Non-Peptide GPIIb/IIIa Inhibitors. 20. Centrally Constrained Thienothiophene α-Sulfonamides Are Potent, Long Acting in Vivo Inhibitors of Platelet Aggregation

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The synthesis and pharmacology of **4**, a potent thienothiophene non-peptide fibrinogen receptor antagonist, are reported. Compound **4** inhibited the aggregation of human gel-filtered platelets with an IC_{50} of 8 nM and demonstrated an 8-fold improvement in affinity for isolated GPIIb/IIIa receptors over analogues possessing an isoindolinone backbone. Flow cytometry studies revealed that the binding of **4** to resting platelets is a diffusion-controlled process ($k_{on} = 3.3 \times 10^6$ M^{-1} s^{-1}) and that **4** binds to dog and human platelets with comparable affinity ($K_d = 0.04$ and 0.07 nM, respectively). Ex vivo platelet aggregation in dogs was completely inhibited by an iv dose of 5 mg/kg, and an oral dose of 50–90 mg/kg followed by low daily doses of 10 mg/kg was sufficient to maintain ~80% inhibition of ex vivo platelet aggregation over several days. Inhibition of ADP-induced platelet aggregation in anesthetized dogs at 77 ± 7% resulted in a moderate 2.5-fold increase in bleeding time, while complete inhibition (100%) resulted in an approximately 10-min bleeding time. Additional doses were required to increase the bleeding time to the maximum time allowed in the protocol (15 min), thus indicating a potentially useful and safe separation of efficacy and bleeding time.

Introduction

The binding of fibrinogen to platelet glycoprotein GPIIb/IIIa (fibrinogen receptor) represents a final pathway in both hemostasis and pathologically critical thrombosis,[1] and can be inhibited by proteins or peptides containing an RGD tripeptide or its synthetic mimetics.[2,3] We have found two key structural modifications of some RGD mimics that have led to improved potency. First, α-carbon substitution of a tyrosine-like lead compound with sulfonamides improved potency from the micromolar to the low-nanomolar level. The potent *n*-butylsulfonamide intravenous therapeutic Aggrastat (**1**) (Figure 1) demonstrates the potency-enhancing affect of the α-sulfonamide moiety in this series.[4,5]

Second, we proposed that potent inhibitors could be prepared by incorporation of an element of geometric (structural) constraint at the center of the molecule to direct the vectors of the N- and C-terminal chains. In practice, an isoindolinone moiety proved to be an effective "central constraint", and the nanomolar inhibitor **2** was found to be orally active.[6,7] Recently we have outlined how the combination of these two key elements of structure led to potent, orally active GPIIb/IIIa inhibitors such as the α-pyridylsulfonamide isoindolinone **3**.[8] Compound **3** has a binding affinity for isolated receptors[9] similar to that of **1** and greater than that of **2** and demonstrates greatly improved oral activity in dogs when compared to **1** and **2**. The success of the isoindolinone central constraint prompted us to screen other types of bicyclic central constraints for potency and optimal oral profile. This paper describes the substitution of the isoindolinone central constraint with a thienothiophene central constraint, the additional
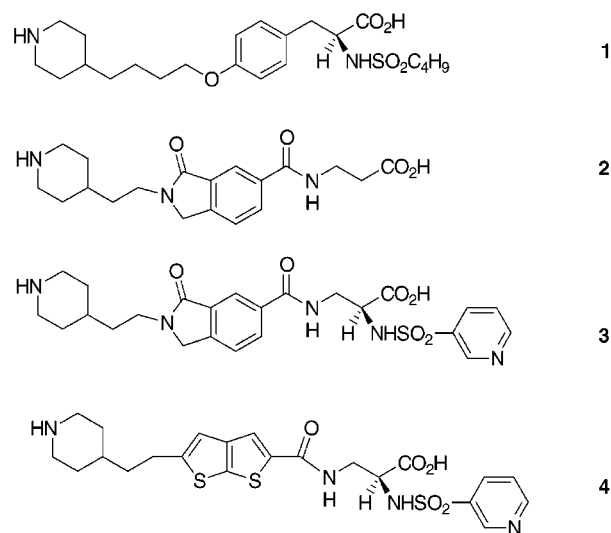

**Figure 1.** Small molecule inhibitors of GPIIb/IIIa.

improvements in potency associated with that substitution,[10] and how this approach led to the identification of **4** as an oral fibrinogen receptor antagonist suitable for once-a-day dosing in dogs.

Chemistry

We chose to combine the N-terminal and central portions of analogues first, so as to allow for convenient derivatization at the C-terminus. Construction of the substituted thienothiophenes **9** and **11** proceeded from initial alkylation of the [2,3-*b*]- and [3,2-*b*]thienothiophene central constraints with iodide **5**. An example of our initial synthetic approach is outlined in Scheme 1.

Scheme 1[a]
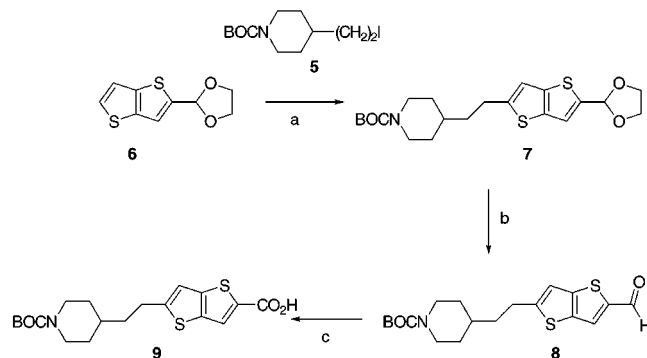
[a] (a) THF/*n*-butyllithium/**5**/−78 °C (45%); (b) TFA/acetone/5 min (93%); (c) NaClO$_2$/*tert*-butanol/NaH$_2$PO$_4$/H$_2$O/2-methyl-2-butene (83%).

Scheme 2[a]
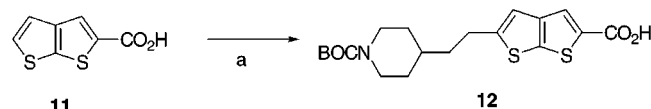
[a] (a) 10:1 THF/HMPA, *n*-butyllithium/**5**/−78 °C (80%).

N-BOC-piperidinylethyl iodide (**5**) was prepared from *N*-BOC-piperidinylethyl alcohol[5] using standard conditions. The lithio anion of the [3,2-*b*]thienothiophene acetal **6**[11] or its [2,3-*b*] analogue was generated at −78 °C with *n*-butyllithium, and the alkylation proceeded in 45% yield. The acetal **7** was then converted to the acid **9** in high yield through deprotection with TFA followed by oxidation with sodium hypochlorite (77% for two steps). A [2,3-*b*]thienothiophene analogue of **9** possessing a three-carbon linker between the piperidine and thienothiophene moieties was prepared in a manner similar to that used for **9**, using the appropriate *N*-BOC-piperidinylpropyl iodide[5] (compound **10**, not shown).

While this sequence worked well, we sought a way to reduce the number of transformations required. In the [2,3-*b*] series, direct alkylation of the appropriate [2,3-*b*] carboxylic acid **11** made further adjustment of the fragment's oxidation state unnecessary (Scheme 2). The solubility and reactivity of the thienothiophene acid mono- and dianions proved to be problematic but could be improved by using HMPA as a cosolvent in the reaction. In the absence of HMPA, greater than 50% of the alkylation took place at the kinetically more reactive 3-position of the thienothiophene. In the presence of HMPA, lithiation ortho to the carboxylate was disrupted, the desired 5-anion predominated, and **12** was obtained in 80% yield.

An analogue possessing only one methylene unit between the piperidine and thienothiophene moieties was prepared as outlined in Scheme 3. Commercially available 4-carboxypiperidine was protected and the acid converted in two steps to aldehyde **15**. Addition of the lithium anion of 2-(2-dioxolanyl)thieno[2,3-*b*]thiophene[11] gave alcohol **16**. The aldehyde was then unmasked and converted to the acid **18**. Reduction of the secondary alcohol to **19** with triethylsilyl hydride was accompanied by loss of the BOC group, which was reinstalled to give **20**.

The 2,3-diaminopropanoic acid derivatives **26** were prepared from commercially available **21** through a sequence of esterification, BOC protection of the 3-ami-

Scheme 3[a]
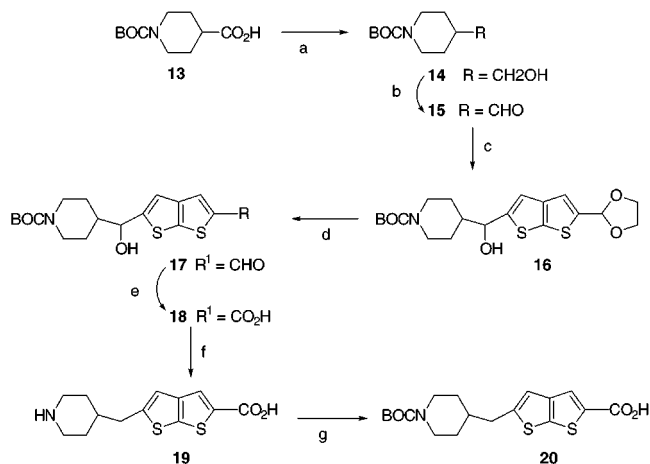
(a) 1 M BH$_3$/THF, 0 °C; (b) PCC/CH$_2$Cl$_2$ (69% for two steps); (c) *n*-BuLi, 78 °C, 2-(dioxolanyl)thieno[2,3-*b*]thiophene[11] (77%); (d) 1 N KHSO$_4$ (98%); (e) 2-methyl-2-butene, NaClO$_2$ (76%); (f) Et$_3$SiH/TFA; (g) BOC$_2$O/TEA/THF/H$_2$O.

no group, deprotection of the 2-amino group, sulfonylation, and deprotection of the 3- amino group (Scheme 4).
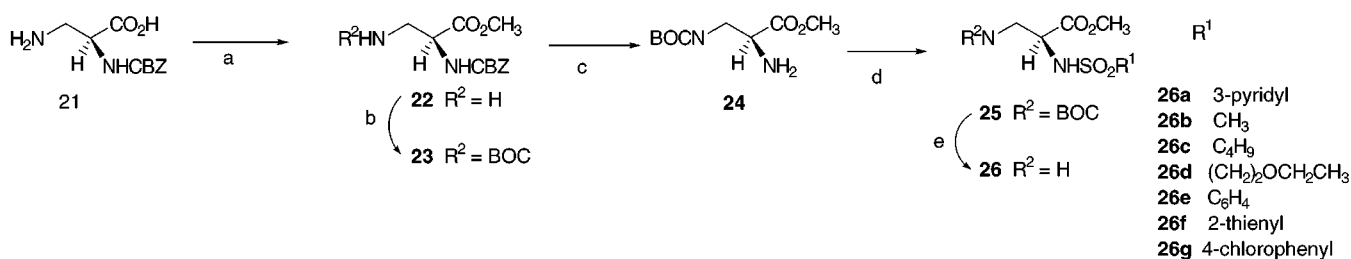
The *R*-isomer of **26a** (**26h**) was accessed through Hoffman rearrangement of 2-(*R*)-*N*-CBZ-asparagine to give the *R*-isomer of **21** which was then derivatized as shown in Scheme 4. The (*R*)-phenylsulfonamide analogue was prepared by Hoffman rearrangement of 2-(*R*)-*N*-(phenylsulfonyl)asparagine to give 2-(*R*)-*N*-phenylsulfonyl-3-aminopropionic acid (**26i**), which was used without further derivatization.

