Non-Peptide Glycoprotein IIb/IIIa Inhibitors. 17. Design and Synthesis of Orally Active, Long-Acting Non-Peptide Fibrinogen Receptor Antagonists

Benny C. Askew,*[,†] Rodney A. Bednar,[‡] Bohumil Bednar,[‡] David A. Claremon,[†] Jacquelynn J. Cook,[‡] Charles J. McIntyre,[†] Cecila A. Hunt,[†] Robert J. Gould,[‡] Robert J. Lynch,[‡] Joseph J. Lynch, Jr.,[‡] Stanley L. Gaul,[‡] Maria T. Stranieri,[‡] Gary R. Sitko,[‡] Marie A. Holahan,[‡] Joan D. Glass,[‡] Terrence Hamill,[‡] Lynn M. Gorham,[§] Thomayant Prueksaritanont,[§] John J. Baldwin,[†,||] and George D. Hartman[†]

Merck Research Laboratories, Departments of Medicinal Chemistry, Pharmacology, and Drug Metabolism, West Point, Pennsylvania 19486

Received November 27, 1996[⊗]

The synthesis and pharmacological evaluation of **5** (L-738,167), a potent, selective non-peptide fibrinogen receptor antagonist is reported. Compound **5** inhibited the aggregation of human gel-filtered platelets with an IC_{50} value of 8 nM and was found to be >33000-fold less effective at inhibiting the attachment of human endothelial cells to fibrinogen, fibronectin, and vitronectin than it was at inhibiting platelet aggregation. *Ex vivo* platelet aggregation was inhibited by >85% 24 h after the oral administration of **5** to dogs at 100 μg/kg. The extended pharmacodynamic profile exhibited by **5** appears to be a consequence of its high-affinity binding to GPIIb/IIIa on circulating platelets and suggests that **5** is suitable for once-a-day dosing.

Introduction

When a blood vessel is damaged acutely by trauma induced by clinical interventions such as angioplasty, or more chronically by the pathophysiological processes of atherosclerosis, platelets become activated and adhere to the site of injury and to each other. This activation, adherence, and aggregation may lead to the formation of occlusive thrombi in the vessel lumen resulting in acute thrombotic disorders.[1−4] The final obligatory step in platelet aggregation, regardless of the activating signal, is the binding of fibrinogen to activated glycoprotein IIb/IIIa (GPIIb/IIIa) on the surface of activated platelets.[5−7] Cyclic peptides containing the tripeptide sequence Arg-Gly-Asp (RGD) have been demonstrated to be potent inhibitors of platelet aggregation;[8−13] therefore, small molecules and peptidomemetics that feature elements of this tripeptide sequence have been pursued as inhibitors of platelet aggregation.[14] Design strategies employed by several groups use rigid central constraints to direct the vectors of the pharmacophoric moieties: a guanidine or guanidine surrogate mimicking the arginyl side chain and a carboxylic acid representing the side chain carboxylic acid of aspartic acid.[15−18]

Researchers at these and other laboratories have reported that incorporation of sulfonamido or carbamoyl moieties α to the carboxylate resulted in increased *in vitro* potency and improvements in the *in vivo* profiles of centrally constrained analogs.[16a,17,18] Recently, we reported that incorporation of an α-*n*-butanesulfonamide moiety on the centrally constrained pyrazolopiperazinone **1** to give **2** (L-734,115) resulted in a 28-fold increase in *in vitro* antiaggregatory potency (Table 1) and afforded a compound that produced profound activity following oral administration to dogs and primates.[17c] In this report we describe modification of the homologous pyrazolodiazepinone **3** to provide **5** (L-738,167), a potent, selective fibrinogen receptor antagonist that displays an extremely long pharmacodynamic profile following intravenous or oral administration in dogs.

Chemistry

The pyrazolopiperazinone analog **1** was prepared as illustrated in Schemes 1 and 2. The N-terminal piperidine moiety utilized in all of the analogs was derived from commercially available alcohol **6** (Scheme 1). Following protection of **6** with Boc_2O, the resulting alcohol was converted to the iodide **7** using conditions described by Garegg.[19] Reaction of **7** with excess NaN_3 in DMSO and reduction of the resulting azide afforded the amine **8** in 98% yield. Commercially available 3,5-pyrazoledicarboxylic acid (**9**) served as the key synthon of the bicyclic central constraints. Reaction of **9** with methanolic HCl afforded the diester **10**, which was alkylated with excess 1,2-dibromoethane or 1-bromo-3-chloropropane to give the alkyl halides **11a** and **11b**. Reaction of **8** with **11a** and saponification of the resulting ester afforded the pyrazolopiperazinone **12b** in 49% yield. Coupling **12b** with β-alanine *tert*-butyl ester and deprotection of the resulting doubly protected compound with hydrogen chloride gas in ethyl acetate afforded **1** (Scheme 2).

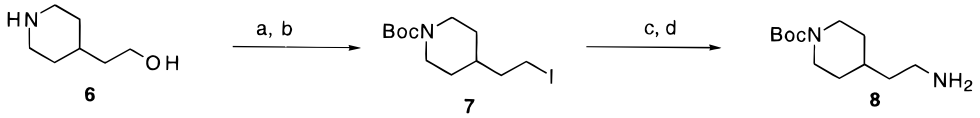
Since analogous preparation of the pyrazolodiazepinone analog **3** was problematic due to intramolecular cyclization of the chloride **11b** to a bicyclic pyrazolium salt,[16d] an alternate route to **3** was developed (Scheme 3). Reaction of **11b** with excess NaN_3 afforded the corresponding azide which was converted to the pyrazolodiazepinone **13** in 95% yield by treatment with palladium on carbon under a hydrogen atmosphere. Alkylation of **13** with the iodide **7**, followed by saponification, afforded the acid **14b.** Conversion of **14b** to **3** was carried out as illustrated in Scheme 2 for the synthesis of **1**.

Compounds **2**, **4**, and **5** were prepared from intermediates **12b** and **14b** as illustrated in Scheme 5. The α-sulfonamido-β-alanines **17a** and **17b**, prepared from L-asparagine as depicted in Scheme 4, were coupled with **12b** and **14b** without carboxylate protection using the

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Department of Drug Metabolism.
|| Present address: Pharmacopia, Princeton, NJ 08540.
⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1997.

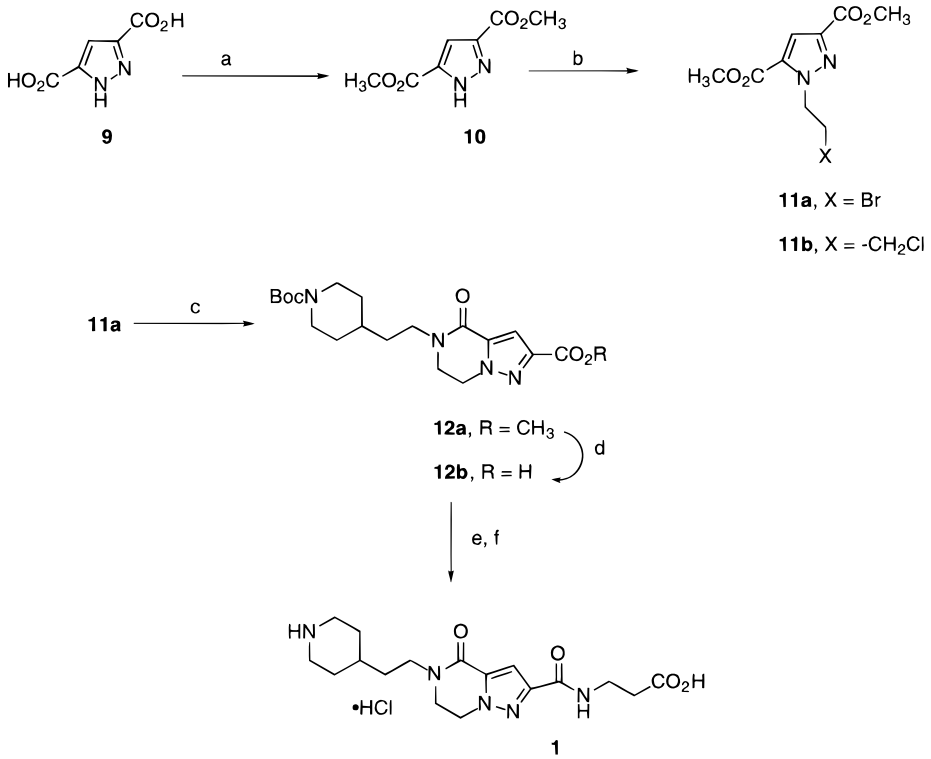
S0022-2623(96)00811-4 CCC: $14.00

Scheme 1



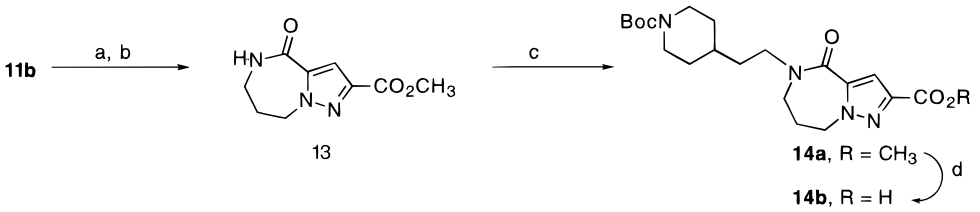
[a] (a) Boc_2O, CH_2Cl_2; (b) I_2, Ph_3P, imidazole, toluene; (c) NaN_3, DMSO; (d) H_2, Pd/C, MeOH.

Scheme 2



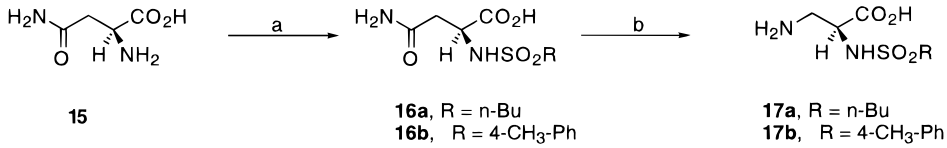
[a] (a) HCl, MeOH; (b) $Br(CH_2)_2Br$ or $Br(CH_2)_3Cl$, CH_3CN, K_2CO_3; (c) **8,** CH_3CN, Et_3N; (d) LiOH, THF/H_2O; (e) β-alanine *t*-Bu ester·HCl, EDC, Et_3N, CH_2Cl_2; (f) HCl, EtOAc.

Scheme 3



[a] (a)NaN_3, DMSO; (b) H_2, Pd/C, MeOH; (c) NaH, **7**, DMF; (d) LiOH, THF/H_2O.

