Design, Synthesis, and Activity of 2,6-Diphenoxypyridine-Derived Factor Xa Inhibitors[1,2]

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A novel series of 2,6-diphenoxypyridines has been designed to inhibit factor Xa, a serine protease strategically located in the coagulation cascade. The evolution from the photochemically unstable bisamidine (*Z*,*Z*)-BABCH to potent bisamidine compounds with a pyridine heterocycle as the core scaffold has been achieved. The most potent compound in the series, **6h**, has a K_i for human factor Xa of 12 nM. The selectivity of **6h** against bovine trypsin and human thrombin was greater than 90- and 1000-fold, respectively. Two proposed modes of binding of **6h** to factor Xa are made based on the crystal structures of **6h** by itself and of **6h** bound to bovine trypsin.

Introduction

There is a large unmet clinical need for orally active antithrombotics. The only agents available, aspirin and coumadin, have problems with the control of bleeding and subsequent safety.[3] Efforts to find new antithrombotics have been directed at specifically inhibiting a single serine protease along the coagulation cascade.[4] Many of those efforts have concentrated at inhibiting thrombin, the final serine protease along the cascade and the enzyme directly responsible for the conversion of fibrinogen to fibrin.[5] Another enzyme along the coagulation cascade, factor Xa (FXa), has been the subject of intensive research that has culminated in the discovery of nonpeptidic small-molecule inhibitors of FXa (Figure 1).[6] FXa is strategically located at the juncture of the intrinsic and extrinsic arms of the cascade, and preclinical data indicates that inhibition of this key enzyme would control the coagulation process and minimize bleeding problems. Specifically, Sitko has directly compared the parentally administered thrombin inhibitor hirudin with the FXa inhibitor tick anticoagulant peptide, and the data suggests the FXa inhibitor to have a superior therapeutic ratio.[7] In addition, due to the amplification nature of the coagulation cascade, a small amount of FXa produces a large amount of thrombin, leading to the belief that inhibition of FXa may be more efficient than inhibition of thrombin. For these reasons we initiated a program to discover potent, selective, and orally active inhibitors of FXa.

We have recently reported on (*Z*,*Z*)-BABCH (2,7-bis(amidinobenzylidine)cycloheptan-1-one, **4**), a FXa inhibitor that is potent and selective against two other serine proteases (FXa K_i, 0.66 nM; thrombin K_i, 530 nM; bovine trypsin K_i, 33 nM).[8] Those studies demonstrated that the double-bond geometry is critical for FXa activity, the corresponding (*Z*,*E*)- and (*E*,*E*)-isomers being much less potent than the (*Z*,*Z*)-isomer. The potency and selectivity of the conformationally restricted inhibitor **4** was very encouraging, and we theorized that the U-shape of the molecule and the resulting positioning of the amidines were critical for the FXa potency. While the benzamidine moieties are structurally constrained about the (*Z*)-olefins, the photolytic lability of inhibitor **4** may limit its utility as an anticoagulant. Inhibitor **4** readily isomerizes to a mixture of double-bond isomers in light. We embarked on synthetic efforts to explore alternate scaffolds to the cycloheptadienone motif that could adopt a U-shaped conformation and would be more chemically stable and amenable to a final drug candidate (Figure 2). Herein, we describe the utilization of 2,6-diphenoxypyridines as a scaffold that allows the benzamidines to adopt a position similar to those of **4**, leading to potent and selective inhibitors of FXa.

Chemistry

Compounds containing a pyridine, pyrazine, or pyrimidine as the central scaffold and two of the same amidinophenoxy moieties were prepared by the reaction of 2 equiv of a cyanophenol and excess base with the appropriately substituted dihaloheterocycle. (Scheme 1 illustrates the preparation of pyridine analogues. Pyrazines and pyrimidines are prepared in an analogous manner.) Conversion of the nitriles to the amidines was carried out using the Pinner reaction.[9] Groups on the central scaffold were modified appropriately prior to imidate formation. Unsymmetrical compounds were prepared by reacting 1 equiv of the phenol at lower temperatures and elevating the temperature for the second addition. The unsymmetrical compounds could be prepared in a single reaction vessel. Attempts at preparing compounds with nitrogen linking the central scaffold with the benzamidine by adding 2 equiv of the corresponding primary cyanoaniline under similar conditions were unsuccessful. Regardless of the number of equivalents of aniline used, only the compound resulting from a single addition was isolated. Presumably, formation of the nitrogen anion stabilized by two aromatic groups renders the remaining electrophilic center of the pyridine much less reactive. The intermediate monoadduct was methylated on nitrogen and the second equivalent of cyanoaniline added smoothly.

The compounds containing a phenyl group as the central scaffold were prepared by a reverse addition

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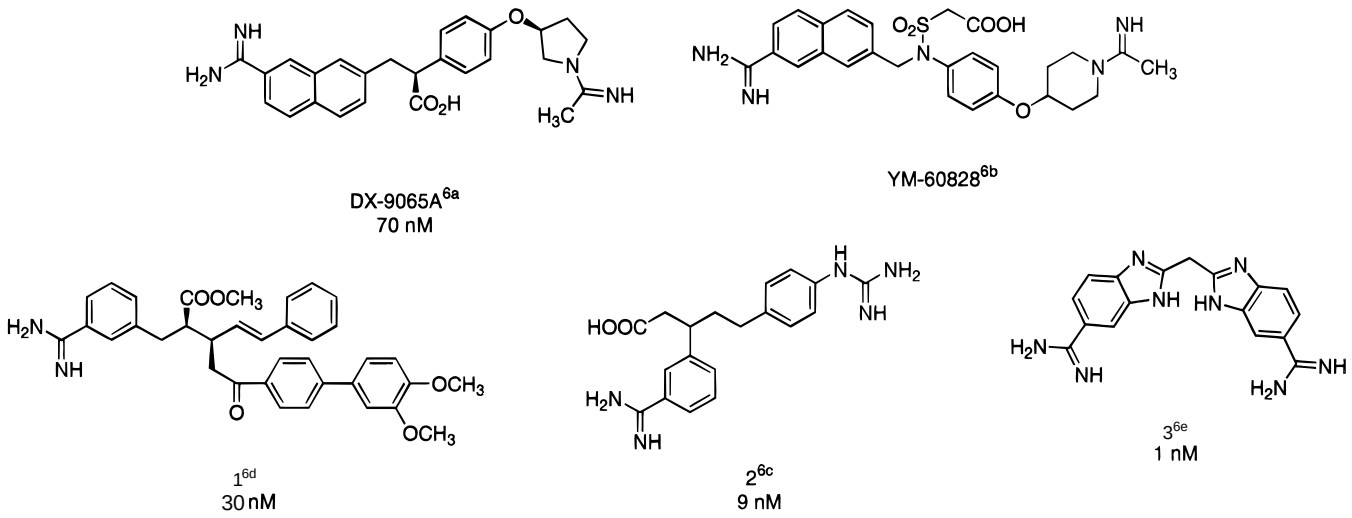


Figure 1. Structures of nonpeptidic FXa inhibitors.

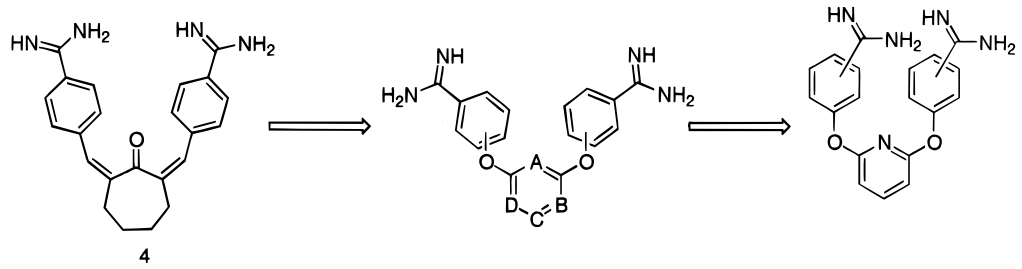


Figure 2.

