Biphenylcarboxamide Derivatives as Antagonists of Platelet-Activating Factor

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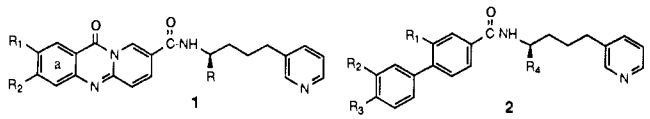
A series of *N*-[4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamides was prepared, and the compounds were evaluated for platelet-activating factor (PAF) antagonist activity in a binding assay employing washed, whole dog platelets and in vivo for their ability to inhibit PAF-induced bronchoconstriction in the guinea pig. The inclusion of a methyl group in the *R* configuration on the side-chain carbon adjacent to the carboxamide nitrogen atom of these derivatives resulted in a marked enhancement of potency in the binding assay for compounds unsubstituted in the biphenyl 2-position and, more importantly, in improved oral bioavailability. Previous work with related pyrido[2,1-*b*]-quinazoline-8-carboxamides suggests that the presence of such an alkyl group improves bioavailability by rendering the resulting compounds resistant to degradation by liver amidases. The most interesting compounds to emerge from this work are (*R*)-2-bromo-3′,4′-dimethoxy-*N*-[1-methyl-4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (**33**) and (*R*)-2-butyl-3′,4′-dimethoxy-*N*-[1-methyl-4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (**40**) each of which inhibits PAF-induced bronchoconstriction in the guinea pig by >55%, 6 h after an oral dose of 50 mg/kg.

Platelet-activating factor (PAF) is an ether phospholipid which has been receiving increasing attention lately as a potential mediator of asthma[1-4] and inflammation.[5] An intensive effort to find drugs which attenuate the effects of PAF using a variety of in vitro screening techniques has resulted in the discovery of a number of specific PAF antagonists,[6-9] some of which are currently undergoing clinical trial. In our own work, we have employed a binding assay using washed, whole dog platelets to find new lead compounds.[10,11]

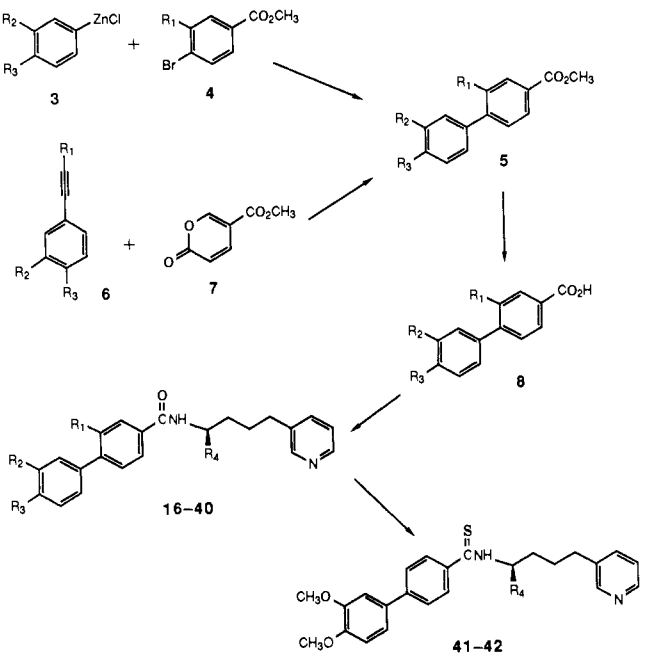
Recently pyridoquinazolinecarboxamides of the general structure **1** have been shown to be relatively potent in-



hibitors of platelet-activating factor binding to dog platelets and to exhibit PAF-antagonist activity in vivo.[12] Since the *N*-[4-(3-pyridinyl)butyl]carbamoyl moiety was found to be a key feature of these molecules, we have chosen to focus our search for new PAF antagonists on variations of the tricyclic portion. Preliminary experiments in which the pyridoquinazoline ring system was partially reduced or replaced with a monocyclic aromatic ring suggested that the ring marked "a" in **1** contributes importantly to binding, particularly when conjugated to the carboxamide. We thus hypothesized that the acceptor region of the PAF receptor comprises at minimum, a hydrophobic area which associates with the aromatic ring marked "a", a hydrogen-bond donor which interacts with the amide of **1**, and a π donor which interacts with the electron-deficient pyridine ring. Studies with Dreiding models indicated that the 1,1′-biphenyl-4-carboxamide derivatives **2** fulfill the requirements of this model as they possess a suitably located aromatic ring connected through a conjugated π system to a substituted carboxamido group and thus might represent a new class of PAF antagonists.

In this paper, we report the synthesis of a number of biphenylcarboxamide derivatives of general structure **2** and their evaluation as PAF antagonists. Several of these compounds, particularly those in which R_1 is hydrogen or lower alkyl, R_2 and R_3 are methoxy, and R_4 is methyl are potent inhibitors of PAF binding to dog platelets and effectively block PAF-induced bronchospasm in the guinea pig after intravenous or oral administration.

Scheme I



Chemistry

The carboxamides **16-40** shown in Table I were generally prepared by coupling of a suitably activated biphenylcarboxylic acid derivative **8** with the appropriate 3-pyridinylbutanamines.[12,13] Activation was achieved

(1) Morley, J. *Agents Actions* **1986**, *19*, 100.
(2) Page, C. P. *J. Allergy Clin. Immunol.* **1988**, *81*, 144.
(3) Barnes, P. J. . *Allergy Clin. Immunol.* **1988**, *81*, 152.
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(5) Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. *Pharmacol. Rev.* **1987**, *39*, 97.
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(7) Godfroid, J. J.; Braquet, P. *Trends Pharmacol. Sci.* **1986**, *7*, 397.
(8) Saunders, R. N.; Handley, D. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 237.
(9) Casals-Stenzel, J.; Muacevic, G.; Weber, K.-H. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 974.
(10) Janero, D. R.; Burghardt, B.; Burghardt, C. *Thombosis Res.* **1988**, *50*, 789.
(11) Janero, D. R.; Burghardt, B.; Burghardt, C. *J. Pharmacol. Methods* **1988**, *20*, 237.
(12) Tilley, J. W.; Burghardt, B.; Burghardt, C. Mowles, T. F.; Leinweber, F.-J.; Klevans, L.; Young, R.; Hirkaler, G.; Fahrenholtz, K.; Zawoiski, S.; Todaro, L. *J. Med. Chem.* **1988**, *31*, 466.

