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Original paper

Non-acidic anti-inflammatory compounds: activity of *N*-(4,6-dimethyl-2-pyridinyl) benzamides and derivatives

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Summary – The inhibition of the carragenin-induced rat-paw edema by previously synthesized *N*-(4,6-dimethyl)-2-pyridinyl) benzamides was evaluated. Amoung the 29 tested compounds, secondary benzamides **1, 12** and tertiary benzamide **60** exhibited a significant anti-inflammatory activity. It prompted us to envision a pharmacomodulation in this series by structural modifications on the homocycle, the amide function and the heterocycle. Although benzamide **38,** acetamide **50** and benzylamine **56** elicited marked inhibitory activity, none was more active than *N*-(4,6-dimethyl-2-pyridinyl) benzamide **1.**

The mechanism of the anti-inflammatory action of **1** was investigated. The results showed that this molecule reduced eicosanoid biosynthesis but was unable to reduce cyclooxygenase or lipoxygenase activities. Although it did not directly block phospholipase activity, however, an inhibitory process at this level is likely.

Résumé – **Divers *N*-(4,6-diméthyl-2-pyridinyl) benzamides ont été expérimentés vis-à-vis de l'œdème à la carragénine.** *L'activité anti-inflammatoire notable manifestée par les benzamides secondaires **1** et **12** et le benzamide tertiaire **60** nous a incités à poursuivre la pharmacomodulation du modèle structural par des modifications au niveau de l'homocycle, de la fonction amide et de l'hétérocycle.*

*Trois composés, le benzamide **38**, l'acétamide **50** et la benzylamine **56**, se sont révélés actifs; le pourcentage d'inhibition reste cependant inférieur à celui exercé par le* N-*(4,6-diméthyl-2-pyridinyl) benzamide **1**.*

*L'étude du mécanisme d'action anti-inflammatoire de **1** a permis de montrer que cette molécule réduit la biosynthèse des icosanoïdes, sans cependant diminuer les activités cyclooxygénasique et 5-lipoxygénasique.*

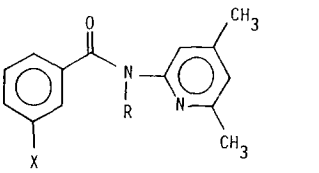
Bien qu'une action inhibitrice directe à l'égard de la phospholipase A_2 soit exclue, une interférence avec un processus de régulation à ce niveau peut être envisagée.

***N*-(2-pyridinyl)benzamide derivatives / non-acidic anti-inflammatory agents / eicosanoid biosynthesis inhibition**

Introduction

It was previously shown that some benzamide molecules with 2-aminopyridine structure exhibit anti-ulcerogenic and sedative [1] or anti-inflammatory properties [2]. These results and our work on *N*-pyridinyl-phthalimides derivatives [3] prompted us to determine the possible anti-inflammatory activity of the previously reported *N*-(4,6-dimethyl-2-pyridinyl) benzamides **1**–**23** and **60**–**63** [4, 5].

The anti-inflammatory activity elicited by secondary amides **1, 12,** and tertiary amide **60** led us to consider a pharmacomodulation by means of various structural modifications on the homocycle, the amide function and the pyridinic heterocycle. Finally, studies were carried out on the mechanism of action of the most active derivative **1.**



X = H, R = H : *1*
= Br, = H : *12*
= H = C_2H_5 : *60*

Chemistry

Like benzamides **1**–**23,** secondary benzamides **24**–**38** and **50, 51, 53** were prepared by acid chloride or anhydride

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(50–51) action on the corresponding primary amine [4] (Scheme 1, method A). Because of difficulties arising from alkylation of benzamide **1** [4], tertiary amides **60–63** were prepared by benzoylation of secondary amines **54** and **55** (Scheme 1, methods B and A). As before, the reduction of tertiary benzamides **60** and **61** by $LiAlH_4$ gave tertiary amines **64** and **65**.

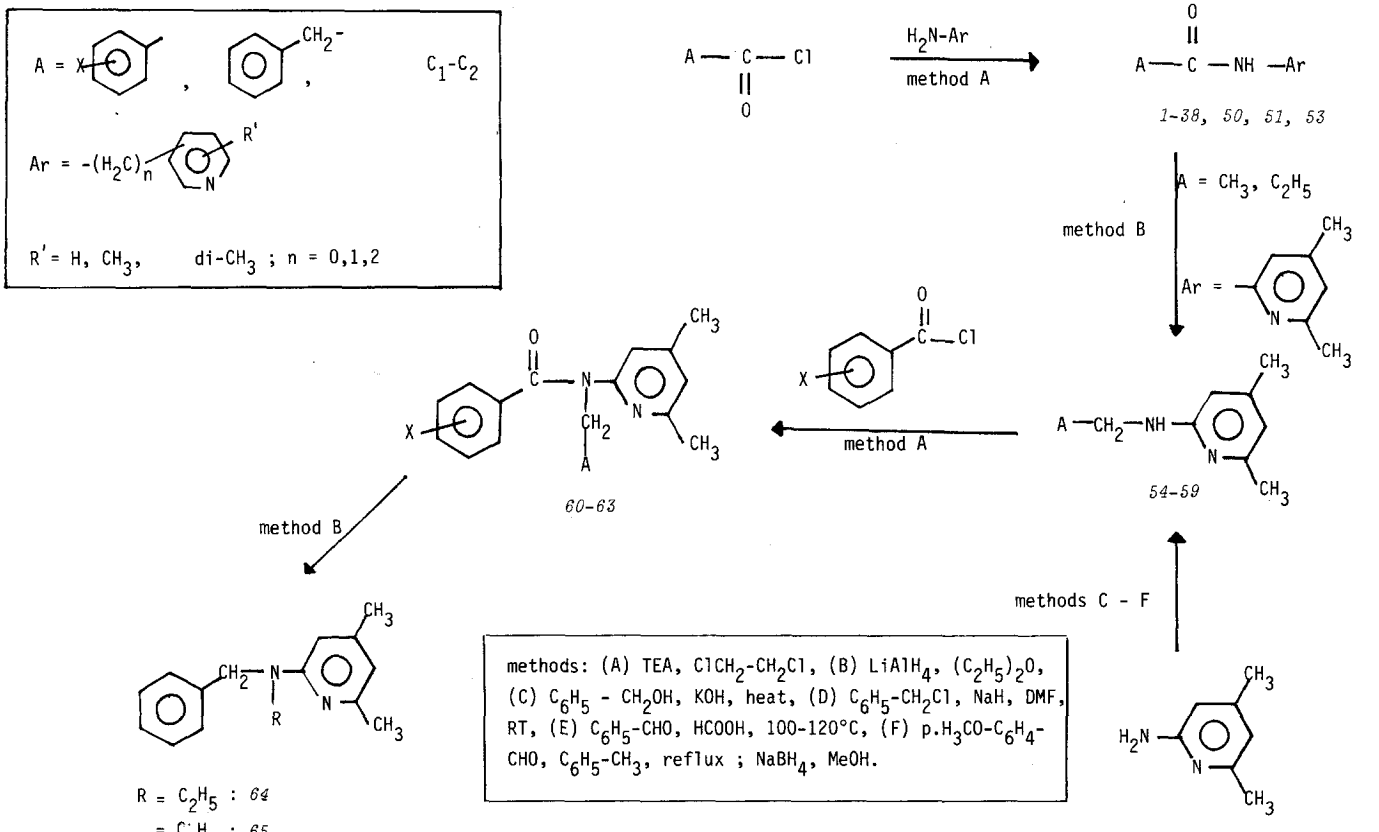
Benzylation of 2-amino-4,6-dimethylpyridine could be performed using benzylic alcohol [14] (method C), benzyl bromide [15] (method D) or, with a better yield, the Leuckart–Wallack reaction [16] (method E); in the case of **59,** reduction of the imine by sodium borohydride [17] gave an interesting variant.