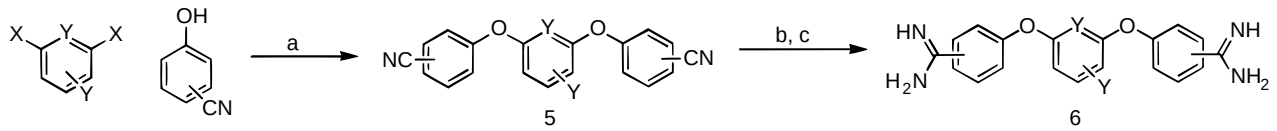
sequence (Scheme 2).[10] Reaction of the appropriately substituted resorcinol with 4-fluorobenzonitrile and sodium hydride gave the desired ethers. Pinner reaction of the nitriles produced the bisamidines. Nitrogen-substituted phenyl derivatives were prepared by the method outlined in Scheme 1, in that phenols were added to 2,4-difluoronitrobenzene.

The pyrazinone **9** was prepared in two steps from the pyrazine **7** (Scheme 3).[11] Reaction of the pyrazine **7** with mCPBA gives the pyrazine *N*-oxide **8**. Treatment of **8** with acetic anhydride causes a rearrangement to the intermediate acetoxypyrazine, which hydrolyzes to the pyrazinone **9** on workup.

Results and Discussion

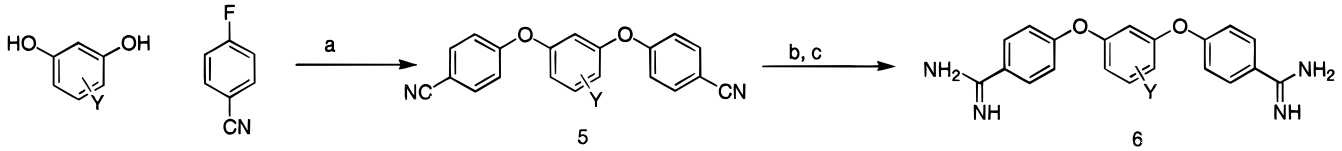
Compounds were initially screened against human FXa to determine potency. Screening against human thrombin (FIIa) for selectivity within the coagulation cascade and bovine trypsin for general specificity against serine proteases was carried out for the more potent

Scheme 1[a]



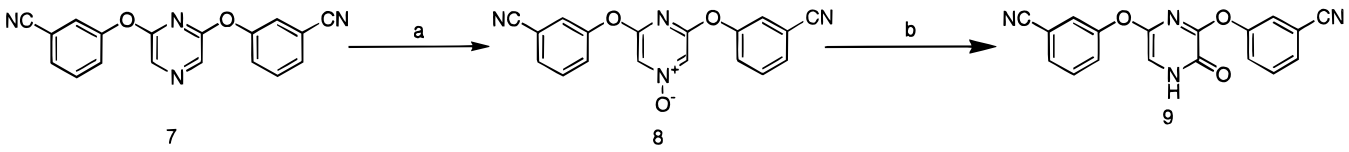
[a] Conditions: (a) NaH, DMF, 90 °C, 24 h or K_2CO_3/DMSO, 16 h, 90 °C; (b) HCl/EtOH, 16 h; (c) NH_3/EtOH, 2 h, 100 °C.

Scheme 2[a]



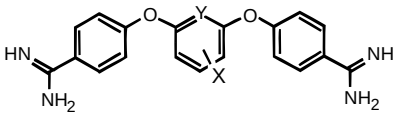
[a] Conditions: (a) K_2CO_3/DMSO, 16 h, 90 °C; (b) HCl/EtOH, 16 h; (c) NH_3/EtOH, 2 h, 100 °C.

Scheme 3[a]



[a] Conditions: (a) H_2O_2/AcOH, 80 °C, 2 days; (b) TFA/DMF, 4 h.

Table 1. Activity of Para-Substituted Bis(amidines)



6

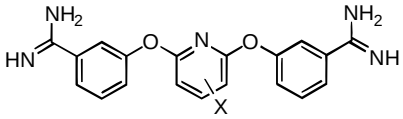
			K_i (nM)		
compd	Y	X	FXa	FIIa	trypsin
4			0.66	530	33
6a	N	H	400	550	2800
6b	N	3,5-Cl	620	1400	2700
6c	CH	H	970	4900	1000
6d	CH	3,5-Cl	1100	2500	1800

compounds (FXa K_i < 1 μM). The activities of the three serine proteases were determined as the initial rate of the cleavage of peptide *p*-nitroanilide by the enzyme. All substrate concentrations used are equal to their K_m values under the present assay conditions.

Studies were initiated by preparing the compounds in which a pyridine and phenyl were substituted for the cycloheptanone and the linkages were changed from an olefin to an ether, compounds **6a** and **6c**, respectively (Table 1). These modifications resulted in a large decrease in potency and selectivity, presumably due to the loss of conformational rigidity. Substitutions were made on the central ring in an attempt to make the conformation in which both phenyl groups aligned in a parallel fashion to one another more energetically favorable and more similar to that in **4**. The substituted pyridine and phenyl analogues, **6b** and **6d**, respectively, showed no significant improvement compared to their unsubstituted analogues. The difference in the molecular orbital hybridization of the linking ether of **6a**−**d** from the olefin of **4** results in different dihedral angles between the benzamidines and the central template. Consequently, in a U-shaped conformation the two amidine moieties of **6a**−**d** would be pointing into one another, forcing the phenyl rings to skew. Even if these benzamidine groups were forced to get closer due to steric interactions with substituents on the central template, the individual amidines would be forced to bend away from one another.

We next studied meta-substituted compounds (Table 2, DX-9065A is included for comparison). Unlike the para-substituted benzamidines in which the positioning of the amidine is dependent solely on rotation about the pyridine ether bond, the meta-substituted benzamidines have more possible positions due to the additional possibility of rotation about the ether benzamidine bond. Compounds of this class were more potent than the para-substituted analogues, and selectivity toward FIIa has been improved. The potency results were opposite to those found for analogues of **4** in which the compound where the amidines are in the meta position were less potent than **4** by almost a factor of 10.[8] As in the para-substituted compounds, substitution adjacent to the ether linkages with chlorine, **6f**, did not enhance potency. However, the bisfluoro analogues, **6g** and **6h**, showed a 2- and 4-fold increase in potency compared with **6e**. The primary amide **6r** is interesting because of the lower activity, especially as compared to the tertiary amide **6t** that differs only by the presence of

Table 2. Activity of Meta-Substituted Bis(amidines)



6

		K_i (nM)		
compd	X	FXa	FIIa	trypsin
DX-9065a		22	>5000	190
6e	H	53	14000	1100
6f	3,5-Cl	130	>5000	2100
6g	3,5-F	24	37000	1200
6h	3,5-F-4-Me	13	22000	810
6i	3,5-F-4-OMe	23	>5000	1100
6j	3-NH_2	53	>5000	1300
6k	3-NHAc	52	>5000	1200
6l	3-$NHSO_2Me$	130	>5000	1900
6m	3-NHCONHPh	62	3400	100
6n	3-NHCONHMe	59	>5000	840
6o	3-NHCOPh	120	3400	260
6p	3-$NHCOGlyNH_2$	110	>5000	280
6q	3-CF_3	110	>5000	780
6r	3-$CONH_2$	600	>5000	1200
6s	3-CONHMe	230	>5000	1400
6t	3-$CONMe_2$	32	>5000	780
6u	3-$COGlyNH_2$	690	>5000	2000
6v	3-COOEt	190	>5000	920
6w	3-COOH	95	>100000	2200
6x	4-$CONH_2$	42	6000	780
6y	4-CONHMe	60	4200	750
6z	4-COOEt	34	11000	800
6aa	4-COOH	59	27000	570

