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FORMAMIDINES WITH ANTINOCICEPTIVE AND ANTIINFLAMMATORY ACTIVITIES

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SUMMARY — *Pursuing our investigations on 7-amino-2,3-polymethyleneindole derivatives, a set of 7-(dimethylaminomethylene)-amino-2,3-polymethyleneindoles, together with some other aryl or cycloalkyl substituted formamidines, were prepared and tested for analgesic and antiinflammatory activities. Several compounds resulted endowed with one or both of these activities; the indole derivatives* **1** *and* **2** *exhibited a good degree of both of them.*

RIASSUNTO — *Proseguendo lo studio dei derivati 7-ammino-2,3-polimetilenindolici sono stati preparati alcuni 7-(dimetilamminometilen)ammino-2,3-polimetilenindoli nonché alcune altre amidine recanti spezzoni aromatici o alicicli-ci. I composti sono stati studiati per quanto concerne le attività analgesica e antiinfiammatoria. Svariati prodotti sono risultati dotati dell'una e/o dell'altra attività; appaiono interessanti i derivati indolici* **1** *e* **2** *che presentano ad elevato livello le due attività.*

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Introduction

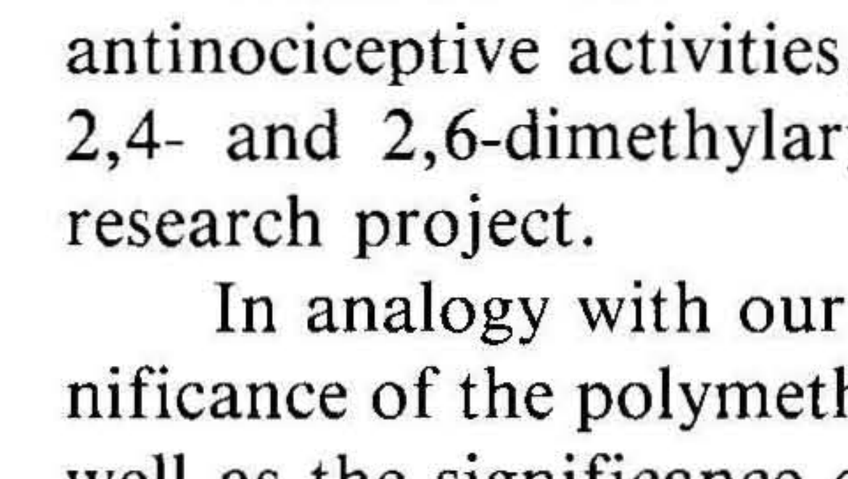
Several compounds bearing an amidine group seem to be involved in different biological and pharmacological areas (1), and recently a particular importance has been attained by compounds containing a formamidine moiety joined with an aromatic nucleus.

Thus N'-(imidazolyl)phenyl-N''-alkylformamidines (2) show potent inhibition on hystamine receptors H-2 (bisfentidine), whereas, N'-(4-arylmethylamino)-2,6-dichlorophenyl-N'', N''-dimethylformamidines display different levels of antihypertensive and diuretic activities (3).

On such a basis we deemed interesting to study the effects of the introduction of a formamidine function on position 7 of certain 2,3-polymethyleneindoles **B**, which since a long time are object of our investigation. In fact, several 7-(N-acyl)amino derivatives of general formula **A** exhibited different levels, sometimes rather high, of antiinflammatory (4,5) and analgesic (6) activities.

