Articles

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

Mimi L. Quan,* Ann Y. Liauw, Christopher D. Ellis, James R. Pruitt, David J. Carini, Lori L. Bostrom, Peggy P. Huang, Kimberly Harrison, Robert M. Knabb, Martin J. Thoolen, Pancras C. Wong, and Ruth R. Wexler

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

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Thrombosis is a major cause of mortality in the industrialized world. 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 prothrombin activation. We report a series of novel biaryl-substituted isoxazoline derivatives in which the biaryl moiety was designed to interact with the S_4 aryl-binding domain of the FXa active site. Several of the compounds herein have low nanomolar affinity for FXa, have good in vitro selectivity for FXa, and show potent antithrombotic efficacy in vivo. The three most potent compounds (**33**, **35**, and **37**) have inhibition constants for human FXa of 3.9, 2.3, and 0.83 nM, respectively, and ID_{50}'s ranging from 0.15 to 0.26 μmol/kg/h in the rabbit arterio-venous thrombosis model.

Introduction

Intravascular clot formation is an important factor in a number of cardiovascular diseases such as myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism, and ischemic stroke. Therefore, the prevention of blood coagulation has become a major target for new therapeutic agents. Most of the research in the past decade has been focused on thrombin inhibition. More recently, inhibition of factor Xa (FXa), the enzyme directly responsible for prothrombin activation, has emerged as an attractive target.[2]

FXa is the serine protease in the blood coagulation cascade that converts prothrombin to thrombin. FXa holds the central position linking the intrinsic and extrinsic activation mechanisms in the final common pathway of coagulation.[3] Inhibition of FXa interrupts both the intrinsic and extrinsic activation pathways of thrombin production. This process involves signal amplification, with one molecule of FXa activating many molecules of prothrombin to thrombin.[4] Therefore, inhibition of FXa may be more effective than inhibition of thrombin. In addition, a recent study by Harker[5] showed that FXa inhibitors may have less bleeding risk than thrombin inhibitors in a baboon model of thrombosis. In view of the favorable safety−efficacy ratio in this primate model and the central position of FXa in the coagulation cascade, we believe it is logical to design small-molecule FXa inhibitors as antithrombotic agents.

Most of the nonpeptide FXa inhibitors reported in the literature are dibasic compounds (representative examples are shown in Chart 1). Tidwell[6] has published a series of bisamidines, represented by 1,2-bis(5-amidino-2-benzofuranyl)ethane (DABE, **1**), which is a selective inhibitor of FXa (K_i = 570 nM, bovine FXa). Stürzebecher et al.[7,8] have reported a series of bisamidines with the most potent compound being 2,7-bis-(4-amidinobenzylidene)cycloheptan-1-one (BABCH, **2**) with a FXa K_i of 13 nM. More recently, Nagahara et al.[9,10] have reported a series of dibasic (amidinoaryl)-propanoic acid derivatives from which DX-9065a (**3**) was selected for clinical studies. DX-9065a is a selective FXa inhibitor, with a K_i of 41 nM, that does not inhibit thrombin at a concentration of 2000 μM.[9,10] YM-60828 (**4**), a compound structurally related to DX-9065a, has been reported to have a K_i value of 1.3 nM for FXa and has demonstrated good antithrombotic efficacy in animal models of thrombosis.[11−13] Maduskuie et al. have published a series of novel bisphenylamidines with the most potent compound having a K_i value of 9 nM for FXa and 400-fold selectivity over thrombin.[14]

We have recently reported on a series of bisbenzamidine isoxazoline derivatives such as compound **5** as FXa inhibitors.[15] Compound **5** inhibited FXa with a K_i of 94 nM and showed in vivo efficacy (ID_{50} = 1.6 mg/kg/h) in a rat vena cava thrombosis model.[15,16] As dibasic compounds, we expected that compounds such as **5** might not have adequate oral absorption or pharmacokinetics[17] to be useful as oral antithrombotic agents. To reduce the basicity of these compounds, our strategy was to replace one of the amidine groups with a neutral species. To date, there have been very few reports on monobasic FXa inhibitors.[18] Herein we report a series of isoxazoline derivatives which were designed to place a biaryl moiety into the S_4 pocket (aryl-binding domain) of the FXa active site.

Chemistry

The synthesis of this series of compounds is exemplified by the preparation of **15** (SF303**)** shown in Scheme 1. 3-Cyanobenzaldehyde (**6**) was converted to the oxime **7** by reaction with hydroxylamine hydrochloride in pyridine and ethanol in 93% yield. The oxime was

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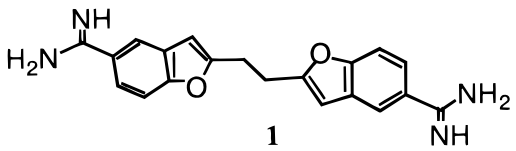
Chart 1. FXa Inhibitors



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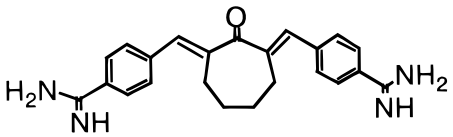

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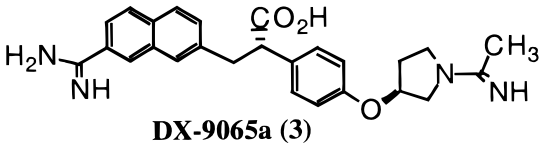
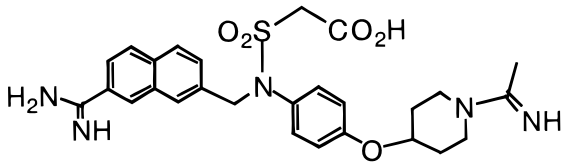
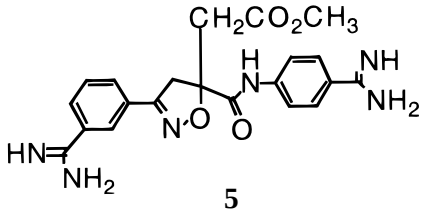


