Design and Synthesis of Isoxazoline Derivatives as Factor Xa Inhibitors. 2[1]

Mimi L. Quan,* Christopher D. Ellis, Ann Y. Liauw, Richard S. Alexander, Robert M. Knabb, Gilbert Lam, Matthew R. Wright, Pancras C. Wong, and Ruth R. Wexler

DuPont Pharmaceuticals Company, Experimental Station, P.O. Box 80500, Wilmington, Delaware 19880-0500

Received July 9, 1998

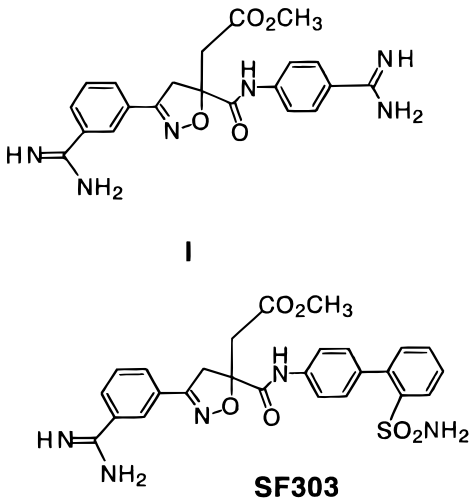
Intravascular clot formation is an important factor in a number of cardiovascular diseases. Therefore, the prevention of blood coagulation has become a major target for new therapeutic agents. One attractive approach is the inhibition of factor Xa (FXa), the enzyme directly responsible for thrombin activation. Herein we report a series of isoxazoline derivatives which are potent FXa inhibitors. Optimization of the side chain at the quaternary position of the isoxazoline ring led to SK549 which showed subnanomolar FXa potency (K_i 0.52 nM). SK549 shows good selectivity for FXa compared to thrombin and trypsin, potent antithrombotic effect in the rabbit arterio-venous thrombosis model, and improved pharmacokinetics relative to other compounds evaluated from this series.

Introduction

Factor Xa (FXa) is a critical enzyme in the blood coagulation cascade that converts prothrombin to thrombin. It holds the central position at the point of convergence of the intrinsic and extrinsic activation mechanisms in the final common pathway of coagulation.[2] Inhibition of FXa interrupts both the intrinsic and extrinsic activation pathways of thrombin production. FXa in combination with FVa and calcium on a phospholipid surface forms the prothrombinase complex which generates thrombin via proteolysis of prothrombin. This activation of thrombin by FXa is a highly amplified process.[3] As a result, inhibition of FXa may be more effective than direct inhibition of thrombin in interrupting the coagulation cascade. It was also shown recently by Harker[4] that FXa inhibitors may have less bleeding risk than thrombin inhibitors in a baboon thrombosis model. Therefore, FXa inhibition may have a more favorable efficacy−safety ratio than thrombin inhibition. Consequently, FXa has emerged as an attractive target enzyme for new therapeutic agents with potential for treatment of arterial and venous thrombosis.[5]

Most of the nonpeptide FXa inhibitors in the literature are compounds with two basic groups.[6−14] There have been very few monobasic FXa inhibitors reported to date.[15] We have recently reported on a series of bisbenzamidine isoxazoline derivatives[16] with structures such as **I** (Chart 1) and a series of monobasic substituted biaryl isoxazoline derivatives such as SF303 (Chart 1) which are potent FXa inhibitors.[17] SF303 inhibited FXa with low nanomolar affinity (K_i = 6.3 nM) and showed good in vivo efficacy in the rabbit arterio-venous (A-V) shunt thrombosis model.[17] Concerns about in vivo stability of the ester side chain prompted us to pursue nonhydrolyzable ester replacements with the goal of improving the in vivo antithrombotic profile of these compounds.

Chart 1



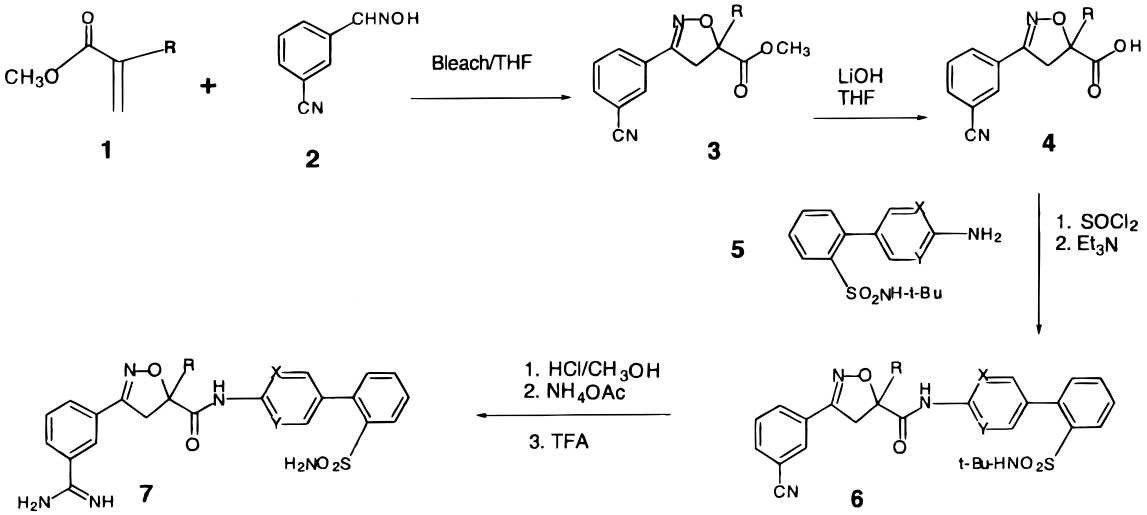
Chemistry

A general synthesis of this series of compounds is shown in Scheme 1. 3-Cyanobenzene oxime (**2**) was oxidatively chlorinated with bleach and then dehydrochlorinated to generate the nitrile oxide in situ. Cycloaddition of the nitrile oxide with 2-substituted acrylic acid ester **1** afforded isoxazoline **3**. The ester group of **3** was hydrolyzed to acid **4**, which was then coupled with the biaryl derivative **5** to give amide **6**. The cyano group of **6** was converted to a benzamidine using a Pinner sequence.[18] Deprotection of the *tert*-butyl group produced the final targets. Biaryl intermediate **5** was prepared as shown previously.[17] When R is hydrogen, methyl, and trifluoromethyl, the starting acrylates were purchased from commercial sources. When R is benzyl, the acrylate was prepared from iodobenzene following the methods described by Knoechel.[19,20] Iodobenzene was converted to phenylzinc iodide with zinc dust. The iodide was reacted with LiCl/CuCN to form a copper complex, which was then reacted with methyl 2-(bromomethyl)acrylate (**8**) to give methyl 2-benzylacrylate.

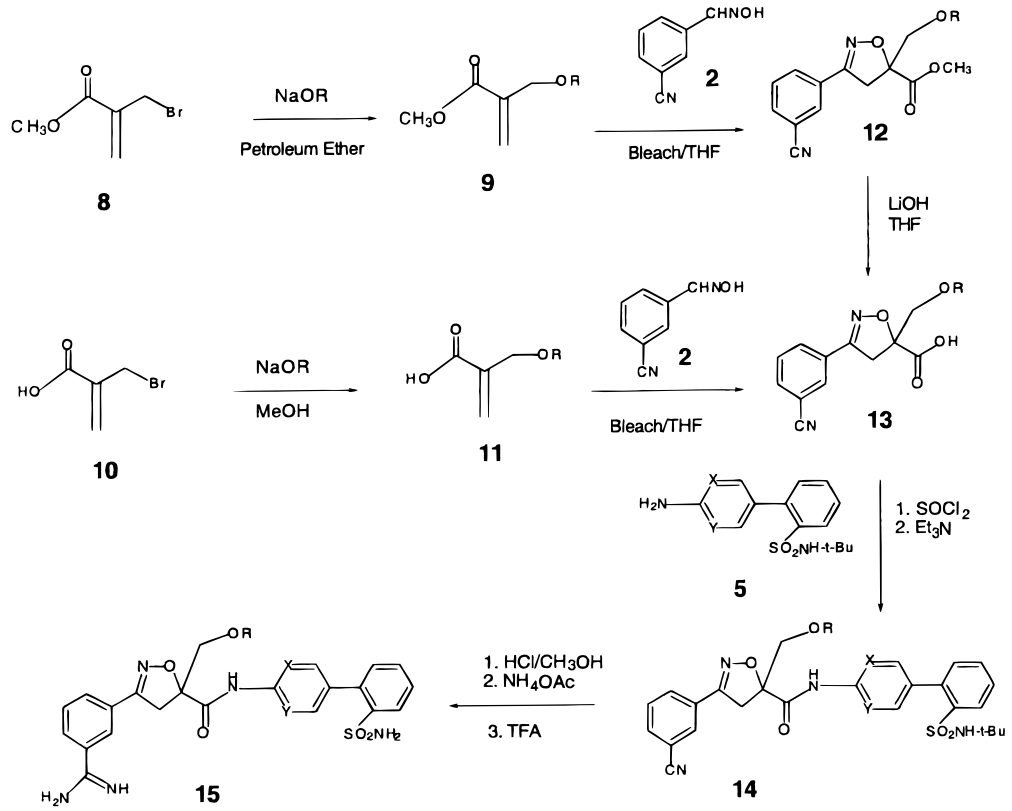
The syntheses of the ether analogues are shown in Scheme 2. Methyl 2-(bromomethyl)acrylate (**8**) was alkoxylated with sodium alkoxide in petroleum ether

10.1021/jm980406a CCC: $18.00
Published on Web 07/15/1999

Scheme 1. General Synthesis



Scheme 2. Synthesis of the Ether Analogues

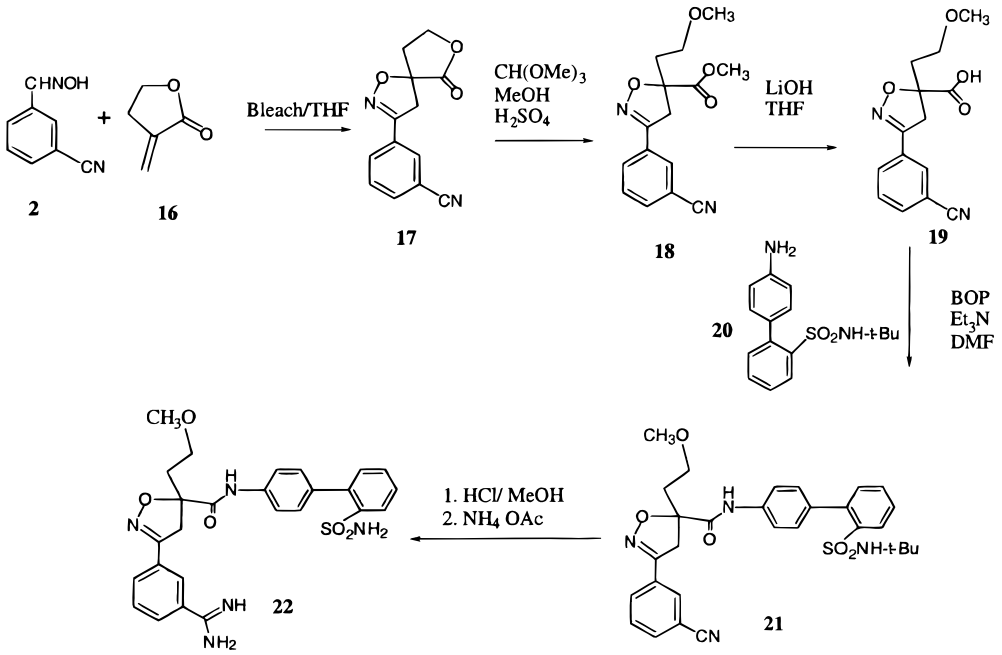


to give methyl 2-(alkoxymethyl)acrylate (**9**). When R is either *n*-propyl or *i*-pentyl, (bromomethyl)acrylic acid **10** was employed as the starting material instead of the methyl ester. Both the alkoxyacrylate **9** and alkoxyacrylic acid **11** underwent 2+3 cycloaddition with oxime **2** to give isoxazolines **12** and **13,** respectively. Compound **12** was hydrolyzed to **13**, which was then carried on to produce the final products as shown.

