Structure-Based Design of Novel Guanidine/Benzamidine Mimics: Potent and Orally Bioavailable Factor Xa Inhibitors as Novel Anticoagulants

Patrick Y. S. Lam,* Charles G. Clark, Renhua Li, Donald J. P. Pinto, Michael J. Orwat, Robert A. Galemmo, John M. Fevig, Christopher A. Teleha, Richard S. Alexander, Angela M. Smallwood, Karen A. Rossi, Matthew R. Wright, Stephen A. Bai, Kan He, Joseph M. Luettgen, Pancras C. Wong, Robert M. Knabb, and Ruth R. Wexler

Bristol-Myers Squibb Company, P.O. Box 5400, Princeton, New Jersey 08542-5400

Received December 20, 2002

As part of an ongoing effort to prepare orally active factor Xa inhibitors using structure-based drug design techniques and molecular recognition principles, a systematic study has been performed on the pharmacokinetic profile resulting from replacing the benzamidine in the P1 position with less basic benzamidine mimics or neutral residues. It is demonstrated that lowering the pK_a of the P1 ligand resulted in compounds (3-benzylamine, **15a**; 1-aminoisoquinoline, **24a**; 3-aminobenzisoxazole, **23a**; 3-phenylcarboxamide, **22b**; and 4-methoxyphenyl, **22a**) with improved pharmacokinetic features mainly as a result of *decreased clearance, increased volume of distribution, and enhanced oral absorption*. This work resulted in a series of potent and orally bioavailable factor Xa inhibitors that ultimately led to the discovery of SQ311, **24a**. SQ311, which utilizes a 1-aminoisoquinoline as the P1 ligand, inhibits factor Xa with a K_i of 0.33 nM and demonstrates both good in vivo antithrombotic efficacy and oral bioavailability.

Introduction

Arginine plays an important role in many inhibitor/ligand and macromolecule interactions. Consequently, guanidine- and benzamidine-containing compounds have been extensively utilized in the rational design of biologically active compounds. Some representative examples that are illustrated in Figure 1 include factor Xa (FXa) inhibitors (**1**),[2] glycoprotein IIb/IIIa integrin antagonists (**2**),[3] nitric oxide synthase inhibitors (**3**),[4] urokinase inhibitors (**4**),[5] and oligoarginines as antagonists in HIV TAT/TAR interactions (**5**).[6] However, because of the high basicity of the guanidine (pK_a = 13) and benzamidine (pK_a = 11.6), which renders these compounds to be very polar, it has been a challenge to identify compounds with good pharmacokinetic profiles from these series. The high basicity of guanidine/benzamidine groups has translated into a high desolvation cost during passive absorption through the epithelial layer of the gut wall, which results in poor oral absorption. Overall, the short duration of action and poor oral absorption have precluded the development of these compounds as oral agents. As such, many pharmaceutical companies have invested significant efforts to identify less basic and neutral mimics to try to improve the pharmacokinetic properties of these compounds.

Herein we report on our systematic and comprehensive search for novel benzamidine mimics with reduced basicity that arose from our efforts to develop orally active FXa inhibitors suitable for clinical development. FXa was chosen as the target because it is the crucial enzyme at the convergent point of the intrinsic and extrinsic coagulation pathways.[7,8] Together with FVa, calcium, and phospholipids, a prothrombinase complex is formed, which converts prothrombin to thrombin. Thrombin, in turn, converts fibrinogen to fibrin and activates platelets, eventually leading to the formation of thrombus or blood clots. Inhibition of FXa has been demonstrated to result in antithrombotic efficacy in both animal models[9] and clinical settings.[10] Thus, FXa has emerged as a promising target for the discovery of oral anticoagulants.[11,12]

Our ability to design less basic and neutral P1 ligands was facilitated by the availability of a large number of X-ray structures of trypsin-like enzyme/inhibitor complexes.[11] The strategy employed was to drive the potency of our inhibitor to the picomolar range and then replace the benzamidine moiety with a less basic or neutral P1 ligand.

More specifically we recently reported the design and synthesis of SN429, a picomolar pyrazole-based FXa inhibitor (Figure 2) containing a benzamidine in the P1 position.[2,14] However SN429 is only 4% orally bioavailable and has a short half-life (iv $t_{1/2}$ of 0.82 h in dogs, typical of benzamidine-containing compounds). A comprehensive effort was initiated to search for potent and orally bioavailable benzamidine mimics. The high potency of the pyrazole (FXa K_i = 13 pM) afforded us the opportunity of being able to sacrifice 1−2 orders of magnitude in affinity during the process of benzamidine replacement while maintaining subnanomolar FXa inhibition. This work culminated in a subnanomolar FXa inhibitor with a 1-aminoisoquinoline as the P1 moiety that demonstrated improved pharmacokinetic properties. During the course of our investigations, several other research groups have also reported *subnanomolar* benzamidine mimics with improved pharmacokinetic features.[11b,c,13]

* Author to whom correspondence should be addressed [telephone (609) 818-5720; fax (609) 818-3550; e-mail patrick.lam@bms.com].

10.1021/jm020578e CCC: $25.00
Published on Web 09/17/2003

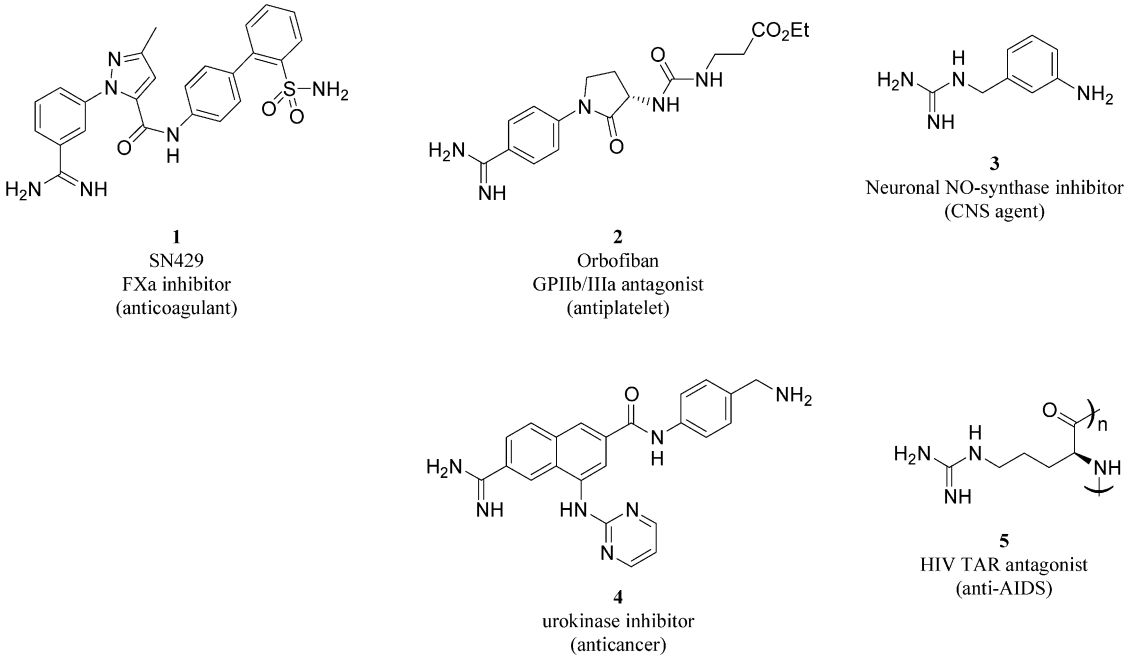


Figure 1. Literature examples of guanidine/benzamidine-containing compounds.

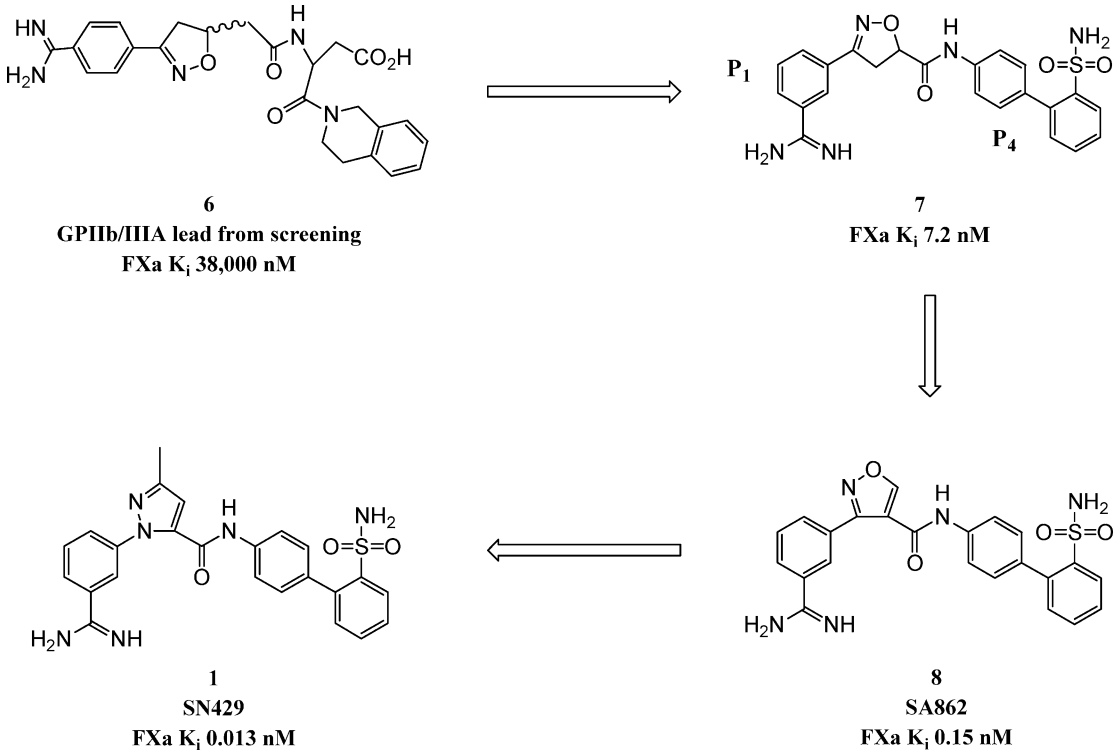


Figure 2. Factor Xa inhibitor design.

Design

The interaction of the benzamidine group of the inhibitors with the carboxylate of Asp189 located at the bottom of the S1 of the FXa pocket is a reinforced interaction consisting of two components (Figure 3).[15] The first is the Coulombic interaction of positive and negative charges (ion pairing), and the second is the bidentate hydrogen-bonding interaction. In general, a 1−2 orders of magnitude loss in K_i will result if one of the two components is lost. When both components are lost, there is usually a decrease in K_i of 3−4 orders of magnitude. Thus, in the design of potent benzamidine mimics, it is preferable to maintain both components in order to minimize the loss in binding affinity.

Figure 3 presents a representative series of designed benzamidine mimics and nonbenzamidines, which were synthesized and evaluated, in the order of decreasing pK_a. To maintain the Coulombic interaction of the mimic with Asp189, one would prefer the mimic to have a pK_a above the physiological pH of 7.4 so that at least 50% of the protonated mimic is available for binding. However, because of the phenomenon of pK_a perturbation as a result of ion-pairing interaction,[16] a base with a pK_a 1−2 units lower than 7.4 may also be sufficiently

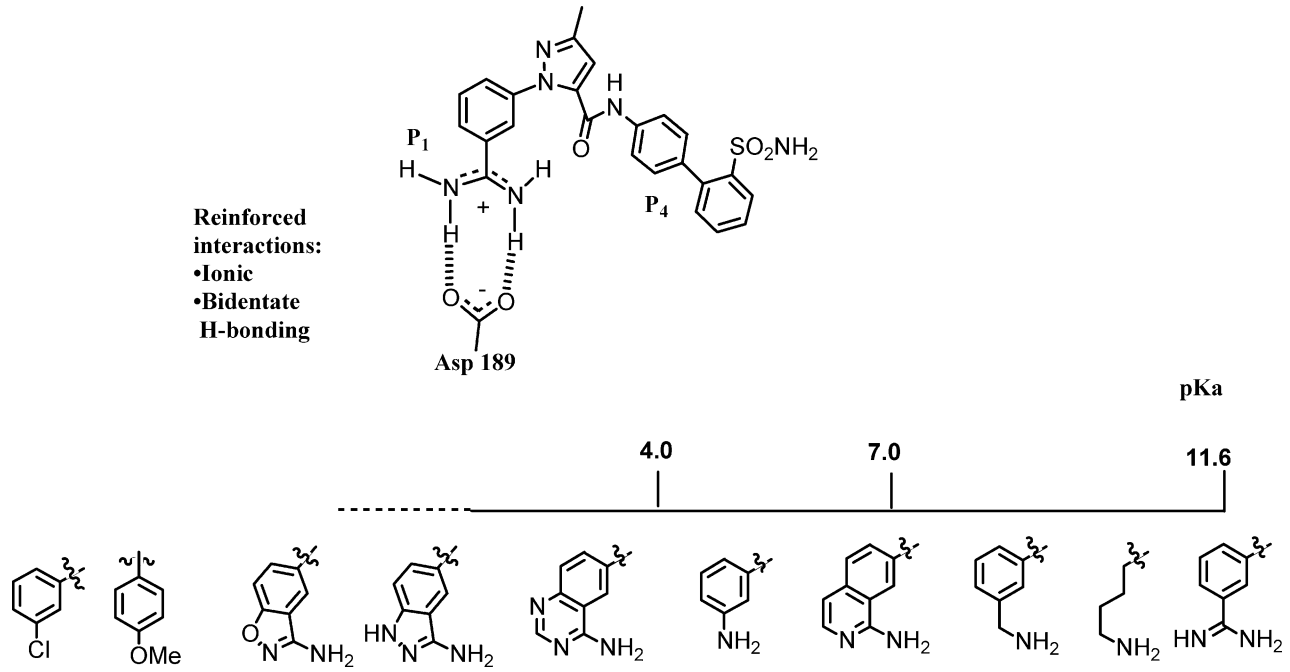
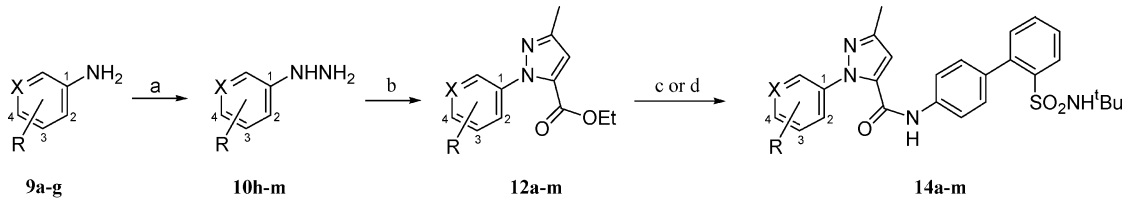
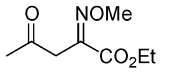


Figure 3. Design of guanidine/benzamidine mimics.

