IL FARMACO, 48 (9), 1279-1289; 1993

AMIDES AND FORMAMIDINES WITH ANTINOCICEPTIVE ACTIVITY (NOTE I)

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SUMMARY — *Pursuing our investigations on N-aryl or N-cycloalkyl substituted amides and formamidines with antiinflammatory and/or analgesic activity, two set of cycloalkyl substituted amides and formamidines were prepared and tested for analgesic activity against a chemical stimulus. Some compounds, particularly the amides* ***7, 11, 17*** *and the formamidine* ***35*** *proved to be endowed with this activity.*

RIASSUNTO — *Proseguendo lo studio di ammidi e formammidine recanti residui arilici o cicloalchilici e dotate di attività antiinfiammatoria e/o analgesica sono state preparate due serie di derivati ammidici e formammidinici recanti spezzoni aliciclici. I composti sono stati saggiati per la ricerca dell'attività analgesica contro stimolo chimico. Alcuni prodotti sono risultati attivi; appaiono interessanti le ammidi* ***7, 11,*** *e* ***17*** *e l'ammidina* ***35****.*

Introduction

As development of former studies on amido derivatives (1,2) in a recent note (3) were described the antiinflammatory and antinociceptive activities of some 7-(dimethylaminomethylene)-aminoindole derivatives, variously alkylated in positions 3 or 2 and 3 and of the analogous derivatives of simple aromatic and alicyclic amines. With the aim of a more accurate eva-

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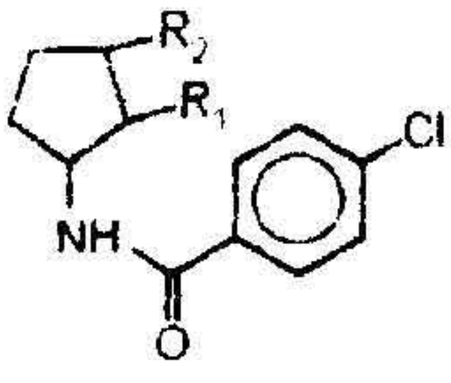
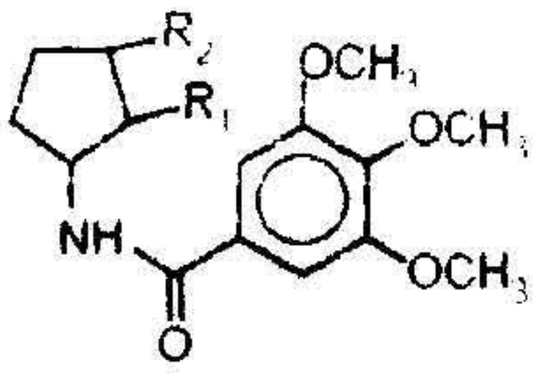
luation of the contribution to peripheral analgesic activity of the moieties in which the molecule can be formally split up, two large sets of amides and dimethylaminomethylene derivatives of cyclopentyl (Scheme 1) and cyclopentenylamine (Scheme 2) were prepared, utilizing the acyl residues present in the more active compounds, previously described (1,2).

Scheme 1



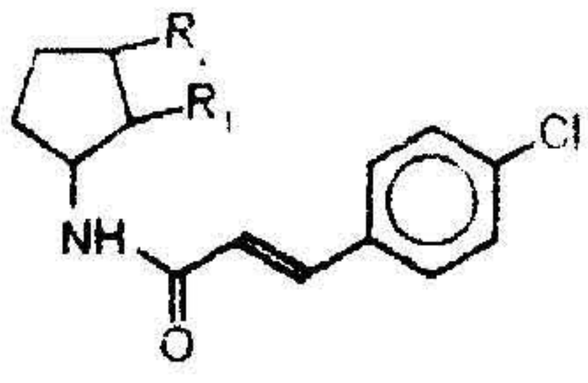
(I)

Ia = **1**
Ib = **2**
Ic = **3**
Id = **4**
Ie = **5**



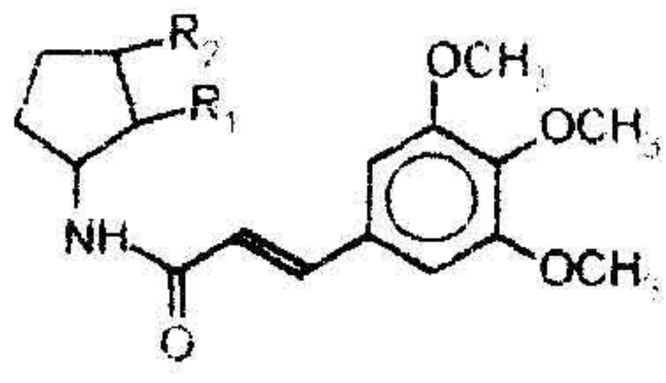
(II)