An attempt at chemical reduction of the nitrogen heterocycle of benzamide **1** by sodium in ethanol failed, as did catalytic methods: Ni Raney W-2 in acetic acid at 100°C under 100 at. Pd/C in hydrochloric acid and/or acetic acid [18] at atmospheric pressure or under 4–5 at. Use of the second catalyst in methanol at 60°C under 100 at. gave starting material **1** (75%) and a mixture of reduced compounds, from which only cyclohexylcarboxamide, as evidenced by ^1H NMR and mass spectra, could be isolated at a low yield (10%). However, platinum oxide in acetic acid [21] gave the product resulting from reduction of only the benzene nucleus, compound **52** (method G), whereas in the case of *N*-[(2-pyridinyl) methyl] benzamide **37** the same experimental conditions led to reduction of the heterocycle giving *N*-[(2-piperidinyl) methyl] benzamide **39** (see *Experimental protocols*).

As various attempts at catalytic reduction of 2-amino-4,6-dimethylpyridine before amidification also failed, we were led to undertake reduction of the benzamides by an indirect pathway, as shown in Scheme 2. Quaternization occurred by simple refluxing in acetonitrile with the *N*-(3-pyridinyl) and *N*-(4-pyridinyl) benzamides **34, 35** and the *N*-[(2-pyridinyl) methyl] benzamide **37** (methods H and I) but required prolonged heating at 100°C in a reactor in the case of *N*-(4,6-dimethyl-2-pyridinyl) benzamide **1.** The iodomethylate of **1,** contrary to the pyridinium salts **40–42** which are easily reduced to tetrahydro-pyridines **(43–45),** was inert vis-à-vis sodium borohydride. The anhydro-base **49** [24], isolated after passage in alkali medium (method J), resist catalytic hydrogenation at atmospheric pressure in the presence of Pd/C or PtO_2 (method G). The reduction carried out in the presence of the first catalyst, at 100°C under 50 at. in acetic medium, gave benzamide and 1,2,4-trimethylpiperidine [25, 26] whose formation may be interpreted by reduction of the heterocycle and hydrogenolysis of the acylimine.

Pharmacology

The anti-inflammatory activity of benzamide molecules was assessed by carragenin-induced rat-paw edema inhibition. The mechanism of action of the most efficient derivative **1** was also studied. Prostanoid biosynthesis inhibition was investigated first using techniques which



Scheme 1.

can reveal possible blockade of phospholipase (PLA_2) and cyclooxygenase activities (spontaneously contracting pregnant rat uterus, prostacyclin biosynthesis by rat aortic tissue *in vitro*). Since derivative **1** can reduce eicosanoid biosynthesis, techniques capable of assessing specific inhibition of cyclooxygenase activity (guinea-pig lung homogenate; arachidonic acid (AA)-induced bronchospasm in anesthetized guinea-pig), 5-lipoxygenase activity (isolated guinea-pig trachea) and PLA_2 activity (^{3}H-AA release from cultured macrophages) were employed. Indomethacin, dexamethasone, nordihydroguaiaretic acid (NDGA), mepacrine were used as reference drugs (see *Methods* in *Experimental protocols*).

Results and Discussion

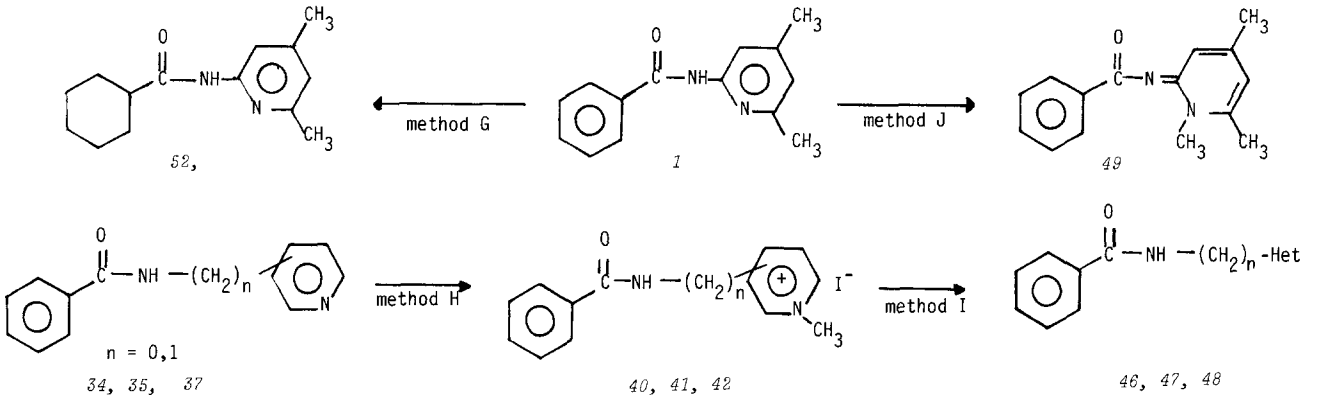
Among the studied benzamides with one or two substituents in the benzene ring, only 3-bromo derivative **12** elicited an anti-inflammatory effect similar to that noted with **1**. The total result shows that, except for **5** and **6**, the activity increased in the following order: disubstituted derivative < 2-mono-substituted < 4-mono- < 3-mono. A tentative correlation between the lipophilicity of these molecules, expressed by their R_M [27], and the percentage of edema inhibition gave no satisfactory result.

Aliphatic amides **50, 51,** cyclohexylcarboxamide **52** and phenylacetamide **53** induced edema inhibition of 50% or more. The lack of activity of *N*-pyridinyl-benzamides structurally related to **1** shows the importance of the position of the nitrogen and/or methyl groups: **30–36.** Among the molecules with *N*-pyridinyl-alkyl structure, the *N*-[(2-pyridinyl)-2-ethyl] benzamide **38** had a marked activity but not the *N*-(2-picolinyl) benzamide **37** which, in addition, was toxic. The heterocycle reduction of **34, 35** and **37** failed to elicit any significant effect, except for **48**.

