Structure–Activity Relationships Associated with 3,4,5-Triphenyl-1*H*-pyrazole-1-nonanoic Acid, a Nonprostanoid Prostacyclin Mimetic

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A series of phenylated pyrazoloalkanoic acid derivatives were synthesized and evaluated as inhibitors of ADP-induced human platelet aggregation. 3,4,5-Triphenyl-1*H*-pyrazole-1-nonanoic acid (**8d**), with an IC_{50} of 0.4 μM, was the most potent inhibitor identified in this study. Biochemical studies determined that **8d** increased intraplatelet cAMP accumulation and stimulated platelet membrane-bound adenylate cyclase in a concentration-dependent fashion. Displacement of [^{3}H]iloprost by **8d** from platelet membranes indicated that the platelet prostacyclin (PGI_2) receptor is the locus of biological action. Structure–activity studies demonstrated that the minimum structural requirements for binding to the platelet PGI_2 receptor and inhibition of ADP-induced platelet aggregation within this series are a vicinally diphenylated pyrazole substituted with an ω-alkanoic acid side chain eight or nine atoms long. Potency depended upon both side-chain length and its topological relationship with the two phenyl rings.

Blood platelet activation has been implicated in a number of pathophysiological conditions including tumor cell metastasis, asthma, migraine, atherosclerosis, and, most prominently, thrombosis.[1] While venous thrombosis is associated with the activation of both platelets and the coagulation cascade, arterial thrombi are composed almost entirely of platelet aggregates.[2] Recent clinical studies with inhibitors of blood platelet aggregation have demonstrated a reduction in the incidence of occlusive vascular events in both healthy individuals[3] and those at risk.[4–6] Aspirin, dipyridamole, and ticlopidine have been used to establish a clinical role for platelet aggregation inhibitors but none of these agents satisfies the criteria demanded of the ideal antiplatelet drug.[7,8]

Platelet activation involves adhesion, shape change, aggregation, and the release of the contents of intracellular storage granules, which occurs in response to a variety of different agonists.[9] Combinations of stimulating agents, acting synergistically, are likely to be responsible for occlusive vascular events in vivo, but this may depend upon the underlying pathological condition. Elevation of intraplatelet cAMP concentration is associated with inhibition of activation in response to most stimuli[10] and this may be accomplished either by inhibition of cAMP phosphodiesterase[11] or stimulation of adenylate cyclase.[12] Prostacyclin (PGI_2) (1) was identified in 1976[13–15] as the most powerful endogenous stimulator of blood platelet adenylate cyclase; it binds to and activates receptors that also recognize PGE_1 but are distinct from those that bind PGD_2 or adenosine.[12] Although PGI_2 is available to the clinician,[16] its utility is limited, in part, by inherent chemical instability. This is due to the incompatibility of a strained enol ether moiety and pendant carboxylic acid, which is capable of intramolecularly catalyzing hydrolytic decomposition.[17] Attempts to develop analogues of PGI_2 as potential therapeutic agents focused initially upon modifying or stabilizing the labile enol ether functionality while subsequent studies were directed toward identifying

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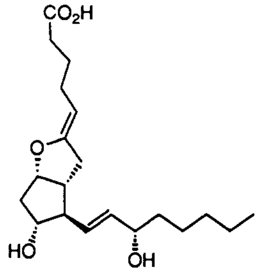
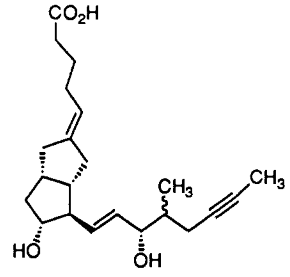
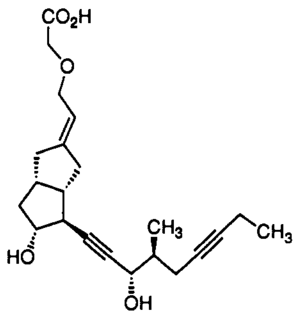


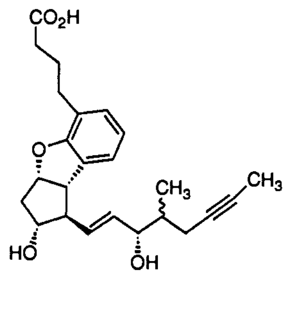
1, PROSTACYCLIN



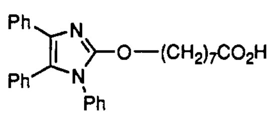
2, ILOPROST



3, CICAPROST



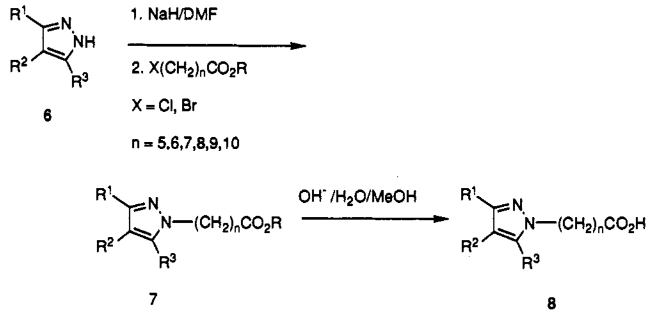
4, BERAPROST



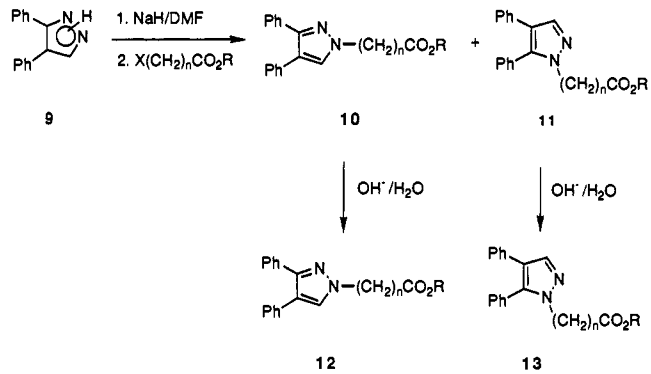
5, OCTIMIBATE

agents with improved oral bioavailability and pharmacokinetic properties.[11b,18] Iloprost (2),[19] cicaprost (3),[20] and beraprost (4)[21] are representatives of this structural class that have advanced into clinical trials. All of these compounds are patterned after the natural substance and retain the functionality and architectural complexity of PGI_2. We[22] and others[23] have recently demonstrated that the triphenylated imidazole derivative octimibate (5) inhibits platelet function by binding to the platelet PGI_2 receptor and stimulating adenylate cyclase. We were intrigued by this observation since octimibate is significantly less complex and structurally quite distinct from PGI_2 and its close analogues and presents an unusual template from which it may be possible to design potent, orally-effective PGI_2 mimetics. As part of an effort to elucidate the structural elements of octimibate that are responsible for platelet inhibitory activity, we synthesized and evaluated a series of phenylated pyrazoloalkanoic acid derivatives. We report herein the results of this investigation which identifies the minimal structural features essential for effective PGI_2 mimicry.