IIa = **7**
IIb = **8**
IIc = **9**
IId = **10**
IIe = **11**



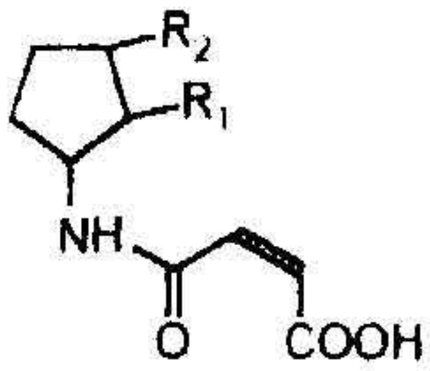
(III)

IIIa = **13**
IIIb = **14**
IIIc = **15**
IIId = **16**
IIIe = **17**



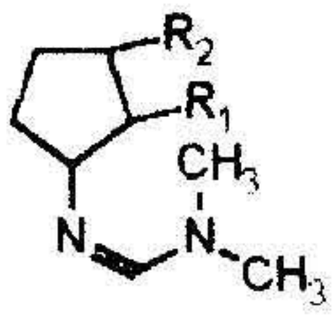
(IV)

IVa = **19**
IVb = **20**
IVc = **21**
IVd = **22**
IVe = **23**



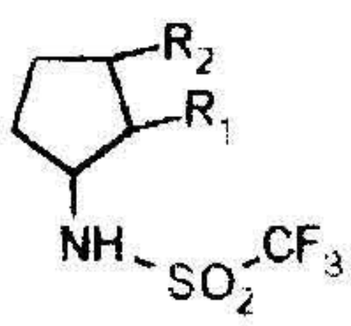
(V)

Va = **25**
Vb = **26**
Vc = **27**
Vd = **28**
Ve = **29**



(VI)

VIa = **31**[3]
VIb = **32**[3]
VIc = **33**
VId = **34**
VIe = **35**



(VII)

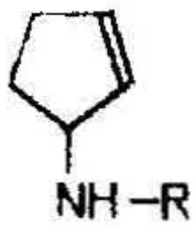
VIIa = **37**[2]
VIIb = **38**
VIIc = **39**
VIId = **40**
VIIe = **41**

a : R_1 , R_2 = H ; b : R_1 = OH, R_2 = H ; c : R_1 = Cl, R_2 = H

d : R_1 = CH_3, R_2 = H ; e : R_1 = H, R_2 = CH_3

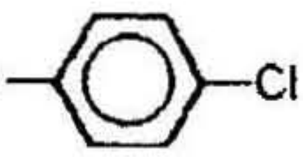
(2), (3) **see references**

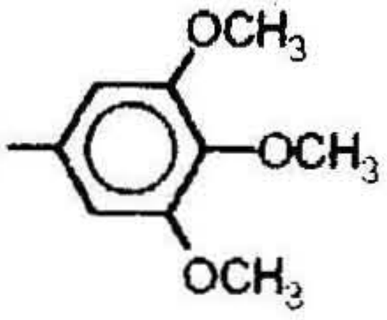
Scheme 2

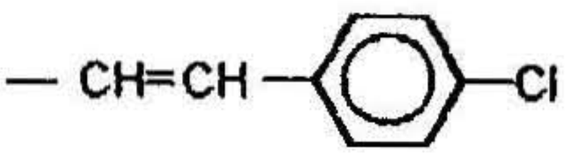


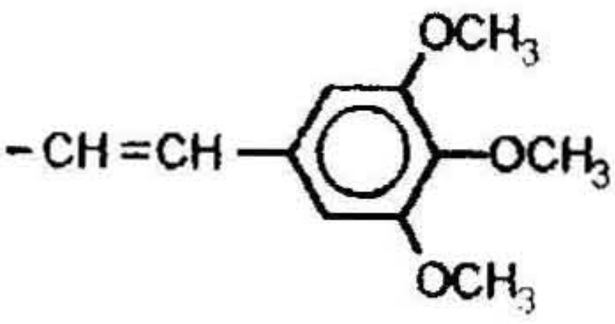
(VIII)

VIIIa = 6
VIIIb = 12
VIIIc = 18
VIIId = 24[4]
VIIIe = 30
VIIIf = 36[3]

a : R = 

b : R = 

c : R = 

d : R = 

e : R = —CO—CH=CH—COOH

f : R =

(3), (4) see references

Chemistry

The preparation of amides **1-24** was performed by stirring the benzene solution of the suitable amino compound with an excess of appropriate acyl halide at room temperature, in the presence of triethylamine.