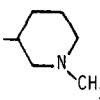
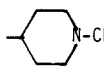
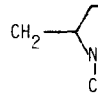
Finally, *N*-ethylation of **1** led to derivative **60** with similar activity (80.8 ± 3.2% of inhibition) but conversion to 2-benzoylimino-1,2-dihydropyridine **49** markedly decreased the effect (24.4 ± 4.2%, $P \leq 0.001$ compared to **1**, $N = 8$ for each group).

Compound **1** which showed the highest *in vivo* activity, had an ID_{50} of 35.2 mg·kg^{-1} (I = 33.85D−70.55; $r = 0.97$). Its LD_{50} *(p.o.)* was 1830 mg·kg^{-1} (1440–2324, calculated according to the method of Lichtfield and Wilcoxon) indicated a wide therapeutic index between anti-inflammatory and toxic doses. In the same experimental conditions, the ID_{50} of dexamethasone, used as a reference drug, was 10.0 mg·kg^{-1} (I% = 17.73D + 8.99; $r = 0.96$).

Benzamide **1,** devoid of spasmolytic activity when evaluated on $BaCl_2$-induced contractions of isolated guinea pig ileum, had a marked inhibitory effect on isolated pregnant rat uterus contractions ($IC_{50} = 7.8 \cdot 10^{-7}$ M; indomethacin and dexamethasone showed recpectively IC_{50} of $7.6 \cdot 10^{-8}$ M and $9.1 \cdot 10^{-6}$ M in the same experimental condition: Fig. 1). This molecule also reduced prostacyclin biosynthesis by rat aortic tissue ($IC_{50} = 2.3 \cdot 10^{-4}$ M and $2.7 \cdot 10^{-5}$ M for indomethacin). As these results indicated

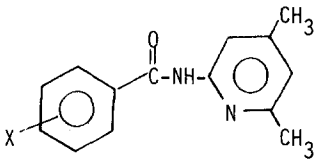


methods (G) H_2, PtO_2, CH_3COOH, (H) CH_3I, CH_3CN, reflux
(I) $NaBH_4$, CH_3OH ; H_2, 5 % Pd-C, C_2H_5OH,
(J) CH_3I, CH_3CN at 100°C; $NaHCO_3$

N°	$-(CH_2)_n$-Het
46	
47	
48	

Scheme 2.

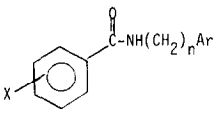
Table I. Anti-inflammatory activity of secondary benzamides **1**–**23**[a].

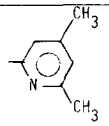
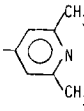
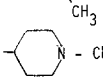


N°	X	Formula	Carragenin-induced rat-paw edema	
			N [b]	inhibition % [c]
1	H	$C_{14}H_{14}N_2O$	(10)	84.7 ± 5.4
2	4-NO_2	$C_{14}H_{13}N_3O_3$	(7)	54,4 ± 12.5
3	3-NH_2	$C_{14}H_{15}N_3O$	(10)	53.8 ± 8.9
4	4-NH_2	$C_{14}H_{15}N_3O$	(10)	63.3 ± 6.4
5	3-$NHCOCH_3$	$C_{16}H_{17}N_3O_2$	(10)	44.5 ± 7.5
6	4-$NHCOCH_3$	$C_{16}H_{17}N_3O_2$	(10)	53.3 ± 7.7
7	2-OH	$C_{14}H_{14}N_2O_2$	(10)	0
8	4-F	$C_{14}H_{13}FN_2O$	(10)	60.4 ± 8.4
9	2-Cl	$C_{14}H_{13}ClN_2O$	(10)	28.1 ± 7.6
10	3-Cl	$C_{14}H_{13}ClN_2O$	(10)	61.1 ± 2.5
11	4-Cl	$C_{14}H_{13}ClN_2O$	(10)	54.5 ± 5.5
12	3-Br	$C_{14}H_{13}BrN_2O$	(10)	78.3 ± 3.4
13	4-Br	$C_{14}H_{13}BrN_2O$	(10)	38.3 ± 8.6
14	3-CH_3	$C_{15}H_{16}N_2O$	(10)	47.5 ± 8.4
15	3-CF_3	$C_{15}H_{13}F_3N_2O$	(10)	65.8 ± 6.2
16	2-OCH_3	$C_{15}H_{16}N_2O_2$	(10)	33.0 ± 5.7
17	3-OCH_3	$C_{15}H_{16}N_2O_2$	(8)	61.3 ± 7.9
18	4-OCH_3	$C_{15}H_{16}N_2O_2$	(10)	48.3 ± 8.2
19	4-OC_2H_5	$C_{16}H_{18}N_2O_2$	(10)	45.0 ± 6.1
20	4-SCH_3	$C_{15}H_{16}N_2OS$	(10)	45.0 ± 8.4
21	2-OCH_3,5-Cl	$C_{15}H_{15}ClN_2O_2$	(10)	12.4 ± 3.7
22	2-OCH_3, 5-SO_2CH_3	$C_{16}H_{18}N_2O_4S$	(10)	15.2 ± 0.1
23	2-OCH_3, 5-$SO_2C_2H_5$	$C_{17}H_{20}N_2O_4S$	(10)	36.8 ± 5.5

[a]The physical properties of these compounds have been previously described in [4]. They were synthesized by method A except for compound **7** obtained by aminolysis of phenyl salicylate.
[b]No. of animals.
[c]Means ± SEM.

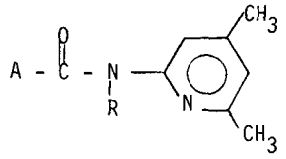
Table II. Physical properties and anti-inflammatory activity of secondary benzamides **24–38** and **46–48.**

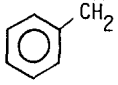
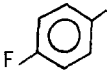


