Orally Active Isoxazoline Glycoprotein IIb/IIIa Antagonists with Extended Duration of Action

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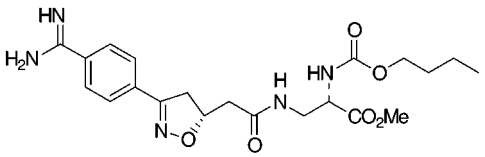
Modification of the α-carbamate substituent of isoxazoline GPIIb/IIIa ($\alpha_{IIb}\beta_3$) antagonist DMP 754 (**7**) led to a series of α-sulfonamide and α-sulfamide diaminopropionate isoxazolinylacetamides which were found to be potent inhibitors of in vitro platelet aggregation. Aryl- and heteroaryl-α-sulfonamide groups, in conjunction with (5*R*)-isoxazoline (2*S*)-diaminopropionate stereochemistry, were found to impart a pronounced duration of antiplatelet effect in dogs, potentially due to high affinity for unactivated platelets. Isoxazolylsulfonamide **34b** (DMP 802), a highly selective GPIIb/IIIa antagonist, demonstrated a prolonged duration of action after iv and po dosing and high affinity for resting and activated platelets. The prolonged antiplatelet profile of DMP 802 in dogs and the high affinity of DMP 802 for human platelets may be predictive of clinical utility as a once-daily antiplatelet agent.

Introduction

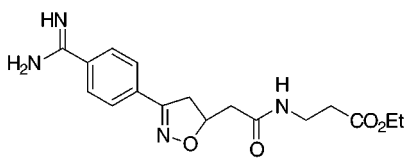
Arterial occlusive disorders remain a major cause of morbidity and mortality despite significant advances in their diagnosis and treatment. Occlusion of arteries can occur in association with atherosclerotic processes or as a result of vessel wall insult caused by invasive coronary intervention.[1] Arterial thrombosis is largely platelet-mediated and is dependent upon platelet adhesion, activation, and aggregation. Antiplatelet agents such as aspirin and ticlopidine, which predominantly target platelet activation pathways, have found widespread use in the treatment and prevention of arterial thrombosis. However, because of the variety of means through which platelets can be activated, the interruption of any single platelet agonistic pathway leads to limited efficacy.[2]

Independent of the means by which platelets become activated, the final and obligatory step in the formation of platelet aggregates involves the cross-linking of platelets by plasma fibrinogen or other matrix proteins. This process is mediated primarily by the platelet fibrinogen receptor, glycoprotein IIb/IIIa (GPIIb/IIIa, $\alpha_{IIb}\beta_3$), which becomes competent to bind fibrinogen upon platelet activation.[3] Proposals regarding the mechanism by which fibrinogen interacts with GPIIb/IIIa have implicated the RGD sequences located in the α-chain[4] and residues 401−411 in the γ-chain.[5] Small molecules designed as mimetics of the Arg-Gly-Asp sequence present in the fibrinogen α-chain have proven to be potent and specific antagonists of the fibrinogen−GPIIb/IIIa interaction in vitro. Such agents feature appropriately oriented Arg-guanidine mimics and carboxylate groups appended to a variety of peptidic and nonpeptidic core structures.[6,7] Peptide and peptidomimetic antagonists of GPIIb/IIIa have been shown to effectively block in vitro or ex vivo platelet aggregation as well as arterial thrombosis in various animal models.[8] Several orally active nonpeptide GPIIb/IIIa antagonists (Figure 1) are in advanced stages of clinical evaluation.[9−15] Results from clinical trials of GPIIb/IIIa antagonists suggest that substantial inhibition of platelet aggregation may be achieved without unduly compromising hemostasis and that GPIIb/IIIa blockade may lower the risk of thrombotic events in the context of unstable angina and following coronary angioplasty.[16−18]

We recently reported that isoxazolinylacetamides of β-alanine are highly effective frameworks for the display of the basic and acidic functionality requisite to high GPIIb/IIIa affinity[19] and that the α-carbamate diaminopropionate **1** (DMP 754)[20−22] provides high oral potency and prolonged antiplatelet effects in dogs as compared to the α-unsubstituted analogue **2** (XR300).[19] The use of N-α-substituted diaminopropionates in other



DMP 754, **1**



XR300, **2**

series of GPIIb/IIIa antagonists has been recently reported.[23−31] The use of α-sulfonamide substituents in GPIIb/IIIa antagonists containing piperidine Arg replacements has led to antiplatelet agents with extended (≥8 h) duration of action.[26−29] In other GPIIb/IIIa

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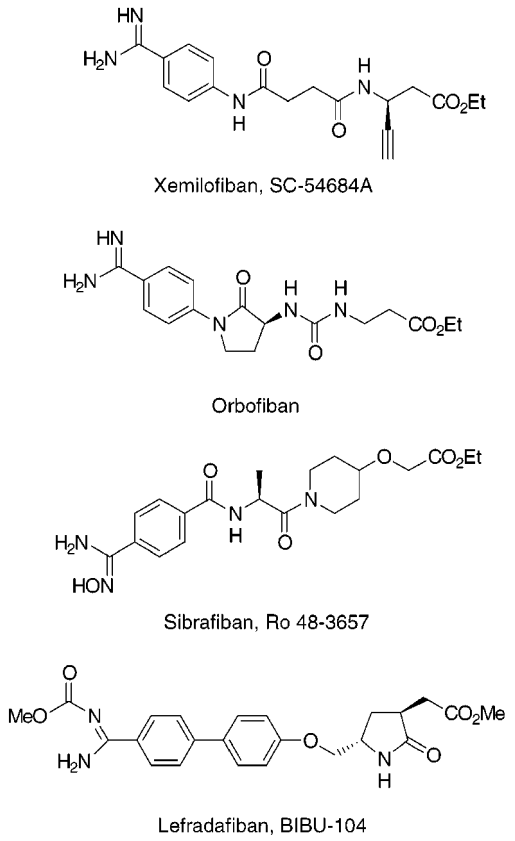


Figure 1. Representative oral nonpeptide GPIIb/IIIa antagonists in clinical development.

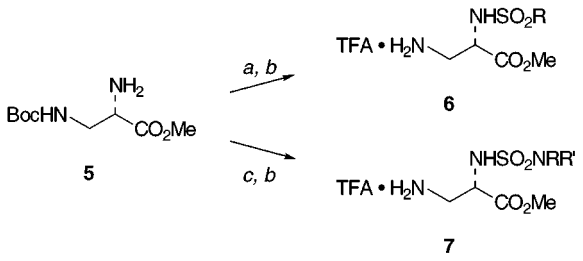
antagonists featuring heterocyclic amidines as Arg mimics, prolonged plasma levels of active species were not demonstrated.[31] In our search for GPIIb/IIIa antagonists with the potential for use in chronic therapy, we have sought agents with pharmacologic profiles consistent with once-daily administration. Following oral administration to dogs and rhesus monkeys, **1** was found to provide >60−90% inhibition of platelet aggregation for 12 h. Minimal antiplatelet effect remained at 24 h in these species. In the baboon, substantial antiplatelet inhibition was extended to ≥24 h.[20−22] Because of uncertainty over the predictability of the various animal species with respect to clinical pharmacodynamics, our efforts to identify GPIIb/IIIa antagonists showing extended duration of action continued. This report describes further investigations into the effect of α-substitution on the duration of antiplatelet activity and the identification of **34b** (DMP 802), a potent, orally active isoxazolinyl diaminopropionate bearing an α-heterocyclic sulfonamide substituent which demonstrates 24-h duration of action in dogs.

Chemistry

Synthesis of Isoxazolinylacetic Acids. (*R,S*)-(4-Cyanophenyl)isoxazolinylacetic acid (**3**), the corresponding (*R*)- and (*S*)-enantiomers, and the (*R,S*)-(4-*N*-Boc-amidinophenyl)isoxazolinylacetic acid (**4**) were synthesized as described previously.[19,20]

***N*^{2}-Sulfonyldiaminopropionate Derivatives.** Alkyl-, aryl-, and heteroarylsulfonyl chlorides were available from commercial sources or were prepared using literature methods. Sulfamoyl chlorides were prepared using the methods of Kloek and Leschinsky[32] or Audrieth and Sveda.[33] Methyl N^2-sulfonyl- (**6**) and N^2-(aminosulfonyl)-2,3-diaminopropionates (**7**) were prepared from methyl N^3-(*tert*-butyloxycarbonyl)-2,3-diaminopropionate (**5**)[24] and the appropriate sulfonyl or sulfamoyl chlorides, as shown in Scheme 1.

Scheme 1. Preparation of Sulfonamide and Sulfamide Diaminopropionates[a]



[a] Reagents: (a) RSO_2Cl, Et_3N, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) $RR'NSO_2Cl$, Et_3N, CH_2Cl_2.

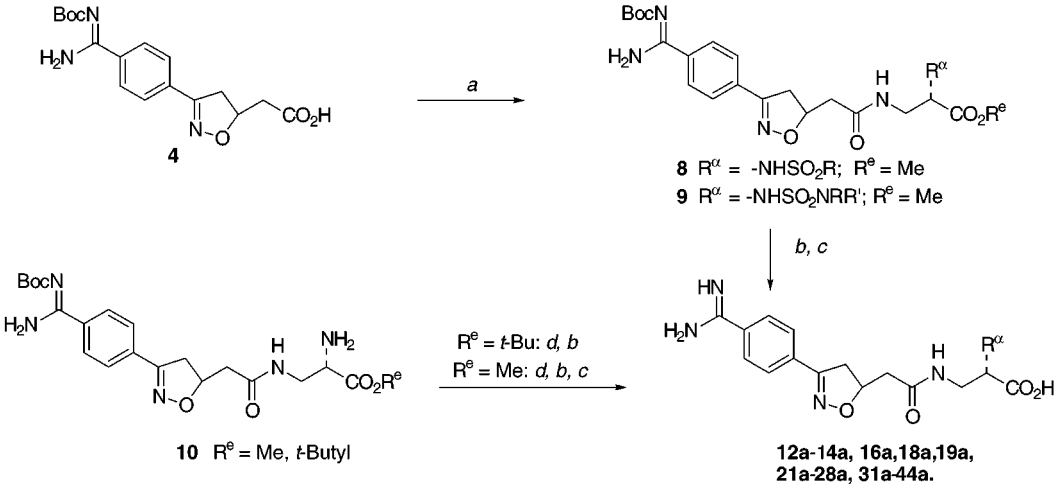
Isoxazolinylacetamides and Elaboration of Final Products. Coupling of Boc-amidine isoxazolinylacetic acids **4** and various methyl diaminopropionates provided the desired isoxazolinylacetamides **8** and **9**, which upon deprotection gave the free amidine carboxylates **12a**−**14a**, **16a**, **18a**, **19a**, **21a**−**28a**, and **31a**−**44a**[34] (Scheme 2).[20] Alternatively, the desired sulfonamides and sulfamides could be obtained by derivatization of the free amine of Boc-amidine ester **10** with appropriate sulfonyl and sulfamoyl chlorides. The methyl ester derivative of Boc-amidine **10** was prepared as previously described.[20] Coupling of the *tert*-butyl ester of N^2-Cbz-diaminopropionate[35] with the protected isoxazolinyl acetate **4** using TBTU provided the *tert*-butyl ester of **10**. The presence of the *tert*-butyl ester allowed the simultaneous deprotection of the amidine and ester groups at the end of the synthesis. Analogous use of the scalemic nitrile acids **3**, followed by a Pinner amidine synthesis, allowed access to the individual isoxazoline diaminopropionate diastereomers (Scheme 3).

Selective methylation of the sulfonamide nitrogen of nitrile ester **11** was carried out under Mitsunobu conditions, as shown in Scheme 4, which provided a route to the N-methylated sulfonamide **29b**. Coupling of methyl N^2-(*m*-tolylsulfonamido)diaminopropionate with 3-(4-*N*-Bocamidinophenyl)isoxazolyl-5-acetic acid,[20] deprotection of the amidine, and saponification provided the isoxazole analogue **30** (Scheme 5). Separation of the isoxazoline C5 diastereomers of the final products using chiral chromatography[20] provided an alternative means of obtaining the individual isomers. The stereochemical assignment was confirmed in the case of **34b** (DMP 802) by X-ray crystallographic analysis.