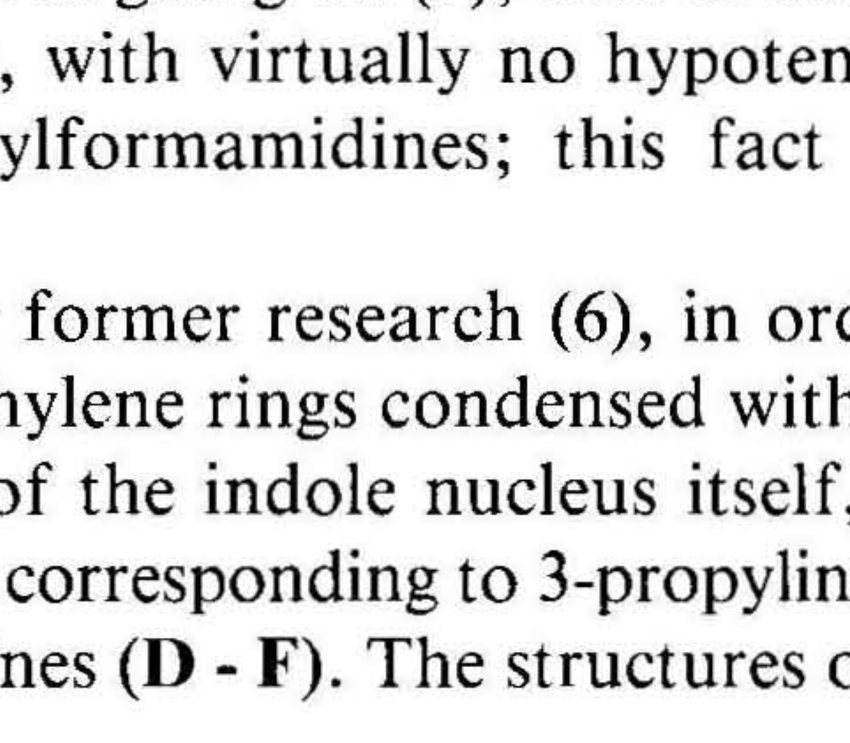
While our research was going on (7), Gall *et al.* (8) observed very high antinociceptive activities, with virtually no hypotensive effects, in certain 2,4- and 2,6-dimethylarylformamidines; this fact further validated our research project.

In analogy with our former research (6), in order to evaluate the significance of the polymethylene rings condensed with the indole nucleus, as well as the significance of the indole nucleus itself, we prepared also the 7-formamidine derivative corresponding to 3-propylindole (**C**) and some aryl- and cycloalkylformamidines (**D - F**). The structures of these compounds are illustrated in Scheme 1.

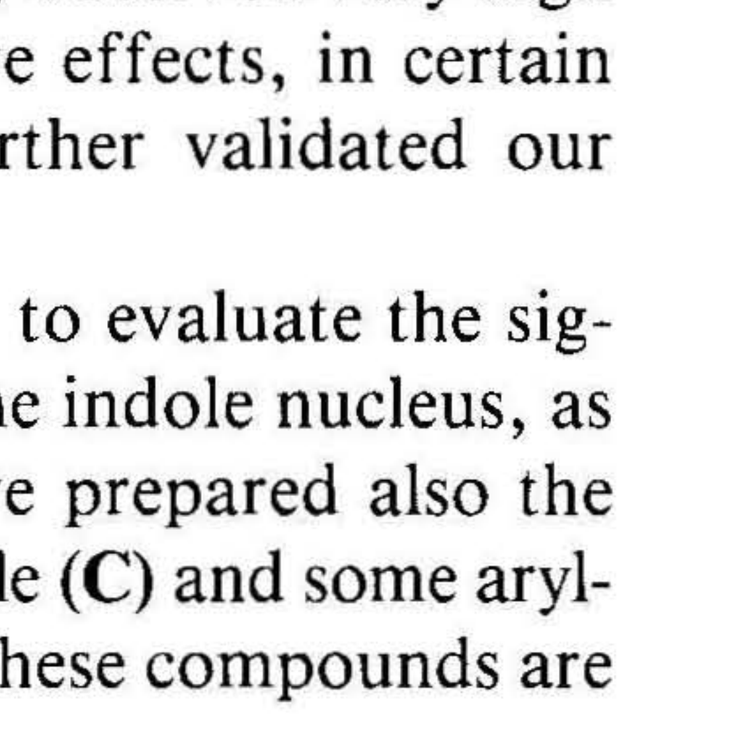


'A'

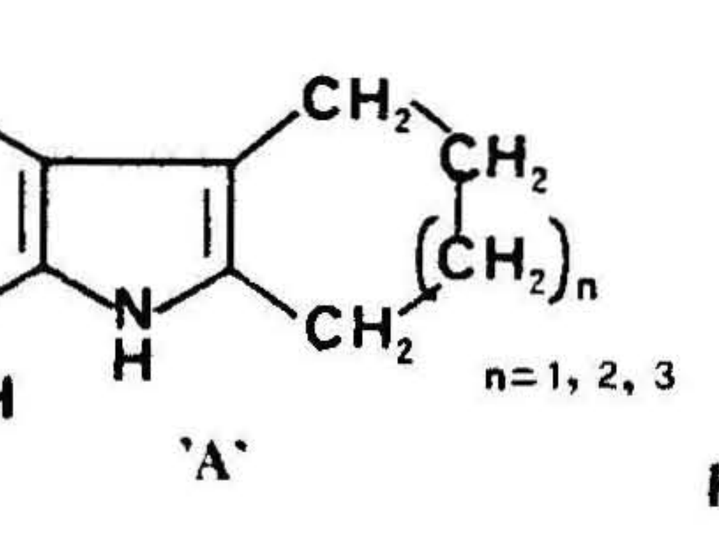
Z= CO-R; CO-CF3
SO2-CF3



'B'

