SYNTHESIS OF AMIDES WITH ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES

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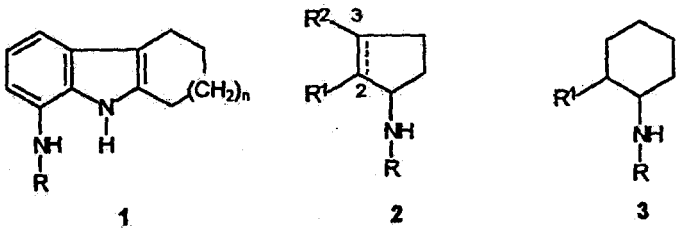
Summary – A series of N-Aroyl-cyclohexyl- and cyclohexenylamides 3- or 4-methylsubstituted were synthesized and evaluated for their anti-inflammatory and analgesic potencies, and gastrointestinal irritation liability. One compound, N-benzoyl-4-methyl-cyclohexylamide **6a**, possessed an anti-inflammatory activity comparable to that of indomethacin.

INTRODUCTION

In previous studies interesting anti-inflammatory and analgesic activities were found in some indole[1,2,3,4] (**1**) and cycloalkane or cycloalkene[5,6] (**2** and **3**) 1-(N-acyl)amino derivatives (Figure 1).



1 **2** **3**

n = 1,2,3

R = p-Cl-C_6H_4-CO; 3,4,5-$(CH_3O)_3$-C_6H_2-CO; p-Cl-C_6H_4-CH=CH-CO
3,4,5-(CH_3O)-C_6H_2-CH=CH-CO; HOOC-CH=CH-CO

R^1 = H; CH_3; OH; Cl

R^2 = H; CH_3

C_2C_3 = single bond; double bond

Fig. 1

Such activities were strongly influenced by the nature of the acyl moiety bound to the amino group and, in case of compounds **2** and **3**, by the substituents on cycloalkane or cycloalkene ring.

In order to acquire new insights on structure-activity relationships of this new class of compounds, in the present paper we describe the synthesis and the pharmacological evaluation of new derivatives **4a-f**, **5a-f** and **6a-f** (Figure 2), in which, respect to compounds of general structure **3**, the substituent on the ring, the methyl moiety, was shifted from position 2 to position 3 or 4, and a double bond between carbons 2-3 of the cycloalkane ring, in the case of compounds **4a-f**, was also introduced.

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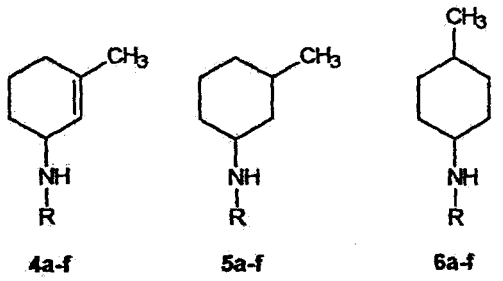


4a-f **5a-f** **6a-f**

a R = p-Cl-C_6H_4-CO-
b R = 3,4,5-$(CH_3O)_3$-C_6H_2-CO-
c R = C_6H_5-CH=CH-CO-
d R = p-Cl-C_6H_4-CH=CH-CO-
e R = 3,4,5-$(CH_3O)_3$-C_6H_2-CH=CH-CO-
f R = 3,4-(CH_2O_2)-C_6H_3-CH=CH-CO-

Fig. 2

CHEMISTRY

The preparation of amides **4a-f**, **5a-f**, and **6a-f** was performed by stirring the suitable amino compound **6a-c** with an excess of the appropriate acyl chloride, in benzene at room temperature, in the presence of triethylamine (Scheme 1).

The characteristics of compounds **4a-f**, **5a-f**, and **6a-f** are described in Table I.

The structure of the obtained compounds was generally supported by IR, GC-MS (Table II), and ^{1}H NMR (Table III) spectra.

