Novel Thiazole-Based Heterocycles as Selective Inhibitors of Fibrinogen-Mediated Platelet Aggregation

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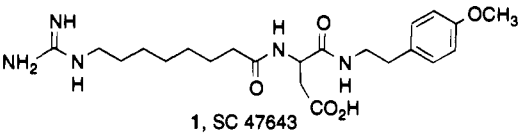
Received August 12, 1994®

The synthesis and biological activity of novel thiazole-based heterocycles as inhibitors of thrombin-induced human platelet aggregation are described. Further evaluation of selected compounds show they inhibit platelet aggregation as stimulated by a variety of agonists. The more active compounds also were found to inhibit fibrinogen binding to platelets. To further delineate the mechanism of action of these compounds, direct binding studies with the purified glycoprotein (GP) IIb/IIIa receptor were performed. Flow cytometry analyses of **24** and **32** indicate that these compounds block the activation process of the GPIIb/IIIa receptor without denaturing the integrin receptor. On the basis of these studies, **32** exhibited the best profile as a novel nonpeptide inhibitor of fibrinogen-mediated platelet aggregation.

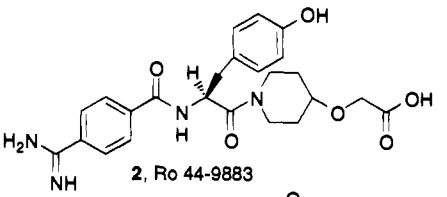
Inappropriate aggregation of blood platelets is a significant contributor to vasoocclusive thrombosis, a process producing ischemic cardiovascular, cerebrovascular, and peripheral vascular disease.[1,2] The pathology emerges from the inability of platelets to differentiate between a damaged normal artery which requires the arrest of hemorrhage to maintain hemostasis and ruptured atheromatous plaque with the resultant formation of a platelet "plug". Fibrinogen binds in a dose-dependent, saturable, and reversible manner to the platelet membrane glycoprotein (GP) IIb/IIIa in response to various platelet activators such as vascular injury, thrombin, ADP, or collagen.[3] This fibrinogen–platelet interaction forms the matrix of the platelet plug.

The discovery of the Arg-Gly-Asp (RGD) sequence, found in the two α chains of fibrinogen,[4] as an inhibitor of the fibrinogen–platelet GPIIb/IIIa receptor interaction,[5,6] has sparked an enormous synthetic effort toward the development of RGD-based peptides[7–11] and nonpeptide GPIIb/IIIa receptor antagonists.[12–15] One of the first peptidomimetics of RGD found to be capable of blocking platelet–fibrinogen interactions was SC 47643 (**1**).[12] Compound **1** contains only one natural amino acid, aspartate, and has no natural peptide bonds. The benzamidino acid Ro 44-9883 (**2**)[13] and the sulfonamido derivative L-700,462 (**3**)[14] have been shown to effectively inhibit fibrinogen-mediated platelet aggregation in the low nanomolar concentrations.

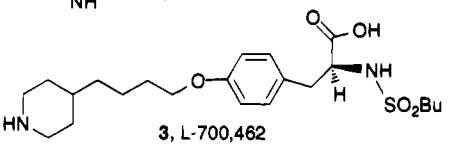
As part of our research program investigating cardiovascular diseases,[16–18] we discovered a novel series of thiazole-based heterocycles as inhibitors of fibrinogen-mediated platelet aggregation induced by numerous agonists. In this study, we report the synthesis and biological activity of these novel agents.



1, SC 47643



2, Ro 44-9883



3, L-700,462

Chemistry

The preparation of the various substituted pyrazolothiazoles is outlined in Scheme 1. Ethyl (ethoxymethylene)trifluoroacetoacetate (**4**)[19] was condensed with thiosemicarbazide affording the thioamide **5**, obtained as a 1:1 mixture of the 3- and 5-substituted positional isomers. Treatment of **5** with the appropriately substituted phenacyl bromide gave exclusively pyrazolothiazole **6**, derived from the condensation with the 5-substituted isomer. No product is observed as a result of condensation with the 3-substituted isomer. Saponification of **6** produced acid **7**. Acid **7** was reacted with carbonyldiimidazole and then an appropriately substituted alkylamine to give the pyrazolothiazole compounds described in Table 1.

The reaction of the activated acid with diaminopropane produced **24** in extremely low yield (<20%). Therefore, the preparation of larger quantities of **24** required an alternative synthetic route. Treatment of acid **7** with carbonyldiimidazole followed by 3-bromopropylamine provided the intermediate bromide **8** (Scheme 2). Displacement of the bromide with sodium azide followed by reduction of the resulting azide using aqueous triphenylphosphine gave **24** in 95% yield.[20] Amine **24** was converted to the dimethylformamide derivative **25** using dimethylformamide dimethyl acetal. Treatment of **24** with 1-guanyl-3,5-dimethylpyrazole nitrate in excess triethylamine provided the guanidino derivative **26**.[21] Likewise, displacement of the inter-

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® Abstract published in *Advance ACS Abstracts*, December 1, 1994.

