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AMIDES AND FORMAMIDINES WITH ANTINOCICEPTIVE ACTIVITY (NOTE II)

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SUMMARY — *Forty amides, formamidines and trifluoromethylsulfonylamides bearing on the nitrogen a cyclohexyl residue, eventually 2-substituted, were prepared and tested for analgesic activity against a chemical stimulus. Good activity was exhibited by amides* ***9, 11*** *and* ***28****, by formamidine* ***34****, as well as by triflylamide* ***40****. Eleven additional compounds exhibited a moderate activity.*

RIASSUNTO — *Si riporta la sintesi di numerosi derivati ammidici, formammidinici e trifluorometilsolfonammidici, recanti sull'azoto un residuo cicloesilico, eventualmente 2-sostituito, che sono stati saggiati per la ricerca dell'attività analgesica contro stimolo chimico. Buona attività è stata mostrata dalle ammidi,* ***9, 11*** *e* ***28****, dalla formammidina* ***34*** *e dalla triflilammide* ***40****. Altri undici composti hanno mostrato moderata attività.*

Introduction

During our researches on new structures endowed with antiinflammatory and/or analgesic activities (1-4), the peripheral antinociceptive activity of some N-substituted cyclopentylamine derivatives was revealed.

Such activity was strongly influenced by the nature of the substituents on the nitrogen and on the cyclopentane ring, so that on changing the former the structural requirements of the latter for the best activity changed too.

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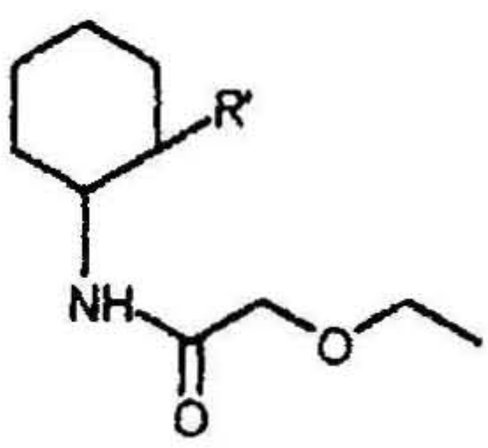
In an attempt to further support those observations some sets of cyclohexylamine derivatives were prepared and screened for antinociceptive activity against phenylquinone induced writhings.

The prepared compounds are listed in Scheme 1.

Scheme 1



(I)

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Ib = 2
Ic = 3
Id = 4
Ie = 5

(II)

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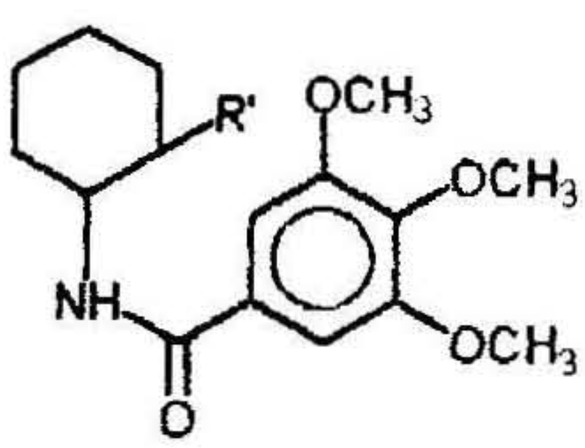
IIb = 7
IIc = 8
IId = 9
IIe = 10



(III)

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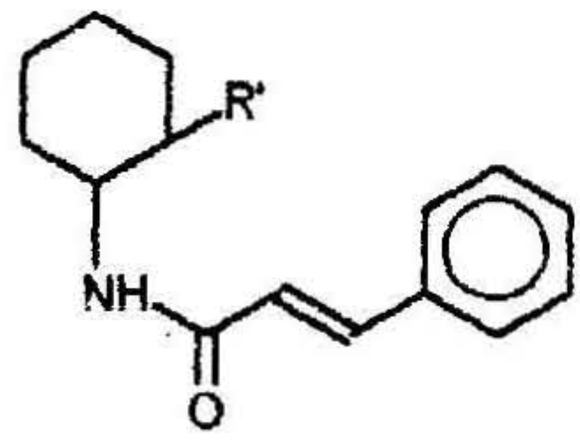