The maleamic acids **25-30**, the formamidines **33-35** and the trifluoromethylsulfonyl amides **38-41** were prepared as already described (1,2,3)

The characteristics of compounds **1-41** are listed in Table I.

The structures of the obtained compounds was generally supported by elemental analysis and NMR spectra (Table II); for the derivative **24**, already known (4), by the agreement of its physical characteristics with bibliographic data, while the formamidines **31-36** and trifluoromethylsulfonyl amides **37-41** were characterized through GC-MS spectra (Table III).

The starting amines were prepared according to the literature: (±)trans-2-aminocyclopentanol through ammonolysis of cyclopentene oxide (5,6), (±)cis-2-chlorocyclopentylamine by the action of phosphorus pentachloride on the hydrochloride of the preceeding amine (5,7), (±)trans-2-methylcyclopentylamine through the reduction of 2-methylcyclopentanone oxime (8) with sodium and alcohol, and (±)3-methyl-cyclopentylamine through catalitic reduction (8) of 3-methylcyclopentanone oxime; at last 3-aminocyclopentene through the reaction of cyclopentadiene with dry hydrogen chloride followed by ammonolysis of the resulting chlorocyclopentene (9).

TABLE I

Characteristics of compounds **1-41**

Comp.	Formula	M.W.	Analyses	m.p. or b.p.(mm Hg) °C	Yield %	Reaction time	Method of purification
1	$C_{12}H_{14}NOCl$	223	CHN	166-168	51	20'	A
2	$C_{12}H_{14}NO_2Cl$	239	CHN	167-160	64	1h	B
3	$C_{12}H_{13}NOC_{12}$	258	CHN	149-151	78	1h	A
4	$C_{13}H_{16}NOCl$	237	CHN	138-140	85	30'	A
5	$C_{13}H_{16}NOCl$	237	CHN	135-136	66	1h,30'	C
6	$C_{12}H_{12}NOCl$	221	CHN	200-202$_{(d)}$	47	1h,30'	C
7	$C_{15}H_{21}NO_4$	279	CHN	179-180	68	30'	A
8	$C_{15}H_{21}NO_5$	295	CHN	145-146	60	1h	B
9	$C_{15}H_{20}NO_4Cl$	313	CHN	159-160	60	30'	C
10	$C_{16}H_{23}NO_4$	293	CHN	182-184	65	30'	A
11	$C_{16}H_{23}NO_4$	293	CHN	157-159	52	1h,30'	A
12	$C_{15}H_{19}NO_4$	277	CHN	140-141	41	1h	C
13	$C_{14}H_{16}NOCl$	282	CHN	170-172	74	20'	C
14	$C_{14}H_{16}NO_2Cl$	265	CHN	192-193	67	30'	C
15	$C_{14}H_{15}NOC_{12}$	284	CHN	168-169	62	2h	D
16	$C_{15}H_{18}NOCl$	263	CHN	44-46	67	20'	C
17	$C_{15}H_{18}NOCl$	263	CHN	140-142	50	30'	C
18	$C_{14}H_{14}NOCl$	247	CHN	202-203	52	2h	C
19	$C_{17}H_{23}NO_4$	305	CHN	178-179	75	30'	A
20	$C_{17}H_{23}NO_4$	321	CHN	198-200	55	1h	E
21	$C_{17}H_{22}NO_4Cl$	339	CHN	166-167	60	30'	A
22	$C_{18}H_{25}NO_4$	319	CHN	189-190	60	1h	A
23	$C_{18}H_{25}NO_4$	319	CHN	161-162	65	30'	A
25	$C_9H_{13}NO_3$	183	CHN	123-124	60	2h	A
26	$C_9H_{13}NO_4$	199	CHN	110-111	76	1h	A
27	$C_9H_{12}NO_3Cl$	217	CHN	47-49	70	1h	A
28	$C_{10}H_{15}NO_3$	197	CHN	63-64	65	1h	A
29	$C_{10}H_{15}NO_3$	197	CHN	75-77	65	1h	A
30	$C_9H_{11}NO_3$	181	CHN	105-106	57	1h	A
33	$C_8H_{15}N_2Cl$	174	GC-MS	82 (3 mm Hg)	86	40'	F
34	$C_9H_{18}N_2$	154	GC-MS	50 (4 mm Hg)	49	1h	F
35	$C_9H_{18}N_2$	154	GC-MS	106 (12mmHg)	93	50'	F
38	$C_6H_{10}NO_3SF_3$	233	GC-MS	115(0.2mmHg)	45		F
39	$C_6H_9NO_2SClF_3$	251	GC-MS	110(0.4mmHg)	50		F
40	$C_7H_{12}NO_2SF_3$	231	GC-MS	80(0.6mmHg)	50		F
41	$C_7H_{12}NO_2SF_3$	231	GC-MS	70 (0.6mmHg)	20		F