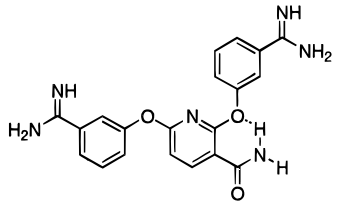
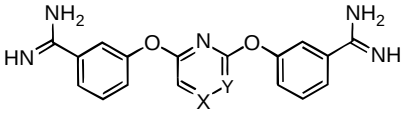


Figure 3. Potential intramolecular hydrogen bonding.

two methyl groups. The same loss of activity is seen with the secondary amide **6s**. We theorized that the primary and secondary amides could be in plane with the attached aromatic ring and have an intramolecular hydrogen bond between the amide N−H and the ether linkage resulting in a conformational restriction of the benzamidine (Figure 3). The restricted relationship of the groups is supported by a crystal structure of a similarly substituted system.[12] The tertiary amide could not interact with the ether in this manner, and the benzamidine is allowed to adopt a conformation independent of the ring substituent. Compounds **6j**−**p** were originally prepared to have an intramolecular hydrogen bond between the ether and nitrogen substituent, to force the benzamidine group away from the substitution of the central scaffold. Unlike the case of the amide **6r**, this substitution pattern did not have an effect on activity. The difference in the resulting ring size (five versus six) and consequently the angle at which the benzamidine radiates may account for the difference in activity. All of the 4-substituted compounds, **6x**−**aa**, have similar potency to the unsubstituted analogue, **6e**. In subsequent studies we have taken advantage of this result by changing the substituent in the 4-position to

Table 3. Activity of Alternate Heterocycles

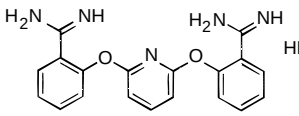


12

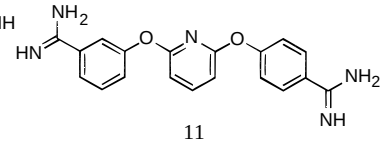
compd	X	Y	K_i (nM) FXa	FIIa	trypsin
12a	N	CH	51	>5000	970
12b	CH	N	120	>5000	760
12c	N^+O^-	CH	180	>5000	1300
12d	NH	CO	190	>5000	760

modulate the basicity of the molecule without a loss of inhibitory activity.

In addition to preparing meta- and para-substituted bisamidines, we also prepared compounds **10** and **11** to further explore the positioning of the amidines. The ortho-substituted compound **10** did not inhibit FXa or the other two proteases at the highest concentration tested (5 μM). This result was not surprising since the ortho substituents force the two aromatic moieties away from one another causing the molecule to be in an extended conformation. Unsymmetrical compound **11** has similar FXa potency to **6e** (K_i, 130 nM), but the selectivity toward thrombin (thrombin K_i, 1200 nM; trypsin K_i, 1900 nM) has been decreased. The selectivity difference associated with this modification illustrates a potential difference in the binding sites of FXa and thrombin, and in future studies, the substitution pattern of the amidines in **6e** was retained to take advantage of that presumed difference.

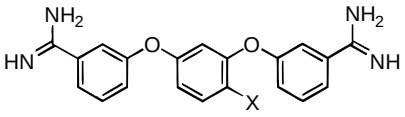


10
K_i >5000 nM



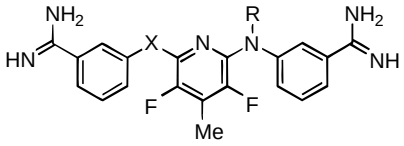
11
K_i =130 nM

To further explore the effects of modifications to the central portion of the template, substituted phenyl and alternate heterocycles were investigated. Changing the central template to either pyrimidine (**12a**), pyrazine (**12b**), or pyrazine *N*-oxide (**12c**) resulted in no enhancement of inhibitory potency (Table 3). The pyrazinone **12d** was prepared in an attempt to again restrict one of the benzamidines with a hydrogen bond between the ether linkage and the hydroxy tautomer of the pyrazinone. Unlike the results seen earlier when comparing **6j**−**n** with **6e**, there is a 4-fold potency difference between **12a** and **12d**. 3-Nitro- and amino-substituted phenyl derivatives were also prepared (**13**). Both compounds lost significant inhibitory activity when compared with their heterocyclic counterparts (K_i, 300 and 1100 nM for **13a** and **13b**, respectively).



13a X=NO_2, K_i=300 nM
13b X=NH_2, K_i=1100 nM

Table 4. Activity of Compounds with Nitrogen Linkers

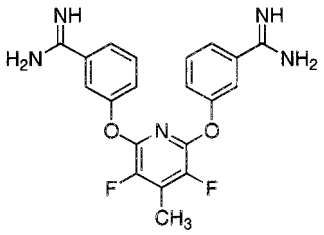


14

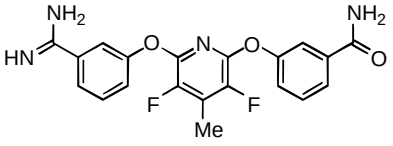
compd	X	R	K_i (nM) FXa	FIIa	trypsin
14a	O	H	660	>5000	3100
14b	O	Me	12	1900	690
14c	NMe	Me	98	360	360

Replacement of the ether linkages with nitrogens had a surprising effect on both potency and selectivity. Replacement of one oxygen of **6h** with a nitrogen (**14a**) reduced potency by more than 50-fold (Table 4). Replacement of the oxygen with an *N*-methyl group (**14b**) gave a compound of equivalent potency, but the potency for thrombin had been increased. Replacement of both oxygens with an *N*-methyl group (**14c**) gave a compound with decreased potency and little selectivity.

Compound **15** was isolated as an impurity in the preparation of **6h** and was found to have a K_i of 280 nM against FXa and >5000 nM against both FIIa and trypsin. The 20-fold loss of potency for this compound illustrates the importance of the second amidine. On the other hand, it also illustrates that selective compounds with a single amidine can be prepared with the potential for potency optimization. Further work on compounds containing a single amidine will be reported in a future publication.



6h



15

Models. The structure of aryloxypyridines is known to adopt a conformation in the solid state where the angle between the pyridine and phenyl ring is nearly perpendicular.[13] This is explained by allowing maximal conjugation between the more electron-rich pyridine ring and the ether oxygen. In addition, the phenyl ring adopts the conformation syn to the pyridine nitrogen. The syn conformation is explained by the absence of steric effects between the pyridine nitrogen and the phenyl. There is no loss of steric interactions with a biphenyl ether, and therefore, the U-shaped conformation is not as favorable as in a compound with a heteroatom adjacent to the ether linkage. Additionally, repulsion between the lone pairs of the pyridine nitrogen

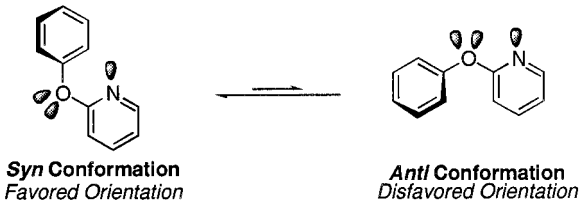


Figure 4. Conformations of phenoxypyridines.

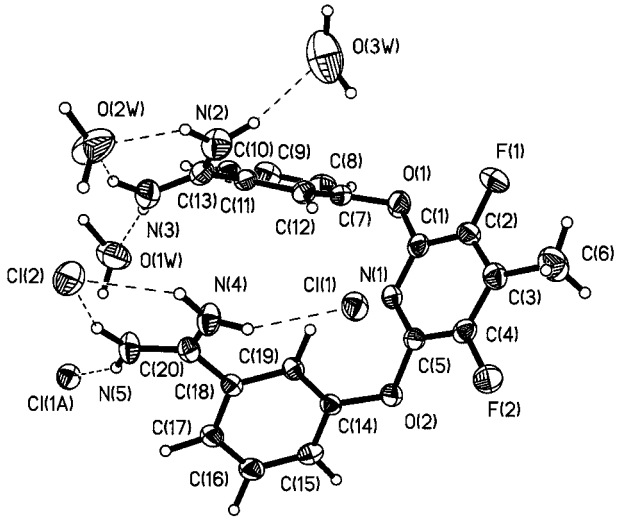


Figure 5. Thermal ellipsoid plot of **6h**.