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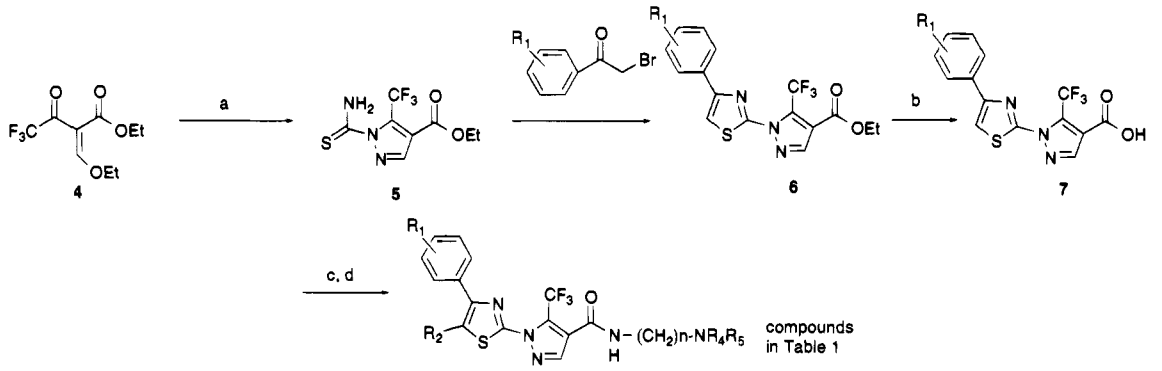
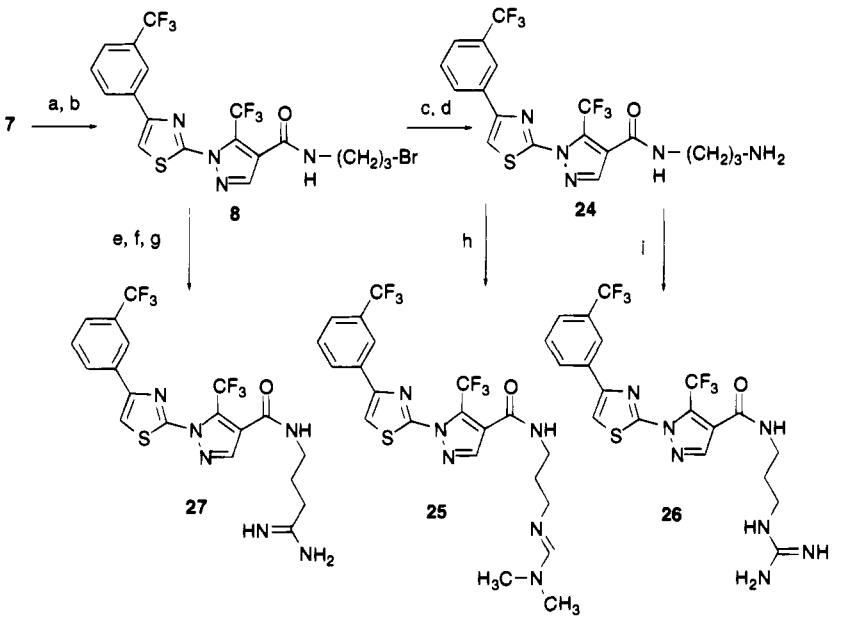
Scheme 1[a]



[a] (a) Thiosemicarbazide, EtOH; (b) KOH, EtOH; (c) CDI, DMF; (d) $NH_2(CH_2)_nNR_4R_5$.

Scheme 2[a]



[a] (a) CDI, DMF; (b) $NH_2(CH_2)_3Br$; (c) NaN_3, DMF; (d) Ph_3P/H_2O; (e) NaCN, DMF; (f) HCl(g), EtOH; (g) NH_3, MeOH; (h) DMF dimethyl acetal; (i) 1-guanyl-3,5-dimethylpyrazole nitrate, Et_3N.

mediate bromide with sodium cyanide gave the corresponding nitrile which was converted to the amidine **27** via preparation of the imidate.

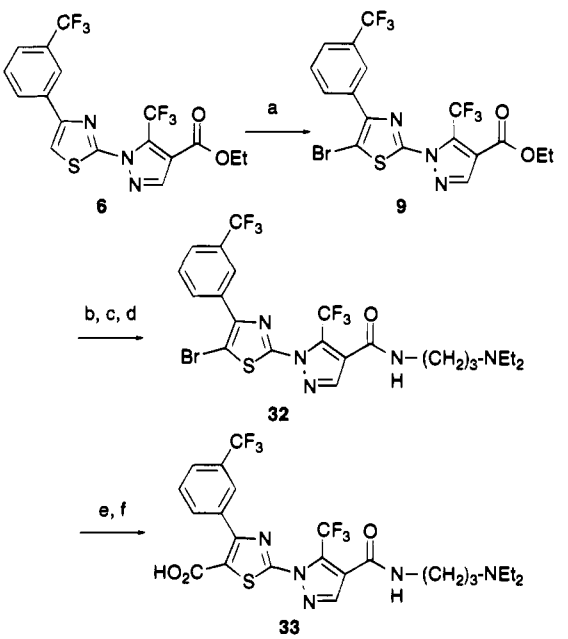
Thiazole **6** was brominated utilizing *N*-bromosuccinimide and catalytic AIBN to give **9** (Scheme 3). Compound **9** was converted to **32** using previously described methodology. Carboalkoxylation of **32** using catalytic bis(triphenylphosphine)palladium chloride[22] in methanol in the presence of carbon monoxide and triethylamine produced the intermediate ester in 52% yield which was readily saponified using methanolic sodium hydroxide to give carboxylic acid **33**.

Results and Discussion

The compounds in this study were evaluated for their ability to inhibit thrombin-induced human platelet aggregation as well as [^{125}I]fibrinogen binding to activated platelets (Table 1).[23,24] Selected compounds were further evaluated for their effects on human platelet aggregation induced by ADP, collagen, and arachidonic acid (Table 2).

Our early lead compound, **10**, inhibits thrombin-induced human platelet aggregation with an $IC_{50} = 30$ μM. Although this activity is much weaker than that of the RGD mimetics, we were surprised to find that **10** also inhibits [^{125}I]fibrinogen binding to activated

Scheme 3[a]



[a] (a) NBS, AIBN; (b) KOH, EtOH; (c) CDI, DMF; (d) $NH_2(CH_2)_3NEt_2$; (e) $(Ph_3P)Cl_2Pd(II)$, CO, MeOH, Et_3N, 100 °C; (f) NaOH, MeOH.

platelets with an $IC_{50} = 50\ \mu M$. A systematic structure–activity relationship (SAR) program aimed at increasing the potency of **10** by studying substituent effects on the aromatic and thiazole rings as well as modifications to the aminoalkyl side chain was initiated. Substituent effects on the aromatic ring were first studied with the side chain maintained as the *N*-[3-(diethylamino)propyl]-amide. A 2–5-fold increase in potency is observed in platelet aggregation inhibition with either a methoxy (**11**), methyl (**12**), chloro (**13**), or trifluoromethyl (**14**) substitution in the para-position. However, activity in the fibrinogen binding assay is diminished, indicating activity seems sensitive to bulk at the para-position. A methyl group (**15**) in the meta-position is essentially equipotent to **10** in both assays. Surprisingly, a trifluoromethyl group (**16**) in the meta-position enhances potency 5-fold in platelet aggregation and, more importantly, enhances potency 10-fold in fibrinogen binding, with an $IC_{50} = 4.1\ \mu M$.

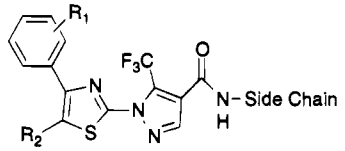
Altering the diethylamino terminus while maintaining R_1 as a meta-substituted trifluoromethyl group was next examined. Although the piperidine **17** is half as active as **16** as an inhibitor of human platelet aggregation, potency is diminished more dramatically for inhibition of fibrinogen binding. Secondary amines such as cyclohexylamino (**18**), isopropylamino (**19**), or propylamino (**20**) groups are essentially equipotent to **16** in both assays. The primary amine **24** is half as active as **16** in both assays. Interestingly, imidazole **21**, pyrrolidinone **22**, and aniline **23** all have greatly diminished activity in both assays of less than 20% inhibition at 50 μM. Formamidino- (**25**), guanidino- (**26**), and amidino- (**27**) substituted derivatives display comparable potency to **16**.