Scheme 4



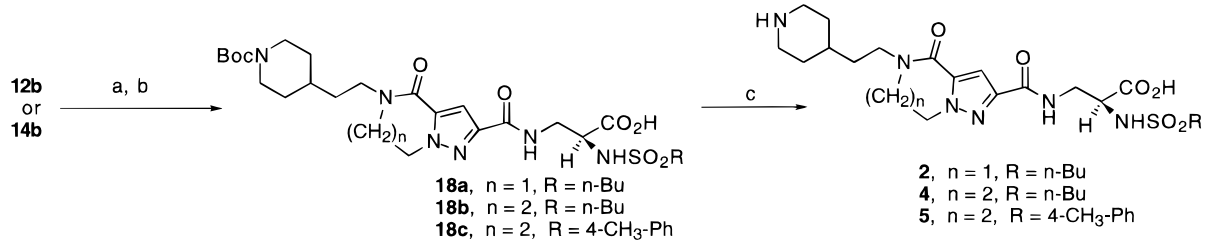
[a] (a) RSO_2Cl, NaOH, dioxane/H_2O; (b) Br_2, NaOH, H_2O.

mixed anhydride method. This protocol avoids the racemization observed when alkyl esters of **17a** were used to prepare **2**.[17c] The resulting analogs, **18a**−**c**, were treated with HCl to effect removal to the Boc protecting group and then purified by ion exchange chromatography to afford analytically pure **2**, **4**, and **5**.

Results and Discussion

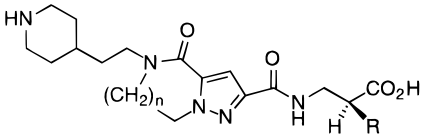
As we previously reported, incorporation of an (*S*)-α-*n*-butanesulfonamide moiety on the centrally constrained pyrazolopiperidinone **1** resulted in a 28-fold increase in potency and afforded **2**, a compound that produced profound antiaggregatory activity following oral administration to dogs and primates.[17c] Similar modification of the homologous pyrazolodiazepinone **3** afforded **4** and **5** which also inhibited ADP mediated aggregation of human gel-filtered platelets at low nanomolar concentrations (Table 1). Since the platelet concentration in the *in vitro* aggregation assay is adjusted to 2×10^8 platelets/mL and platelets from

Scheme 5



[a] (a) *i*-BuOCOCl, *N*-methylmorpholine, THF; (b) **17a** or **17b**, THF/H_2O; (c) HCl, EtOAc.

Table 1. *In Vitro* Potency of Analogs **1**−**5** and **22**



compd	*n*	R	plaggin IC_{50} (nM)[a]	K_d (nM)[b]	ED_{50} (nM)[c]
1	1	H	250	590	72
2	1	$NHSO_2(CH_2)_3CH_3$	9	1.8	0.12
3	2	H	470	680	46
4	2	$NHSO_2(CH_2)_3CH_3$	10	1.4	0.12
5	2	$NHSO_2Ph$-4-CH_3	8	1.1	0.08
22			32	600	5

[a] Inhibition of aggregation of human gel-filtered platelets (GFP) was measured by a light transmittance method at 37 °C with 2 × 10^8 platelets/mL, 0.1 mg/mL human fibrinogen, and 1 mM $CaCl_2$.[16a] Aggregation was initiated by adding 10 μM ADP after all the other components were added. The rate of aggregation in the absence of inhibitor served as the control, and values reported are the concentration necessary to inhibit the rate of aggregation by 50%. At least two determinations were made for each compound, and values typically varied by <20%. [b] Equilibrium binding constant determined for the displacement of the fluroescent ligand **19** (L-736,622) from purified resting GPIIb/IIIa. For details, see the *In Vitro* Pharmacology section of the Experimental Section. [c] Measured by competition with **21** ([^{125}I]L-692,884) for binding to purified GPIIb/IIIa activated by coating onto yttrium silicate Scintillation Proximity Assay Fluoromicrospheres. For details, see the *In Vitro* Pharmacology section of the Experimental Section.

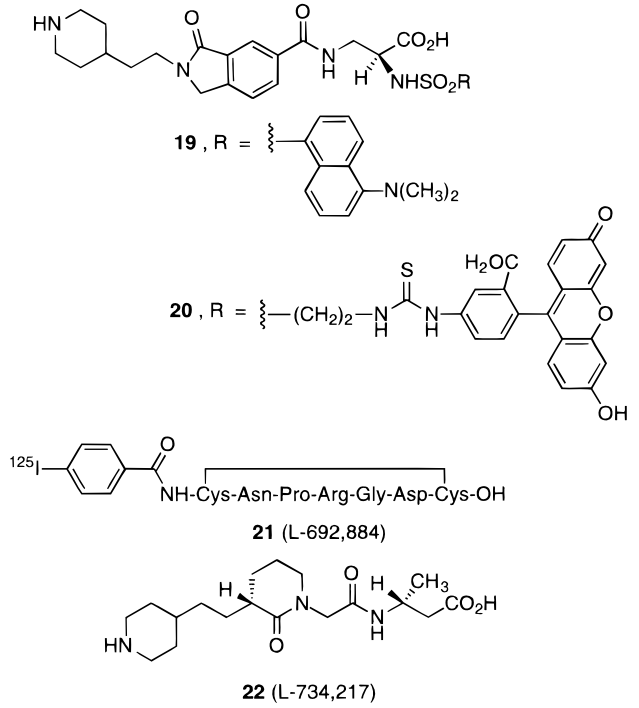


Figure 1.

different donors can contain 20000−100000 receptors per platelet, the minimum concentration of drug required to occupy 50% of the platelet receptors is 4−16 nM. Therefore, the IC_{50} values listed in Table 1 for analogs **2**, **4**, and **5** are at the lower limit of the *in vitro* aggregation assay and do not reflect their intrinsic potency.

To assess the intrinsic potency of these compounds, binding assays using the activated and unactivated forms of purified GPIIb/IIIa[29] were developed. Displacement of the fluorescent fibrinogen receptor antagonist **19**[20] (Figure 1) from both the activated and unactivated forms of human GPIIb/IIIa solubilized in Triton X-100 micelles was used to measure the equilibrium binding of compounds. Table 1 lists the equilibrium dissociation constants, K_D values, for binding to the unactivated form of GPIIb/IIIa. Equilibrium dissociation constants of 0.9 and 1.1 nM were obtained for binding of **5** to activated and unactivated forms, respectively. However, due to the high concentration of GPIIb/IIIa required in this fluorescence-based assay, K_D values can only be accurately determined down to the low nanomolar range.[20,21] Therefore, the K_D values measured for **2**, **4**, and **5** likely do not reflect the intrinsic binding affinity of these potent compounds.

To extend the sensitivity of these binding assays, ED_{50} values were calculated from competitive binding between compounds of interest and the ^{125}I-labeled fibrinogen receptor antagonist **21** ([^{125}I]L-692,884) to purified GPIIb/IIIa activated by coating onto yttrium silicate scintillation proximity assay fluoromicrospheres. The ED_{50} value of 0.08 nM for **5** listed in Table 1 provides an estimate of **5**'s intrinsic affinity for an activated form of GPIIb/IIIa. The equilibrium dissociation constant for binding of **5** to resting human platelets was measured directly using ^{3}H-labeled **5**[22] and indirectly by displacement of the fluorescent ligand **20** by flow cytometry,[23] yielding values of 0.2 and 0.11 nM, respectively. These data indicate that **5** binds to both forms of GPIIb/IIIa with high affinity and differs from compound **22** (L-734,217,[24] Figure 1), which has K_D values of 600 and 5 nM for binding to unactivated and activated GPIIb/IIIa, respectively.[21]

The *in vivo* consequences of high-affinity binding to GPIIb/IIIa on circulating platelets were evaluated by comparing the intravenous and oral profiles of compounds **2**, **4**, and **5** with those of **22**, an orally active compound with low affinity for resting platelets.[21] Administration of **22** to dogs as a 30 μg/kg iv bolus elicited >90% inhibition of the extent of ADP-induced platelet aggregation between 1 and 15 min, with platelet aggregation returning to baseline levels by 120 min (Figure 2). Intravenous administration of compounds **2**, **4**, and **5** at a 3-fold lower dose (10 μg/kg), elicited peak

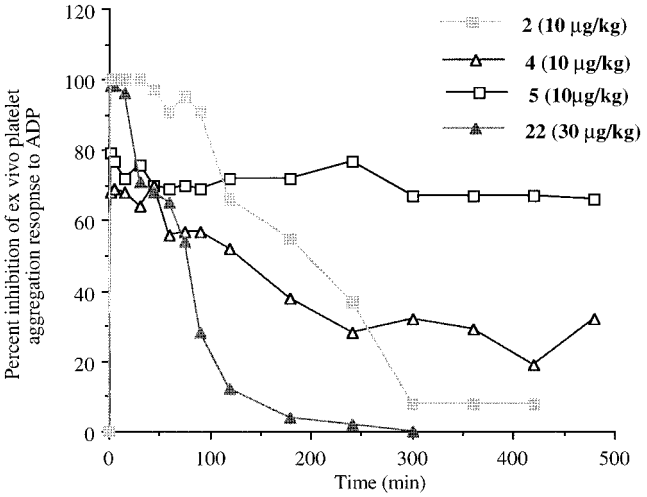


Figure 2. Effect of **2**, **4**, **5** (10 μg/kg iv), and **22** (30 μg/kg iv) on *ex vivo* platelet aggregation responses to ADP (10 μM + 1 μM epinephrine) expressed as percent inhibition over time (min) in conscious dogs. Data are mean for $n = 4$ (**2**, **4**, and **5**) and $n = 8$ (**22**); error bars are not shown for clarity.

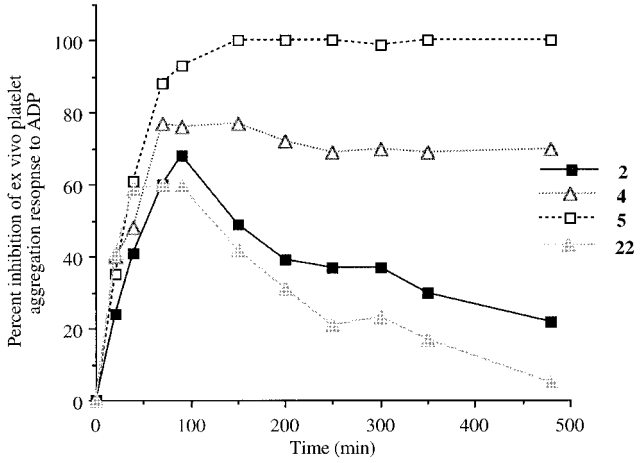


Figure 3. Effect of **2**, **4**, **5** (0.10 mg/kg po gavage), and **22** (0.20 mg/kg po gavage) on *ex vivo* platelet aggregation responses to ADP (10 μM + 1 μM epinephrine) expressed as percent inhibition over time (min) in conscious dogs. Data are mean for $n = 4$ (**2**, **4**, and **5**) and $n = 8$ (**22**); error bars are not shown for clarity.

