[(3-Pyridylalkyl)piperidylidene]benzocycloheptapyridine Derivatives as Dual Antagonists of PAF and Histamine

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A series of [(3-pyridylalkyl)piperidylidene]- and (nicotinoylpiperidylidene)benzocycloheptapyridine derivatives, **Ia,b**, were prepared and evaluated for PAF antagonist and H_1 antihistamine activity. PAF antagonist activity was investigated by the in vitro PAF-induced platelet aggregation assay (PPA) and the in vivo PAF-induced hypotension test in rats (PH) and mortality test in mice (PM). For the evaluation of H_1 antihistamine activity, the in vitro histamine-induced contraction of the guinea-pig ileum assay (HC) and the in vivo histamine-induced hypotension test (HH) in normotensive rats were used. The potential antiallergic activity of the compounds was evaluated using the active anaphylactic shock test in mice. These compounds are structurally related to loratadine (1) and were generated by replacement of the ethoxycarbonyl group of 1 with substituted 3-pyridylmethyl and nicotinoyl moieties. Both anti-PAF and H_1 antihistamine activities have shown a high dependence on the exact nature and position of the substituent in the pyridine ring. Optimum structure **19** (UR-12592) incorporating a (5-methyl-3-pyridyl)methyl radical displayed an unique dual activity inhibiting both PAF-induced effects (PPA, $IC_{50} = 3.7\ \mu M$; PH, $ID_{50} = 0.44$ mg/kg iv; PM, $ID_{50} = 1.9$ mg/kg po) and histamine-induced effects (HC, $IC_{50} = 3.9$ nM; HH, $ID_{50} = 1.4$ mg/kg iv). Furthermore, **19** was highly active in the passive cutaneous anaphylactic shock in rats ($ID_{50} = 1.2$ mg/kg po) and strongly protected mice and rats from mortality induced by endotoxin ($ID_{50} = 1.2$ and 0.5 mg/kg iv, respectively). Compound **19** showed itself to be devoid of CNS depressant effects, neither modifying spontaneous motor activity nor prolonging barbiturate-sleeping time in mice at a dose of 100 mg/kg po, and is now under development.

Introduction

Allergic and inflammatory diseases are complex, multifactorial processes that involve formation and/or release of many distinct mediators.[1] Histamine is released from mast cells and basophils by antigenic stimulation causing smooth muscle contraction, increased vascular permeability, and mucus formation.[2] PAF is known to provoke bronchoconstriction and increased vasopermeability like histamine and may also be responsible for bronchial hyperreactivity.[3] PAF is also a very potent chemotactic stimulus for eosinophils[4] and is believed to be involved in inducing shock and mediating inflammatory disease. Moreover, PAF and histamine are known to complement each other in vivo, as histamine is a mediator of early response, being released from preformed reservoirs in mast cells, while PAF, which can be regarded as a late-response mediator, is mainly synthesized de novo.[5] Furthermore, each mediator is able to promote the release of the other in some tissues and cells.[6] It thus seems reasonable to infer that the blockade of both PAF and histamine may be of greater clinical efficacy than the blockade of only one or the other, and this is the premise behind the search for new chemical entities which possess a dual activity.

Piwinski and co-workers[7] have recently reported that the replacement of the ethoxycarbonyl group of loratadine (**1**), a known H_1 antihistamine presently in clinical use, by an acetyl group to give SCH-37370 (**2**) conferred

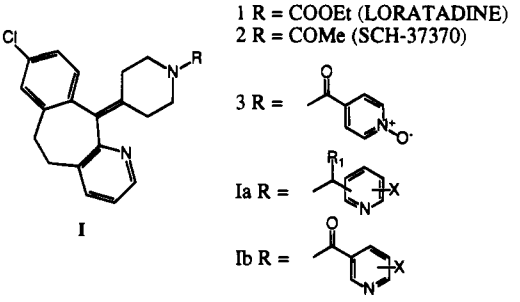

Figure 1.

it a dual character, that is, it retained some antihistamine activity and also possessed an additional PAF antagonist activity not found in the parent compound **1**. Although **2** displays well-balanced dual activity, compounds with increased potencies could be desirable.

While considerable work has been done on the tetracyclic nucleus of **2**,[8] the nature of the acyl substituent remains less explored. The present paper deals with synthesis and PAF antagonist and H_1 antihistamine activity evaluation of two series of benzocycloheptapyridines: 3-pyridylalkyl derivatives **Ia** and nicotinoyl derivatives **Ib**. Exploration of the effect of different pyridine substituents in the two mentioned series led to the discovery of **19**, a more potent dual antagonist. This compound was chosen for further preclinical evaluation.

