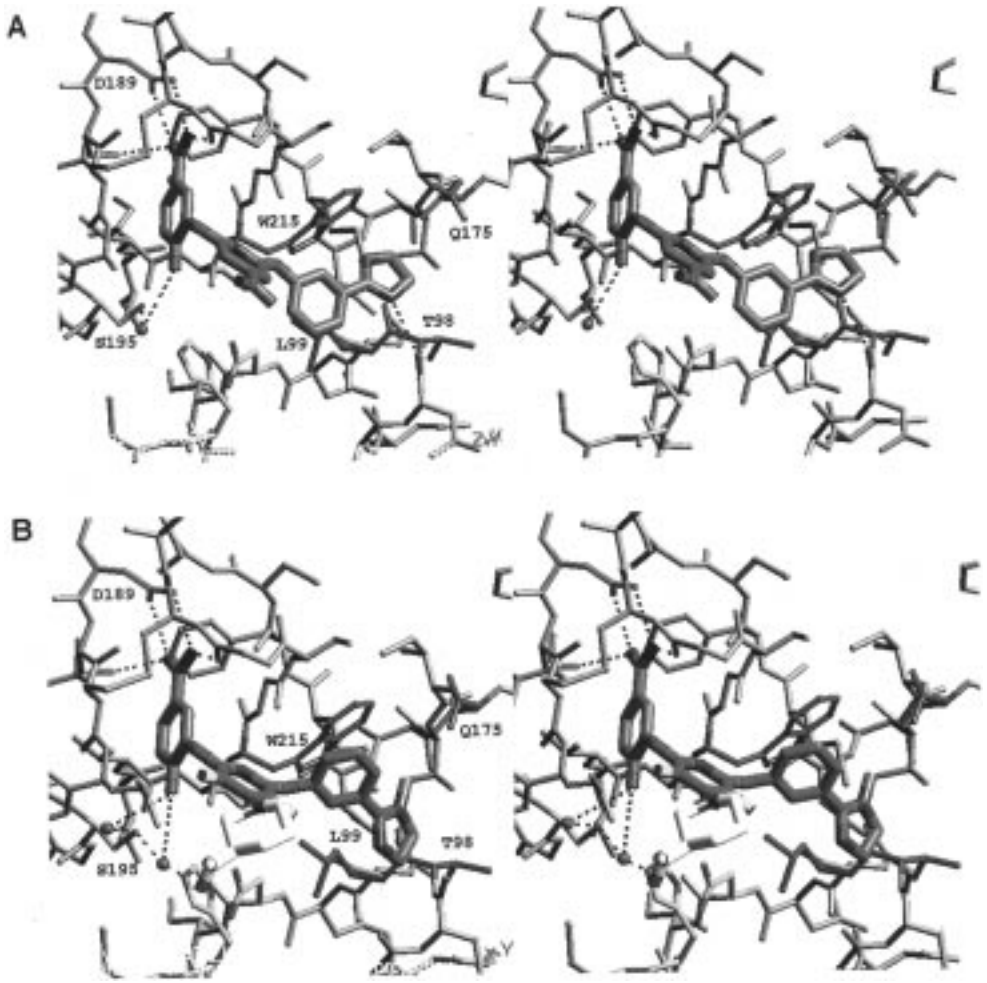


Figure 1. Stereoviews of the X-ray crystal structures in which (A) inhibitor **23** or (B) inhibitor **25**[14] is bound in the active site of bovine trypsin. The principle interactions between each inhibitor and the relevant residues are shown. Asp-189 (blue) is at the bottom of the S1 pocket. Thr-89, Leu-99, Gln-175, and Trp-215 (all shown in purple) surround the S4 pocket. The catalytic triad is formed by residues Ser-195, His-57, and Asp-102, highlighted in orange.

