Pyrido[2,1-*b*]quinazolinecarboxamide Derivatives as Platelet Activating Factor Antagonists

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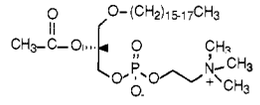
A series of *N*-[(heteroaryl)alkyl]pyrido[2,1-*b*]quinazolines were evaluated for their ability to inhibit the binding of radiolabeled platelet activating factor (PAF) to its receptor on dog platelets. The most potent compounds in this series were found to be pyrido[2,1-*b*]quinazoline-8-carboxamides possessing a four- or six-carbon chain between the carboxamide nitrogen atom and a 3-pyridinyl or 5-pyrimidinyl moiety. Since earlier metabolism studies with pyridoquinazolinecarboxamides suggest that the carboxamide moiety is labile to hydrolysis in vivo, attempts were made to find isosteric replacements for this group. The substitutions examined led to a loss of activity; however, insertion of a methyl group on the carbon atom α to the carboxamide nitrogen led to an enantioselective enhancement of potency. (*R*)-2-(1-Methylethyl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (**34**) was more potent than the corresponding *S* enantiomer in the PAF binding assay and was also shown to be more resistant to degradation by amidases present in whole liver homogenates obtained from guinea pig, dog, and squirrel monkey. The corresponding *rac*-2-(1-methylethyl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (**33**) was found to inhibit transient PAF-induced thrombocytopenia and decreases in blood pressure in guinea pigs after intravenous or oral administration and to have a duration of action of >5 h after an oral dose of 200 mg/kg. Compound **33** thus represents the prototype of a new class of orally active PAF antagonists.

Platelet activating factor (PAF) **1**[1] is a phospholipid ether with a 16–18 carbon alkyl chain,[2] which is released from a variety of cell types including stimulated platelets, neutrophils, basophils, macrophages, and vascular endothelial tissue.[3] It has a myriad of biological effects. In addition to promoting platelet aggregation, PAF induces smooth muscle contraction, increased vascular permeability, hypotension, bronchoconstriction, and cardiac contractility changes.[4-6] A mediator role for PAF has been proposed in several human disease states,[5-7] resulting in an intensive search for compounds capable of blocking the effects of PAF. Membrane binding assays have been widely used to assist this effort and have resulted in the identification of a number of PAF analogues as well as several seemingly unrelated compounds[8] as PAF antagonists.



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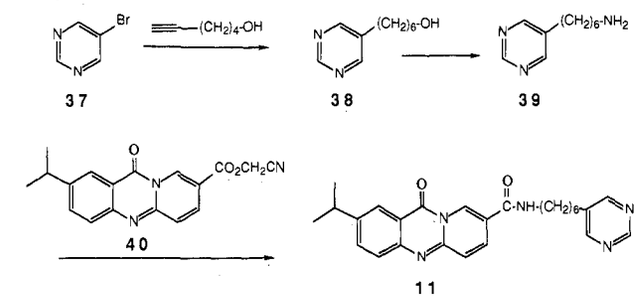
In our efforts to identify novel PAF antagonists, we have relied on a binding assay using whole, washed dog platelets. Using this system, we have found that several members of a series of pyrido[2,1-*b*]quinazoline derivatives that had originally been prepared as spasmolytic agents[9,10] were potent inhibitors of PAF binding. Additionally, one compound, **33**, effectively inhibits PAF-induced changes in blood pressure and platelet aggregation in the guinea pig after intravenous or oral administration. In this paper, we describe these studies and summarize the structure–activity relationships among compounds of this series.

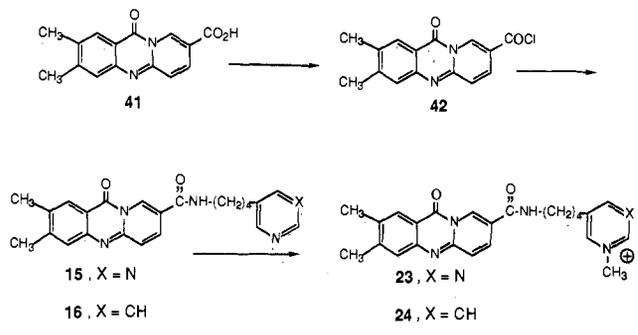
Chemistry

Most of the compounds listed in Tables I and II have been previously described.[9,10] The pyrimidinehexanamine **39** was prepared by a palladium-catalyzed coupling of 5-bromopyrimidine (**37**) with 5-hexyn-1-ol followed by reduction to the alcohol **38** and conversion to the corresponding amine as shown in Scheme I. Amide bond formation to give **11** was effected by reaction with the cyanomethyl ester **40**.[9]

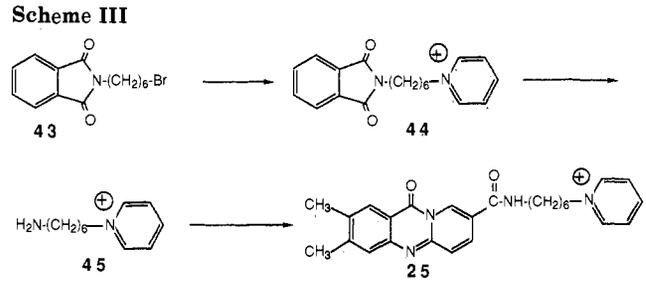
Scheme I



Scheme II



Scheme III



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§ Department of Drug Metabolism.

(1) Benveniste, J.; Henson, P. M.; Cochrane, C. G. *J. Exp. Med.* **1972**, *136*, 1356. Benveniste, J.; Tence, M.; Varenne, P.; Bidault, J.; Boullet, C.; Polonsky, J. *C. R. Seances Acad. Sci. Ser. D.* **1979**, *289*, 1037. Demopoulos, C. A.; Pinckard, R. N.; Hananhan, D. J. *J. Biol. Chem.* **1979**, *254*, 9355.
(2) Ramesha, C. S.; Pickett, W. C. *J. Immunol.* **1987**, *138*, 1559.