Chemistry

Most of the compounds of Table **1** were prepared by alkylation of decarbethoxyloratadine (**4**)[7] with the cor-

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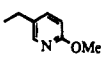
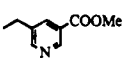
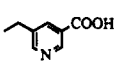
Table 1. PAF Antagonist, Antihistamine, and Antiallergic Activities of Benzocycloheptapyridines of Formula **Ia**

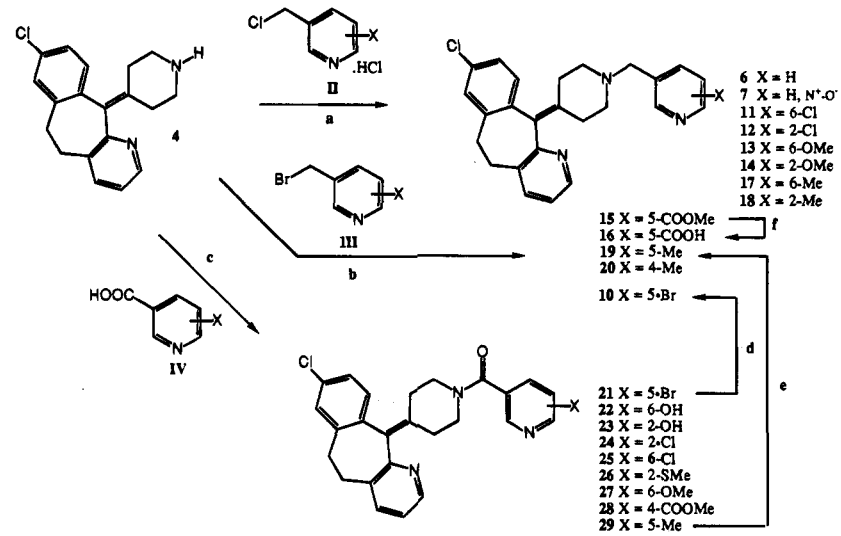
comp	R	PAF antagonist activity: PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv	PAF-induced mortality ID_{50},[c] mg/kg po	Antihistamine and antiallergic activity: AntiH1 activity ileum IC_{50},[d] μM	Histamine-induced hypotension % inh at 10 mg/kg iv	A.A.S. ID_{50}[f] mg/kg po
5		1.8 (1.2-2.7)	>5	30	0.094 (0.059-0.15)	85.1	6.1
6		4.0 (3.4-4.7)	>5	30	0.093 (0.067-0.13)	83.6	6.7
7		14 (10-19)	>5	>30			56% inh at 30 mg/kg
8		8.2 (6.4-9.8)	>5	>30			>30
9		34 (24-50)	>5	>30	0.36 (0.16-0.79)	95.4	>30
10		0.72 (0.57-0.91)	>5	10-30	0.28 (0.24-0.34)	61.9	17
11		>100	>5	20-30			6.9
12		>100	>5	>30	0.35 (0.19-0.66)		4.5
13		37 (22-61)		<30	0.38 (0.27-0.53)		5.9
14		6.0 (4.3-8.5)	>5				17
15		>100	>5		0.19 (0.11-0.33)		
16		85 (65-112)	>5	>30	0.35 (0.29-0.43)		6.5
17		9.0 (4.4-18)	>5	30	0.037 (0.022-0.061)	96.4	12
18		13 (4.4-39)	>5	>30	0.27 (0.23-0.32)		50% inh at 30 mg/kg
19		3.7 (2.8-4.8)	0.44 (0.31-0.65)	1.9 (0.8-4.6)	0.0039 (0.0032-0.0049)	89.0 1.4[e] (0.9-2.0)	13
20		15 (12-19)		10-30	0.035 (0.018-0.068)		56% inh at 30 mg/kg
1 (LORATADINE)		135 (49-375)	>5	>30	0.29 (0.17-0.48)	66.3 4.7[e] (2.1-10)	19
2 (SCH-37370)		0.84 (0.70-0.93)	2.0 (1.1-3.7)	31 (18-53)	0.10 (0.079-0.14)	55.1 1.9[e] (1.1-3.6)	19
3		0.22 (0.19-0.25)	1.9	>30	0.21 (0.17-0.26)	46.2	12
WEB-2086		0.091 (0.071-0.12)	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%. [d] Concentration required to inhibit histamine-induced ileum contraction by 50%. Parentheses contain 95% confidence limits. [e] Dose required to reduce the lowering of the arterial blood pressure caused by histamine by 50%. Parentheses contain 95% confidence limits. [f] Dose required to inhibit mortality in sensitized animals by 50%.

responding substituted (chloromethyl)- or (bromomethyl)pyridine derivatives **II** and **III** (methods A and B, respectively). The chloro derivatives **II** were obtained by treatment of the corresponding 3-(hydroxymethyl)-pyridines with thionyl chloride in CH_2Cl_2. The same methodology was employed to prepare the alkylating agents for compounds **8** and **9**, the 3-(1- and 2-chloroethyl)pyridines. The (hydroxyalkyl)pyridines are commercial or were made according to published procedures.[9]