† Chemistry Research Department.
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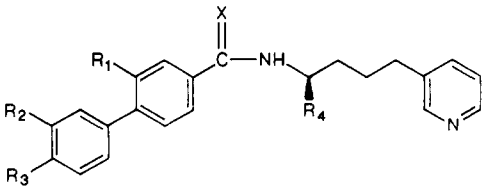
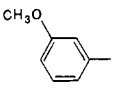
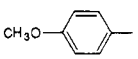
Table I. Data for *N*-[4-(3-Pyridinyl)butyl]-1,1′-biphenyl-4-carboxamides

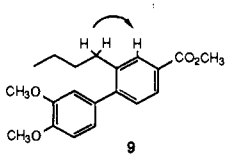


no.	R_1	R_2	R_3	R_4	X	method	% yield	mp, °C	solvent	$[\alpha]_D$[a]	formula	anal.	inhibn of PAF binding:[b] IC_{50}, nM
16	H	H	H	H	O	A	61	158–160	iPrOH–Et_2O		$C_{22}H_{22}N_2O\cdot HCl$	C, H, N, Cl	630
17	F	F	H	H	O	B	31	161–162	EtOH–Et_2O		$C_{22}H_{21}FN_2O\cdot HCl$	C, H, N, Cl	2100
18	H	CH_3	H	H	O	B	44	137–138	EtOH–Et_2O		$C_{23}H_{24}N_2O\cdot HCl$	H, N, Cl; C[c]	500
19	H	CH_3O	H	H	O	A	89	113–114	EtOH–Et_2O		$C_{23}H_{24}N_2O_2\cdot HCl$	C, H, N, Cl	280
20	H	CH_3O	H	CH_3	O	C	79	89–90	EtOAc–hex.	−44.18	$C_{24}H_{26}N_2O_2$	C, H, N	20
21	H	C_7H_7O	H	H	O	B	69	oil			$C_{29}H_{28}N_2O_2\cdot 0.5H_2O$	C, H, N	700
22	H	OH	H	H	O	D	58	151.5–152.5	CH_3CN		$C_{22}H_{22}N_2O_2$	C, H, N	300
23	H	CH_3	CH_3	H	O	C	65	95–98	EtOAc–hex.		$C_{24}H_{26}N_2O$	C, H, N	200
24	H	CH_3O	CH_3O	H	O	B	34	138–139.5	EtOH		$C_{24}H_{26}N_2O_3$	C, H, N	400
25	H	CH_3O	CH_3O	CH_3	O	C	62	156–159	EtOAc	−45.97	$C_{25}H_{28}N_2O_3$	C, H, N	15
26		H	H	H	O	C	93	foam			$C_{28}H_{26}N_2O\cdot 0.25H_2O$	C, H, N, H_2O	5
27		H	H	CH_3	O	C	77	75–77	Et_2O–hex.	−39.71	$C_{29}H_{28}N_2O$	C, H, N	50
28		CH_3O	H	H	O	C	96	oil			$C_{30}H_{30}N_2O_3\cdot 0.2H_2O$	C, H, N, H_2O	200
29		H	CH_3O	H	O	C	91	oil			$C_{30}H_{30}N_2O_3\cdot 0.25H_2O$	C, H, N, H_2O	125
30	NO_2	CH_3O	CH_3O	H	O	C	83	134–136	EtOAc		$C_{24}H_{25}N_3O_5$	C, H, N	200
31	NO_2	CH_3O	CH_3O	CH_3	O	C	87	144–145	EtOAc	−34.79	$C_{25}H_{27}N_3O_5$	C, H, N	200
32	Br	CH_3O	CH_3O	H	O	C	86	98–100	EtOAc–hex.		$C_{24}H_{25}BrN_2O_3$	C, H, N, Br	220
33	Br	CH_3O	CH_3O	CH_3	O	C	74	125–127	EtOAc–hex.	−33.48	$C_{25}H_{27}BrN_2O_3$	C, H, N, Br	180
34	CH_3O	CH_3O	CH_3O	H	O	C	87	119–121	EtOAc		$C_{25}H_{28}N_2O_4$	C, H, N	300
35	HC≡C	CH_3O	CH_3O	CH_3	O	C	83	135–137	EtOAc–hex.	−44.15	$C_{27}H_{26}N_2O_3$	C, H, N[d]	5
36	C_2H_5	CH_3O	CH_3O	CH_3	O	E	87	oil		−32.05	$C_{27}H_{32}N_2O_3$	C, H, N	4
37		CH_3O	CH_3O	CH_3	O	C	93	oil		−33.62	$C_{28}H_{32}N_2O_3$	C, H, N	80
38	*n*-C_3H_7	CH_3O	CH_3O	CH_3	O	E	95	oil		−35.05	$C_{28}H_{34}N_2O_3$	C, H, N	50
39	*n*-C_4H_9	CH_3O	CH_3O	H	O	C	87	oil			$C_{28}H_{34}N_2O_3\cdot 0.4H_2O$	C, H, N	18
40	*n*-C_4H_9	CH_3O	CH_3O	CH_3	O	C	37	84–86	Et_2O	−38.66	$C_{29}H_{36}N_2O_3$	C, H, N	4
41	H	CH_3O	CH_3O	H	S	F	78	136–138	MeOH		$C_{24}H_{26}N_2O_2S$	C, H, N, S	140
42	H	CH_3O	CH_3O	CH_3[e]	S	F	80	oil			$C_{25}H_{28}N_2O_2S$	C, H, N, S[f]	60

[a] Rotations were determined in a 1% ethanol solution at 25 °C. [b] IC_{50} values were determined by linear-regression analysis; the correlation coefficient for each regression line was >0.95. [c] C: calcd, 80.20; found, 79.23. [d] C: calcd, 75.68; found, 75.01. [e] Racemic. [f] C: calcd, 71.40; found, 71.87.

either via the acid chlorides (methods A and B) or by the use of diphenyl phosphorazidate (method C). The hydroxyl-substituted compound **22** was prepared by catalytic hydrogenation of the corresponding benzyl ether **21** over palladium on carbon (method D). Saturation of the side chains of **35** and **37** to give **36** and **38**, respectively, was also achieved by catalytic hydrogenation (method E). The thioamides **41** and **42** were obtained from the corresponding amides through the action of phosphorus pentasulfide in pyridine (method F).

