Design, Synthesis, and Biological Evaluation of Potent and Selective Amidino Bicyclic Factor Xa Inhibitors

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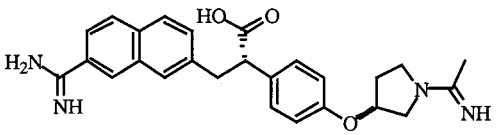
Thrombotic diseases are a major cause of death and morbidity. Factor Xa (fXa) plays a vital role in the regulation of normal homeostasis and abnormal intravascular thrombus development in the blood coagulation cascade. A novel series of fXa inhibitors incorporating an amidino 6,5-fused bicyclic moiety at the P1 position has been designed and synthesized based on molecular modeling studies. Structure−activity relationship (SAR) studies have led to selective subnanomolar fXa inhibitors. The most potent fXa inhibitor in this series (**72**, SE170) has a potent inhibition constant (K_i = 0.3 nM), is 350-fold selective for fXa over trypsin, and also shows good in vivo efficacy in a rabbit arterio-venous thrombosis model (ID_{50} = 0.14 μmol/kg/h). An X-ray crystal structure of **72** complexed to bovine trypsin was completed, and a binding mode of **72** with fXa has been proposed based on modeling with human des-Gla-fXa.

Introduction

Thrombotic diseases, including stroke, deep vein thrombosis, and pulmonary embolism, are a major cause of death and morbidity. Recent understanding of the causes and mechanisms of thromboembolic disorders has grown rapidly. Current antithrombotic drugs have their merits and limitations. Heparin, low-molecular-weight heparins, and recombinant hirudin all lack oral bioavailability.[2] Coumadin (warfarin), the only marketed oral anticoagulant agent, is being used in a growing number of antithrombotic indications; however, it requires individual dose titration and periodic monitoring.[3] Therefore, new orally active anticoagulants, which offer significant advantages over the agents presently in use, are needed for prevention and treatment of thrombotic diseases.

Factor Xa (fXa) plays a vital role in the regulation of normal homeostasis and abnormal intravascular thrombus development in the blood coagulation cascade.[4] fXa, a trypsin-like serine protease, is located at the convergent point of the intrinsic (activated by surface contact) and extrinsic (activated by vessel injury) pathways of coagulation.[5] The major role of fXa is the generation of thrombin from prothrombin.[6] Thrombin, as the last serine protease in the cascade, has several important procoagulant functions including converting fibrinogen to fibrin for clot formation and stimulating platelet aggregation.[7] Most of the research in the past decade has been focused on thrombin inhibition. Recent research indicates that the prothrombinase complex is unaffected by direct thrombin inhibitors;[8] by contrast, inhibition of fXa should prevent continuing production of thrombin while maintaining a basal level of thrombin activity necessary for primary homeostasis. In addition, one molecule of fXa generates many molecules of thrombin,[9] suggesting that inhibition of fXa may be more efficacious than inhibition of thrombin. Moreover, since fXa inhibitors affect coagulation specifically but not platelet function, this mechanism should have less potential to increase the risk of abnormal bleeding. Recent in vivo studies with small-molecule fXa inhibitors have demonstrated that fXa inhibitors are indeed efficacious anticoagulants with a minimal effect upon bleeding.[10] As a result of these investigations, the inhibition of fXa has begun to emerge as an attractive strategy for the discovery of novel antithrombotic agents.[11]



DX-9065a, 1

Figure 1. Strucutre of DX-9065a (**1**).

At the initiation of our program, Daiichi's DX-9065a (**1**; Figure 1) was reported as a potent nonpeptide fXa inhibitor.[12] Since that time, more amidines have been reported as fXa inhibitors.[13] An important feature common to amidine-based, trypsin-like serine protease inhibitors is that the highly basic amidino group remains completely protonated under most physiological conditions. Therefore, amidines are capable of forming a symmetrical bidentate charge interaction with the carboxylate side chain of Asp_{189} in the primary specificity pocket (S1) of the serine proteases.[14] In this paper, we describe our progress toward the rational design and synthesis of novel series of amidinobenzimidazole, amidinoindole, and amidinoindazole derivatives as potent and specific fXa inhibitors.

Chemistry

The preparative route to amidinobenzimidazole esters **12**−**15** is shown in Scheme 1. Ethyl 2-[2-(3-cyanophe-

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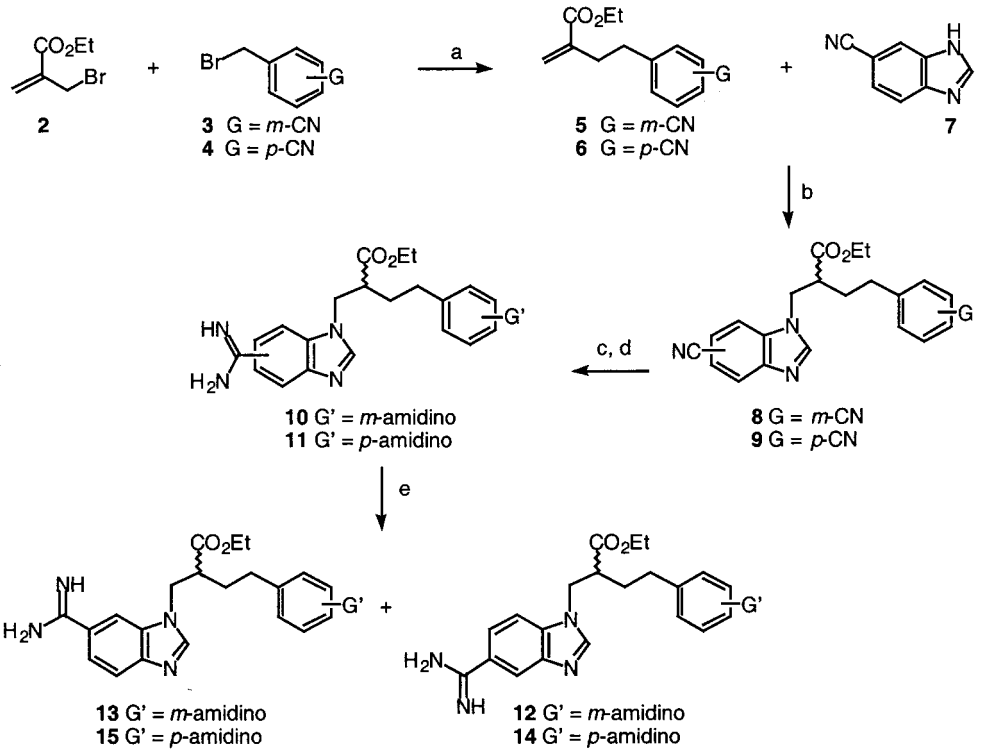
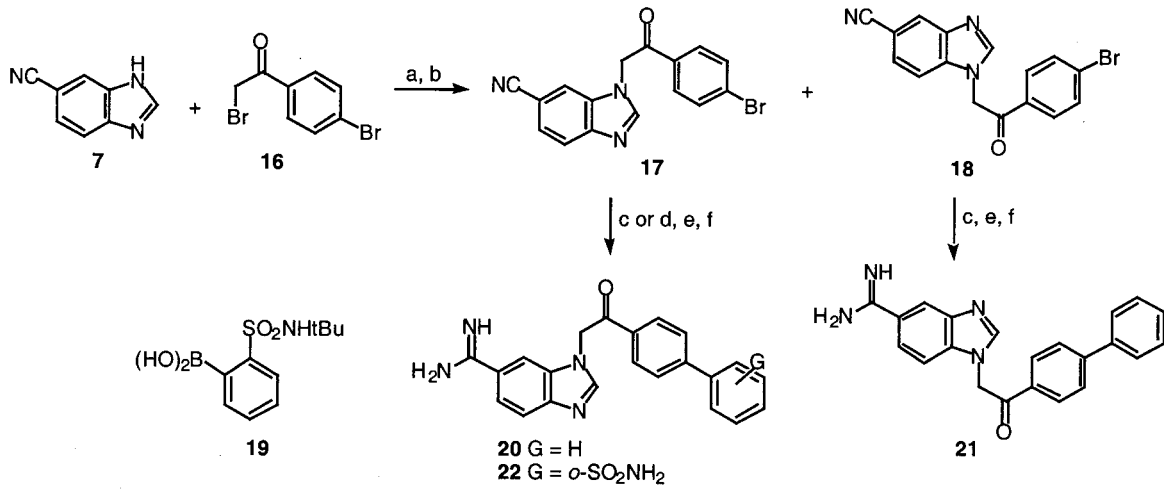
Scheme 1. Synthesis of Amidinobenzimidazole Ester Derivatives[a]



[a] Reagents: (a) Zn, CuCN, 2 equiv LiCl; (b) K_2CO_3, DMF, 110 °C; (c) HCl(g), EtOH; (d) $(NH_4)_2CO_3$, EtOH; (e) HPLC separation.

Scheme 2. Synthesis of Amidinobenzimidazole Ketone Derivatives[a]

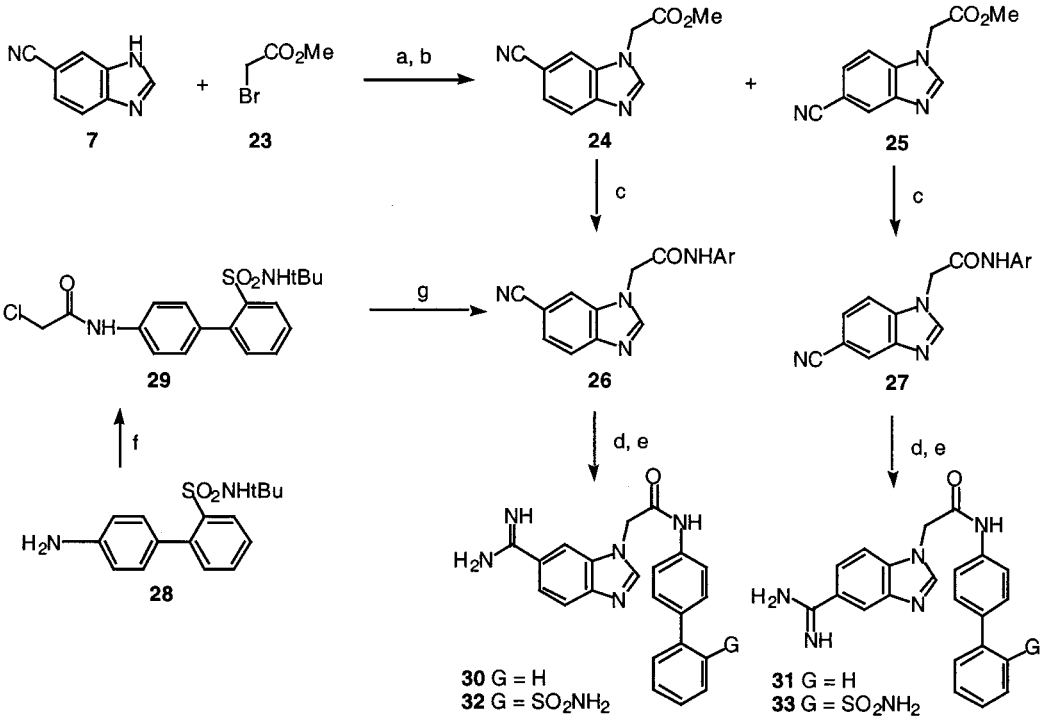


[a] Reagents: (a) NaH, THF; (b) HPLC separation; (c) phenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, THF−H_2O; (d) **19**, $Pd(PPh_3)_4$, 2 M Na_2CO_3, THF−H_2O; (e) HCl(g), EtOH; (f) $(NH_4)_2CO_3$, EtOH.

nyl)ethyl]acrylate (**5**) and ethyl 2-[2-(4-cyanophenyl)-ethyl]acrylate (**6**) were prepared, respectively, from a carbon−carbon coupling reaction of ethyl (bromomethyl)acrylate (**2**) with either a *m*- or *p*-cyanotolyl copper reagent, which in turn was prepared from the reaction of the corresponding bromide (**3** or **4**) with zinc, followed by treatment with copper(I) cyanide and lithium chloride. Michael addition of 5(6)-cyanobenzimidazole (**7**)[15] to **5** or **6** gave the regioisomeric mixture **8** or **9**, respectively. Subsequent conversion of cyano precursors **8** and **9** to the corresponding amidino compounds **10** and **11** was carried out under standard Pinner reaction conditions by bubbling hydrogen chloride gas into a solution of the cyano compound in ethanol and subsequent amination of the formed imidate by treatment with ammonium carbonate in ethanol. The regioisomeric mixtures **8**−**11** could be separated on HPLC either at the cyano precursor stage or at the amidino stage; however, separation of the cyano precusors **8** and **9** proved to be more facile than that of amidino derivatives **10** and **11**. The structures of the four compounds **12**−**15** were confirmed by long-range proton−carbon correlation NMR spectroscopy.

The synthesis of the substituted amidinobenzimidazole ketones **20**−**22** as shown in Scheme 2 began with the alkylation of **7** with 2′,4-dibromoacetophenone (**16**) in the presence of NaH. HPLC separation of the two regioisomers provided 6-cyano-substituted isomer **17** and 5-cyano-substituted isomer **18**. Suzuki coupling reactions of **17** with phenylboronic acid or 2-(*tert*-

Scheme 3. Synthesis of Amidinobenzimidazole Amide Derivatives[a]



[a] Reagents: (a) K_2CO_3, 18-crown-6, THF; (b) HPLC separation; (c) 4-aminobiphenyl or **28**, $AlMe_3$, CH_2Cl_2; (d) HCl(g), EtOH; (e) $(NH_4)_2CO_3$, EtOH; (f) α-chloroacetyl chloride, K_2CO_3, CH_3CN; (g) K_2CO_3, DMF, **7**.

butylaminosulfonyl)phenylboronic acid (**19**),[16] and **18** with phenylboronic acid, followed by the standard conversion from a cyano to an amidine group as described above, gave the desired amidino ketones **20**−**22**, respectively.