DX-9065a (3)



YM-60828 (4)



5

chlorinated with bleach in THF and then dehydrochlorinated to generate the nitrile oxide in situ. [2+3] Cycloaddition with itaconic acid monomethyl ester (**8**) generated the isoxazoline **9** in 74% yield. The 2-(*tert*-butylaminosulfonyl)benzeneboronic acid (**11**) was prepared from sulfonamide **10** by reaction with *n*-butyllithium and triisopropyl borate followed by quenching with hydrochloric acid. Suzuki coupling of the boronic acid **11** with 4-bromoaniline (**12**) using tetrakis(triphenylphosphine)palladium(0) and aqueous sodium carbonate in toluene at reflux produced the biphenyl intermediate **13**. The acid **9** was converted to the acyl chloride with thionyl chloride, and the acid chloride was then coupled with the biphenyl derivative **13** in triethylamine and methylene chloride to give compound **14**. TFA deprotection of the *tert*-butyl group afforded the free sulfonamide. The cyano group was converted to the imidate with HCl and methanol and was then reacted with ammonium acetate in methanol to give the benzamidine **15** (SF303). Other biaryl intermediates were synthesized via palladium-catalyzed coupling of the appropriately substituted 4-bromoaniline with the requisite arylboronic acid employing standard Suzuki reaction conditions.[19] The pyridyl and pyrimidyl analogues listed in Tables 2 and 3 were prepared similarly as described above using 5-amino-2-bromopyridine, 2-amino-5-bromopyridine, and 2-amino-5-bromopyrimidine as starting materials.

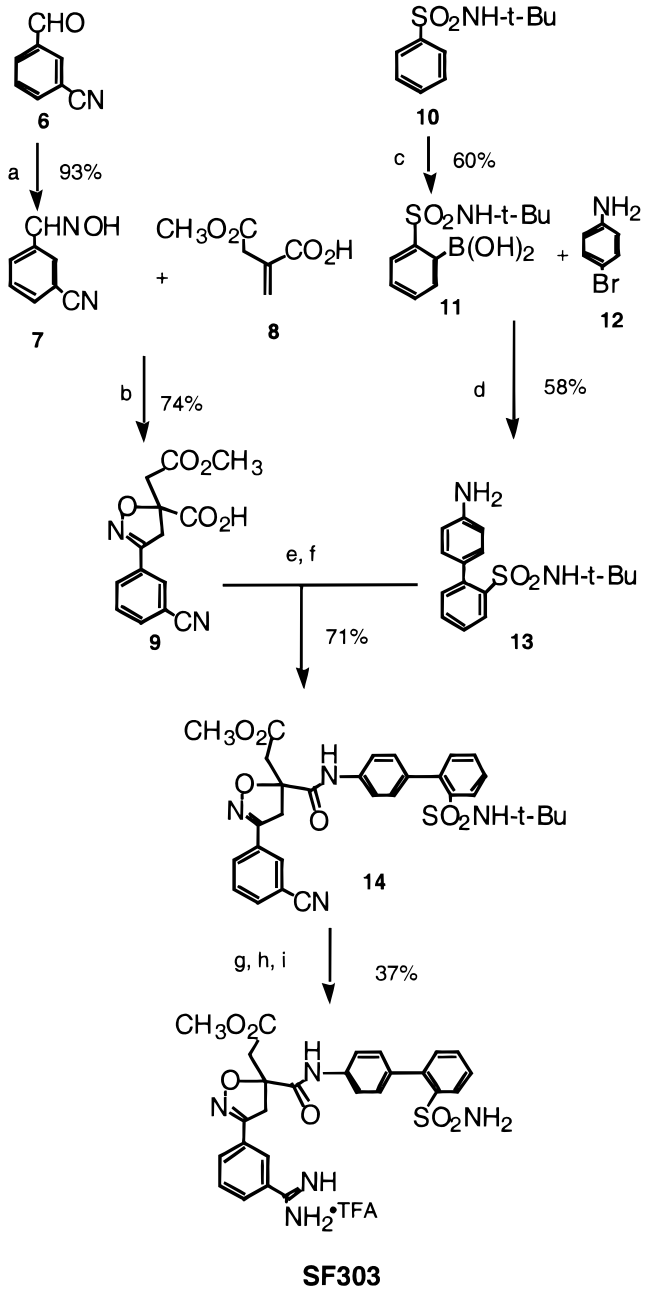
All compounds described above were prepared as racemic mixtures. The two enantiomers of compound **9** were separated by chiral HPLC to enable the synthesis of the final compounds as single pure enantiomers (Table 3). The enantiomers of **9** were then carried on independently using the methods described in Scheme 1 to give the final products each in greater than 95% ee.

Results and Discussion

In our earlier thrombin inhibitor work we have shown that the P_2P_3 peptide portion of DuP 714 (Chart 2) could be replaced with a substituted biaryl group.[20] The X-ray crystal structures of these inhibitors bound in the thrombin active site indicated that the terminal phenyl ring is at a similar location to the phenylalanine side chain of DuP 714 and forms an edge-to-face interaction with Trp^{215}.[20] In FXa the S_4 pocket, or aryl-binding site,[21] is largely hydrophobic and is lined with three aromatic residues: Trp^{215}, Tyr^{99}, and Phe^{174} as compared with Trp^{215}, Leu^{99}, and Ile^{174} found in the thrombin distal hydrophobic binding pocket. Molecular modeling studies of compound **5** employing the crystal structure of des(1−45) FXa[21] indicated that the *m*-amidine group fits in the S_1 pocket and forms hydrogen-bonding and ionic interactions with Asp^{189} of FXa and the *p*-amidine binds in the "aryl-binding site".[15] We decided to employ the biaryl as a replacement for the *p*-benzamidine. We were gratified to find that the biphenyl analogue **16** was only 2-fold less potent than bisbenzamidine **5** (Table 1).