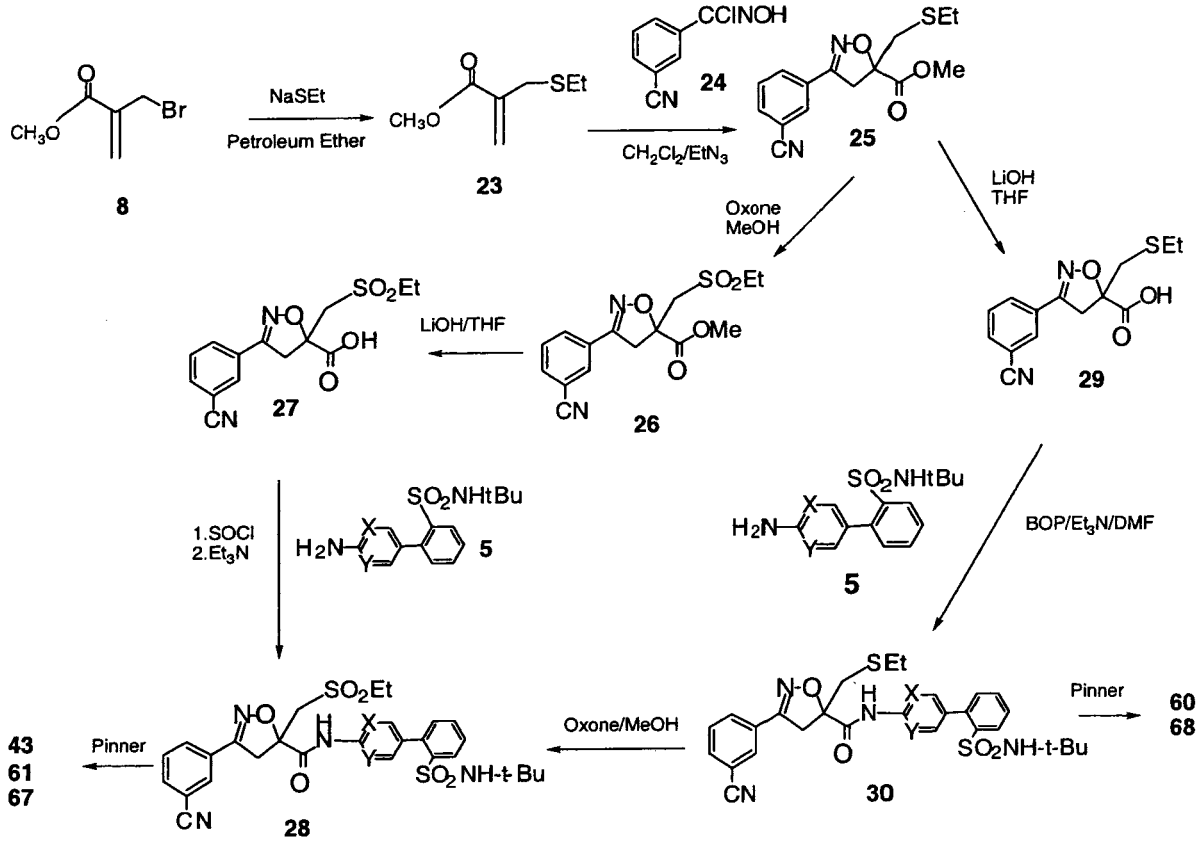
Compound **22** with a methoxyethyl side chain was prepared as shown in Scheme 3. α-Methylene-γ-butyrolactone (**16**) was reacted with 3-cyanobenzene oxime (**2**) as described in Scheme 1 to give isoxazoline **17**. The lactone ring was opened with trimethyl orthoformate in methanol and a catalytic amount of H_2SO_4 to give compound **18**. Hydrolysis of the methyl ester afforded acid **19**, which was coupled with biphenyl aniline **20** using BOP/Et_3N/DMF to generate **21**. The latter compound was carried on to produce **22** by a Pinner sequence.

The syntheses of the sulfide and sulfone analogues are shown in Scheme 4. Methyl 2-(bromomethyl)acrylate (**8**) was treated with sodium ethyl sulfide. The resulting compound **23** underwent cycloaddition with the chloro oxime **24** to give the isoxazoline **25**. At this stage, either the ethyl sulfide could be oxidized to the sulfone **26** or the methyl ester could be hydrolyzed to the acid **29**. Each intermediate was then carried on to the final products by the methods shown in Scheme 4.

Scheme 3. Synthesis of Compound **22**

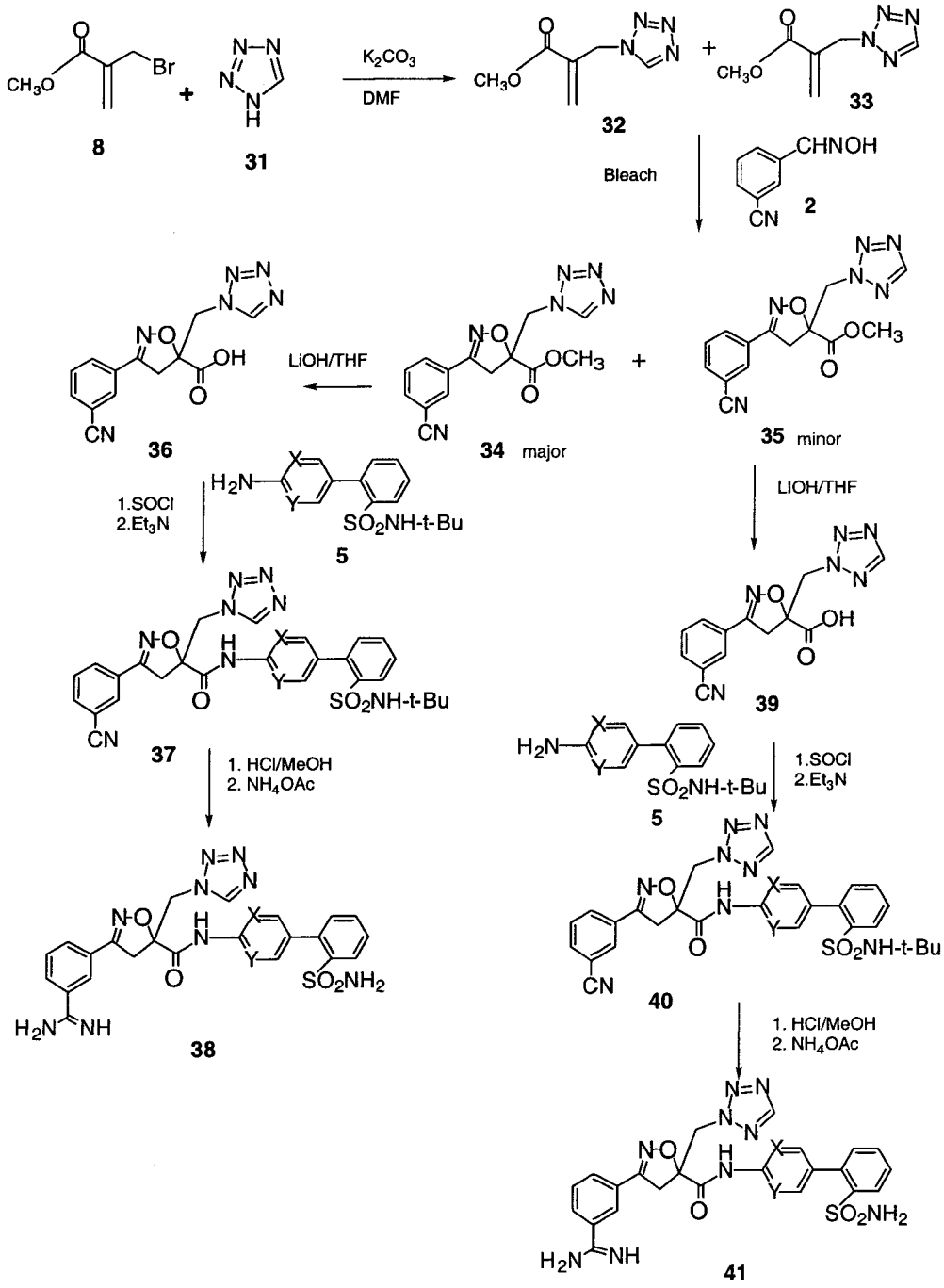


Scheme 4. Synthesis of the Sulfide and Sulfone Analogues



The syntheses of the tetrazole analogues are shown in Scheme 5. Alkylation of the methyl 2-(bromomethyl)-acrylate (**8**) with *1H*-tetrazole in K_2CO_3/DMF gave both the 1-alkylated (**32**) and 2-alkylated (**33**) products. This mixture was subjected to the cycloaddition reaction, and the resulting products were separated to give the 1-alkylated isomer **34** as major product and 2-alkylated isomer **35** as minor product (3.2:1 ratio). Each methyl ester was independently hydrolyzed and coupled with the biaryl intermediate **5**. The *tert*-butyl protecting group was removed in the Pinner reaction while generating the imidate. To obtain the negative enantiomers listed in Table 5, the two enantiomers of compound **36** were separated by chiral HPLC, and the (−)-enantiomer was then carried on following the same methods described above to give the final products in greater than

Scheme 5. Synthesis of the Tetrazole Analogues



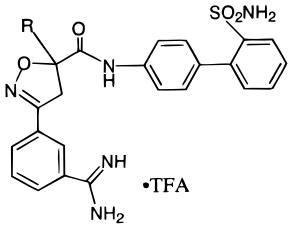
95% ee. The triazole **54** was prepared by employing analogous chemistry to that utilized for the preparation of the tetrazoles.

Results and Discussion

Due to concern over potential metabolic instability of the ester functionality in SF303, the corresponding acid **42** was prepared and was found to be 3-fold less potent than SF303 in FXa affinity (Table 1). While carboxylic acid **42** is less potent, enhanced selectivity for FXa compared to thrombin is observed. Although the role of the substituent at the quaternary center is not well-understood, molecular modeling studies indicate that the ester carbonyl group might form a hydrogen bond with Gln^{192} of FXa. The enhanced selectivity of the carboxylic acid **42** for FXa over thrombin may be explained by the charge repulsion between the carboxylic acid group and Glu^{192} of thrombin.

Pharmacokinetic studies of the ester (SF303) and the corresponding acid (**42**) dosed in rabbits at 5 mg/kg intravenously showed both compounds to have low clearance (0.21 and 0.28 L/kg/h, respectively) with apparent elimination half-lives of 0.92 and 1.54 h, respectively (Table 1). Although SF303 showed good in vivo potency in the rabbit A-V shunt model, LC−MS−MS analysis of the rabbit plasma samples indicated a significant amount of acid **42** present. Acid **42** was found to be substantially less potent in vivo compared to SF303 in the rabbit A-V shunt model (Table 1). In addition, the ratio of SF303 to **42** increased significantly with time. A metabolite screening study of SF303 in which rabbits were dosed intravenously at 10 mg/kg did

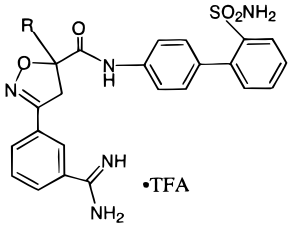
Table 1. Comparison of the Compounds with an Ester and Acid Side Chain[a]



compd	R	K_i (nM) FXa	thrombin	trypsin	rabbit Cl (L/kg/h)	rabbit $t_{1/2}$ (h)	A-V shunt ID_{50} (μmol/kg/h)
SF303	CO_2CH_3	6.3	3100	110	0.21	0.92	0.60
42	CO_2H	20	>21000	630	0.28	1.57	>4.8

[a] All compounds shown in this table are racemic. FXa, thrombin, and trypsin K_i's were obtained using human purified enzymes. PK data were determined in rabbits at 5 mg/kg by anti-FXa assay. The A-V shunt ID_{50} values were determined in rabbits.

Table 2. Replacement of the Ester Group in the Biphenyl Series[a]



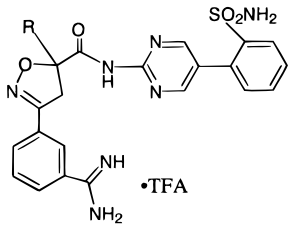
compd	R	K_i (nM) FXa	thrombin	trypsin
SF303	$CH_2CO_2CH_3$	6.3	3100	120
43	$CH_2SO_2CH_2CH_3$	3.5	2000	72
22	$CH_2CH_2OCH_3$	5.0	4400	98
44	CH_2OCH_3	3.4	2400	70
45	$CH_2OCH_2CH_3$	3.5	3100	70
46	CH_2O-*n*-Pr	2.9	4300	90
47	CH_2O-*i*-Pr	4.3	4500	120
48	CH_2O-*n*-Bu	13.0	4000	113
49	CH_2O-*i*-amyl	37	3900	140
50	H	7.2	3900	83
51	CH_3	11	5800	120
52	CF_3	23	6100	310
53	CH_2Ph	8.5	1300	80
54	CH_2-1-(1,2,4-triazole)	1.7	3400	47
55	CH_2-1-tetrazole	1.6	900	91
56	CH_2-2-tetrazole	1.6	2900	91

[a] All compounds shown are racemic. FXa, thrombin, and trypsin K_i's were obtained using human purified enzymes.

not show any metabolites other than **42**. In vitro hydrolysis of SF303 was conducted in both rabbit and human plasma, and the half-lives were determined to be 23 and 17 min, respectively. Due to the reduced potency of **42**, our strategy was to find a replacement for the ester side chain with equal or better FXa potency, which lacked the potential for hydrolysis to the carboxylic acid.