Results and Discussion

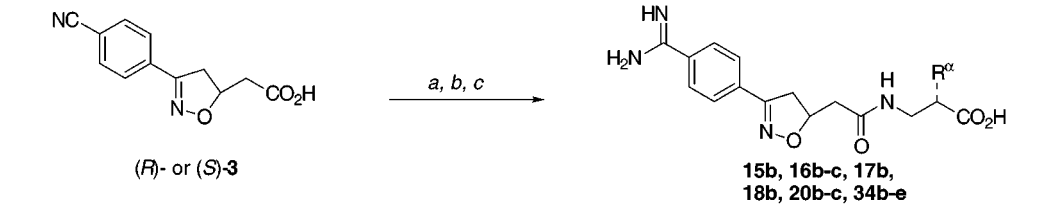
The sulfonamides **12**−**39** and sulfamides **40**−**44** were assessed for their ability to inhibit ADP-stimulated platelet aggregation in platelet-rich plasma (PRP).[36] α-Sulfonamide and α-sulfamide functionality at the carboxy-terminus was found to provide GPIIb/IIIa antagonists with submicromolar in vitro potency; the majority of the compounds in this series showed IC_{50}'s in this assay ≤100 nM. As shown by the entries in Tables 1−3, substituents with a variety of steric and electronic properties were tolerated at the α-position. As demonstrated by the activities of **29b** and **20b**,

Scheme 2. Synthesis of (*R*,*S*)-Isoxazolinyl Diaminopropionate Acetamides[a]



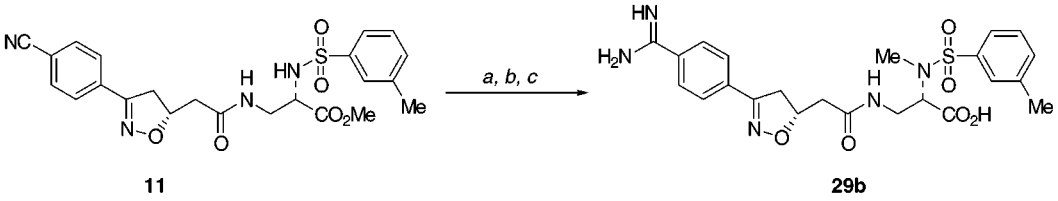
[a] Reagents: (a) **6** or **7**, TBTU, Et_3N; (b) TFA, CH_2Cl_2; (c) LiOH or 6 N HCl; (d) RSO_2Cl or $RR'NSO_2Cl$, Et_3N, CH_2Cl_2.

Scheme 3. Chiral Synthesis of Isoxazolinyl Diaminopropionate Acetamides[a]



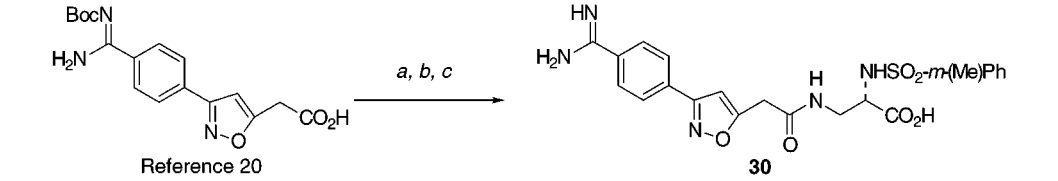
[a] Reagents: (a) **6** or **7**, TBTU, Et_3N, DMF; (b) HCl (anhydrous), MeOH, 0 °C, then NH_3 or $(NH_4)_2CO_3$ or NH_4OAc, MeOH, rt; (c) LiOH, 6 N HCl, or rabbit liver esterase.

Scheme 4. Synthesis of *N*-Methylsulfonamide **29b**[a]



[a] Reagents: (a) DEAD/Ph_3P/MeOH (1:1:1), THF; (b) HCl (anhydrous), MeOH, 0 °C, then NH_3, MeOH, rt; (c) LiOH.

Scheme 5. Synthesis of Isoxazolyl Diaminopropionate Acetamide **30**[a]



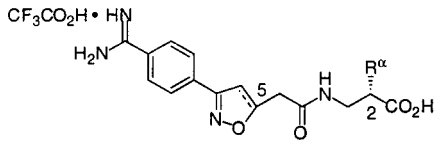
[a] Reagents: (a) methyl N^2-(*m*-tolylsulfonamido)diaminopropionate, TBTU, Et_3N, DMF; (b) TFA−CH_2Cl_2 (1:1), rt; (c) LiOH, H_2O−MeOH.

N-methylation of the sulfonamide caused a loss of potency, as observed in the previously reported α-carbamate series.[20]

The effect of stereochemistry at the isoxazolinyl C5 center on in vitro activity (PRP assay) was studied. In the case of the *m*-tolylsulfonamides, the (*R*)- and (*S*)-C5 diastereomers **20b**,**c**, as well as the planar isoxazole **30**, showed similar activity. In the case of the heterocyclic sulfonamide **34**, the effect of the diaminopropionate stereochemistry was also investigated. With the (*R*)-isoxazoline isomers **34b** (DMP 802) and **34d**, both diaminopropionate configurations gave highly active GPIIb/IIIa antagonists. In the (*S*)-isoxazoline series, the (*S*)-diaminopropionate isomer **34c** demonstrated PRP activity comparable to that of DMP 802 and **34d**, whereas the (*S*),(*R*)-diastereomer **34e** was significantly less potent. This pattern of activity in the PRP assay was previously noted for the diastereomers of DMP 754.[20] New α-substituents were generally tested as (*S*)-diaminopropionate isoxazoline C5 diastereomeric mixtures.

With respect to the structure of the α-substituent, the potent inhibition of platelet function observed in the PRP assay across this series did not allow the development of definitive structure−activity relationships. With the objective of identifying compounds with extended duration of action, we distinguished the potent IIb/IIIa antagonists of the present study by assessing their in

Table 1. In Vitro Potencies of Sulfonamides **12**−**30**



Compound	R^α	5	IC_{50} ± SEM, μM, hPRP[a]
DMP 754	-	(*R*)-	0.050 ± 0.0028[b]
12a	$NHSO_2(CH_2)_2CH_3$	(*R,S*)-	0.017 ± 0.0038
13a	$NHSO_2(CH_2)_3CH_3$	(*R,S*)-	0.042 ± 0.016
14a	$NHSO_2CH_2Ph$	(*R,S*)-	0.039 ± 0.0092
15b	$NHSO_2Ph$	(*R*)-	0.190 ± 0.018
16a	$NHSO_2(2\text{-}CH_3)Ph$	(*R,S*)-	0.041 ± 0.0087
16b	$NHSO_2(2\text{-}CH_3)Ph$	(*R*)-	0.130 ± 0.040
16c	$NHSO_2(2\text{-}CH_3)Ph$	(*S*)-	0.110 ± 0.025
17b	$NHSO_2(2\text{-}CF_3)Ph$	(*R*)-	0.089 ± 0.016
18a	$NHSO_2(2\text{-}Br)Ph$	(*R,S*)-	0.036 ± 0.010
18b	$NHSO_2(2\text{-}Br)Ph$	(*R*)-	0.036 ± 0.013
19a	$NHSO_2(2\text{-}Ph)Ph$	(*R,S*)-	0.080 ± 0.0048
20b	$NHSO_2(3\text{-}CH_3)Ph$	(*R*)-	0.025 ± 0.010[c]
20c	$NHSO_2(3\text{-}CH_3)Ph$	(*S*)-	0.035 ± 0.011[d]
21a	$NHSO_2(3\text{-}CF_3)Ph$	(*R,S*)-	0.120 ± 0.025
22a	$NHSO_2(3\text{-}Br)Ph$	(*R,S*)-	0.060 ± 0.0012
23a	$NHSO_2(4\text{-}CH_3)Ph$	(*R,S*)-	0.037 ± 0.011
24a	$NHSO_2(4\text{-}CF_3)Ph$	(*R,S*)-	0.170 ± 0.012
25a	$NHSO_2(4\text{-}n\text{-}Pr)Ph$	(*R,S*)-	0.130 ± 0.022
26a	$NHSO_2(4\text{-}Br)Ph$	(*R,S*)-	0.051 ± 0.014
27a	$NHSO_2$-1-naphthyl	(*R,S*)-	0.055 ± 0.0012
28a	$NHSO_2$-2-naphthyl	(*R,S*)-	0.14[e]
29b	$N(CH_3)SO_2(3\text{-}CH_3)Ph$	(*R*)-	0.095 ± 0.010
30	$NHSO_2(3\text{-}CH_3)Ph$	isoxazole	0.069 ± 0.0061

[a] Inhibition of ADP-induced platelet aggregation was determined in three donors. See ref 36 for protocol. [b] IC_{50} determined for free acid of DMP 754.[21] [c] See refs 42 and 43. [d] Determined for HCl salt. [e] Result of one determination.

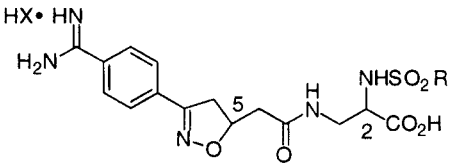
vivo profile in dogs. Selected compounds having an IC_{50} less than approximately 100 nM in the PRP assay were assessed for their ability to inhibit ex vivo platelet aggregation in dogs after administration of an iv bolus dose of 0.025 mg/kg (Table 4). Using this protocol, the reference α-carbamate **1** (DMP 754), as the free acid,[20] completely inhibited ex vivo aggregation at 1 h (>99%) and showed approximately 40% inhibition at 5 h. The antiplatelet activity at the 5 h time point proved useful in differentiating the compounds of the present study. Thus, α-*n*-alkylsulfonamides **12a** and **13a**[20] and the isobutylsulfamide **41a** were found to have in vivo duration in dogs comparable to that of the free acid of **1** (Table 4). However, the α-aryl-, heteroaryl-, and benzylsulfonamides showed enhanced antiplatelet effects at 5 h postdose. In some cases, complete inhibition of ex vivo platelet aggregation was present for ≥24 h. Prolonged in vivo duration was often associated with ortho substitution, as seen with the *o*-methyl- (**16a**), *o*-bromo- (**18a**), and *o*-phenyl- (**19a**) phenylsulfonamides and the quinolinylsulfonamide **39a** (Table 4). The antiplatelet activity of *o*-tolylsulfonamide **16a** was followed for several days; significant inhibition of ex vivo aggregation was still present at 96 h (Figure 2). Ortho substitution, however, was not essential to prolonged antiplatelet action, as demonstrated by thiazolylsulfonamide (**37a**).

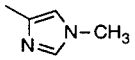
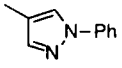
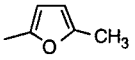
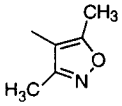
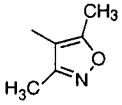
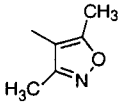
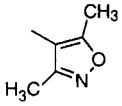
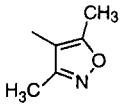
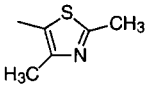
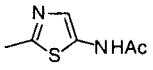
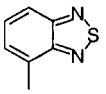
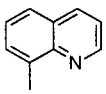
As previously discussed, the isoxazoline C5 configuration did not appear to strongly influence the in vitro activity (PRP); however, the stereochemistry at this center was found to affect duration of action in vivo. In the (*S*)-diaminopropionate *m*-tolylsulfonamide series, the (5*R*)-isoxazoline isomer **20b** showed 94% inhibition of ex vivo platelet aggregation at 5 h, while the (5*S*)-isomer **20c** demonstrated 64% inhibition at the same time point. Interestingly, the planar isoxazole analogue **30**, while essentially equipotent in the PRP aggregation assay, provided only 19% ex vivo antiplatelet activity at 5 h (Figure 3). The effects of both isoxazoline and diaminopropionate stereochemistry were studied among the isomers of the dimethylisoxazolesulfonamide (DMP 802) series (**34b**−**34e**). The (5*R*)-isoxazoline (2*S*)-diaminopropionate diastereomer proved superior in terms of in vivo profile (Figure 4) although the (5*S*),(2*S*)- (**34c**) and (5*R*),(2*R*)- (**34d**) isomers had comparable in vitro activity.