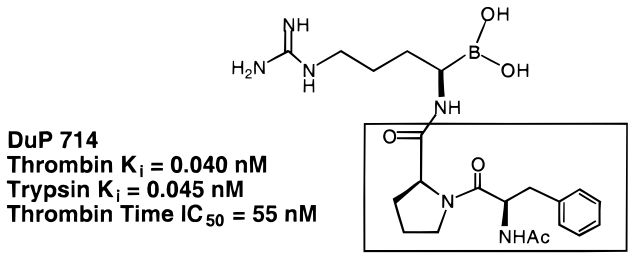
To further improve affinity for FXa in this series, we investigated the effect of substitution on the terminal ring of the biphenyl (Table 1). Substitution at the 2′-position of the terminal phenyl ring produced more potent compounds for FXa than compounds substituted at the 3′-position. Our modeling suggests that the 3′-substituted analogues are less potent because of steric conflict with the backbone carbonyls of Lys^{96} and Glu^{97}. For 2′-substituted compounds, both the methoxy and methyl ester compounds (**22** and **25**, respectively) are more potent than the corresponding methyl derivative (**18**) suggesting that substituents capable of engaging in a hydrogen bond interaction enhance potency. Both the sulfonamide and methyl sulfone compounds (**15** and **26**) show a further 5−10-fold enhancement in potency

Scheme 1. Synthesis of SF303[a]



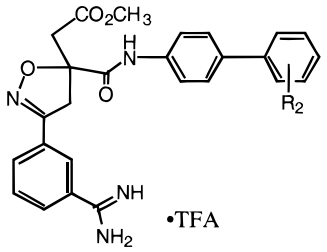
[a] (a) $NH_2OH{\cdot}HCl$, pyridine, ethanol; (b) bleach, THF; (c) *n*-BuLi, $B(O\text{-}i\text{-}Pr)_3/H^+$; (d) $Pd(PPh_3)$, aq Na_2CO_3, toluene, reflux; (e) $SOCl_2$, CH_3CN, reflux; (f) Et_3N, CH_2Cl_2; (g) TFA; (h) HCl, CH_3OH; (i) NH_4OAc, CH_3OH.

Chart 2. Structure of DuP 714



compared to the other 2′-substituted compounds. Molecular modeling studies based on the X-ray structure of FXa[21] indicate that the sulfonyl oxygens of the sulfonamide and methyl sulfone substituents are positioned to form a hydrogen bond with the OH group of Tyr^{99}, which could contribute to this further improvement of the FXa affinity. Figure 1 shows **15** modeled in the FXa active site.[22] In this model the *m*-benzamidine group is in the S_1 specificity pocket and forms a salt bridge with Asp^{189} of FXa similar to that of the bisbenzamidine compounds.[16] The carbonyl group of the ester forms a hydrogen bond with Gln^{192}. The biaryl moiety extends to the "aryl pocket" formed by Phe^{174}, Trp^{215}, and Tyr^{99} of FXa, where the terminal ring forms an edge-to-face interaction with Trp^{215} of FXa. The sulfonamide substituent is in close proximity to the OH group of Tyr^{99}. There is no interaction observed between the inhibitor and Ser^{195} of the enzyme. This might contribute to the improved selectivity of this series of compounds.

Table 1. Effects of Substitution on the Biphenyl Terminal Ring[a]



compd	R^2	K_i (nM) FXa	thrombin	trypsin
5		94	16000	480
16	H	220	3500	470
17	3′-CH_3	240	2100	not tested
18	2′-CH_3	210	1800	390
19	3′-CF_3	240	1200	570
20	2′-CF_3	28	1400	230
21	3′-OCH_3	200	2700	390
22	2′-OCH_3	62	2800	310
23	3′-SO_2NH_2	68	3100	340
15 (SF303)	2′-SO_2NH_2	6.3	3100	110
24	2′-SCH_3	66	1200	260
25	2′-CO_2CH_3	53	2100	300
26	2′-SO_2CH_3	4.2	600	100

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

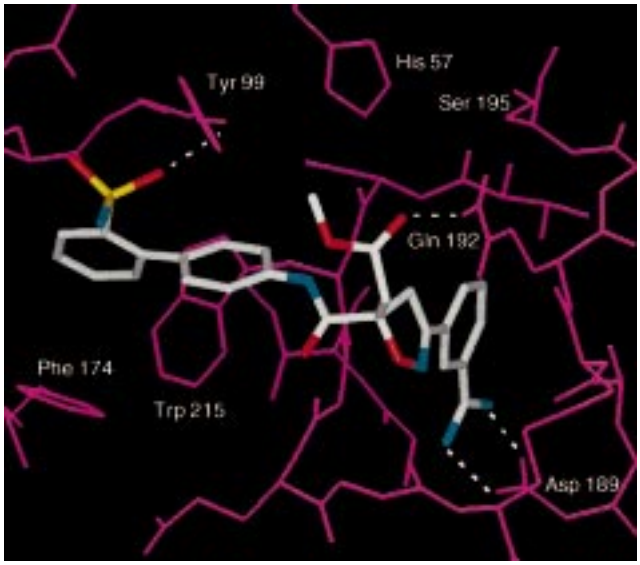
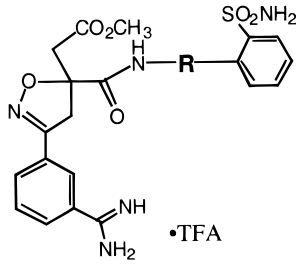
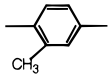
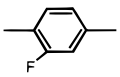
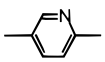
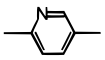
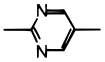


Figure 1. SF303 modeled in the FXa active site.