With the appropriate derivatized thienothiophene acids **9**, **10**, **12**, and **20** and 2,3-diaminopropionic acid α-sulfonamides **26** in hand, coupling proceeded cleanly to give **27**. Deprotection of the coupled products was accomplished with different methods. In method A, the C-terminal methyl ester and the N-terminal BOC were removed in one step with aqueous acid to give the final product (illustrated for **4**, Scheme 5). This method was superior to method B (basic hydrolysis of the ester, followed by BOC removal with HCl/EtOAc) as it minimized racemization at the α-stereocenter (see Experimental Section).
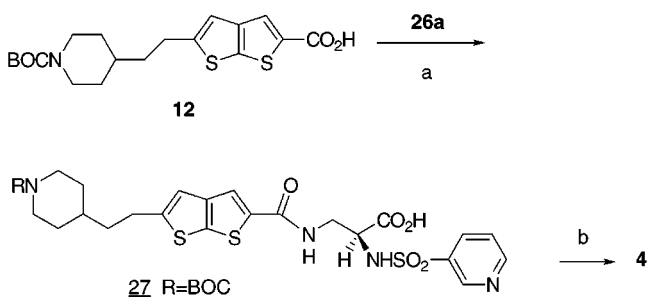
Results and Discussion

In our efforts to screen other types of bicyclic central constraints for potency and optimal oral profile, we first considered the physical characteristics of the isoindolinone lead compounds. The isoindolinone group contributes polarity to the overall structure; the isoindolinone analogues of **3**, for example, commonly exhibit log P values of −3 or less. The N-terminal amide may be important for hydrogen-binding interactions. The vectors of the side chains are not directed in a perfectly linear fashion.[12] We decided to consider other bicyclic systems as central constraints that would differ from the isoindolinone in their polarity and side-chain-directing characteristics.

Thienothiophenes offer a number of interesting features as bicyclic central constraints. First, the vectors of the side chains in a 2,5-substituted thienothiophene would be similar to, but more linearly disposed than, those of the isoindolinone. The absence of the N-

Scheme 4[a]

26a 3-pyridyl
26b CH$_3$
26c C$_4$H$_9$
26d (CH$_2$)$_2$OCH$_2$CH$_3$
26e C$_6$H$_4$
26f 2-thienyl
26g 4-chlorophenyl

[a] (a) MeOH/SOCl$_2$ (99%); (b) di-*tert*-butyl dicarbonate/NaHCO$_3$/CH$_2$Cl$_2$ (95%); (c) H$_2$, 10% Pd/C, EtOH (99%); (d) R^{1}SO$_2$Cl/CH$_2$Cl$_2$/pyridine ($\sim$60%); (e) HCl/EtOAc, $-$70 to 0 °C (100%).

Scheme 5[a]

[a] (a) THF/BOP/NMM, $-$40 °C to room temperature (87%); (b) 6 N HCl/dioxane (71%).

Table 1. Comparison of Backbone Potencies

no.	R	inhibition of platelet aggregation IC$_{50}$ (nM)[a]
2		27
28		410
29		500

[a] Inhibition of aggregation of human gel-filtered platelets (GFP) was measured by a light transmittance method at 37 °C.[5a] Human gel-filtered platelets were adjusted to a concentration of 2×10^8/mL and mixed with 0.1 mg/mL human fibrinogen, 1 mM CaCl$_2$, and the compound of interest. Aggregation was then initiated by addition of the agonist (10 mM adenosine diphosphate (ADP)). Inhibition of platelet aggregation was determined by comparison of light transmittance values for the control and subject samples. The IC$_{50}$ was determined as the concentration necessary to inhibit the change in light transmittance by 50%. At least two determinations were made for each compound, and the IC$_{50}$ values were calculated by fitting to a four-parameter equation. The average standard error of the IC$_{50}$ determinations was $\pm$20%.

terminal amide provided an opportunity to test our assumptions about the role of this functional group in determining potency. Also, the [2,3-*b*] and [3,2-*b*] regioisomers provided an additional probe of polar heteroatom interactions in the central region. The thienothiophene substitution was therefore studied in both substituted and unsubstituted α-carbon analogues to provide comparison of the potency-contributing abilities of the two types of central constraints and to test the effect of α-sulfonamide substitution in this series.

The α-unsubstituted isoindolinone **2** inhibits ADP-induced platelet aggregation with an IC$_{50}$ of 27 nM (Table 1).[6] Both the [2,3-*b*] (**28**) and [3,2-*b*] (**29**) thienothiophenes were less potent, with IC$_{50}$ values of 410 and 500 nM, respectively. A moderate change in polarity was observed (log *P* **28** was $-$2.3 versus $<-$3 for **2**). These results suggested that the overall polarity of the structures was due to the presence of the polar C- and N-terminal substituents and that the presence of the N-terminal amide bond and the side-chain vectors in the isoindolinone compounds may be important binding features for α-unsubstituted compounds that are lacking in thienothiophene backbone compounds.

However, when the thienothiophene central constraints were combined with α-sulfonamide substitution, very potent compounds resulted (Tables 2 and 3). Inhibition of platelet aggregation for both [2,3-*b*]- and [3,2-*b*]thienothiophenes substituted with either alkylsulfonamides (**30**−**32**, **40**−**42**) or arylsulfonamides (**33**−**37**, **43**−**45**) occurred at low-nanomolar concentrations, comparable to the results obtained for α-arylsulfonamide compounds in the isoindolinone series (**3**, IC$_{50}$ = 15 nM, for example). The 3-pyridylsulfonamide **4** showed an IC$_{50}$ of 8 nM. The *R*-enantiomers of both the 3-pyridyl and phenyl analogues appeared slightly less potent (**37** and **34**, 2- and 4-fold, respectively) than the *S*-enantiomers. That the piperidinylethyl side chain was still a necessary requirement for optimal potency was shown
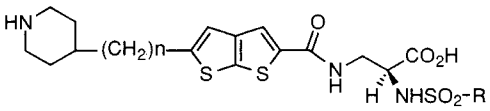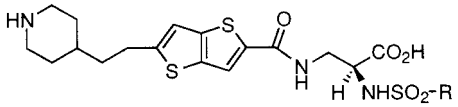
by the preparation of the despiperidinylethyl analogue of **4**, **46** (structure not shown), which was $\sim$1000-fold less potent inhibitor of platelet aggregation (IC$_{50}$ of **46** = 1.6 μM). The integrin specificity of compounds **4** and **30**−**45** was determined by comparison of their inhibition of fibrinogen binding to platelets (Tables 2 and 3) to their inhibition of fibrinogen, vitronectin, and fibronectin binding to HUVEC (human umbilical vein endothelial cells),[13] for which the compounds showed IC$_{50}$'s greater than 200 μM. Compound **4** was at least 35 000-fold selective between platelet and HUVEC fibrinogen receptors. Thus the thienothiophene central constraints, despite their lack of potency as α-unsubstituted analogues, were shown to combine in a synergistic manner with the α-sulfonamide substitution to give very potent and selective analogues.

This result was rewarding, since many but not all RGD mimetics demonstrate a large enhancement in potency upon substitution at the α-carbon.[14] Without X-ray data to examine the different binding geometries of different series, it is difficult to explain why this is so. However, empirically one can draw a distinction between compounds that are more peptide-like in structure and that have more flexibility in their backbone in the region next to the C-terminal amide bond (the glycine-mimicking portion) and those that are more

Table 2. Sulfonamide Derivatives of [2,3-*b*]Thienothiophenes

no.	n	R	inhibition platelet aggregation IC_{50} (nM)[a]	SPA-A ED_{50} (nM)[b]	flow cytometry K_D (nM)[c]	fold difference K_D/ED_{50}[d]
1		see Figure 1	11	0.97	15	15
2		see Figure 1	27	13	550	42
3		see Figure 1	15	0.38	2.2	5.7
30	2	CH₃	15	1.2	52	43
31	2	(CH₂)₃CH₃	32	0.12	5.6	46
32	2	(CH₂)₂OCH₂CH₃	7	0.13	1.2	9.2
33	2	C₆H₅	8	0.03	0.023	0.76
34	2	C₆H₅ (*R*-isomer)	36			
35	2	4-ClC₆H₅	11	0.09	0.026	0.28
36	2	2-thienyl	9	0.04	0.15	3.75
4	2	3-pyridyl	8	0.047	0.07	1.48
37	2	3-pyridyl (*R*-isomer)	19	0.47	28	59
38	0	3-pyridyl	21	0.45	22	48
39	3	3-pyridyl	23	0.07	17	242

[a] See Table 1. [b] Measured by competition with [¹²⁵I]L-692,884 for binding to purified GPIIb/IIIa activated by coating onto yttrium silicate scintillation proximity assay fluomicrospheres. An average standard error of mean of ±20% was obtained for all compounds. For details, see the In Vitro Pharmacology section of the Experimental Section. [c] Measured by displacement of the fluorescent ligand L-762,745 from human platelets by flow cytometry from at least three different platelet donors. The standard deviation of the measurements was for all compounds <20%. The value for **4** is 0.07 ± 0.006 nM (mean ± STD) with *n* = 20. For details, see the In Vitro Pharmacology section of the Experimental Section. [d] Fold difference in activity as determined by ratio of flow cytometry K_D divided by SPA-A ED_{50}.

Table 3. Sulfonamide Derivatives of [3,2-*b*]Thienothiophenes

no.	R	inhibition platelet aggregation IC_{50} (nM)[a]	SPA-A ED50 (nM)[b]	flow cytometry K_D (nM)[c]	fold difference K_D/ED_{50}[d]
1	see Figure 1	11	0.97	15	15
2	see Figure 1	27	13	550	42
3	see Figure 1	15	0.38	0.2	0.5
40	CH₃	14			
41	(CH₂)₃CH₃	10	0.21	3.0	14
42	(CH₂)₂-OCH₂CH₃	10	0.27	1.1	4
43	C₆H₅	7	0.08	0.15	1.8
44	2-thienyl	13	0.04	0.092	2.3
45	3-pyridyl	15	0.11	0.08	0.72

[a] See Table 1. [b] Measured by competition with [¹²⁵I]L-692,884 for binding to purified GPIIb/IIIa activated by coating onto yttrium silicate scintillation proximity assay fluomicrospheres. An average standard error of mean of ±20% was obtained for all compounds. For details, see the In Vitro Pharmacology section of the Experimental Section. [c] Measured by displacement of the fluorescent ligand L-762,745 from human platelets by flow cytometry. The standard deviation of the measurements was for all compounds <20%. For details, see the In Vitro Pharmacology section of the Experimental Section. [d] Fold difference in activity as determined by ratio of flow cytometry K_D divided by SPA-A ED_{50}.

constrained, usually by a flat, aromatic ring, in the same region. The first group tends not to benefit as much from α-substitution as the latter does. Conversely, substitution at the β-carbon that benefits the first group does

not benefit the second group as much.[7i] The work of Gartner and co-workers in the RGD peptides indicated that the glycine portion is sensitive to substitution and that bulky substitution is especially detrimental to potency.[15] The different steric demands that arise from more or less flexibility in the center of the molecule may have a profound effect on the accessibility of binding pocket(s) to the substituents at the C-terminus.

We found the high potency of all the thienothiophene α-sulfonamide analogues, despite their obvious differences in structural features, an interesting result. Previous work in the isoindolinone series[8] had suggested that the potency of tightly binding α-substituted compounds was not being accurately assessed by the in vitro platelet aggregation assay.[16] To better measure the intrinsic differences in potency between very potent compounds, we have isolated the unactivated form of platelet receptor GPIIb/IIIa and have developed an assay based on purified receptor. In this assay, ED_{50} values are calculated from the competitive binding between compounds of interest and the fibrinogen receptor antagonist [¹²⁵I]L-692,884 to purified GPIIb/IIIa activated by coating onto yttrium silicate scintillation proximity assay fluomicrospheres (SPA-A assay, see In Vitro Pharmacology, Experimental Section). Binding measurements with fibrinogen indicate that the SPA-A assay provides a close estimate of binding affinity for an *activated* form of GPIIb/IIIa.[17]

Using the more sensitive SPA-A assay, the α-3-pyridylsulfonamide **3** was shown to be 34-fold more potent than **2** (ED_{50} = 0.38 versus 13 nM). The thienothiophene analogues also proved to be more potent than **2**, and many were revealed to be more potent than **3** (Table 2). In addition, it became apparent that analogues at the sulfonamide side chain exert a structural influence on potency that closely parallels the results seen in the isoindolinone series.[8] While the methylsulfonamide **30** was approximately 3-fold less potent than **3** (ED_{50} = 1.2 vs 0.38 nM), longer alkyl chain length was associated with improved potency, (*n*-butylsulfonamide **31** ED_{50} = 0.12 nM, a 2-fold improvement), and incorporation of an oxygen in the chain did not negatively affect potency (**32**, ED_{50} = 0.13 nM). Arylsulfonamides **33**−**39** were, in general, even more potent than the alkylsulfonamides (ED50's = 0.03−0.09 nM), and direct comparison of **3** and **4** reveals the contribution of the [2,3-*b*]thienothiophene backbone: **4** was ∼8-fold more potent than **3** (ED_{50} = 0.047 vs 0.38 nM). Similarly, the phenylsulfonamide **33** was ∼12-fold more potent than **3**.[10] *Thus the combination of the α-arylsulfonamides and the [2,3-b]thienothiophene backbone acts in a synergistic manner to give compounds with approximately 10-fold improvements in potency over the isoindolinone series.*

The more sensitive assay also allowed a better differentiation between the *S*- and *R*-enantiomers of **4**. The *R*-enantiomer **37** was shown to be approximately 10-fold less potent than the *S*-enantiomer **4** (ED_{50} = 0.42 vs 0.047 nM, respectively). Also, longer separation of the N-terminal piperidine from the central constraint appeared to be tolerated better than chain shortening, as was shown by comparison of **38** and **39** (ED_{50} = 0.07 vs 0.45 nM, respectively). The [3,2-*b*]thienothiophene backbone appears to be somewhat less potency-enhanc-

ing than is the [2,3-*b*] central constraint, as shown by compounds **42** and **45** (ED_{50} = 0.27 and 0.11 nM, respectively).