The new biphenyl esters **5** required for this work were generally prepared by the palladium-catalyzed coupling of the arylzinc chlorides **3** with the 4-bromobenzoates **4** (method G).[14] For the biphenyl derivatives in which R_1 was aryl or butyl, a Diels–Alder reaction[15] between the acetylenes **6** and methyl coumalate (**7**) (method H) was employed as indicated in Scheme I. When R_1 was butyl, the regiochemistry of the major product, **9**, was determined



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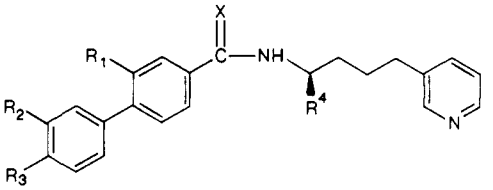
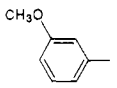
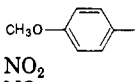
(15) Reed, J. A.; Schilling, C. L., Tarvin, R. F., Rettig, T. A., Stille, J. K. *J. Org. Chem.* **1969**, *34*, 2188.

Table II. PAF-Antagonist Activity of *N*-[4-(3-Pyridinyl)butyl]-1,1′-biphenyl-4-carboxamides



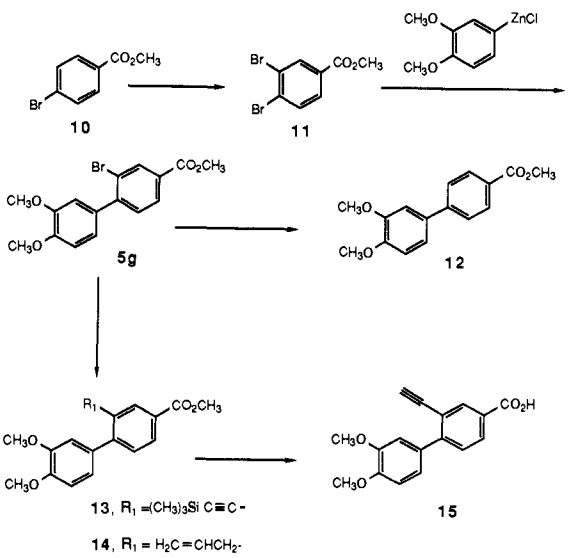
						guinea pig bronchoconstriction assay				
								% inhibn, 50 mg/kg, po		
no.	R_1	R_2	R_3	R_4	X	% inhibn, 1 mg/kg, iv[a]	ID_{50}, mg/kg, iv[a,b]	2 h	6 h	ID_{50}, mg/kg, po[b,c]
16	H	H	H	H	O	42 ± 7				
18	H	CH_3	H	H	O	0 ± 11				
19	H	CH_3O	H	H	O	70 ± 10	0.51	55 ± 17		50
20	H	CH_3O	H	CH_3	O	74 ± 6	0.45	44 ± 13		
22	H	OH	H	H	O	7 ± 7				
23	H	CH_3	CH_3	H	O	0 ± 4				
24	H	CH_3O	CH_3O	H	O	8 ± 3				
25	H	CH_3O	CH_3O	CH_3	O	65 ± 9	0.62	88 ± 5	29 ± 11	30
26		H	H	H	O	21 ± 4	3.2	33 ± 12		
27		H	H	CH_3	O	31 ± 4				
28		CH_3O	H	H	O	12 ± 6				
29		H	CH_3O	H	O	26 ± 6				
30	NO_2	CH_3O	CH_3O	H	O	81 ± 15	0.76	62 ± 15	4 ± 4	39
31	NO_2	CH_3O	CH_3O	CH_3	O	97 ± 0.7	0.19	89 ± 8	36 ± 14	22
32	Br	CH_3O	CH_3O	H	O	81 ± 10	0.40	74 ± 12	1 ± 2	19
33	Br	CH_3O	CH_3O	CH_3	O	98 ± 0.7	0.40	97 ± 1	59 ± 21	14
34	CH_3O	CH_3O	CH_3O	H	O	58 ± 10	0.52	64 ± 12	13 ± 9	
35	$HC\equiv C$	CH_3O	CH_3O	CH_3	O	22 ± 10				
36	C_2H_5	CH_3O	CH_3O	CH_3	O	93 ± 1	0.34	89 ± 5	23 ± 7	24
37		CH_3O	CH_3O	CH_3	O	80 ± 11	0.33	93 ± 3	26 ± 7	24
38	nC_3H_7	CH_3O	CH_3O	CH_3	O	28 ± 12				
39	nC_4H_9	CH_3O	CH_3O	H	O	6 ± 4				
40	nC_4H_9	CH_3O	CH_3O	CH_3	O	55 ± 12	0.80	91 ± 1	55 ± 18	25
41	H	CH_3O	CH_3O	H	S	8 ± 11				
42	H	CH_3O	CH_3O	CH_3	S	86 ± 7	0.31	37 ± 9		

[a] One-minute pretreatment time. [b] ID_{50} values were determined by linear-regression analysis; the correlation coefficient for each regression line was >0.95. [c] Two-hour pretreatment time.

by the observation of an NOE between the methylene protons and the aromatic proton indicated by the arrow in the structure. Base-catalyzed hydrolysis of the esters **5** gave the acids **8** used in the coupling reactions (method H).