Shortening the methylene spacer in the side chain to two (**28**) methylene units increases potency slightly in the platelet aggregation assay. Lengthening the side chain to four (**29**) or five (**30**) units also enhances potency slightly as compared to **24** as inhibitors of platelet aggregation. However, activity is diminished in the fibrinogen binding assay for **28**–**30**. Lengthening the methylene spacer to seven units (**31**) severely attenuates activity in both the platelet aggregation and fibrinogen binding assays. It is interesting to note the fall off of activity for **31**. This may be due to the increased lipophilicity of **31** which may prevent its interaction with activated platelets.

Substituent effects on the thiazole ring were studied while maintaining the *N*-[3-(diethylamino)propyl]amide side chain and a 3-(trifluoromethyl)phenyl group. Substitution on the thiazole ring greatly influences activity. A carboxy group (**33**) reduces activity to 30% at 50 μM. The reduced solubility of the carboxylic acid derivative **33** may account for this diminished activity. A bromo group (**32**) significantly enhances potency by 4-fold as compared to **16** and **24** and 15-fold as compared to the initial lead **10**! On the basis of these SAR studies, **32** has the best profile with an $IC_{50}30 = 2.7\ \mu M$ in the thrombin-induced human platelet aggregation assay and an $IC_{50} = 1.3\ \mu M$ in the fibrinogen binding assay.

Direct binding studies to purified GPIIb/IIIa were performed using **24** and **32** to delineate the mechanism of action of this novel class of compounds. Compounds **24** and **32** failed to compete with either biotinylated

Table 1. Chemical Properties and Biological Data for Various Pyrazolothiazole Derivatives



no.	R_1	R_2	side chain	mp, °C	formula[a]	aggregation[b]	binding[c]
10	H	H	$(CH_2)_3NEt_2$	88–90	$C_{21}H_{24}F_3N_5OS$[d]	30 μM	50 μM
11	4-MeO	H	$(CH_2)_3NEt_2$	87–89	$C_{22}H_{26}F_3N_5O_2S$[e]	12.5 μM	57%
12	4-Me	H	$(CH_2)_3NEt_2$	86–88	$C_{22}H_{26}F_3N_5OS$	8.2 μM	70 μM
13	4-Cl	H	$(CH_2)_3NEt_2$	98–100	$C_{21}H_{23}ClF_3N_5OS$	6.8 μM	45 μM
14	4-CF_3	H	$(CH_2)_3NEt_2$	105–106	$C_{22}H_{23}F_6N_5OS$	9.1 μM	40 μM
15	3-Me	H	$(CH_2)_3NEt_2$	86–88	$C_{22}H_{26}F_3N_5OS$	25 μM	50 μM
16	3-CF_3	H	$(CH_2)_3NEt_2$	118–120	$C_{22}H_{23}F_6N_5OS$	5.6 μM	4.1 μM
17	3-CF_3	H	$(CH_2)_3N$-C-C_5H_{10}	126–128	$C_{23}H_{23}F_6N_5OS$	10.9 μM	30 μM
18	3-CF_3	H	$(CH_2)_3NH$-C-C_6H_{11}	130–132	$C_{24}H_{26}F_6N_4OS{\cdot}0.5C_2H_2O_4$	6.1 μM	25 μM
19	3-CF_3	H	$(CH_2)_3NH$-*i*-Pr	154–155	$C_{21}H_{21}F_6N_5OS$	6.7 μM	11.2 μM
20	3-CF_3	H	$(CH_2)_3NHPr$	136–138	$C_{21}H_{21}F_6N_5OS$	14.3 μM	10 μM
21	3-CF_3	H	$(CH_2)_3$(N)imidazole	165–168	$C_{21}H_{16}F_6N_6OS$	33%	0%
22	3-CF_3	H	$(CH_2)_3$(N)pyrrolidinone	162–165	$C_{22}H_{19}F_6N_5O_2S$	0%	0%
23	3-CF_3	H	$(CH_2)_3NHC_6H_5$	147–149	$C_{24}H_{19}F_6N_5OS$	18%	0%
24	3-CF_3	H	$(CH_2)_3NH_2$	263–265	$C_{18}H_{15}F_6N_5OS{\cdot}HCl$	14.0 μM	12.3 μM
25	3-CF_3	H	$(CH_2)_3N{=}CHNMe_2$	160–163	$C_{21}H_{20}F_6N_6OS$	13.7 μM	15.6 μM
26	3-CF_3	H	$(CH_2)_3NHC{=}(NH)NH_2$	198–201	$C_{19}H_{17}F_6N_7OS{\cdot}HNO_3$	11.8 μM	17.5 μM
27	3-CF_3	H	$(CH_2)_3C{=}(NH)NH_2$	242–244	$C_{19}H_{16}F_6N_6OS{\cdot}HCl$	13 μM	23 μM
28	3-CF_3	H	$(CH_2)_2NH_2$	264–266	$C_{17}H_{13}F_6N_5OS{\cdot}HCl$	11.2 μM	44%
29	3-CF_3	H	$(CH_2)_4NH_2$	259–262	$C_{19}H_{17}F_6N_5OS{\cdot}HCl$	4.8 μM	18.3 μM
30	3-CF3	H	$(CH_2)_5NH_2$	223–225	$C_{20}H_{19}F_6N_5OS{\cdot}HCl$	11.5 μM	61%
31	3-CF3	H	$(CH_2)_7NEt_2$	103–106	$C_{26}H_{31}F_6N_5OS$	35%	0%
32	3-CF_3	Br	$(CH_2)_3NEt_2$	99–101	$C_{22}H_{22}BrF_6N_5OS$	2.7 μM	1.3 μM
33	3-CF_3	CO_2H	$(CH_2)_3NEt_2$	197–203	$C_{23}H_{23}F_6N_5O_3S{\cdot}HCl$	30%	NT
RGDS						30.0 μM	7.9 μM
1, SC 47643						7.9 μM	9.0 μM

[a] All compounds exhibited satisfactory (±0.4%) elemental analyses for C, H, N unless otherwise noted. [b] Inhibition of thrombin-induced platelet aggregation is reported as IC_{50} or percent inhibition at 50 μM. The mean standard error is <5% ($N = 3$). [c] Inhibition of fibrinogen binding to activated platelets is reported as IC_{50} or percent inhibition at 50 μM. The mean standard error is <5% ($N = 3$). [c] C: calcd, 55.86; found, 55.31. [e] C: calcd, 54.87; found, 54.35. [f] C: calcd, 44.41; found, 43.95.