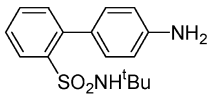
Scheme 1[a]



	X	R
9a	CH	4-OMe
9b	CH	H
9c	C	3-CN
9d	C	3-SMe
9e	C	3-SO_2NH^tBu
9f	C	3-CN; 4-F
9g	C	4-CN; 3-F
10h	C	3-Cl
10i	C	3-Br
10j	CH	2-NO_2
10k	C	3-NO_2
10l	CH	4-NO_2
10m	N	H



11



13

[a] Reagents: (a) (i) $NaNO_2$, HCl, 0 °C; (ii) $SnCl_2$; (b) EtOH, **11**, reflux; (c) (i) 3 N NaOH, dioxane, (ii) $(ClCO)_2$, DMF or BOP, TEA, CH_2Cl_2, then **13**; (d) $AlMe_3$, **13**, CH_2Cl_2.

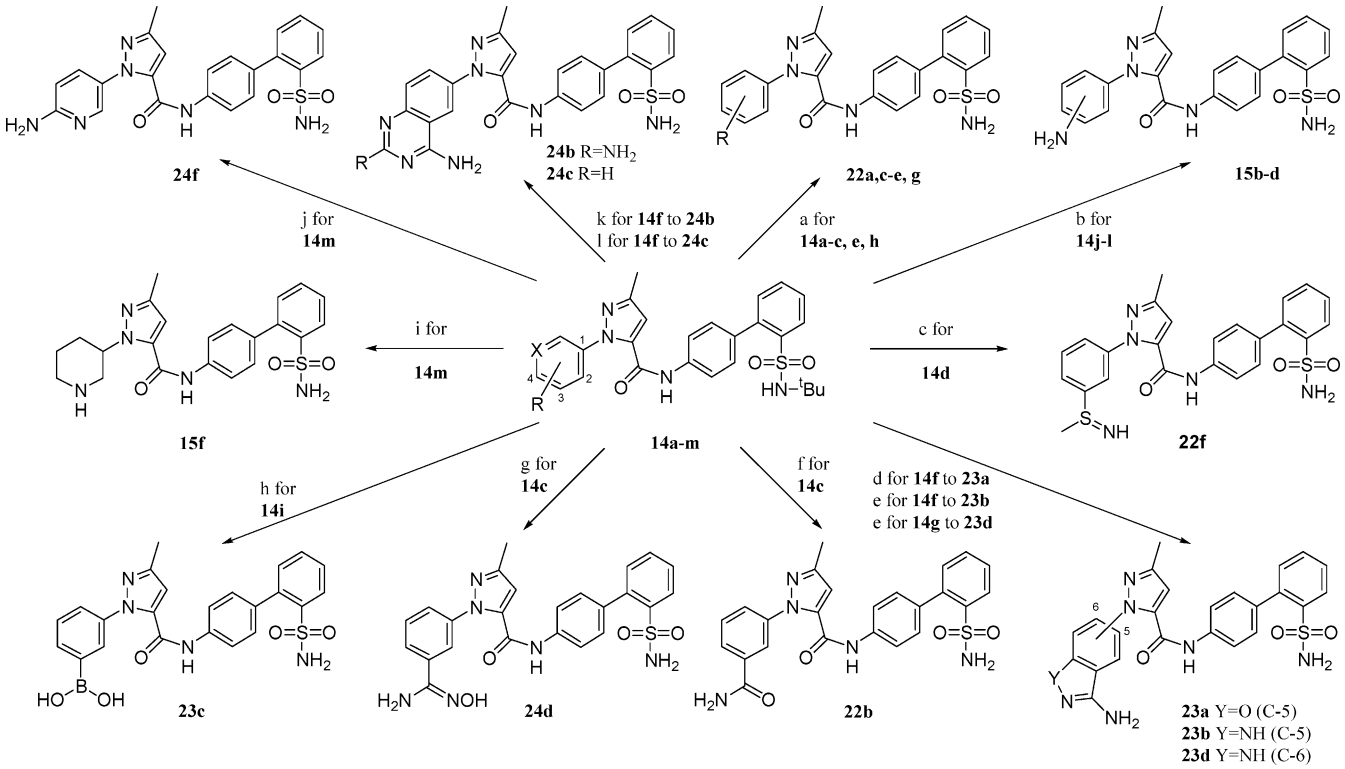
protonated when it is involved in an ion-pairing interaction with Asp189.

Benzamidine mimics were also explored with the goal of increasing selectivity against other trypsin-like proteases. Trypsin was used as a prototypical example for other trypsin-like proteases. One unique structural feature of FXa (and also thrombin) as compared with other trypsin-like enzymes is that FXa (and also thrombin) contains Ala190 at the bottom of the S1 pocket. In contrast, several trypsin-like enzymes, such as trypsin, FIXa, and FVIIa, contain Ser190.[17] Hence, our strategy was to take advantage of the larger S1 specificity pocket in FXa (one oxygen-atom size difference) and introduce selectivity against trypsin-like proteases by preparing FXa inhibitors with a larger P1 moiety. The Ala190 of the S1 pocket is positioned close to the 4-position of the P1 phenyl group of the FXa inhibitors. Therefore, 4-substituted phenyl P1 moieties were designed to introduce selectivity against trypsin.

Synthesis

The synthesis of pyrazoles **14a**−**m** was performed according to the procedures shown in Scheme 1 beginning either from the commercially available anilines **9a**−**g** or from hydrazines **10h**−**m**. Anilines **9a**−**g**[18] were converted to their hydrazine analogues via diazotization and stannous chloride reduction to give compounds **10a**−**g**. The hydrazines **10a**−**m** were next condensed with ethyl 2-(methoxyimino)-4-oxopentanoate **11**[2] to give pyrazole esters **12a**−**m** in a regioselective manner.

Scheme 2[a]



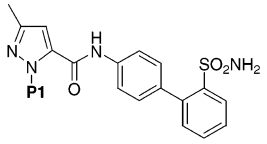
[a] Reagents: (a) TFA, reflux; (b) (i) TFA, reflux, (ii) Pd(C), H_2, EtOH; (c) (i) chloramine-T, then TFA, (ii) H_2SO_4; (d) (i) acetone oxime, *t*-BuOK, DMF, (ii) 6 N HCl aq, EtOH, 80 °C; (e) (i) $H_2NNH_2 \cdot H_2O$, *n*-BuOH, 105 °C, (ii) TFA, reflux; (f) H_2SO_4; (g) (i) HCl, MeOH, (ii) H_2NOH; (h) (i) NaH, THF, (ii) *n*-BuLi, −78 °C, then $(i\text{-PrO})_3B$, (iii) HCl aq (i) (i) *m*-CPBA, acetone, (ii) PtO_2, H_2, EtOH, AcOH, (iii) TFA, reflux; (j) (i) *m*-CPBA, acetone, (ii) TsCl, pyridine, (iii) ethanolamine, 50 °C, (iv) TFA, reflux; (k) (i) NaH, $H_2NC(=NH)NH_2 \cdot HCl$, DMAC, reflux, (ii) TFA, reflux; (l) (i) $HC(=NH)NH_2 \cdot AcOH$, DMAC, reflux, (ii) TFA, reflux.

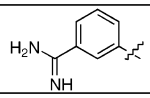
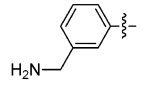
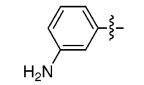
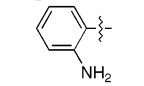
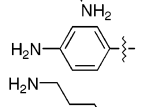
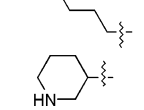
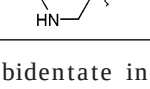
The pyrazole ester was coupled with biarylaniline **13** via the Weinreb procedure[19] to afford pyrazole amides **14a**−**m**. Alternatively, ester hydrolysis yielded the pyrazole carboxylic acid, which was activated with the BOP reagent and coupled with biarylaniline **13** to furnish pyrazole amides **14a**−**m**. On the other hand, the pyrazolecarboxylic acid was also converted to the acyl chloride prior to coupling with biarylaniline **13**.

Scheme 2 describes the conversion of pyrazoles **14a**−**m** to the inhibitors found in Tables 1−4. The synthesis of basic inhibitor **15a** has been reported by our research group earlier.[2] Inhibitors **15b**−**d** of Table 1 were obtained from the corresponding aryl nitro compounds by sulfonamide deprotection in hot trifluoroacetic acid followed by hydrogenation over palladium on carbon. Butylamine **15e** was synthesized as described in Scheme 3. More specifically, protection of amine **16**, followed by unmasking of the aldehyde gave **18**. The aldehyde was reductively aminated to give hydrazine **20**, which followed by condensation and deprotection resulted in pyrazole **15e**. Piperidinyl inhibitor **15f** resulted from the N-oxidation of pyridine **14m**, followed by reduction over Pt_2O and deprotection.

The neutral inhibitors **22a**,**c**−**e**,**g** of Table 2 were prepared in a straightforward manner by removal of the sulfonamide *tert*-butyl group using trifluoroacetic acid heated to reflux. Amide **22d** was prepared via removal of the sulfonamide *tert*-butyl group and concomitant nitrile hydrolysis using concentrated sulfuric acid. 3-Methylsulfinimidoylphenyl **22f** was synthesized from methylthiophenyl pyrazole **14d** by sulfimidation with chloramine-T, followed by acidic hydrolysis.

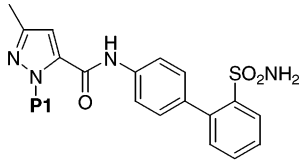
Table 1. Basic P1



	P1	pKa	FXa K_i nM	Thrombin K_i nM	Trypsin K_i nM
1 SN429		10.7	0.013	300	16
15a		8.8	2.7	>21000	248
15b		4.7	63	>21000	>1600
15c		4.7	600	>21000	-
15d		4.7	3000	>21000	-
15e		10	12000	>21000	-
15f		10	21000	>21000	-

The bidentate inhibitors **23a**,**b**,**d** in Table 3 were prepared from the regioisomeric cyanofluorophenylpyrazoles **14f** and **14g**. Aminobenzisoxazole **23a** was synthesized using acetone oxime and base to replace aryl fluoride followed by acidic ring closure and accompanying sulfonamide deprotection. Aminoindazoles **23b** and

Table 2. Neutral P1



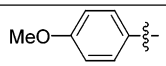
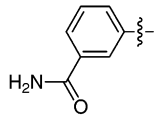
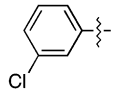
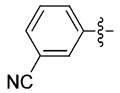
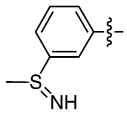
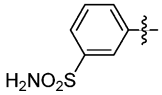
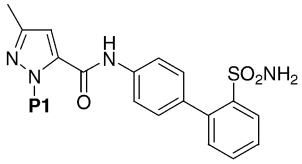
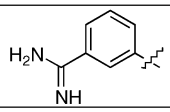
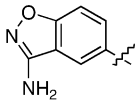
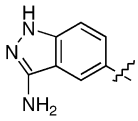
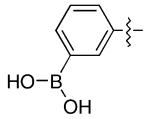
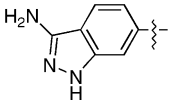
	P1	FXa K_i nM	Thrombin K_i nM	Trypsin K_i nM
22a		11	>21000	>1600
22b		19	>21000	>1600
22c		37	>21000	>1600
22d		198	>21000	>1600
22e		310	>21000	-
22f		2700	>21000	-
22g		5000	>21000	-

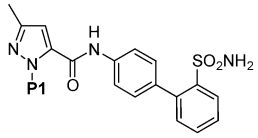
Table 3. Bidentate Hydrogen-Bonding P1

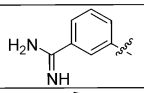
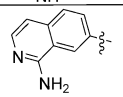
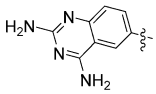
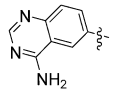
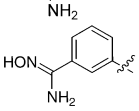
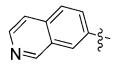
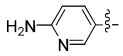


	P1	FXa K_i nM	Thrombin K_i nM	Trypsin K_i nM
1 SN429		0.013	300	16
23a		1.4	>21000	>1600
23b		29	>21000	-
23c		61	>21000	>1600
23d		2800	>21000	>1600

23d were prepared from heating hydrazine hydrate with **14f** or **14g**, respectively, in *n*-butanol followed by sulfonamide deprotection. Phenylboronic acid **23c** was obtained from the bromide **14i** via lithiation, followed by quenching with borate and aqueous hydrolysis.