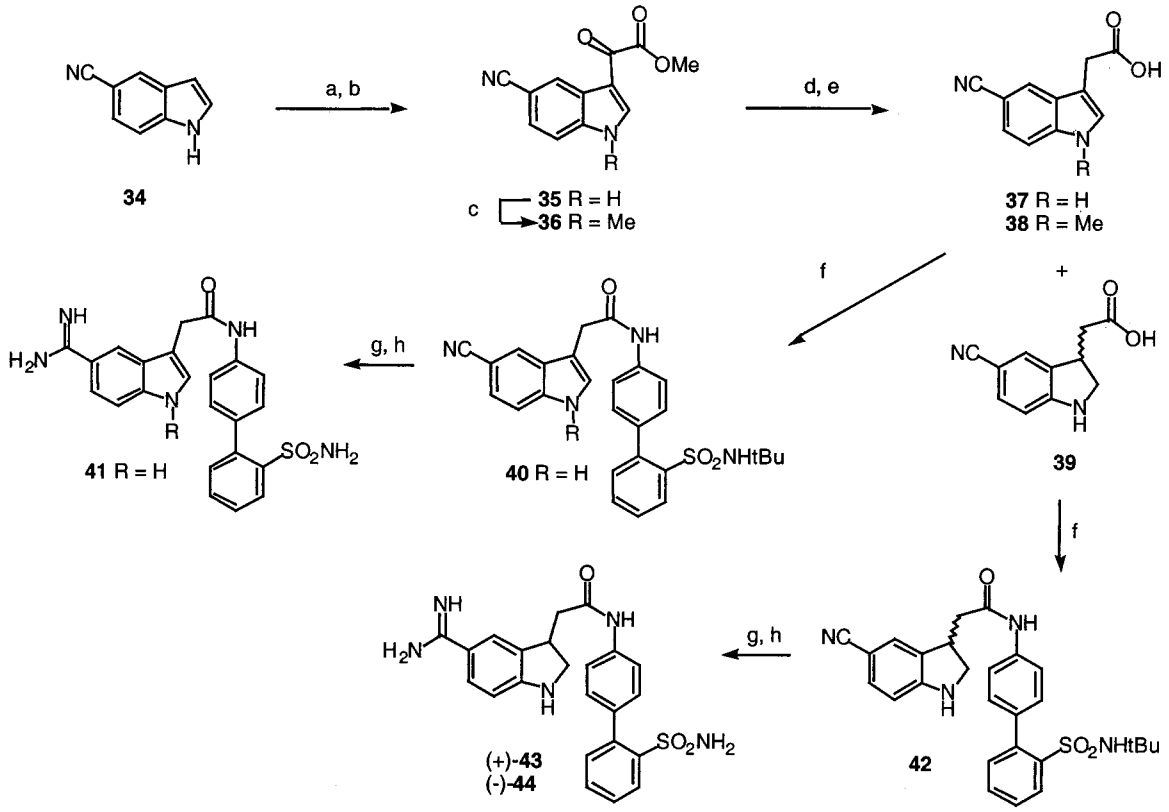
Two routes were employed to make the substituted amidinobenzimidazole amides **30**−**33** as shown in Scheme 3. Alkylation of **7** with methyl 2-bromoacetate (**23**), followed by separation on HPLC, afforded the 6-cyano-substituted isomer **24** and 5-cyano-substituted isomer **25**. Weinreb reaction of ester **24** or **25** with the aluminum salt of commercial 4-aminobiphenyl or *N*-(*tert*-butyl)-4′-amino[1,1′-biphenyl]-2-sulfonamide (**28**)[16] provided amides **26** and **27**, respectively. The amides **26** and **27** were also prepared by alkylation of **7** with 2-chloroamide **29**, which was obtained from acylation of **28** with 2-chloroacetyl chloride, followed by HPLC isolation. The latter route is preferred. Conversion of the cyano intermediates **26** and **27** to the amidines **30**−**33** was carried out under reaction conditions described earlier.

The synthesis of the amidinoindole derivatives is exemplified by the preparation of **41**, while the preparation of the amidinoindoline derivatives is exemplified by the preparation of **43** and **44** (Scheme 4). 5-Cyanoindole (**34**) was converted to methyl (5-cyano-1*H*-indol-3-yl)(oxo)acetate (**35**) by treatment with oxalyl chloride and then methanol. *N*-Methylation of indole **35** with methyl iodide in the presence of NaH provided methyl (5-cyano-1-methyl-1*H*-indol-3-yl)(oxo)acetate (**36**). Reduction of **35** with triethylsilane in trifluoroacetic acid, followed by saponification with KOH in MeOH, provided (5-cyano-1*H*-indol-3-yl)acetic acid (**37**) and (5-cyano-2,3-dihydroindol-3-yl)acetic acid (**39**). Similarly, reduction of **36** followed by saponification provided **38**. The reaction of acid **37** or **39** with aniline **28** in DMF in the presence of the BOP coupling reagent and triethylamine provided the cyano intermediates **40** and **42**, respectively. These intermediates were subjected to the standard Pinner reactions and aminations, followed by HPLC purification, to give amidinoindole **41** and amidinoindolines **43** and **44**, respectively.

The synthesis of the amidinoindazole derivative **50**, shown in Scheme 5, was accomplished by alkylation of 6-nitroindazole (**45**) with methyl 2-bromoacetate, followed by hydrogenation of the nitro group to form methyl (6-amino-1*H*-indazol-1-yl)acetate (**46**). Diazotization of **46** followed by quenching with CuCN/NaCN and then saponification provided (6-cyano-1*H*-indozol-1-yl)acetic acid (**47**). Coupling of acid **47** with 2-(6-amino-3-pyridinyl)-*N*-(*tert*-butyl)benzenesulfonamide (**48**),[16] followed by the standard Pinner reaction and amination, produced **50**.

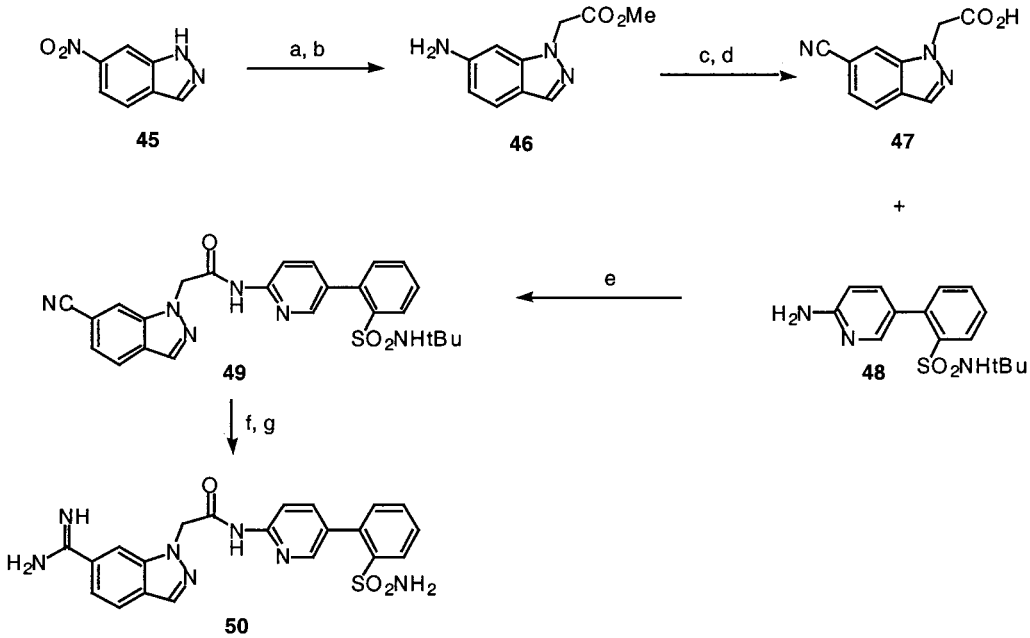
Generally, the 4-aminobiphenyl intermediates were prepared via a palladium-catalyzed coupling reaction (Scheme 6).[17] A Suzuki coupling reaction of 4-bromonitrobenzene (**51**) with **19** provided nitrobiphenyl **52**. Alkylation of the sulfonamide NH of **52** with methyl iodide, ethyl bromide, or propyl bromide in the presence of NaH or K_2CO_3, followed by hydrogenation in the presence of 10% palladium on carbon, afforded **53**−**55**. Removal of the *tert*-butyl group of **52** with TFA, followed by dialkylation of the sulfonamide with methyl iodide in the presence of NaH and then hydrogenation, gave **56**. The 4-aminobiphenyl intermediates **58**−**62** were synthesized via Suzuki reactions of appropriately substituted 4-bromoaniline **57** with **19**. *N*-(*tert*-Butyl)-4′-(methylamino)[1,1′-biphenyl]-2-sulfonamide (**63**) was synthesized by acylation of **28** with ethyl formate, followed by reduction with LAH.

Scheme 4. Synthesis of Amidinoindole Amide Derivatives[a]



[a] Reagents: (a) oxalyl chloride, Et_2O; (b) MeOH; (c) NaH, MeI; (d) TFA, Et_3SiH; (e) KOH, MeOH; (f) **28**, BOP, Et_3N, DMF; (g) HCl(g), MeOH; (h) $(NH_4)_2CO_3$, MeOH.

Scheme 5. Synthesis of Amidinoindazole Amide Derivatives[a]

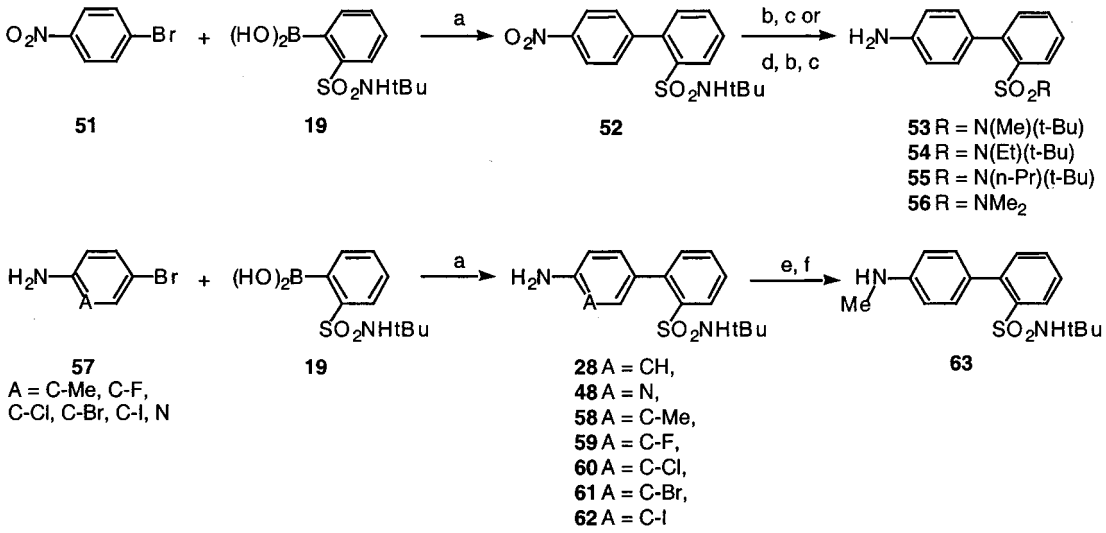


[a] Reagents: (a) $BrCH_2CO_2Me$, K_2CO_3, DMF; (b) H_2, 10% Pd−C; (c) 0.5 N HCl, $NaNO_2$, Na_2CO_3, CuCN, NaCN, 0 °C; (d) KOH, MeOH−H_2O; (e) BOP, Et_3N; (f) HCl(g), EtOH; (g) $(NH_4)_2CO_3$, EtOH.

Results and Discussions

Modeling Study. At the initiation of our program the crystal structure of human des-Gla-fXa dimer[18] was used in our molecular modeling studies and Daiichi's DX-9065a (**1**) was chosen as a model for our inhibitor design. The key features of a proposed binding mode of DX-9065a docked in fXa, which were subsequently confirmed by the crystal structure of the fXa/DX-9065a complex,[19] include four key interactions (Figure 2). First, the naphthyl amidine binds via a symmetrical bidentate charge interaction with a single Asp_{189} at the bottom of the S1 pocket; however, only Asp_{189} oxygen twin (naphthamidine) arrangement was observed in the crystal structure of the fXa/DX-9065a complex. Second, the

Scheme 6. Synthesis of Aminobiphenyls[a]



[a] Reagents: (a) $Pd(PPh_3)_4$, 2 M Na_2CO_3, THF−H_2O, reflux, 4−18 h; (b) MeI, EtBr or PrBr; K_2CO_3 or NaH; (c) H_2, 10% Pd−C; (d) TFA; (e) HCO_2Et, reflux, 2 h; (f) LAH.

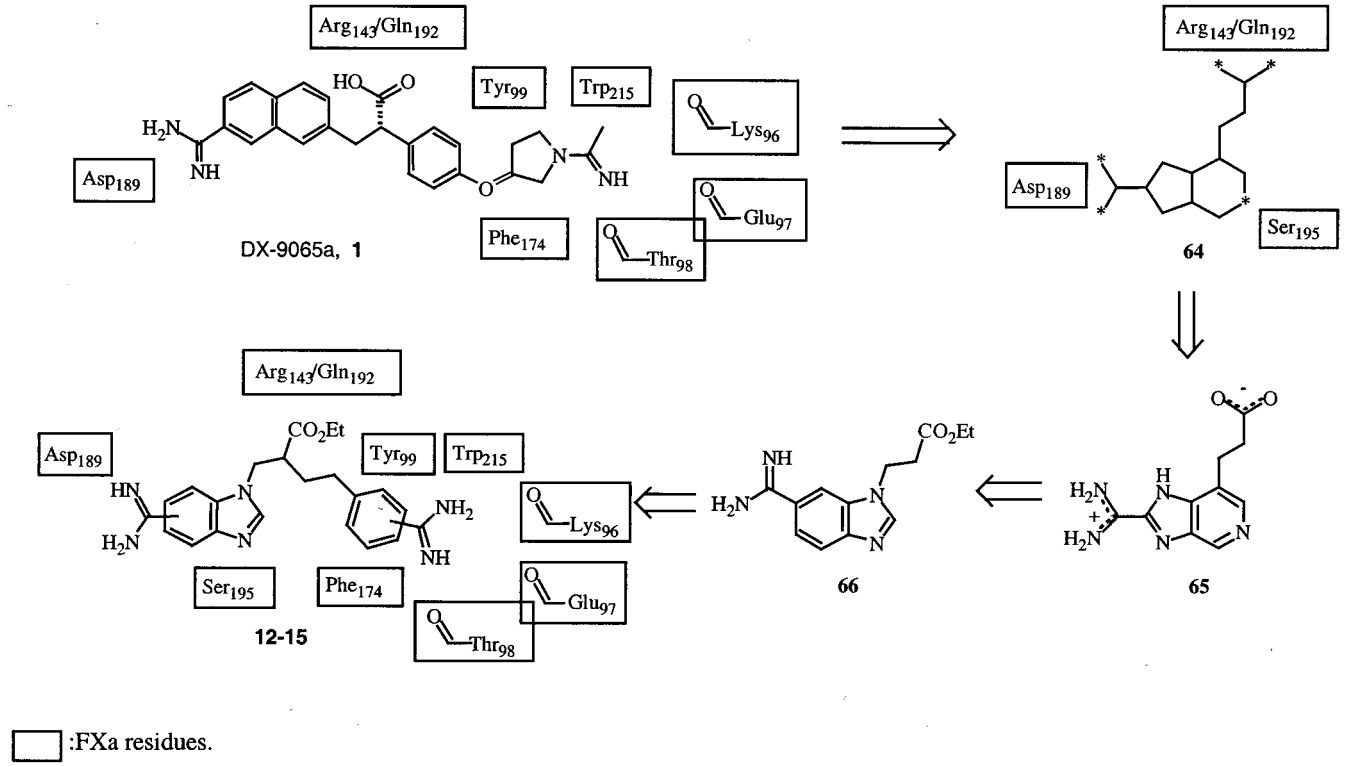
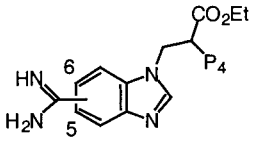


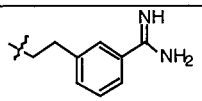
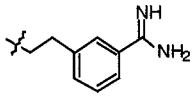
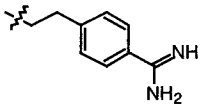
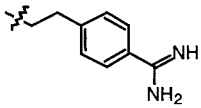
Figure 2. Modeling studies of DX-9065a in fXa and design of our initial fXa inhibitors.

pyrrolidine moiety sits in the aryl-binding pocket, which is formed by residues Phe_{174}, Trp_{215}, and Tyr_{99}. Third, the acetimidoyl functionality binds in the cation hole at the back of the S4 pocket composed of the carbonyl oxygens of Lys_{96}, Thr_{98}, and Glu_{97} and the side chain carboxylate of Glu_{97}, which binds the basic charged group. Fourth, the carboxylic acid, which was believed to be responsible for the specificity for fXa over thrombin, forms a hydrogen bond with the side chain of Arg_{143} and Gln_{192}. This binding model was used to design our more rigid and tighter binding inhibitors.