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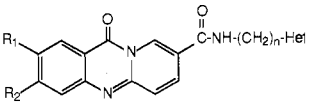
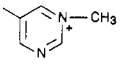
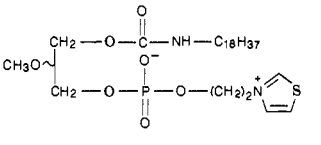
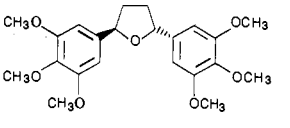
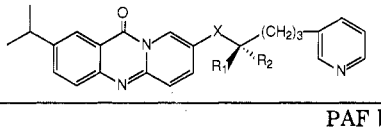
Table I. 2-Isopropyl-*N*-[(heteroaryl)alkyl]-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamides



compd	R_1	R_2	*n*	Het	PAF binding: IC_{50}, μM[a]
2	$(CH_3)_2CH$	H	2		2.5
3	$(CH_3)_2CH$	H	3		>1 (5)
4	$(CH_3)_2CH$	H	5		>1 (9)
5	$(CH_3)_2CH$	H	4		0.35
6	$(CH_3)_2CH$	H	6		0.40
7	$(CH_3)_2CH$	H	7		>1 (19)
8	$(CH_3)_2CH$	H	4		5.0
9	$(CH_3)_2CH$	H	4		1.3
10	$(CH_3)_2CH$	H	4		0.45
11	$(CH_3)_2CH$	H	6		0.20
12	$(CH_3)_2CH$	H	4		3.5
13	H	H	4		1.8
14	CH_3	H	4		0.50
15	CH_3	CH_3	4		0.10
16	CH_3	CH_3	4		0.25
17	HO	H	4		1.2
18	CH_3O	H	4		1.0
19	Br	H	4		0.70
20	$(CH_3)_2CHO$	H	4		0.60
21	H	Cl	4		1.0
22	H	CH_3O	4		0.37
23	CH_3	CH_3	4		>1 (0)
24	CH_3	CH_3	4		>1 (0)
25	CH_3	CH_3	6		>1 (9)
26	CH_3	CH_3	4		> (4)
27 (CV-3988)					0.60
28 (L652,731)					0.18

[a] For inactive compounds, the number in parentheses is the percent inhibition of specific binding observed at a concentration of 1.0 μM.

Table II.
2-Isopropyl-8-substituted-11-oxo-11*H*-pyrido[2,1-*b*]quinazolines



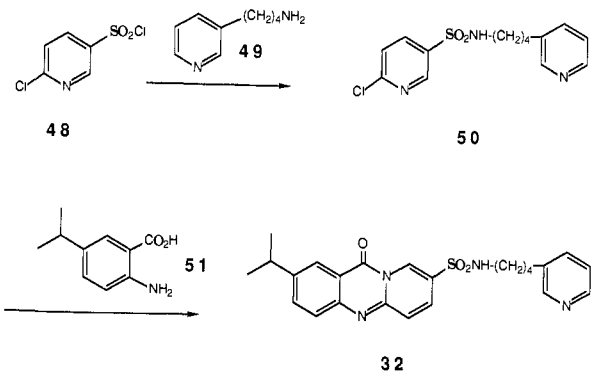
compd	X	R_1	R_2	PAF binding: IC_{50}, μM[a]
29	$(CH_2)_2$	H	H	>1 (15)
30	CH_2NH	H	H	>1 (15)
31	CO_2	H	H	>1 (16)
32	SO_2NH	H	H	>1 (7)
33[b]	CONH	CH_3	H	0.40
34	CONH	CH_3	H	0.25
35	CONH	H	CH_3	0.60
36	CONH	CH_3	CH_3	1.2

[a] For inactive compounds, the number in parentheses is the percent inhibition of specific binding observed at a concentration of 1.0 μM. [b] Racemic.

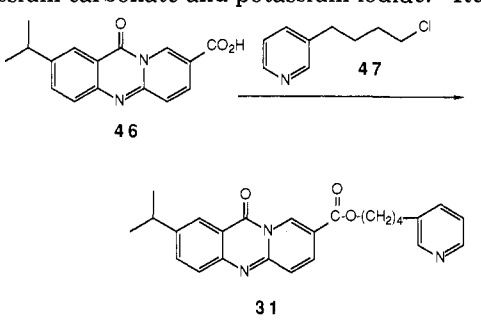
The 2,3-dimethylpyridoquinazolinecarboxylic acid **41** was converted to the relatively stable acid chloride **42** by treatment with thionyl chloride. Treatment with 5-pyrimidinebutanamine[9] afforded the corresponding amide **15**. In order to test a hypothesis that a pyridinium moiety resulting from protonation of the pyridine ring of the active species might be mimicking the choline ammonium ion of PAF, the quaternary analogues **23** and **24** were prepared by treatment of **15** and **16**, respectively, with iodomethane (Scheme II). In addition, the (bromohexyl)phthalimide **43** was reacted with pyridine to give a high yield of the pyridinium salt **44**. Hydrolysis with hydrobromic acid provided the amine **45**, which was condensed with the acid chloride **42** to give the amide **25** (Scheme III).

(3) Vargaftig, B. B.; Benveniste, J. *Trends Pharmacol. Sci.* **1983**, *4*, 341.
(4) Braquet, P.; Vargaftig, B. B. *Transplant. Proc.* **1986**, *18* (Suppl 4), 10.
(5) Morley, J. *Agents Actions* **1986**, *15*, 100.
(6) Synder, F. *Med. Res. Rev.* **1985**, *5*, 107. Venuti, M. C. *Annu. Rep. Med. Chem.* **1985**, *20*, 193.
(7) McManus, L. *Pathol. Immunopathol. Res.* **1986**, *5*, 104.
(8) Braquet, P.; Godfroid, J. J. *Trends Pharmacol. Sci.* **1986**, *7*, 368 and 397.
(9) Tilley, J. W.; Levitan, P.; Lind, J.; Welton, A. F.; Crowley, H. J.; Tobias, L. D.; O'Donnell, M. *J. Med. Chem.* **1987**, *30*, 185.
(10) Tilley, J. W.; Coffen, D. L.; Schear, B. H.; Lind, J. *J. Org. Chem.* **1987**, *52*, 2469.

Scheme IV



We were also interested in defining the contribution of the amide moiety of the compounds in Table I to PAF binding and thus prepared the analogues shown in Table II. The ester **31** resulted from the direct condensation of the pyridoquinazolinecarboxylic acid **46**[11] with the (chlorobutyl)pyridine **47** in dimethylformamide in the presence of potassium carbonate and potassium iodide. Reaction



of the pyridinesulfonyl chloride **48** with 3-pyridinebutanamine (**49**) afforded the sulfonamide **50**, which was condensed with 5-isopropylanthranilic acid (**51**) in a thermal reaction to yield the corresponding pyridoquinazoline **32** (Scheme IV).

