Synthesis and Structure-Activity Relationships of 1-Acyl-4-((2-methyl-3-pyridyl)cyanomethyl)piperazines as PAF Antagonists

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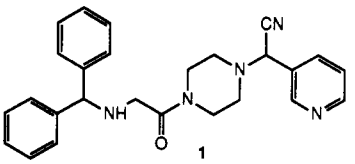
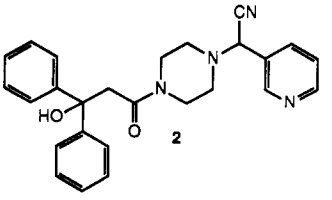
A second generation of (cyanomethyl)piperazines, 1-acyl-4-((2-methyl-3-pyridyl)cyanomethyl)-piperazines, with increased oral activity was prepared and evaluated in vitro in a PAF-induced platelet aggregation assay (PAG) and in vivo in a PAF-induced hypotension test in normotensive rats (HYP). Oral activity was ascertained through a PAF-induced mortality test in mice (MOR). Attachment of a methyl group at position 2 of our earlier pyridine derivatives resulted in an improvement of 1 order of magnitude or greater in the ID_{50} of the oral test. Three different types of acyl substituents of similar potency emerge from this work: *N*-(diphenylmethylamino)acetyl, 3-substituted 3-hydroxy-3-phenylpropionyl, and N-substituted 3-amino-3-phenylpropionyl groups. The most interesting compounds, **26** (UR-12460, PAG IC_{50} = 0.040 μM, HYP, ID_{50} = 0.021 mg/kg iv, MOR, ID_{50} = 0.30 mg/kg po) and **58** (UR-12519, PAG IC_{50} = 0.041 μM, HYP, ID_{50} = 0.015 mg/kg iv, MOR, ID_{50} = 0.044 mg/kg po), compare favorably with WEB-2086. Compounds **26** and **58** were also tested in active anaphylactic shock (AAS) and endotoxin-induced mortality (EIM) tests. On the basis of these data, compounds **26** and **58** have been selected for further pharmacological development.

Introduction

We described[1] the preparation and pharmacological evaluation of a novel series of potent, orally active PAF antagonists exemplified by 1-((*N*-(diphenylmethyl)amino)acetyl)-4-((3-pyridyl)cyanomethyl)piperazine (**1**) and 1-(3-hydroxy-3,3-diphenylpropionyl)-4-((3-pyridyl)cyanomethyl)piperazine (**2**). Our results showed that the PAF antagonist character of these compounds largely stems from the presence of the cyano group and from the nature of the aromatic ring, 3-pyridine being optimal. Among the *N*-acyl substituents successfully introduced, 3,3-diphenylpropionyl provided the most active compounds. Addition of an amine or hydroxy group on that acyl group, leading to compounds **1** and **2**, produced further improvement in oral activity.



1



2

In our ongoing investigation, we have faced the problem of allergic reactions after prolonged handling of pyridine-3-carboxaldehyde, the starting material for ((3-pyridyl)-cyanomethyl)piperazines and other known PAF antagonists.[2-4] In an attempt to overcome this problem, we tried a new synthetic route avoiding the use of pyridine-3-carboxaldehyde, the Sommelet-Hauser rearrangement of cyanomethylammonium salts to cyanomethylpyridines. This reaction leaves a methyl group in position 2 of the pyridine ring, and we observed that this modification was frankly beneficial for oral activity. Thus, by this serendipitous change, we obtained compounds which not only retained the potent in vitro and intravenous activity of the reported compounds, but also showed improved oral activity. Here we report the synthesis and PAF antagonist activity of this novel series of 1-acyl-4-((2-methyl-3-pyridyl)cyanomethyl)piperazines with further structural modifications in the acyl substituent.

Chemistry

The (cyanomethyl)pyridines **22**, **24**, **26-33**, **35-43**, **45-55**, **61-65**, **76**, **77**, **80**, **82**, **89**, and **91** listed in Tables III-VII were obtained by reaction of the appropriate arylalkanoic acid and amine **3a** via DCC coupling in the presence of HOBT (method A, Scheme I). To synthesize amine **3a** we applied the Sommelet-Hauser rearrangement of ((2-picolyl)cyanomethyl)ammonium salts described by Sanders for nicotine analogues.[5] As shown in Scheme I, treatment of 2-picolyl chloride with an excess of piperazine monohydrochloride, followed by BOC-protection of the secondary amino group, gave 1-BOC-4-(2-picolyl)piperazine (**5**) in 88% yield. Alkylation of piperazine nitrogen with bromoacetronitrile gave quaternary salt **6a** (74%) accompanied by minor amounts of the alkylated pyridine **7a** (**6a**/**7a**, 8:2). Several conditions were tried to bring about the rearrangement of quaternary salt **6a**, since the first attempts with the usual reagents, NaH or KOBu^t in THF-DMSO, gave poor yields. NaOH aqueous solution (1 N) in THF was very effective, but concommitant hydrolysis of the cyano group was detected. DBU in CH_3CN proved to be the system of choice, and 1-BOC-4-((2-methyl-3-pyridyl)cyanomethyl)piperazine (**8a**) was obtained in 55% yield. Finally, deprotection of **8a** with HCl in dioxane gave amine **3a** in 99% yield. The 2-ethylpyr-

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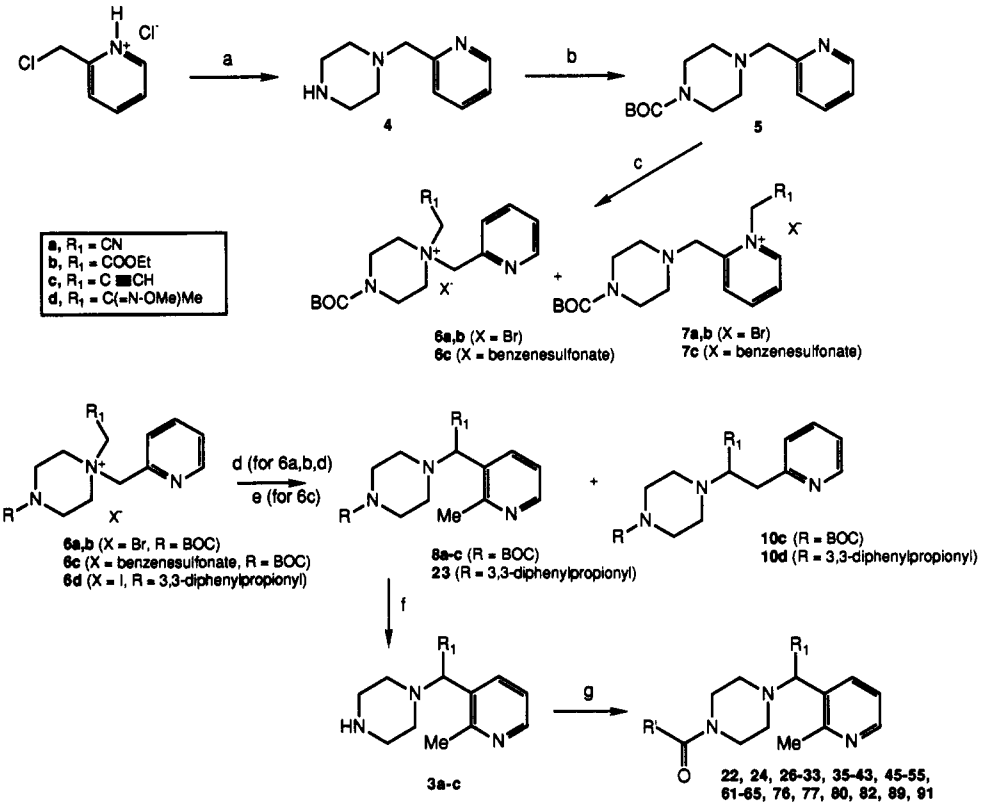
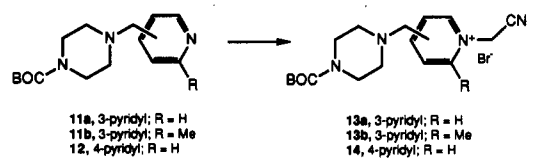
Scheme I[a]



[a] Key: (a) piperazine monochlorhydrate, H_2O, room temperature, 18 h; (b) BOC_2O, 1 N NaOH/THF, room temperature, 45 min; (c) $BrCH_2CN$, acetone, room temperature, 48 h; (d) DBU, CH_3CN, room temperature, 18h; (e) $KOBu^t$, DMSO/THF, room temperature, 18 h; (f) 6.5 N $HCl_{(g)}$/dioxane, $CHCl_3$, room temperature, 1 h; (g) R'COOH, DCC, HOBT, DMF, room temperature, 18 h (method A).

Scheme II[a]



[a] Key: (a) $BrCH_2CN$, CH_3CN, room temperature, 48 h.

idine analogue of **3a** was obtained by a similar sequence, starting from 1-(1-(2-pyridyl)ethyl)piperazine.

Attempts to generate 3- and 4-methylpyridine analogues of amine **3a** in the same way did not meet with success because quaternization of 1-BOC-4-(4-picolyl)piperazine (**12**) and 1-BOC-4-(3-picolyl)piperazine (**11a**) took place only at the pyridine nitrogen (Scheme II). More surprisingly, we found the same preference in the more steric congested 2-methylpyridine derivative **11b** whose transformation would have afforded a dimethyl derivative.

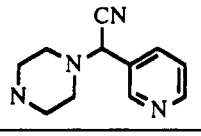
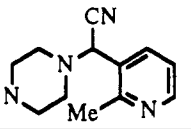
We also studied alternative substitutes for the cyano group using the same synthetic approach. With an ethoxycarbonyl group, the alkylation step was less selective (**6b**/**7b** 6:4, 28%), and rearrangement to **8b** gave a 43% yield. Poor selection was also observed in the alkylation of **5** to (ethynylimino)- and (ethoxyimino)acetate derivatives **6c** and **6d**. Moreover, the rearrangement of **6c** and **6d** produced a 1:1 mixture of the Sommelet–Hauser and Stevens products[5,6] (**8c**/**10c** and **23**/**10d**, respectively), which had not been observed in the sequence with a cyano or ethoxycarbonyl group. Alcohol **25** (Table II) was derived from **22** by $NaBH_4$ reduction.

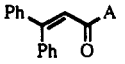
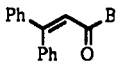
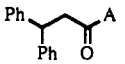
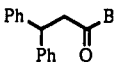
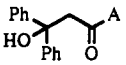
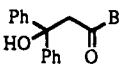
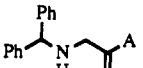
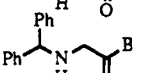
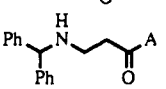
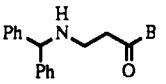
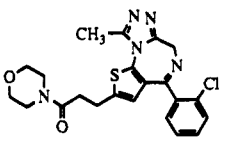
The required arylalkanoic acids were available through a variety of methods and conditions outlined in Schemes III–V. The carboxylic acids have the same numbering as the final product in whose preparation they were employed, followed by an "A". (Aralkylamino)acetic and -propanoic acids **26A** and **35A–40A** were obtained by reductive amination of benzophenone and other aromatic ketones with different amino acids[7] (method B, Scheme III). The absence of racemization in the condensation of D- and L-alanine was verified by NMR analysis of the corresponding Mosher amides. An alternative route for the preparation of (aralkylamino)acetic acids was used for **41A**: alkylation of amine **15** with *tert*-butyl bromoacetate followed by ester hydrolysis. Amine **15** was obtained by Curtius rearrangement of 2,2-diphenylpropionyl chloride. Alkylation of sulfonamide **16** with ethyl bromoacetate and subsequent ester hydrolysis provided **32A**. Reductive amination of (diphenylmethyl)amine with glyoxylic acid in formic acid gave formylated amino acid **33A**. However, its acetylated analogue **34** (Table III) was derived from final product **26** by treatment with Ac_2O in the presence of pyridine.