Table 4. Basic Bidentate Hydrogen-Bonding P1



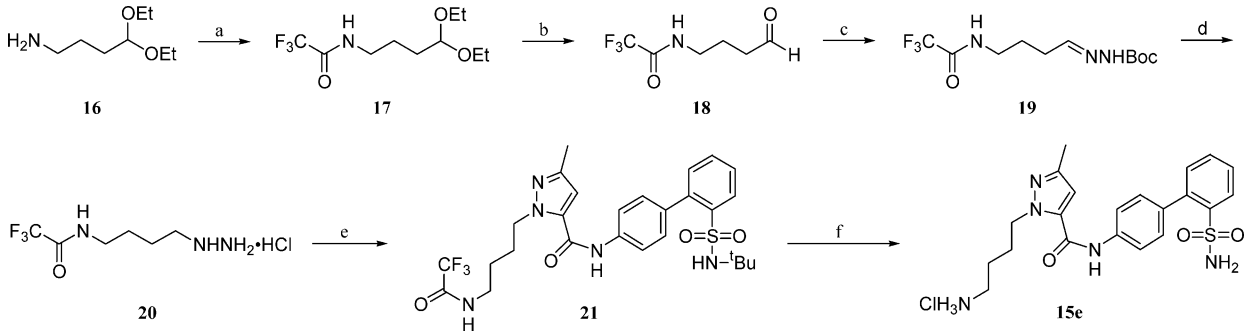
	P1	pKa	FXa K_i nM	Thrombin K_i nM	Trypsin K_i nM
1 SN429		10.7	0.013	300	16
24a SQ311		6.7	0.33	6500	2600
24b		~8.1	16	>21000	>1600
24c		~5.7	29	>21000	>1600
24d		~7	19	>21000	>1600
24e		5.4	370	>21000	>1600
24f		~7	1300	>2100	-

The basic inhibitors **24a** and **24e** were synthesized according to Scheme 4. 7-Aminoisoquinoline **25** was converted to the hydrazine **26** via diazotization and stannous chloride reduction followed by condensation with ethyl 2-(methoxyimino)-4-oxopentanoate, **11**, to give isoquinolinepyrazole ester **27**. Amino and diaminoquinazolines **24b**,**c** were synthesized from **14f** via displacement and condensation with either formamidine or guanidine, respectively. Benzamidoxime **24d** was prepared from **14c** via Pinner reaction with hydroxylamine. Aminopyridine **24f** was the result of amination of pyridine **14m**.

Results and Discussion

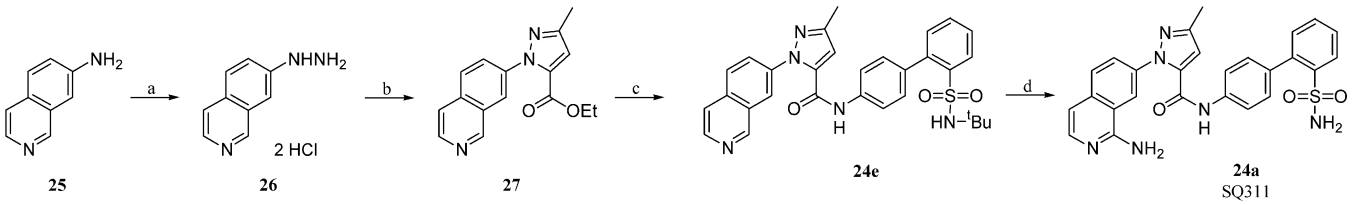
Table 1 summarizes a list of basic amine P1 moieties that were examined as benzamidine mimics. Benzylamine **15a**[2] was the most potent FXa inhibitor in this series (FXa K_i of 2.7 nM compared to 0.013 nM for SN429, **1**), having a loss of only 200-fold binding affinity as compared with compound **1**. Among the anilines, the 3-anilino derivative **15b** was the most potent but was 20-fold less potent than the corresponding *m*-benzylamine **15a**. All of the remaining amines that were evaluated were determined to be significantly less potent. Surprisingly, *n*-butylamine **15e** was found to be ineffective with a FXa K_i of 12 μM. We speculate that this is a result of the steric congestion of the 1,2-regiochemistry on the pyrazole ring, leading to a distortion of the binding conformation of the flexible P1 alkyl side chain of **15e**. Finally, piperidine **15f** was also determined to be a very weak inhibitor, probably because the piperidine nitrogen is not close enough to interact with Asp189.

Scheme 3[a]



[a] Reagents: (a) TFAA, pyr, CH_2Cl_2; (b) HCO_2H, H_2O, $CHCl_3$; (c) NH_2NHBoc, PhH, reflux; (d) (i) BH_3·THF, (ii) HCl; (e) EtOH, **11**, reflux; (f) (i) TFA, reflux, (ii) K_2CO_3, MeOH aq, (iii) HCl.

Scheme 4[a]



[a] Reagents: (a) (i) $NaNO_2$, HCl, 0 °C, (ii) $SnCl_2$; (b) AcOH, **11**, reflux; (c) $AlMe_3$, **13**, CH_2Cl_2; (d) (i) *m*-CPBA, acetone, (ii) TsCl, pyridine, (iii) ethanolamine, (iv) TFA, reflux.

A series of pyrazole inhibitors with neutral non-benzamidine P1 ligands were explored (Table 2). It was found that **22a** with a *p*-methoxyphenyl P1 moiety has a FXa K_i of 11 nM, a loss of only 850-fold as compared with benzamidine **1** (SN429). This was the most potent neutral P1 ligand in our pyrazole series of FXa inhibitors.[20] Compound **22a** resulted from a P1 screen of phenols and anisoles, which was initiated on the basis of the analysis of our X-ray structure of FXa/r-tick anticoagulant protein.[21] The details of the design will be published elsewhere.[20] Other compounds containing neutral non-benzamidine P1 ligands, which were found to be relatively potent inhibitors, were the 3-carboxamidophenyl derivative **22b** and the 3-chlorophenyl derivative **22c** (FXa affinities of 19 and 37 nM, respectively). Compounds that utilize the 3-cyanophenyl P1 **22e**, 3-sulfiniminephenyl P1 **22f**, and 3-sulfonamidophenyl P_1 **22g** ligands were weaker FXa binders than **22d**, which has an unsubstituted phenyl group in the P1 position.

A series of benzamidine mimics capable of forming bidentate hydrogen bonding were designed (Table 3) to be able to achieve two hydrogen-bonding interactions with Asp189 similar to the benzamidine and hence were anticipated to be potent FXa inhibitors. Indeed, 3-aminobenzisoxazole, **23a**, was found to be a potent FXa inhibitor with a K_i of 1.4 nM, a loss of only 110-fold compared with benzamidine **1**. 3-Aminoindazole, **23b**, was determined to be a weaker FXa binder, with a K_i of 29 nM. Pyrazole **23c**, with phenylboronic acid in the P1 position, was a surprisingly weak inhibitor (K_i of 61 nM) even though it contains the bidentate hydrogen-bond donor feature. This may be because phenylboronic acid prefers to exist in the form of a negatively charged borate and as a result cannot interact favorably with Asp189. Not surprisingly, aminoindazole **23d**, with a K_i of 2800 nM, was found to be a much poorer binder than the corresponding **23b**, which contains the correct regiochemistry.

Combining the bidentate hydrogen-bonding feature with the basic amine feature, a series of interesting benzamidine mimics were designed (Table 4). It was anticipated this combination ought to provide the best FXa affinity. Indeed, 1-aminoisoquinoline **24a** (SQ311) was found to be a subnanomolar inhibitor (FXa K_i of 0.33 nM), showing only a 25-fold loss in affinity relative to the parent benzamidine **1** (K_i = 0.013 nM). Thus, 1-aminoisoquinoline is the best benzamidine mimic in this series in terms of FXa affinity. In addition, trypsin selectivity was improved (7900- versus 1200-fold for **1**). We believe this is due to the negative steric interaction incurred for the fused bicyclic P1 as a result of the replacement of Ala190 in FXa with Ser190 in trypsin. The addition of nitrogens as in 1-aminoquinazoline **24c** or 1,3-diaminoquinazoline **24b** yielded weaker inhibitors with FXa K_i of 29 and 16 nM, respectively. The importance of the amino group of 1-aminoisoquinoline P1 is evidenced by the weaker binding affinity of isoquinoline **24e** (FXa K_i of 370 nM). Benzamidoxime **24d** was found to be 1000-fold less potent than benzamidine **1**. The significantly reduced FXa affinity may be a result of the intramolecular hydrogen bonding of the amidoxime functional group, preventing a favorable interaction in a bidentate fashion with Asp189. 4-Aminopyridine **24f**, a benzamidine mimic used frequently in the design of thrombin inhibitors,[13b] was ineffective in our case.

To confirm our design strategy, the X-ray structure of the complex of **24a** (SQ311) bound in the active site of thrombin was solved (Figure 4).[22] In the S1 pocket, 1-aminoisoquinoline was determined to interact with Asp189 in a bidentate fashion (2.5 and 2.9 Å) as designed. This represents the first published 1-aminoisoquinoline (or the analogous aminopyridine) P1 interaction with Asp189 in the serine protease literature where the interaction is truly bidentate, precisely mimicking the guanidine/carboxylate bidentate interaction in nature. The pK_a of **24a** was measured at 6.7.

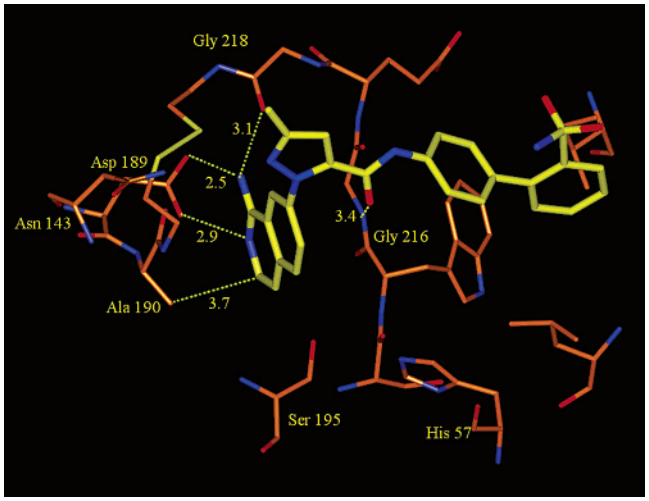


Figure 4. Crystal structure of **24a** (SQ311) in thrombin.

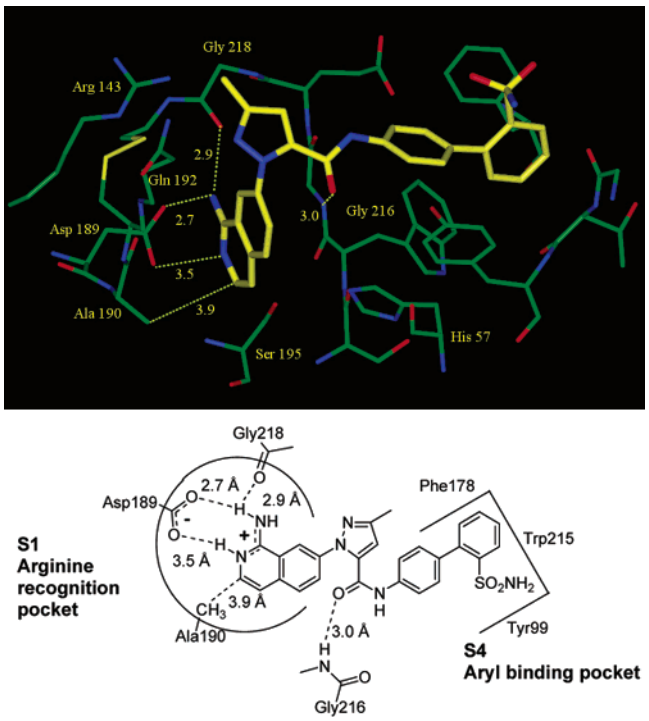
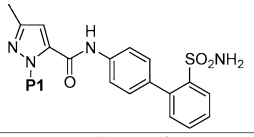


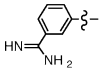
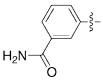
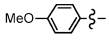
Figure 5. (a, top) Model of **24a** (SQ311) in FXa. (b, bottom) Model of aminoisoquinoline SQ311 binding at the FXa active site.

With a pK_a of 6.7, only 13% of the 1-aminoisoquinoline is protonated at the physiological pH of 7.4. The high potency and the bidentate interaction of the 1-aminoisoquinoline P1 group, as seen from the X-ray complex, implies that the 1-aminoisoquinoline is probably fully protonated in its interaction with Asp189, due to ion-pairing perturbation as described under Design.[16] In the crystal structure, the 1-aminoisoquinoline ring and the pyrazole ring are found to form a dihedral angle of 73°. The amide carbonyl accepts a hydrogen bond (3.4 Å) from the backbone N−H of Gly216. The biaryl P4 moiety is situated in the S4 hydrophobic aryl-binding pocket similar to **1**, as detailed in our earlier published work.[2]

Because the S1 pockets of FXa and thrombin are similar, a model of **24a**/FXa complex was obtained (Figure 5a).[15] The interaction of **24a** with the FXa active site is similar to that of the X-ray structure of the **24a**/thrombin complex. The distance between the 4-carbon of 1-aminoisoquinoline and the methyl carbon of Ala190 is 3.9 Å. This is approximately the van der Waals contact distance and as anticipated introduces selectivity against trypsin, because trypsin has a slightly smaller S1 pocket because of Ser190. Thus, for the conversion of benzamidine (SN429) to 1-aminoisoquinoline (**24a**), a loss of 25-fold in FXa K_i and a 22-fold loss in thrombin K_i were oberved (Table 4). In contrast, the identical conversion gave a bigger loss of 163-fold in trypsin K_i, indicating better selectivity of **24a** against trypsin.