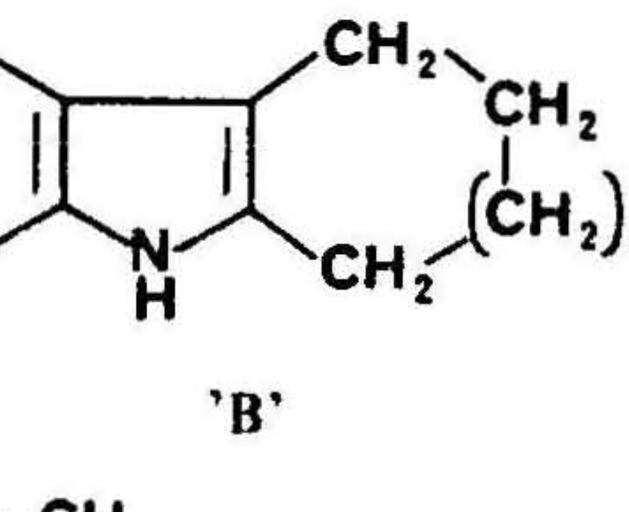


'C'



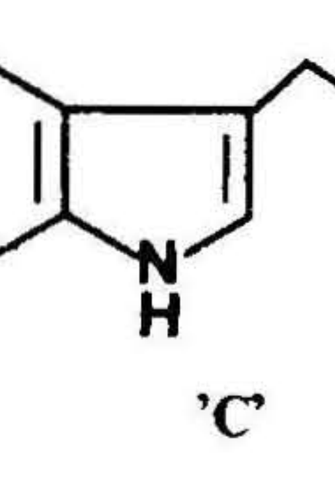
R = H
OCH3
CF3

'D'



R = H
OH

'E'



'F'

The arylformamidines **D** were already known, but no data on their pharmacological activities were available.

In the present paper we describe the results of the investigation on antinociceptive and antiinflammatory activities, while those concerning possible antihypertensive and diuretic activities will be the object of a forthcoming paper.

Chemistry

The preparation of formamidines **B - F** was effected by refluxing the benzene solution of the suitable amino compounds with an excess of N,N-dimethylformamide dimethylacetal:

The structure of the obtained compounds is supported by elemental analyses and IR and NMR spectra and, in the case of alicyclic compounds **E** and **F**, also by mass spectra (see Tables I and II).

The required 7-amino-2,3-polymethyleneindoles and 7-amino-3-propylindole were prepared as already described by us (6,9). The alicyclic amines were prepared according to the literature: (+) *trans*-2-aminocyclopentanol through ammonolysis of cyclopentene oxide (**10, 11**) and 3-aminocyclopentene through the reaction of cyclopentadiene with dry hydrogen chloride followed by the ammonolysis of the resulting chlorocyclopentene (12).

Chemical Experimental Section

Melting points were determined using a Kofler apparatus and were not corrected. Elemental analyses (C, H, N) were perfomed at the Microanalytical Laboratory of the Department of Pharmaceutical Sciences, Padua University, and the analytical results for the elements indicated were within $\pm 0.3\%$ of the calculated values.

IR spectra were recorded with a Perkin-Elmer mod. 298 Spectrophotometer using liquid samples as films and including solid samples in KBr pellets; NMR spectra were taken on a Varian XL 200 spectrometer, using $CDCl_3$ as solvent with TMS as internal standard. Mass spectra were obtained on a HP 5970A apparatus, using a capillary column SE 30 of 12 m length; programmed temperature was from 50°C to 270°C; detector temp. was 280°C; the carrier gas was helium at 99,9998% purity.

*7-(Dimethylamino)methylene amino-2,3-polymethyleneindoles (**B**) and 7-(dimethylamino)methylene amino-3-propylindole (**C**).*

In a boiling solution of 5-7 mmoles of 7-amino-2,3-polymethyleneindoles or 7-amino-3-propylindole in 15-25 ml of benzene were dropped rapidly 0.80 - 1.15 ml (stoichiometric quantity + 20% excess) of dimethylformamide dimethylacetal and the mixture was further refluxed for 0.5 to 1 hour.