As shown by the in vitro data discussed above, the PRP assay did not distinguish the compounds in the present series from α-carbamates such as **1** (DMP 754).[20] The limitations of PRP aggregation assays include a sensitivity limited by the concentration of GPIIb/IIIa receptors in the assay mixture (~20−40 nM),[27] as well as the inability to distinguish the effects of protein binding from inherent GPIIb/IIIa affinity. Recently, certain GPIIb/IIIa antagonists having diaminopropionate carboxy-termini have been shown to possess considerable affinity for the unactivated platelet GPIIb/IIIa receptor, a further property not assessed by the PRP assay.[20−22,27,37] Affinity for unactivated GPIIb/IIIa receptors and resting platelets has been proposed as a mechanism to decrease clearance and prolong antiplatelet effects of GPIIb/IIIa antagonists.[27,38,39] Analogous target-mediated clearance of ACE inhibitors from plasma[40] and of benzodiazepines from cerebrospinal fluid[41] has been reported. The inhibition of aggregation in platelet-rich dog plasma, as well as the dissociation constants for activated and unactivated dog platelets, was measured for the stereoisomers of **34** (Table 5) (see Experimental Section). Significantly, **34b** (DMP 802), the (5*R*)-isoxazoline (2*S*)-diaminopropionate isomer, showed the highest affinity for unactivated dog platelets and the longest duration of action in dogs. DMP 802 also demonstrated higher affinity for dog platelets than the free acid of **1** (DMP 754).

Selected sulfonamides demonstrating persistent antiplatelet activity after iv dosing were compared for antiplatelet effects in dogs after oral doses of 0.1 mg/kg as the (5*R*),(2*S*)-isomers. Prolonged antiplatelet activity was shown by the arylsulfonamides **16b, 18b**, and **20b**[42,43] and by the isoxazolylsulfonamide **34b** (DMP 802) (Table 6). Improved potency and prolonged dura-

Table 2. In Vitro Potencies of Heterocyclic Sulfonamides **31**−**39**



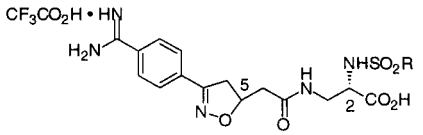
Cpd. No.	R	5	2	HX	IC_{50} ± SEM, μM hPRP[a]
DMP 754	-	(*R*)-	(*S*)-	TFA	0.050 ± 0.0028[b]
31a		(*R,S*)-	(*S*)-	TFA	0.110 ± 0.046
32a		(*R,S*)-	(*S*)-	TFA	0.072 ± 0.0093
33a		(*R,S*)-	(*S*)-	TFA	0.160 ± 0.0088
34a		(*R,S*)-	(*S*)-	TFA	0.034 ± 0.011
34b		(*R*)-	(*S*)-	MsOH	0.029 ± 0.0042
34c		(*S*)-	(*S*)-	MsOH	0.029 ± 0.012
34d		(*R*)-	(*R*)-	MsOH	0.023 ± 0.0059
34e		(*S*)-	(*R*)-	MsOH	0.120 ± 0.034
35a		(*R,S*)-	(*S*)-	TFA	0.024 ± 0.0087
36a		(*R,S*)-	(*S*)-	TFA	0.036 ± 0.012
37a		(*R,S*)-	(*S*)-	TFA	0.053 ± 0.014
38a		(*R,S*)-	(*S*)-	TFA	0.091 ± 0.0048
39a		(*R,S*)-	(*S*)-	TFA	0.081 ± 0.0019

[a,b] See corresponding footnotes for Table 1.

tion of action with respect to DMP 754 were observed in dogs, particularly with sulfonamides **16b**, **20b,** and DMP 802. However, the >96-h duration of antiplatelet activity of **16b** was deemed undesirable. In reviewing the physical properties of the orally active analogues **20b** and DMP 802, the solubility of DMP 802, a crystalline mesylate salt, was found to be 18 mg/mL at pH 1.7 and 0.82 mg/mL at pH 5−7, which was considered sufficient for consistent oral dosing.

Further characterization of **34b** (DMP 802) revealed a high selectivity for the GPIIb/IIIa receptor over the $\alpha_4\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$ integrins in vitro (Table 7). The affinity (K_d) of DMP 802 for resting human platelets[44] was found to be higher (0.57 nM) and the K_{off} longer (32 min) than those of the free acid of DMP 754[22] (2.5 nM and 7 min, respectively), potentially predictive of a prolonged antiplatelet effect in vivo. In dogs, DMP 802 demonstrated a long duration of ex vivo antiplatelet effect after oral doses of 0.05 and 0.10 mg/kg (Table 6, Figure 5) with significant antiplatelet activity still present at 24 h. The bioavailability of DMP 802 in dogs after doses of 0.025 mg/kg iv and 0.1 mg/kg po was found to be 14.9% with a terminal half-life after oral dosing of 13−17 h.[44] Clearance was dose-dependent,

Table 3. In Vitro Potencies of Sulfamides **41**−**45**



Cpd No.	R	5	IC_{50} ± SEM, μM hPRP[a]
DMP 754	-	(*R*)-	0.050 ± 0.0028[b]
40a	$NHCH(CH_3)_2$	(*R,S*)-	0.051 ± 0.0075
41a	$NH\ CH_2CH(CH_3)_2$	(*R,S*)-	0.052 ± 0.0049
42a	$N(CH_3)CH_2Ph$	(*R,S*)-	0.086 ± 0.018
43a	$NH(CH_2)_2Ph$	(*R,S*)-	0.070 ± 0.0027
44a	$NH(4\text{-}CH_3)Ph$	(*R,S*)-	0.072 ± 0.0035

[a,b] See corresponding footnotes for Table 1.

increasing from 0.55 to 4.5 mL/min/kg, after iv doses of 0.025 and 0.4 mg/kg, respectively, while the apparent volume of distribution at steady state increased from 0.8 to 4.2 L/kg over the same dose range. This nonlinear pharmacokinetic profile[44] may reflect a role for platelet-mediated clearance at low plasma concentrations.

Conclusions

Modification of the α-carbamate substituent of the isoxazoline GPIIb/IIIa antagonist **1** (DMP 754) led to a series of α-sulfonamide and α-sulfamide diaminopropionate derivatives, which were examined for their in vitro potency and duration of ex vivo antiplatelet activity in dogs. Most compounds showed high potency in the platelet aggregation assay, demonstrating a wide tolerance for diverse steric and electronic substituent properties at the α-position for the GPIIb/IIIa receptor. However, considerable differences with respect to in vivo duration of antiplatelet action in dogs were observed. In the case of the stereoisomers of **34**, potent and long duration antiplatelet effects after iv dosing were found in the (5*R*)-isoxazoline (2*S*)-diaminopropionate isomer **34b** (DMP 802), which demonstrated the highest affinity for unactivated platelets. DMP 802 was also found to provide prolonged antiplatelet activity after oral administration in dogs. The prolonged antiplatelet profile in dogs and the high affinity of DMP 802 for human platelets may be predictive of clinical utility as a long-acting antiplatelet agent. DMP 802 has been selected for clinical evaluation as an oral GPIIb/IIIa antagonist for the treatment and prevention of arterial thrombosis.

Experimental Section

General Experimental. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Unless otherwise stated, preparative HPLC separations were accomplished on a Vydac C18 column operated at room temperature and eluted at a 10 mL/min flow rate, using a linear gradient of 100% H_2O containing 0.05% TFA−20% H_2O/MeCN containing 0.05% TFA over 50 min, with UV detection at 254 nm. ^1H and ^{13}C NMR data were obtained using a Varian Unity 300, Unity 400, or VXR400 spectrometer and were referenced to TMS or residual HOD. Mass spectral data were obtained on either VG 70-VSE (FAB, high-res FAB, high-res DCI) or Finnigan MAT 8230 (DCI) mass spectrometers. Combustion analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ. Solvents and reagents were used as purchased from commercial sources unless otherwise stated. The yields quoted in this paper were isolated yields.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3,5-dimethyl-4-isoxazolyl)-sulfonyl]-L-alanine Trifluoroacetate (34a). This compound was synthesized in an analogous manner to that used for **25b** in Xue et al.:[20] ^1H NMR (300 MHz, DMSO-d_6) δ 9.72 (bs, 2H), 9.29 (bs, 2H), 8.25 (bm, 1H), 8.16 (m, 1H), 7.87 (s, 4H), 5.03 (m, 1H), 3.78 (bs, 1H), 3.6−3.1 (m, 4H) 2.55 (s, 3H), 2.34 (s, 3H), 2.62−2.38 (m, 2H); HRMS (FAB) *m/z* 493.1487 [$(M + H)^+$ calcd for $C_{20}H_{25}N_6O_7S_1$ 493.1505]; 96.2% purity by HPLC.

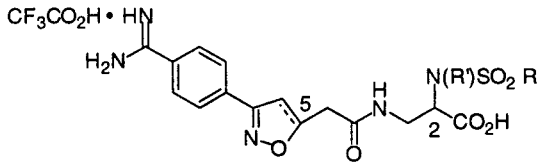
(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3,5-dimethyl-4-isoxazolyl)sulfonyl]-L-alanine Methanesulfonate (34b). To a solution of methyl N^3-Boc-(*S*)-2,3-diaminopropionate (**5**) (22.05 g, 101 mmol) in CH_2Cl_2 (300 mL) at 0 °C was added 3,5-dimethylisoxazole-4-sulfonyl chloride (20.0 g, 102 mmol). A solution of Et_3N (16.2 mL, 116 mmol) in CH_2Cl_2 (50 mL) was added over 30 min and the resulting mixture allowed to warm to room temperature overnight (18 h). The mixture was washed with 0.1 M HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification using flash chromatography (0−8% MeOH−$CHCl_3$) gave 31.56 g (83%) of the desired sulfonamide as a viscous oil: ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 (bs, 1H), 5.04 (m, 1H), 3.97 (bs, 1H), 3.66 (s, 3H), 3.50 (m, 2H), 3.15 (bq, J = 7.3 Hz, 1H), 2.62 (s, 3H), 2.42 (s, 3H), 1.43 (s, 9H).

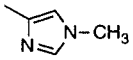
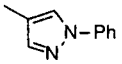
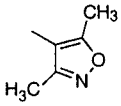
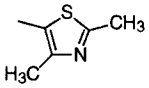
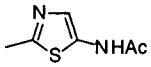
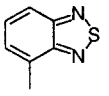
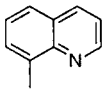
Methyl N^2-(3,5-dimethylisoxazol-4-ylsulfonyl)-N^3-Boc-(*S*)-2,3-diaminopropionate (31.56 g, 83.62 mmol) in 4 M HCl/dioxane (100 mL, 400 mmol) was stirred at room temperature for 4 h and then concentrated in vacuo to an oil. Trituration with ether (3 × 10 mL) followed by drying under vacuum afforded 28.24 g of the desired amine, still containing 30 mol % residual dioxane (75% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (bs, 3H), 8.05 (bs, 1H), 4.47 (bs, 1H), 3.64 (m, 2H), 3.50 (m, 2H), 3.58 (s, 3H), 3.13 (m, 1H), 2.61 (s, 3H), 2.43 (s, 3H).

To a suspension of 3-(4-cyanophenyl)isoxazolin-5(*R*)-ylacetic acid (20.72 g, 90.0 mmol) and methyl N^2-(3,5-dimethylisoxazol-4-ylsulfonyl)-(*S*)-2,3-diaminopropionate hydrochloride (28.24 g, 70% purity, 63.0 mmol) in DMF (200 mL) was added TBTU (28.90 g, 90 mmol). The mixture was cooled to 0 °C, Et_3N (31.4 mL, 225 mmol) was added dropwise, and the resulting mixture was allowed to warm to room temperature overnight (18 h). Upon dilution with EtOAc (500 mL) the organic layer was extracted with H_2O (4 × 200 mL), saturated $NaHCO_3$ (100 mL), and saturated NaCl (100 mL), dried ($MgSO_4$), and filtered. Concentration in vacuo afforded 25.06 g (81%) of the desired amide: ^1H NMR (300 MHz, DMSO-d_6) δ 8.77 (bs, 1H), 8.22 (t, J = 5.9 Hz, 1H), 5.02 (m, 1H), 3.98 (t, J = 7.0 Hz, 1H), 3.55 (dd, J = 17.2, 10.6 Hz, 1H), 3.48 (s, 3H), 3.42 (m, 1H), 3.16 (m, 2H), 2.54 (s, 3H, partially coincident with m, 1H and DMSO-d_5), 2.37 (dd, J = 14.6, 7.0 Hz, 1H), 2.33 (s, 3H).