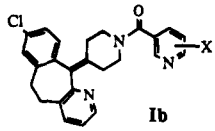
Bromides **III** were prepared by bromination of 3,4- and 3,5-lutidine and 3-(methoxycarbonyl)-5-methylpyr-

Scheme 1[a]



[a] (a) Et_3N, CH_2Cl_2, room temperature, 18 h, method A; (b) DMAP, Et_3N, $CCl_4/CHCl_3$, room temperature, 18 h, method B; (c) DCC, HOBT, DMF, room temperature, 18 h, method C; (d) $LiAlH_4$, THF, room temperature, 18 h; (e) (1) $POCl_3$, 50 °C, 3 h; (2) $NaBH_4$, DME, room temperature, 2.5 h; (f) K_2CO_3, $MeOH/H_2O$, reflux, 12 h.

Table 2. PAF Antagonist, Antihistamine, and Antiallergic Activities of Benzocycloheptapyridines of Formula **Ib**



		PAF antagonist activity			antihistamine and antiallergic activity		
compd	X	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	anti-H_1 activity ileum, IC_{50},[d] μM	histamine-induced hypotension, % inh at 10 mg/kg iv	AAS ID_{50},[f] mg/kg po
21	5-Br	3.4 (2.3–5.0)	>5	>30			>30
22	6-OH	1.5 (1.1–2.0)	1.5 (0.88–2.5)	>3	0.39 (0.19–0.78)	67.0	>30
23	2-OH	>100		>30			56% inh at 10 mg/kg
24	2-Cl	>100			0.13 (0.076–0.22)		>30
25	6-Cl	>100	>1	20 (10–40)	0.064 (0.063–0.066)		1.9
26	2-SMe	20 (15–27)	>5	>30			50% inh at 10 mg/kg
27	6-OMe	>100	>5	30	0.034 (0.023–0.050)		5.5
28	4-COOMe	17 (12–24)	>5	>30			11
29	5-Me	3.1 (1.8–5.4)	>5		0.35 (0.25–0.48)		12

[a–f] See footnotes in Table 1.

idine with NBS in CCl_4 in the presence of AIBN.[10] The carboxylic acid **16** was obtained by saponification of the ester **15**.

The nicotinoyl derivatives of Table 2 were obtained by acylation of **4** with the appropriate carboxylic acid **IV** using HOBT and DCC in DMF (method C). Finally, the reduction of amide **21** with $LiAlH_4$ led to the pyridylmethyl derivative **10**. Chemical and analytical data for all new compounds are provided in Table 3.

For large-scale syntheses of **19**, the following route was chosen, acylation of **4** with 5-methylnicotinic acid[11,12] to give **29** followed by reduction with $POCl_3/NaBH_4$.[13]

Discussion

Compounds were evaluated for PAF antagonist activity using the in vitro PAF-induced platelet aggregation assay,[14] the in vivo PAF-induced hypotension test in normotensive rats,[15] and the PAF-induced mortality test in mice.[16] In the in vivo tests, an initial dose of 5 mg/kg iv (hypotension test) or 30 mg/kg po (mortality test) of the compounds was administered before PAF injection. Only when more than 50% inhibition of mortality or of the hypotensive response was reached were more doses tested to obtain the ID_{50} values. For the evaluation of H_1 antihistamine activity, we used the in vitro histamine-induced contraction of the guinea-pig ileum[17] and the in vivo histamine-induced hypotension test in normotensive rats.[1] In the latter, an initial dose of 10 mg/kg iv was administered before histamine injection and percent inhibition was recorded. Additionally, the potential antiallergic activity of the compounds was evaluated using the active anaphylactic shock (AAS) test in mice.[18] Loratadine (**1**), SCH-37370 (**2**), and a known PAF antagonist, WEB-2086, were used as reference compounds. Also *N*-oxide isonicotinoyl derivative **3** reported in patent literature was synthesized and used as a reference compound because of its structural similarity to our compounds.[19] The results of these