A series of compounds in which the ester functionality of SF303 has been replaced with a variety of substituents is shown in Table 2. Ethylsulfonylmethyl derivative **43** gave a slight improvement in potency compared to SF303. Reducing the carbonyl group of SF303 to a methylene group (**22**) did not affect the FXa affinity indicating that the carbonyl group is not crucial for FXa affinity. Reducing the length of the side chain to methoxymethyl resulted in a compound (**44**) with similar FXa activity compared to **22**. Ethoxymethyl, *n*-propoxymethyl, and *i*-propoxymethyl substitutions also resulted in compounds with similar affinity for FXa compared to **22**, but compounds bearing larger alkoxy groups such as a *n*-butoxymethyl **(48)** and an *i*-pentoxymethyl **(49)** functionality resulted in significantly reduced FXa affinity.

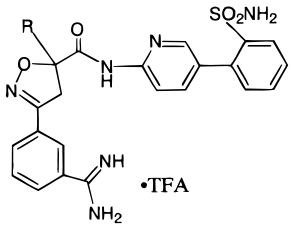
Table 3. Replacement of the Ester Group in the Pyrimidyl Series[a]



compd	R	K_i (nM) FXa	thrombin	trypsin
57	$CH_2CO_2CH_3$	4.3	14000	330
58	CH_2OCH_3	9.9	16000	390
59	$CH_2OCH_2CH_3$	6.8	>21000	330
60	$CH_2SCH_2CH_3$	2.4	10000	350
61	$CH_2SO_2CH_2CH_3$	5.3	13000	290
62	CH_2-1-tetrazole	1.3	7700	500

[a] All compounds shown are racemic. FXa, thrombin, and trypsin K_i's were obtained using human purified enzymes.

Table 4. Replacement of the Ester Group in the Pyridyl Series[a]

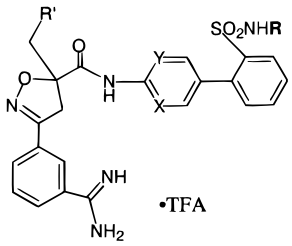


compd	R	K_i (nM) FXa	thrombin	trypsin
63	$CH_2CO_2CH_3$	2.3	3900	52
64	CH_3	4.1	8100	41
65	CH_2OCH_3	2.5	7400	45
66	$CH_2OCH_2CH_3$	2.8	8400	40
67	$CH_2SCH_2CH_3$	2.2	2800	54
68	$CH_2SO_2CH_2CH_3$	1.7	5800	72
69	CH_2-1-tetrazole	0.17	1100	31

[a] All compounds shown are racemic. FXa, thrombin, and trypsin K_i's were obtained using human purified enzymes.

Removal of the acetic acid ester moiety entirely resulted in a compound (**50**) with similar potency and selectivity to SF303. This is in contrast to previous observations in the bisbenzamidine series where the compound lacking the ester functionality was 3-fold less potent.[16] Methyl-, trifluoromethyl-, and benzyl-substituted compounds (**51**−**53**, respectively) had lower affinity for FXa. However, heterocyclic substitution employed to hydrogen bond with Gln^{192} of FXa, such as the triazole **54** and tetrazoles **55** and **56**, improved FXa affinity by approximately 4-fold. While the selectivity for FXa over trypsin remained similar for triazole **54**, the selectivity was improved significantly for the two tetrazole compounds. Interestingly, the triazole and 2-tetrazole were more selective for FXa over thrombin than the 1-tetrazole. In addition to the data shown in

Table 5. (−)-Enantiomers of Potent Compounds[a]



compd	R′	X, Y	R	K_i (nM) FXa	thrombin	trypsin
70	OCH_2CH_3	CH, CH	H	3.3	2100	86
71	OCH_2CH_3	CH, N	H	0.8	5000	25
72 (SK549)	tetrazol-1-yl	CH, CH	H	0.52	400	45
73	tetrazol-1-yl	CH, CH	CH_3	0.92	100	49
74	tetrazol-1-yl	CH, CH	$CH_2CH_2CH_3$	2.1	70	49
75	tetrazol-1-yl	CH, CCH_3	H	1.0	300	200
76	tetrazol-1-yl	CH, CF	H	0.44	500	110
77	tetrazol-1-yl	CH, CCl	H	0.15	300	47
78	tetrazol-1-yl	N, N	H	0.32	3000	120
79 (SM084)	tetrazol-1-yl	CH, N	H	0.11	900	18

[a] FXa, thrombin, and trypsin K_i's were obtained using human purified enzymes.

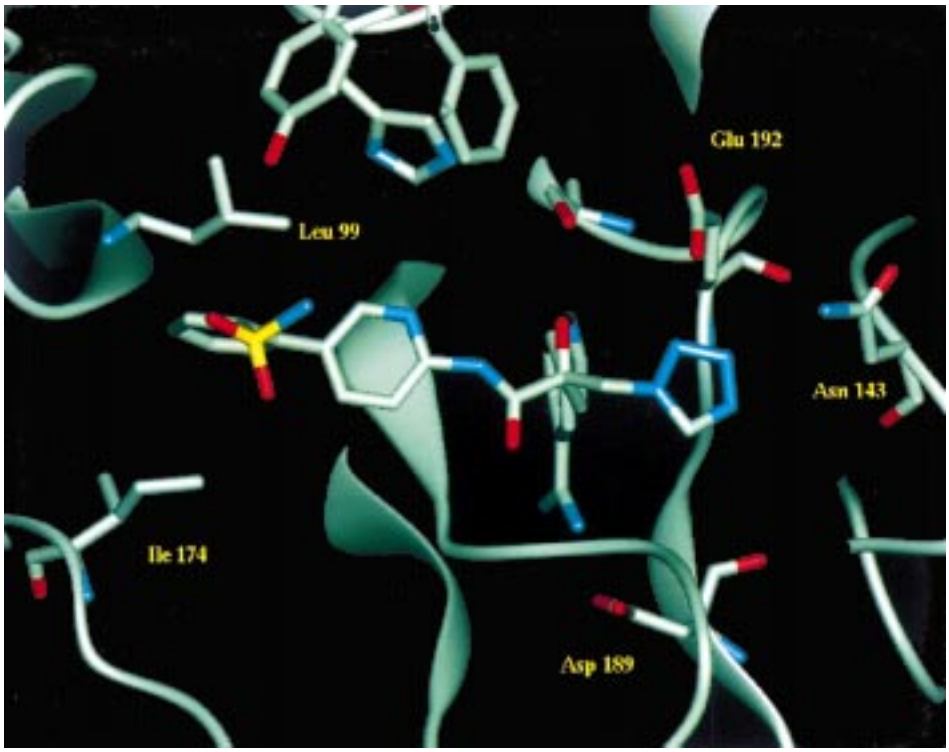


Figure 1. Crystal structure of SM084 in thrombin active site.

Table 2, high selectivity against a panel of serine proteases including plasmin, tPA, activated protein C, and FI was observed for these compounds.

The same overall trends observed in the biphenylsulfonamide series (Table 2) were also seen in the pyrimidinylphenylsulfonamide series (Table 3) and the pyridinylphenylsulfonamide series (Table 4). However, the methoxymethyl and ethoxymethyl compounds were somewhat less potent in the pyrimidinyl series. In most cases, the pyrimidinyl compounds showed improved selectivity for FXa over trypsin, while the pyridinyl compounds showed enhanced potency compared to the biphenyl derivatives.[17]

We have previously demonstrated that the (−)-isomer is more active than the corresponding (+)-isomer for a given set of enantiomers.[17] Therefore, the (−)-enantiomers of several of the more potent compounds from Tables 2−4 were prepared and evaluated (Table 5). Compound **70** with an ethoxymethyl side chain shows low nanomolar affinity for FXa, while the pyridyl analogue **71** exhibits a 4-fold enhancement in improved FXa affinity. Biphenylsulfonamide **72** (SK549) has a K_i of 0.52 nM for FXa and good selectivity for FXa compared to thrombin and trypsin. Alkylation of the sulfonamide resulted in a 2−4-fold reduction in FXa affinity (**73**, **74**). While *o*-methyl substitution resulted in a modest decrease in FXa affinity (**75**), *o*-chloro substitution resulted in a 3-fold enhancement of FXa potency (**77**). Both the pyrimidyl (**78**) and pyridinyl (**79**, SM084) analogues of SK549 are more potent FXa

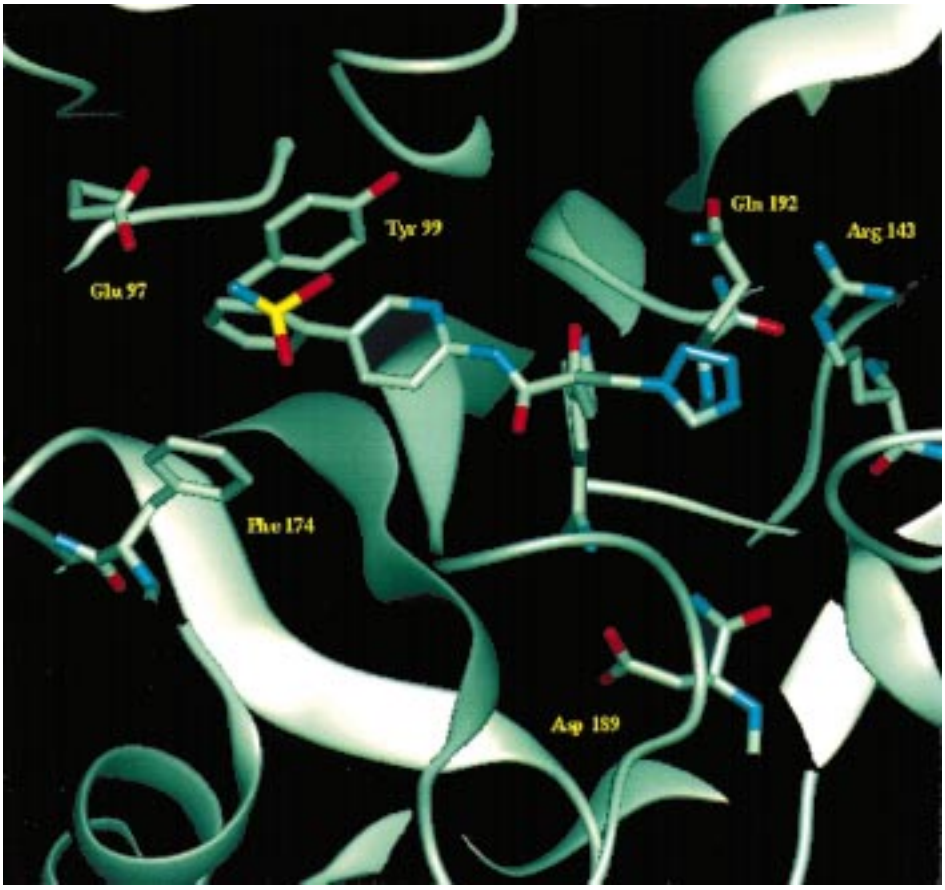


Figure 2. SM084 modeled in the FXa active site.

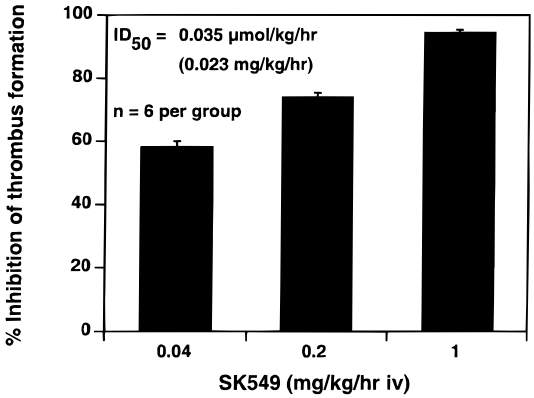


Figure 3. Antithrombotic effect of SK549 in the rabbit A-V shunt thrombosis model.