The ether **27A** and thioether **28A** were obtained by condensation of diphenylmethanol with glycolic acid and mercaptoacetic acid, respectively, in TFA. The sulfoxide and sulfone analogues (**29A** and **30A**) were derived from sulfide **28A** by oxidation with H_2O_2 under different conditions.[8,9]

To prepare the 3-hydroxypropanoic acids **42A** and **45A–48A**, the appropriate carboxylic acids were treated with 2 equiv of LDA, and the resulting dianion was reacted with the corresponding aromatic ketone[10] (method C, Scheme IV). Reaction of hydroxy acid **17**[11] with DAST provided fluorinated anhydride **18** which was used directly in the acylation of amine **3a**. *cis*- and *trans*-3-(trifluoromethyl)cinnamic acids **54A** and **55A** were obtained from trifluoroacetophenone through a Wittig reaction with

Table I. Comparison of 3-Pyridyl Derivatives with 2-Methyl-3-pyridyl Derivatives

A =  B = 

compd		PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced mortality ID_{50},[c] mg/kg po
53a		0.010 (0.0068–0.015)	0.049 (0.032–0.076)	30–50
53		0.026 (0.019–0.036)	0.038 (0.031–0.046)	2.2 (1.3–3.5)
49a		0.020 (0.016–0.024)	0.013 (0.0062–0.026)	12 (8–19)
49		0.018 (0.016–0.020)	0.015 (0.008–0.029)	0.90 (0.51–1.6)
2		0.021 (0.015–0.029)	0.061 (0.045–0.082)	4.7 (2.7–8.0)
42		0.017 (0.013–0.022)	0.039 (0.032–0.047)	0.69 (0.28–1.7)
1		0.0091 (0.0091–0.0091)	0.023 (0.019–0.026)	5.9 (4.4–7.9)
26		0.040 (0.030–0.053)	0.021 (0.015–0.031)	0.30 (0.160–0.54)
40a		0.053 (0.038–0.074)	0.025 (0.011–0.053)	5.4 (2.4–12)
40		0.12 (0.10–0.14)	0.044 (0.020–0.100)	0.62 (0.16–2.4)
WEB-2086[d]		0.091 (0.071–0.120)	0.17 (0.12–0.27)	0.97 (0.38–2.5)

[a] Concentration required to inhibit PAF-induced maximum aggregation by 50%. Parentheses contain 95% confidence limits. [b] Dose required to reduce the lowering of the arterial blood pressure caused by PAF by 50%. Parentheses contain 95% confidence limits. [c] Dose required to inhibit PAF-induced mortality by 50%. Parentheses contain 95% confidence limits. [d] WEB-2086 was kindly provided by Boehringer Ingelheim.

triethyl phosphonoacetate followed by chromatographic separation and ester hydrolysis. Assignment of the cis and trans stereochemistry was based on their proton NMR spectra through correlation of the observed chemical shifts for the ethylenic proton and the values calculated by addition of tabulated substituents' contributions on ethylene derivatives.[12] The double bond of **54A** was hydrogenated to produce **52A**. Acids **31A**, **43A**, and **50A** were prepared by described procedures.[13]

β-Amino acid derivatives **65A**, **76A**, and **80A** were synthesized from 3-amino-3-phenylpropanoic acid by acylation/alkylation sequences under the conditions outlined in Scheme V. Condensation of 3-amino-3-phenylpropanoic acid with 2,5-diethoxytetrahydrofuran provided pyrrole derivative **91A**.

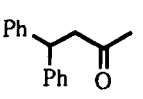
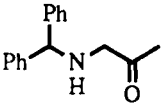
N-Alkyl-β-amino acids **63A**, **82A**, and **89A** were prepared as shown in Scheme V employing the reductive amination of ethyl benzoylacetate with aniline and methylamine as the key step.[14]

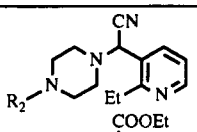
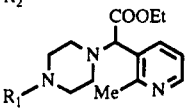
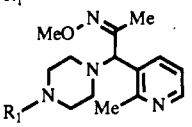
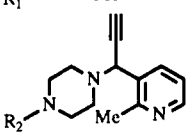
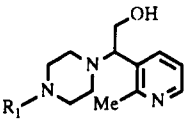
An alternative route based on the functionalization of the primary amino group of common intermediate **81** was used to prepare the remaining compounds carrying an N-substituted 3-amino-3-phenylpropionyl moiety (Scheme VI). Compound **81** was prepared by DCC coupling of amine **3a** with *N*-(*tert*-butoxycarbonyl)-3-amino-3-phenylpropionic acid **61A** followed by N-deprotection. Amides **69–73** were prepared from **81** by method A described above. Amides **68** and **74** were obtained via the corresponding acyl chloride and anhydride respectively. Reaction of **81** with succinic anhydride followed by dehydration in Ac_2O yielded the imide **75** (Table VI). Carbamates **56–60** were obtained by reaction of **81** with the corresponding alkyl chloroformate in the presence of Et_3N and sulfonamides **78** and **79** by reaction with phenyl and methylsulfonyl chloride, respectively, under similar conditions. Reductive amination of **81** with benzaldehyde provided amine **83**. Alkylation of **81** with the appropriate alkyl chlorides produced amines **84**, **86**, and **87** and dialkylation of **81** with 1-chloro-4-bromobutane gave **90**. Finally, urea **66** was prepared by reaction of **81** with trimethylsilyl isocyanate.

Results and Discussion

The (cyanomethyl)pyridines **21–91** were evaluated for PAF antagonist activity using the in vitro PAF-induced platelet aggregation assay[15] and the in vivo PAF-induced

Table II. Characterization and PAF Antagonist Activity of Compounds 21–25

R_1=  R_2 = 

compd		PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced mortality ID_{50},[c] mg/kg po	mp, °C	solvents[d]	formula[e]	anal.[f]
21		0.038 (0.031–0.045)	0.063 (0.054–0.074)	0.70 (0.30–1.7)	65–67	E	$C_{28}H_{31}N_5O \cdot 1/2H_2O$	C, H, N
22		15.5 (10.5–22.9)		>30	130–133	E	$C_{29}H_{33}N_3 \cdot 2HCL \cdot 1.4H_2O$	C, H, N
23		7.8 (4.9–12.7)		>3	50–54	E	$C_{29}H_{34}N_4O_2 \cdot H_2O$	C, H, N
24		0.86 (0.59–1.25)		>3	<30	E	$C_{28}H_{30}N_4O \cdot 3/4H_2O \cdot 1/2Et_2O$	C, H,[g] N[h]
25		4.0 (2.9–5.6)		>3	55–60	E	$C_{27}H_{31}N_3O_2 \cdot 5/4H_2O$	C, H, N

[a–c] See footnotes in Table I. [d] Solvents: A = acetonitrile, Cl = chloroform, D = dichloromethane, H = hexane, E = trituration with ether, EA = ethyl acetate. [e] Empirical formula with amount of water of hydration. [f] Analytical results for the indicated elements are within ±0.4% of the calculated values unless indicated otherwise. [g] H: calcd, 6.52; found, 7.01. [h] N: calcd, 11.45; found 10.59.

hypotension test in normotensive rats.[16] Oral activity was tested through the PAF-induced mortality test in mice.[17] In the latter, initial oral doses of 3 mg/kg of the test compounds were administered before PAF injection. Only when more than 50% inhibition of mortality was achieved were more doses tested in order to obtain ID_{50} values.

The effect of the attachment of a methyl group at position 2 of the pyridine was tested on the methylated analogues of five compounds with different acyl substituents chosen from the most active compounds of the reported series (Table I). Comparison between the unsubstituted and 2-methyl-substituted analogues showed an improvement of 1 order of magnitude or greater in the oral test for 2-methyl derivatives, whereas the results in the aggregation and hypotension tests scarcely changed. This effect seemed to be quite general and independent of the nature of the acyl substituent, leading us to expect that the established SAR for the unsubstituted series reported would be roughly applicable to the 2-methyl analogues.

On the basis of these findings, our search for more potent orally active compounds focused on the preparation of (a) compounds bearing new functions in place of the cyano group, now accessible by Sommelet–Hauser rearrangement (Table II), (b) compounds generated by acyl substituent modification of the most potent two 2-methyl derivatives **42** and **26** (Tables III and IV), and (c) N-substituted 3-amino-3-phenylpropanoyl derivatives **56–91** featuring a new type of acyl substituent (Tables V–VII).

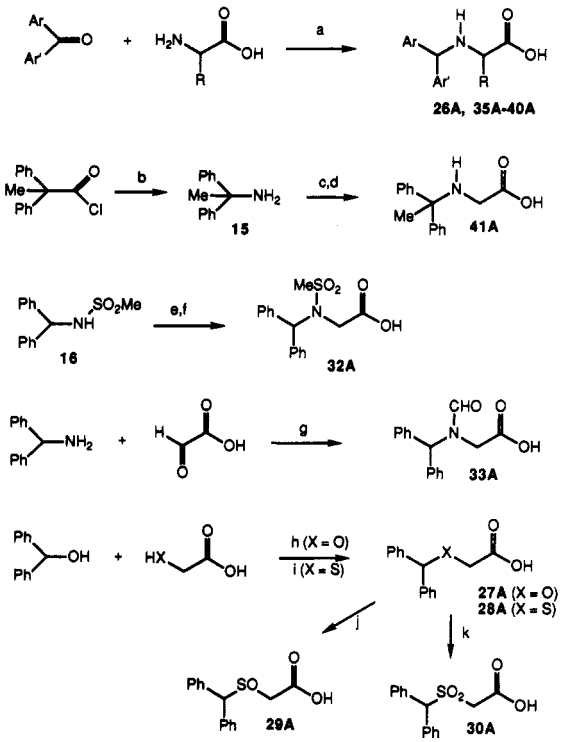
The results from this study as well as our early ones[1] demonstrate that the cyano group has an essential role in activity. Indeed, we observed only decreased potencies when we tried new functions similar to the cyano group with respect to either their electronic nature or their steric requirements (ethoxycarbonyl **22**, (methoxyimino)acetonyl **23**, ethynyl **24**, and hydroxymethyl **25**, Table II). Regarding the size of the pyridine substituent, ethyl may replace methyl (**21** versus **26**) without significant variation in the results of the three screening tests.

Table III shows data for compounds in which the amino group present in the (*N*-(diphenylmethyl)amino)acetyl radical of compound **26** was replaced by other heteroatom-containing functions (**27–31**), as well as compounds with additional substitution either at the amino group (**32–34**) or at the α-position of the carbonyl group (**35–37**). The diphenylmethyl moiety was also varied (**38**, **39**, and **41**). These modifications on the structure of **26** led to compounds of comparable PAF antagonist potency but with lesser oral activity.

Additional changes effected around the 3-hydroxy-3,3-diphenylpropanoyl substituent of **42** are shown in Table IV. Substitution of the hydroxy group by ethoxycarbonyl group (**43**) or by fluorine (**44**) did not improve activity. However, replacement of a phenyl group by a 3-pyridine ring (**46**) or by a trifluoromethyl group (**47**) led to compounds of oral activity comparable to **42**. By changing a phenyl group for a trifluoromethyl group on diphenylpropanoate **49** and diphenylpropenoate **53**, compounds **52**, **54**, and **55** with diminished oral activity were obtained.