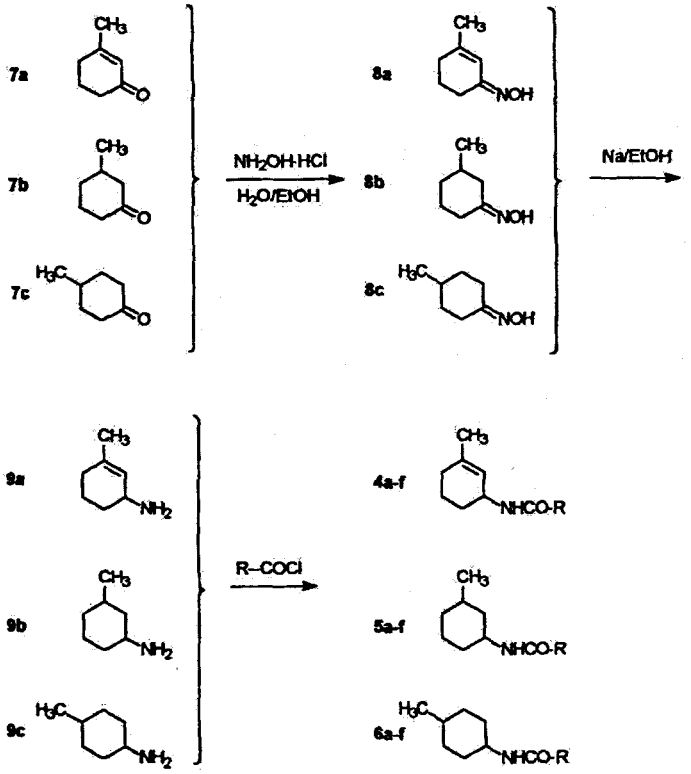
Starting amines **9a-c** were prepared reducing the ketoximes of 3-methyl-cyclohexenone, 3-methyl-, and 4-methyl- cyclohexanone **8a-c** with sodium in anhydrous ethanol[7,8], respectively.

The oxime **8a** was prepared by the direct action of hydroxylamine hydrochloride on the 3-methyl-ciclohexenone **7a** according to Adams[9].

The ketone **7a** was obtained according to an experimental procedure already described[10].

On the contrary, the oximes **8b,c** were prepared according to Lachman[11] starting from 3-methyl-

SCHEME 1



and 4-methyl-cyclohexanone **7b,c** which were commercially available.

In Table IV the characteristics of compounds **7a, 8a-c** and **9a-c** are described.

TABLE I - Characteristic data of compounds **4a-f, 5a-f** and **6a-f**

Comp.	Formula	M.W.	M.P. (°C)	Yield %	Method of purification
4a	$C_{14}H_{16}NOCl$	249	195-197	45	B
4b	$C_{17}H_{23}NO_4$	305	168-170	49	G
4c	$C_{16}H_{19}NO$	241	131-133	77	A
4d	$C_{16}H_{18}NOCl$	275	179-182	45	H
4e	$C_{19}H_{25}NO_4$	331	169-172	52	C
4f	$C_{17}H_{19}NO_3$	285	151-153	96	C-E
5a	$C_{14}H_{16}NOCl$	251	176-178	90	E
5b	$C_{17}H_{25}NO_4$	307	187-189	96	E
5c	$C_{16}H_{21}NO$	243	144-145	65	D-F
5d	$C_{16}H_{20}NOCl$	277	200-202	79	C-E
5e	$C_{19}H_{27}NO_4$	277	189-191	68	C-E
5f	$C_{17}H_{21}NO_3$	287	161-163	66	F
6a	$C_{14}H_{16}NOCl$	251	235-236	98	C
6b	$C_{17}H_{25}NO_4$	307	209-211	67	E
6c	$C_{16}H_{21}NO$	243	199-201	73	G
6d	$C_{16}H_{20}NOCl$	277	252-253	89	C-E
6e	$C_{19}H_{27}NO_4$	333	220-222	85	E
6f	$C_{17}H_{21}NO_3$	287	229-231	42	E

A, B) Chromatography (silica gel column, eluant: benzene-acetone 8/2 and 9/1). **C)** Flash chromatography (silica gel column, eluant: benzene-acetone 8/2). **D)** Flash chromatography (silica gel column, eluant: petroleum ether- ethyl acetate 7/3). **E)** Crystallization (ethanol). **F)** Crystallization (ethanol/water). **G)** Crystallization (methanol). **H)** Analyzed as pure compound.

Among the acyl chlorides, most of them were commercially available, except 3,4-methylenedioxycinnamoyl chloride which was prepared according to the procedure described by Koo[13].