When the conversion of the amino compounds to formamidine derivatives [as detected through TLC on silica using benzene-aceton (4:1) as eluent] was practically completed, the reacting mixture was cooled and the precipitate was collected and crystallized from benzene **B** or aqueous ethanol **C**.

All data concerning these compounds are collected in Tables I and II.

*N',N''-Dimethyl-N''-(3-R)arylformamidines (**D**) and N',N'-dimethyl-N''-cycloalkylformamidines (**E, F**).*

These formamidines were prepared under the above mentioned conditions, but after 30 to 60 min of reflux the solvent was removed under reduced pressure and the residue was distilled *in vacuo* (bulb to bulb). All data concerning these compounds are collected in Tables I and II. The boiling points of aryl formamidines **D** are in agreement with those reported in the literature (13, 14, 15).

TABLE I

*Formamidine derivatives **1-10***

Comp.	Structure	Formula	Molecular weight	Analyses	m.p. or b.p. (mmHg) °C	Yield %
1	B; n=1	$C_{16}H_{19}N_3$	241.3	C,H,N	205-207	75
2	B; n=2	$C_{16}H_{21}N_3$	255.3	C,H,N	181-183	73
3	B; n=3	$C_{17}H_{23}N_3$	269.4	C,H,N	144-145	83
4	C	$C_{14}H_{19}N_3$	229.3	C,H,N	119-120	72
5 [a]	D; R=H	$C_9H_{12}N_2$	148.2	C,H,N	78-80 (0.1)	70
6 [b]	D; R=OCH_3	$C_{10}H_{14}N_2O$	178.2	C,H,N	164 (12)	70
7 [c]	D; R=CF_3	$C_{10}H_{11}F_3N_2$	216.2	C,H,N	93-95 (12)	70
8	E; R=H	$C_8H_{16}N_2$	140.2	C,H,N	59-60 (6)	70
9	E; R=OH	$C_8H_{16}N_2O$	156.2	C,H,N	85 (7)	74
10	F	$C_8H_{14}N_2$	138.2	C,H,N	60 (10)	30

(a) known [13]; (b) known [14]; (c) known [15]

TABLE II

Spectral data of compounds ***1-4*** and ***8-10***

Comp.	IR, cm-1 (in KBr or film) NH indole	IR, cm-1 (in KBr or film) -C=N-	^{1}H-NMR,δ (CDCl3)	GC-Mass Retention time (min)	GC-Mass Most important fragments M+/z
1	3126	1630	1.75 (m, 4H, $2CH_2$); 2.55 (m, 4H, $2CH_2$); 2.85 (s, 6H, $2CH_3$); 6.85 (m, 3H arom.); 7.55 (s, 1H, -CH=); 9.05 (s, 1H, NH indole)		
2	3120	1630	1.65 (m, 6H, $3CH_2$); 2.75 (m, 4H, $2CH_2$); 3.00 (s, 6H, $2CH_3$); 6.85 (m, 3H arom.); 7.60 (s, 1H, -CH=); 8.95 (s, 1H, NH indole)		
3	3199	1626	1.50 (m, 8H, $4CH_2$); 2.70 (m, 4H, $2CH_2$); 3.00 (s, 6H, $2CH_3$); 6.75 (m, 3H arom.); 7.60 (s, 1H, -CH=); 8.95 (s, 1H, NH indole		
4	3130	1640	1.00 (t, 3H, CH_2-$\underline{CH_3}$); 1.75 (m, 2H, CH_2); 2.70 (t, 2H, CH_2); 3.00 (s, 6H, $2CH_3$); 6.90 (m, 4H arom.); 7.65 (s, 1H, -CH=); 8.85 (s, 1H, NH indole)		
8		1650	1.60 (m, 8H, $4CH_2$); 2.80 (s, 6H, $2CH_3$); 3.4 (m, 1H, CH-N); 7.25 (s, 1H, -CH=)		
9		1640	1.75 (m, 6H, $3CH_2$); 2.85 (s, 6H, $2CH_3$); 3.2 (m, 1H, CH-N); 3.35 (s, 1H, OH); 3.80 (m, 1H, $\underline{CH}$-OH); 7.25 (s, 1H, -CH=)	5.93	44 [$N(CH_3)_2$]; 57 [CH-$N(CH_3)_2$]; 71 [N=CH-$N(CH_3)_2$]; 85 [C_5H_8OH]; 99 [N-C_5H_8OH]; 156 [M^+]
10		1640	1.95 (m, 4H, $2CH_2$); 2.80 (s, 6H, $2CH_3$); 4.25 (m, 1H, CH-N); 5.50-5.75 (m, 2H, -CH=CH-); 7.20 (s, 1H, -CH=)	12.10	44 [$N(CH_3)_2$]; 57 [CH-$N(CH_3)_2$]; 67 [C_5H_7]. 71 [N=CH-$N(CH_3)_2$]; 138 [M^+]