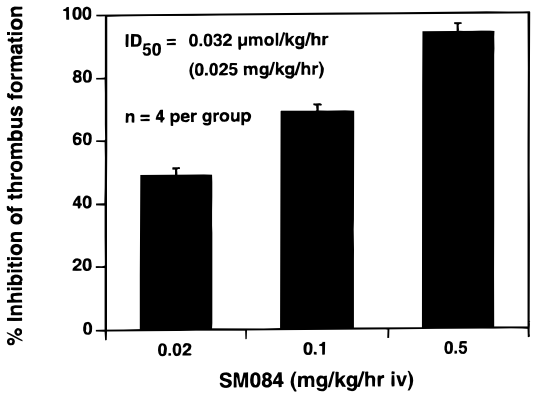


Figure 4. Antithrombotic effect of SM084 in the rabbit A-V shunt thrombosis model.

inhibitors than SK549. This is in agreement with the trend previously observed for the corresponding esters.[17]

To determine the binding mode of these inhibitors, an X-ray crystal structure of **79** (SM084) complexed to human thrombin was obtained (*R*-factor 0.194, crystals diffracted to 2.2 Å, methods identical to those used in Weber et al.).[21] As anticipated, it was observed that the inhibitor binds to thrombin by filling the S_1 specificity pocket and interacting with the nonprimed portion of the active site (Figure 1), with the benzamidine group occupying the S_1 pocket and interacting with the side chain of Asp^{189} in a bidentate manner (2.7 Å). The biaryl moiety is located in the S_2/S_3 site wherein the terminal phenyl ring forms an edge-to-face interaction with Trp^{215} of thrombin. There are no hydrogen bonds observed between the protein and the isoxazoline ring of the inhibitor. The tetrazole group is found in close proximity to the side chain of Glu^{192} (3.4 Å). It is not surprising that **79** (SM084) and related analogues show relatively poor thrombin activity since it is unlikely that either Glu^{192} or the tetrazole nitrogens are protonated; there-

Table 6. Pharmacokinetic and Efficacy Data of Selective Compounds in Rabbits[a]

compd	FXa K_i (nM)	CL (L/h/kg)	$t_{1/2}$ (h)	ID_{50} (µmol/kg/h)
70	3.3	0.49	0.63	0.74
71	0.8	1.30	0.60	0.80
77	0.15	0.80	0.80	0.25
72 (SK549)	0.52	0.3	0.6	0.035
79 (SM084)	0.11	0.5	0.7	0.032

[a] FXa K_i's were obtained using purified human enzyme. PK data were determined in rabbits at 5 mg/kg by LC/MS/MS. ID_{50} was determined in rabbits using A-V shunt method.

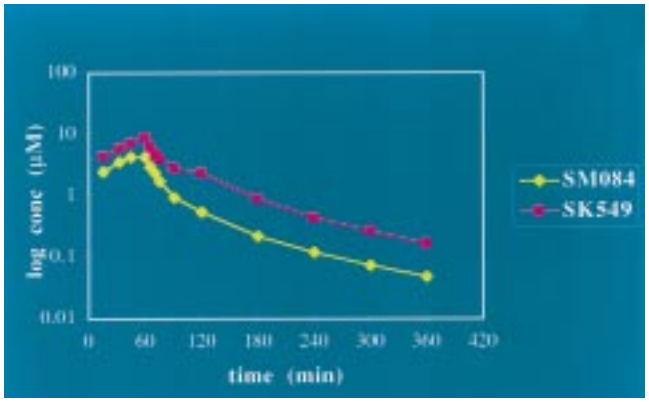


Figure 5. PK profiles of SK549 and SM084 in dogs at 5 mg/kg.

fore they are not likely to engage in a hydrogen bond. This crystal structure of **79** (SM084) revealed that the (−)-enantiomer, or the more potent enantiomer, is in the *S*-configuration. When **79** (SM084) in this same orientation was modeled in the FXa active site[22] (Figure 2) and then refined further by energy minimization,[23] the tetrazole group was found to be in close proximity to residues Gln^{192} and Arg^{143}. The observed increase in potency for **79** (SM084) and other tetrazole-containing compounds is likely to be because the tetrazole can engage in hydrogen-bonding interactions with Gln^{192} and/or possibly Arg^{143}.

In vivo efficacy for selected compounds were studied in the rabbit A-V shunt thrombosis model.[17] The compounds were administered by intravenous infusion, and the antithrombotic effect was then expressed as the ID_{50}, a dose which reduced the clot weight by 50%. As shown in Table 6, **72** (SK549) and **79** (SM084) have similar efficacy and are more potent than the other three compounds. Both compounds inhibited thrombus formation in a dose-dependent fashion, and the ID_{50}'s for inhibition of thrombus formation in this model for **72** (SK549) and **79** (SM084) were found to be 0.035 and 0.032 μmol/kg/h, respectively (Figures 3 and 4). Recombinant tick coagulant peptide (rTAP) was also studied in this model, and the ID_{50} was found to be 0.009 μmol/kg/h. SK549 and SM084 are only 3-fold less potent than rTAP in the rabbit A-V shunt thrombosis model.

The pharmacokinetic data for representative compounds studied in rabbits dosed intravenously at 5 mg/kg given as a 1-h infusion are also shown in Table 6. Compound **72** (SK549) bearing a tetrazole side chain shows the lowest clearance of the compounds evaluated. In general, the biphenyl compounds **70** and **72** (SK549) show lower clearance than the corresponding pyridyl analogues **71** and **79** (SM084). Although the chloro analogue **77** has higher affinity than SK549, unfortunately it is cleared more rapidly which is in agreement with it being less potent in the rabbit A-V shunt model.

The pharmacokinetic profiles of both SK549 and SM084 were further studied in dogs dosed intravenously at 5 mg/kg given as a 1-h infusion (Figure 5). The total body clearance of SM084 (1.3 L/h/kg) was greater than that of SK549 (0.7 L/h/kg), and the half-life of SK549 was slightly longer (1.6 h) than that of SM084 (1.1 h). For both compounds, the total body clearances in dogs was approximately twice that observed in rabbits on a weight-normalized basis. Overall, SK549 has a more favorable pharmacokinetic profile than SM084 due to its lower total body clearance and greater residence time.

Conclusion

We have replaced the labile ester functionality in SF303 and prepared compounds with improved in vitro and in vivo potency. Among these compounds, SK549 has subnanomolar affinity and good selectivity for FXa compared to thrombin and trypsin. SK549 has relatively low clearance in both rabbits and dogs with β-phase half-lives of 0.6 and 1.6 h, respectively. SK549 also showed potent antithrombotic effect in the rabbit arterio-venous thrombosis model and is only 3-fold less potent than rTAP in this model.

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 7.0. The compounds were run at 10, 3.16, 1.0, 0.316, 0.1, 0.0316, 0.01, and 0.00316 μM. Plates were read for 30 min at 405 nm. Rates were 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: $K_i = 1000 \times$ inhibitor concentration/$(((K_m + S) - S \times \mathrm{ACT})/(\mathrm{ACT} \times K_m) - 1)$, where S is substrate concentration and ACT is % enzyme activity for inhibitor. All compounds were tested in duplicate studies and were compared with the same internal standards. These assays are described in detail in refs 24 and 25.

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 (i.d. = 7.9 mm) and an inner piece of 2.5-cm tubing (i.d. = 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. Forty minutes later, the shunt was disconnected and the silk thread covered with thrombus was weighed. The compound or saline vehicle was given as continuous iv infusion 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.

Physical Methods. IR spectra were determined with a Perkin-Elmer 1600 series FTIR. NMR spectra were determined with a Varian VXR-300a. Microanalyses were performed by Quantitative Technologies Inc. and were within ≤0.4% of the calculated values. Mass spectra were obtained on a HP 5988A MS/HP particle beam interface. Chromatography was done using EM Science silica gel 60. HPLC was performed on a Rainin Dynamax SD200 using a C_{18} reverse-phase column with CH_3CN/H_2O (containing 0.05% TFA) as mobile phase.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Trifluoroacetic Acid Salt (±) (42). 5-Isoxazoleacetic acid, 3-[3-(aminoiminomethyl)phenyl]-5-[[[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, methyl ester, trifluoroacetic acid salt (±) (SF303)[6] (0.20 g, 0.37 mmol) was dissolved in 10 mL of methanol. To it was added aqueous LiOH (0.75 mL of 1 M solution). The mixture

was stirred at room temperature for 1 h. The solvent was removed. The residue was purified by HPLC (C18 reverse phase) eluted with 0.05% TFA in H_2O/CH_3CN to give 146.6 mg of the benzamidine TFA salt (62%). MS (ES^+):: 522.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.20 (m, 2H); 3.78−4.02(m, 2H); 7.21 (s, 1H); 7.30 (t, 2H); 7.57 (m, 4H); 7.72 (m, 1H); 7.89 (d, 1H); 8.01 (d, 1H); 8.08 (s, 1H); 8.10 (s, 2H); 9.12 (bs, 2H); 9.42 (bs, 2H); 10.27 (s, 1H). Anal. TFA salt ($C_{27}H_{24}F_3N_5O_8S$) C, H, N.

3-(3-Cyanophenyl)-5-benzylisoxazoline-5-carboxylic Acid (±) (4a). Zinc dust (3.65 g, 55.8 mmol) was placed in a three-neck round-bottom flask together with 30 mL of DMF. Dibromoethane (0.41 mL, 4.74 mmol) was added. The mixture was heated at 65 °C under N_2 for 1 min and then cooled to room temperature. To it was added chlorotrimethylsilane (0.50 mL, 4.0 mmol). The resulting mixture was stirred at room temperature for 15 min and then warmed to 55 °C. A solution of iodobenzene (2.55 mL, 22.3 mmol) in 30 mL of DMF was added slowly over 40 min. The reaction mixture was stirred at 55 °C under N_2 for 22 h. The mixture was cooled to room temperature and cannulated to a solution of LiCl (1.89 g, 44.68 mmol) and CuCN (2.0 g, 22.3 mmol) in 30 mL of THF at −70 °C. The mixture was warmed to 0 °C, stirred at 0 °C for 15 min, and then cooled back to −70 °C, and methyl 2-(bromomethyl)acrylate (**8**) (2.16 mL, 18.0 mmol) was added. The mixture was allowed to warm to 0 °C and stirred at 0 °C for 2 h. The reaction mixture was then quenched with saturated aqueous NH_4Cl. The THF was removed, and the mixture was extracted with EtOAc. The EtOAc solution was washed with brine, dried over $MgSO_4$, and concentrated to a light-yellow oil. The resulting oil and 3-cyanobenzaldehyde oxime (**2**)[6] (2.92 g, 20.0 mol) were dissolved in 100 mL of THF. Bleach (75 mL of 0.67 M aqueous solution) was added dropwise at room temperature under N_2. The mixture was stirred for 2 h, and the solvent was removed. The residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to a yellow solid. MS (CI-NH_3): 321 $(M + H)^+$; 338 $(M + NH_4)^+$. The above solid was dissolved in 50 mL of THF, and LiOH (30 mL of 1 M aqueous solution) was added. The mixture was stirred at room temperature under N_2 for 1 h. The solvent was removed, and the residue was diluted with H_2O. It was extracted with EtOAc, and this EtOAc solution was discarded. The resulting aqueous solution was acidified with concentrated HCl and extracted with EtOAc. This EtOAc solution was washed with brine, dried over $MgSO_4$, and concentrated to give a light-yellow solid (2.70 g, 40%). MS (ES^+): 307.3 $(M + H)^+$. ^1H NMR ($CDCl_3$): δ 3.20−3.80 (m, 4H); 7.18−7.28 (m, 5H); 7.40−7.88 (m, 4H).

Methyl 3-(3-Cyanophenyl)-5-(methoxymethyl)isoxazoline-5-carboxylate (±) (12). Methyl (2-bromomethyl)acrylate (**8**) (1.04 g, 5.82 mmol) was dissolved in 20 mL of petroleum ether, and K_2CO_3 (0.88 g, 6.40 mmol) was added, followed by NaOMe (1.3 mL of 25 wt % NaOMe in MeOH). The mixture was stirred at room temperature under N_2 for 12 h. The solid was filtered off. The filtrate was concentrated to a colorless oil. This oil and 3-cyanobenzaldehyde oxime (**2**) (0.85 g, 5.82 mol) were dissolved in 30 mL of THF. Bleach (15 mL of 0.67 M aqueous solution) was added dropwise at room temperature under N_2. The mixture was stirred for 3 h, and the solvent was removed. The residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification by chromatography on silica gel with 5% EtOAc in CH_2Cl_2 afforded 0.57 g of the desired product (36%). MS (CI-NH_3): 275 $(M + H)^+$; 292 $(M + NH_4)^+$. ^1H NMR ($CDCl_3$): δ 3.44 (s, 3H); 3.51−3.85 (m, 4H); 3.84 (s, 3H); 7.53 (t, 1H); 7.71 (d, 1H); 7.92 (s, 1H); 7.93 (d, 1H).