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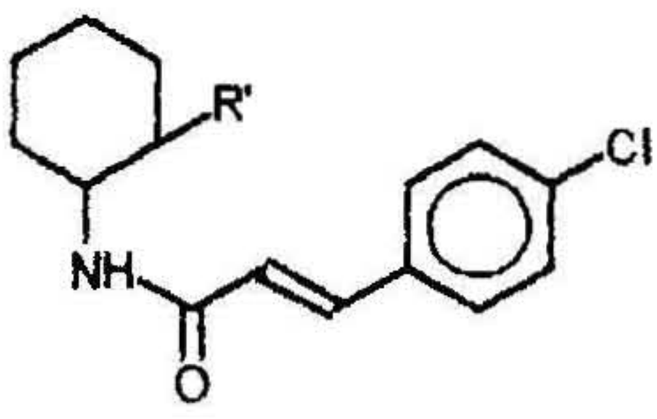
IVe = 20



(V)

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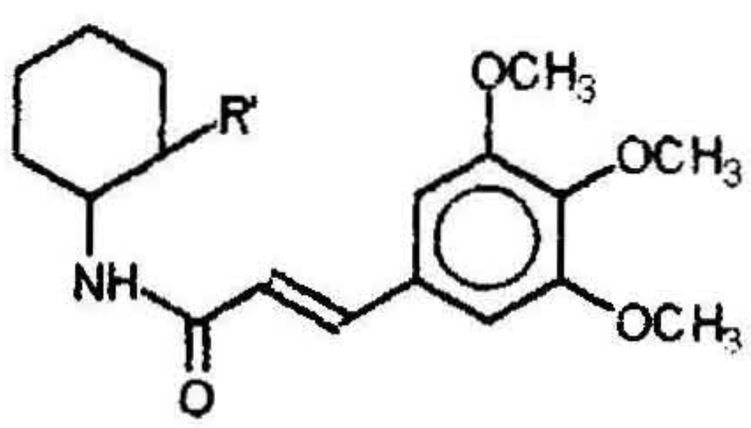
Vb = 22
Vc = 23
Vd = 24
Ve = 25



(VI)

VIa = 26
VIb = 27
VIc = 28
VId = 29
VIe = 30

(VII)

VIIa = 31
VIIb = 32
VIIc = 33
VIId = 34
VIIe = 35

(VIII)

VIIIa = 36
VIIIb = 37
VIIIc = 38
VIIId = 39
VIIIe = 40

a = R' = H
b = R' = CH_3
c = R' = t-(C_4H_9)
d = R' = $CH_2C_6H_5$
e = R' = OH

Chemistry

The preparation of amides **1-30** was effected by stirring the benzene solution of the suitable amino compound with an excess of appropriate acyl halide, in the presence of triethylamine at room temperature. The formamidines **31-35** and the trifluoromethylsulfonylamides **36-40** were prepared, as already described (1-4), by reacting the 2-substituted cyclohexylamine with dimethylformamide dimethylacetal and trifluoromethylsulfonic anhydride respectively.

The characteristics of compounds **1-40** are collected in Table I.

The structure of the obtained compounds was generally supported by elemental analysis and NMR spectra (Table II); in the case of formamidines and trifluoromethylsulfonylamides by GC-MS spectra (Table III).

Of the required starting amines cyclohexylamine and 2-methylcyclohexylamine were commercial products; 2-*tert*-butyl- and 2-benzylcyclohexylamine were prepared reducing the oximes of 2-*tert*-butyl- and 2-benzyl-cyclohexanone, respectively with sodium and absolute ethanol (5,6). 2-*tert*-butylcyclohexanone was commercially available, while 2-benzylcyclohexanone was prepared through the alkylation (7) of 1-pyrrolidino-1-cyclohexene (8). The oximes were prepared according to (9). At last (±)trans-2-aminocyclohexanol was prepared through ammonolysis of cyclohexene oxide (10,11).

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. Mass spectra (**31-40**) were obtained on a HP 5970A apparatus, using a capillary column HP1 of 25m length; programmed temperature was from 150°C to 300°C (10°C/min), detector temp. was 300°C and the carrier gas was Helium of 99.9998% purity, at 10 psi pressure.