We had, therefore, identified a series of compounds that showed high affinity for a purified, *activated* form of the receptor. The affinity of thienothiophene analogues for *resting* human platelets was then measured by flow cytometry using competitive displacement of fluorescein-containing isoindolinone GPIIb/IIIa antagonist L-762,745.[9,18] Similar trends in potency were observed on resting platelets, but the differences were more pronounced (Tables 2 and 3). For example, **4** was 31-fold more potent than **3** on resting platelets (as compared to 8-fold more potent against the activated receptor), and **33** was 95-fold more potent than **3** (vs 12-fold). Interestingly, the *R*-enantiomer **37**, which is 10-fold less potent than **4** in binding to activated receptor, is 400-fold less potent than **4** in binding to resting platelets. The longer chain analogue **39**, which is well-tolerated against the activated receptor (only a 1.5-fold decrease relative to **4**) compared to the short chain analogue **38** (9.5-fold decrease), is similarly as poor as the short chained analogue **38** in resting platelets (242- and 312-fold decreases, respectively). Thus, these variations in structure seem to have more effect on the potency against resting platelets than against an activated form of the receptor.

Recently, we have demonstrated that GPIIb/IIIa antagonists can be separated into two classes[15] based on their affinity for resting and activated forms of GPIIb/IIIa. One class binds to both resting and activated forms of purified GPIIb/IIIa and resting and activated platelets, with similar K_D values. The other class of compounds binds with much higher affinity to the activated form of GPIIb/IIIa (purified or on platelets) as compared with the resting form.[19] Thienothiophene analogues can also be separated into these two classes. Compounds in the first class, represented by arylsulfonamide compounds **4, 33, 35**, and **43**−**45**, have similar ED_{50} and K_d values indicating similar affinity for both forms of the receptor (see fold difference columns, Tables 2 and 3). The affinity of the second class of compounds (which includes the alkylsulfonamides and length-varied analogues) for the resting form of the receptor is at least 5-fold less than the affinity for the activated form of the receptor.[20] Previous experiments suggested that the binding site for RGD peptides on resting platelets may resemble a narrow cavity buried 10−20 Å below the surface of the receptor.[21] Thus, the differences in affinities of 3-pyridyl derivatives **4** and **37**−**39** (Table 2) in binding to the resting and activated forms of the receptor may indicate that the activation of the receptor results in an increase in the size of the binding cavity or exposure of the binding site to a position closer to the surface of the receptor.

The high affinity of **4** for both resting and activated forms of the receptor suggested a unique potential of this compound in blocking GPIIb/IIIa. Compared to the previously reported, very potent pyrazolodiazepinone analogue L-738,167,[7j,22] **4** is approximately 1.5-fold more potent against resting human platelets. Therefore, we became interested in further characterization of the binding parameters of this compound to human platelets. In addition to the equilibrium dissociation constant
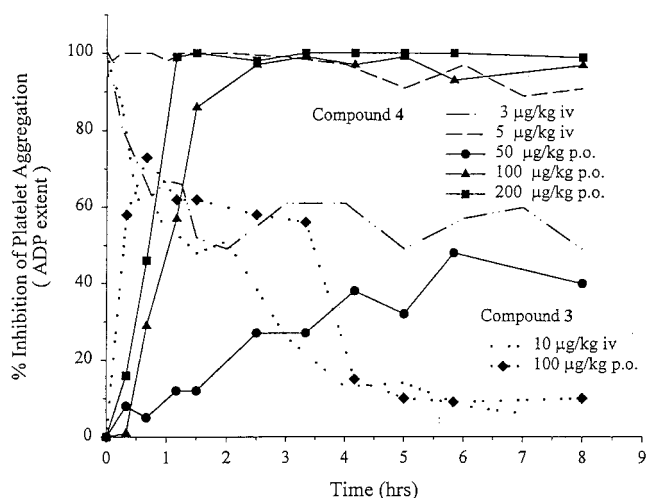

Figure 2. Single-dose intravenous and oral administration of **3** and **4** to conscious dogs and effects on inhibition of ex vivo ADP-stimulated platelet aggregation (PRP, n = 2−5/group).

(Table 2), the flow cytometric measurements[18] provided the dissociation rate constant k_{off} of 2.3×10^{-4} s^{-1}, which gives the $t_{1/2}$ for dissociation of the compound from the receptor of about 50 min. Using these binding parameters one can calculate the association rate constant k_{on} as 3.3×10^{6} M^{-1} s^{-1}. *This value for k_{on} indicates that binding of **4** to the resting form of the receptor is a diffusion-controlled process.*

Flow cytometric measurements of the dissociation constant on resting dog platelets provided a K_d value of 0.04 nM. This value indicates that the affinity of **4** for receptors on resting dog platelets is comparable to the affinity for the receptors on resting human platelets (K_d = 0.07 nM). The high affinity of **4** for nonhuman platelets has made this compound a useful tool for the exploration of platelet function.

Single-Dose Intravenous and Oral Administration of 4 to Conscious Dogs. The effects of the single-dose intravenous bolus administration of 3 and 5 μg/kg and the oral administration of 50, 100, and 200 μg/kg **4** on ex vivo platelet aggregation responses to ADP (maximum extent of aggregation) in conscious dogs are depicted in Figure 2 and compared to the iv and po traces for **3**. For **4**, a potent, dose-related effect was observed with iv and oral administration; *complete inhibition of platelet aggregation was achieved with the extremely low dose of 5 mg/kg iv.* In addition, similar effects were observed with the more potent platelet agonist, collagen (data not shown). Error bars have been omitted from Figure 2 for clarity. It should be noted, however, that there was variability among animals with the lowest iv (inhibition = 61± 21 at 3 mg/kg at 4 h postdose, approximately steady state, compound **4**) and po (peak inhibition = 48 ± 15 at 50 mg/kg, compound **4**) doses presumably due to the steep dose reponse with these compounds. With increasing antiplatelet effects (90−100% inhibition) at the higher doses, results were very consistent (peak inhibition = 99 ± 6 at 100 mg/kg po, compound **4**) among dogs. When high levels of inhibition were achieved, the effects were maintained for the duration of the 8-h protocol, suggesting extended duration of activity. These results suggest minimal dose requirement as well as low frequency of administration.

In comparison, the iv administration of **3** at 10 mg/kg also resulted in 100% inhibition, but this activity decreased rapidly over 8 h.

It is likely that the very high affinity of **4** for GPIIb/IIIa on resting platelets produces the long duration of effect observed in dogs. However, the high affinity of **4** cannot fully explain the long duration since the $t_{1/2}$ for dissociation of the drug from the receptors is only 50 min. Therefore, the mechanism of long duration for this highly potent fibrinogen receptor antagonist must reflect involvement of other parameters in the clearance of the drug from the blood. The first and most critical parameter for high occupancy of the receptors by the drug is the concentration of receptors, typically 20−40 nM, which is more than 100-fold higher than the dissociation constant of the drug. Thus, **4** titrates the receptors, and once all receptors are occupied (a fast event since the on-rate for **4** is diffusion-controlled), the excess of free drug is cleared from the blood with a $t_{1/2}$ of ∼30 min.[7j] The critical part of the mechanism of clearance of the drug from blood for a long duration compound is the competition between the clearance of free drug and rebinding of the drug to unoccupied receptors. Since the rebinding of the drug is much faster than the clearance of the free drug, the resulting duration of the drug is much longer than what would be predicted purely on the off-rate. Thus, the duration of action for fibrinogen receptor antagonists depends on the dissociation constant and the rate of the clearance of the free drug from the plasma. If the clearance of free drug is similar for different low-molecular-weight FRAs, the critical parameter to compare pharmacodynamic duration for different FRAs would be the dissociation constant. And indeed **3**, which has a dissociation constant 31-fold higher than that for **4** (Table 2), has a much shorter duration of action when compared with the highly potent **4** (Figure 2).

Once-Daily Oral Administration of 4 for 6 Days to Conscious Dogs by Gastric Lavage: Maintenance of Inhibition of Platelet Function. To determine if the extended duration of **4** would permit a once-daily dosing regime without loss of efficacy, trough levels of inhibition of the extent of ADP-induced ex vivo platelet aggregation were monitored for 6 days of once daily oral dosing (a single bolus followed by low-dose maintenance) and for 12 days following the termination of dosing. The results of this study demonstrated that despite normal variability among individual animals, submaximal levels of inhibition of platelet function could be maintained with low (10 mg/kg) daily doses of compound. Results for two groups of dogs are shown in Figure 3: the maintenance group (six once-daily doses) received 6 days of dosing and is compared to a separate group of dogs that received a single dose only. A clear benefit of the low, daily dose can be seen in the maintenance group in comparison to the control group by day 4 of the study.

Relationship between Effects of Intravenous 4 on ex Vivo Platelet Aggregation Responses and Bleeding Times in Anesthetized Dogs. The effect of increasing iv bolus doses of **4** on template bleeding times relative to inhibition of the extent of ex vivo platelet aggregation induced by ADP in anesthetized dogs is depicted in Figure 4. Inhibition of platelet aggregation
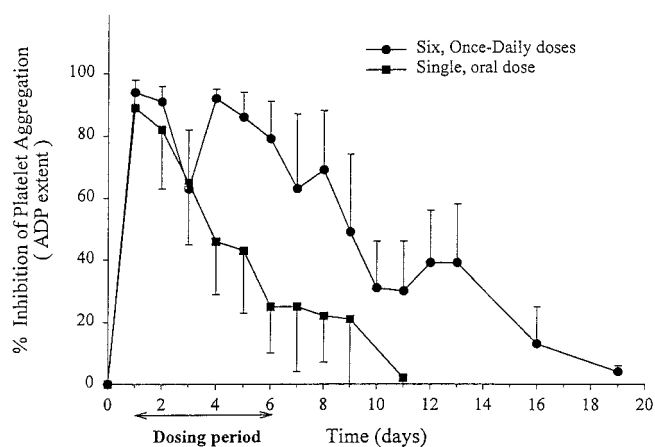

Figure 3. Once-daily oral administration of **4** for 6 days to conscious dogs and effects on inhibition of ex vivo ADP-stimulated platelet aggregation (PRP). Individualized loading doses of 50−90 μg/kg **4** were followed by individualized doses of 0, 10, 20, or 30 μg/kg doses for days 2−3 and 10 μg/kg for days 4−6. For further details, see Experimental Section.
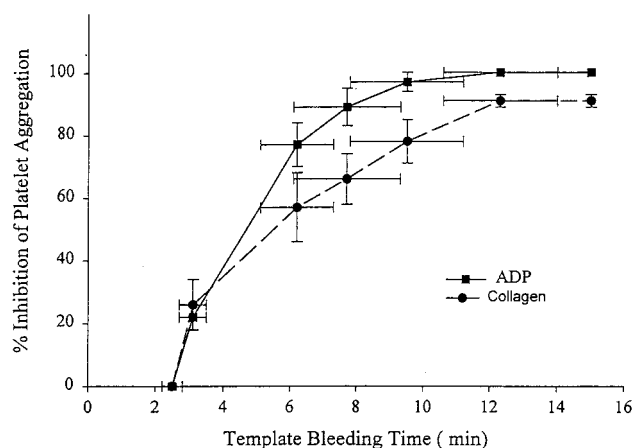

Figure 4. Ex vivo platelet aggregation and template bleeding time relationship. Effect of **4** on inhhibition of ex vivo ADP-stimulated platelet aggregation (PRP) and simultaneous measurement of effect of **4** on buccal mucosal template bleeding time.

of 77 ± 7% corresponded to a moderate (2.5-fold base) elevation in bleeding time. Although several bolus infusions could achieve complete inhibition of ADP-induced platelet aggregation and were accompanied by approximately 10-min template bleeding time, additional infusions beyond these were required for the prolongation of bleeding time to the maximum time allowed in this protocol (15 min). Figure 4 shows that additional doses were required to inhibit aggregation by the more potent agonist collagen. This could suggest that additional GP IIb/IIIa receptors exposed upon activation by the more potent agonist require increased inhibitor concentrations to block aggregation and that their additional exposure is associated with elevated bleeding. For example, 80% inhibition of ADP-induced aggregation is accompanied by bleeding times of ∼6 min, whereas 80% of collagen-induced aggregation results in ∼10-min bleeding times. This separation between efficacy, as indicated by inhibition of aggregation, and bleeding time prolongation suggests the potential for safe, efficacious therapy with this compound.