PHARMACOLOGY

All compounds **4a-f, 5a-f** and **6a-f** were subjected to a series of *in vivo* tests in order to evaluate their pharmacological activity. The anti-inflammatory activity was studied by means of the carrageenan rat paw edema assay, whereas the acetic acid writhing test was used to assess the analgesic activity in mice. Higher doses were administered to rats in order to study the irritative and ulcerogenic action on the mucosa of the stomach and small intestine up to the distal ileum. Indomethacin was included in all tests as reference drug.

RESULTS AND DISCUSSION

As can be noted (Table V), compounds **4b,c** and **6a,b** showed an interesting and dose-depending anti-inflammatory activity, compounds **5b,d,e** and **6c** showed a lower activity, whereas all the other compounds resulted moderately or poorly active. It is worth to note that **6a** at 40 mg/kg was significantly more active than indomethacin at 5 mg/kg.

These results were substantially confirmed by acetic acid writhing test, used to assess the analgesic activity (Table VI).

In fact, best results were shown by compounds **4b,c** and **6a**, and also **5b,d** and **6c** showed a significant activity in this test. The main exceptions were **6b**, that resulted almost lacking analgesic activity, and compounds **4a** and **6d**, that on the contrary showed a moderate analgesic activity, although they were almost inactive in rat paw edema test.

Concerning the ulcerogenic potency (Table VII), all compounds showed to be much less ulcerogenic than Indomethacin (at 5 mg/kg) especially in view of the severe dose used (100 mg/kg). Anyway it does not always correspond to the anti-inflammatory and/or analgesic effect. In fact, the ulcerogenic potency of the most active compounds (**4b,c** and **6a**) was not significantly different from the potency of compounds that were completely lacking in anti-inflammatory and analgesic activities.

These pharmacological results allow us to draw some preliminary conclusions on structure-activity relationships.

As regards the acyl moiety, the anti-inflammatory and analgesic activities were mainly linked to the 4-chlorobenzoyl (**6a**) and trimetoxybenzoyl residues (**4b, 5b, 6b**). Among the derivatives bearing an (un)substituted cynnamoyl moiety, best results were obtained by compounds without substituents on the phenyl ring (**4c, 6c**), although the activity of some of the others was not negligible (**5d, 6d**).