Table 2. Effects of Modification on the Biphenyl Ring[a]



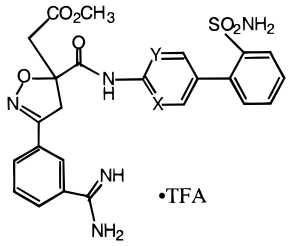
compd	R	K_i (nM) FXa	thrombin	trypsin
15 (SF303)		6.3	4900	110
27		8.8	3100	300
28		4.2	3800	120
29		19	10800	180
30		0.96	3900	25
31		4.3	12000	336

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

As reported previously, in targets focused at thrombin inhibition, we found that addition of a methyl group to the ortho position on the proximal phenyl ring (ring closest to the amide functionality) resulted in a 2-fold increase in thrombin affinity, while addition of a fluorine at this position showed little to no effect on thrombin affinity.[20] In addition, replacement of the proximal phenyl ring with a six-membered aromatic ring containing nitrogen resulted in a 15−20-fold decrease in thrombin affinity. The same modifications to the proximal phenyl ring were examined in this series of isoxazolines (Table 2). The addition of a methyl or fluoro substituent (**27** and **28**) at the "ortho" position of the proximal phenyl ring resulted in little to no change in FXa affinity compared to the unsubstituted phenyl derivative. A 3-fold decrease in both FXa and thrombin affinity was observed for the 3-pyridyl compound **29**; however, we were gratified to see a 6-fold increase in FXa affinity with the 2-pyridyl derivative **30**, resulting in the first subnanomolar FXa inhibitor in this series. The pyrimidyl analogue **31** was comparable in affinity to the biphenyl derivative **15**; however, interestingly the selectivity for FXa versus both thrombin and trypsin improved significantly.

Three sets of enantiomers were prepared (Table 3). In each case, the (−)-enantiomer has higher affinity for FXa than the (+)-enantiomer as shown by their K_i values. In vivo the (−)-enantiomers were also more potent when studied in a rabbit arterio-venous (A-V) shunt thrombosis model. Thrombosis was induced by an A-V shunt device containing a piece of silk thread. The A-V shunt was connected between the femoral artery and vein. Anesthetized rabbits were treated with either the vehicle or test compound given as a continuous intravenous infusion started 60 min before thrombus induction. The end point measured was the clot weight. The antithrombotic effect was expressed as the ID_{50}, a dose which reduced the clot weight by 50%. The (−)-enantiomers inhibited thrombus formation in a dose-dependent fashion with ID_{50}'s ranging from 0.15 to 0.26 μmol/kg/h (Table 3). The antithrombotic effect of **35** (SF324) in the rabbit A-V shunt thrombosis model is illustrated in Figure 2. In this model, the ID_{50} for this compound was 0.15 μmol/kg/h.

Table 3. Comparison of Enantiomers[a]



compd	X, Y	K_i (nM) FXa	thrombin	trypsin	rabbit A-V shunt ID_{50} (μmol/kg/h)
15 (SF303) (±)	CH, CH	6.3	3100	121	0.60
32 (+)	CH, CH	6.8	>21000	300	2.00
33 (−)	CH, CH	3.9	2400	98	0.20
31 (±)	N, N	4.3	14000	330	0.40
34 (+)	N, N	12	>21000	710	>1.50
35 (SF324) (−)	N, N	2.3	8900	170	0.15
33 (±)	N, CH	0.96	3900	25	0.30
36 (+)	N, CH	7.0	18000	130	0.60
37 (−)	N, CH	0.83	2900	17	0.26

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

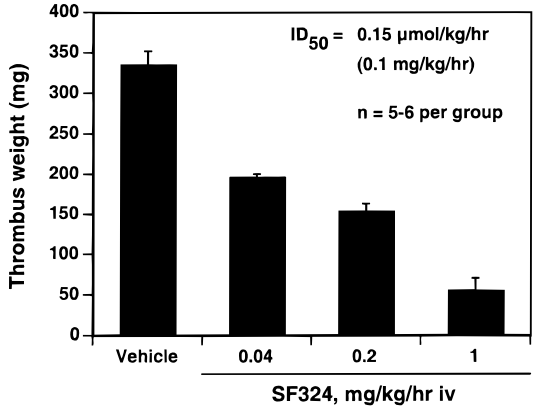


Figure 2. Antithrombotic effect of SF324 in rabbit A-V shunt thrombosis model.

As shown in Tables 1−3, these compounds have good selectivity for FXa over thrombin and moderate selectivity over trypsin. In addition to the data shown, high selectivity against a panel of serine proteases including plasmin, tPA, FVIIa, FIXa, and FI was also observed for these compounds. For example, **15** has K_i values of 5 600 nM for FVIIa, >12 000 nM for FIXa, >20 000 nM for tPA, and 2 500 nM for plasmin and an IC_{50} of 680 000 nM for FI. We believe this series of compounds exhibits improved efficacy and selectivity over our earlier FXa inhibitors due to their monobasic nature and the absence of an electrophilic functional group.

Conclusion

We have designed and synthesized a series of biaryl-substituted isoxazoline derivatives as FXa inhibitors with low to subnanomolar affinity against FXa. This is one of the few monobasic series of FXa inhibitors lacking an electrophilic functional group which has been described to date. These compounds exhibit good selectivity for FXa compared to thrombin and trypsin and show potent antithrombotic effect in the rabbit A-V shunt thrombosis 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/((($K_m + S$) $- S \times$ ACT)/(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. The detailed methodology of the assay is described in refs 23 and 24.