A) Crystallization (ethanol-water 1/1). B) Chromatography (silica gel column, eluent : benzene - aceton 3/2). C) Crystallization (ethanol). D) Chromatography (silica gel column, eluent: Petroleum ether-diethyl ether 3/2). E) Crystallization (tetrahydrofuran-methanol). F) Distillation under vacuum (bulb to bulb)

TABLE II

^{1}H-NMR spectra collected for molecules with similar structures

Comp. Solvent	δ
1 - 6 $CDCl_3$	0.95-1.1 (d, CH_3); 1.2- 2.8 (m, CH_2); 3.3 (s, OH for the product **2**); 4 - 5 (m, CH); 5.5 (m, CH=CH for the product **6**); 5.6 - 6.6 (s, NH); 7 - 8 (m, aromatics).
7 - 12 $CDCl_3$	0.8-1 (m, CH_3); 1-3 (m, CH_2); 3.3-4 (s, OCH_3); 4.6 (s, OH for the product **8**); 4.1-5.2 (m, CH); 5.4-6 (m, CH=CH for the product **12**); 6-6.7 (s, NH); 7-8 (m, aromatics).
13 - 18 $CDCl_3$/DMSO	0.8-1 (m, CH_3); 1-2 (m, CH_2); 3.8-5.2 (m, CH); 4.7 (s, OH for the product **14**); 5.7 (m, CH=CH for the product **18**); 6.4-6.8 (d, CH=CH cinnamic); 6.1-7.9 (s, NH); 7-8 (m, aromatics).
19 - 23 $CDCl_3$	0.7-1.2 (m, CH_3); 1.3-3 (m, CH_2); 3.3-4 (s, OCH_3); 4.5 (s, OH for the product **20**); 6.2-6.6 (m, CH=CH cinnamic); 6.3-7.8 (s, NH); 7-8 (m, aromatics).
25 - 30 $CDCl_3$/DMSO	0.8-1.2 (m, CH_3); 1.3-2.1 (m, CH_2); 3.5-4 (m, CH); 4.6 (s, OH alcholic for the product **26**); 5-5.7 (m, CH=CH for the product **30**); 5.9-6.4 (s, NH); 5.8-6.5 (m, CH=CH); 8-9 (s, OH acid).
33 - 35 $CDCL_3$	0.86-1.05 (d, CH_3); 1.75-2 (m, CH-R and CH_2); 2.8-2.9 (m, CH_3); 3.5-4.2 (m, CH-N); 7.25-7.27 (s, CH=N).
38 - 41 $CDCl_3$/DMSO	0.95-1.1 (d, CH_3); 1.2-2.2 (m, CH_2); 3.4-4.4 (m, CH); 3.85 (s, OH for the product **34**); 3.25-5.15 (s, NH).

TABLE III

*GC-MASS spectra of compounds **33-35** and **38-41***

Products	t_r (min)	Most Important fragments (M^+/e)
33	12,96	174 : (M^+); 176 : $(M+2)^+$; 139 : (M^+ - Cl•); 111 : (139 - CH_2=CH_2); 57: (CH-N-$(CH_3)_2$)
34	9,08	154 : (M^+) ; 139 : (M^+ - CH_3•) ; 111 : (139 - CH_2=CH_2)
35	9,19	same fragmentation of **34**
38	5,18	233 : (M^+) ; 133 : (M^+ - SO_2-CF_3); 116 : (133 - OH•)
39	4,28	251 : (M^+) ; 253 : $(M+2)^+$; 189 : (M^+ - CHCl=CH_2) ; 162 : (189 - - •CH=CH_2)
40	5,20	231: (M^+) ; 189 : (M^+ - CH_3-CH=CH_2)
41	5,42	same fragmentation of **40**

Chemical experimental section

Melting points were determined using a Kofler apparatus and were not corrected.