Table 2. Effects of Various Agonists on Various Pyrazolothiazoles

compd	agonist (IC_{50}, μM)[a]		
	thrombin	collagen	arachidonic acid
16	5.6	2.5	0.4
17	10.9	3.0	12
20	14.3	3.5	4.1
24	14.0	3.0	3.7
29	4.8	0.1	4.5
32	2.7	3.5	2.1
aspirin	>100	>100	5.0
SC 47643	10.2	8.2	2.4
RGDS	30	16.4	13

[a] Inhibition of agonist-induced platelet aggregation is reported as IC_{50}, μM. The mean standard error is <5% ($N = 3$).

fibrinogen or fibronectin for binding sites on purified GPIIb/IIIa (Figure 1). We were surprised at this finding, since the compounds are potent inhibitors of platelet aggregation and displace radiolabeled fibrinogen bound to platelets. These results indicate that **24** and **32** do not bind to the GPIIb/IIIa receptor, which distinguishes them from the RGD-derived inhibitors of fibrinogen-mediated platelet aggregation. In some manner, these novel thiazole-based heterocycles affect the activated platelets, rendering them incapable of binding to fibrinogen, thereby blocking platelet aggregation.

In an effort to further explore the mechanism of inhibition of platelet activation, possibly via interference of conformational changes of the GPIIb/IIIa receptor, flow cytometry analysis (FACS) of platelet surface GPIIb/IIIa with conformation-specific monoclonal antibodies in nonactivated as well as activated platelets was performed with **24** and **32**. Results from the histograms (Figure 2) reveal that, at a concentration of 50 μM, these compounds block normal α granule secretion from platelets in response to phorbol myristate acetate (PMA). This is evidenced by a lowered expression of thrombospondin, which is assessed by a decrease of FITC-TSPI antibody on the platelet surface. These results indicate a possible interference with signal transduction pathways necessary for proper exposure of GPIIb/IIIa fibrinogen binding sites. Moreover, there is not a direct interaction of these compounds with GPIIb/IIIa as manifested by no change in the conformation of the receptor using anti-LIBS-1. Conformational change of the GPIIb/IIIa receptor can be observed with the GRGDSP peptide.

A dose–response flow cytometry study was performed with **32** (Figure 3). This compound blocks GPIIb/IIIa receptor activation at a concentration of 30 μM, which is assessed by FITC-PAC1 antibody binding to ADP-activated platelets. However, at this same concentration it does not alter thrombospondin release. These results suggest that, at lower concentrations, **32** may affect the GPIIb/IIIa receptor activation without altering signal transductions. Neither **24** nor **32** were found to alter or denature the GPIIb/IIIa integrin receptor, as indicated by the equal binding of FITC-Ab15 antibody which recognizes the GPIIb/IIIa receptor in all conformational states without affecting fibrinogen binding.

Conclusion

We have discovered a novel class of thiazole-based heterocycles, which inhibit fibrinogen-mediated platelet aggregation (IC_{50} < 10 μM) in response to a variety of agonists. Unlike the platelet aggregation inhibitors derived from the RGD sequence, these compounds do not bind to the integrin GPIIb/IIIa receptor. Compounds **24** and **32** block the activation process of the GPIIb/IIIa receptor, yet do not alter or denature the receptor. It is possible that these compounds produce the observed inhibition of fibrinogen-mediated platelet aggregation by interfering with the signal transduction pathways that lead to the release of the α granule content from platelets, which is a prerequisite for exposure of GPIIb/IIIa fibrinogen binding sites. The precise mechanism of action by which these compounds block fibrinogen binding to activated platelets thereby inhibiting platelet aggregation is a subject of further investigation.

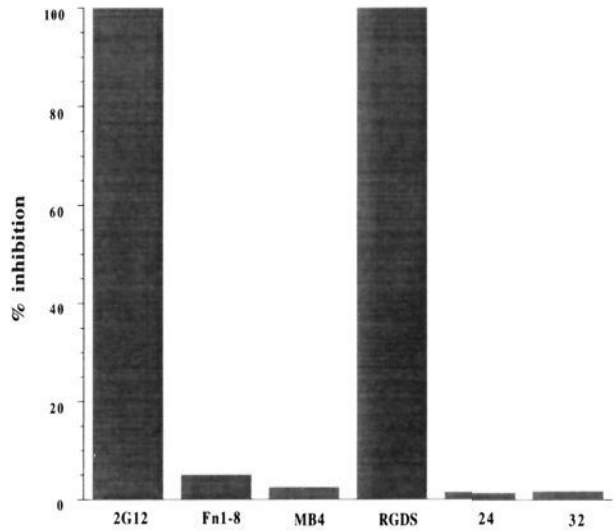


Figure 1. Inhibition of biotinylated-fibrinogen binding to GPIIb/IIIa. The binding of fibrinogen was examined by the solid phase ligand binding assay described in the Experimental Section. Binding was detected as the absorbance at 490 nm and is represented as the average of triplicate data points. Ab 2G12 (50 μg/mL), anti-GPIIb/IIIa; Ab MB4 (50 μg/mL), IgG negative control; Ab Fn 1-8 (50 μg/mL), antifibronectin; RGDS (50 μM); **24** and **32** (50 μM).

Experimental Section

All compounds were homogeneous by TLC analysis and had spectral properties consistent with their assigned structures. Melting point determinations were performed on a Thomas Hoover capillary melting point apparatus and are uncorrected. The ^{1}H NMR were determined relative to TMS on a GE QE-300 spectrometer. Microanalyses were performed on a Perkin Elmer model 240c elemental analyzer and mass spectra were determined on a Finnigan Mat 8230 spectrometer using desorption chemical ionization techniques. Silica gel, 230–400 mesh, was used for both flash and medium pressure chromatography.

Ethyl (Ethoxymethylene)trifluoroacetatoacetate (4). A mixture of ethyl trifluoroacetatoacetate (100 g, 0.54 mol) and ethyl orthoformate (135 mL, 0.81 mol) in acetic anhydride (153 mL, 1.62 mol) was heated at 120 °C for 2 h and then at 140 °C for 5 h, during which time the volatile products were distilled through a short column. The remaining liquid was distilled under reduced pressure to give 83 g (64%) of **4** as a pale yellow oil: bp 85 °C (1.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.75 (s, 1H), 4.21–4.40 (m, 4H), 1.45–1.29 (m, 6H).