Table 3. Chemical Data for Benzocycloheptapyridines of Tables 1 and 2

compd	method	% yield[a]	mp, °C[b] (cryst solvent)	formula[c]	compd	method	yield[a]	mp °C[b] (cryst solvent)	formula[c]
5	A	76	58–62	$C_{25}H_{24}ClN_3 \cdot {}^5/_2H_2O$	**18**	A	75	82–86	$C_{26}H_{26}ClN_3 \cdot H_2O$
6	A	70	54–58	$C_{25}H_{24}ClN_3 \cdot H_2O$	**19**			213–217 (A)	$C_{26}H_{26}ClN_3 \cdot 3HCl$
7	A	44	90–93	$C_{25}H_{24}ClN_3O \cdot {}^3/_2H_2O$	**20**	B	14	96–100	$C_{26}H_{26}ClN_3 \cdot {}^3/_2H_2O$
8	A	20	210–212 (A)	$C_{26}H_{26}ClN_3 \cdot 3HCl \cdot 3H_2O$	**21**	C	92	97–101	$C_{25}H_{21}BrClN_3O \cdot {}^1/_2H_2O$
9	A	50	162–165 (A)	$C_{26}H_{26}ClN_3 \cdot 3HCl \cdot 3H_2O$	**22**	C	39	246–250 (B)	$C_{25}H_{22}ClN_3O_2$
10			58–62	$C_{25}H_{23}BrClN_3 \cdot {}^1/_2H_2O$	**23**	C	28	141–143 (C)	$C_{25}H_{22}ClN_3O_2 \cdot {}^3/_2H_2O$
11	A	61	61–64	$C_{25}H_{23}Cl_2N_3 \cdot {}^3/_2H_2O$	**24**	C	45	124–128	$C_{25}H_{21}Cl_2N_3O \cdot H_2O$
12	A	37	57–60	$C_{25}H_{23}Cl_2N_3 \cdot H_2O$	**25**	C	17	103–107	$C_{25}H_{21}Cl_2N_3O \cdot H_2O$
13	A	78	61–64	$C_{26}H_{26}ClN_3O$	**26**	C	37	104–108	$C_{26}H_{24}ClN_3OS \cdot {}^5/_2H_2O$
14	A	78	59–60	$C_{26}H_{26}ClN_3O \cdot {}^1/_2H_2O$	**27**	C	29	83–87	$C_{26}H_{24}ClN_3O_2 \cdot H_2O$
15	B	87	145–149 (A)	$C_{27}H_{26}ClN_3O_2 \cdot 3HCl \cdot {}^1/_2H_2O$	**28**	C	62	100–102	$C_{27}H_{24}ClN_3O_3 \cdot H_2O$
16			218–222	$C_{26}H_{24}ClN_3O_2 \cdot {}^5/_2H_2O$	**29**	C	44	123–126 (C)	$C_{26}H_{24}ClN_3O \cdot H_2O$
17	A	87	207–211 (A)	$C_{26}H_{26}ClN_3 \cdot 3HCl \cdot {}^7/_2H_2O$					

[a] Yields of compounds were not optimized. [b] A, EtOAc/ether; B, acetonitrile; C, EtOAc. All other compounds were purified by chromatography. [c] Satisfactory elemental analyses (±0.4%) were obtained for C, H, and N for all compounds except for the following products [compound, element, calcd (found value)]: **5**, H, 6.54 (5.73); **11**, H, 5.65 ((4.94); **15**, N, 7.27(7.96); **20**, H, 6.59 (5.97); **25**, N, 8.97 (9.69); **26**, H, 5.76 (4.88).

screening tests are gathered in Tables 1 (series **Ia**) and 2 (series **Ib**).

In Vitro Antihistamine and PAF Antagonism. The unsubstituted 3- and 4-pyridylmethyl derivatives **5** and **6** were 3-fold more active than loratadine (**1**) and equipotent to **2** in the histamine-induced contraction in the guinea-pig ileum test. Compound **9** with an enlarged alkyl chain and the following substituted 3-pyridylmethyl derivatives 5-Br (**10**), 2-Cl (**12**), 6-MeO (**13**), 5-MeOOC (**15**), and 5-HOOC (**16**) showed activities similar to that of **1**. More potent compounds arised from the methyl substitution on the 3-pyridine. Whereas **18** (2-Me) was as active as **1**, **17** (6-Me) and **20** (4-Me) were 3-fold and **19** (5-Me) was 25-fold more active than **2**. Among the tested amides, a 3-fold improvement was observed for **27** (6-MeO) compared with **2**.

As occurred with the in vitro H_1 antihistamine activity, the results in the PAF-induced platelet aggregation test are highly dependent on the precise nature and position of the substituent in the pyridine. 3-Pyridylmethyl derivative **10** (5-Br) was equipotent to **2**, and the unsubstituted 3- and 4-pyridylmethyl derivatives **5** and **6**, **19** (5-Me), and the amides **21** (5-Br), **22** (6-OH), and **29** (5-Me), were 2–4-fold less active than **2**.

On the basis of the in vitro data, we have compounds with increased antihistamine activity compared with **2** but slightly less active in the PAF-induced aggregation test.

In Vivo Pharmacology. The greater potency of **19** over that of the reference compounds **1** and **2** in the antihistamine in vitro test is also observed in the in vivo histamine-induced hypotension test.