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 obtained with a Perkin-Elmer 1600 series FTIR. NMR spectra were obtained 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 C18 reverse-phase column with CH_3CN/H_2O (containing 0.05% TFA) as mobile phase.

3-Cyanobenzaldehyde Oxime (7).[25] 3-Cyanobenzaldehyde (**6**) (25.0 g, 0.19 mol) and hydroxylamine hydrochloride (16.6 g, 0.24 mol) were dissolved in 100 mL of pyridine and 100 mL of ethanol. The mixture was stirred at room temperature under N_2 for 12 h. The mixture was concentrated to one-half of its volume, and 200 mL of water was added. A white precipitate formed. It was filtered and dried to afford 25.9 g of the oxime (93%). ^{1}H NMR (DMSO-d_6): δ 7.61 (t, 1H); 7.85 (d, 1H); 7.96 (d, 1H); 8.00 (s, 1H); 8.21 (s, 1H); 11.61 (s, 1H).

3-(3-Cyanophenyl)-5-(carbomethoxymethyl)isoxazoline-5-carboxylic Acid (±) (9). 3-Cyanobenzaldehyde oxime (**7**) (26.9 g, 0.18 mol) and itaconic acid monomethyl ester (**8**) (31.8 g, 0.22 mol) were dissolved in 600 mL of THF. Bleach (467 mL of 0.67 M aqueous solution) was added dropwise to the above mixture at room temperature. The reaction mixture was then stirred at room temperature under N_2 for 12 h. The THF was removed in vacuo. The aqueous mixture was diluted with aqueous NaOH (1 N) and then extracted with ethyl acetate. After residual organic solvents were removed from the aqueous mixture, it was acidified with concentrated HCl. A white precipitate formed, and it was filtered and dried to give 39.4 g of the desired product (74%). ^{1}H NMR (DMSO-d_6): δ 3.12 (m, 2H); 3.63 (s, 3H); 3.66 (d, 1H); 3.95 (d, 1H);7.68 (t, 1H); 7.85 (d, 1H); 7.95 (d, 1H); 8.04 (d, 1H); 8.12 (s, 1H).

2-(*tert*-Butylaminosulfonyl)phenylboronic Acid (11). To a solution of *N*-*tert*-butylbenzenesulfonamide (34.0 g, 0.16 mol) in 500 mL of THF under N_2 was added 160 mL (0.36 mol) of 2.25 M *n*-butyllithium in hexane over 35 min, keeping the temperature between 0−2 °C. The reaction mixture was allowed to warm to room temperature over 1.5 h, during which time a thick precipitate formed. Triisopropyl borate (46 mL, 0.20 mol) was added, keeping the temperature below 35 °C. After 1 h, the reaction mixture was cooled, 1 N HCl (260 mL) was added, and the mixture was stirred for 30 min. After dilution with 520 mL of water, the mixture was extracted with 3 × 400 mL of ether. The combined organic extracts were extracted with 3 × 250 mL of 1 N NaOH. The aqueous extracts were acidified to pH 1 with 6 N HCl and then extracted with 3 × 250 mL of ether. The ether extracts were washed with 250 mL of brine and dried over $MgSO_4$, and the solvents were removed in vacuo to yield 45 g of a thick oil. After addition of toluene (45 mL), the mixture was agitated for 1 h on the rotary evaporator. A small quantity of solid formed, which was used to induce partial solidification of the remaining crude product. Additional toluene (150 mL) was added, and the mixture was reduced to one-half volume in vacuo, keeping the temperature 0−10 °C. The resulting precipitate was collected and washed with hexane and then dried under vacuum to give 24.6 g (60%) of the title compound as white crystals. Mp: 118−119 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (s, 9H); 5.13 (s, 1H); 6.29 (br s, 2H); 7.53 (m, 2H); 7.82 (d, 1H); 8.00 (d, 1H).

2′-(*tert*-Butylaminosulfonyl)-4-amino[1,1′-biphenyl] (13). A mixture of 4-bromoaniline (3.44 g, 0.020 mol), 2-(*tert*-butylaminosulfonyl)phenylboronic acid (**11**) (5.14 g, 0.020 mol), tetrakis(triphenylphosphine)palladium(0) (1.16 g, 0.001 mol), tetrabutylammonium bromide (0.32 g, 0.001 mol), and aqueous sodium carbonate (20 mL of 2 M) was refluxed with 180 mL of benzene under N_2 for 5.5 h. After cooling, the mixture was diluted with methylene chloride and water. The two phases were separated, and the organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated. The resulting solid was chromatographed on silica gel with EtOAc/hexane (1:2) to afford 3.54 g of the desired biphenyl (58%). MS (ES^+): 305.3 $(M + H)^+$. ^{1}H NMR ($CDCl_3$): δ 0.99 (s, 9H); 3.72 (br s, 1H); 3.83 (br s, 2H); 6.76 (d, 1H); 7.27 (d, 1H); 7.33 (d, 2H); 7.43 (t, 1H); 7.53 (t, 1H); 8.14 (d, 1H).

5-Isoxazoleacetic Acid, 3-(3-Cyanophenyl)-5-[[[2′-(*tert*-butylaminosulfonyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester (14). 3-(3-Cyanophenyl)-5-(carbomethoxymethyl)isoxazoline-5-carboxylic acid (**9**) (0.50 g, 1.73 mmol) was refluxed with 10 mL of acetonitrile and 0.76 mL (10.4 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 evaporating to dryness. The resulting solid was dissolved in 20 mL of THF, and 2′-(*tert*-butylaminosulfonyl)-4-amino[1,1′-biphenyl] (**13**) (0.43 g, 1.40 mmol) was added, followed by triethylamine (1.5 mL, 10.4 mmol). The reaction mixture was stirred at room temperature, and the reaction was completed in less than 30 min. The mixture was diluted with ethyl acetate, 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 0.57 g of the desired product (71%). MS (ES^+): 575.2, $(M + H)^+$. ^{1}H NMR ($CDCl_3$): δ 0.95 (s, 9H); 3.03 (d, 1H); 3.27 (d, 1H); 3.60 (d, 1H); 3.66 (s, 3H); 3.78 (d, 1H);

7.19 (d, 1H); 7.39−7.71 (m, 8H); 7.83 (d, 1H); 7.92 (s, 1H); 8.09 (d, 1H); 8.68 (s, 1H).