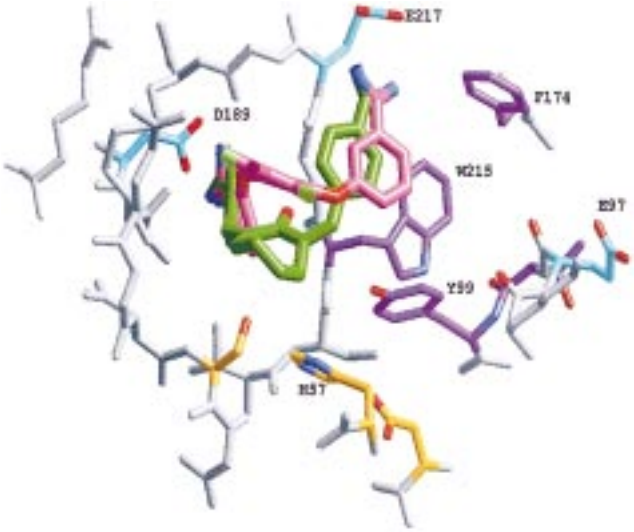


Figure 6. Proposed mode of binding of **6h** and **4** in FXa.

and the ether oxygen would cause an anti relationship between the electrons to be favored and again favor the U-shaped conformation (Figure 4). Consistent with the U-shaped conformation being preferred, the phenyl analogues (**6c**, **6d**, and **13b**) are not as potent as their heterocyclic counterparts (**6a**, **6b**, and **6j**, respectively).

The crystal structure of **6h** was determined by X-ray crystallography (Figure 5). The structure shows the two phenyls skewed slightly from parallel, with both amidines on the same side of the molecule. This conformation places the amidine moieties in close proximity to those initially modeled for inhibitor **4**. However, the relationship of the benzamidines in the crystal structure could be biased by the counterions in the solid state.

Two potential modes of binding were developed by using X-ray coordinates published by Brandstetter et al. for FXa.[14] One proposed mode of binding of **4** to FXa has one amidine binding through a salt bridge to Asp-189 in the P_1 pocket (Figure 6). This interaction is consistent with the interaction of other benzamidine-derived inhibitors bound to related serine proteases. The second amidine can bind through a second salt bridge to Glu-217. The structure of **6h** in FXa corresponds with the solid-state structure, and a model of compound **4** has been inserted for comparison purposes. Substituents on the pyridine are oriented into solvent and are not expected to have an effect on FXa binding as observed experimentally. An alternate mode of binding of **6h** to FXa could be similar to that found for DX-9065a in the crystal structure with FXa (Figure 7).[14] DX-9065a has one amidine binding through a salt bridge to Asp-189 in the S1 pocket, but the second aryl amidine is located in the S4 pocket. An extended conformation of **6h** has been modeled into this structure. This mode of binding is supported by the X-ray structure of **6h** cocrystallized with bovine trypsin (Figure 8). When extrapolating this mode of binding of **6h** to FXa, two points must be kept

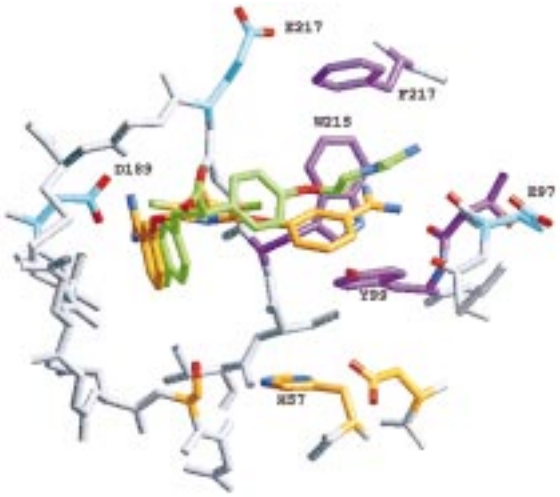


Figure 7. Overlapping mode of binding of **6h** in the crystal structure of DX-9065a and FXa.

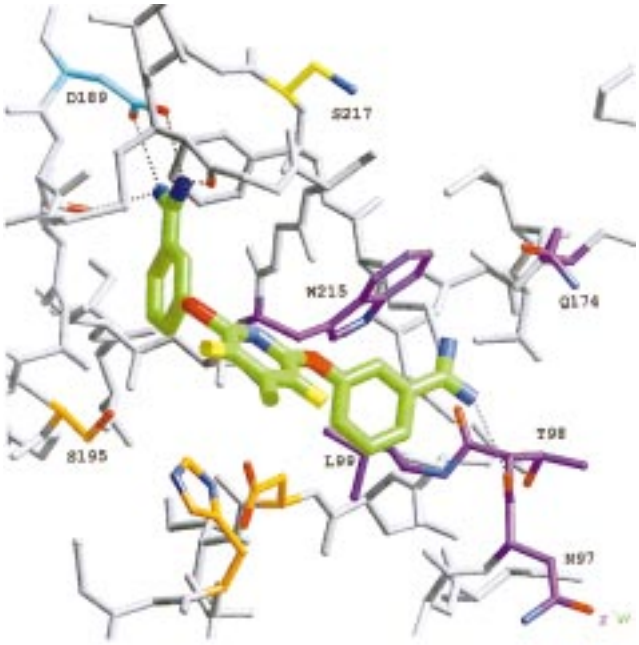


Figure 8. Crystal structure of **6h** in trypsin.

in mind: first, the large difference in binding energy found for **6h** in FXa and bovine trypsin (13 versus 810 nM) and second, in trypsin, the amino acid that replaces Glu-217 of FXa in the binding pocket is a serine. Therefore, the conformation of **6h** binding illustrated in Figure 8 would not be as highly favorable in FXa as in trypsin. Additionally, the binding mode illustrated in Figure 6 would not be as favorable in trypsin as FXa.

Conclusion

Beginning with the potent, selective, and sterically constrained FXa inhibitor **4**, a novel series of potent inhibitors of human FXa have been designed and prepared that show selectivity over two related serine proteases, human thrombin and bovine trypsin. Two potential modes of binding are proposed, one that is consistent with the X-ray crystal structure of an inhibitor **6h** and a second model based on the crystal structure of **6h** bound to bovine trypsin. Further efforts directed toward the replacement of one of the benzamidines, identification of additional sites of binding, and optimization of related inhibitors with oral availability will be presented in future publications.

Experimental Section

All melting points were taken on a capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ, and results were within ±0.4% of the calculated values. NMR spectra were recorded with a Varian XL-300 spectrometer and were consistent with the assigned structures.

Human FXa and human thrombin were from Enzyme Research Lab., South Bend, IN, and bovine trypsin was from Boehringer Mannheim Corp., Indianapolis, IN. All peptide-*p*-nitroanilide substrates were purchased from Pharmacia Hepar Inc., Franklin, OH. TrisHCl, NaCl, and $CaCl_2$ were from J. T. Baker Inc., Jackson, TN, and poly(ethylene glycol) 6000 was from BDH Laboratory Supplies, Poole, England.