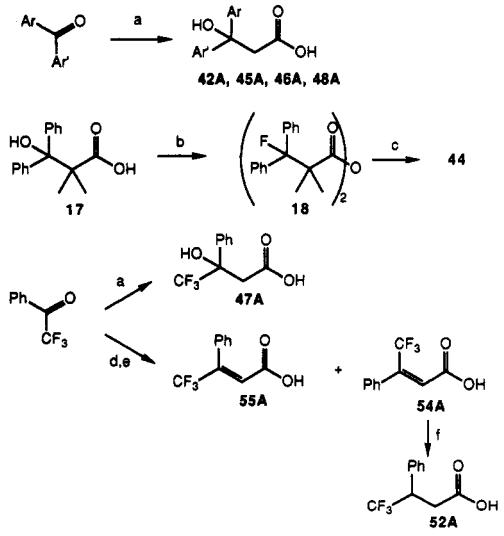
Compound **47** is particularly interesting as it is the first highly active compound among the ((3-pyridyl)cyanomethyl)piperazines carrying only one phenyl group in the acyl substituent. This suggested the idea of preparing phenylpropanoyl derivatives with electron-withdrawing groups at the 3-position, and we obtained excellent results with substituted amino groups. We tested five types of functional groups as substituents: carbamates, sulfonamides, ureas, amides, and amines. Within each function, we prepared either cyclic or acyclic lower alkyl or aromatic derivatives. Most of the compounds again showed potent

Scheme III[a]



[a] Key: (a) $NaBH_3CN$, H_2O/MeOH, reflux, 18h (method B); (b) NaN_3, acetone/H_2O, 10 °C, 1 h; (c) $BrCH_2COOBu^t$, K_2CO_3, DMF, 80 °C, 12 h; (d) K_2CO_3, MeOH/H_2O, reflux 2 h; (e) $BrCH_2COOEt$, NaH, DMF, 60 °C, 18 h; (f) 5% NaOH/THF, 70 °C, 1 h; (g) HCOOH, 70 °C, 90 min; (h) $HOCH_2COOH$, TFA, room temperature, 24 h; (i) $HSCH_2COOH$, TFA, room temperature, 30 min; (j) 30% wt H_2O_2, MeOH, $^iPrOH/H_2SO_4$(cat), room temperature, 1 h; (k) 30% wt H_2O_2, AcOH, room temperature, 18 h.

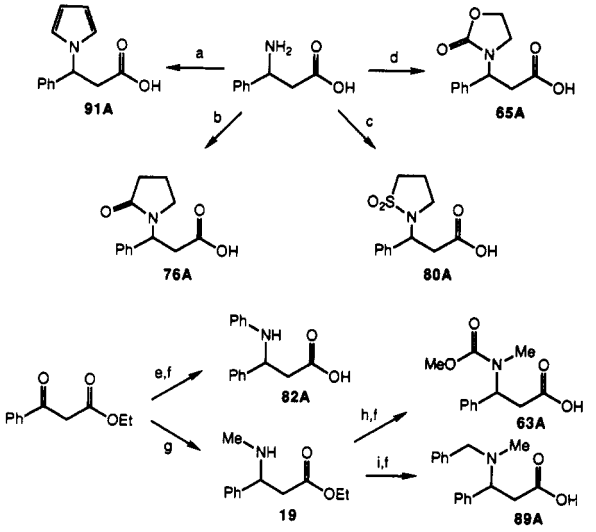
Scheme IV[a]



[a] Key: (a) HOAc, LDA (2 mol), THF, room temperature, 18 h (method C); (b) DAST, CH_2Cl_2, 0 °C, 2 h; (c) **3a**, pyr, room temperature, 18 h, then, 60 °C, 2 h; (d) $(EtO)_2P(O)CH_2COOEt$, NaH, DME, reflux, 1 h; (e) K_2CO_3, MeOH/H_2O, reflux 2h; (f) H_2, 5% Pd/C, MeOH.

PAF antagonist activity but behaved differently in the oral test. Within the carbamate and urea groups (Table V), we observed good correlation between the size of the alkyl substituent and oral activity. Practically all compounds with a cyclic or acyclic lower alkyl carbamate (**58**, **59**, **61**, **63**, and **65**) showed ID_{50} around 1 mg/kg or less in the mortality test, whereas compounds bearing a second

Scheme V[a]



[a] Key: (a) 2,5-dimethoxytetrahydrofuran, HOAc, reflux, 1 h; (b) (1) $Cl(CH_2)_3COCl$, 0.5 N NaOH/THF, room temperature, 18 h; (2) $KOBu^t$, THF, room temperature, 1 h; (c) (1) $Cl(CH_2)_3SO_2Cl$, 1 N NaOH, THF, room temperature, 18 h; (2) NaOEt, EtOH, reflux, 18 h; (d) (1) $Br(CH_2)_2OCOCl$, 1 N NaOH/THF, room temperature, 18 h; (2) NaH, DMF, 80 °C, 18 h; (e) $PhNH_2$·HCl, $NaBH_3CN$, MeOH, room temperature, 18 h; (f) K_2CO_3, MeOH/H_2O, reflux 2 h; (g) $MeNH_2$·HCl, $NaBH_3CN$, MeOH, room temperature, 48 h; (h) ClCOOMe, Et_3N, CH_2Cl_2, room temperature, 18 h; (i) $PhCH_2Br$, K_2CO_3, MeCOEt, 70 °C, 18 h.

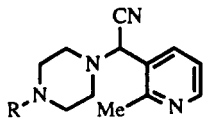
phenyl group in the nitrogen substituent performed more poorly in the oral test. The most active compound ever evaluated in our mortality test was methyl carbamate **58** with an ID_{50} of 0.044 mg/kg. The same preference for the lower alkyl substituent was observed in the amide and sulfonamide group (Table VI). Again the most active compound in the oral test was the acetamide **74**, with results very similar to the 2-pyrrolidone **76**. Benzamide **68**, although very active in the platelet aggregation test, showed impaired oral activity. The negative effect of the phenyl group, however, is compensated for by the presence of a pyridine nitrogen in the nicotinoyl analogue **69**. Interestingly, transposing the amide substituents in compound **74** (to give **77**) decreased in vitro activity. Phenylamine **82** or benzylamine **83** were the best compounds in the amine group (Table VII).

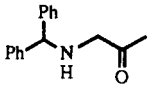
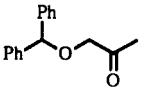
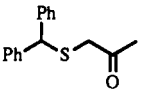
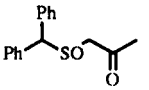
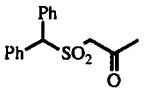
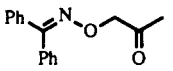
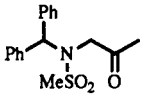
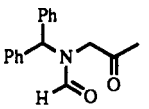
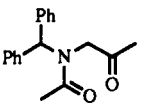
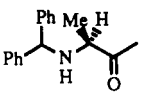
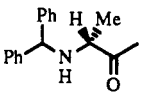
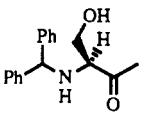
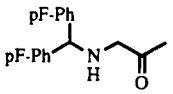
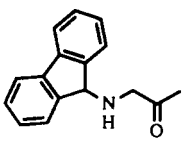
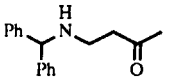
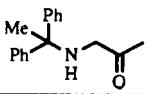
At this point, compounds **26** and **58** were selected for profiling in additional pharmacological models, i.e., anaphylactic shock (AAS) in mice and endotoxin-induced mortality (EIM) in mice and rats. These models are good tools for assessing the potential therapeutic usefulness of a PAF antagonist, as they mimic pathological conditions in which PAF may be involved.[18] Table VIII shows the in vivo profiles of **26** and **58** in these tests compared with that of WEB-2086. Overall, **26** and **58** showed similar oral activities and **58** was slightly more active than **26** when administered intravenously. Besides, they also compare favorably with WEB-2086, being somewhat more potent by intravenous route.

Conclusion

The 1-acyl-4-((2-methyl-3-pyridyl)cyanomethyl)piperazines reported here are second-generation (cyanomethyl)-piperazine PAF antagonists with increased oral activity. Three different types of similarly potent acyl substituents emerge from this work: (*N*-(diphenylmethyl)amino)acetyl, 3-substituted 3-hydroxy-3-phenylpropionyl, and N-sub-

Table III. Characterization and PAF Antagonist Activity of Compounds 26–41

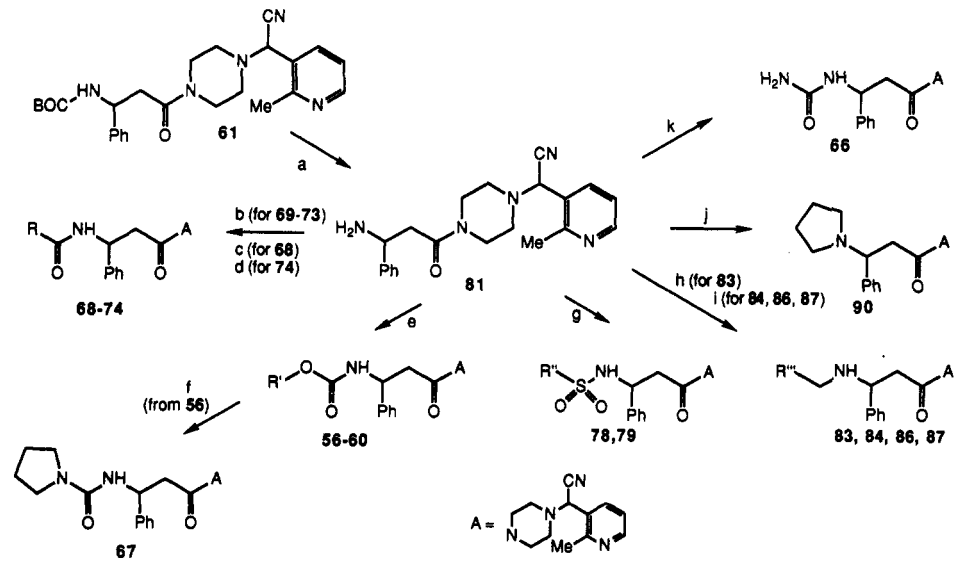


compd	R	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced mortality ID_{50},[c] mg/kg po	mp, °C	solvents[d]	formula[e]	anal.[f]
26		0.040 (0.030–0.053)	0.021 (0.015–0.031)	0.30 (0.16–0.54)	164–168	A	$C_{27}H_{29}N_5O$	C, H, N
27		0.042 (0.029–0.060)	0.048 (0.032–0.071)	>3	55–57	E	$C_{27}H_{28}N_4O_2$	C, H, N
28		0.079 (0.063–0.099)	0.058 (0.020–0.172)	>3	183–184	EA	$C_{27}H_{28}N_4OS$	C, H, N
29		0.044 (0.033–0.058)	0.034 (0.023–0.051)	>3	94–98	E	$C_{27}H_{28}N_4O_2S\cdot 7/4H_2O$	C, H, N
30		0.026 (0.017–0.038)	0.11 (0.09–0.14)	>3	101–105	E	$C_{27}H_{28}N_4O_3S$	C, H, N
31		0.087 (0.049–0.150)	0.071 (0.040–0.127)	>3	68–72	EA/Et_2O	$C_{27}H_{27}N_5O_2$	C, H, N
32		0.082 (0.071–0.094)	0.083 (0.071–0.098)	>3	97–100	E	$C_{28}H_{31}N_5O_3S\cdot 5/4H_2O\cdot 1/3Et_2O$	C, H, N
33		0.030 (0.021–0.041)	0.016 (0.008–0.036)	1.5 (1.1–2.1)	87–92	E	$C_{28}H_{29}N_5O_2\cdot 3/2H_2O$	C, H, N
34		0.020 (0.017–0.024)	0.039 (0.027–0.056)	1–2	88–95	E	$C_{29}H_{31}N_5O_2\cdot H_2O$	C, H, N
35		0.013 (0.009–0.019)	0.025 (0.016–0.038)	1.6 (0.61–8.4)	64–69	E	$C_{28}H_{31}N_5O\cdot H_2O$	C, H, N
36		0.030 (0.023–0.039)		>3	79–83	E	$C_{28}H_{31}N_4O\cdot 1/2H_2O$	C, H, N
37		0.034 (0.027–0.044)	0.020 (0.016–0.025)	2.4 (0.67–8.4)	75–80	E	$C_{28}H_{31}N_4O_2\cdot 1/4H_2O$	C, H, N
38		0.15 (0.11–0.20)	0.049 (0.028–0.087)	2–3	68–73	E	$C_{27}H_{27}F_2N_5O\cdot 1/2H_2O$	C, H, N
39		0.050 (0.035–0.073)	0.085 (0.056–0.130)	>3	68–73	E	$C_{27}H_{27}N_5O\cdot 3/2H_2O$	C, H, N
40		0.12 (0.10–0.14)	0.044 (0.020–0.100)	0.62 (0.16–2.4)	54–57	E	$C_{28}H_{31}N_5O\cdot 1/4H_2O$	C, H, N
41		0.086 (0.058–0.13)	0.057 (0.040–0.082)	>3	66–70	EA/Et_2O	$C_{28}H_{31}N_5O\cdot 1/2H_2O$	C, H, N

[a–c] See footnote Table I. [d–f] See footnote Table II.

stituted 3-amino-3-phenylpropionyl. After a careful structural study around the (*N*-(diphenylmethyl)amino)acetyl group, compound **26** was confirmed as optimal for oral activity. In the second group, we found that one of the phenyl groups of **42** can be successfully changed for a trifluoromethyl group (**47**). Quite a few highly active

Scheme VI[a]



[a] Key: (a) 6.5 N $HCl_{(g)}$/dioxane, $CHCl_3$, room temperature, 1 h; (b) RCOOH, method A; (c) RCOOCl, Et_3N, CH_2Cl_2, room temperature, 18 h; (d) Ac_2O, pyr, room temperature, 18 h; (e) ClCOOR', Et_3N, CH_2Cl_2, room temperature, 18 h; (f) pyrrolidine, CH_3CN, reflux, 5h; (g) R''SO_2Cl, Et_3N, CH_2Cl_2, room temperature, 18 h; (h) R'''CH_2Cl, Et_3N, $CHCl_3$, room temperature, 18 h; (i) PhCHO, $NaBH_3CN$, MeOH, pH = 6–8, room temperature, 48 h; (j) $Br(CH_2)_4Cl$, Et_3N, DMAP, CH_2Cl_2, room temperature, 18 h; (k) Me_3SiNCO, THF, 60 °C, 2 h.

structures are found in the third type of acyl substituent: methyl carbamate **58**, acetamide **74**, phenylamine **82**, and benzylamine **83**. All of them compare favorably with the reference compound WEB-2086. Compounds **26** and **58** have been selected for further pharmacological and toxicological trials. Chemical stability studies are in progress.

