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Original paper

Aminoacylates and aminocarbamates of 2-substituted 4-hydroxymethyl 1,3-dioxolans as ammonium salts. A new series of PAF antagonists

C Broquet[1*], E Auclair[1], N Blavet[1], C Touvay[1], P Braquet[2**]

[1]*1 av des Tropiques, 91952 Les Ulis, Cedex;*
[2]*Institut H Beaufour, 17, avenue Descartes, 92350 Le Plessis-Robinson, France*

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Summary — The synthesis and biological activity of some aminoacylate and aminocarbamate ammonium salts of 2-substituted 4-hydroxymethyl 1,3-dioxolans are described. The compounds were obtained as mixtures of diastereoisomeric racemates. *In vitro*, platelet aggregation (PRP) induced by PAF was antagonized by all derivatives. *In vivo* (in the guinea pig, iv), the PAF-induced bronchoconstriction, thrombocytopenia and leukopenia, were inhibited by most of them. Among the synthesized compounds, two highly potent and specific PAF antagonists: BN 52111 and BN 52115 have been identified.

Résumé — Aminoacylates et aminocarbamates d'hydroxyméthyl-4 dioxolanes-1,3 substitués en position 2. Une nouvelle série d'antagonistes du PAF. *Les auteurs décrivent la synthèse et les propriétés biologiques d'aminoacylates et aminocarbamates de 4-hydroxyméthyl-1,3-dioxolanes. Ces composés sous forme de mélanges de diastéréoisomères antagonisent* in vitro *l'agrégation plaquettaire (PRP) induite par le PAF.* In vivo, *la plupart d'entre eux inhibent la bronchoconstriction, la thrombocytopénie et la leukopénie provoquées par le PAF. Dans cette série, deux composés: les BN 52111 et BN 52115 se sont révélés particulièrement actifs en tant qu'antagonistes spécifiques du PAF.*

PAF antagonists / synthesis / aggregation / bronchoconstriction / thrombocytopenia / leukopenia

Introduction

Platelet-activating factor (PAF) is an endogenous ether phospholid mediator with a wide range of biological actions [1]. It is generated in inflammatory and allergic responses [2]. PAF promotes aggregation of platelets and neutrophils, induces bronchoconstriction, hypotension and increases vascular permeability [3-5].

Since the first molecule described, CV 3988 [6], a great number of structurally different PAF antagonists have been reported [7–8].

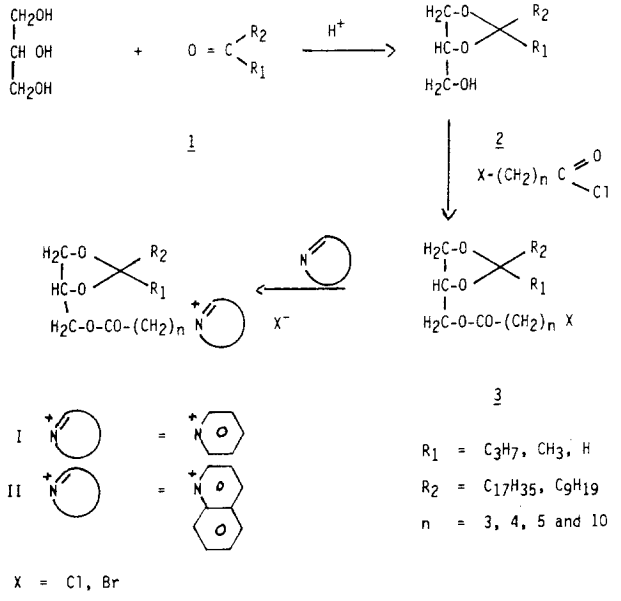
These have been classified as: (i) charged PAF-like antagonists, with an open chain (CV 3988) or cyclic structure (SRI 63073 [10]); (ii) natural products from plants (BN 52021, Kadsurenone [11]); and (iii) synthetic polycyclic compounds (WEB 2086 [12]).

We report here the synthesis and biological activity of new PAF-related antagonists with a constrained framework and summarize the structure–activity relationship in this series.