Regarding the PAF-induced hypotension test, only amine **19** (5-Me) and amide **22** (6-OH) showed more than 50% inhibition at a dose of 5 mg/kg. While **22** showed a lack of activity in the two oral tests probably due to a low bioavailability, compound **19** was 3-fold more potent as a PAF antagonist in the hypotension test and 15-fold more potent in the PAF-induced mortality test than the reference compound **2**. Compound **19** was significantly more active as a PAF antagonist in vivo than what could be expected from the result of the aggregation test. Thus, while it was 40-fold less potent than WEB-2086 in the in vitro test, in vivo it showed only half of its potency. This discrepancy could be attributed to a different selectivity of WEB-2086 and **19** for platelet and endothelial cell PAF receptors and/or to a different pharmacokinetic profile (e.g., presence of active metabolites) favoring **19** in the in vivo tests.

Table 4. Activity in the Passive Cutaneous Anaphylaxis Test in Rats

	ID_{50},[a] mg/kg po
19	1.2
1 (loratadine)	2.4
2 (SCH-37370)	10–20
WEB-2086	>20

[a] Dose required to inhibit antigen-induced increased permeability by 50%.

Table 5. Activity in the Endotoxin-Induced Mortality Test in Rats and Mice

	ID_{50},[a] mg/kg iv	
	mice	rats
19	1.2	0.5
1 (loratadine)	>10	>5
2 (SCH-37370)	>10	>5
WEB-2086	1.7	0.5
diphenhydramine	>10	>5

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

Series **Ia,b** were additionally examined in vivo in the active anaphylactic shock test in mice. Most of the compounds showed excellent results in this oral test. Amide **25** exhibited the highest activity with an ID_{50} of 1.9 mg/kg. Compound **19** proved to be slightly more active than reference compounds **1** and **2** and equipotent to **3**.

Compound **19** was further assayed in other pharmacological tests. It proved to be very active in the passive cutaneous anaphylactic shock in the rat,[20] a model that is histamine-dependent in this species (Table 4). Thus, **19** was 2-fold more potent than **1** and about 10-fold more potent than **2**, while WEB-2086 was ineffective. Compound **19** also strongly protected mice and rats from mortality induced by endotoxin,[21] a PAF-dependent model, whereas **2** and pure antihistamines (diphenhydramine and **1**) did not show efficacy (Table 5). In this test, compound **19** compares well with WEB-2086, in agreement with its good in vivo profile as a PAF antagonist.

In order to evaluate whether **19** had effects on the CNS, spontaneous motor activity test[22] and barbiturate-sleeping time test[23] in mice were run. Compound **19** and the "nonsedative" antihistamines **1** and terfenadine did not affect spontaneous motor activity in mice at a

Table 6. Effects of CNS: Spontaneous Motor Activity (SMA) Test in Mice and Barbiturate-Sleeping Time (BST) Test in Mice

	dose, mg/kg po	SMA % relative to control	SMA P^c	BST $X \pm$ SEM, min	BST P^c
control		100		113 ± 10	
19	100	97	NS[a]	138 ± 26	NS[a]
1 (loratadine)	100	106	NS[a]	320 ± 35	<0.001
2 (SCH-37370)	100	NT[b]		296 ± 35	<0.001
terfenadine	100	90	NS[a]	133 ± 26	NS[a]
diazepam	10	54	<0.001	NT[b]	

[a] Not significant. [b] Not tested. [c] Probability with respect to control values.

dose of 100 mg/kg po (Table 6). In the same conditions, a positive control, diazepam, showed a 46% reduction of activity at a dose of 10 mg/kg. Compound **19** and terfenadine did not prolong sleeping time induced by sodium pentobarbital (Table 6) at a dose of 100 mg/kg. However, compounds **1** and **2** produced an important potentiation of the barbiturate effect, with a near 3-fold increase of the sleeping time.

In conclusion, we can say that both H_1 and PAF antagonist activities of these series have shown a high dependence on the exact nature and position of the substituent in the pyridine. The (5-methyl-3-pyridyl)-methyl radical of **19** proved to be the optimum radical tested. Further pharmacological development has revealed compound **19** (UR-12592) to be a potent dual PAF–histamine antagonist in vivo, surpassing **2** (SCH-37370). This compound, which is devoid of CNS depressant effects, is now under development.

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. 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. THF, diethyl ether, and toluene were distilled from sodium metal/benzophenone ketyl. $CHCl_3$ was passed through an alumina column. CH_2Cl_2 and Et_3N were distilled from calcium hydride. DMSO and DMF 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.