N°	X	n	Ar	Formula	Synth. method	Yield %	mp °C	Recrystn Solv.	Carrageenin-induced rat-paw edema	
									N	Inhibition %
24	4-CN	0		$C_{15}H_{13}N_3O$	A	81	152	EtOH/H_2O	(6)	57.3 ± 12.4
25	2,4-di-Cl	0	"	$C_{14}H_{12}Cl_2N_2O$	A	80	120	*i*-Pr_2O	(10)	52.6 ± 5.5
26	2,6-di-Cl	0	"	$C_{14}H_{12}Cl_2N_2O$	A	72	200	EtOH	(9)	0
27	3,4-di-Cl	0	"	$C_{14}H_{12}Cl_2N_2O$	A	85	148	*i*-Pr_2O	(10)	36.2 ± 7.6
28	3,4-di-CH_3	0	"	$C_{16}H_{18}N_2O$	A	75	176	EtOH/H_2O	(10)	40.6 ± 7.6
29	3,5-di-CH_3	0	"	$C_{16}H_{18}N_2O_2$	A	89	132	EtOH/H_2O	(10)	43.9 ± 7.2
30	H	0		$C_{12}H_{10}N_2O$	A	73	82[a]	EtOH/H_2O	(8)	0
31	H	0		$C_{13}H_{12}N_2O$	A	67	222[b]	EtOH/H_2O	(8)	34.6 ± 10.7
32	H	0		$C_{13}H_{12}N_2O$	A	69	116[c]	EtOH/H_2O	(8)	23.6 ± 7.1
33	H	0		$C_{13}H_{12}N_2O$	A	70	160[d]	EtOH/H_2O	(10)	28.1 ± 7.7
34	H	0		$C_{12}H_{10}N_2O$	A	76	112[e]	*i*-Pr_2O	(7)	toxic
35	H	0		$C_{12}H_{10}N_2O$	A	73	208[f]	EtOH	(7)	toxic
36	H	0		$C_{14}H_{14}N_2O$	A	65	121	*i*-Pr_2O	(10)	0
37	H	1		$C_{13}H_{12}N_2O$	A	63	78[g]	EtOH/H_2O	(9)	toxic
38	H	2	"	$C_{14}H_{14}N_2O$	A	85	70[h]	*i*-Pr_2O	(8)	71.9 ± 11.8
46	H	0		$C_{13}H_{18}N_2O$	I	79	90	*i*-Pr_2O	(8)	39.3 ± 9.9
47	H	0		$C_{13}H_{18}N_2O$	I	60	161[i]	*i*-Pr_2O	(8)	0
48	H	1		$C_{14}H_{20}N_2O$	I	75	94	*i*-Pr_2O	(7)	53.2 ± 11.3

[a]Lit. [6] mp 82–84°C; [b]lit. [7] mp 127–128°C; [c]lit. [8] mp 112.5–113°C; [d]Lit. [9] mp 102–104°C; [e]lit. [10] mp 113–114°C; [f]lit. [10] mp 207–209°C; [g]lit. [11] mp 53°C; [h]lit. [12] mp 73–74°C; [i]lit [20] mp 164–165°C.

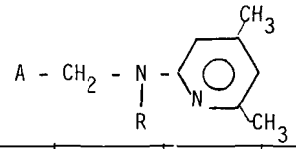
Table III. Physical properties and anti-inflammatory activity of secondary amides **50–53** and tertiary *N*-(4,6-dimethyl-2-pyridinyl) benzamides **60–63.**

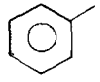
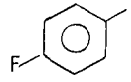
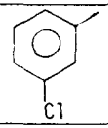
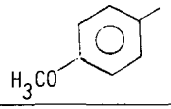
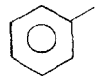
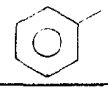


N°	A	R	Formula	Synth. method.	Yield %	mp, °C	Recrystn solv.	Carragenin-induced rat-paw edema	
								N	Inhibition %
50	CH_3	H	$C_9H_{12}N_2O$	A	81	160[a]	EtOH/H_2O	(10)	70.2 ± 8.5
51	C_2H_5	H	$C_{10}H_{14}N_2O$	A	80	112	*i*-Pr_2O	(8)	56.1 ± 7.2
52		H	$C_{14}H_{20}N_2O$	G	60	138	*i*-Pr_2O	(10)	50.7 ± 3.9[b]
53		H	$C_{15}H_{16}N_2O$	A	74	114	*i*-Pr_2O	(8)	51.1 ± 8.3
60		C_2H_5	$C_{16}H_{18}N_2O$	B[c]				(8)	80.8 ± 3.2
61		C_3H_7	$C_{17}H_{20}N_2O$	B				(7)	0
62		C_2H_5	$C_{16}H_{17}FN_2O$	B				(8)	66.8 ± 9.8
63		C_2H_5	$C_{16}H_{17}ClN_2O$	B				(6)	33.5 ± 10.3

[a]Lit. [13] mp 150–158°C; [b] tested at 100 mg·kg^{-1}, toxic at 200 mg·kg^{-1}; [c]compounds **60–63** have been previously described in [4].

Table IV. Physical properties and anti-inflammatory activity of 2-amino-4,6-dimethylpyridine derivatives **54–59** and **64–65.**



N°	A	R	Formula	Synth method	Yield	mp, °C	Recrystn solv	Carrageenin-induced rat-paw edema	
								N	inhibition %
54	CH_3	H	$C_9H_{14}N_2$	B[a]				(7)	31.9 ± 13.0
55	C_2H_5	H	$C_{10}H_{16}N_2$	B				(6)	21.8 ± 10.7
56		H	$C_{14}H_{16}N_2$	B C D E	93 25 54 85	78	*i*-Pr_2O	(8)	74.2 ± 3.2
57		H	$C_{14}H_{15}FN_2$	B	71	72	*i*-Pr_2O	(8)	65.5 ± 7.8
58		H	$C_{14}H_{15}ClN_2$	B	92	53	*i*-Pr_2O	(8)	32.4 ± 8.3
59		H	$C_{15}H_{18}N_2O$	F	48	152	*i*-Pr_2O	(7)	0
64		C_2H_5	$C_{16}H_{20}N_2$	B	64	*b*		(5)	0
65		C_3H_7	$C_{17}H_{22}N_2$	B	70	*c*		(7)	28.5 ± 11.0

[a]Compounds **54** and **55** have been previously described in [4]; [b]Eb / 0.1 mn: 78–80, n_D^{21} 1.540; [c]Eb / 0.1 mm: 98–100, n_D^{21} 1.537.

that this *N*-pyridinyl-benzamide can reduce eicosanoid production, we were led to investigate its possible inhibitory effects on cyclooxygenase, 5-lipoxygenase and phospholipase A_2 activities.

This compound did not inhibit lung homogenate cyclooxygenase activity even at 100 μM when a method similar to that described by Vane [28] was used. Experiments *in vivo* (29) were also performed by *i.v.* administration of AA in the anesthetized guinea pig. In these conditions the benzamide (50 $\mu M \cdot kg^{-1}$ *i.p.*) was unable to block the bronchospasm induced by thromboxane A_2 production contrary to indomethacin which completely inhibited the effect at 7 $\mu M \cdot kg^{-1}$ *i.p.*

The guinea-pig isolated trachea technique was used to assess a possible effect on 5-lipoxygenase activity. Contractions on the organ in the presence of AA are due to leukotriene C_4 and D_4 biosynthesis [30]. The studied molecule was unable to block leukotriene production or release at concentrations up to 5.10^{-4} M. In the same conditions, NDGA showed a marked inhibition at 10^{-5} M.

The preceding results suggest an inhibitory effect interfering with the eicosanoid biosynthesis process before the cyclooxygenase or lipoxygenase step, indicating a possible action at phospholipasic level.