4,4′-[1,3-Benzenediylbis(oxy)]bis(benzonitrile) (5, X = H, Y = CH). Resorcinol (1.1 g, 9.8 mmol) and 4-fluorobenzonitrile (2.4 g, 20 mmol) were dissolved in DMSO (6 mL). K_2CO_3 (2.4 g, 17 mmol) was added. After being heated with an oil bath at 100 °C for 16 h, the reaction was quenched with water and extracted with EtOAc. The organic layer was washed with 1 N NaOH, water, and brine and dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was chromatographed on silica (50 g) with 20/1 CH_2Cl_2/Hex. Recrystallization from EtOAc/Hex gave 0.51 g (17%) of **5** (X = H, Y = CH): NMR (DMSO-d_6) δ 7.64 (d, 4 H), 7.42 (t, 1 H), 7.05 (d, 4 H), 6.91 (dt, 2 H), 6.78 (t, 1H).

3,3′-[3,5-Difluoro-4-methyl-2,6-pyridinediylbis(oxy)]bis(benzonitrile) (5, X = 3,5-F-4-Me, Y = N). Sodium hydride (0.51 g, 9.5 mmol) was slurried in DMF (5 mL). The slurry was cooled in an ice bath, and 3-cyanophenol (1.44 g, 12 mmol) was added. After the mixtured stirred for 10 min, the ice bath was removed and 2,3,5,6-tetrafluoro-4-methylpyridine (1.0 g, 6.0 mmol) was added. After being heated with an oil bath at 90 °C for 20 h, the reaction was quenched with water and the solid was collected by filtration. Recrystallization from EtOAc/Hex gave 0.80 g (39%) of **5** (X = 3,5-F-4-Me, Y = N): NMR (DMSO-d_6) δ 7.48 (m, 4 H), 7.24 (m, 4 H).

3,3′-[3,5-Difluoro-4-methyl-2,6-pyridinediylbis(oxy)]bis[benzenecarbox(imide)amide], 2 Trifluoroacetic Acid Salt (6h). Compound **5** (X = 3,5-F-4-Me, Y = N; 0.38 g, 1.1 mmol) was slurried in EtOH (15 mL) and cooled in an ice bath. After the solution was saturated with HCl(g), the reaction vessel was sealed. After the mixture stirred overnight, the seal was broken and the solvent was removed in vacuo. The residue was triturated with Et_2O, and the solvent was decanted off. After the remaining solvent was removed in vacuo, the residue was dissolved in EtOH (6 mL) and cooled in a dry ice bath, and nitrogen was condensed in the reaction. The reaction vessel was sealed and heated in an oil bath at 100 °C for 2 h. The reaction was then cooled in a dry ice bath, and the pressure was released. The residue was purified by HPLC using a Dynamax column and 20−80% gradient of CH_3CN in H_2O with 0.1% TFA. After most of the solvent was removed in vacuo, a solid precipitated. The solid was isolated by suction filtration and dried to give **6h** (0.26 g, 38%): mp >210 °C; NMR (DMSO-d_6) δ 9.3 (br s, 8 H), 7.6 (m, 4 H), 7.54 (m, 4 H), 2.4 (m, 3H). Anal. ($C_{20}H_{17}N_5O_2F_2$·2TFA) C, H, N.

A second fraction was lyophilized and gave **15** (36 mg, 6%): mp >200 °C; NMR (DMSO-d_6) δ 9.3 (s, 2H), 9.05 (s, 2H), 8.0 (s, 1H), 7.5 (m, 6H), 7.42 (t, 1H), 7.22 (d, 2H), 2.4 (m, 3H). Anal. ($C_{20}H_{16}N_4O_3F_2$·1.1TFA) C, H, N.

4,4′-[2,6-Pyridinediylbis(oxy)]bis(benzenecarboximidamide), dihydrochloride (6a):[15] NMR (300 MHz, DMSO) δ 9.25 (s, 4H), 9.0 (s, 4H), 8.58 (s, 1H), 7.8 (d, 4H), 7.4 (d, 4H). Anal. ($C_{19}H_{17}N_5O_2$·2HCl·2.6H_2O) C, H, N.

4,4′-[3,5-Dichloro-2,6-pyridinediylbis(oxy)]bis(benzenecarboximidamide), trifluoroacetic acid salt (6b): NMR (300 MHz, DMSO-d_6) δ 9.4 (s, 4H), 9.2 (s, 4H), 8.1 (t, 1H), 7.95 (d, 4H), 7.42 (s, 1H), 7.3 (d, 4H). Anal. ($C_{19}H_{15}N_5O_2Cl_2$·2.5TFA) C, H, N.

4,4′-[1,3-Benzenediylbis(oxy)]bis(benzenecarboximidamide), 2 trifluoroacetic acid salt (6c):[10] NMR (DMSO-d_6) δ 8.3 (s, 4H), 8.1 (s, 4H), 7.88 (d, 4H), 7.58 (t, 1H), 7.28 (d, 4H), 7.02 (d, 2H), 6.95 (s, 1H); IR 1665, 1190. Anal. ($C_{20}H_{18}N_4O_2$·2TFA) C, H, N.

4,4′-[2,4-Dichloro-1,5-benzenediylbis(oxy)]bis(benzenecarboximidamide), dihydrochloride, 0.8 hydrate (6d): NMR (300 MHz, DMSO-d_6) δ 9.2 (br, 8H), 8.2 (s, 1H), 7.9 (d, 4H), 7.4 (d, 4H), 6.96 (d, 2H). Anal. ($C_{20}H_{16}N_4O_2Cl_2$·2HCl·0.8H_2O) C, H, N.

3,3′-[2,6-Pyridinediylbis(oxy)]bis(benzenecarboximidamide), dihydrochloride (6e): NMR (DMSO-d_6) δ 9.45 (s, 4H), 9.3 (s, 4H), 8.02 (t, 1H), 7.4−7.8 (m, 8H), 6.90 (d, 2H). Anal. ($C_{19}H_{17}N_5O_2$·2HCl·2.6H_2O) C, H, N.

3,3′-[3,5-Dichloro-2,6-pyridinediylbis(oxy)]bis(benzenecarboximidamide), trifluoroacetic acid salt (6f): NMR (300 MHz, DMSO-d_6) δ 9.2 (br s, 8H), 8.6 (s, 1H), 7.4−7.7 (m, 8H). Anal. ($C_{19}H_{15}N_5O_2F_2$·2TFA) C, H, N.

3,3′-[3,5-Difluoro-2,6-pyridinediylbis(oxy)]bis(benzenecarboximidamide), trifluoroacetic acid salt (6g): NMR (300 MHz, DMSO-d_6) δ 9.55 (br s, 4H), 9.4 (br s, 4H), 8.5 (t, 1H), 7.7 (m, 4H), 7.55 (m, 4H). Anal. ($C_{19}H_{15}N_5O_2F_2$·2TFA) C, H, N.

3,3′-[3,5-Difluoro-4-methoxy-2,6-pyridinediylbis(oxy)]bis(benzenecarboximidamide), 2 trifluoroacetic acid salt (6i): NMR (DMSO-d_6) δ 9.3 (br s, 4H), 9.2 (br s, 4H), 7.6 (m, 4H), 7.5 (m, 4H), 4.3 (s, 3H), 2.3 (m, 3H). Anal. ($C_{20}H_{17}N_5O_3F_2$·2TFA) C, H, N.

3,3′-[3-Amino-2,6-pyridinediylbis(oxy)]bis(benzenecarboximidamide), 2 trifluoroacetic acid salt (6j): NMR (DMSO-d_6) δ 9.5 (br, 4H), 9.2 (br, 4H), 7.3−7.7 (m, 9H), 6.8 (d, 1H). Anal. ($C_{19}H_{18}N_6O_2$·2.4TFA) C, H, N.

***N*-[2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridin-3-yl]acetamide, 2 trifluoroacetic acid salt (6k):** NMR (DMSO-d_6) δ 9.8 (s, 1H), 9.3 (m, 4H), 9.25 (m, 4H), 8.4 (d, 1H), 7.4−7.7 (m, 8H), 6.9 (d, 1H), 2.15 (s, 3H). Anal. ($C_{21}H_{20}N_6O_3$·2.5TFA) C, H, N.