Table 5. Relationship of pK_a and Pharmacokinetic Parameters in Dogs



		pKa	HPLC logP	Clearance L/h/kg	V_{dss} L/kg	$T_{1/2}$ h, *iv*	$T_{1/2}$ h, *po*	F%
1 SN429		10.7	1.5	0.67	0.29	0.82	1.3	4
17a SQ808		8.8	1.7	0.42	1.6	3.9	9.3	13
24a SQ311		6.7	2.6	0.33	1.2	3.4	4.4	13
23a		<2.3	2.3	0.3	0.7	2.8	3.2	26
22b		<0	2.1	0.28	0.9	3.1	3.9	46
22a		<0	2.9	0.09	0.52	4.5	4.5	48

Pharmacokinetics

A representative set of less basic and neutral benzamidine replacements were evaluated in dog pharmacokinetic studies and compared to benzamidine **1**. These compounds are listed in order of decreasing pK_a in Table 5. The compounds were dosed both intravenously and orally. All of the non-benzamidines have lower clearances, ranging from 0.42 (**15a**) to 0.09 (**22a**) L/h/kg relative to benzamidine **1** (0.67 L/h/kg). Also shown in Table 5, lowering the pK_a also resulted in a concomitant increase in lipophilicity as measured by log*P*.[23] The volume of distribution at steady state (V_{dss}) of benzamidine **1** is very small (0.29 L/kg). As might be expected on the basis of the higher log*P* values, the non-benzamidines have larger volumes of distribution at steady state (V_{dss}), ranging from 0.52 L/kg (**22a**) to 1.6 L/kg (**15a**). The combined effect of smaller clearance and larger V_{dss} of non-benzamidines results in longer iv half-lives (2.8−4.5 h), compared with **1** (0.82 h). As can be seen in Figure 6a, where the pharmacokinetics following intravenous dosing are normalized to a dose of 1 mg/kg, the compounds separate into three clusters. The neutral non-benzamidines have the best pharmacokinetic profile, followed by the moderately basic benzamidine mimics. The highly basic benzamidine **1** has the poorest exposure in plasma and shortest half-life.

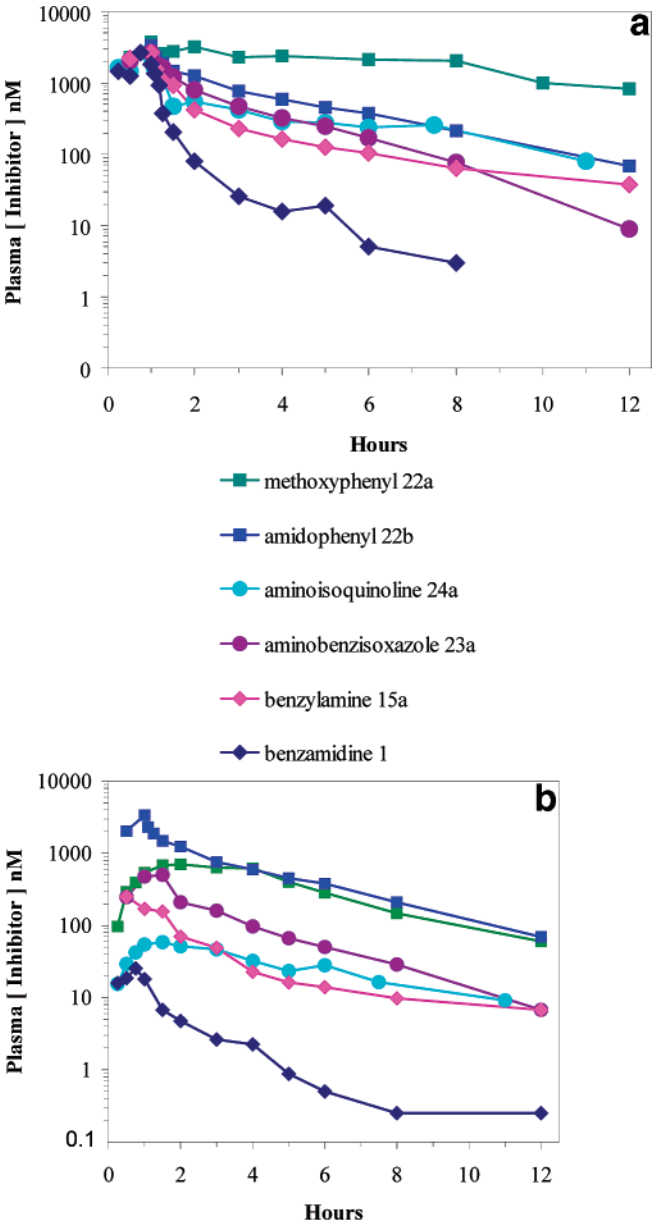


Figure 6. Pharmacokinetics in dogs: (a) intravenous dosing normalized to 1 mg/kg; (b) oral dosing normalized to 1 mg/kg.

In terms of oral absorption, benzamidine **1** is only 4% bioavailable, typical of benzamidine-containing compounds. On the other hand, all of the non-benzamidines have higher oral bioavailabilities ranging from 13% (**15a** and **24a**) to 48% (**22a**) due to a lower desolvation cost, leading to higher lipid permeability, as seen from the higher log*P* of the nonbenzamidines. The $t_{1/2}$ (po) values of the non-benzamidine compounds range from 3.2 h (**23a**) to 9.3 h (**15a**), substantially longer than that of benzamidine **1** (1.3 h). The normalized pharmacokinetics for dogs dosed orally are shown in Figure 6b. To a first approximation, the pharmacokinetics improve as the pK_a is decreased.[24] Thus, the pharmacokinetic features improve as one lowers the pK_a from benzamidines to less basic benzamidine mimics to the neutral nonbenzamidines. This improvement is mainly a result of three parameters: the decrease in clearance and the increases in V_{dss} and oral absorption as a result of the respective decreasing polarity and desolvation cost upon lowering pK_a.

Antithrombotic Efficacy

Among the various benzamidine mimics and non-benzamidines in this study, the aminoisoquinoline, **24a** (SQ311), was chosen for further evaluation on the basis of its sub-nanomolar FXa K_i (0.33 nM), excellent selectivity profile, good human protein binding (89%), and improved pharmacokinetic profile relative to benzamidine **1**. In a rabbit arterial−venous (AV) shunt antithrombosis model,[9] **24a** inhibits thrombus formation with an ID_{50} of 1.2 μmol/kg/h and with an IC_{50} (concentration required to inhibit thrombus formation by 50%) of 690 nM. Thus, **24a** is an effective antithrombotic agent in the rabbit AV shunt thrombosis model.

Summary

In summary, we have performed a systematic study of the effect of lowering the pK_a of the benzamidine P1 ligand by employing benzamidine mimics and non-benzamidines as it relates both to potency and to the pharmacokinetic profile of the compounds examined. We have demonstrated that lowering the pK_a improves the pharmacokinetic features mainly as a result of three parameters: *the decrease in clearance and the increases in V_{dss} and oral absorption* as a result of the associated decreasing polarity and desolvation cost. As a result of this work a series of potent and orally bioavailable benzamidine replacements have been identified. Further optimization to potential clinical candidates is in progress and will be reported elsewhere.[2,20] Among these benzamidine mimics and non-benzamidines, 1-aminoisoquinoline SQ311, **24a**, was shown to be a highly potent and selective orally bioavailable inhibitor of FXa.

Experimental Section

Synthesis. All reactions were run under an atmosphere of dry nitrogen unless otherwise noted. Solvents and reagents were obtained from commercial vendors in the appropriate grade and used without further purification unless otherwise indicated. NMR spectra were obtained on VXR or Unity 300 MHz instruments (Varian Instruments, Palo Alto, CA) with chemical shift in parts per million downfield from TMS as an internal reference standard. ^{1}H assignment abbreviations are as follows: singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), broad singlet (bs), doublet of doublets (dd), doublet of triplets (dt), and multiplet (m). Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. Mass spectra were measured with an HP 5988A mass spectrometer with particle beam interface using NH_3 for chemical ionization or a Finnigan MAT 8230 mass spectrometer with NH_3-DCI or VG TRIO 2000 for ESI. High-resolution mass spectra were measured on a VG 70-VSE instrument with NH_3 ionization. Flash chromatography was done using EM Science silica gel 60 (230−400 mesh). Preparative thin-layer chromatography was done on EM Science 60 plates F_{254} (2 mm; 20 × 20 cm). HPLC purification was performed on a Jasco 900 series instrument using a C18 reverse phase column with acetonitrile/water (containing 0.05% TFA) as a mobile phase. All compounds were found to be >97% pure by HPLC analysis unless otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Compounds **11**,[2] **13**,[14b] and **22a**,**d**[20] were obtained as described previously.

Representative Procedure for Pyrazole Intermediates 14a−m. Ethyl 1-(3-Chlorophenyl)-3-methyl-1*H*-pyrazole-carboxylate (12h). Ethyl 2-(methoxyimino)-4-oxopentanoate, **11** (217 mg, 1.16 mmol), and 3-chlorophenylhydrazine hydrochloride, **10h** (416 mg, 2.32 mmol), in absolute ethanol (15 mL) were heated at reflux for 5 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in

dichloromethane and applied to a silica gel column. Elution with a gradient of hexane to 4:1 hexane/ethyl acetate gave 305 mg (99%) of ethyl 1-(3-chlorophenyl)-3-methyl-1*H*-pyrazole-5-carboxylate, **12h**: ^{1}H NMR ($CDCl_3$) δ 7.45−7.26 (m, 4H), 6.82 (s, 1H), 4.26 (dd, 2H, J = 7 Hz), 2.35 (s, 3H), 1.26 (t, 3H, J = 7 Hz); MS, 265.1 $(M + H)^+$.

***N*-{2′-[(*tert*-Butylamino)sulfonyl]-1,1′-biphenyl-4-yl}-1-[3-chlorophenyl]-3-methyl-1*H*-pyrazole-5-carboxamide (14h).** To a solution of 4′-amino-*N*-(*tert*-butyl)-1,1′-biphenyl-2-sulfonamide, **13** (115 mg, 0.378 mmol), in dichloromethane (5 mL) at ambient temperature was added dropwise trimethylaluminum (0.38 mL, 2.0 M in hexane, 0.756 mmol). This mixture was stirred for 15 min at ambient temperature, and then **12h** (100 mg, 0.378 mmol) in dichloromethane (5 mL) was added. The reaction was heated to 40 °C and stirred for 18 h. The reaction was cooled and carefully added to 20 mL of a 10% methanol/chloroform solution. The solvent volume was reduced to ∼2 mL, and the residue was purified by preparative TLC (eluent 50% ethyl acetate/hexane) to give 170.5 mg (70%) of the title compound as a colorless solid: ^{1}H NMR (CD_3OD) δ 8.10 (d, 1H, J = 8.7 Hz), 7.64−7.31 (m, 11H), 6.84 (s, 1H), 2.37 (s, 3H), 1.01 (s, 9H). Anal. Calcd ($C_{27}H_{26}N_4O_3SCl\cdot H_2O$) C, H, N.

1-[3-(Aminomethyl)phenyl]-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide, Trifluoroacetic Acid Salt (15a). 15a was prepared according to the method of Pinto et al.[2]

1-(3-Aminophenyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (15b). 14k was synthesized from **10k** according to the procedure described for the conversion of **10h** to **14h**. A solution of **14k** in trifluoroacetic acid (5 mL) was heated to reflux for 1 h. The reaction was cooled to ambient temperature, and the solvent was concentrated under reduced pressure. The residue was taken up in ethyl acetate and washed with saturated aqueous sodium bicarbonate, water, and brine, dried over sodium sulfate, and filtered, and the solvent was evaporated under reduced pressure. *N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1-(3-nitrophenyl)-1*H*-pyrazole-5-carboxamide was judged to be of sufficient purity and used without purification. To this compound (64.3 mg, 0.135 mmol) in absolute ethanol (5 mL) under N_2 was added 5% Pd on carbon (60 mg), and the flask was evacuated and charged with H_2 (1 atm) via a double-walled latex balloon. After 3 h, the reaction was judged to be complete by TLC, the flask was purged with N_2, and the contents were filtered through a pad of Celite. The pad was washed with methanol and the solvent concentrated under reduced pressure. The residue was purified by preparative TLC (eluent 10% methanol/chloroform) to give 50 mg (83%) of the title compound as a colorless solid: ^{1}H NMR (CD_3OD) δ 8.0 (d, 1H, J = 7.8 Hz), 7.61−7.58 (m, 3H), 7.50 (t, 1H, J = 7.8 Hz), 7.38 (d, 2H, J = 6.9 Hz), 7.31 (d, 1H, J = 7.5 Hz), 7.13 (t, 1H, J = 8.1 Hz), 6.78 (s, 1H), 6.72−6.69 (m, 3H), 2.33 (s, 3H). HRMS calcd 448.1443; found 448.144 for $C_{23}H_{22}N_5O_3S$ $(M + H)^+$.

1-(2-Aminophenyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (15c). 15c was synthesized from **10j** according to the procedure described for the conversion of **10k** to **15b**: ^{1}H NMR (CD_3OD) δ 8.13 (d, 1H, J = 7.5 Hz), 8.04 (d, 1H, J = 8.1 Hz), 7.58−7.28 (m, 6H), 7.17 (d, 2H, J = 8.4 Hz), 6.93 (s, 1H), 6.75 (d, 2H, J = 9.0 Hz), 2.47 (s, 3H); LRMS (ESI+), 448.12 $(M + H)^+$(60%), 470.16 $(M + Na)^+$(100%).

1-(4-Aminophenyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide, Trifluoroacetic Acid Salt (15d). 15d was synthesized from **10l** according to the procedure described for the conversion of **10k** to **15b**: mp 234.9 °C; ^{1}H NMR (CD_3OD) δ 8.10 (dd, 1H, J = 7.8, 1.2 Hz), 7.63−4.49 (m, 4H), 7.40−7.31 (m, 3H), 7.15 (d, 2H, J = 8.7 Hz), 6.75−7.70 (m, 3H), 2.34 (s, 3H); LRMS (ESI+), 448.4 $(M + H)^+$(100%). HRMS calcd 448.1443; found 448.1444 for $C_{23}H_{22}N_5O_3S$ $(M + H)^+$.