Scheme I



Scheme II



Chemistry

Exposure of 3,4,5-triphenyl-,[24] 3,5-diphenyl-4-(phenylmethyl)-,[27] 3,5-diphenyl-4-ethyl-,[27] 3,5-diphenyl-,[28] and

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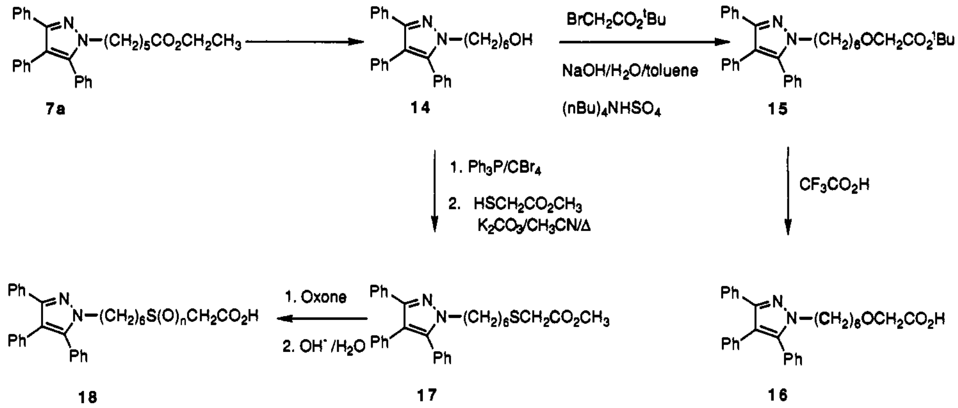
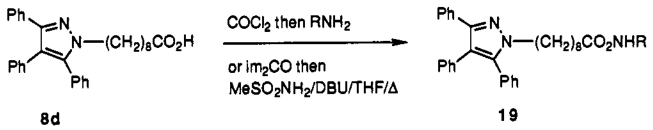
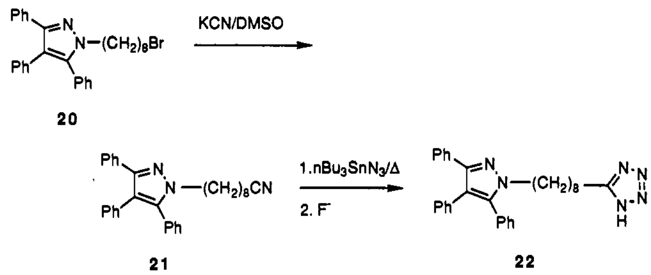
Scheme III



Scheme IV



Scheme V



4-phenyl-1*H*-pyrazole[29] to sodium hydride in DMF produced the corresponding sodium salts, which were alkylated with the ester of an ω-haloalkanoic acid[30] to provide esters **7a–j** (Scheme I). Saponification afforded the target carboxylic acids **8a–j**. Alkylation of diphenylpyrazole **9**[33] provided mixtures of isomeric pyrazoles **10** and **11**, which were separated by flash column chromatography (Scheme II). The more mobile esters constituted the major reaction product and were identified as the 3,4-diphenyl-substituted isomers **10** after examination of NMR spectral data. In $CDCl_3$, the pyrazole ring proton of the major products **10** is shifted upfield and the NCH_2 protons appear downfield relative to the corresponding protons of the minor products **11**.[35,36] Confirmation was obtained by measuring long range $^1H-^{13}C$ coupling constants in the fully-coupled ^{13}C NMR spectrum of **10c** and **11c**. The proton-bearing pyrazole ring carbon of **10c** resonates as a doublet of triplets at δ 128.97 with coupling constants of 183.99, 3.11, and 2.28 Hz. This signal collapsed to a doublet, J = 184 Hz upon irradiation of the NCH_2 protons at δ 4.14. In contrast, the corresponding ring carbon atom of **11c** appears as a doublet at δ 137.53, J = 188.92 Hz. Alkaline hydrolysis of **10** and **11** provided acids **12** and **13**, respectively.

The introduction of heteroatoms β to the carboxylic acid moiety of **8** was accomplished as depicted in Scheme III and began with reduction of ester **7a**, using $LiAlH_4$ in Et_2O, to provide alcohol **14**. Alkylation of **14** with *tert*-butyl bromoacetate under phase-transfer catalysis[20a] furnished ester **15**, which was converted to acid **16** upon dissolution in CF_3CO_2H. Bromination of **14** and treatment of the resultant bromide with methyl mercaptoacetate and K_2CO_3 in CH_3CN at reflux afforded ester **17**, which was hydrolyzed to acid **18a**. Oxidation of **17** to the corresponding sulfoxide and sulfone was accomplished using Oxone, the former produced selectively at −10 °C with limited reagent and brief exposure.[37] Alkaline hydrolysis gave acids **18b** and **18c**.

Amides **19a** and **19b** were obtained from acid **8d** by sequential treatment with $(COCl)_2$ and either NH_4OH or CH_3NH_2, respectively, and sulfonamide **19c** was prepared from **8d** using a published procedure[38] (Scheme IV). Tetrazole **22** was synthesized from bromide **20** as depicted in Scheme V and proceeded through the intermediacy of nitrile **21**. Admixture of **21** and tri-*n*-butyltin azide at 140

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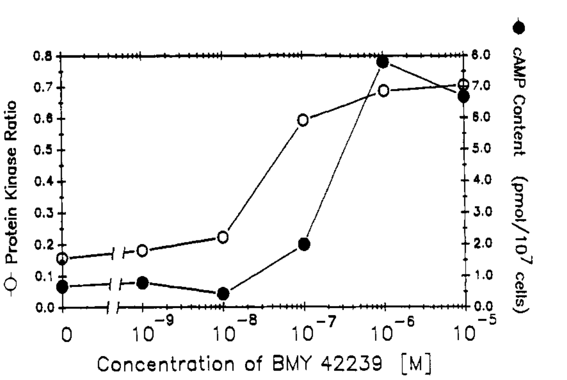

Figure 1. Effects of BMY 42239 (**8d**) on platelet cAMP levels (●) and cAMP-dependent protein kinase activity ratio (○). Human platelets were isolated by differential centrifugation and treated with the indicated concentrations of **8d** for 10 min prior to removing aliquots for cAMP determination and assay of the cAMP-dependent protein kinase ratio. The cAMP levels were determined by RIA using a commercially-available kit, and the cAMP kinase ratio was used as an indication of the activation of the cAMP-dependent protein kinase in the cell.[56] Both the cAMP and the protein kinase ratio determinations were obtained from the same drug-treated samples. The cAMP determinations are the average of duplicate determinations and the cAMP-dependent protein kinase measurements represent the average of triplicate determinations of a single representative experiment.

°C followed by treatment with potassium fluoride[39] furnished the crystalline tetrazole **22** in 73% overall yield.

The compounds prepared as part of this study are listed in Table I along with relevant physicochemical data.