***N*-[2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridin-3-yl]methanesulfonamide, 2 trifluoroacetic acid salt (6l):** NMR (DMSO-d_6) δ 9.6 (s, 1H), 9.4 (m, 8H), 8.4 (d, 1H), 7.95 (d, 1H), 7.5−7.7 (m, 8H), 6.9 (d, 1H), 3.1 (s, 3H). Anal. ($C_{21}H_{20}N_6O_4S$·3.5TFA) C, H, N.

***N*-[2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridin-3-yl]-*N′*-phenylurea, 2 trifluoroacetic acid salt (6m):** NMR (DMSO-d_6) δ 9.2 (s, 1H), 9.1 (br, 8H), 8.6 (m, 2H), 7.3−7.7 (m, 10H), 7.26 (t, 2H) 6.95 (t, 1H), 6.82 (d, 1H); IR 1672, 1205; mp 159−160 °C. Anal. ($C_{26}H_{23}N_7O_3$·2TFA) C, H, N, F.

***N*-[2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridin-3-yl]-*N′*-methylurea, dihydrochloride (6n):** NMR (DMSO-

d_6) δ 9.8 (br, 8H), 8.6 (d, 1H), 8.5 (s, 1H), 7.7 (m, 2H), 7.5−7.7 (m, 5H), 7.4 (m, 1H), 6.90 (m, 1H), 6.85 (d, 1H), 2.7 (d, 3H); IR 1679, 1231; mp 205−206 °C. Anal. ($C_{21}H_{21}N_7O_3$·2HCl·H_2O) C, H, N.

***N*-[2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridin-3-yl]benzamide, 2 trifluoroacetic acid salt (6o):** NMR (DMSO-d_6) δ 10.2 (s, 1H), 9.3 (s, 4H), 9.4 (m, 4H), 8.2 (d, 1H), 8.0 (d, 1H), 7.6 (m, 12H), 6.95 (d, 1H); mp 269−271 °C. Anal. ($C_{26}H_{22}N_6O_3$·2TFA·0.3H_2O) C, H, N.

***N*-[[[2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridin-3-yl]amino]carboxy]glycinamide, 2 trifluoroacetic acid salt (6p):** NMR (DMSO-d_6) δ 9.4 (m, 8H), 8.75 (s, 1H), 8.65 (d, 1H), 7.4−7.7 (m, 9H), 7.1 (m, 2H), 6.9 (d, 1H), 3.8 (d, 2H); mp 129−130 °C. Anal. ($C_{22}H_{22}N_8O_4$·2.5TFA·1.5H_2O) C, H, N.

3,3′-[3-(Trifluoromethyl)-2,6-pyridinediylbis(oxy)]bis(benzenecarboximidamide), dihydrochloride (6q): NMR (DMSO-d_6) δ 9.4 (s, 4H), 9.2 (s, 4H), 8.3 (d, 1H), 7.7 (m, 4H), 7.6 (d, 4H), 7.0 (m, 1H). Anal. ($C_{20}H_{16}N_5O_2$·2HCl·H_2O·0.25NH_4Cl) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridine-3-carboxamide, 2 trifluoroacetic acid salt (6r): NMR (DMSO-d_6) δ 9.45 (br s, 4H), 9.35 (br s, 4H), 8.4 (d, 1H), 7.4−7.9 (m, 10H), 6.95 (d, 1H). Anal. ($C_{20}H_{18}N_6O_3$·2.5TFA) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]-*N*-methylpyridine-3-carboxamide, 2 trifluoroacetic acid salt (6s): NMR (DMSO-d_6) δ 9.6 (m, 4H), 9.4 (br s, 4H), 8.65 (d, 1H), 8.5 (m, 1H), 7.7−8.0 (m, 8H), 7.2 (d, 1H), 2.85 (d, 3H). Anal. ($C_{21}H_{20}N_6O_3$·2.4TFA) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]-*N*,*N*-dimethylpyridine-3-carboxamide, 2 trifluoroacetic acid salt (6t): NMR (DMSO-d_6) δ 9.4 (br s, 4H), 9.3 (br s, 4H), 8.0 (d, 1H), 7.6 (m, 8H), 6.9 (d, 1H), 3.0 (d, 6H); mp 180−183 °C. Anal. ($C_{22}H_{22}N_6O_3$·2.4TFA) C, H, N.

***N*-[[2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridin-3-yl]oxomethyl]glycinamide, 2 trifluoroacetic acid salt (6u):** NMR (DMSO-d_6) δ 9.3 (s, 2H), 9.25 (s, 2H), 9.05 (s, 4H), 8.5 (d, 1H), 7.4−7.7 (m, 6H), 6.85 (d, 1H) 4.0 (d, 2H). Anal. ($C_{22}H_{21}N_7O_4$·2.3TFA·0.7H_2O) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridine-3-carboxylic acid, ethyl ester, 2 trifluoroacetic acid salt (6v): NMR (DMSO-d_6) δ 9.35 (br s, 4H), 9.1 (br s, 4H), 8.4 (d, 1H), 7.6 (m, 4H), 7.5 (m, 4H), 6.9 (d, 1H), 4.3 (q, 2H), 1.3 (t, 3H). Anal. ($C_{22}H_{21}N_5O_4$·2.15TFA) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridine-3-carboxylic acid, dihydrochloride (6w): NMR (DMSO-d_6) δ 9.5 (br s, 4H), 9.35 (br s, 4H), 8.45 (d, 1H), 7.7 (m, 2H), 7.6 (m, 2H), 7.5 (m, 4H), 6.95 (d, 1H). Anal. ($C_{20}H_{17}N_5O_4$·2HCl·2.8H_2O) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridine-4-carboxamide, 2 trifluoroacetic acid salt (6x): NMR (DMSO-d_6) δ 9.3 (s, 4H), 9.1 (s, 4H), 8.3 (s, 1H), 7.8 (s, 1H), 7.65 (m, 4H), 7.55 (m, 4H), 7.2 (s, 2H). Anal. ($C_{20}H_{18}N_6O_3$·2TFA·1.5H_2O) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]-*N*-methylpyridine-4-carboxamide, 2 trifluoroacetic acid salt (6y): NMR (DMSO-d_6) δ 9.35 (br s, 4H), 9.2 (br s, 4H), 8.85 (m, 1H), 7.5−7.7 (m, 8H), 7.2 (s, 2H), 2.9 (d, 3H). Anal. ($C_{21}H_{20}N_6O_3$·2TFA·1.5H_2O) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridine-4-carboxylic acid, dihydrochloride (6z): NMR (DMSO-d_6) δ 9.5 (s, 4H), 9.35 (s, 4H), 7.7 (m, 4H), 7.6 (m, 4H), 7.2 (s, 2H), 4.4 (q, 2H), 1.38 (t, 3H); IR 1670, 1187 cm^{-1}. Anal. ($C_{22}H_{21}N_5O_4$·2TFA) C, H, N.

2,6-Bis[3-[amino(imino)methyl]phenoxy]pyridine-4-carboxylic acid, dihydrochloride (6aa): NMR (DMSO-d_6) δ 9.4 (br s, 4H), 9.2 (br s, 4H), 7.4 (m, 4H), 7.3 (m, 4H), 7.2 (s, 2H). Anal. ($C_{20}H_{17}N_5O_4$·2HCl·0.2H_2O) C, H, N.

2,2′-[3,5-Dichloro-2,6-pyridinediylbis(oxy)]bis(benzenecarboximidamide), 2 trifluoroacetic acid salt (10): NMR (300 MHz, DMSO-d_6) δ 10.3 (br, 8H), 8.1 (t, 1H), 7.65 (m, 2H), 7.55 (t, 2H), 7.25 (m, 2H), 7.1 (m, 4H); IR 1672, 1202. Anal. ($C_{19}H_{17}N_5O_2$·2.4TFA) C, H, N.