1-(4-Aminobutyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide, Hydrochloride (15e). To a solution of 4,4-diethoxy-1-butanamine, **16** (8.05 g, 50 mmol), in methylene chloride (10 mL) cooled to 0 °C was added pyridine (4.48 mL, 55 mmol); trifluoroacetic anhydride (7.77 mL, 55 mmol) was then added dropwise via an addition funnel. After the addition, the reaction was slowly warmed to room temperature and stirred for an additional 3 h. The reaction mixture was then partitioned between methylene chloride (80 mL) and water (40 mL), washed with 1 N HCl (30 mL) and brine (30 mL), dried over sodium sulfate, filtered, and concentrated to give 12.3 g (96%) of *N*-(4,4-diethoxybutyl)-2,2,2-trifluoroacetamide, **17**. This compound (12.3 g, 47.8 mmol) was dissolved in chloroform (20 mL), and 85% formic acid (5 mL) was added. The reaction mixture was stirred at room temperature for 14 h, after which the reaction mixture was partitioned between chloroform (80 mL) and water (40 mL), washed once with brine (40 mL), dried over sodium sulfate, filtered, and concentrated to give 7.0 g (80%) of 2,2,2-trifluoro-*N*-(4-oxobutyl)acetamide, **18**. This compound (5.0 g, 27.2 mmol) was dissolved in benzene (50 mL), and *tert*-butyl carbazate (3.6 g, 27.2 mmol) was added. The reaction was brought to reflux and stirred for 14 h. The reaction was cooled to room temperature, the benzene was evaporated under reduced pressure to ∼20 mL, and the resulting white solid that precipitated was isolated by filtration. The solids were washed once with diethyl ether (20 mL) and air-dried to give 5.5 g (68%) of *tert*-butyl 2-{4-[(trifluoroacetyl)amino]butylidene}-hydrazinecarboxylate, **19**. This compound (5.0 g, 16.8 mmol) was added to 18 mL of a borane THF complex (1 M). The reaction mixture was stirred at room temperature for 14 h, and the solvent was removed under reduced pressure. The residue was purified via flash chromatography on silica gel using 1:1 ethyl acetate/hexane to give 3.2 g (95%) of *tert*-butyl 2-{4-[(trifluoroacetyl)amino]butyl}hydrazinecarboxylate. This compound (3.2 g, 16 mmol) was dissolved in methanol (20 mL), cooled to 0 °C, and HCl gas was bubbled through the solution for 10 min. The solution was slowly warmed to room temperature and stirred for another 10 min. The solvent was evaporated to give 2.5 g of 2,2,2-trifluoro-*N*-(4-hydrazinobutyl)-acetamide hydrochloride, **20**, as a brown oil (63%): LRMS (AP+), 200.1 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 7.06 (bs, 1H), 4.45 (bs, 2H), 3.39 (q, 2H), 3.54 (t, 2H), 1.82−1.76 (m, 2H), 1.64−1.58 (t, 2H). This compound was converted to **21** according to the procedure for the conversion of **14h** to **22c**. Compound **21** (250 mg, 0.431 mmol) was dissolved in trifluoroacetic acid (4 mL) and brought to reflux for 1.5 h. The solution was cooled to ambient temperature and the solvent evaporated under reduced pressure. The residue was taken up in ethyl acetate and saturated sodium bicarbonate, the phases were separated, and the organic phase was washed once with water, dried over sodium carbonate, filtered, and evaporated to give 200 mg (89%) of the corresponding deprotected sulfonamide. This compound (200 mg, 0.382 mmol) was dissolved in methanol (3 mL), and potassium carbonate (211 mg, 1.52 mmol) and water (3 mL) were added and stirred for 14 h. The methanol was evaporated, and ethyl acetate and water were added; the phases were separated, and the organic phase was washed with water and brine, dried over sodium sulfate, filtered, and evaporated. The residue was purified by silica gel flash chromatography to give 80 mg (49%) of 1-(4-aminobutyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide, which was converted to its hydrochloride salt by treating a methanol solution with 1 M HCl in ether followed by evaporation to give the title compound: ^{1}H NMR ($CDCl_3$) δ 8.12 (d, 1H, J = 8.1 Hz), 7.68 (d, 2H, J = 8.4 Hz), 7.59 (td, H, J = 7.2, 1.1 Hz), 7.52−7.43 (m, 3H), 7.34−7.30 (m, 2H), 6.57 (s, 1H), 4.49 (t, 2H, J = 7.2 Hz), 2.78 (bs, 4H), 2.65 (t, 2H, J = 7.2 Hz), 2.29 (s, 3H), 1.85 (p, 2H, J = 7.5 Hz), 1.44 (p, 2H, J = 7.5 Hz); LRMS (ESI+), 428.3 $(M + H)^+$(100%). HRMS calcd 428.1756; found 428.173774 for $C_{21}H_{26}N_5O_3S$ $(M + H)^+$.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1-(3-piperdinyl)-1*H*-pyrazole-5-carboxamide (15f). 14m** was synthesized from **10m** according to the procedure described for the conversion of **10h** to **14h**. To a solution of **14m** (0.5 g, 1.02 mmol) in acetone (20 mL) was added *m*-chloroperbenzoic

acid (0.28 g of 70% grade, 1.12 mmol), and the reaction was brought to reflux for 1.5 h. The reaction was cooled to ambient temperature and solvent removed under reduced pressure. The residue was taken up in ethyl acetate and saturated sodium bicarbonate, and the phases were separated. The organic phase was washed with saturated sodium bicarbonate and water, dried over sodium sulfate, and filtered, and the solvent was removed under reduced pressure to give 470 mg (91%) of the *N*-oxide of sufficient purity to be used without purification. This compound (50 mg, 0.099 mmol) was subjected to hydrogenation in absolute ethanol/acetic acid 5:1 (6 mL) with PtO_2 (10 mg, 0.044 mmol) under 20 lb/in.^{2} of H_2 for 14 h. The reaction was filtered through a pad of Celite, the pad washed with methanol, and the filtrate evaporated. The residue was taken up in ethyl acetate and saturated sodium bicarbonate, and the phases were separated. The organic phase was washed with water, dried over sodium sulfate, and filtered, and the solvent was removed under reduced pressure. The residue was subjected to silica gel flash column chromatography eluting with a gradient of 10−20% methanol in chloroform to give 30 mg (61%) of the piperdinyl compound. A solution of this compound (30 mg, 0.061 mmol) in trifluoroacetic acid (2 mL) was heated to reflux for 1 h. The reaction was allowed to cool to ambient temperature, and the solvent was concentrated under reduced pressure. The residue was taken up in ethyl acetate and washed with saturated aqueous sodium bicarbonate and water, dried over sodium sulfate, and filtered, and the solvent was evaporated under reduced pressure to give 17 mg (64%) of the title compound: ^{1}H NMR (CD_3OD) δ 8.02 (dd, 1H, J = 7.8, 1.2 Hz), 7.62 (d, 2H, J = 8.4 Hz), 7.53 (td, 1H, J = 7.5, 1.5 Hz), 7.44 (td, 1H, J = 7.8, 1.5 Hz), 7.34 (d, 2H, J = 8.7 Hz), 7.26 (dd, 1H, J = 7.5, 1.5 Hz), 6.73 (s, 1H), 5.40 (m, 1H), 3.45 (d, 2H, J = 5.7 Hz), 3.14−3.08 (m, 2H), 2.23 (s, 3H), 2.14 (dd, 2H, J = 37.2, 5.4 Hz), 1.87 (m, 1H), 1.74 (m, 1H); LRMS (ESI+), 440.1 (M + H)$^+$(100%). HRMS calcd 440.1756; found 440.176811 for $C_{22}H_{26}N_5O_3S$ (M + H)$^+$.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1-(3-methoxyphenyl)-1*H*-pyrazole-5-carboxamide (22a). 22a** was prepared according to the method of Pruitt.[20]

1-[3-(Aminocarbonyl)phenyl]-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (22b). 14c was synthesized from **9c** according to the procedure described for the conversion of **10h** to **14h**. To **14c** (185 mg, 0.361 mmol) was added concentrated H_2SO_4 (5 mL) at ambient temperature and the nitrile dissolved slowly. After 24 h, ice and water were added and the precipitated solids were isolated by filtration. The solids were taken up in ethyl acetate and water. The phases were separated, and the aqueous fraction was extracted three times with ethyl acetate. The combined organic fraction was washed with water, saturated sodium bicarbonate, and brine, dried over magnesium sulfate, and filtered, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (gradient elution 1−10% methanol in methylene chloride) and afforded 88 mg (52%) of the title compound: ^{1}H NMR (DMSO-d_6) δ 10.63 (s, 1H), 8.12 (s, 1H), 8.04 (m, 2H), 7.90 (m, 1H), 7.69 (d, 2H, J = 8.4 Hz), 7.62−7.52 (m, 5H), 7.36 (d, 2H, J = 8.4 Hz), 7.32 (m, 1H), 7.24 (s, 2H), 6.93 (s, 1H), 2.50 (s, 3H); LRMS (ESI+), 476.3 (M + H)$^+$. HRMS calcd 476.1392; found 476.1392 for $C_{24}H_{22}O_3N_5S$ (M + H)$^+$. Anal. Calcd ($C_{24}H_{21}O_4N_5S$) C, H, N.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-1-[3-chlorophenyl]-3-methyl-1*H*-pyrazole-5-carboxamide (22c).** A solution of **14h** (28.0 mg, 0.0536 mmol) in trifluoroacetic acid (3 mL) was heated at reflux for 1 h. The reaction was evaporated, taken up in ethyl acetate, and washed with 1 N sodium hydroxide solution. The organic solution was dried over sodium sulfate, filtered, and evaporated. The residue was purified by preparative TLC (eluent 10% methanol/chloroform) to give 17.5 mg (70%) of the title compound as a colorless solid: ^{1}H NMR (CD_3OD) δ 8.09 (d, 1H, J = 8.1 Hz), 7.64−7.51 (m, 6H), 7.48−7.41 (m, 6H), 7.30 (d, 1H, J = 8.1 Hz), 2.37 (s, 3H); LRMS (ES+) 467.2 (M + H)$^+$ (100%). HRMS calcd 467.0945; found 467.0915 for $C_{23}H_{19}N_4O_3SCl$ (M + H)$^+$.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1-phenyl-1*H*-pyrazole-5-carboxamide (22d). 22d** was prepared according to the method of Pruitt.[20]

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-1-[3-cyanophenyl]-3-methyl-1*H*-pyrazole-5-carboxamide (22e). 22e** was synthesized from **9c** according to the procedure for the conversion of **10h** to **22c**: ^{1}H NMR ($CDCl_3$) δ 8.17 (dd, 2H, J = 8.1,1.5 Hz), 7.86 (m, 3H), 7.66 (d, 2H, J = 8.8 Hz), 7.61 (m, 4H), 7.50 (d, 2H, J = 8.4 Hz), 7.35 (dd, 1H, J = 7.3, 1.5 Hz), 6.72 (s, 1H), 4.24 (s, 2H), 1.56 (s, 3H); LRMS (ESI+) 458.18 (M + H)$^+$, 480.13 (M + Na)$^+$. HRMS calcd 458.1287; found 458.1274 for $C_{24}H_{20}O_3N_5S_1$ (M + H)$^+$. Anal. Calcd ($C_{24}H_{19}O_3N_5S\cdot 0.1H_2O$) C, H, N.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1-[3-(methylsulfinimidoyl)phenyl]-1*H*-pyrazole-5-carboxamide, Trifluoroacetic Acid Salt (22f). 14d** was synthesized from **9d** according to the procedure described for the conversion of **10h** to **14h**. To a solution of **14d** (300 mg, 0.56 mmol) in 10 mL of methanol was added chloramine-T hydrate (153 mg, 0.67 mmol), and the resulting solution was stirred at 60 °C for 1 h. The reaction was allowed to cool and then was diluted with ethyl acetate, washed with 1 N sodium hydroxide and brine, dried over magnesium sulfate, and concentrated to afford 250 mg (63%) of an approximate 1:1 mixture of two products. This mixture was dissolved in 5 mL of trifluoroacetic acid and was stirred at 80 °C for 15 min. After cooling to ambient temperature, the reaction was concentrated in vacuo, and the residue was purified by HPLC and lyophilized to afford 45 mg (20%) of the desired *N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1-(3-{methyl[(4-methylphenyl)sulfonyl]-sulfinimidoyl}phenyl)-1*H*-pyrazole-5-carboxamide: ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 1H), 8.10 (d, 1H, J = 7.0 Hz), 7.80 (s, 1H), 7.68−7.60 (m, 4H), 7.59−7.51 (m, 2H), 7.50−7.44 (m, 2H), 7.37 (d, 2H, J = 8.1 Hz), 7.28 (m, 1H), 7.16 (d, 2H, J = 8.1 Hz), 6.78 (s, 1H), 4.64 (s, 2H), 2.82 (s, 3H), 2.32 (s, 3H), 2.29 (s, 3H); LRMS (ES+), 648 (M + H)$^+$. To this compound (30 mg, 0.046 mmol) was added 0.10 mL of concentrated H_2SO_4, and this reaction mixture was stirred at ambient temperature until it was homogeneous. Ether was added, and a solid precipitated out of solution. The solvent was decanted, and the solid was further triturated with ether and dried in vacuo. The solid was then purified by HPLC and lyophilized to afford 20 mg (71%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 10.65 (s, 1H), 8.15 (s, 1H), 7.99 (dd, 1H, J = 8.1, 1.5 Hz), 7.93−7.89 (m, 1H), 7.79−7.70 (m, 2H), 7.66 (d, 2H, J = 8.8 Hz), 7.61−7.53 (m, 2H), 7.33 (d, 2H, J = 8.8 Hz), 7.28−7.25 (m, 1H), 7.01 (s, 1H), 6.69 (s, 1H), 3.34 (s, 3H), 2.31 (s, 3H); LRMS (ES)$^+$, 494.0 (M + H)$^+$. HRMS (ES+) calcd 494.1321; found 494.1323 for $C_{24}H_{24}N_5O_3S_2$ (M + H)$^+$.