Results and Discussion

The target compounds were evaluated as inhibitors of ADP-induced aggregation of human platelets in platelet-rich plasma (PRP) using the previously described experimental protocol[22] and the results are presented in Table I. In this assay, PGI_2, iloprost, and octimibate (**5**) exhibit IC_{50}'s of 8 nM, 2 nM, and 1.02 μM, respectively. 3,4,5-Triphenyl-1*H*-pyrazole-1-nonanoic acid (**8d**), BMY 42239, inhibits ADP-induced platelet aggregation with an IC_{50} of 0.4 μM and is 2-fold more effective than octimibate (**5**). When collagen was employed as the stimulus, **8d** prevented platelet aggregation with an IC_{50} of 0.15 μM compared to an IC_{50} of 1.40 μM for octimibate under the same conditions.[22] The biochemical properties of pyrazole **8b** were investigated in some detail in order to establish the mode of action for this series of platelet aggregation inhibitors. Exposure of human platelets to **8d** resulted in a concentration-dependent increase in intracellular cAMP levels and activation of the cAMP-dependent protein kinase (Figure 1). Pyrazole **8d** is a weak inhibitor of a crude human platelet cAMP phosphodiesterase preparation with an IC_{50} = 10 μM,[40] which is significantly higher than the concentrations necessary to inhibit platelet aggregation and increase cAMP levels. As shown in Figure 2, **8d** stimulates adenylate cyclase in platelet membranes in a dose-dependent fashion. Maximal stimulation occurs between 0.1 and 1 μM and is 70–75% of the maximum effect observed for PGE_1. PGE_1 activates platelet adenylate cyclase by binding to the PGI_2 receptor and stimulates the enzyme to levels similar to that maximally attained by iloprost.[40] Compared to PGE_1, **8d** is therefore a partial agonist as a stimulant of platelet adenylate cyclase, a property shared

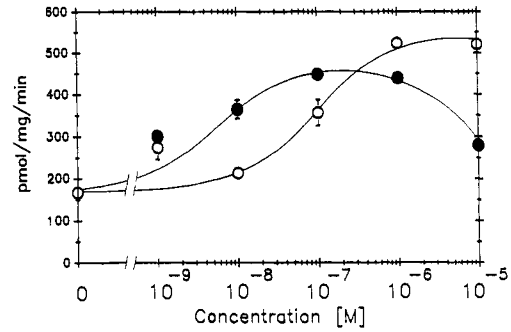

Figure 2. Stimulation of human platelet adenylate cyclase activity by **8d** (●) and PGE_1 (○). Adenylate cyclase activity, determined in the presence of 10 μM GTP and the indicated concentrations of **8d** or PGE_1, was performed as previously described.[22] Each point represents the mean ± standard deviation of triplicate determinations within a representative experiment.

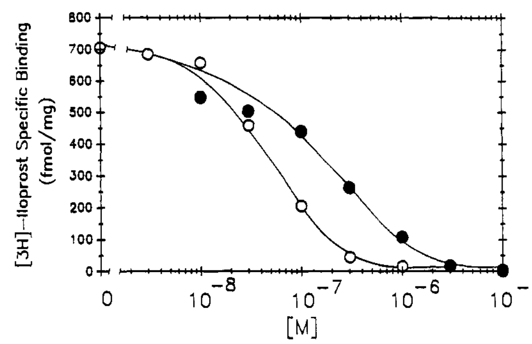

Figure 3. Effects of **8d** (●) and cold iloprost (○) on [^{3}H]iloprost binding to isolated platelet membranes. Binding studies were performed using 5 nM [^{3}H]iloprost at 0–4 °C, as described previously.[22] Each point represents the average of duplicate determinations within a representative experiment.

with octimibate.[22,23] Radioligand binding studies were used to determine the site of action of **8d** on the platelet membrane. Pyrazole **8d** displaces [^{3}H]iloprost from platelet membranes in a concentration-dependent manner as depicted in Figure 3. The IC_{50} for displacement of [^{3}H]iloprost by **8d** is 160 nM, which compares with IC_{50}'s of 29 nM for unlabeled iloprost and 500 nM for octimibate under similar conditions.[22] Pyrazole **8d** displaced [^{3}H]PGE_1 from human platelet membranes but did not significantly alter [^{3}H]PGD_2 binding at 1 μM (supplemental material). However, **8d** exhibited weak affinity for the platelet thromboxane (TXA_2) receptor and reduced [^{3}H]SQ 29548[41] binding to platelet membranes by 50% at a concentration of 8 μM.[40]

Concentrations of **8d** above 1 μM are associated with reduced stimulation of adenylate cyclase compared to the maximal effect, a phenomenon also observed with octimibate and for which we do not have a satisfactory explanation. This may be a nonspecific effect resulting from membrane disruption at these high concentrations as a consequence of the detergentlike nature of **8d**. Alternatively, **8d** may bind to and activate a prostanoid receptor linked through G_i to platelet adenylate cyclase as has been postulated for PGI_2 itself.[42]

Although **8d** exhibits high affinity for the platelet PGI_2 receptor and is an effective stimulant of adenylate cyclase,

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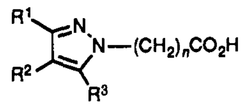
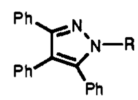
Table I



compd	R^1	R^2	R^3	*n*	mp, °C	anal.[a]	IC_{50}, μM: inhibition of ADP-induced human platelet aggregation	IC_{50}, μM: displacement of [^{3}H]iloprost from human platelet membranes
8a	Ph	Ph	Ph	5	140–142	$C_{27}H_{26}N_2O_2$	>78	
8b	Ph	Ph	Ph	6	136–138	$C_{28}H_{28}N_2O_2$	22.4	
8c	Ph	Ph	Ph	7	101–102.5	$C_{29}H_{30}N_2O_2 \cdot 0.05H_2O$	3.4	
8d	Ph	Ph	Ph	8	112–114	$C_{30}H_{32}N_2O_2$	0.4	0.16
8e	Ph	Ph	Ph	9	oil	$C_{31}H_{34}N_2O_2 \cdot 0.2H_2O$	5.5	
8f	Ph	Ph	Ph	10	81.5–84	$C_{32}H_{36}N_2O_2$	18.6	
8g	Ph	H	Ph	8	oil	$C_{24}H_{28}N_2O_2$	>66	>10
8h	Ph	$PhCH_2$	Ph	8	oil	$C_{31}H_{34}N_2O_2 \cdot 0.3H_2O$	>68	
8i	Ph	C_2H_5	Ph	8	oil	$C_{26}H_{32}N_2O_7$	>79	
8j	H	Ph	H	8	119–121	$C_{18}H_{24}N_2O_2 \cdot 0.1H_2O$	>105	>10
12a	Ph	Ph	H	6	80–84	$C_{22}H_{24}N_2O_2$	>92	
12b	Ph	Ph	H	7	108–110	$C_{23}H_{26}N_2O_2 \cdot 0.1H_2O$	>85	
12c	Ph	Ph	H	8	83–85	$C_{24}H_{28}N_2O_2 \cdot 0.2H_2O$	4.5	0.35
12d	Ph	Ph	H	9	107–110	$C_{25}H_{30}N_2O_2 \cdot 0.15H_2O$	>81	
13a	H	Ph	Ph	6	96–103	$C_{22}H_{24}N_2O_2$	>92	
13b	H	Ph	Ph	7	88–90	$C_{23}H_{26}N_2O_2$	1.5	0.16
13c	H	Ph	Ph	8	oil	$C_{24}H_{28}N_2O_2$	5.8	0.7
13d	H	Ph	Ph	9	72–74	$C_{25}H_{30}N_2O_2$	>82	



compd	R	mp, °C	anal.[a]	IC_{50}, μM: inhibition of ADP-induced human platelet aggregation
7d	$(CH_2)_8CO_2CH_3$	oil	$C_{31}H_{34}N_2O_2$	4.3
15	$(CH_2)_6OCH_2CO_2{}^tBu$	69–72	$C_{33}H_{38}N_2O_3$	>62
16	$(CH_2)_6OCH_2CO_2H$	92–94	$C_{29}H_{30}N_2O_3$	0.44
18a	$(CH_2)_6SCH_2CO_2H$	92–97	$C_{29}H_{30}N_2O_2S$	0.87
18b	$(CH_2)_6S(O)CH_2CO_2H$	132.5–134.5	$C_{29}H_{30}N_2O_3S \cdot 0.1H_2O$	6.5
18c	$(CH_2)_6SO_2CH_2CO_2H$	153.5–155	$C_{29}H_{30}N_2O_4S$	22
19a	$(CH_2)_8CONH_2$	104–107	$C_{30}H_{33}N_3O$	33
19b	$(CH_2)_8CONHCH_3$	68–71	$C_{31}H_{35}N_3O$	>68
19c	$(CH_2)_8CONHSO_2CH_3$	88–90	$C_{31}H_{35}N_3O_3S$	5.9
21	$(CH_2)_8CN$	79.5–80.5	$C_{30}H_{31}N_3 \cdot 0.5H_2O$	>72
22	$(CH_2)_8CN_4H$	158–160	$C_{30}H_{32}N_6$	1.04