Experimental Section

A. Chemistry. Melting points were determined with a Mettler FP 80 central processor melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^1H NMR (80 MHz) and ^{13}C NMR (20.1 MHz) spectra were recorded on a Brücker AC80 spectrometer and are reported in ppm on the δ scale from the indicated reference. Mass spectra were measured on an HP-5988 quadrupole mass spectrometer. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Combustion analyses were performed with a Carlo Erba 1106 analyzer. Liquid chromatography was performed with a forced flow (flash chromatography) of the indicated solvent system on SDS silica gel Chromagel 60 a C.C. (230–400 mesh). When necessary, solvents and reagents were dried prior to use. Tetrahydrofuran, diethyl ether, and toluene were distilled from sodium metal/benzophenone ketyl. $CHCl_3$ was passed through an alumina column. Dichloromethane and triethylamine were distilled from calcium hydride. Dimethyl sulfoxide and dimethylformamide were distilled under reduced pressure from calcium hydride and stored over activated 4-Å molecular sieves. Unless otherwise specified, all nonaqueous reactions were conducted under a rigourously dried argon atmosphere, using oven-dried glassware.

C-18-PAF-acether was synthesized from (S)-batyl alcohol[19] following a published procedure.[20]

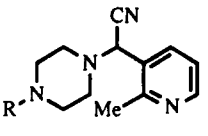
1-((2-Pyridyl)methyl)piperazine (4). To a solution of piperazine monochlorohydrate (352 g, 2.87 mol) in water (1 L) was added a solution of 2-(chloromethyl)pyridine monochlorohydrate (236 g, 1.44 mol) in water (400 mL), and the mixture was stirred for 18 h. It was then extracted with AcOEt (3 × 400 mL), and the aqueous layer was basified by addition of solid NaOH (60 g) and extracted with $CHCl_3$ (2 × 200 mL). Additional solid NaOH (20 g) was added, and the aqueous was extracted again with $CHCl_3$. Finally, additional solid NaOH (80 g) was added to bring the pH to 10, and the solution was extracted with $CHCl_3$ (4 × 200 mL). The organic phases of the extractions at pH = 10 were dried (Na_2SO_4) and evaporated to give a brown solid (224 g, 88%) which was used directly in the next step: IR (film) ν 3267, 3002, 2933, 2807, 1585, 1564, 1469, 1427, 1316, 1141, 757 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.55 (broad d, J = 4.8 Hz, 1H, pyr), 7.24 (m, 3H, pyr), 3.64 (s, 2H, CH_2N), 2.95 (m, 4H, pip), 2.56 (m, 5H, pip, NH).

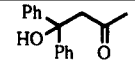
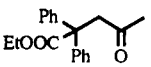
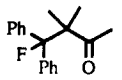
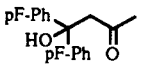
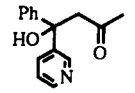
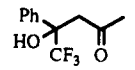
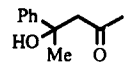
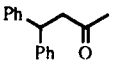
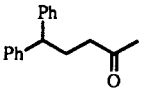
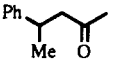
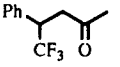
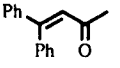
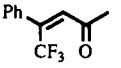
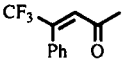
1-(*tert*-Butoxycarbonyl)-4-((2-pyridyl)methyl)piperazine (5). To a cooled solution (0 °C) of **4** (223 g, 1.26 mol) in THF (750 mL) was added 1.5 N NaOH aqueous solution (1.2 L). BOC_2O (275 g, 1.26 mol) was then added, and the mixture was stirred for 30 min. The cooling bath was removed and stirring continued at room temperature for 24 h. The organic solvent was removed under reduced pressure, and the residue was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4) and evaporated to give a brown oil (342 g, 98%). An analytical sample was obtained by crystallization from hexane: IR (film) ν 2970, 2805, 1691, 1416, 1361, 1243, 1121, 1004, 757 cm^{-1}; ^1H NMR ($CDCl_3$) δ ppm (TMS) 8.56 (broad d, J = 4.4 Hz, 1H, pyr), 7.70 (m, 1H, pyr), 7.40 (m, 1H, pyr), 7.16 (dd, J = 7.2, 4.4 Hz, 1H, pyr), 3.66 (s, 2H, CH_2N), 3.46 (m, 4H, pip), 2.45 (m, 4H, pip), 1.46 (s, 9H, CH_3).

1-(*tert*-Butoxycarbonyl)-4-(cyanomethyl)-4-((2-pyridyl)methyl)piperazinium Bromide (6a). Bromoacetonitrile (33.6 mL) was added to a cooled solution (0 °C) of **5** (127 g, 0.468 mol) in acetone (350 mL), and the mixture was stirred for 48 h. The solid formed was separated, washed with acetone, and after drying (170 g, 92%), used directly in the following step. The NMR analysis of this solid showed a mixture 80:20 of **6a**/**7a** (**7a**, δ 6.75 pyrCH_2CN). Recrystallization of a sample from acetone/Et_2O provided an analytical sample of **6a** as a white solid: mp 140–141 °C; IR (KBr) ν 3427, 2974, 2923, 1747, 1685, 1422, 1275, 1146, 877 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.68 (broad d, J = 4.7 Hz, 1H, pyr), 8.18 (broad d, J = 7.5 Hz, 1H, pyr), 7.85 (d de t, J = 7.5, 1.6 Hz, 1H, pyr), 7.46 (dd, J = 7.5, 4.7 Hz, 1H pyr), 5.91 (s, 2H, CH_2pyr), 5.42 (s, 2H, CH_2CN), 4.32–3.84 (complex signal, 8H, pip), 1.47 (s, 9H, CH_3). Anal. ($C_{17}H_{25}N_4O_2Br$) C, H, N.

1-(*tert*-Butoxycarbonyl)-4-((2-methyl-3-pyridyl)cyanomethyl)piperazine (8a). DBU (68.35 mL, 0.426 mol) was added to a solution of the crude obtained in the last step (170 g) in CH_3CN (500 mL). The mixture was stirred at room temperature for 18 h. After the solvents were removed, the residue was crystallized from CH_3CN to yield 70 g (65%) of a creamy solid: mp 186.6–186.7 °C; ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 8.53 (dd, J = 4.9, 1.7 Hz, 1H, pyr), 7.82 (dd, J = 7.8, 1.7 Hz, 1H, pyr), 7.21 (dd, J = 7.8, 4.9 Hz, 1H, pyr), 4.90 (s, 1H, CHCN), 3.43 (m, 4H, pip), 2.55 (m, 7H, pip, CH_3 pyr), 1.46 (s, 9H, CH_3); ^{13}C NMR (20.15 MHz, $CDCl_3$) δ (TMS) 157.96 (C), 154.90 (C), 149.52 (CH), 136.08 (CH), 126.41 (C), 120.93 (CH), 114.18 (C), 79.98 (C), 60.09

Table IV. Characterization and PAF Antagonist Activity of Compounds 42–55



compd	R	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced mortality ID_{50},[c] mg/kg po	mp °C	solvents[d]	formula[e]	anal.[f]
42		0.017 (0.013–0.022)	0.039 (0.032–0.047)	0.69 (0.28–1.7)	192–194	A	$C_{27}H_{28}N_4O_2 \cdot 1/4H_2O$	C, H, N
43		0.046 (0.028–0.075)	0.12 (0.06–0.22)	>3	88–91	E	$C_{30}H_{32}N_4O_3 \cdot 1/4H_2O$	C, H, N
44		0.026 (0.012–0.056)	0.34 (0.18–0.63)	>3	68–72	E	$C_{29}H_{31}FN_4O \cdot H_2O$	C, H, N
45		0.075 (0.060–0.090)	0.051 (0.025–0.103)	>3	72–76	E	$C_{27}H_{26}F_2N_4O_2 \cdot 1/2H_2O$	C, H, N
46		0.032 (0.023–0.044)	0.042(0.026–0.067)	1.0 (0.8–1.3)	101–105	E	$C_{26}H_{27}N_5O_2 \cdot 1/2H_2O \cdot 1.3Et_2O$	C, H, N
47		0.014 (0.011–0.018)	0.013 (0.008–0.019)	0.44 (0.29–0.68)	72–75	E	$C_{22}H_{23}F_3N_4O_2$	C, H, N
48		0.036 (0.021–0.062)	0.029 (0.019–0.044)	>3	58–62	E	$C_{22}H_{26}N_4O_2 \cdot 1/4H_2O$	C, H, N
49		0.018 (0.016–0.020)	0.015 (0.008–0.029)	0.90 (0.51–1.6)	70–73	E	$C_{27}H_{28}N_4O \cdot 1/4H_2O$	C, H, N
50		0.080 (0.069–0.120)	0.028 (0.016–0.049)	>3	65–71	E	$C_{28}H_{30}N_4O$	C, H, N
51		0.032 (0.021–0.048)	0.039 (0.026–0.058)	>3	105–109	E	$C_{22}H_{26}N_4O \cdot 1/4H_2O$	C, H, N
52		0.012 (0.0083–0.018)	0.023 (0.020–0.027)	>3	58–62	E	$C_{22}H_{23}F_3N_4O \cdot 1/2H_2O$	C, H, N
53		0.026 (0.019–0.036)	0.038 (0.031–0.046)	2.2 (1.3–3.5)	76–79	E	$C_{27}H_{26}N_4O \cdot 1/2H_2O$	C, H, N
54		0.12 (0.078–0.18)		>3	212–213	E	$C_{22}H_{21}F_3N_4O \cdot 1/4H_2O$	C, H, N
55		0.055 (0.041–0.073)	0.12 (0.10–0.15)	>3	152–155	E	$C_{22}H_{21}F_3N_4O$	C, H, N

[a–c] See footnote Table I. [d–f] See footnote Table II.

(CHCN), 49.29 (CH_2), 43.24 (CH_2), 28.33 (CH_3), 21.96 (CH_3 pyr). Anal. ($C_{17}H_{24}N_4O_2$) C, H, N.