1-[3-(Aminosulfonyl)phenyl]-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (22g). 22g was synthesized from **9e** according to the procedure described for the conversion of **10h** to **22c**: ^{1}H NMR (CD_3OD) δ 8.08 (dd, 1H, J = 7.8, 0.9 Hz), 7.65−7.30 (m, 15H), 6.88 (s, 1H), 2.37 (s, 3H); LRMS (ES+), 512.07 (M + H)$^+$(20%), 534.07 (M + Na)$^+$(100%).

1-(3-Amino-1,2-benzisoxazol-5-yl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (23a). 14f was synthesized from **9f** according to the procedure described for the conversion of **10h** to **14h**. To a solution of acetone oxime (86 mg, 1.18 mmol) in DMF (6 mL) was added sodium *tert*-butoxide (1 M in THF, 1.18 mL). The mixture was stirred at room temperature for 30 min followed by the addition of a solution of **14f** (0.21 g, 0.39 mmol) in DMF (4 mL). The reaction was stirred at room temperature for 5 h. The reaction mixture was then partitioned between ethyl acetate and aqueous HCl (1.4 M), washed once with aqueous HCl (1.4 M), four times with water, and once with brine, dried over sodium sulfate, filtered, and concentrated. Flash chromatography (30% ethyl acetate/hexane) gave *N*-{2′-[(*tert*-butylamino)sulfonyl]-1,1′-biphenyl-4-yl}-1-(3-cyano-4-isopropylideneaminooxyphenyl)-3-methyl-1*H*-pyrazole-5-carboxamide (0.19 g, 81% yield) in sufficient purity to be used without purification. This compound (0.19 g, 0.32 mmol) was

dissolved in ethanol (4 mL), and to the solution was added 6 N HCl (4 mL). The reaction mixture was stirred at 80 °C for 3 h and cooled to room temperature. The white precipitate that formed was filtered and recrystallized from methanol to give 0.14 g (80%) of the title compound: ^{1}H NMR (CD_3OD) δ 8.17 (d, 1H, J = 1.8 Hz), 8.07 (dd, 1H, J = 7.9, 1.2 Hz), 7.94 (dd, 1H, J = 9.0, 1.2 Hz), 7.64 (d, 2H, J = 8.7 Hz), 7.61−7.50 (m, 3H), 7.38 (d, 2H, J = 8.4 Hz), 7.30 (dd, 1H, J = 7.5, 1.0 Hz), 6.97 (s, 1H), 2.38 (s, 3H); LRMS (ESI+), 489.1 $(M + H)^+$(100%). Anal. Calcd ($C_{24}H_{20}N_6O_4S_1 \cdot H_2O$) C, H.

1-(3-Amino-1*H*-indazol-5-yl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (23b). 14f was synthesized from **9f** according to the procedure described for the conversion of **10h** to **14h**. To a solution of **14f** (95 mg, 0.179 mmol) in *n*-butanol (5 mL) was added hydrazine monohydrate (0.3 mL) and the solution brought to reflux for 4 h. The reaction was cooled to ambient temperature and partitioned between ethyl acetate and water. The organic phase was washed once with water, dried over sodium sulfate, and filtered, and the solvent was evaporated under reduced pressure. The residue was subjected to silica gel flash column chromatography eluting with 10% methanol in chloroform to give 50 mg (52%) of 1-(3-amino-1*H*-indazol-5-yl)-*N*-{2′-[(*tert*-butylamino)sulfonyl]-1,1′-biphenyl-4-yl}-3-methyl-1*H*-pyrazole-5-carboxamide. This compound (45 mg, 0.083 mmol) was treated with trifluoroacetic acid (3 mL) at reflux for 1 h. The solvent was evaporated and the residue taken up in ethyl acetate and saturated sodium bicarbonate. The organic phase was washed twice with water, dried over sodium sulfate, filtered, and evaporated to yield 31 mg (77%) of the title compound: ^{1}H NMR (CD_3OD) δ 8.07 (dd, 1H, J = 7.5, 1.2 Hz), 7.76 (s, 1H), 7.59−7.54 (m, 3H), 7.50 (dd, 1H, J = 7.5, 1.5 Hz), 7.35 (d, 4H, J = 7.8 Hz), 7.29 (dd, 1H, J = 7.5, 1.2 Hz), 6.81 (s, 1H), 2.36 (s, 3H); LRMS (ESI+), 488.1 $(M + H)^+$(100%). HRMS calcd 448.1505; found 448.150485 for $C_{24}H_{22}N_7O_3S$ $(M + H)^+$. Anal. Calcd ($C_{24}H_{21}N_7O_3S_1 \cdot H_2O \cdot$MeOH) C, H.

3-[5-({[Aminosulfonyl)-1,1′-biphenyl-4-yl]amino}-carbonyl)-3-methyl-1*H*-pyrazol-1-yl]phenylboronic acid (23c). 14i was synthesized from **10i** according to the procedure described for the conversion of **10h** to **14h**. To a solution of **14i** (100 mg, 0.196 mmol) in THF (15 mL) was added sodium hydride (47 mg of 60% dispersion in oil, 1.2 mmol) and the reaction stirred at ambient temperature for 20 min. The reaction was cooled to −78 °C, and *n*-butyllithium (0.25 mL of 1.6M in hexanes, 0.40 mmol) was added dropwise followed by stirring at this temperature for 30 min and warming to 0 °C for 10 min, followed by cooling again to −78 °C. Triisopropyl borate (0.11 mL, 0.49 mmol) was next added and the cooling bath removed, allowing the reaction to warm to ambient temperature for 14 h. The reaction was cooled to 0 °C, 10% HCl (5 mL) was added, and the reaction was allowed to warm to ambient temperature and stirred for 1.5 h. The reaction was quenched into an excess of saturated sodium bicarbonate, ethyl acetate was added, and the phases were separated. The organic phase was washed once with water and brine, dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was subjected to preparative TLC to give 35 mg (37%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 8.13 (d, 1H, J = 8.1 Hz), 7.75 (s, 2H), 7.59−7.39 (m, 11H), 6.75 (s, 1H), 2.40 (s, 3H); LRMS (ESI+), 477.1 $(M + H)^+$(40%), 499.0 $(M + Na)^+$(100%). HRMS calcd 533.1666; found 533.167468 for glycerol ester $C_{26}H_{26}BN_4O_6S$ $(M + H)^+$.

1-(3-Amino-1*H*-indazol-6-yl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (23d). 14g was synthesized from **9g** according to the procedure described for the conversion of **10h** to **14h**. **23d** was synthesized from **14g** according to the procedure for the conversion of **14f** to **23b**: ^{1}H NMR (CD_3OD) δ 8.12 (d, 1H, J = 7.8 Hz), 7.75−7.31 (m, 8H), 7.11 (d, 1H, J = 7.8 Hz), 6.83 (s, 1H), 2.41 (s, 3H); LRMS (ESI+), 510.1 $(M + Na)^+$(100%).

1-(1-Amino-7-isoquinolyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide, Methanesulfonic Acid Salt (24a). *N*-{2′-[(*tert*-Butylmino)sulfonyl]-1,1′-biphenyl-4-yl}-1-(7-isoquinolyl)-3-methyl-1*H*-pyrazole-5-carboxamide, **24e** (3.50 g, 6.49 mmol), was dissolved in 60 mL of acetone to which was added *m*-chloroperbenzoic acid (70%) (1.86 g, 7.55 mmol), and the reaction was allowed to stir at ambient temperature. Upon completion, as determined by TLC, the solvent was removed under reduced pressure and the residue taken up in 100 mL each of ethyl acetate and saturated sodium bicarbonate. The phases were separated, and the organic layer was dried over sodium sulfate, filtered, and evaporated to give the *N*-oxide as a light pink solid in quantitative yield and of sufficient purity for the next step: MS (ES+), 556.20 $(M + H)^+$ (15%), 578.21 $(M + Na)^+$ (100%).

The *N*-oxide was dissolved in 110 mL of anhydrous pyridine, tosyl chloride (1.64 g, 8.63 mmol) was added in three equal portions, and the reaction was allowed to stir at ambient temperature. The pyridine was removed under reduced pressure, and to the residue was added 45 mL of ethanolamine; the reaction was stirred at ambient temperature. Upon completion, as determined by TLC, the reaction was poured onto cracked ice, and the solids were isolated by filtration and dried under vacuum to yield 2.33 g (65% yield) of a mixture of the 1-aminoisoquinoline (major) and 4-aminoisoquinoline (minor) products as a tan solid: MS (ES+) 555.22 $(M + H)^+$ (100%). HRMS calcd 555.2178; found 555.21858 for $C_{30}H_{30}N_6O_3S$ $(M + H)^+$.

To 20 mL of trifluoroactic acid was added the mixture of the aminoisoquinoline compounds and the reaction brought to reflux for 4 h. The solvent was removed under reduced pressure and the residue made basic with cold aqueous sodium carbonate, extracted with ethyl acetate (3 × 40 mL), dried over sodium sulfate, and evaporated. The tan solid was purified by silica gel flash column chromatography eluting with 15% MeOH/$CHCl_3$ to give 1.60 g (76% yield) of the title compound as a light tan solid.

The product was then treated with 1 equiv of methanesulfonic acid in THF. Evaporation of the solvent gave **24a**: mp 195 °C; ^{1}H NMR (CD_3OD) δ 8.27 (s, 1H), 8.07 (d, 1H, J = 7.5 Hz), 7.80−7.68 (m, 6H), 7.62−7.49 (m, 5H), 7.36 (d, 2H, J = 8.4 Hz), 7.29 (d, 1H, J = 7.5 Hz), 7.02 (d, 1H, J = 6.0 Hz), 6.91 (s, 1H), 2.38 (s, 3H); LRMS (ESI+), 499.14 $(M + H)^+$ (100%). HRMS calcd 499.1552; found 499.1535 for $C_{26}H_{22}N_6O_3S$ $(M + H)^+$. Anal. Calcd ($C_{26}H_{22}N_6O_3S \cdot$MsOH$\cdot 3H_2O$) C, H, N.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-1-(2,4-diamino-6-quinazolinyl)-3-methyl-1*H*-pyrazole-5-carboxamide, Trifluoroacetic Acid Salt (24b). 14f** was synthesized from **9f** according to the described procedure for the conversion of **10h** to **14h**. To a solution of **14f** (60 mg, 0.112 mmol) in dimethylacetamide (5 mL) was added guanidine hydrochloride (40 mg, 0.419) and sodium hydride (25 mg of a 60% dispersion in oil, 1.7 mmol), and the reaction was brought to reflux for 14 h. The reaction was cooled to ambient temperature, methanol was added (2 mL), and the reaction was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic phase was dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by preparative TLC (eluent 10% methanol/chloroform) to give 15 mg (24%) of the 2,4-diamino-6-quinazolinyl compound. This compound (15 mg, 0.026 mmol) in trifluoroacetic acid (2 mL) was heated to reflux for 1 h. The reaction was allowed to cool to ambient temperature, and the solvent was concentrated under reduced pressure. The residue was taken up in ethyl acetate and washed with saturated aqueous sodium bicarbonate and water, dried over sodium sulfate, and filtered, and the solvent was evaporated under reduced pressure to give 6 mg (44%) of *N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-1-(2,4-diamino-6-quinazolinyl)-3-methyl-1*H*-pyrazole-5-carboxamide. The residue was purified by reverse phase HPLC and lyophilized to give the title compound: ^{1}H NMR (CD_3OD) δ 8.31 (d, 1H, J = 2.4 Hz), 8.10 (d, 1H, J = 7.8 Hz), 7.87 (d, 1H, J = 8.7 Hz), 7.66−7.32 (m, 13H), 6.99 (s, 1H), 2.40 (s, 3H); LRMS (ESI+), 515.2 $(M + H)^+$ (100%). HRMS calcd 515.1614; found 515.1604 for $C_{25}H_{22}N_8O_3S$ $(M + H)^+$.

1-(4-Amino-6-quinazolinyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide, Trifluoroacetic Acid Salt (24c). 14f was synthesized from **9f**

according to the procedure described for the conversion of **10h** to **14h**. To a solution of **14f** (53 mg, 0.10 mmol) in dimethylacetamide (5 mL) was added formamidine acetate (104 mg, 1.00 mmol), and the reaction was heated to 120 °C for 14 h. The reaction was cooled to ambient temperature, ethyl acetate and saturated aqueous sodium bicarbonate were added, and the phases were separated. The organic phase was dried over sodium sulfate and filtered and the solvent evaporated under reduced pressure to give 30 mg (54%) of the 4-amino-6-quinazolinyl compound. This compound (30 mg, 0.054 mmol) in trifluoroacetic acid (3 mL) was heated to reflux for 1 h. The reaction was allowed to cool to ambient temperature, and the solvent was concentrated under reduced pressure. The residue was purified by reverse phase HPLC and lyophilized to afford 18 mg (54%) of the title compound: ^{1}H NMR (CD_3OD) δ 10.63 (s, 1H), 8.80 (s, 1H), 8.62 (d, 1H, J = 2.1 Hz), 8.25 (s, 2H), 8.04 (d, 2H, J = 6.6 Hz), 7.84 (d, 1H, J = 9 Hz), 7.71 (d, 2H, J = 8.7 Hz), 7.60−7.53 (m, 2H), 7.36 (d, 2H, J = 8.7 Hz), (d, 2H, J = 8.7 Hz), 7.30 (dd, 1H, J = 7.2, 1.5 Hz), 7.19 (s, 2H), 7.09 (s, 1H), 2.39 (s, 3H); LRMS (ESI+), 500.2 $(M + H)^+$ (100%). HRMS calcd 500.1505 $(M + H)^+$; found 500.1485 for $C_{25}H_{22}N_7O_3S$ $(M + H)^+$.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-1-{3-[(hydroxyamino)(imino)methyl]phenyl}-3-methyl-1*H*-pyrazole-5-carboxamide, Trifluoroacetic Acid Salt (24d). 14c** was synthesized from **9c** according to the procedure described for the conversion of **10h** to **14h**. To a solution of **14c** (184 mg, 0.403 mmol) in absolute ethanol (2 mL) was added hydroxylamine hydrochloride (100 mg, 0.145 mmol) and sodium carbonate (72 mg, 0.68 mmol) and the reaction heated to reflux for 14 h. The reaction was filtered and the filtrate purified by reverse phase HPLC to give 54 mg (27%) of the desired compound after removal of the solvent: ^{1}H NMR (DMSO-d_6) δ 10.65 (s, 1H), 8.04 (dd, 1H, J = 7.7, 1.5 Hz), 7.85 (s, 1H), 7.70 (d, 2H, J = 8.4 Hz), 7.62 (m, 6H), 7.37 (d, 2H, J = 8.4 Hz), 7.32 (dd, 2H, J = 7.3, 1.5 Hz), 7.27 (s, 2H), 6.98 (s, 1H), 2.34 (s, 3H); LRMS (ESI+), 491.2 $(M + H)^+$(100%), 513.2 $(M + Na)^+$(50%); HPLC purity >97%. HRMS calcd 491.1501; found 491.1490 for $C_{24}H_{22}N_6O_4S$ $(M + H)^+$. Anal. Calcd ($C_{24}H_{22}N_6O_4S$·TFA·0.7H_2O) C, H, N.