TABLE II - GC-MASS spectra of compounds **4a-f**, **5a-f** and **6a-f**

GC-MS			IR	
Products	t_r (min)	Most Important Fragments (M^+/z)	ν N-H (cm^{-1})	ν C=O (cm^{-1})
4a	19.4	249: (M^+); 156: [p-Cl-C_6H_4-CO-NH_2]; 138: [p-Cl-C_6H_4-CO]; 111: [$C_7H_{13}N$].	3280	1640
4b	7.2	305: (M^+); 211: [$(CH_3O)_3$-C_6H_2-CO-NH_2]; 195:[$(CH_3O)_3$-C_6H_2-CO]; 110: [$C_7H_{13}N$].	3260	1650
4c	21.4	241: (M^+); 146: [C_6H_5-CH=CH-CO-NH_2]; 131: [C_6H_5-CH=CH-CO]; 103: [C_6H_5-CH=CH]; 110: [$C_7H_{13}N$].	3270	1625
4d	15.9	275: (M^+); 182: [p-Cl-C_6H_4-CH=CH-CO-NH_2]; 165: [p-Cl-C_6H_4-CH=CH-CO]; 137: [p-Cl-C_6H_4-CH=CH]; 110: [$C_7H_{13}N$].	3250	1640
4e	18.9	331: (M^+); 236: [$(CH_3O)_3$-C_6H_2-CH=CH-CO-NH_2]; 221: [$(CH_3O)_3$-C_6H_2-CH=CH-CO]; 193: [$(CH_3O)_3$-C_6H_2-CH=CH]; 110: [$C_7H_{13}N$].	3260	1650
4f	17.9	285: (M^+); 190: [3,4-(CH_2O_2)-C_6H_3-CH=CH-CO-NH_2]; 175: [3,4-(CH_2O_2)-C_6H_3-CH=CH-CO]; 147: [3,4-(CH_2O_2)-C_6H_3-CH=CH]; 110: [$C_7H_{13}N$].	3280	1620
5a	12.3	251: (M^+); 156: [p-Cl-C_6H_4-CO-NH_2]; 139: [p-Cl-C_6H_4-CO]; 111: [$C_7H_{11}N$].	3280	1620
5b	17.9	307: (M^+); 211: [$(CH_3O)_3$-C_6H_2-CO-NH_2]; 195: [$(CH_3O)_3$-C_6H_2-CO]; 112: [$C_7H_{15}N$].	3260	1645
5c	15.4	243: (M^+); 147: [C_6H_5-CH=CH-CO-NH_2]; 131: [C_6H_5-CH=CH-CO]; 103: [C_6H_5-CH=CH]; 112: [$C_7H_{15}N$].	3270	1620
5d	15.8	277: (M^+); 180: [p-Cl-C_6H_4-CH=CH-CO-NH_2]; 165: [p-Cl-C_6H_4-CH=CH-CO]; 137: [p-Cl-C_6H_4-CH=CH]; 112: [$C_7H_{15}N$].	3230	1640
5e	18.6	333: (M^+); 238: [$(CH_3O)_3$-C_6H_2-CH=CH-CO-NH_2]; 221: [$(CH_3O)_3$-C_6H_2-CH=CH-CO]; 193: [$(CH_3O)_3$-C_6H_2-CH=CH]; 112: [$C_7H_{15}N$].	3270	1645
5f	17.6	287: (M^+); 190: [3,4-(CH_2O_2)-C_6H_3-CH=CH-CO-NH_2]; 175: [3,4-(CH_2O_2)-C_6H_3-CH=CH-CO]; 147: [3,4-(CH_2O_2)-C_6H_3-CH=CH]; 112: [$C_7H_{15}N$].	3270	1640
6a	7.1	251: (M^+); 156: [p-Cl-C_6H_4-CO-NH_2]; 139: [p-Cl-C_6H_4-CO]; 112: [$C_7H_{15}N$].	3260	1640
6b	16.4	307: (M^+); 211: [$(CH_3O)_3$-C_6H_2-CO-NH_2]; 195: [$(CH_3O)_3$-C_6H_2-CO]; 112: [$C_7H_{15}N$].	3240	1650
6c	8.6	243: (M^+); 147: [C_6H_5-CH=CH-CO-NH_2]; 131: [C_6H_5-CH=CH-CO]; 103: [C_6H_5-CH=CH]; 112: [$C_7H_{15}N$].	3280	1620
6d	17.4	277: (M^+); 180: [p-Cl-C_6H_4-CH=CH-CO-NH_2]; 165: [p-Cl-C_6H_4-CH=CH-CO]; 137: [p-Cl-C_6H_4-CH=CH]; 112: [$C_7H_{15}N$].	3280	1630
6e	13.6	333: (M^+); 236: [$(CH_3O)_3$-C_6H_2-CH=CH-CO-NH_2]; 221: [$(CH_3O)_3$-C_6H_2-CH=CH-CO]; 193: [$(CH_3O)_3$-C_6H_2-CH=CH]; 112: [$C_7H_{15}N$].	3260	1650
6f	11.9	287: (M^+); 190: [3,4-(CH_2O_2)-C_6H_3-CH=CH-CO-NH_2]; 175: [3,4-(CH_2O_2)-C_6H_3-CH=CH-CO]; 147: [3,4-(CH_2O_2)-C_6H_3-CH=CH]; 112: [$C_7H_{15}N$].	3280	1640

Concerning the substituted cycloaliphatic moiety, it is not easy to establish the best position of the methyl group on the ring. In fact some evidences seem to indicate position 4: for example, in case of **6a**, shifting the methyl group to position 3 led to compounds with diminished activity (**4a** and **5a**). In other cases the introduction of a double bond between position 2 and 3 avoids this decrease in activity. For example, **5b** was less active than **6b** (at least in rat paw edema test), but **4b** had the same or even a greater pharmacological potency compared to **6b**; besides, **4c** was significantly more active than **6c** and **5c** in both pharmacological tests.

In conclusion, this series of amides provide information for the development of new interesting and selective anti-inflammatory and analgesic compounds with low ulcerogenic activity. A more detailed pharmacological characterization is required to establish their action mechanism.

Further studies concerning structure activity relationship are planned to establish definitively the ideal position, bulk and chemical nature of the substituents on the cycloaliphatic ring.