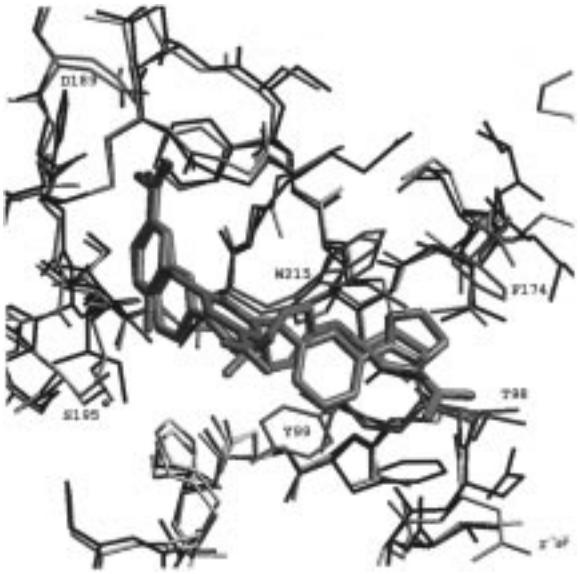
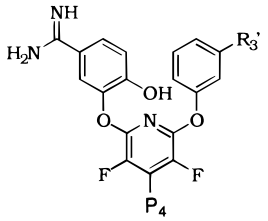


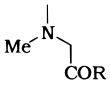
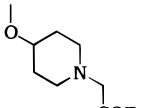
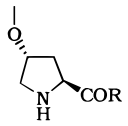
Figure 2. Overlay of inhibitor **23** (with green carbons) bound to bovine trypsin (gray) and DX-9065a (with blue carbons) bound in factor Xa (black). Asp-189 (blue) is at the bottom of the S1 pocket. Thr-89, Tyr/Leu-99, Phe-174/Gln-175, and Trp-215 (all shown in purple) surround the S4 pocket. The catalytic triad is formed by residues Ser-195, His-57, and Asp-102, highlighted in orange.

crystal structures, the amidine forms a salt bridge with Asp-189,[17] while the 6-hydroxyl group forms a hydrogen-bond with a water molecule, which in turn is hydrogen bonded to the hydroxyl group of the catalytic triad Ser-195. Two slightly different conformations are seen for distal phenyl groups containing either 2(1*H*)-imidazoline **23** (Figure 1A) or 1-methyl-(1*H*)-imidazoline (**25** in Figure 1B). Both conformations bind to the arylbinding site as defined by Bode[18] which exists as a shallow groove formed by Leu-99, Gln-175, and Trp-214.[19] However, addition of a methyl group to the 1-position of the 2(1*H*)-imidazoline ring in **25** induces a 75° rotation of the distal phenyl group around the distal oxygen−C1 bond which appears to disrupt the hydrogen bond between the 2(1*H*)-imidazoline ring of **23** and the carbonyl oxygen of Asn-97 in trypsin (Figure 1A,B). The different conformations observed for the distal phenyl groups of **23** and **25** may explain the enhanced potency of inhibitors **18**, **20**, **21** and **24** which all share a conformational bias toward a nonplanar orientation between the 3-substituent (R_3') and the distal phenyl ring (Table 2).

Once the optimal substituents for potency and selectivity were identified in the proximal and distal phenoxy groups, the core pyridine ring then became the focus of the optimization effort. Amino acid derivatives were

Table 3. In Vitro and in Vivo Optimization of 2,6-Diaryloxypyridine FXa Inhibitors



				Enzyme Inhibition (K_i, nM)			PT Extension (Human)	Inhibitor Plasma Concentration (μM):[b] Oral Dosing in Dogs		
Entry	**P_4**	**R**	**R_3'**	**FXa**	**FIIa**	**Trypsin**	**(nM)[a]**	**1.0 h**	**2.0 h**	**4.0 h**
26		**OH**	$CONMe_2$	0.41	4500	1100	1000	0.3	0.4	0.0
2		**OH**	1-Me-2-(1H)-Imidazoline	0.11	2000	280	230	3.0	2.1	0.7
27		**OEt**	1-Me-2-(1H)-Imidazoline	0.26	630	240	630	0.2	0.2	0.1
28		**OH**	$CONMe_2$	0.65	192	770	850	0.3	0.1	0.0
29		**OH**	1-Me-2-(1H)-Imidazoline	0.24	125	330	220	5.5	3.8	1.2
30		**OEt**	1-Me-2-(1H)-Imidazoline	0.31	190	300	240	0.3	0.3	0.2
31		**OEt**	$CONMe_2$	0.71	280	740	1400	0.3	0.2	0.1
32		**OH**	1-Me-2-(1H)-Imidazoline	0.33	250	310	670	3.2	2.2	0.8
33		**OEt**	1-Me-2-(1H)-Imidazoline	0.43	140	330	300	3.5	3.4	2.6