Summary

Compound **4** is a potent inhibitor of in vitro and in vivo platelet aggregation and demonstrated an 8-fold improvement in affinity for isolated GPIIb/IIIa receptors over analogues possessing an isoindolinone backbone and a one-and 1.5-fold improvement over the pyrazolo-diazepinone-containing compound L-738,167. The very tight binding of **4** revealed by flow cytometry studies makes **4** a useful tool for the study of platelet function. Low doses of **4** were sufficient to maintain ∼80% inhibition of ex vivo platelet aggregation over several days in dogs. Significant levels of inhibition of ADP-induced platelet aggregation resulted in a moderate 2.5-fold increase in bleeding time, while additional doses were required to increase the bleeding time to the maximum time allowed in the protocol (15 min), thus indicating a potentially useful and safe separation of efficacy and bleeding time.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian XL-300 or Varian XR-400 spectrometer as noted. Chemical shifts are reported in parts per million relative to tetramethylsilane as the internal standard. Elemental analyses for carbon, hydrogen, and nitogen were determined with Leeman Labs CE440 elemental analyzer and are within ±0.4% of the theoretical value unless noted otherwise. Low-mass spectra were recorded with a Vg Micromass MM7035 analytical spectrometer. Normal phase silica gel (EM Science silica gel 60 (230−400 mesh)) was used for chromatography. HPLC separations were done using a semipreparative Vydac protein and peptide C$_{18}$ column (cat. #218TP1022). TLCs were developed with ninhydrin or iodine stain. All starting materials and solvents were commercially available and used as received. Yields are unoptimized.

2-[*N*-[(*tert*-Butyloxy)carbonyl]piperidinyl]ethyl Iodide (5). 2-[*N*-[(*tert*-Butyloxy)carbonyl]amino]ethyl alcohol[5] (68.8 g, 0.3 mol), diphenylchlorophosphine (86.04 g, 0.39mol), and imidazole (61.27 g, 0.9 mol) were placed in a 3-L flask equipped with an argon line and an overhead stirrer, dissolved in 1000 mL of toluene, and cooled to 0 °C. Iodine (99 g, 0.39 mol) was added in portions and the reaction stirred for 1.5 h. The reaction mixture was added to 1000 mL of saturated Na$_2$CO$_3$ solution; the organic layer was separated and washed with 250 mL of saturated Na$_2$CO$_3$ solution. The combined Na$_2$CO$_3$ washes were extracted with 300 mL of toluene. The organic layers were combined and washed successively with 2 × 250 mL of 5% Na$_2$S$_2$O$_3$, 250 mL of H$_2$O, 3 × 250 mL of 10% KHSO$_4$, and 200 mL of brine, dried (Na$_2$SO$_4$), filtered, and evaporated. The crude product was passed through a silica gel pad using 4000 mL of 20% EtOAc/hexanes as an eluent. The solution was concentrated to give 82 g of **5** as a viscous oil (80%). R_f (40% EtOAc/hexanes): 0.6. NMR (300 MHz, CDCl$_3$): δ 4.1 (d, J = 12.8 Hz, 2H), 3.25 (t, J = 7.2 Hz, 2H), 2.7 (t, J = 12.8 Hz, 2H), 1.8 (m, 2H), 1.65 (m, 3H), 1.5 (s, 3H), 1.15 (m, 2H).

Preparation of Intermediate Acid 9. 5-[2-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]ethyl]thieno[3,2-*b*]-thiophene-2-carboxaldehyde Ethylene Glycol Acetal (7). A solution of thieno[3,2-*b*]thiophene-2-carboxaldehyde ethylene glycol Acetal[11] (0.212 g, 1.0 mmol) in THF (10 mL) cooled to −78 °C was treated with *n*-butyllithium (1.1 mmol in hexane) with stirring for 2 h. A solution of **5** (0.339 g 0.96 mmol) in THF (2 mL) was added, and the resulting solution was allowed to slowly warm to room temperature over 16 h. The solvent was removed, the residue was quenched with ether/H$_2$O, and the organic phase was separated, dried (MgSO$_4$), and concentrated. The residue was purified using flash chromatography on silica gel eluting with 10−20% EtOAc/hexane to give pure **7** (0.207 g, 45%). NMR (300 MHz, CDCl$_3$): δ 7.26 (s, 1H), 6.91

(s, 1H), 6.14 (s, 1H), 4.08 (m, 8H), 2.90 (t, J = 8 Hz, 2H), 2.65 (app. t, J = 13 Hz, 2H), 1.70 (m, 5H), 1.46 (s, 9H), 1.14 (m, 2H).

5-[2-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]ethyl]-thieno[3,2-*b*]thiophene-2-carboxaldehyde (8). A solution of **7** (0.458 g, 1.08 mmol) in acetone (20 mL) was treated with 2.2 g of trifluoroacetic acid for 5 min and then promptly diluted with EtOAc (100 mL). This solution was extracted with saturated Na$_2$CO$_3$ solution (25 mL) and H$_2$O, dried (MgSO$_4$), and concentrated to give **8** (0.38 g, 93%). NMR (300 MHz, CDCl$_3$): δ 9.93 (s, 1H), 7.86 (s, 1H), 7.03 (s, 1H), 4.10 (m, 4H), 2.96 (t, J = 8 Hz, 2H), 2.69 (app. t, J = 13 Hz, 2H), 1.71 (m, 4H), 1.45 (s, 9H), 1.15 (m, 2H).

5-[2-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]ethyl]-thieno[3,2-*b*]thiophene-2-carboxylic Acid (9). A solution of **8** (0.046 g, 0.1 mmol) in *tert*-butyl alcohol (3 mL) at room temperature was treated with a solution of NaClO$_2$ (0.1 g, 1.1 mmol) and NaH$_2$PO$_4$·H$_2$O (0.1 g, 0.72 mmol) in 1 mL of H$_2$O and 1 mL of 2-methyl-2-butene with stirring at room temperature for 16 h. The reaction mixture was concentrated and partitioned between EtOAc (10 mL) and 3 mL of 1 N NaHSO$_4$. The organic phase was dried (MgSO$_4$), and the solvent was removed. The residue was triturated with hexane to give pure **9** (0.040 g, 83%). NMR (300 MHz, CDCl$_3$): δ 7.99 (s, 1H), 7.00 (s, 1H), 4.10 (bd, J = 13 Hz, 2H), 2.93 (t, J = 8 Hz, 2H), 2.69 (app. t, J = 13 Hz, 2H), 1.70 (m, 3H), 1.46 (s, 9H), 1.18 (m, 2H).

5-[2-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]prop-yl]thieno[2,3-*b*]thiophene-2-carboxylic Acid (10). Prepared as described for **9** except that 3-[*N*-[(*tert*-butyloxy)-carbonyl]amino]propyl iodide[5] was used to alkylate thieno[2,3-*b*]thiophene-2-carboxaldehyde ethylene glycol acetal.[11] NMR (300 MHz, CDCl$_3$): δ 7.99 (s, 1H), 6.95 (s, 1H), 4.08 (bd, J = 13 Hz, 2H), 2.87(app. t, J = 7 Hz, 2H), 2.67 (app.t, J = 14 Hz, 2H), 1.7 (m, 4H), 1.45 (s, 9H), 1.4 (m, 3H), 1.1 (m, 2H).

Preparation of Intermediate Acid 12. 2-Carboxythieno-[2,3-*b*]thiophene (11). A suspension of methylthieno[2,3-*b*]-thiophene-2-carboxylic acid[11] (10 g, 0.05 mol) in THF/CH$_3$OH/H$_2$O (1:1:1, 300 mL) was treated with LiOH·H$_2$O (4.2 g, 0.1 mol) over 24 h. The THF was then removed and the residue acidified. The resultant precipitate was collected by vacuum filtration, the filtrate was reacidified, and a second crop of precipitate was collected. The combined solids were dried on a drying pistol to yield 5.1 g (55%) of **11**. The filtrate was extracted with EtOAc, dried (MgSO$_4$), and concentrated. The residue was suspended in EtOAc, filtered, and the solids washed with hexanes to obtain an additional 4.2 g (45%) of **11** as a white solid (combined yield, 100%). R_f (97/3/1 CHCl$_3$/CH$_3$OH/HOAc): 0.29. NMR (400 MHz, CDCl$_3$): δ 7.90 (s, 1H), 7.36 (d, J = 5 Hz, 1H), 7.23 (d, J = 5 Hz, 1H).

5-[2-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]ethyl]-thieno[2,3-*b*]thiophene-2-carboxylic Acid (12). Acid **11** (5 g, 0.027 mol) was dissolved in THF (190 mL) and HMPA (19 mL, 0.11 mol) and cooled to −78 °C under argon. *n*-Butyl-lithium (25.6 mL, 2.12 M in hexanes, 0.054 mol) was added dropwise and the reaction was stirred at −78 °C for 1 h. Iodide **5** (9.3 g, 0.027 mol) in 10 mL of THF was added dropwise with vigorous stirring while the temperature of the reaction was kept below −65 °C. The reaction was allowed to warm slowly to room temperature overnight and then concentrated and the residue suspended in H$_2$O. The solution was acidified with 10% KHSO$_4$ and extracted with 5 × 50 mL of CH$_2$Cl$_2$. The organic extracts were washed with dilute sodium thiosulfate solution and brine, dried over MgSO$_4$, filtered, and evaporated to give 24 g of a dark oil that was suspended/dissolved in 10:1 Et$_2$O/EtOAc and washed three times with 10% KHSO$_4$ and brine and evaporated to give 11.7 g of a tan solid. The solid was triturated with 1% EtOAc/hexanes and the solid collected and dried under vacuum to give 8.66 g of **12** as a pale tan solid, 94% pure by HPLC (80% yield). This material was used directly in the next step without further purification. R_f (9:0.5:0.5 CH$_2$Cl$_2$/MeOH/HOAc): 0.66. NMR (400 MHz, CD$_3$-OD): δ 7.8 (s, 1H), 7.0 (s, 1H), 4.0 (d, J = 13 Hz, 2H), 2.9 (t,

$J = 7$ Hz, 2H), 2.7 (bt, 2H), 1.7 (bd, 2H), 1.6 (m, 2H) 1.5 (m, 1H), 1.44 (s, 9H), 1.1 (m, 2H).

Preparation of Intermediate Acid 20. N-[(*tert*-Butyloxy)carbonyl]piperidine-4-carboxylic Acid (13). A solution of Piperidine-4-carboxylic acid (12.9 g, 100 mmol) and NaOH (4.0 g, 100 mmol) in 100 mL of H_2O was diluted with 100 mL of dioxane and cooled to 0 °C. Di-*tert*-butyl dicarbonate (24.0 g, 110 mmol) was added, followed by an additional 30 mL of dioxane and 130 mL of H_2O. The mixture was stirred at 0 °C for 30 min and then at ambient temperature for 2 h. Triethylamine (10 g, 100 mmol) and di-*tert*-butyldicarbonate (10 g, 46 mmol) were added, and stirring was continued over 18 h. The dioxane was removed in vacuo and the residue partitioned betweened EtOAc and 10% KHSO$_4$. The organic phase was dried (MgSO$_4$), filtered, and concentrated. The residue was triturated with hexanes and collected by filtration to afford 21 g (92%) of **13** as a white solid. NMR (300 MHz, CDCl$_3$): δ 4.02 (bd, $J = 13$ Hz, 2H), 2.86 (app. t, $J = 12.5$ Hz, 2H), 2.49 (m, 1H), 1.93 (m, 2H), 1.66 (m, 2H), 1.46 (s, 9H).