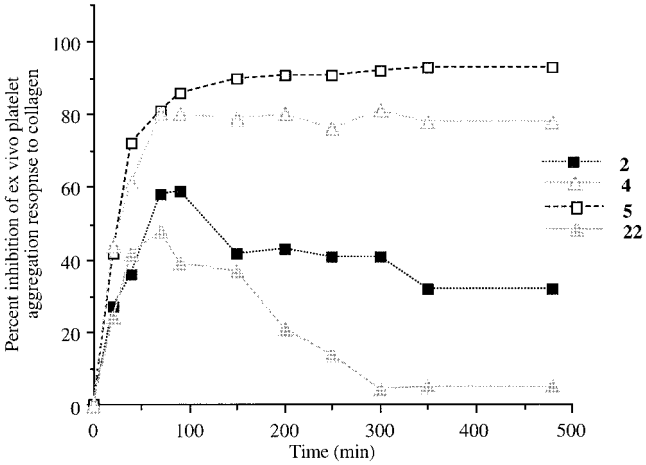


Figure 4. Effect of **2**, **4**, **5** (0.10 mg/kg po gavage), and **22** (0.20 mg/kg po gavage) on *ex vivo* platelet aggregation responses to collagen (10 μg/mL + 1 μM epinephrine) expressed as percent inhibition over time (min) in conscious dogs. Data are mean for $n = 4$ (**2**, **4**, and **5**) and $n = 8$ (**22**); error bars are not shown for clarity.

inhibition of platelet aggregation at 1 min, with values of 100%, 71± 11%, and 79 ± 13% observed for **2**, **4**, and **5**, respectively. Administration of **2** resulted in complete inhibition of platelet aggregation between 1 and 30 min, with platelet aggregation returning to within 8% of baseline levels by 300 min. Significant inhibition of *ex vivo* platelet function was maintained throughout the 8 h protocol following administration of either **4** or **5**. In the case of compound **5**, the iv pharmacodynamic response was essentially flat, with 67 ± 21% inhibition observed at the end of the 8 h experiment.

Differences in the pharmacodynamic profiles of compounds **2**, **4**, **5**, and **22** were also apparent following oral administration. Aqueous solutions of each compound (5 mL volume with sterile water as vehicle) were administered to mongrel dogs by gastric lavage at doses of 100 μg/kg (**2**, **4**, and **5**) or 200 μg/kg (**22**). At specified time points, blood was withdrawn and the derived platelet-rich plasma used to determine *ex vivo* platelet aggregation in response to ADP and collagen. The inhibition of ADP-mediated platelet aggregation following oral administration of **2**, **4**, **5**, and **22** is depicted in Figure 3. For each compound, the inhibition of collagen induced aggregation followed a similar time course, however, peak levels of inhibition were typically lower (Figure 4). Peak inhibition of ADP-induced platelet aggregation (60 ± 12%) occurred 70 min following the administration of **22** with platelet function returning to baseline levels by 480 min. Compounds **2**, **4**, and **5** each inhibited ADP-mediated platelet aggregation by greater than 65% at the 100 μg/kg dose. However, there were striking differences between compounds in terms of both the peak antiaggregatory response and the duration of the effect. Peak inhibition of platelet aggregation (65 ± 5%) occurred 90 min following the administration of **2** with platelet function returning to within 21 ± 3% of baseline by 480 min. Both of the pyrazolodiazepinone analogs, **4** and **5**, produced higher peak levels of inhibition and the effect remained stable through the end of the 8 h experimental protocol. For compound **4**, peak inhibition of 76 ± 8% occurred 75 min following dosing and inhibition remained at 68 ± 9% at 8 h. Maximal inhibition (100%) occurred 150 min following dosing of **5** and was maintained through the end of the experiment. The duration of antiplatelet activity following oral administration of crystalline **5** in gelatin capsules was evaluated in an additional experiment. Platelet aggregation was inhibited by 87 ± 7% 24 h after the administration of **5** to conscious dogs ($n = 4$) at 100 μg/kg, suggesting that its pharmacodynamic profile would allow once-a-day dosing.[27]

The differences in the pharmacodynamic profiles of compounds **2**, **4**, **5**, and **22** are likely a result of their differing affinities for GPIIb/IIIa on resting platelets. Compounds **2**, **4**, and **5** bound tightly to isolated, unactivated GPIIb/IIIa with K_D values of 1.8, 1.4, and 1.1 nM, respectively, while **22** had modest affinity with $K_D = 600$ nM. Since **22** has modest affinity for GPIIb/IIIa on resting platelets, its pharmacodynamic profile is determined largely by the rate of clearance of circulating drug.[25] However, because **5** binds to resting platelets with high affinity, the pharmacodynamic profile of this compound is governed by the rate of clearance of the platelet bound drug rather then the clearance of free drug from plasma.[26b] When ^{3}H-**5** was

Table 2. Inhibition of Platelet Aggregation and HUVEC[a] Attachment to Fibrinogen (F_G), Vitronectin (V_N), and Fibrinogen (F_N) by **5**

plaggin IC_{50} (nM)	HUVEC IC_{50} (nM)		
	F_G	V_N	F_N
8	$>3 \times 10^5$	$>3 \times 10^5$	$>3 \times 10^5$

[a] HUVEC-human umbilical vein endothelial cells. Experimental details of this assay have been previously reported.[16a,28]

administered iv at doses ranging from 3 to 250 μg/kg, the pharmacokinetic parameters were dose-dependent; both the clearance and volume of distribution increased markedly with increasing doses.[26b] Measurement of the concentration of **5** in whole blood (WB) and platelet-rich (PRP) and platelet-poor plasma (PPP) following iv administration at several doses indicated that **5** bound tightly to circulating platelets. Following administration of **5** doses exceeding the platelet binding capacity (70 nM in canine whole blood), excess free drug was rapidly eliminated, with a half-life ($t_{1/2}$) of approximately 0.5 h. However, the platelet bound **5** was slowly eliminated from the PRP compartment and the terminal half-life ($t_{1/2}$) was independent of the dose given, with a mean value of 96 h. Full details of the pharmacokinetics of **5** are reported elsewhere.[26b]

Intrinsic potency differences between **2**, **4**, and **5** are likely masked since the fluorescence-based assay can only accurately determine K_D values down to the low nanomolar range.[20,21] The greater potency of **4** compared **2** is likley a consequence of the larger ring of **4**. The added flexibility may allow **4** to adopt conformations that maximize the interactions between GPIIb/IIIa, its charged termini, and potency-enhancing α-sulfonamide moiety. The apparent increase in potency of **5** relative to **4** is in agreement with results from an earlier, less potent series of centrally constrained α-sulfonamides in which arenesulfonamide analogs were generally 4−10-fold more potent than alkanesulfonamides.[20]

Since the platelet fibrinogen receptor is a member of the integrin superfamily of receptors, specificity for GPIIb/IIIa over other RGD-binding integrins is believed to be an important requirement for safety in an oral antiplatelet agent. The specificity of **5** was evaluated by determining IC_{50} values for the inhibition of human umbilical vein endothelial cell (HUVEC) attachment to fibrinogen-, vitronectin-, and fibronectin-coated surfaces.[28] The results summarized in Table 2 demonstrate that **5** is >33000-fold less effective at inhibiting cell attachment than at inhibiting platelet aggregation.

In summary, we have reported the discovery of **5** (L-738,167), a potent, selective fibrinogen receptor antagonist that displays an extended pharmacodynamic profile following intravenous and oral administration in dogs. Platelet aggregation was inhibited by 87 ± 7% 24 h after the administration of **5** to conscious dogs at 100 μg/kg. The unprecedented pharmacodymanic profile exhibited by **5** appears to a consequence of its tight binding to circulating platelets and may enable clinically useful levels of inhibition of platelet aggregation to be maintained with small amounts of compound given once a day.

Experimental Section

Unless otherwise noted, starting materials were obtained from commercial sources and used without purification. Silica gel (E. Merck, 230−400 mesh) was used for flash column chromatography, and silica gel plates (E. Merck) were used for analytical thin-layer chromatography. All NMR spectra were recorded on a Varian XL-300 spectrometer, and chemical shifts are reported in parts per million relative to internal tetramethylsilane. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Enantiomeric purities were determined by HPLC using the following conditions: chiral AGP column (4.0 × 100 mm); flow rate 1.00 mL/min; 100% pH = 3.0, 20 mM NaH_2PO_4 buffer; UV detection at 255 nm. Under these conditions baseline separation between **5** and its (*R*) enantiomer was obtained (retention time for **5** = 6.71 min; (*R*) enantiomer = 10.72 min) and a detection limit of 0.1% of the (*R*) isomer was established. The enantiomer of **5** was prepared as described for **5** with the amino acid portion being derived from D-asparagine.

2-(*N*-Boc-piperidin-4-yl)ethyl Iodide (7). A solution of 4-piperidineethanol (**6**) (13.0 g, 100 mmol) was dissolved in CH_2Cl_2 (250 mL), cooled to 0 °C, and treated with di-*tert*-butyl dicarbonate (22.19 g, 100 mmol). The solution was warmed to room temperature and stirred for 4 h. The solution was then washed with water, 10% $KHSO_4$, and brine, dried (Na_2SO_4), filtered, and concentrated to give 2-(*N*-Boc-piperidin-4-yl)ethanol (21.4 g, 98%) as a colorless oil: TLC R_f = 0.25 (40% ethyl acetate/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (bd, 2H), 3.70 (t, 2H), 2.7 (t, 2H), 1.8−1.6 (m, 5H), 1.48 (s, 9H), 1.1 (m, 2H).

A solution of the above alcohol (10.42 g, 48 mmol) in 200 mL of toluene was treated with imidazole (4.66 g, 68.0 mmol), triphenylphosphine (15.24 g, 50 mmol), and iodine (12.2 g, 48 mmol). The resulting mixture was heated at 80 °C for 2.5 h, then filtered, and concentrated. Flash chromatography of the residue on silica gel (10% ethyl acetate/hexanes) afforded **7** (15.8 g, 97%) as a colorless oil: TLC R_f = 0.45 (40% ethyl acetate/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (bd, 2H), 3.19 (t, 2H), 2.65 (t, 2H), 1.8−1.6 (m, 5H), 1.45 (s, 9H), 1.1 (m, 2H).