[a] Concentration which gives a 2-fold extension.[23] [b] All doses were 10 mg/kg po ($n = 3$) unless otherwise indicated. Plasma concentrations determined by FXa inhibition assay.[20]

introduced into the 4-position of the pyridine nucleus to optimize the in vitro potency and selectivity and in vivo pharmacokinetic properties of the 2,6-diphenoxypyridine template. Three representative amino acids (sarcosine, 4-hydroxypiperdine acetic acid, and 4(*R*)-hydroxy-2(*S*)-proline) are illustrated in Table 3 with variations of the distal ring substituent (R_3') and the amino acid carboxylate substitution (acids and esters). Two important SAR trends emerged from these data. First, the incorporation of amino acids into the 4-position of the pyridine nucleus enhanced the FXa inhibitory activity by 10-fold without loss of selectivity. In addition, the FXa inhibitory activity for this series appeared to be independent of the amino acid carboxylate substitution in that both the carboxylic acids and the corresponding esters were equipotent (**2** vs **27**, **29** vs **30**, and **32** vs **33**). In this final optimization step, introduction of amino acid derivatives into the 4-position of the pyridine nucleus afforded sub-nanomolar FXa inhibitors with excellent selectivity against related serine proteases and potent in vitro anticoagulant activities.

The plasma levels following oral dosing of these advanced FXa inhibitors were determined in pharmacokinetic studies in the dog. Conscious beagles (10−16 kg) were dosed via oral gavage with homogeneous solutions of inhibitors dissolved in aqueous acid solutions. Inhibitor concentrations were determined at various time points with an established ex vivo FXa inhibition activity assay.[20] As outlined in Table 3, several of the compounds (entries **2**, **29**, **32**, and **33**) reached and sustained micromolar concentration levels for up to 4 h. Surprisingly, FXa inhibitors with the highly basic 3-imidazoline substituent ($pK_a = 10.6$) in the distal phenyl ring showed higher plasma levels upon oral administration than analogues with neutral groups. For example, inhibitors containing the *N*,*N*-dimethylcarboxamide moiety (**26**, **28**, and **31**) in the distal phenyl ring displayed negligible plasma levels at all time points. A strategy to enhance the oral bioavailability of these inhibitors through increasing their overall lipophilicity by introduction of ester functionalities provided mixed results (**27** or **30** vs **33**).[21]

Selected 2,6-diphenoxypyridines were evaluated in pharmacokinetic studies in several additional species including rat, rabbit, guinea pig, hamster and mouse.[22] Pharmacokinetic studies were completed with inhibitors **2**, **29**, and **33** in conscious, restrained nonhuman primates, baboons and cynomolgus monkeys (Table 4), with the expectation that profiles would be similar to humans. In contrast to the results obtained in dogs, inhibitor **2** demonstrates a superior profile in these nonhuman primates and appears to have a much longer absorption phase than in dogs. For example, maximum plasma levels of **2** after oral dosing were not observed until after 4 h in the baboon and persisted for up to 6 h when measured by plasma level or anticoagulant activity. Anticoagulant activity was assessed using prothrombin time (PT)[23] or activated clot time (ACT) measurements. After administration of a single 10 mg/kg oral dose in baboon, the PT was extended 2.0-, 3.1-,

Table 4. Plasma Kinetics of Selected 2,6-Diaryloxypyridines in Nonhuman Primates Following Oral Dosing[a]

		plasma concentration (μM)[b]					
entry	species	n[c]	1 h	2 h	3 h	4 h	6 h
2	baboon	4	0.3	0.6	0.9	1.1	1.0
2	cynomolgus monkey	3	0.2	0.5	0.7	1.2	
29	baboon	3	0.1	0.0	0.1	0.2	0.7
29	cynomolgus monkey	3	0.1	0.0	0.0		
33	baboon	2	0.2	0.3	0.6	0.5	0.3
33	cynomolgus monkey	3	0.0	0.0	0.0		