[a] Elemental analyses for C, H, and N were within ±0.4% of the theoretical values.

being only 5–10-fold less potent than iloprost, it is approximately 200-fold weaker than iloprost as an inhibitor of ADP-induced platelet aggregation in PRP. This finding is most likely a consequence of **8d** binding to the plasma proteins present in the latter assay but not the former two, which would reduce the effective concentration of the free drug in solution. This phenomenon was observed with octimibate, which is a markedly more potent inhibitor of induced platelet aggregation in washed platelets compared to PRP,[23] and a similar effect has been reported for a renin inhibitor.[43]

Pyrazole **8d** inhibits ADP-induced aggregation of rabbit and rat platelets less effectively than human platelets with IC_{50}'s of 5.5 and 0.8 μM, respectively, a pattern of species dependence similar to that documented for octimibate.[22,23] Nevertheless, pyrazole **8d** demonstrated significant antithrombotic activity in a rabbit model of thrombosis following oral administration. In this model, where platelet-dependent thrombus formation is induced in the microcirculation of the ear of a conscious rabbit using a laser,[44] **8d** reduced thrombus formation by 55% 2 h following a dose of 10 mg/kg po. In contrast, octimibate, at a dose of 30 mg/kg po, provided only 39% protection in this model while PGI_2 was not effective orally but inhibited thrombosis as long as an iv infusion of 0.1 μg/kg per min was maintained.[40]

The structure–activity studies associated with **8d** presented in Table I demonstrate that the nonanoic acid side chain is the optimal length, since homologation in either direction results in a 10-fold reduction in potency. Abbreviation of the chain length by two carbon atoms (**8b**) results in a further decrease in activity and hexanoate **8a** is devoid of significant platelet inhibitory effect. Both 3,5-diphenyl-1*H*-pyrazole-1-nonanoic acid (**8g**) and 4-phenyl-1*H*-pyrazole-1-nonanoic acid (**8j**) are inactive as platelet aggregation inhibitors, and neither compound binds appreciably to the PGI_2 receptor, indicating that they do not function as antagonists. Substitution of the

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4-phenyl ring of **8d** by a benzyl (**8h**) or ethyl (**8i**) group also gave inactive compounds, demonstrating a specific requirement for a phenyl ring at this position.

3,4-Diphenyl-1*H*-pyrazole-1-nonanoic acid (**12c**) and its 4,5-diphenyl isomer, **13c**, inhibit ADP-induced platelet aggregation with similar efficacy but are 10-fold weaker than **8d**, which correlates with reduced affinity for the PGI_2 receptor. The 3,4-diphenylated pyrazole derivatives **12** display a similar structure–activity profile to the triphenylated series **8** with regard to the effects of variation of side-chain length. However, in the isomeric 4,5-diphenyl pyrazole series **13**, reduction of the side chain length by a single carbon atom provided a compound, **13b**, with enhanced activity compared to **13c**, while further truncation gave a weakly active compound, **13a**.

Modifications of the side chain terminus region were explored in an attempt to identify agents that might exhibit increased resistance to β-oxidative degradation in vivo. Introduction of an oxygen atom β to the carboxylate moiety (**16**) led to only a marginal reduction in potency compared to the prototype **8d**, which parallels structure–activity relationships associated with PGI_2 agonists of a more classical structure.[20] A sulfur atom at this site resulted in a 2-fold diminution in potency (**18a**), and increasing the oxidation state of the sulfur to that of a sulfoxide (**18b**) and sulfone (**18c**) led to further reductions in inhibitory activity relative to **18a**.

An acidic proton at the side-chain terminus appears to be an essential requirement for effective platelet inhibitory activity. Methyl ester **7d** is 10-fold less potent than the corresponding acid **8d**, and the activity observed for **7d** is presumably the result of significant plasma esterase-mediated cleavage to **8d** during the 3-min incubation period of drug in PRP prior to the addition of the agonist. The inactivity associated with *tert*-butyl ester **15**, which would be expected to be less readily unmasked to acid **16**, provides support for this contention. The primary amide **19a** is almost 100-fold weaker than **8a** while the methylated amide **19b** is inactive. Acylated sulfonamide **19c** is 15-fold less potent than **8d**, demonstrating that this carboxylic acid isostere, which has been previously incorporated into prostanoids with some success,[45] is moderately effective in these PGI_2 mimetics. However, the tetrazole moiety does function as an effective carboxylic acid isostere and **22** is less than 3-fold weaker than **8d** as an inhibitor of ADP-induced platelet aggregation.

A pharmacophore for platelet PGI_2 receptor agonism within this series of pyrazoles can readily be deduced from the data presented in Table I. Two phenyl rings bound to vicinal atoms of the heterocycle appear to be fundamental, and this is optimal when separated from a carboxylic acid moiety, or its surrogate, by a chain of seven or eight atoms in length. The functional equivalence of the pyrazole ring of **8d** with the more basic imidazole ring of octimibate suggests that the role of the heterocycle may be that of a scaffold on which the pharmacophoric elements are arranged. The conformational flexibility inherent in the alkanoic acid side chain of the compounds listed in Table I limits the reliable application of molecular modeling studies that might provide insight into the topographical relationships between the key structural elements. These studies await the identification of more rigid molecules with this kind of biological activity. However, the structure–activity observations do allow some suggestion pertaining to the topological relationships for this

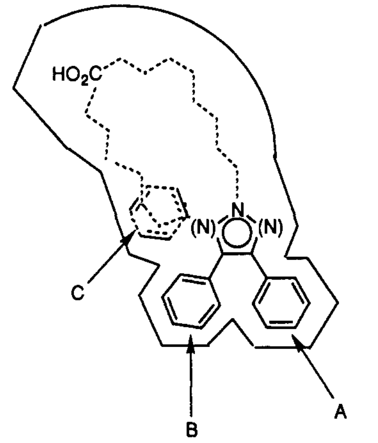
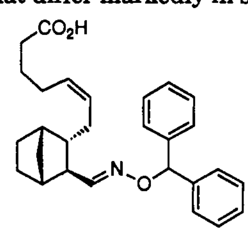


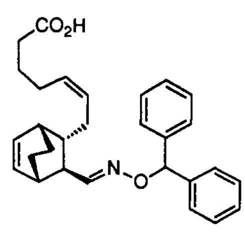
Figure 4. A topological descriptor of that portion of the PGI_2 receptor occupied by pyrazole derivatives presented in Table I. The phenyl rings are depicted as coplanar with the heterocyclic ring for purposes of illustration only and are not intended to suggest conformational preferences.

class of prostacyclin mimetic and this is summarized in Figure 4. The three phenyl rings of octimibate (**5**) and **8d** presumably occupy a hydrophobic cavity of the platelet PGI_2 receptor that can be conveniently divided into three distinct regions, designated A, B, and C. Occupation of sites A and B appears to be crucial for binding to the PGI_2 receptor and transmission of the signal leading to activation of adenylate cyclase. 3,4-Diphenyl-1*H*-pyrazole-1-nonanoic acid (**10c**) leaves site C unfilled and this presumably accounts for the 10-fold reduction in potency compared to **8d**. The SAR associated with the isomeric acids **11b** and **11c** suggests that the carboxylic acid binding site is proximate to the region C, which is occupied by the side chain atoms of **11b** and **11c** when their phenyl rings are accommodated in sites A and B.