***N*-[2′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]-1-(7-isoquinolyl)-3-methyl-1*H*-pyrazole-5-carboxamide (24e).** 7-Aminoisoquinoline[25] (6.26 g, 43.4 mmol), **25**, was added to 40 mL of concentrated hydrochloric acid at 0 °C. Sodium nitrite (3.0 g, 43.4 mmol) was dissolved in 15 mL of water, cooled to 0 °C, and added dropwise to the solution of **25** in HCl. The reaction was stirred for 30 min at 0 °C. Stannous chloride dihydrate (29.3 g, 130.2 mmol, 3 equiv) was dissolved in 25 mL of concentrated hydrochloric acid, and the solution was cooled to 0 °C and added dropwise to the solution containing **25** and allowed to stand at 0 °C for 1 h. The resulting precipitate was isolated by filtration and washed with 100 mL of ice-cold brine followed by 100 mL of a 2:1 petroleum ether/ethyl ether solution. The brown solid was dried under dynamic vacuum to give **26**. The isoquinoline salt **26** (9.0 g, 26 mmol) was suspended in 100 mL of glacial acetic acid, and ethyl 2-(methoxyimino)-4-oxopentanoate, **11** (4.0 g, 21.3 mmol), was added dropwise. The reaction was heated to reflux for 14 h, after which the acetic acid was evaporated; to the residue was added 100 mL of water, and the suspension was cooled to 0 °C and neutralized by the addition of solid sodium bicarbonate. The solution was extracted with ethyl acetate (6 × 50 mL), dried over sodium sulfate, and evaporated to give the title compound as a brownish solid (5.15 g, 86% yield), which was >85% of the desired pyrazole regioisomer. This material was purified by silica gel flash chromatography eluting with 5% methanol in chloroform to give **27**: ^{1}H NMR ($CDCl_3$) δ 9.29 (s, 1H,), 8.58 (s, 1H, J = 5.9 Hz), 8.05 (d, 1H, J = 2.0 Hz), 7.89 (d, 1H, J = 8.8 Hz), 7.75 (dd, 1H, J = 8.8, 2.2 Hz), 7.70 (d, 1H, J = 5.9 Hz), 6.89 (s, 1H), 4.24 (q, 2H, J = 7.1 Hz), 2.40 (s, 3H), 1.24 (t, 3H, J = 7.1 Hz); MS (ES+), 282.1 $(M + H)^+$ (100%).

To a solution of 4′-amino-*N*-(*tert*-butyl)-1,1′-biphenyl-2-sulfonamide, **13** (2.19 g, 7.19 mmol), in 100 mL of anhydrous dichloromethane under an atmosphere of nitrogen was added dropwise trimethylaluminum (10.9 mL, 21.6 mmol, 2 M in hexane). The solution was stirred for 30 min at ambient temperature, and then ethyl 1-(isoquinolyn-7′-yl)-3-methyl-5-pyrazole carboxylate (2.02 g, 7.19 mmol), **27**, in 70 mL of anhydrous dichloromethane was added dropwise; the reaction warmed to 40 °C and allowed to stir for 14 h. The reaction was cooled to 0 °C and carefully quenched with 50 mL of 1 N hydrochloric acid, diluted with 50 mL of water, and made basic with solid sodium carbonate. The phases were separated, and the aqueous fraction was extracted with dichloromethane (3 × 30 mL), dried over sodium sulfate, and evaporated to give *N*-{2′-[(*tert*-butylamino)sulfonyl]-1,1′-biphenyl-4-yl}-1-(7-isoquinolyl)-3-methyl-1*H*-pyrazole-5-carboxamide (3.50 g, 90% yield), **24e**, as a brown solid of sufficient purity for the next step. The material was further purified by silica gel flash chromatography eluting with 5% methanol in chloroform: MS (ES+), 540.22 $(M + H)^+$ (100%). **24e** (26.3 mg, 0.0487 mmol) in trifluoroacetic acid (3 mL) was heated to reflux for 1 h. The reaction was allowed to cool to ambient temperature and the solvent concentrated under reduced pressure. The residue was taken up in ethyl acetate and washed with saturated aqueous sodium bicarbonate and water, dried over sodium sulfate, and filtered, and the solvent was evaporated under reduced pressure. The residue was purified by preparative TLC (eluent 10% methanol/chloroform) to give 14.4 mg (61%) of the title compound: ^{1}H NMR (CD_3OD) δ 9.27 (s, 1H), 8.46 (d, 1H, J = 6.0 Hz), 8.18 (s, 1H), 8.09−8.01 (m, 2H), 7.90−7.86 (m, 2H), 7.63−7.46 (m, 4H), 7.37 (d, 2H, J = 8.4 Hz), (dd, 1H, J = 7.8, 1.5 Hz), 6.92 (s, 1H), 2.40 (s, 3H); LRMS (ESI+), 484.12 $(M + H)^+$(100%). HRMS calcd 484.1443; found 484.143007 for $(M + H)^+$.

1-(2-Amino-5-pyridyl)-*N*-[2′-(aminosulfonyl)-1,1′-biphenyl-4-yl]-3-methyl-1*H*-pyrazole-5-carboxamide (24f). 14m was synthesized from **10m** according to the procedure described for the conversion of **10h** to **14h**. **14m** was oxidized by *m*-CPBA according to the procedure for **15f**. The *N*-oxide (110 mg, 0.218 mmol) was dissolved in anhydrous pyridine (4 mL), tosyl chloride (54 mg, 0.283 mmol) was added, and the reaction was allowed to stir at ambient temperature. The pyridine was removed under reduced pressure, and to the residue was added ethanolamine (4 mL); the reaction was stirred at 50 °C for 24 h. The reaction was purified by silica gel flash chromatography eluting with 7% methanol in chloroform to give 75 mg (67%) of the 2-aminopyridin-5-yl compound. A solution of this compound (40 mg, 0.077 mmol) in trifluoroacetic acid (3 mL) was heated to reflux for 1 h. The reaction was allowed to cool to ambient temperature, and the solvent was concentrated under reduced pressure. The residue was taken up in ethyl acetate, washed with saturated aqueous sodium bicarbonate and water, dried over sodium sulfate, and filtered, and the solvent was evaporated under reduced pressure to give 29 mg (84%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 1H, J = 7.2 Hz), 7.83 (d, 1H, J = 2.4 Hz), 7.63 (d, 2H, J = 8.4 Hz), 7.65−7.52 (m, 2H), 7.39 (dd, 1H, J = 9.0, 3.0 Hz), 7.30 (d, 2H, J = 8.7 Hz), 7.20 (s, 2H), 6.82 (s, 1H), 6.44 (d, 1H, J = 8.7 Hz), 6.18 (s, 2H), 2.26 (s, 3H); LRMS (ESI+), 449.1 $(M + H)^+$(100%). HRMS calcd 449.1395; found 449.139317 for $C_{22}H_{21}N_6O_3S$ $(M + H)^+$.

Pharmacokinetic Study. Compounds were administered intravenously over 60 min to two dogs or orally to two dogs (0.5 mg/kg iv, 0.2 mg/kg po) in an N-in-1 format. The formulation used for both intravenous and oral dosing was 70% water, 10% propylene glycol, 10% ethanol, and 10% *N*,*N*-dimethylacetamide. Blood samples were collected into sodium citrate tubes predose and at 0.5, 1, 1.1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, and 12 h for the intravenous dosingg or predose and at 1, 2, 3, 4, 5, 6, 8, and 12 h for oral dosing. Plasma concentrations for the compounds were determined by LC-MS/MS using multiple reaction monitoring in positive electrospray ionization mode on a Micromass Quattro Ultima mass spectrometer coupled with a Shimadzu HPLC system and an HTC PAL autosampler. Each compound was separated on a Supelco Discovery C18 column. The quantitation limit was 1 nM for all compounds.

The pharmacokinetic analyses were carried out using WinNonlin software (Pharsight Co.).

References

(1) This work was presented in part at the 220th National Meeting of the American Chemical Society, Washington, DC, Aug 19−24, 2000, *MEDI-329*. Lam, Patrick Y. S. "Structure-based Design and Discovery of Orally-bioavailable Potent Nonbenzamidine Factor Xa Inhibitors", presented at the "New Advances in Cardiovascular Therapeutics" symposium.

(2) First pyrazole clinical example: Pinto, D. J.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Wong, P. C.; Knabb, R. M.; Luettgen, J. M.; Aungst, B. J.; Li, L.; Wright, M.; Jona, J. A.; Wexler, R. R.; Lam, P. Y. S. The Discovery of DPC 423, a Highly Potent, Selective and Orally Bioavailable Inhibitor of Blood Coagulation Factor Xa. *J. Med. Chem.* **2001**, *44*, 566−578.

(3) Merlos, M.; Leeson, P. A.; Castaner. Orbofiban Acetate. *J. Drugs Future* **1998**, *23*, 1190−1198.

(4) (a) Collins, J. L.; Shearer, B. G.; Oplinger, J. A.; Lee, S.; Garvey, E. P.; Salter, M.; Duffy, C.; Burnette, T. C.; Furfine, E. S. *N*-Phenylamidines as Selective Inhibitors of Human Neuronal Nitric Oxide Synthase: Structure−Activity Studies and Demonstrations of In Vivo Activity. *J. Med. Chem.* **1998**, *41*, 2858−2871. (b) Huang, H.; Martasek, P.; Roman, L. J.; Silverman, R. B. Synthesis and evaluation of peptidomimetics as selective inhibitors and active site probes of nitric oxide synthases. *J. Med. Chem.* **2000**, *43*, 2938−2945.

(5) McClellan, W. J.; Rockway, T. W.; Mantei, R.; Geyer, A.; Wendt, M.; Weitzberg, M.; Zhao, X.; Sauer, D.; Bruncko, M.; Dalton, C.; Kaminski, M.; Nienaber, V.; Stewart, K.; Giranda, V.; Butler, C.; Klinghofer, V.; Joseph, M. Studies directed toward naphthamidine containing urokinase inhibitors with improved pharmacokinetic properties. *Abstr. Pap.—Am. Chem. Soc.* **2001**, *221*, MEDI-294.

(6) Tamilarasu, N.; Huq, I.; Rana, T. M. High Affinity and Specific Binding of HIV-1 TAR RNA by a Tat-Derived Oligourea. *J. Am. Chem. Soc.* **1999**, *121*, 1597−1598.

(7) (a) Rosenberg, J. S.; Beeler, D. L.; Rosenberg, R. D. Activation of human prothrombin by highly purified human factor V and FXa in presence of human antithrombin. *J. Biol. Chem.* **1995**, *250*, 1607−1617. (b) Davre, E. W.; Fujikawa, K.; Kisiel, W. The coagulation cascade: Initiation, maintenance and regulation. *Biochemistry* **1991**, *30*, 10363−10370. (c) Rosenberg, R. D.; Damus, P. S. Purification and mechanism of action of human antithrombin-heparin cofactor. *J. Biol. Chem.* **1973**, *248*, 6498−6505.

(8) Mann, K. G.; Nesheim, M. E.; Church, W. R.; Haley, P.; Krishnaswamy, S. Surface-dependent enzyme complexes. *Blood* **1990**, *76*, 1−16.

(9) (a) Wong, P. C.; Quan, M. L.; Crain, E. J.; Watson, C. A.; Wexler, R. R.; Knabb, R. M. Nonpeptide Factor Xa Inhibitors: I. Studies with SF303 and SK549, a New Class of Potent Antithrombotics. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 351−357. (b) Wong, P. C.; Crain, E. J.; Knabb, R. M.; Meade, R. P.; Quan, M. L.; Watson, C. A.; Wexler, R. R.; Wright, M. R.; Slee, A. M. Nonpeptide Factor Xa Inhibitors: II. Antithrombotic Evaluation in a Rabbit Model of Electrically Induced Carotid Artery Thrombosis. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 212−218.

(10) Murayama, N.; Tanaka, M.; Kunitada, S.; Yamada, H.; Inoue, T.; Terada, Y.; Fujita, M.; Ikeda, Y. Tolerability, Pharmacokinetics, and Pharmacodynamics of DX-9065a, a New Synthetic Potent Anticoagulant and Specific Factor Xa Inhibitor, in Healthy Male Volunteers. *Clin. Pharmacol. Ther.* **2000**, *66*, 258−264.