Chemistry

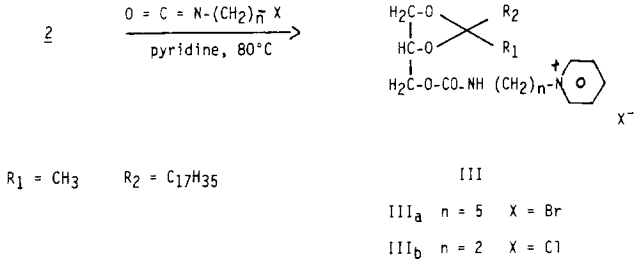
The syntheses of 2-substituted 4-ammonioacyl and ammoniocarbamoyl hydroxymethyl 1,3-dioxolan halogenides, as mixtures of diastereoisomeric racemates, were accomplished as illustrated in schemes 1 and 2. These syntheses are convenient methods for a multigram preparation. 2-substituted 4-hydroxymethyl 1,3-dioxolans **2** were obtained as previously described by reacting the corresponding carbonyl compound **1** with glycerol in the presence of *p*-toluene sulfonic acid [9]. Esterification of **2** by ω-halogeno acyl chloride yielded 2-substituted 4-(ω-halogenoacyl) oxymethyl 1,3-dioxolans, **3** which were converted to **I** or **II** by treatment with pyridine or quinoline.

*Correspondence and reprints
**General correspondence



Scheme 1.

Condensation of **2** with ω-halogeno alkyl isocyanate in the presence of pyridine directly gave the corresponding carbamate **III**.



Scheme 2.

Physicochemical properties of the intermediates are summarized in table I.

Physicochemical properties and mass spectra for the terminal compounds are listed in table II.

Biological results and Discussion

The compounds were tested *in vitro* for their ability to inhibit platelet aggregation induced by PAF, A 23187, arachidonic acid (AA) and adenosine diphosphate (ADP) in platelet-rich plasma (PRP), and *in vivo* for their ability to inhibit PAF-induced bronchoconstriction, thrombocytopenia and leukopenia.

Table I. Physicochemical properties of synthetic intermediates.

	R_1	R_2	n	X	*rf (TLC)*	*mp (°C)*
2_1	H	$C_{17}H_{35}$			0.17[a]	56–57 [21]
2_2	CH_3	$C_{17}H_{35}$			0.18[a]	viscous
2_3	C_3H_7	$C_{17}H_{35}$			0.23[a]	"
2_4	CH_3	C_9H_{19}			0.15[a]	" [22]
3a	H	$C_{17}H_{35}$	3	Cl	0.37[a]	"
3b	CH_3	$C_{17}H_{35}$	3	Cl	0.45[b]	39–40
3c	C_3H_7	$C_{17}H_{35}$	3	Cl	0.38[b]	viscous
3d	CH_3	$C_{17}H_{35}$	4	Cl	0.40[b]	"
3e	CH_3	$C_{17}H_{35}$	5	Br	0.57[b]	"
3f	C_3H_7	$C_{17}H_{35}$	5	Br	0.40[c]	"
3g	CH_3	$C_{17}H_{35}$	10	Br	0.26[c]	52
3h	CH_3	C_9H_{19}	5	Br	0.50[b]	viscous

Eluent system petroleum ether/ether [a]70:30; [b]80:20; [c]85:15 (v/v).

None of the synthesized compounds (**I**, **II** and **III**) shows agonistic activity *in vitro* at 10^{-3} M and *in vivo* at 1 mg / kg. In contrast, all have antagonistic activity on the effects induced by PAF and no effects towards the other stimulatory agents.

Platelet aggregation in vitro *(tables III, IV)*

The antagonistic activity of the compounds against PAF occurs between 31 and 0.3 μM. None of them exhibits any inhibitory effect on platelet aggregation induced by A 23187, AA or ADP at 10^{-4} M.