Into a solution of methyl N^2-(3,5-dimethylisoxazol-4-ylsulfonyl)-N^3-[3-(4-cyanophenyl)isoxazolin-5(*R*)-ylacetyl]-(*S*)-2,3-diaminopropionate (25.06 g, 51.17 mmol) in MeOH (anhydrous, 750 mL) at 0 °C was bubbled HCl gas for 3 h. The resulting solution was allowed to warm to room temperature overnight (18 h), after which the solvent was evaporated in vacuo. The oily residue was triturated with ether (3 × 100 mL) and the resulting solid placed under vacuum until constant weight was achieved. The crude imidate was then dissolved in MeOH (1 L) and ammonium acetate (20.0 g, 259 mmol) added. The resulting mixture was stirred at room temperature for 18 h and then concentrated in vacuo. The residue was then crystallized from EtOH, giving 21.75 g of crude amidine. A portion of this material (8.5 g) was purified using flash chromatography (20% MeOH−EtOAc) to give 3.77 g (33%) of pure (by analytical HPLC) amidine: ^1H NMR (300 MHz, DMSO-d_6) δ 8.26 (m, 1H), 7.86 (m, 4H), 5.01 (m, 1H), 3.96 (t, J = 6.6 Hz, 1H), 3.56 (dd, J = 17.2, 10.6 Hz, 1H), 3.48 (s, 3H, coincident with m, 1H), 3.18 (m, 2H), 2.53 (s, 3H, partially coincident with m, 1H and DMSO-d_5), 2.54 (s, 3H), 2.36 (dd, J = 14.6, 7.0 Hz, 1H), 2.32 (s, 3H), 1.74 (s, 3H).

Table 4. Ex Vivo Inhibition of Platelet Aggregation in Dogs after iv Dosing



Cpd. No.	R	R'	5	2	Inhibition of ex vivo aggregation[a] 1h	5h	24h
DMP 754	-	-	(*R*)-	(*S*)-	100	38	nd
12a	$(CH_2)_2CH_3$	H	(*R,S*)-	(*S*)-	98	24	nd
13a	$(CH_2)_3CH_3$	H	(*R,S*)-	(*S*)-	97	34	nd
14a	CH_2Ph	H	(*R,S*)-	(*S*)-	100	90	26
16a	2-(CH_3)Ph	H	(*R,S*)-	(*S*)-	90	97	99
17a	2-(CF_3)Ph	H	(*R,S*)-	(*S*)-	90	85	45
18a	2-BrPh	H	(*R,S*)-	(*S*)-	100	90	100[b]
19a	2-PhPh	H	(*R,S*)-	(*S*)-	97	69	65
20b	3-(CH_3)Ph	H	(*R*)-	(*S*)-	89	94	24
20c	3-(CH_3)Ph	H	(*S*)-	(*S*)-	100	64	16
25a	4-(*n*-Pr)Ph	H	(*R,S*)-	(*S*)-	90	16	nd
28a	2-naphthyl	H	(*R,S*)-	(*S*)-	100	62	nd
29b	3-(CH_3)Ph	CH_3	(*R*)-	(*S*)-	94	39	nd
30	3-(CH_3)Ph	H	isoxazole	(*S*)-	40	19	nd
31a		H	(*R,S*)-	(*S*)-	98	36	nd
32a		H	(*R,S*)-	(*S*)-	99	4	nd
34a		H	(*R,S*)-	(*S*)-	98	69	33
36a		H	(*R,S*)-	(*S*)-	82	54	8
37a		H	(*R,S*)-	(*S*)-	94	86	68
38a		H	(*R,S*)-	(*S*)-	96	67	0
39a		H	(*R,S*)-	(*S*)-	97	93	67
41a	$NHCH_2CH(CH_3)_2$	H	(*R,S*)-	(*S*)-	94	48	nd

[a] $n = 2$. [b] Tested at 0.05 mg/kg.

To a solution of methyl N^2-(3,5-dimethylisoxazol-4-ylsulfonyl)-N^3-[3-(4-amidinophenyl)isoxazolin-5(*R*)-ylacetyl]-(*S*)-2,3-diaminopropionate HOAc salt (3.77 g, 6.65 mmol) in 0.1 N Hepes buffer (pH 7, 140 mL, 48 mmol) was added rabbit liver esterase (3.6 M crystalline suspension in ammonium sulfate, 3500 units; Sigma). The resulting solution was incubated at 37 °C. After 3 days, an additional 2000 units of esterase was added and the pH was adjusted from 5.2 to 7.2, causing a substantial amount of zwitterion to precipitate. After 2 additional days the precipitate was collected by filtration, washed with cold H_2O (50 mL), and dried under vacuum until constant weight was achieved to yield 1.850 g of pure zwitterion. Protein was removed from the filtrate by ultra filtration (Amicon YM-10 membrane), and the filtrate was then concentrated in vacuo and lyophilized. Purification using a reverse-phase silica column (5 × 9.5 cm in H_2O; crude product loaded as an aqueous solution followed by elution with H_2O (1000 mL) and by 500 mL each of 20%, 30%, and 40% CH_3CN-H_2O). Frac-

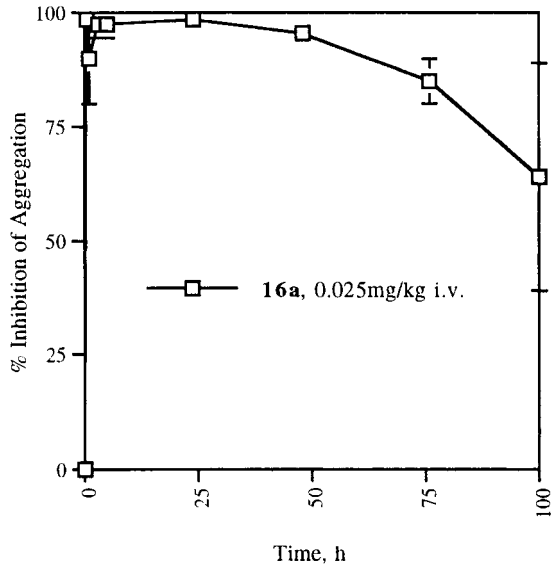


Figure 2. Inhibition of ADP (100 μM)-mediated ex vivo platelet aggregation in the canine dosed intravenously (bolus) with 0.025 mg/kg **16a** ($n = 2$).

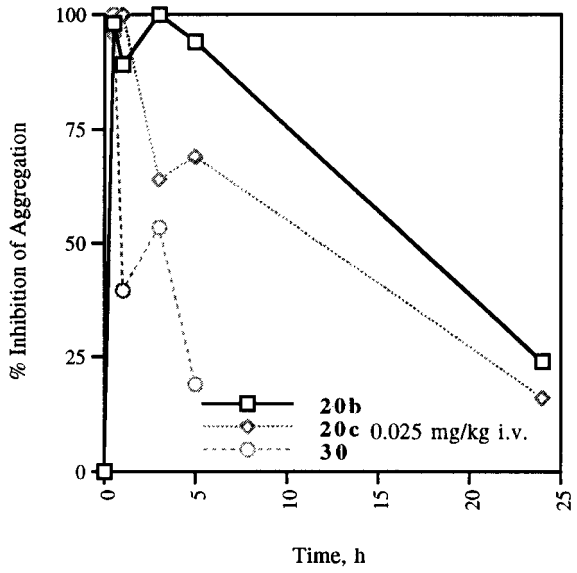


Figure 3. Inhibition of ADP (100 μM)-mediated ex vivo platelet aggregation in the canine dosed intravenously (bolus) with 0.025 mg/kg **20b**,**c** and **30** ($n = 2$).

tions containing the desired product were pooled, acetonitrile was removed, and the aqueous solution was lyophilized to yield an additional 900 mg of pure zwitterion to afford a combined yield of 2.75 g (82%). To a solution of the zwitterion (2.75 g, 5.43 mmol) in 50% CH_3CN-H_2O (135 mL) was added methanesulfonic acid (0.57 g, 5.97 mmol). The reaction mixture was stirred at room temperature for 1 h, resulting in a clear solution. Solvent was removed in vacuo and the residue placed under vacuum for several hours. The crude mesylate was dissolved in hot acetone and H_2O until the solution was clear (120 mL total volume). After hot filtration the solution was allowed to cool to room temperature and then refrigerated for 24 h. A white precipitate was filtered and dried under vacuum, affording 1.72 g (52%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.43 (bs, 2H), 9.17 (bs, 2H), 8.60 (d, $J = 9.5$ Hz, 1H), 8.27 (t, $J = 5.8$ Hz, 1H), 7.92−7.85 (m, 4H), 5.06−5.00 (m, 1H), 3.95−3.87 (m, 1H), 3.57 (dd, $J = 17.2$, 10.6 Hz, 1H), 3.51−3.44 (m, 1H), 3.22 (dd, $J = 17.6$, 7.7 Hz, 1H), 3.14−3.05 (m, 1H), 2.60−2.55 (m, 1H, coincident with 2.55 (s, 3H)), 2.41−2.35 (m, 1H, coincident with 2.35 (s, 3H)), 2.34 (s, 3H) (carboxylic acid proton not observed); $[\alpha]^{25}_D = -51.48°$ ($c = 0.338$, MeOH); MS (ESI) m/z 493 [$(M + H)^+$, 100]. Anal. ($C_{20}H_{24}N_5O_7S\cdot CH_3SO_3H$) C, H, N, S.

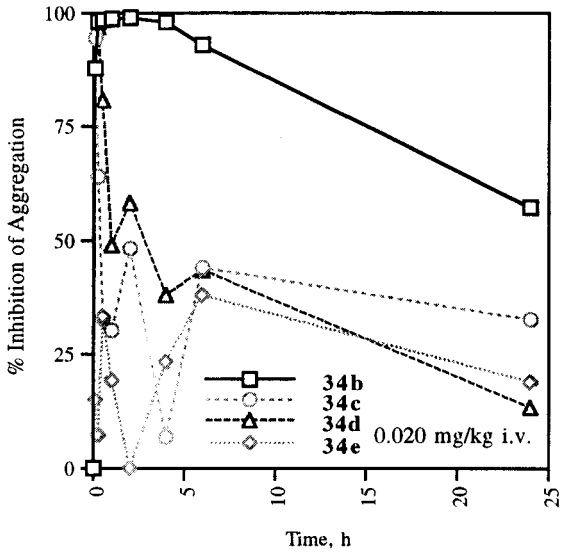


Figure 4. Inhibition of ADP (100 μM)-mediated ex vivo platelet aggregation in the canine dosed intravenously (bolus) with 0.020 mg/kg stereoisomers of **34** ($n = 2$).

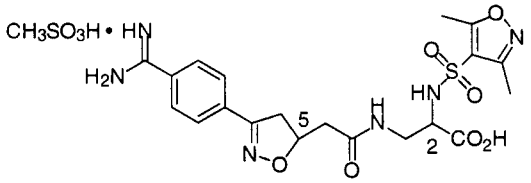
(*S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3,5-dimethyl-4-isoxazolyl)sulfonyl]-L-alanine Methanesulfonate (34c). This compound was synthesized in an analogous manner to that used for **34b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.05 (bs, 1H), 9.44 (bs, 2H), 9.18 (bs, 2H), 8.59 (d, $J = 9.9$ Hz, 1H), 8.24 (t, $J = 5.9$ Hz, 1H), 7.92−7.85 (m, 4H), 5.04−4.98 (m, 1H), 3.94−3.86 (m, 1H), 3.54 (dd, $J = 17.2$, 10.6 Hz, 1H), 3.39−3.29 (m, 2H), 3.29−3.20 (m, 2H), 2.54 (s, 3H), 2.41 (dd, $J = 13.9$, 7.3 Hz, 1H), 2.35 (s, 3H), 2.34 (s, 3H); $[\alpha]^{25}_D = +37.57°$ ($c = 0.338$, MeOH); MS (ESI) m/z 515 [$(M+Na)^+$, 100], 493 [$(M + H)^+$, 60]. Anal. ($C_{20}H_{24}N_5O_7S\cdot CH_3SO_3H\cdot H_2O$) C, H, N, S.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3,5-dimethyl-4-isoxazolyl)sulfonyl]-D-alanine Methanesulfonate (34d). This compound was synthesized in an analogous manner to that used for **34b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.44 (bs, 2H), 9.17 (bs, 2H), 8.59 (d, $J = 9.5$ Hz, 1H), 8.23 (t, $J = 5.5$ Hz, 1H), 7.92−7.86 (m, 4H), 5.04−4.98 (m, 1H), 3.92−3.88 (m, 1H), 3.54 (dd, $J = 17.2$, 10.6 Hz, 1H), 3.38−3.30 (m, 2H), 3.29−3.29 (m, 2H), 2.54 (s, 3H), 2.41 (dd, $J = 14.3$, 7.7 Hz, 1H), 2.34 (s, 3H), 2.34 (s, 3H) (carboxylic acid proton not observed); $[\alpha]^{25}_D = -42.77°$ ($c = 0.346$, MeOH); MS (ESI) m/z 515 [$(M+Na)^+$, 100], 493 [$(M + H)^+$, 55]. Anal. ($C_{20}H_{24}N_5O_7S\cdot CH_3SO_3H$) C, H, N, S.