3-(3-Cyanophenyl)-5-(methoxymethyl)isoxazoline-5-carboxylic Acid (±) (13). Methyl 3-(3-cyanophenyl)-5-(methoxymethyl)isoxazoline-5-carboxylate (±) (**12**) (0.57 g, 2.08 mmol) was dissolved in 50 mL of THF, and LiOH (05.0 mL of 0.5 M aqueous solution) was added. The mixture was stirred at room temperature under N_2 for 30 min, and an additional 2.0 mL of LiOH was added. The mixture was stirred for 5 min. The solvent was removed; the residue was diluted with H_2O and then acidified with concentrated HCl. Following extraction, the EtOAc solution was washed with brine, dried over $MgSO_4$, and concentrated to afford a white solid (0.54 g, 100%). ^1H NMR (DMSO-d_6): δ 3.32 (s, 3H); 3.53−3.80 (m, 4H); 7.67 (t, 1H); 7.94 (d, 1H); 8.03 (d, 1H); 8.11 (s, 1H); 13.45 (bs, 1H).

5-Isoxazolecarboxamide, 3-(3-Cyanophenyl)-*N*-[5-[2′-(*tert*-butylaminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(3-methoxymethyl)-, (±) (14). 3-(3-Cyanophenyl)-5-(methoxymethyl)isoxazoline-5-carboxylic acid (±) (**13**) (1.03 g, 3.96 mmol) was refluxed with 30 mL of acetonitrile and 2.9 mL (39.6 mmol) of thionyl chloride for 1 h under N_2. The solvent was removed in vacuo. Residual thionyl chloride was removed by adding toluene and then evaporated to dryness. The resulting solid was dissolved in 30 mL of CH_2Cl_2, and 2′-(*tert*-butylaminosulfonyl)phenyl-4-amino-2-pyridine (**5**) (0.97 g, 3.17 mmol) was added, followed by triethylamine (3.2 mL, 23.76 mmol). The reaction mixture was stirred at room temperature, and the reaction was completed in less than 30 min. The mixture was diluted with CH_2Cl_2, and the solution was washed with water and brine. It was dried over $MgSO_4$ and concentrated. The crude product mixture was chromatographed on silica gel eluted with methylene chloride/ethyl acetate (9:1) to give 0.90 g of the desired product (42%). MS (ES^+): 548.3 $(M + H)^+$. ^1H NMR ($CDCl_3$): δ 1.05 (s, 9H); 3.49 (s, 3H); 3.57−4.01 (m, 5H); 7.28 (s, 1H); 7.50−7.62 (m, 3H); 7.74 (d, 1H); 7.87−7.98 (m, 3H); 8.18 (d, 1H); 8.25 (d, 1H); 8.35 (d, 1H); 9.29 (bs, 1H).

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(methoxymethyl)-, (±) (65). 5-Isoxazolecarboxamide, 3-(3-cyanophenyl)-*N*-[5-[2′-(*tert*-butylaminosulfonyl)-phenyl]-2-pyridinyl]-4,5-dihydro-5-(3-methoxymethyl)-, (±) (**14**) (0.90 g, 1.64 mmol) was refluxed with 15 mL of trifluoroacetic acid under N_2 for 1 h. The TFA was removed in vacuo, and the residue was dried under vacuum to give 1.03 g of white solid. MS (ES^+): 492.2 $(M + H)^+$. This solid was dissolved in 40 mL of $CHCl_3$ and 12 mL of MeOH. The reaction mixture was cooled in an ice bath, and HCl gas was bubbled in for 15 min until the solution was saturated. The mixture was sealed and placed at 0 °C for 12 h. The solvents were removed in vacuo, and the resulting solid was dried under vacuum. The imidate formed above was added with 1.0 g (12.97 mmol) of ammonium acetate and 20 mL of methanol. The mixture was sealed and stirred at room temperature for 12 h. The solvents were removed. The crude benzamidine was purified by HPLC (C18 reverse phase) eluted with 0.05% TFA in H_2O/CH_3CN to give 0.61 g of the benzamidine TFA salt (60%). MS (ES^+): 509.2 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.39 (s, 3H); 3.78−4.03(m, 4H); 7.35−7.45 (m, 3H); 7.60−7.76 (m, 3H); 7.82−7.94 (m, 2H); 8.02−8.16 (m, 4H); 8.35 (bs, 1H); 9.32 (s, 2H); 9.43 (s, 2H); 9.71 (s, 1H). High-resolution MS ($C_{24}H_{24}N_6O_5S$): calcd, 509.1607; found, 509.1611. HPLC purity 99%.

1,7-Dioxa-2-azaspiro[4.4]non-2-en-6-one, 3-(Cyanophenyl)- (17). α-Methylene-γ-butyrolactone (**16**) (1.94 g, 19.8 mmol) and 3-cyanobenzene oxime (**2**) (2.89 g, 19.8 mmol) were added together with 100 mL of THF. Bleach (50 mL of 0.67 M solution) was added dropwise to the above mixture. The resulting mixture was stirred at room temperature under N_2 for 2 h. The THF was removed. The mixture was diluted with water and extracted with EtOAc. The combined organic solution was washed with brine, dried over $MgSO_4$, and concentrated. It was recrystallized from CH_2Cl_2 and hexane to give 3.83 g of a white solid. MS (DCI-NH_3): 260 $(M+NH_4)^+$. ^1H NMR ($CDCl_3$): δ 2.39−2.52 (m, 1H); 2.71−2.80 (m, 1H); 3.38 (d, 1H); 3.90 (d, 1H); 4.43−4.59 (m, 2H); 7.58 (t, 1H); 7.75 (d, 1H); 7.94 (s, 1H); 7.96 (d, 1H).

Methyl 3-(3-Cyanophenyl)-5-(methoxyethyl)isoxazoline-5-carboxylate (±) (18). Compound **17** (1.00 g, 4.12 mmol) was dissolved in 40 mL of anhydrous methanol. To it were added trimethyl orthoformate (0.9 mL, 8.24 mmol) and a catalytic amount (6 drops) of concentrated H_2SO_4. The mixture was refluxed under N_2 for 24 h. The solvent was removed. The residue was dissolved in EtOAc and washed with

saturated $NaHCO_3$ and brine. It was dried over $MgSO_4$ and purified by chromatography on silica gel eluted with 5% EtOAc in CH_2Cl_2 to give 0.67 g of the desired product (56%). MS (ES^+): 289 $(M + H)^+$. ^1H NMR ($CDCl_3$): δ 2.33 (t, 2H); 3.27 (s, 3H); 3.48−3.60 (m, 3H); 3.80−3.88 (m, 4H); 7.55 (t, 1H); 7.72 (d, 1H); 7.90 (s, 1H); 7.92 (d, 1H).

3-(3-Cyanophenyl)-5-(methoxyethyl)isoxazoline-5-carboxylic Acid (±) (19). Methyl 3-(3-cyanophenyl)-5-(methoxyethyl)isoxazoline-5-carboxylate (±) (**18**) (0.48 g, 1.66 mmol) was dissolved in 10 mL of THF. LiOH (2 mL of 1 M aqueous solution) was added. The mixture was stirred at room temperature under N_2 for 0.5 h. The THF was removed. The mixture was diluted with water, acidified with concentrated HCl, and extracted with EtOAc. The combined organic solution was washed with brine, dried over $MgSO_4$, and concentrated to a white solid (0.42 g, 92% yield). ^1H NMR (DMSO-d_6): δ 2.18 (t, 2H); 3.19 (s, 3H); 3.44 (t, 2H); 3.55−3.84 (m, 2H); 7.68 (t, 1H); 7.79 (d, 1H); 8.03 (d, 1H); 8.10 (s, 1H); 13.29 (bs, 1H).

5-Isoxazolecarboxamide, 3-(3-Cyanophenyl)-*N*-[[5-[2′-(*tert*-butylaminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(methoxyethyl)-, (±) (21). 3-(3-Cyanophenyl)-5-(methoxyethyl)isoxazoline-5-carboxylic acid (±) (**19**) (0.40 g, 1.46 mmol) was dissolved in 10 mL of DMF. (Benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (0.65 g, 1.46 mmol) was added followed by 2′-(*tert*-butylaminosulfonyl)-4-amino[1,1′-biphenyl] (**20**) (0.44 g, 1.46 mmol) and triethylamine (0.31 mL, 2.19 mmol). The mixture was heated at 50 °C under N_2 for 24 h. The reaction mixture was cooled and poured into water. It was then extracted with EtOAc. The combined organic solution was washed with brine, dried over $MgSO_4$, and concentrated. Purification by chromatography (silica gel, 5−10% EtOAc in CH_2Cl_2) gave 0.30 g of the desired product (37%). MS (ES^+): 561.5 $(M + H)^+$. ^1H NMR ($CDCl_3$): δ 1.01 (s, 9H); 2.30−2.55 (m, 2H); 3.30 (s, 3H); 3.60−3.94 (m, 5H), 7.25−7.34 (m, 2H), 7.42−7.60 (m, 5H), 7.67−7.76 (m, 2H); 7.91 (d, 1H); 7.98 (s, 1H); 8.17 (d, 1H); 8.76 (s, 1H).

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(methoxyethyl)-, Trifluoroacetic Acid Salt (±) (22). 5-Isoxazolecarboxamide, 3-(3-cyanophenyl)-*N*-[2′-(*tert*-butylaminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(methoxyethyl)-, (±) (**21**) (0.30 g, 0.54 mmol) was dissolved in anhydrous $CHCl_3$ (20 mL) and anhydrous CH_3OH (5 mL). It was cooled to 0 °C, and HCl gas was bubbled in until the solution was saturated (about 15 min). The reaction mixture was sealed and placed in a refrigerator for 12 h. The solvents were removed. The resulting solid was dried under vacuum. The imidate formed above was dissolved in 20 mL of anhydrous CH_3OH. Ammonium acetate (0.41 g, 5.4 mmol) was added. The mixture was sealed and stirred at room temperature for 12 h. The solvent was removed. The solid was dissolved in CH_3CN/H_2O/TFA and purified by HPLC (C_{18} reverse-phase column, 0.5% TFA in H_2O/CH_3CN) to give the desired TFA salt (0.16 g). MS (ES^+): 523.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 2.22−2.40 (m, 2H); 3.21 (s, 3H); 3.42−3.58 (m, 2H); 3.60−4.03 (q, 2H); 7.18−7.32 (m, 3H), 7.49−7.60 (m, 2H), 7.64−7.76 (m, 3H); 7.86 (d, 1H); 7.97 (d, 1H); 8.01−8.08 (m, 2H); 9.12 (s, 2H); 9.38 (s, 2H), 10.13 (s, 1H). Anal. ($C_{26}H_{27}N_5O_5S$·TFA·0.5H_2O) C, H, N.

3-(3-Cyanophenyl)-5-(ethylthiomethyl)isoxazoline-5-carboxylic Acid (±) (29). Methyl 2-(bromomethyl)acrylate (**8**) (5.00 g, 27.9 mmol) was dissolved in 150 mL of petroleum ether. NaSEt (2.61 g, 27.9 mmol) was added. The mixture was stirred at room temperature under N_2 for 12 h. The solid was filtered off. The filtrate was concentrated to methyl 2-(ethylthiomethyl)acrylate (**23**) (4.03 g, 90%). ^1H NMR ($CDCl_3$): δ 1.22 (t, 3H); 2.48 (q, 2H); 3.38 (s, 2H); 3.77 (s, 3H); 5.63 (s, 1H); 6.19 (s, 1H).

Compound **23** (2.99 g, 18.1 mmol) and 3-cyanobenzaldehyde chloroxime (**24**) (3.27 g, 18.1 mmol) were dissolved in CH_2Cl_2 (150 mL). To the above mixture was added dropwise a solution of triethylamine (2.52 mL, 18.1 mmol) in 50 mL of CH_2Cl_2. The resulting mixture was stirred at room temperature under N_2 for 0.5 h. The mixture was washed with water and brine, dried over $MgSO_4$, and concentrated to a light-yellow oil (7.40 g). This oil was dissolved in 150 mL of THF. LiOH (27 mL of 1 M aqueous solution) was added. The mixture was stirred at room temperature under N_2 for 0.5 h. The THF was removed. The mixture was diluted with water and extracted with EtOAc. The resulting aqueous solution was acidified with concentrated HCl and then extracted with EtOAc. This EtOAc solution was washed with brine, dried over $MgSO_4$, and concentrated to a light-yellow solid (4.14 g, 79%). MS (DCI-NH_3): 308 $(M + NH_4)^+$. ^1H NMR (DMSO-d_6): δ 1.12 (t, 3H); 2.59 (q, 2H); 3.08 (q, 2H); 3.51−3.89 (q, 2H); 7.74 (t, 1H); 7.92 (d, 1H); 8.01 (d, 1H); 8.10 (s, 1H), 13.48 (bs, 1H).