[a] For both primate species an oral dose of 10 mg/kg in either 2% carboxymethylcellulose (cynomolgus monkeys) or 1.5% carboxymethylcellulose and 1% Tween 80 (baboons) was administered via a nasogastric tube. [b] Plasma concentration determined via HPLC (cynomolgus monkey) or FXa inhibition assay (baboon). [c] Number of animals.

and 3.5-fold at 1, 3, and 6 h, respectively. Following these encouraging results, plasma levels of **2** were determined in both primate species, following an intravenous dose of 0.5 mg/kg in 6% DMSO administered via the cephalic vein ($n = 3$). The beta-phase elimination half-life is 1.5−2 h and appears to be independent of whether plasma concentrations were determined by HPLC or FXa inhibition analysis. The close correlation found by comparing HPLC analysis of the parent compound to the FXa activity assay suggests the absence of circulating metabolites.

In this communication, we have outlined our systematic synthetic approach for transforming the photochemically unstable FXa inhibitor (*Z*,*Z*)-BABCH **(1)** into a synthetically viable inhibitor template derived from 2,6-diphenoxypyridine. While the initial inhibitors in this series demonstrated modest FXa inhibitory potency, optimization efforts led to a 1000-fold enhancement in FXa inhibitory activity while maintaining excellent selectivity against the related proteases thrombin and trypsin. On the basis of the FXa inhibitory potency, selectivity, and oral bioavailability data in the dog and nonhuman primates, compound **2** has been chosen for further preclinical evaluation. The medicinal chemistry, biochemistry, pharmacokinetics and pharmacology of this highly potent, selective, and orally active FXa inhibitor will be reported in detail in future publications.

Acknowledgment. We thank Dr. Michael Hildebrand, Schering AG, for his help with the primate studies and Drs. Les Browne, William Dole, and John Morser for their support and encouragement of this effort.

Supporting Information Available: Elemental analyses and NMR spectral data (8 pages). Ordering information is given on any current masthead page.

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(1) This manuscript is dedicated to the memory of our friend and mentor Allan D. Rudzik, Ph.D.

(2) Shaw, K. J.; Guilford, W. J.; Dallas, J. L.; Koovakaat, S. K.; Liang, A.; Light, D. R.; Morrissey, M. M. (*Z*,*Z*)-2,7-Bis(4-amidinobenzylidene)cycloheptan-1-one: Identification of a Highly Active Inhibitor of Blood Coagulation Factor Xa. *J. Med. Chem.* **1998**, *41*, 3551−3556.

(3) The following abbreviations are used: BABCH, 2,7-bis(4-amidinobenzylidene)cycloheptan-1-one; FXa, human factor Xa; IIa, human thrombin; trypsin, bovine cationic trypsin; PT, prothrombin time; ACT, activated clotting time.