Pharmacology

Materials and methods

For the detection of antinociceptive properties, female Swiss mice (Nossan) were used. The animals, weighing 18-22 g, were divided in groups of ten and stabulated at constant temperature and humidity (21°C ± 1°C; 60 ± 5%) with alternating 12-hour periods of light and dark.

Antiinflammatory activity was evaluated on male albino Wistar-Nossan rats (weight: 120-140 g).

In all cases the animals were fed with Nossan feed in pellets and *aqua fontis ad libitum*.

Antinociceptive activity against a chemical stimulus (peripheral analgesia).

This type of analgesia, which involves receptors μ and χ, was evaluated through the inhibition of writhings induced by the *i.p.* injection of phenylquinone (2 mg/kg as a 0.02% solution in 5% ethanol).

The test compounds were administered *per os* at the dose of 0.167 mmol/kg suspended in a 10% arabic gum solution.

The animals were dosed 15, 30, 60, and 120 minutes before the phenylquinone injection.

For every point three groups of 10 mice each were used. For each experiment were employed two groups (one at the beginning and one at the end) of control animals receiving only the quinone *i.p.* and the arabic gum *per os*.

After the injection of phenylquinone the animals were introduced into a glass cylinder and the writhings were recorded for 20 min starting from the fifth min after injection.

The results are expressed as the percent variation in writhings number compared to that of the control animals.

Antinociceptive activity against a thermic stimulus (central analgesia).

To evaluate this kind of analgesia, typically overspinal and involving the μ and δ receptors, we used the Eddy and Leimbach hot-plate test (16).

The plate was kept at 55 ± 0.5°C and the reaction time (in seconds) was determined on the basis of licking, raising of hind paws, jumping and escape reaction. The animals with a reaction time superior to 15 sec or inferior to 10 sec were discarded. The reaction time was measured thrice before the administration of the test compounds and 15, 30, 60, 90, 120, 150, 210 and 270 minutes after the dosing. The test compounds were administered p.o. at the dose of 0.167 mmol/kg, suspended in a 10% solution of arabic gum.

Antiinflammatory activity

Antiinflammatory activity was evaluated by means of the already described (5) inhibition of the carrageenan-induced hind paw edema in rats.

One hour before the injection of carrageenan the test compounds were administered *per os* at the dose of 1 mmol/kg suspended in about 10 ml/kg of a 5% arabic gum solution. The control animals received only arabic gum. The rats had been fasting from 12 hours, but with free access to water. The activity was evaluated by the edema volume at the 1st, 2nd, 3rd, and 4th hour after treatment with carrageenan and results are expressed as mean percent variation of edema volume com-

pared to that in control animals. Each value corresponds to the mean of six animals. Indomethacin at the dose of 0.028 mmol/kg (10 mg/kg) was used for comparison.

All animals were observed for 48 hours after administration of test compounds.

Results and Discussion

Depending on the quantity available for each compound the tests were not performed for all of them. The antinociceptive activity against a chemical stimulus was determined on all compounds except one (**4**). The antinociceptive activity against a thermic stimulus was investigated only with the four compounds **1, 2, 7** and **10**, wich resulted the most active in the foresaid test. The inhibitory activity against the carrageenan edema was tested on all compounds except compounds **9** and **10**. The results are illustrated in Tables III-IV.

Even if the compounds have been tested at a single dose, some indications have been provided by these preliminary assays.

It is worthy of note that all the three 2,3-polymethyleneindole derivatives (**1, 2, 3**) exhibited analgesic activity against a chemical stimulus, although of different degree and duration. For the remaining aromatic and alicyclic compounds the activity appeared to be erratic, depending on the nature of substituents (compounds **7** and **10**).