4-Carboethoxy-5-(trifluoromethyl)pyrazole-1-thioamide (5). To a −15 °C solution of thiosemicarbazide (34.4 g, 0.38 mol) in EtOH (500 mL) was slowly added **4** (70 g, 0.38 mol). The temperature was not allowed to exceed −10 °C during the addition. The mixture was slowly warmed to room temperature and stirred for 1 h. The solvent was removed, and the residual oil was added to a 1 N hydrochloric acid solution (500 mL). The resulting precipitate was collected by

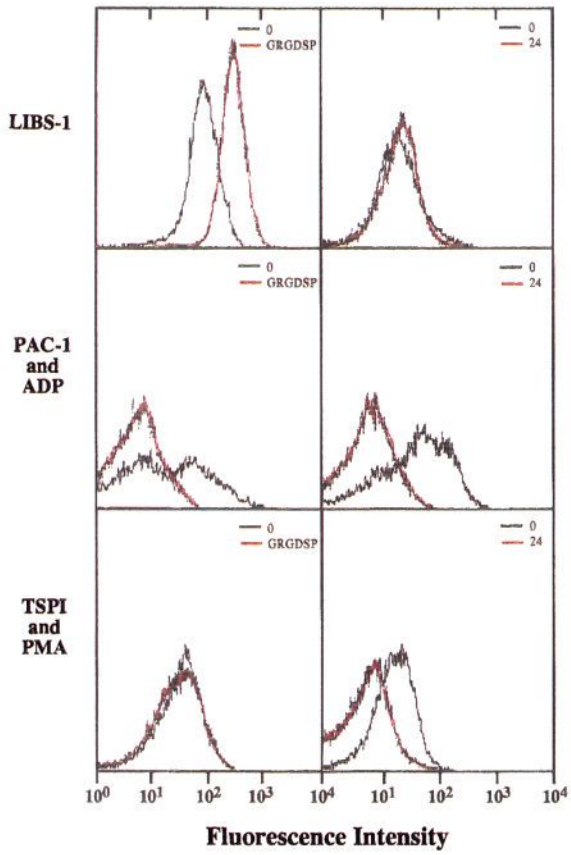


Figure 2. Flow cytometry analysis of platelets in whole blood. Shown are histograms obtained after data analysis. The reporting antibody is indicated at the left-hand column. Each panel contains histograms where the red line represents antibody binding to platelets exposed to GRGDSP (100 μM) or **24** (50 μM). The ADP and PMA were added at a final concentration of 50 μM and 50 nM, respectively. Similar results were obtained with **32**.

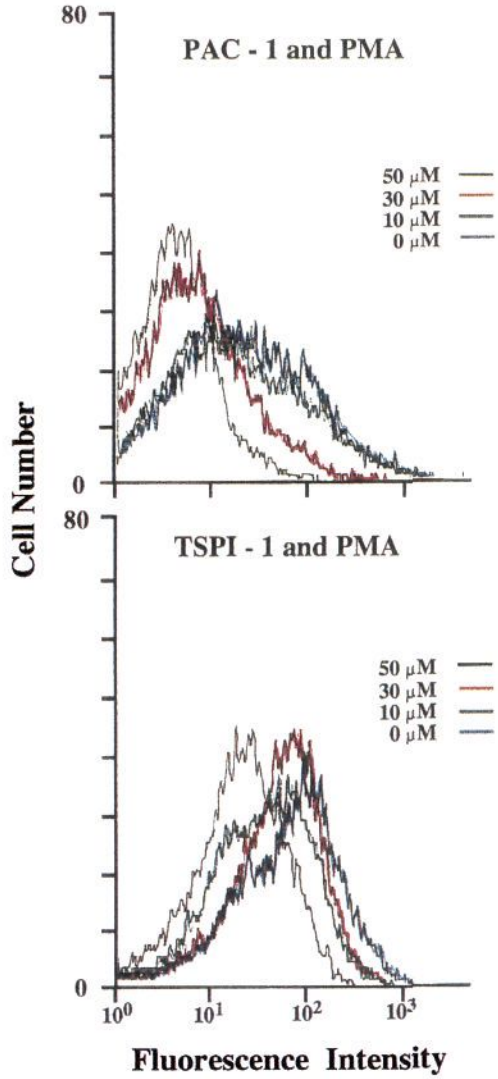


Figure 3. Flow cytometry analysis of platelets in whole blood treated with different concentrations of **32**. Effect of PAC-1 and TSP-1 binding to PMA-stimulated platelets at various concentrations (0–50 μM) of **32**.

filtration to give 73.8 g (73%) of **5**, as a 1:1 mixture of 3- and 5-trifluoromethyl-substituted positional isomers: mp 96–98 °C; IR (KBr) 3392, 3289, 1725 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1H), 7.29 (br s, 1H), 7.05 (s, 1H), 6.59 (br s, 1H), 4.27 (m, 4H), 1.31 (m, 6H).

2-[4-Carboethoxy-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole (6a; R_1 = 3-CF_3, R_2 = H). Bromine (15 mL, 0.29 mol) was added to an ether solution (300 mL) of 3-(trifluoromethyl)acetophenone (50 g, 0.27 mol) at room temperature. The dark mixture was stirred for 3 h and then poured slowly into an aqueous saturated sodium bicarbonate solution (300 mL). The organic layer was separated, dried, and concentrated to give 3-(trifluoromethyl)-phenacyl bromide as a clear oil (73 g, 99%): ^{1}H NMR ($CDCl_3$) δ 8.24 (s, 1H), 8.18 (d, J = 2.6 Hz, 1H), 7.86 (d, J = 2.6 Hz, 1H), 7.66 (t, J = 2.6 Hz, 1H), 4.94 (s, 2H).

A mixture of **5** (12.0 g, 45 mmol) and 3-(trifluoromethyl)-phenacyl bromide (11.9 g, 45 mmol) in EtOH (200 mL) was stirred at reflux for 3 h. The reaction mixture was cooled to room temperature, and the resulting precipitate was collected by filtration to give 16.1 g (82%) of **6a**: mp 82–84 °C; IR (KBr) 1733 cm^{-1}; MS 436 (MH^+); ^{1}H NMR ($CDCl_3$) δ 8.11 (m, 3H), 7.60 (m, 3H), 4.41 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H). Anal. ($C_{17}H_{11}F_6N_3O_2S$) C, H, N.