EXPERIMENTAL

A) CHEMISTRY

Precoated silica gel Merck 60 F254 plates were used for thin layer chromatography: detection of components was made by UV light (254 nm) and/or treatment with iodine vapors. Chromatographic and flash-chromatographic separations were performed in columns packed with silica gel 60 (Merck 70-230 mesh ASTM) and in columns packed with silica gel 60 (Merck 230-400 mesh ASTM), respectively. Melting

TABLE III - ^{1}H NMR spectra of compounds **4a-f, 5a-f** and **6a-f**

Comp.	δ ppm
4a	0.9-0.94: (d, 3H CH_3); 0.75-2.1: (m, 6H cycloal.); 3.85-4: (m, 1H CH-N); 5.4: (s, 1H CH=); 5.95-6.05: (d, 1H NH); 7.31-7.75: (m, 4H aromatics).
4b	0.8-2.1: (m, 7H cycloal.); 0.9-0.95: (d, 3H CH_3); 3.87: (s, 9H $3OCH_3$); 3.8-4: (m, 1H CH-N); 5.24: (s, 1H CH=); 6-6.1: (d, 1H NH); 6.99: (s, 2H aromatics).
4c	0.9-0.94: (d, 3H CH_3); 0.75-2.1: (m, 6H cycloal.); 3.85-4: (m, 1H CH–N); 5.38: (s, 1H CH=); 5.75-5.85: (d, 1H NH); 6.3-6.4: (d, 1H CO-CH=); 7.6-7.7: 7.3-7.55: (m, 5H aromatics); 7.6-7.7: (d, 1H Ph-CH=).
4d	0.88-0.91: (d, 3H CH_3); 0.75-2.1: (m, 6H cycloal.); 3.75-3.9: (m, 1H CH-N); 5.38: (s, 1H CH=); 5.75-5.85: (d, 1H NH); 6.35-6.4: (d, 1H CO-CH); 7.25-7.45: (m, 4H aromatics); 7.53-7,6: (d, 1H, Ph-CH=).
4e	0.88-0.91: (d, 3H CH_3); 0.75-2.1: (m, 6H cycloal.); 3.85: (s, 9H $3OCH_3$); 3.8-3.91: (m, 1H CH-N); 5.36: (s, 1H CH=); 5.6-5.65: (d, 1H NH); 6.25-6.3: (d, 1H CO-CH=); 6.7: (s, 2H aromatics); 7.45-7.5: (d, 1H Ph-CH=).
4f	0.87-0.9: (d, 3H CH_3); 0.72-2.05: (m, 6H cycloal.); 3.8-3.95: (m, 1H CH-N); 5.36: (s, 1H CH=); 5.65-5.75: (d, 1H NH); 5.96: (s, 2H O-CH_2-O); 6.18-6.23: (d, 1H CO-CH=); 6.7-7: (m, 3H aromatics); 7.5-7.54: (d, 1H Ph-CH).
5a	0.88-0.95: (d, 3H CH_3); 0.65-2.1: (m, 9H cycloal.); 3.8-4: (m, 1H CH-N); 6.1-6.3: (d, 1H NH).
5b	0.88-0.95: (d, 3H CH_3); 0.65-2.1: (m, 9H cycloal.); 3.78-3.9: (s, 9H $3OCH_3$); 3.8-4: (m, 1H CH-N); 6-6.1: (d, 1H NH); 6.9-7: (s, 2H aromatics).
5c	0.85-0.95: (d, 3H CH_3); 0.7-2.1: (m, 9H cycloal.); 3.8-4: (m, 1H CH-N); 5.85-6: (d, 1H NH); 6.38-6.5: (d, 1H CO-CH=); 7.25-7.38: (m, 5H aromatics); 7.58-7.65: (d, 1H =CH).
5d	0.85-0.95: (d, 3H CH_3); 0.75-2.1: (m, 9H cycloal.); 3.8-4: (m, 1H CH-N); 5.6-5.75: (d, 1H NH); 6,3-6,4: (d, 1H CO-CH); 7.25-7.45: (m, 4H aromatics); 7.5-7.6: (d, 1H CH=).
5e	0.85-0.95: (d, 3H CH_3); 0.70-2.1: (m, 9H cycloal.); 3.8-4: (s, 9H $3OCH_3$); 5.6-5.75: (d, 1H NH); 6.25-6.35: (d, 1H CO-CH=); 6.7: (s, 2H aromatics); 7.48-7.53: (d, 1H =CH).
5f	0.85-0.95: (d, 3H CH_3); 0.65-2.1: (m, 9H cycloal.); 3.85-4: (m, 1H CH-N); 5.78-5.86: (d, 1H NH); 5.9-6.1: (s, 2H O-CH_2-O); 6.18-6.3: (d, 1H CO-CH=); 6.7-7: (m, aromatics); 7.46-7.54: (d, 1H =CH).
6a	0.85-0.95: (d, 3H CH_3); 1-2.1: (m, 9H cycloal.); 3.8-3.98: (m,1H CH-N); 5.9-6: (d, 1H NH); 7.3-7.7: (m, 4H aromatics).
6b	0.8-0.9: (d, 3H CH_3); 1-2.1: (m, 9H cycloal.); 3.85: (s, 9H $3OCH_3$);3.75-3.95: (m, 1H CH-N); 5.95-6.05: (d, 1H NH); 6.95: (s, 2H aromatics).
6c	0.87-0.89: (d, 3H CH_3); 1-2.1: (m, 9H cycloal.); 3.75-3.95: (d, 1H CH-N); 5.65-5.75: (d, 1H NH); 6.35-6.45: (d, 1H CO-CH=); 7.3-7.5: (m, 5H aromatics); 7.55-7.65: (d, 1H =CH).
6d	0.88-0.91: (d, 3H CH_3); 1-2.1: (m, 9H cycloal.); 3.75-3.9: (m, 1H CH-N); 5.45-5.55: (d, 1H NH); 6.3-6.35: (d, 1H CO-CH=); 7.25-7.45: (m, 4H aromatics); 7.59-7.6: (d, 1H =CH).
6e	0.85-0.95: (d, 3H CH_3); 1-2.1: (m, 9H cycloal.); 3.85: (s, 9H $3OCH_3$); 3.65-4: (m, 1H CH-N); 5.65-5.75: (d, 1H NH); 6.25-6.35: (d, 1H CO-CH=); 6.72: (s, 2H aromatics); 7.48-7.56: (d, 1H =CH).
6f	0.85-0.95: (d, 3H CH_3); 1-2.1: (m, 9H cycloal.); 3.75-3.9: (m, 1H CH-N); 5.41-5.71: (d, 1H NH); 5.9: (s, 2H O-CH_2-O); 6.1-6.2: (d, 1H CO-CH=); 6.7-6.9: (m, 3H aromatics); 7.4-7.5: (d, 1H =CH).