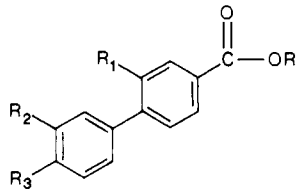
Methyl 3,4-dibromobenzoate (**11**) was obtained by the bromination of methyl 4-bromobenzoate (**10**) with *N*-bromosuccinimide in sulfuric acid. When the palladium-catalyzed coupling reaction was carried out with **11** and the arylzinc derived from 3,4-dimethoxybromobenzene, the reaction proceeded regioselectively to give the 2-bromo-1,1′-biphenyl derivative **5f** in 53% yield. The identity of the product was confirmed by reductive debromination to **12**, which we had previously prepared directly from **10** (Scheme II). Palladium-catalyzed coupling of **5f** with (trimethylsilyl)acetylene and allyltributyltin led to the analogues **13** and **14**, respectively. Hydrolysis of **13** with sodium hydroxide served to cleave both the silyl and methyl ester groups to give the corresponding acid **15**. Physical chemical data for the new biphenyl esters and acids prepared by the above methods are summarized in Table III.

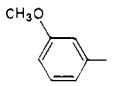
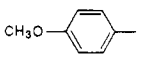
Scheme II



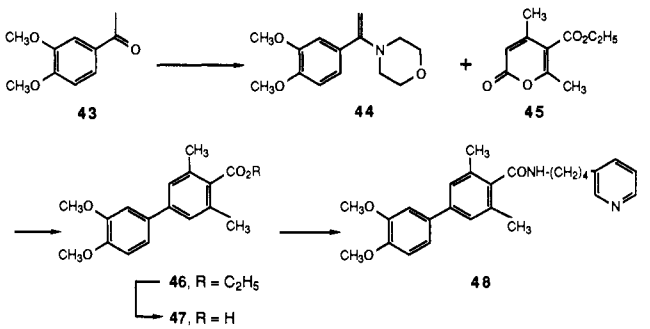
The 3,5-dimethylbiphenyl-4-carboxylic acid **47** was prepared as shown in Scheme III, employing as a key step an inverse electron demand Diels–Alder reaction between the enamine **44** and ethyl isodehydracetate (**45**).[16] Con-

Table III. Data for 1,1-Biphenyl-4-carboxylates



no.	R_1	R_2	R_3	R	method	% yield	mp, °C	solvent	formula	anal.
5a	H	C_7H_7O	H	CH_3	G	42	105–106	EtOAc	$C_{21}H_{18}O_3$	C, H
5c	H	CH_3O	CH_3O	CH_3	G	58	130–131	EtOH	$C_{16}H_{16}O_4 \cdot 0.05H_2O$	C, H, H_2O
5f	NO_2	CH_3O	CH_3O	CH_3	G	70	127–129	EtOAc–hex.	$C_{16}H_{15}NO_6$	C H, N
5g	Br	CH_3O	CH_3O	CH_3	G	53	101–102	EtOAc–hex.	$C_{16}H_{15}BrO_4$	C, H, Br
5h	CH_3O	CH_3O	CH_3O	CH_3	G	47	88–89	EtOAc–hex.	$C_{17}H_{18}O_5$	C, H
5i	n-C_4H_9	CH_3O	CH_3O	CH_3	H	63	oil		$C_{20}H_{24}O$	C, H
8a	H	C_7H_7O	H	H	I	52	185–186	EtOH	$C_{20}H_{16}O_3$	C, H
8b	H	CH_3	CH_3	H	I	50	208–209	EtOAc	$C_{15}H_{14}O_2$	C, H
8c	H	CH_3O	CH_3O	H	I	55	221–222	EtOAc	$C_{15}H_{14}O_4$	C, H
8d		CH_3O	H	H	H, I	89	120–123	EtOAc–hex.	$C_{21}H_{18}O_4$	C, H
8e		H	CH_3O	H	H, I	65	191–193	EtOAc–hex.	$C_{21}H_{18}O_4$	C, H
8f	NO_2	CH_3O	CH_3O	H	I	65	260–263	HOAc–EtOAc	$C_{15}H_{13}NO_6$	C, H, N
8g	Br	CH_3O	CH_3O	H	I	79	199–201	CH_2Cl_2–hex.	$C_{15}H_{13}BrO_4$	C, H, Br
8h	CH_3O	CH_3O	CH_3O	H	I	90	193–194	MeOH–EtOAc–H_2O	$C_{16}H_{16}O_5$	C, H
8i	n-C_4H_9	CH_3O	CH_3O	H	I	65	122–127 dec	cyclohex	$C_{19}H_{22}O_4$	C, H

Scheme III

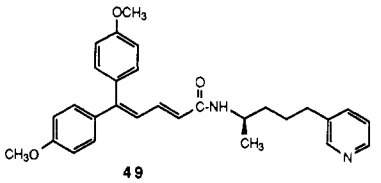


version of the hindered acid **47** to the corresponding *N*-pyridinylbutyl carboxamide **48** was achieved through the intermediacy of the acid chloride.

Results and Discussion

The compounds listed in Table I were evaluated for PAF-antagonist activity with a binding assay employing washed dog platelets as previously described;[10-12] the binding data are summarized in Table I. The initial series of compounds prepared in this work were unsubstituted in the biphenyl 2-position (R_1 = H). Among these derivatives, variation of the substituents in the 3′- and 4′-positions has only a modest effect on potency as was observed previously with pyridoquinazolinecarboxamides.[12] In the latter series, introduction of a methyl group in the R configuration on the side chain adjacent to the carboxamide nitrogen atom resulted in an increase in oral activity due to improved stability to liver amidases and a modest increase in potency in the PAF-binding assay. Surprisingly, comparison of **19** with **20**, and **24** with **25** indicate that similar incorporation of a methyl group in the biphenyl series led to a 10–20-fold increase in potency in the binding assay. A similar, although less pronounced, effect was observed for the pair of thioamide analogues **41** and **42**.