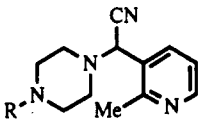
1-((2-Methyl-3-pyridyl)cyanomethyl)piperazine (3a). To a cooled solution (0 °C) of **8a** (41.8 g, 0.13 mol) in $CHCl_3$ (330 mL) was added dropwise 6.5 N HCl(g)/dioxane solution (256 mL). After the addition was completed, the cooling bath was removed, and the mixture was stirred at room temperature for 1 h. It was then evaporated, and the residue was partitioned between 1 N NaOH (140 mL) and $CHCl_3$. The aqueous phase was reextracted twice with $CHCl_3$, and the combined organic extracts were dried (Na_2SO_4) and evaporated to give 30 g of a creamy solid (99%): IR (film) ν 3269, 2944, 2910, 1569, 1438, 1322, 1119, 1101 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.52 (dd, J = 4.8, 1.4 Hz, 1H, pyr), 7.83 (dd, J = 7.8, 1.4 Hz, 1H, pyr), 7.19 (dd, J = 7.8, 4.8 Hz, 1H, pyr), 4.84 (s, 1H, CHCN), 2.88 (m, 4H, pip), 2.64 (s, 3H, CH_3), 2.58 (m, 4H, pip), 1.75 (s, 1H, NH).

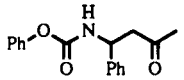
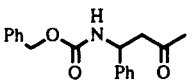
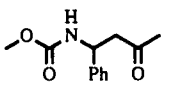
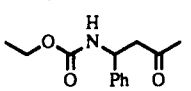
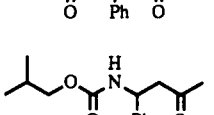
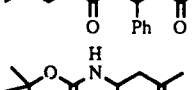
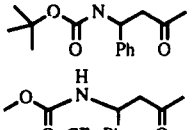
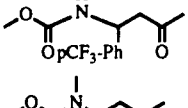
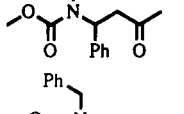
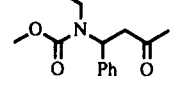
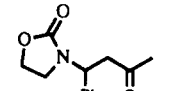
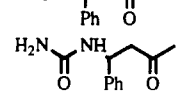
1-(*tert*-Butoxycarbonyl)-4-((ethoxycarbonyl)methyl)-4-((2-pyridyl)methyl)piperazinium Bromide (6b). Ethyl bromoacetate (32 mL) was added to a solution of **5** (80.4 g, 0.29 mol) in CH_3CN (80 mL), and the mixture was stirred for 48 h. After the solvents were removed, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH 5%) to yield an oil (36 g, 28 %): IR (film) ν 3417, 2973, 1738, 1691, 1485, 1469 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.65 (broad d, J = 4.6 Hz, 1H, pyr), 8.09 (m, 1H, pyr), 7.83 (t of d, J = 1.7, 7.5 Hz, 1H, pyr), 7.31 (m, 1H, pyr), 5.50 (s, 2H, CH_2pyr), 4.98 (s, 2H, CH_2COOEt), 4.4–3.7 (complex signal, 10H, pip, OCH_2CH_3), 1.39 (s, 9H, $C(CH_3)_3$), 1.30 (t, J = 6.8 Hz, 3H, OCH_2CH_3).

1-(*tert*-Butoxycarbonyl)-4-((2-methyl-3-pyridyl)(ethoxycarbonyl)methyl)piperazine (8b) was prepared as described above for **8a** by rearrangement of **6b**. After purification by chromatography on silica gel ($CHCl_3$/MeOH 2%), a colorless oil was obtained (43% yield): IR (film) ν 2971, 1735, 1691, 1416 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.43 (dd, J = 4.6, 1.7 Hz, 1H, pyr), 7.86 (dd, J = 7.5, 1.7 Hz, 1H, pyr), 7.15 (dd, J = 7.8, 4.8 Hz, 1H, pyr), 4.28 (s, 1H, CHCOOEt), 4.07 (q, J = 6.8 Hz, 2H, OCH_2CH_3), 3.40 (m, 4H, pip), 2.65 (s, 3H, CH_3), 2.46 (m, 4H, pip), 1.44 (s, 9H, $C(CH_3)_3$), 1.20 (t, J = 6.8 Hz, 3H, OCH_2CH_3).

1-(*tert*-Butoxycarbonyl)-4-(2-propyn-1-yl)-4-((2-pyridyl)-methyl)piperazinium Phenylsulfonate (6c). To a (0 °C) cooled solution of **5** (8 g, 0.029 mol) in CH_3CN (10 mL) was added propargyl phenylsulfonate (4.55 mL), and the solution was stirred at room temperature for 72 h. After concentration to dryness, the resulting residue was purified by chromatography on silica

Table V. Characterization and PAF Antagonist Activity of Compounds 56–67



compd	R	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced mortality ID_{50},[c] mg/kg po	mp, °C	solvents[d]	formula[e]	anal.[f]
56		0.016 (0.012–0.021)	0.036 (0.020–0.063)	>3	97–103	E	$C_{28}H_{29}N_5O_3 \cdot 1/2H_2O$	C, H, N
57		0.022 (0.013–0.038)	0.018 (0.012–0.027)	>3	73–76	E	$C_{29}H_{31}N_5O_3 \cdot 1/4H_2O$	C, H, N
58		0.041 (0.031–0.055)	0.015 (0.011–0.021)	0.044 (0.023–0.091)	150–151	EA	$C_{23}H_{27}N_5O_3 \cdot 1/2H_2O$	C, H, N
59		0.014 (0.012–0.016)	0.025 (0.020–0.031)	0.23 (0.07–0.67)	78–84	E	$C_{24}H_{29}N_5O_3 \cdot 1/2H_2O$	C, H, N
60		0.036 (0.021–0.061)	0.017 (0.010–0.028)	>3	70–74	E	$C_{26}H_{33}N_5O_3 \cdot 1/2H_2O$	C, H, N
61		0.056 (0.049–0.064)	0.049 (0.030–0.081)	1.2 (0.63–2.2)	88–90	E	$C_{26}H_{33}N_5O_3 \cdot 1/4H_2O$	C, H, N
62		0.012 (0.011–0.013)	0.014 (0.012–0.017)	1.6 (0.46–5.6)	90–94	E	$C_{24}H_{26}F_3N_5O_3 \cdot 1/2H_2O$	C, H, N
63		0.020 (0.018–0.022)	0.036 (0.030–0.043)	1–2	68–72	E	$C_{24}H_{29}N_5O_2 \cdot 3/2H_2O$	C, H, N
64		0.068 (0.057–0.081)	0.066 (0.054–0.081)	1–3	88–92	E	$C_{30}H_{33}N_5O_3 \cdot 3/4H_2O$	C, H, N
65		0.063 (0.030–0.10)	0.029 (0.018–0.047)	0.3–1	87–90	E	$C_{24}H_{27}N_5O_3 \cdot 3/2H_2O$	C, H, N
66		0.47 (0.40–0.64)		>3	115–118	E	$C_{22}H_{28}N_6O_2 \cdot 5/4H_2O$	C, H, N
67		0.064 (0.048–0.085)	0.055 (0.036–0.084)	0.97 (0.33–2.8)	98–101	E	$C_{26}H_{32}N_6O_2 \cdot 7/4H_2O$	C, H, N

[a–c] See footnote Table I. [d–f] See footnote Table II.

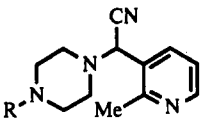
gel ($CHCl_3$/MeOH 10%) to yield 5.9 g (43%) of **6c** as a dark oil: IR (film) ν 3443, 3197, 2973, 2122, 1691, 1585, 1416, 1213, 1192, 1016 cm^{-1}; ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 8.61 (broad d, J = 5 Hz, 1H, pyr), 7.90 (m, 4H), 7.30 (m, 4H), 5.12 (s, 2H, CH_2pyr), 4.76 (d, J = 2.1 Hz, 2H, CH_2CCH), 3.89 (s, 8H, pip), 2.88 (t, J = 2 Hz, 1H, CCH), 1.46 (s, 9H, CH_3).

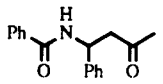
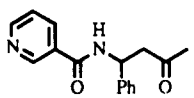
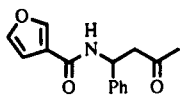
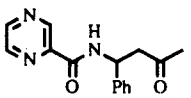
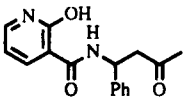
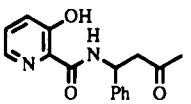
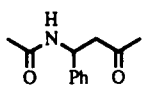
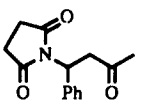
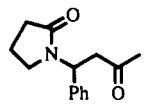
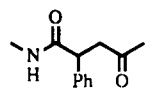
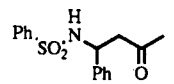
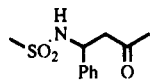
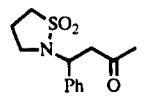
1-(*tert*-Butoxycarbonyl)-4-(1-(2-methyl-3-pyridyl)-2-propyn-1-yl)piperazine (8c). $KOBu^t$ (1.25 g) was added to a cooled solution (−10 °C) of **6c** (3 g, 6.8 mmol) in THF (15 mL) and DMSO (3 mL), and the mixture was stirred at room temperature for 18 h. After addition of water, extraction with $CHCl_3$, and evaporation of the solvents, 1.3 g of a crude were obtained. This crude was purified by chromatography on silica gel (EtOAc) to give 250 mg (12%) of **8c** and 300 mg (14%) of **10c** as oils. **8c**: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.42 (dd, J = 4.8, 1.5 Hz, 1H, pyr), 7.89 (dd, J = 7, 1.5 Hz, 1H, pyr), 7.16 (dd, J = 7, 4.8 Hz, 1H, pyr), 4.71 (d, J = 2 Hz, 1H, $CHCCH$), 3.40 (m, 4H, pip), 2.66 (s, 3H, CH_3 pyr), 2.65 (d, J = 2 Hz, 1H, CCH), 2.47 (m, 4H, pip), 1.46 (s, 9H, CH_3 (BOC)). **10c**: ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 8.53 (broad d, J = 4.6 Hz, 1H, pyr), 7.61 (t of d, J = 7.7, 1.9 Hz, 1H, pyr), 7.16 (broad t, J = 7 Hz, 1H, pyr), 3.98 (t of d, J = 2.2, 7.8 Hz, 1H, $CHCCH$), 3.49 (m, 4H, pip), 3.12 (m, 2H, CH_2pyr), 2.65 (m, 4H, pip), 2.26 (d, J = 2.1 Hz, 1H, CCH), 1.47 (s, 9H, CH_3 (BOC)).

1-(3,3-Diphenylpropionyl)-4-(2-(methoxyimino)propyl)-4-((2-pyridyl)methyl)piperazinium Iodide (6d). To a cooled mixture (ice bath) of 1-(3,3-diphenylpropionyl)-4-(2-pyridylmethyl)piperazine (1.675 g, 2.6 mmol) and KI (0.44 g) in CH_3CN (15 mL) was added chloroacetone O-methyloxime (0.316 g, 2.6 mmol) in CH_3CN (1 mL) dropwise. The mixture was stirred for 48 h and then concentrated to dryness. The resulting crude was purified by chromatography on silica gel ($CHCl_3$/MeOH 5%) to give 0.33 g (21%) of **6d**: IR (film) ν 3483, 3019, 2931, 1642, 1585, 1433, 1246, 1045, 1032, 753, 736, 702 cm^{-1}; ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 8.63 (broad d, J = 3.9 Hz, 1H, pyr), 8.08 (broad d, J = 8 Hz, 1H, pyr), 7.82 (d of t, J = 8, 1.7 Hz, 1H, pyr), 7.23 (m, 11H, pyr+Ph), 5.20 (s, 2H, CH_2pyr), 4.57 (m, 3H, Ph_2CH, $CH_2C{=}N$), 3.41–3.95 (complex signal, 11H, OCH_3, pip), 3.11 (d, J = 7.5 Hz, 2H, Ph_2CHCH_2), 2.13 (s, 3H, CH_3).