α-Methyl-3-pyridinebutanamine (**52**) was resolved via the mandelic amides **53** and **54** to give the corresponding (*R*)- and (*S*)- amines **55** and **56**, respectively, as described in the Experimental Section. The absolute configuration of **56** was determined by X-ray crystallographic analysis of the corresponding amide **54**, which allowed us to relate the configuration at the amine-bearing chiral center to the known configuration of the mandelic acid component.

Results and Discussion

A radioreceptor binding assay utilizing washed dog platelets as the receptor source and [^{3}H]PAF as the ligand was developed in our laboratories to evaluate new compounds as potential PAF antagonists.[12] Comparing the isopropyl-substituted pyridoquinazoline derivatives **2–7** in Table I for potency in the PAF binding assay, it is apparent that the compounds with four- and six-carbon chains between the carboxamide nitrogen atom and the pyridine ring are the most interesting. For comparative purposes, a variety of analogues with four-carbon chains and differing heteroaromatic species were investigated. Among the positionally isomeric pyridine derivatives, the order of potency with respect to the position of side chain substitution is 3 > 4 > 2. The 5-substituted pyrimidines

Table III. Relative Rate of Hydrolysis of PAF Antagonists in Whole Liver Homogenates[a]

	species		
compd	guinea pig	squirrel monkey	dog
5	1.0 (n = 1)		
33	0.35 ± 0.18 (n = 5)	0.49 ± 0.18 (n = 4)	1.9 ± 0.7 (n = 3)
34	0.063 ± 0.015 (n = 3)	<0.01 (n = 2)	<0.01 (n = 1)
35	0.50 ± 0.07 (n = 3)	0.26 (n = 2)	6.8 (n = 1)
36	<0.01 (n = 1)		

[a] Nanomoles of **46** formed/10 min with 21 mg of fresh liver and 0.4 mM substrate.

10 and **11** in which the heteroaromatic nitrogen atoms and point of side-chain attachment are in a meta relationship are equipotent to the corresponding 3-substituted pyridines **4** and **6**, respectively. The more basic imidazole **12**, which also has a *m*-heteroaromatic nitrogen atom was approximately 1 order of magnitude less active in this assay, and the phenyl derivative **26** was inactive. Since PAF has a quaternary nitrogen atom, we also prepared several quaternized analogues of the more potent species, but all were inactive in the PAF binding assay.

Compounds **13–22** were examined to compare the effect of substitution in the pyridoquinazoline 2- and 3-positions on potency. These limited results indicate that such substitutions contribute only modestly to overall potency and are not positionally specific. The data in Table I include the results obtained in this assay with the previously reported PAF antagonists CV-3988 (**27**)[13] and L-652,731 (**28**),[14] which have IC_{50} values of 0.70 and 0.18 μM, respectively, indicating that the compounds reported herein compare favorably in potency.

Our previous experience with the pyridoquinazolinecarboxamide **12** suggests that related compounds may be subject to the action of amidases in vivo,[15] therefore, we were interested in designing modifications to the amide moiety of the PAF antagonist **5**, which would render it more metabolically stable. Compounds **29–32** in Table II represent attempts to find isosteric replacements for the amide, all of which led to a loss of activity. Addition of a single methyl group α to the carboxamide nitrogen atom (compound **33**) did not attenuate potency in the PAF binding assay whereas the corresponding α,α-dimethyl derivative **36** had only about one-third the potency of **5**. Comparison of the *R* and *S* enantiomers of **33–35** revealed that PAF-binding inhibition is enantioselective with the *R* enantiomer being the more potent by a factor of about 2.5.

In order to determine whether inclusion of α-methyl group would have an effect on hydrolysis rates, studies were carried out in liver homogenates derived from guinea pig, squirrel monkey, and dog in which the initial rates of formation of the pyridoquinazolinecarboxylic acid **46** from the amides **5** and **33–36** were compared. Since preliminary experiments indicated that amidase activity was present in both the particulate and supernatant fractions of rat liver, further work was carried out on whole liver homogenates. Reaction rates were linear over the first 15 min of incubation and tended to fall after longer periods,

(11) Tilley, J. W.; LeMahieu, R. A.; Carson, M.; Kierstead, R. W.; Baruth, H. W.; Yaremko, B. *J. Med. Chem.* **1980**, *23*, 92.
(12) Mowles, T. F.; Burghardt, B.; Tsien, W. H.; Sheppard, H. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 1459. Janero, D. R.; Burghardt, B.; Burghardt, C., submitted for publication in *Thromb. Res.*
(13) Terashita, Z.-i.; Tsushima, S.; Yoshioka, Y.; Nomura, H.; Inada, Y.; Nishikawa, K. *Life Sci.* **1983**, *32*, 1975.
(14) Biftu, T.; Gamble, N. F.; Doebber, T.; Hwang, S.-B.; Shen, T.-Y.; Snyder, J.; Springer, J. P.; Stevenson, R. *J. Med. Chem.* **1986**, *29*, 1917.
(15) Strojny, N.; Puglisi, C. V.; de Silva, J. A. F. *J. Chromatogr.* **1984**, *336*, 301.

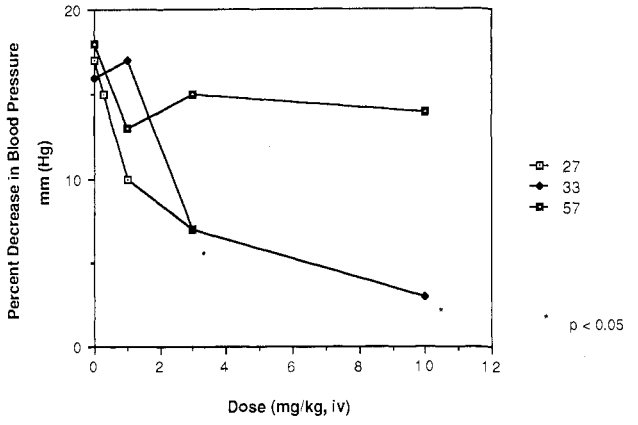


Figure 1. Effect of iv drug treatment on PAF-induced blood pressure decrease.