Comparison of the structures of these biphenyl derivatives with that of the 5,5-bis(4-methoxyphenyl)pentadienamide **49**, which is an orally active PAF antagonist also



being investigated in our laboratories at the time of this work,[17] suggested that hydrophobic region of the binding site would tolerate additional bulk and prompted us to prepare a number of analogues bearing a substituent in the 2-position. The first such compound, the diarylbenzamide **26**, was among the most potent members of this series in the PAF-binding assay. Surprisingly, the introduction of methoxy substituents on the aromatic rings or a methyl group on the side chain led to a marked decrease in binding affinity. We interpret this result to indicate that the extra aromatic ring has forced a change in the mode of binding such that new steric constraints are encountered. Compounds **30–34**, in which a nitro, bromo, or methoxy moiety is present in the biphenyl 2-position, were approximately equipotent to the corresponding unsubstituted analogue **24**, but they were also insensitive to the presence or absence of an alkyl group on their side chains. The high-affinity binding observed with the alkyl-substituted analogues **35–40** indicates that saturated and unsaturated alkyl groups of up to four carbon atoms in the 2-position are well tolerated. Finally, in order to investigate whether steric hindrance sufficient to prevent coplanarity between the carboxamide moiety and the aromatic system would interfere with binding, **48**, the 3,5-dimethyl analogue of **24** was synthesized and found to have an IC_{50} of 60 nM.

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(17) Guthrie, R. W.; Kaplan, G.; Mennona, F.; Tilley, J. W.; Kierstead, R. W.; Mullin, J.; Zawoiski, S.; LeMahieu, R. A.; O'Donnell, M.; Crowley, H.; Yaremko, B.; Welton, A. F. *J. Med. Chem.*, following paper in this issue.

The compounds of Table I which had binding IC_{50}s of ≤500 nM were further evaluated in guinea pigs for their ability to prevent PAF-induced bronchoconstriction. In this model, guinea pigs were administered 1 mg/kg of the drug substance 1 min prior to intravenous challenge with a maximally constrictory dose of PAF (1μg/kg) and the ability of the drug to inhibit the ensuing bronchoconstriction relative to control animals was determined. Compounds which caused a ≥50% inhibition of the response were further evaluated at multiple doses to determine an intravenous ID_{50} and were tested at a trial dose of 50 mg/kg, orally, 2 hours prior to PAF challenge. Oral ID_{50} values and the percent inhibition 6 h after a 50 mg/kg po dose were also determined for compounds which caused a ≥50% inhibition of the bronchoconstriction response in the initial oral screen.

The data in Table II reveal that of the amides and the thioamides for which R_1 is hydrogen, **20**, **25**, and **42**, each of which incorporate a side-chain methyl group and are potent in the binding assay, and **19**, which was less potent in the binding assay, all showed reasonable activity after intravenous administration. Compound **25** was also effective 2 h after oral dosing, with an oral ID_{50} of 30 mg/kg, comparable to the more potent compounds of this series. None of the diarylbenzamides was of interest based on this assay, but the analogues of **25** which bear nonaromatic substituents in the 2-position (**30-38**, **40**) had comparable activity to each other after intravenous dosing (ID_{50} 0.19–0.80 mg/kg) and 2 h after oral dosing (ID_{50} 14–39 mg/kg). Among the 2-nitro (**30** and **31**) and 2-bromo (**32** and **33**) pairs for which both the unsubstituted and methyl bearing side chains were prepared, it is apparent that the methyl group confers a slight enhancement of oral activity at the 2-h time point as reflected in lower ID_{50} values and a more marked enhancement at the 6-h time point. The influence of the side-chain methyl was even more evident in the case of the pair of 2-butyl derivatives **39** and **40** in which the linear-chain compound **39** was inactive in marked contrast to its branched-chain homologue. The two most interesting compounds to emerge from this work are the 2-bromo and 2-butyl compounds, **33** and **40**, respectively, both of which inhibit PAF-induced bronchoconstriction by >55%, 6 h after an oral dose of 50 mg/kg.

We conclude from the present work that the PAF-binding assay employing dog platelets is useful for the identification of potential PAF antagonists, but not for predicting relative potencies of compounds in the guinea pig bronchoconstriction assay. The in vitro and in vivo activity of the 2-substituted analogues implies that coplanarity and conjugation of the biphenyl aromatic rings is not essential. Inclusion of an alkyl group on the chain α to the carboxamide nitrogen atom improves bioavailability, presumably by inhibition of degradation, as predicted based on our prior work.[12] An in-depth study of the effects of various side-chain alkyl groups in a related series of pentadienyl carboxamides is presented in the accompanying manuscript.[17]

Experimental Section

Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. Proton magnetic resonance spectra were taken on a Varian XL-100, XL-200, or XL 400 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. Microanalyses were obtained for C, H, N, Br, Cl, and F and were within ±0.4% of the calculated values except as indicated. Preparative high-pressure liquid chromatography (HPLC) was performed with silica gel Prep-Pak 500 cartridges on a Water Associates Prep LC 500A. 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 vacuum using 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. The PAF used in the in vivo studies was that designated γ-*O*-alkyl-β-acetyllecithin available from Calbiochem.

3,4-Dibromobenzoic Acid Methyl Ester (11). A solution of 28.63 g (0.133 mol) of methyl 4-bromobenzoate (**10**) in 125 mL of 96% sulfuric acid was cooled in an ice bath and 24.0 g (0.135 mol) of *N*-bromosuccinimide was added in small portions over 30 min. The mixture was allowed to warm to room temperature overnight and was poured onto ice. The resulting suspension was extracted wtih ether (3 × 300 mL), and the combined organic layers were washed successively with 100-mL portions of 10% sodium thiosulfate, water, saturated sodium bicarbonate, and brine and were dried ($MgSO_4$) and concentrated. The residue was recrystallized twice from ethyl acetate–hexane to give 27.3 g (70%) of **11**, mp 61–64 °C.

2-Bromo-3′,4′-dimethoxy-1,1′-biphenyl-4-carboxylic Acid Methyl Ester (5g). A solution of 25 mL (40.7 mmol) of 1.6 M butyllithium in hexane in 25 mL of THF was cooled in a dry ice–acetone bath and 5.3 mL (8.8 g, 40.7 mmol) of 3,4-dimethoxybromobenzene was added dropwise with mechanical stirring. The reaction mixture was maintained at −78 °C for 1 h and a solution of 5.6 g (40.7 mmol) of freshly fused zinc chloride in 25 mL of THF was added in a slow stream via a cannula and the resulting mixture was allowed to warm to room temperature over 40 min. In a separate flask, 0.70 g (1.0 mmol) of bis(triphenylphopshine)palladium dichloride was suspended in 20 mL of THF and 1.5 mL (2.1 mmol) of DiBAL in hexane was added. Compound **11** (10 g, 34.0 mmol) was added to the dark mixture immediately followed by transfer of the 3,4-dimethoxyphenylzinc solution. The resulting reaction mixture was allowed to stir at room temperature for 2 h and was diluted with 300 mL of ether. The organic solution was washed with 3 × 50 mL of 1 N HCl, 2 × 50 mL of water, and 1 × 50 mL of saturated brine and was dried ($MgSO_4$). The residue obtained from evaporation was purified by preparative HPLC, with 80% hexane–ethyl acetate as eluant, to give 6.30 g (53%) of **5g**, mp 101–102 °C. Anal. ($C_{16}H_{15}BrO_4$): C, H, Br.