The Sprout program[20] was used as a de novo design technique to produce frameworks as starting points in the design of our initial fXa inhibitors. This program assesses the active site for receptor "target" points. The selected interest points are required to interact with the proposed inhibitors. The Sprout program does an exhaustive search based on electrostatic interactions by selecting templates to connect these points and then generates hundreds of skeletons. The S1 pocket of fXa was chosen for template design since it is a well-defined region with a short distance to span in order to join the target points. The relevant electrostatic target points for the S1 pocket of fXa, based on the modeling results of the des-Gla-fXa/DX-9065a complex, include Asp_{189} (deep in the S1 pocket), Ser_{195} (at the active site), and Arg_{143}/Gln_{192} (hovering over the S1 pocket) (Figure 2). The Sprout-generated skeleton **64** was chosen by an

Table 1. SAR of Amidinobenzimidazole Ester Derivatives with Basic P4 Fragments



No.	Amidino	P4	Ki (nM)		
			fXa[a]	Thrombin[a]	Trypsin[a]
12	5		140	2,000	220
13	6		700	1,900	820
14	5		2,200	> 21,000	--
15	6		11,900	> 21,000	--

[a] Human purified enzymes were used. Values are averages from multiple determinations ($n \geq 2$) and the standard deviations were <20% of the mean.

evaluation of its tight fit in the active site and for its rigidity and size. Skeleton **64** was transformed to compound **65**, which was reversed to ethyl 3-(6-amidino-1*H*-benzimidazol-1-yl)propionate (**66**) because the structure offered more synthetic flexibility. Subsequently, **66** was optimized in the S1 pocket prior to expansion into the S4 pocket, through energy minimization using Sprout and 3D database searching. When **66** was modeled in the S1 pocket of fXa, **66** formed favorable interactions with all four electrostatic targets (Asp_{189}, Arg_{143}, Gln_{192}, and Ser_{195}) in the S1 pocket. To maximize the interaction with the aryl pocket and the "cation hole" in the S4 pocket of fXa, a benzamidine group as the P4 fragment was placed in the center of this area. We modeled different length carbon chains as a tether for connecting the P1 and P4 fragments without disturbing the orientation of the fragments in their respective pockets. A two-carbon chain connecting the α-position of the ester group of **66** to either the *meta*- or *para*-position of the benzamidine was found to be an optimal tether.

The modeling studies led to the design and synthesis of four initial compounds, **12**−**15** (Table 1). Compound **12** was found to be the most potent fXa inhibitor (K_i = 140 nM) among the four, with **15** being approximately 85-fold less potent (K_i = 11 900 nM) than **12**. Compounds **12** and **13** containing a 3-benzamidine moiety as the P4 fragment are more potent than **14** and **15** containing a 4-benzamidine moiety. This observation is similar to our earlier findings and those reported in the literature, in which 3-substituted benzamidines are preferred over 4-substituted benzamidines.[18,21]

Since compounds **12**−**15** have two basic moieties, we were concerned that these bisamidines would not have adequate oral absorption or appropriate pharmacokinetics to be useful as oral antithrombotic agents.[22] Therefore, our strategy was to replace the P4 benzamidine group with a neutral fragment to attenuate the basicity of the compounds (Figure 3). Our modeling suggested that the benzamidine P4 fragment could be replaced by a variety of aryl moieties, such as a functionalized *p*-biphenyl. In addition, introduction of a hydrogen-bond donor/acceptor on the *ortho*-position of the distal phenyl ring ought to be capable of additional hydrogen-bonding interactions with Tyr_{99} of fXa. This strategy was found to be successful in our earlier series of fXa inhibitors when we replaced the P4 benzamidine moiety in **67** with a biphenylsulfonamide in **68**.[16] The modeling also suggested that the carboxylate in **12** could be replaced with a carbonyl as the linking functionality in **69** to eliminate the chiral center. In so doing, these inhibitors ought to also have an additional hydrogen-bond interaction between the carbonyl oxygen and the Gly_{219} NH of fXa.

SAR Evaluation. Initially, we were gratified to find that the 6-amidinobenzimidazole derivative with a biphenyl P4 fragment (**20**) shown in Table 2 had similar potency and selectivity to benzamidine **12**. As anticipated based on our previous results, these data suggested that indeed a neutral biphenyl without cationic character could replace the pharmacokinetically undesirable amidine moiety. For comparison 5-amidinobenzimidazole derivative **21** was also prepared. The 6-isomer **20** (fXa K_i = 120 nM) was significantly more potent than the 5-isomer **21** (fXa K_i = 2,700 nM). We therefore focused on the preparation of 6-amidinobenzimidazole derivatives, employing **20** as the starting point to systematically introduce changes into the P4 moiety. We had previously shown that the *o*-biphenylsulfonamide is an optimal P4 substituent.[16] Unfortunately, as shown by **22** (fXa K_i = 240 nM), an *o*-sulfonamide on the distal phenyl ring of this series showed 2-fold weaker affinity than the unsubstituted biphenyl **20**. To improve the potency, we thought to extend the tether from a ketone group to an amide group to allow the biphenyl ring to sit deeper into the S4 pocket. To initially test this

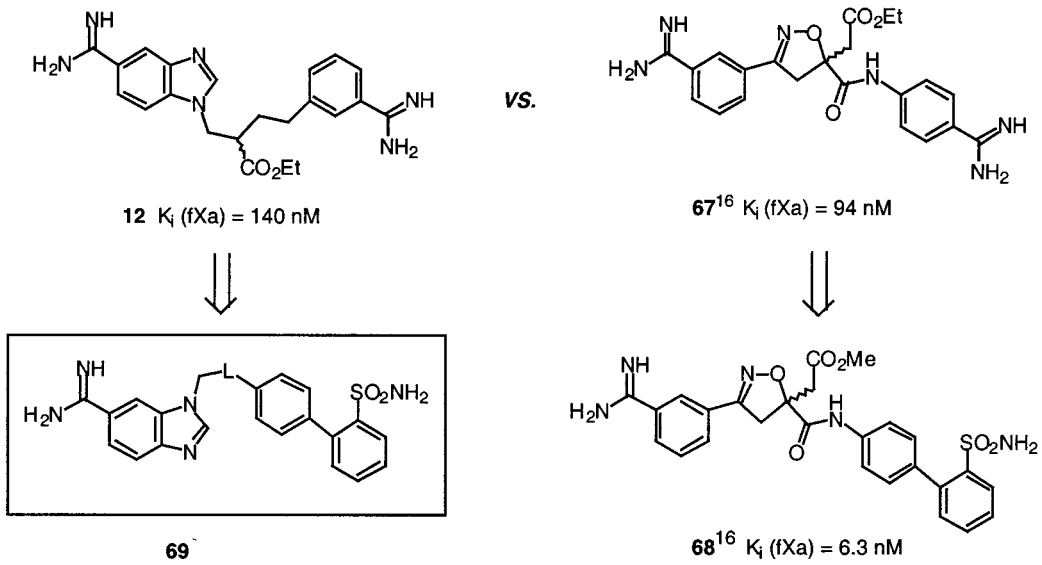
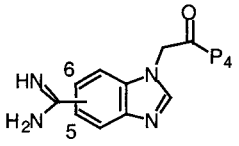
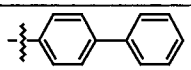
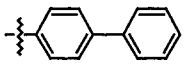
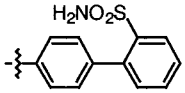
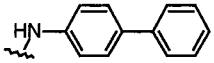
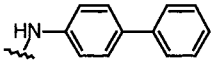
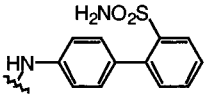
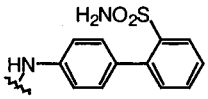


Figure 3. Introduction of neutral P4 fragments into amidinobenzimidazoles.

Table 2. SAR of Amidinobenzimidazole Derivatives with Neutral P4 Fragments



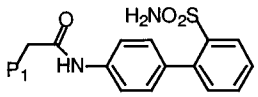
No.	Amidino	P4	Ki (nM)		
			fXa[a]	Thrombin[a]	Trypsin[a]
20	6		120	1,200	320
21	5		2,700	> 21,000	--
22	6		240	2,800	300
30	6		220	4,700	480
31	5		3,900	12,500	>2,500
32	6		**2.4**	**2,200**	**47**
33	5		400	2,700	130

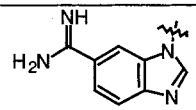
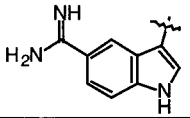
[a] Human purified enzymes were used. Values are averages from multiple determinations ($n \geq 2$) and the standard deviations were <20% of the mean.

hypothesis, we prepared a pair of amides, the 6-amidinobenzimidazole **30** (fXa K_i = 220 nM) and 5-amidinobenzimidazole **31** (fXa K_i = 3900 nM), which did not show improved potency. Fortunately, when the *o*-sulfonamide group was introduced on the distal phenyl ring in this series, the 6-substituted isomer **32** (fXa K_i = 2.4 nM) and also the corresponding 5-isomer **33** (fXa K_i = 400 nM) showed significant improvement in potency for fXa relative to unsubstituted biphenyl derivatives **30** and **31**. We believe that the *o*-sulfonamide on the distal phenyl ring in the P4 fragment causes the two rings of the biphenyl to be more orthogonal due to steric bulk. The orthogonal arrangement allows the distal phenyl ring to form a stronger "two-sandwich" π-interaction with residues Phe_{174} and Tyr_{99} in the S4 pocket of fXa. In addition, our model suggests that the sulfonamide may be interacting with Tyr_{99} to further enhance fXa potency.

As the modeling suggested, the benzimidazole P1 moiety could be replaced with other 6,5-fused bicyclic templates, such as indole, with good retention of fXa potency. The use of 6-amidinoindole as the P1 moiety

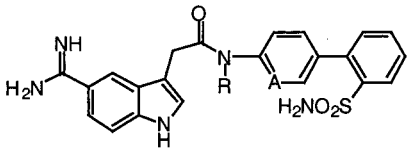
Table 3. Comparison of Selectivity and Pharmacokinetics[a] of Amidinobenzimidazole **32** and Amidinoindole **41**



No.	P1	Ki (nM) fXa[b]	Thrombin[b]	Trypsin[b]	Cl (L/h/kg)	$T_{1/2}$ (h)	V_{dss} (L//kg)	C_{max} (μM)
32		2.4	2,200	47	4.4	0.96	3.04	3.85
41		2.1	1,600	160	2.0	1.22	0.7	8.7

[a] Dosed intravenously in rabbit for 60 min at a dose of 10 mg/kg/h. [b]Human purified enzymes were used. Values are averages from multiple determinations ($n \geq 2$) and the standard deviations were <20% of the mean.

Table 4. SAR of P4 Fragments in Amidinoindole Series



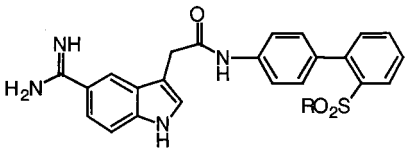
No.	R	A	Ki (nM) fXa[a]	Thrombin[a]	Trypsin[a]
41	H	C-H	2.1	900	180
70	Me	C-H	41	14,000	600
71	H	N	0.92	2,100	150
72	H	C-Br	**0.32**	**200**	**120**
73	H	C-I	0.6	200	240
74	H	C-Cl	0.7	500	140
75	H	C-Me	2.3	1,000	340
76	H	C-F	3.1	2,100	270

[a] Human purified enzymes were used. Values are averages from multiple determinations ($n \geq 2$) and the standard deviations were <20% of the mean.

has been shown to successfully result in potent thrombin inhibitors.[24] As shown in Table 3, both indole **41** (fXa K_i = 2.1 nM) and benzimidazole **32** have similar affinity for fXa, but indole **41** has the advantage of being more selective for fXa over trypsin. Indole **41** and benzimidazole **32** were evaluated in pharmacokinetic studies in rabbits via an intravenous infusion at a dose of 10 mg/kg/h over 60 min. Indole **41** exhibited the better pharmacokinetic profile, having a lower clearance and a slightly longer half-life than benzimidazole **32**. As a result of these studies, further optimization was pursued in the indole series.

Further efforts to define the SAR included manipulation of the P4 side chain by alkylation of the amide NH and exploration of biphenyl replacements (see Table 4). The *N*-methylamide **70** was 20-fold less active than **41**. As anticipated, based on earlier results with the isoxazoline series[16], replacement of the proximal phenyl ring with a pyridyl ring (**71**) resulted in a 2-fold enhancement in potency. To further improve the potency and block possible amide bond cleavage in the indole series, halogen and alkyl substituents were introduced into the *ortho*-position of the proximal phenyl ring (Table 4). Introduction of a bromo (**72**), iodo (**73**), or chloro (**74**) substituent in the *ortho*-position of the proximal ring also afforded compounds with subnanomolar fXa inhibition and good selectivity (>200-fold) against related serine proteases (thrombin and trypsin). Significantly, bromide **72** showed a 6-fold improvement in fXa affinity (fXa K_i = 0.32 nM) and had improved selectivity (375-fold selectivity over trypsin). Introduction of a methyl or fluoro substituent (**75** and **76**) in the *ortho*-position resulted in little change in fXa affinity and selectivity of fXa over trypsin, but the fluoro analog (**76**) showed better selectivity against thrombin compared to the unsubstituted biphenyl derivative of this series (**41**).

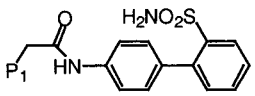
Table 5. Substitution in Distal Phenyl Ring



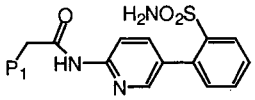
No.	R	K_i (nM)		
		fXa[a]	Thrombin[a]	Trypsin[a]
41	NH_2	2.1	900	180
77	NHMe	3.4	900	120
78	NHEt	4.1	800	160
79	NMe_2	7.0	1,700	390
80	NHn-Pr	8.0	400	180

[a] Human purified enzymes were used. Values are averages from multiple determinations ($n \geq 2$) and the standard deviations were <20% of the mean.