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 (±) (15, SF303). Compound **14** (1.12 g, 1.95 mmol) was refluxed with 25 mL of trifluoroacetic acid under N_2 for 0.5 h. The TFA was removed in vacuo; the residue was dissolved in methylene chloride and then precipitated with ether to give 1.0 g of white solid (99%). MS (ES^+): 519.2 $(M + H)^+$. ^1H NMR ($CDCl_3$): δ 3.14 (d, 1H); 3.40 (d, 1H); 3.76 (s, 3H); 3.85 (dd, 2H); 4.40 (br s, 2H); 7.35 (d, 1H); 7.48−7.80 (m, 8H); 7.83 (d, 1H); 8.01 (s, 1H); 8.18 (d, 1H); 8.82 (s, 1H).

The sulfonamide (1.2 g, 1.93 mmol) was dissolved in 90 mL of $CHCl_3$ and 20 mL of MeOH. The reaction mixture was cooled in an ice bath, and HCl gas was bubbled in for 30 min until the solution was saturated. The mixture was sealed and placed at O °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 0.92 g (12.0 mmol) of ammonium acetate and 30 mL of methanol. The mixture was sealed and stirred at room temperature for 12 h. The solvent was removed. The crude benzamidine was purified by HPLC (C18 reverse phase) eluted with 0.05% TFA in H_2O/CH_3CN to give 0.47 g of the benzamidine TFA salt (37%). MS (ES^+): 536.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.20 (m, 2H); 3.48 (s, 3H); 3.70−4.01 (m, 2H); 7.20−7.32 (m, 4H); 7.52 (m, 2H); 7.72 (d, 2H); 7.88 (d, 1H); 7.98 (d, 1H); 8.05 (d, 1H); 8.07 (s, 1H); 9.24 (s, 2H); 9.40 (s, 2H); 10.05 (s, 1H). High-resolution MS ($C_{26}H_{25}N_5O_6S$): calcd, 536.1604; found, 536.1599. HPLC purity 95%.

The following compounds were prepared by the same methods described above for the synthesis of **15** (SF303) using appropriate starting materials.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (16). MS (ES^+): 457.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.18−3.35 (m, 2H); 3.61 (s, 3H); 3.80−4.10 (m, 2H); 7.34 (t, 2H); 7.45 (t, 2H); 7.60−7.94 (m, 7H); 8.08 (m, 2H); 9.03 (s, 2H); 9.42 (s, 2H); 10.10 (s, 1H). High-resolution MS ($C_{26}H_{24}N_4O_4$): calcd, 457.1876; found, 457.1893. HPLC purity 95%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[3′-methyl[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (17). High-resolution MS ($C_{27}H_{27}N_4O_4S$): calcd, 471.2032; found, 471.2049. HPLC purity 95%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-methyl[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (18). MS (ES^+): 471.5 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 2.23 (s, 3H); 3.18−3.32 (m, 2H); 3.64 (s, 3H); 3.83−4.03 (m, 2H); 7.16−7.30 (m, 6H); 7.60−7.79(m, 3H); 7.92 (d, 1H); 8.08 (m, 2H); 9.25 (bs, 2H); 9.43 (bs, 2H); 10.07 (bs, 1H). Anal. ($C_{27}H_{27}N_4O_4 \cdot 1.2TFA \cdot 1H_2O$) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[3′-(trifluoromethyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (19). MS (ES^+): 525.2 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.19−3.33 (m, 2H); 3.62 (s, 3H); 3.83−4.04 (m, 2H); 7.70−7.76 (m, 5H); 7.85−7.98 (m, 5H); 8.08 (m, 2H); 9.28 (bs, 4H); 10.17 (bs, 1H). Anal. ($C_{27}H_{24}F_3N_4O_4 \cdot 1TFA \cdot 0.5CH_3CN$) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-(trifluoromethyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (20). MS (ES^+): 525.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.12−3.32 (m, 2H); 3.59 (s, 3H); 3.76−4.03 (m, 2H); 7.23 (d, 2H); 7.34 (d, 1H); 7.59 (t, 1H); 7.67−7.79 (m, 5H); 7.87 (d, 1H); 8.05 (m, 2H); 9.04 (s, 2H); 9.38 (s, 2H); 10.10 (s, 1H). High-resolution MS ($C_{27}H_{23}F_3N_4O_4$): calcd, 525.1736; found, 525.1733. HPLC purity 97%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[3′-methoxy[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (21). MS (ES^+): 487.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.18−3.32 (m, 2H); 3.61 (s, 3H); 3.81 (s, 3H); 3.84−4.04 (m, 2H); 6.90 (d, 1H); 7.22 (d, 1H); 7.36 (t, 1H); 7.65 (m, 3H); 7.73 (t, 1H); 7.81 (d, 2H); 7.91 (d, 1H); 8.09 (m, 2H); 9.06 (bs, 2H); 9.42 (bs, 2H); 10.01 (s, 1H). High-resolution MS ($C_{27}H_{27}N_4O_5$): calcd, 487.1981; found, 487.1998. HPLC purity 96%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-methoxy[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (22). MS (ES^+): 487.3 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.18−3.32 (m, 2H); 3.62 (s, 3H); 3.75 (s, 3H); 3.82−4.03 (m, 2H); 7.01 (t, 1H); 7.10 (d, 1H); 7.26−7.32 (m, 2H); 7.42 (d, 2H); 7.72 (m, 3H); 7.91 (d, 1H); 8.10 (m, 2H); 9.09 (bs, 2H); 9.43 (bs, 2H); 10.06 (s, 1H). High-resolution MS ($C_{27}H_{27}N_4O_5$): calcd, 487.1981; found, 487.1990. HPLC purity 96%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[3′-(aminosulfonyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (23). MS (ES^+): 536.5 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.18−3.33 (m, 2H); 3.62 (s, 3H); 3.83−4.04 (m, 2H); 7.21 (bs, 2H); 7.60−7.76 (m, 5H); 7.86−7.92 (m, 4H); 8.10 (m, 3H); 9.20 (bs, 2H); 9.42 (bs, 2H); 10.16 (bs, 1H). Anal. ($C_{26}H_{26}N_5O_5S \cdot 1.15TFA \cdot 1.6H_2O$) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-(thiomethyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (24). MS (ES^+): 503.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 2.36 (s, 3H); 3.19−3.32 (m, 2H); 3.62 (s, 3H); 3.83−4.04 (m, 2H); 7.18 (m, 2H); 7.33 (m, 4H); 7.75 (m, 3H); 7.91 (d, 1H); 8.09 (m, 2H); 9.09 (s, 2H); 9.42 (s, 2H); 10.10 (s, 1H). High-resolution MS ($C_{27}H_{26}N_4O_4S$): calcd, 503.1713; found, 503.1718. HPLC purity 92%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-(carbomethoxy)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (25). MS (ES^+): 515.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.20 (m, 2H); 3.57 (s, 3H); 3.58 (s, 3H); 3.79−4.00 (m, 2H); 6.52 (d, 1H); 7.21 (d, 2H); 7.41 (m, 2H); 7.57 (m, 1H); 7.71 (m, 4H); 7.87 (d, 1H); 8.06 (m, 2H); 8.88 (s, 2H); 9.39 (s, 2H); 10.06 (s, 1H). High-resolution MS ($C_{28}H_{26}N_4O_6$): calcd, 515.1931; found, 515.1910. HPLC purity 95%.