Compound **1**, 15 min after administration, exhibited strong antinociceptive activity which reached maximal intensity (-80%) after 30 min and then decreased slowly. Compound **2** presented a slow onset of activity which, however, growed steadily for the whole duration of the experiment; thus, 2 h after administration it produced 87% inhibition of writhings. The different behaviours may be attributed to a remarkable difference in the kinetics of absorption and/or of metabolic activation. Compound **3** gave rise to an alternating response. In fact, after only 30 min it exhibited a strong inhibitory activity that was of short duration; actually after 60 min the activity was over but it was then progressively restored reaching 38% inhibition of writhings 2 h after administration.

Concerning the arylformamidines, a short lived analgesic activity was observed in the unsubstituted compound and only a late activity in the 3-methoxyderivative; on the contrary, compound **7**, bearing a strong electron-withdrawing substituent, exhibited a strong activity with rapid onset and long duration, which was comparable with that of compound **1**. Even if with some reservations due to the different methodologies used, the activity of N', N'-dimethyl-N''-(3-trifluoromethyl)phenylformamidine appeared to be comparable with that of all the variously substituted arylformamidines described by Gall *et al* (8), with the exception of the 2,4- and 2,6-dimethyl derivatives.

Among the alicyclic derivatives a short lasting hyperalgesic activity was observed for the unsubstituted compound and a fleeting analgesic activity

TABLE III

Antinociceptive activity against a chemical stimulus (phenylquinone)

Comp.	Dose mg/Kg p.os (a)	Percent variation of writhings during 20 min compared with control animals (b). Reading was started 5 min after phenylquinone injection; the irritant was introduced after the indicated time from compounds administration.			
		15'	30'	60'	120'
1	40.2	-60.3	-80.1	-64.7 Δ	-51.2
2	42.5	-15.3	-30.0	-65.3 Δ	-87.3
3	44.9	+7.4	-52.3	-6.0	-38.2
5	24.7	-9.3	-38.8	+21.3	+9.4
6	29.7	-3.9	-1.1	+1.1	-35.1
7	36.0	-32.7	-71.9	-56.1	-53.4
8	23.4	+37.8	+1.2	+9.6	+5.3
9	26.0	-3.5	-22.2	+13.6	+12.5
10	23.0	-25.4	-18.8	-45.7	-7.5

(a) corresponding to ~ 0.167 nmoles/Kg

(b) inhibition (-); increase (+)

for the hydroxyderivative **9**. It is worthy of note that the activity of the unsaturated compound **10** was quite long lasting though at only moderate level (-46% at 60 min): this could indicate that the presence of an aromatic moiety is not necessary for the expression of analgesic activity.

Compounds **1, 2, 7** and **10** which proved to be the most active in the peripheral analgesia test, were also tested for central analgesia. None of them resulted active in the hot plate test, at least at the used dose of 0.167 mmol/kg; on the contrary, some contraction of the reaction time was observed, which normally lasted for several hours.

For what concerns the antiinflammatory activity, we noted that, at the oral dose of 1 mmol/kg, all the tested compounds exhibited a significant inhibition of edema ($\geqslant$30%) at the 3rd hour after the carrageenan injection. However, only the indole derivatives **1, 2, 3, 4** exhibited a rather strong activity, spanning the whole experimentation time. At the indicated dose, these compounds showed an activity which was equal to or higher than that of indomethacin at the dose of 10 mg/kg, and was also comparable to that of phenylbutazone (17) at the same dose of 1 mmol/kg (308 mg/kg).

TABLE IV

Antiinflammatory activity in the carrageenan induced rat paw edema

Compound	Dose mg.Kg p.os. (a)	Percent variation of edema volume at the following times (hours) after carrageenan injection (b)			
		1	2	3	4
1	241	-40	-58	-69	-79
2	255	-18	-51	-65	-63
3	269	-18	-60	-53	N.D.
4	229	N.D.	-77	-74	-72
5	148	-4	-13	-33	-21
6	178	-7	-21	-34	-25
7	216	-3	-6	-33	-27
8	140	-6	-25	-29	N.D.
indomethacin	10	-9	-58	-64	-43

(a) Corresponding to 1 mmole/Kg

(b) Inhibition (-) or increase (+) compared to the edema volume of control animals

N.D. Not determined

On doubling the dose of polymethyleneindole derivatives any sign of toxicity did not appear during the whole time (48 h) of observation, while antiinflammatory activity was only slightly increased.

Concluding, we can remark that compounds **1** and **2** appeared to be the most interesting, having shown a good degree of both peripheral antinociceptive and antiinflammatory activity, therefore their pharmacological profile will be deeply examined. Moreover, these results support our interest on the 7-aminoindole derivatives, whose pharmacogenic potentialities will be further investigated.

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