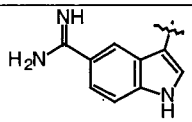
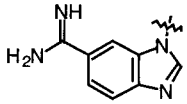
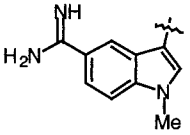
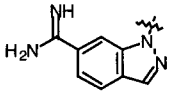
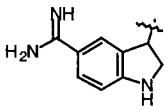
Table 6. SAR of Amidino 6,5-Fused Bicyclic fXa Inhibitors



32, 41, 43, 44



71, 81, 50

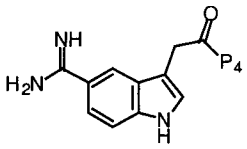
No.	P_1	K_i (nM)		
		fXa[a]	Thrombin[a]	Trypsin[a]
41		2.1	900	180
71		0.92	2,100	87
32		2.4	2,200	47
81		89	21,000	1,500
50		3.1	6,800	41
43 (+) 99%ee		0.71	1,500	140
44 (-) 99%ee		52	17,000	400

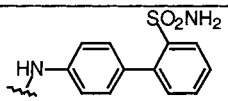
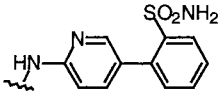
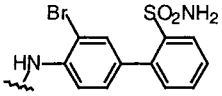
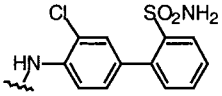
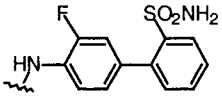
[a] Human purified enzymes were used. Values are averages from multiple determinations ($n \geq 2$) and the standard deviations were <20% of the mean.

N-Alkylation of the sulfonamide was pursued in an attempt to improve the pharmacokinetic profile. More specifically the rationale for alkylation of the sulfonamide NH_2 with methyl (**77**), ethyl (**78**), dimethyl (**79**), and *n*-propyl (**80**) as illustrated in Table 5 was to increase the lipophilicity of the compounds in an attempt to increase the volume of distribution (V_{dss}), which would result in an increased duration of action. Unfortunately, as the size of the alkyl group on the sulfonamide was increased, the fXa inhibitory activity decreased. Additionally, the selectivity of fXa over thrombin and trypsin of these compounds was also lower than that of the parent **41**. Therefore, the *N*-alkylated derivatives were not evaluated further.

Efforts to further explore the P1 group are shown in Table 6. The *N*-methylated indole **81** showed a 200-fold decrease in affinity for fXa compared with **71**. In an effort to optimize the amidine interaction in the S1 pocket, additional heterobicyclic amidines were evaluated. Amidinoindazole **50** was found to be 2-fold less

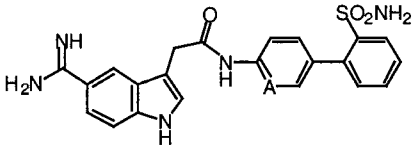
Table 7. Dog Pharmacokinetics of Selective Amidinoindole fXa Inhibitors



No.	P_4	fXa K_i (nM)	Dog iv dose (mg/kg/h)	$T_{1/2}$ (h)	Cl (L/h/kg)	V_{dss} (L/kg)	C_{max} (μM)
41		2.1	1.0[a]	0.79	1.22	0.38	1.51
71		0.44	10.0	0.4	3.7	1.9	2.2
72		0.32	1.0[a]	1.8	1.34	0.67	1.43
74		0.7	1.0[a]	1.75	3.0	2.01	0.65
76		3.1	1.0[a]	2.5	1.1	0.87	1.63

[a] From *N*-in-1 results.

Table 8. A-V Shunt Results of Select Amidinoindole fXa Inhibitors



No.	A	Human fXa Ki (nM)	Rabbit fXa Ki (nM)	Rabbit AV Shunt ID50 (μmol/kg/h)	Number of replications
72 (SE170)	C-Br	0.32	0.9	0.14 ± 0.02	4
71	N	0.43	0.93	0.3 ± 0.12	5
74	C-Cl	0.7	0.79	0.35 ± 0.05	3
41	C-H	2.1	6	1.7 ± 0.2	4

potent than the corresponding indole **71**. Amidinoindoline (+)-**43** showed a 3-fold enhancement in potency relative to the corresponding indole **41**, while the negative isomer (−)-**44** is approximately 25-fold less potent than **41**.

Pharmacokinetic Analysis. Representative indoles including **71, 72, 74,** and **76** were evaluated in pharmacokinetic studies following intravenous administration in the dog and compared to parent indole **41** (Table 7). The halogenated compounds **72**, **74**, and **76** all were shown to have longer duration of action than **41**. However, only fluoro analog **76** had a $t_{1/2} > 2$ h. Overall, **76** demonstrated the best pharmacokinetic profile in the series with the lowest clearance (Cl = 1.1 L/kg/h), the longest $t_{1/2}$ (2.5 h), and an increase in V_{dss} (0.87 L/kg) compared to the lead **41**. However, **76** is 10-fold less potent than bromide **72** (K_i values 3.1 nM vs 0.32 nM). Bromide **72** and chloride **74** both showed increased $t_{1/2}$ (1.8 and 1.75 h) compared to **41** ($t_{1/2}$ = 0.79 h) due to the larger V_{dss} (0.67 and 2.01 L/kg), despite having similar clearance (1.34 L/h/kg vs 1.22 L/h/kg). Even though pyridine **71** showed an increase in V_{dss} (1.9 L/kg), it had poorer exposure than the other compounds evaluated because of increased clearance (3.7 L/h/kg).

Antithrombotic Afficacy Study. The antithrombotic efficacy of indoles **41**, **71, 72,** and **74** was evaluated in a rabbit arterio-venous thrombosis model[25] (Table 8). The compounds were administered by intravenous infusion, and the antithrombotic effect was expressed as the ID_{50} (dose which reduced the clot weight by 50%). As anticipated based on the in vitro potency, bromide **72** was the most potent compound with an ID_{50} of 0.14 μmol/kg/h. Pyridine **71** (ID_{50} = 0.3 μmol/kg/h) and

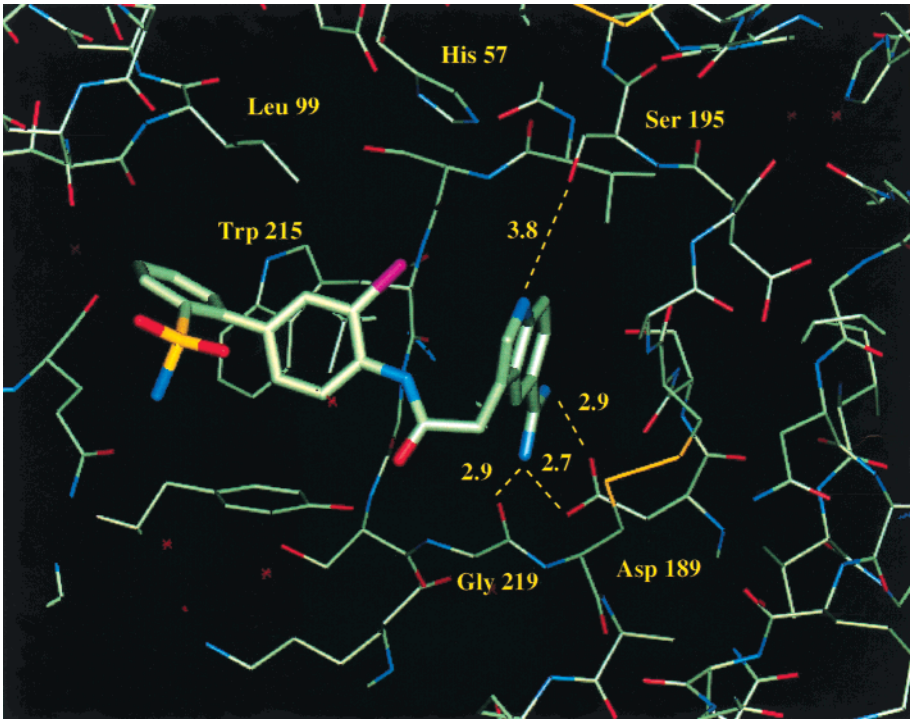


Figure 4. X-ray structure of **72** (SE170)/trypsin complex. Structure of **72** (SE170; thick bonds) complexed to bovine trypsin (thin bonds). Selected active site residues are labeled. The inhibitor binds in both the P1 pocket and the P4 binding site.

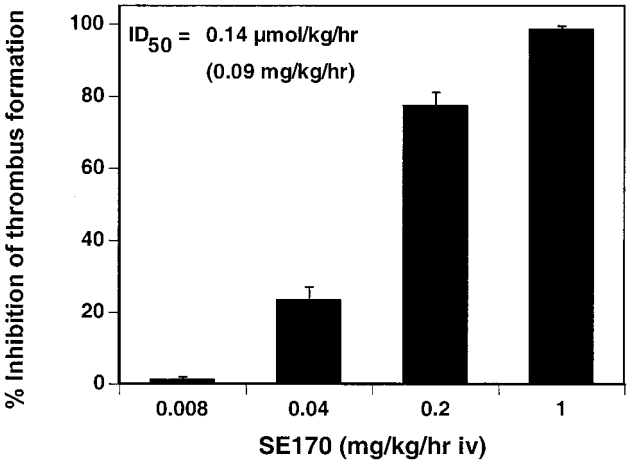


Figure 5. **72** (SE170) in rabbit A-V shunt model. Antithrombotic effect (expressed as % inhibition of thrombus formation) of SE170 at 0.008−1 mg/kg/h in rabbit arterio-venous shunt model; $n = 3-4$/group and means ± SE.

chloride **75** ($ID_{50} = 0.35$ μmol/kg/h) also showed better in vivo efficacy than the lead **41** ($ID_{50} = 1.7$ μmol/kg/h).

X-ray Analysis of 72/Trypsin Complex. Difficulties were encountered in obtaining an X-ray crystal structure of fXa bound with an inhibitor. Instead, because of the large degree of homology between fXa and trypsin,[26] the structure of **72** (SE170) complexed to bovine trypsin was determined (Figure 4). The structure has been refined to a crystallographic *R*-factor of 0.184 at 2.0 Å resolution. The binding of the inhibitor includes interactions with both the S1 specificity pocket and the S4 aryl-binding pocket. No major reorientations of the active site residues were observed upon inhibitor binding. As expected, the nitrogens of the amidine group are in close contact (2.8 and 2.9 Å) with the side chain of the carboxylic acid oxygens of Asp_{189}. Interestingly, there is a 3.8 Å distance between the nitrogen of the indole ring and the side chain hydroxyl of Ser_{195}. This distance is too long to be considered a hydrogen bond but may be indicative of a weakly polar interaction. A hydrogen bond is observed between the carbonyl oxygen of **72** and the backbone amide oxygen of Gly_{219}. Only one orientation is observed for the bromine on the proximal phenyl ring and for the sulfonamide on the distal phenyl ring. The biphenyl moiety is located in the aryl-binding pocket (S4). This region includes the side chains of Trp_{215} and Leu_{99}.

Superimposing the inhibitor coordinates on the structure of fXa, an overlap between the van der Waals surfaces of the bromine and Tyr_{99} is observed. This interaction may lead to the greater selectivity and affinity of this molecule for fXa, as thrombin and trypsin both have a leucine in position 99.

Conclusion

On the basis of molecular modeling studies employing the active site coordinates of the fXa dimer, we have designed and synthesized a novel class of potent and selective fXa inhibitors. These inhibitors employ amidine-substituted 6,5-fused heterocycles, such as amidinobenzimidazole and amidinoindole, to occupy the S1 site of fXa. The X-ray structure of the **72**/trypsin complex supports the proposed binding model. These compounds demonstrated potent inhibitory fXa activity with the most potent compounds showing subnanomolar affinity. These inhibitors also maintained high selectivity for fXa versus thrombin and trypsin.

Experimental Section

Enzyme Affinity Assays. fXa, thrombin, and trypsin K_i's were obtained from human purified enzymes. All assays were run in microtiter plates using a total volume of 250 μL in 0.1 M sodium phosphate buffer containing 0.2 M NaCl and 0.5% poly(ethylene glycol) 6000 at pH 7.4. The compounds were run at 10, 3.16, 1.0, 0.316, 0.1, 0.0316, 0.01, and 0.00316 μM. The

substrate for fXa and trypsin was S2222 and the substrate for thrombin was S2366. Plates were read for 30 min at 405 nm. Rate of substrate hydrolysis was determined for the controls (no inhibitor) and for the inhibitors. Percent (%) enzyme activity was determined from these rates and used in the following formula to determine K_i: $K_i = 1000 \times I(((K_m + S) - S \times \mathrm{ACT})/(\mathrm{ACT} \times K_m) - 1)$; where I = inhibitor concentration, K_m = substrate concentration that yields half-maximal velocity, S = substrate concentration, and ACT = % enzyme activity in the presence of inhibitor. All compounds were tested in duplicate studies and were compared with the same internal standards. Standard deviation of all assays was less than 20%. The detailed methodology is described in ref 23.

Pharmacokinetic Studies. Plasma samples (100 μL) were prepared by protein precipitation with acetonitrile (750 μL). The supernatant was dried under nitrogen and reconstituted in 100 μL of acetonitrile:water (5:95 v/v). Plasma levels were determined by liquid chromatography−tandem mass spectrometry. The instrument used was a Sciex (Thornhill, Ontario) model API III+ tandem mass spectrometer interfaced with a turbo ion spray ionization source. The limit of quantitation was 5 nM. Plasma concentration−time data were fitted to a noncompartment kinetic model using Winnonlin pharmacokinetic program (Scientific Consulting, Inc., Cary, NC). Plasma half-lives were calculated using nonlinear regression. The areas under the concentration curve (AUC) were calculated by trapezoidal approximation. The systemic plasma clearance (Cl) was calculated from the concentration−time data using the standard formula: $\mathrm{Cl} = \mathrm{dose}/\mathrm{AUC}_{0\rightarrow\infty}$. The apparent volume of distribution at steady state (V_{dss}) was calculated using statistical moment analysis by the equation: $V_{dss} = \mathrm{Cl} \times \mathrm{MRT}$, where MRT is the mean residence time, calculated as: $\mathrm{MRT} = \mathrm{AUMC}_{0\rightarrow\infty}/\mathrm{AUC}_{0\rightarrow\infty}$, where AUMC is the area under the first moment curve.

Arterio-Venous Shunt Thrombosis Model. New Zealand rabbits (2−4 kg) were anesthetized with ketamine (50 mg/kg im) and xylazine (10 mg/kg im). These anesthetics were supplemented as needed. The femoral artery, jugular vein, and femoral vein were isolated and catheterized. A saline-filled arterio-venous (A-V) shunt device was connected between the femoral arterial and the femoral venous cannulae. The A-V shunt device consisted of an outer piece of 8-cm Tygon tubing (internal diamter = 7.9 mm) and an inner piece of 2.5-cm tubing (internal diamter = 4.8 mm). The A-V shunt also contained an 8-cm-long 2-0 silk thread (Ethicon, Somerville, NJ). Blood flowed from the femoral artery via the A-V shunt into the femoral vein. The exposure of flowing blood to a silk thread induced the formation of a significant thrombus. After 40 min, the shunt was disconnected and the silk thread covered with thrombus was weighed. The compounds or saline vehicle was given as continuous iv infusions via the jugular vein starting 1 h before blood was circulated in the shunt and continuing throughout the experiment (i.e. 100 min). The percentage inhibition of thrombus formation was determined for each treatment group. The ID_{50} values (dose which produced 50% inhibition of thrombus formation) were estimated by linear regression. Variation of antithrombotic effect was less than 10%. See detailed description in ref 25.