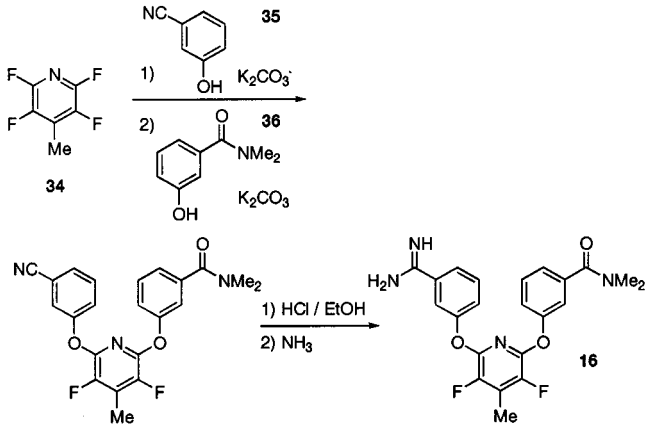
(4) Published examples of nonpeptide FXa inhibitors include both dibasic and monobasic templates. Dibasic templates: (a) Nagahara, T.; Yokoyama, Y.; Inamura, K.; Katakura, S.-I.; Komoriya, S.; Yamaguchi, H.; Hara, T.; Iwamoto, M. Dibasic (Amidinoaryl)-propanoic Acid Derivatives as Novel Blood Coagulation Factor Xa Inhibitors. *J. Med. Chem.* **1994**, *37*, 1200−1207. (b) Sato, K.; Kawasaki, T.; Hisamichi, N.; Taniuchi, Y.; Hirayama, F.; Koshio, H.; Matsumoto, Y. Antithrombotic Effects of YM-60828, A Newly Synthesized Factor Xa Inhibitor, in Rat Thrombosis Models and Its Effects on Bleeding Time. *Br. J. Pharm.* **1998**, *123*, 92−96. (c) 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. Identification and Initial Structural-Activity Relationships of a Novel Class of Nonpeptide Inhibitors of Blood Coagulation Factor Xa. *J. Med. Chem.* **1998**, *41*, 437−450. (d) Maduskuie, T. P.; McNamara, K. J.; Ru, Y.; Knabb, R. M.; Stouten, R. W. Rational Design and Synthesis of Novel, Potent Bis-phenylamidine Carboxylate Factor Xa Inhibitors. *J. Med. Chem.* **1998**, *41*, 53−62. Monobasic templates: (e) Katz, B. A.; Clark, J. M.; Finer-Moore, J. S.; Jenkins, T. E.; Johnson, C. R.; Ross, M. J.; Luong, C.; Moore, W. R.; Stroud, R. M. Design of Potent Selective Zinc-mediated Serine Protease Inhibiors. *Nature* **1998**, *391*, 608−612. In addition, novel peptide FXa inhibitors have recently been described: (f) Ostrem, J. A.; Al-Obeidi, F.; Safar, P.; Safarova, A.; Stringer, S. K.; Patek, M.; Cross, M. T.; Spoonamore, J.; LoCascio, J. C.; Kasireddy, P.; Thorpe, D. S.; Sepetov, N.; Lebl, M.; Wildgoose, P.; Strop, P. Discovery of a Novel, Potent, and Specific Family of Factor Xa Inhibitors via Combinatorial Chemistry. *Biochemistry* **1998**, *37*, 1053−1059.

(5) (a) Phillips, G. B.; Davey, D. D.; Guilford W. J.; Eagen, K.; Ng, H.; Pinkerton, M.; Koovakkat, S.; Wu, S.; Xu, W.; Liang, A.; Trinh, L.; Hinchman, J.; Post, J.; Whitlow, M.; Morrissey, M. M. Design, Synthesis, and Biological Activity of Novel Factor Xa Inhibitors − 2. 2,6-Diphenoxypyridine Inhibitors. Presented at the 215th American Chemical Society National Meeting, March 29−April 2, 1998, Dallas, TX, Abstract 122. (b) Buckman, B. O.; Mohan, R.; Liang, A.; Trinh, L.; Morrissey, M. M. Design, Synthesis, and Biological Activity of Novel Factor Xa Inhibitors − 6. Bicyclic Pyrimidine Analogues of 2,6-Diphenoxypyridine Inhibitors. Presented at the 215th American Chemical Society National Meeting, March 29−April 2, 1998, Dallas, TX, Abstract 126. (c) Griedel, B. D.; Arnaiz, D. O.; Sakata, S. T.; Shaw, K. J.; Zhao, Z.; Dallas, J.; Koovakkat, S.; Whitlow, M.; Liang, A.; Trinh, L.; Hinchman, J.; Post, J.; Ho, E.; Subramanyam, B.; Vergona, R.; Walters, W.; White; Sullivan, M. E.; Morrissey, M. M. Design, Synthesis, and Biological Activity of Novel Factor Xa Inhibitors − 9. Benzimidazole-Based Analogues. Presented at the 215th American Chemical Society National Meeting, March 29−April 2, 1998, Dallas, TX, Abstract 129. (d) Chou, Y.-L.; Guilford W. J.; Koovakkat; Mohan, R.; Wu, S. C.; Liang, A.; Trinh; Morrissey, M. M. Design, Synthesis, and Biological Activity of Novel Factor Xa Inhibitors − 10. Optimization of Dibenzyl Cyclic Urea Analogues. Presented at the 215th American Chemical Society National Meeting, March 29−April 2, 1998, Dallas, TX, Abstract 130.