1-(3,3-Diphenylpropionyl)-4-(1-(2-methyl-3-pyridyl)-2-(methoxyimino)propyl)piperazine (23) was prepared as de-

Table VI. Characterization and PAF Antagonist Activity of Compounds 68–80



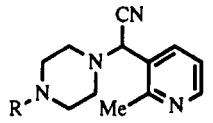
compd	R	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced mortality ID_{50},[c] mg/kg po	mp, °C	solvents[d]	formula[e]	anal.[f]
68		0.0091 (0.0062–0.014)	0.056 (0.048–0.065)	>3	103–105	E	$C_{28}H_{29}N_5O_2 \cdot H_2O$	C, H, N
69		0.0089 (0.0065–0.012)	0.029 (0.025–0.033)	0.41 (0.28–0.61)	89–93	E	$C_{27}H_{28}N_6O_2 \cdot 2H_2O$)	C, N, H[g]
70		2.6 (1.8–3.9)			74–76	EA/H	$C_{26}H_{27}N_5O_3 \cdot 1/2H_2O \cdot 1.3EtOAc$	C, H, N
71		0.82 (0.54–1.23)			94–97	EA/Et_2O	$C_{26}H_{27}N_7O_2 \cdot 3/4H_2O \cdot 1.3EtOAc$	C, H, N
72		0.029 (0.024–0.035)	0.12 (0.080–0.17)	>3	129–134	EA/A/Et_2O	$C_{27}H_{28}N_6O_3 \cdot 3/2H_2O \cdot 1/3EtOAc$	C, H, N
73		0.13 (0.11–0.15)	0.036 (0.023–0.055)	2–3	89–92	EA/Et_2O	$C_{27}H_{28}N_6O_3 \cdot 5/4H_2O \cdot 1.3EtOAc$	C, H, N
74		0.031 (0.028–0.051)	0.035 (0.029–0.043)	0.090 (0.050–0.17)	88–92	E	$C_{23}H_{27}N_5O_2 \cdot H_2O$	C, H, N
75		0.069 (0.056–0.084)	0.042 (0.028–0.063)	0.71 (0.29–1.8)	90–95	E	$C_{25}H_{27}N_5O_3 \cdot H_2O$	C, H, N
76		0.030 (0.018–0.050)	0.026 (0.018–0.039)	0.13 (0.054–0.32)	172–175	EA	$C_{25}H_{29}N_5O_2 \cdot 1/2H_2O$	C, H, N
77		15.2 (8.4–27.3)	0.050 (0.030–0.083)	0.78 (0.13–4.8)	104–108	E	$C_{23}H_{27}H_5O_2 \cdot 5/4H_2O$	C, H, N[h]
78		0.018 (0.015–0.022)	0.04 (0.036–0.045)	>3	85–86	E	$C_{27}H_{29}N_5O_3S \cdot 1/2H_2O$	C, H, N
79		0.021 (0.103–0.032)	0.017 (0.010–0.029)	1.0 (0.38–2.9)	79–83	E	$C_{22}H_{27}N_5O_3S \cdot 1/2H_2O$	C, H, N
80		0.17 (0.10–0.27)	0.048 (0.032–0.071)	>3	85–87	E	$C_{24}H_{29}N_5O_3S \cdot 3H_2O$	C, N, H[i]

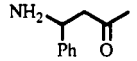
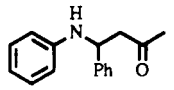
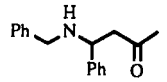
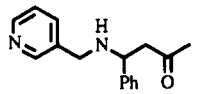
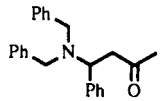
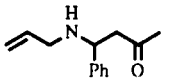
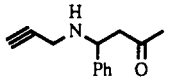
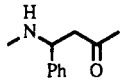
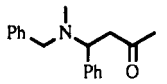
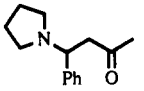
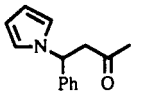
[a–c] See footnote Table I. [d–f] See footnote Table II. [g] H: calcd, 6.39; found, 5.75. [h] N: calcd, 16.36; found, 15.71. [i] H: calcd, 6.76; found, 5.84.

scribed above for **8a** by rearrangement of **6d**. After purification by chromatography on silica gel (EtOAc), 23 mg (10%) of **23** and 15 mg (7%) of **10d** were obtained. **23**: mp 49.7–54.3 °C; ^{1}H NMR (80MHz, $CDCl_3$) δ ppm (TMS) 8.39 (dd, J = 4.5, 1.6 Hz, 1H, pyr), 7.84 (dd, J = 7.3, 1.6 Hz, 1H, pyr), 7.23 (m, 11H, pyr, Ph), 4.66 (t, J = 7.5 Hz, 1H, Ph_2CH), 4.00 (s, 1H, CHN), 3.85 (s, 3H, OCH_3), 3.45 (m, 4H, pip), 3.00 (d, J = 7.4 Hz, 2H, CH_2CO), 2.59 (s, 3H, CH_3pyr), 2.20 (m, 4H, pip), 1.63 (s, 3H, $CH_3C{=}N$). Anal. ($C_{29}H_{34}N_4O_2 \cdot H_2O$) C, H, N. **10d**: ^{1}H NMR (80 MHz, $CDCl_3$) δ ppm (TMS) 8.50 (dd, J = 4.5, 1.6 Hz, 1H, pyr), 7.58 (t of d, J = 7.7, 1.6 Hz, 1H, pyr), 7.23 (m, 11H, pyr, Ph), 4.65 (t, J = 7.5 Hz, 1H, $CHCH_2$), 3.74 (s, 3H, OCH_3), 3.6–3.15 (complex signal, 7H, pip, $CHCH_2$pyr), 3.00 (d, J = 7.4 Hz, 2H, CH_2CO), 2.35 (m, 4H, pip), 1.76 (s, 3H, $CH_3C{=}N$).

(*R*)-2-(*N*-(Diphenylmethyl)amino)propanoic Acid (36A). Method B. A solution of D-alanine (4.45 g, 50 mmol) in water (10 mL) was added to a solution of benzophenone (9.1 g, 50 mmol) in MeOH (100 mL). $NaBH_3CN$ (4.7 g, 75 mmol) was added in batches to this mixture heated to 90 °C. After being refluxed for 18 h, the mixture was concentrated, and water (100 mL) and 10% NaOH (20 mL) were added. The resulting solution was washed with Et_2O and adjusted to pH 5 with 10% HCl aqueous solution. The precipitated solid was collected and dried (2.6 g, 20%): mp 204–206 °C; IR(KBr) ν 3500–2500, 1614, 1583, 1565, 1387, 1360 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.39 (broad s, 10 H), 5.21 (s, 1H, Ph_2CH), 3.80 (s, NH, OH), 3.38 (q, J = 7.3 Hz, 1H, $CHCH_3$), 1.40 (d, J = 7.2 Hz, 3H, $CHCH_3$). Anal. ($C_{16}H_{17}NO_2 \cdot 3/2H_2O$) C, H, N.

Table VII. Characterization and PAF Antagonist Activity of Compounds 81–91



compd	R	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced morality ID_{50},[c] mg/kg po	mp, °C	solvents[d]	formula[e]	anal./
81		0.74 (0.55–0.99)	0.92 (0.72–1.18)	>3	40–46	E	$C_{21}H_{25}N_5O\cdot 3/4H_2O$	C, H, N
82		0.028 (0.020–0.041)	0.042 (0.037–0.048)	0.27 (0.074–0.98)	158–162	E	$C_{27}H_{29}N_5O\cdot 1.2H_2O$	C, H, N
83		0.0091 (0.0057–0.0150)	0.035 (0.024–0.051)	1.2 (0.80–2.0)	68–72	E	$C_{28}H_{31}N_5O\cdot 1/2H_2O$	C, H, N
84		0.075 (0.064–0.088)	0.029 (0.020–0.042)	>3	58–64	E	$C_{27}H_{30}N_6O\cdot 3/4H_2O$	C, H, N
85		0.041 (0.028–0.060)	0.068 (0.053–0.088)	>3	79–90	E	$C_{35}H_{37}N_5O\cdot 1.2H_2O$	C, H, N
86		0.018 (0.015–0.022)		1.0 (0.94–1.2)	155–159	EA/Et_2O	$C_{24}H_{29}N_5O\cdot 3HCl\cdot H_2O$	C, H, N
87		0.27 (0.20–0.37)		2–3	60–63	E	$C_{22}H_{27}N_5O\cdot 1/2H_2O$	C, H, N
88		0.011 (0.0093–0.014)	0.067 (0.052–0.087)	1–3	38–42	E	$C_{22}H_{27}N_5O\cdot 1/2H_2O$	C, H, N
89		0.020 (0.016–0.025)	0.024 (0.015–0.040)	>3	152–155	EA/Et_2O	$C_{29}H_{33}N_5O\cdot 2HCl\cdot 2H_2O$	C, H, N
90		0.018 (0.018–0.020)	0.22 (0.13–0.36)		58–61	E	$C_{25}H_{31}N_5O\cdot 1/2H_2O$	C, H, N
91		0.35 (0.31–0.41)	0.014 (0.012–0.017)	0.3–1	71–72	E	$C_{25}H_{27}N_5O\cdot 3/4H_2O$	C, H, N

[a–c] See footnote Table I. [d–f] See footnote Table II.

Table VIII. In Vivo Activity in Active Anaphylactic Shock (AAS) and Endotoxin-Induced Mortality (EIM) Tests

	AAS (mice) ID_{50},[a] mg/kg		EIM (mice) ID_{50},[b] mg/kg		EIM (rats) ID_{50},[b] mg/kg	
compd	iv	po	iv	po	iv	po
26	0.51	2.7	1.4	9.0	0.28	1.4
58	0.19	2.7	1.7	7.5	0.11	1.0
WEB-2086	1.7	0.8	1.7	13.2	0.47	1.4

[a] Dose required to inhibit antigen-induced mortality by 50%. [b] Dose required to inhibit endotoxin-induced mortality by 50%.

The absence of racemization was verified by preparation of the **α-methylbenzylamide** with D-(+)-α-methylbenzylamine: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.17 (broad s, 16 H), 5.05 (qint, J = 1.8 Hz, 1H), 4.63 (s, 1H, Ph_2CH), 3.08 (q, J = 7.1 Hz, 1H), 1.92 (s, 1H), (s, 1H), 1.38 (d, J = 7.1 Hz, 3H), 1.22 (d, J = 7.1 Hz, 3H).

The procedure was repeated with L-alanine to give **(*S*)-2-(diphenylmethylamino)propanoic acid (35A)** as a white solid: mp 198–199 °C. Anal. $C_{16}H_{17}NO_2\cdot H_2O$: C, H, N. **α-Methylbenzylamide**: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.28 (broad s, 16 H), 5.10 (qint, J = 1.8 Hz, 1H), 4.79 (s, 1H, Ph_2CH), 3.21 (q, J = 7.1 Hz, 1H), 1.99 (s, 1H), 1.42 (d, J = 7.1 Hz, 3H), 1.31 (d, J = 7.1 Hz, 3H).

***N*-Formyl-*N*-(diphenylmethyl)glycine (33A).** A mixture of (diphenylmethyl)amine (5 g, 27 mmol) and glyoxylic acid monohydrate (5.4 g, 59 mmol) in formic acid (25 mL) was held at 70 °C for 90 min. After the solvent was removed, the residue was partitioned between water and $CHCl_3$ and the aqueous phase reextracted twice with $CHCl_3$. The organic phases were dried and evaporated to give an orange solid (7.2 g) which was purified by flash chromatography ($CHCl_3$/MeOH, 3%) to afford a white solid (5.3 g, 74%). An analytical sample was obtained by crystallization from EtOAc/hexane: mp 117–118 °C; IR(KBr) ν 3200–3500, 1730, 1658, 1631, 1388 cm^{-1}; ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 8.13 (s, 1H, CHO), 7.30 (m, 10H), 6.58 (m, OH), 5.90 (s, 1H, Ph_2CH), 4.12 (s, 2H, CH_2CO). Anal. ($C_{16}H_{15}NO_3$) C, H, N.