5-Isoxazolecarboxamide, 3-(3-Cyanophenyl)-*N*-[5-[2′-(*tert*-butylaminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethylthiomethyl)-, (±) (30). 3-(3-Cyanophenyl)-5-(ethylthiomethyl)isoxazoline-5-carboxylic acid (±) (**29**) (0.50 g, 1.72 mmol) was dissolved in 10 mL of DMF. BOP (1.14 g, 2.58 mmol) was added followed by 2′-[(*tert*-butylaminosulfonyl)-phenyl]-4-aminopyridine (0.80 g, 2.62 mmol) and triethylamine (0.36 mL, 2.58 mmol). The mixture was heated at 50 °C under N_2 for 12 h. The reaction mixture was cooled, poured into water, and then extracted with EtOAc. The combined organic solution was washed with brine and dried over $MgSO_4$. It was concentrated and purified by chromatography (silica gel, 0−10% EtOAc in CH_2Cl_2) to give 0.43 g of the off-white solid (43%). MS (ES^+): 578.4 $(M + H)^+$. ^1H NMR ($CDCl_3$): δ 1.05 (s, 9H); 1.25 (t, 3H); 2.76 (q, 2H); 3.18−3.37 (m, 2H), 3.62 (bs, 1H), 3.80 (q, 2H), 7.57 (m, 3H); 7.75 (d, 1H); 7.90 (t, 2H); 7.95 (s, 1H); 8.16 (d, 1H); 8.23 (d, 1H); 8.37 (s, 1H); 9.25 (s, 1H).

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(methoxyethyl)-, Trifluoroacetic Acid Salt, (±) (67). 5-Isoxazolecarboxamide, 3-(3-cyanophenyl)-*N*-[5-[2′-(*tert*-butylaminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethylthiomethyl)-, (±) (**30**) (0.43 g, 0.74 mmol) was refluxed with 10 mL of trifluoroacetic acid under N_2 for 1 h. The TFA was removed in vacuo; the residue was dissolved in CH_2Cl_2 and precipitated with ether to give 0.56 g of an off-white solid. MS (ES^+): 522.4 $(M + H)^+$. This solid was dissolved in 40 mL of $CHCl_3$ and 10 mL of MeOH. The reaction mixture was cooled in an ice bath, and HCl gas was bubbled in for 15 min until the solution was saturated. The mixture was sealed and placed at 0 °C for 12 h. The solvents were removed in vacuo, and the resulting solid was dried under vacuum. The imidate formed above was added with 0.35 g (4.54 mmol) of ammonium acetate and 20 mL of methanol. The mixture was sealed and stirred at room temperature for 12 h. The solvents were removed. The crude benzamidine was purified by HPLC (C18 reverse phase) eluted with 0.05% TFA in H_2O/CH_3CN to give 0.26 g of the benzamidine TFA salt (46%). MS (ES^+): 539.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.21 (t, 3H); 2.70 (q, 2H); 3.30−3.42 (q, 2H), 3.78−4.05 (q, 2H), 7.38 (d, 1H); 7.42 (s, 2H); 7.63 (t, 2H); 7.74 (t, 1H); 7.85 (m, 2H); 8.10 (m, 3H); 8.33 (s, 1H); 9.02 (s, 2H). 9.41 (s, 2H); 9.91 (s, 1H). Anal. ($C_{25}H_{26}N_6O_4S_2$·TFA) C, H, N.

5-Isoxazolecarboxamide, 3-(3-Cyanophenyl)-*N*-[5-[2′-(*tert*-butylaminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethylsulfonylmethyl)-, (±) (28). 5-Isoxazolecarboxamide, 3-(3-cyanophenyl)-*N*-[5-[2′-(*tert*-butylaminosulfonyl)-phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethylthioymethyl)-, (±) (**30**) was oxidized with oxone in methanol to give the title compound. MS (ES^+): 610.4 $(M + H)^+$. ^1H NMR ($CDCl_3$): δ 1.08 (s, 9H); 1.45 (t, 3H); 3.38 (m, 2H); 3.73 (m, 2H), 3.85−3.95 (m, 3H), 4.23 (d, 1H), 7.37 (d, 1H); 7.50−7.65 (m, 3H); 7.77 (d, 1H); 7.85−7.94 (m, 2H); 7.99 (s, 1H); 8.18 (d, 1H); 8.25 (d, 1H); 8.38 (s, 1H); 9.20 (s, 1H).

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethylsulfonylmethyl)-, Trifluoroacetic Acid Salt, (±) (68). The title compound was prepared by the procedure described for compound **67** from 5-isoxazolecarboxamide, 3-(3-cyanophenyl)-*N*-[5-[2′-(*tert*-butylaminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethylsulfonylmethyl)-, (±) (**28**).

MS (ES^+): 571.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.29 (t, 3H); 3.25 (q, 2H); 3.98−4.27 (m, 4H), 7.38 (d, 1H); 7.43 (s, 2H); 7.60−7.78 (m, 3H); 7.82−7.94 (m, 2H); 8.02 (m, 3H); 8.14 (s, 1H); 8.35 (s, 1H); 9.19 (s, 2H). 9.48 (s, 2H); 9.91 (s, 1H). Anal. ($C_{25}H_{26}N_6O_6S_2 \cdot 3TFA \cdot 2.3H_2O$) C, H, N.

3-(3-Cyanophenyl)-5-carbomethoxy-5-(1*H*-tetrazol-1-ylmethyl)isoxazoline (±) (34). Methyl 2-(bromomethyl)-acrylate (**8**) (2.5 g, 14.0 mmol) was added to a mixture of 1*H*-tetrazole (0.89 g, 14.0 mmol) and K_2CO_3 in 50 mL of DMF. The mixture was stirred at room temperature under N_2 for 12 h. The mixture was poured into water and extracted with EtOAc. The combined organic solution was washed with brine, dried over $MgSO_4$, and then concentrated to give 1.63 g of methyl 2-(1*H*-tetrazol-ylmethyl)acrylate. This crude product mixture and 3-cyanobenzaldehyde oxime (1.42 g, 9.69 mmol) were dissolved in THF (50 mL). Bleach (25 mL of 0.67M solution) was added dropwise to the above mixture. The resulting mixture was stirred at room temperature under N_2 for 3 h. The THF was removed. The mixture was diluted with water and extracted with EtOAc. The combined organic solution was washed with brine, dried over $MgSO_4$, and concentrated. It was purified by chromatography (silica gel, 5−15% EtOAc in CH_2Cl_2) to give 1.61 g of the desired product and 0.50 g of the regioisomer 3-(3-cyanophenyl)-5-carbomethoxy-5-(1*H*-tetrazol-2-ylmethyl)isoxazoline (**35**). MS (ES^+): 313.1 $(M + H)^+$. ^1H NMR (DMSO-d_6): d 3.78 (s, 3H); 3.80−4.10 (q, 2H); 5.09−5.20 (q, 2H); 7.68 (t, 1H); 7.98 (d, 1H); 8.07 (s, 1H); 9.45 (s, 1H).

3-(3-Cyanophenyl)-5-(1*H*-tetrazol-1-ylmethyl)isoxazoline-5-carboxylic Acid (±) (36). LiOH (12 mL of 0.5 M aqueous solution) was added to a stirring solution of 3-(3-cyanophenyl)-5-carbomethoxy-5-(1*H*-tetrazol-1-ylmethyl)isoxazoline (**34**) (1.60 g, 5.12 mmol) in 75 mL of THF. The mixture was stirred at room temperature under N_2 for 1 h. The THF was removed, and the mixture was diluted with water, acidified with concentrated HCl, and extracted into EtOAc. The combined organic solution was washed with brine, dried over $MgSO_4$, and concentrated to give a white solid (1.54 g). MS (ES^+): 299 $(M + H)^+$. ^1H NMR (DMSO-d_6): d 3.70−4.02 (q, 2H); 5.02−5.18 (q, 2H); 7.67 (t, 1H); 7.97 (d, 1H); 8.04 (s, 1H); 9.42 (s, 1H).

5-Isoxazolecarboxamide, 3-(3-Cyanophenyl)-*N*-[[5-[2′-(*tert*-butylaminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, (±) (37). 3-(3-Cyanophenyl)-5-(1*H*-tetrazol-1-ylmethyl)isoxazoline-5-carboxylic acid (±) (**36**) (0.55 g, 1.84 mmol) was refluxed with CH_3CN (20 mL) and $SOCl_2$ (1.34 mL, 18.4 mmol) under N_2 for 1 h. The solvent was removed, and residual $SOCl_2$ was removed by dissolving in toluene and then removing the solvent to dryness. The resulting solid was dissolved in CH_2Cl_2 (20 mL). 2′-(*tert*-Butylaminosulfonyl)-4-amino[1,1′-biphenyl] (0.28 g, 0.92 mmol) was added followed by Et_3N (1.5 mL, 18.4 mmol). The mixture was stirred at room temperature under N_2 for 0.5 h. It was diluted with CH_2Cl_2 and washed with water and brine. It was dried over $MgSO_4$ and concentrated. The desired product was then purified by chromatography (silica gel, 20% EtOAc in CH_2Cl_2) to give 0.59 g of an off-white solid. MS (ES^+): 585.2 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.01 (s, 9H); 3.90−4.10 (q, 2H); 5.08−5.16 (q, 2H); 6.70 (s, 1H), 7.24−7.38 (m, 3H), 7.50−7.77 (m, 5H), 7.98−8.03 (m, 3H); 8.12 (s, 1H); 9.42 (s, 1H).

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (±) (55). 5-Isoxazolecarboxamide, 3-(3-cyanophenyl)-*N*-[2′-(*tert*-butylaminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)- (±) (**37**) (0.41 g, 0.70 mmol) was dissolved in anhydrous $CHCl_3$ (20 mL) and anhydrous CH_3OH (5 mL). The mixture was cooled at 0 °C, and HCl gas was bubbled in until the solution was saturated (about 15 min). The reaction mixture was sealed and placed in a refrigerator for 12 h. The solvents were removed. The resulting solid was dried under vacuum. The imidate formed above was dissolved in 20 mL of anhydrous CH_3OH. Ammonium acetate (0.55 g, 7.0 mmol) was added. The mixture was sealed and stirred at room temperature for 12 h. The solvent was removed. The solid was dissolved in CH_3CN/H_2O/TFA and purified by HPLC (C_{18} reverse-phase column, 0.5% TFA in H_2O/CH_3CN) to give the desired TFA salt (0.15 g). MS (ES^+): 546.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.89−4.16 (q, 2H); 5.13−5.31 (q, 2H); 7.22−7.48 (m, 5H), 7.52−7.78 (m, 5H), 7.91 (d, 1H); 8.00−8.08 (m, 3H); 9.12 (s, 2H); 9.41 (s, 2H); 9.43 (s, 1H). Anal. ($C_{25}H_{23}N_9O_4S \cdot 1.5TFA \cdot H_2O$) C, H, N.

The following compounds were prepared by the same methods described above using appropriate starting materials.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-, Trifluoroacetic Acid Salt (±) (50). MS (ES^+): 464.2 $(M + H)^+$. ^1H NMR (CH_3OH-d_4): δ 3.84 (dd, 2H), 5.40 (dd, 1H), 7.34 (d, 1H), 7.40 (d, 2H), 7.52 (t, 1H), 7.58 (t, 1H), 7.66 (d, 2H), 7.72(d, 1H), 7.86 (d, 1H), 8.08 (d, 2H), 8.16 (d, 1H).