2-[4-Carboxy-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole (7a; R_1 = 3-CF_3, R_2 = H). To a mixture of **6a** (16.0 g, 37.0 mmol) in EtOH (200 mL) was added slowly potassium hydroxide (3.1 g, 55.0 mmol). The mixture was then heated to reflux and stirred for 3 h. The reaction mixture was cooled to room temperature and then concentrated. The residual semisolid was dissolved in H_2O and acidified by dropwise addition of 12 M hydrochloric acid. The resulting precipitate was collected by filtration and then recrystallized from hot toluene to give 14.5 g (96%) of **7a**: mp 184–186 °C; IR (KBr) 3000 (br), 1706 cm^{-1}; MS 408 (MH^+); ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.64 (m, 2H), 7.26 (s, 2H). Anal. ($C_{15}H_7F_6N_3O_2S$) C, H, N.

2-[4-[[3-(*N*,*N*-Diethylamino)propyl]carbamoyl]-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]-thiazole (16). A mixture of **7a** (0.18 g, 0.44 mmol) and carbonyldiimidazole (0.10 g, 0.63 mmol) in dimethylformamide (5 mL) was stirred at room temperature for 30 min and then treated with 3-(*N*,*N*-diethylamino)propylamine (0.42 mL, 2.66 mmol). The mixture was stirred for 2 h, poured into H_2O (50 mL), and stirred overnight. The resulting precipitate was collected by filtration to give 0.21 g (92% yield) of **16**: mp 118–120 °C; IR (KBr) 3306, 1632 cm^{-1}; MS 520 (MH^+); ^{1}H NMR (DMSO-d_6) δ 10.34 (br s, 1H), 8.96 (t, J = 5.6 Hz, 1H), 8.46 (s, 1H), 8.33 (m, 3H), 7.77 (m, 2H), 3.34 (m, 2H), 3.09 (m, 6H), 1.92 (m, 2H), 1.23 (t, J = 7.21 Hz, 6H). Anal. ($C_{22}H_{23}F_6N_5OS$) C, H, N.

2-[4-[(3-Aminopropyl)carbamoyl]-5-(trifluoromethyl)-pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole Monohydrochloride (24). A mixture of **7a** (4.7 g, 11.6 mmol) and carbonyldiimidazole (2.8 g, 17.3 mmol) in dimethylformamide (30 mL) was stirred at room temperature for 30 min and then treated with 3-bromopropylamine hydrobromide (12.7 g, 58 mmol). The mixture was stirred for 2 h, poured into H_2O (100 mL), and stirred overnight. The resulting precipitate was collected by filtration to give 6.1 g (100% yield) of **8**: mp 187–

190 °C; IR (KBr) 3294, 1633 cm^{-1}; MS 528 (MH^+); ^1H NMR (DMSO-d_6) δ 8.82 (m, 1H), 8.45 (s, 1H), 8.30 (m, 3H), 7.77 (m, 2H), 3.61 (t, J = 2.2 Hz, 2H), 3.38 (m, 2H), 2.06 (p, J = 2.2 Hz, 2H). Anal. ($C_{18}H_{13}BrF_6N_4OS$) C, H, N.

A mixture of **8** (5.0 g, 9.5 mmol) and sodium azide (3.0 g, 47.0 mmol) in dimethylformamide (10 mL) was heated to 90 °C for 2 h, cooled to room temperature, poured into H_2O (50 mL), and stirred overnight. The resulting precipitate was collected by filtration to give 4.6 g (100%) of azide: mp 158–160 °C; MS 490 (MH^+); ^1H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.92 (s, 1H), 7.60 (m, 3H), 6.19 (br s, 1H), 3.55 (q, J = 2.2 and 2.0 Hz, 2H), 3.47 (t, J = 2.1 Hz, 2H), 1.92 (m, 1H).

A mixture of the above azide (1.0 g, 2.0 mmol) and triphenylphosphine (1.18 g, 4.5 mmol) in dimethylformamide (10 mL) and H_2O (1 mL) was stirred at room temperature overnight. The mixture was concentrated and the residual semisolid diluted with acetone and then filtered through a short silica gel column using acetone/MeOH/Et_3N (7:2:1) as the eluant. Recrystallization from ethyl acetate/ether gave 0.9 g (95%) of **24** which was converted to the hydrochloride salt: mp 263–265 °C; IR (KBr) 3300, 2080, 1648 cm^{-1}; MS 464 (M^+); ^1H NMR (DMSO-d_6) δ 8.92 (m, 1H), 8.46 (s, 1H), 8.28 (m, 3H), 7.96 (br s, 2H), 7.76 (m, 2H), 3.50 (m, 2H), 3.30 (s), 2.86 (t, J = 2.5 Hz, 2H), 1.82 (m, 2H). Anal. ($C_{18}H_{15}F_6N_5OS$·HCl) C, H, N.

2-[4-[[3-(*N*,*N*-Dimethuylformamidinyl)propyl]carbamoyl]-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole (25). A mixture of **24** (1.0 g, 2.2 mmol) and dimethylformamide dimethyl acetal (0.53 g, 4.5 mmol) in MeOH (20 mL) was stirred at reflux for 6 h and concentrated. The resulting residue was purified by column chromatography using triethylamine/acetone (2:8) as an eluant and recrystallized from MeOH/ether to give 1.1 g of **25** (96%): mp 160–163 °C; MS 519 (MH^+). Anal. ($C_{21}H_{20}F_6N_6OS$) C, H, N.

2-[4-[(3-Guanidinopropyl)carbamoyl]-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole Nitrate (26). A mixture of **24** (1.35 g, 2.9 mmol), triethylamine (1.2 mL, 8.7 mmol), and 1-guanyl-3,5-dimethylpyrazole nitrate (1.17 g, 5.8 mmol) in dimethylformamide (30 mL) was stirred at 55 °C for 4 h and cooled to room temperature. The mixture was concentrated and the resulting oily residue triturated with ether (5 mL). The semisolid was diluted with H_2O (50 mL), and the resulting precipitate was collected by filtration. Recrystallization from MeOH/ether gave 1.2 g (73%) of **26**: mp 198–201 °C; IR (KBr) 3200, 1650 cm^{-1}; MS 506 (MH^+); ^1H NMR (DMSO-d_6) δ 8.78 (t, J = 1.8 Hz, 1H), 8.45 (s, 1H), 8.29 (m, 3H), 7.77 (m, 2H), 7.40 (t, J = 1.8 Hz, 1H), 7.15 (br s, 4H), 3.36 (s), 3.29 (q, J = 2.2 and 2.0 Hz, 2H), 3.19 (q, J = 2.2 and 2.0 Hz, 2H), 1.73 (m, 2H). Anal. ($C_{19}H_{17}F_6N_7OS$·HNO_3) C, H, N.