points were determined with a Kofler hot stage microscope and are uncorrected.

IR spectra were recorded on a Perkin-Elmer mod. 298 spectrophotometer, including solid samples in KBr pellets and liquid samples as films. The ^{1}H NMR measurements were performed on a Varian XL 200 Spectrometer, using $CDCl_3$ as solvent and tetramethyl silane (TMS) as internal standard. Chemical shifts (δ) are expressed in p.p.m. downfield from tetramethylsilane.

GC/MS spectra were obtained on a HP 5970A apparatus, equipped with a capillary column HP-5 (25mx0.2 mmx0.11 μm). Programmed temperature ranged from 100 °C to 300 °C (10 °C/min), detector temperature was set at 300 °C and carrier gas was helium at 10 psi of pressure.

Commercially available solvents and chemicals were usually used for syntheses.

3-METHYLCYCLOHEX-2-ENONE, 7a

It was prepared by treating ethylacetoacetate, powdered paraformaldehyde and piperidine according to the literature[10].

Physical and spectral data are reported in Table IV.

3-METHYLCYCLOHEX-2-ENONE OXIME, 8a[9]

13.8 g (0.199 mol) of hydroxylamine hydrochloride and 20.7 g (0.152 mol) of sodium acetate crystals ($Na\ C_2\ H_3\ O_2$-H_2O) were dissolved in 55 ml of water in two-necked round bottomed flask, fitted with a thermometer and a mechanical stirrer.

The solution was then warmed up to about 40 °C and 13.8 g (0.126 mol) of 3-methylcyclohex-2-enone were added. The mixture was stirred for 30 min. White solid product was filtered

off and purified by recrystallization from hot water to give **8a** (8.6 g, 55 %), m.p. 48-50 °C.

Physical and spectral data are reported in Table IV.