3,4′-[Pyridine-2,6-diylbis(oxy)]bis(benzenecarboximidamide), dihydrochloride (11): NMR (300 MHz, DMSO-d_6) δ 9.4 (br s, 8H), 8.1 (t, 1H), 7.9 (d, 2H), 7.7−7.5 (m, 4H), 7.4 (d, 2H), 6.9 (dd, 2H). Anal. ($C_{19}H_{17}N_5O_2$·2HCl·1.2H_2O) C, H, N.

3,3′-[Pyrazine-2,6-diylbis(oxy)]bis(benzenecarboximidamide), 2.7 trifluoroacetic acid salt (12a): NMR (300 MHz, DMSO-d_6) δ 9.4 (br s, 8H), 8.4 (s, 2H), 7.7−7.5 (m, 8H). Anal. ($C_{18}H_{16}N_6O_2$·2.7TFA) C, H, N.

3,3′-[Pyrimidine-2,4-diylbis(oxy)]bis(benzenecarboximidamide), dihydrochloride (12b): NMR (300 MHz, DMSO-d_6) δ 9.4 (br s, 8H), 8.6 (d, 1H), 7.7−7.5 (m, 8H), 7.05 (d, 1H). Anal. ($C_{18}H_{16}N_6O_2$·2HCl·H_2O) C, H, N.

3,3′-[(Pyrazine-2,6-diyl 4-oxide)bis(oxy)]bis(benzenecarboximidamide), 2 trifluoroacetic acid salt (12c): NMR (300 MHz, DMSO-d_6) δ 9.4 (br s, 8H), 8.15 (s, 2H), 7.7−7.5 (m, 8H). Anal. ($C_{18}H_{16}N_6O_3$·2TFA) C, H, N.

3,3′-[(3,4-Dihydro-3-oxopyrazine-2,6-diyl)bis(oxy)]bis(benzenecarboximidamide), dihydrochloride, ammonium chloride, 0.4 ethyl alcohol, hydrate (12d): NMR (300 MHz, DMSO-d_6) δ 9.4 (s, 4H), 9.3 (m, 4H), 7.3−7.7 (m, 10H); IR 3119, 1680 cm^{-1}; MS (M + 1) 235. Anal. ($C_{18}H_{16}N_6O_3$·2HCl·H_2O·NH_3Cl) C, H, N.

3,3′-[4-Nitro-1,3-phenylenebis(oxy)]bis(benzenecarboximidamide), trifluoroacetic acid salt, 0.5 hydrate (13a): NMR (DMSO-d_6) δ 9.4 (s, 4H), 9.3 (m, 4H), 8.25 (d, 1H), 7.7 (m, 5H), 7.6 (m, 2H), 7.5 (m, 1H), 7.0 (m, 1H), 6.9 (s, 1H). Anal. ($C_{20}H_{17}N_5O_4$·$C_4H_2F_6O_4$·0.5H_2O) C, H, N.

3,3′-[4-Nitro-1,3-phenylenebis(oxy)]bis(benzenecarboximidamide), trifluoroacetic acid salt, 0.5 hydrate (13b): NMR (DMSO-d_6) δ 9.4 (m, 8H), 7.4−7.7 (m, 5H), 7.3 (m, 3H), 7.05 (d, 1H), 6.86 (dd, 1H), 6.73 (t, 1H). Anal. ($C_{20}H_{19}N_5O_2$·2.7$C_2HF_3O_2$·2.5H_2O) C, H, N.

3-[[3,5-Difluoro-6-[[3-[amino(imino)methyl]phenyl]amino]-4-methylpyridin-2-yl]oxy]benzenecarboximidamide, 2 trifluoroacetic acid salt (14a): NMR (DMSO-d_6) δ 9.35 (s, 2H), 9.2 (s, 4H), 8.95 (s, 2H), 7.5−7.8 (m, 6H), 7.25 (m, 2H), 2.35 (s, 3H). Anal. ($C_{20}H_{18}N_6OF_2$·2TFA·2H_2O) C, H, N.

3-[[3,5-Difluoro-6-[[3-[amino(imino)methyl]phenyl]-methylamino]-4-methylpyridin-2-yl]oxy]benzenecarboximidamide, 2 trifluoroacetic acid salt (14b): NMR (DMSO-d_6) δ 9.35 (s, 2H), 9.3 (s, 2H),9.15 (s, 2H), 9.05 (s, 2H), 7.6 (m, 4H), 7.4 (m, 3H), 7.25 (m, 1H), 7.2 (d, 1H),3.25 (s, 3H), 2.3 (s, 3H). Anal. ($C_{21}H_{20}N_6OF_2$·2TFA) C, H, N.

3,3′-[3,5-Difluoro-4-methyl-2,6-pyridinediylbis(methylamino)]bis(benzenecarboximidamide), 2 Trifluoroacetic Acid Salt (14c). 3-Cyanoaniline (1.7 g, 15 mmol) was dissolved in DMSO (100 mL) and sodium hydride (1.2 g, 30 mmol, 60% dispersion in oil) was added. After cessation of gas evolution, the mixture was stirred at ambient temperature for an additional 30 min. At that time 2,3,5,6-tetrafluoro-4-methylpyridine (2.0 g, 12 mmol) was added in portions. The mixture was then stirred vigorously at ambient temperature for 1 h and then at 60 °C for 16 h. The mixture was then cooled to ambient temperature, carefully quenched with water (800 mL), and partitioned with ethyl acetate (800 mL). The organic extract was then washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to a black tar. This was dissolved in methylene chloride and filtered through a short column of silica gel. The resulting filtrate was concentrated in vacuo to afford 3.2 g of a brown oil. The oil (1.0 g) was dissolved in acetonitrile (50 mL). Iodomethane (0.79 g, 5.6 mmol) followed by sodium hydride (0.30 g, 7.4 mmol, 60% dispersion in oil) were added. The mixture was stirred at ambient temperature for 30 min and then quenched by addition of water (400 mL). This was extracted with ethyl acetate (2 × 300 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to a brown oil. This was filtered through a short column of silica gel with 10% ethyl acetate in hexanes to afford 1.12 g of a yellow oil. The oil (dissolved in 5 mL of DMSO) was added to a solution of 3-cyanoaniline (590 mg, 5 mmol) in anhydrous DMSO (40 mL) and sodium hydride (420 mg, 10.4 mmol, 60% dispersion in oil) after stirring at ambient temperature for 30 min. The mixture was stirred at ambient temperature for 90 min, then carefully quenched with water (400 mL), and partitioned with EtOAc. The organic extract

was then washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to a black oil. Purification by flash column chromatography through a column of silica gel with 10% ethyl acetate in hexanes afforded 524 mg of a yellow solid. The solid (200 mg, 0.53 mmol) in 10 mL of acetonitrile was treated with iodomethane (113 mg, 0.80 mmol), followed by sodium hydride (42 mg, 1.1 mmol, 60% dispersion in oil). The resultant mixture was stirred vigorously at ambient temperature for 3 h and then quenched by careful addition of 30 mL of water. This was extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to a brown oil. Purification by flash column chromatography through a column of silica gel with 15% ethyl acetate in hexanes afforded 150 mg of a yellow oil. Standard conversion to the amidine followed by purification by prep-HPLC afforded 85 mg of **14c** as a white solid: NMR (DMSO-d_6) δ 9.3 (br s, 4H), 9.2 (br s, 4H), 7.5 (m, 4H), 7.4 (d, 2H), 7.3 (d, 2H), 3.4 (s, 6H), 2.2 (s, 3H). Anal. ($C_{22}H_{23}N_7F_2$·2.4TFA) C, H, N.

3-[[6-[3-[Amino(imino)methyl]phenoxy]-3,5-difluoro-4-methylpyridin-2-yl]oxy]benzenecarboxamide, trifluoroacetic acid salt (15): NMR (300 MHz, $CDCl_3$) δ 9.3 (s, 2H), 9.05 (s, 2H), 8.0 (s, 1H), 7.5 (m, 6 H), 7.42 (t, 1H), 7.22 (d, 2H), 2.4 (m, 3H). Anal. ($C_{23}H_{26}N_7F_2$·2.4TFA) C, H, N.