Radioenzymatic determination of *in vitro* PLA_2 activity in the presence of benzamide **1** showed that this derivative had no direct inhibitory effect on the enzyme. In the same experimental conditions, indomethacin at 0.2 mM induced

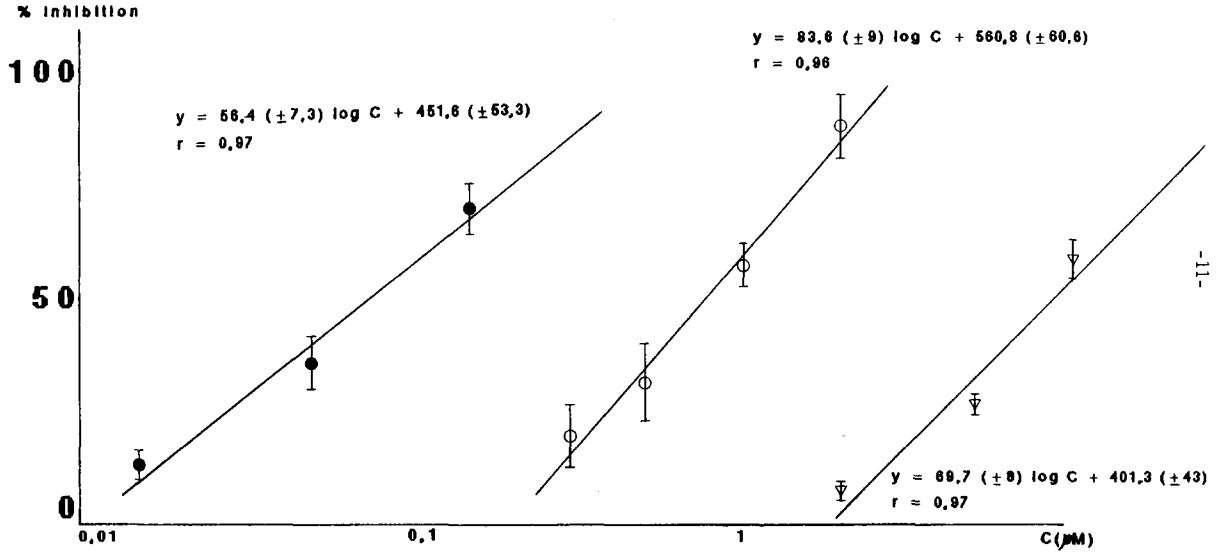


Fig. 1. Inhibitory effects of indomethacin (●), benzamide **1** (○) and dexamethasone (▽) on spontaneous contraction of isolated pregnant rat uterus.

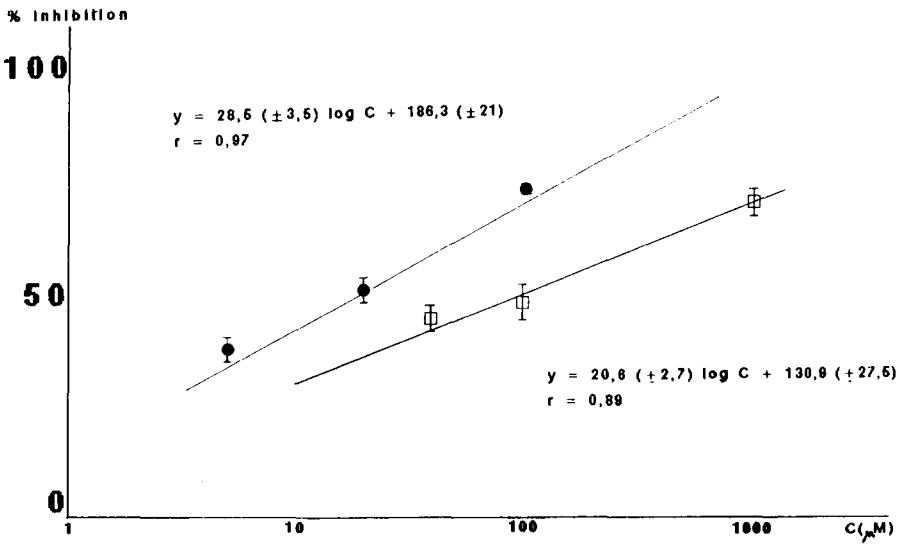


Fig. 2. Inhibitory effect of benzamide **1** and mepacrine on ^{3}H-AA release from cultured mouse peritoneal macrophages. □: benzamide 1; ●: mepacrine; Y: linear regression data; m ± SEM; r: regression coefficient.

a clear reduction of enzymatic activity (decrease of 34.9 ± 8.1%, $N = 4$); this result is in agreement with data reported by Franson *et al.* [31].

The mouse peritoneal macrophage technique [32], enabling membrane AA release to be assessed, also showed that in our conditions benzamide **1** blocked ^{3}H-AA release: IC_{50} = 0.12 mM and 17 μM for mepacrine as reference drug (Fig. 2). This result confirms the inhibitory activity of the studied molecule on eicosanoid production, despite its lack of effect on cyclooxygenase and 5-lipoxygenase. It is thus possible that the stimulatory effect of a mechanism incorporating fatty acids into membrane phospholipids may be involved or that, mainly because of the rapidity of the blocking effect on eicosanoid production, there may be inhibition of eicosanoid release from cellular membrane through disturbance at the phospholipasic A_2 level. In effect, it can be noted that glucocorticoids, which act *via* protein biosynthesis, only elicit their inhibitory effect slowly on spontaneous contractions of isolated rat pregnant uterus 20 min after contact with the organ [33], whereas the studied benzamide acts very quickly. A mechanism of action on one or more systems controlling membrane PLA_2 activity is likely.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Boiling points were determined by microdistillation. Structures of the described amides and their reduction products were supported by IR, ^{1}H NMR spectra and microanalytical data. IR spectra were run with KBr pellets or as a thin film on a NaCl disk with a Beckman IR 4230 grating infrared spectrophotometer. ^{1}H NMR spectra were recorded on a Varian EM 360, a Varian XL 100 or a Brucker AC 100 spectrometer using $CDCl_3$ or Me_2SO d_6 as solvent; chemical shifts are reported in parts per million (δ) from internal Me_4Si. Microanalyses were performed on an F and M Hewlett–Packard apparatus. Mass spectra were recorded on a double beam Varian Mat 112 spectrometer; ionisation energy was 70 eV. The spectra obtained confirm the proposed structures. Analytical TLC was performed on precoated silica gel aluminium plates (0.2 mm, GF 254, E. Merck); the spots were located by UV illumination. Evaporations were made *in vacuo* (rotating evaporator). Sodium sulfate was always used as the drying agent. Crude products were routinely passed through short columns of silica gel with an appropriate mixture of dichloromethane and ethanol.