(*S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3,5-dimethyl-4-isoxazolyl)sulfonyl]-D-alanine Methanesulfonate (34e). This compound was synthesized in an analogous manner to that used for **34b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.44 (bs, 2H), 9.18 (bs, 2H), 8.59 (d, $J = 9.9$ Hz, 1H), 8.27 (t, $J = 5.9$ Hz, 1H), 7.92−7.85 (m, 4H), 5.08−4.98 (m, 1H), 3.93−3.89 (m, 1H), 3.56 (dd, $J = 17.6$, 10.6 Hz, 1H), 3.51−3.45 (m, 1H), 3.22 (dd, $J = 17.6$, 7.7 Hz, 1H), 3.13−3.06 (m, 1H), 2.60−2.54 (m, 1H, coincident with 2.54 (s, 3H)), 2.41−2.34 (m, 1H, coincident with 2.34 (s, 6H)) (carboxylic acid proton not observed); $[\alpha]^{25}_D = +48.82°$ ($c = 0.340$, MeOH); MS (ESI) m/z 515 [$(M+Na)^+$, 100], 493 [$(M + H)^+$, 50]. Anal. ($C_{20}H_{24}N_5O_7S\cdot CH_3SO_3H$) C, H, N, S.

***tert*-Butyl 3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-L-alanine (10).** The intermediate *tert*-butyl 3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(phenylmethoxy)carbonyl]-L-alanine was prepared by the coupling reaction (TBTU) of **4** with *tert*-butyl *N*-[(phenylmethoxy)carbonyl]-L-alanine[45] in an

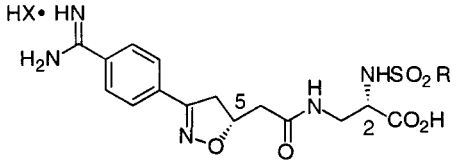
Table 5. Dog Platelet Affinities of Isoxazolyl Sulfonamides **34**

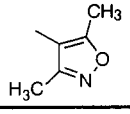


			IC_{50} ± SEM, μM	Kd ± SEM, μM	
Cpd. No.	5	2	dog PRP [a]	Activated	Unactivated
DMP 754	(*R*)-	(*S*)-	0.027[b]	0.00283 ± 0.001	0.00236 ± 0.00139
34b	(*R*)-	(*S*)-	0.023 ± 0.0061	0.0006 ± 0.0002	0.0009 ± 0.00019
34c	(*S*)-	(*S*)-	0.021 ± 0.0038	0.0027 ± 0.0013	0.0037 ± 0.0013
34d	(*R*)-	(*R*)-	0.025 ± 0.0077	0.0059 ± 0.0016	0.0241 ± 0.0160
34e	(*S*)-	(*R*)-	0.170 ± 0.0033	0.1864 ± 0.1846	0.2528 ± 0.1271

[a] See corresponding footnote for Table 1. [b] See ref 21b.

Table 6. Inhibition of Platelet Aggregation in Dogs after Oral Dosing of **16b**, **18b**, **20b**, and **34b**



		Dose				Inhibition of ex vivo aggregation		
Cpd. No.	R	mg/kg	HX	5	2	1h	6h	24h
DMP 754	-	0.3	-	(*R*)-	(*S*)-	54	76	11[a]
16b	2-(CH_3)Ph	0.1	TFA	(*R*)-	(*S*)-	39	92	65
18b	2-BrPh	0.1	TFA	(*R*)-	(*S*)-	29[b]	73	40[c]
20b	3-(CH_3)Ph	0.1	TFA	(*R*)-	(*S*)-	78	94	60[c]
34b		0.1	MsOH	(*R*)-	(*S*)-	54	88	75[c]

[a] $n = 3$.[20] [b] Inhibition measured at 2 h. [c] $n = 2$.

Table 7. Selectivity of **34b** (DMP 802) for GPIIb/IIIa[44]

Integrin	Method	% Inhibition/IC_{50}
GPIIb/IIIa	PRP	0.029 ± 0.0042 μM
GPIIb/IIIa	gel-purified platelet−Fg binding	0.012 ± 0.003 μM
GPIIb/IIIa	ELISA	0.00021 ± 0.00005 μM
$\alpha_v\beta_3$	ELISA	>10 μM
$\alpha_v\beta_5$	SK−BR-3 cell−Vn-mediated adhesion	<15% @ 10 μM
$\alpha_4\beta_1$	Jurkat-Fn CS-1 adhesion	>100 μM
$\alpha_5\beta_1$	ELISA	<5% @ 10 μM

analogous manner to that used for **34b** to yield the desired compound in 75% yield as a colorless glass: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.0, 2H), 7.33 (m, 5H), 6.43 (m, 1H), 5.76 (bt, J = 8.0 Hz, 1H), 5.08 (s, 2H), 5.05 (m, 1H), 4.35 (m, 1H), 3.65 (m, 2H), 3.45 (m, 1H), 3.10 (m, 1H), 2.65 (m, 1H), 2.48 (m, 1H), 1.56 (s, 9H), 1.47 (s, 9H); MS (ESI) m/z 624 [M + H)$^+$, 100].

tert-Butyl 3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-L-alanine (**10**) was prepared from *tert*-butyl 3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(phenylmethoxy)carbonyl]-L-alanine by catalytic reduction (10% Pd/C, EtOH, H_2, 1 atm, 18 h) and purified by flash chromatography (10% MeOH/CH_2Cl_2) to provide **10** as a glass in 64% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 8.0 Hz, 2H), 7.69 (d, J = 8.0 Hz, 2H), 6.43 (bt, J = 7.0 Hz, 1H), 5.12 (m, 1H), 3.66 (m, 1H), 3.54 (m, 1H), 3.47 (m, 1H), 3.29 (m, 1H), 3.29 (m, 1H), 3.19 (m, 1H), 2.71 (m, 1H), 2.58 (m, 1H), 1.65 (bs, 2H), 1.56 (s, 9H), 1.48 (s, 9H); MS (ESI) m/z 490.3 [(M + H)$^+$, 100].

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(propylsulfonyl)-L-alanine Trifluoroacetate (12a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.45 (s, 1H), 9.34 (s, 1H), 8.23−8.21 (m, 1H), 7.92−7.86 (m, 4H), 5.07−5.02 (m, 1H), 3.96 (m, 1H), 3.56 (dd, J = 17.2, 10.2 Hz, 1H, partially coincident with m, 1H), 3.28−3.15 (m, partially coincident with H_2O, 2H), 3.01−2.94 (m, 2H), 2.61 (dd, J = 14.6, 6.6 Hz, 1H), 2.46 (dd, J = 14.6, 7.7 Hz, 1H, partially coincident with solvent), 1.75−1.65 (m, 2H), 0.95 (t, J = 7.7 Hz, 3H) (amidine protons not observed); MS (ESI) m/z 440 [(M + H)$^+$, 100]. Anal. ($C_{18}H_{25}N_5O_6S\cdot1.3CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(phenylmethyl)sulfonyl]-L-alanine Trifluoroacetate (14a). This compound was syn-

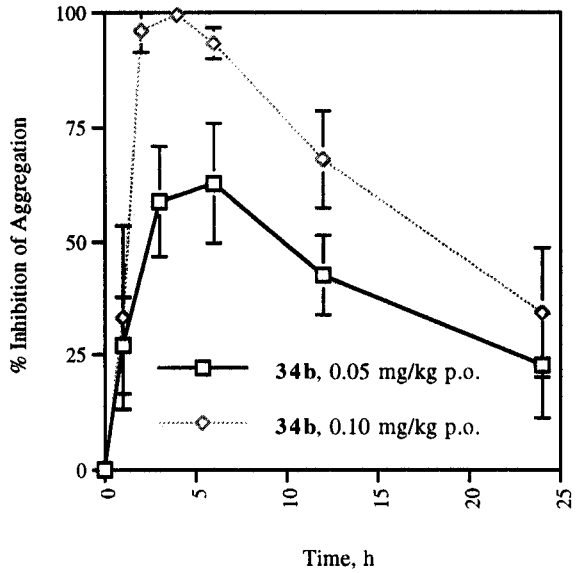


Figure 5. Inhibition of ADP (100 μM)-mediated ex vivo platelet aggregation in the canine dosed orally with **34b** (n = 2).

thesized in an analogous manner to that used for **34a**. The ester was cleaved using the LiOH protocol described in the preparation of **22** in Xue et al.:[20] ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 2H), 9.21 (s, 2H), 8.16 (q, J = 5.5 Hz, 1H), 7.86 (d, J = 2.2 Hz, 4H), 7.58 (m, 1H), 7.35−7.40 (m, 5H), 5.04 (m, 1H), 4.36 (s, 2H), 4.01 (q, J = 5.5 Hz, 1H), 3.60−3.47 (m, 2H), 3.27−3.19 (m, 2H), 2.61 (dd, J = 6.6, 14.6 Hz, 1H), 2.42−2.48 (m, 1H); MS (ESI) m/z 488 [(M + H)$^+$, 100]. Anal. ($C_{22}H_{25}N_5O_6S\cdot 1.7CF_3CO_2H$) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(phenylsulfonyl)-L-alanine Trifluoroacetate (15b). This compound was synthesized in an analogous manner to that used for **34b.** The ester was hydrolyzed and product purified following the procedure for **22** in Xue et al.:[20] ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 2H), 9.23 (s, 2H), 8.44 (d, J = 9.5 Hz, 1H), 8.23 (t, J = 5.8 Hz, 1H), 7.88 (s, 4H), 7.76 (dd, J = 1.5, 8.1 Hz, 2H), 7.54−7.66 (m, 3H), 5.0 (m, 1H), 3.99 (q, J = 7 Hz, 1H), 3.56 (dd, J = 10.4, 17.2 Hz, 1H), 3.39 (dd, 1H), 3.33−3.1 (m, 2H), 2.57−2.48 (m, 1H, coincident with DMSO-d_6), 2.35 (dd, J = 7.3, 14.5 Hz, 1H); MS (ESI) m/z 474 [(M + H)$^+$, 100]. Anal. ($C_{21}H_{23}N_5O_6S\cdot 0.5CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-methylphenyl)sulfonyl]-L-alanine Trifluoroacetate (16a). The title compound was prepared and purified following the general method outlined for **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.48 (bs, 2H), 9.04 (bs, 2H), 7.91−7.8 (d, J = 8.0 Hz, 1H), 7.69−7.60 (m, 2H), 7.48−7.18 (m, 5H), 6.50−6.31 (m, 1H), 5.10 (m, 1H), 4.05−3.97 (m, 1H), 3.66−3.52 (m, 1H), 3.40−3.30 (m, 1H), 3.01−2.98 (dd, J = 7.9, 16 Hz, 1H), 2.79 (m, 1H), 2.65−2.50 (m, 4H) (amidine protons and carboxylic acid proton not observed); MS (ESI) m/z 488 [(M + H)$^+$, 100]; HRMS (FAB) m/z 488.1603 [(M + H)$^+$ calcd for $C_{22}H_{25}N_5O_6S$ 488.1604]. Anal. ($C_{22}H_{25}N_5O_6S\cdot 1.1CF_3CO_2H$) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-methylphenyl)sulfonyl]-L-alanine Trifluoroacetate (16b). This compound was synthesized in an analogous manner to that used for **34b**. The ester was hydrolyzed and product purified following the procedure for **22** in Xue et al.:[20] ^{1}HNMR (300 MHz, DMSO-d_6) δ 11.3 (s, 1H), 9.45 (bs, 2H), 9.17 (bs, 2H), 7.87 (s, 4H), 7.84−7.81 (d, J = 7.8 Hz, 1H), 7.54 (m, 2H), 7.51−7.40 (m, 2H), 5.06−4.97 (m, 1H), 3.93−3.85 (m, 1H), 3.60−3.19 (m, 4H), 2.61−2.58 (m overlap, 4H), 2.45−2.41(m, 1H); MS (ESI) m/z 488 [(M + H)$^+$, 100]; HRMS (FAB) m/z 488.1588 [(M + H)$^+$ calcd for $C_{22}H_{25}N_5O_6S$ 488.1604].