(i) It is of interest to note the effect of chain hydrophobicity for R_1 and R_2 on IC_{50}. $\mathbf{I_e}$ ($R_1 = C_{17}H_{35}$) is $\approx$ 3.5 times more potent than $\mathbf{I_h}$ ($R_1 = C_9H_{19}$). The difference is less pronounced for R_2 (compounds $\mathbf{I_a}$ to $\mathbf{I_c}$). (ii) The length of the acyl chain seems to be more important. For n = 3 and 4 (compounds $\mathbf{I_b}$ and $\mathbf{I_d}$) no significant difference in IC_{50} is observed and the best results are obtained with n = 5 ($\mathbf{I_e}$); with n = 10 ($\mathbf{I_g}$) the antagonistic activity dramatically decreases. (iii) The nature of the charge transfer group (pyridinium or quinolinium) does not significantly affect the IC_{50} activity. (iv) All compounds are diastereoisomeric mixtures, but for BN 52115 ($\mathbf{II_e}$) one racemic diastereoisomer could be separated using HPLC; concerning platelet aggregation antagonism effect, there is no significant difference between diastereoisomeric mixture and one purified isomer. (v) The change from ester moiety (BN 52111) to an acylcarbamoyl moiety with the same length of acyl chain ($\mathbf{III_a}$) does not lead to significant decrease in IC_{50}. However, the PAF antagonist activity is strongly influenced by the chain length: $\mathbf{III_a}$ (n = 5) is $\approx$ 25 times more active than $\mathbf{III_b}$ (n = 2). Similar results have been reported by Takani *et al* [13] on PAF antagonists having a carbamoyl group in position 3. (vi) The most active compounds were tested against

Table II. Physicochemical properties of terminal compounds.

	Names	R_1	R_2	*n*	*X*	*rf*[a] *(TLC)*	*mp (°C)*	*Mass spectrum* *M-x*	*A*[b]
I_a		H	$C_{17}H_{35}$	3	Cl	0.16	viscous	490	166
I_b	BN 52102	CH_3	$C_{17}H_{35}$	3	Cl	0.28	84–85	504	166
I_c	BN 52109	C_3H_7	$C_{17}H_{35}$	3	Cl	0.26	viscous	532	166
I_d	BN 52107	CH_3	$C_{17}H_{35}$	4	Cl	0.23	102 (dec)	518	178
I_e	BN 52111	CH_3	$C_{17}H_{35}$	5	Br	0.36	78	532	194
I_f	BN 52121	C_3H_7	$C_{17}H_{35}$	5	Br	0.44	76	560	194
I_g		CH_3	$C_{17}H_{35}$	10	Br	0.45	92	602	264
I_h	BN 52120	CH_3	C_9H_{19}	5	Br	0.30	viscous	420	194
II_a		H	$C_{17}H_{35}$	3	Cl	0.48	viscous	540	216
II_b	BN 52105	CH_3	$C_{17}H_{35}$	3	Cl	0.55	180 (dec)	554	216
II_e	BN 52115	CH_3	$C_{17}H_{35}$	5	Br	0.60	170 (dec)	582	244
III_a	BN 52132	CH_3	$C_{17}H_{35}$	5	Br	0.36	viscous	547	
III_b		CH_3	$C_{17}H_{35}$	2	Cl	0.14	viscous	505	

[a]Eluent system $CHCl_3$/MeOH, 70:30, v/v.

[b]A = HO-OC-(CH_2)n - 

Table III. Platelet aggregation antagonist effect (PRP) in comparison with other PAF antagonists.

Names[a]	IC_{50} *μM*[b] *(PAF 2.5 nM)*
BN 52021	0.3
Web 2086 (11)	0.2
CV 3988 (6)	30.5
Kadsurenone (10)	1
I_a	7
I_b BN 52102	2.2
I_c BN 52109	3.04
I_d BN 52107	5
I_e BN 52111	0.4
I_f BN 52121	1.73
I_g	31
I_h BN 52120	1.46
II_a	3.1
II_b BN 52105	1.2
II_e BN 52115*	0.3
III_a BN 52132	0.64
III_b	17

[a]See table II for formula. [b]See *Experimental protocols* for details. *One racemic diastereoisomer was isolated (see Chemical section for details); its IC_{50} was 0.24 μM.

other aggregating agents (table IV). All compounds are more active on the aggregation induced by PAF than on aggregation induced by ADP. All are ineffective on aggregation induced by AA or A23187.

Antagonistic effects in vivo *(table V)*

Only the most active antagonists were tested *in vivo*. All selected compounds, except BN 52120 **(I_h)** inhibit bronchoconstriction, thrombocytopenia and leukopenia induced by PAF in the guinea pig. The alkyl chain length seems to play a role in the *in vivo* PAF antagonistic activity. For instance, BN 52120 (R_1: C_9H_{19}) is virtually inactive. Indeed, the lipophilic group R_1 in this series of compounds is suggested to fit into the hydrophobic area of the membrane receptor, competitively with the lipophilic moiety of PAF molecule [7]. No close relationship between the *in vitro* antagonistic activity against PAF-induced platelet aggregation and *in vivo* inhibition of bronchoconstriction induced by the mediator is observed. Similar results have also been obtained in our laboratory with PAF antagonists belonging to an other chemical series (Braquet *et al*, unpublished results). Up to now, no available explanation is given for such a difference in the *in vitro* and *in vivo* PAF antagonistic activity.