C18–PAF–acether was synthesized from (*S*)-batyl alcohol[24] following a published procedure.[25]

5-Methylnicotinic Acid. To a solution of $KMnO_4$ (1.4 kg, 8.9 mol) in water (11 L) was added 3,5-lutidine (500 mL, 4.45 mol) at such a rate that the temperature was maintained between 45 and 60 °C. The mixture was stirred at 45 °C for 20 h; the precipitate was filtered through Celite and washed with hot water. HCl (12 N) was added to the filtrate to bring the pH to 1–2, and the resulting solution was continously extracted with $CHCl_3$ for 3 days to give a white solid (250 g, 41%): mp 215–216 °C (lit.[12] mp 214–216 °C).

8-Chloro-11-[1-[(6-methyl-3-pyridyl)methyl]-4-piperidylidene]-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (17). Method A. To a cooled (0 °C) solution of **4** (1 g, 3.2 mmol) and Et_3N (1.2 mL, 8.7 mmol) in CH_2Cl_2 (20 mL) was added 3-(chloromethyl)-6-methylpyridine monohydrochloride (4.87 mmol). The mixture was stirred at room temperature for 18 h. After diluting with CH_2Cl_2, the solution was washed with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo to an oil (1.3 g) which was chromatographed on silica gel ($CHCl_3$:MeOH:NH_3, 60:2:0.2) to give a white solid (1.13 g, 87%): ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.38 (d, J = 2.6 Hz, 2H), 7.56 (dd, J_a = 8.2 Hz, J_b = 2 Hz, 1H), 7.41 (dd, J_a = 7.8 Hz, J_b = 1.5 Hz, 1H), 7.12 (m, 5H), 3.47 (s, 2H), 3.3 (m, 2H), 3.0–2.1 (m, 13H). The product was dissolved in EtOAc and converted to its hydrochloride by treatment with $HCl_{(g)}/Et_2O$ solution to give a white solid: mp 207–211 °C. Anal. ($C_{26}H_{26}ClN_3 \cdot 3HCl \cdot {}^7/_2H_2O$) C, H, N.

8-Chloro-11-[1-[(5-methyl-3-pyridyl)methyl]-4-piperidylidene]-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (19). Method B. To a solution of 3,5-lutidine (11.4 mL, 0.1 mol) in CCl_4 (400 mL) was added NBS (21.6 g, 0.12 mol) followed by α,α′-azoisobutyronitrile (0.16 g, 0.1 mmol). The mixture was stirred at reflux for 2 h. Then, the mixture was cooled, and succinimide was removed by filtration. To the filtrate diluted with $CHCl_3$ (30 mL) were added **4** (14 g, 0.045 mol), DMAP (0.5 g), and Et_3N (9 mL, 0.064 mol). The resulting mixture was stirred at room temperature for 18 h. After diluting with CH_2Cl_2, the solution was washed with 0.5 N $NaHCO_3$ solution and water. The organic phase was dried over anhydrous Na_2SO_4, and the solvent was removed. The residue was chromatographed on silica gel ($CHCl_3$:MeOH:NH_3, 60:2:0.2) to afford a creamy solid (7.4 g, 41%): mp 58–61 °C; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.39 (m, 3H), 7.48 (m, 1H), 7.37 (m, 1H), 7.12 (m, 4H), 3.45 (s, 2H), 3.36 (m, 2H), 3.1–2.1 (m, 13H); ^{13}C NMR (20.15 MHz, $CDCl_3$) δ (TMS) 157.20 (C), 148.93 (CH), 147.46 (CH), 146.48 (CH), 139.50 (C), 138.56 (C), 137.06 (CH), 133.3 (C), 132.54 (C), 130.67 (CH), 128.80 (CH), 125.85 (CH), 121.92 (CH), 59.84 (CH_2), 54.63 (CH_2), 31.70 (CH_2), 31.32 (CH_2), 30.80 (CH_2), 30.56 (CH_2), 18.14 (CH_3); MS (EI) m/e 311 (34), 309 (100), 280 (20), 266 (15), 245 (15), 230 (10), 107 (72), 106 (28). The corresponding hydrochloride was prepared by treatment of a solution of **19** in EtOAc with $HCl_{(g)}/Et_2O$ solution (90%): mp 213–217 °C. Anal. ($C_{26}H_{26}ClN_3 \cdot 3HCl$) C, H, N.

Alternatively, the title compound was prepared as follows. A solution of **29** prepared as described below (26 g, 0.06 mol) in $POCl_3$ (130 mL) was stirred at 50 °C for 3 h. The excess of $POCl_3$ was then removed under vacuum, and the resulting solid was suspended in DME (500 mL). The solution was cooled in ice and treated with $NaBH_4$ (14.56 g, 0.39 mol). The reaction mixture was warmed to 20 °C, stirred for 2.5 h, and cooled in ice, and 10% HCl solution (260 mL) was added dropwise. DME was eliminated, water (1 L) was added, and the mixture was refluxed for 20 min. After extraction with ether, enough 5 N NaOH was added to bring the pH to 8–9 and the aqueous solution was extracted with EtOAc. The organic phase was dried over anhydrous $NaSO_4$, and the solvent was removed. The residue was chromatographed on silica gel ($CHCl_3$:MeOH:NH_3, 60:2:0.2) to afford **19** as a white solid (17.8 g, 72%).