Compound **72** (SE170) given at 0.008−1 mg/kg/h produced a dose-dependent antithrombotic effect with an ID_{50} of 0.14 μmol/kg/h (Figure 5).

Computational Molecular Modeling. DX-9065a was manually placed into the fXa active site using Insight II (Molecular Simulations, Inc., San Deigo, CA). The protein was held rigid while the inhibitor was minimized using the cvff force field. After the four target points (Asp_{189}, Ser_{195}, Arg_{143}/Gln_{192}, and Ser_{195}) were selected in Sprout, templates to join these target points were selected. A basic set using a single point, double bond, single bond, phenyl ring, and unsaturated five-membered ring were selected. No atom types are assigned during this phase of the Sprout calculation. A maximum of three fused rings and a total of two six-membered rings and two five-membered rings were allowed in the templates. Additionally, the longest carbon chain was set to a length of three atoms. The run resulted in thousands of skeletons. These were manually reduced to approximately 200 that were "reasonable". Manual assignment of atom types in a few of these skeletons was performed to allow for interactions with specific residues. Once skeletons were atom-typed, these structures were placed into Insight II for further evaluations. The protein was held rigid while the ligands were minimized using the cvff force field. The starting structures of the potentially inhibitory molecules for fXa were directly chosen from the Sprout results.

Chemical and Physical Methods. All reactions detailed below were carried out under inert gas in oven-dried glassware unless otherwise indicated. Solvents and reagents were obtained from commercial vendors in the appropriate grade and used without further purification unless otherwise indicated. Proton and carbon NMR spectra were obtained on VXR or Unity 300 or 400 MHz instruments (Varian Instruments, Palo Alto) with TMS as an internal reference standard. Mass spectra were measured with a Hewlett-Packard 5988A mass spectrometer with particle beam interface using NH_3 for chemical ionization, a Finnigan "Navigator-aQa" system under atmospheric pressure chemical ionization (APcI) conditions, or a Micromass Platform II system under electrospray ionization (ESI) conditions. High-resolution mass spectra were obtained either on a VG70-VSE mass spectrometer under NH_3-CI conditions or on a Finnigan MAT95S mass spectrometer under ESI conditions. Melting points were determined on a Mettler SP61 apparatus and are uncorrected. All compounds were determined to be homogeneous by TLC, elemental analysis, and/or HPLC. Analytical HPLC was performed on a C18 column using linear gradients of CH_3CN/H_2O/0.05% TFA from 90% water and 0.05% TFA in CH_3CN to 0.05% TFA in CH_3CN over 30 min. Preparative reverse-phase HPLC was performed on a C18 column using gradients with compositions of CH_3CN/H_2O/0.05% TFA over 45 min. Elemental analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ.

Ethyl 2-[2-(3-Cyanophenyl)ethyl]acrylate (5) and Ethyl 2-[2-(4-Cyanophenyl)ethyl]acrylate (6). To a stirred suspension of zinc powder (1.43 g, 22 mmol) in THF (10 mL) was added a catalytic amount of 1,2-dibromoethane (0.2 g) at room temperature, and the mixture was stirred for 30 min. A solution of 3-cyanobenzyl bromide (**3**) or 4-cyanobenzyl bromide (**4**) (3.92 g, 20 mmol) in THF (25 mL) was slowly added at a rate of 1 drop every 5 s at 5−10 °C. The resulting mixture was stirred for 3 h at that temperature and then transferred into a solution of CuCN (1.82 g, 20 mmol) and LiCl (1.68 g, 40 mmol) in THF (20 mL) at −78 °C. The resulting mixture was warmed to −20 °C, stirred for 20 min, and then re-cooled to −78 °C. After ethyl 2-(bromomethyl)acrylate (**2**) (3.86 g, 20 mmol) was slowly added, the mixture was stirred at −78 °C for 2 h and then warmed to room temperature overnight. Ether (100 mL) and aqueous saturated NH_4Cl (50 mL) were added, and the mixture was filtered. The filtrate was washed with water and brine and dried over $MgSO_4$. Concentration gave a residue, which was purified by column chromatography with a gradient of CH_2Cl_2−EtOAc to give pure **5** (3.6 g, 78.6%) and **6** (1.2 g, 26.2%), respectively.

Ester 5. ^{1}H NMR ($CDCl_3$): δ 7.51−7.36 (m, 4H), 6.17 (s, 1H), 5.48 (d, J = 1.1 Hz, 1H), 4.22 (q, J = 7.3 Hz, 2H), 2.84 (dd, J = 8.4 Hz, J = 7.0 Hz, 2H), 2.61 (dd, J = 8.4 Hz, J = 7.0 Hz, 2H),1.32 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 166.80, 142.85, 139.41, 133.14, 132.05, 129.85, 129.17, 118.94, 112.43, 60.79, 34.50, 33.57, 14.22. LRMS: 247 $(M + NH_4)^+$.

Ester 6. ^{1}H NMR ($CDCl_3$): δ 7.58 (dd, J = 8.4 Hz, J = 1.8 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 6.17 (d, J = 1.1 Hz, 1H), 5.48 (dd, J = 2.6 Hz, J = 1.1 Hz, 1H), 4.22 (q, J = 7.3 Hz, 2H), 2.86 (dd, J = 8.6 Hz, J = 7.1 Hz, 2H), 2.61 (dd, J = 8.6 Hz, J = 7.0 Hz, 2H),1.32 (t, J = 7.0 Hz, 3H). MS: 247 $(M + NH_4)^+$.

Ethyl 2-(3-Amidinophenyl)ethyl-3-(5-amidinobenzimidazol-1-yl)propionate (12) and Ethyl 2-(3-Amidinophenyl)ethyl-3-(6-amidinobenzimidazol-1-yl)propionate (13). A mixture of **7**[15] (288 mg, 2 mmol), **5** (438 mg, 2 mmol), and

K_2CO_3 (276 mg, 2 mmol) in DMF (10 mL) was heated at 90 °C for 16 h. The mixture was diluted with EtOAc (150 mL), washed with 1 N HCl and brine, and dried over $MgSO_4$. After filtration and concentration, the residue was purified by column chromatography with a gradient of CH_2Cl_2−EtOAc to give a mixture of two regioisomers **8** (570 mg, 76.4%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 8.13−7.36 (m, 8H), 4.55 (dd, J = 14.3 Hz, J = 9.2 Hz, 1H), 4.28 (dd, J = 14.3 Hz, J = 5.5 Hz, 1H), 4.07 (q, J = 7.0 Hz, 2H), 3.00−2.91 (m, 1H), 2.80−2.64 (m, 2H), 2.18−2.07 (m, 1H), 1.92−1.82 (m, 1H), 1.12 (t, J = 7.0, 3H).

Compound **8** (570 mg, 1.53 mmol) in anhydrous EtOH (10 mL) was treated with HCl (gas) for 15 min at 0 °C and then stirred at room temperature for 16 h. After evaporation of excess HCl (gas) and EtOH, the residue was dissolved in anhydrous EtOH (10 mL), and treated with $(NH_4)_2CO_3$ (5 equiv) at room temperature for 24 h. Concentration gave a residue, which was purified on preparative TLC plates with 10% MeOH in CH_2Cl_2 to give **10** (400 mg, 65.4%). Mp: 160−165 °C. ESIMS: 204.2 $(M + 2H)^{+2}$. HRMS: 407.2199 (obsd), 407.2195 (calcd) for $C_{22}H_{26}N_6O_2$.

Compound **10** containing two regioisomers was further separated by HPLC on a Zorbax protein 10 silica column (2" × 25 cm) with 90% CH_3CN/10% H_2O/0.2% TFA at flow rate of 60 mL/min to give **12** and **13**. The two regioisomers, as well as the following pair of **14** and **15**, were identified by long-range proton−carbon correlation spectroscopy. These four compounds were shown 100% pure on reversed analytical column and also on Zorbax silica analytical column with 90% CH_3CN/10% H_2O/0.2% TFA at flow rate of 1 mL/min.

Propionate 12. ^{1}H NMR (CD_3OD): δ 8.36 (s, 1H), 8.17 (s, 1H), 7.75−7.72 (m, 2H), 7.63 (bs, 2H), 7.50−7.48 (m, 2H), 0.4.66 (dd, J = 9.5 Hz, J = 14.3 Hz, 1H), 4.55 (dd, J = 5.5 Hz, J = 14.2 Hz, 1H), 4.02−3.92 (m, 2H), 3.14−3.08 (m, 1H), 2.81 (t, J = 7.0 Hz, 2H), 2.19−1.93 (m, 2H), 1.04 (t, J = 7.0 Hz, 3H). ESIMS: 407 $(M + H)^+$.

Propionate 13. ^{1}H NMR (CD_3OD): δ 8.37 (s, 1H), 8.10 (s, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.65−7.62 (m, 2H), 7.55−7.46 (m, 2H), 4.68 (dd, J = 9.5 Hz, J = 14.3 Hz, 1H), 4.56 (dd, J = 5.5 Hz, J = 14.2 Hz, 1H), 4.04−3.94 (m, 2H), 3.24−3.18 (m, 1H), 2.83 (t, J = 7.0 Hz, 2H), 2.19−1.95 (m, 2H), 1.05 (t, J = 7.0 Hz, 3H). ESIMS: 407 $(M + H)^+$.

Ethyl 2-(4-Amidinophenyl)ethyl-3-(5-amidinobenzimidazol-1-yl)propionate (14) and Ethyl 2-(4-Amidinophenyl)ethyl-3-(6-amidinobenzimidazol-1-yl)propionate (15). By using the same method as described for compounds **12** and **13**, Michael addition of **6** (492 mg, 2 mmol) to **7** (286 mg, 2 mmol), followed by Pinner reaction and amination gave compound **11** (100 mg, 13% for two steps). Mp: 230 °C dec. HRMS: 407.2200 (obsd), 407.2195 (calcd) for $C_{22}H_{26}N_6O_2$. Compound **11** was further separated by HPLC on a chiral OJ column with CO_2/MeOH/TEA (80/20/0.1) to give **14** and **15**.

Propionate 14. ^{1}H NMR (DMSO-d_6): δ 9.43−9.08 (m, 6H), 7.74−7.65 (m, 2H), 7.40−7.38 (m, 2H), 7.35−7.00 (m, 4H), 4.67−4.55 (m, 2H), 4.06 (bs, 2H), 3.48 (bs, 2H), 3.20 (bs, 1H), 2.70 (bs, 2H), 1.00 (bs, 3H). ESIMS: 407.2 $(M + H)^+$.

Propionate 15. ^{1}H NMR (DMSO-d_6): δ 9.23−9.12 (m, 6H), 8.41 (s, 1H), 8.21 (s, 1H), 7.84−7.82 (m, 1H), 7.74 (d, J = 8.1 Hz, 2H), 7.41 (d, J = 7.8 Hz, 2H), 7.24 (bs, 1H), 4.58−4.56 (m, 2H), 3.95−3.89 (m, 2H), 3.10−3.00 (m, 1H), 2.73−2.71 (m, 2H), 1.90−1.88 (m, 2H), 0.98−0.96 (m, 3H). ESIMS: 407 $(M + H)^+$.

1-[2-(4-Bromophenyl)-2-oxoethyl]-1*H*-benzimidazole-6-carbonitrile (17) and 1-[2-(4-Bromophenyl)-2-oxoethyl]-1*H*-benzimidazole-5-carbonitrile (18). To a suspension of NaH (60% in mineral oil, 1.92 g, 48 mmol) in THF (80 mL) was added **7** (5.14 g, 36 mmol) and the mixture was stirred at room temperature for 1 h. 2,4′-Dibromoacetophenone (**16**) (10 g, 36 mmol) was added to the reaction mixture, and the resulting mixture was stirred under reflux for 2 h. The mixture was diluted with EtOAc (200 mL), washed with water, dried over $MgSO_4$, and concentrated to give a mixture of two regioisomers (11 g, 90%). The mixture was isolated by HPLC on a chiralcel OJ column with MeOH/CO_2 (20%/80%) to give **17** and **18**.

6-Carbonitrile 17. ^{1}H NMR ($CDCl_3$): δ 8.35 (s, 1H), 8.11 (dd, J = 1.1 Hz, J = 0.7 Hz, 1H), 8.08 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H), 7.75 (dd, J = 8.4 Hz, J = 0.7 Hz, 1H), 7.56 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 6.16 (s, 2H). ESIMS: 340/342 $(M + H)^+$.

5-Carbonitrile 18. ^{1}H NMR ($CDCl_3$): δ 8.31 (s, 1H), 8.13 (t, J = 0.7 Hz, 1H), 8.07 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H), 7.75 (dd, J = 8.4 Hz, J = 0.7 Hz, 1H), 7.57 (dd, J = 8.4 Hz, J = 1.1 Hz, 1H), 6.15 (s, 2H). ESIMS: 340/342 $(M + H)^+$.

1-[2-[2′-(Aminosulfonyl)[1,1′-biphenyl]-4-yl]-2-oxoethyl]-1*H*-benzimidazole-6-carboximidamide (22). Compounds **20**−**22** were prepared as described in the following procedures. To a solution of **17** (200 mg, 0.59 mmol) and 2-(*tert*-butylaminosulfonyl)phenylboronic acid (**19**) (1.52 g, 0.59 mmol) in THF (9 mL) was added Na_2CO_3 (2 M in water, 9 mL), and the mixture was purged with nitrogen. To the mixture was added $Pd(PPh_3)_4$ (60 mg, 0.05 mmol), and the resulting mixture was refluxed for 16 h. The mixture was diluted with EtOAc (200 mL), washed with water and brine, dried over $MgSO_4$, and concentrated. The resulting residue was crystallized from EtOAc two times and further purified on preparative TLC plates with 50% EtOAc in CH_2Cl_2 to provide 1*N*-(4′-(*o*-*tert*-butylaminosulfonylphenyl)phenylcarbonyl)methyl-6-cyanobenzimidazole (270 mg, 97%) as a white solid. This solid was further modified to the amidine as described above, followed by purification on preparative HPLC to provide **22** (14 mg, 5.6%) and 1-[2-[2′-(*tert*-butylaminosulfonyl)[1,1′-biphenyl]-4-yl]-2-oxoethyl]-1*H*-benzimidazole-6-carboximidamide (*tert*-butyl analogue of **22**) (100 mg, 31%). For **22**. Mp: 126−128 °C. ^{1}H NMR (CD_3OD): δ 8.55 (bs, 1H), 8.18 (d, J = 8.4 Hz, 2H), 8.13 (dd, J = 7.7 Hz, J = 1.4 Hz, 1H), 8.09 (s, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.71 (dd, J = 8.4 Hz, J = 1.4 Hz, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.60 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H), 7.36 (dd, J = 7.3 Hz, J = 1.4 Hz, 1H), 6.13 (s, 2H). MS: 217.7 $(M + 2H)^{+2}$. HRMS: 434.1303 (obsd), 434.1287 (calcd) for $C_{22}H_{20}N_5O_3S$ $(M + H)^+$. For the *tert*-butyl analogue of **22**. Mp: 118−120 °C. ^{1}H NMR (CD_3OD): δ 8.60 (bs, 1H), 8.19 (d, J = 8.4 Hz, 2H), 8.13 (dd, J = 7.7 Hz, J = 1.4 Hz, 1H), 8.09 (s, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.76 (dd, J = 8.4 Hz, J = 1.4 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.63 (dd, J = 7.7 Hz, J = 1.5 Hz, 1H), 7.60 (dd, J = 7.7 Hz, J = 1.4 Hz, 1H), 7.34 (dd, J = 7.7 Hz, J = 1.4 Hz, 1H), 6.14 (s, 2H), 1.09 (s, 9H); MS: 282.8 $(M + 2H)^{+2}$. Anal. Calcd for $C_{26}H_{27}N_5O_3S$·1.89TFA·$1H_2O$·0.23HCl: C, 49.19; H, 4.31; N, 9.62; F, 14.10; S, 4.41; Cl, 1.22. Found: C, 49.14; H, 4.24; N, 9.60; F, 14.44; S, 4.17; Cl, 1.12.