*N-acyl- or N-aroylcyclohexylamides 2 monosubstituted (**1-30**)*

A stoichiometric quantity of the required acyl halide, increased of 25%, dissolved in 10-15 ml of benzene, was dropped 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 varying from 30 minutes to 2 hours (Table I); then the reaction 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 on silica gel (Table I).

TABLE I

Characteristics of compounds ***1-40***

Comp.	Formula	M.W.	Analyses	m.p. or b.p.(mm Hg) °C	Yield %	Reaction time	Method of purification
1	$C_{10}H_{19}NO_2$	185	CHN	58-59	36	2h	A
2	$C_{11}H_{21}NO_2$	199	CHN	100 /1	40	2h	B
3	$C_{14}H_{27}NO_2$	241	CHN	48-49	30	2h	A
4	$C_{17}H_{25}NO_2$	275	CHN	73-75	40	2h	A
5	$C_{10}H_{19}NO_3$	201	CHN	120-130/1	45	2h	B
6	$C_{13}H_{16}NOCl$	238	CHN	179-180	78	30'	A
7	$C_{14}H_{18}NOCl$	252	CHN	181-182	72	1h	A
8	$C_{17}H_{24}NOCl$	294	CHN	210-211	75	1h	A
9	$C_{20}H_{22}NOCl$	328	CHN	173-174	52	1h	A
10	$C_{13}H_{16}NO_2Cl$	253	CHN	173-175	62	1h	A
11	$C_{16}H_{23}NO_4$	293	CHN	180-181	75	30'	A
12	$C_{17}H_{25}NO_4$	307	CHN	196-198	40	1h	A
13	$C_{20}H_{31}NO_4$	249	CHN	225-226	44	1h	A
14	$C_{23}H_{29}NO_4$	383	CHN	208-210	47	1h	A
15	$C_{16}H_{23}NO_5$	309	CHN	174-175	51	1h	A
16	$C_{15}H_{19}NO$	229	CHN	180-181	43	1h	C
17	$C_{16}H_{21}NO$	243	CHN	162-164	50	1h	A
18	$C_{19}H_{27}NO$	285	CHN	218-219	60	1h	A
19	$C_{22}H_{25}NO$	319	CHN	191-192	46	1h	A
20	$C_{15}H_{19}NO_2$	245	CHN	122-124	40	1h	A
21	$C_{15}H_{18}NOCl$	264	CHN	203-204	75	1h	A
22	$C_{16}H_{20}NOCl$	278	CHN	195-196	60	1h	A
23	$C_{19}H_{26}NOCl$	320	CHN	184-186	30	1h	A
24	$C_{22}H_{24}NOCl$	354	CHN	180-182	50	1h	A
25	$C_{15}H_{18}NO_2Cl$	280	CHN	195-197	40	1h	D
26	$C_{18}H_{25}NO_4$	319	CHN	205-206	57	1h	C
27	$C_{19}H_{27}NO_4$	333	CHN	208-210	59	1h	C
28	$C_{22}H_{33}NO_4$	375	CHN	74-75	60	1h	A
29	$C_{25}H_{31}NO_4$	409	CHN	186-188	45	1h	D
30	$C_{18}H_{25}NO_5$	335	CHN	168-170	45	1h	D

(continue)

A) Crystallization (ethanol). B) Chromatography (silica gel column, eluent : benzene - aceton 8/2). C) Distillation under vacuum (bulb to bulb). D) Crystallization (ethanol-water 1/1).

continuation TABLE I

Comp.	Formula	M.W.	Analyses	m.p. or b.p.(mm Hg) °C	Yield %	Reaction time	Method of purification
31	$C_9H_{18}N_2$	154	GC-MS	58/5	64	1h	B
32	$C_{10}H_{20}N_2$	168	GC-MS	80-90/7	70	1h	B
33	$C_{13}H_{26}N_2$	210	GC-MS	95-100/3	65	1h	B
34	$C_{16}H_{24}N_2$	244	GC-MS	135-145/5	34	1h	B
35	$C_9H_{18}N_2O$	170	GC-MS	117-119	52	1h	B
36	$C_7H_{12}NO_2SF_3$	231	GC-MS	64-65	37	1h	D
37	$C_8H_{14}NO_2SF_3$	245	GC-MS	73-74	24	1h	D
38	$C_{11}H_{20}NO_3SF_3$	287	GC-MS	96-97	38	1h	D
39	$C_{14}H_{18}NO_2SF_3$	321	GC-MS	90-92	26	1h	D
40	$C_7H_{12}NO_3SF_3$	247	GC-MS	96-98	50	1h	D

A) Crystallization (ethanol). B) Chromatography (silica gel column, eluent : benzene - aceton 8/2). C) Distillation under vacuum (bulb to bulb). D) Crystallization (ethanol-water 1/1).