2-(*N*-Boc-piperidin-4-yl)ethylamine (8). A solution of iodide **7** (10.5 g, 31.0 mmol) in DMSO (100 mL) was treated with NaN_3 (4.0 g, 62.0 mmol) and stirred at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate (200 mL), washed with H_2O (2 × 100 mL) and brine (100 mL), then dried over Na_2SO_4, filtered, and concentrated to give the azide in quantitative yield: TLC R_f = 0.48 (40% ethyl acetate/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (bd, 2H), 3.21 (t, 2H), 2.65 (t, 2H), 1.8−1.6 (m, 5H), 1.45 (s, 9H), 1.1 (m, 2H). A solution of the above azide (7.40 g, 31.0 mmol) in methanol was treated with 10% Pd on C (1.2 g) and the mixture stirred under a H_2-filled balloon for 12 h. The catalyst was removed by filtration through Celite and the filtrate concentrated to afford **8** in quantative yield (6.65 g, 31.0 mmol): TLC R_f = 0.36 (10% methanol/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (bd, 2H), 3.26 (t, 2H), 2.75 (bs, 2H), 2.64(t, 2H), 1.8−1.6 (m, 5H), 1.47 (s, 9H), 1.23 (m, 2H).

Dimethyl Pyrazole-3,5-dicarboxylate (10). A solution of pyrazole-3,5-dicarboxylic acid (**9**) (75 g, 431 mmol) in 1 L of anhydrous methanol was placed in a 2-L three-neck flask equipped with a gas inlet, vertical condenser, and $CaCl_2$ drying tube. A stream of anhydrous HCl gas was rapidly passed through the solution causing it to reflux vigorously. The HCl addition was continued at this rate for 30 min after which the solution was heated at reflux for 2 h and cooled and the solvent removed at reduced pressure. The resulting white solid was treated with 600 mL of saturated $NaHCO_3$ and extracted into CH_2Cl_2 (3 × 500 mL). The pooled extracts were dried (Na_2SO_4), filtered, and concentrated at reduced pressure. The resulting white solid was recrystallized from methanol with the addition of anhydrous ether to give **10** (75.0 g, 95%) as a white solid: TLC R_f = 0.45 (60% ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.38 (s, 1H), 7.18 (br s, 1H), 3.98 (s, 3H), 3.93 (s, 3H).

Dimethyl 1-(2-Bromoethyl)pyrazole-3,5-dicarboxylate (11a). To solution of **10** (5.0 g, 27.2 mmol) in 150 mL of anhydrous acetonitrile were added K_2CO_3 (5.2 g, 40.0 mmol) and 1,2-dibromoethane (25.0 mL, 291 mmol). The resulting mixture was heated at reflux under Ar. After 25 min, TLC analysis (silica, 70:30 EtOAc/hexane) showed that no **10**

remained. The reaction suspension was cooled and filtered and the filtrate evaporated to dryness at reduced pressure and placed on a high vacuum line for 12 h. The resulting white solid was recrystallized from hexane containing 10% ethyl acetate, giving 5.61 g of a white solid (71%): ^{1}H NMR ($CDCl_3$) δ 7.38 (s, 1H); 5.03 (t, J = 8.2 Hz, 2H), 3.98 (s, 3H), 3.93 (s, 3H), 3.75 (t, J = 8.5 Hz, 2H).

Dimethyl 1-(3-Chloropropyl)pyrazole-3,5-dicarboxylate (11b). Dimethyl pyrazole-3,5-dicarboxylate (**10**) (5.0 g, 27.2 mmol) in 150 mL of anhydrous acetonitrile was treated with K_2CO_3 (5.2 g, 40.0 mmol) and 1-bromo-3-chloropropane (10.8 mL, 109 mmol). The resulting mixture was heated to reflux under Ar. After 25 min, the reaction suspension was cooled and filtered and the fitrate evaporated to dryness at reduced pressure and placed on a high vacuum line for 12 h. The resulting white solid was recrystallized from hexane containing 10% ethyl acetate, giving **11b** (5.52 g, 78%) as a white solid: TLC R_f = 0.60 (50% ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.38 (s, 1H), 4.95 (t, J = 8.2 Hz, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.75 (t, J = 8.5 Hz, 2H), 2.51 (m, 2H).

Methyl [4,5,6,7-Tetrahydro-4-oxo-5-[2-(*N*-Boc-piperidin-4-yl)ethyl]-4*H*-pyrazolo[1,5-*a*][1,4]pyrazine-2carboxylate (12a). A solution containing **11a** (14.0g, 48.0 mmol), *N*,*N*-diisopropylethylamine (25 mL, 144 mmol), Boc-4-(aminoethyl)piperdine (**8**) (12.0 g, 52.6 mmol), and potassium iodide (2.39 g, 0.3 mmol) in 250 mL of CH_3CN was refluxed under N_2 for 4.5 h, cooled, filtered, and evaporated at reduced pressure. The resulting yellow residue was chromatographed on silica gel using EtOAc as eluent to give 9.53 g of an off-white crystaline solid (49%): ^{1}H NMR ($CDCl_3$) δ 7.15 (s, 1H), 4.29(t, J = 7.0 Hz, 2H), 3.93 (br d, J = 12 Hz, 2H), 3.76 (s, 3H), 3.61(t, J = 5.3 Hz, 2H), 3.42 (t, J = 7.3 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 1.55 (d, J = 12.5 Hz, 2H), 1.38 (m, 2H), 1.33−1.25 (m, 1H), 1.27 (s, 9H), 1.01 (m, 2H).

4,5,6,7-Tetrahydro-4-oxo-5-[2-(*N*-Boc-piperidin-4-yl)-ethyl]-4*H*-pyrazolo[1,5-*a*][1,4]pyrazine-2-carboxylic Acid (12b). A solution contaning LiOH (140 mg, 3.35 mmol) in 10 mL of H_2O was added to a solution of the **12a** (908 mg, 2.23 mmol) in 10 mL of CH_3OH, and the resulting mixture was heated to 60 °C for 2.5 h and then cooled and the CH_3OH removed at reduced pressure. The remaning aqueous phase was acidified with 10% aqueous citric acid and extracted with CH_2Cl_2 (2 × 50 mL). The pooled organic extracts were dried over Na_2SO_4 and then evaporated, giving 883 mg of a white solid (98.7%): ^{1}H NMR ($CDCl_3$) δ 7.43 (s, 1H), 4.48 (t, J = 7.0 Hz, 2H), 4.01 (br d, J = 12 Hz, 2H), 3.77 (t, J = 5.3 Hz, 2H), 3.51 (t, J = 7.3 Hz, 2H), 2.71 (t, J = 8.3 Hz, 2H), 1.72 (d, J = 12.5 Hz, 2H), 1.53 (m, 2H), 1.42−1.37 (m, 1H), 1.35 (s, 9H), 1.10 (m, 2H).

3-[[[4-Oxo-5-(2-piperidin-4-yl-ethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazin-2-yl]carbonyl]amino]propionic Acid Hydrochloride (1). A solution containing **12b** (79 mg, 0.20 mmol), β-alanine *tert*-butyl ester hydrochloride (40 mg, 0.22 mmol), and EDC (41.5 mg. 0.22 mmol) in CH_2Cl_2 (20 mL) was treated with Et_3N (30.8 μL, 0.22 mmol) and stirred at room temperature for 13.5 h. the resulting solution was washed successively with 10% citric acid, H_2O, saturated $NaHCO_3$, and brine (10 mL each), dried over Na_2SO_4, and evaporated to afford the doubly protected intermediate as a glass (88.5 mg, 98%): ^{1}H NMR ($CDCl_3$) δ 7.38 (t, 1H), 7.31 (s, 1H), 4.48 (t, J = 7.0 Hz, 2H), 4.01 (br d, J = 12 Hz, 2H), 3.77 (t, J = 5.3 Hz, 2H), 3.73 (t, 2H), 3.58 (t, J = 7.3 Hz, 2H), 2.71 (m, 4H), 1.72 (d, J = 12.5 Hz, 2H), 1.53 (m, 2H), 1.42−1.37 (m, 1H), 1.35 (s, 9H), 1.28 (s, 9H), 1.10 (m, 2H). The above ester was dissolved in EtOAc (10 mL) cooled to 0 °C and treated with anhydrous HCl gas for 15 min. After being stirred at 0 °C for 1 h, the resulting suspension was diluted with anhydrous ether (25 mL), filtered, and dried under vacuum to afford **1** as a hydrochloride salt: ^{1}H NMR (DMSO-d_6) δ 8.60 (br s, 1H), 8.38 (br s, 1H), 8.18 (t, 1H), 7.00 (s, 1H), 4.40 (t, J = 7.0 Hz, 2H), 3.78 (t, J = 5.3 Hz, 2H), 3.5−3.1 (m, 6H), 2.81 (m, 2H), 1.72 (d, J = 12.5 Hz, 2H), 1.53 (m, 2H), 1.37 (m, 1H), 1.10 (m, 2H); FAB MS (MH^+ = 364). Anal. ($C_{17}H_{25}N_5O_4$·HCl·0.6H_2O) C, H, N.

Methyl 5,6,7,8-Tetrahydro-4-oxo-4-H-pyrazolo[1,5-*a*]-[1,4]diazepine-2-carboxylate (13). A solution of **11b** (10 g, 38.5 mmol) in 100 mL of DMSO was treated with NaN_3 (8.83 g, 138.0 mmol), and the mixture was stirred at 25 °C for 16 h. The reaction mixture was then diluted with 100 mL of H_2O and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water (2 × 100 mL) and brine (1 × 100 mL), dried over Na_2SO_4, and concentrated to give dimethyl 1-(3-azidopropyl)pyrazole-3,5-dicarboxylate (9.8 g, 95%) as a colorless oil: TLC R_f = 0.65 (50% ethyl acetate/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.38 (s, 1H), 4.95 (t, J = 8.2 Hz, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.75 (t, J = 8.5 Hz, 2H), 2.51 (m, 2H). A solution of the above azide (8.5 g, 32.5 mmol) in 100 mL of MeOH saturated with HCl gas was treated with 500 mg of 10% Pd on C and the mixture shaken on a Parr hydrogenator at 45 psi for 5 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated to give a colorless oil. This oil was dissolved in 250 mL of MeOH, treated with Et_3N (6.70 mL, 48.8 mmol), refluxed for 1.5 h, and then concentrated to a volume of 50 mL. The resulting white solid was filtered to give **13** (6.53 g, 91%). mp 220−221 °C; TLC R_f = 0.40 (10% methanol/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 1H); 6.42 (br t, 1H); 4.58 (t, J = 8.0 Hz, 2H), 3.95 (s, 3H), 3.39 (m, 2H), 2.31 (m, 2H).