3′,4′-Dimethoxy-2-[(trimethylsilyl)ethynyl]-1,1′-biphenyl-4-carboxylic Acid Methyl Ester (13). A solution of 702 mg (2.0 mmol) of **5g** in 7 mL of DMF and 2 mL of triethylamine was deoxygenated with argon for 20 min and 0.50 mL (3.5 mmol) of (trimethylsilyl)acetylene followed by 70 mg (0.10 mmol) of bis(triphenylphosphine)palladium dichloride were added all at once. The bath temperature was raised to 80–85 °C for 2 h and the mixture was allowed to cool. The dark solution was diluted with ethyl acetate, washed with water and saturated potassium carbonate solution, dried, and concentrated. The residue was chromatographed over 100 g of silica gel, with 4:1 hexane–ethyl acetate as eluant, and recrystallized from hexane to afford 391 mg (53%) of **13**, mp 93–96 °C. Anal. ($C_{21}H_{24}O_4Si$): C, H.

3′,4′-Dimethoxy-2-(2-propenyl)-1,1′-biphenyl-4-carboxylic Acid Methyl Ester (14). A solution of 702 mg (2.0 mmol) of **5g** and 0.65 mL (2.13 mmol) of allyltributyltin in 6 mL of DMF was deoxygenated with argon and 70 mg (0.10 mmol) of bis(triphenylphosphine)palladium dichloride was added. The bath temperature was raised to 100 °C for 1 h and the mixture was allowed to cool. It was diluted with ethyl acetate and washed with water and saturated potassium carbonate, dried, and concentrated. The residue was chromatographed over 100 g of silica gel with 4:1 hexane-ethyl acetate as eluant to give 602 mg (93%) of **14**, which solidified on drying, mp 99–103 °C. Anal. ($C_{19}H_{20}O_4$): C, H.

3′,4′-Dimethoxy-2-ethynyl-1,1′-biphenyl-4-carboxylic Acid (15). A solution of 704 mg (1.91 mmol) of **13** in 25 mL of ethanol was treated with 3 mL of 2.5 N NaOH. After 18 h at room temperature, the mixture was concentrated, and the residue was dissolved in water and filtered. The filtrate was acidified with

HCl and the precipitated material was collected and recrystallized from ethanol to give 387 mg (72%) of **15**, mp 226–227 °C. Anal. ($C_{17}H_{14}O_4$): C, H.

Method A. *N*-[4-(3-Pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (16). A suspension of 5.0 g (25.2 mmol) of biphenyl-4-carboxylic acid in 20 mL of dichloromethane and 0.5 mL of DMF was treated with 2.1 mL (28.8 mmol) of thionyl chloride and the resulting mixture was heated to reflux until a clear solution was obtained. The mixture was cooled to room temperature and 7.8 g (51.9 mmol) of 3-pyridinebutanamine was slowly added. The reaction mixture was stirred for 10 min, was diluted with 150 mL of dichloromethane and was washed with 50 mL of 1 N sodium hydroxide. The organic layer was dried and concentrated. The residue was crystallized from ethyl acetate–hexane with a charcoal treatment to yield 5.0 g (61%) of **16**, mp 122.5–126 °C. Anal. ($C_{22}H_{22}N_2O$): C, H, N. The hydrochloride salt was recrystallized from 2-propanol–ether, mp 158–160 °C. Anal. ($C_{22}H_{22}N_2O$): C, H, N, Cl.

Method B. 3′-(Phenylmethoxy)-*N*-[4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (21). To a suspension of 7.9 g (26 mmol) of 3′-(phenylmethoxy)-1,1′-biphenyl-4-carboxylic acid (**5a**) in 50 mL of toluene was added a solution of 6.8 mL (78 mmol) of oxalyl chloride in 20 mL of toluene. The mixture was heated to reflux for 18 h, and the mixture was concentrated to dryness with a vacuum pump. The residue was dissolved in 40 mL of dry dichloromethane and a solution of 5.08 g (34 mmol) of 3-pyridinebutanamine in 20 mL of dry pyridine was added dropwise. The mixture was heated to reflux for 18 h and was concentrated. The residue was dissolved in ethyl acetate and was washed with dilute K_2CO_3, water, and brine and was dried. The crude product was purified by preparative HPLC, wtih 95:5 dichloromethane–methanol as eluant, to give 7.8 g (69%) of **21** as a light yellow oil. Anal. ($C_{29}H_{28}N_2O\cdot0.4H_2O$): C, H, N, H_2O.

Method C. (*R*)-2-Ethynyl-3′,4′-dimethoxy-*N*-[1-methyl-4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (35). A solution of 459 mg (1.63 mmol) of **15** in 8 mL of DMF was cooled in an ice bath and treated with 0.25 mL of triethylamine and 0.38 mL (1.8 mmol) of diphenyl phosphorazidate. After 1 h, 0.3 g (1.6 mmol) of (*R*)-α-methyl-3-pyridinebutanamine was added and the reaction mixture was allowed to warm to room temperature over 48 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried, and concentrated. The residue was chromatographed over 50 g of silica gel, with ethyl acetate as eluant, and the product was crystallized from ethyl acetate–hexane to give 576 mg (83%) of **35**, mp 135–137 °C, $[\alpha]_D$-44.15° (c = 0.9875, ethanol). Anal. ($C_{27}H_{28}N_2O_3$): C, N, N.