TABLE II

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

Comp.	δ ppm
1-5	0.9-1(m, CH_3t-butyl); 1.1-1.3(t, CH_3);1-2(m, CH_2); 2.5-3.7(q, CH_2-); 3.7-3.8(s, OCH_2CO); 6.2-7.4(m, aromatics).
6-10	0.98-0.95(d, CH_3); 1.2-2.2(m, CH_2); 3.4(s, OH for the product **10**); 4.5(m,CH); 5.8(s, NH); 7.2-7.7(m, aromatics).
11-15	0.95-0.99(m, CH_3); 1-3(m, CH_2); 3.8-3.9(s, OCH_3); 4.7(s, OH for the product **15**); 4-4.5(m, CH); 5.9-6.1(s, NH); 6.9-7.3(m, aromatics).
16-20	0.94-0.96(m, CH_3); 1.1-2(m, CH_2); 4.6(s, OH for the product **20**); 6.4-6.8(d, CH=CH); 5.5-5.9(s, NH); 7-7.5(m, aromatics).
21-25	0.94-0.96(m, CH_3); 1.1-2.1(m, CH_2); 3.7-4.5(m, CH); 4.7(s, OH for the product **25**); 5.5-5.9(s, NH); 6.2-6.7(d, CH=CH); 7-7.7(m, aromatics).
26-30	0.8-1.2(m, CH_3); 0.9-2(m, CH_2); 3.7-3.8(s, OCH_3); 4.7(s, OH for the product **30**); 5.5-6(s, NH); 6-6.5(m, CH=CH); 6.7-7.8(m, aromatics).

Solvent used $CDCl_3$; a mixture $CDCl_3$-DMSO (1:1) for the product **21-25**.

TABLE III

GC-MASS spectra of compounds ***31-40***

Products	t_r (min)	Most Important fragments (M^+/e)
31	8.76	154 : (M^+) ; 97 :(N-C_6H_{11}) ; 57 : [CH-N$(CH_3)_2$]; 44 :[N-$(CH_3)_2$]
32	9.08	168 :(M^+) ;97 : (2 methylcycloexyle) ; 71 :[N=CH-N$(CH_3)_2$] ; 57 :[CH-N$(CH_3)_2$]; 44 :[N-$(CH_3)_2$]
33	9.94	210 : (M^+) ; 84 :[CH_2-CH_2-C$(CH_3)_3$]; 57 : [CH-N$(CH_3)_2$] ; 44 :[N-$(CH_3)_2$]
34	14.69	244 : (M^+) ; 111 :[CH_2=CH-CH-N=CH-N$(CH_3)_2$]; 91 :(CH_2-C_6H_5); 57 :[CH-N$(CH_3)_2$] ; 44 :[N-$(CH_3)_2$]
35	3.42	170 : (M^+) ; 99 : ($C_6H_{11}OH$) ; 71 :[N=CH-N$(CH_3)_2$] ; 57 :[CH-N$(CH_3)_2$] ; 44 :[N-$(CH_3)_2$]
36	5, 69	231 : (M^+) ; 188 : (CH2=CH-CH_2-NHSO_2CF_3) ; 83 : (cyclohexyle) ; 69 :(CF_3) ; 64 :(SO_2); 55 :(CH_2=CH-CH-NH)
37	5.99	245 : (M^+) ; 202 :(CH_3-CH=CH-NHSO_2CF_3) ; 96:(cyclohexylamine); 69 :(CF_3); 64 :(SO_2); 55 : (CH_2=CH-CH-NH)
38	4.06	287 : (M^+) ; 57: [C$(CH_3)_3$] ; 55 : (CH_2=CH-CH-NH) ; 41 : (CH_2-CH=CH_2)
39	9.13	321 : (M^+) ; 188 :(CH_2=CH-CH_2-NHSO_2CF_3); 91 :(CH_2-C_6H_5); 69 : (CF_3) ; 64 :(SO_2); 41 :(CH_2-CH=CH_2)
40	3.19	247 : (M^+) ; 114 :(NH-$C_6H_{10}OH$); 97 : (NH-C_6H_{10}); 69 : (CF_3); 64 :(SO_2); 57 : (CH_2-CH=CHOH) ; 55 :(CH_2=CH-CH-NH)