(*N*-(Diphenylmethyl)-*N*-(methylsulfonyl)amino)acetic Acid (32A). To a suspension of 50% NaH (0.7 g, 0.014 mol) in DMF (10 mL) was added dropwise a solution of *N*-(diphenylmethyl)methanesulfonamide (16) (3.36 g, 0.018 mol) in DMF (5 mL). After the addition was completed, ethyl bromoacetate (1.5 mL, 0.014 mol) was added, and the mixture was stirred at 60 °C for 18 h. DMF was removed, and the resulting residue was worked up with phosphate buffer and EtOAc and extracted two more times with EtOAc. The organic phase was dried (Na_2SO_4) and the solvent evaporated to afford a crude product (4 g) that was purified by chromatography on silica gel (hexane:EtOAc, 20%) to give 1.47 g (30%) of ethyl (*N*-diphenylmethyl)-*N*-(methyl-

sulfonyl)amino)acetate. To a solution of this compound (1.47 g) in THF (5 mL) was added a 5% NaOH aqueous solution (7 mL), and the mixture was stirred at 70 °C for 1 h. Water (10 mL) was added, and the resulting solution was extracted with Et_2O. The aqueous phase was acidified with 1 N HCl and extracted with $CHCl_3$. The organic phase was dried (Na_2SO_4), and the solvents were removed to afford a crude (0.9 g) that was purified by chromatography on silica gel ($CHCl_3$/MeOH, 5%) to give 0.98 g (73%) of **32A**: mp 153–154 °C: IR(KBr) ν 3500–2400, 1723, 1313, 1231, 1165, 1145, 1063, 941 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.96 (complex signal, 1H, COOH), 7.31 (s, 10H, Ph), 6.32 (s, 1H, Ph_2CH), 4.11 (s, 2H, CH_2CO), 3.05 (s, 3H, SO_2CH_3). Anal. ($C_{16}H_{17}NO_4S \cdot 1/4H_2O$), C, H, N, S.

((Diphenylmethyl)thio)acetic Acid (28A). A mixture of diphenylmethanol (1.85 g, 10 mmol) and mercaptoacetic acid (0.7 mL, 10 mmol) in TFA (10 mL) was stirred at room temperature for 0.5 h. Upon removal of the solvent, a white solid was obtained (quantitative yield): mp 123–124 °C; IR (KBr) ν 3100–2500, 1692, 1485, 1446, 1301, 1138 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.46 (m, 1H), 7.31 (m, 10H), 5.41 (s, 1H, Ph_2CH), 3.08 (s, 2H, CH_2CO). Anal. ($C_{15}H_{14}O_2S \cdot 1/4H_2O$): C, H, N, S.

(Diphenylmethoxy)acetic acid (27A) was prepared as described for **28A**, starting from glycolic acid with a reaction time of 24 h: IR (KBr) ν 3200–2500, 1721, 1488, 1445, 1244 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.86 (m, 1H), 7.27 (m, 10H), 5.53 (s, 1H, Ph_2CH), 4.11 (s, 2H, CH_2CO).

((Diphenylmethyl)sulfinyl)acetic Acid (29A). To a solution of **28A** (1g, 3.8 mmol) in MeOH (10 mL) was added 0.9 mL of a solution prepared mixing 2-propanol (3g) and 96% H_2SO_4 (0.15 mL). After the solution was stirred for 5 min, 30% wt H_2O_2 (0.82 mL) was added, and the mixture was stirred at room temperature for 1 h. It was then poured into saturated aqueous NaCl solution (20 mL) and extracted with $CHCl_3$ (3 × 20 mL). The combined extracts were dried (Na_2SO_4) and evaporated to give a crude which was purified by flash chromatography ($CHCl_3$/MeOH, 5%) to afford a white solid (0.7 g, 64%): IR (KBr) ν 3100–2500, 1718, 1447, 1411, 1210 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.42 (m, 10H), 5.27 (s, 1H, Ph_2CH), 4.07 (s, 1H), 3.46 (s, 2H, CH_2CO); mass spectrum m/z 167 ($C_{13}H_{13}$), 105 ($C_2H_3SO_3$). Anal. ($C_{15}H_{14}SO_3 \cdot H_2O$) C, H, N.

((Diphenylmethyl)sulfonyl)acetic Acid (30A). To a suspension of **28A** (1 g, 3.8 mmol) in acetic acid (6 mL) was added 30% wt H_2O_2, and the resultant solution was stirred overnight at room temperature. After removal of the solvents, the residue was partitioned between water and $CHCl_3$. The organic phase was separated, dried (Na_2SO_4), and evaporated to give a crude that was purified by chromatography on silica gel ($CHCl_3$/MeOH, 5%) to give 0.6 g (54%) of product: IR (KBr) ν 3200–2500, 1726, 1490, 1448, 1308, 1219 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.70 (m, 4H), 7.36 (m, 6H), 6.02 (s, 1H, Ph_2CH), 3.95 (s, OH), 3.82 (s, 2H, CH_2CO); mass spectrum m/z 167 ($C_{13}H_{13}$), 123 ($C_2H_3SO_4$).

3-Fluoro-3,3-diphenyl-2,2-dimethylpropanoic Anhydride (18). (Diethylamino)sulfur trifluoride (DAST) (0.6 mL, 4.5 mmol) was added to a cooled solution (0 °C) of 3-hydroxy-3,3-diphenyl-2,2-dimethylpropanoic acid (17)[11] (1g, 3.7 mmol) in CH_2Cl_2 (15 mL), and the mixture was stirred at 0 °C for 2 h. Then, cooled water (15 mL) was added and the mixture stirred for 0.5 h. It was then extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated in vacuo to an oil (1g, quantitative yield), which was used directly in the following step: IR(KBr) ν 2975, 1823, 1487, 1254, 1180 cm^{-1}; ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 7.37 (m, 10H), 1.47 (broad s, 3H), 1.22 (broad s, 3H).

3-Hydroxy-3-phenyl-3-(trifluoromethyl)propanoic Acid (47A). Method C. To a cooled solution (ice bath) of 1.6 M n-BuLi in hexane (40 mL) in THF (90 mL) was added dropwise diisopropylamine (9.45 mL), and the solution was stirred for 5 min. Keeping the solution at 0 °C, acetic acid (1.92 mL, 33.6 mmol) was added dropwise and the mixture was stirred for 10 min and then heated to 50 °C, held at that temperature for 30 min, and finally allowed to cool. A solution of 2,2,2-trifluoroacetophenone (4.76 mL, 33.6 mmol) in THF (15 mL) was then added at 0 °C, and the mixture was stirred at room temperature overnight. Finally, Et_2O (150 mL) and water (50 mL) were added, and the aqueous phase was separated, acidified with 1 N HCl, and extracted three times with EtOAc. After removal of the solvents, 3.88 g (49%) of crude **47A** was obtained: IR (KBr) ν 3700–2300, 1702, 1447, 1420, 1265, 1163, 1074, 700 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.0 (complex signal, 2H, COOH, OH), 7.38 (m, 5H, Ph), 3.2 (s, 2H, CH_2CO).

***cis*- and *trans*-3-Phenyl-3-(trifluoromethyl)propenoic Acid (54A and 55A).** To a cooled (0 °C) suspension of 50% NaH (1.5 g, 31.2 mmol) in DME (50 mL) was added triethyl phosphonoacetate (7.1 mL, 30.4 mmol), and the resulting mixture was stirred for 0.5 h at room temperature. Then, 2,2,2-trifluoroacetophenone (5 mL, 35.6 mmol) was added dropwise, and the resulting mixture was refluxed for 1 h. After removal of the solvent, the crude was partitioned between water and Et_2O. The organic solution was dried over Na_2SO_4 and evaporated, affording 8.9 g of a crude that was purified by chromatography on silica gel (hexane/EtOAc, 2%) to give 3.54 g (41%) of ethyl *cis*-3-phenyl-3-(trifluoromethyl)propenoate and 4.0 g (46%) of ethyl *trans*-3-phenyl-3-(trifluoromethyl)propenoate. **Cis isomer**: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.33 (s, 5H, Ph), 6.59 (s, 1H, CH), 4.00 (q, J = 7.1 Hz, 2H, CH_2CH_3), 1.00 (t, J = 7.2 Hz, 3H, CH_2CH_3). **Trans isomer**: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.40 (s, 5H, Ph), 6.32 (s, 1H, CH), 4.30 (q, J = 7.1 Hz, 2H, CH_2CH_3), 1.34 (t, J = 7.2 Hz, 3H, CH_2CH_3). Cis and trans isomers were treated separately with 5 equiv of K_2CO_3 in MeOH/H_2O at 60 °C for 2 h to give **54A** (80%) and **55A** (90%), respectively. **54A (cis isomer)**: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.33 (m, 1H, OH), 7.35 (m, 5H, Ph), 6.57 (s, 1H, CH). **55A (trans isomer)**: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 10.26 (m, 1H, OH), 7.41 (m, 5H, Ph), 6.35 (s, 1H, CH).

3-Phenyl-3-(trifluoromethyl)propanoic Acid (52A). A mixture of **55A** (0.7 g, 3.5 mmol) and Pd/C 5% (0.23 g) in MeOH (40 mL) was hydrogenated at atmospheric pressure for 18 h. The insoluble material was removed by filtration and the filtrate evaporated to give 0.7 g (92%) of a white solid: ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 7.32 (m, 5H, Ph), 6.57 (m, OH), 3.80 (d of t, J = 9.0, 5.8 Hz, 1H, $CHCF_3$), 2.98 (m, 2H, CH_2CO).

3-Phenyl-3-(1-pyrrolyl)propanoic Acid (91A). To a solution of 3-amino-3-phenylpropanoic acid (1.5 g, 8.8 mmol) in glacial AcOH (20 mL) was added 2,5-dimethoxytetrahydrofuran (1.15 mL, 8.8 mmol). The mixture was then refluxed for 1 h. After removal of the solvents, the crude was purified by chromatography on silica gel (hexane/ethyl acetate, 20%) to give an oil (1.04 g, 55%): ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 10.87 (s, 1H, OH), 7.22 (m, 5H, Ph), 6.72 (t, J = 2 Hz, 2H, pyrr), 6.16 (t, J = 2 Hz, 2H, pyrr), 5.63 (t, J = 7.6 Hz, 1H, CHN), 3.22 (d, J = 7.7 Hz, 2H, CH_2CO).

3-(2-Oxo-3-oxazolidinyl)-3-phenylpropanoic Acid (65A). To a cooled (0 °C) solution of 3-amino-3-phenylpropanoic acid (1.5 g, 8.8 mmol) in THF (11 mL), water (11 mL), and 2 N NaOH (11 mL) was added 2-bromoethyl chloroformate (0.95 mL, 8.8 mmol). The mixture was stirred at room temperature for 18 h and then acidified with 5 N HCl and extracted with EtOAc. The organic phase was separated and dried over Na_2SO_4, and the solvent was removed. The resulting crude was dissolved in DMF (25 mL) and cooled to 0 °C. To that solution was added 50% NaH (0.63 g, 16 mmol), and the resulting mixture was heated at 80 °C for 18 h. After the solvents were removed, 0.5 N HCl and EtOAc were added. The organic phase was separated, dried over Na_2SO_4, and evaporated to give a crude that was purified by chromatography on silica gel eluting with EtOAc to afford 0.75 g of (38%) of **65A** as a white solid: ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.11 (s, 1H, OH), 7.34 (m, 5H, Ph), 5.41 (dd, J = 8.9, 6.7 Hz, 1H, CHN), 4.36 (m, 2H), 3.55 (m, 2H), 3.12 (m, 2H).

3-Phenyl-3-(phenylamino)propanoic Acid (82A). To a solution of aniline hydrochloride (3.37 g, 26 mmol) and ethyl benzoylacetate (5 mL, 26 mmol) in MeOH (70 mL) was added $NaBH_3CN$ (1.75 g). After the solution was stirred at room temperature for 18 h, the solvent was removed, and 0.5 N HCl and Et_2O were added. The aqueous phase was separated, basified with 1 N NaOH, and extracted with $CHCl_3$. The organic phase was separated, dried, and evaporated to give a crude that was purified by chromatography on silica gel (hexane/EtOAc, 5%) to afford 4.3 g (62%) of ethyl 3-phenyl-(3-phenylamino)propanoic acid. This compound was subjected to basic hydrolysis (following the procedure described for **54A**) to **82A**: mp 110–111 °C; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.10 (m, 5H, Ph), 6.63 (m, 3H, Ph), 4.85 (t, J = 6.3 Hz, 1H, CHN), 4.05 (m, 2H, OH, NH), 2.85 (d, J = 6.5 Hz, 2H, CH_2CO).