Methyl 5,6,7,8-Tetrahydro-4-oxo-5-[2-(*N*-Boc-piperidin-4-yl)ethyl]-4*H*-pyrazolo[1,5-*a*][1,4]diazepine-2-carboxylate (14a). A solution of **13** (17.5 g, 83.0 mmol) in 150 mL of DMF was treated with 60% NaH (3.6 g, 91 mmol), and the mixture was stirred under N_2 at −15 °C for 30 min. This mixture was treated dropwise with a solution of **7** (28.3 g, 83.0 mmol) in 50 mL of DMF over 20 min. The resulting solution was stirred for 30 min at −15 °C, then warmed to room temperature, and stirred overnight. The DMF was removed at reduced pressure and the residue dissolved in ethyl acetate, filtered, and chromatographed on silica gel using ethyl acetate as eluent to afford **14a** (29.8 g, 85.5%) as a colorless glass: TLC R_f = 0.50 (ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (s, 1H), 4.50 (t, J = 7.0 Hz, 2H), 3.93 (br d, J = 12 Hz, 2H), 3.94 (s, 3H), 3.61 (t, J = 5.3 Hz, 2H), 3.42 (t, J = 7.3 Hz, 2H), 2.7 (br t, J = 6.3 Hz, 2H), 2.3 (m, 2H), 1.55 (d, J = 12.5 Hz, 2H), 1.38 (m, 2H), 1.33−1.25 (m, 1H), 1.27 (s, 9H), 1.01 (m, 2H).

5,6,7,8-Tetrahydro-4-oxo-5-[2-(*N*-Boc-piperidin-4-yl)-ethyl]-4*H*-pyrazolo[1,5-*a*][1,4]diazepine-2-carboxylic Acid (14b). A solution of **14a** (16.6 g, 39.5 mmol) in 100 mL of THF was treated with 1 N LiOH (43.5 mL, 43.0 mmol). The resulting solution was stirred at room temperature for 18 h, and then THF was removed at reduced pressure. The remaining aqueous phase was acidified with 10% $KHSO_4$, and the resulting white solid was filtered, washed with H_2O, and dried to give **14b** (16.0 g, 99%): TLC R_f = 0.30 ($CHCl_3$/CH_3OH/NH_4-OH, 94:5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 1H), 4.52 (t, J = 7.0 Hz, 2H), 4.12 (br d, J = 12 Hz, 2H), 3.94 (s, 3H), 3.61 (t, J = 5.23 Hz, 2H), 3.42 (t, J = 7.3 Hz, 2H), 2.7 (br t, J = 6.3 Hz, 2H), 2.3 (m, 2H), 1.55 (d, J = 12.5 Hz, 2H), 1.38 (m, 2H), 1.33−1.25 (m, 1H), 1.27 (s, 9H), 1.01 (m, 2H).

3-[[4-Oxo-5-(2-piperidin-4-yl-ethyl)-5,6,7,8-tetrahydropyrazolo[1,5-*a*]diazepin-2-yl]amino]propionic Acid Hydrochloride (3). Prepared from **14b** in a manner similar to that described above for the preparation of **1**: ^{1}H NMR (DMSO-d_6) δ 8.80 (br s, 2H), 8.18 (t, 1H), 6.98 (s, 1H), 4.45 (t, J = 7.0 Hz, 2H), 3.52 (t, J = 5.3 Hz, 2H), 3.5−3.41 (m, 2H), 3.25 (d, J = 12.7 Hz, 2H), 2.84 (m, 2H), 2.20 (t, 2H), 2.21 (m, 2H), 1.85 (d, J = 12.5 Hz, 2H), 1.53 (m, 3H), 1.38 (m, 2H). Anal. ($C_{18}H_{27}N_5O_4$·HCl·0.65H_2O) C, H, N.

***N*-(*n*-Butylsulfonyl)-L-asparagine (16a).** A solution containing L-asparagine (6.45g, 48.9 mmol) and NaOH (2.0 g, 50.0 mmol) in 100 mL of 50% aqueous dioxane was cooled to 0 °C in an ice bath. To this rapidly stirred mixture were added alternately a solution of NaOH (2.2 g, 55.0 mmol) in 50 mL of water and neat *n*-butanesulfonyl chloride (7.0 mL, 53.9 mmol) over a period of 30 min. After the mixture was mixed at 0 °C for 30 min, HPLC analysis showed the reaction to be approximately 60% complete. Neither longer reaction times nor the use of additional butanesulfonyl chloride resulted in increased product formation. The reaction solution was concentrated to a volume of 50 mL at reduced pressure, and the aqueous residue was cooled, acidified with concentrated

HCl, and extracted into ethyl acetate (3 × 100 mL). The organic extracts were dried over Na_2SO_4 and concentrated to a volume of approximately 50 mL, anhydrous ether (50 mL) was added, and the resulting white precipitate was isolated by vacuum filtration to give **16a** (5.31 g, 43.1%): mp 154−155 °C; ^{1}H NMR (DMSO-d_6) δ 11.9 (br s, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.46 (br s, 1H), 7.01 (br s, 1H), 4.14 (m, 1H), 3.00 (t, J = 7.8 Hz, 2H), 2.62−2.38 (m, 2H), 1.65 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H).

***N*-*p*-Toluenesulfonyl-L-Asparagine (16b).** L-Asparagine (10.0g, 75.7 mmol) was placed in a 500 mL round bottom flask equipped with a magnetic stir bar and an addition funnel. NaOH (1 N, 170 mL, 166.5 mmol) was added along with 50 mL of dioxane. *p*-Toluenesulfonyl chloride (15.88 g, 83.27 mmol) dissolved in dioxane (50 mL) was added to the reaction mixture with vigorous stirring. The reaction mixture was then stirred for 2 h at room temperature, cooled to 0 °C, and acidified to pH 2−3 with hydrochloric acid (concentrated). The product **16b** formed as a white crystalline solid (20.3 g, 94%) that was collected by filtration: TLC R_f 0.55 (10:0.1 CH_3OH:NH_4OH); ^{1}H NMR (DMSO-d_6) δ 7.91 (d, J = 8.79 Hz, 1H), 7.64 (d, J = 8.06 Hz, 2H), 7.32 (s, d (overlapping), J = 8.06 Hz, 3H), 6.87 (s, br, 1H), 4.03 (m, 1H), 2.49 (m, 1H), 2.43 (d, d, J = 7.08, 15.38 Hz, 1H), 2.35 (s, 3H), 2.21 (dd, J = 6.11, 15.38 Hz, 1H).

2(*S*)-[(*n*-Butylsulfonyl)amino]-*β*-alanine (17a). A solution containing NaOH (6.04 g, 151 mmol) in 50 mL of H_2O was cooled to 0 °C, and bromine (1.40 mL, 26.9 mmol) was added. The resulting solution was stirred at 0 °C for 5 min. Next, a cooled solution of **16a** (5.23 g, 20.7 mmol) and NaOH (1.66 g, 41.4 mmol) in 15 mL of H_2O was added at once and mixture stirred at 0 °C for 5 min and then heated to 80 °C for 15 min. The solution was then cooled to 0 °C, neutralized with 12 N HCl (9.5 mL), and stirred until gas evolution ceased. None of the amino acid **12** crystallized on standing, so the solution was then made basic by the addition of 2 N NaOH and 20 mL of THF was added along with di-*tert*-butyl dicarbonate (9.0 g, 41.4 mmol). After the solution was stirred overnight at 25 °C the THF was removed at reduced pressure and the basic aqueous phase extracted ethyl acetate (2 × 50 mL). The aqueous phase was then made acidic with 10% $KHSO_4$ and extracted with ethyl acetate (3 × 100 mL). The pooled acidic extracts were dried over Na_2SO_4, filtered, and evaporated, giving a white solid (5.64 g, 84%): mp, 111−112 °C; ^{1}H NMR (DMSO-d_6) δ 12.1 (br s, 1H), 7.44 (d, J = 9.0 Hz, 1H); 6.89 (m, 1H), 4.91 (m, 1H), 3.3 −3.05 (m, 2H), 2.93 (t, J = 7.7 Hz, 2H), 1.65 (m, 2H), 1.40 (m, 2H), 1.33 (s, 9H), 0.86 (t, J = 7.3 Hz, 3H). A solution of this Boc sulfonamide (3.83 g, 11.8 mmol) in 200 mL of ethyl acetate was cooled to 0 °C and treated with HCl gas for 5 min. The solution was then warmed to 25 °C and stirred for 30 min, then concentrated at reduced pressure to 50% of its volume, and diluted with 100 mL of ether. The resulting white solid was collected by vacuum filtration, giving **17a** (2.70 g, 92%): mp, 122−125 °C; ^{1}H NMR (DMSO-d_6) δ 8.20 (br s, 2H), 7.78 (d, J = 9.0 Hz, 1H); 4.21 (m, 1H), 3.2−3.05 (m, 3H), 2.93 (m, 1H), 1.65 (m, 2H), 1.40 (m, 2H), 0.87 (t, J = 7.3 Hz, 3 H).

2-(*S*)-(*p*-Toluenesulfonylamino)-*β*-alanine (17b). Bromine (5.03 mL, 97.5 mmol) was added to a chilled solution (−15 °C) of sodium hydroxide (21 g, 525 mmol) in water (300 mL) at such a rate as to keep the temperature below 0 °C. The solution was mixed for 10 min, and then a cold (0 °C) solution of *N*-*p*-toluenesulfonyl-L-asparagine (**16b**) (21.5 g, 75.0mmol) in 40 mL of 10% NaOH was added in a single portion to the sodium hypobromite solution. This mixture was stirred with cooling for 20 min, placed in an oil bath, and heated at 80−90 °C for 40 min. The solution was then cooled in an ice bath and adjusted to pH 7 by adding hydrochloric acid (concentrated) dropwise. The resulting white solid was isolated by vacuum filtration and then dried in a vacuum oven to afford **17b** (14.2 g, 73.6%): TLC R_f = 0.40 (10:0.1 CH_3OH:NH_4OH); ^{1}H NMR (DMSO-d_6) δ 8.2−7.2 (br, 2H, (NH,COOH)), 7.70 (d, J = 8.18 Hz, 2H), 7.38 (d, J = 8.18 Hz, 2H), 3.7−3.0 (br, 2H, (NH_2)), 3.12 (q, J = 4.76 Hz, 1H), 2.99 (d, d, J = 4.64, 11.96 Hz, 1H), 2.79 (d, d, J = 9.52, 11.96 Hz, 1H). 2.36 (s, 3H).