(*S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-methylphenyl)sulfonyl]-L-alanine Trifluoroacetate (16c). This compound was synthesized in an analogous manner to that used for **34b**. The ester was hydrolyzed and product purified following the procedure for **22** in Xue et al.:[20] ^{1}HNMR (300 MHz, DMSO-d_6) δ 11.2 (s, 1H), 9.50 (bs, 2H), 9.20 (bs, 2H), 7.80 (s, 4H), 7.75 (d, J = 8.0 Hz, 1H), 7.45−7.41 (m, 2H), 7.36−7.28 (m, 2H), 4.98−4.92 (m, 1H), 3.80 (dq, J = 7.9, 16 Hz, 1H), 3.55−3.38 (m, 2H), 3.15−3.00 (m, 2H), 2.51 (s, 3H), 2.48−2.42 (m, 1H), 2.25−2.18 (m, 1H); MS (ESI) m/z 488 [(M + H)$^+$, 100]; HRMS (FAB) m/z 488.1613 [(M + H)$^+$ calcd for $C_{22}H_{25}N_5O_6S$ 488.1604]. Chiral HPLC analysis (SFC, Chiralcel OD; 0.46 × 25 cm; 30 °C; 2.0 mL/min flow rate; 0.1% TFA, 22% MeOH, 78% CO_2; 280 nM; 150 atm) showed >99% de with respect to the (*S*,*S*)-diastereomer and >98% chemical purity.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[2-(trifluoromethyl)phenyl]-sulfonyl]-L-alanine Trifluoroacetate (17b). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (300 MHz, D_2O) δ 7.95 (m, 1H), 7.79 (m, 1H), 7.62 (m, 6H), 4.94 (m, 1H), 3.98−3.87 (m, 1H), 3.55−3.34 (m, 2H), 3.29−3.04 (m, 2H), 2.48−2.25 (m, 2H) (amidine protons, carboxylic acid proton, amide proton, and sulfonamide proton not observed); MS (ESI) m/z 542 [(M + H)$^+$, 100]. Anal. ($C_{22}H_{22}F_3N_5O_6S\cdot 1.3CF_3CO_2H\cdot 0.4H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-bromophenyl)sulfonyl]-L-alanine Trifluoroacetate (18a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (bs, 2H), 9.13 (bs, 2H), 8.22 (m, 2H), 8.0 (dd, J = 1.8 Hz, 1H), 7.88 (s, 4H), 7.83 (m, 1H), 7.53 (m, 2H), 4.99 (m,1H), 3.99 (m, 1H), 3.5 (m, 2H), 3.2 (m, 2H), 2.45 (m, 2H); HRMS (FAB) m/z 552.0552 [(M + H)$^+$ calcd for $C_{21}H_{23}N_5O_6S_1Br_1$ 552.0552]. Anal. ($C_{21}H_{22}BrN_5O_6S\cdot 1.6CF_3CO_2H$) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-bromophenyl)sulfonyl]-L-alanine Trifluoroacetate (18b). This compound was synthesized in an analogous manner to that used for **34b**. The ester was hydrolyzed and product purified following the procedure for **22** in Xue et al.:[20] ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (bs, 2H), 9.13 (bs, 2H), 8.21 (m, 2H), 8.0 (dd, J = 2.2, 1.8 Hz, 1H), 7.88 (s, 4H), 7.85 (m, 1H), 7.53 (m, 2H), 5.01 (m, 1H), 3.99 (m, 1H), 3.5 (m, 2H), 3.2 (m, 2H), 2.45 (m, 2H); HRMS (FAB) m/z 552.0534 [(M + H)$^+$ calcd for $C_{21}H_{23}N_5O_6S_1Br_1$ 552.0552]. Anal. ($C_{21}H_{22}BrN_5O_6S\cdot 1.7CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-([1,1′-biphenyl]-2-ylsulfonyl)-L-alanine Trifluoroacetate (19a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (bs, 2H), 9.20 (bs, 2H), 8.15 (m, 1H), 8.0 (d, J = 7.0 Hz, 1H), 7.87 (s, 4H), 7.63 (m, 3H), 7.39 (s, 5H), 7.3 (d, J = 7.3 Hz, 1H), 4.99 (m, 1H), 3.79 (m, 1H), 3.5 (m, 2H), 3.2 (m, 2H), 2.45 (m, 2H); HRMS (FAB) m/z 550.1753 [(M + H)$^+$ calcd for $C_{27}H_{28}N_5O_6S_1$ 550.1760]. Anal. ($C_{27}H_{27}N_5O_6S\cdot 1.2CF_3CO_2H$) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3-methylphenyl)sulfonyl]-L-alanine Trifluoroacetate (20b). This compound was synthesized in an analogous manner to that used for **34b**. The ester was hydrolyzed following the procedure for **22** in Xue et al.:[20] ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.35 (bs, 2H), 9.28 (bs, 2H), 8.14 (m, 1H), 8.00 (bs, 1H), 7.87 (m, 4H), 7.60 (m, 2H), 7.42 (m, 2H), 4.94 (m, 1H), 3.82 (m, 1H), 3.54 (m, 1H), 3.40 (m, overlaps with H_2O peak, 1H), 3.20 (m, 1H), 3.15 (m, 1H), 2.58 (m, 1H), 2.37 (s, 3H), 2.33 (m, 1H) (carboxylic acid proton not observed); HRMS (FAB) m/z 488.1605 [(M + H)$^+$ calcd for $C_{22}H_{26}N_5O_6S$ 488.1604]. Anal. ($C_{22}H_{25}N_5O_6S\cdot CF_3CO_2H$) C, H, N, S. Chiral HPLC analysis (SFC, Chiralcel OD; 0.46 × 25 cm; 30 °C; 2.0 mL/min flow rate; 0.1% TFA, 22% MeOH, 78% CO_2; 280 nM; 150 atm) showed >99% de with respect to the (*S*,*S*)-diastereomer and >98% chemical purity.

(*S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3-methylphenyl)sulfonyl]-L-alanine Trifluoroacetate (20c). This compound was synthesized in an analogous manner to that used for **34b**. The ester was hydrolyzed following the procedure for **22** in Xue et al.:[20] ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.81 (s, 1H), 9.36 (bs, 2H), 9.14 (bs, 2H), 8.12 (m, 1H), 7.86 (m, 4H), 7.60 (m, 2H), 7.42 (m, 2H), 4.94 (m, 1H), 3.82 (m, 1H), 3.52 (m,, 1H), 3.30 (m, overlaps with H_2O, 1H), 3.20 (m, 2H), 2.73 (m, 1H), 2.37 (s, 3H), 2.33 (m, 1H) (sulfonamide proton not observed); MS (ESI) *m*/*z* 488 [(M + H)$^+$, 100]; HRMS (FAB) *m*/*z* 488.1603 [(M + H)$^+$ calcd for $C_{22}H_{26}N_5O_6S$ 488.1604]; 99.3% pure by HPLC.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[3-(trifluoromethyl)phenyl]-sulfonyl]-L-alanine Trifluoroacetate (21a). This compound was synthesized in an analogous manner to that used for **34a**. The ester was cleaved using the LiOH protocol described in the preparation of **22** in Xue et al.:[20] ^{1}H NMR (400 MHz, D_2O) δ 7.97 (s, 1H), 7.88 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.69−7.55 (m, 5H), 4.95−4.90 (m, 1H), 3.87−3.80 (m, 1H), 3.52−3.33 (m, 2H), 3.22−3.06 (m, 2H), 2.41−2.29 (m, 2H) (amidine protons, amide proton, sulfonamide proton, and carboxylic acid proton not observed); MS (ESI) *m*/*z* 542 [(M + H)$^+$, 100]. Anal. ($C_{22}H_{22}F_3N_5O_6S\cdot1.5CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3-bromophenyl)sulfonyl]-L-alanine Trifluoroacetate (22a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (bs, 2H), 9.1 (bs, 2H), 8.43 (m, 1H), 8.22 (m, 1H), 7.91 (s, 1H), 7.88 (s, 4H), 7.83 (m, 1H), 7.77 (d, J = 8 Hz, 1H), 7.53 (m, 1H), 5.0 (m, 1H), 3.95 (m, 1H), 3.6−3.0 (m, 4H), 2.6−2.3 (m, 2H); HRMS (ESI) *m*/*z* 552.0544 [(M + H)$^+$ calcd for $C_{21}H_{23}BrN_5SO_6$ 552.0552]. Anal. ($C_{21}H_{22}BrN_5SO_6\cdot1.6CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(4-methylphenyl)sulfonyl]-L-alanine Trifluoroacetate (23a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (bs, 2H), 9.15 (bs, 2H), 8.19−8.08 (m, 2H), 7.97 (d, J = 1.5 Hz, 4H), 7.66 (d, J = 8.1, 2H), 7.35 (dd, J = 8.1, 3.7 Hz, 2H), 5.02−4.92 (m, 1H), 3.91−3.83 (m, 1H), 3.60−3.48 (m, 1H), 3.32−3.04 (m, coincident with H_2O, 4H), 2.56−2.45 (m, coincident with solvent, 1H), 2.39−2.27 (m, 4H); MS (ESI) *m*/*z* 488 [(M + H)$^+$, 100]. Anal. ($C_{22}H_{25}N_5O_6S\cdot1.5CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[4-(trifluoromethyl)phenyl]-sulfonyl]-L-alanine Trifluoroacetate (24a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.6−9.4 (bs, 2H), 9.26 (s, 2H), 8.08−8.05 (m, 1H), 7.96 (AB quartet, Δ = 23.2 Hz, J = 7.6 Hz, 4H), 7.86−7.84 (m, 4H), 5.01−4.96 (m, 1H), 3.80−3.78 (m, 1H), 3.56−3.48 (m, 2H), 3.42−3.12 (m, partially coincident with H_2O, 3H), 2.54 (dd, J = 14.4, 6.1 Hz, 1H), 2.40−2.31 (m, 1H); HRMS (ESI) *m*/*z* 542.1313 [(M + H)$^+$ calcd for $C_{22}H_{22}F_3N_5O_6S$ 542.1321].