Method D. 3′-Hydroxy-*N*-[4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (22). A solution of 7.8 g (0.018 mmol) of **21** in 150 mL of ethanol was hydrogenated over 0.8 g of 10% palladium on carbon. The product was crystallized from acetonitrile to give 3.6 g (58%) of **22**, mp 150–151.5 °C. The analytical sample was obtained from acetonitrile, mp 151.5–152.5 °C. Anal. ($C_{22}H_{22}N_2O_2$): C, H, N.

Method E. (*R*)-2-Ethyl-3′,4′-dimethoxy-*N*-[1-methyl-4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (36). A suspension of 435 mg of **35** in 15 mL of ethanol was hydrogenated over 45 mg of 10% palladium on carbon and the product was chromatographed over 50 g of silica gel with ethyl acetate as eluant to give 381 mg of **36** as a colorless oil. Anal. ($C_{27}H_{32}N_2O_3$): C, H, N.

Method F. 3′,4′-Dimethoxy-*N*-[4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carbothioamide (41). A solution of 2.8 g (7.20 mmol) of **24** and 1.75 g (8.00 mmol) of phosphorous pentasulfide in 200 mL of dry pyridine was heated to reflux for 1.5 h. After cooling, the reaction mixture was concentrated under high vacuum. The residue was taken up in dichloromethane, filtered, washed several times with water, and dried ($MgSO_4$). Concentration afforded 3.6 g of crude product, which was purified by HPLC, with 19:1 dichloromethane–methanol as eluant, followed by crystallization from methanol to give 2.2 g (76%) of **41**, mp 138–139.5 °C. The filtrate afforded an additional 0.30 g (10%), mp 136–138 °C. Anal. ($C_{24}H_{26}N_2O_2S$): C, H, N, S.

Method G. 3′,4′-Dimethoxy-2-nitro-1,1′-biphenyl-4-carboxylic Acid Methyl Ester (5f). A solution of 2.55 mL (20 mmol) of 3,4-dimethoxybromobenzene in 50 mL of dry THF was cooled in a dry ice–acetone bath and 14 mL (22.4 mmol) of a 1.6 M solution of *n*-butyllithium in hexane was added slowly. After 30 min, a solution of 2.75 g (20 mmol) of freshly fused zinc chloride in 50 mL of dry THF was added via a double-tipped syringe needle and the resulting mixture was allowed to warm to room temperature over 45 min.

Simultaneously, 350 mg (0.50 mmol) of bis(triphenylphosphine)palladium dichloride was suspended in 30 mL of dry THF and was treated with 1.0 mL (1.0 mmol) of a 1 M solution of diisobutylaluminum hydride in toluene. After 20 min, 3.90 g (15 mmol) of methyl 3-bromo-4-nitrobenzoate[12] was added and the arylzinc chloride solution prepared above was transferred in via a double-tipped syringe needle. The resulting mixture was stirred 1.5 h, concentrated, and diluted to 300 mL with ethyl acetate. The solution was washed successively with 100-mL portions of 1 N HCl, water, 1 N NaOH, and brine and was dried. The residue obtained after concentration was purified by HPLC, with 4:1 ethyl acetate–hexane as eluant, followed by crystallization from ethyl acetate–hexane to afford 3.16 g (66%) of **5f**, mp 128–130 °C. Anal. ($C_{16}H_{15}NO_6$): C, H, N.

Method H. 3,4-Bis(4-methoxyphenyl)benzoic Acid (8e). A mixture of 9.52 g (0.040 mol) of bis(4-methoxyphenyl)acetylene and 6.16 g (0.040 mol) of methyl coumalate in 75 mL of toluene was heated for 24 h at 250 °C in an autoclave under 50 psi of N_2. The crude product was filtered through a plug of coarse silica gel and was purified by HPLC, eluting with toluene to give 4.60 g (33%) of methyl 3,4-bis(4-methoxyphenyl)benzoate as an oil. This material was dissolved in 100 mL of methanol and 26 mL (0.026 mol) of 1.0 N NaOH and was heated to reflux for 18 h. The cooled reaction mixture was acidified with HCl and was extracted wtih ethyl acetate. The organic layer was dried and concentrated, and the residue was crystallized from ethyl acetate–hexane to give 2.82 g (65%) of **8e**, mp 191–193 °C. Anal. ($C_{21}H_{18}O_4$): C, H.

Method I. 3′,4′-Dimethoxy-2-nitro-1,1-biphenyl-4-carboxylic Acid (8f). A mixture of 3.00 g (9.5 mmol) of **5f** in 100 mL of ethanol was heated to a bath temperature of 60 °C to effect complete dissolution and 2.0 mL of 30% NaOH was added. After 2 h, TLC indicated that the starting material had been consumed. The reaction mixture was cooled, concentrated, and diluted with 100 mL of water and filtered. The filtrate was acidified with 6 N HCl and the precipitate was collected to give 2.35 g (82%) of **8f**, mp 263–265 °C. Anal. ($C_{15}H_{13}NO_6$): H, N; C: calcd, 59.41; found, 58.96.

3′,4′-Dimethoxy-3,5-dimethyl-1,1′-biphenyl-4-carboxylic Acid (47). A solution of 6.4 g (35.5 mmol) of 3,4-dimethoxyacetophenone (**43**) and 12.3 mL (142 mmol) of morpholine in 100 mL of dry toluene was cooled to −15 °C and a solution of 1.95 mL (17.8 mmol) of titanium tetrachloride in 50 mL of toluene was added dropwise, and the temperature was maintained at <−10 °C. Upon completion of the addition, the mixture was allowed to stand overnight, was filtered through a pad of Celite, and was concentrated to dryness with a rotary evaporator using an oil pump to give 8.8 g of crude enamine **44**.

A mixture of 8.8 g (35.5 mmol) of the above enamine and 7.41 g (35.5 mmol) of ethyl isodehydracetate (**45**) was heated rapidly to 140 °C and then slowly to 160 °C. After 6 h, the mixture was cooled to room temperature and the resulting brown oil was dissolved in 150 mL of ether and washed successively with 50 mL of 1 N HCl, 50 mL of water, and 50 mL of brine. Drying ($MgSO_4$) and concentration gave 8.5 g (85%) of methyl 3′,4′-dimethoxy-3,5-dimethyl-1,1′-biphenyl-4-carboxylate (**46**), mp 91–92 °C.