3-METHYL- and 4-METHYLCYCLOHEXANONE OXIME, 8b,c

These compounds were prepared by using the procedure already described by Lachman[11]: yields, m.p., IR spectral data are reported in Table IV.

3-METHYLCYCLOHEX-2-EN-1-AMINE, 9a[7,8]

To a solution of 3.36 g (0.027 mol) 3-methylcyclohex-2-enone oxime **8a**, in 56 ml of boiling anhydrous ethanol 6.5 g (0.283 mol) of clean sodium (previously cut into small pieces) were added at a controlled rate for obtaining that the reaction, although vigorous, remains under control. When sodium has reacted, the reaction mixture was cooled and diluted with aqueous ethanol solution (1/1) and then it was treated with concentrated hydrochloric acid, with stirring until the solution was acid to litmus.

The white precipitate, in the cooled reaction mixture, was filtered off and the solvent evaporated under vacuum to leave a viscous oil, which was washed with diethyl ether discarding the ether layer. The aqueous layer was basified with 2N NaOH solution and extracted with diethyl ether which was dried with anhydrous sodium sulfate, and evaporated under vacuum to leave a oily residue. The crude oil was distilled on a Kugelrohr apparatus under vacuum (bp 45-65 °C, 0.5 mm Hg) to yield 1.76 g (59%) of the 3-methylcyclohex-2-en-1-amine **9a**, as a clear liquid (Table IV).

TABLE IV - Characteristics of intermediate products

Comp.	MW	m.p or b.p./(mm Hg) °C	Yield %	IR (cm^{-1})
7a	110	95/25	44	1680 (ν C=O)
8a	125	48-50	55	3240(ν OH); 1700(ν C=N)
8b	127	110-116/15	93	3260(ν OH); 1650(ν C=N)
8c	127	90-100/5	85	3210(ν OH); 1690(ν C=N)
9a	111	45-65/0.5	59	3340-3260(ν NH_2);1570(ν NH_2)
9b	113	50-70/10[12]	62	3320-3260(ν NH_2);1580(ν NH_2)
9c	113	50-70/10[12]	70	3320-3260(ν NH_2);1580(ν NH_2)

[12] Lit: 151 °C/730 mmHg.
[12] Lit: 148 °C/717 mmHg.

3-METHYL- AND 4-METHYL-CYCLOHEXYLAMINE, 9b,c

Compounds **9b,c** were prepared as reported for derivative **9a**, starting from the suitable 3-methyl- and 4-methyl-cyclohexanone oxime **8b,c**[7,8].

Physical analytical and spectroscopic data are reported in Table IV.

N-AROYLCYCLOHEXYL- AND N-AROYLCYCLOHEXENYL-AMIDES 3 OR 4 MONOSUBSTITUTED, 4a-f, 5a-f AND 6a-f

The appropriate amine (5 mmol), dissolved in anhydrous benzene (30 ml), was dropwise added to a solution of acyl chloride (a stoichiometric quantity, increased of 10-20%) and stirred in the same solvent (10-15 ml).

The mixture was stirred at room temperature for a time varying from 30 minutes to 2 hours; at this time, the reaction was considered completed, after having monitored the disappearance of starting materials by TLC analysis (benzene-acetone 8:2 v/v eluant). It 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

TABLE V - Carrageenan rat paw edema: anti-inflammatory activity

Compound	Dose	% Edema inhibition relative to control at:			
	(mg/kg po)	3rd h	ED_{50} (mg/kg)	4th h	ED_{50} (mg/kg)
Indomethacin	5	-63		-68	
4a	40	-22		-17	
	40	-61		-67	
4b	20	-50	*24.4*	-56	*18.5*
	10	-24	*(19.5-30.6)*	-33	*(14.4-23.7)*
	40	-46		-54	
4c	20	-28		-37	*33.9*
	10	-10		-21	*(26.6-43.3)*
4d	40	-13		- 7	
4e	40	-17		-30	
5a	40	-22		-15	
5b	40	-44		-38	
5c	40	-14		-10	
5d	40	-48		-42	
5e	40	-30		-38	
	40	-82		-84	
6a	20	-46	*20.9*	-53	*17.7*
	10	-16	*(18.1-24.1)*	-26	*(15.5-20.3)*
	40	-58		-64	
6b	20	-34	*31.8*	-26	*31.2*
	10	- 8	*(26.9-37.6)*	- 7	*(26.9-36.1)*
6c	40	-45		-37	
6d	40	-33		-29	
6e	40	-33		-29	
6f	40	-17		-14	