(6) The MOPAC progam available in the Cerius2 molecular modeling package, Molecular Simulations, Inc., was used for AM1 semiempirical calculations to study the relative energies for torsion about the C−O−C bonds of 2-phenoxypyridine. A one-dimensional torsional potential was generated for the C−O−C torsion from 0 to 180° in steps of 10°. The energy differences for two cases, 2-phenoxypyridine and 3-chloro-2-phenoxypyridine, are 4.7 and 7.4 kcal/mol, respectively. The energy for the torsion angle of 0° are lower in both cases. Stewart, J. J. P. MOPAC: A General Molecular Orbital Package (Version 6.0), Frank J. Seiler Research Laboratory, U.S. Air Force Academy, Colorado Springs, CO 80840.

(7) Drendel, W. B.; Sundaralingam, M. Structures of 1,4-Bis(3,5-dichloro-2-pyridyloxy)benzene (1), $C_{16}H_8Cl_4N_2O_2$, 1,4-Bis(5-chloro-2-pyridyloxy)benzene (2), $C_{16}H_{10}Cl_2N_2O_2$ and 1,4-Bis(3-chloro-2-pyridyloxy)benzene (3), $C_{16}H_{10}Cl_2N_2O_2$. *Acta Crystallogr.* **1985**, *C41*, 950−953.

(8) (a) All new compounds gave physical and spectroscopic data consistent with their structure. See Supporting Information for details. (b) The general synthesis of the FXa inhibitors described herein is outlined in the synthesis of compound **16**: Pyridine **34** was coupled first with phenol **35**, then with phenol **36** in the presence of a mild base (K_2CO_3). The resulting nitrile was converted to an amidine by careful treatment with ethanolic hydrochloric acid, followed by ammonia to give compound **16**.



(9) Commercial purified serine proteases, human factor Xa and thrombin (Enzyme Research Laboratories, Inc., South Bend, IN), and bovine cationic trypsin (Boehringer Mannheim, Corp., Indianapolis, IN) were assayed in 150 mM NaCl, 2.5 mM $CaCl_2$, 50 mM Tris-HCl, pH 7.5, and 0.1% PEG 6000 in a final assay volume of 200 μL at room temperature with Chromogenix substrates (Kabi Pharmacia Hepar, Inc., Franklin, OH). The final concentrations of enzyme and substate were: Factor Xa (1 nM) and S-2222 (164 μM), thrombin (16 nM) and S-2302 (300 μM), and trypsin (16 nM) and S-2266 (127 μM). Inhibition mechanism is competitive; therefore a substrate concentration equal to K_m was used to assay varying concentrations of the inhibitor to determime the K_i. K_i's of very potent inhibitors (<3 nM) were determined by fitting data obtained at 0.3 and 1 nM FXa to a modification of the Morrison equation to correct for the proportion of inhibitor bound to the enzyme relative to the free (a) Jordan, S. P.; Waxman, L.; Smith, D. E.; Vlasuk, G. P. Kinetic Analysis of the Recombinant Inhibitor with Blood Coagulation Factor Xa. *Biochemistry* **1990**, *29*, 11095−11100. (b) Morrison, J. F. Kinetics of the reversible inhibition of enzyme-catalysed reactions by tight-binding inhibitors. *Biochim. Biophys. Acta* **1969**, *185*, 269−286.

(10) The inhibitory activity of this new structural element was regiospecific since a hydroxyl moiety introduced into the 2-position of the proximal phenyl ring resulted in the complete loss of inhibitory activity (data not shown).

(11) Stubbs, M. T.; Huber R.; Bode, W. Crystal Structures of Factor Xa Specific Inhibitors in Complex with Trypsin: Structural Grounds for Inhibition of Factor Xa and Selectivity Against Thrombin. *FEBS Lett.* **1995**, *375*, 103−107.

(12) Brandstetter, H; Kühne, A.; Bode, W.; Huber, R.; von der Saal, W.; Wirthensohn, K.; Engh, R. A. X-ray Structure of Active Site-inhibited Clotting Factor Xa − Implications for Drug Design and Substrate Recognition. *J. Biol. Chem.* **1996**, *271*, 29988−29992.