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-methyl-, Trifluoroacetic Acid Salt (±) (51). MS (ES^+): 478.2 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.76 (s, 3H); 3.54 (d, 1H); 4.09 (d, 1H); 7.22−7.38 (m, 4H); 7.52−7.65 (m, 2H), 7.68−7.80 (m, 2H), 7.91 (d, 1H); 7.99−8.12 (m, 3H); 9.38 (s, 2H); 9.44 (s, 2H); 10.21 (s, 1H). Anal. ($C_{24}H_{23}N_5O_4S \cdot 1.5TFA \cdot 0.5H_2O$) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(trifluoromethyl)-, Trifluoroacetic Acid Salt (±) (52). MS (ES^+): 532.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 4.36 (q, 2H); 7.25 (bs, 2H); 7.30 (d, 1H), 7.38 (d, 2H), 7.59 (m, 2H); 7.78 (m, 3H); 7.97 (d, 1H); 8.01 (d, 1H); 8.15 (d, 1H); 8.19 (s, 1H); 9.15−9.41 (bs, 4H); 10.02 (s, 1H). High-resolution MS ($C_{24}H_{20}F_3N_5O_4S$): calcd, 532.1266; found, 532.1288. HPLC purity 98%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(methoxymethyl)-, Trifluoroacetic Acid Salt (±) (44). MS (ES^+): 508.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.35 (s, 3H); 3.60−3.80 (m, 2H); 3.90−3.99 (m, 2H); 7.22−7.38 (m, 4H); 7.52−7.64 (m, 2H), 7.69−7.82 (m, 2H), 7.90 (d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.12 (s, 2H); 9.42 (s, 2H); 10.17 (s, 1H). Anal. ($C_{25}H_{25}N_5O_5S \cdot 1.5TFA$) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(ethoxymethyl)-, Trifluoroacetic Acid Salt (±) (45). MS (ES^+): 522.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.12 (t, 3H); 3.55−4.02 (m, 6H); 7.22−7.38 (m, 4H); 7.52−7.64 (m, 2H), 7.69−7.82 (m, 2H), 7.90 (d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.18 (s, 2H); 9.41 (s, 2H); 10.16 (s, 1H). Anal. ($C_{26}H_{27}N_5O_5S \cdot TFA \cdot H_2O$) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(*n*-propoxymethyl)-, Trifluoroacetic Acid Salt (±) (46). MS (ES^+): 536.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 0.85 (t, 3H); 1.50 (q, 2H); 3.45−4.02 (m, 6H); 7.20−7.37 (m, 4H); 7.52−7.65 (m, 2H), 7.70−7.80 (m, 2H), 7.90 (d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.04 (s, 2H); 9.42 (s, 2H); 10.17 (s, 1H). High-resolution MS ($C_{27}H_{29}F_3N_5O_5S$): calcd, 536.1968; found, 536.1965. HPLC purity 97%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(*i*-propoxymethyl)-, Trifluoroacetic Acid Salt (±) (47). MS (ES^+): 536.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.15 (t, 6H); 1.50 (q, 2H); 3.60−4.02 (m, 5H); 7.20−7.37 (m, 4H); 7.52−7.65 (m, 2H), 7.70−7.80 (m, 2H), 7.90 (d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.04 (s, 2H); 9.42 (s, 2H); 10.17 (s, 1H). Anal. ($C_{27}H_{29}N_5O_5S \cdot 1.3TFA \cdot 2H_2O$) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(*n*-butoxymethyl)-, Trifluoroacetic Acid Salt (±) (48). MS (ES^+): 550.5 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 0.84 (t, 3H); 1.30 (m, 2H); 1.49 (m, 2H); 3.48−4.01 (m, 6H); 7.20−7.37 (m, 4H); 7.52−7.65 (m, 2H), 7.70−7.80 (m, 2H), 7.90

(d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.08 (s, 2H); 9.42 (s, 2H); 10.17 (s, 1H). Anal. ($C_{28}H_{29}N_9O_4S$·1.1TFA·0.5H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(*i*-pentoxymethyl)-, Trifluoroacetic Acid Salt (±) (49). MS (ES^+): 564.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.03 (d, 6H); 1.40 (m, 2H); 1.63 (m, 1H); 3.55−4.02 (m, 6H); 7.22−7.38 (m, 4H); 7.52−7.64 (m, 2H), 7.69−7.82 (m, 2H), 7.90 (d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.18 (s, 2H); 9.41 (s, 2H); 10.16 (s, 1H). High-resolution MS ($C_{29}H_{33}N_5O_5S$): calcd, 564.2281; found, 564.2274. HPLC purity 100%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(ethylsulfonylmethyl)-, Trifluoroacetic Acid Salt (±) (43). MS (ES^+): 570.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.27 (t, 3H); 3.28 (m, 2H); 3.92−4.14 (m, 4H); 7.22−7.38 (m, 4H); 7.52−7.64 (m, 2H), 7.69−7.82 (m, 2H), 7.90 (d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.21 (s, 2H); 9.43 (s, 2H); 10.15 (s, 1H). Anal. ($C_{26}H_{27}N_5O_6S_2$·TFA·H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-benzyl-, Trifluoroacetic Acid Salt (±) (53). MS (ES^+): 554.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.30−3.60 (m, 4H); 4.02 (d, 2H); 7.20−7.38 (m, 9H); 7.52−7.74 (m, 4H), 7.87 (d, 1H); 7.98−8.07 (m, 3H); 9.12 (s, 2H); 9.39 (s, 2H); 10.13 (s, 1H). Anal. ($C_{30}H_{27}N_5O_4S$·1.3TFA·2.5H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-triazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (±) (54). MS (ES^+): 545.1 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.87−4.05 (q, 2H); 4.85−4.99 (q, 2H); 7.23 (s, 2H); 7.30 (t, 1H), 7.35 (d, 2H); 7.59 (m, 2H); 7.72 (m, 3H); 7.90 (d, 1H); 7.99 (s, 1H); 8.02 (m, 1H); 8.04 (s, 2H); 8.56 (s, 1H); 9.15 (s, 2H); 9.41 (s, 2H); 10.18 (s, 1H). Anal. ($C_{26}H_{24}N_8O_4S$·1.5TFA) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-2-ylmethyl)-, Trifluoroacetic Acid Salt (±) (56). MS (ES^+): 546.5 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.94−4.18 (q, 2H); 5.41−5.56 (q, 2H); 7.24−7.37 (m, 4H); 7.52−7.63 (m, 2H), 7.70−7.77 (m, 2H); 7.92 (d, 1H); 8.01−8.12 (m, 3H); 9.03 (s, 1H); 9.18(s, 2H); 9.43 (s, 2H); 10.21 (s, 1H). Anal. ($C_{25}H_{23}N_9O_4S$·TFA·H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2-(aminosulfonyl)phenyl]-2-pyrimidinyl]-4,5-dihydro-5-(methoxymethyl)-, Trifluoroacetic Acid Salt (±) (58). MS (ES^+): 510.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.39 (s, 3H); 3.64−4.02 (m, 4H); 7.43 (d, 1H); 7.52 (s, 1H), 7.63−7.75 (m, 2H), 7.90 (d, 1H); 8.07−8.15 (m, 3H); 8.68 (s, 2H); 9.05 (s, 2H); 9.41 (s, 2H); 10.09 (s, 1H). High-resolution MS ($C_{23}H_{23}N_7O_5S$): calcd, 510.1560; found, 510.1570. HPLC purity 100%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyrimidinyl]-4,5-dihydro-5-(ethoxymethyl)-, Trifluoroacetic Acid Salt (±) (59). MS (ES^+): 524.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.13 (t, 3H); 3.53−4.08 (m, 6H); 7.43 (d, 1H); 7.52 (s, 1H), 7.63−7.75 (m, 2H), 7.90 (d, 1H); 8.07−8.15 (m, 3H); 8.68 (s, 2H); 9.03 (s, 2H); 9.41 (s, 2H); 10.10 (s, 1H). High-resolution MS ($C_{24}H_{25}N_7O_5S$): calcd, 524.1716; found, 524.1730. HPLC purity 98%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyrimidinyl]-4,5-dihydro-5-(ethylthiomethyl)-, Trifluoroacetic Acid Salt (±) (60). MS (ES^+): 540.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.18 (t, 3H); 2.65 (q, 2H); 3.20−3.42 (m, 2H); 3.68−4.07 (m, 2H); 7.38−7.42 (m, 1H); 7.49 (s, 2H); 7.62−7.73 (m, 3H), 7.87 (d, 1H); 8.01−8.12 (m, 3H); 8.64 (s, 2H); 9.15 (s, 2H); 9.41 (s, 2H); 10.27 (s, 1H). Anal. ($C_{24}H_{27}N_7O_4S_2$·2TFA) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyrimidinyl]-4,5-dihydro-5-(ethylsulfonylmethyl)-, Trifluoroacetic Acid Salt (±) (61). MS (ES^+): 572.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.28 (t, 3H); 3.25 (q, 2H); 3.95−4.24 (m, 4H); 7.45 (d, 1H); 7.52 (s, 2H), 7.63−7.78 (m, 3H); 7.92 (d, 1H); 8.02−8.13 (m, 3H); 8.68 (s, 2H); 9.05 (s, 2H); 9.43 (s, 2H); 10.29 (s, 1H). High-resolution MS ($C_{24}H_{25}N_7O_6S_2$): calcd, 572.1386; found, 572.1371. HPLC purity 98%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyrimidinyl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (±) (62). MS (ES^+): 548.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.85−4.22 (m, 4H); 5.20−5.41 (m, 2H); 7.45 (d, 1H); 7.52 (s, 2H), 7.63−7.78 (m, 3H); 7.92 (d, 1H); 8.02−8.13 (m, 3H); 8.70 (s, 2H); 9.09 (s, 2H); 9.42 (s, 2H); 9.45 (s, 1H); 10.57 (s, 1H). High-resolution MS ($C_{23}H_{21}N_{11}O_4S$): calcd, 548.1577; found, 548.1563. HPLC purity 80%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-methyl-, Trifluoroacetic Acid Salt (±) (64). MS (ES^+): 479.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.77 (s, 3H); 3.55−4.02 (q, 2H); 7.32 (m, 1H); 7.37 (s, 1H), 7.60 (m, 1H); 7.68 (t, 1H); 7.82 (m, 2H); 8.00 (m, 1H); 8.01 (s, 1H); 8.05 (d, 2H); 8.28 (s, 1H); 9.02 (bs, 2H); 9.38 (bs, 2H); 9.81 (s, 1H). Anal. ($C_{23}H_{22}N_6O_4S$·1.5TFA) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethoxymethyl)-, Trifluoroacetic Acid Salt (±) (66). MS (ES^+): 523.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.15 (t, 3H); 3.55−4.08 (m, 6H); 7.36 (d, 1H); 7.41 (s, 2H), 7.60−7.78 (m, 3H); 7.83−7.94 (m, 2H); 8.03−8.15 (m, 4H); 8.33 (s, 1H); 9.07 (bs, 2H); 9.41 (bs, 2H); 9.73 (s, 1H). High-resolution MS ($C_{25}H_{26}N_6O_5S$): calcd, 523.1764; found, 523.1768. HPLC purity 99%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (±) (69). MS (ES^+): 547.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.87−4.21 (q, 2H); 5.22−5.40 (q, 2H); 7.38 (m, 1H); 7.43 (s, 2H), 7.61−7.76 (m, 3H); 7.83−7.94 (m, 2H); 7.99−8.08 (m, 4H); 8.14 (d, 1H); 9.11 (bs, 2H); 9.43 (bs, 2H); 9.48 (s, 1H); 10.15 (s, 1H). High-resolution MS ($C_{24}H_{22}N_{10}O_4S$): calcd, 547.1635; found, 547.1624. HPLC purity 97%.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(ethoxymethyl)-, Trifluoroacetic Acid Salt (−) (70). MS (ES^+): 522.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.12 (t, 3H); 3.55−4.02 (m, 6H); 7.22−7.38 (m, 4H); 7.52−7.64 (m, 2H), 7.69−7.82 (m, 2H), 7.90 (d, 1H); 8.02 (d, 1H); 8.07−8.10 (m, 2H); 9.18 (s, 2H); 9.41 (s, 2H); 10.16 (s, 1H). Anal. ($C_{26}H_{27}N_5O_5S$·1.25TFA·1.2H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(ethoxymethyl)-, Trifluoroacetic Acid Salt (−) (71). MS (ES^+): 523.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 1.15 (t, 3H); 3.55−4.08 (m, 6H); 7.36 (d, 1H); 7.41 (s, 2H), 7.60−7.78 (m, 3H); 7.83−7.94 (m, 2H); 8.03−8.15 (m, 4H); 8.33 (s, 1H); 9.07 (bs, 2H); 9.41 (bs, 2H); 9.73 (s, 1H). Anal. ($C_{25}H_{26}N_6O_5S$·2TFA·0.9H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (SK549, 72). Optical rotation −107.01° (acetonitrile, 0.114 g/mL, 25 °C); 99% ee. MS (ES^+): 546.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.89−4.16 (q, 2H); 5.13−5.31 (q, 2H); 7.22−7.48 (m, 5H), 7.52−7.78 (m, 5H), 7.91 (d, 1H); 8.00−8.08 (m, 3H); 9.12 (s, 2H); 9.41 (s, 2H); 9.43 (s, 1H); 10.22 (s, 1H). Anal. ($C_{25}H_{23}N_9O_4S$·TFA) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(methylaminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (73). Optical rotation −82.73° (1:1 acetonitrile/H_2O, 0.220 g/mL, 25 °C); 99% ee. MS (ES^+): 560.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 2.40 (d, 3H); 3.90−4.10 (q, 2H); 5.15−5.35 (q, 2H); 7.22−7.48 (m, 5H), 7.52−7.78 (m, 5H),