5-Isoxazoleacetic Acid, 3-[3-(aminoiminomethyl)phenyl]-5-[[[2′-(methylsulfonyl)[1,1′-biphenyl]-4-yl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (26). MS (ES^+): 535.4 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 2.78 (s, 3H); 3.16−3.29 (m, 2H); 3.59 (s, 3H); 3.79−4.01 (m, 2H); 7.33 (m, 4H); 7.70 (m, 5H); 7.87 (d, 1H); 8.05 (m, 3H); 8.99 (s, 2H); 9.38 (s, 2H); 10.13 (s, 1H). High-resolution MS ($C_{27}H_{26}N_4O_6S$): calcd, 535.1651; found, 535.1646. HPLC purity 95%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-(aminosulfonyl)-3-methyl[1,1′-biphenyl]-4-yl]-amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (27). MS (ES^+): 550.5 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 2.21 (s, 3H); 3.19−3.31 (m, 2H); 3.63 (s, 3H); 3.91−3.99 (m, 2H); 7.23 (m, 3H); 7.39 (d, 4H); 7.57 (m, 2H); 7.75 (m, 1H); 7.91 (d, 1H); 8.01 (d, 1H); 8.06 (t, 1H); 8.10 (bs, 1H); 9.08 (s, 2H); 9.41 (s, 2H); 9.56 (bs, 1H). High-resolution MS ($C_{27}H_{27}N_5O_6S$): calcd, 550.1760; found, 550.1742. HPLC purity 97%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[2′-(aminosulfonyl)-3-fluoro[1,1′-biphenyl]-4-yl]-amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (28). MS (ES^+): 554.2 $(M + H)^+$. ^1H NMR (DMSO-d_6): δ 3.21−3.37 (m, 2H); 3.64 (s, 3H); 3.85−3.99 (m, 2H); 7.21 (d, 1H); 7.33 (d, 1H); 7.41 (s, 2H); 7.61 (m, 2H); 7.73 (m, 2H); 7.91 (d, 1H); 8.03 (d, 1H); 8.09 (d, 1H); 8.11 (bs, 2H); 9.07 (s, 2H); 9.43 (s, 2H); 9.71 (bs, 1H). High-resolution MS ($C_{26}H_{24}FN_5O_6S$): calcd, 554.1510; found, 554.1517. HPLC purity 95%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[5-[2′-(aminosulfonyl)phenyl]-3-pyridinyl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (29). MS (ES^+): 537.2 $(M + H)^+$. ^1H NMR

(DMSO-d_6): δ 3.12−3.37 (m, 2H); 3.59 (s, 3H); 3.78−4.08 (m, 2H); 7.41 (bs, 2H); 7.50−7.76 (m, 5H); 7.86 (d, 1H); 7.97−8.10 (m, 3H); 8.22 (m, 1H); 8.98 (d, 1H); 9.10 (s, 2H); 9.38 (s, 2H); 10.42 (s, 1H). Anal. ($C_{25}H_{24}N_6O_6S$·2TFA·0.5H_2O) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[5-[2′-(aminosulfonyl)phenyl]-2-pyridinyl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (30). MS (ES^+): 537.3 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.22−3.45 (m, 2H); 3.62 (s, 3H); 3.80−4.13 (m, 2H); 7.38 (d, 1H); 7.42 (bs, 2H); 7.58−7.60 (m, 3H); 7.86 (d, 1H); 7.96 (d, 1H); 8.08 (m, 2H); 8.15 (s, 1H); 8.32 (s, 1H); 9.35 (s, 2H); 9.52 (s, 2H); 9.80 (s, 1H). Anal. ($C_{25}H_{24}N_6O_6S$·TFA·2H_2O) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(aminoiminomethyl)phenyl]-5-[[[5-[2′-(aminosulfonyl)phenyl]-2-pyrimidinyl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (±) (31). MS (ES^+): 538.2 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.20−3.40 (m, 2H); 3.60 (s, 3H); 3.78−4.08 (m, 2H); 7.38 (d, 1H); 7.42 (bs, 2H); 7.58−7.60 (m, 3H); 7.86 (d, 1H); 8.00−8.10 (m, 3H); 8.62 (s, 2H);9.02 (s, 2H); 9.38 (s, 2H); 10.06 (s, 1H). Anal. ($C_{24}H_{23}N_7O_6S$·1.3TFA·0.8H_2O) C, H, N.