(13) Several active site amino acid side chains differ between human FXa and bovine trypsin which may alter the environment near the aryl binding site. For example, Glu-97 (FXa) is replaced by Asn (trypsin), Tyr-99 by Leu, Glu-217 by Ser, and the side chain of Phe-174 occupies a position similar to that of Gln-175 in trypsin. Therefore, subtle differences in the active site of FXa and trypsin may lead to unique binding orientations for this series of inhibitors in these related enzymes. The binding mode for this class of inhibitors with FXa can only be proven unambiguously once the requisite FXa−inhibitor complexes are formed and their structures determined experimentally.

(14) Inhibitor **25** (K_i (FXa) = 0.11 nM; K_i (trypsin) = 165 nM) is an analogue of **24** (Table 2) in which the carboxy group at P4 is replaced by an *N*-[3-carboxypiperidinyl] group.

(15) Whitlow, M.; Seto, M.; Koovakkat, S. Structural Studies of Factor Xa Inhibitors. Presented at the 215th American Chemical Society National Meeting, March 29−April 2, 1998, Dallas, TX, Abstract 133.

(16) The crystal structure of bovine trypsin−inhibitor **10** complex revealed that related bis amidine FXa inhibitors bound in a conformation similar to that of **23** and **25**.

(17) The numbering used for trypsin residues is based on topological equivalence to chymotrypsin: Stubbs, M. T.; Bode, W. A. Player of Many Parts: The Spotlight Falls on Thrombin's Structure. *Thromb. Res.* **1993**, *69*, 1−58.

(18) Bode, W., Turk, D. and Karshikov, A. The Refined 1.9Å X-ray Crystal Structure of D-Phe-Pro-Arg Chloromethylketone-inhibited Human a-Thrombin. Structure Analysis, Overall Structure, Electrostatic Properties, Detailed Active Site Geometry, Structure Function Relationships. *Protein Sci.* **1992**, *1*, 426−471.

(19) These residues make up the aryl binding site (S4) which is a continuation of an apolar binding subsite starting at S2. Pharmacophores on nonpeptide inhibitors do not necessarily map directly to the peptide side chains which define the S2 to S4 subsites. (a) Padmanabhan, K.; Padmanabhan, K. P.; Tulinsky, A.; Park, C. H.; Bode, W.; Huber, R.; Blankenship, D. T.; Cardin, A. D.; Kisiel, W. Structure of Human Des(1−45) Factor Xa at 2.2Å Resolution. *J. Mol. Biol.* **1993**, *232*, 947−966. (b) Rezaie, A. R. Role of Leu99 of Thrombin in Determining the P2 Specificity of Serpins. *Biochemistry* **1997**, *36*, 7437−7446.

(20) Hara, T.; Yokoyama, A.; Ishihara, H.; Yokoyama, Y.; Nagahara, T.; Iwamoto, M. *Thromb. Hemostasis* **1994**, *71*(3), 314−319.

(21) The FXa activity assay employed to determine plasma inhibitor levels ex vivo would not discriminate between a specific carboxylic ester and the corresponding carboxylic acid which resulted from ester hydrolysis in vivo. However, the inhibitor plasma concentrations for **27**, **30**, and **33** in Table 3 reflect the total concentration of these specific ester/acid pairs since the K_i values for FXa inhibition with the carboxylic acid/ester pairs (**2** and **27**, **29** and **30**, **32** and **33**) are nearly identical and are well below the concentration of FXa in the assay (3 nM).

(22) Subramanyam, B., Ho, E., Walters, J., Cheesman, S. Guilford, W., Vergona, R., White, K., Phillips, G., Davey, D., M. Morrissey, Light, D., Morser, J., Sullivan, M. The Pharmacokinetics of BX-807834 (BX), A Potent, Selective Factor Xa Inhibitor in Animals. Presented at the FASEB Experimental Biology 98 Meeting, April 18−22, 1998, San Francisco, CA, Abstract 4169.

(23) Prothrombin times (PT) were determined using either pooled lyophylized human plasma (SARP, Helena Laboratories, Beaumont, TX) or non human primate plasma samples, as indicated, and were performed using Thromboplastin IS (Baxter Healthcare Corporation, Dade Division, Miami, FL) in an MLA Electra 900C coagulation analyzer (Medical Laboratory Automation, Inc., Pleasantville, NY).

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