A very significant antagonism of PAF activity (60 mg / kg) is noted on the bronchoconstriction in the guinea pig at 5.1 and 0.5 mg/kg, between 54 and 95%. BN 52111 **(I_e)** is still active at 0.1 mg / kg (45%). With regard to the thrombocytopenia induced by PAF, the protection is very marked, between 43–94%, at 1 mg / kg. At 0.1 mg/kg, BN 52111 is still active (62.5%). Concerning leukopenia induced by PAF, the antagonistic effect is less clear; it is highest for BN 52111 and BN 52121 at 1 mg / kg.

Conclusion

Aminoacylates and aminocarbamates of 2-substituted 4-hydroxymethyl 1,3-dioxolan are specific very potent PAF antagonists. No significant differences are ob-

Table IV. *In vitro* agonistic effect and antagonistic activities of selected compounds against other inducers of platelet aggregation.

Names[a]	*Agonistic effect % aggregation at 10^{-3} M*	*Antagonistic effects at 10^{-4} M % of change from control (DMSO)*		
		ADP 2.5 μM	*AA 0.5 mM*	*A23187 5 μM*
BN 52021	0	–28	– 2	–5
BN 52102 (**Ib**)	0	–34	–10	–9
BN 52109 (**Ic**)	0	–28	– 1	–5
BN 52107 (**Id**)	0	–28	– 2	–3
BN 52111 (**Ie**)	0	–24	– 2	–2
BN 52121 (**If**)	0	–23	+ 3	+1
BN 52120 (**Ih**)	0	– 8	+ 3	+3
BN 52105 (**IIb**)	0	–26	– 4	–2
BN 52115 (**IIe**)	0	–27	– 8	–6
BN 52132 (**IIIa**)	0	–12	+ 7	0

[a]See table II for formulas. [b]See *Experimental protocols* for details. [c](–) Inhibition. [d](+) Enhancement.

Table V. *In vivo*, antagonistic effects of selected compounds against bronchoconstriction, thrombocytopenia and leukopenia induced by PAF in the guinea pig.

Compounds	*Dose mg/kg iv*	*Bronchoconstriction % protection*		*Thrombocytopenia % protection*		*Leukopenia % protection*	
BN 52021	3	94.0	***	56.1	**	17.3	NS
	1	54.0	***	9.4	NS	6.5	NS
BN 52102 (I_b)	5	94.9	***	77.1	***	58.6	*
	1	61.3	***	43.0	*	29.2	NS
BN 52107 (I_d)	5	73.5	**	68.0	**	36.1	NS
BN 52111 (I_e)	5	95.6	***	93.9	***	59.6	*
	1	76.1	***	83.2	***	91.4	***
	0.5	70.4	***	71.9	***	34.6	NS
	0.1	45.1	**	56.1	**	41.8	NS
BN 52121 (I_f)	1	83.5	***	80.1	***	87.0	**
	0.5	54.0	***	65.5	***	60.6	*
BN 52120 (I_h)	1	14.1	NS	no effect		no effect	
BN 52105 (II_b)	5	86.8	***	62.0	**	24.8	NS
BN 52115 (II_e)	1	91.1	***	70.42	***	41.0	*
	0.5	72.4	***	59.9	**	58.9	*
	0.1	11.5	NS	21.7	NS		
BN 52132 (III_a)	1	57.0	***	62.8	***	36.0	***
	0.5	68.0	***	45.2	***	25.4	***

See *Experimental protocols* for details, PAF 60 ng/kg iv. $N = 4$–6 animals. NS = Not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

served in PAF antagonistic activity *in vitro* and inhibitory effects *in vivo* except for BN 52120 ($\mathbf{I_h}$). BN 52111 ($\mathbf{I_e}$) and BN 52115 ($\mathbf{II_e}$) proved to be the most active compounds in this series.

Experimental protocols

Chemistry

Solvents were all reagent grade and were used without purification. TLC was performed on silica gel 60 F_{254} plates (Merck) and the spots were visualized by UV light and iodine. Column chromatography was performed on silica gel (Merck, particle size 0.063–0.200 for normal chromatography and middle particle size (15 μm) for flash chromatography) without any special treatment.