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 (+) (32). Optical rotation +51° (acetonitrile, 0.100 g/mL, 25 °C); >99% ee. MS (ES^+): 536.2 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.10−3.22 (m, 2H); 3.58 (s, 3H); 3.78−4.05 (m, 2H); 7.17−7.34 (m, 6H); 7.48−7.61 (m, 2H); 7.64−7.75 (m, 2H); 7.85 (d, 1H); 7.98 (d, 1H); 8.05 (m, 2H); 9.13 (s, 2H); 9.40 (s, 2H); 10.05 (s, 1H). High-resolution MS ($C_{26}H_{25}N_5O_6S$): calcd, 536.1638; found, 536.1598. HPLC purity 96%.

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 (−) (33). Optical rotation −63.2° (acetonitrile, 0.242 g/mL, 25 °C); >98% ee. MS (ES^+) 536.2 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.25 (m, 2H); 3.60 (s, 3H); 3.92−4.03 (m, 2H); 7.22 (bs, 2H); 7.35 (m, 5H); 7.58 (m, 2H); 7.76 (m, 3H); 7.91 (d, 1H); 8.05 (d, 1H); 8.09 (m, 2H); 9.08 (s, 2H); 9.42 (s, 2H); 10.10 (s, 1H). Anal. ($C_{26}H_{25}N_5O_6S$·TFA) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[5-[2′-(aminosulfonyl)phenyl]-2-pyrimidinyl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (+) (34). Optical rotation +49.38° (acetonitrile, 0.162 g/mL, 25 °C); 95% ee. MS (ES^+): 538.2 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.28−3.45 (m, 2H); 3.65 (s, 3H); 3.80−4.10 (m, 2H); 7.44 (m, 1H); 7.52 (bs, 2H); 7.58−7.64 (m, 3H); 7.92 (d, 1H); 8.03−8.15 (m, 3H); 8.68 (s, 2H); 9.13 (s, 2H); 9.44 (s, 2H); 10.12 (s, 1H). Anal. ($C_{24}H_{23}N_7O_6S$·1.3TFA·1H_2O) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[5-[2-(aminosulfonyl)phenyl]-2-pyrimidinyl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (−) (35, SF324). Optical rotation −47.01° (acetonitrile, 0.134 g/mL, 25 °C); 95% ee. MS (ES^+): 538.2 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.28−3.45 (m, 2H); 3.65 (s, 3H); 3.80−4.10 (m, 2H); 7.44 (m, 1H); 7.52 (bs, 2H); 7.58−7.64 (m, 3H); 7.92 (d, 1H); 8.03−8.15 (m, 3H); 8.68 (s, 2H); 9.13 (s, 2H); 9.44 (s, 2H); 10.12 (s, 1H). Anal. ($C_{24}H_{23}N_7O_6S$·1TFA·0.5H_2O) C, H, N.

5-Isoxazoleacetic Acid, 3-[3-(aminoiminomethyl)phenyl]-5-[[[5-[2-(aminosulfonyl)phenyl]-2-pyridinyl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (+) (36). Optical rotation +53.15° (acetonitrile, 0.222 g/mL, 25 °C); 84% ee. MS (ES^+): 537.3 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.22−3.45 (m, 2H); 3.60 (s, 3H); 3.80−4.15 (m, 2H); 7.38 (d, 1H); 7.42 (bs, 2H); 7.58−7.60 (m, 3H); 7.86 (d, 1H); 7.96 (d, 1H); 8.08 (m, 2H); 8.15 (s, 1H); 8.32 (s, 1H); 9.35 (s, 2H); 9.52 (s, 2H); 9.80 (s, 1H). High-resolution MS ($C_{25}H_{24}N_6O_6S$): calcd, 537.1556; found, 537.1559. HPLC purity 98%.

5-Isoxazoleacetic Acid, 3-[3-(Aminoiminomethyl)phenyl]-5-[[[5-[2-(aminosulfonyl)phenyl]-2-pyridinyl]amino]carbonyl]-4,5-dihydro-, Methyl Ester, Trifluoroacetic Acid Salt (−) (37). Optical rotation −59.64° (acetonitrile, 0.166 g/mL, 25 °C); 99% ee. MS (ES^+): 537.3 $(M + H)^+$. ^{1}H NMR (DMSO-d_6): δ 3.22−3.45 (m, 2H); 3.60 (s, 3H); 3.80−4.15 (m, 2H); 7.38 (d, 1H); 7.42 (bs, 2H); 7.58−7.60 (m, 3H); 7.86 (d, 1H); 7.96 (d, 1H); 8.08 (m, 2H); 8.15 (s, 1H); 8.32 (s, 1H); 9.35 (s, 2H); 9.52 (s, 2H); 9.80 (s, 1H). High-resolution MS ($C_{25}H_{24}N_6O_6S$): calcd, 537.1556; found, 537.1548. HPLC purity 98%.

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