Commercially available heterocyclic amines were used without further purification. 4-Amino-2,6-dimethylpyridine was obtained from 2,6-dimethylpyridine by successive *N*-oxydation, nitration [21] and reduction [22]; catalytic hydrogenation by W-2 Raney nickel, in an acetic acid–methanol (2/5) mixture [23] gave better results (55% yield) than chemical reduction by sodium dithionite [22]. The following experimental procedures are illustrative of the general procedures used to synthesize all of the compounds.

Method A: N-*(4,6-dimethyl-2-pyridinyl)-4-cyanobenzamide* ***24***
To a cold solution of 2-amino-4,6-dimethylpyridine (4.28 g, 35 mmol) and triethylamine (7 ml) in dichloroethane (35 ml) was added dropwise a solution of p. cyanobenzoyl chloride (5.8 g, 35 mmol) in dichloroethane (20 ml) during a period of 0.25 h. Stirring was continued for 1 h at room temperature and for 3 h at 60°C. After evaporation of the reaction mixture to dryness, the residue was washed with water, neutralized by $NaHCO_3$ and extracted with methylene chloride. The organic layer was washed, dried and evaporated to give 7.66 g (87.1% yield) of crude **24.** After column chromatography, crystallization from EtOH–H_2O gave 7.14 g (81.2% yield) of pure **24** as white crystals: mp 152°C; IR (KBr) 3440 (ν_{NH}), 2240 ($\nu_{C\equiv N}$), 1675 ($\nu_{C=O}$); ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, $\gamma-CH_3$), 2.43 (s, 3H, $\alpha-CH_3$), 6.38 (s, 1H, $H_{\beta'}$), 7.87 (d, 2H, J = 7.0 Hz, H_2, H_6), 8.10 (s, 1H, H_β), 8.13 (d, 2H, H_3, H_5), 9.1 (br.s, 1H, NH). Anal. ($C_{15}H_{13}N_3O$) C, H, N.

Method B: 2-(4-fluorobenzylamino)-4,6-dimethylpyridine ***57***
A solution of *N*-(4,6-dimethyl-2-pyridinyl)-4-fluorobenzamide **8** (2.46 g, 10 mmol) in dry ether (100 ml) was reduced by lithium aluminium hydride (0.38 g, 10 mmol). After 3 h of heating, excess hydride was destroyed by progressive addition of ice-water. The two phases were separated and the organic phase was washed (water), dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by column chromatography. By recrystallization from diisopropyl ether 1.62 g (70.6 % yield) of pure **57** was obtained: mp 72°C, IR (KBr) 3260 (ν_{NH}), 2950, 2920 (ν_{CH_2}), 1010 (ν_{C-F}); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, $\gamma-CH_3$), 2.37 (s, 3H, $\alpha-CH_3$), 4.43 (d, 2H, ^3J = 6 Hz, CH_2), 4.90 (t, 1H, NH) 6.0 (s, 1H, $H_{\beta'}$), 6.37 (s, 1H, H_β), 7.17 (m, 4H, arom. H). Anal. ($C_{14}H_{15}FN_2$) C, H, N.

Method C: 2-benzylamino-4,6-dimethylpyridine ***56***
A mixture of 2-amino-4,6-dimethylpyridine (6.1 g, 50 mmol), benzylic alcohol (7.6 g, 70 mmol) and potassium hydroxide (pellets, 0.5 g) was placed in a round-bottomed flask equipped with a short distillation column, and progressively heated up to 150°C. Water distilled and the reaction became exothermic. After 2 h contact at 200–220°C, the reaction mixture was cooled, extracted by methylene chloride and washed with water. The organic phase was dried and evaporated *in vacuo*. The crude product was purified by column chromatography using first methylene chloride and then a 95/5 methylene chloride–ethanol solvent system. The collected product **56** was recrystallized from diisopropyl ether to afford 2.63 g (24.8% yield) of pure **56:** mp 78°C; IR (KBr) 3260 (ν_{NH}), 3060, 3030 ($\nu_{arom.\ CH}$), 2950, 2920 ($\nu_{aliph.\ CH}$), 1610, 1585, 1575 ($\nu_{C=C}$); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, $\gamma-CH_3$), 2.40 (s, 3H, $\alpha-CH_3$), 4.53 (d, 2H, ^3J = 6.0 Hz, CH_2), 4.97 (t, 1H, NH), 6.13 (d, 1H, $H_{\beta'}$), 6.43 (d, 1H, H_β), 7.50 (m, 5H, arom. H). Anal. ($C_{14}H_{16}N_2$) C, H, N.

Method D
2-Amino-4,6-dimethylpyridine (2.4 g, 20 mmol) was dissolved in DMF (20 ml). NaH mineral oil dispersion (50% sodium hydride, 1.1 g, 22 mmol) was rinsed with dry toluene and gradually poured into the stirred solution. After 15 min, benzyl bromide (3.8 g, 22 mmol), dissolved in DMF, was added. Stirring was continued for one night, at room temperature, after which the reaction mixture was poured into water. The organic layer was extracted with methylene chloride and the combined extracts were washed with water, dried (Na_2SO_4) and evaporated to give the crude product. This was purified by column chromatography as previously described to give 2.28 g (53.8% yield) of **56.**

Method E
A mixture of benzaldehyde (5.3 g, 50 mmol) 2-amino-4,6-dimethylpyridine (6.1 g, 50 mmol) and formic acid (2.3 g, 50 mmol) was heated at 110–120°C for 36 h. The cooled reaction mixture was acidified with N–HCl and extracted with ether. The aqueous layer was alkalinized with 5N–NaOH and washed with water, dried (Na_2SO_4) and evaporated. After the usual workup **56** was obtained in a 84.7% yield.

Method F: 2-(4-methoxybenzylamino)-4,6-dimethylpyridine ***59***
A solution of 2-amino-4,6-dimethylpyridine (6.1 g, 50 mmol) and *p*-anisaldehyde (6.8 g, 50 mmol) in dry toluene (25 ml) was heated under reflux in a round-bottomed flask equipped with a Dean–Stark; 0.6 ml water was collected. The yield in imine (66%) was confirmed by the ^{1}H NMR spectrum of the crude product: ($CDCl_3$) δ 2.33 (s, 3H, $\gamma-CH_3$), 2.50 (s, 3H, $\alpha-CH_3$), 3.87 (s, 3H, OCH_3), 7.0 (m, 2H, H_3, H_5), 7.87 (m, 2H, H_2, H_6), 9.07 (s, 1H, CH=N). After complete evaporation of toluene, the organic layer was dissolved in methanol (50 ml). Sodium borohydride (2.0 g) was progressively added to the stirred solution. After 0.5 h of stirring at room temperature, the methanol was evaporated. The resulting residue was acidified by dilute acetic acid and extracted by methylene chloride. The organic phase was acidified by N–HCl. The aqueous acidic phase was alkalinized by N–NaOH and extracted several times by methylene chloride. The combined organic extracts were washed with water, dried and evaporated in vacuo. The resulting oil was purified by silica gel column chromatography using first methylene chloride and then a 98/2 methylene chloride-ethanol solvent

system. Elution afforded 6.63 g (54.8% yield) of **59** as a pale yellow oil. This was crystallized from diisopropyl ether and recrystallized from ethanol water to afford 5.8 g (47.9% yield) of **59** as white crystals: mp 152°C; IR (KBr) 3370 (ν_{NH}), 2850 (ν_{OCH_3}); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, $\gamma-CH_3$), 2.58 (s, 3H, $\alpha-CH_3$), 3.80 (s, 3H, OCH_3), 4.41 (d, 2H, CH_2), 6.24 (s, 1H, $H_{\beta'}$), 6.39 (s, 1H, H_β), 6.89 (d, 2H, H_3, H_5), 7.28 (d, 2H, H_2, H_6), 7.15 (m, 1H, NH). Anal. ($C_{15}H_{18}N_2O$), C, H, N.