1-[2-[(1,1′-Biphenyl)-4-yl]-2-oxoethyl]-1*H*-benzimidazole-6-carboximidamide (20). Mp: 155−157 °C. ^{1}H NMR (CD_3OD): δ 8.44 (s, 1H), 8.23 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 1.1 Hz, 1H), 7.91 (d, J = 8.8 Hz, 1H), 7.88 (dd, J = 8.4, 2H), 7.72 (dd, J = 8.4 Hz, J = 1.1 Hz, 4H), 7.52−7.41 (m, 3H), 6.10 (s, 2H). LRMS: 355 $(M + H)^+$. HRMS: 355.1559 (calcd), 355.1554 (obsd). Anal. Calcd for $C_{22}H_{18}N_4O$·0.7TFA·1.2HCl·$1.2H_2O$: C, 56.26; H, 4.50; N, 11.21; Cl, 8.52. Found: C, 56.37; H, 4.86; N, 11.09; Cl, 8.54.

1-[2-[(1,1′-Biphenyl)-4-yl]-2-oxoethyl]-1*H*-benzimidazole-5-carboximidamide (21). Mp: 260−261 °C. ^{1}H NMR (CD_3OD): δ 8.41 (s, 1H), 8.22 (s, 1H), 8.20 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 8.4 Hz, 2H), 7.73−7.70 (m, 4H), 7.51−7.41 (m, 3H), 6.10 (s, 2H). MS: 355.2 $(M + H)^+$. HRMS: 355.1559 (calcd), 355.1538 (obsd). Anal. Calcd for $C_{22}H_{18}N_4O$·1.5TFA·0.08HCl·$1H_2O$: C, 54.96; H, 3.98; N, 10.22; Cl, 0.52. Found: C, 54.65; H, 3.87; N, 10.56; Cl, 0.45.

2-(6-Amidinobenzimidazol-1-yl)-*N*-[2′-(aminosulfonyl)-(1,1′-biphenyl)-4-yl]acetamide (32) and 2-(5-Amidinobenzimidazol-1-yl)-*N*-[2′-(aminosulfonyl)(1,1′-biphenyl)-4-yl]-acetamide (33). Compounds **30**−**33** were prepared as described in the following procedures. To a suspension of K_2CO_3 (331 mg, 2.4 mmol) in CH_2Cl_2 (10 mL) were added 4′-amino(1,1′-biphenyl)-2-*N*-(*tert*-butylamino)sulfonamide (**28**) (608 mg, 2 mmol) and α-chloroacetyl chloride (271 mg, 2.4 mmol), and the resulting mixture was stirred at room temperature for 16 h. The mixture was quenched with water (20 mL) and diluted

with EtOAc (100 mL). The organic layer was dried over $MgSO_4$ and concentrated to give *N*-[2-(*tert*-butylaminosulfonyl)(1,1′-biphenyl)-4′-yl]-2-chloroacetamide (**29**) (700 mg, 92%) as a white solid. To a suspension of K_2CO_3 (552 mg, 4 mmol) in DMF (10 mL) were added **7** (286 mg, 2 mmol) and **29** (760 mg, 2 mmol), and the mixture was stirred at room temperature for 16 h. As described above, workup and purification on preparative TLC plates, followed by preparative HPLC, provided **26** (240 mg, 56%) and **27** (160 mg, 37%). The two regioisomers were further modified to the amidines followed by purification on preparative HPLC to give **32** and **33** in varied yields (15−50%), respectively.

Acetamide 32. Mp: 134−136 °C. ^{1}H NMR (CD_3OD): δ 8.73 (bs, 1H), 8.15 (s, 1H), 8.10 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 7.4 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.60 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H), 7.52 (td, J = 7.6, J = 1.4 Hz, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.32 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H), 5.36 (s, 2H). ^{13}C NMR (CD_3OD): δ 168.79, 166.75, 143.05, 141.48, 138.93, 137.50, 133.63, 132.92, 131.28, 128.75, 128.59, 124.63, 123.35, 120.96, 120.53, 112.80, 47.51. HRMS: 449.1401 (obsd), 449.1396 (calcd) for $C_{22}H_{21}N_6O_3S$ $(M + H)^+$. Anal. Calcd for $C_{22}H_{20}N_6O_3S \cdot 2.05TFA \cdot 0.15HCl \cdot 1.5H_2O$: C, 43.86; H, 3.55; N, 11.76; F, 16.35; S, 4.49; Cl, 0.74. Found: C, 43.92; H, 3.35; N, 12.00; F, 16.55; S, 4.15; Cl, 0.71.

Acetamide 33. Mp: 254 °C dec. ^{1}H NMR (CD_3OD): δ 8.55 (s, 1H), 8.22 (s, 1H), 8.08 (d, J = 6.6 Hz, 1H), 7.83−7.75 (m, 2H), 7.62 (d, J = 8.8, 2H), 7.59−7.52 (m, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 7.4 Hz, 1H), 5.33 (s, 2H). ^{13}C NMR (DMSO-D6): δ 165.73, 164.97, 147.72, 142.58, 142.19, 139.43, 138.17, 137.63, 135.23, 132.31, 131.35, 129.69, 127.39, 127.23, 122.20, 121.03, 120.96, 120.17, 118.21, 118.12, 111.24, 47.51. HRMS: 449.1414 (obsd), 449.1396 (calcd) for $C_{22}H_{21}N_6O_3S$ $(M + H)^+$. Anal. Calcd for $C_{22}H_{20}N_6O_3S \cdot 1.8TFA \cdot 0.25HCl \cdot 1H_2O$: C, 45.16; H, 3.56; N, 12.34; F, 15.07; S, 4.71; Cl, 1.30. Found: C, 45.38; H, 3.57; N, 12.49; F, 15.19; S, 4.67; Cl, 1.20.

2-(6-Amidinobenzimidazol-1-yl)-*N*-[(1,1″-biphenyl)-4-yl]acetamide (30). ^{1}H NMR (CD_3OD): δ 8.47 (bs, 1H), 8.08 (s, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 9.1 Hz, 2H), 7.52−7.48 (m, 4H), 7.32 (t, J = 7.3 Hz, 2H), 7.21 (t, J = 7.3 Hz, 1H), 5.28 (s, 2H). LRMS: 370.2 $(M + H)^+$.

2-(5-Amidinobenzimidazol-1′-yl)-*N*-[(1,1″-biphenyl)-4-yl]acetamide (31). ^{1}H NMR (CD_3OD): δ 8.47 (bs, 1H), 8.21 (s, 1H), 7.77 (td, J = 8.1 Hz, J = 4.0 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.57 (dd, J = 8.1 Hz, J = 1.1 Hz, 4H), 7.39 (t, J = 7.3 Hz, 2H), 7.29 (t, J = 7.3 Hz, 1H), 5.31 (s, 2H). LRMS: 370.2 $(M + H)^+$.

Methyl (5-Cyano-1*H*-indol-3-yl)(oxo)acetate (35). To a stirred solution of oxalyl chloride (103.8 g, 818 mmol) in dry Et_2O (500 mL) was added 1*H*-indole-5-carbonitrile (**34**) (49.8 g, 350 mmol) in small portions over 15 min at −10 °C, and the resulting mixture was stirred at room temperature for 3.5 h. The mixture was filtered, and the residue was collected and rinsed with Et_2O. After dry MeOH (400 mL) was added in, the resulting mixture was stirred at room temperature for 18 h and filtered. The solids were collected and rinsed with MeOH and Et_2O to afford **35** (74.9 g, 94%). Mp: 291−292 °C. ^{1}H NMR (DMSO-d_6): δ 12.85 (bs, 1H), 8.69 (d, J = 3.3 Hz, 1H), 8.65 (d, J = 3.3 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.70 (dd, J = 8.4 Hz, J = 1.4 Hz, 1H), 3.91 (s, 2H). LRMS: 229 $(M + H)^+$.

(5-Cyano-1*H*-indol-3-yl)acetic Acid (37) and (5-Cyano-2,3-dihydro-1*H*-indol-3-yl)acetic Acid (39). To a solution of triethylsilane (186.7 mL, 1167 mmol) in trifluoroacetic acid (250 mL) at −10 °C was added **35** (76.16 g, 334 mmol) in small potions over 25 min, and the resulting mixture was stirred at room temperature for 20 h. The mixture was concentrated to remove the excess solvent and reagent, and the residue was neutralized with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 300 mL). The organic layer was concentrated and purified by column chromatography eluted with MeOH in CH_2Cl_2 (0−5%) to provide methyl (5-cyano-1*H*-indol-3-yl)acetate (16.18 g, 22.6%) and methyl (5-cyano-2,3-dihydro-1*H*-indol-3-yl)acetate (30 g, 41.6%). To a solution of methyl (5-cyano-2,3-dihydro-1*H*-indol-3-yl)acetate (3 g, 13.9 mmol) in MeOH (60 mL) was added another solution of NaOH (1.56 g, 27.7 mmol) in water (15 mL), and the resulting mixture was stirred at room temperature for 4 h and then concentrated. The residue was dissolved in water (100 mL), and the resulting mixture was extracted with Et_2O (2 × 50 mL). The aqueous layer was acidified to pH 1 with 6 N HCl and extracted with Et_2O (4 × 50 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to afford **39** (2.53 g, 90%). Mp: 120−121 °C. ^{1}H NMR (CD_3OD): δ 7.30−7.26 (m, 2H), 6.53 (d, J = 8.1 Hz, 1H), 3.80 (t, J = 9.2 Hz, 1H), 3.69−3.59 (m, 1H), 3.33−3.28 (m, 1H), 2.71 (dd, J = 16.3 Hz, J = 6.0 Hz, 1H), 2.53 (dd, J = 16.1 Hz, J = 8.4 Hz, 1H). ^{13}C NMR (CD_3OD): δ 174.08, 156.03, 133.24, 131.94, 127.18, 120.38, 107.68, 97.44, 52.52, 38.40, 37.29. LRMS: 199 $(M - H)^+$. Anal. Calcd for $C_{11}H_8N_2O_2$: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.41; H, 4.94; N, 13.65.

By using the same procedure, methyl (5-cyano-1*H*-indol-3-yl)acetate was saponified in KOH/MeOH at room temperature over 18 h to provide **37**. Mp: 196.5−198.5 °C. ^{1}H NMR (CD_3OD): δ 7.89 (s, 1H), 7.45 (d, 1H, J = 8.6 Hz), 7.38 (d, 1H, J = 8.6 Hz), 7.28 (s, 1H), 3.78 (s, 2H). Anal. Calcd for $C_{11}H_{10}N_2O_2$: C, 66.00; H, 4.04; N, 13.99. Found: C, 65.71; H, 4.24; N, 13.94.

(5-Cyano-1-methyl-1*H*-indol-3-yl)acetic Acid (38). To a solution of **35** (4 g, 17.5 mmol) in DMF (50 mL) was added NaH (95%, 0.5 g, 21 mmol), and the mixture was stirred at room temperature for 20 min. Methyl iodide (2.5 g, 17.5 mmol) was added, and the resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc, washed with water (4×), dried over $MgSO_4$, and concentrated to give a crude of methyl (5-cyano-1-methyl-1*H*-indol-3-yl)(oxo)-acetate (**36**; 6.98 g). The crude was reduced with triethylsilane and by saponified in NaOH and aqueous methanol as described for example **37** to provide **38**. ^{1}H NMR (CD_3OD): δ 7.97 (t, J = 0.7 Hz, 1H), 7.43 (d, 1H, J = 8.6 Hz), 7.37 (d, 1H, J = 8.6 Hz), 7.29 (s, 1H), 3.81 (s, 3H), 3.74 (d, J = 0.7 Hz, 2H). LRMS: 215.1 $(M + H)^+$.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)(1,1′-biphenyl)-4-yl]acetamide (41). To a stirred solution of **37** (281 mg, 1.4 mmol) and BOP (928 mg, 2.1 mmol) in DMF (10 mL) were added **28** (640 mg, 2.1 mmol) and Et_3N (212 mg, 2.1 mmol). After being heated at 50 °C for 18 h, the reaction was diluted with water, extracted with EtOAc, and washed with 10% HCl (3×), 5% NaOH (2×), and brine (2×). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo to afford **40** (370 mg, 54%). A solution of **40** (370 mg, 0.76 mmol) in TFA (10 mL) was refluxed for 2 h. Concentration and purification via silica gel chromatography using 100% EtOAc as the eluant, afforded the des-*tert*-butyl cyano intermediate (280 mg, 86%). This cyano intermediate (880 mg, 2 mmol) was dissolved in dry MeOH (5 mL) and MeOAc (10 mL) at 0 °C, and the solution was saturated with HCl (gas). The resulting solution was stirred at room temperature for 18 h. The mixture was concentrated in vacuo, redissolved in dry MeOH, and solid $(NH_4)_2CO_3$ (672.0 mg) was added. The reaction flask was sealed, and the mixture was stirred at room temperature for 18 h. The resulting suspension was filtered through Celite, and the solids were rinsed with dry MeOH. The filtrate was concentrated in vacuo, followed by purification on preparative HPLC to afford **41** (450 mg, 50%). ^{1}H NMR (CD_3OD): δ 8.22 (s, 1H), 8.06 (dd, J = 7.7 Hz, J = 1.5 Hz, 1H), 7.60−7.54 (m, 6H), 7.43 (s, 1H), 7.36 (d, J = 8.8 Hz, 2H), 7.30 (d, J = 7.7 Hz, 1H), 3.91 (s, 2H). HRMS: 448.1437 (obsd), 448.1443 (calcd) for $C_{23}H_{22}N_5O_3S$ $(M + H)^+$. Anal. Calcd for $C_{23}H_{21}N_5O_3S \cdot 1.15TFA \cdot 1.4H_2O$: C, 50.32; H, 4.16; N, 11.60; F, 10.85; S, 5.31. Found: C, 50.59; H, 3.91; N, 11.57; F, 10.57; S, 4.92.