EP 035 (**23**) and EP 157 (**24**) have been described as PGI_2 mimetics that differ markedly in structure from the



23, EP 035



24, EP 157

natural prostanoid.[46] The biochemical profile of **23** and **24** bears a striking resemblance to that described for octimibate[22,23] and **8d**, and some structural homology is also apparent. The benzhydryl oxime moiety of **23** and **24** presents two phenyl rings in a geminally-disposed ar-

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rangement that, from an inspection of molecular models, is structurally analogous to the vicinally-diphenylated heterocycles described above. The bicyclic rings of **23** and **24** presumably provide some degree of stereodefinition and also function as spacers between the benzhydryl and carboxylic acid moieties.

The relationship between that part of the platelet PGI_2 receptor to which the natural ligand and its structurally similar analogues bind and that described by Figure 4 is not obvious. There appears to be little structural homology between the two classes of compound. Indeed, only the carboxylic acid moiety is a common feature and the extent of overlap of the remainder of the two classes or molecule is a matter of speculation. However, the hydrophobic phenyl rings of **5**, **8d**, **23**, and **24**, may occupy a region of the PGI_2 receptor filled in part by the ω-side chain of the natural ligand and its close relatives, which has been shown to be tolerant of quite wide structural variation.[18] Such an alignment would allow the C-14–C15 unsaturation of **1–4** and the π-systems of **5**, **8d**, **23**, and **24** to overlap.

In addition to pharmacokinetic problems, therapeutic application of PGI_2 and its mimetics has been limited by the incidence of side effects, most notably hypotension, facial flushing, and nausea.[47] These problems are presumably the result of activation of PGI_2 receptors located on tissues other than platelets. Tissue-selective PGI_2 agonists remain an important target of prostaglandin research, and although there is some suggestion of the existence of receptor subtypes,[48] this data must be interpreted with caution due to complications arising from variation of response across species.[49] The species-dependent effects of **5**, **8d**, **23**, and **24** suggest heterogeneity of platelet PGI_2 receptors,[50] and the possibility of receptor subtypes within species limits the predictive value of studies of the hypotensive effects of this class of compound in traditional laboratory animals. Nonhuman primates appear to be an acceptable species with which in vitro and in vivo studies may be conducted with some confidence in the predictive value of the likely effect in humans. None of the compounds described in this report has been evaluated in this fashion. However, studies of this nature have been conducted with **5**[51] and **24**,[52] and the results suggest that neither is able to effectively differentiate the platelet PGI_2 receptor from that in vascular tissue.

In summary, we have described the synthesis and SAR associated with a series of architecturally simple and novel nonprostanoid PGI_2 mimetics and defined the minimum requirements for expression of biological activity. Although these compounds exhibit reasonable affinity for the platelet PGI_2 receptor, they are less potent than PGI_2 and closely related analogues as inhibitors of blood platelet aggregation in PRP. However, the structural simplicity and absence of functionality previously considered essential for effective PGI_2 mimicry suggest that this class of agonist is worthy of further study in an effort to identify more potent agents with improved oral activity and, possibly, tissue specificity.

Experimental Section

Melting points were recorded on a Thomas-Hoover capillary apparatus and are uncorrected. Proton (^{1}H NMR) and carbon (^{13}C) nuclear magnetic resonance spectra were recorded on a Bruker AM FT instrument operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. All spectra were recorded using tetramethylsilane as an internal standard, and signal multiplicity is designated according to the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Infrared (IR) spectra were obtained using a Perkin-Elmer 1800 FT IR, scanning from 4000 to 400 cm^{-1} and calibrated to the 1601 cm^{-1} absorption of a polystyrene film. Mass spectral data were obtained on a Finnigan Model 4500 GC/MS using chemical ionization (isobutane) procedures. Analytical samples were dried in vacuo at 78 °C or in the presence of P_2O_5 at room temperature for at least 12 h. Elemental analyses were provided by Bristol-Myers Squibb's Analytical Chemistry Department or Oneida Research Services (Whitesboro, NY).

Methyl 3,4,5-Triphenyl-1*H*-pyrazole-1-nonanoate (7d). NaH (588 mg of a 60% dispersion in mineral oil, 13 mmol) was washed twice with hexane and covered with DMF (45 mL). 3,3,5-Triphenyl-3*H*-pyrazole (3.0 g, 10 mmol) was added and the mixture stirred at 100 °C under N_2 for 0.5 h before being cooled to room temperature. Methyl 9-bromononanoate[30,31] (2.80 g, 11 mmol) in DMF (2 mL) was added dropwise and the mixture stirred for 2 h before being poured onto H_2O and extracted with Et_2O (3 × 100 mL). The extracts were washed with H_2O (3 × 100 mL), dried over Na_2SO_4, and concentrated. Chromatography on silica gel using hexane and Et_2O (2:1) as eluent gave **7d** (4.72 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.06 (8 H, bs), 1.41 (2 H, t, J = 7 Hz), 1.68 (2 H, bs), 2.11 (2 H, t, J = 7.5 Hz, $CH_2CO_2CH_3$), 3.47 (3 H, s, CO_2CH_3), 3.90 (2 H, t, J = 7 Hz, NCH_2), 6.83–7.32 (15 H, m); MS m/z 467 (MH^+). Anal. ($C_{31}H_{34}N_2O_2$) C, H, N.

3,4,5-Triphenyl-1*H*-pyrazole-1-nonanoic Acid (8d). A mixture of **7d** (47.23 g, 0.1 mol), 5 N NaOH solution (60.88 mL, 0.3 mol), and MeOH (600 mL) was heated at reflux for 0.5 h. The solvent was evaporated, and the residue diluted with H_2O and 2 N HCl until pH = 1 and extracted with CH_2Cl_2. The extracts were washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was dissolved in CH_2Cl_2 (150 mL) and diluted with hexane to precipitate **8d** (39.03 g, 85%): mp 112–113 °C; IR (KBr) 1715 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.13 (8 H, m), 1.43 (2 H, t, J = 6.5 Hz), 1.71 (2 H, t, J = 6 Hz), 2.12 (2 H, t, J = 7 Hz, CH_2CO_2H), 3.99 (2 H, t, J = 7 Hz, NCH_2), 7.00–7.59 (15 H, m); MS m/z 453 (M^+). Anal. ($C_{30}H_{32}N_2O_2$) C, H, N.