IR spectra were recorded on a Perkin–Elmer 1420 spectrometer, ^{1}H NMR spectra on a R 12B (60 MHz) or a Brucker (500 MHz) in $CDCl_3$ with Me_4Si (TMS) as internal standard [14]. Chemical ionisation mass spectra were obtained at 220°C on a modified AEI spectrometer [15]. Elemental analyses of final products were consistent with the proposed structures (C, H, N, X) [15].

Carbonyl compounds ***1***

Octadecanal was obtained by oxidation of the mesylate of octadecanol with DMSO in the presence of $NaHCO_3$ according to Mahadevan [16].

Nonadecanone was synthesized from ethyl sodio acetoacetate and hexadecyl iodide according to a previously described procedure [17] (mp 55–56° from EtOH). Propyl heptadecyl ketone was obtained from ethyl sodium butyryl acetate and hexadecyl iodide (mp 56° from EtOH). Undecanone was obtained from commercial sources.

The ω-halogeno acyl chlorides required are commercially available (Aldrich) and used without any purification.

General syntheses

2-Substituted 4-hydroxymethyl 1,3-dioxolans ***2***
A mixture of appropriate **1** (40 mmol), twice distilled glycerol (65 mmol) and 0.6 g of *p*-toluene sulfonic acid in 120 ml of dry toluene was stirred under reflux for 12 h; the resulting water was eliminated using a Dean–Stark apparatus. After cooling, the organic phase was washed with 30 ml of aqueous solution of KOH (5%), then with the minimum amount of water. After drying (Na_2SO_4), the solvent was evaporated *in vacuo* to give an oil. Flash chromatography using petroleum ether/ether (95:5, then 85:15 v/v) as eluent gave the dioxolan (yield 75–80%). IR (film) 3500 (OH); 1100–1060 (C–O) cm^{-1}.

2-Substituted 4-(ω-halogenoacyl oxy-methyl) 1,3-dioxolans ***3***
2 (18 mmol) and Et_3N (45 mmol) in 15 ml of ethanol-free $CHCl_3$ were added dropwise to a solution of ω-halogeno acyl chloride (20 mmol) in 10 ml of the same solvent at 0°C. The mixture was stirred for 15 h at room temperature.

After addition of 20 ml of $CHCl_3$, the organic phase was washed with NaOH (1 N), then with water and dried (Na_2SO_4). The solvent was eliminated *in vacuo*. Chromatography on silica gel (Flash chromatography) using petroleum ether/ether (95:5, v/v) as eluent gave **3** (yield 75–85%). IR (film) 1740 (C = O); 1180–1160 (C–O–C) cm^{-1}. ^{1}H NMR 60 MHz $CDCl_3$ (TMS) δ ppm. 0.9 (t, 3H, CH_3); 1.3 (m, CH_2 of the chain, CH_3); 2.1 (m, 2H, $CO–CH_2–CH_2$); 2.5 (t, 2H, $CO–CH_2$); 3.5–3.6 (m, 4H, CH_2O and CH_2X); 4.2 (m, 3H, CH_2OCO and HCO). For $\mathbf{3_a}$ instead of CH_3 at 1.3, 2 multiplets centered on 4.9 (1H).

2-Substituted 4-[ω-(N-pyridinium)acyloxy-methyl] 1,3-dioxolan halogenides ***I***
A solution of 3 (10 mmol) in 30 ml of dry pyridine was stirred at 80°C, under N_2, for 24 h. Pyridine was eliminated *in vacuo* and the residue was purified by chromatography on silica gel with $CHCl_3$, then $CHCl_3$ / MeOH (90:10 then 80:20, v/v) as eluent to yield **I** (70–75%).

2-Substituted 4-[ω-(N-quinolinium)acyloxy-methyl] 1,3-dioxolan halogenides ***II***
3 (10 mmol) dissolved in a mixture of quinoline (20 ml) and DMSO (20 ml) was heated at 80°C with stirring for 3 days, under N_2. Quinoline and DMSO were distilled under reduced pressure and the black residue was chromatographed on a silica gel column: eluent successively, $CHCl_3$ then $CHCl_3$ / MeOH (95:5 then 90:10 v/v) to give **II** (yields 48–51%).