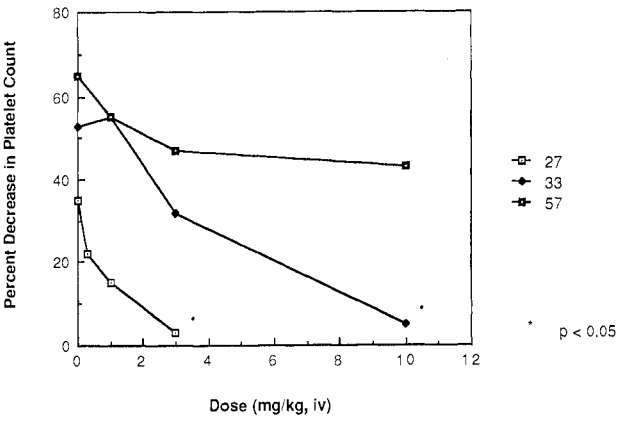
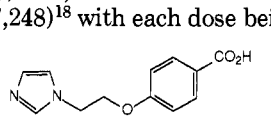


Figure 2. Effect of iv drug treatment on PAF-induced thrombocytopenia.

presumably due to instability of the amidase preparation. Thus, for comparison purposes, incubations were carried out for 10 min at an enzyme saturating concentration of 0.4 mM of substrate, and the amount of **46** that had formed was determined by HPLC as previously described.[15]

From the results in Table III, it is apparent that the racemic (**33**) and (*S*)-α-methyl (**35**) amides are hydrolyzed at substantially the same initial rates in the guinea pig and squirrel monkey and somewhat more slowly than the parent compound **5**, which lacks the α-methyl group. A dramatic contrast is seen with the *R* enantiomer **34**, which is only slowly hydrolyzed by guinea pig liver and at a nondetectable rate by squirrel monkey and dog liver homogenates. Thus, we conclude that the hydrolysis observed with the racemate **33** is due to the presence of the *S* enantiomer and have used this racemic material in our further studies.

For the evaluation of PAF antagonists, we have developed in animal model designed to assess a drug's ability to prevent PAF-induced platelet aggregation and blood pressure lowering in the guinea pig.[16] In this species, low doses of PAF cause a transient, reversible thrombocytopenia and decrease in blood pressure. Guinea pigs were anesthetized with urethane and were cannulated in the left jugular vein for intravenous drug administration and in the right carotid artery for blood sample withdrawal for platelet counting. In the intravenous procedure, the left femoral artery was cannulated for blood pressure measurements and animals were challenged with PAF. Groups of four animals were given an initial dose of PAF (50 ng/kg) to obtain control responses and were rechallenged with PAF 15 min after each iv drug dose. The animals were given ascending doses of the reference PAF antagonist **27** (CV 3988),[13] **33**, or the thromboxane synthase inhibitor **57** (UK-37,248)[18] with each dose being given 15 min



57

after the previous challenge with PAF. Blood samples were withdrawn at 15, 30, 60, and 120 s after PAF challenge and counted for number of platelets. The maximum decreases in platelet count and blood pressure observed during this interval was recorded and used in the statistical analysis. The reference PAF-antagonist **27** was inactive under this protocol but did give a significant response when given 2 min prior to challenge, suggesting that it has a short duration of action. The data were analyzed for statistical significance with Student's paired *t* test, and the results are summarized in Figures 1 and 2.

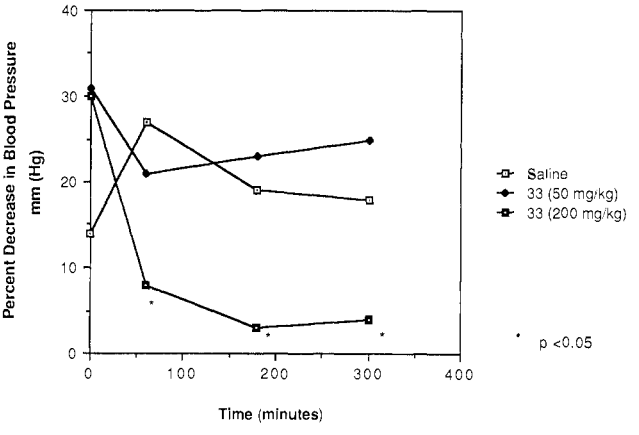


Figure 3. Effect of oral **33** on PAF-induced blood pressure decrease.

None of the drugs tested caused significant changes in base-line platelet counts or blood pressure. Neither saline nor the thromboxane synthase inhibitor **57** affected the response of either parameter to PAF challenge whereas both **27** and **33** significantly attenuated both at the highest doses examined, indicating that these responses to PAF are not thromboxane-mediated.

For oral studies, the carotid and jugular cannulas were exteriorized, and the animals were allowed to recover from sodium pentobarbital anesthesia. Blood pressure was monitored via the carotid cannula, which was also used for blood sampling. The animals were given three PAF challenges at 30-min intervals, the first two to establish a consistent response and the third to serve as a control. Thirty minutes after the third challenge, the animals were given saline or **33** (50 or 200 mg/kg) po, and they were rechallenged 60, 180, and 300 min after dosing. The results in, figures 3 and 4 indicate that at 50 mg/kg, **33** significantly attenuated platelet aggregation after 1 h whereas at 200 mg/kg, significant efforts on both platelet aggregation and the blood pressure response to PAF were observed 1, 3, and 5 h after dosing.

In conclusion, we have described a series of novel PAF antagonists, key features of which include an optionally

(16) Burghardt, C.; Heller, F.; Mowles, T. F. *Pharmacologist* **1983**, *25*, 734.
(17) Randall, M. J.; Parry, M. J.; Hawkeswood, E.; Cross, P. E.; Dickinson, R. P. *Thromb. Res.* **1981**, *23*, 145.
(18) Main, P.; Fiske, S.; Hull, S.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M. MULTAN (1982), University of York and University of Louvain.

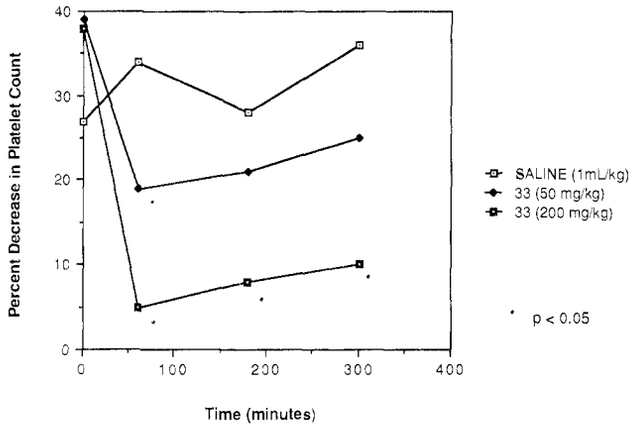


Figure 4. Effect of oral **33** on PAF-induced thrombocytopenia.

substituted pyrido[2,1-*b*]quinazoline nucleus connected via an amide linkage and a four- or six-carbon methylene bridge to a 3-pyridinyl or 5-pyrmidinyl moiety. We have further discovered that while a metabolically labile amide moiety is required for activity, inclusion of a methyl group on the carbon adjacent to the amide nitrogen atom stereospecifically enhances potency in the PAF binding assay and renders the molecule resistent to the action of liver amidases with the *R* enantiomer favored for both activities. Since the compounds described in this work are both inhibitors of thromboxane synthase and spasmolytic agents,[9] in vivo evaluation for their effects on PAF-induced symptomatology were limited to demonstration that **33** is orally active in preventing PAF-induced platelet aggregation and blood pressure decreases in the guinea pig. Our application of the present findings to the design of more specific and highly bioavailable PAF antagonists will be the subject of future papers in this series.