2-[4-[(3-Amidonopropyl)carbamoyl]-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole Hydrochloride (27). A mixture of **8** (5.7 g, 10.8 mmol) and sodium cyanide (2.6 g, 54.0 mmol) in dimethylformamide (20 mL) was stirred at room temperature overnight. The mixture was poured into H_2O (50 mL), and the resulting precipitate was collected by filtration to give 5.2 g (100%) of nitrile: MS 474 (MH^+). Gaseous hydrogen chloride was bubbled into a −10 °C $CHCl_3$ (150 mL) solution containing the above nitrile (1.0 g, 2.1 mmol) and EtOH (3.4 mL) until saturated. The resultant mixture was stirred overnight at −10 °C and then concentrated. Ether (50 mL) was added, and the intermediate imidate was collected by filtration. The crude imidate was added to a saturated methanolic ammonia solution (50 mL), heated to reflux, and stirred for 3 h. The mixture was cooled to room temperature, concentrated, and diluted with ether (50 mL), and the resulting precipitate was collected by filtration. The crude amidine was dissolved in dimethylformamide (2 mL) and added to a 2 N hydrochloric acid solution. The resulting solid was collected by filtration to give 1.8 g (33%) of **27**: mp 242–244 °C; MS 491 (MH^+). Anal. ($C_{19}H_{16}F_6N_6OS$·HCl) C, H, N.

5-Bromo-2-[4-[[3-(*N*,*N*-diethylamino)propyl]carbamoyl]-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)-phenyl]thiazole (32). A mixture of **6a** (6.0 g, 14.0 mmol), *N*-bromosuccinimide (2.5 g, 14.0 mmol), and a catalytic amount of AIBN in $CHCl_3$ (80 mL) was stirred at reflux for 8 h. The mixture was cooled to room temperature and poured into saturated sodium bicarbonate solution (75 mL) and the organic layer separated, dried, and concentrated to give 7.1 g (98%) of **9**: mp 66–68 °C; IR (KBr) 1746 cm^{-1}; MS 516 (MH^+); ^1H NMR (DMSO-d_6) δ 8.47 (s, 1H), 8.23 (m, 2H), 7.86 (m, 2H), 4.33 (q, J = 7.07 Hz, 2H), 1.32 (t, J = 7.11 Hz, 2H). Anal. ($C_{17}H_{10}BrF_6N_3O_2S$) C, H, N.

A mixture of **9** (7.0 g, 13.6 mmol) and potassium hydroxide (1.0 g, 20 mmol) in EtOH (100 mL) was stirred at reflux for 3 h, cooled to room temperature, and concentrated. The reaction mixture was diluted with H_2O (100 mL) and acidified by dropwise addition of 12 M hydrochloric acid, and the resulting precipitate was collected by filtration to give the desired acid (5.8 g, 88%): mp 166–169 °C; MS 486 (MH^+); ^1H NMR (DMSO-d_6) δ 8.37 (s, 1H), 8.24 (m, 2H), 7.85 (m, 3H).

A mixture of the above acid (3.7 g, 7.8 mmol) and carbonyldiimidazole (1.5 g, 9.4 mmol) in dimethylformamide (15 mL) was stirred at room temperature for 30 min and then treated with 3-(*N*,*N*-diethylamino)propylamine (6.2 g, 38 mmol). The mixture was stirred at room temperature for 2 h and poured into H_2O (80 mL), and the resulting precipitate was collected by filtration to give 3.3 g (71%) of **32**: mp 99–101 °C; IR (KBr) 3288, 1639 cm^{-1}; MS 598 (MH^+); ^1H NMR (DMSO-d_6) δ 8.73 (m, 1H), 8.28 (m, 3H), 7.86 (m, 2H), 3.34 (m, 4H), 3.26 (m, 2H), 2.51 (m, 2H), 1.62 (m, 2H), 0.96 (t, 6H). Anal. ($C_{22}H_{22}BrF_6N_5OS$) C, H, N.

5-Carboxy-2-[4-[[3-(*N*,*N*-diethylamino)propyl]carbamoyl]-5-(trifluoromethyl)pyrazol-1-yl]-4-[3-(trifluoromethyl)phenyl]thiazole (33). A solution of **32** (2.4 g, 4.0 mmol), triethylamine (0.87 mL, 6.25 mmol), and bis(triphenylphosphine)palladium(II) chloride (1.4 g, 2.0 mmol) in MeOH (30 mL) was heated in a stainless steel Parr pressure reactor pressurized with CO (130 psi) at 100 °C for 2 days. The resulting mixture was cooled to room temperature, poured into H_2O (100 mL), extracted with EtOAc, dried, and concentrated. The crude oil was purified by flash chromatography (8:1:1 acetonitrile:MeOH:triethylamine) to give 1.2 g (52%) of the methyl ester: mp 122–124 °C; IR (KBr) 3282, 1701, 1647 cm^{-1}; MS 578 (MH^+); ^1H NMR (DMSO-d_6) δ 8.74 (m, 1H), 8.32 (s, 1H), 8.23 (m, 1H), 8.15 (d, J = 7.86 Hz, 1H), 7.86 (d, J = 7.84 Hz, 1H), 7.75 (m, 1H), 3.83 (s, 3H), 3.38 (q, J = 7.00 Hz, 4H), 3.25 (m, 2H), 2.45 (m, 2H), 1.61 (m, 2H), 0.95 (t, J = 7.08 Hz, 6H).

A solution of the above methyl ester (0.60 g, 1.0 mmol) in MeOH (10 mL) was treated with 10% methanolic sodium hydroxide (5 mL) and stirred at reflux for 2 h. The mixture was cooled to room temperature, acidified to pH 5 with 12 M hydrochloric acid, and concentrated. The resulting solid was recrystallized from acetone to give 0.45 g (75%) of **33**: mp 197–203 °C; IR (KBr) 3409, 1670 cm^{-1}; MS 564 (MH^+); ^1H NMR (DMSO-d_6) δ 8.96 (m, 1H), 8.64 (s, 1H), 8.46 (d, J = 7.3 Hz, 1H), 8.19 (s, 1H), 7.64 (m, 2H), 3.34 (m, 4H), 3.07 (m, 2H), 2.50 (m, 2H), 1.89 (m, 2H), 1.19 (m, 6H). Anal. ($C_{23}H_{23}F_6N_5O_3S$·HCl) C, H, N.