Methyl 3,4-Diphenyl-1*H*-pyrazole-1-nonanoate (10c) and Methyl 4,5-Diphenyl-1*H*-pyrazole-1-nonanoate (11c). NaH (945 mg of a 60% dispersion, 23 mmol) was washed with hexane (3×) and covered with DMF (60 mL), and **9** (4.00 g, 18 mmol) was added. After stirring at room temperature for 20 min, methyl 9-bromononanoate (5.02 g, 20 mmol) was added and stirring continued for 2 h. The mixture was diluted with H_2O and extracted with Et_2O (3×), and the extracts were washed with H_2O (3×), dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica using hexane/Et_2O (2:1) as eluent to give **10c** (4.43 g, 62%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.20–1.50 (8 H, m), 1.63 (2 H, m), 1.94 (2 H, m), 2.29 (2 H, t, J = 7 Hz, $CH_2CO_2CH_3$), 3.64 (3 H, s), 4.14 (2 H, t, J = 7 Hz, NCH_2), 7.20–7.40 (8 H, m), 7.50–7.60 (2 H, m); MS m/z 391 (MH^+). Anal. ($C_{25}H_{30}N_2O_2$) C, H, N.

Further elution gave a mixed fraction (1.00 g, 14%) followed by **11c** (1.00 g, 14%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.10–1.35 (8 H, m), 1.55 (2 H, quintet, J = 7 Hz), 1.74 (2 H, quintet, J = 7

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Hz), 2.25 (2, H, t, J = 7.5 Hz, $CH_2CO_2CH_3$), 3.63 (3 H, s), 3.99 (2 H, t, J = 7 Hz, NCH_2), 7.00–7.55 (10 H, m), 7.75 (1 H, s, pyrazole ring H); MS m/z 391 (MH^+). Anal. ($C_{25}H_{30}N_2O_2$) C, H, N.

3,4-Diphenyl-1*H*-pyrazole-1-nonanoic Acid (12c). Hydrolysis of **10c** (3.00 g, 7.7 mmol), as described for **8d**, gave **12c** (2.23 g, 77%): mp 83–85 °C; ^1H NMR ($CDCl_3$) δ 1.20–1.50 (8 H, m), 1.61 (2 H, quintet, J = 7 Hz), 1.92 (2 H, quintet, J = 7 Hz), 2.31 (2 H, t, J = 7.5 Hz, CH_2CO_2H), 4.15 (2 H, t, J = 7 Hz, NCH_2), 7.15–7.30 (8 H, m), 7.40 (1 H, s, pyrazole ring H), 7.40–7.60 (2 H, m); MS m/z 377 (MH^+). Anal. ($C_{24}H_{28}N_2O_2 \cdot 0.2H_2O$) C, H, N.

4,5-Diphenyl-1*H*-pyrazole-1-nonanoic Acid (13c). Hydrolysis of **11c** (850 mg, 2 mmol) gave **13c** (800 mg, 97%) as an oil after chromatography on silica using Et_2O as eluent: ^1H NMR ($CDCl_3$) δ 1.20–1.40 (8 H, m), 1.59 (2 H, quintet, J = 7 Hz), 1.74 (2 H, quintet, J = 7 Hz), 2.31 (2 H, t, J = 7.5 Hz, CH_2CO_2H), 4.00 (2 H, t, J = 7 Hz, NCH_2), 7.00–7.60 (10 H, m), 7.79 (1 H, s, pyrazole ring H); MS m/z 377 (MH^+). Anal. ($C_{24}H_{28}N_2O_2$) C, H, N.

3,4,5-Triphenyl-1*H*-pyrazole-1-hexanol (14). Ethyl 3,4,5-triphenyl-1*H*-pyrazole-1-hexanoate (**7a**) (9.00 g, 20 mmol) in Et_2O (50 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (780 mg, 20 mmol) in Et_2O (200 mL). After 15 min, water was added dropwise until the salts coagulated. The ethereal layer was decanted, the residue washed with Et_2O (2×), and the organic phase dried over Na_2SO_4. Evaporation of the solvent left **14** (8.13 g, 100%). An analytical sample recrystallized from Et_2O/hexane had mp 76–78 °C: ^1H NMR ($CDCl_3$) δ 1.25 (4 H, m), 1.46 (2 H, quintet, J = 6 Hz), 1.60 (1 H, t, J = 5 Hz, OH), 1.85 (2 H, m), 3.54 (2 H, q, J = 5 Hz, CH_2OH), 4.07 (2 H, t, J = 7 Hz, NCH_2), 7.00–7.50 (15 H, m); MS m/z 397 (MH^+). Anal. ($C_{27}H_{28}N_2O$) C, H, N.

1,1-Dimethylethyl [[6-(3,4,5-Triphenyl-1*H*-pyrazol-1-yl)-hexyl]oxy]acetate (15). A mixture of **14** (5.00 g, 12 mmol), *tert*-butyl bromoacetate (4.92 g, 4.10 mL, 25 mmol), nBu_4NHSO_4 (0.4 g), 50% aqueous NaOH solution (80 mL), and toluene (80 mL) was stirred vigorously at room temperature. After 18 h, the organic phase was separated, the aqueous layer was extracted twice with Et_2O, and the combined extracts were dried over Na_2SO_4 and concentrated. Chromatography on silica (hexane/Et_2O 2:1) afforded **15** (5.84 g, 95%) that slowly crystallized to a white solid: mp 69–72 °C; ^1H NMR ($CDCl_3$) δ 1.24 (4 H, m), 1.46 (9 H, s, $C(CH_3)_3$), 1.53 (2 H, m), 1.88 (2 H, m), 3.44 (2 H, t, J = 6.5 Hz, OCH_2), 3.91 (2 H, s, OCH_2CO_2-tBu), 4.07 (2 H, t, J = 7 Hz, NCH_2), 6.90–7.50 (15 H, m); MS m/z 511 (MH^+). Anal. ($C_{33}H_{38}N_2O_3$) C, H, N.

[[6-(3,4,5-Triphenyl-1*H*-pyrazol-1-yl)hexyl]oxy]acetic Acid (16). A solution of **15** (4.30 g, 8 mmol) in CF_3CO_2H (25 mL) was stirred at room temperature for 40 min before being concentrated. The residue was dissolved in Et_2O and diluted with hexane to furnish **16** (3.20 g, 83%): mp 92–94 °C; ^1H NMR ($CDCl_3$) δ 1.12 (4 H, m), 1.55 (2 H, quintet, J = 7 Hz), 1.83 (2 H, quintet, J = 7 Hz), 3.47 (2 H, t, J = 6.5 Hz, OCH_2), 4.04 (2 H, s, OCH_2CO_2H), 4.10 (2 H, t, J = 7 Hz, NCH_2), 6.95–7.55 (15 H, m), 8.93 (1 H, bs, CO_2H); MS m/z 455 (MH^+). Anal. ($C_{29}H_{30}N_2O_3$) C, H, N.