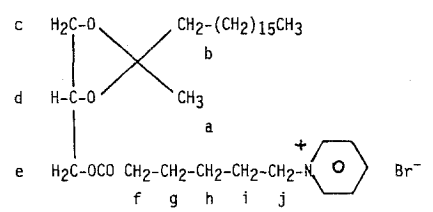
IR 3400 (H_2O); 1740 (C= O); 1200–1180 (COC) cm^{-1} for **I**, 1630 (pyridinium); for **II**, 1650, 1630, 1600 cm^{-1} (quinolinium).

The mixture of the two racemic diastereoisomers was studied for BN 52111 and BN 52115 by ^{1}H NMR (500 MHz). At 1.35 ppm, a doublet (apparent *J* 12 Hz) was observed on the spectra for the ratio 50/50. HPLC was conducted on a Waters 510 high pressure liquid chromatograph, equipped with a differential refractomer and using a Waters porasil column (3.9 mm X 30 cm) and CH_2Cl_2/MeOH as eluent (flow rate 2.5 ml/min). The two racemic diastereoisomers eluted very close to each other and it was very difficult to separate them cleanly. For BN 52111 shouldering was observed (T_R 4.4 min), but the BN 52115 preparation showed the presence of two very close peaks: T_R 2.9 and 3.1 min. Only the second product could be obtained pure but in very small quantities. ^{1}H NMR data showed a singlet at 1.35 ppm (3H) indicating the presence of one racemic diastereoisomer.

2-Heptadecyl 2-methyl 4-[5'-(N-pyridinium)pentyl carbamoyl methyl] 1,3-dioxolan bromide ***IIIa***
A mixture of 2 (14 mmol), 5-bromopentyl isocyanate [18] (18 mmol) and 30 ml of pyridine was heated for 2 days at 80°C under N_2. Pyridine was eliminated *in vacuo* and the residue was dissolved in $CHCl_3$, washed with water, dried (Na_2SO_4) and the solvent was evaporated. Chromatography on silica gel with $CHCl_3$ then $CHCl_3$ / MeOH (95:5 then 90:10 v/v) as eluent yielded $\mathbf{III_a}$ (40%). IR 3350 (NH); 1720 (CONH); 1640 (pyridine)cm^{-1}.

2-Heptadecyl 2-methyl 4-(2'-(N-pyridinium) ethyl carbamoyl methyl) 1,3-dioxolan chloride ***III_b***
It was obtained using 2-chloroethyl isocyanate (Aldrich) instead of 5-bromopentyl isocyanate (yield 25%).

^{1}H NMR 500 MHz $CDCl_3$ TMS, δ; for $\mathbf{I_e}$



0.85 (t, 3H, CH_3); 1.25 (large s, 30H, $(CH_2)_{15}$); 1.35 (d, 3H, CH_{3a}, J = 12 Hz, diastereoisomeric mixture); 1.45 (m, 2H, CH_{2h}); 1.6 (m, 4H, CH_{2b} and CH_{2g}); 2.1 (m, 2H, CH_{2i}); 2.35 (d, 2H, CH_{2f}); 3.70 and 4.35 (2m, 2H, CH_{2c} diastereoisomeric mixture); 4.1 (m, 3H, CH_{2e} and CH_d); 5.05 (t, 2H, CH_2–N^+); pyridinium 8.1 (t, 2H, Hβ); 8.6 (d, 1H, H); 9.5 (d, 2H, Hα).
I_a instead of CH_3 at 1.35 2 multiplets centered on 4.9 for 1H.
II_e quinolinium instead of pyridinium. 5.5 (t, 2H, CH_2N); 7.90–8.40 (5H); 9.10 (1H); 10.70 (1H, H_a).
III_a carbamate instead of ester; instead of CH_2 at 2.35, 3.25 (d, 2H, CONH–CH_2); 5.6 (NH). **III_b** 3.55 (m, 2H, CONHCH_2); 5.35 (t, 2H, CH_2N^+).

Biological methods

Platelet aggregation in platelet rich plasma (PRP) according to [19]
Blood samples were collected in citrate buffer (3.8%, pH 7.4) from auricular artery of New Zealand rabbit (≈2.5 kg). PRP was obtained by centrifugation of blood at 120 *g*, for 15 min at room temperature.

Platelet aggregation was monitored on a chrono-log Coultronics aggregometer, which determines the transmission percentage corresponding to the maximum of the peak before the desaggregation. The tested sample was prepared in DMSO.