[N-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]methyl Alcohol (14). A solution of **13** (21.2 g, 92.3 mmol) in 250 mL of THF was cooled to 0 °C and treated with borane–THF (1.0 M, 277 mL, 277 mmol) over 1 h. After an additional 1 h at 0 °C, 1 N NaOH (305 mL, 305 mmol) was added, keeping the temperature between 5 and 10 °C. The cooling bath was then removed and the reaction allowed to reach ambient temperature, at which time TLC analysis showed all **13** was consumed. The THF was removed in vacuo and the residue extracted with Et$_2$O, washed with brine, dried (MgSO$_4$), filtered, and concentrated to give **14** (21.5 g, >100%) as an oil. NMR (300 MHz, CDCl$_3$): δ 4.12 (app. d, $J = 13$ Hz, 2H), 3.50 (d, $J = 6$ Hz, 2H), 2.70 (app. t, $J = 13$ Hz, 2H), 1.65 (m, 3H), 1.46 (s, 9H), 1.14 (m, 2H).

N-[(*tert*-Butyloxy)carbonyl]piperidine-4-carboxaldehyde (15). A solution of **14** (2.15 g, 10 mmol) in 17 mL of CH$_2$-Cl$_2$ was added in one portion to a stirred suspension of PCC (3.23 g, 15 mmol), freshly fused NaOAc (1.23 g, 15 mmol), and 2 g of powdered 3 Å molecular sieves. Stirring was continued for 2 h; then the reaction was triturated with Et$_2$O and decanted onto a column of SiO$_2$. The eluant was collected and concentrated. Chromatography (SiO$_2$, 30% EtOAc/hexanes) gave **15** (1.46 g, 69%). NMR (300 MHz, CDCl$_3$): δ 9.67 (s, 1H), 3.9 (app. d, $J = 13$ Hz, 2H), 2.93 (m, 2H), 2.43 (m, 1H), 1.89 (m, 2H), 1.56 (m, 2H), 1.46 (s, 9H).

5-[[N-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]hydroxymethyl]thieno[2,3-*b*]thiophene-2-carboxaldehyde Ethylene Glycol Acetal (16). A solution of 2-(dioxolanyl)thieno[2,3-*b*]thiophene[11] (0.212 g, 1 mmol) in 10 mL of THF was cooled to −78 °C. *n*-Butyllithium (2.01 M in hexanes, 0.50 mL, 1 mmol) was added dropwise, and the resulting mixture stirred at −78 °C for 2 h. A solution of **15** (0.213 g, 1 mmol) in 1 mL of THF was added, and the mixture stirred at −78 °C for 3.5 h. The cooling bath was then allowed to expire and the mixture to gradually warm to ambient temperature over 18 h. The reaction was poured into EtOAc, washed with water, dried (MgSO$_4$), filtered, and concentrated. Chromatography (SiO$_2$, 46% EtOAc/hexanes) afforded **16** (0.32 g, 77%). NMR (300 MHz, CDCl$_3$): δ 7.22 (s, 1H), 7.01 (s, 1H), 6.13 (s, 1H), 4.62 (m, 1H), 4.10 (m, 6H), 2.66 (m, 2H), 2.25 (m, 1H), 2.03 (m, 1H), 1.79 (m, 1H), 1.46 (s, 9H), 1.22 (m, 2H).

5-[[N-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]hydroxymethyl]thieno[2,3-*b*]thiophene-2-carboxaldehyde (17). A solution of **16** (0.32 g, 0.77 mmol) in 50 mL of EtOAc and 20 mL of THF was treated with 1 N KHSO$_4$ (25 mL) over 48 h. The layers were then separated, and the organic phase was washed with H$_2$O and saturated NaHCO$_3$, dried (MgSO$_4$), filtered, and concentrated to afford **17** (0.29 g, 98%). NMR (300 MHz, CDCl$_3$): δ 9.91(s, 1H), 7.82 (s, 1H), 7.15 (s, 1H), 4.69 (d, $J = 7.5$ Hz, 1H), 4.18 (m, 2H), 3.68 (m, 2H), 2.00 (m, 1H), 1.79 (m, 2H), 1.45 (s, 9H), 1.26 (m, 2H).

5-[[N-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]hydroxymethyl]thieno[2,3-*b*]thiophene-2-carboxylic Acid (18). To a solution of **17** (0.289 g, 0.759 mmol) in 19 mL *tert*-butyl alcohol (Aldrich) was added 2-methyl-2-butene (19 mL)

followed by a solution of NaClO$_2$ (0.76 g, 8.4 mmol) and NaHPO$_4$ (0.76 g, 5.5 mmol) in 7.6 mL of H$_2$O. The mixture was stirred over 18 h, then the organic phase was separated, and the solvent was removed. The residue was partitioned between EtOAc and KHSO$_4$ and the organic phase washed with water, dried (MgSO$_4$), filtered, and concentrated to yield crude **18** (0.23 g, 76%). NMR (300 MHz, DMSO): δ 7.72 (s, 1H), 7.14 (s, 1H), 4.59 (d, $J = 6$ Hz, 1H), 3.95 (m, 2H), 2.61 (m, 1H), 1.71 (m, 3H), 1.11 (s, 9H), 1.0 (m, 3H).

5-[(Piperidin-4-yl)methyl]thieno[2,3-*b*]thiophene-2-carboxylic Acid (19). A mixture of **18** (0.141 g, 0.456 mmol) and triethylsilane (1.0 g, 8.6 mmol) was treated with 3 mL of TFA and capped with a balloon to trap the CO$_2$ released while stirring over 18 h. The TFA was then removed in vacuo and the residue triturated with Et$_2$O to give crude **19** containing residual triethylsilane and Et$_2$O. This material was taken on without further purification. NMR (300 MHz, CD$_3$OD): δ 7.84 (s, 1H), 7.10 (s, 1H), 3.48 (d, $J = 7$ Hz, 2H), 2.99 (m, 4H), 1.96 (m, 3H).

5-[N-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]methyl]-thieno[2,3-*b*]thiophene-2-carboxylic Acid (20). To a suspension of crude **19** (0.357 mmol) and triethylamine (1 mL, 7 mmol) in 20 mL of THF was added 5 mL of H$_2$O. The resultant solution was treated with di-*tert*-butyl dicarbonate (0.15 g, 0.68 mmol) for 18 h. The THF was removed in vacuo, and the residue was partitioned between 1 N KHSO$_4$ and EtOAc. The organic phase was dried (MgSO$_4$), filtered, and concentrated to yield 0.226 g of crude **20** containing residual triethylsilane and Et$_2$O. This material was used to prepare **38** without further purification.

General Method for the Preparation of 2-(S)-Sulfonamide 3-Aminopropionic Acid Methyl Ester (26). Methyl 2-(S)-[N-[(Benzyloxy)carbonyl]amino]-3-aminopropionate Hydrochloride (22). To a cooled suspension of 2-(S)-[N-[(Benzyloxy)carbonyl]amino]-3-aminopropionic acid (21) (Fluka) (10 g, 0.042 mol) in 150 mL of CH$_3$OH was added 5.47 g (0.046 mol) of thionyl chloride over 20 min. The resulting solution was allowed to stir at room temperature overnight. After ~18 h, the solvent was removed in vacuo, and the residual solid was stirred with 150 mL of Et$_2$O for 0.5 h. The resulting white solid was collected and air-dried to give 11.98 g (99%) of ester **22**. Mp: 168.5−171 °C. R_f (CHCl$_3$ saturated with ammonia): 0.37. NMR (400 MHz, CD$_3$OD): δ 7.33 (m, 5H), 5.13 (s, 2H), 4.50 (dd, $J = 5, 9$ Hz, 1H), 3.71 (s, 3H), 3.43 (dd, $J = 5, 13$ Hz, 1H), 3.21 (dd, $J = 9, 13$ Hz, 1H).

Methyl 2-(S)-[N-[(Benzyloxy)carbonyl]amino]-3-[N-[(*tert*-butyloxy)carbonyl]amino]propionate (23). To a two-phase mixture of CH$_2$Cl$_2$ (500 mL) and saturated NaHCO$_3$ solution (300 mL) was added 28.87 g (0.10 mol) of **22**. After a few minutes, 21.83 g (0.10 mol) of di-*tert*-butyl dicarbonate was added in one portion, and the resulting mixture was stirred at room temperature for 4 h. The CH$_2$Cl$_2$ layer was then separated from the aqueous layer, and the aqueous layer was extracted with 300 mL of CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried and the solvent removed in vacuo to provide the product as a viscous oil. Trituration of this oil with 300 mL of hexane gave 33.54 g (95%) of **23** as a white solid. Mp: 85−87 °C. R_f (5% MeOH/CH$_2$Cl$_2$): 0.68. NMR (400 MHz, CDCl$_3$): δ 7.35 (s, 5H), 5.78 (bs, 1H), 5.11 (s, 2H), 4.83 (bs, 1H), 4.41 (bs, 1H), 3.76 (s, 3H), 3.55 (bs, 2H), 1.41 (s, 9H).

Methyl 2-(S)-Amino-3-[N-[(*tert*-butyloxy)carbonyl]amino]propionate (24). To a solution of **23** (6.60 g, 0.0187 mol) in 150 mL of EtOH was added 0.5 g of 10% Pd/C. The resulting mixture was hydrogenated under balloon pressure at room temperature for 4 h. The catalyst was filtered off and the solvent removed in vacuo to provide 4.05 g (99%) of **24** as a viscous oil. R_f (10% MeOH/CHCl$_3$/NH$_3$): 0.6. NMR (400 MHz, CDCl$_3$): δ 5.0 (bs, 1H), 3.72 (s, 3H), 3.56 (t, $J = 5.7$ Hz, 1H), 3.46 (m, 1H), 3.23 (m, 1H), 1.55 (bs, 2H), 1.42 (s, 9H).

Methyl 2-(S)-[N-(3-Pyridylsulfonyl)amino]-3-[N-[(*tert*-butyloxy)carbonyl]amino]propionate (25). 3-Pyridine-sulfonic acid (30 g, 0.188 mol) was added to PCl$_5$ (46.8 g, 0.225 mol), suspended in 150 mL of toluene, and heated to reflux

overnight. The suspension was cooled and concentrated to yield a yellow oil, which was diluted with benzene, filtered through a pad of Celite, and concentrated to give 30.7 g (92%) of 3-pyridinesulfonyl chloride as a yellow oil, which was used in the next step without purification. NMR (300 MHz, CDCl$_3$): δ 9.27 (s, 1H), 8.98 (d, J = 4 Hz, 1H), 8.35 (d, J = 8 Hz, 1H), 7.62 (dd, J = 4, 8 Hz, 1H).

Amine **24** (16.8 g, 0.077 mole) dissolved in 330 mL of CH$_2$-Cl$_2$ was treated with 3-pyridinesulfonyl chloride (20.6 g, 0.116 mol) and pyridine (12.5 mL, 0.154 mol), and the reaction was stirred for 21 h. The reaction was concentrated, adsorbed to silica and chromatographed with a gradient of 30−70% acetone/hexanes to give crude **25** which was swished with hot EtOAc, cooled, and filtered to give **25** (17.5 g, 63%) as a pale-yellow solid. R_f (30% acetone/hexanes): 0.29. NMR (300 MHz, CDCl$_3$): δ 9.0 (s,1H), 8.8 (d, J = 4 Hz, 1H), 8.6 (d, J = 4 Hz, 1H), 8.1 (d, J = 8 Hz, 1H), 7.45 (dd, J = 4, 8 Hz, 1H), 7.3 (m, 1H), 4.1(m, 1H), 4.1 (s, 3H), 3.4−3.5 (m, 2H), 1.4 (s, 9H).

Methyl 2-(S)-[N-(3-Pyridylsulfonyl)amino]-3-amino-propionate (26a). A suspension of **25** (17.5 g, 0.049 mol) in 200 mL of EtOAc was cooled to −78 °C. HCl gas was bubbled through the solution for 10 min, and the solution was then placed in an ice bath. After stirring for 40 min at 0 °C, no starting material could be detected by TLC. The solution was concentrated, first at room temperature and then at 40 °C to yield 16 g (100%) of **26a** as an off-white solid. R_f (9:1:1 EtOH/H$_2$O/NH$_4$OH): 0.34. NMR (300 MHz, CD$_3$OD) d 9.3(s, 1H), 9.0 (dd, J = 1, 5 Hz, 1H), 8.9 (d, J = 9 Hz, 1H), 8.2 (dd, J = 5, 9 Hz, 1H), 4.6 (dd, J = 5, 9 Hz, 1H), 3.6 (s, 3H), 3.5 (dd, J = 5, 13 Hz, 1H), 3.3 (dd, J = 9, 13 Hz, 1H).