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(4-propylphenyl)sulfonyl]-L-alanine Trifluoroacetate (25a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.9−12.5 (bs, 1H), 9.31 (s, 2H), 9.22 (s, 2H), 8.05 (dt, J = 15.7, 5.7 Hz, 1H), 7.86 (d, J = 2.0 Hz, 4H), 7.67 (dd, J = 8.2, 1.4 Hz, 2H), 7.35 (dd, J = 8.2, 4.1 Hz, 2H), 5.00−4.94 (m, 1H), 3.78 (s, 1H), 3.56−3.49 (m, 2H), 3.41−3.36 (m, 0.5H), 3.23−3.16 (m, partially coincident with H_2O, 2H), 3.12−3.07 (m, 0.5H), 2.60 (q, J = 7.8 Hz, 2H), 2.55−2.47 (m, partially coincident with solvent), 2.38−2.30 (m, 1H), 1.63−1.54 (m, 2H), 0.87 (dt, J = 7.3, 3.8 Hz, 3H); MS (ESI) *m*/*z* 516 [(M + H)$^+$, 100]. Anal. ($C_{24}H_{29}N_5O_6S\cdot1.4CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(4-bromophenyl)sulfonyl]-L-alanine Trifluoroacetate (26a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (bs, 2H), 9.09 (bs, 2H), 8.36 (m, 1H), 8.18 (m, 1H), 7.88 (s, 4H), 7.78 (m, 2H), 7.7 (m, 2H), 5.02 (m, 1H), 3.93 (m, 1H), 3.7−3.0 (m, 4H), 2.6−2.3 (m, 2H); HRMS (ESI) *m*/*z* 552.0537 [(M + H)$^+$ calcd for $C_{21}H_{23}BrN_5SO_6$ 552.0552]. Anal. ($C_{21}H_{22}BrN_5SO_6\cdot2.0CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(1-naphthalenylsulfonyl)-L-alanine Trifluoroacetate (27a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42−9.26 (bs, 1H), 8.63 (d, J = 8.4 Hz, 1H), 8.22−8.14 (m, 2H), 8.07−7.99 (m, 2H), 7.88−7.84 (m, 4H), 7.73−7.59 (m, 3H), 4.95−4.87 (m, 1H), 3.78−3.69 (m, 1H), 3.54−3.08 (m, partially coincident with H_2O, 3H), 3.60−3.48 (m, 1H), 2.46−2.12 (m, 2H) (amidine protons not observed); MS (ESI) *m*/*z* 524 [(M + H)$^+$, 100]. Anal. Calcd for $C_{25}H_{25}N_5O_6S\cdot CF_3CO_2H$: C, 50.86; H, 4.11; N, 10.98. Found: C, 50.55; H, 4.54; N, 11.36.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(2-naphthalenylsulfonyl)-L-alanine Trifluoroacetate (28a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (500 MHz, CD_3OD) δ 8.43 (s, 1H), 8.01 (m, 2H), 7.95 (m, 3H), 7.90−7.82 (m, 3H), 7.65−7.58 (m, 2H), 5.11 (m, 1H), 3.85−3.81 (m, 1H), 3.68−3.53 (m, 2H), 3.43 (m, 1H), 3.32 (m, 1H), 2.69 (m, 1H), 2.48 (m, 1H) (amidine protons, amide proton, sulfonamide proton, and carboxylic acid proton not observed); MS (ESI) *m*/*z* 524 [(M + H)$^+$, 100]. Anal. ($C_{25}H_{25}N_5O_6S\cdot1.6CF_3CO_2H$) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-methyl-*N*-[(3-methylphenyl)-sulfonyl]-L-alanine Trifluoroacetate (29b). To an ice-cold (0 °C) dry THF (5 mL) solution of nitrile **11** (0.82 g, 1.69 mmol) were added sequentially diethylacetylene dicarboxylate (0.35 g, 2.03 mmol), triphenylphosphine (0.53 g, 2.03 mmol), and dry MeOH (0.07 g, 2.03 mmol). The reaction mixture was stirred at this temperature for 2 h at which point another equivalent of DEAD, triphenylphosphine, and MeOH were added. The reaction was then allowed to warm to room temperature and stirred for 18 h. Evaporation of the solvents afforded a crude mixture which was subjected to silica gel column chromatography (ethyl acetate:hexane, 7:3) to afford desired product (0.45 g, 53%): mp 148−149 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 7.9 Hz, 3H), 7.46 (m, 1H), 7.41 (d, J = 8.2 Hz, 2H), 6.07 (m, 1H), 5.21 (m, 1H), 4.80 (dd, J = 4.8, 10.6 Hz, 1H), 3.81 (m, 1H), 3.56 (s, 3H), 3.43 (m, 2H), 3.25 (dd, J = 7.40, 17.4 Hz, 1H), 2.80 (m, 1H), 2.77 (m 3H), 2.56 (dd, J = 7.7, 15.1 Hz, 1H), 2.44 (s, 3H); IR (KBr) cm^{-1} 3340, 2224, 1726, 1644, 1610, 1596, 1534, 14440, 1402, 1366, 1300, 1284, 1212, 1144, 1012, 934, 918, 896, 844, 812, 690, 586; MS (ESI) *m*/*z* 499 [(M + H)$^+$, 48]; HRMS (FAB) *m*/*z* 499.1649 [(M + H)$^+$ calcd for $C_{24}H_{27}N_4O_6S$ 499.1651].

The benzonitrile (0.45 g, 0.90 mmol) obtained above was then subjected to the Pinner amidine reaction sequence described previously to obtain crude amidine which was saponified with LiOH (0.08 g, 1.80 mmol) in THF/H_2O (4:1) (5 mL) to afford crude product which was purified via reverse-phase HPLC (gradient flow acetonitrile:H_2O containing 0.05% TFA). Lyophilization afforded **29b** as colorless crystals (0.12 g, 26%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.2 (s, 1H), 9.5 (bs, 2H), 9.1 (bs, 2H), 7.98−7.90 (dd, J = 2.4, 8 Hz, 2H), 7.88−7.82 (dd, J = 2.4, 8.0 Hz, 2H), 7.64 (m, 2H), 7.41 (d, J = 8.0 Hz, 2H), 5.15 (m, 1H), 4.81 (m, 1H), 3.70−3.59 (m, 3H), 3.44−3.38 (m, 1H), 2.84 (s, rotomer,3H), 2.79−2.70 (dd, J = 8.0, 16.0 Hz, 1H), 2.58−2.45 (m, 1H), 2.43 (s, 3H); MS (ESI) *m*/*z* 502 [(M + H)$^+$, 100]; HRMS (FAB) *m*/*z* 502.1912 [(M + H)$^+$ calcd for $C_{23}H_{27}N_5O_6S$ 502.1917]. Anal. Calcd for $C_{23}H_{27}N_5O_6S\cdot CF_3CO_2H$: C,48.78; H, 4.58; N, 11.38; F, 9.26. Found: C, 48.35; H, 4.73; N, 11.6; F, 9.82.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-5-isoxazolyl]-acetyl]amino]-*N*-[(3-methylphenyl)sulfonyl]-L-alanine Trifluoroacetate (30). This compound was synthesized in an analogous fashion to compound **55** in Xue et al.:[20] ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.37 (bs, 2H), 9.29 (bs, 2H), 8.42 (m, 1H), 8.1 (d, J = 8.8 Hz, 2H), 8.05 (m, 1H), 7.92 (d, J = 8.4 Hz,

2H), 7.59 (m, 2H), 7.42 (m, 2H), 6.99 (s, 1H), 3.85 (m, 1H), 3.7 (ABquart, $\Delta = 16.5$, 23.4 Hz, 2H), 3.16 (d, $J = 4.8$ Hz, 2H), 2.37 (s, 3H); HRMS (FAB) *m/z* 486.1436 [$(M + H)^+$ calcd for $C_{22}H_{24}N_5O_6S$ 486.1447]. Anal. ($C_{22}H_{23}N_5O_6S \cdot 1.2CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(1-methyl-1*H*-imidazol-4-yl)-sulfonyl]-L-alanine Trifluoroacetate (31a). This compound was synthesized in an analogous fashion to compound **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.8 (bs, 2H), 9.33 (bs, 2H), 8.08 (dt, $J = 16.8$, 5.7 Hz, 1H), 7.86/7.85 (2s, 4H), 7.77 (s, 1H), 7.73 (s, 1H), 7.35 (m, 1H), 5.01 (m, 1H), 3.77 (m, 1H), 3.68/3.67 (2s, 3H), 3.6−3.2 (m, 4H), 2.55 (m, 1H), 2.41 (m, 1H); HRMS (FAB) *m/z* 478.1503 [$(M + H)^+$ calcd for $C_{19}H_{24}N_7O_6S_1$ 478.1509]; >99% purity by HPLC.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(1-phenyl-1*H*-pyrazol-4-yl)-sulfonyl]-L-alanine Trifluoroacetate (32a). This compound was synthesized in an analogous fashion to compound **34a**: ^{1}H NMR (300 MHz, CD_3OD) δ 8.69 (s, 1H), 8.24 (m, 1H), 8.31 (m, 1H), 7.93−7.76 (m, 7H), 7.49 (t, $J = 7.3$ Hz, 2H), 7.37 (t, $J = 7.3$ Hz, 1H), 5.11 (m, 1H), 4.17 (m, 1H), 3.77−3.44 (m, 2H), 3.47−3.24 (m, 2H), 2.70 (m, 1H), 2.49 (dd, $J = 7.7$, 14.3 Hz, 1H) (amidine protons not observed); HRMS (FAB) *m/z* 540.1649 [$(M + H)^+$ calcd for $C_{24}H_{25}N_7O_6S$ 540.1665]. HPLC analysis (Vydac C18; 0.46 × 25 cm; ambient temperature; 1.0 mL/min flow rate; gradient of 0.05% TFA, 90% H_2O, 10% CH_3CN to 0.05% TFA, 10% H_2O, 90% CH_3CN over 20 min; 254 nM) showed >99% chemical purity.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(5-methyl-2-furanyl)sulfonyl]-L-alanine Trifluoroacetate (33a). This compound was synthesized in an analogous fashion to compound **34a**: ^{1}H NMR (300 MHz, CD_3OD) δ 8.41 (m, 1H), 8.32 (m, 1H), 7.93 (dd, $J = 1.1$, 8.8 Hz, 2H), 7.83 (dd, $J = 1.8$, 6.6 Hz, 2H), 6.88 (d, $J = 3.3$ Hz, 1H), 6.14 (m, 1H), 5.10 (m, 1H), 4.15 (m, 1H), 3.77−3.40 (m, 2H), 3.36−3.12 (m, 2H), 2.70 (m, 1H), 2.51 (m, 1H), 2.32 (s, 3H) (amidine protons and carboxylic acid proton not observed). Anal. ($C_{20}H_{23}N_5SO_7 \cdot 2.0CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(2-thienylsulfonyl)-L-alanine Trifluoroacetate (35a). This compound was synthesized in an analogous manner to that used for **34a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85−7.77 (m, 5H), 7.61 (bs, 1H), 7.57 (d, $J = 1.0$ Hz, 1H), 7.12 (dd, $J = 4.9$, 3.9 Hz, 1H), 5.04−4.96 (m, 1H), 3.54−3.15 (m, 6H), 2.65−2.53 (m, 1H), 2.46−2.41 (m, partially coincident with solvent, 1H) (amidine protons and carboxylic acid not observed); MS (ESI) *m/z* 480 [$(M + H)^+$, 100]. Anal. ($C_{19}H_{21}N_5O_6S_2 \cdot 0.7CF_3CO_2H \cdot 1.6H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2,4-dimethyl-5-thiazolyl)-sulfonyl]-L-alanine Trifluoroacetate (36a). This compound was synthesized in an analogous fashion to compound **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.48 (bs, 2H), 9.34 (bs, 2H), 8.46 (bs, 1H), 8.18 (m, 1H), 7.88 (s, 4H), 5.01 (m, 1H), 3.89 (bs, 1H), 3.6−3.0 (m, 4H), 2.61/2.60 (2s, 3H), 2.48 (s, 3H), 2.64−2.37 (m, 2H); HRMS (FAB) *m/z* 509.1265 [$(M + H)^+$ calcd for $C_{20}H_{25}N_6O_6S_2$ 509.1277]. Anal. ($C_{20}H_{24}N_6O_6S_2 \cdot 1.4CF_3CO_2H$) C, H, N.

***N*-[[2-(Acetylamino)-5-thiazolyl]sulfonyl]-3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]-amino]-L-alanine Trifluoroacetate (37a).** This compound was synthesized in an analogous fashion to compound **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.5 (bs, 1H), 9.51 (bs, 2H), 9.36 (bs, 2H), 8.20 (m, 2H), 7.88 (s, 4H), 5.0 (m, 1H), 3.84 (bs, 1H), 3.57−3.07 (m, 4H), 2.44 (s, 3H), 2.56−2.35 (m, 2H), 2.15 (s, 3H); HRMS (FAB) *m/z* 552.1339 [$(M + H)^+$ calcd for $C_{21}H_{26}N_7O_7S_2$ 552.1335]; 97.8% purity by HPLC.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(2,1,3-benzothiadiazol-4-yl-sulfonyl)-L-alanine Trifluoroacetate (38a). This compound was synthesized in an analogous fashion to compound **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (s, 2H), 9.05 (s, 2H), 8.36 (dd, $J = 2.6$, 9.2 Hz, 1H), 8.31−8.25 (m, 1H), 8.20−8.12 (m, 2H), 7.88−7.81 (m, 5H), 5.05 (m, 1H), 4.38 (m, 1H), 3.59−3.39 (m, 2H), 3.24−3.09 (m, 2H), 2.54−2.43 (m, 1H), 2.37−2.31 (m, 1H); HRMS (FAB) *m/z* (532.1080) [$(M + H)^+$ calcd for $C_{21}H_{22}N_7S_2O_6$ 532.1073]. Anal. ($C_{21}H_{21}N_7S_2O_6 \cdot 1.7CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(8-quinolinylsulfonyl)-L-alanine Trifluoroacetate (39a). This compound was synthesized in an analogous fashion to compound **34a**: ^{1}H NMR (400 MHz, CD_3OD) δ 9.03 (m, 1H), 8.42 (m, 1H), 8.36 (dd, $J = 1.2$, 7.1 Hz, 1H), 8.19 (dd, $J = 1.2$, 8.3 Hz, 1H), 7.95 (m, 2H), 7.84 (m, 2H), 7.69 (dt, $J = 7.3$, 0.7 Hz, 1H), 7.63 (m, 1H), 5.13 (m, 1H), 4.41 (m, 1H), 3.70−3.53 (m, 2.5H), 3.38−3.29 (m, 0.5H), 2.75−2.69 (m, 1H), 2.54−2.48 (m, 1H) (amidine protons, amide proton, sulfonamide proton, and carboxylic acid proton not observed); HRMS (FAB) *m/z* 525.1556 [$(M + H)^+$ calcd for $C_{24}H_{25}N_6O_6S$ 525.1572]. Anal. ($C_{24}H_{24}N_6O_6S \cdot 3CF_3CO_2H \cdot 1.1H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[(2-methylethyl)amino]sulfonyl]-L-alanine Trifluorocetate (40a). Following the procedure of Kloek and Leschinsky,[32] nitromethane (80 mL) was stirred and treated with concentrated sulfuric acid (12.5 mL) and then with 30% fuming sulfuric acid (12.5 mL). The resulting solution was stirred on ice until below room temperature; then isopropyl isocyanate (25 mL, 250 mmol) was added over 15 min. The mixture was stirred for 5 min, heated to reflux for 35 min, and then cooled to room temperature. After stirring for several hours, the precipitate was collected by filtration, washed with H_2O, and dried to provide isopropylsulfamic acid (6.08 g, 17%) as a sticky white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (bs, 2H), 3.48 (septet, $J = 7.0$ Hz, 1H), 1.24 (d, $J = 7.0$ Hz, 6H).