1-(3-(*N*-(*tert*-Butoxycarbonyl)amino)-3-phenylpropanoyl)-4-((2-methyl-3-pyridyl)cyanomethyl)piperazine (61). Method A. To a cooled (0 °C) solution of **61A** (8.6 g, 32.4 mmol), amine **3a** (7g, 32.4 mmol), and HOBT (4.6 g, 34 mmol) in DMF (200 mL) was added DCC (7 g, 34 mmol). The mixture was stirred at room temperature for 18 h. The solvents were then removed in vacuo, and the residue was stirred with EtOAc. The white solid formed was removed, and the organic solution was washed with saturated $NaHCO_3$ solution, water, and brine, dried, and evaporated. The residue (15.6 g) was purified by flash chromatography (EtOAc) to afford a white solid (12.9 g, 86%): mp 88–90 °C; IR (film) ν 3365, 2971, 2923, 1703, 1631, 1438, 1244, 1164, 998, 700 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.53 (dd, J = 4.8, 1.3 Hz, 1H, pyr), 7.78 (broad d, J = 7.7 Hz, 1H, pyr), 7.30 (s, 5H, Ph), 7.20 (d of d, J = 7.7, 4.8 Hz, 1H, pyr), 6.09 (broad d, 1H, NH), 5.04 (m, 1H, C*H*NH), 4.84 (s, 1H, CHCN), 3.52 (m, 2H, pip), 3.23 (m, 2H, pip), 2.86 (d of d, J = 5.4Hz, J = 9.5Hz, 2H, CH_2CO), 2.60 (s, 3H, CH_3 pyr), 2.41 (m, 4H, pip), 1.41 (s, 9H, CH_3 (BOC)). Anal. ($C_{26}H_{33}N_5O_3 \cdot 1/4H_2O$) C, H, N.

1-(3-Phenyl-3-aminopropanoyl)-4-((2-methyl-3-pyridyl)-cyanomethyl)piperazine (81) was obtained as a white solid (98% yield) by N-deprotection of **61** (following the procedure described above for **8a**): mp 40–46 °C; IR (film) ν 3600–3100, 3021, 2908, 1631, 1438, 1224, 1126, 998, 701 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.53 (broad d, J = 4.8 Hz, 1H, pyr) 7.80 (broad d, J = 7.8 Hz, 1H, pyr), 7.35 (s, 5H, Ph), 7.21 (dd, J = 7.8, 4.8 Hz, 1H, pyr), 4.88 (s, 1H, CHCN), 4.52 (t, J = 6.3 Hz, 1H, C*H*NH_2), 3.59 (m, 2H, pip), 3.36 (m, 2H, pip), 2.62 (s, 3H, CH_3-pyr), 2.50 (m, 6H, pip, CH_2CO), 1.96 (s, 2H, NH_2). Anal. ($C_{21}H_{25}N_5O \cdot 3/4H_2O$) C, H, N.

1-(3-(*N*-Benzylamino)-3-phenylpropanoyl)-4-((2-methyl-3-pyridyl)cyanomethyl)piperazine (83). To a solution of amine **81** (13.7 g, 37.7 mmol) and benzaldehyde (4.1 mL, 37.7 mmoL) in MeOH (100 mL) were added a few drops of 2 N $HCl_{(g)}$/MeOH solution to achieve pH = 6–8. Then, $NaBH_3CN$ (2.4 g, 37.7 mmol) was added in portions, and the resulting solution was stirred at 25 °C for 24 h. Additional 2 N $HCl_{(g)}$/MeOH solution was added to hold the pH within a range of 6–8. This was followed by additional $NaBH_3CN$ (1.2 g, 18 mmol) and the stirring continued for 48 h. After removal of the MeOH, the residue was partitioned between 0.1 N NaOH and $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH 2%) to yield an oil (7.5 g, 44 %) which was triturated with Et_2O to yield a white solid: mp 68–72 °C; IR (film) ν 3312, 3019, 2822, 1631, 1437, 1224, 1126, 998, 752, 700 cm^{-1}; ^1H NMR (80MHz, $CDCl_3$) δ (TMS) 8.52 (dd, J = 5Hz, J = 1.4Hz, 1H, pyr), 7.78 (broad d, J = 8.0Hz, 1H, pyr), 7.32 (m, 11H, pyr+Ph), 4.84 (s, 1H, CHCN), 4.19 (t, J = 6.9Hz, 1H, C*H*NH), 3.77 (complex signal, 4H, CH_2NH, pip), 3.43 (m, 2H, pip), 2.55 (complex signal, 10H, pip, CH_2CO, CH_3-pyr, NH). Anal. ($C_{28}H_{31}N_5O \cdot 1/2H_2O$) C, H, N.

1-(3-(*N*-(Methoxycarbonyl)amino)-3-phenylpropanoyl)-4-((2-methyl-3-pyridyl)cyanomethyl)piperazine (58). To a solution of **81** (0.5 g, 1.3 mmol) and Et_3N (0.29 mL) in CH_2Cl_2 (10 mL) was added methyl chloroformate (0.12 mL, 1.5 mmol), and the mixture was stirred at room temperature for 12 h. The resulting solution was diluted with CH_2Cl_2 and treated with 0.1 N NaOH. The organic phase was dried over Na_2SO_4 and evaporated. Purification of the residue (0.49 g) by chromatography on silica gel (EtOAc) gave 0.40 g (68%) of **58**. Crystallization from EtOAc gave a white solid: mp 150–51 °C; IR(KBr) ν 3308, 2945, 1712, 1625, 1438, 1247 cm^{-1}; ^1H NMR (80 MHz, CD_3Cl) δ (TMS) 8.53 (broad d, J = 4 Hz, 1H, pyr), 7.78 (broad d, J = 8.5 Hz, 1H, pyr), 7.30 (m, 6H, pyr, Ph) 6.28 (m, 1H, NH), 5.08 (q, J = 7.2 Hz, 1H, CHN) 4.83 (s, 1H, CHCN), 3.64 (s, 3H, OCH_3), 3.60 (m, 2H, pip), 3.24 (m, 2H, pip), 2.86 (dd, J = 10, 5.5 Hz, 2H, CH_2CO), 2.59 (s, 3H, CH_3 pyr), 2.41 (m, 4H, pip). Anal. ($C_{23}H_{27}N_5O_3 \cdot 1/2H_2O$) C, H, N.

1-(3-(*N*-(Phenoxycarbonyl)amino)-3-phenylpropanoyl)-4-((2-methyl-3-pyridyl)cyanomethyl)piperazine 56 was prepared as described above for **58** by reaction of **81** with phenyl chloroformate: mp 97–103 °C. Anal. ($C_{28}H_{29}N_5O_3 \cdot 1/2H_2O$) C, H, N.

1-(3-(*N*-(1-Pyrrolidinylcarbonyl)amino)-3-phenylpropanoyl)-4-((2-methyl-3-pyridyl)cyanomethyl)piperazine (67). A mixture of **56** (0.2 g, 0.4 mmol) and pyrrolidine (1 mL) in CH_3CN (2 mL) was heated at reflux for 5 h. After the solvents were removed, the residue was partitioned between 0.1 N NaOH and $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH 3%) to yield a white solid (0.12 g, 66%): mp 98–101 °C. Anal. ($C_{26}H_{32}N_6O_2 \cdot 7/4H_2O$), C, H, N.

B. Biological Methods. Inhibition of Platelet Aggregation in Vitro. Platelet-aggregation studies were done by the method of Born.[15] Blood was collected in 3.16% sodium citrate (1 volume for 9 volume of blood) by cardiac puncture from male New Zealand rabbits (2–2.5 kg body weight). Platelet rich plasma (PRP) was prepared by centrifuging the blood at 250*g* for 10 min at 4 °C. The PRP was diluted with platelet-poor plasma obtained by further centrifuging at 3000*g* for 10 min. The platelet number was adjusted to 3.5×10^5 cells/mm^3. Platelet aggregation was induced by C18-PAF (1.5×10^{-8} M) and measured with a dual-channel aggregometer Chrono-log 500. Activity of the inhibitors was expressed as the IC_{50} value, *i.e.*, the concentration required to inhibit platelet aggregatory response by 50%. The values shown in the tables were calculated by linear regression from a single experimental curve with no less than four data points, each point being the mean of the percentage inhibition at a given concentration obtained from one to three independent experiments.

Inhibition of PAF-Induced Hypotension in Normotensive Rats. Hypotension studies were performed as described by Baranes.[16] Male Sprague–Dawley rats, weighing 180–220 g, were anesthetized with sodium pentobarital (50 mg/kg, ip). Blood pressure was recorded from the left carotid artery using a Beckman pressure transducer coupled to a Beckman R611 polygraph. Right and left femoral veins were catheterized to inject PAF (0.5 μg/kg) or the test compound. Test compounds were administered by intravenous injection (1 mL/kg, dissolved in saline) 3 min before PAF injection. Control animals received only the vehicle. Blood pressure was monitored and percentage inhibition of PAF-induced hypotension with respect to controls was calculated. The results were expressed as ID_{50} values, *i.e.*, the dose of test compound required to inhibit the PAF-induced hypotension by 50%. The results were calculated by linear regression from a single experimental curve with no fewer than four points, each point being the mean of the percentage inhibition at a given dose obtained from two or more independent experiments.

Inhibition of PAF-Induced Mortality in Mice.[17] Groups of 10 male Swiss mice weighing 22–26 g were used. One hundred μg/kg of C18-PAF plus 1mg/kg of propanolol was administered through a lateral tail vein 60 min after po administration of the test compounds (20 mL/kg) or 1% Tween 80 (control group). The animals were observed 2 h after the PAF injection. Following this protocol we obtained a consistent mortality of 70–100% in the control group. Percent inhibition of mortality due to treatment in comparison with the control group was calculated. Results were given as ID_{50} values, *i.e.*, the dose required to inhibit PAF-induced mortality by 50%. The results were calculated by linear regression from a single experimental curve with no fewer than four data points.

Inhibition of Active Anaphylactic Shock in Mice.[21] Groups of 10 male Swiss mice weighing 30 g were used. Animals were sensitized by ip injection of 1 mL of saline containing 1 mg of bovine serum albumin (BSA) and *B. pertussis* antigen (Difco, 1:25 v/v). The challenge was made 14 days after sensitization. Anaphylactic shock was caused by 1 mg/kg iv of BSA (in saline) as antigen plus 1 mg/kg of propanolol (administered 20 min before BSA) in a volume of 10 mL/kg through a tail vein. The compounds were administered 5 min (iv) or 30 min (po) prior to the BSA challenge. The survival rate was recorded 60 min after BSA had been injected. Following this protocol we obtained a consistent mortality of 80–100% in the control group. The results were expressed as ID_{50} values, i.e., the dose of the test compound required to inhibit mortality by 50%. The results were calculated by linear regression from a single experimental curve with no fewer than four data points.

Inhibition of Endotoxin-Induced Mortality in Mice and Rats.[22] Groups of 10 male Swiss mice weighing 22–26 g or male Sprague–Dawley rats weighing 150–175 g were used. Twenty mg/kg of endotoxin from *E. coli* 0111:B4 (mice) or 5 mg/kg of

endotoxin from *E. coli* 0127:B8 (rats) was injected through a lateral tail vein 5 min after iv or 30 min after po administration of the test compounds. Vehicle (saline or 1% Tween 80, for iv and po administration, respectively) was administered to control animals. Mortality was recorded 7 days after endotoxin injection. Following this protocol we obtained a consistent mortality of 80–100% in the control group. The results were expressed as ID_{50} values, *i.e.*, the dose of the test compound required to inhibit mortality by 50%. The results were calculated by linear regression from a single experimental curve with no fewer than four data points.

Statistics. Statistical analyses of pharmacological data were made using a standard pharmacology program implemented on an IBM PC.[23]

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