Methyl [[6-(3,4,5-Triphenyl-1*H*-pyrazol-1-yl)hexyl]-thio]acetate (17). Br_2 (3.44 g, 21 mmol) was added dropwise to a stirred solution of Ph_3P (5.65 g, 21 mmol) in dry DMF. After 0.5 h, **14** (7.11 g, 18 mmol) in dry DMF (45 mL) was added in one portion and the mixture stirred at room temperature for 20 min. The mixture was diluted with Et_2O (500 mL), washed with H_2O (2×) and brine (2×), dried over $MgSO_4$, and concentrated. Chromatography of the residue on a column of silica gel using hexane/EtOAc (9:1) as eluent gave 1-(6-bromohexyl)-3,4,5-triphenyl-1*H*-pyrazole (6.98 g, 84%): ^1H NMR ($CDCl_3$) δ 1.16–1.45 (4 H, m), 1.70–1.95 (4 H, m), 3.32 (2 H, t, J = 7 Hz, CH_2Br), 4.06 (2 H, t, J = 7 Hz, NCH_2), 6.95–7.55 (15 H, m); MS m/z 459, 461 (MH^+). A mixture of the bromide (6.53 g, 14 mmol), methyl mercaptoacetate (1.66 g, 15 mmol), K_2CO_3 (2.26 g, 16.5 mmol), KI (catalyst quantity), and CH_3CN (150 mL) was heated at reflux for 4 h. The mixture was filtered and concentrated, and the residue chromatographed on silica gel (hexane/EtOAc 4:1 as eluent) to give **17** (6.47 g, 91%) as an oil: ^1H NMR ($CDCl_3$) δ 1.28 (4 H, m), 1.53 (2 H, quintet, J = 7.5 Hz), 1.84 (2 H, quintet, J = 7.5 Hz), 2.55 (2 H, t, J = 7 Hz, CH_2S), 3.17 (2 H, s, $SCH_2CO_2CH_3$), 3.70 (3 H, s, CO_2CH_3), 4.06 (2 H, t, J = 7.5 Hz, NCH_2), 7.00–7.50 (15 H, m); MS m/z 485 (MH^+). Anal. ($C_{30}H_{32}N_2O_2S$) C, H, N.

[[6-(3,4,5-Triphenyl-1*H*-pyrazol-1-yl)hexyl]thio]acetic Acid (18a). A mixture of **17** (1.01 g, 2 mmol), 3 N NaOH (2.1 mL, 6 mmol), and MeOH (125 mL) was heated at reflux for 20 min. The solvent was removed, and the residue diluted with 1 N HCl solution and extracted with CH_2Cl_2. The extracts were dried (Na_2SO_4) and concentrated to leave **18a** (0.94 g, 96%): mp 92–97 °C; ^1H NMR ($CDCl_3$) δ 1.20–1.50 (4 H, m), 1.59 (2 H, quintet, J = 7 Hz), 1.83 (2 H, quintet, J = 7 Hz), 2.61 (2 H, t, J = 7 Hz, CH_2S), 3.18 (2 H, s, CH_2CO_2H), 4.10 (2 H, t, J = 7.5 Hz, NCH_2), 6.95–7.50 (15 H, m), 9.72 (1 H, bs, CO_2H); MS m/z 471 (MH^+). Anal. ($C_{29}H_{30}N_2O_2S$) C, H, N.

[[6-(3,4,5-Triphenyl-1*H*-pyrazol-1-yl)hexyl]sulfinyl]acetic Acid (18b). Oxone (3.30 g, 5 mmol) was added in one portion to a stirred mixture of **17** (2.08 g, 4.3 mmol) in MeOH (100 mL) and H_2O (50 mL) maintained at −10 °C. After 45 min, the mixture was diluted with H_2O and extracted with $CHCl_3$. Chromatography of the residue on silica (EtOAc/hexane 7:3 as eluent) gave the sulfoxide ester (1.84 g, 85%), mp 70.5–71.5 °C [Anal. ($C_{30}H_{32}N_2O_3S \cdot 0.1H_2O$) C, H, N], of which 1.06 g (2.1 mmol) was heated at reflux with 3 N NaOH (2.1 mL, 6.3 mmol) and MeOH (50 mL) for 10 min. The mixture was concentrated, made pH = 1 with 1 N HCl, and extracted with CH_2Cl_2 to give **18b** (0.98 g, 95%) as a white foam: mp 132.5–134.5 °C; ^1H NMR ($CDCl_3$) δ 1.25–1.40 (4 H, m), 1.65–1.85 (4 H, m), 2.75–2.95 (2 H, m, $CH_2S(O)$), 3.68 (2 H, s, CH_2CO_2H), 4.10 (2 H, t, J = 7.5 Hz, NCH_2), 6.75–7.50 (15 H, m), 9.82 (1 H, bs, CO_2H); MS m/z 443 ($MH^+ - CO_2H$). Anal. ($C_{29}H_{30}N_2O_3S \cdot 0.1H_2O$) C, H, N.

[[6-(3,4,5-Triphenyl-1*H*-pyrazol-1-yl)hexyl]sulfonyl]acetic Acid (18c). Oxone (3.80 g, 6 mmol) suspended in water (20 mL) was added slowly to a solution of **17** (1.00 g, 2 mmol) in MeOH (20 mL) maintained at 0 °C. The mixture was warmed to room temperature and stirred for 5.5 h before being diluted with H_2O and extracted with Et_2O. The Et_2O layer was dried (Na_2SO_4) and concentrated and the residue chromatographed on silica gel (hexane/CH_2Cl_2 25:1 as eluent) to give the sulfonyl ester (1.17 g, 91%), mp 92.5–94.5 °C [Anal. ($C_{30}H_{32}N_2O_4S$) C, H, N], of which 0.94 g (2 mmol) was heated at reflux with 3 N NaOH (2.43 mL, 7 mmol) and MeOH (100 mL) for 20 min. The solvent was removed, and the residue diluted with 1 N HCl and extracted with CH_2Cl_2 to give a foam. Recrystallization from CH_2Cl_2/hexane furnished **18c** (0.80 g, 88%): mp 153.5–155 °C; ^1H NMR ($CDCl_3$) δ 1.20–1.40 (4 H, m), 1.65–1.85 (4 H, m), 3.14 (2 H, t, J = 7 Hz, CH_2SO_2), 3.25 (2 H, m, CH_2CO_2H), 4.02 (2 H, t, J = 7.5 Hz, NCH_2), 6.90–7.40 (15 H, m); MS m/z 459 (MH^+). Anal. ($C_{29}H_{30}N_2O_4S$) C, H, N.

3,4,5-Triphenyl-1*H*-pyrazole-1-nonanamide (19a). Oxalyl chloride (0.42 g, 0.29 mL, 3.3 mmol) was added dropwise to a solution of **8d** and a catalytic amount of DMF in dry THF (15 mL) maintained at 0 °C under N_2. After 30 min, the mixture was warmed to room temperature, stirred 30 min, and concentrated to leave a yellow solid which was dissolved in dry THF. Concentrated NH_4OH solution (specific gravity = 0.90, 2 mL) was added and the mixture stirred for 20 min before being poured onto H_2O and extracted with CH_2Cl_2. The residual solid was recrystallized from CH_2Cl_2/hexane to give **19a** (0.80 g, 80%): mp 104–107 °C; ^1H NMR ($CDCl_3$) δ 1.00–1.20 (8 H, bs), 1.57 (2 H, quintet, J = 7 Hz), 1.83 (2 H, q, J = 7 Hz), 2.15 (2 H, t, J = 7 Hz, CH_2CONH_2), 4.05 (2 H, t, J = 7 Hz, NCH_2), 5.52–5.72 (2 H, bs, NH_2), 6.90–7.70 (15 H, m); MS m/z 452 (MH^+). Anal. ($C_{30}H_{33}N_3O$) C, H, N.