Platelet Aggregation. The percentage of platelet aggregation was calculated as an increase in light transmission of compound-treated platelet concentrate vs control-treated platelet concentrate. Blood was obtained from drug free, normal donors into tubes containing 0.13 M sodium citrate. Platelet rich plasma (PRP) was collected by centrifugation of whole blood at 200*g* for 10 min at 25 °C. The PRP (5 mL) was gel filtered through Sepharose 2B (bed volume 50 mL), and the platelet count was adjusted to 2×10^7 platelets/sample. The following constituents were added to a siliconized cuvette to a final volume of 500 μL: concentrated platelet filtrate in Tyrode's buffer (0.14 M NaCl, 2.7 mM KCl, 0.012 M $NaHCO_3$, 0.76 mM Na_2HPO_4, 5.5 mM glucose, 2 mg/mL BSA, and 5.0 mM HEPES; pH 7.4), 50 μL of 20 μM calcium, and 50 μL of the test compound. Aggregation was monitored in a BIODATA aggregometer for 3 min following the addition of agonist (thrombin, 50 μL of 1 unit/mL).

Binding of [^{125}I]Fibrinogen to Platelets. [^{125}I]Fibrinogen binding to activated platelets was a modification of a literature procedure described by Bennett.[23,24] Briefly, 40 mL of PRP were centrifuged at 120*g* to remove contaminating red cells. Aspirin (50 μM) was added, and the PRP was incubated at 37 °C for 20 min.. The pH of the PRP was adjusted to 6.5 with

3.8% sodium citrate, and 0.9 μM PGE_1 was added. Platelets were concentrated by a 300*g* centrifugation (10 min), and the pellet was resuspended in 4 mL of Tyrode's buffer. The platelet suspension (4 mL) was gel filtered through a Sepharose 2B column (bed volume 50 mL). The platelet count was adjusted to 1×10^8 platelets/200 μL. The binding reaction was performed in poly(styrene) tubes (final volume 500 μL). Reagents were added in the following order: 30 μL of Tyrode's buffer, 50 μL of 2.0 μM $CaCl_2$, and 50 μL of 1.0 unit/mL thrombin. The platelet suspension was then added, and the mixture was allowed to incubate at room temperature for 2 min. Hirudin (50 μL, 5 unit/mL) was immediately added to inhibit the catalytic activity of thrombin. Various concentrations of the compound to be tested (100 μL) with the competing radioligand [^{125}I]fibrinogen (final concentration of 0.1 μM) were added. The mixture was incubated for 10 min at room temperature. To terminate the binding reaction, the platelets were sedimented (10000*g* for 3 min) through silicone oil (3:1 hi-phenol 550/methyl silicone 200; W.F.Nye, Inc., New Bedford, MA) in an Eppendorf centrifuge. The tips of the centrifuge tubes containing the pelleted platelets were cut off and counted for [^{125}I]fibrinogen associated with the stimulated platelets. The amount of platelet radioactivity measured in the presence of nonlabeled fibrinogen (4 mg/mL) was considered the nonspecific binding. All samples were repeated in triplicate. Data is expressed as the percent of inhibition of specifically bound [^{125}I]fibrinogen in the presence of the compounds compared to control (fibrinogen alone).

Solid Phase-Purified Glycoprotein IIb/IIIa Binding Assay. A 96-well Immulon-2 microtiter plate (Dynatech-Immulon) was coated with 50 μL/well of RGD-affinity purified GPIIb/IIIa (effective range 0.5–10 μg/mL) in 10 mM HEPES, 150 μM NaCl, and 1 mM $MgCl_2$ at pH 7.4. The plate was covered and incubated overnight at 4 °C. The GPIIb/IIIa solution was discarded, and 150 μL of 5% BSA was added and the mixture incubated at room temperature for 1–3 h. The plate was washed extensively with modified Tyrode's buffer. Biotinylated fibrinogen (25 μL/well) at 2 × final concentration was added to the wells that contained the test compounds (25 μL/well) at 2 × final concentration. The plate was covered and incubated at room temperature for 2–4 h. In a separate tube containing 5 mL of modified Tyrode's buffer, 1 drop of reagent A (Vecta Stain ABC horseradish peroxidase kit, Vector Laboratories, Inc.) and 1 drop of reagent B were added with mixing and allowed to stand. The ligand solution was discarded and the plate washed (5 × 200 μL/well) with modified Tyrode's buffer. Vecta Stain HRP–biotin–avidin reagent (50 μL/well, as prepared above) was added and the mixture incubated at room temperature for 15 min. The Vecta Stain solution was discarded, and the wells were washed (5 × 200 μL/well) with modified Tyrode's buffer. Developing buffer (10 mL of 50 mM citrate/phosphate buffer, pH 5.3, 6 mg of *o*-phenylenediamine, and 6 μL of 30% H_2O_2; 50 μL/well) was added and the mixture incubated at room temperature for 3–5 min; then 2 N H_2SO_4 (50 μL/well) was added. The absorbance was read at 490 nM.

Flow Cytometry Analysis. Antibodies. Preparation and characterization of the antibodies LIBS, TSPI, Ab-62, and Ab15 have been previously described.[25] PAC-1 was generously provided by Dr. Sanford J. Shattil, University of Pennsylvania, Philadelphia. Prior to their use in flow cytometry, these antibodies were conjugated with fluorescein isothiocyanate (FITC). Fluorescein isothiocyanate antibodies were prepared as described to achieve a fluorescein/protein molar ratio between 3 and 6.[26]

Flow Cytometry Analysis. Blood was obtained by taking venous blood from normal volunteers who had not taken medications for at least 10 days. Blood was anticoagulated in the presence of 1/10 volume of 3.8% sodium citrate. Immediately, 5 μL aliquots of whole blood were added to poly(propylene) tubes containing the FITC-conjugated monoclonal antibody (10^{-9}–10^{-6} M) in Tyrode's buffer (2 mM $MgCl_2$, 137.5 mM NaCl, 12 mM $NaHCO_3$, 2.6 mM KCl, and 1% bovine serum albumin, pH 7.4). Samples were incubated at room temperature for 30 min and then diluted with 0.6 mL of Tyrode's buffer and analyzed on a Becton Dickinson FACStar flow cytometer. Light scatter and fluorescence data were obtained from 10 000 platelets/sample with gain settings in the logarithmic mode, and the data were analyzed with a Lysys II program (version 1.0; Becton Dickinson, Mountain View, CA). Results are expressed with histograms where the log of fluorescence intensity is in arbitrary units on the abscissa and platelet number is on the ordinate.

Acknowledgment. We wish to thank the RWJPRI Analytical Chemistry Department staff for microanalytical determinations as well as NMR and IR measurements and Ms. J. Lieuallen for the reproduction of the histograms.

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JM9405349