N,N-Dimethyl-N'-cyclohexylformamidines 2 monosubstituted ***(31-35)***

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)cyclohexylamides 2 monosubstituted ***(36-40)***

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 a substantial amount of tar.

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 ad libitum*.

Antinociceptive activity against a chemical stimulus (peripheral analgesia)

This type of analgesia 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 were employed 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 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 three most active compounds (**11,28,34**) was measured 15, 60 and 120 minutes after the drug (0.167 mmoles/kg; *p.o.*) administration (Table V).

Results and Discussion

The results of the writhing test on 31 of the 40 compounds described are shown in Tables IV and V. Nine compounds were not tested due to the scarcety of material.

Of the tested compounds three (**11,28,34**) exhibited a good activity with writhing inhibition going from 56 to 65%; other thirteen compounds showed a moderate activity with writhing inhibition ranging between 25 and 44%. The remaining compounds were poorly active or completely inactive, but only one compound was endowed with hyperalgesic activity increasing the writhings for 28.7%. These results already indicate a clear difference between these substances and the cyclopentylderivatives previously studied (4), only a few of which were active as analgetics and many were characterized by hyperalgesic activity. Moreover mortality was never observed at the tested dose (0.167 mmoles/kg), thus the triflylcyclohexylamides differed strikingly from the corresponding cyclopentylderivatives previously studied.

Although active compounds were found in all the groups of cyclohexylamine derivatives now considered, the active compounds were mainly der-

TABLE IV

Antinociceptive activity: phenylquinone writhing test (a)

Comp.	**1**	**3**	**4**	**6**	**7**	**8**	**9**	**11**
Var. %(b)	-6.0	-29.4	-31.3	-4.5	+8.0	-15.3	- 43.8	- 58.6
Comp:	**12**	**13**	**14**	**15**	**17**	**18**	**19**	**21**
Var. %	-25.6	-14.4	-28.5	-32.5	-8.5	-29.1	-24.6	-12.4
Comp.	**22**	**24**	**26**	**27**	**28**	**30**	**31**	**32**
Var. %	-5.4	-9.1	+7.3	-6.5	-64.7	-8.3	-36.1	+28.7
Comp.	**33**	**34**	**35**	**37**	**38**	**39**	**40**	
Var. %	-31.9	-55.7	-26.9	-29.6	+3.4	-13.7	-40.0	

(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.

TABLE V

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

% Inhibition			
time / comp.	**15'**	**60'**	**120'**
11	**+1.9**	**-58.6**	**-33.5**
28	**-11.2**	**-64.7**	**-31.5**
34	**-63.0**	**-55.7**	**-2.6**

ived from 2-benzyl- and 2-*tert*-butylcyclohexylamine. For what concerns the acyl moiety, the activity was mainly bound to the trimethoxybenzoic and the dimethylformamidinic residues; however, apart from the 4-chlorocinnamoyl residue, the remaining acyl or aroyl residues gave some active compounds.

It should be observed once more that on changing the substituents on the alicyclic ring the structural requirements for the acyl or aroyl residue to give the best activity were also changing, so that the overall correlation between structure or physical properties and activity resulted rather elusive. Good activity was found for the highly lipophilic N-(3,4,5-trimethoxycinnamoyl)-2-benzylcyclohexylamine (**28**) and for the rather hydrophilic N-(triflyl)-2-hydroxycyclohexylamine (**40**).

Finally, on the three most active compounds (**11,28,34**) the temporal variation of activity was investigated (Table V).

Compounds **11** and **28** exhibited the maximal activity after 60 minutes from administration but this activity was slowly decreasing, remaining moderate after 2 hours.

On the contrary the amidine **34** showed a rapid onset of activity being highly active already after 15 minutes; such activity was still present after one hour but ceased completely during the next hour.

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