8-Chloro-11-[1-(5-methylnicotinoyl)-4-piperidylidene]-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (29). Method C. To a cooled (0 °C) mixture of **4** (80.6 g, 0.26 mol), 5-methylnicotinic acid (35.7 g, 0.26 mol), and HOBT (34.6 g, 0.256 mol) in DMF (400 mL) was added DCC (53.7 g, 0.26 mol). After the solution stirred at room temperature for 18 h, the solvent was removed under vacuum and the residue was stirred with EtOAc. The insoluble material was filtered off and washed with EtOAc (300 mL). The filtrates were washed with saturated $NaHCO_3$ solution and water, and the organic phase was dried over anhydrous Na_2SO_4 and concentrated to half of the volume. The white solid formed was separated and dried (72.8 g, 65%): mp 123–126 °C; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.40 (m, 3H), 7.56 (m, 1H), 7.44 (m, 1H), 7.13 (m, 5H), 3.8–2.1 (m, 12H), 2.30 (s, 3H). Anal. ($C_{26}H_{24}ClN_3O \cdot H_2O$) C, H, N.

8-Chloro-11-[1-(5-bromonicotinoyl)-4-piperidylidene]-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (21). This compound was prepared following method C by reaction of **4** with 5-bromonicotinic acid: mp 97–101 °C; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.60 (d, J = 2 Hz, 1H), 8.57 (d, J = 1.6

Hz, 1H), 8.40 (m, 1H), 7.90 (m, 1H), 7.48 (d, J = 7.7 Hz, 1H), 7.14 (m, 4H), 3.36 (m, 4H), 3.0–2.3 (m, 8H). Anal. ($C_{25}H_{21}BrClN_3O \cdot {}^1/_2H_2O$) C, H, N.

8-Chloro-11-[1-[(5-bromo-3-pyridyl)methyl]-4-piperidylidene]-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (10). To a cooled (0 °C) suspension of $LiAlH_4$ (0.12 g, 3.2 mmol) in THF (5 mL) was added dropwise **21** (0.84 g, 1.7 mmol) in THF (3 mL). The resulting mixture was stirred at room temperature overnight, cooled with an ice bath, and treated with water (0.17 mL) and THF (0.35 mL) followed by 15% NaOH solution (0.17 mL) and water (0.46 mL). The precipitate formed was filtered and washed with THF, and the filtrates were concentrated in vacuo. $CHCl_3$ was added and the resulting solution dried over anhydrous $NaSO_4$. After solvent removal, the residue was chromatographed on silica gel ($CHCl_3$:MeOH, 3%) to give a white solid (0.22 g, 37%): mp 58–62 °C; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.55 (d, J = 1.5 Hz, 1H), 8.39 (m, 2H), 7.86 (s, 1H), 7.42 (d, J = 7.5 Hz, 1H), 7.12 (m, 4H), 3.49 (m, 2H), 3.36 (m, 2H), 3.0–2.1 (m, 10H). Anal. ($C_{25}H_{23}BrClN_3 \cdot {}^1/_2H_2O$) C, H, N.

B. Biological Methods: Inhibition of Platelet Aggregation in Vitro. Platelet aggregation studies were done by the method of Born.[14] Blood was collected in 3.16% sodium citrate (1 vol/9 vol 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, Chronolog 500. Activity of the inhibitors is 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 Mortality in Mice.[16] Groups of 10 male Swiss mice weighing 22–26 g were used; 100 μg/kg C18–PAF plus 1 mg/kg 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 are 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.[18] 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 *Bordetella pertussis* antigen (Difco; 1:25, v/v). The challenge was made 14 days after sensitization. Anaphylactic shock was caused by 1 mg/kg iv BSA (in saline) as antigen plus 1 mg/kg propanolol (administered 20 min before BSA), in a volume of 10 mL/kg through a tail vein. The compounds were administered orally 30 min 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 are 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 Histamine-Induced Contraction in Guinea-Pig Ileum. This test was performed according to the method of Magnus.[17] Male Dunkin–Hartley guinea pigs (bw 300–350 g), fasted overnight, were used. Animals were stunned, the abdomen was opened, and 4 cm long ileum sections were cut off. The sections were placed in a petri dish containing Tyrode's solution at 37 °C and continuously bubbled with carbogen. The ileum fragments were washed with Tyrode's solution and then transferred to an organ bath. Ileum contraction was measured using an isometric transducer. The initial load was 1 g. After a stabilization period of 20 min in which the organ was immersed in Tyrode's solution at 37 °C continuously bubbled with carbogen, noncumulative stimuli with submaximal doses of histamine (5×10^{-7} M) were given. The contraction in absence or presence (5 min incubation) of the test compounds was recorded. The activities of the antagonists are expressed as IC_{50} values, that is to say, the concentration of the drug required to inhibit histamine-induced contraction by 50%.