3,3′-[(2,6-Pyrazinediyl 4-oxide)bis(oxy)]bis(benzonitrile) (8). 3,3′-[2,6-Pyrazinediylbis(oxy)]bis(benzonitrile) (**7**; 2.7 g, 8.6 mmol) was slurried in AcOH (10 mL). Hydrogen peroxide (2 mL of 30%) was added, and the reaction was heated at 80 °C for 20 h. AcOH (10 mL) and H_2O_2 (6 mL) were added. After heating for 24 h, the reaction was cooled to ambient temperature, and water was added. The resulting solid was collected by filtration to give 0.54 g (35%) of **8**: NMR ($CDCl_3$) δ 7.80 (s, 2H), 7.55 (m, 4H), 7.35 (m, 4H).

3,5-Bis(3-cyanophenoxy)-2-pyrazinone (9). 3,3′-[2,6-Pyrazinediyl 4-oxide)bis(oxy)]bis(benzonitrile) (**8**) was slurried in DMF (10 mL). Trifluoroacetic acid (3.4 g, 16 mmol) was added and the solution cleared. After stirring for 4 h the solvent was removed in vacuo, water was added, and the resulting solid was collected by suction filtration to give 0.35 g (64%) of **9**: NMR ($CDCl_3$) δ 7.52 (m, 2H), 7.44 (m, 4H), 7.20 (m, 2H), 6.98 (s, 1H).

Enzyme Assay Procedures.[16] The activities of human FXa, human thrombin, and bovine trypsin were determined as the initial rate of the cleavage of peptide *p*-nitroanilide by the enzyme. The assay was performed at room temperature in flat-bottom microtiter plates in a final volume of 200 μL. The reaction mixture consisted of 50 mM TrisHCl (pH 7.5), 150 mM NaCl, 2.5 mM $CaCl_2$, and 0.1% poly(ethylene glycol) 6000, with enzyme and substrate at the following concentrations: (1) FXa assay, 1 nM FXa and 164 μM S2222; (2) thrombin assay, 16 nM thrombin and 300 μM S2302; and (3) trypsin assay, 16 nM bovine trypsin and 127 μM S2266. All substrate concentrations used are equal to their K_m values under the present assay conditions. Controls without the test compounds or with a reference compound were also run in each assay plate. Enzyme was incubated with test compounds for 10 min, the reaction was then started by the addition of the substrate. Reaction rates were determined by measuring the rate of the absorbance change at 405 nm in a ThermoMax microplate reader (Molecular Devices Corp., Sunnyvale, CA).

Data Analysis Methods. IC_{50} values for compounds were obtained by log−logit analysis with a computer spreadsheet. Since the inhibition mechanism is competitive, K_i values were then obtained by dividing the IC_{50} values by a factor of $1 + [S]/K_m$. K_i values are the mean of multiple determinations ($n \geq 2$). Standard deviations are <30% of the mean.

Supporting Information Available: Table of crystal data and structure refinement for **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Some of this data has been previously reported: 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.; 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.

(2) A communication introducing this material has been published: Phillips, G. B.; Buckman, B. O.; Davey, D. D.; Eagen, K. A.; Guilford, W. J.; Hinchman, J.; Ho, E.; Koovakkat, S.; Liang, A.; Light, D. R.; Mohan, R.; Ng, H. P.; Post, J.; Show, K.; Smith, D.; Subramanyam, B.; Sullivan, M. E.; Trinh, L.; Vergona, R.; Walters, J.; White, K.; Whitlow, M.; Wu, S.; Xu, W.; Morrissey, M. M. 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. *J. Med. Chem.* **1998**, *41*, 3557−3562.

(3) Hermann, J.-P. R.; Kutryk, M. J. B.; Serruys, P. W. Clinical trials of direct thrombin inhibitors during invasive procedures. *Thromb. Haemostasis* **1997**, *78*, 367−376.

(4) Kocis, P. New trends in medicinal chemistry. *Drugs Future* **1995**, *20*, 173−183.

(5) (a) Kimball, S. D. Thrombin active site inhibitors. *Curr. Pharm. Des.* **1995**, *1*, 441−468. (b) Katakura, S.; Nagayasu, T.; Hara, T.; Iwamoto, M. A novel factor Xa inhibitor: structure activity relationships and selectivity between factor Xa and thrombin. *Biochem. Biophys. Res. Commun.* **1993**, *197*, 965−972.

(6) Published examples of nonpeptide FXa inhibitors: (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) Maduskuie, T. P.; McNamara, K. J.; Ru, Y.; Knabb, R. M.; Stouten, P. F. W. Rational design and synthesis of novel, potent bis-phenylamidine carboxylate factor Xa inhibitors. *J. Med. Chem.* **1998**, *41*, 53−62. (d) 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 non-peptide inhibitors of blood coagulation factor Xa. *J. Med. Chem.* **1998**, *41*, 437−450. (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 inhibitors. *Nature* **1998**, *391*, 608−612. (f) Ripka, W. C.; Vlasuk, G. P. Antithrombotic/Serine Proteases. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1997; pp 71−89.

(7) Sitco, G. R.; Ramjit, D. R.; Stabilito, I. I.; Lehman, D.; Lynch, J. J.; Vlasuk, G. P. Conjunctive enhancement of enzymatic thrombolysis and prevention of thrombotic reocclusion with the selective fXa inhibitor, Tick anticoagulant peptide: Comparison to hirudin and heparin in a canine model of acute coronary artery thrombosis. *Circulation* **1992**, *85*, 805−815.

(8) Shaw, K. J.; Guilford, W. J.; Dallas, J. L.; Koovakkat, S. K.; McCarrick, M. A.; 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.

(9) Sandler, S. R.; Karo, W. *Org. Funct. Group Prep.* **1972**, *3*, 217.

(10) Chauhan, P. M. S.; Iyer, R. N.; Bhakuni, D. S.; Shankhdhar, V.; Guru, P. Y.; Sen, A. B. Antiparasitic agents: Part VI−Synthesis of 1, 2-, 1, 3-, and 1,4-bis(4-substituted aryloxy)benzenes and their biological activities. *Indian J. Chem.* **1988**, *27B*, 38−42.

(11) Ohta, A.; Imazeki, A.; Itoigawa, Y.; Yamada, H.; Suga, C.; Takagai, C.; Sano, H.; Watanabe, T. Reactions of the monoxides of 2,6-disubstituted pyrazines with phosphoryl chloride and acetic anhydride. *J. Heterocycl. Chem.* **1983**, *20*, 311−320.

(12) Pepe, G.; Pfefer, G.; Boistelle, R.; Marchal, P. Diflufenican, N-(2,4-Difluorophenyl)-2-(3-(trifluoromethyl)phenoxy)-3-pyridinecarboxamide. C19H11F5N2O2. *Acta Crystallogr., Sect. C (Cr. Str. Comm.)* **1995**, *51*, 2671.

(13) Drendel, W. B.; Sundaralingam, M. Structures of 1,4-bis(3,5-dichloro-2-pyridyloxy)benzene, 1,4-bis(5-chloro-2-pyridyloxy)benzene, and 1,4-bis(3-chloro-2-pyridyloxy)benzene. *Acta Crystallogr.* **1985**, *C41*, 950−953.

(14) 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. *J. Med. Chem.* **1996**, *271*, 29988−29992.

(15) Delia, T. J.; Nagarajan, A.; Queener, S. F.; Bartlett, M. S. Ring-based analogues of pentamidine versus *P. carinii* pneumonia in culture. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2367−2370.

(16) Lottenberg, R.; Christensen, U.; Jackson, C. M.; Coleman, P. L. *Methods Enzymol.* **1981**, *80*, 341−361.

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