The following compounds (**70**, **71**, **77**−**81**) were prepared by the procedure described for the preparation of **41**, and the yields of the final step were in the range of 5−60%.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)(1,1′-biphenyl)-4-yl]-*N*-methylacetamide (70). LRMS: 462 $(M + H)^+$. ^{1}H NMR (CD_3OD): δ 8.14 (dd, J = 7.9 Hz, J = 1.1 Hz, 1H), 8.09 (s, 1H), 7.66 (t, J = 7.7 Hz, 1H), 7.58 (t, J = 7.5 Hz, 1H), 7.54−7.49 (m, 4H), 7.37 (d, J = 7.7 Hz, 1H), 7.27 (d, J =

8.3 Hz, 2H), 7.00 (s, 1H), 3.79 (s, 2H), 3.31 (s, 3H). ^{13}C NMR (CD_3OD): δ 173.62, 169.34, 144.46, 143.26, 141.69, 141.11, 133.44, 132.92, 132.10, 129.15, 128.67, 128.23, 127.84, 121.36, 119.25, 113.20, 110.99, 38.02, 32.41. Anal. Calcd for $C_{24}H_{23}N_5O_3S\cdot1.3TFA\cdot1.4H_2O\cdot0.4CH_3CN$: C, 50.01; H, 4.32; N, 11.41; F, 11.04; S, 4.84. Found: C, 50.20; H, 3.97; N, 11.15; F, 10.98; S, 4.45.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[5-[2-(aminosulfonyl)-phenyl]-2-pyridinyl]acetamide (71). ^{1}H NMR (CD_3OD): δ 8.30 (s, 1H), 8.23 (s, 1H), 8.11 (dd, J = 7.7 Hz, J = 1.5 Hz, 1H), 8.03 (d, J = 8.8 Hz, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.68−7.53 (m, 4H), 7.49 (s, 1H), 7.37 (d, J = 7.7 Hz, 1H), 4.04 (s, 2H). HRMS: 449.1396 (calcd), 449.1391 (obsd) for $C_{22}H_{21}N_6O_3S$ $(M + H)^+$. Anal. Calcd for $C_{22}H_{20}N_6O_3S\cdot2TFA\cdot1.5H_2O$: C, 44.39; H, 3.58; N, 11.94; F, 16.20; S, 4.56. Found: C, 44.61; H, 3.39; N, 11.93; F, 16.25; S, 4.23.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-[(methylamino)sulfonyl](1,1′-biphenyl)-4-yl]acetamide (77). ^{1}H NMR (CD_3OD): δ 8.22 (s, 1H), 7.99 (d, J = 8.01 Hz, 1H), 7.64−7.49 (m, 6H), 7.44 (s, 1H), 7.36−7.30 (m, 3H), 3.91 (s, 2H), 2.34 (s, 3H). HRMS: 462.1600 (calcd), 462.1591 (obsd) for $C_{24}H_{24}N_5O_3S$ $(M + H)^+$. Anal. Calcd for $C_{24}H_{23}N_5O_3S\cdot1.2TFA\cdot2H_2O$: C, 49.98; H, 4.48; N, 11.04; S, 5.05; F, 10.78. Found: C, 49.80; H, 4.03; N, 11.00; S, 5.15; F, 10.64.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-[(ethylamino)sulfonyl](1,1′-biphenyl)-4-yl]acetamide (78). ^{1}H NMR (CD_3OD): δ 8.23 (s, 1H), 8.01 (d, J = 7.7 Hz, 1H), 7.63−7.48 (m, 6H), 7.44 (s, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 7.3 Hz, 1H), 3.92 (s, 2H), 2.70 (q, J = 7.3 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H). HRMS: 476.1759 (calcd), 476.1759 (obsd) for $C_{25}H_{26}N_5O_3S$ $(M + H)^+$. Anal. Calcd for $C_{25}H_{25}N_5O_3S\cdot1.5TFA\cdot0.8H_2O$: C, 51.36; H, 4.36; N, 10.69. Found: C, 51.20; H, 4.19; N, 10.88.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-[(dimethylamino)-sulfonyl](1,1′-biphenyl)-4-yl]acetamide (79). ^{1}H NMR (CD_3OD): δ 8.22 (s, 1H), 8.00 (dd, J = 8.0 Hz, J = 1.4 Hz, 1H), 7.64−7.28 (m, 10H), 3.91 (s, 2H), 2.37 (s, 6H). HRMS: 476.1556 (calcd), 476.1752 (obsd) for $C_{25}H_{26}N_5O_3S$ $(M + H)^+$. Anal. Calcd for $C_{25}H_{25}N_5O_3S\cdot1.5TFA\cdot2H_2O\cdot2MeOH$: C, 48.19; H, 5.32; N, 9.37; F, 11.43. Found: C, 48.25; H, 5.42; N, 9.28; F, 11.51.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-[(propanylamino)-sulfonyl](1,1′-biphenyl)-4-yl]acetamide (80). ^{1}H NMR (CD_3OD): δ 8.22 (s, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.62−7.48 (m, 6H), 7.44 (s, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 7.43 Hz, 1H), 3.92 (s, 2H), 2.60 (t, J = 7.3 Hz, 2H), 1.31 (q, J = 7.3 Hz, 2H), 0.75 (t, 3H). HRMS: 490.1913 (calcd), 490.1910 (obsd) for $C_{26}H_{28}N_5O_3S$ $(M + H)^+$.

2-(5-Amidino-1-methylindol-3-yl)-*N*-[5-[2-(aminosulfonyl)phenyl]-2-pyridinyl]acetamide (81). ^{1}H NMR (CD_3OD): δ 8.29 (s, 1H), 8.22 (s, 1H), 8.10 (d, J = 6.6 Hz, 1H), 8.00 (d, J = 6.6 Hz, 1H), 7.91 (dd, J = 7.7 Hz, J = 2.6 Hz, 1H), 7.64−7.57 (m, 4H), 7.43 (s, 1H), 7.35 (d, J = 6.2 Hz, 1H), 4.00 (s, 3H), 3.87 (s, 2H). HRMS: 463.1552 (calcd); 463.1570 (obsd) for $C_{23}H_{23}N_6O_3S$ $(M + H)^+$.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)-3-bromo(1,1′-biphenyl)-4-yl]acetamide (**72**). Compounds **72**−**76** were made as described in the following procedures. To a solution of **37** (1 mmol, 0.2 g) in anhydrous CH_3CN (10 mL) was added thionyl chloride (0.3 mL, 4 mmol). The reaction mixture was warmed at 50 °C for 10 min and then allowed to cool to room temperature and stirred for 2 h. The solvent and excess thionyl chloride were removed in vacuo, and the residue was dried in vacuo. To this residue was added a mixture of an aniline (**58**−**62**) (1.0 equiv) and Et_3N (0.14 mL, 1.0 equiv; 2.0 equiv for the HCl salt) in anhydrous CH_2Cl_2 (10 mL). This reaction mixture was stirred at room temperature for 2 h, then concentrated and purified by flash chromatography on a silica gel column eluted with hexane:EtOAc (3:1) to give pure amides in about 50% yields. A solution of the amide intermediate (1.24 mmol, 0.7 g) in anhydrous MeOAc (15 mL) and anhydrous MeOH (0.5 mL) was saturated with dry HCl (gas) at −20 °C for 20 min. The reaction mixture was left at room temperature for 18 h, and was evaporated to remove any residual HCl and the solvents. To the imidate in anhydrous MeOH (15 mL) was added $(NH_4)_2CO_3$ (10 equiv), and the reaction mixture was allowed to stir at room temperature for 24 h. This final reaction mixture was evaporated and purified on preparative HPLC. After lyophilization, pure amidine products were obtained in varied yields (15−50%). For **72**: ^{1}H NMR (CD_3OD): δ 8.22 (s, 1H), 8.07 (d, J = 7.7 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.52−7.61 (m, 6H), 7.36 (d, J = 8.2 Hz, 1H), 7.27 (d, J = 7.3 Hz, 1H), 4.01 (s, 2H). HRMS: 526.0548 (calcd), 526.0538 (obsd) for $C_{23}H_{21}N_5SO_3Br$ $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}N_5SO_3Br\cdot1.3TFA\cdot2H_2O$: C, 43.27; H, 3.59; N, 9.98; S, 4.51; F, 10.43. Found: C, 43.20; H, 3.37; N, 9.85; S, 4.27; F, 10.29.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)-3-iodo(1,1′-biphenyl)-4-yl]acetamide (**73**). ^{1}H NMR (CD_3OD): δ 8.15 (s, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.76 (s, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.55−7.40 (m, 3H), 7.32 (m, 2H), 7.20 (t, J = 7.33 Hz, 2H), 3.90 (s, 2H). HRMS: 574.0410 (calcd), 574.0428 (obsd) for $C_{23}H_{21}N_5SO_3I$ $(M + H)^+$.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)-3-chloro(1,1′-biphenyl)-4-yl]acetamide (**74**). ^{1}H NMR (CD_3OD): δ 8.22 (s, 1H), 8.08 (d, J = 7.69 Hz, 1H), 7.85 (d, J = 8.42 Hz, 1H), 7.5−7.6 (m, 5H), 7.45 (s, 1H), 7.31 (t, J = 8.42 Hz, 2H), 4.01 (s, 2H). HRMS: 482.1054 (calcd), 482.1039 (obsd) for $C_{23}H_{21}N_5SO_3Cl$ $(M + H)^+$.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)-3-methyl(1,1′-biphenyl)-4-yl]acetamide (75). ^{1}H NMR (CD_3OD): δ 8.25 (s, 1H), 8.09 (d, J = 8.05 Hz, 1H), 7.56 (m, 3H), 7.49 (t, J = 8.79 Hz, 2H), 7.32 (d, J = 8.05 Hz, 1H), 7.25 (m, 3H), 3.96 (s, 2H), 2.12 (s, 3H). HRMS: 462.1600 (calcd), 462.1586 (obsd) for $C_{24}H_{24}N_5O_3S$ $(M + H)^+$.

2-(5-Amidino-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)-3-fluoro(1,1′-biphenyl)-4-yl]acetamide (76). ^{1}H NMR (CD_3OD): δ 8.21 (s, 1H). 8.15 (d, J = 7.69 Hz, 1H), 7.85 (t, J = 8.06 Hz, 1H), 7.5−7.6 (m, 4H), 7.45 (s, 1H), 7.3 (d, J = 7.69 Hz, 1H), 7.25 (d, J = 9.52 Hz, 1H), 7.18 (d, J = 9.52 Hz, 1H), 3.98 (s, 2H). HRMS: 466.1349 (calcd), 466.1338 (obsd) for $C_{23}H_{21}N_5SO_3F$ $(M + H)^+$. Anal. Calcd for $C_{23}H_{20}N_5SO_3F\cdot2TFA\cdot0.15H_2O$: C, 46.20; H, 3.63; N, 10.21; F, 16.88. Found: C, 46.14; H, 3.46; N, 10.51; F, 16.98.

2-(5-Amidino-2,3-dihydro-1*H*-indol-3-yl)-*N*-[2′-(aminosulfonyl)(1,1′-biphenyl)-4-yl]acetamide (43, 44). To a solution of **39** (5.0 g, 25 mmol) in DMF (25 mL) was added BOP (16.41 g, 37 mmol), and the resulting solution was stirred at room temperature for 30 min. To this solution was added **28** (11.29 g, 37 mmol) followed by Et_3N (5.2 mL, 37 mmol), and the reaction mixture was heated at 60 °C for 18 h. The mixture was poured into water and extracted with EtOAc (4×). The organic extracts were washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography on a silica gel column (300 g) eluted with hexane:EtOAc (2:1) to give the racemic coupled product (3 g, 25%). The isomers were then separated by HPLC on a chiral column eluted with 95% MeOH, 5% H_2O, and 0.1% TEA at 7 mL/min and monitored at 230 nm to afford the (−)-enantiomer ((−)-**42**, 1.49 g) and the (+)-enantiomer ((+)-**42**, 1.49 g). For (−)-**42**. LRMS: 489 $(M + H)^+$ and 511 $(M + Na)^+$. [α]: −52.00° (c = 0.400 g/dL in methanol at 25 °C). For (+)-**42**. LRMS: 489 $(M + H)^+$ and 511 $(M + Na)^+$. [α]: +49.75° (c = 0.400 g/dL in methanol at 25 °C). ^{1}H NMR ($CDCl_3$): δ 8.16 (d, J = 7.9 Hz, 1H), 7.79 (s, 1H), 7.62 (m, 2H), 7.56 (m, 2H), 7.46 (m, 2H), 7.34 (m, 2H), 6.55 (d, J = 8.8 Hz, 1H), 3.93 (m, 1H), 3.68 (s, 1H), 3.49 (m, 1H), 2.83−2.62 (m, 2H), 1.02 (s, 9H).

The same procedure as described in **41** was used to make (+)-**43** from (+)-**42** and (−)-**44** from (−)-**42**.

Acetamide (+)-43. ^{1}H NMR (CD_3OD): δ 8.08 (d, J = 7.3 Hz, 1H), 7.65−7.30 (m, 9H), 6.60 (d, J = 8.1 Hz, 1H), 3.90−3.72 (m, 2H), 3.45 (bs, 1H), 2.83−2.68 (m, 2H); [α]: +38.50° (c = 0.200 g/dL in methanol). HRMS: 450.1600 (calcd), 450.1596 (obsd) for $C_{23}H_{24}N_5O_3S_1$ $(M + H)^+$. Anal. Calcd for $C_{23}H_{23}N_5O_3S_1\cdot1.2TFA\cdot0.5\ H_2O$: C, 51.24; H, 4.27; N, 11.76. Found: C, 51.38; H, 4.07; N, 11.58.

Acetamide (−)-44. ^{1}H NMR (CD_3OD): δ 8.08 (d, J = 7.3 Hz, 1H), 7.65−7.47 (m, 6H), 7.37 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 7.0 Hz, 1H), 6.62 (d, J = 8.8 Hz, 1H), 3.89−3.77 (m, 2H), 3.44 (bs, 1H), 2.85−2.62 (m, 2H). [α]: +45.05 (c = 0.202 g/dL in methanol). HRMS: 450.1600 (calcd), 450.1596 (obsd) for

$C_{23}H_{24}N_5O_3S_1$ $(M + H)^+$. Anal. Calcd for $C_{23}H_{23}N_5O_3S_1 \cdot 1.3TFA$: C, 51.44; H, 4.10; N, 11.72; F, 12.40. Found: C, 51.12; H, 4.10; N, 11.49; F, 12.40.