2(*S*)-[(*n*-Butylsulfonyl)amino]-3-[[[4,5,6,7-tetrahydro-4-oxo-5-[2-(*N*-Boc-piperidin-4-yl)ethyl]-4*H*-pyrazolo[1,5-*a*][1,4]pyrazin-2-yl]carbonyl]amino]propionic Acid (18a). Isobutyl chloroformate (1.75 mL, 13.35 mmol) was added to a cooled solution (0 °C) containing **12a** (4.98 g, 12.72 mmol) and *N*-methylmorpholine (1.53 mL, 14.00 mmol) in 100 mL of THF. This mixture was stirred under an atmosphere of dry nitrogen. After the reaction continued for 1 h, HPLC analysis of an aliquot indicated that the reaction was >90% complete. The *N*-methylmorpholine hydrochloride was removed by filtration and the filtrate poured into a solution containing **17a** hydrochloride (4.30 g, 16.54 mmol), *N*,*N*-diisopropylethylamine (4.27 mL, 33.10 mmol), THF (60 mL), and H_2O (20 mL). HPLC analysis indicated that the mixed anhydride was consumed within 15 min with an 83% conversion to product the remainder being hydrolyzed to starting acid. The THF was then removed from the reaction solution at reduced pressure and the remaining aqueous portion acidified with saturated $NaHSO_4$ and extracted with ethyl acetate (3 × 200 mL). Pooled extracts were dried over Na_2SO_4, filtered, and concentrated, giving a red oil from which a white solid formed on standing. Solid **18a** was collected by vacuum filtration (4.45 g, 58.8%); additional material was obtained from the filtrate in a second crop (total yield = 65.6%): ^{1}H NMR (DMSO-d_6) δ 8.31 (t, J = 6 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.01 (s, 1H), 4.43 (t, J = 6.6 Hz, 2H), 4.11 (m, 1H), 3.92 (d, J = 12 Hz, 2H), 3.80 (t, J = 6.6 Hz, 2H), 3.51 (t, J = 7.3 Hz, 2H), 3.65 (m, 2H), 3.51 (t, J = 6.8 Hz, 2H), 2.96 (t, J = 7.2 Hz, 2H), 1.70 (d, J = 11 Hz, 2H), 1.53 (m, 2H), 1.60−1.49 (overlaping m, 5H), 1.40 (s, 9H), 1.28 (q, J = 7.1 Hz, 2H), 1.05 (m, 2H), 0.79 (t, J = 7.1 Hz, 3H).

2(*S*)-[(*n*-Butylsulfonyl)amino]-3-[[[4,5,6,7-tetrahydro-4-oxo-5-(2-piperidin-4-ylethyl)-4*H*-pyrazolo[1,5-*a*][1,4]-diazepin-2-yl]carbonyl]amino]propionic Acid (2). A solution contaning **18a** (278 mg, 0.437 mmol) in 30 mL of ethyl acetate was cooled to 0 °C and HCl gas bubbled through for 3 min. The reaction mixture was warmed to room temperature, stirred for 30 min, and then taken to dryness on a rotary evaporator. The resulting hydrochloride salt was subjected to ion exchange chromatography using Dowex 50 × 8-200 resin with ammonium hydroxide:acetonitrile:water (50:25:25) as eluent. Fractions containing **2** were combined and then concentrated at high vacuum, and the resulting white foam was dried for 8 h under high vacuum and then recrystallized from water to afford **2** (223 mg, 95.3%) as an analytically pure crystaline solid: mp 283 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.95 (br s, 1H), 8.33 (t, J = 5.7 Hz, 1H), 7.64 (d, J = 9 Hz, 1H), 7.02 (s, 1H), 4.35 (t, J = 5.1 Hz, 2H), 4.10 (m, 1H), 3.81 (t, J = 5.2 Hz, 2H), 3.6−3.4 (m, 4H), 3.21 (d, J = 10.5 Hz, 2H), 2.95 (t, J = 7.8 Hz, 2H), 2.81 (br m, 2H), 1.96 (d, J = 11 Hz, 2H), 1.62−1.2 (overlaping multiplets, 9H), 0.80 (t, J = 7.3 Hz, 3H); FAB-MS *m*/*e* 499 $(M + H)^+$; enantiomeric excess >99.8% by HPLC. Anal. ($C_{21}H_{34}N_6O_6S\cdot3.0H_2O$) C, H, N.

2(*S*)-[(*n*-Butylsulfonyl)amino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-(2-piperidin-4-ylethyl)-4*H*-pyrazolo[1,5-*a*][1,4]-diazepin-2-yl]carbonyl]amino]propionic Acid (4). Prepared from **14b** and **17a** in a manner similar to that described above for the preparation of **2**: ^{1}H NMR (DMSO-d_6) δ 8.95 (br s, 1H), 8.33 (t, J = 5.7 Hz, 1H), 7.64 (d, J = 9 Hz, 1H), 7.02 (s, 1H), 4.35 (t, J = 5.1 Hz, 2H), 4.10 (m, 1H), 3.81 (t, J = 5.2 Hz, 2H), 3.6−3.4 (m, 4H), 3.21 (d, J = 10.5 Hz, 2H), 2.95 (t, J = 7.8 Hz, 2H), 2.81 (br m, 2H), 1.96 (d, J = 11 Hz, 2H), 1.62−1.2 (overlaping multiplets, 9H), 0.80 (t, J = 7.3 Hz, 2H); FAB-MS (MH^+ = 513); enantiomeric excess = 99.8%. Anal. ($C_{22}H_{36}N_6O_6S\cdot2.5H_2O$) C, H, N.

2-(S)-(*p*-Toluenesulfonylamino)-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(*N*-Boc-piperidin-4-yl)ethyl]-4*H*-pyrazolo[1,5-*a*][1,4]diazepin-2-yl]carbonyl]amino]propionic acid (18c). A solution of **14b** (5.0 g, 12.3 mmol) in THF (150 mL) was cooled to 0 °C, and *N*-methylmorpholine (2.11 mL, 19.2 mmol) was added via syringe. After being mixed for 20 min, isobutyl chloroformate (2.38 mL, 18.2 mmol) was added dropwise via syringe. After 0.5 h, HPLC analysis indicated that **14b** had been >95% consumed. The amine **17b** (7.00 g, 27.1 mmol), THF (125 mL), and *N*,*N*-diisopropylethylamine (4.71 mL, 27.1 mmol) were combined in a 500 mL round bottom flask with a magnetic stir bar. Water was added in small portions until a

clear solution resulted. The resulting solution was cooled in an ice bath. The mixed anhydride suspension was added in a single portion to the solution of **17b** with vigorous mixing. After 20 min, HPLC analysis of the crude reaction solution indicated that the coupled product had formed in 86% yield. At this time there was no evidence from HPLC of any mixed anhydride remaining in the reaction solution. The reaction solution was concentrated to remove THF. The remaining aqueous material was acidified with 10% $KHSO_4$. The resulting percipitate was filtered to give a white solid. This material was subjected to flash column chromatography using silica (EM Science, 230−400 mesh, 10 × 20 cm). The column was eluted with methylene chloride:methanol:ammonium hydroxide (98:2:0.2, 95:5:0.5, 90:10:1, and then 85:15:1.5) to give pure **18c** (6.2 g, 78%) as a white solid: TLC R_f = 0.30 (85:15:1.5 CH_2Cl_2:CH_3OH:NH_4OH); ^{1}H NMR (DMSO-d_6) δ 8.23 (q, J = 3.40 Hz, 1H), 7.64 (d, J = 8.20 Hz, 2H), 7.32 (d, J = 8.20 Hz), 7.2−7.0 (br, 1H), 6.86 (s, 1H), 4.36 (t, J = 6.70 Hz, 2H), 3.89 (d, br, J = 12.21 Hz, 2H), 3.59 (m, 1H), 3.47 (t, J = 7.08 Hz, 2H), 3.5−3.1 (m, br, 5H), 2.8−2.6 (br, 2H), 2.33 (s, 3H), 2.17 (t, J = 6.47 Hz, 2H), 1.66 (d, br, J = 11.97 Hz, 2H), 1.55−1.45 (m, br, 3H), 1.37 (s, 9H), 1.1−0.9 (m, br, 2H).

2(*S*)-(*p*-Toluenesulfonylamino)-3-[[[5,6,7,8-tetrahydro-4-oxo-5-(2-piperidin-4-ylethyl)-4*H*-pyrazolo[1,5-*a*][1,4]-diazepin-2-yl]carbonyl]amino]propionic Acid (5). The above Boc-protected material **18c** (7.42 g, 11.48 mmol) was placed in a 1 L round bottom flask equipped with a magnetic stir bar. Methylene chloride (300 mL) was added, and the reaction mixture was cooled to 0−5 °C. Hydrogen chloride was bubbled through the suspension with stirring. After 30 min the contents of the reaction flask were concentrated, and the resulting hydrochloride of **5** was collected by filtration. This hydrochloride salt was subjected to ion exchange chromatography using Dowex 50 × 8-200 ion exchange resin (110 g, 4.11 mequiv/g) using ammonium hydroxide:acetonitrile:water (50:25:25) as eluent. Fractions containing **5** were combined and then concentrated at high vacuum, and the resulting white foam was dried for 8 h under high vacuum and then recrystallized from water to afford **5** (7.43 g, 77.6%) as a white solid: mp 187 °C; TLC R_f 0.55 (10:0.1 CH_3OH:NH_4OH); ^{1}H NMR (DMSO-d_6) δ 9.0−8.5 (br, 1H), 8.17 (m, 1H), 7.67 (d, J = 8.18 Hz, 2H), 7.32 (d, J = 8.18 Hz, 2H), 6.89 (s, 1H), 4.38 (t, J = 6.84 Hz, 2H), 3.75−3.65 (m, br, 1H), 3.46 (t, br, 2H), 3.5−3.1 (m, br, 8H, H_2O), 2.77 (t, br, J = 11.36, 2H), 2.35 (s, 3H), 2.17 (t, J = 6.47 Hz, 2H), 1.80 (d, br, J = 12.7 Hz, 2H), 1.53−1.42 (m, br, 3H), 1.33−1.24 (m, br, 2H); FAB-MS (MH = 547$^+$); enantiomeric excess = 99.8%. Anal. ($C_{25}H_{34}N_6O_6S\cdot3.0H_2O$ C, H, N.

In Vitro Pharmacology. Purification of Human GP IIb/IIIa. GPIIb/IIIa was purified from outdated human platelets by passing platelet lysates sequentially over concanavalin A affinity, Sepharose 4B-hexyl-RGDS affinity and Sephacryl S-300HR size exclusion columns by a modification of the method of Kouns *et al.*[29] GPIIb/IIIa fractions which bound to the RGDS-affinity column bound fibrinogen (activated form), while the fractions purified on Sephacryl S-300HR did not (unactivated form).