Without further purification, a mixture of this material (4.88 g, 35 mmol) in benzene (70 mL) was treated with phosphorus pentachloride (7.70 g, 37 mmol), and the mixure was heated at reflux for 60 min. After cooling and concentration, the residue was purified by distillation to provide isopropylsulfamyl chloride (1.21 g, 22%) as a colorless liquid: bp 59−60 °C (0.33 Torr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.53 (bs, 1H), 3.90 (septet, $J = 7.0$ Hz, 1H), 1.36 (d, $J = 7.0$ Hz, 6H); (lit. bp 59−61 °C (0.25−0.3 Torr).[46]

This intermediate was elaborated to **40a** following the procedure for **44a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.49 (bs, 2H), 9.24 (bs, 2H), 8.19 (m, 1H), 7.89 (m, 4H), 7.12 (bt, $J = 8.0$ Hz, 1H), 6.83 (m, 1H), 5.04 (m, 1H), 3.81 (m, 1H), 3.7−3.2 (m, 6H), 2.60 (m, 1H), 2.45 (m, 1H), 1.08 (m, 6H); MS (ESI) *m/z* 455.2 [$(M + H)^+$, 100]. Anal. ($C_{18}H_{26}N_6O_6S \cdot 1.8TFA \cdot 1.2H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[(2-methylpropyl)amino]sulfonyl]-L-alanine Trifluoroacetate (41a). Following the procedure of Audrieth and Sveda,[33] a solution of isobutylamine (29.8 mL, 300 mmol) in CH_2Cl_2 (250 mL) was cooled to −10 °C. Chlorosulfonic acid (6.65 mL, 100 mmol) was added over 30 min, at a rate to keep the temperature below 10 °C. The mixture was then warmed to room temperature and concentrated. The residue was slurried in a small amount of H_2O, filtered, rinsed with ether, and dried to provide the isobutylamine salt of isobutylsulfamic acid (26.3 g) as a sticky white solid. This material was stirred in benzene (200 mL), treated with phosphorus pentachloride (20.8 g, 100 mmol), and heated at reflux for 2 h. The mixture was cooled and concentrated, and the residue was taken up in CH_2Cl_2 and filtered. The filtrate was concentrated and purified by vacuum distillation to provide isobutylsulfamyl chloride (8.68 g, 51%) as a colorless liquid: bp 77−85 °C (0.3 Torr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.87 (bs, 1H), 3.14 (t, $J = 7.0$ Hz, 2H), 1.95 (m, $J = 7.0$ Hz, 1H), 1.02 (d, $J = 7.0$ Hz, 6H).

A solution of **5** (421 mg, 1.93 mmol) in CH_2Cl_2 (12 mL) was stirred on ice and treated with triethylamine (0.40 mL, 2.89 mmol) and isobutylsulfamyl chloride (397 mg, 2.31 mmol). The mixture was warmed to room temperature, stirred for 14.5 h, and then concentrated. The residue was purified by flash

chromatography (chloroform:2-propanol, 95:5) to provide the intermediate (632 mg, 93%) as a white solid: mp 122−124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.43 (bd, J = 8.0 Hz, 1H), 4.95 (bt, J = 8.0 Hz, 1H), 4.48 (bt, J = 6.0 Hz, 1H), 4.11 (m, 1H), 3.80 (s, 3H), 3.55 (m, 2H), 2.87 (m, J = 6.5 Hz, 2H), 1.79 (m, J = 6.5 Hz, 1H), 1.43 (s, 9H), 0.93 (d, J = 6.5 Hz, 6H); MS (NH_3-CI) m/z 371 [$(M + NH_4)^+$, 7), 354 [$(M + H)^+$, 16), 298 [100]; $[\alpha]^{25}_D$ = +24.52° (c = 0.416, $CHCl_3$).

The material was further elaborated to **41a** as in **34a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.46 (bs, 2H), 9.26 (bs, 2H), 7.99 (bs, 1H), 7.83 (m, 4H), 6.94 (bs, 1H), 6.49 (bs, 1H), 5.00 (m, 1H), 3.7−3.1 (m, 6H), 2.65 (2m, 3H), 2.43 (m, 1H), 1.67 (m, 1H), 0.85 (d, J = 6.5 Hz, 6H); MS (ESI) m/z 469.2 [$(M + H)^+$, 100]. Anal. ($C_{19}H_{28}N_6O_6S$·1.25TFA·1.5H_2O) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[(4-methylphenyl)amino]-sulfonyl]-L-alanine Trifluoroacetate (44a). Following the procedure of Audrieth and Sveda,[34] a solution of *p*-toluidine (39.45 g, 368 mmol) in chloroform (250 mL) was cooled to −5 °C and treated dropwise over 1.5 h with chlorosulfonic acid (8.1 mL, 122 mmol). The mixture was warmed to room temperature, stirred for 20 min, and then filtered, and the solid was washed with CH_2Cl_2 and dried. The residue was dissolved in a solution of sodium carbonate (19.4 g, 183 mmol) in H_2O (600 mL), and the resulting mixture was extracted twice with ether. The aqueous phase was concentrated, and the solid residue was extracted with boiling ethanol (500 mL). The filtrate was concentrated to about 200 mL, cooled, and filtered to provide sodium 4-methylphenylsulfamate (13.49 g, 53%) as white plates: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.64 (s, 1H), 6.95 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 8.0 Hz, 2H), 2.17 (s, 3H).

This salt (4.18 g, 20 mmol) was treated with phosphorus pentachloride (4.17 g, 20 mmol) in benzene (65 mL) at reflux for 24 h. The mixture was filtered, and the filtrate was concentrated to provide 4-methylphenylsulfamyl chloride (4.0 g, 98%) as a brown liquid, used without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (bs, 1H), 7.25 (s, 4H), 2.39 (s, 3H).

A solution of **10** (R^e = ^{t}Bu) (130 mg, 266 μmol) in CH_2Cl_2 (3 mL) was cooled on ice and treated with triethylamine (46 μL, 332 μmol) and 4-methylphenylsulfamyl chloride (66 mg, 319 μmol). The mixture was stirred at room temperature for 24 h and then was concentrated. The residue was purified by flash chromatography (CH_2Cl_2:EtOAc, 2:8) to provide the intermediate (68 mg, 39%) as a glass: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (m, 2H), 7.51 (t, J = 8.0 Hz, 2H), 7.08 (s, 4H), 6.67 (m, 1H), 5.90 (bs, 1H), 5.11 (m, 1H), 4.07 (m, 1H), 3.66 (m, 1H), 3.5−3.3 (m, 2H), 3.04 (m, 1H), 2.60 (m, 1H), 2.47 (m, 1H), 2.26 (s, 3H), 1.80 (m, 2H), 1.56 (s, 9H), 1.37 (s, 9H); MS (ESI) m/z 659.3 [$(M + H)^+$, 100].

The intermediate (66 mg, 100 μmol) in CH_2Cl_2 (3 mL) was treated with trifluoroacetic acid (2 mL) and stirred at room temperature for 4 h. The solution was diluted with ether, and the resulting precipitate was collected by filtration and dried to provide **44a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (bs, 1H), 9.40 (bs, 2H), 9.20 (bs, 2H), 7.97 (bt, J = 7.0 Hz, 1H), 7.90 (s, 4H), 7.77 (m, 1H), 7.05 (m, 4H), 4.94 (m, 1H), 3.90 (m, 1H), 3.52 (m, 1H), 3.25 (bt, J = 7.0 Hz, 1H), 3.10 (m, 1H), 2.5−2.2 (m, 3H), 2.20 (2s, 3H); MS (ESI) m/z 503.2 [$(M + H)^+$, 100]. Anal. ($C_{22}H_{26}N_6O_6S$·1.2TFA) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[methyl(phenylmethyl)amino]sulfonyl]-L-alanine Trifluoroacetate (42a). The sulfamyl chloride was prepared as in example **44a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.4−7.3 (m, 5H), 4.38 (bs, 2H), 2.85 (s, 3H). This sulfamyl chloride was elaborated to **42a** following the procedure outlined for **44a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.05 (bs, 1H), 9.38 (bs, 2H), 9.27 (bs, 2H), 8.25 (m, 1H), 7.87 (s, 4H), 7.79 (bt, J = 9.0 Hz, 1H), 7.33 (m, 5H), 5.05 (m, 1H), 4.19 (s, 2H), 3.95 (bm, 1H), 3.56 (m, 1H), 3.4−3.2 (m, 2H), 2.62 (m, 1H), 2.55 (s, 3H), 2.45 (m, 1H); MS (ESI) m/z 517.2 [$(M + H)^+$, 100]. Anal. ($C_{24}H_{28}N_6O_6S$·TFA·1.2H_2O) C, H, N, S.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[(2-phenylethyl)amino]sulfonyl]-L-alanine Trifluoroacetate (43a). The sulfamyl chloride was prepared as in **44a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.4−7.1 (m, 5H), 5.58 (bs, 1H), 3.58 (q, J = 7.5 Hz, 2H), 2.97 (t, J = 7.5 Hz, 2H). The sulfamoyl chloride was further elaborated as in **44a** to give **43a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (bs, 2H), 9.20 (bs, 2H), 8.15 (bq, J = 8.0 Hz, 1H), 7.88 (s, 4H), 7.3−7.15 (m, 6H), 7.05 (bs, 1H), 5.03 (m, 1H), 3.85 (m, 1H), 3.54 (m, 1H), 3.5−3.2 (m, 2H), 3.20 (m, 1H), 3.04 (m, 2H), 2.74 (m, 2H), 2.59 (m, 1H), 2.42 (m, 1H); MS (ESI) m/z 517.2 [$(M + H)^+$, 100]. Anal. ($C_{24}H_{28}N_6O_6S$·1.1TFA) C, H, N.

Binding Assay. Serial dilutions of experimental compounds were made in platelet buffer containing NaCl (140 mM), KCl (2.5 mM), $MgCl_2$ (1 mM), HEPES (50 mM), glucose (5.5 mM), and BSA (1 mg/mL). Compounds were incubated in the presence of 3 nM [^{3}H]DMP 728 containing epinephrine−thrombin−arachadonic acid−collagen platelet activation mix or [^{3}H]SA202 with 2.5 × 10^5 platelets in platelet buffer having a total volume of 150 μL/well in a 96-well polypropylene plate. Following 30 min of incubation at 37 °C, reaction mixtures were harvested onto poly(ethylenimine) (PEI)-soaked GF/B unifilters using a Packard harvester. Filters were rinsed twice with ice-cold PBS and placed in a drying oven at 37 °C for 15 min. Subsequently, 40 μL of Packard Microscint Cocktail was added to each filter well, and the plates were sealed and counted in a Packard Topcount. IC_{50} values were calculated using the Michaelis−Menton equation.

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