A suspension of 9.5 g (30.2 mmol) of **46** in 50 mL of 2 N NaOH and 100 mL of ethanol was heated to reflux overnight. After cooling, the reaction mixture was poured onto ice and the precipitated strating material was collected. The filtrate was washed with ether, acidified and extracted with ether, dried ($MgSO_4$), and concentrated to give 2.0 g of a white solid, which was recrystallized from ethanol–water to give 1.4 g (16%) of **47**, mp 175–176 °C, then resolidifies, mp 186.5–187 °C. Anal. ($C_{17}H_{18}O_4$): C, H.

3′,4′-Dimethoxy-3,5-dimethyl-*N*-[4-(3-pyridinyl)butyl]-1,1′-biphenyl-4-carboxamide (48). A solution of 0.50 g (0.0017 mmol) of **47** in 10 mL of thionyl chloride was heated to reflux for 20 min, concentrated, diluted with 10 mL of toluene, and evaporated to dryness. The residue was suspended in 5 mL of DMF, treated with 0.51 g (0.0034 mol) of 3-pyridinebutanamine, and allowed to stir for 30 min at 25 °C. The reaction mixture

was diluted to 50 mL with ethyl acetate, was washed successively with water, dilute sodium hydroxide, and brine, dried, and concentrated. Chromatography over 50 g of silica gel, with ethyl acetate as eluant, and recrystallization of the product from ethyl acetate–hexane afforded 0.40 g (56%) of 48, mp 117–119 °C. Anal. ($C_{26}H_{30}N_2O_3$): C, H, N.

PAF-Binding Assay.[10,11] [^{3}H]PAF was obtained from the New England Nuclear Company. 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 minutes 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 recentrifugation. The washed platelet preparation was adjusted to 2×10^7 platelets/0.05 mL in 0.1% BSA–PBS. Platelet counting was done using a Royco Cell-Crit 921.

To a 0.40-mL Microfuge tube containing 0.05 mL of silicone oil was added buffer and a PAF standard or a test drug to bring the aqueous volume to 0.15 mL. A solution (0.05 mL) 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 which significantly inhibited specific PAF binding were reevaluated at three or more logarithmically spaced concentrations and IC_{50} values were determined by linear regression of log plots of concentration vs specific inhibition. The correlation coefficient for the regression line of each antagonist was always greater than 0.95.

In Vivo PAF-Induced-Bronchoconstriction Assay. Male guinea pigs (Hartley strain, Charles River) weighing 400–600 g were anesthetized with urethane (2 g/kg) given intraperitoneally and a polyethylene cannula was inserted into the jugular vein for intravenous drug administration. Tracheal pressure (centimeters of water) was recorded from a Statham pressure transducer (P 32 AA). Propanolol was administered 5 min before PAF challenge. Two minutes later, spontaneous breathing was arrested with succinylcholine chloride (1.2 mg/kg) administered intravenously, and the animals were ventilated with a Harvard Model 680 small-animal respirator set at 40 breaths/min and a 4.0-cm^3 stroke volume.

For intravenous drug dosing, test drug or vehicle were administered through the cannula into the jugular vein 1 min before the animals were challenged with a maximum constrictory dose of PAF (1 μg/kg) given intravenously. The change in tracheal pressure was averaged for four control and four drug-treated animals and the percent inhibition was calculated. For oral drug dosing, animals were dosed with the test compound or vehicle at the appropriate interval prior to intravenous challenge with PAF as noted above. ID_{50} values for active compounds were determined by linear regression of log dose–response curves generated by at least three doses that caused statistically significant inhibition of the PAF-induced bronchoconstriction of between 10 and 90%. The correlation coefficient for the regression line of each antagonist was always greater than 0.95.

Acknowledgment. We thank members of the Physical Chemistry Department, Hoffmann-La Roche, Inc., for determination of the spectral and microanalytical data for the compounds reported herein. We also thank Dr. R. W. Kierstead for his continued support and encouragement during the course of these studies.

Pentadienyl Carboxamide Derivatives as Antagonists of Platelet-Activating Factor

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A series of *N*-[4-(3-pyridinyl)butyl]-5,5-disubstituted-pentadienamides was prepared and evaluated for PAF-antagonist activity. Compounds were assayed in vitro in a PAF-binding assay employing washed, whole dog platelets as the receptor source and in vivo after intravenous or oral administration for their ability to prevent PAF-induced bronchoconstriction in guinea pigs. Criteria required for good oral activity in the latter model include an (*E*,-*E*)-5-phenyl-2,4-pentadienamide, a second phenyl or a four- or five-carbon alkyl moiety in the 5-position of the diene, and an (*R*)-[1-alkyl-4-(3-pyridinyl)butyl] substituent on the carboxamide nitrogen atom. The alkyl substituent on this side chain can be methyl, ethyl, or cyclopropyl. Two members of this series, [*R*-(*E*)]-5,5-bis(4-methoxyphenyl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-2,4-pentadienamide (**31**) and [*R*-(*E*,*E*)]-5-(4-methoxyphenyl)-*N*-[1-methyl-4-(3-pyridinyl)butyl]-2,4-decadienamide (**58**), were selected for further pharmacological evaluation. Both were found to be substantially longer acting after oral administration than the corresponding *S* enantiomers in the guinea pig bronchoconstriction assay. A second in vivo model used to evaluate PAF antagonists determines the ability of test compounds to decrease the area of skin wheals induced by an intradermal injection of PAF. In this model, using both rats and guinea pigs, compounds **31** and **58** were found to be as active as the reference PAF antagonist 3-[4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-2-yl]-1-(4-morpholinyl)-1-propanone (**45**).

We have recently described the preparation and evaluation of two series of novel platelet-activating-factor (PAF) antagonists typified by the pyridoquinazolinecarboxamide **1**[1] and the biphenylcarboxamide **2**.[2] Key elements of these compounds were shown to be the aromatic ring marked "a", the carboxamide moiety, and the 3-substituted pyri-

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