Elemental analyses (C,H,N) were performed at the Micro-analytical Laboratory of the Department of Pharmaceutical Sciences, Padua University, and the analytical results for the elements indicated were within ±0.3% of the calculated values.

^{1}H-NMR spectra were taken on a Varian VXR 300 (at the CNR laboratories; Sassari), using $CDCl_3$ or DMSO as solvents with TMS as internal standard. GC-Mass spectra were obtained on a HP 5970A apparatus, using a capillary column SE30 of 12 m length (**34-35**) and SE52 of 25m length (**38-41**); programmed temperature was from 80°C to 180°C (5°C/min.), detector temp. was 280°C and the carrier gas was Helium of 99.9998% purity, at 10 psi pressure.

*N-Aroylcyclopentylamines 2 or 3 monosubstituted (**1-23**)*

A stoichiometric quantity plus a 25% excess of the desired acyl halide, dissolved in 10-15 ml of benzene, was dropped slowly into a solution of 5 mmoles of amine and 6.5 mmoles of TEA in 30 ml of benzene. The mixture was stirred at room temperature for a time variable from 20 minutes to 2 hours (Table I); then the reacting mixture was filtered and the benzene solution was shaken with a solution of $NaHCO_3$ (5%) and subsequently with water. After benzene removal, pure compounds were obtained generally by crystallization of the residue from alcohol or hydroalcoholic mixtures, whereas in some cases it was necessary a chromatography (Table I) on silica gel.

*N-(2/3-R-cyclopentyl)- and N-(cyclopentenyl)-maleamic acids (**25-30**)*

In a solution of 1 mmole of amine in 10 ml of tetrahydrofuran, free from peroxides, 1 mmole of maleic anhydride dissolved in 20 ml of the same solvent was dropped. The mixture was further stirred, at room temperature, for 1 or 2 hour (Table I). Finally the solvent was removed under reduced pressure and the residue was crystallized as indicated in Table I.

*N,N-Dimethyl-N' cycloalkylformamidines 2 or 3 monosubstituted (**33-35**)*

A stoichiometric quantity plus a 20% excess of dimethylformamide dimethylacetal was dropped rapidly into a boiling solution of 5 mmoles of amine in 15 ml of benzene and the mixture was further refluxed for 0.5 to 1 hour. The solvent was removed under reduced pressure and the crude oils were distilled under vacuum (Table I).

*(N-Trifluoromethylsulfonyl)cyclopentylamides 2 or 3 monosubstituted (**38-41**)*

A solution of 5 mmoles of amine in 30 ml of dichloromethane was cooled, under stirring, with a suitable immersion cooler (SEDAS DF 100). When the solution's temperature reached −30°C a stoichiometric quantity of triethylamine was added and when the temperature reached −80°C the stoichiometric quantity plus a 10% excess of trifluoromethylsulfonic anhydride was slowly dropped. Finally the temperature was allowed to rise slowly to room value and the solvent was removed under reduced pressure. The crude oils were distilled under vacuum (Table I) leaving back the triethylamine trifluoromethylsulfonate.

Pharmacology

Materials and methods

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

The animals were fed with Nossan feed in pellets and *aqua fontis and libitum.*

Antinociceptive activity against a chemical stimulus (peripheral analgesia)

This type of analgesia, which seems to involve 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 60 minutes before the phenylquinone injection.

For every point three groups of 10 mice each were used. For each experiment 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* were employed.

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

The results are expressed as a percent variation in writhings number compared to that of the control animals (Table IV).

In order to detect any gross overall effect of the absorption-elimination rate and/or of metabolic activation, the antinociceptive activity of the four most active compounds (**7, 11, 17, 35**) was also measured 15, 30 and 120 minutes after the drug (0.167 mmoles/kg; *p.o.*) administration (Table V).

Moreover, having observed that in all cases the peak of activity occurred at 60 minutes from administration, the dependence of activity from dose at that time was investigated (Table VI).

Results and Discussion

The results of the writhing test on the newly prepared compounds are illustrated in Tables IV, V and VI. For comparison, in Table IV are included also the results relative to a few correlated compounds previously described (2,3).

From Table IV it is evident that, apart from compounds **36** and **37** already described, only four compouns (**7,11,17,35**) exhibited a fairly good activity, while six more (**2,4,8,10,16,19**) were just moderately active, being more or less comparable with acetanilide given at the same dosage (2), tak-

ing into account that acetanilide was tested against formic acid induced writhings. The remaining compounds were poorly active or completely inactive; some of them (**3,6,9,15,27**) were even endowed with strong hyperalgesic activity.