Fluoresecence-Based Assay of Affinity toward Triton X-100 Solublized GP IIb/IIIa. Competitive fluorescent displacement binding measurements were done with unactivated and activated forms of GP IIb/IIIa (typically 600 nM) solubilized in Triton X-100 buffer (0.1% Triton X-100, 20 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) and 1 μM of **19** (L-736,622) at room temperature. Changes in the fluorescence of the solution upon addition of nonfluorescent fibrinogen receptor antagonists were recorded and, after correction for the fluorescence of GPIIb/IIIa and subtraction of the fluorescence of L-736,622 in the buffer, were plotted as percent of fluorescence of the initial solution (nonfluorescent ligand present). The values of K_D were calculated from displacement measurement using the K_D value of L-736,622 of 3.7 nM obtained in stopped-flow measurements.[17,20]

Scintillation Proximity Based Assay for Affinity of an Activated Form of GP IIb/IIIa. Purified GPIIb/IIIa was activated by coating onto yttrium silicate Scintillation Proximity Assay Fluoromicrospheres (Amersham RPN 143) and is abbreviated GP IIb/IIIa/SPA. The binding of the ^{125}I-labeled RGD-containing heptapetide **21** ([^{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-containing **21** to GP IIb/IIIa/SPA at pH 7.5 (20 mM HEPES, 0.15 M NaCl, room temperature) with ∼0.3 nM of IIb/IIIa/SPA, ∼0.3 nM of **21**, and a wide range of concentrations of the competing nonradiolabeled compounds. After equilibration, the bound CPM was measured and the ED_{50} value determined by a nonlinear least-squares fit of $CPM = (B_{max} - B_{min})/(1 + (I/ED_{50})^B + B_{min}$, 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 [^{3}H]-5 to Platelets. Human gel filtered platelets (10 mL of 2 × 10^6 platelets/mL) were incubated at room temperature in a platelet buffer (137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.0 mM $CaCl_2$, 3.3 mM NaH_2PO_4, 3.8 mM HEPES, containing 1% glucose and 1% BSA at pH 7.4) with 14 concentrations of [^{3}H]-**5** (0.02−10 nM; radiolabel diluted 10-fold with unlabeled **5**) in the presence or absence of 10 μM unlabeled **5** for 3 h to attain equilibrium. Platelets were then pelleted by centrifugation (5000*g* for 10 min in a fixed angle rotor) and the supernatant decanted. Following an additional centrifugation (5000*g* for 5 min) the remaining supernatant was pipetted off, and the platelet pellet was resuspendended in 60 μL of 10 μM unlabeled **5** and allowed to stand overnight to dissociate all bound radiolabel. An aliquot (50 μL) was counted in 4.5 mL of Readysafe scintillation cocktail and counted on a β counter. The specific binding (CPM_{Bound}) was determined from the difference in bound counts in the absence and presence of a large excess of unlabeled **5**. The concentration of total [^{3}H]-**5** required to produce half-maximal binding ($K_{1/2}$) was obtained by a nonlinear least-squares fit of the binding isotherm to $CPM_{Bound} = B_{max}/(1 + K_{1/2}/[[^3H]\text{-}\mathbf{5}]$ where B_{max} is the maximum amount of specific binding. The concentration of GPIIb/IIIa receptors were determined from the value of B_{max} and the measured specific radioactivity of [^{3}H]-**5**. The concentration of free [^{3}H]-**5** required to produce half-maximal binding (K_D) was calculated from the observed $K_{1/2}$ after correction by subtraction of 50.

***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 instutional aminal care and use committiee (MRL-20P).

Single Dose Intravenous Administration of Compounds to Conscious Dogs. Conscious purpose-bred, laboratory-raised dogs of each sex were administered compounds intravenously in sterile saline. During these studies, dogs rested comfortably in nylon slings. At specified time points, blood samples (6 mL) were drawn from either the saphenous or cephalic veins (0.38% sodium citrate, final concentration). Whole blood platelet counts were determined (1 mL), and the remainder of the sample was used for the preparation of PRP for *ex vivo* platelet aggregation as described below. For each treatment group, blood samples were obtained before compound administration (baseline) and at 1, 5, 15, 30, 45, 60, 75, 90,120, 180, 240, 300, 360, 420, and 480 min after dosing.

Measurement of *ex Vivo* Platelet Aggregation. Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 150*g* for 5 min, and the platelet count was adjusted to 2 × 10^8 platelets/mL with time matched platelet-poor plasma (PPP). PRP (300 μL, 2 × 108 platelets/mL) was incubated at 37 °C for 3 min prior to the addition of agonists. 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 and was initiated by the addition of 10 μM ADP + 1 μM epinephrine or 10 μg/mL collagen + 1 μM epinephrine. Epinephrine was used to enhance the aggregation response of canine platelets to the other agonists. 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 a compound on the extent and rate of platelet aggregation is expressed as the percent inhibition using the baseline, pretreatment response as 100%.

Single Dose Oral Administration of Compounds to Conscious Dogs by Gastric Lavage or Capsule. Conscious purpose-bred, laboratory-raised dogs of each sex were administered compounds orally by gastric lavage in aqueous solution (5 mL volume with sterile water as vehicle) or orally in gelatin capsules. During these studies, dogs rested comfortably in nylon slings. At specified time points, blood samples (6 mL) were drawn from either the saphenous or cephalic veins (0.38% sodium citrate, final concentration). Whole blood platelet counts were determined (1 mL), and the remainder of the sample was used for the preparation of PRP for *ex vivo* platelet aggregation as described above. For each treatment group, blood samples were obtained before compound administration (baseline) and at 20, 40, 70, 90, 150, 200, 250, 300, 350, and 480 min after compound administration.

References

(1) (a) Falk, E. Unstable Angina with Fatal Outcome, Dynamic Coronary Thrombosis leading to Infarction and/or Sudden Death: Autopsy Evidency of Rerecurrent Mural Thrombosis with Perpheral Embolism Culminating in Total Vascular Occlusion. *Circulation* **1985**, *71*, 699. (b) Colman, R. W.; Marder, V. J.; Salzman, E. W.; Hirsch, J. *Hemostatis and Thrombosis: Basic Principles and Clinical Practices;* Lippincott Publishers: Philadelphia, PA.

(2) Levin, D. C; Fallon, J. T.; Significance of the Angiographic Morphology of Localized Coronary Stenosis: Histopathologic Correlations. *Circulation* **1982**, *66*, 316−320.

(3) Fitzgerald, D. J.; Roy, L.; Catella, F.; Fitzgerald, G. G. Platelet Activation in Unstable Cornary Disease. *N. Engl. J. Med.* **1986**, *315*, 983−989.

(4) Splawinski, B; Kuzniar, J.; Splawinski, J. Platelet Aggregation and Deaggregation in Unstable Cornary Disease. *Med. Actual.* **1991**, *27*, 83−106.

(5) Marguerie, G. A.; Plow, E. F.; Edginton, T. S. Human Platelets Possess an Inducible and Saturable Receptor Specific for Fibrinogen. *J. Biol. Chem.* **1979**, *254*, 5357−5363.

(6) Peerscke, E. I.; Zucker, M. B.; Grant, R. A.; Egan, J. J.; Johnson, M. M. Correlation Between Fibrinogen Binding to Human Platelets and Platelet Aggregability. *Blood* **1980**, *55*, 841−847.

(7) Hawiger, J.; Klozewiak, M.; Bednarek, M. A.; Timmonms, S. Platelet Receptor Recognition Domains on the a Chain of Human Fibrinogen: Structure, Function and Analysis. *Biochemistry* **1989**, *28*, 2909−2914.

(8) Plow, E. F.; Piersbascher, M. D.; Ruoslathi, E.; Margurie, G. A.; Ginsberg, M. H. The Effect of Arg-Gly-Asp-Containing Peptides on Fibrinogen and von Willebrand Factor binding to Platelets. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 8057−8061.

(9) Gan, Z.-R.; Gould, R. G.; Jacobs, J. W.; Freidman, P. A.; Polokoff, M. A. Echistatin, A Potent Platelet Aggregation Inhibitor from the Venom of the Viper, *Echis Carinatus. J. Biol. Chem.* **1988**, *263*, 19827−19823.

(10) Gould, R. J. ; Polokoff, M. A.; Freidman, P. A.; Huang, T.-F.; Holt, J. C.; Cook, J. J.; Niewiarowski, S. Disintegrins, : a Family of Integrin Inhibitory Proteins from Viper Venoms. *Proc. Soc. Exp. Biol. Med.* **1990**, *195*, 168−171.

(11) Nutt, R. F.; Brady, S. F.; Sisko, J. T.; Ciccarone, T. M.; Colton, C. D.; Levy, M. R.; Gould, R. J.; Zhang, G.; Friedman, P. A.; Veber, D. F. Structure-and Conformation-Activity Studies Leading to Potent Fibrinogen Receptor Antagonists Containing Arg-Gly-Asp. In *Peptides 1990 (proceedings of the 21st European Peptide Symposium);* Giralt, E., Andreu, D., Eds.; ESCOM: Leiden, 1991; pp 784−786.

(12) Barker, P. L.; Bullens, S.; Bunting, S.; Burdick, D. J.; Chan, K. S.; Diesher, T.; Eigenbrot, C.; Gadek, T. R.; Gantoz, R.; Lipari, M. T.; Muir, C. D.; Pitti, R. M.; Padua, A.; Quan, C. Stanley, M. Stuble, M.; Tom, J. Y. K.; Burnier, J. P. Cyclic RGD Peptide Analogues as Antiplatelet Antithrombotics. *J. Med. Chem.* **1992**, *35*, 2040−2048.

(13) Cheng, S.; Craig, W. S.; Mullen, D.; Tschopp, J. F.; Dixon, D.; Piersbascher, M. D. Design and Synthesis of Novel Cyclic RGD-containing Peptides as Highly Potent and Selective Integrin $\alpha II_b\beta_3$ Antagonists. *J. Med. Chem.* **1994**, *37*, 1−9.

(14) For recent reviews, see: (a) Weller, T. Alig, L. Hureler-Muller, M.; Kouns, W. C.; Steiner, B. Fibrinogen Receptor Antagonists: A Novel Class of Promising Antithrombotics. *Drugs Future* **1994**, *19*, 461−476. (b) Austel, V.; Himmelsbach, F.; Muller, T. Non-peptide Fibrinogen Receptor Antagonists. *Drugs Future* **1994**, *19*, 461−476. (c) Cook, N. S.; Kottirisch, G. Zerwes, H.-G. Platelet Glycoprotein IIb/IIIa Antagonists. *Drugs Future* **1994**, *19*, 135.159.