(11) For reviews and references see: (a) Vacca J. P. Thrombosis and Coagulation. *Annu. Rep. Med. Chem.* **1998**, *33*, 81−90. (b) Fevig, J. M.; Wexler, R. R. Anticoagulants: Thrombin and Factor Xa inhibitors. *Annu. Rep. Med. Chem.* **1999**, *34*, 81−100. (c) Zhu, B.-Y.; Scarborough, R. M. Factor Xa Inhibitors: Recent Advances in Anticoagulant Agents. *Annu. Rep. Med. Chem.* **2000**, *35*, 83−102. (d) Sanderson, P. E. J. Anticoagulants: Inhibitors of Thrombin and Factos Xa. *Annu. Rep. Med. Chem.* **2001**, *36*, 79−98.

(12) Currently there are no oral antithrombotic agents on the market based solely on FXa inhibition. More than 20 companies worldwide are performing research with the aim of producing orally bioavailable FXa inhibitors as clinical agents.

(13) For review specifically on nonbenzamidines see: (a) Menear, K. Progress Towards the Discovery of Orally Active thrombin Inhibitors. *Curr. Med. Chem.* **1998**, *5*, 457−468. (b) Rewinkel, J. B. M.; Adang, A. E. P. Strategies and Progress Towards the Ideal Orally Active Thrombin Inhibitor. *Curr. Pharm. Design* **1999**, *5*, 1043−1075. (c) Peterlin-Masic, L.; Kikelj, D. Arginine Mimetics. *Tetrahedron* **2001**, *57*, 7073−7105. It is our belief that for benzamidine mimics to be useful as anticoagulants in clinics, they should have subnanomolar FXa K_i. For examples of papers containing nonbenzamidne thrombin and FXa inhibitors that are subnanomolar in K_i and have demonstrated good oral bioavailability see: (d) Coburn, C. A.; Rush, D. M.; Williams, P. D.; Homnick, C.; Lyle, E. A.; Lewis, S. D.; Lucas, B. J., Jr.; Muzio-Mower, J. M. D.; Juliano, M.; Krueger, J. A.; Vastag, K.; Chen, I.-W.; Vacca, Joseph P. Bicyclic pyridones as potent, efficacious and orally bioavailable thrombin inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1069−1072 and references cited therein. (e) Masters, J. J.; Franciskovich, J. B.; Tinsley, J. M.; Bailey, B. D.; Beight, D. W.; Brown, R. F.; Buben, J.; Chouinard, M. L.; Craft, T. J.; Daniels, W. D.; Dunwiddie, C. T.; Ficorilli, J. V.; Froelich, L. L.; Gifford-Moore, D. S.; Goodson, T., Jr.; Harms, C. S.; Herron, D. K.; Jackson, C. V.; Jakubowski, J. A.; Joseph, S. P.; Klimkowski, V. J.; Kurz, K. D.; McCowan, J. R.; McGill, J. M., III; Mendel, D.; Muegge, L. P.; Murphy, A. T.; Ratz, A. M.; Shetler, T. J.; Sawyer, J. S.; Smallwood, J. K.; Smith, G. F.; Smith, T.; Tebbe, A. L.; Toth, J. E.; Towner, R. D.; Trankle, W. G.; Weir, L. C.; Wiley, M. R.; Wilson, A.; Yee, Y. K. Aryl 1,2-diamino- and anthranilate-based inhibitors of human factor Xa**.** *Abstr. Pap.—Am. Chem. Soc.* **2000**, *220*, MEDI-328. (f) Kochanny, M. J.; Adler, M.; Cheeseman, S.; Chou, Y.-L.; Davey, D. D.; Eagen, K. A.; Ewing, J.; Fitch, R.; Griedel, B. D.; Karanjawala, R.; Lee, W.; Lentz, D.; Liang, A.; Morrissey, M. M.; Phillips, G. B.; Post, J.; Sacchi, K. L.; Sakata, S. T.; Shaw, K. J.; Snider, R. M.; Subramanyam, B.; Trinh, L.; Vergona, R.; Walters, J.; Wang, Y.-X.; White, K. A.; Whitlow, M.; Wu, S. C.; Ye, B.; Zhao, Z. Development of highly potent, selective, and orally available nonamidine factor Xa inhibitors. *Abstr. Pap.—Am. Chem. Soc.* **2001**, *221*, MEDI-016. (g) Adler, M.; Kochanny, M. J.; Ye, B.; Rumennik, G.; Light, D. R.; Biancalana, S.; Whitlow, M. Crystal Structures of Two Potent Nonamidine Inhibitors Bound to Factor Xa. *Biochemistry* **2002**, *41*, 15514−15523. (h) Sanderson, P. E. J.; Cutrona, K. J.; Savage, K. L.; Naylor-Olsen, A. M.; Bickel, D. J.; Bohn, D. L.; Clayton, F. C.; Krueger, J. A.; Lewis, S. D.; Lucas, B. J.; Lyle, E. A.; Wallace, A. A.; Welsh, D. C.; Yan, Y. 3-Amino-4-sulfonylpyridinone acetamide and related pyridothiadiazine thrombin inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1441−1444. (i) Burgey, C. S.; Robinson, K. A.; Lyle, T. A.; Sanderson, P. E. J.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Singh, R.; Miller-Stein, C.; White, R. B.; Wong, B.; Lyle, E. A.; Williams, P. D.; Coburn, C. A.; Dorsey, B. D.; Barrow, J. C.; Stranieri, M. T.; Holahan, M. A.; Sitko, G. R.; Cook, J. J.; McMasters, D. R.; McDonough, C. M.; Sanders, W. M.; Wallace, A. A.; Clayton, F. C.; Bohn, D.; Leonard, Y. M.; Detwiler, T. J., Jr.; Lynch, J. J., Jr.; Yan, Y.; Chen, Z.; Kuo, L.; Gardell, S. J.; Shafer, J. A.; Vacca, J. P. Metabolism-Directed Optimization of 3-Aminopyrazinone Acetamide Thrombin Inhibitors. Development of an Orally Bioavailable Series Containing P1 and P3 Pyridines. *J. Med. Chem.* **2003**, *46*, 461−473.

(14) (a) Quan, M. L.; Pruitt, J. R.; Ellis, C. D.; Liauw, A. Y.; Galemmo, R. A.; Stouten, P. F. W.; Wityak, J.; Knabb, R. M.; Thoolen, M. J.; Wong, P. C.; Wexler, R. R. Design and Synthesis of Isoxazoline Derivatives as Factor Xa Inhibitors. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 2813−2818. (b) Quan, M. J.; Liauw, A. Y.; Ellis, C. D.; Pruitt, J. R.; Bostrom, L. L.; Carini, D. J.; Huang, P. P.; Harrison, K.; Knabb, R. M.; Thoolen, M. J.; Wong, P. C.; Wexler, R. R. Design and Synthesis of Isoxazoline Derivatives as Factor Xa Inhibitors. 1. *J. Med. Chem.* **1999**, *42*, 2752−2759. (c) Quan, M. J.; Ellis, C. D.; Liauw, A. Y.; Alexander, R.; Knabb, R. M.; Lam, G. N.; Wong, P. C.; Wexler, R. R. Design and Synthesis of Isoxazoline Derivatives as Factor Xa Inhibitors. 2. *J. Med. Chem.* **1999**, *42*, 2760−2773. (d) Pruitt, J. R.; Pinto, D. J.; Quan, M. L.; Estrella, M. J.; Bostrom, L. L.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. Isoxazolines and Isoxazoles as Factor Xa Inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 685−689. (e) Fevig, J. M.; Pinto, D. J.; Han, Q.; Quan, M. L.; Pruitt, J. R.; Jacobson, I. C.; Galemmo, R. A., Jr.; Wang, S.; Orwat, M. J.; Bostrom, L. L.; Lam, P. Y. S.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. Synthesis and SAR of Benzamidine Factor Xa Inhibitors Containing a Vicinal-Substituted Heterocyclic Core. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 641−645. (f) Quan, M. L.; Wexler, R. R. The Design and Synthesis of Noncovalent Factor Xa Inhibitors. *Curr. Top. Med. Chem.* **2001**, *1*, 131−149.

(15) Energy-minimized molecular models were performed using the Insight II program developed by Molecular Simulations Inc. molecular modeling program (version 97.2). For similar models refer to: Padmanabhan, K.; Padmanabhan, K. P.; Tulinsky, A.; Park, C. H.; Bode, W.; Blankenship, D. T.; Cardin, A. D.; Kisiel, Structure of human Des (1−45) factor Xa at 2.2 Å resolution W. *J. Mol. Biol.* **1993**, *232*, 947−966.

(16) A good example is the crucial ion-pairing interaction of Asp29/Arg8′ or Asp29′/Arg8 at the dimeric interface of HIV protease. The pK_a of Asp29 was titrated to be 2.1 instead 3.7, the pK_a of normal aspartic acid. This pK_a suppression of aspartic acid is attributed to the ion-pairing effect, which suppresses the pK_a of the acid partner and elevates the pK_a of the base partner. See: Yamazaki, T.; Nicholson, L. K.; Torchia, D. A.; Wingfield, P.; Stahl, S. J.; Kaufman, J. D.; Eyermann, C. J.; Hodge, C. N.; Lam, P. Y. S.; Ru, Y.; Jadhav, P. K.; Chang, C.-H.; Weber, P. C. NMR

and X-ray Evidence That the HIV Protease Catalytic Aspartyl Groups Are Protonated in the Complex Formed by the Protease and a Non-Peptide Cyclic Urea-Based Inhibitor. *J. Am. Chem. Soc.* **1994**, *116*, 10791−10792.

(17) Mann, Kenneth G.; Lorand, Laszlo. Blood coagulation. *Methods Enzymol.* **1993**, 222 (Proteolytic Enzymes in Coagulation, Fibrinolysis, and Complement Activation, Part A), 121−123.

(18) Compound **9e** was obtained from 3-nitro-benzenesulfonyl chloride via reaction with *tert*-butylamine/TEA/CH_2Cl_2 then reduction Pd(C)/H_2/EtOH.

(19) Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *48*, 4171−4174.

(20) A comprehensive list of neutral non-benzamidines with *p*-methoxyphenyl being the best neutral non-benzamidine can be found in: Pruitt, J. R.; Galemmo, R. A., Jr.; Lam, P. Y. S.; Pinto, D. J.; Dominguez, C.; Alexander, R. S.; Rossi, K. A.; Wells, B. L.; Drummond, S.; Bostrom, L. L.; Wong, P. C.; Wright, M. R.; Bai, S.; Knabb, R. M.; Wexler, R. R. Discovery of 2-(2-Aminomethyl-phenyl)-5-trifluoromethyl-2H-pyrazole-3-carboxylic acid (3-fluoro-2′-sulfamoyl-biphenyl-4-yl)-amide (DPC602), a Potent, Selective and Orally Bioavailable Factor Xa Inhibitor. *J. Med. Chem.* **2003**, in press.

(21) Wei, A.; Alexander, R. S.; Duke, J.; Ross, H.; Rosenfeld, S. A.; Chang, C.-H. Unexpected Binding Mode of Tick Anticoagulant Peptide Complexed to Bovine Factor Xa. *J. Mol. Biol.* **1998**, *283*, 147−154.

(22) Human α-thrombin was purchased from Enzyme Research Labs and used without further purification. Acetylhirudin (54−65) sulfated was purchased from Bachem (catalog no. H-7415). The thrombin−hirudin complex was prepared and concentrated to 10 mg/mL. This complex was diluted in half with 41 mM SQ311 in 0.375 M NaCl, 0.05M sodium phosphate, pH 7.3, 0.02% NaN_3, and 4% DMSO. Crystallization conditions were similar to those described by Skrzypczak-Jankun et al. (Skrzypczak-Jankun, E.; Carperos, V. E.; Ravichandran, K. G.; Tulinsky, A.; Westbrook, M.; Maraganore, J. M. Structure of the hirugen and hirulog 1 complexes of α-thrombin. *J. Mol. Biol.* **1991**, *221*, 1379). Crystals were grown using hanging drop vapor diffusion from 27 to 30% PEG 8000 buffered with 100 mM sodium phosphate in the reservoir. Crystal growth was initiated by macroseeding with small thrombin−hirudin crystals. Crystals of a usable size grew in 1 week. Data were collected on an R Axis II image plate mounted on a Rigaku RU200 rotating anode. Data were processed with the HKL suite (Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. *Methods Enzymol.* **1997**, *276* (Macromolecular Crystallography, Part A), 307−326.) Crystals diffracted to 2.1 Å with an *R*-merge of 7.4%. Unambiguous density for the inhibitor was observed in the active site. The inhibitor was built using the program Quanta (Molecular Structure Inc.). Data were refined using the program CNX (Molecular Structure Inc.) with a crystallographic *R* factor of 21.0% with an *R*-free of 25.8%.

(23) HPLC log*P* was performed by J. Gerry Everlof: Partition coefficients were determined using reversed-phase high-performance liquid chromatography (RP-HPLC). A series of standards with known literature Log *P* values were eluted through a C18 column using an isocratic methanol/water mobile phase. There is a linear correlation between the Log *K*′ and Log *P*. In reversed-phase liquid chromatography the compounds' distribution between the mobile and stationary phase is governed by hydrophobic forces; these parameters can be regarded as a measure of the compounds' lipophilicity.

(24) The normal correlation of increasing oral absorption with increasing Caco2 permeability cannot be performed here due to the relatively low water solubility of the less polar inhibitors, preventing permeability measurements.

(25) 7-Aminoisoquinoline **25** was obtained from 3,4-dihydroisoquinoline according to the method of McCoubrey (Isoquinolines. Part III. The Nitration of 3,4-Dihydro- and 1,2,3,4-Tetrahydroisoquinolines *J. Chem. Soc.* **1951** 2851−2853). 3,4-Dihydroisoquinoline was obtained from commercially available 1,2,3,4-tetrahydroisoquinoline according to the method of Yamazaki (Oxidation of secondary amines with nickel sulfate-potassium persulfate [$NiSO_4$−$K_2S_2O_8$]. *Chem. Lett.* **1992**, *5*, 823−826).

JM020578E