TABLE VI - Acetic acid writhing test: analgesic activity

Compound	Dose (mg/kg po)	Mean No of writhes in 25 min period after treatment ± SE	% Decrease relative to controls
Controls	-	47.3 ± 4.1	-
Indomethacin	5	23.0 ± 3.9	-51
4a	40	31.3 ± 3.8	-34
4b	40	24.9 ± 4.2	-47
4c	40	26.1 ± 2.8	-45
4d	40	39.6 ± 5.1	-16
4e	40	36.7 ± 3.7	-22
5a	40	33.8 ± 5.2	-28
5b	40	35.7 ± 3.6	-24
5c	40	38.6 ± 4.1	-18
5d	40	37.1 ± 2.8	-22
5e	40	41.3 ± 2.8	-13
6a	40	25.1 ± 3.3	-47
6b	40	41.2 ± 4.5	-13
6c	40	35.8 ± 2.9	-24
6d	40	29.7 ± 5.1	-37
6e	40	37.5 ± 4.2	-21
6f	40	36.7 ± 4.3	-22

TABLE VII - Induction of gastric lesions in rats

Compound	Dose (mg/kg po)	6th h after treatment, animals with:	
		Hyperaemia (%)	Ulcers (%)
Indomethacin	5	80	60
4a	100	60	30
4b	100	50	40
4c	100	70	50
4d	100	40	20
4e	100	80	20
5a	100	20	20
5b	100	50	30
5c	100	30	20
5d	100	60	40
5e	100	50	40
6a	100	50	30
6b	100	40	30
6c	100	30	20
6d	100	40	20
6e	100	60	30
6f	100	30	30

hydro-alcoholic mixture. For a complete purification, whereas in some cases a chromatography on silica gel column with benzene-acetone 8:2 as eluant was necessary, in other cases a Flash chromatography was carried out using silica gel 60 (Merck 230-400 mesh ASTM). The appropriate fractions were collected and evaporated to yield the desired products (table I).

All compounds exhibited IR, GC/Mass, and ^{1}H NMR spectra consistent with those of the structures assigned (Tables II and III).

B) PHARMACOLOGY

Tested compounds were administered orally by gavage in 1% methylcellulose suspension, using first a dose of 40 mg/kg and then lower doses if a significant activity was found.

Gastric ulcerogenic action was studied in rats which were treated orally with high doses (100 mg/kg).

Indomethacin was included in all tests for comparison purposes at the dose level of 5 mg/kg.

The following experimental procedures were employed:

ANTI-INFLAMMATORY ACTIVITY

Paw edema inhibition test[14] was used on rats. Groups of 5 rats of both sexes (body weight 180-250 g), pregnant females excluded, were given a dose of a test compound. Thirty min later 0.2 ml of 1% carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw. The paw volume was measured by a water plethysmometer Socrel and then measured again 1, 2, 3, 4 h later. The mean increase of paw volume at each time interval was compared with that of the control group (5 rats treated with carrageenan, but not treated with test compounds) at the same time intervals and percent inhibition values were calculated. Experimental results at 3rd and 4th hours are listed in Table V.

ANALGESIC ACTIVITY

Acetic acid writhing test[15] was used on mice. Groups of 5 mice (body weight 20-30 g) of both sexes, pregnant females excluded, were given a dose of a test compound. Thirty min later the animals were injected intraperitoneally with 0.25 ml/mouse of 0.5% acetic acid solution and writhes were counted during the following 25 min. The mean number of writhes for each experimental group and percent decrease compared with the control group (5 mice not treated with test compounds) were calculated. Experimental results are listed in Table VI.

ULCEROGENIC ACTION

Groups of 10 rats (body weight 180-250 g) of both sexes, pregnant females excluded, were treated with an oral dose of a test compound, except the control group[16]. All animals were sacrificed 6 h after dosing and their stomachs and small intestines were examined using a 2×2 binocular magnifier, to assess the incidence of hyperemia and ulcers. All the ulcers >0.5 mm were recorded. Experimental results are listed in Table VII.

This work was partially supported by M.U.R.S.T. (60 %).

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Received September 18, 1996; accepted October 30, 1996