Inhibition of PAF- and Histamine-Induced Hypotension in Normotensive Rats.[15,1] Male Sprague–Dawley rats, weighing 180–220 g, were anesthetized with sodium pentobarbital (50 mg/kg ip). Blood pressure was recorded from the left carotid artery using a Statham pressure transducer coupled to a Beckman R611 recorder. Right and left femoral veins were catheterized to inject the test compound and PAF (0.5 μg/kg) or histamine (25 μg/kg). Test compounds were administered by intravenous injection (1 mL/kg, dissolved in saline) 3 min before PAF or histamine injection. Blood pressure was monitored, and percentage inhibition of PAF- or histamine-induced hypotension with respect to controls was calculated. The results are expressed as ID_{50} values, i.e., the dose of test compound required to inhibit hypotension by 50%, or as percentage inhibition at a given dose of test compound. The ID_{50} values 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.

Passive Cutaneous Anaphylaxis in Rats.[20] **(a) Generation of antiserum.** Groups of male Sprague–Dawley rats were sensitized by subcutaneous administration of 10 mg/kg ovalbumin plus 1 g/kg aluminum hydroxide accompanied with intraperitoneal injection of 5 mL/kg *B. pertussis* antigen (Difco) diluted 1:5, v/v, in saline. Blood was withdrawn by cardiac puncture 2 weeks later, and serum was obtained by centrifugation. Serum aliquots were stored frozen at −70 °C until use. **(b) Induction of Dermal Response.** Male Sprague–Dawley rats weighing about 250 g were randomized into groups of five, and their dorsal skin was shaved. Diluted antiserum (1:5, v/v, in saline) was then injected intradermally in 100 μL volumes into six zones of the dorsal skin. Two days later, the rats were anesthetized with sodium pentobarbital (50 mg/kg ip); 0.5 mL of a mixture of ovalbumin (10 mg/mL) and Evans blue dye (10 mg/mL) in saline was injected through the femoral vein. Test compounds or vehicle (1% Tween 80 in distilled water) were administered orally 60 min before antigen injection. **(c) Measurement of Plasma Extravasation.** Animals were killed 30 min after antigen injection. The dorsal skin was then removed, and the blue zones were cut off. Dye was extracted with a mixture of 70:30, v/v, acetone/0.5%, w/v, sodium sulfate in distilled water and later read in a 550 Perkin-Elmer spectrophotometer at 620 nm. Percentage inhibition of plasma extravasation with respect to control values was calculated for each experiment. The ID_{50} values 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 three or more independent experiments.

Endotoxin-Induced Mortality in Mice and Rats.[21] Groups of 10 fasting male Swiss mice weighing 22–26 g or male Sprague–Dawley rats weighing 125–150 g were used; 20 mg/kg endotoxin from *Escherichia coli* 0111:B4 (mice) or 5 mg/kg endotoxin from *E. coli* 0127:B8 (rats) was administered through a lateral tail vein 5 min after iv injection of the test compounds. Vehicle (saline) was administered to control groups. Mortality was recorded at 7 days after endotoxin injection. Following this protocol, we obtained a consistent mortality of 80–100% in the control group. Percentage inhibition of mortality due to the treatments in comparison with the control group was calculated. The results are 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.

Spontaneous Locomotor Activity in Mice.[22] Male Swiss mice weighing 22–26 g were used in groups of three animals. Thirty minutes after an oral dose of test compounds (or vehicle in control groups), the animals were placed in plastic cages (22 × 22 × 14.5 cm) located in a sound-proof room. Five minutes later, movement was measured for 25 min by means of a Panlab actimeter. Not less than four groups of three animals for each compound were used. Activity counts for treated groups were averaged and are expressed in percentage compared to those for controls.

Barbiturate-Induced Narcosis in Mice.[23] Male Swiss mice weighing 22–26 g were used. Sixty minutes after an oral dose of test compounds (or vehicle in control groups), each animal was given a subcutaneous injection of sodium pentobarbital (35 mg/kg). The time from the loss of the righting reflex until its recovery was monitored for each animal. Results are given as the mean sleeping time and its standard error for each group of treatment.

Statistics. Analyses of pharmacological data (i.e., IC_{50} or ID_{50} values and their 95% confidence limits) were made using a standard pharmacology program.[26] Statistical comparisons were made by Bonferroni's test using the INSTAT program.

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