7.91 (d, 1H); 8.00−8.08 (m, 3H); 9.15 (s, 2H); 9.45 (s, 2H); 9.49 (s, 1H); 10.22 (s, 1H). Anal. ($C_{26}H_{25}N_9O_4S$·1.1TFA·0.6H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(*n*-propylaminosulfonyl)[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (74). Optical rotation −96.74° (acetonitrile, 0.184 g/mL, 25 °C); 99% ee. MS (ES^+): 588.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 0.87 (t, 3H); 1.54 (m, 2H); 2.66 (q, 2H); 3.88−4.17 (q, 2H); 5.13−5.32 (q, 2H); 7.28−7.37 (m, 4H), 7.54−7.78 (m, 5H), 7.91 (d, 2H); 8.02−8.08 (m, 2H); 9.08 (s, 2H); 9.42 (s, 2H); 9.45 (s, 1H); 10.22 (s, 1H). Anal. ($C_{28}H_{29}N_9O_4S$·1.1TFA·0.5H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)-3-fluoro[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (76). Optical rotation −84.69° (acetonitrile, 0.294 g/mL, 25 °C); 99% ee. MS (ES^+): 564.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.88−4.07 (q, 2H); 5.10−5.29 (q, 2H); 7.17 (d, 1H); 7.23−7.33 (m, 2H), 7.38 (s, 2H); 7.47 (t, 1H), 7.52−7.62 (m, 2H); 7.70 (t, 1H); 7.89 (d, 1H); 7.97−8.05 (m, 3H); 9.10 (s, 2H); 9.40 (s, 2H); 9.41 (s, 1H); 9.92 (s, 1H). Anal. ($C_{25}H_{22}FN_9O_4S$·1.1TFA·0.55H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)-3-chloro[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (77). Optical rotation −85.42° (acetonitrile, 0.240 g/mL, 25 °C); 99% ee. MS (ES^+): 580.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.90−4.12 (q, 2H); 5.15−5.37 (q, 2H); 7.31−7.45 (m, 4H), 7.51 (d, 1H); 7.59−7.78 (m, 4H); 7.92 (d, 1H); 8.01−8.12 (m, 3H); 9.08 (s, 2H); 9.43 (s, 2H); 9.48 (s, 1H); 9.78 (s, 1H). Anal. ($C_{25}H_{22}ClN_9O_4S$·1TFA·0.5H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)-3-methyl[1,1′-biphenyl]-4-yl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (75). Optical rotation −75.6° (acetonitrile, 0.344 g/mL, 25 °C); 99% ee. MS (ES^+): 560.1 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 2.06 (s, 3H); 3.92−4.04 (q, 2H); 5.16−5.28 (q, 2H); 7.22 (s, 3H), 7.26 (s, 2H), 7.30 (m, 1H); 7.58 (m, 2H); 7.75 (t, 1H); 7.92 (d, 1H); 8.02 (m, 1H); 8.05 (bs, 1H); 9.02 (s, 2H); 9.42 (s, 2H); 9.45 (s, 1H); 9.71 (s, 1H). Anal. ($C_{26}H_{25}N_9O_4S$·1.3TFA) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyrimidinyl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (78). Optical rotation −74.2° (acetonitrile, 0.090 g/mL, 25 °C); 99% ee. MS (ES^+): 548.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.85−4.22 (m, 4H); 5.20−5.41 (m, 2H); 7.45 (d, 1H); 7.52 (s, 2H), 7.63−7.78 (m, 3H); 7.92 (d, 1H); 8.02−8.13 (m, 3H); 8.70 (s, 2H); 9.09 (s, 2H); 9.42 (s, 2H); 9.45 (s, 1H); 10.57 (s, 1H). Anal. ($C_{23}H_{21}N_{11}O_4S$·1.4TFA·H_2O) C, H, N.

5-Isoxazolecarboxamide, 3-[3-(Aminoiminomethyl)-phenyl]-*N*-[[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]-4,5-dihydro-5-(1*H*-tetrazol-1-ylmethyl)-, Trifluoroacetic Acid Salt (−) (SM084, 79). Optical rotation −84.00° (acetonitrile, 0.150 g/mL, 25 °C); 99% ee. MS (ES^+): 547.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.87−4.21 (q, 2H); 5.22−5.40 (q, 2H); 7.38 (m, 1H); 7.43 (s, 2H), 7.61−7.76 (m, 3H); 7.83−7.94 (m, 2H); 7.99−8.08 (m, 4H); 8.14 (d, 1H); 9.11 (bs, 2H); 9.43 (bs, 2H); 9.48 (s, 1H); 10.15 (s, 1H). Anal. ($C_{24}H_{22}N_{10}O_4S$·1.95TFA) C, H, N.

Acknowledgment. We thank D. McCall, J. M. Luettgen, S. Spitz, A. Smallwood, R. Bernard, E. Crain, C. Watson, and R. F. Carney for their technical assistance. We would also like to thank A. M. Blum, N. C. Caputo, and A. J. Mical for chiral separations and Dr. B. J. Aungst for the in vitro hydrolysis of SF303.

References

(1) This work was partially presented at the 215th ACS National Meeting: Quan, M. L. Design and Synthesis of Isoxazoline Derivatives as Factor Xa Inhibitors. 215th ACS National Meeting Dallas, TX, March 29−April 2, 1998, Abstract 202.

(2) Davre, E. W.; Fujikawa, K.; Kisiel, W. The coagulation cascade: initiation, maintenance and regulation. *Biochemistry* **1991**, *30*, 10363−10370.

(3) Elodi, S.; Varadi, K. Optimization of conditions for the catalytic effect of the factor IXa-factor VIII complex: probable role of the complex in the amplication of blood coagulation. *Thromb. Res.* **1979**, *15*, 617−629.

(4) Harker, L. A.; Hanson, S. R.; Kelly, A. B. Antithrombotic Strategies Targeting Thrombin Activities, Thrombin Receptors and Thrombin Generation. *Thromb. Haemostasis* **1997**, *78*, 736−741.

(5) Ripka, W. C.; Vlasuk, G. P. Antithrombotics/Serine Proteases. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1997, Vol. 32, pp 71−89.

(6) Tidwell, R. R.; Webster, W. P.; Shaver, S. R.; Geratz, J. D. Strategies for Anticoagulation with Synthetic Protease Inhibitors. Xa Inhibitors Versus Thrombin Inhibitors. *Thromb. Res.* **1980**, *19*, 339−349.

(7) Stürzebecher, J.; Markwardt, F.; Walsmann, P. Synthetic Inhibitors of Serine Proteinases XIV. Inhibition of Factor Xa by Derivatives of Benzamidine. *Thromb. Res.* **1976**, *9*, 637−646.

(8) Stürzebecher, J.; Markwardt, F.; Walsmann, P. Synthetic Inhibitors of Serine Proteinases XXIII. Inhibition of Factor Xa by Diamidines. *Thromb. Res.* **1980**, *17*, 545−548.

(9) Nagahara, T.; Yukoyama, Y.; Inamura, K.; Katakura, S.; Komoriya, S.; Yamaguchi, H.; Hara, T.; Iwamoto, M. Dibasic (amidinoaryl)propanoic Acid Derivatives as Novel Blood Coagulation Factor Xa Inhibitors. *J. Med. Chem.* **1994**, *37*, 1200−1207.

(10) Nagahara, T.; Yukoyama, Y.; Inamura, K.; Katakura, S.; Komoriya, S.; Yamaguchi, H.; Hara, T.; Iwamoto, M. Design, Synthesis and Biological Activities of Orally Active Coagulation Factor Xa Inhibitors. *Eur. J. Med. Chem.* **1995**, *30*, 139s.

(11) Sato, K.; Kawasaki, T.; Taniuchi, Y.; Hisamichi, N.; Koshio, H.; Matsumoto, Y. YM-60828, a Novel Factor Xa Inhibitor: Separation of its Antithrombotic Effects from its Prolongation of Bleeding Time. *Eur. J. Pharmacol.* **1997**, *339*, 141−146.

(12) Taniuchi, Y.; Sakai, Y.; Hisamichi, N.; Kayama, M.; Mano, Y.; Sato, K.; Hirayama, F.; Koshio, H.; Matsumoto, Y.; Kawasaki, T. Biochemical and Pharmacological Characterization of YM-60828, a Newly Synthesized and Orally Active Inhibitor of Human Factor Xa. *Thromb. Haemostasis* **1998**, *79*, 543−548.

(13) Sato, K.; Kawasaki, T.; Hisamichi, N.; Taniuchi, Y.; Hirayama, F.; Koshio, H.; Matsumoto, Y. Antithrombotic Effects of YM-60828, a Newly Synthesized Factor Xa Inhibitor, in Rat Thrombosis Model and its Effects on Bleeding Time. *Br. J. Pharmacol.* **1998**, *123*, 92−96.

(14) Maduskuie, T. P., Jr.; McNamara, K. J.; Ru, Y.; Knabb, R. M.; Stouten, P. F. W. Rational Design and Synthesis of Novel, Potent Bis-Phenylamidine Carboxylate Factor Xa Inhibitors. *J. Med Chem.* **1998**, *41*, 53−62.

(15) At the time this work was carried out, there were no published reports on monobasic FXa inhibitors. Subsequently, the following manuscripts have been published: (a) Klein, S. I.; Czekaj, M.; Gardner, C. J.; Guertin, K. R.; Cheney, D. L.; Spada, A. P.; Bolton, S. A.; Brown, K.; Colussi, D.; Heran, C. L.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. Identification and Initial Structure−Activity Relationships of a Novel Class of Nonpeptide Inhibitors of Blood Coagulation Factor Xa. *J. Med. Chem.* **1998**, *41*, 437−450. (b) Phillips, G. B.; Buckman, B. O.; Davey, D. D.; Eagen, K. A.; Guilford, W. J.; Hinchman, J.; Ho, E.; Koovakkar, A. L.; Light, D. R.; Mohan, R.; Ng, H. P.; Post, J. M.; Shaw, K. J.; Smith, D.; Subramanyam, B.; Sullivan, M. E.; Trinh, L.; Verona, R.; Walters, J.; White, K.; Whitlow, M.; Wu, S.; Xu, W.; Morrissey, M. M. Discovery of N-[2-[5-[Amino(imino)methyl]-2-hydro-1-methyl-1H-imidazol-2-yl)phenoxy]pyridin-4-yl]-*N*-methylglycine (ZK-807834): A Potent, Selective, and Orally Active Inhibitor of the Blood Coagulation Enyme Factor Xa. *J. Med. Chem.* **1998,** *41*, 3557−3562.

(16) Quan, M. L.; Pruitt, J. R.; Ellis, C. D.; Liauw, A. Y.; Galemmo, R. A., Jr.; 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.

(17) Quan, M. L.; Liauw, A. Y.; Ellis, C. D.; Pruitt, J. R.; Bostrom, L. L.; Carini, D. J.; Hung, P. P.; Moody, 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.

(18) Pinner, A. *Die Imidoaether und ihre Derivate*; Oppenheim: Berlin, 1892; pp 1−85.

(19) Majid, T. N.; Knoechel, P. A New Preparation of Highly Functionalized Aromatic and Heteroaromatic Zinc and Copper Organometallics. *Tetrahedron Lett.* **1990**, *31*, 4413−4416.

(20) Knoechel, P.; Yeh, M. C. P.; Berk, S. C.; Telbert, J. Synthesis and Reactivity Toward Acyl Chlorides and Enones of the New highly Functionalized Copper Reagents RCu(CN)ZnI. *J. Org. Chem.* **1988**, *53*, 2390−2393.

(21) Weber, P. C.; Lee, S.; Lewandowski, F. A.; Schadt, M. C.; Chang, C.; Kettner, C. A. Kinetic and Crystallographic Studies of Thrombin with Ac-(D)Phe-Pro-boroArg-OH and its Lysine, Amidine, Homolysine and Ornithine Analogues. *Biochemistry* **1995**, *34*, 3750−3757.
(22) Padmanabhan, K.; Padmanabhan, K. P.; Tulinsky, A.; Park, C. H.; Bode, W.; Huber, R.; Blankenship, D. T.; Cardin, A. D.; Kiesel, W. Structure of Human Des(1−45) Factor Xa at 2.2 Å Resolution. *J. Mol. Biol.* **1993**, *232*, 947−966.
(23) The model was generated by minimizing the conformation of **79** (SM084) in the active site of FXa using Discover with the CFF95 force field (MSI, San Diego, CA) until the rms force was smaller than 0.001 kcal/mol Å. The minimizations were carried out keeping the protein rigid and the inhibitor completely flexible.
(24) Knabb, R. M.; Kettner, C. A.; Timmermans, P. B. M. W. M.; Reilly, T. M. In Vivo Characterizations of a Synthetic Thrombin Inhibitor. *Thromb. Haemostasis* **1992**, *67*, 56−59.
(25) Kettner, C. A.; Mersinger, L. J.; Knabb, R. M. The Selective Inhibition of Thrombin by Peptides of Boroarginine. *J. Biol. Chem.* **1990**, *265*, 18289−18297.

JM980406A