Methyl 2-(S)-[N-(Methylsulfonyl)amino]-3-aminopro-pioate Hydrochloride (26b). The procedure for **26a** was used except that methanesulfonyl chloride was used in the sulfonylation step. NMR (400 MHz, CD$_3$OD): δ 4.46 (dd, J = 4.7, 9 Hz, 1H), 3.84 (s, 3H), 3.43 (dd, J = 4.7, 13 Hz, 1H), 3.10 (dd, J = 9, 13 Hz, 1H), 3.06 (s, 3H).

Methyl 2-(S)-[N-(n-Butylsulfonyl)amino]-3-aminopro-pionate Hydrochloride (26c). The procedure for **26a** was used except that n-butanesulfonyl chloride was used in the sulfonylation step. R_f (9:1 EtOH/H$_2$O): 0.6. NMR (300 MHz, CDCl$_3$): δ 8.1 (bs, 2H), 7.2 (m, 1H), 4.65 (m, 1H), 3.82 (s, 3H), 3.65 (m, 1H), 3.54 (m, 1H), 3.20 (bs, 2H), 1.8 (m, 2H), 1.45 (m, 2H), 0.95 (t, J = 7 Hz, 3H).

Methyl 2-(S)-[N-(Ethoxylethylsulfonyl)amino]-3-ami-nopropionate Hydrochloride (26d). The procedure for **26a** was used except that ethoxyethanesulfonyl chloride, prepared from oxidation of the commercially available sulfide with chlorine,[23] was used in the sulfonylation step. NMR (300 MHz, CD$_3$OD): δ 3.89 (m, 5H), 3.65−3.76 (m, 6H), 1.19 (t, J = 7 Hz, 3H).

Methyl 2-(S)-[N-(Phenylsulfonyl)amino]-3-aminopro-pionate Hydrochloride (26e). The procedure for **26a** was used except that benzenesulfonyl chloride was used in the sulfonylation step. R_f (10% MeOH/CHCl$_3$ saturated with NH$_3$): 0.4. NMR (300 MHz, CD$_3$OD): δ 7.88 (m, 2H), 7.60 (m, 3H), 4.24 (m, 1H), 3.40 (s, 3H), 3.36 (m, 1H), 3.30 (m, 2H), 3.09 (m, 1H).

Methyl 2-(S)-[N-(2-Thienylsulfonyl)amino]-3-amino-propionate Hydrochloride (26f). The procedure for **26a** was used except that 2-thiophenesulfonyl chloride was used in the sulfonylation step. NMR (300 MHz, CD3OD): δ 7.86 (m, 1H), 7.66 (m, 1H), 7.17 (m, 1H), 4.33 (dd, 1H), 3.54 (s, 3H), 3.37 (dd, 1H), 3.12 (dd, 1H).

Methyl 2-(S)-[N-(4-Chlorophenylsulfonyl)amino]-3-aminopropionate Hydrochloride (26g). The procedure for **26a** was used except that 4-chlorobenzenesulfonyl chloride was used in the sulfonylation step. NMR (300 MHz, CD$_3$OD): δ 7.85 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 4.27 (dd, 1H), 3.47 (s, 3H), 3.35 (dd, 1H), 3.08 (dd, 1H).

Preparation of 2-(R)-Sulfonamide 3-aminopropionic Acids 26h,i. Methyl 2-(R)-N-[(3-Pyridylsulfonyl)amino]-3-aminopropionate (26h). 2-(R)-[N-[(Benzyloxy)carbonyl]-amino]asparagine was treated with bis(trifluoroacetoxy)phenyl iodide and pyridine using the method of Waki[24] to afford 2-(R)-

[N-[(benzyloxy)carbonyl]amino]-3-amino propionic acid. This was taken to the (R)-3-pyridylsulfonamide derivative **26h** as described for **26a**. NMR (300 MHz, CD$_3$OD): δ 9.25(s, 1H), 9.02 (d, J = 5 Hz, 1H), 8.75 (d, J = 9 Hz, 1H), 8.06 (dd, J = 5, 9 Hz, 1H), 4.51 (dd, J = 5, 9 Hz, 1H), 3.55 (s, 3H), 3.45 (dd, J = 5, 13 Hz, 1H), 3.16 (dd, J = 9, 13 Hz, 1H).

2-(R)-[N-(Phenylsulfonyl)amino]-3-aminopropionic Acid (26i). A solution of d-asparagine·HCl (4.31 g, 25.6 mmol) and NaOH (2.10 g, 50.0 mmol) in 20 mL of H$_2$O and 20 mL of dioxane was cooled to 0 °C. Benzenesulfonyl chloride (3.65 mL, 28.5 mmol) was added, followed by an additional 1.14 g (28.5 mmol) of NaOH in 20 mL of H$_2$O. After 30 min at 0 °C, the dioxane was removed in vacuo. The residue was basified with 20% NaOH to pH 10 and washed with EtOAc. The aqueous layer was then acidified with concentrated HCl and cooled to 0 °C. The resultant precipitate was collected by vacuum filtration and dried in a drying pistol for 18 h to yield 2.20 g (32%) of 2-(R)-[N-(phenylsulfonyl)amino]asparagine as a white solid. NMR (300 MHz, D$_2$O + NaOD): δ 7.63 (m, 5H), 3.98 (m, 1H), 2.64 (m, 1H), 2.49 (dd, J = 9, 15 Hz, 1H).

To a solution of NaOH (2.40 g, 60 mmol) in 10 mL of H$_2$O at 0 °C was added 0.54 mL (10 mmol) of bromine. After 5 min at 0 °C, a solution of 2-(R)-[N-(phenylsulfonyl)amino]aspar-agine (2.20 g, 8.08 mmol) and NaOH (0.68 g, 17 mmol) in 5 mL H$_2$O was added, and the solution stirred for 15 min at 0 °C. The reaction was then heated to 90 °C for 30 min, cooled to 0 °C, and neutralized with concentrated HCl. The resultant precipitate was collected by vacuum filtration to yield 1.48 g (65%) of the hydrochloride salt of **26i** as a white solid. NMR (300 MHz, D$_2$O + NaOD): δ 7.85 (m, 2H), 7.61 (m, 3H), 3.55 (dd, J = 4.7, 7.5 Hz, 1H), 2.82 (dd, J = 4.7, 13 Hz, 1H), 2.65 (dd, J = 7.5, 13 Hz, 1H).

Method for Preparation of Coupled Intermediates. Methyl 2-(S)-[N-(3-Pyridylsulfonyl)amino]-3-[[2-carbo-nyl-5-[2-[N-[($tert$-butyloxy)carbonyl]piperidin-4-yl]ethyl]-thieno[2,3-b]thiopheneyl]amino]propionate (27). Compound **12** (8.5 g, 0.0215 mol), amine **26a** (R = 3-pyridyl, 7.36 g, 0.025 mol), and BOP reagent (14.73 g, 0.033 mol) were suspended in 150 mL of CH$_3$CN and cooled to −40 °C. N-Methylmorpholine (7.05 mL, 0.075 mol) was added, the cold bath was removed, and the reaction mixture was stirred for 16 h. The resulting homogeneous solution was concentrated to one-half its original volume, diluted with 300 mL EtOAc, and washed successively with 10% KHSO$_4$, H$_2$O, saturated NaHCO$_3$, and brine, dried (Na$_2$SO$_4$), filtered, and concentrated. The crude product was chromatographed on SiO$_2$ using a gradient (15−45% acetone/hexanes) to give 12 g (87%) of **27**. R_f (45% acetone/hexanes): 0.28. NMR (300 MHz, CDCl$_3$): δ 9.0 (s,1H), 8.6 (d, J = 4 Hz, 1H), 8.1 (d, J = 8 Hz, 1H), 7.5 (s, 1H), 7.4 (m, 1H), 7.3 (dd, J = 4, 8 Hz, 1H), 7.2 (m, 1H), 6.8 (s, 1H), 4.3 (m, 1H), 4.0−4.1 (bs, 2H), 3.9 (m, 2H), 3.6 (s, 3H), 2.9 (m, 2H), 2.7 (bt, 2H), 1.6−1.5 (m, 5H), 1.45 (s, 9H), 1.1 (m, 2H).

Method A for Deprotection of Coupled Intermediates. 2-(S)-[N-(3-Pyridylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]amino]-propionic Acid (4). Compound **27** (17.3 g, 0.027 mol) was dissolved in 900 mL of 6 N HCl and stirred for 20 h at which time HPLC analysis indicated all **27** was consumed. The solvent was removed to yield 16.4 g of yellow solid hydrochlo-ride salt. Addition of 500 mL of H$_2$O to the crude product resulted in dissolution and then crystallization. The crystalline product was filtered off to give 11 g of a wet solid which was dried in a drying pistol overnight at room temperature to yield 6.21 g of **4** as the free base. The filtrate was concentrated to dryness to yield 7.5 g, which was dissolved in 70 mL of H$_2$O. One equivalent of 1 N NaOH (12 mL) was added slowly dropwise while the solution was stirred vigorously, and a crystalline precipitate formed. After the mixture cooled for 1 h, the solid was collected and dried on the drying pistol overnight at room temperature to yield 3.87 g of **4** as the free base. The combined yield of the two crops was 10.08 g (71%). Mp 245−247 °C (>99% ee (CHIRAL AGP 100 × 4.0 mm, Advanced Separation Technology)). R_f (9:1:1 EtOH/H$_2$O/NH$_4$-

OH): 0.31. NMR (300 MHz, D$_2$O + NaOD): δ 8.7 (s,1H), 8.0 (d, J = 8 Hz, 1H), 7.7 (d, J = 5 Hz, 1H), 7.2 (s, 1H), 6.8 (dd, J = 5, 8 Hz, 1H), 6.8 (s, 1H), 4.8 (dd, J = 2, 3 Hz,1H), 4.7 (bd, 2H), 3.1 (m, 2H), 2.8 (bd, 2H), 2.6 (bt, 2H), 2.1 (bt, 2H), 1.6 (m, 4H), 1.1 (m, 1H), 1.9 (m, 2H). Exact mass (FAB, m + 1): calcd, 523.1144; found, 523.1163. Anal. (C$_{22}$H$_{26}$N$_4$O$_5$S$_3$·1.95 H$_2$O) C, H, N.

2-(S)-[N-(Methylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]amino]-propionic Acid Hydrochloride (30). Acid **12** and amine **26b** were coupled as described for **27**. The coupled intermediate was deprotected using method A as described for **4** except that the crystallization step was omitted (50% for two steps). R_f (9:1:1 EtOH/H$_2$O/NH$_4$OH): 0.47. NMR (400 MHz, D$_2$O): δ 7.56 (s, 1H), 6.85 (s, 1H), 4.23 (dd, J = 5, 8 Hz, 1H), 3.71 (dd, J = 5, 14 Hz, 1H), 3.45 (dd, J = 8, 14 Hz, 2H), 3.24 (bd, J = 13 Hz, 2H), 2.93 (s, 3H), 2.74 (m, 4H), 1.78 (bd, J = 14 Hz, 2H), 1.50 (m, 3H), 1.25 (m, 2H). Exact mass (FAB, m + 1): calcd, 460.1035; found, 460.1028. Anal. (C$_{18}$H$_{25}$N$_3$O$_6$S$_3$·1.8 HCl· 0.25H$_2$O).

2-(S)-[N-(2-Ethoxyethylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]ami-no]propionic Acid Hydrochloride (32). Acid **12** and amine **26d** were coupled as described for **27**. The coupled intermediate was deprotected using Method A as described for **30** (38% for two steps). NMR (300 MHz, D$_2$O): δ 7.62 (s, 1H), 6.80 (s, 1H), 4.27 (dd, J = 4.6, 9 Hz, 1H), 3.75 (m, 3H), 3.50 (m, 1H), 3.34 (m, 6H), 2.73 (bt, J = 12 Hz, 2H), 2.60 (bs, 2H), 1.75 (bd, 2H), 1.20−1.53 (m, 5H), 0.99 (t, J = 6 Hz, 3H). Exact mass (FAB, m + 1): found, 518.1453; found, 518.1444. Anal. (C$_{21}$H$_{31}$N$_3$O$_6$S$_3$·1.25HCl·0.10Et$_2$O) C, H, N.