The trifluoromethylsulfonyl derivatives were rather more toxic than the other tested compounds; thus at the dose of 0.167 mmoles/kg (used for all compounds) mortality was observed and it was impossible to establish the eventual antinociceptive activity. The shortage of material prevented to test these compounds at lower doses.

With regard to structure-activity relationships, only a few preliminary observations may be warranted.

The active compounds were mainly derived from cyclopentyl and 2- or 3-methylcyclopentylamines; the 3-methyl derivatives were generally more active than the 2-substituted isomers.

The 2-hydroxy- and still more the 2-chloro-cyclopentylamido derivatives were only occasionally endowed with modest activity, being generally inactive or even endowed with hyperalgesic activity. Similarly, all but one of the cyclopentenyl derivatives were inactive or hyperalgesic. Thus the good analgesic activity of the cyclopentenylamidine **36**, previously described, represents an exception that is difficult to explain.

Considering the acyl moieties, the 3,4,5-trimethoxybenzoyl one was the more commonly present in active compounds, with the due reservation concering the trifluromethylsufonyl residue, since the corresponding derivatives might be active at lower doses than that presently used, which proved toxic. The maleyl derivatives were poorly active or inactive, nevertheless the very strong hyperalgesic activity of the 2-chlorocyclopentylmaleamic acid (**27**) is somewhat surprising.

The antinociceptive activity of the four most active compounds (Table V) reached the maximal intensity after 60 minutes from the oral administration. It is worth noting that compounds **17** and **35** presented a rapid onset of activity, differing from the trimethoxybenzoyl derivatives **7** and **11**, the activity of which increased slowly. Compound **17** exhibited a moderate level of activity also after two hours.

Unaccountable was the variation of activity with a growing dose from 1/24 mmoles to 1 mmole/kg (Table VI).

Compounds **7,11** and **35** presented a maximum of activity, respectively, with a dose equal to 1/12 and 1/6 of mmoles/kg; a further increase of the dose resulted in a decrease of activity. However, in the case of compounds **17** the activity augmented in the whole span of doses investigated, although such increase of activity was very modest with doses from 1/6 to 1 mmole/kg.

A more appropriate discussion of the present results will be possible after completion of the screening of another set of similar derivatives.

TABLE IV

Antinociceptive activity: phenylquinone writhing test (a)

Comp.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Var. %(b)	-2.1	-25.8	+23.8	-19.4	-3.1	+37.2	-33.3	-18.3
Comp:	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Var. %	+26.4	-22.2	-33.1	-5.5	-9.8	-10.6	+40.7	-20.0
Comp.	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Var. %	-43.2	-5.6	-22.4	+0.8	-7.6	-12.8	-5.1	+0.4
Comp.	**25**	**26**	**27**	**28**	**29**	**30**	**31**(3)	**32**(3)
Var. %	-13.2	-13.3	+84.5	-7.2	-6.5	+0.3	+9.9	+13.6
Comp.	**33**	**34**	**35**	**36**(3)	**37**(2) (c)	**38**	**39**	**40** **41**
Var. %	-14.2	+14.0	-32.3	-45.7	-56.7	(*)	(*)	(*)

(a) Dose: 0.167 mmoles/kg
(b) Var. %: percent variation of writhings during 20 min. compared with control animals (inhibition-, increase+). Reading was started 5 min. after phenylquinone injection; the irritant was introduced 60 min. after compounds administration.
(c) in reference (2), formic acid was used as irritant.
(*) about half of the animals died during the experiment.

TABLE V

Wrighings inhibition at different time from drug administration (0.167 mmoles/kg)

	% Inhibition			
time / comp.	15'	30'	60'	120'
7	6.2	14.9	33.3	4.0
11	- 1.2	6.0	33.1	14.8
17	33.0	26.8	43.2	23.7
35	19.2	11.8	32.3	16.9

TABLE VI

Writhings inhibition with different doses at 60 min. from drug administration

	% Inhibition			
dose mmol/kg / comp	0.0416	0.083	0.167	1
7	12.1	44.4	33.3	17.4
11	-5.4	-14.8	33.1	30.4
17	1.2	24.2	43.2	49.4
35	0.3	6.0	32.3	13.8

Acknowledgement

The financial support from the M.U.R.S.T. is gratefully acknowledged.

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Received June 2, 1993; accepted June 21, 1993.