Experimental Section

Melting points were taken on a Büchi 510 melting points apparatus and are uncorrected. Proton magnetic resonance spectra were taken on a Varian XL-100 or XL-200 spectrometer, infrared spectra were obtained on a Beckman IR-9 or IR-12 spectrometer, and mass spectra were taken on a CEC 21-110 mass spectrometer at 70 eV. NMR, IR, and MS data were recorded for each compound reported and were consistent with the assigned structures. Preparative high-pressure liquid chromatography (HPLC) was performed on silica gel Prep-Pak 500 cartridges with a Waters Associates Prep LC 500A instrument. Gas chromatography was performed on a Hewlett-Packard 5710A instrument equipped with a flame ionization detector and a 75 m × 0.3 mm chirasil-val capillary column. Amines were analyzed as their *N*-pentafluoroproprionyl derivatives. Dry dichloromethane was distilled from P_2O_5, DMF was dried over Linde 3A sieves, and triethylamine was distilled from calcium hydride. Concentration refers to evaporation under aspirator pressure with a Büchi rotary evaporator. Bulb-to-bulb distillations were carried out with a Büchi Kugelrohr oven at the indicated air bath temperatures and pressures; distillation was continued until the distillation pot was dry. Except where noted otherwise, drying refers to the drying of combined extracts over potassium carbonate.

5-Pyrimidinehexanol (38). A solution of 23.8 g (0.15 mol) of 5-bromopyrimidine (**37**) and 17.7 g (0.18 mol) of 5-hexyn-1-ol in 60 mL of triethylamine and 240 mL of dichloromethane was treated with 2.1 g (0.003 mol) of bis(triphenylphosphine)palladium dichloride under an argon atmosphere. The resulting mixture was heated to reflux for 3 h, allowed to cool, diluted with dichloromethane, and washed with water. The aqueous layer was saturated with potassium carbonate and extracted with dichloromethane. The combined organic layers were dried (K_2CO_3) and concentrated. The residue was purified by chromatography on 400 g of silica gel, with ethyl acetate as eluent, to give 25.3 g of 6-(5-pyrimidinyl)-5-hexyn-1-ol, which was used directly in the next step.

A solution of 25.3 g (0.146 mol) of 6-(5-pyrimidinyl)-5-hexyn-1-ol in 300 mL of ethanol was hydrogenated over 1.5 g of 10% palladium on carbon. After hydrogen uptake ceased, the mixture was filtered and concentrated to a colorless oil, which was purified to bulb-to-bulb distillation, giving 22.6 g (84%) of **38**, bp 185–195 °C (0.35 mm). Anal. ($C_{10}H_{16}N_2O$) C, H, N.

5-Pyrimidinehexanamine (39). To an ice-cold solution of 20.6 (0.114 mol) of **38** in 80 mL of dry dichloromethane was added a solution of 12.3 mL (20.4 g, 0.17 mol) of thionyl chloride in 40 mL of dry dichloromethane over 20 min. The resulting mixture was allowed to warm to room temperature over 1 h and was heated to reflux for 1 h. The mixture was concentrated, diluted with toluene, and concentrated. The residue was taken up in dichloromethane and washed successively with saturated potassium carbonate and saturated sodium chloride and was dried. Concentration afforded 22.3 g (0.112 mol) of crude chloride, which was dissolved in 180 mL of DMF, and 41.6 g (0.225 mol) of potassium phthalimide and 1.8 g (0.011 mol) of potassium iodide were added. The resulting mixture was heated to reflux for 1 h, diluted with 300 mL of water, and extracted with 3 × 100 mL of dichloromethane. The combined organic layers were washed with water, dried, and concentrated to give 35.6 g of a solid. Two recrystallizations from ethyl acetate–hexane afforded 24.7 g (71%) of 2-[6-(5-pyrimidinyl)hexyl]-1*H*-isoindole-1,3-dione, mp 124–126.5 °C. Anal. ($C_{18}H_{19}N_3O_2$) C, H, N.

A solution of 23.2 g (0.075 mol) of 2-[6-(5-pyrimidinyl)-hexyl]-1*H*-isoindole-1,3-dione in 320 mL of ethanol and 14.6 mL (0.30 mol) of hydrazine hydrate was heated to reflux for 3 h and was cooled. The precipitated phthalimide was filtered, and the filtrate was concentrated. The resulting oil was taken up in dichloromethane and was washed with three portions of 2.5 N NaOH, dried, and concentrated. The residue was purified by bulb-to-bulb distillation to give 12.0 g (90%) of **39**, bp 120–128 °C (0.6 mm). Anal. ($C_{10}H_{17}N_3$) C, H, N.

2-(1-Methylethyl)-11-oxo-*N*-[6-(5-pyrimidinyl)hexyl]-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (11). A solution of 3.6 g (0.011 mol) of 2-(1-methylethyl)-11-oxo-1*H*-pyrido[2,1-*b*]qauinazoline-8-carboxylic acid cyanomethyl ester[9] (**40**) and 4.0 g (0.0223 mol) of **39** in 45 mL of DMF was heated to a bath temperature of 35 °C for 18 h. The cooled reaction mixture was diluted with water and extracted with dichloromethane. The organic layer was washed with water, dried, and concentrated. The residue was recrystallized from acetonitrile to give 3.1 g (63%) of 11, mp 132–134 °C. Anal. ($C_{26}H_{29}N_5O_2$) C, H, N.

2,3-Dimethyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carbonyl Chloride Hydrochloride (42). A suspension of 22 g (0.080 mol) of 2,3-dimethyl-11-oxo-11*H*-pyrido[2,1-*b*]-quinazoline-8-carboxylic acid (**41**) in 500 mL of thionyl chloride was heated to reflux for 3 h and allowed to stand overnight. The excess solvent was removed by evaporation, and the residue was taken up in toluene and concentrated three times to remove excess thionyl chloride. The resulting solid was triturated with ether and collected to give 23.8 g (92%) of **42** as a yellow powder, which was used as is.