Agonistic effect. Various concentrations of PAF or different samples (40 μl) were added to 380 μl of incubated and stirred PRP. Aggregation induced by different compounds was compared to that obtained with PAF which produced 100% aggregation at 2.5 nM.

Antagonistic effect. Various concentrations of samples were added to incubated and stirred PRP 2 min before addition of PAF (2.5 nM) or other aggregating agents: AA (0.5 mM); ADP (2.5 μM); A 23187 (5 μM).

The concentration giving 50% inhibition of PAF-induced aggregation (IC_{50}) was determined (control: pure DMSO); 5 tests made on 5 different rabbits allowed calculation of the IC_{50}, using the linear regression test.

Bronchoconstriction, thrombocytopenia and leukopenia in the guinea pig
Male Hartley guinea pigs (450–500 g) were anesthetized with ethyl carbamate (1.25 mg/kg; ip) tracheotomized and ventilated by means of a respiratory pump (Ugo Basile) (70–80 strokes/min, 1 ml air/100 g/breath).

To abolish spontaneous respiration, a pneumothorax was performed. The jugular vein was cannulated for administration of drugs and the carotid artery for blood sampling. Resistance to lung inflation was measured according to the Konzett–Rössler overflow technique [20] by a bronchial transducer (Ugo Basile) (10 cm of H_2O).

Bronchoconstriction was induced by intravenous injection of PAF (60 ng/kg). The animal received one dose of antagonist compound iv, 5 min before PAF in NaCl 0.1 M (0.1 ml/kg).

The bronchoconstriction was compared to that obtained with a control group. Percent inhibition was calculated for each dose. One min before and after PAF injection, blood was collected for platelet and leukocyte counting with a Coulter counter ZBI.

References

1 Winslow CM, Lee ML (1987) *New Horizons in Platelet-Activating Factor Research.* Wiley, New York; Braquet P, Touqui L, Shen TY, Vargaftig BB (1987) *Perspectives in Platelet-Activating Factor Research. Pharmacol Rev* 97
2 Vargaftig BB, Chignard M, Benveniste J, Lefort J, Wal F (1981) *Ann NY Acad Sci* 370, 119
3 Braquet P, Vargaftig BB' (1986) *Transplant Proc* 18 (suppl 4), 10
4 Morley J (1986) *Agents Actions* 19, 100
5 Snyder F (1985) *Med Res Rev* 5, 107
6 Terashita Z, Tsushima S, Yoshioka Y, Nomura H, Imada Y, Nishikawa K (1983) *Life Sci* 32, 1975
7 Braquet P, Godfroid JJ (1986) *Trends Pharmacol Sci* 7, 397
8 Saunders RN, Handley DA (1987) *Ann Rev Pharmacol Toxicol* 27, 237
9 Showler AJ, Darley PA (1987) *Chem Rev* 67, 427
10 Lee ML, Winslow CM, Jaeggic C *et al* (1985) *Prostaglandins* 30, 690
11 Shen TY, Hwang SB, Chang MN, Doebber TW, Lam MH, Wu MS, Wang X, Hang GQ, Li RZ (1985) *Proc Natl Acad Sci USA* 82, 672
12 Cazals-Stenzel J, Muacevic G, Weber KH (1986) *Naunyn-Schmiedebergs Arch Pharmacol* 334 (suppl) R 44
13 Takani M, Yoshioka Y, Tasaka A, Terashita Z, Imura Y, Nishikawa K, Tsushima S (1989) *J Med Chem* 32, 56
14 CEN/Saclay, Département de Physico-Chimie 91191 Gif-sur-Yvette Cedex, France
15 Institut de Chimie des Substances Naturelles (CNRS) 91190 Gif-sur-Yvette Cedex, France
16 Mahadevan V, Philipps F, Lundberg WO (1966) *Lipids* 1, 183
17 Saville WB, Shearer G (1925) *J Chem Soc* 127, 591
18 Kricheldorf HR (1972) *Synthesis* 551
19 Born GVR, Cross MJ (1963) *J Physiol (London)* 168 (20), 178; Konzett H, Rossler R (1940) *Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol* 195, 71
20 Piantadosi C, Anderson CE, Brecht EA, Yarbro CL (1958) *J Am Chem Soc* 80, 6613
21 Nelson F (1961) *Arch Intern Pharmacodyn* 33, 327