(15) (a) Ku, T. W.; Ali, F. E.; Barton, L. S.; Bean, J. W.; Bondinell, W. E.; Burges, J. L. Callahan, J. F.; Calco, R. R.; Chen. L.; Eggleston, D. S.; Gleason, J. G.; Huffman, W. F.; Hwang, S. M.; Jakas, D. R.; Karash, C. B.; Keenan, R. M.; Kopple, K. D.; Miller W. H.; Newlander, K. A.; Nichols, A.; Parker, M. F.; Peishoff, C. E.; Samanen, J. M. ; Uzinskas, I.; Venslavsky, J. W. Direct Design of a Potent Non-peptide Fibrinogen Receptor Antagonist Based on the Strucutre and Conformation of a Highly Constrained Cyclic RGD Peptide. *J. Am. Chem. Soc.* **1993**, *115*, 8861−8862. (b) McDowell, R. S.; Gadek, T. R.; Barker, P. L.; Burdick, D. J.; Chan, K. S.; Quan, C. L.; Skelton, N.; Struble, M.; Thorsett, E. D.; Tischler, M.; Tom. J. Y.; Webb, T. R.; Burnier, J. P. From peptide to non-peptide. 1. The Elucidation of a Bioactive Conformation of the Arginine-Glycine-Aspartic Acid Recognition Sequence. *J. Am. Chem. Soc.* **1994**, *116*, 5069−5076. (c) McDowell, R. S.; Blackburn, B. K.; Gadek, T. R.; McGee, L. R.; Rawson, T.; Reynolds, M. E.; Robarge, K. D. Sommers, T. C.; Thorsett, E. D.; Tischler, M.; Webb, T. R.;Venuti, M. C. From Peptide to Non-Peptide. 2. The *De Novo* Design of Potent, Non-Peptidal Inhibitors of Platelet Aggregation Based on a Benzodiazepinedione Scaffold. *J. Am. Chem. Soc.* **1994**, *116*, 5077−5083. (d) Ku, T. W.; Miller W. H.; Bondinell, W. E.; Erhard, K. F.; Keenan, R. M.; Nichols, A.; Peishoff, C. E.; Samanen, J. M.; Wong, A. S.; Huffman, W. F.; Potent Non-Peptide Fibrinogen Receptor Antagonists Which Present an Alternate Pharmacophore. *J. Med. Chem.* **1995**, *38*, 9−12. (e) Stilz, H. U.; Jablonka, B.; Just, M.; Knolle, J.; Paulus, E. F.; Zoller, G. Discovery of an Orally Active Non-Peptide Fibrinogen Receptor Antagonist. *J. Med. Chem.* **1995**, *39*, 2118−2122. (f) Weller, T.; Alig, L. Beresini, M.; Blackburn, B.; Bunting, S.; Hadváry, P.; Müller, H. M.; Knopp, D.; Levet-Traift, B.; Lipari, M. T.; Modi, N. B.; Müller, M.; Refino, C. J.; Schmitt, M.; Schönholzer, P.; Weiss, S.; Steiner, B. Orally Active Fibrinogen Receptor Antagonists. 2. Amidoximes as Prodrugs of Amidines. *J. Med. Chem.* **1996**, *39*, 3139−3147.

(16) (a) Egbertson, M. S.; Chang, C. T. C.; Duggan, M. E.; Gould, R. J.; Halczenko, W.; Hartman, G. D.; Laswell, W. L.; Lynch, J. J., Jr.; Manno, P. D.; Naylor, A. M.; Prugh, J. D.; Ramjit, D. R.; Sitko, G. R.; Smith, R. S.; Turchi, L. M.; Zhang, G. X. Non-Peptide Fibrinogen Receptor Antagonists. 2. Optimization of a Tyrosine Template as a Mimic of Arg-Gly-Asp. *J. Med. Chem.* **1994**, *37*, 2535−2551. (b) Egbertson, M. S.; Naylor, A. M.; Hartman, G. D.; Cook, J. J.; Gould, R. J.; Holohan M. A. Non-Peptide Fibrinogen Receptor Antagonists. 3. Design and Discovery of a Centrally Constrained Inhibitor. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1835−1840. (c) Naylor, A. M.; Egbertson, M. S.; Vasallo, L. M.; Birchenough, L. A.; Zhang, G. X.; Gould, R. J. Non-Peptide Fibrinogen Receptor Antagonists. 4. Proposed Three-Diminisional Binding Requirements In Centrally Constrained Inhibitors. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1841−1845. (d) Askew, B. C.; McIntyre, C. J.; Hunt, C. A.; Claremon, D. A.; Gould, R. J.; Lynch, R. J. Armstrong, D. J. Non-Peptide Glycoprotein IIb/IIIa Inhibitors. 8. Design and Synthesis Centrally Constrained Non-Peptide Fibrinogen Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1995**, *5* , 475−480.

(17) (a) Egbertson, M. S.; Hartman, G. D.; Gould, R. J.; Bednar, B; Bednar, R. A.; Cook, J. J.; Gaul, S. L.; Holahan, M. A.; Libby, L. A.; Lynch, J. J.; Lynch, R. J.; Sitko, G. R.; Stranieri, M. T.; Vassallo, L. M. Non-Peptide GPIIb/IIIa Inhibitors. 10. Centrally Constrained Alpha-Sulfonamides are Potent Inhibitors of Platelet Aggregation. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2519−2524. (b) Halczenko, W.; Cook, J. J.; Holahan, M. A.; R. J.; Sitko, G. R.; Stranieri, M. T.; Zhang, G.; Lynch, R. J.; Lynch, J. J.; Gould, R. J.; Hartman, G. D. Non-Peptide GPIIb/IIIa Inhibitors. 11. Potent and Orally Active Centrally Constrained Thieno[2,3-*c*]-Pyridinones. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2771−2776. (c) Askew, B. C.; McIntyre, C. J.; Hunt, C. A.; Claremon, D. A.; Gould, R. J.; Lynch, R. J. Armstrong, D. J. Non-Peptide Glycoprotein IIb/IIIa Inhibitors. 13. Design and Synthesis of Orally Active Pyrazolopiperazinone Non-Peptide Fibrinogen Receptor Antagonists. *Bioorg. Med. Chem. Lett.*, in press.

(18) Xue, C.-B.; Raafalski, M.; Roderick, J.; Eyermann, C. J.; Mousa, S.; Olsen, R. E.; DeGrado, W. F., Design, Synthesis and In Vitro Activities of a Series of Benzimidazole/Benzoxazole Glycloprotein IIb/IIIa Inhibitors. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 339−344.

(19) Garegg, P. J.; Samuelsson, B. Novel Reagent System for Converting a Hydroxy-group into an Iodo-group in Carbohydrates with Inversion of Configuration. *J. Chem. Soc., Chem Commun.* **1979**, 978.

(20) Egbertson, M. S.; Bednar, B.; Bednar, R. A.; Hartman, G. D.; Gould, R. J.; Lynch, R. J.; Vassallo, L. M.; Young, S. D. Non-Peptide Glycoprotein IIb/IIIa Inhibitors. 9. Centrally Constrained Alpha-Sulfonamides are Useful Tools for Exploring Platelet Receptor Function. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1415−1420.

(21) Bednar, R. A.; Gaul, S. L.; Hamill, T.; Egbertson, M. S.; Hartman, G. D.; Gould, R. J.; Bednar, B. Selectivity of GP IIb/IIIa Antagonists in Binding to Activated and Unactivated Forms of Purified GP IIb/IIIa and to Resting and Activated Platelets. *Blood*, in press.

(22) The synthesis of [^{3}H]-**5** will be published elsewhere. For a description of its use in measuring binding affinities to resting human platelets see the *In Vitro* Pharmacology section of the Experimental Section.

(23) Bednar, B.; Cunnigham, M. E.; McQueney, P. A.; Egbertson, M. S.; Askew, B. C.; Bednar, R. A.; Hartman, G. D.; Gould, R. J. Flow Cytometric Measurement of Kinetic and Equilibrium Binding Parameters of RGD Ligands in Binding to GPIIb/IIIa on Platelets. *Cytometry*, in press.

(24) Duggan, M. E.; Naylor-Olsen, A. M.; Perkins, J. J.; Anderson, P. S.; Chang, C. T.-C.; Cook, J. J.; Gould, R. G.; Ihle, N. C.; Hartman, G. D.; Lynchn J. J.; Lynch, R. J.; Manno, P. D.; Schaffer, L. W.; Smith, R. L. Non-Peptide Fibrinogen Receptor Antagonists. 7. Design and Synthesis of a Potent, Orally Active Fibrinogen Receptor Antagoniost. *J. Med. Chem.* **1995**, *38*, 3332−3341.

(25) Prueksaritanont, T.; Stranieri, M. T.; Hand, E.l.; Ellis, J. D.; Holahan, M. A.; Sitko, G. R.; Cook. J. J. Effects of Pentobarbital on the Pharmacokinetics and Pharmacodynamics of L-734,217, a Potent Fibrinogen Receptor Antagonist in Dogs. *Biopharm. Drug Disp.*, in press.

(26) (a) Bednar, R. A.; Gaul, S. L.; Cook, J. J.; Askew, B. C.; Hartman, G. D.; Gould, R. J.; Bednar, B.; Novel Mechanism for Long Acting GP IIb/IIIa Antagonists. *Circulation* **1996**, *94*, I−98 (b) Prueksaritanont, T.; Gorham, L. M.; Naue, J. A.; Hamill. T. Askew, B. C.; Vyas, K. P.; Disposition of L-738,167, a Potent and Long Acting Fibrinogen Receptor Antagonist in Dogs: Dose-dependent Pharmacokinetics. *Drug Metab. Dispos.* **1997**, *25*, 355−361.

(27) (a) Cook, J. J.; Sitko, G. R.; Holahan, M. A.; Stranieri, M. T.; Glass, J. D. Askew, B. C.; McIntyre, C. J.; Claremon, D. A.; Baldwin, J. J.; Hartman, G. D.; Gould, R.J.; Lynch, J. J. Non-Peptide Glycoprotein IIb/IIIa Inhibitors. 14. Oral Antithrombotic Efficacy of L-738,167 in a Conscious Canine Model of Coronary Artery Electrolytic Injury. *Circulation*, in press. (b) Cook, J. J.; Sitko, G. R.; Holahan, M. A.; Stranieri, M. T.; Glass, J. D. Askew, B. C.; Hartman, G. D.; Gould, R. J.; Lynch, J. J. Non-Peptide Glycoprotein IIb/IIIa Inhibitors. 15. Antithrombotic Efficacy of L-738,167, A Long-Acting GPIIb/IIIa Antagonist, Correlates with Inhibition of ADP-Induced Platelet Aggregation But Not With Bleeding Time Prolongation. *J. Pharmacol. Exp. Ther.*, in press.

(28) Albeida, S. M.; Daise, M.; Levine, E. M.; Buck, C. A. Identification and Chacterization of Cell-Stratum Adhesion Reseptors on Cultured Human Endothelial Cells. *J. Clin. Invest.* **1989**, *83*, 1992.

(29) Kouns, W. C.; Hadvary, P.; Haering, P.; Steiner, B.; Conformational Modulation of Purified Glycoprotein GPIIb-IIIa Allows Proteolytic Generation of Active Fragments from Either Active or Inactive GP IIb-IIIa. *J. Biol. Chem.* **1992**, *267*, 18844−18851.

JM9608117