2,3-Dimethyl-11-oxo-*N*-[4-(5-pyrimidinyl)butyl]-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (15). To an ice-cold solution of 3.02 g (0.020 mol) of 5-pyrimidinebutanamine and 5 mL of triethylamine in 125 mL of dichloromethane was added 5.74 g (0.020 mol) of **42** in portions. After 24 h, the precipitate was collected to give 4.4 g, mp 199–202 °C. The solid was dissolved in 125 mL of dichloromethane, filtered with the aid of Celite, and concentrated to 40 mL to afford 3.3 g (41%) of **15**, mp 203–205 °C. Anal. ($C_{23}H_{23}N_5O_2$) C, H, N.

5-[4-[[(2,3-Dimethyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazolin-8-yl)carbonyl]amino]butyl]-1-methylpyrimidinium chloride hydrochloride (23) was prepared by using the procedure described below for **24**. From 1.5 g (3.74 mmol) of **15** and 10 mL of iodomethane was obtained 2.9 g of crude product. The crude product was dissolved in water and passed through 40 mL of Dowex 1-X8 ion-exchange resin in the chloride form. The solvent was removed by evaporation, and the residue was crystallized from ethanol and then from methanol to give 0.55 g (30%) of **23**, mp 264–260 °C. Anal. ($C_{24}H_{26}ClN_5O_2 \cdot HCl$) C, H, N, Cl.

3-[4-[[(2,3-Dimethyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazolin-8-yl)carbonyl]amino]butyl]-1-methylpyridinium Iodide Methanolate (24). A solution of 2.2 g (5.49 mmol) of **16** in 10

mL of iodomethane was stirred for 1 week. The solvent was allowed to evaporate, and the residue was dissolved in methanol, treated with activated charcoal, filtered, and allowed to cool to give 2.3 g (77%) of **24**, mp 129 °C dec. Two further recrystallizations from methanol afforded 1.25 g (42%), mp 133–136 °C. Anal. ($C_{25}H_{27}IN_4O_2 \cdot CH_3OH$) C, H, N.

1-[6-[[(2,3-Dimethyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazolin-8-yl)carbonyl]amino]hexyl]pyridinium Chloride Hemihydrate (25). A mixture of 2.8 g (8.2 mmol) of **45** and 2.4 g (8.3 mmol) of **42** in 5 mL of triethylamine and 100 mL of dry dichloromethane was stirred 18 h at room temperature. The resulting solid was collected, dissolved in water, and passed through a column of Dowex 1-X8 ion-exchange resin in the chloride form. Concentration of the eluate gave 2.15 g of a solid, which was recrystallized from 2-propanol to give 1.7 g (45%) of **25**, mp 215–217 °C dec. Anal. ($C_{26}H_{29}ClN_4O_2 \cdot 0.5H_2O$) C, H, N, Cl.

2,3-Dimethyl-11-oxo-*N*-(4-phenylbutyl)-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (26). A suspension of 15.0 g (0.056 mol) of 2,3-dimethyl-11-oxo-11*H*-pyrido[2,1-*b*]-quinazoline-8-carboxylic acid (**41**) and 4.9 mL (0.077 mol) of chloroacetonitrile in 150 mL of DMF over 10.2 g (0.074 mol) of potassium carbonate was heated to a bath temperature of 60–65 °C for 18 h. The cooled reaction mixture was diluted with water, and the resulting precipitate was collected and washed with water to afford 14.45 g of a yellow solid, mp 173–177 °C. Recrystallization from acetonitrile gave 8.7 g (56%) of [[(2,3-dimethyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazolin-8-yl)carbonyl]oxy]acetonitrile, mp 180–182 °C. Anal. ($C_{17}H_{13}N_3O_3$) C, H, N.

A solution of 6.4 g (0.021 mol) of the above cyanomethyl ester and 5.07 g (0.034 mol) of 4-phenylbutanamine in 75 mL of dry DMF was heated to a bath temperature of 65 °C for 24 h. The cooled reaction mixture was filtered and diluted with water to give 7.7 g of a yellow precipitate. Recrystallization from ethanol and then toluene afforded 4.2 g (50%) of **26**, mp 195–197 °C. Anal. ($C_{25}H_{25}N_3O_2$) C, H, N.

1-(6-Aminohexyl)pyridinium Bromide Hydrobromide (45). A solution of 4.6 g (14.8 mmol) of 2-(6-bromohexyl)-1,3-dioxo-1*H*,3*H*-isoindole in 10 mL of dry pyridine was heated to reflux for 3 h. The hot mixture was filtered with the aid of Celite, and the filtrate was cooled to deposit 4.75 g (94%) of **44**, mp 148–151 °C. This material was taken up in 25 mL of 48% hydrobromic acid and heated to reflux for 2 h. The mixture was cooled in an ice bath, and the precipitated phthalic acid was filtered to give 1.7 g, mp 206 °C dec. The aqueous filtrate was concentrated, and the residue was recrystallized from 2-propanol to give 3.0 g (75%) of **45**, mp 156–158 °C. Anal. ($C_{11}H_{19}BrN_2 \cdot HBr$) C, H, N, Br.

2-(1-Methylethyl)-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid 8-[4-(3-Pyridinyl)butyl ester] (31). A suspension of 0.70 g (2.5 mmol) of 2-(1-methylethyl)-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic acid (**46**),[11] 0.39 g (2.9 mmol) of potassium carbonate, and 0.50 g (2.9 mmol) of 4-(3-pyridinyl)butyl chloride in 12 mL of DMF was heated to a bath temperature of 50 °C for 18 h. The cooled reaction mixture was diluted with ethyl acetate and washed with water and saturated sodium chloride, dried (K_2CO_3), and concentrated. The residue was chromatographed on 75 g of silica gel with ethyl acetate as eluent to give 0.33 g (32%) of **31**, mp 95–96 °C. Anal. ($C_{25}H_{25}N_3O_3$) C, H, N.

2-Chloro-*N*-[4-(3-pyridinyl)butyl]pyridine-5-sulfonamide (50). To a solution of 10.0 g (0.0472 mol) of 2-chloropyridine-5-sulfonyl chloride in 100 mL of dry dichloromethane was added 7.1 g (0.0472 mol) of 3-pyridinebutanamine **49**[9] and 6.6 mL (0.0472 mol) of triethylamine. After 18 h, the mixture was concentrated and the residue was crystallized from dichloromethane and triethylamine to afford 10.9 g (68%) of **50**, mp 105–107 °C. Anal. ($C_{14}H_{16}ClN_3O_2S$) C, H, Cl, N, S.