***N*-(Methylsulfonyl)-3,4,5-triphenyl-1*H*-pyrazole-1-nonanamide (19c).** A mixture of 1,1′-carbonyldiimidazole (0.39 g, 2.4 mmol) and **8d** (1.0 g, 2.2 mmol) in dry THF (10 mL) was stirred at room temperature under N_2 for 0.5 h and at reflux for 0.5 h. After cooling, methanesulfonamide (0.21 g, 2.2 mmol) was added followed, after 10 min by DBU (0.336 g, 0.34 mL, 2.2 mmol). The mixture was stirred for 16 h, poured onto 2 N HCl solution, and extracted with CH_2Cl_2. The residue was chromatographed on silica gel (Et_2O/hexane 4:1 as eluent) to give **19c** (1.00 g, 80%): mp 88–90 °C; ^1H NMR ($CDCl_3$) δ 1.22 (8 H, m), 1.54 (2 H, quintet, J = 7 Hz), 1.84 (2 H, quintet, J = 7 Hz), 2.18 (2 H, t, J = 7.5 Hz, CH_2CO), 3.21 (3 H, s, SO_2CH_3), 4.08 (2 H, t, J = 7.5 Hz, NCH_2), 6.90–7.60 (15 H, m), 9.64 (1 H, bs, $NHSO_2$); MS m/z 530 (MH^+). Anal. ($C_{31}H_{35}N_3O_3S$) C, H, N.

3,4,5-Triphenyl-1*H*-pyrazole-1-nonanenitrile (21). A mixture of **20** (2.70 g, 5.5 mmol), KCN (0.40 g, 6.1 mmol), and

DMF (30 mL) was stirred at 70 °C under N_2 for 58 h before being diluted with H_2O and extracted with CH_2Cl_2. The extracts were washed with H_2O, dried (Na_2SO_4), and concentrated to leave an oil. Chromatography on silica gel (EtOAc/hexane/Et_3N 20:79:1 as eluent) gave **21** (1.27 g, 52%): mp 79.5–80.5 (CH_2Cl/hexane); ^1H NMR ($CDCl_3$) δ 1.24 (8 H, bs), 1.60 (2 H, quintet, J = 7 Hz, CH_2CN), 4.07 (2 H, t, J = 7 Hz, NCH_2), 6.95–7.60 (15 H, m); MS m/z 434 (MH^+). Anal. ($C_{30}H_{31}N_3{\cdot}0.5H_2O$) C, H, N.

5-[8-(3,4,5-Triphenyl-1*H*-pyrazol-1-yl)octyl]-2*H*-tetrazole (22). A mixture of **21** (1.25 g, 2.9 mmol) and $(nBu)_3SnN_3$ (1.15 g, 3.5 mmol) was stirred at 140 °C under N_2. After 2.5 h, the mixture was cooled, diluted with EtOAc, and washed with 0.5 N HCl (3×) and NaCl solutions. The solvent was evaporated, the residue dissolved in CH_2Cl_2, and a concentrated aqueous solution of KF added. The mixture was stirred for 24 h and extracted with CH_2Cl_2, and the residue recrystallized from hexane/CH_2Cl_2 (2:1) to give **22** (1.00 g, 73%): mp 158–160 °C; ^1H NMR ($CDCl_3$) δ 1.14 (8 H, m), 1.60 (2 H, t, J = 7 Hz), 1.79 (2 H, t, J = 7 Hz), 2.74 (2 H, t, J = 7 Hz, CH_2-tetrazole), 4.10 (2 H, t, J = 7 Hz, NCH_2), 6.90–7.50 (15 H, m); MS m/z 477 (MH^+). Anal. ($C_{30}H_{32}N_6$) C, H, N.

Blood Platelet Aggregometry. Platelet-rich plasma was prepared from human blood drawn into syringes containing $^1/_{10}$ volume of 3.8% sodium citrate. The blood was then subjected to centrifugation for 10 min at 140*g* and the platelet-rich plasma decanted. The test compound was dissolved in DMSO (5 μL) and added to PRP (0.9 mL) 3 min prior to the addition of ADP (5.86 μM). The aggregometer method of Born,[53] as modified by Mustard et al.,[54] was employed to measure platelet aggregation. Vehicle control trials were performed and compared with the extent of aggregation induced in PRP containing various concentrations of the test compounds. Dose–response curves were thus obtained and IC_{50} values determined. The data presented in Table I are the results of single determinations or the average of duplicates. Rabbit and rat PRP were prepared in a similar fashion,[55] and ADP in a final concentration of 29.3 μM was employed as the agonist.

Laser-Induced Thrombosis in Rabbits. This model, which has been described in detail[44,55] uses a ruby-laser flash to induce a small thrombus in the microcirculation of the ear of an English lop-ear rabbit. The mean thrombus area (μM^2) obtained for 10 trials in each rabbit served as a control value. The test compound was administered orally as a suspension in water and Tween 20, and the experiment repeated 2 h later. Drug efficacy was determined from a comparison of pre- and postdose mean thrombus areas. The results presented are an average of experiments conducted in five rabbits. BMY 42239 (**8d**) provided 55 ± 3% inhibition at a dose of 10 mg/kg po and octimibate (**5**) provided 39 ± 3% inhibition at a dose of 30 mg/kg po.

Radioligand Binding Studies. Radioligand binding assays were performed in 200-μL volumes containing 200 μg of platelet plasma membranes. The isolated membranes were added to a buffer composed of 10 mM $MgCl_2$, 1 mM EGTA, and 50 mM Tris/HCl (pH 7.4) with either 5 nM [^{3}H]iloprost or 5 nM [^{3}H]-PGD_2. The membranes were incubated at 0–4 °C for 90–120 min. After incubation, 5 mL of ice-cold 50 mM Tris/HCl (pH 7.4) was added, the tubes were vortexed, and the samples were rapidly filtered through presoaked Whatman GF/C filters. The filters were then washed four times with 5 mL of ice-cold 50 mM Tris/HCl (pH 7.4), blotted dry on absorbent paper, and counted in a scintillation counter. The specific binding was greater than 90% for [^{3}H]iloprost and 60% for [^{3}H]PGD_2 as determined using excess (10 μM iloprost and 100 μM PGD_2) cold ligand.

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Supplementary Material Available: A graph of the effect of **8d**, SQ 27986, and unlabeled PGD_2 on [^{3}H]PGD_2 binding to isolated platelet membranes (1 page). Ordering information is given on any current masthead page.

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Synthesis and Biologic Activity of 2′-Fluoro-2-halo Derivatives of 9-β-D-Arabinofuranosyladenine[1]

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The synthesis of 2-halo-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenines (**4b** and **4d**) by coupling the 2,6-dihalopurine with 3-acetyl-5-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide (**2**) followed by replacement of the 6-halogen with concomitant removal of the acyl blocking groups is described. 2-Fluoroadenine derivative **4g** had to be prepared by the diazotization–fluorination of 2-aminoadenine nucleoside **4e**. All three nucleosides provided good increases in life span of mice inoculated with P388 leukemia. The best results were obtained when the compounds were administered q3h×8 on days 1, 5, and 9 after implantation of the leukemia cells. The 2′,3′-dideoxynucleoside **5b**, prepared by deacetylation of **4f** and deoxygenation of the resultant **4h** followed by removal of the benzoyl group of **5a**, was slightly active against HIV in cell culture.

Fludarabine phosphate (9-β-D-arabinofuranosyl-2-fluoroadenine 5′-*O*-phosphate, F-*ara*-AMP, **1**) has shown activity in a number of human cancers in Phase I and II clinical trials.[3] It has group C status at the present time

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