2-(R)-[N-(3-Pyridylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]amino]-propionic Acid (37). Acid **12** and amine **26h** (*R*-isomer) were coupled as described for **27**. The coupled intermediate was deprotected using method A as described for **30** (45% for two steps) (>99% ee (CHIRAL AGP 100 × 4.0 mm, Advanced Separation Technology)). NMR (300 MHz, D$_2$O+NaOD): δ 8.74 (s, 1H), 7.98 (d, J = 8 Hz, 1H), 7.81 (d, J = 5 Hz, 1H), 7.30 (s, 1H), 6.92 (m, 1H), 6.81 (s, 1H), 3.70 (m, 2H), 3.10 (m, 1H), 2.81 (bd, J = 12 Hz, 2H), 2.50 (t, J = 7 Hz, 2H), 2.26 (t, J = 11.5 Hz, 2H), 1.44 (m, 4H), 1.10 (m, 1H), 0.92 (m, 2H). Exact mass (FAB, m + 1): calcd, 523.1144; found, 523.1135. Anal. (C$_{22}$H$_{26}$N$_4$O$_5$S$_3$·2.00 HCl·0.30H$_2$O) C, N; H: calcd, 9.32; observed, 8.80.

2-(S)-[N-(3-Pyridylsulfonyl)amino]-3-[[2-carbonyl-5-[(piperidin-4-yl)methyl]thieno[2,3-b]thiopheneyl]amino]-propionic Acid Hydrochloride (38). Acid **20** and amine **26a** were coupled as described for **27**. The coupled intermediate was deprotected using method A as described for **30** (29% for two steps). NMR (300 MHz, CD$_3$OD): δ 8.86 (m, 2H), 8.05 (m, 1H), 7.72 (s, 1H), 7.10 (s, 1H), 4.43 (dd, J = 5, 9 Hz, 1H), 3.81 (dd, J = 5, 14 Hz, 1H), 3.53 (dd, J = 9, 14 Hz, 1H), 3.39 (bd, J = 13 Hz, 2H), 2.92 (m, 4H), 1.98 (m, 3H), 1.48 (m, 2H). Anal. (C$_{21}$H$_{24}$N$_4$O$_5$S$_3$·2HCl·H$_2$O) C, H, N.

2-(S)-[N-(3-Pyridylsulfonyl)amino]-3-[[2-carbonyl-5-[3-(piperidin-4-yl)propyl]thieno[2,3-b]thiophenyl]amino]-propionic Acid (39). The acid **10** and amine **26a** were coupled as described for **27**. The coupled intermediate was deprotected using method A as described for **30** (36% for two steps). NMR (300 MHz, CD$_3$OD): δ 9.24 (s, 1H), 8.84 (m, 1H), 8.02 (m, 1H), 7.69 (s, 1H), 7.05 (s, 1H), 4.42 (dd, J = 5, 8.5 Hz, 1H), 3.81 (dd, J = 5, 14 Hz, 1H), 3.52 (dd, J = 8.5, 14 Hz, 1H), 3.36 (bd, J = 13 Hz, 2H), 2.93 (m, 4H), 1.95 (bd, J = 13 Hz, 2H), 1.70 (m, 2H), 1.39 (m, 4H). Anal. (C$_{23}$H$_{28}$N$_4$O$_5$S$_3$·2.55HCl·0.05H$_2$O) C,H,N.

2-(S)-[N-(Methylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[3,2-b]thiopheneyl]amino]-propionic Acid Hydrochloride (40). Acid **9** and amine **26b** were coupled as described for **27**. The coupled intermediate was deprotected using method A as described for **30** (50% for two steps). NMR (400 MHz, D$_2$O): δ 7.70 (s, 1H), 6.98 (s, 1H), 4.18 (dd, J = 5, 8.5 Hz, 1H), 3.72 (dd, J = 5, 14 Hz, 1H), 3.46 (dd, J = 8.5, 14 Hz, 1H), 3.26 (bd, J = 12.5 Hz, 2H), 2.93 (s,

3H), 2.84−2.74 (m, 4H), 1.81 (m, 2H), 1.57 (m, 3H), 1.26 (m, 2H). Exact mass (FAB, m + 1): calcd, 460.1035; found, 460.1017. Anal. (C$_{18}$H$_{25}$N$_3$O$_5$S$_3$·1.65HCl) C, H, N.

2-(S)-[N-(2-Ethoxyethylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethylthieno[2,3-b]thiopheneyl]ami-no]propionic Acid Hydrochloride (42). Acid **9** and amine **26d** were coupled as described for **27**. The coupled intermediate was deprotected using method A as described for **30** (42% for two steps). NMR (300 MHz, D$_2$O): δ 7.78 (s, 1H), 6.90 (s, 1H), 4.32 (m, 1H), 3.73 (m, 3H), 3.40 (m, 7H), 2.78 (m, 4H), 1.85 (bd, 2H), 1.6−1.24 (m, 5H), 1.02 (t, 3H). Exact mass (FAB, m + 1): calcd, 518.1453; found, 518.1452. Anal. (C$_{21}$H$_{31}$N$_3$O$_6$S$_3$· 1.50HCl) C, H, N.

2-(S)-[N-(3-Pyridylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[3,2-b]thiopheneyl]amino]-propionic Acid Hydrochloride (45). Acid **9** and amine **26a** were coupled as described for **27**. The coupled intermediate was deprotected using method A as described for **30** (34% for two steps). NMR (300 MHz, D$_2$O + NaOD): δ 8.68 (s, 1H), 7.97 (d, 1H), 7.83 (d, 1H), 7.39 (s, 1H), 7.01 (m, 2H), 3.73 (m, 1H), 3.61 (dd, 1H), 3.19 (dd, 1H), 2.85 (m, 4H), 2.36 (t, 3H), 1.58 (m, 4H), 1.30 (m, 1H), 1.03 (m, 2H). Exact mass (FAB, m + 1): calcd, 523.1144; found, 523.1135. Anal. (C$_{22}$H$_{26}$N$_4$O$_5$S$_3$· 1.90HCl·1.05H$_2$O) C,H,N.

Method B for Deprotection of Coupled Intermediates. 2-(S)-[N-(n-Butylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(pi-peridin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]amino]pro-pionic Acid (31). Acid **12** and amine **26c** were coupled as described for **27**. The coupled intermediate was deprotected with LiOH in 1:1:1 THF/H$_2$O/MeOH as described for **11**; then the BOC group was removed with HCl-saturated EtOAc at 0 °C as described for **26a**. The HCl salt was converted to the free base by recrystallization from water as described for **4** (71% for three steps) NMR (300 MHz, CD$_3$OD): δ 7.76 (s, 1H), 7.05 (s, 1H), 4.30 (m, 1H), 3.82 (m, 1H), 3.56 (m, 1H), 3.00 (m, 3H), 2.00 (bd, J = 12 Hz, 2H), 1.72 (m, 4H), 1.48 (m, 4H), 0.85 (t, J = 7 Hz, 3H). Anal. (C$_{21}$H$_{31}$N$_3$O$_5$S$_3$·0.5H$_2$O) C, H, N.

2-(S)-[N-(Phenylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]amino]-propionic Acid (33). Acid **12** and amine **26e** were coupled as described for **27**. The coupled intermediate was deprotected as described for **31** and recrystallized from water (60% for three steps). NMR (300 MHz, DMSO-d$_6$): δ 8.61 (m, 2H), 7.79 (m, 3H), 7.49 (m, 3H), 7.14 (s, 1H), 3.20 (m, 3H), 2.80 (m, 4H), 1.82 (d, 2H), 1.58 (m, 3H), 1.30 (m, 2H). Anal. (C$_{23}$H$_{27}$N$_3$O$_5$S$_3$) C, H, N.

2-(R)-[N-(Phenylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]amino]-propionic Acid (34). Acid **12** in DMF was activated with CDI in DMF and then treated with amine **26i** and N,N-diisopro-pylethylamine to afford the coupled BOC acid intermediate. The BOC group was removed with HCl-saturated EtOAc at 0 °C as described for **26a**. The crude product was chromato-graphed on silica eluting with 9:1:1 EtOH/H$_2$O/NH$_4$OH to give the free base (25% for two steps). NMR (300 MHz, D$_2$O + NaOD): δ 7.57 (d, J = 7.5 Hz, 2H), 7.27 (s, 1H), 7.00 (m, 2H), 6.88 (s, 1H), 6.81 (m, 1H), 3.60 (m, 2H), 3.05 (m, 1H), 2.80 (m, 2H), 2.78 (m, 2H), 2.28 (m, 2H), 1.48 (m, 4H), 1.20 (m, 1H), 0.92 (m, 2H). Exact mass (FAB, m + 1): calcd, 522.1191; found, 522.1200. Anal. (C$_{23}$H$_{27}$N$_3$O$_5$S$_3$·1.35 H$_2$O·0.55SiO$_2$) C,H,N.

2-(S)-[N-(4-Chlorophenylsulfonyl)amino]-3-[[2-carbo-nyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]-amino]propionic Acid Hydrochloride (35). Acid **12** and amine **26g** were coupled as described for **27**. The coupled intermediate was deprotected as described for **31** (80% for three steps). NMR (300 MHz, CD$_3$OD): δ 7.77 (d, J = 9 Hz, 2 Hz), 7.59 (s, 1H), 7.35 (d, J = 9 Hz, 2H), 7.05 (s, 1H), 4.20 (dd, J = 5, 9 Hz, 1H), 3.71 (dd, J = 5, 14 Hz, 3H), 3.38 (m, 3H), 2.98 (m, 4H), 2.00 (bd, J = 14 Hz, 2H), 1.73 (m, 3H), 1.45 (m, 2H). Anal. (C$_{23}$H$_{26}$ClN$_3$O$_5$S$_3$·HCl·0.10EtOAc·0.95H$_2$O) C, H, N.

2-(S)-[N-(2-Thienylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-b]thiopheneyl]amino]-propionic Acid Hydrochloride (36). Acid **12** and amine **26f** were coupled as described for **27**. The coupled intermediate

was deprotected as described for **31** and then chromatographed on silica eluting with 9:1:1 EtOH/H_2O/NH_4OH to give the free base (72% for three steps). NMR (300 MHz, CD_3OD): δ 7.68 (s, 1H), 7.61 (m, 2H), 7.05 (s, 1H), 7.00 (m, 1H), 4.20 (dd, $J =$ 5, 8 Hz, 1H), 3.75 (dd, $J =$ 5, 13.5 Hz, 1H), 3.52 (dd, $J =$ 8, 13.5 Hz, 1H), 3.37 (bd, $J =$ 12 Hz, 2H), 2.96 (m, 4H), 1.99 (bd, $J =$ 11 Hz, 2H), 1.72 (m, 3H), 1.43 (m, 2H). Exact mass (FAB, $m + 1$): calcd, 528.0750; found, 528.0771. Anal. ($C_{21}H_{25}N_3O_5S_3$·HCl·0.60EtOAc·0.85SiO_2) C, H, N.

2-(*S*)-[*N*-(*n*-Butylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[3,2-*b*]thiopheneyl]amino]-propionic Acid Hydrochloride (41). Acid **9** and amine **26c** were coupled as described for **27**. The coupled intermediate was deprotected as described for **31** (56% for three steps). NMR (300 MHz, CD_3OD): δ 7.86 (s, 1H), 7.13 (s, 1H), 4.32 (dd, $J =$ 5, 9 Hz, 1H), 3.82 (dd, $J =$ 5, 18.5 Hz, 1H), 3.58 (dd, $J =$ 9 Hz, 1H), 3.32 (m, 2H), 3.00 (m, 6H), 2.00 (m, 3H), 1.72 (m, 5H), 1.30 (m, 6H), 0.86 (t, $J =$ 7 Hz, 3H). Anal. ($C_{21}H_{31}N_3O_5S_3$·HCl) C, H, N.