Method G: N-*(4,6-dimethyl-2-pyridinyl) cyclohexylcarboxamide* ***52***

To a solution of *N*-(4,6-dimethyl-2-pyridinyl) benzamide **1** (1 g, 4.4 mmol) in acetic acid (30 ml) was added PtO_2 (0.100 g). The mixture was stirred under an H_2 atmosphere at room temperature and pressure. When consumption of hydrogen ceased, the catalyst was filtered off and the solvent evaporated *in vacuo*. The residue was neutralized by $NaHCO_3$; the resulting solid was filtered, washed with water and dried: 0.755 g (73.5% yield). Purification was accomplished by column chromatography on silica gel using a 9/1 methylene chloride–ethanol solvent system. The collected product was recrystallized from diisopropyl ether to afford 0.620 g (60.3% yield) of **52** as cream-colored crystals: mp 188°C; IR (KBr) 3240 (ν_{NH}), 3060 ($\nu_{arom.\ CH}$), 2920, 2850 ($\nu_{aliph.\ CH}$), 1610, 1570 ($\nu_{C=C}$), 1540 (δ_{NH}), 1280 (amide III); ^{1}H NMR ($CDCl_3$) δ 1.10–2.20 (m, 11H, CH_2, CH), 2.33 (s, 3H, $\gamma-CH_3$), 2.43 (s, 3H, $\alpha-CH_3$), 6.76 (d, 1H, $J_{\beta'\beta}$ = 1.3 Hz, H_β), 7.96 (d, 1H, H_β), 8.14 (s, 1H, NH). Anal. ($C_{14}H_{20}N_2O$) C, H, N.

N-[((2-Piperidinyl) methyl)]benzamide **39** was similarly prepared from *N*[(2-pyridinyl) methyl] benzamide **37** in 64% yield: mp 100°C after recrystallization from diisopropyl ether; IR (KBr) 3340, 3240 (ν_{NH}), 1640 ($\nu_{C=O}$); ^{1}H NMR ($CDCl_3$) δ 1.0–1.78 (m, 6H, CH_2), 1.83–2.33 (m, 2H, piperid. CH_2–NH), 2.83 (br.s, 1H, piperid. NH), 2.7–3.2 (m, 1H, CH), 3.2–3.5 (m, 2H, CH_2–NH), 7.1 (br.s, 1H, NH), 7.47–7.77 (m, 3H, H_3, H_4, H_5), 7.83–8.10 (m, 2H, H_2, H_6). Anal. ($C_{13}H_{18}N_2O$) C, H, N.

*Method H: 4-benzamido-*N*-methylpyridinium iodide* ***41***

A solution of *N*-(4-pyridinyl) benzamide **35** (3.96 g, 20 mmol) and methyl iodide (5.7 g, 40 mmol) in acetonitrile (30 ml) was heated under reflux during 1 h. The solvent was evaporated under reduced pressure. Recrystallization of the residue from ethanol afforded 6.53 g (96% yield) of the pure compound **41** as yellow crystals: mp 232°C; IR (KBr) 3180 (ν_{NH}), 2830 (ν_{N-CH_3}), 1685 ($\nu_{C=O}$); ^{1}H NMR ($(CD_3)_2SO$) δ 4.28 (s, 3H, $N-CH_3$), 7.56–7.69 (m, 3H, H_3, H_4, H_5), 7.97–8.12 (m, 2H, H_2, H_6), 7.34 (d, 2H, $J_{\beta\alpha}$ = 7.2 Hz, H_α), 8.67 (d, 2H, H_β), 11.52 (s, 1H, NH).

3-Benzamido-*N*-methylpyridinium iodide **40** and 2-benzamidomethyl-*N*-methyl-pyridinium iodide **42** were similarly prepared from the secondary benzamides **34** *and* **37. 40:** yield 96.2%; mp 212°C (Et_2O). **42:** yield 97%; mp 176°C (i-Pr_2O).

Method I: N-*(1-methyl-4-piperidinyl) benzamide* ***47***

To a stirred solution of **41** (5.1 g, 15 mmol) in methanol (30 ml) was added portionwise $NaBH_4$ (1.35 g, 36 mmol). The reaction mixture was heated under reflux for 3 h. The solvent was distilled off under reduced pressure and the residue was solubilized into dichloromethane. The organic phase was washed with water, dried and evaporated to give the crude product as a viscous oil. Distillation (Eb/0.1 mm: 140°C) afforded 2.53 g (78% yield) of pure *N*-(1-methyl-1,2,5,6-tetrahydro-4-pyridinyl) benzamide **44:** IR (KBr) 3300 (ν_{NH}), 2780 (ν_{N-CH3}), 1635 ($\nu_{C=O}$); ^{1}H NMR ($CDCl_3$) 2.33–2.73 (m, 4H, CH_2-CH_2), 2.43 (s, 3H, CH_3), 3.0–3.20 (m, 2H, CH_2-CH), 6.33 (t, 1H, H_β), 7.43–7.67 (m, 3H, H_3, H_4, H_5), 7.77–8.0 (m, 2H, H_2, H_6).

To a solution of **44** (2.16 g, 10 mmol) in ethanol (60 ml) was added palladium on charcoal (10%, 60 mg). The mixture was stirred under an H_2 atmosphere. When consumption of hydrogen ceased, the catalyst was filtered off and the solvent evaporated under reduced pressure. The resulting solid was recrystallized from diisopropyl ether to afford 1.31 g (60% yield) of **47** as pale yellow crystals: mp 150°C; IR (KBr) 3320 (ν_{NH}), 2780 (ν_{CH_2}), 1635 ($\nu_{C=O}$); ^{1}H NMR ($CDCl_3$) δ 1.7–2.3 (m, 4H, CH_2), 2.33 (s, 3H, CH_3), 2.47–3.17 (m, 4H, CH_2-N), 4.0 (m, 1H, CH), 6.27 (s, 1H, NH), 7.40–7.67 (m, 3H, H_3, H_4, H_5), 7.77–8.0 (m, 2H, H_2, H_6). Anal. ($C_{13}H_{18}N_2O$) C, H, N.