2-(1-Methylethyl)-11-oxo-*N*-[4-(3-pyridinyl)butyl]-11*H*-pyrido[2,1-*b*]quinazoline-8-sulfonamide (32). A solution of 5.0 g (0.0158 mol) of **50** in 10 mL of 2 N HCl was concentrated to dryness. The residue was added to a mixture of 4.3 g (0.0238 mol) of 2-amino-5-(1-methylethyl)benzoic acid and 0.16 g (0.96 mmol) of potassium iodide in 10 mL of triglyme, and the resulting reaction mixture was heated to a bath temperature of 160 °C for 1.5 h. The cooled reaction mixture was applied directly to a column of 400 g of silica gel with 94:5:1 dichloromethane–methanol–triethylamine as eluent. The product-containing fractions were combined, rechromatographed, and recrystallized from acetonitrile to afford 0.72 g (10%) of **32**, mp 155–158 °C. Anal. ($C_{24}H_{26}N_4O_3S$) C, H, N, S.

(*R*,*R*)-α-Hydroxy-*N*-[1-methyl-4-(3-pyridinyl)butyl]-benzeneacetamide (53) was prepared analogously to **54** below. From 3.2 g (19.5 mmol) of **52**, 3.00 g (19.7 mmol) of (*R*)-mandelic acid, 4.2 mL (19.5 mmol) of diphenyl phosphorazidate, and 2.7 mL (19.5 mmol) of triethylamine was obtained 0.60 g (10%) of **53**: mp 145–146 °C; $[\alpha]_D$ −17.85° (ethanol, *c* 0.9465). Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

(*S*,*S*)-α-Hydroxy-*N*-[1-methyl-4-(3-pyridinyl)butyl]-benzeneacetamide (54). A solution of 3.00 g (19.7 mmol) of (*S*)-mandelic acid, 4.21 mL (19.5 mmol) of diphenyl phosphorazidate, and 2.7 mL (19.5 mmol) of triethylamine in 20 mL of ice-cold DMF was stirred for 15 min, and 3.20 g (19.5 mmol) of **52** was added over 2 min. The mixture was stirred at 0 °C for 1 h and allowed to warm to room temperature overnight. The mixture was diluted with 150 mL of ethyl acetate, washed with 4 × 25 mL of water, dried, and concentrated. The residue was crystallized four times from ethyl acetate to give 0.47 g (8%): mp 145–146 °C; $[\alpha]_D$ +17.07° (ethanol, *c* 0.8436). A sample of this material was recrystallized from ethanol and submitted for X-ray crystallographic analysis. Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

(*R*)-α-Methyl-3-pyridinebutanamine (55). A solution of 0.60 g (2.01 mmol) of **53** in 5 mL of 6 N HCl was heated at reflux for 8 h. The cooled reaction mixture was made basic with 30% NaOH solution and extracted 3 × 20 mL of ethyl acetate. The combined organic layers were dried, concentrated, and purified by bulb-to-bulb distillation to give 0.32 g (92%) of **55**: bp 90–95 °C (0.22 mm); $[\alpha]_D$ −4.76° (ethanol, *c* 0.9213), which consisted of a single enantiomer by GLPC analysis. Anal. ($C_{10}H_{16}N_2$) C, H, N.

(*S*)-α-Methyl-3-pyridinebutanamine (56). A solution of 0.44 g (1.47 mmol) of **54** in 5 mL of 6 N HCl was heated to reflux for 8 h. The cooled reaction mixture was made basic with 30% NaOH solution and extracted 3 × 20 mL with ethyl acetate. The combined extracts were dried, concentrated, and purified by bulb-to-bulb distillation to afford 0.24 g (98%) of **54**: bp 90–95 °C (0.2 mm); $[\alpha]_D$ +3.40° (ethanol, *c* 0.9424), which consisted of a single enantiomer by GLPC analysis and was used as is. Anal. ($C_{10}H_{16}N_2$) Calcd: C, 73.13; H, 9.82; N, 17.06. Found: C, 72.08; H, 9.76; N, 16.71.

(*R*)-2-(1-Methylethyl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (34). A solution of 0.48 g (1.7 mmol) of **46**, 0.37 mL (1.7 mmol) of diphenylphosphoryl azide, and 0.24 mL (1.7 mmol) of triethylamine in 3 mL of DMF was cooled in an ice bath for 1 h, and a solution of 0.26 g (1.58 mmol) of **55** in 2 mL of DMF was added. The reaction mixture was allowed to warm to room temperature overnight and was diluted with 100 mL of ethyl acetate. The organic solution was washed with water and saturated $NaHCO_3$, dried, and concentrated. The residue was chromatographed over 50 g of silica gel, with 5% methanol–ethyl acetate as eluent. The product thus obtained was recrystallized from ethyl acetate–hexane to give 0.30 g (44%) of **34**: mp 115–117 °C; $[\alpha]_D$ −64.19° (ethanol, *c* 0.7415). Anal. ($C_{26}H_{28}N_4O_2$) C, H, N.

(*S*)-2-(1-Methylethyl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxamide (35) was prepared similarly to **34** above. With 0.15 g (0.91 mmol) of **56**, 0.282 g (1.00 mmol) of **46**, 0.215 mL of diphenyl phosphorazidate, and 0.14 mL (1.0 mmol) of triethylamine as starting material, there was obtained 0.164 g (45%) of **35**: mp 115–117 °C; $[\alpha]_D$ +62.36° (ethanol, *c* 0.5805). Anal. ($C_{26}H_{28}N_4O_2$) C, H, N.

PAF Binding Assay.[12] [^{3}H]PAF was obtained from the New England Nuclear Co. Platelet-rich plasma was prepared by centrifugation of citrate-treated dog blood. Acidification to pH 6.5 with 0.15 M citric acid and centrifugation for 10 min at 1000*g* yielded a platelet-rich pellet, which was then washed by resuspension in phosphate-buffered saline, pH 7.3 (PBS), containing 1 mM EDTA and recentrifuged. The washed platelet preparation was adjusted to 2 × 10^7 platelets/0.05 mL in 0.1% BSA–PBS. Platelet counting was done with a Royco Cell-Crit 921 instrument.