2-(*S*)-[*N*-(Phenylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[3,2-*b*]thiopheneyl]amino]-propionic Acid Hydrochloride (43). Acid **9** and amine **26e** were coupled as described for **27**. The coupled intermediate was deprotected as described for **31** (65% for three steps). NMR (300 MHz, CD_3OD): δ 7.81 (m, 2H), 7.69 (s, 1H), 7.40 (m, 3H), 7.12 (s, 1H), 4.15 (m, 1H), 3.71 (dd, $J =$ 5, 13.5 Hz, 1H), 3.36 (m, 3H), 2.98 (m, 7H), 2.00 (m, 3H), 1.72 (m, 3H), 1.42 (m, 3H). Anal. ($C_{23}H_{27}N_3O_5S_3$·HCl) C, H, N.

2-(*S*)-[*N*-(2-Thienylsulfonyl)amino]-3-[[2-carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[3,2-*b*]thiopheneyl]amino]-propionic Acid Hydrochloride (44). Acid **9** and amine **26f** were coupled as described for **27**. The coupled intermediate was deprotected as described for **31** and then chromatographed on silica eluting with 9:1:1 EtOH/H_2O/NH_4OH to give the free base (60% for three steps). NMR (300 MHz, CD_3OD): δ 7.76 (s, 1H), 7.61 (m, 2H), 7.12 (s, 1H), 6.98 (m, 1H), 4.24 (dd, $J =$ 5, 8 Hz, 1H), 3.75 (dd, $J =$ 5, 13.5 Hz, 1H), 3.52 (dd, $J =$ 8, 13.5 Hz, 1H), 3.38 (bd, $J =$ 12.5 Hz, 2H), 3.00 (m, 4H), 2.01 (m, 2H), 1.75 (m, 3H), 1.44 (m, 2H). Exact mass (FAB, $m + 1$): calcd, 528.0750; found, 528.0764. Anal. ($C_{21}H_{25}N_3O_5S_3$·HCl·1.15H_2O·0.75SiO_2) C, H, N.

Method C for Deprotection of Coupled Intermediates. 3-[[2-Carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[2,3-*b*]-thiopheneyl]amino]propionic Acid Hydrochloride (28). *tert*-Butyl-3-aminopropionic acid was coupled with **12** as described for **27**. The protecting groups were removed in one step with HCl-saturated EtOAc at 0 °C as described for **26a** (62% for two steps). NMR (300 MHz, CD_3OD): δ 7.73 (s, 1H), 7.05 (s, 1H), 3.62 (t, 2H), 3.38 (bd, 2H), 2.95 (m, 4H), 2.62 (t, 2H), 2.01 (bd, 2H), 1.74 (m, 3H), 1.95 (m, 2H). Anal. ($C_{17}H_{22}N_2O_3S_2$·HCl·0.6H_2O) C, H, N.

3-[[2-Carbonyl-5-[2-(piperidin-4-yl)ethyl]thieno[3,2-*b*]-thiopheneyl]amino]propionic Acid Hydrochloride (29). Prepared as described for **28** except that **9** was used in the coupling step (58% for two steps). NMR (400 MHz, CD_3OD): δ 7.81 (s, 1H), 7.12 (s, 1H), 3.61 (t, $J =$ 7 Hz, 2H), 3.37 (bd, $J =$ 12.5 Hz, 2H), 3.0 (m, 4H), 2.62 (t, $J =$ 7 Hz, 2H), 2.00 (bd, $J =$ 14 Hz, 2H), 1.75 (m, 3H), 1.41 (m, 2H). Anal. ($C_{17}H_{22}N_2O_3S_2$·HCl·0.35H_2O) C, H, N.

2-(*S*)-[*N*-(3-pyridylsulfonyl)amino]-3-[(2-carbonylthieno-[2,3-*b*]thiopheneyl]amino]propionic Acid (46). Coupled as described for **27**, deprotected as described for **31**, then chromatographed in 45:1:1 EtOH/H_2O/NH_4OH, and further purified by preparative HPLC eluting with 95:5 0.1% TFA in H_2O/CH_3CN (25% for two steps). R_f (45:1:1 EtOH/H_2O/NH_4OH): 0.45. NMR (300 MHz, D_2O + NaOD): δ 8.78 (s, 1H), 8.09 (d, $J =$ 7.5 Hz, 1H), 7.95 (d, $J =$ 5 Hz, 1H), 7.56 (d, $J =$ 5, Hz, 1H), 7.38 (s, 1H), 7.31 (d, $J =$ 5 Hz, 1H), 7.17 (m, 1H), 3.98 (m, 1H), 3.65 (m, 1H), 3.32 (m, 1H). Exact mass (FAB, $m + 1$): calcd, 412.0096; found, 412.0098. Anal. ($C_{15}H_{13}N_3O_5S_3$·0.55H_2O) C, H, N.

In Vitro Pharmacology: Scintillation Proximity-Based Assay for Affinity of an Activated Form of GPIIb/IIIa. GPIIb/IIIa was purified from outdated human platelets by passing platelet lysates sequentially over concanavalin A affinity, Sepharose 4B-hexyl-RGDS, affinty and Sephacryl S-300HR size exclusion columns by a modification of the method of Kouns et al.[25] Purified GPIIb/IIIa was coated onto yttrium silicate scintillation proximity assay fluomicrospheres (Amersham RPN 143) and is abbreviated IIb/IIIa/SPA. The binding of the RGD-containing heptapeptide [^{125}I]L-692,884 (New England Nuclear, NEX-330) to the yttrium silicate-containing IIb/IIIa/SPA is detectable, without the necessity of separation of bound from free, in a Top Count scintillation counter. The ED_{50} for a nonradiolabeled compound was determined by competition with the binding of [^{125}I]L-692,-884 to IIb/IIIa/SPA at pH 7.5 (20 mM HEPES, 0.15 M NaCl, room temperature) with ~0.3 nM IIb/IIIa/SPA, ~0.3 nM [^{125}I]L-692,884, and a wide range of concentrations of the competing nonradiolabeled compounds. After equilibration, the bound CPM were measured and the ED_{50} value was determined by nonlinear least-squares fit to CPM = $(B_{max} - B_{min})/(1 + (I/ED_{50})^B) + B_{min}$, where I is the concentration of the test compound, B is the Hill slope, B_{max} is the maximum binding observed without the test compound, and B_{min} is the nonspecific binding signal. The average standard error of mean for ED_{50} determinations was ± 20%.

Equilibrium Dissociation Constant (K_D) for Competitive Binding of Analogues with Fluorescent Ligand L-762,745. Fluorescein-containing L-762,745[9,18] in flow cytometric buffer at a final concentration of 110 nM was added to wells of a 96-well microtiter plate that contained test compounds at concentrations ranging from 0.05 to 1000 nM. GFP were then added to each well to give a final concentration of 2×10^6 cells/mL, and the microtiter plate was incubated for 2 h at room temperature. The samples were analyzed in a FACScan, as described previously.[17] The flow cytometric measurements of the affinity of the compounds for the receptors on resting platelets were measured from at least three different platelet donors. The standard deviation of the measurements was for all compounds <20%. The value for **4** is 0.07 ± 0.006 nM (mean ± STD) with $n = 20$.

In Vivo Pharmacology. All animals were cared for under the standards of the NIH Guide for the Care and Use of Laboratory Animals. All studies were reviewed by an institutional animal care and use committee (MRL-20P).

Single-Dose Intravenous and Oral Administration of 4 to Conscious Dogs. The potency of antiplatelet activity of **4** was assessed following single-dose intravenous administration. Two groups of conscious, purpose-bred mongrel dogs of either sex (10.5−13 kg) were administered **4** as an intravenous bolus (vehicle = saline) of 3 ($n = 3$) or 5 ($n = 3$) μg/kg. In addition, oral antiplatelet activity of **4** was assessed following single-dose oral administration in aqueous solution by gastric lavage to conscious dogs. Three groups of dogs of either sex (9.2−12.7 kg) were administered **4** orally (5-mL volume, vehicle = sterile water): 50 ($n = 5$), 100 ($n = 4$); 200 ($n = 2$) μ/kg. For all treatment groups described above, blood samples were obtained before compound administration (baseline) and at multiple timepoints up to 8 h after compound administration from either saphenous or cephalic veins (0.38% sodium citrate, final concentration) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts, as described below.

Once-Daily Oral Administration of 4 for 6 Days to Conscious Dogs by Gastric Lavage: Maintenance of Inhibition of Platelet Function. A study in dogs was conducted to determine if submaximal but therapeutic levels of inhibition of platelet aggregation could be maintained with once-daily oral doses of **4**. A group of conscious, purpose-bred mongrel dogs of either sex ($n = 4$, 7.6−11.0 kg) was administered individualized loading doses of 50−90 μg/kg **4** on the first day of treatment, followed by individualized daily doses of 0, 10, 20, or 30 μg/kg for 2 days and identical daily doses of 10 μg/kg for 3 additional days, 6 total days of dosing. Compound **4** was administered in solution by gastric lavage (5-mL volume with sterile water as vehicle and 10-mL flush with sterile water). Blood samples were obtained prior to the first oral dose

(baseline) at 120, 180, 240, and 330 min after the first oral dose and at 30 min before and 60 min after compound administration on succeeding days (i.e., 23.5 h after dosing on the previous day) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts, as described below. Blood samples were also obtained over the next 12 days following termination of dosing for these measurements. A separate, control group of four dogs was studied for daily effects on ex vivo platelet aggregation following one, single oral dose for comparison to the multiple-dose group.

Relationship between Acute Effects of Intravenous 4 on ex Vivo Platelet Aggregation Responses and Bleeding Times in Anesthetized Dogs. The relationship between the inhibition of platelet aggregation and the prolongation of bleeding time was investigated during the intravenous administration of increasing doses of **4** to the anesthetized dog. Two groups of pentobarbital anesthetized dogs were evaluated for ex vivo inhibition of platelet aggregation and template bleeding time: **4** (3–6 increasing doses, iv bolus, $n = 6$) and vehicle (5 mL of saline, 4 bolus infusions, $n = 4$). Purpose-bred mongrels of either sex (9.2–14.8 kg) were anesthetized with 35 mg/kg iv sodium pentobarbital, intubated, ventilated with room air, and instrumented with arterial catheters for blood withdrawal and venous catheters for the infusion of experimental agents and supplemental anesthesia. Prior to the administration of the first bolus of **4** or vehicle, a blood sample (5 mL) was taken to determine the baseline platelet aggregation response to ADP and collagen as described below, and buccal mucosal bleeding time was measured using a SIM-PLATE bleeding time device (Organon Teknika Corp., Durham, NC) as described below. One minute following each bolus, a blood sample was obtained and a bleeding time measurement was initiated. A second blood sample was taken at the termination of the bleeding time measurement. For each individual dose, the percent inhibition of aggregation was averaged from the "pre" and "post" bleeding time samples, and this value was used to represent the effect on platelet aggregation for that particular dose and measured bleeding time. This was repeated for 3–6 iv bolus doses of **4** (0.5 μg/kg followed by 0.5 or 1.0 μg/kg) or for 4 iv bolus infusions of vehicle. In the **4** treatment group the final dose was not predetermined but was the dose at which template bleeding time exceeded the maximum limit which was set at 15 min.

Ex Vivo Platelet Aggregation Responses to ADP and Collagen in PRP and Whole Blood Platelet Counts. Blood was withdrawn into sodium citrate (0.38%, final concentration). Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 150g for 5 min, and the platelet concentration was adjusted to 2×10^8 platelets/mL with time-matched platelet-poor plasma (PPP). PRP (300 μL, 2×10^8 platelets/mL) was incubated at 37 °C for 3 min prior to the addition of agonist. Platelet aggregation was measured by the change in light transmittance (PPP represents 100%) under stirring conditions (1100 rpm) at 37 °C in a Biodata platelet aggregation profiler, model PAP-4. Aggregation was initiated by the addition of 10 μM ADP + 1 μM epinephrine or 10 μg/mL collagen + 1 μM epinephrine. The extent of aggregation is reported as the peak percent of aggregation achieved based on a maximum of 100%, and the maximum slope represents the maximum sustained rate of the aggregatory response. The effect of **4** treatment on the extent and rate of aggregation is expressed as the percent inhibition using the baseline, pre-treatment aggregation response (day 1 in mulitple day maintenance studies) as 100%. Whole blood platelet counts were determined using an automated hematology analyzer (Serono-Baker Diagnostics, Allentown, PA).

Buccal Mucosal Template Bleeding Times. Buccal mucosal template bleeding times were measured with a SIMPLATE bleeding time device (Organon Teknika Corp., Durham, NC). Uniform incisions were made on the mucous membrane of the inner upper lip of the dog, and the duration of bleeding was timed to a maximum of 15 min.

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