(6-Cyano-1*H*-indazol-1-yl)acetic Acid (47). To a solution of 6-nitro-1*H*-indazole (**45**) (10 g, 88.5 mmol) in DMF (50 mL) were added K_2CO_3 (30.55 g, 221 mmol) and methyl 2-bromoacetate (8.37 mL, 88 mmol), and the resulting mixture was stirred at room temperatureerature for 18 h. The reaction was quenched with water, and the precipitate was collected, washed with water, and dried by air. The solid was dissolved in hot EtOAc, decolorized with charcoal, filtered through Celite, and concentrated to give methyl 2-(6′-nitroindazol-1′-yl)acetate (7.01 g, 34%). The nitro compound (3 g, 12.8 mmol) was dissolved in MeOH (70 mL) and $CHCl_3$ (20 mL) and treated with hydrogen at 1 atm in the presence of 10% Pd/C (0.7 g) at room temperature for 4 h. After additional 20% $Pd(OH)_2/C$ (0.5 g) was added, the reaction was further treated with hydrogen at room temperature for 3 h. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by flash chromatography with a gradient mixture of 0% to 100% of EtOAc in $CHCl_3$ to provide methyl (6-amino-1*H*-indazol-1-yl)acetate (**46**) (1.33 g, 51%). To a solution of **46** (1.33 g, 6.4 mmol) in 2.5 N HCl (20 mL) at 0 °C was added $NaNO_2$ (0.44 g, 6.4 mmol), and the mixture was stirred for 1 h. The reaction was neutralized with saturated Na_2CO_3 and then was added to a solution of CuCN (0.75 g) and NaCN (0.632 g) in water (12 mL) and EtOAc (24 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h and then warmed to room temperature for 1 h. The resulting mixture was extracted with EtOAc, and the organic extracts were concentrated. The residue was purified by flash chromatography on silica gel with EtOAc:hexane (4:1) to provide methyl 2-(6′-cyanoindazol-1′-yl)eacetate (0.64 g, 46%). The ester (0.63 g, 2.95 mmol) was saponified in MeOH (10 mL) and H_2O (10 mL) with KOH (215 mg, 3.84 mmol) at room temperature for 1.5 h. The solution then was concentrated in vacuo, diluted with water, extracted with EtOAc, and then acidified to pH 3 with 1 N HCl. The resulting orange participate was filtered and dried under high vacuum to afford **47**. Mp: 189−191 °C. ^{1}H NMR (CD_3OD): δ 8.17 (s, 1H), 8.08 (s, 1H), 7.95 (dd, J = 8.4 Hz, J = 1.1 Hz, 1H), 7.40 (dd, J = 8.4 Hz, J = 1.1 Hz, 1H), 5.30 (s, 1H). LRMS: 202 $(M + H)^+$.

2-(6-Amidino-1*H*-indazol-3-yl)-*N*-[5-[2-(aminosulfonyl)-phenyl]-2-pyridinyl]acetamide (50). The same procedure as described in **41** was used to make **50** from the coupling of **47** with **48**, followed by Pinner reaction and amination, to form **50**. ^{1}H NMR (CD_3OD): δ 8.34 (d, J = 2.6 Hz, 1H), 8.26 (s, 1H), 8.15 (s, 1H), 8.10 (dd, J = 7.7 Hz, J = 1.5 Hz, 1H), 8.03 (d, J = 8.4 Hz, 2H), 7.81 (dd, J = 8.8 Hz, J = 2.2 Hz, 1H), 7.65 (dd, J = 7.3 Hz, J = 1.1 Hz, 1H), 7.60 (dd, J = 6.3 Hz, J = 1.5 Hz, 1H), 7.55 (dd, J = 7.3 Hz, J = 1.5 Hz, 1H), 7.34 (dd, J = 7.7 Hz, J = 1.5 Hz, 1H), 5.52 (s, 2H). HRMS: 450.1348 (calcd), 450.1347 (obsd) for $C_{21}H_{20}N_7O_3S_1$ $(M + H)^+$. Anal. Calcd for $C_{21}H_{19}N_7S_1O_3 \cdot 1.8TFA \cdot 1.5H_2O$: C, 43.34; H, 3.52; N, 14.38, F, 4.70. Found: C, 43.28; H, 3.46; N, 14.18, F, 4.78.

***N*-(*tert*-Butyl)-4′-nitro[1,1′-biphenyl]-2-sulfonamide (52).** To a solution of 4-bromonitrobenzene (**51**) (8.16 g, 40.6 mmol) and **19** (10.43 g, 40.6 mmol) in THF (400 mL) was added aqueous sodium carbonate (8.6 g in 200 mL of water). The mixture was stirred at room temperature for 30 min while being degassed with nitrogen, and then $Pd(PPh_3)_4$ (1.0 g, 0.87 mmol) was added. The reaction mixture was refluxed for 18 h and filtered through Celite. The filtrate was concentrated. The residue was diluted with water, and the resulting mixture was extracted with EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, and concentrated. The residue was recrystallized from EtOAc to give **52** (6.83 g, 50.4%). ^{1}H NMR ($CDCl_3$) δ 8.3−7.2 (m, 8H), 3.6 (s, 1H), 1.2 (s, 9H). LRMS: 335 $(M + H)^+$.

4′-Amino-*N*-dimethyl[1,1′-biphenyl]-2-sulfonamide (56). A solution of **52** (6.83 g, 20.4 mmol) in CH_2Cl_2 (15 mL) was treated with TFA (20 mL) under reflux for 3 h and then concentrated to give a crude sulfonamide (5.87 g). ^{1}H NMR ($CDCl_3$): δ 8.31 (d, J = 8.8 Hz, 2H), 8.20 (dd, J = 7.7 Hz, J = 1.1 Hz, 1H), 7.69−7.60 (m, 3H), 7.58 (td, J = 7.7 Hz, J = 1.4 Hz, 1H), 7.33 (dd, J = 7.3 Hz, J = 1.4 Hz, 1H), 4.42 (s, 2H).

To a solution of the crude product (1.0 g, 3.59 mmol) in DMF (20 mL) at 0 °C was added NaH (95%, 0.181 g, 7.2 mmol). After the mixture was stirred for 15 min, methyl iodide (0.47 mL, 7.53 mmol) was added, and the resulting mixture was stirred at room temperature for 18 h. The mixture was extracted with EtOAc, and the combined extracts were washed with water (5×), dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography eluted with hexane and EtOAc (1:1) to give a *N*,*N*-dimethylsulfonamide (725 mg, 66%). ^{1}H NMR ($CDCl_3$): δ 8.28 (d, J = 8.4 Hz, 2H), 8.08 (d, J = 7.7 Hz, 1H), 7.64 (t, J = 7.3 Hz, 1H), 7.61−7.56 (m, 3H), 7.29 (t, J = 7.3 Hz, 1H), 2.47 (s, 6H). LRMS: 324 $(M + H)^+$.

A solution of the intermediate (725 mg, 2.37 mmol) in MeOH (20 mL) and CH_2Cl_2 (15 mL) was treated with hydrogen at one atmosphere in the presence of 10% Pd/C (250 mg) at room temperature for 2 h. The resulting mixture was then filtered through Celite and concentrated to provide **56** (0.56 g, 85.6%). ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H), 7.53 (td, J = 7.3 Hz, J = 1.4 Hz, 1H), 7.44 (td, J = 7.3 Hz, J = 1.4 Hz, 1H), 7.28 (dd, J = 7.7 Hz, J = 1.4 Hz, 1H), 7.26 (s, 1H), 7.22 (dd, J = 8.4 Hz, 2H), 6.75 (d, J = 8.4 Hz, 2H), 2.38 (s, 6H). LRMS: 277.4 $(M + H)^+$.

Aminobiphenyls 53−55. Compounds **53**−**55** were prepared as described for the synthesis of **56**. To a solution of **52** (828 mg, 2.48 mmol) in DMF (8 mL) at 0 (C was added NaH (95%, 71 mg, 2.98 mmol). After the mixture was stirred for 15 min, bromopropane (0.27 mL, 2.97 mmol) was added, and the resulting mixture was stirred at room temperature for 18 h. The mixture was worked up, and the nitro intermediate was hydrogenated as described for preparation of **56** to give **55** (651 mg, 75.9% for two steps).

4′-Amino-*N*-propanyl-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (55). ^{1}H NMR ($CDCl_3$): δ 8.14 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H), 7.55−7.21 (m, 5H), 6.76 (d, J = 8.8 Hz, 2H), 2.90 (q, J = 7.0 Hz, 2H), 1.13 (t, J = 7.0 Hz, 3H), 1.22 (s, 9H). LRMS: 347.2 $(M + H)^+$.

4′-Amino-*N*-methyl-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (53). LRMS: 319.2 $(M + H)^+$.

4′-Amino-*N*-ethyl-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (54). ^{1}H NMR ($CDCl_3$): δ 8.00 (dd, J = 8.1 Hz, J = 1.4 Hz, 1H), 7.47−7.21 (m. 5H), 6.73 (d, J = 8.4 Hz, 2H), 2.71 (t, J = 7.7 Hz, 2H), 1.57−1.51 (m, 2H), 1.22 (s, 9H), 0.73 (t, J = 7.7 Hz, 3H). LRMS: 333.2 $(M + H)^+$.

Aminobiphenyls 58−62. In a general procedure, a mixture of 4-bromo-2-X-aniline (X = halide or methyl) (**57**) (10 mmol), 2-(*tert*-butylaminosulfonyl)phenylboronic acid (**19**; 2.57 g, 10 mmol), $Pd(PPh_3)_4$ (0.23 g, 0.2 mmol), and Na_2CO_3 (3.18 g, 30 mmol) in THF (100 mL) and water (50 mL) was stirred at room temperature for 30 min while degassed with nitrogen. The reaction mixture was then heated at reflux for 18 h and filtered through Celite. The filtrate was evaporated in vacuo and the residue was diluted with water. The resulting mixture was extracted with EtOAc. The organic extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography on a silica gel column eluted with hexane:EtOAc (2.5:1) (or hexane for the *o*-F compound) to give pure products in 50−60% yields.

4′-Amino-3′-methyl-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (58). ^{1}H NMR (acetone-d_6): δ 8.07 (d, J = 7.9 Hz, 1H), 7.60 (t, J = 7.3 Hz, 1H), 7.5 (t, J = 7.7 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.15 (m, 2H), 6.78 (d, J = 8.1 Hz, 1H), 4.72 (bs, 2H), 4.24 (bs, 1H), 2.20 (s, 3H), 0.98 (s, 9H). LRMS: 319 $(M + H)^+$ and 336 $(M + NH_4)^+$.

4′-Amino-3′-fluoro-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (59). ^{1}H NMR (DMSO-d_6): δ 7.96 (d, J = 7.8 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 7.03 (d, J = 12.9 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.74(t, J = 9.2 Hz, 1H), 6.45 (s, 1H), 5.24 (s, 2H), 0.95 (s, 9H). HRMS: 323.1230 (calcd), 323.1215 (obsd) for $C_{16}H_{20}N_2SO_2F$ $(M + H)^+$.

4′-Amino-3′-chloro-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (60). ^{1}H NMR ($CDCl_3$): δ 8.14 (dd, J = 7.9 Hz, J =

1.3 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.45 (t, J = 7.9 Hz, 1H), 7.36 (d, J = 2.2 Hz, 1H), 7.29−7.25 (m, 2H), 6.83 (d, J = 8.0 Hz, 1H), 1.03 (s, 9H). LRMS: 339 $(M + H)^+$ and 356 $(M + NH_4)^+$.

4′-Amino-3′-bromo-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (61). ^{1}H NMR (CD_3OD): δ 8.07 (dd, J = 7.8 Hz, J = 1.2 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.48 (dd, J = 7.9 Hz, J = 1.3 Hz, 1H), 7.45 (d, J = 2.0 Hz, 1H), 7.28 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H), 7.20 (dd, J = 8.2 Hz, J = 2.1 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 1.02 (s, 9H). LRMS: 383/385 $(M + H)^+$.

4′-Amino-3′-iodo-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (62). ^{1}H NMR (CD_3OD): δ 8.07 (dd, J = 8.1 Hz, J = 1.3 Hz, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.59 (td, J = 7.6 Hz, J = 1.5 Hz, 1H), 7.48 (td, J = 7.7 Hz, J = 1.5 Hz, 1H), 7.30 (dd, J = 7.6 Hz, J = 1.5 Hz, 1H), 7.23 (dd, J = 8.3 Hz, J = 2.0 Hz, 1H), 6.84 (d, J = 8.3 Hz, 1H), 1.03 (s, 9H). LRMS: 431 $(M + H)^+$ and 448 $(M + NH_4)^+$.

4′-(Methylamino)-*N*-(*tert*-butyl)[1,1′-biphenyl]-2-sulfonamide (63). A solution of **28** (2 g, 6.58 mmol) in ethyl formate (30 mL) was refluxed for 2 h. The mixture was concentrated in vacuum to afford a formamide intermediate (2.24 g) as a white solid. To a stirred solution of the above formamide in THF (70 mL) at 0 °C was added LAH (1 M in THF, 20.25 mL, 20.3 mmol), and the resulting mixture was stirred at room temperature for 3 h. The reaction was quenched with Et_2O and water (1:1), filtered through Celite, and purified by silica gel chromatography with $CHCl_3$ to **63** (1.76 g, 48% for two steps). ^{1}H NMR (CD_3OD): δ 8.04 (dd, J = 8.0, J = 1.1 Hz, 1H), 7.56 (td, J = 7.7 Hz, J = 1.1 Hz, 1H), 7.43 (td, J = 7.7 Hz, J = 1.1 Hz, 1H), 7.30 (dd, J = 7.7 Hz, J = 1.1 Hz, 1H), 7.26 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 2.79 (s, 3H), 0.95 (s, 9H). LRMS 319 $(M + H)^+$.

X-ray Materials and Methods. Bovine trypsin was purchased from Worthington (cat. #3707) and used without further purification. Crystals were grown by vapor diffusion using 20-μL hanging drops containing 10−30 mg/mL β-trypsin, 35 mM Tris pH 7.5, 2.5 mM benzamidine, 100 mM ammonium sulfate, and 6−12% W/V PEG 8,000. The drops were equilibrated at 5 °C over 50 mM Tris pH 7.5, 200 mM ammonium sulfate, and 12−24% W/V PEG 8,000. Crystals appeared after 1 week. Benzamidine was removed by letting the crystals soak overnight in a stabilizing solution containing 50 mM Tris pH 7.5, 200 mM ammonium sulfate, and 20% W/V PEG 8,000. The crystals were transferred to a solution containing 20 mM sodium phosphate pH 7.5, 20% PEG 8,000 and 0.1% glutaraldehyde for 30 min to cross-link. These crystals were then transferred to a solution containing the inhibitor. The inhibitor solution was prepared by first dissolving 1 mg of inhibitor in 5 μL of DMSO. This was followed by a 40-fold dilution of the inhibitor/DMSO solution into the first stabilizing solution. Data were collected 1 week after inhibitor addition.

A crystal of the trypsin−inhibitor complex was mounted and sealed in a glass capillary. An R-AXIS image plate detector was used for X-ray data acquisition. A Rigaku RU-200 rotating anode X-ray generator operating at 50 kV/100 mA equipped with a graphite monochromator was used for data collection. The trypsin data were collected at 4 °C using an Enraf Nonius cooling device. Data frames of 2° rotation about the spindle axis, ϕ, were collected, with exposure times of 30 min/frame, for total angular rotation ranges about ϕ of 90°. Data were processed using the Raxis data processing software (Molecular Structure Corp.). Crystals grew in space group $P2_12_12_1$ with unit cell parameters: a = 54.8 Å, b = 58.9 Å, c = 67.3 Å. Data greater than 1σ were used in refinement and were 89.7% complete. The XPLOR[27] program was used for crystallographic refinement. Simulated annealing (at a maximum temperature of 3000 °C) was followed by *B*-factor refinement. The refined coordinates of trypsin[28] were used to calculate the initial phases for the enzyme−inhibitor structure. The inhibitor was built with the program QUANTA (Molecular Simulations Inc.). No major adjustments to the protein model were needed during the course of the refinements. The final crystallographic *R*-factor was 18.4%.

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