To a 0.40-mL Microfuge tube containing 0.05 mL of silicone oil were added buffer and a PAF standard or a test drug to bring the aqueous volume to 0.15 mL. Next, 0.05 mL of a solution of [^{3}H]PAF (10 000 cpm, 45 Ci/mM) in ethanol was added followed

by 2×10^7 dog platelets. After mixing, incubation for 10 min at room temperature, and centrifugation for 1 min in a Beckman Microfuge B (8000*g*), the pellet was removed by clipping off the tip of the tube, and the platelets were washed out of the tip with 0.20 mL of 50% methanol. For counting, 10 mL of Aquasol was added, and the radioactivity in the samples was determined with a Searle Mark III liquid scintillation counter linked to an Iso-Data microprocessor.

Experiments were run in triplicate; compounds were initially evaluated at a concentration of 1 μM, and percent specific inhibition was determined. Those drugs that significantly inhibited specific PAF binding were reevaluated at three or more logarithmically spaced concentrations and IC_{50} values were determined from plots of specific inhibition vs concentration. The data for most of the active compounds shown in Tables I and II were confirmed by independent, duplicate determinations.

In Vitro Metabolism Studies. Male guinea pigs (Hartley strain) and Sprague–Dawley rats were obtained from Charles River Breeding Labs. Squirrel monkeys were caught wild and obtained from Charles River Primate Corp.; livers were taken immediately after a 50 mg im overdose of ketamine. Beagles of both sexes, obtained from White Eagle Farm, were killed by iv injection with sodium pentobarbital.

Differential fractionation of rat liver homogenate in 0.25 M sucrose provided nuclear, mitochondrial, and 9000*g* supernatant fractions, each of which contained amidase activity, and thus, further work was conducted with whole liver homogenates. In preliminary experiments with **33**, using liver obtained from guinea pigs, dogs, and monkeys, it was established that rates of hydrolysis were linear with incubation times up to 15 min and tended to fall at longer incubation times presumably due to instability of the amidases with respect to time. To compare hydrolysis rates catalyzed by liver homogenates from different species, it was necessary to demonstrate that the amidase reaction rates were saturated with respect to substrate concentration. While with dog and squirrel monkey liver homogenates, successive doubling of the substrate concentration from 0.1 mM prompted only small increases in initial hydrolysis rates; the reaction with guinea pig liver was not fully saturated at 0.4 mM. Because of the limited solubility of the substrates, a final concentration of 0.4 mM was employed in these studies.

The standard reaction mixtures contained 40 mM potassium phosphate buffer, pH 7.4, 0.4 mM substrate, and 0.1 mL of liver homogenate, equivalent to 21 mg of fresh liver, in a final volume of 1.0 mL. Incubations were carried out at 37 °C for 10 min, and 1.5 mL of acetonitrile–methanol (9:1) was added. After 10 min at 0 °C, the mixtures were centrifuged, and the clear supernatants were assayed by HPLC for the presence of **46** as previously described.[15] Unincubated controls as well as mixtures without substrate were run in parallel with each experiment. The results are presented in Table III as the number of nmol of **46** formed in 10 min per 21 mg of fresh liver.

In Vivo Evaluation. Male Hartley guinea pigs weighing between 500 and 900 g were provided standard guinea pig food and tap water ad libitum up to the time of each experiment. A stock solution of 2 mM PAF in ethanol was kept at −70 °C prior to use. Dilutions were made in Tris buffer, pH 7.4, and 0.1% BSA to a concentration of 10^{-6} M for injection into the animals.

A. Intravenous Procedure. Animals were anesthetized with urethane (1.6 g/kg, ip), and catheters (PE50) were introduced into the right carotid and left femoral arteries for the purpose of withdrawing blood samples and monitoring systemic arterial blood pressure. The left jugular vein was also cannulated (PE10) for intravenous drug injection and for introducing the PAF challenge (50 ng/kg). Three cumulative intravenous doses of normal saline (1 mL/kg × 3), **57** (1.0, 3.0, and 10.0 mg/kg), **27** (0.3, 1.0, and 3.0 mg/kg), and **33** (1.0, 3.0, and 10.0 mg/kg) were examined in this phase of the study. Four animals were used for each of the compounds except for **33** where five animals were employed. Arterial blood pressure was monitored continuously throughout the experiment. Blood samples (0.25 mL) were withdrawn for platelet counting 30 s prior to PAF and at 15, 30, 60, and 120 s after PAF. Before the withdrawal of blood, the carotid artery catheter was filled with sodium citrate (3.8%) in normal saline to prevent clotting of the blood in the collection tube. After each collection period, the blood samples were counted for number of platelets on a Baker Instruments series 810 whole blood platelet analyzer. A decrease in platelet count following the injection of PAF was used as an indication of platelet aggregation.

Fifteen minutes after the PAF challenge, intravenous drug dosing began. Animals were dosed with a drug at 30-min intervals. Fifteen minutes after each drug dose, the animals were challenged with PAF. In a separate experiment, four animals were treated with cumulative doses of **27** and challenged with PAF 2 min after each drug dose to compensate for its apparent short duration of action. Data were analyzed for significance by using the paired *t* test.

B. Oral Procedure. Animals were anesthetized with sodium pentobarbital (35 mg/kg, ip). The carotid artery and jugular veins were cannulated in the same manner as in the intravenous protocol. The femoral artery, however, was not cannulated. The cannulas were exteriorized at the base of the neck, and the animals were allowed to recover and regain consciousness in a special holding cage that maintains catheter patency without restricting movement. At least 18 h after surgery, the conscious animal was placed in a small cage, and the carotid catheter was connected to a swivel, which was attached to a pressure transducer. The swivel allows freedom of movement while recording arterial blood pressure. The animals were challenged with PAF (120 ng/kg, iv) three times at 30-min intervals. The first and second PAF challenges were used to establish a consistent response to PAF. The third PAF challenge was used as the control. Control data consisted of continuously monitored direct arterial blood pressure and arterial blood samples (0.35 mL) taken at 15, 30, and 45 s after PAF. Platelet counts were determined in the same manner as described previously. Arterial blood pressure was not recorded during the collection of blood samples. Thirty minutes after the third PAF challenge (control), the animals were orally dosed with either saline (1 mL/kg) or **33** (50 or 200 mg/kg). Animals were then challenged with PAF at 60, 180, and 300 min after receiving the drug.

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Supplementary Material Available: Details of the X-ray crystallographic analysis of **54**, a perspective drawing, and tables listing the final atomic parameters, the final anisotropic thermal parameters, bond lengths, and bond angles for **54** (6 pages). Ordering information is given on any current masthead page.