Reduction of **40** by sodium borohydride afforded the 1,2,5,6-tetrahydroderivative **43** in 95.8% yield: mp 51°C (*i*-Pr_2O). Catalytic reduction of **43** gave *N*-(1-methyl-3-piperidinyl) benzamide **46** in 79% yield, mp 90°C. Anal. ($C_{13}H_{18}N_2O$) C, H, N. In the same way, iodide **42** was reduced into the corresponding tetrahydro derivative **45;** it was obtained in 93.5% yield after distillation (Eb/0.1 mm Hg: 143–145°C) as an oil.

Catalytic reduction afforded *N*-[(1-methyl-2-piperidinyl) methyl] benzamide **48** in a 74.9% yield after recrystallization from diisopropyl ether: mp 94°C. Anal. ($C_{14}H_{20}N_2O$) C, H, N.

Pharmacology

Drugs

The drugs used in *in vitro* experiments were indomethacin, arachidonic acid, nordihydroguaiaretic acid, dipalmitoylphosphatidylcholine (Sigma, USA), dexamethasone sodium phosphate (MSD), bromophenacyl bromide (Janssen Pharmaceutica), trichlorethylene (CPF, France); all were expressed as base molecules ; dexamethasone acetate was used as a reference drug *in vivo* experiment. D-Tubocurarine hydrochloride (Laboratoire Lebrun, France) and urethan (Merck, FRG) were used in other tests.

Animals

Wistar CF male (150 ± 10 g) and female rats and male guinea pigs (300–400 g) were used; food and water were given *ad libitum,* unless otherwise stated.

Carragenin-induced rat-paw edema

The inhibitory activity of the studied molecules on carragenin-induced rat-paw edema was determined according to the method of Winter *et al.* [34] with slight modification. The drugs were orally administered (200 mg·kg^{-1}) 4 h before injection of 0.05 ml of a 1% suspension of carragenin in saline into the subcutaneous tissues of one hind paw. The other hind paw was injected identically with 0.05 ml of a saline solution. Since the hydration state of animals can modify the intensity of swelling, rats were fasted 24 h before experiment, and water (1.5 ml/100 g body weight) was orally administered twice (20 h and 4 h) before injections. Volumes of both hind paws of control and treated animals were measured with a plethysmograph 3 h after injections. Rats were kept in the same experimental conditions.

Inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls. The mean of the obtained values was then calculated.

Spontaneous contracting pregnant rat uterus

In vitro spontaneous contractile activity of rat uterus was induced by marked prostaglandin synthesis and release from this tissue [33]. Uteri were removed from pregant rate (17–21 days) weighing 300 ± 50 g, and strips were suspended in a 20 ml organ bath with Krebs solution at 37°C, gassed with 95% O_2, 5% CO_2. The bath fluid was changed every 20 min. Two h later, concentrations were recorded isotonically, and drugs were added immediately after washout with each concentration remaining in contact with the organ for 2 cycles of 20 min. Tissue responses were evaluated as the product of the number and mean height of contractions during the last 15 min period of the second cycle. Control values are those obtained in the absence of drug. IC_{50} were calculated from inhibition percentages compared to control means.

Prostacyclin production by rat aortic tissue

The method used has been previously described by Darius and Schör [35]. Control determinations were performed with the vehicle of the molecule (ethanol: water 1/10).

Cyclooxygenase activity

Cyclooxygenase activity was studied using two techniques; the first was the method described by Vane [28], involving cyclooxygenase activity of guinea pig lung homogenate. The following modifications were introduced: substitution of Bücher buffer by 0.25 M-sucrose solution, and additional centrifugation (5 min, 900 *g*) of the incubation medium in order to prevent baseline contracting effects on rat colon.

The second method, similar to that described by Greenberg *et al.* [29], involves arachidonic acid-induced bronchoconstriction in the anesthetized guinea pig after *i.v.* administration. Indomethacin pretreatment antagonizes this *in vivo* effect. Male guinea pigs were anesthetized with urethan (1.5 g·kg^{-1} *i.p.*), curarized (*d*-tubocurarine 1 mg·kg^{-1} *i.p.*) and placed under artificial ventilation (10 ml; 60 s/min). Bronchomotricity was then measured, according to the method of Halpern [36] with a Beckman transducer (0–400 mmHg) and recorded on a Beckman R611 polygraph. Airway sensitivity was tested by previous administration of histamine (5 μg·kg^{-1} *i.v.*). Arachidonic acid injection was then performed (0.25–0.75 mg·kg^{-1} *i.v.*) immediately before and 30 min after drug administration.

5-Lipoxygenase activity
The technique used has been previously described [30]. Arachidonic acid-induced contractions were isotonically recorded with an Ugo-Basile transducer device. The calcium concentration was kept constant (2.2 mM), and contractions were measured 15 min after arachidonic acid addition.

Radioenzymatic determination of in vitro *phospholipase A_2 activity*
The enzymatic activity of PLA_2 from *Naja naja* venom was estimated by 1-stearoyl-2 $^{14}C_1$-arachidonylphosphatidylcholine hydrolysis [37]: a direct inhibitory effect induced a decrease of ^{14}C-AA release. To the reaction medium (250 μl Tris buffer, pH 7.2, 10 mM $CaCl_2$) in test tubes were added 17 mM of radiolabeled phospholipid (50 mCi·mmol^{-1}), 0.5 μg of PLA_2 and 2.10^{-4} mM of the studied drug. After incubation (10 min, 37°C) the reaction was stopped by addition of 1.5 ml of chloroform-methanol (1:2 v/v) and lipids were extracted twice with chloroform after acidification with 25 μl 0.5 N−HCl. The chloroformic extracts were concentrated by evaporation under reduced pressure and then separated by TLC [38]. The spotting zones corresponding to phospholipids and ^3H-AA were cut, scratched and placed in scintillation vials to determine radioactivity (LKB instrument). Benzamide **1** and indomethacin were added to the reaction medium in hydro-ethanolic solution (85:15 v/v) in a 25μl volume.

Arachidonic acid release from cultured mouse peritoneal macrophages
The technique used was that described by Bonney *et al.* [32] with slight modifications: the culture medium was RPMI 1640 (GIBCO) with glutamine, and the stimulation of ^3H-AA release was induced by calcium ionophore A 23187 10^{-5} M added under a 25 μl volume. After 2 h incubation, 50 μl of the culture supernatant were treated by TLC with a solvent medium composed as follows: benzene, dioxane, acetic acid, formic acid, (82:14:1:1 v/v). After drying, the spotting zones were treated as previously described for radioenzymatic evaluation. In these conditions, 95% of the radioactivity released in the medium corresponded to ^3H-AA.

Since the studied drug was already dissolved in ethanolic solution (25 μl), control determinations were done in the presence of 25μl of ethanol for the incubation time.

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